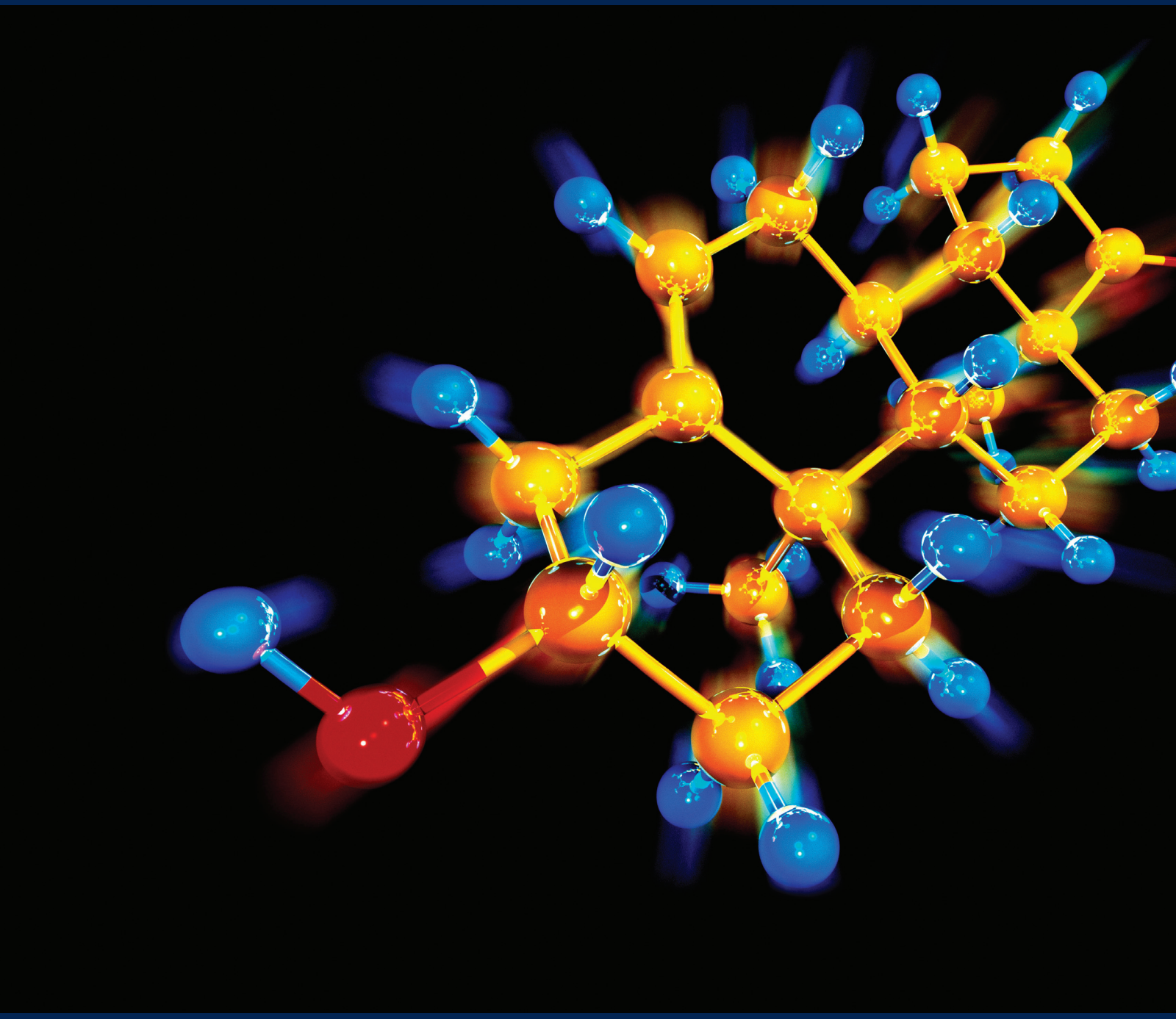
Journal of Chemistry

Five-Membered Nitrogen Heterocyclic Compounds

Guest Editors: Ahmad Sazali Hamzah, Zurina Shaameri, and Suleyman Goksu



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Editorial

Five-Membered Nitrogen Heterocyclic Compounds

Ahmad Sazali Hamzah,[1] Zurina Shaameri,[1] and Suleyman Goksu[2]

[1] *Institute of Science, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*
[2] *Chemistry Department, University of Attartuk, 25400 Erzurum, Turkey*

Correspondence should be addressed to Ahmad Sazali Hamzah; asazali@salam.uitm.edu.my

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Five-membered nitrogen heterocycles with a pyrrolidine moiety or pyrrolidine derivatives are often found in many bioactive molecules. Pyrrolidine, which is also known as tetrahydropyrrole, is a cyclic secondary amine with a five-membered heterocycle containing carbon atoms and nitrogen. Pyrrolidine can be prepared from 1,4-diaminobutanes by the action of acids. Furthermore, it can act as a strong base, and it is often used in homogeneous nonaqueous reactions.

Major fractions of organic compounds isolated from nature are comprised of nitrogen heterocycles. Such five-membered nitrogen heterocycles with a pyrrolidine ring system include nicotine, tryptamine, and vinblastine and possess considerable biochemical, pharmaceutical, and agricultural importance. These natural compounds may have hydroxyproline, 2-pyrrolidone, streptopyrrolidine, or diphenylprolinol rings as part of their well-defined conformations.

Apart from having significant structural features in many naturally occurring bioactive natural products, these ring systems can act as versatile intermediates towards the synthesis of more complex medicinally important compounds such as aniracetam, doxapram, cotinine, clausenamide, lactacystin, detoxine, and codonopsinine. These compounds have received much attention lately due to their diverse medicinal properties such as antibacterial, antibiotics, antitumor, and cytotoxic effects.

Constructing highly functionalized heterocyclic compounds would seem to be essential and significant prior to furnishing many of these biologically active natural constituents. Preparing enantiopure polysubstituted pyrrolidine derivatives can even be a more challenging task. A number of stereoselective methods for the synthesis of polysubstituted pyrrolidines have been reported in the last decade. This is due to the fact that the demand for enantiomerically pure drugs, agrochemicals, and food additives is rising, because pure enantiomers are often more target specific and have fewer side effects than racemic mixtures. Furthermore, the global market for chiral drugs alone currently stands at USD 100 billion and steadily grows at the rate of 9% per annum.

During the synthesis of a complex product consisting of several steps, it is essential for practical and economical reasons to introduce the proper stereochemistry in an early stage of the synthesis. This can be achieved by utilizing chiral building blocks that are enantiomerically pure and have functionalities that allow them to be transformed in the desired products. Nevertheless, stereocontrolled synthetic strategies will also have to be employed to acquire the potential products.

Diverse synthetic knowledge and chemical possibilities or transformations would be inevitably explored when conducting research in such area. During such synthetic processes, novel pharmacological agents beneficial in curing human diseases as well as new scientific findings may well be generated.

Research accounts in the field of organic chemistry and synthesis of five-membered nitrogen heterocyclic compounds (both aromatic and nonaromatic), as well as natural products with such heterocyclic systems, submitted and accepted in this publication complement the required standards and features of the journal.

The articles generally contain definitive and comprehensive reports of significant findings obtained *via* original work in heterocyclic chemistry. Some display novel synthetic methodology towards active heterocyclic materials which include efficient route, reproducible methodology, stereospecific materials, high product yields, and enantiomeric excess. Some works had also reported on findings from biological

studies. Scientifically and fundamentally sound manuscripts with sufficient scientific data and evidence are highly considered.

Ahmad Sazali Hamzah
Zurina Shaameri
Suleyman Goksu

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Research Article

Synthesis, Characterization, and Biological Activity of Novel Schiff and Mannich Bases of 4-Amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione

Uzma Yunus, Moazzam H. Bhatti, Naima Rahman, Nosheen Mussarat, Shazia Asghar, and Bilal Masood

Department of Chemistry, Allama Iqbal Open University, Islamabad 44000, Pakistan

Correspondence should be addressed to Uzma Yunus; uzma_yunus@yahoo.com

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The present work describes the syntheses and antimicrobial activity studies of a series of novel Schiff bases (**4a–4i**) and their Mannich bases (**5a–5h**) starting from 4-amino-3-(N-phthalimido-methyl)-1,2,4-triazole-5-thione (**3**). All the synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, and MS. All the synthesized compounds were screened for four Gram-negative strains, one Gram-positive strain of bacteria, and one diploid fungal strain. In general the antimicrobial activity increased remarkably on the introduction of azomethine functionality in parent triazole (**3**). The antimicrobial activity further improved when morpholine group was added to them except for *Enterobacter cloacae*, where loss of activity was observed. The results are promising and show that the fine tuning of the structures (**5a, 5b, 5e, 5f,** and **5h**) can lead to some new antimicrobial compounds.

1. Introduction

Many of the antibiotics presently in use are becoming ineffective due to the emergence of resistant microbial strains. It proves that the microbes are more intelligent than what is being anticipated by human beings, as they quickly develop mechanisms to intercept the antibiotic, thus making them ineffective. This situation demands the development of new antimicrobial agents which can deprive the microbes of their pathogenicity by novel multisite mechanisms of action [1–4]. The 1,2,4-triazole nucleus is the main structural unit of many medicines currently in market. Ribavirin (**1**), letrozole (**2**), fluconazole (**3**), itraconazole (**4**), and anastrozole (**5**) are a few to name which are currently in use as medicines (Figure 1). Many other 1,2,4-triazole derivatives are also known to possess pharmacological activities like antitubercular, anticonvulsant, anti-inflammatory, and analgesic activities [5–14]. It has been reported that triazoles are less susceptible to metabolic degradation and have higher target specificity and wider spectrum of activities as compared to imidazoles [15, 16]. Many heterocyclic systems having azomethine functionality are known to possess cytotoxic, antimicrobial, anticancer, and antifungal activities [17–21].

The literature reveals that the presence of morpholine or piperazine ring on a heterocyclic system contributes to enhanced pharmacological activities in many cases [22–24]. This could be attributed to the increased solubility of the compounds in aqueous solvents because of the formation of aminium salt [25]. These wide applications of 1,2,4-triazole Schiff and Mannich bases motivated us to synthesize new derivatives of 4-amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione and to test their potential as antibacterial and antifungal agents.

2. Experimental

General Comments. All reagents were purchased from Acros, Fluka, and Aldrich and used without further purification. All reactions were performed in standard glassware. All reactions were monitored by TLC plates precoated with silica gel Si 60 F_{254} from Merck and were visualized under UV lamp at $\lambda = 254$ nm. Melting points were determined with electrothermal apparatus, Gallenkamp and are uncorrected. IR spectra were recorded on PerkinElmer spectrophotometer by ATR technique. ^{1}H-NMR and ^{13}C-NMR spectra were

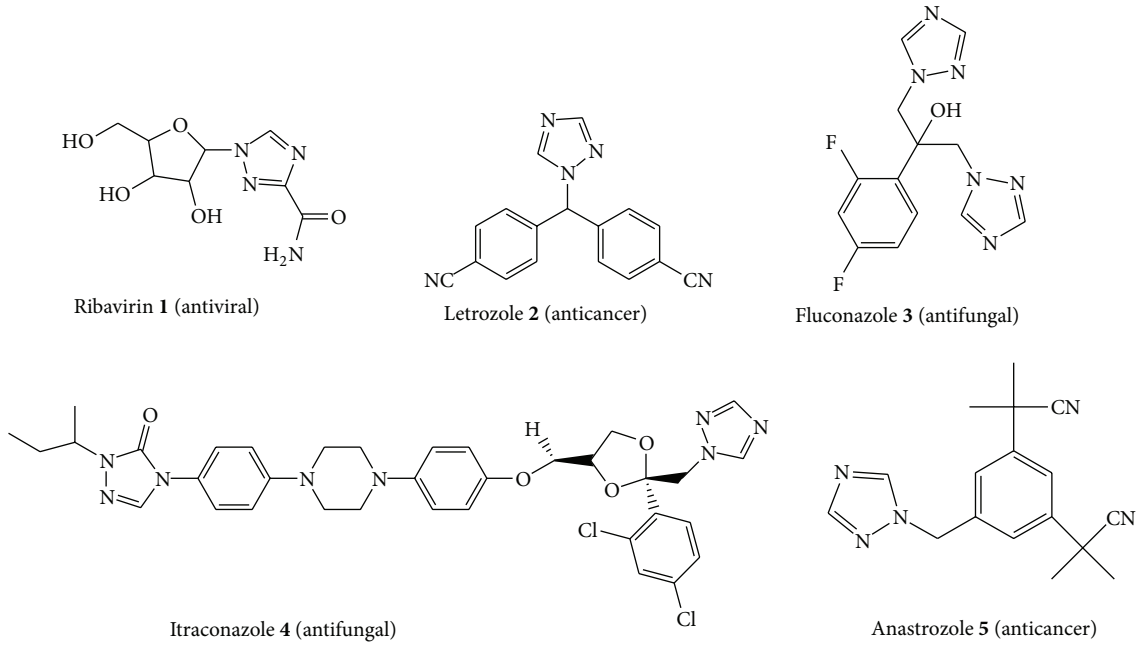

Figure 1: Some 1,2,4-triazole based medicine currently in use.

recorded on Burker 300 MHz/400 MHz instruments and 75 MHz/100 MHz instruments, respectively; the solvent used is specified along with data. Mass spectra were recorded on Jeol mass spectrometer in electron ionization mode. Leco 3200CHNS analyzer was used for elemental analysis. The thiocarbohydrazide (**2**) and the 4-amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**3**) were synthesized by the previously reported method [26, 27]. All manipulations of microbial activity were performed in laminar flow chamber (LFC) with disposable surgical gloves, all standard biosafety measures were taken, and contaminated materials after experimentation were collected in autoclave bags and autoclaved at 120°C before disposal.

*2.1. 4-Amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**3**).* To a clean test tube were added 0.01 mol (2.05 g) of N-phthaloylglycine and 0.01 mol (1.06 g) of thiocarbohydrazide, and the test tube was kept in a preheated oil bath. The molten mixture was kept at 145°C for 25 minutes. On cooling the mixture a solid mass was formed which was triturated with ethanol and filtered. The crude solid was recrystallized in acetonitrile and ethanol (1 : 1). Yield: 69%, white crystals, m.p. 189–192°C, IR (ATR) (ν cm^{-1}), 3305.08, 3152.47 (NH), 2982.12 (Ar-H), 2952.46 (CH_2), 1767.06 (cyclic amide), 1605 (C=N), 1145 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 13.98 (b, 1H, NH), 7.86–7.94 (m, 4H, C_6H_4), 5.63 (s, 2H, NH_2), 4.84 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.53 (C=S), 167.24 (C=O), 147.81 (N=C), 135.24 (N=C), (131.91, 123.86 (Ar-C)), 32.99 (CH_2). MS-E1 (*M/z*), Relative intensity: 275.1 (M+1, 100%), 259.1 (65%), 160.1 (80%), 130.1 (30%), 104.0 (45%), 77.1 (30%); $C_{11}H_9N_5O_2S$: Calculated: C, 47.99; H, 3.30; N, 25.44; S, 11.65. Found: C, 48.10; H, 3.35; N, 25.49; S, 11.85.

*2.2. General Procedure for Synthesis of Schiff Bases of 1,2,4-Triazole (**3**) (**4a–4i**).* Aromatic aldehyde (15 mmol) was dissolved in 8.0 mL of glacial acetic acid, and 10 mmol of 1,2,4-triazole (**3**) was added. The reaction mixture was refluxed for 25 minute to one hour in a preheated oil bath. The mixture was cooled, and the solid formed was filtered and washed with cold ethanol. The solid was recrystallized from ethanol and Schiff bases (**4a–4i**) were obtained in good to excellent yields.

*2.3. 4-(Benzylidene)amino-3-(N-phthalimidomethyl)-1,2,4-trazole-5-thione (**4a**).* Yield 42.8%, Yellow crystals m.p. 198–200°C. IR (ATR, ν cm^{-1}): 3283.40, 3156.39 (N–H), 3012.31 (Ar-H), 2996.00 (CH_2), 1770.75 (cyclic amide), 1599.79 (C=N), 1243.82 (C–N), 1120.06 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 14.02 (s, 1H, NH), 9.92 (s, 1H, N=CH), 7.88 (m, 4H, Ph), 7.80 (d, 2H, J = 7.2 Hz, Ph), 7.59 (m, 1H, Ph), 7.50 (m, 2H, Ph), 4.99 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 167.47 (C=S), 164.39 (C=O), 162.48 (N=CH), 146.52 (C=N), (135.33, 133.23, 132.29, 131.81, 129.52, 129.10, 123.90 (Ar-C)), 33.17 (CH_2). MS-E1: (*m/z*, Relative intensity, %): 363.3 (20%), 260.2 (100%), 242.2 (24%), 228.2 (19%), 203.2 (5%), 183.2 (15.9%), 160.2 (28.8%), 148.2 (18.2%), 130.1 (24%), 104.1 (87%), 89.1 (7.1%), 76.1 (45%). $C_{18}H_{13}N_5O_2S$: Calculated: C, 59.49; H, 3.61; N, 19.27; S, 8.82. Found: C, 59.48; H, 3.66; N19.30; S, 8.84.

*2.4. 4-(2-Hydroxybenzylidene)amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**4b**).* Yield 73%, white crystals, m.p. 239–241°C. IR (ATR, ν cm^{-1}): 3216.31 (O–H), 3055.35 (Ar-H), 2997.4 (CH_2), 1774.02 (cyclic amide), 1601.09 (C=N), 1253.65 (C–N), 1119.56 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 13.95 (s, 1H, NH), 10.40 (s, 1H, OH), 10.13 (s, 1H, N=CH), 7.89 (m, 4H, Ph), 7.70 (dd, 1H, J = 7.9, 1.5 Hz,

Ph), 7.40 (dt, 1H, J = 7.8 Hz, 1.5 Hz, Ph), 6.94 (d, 1H, J = 8.1 Hz, Ph), 6.85 (t, 1H, J = 7.8 Hz, Ph), 4.97 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 167.49 (C=S), 162.37 (C=O), 160.72 (N=CH), 158.90 (C–OH), 146.56 (C=N), (135.31, 134.80, 131.82, 127.28, 123.88, 119.88, 118.62, 117.03 (Ar-C)), 33.19 (CH_2). MS-E1 (m/z, Relative intensity, %): 379.3 (M^+, 2.7%), 347.4 (2.8%), 330.3 (5.2%), 260.2 (100%), 242.2 (8.6%), 228.2 (27.1%), 160.2 (53.4%), 148.2 (32.5%), 130.1 (57.3%), 119.1 (84.2%), 104.1 (85.3%), 91 (88%). $C_{18}H_{13}N_5O_3S$: Calculated: C, 56.98; H, 3.45; N, 18.46; S, 8.45. Found: C, 57.07; H, 3.54; N, 18.57; S, 8.57.

*2.5. 3-(N-Phthalimidomethyl)-4-(3-pyridine)amino-1,2,4-triazole-5-thione (**4c**).* Yield 76.05%, off-white crystals, m.p. 219–221°C. IR (ATR, ν cm^{-1}): 3041.73 (Ar-H), 2905.69 (CH_2), 1771.38 (cyclic amide), 1591.89 (C=N), 1302.07 (C–N), 1112.59 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 14.07 (s, 1H, NH), 10.13 (s, 1H, N=CH), 8.95 (d, 1H, J = 1.8 Hz, Ph), 8.75 (dd, 1H, J = 4.8, 1.5 Hz, Ph), 8.22 (d, 1H, J = 8.1 Hz, Ph), 7.88 (m, 4H, Ph), 7.55 (dd, 1H, J = 7.8 Hz, 4.8 Hz, Ph), 5.01 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.0 (C=S), 162.05 (C=O), 160.88 (HC=N), 152.0 (Py-C), 151.0 (Py-C), 149.0 (C=N), (133.7, 132.2, 130.4, 123.9, 123.6, 123.0 (Ar-C)), 33.5 (CH_2). MS-E1 (m/z, Relative intensity, %): 364.0 (12.8%), 260.0 (100%), 242.0 (8.1%), 228.0 (4.6%), 183.0 (6.4%), 160.0 (16.9%), 148.0 (11.1%), 130.0 (16.1%), 104.0 (46.7%). $C_{17}H_{12}N_6O_2S$: Calculated: C, 56.04; H, 3.32; N, 23.06; S, 8.80. Found: C, 56.16; H, 3.45; N 23.10; S, 8.82.

*2.6. 4-(3-Nitrobenzylidine)amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**4d**).* Yield 48%, yellow powder, m.p. 258–260°C. IR (ATR, ν cm^{-1}): 3200 (N–H), 3041.73 (Ar-H), 2919 (CH_2), 1770.38 (cyclic amide), 1591.89 (C=N), 1302.07 (C–N), 1112.59 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 14.06 (s, 1H, NH), 10.22 (s, 1H, N=CH), 8.61 (s, 1H, Ph), 8.40 (d, J = 6.8 Hz, 1H, Ph), 8.24 (d, J = 6.5 Hz, 1H, Ph), 7.85 (m, 5H, Ph), 5.01 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 166.87 (C=S), 162.08 (C=O), 160.84 (N=CH), 148.21 (C=N), (146.09, 134.71, 134.61, 133.58, 131.23, 130.66, 126.65, 123.29, 122.47 (Ar-C)), 32.58 (CH_2). MS-E1: m/z, (Relative intensity, %): 407.9 (7.3%), 260.0 (100%), 242 (5.1%), 228.0 (3.5%), 160.0 (13.3%), 148.0 (17.3%), 130.0 (11.0%), 104.0 (26.2%). $C_{18}H_{12}N_6O_4S$: Calculated: C, 52.94; H, 2.96; N, 20.58; S, 7.85. Found: C, 53.0; H, 3.10; N, 20.78; S, 8.02.

*2.7. 4-(4-Chlorobenzylidine)amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**4e**).* Yield 64.52%, white crystals, m.p. 241–244°C. IR (ATR, ν cm^{-1}): 3035.73 (Ar-H), 2956 (CH_2), 1772.18 (cyclic amide), 1611.24 (C=N), 1306.75 (C–N), 1118.19 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 13.99 (s, 1H, NH), 9.99 (s, 1H, N=CH), 7.84 (m, 4H, Ph), 7.81 (d, 2H, J = 8.4 Hz, Ph), 7.57 (d, 2H, J = 8.4 Hz, Ph), 4.97 (s, 2H, CH_2). ^{13}C NMR: (100 MHz, DMSO-d_6, δ ppm): 166.87 (C=S), 162.05 (C=O), 162.02 (N=CH), 145.99 (C=N), (137.29, 134.74, 131.25, 130.73, 130.14, 129.12, 123.32 (Ar-C)), 32.56 (CH_2). MS-E1: (m/z, Relative intensity, %): 397.3 (14.1%), 260.2 (100%), 242.2 (23.1%), 228.2 (14.7%), 183.2 (15.7%), 160.2 (26.6%), 148.1 (15%), 137.1 (77.6%), 104.1 (61.7%), 76.1 (24.8%). $C_{18}H_{12}ClN_5O_2S$: Calculated: C, 54.34; H, 3.04; N, 17.60; S, 8.06. Found: C, 54.45; H, 3.16; N, 17.62; S, 8.08.

*2.8. 4-(4-Bromobenzylidine)amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**4f**).* Yield 66.13%, white crystals, m.p. 215–217°C. IR (ATR, ν cm^{-1}): 3201 (N–H), 3041.73 (Ar-H), 2950.09 (CH_2), 1772.54 (cyclic amide), 1620.89 (C=N), 1302.07 (C–N), 1112.07 (C=S), 710 (C–Br). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) 14.04 (s, 1H, NH), 9.73 (s, 1H, N=CH), 7.79 (m, 4H, Ph), 7.57 (d, 2H, J = 8.4 Hz, Ph), 7.40 (d, 2H, J = 8.4 Hz, Ph), 5.0 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 167.12 (C=S), 163.68 (C=O), 159.10 (N=CH), 144.94 (C=N), (134.39, 132.24, 131.85, 131.32, 130.08, 127.15, 123.75 (Ar-C)), 32.88 (CH_2). MS-E1: (m/z, Relative intensity, %): 442.9 (3.7%), 274.0 (100%), 260.0 (84.1%), 241 (16.2%), 182.9 (28.7%), 148.0 (26.3%), 127.0 (51.1%), 104 (47.4%). $C_{18}H_{12}BrN_5O_2S$: Calculated: C, 48.88; H, 2.73; N, 15.8; S, 7.25. Found: C, 49.00; H, 2.88; N, 15.86; S, 7.29.

*2.9. 3-(N-Phthalimidomethyl)-4-(4-pyridine)amino-1,2,4-triazole-5-thione (**4g**).* Yield 48.02%, amorphous solid, m.p. 217–218°C. IR (ATR, ν cm^{-1}): 3012.18 (Ar-H), 2896.83 (CH_2), 1771.78 (cyclic amide), 1603.02 (C=N), 1268.54 (C–N), 1119.49 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 13.90 (s, 1H, NH), 9.70 (s, 1H, N=CH), 8.66 (d, 2H, J = 7.9, Ph), 7.98 (d, 2H, J = 7.9 Hz, Ph), 7.80 (m, 4H, Ph), 4.98 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 168.20 (C=S), 162.70 (C=O), 159.00 (N=CH), 152.20 (Py-C), 149.00 (Py-C), 144.30 (C=N), (132.2, 123.70, 123.10, 120.41 (Ar-C)), 37.23 (CH_2). MS-E1 (m/z, Relative intensity, %): 364.0 (10.8%), 260.0 (100%), 242.0 (6.0%), 228.0 (3.1%), 183.0 (5.1%), 160.0 (12.9%), 148.0 (6.8%), 130.0 (8.6%), 104.0 (34.3%). $C_{17}H_{12}N_6O_2S$: Calculated: C, 56.04; H, 3.32; N, 23.06; S, 8.80. Found: C, 56.13; H, 3.51; N, 23.10; S, 8.81.

*2.10. 4-(4-Methoxybenzylidine)amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**4h**).* Yield 36% white crystals, m.p. 239°C. IR (ATR, ν cm^{-1}): 3200 (Ar-H), 2919 (sp3 C–H), 1767 (C=O of anhydride), 1602 (C=N), 1279 (C=S) and 1255 (C–O). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 13.91 (s, 1H, NH), 9.69 (s, 1H, N=CH), 7.87 (m, 4H, Ph), 7.7 (d, 2H, J = 8.8 Hz, Ph), 7.33 (2H, J = 8.8 Hz, Ph), 4.94 (s, 2H, CH_2), 3.83 (s, 3H, O–CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 166.87 (C=S), 163.95 (C=O), 162.84 (N=CH), 161.99 (C–OCH_3), 145.78 (C=N), (134.72, 131.26, 130.54, 124.16, 123.30, 114.46 (Ar-C)), 55.47 (O–CH_3), 32.61 (CH_2), MS-E1: (m/z, Relative intensity, %): 393.4 (26.0%), 361.4 (4.7%), 260.2 (100%), 242.2 (8.7%), 228.3 (17.8%), 186.2 (9.8%), 160.2 (32.2%), 148.2 (10.8%), 133.1 (100%), 104 (66.4%). $C_{19}H_{15}N_5O_3S$: Calculated: C, 58.01; H, 3.84; N, 17.80; S, 8.15. Found: C, 58.10; H, 4.01; N, 17.85; S, 8.20.

*2.11. 4-(4-Fluorobenzylidine)amino-3-(N-phthalimidomethyl)-1,2,4-triazole-5-thione (**4i**).* Yield 35%, light yellow crystals, m.p. 231–233°C. IR (ATR, ν cm^{-1}): 3200 (Ar-H), 2912 (sp^3 C–H), 1770 (C=O of anhydride), 1597 (C=N), 1279 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 14.00 (s, 1H, NH),

9.93 (s, 1H, N=CH), 7.85 (m, 6H, Ph), 7.33 (t, 2H, J = 8.4 Hz, Ph), 4.98 (s, 2H, CH_2). ^{13}C NMR: (75 MHz, DMSO-d_6, δ ppm): 166.91 (C=S), 164.62 (d, $^1J_{C-F}$ = 249 Hz, C–F), 162.43 (C=O), 162.02 (N=CH), 145.99 (C=N), 134.76, 131.27, 131.17 ($^3J_{C-F}$ = 15 Hz), 128.46, 123.35, 116.21 ($^2J_{C-F}$ = 21.7 Hz), (Ar-C), 32.62 (CH_2). MS-E1 (*m/z*, Relative intensity, %): 381.0 (22.4%), 260.0 (100%), 242.0 (10.1%), 228.0 (4.5%), 183.0 (7.7%), 160.0 (18.4%), 148.0 (9.1%), 132.0 (9.3%), 104.0 (36.6%). $C_{18}H_{12}FN_5O_2S$: Calculated: C, 56.69; H, 3.17; N, 18.36; S, 8.41. Found: C, 56.74; H, 3.37; N, 18.39; S, 8.44.

*2.12. General Procedure for the Synthesis of Mannich Bases (**5a–5h**).* The corresponding Schiff bases (**4a–4h**) (10 mmol) were dissolved in acetonitrile: dioxane (2 : 1) at RT. Then, a solution of formaldehyde (37%, 0.5 mL) and morpholine (10 mmol) in ethanol was added dropwise with vigorous stirring. The reaction mixture was stirred at RT for 2 hours and left over night. The solid product was filtered and washed with ethanol and recrystallized from acetonitrile: dioxane (2 : 1) to yield title compound.

*2.13. 4-(Benzylideneamino)-1-(morpholinomethyl)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5a**).* Yield 72%, m.p. 208–210°C IR (ATR, ν cm^{-1}) 2958 (CH_2, asym), 2855 (CH_2, sym), 1771 (cyclic amide), 1591 (C=N), 1112 (C=S). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 10.50 (s, 1H, N=CH), 7.91 (dd, 2H, J = 5.7 Hz, J = 3.0 Hz, Ph), 7.80 (m, 2H, Ph), 7.77 (dd, 2H, J = 5.7 Hz, J = 3.0 Hz, Ph), 7.44–7.57 (m, 3H, Ph), 5.10 (s, 4H, 2 × CH_2, N–CH_2–N, N–CH_2–C), 3.69 (b, 4H, morpholine), 2.79 (b, 4H, CH_2, morpholine). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 167.20 (C=S), 163.70 (C=O), 161.06 (N=CH), 145.05 (C=N), (134.40, 132.52, 132.28, 131.87, 128.93, 128.88, 123.73 (Ar-C)), 69.08 (N–CH_2–N), (66.85, 50.68 (CH_2, morpholine)), 32.96 (CH_2). MS-E1: (*m/z*, Relative intensity, %): 462.1 (M+1, 20.6%), 363.0 (10.5%), 260.0 (76.6%), 242.0 (4.6%), 228.0 (5.7%), 185 (4.9%), 160.0 (27.5%), 100.1 (100%). $C_{23}H_{22}N_6O_3S$: Calculated: C, 59.73; H, 4.79; N, 18.17; S, 6.93. Found: C, 59.82; H, 4.85; N, 18.20; S, 6.99.

*2.14. 4-((2-Hydroxybenzylidene)amino)-1-(morpholinomethyl)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5b**).* Yield 74%, m.p. 214–216°C. IR (ATR, ν cm^{-1}): 2942 (CH_2, asym), 2849 (CH_2, sym), 1774 (cyclic amide), 1618 (C=N), 1111 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm) 10.43 (s, 1H, N=CH), 10.00 (s, 1H, OH), 7.88 (m, 4H, Ph), 7.66 (dd, 1H, J = 8.1 Hz, 1.5 Hz, Ph), 7.40 (t, 1H, J = 7.6 Hz, Ph), 6.94 (d, 1H, J = 8.1 Hz, Ph), 6.83 (t, 1H, J = 7.5 Hz, Ph), 5.03 (s, 2H, N–CH_2–N), 5.02 (s, 2H, N–CH_2–C), 3.53 (b, 4H, CH_2, morpholine), 2.64 (b, 4H, CH_2, morpholine). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 167.48 (C=S), 163.17 (C=O), 161.99 (N=CH), 159.00 (C–OH), 145.35 (C=N), (135.30, 134.98, 131.82, 127.24, 123.90, 119.86, 118.41, 117.05 (Ar-C)), 69.00 (N–CH_2–N), (66.51, 50.63 (CH_2, morpholine)), 32.27 (CH_2). MS-E1 (*m/z*, Relative intensity, %): 478 (M^+, 3%), 359 (5%), 260 (31.8%), 185 (3.8%), 160 (12%), 119 (18.1%), 110.1 (100%). $C_{23}H_{22}N_6O_4S$: Calculated: C, 57.73; H, 4.63; N, 17.56; S, 6.70. Found: C, 57.8; H, 4.73; N, 17.57; S, 6.79.

*2.15. 1-(Morpholinomethyl)-4-((pyridin-3-ylmethylene)amino)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5c**).* Yield 69% m.p. 188–190°C IR (ATR, ν cm^{-1}): 2953 (CH_2, asym), 2848 (CH_2, sym), 1772 cyclic amide, 1591 (C=N), 1115 (C=S). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 10.13 (s, 1H, N=CH), 8.94 (s, 1H, Ph), 8.73 (dd, 1H, J = 6.2 Hz, 1.5 Hz, Ph), 7.93–7.83 (m, 5H, Ph) 5.07 (s, 2H, N–CH_2–N), 5.03 (s, 2H, N–CH_2–C), 3.64 (b, 4H, CH_2, morpholine), 2.71 (b, 4H, CH_2, morpholine), ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 167.13 (C=S), 163.75 (C=O), 157.17 (N=CH), 152.98 (Py-C), 150.62 (Py-C), 145.00 (C=N), (134.82, 134.45, 131.86, 128.57, 123.86, 123.73 (Ar-C)), 69.17 (N–CH_2–N), (66.87, 50.73 (CH_2, morpholine)), 32.85 (CH_2). MS-E1 (*m/z*, Relative intensity, %): 463 (M+1, 7.5%), 363.9 (5.0%), 260.0 (40.0%), 228.0 (5.3%), 160.0 (14.0%), 100.1 (100%). $C_{22}H_{21}N_7O_3S$: Calculated: C, 57.01; H, 4.57; N, 21.15; S, 6.92. Found: C, 57.10; H, 4.68; N, 21.20; S, 7.02.

*2.16. 1-(Morpholinomethyl)-4-((3-nitrobenzylidene)amino)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5d**).* Yield 80% m.p. 193–195°C (decomposed), IR (ATR, ν cm^{-1}): 2940 (CH_2, asym), 2840 (CH_2, sym), 1774 (amide), 1610 (C=N), 1115 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 10.01 (s, 1H, N=CH), 8.62 (t, 1H, J = 1.5 Hz, 1.8 = Hz, Ph), 8.42 (ddd, 1H, J = 8.1, 2.1 and 0.6 Hz, Ph), 8.23 (d, 1H, J = 7.8 Hz, Ph), 7.78–7.92 (m, 4H, Ph) 7.79 (d, 1H, J = 8.2 Hz, Ph), 5.07 (2H, N–CH_2–N), 5.06 (s, 2H, N–CH_2–C), 3.54 (b, 4H, CH_2, morpholine), 2.66 (b, 4H, CH_2, morpholine). ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 167.46 (C=S), 163.20 (C=O), 162.93 (N=CH), 148.71 (C=N), (145.42, 135.31, 133.91, 131.76, 131.25, 127.43, 123.91, 123.13 (Ar-C)), 69.12 (N–CH_2–N), (66.51, 50.62 (CH_2, morpholine)), 33.20 (CH_2). MS-E1: (*m/z*, Relative intensity, %): 507. (M^+, 1.9%), 408.0 (2.5%), 260.0 (21.6%), 185.0 (2.7%), 160.0 (12.2%), 130.0 (6.1%), 100.1 (100%). $C_{23}H_{21}N_7O_5S$: Calculated: C, 54.43; H, 4.17; N, 19.32; S, 6.32. Found: C, 54.53; H, 4.28; N, 19.35; S, 6.33.

*2.17. 4-((4-Chlorobenzylidene)amino)-1-(morpholinomethyl)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5e**).* Yield 79% m.p. 178–180°C IR (ATR, ν cm^{-1}): 2958 (CH_2, asym), 2855 (CH_2, sym), 1771 (cyclic amide), 1593 (C=N), 1112 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 9.85 (s, 1H, N=CH) 7.88 (m, 4H, Ph), 7.80 (d, 2H, J = 8.7 Hz, Ph), 7.57 (d, 2H, J = 8.4 Hz, Ph), 5.02 (s, 4H, 2 × CH_2, N–CH_2–N, N–CH_2–C), 3.52 (b, 4H, CH_2, morpholine), 2.63 (b, 4H, CH_2, morpholine). ^{13}C NMR (300 MHz, DMSO-d_6, δ ppm): 166.95 (C=S), 163.62 (C=O), 162.73 (N=CH), 144.84 (C=N), (137.53, 134.84, 131.30, 130.60, 130.21, 129.21, 123.43 (Ar-C)), 68.60 (N–CH_2–N), (66.01, 50.14 (CH_2, morpholine)), 32.69 (CH_2). MS-E1: (*m/z* Relative intensity, %): (496.0, M^+, 5.7%), 396.9 (5.1%), 260.0 (56.5%), 242.0 (2.7%), 228.0 (3.5%), 185.0 (4.1%), 160.0 (20.8%), 137.0 (29.0%), 100.1 (100%). $C_{23}H_{21}ClN_6O_3S$: Calculated: C, 55.59; H, 4.26; N, 16.91; S, 6.45. Found: C, 55.74; H, 4.37; N, 16.99; S, 6.47.

*2.18. 1-(Morpholinomethyl)-4-((4-bromobenzylidene)amino)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5f**).* Yield 39% m.p. 189–191°C IR (ATR, ν cm^{-1}): 2958

(CH_2, asym), 2854 (CH_2, sym), 1772, 1719 (cyclic amide), 1607 (C=N), 1112 (C=S). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 10.56 (s, 1H, N=CH), 7.86 (dd, 2H, J = 5.4 Hz, 3.0 Hz, Ph), 7.74 (dd, 2H, J = 5.4 Hz, 3.0 Hz, Ph), 7.66 (d, 2H, J = 8.4 Hz, Ph), 7.56 (d, 2H, J = 8.4 Hz, Ph), 5.05 (s, 2H, N–CH_2–N), 5.04 (s, 2H, N–CH_2–C), 3.63 (b, 4H, CH_2, morpholine), 2.73 (b, 4H, CH_2, morpholine), ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 167.12 (C=S), 163.68 (C=O), 159.10 (N=CH), 144.94 (C=N), (134.39, 132.24, 131.85, 131.85, 131.32, 130.08, 127.15, 123.75, 123.68 (Ar-C)), 69.12 (N–CH_2–N), (66.84, 50.70 (CH_2, morpholine)), 32.88 (CH_2). MS-E1 (*m/z*, Relative intensity, %): 540 (M^+, 3.1%), 441 (3.1%), 260.0 (84.4%), 242.0 (3.7%), 182.9 (22.6%), 160.0 (26.7%), 100.1 (100%). $C_{23}H_{21}BrN_6O_3S$: Calculated: C, 51.02; H, 3.91; N, 15.52; S, 5.92. Found: C, 51.00; H, 3.99; N, 15.59; S, 6.02.

*2.19. 1-(Morpholinomethyl)-4-((pyridin-4-ylmethylene)amino)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5g**).* Yield 69% m.p. 201–203°C IR (ATR, ν cm^{-1}): 2953 (CH_2, asym), 2848 (CH_2, sym), 1770 (cyclic amide), 1602 (C=N), 1112 (C=S). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 10.10 (s, 1H, N=CH), 8.65 (d, 2H, J = 7.8 Hz, Ph), 7.97 (d, 2H, J = 7.8 Hz, Ph), 7.80 (m, 4H, Ph), 5.07 (s, 2H, N–CH_2–N), 5.03 (s, 2H, N–CH_2–C), 3.64 (b, 4H, CH_2, morpholine), 2.71 (b, 4H, CH_2, morpholine). ^{13}C NMR (300 MHz, $CDCl_3$, δ ppm): 167.23 (C=S), 163.72 (C=O), 157.17 (N=CH), 152.97 (Py-C), 149.02 (Py-C), 145.40 (C=N), (133.82, 134.54, 131.80, 128.77, 122.96, 123.73 (Ar-C)), 70.02 (N–CH_2–N), (66.89, 50.77 (CH_2, morpholine)), 32.65 (CH_2). MS-E1 (*m/z*, Relative intensity, %): 463 (M+1), 363.9 (5.8%), 260.0 (72.4%), 228.0 (2.8%), 160.0 (32.2%), 104 (26.8%), 100.1 (100%). $C_{22}H_{21}N_7O_3S$: Calculated: C, 57.01; H, 4.57; N, 21.15; S, 6.92. Found: C, 57.09; H, 4.63; N, 21.26; S, 7.03.

*2.20. 4-((4-Methoxybenzylidene)amino)-1-(morpholinomethyl)-3-(N-phthalimidomethyl)-1,4-dihydro-5H-1,2,4-triazole-5-thione (**5h**).* Yield 82% m.p. 218–220°C IR (ATR, ν cm^{-1}): 2939 (CH_2, asym), 2840 (CH_2, sym), 1770 (amide), 1598 (C=N), 1171 (C=S). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 10.21 (s, 1H, N=CH), 7.86 (dd, 2H, 5.4 Hz and 3.3 Hz, Ph), 7.72–7.74 (m, 4H, Ph), 6.93 (d, 2H, J = 8.4 Hz, Ph), 5.04 (s, 4H, 2 × CH_2, N–CH_2–N, N–CH_2–C), 3.85 (s, 3H, OCH_3), 3.65 (b, 4H, CH_2, morpholine), 2.73 (b, 4H, CH_2, morpholine), ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 167.15 (C=S), 163.68 (C=O), 162.64 (N=CH), 161.99 (C–OCH_3), 144.85 (C=N), (134.38, 134.31, 131.93, 130.79, 124.83, 123.74, 123.65, 114.38 (Ph-C)), (69.14, 66.87 (CH_2, morpholine)), 55.48 (O–CH_3), 50.71 (CH_2), 32.9. MS-E1: (*m/z*, Relative intensity, %): 492 (M^+, 5.9%), 393.0 (9.6%), 358.0 (3.8%), 260.0 (57.2%), 228.0 (2.9%), 185.0 (3.1%), 160.0 (23%), 133.0 (71.1%), 100.1 (100%). $C_{24}H_{24}N_6O_4S$: Calculated: C, 58.52; H, 4.91; N, 17.06; S, 6.51. Found: C, 58.59; H, 4.92; N, 17.08; S, 6.53.

3. Microbiology

The antimicrobial activity of the compounds **3**, **4a–4i,** and **5a–5h** was tested on one Gram-positive strain (*Staphylococcus aureus*) ATCC25923, four Gram-negative strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Enterobacter cloacae* 13047, and *Klebsiella pneumoniae* ATCC 13883), and one diploid fungus (*Candida albicans* ATCC SC5314). Levofloxacin, amikacin, and fluconazole were used as standards. Filter paper disc method was used to evaluate the microbial activity.

3.1. Methodology. 10 gm of nutrient agar broth was dissolved in 400 mL of distilled water. The media was autoclaved at 120°C for 2 hrs. The media was poured in sterilized petri plates up to 40 mL and then covered the plates with lids. The agar was allowed to set and harden. Filter papers of 5 mm diameter were cut and dipped in dilution of 100 μg/mL of each sample that is, **3**, **4a–4i**, **5a–5h**, and standards, and were placed on seeded agar. All stock solutions of tested compounds were made in DMSO. The bacterial culture was kept at 37°C, and fungal plates were kept at 18°C for 3-4 days. After incubation, the diameter of clear zone around the discs was measured and compared against zone of inhibition formed by solutions of known concentrations of standard antibiotics. All the tests were carried out in triplicate, and the results were averaged out.

4. Results and Discussion

4.1. Chemistry. The 1,2,4-triazole (**3**) was synthesized by the fusion of N-phthaloylglycine and thiocarbohydrazide by the method reported earlier by our group [27]. The Schiff bases were synthesized by refluxing the triazole (**3**) with corresponding aldehydes in glacial acetic acid; Figure 2 describes the synthetic scheme. All the synthesized compounds were characterized by spectroscopic analysis. In the H NMR spectra of Schiff bases (**4a–4i**), the two protons of CH_2 group of glycine gave a singlet in the range of 4.90–5.10 ppm. The most downfield signal around 14 ppm (NH) proves that the triazole exists in thione form when in solution. The proton of azomethine linkage (N=CH) gave a singlet downfield around 9.9–10.2 ppm. The chemical shifts for the four protons of phthalimido group are in the range of 7.89–7.79 ppm as a multiplet due to AA′BB′ spin system, whereas the para-disubstituted Schiff bases (**4f–4i**) give a doublet for each proton characteristic of an AB spin system in the range of 8.66–7.33 ppm. In C-13, the signal around 163 ppm is of carbonyl carbon of imide (C=O), and most down field signals around 167 ppm are for thione form (C=S). All other carbons are also well justified. The molecular ion could be seen in all the Schiff bases (**4a–4i**). In the IR spectra, characteristic absorption bands are visible for C=S and C=N groups in the range of 1110–1120 cm^{-1} and 1590–1600 cm^{-1}, respectively. The absorption for the C=O of cyclic amide appears around 1770 cm^{-1} in all compounds.

The Schiff bases (**4a–4h**) were reacted with formaldehyde in presence of morpholine to obtain the corresponding Mannich bases (**5a–5h**). The ^{1}H NMR spectra showed identical chemical shifts for the two methylene protons of phthalimidomethylene and the two morpholino methylene protons. The four protons either appeared as a one singlet in case of **5a**, **5e**, and **5h** or two singlets with a chemical shift

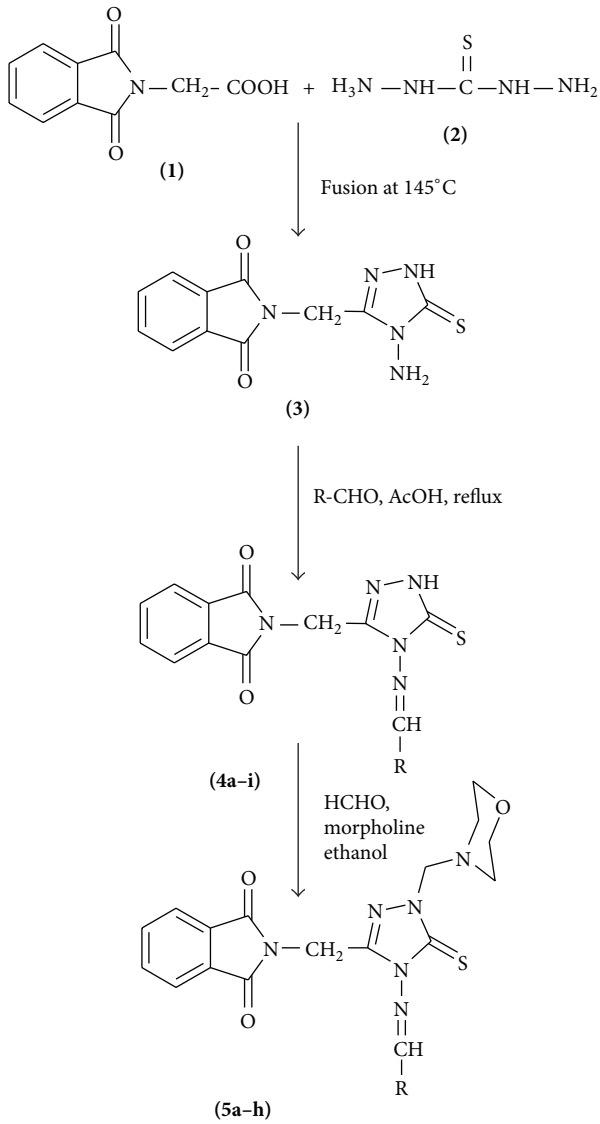

Figure 2: Scheme for the synthesis of (**5a–5h**).

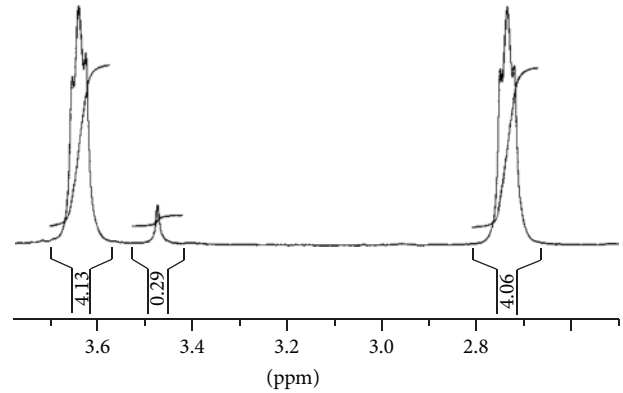

Figure 3: ^{1}H NMR signals for methylene protons of morpholine group.

difference of only 0.01–0.03 ppm as in **5d**, **5f**, and **5g**. The methylenes of morpholine appeared as a set of two broad signals as shown in Figure 3. The methylenes of morpholine constitute A_2M_2 spin system which gives complex second order splitting pattern and is not easy to interpret. In case of morpholine ring, the rapid ring flipping at room temperature makes axial and equatorial protons of morpholine ring almost equivalent; therefore a broad signal showing some splitting as well is observed; however, it cannot be called a triplet as neither line intensity nor coupling constant values are justified for triplet. The C-13 data IR and mass spectral data are consistent with the structure.

4.2. Antimicrobial Activity Test. The antimicrobial activity of the 1,2,4-triazole (**3**) and the nine Schiff bases (**4a–4i**) derived from triazole (**3**) were tested on a Gram-positive strain (*Staphylococcus aureus*), four Gram-negative strains, (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Klebsiella pneumoniae*), and one diploid fungus (*Candida albicans*). Levofloxacin, amakacine, and fluconazole were used as standards. A comparison of the microbial activity of the triazole (**3**) and its Schiff base derivatives (**4a–4i**) has been made. Furthermore, the Mannich bases (**5a–5h**) were also screened against the above mentioned strains. The results are shown in Table 1. The triazole (**3**) did not show any significant activity against the strains mentioned here; however, the introduction of azomethine linkage in all cases has improved the antimicrobial activity exceptions being **4h** and **4i**, where the activity remained almost the same. The best activity comparable to Levofloxacin was shown by **4b** and **4e** against *Enterobacter cloacae*, whereas compounds (**4a**, **4c**, **4d,** and **4g**) have displayed moderate activity against the same bacterial strain. **4a** and **4b** have demonstrated good activity and **4c**, **4g,** and **4f** moderate activity against *Klebsiella pneumoniae* with reference to levofloxacin and amikacin. Compounds (**4b**, **4c**, **4d**, **4f**, and **4g**) have displayed moderate activity against fungal strain, *Candida albicans*, in comparison to fluconazole. It has been observed [23] that the introduction of morpholine or piprazine ring increases the antimicrobial activity in many heterocyclic systems. For instance, itraconazole, eperezolid, and linezolid antibiotics possess a morpholine or piperazine ring. These ring functions increase the solubility in aqueous solvents when transformed into iminium salts, thus increasing the bioavailability of the compound. Keeping this in mind a morpholine group was introduced in compounds (**4a–4h**) the resulting Mannich bases (**5a–5h**) were also tested against the above mentioned six strains. In general antimicrobial activity in all cases significantly increased except for *Enterobacter cloacae,* where loss of activity was observed. Compound (**5h**) showed comparable activity to levofloxacin against *Escherichia coli* and *Klebsiella pneumoniae*. **5b**, **5d**, **5e**, and **5f** showed activity very close to levofloxacin against *Pseudomonas aeruginosa*. **4d** showed compareable activity to levofloxacin, however, its Mannich

Table 1: In vitro antimicrobial screening of compounds (mm) conc. 100 μg/mL.

Compound no.	Microorganisms and inhibition zone (mm)					
	Escherichia coli	*Pseudomonas aeruginosa*	*Enterobacter cloacae*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Candida albicans*
3	7.00	8.00	9.68	9.40	7.00	9.95
4a	10.25	8.60	11.65	12.35	9.10	10.00
4b	8.52	9.65	14.42	11.20	8.10	11.75
4c	6.75	8.10	8.00	10.20	7.25	11.25
4d	7.38	9.30	9.60	9.83	8.00	10.76
4e	7.30	8.37	12.20	9.42	7.67	9.72
4f	8.21	8.43	9.33	10.70	9.42	10.45
4g	7.22	10.90	7.30	7.10	7.10	12.30
4h	6.55	6.95	7.30	7.44	8.0	7.82
4i	7.1	8.11	8.63	7.50	7.43	10.28
5a	13.2	13.20	—	11.10	12.60	14.30
5b	13.0	14.10	—	11.70	11.60	15.10
5c	7.00	10.00	9.00	11.00	11.00	13.20
5d	11.60	13.80	—	12.40	14.00	12.40
5e	12.50	14.80	—	12.00	14.10	13.70
5f	11.30	14.10	—	11.10	12.20	13.20
5g	—	—	—	—	10.00	12.20
5h	14.70	11.40	—	13.20	14.20	14.60
Levofloxacin	16.50	13.85	14.80	13.95	18.32	—
Fluconazole	—	—	—	—	—	17.25
Amikacin	17.70	12.75	16.30	14.20	19.20	—

base lost all activity. **5d**, **5e**, and **5h** showed significant increase in antimicrobial activity against *S. aureus*.

All compounds (**5a–5h**) showed improved activity against fungal strain *Candida albicans*; however, **5b** demonstrated best activity, and **5a** and **5h** also showed good activity. The results show that the compound **5h** is the most promising among the tested compounds. Our results strengthen the earlier findings by others that the presence of morpholine ring in heterocyclic molecules increases the antimicrobial activity. In conclusion the Mannich bases with electron donating substituents (–OH, $-OCH_3$) on phenyl ring **5b** and **5h** or with halogens on phenyl ring (**5e** and **5f**) showed best activity among the tested compounds. The results are promising and show that the fine tuning of the structures can lead to some new antibacterial compounds.

5. Conclusion

A series of novel Schiff bases and their Mannich bases were synthesized to study the structure activity relationship. All the synthesized compounds were screened for their antimicrobial activities against six strains. In general the antimicrobial activity increased remarkably on the introduction of azomethine functionality in parent triazole (**3**). The antimicrobial activity further improved when morpholine group was added to them except for *Enterobacter cloacae*, where loss of activity was observed. The results show that the compound (**5h**) is the most promising among the tested compounds. Our results strengthen the earlier findings by others that the presence of morpholine ring in heterocyclic molecules increases the antimicrobial activity. In conclusion the Mannich bases with electron donating substituents (–OH, $-OCH_3$) on phenyl ring (**5b** and **5h**) or with halogens on phenyl ring (**5e** and **5f**) showed the best activity among the tested compounds. The results are promising and show that the fine tuning of the structures (**5a**, **5b**, **5e**, **5f**, and **5h**) can lead to some new antimicrobial compounds.

References

[1] M. Koca, S. Servi, C. Kirilmis et al., "Synthesis and antimicrobial activity of some novel derivatives of benzofuran: part 1. Synthesis and antimicrobial activity of (benzofuran-2-yl)(3-phenyl-3-methylcyclobutyl) ketoxime derivatives," *European Journal of Medicinal Chemistry*, vol. 40, no. 12, pp. 1351–1358, 2005.

[2] C. G. Bonde and N. J. Gaikwad, "Synthesis and preliminary evaluation of some pyrazine containing thiazolines and thiazolidinones as antimicrobial agents," *Bioorganic & Medicinal Chemistry*, vol. 12, no. 9, pp. 2151–2161, 2004.

[3] D. Yu and G. Huiyuan, "Synthesis and antibacterial activity of linezolid analogues," *Bioorganic & Medicinal Chemistry Letters*, vol. 12, no. 6, pp. 857–859, 2002.

[4] V. J. Ram and A. J. Vlietinck, "Chemotherapeutical agents. VII. Synthesis and pesticidal activities of sulphides and sulphones

derived from bis[4-aryl-1, 2, 4-triazoline-5-thione-3-yl]alkane and 5-phenyl-1, 3, 4-oxadiazole-2-thione," *Journal of Heterocyclic Chemistry*, vol. 25, no. 1, pp. 253–256, 1988.

[5] R. W. Sidwell, J. H. Huffman, G. P. K. Lois et al., "Broad-spectrum antiviral activity of virazole: 1-β-D-ribofuranosyl-1, 2,4-triazole-3-carboxamide," *Science*, vol. 177, no. 4050, pp. 705–706, 1972.

[6] J. T. Witkowski, R. K. Robins, G. P. Khare, and R. W. Sidwell, "Synthesis and antiviral activity of 1,2,4-triazole-3-thiocarboxamide and 1,2,4-triazole-3-carboxamidine ribonucleosides," *Journal of Medicinal Chemistry*, vol. 16, no. 8, pp. 935–937, 1973.

[7] M. Clemons, R. E. Coleman, and S. Verma, "Aromatase inhibitors in the adjuvant setting: bringing the gold to a standard?" *Cancer Treatment Reviews*, vol. 30, no. 4, pp. 325–332, 2004.

[8] S. Budavari, *The Merck Index*, Merck, White House Station, NJ, USA, 12th edition, 1996.

[9] J. Haber, "Present status and perspectives on antimycotics with systemic effects," *Journal of Czech Physicians*, vol. 140, no. 19, pp. 596–604, 2001.

[10] K. Walczak, A. Gondela, and J. Suwiński, "Synthesis and antituberculosis activity of N-aryl-C-nitroazoles," *European Journal of Medicinal Chemistry*, vol. 39, no. 10, pp. 849–853, 2004.

[11] B. S. Holla, K. N. Poojary, B. S. Rao et al., "New bis-aminomercaptotriazoles and bis-triazolothiadiazoles as possible anticancer agents," *European Journal of Medicinal Chemistry*, vol. 37, no. 6, pp. 511–517, 2002.

[12] B. S. Holla, B. Veerendra, M. K. Shivananda et al., "Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles," *European Journal of Medicinal Chemistry*, vol. 38, no. 7-8, pp. 759–767, 2003.

[13] M. Amir and K. Shikha, "Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-[(2,6-dichloroanilino) phenyl]acetic acid derivatives," *European Journal of Medicinal Chemistry*, vol. 39, no. 6, pp. 535–545, 2004.

[14] A. Almasirad, S. A. Tabatabai, M. Faizi et al., "Synthesis and anticonvulsant activity of new 2-substituted-5-[2-(2-fluorophenoxy)phenyl]-1,3,4-oxadiazoles and 1,2,4-triazoles," *Bioorganic & Medicinal Chemistry Letters*, vol. 14, no. 24, pp. 6057–6059, 2004.

[15] K. El Akri, K. Bougrin, J. Balzarini, A. Faraj, and R. Benhida, "Efficient synthesis and in vitro cytostatic activity of 4-substituted triazolyl-nucleosides," *Bioorganic & Medicinal Chemistry Letters*, vol. 17, no. 23, pp. 6656–6659, 2007.

[16] M. S. Karthikeyan, B. S. Holla, and N. S. Kumari, "Synthesis and antimicrobial studies of novel dichlorofluorophenyl containing aminotriazolothiadiazines," *European Journal of Medicinal Chemistry*, vol. 43, no. 2, pp. 309–314, 2008.

[17] M. T. H. Tarafder, A. Kasbollah, N. Saravanan et al., "S-methyldithiocarbazate and its Schiff bases: evaluation of bondings and biological properties," *The Journal of Biochemistry, Molecular Biology and Biophysics*, vol. 6, no. 2, pp. 85–91, 2002.

[18] P. Vicini, A. Geronikaki, M. Incerti et al., "Synthesis and biological evaluation of benzo[d]isothiazole, benzothiazole and thiazole Schiff bases," *Bioorganic & Medicinal Chemistry*, vol. 11, no. 22, pp. 4785–4789, 2003.

[19] O. Bekircan, B. Kahveci, and M. Küçük, "Synthesis and anticancer evaluation of some new unsymmetrical 3,5-diaryl-4H-1,2,4-triazole derivatives," *Turkish Journal of Chemistry*, vol. 30, no. 1, pp. 29–40, 2006.

[20] O. Bekircan and H. Bektas, "Synthesis of Schiff and Mannich bases of isatin derivatives with 4-amino-4,5-dihydro-1H-1,2,4-triazole-5-ones," *Molecules*, vol. 13, no. 9, pp. 2126–2135, 2008.

[21] B. Kahveci, O. Bekircan, and Ş. A. Karaoğlu, "Synthesis and antimicrobial activity of some 3-alkyl-4-(arylmethyleneamino)-4,5-dihydro-1H-1,2,4-triazol-5-ones," *Indian Journal of Chemistry B*, vol. 44, no. 12, pp. 2614–2617, 2005.

[22] T. Plech, M. Wujec, A. Siwek et al., "Synthesis and antimicrobial activity of thiosemicarbazides, s-triazoles and their Mannich bases bearing 3-chlorophenyl moiety," *European Journal of Medicinal Chemistry*, vol. 46, no. 1, pp. 241–248, 2011.

[23] H. Bayrak, A. Demirbas, S. A. Karaoglu, and N. Demirbas, "Synthesis of some new 1,2,4-triazoles, their Mannich and Schiff bases and evaluation of their antimicrobial activities," *European Journal of Medicinal Chemistry*, vol. 44, no. 3, pp. 1057–1066, 2009.

[24] M. Ashok, B. S. Holla, and B. Poojary, "Convenient one pot synthesis and antimicrobial evaluation of some new Mannich bases carrying 4-methylthiobenzyl moiety," *European Journal of Medicinal Chemistry*, vol. 42, no. 8, pp. 1095–1101, 2007.

[25] M. S. Karthikeyan, D. J. Prasad, B. Poojary et al., "Synthesis and biological activity of Schiff and Mannich bases bearing 2,4-dichloro-5-fluorophenyl moiety," *Bioorganic & Medicinal Chemistry*, vol. 14, no. 22, pp. 7482–7489, 2006.

[26] L. F. Audrieth, E. S. Scott, and P. S. Kippur, "Hydrazine derivatives of the carbonic and thiocarbonic acids. I. The preparation and properties of thiocarbohydrazide," *The Journal of Organic Chemistry*, vol. 19, no. 5, pp. 733–741, 1954.

[27] U. Yunus, M. K. Tahir, M. H. Bhatti, N. Yousaf, and M. Helliwell, "2-(4-Amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-ylmethyl)isoindoline-1, 3-dione," *Acta Crystallographica E*, vol. 64, no. 2, pp. o476–o477, 2008.

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Review Article

Imidazole and Triazole Coordination Chemistry for Antifouling Coatings

Markus Andersson Trojer,[1] Alireza Movahedi,[1] Hans Blanck,[2] and Magnus Nydén[1,3]

[1] *Applied Surface Chemistry, Department of Chemical and Biological Engineering, Chalmers University of Technology, 412 96 Göteborg, Sweden*
[2] *Department of Biological and Environmental Sciences, University of Gothenburg, 405 30 Göteborg, Sweden*
[3] *Ian Wark Research Institute, University of South Australia, Adelaide, SA 5001, Australia*

Correspondence should be addressed to Markus Andersson Trojer; markus.andersson@chalmers.se

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Fouling of marine organisms on the hulls of ships is a severe problem for the shipping industry. Many antifouling agents are based on five-membered nitrogen heterocyclic compounds, in particular imidazoles and triazoles. Moreover, imidazole and triazoles are strong ligands for Cu^{2+} and Cu^{+}, which are both potent antifouling agents. In this review, we summarize a decade of work within our groups concerning imidazole and triazole coordination chemistry for antifouling applications with a particular focus on the very potent antifouling agent *medetomidine*. The entry starts by providing a detailed theoretical description of the azole-metal coordination chemistry. Some attention will be given to ways to functionalize polymers with azole ligands. Then, the effect of metal coordination in azole-containing polymers with respect to material properties will be discussed. Our work concerning the controlled release of antifouling agents, in particular medetomidine, using azole coordination chemistry will be reviewed. Finally, an outlook will be given describing the potential for tailoring the azole ligand chemistry in polymers with respect to Cu^{2+} adsorption and $Cu^{2+} \rightarrow Cu^{+}$ reduction for antifouling coatings without added biocides.

1. Introduction

A major problem for the shipping industry is fouling of marine organisms, such as algae, mussels, and barnacles on ship hulls [1–5]. As the ship moves through the water, the hull is subjected to *form drag* and *skin friction drag*. Since fouling increases the surface roughness, the skin friction drag is increased, which subsequently results in increased fuel consumption and reduced manoeuvrability [3]. Traditional countermeasures against marine fouling are coatings containing antifouling toxicants that have had severe environmental consequences on marine ecosystems. The most well-known examples are organometallic paint systems (comprising lead, arsenic, mercury, etc.) which met the market in the early 1960s. The notorious tributyltin self-polishing coating (TBT-SPC) was developed in the 1970s [1, 6]. The reason for the success of the TBT-SPC paints is that the rate of release can be controlled on a molecular level. Generally, these paints are based on acrylic or methacrylic copolymers. The active substance (TBT) is covalently attached to the carboxylic group of the polymer, which is hydrolytically instable in slightly alkaline conditions (as in seawater) [1, 4, 6]. Toxic copper pigment is also included in the TBT-SPC paint systems, which in combination with TBT, provides protection against the entire spectrum of fouling organisms since some algae are tolerant to TBT but not to copper and vice versa [7]. As the use of TBT containing paints increased, marine life in the vicinity of harbours and marinas was severely affected [8, 9]. The hazardous nature of TBT for the marine environment is ascribed to its weakening of fish immune systems, causing imposex and sterility in female gastropods (at such low concentration as 1 ng/L) as well as its bioaccumulative properties in mammals [1, 4, 6]. Starting with TBT ban for pleasure craft in France 1982, TBT-containing paint systems were successively phased out in the 1990s

and finally banned by IMO (the International Maritime Organization) [10, 11].

A number of tin-free alternatives have been developed due to the restrictive legislation regarding TBT paints. These can crudely be divided into *biocide-release* and *biocide-free* antifouling systems. Biocide-free approaches are dominated by paint systems called *foul-release coatings*. The antifouling mechanism lies in the low surface energy and smoothness of the coating. The binders in these coatings are silicone polymers, for example, PDMS (polydimethylsiloxane) and to some extent fluoropolymers, with both possessing very low surface energy.

In tin-free SPC paints, tin is replaced by other elements such as copper, zinc, and silicon. Even though the side group of a few tin-free SPC paint binders also possess biocidal properties, no alternative is nearly as effective as TBT [4, 6]. To provide full and long-term fouling protection, it is now necessary to add other biocides, so called *boosters*, generally in combination with copper. The most frequently employed boosters are conventional agrochemicals, some of which, for example, irgarol, have been under scrutiny due to low rate of biodegradation as well as toxicity towards nontarget organisms [1, 4, 6, 10–18]. Since also the use of copper has been under debate recently, a number of other pigments are used such as Zn(II), Fe(III), and Ti(IV) oxides [1, 3, 4, 6].

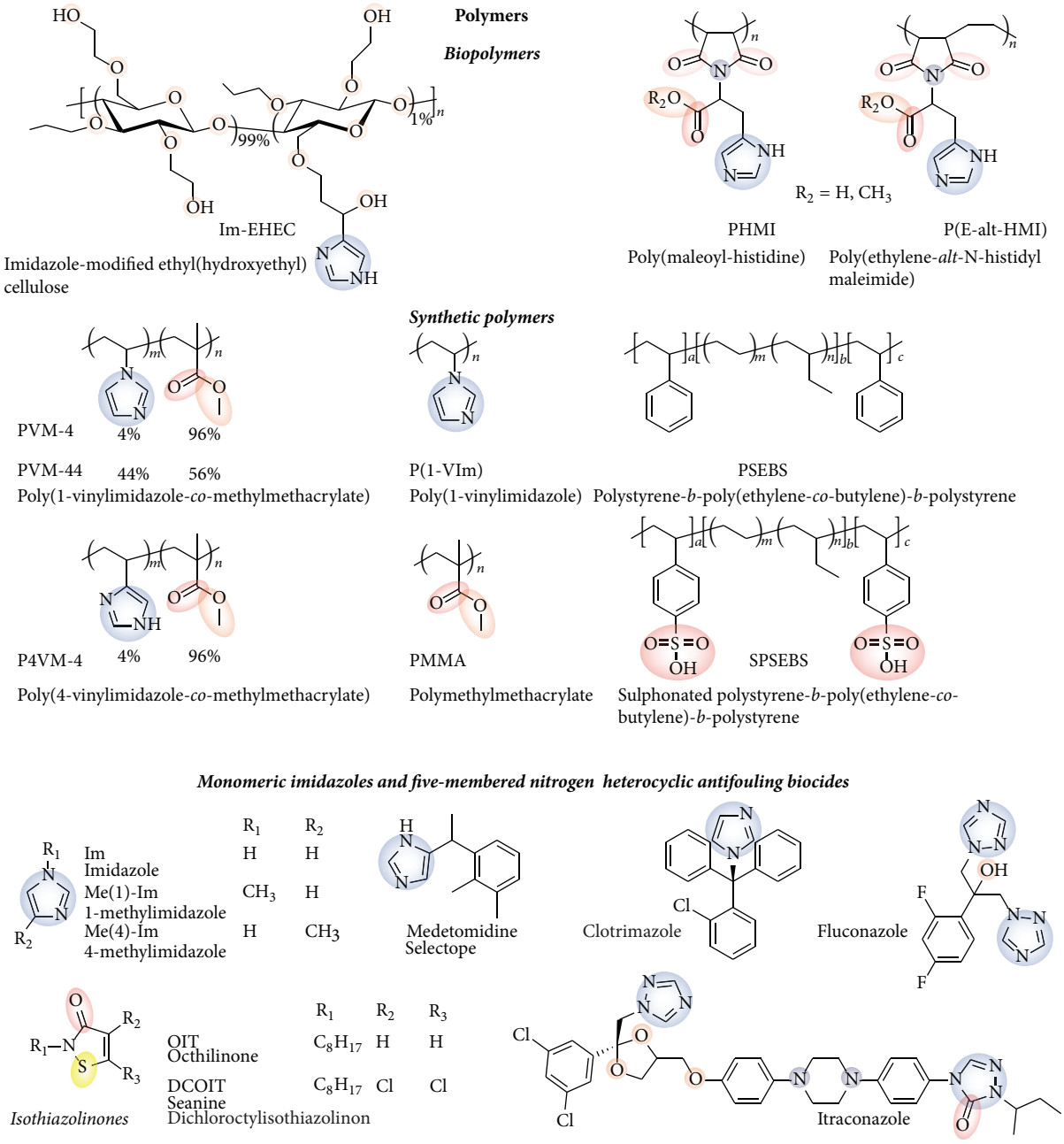
A generic problem with tin-free SPC paints is premature leakage of the booster biocides [3]. These are not anchored to the binder, as TBT is, and are hence subjected to free diffusion within the paint matrix. The immediate consequence of premature leakage is loss of antifouling effect prior to the hydrolysis-dependent lifetime of the coating.

A lot of research and development has been conducted on SPC paints from an environmental perspective. For instance, the antifouling substances secreted by marine organism, that is, *natural biocides*, have recently attracted attention from the scientific community. The antifouling mechanisms of these natural biocides are generally much more intricate and specific compared to those of conventional booster biocides [3, 4, 6]. Some examples of natural antifoulants are worth mentioning. Furanon, originating from red seaweed, is for instance 100 times more effective than TBT as antifoulant against barnacle [3]. Bufalin (a toad poison) is for example, considered to be 100 times more toxic than TBT and 6000 times more efficacious against barnacles [6]. Most substances are found in the phyla *Porifera* (sponges), *Algae*, *Cnidaria* (e.g., corals, sea anemones, and hydroids), *Echinodermata* (e.g., sea urchins), *Bryozoa* (moss animals), and bacteria [3, 6]. Many antimicrobial substances are based on five-membered aromatic heterocycles such as Seanine and OIT (see Scheme 1). Moreover, triazole-based biocides represent a wide spectrum of in particular antifungals such as fluconazole and itraconazole (see Scheme 1) [19–27]. The imidazole fungicide clotrimazole (see Scheme 1) used as human and veterinary pharmaceutical has been found in the marine environment at concentrations affecting sterol metabolism in marine microalgal communities [28]. The interdisciplinary research program *Marine Paint* [2], in which our group has been a part, has since 2001 been investigating the substance medetomidine [29] (see Scheme 1) which is based on imidazole for antifouling purposes [30–32]. Medetomidine is exceptionally efficient against fouling barnacles [33], which is one of the most problematic foulants in the north Atlantic, yet, without seriously affecting nontarget organisms [34–37].

In this review, we will summarize a decade of research concerning azole (primarily imidazole) coordination chemistry for antifouling coatings. The work spans a variety of chemistry disciplines including molecular modelling [38, 39], surface and colloidal chemistry [40–43], controlled release [30, 31, 40–42, 44–46], coordination chemistry [39, 40, 47], polymer synthesis [47–50], and material science [39, 47]. Some references from other groups will be provided; however, it is important to note that very few studies have addressed azole coordination for antifouling purposes, and the area is still rather unexplored. The azole coordination approaches implemented by us with respect to antifouling coatings can be divided into three main categories: (1) antifoulant immobilization, (2) triggered release and (3) Cu^{2+}/Cu^{+} mediated antifouling as described later.

1.1. Antifoulant Immobilization. Booster biocides, including the azole-based molecules already mentioned, are typically small molecules which are molecularly dispersed in the antifouling paint [3, 51]. As a consequence, the biocides leach out from the coating within a couple of weeks (diffusive release from thin coatings ~100 μm) [52]. This results in a premature loss of fouling protection which is a severe problem since the desired lifetime of a marine coating is ~5 years [3, 4]. However, when the biocides are immobilized on macromolecules or colloids (with negligible diffusion coefficients given there large sizes), the effective diffusion coefficient will be reduced by the magnitude of the stability constant for immobilization [53]. We have explored the possibility to immobilize the very efficient antifoulant medetomidine on polymers and nanoparticles by exploiting the strong and specific coordination chemistry of the molecule's imidazole moiety. This is treated in Section 5.

1.2. Triggered Release and Imidazole-Cu^{2+} Coordination. The approach discussed in the previous section is obviously only applicable for azole-based biocides/antifoulants. Most booster biocides do not contain a strong ligand moiety for coordination chemistry. We have therefore developed more generic release systems based on polymeric poly(methyl methacrylate) microcapsules [52–60] with some novel advances involving ultrathin polyelectrolyte multilayers for *sustained release* [53, 60–66]. However, in order to maintain protection against fouling, it is important that the biocide concentration reaches high enough concentrations at the coating surface (see Scheme 2). Consequently, it would be beneficial if the release of the antifouling substance could be triggered by some physical or chemical parameters at the hull-water interface. We have explored the possibility to achieve *triggered release* by incorporating salt in the otherwise rather hydrophobic microcapsule shell using azole coordination chemistry. The salt will initiate swelling of the shell by increasing the free volume of the polymer [54] and hence

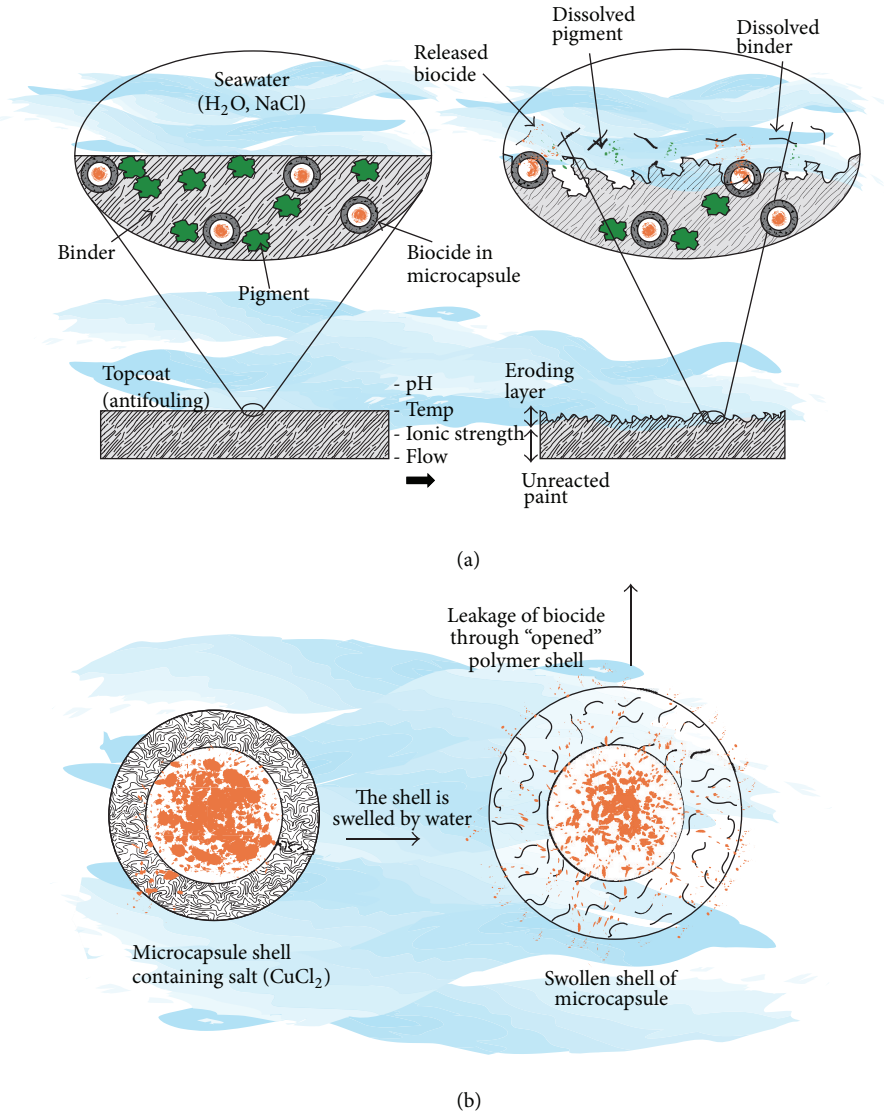


Scheme 1: Molecular structure, names, and abbreviations of polymers used for azole coordination and low molecular weight imidazole containing substances and five-membered nitrogen heterocyclic biocides investigated in this review. Potential nitrogen coordinating borderline ligands (imidazole, triazole) or hard ligands (amines), oxygen coordinating hard ligands, and sulphur coordinating soft ligands are marked with blue, purple, red, and yellow, respectively. Weak ligands such as organic halides have not been marked.

trigger the release from the microcapsule shell as a function of contact with water or moisture (see Scheme 2). The functionalization of polymers with azoles (mainly imidazole) is treated in Section 3, and the material properties and water sorption with respect to the presence of metal ions (in particular Cu^{2+} and Zn^{2+}) in the polymer matrix are treated in Section 4.

The azole coordination has also been used to trigger the release of medetomidine. This is treated in Section 5.1.

1.3. Towards Cu^{2+}/Cu^{+} Catalysis in Novel Biocide-Free Marine Coatings. Copper is a potent antifouling biocide for in particular marine organisms [67, 68]. Yet, copper is also a trace element and constitutes an essential part of many enzymes where redox reactions are the central function. However, excess copper concentrations are lethal for the organism since vital biological processes (disruption of cell homeostasis such as pH balance, membrane potential, and osmosis) are affected *via* the inactivation of enzymes and by the precipitation



Scheme 2: Triggered release mechanism for (a) a self-polishing paint and (b) containing microcapsules.

cytoplasmic proteins into metal-protein aggregates [69, 70]. Many studies are pointing towards the fact that copper crosses the biological membranes as Cu^+ which is subsequently the biologically most active oxidation state [69]. However, Cu^+ is unstable in an aqueous environment and disproportionates to Cu^{2+} and Cu^0 [71, 72]. Moreover, only free ions of Cu^{2+} and Cu^+ are considered to be bioavailable [73–75]. A lot of recent focus in the catalysis and microbiology community is addressing Cu^+ stabilization by altering the Cu^{2+}/Cu^+ redox potential via the proper choice of ligand environment, both with respect to chemistry and geometry (see Section 2) [76–79]. Here, the ligand environment in copper proteins, including both the Cu(I) [77–79] and the Cu(II) [79–94] oxidation statse, is a continuous source of inspiration.

Encouraged by our findings concerning azole-Cu^{2+} coordination mentioned in the previous Section and recent advances in Cu^{2+}/Cu^+ stabilization, we have developed a generic concept of *biocide-free* antifouling coatings. By incorporating azole-type ligands in the antifouling coating, the pool of free and sequestered Cu^{2+} in the marine environment may be absorbed. It is important that the interaction is strong enough since a large fraction of the dissolved copper in the sea is associated with organic ligands. Typically, weak ligands are considered to have stability constants of the order 10^9 and strong ligands of the order 10^{13} [73–75]. However, this concept is still feasible given the fact that azole-type ligands have stability constants for copper coordination ranging between 10^{14}–10^{20} depending on chelate properties and chemistry as discussed in Sections 2 and 6 and the references within. Furthermore, regarding the antifouling coating, the presence of strongly Cu^+ stabilizing ligands, for example, nitriles and sulphur-containing ligands, may catalyse the reduction of Cu^{2+} to Cu^+. This will produce high concentrations of Cu^{2+} and Cu^+, both of which are efficient antifouling agents

[67, 68], at the coating interface without any net addition of biocides to the environment (see Scheme 3). The azole specificity towards Cu^{2+} is treated in Section 4.1, and the Cu^{+} stabilization including our on-going work with the triazole ligand is briefly discussed in Section 6.2.

2. Theoretical Considerations of Imidazole and Triazole Coordination Chemistry

In this work, the coordination of azole ligands (mainly imidazole) by Cu^{2+}, Cu^{+}, and Zn^{2+} ions has been investigated as presented in Scheme 4. It is therefore pertinent to describe the fundamentals for the strong interaction between the previously mentioned species. In the following subsections, the azole coordination chemistry will be briefly reviewed in terms of coordination geometry, molecular orbitals and electronic structure, covalency, aromaticity, and so forth.

2.1. HSAB Principles and σ-Interaction. The strong metal-ligand bonding between the metal ions Zn^{2+}, Cu^{+} (diamagnetic d^{10} full d-shell configuration), Cu^{2+} (paramagnetic d^{9} configuration), and the imidazole or triazole ligand (see Scheme 5) may be qualitatively explained by HSAB (Hard Soft Acid Base) principles [95, 98, 99]. To begin with, only the electron lone pair donation from the base (ligand) to the acid (metal), σ-bonding, will be considered.

Hard bases preferentially bond to hard acids via ionic bonding, whereas soft bases favourably bond to soft acids via covalent bonding [98, 99]. In the ligand field depiction (see Scheme 7) this implies that the energy levels of the metal (M) and ligand (L) orbitals (the dσ- and σ-levels) will be similar [100, 101]. The ensuing M-L molecular orbitals will have almost equivalent proportions of metal and ligand character, respectively, and the electrons spend their time at the metal and ligand equally [100]. Ionic bonding denotes large orbital energy differences with essentially no electron sharing [98–100].

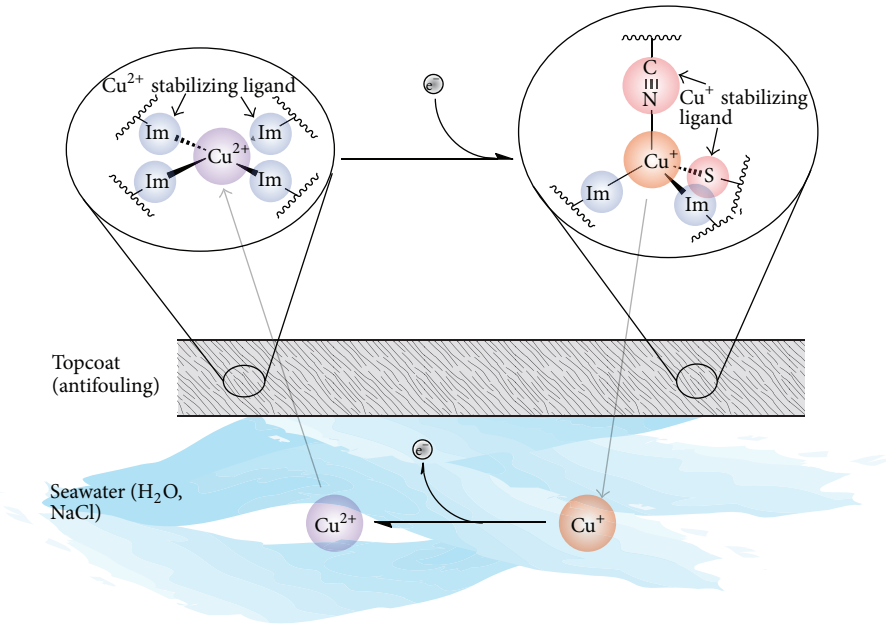
Hard acids and bases are small, hard to polarize with low and high electronegativity, respectively [98, 99]. They possess large HOMO-LUMO (Highest Occupied Molecular Orbital, Lowest Unoccupied Molecular Orbital) gaps, favouring ionic bonding [100]. Soft acids and bases are large and polarizable with intermediate electronegativity and with small HOMO-LUMO gaps [98, 99]. Note that Cu^{+} is considered a soft acid and that triazole is softer than imidazole. A high oxidation state usually renders the transition metal hard, whereas the soft has low oxidation state (≤ 2) [100]. With regard to bases, unsaturation usually makes them softer [99]. For regular σ-donors, the coordinate bond may be viewed as polar covalent, especially with regards to borderline acids and bases (see below) [99, 100]. The selectivity of different metal-ligand pairs in polymers is treated in Section 4.1.

2.2. π-Interaction. π-bonding is another important interaction (besides σ-bonding) in transition metal complexes (see Schemes 5 and 7) [100]. Ligands with more than one lone pair, labelled π-donors or π-bases (see Schemes 5 and 7), for example, Cl^{-} and RO^{-}, may donate additional electron density to a metal d orbital (π-acid) of proper symmetry (see Scheme 7, π-bond) [100]. This interaction is typically considered hard and destabilizes the metal d-orbitals but will be favourable for hard, electropositive (especially d^{0}) transition metals [100]. Certain ligands, labelled π-acceptor or π-acid have low-lying vacant LUMOs of proper symmetry with regard to the metal dπ-orbitals (π-base) susceptible for metal to ligand π-bonding (back donation), an interaction typically considered soft. This interaction may be favourable since it alleviates the metal of some electron density in the coordination compound. For unsaturated ligands, the anti-bonding π^{*}-orbital typically accepts electron from the metal dπ-orbitals, which is favourable for electron-rich metals in low oxidation states with high energy d-orbitals that are basic in character [100].

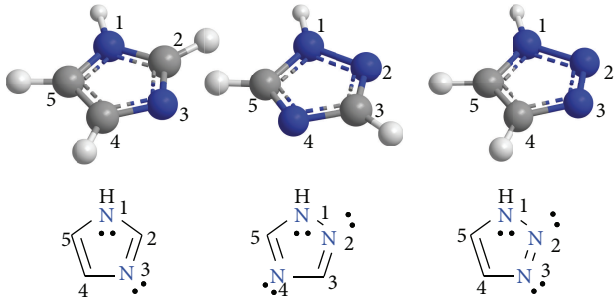
2.3. Coordination Geometry. Imidazole is coordinated by Cu^{2+} in an essentially square planar geometry $Cu(Im)_4{}^{2+} \cdot X^{-}{}_2$, similar to pyridine (see Schemes 6(a) and 6(c)), with the imidazole rings occupies coplanar positions with respect to Cu(II) [95]. This favours π-interaction between the imidazole ring orbitals and metal dπ orbitals and locates the electron hole in a distinctly separated orbital (see Scheme 6(a)) [79, 95]. In the case of Zn^{2+} or Cu^{+}, tetrahedral geometry is frequently encountered (common for d^{10} metals [101]), $Zn(Im)_4{}^{2+} \cdot X^{-}{}_2$ (see Scheme 7(b)). Octahedral geometry (see Scheme 5(c)), $Zn(Im)_6{}^{2+} \cdot X^{-}{}_2$, is also possible for Zn(II) but not for Cu(I). The coordination is less dependent on electronic factors for Zn(II) and more on steric factors in contrast to Cu^{2+} complexes [95, 101]. As an example, Zn^{2+} with Cl^{-} as counterion coordinates unsubstituted imidazole in octahedral geometry of O_h symmetry $[Zn(Im)_6]^{2+}$ (see Section 6.1) [38]. However, the addition of a methyl group at position 1 or 4 on the imidazole ring, which gives a slight steric hindrance, results in the formation of pseudotetrahedral complexes $[Zn(Me\text{-}Im)_2Cl_2]$ of C_{2v} symmetry, even at large excess of imidazole ligands [38]. It should be noted that the nature of the counterion has an effect on the type of complex formed and that octahedral (O_h symmetry) and tetrahedral (T_d symmetry) complexes of Zn^{2+} and methylated imidazoles have been reported [95].

The coordination geometries for triazole ligands are more complicated. Since triazole is *bidentate*, with the possibility to bind to two metal centers, the coordination usually results in polymeric (1D, 2D or 3D) structures [102–105]. This is a popular method to produce metal-organic frameworks which is also possible for imidazole ligands at sufficiently high pH [38, 95]. However, this is outside the scope of this review and will not be further treated. Mononuclear coordinate complexes comprising triazole ligands do exist provided that the triazole is substituted with sufficiently bulky groups. In these situations, the triazole displays the same type of coordination geometries as the imidazole ligand [102–104] (see Scheme 6).

2.4. Comparison with Other Nitrogen Ligands. Azoles and other unsaturated nitrogen ligands, such as pyridine, are categorized as borderline bases, while Cu^{2+} and Zn^{2+} are borderline acids in contrast to Cu^{+} which is soft [95, 98, 99].



Scheme 3: Mechanism for ligand-mediated absorption and redox reaction.



Scheme 4: Structure and numbering of the imidazole 1,2,4-triazole and 1,2,3-triazole. N_1 is labelled pyrrole nitrogen and N_3 (imidazole) and N_2/N_4 (triazole) are labelled pyridine nitrogen. Note that N_1 is also sp^2 hybridized and the free electron pair is donated to the ring π-orbital system in order to obtain aromaticity. The lone electron pair of N_3 is, however, free rendering of this nitrogen basic and the predominant site for coordination [95].

Consequently, these borderline acid-base pairs form favourable bonds with bond properties which are intermediate to those of soft and hard acid-base pairs [98–100]. Imidazole is generally considered to be a moderate σ-donor and a weak π-acceptor, with σ-donor and π-acceptor abilities in between saturated amines such as ammonia (NH_3) and unsaturated amines such as pyridine [95]. This positions imidazole above oxygen donors and below ammonia and pyridine in the spectrochemical series [95, 106]. In rare cases, π-donor abilities from the imidazole-ring π-orbital have been reported [107, 108].

The series of nitrogen donor ligands with increasing basicity follows the order NH_3 > imidazole > pyridine, whereas the order of increasing π-acceptor ability is the reversed [95]. For Cu(II) and Zn(II), the bond strength follows the order imidazole > NH_3 > pyridine, reflecting a dominating importance of σ-bonding but also a significant contribution from π-bonding [95].

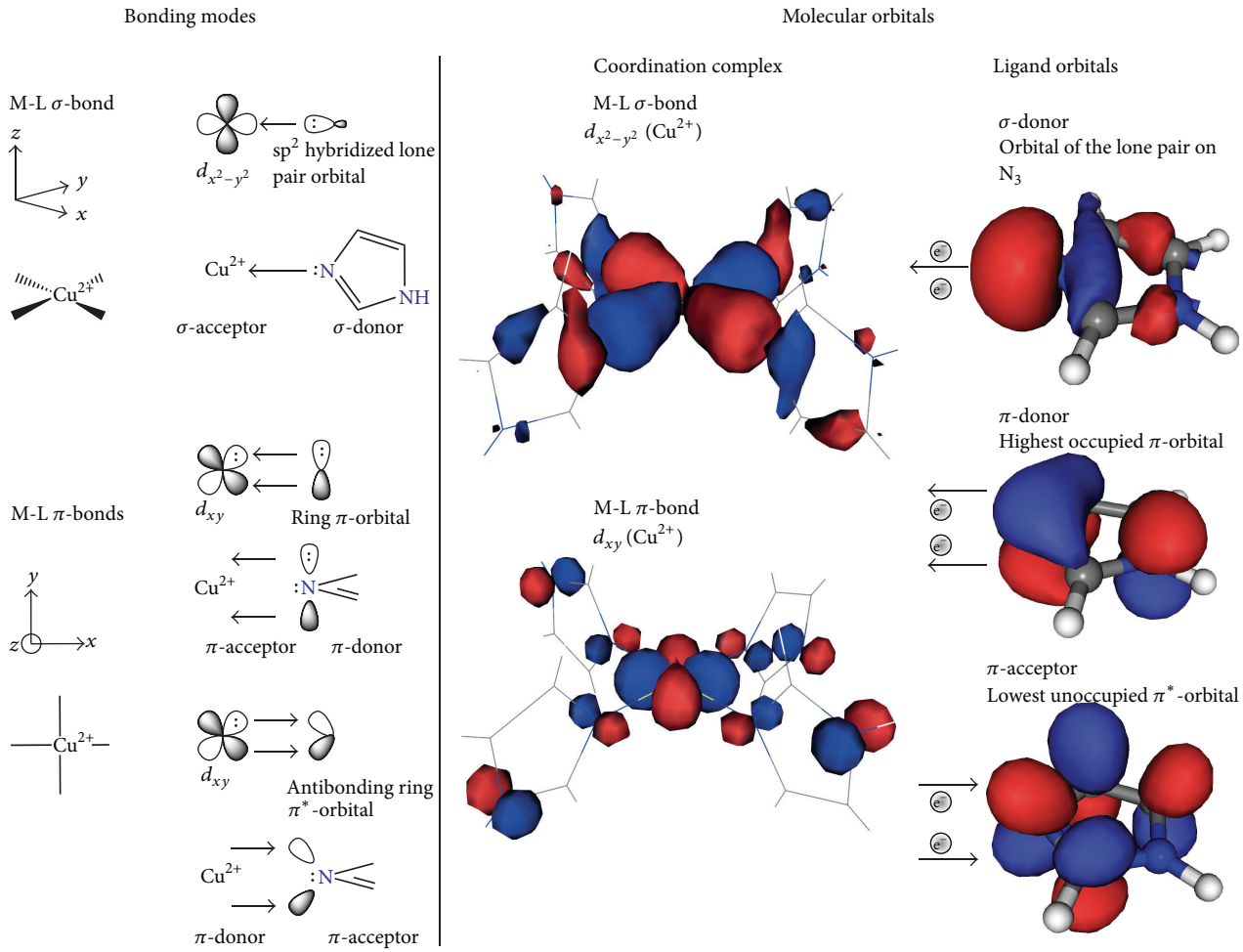
Triazoles are softer ligands than the corresponding imidazoles with stronger π-acceptor interaction and weaker σ-donor (basicity) interaction [109]. For azoles, the series of increasing σ-basicity and decreasing π-acidity follows the order 1,2,4-triazole > pyrazole > thiazole ≫ imidazole [110]. Consequently, triazole ligands are better than imidazole in stabilizing Cu^+ in favour of Cu^{2+}.

3. Functionalization of Polymers

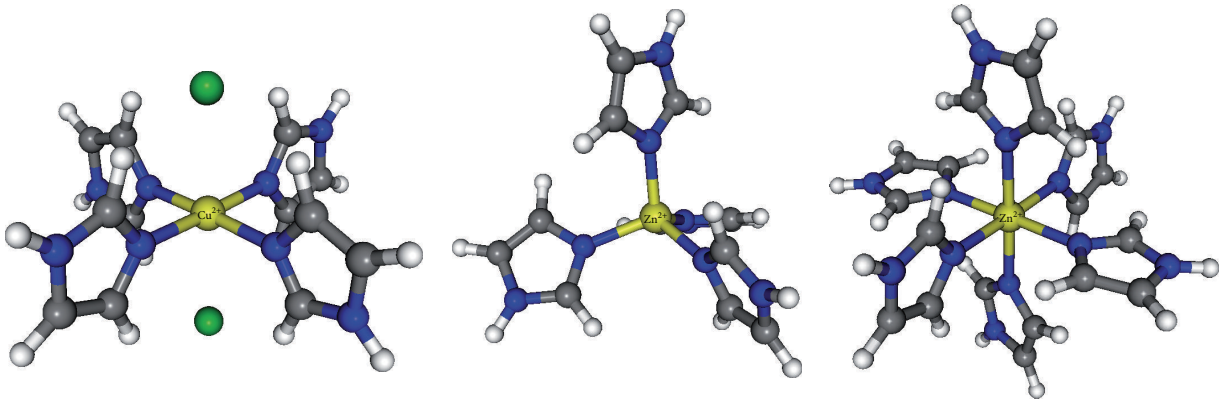
For the antifouling coatings containing azole ligands as described in Sections 1.2 and 1.3, it is necessary to develop suitable synthetic routes in order to functionalize polymers with azole ligands. All polymers and low molecular weight imidazole containing substances used in this work are presented in Scheme 1. We have investigated both N- and C-substituted azoles. The attachment position of the polymer backbone (the substituent) on the azole ring has important consequences for the coordination chemistry as described in Section 6.1.

3.1. Imidazole Functionalization. A popular technique to functionalize polymers is to graft functional groups onto an active site on the polymer backbone [111–116]. It is often easier to polymerize a smaller monomer with an active site than a large bulky monomer with the functional group already attached. This type of functionalization is sometimes called *postpolymerization modification* [115, 116].

An epoxide route was used in order to functionalize ethyl(hydroxyethyl)cellulose (EHEC) with imidazole groups [49]. In short, the procedure involved 4 steps: protection of

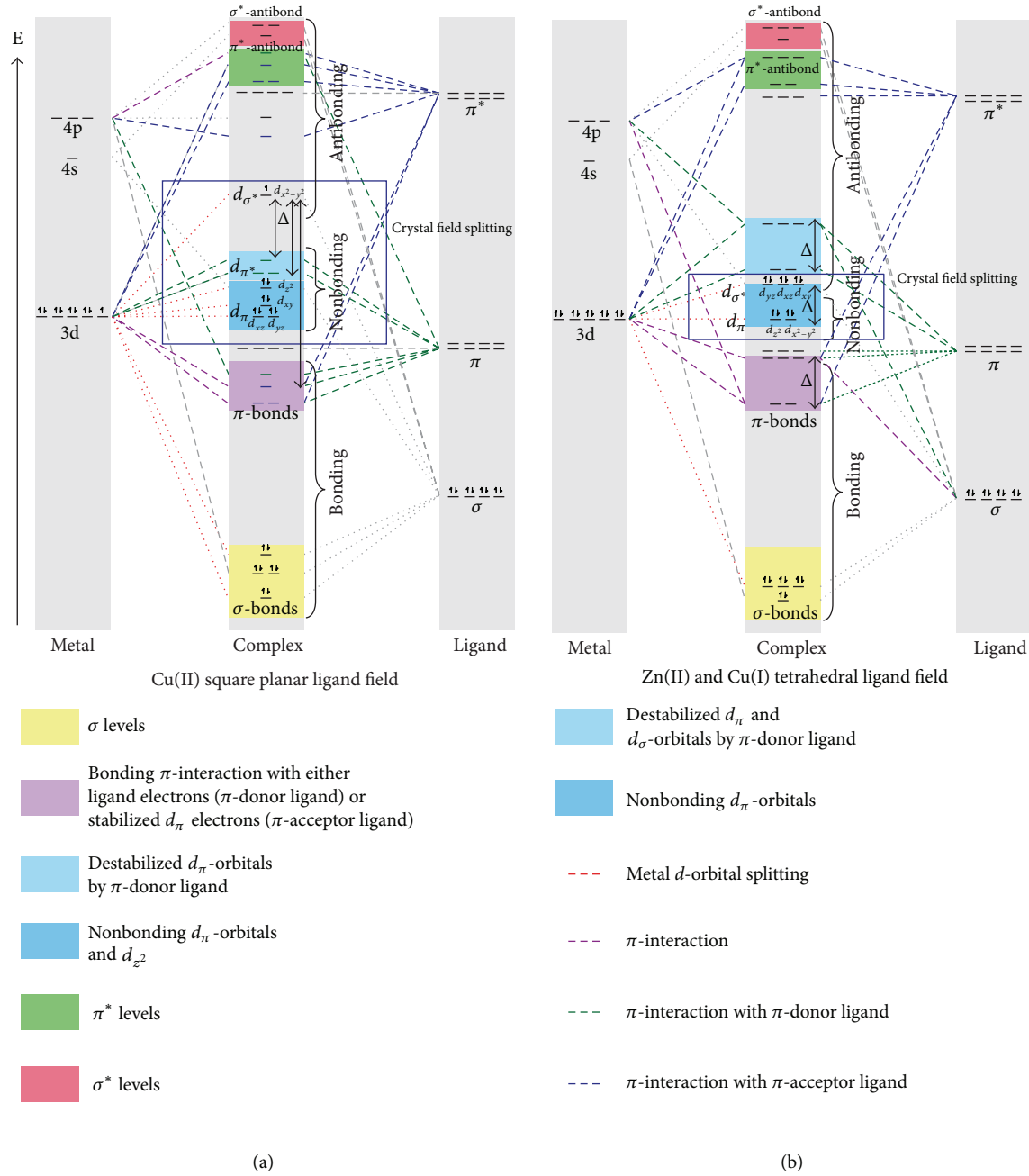


Scheme 5: Coordinate bonding modes (left) and molecular orbitals (right) envisaged for azole-Cu(II) in square planar geometry as obtained from DFT calculations [38]. The bonding principles are generic and applied also for Zn(II) and Cu(I) complexes.



(a) (b) (c)

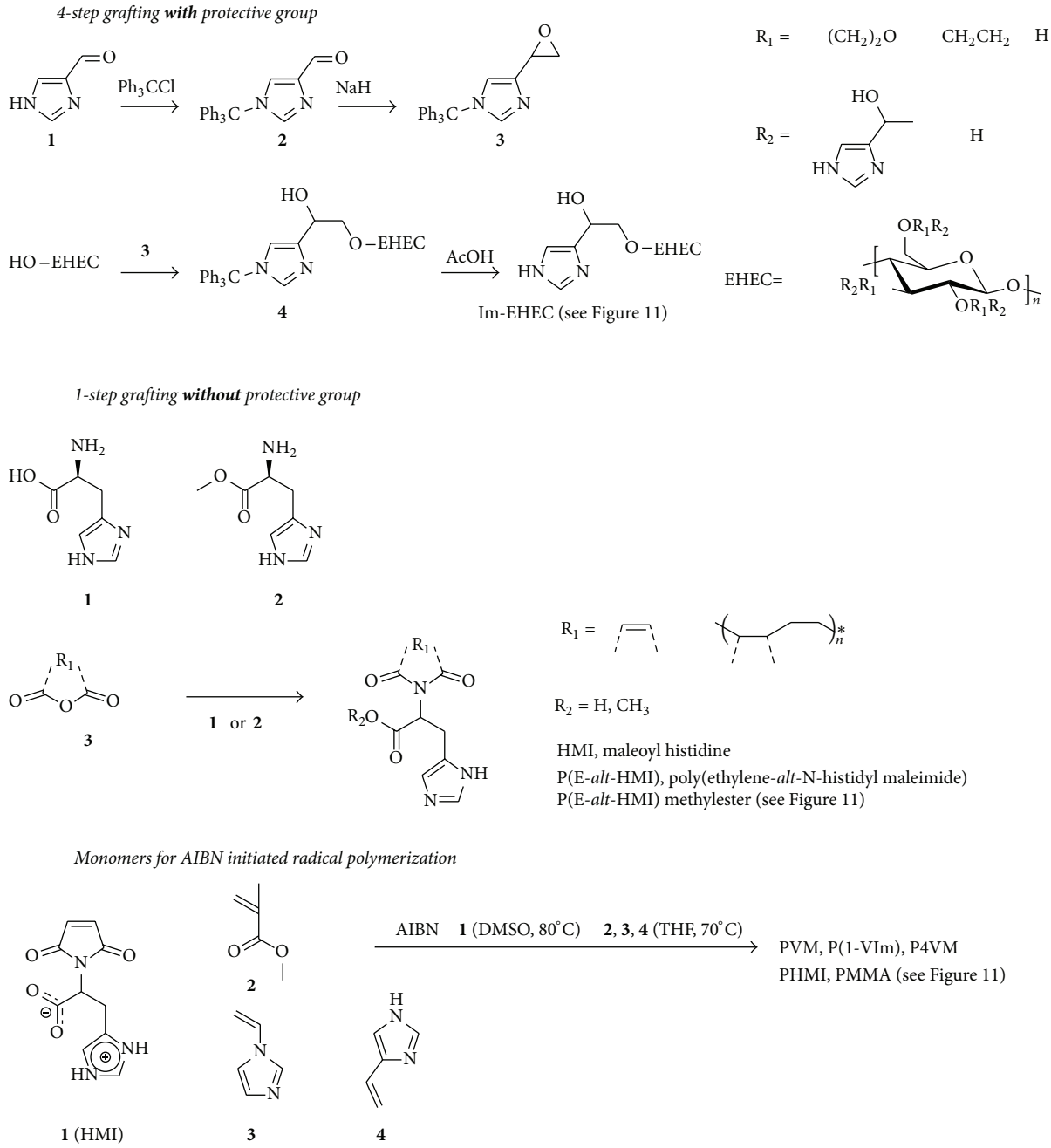
Scheme 6: Coordination geometries for imidazole-Cu(II) or -Zn(II) complexes [38]. (a) Square planar $[Cu(Im)_4]^{2+}$. (b) Tetrahedral $[Zn(Im)_4]^{2+}$. (c) Octahedral $[Zn(Im)_6]^{2+}$.



Scheme 7: (a) Molecular orbitals and ligand field splitting of Cu(II) in a square planar complex and of (b) Zn(II) or Cu(I) in a tetrahedral complex [96]. (a) With regard to cupric complexes, there is possibility for weakly bound counterions or coordinating solvent molecules in axial position and the geometry is then labelled tetragonal (distorted octahedral symmetry). However, the ligand field splitting will be similar in tetragonal and square planar symmetry if one accounts for the important 4s-3dz^2 mixing, which effectively lowers the metal dz^2 orbital in both symmetries [97]. (b) The ligand field splitting in tetrahedral complexes is the inverse of an octahedral one but with some mixing of the d_σ and p-orbitals. The situation with π-interaction is, however, more complicated since the π-interacting ligand orbitals may form both σ- and π-bonds, and the effect on the ligand field splitting will be a reflection of both bonding modes [96, 97].

the pyrrole nitrogen, reduction of the aldehyde to epoxide, epoxide opening and attachment to the polymer backbone, and finally removal of the protective group (see Scheme 8). The modified polymer and its interaction with Cu^{2+} are evaluated in Section 4.2.2.

We have also developed a synthesis procedure to attach the biological amino acid histidine to a polymer backbone as well as to a polymerizable monomer using maleimide chemistry [48]. The synthetic routes comprised both *grafting onto* by maleimide bond formation on a maleic anhydride polymer (see Scheme 8) and conventional radical polymerization (see Scheme 8). The successful grafting of histidine onto P(E-alt-MAh) (poly(ethylene-alt-maleicanhydride)) was confirmed by the formation of the maleimide bond. The DS (degree of



Scheme 8: Imidazole functionalization of polymers and monomers [48, 49] and monomers used for AIBN initiated radical polymerization [39, 47, 48].

substitution) ranging from 80–90% was estimated from NMR spectra and elemental analysis. In conclusion, imide bond formation between amine and anhydride is an efficient way of grafting histidine onto a polymer backbone [48].

Radical or anionic polymerization of maleimide monomers is an established synthesis procedure, thoroughly investigated [120–128]. Since the maleimide group is strongly electron withdrawing, effectively reducing the electron density at the maleic double bond, it is custom to copolymerize it with for example, styrene or other monomers containing electron donating groups. Such a copolymerization results in rather alternating copolymers [120]. This copolymerization technique is also common for polymerization of maleic anhydride, which is not possible to homopolymerize [120]. However, it is still possible to homopolymerize maleimides, even though the reaction rate is very slow [125]. The reaction rate in this case is additionally decreased since the histidine side group renders the monomer large and bulky [122, 129]. Both routes were successful, but the maleimide route facilitates both grafting onto and radical polymerization.

Furthermore, the maleimide route is conveniently carried out in one step without the necessity of protective groups [48].

Another way of functionalizing polymers is to copolymerize with the monomer of the polymer with a small fraction a functional comonomer. We have functionalized PMMA with imidazole moieties by simple copolymerization using AIBN as initiator (see Scheme 8). Copolymers of MMA (methylmethacrylate) and 1-VIm (1-vinylimidazole or N-vinylimidazole) (poly(1-VIm-*co*-MMA, or poly(N-VIm-*co*-MMA) abbreviated *PVM*) [47] as well as 4-VIm (4-vinylimidazole) (poly(4-VIm-*co*-MMA) were polymerized using conventional AIBN radical polymerization (see Scheme 8) with the rationale of functionalizing PMMA which is the most commonly used microcapsule shell material [47, 57–59, 63] (see Section 1.1). The polymerization results in statistical copolymers with a higher reactivity ratio for MMA compared with 1-VIm as demonstrated by Wu et al. [130].

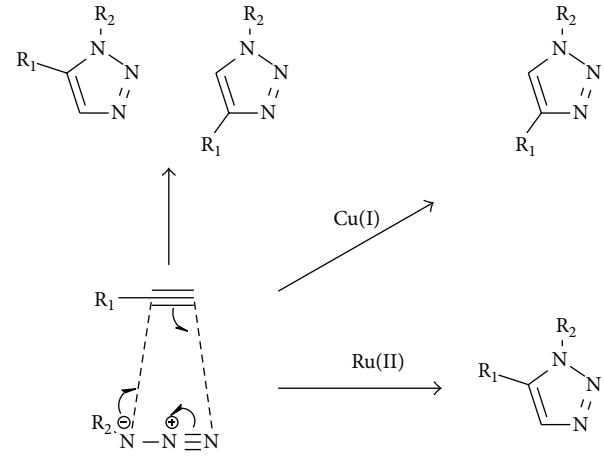
3.2. Triazole Functionalization and Click Chemistry. In conformity with the vinylimidazole-based polymer, polymers based on vinyltriazoles (1-vinyl-1,2,4-triazole [131], 1-vinyl-1,2,3-triazole [132], and 4-vinyl-1,2,3 triazole [132]) may be prepared by conventional AIBN-initiated radical polymerization [131, 132]. These polymers have found use as heavy metal binding resins for waste water treatments [131] and proton conductors (C-substituted 4-vinyl-1,2,3 triazole) [132].

However, triazole synthesis is most synonymous with the copper catalyzed alkyne-azide huisgen cycloaddition (CuAAC) reaction [117] (see Scheme 9). This reaction is a well-known example of a class of chemical reactions called "click reactions" defined for the first time by Kolb et al. [133]. Click reactions are characterized by high quantitative yields and tolerance towards the use of functional groups. The reactions are also relatively insensitive to the solvent system. Consequently, these characteristics make click reactions a very good tool for polymer synthesis and modifications.

It is obvious that the CuAAC reaction can be used for triazole functionalization of azide or alkyne containing polymers which has been investigated by us [50]. However, the CuAAC reaction can also be used to synthesize triazole-based vinyl monomers. Thibault synthesized vinyl containing triazole monomers (R_1 = vinyl in Scheme 9) which were polymerized *via* reversible addition chain-transfer (RAFT) reaction [134]. Using this methodology, a variety of monodisperse polymers carrying different functional group were synthesized [134].

4. Properties of Coordinated Polymers

The coordination of polymers carrying ligand sites by metal ions has a profound effect on the polymeric material [135–139], which includes properties and functions such as optical, electrical, rheological, molecular self-assembly, and solubility properties [137–141]. The basis for the majority of properties and functions relies on the specificity and strength of coordination for a given set of metal ion and ligand. Therefore, regarding the use of these materials in antifouling coatings (triggered release or Cu^{2+} sorption/redox reaction), it is important to understand the details of the coordinate interactions on both a microscopic and a macroscopic levels.



Scheme 9: Dipolar 1,3-cycloaddition of an azide and an alkyne. The regular Huisgen 1,3 dipolar cycloaddition between an azide and an alkyne results in a mixture of 1,4- and 1,5 substituted triazoles [117]. The CuAAC reaction is stereospecific and yields 1,4 substituted triazoles [118]. The reaction can also be catalysed by Ru(II) which yields 1,5 substituted triazoles [119].

In our group, the coordinate interaction of the materials has been characterized using EPR, vibrational spectroscopy (far-FTIR and mid-FTIR), *ab initio*, and DFT (Density Functional Theory) calculations. The material properties have been characterized using mainly DSC (differential scanning calorimetry) and vibrational spectroscopy.

4.1. Bond Strength and Selectivity. Regarding the PVM copolymers discussed in Section 3.1, the polymer contains two possible metal-coordinating ligands: the imidazole and the ester groups (see Scheme 1). The carbonyl group is regarded as a hard ligand with the oxygen atom as the ligating group, although with a theoretical possibility of acting as a soft π-bond donor [100]. However, the metal ions exclusively coordinate the imidazole ligand of the polymer [47]. This is evident since only imidazole bands in FTIR are altered upon coordination (see Figure 1) [39, 47]. Regarding copper complexes, EPR affirms this fact since complexation of PMMA results in no signal (see Figure 2(c)). The EPR results concern only Cu^{2+} complexes. Zn^{2+} is diamagnetic and hence EPR silent. Furthermore, the environment appears to be square planar (or tetragonal) due to the resemblance between the EPR spectra of square planar $[Cu(Me(1)\text{-}Im)_4]^{2+}$ and PVM-4-Cu^{2+}. This result is quite spectacular since the enforced coordination symmetry results in a high entropic penalty for the polymer chain. It should be noted that the EPR parameters and spectrum of PVM-4-Cu^{2+} are especially similar to imidazole methylated at position 1 (Me(1)-Im) coordinated by Cu^{2+} (see Figure 2) [38, 47].

Publications on polymers containing the C_4 substituted 4-vinylimidazole are rare in comparison with N_1 substituted 1-vinylimidazole, yet interesting due to the unsubstituted pyrrole nitrogen (see Schemes 1 and 8). Note that biological imidazole moieties, such as histidine, are never N_1-substituted [38] (see Section 6.1). Sato et al. have studied homopolymers

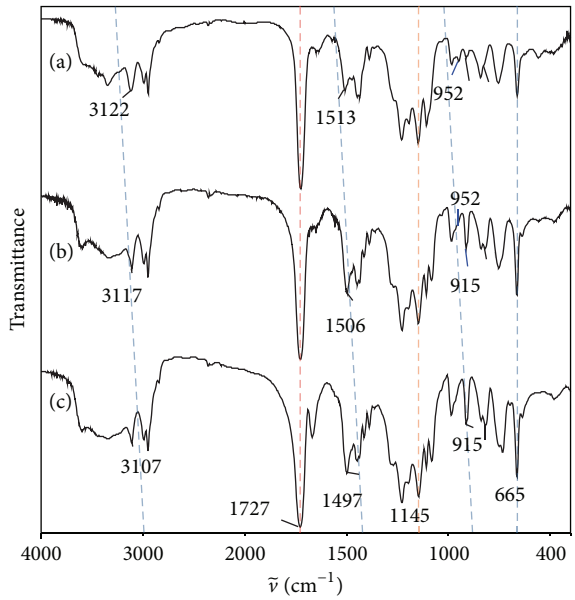


Figure 1: FTIR spectra of PVM-44 (c) coordinated by Cu^{2+} (a) or Zn^{2+} (b) [47]. As a result of complexation, some FTIR bands of the imidazole (marked blue) are shifted, in particular the in-plane skeletal vibrations of the imidazole ring. However, the carbonyl stretch of the MMA methylester (marked red) remains unaltered.

of poly(4(5)-vinylimidazole) [142–144] which appeared to be coordinated by Cu^{2+} in a square planar/tetragonal geometry. The copolymers investigated by us, poly(4(5)-VIm-*co*-MMA), appeared to form stronger bonds than the corresponding P(1-VIm-*co*-MMA) polymers as manifested by a complete insolubility of the resulting metal complex. In contrast, the metal complexes of P(1-VIm-*co*-MMA) were soluble in strongly coordinating solvents, for example, DMSO, MeCN, or DMF [47].

4.2. Coordinate Crosslinks. The coordinate bonds between the imidazole ligand and the metal ions generate crosslinks between the polymer chains [47]. The crosslinks cause the glass transition temperature, T_g, to increase since the chain segments in proximity of a crosslink are stiffened due to the reduced rotational freedom (see Figure 3) [47, 145]. The stiffness depends on the strength of the crosslink. Hence, the increase of T_g will depend on both the number of effective crosslinks as well as the strength of the crosslinking bond [47, 135, 136, 146]. Regarding the polymers, the increase of T_g is more pronounced for PVM-44 than PVM-4 due to the larger number of possible crosslinks. However, when the effect of the particular metal ion is inspected, it is observed that Cu^{2+} raises T_g more than Zn^{2+} for PVM-4 while the opposite holds for PVM-44. This may appear contradictory but is explained by coordination bond strength and coordination number. The bond strength between imidazole and Cu^{2+} which is stronger than the bond strength between imidazole and Zn^{2+} which is most important for PVM-4 with a *low number of imidazole ligands*. However, the total number of possible

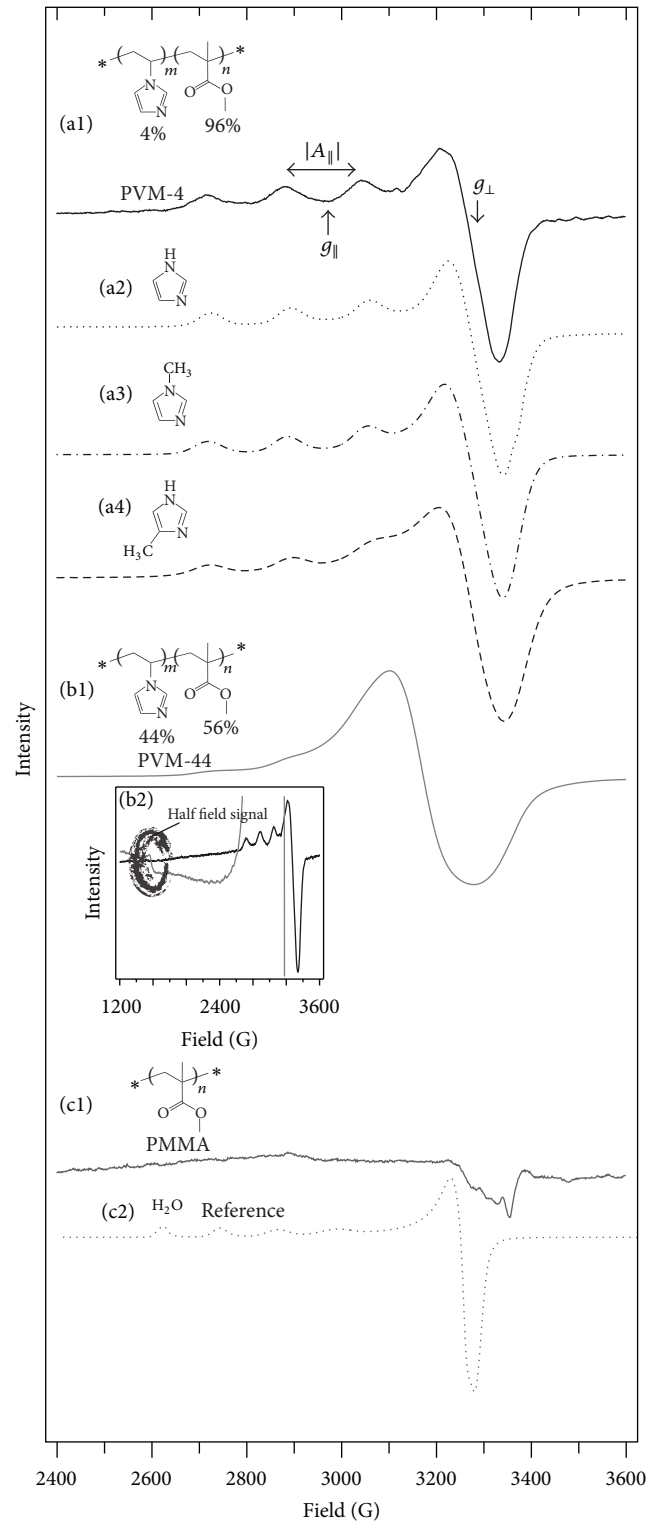


Figure 2: Cu^{2+} EPR spectra in MeCN of (a1) PVM-4, (a2) Im, (a3) Me(1)-Im, (a4) Me(4)-Im, (b1) PVM-44, (b2) PVM-44 half field signal, (c1) PMMA, and (c2) H_2O [38, 47]. The paramagnetic centers, that is, the Cu(II) ions, of PVM-44 are closer together compared to PVM-4 which is reasonable due to the larger fraction of imidazole ligands per polymer chain. To be more precise, the coordination complexes are separated by less than 20 Å (see Figure 2(b2)) which is indicated by the EPR half field signal (see Figure 2(b2)).

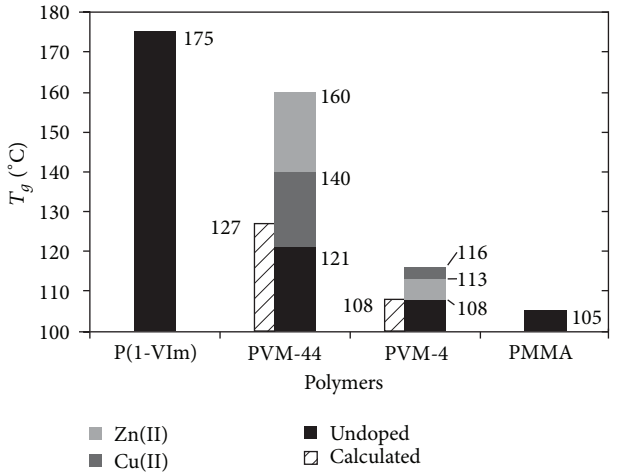


Figure 3: Glass transition of polymers and polymer complexes [47].

crosslinks may be higher for Zn^{2+} complexes since this metal allows for octahedral geometry (see Section 2.3), whereas Cu^{2+} is restricted to square planar geometry. This explains why PVM-44 with a *high number of imidazole ligands* coordinated to Zn^{2+} acquires a higher T_g than PVM-44 coordinated to Cu^{2+} despite a weaker coordinate bond.

4.2.1. Hygroscopicity and Metal Coordination. The hygroscopicity of a PVM polymer containing a metal ion salt is highly dependent on the fraction of imidazole containing monomers. In addition, the water molecules can crosslink two imidazole groups of a PVM polymer in a similar manner as the metal cation. The hydrogen-bond interaction is too weak to alter the thermal properties of the polymer, yet, the change of the polarization of the water molecules is evident by the blue shift of the δ(HOH) water bending vibration (see Figure 4). However, when metal ions are introduced, this population of bridged water molecules disappears and is replaced by bridging metal ions (see Figure 4) [39, 47]. For PVM-4, with a low fraction of imidazole ligands, the introduction of metal ions results in a significant increase of solitary water as indicated by the area of the ν(OH) water stretching vibrations [39]. However, for PVM-44 with a high fraction of imidazole ligands, the metal ion coordination results in a decrease of the amount of sorbed water [39]. Note that this polymer is already rather polar due to the high fraction of N-VIm monomers. Consequently, the triggered release effect discussed in Section 1.2 is more applicable for hydrophobic polymers which are functionalized with crosslinking metal ions.

4.2.2. Coordination-Mediated Multilayer Assembly. The strong coordinate cross-links discussed previously can be used to assemble polymer multilayers. Polymer multilayers are most synonymous with the polyelectrolyte layer-by-layer technique which has received a lot of attention during the last decade [64]. However, coordination chemistry is a feasible tool to assemble multilayers with anisotropic properties as provided by the restricted coordination geometry of a given metal ion. Hedin et al. studied the bilayer formation of the imidazole functionalized biopolymer Im-EHEC (see Scheme 1) using QCM-D [49]. Although Im-EHEC was functionalized with only 1% imidazole moieties per monomer unit, the introduction of copper resulted in a slight crosslinking of the adsorbed polymer layer, and the subsequent addition of Im-EHEC solution gave an additional adsorbed monolayer of Im-EHEC (see Figure 5).

5. Antifoulant Immobilization and Controlled Release

In the following subsections, ways to immobilize and control the release of antifouling agents using coordinate interactions, with a special focus on medetomidine, will be presented. The immobilization of medetomidine has been evaluated using HPLC, NMR, and vibrational spectroscopy as well as monitored *in situ* in model systems using QCM-D and SPR. The controlled release has been monitored from real coatings using liquid scintillation spectrometry (^{14}C_ probed release substances) as well as from model surfaces using the surface sensitive techniques QCM-D and SPR.

5.1. Polymer Coordination. Triazole derivatives represent a wide class of antimicrobial substances (see Scheme 1) [19–27] as mentioned in the Introduction. Their use in antifouling coatings has been claimed in a number of patents [147–150]. As an example, Tanaka and coworkers immobilized antifoulants based on triazole, thiadiazole, and benzotriazole in resins containing a high fraction of carboxylate groups [147].

In a similar manner, Shtykova et al. studied the immobilization of the imidazole containing medetomidine (see Scheme 1) on an alkyd resin using NMR diffusometry [44]. It was found that the self-diffusion coefficient of medetomidine was decreased by more than one order of magnitude due to immobilization of medetomidine on the alkyd resin. It was proposed that the mechanism of immobilization was hydrogen bonding between the protonated imidazolium ion of medetomidine and deprotonated carboxylate groups of the alkyd resin [44].

This concept was further developed by Handa et al. to encompass polymers with very strong Lewis acid sites. Medetomidine was immobilized and released from the sulphonated polymer SPSEBS (see Scheme 1, the nonsulphonated polymer PSEBS was used as a reference) as monitored *in situ* using QCM-D [41]. In the solvent xylene, large amounts of medetomidine were adsorbed almost irreversibly, which was ascribed to a coulombic charge-transfer reaction in the hydrophobic solvent. The interaction was much weaker in artificial seawater with less adsorbed medetomidine and a pronounced release upon rinsing [41]. This system could consequently be used as a triggered release system (see Scheme 2) as discussed in Sections 1.1 and 1.2.

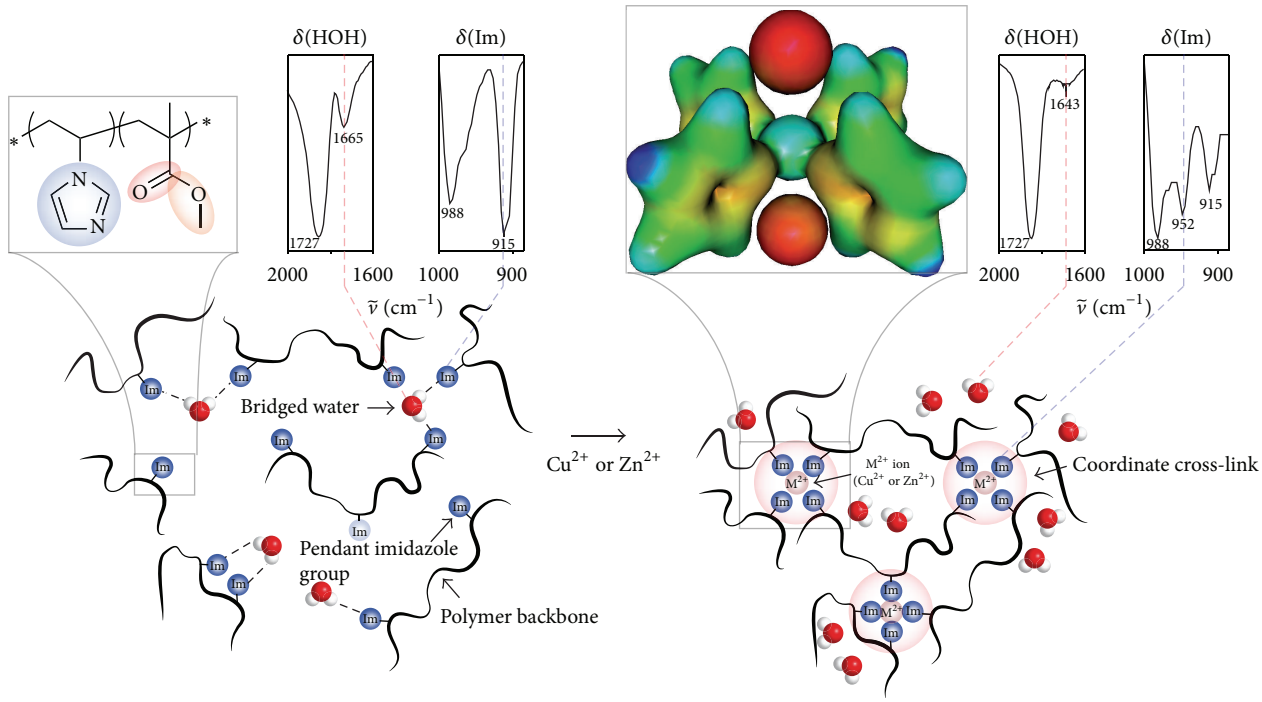


FIGURE 4: Schematic drawing of Im-H_2O-Im bridged water and subsequent replacement by Cu^{2+} or Zn^{2+}, including the most important markers for H_2O and Im, respectively, as well as the electrostatic potential energy surface of the $[Cu(Im)_4X_2]$ complex as obtained from *ab initio* calculations [39].

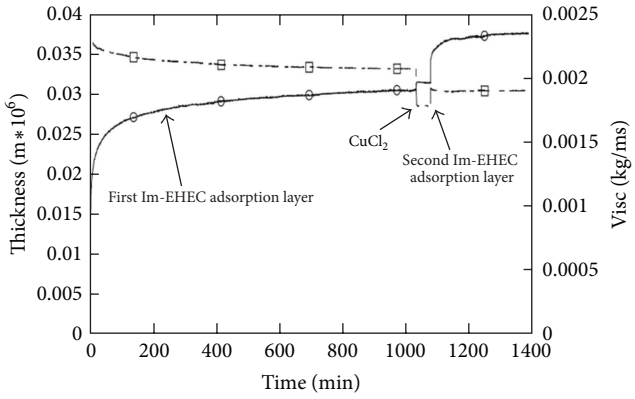


FIGURE 5: Bilayer formation of Im-EHEC (see Scheme 1) with $CuCl_2$ (measured using QCM-D and modeled using a Voight-based viscoelastic model). The second layer is thin and corresponds to a monolayer. Reprinted from [49].

5.2. Metal Ion and Metal Oxide Nanoparticle Coordination. The hydrogen bonds mentioned in the previous section are relatively strong interactions; yet, the strength of coordinate metal-ligand bonds discussed in Section 2 is significantly higher. Therefore, the Nydén group explored the feasibility of using transition metal coordination for medetomidine immobilization. Fant et al. investigated the adsorption and controlled release of medetomidine from PVM polymers (see Scheme 1, PVM-44) coordinated by Cu^{2+} or Zn^{2+} using QCM-D [40]. Cu^{2+} was adsorbed in much higher amounts than Zn^{2+} by the PVM polymer which is in line with the stability constants. Moreover, the metal ion adsorption resulted in a pronounced swelling of the PVM polymer by water which corroborates its feasibility to be used as a material for triggered release applications as discussed in Sections 1.2 and 4.2.1. The subsequent addition of medetomidine resulted in significant adsorption for both PVM coordinated by Cu^{2+} and Zn^{2+} (see Figure 6). Note that the resulting complex contains imidazole ligands from both the PVM polymer and medetomidine. However, the interaction of medetomidine with Zn^{2+} was more reversible than with Cu^{2+} and resulted in a much faster release (see Figure 6). Thus, the release profile may be tailored by coordinating the PVM polymer with mixtures of Zn^{2+} and Cu^{2+} [40].

In addition to polymers coordinated by metal ions, metal oxide nanoparticles are feasible vehicles for antifoulant immobilization using coordination chemistry [45, 46]. Shtykova and coworkers studied the immobilization of some common booster biocides including medetomidine and Seanine (see Scheme 1) on different types of metal oxide nanoparticles (see Figure 7). It was apparent that the booster biocides containing ligand moieties (medetomidine, irgarol, diuron, and Seanine) gave the strongest adsorption. Note that the isothiazolinone Seanine (see Scheme 1) has the possibility to bind *via* hard interaction (carbonyl oxygen) or *via* soft interaction (sulphur free electron pair). Regarding the nitrogen which is of pyrrole type, interaction at this position is only possible at very high pH for isothiazolinones which

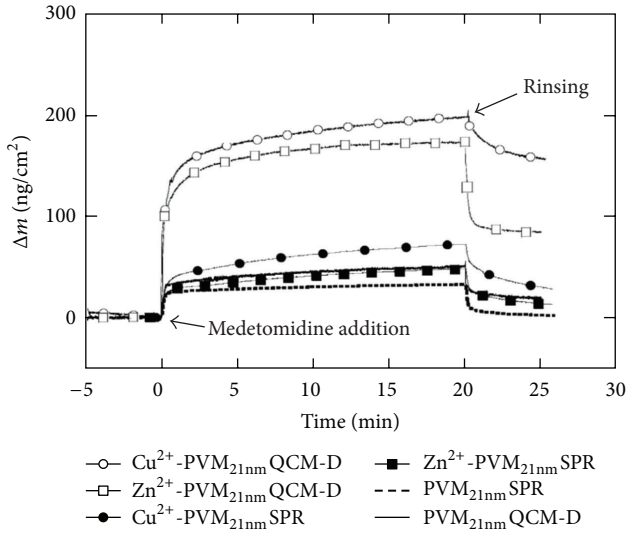


Figure 6: Medetomidine absorption (measured using QCM-D and SPR) to a Cu^{2+} doped, Zn^{2+} doped, and metal free 21 nm thick PVM film. It is apparent that metal coordination significantly enhances medetomidine adsorption and that the coordination to Cu^{2+} is stronger and less reversible. Reprinted from [40]. The combination of QCM-D and SPR measurements can be used to estimate the amount of sorbed solvent in an adsorbed film. The acoustic QCM-D technique probes the entire adsorbed viscoelastic layer, including bound solvent. In contrast, the SPR method measures changes in refractive index at the surface and consequently takes only the mass of the adsorbent into account. The difference in adsorbed mass from QCM-D and SPR measurements can therefore be related to the amount of solvent.

are unsubstituted at position 2. However, the affinity of medetomidine for the metal oxide particles was substantially higher than that of any other biocide. This indicates that the imidazole group of medetomidine is key for its successful immobilization (see Figure 7(a)). The order of increasing interaction strength with the imidazole ligand was slightly different for the metal oxides compared with the corresponding metal ions (see Figure 7(b), and ZnO displays a stronger interaction with medetomidine than CuO) [42]. This is reasonable since the metal centers in the oxides are much more electron-rich compared to the free metal ions. The immobilization of medetomidine on ZnO nanoparticles (which are common pigments in antifouling marine paints) resulted in a significant decrease in the release rate from a varnish coating as compared with medetomidine molecularly dispersed in the same coating (see Figure 7(c)) [42].

6. Effect of Ligand Substituent Position and Ligand Chemistry

The coordinate interaction as described in Section 2 is not only dependent on the type of ligand used but also on the substituents attached at the azole ring. This has been investigated by us in detail using vibrational spectroscopy, EPR, *ab initio*, and DFT calculations and is presented in Section 6.1. In Section 6.2, these results are discussed with respect to Cu^{2+}/Cu^{+} stabilization as presented in Section 1.3.

6.1. "Natural" versus Synthetic Ligands. When inspecting the polymers discussed in Section 3 (see Scheme 1), it is observed that the imidazole ligand is attached to the polymer backbone at either position 1 or 4, and this holds for in principle any arbitrary imidazole-containing polymer. Moreover, regarding imidazole from biological sources, such as histidine, the imidazole ring is always substituted at position 4. This is in contrast to synthetic imidazole-containing polymers where the imidazole ring is usually attached to the backbone at position 1, that is, *via* the pyrrole nitrogen. Subsequently, it may be speculated why nature has chosen this particular position for substituent attachment. To investigate this, the methylated imidazole model compounds Me(1)-Im and Me(4)-Im (see Schemes 1 and 10) were analysed with respect to similarities and differences in coordination strength, coordination geometry, covalency, and so forth [38].

Both compounds display profound covalence in the σ-bond ($\alpha^2 = 71\%$ according to DFT calculations) which is also evident by the hyperfine splitting in the EPR spectrum (see Figure 8) [38]. An even better indication of the high degree of covalence is the π-interaction coefficient (EPR; $\beta^2 \approx [0.65, 0.69]$, *ab initio*; $\beta^2 \approx [0.51, 0.60]$) [38, 151]. Small differences as an effect of position regarding the coordinate interaction are evident since the Me-C_4 bond is slightly more polarizable than the Me-N_1 bond. As a consequence, Me(4)-Im is a stronger base than both Me(1)-Im and Im since the methyl substituent is an electron-donating group (see Scheme 10) [38]. However, the stability constant is lower for the corresponding Me(4)-Im complex compared to Me(1)-Im and Im [38, 152] which suggest a significant π-acceptor interaction in addition to the σ-donor interaction in the coordinate complex [38] as discussed in Section 2. However, much larger effects were obtained by changing the type of substituent as was apparent from *ab initio* calculation. By systematically changing the position of the substituent as well as the substituting group, it was observed that ΔE (the energy of complexation) and υ_a(M-L) (metal-ligand asymmetric stretch vibration) were more affected by the chemistry of the group than its actual position.

The coordination geometries for Me(1)-Im and Me(4)-Im are more or less identical. For instance, the steric hindrance of the methyl group upon coordination is the same for both methylated imidazoles (see Schemes 1 and 10 and Figure 9) since Me(4)-Im actually coordinates as Me(5)-Im [38, 154] due to tautomerism and steric restrictions. As a consequence, the coordination geometries for the methylated imidazole ligands are very similar, both for Cu^{2+} and Zn^{2+} complexes, in contrast to unsubstituted imidazole (see Figure 9). However, an important difference between Me(1)- and Me(4)-Im is the reduced "degrees of freedom" for Me(1)-Im. Me(4)-Im, equivalent to Im or triazoles, contains two potential sites for coordination or protonation. Consequently, Me(4)-Im and Im may, as a function of ligand metal ratio and pH, form coordination polymers which is restricted to Me(1)-Im [38].

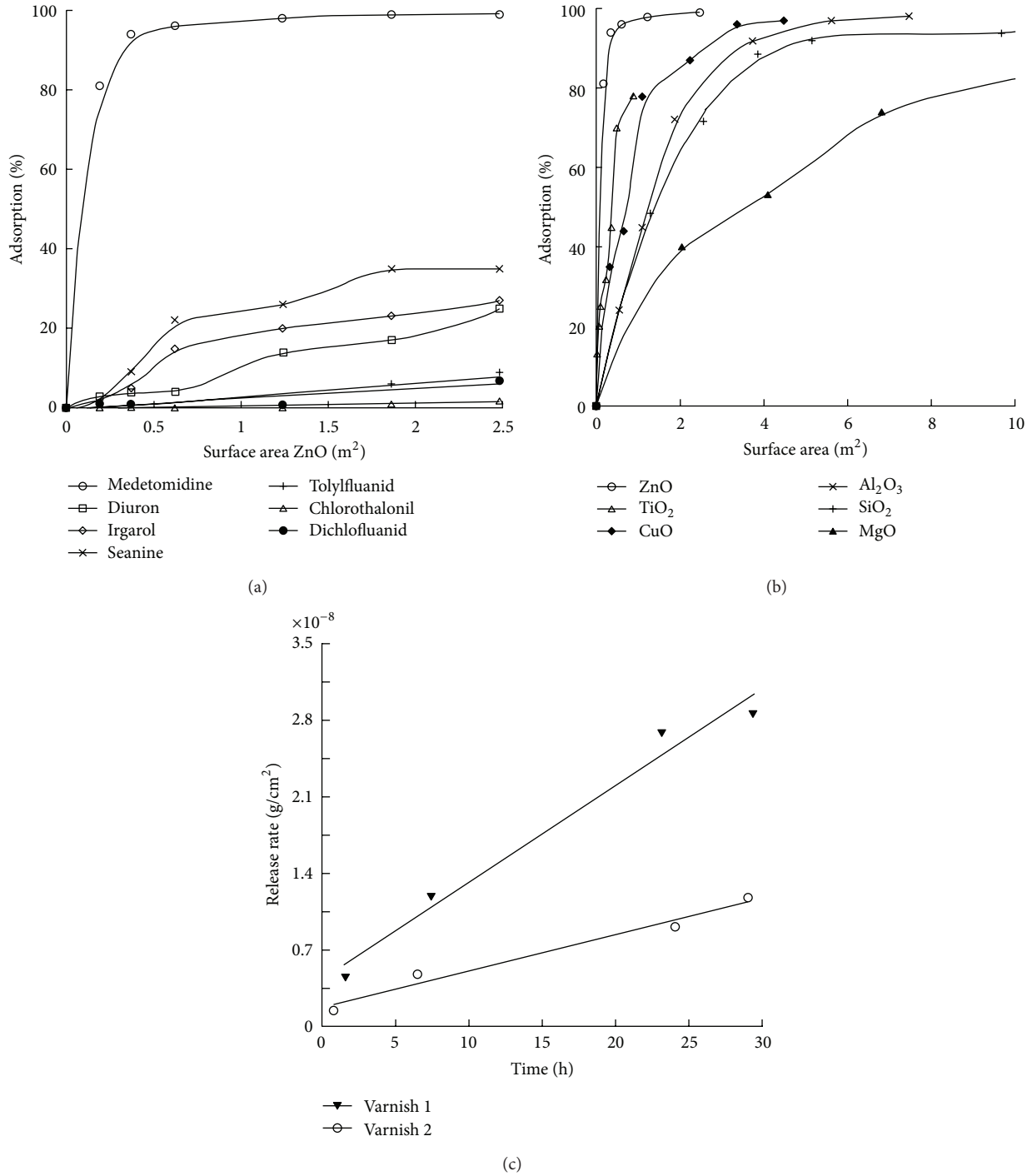
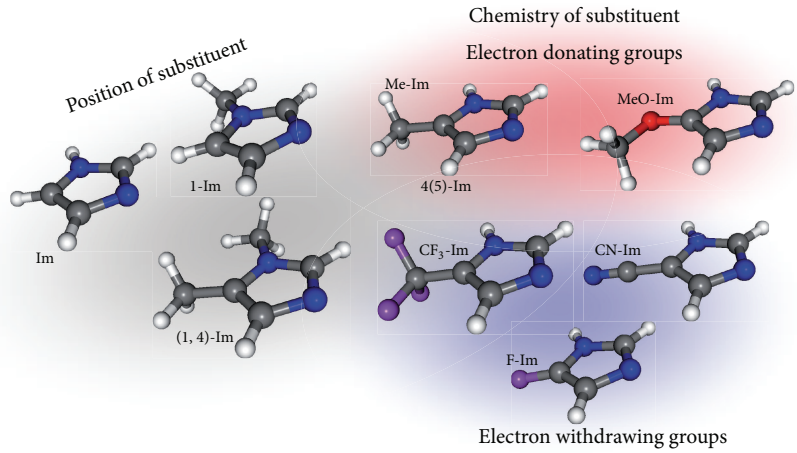
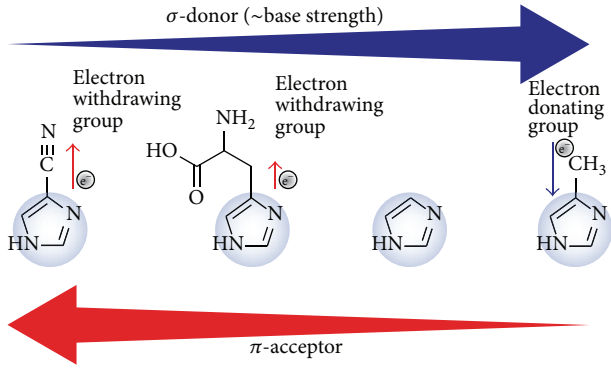


Figure 7: (a) Immobilization efficiency of various booster biocides including medetomidine (see Scheme 1) on ZnO nanoparticles as a function of total particle surface area. (a) Immobilization efficiency of medetomidine on various metaloxide nanoparticles as a function of total particle surface area. (c) Effect on release of free (varnish 1, ▼) and ZnO immobilized (varnish 2, ○) medetomidine from coatings. Reprinted from [42].

If the polymeric imidazole ligands used in this work are compared, that is, 1-VIm and histidine, the *position* of the substituent in the imidazole ring is not the most important factor (see Scheme 11). The *chemical nature* of the substituent, more precisely its electron withdrawing or electron donating abilities, is more important with respect to the coordinate interaction. However, the position of the ring substituent is important when it comes to *bidentate* coordination, the possibility to conduct protons between adjacent imidazole ligands and ring polarization due to coordinating nucleophiles [38].



Scheme 10: Imidazole derivatives with various substituents at different ring positions. The molecules have been geometry-optimized using DFT [38].



Scheme 11: The effect of the substituent with respect to electron donating/accepting capacity on the coordinate interaction.

6.2. Cu^{2+}/Cu^{1+} Stabilization. As mentioned in the introduction, Cu^{+} is highly unstable in water and disproportionates to Cu^{2+} and Cu^{0}. This is a consequence of the hydrate complex where water, which is a hard base (see Section 2.1), strongly stabilizes Cu^{2+} (border line acid) over Cu^{+} (soft acid). However, it is possible to alter the redox potential in favor of Cu^{+} by changing the ligand environment [100, 158]. In Figure 10, 4 mM $CuCl_2$ has been dissolved in water/glycerol (hard ligands) or acetonitrile (MeCN, soft ligand) [38]. The area of the EPR signal is proportional to the concentration of Cu(II) ions. The MeCN system displays a smaller area compared to the water/glycerol system which indicates reduction of Cu(II) to the EPR silent Cu(I). It is well known that nitriles strongly stabilize Cu^{+} in favour of Cu^{2+} [38, 47, 76, 159–161].

However, soft ligands such as nitriles prevent the catalytic ability of Cu^{2+}/Cu^{+} by stabilizing the Cu(I) oxidation state too well [76]. In order to find a proper ligand environment, scientists are once again finding inspiration in biological systems, in particular copper containing enzymes [76, 79]. Regarding the efficient catalytic activity and the electron transfer capability found in type 1 blue copper proteins, the ligand set of the copper complex contains both Cu^{2+} and Cu^{+} stabilizing ligands (histidine and cysteine/methionine resp.) [79, 162]. In addition, the coordination geometry is forced into a distorted tetrahedral symmetry which is an intermediate geometry between square planar/tetragonal (Cu^{2+} coordination) and tetrahedral (Cu^{+} coordination, see Scheme 6) [79]. This has been suggested to facilitate the ease of electron transfer and oxidation/reduction of the copper ion [79, 162].

We are currently investigating triazole-based ligands for Cu^{+} stabilization [50] as described in Section 1.3 with respect to antifouling properties. The polymers are synthesized by a *grafting onto* approach as described for imidazoles [48] in Section 3.1 using the CuAAC click reaction (see Section 3.2). The triazole ligand have, in accordance with imidazole, a very high affinity for Cu^{2+}. Yet, triazole ligands may also stabilize Cu^{+}. Moreover, the coordination geometry can be tuned by the proper spatial distribution of ligands of different chemical nature. By tailoring the ligand environment and the subsequent coordination geometry in triazole containing polymers, our intention is to prepare antifouling hydrophilic polymers [163], inspired by copper enzymes, which may absorb Cu^{2+} from the sea as well as facilitate the $Cu^{2+} \rightarrow Cu^{+}$ reduction.

7. Conclusion and Outlook

Azole coordination chemistry shows great potential with respect to antifouling coatings for a number of reasons. Many antifouling agents contain azole moieties. The imidazole containing biocide medetomidine is promising antifouling agent with respect to protection against fouling of barnacles. The fact that the azole moiety is a ligand can be used to immobilize these biocides on macromolecules or nanoparticle and thus control their release (both sustained and triggered release are possible). Moreover, the polymeric materials used for the purpose of controlling the release of antifouling agents can be given new properties *via* transition

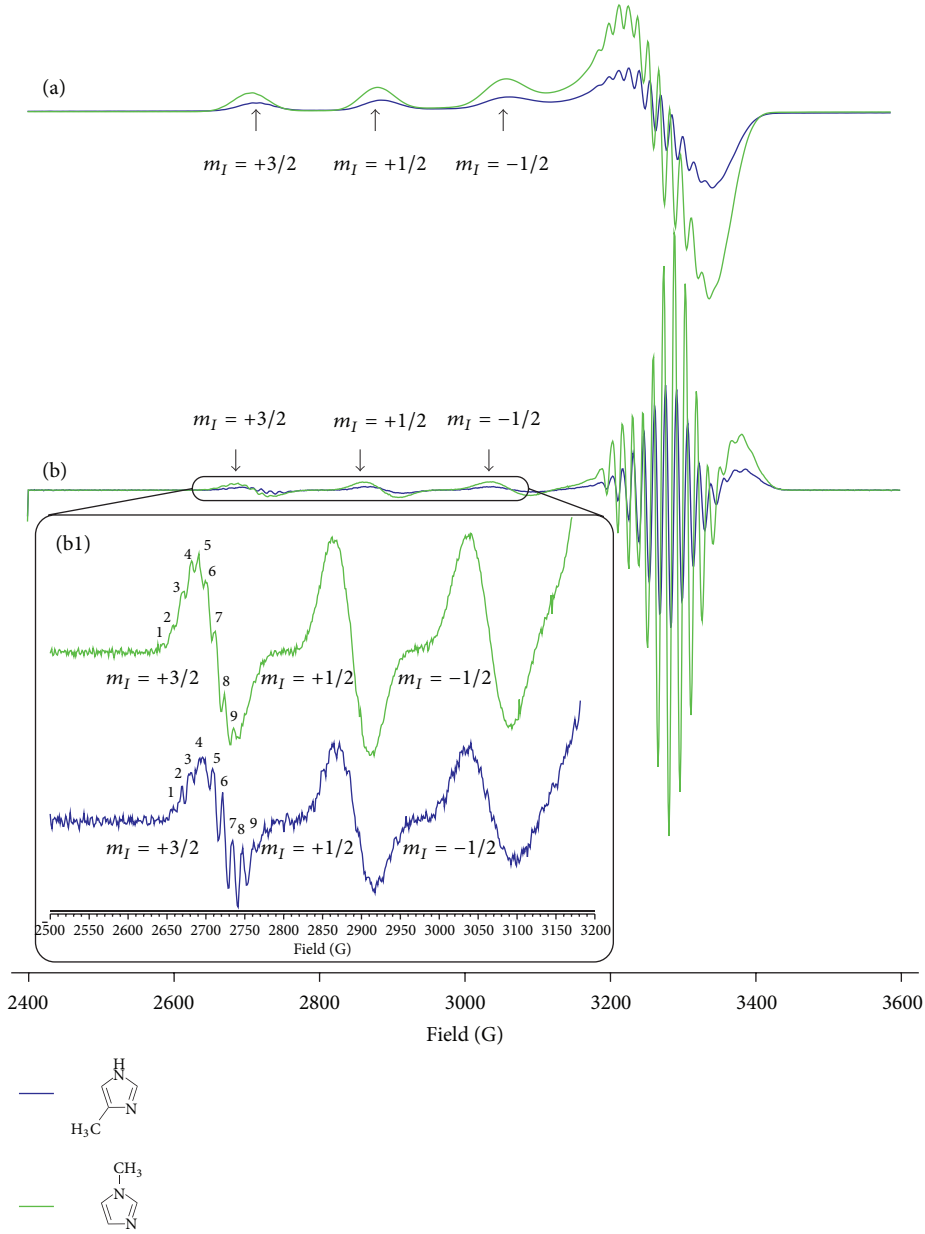


Figure 8: Me-Im in water/glycerol. (a) EPR signal of Me(1)-Im (green) and Me(4)-Im (blue). (b) Derivative of the EPR signal with inset (b1) displaying the fine structure at $m_I = +3/2$ [38]. The fine structure indicates four equivalent ligands. However, some signals appear to be doublets which may be explained by the Cu^{2+} isotope mixture [79, 153].

metal coordination of pendant azole ligands in the polymer. However, the most promising antifouling approach lies in the very strong affinity of azoles for Cu^{2+} which is a potent antifouling biocide. An antifouling coating containing azole ligands can subsequently absorb large amounts of naturally abundant copper and obtain antifouling protection without any net release of biocide. Moreover, the $Cu^{2+} \rightarrow Cu^{+}$ redox potential may be altered towards reduction to Cu^{+}, which is the most bioactive oxidation state. This can be achieved by tailoring the ligand chemistry where triazole ligands are promising for these purposes. We believe that the potential of this bioinspired approach has not been fully explored. A lot of inspiration can be found in nature, in particular copper enzymes. These enzymes are optimised through evolution for copper absorption as well as copper reduction by using a proper choice of ligand chemistry and ligand geometry.

Conflict of Interests

The research in this work has been financed by the foundation for environmental strategic research, MISTRA, which is administrated by the Swedish state. In the paper, the brand names of two antifouling biocides are mentioned; Selectope and Seanine. It is hereby stated that neither of the authors

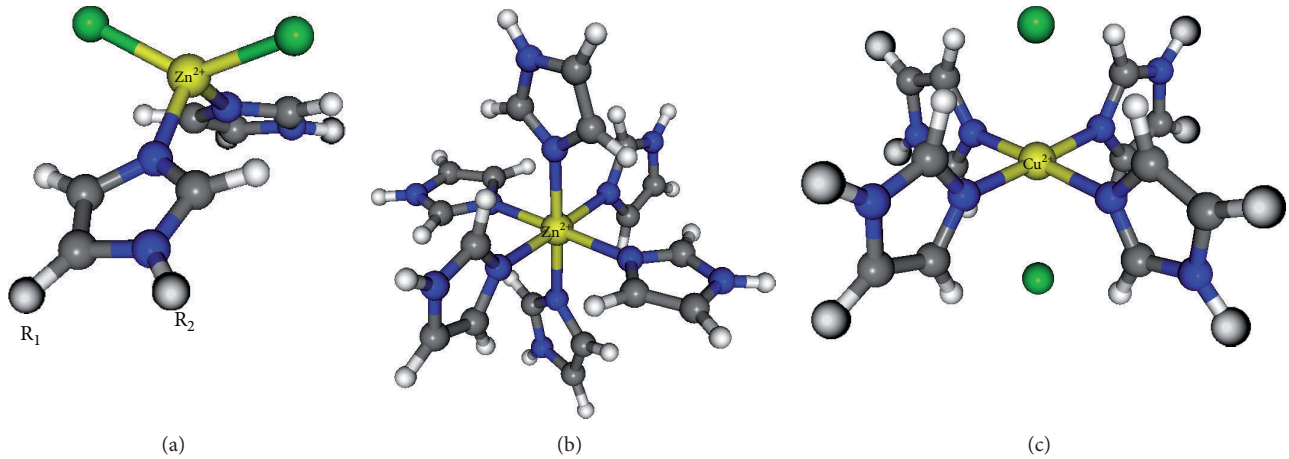


Figure 9: Coordination geometries [38]. (a) Tetrahedral $[Zn(Me\text{-}Im)_2Cl_2]$, (b) octahedral $[ZnIm_6]^{2+}$, and (c) tetragonal $[CuIm_4Cl_2]$ and $[Cu(Me\text{-}Im)_4Cl_2]$. R_1 and R_2 are defined as in Scheme 1. The coordination geometry for Cu^{2+} complexes is tetragonal D4 h symmetry for the methylated imidazoles, equivalent to unsubstituted imidazole. The imidazole ligands are almost perpendicular to the coordination axis and the chlorides are weakly bound axially [38, 154–156]. However, regarding Zn^{2+} complexes, unsubstituted imidazole forms octahedral complexes [38, 155] $[Zn(Im)_6]^{2+}$, whereas the methylated imidazoles form tetrahedral C2v complexes [38, 156, 157] $[Zn(Me\text{-}Im)_2Cl_2]$ despite the large excess of imidazole ligand.

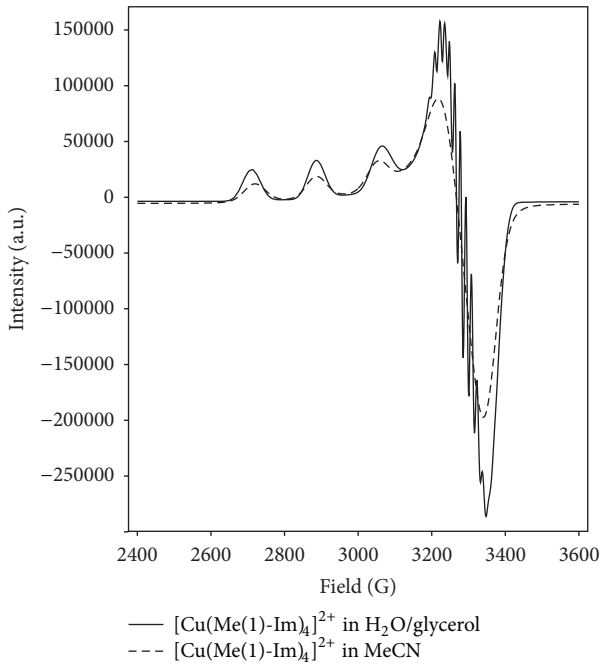


Figure 10: EPR spectra of a tetragonal Cu(II) complex with 1-methylated imidazole in a mixture of water and glycerol (—) and acetonitrile (- - -).

is in any financial relations with the previously mentioned commercial identities. Therefore, no conflict of interests exists.

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References

[1] E. Almeida, T. C. Diamantino, and O. de Sousa, "Marine paints: the particular case of antifouling paints," *Progress in Organic Coatings*, vol. 59, no. 1, pp. 2–20, 2007.

[2] T. Backhaus and Å. Arrhenius, *Marine Paint Final Report 2003–2011*, U.O. Gothenburg, Gothenburg, Sweden, 2012.

[3] L. D. Chambers, K. R. Stokes, F. C. Walsh, and R. J. K. Wood, "Modern approaches to marine antifouling coatings," *Surface and Coatings Technology*, vol. 201, pp. 3642–3652, 2006.

[4] I. Omae, "General aspects of tin-free antifouling paints," *Chemical Review*, vol. 103, no. 9, pp. 3431–3448, 2003.

[5] International Maritime Organization, International Convention on the Control of Harmful Anti-fouling Systems on Ships, 2011, http://www.imo.org .

[6] I. Omae, "Organotin antifouling paints, their alternatives and marine environment," *Kagaku Kogyo*, vol. 54, p. 646, 2003.

[7] C. Hellio and D. Yebra, *Advances in Marine Antifouling Coatings and Technologies*, Woodhead Publishing, 2009.

[8] M. A. Champ and P. F. Seligman, "Research information requirements associated with the environmental fate and effects of compounds," in *Organotin*, pp. 601–614, 1996.

[9] M. A. Champ and P. F. Seligman, *Organotin: Environmental Fate and Effects*, 1996.

[10] H. Blanck and B. Dahl, "Recovery of marine periphyton communities around a Swedish marina after the ban of TBT use in antifouling paint," *Marine Pollution Bulletin*, vol. 36, no. 6, pp. 437–442, 1998.

[11] M. Gustafsson, I. Dahllöf, H. Blanck, P. Hall, S. Molander, and K. Nordberg, "Benthic foraminiferal tolerance to tri-n-butyltin (TBT) pollution in an experimental mesocosm," *Marine Pollution Bulletin*, vol. 40, no. 12, pp. 1072–1075, 2000.

[12] A. Arrhenius, T. Backhaus, F. Groenvall, M. Junghans, M. Scholze, and H. Blanck, "Effects of three antifouling agents

on algal communities and algal reproduction: mixture toxicity studies with TBT, irgarol, and sea-nine," *Archives of Environmental Contamination and Toxicology*, vol. 50, no. 3, pp. 335–345, 2006.

[13] H. Blanck, K. M. Eriksson, F. Grönvall et al., "A retrospective analysis of contamination and periphyton PICT patterns for the antifoulant irgarol 1051, around a small marina on the Swedish west coast," *Marine Pollution Bulletin*, vol. 58, no. 2, pp. 230–237, 2009.

[14] B. Dahl and H. Blanck, "Toxic effects of the antifouling agent Irgarol 1051 on periphyton communities in coastal water microcosms," *Marine Pollution Bulletin*, vol. 32, no. 4, pp. 342–350, 1996.

[15] I. Dahllof, H. Blanck, and P. Hall, "Short-term effects of tri-n-butyl-tin on marine sediment samples using nutrient fluxes as effect indicators," *Environmental Toxicology and Chemistry*, vol. 18, no. 5, pp. 850–857, 1999.

[16] K. M. Eriksson, A. Antonelli, R. H. Nilsson, A. K. Clarke, and H. Blanck, "A phylogenetic approach to detect selection on the target site of the antifouling compound irgarol in tolerant periphyton communities," *Environmental Microbiology*, vol. 11, no. 8, pp. 2065–2077, 2009.

[17] K. M. Eriksson, A. K. Clarke, L.-G. Franzen, M. Kuylenstierna, K. Martinez, and H. Blanck, "Community-level analysis of psbA gene sequences and irgarol tolerance in marine periphyton," *Applied and Environmental Microbiology*, vol. 75, no. 4, pp. 897–906, 2009.

[18] P. Johansson, K. M. Eriksson, L. Axelsson, and H. Blanck, "Effects of seven antifouling compounds on photosynthesis and inorganic carbon use in sugar kelp Saccharina iatissima (Linnaeus)," *Archives of Environmental Contamination and Toxicology*, vol. 63, no. 3, pp. 365–377, 2012.

[19] D. George and S. van Epps, "Benzyl triazole-based aromatase inhibitors for the treatment of breast cancer," *Bioact. Compd. Cl*, p. 275, 2012.

[20] P. Worthington, "Sterol biosynthesis inhibiting triazole fungicides," *Bioactive Heterocyclic Compound Classes*, p. 129, 2012.

[21] R. Kharb, P. C. Sharma, and M. S. Yar, "Pharmacological significance of triazole scaffold," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 26, no. 1, pp. 1–21, 2011.

[22] I. P. Korbila and M. E. Falagas, "Investigational antimicrobial drugs for blood stream infections," *Current Opinion in Investigational Drugs*, vol. 9, no. 8, pp. 871–878, 2008.

[23] R. C. Moellering Jr., J. R. Graybill, J. E. McGowan Jr., and L. Corey, "Antimicrobial resistance prevention initiative: an update: proceedings of an expert panel on resistance," *The American Journal of Medicine*, vol. 120, no. 7, pp. S4–S25, 2007.

[24] M. M. Pearson, P. D. Rogers, J. D. Cleary, and S. W. Chapman, "Voriconazole: a new triazole antifungal agent," *Annals of Pharmacotherapy*, vol. 37, no. 3, pp. 420–432, 2003.

[25] C. W. Roberts, R. McLeod, D. W. Rice, M. Ginger, M. L. Chance, and L. J. Goad, "Fatty acid and sterol metabolism: potential antimicrobial targets in apicomplexan and trypanosomatid parasitic protozoa," *Molecular and Biochemical Parasitology*, vol. 126, no. 2, pp. 129–142, 2003.

[26] D. S. Schiller and H. B. Fung, "Posaconazole: an extended-spectrum triazole antifungal agent," *Clinical Therapeutics*, vol. 29, no. 9, pp. 1862–1886, 2007.

[27] N. Singhal, P. K. Sharma, R. Dudhe, and N. Kumar, "Recent advancement of triazole derivatives and their biological significance," *Journal of Chemical and Pharmaceutical Research*, vol. 3, no. 2, pp. 126–133, 2011.

[28] T. Porsbring, H. Blanck, H. Tjellström, and T. Backhaus, "Toxicity of the pharmaceutical clotrimazole to marine microalgal communities," *Aquatic Toxicology*, vol. 91, no. 3, pp. 203–211, 2009.

[29] i-tech marine paint, about selektope, 2012, http://www.i-tech.se/en/technology_and_product.aspx.

[30] A. Hilvarsson, C. Ohlauson, H. Blanck, and Å. Granmo, "Bioaccumulation of the new antifoulant medetomidine in marine organisms," *Marine Environmental Research*, vol. 68, no. 1, pp. 19–24, 2009.

[31] C. Ohlauson, K. M. Eriksson, and H. Blanck, "Short-term effects of medetomidine on photosynthesis and protein synthesis in periphyton, epipsammon and plankton communities in relation to predicted environmental concentrations," *Biofouling*, vol. 28, no. 5, pp. 491–499, 2012.

[32] M. Dahlström, L. G. E. Mårtensson, P. R. Jonsson, T. Arnebrant, and H. Elwing, "Surface active adrenoceptor compounds prevent the settlement of cyprid larvae of Balanus improvisus," *Biofouling*, vol. 16, no. 2–4, pp. 191–203, 2000.

[33] M. Nydén and K. Holmberg, "Stoppa havstulpanerna," *Kemivärlden Biotech Med Kemisk Tidskrift*, vol. 5, pp. 22–24, 2006.

[34] A. Lennquist, N. Asker, E. Kristiansson et al., "Physiology and mRNA expression in rainbow trout (Oncorhynchus mykiss) after long-term exposure to the new antifoulant medetomidine," *Comparative Biochemistry and Physiology C*, vol. 154, no. 3, pp. 234–241, 2011.

[35] A. Lennquist, M. C. Celander, and L. Foerlin, "Effects of medetomidine on hepatic EROD activity in three species of fish," *Ecotoxicology and Environmental Safety*, vol. 69, no. 1, pp. 74–79, 2008.

[36] A. Lennquist, A. Hilvarsson, and L. Foerlin, "Responses in fish exposed to medetomidine, a new antifouling agent," *Marine Environmental Research*, vol. 69, supplement 1, pp. S43–S45, 2010.

[37] A. Lennquist, L. G. E. Mårtensson Lindblad, D. Hedberg, E. Kristiansson, and L. Förlin, "Colour and melanophore function in rainbow trout after long term exposure to the new antifoulant medetomidine," *Chemosphere*, vol. 80, no. 9, pp. 1050–1055, 2010.

[38] M. Andersson, J. Hedin, P. Johansson, J. Nordstroem, and M. Nyden, "Coordination of imidazoles by Cu(II) and Zn(II) as studied by NMR relaxometry, EPR, far-FTIR vibrational spectroscopy and Ab initio calculations: effect of methyl substitution," *Journal of Physical Chemistry A*, vol. 114, no. 50, pp. 13146–13153, 2010.

[39] M. Andersson Trojer, A. Maartensson, and M. Nyden, "Replacement of H-bonded bridged water by transition metal ions in poly(1-vinylimidazole-co-methylmethacrylate) copolymers: a vibrational spectroscopy study using mid-FTIR, far-FTIR and ab initio calculations," *Vibrational Spectroscopy*, vol. 61, pp. 38–42, 2012.

[40] C. Fant, P. Handa, and M. Nyden, "Complexation chemistry for tuning release from polymer coatings," *Journal of Physical Chemistry B* , vol. 110, no. 43, pp. 21808–21815, 2006.

[41] P. Handa, C. Fant, and M. Nyden, "Antifouling agent release from marine coatings-ion pair formation/dissolution for controlled release," *Progress in Organic Coatings*, vol. 57, no. 4, pp. 376–382, 2006.

[42] L. Shtykova, C. Fant, P. Handa et al., "Adsorption of antifouling booster biocides on metal oxide nanoparticles: effect of different metal oxides and solvents," *Progress in Organic Coatings*, vol. 64, no. 1, pp. 20–26, 2009.

[43] H. Elwing, M. Dahlstrom, T. Arnebrant et al., "Test process for bioactive additives in antifouling paint, comprises quantifying binding ability of test substance to surface coating on flat sensor in aqueous medium or organic solvent 2004-1097, 526684, 20040429," 2005.

[44] L. S. Shtykova, D. Ostrovskii, P. Handa, K. Holmberg, and M. Nydén, "NMR diffusometry and FTIR in the study of the interaction between antifouling agent and binder in marine paints," *Progress in Organic Coatings*, vol. 51, no. 2, pp. 125–133, 2004.

[45] M. Nyden and C. Fant, "Method and use of nanoparticles to bind biocides in paints 2006-SE318, 2006096128, 20060313," 2006.

[46] M. Nyden, C. Fant, K. Holmberg, and L. Swanson, "Method and use of acidified modified polymers to bind biocides in paints 2006-372522, 7531581, 20060310," 2009.

[47] M. Andersson, O. Hansson, L. Oehrstroem, A. Idstroem, and M. Nyden, "Vinylimidazole copolymers: coordination chemistry, solubility, and cross-linking as function of Cu^{2+} and Zn^{2+} complexation," *Colloid and Polymer Science*, vol. 289, no. 12, pp. 1361–1372, 2011.

[48] M. Andersson Trojer, D. Isaksson, and M. Nydén, "Synthesis and polymerisation of maleoyl-L-histidine monomers and addition of histidine to an ethylene-alt-maleic co-polymer," *Journal of Polymer Research*, vol. 19, Article ID 9821, 2012.

[49] J. Hedin, D. Isaksson, M. Andersson, and M. Nydén, "Bi-layer formation of imidazole-modified ethyl(hydroxyethyl)cellulose at a hydrophobic surface as monitored by QCM-D," *Journal of Colloid and Interface Science*, vol. 336, no. 2, pp. 388–392, 2009.

[50] A. Movahedi, N. Kann, K. Moth-Paulsen, and B. M. Nyden, "Synthesis of chelating Cu(I)stabilizing polymeric ligands".

[51] *Marine Paint Annual Report 2008*, Gothenburg, Sweden, 2009.

[52] L. Nordstierna, A. A. Abdalla, M. Masuda, G. Skarnemark, and M. Nydén, "Molecular release from painted surfaces: free and encapsulated biocides," *Progress in Organic Coatings*, vol. 69, no. 1, pp. 45–48, 2010.

[53] "Polymeric core-shell particles: physicochemical properties and controlled release," in *Encyclopedia of Surface and Colloid Science*, P. Somasundaran, Ed., Taylor and Francis, New York, NY, USA, 2013.

[54] J. Bergek, M. Andersson Trojer, A. Mok, and L. Nordstierna, "Controlled release of microencapsulated 2-n-octyl-4-isothiazolin-3-one from coatings: effect of macroscopic and microscopic pores," *Colloids and Surfaces A*. In press.

[55] J. Bergek, M. Andersson Trojer, H. Uhr, and L. Nordstierna, "Controlled release of microencapsulated 2-n-octyl-4-isothiazolin-3-one from coatings: polyelectrolyte multilayers as a global a rate-determining barrier," *Journal of Materials Chemistry B*. Submitted.

[56] A. Enejder, F. Svedberg, L. Nordstierna, and M. Nydén, "Chemical release from single PMMA microparticles monitored by CARS microscopy," in *Multiphoton Microscopy in the Biomedical Sciences*, Proceedings of SPIE, January 2011.

[57] L. Nordstierna, A. A. Abdalla, M. Nordin, and M. Nydén, "Comparison of release behaviour from microcapsules and microspheres," *Progress in Organic Coatings*, vol. 69, no. 1, pp. 49–51, 2010.

[58] L. Nordstierna, A. Movahedi, and M. Nydén, "New route for microcapsule synthesis," *Journal of Dispersion Science and Technology*, vol. 32, no. 3, pp. 310–311, 2011.

[59] B. M. Nyden, L. O. Nordstierna, E. M. Bernad, and A. M. A. A. Abdalla, "Slow releasing microcapsules and microspheres comprising an biocidal substance," 2010-EP56735, 2010133548, 20100517, 2010.

[60] M. Andersson Trojer, L. Nordstierna, M. Nordin, B. M. Nyden, and K. Holmberg, "Physical Chemistry chemical physics," *Encapsulation of Actives For Controlled Release*, 2013.

[61] M. Andersson Trojer, H. Andersson, Y. Li et al., "Charged microcapsules for controlled release of hydrophobic actives. Part III: the effect of polyelectrolyte brush- and multilayers on sustained release," *Physical Chemistry Chemical Physics*, vol. 15, no. 17, pp. 6456–6466, 2013.

[62] M. Andersson Trojer, K. Holmberg, and M. Nydén, "The importance of proper anchoring of an amphiphilic dispersant for colloidal stability," *Langmuir*, vol. 28, no. 9, pp. 4047–4050, 2012.

[63] M. A. Trojer, Y. Li, C. Abrahamsson et al., "Charged microcapsules for controlled release of hydrophobic actives. Part I: encapsulation methodology and interfacial properties," *Soft Matter*, vol. 9, no. 5, pp. 1468–1477, 2013.

[64] M. Andersson Trojer, Y. Li, M. Wallin, K. Holmberg, and M. Nyden, "Charged microcapsules for controlled release of hydrophobic actives Part II: surface modification by LbL adsorption and lipid bilayer formation on properly anchored dispersant layers," *Journal of Colloid and Interface Science*, vol. 409, no. 1, pp. 8–17, 2013.

[65] M. Andersson Trojer, A. Mohamed, and J. Eastoe, "A highly hydrophobic anionic surfactant at the oil-water, water-polymer and oil-polymer interfaces: implications on spreading coefficients, polymer interactions and microencapsulation via internal phase separation," *Colloids and Surfaces A*, vol. 436, no. 5, pp. 1048–1059, 2013.

[66] M. A. Trojer, A. Wendel, K. Holmberg, and M. Nydén, "The effect of pH on charge, swelling and desorption of the dispersant poly(methacrylic acid) from poly(methyl methacrylate) microcapsules," *Journal of Colloid and Interface Science*, vol. 375, no. 1, pp. 213–215, 2012.

[67] E. Ytreberg, J. Karlsson, and B. Eklund, "Comparison of toxicity and release rates of Cu and Zn from anti-fouling paints leached in natural and artificial brackish seawater," *Science of the Total Environment*, vol. 408, no. 12, pp. 2459–2466, 2010.

[68] S. R. Nadella, J. L. Fitzpatrick, N. Franklin, C. Bucking, S. Smith, and C. M. Wood, "Toxicity of dissolved Cu, Zn, Ni and Cd to developing embryos of the blue mussel (Mytilus trossolus) and the protective effect of dissolved organic carbon," *Comparative Biochemistry and PhysiologyC*, vol. 149, no. 3, pp. 340–348, 2009.

[69] S. Niyogi and C. M. Wood, "Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals," *Environmental Science and Technology*, vol. 38, no. 23, pp. 6177–6192, 2004.

[70] A. Viarengo, M. Pertica, G. Mancinelli, B. Burlando, L. Canesi, and M. Orunesu, "In vivo effects of copper on the calcium homeostasis mechanisms of mussel gill cell plasma membranes," *Comparative Biochemistry and Physiology*, vol. 113, no. 3, pp. 421–425, 1996.

[71] L. Ciavatta, D. Ferri, and R. Palombari, "On the equilibrium $Cu^{2+} + Cu(s) \rightleftarrows 2Cu^{+}$," *Journal of Inorganic and Nuclear Chemistry*, vol. 42, no. 4, pp. 593–598, 1980.

[72] M. G. Simmons, C. L. Merrill, L. J. Wilson, L. A. Bottomley, and K. M. Kadish, "The Bis-2,6-[1-(2-imidazol-4-ylethylimino)ethyl]pyridinecopper(I) cation. A synthetic CuI oxygen carrier in solution as a potential model for oxyhemocyanin,"

Journal of the Chemical Society, Dalton Transactions, no. 10, pp. 1827–1837, 1980.

[73] L. Kiaune and N. Singhasemanon, "Pesticidal copper (I) oxide: environmental fate and aquatic toxicity," *Reviews of Environmental Contamination and Toxicology*, vol. 213, pp. 1–26, 2011.

[74] S. J. Brooks and M. Waldock, "The use of copper as a biocide in marine antifouling paints," in *Advances in Marine Antifouling Coatings and Technologies*, pp. 492–521, 2009.

[75] E. Cotou, M. Henry, C. Zeri, G. Rigos, A. Torreblanca, and V. A. Catsiki, "Short-term exposure of the European sea bass Dicentrarchus labrax to copper-based antifouling treated nets: copper bioavailability and biomarkers responses," *Chemosphere*, vol. 89, no. 9, pp. 1091–1097, 2012.

[76] T. R. Chan, R. Hilgraf, K. B. Sharpless, and V. V. Fokin, "Polytriazoles as copper(I)-stabilizing ligands in catalysis," *Organic Letters*, vol. 6, no. 17, pp. 2853–2855, 2004.

[77] Y. Rondelez, M.-N. Rager, A. Duprat, and O. Reinaud, "Calix[6]arene-based cuprous "funnel complexes": a mimic for the substrate access channel to metalloenzyme active sites," *Journal of the American Chemical Society*, vol. 124, no. 7, pp. 1334–1340, 2002.

[78] Y. Rondelez, G. Bertho, and O. Reinaud, "The first water-soluble copper(I) calix[6]arene complex presenting a hydrophobic ligand binding pocket: a remarkable model for active sites in metalloenzymes," *Angewandte Chemie International Edition*, vol. 41, no. 6, pp. 1044–1046, 2002.

[79] T. Vänngård, H. M. Swartz, J. R. Bolton, and D. C. Borg, *Applications of Electron Spin Resonance*, John Wiley and Sons, New York, NY, USA, 1972.

[80] M. Brändén, A. Namslauer, Ö. Hansson, R. Aasa, and P. Brzezinski, "Water-hydroxide exchange reactions at the catalytic site of heme-copper oxidases," *Biochemistry*, vol. 42, no. 45, pp. 13178–13184, 2003.

[81] M. M. Ivkovic-Jensen, G. M. Ullmann, S. Young et al., "Effects of single and double mutations in plastocyanin on the rate constant and activation parameters for the rearrangement gating the electron-transfer reaction between the triplet state of zinc cytochrome c and cupriplastocyanin," *Biochemistry*, vol. 37, no. 26, pp. 9557–9569, 1998.

[82] F. Jacobson, A. Pistorius, D. Farkas et al., "pH dependence of copper geometry, reduction potential, and nitrite affinity in nitrite reductase," *Journal of Biological Chemistry*, vol. 282, no. 9, pp. 6347–6355, 2007.

[83] H. Jansson and O. Hansson, "Competitive inhibition of electron donation to photosystem 1 by metal-substituted plastocyanin," *Biochimica Et Biophysica Acta*, vol. 1777, no. 9, pp. 1116–1121, 2008.

[84] H. Jansson, M. Oekvist, F. Jacobson, M. Ejdebaeck, O. Hansson, and L. Sjoelin, "The crystal structure of the spinach plastocyanin double mutant G8D/L12E gives insight into its low reactivity towards photosystem 1 and cytochrome f," *Biochimica et Biophysica Acta*, vol. 1607, no. 2-3, pp. 203–210, 2003.

[85] D. La Mendola, R. P. Bonomo, S. Caminati et al., "Copper(II) complexes with an avian prion N-terminal region and their potential SOD-like activity," *Journal of Inorganic Biochemistry*, vol. 103, no. 2, pp. 195–204, 2009.

[86] D. La Mendola, D. Farkas, F. Bellia et al., "Probing the copper(II) binding features of angiogenin. Similarities and differences between a N-terminus peptide fragment and the recombinant human protein," *Inorganic Chemistry*, vol. 51, no. 1, pp. 128–141, 2012.

[87] D. La Mendola, A. Magrì, T. Campagna et al., "A doppel α-helix peptide fragment mimics the copper(II) interactions with the whole protein," *Chemistry*, vol. 16, no. 21, pp. 6212–6223, 2010.

[88] D. L. Mendola, A. Magrì, L. I. Vagliasindi, Ö. Hansson, R. P. Bonomo, and E. Rizzarelli, "Copper(II) complex formation with a linear peptide encompassing the putative cell binding site of angiogenin," *Dalton Transactions*, vol. 39, no. 44, pp. 10678–10684, 2010.

[89] D. L. Mendola, A. Magrì, Ö. Hansson, R. P. Bonomo, and E. Rizzarelli, "Copper(II) complexes with peptide fragments encompassing the sequence 122-130 of human doppel protein," *Journal of Inorganic Biochemistry*, vol. 103, no. 5, pp. 758–765, 2009.

[90] S. Modi, M. Nordling, L. G. Lundberg, O. Hansson, and D. S. Bendall, "Reactivity of cytochromes c and f with mutant forms of spinach plastocyanin," *Biochimica et Biophysica Acta*, vol. 1102, no. 1, pp. 85–90, 1992.

[91] K. Olesen, M. Ejdeback, and O. Hansson, "Electron transfer from genetically modified plastocyanin to photosystem 1," in *Proceedings of the 11th International photosynthesis: Photosynthesis mechanisms and effects Congress*, pp. 1597–1600, 1998.

[92] K. Sigfridsson, M. Ejdeback, and M. Sundahl, "Electron transfer in ruthenium-modified spinach plastocyanin mutants," *Archives of Biochemistry and Biophysics*, vol. 351, no. 2, pp. 197–206, 1998.

[93] K. Sigfridsson, S. Young, and O. Hansson, "Electron transfer between spinach plastocyanin mutants and photosystem 1," *European Journal of Biochemistry*, vol. 245, no. 3, p. 805, 1998.

[94] Y. Xue, M. Okvist, O. Hansson, and S. Young, "Crystal structure of spinach plastocyanin at 1. 7 A resolution," *Protein Science*, vol. 7, no. 10, pp. 2099–2105, 1998.

[95] R. J. Sundberg and R. B. Martin, "Interactions of histidine and other imidazole derivatives with transition metal ions in chemical and biological systems," *Chemical Reviews*, vol. 74, no. 4, pp. 471–517, 1974.

[96] C. J. Jones, *D- and F-Block Chemistry*, The Royal Society of Chemistry, Birmingham, England, 2001.

[97] B. N. Figgis and M. A. Hitchman, "Ligand field theory and its applications," *Journal of Chemical Education*, vol. 79, no. 9, p. 1072, 2002.

[98] R. G. Pearson, "Hard and soft acids and bases," *Journal of the American Chemical Society*, vol. 85, no. 22, pp. 3533–3539, 1963.

[99] R. G. Pearson, "Absolute electronegativity and hardness correlated with molecular orbital theory," *Proceedings of the National Academy of Sciences of the USA*, vol. 83, no. 22, pp. 8440–8441, 1986.

[100] R. H. Crabtree, *The Organometallic Chemistry of the Transition Metals*, John Wiley and Sons, New York, NY, USA, 4th edition, 2005.

[101] Y. Jean, *Molecular Orbitals of Transition Metal Complexes*, Oxford University Press, New York, NY, USA, 2005.

[102] K. Liu, W. Shi, and P. Cheng, "The coordination chemistry of Zn(II), Cd(II) and Hg(II) complexes with 1,2,4-triazole derivatives," *Dalton Transactions*, vol. 40, no. 34, pp. 8475–8490, 2011.

[103] W. Ouellette, S. Jones, and J. Zubieta, "Solid state coordination chemistry of metal-1,2,4-triazolates and the related metal-4-pyridyltetrazolates," *CrystEngComm*, vol. 13, no. 14, pp. 4457–4485, 2011.

[104] J. G. Haasnoot, "Mononuclear, oligonuclear and polynuclear metal coordination compounds with 1, 2, 4-triazole derivatives

as ligands," *Coordination Chemistry Reviews*, vol. 200-202, pp. 131–185, 2000.

[105] J. P. Zhang, Y. B. Zhang, J. B. Lin, and X. M. Chen, "Metal azolate frameworks: from crystal engineering to functional materials," *Chemical Reviews*, vol. 112, no. 2, pp. 1001–1033, 2012.

[106] R. G. Pearson, "Hard and soft acids and bases," *Journal of the American Chemical Society*, vol. 85, no. 22, pp. 3533–3539, 1963.

[107] L. M. Epstein, D. K. Straub, and C. Maricondi, "Mossbauer spectra of some porphyrin complexes with pyridine, piperidine, and imidazole," *Inorganic Chemistry*, vol. 6, no. 9, pp. 1720–1724, 1967.

[108] N. S. Rannulu, R. Amunugama, Z. Yang, and M. T. Rodgers, "Influence of s and d orbital occupation on the binding of metal ions to imidazole," *Journal of Physical Chemistry A*, vol. 108, no. 30, pp. 6385–6396, 2004.

[109] D. Reddy, K. J. Akerman, M. P. Akerman, and D. Jaganyi, "A kinetic investigation into the rate of chloride substitution from chloro terpyridine platinum(II) and analogous complexes by a series of azole nucleophiles," *Transition Metal Chemistry*, vol. 36, no. 6, pp. 593–602, 2011.

[110] B. Barszcz, M. Gabryszewski, J. Kulig, and B. Lenarcik, "Potentiometric studies on complexes of silver(I) in solutions. Part 2. Correlation between the stability of the silver(I)-azole complexes and the ligand basicity," *Journal of the Chemical Society, Dalton Transactions*, no. 10, pp. 2025–2028, 1986.

[111] D. Hourdet, J. A. Gadgil, K. Podhajecka, M. V. Badiger, A. Brûlet, and P. P. Wadgaonkar, "Thermoreversible behavior of associating polymer solutions: thermothinning versus thermothickening," *Macromolecules*, vol. 38, no. 20, pp. 8512–8521, 2005.

[112] M. Prabaharan and J. F. Mano, "Chitosan derivatives bearing cyclodextrin cavitiesas novel adsorbent matrices," *Carbohydrate Polymers*, vol. 63, no. 2, pp. 153–166, 2006.

[113] R. Souzy, B. Ameduri, B. Boutevin, G. Gebel, and P. Capron, "Functional fluoropolymers for fuel cell membranes," *Solid State Ionics*, vol. 176, no. 39-40, pp. 2839–2848, 2005.

[114] M. M. Nasef and E. S. A. Hegazy, "Preparation and applications of ion exchange membranes by radiation-induced graft copolymerization of polar monomers onto non-polar films," *Progress in Polymer Science*, vol. 29, no. 6, pp. 499–561, 2004.

[115] Q. Chen and B.-H. Han, "Prepolymerization and postpolymerization functionalization approaches to fluorescent conjugated carbazole-based glycopolymers via click chemistry," *Journal of Polymer Science A*, vol. 47, no. 11, pp. 2948–2957, 2009.

[116] M. A. Gauthier, M. I. Gibson, and H. A. Klok, "Synthesis of functional polymers by post-polymerization modification," *Angewandte Chemie International Edition*, vol. 48, no. 1, pp. 48–58, 2008.

[117] R. Huisgen, "1,3-Dipolar cycloadditions," *Proceedings of the Chemical Society*, pp. 357–369, 1961.

[118] V. V. Rostovtsev, L. G. Green, V. V. Fokin, and K. B. Sharpless, "A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective, "ligation" of azides and terminal alkynes," *Angewandte Chemie International*, vol. 41, no. 14, pp. 2596–2599, 2002.

[119] B. C. Boren, S. Narayan, L. K. Rasmussen et al., "Ruthenium-catalyzed azide-alkyne cycloaddition: scope and mechanism," *Journal of the American Chemical Society*, vol. 130, no. 28, pp. 8923–8930, 2008.

[120] D. Braun and F. Hu, "Polymers from non-homopolymerizable monomers by free radical processes," *Progress in Polymer Science*, vol. 31, no. 3, pp. 239–276, 2006.

[121] R. C. P. Cubbon, "The free radical and anionic polymerization of some N-substituted maleimides," *Polymer*, vol. 6, no. 8, pp. 419–426, 1965.

[122] D. J. T. Hill, L. Y. Shao, P. J. Pomery, and A. K. Whittaker, "The radical homopolymerization of N-phenylmaleimide, N-n-hexylmaleimide and N-cyclohexylmaleimide in tetrahydrofuran," *Polymer*, vol. 42, no. 11, pp. 4791–4802, 2001.

[123] C. Hulubei and S. Morariu, "Synthesis and radical polymerization of N-(3-acetyl-4-carboxy-phenyl)-maleimide," *High Performance Polymers*, vol. 12, no. 4, pp. 525–533, 2000.

[124] T. Oishi, H. Nagata, and H. Tsutsumi, "Synthesis and polymerization of N-substituted maleimides based on L-alanine ester," *Polymer*, vol. 39, no. 17, pp. 4135–4146, 1998.

[125] T. M. Pyriadi and A. S. Hamad, "Preparation of new copolymers of vinyl acetate and N-substituted maleimides," *Polymer*, vol. 37, no. 23, pp. 5283–5287, 1996.

[126] P. O. Tawney, R. H. Snyder, C. E. Bryan et al., "The chemistry of maleimide and its derivatives. I. N-carbamylmaleimide," *Journal of Organic Chemistry*, vol. 25, no. 1, pp. 56–60, 1960.

[127] P. O. Tawney, R. H. Snyder, R. P. Conger, K. A. Leibbrand, C. H. Stiteler, and A. R. Williams, "The chemistry of maleimide and its derivatives. II. Maleimide and N-methylolmaleimide," *Journal of Organic Chemistry*, vol. 26, no. 1, pp. 15–21, 1961.

[128] J. Z. Yang and T. Otsu, "Reactivities of citraconic anhydride, N-alkylcitraconimides, dialkyl citraconates and dialkyl mesaconates in radical copolymerization with vinyl acetate and characterization of the resulting copolymers," *European Polymer Journal*, vol. 27, no. 10, pp. 1081–1086, 1991.

[129] J. M. G. Cowie, *Polymers: Chemistry and Physics of Modern Materials*, CRC Press, 2nd edition, 1991.

[130] K. H. Wu, T. C. Chang, Y. T. Wang, Y. S. Hong, and T. S. Wu, "Interactions and mobility of copper(II)-imidazole-containing copolymers," *European Polymer Journal*, vol. 39, no. 2, pp. 239–245, 2003.

[131] N. L. Mazyar, V. V. Annenkov, V. A. Kruglova et al., "Acid-base properties of poly(1-vinylazoles) in aqueous solution," *Russian Chemical Bulletin*, vol. 49, no. 12, pp. 2013–2017, 2000.

[132] E. N. Danilovtseva, M. A. Chafeev, and V. V. Annenkov, "New polyelectrolytes based on 4-vinyl-1,2,3-triazole and 1-vinylimidazole," *Journal of Polymer Science A*, vol. 50, no. 8, pp. 1539–1546, 2012.

[133] H. C. Kolb, M. G. Finn, and K. B. Sharpless, "Click chemistry: diverse chemical function from a few good reactions," *Angewandte Chemie International Edition*, vol. 40, no. 11, pp. 2004–2021, 2001.

[134] R. J. Thibault, K. Takizawa, P. Lowenheilm et al., "A versatile new monomer family: functionalized 4-vinyl-1,2,3-triazoles via click chemistry," *Journal of the American Chemical Society*, vol. 128, no. 37, pp. 12084–12085, 2006.

[135] L. A. Belfiore and M. P. McCurdie, "Reactive blending via metal-ligand coordination," *Journal of Polymer Science B*, vol. 33, no. 1, pp. 105–124, 1995.

[136] L. A. Belfiore, M. P. McCurdie, and P. K. Das, "Macromolecule-metal complexes: ligand field stabilization and thermophysical property modification," *Polymer*, vol. 42, no. 25, pp. 9995–10006, 2001.

[137] T. Kaliyappan and P. Kannan, "Co-ordination polymers," *Progress in Polymer Science*, vol. 25, no. 3, pp. 343–370, 2000.

[138] R. Hoogenboom, D. Fournier, and U. S. Schubert, "Asymmetrical supramolecular interactions as basis for complex responsive macromolecular architectures," *Chemical Communications*, no. 2, pp. 155–162, 2008.

[139] V. Marin, E. Holder, R. Hoogenboom, and U. S. Schubert, "Functional ruthenium(II)- and iridium(III)-containing polymers for potential electro-optical applications," *Chemical Society Reviews*, vol. 36, no. 4, pp. 6618–6635, 2007.

[140] C. S. K. Mak and W. K. Chan, "Electroluminescence from metal-containing polymers and metal complexes with functional ligands," in *Highly Efficient OLEDs With Phosphorescent Materials*, p. 329, 2008.

[141] H. K. Reimschuessel, "Polymer-metal halide interactions: nylon 6-zirconium tetrafluoride system," *Colloid and Polymer Science*, vol. 260, no. 9, pp. 842–850, 1982.

[142] M. Sato, K. Kondo, and K. Takemoto, "Functional monomers and polymers, 43. ESR study on the copper(II) complexes of polymers containing imidazolyl groups and of their monomeric analogs," *Die Makromolekulare Chemie*, vol. 179, no. 3, pp. 601–610, 1978.

[143] M. Sato, K. Kondo, and K. Takemoto, "Functional monomers and polymers, 56. Effects of ionic strength on the structure of Cu(II). poly(vinylimidazole) and Cu(II). imidazole complexes in aqueous solution," *Die Makromolekulare Chemie*, vol. 180, no. 3, pp. 699–704, 1979.

[144] M. Sato, K. Kondo, and K. Takemoto, "ESR studies on the dimer formation between copper ions in the poly(vinylimidazole) copper(II) complex," *Polymer Bulletin*, vol. 2, no. 5, pp. 305–308, 1980.

[145] A. B. Strong, *Plastics: Materials and Processing*, Pearson Education, New Jersey, NJ, USA, 3rd edition, 2006.

[146] V. A. Berstein and V. M. Egorov, *Differential Scanning Calorimetry of Polymers*, Ellis Horwood, Chichester, UK, 1994.

[147] H. Tanaka, S. Tai, K. Kamijima et al., "Antifouling paint containing compounds to improve viscosity stability and durability of films in seawater 1994-309674, 659848, 19941222," 1995.

[148] U. Gerigk and D. Ventur, "Preparation of thiadiazole compounds as antifouling agents 1992-4230955, 19920916," 1994.

[149] M. Mechtel, W. Podszun, and K. H. Kaesler, "Antifouling agents and coatings and their preparation 1997-EP5626, 9817732, 19971013," 1998.

[150] F. Kunisch, M. Kugler, H. Schrage, and H. L. Elbe, "Antifouling agents comprising benzothiophene-2-carboxamide S, S-dioxide derivs 1995-19534868, 19950920," 1997.

[151] R. Neiman and D. Kivelson, "ESR line shapes in glasses of copper complexes," *The Journal of Chemical Physics*, vol. 35, no. 1, pp. 149–155, 1961.

[152] F. Jiang, J. McCracken, and J. Peisach, "Nuclear quadrupole interactions in copper(II)-diethylenetriamine-substituted imidazole complexes and in copper(II) proteins," *Journal of the American Chemical Society*, vol. 112, no. 25, pp. 9035–9044, 1990.

[153] H. J. Scholl and J. Huettermann, "ESR and ENDOR of copper(II) complexes with nitrogen donors: probing parameters for prosthetic group modeling of superoxide dismutase," *Journal of Physical Chemistry*, vol. 96, no. 24, pp. 9684–9691.

[154] C.-C. Su, H. C. Jan, H. Kuo-Yih et al., "Bonding properties of Cu(II)-imidazole chromophores: spectroscopic and electrochemical properties of monosubstituted imidazole copper(II) complexes. Molecular structure of [Cu(4-methylimidazole)4(ClO_4)$_2$]," *Inorganica Chimica Acta*, vol. 196, no. 2, pp. 231–240, 1992.

[155] B. C. Cornilsen and K. Nakamoto, "Metal isotope effect on metal-ligand vibrations-XII: imidazole complexes with Co(II), Ni(II), Cu(II) and Zn(II)," *Journal of Inorganic and Nuclear Chemistry*, vol. 36, no. 11, pp. 2467–2471, 1974.

[156] J. Reedijk, "Pyrazoles and imidazoles as ligands. II. Coordination compounds of N-methyl imidazole with metal perchlorates and tetrafluoroborates," *Inorganica Chimica Acta*, vol. 3, pp. 517–522, 1969.

[157] C. Perchard and A. Novak, "The low-frequency infrared and Raman spectra of ammonia and imidazole complexes of zinc(II) halides," *Spectrochimica Acta A*, vol. 26, no. 4, pp. 871–881, 1970.

[158] A. B. P. Lever, "Electrochemical parametrization of metal complex redox potentials, using the ruthenium(III)/ruthenium(II) couple to generate a ligand electrochemical series," *Inorganic Chemistry*, vol. 29, no. 6, pp. 1271–1285, 1990.

[159] S. Ahrland, K. Nilsson, and B. Tagesson, "Thermodynamics of the copper(I) halide and thiocyanate complex formation in acetonitrile," *Acta Chemica Scandinavica A*, vol. 37, pp. 193–201, 1983.

[160] R. R. Bessette and J. W. Olver, "Measurement of diffusion coefficients for the reduction of copper(I) and (II) in acetonitrile," *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, vol. 21, no. 3, pp. 525–529, 1969.

[161] D. C. Gould and A. Ehrenberg, "Cu^{2+} in non axial-field: a model for Cu^{2+} in copper enzymes," *European Journal of Biochemistry*, vol. 5, no. 4, pp. 451–455, 1968.

[162] J. T. Rubino and K. J. Franz, "Coordination chemistry of copper proteins: how nature handles a toxic cargo for essential function," *Journal of Inorganic Biochemistry*, vol. 107, no. 1, pp. 129–143, 2012.

[163] J. L. Dalsin and P. B. Messersmith, "Bioinspired antifouling polymers," *Materials Today*, vol. 8, no. 9, pp. 38–46, 2005.

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Research Article

Synthesis of Pyrroloquinones via a CAN Mediated Oxidative Free Radical Reaction of 1,3-Dicarbonyl Compounds with Aminoquinones

Thao Nguyen,[1] Dwayaja Nadkarni,[1] Shilpa Dutta,[1,2] Su Xu,[1] Sanghun Kim,[1] Srinivasan Murugesan,[1] and Sadanandan Velu[1,2,3]

[1] *Department of Chemistry, University of Alabama at Birmingham, 901 14th Street South, Birmingham, AL 35294-1240, USA*
[2] *Center for Biophysical Sciences and Engineering, University of Alabama at Birmingham, 901 14th Street South, Birmingham, AL 35294-1240, USA*
[3] *Comprehensive Cancer Center, University of Alabama at Birmingham, 901 14th Street South, Birmingham, AL 35294-1240, USA*

Correspondence should be addressed to Sadanandan Velu; svelu@uab.edu

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Pyrroloquinone ring systems are important structural units present in many biologically active molecules including a number of marine alkaloids. For example, they are found in a series of marine metabolites, such as tsitsikammamines, zyzzyanones, wakayin, and terreusinone. Several of these alkaloids have exhibited antimicrobial, antimalarial, antifungal, antitumor, and photoprotecting activities. Synthesis of pyrroloquinone unit is the key step in the synthesis of many of these important organic molecules. Here, we present a ceric (IV) ammonium nitrate (CAN) mediated oxidative free radical cyclization reaction of 1,3-dicarbonyl compounds with aminoquinones as a facile methodology for making various substituted pyrroloquinones. 1,3-dicarbonyl compounds used in this study are ethyl acetoacetate, acetylacetone, benzoyl acetone, and *N,N*-dimethyl acetoacetamide. The aminoquinones used in this study are 2-(benzylamino)naphthalene-1,4-dione and 6-(benzylamino)-1-tosyl-1*H*-indole-4,7-dione. The yields of the synthesized pyrroloquinones ranged from 23–91%.

1. Introduction

Pyrroloquinone is a pharmacophore present in many biologically important molecules. For example, a family of marine alkaloids that include zyzzyanones, tsitsikammamine, and wakayin contains the pyrroloquinone skeleton [1–4]. These alkaloids are metabolites of marine sponges *Latrunculia*, *Zyzzya* and ascidian species *Clavelina* [1, 4–6]. In addition to antimicrobial, antimalarial, and antifungal activities, these alkaloids have notable antitumor properties, which are derived from their unique fused ring structure. Tsitsikammamine A derivatives, for example, inhibit indoleamine-2,3-dioxygenase, an important enzyme contributing to tumor immune invasion [7]. Tsitsikammamines A-B also exhibit antimicrobial, antimalarial, and antifungal properties, cytotoxicity, and topoisomerase inhibition [8]. Additionally, marine alkaloid wakayin inhibits topoisomerase II, damages DNA, exhibits strong antimicrobial property against *Bacillus subtilis,* and is potent toward human colon cancer cell lines [1]. Zyzzyanones A-D contain a bispyrroloquinone ring system and have exhibited cytotoxicity against Ehrlich carcinoma cells at micromolar range [2, 3]. Moreover, bispyrroloquinone is also the core structure of the marine fungus metabolite terreusinone (**1**), which is a potent UV-A protectant [9]. The photoprotecting activity of terreusinone is stronger than that of the commercial sunscreen ingredient oxybenzone [10]. For these reasons, pyrroloquinone alkaloids are regarded as a source of new antitumor and dermatological drugs [11–17]. In addition to this, 3-methyl-1H-benz[f] indole-4,9-dione (**3**) is a natural product isolated from the barks of *G. tapis* and *G. uvaroides* containing pyrrolonaphthaquinone ring system. This compound shows

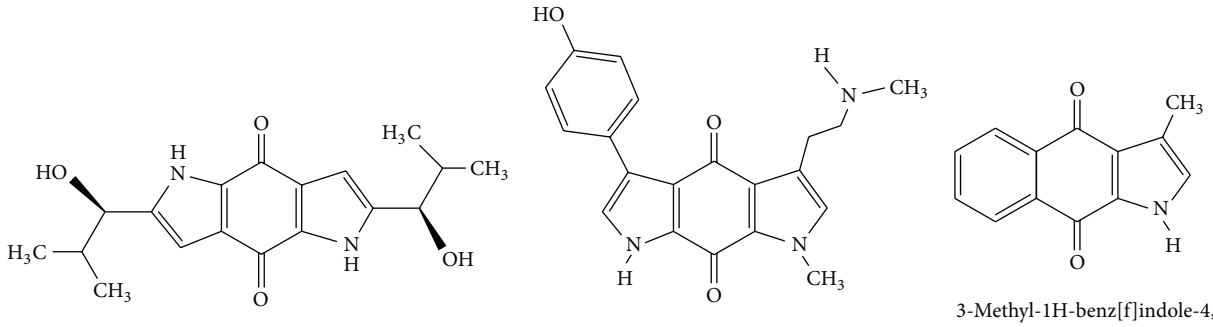


Figure 1: Selected natural products containing pyrroloquinone units.

strong inhibitory effects on platelet-activating factor (PAF) receptor [18]. Selected few examples of the natural products containing pyrroloquinone units are given in Figure 1.

Unfortunately, these natural products are isolated from natural sources only in minute quantities, which impose a limitation on their thorough biological evaluation. Additionally, the unique fused-ring aromatic structure poses a challenge in the total synthesis of these natural products. Several efforts have been made towards achieving the synthesis of these natural products [8, 10, 17, 19]. Our group has recently used an oxidative free radical cyclization reaction as a key step in the synthesis of zyzzyanones and the intermediates [19, 20].

Oxidative free radical reactions facilitated by transition metals have been known to promote carbon-carbon bond formation. In these reactions, the electron transfer between the radical precursor and metal complex generates electrophilic radicals, which ultimately react with alkenes, alkynes, or quinones to form carbon-carbon bonds [21–26]. Among the metal salts that have been investigated in the past two decades for facilitating oxidative free radical cyclization, Mn-$(OAc)_3$ and ceric (IV) ammonium nitrate (CAN) were proven to be the most efficient catalysts. A proposed mechanism of action for these reagents for effecting oxidative free radical cyclization has also been reported [21, 27, 28]. Mn-$(OAc)_3$ and CAN have been extensively used in the synthesis of naphthoquinone, which is an important skeleton of natural products, such as mitosenes, kinamycins, and murrayaquinones [29, 30]. The synthesis of bispyrroloquinone by a CAN mediated oxidative free radical cyclization has been reported from our lab [20]. An Mn $(OAc)_3$ mediated oxidative free radical cyclization leading to the total synthesis of zyzzyanones A-D has also been reported from our lab [19]. As an extension of these studies, herein, we demonstrate the general synthetic utility of CAN mediated oxidative free radical cyclization of various aminoquinones with 1,3-dicarbonyl compounds to form 24 new substituted pyrroloquinones.

2. Results and Discussion

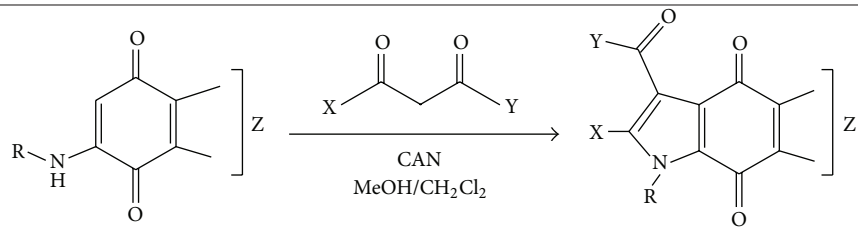
The oxidative free radical reaction leading to the formation of pyrroloquinones is shown in the general Table 1. Substituted benzylaminoquinones such as 2-(benzylamino)naphthalene-1,4-dione (**8**) and 6-(benzylamino)-1-tosyl-1*H*-indole-4,7-dione (**10**) are treated with various 1,3-dicarbonyl compounds in the presence of CAN to afford 24 pyrroloquinone derivatives (**4a-l** and **5a-l**) in moderate to excellent yields.

We used various 2-benzylaminonaphthalene-1,4-diones (**8a–c**) and N-Tosyl-6-benzylaminoindole-4,7-quinones (**10a–c**) as starting substrates for our oxidative free radical reactions yielding pyrroloquinones. Three 2-benzylamino naphthalene-1,4-diones (**8a–c**) used in these studies were prepared as outlined in Scheme 1. Naphthalene-1,4-dione (**7**) was treated with substituted benzyl amines (**6a–c**) in a mixture of MeOH and THF to afford 2-benzylamino naphthalene-1,4-diones (**8a–c**) in 50–86% yield.

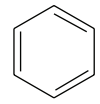
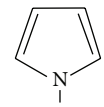
The three N-Tosyl-6-benzylaminoindole-4,7-quinones (**10a–c**) used in these studies were prepared as outlined in Scheme 2. Previously reported [31], N-Tosyl-6-methoxyindole-4,7-quinone (**9**) was treated with substituted benzyl amines (**6a–c**) in a mixture of MeOH and THF to afford N-Tosyl-6-benzylaminoindole-4,7-quinones (**10a–c**) in 70–94% yield.

Yields and the specific reaction conditions used for the oxidative free radical cyclization of the aminoquinones (**8a–c** and **10a–c**) with four 1,3-dicarbonyl compounds are summarized in Figure 4. 1,3-dicarbonyl compounds used in this study are ethyl acetoacetate, acetylacetone, benzoyl acetone and *N*,*N*-dimethyl acetoacetamide. The reactions resulted in the formation of 24 new substituted pyrroloquinones with yields ranging from 23% to 91%. Most of these reactions were carried out in CH_2Cl_2 and MeOH, except for entries 2 and 3 where a combination of CH_2Cl_2 and EtOH was used as solvent. Initially, the reaction of **8a** with 4 equivalents of ethyl acetoacetate yielded the expected product **4a** in good yield (80%, entry 1). However, latter reactions employed fewer equivalents of β-carbonyl compounds, which tended to result in cleaner reactions and made purification easier. Unfortunately, when compound **8b** reacted with ethyl acetoacetate in CH_2Cl_2 and MeOH, the reaction proceeded well, but a mixture of methyl and ethyl esters was obtained due to transesterification. So, a combination of CH_2Cl_2 and EtOH was used in these cases to obtain the products, **4b** and **4c** in 35% and 82%, respectively (entries 2 and 3).

Table 1: General scheme for the oxidative free radical cyclization.

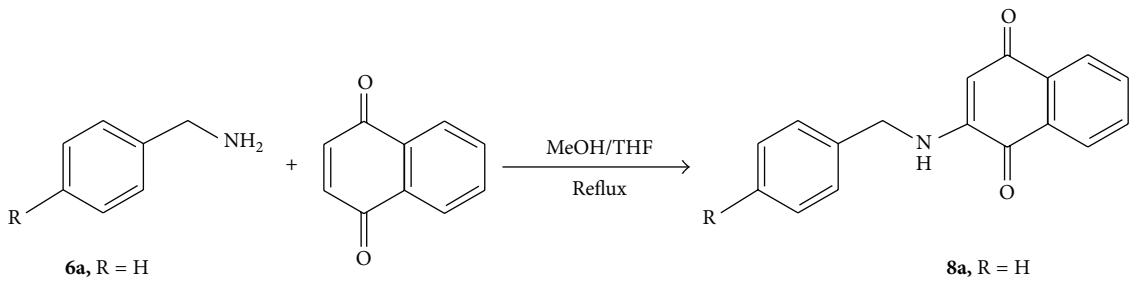


4a-l
5a-l

Z =  or 

I **II** Ts

Compd no.	R	X	Y	Z
4a	Benzyl	CH_3	OCH_2CH_3	**I**
4b	4-Nitrobenzyl	CH_3	OCH_2CH_3	**I**
4c	4-Methoxybenzyl	CH_3	OCH_2CH_3	**I**
4d	Benzyl	CH_3	CH_3	**I**
4e	4-Nitrobenzyl	CH_3	CH_3	**I**
4f	4-Methoxybenzyl	CH_3	CH_3	**I**
4g	Benzyl	CH_3	C_6H_5	**I**
4h	4-Nitrobenzyl	CH_3	C_6H_5	**I**
4i	4-Methoxybenzyl	CH_3	C_6H_5	**I**
4j	Benzyl	CH_3	$N(CH_3)_2$	**I**
4k	4-Nitrobenzyl	CH_3	$N(CH_3)_2$	**I**
4l	4-Methoxybenzyl	CH_3	$N(CH_3)_2$	**I**
5a	Benzyl	CH_3	OCH_2CH_3	**II**
5b	4-Nitrobenzyl	CH_3	OCH_2CH_3	**II**
5c	4-Methoxybenzyl	CH_3	OCH_2CH_3	**II**
5d	Benzyl	CH_3	CH_3	**II**
5e	4-Nitrobenzyl	CH_3	CH_3	**II**
5f	4-Methoxybenzyl	CH_3	CH_3	**II**
5g	Benzyl	CH_3	C_6H_5	**II**
5h	4-Nitrobenzyl	CH_3	C_6H_5	**II**
5i	4-Methoxybenzyl	CH_3	C_6H_5	**II**
5j	Benzyl	CH_3	$N(CH_3)_2$	**II**
5k	4-Nitrobenzyl	CH_3	$N(CH_3)_2$	**II**
5l	4-Methoxybenzyl	CH_3	$N(CH_3)_2$	**II**

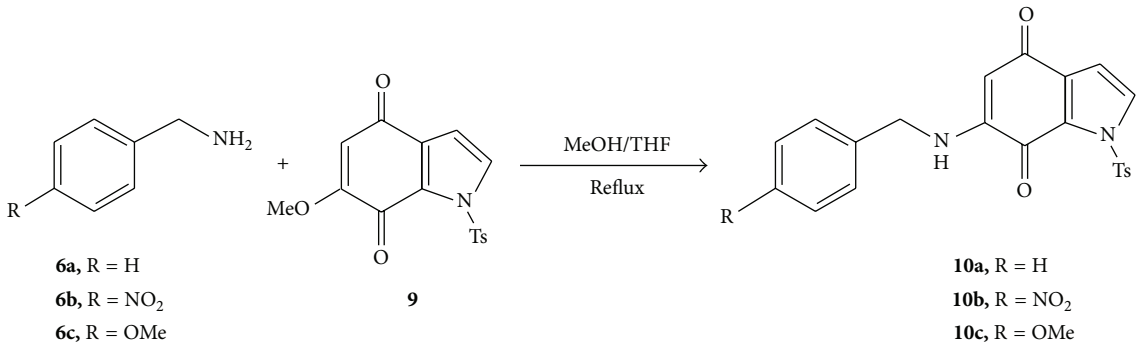


6a, R = H
6b, R = NO_2
6c, R = OMe

7

8a, R = H
8b, R = NO_2
8c, R = OMe

Scheme 1: Synthesis of 2-benzylaminonaphthalene-1,4-diones.



Scheme 2: Synthesis of 6-(benzylamino)-1-tosyl-1H-indole-4,7-quinones.

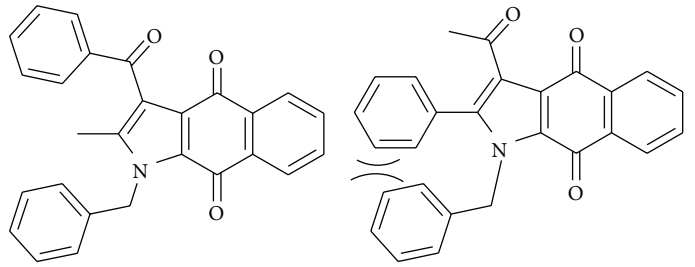


Figure 2: Two possible regioisomers of compound **4g**.

The majority of pyrroloquinones **4a-l** were obtained in good yields regardless of the types of β-carbonyl starting materials used. The products **4a-l** were usually yellow compared to the red orange starting materials **8a–c**. Additionally, we experimented with 1, 1.8, and 4 equivalents of β-carbonyl compounds and found that only 1 equivalent of β-carbonyl compounds was sufficient to bring the reaction to the completion. Highest yields were obtained when the electron donating methoxy group was present on the benzyl substituent (entries 3, 6, 9, and 12, 82–91% yield). In contrast, the presence of nitro group on the benzyl group resulted in significantly lower yields (entries 2, 5, 8, and 11, 31–38% yield). Entries with unsubstituted benzyl substituents resulted in moderate yields (entries 1, 4, 7, and 10, 51–80%). The reactions of **8b** with all four 1,3-dicarbonyl compounds proved to be difficult. This is due to several reasons, firstly, poor solubility of **8b** in CH_2Cl_2 and MeOH which required the usage of triple amount of solvent volume and heating to dissolve the starting material. Secondly, the reaction did not proceed at room temperature as in the other cases and needed refluxing to force the reactions to go to completion. In addition, more CAN (1.5–2.5 equiv) and longer reaction times were required for the reaction to go to completion. Finally, the reactions always resulted in a significant amount of side products, which ultimately led to low product yields.

When 1,3-diketones such as acetylacetone and benzoylacetone are used in these experiments, theoretically, two regioisomeric products are possible. The two possible products (A and B) for the reaction between benzoylacetone and 2-benzylamino naphthalene-1,4-dione mediated by CAN are illustrated in Figure 2. However, only one product (**4g**) was formed in this reaction, and it was proved to be the isomer A by ^{1}H-NMR, ^{13}C-NMR, and NOESY experiments. In the NOESY NMR experiment of compound **4g** as indicated in Figure 3, the methyl group (CH_3; singlet; 2.18 ppm) clearly had a NOESY correlation with the benzyl methylene group (CH_2; singlet; 5.76 ppm). The experiment clearly establishes that the product formed is regioisomer **A**. The absence of the regioisomer **B** is perhaps due to the steric hindrance between the two bulky phenyl groups, which makes the structure significantly more unstable.

Compounds **10a–c** were reacted with the 1,3-dicarbonyl reagents, including ethyl acetoacetate, benzoyl acetone, *N*,*N*-dimethyl acetoacetamide, and acetylacetone. The reactions were carried out in the 1 : 5 ratio mixtures of CH_2Cl_2 and MeOH. The yields and reaction condition to obtain the final products **5a-l** are given in Figure 4. In this study, four equivalents of CAN were necessary for the reactions to complete while less equivalents or absence of CAN resulted in the incomplete or no reactions. The types of 1,3-dicarbonyl reagents did not affect the outcome of the reaction as there were no trends affecting percent yields when different β-carbonyl reagents were used. Interestingly, the reaction of 6-(benzylamino)-1-tosyl-1*H*-indole-4,7-dione with 1,3-diketones is expected to yield two regioisomeric products, but only one product was formed as in the case of 2-(benzylamino)naphthalene-1,4-dione system.

Although the trend is not as strong as in previous 2-(benzylamino)naphthalene-1,4-dione system, the yields of the methoxybenzyl-substituted products **5c, f, i,** and **l** (entries

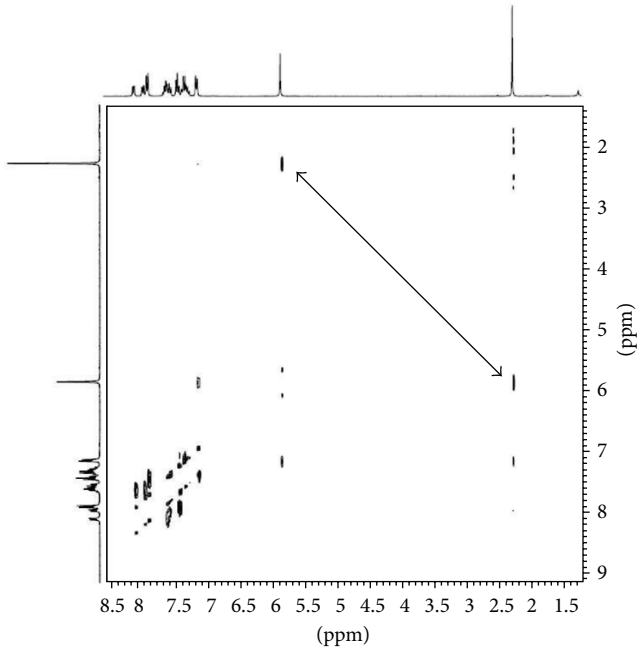


Figure 3: NOESY spectrum of compound **4g**.

15, 18, 21, and 24, 65–71%) are slightly higher than those of the nonsubstituted counterparts **5a, d, g,** and **j** (entries 13, 16, 19, and 22, 67-68%). In addition, the nonsubstituted bispyrroloquinones **5a, d, g,** and **j** were achieved in better yields compared to their equivalent nitrobenzyl substituted compounds **5b, e, h,** and **k** (entries 14, 17, 20, and 23, 52–60%). These results were consistent with our earlier observation in the reactions of 2-benzylaminonaphthalene-1,4-diones.

3. Conclusions

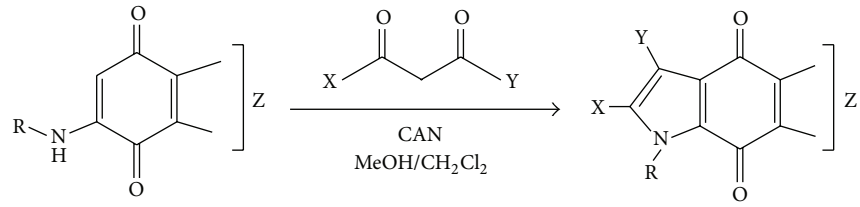
Synthesis of pyrroloquinone unit is the key step in the synthesis of several biologically important organic molecules. A CAN mediated oxidative free radical cyclization reaction of 1,3-dicarbonyl compounds with aminoquinones leading to the formation of various substituted pyrroloquinones is presented. 1,3-dicarbonyl compounds used in this study are acetylacetone, benzoyl acetone, ethyl acetoacetate, and *N*,*N*-dimethyl acetoacetamide. The aminoquinones used in this study are 2-(benzylamino)naphthalene-1,4-dione and 6-(benzylamino)-1-tosyl-1*H*-indole-4,7-dione. The yields of the synthesized pyrroloquinones ranged from 23 to 91%. Interestingly, we found that only one regioisomer was formed even when 1,3-diketones like benzoyl acetone were used. Finally, the majority of the oxidative free radical cyclized products were isolated as yellow solids in good yields.

4. Experimental

4.1. General Considerations. The NMR spectra were recorded on a Bruker DPX 300, DRX 400, or AVANCE 700 spectrometers. Chemical shifts are reported in ppm relative to TMS or $CDCl_3$ as internal standard. The values of chemical shift (δ) and coupling constants J were given in parts per million and in Hz, respectively. Mass spectra were recorded using an Applied Biosystems 4000 Q Trap and Micromass Platform LCC instruments. Thin-layer chromatography was performed with silica gel plates with fluorescent indicator (Whatman, silica gel, UV254, and 25 μm plates) and visualized by UV (wavelengths 254 and 365 nm). The reaction mixture was purified by column chromatography using silica gel (32–63 μm) from Dynamic Absorbent Inc. Melting points were uncorrected and obtained from Mel-Temp II apparatus. Solvents were removed *in vacuo* by using rotatory evaporator. The recrystallization was assisted by Fisher Scientific FS30 sonicator. Anhydrous solvents were purchased in Sure-Seal bottles from Aldrich chemical company. Other reagents were obtained from Aldrich and Acros chemical companies.

*4.1.1. 2-(Benzylamino)naphthalene-1,4-dione **(8a)**.* To a solution of 1,4-naphthoquinone **7** (5.0 g, 31.62 mmol) in THF (50 mL), a solution of benzylamine **6a** (6.91 mL, 63 mmol) in MeOH (50 mL) was added. The reaction was refluxed under N_2 atm for 36 h. Upon the completion of the reaction as indicated by TLC (100% CH_2Cl_2), the solvents were removed *in vacuo*. The residue obtained was dissolved in EtOAc (700 mL) and washed with water (2 × 200 mL), brine (200 mL) and dried over Na_2SO_4. The drying agent was filtered off, and the solution was concentrated under reduced pressure to obtain the crude product which was then purified by chromatography over silica gel using 100% CH_2Cl_2 as eluent to afford compound **8a** as a red solid (6.5 g, 80%); Mp: 137–141°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.37 (d, 2H, J = 5.7 Hz), 5.78 (s, 1H), 6.26 (bs, 1H), 7.25–7.44 (m, 5H), 7.61 (t, 1H, J = 7.5 Hz), 7.73 (t, 1H, J = 7.5 Hz), and 8.01–8.12 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 46.9, 101.9, 126.3, 126.4, 127.8 (2C), 128.3, 129.2 (2C), 130.6, 132.2, 133.7, 134.9, 136.1, 147.9, 182.0, and 183.2; MS (ES+): m/z = 264 [M + H].

*4.1.2. 2-(4-Nitrobenzylamino)naphthalene-1,4-dione **(8b)**.* To a solution of 1,4-naphthoquinone **7** (0.30 g, 1.90 mmol) in THF (7 mL), a mixture of 4-nitrobenzylamine hydrochloride **6b** (0.54 g, 2.84 mmol) and Et_3N (0.383 g, 3.79 mmol) in MeOH and CH_2Cl_2 (1 : 1, 14 mL) was added. The reaction was stirred under N_2 atm overnight at room temperature. After the TLC analysis (EtOAc/hexanes, 1 : 2) showed the completion of the reaction, the solvents were removed *in vacuo*. The residue obtained was dissolved in CH_2Cl_2 (300 mL) and washed with water (2 × 100 mL), brine (100 mL) and dried over Na_2SO_4. The drying agent was filtered off, and the filtrate was concentrated under reduced pressure to obtain the crude product which was purified by chromatography over silica gel using 100% CH_2Cl_2 as eluent to afford compound **8b** as a red solid (0.292 g, 50%); Mp: 225–228°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.54 (d, 2H, J = 8.4 Hz), 5.67 (s, 1H), 6.32 (bs, 1H), 7.45–7.53 (m, 2H), 7.66 (dt, 1H, J_1 = 1.3 Hz, J_2 = 7.7 Hz), 7.75 (dt, 1H, J_1 = 1.3 Hz, J_2 = 7.7 Hz), 8.08 (d, 1H, J = 1.1 Hz), 8.10 (d, 1H, J = 1.1 Hz), and 8.22–8.27 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 46.2, 102.8, 124.5 (2C), 126.6 (2C), 128.1 (2C), 130.6, 132.6, 133.5, 135.2, 143.5, 147.7, 148.0, 181.9, and 183.2; MS (ES+): m/z = 309 [M + H].



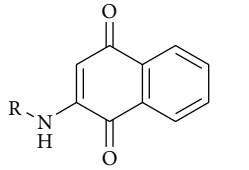
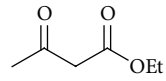
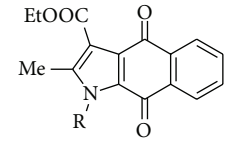
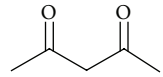
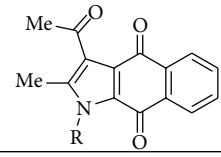
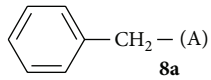
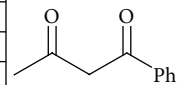
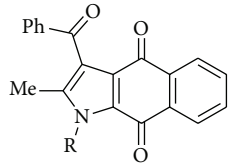
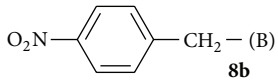
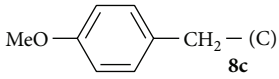
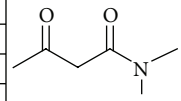
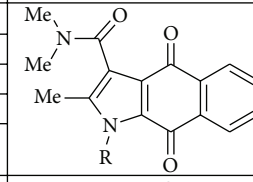
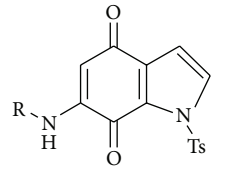
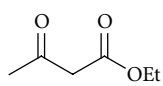
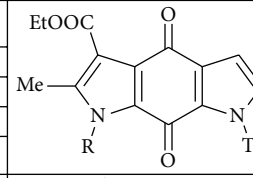
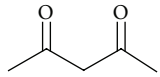
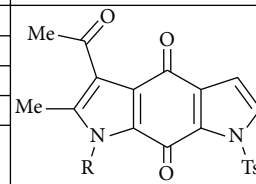
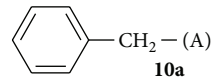
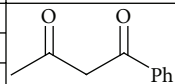
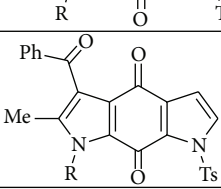
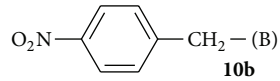
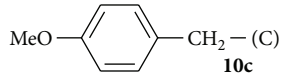
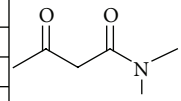
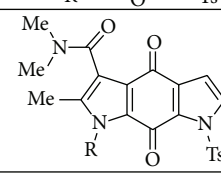
No	Benzylamino Quinone	R	Reagent	CAN (eq)	CH_2Cl_2/MeOH ratio	Compd No	Product	Yield[a] (%)
1		A		3.5	3:7	**4a**		80
2		B		4.5	4:7*†	**4b**		35
3		C		3.5	3:7*	**4c**		82
4		A		3.5	3:7	**4d**		62
5		B		4.5	4:7†	**4e**		23
6		C		3.5	3:7	**4f**		85
7	R =  **8a**	A		3.5	3:7	**4g**		51
8	 **8b**	B		4.5	4:7†	**4h**		38
9		C		3.5	3:7	**4i**		91
10	 **8c**	A		3.5	3:7	**4j**		70
11		B		4.5	3:7†	**4k**		31
12		C		3.5	3:7	**4l**		84
13		A		4	1:5	**5a**		68
14		B		4	1:5	**5b**		60
15		C		4	1:5	**5c**		71
16		A		4	1:5	**5d**		67
17		B		4	1:5	**5e**		58
18		C		4	1:5	**5f**		68
19	R =  **10a**	A		4	1:5	**5g**		67
20	 **10b**	B		4	1:5	**5h**		52
21		C		4	1:5	**5i**		65
22	 **10c**	A		4	1:5	**5j**		67
23		B		4	1:5	**5k**		58
24		C		4	1:5	**5l**		68

Figure 4: CAN-mediated oxidative free radical cyclization reaction of benzylamino quinones yielding substituted N-benzyl pyrroloquinones. [a]isolated yields; *$EtOH/CH_2Cl_2$ mixture was used instead of $MeOH/CH_2Cl_2$ to avoid transesterification; †triple volume of solvents and heating was used to dissolve starting materials.

*4.1.3. 2-[(4-Methoxybenzyl) amino]naphthalene-1, 4-dione **(8c)**.* The compound was prepared using a procedure similar to the one used in the preparation of compound **8a** using 1,4-naphthoquinone **7** (5.0 g, 31.62 mmol) in THF (50 mL) and 4-methoxybenzylamine **6c** (6.15 mL, 47.42 mmol) dissolved in MeOH (50 mL). Compound **8c** was obtained as a red solid (7.95 g, 86%); Mp: 138–141°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.79 (s, 3H), 4.28 (d, 2H), 5.78 (s, 1H), 6.15 (bs, 1H), 6.85–6.92 (m, 2H), 7.19–7.28 (m, 2H), 7.58 (dt, 1H, J_1 = 1.5 Hz, J_2 = 7.6 Hz), 7.71 (dt, 1H, J_1 = 1.5 Hz, J_2 = 7.6 Hz), 8.03 (dd, 1H, J_1 = 1.11 Hz, J_2= 7.7 Hz), and 8.08 (dd, 1H, J_1 = 1.1 Hz, J_2 = 7.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 46.5, 55.5, 101.8, 114.6 (2C), 126.4, 126.5, 128.1, 129.3 (2C), 130.7, 132.2, 133.8, 134.9, 147.9, 159.7, 182.1, and 183.2; MS (ES+): m/z = 294 [M + H].

*4.1.4. Ethyl 1-benzyl-2-methyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indole-3-carboxylate **(4a)**.* To a solution of compound **8a** (0.080 g, 0.30 mmol) and ethyl acetoacetate (0.158 g, 1.21 mmol) in MeOH and CH_2Cl_2 (7 : 3, 10 mL), CAN (0.584 g, 1.06 mmol) was added in four portions at 10 min intervals. After another 10 min of stirring at room temperature, TLC analysis (100% CH_2Cl_2) revealed the completion of the reaction. The solvents were removed *in vacuo*. Water (50 mL) was added to the residue and extracted with CH_2Cl_2 (3 × 30 mL). The combined CH_2Cl_2 layer was washed with water (2 × 30 mL), brine (20 mL) and dried over Na_2SO_4. The drying agent was filtered off, and the filtrate was concentrated under reduced pressure to obtain the crude product which was purified by column chromatography over silica gel using EtOAc/hexanes (1 : 3) as eluent to furnish the product **4a** as a yellow solid (0.091 g, 80%); Mp: 157–160°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (t, 3H, J = 7.2 Hz), 2.40 (s, 3H), 4.43 (q, 2H, J = 7.2 Hz), 5.81 (s, 2H), 7.06 (d, 2H, J = 7 Hz), 7.22–7.36 (m, 3H), 7.60–7.71 (m, 2H), and 8.03–8.19 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 11.2, 14.4, 49.0, 61.4, 114.7, 126.2 (2C), 126.4, 126.5, 126.9, 128.0, 129.2 (2C), 130.5, 133.1, 133.4, 133.5, 134.0, 135.8, 142.5, 164.8, 176.4, and 179.6; MS (ES+): m/z = 374 [M + H].

*4.1.5. Ethyl 1-(4-nitrobenzyl)-4,9-dihydro-2-methyl-4,9-dioxo-1H-benzo[f]indole-3-carboxylate **(4b)**.* Compound **8b** (0.050 g, 0.16 mmol) and ethyl acetoacetate (0.021 g, 0.16 mmol) were dissolved in a mixture of EtOH and CH_2Cl_2 (7 : 3, 33 mL) by heating the solution for 15 min. After the removal of heating, CAN (0.388 g, 0.71 mmol) was added in four installments at 10 min intervals. After 16 h of stirring at room temperature, TLC analysis (100% CH_2Cl_2) showed the completion of reaction. After the solvents were removed *in vacuo*, water (50 mL) was added to the residue and extracted with CH_2Cl_2 (3 × 30 mL). The combined CH_2Cl_2 layer was washed with water (2 × 30 mL), brine (20 mL) and dried over Na_2SO_4. The drying agent was filtered off, and the crude product obtained was purified by column chromatography over silica gel using 100% CH_2Cl_2 and recrystallized with CH_2Cl_2/hexanes to isolate compound **4b** as a yellow solid (0.023 g, 35%); Mp: 96–98°C; ^{1}H NMR ($CDCl_3$, 700 MHz) δ 1.46 (t, 3H, J = 7.0 Hz), 2.44 (s, 3H), 4.47 (q, 2H, J = 7.0 Hz), 5.91 (s, 2H), 7.25 (d, 2H, J = 3.3 Hz), 7.68 (t, 1H, J = 3.2 Hz), 7.71 (t, 1H, J = 3.2 Hz), 8.07 (d, 1H, J = 3.3 Hz), 8.19 (d, 1H, J = 3.2 Hz), and 8.21 (d, 2H, J = 3.3 Hz); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 11.1, 14.4, 48.6, 61.6, 115.2, 124.6 (2C), 126.5 (2C), 127.1, 127.2 (2C), 130.4, 133.1, 133.4, 133.9, 134.0, 142.0, 143.2, 147.9, 164.5, 176.6, and 179.6; MS (ES+): m/z = 419 [M + H].

*4.1.6. Ethyl 1-(4-methoxybenzyl)-4,9-dihydro-2-methyl-4,9-dioxo-1H-benzo[f]indole-3-carboxylate **(4c)**.* To a solution of compound **8c** (0.080 g, 0.27 mmol) in a mixture of EtOH and CH_2Cl_2 (7 : 3, 10 mL), ethyl acetoacetate (0.036 g, 0.27 mmol) was added, and the solution was charged with CAN (0.523 g, 0.96 mmol) in four portions at 10 min intervals and stirred at room temperature for another 10 min. TLC analysis (100% CH_2Cl_2) indicated the reaction was complete. After the solvents were removed under reduced pressure, water (50 mL) was added to the residue. It was extracted with CH_2Cl_2 (3 × 30 mL), washed with water (2 × 30 mL), brine (20 mL) and dried over Na_2SO_4. Drying agent was filtered off, and the filtrate was concentrated *in vacuo* to obtain the crude product, which was purified by column chromatography over silica gel (eluted with 100% CH_2Cl_2) and recrystallized with CH_2Cl_2/hexanes to isolate compound **4c** as a yellow solid (0.090 g, 82%); Mp: 127–129°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (t, 3H, J = 7.1 Hz), 2.43 (s, 3H), 3.77 (s, 3H), 4.44 (q, 2H, J = 7.1 MHz), 5.76 (s, 2H), 6.85 (d, 2H, J = 8.0 Hz), 7.04 (d, 2H, J = 8.0 Hz), 7.67-7.68 (m, 2H), and 8.10–8.15 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.3, 14.4, 48.6, 55.5, 61.4, 114.6 (2C), 114.7, 126.2, 126.5, 126.9, 127.9, 128.0 (2C), 130.4, 133.2, 133.5 (2C), 134.0, 142.4, 159.4, 164.8, 176.4, and 179.7; MS (ES+): m/z = 404 [M + H].

*4.1.7. 3-Acetyl-1-benzyl-2-methyl-1H-benzo[f]indole-4,9-dione **(4d)**.* Compound **8a** (0.080 g, 0.3 mmol) and acetylacetone (0.031 g, 0.3 mmol) were dissolved in a mixture of MeOH and CH_2Cl_2 (7 : 3, 10 mL). The reaction mixture was charged with CAN (0.523 g, 1.06 mmol) in four installments at 10 min intervals. After stirring at room temperature for another 10 min, the reaction was complete as shown by TLC (100% CH_2Cl_2). The solvents were removed under reduced pressure. Water (50 mL) was added to the residue and extracted with CH_2Cl_2 (3 × 30 mL). The combined CH_2Cl_2 layer was washed with water (2 × 30 mL), brine (20 mL) and dried over Na_2SO_4. The drying agent was filtered off, and the solvent was removed *in vacuo*. The crude product thus obtained was purified by column chromatography over silica gel using 100% CH_2Cl_2 and recrystallized with CH_2Cl_2/hexanes to isolate compound **4d** as a yellow solid (0.065 g, 62%); Mp: 203–205°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.35 (s, 3H), 2.73 (s, 3H), 5.81 (s, 2H), 7.06 (d, 2H), 7.22–7.36 (m, 3H), 7.62–7.69 (m, 2H), and 8.04–8.17 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.1, 31.9, 49.0, 123.3, 125.5, 126.5 (3C), 126.6, 126.9, 128.0, 129.2 (2C), 130.0, 133.4, 133.6, 133.7, 135.8, 142.2, 176.3, 180.9, and 199.3; MS (ES+): m/z = 344 [M + H].

*4.1.8. 1-(4-Nitrobenzyl)-3-acetyl-2-methyl-1H-benzo[f]indole-4,9-dione **(4e)**.* Compound **8b** (0.080 g, 0.26 mmol) and acetylacetone (0.026 g, 0.26 mmol) were dissolved in a mixture of MeOH and CH_2Cl_2 (7 : 4, 33 mL) by refluxing for 15 min. The solution was removed from heat and added

with CAN (0.642 g, 1.16 mmol) in four portions at 10 min intervals. After overnight stirring at room temperature, the reaction was complete as indicated by TLC analysis (100% CH_2Cl_2). Upon removing solvents under reduced pressure, water (50 mL) was added and extracted with CH_2Cl_2 (3 × 30 mL). The combined CH_2Cl_2 layer was washed with water (2 × 30 mL), brine (20 mL) and dried over Na_2SO_4. After removing the drying agent, the filtrate was removed *in vacuo* to obtain the crude product, which was purified by column chromatography over silica gel (eluted with 100% CH_2Cl_2) and recrystallized with CH_2Cl_2/hexanes to isolate compound **4e** as a yellow solid (0.023 g, 23%); Mp: 205-206°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.38 (s, 3H), 2.76 (s, 3H), 5.91 (s, 2H), 7.24 (d, 2H, J = 8.1 Hz), 7.60–7.90 (m, 2H), and 8.07–8.22 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.1, 31.9, 48.5, 123.6, 124.5 (2C), 125.8, 126.6, 127.1, 127.2, 129.9 (2C), 133.2, 133.6, 133.7, 133.9, 141.8, 143.1, 147.8, 176.5, 180.7, and 199.1; MS (ES+): m/z = 389 [M + H].

*4.1.9. 1-(4-Methoxybenzyl)-3-acetyl-2-methyl-1H-benzo[f]indole-4,9-dione **(4f)**.* The compound **4f** was prepared following a procedure similar to the one used in the preparation of compound **4d** using compound **8c** (0.080 g, 0.27 mmol), acetylacetone (0.0545 g, 0.54 mmol), and CAN (0.523 g, 0.96 mmol to obtain compound a yellow solid (0.087 g, 85%); Mp: 172-173°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.38 (s, 3H), 2.73 (s, 3H), 3.77 (s, 3H), 5.76 (s, 2H), 6.85 (d, 2H, J = 8.4 Hz), 7.05 (d, 2H, J = 8.4 Hz), 7.68–7.71 (m, 2H), and 8.12–8.17 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.2, 31.9, 48.5, 55.5, 114.3, 114.5, 123.3, 125.5, 126.6, 126.9, 127.8, 128.0, 129.9 (2C), 133.4, 133.5 (2C), 133.7, 142.2, 159.4, 176.4, 180.9, and 199.4; MS (ES+): m/z = 374 [M + H].

*4.1.10. 1-Benzyl-2-methyl-3-(phenylcarbonyl)-1H-benzo[f]indole-4,9-dione **(4g)**.* The compound **4g** was prepared following a procedure similar to the one used in the preparation of compound **4d** using compound **8a** (0.080 g, 0.3 mmol), benzoylacetone (0.050 g, 0.3 mmol), and CAN (0.584 g, 1.06 mmol) to afford a yellow solid (0.063 g, 51%); Mp: 213–215°C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.19 (S, 3H), 5.77 (s, 2H), 7.05 (d, 2H, J = 7.3 Hz), 7.16–7.30 (m, 3H), 7.35 (t, 2H, J = 7.3 Hz), 7.44–7.59 (m, 3H), 7.81 (d, 2H, J = 7.6 Hz), 7.88 (d, 1H, J = 7.2 Hz), and 8.02 (d, 1H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.2, 49.3, 121.7, 126.8 (3C), 126.9, 127.0, 127.1, 128.3, 128.9, 129.5 (2C), 129.7 (2C), 130.0 (2C), 133.6 (2C), 133.7, 134.0, 134.1, 138.7, 141.3, 176.5, 180.2, and 193.4; MS (ES+): m/z = 406 [M + H].

*4.1.11. 1-(4-Nitrobenzyl)-2-methyl-3-(phenylcarbonyl)-1H-benzo[f]indole-4,9-dione **(4h)**.* The compound **4h** was prepared following a procedure similar to the one used in the preparation of compound **4e** using compound **8b** (0.050 g, 0.16 mmol), benzoylacetone (0.026 g, 0.16 mmol), and CAN (0.400 g, 0.73 mmol) to furnish a yellow solid (0.028 g, 38%); Mp: 231-232°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.31 (s, 3H), 5.95 (s, 2H), 7.32 (d, 2H, J = 8.6 Hz), 7.47 (t, 2H, J = 7.6 Hz), 7.58–7.70 (m, 3H), 7.91 (d, 2H, J = 7.8 Hz), 8.00 (d, 1H, J = 7.0), 8.11 (d, 1H, J = 7.0), and 8.25 (d, 2H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.9, 48.6, 121.8, 124.6 (2C), 126.7, 127.0, 127.3 (3C), 128.7 (2C), 129.5, 129.7 (2C), 133.4, 133.5 (2C), 133.6, 133.8, 138.3, 140.6, 143.2, 147.8, 176.4, 179.7, and 192.8; MS (ES+): m/z = 451 [M + H].

*4.1.12. 1-(4-Methoxybenzyl)-2-methyl-3-(phenylcarbonyl)-1H-benzo[f]indole-4,9-dione **(4i)**.* The compound **4i** was prepared following a procedure similar to the one used in the preparation of compound **4d** using compound **8c** (0.080 g, 0.27 mmol), benzoylacetone (0.0885 g, 0.54 mmol), and CAN (0.523 g, 0.96 mmol) to obtain a yellow solid (0.092 g, 91%); Mp: 233–235°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.31 (s, 3H), 3.80 (s, 3H), 5.80 (s, 2H), 6.87–6.91 (m, 2H), 7.12 (d, 2H, J = 8.4 Hz), 7.45 (t, 2H, J = 7.7 Hz), 7.55–7.70 (m, 3H), 7.88–7.91 (m, 2H), 7.99 (dd, 1H, J_1 = 7.3 Hz, J_2 = 1.7 Hz), and 8.15 (dd, 1H, J_1 = 7.3 Hz, J_2 = 1.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.1, 48.7, 55.5, 114.7 (2C), 121.5, 126.8 (2C), 126.9 (2C), 127.9, 128.1 (3C), 128.7 (2C), 129.5, 129.8, 133.4, 133.5 (2C), 133.9, 138.6, 141.0, 159.5, 176.4, 180.0, and 193.3; MS (ES+): m/z = 436 [M + H].

*4.1.13. 1-Benzyl-N,N,2-trimethyl-4,9-dioxo-4,9-dihydro-1H-benzo[f]indole-3-carboxamide **(4j)**.* The compound **4j** was prepared following a procedure similar to the one used in the preparation of compound **4d** using compound **8a** (0.080 g, 0.3 mmol), *N,N*-dimethylacetoacetamide (0.040 g, 0.3 mmol), and CAN (0.584 g, 1.06 mmol) to obtain a yellow solid (0.079 g, 70%); Mp: 163-164°C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.18 (s, 3H), 2.87 (s, 3H), 3.12 (s, 3H), 5.63–5.80 (m, 2H), 7.04 (d, 2H), 7.15–7.30 (m, 3H), 7.59 (t, 2H, J = 3.9 Hz), and 8.04 (t, 2H, J = 3.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.1, 35.3, 38.5, 49.3, 118.4, 125.2 (2C), 126.8 (2C), 127.0, 128.2, 129.4 (3C), 129.9, 133.6 (2C), 134.2, 136.2, 138.8, 166.1, 176.2, and 180.8; MS (ES+): m/z = 373 [M + H].

*4.1.14. 1-(4-Nitrobenzyl)-4,9-dihydro-N,N,2-trimethyl-4,9-dioxo-1H-benzo[f]indole-3-carboxamide **(4k)**.* The compound **4k** was prepared following a procedure similar to the one used in the preparation of compound **4e** using compound **8b** (0.050 g, 0.16 mmol), *N,N*-dimethylacetoacetamide (0.020 g, 0.16 mmol), and CAN (0.400 g, 0.73 mmol) to afford a yellow solid (0.021 g, 31%); Mp: 187–189°C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.29 (s, 3H), 2.97 (s, 3H), 3.22 (s, 3H), 5.87 (s, 2H), 7.28 (d, 2H, J = 8.5 Hz), 7.69 (d, 2H, J = 2.7 Hz), 8.09–8.14 (m, 2H), and 8.21 (d, 2H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.8, 35.2, 38.3, 48.6, 118.5, 124.5 (2C), 125.2, 126.7, 126.8, 127.4 (3C), 129.5, 133.4, 133.6, 133.7, 138.2, 143.2, 147.8, 165.5, 176.1, and 180.4; MS (ES+): m/z = 418 [M + H].

*4.1.15. 1-(4-Methoxybenzyl)-4,9-dihydro-N,N,2-trimethyl-4,9-dioxo-1H-benzo[f]indole-3-carboxamide **(4l)**.* The compound **4l** was prepared following a procedure similar to the one used in the preparation of compound **4d** using compound **8c** (0.080 g, 0.27 mmol), *N,N*-dimethylacetoacetamide (0.0705 g, 0.54 mmol), and CAN (0.523 g, 0.96 mmol) to obtain a yellow solid (0.092 g, 84%); Mp: 224–226°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.27 (s, 3H), 2.93 (s, 3H), 3.19 (s, 3H), 3.75 (s, 3H), 5.63–5.77 (m, 2H), 6.83 (d, 2H, J = 8.7 Hz), 7.01 (d, 2H, J = 8.7 Hz), 7.62–7.68 (m, 2H),

and 8.08–8.13 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.9, 35.0, 38.2, 48.6, 55.4, 114.5 (2C), 118.1, 124.8, 126.5, 126.7, 127.9, 128.1 (3C), 129.5, 133.3 (2C), 134.0, 138.4, 159.3, 165.9, 175.9, and 180.5; MS (ES+): m/z = 403 [M + H].

*4.1.16. 6-Benzylamino-1-tosyl-1H-indole-4,7-dione **(10a)**.* To a solution of 6-methoxy-1-tosyl-1H-indole-4,7-dione **9** (1.2 g, 3.3 mmol) in a mixture of MeOH and THF (1 : 1, 50 mL) at room temperature, a solution of benzyl amine **6a** (0.5 g, 5 mmol) in MeOH (4 mL) was added and stirred at room temperature for 20 hours. TLC analysis (EtOAc/$CHCl_3$, 1 : 1) revealed that the reaction was complete. The solvent was removed under reduced pressure to obtain the crude product as a reddish brown residue. It was purified by column chromatography over silica gel using EtOAc/$CHCl_3$ (1 : 10) as eluent to furnish the pure compound **10a** (1.3 g, 88%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.43 (s, 3H), 4.24 (d, 2H, J = 5.7 Hz), 5.35 (s, 1H), 6.06 (bt, 1H, J = 5.7 Hz), 6.71 (d, 1H, J = 3.0 Hz), 7.15–7.20 (m, 2H), 7.25–7.45 (m, 4H), 7.8 (d, 1H, J = 3.0 Hz), and 7.99 (d, 2H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.8, 47.3, 97.5, 108.6, 127.0, 127.8 (2C), 128.2, 128.9 (2C), 129.0 (2C), 129.8 (2C), 131.3, 134.0, 134.4, 135.7, 146.2, 147.9, 170.2, and 181.4; MS (ES+) m/z 407 [M + H].

*4.1.17. 6-(4-Nitrobenzylamino)-1-tosyl-1H-indole-4,7-dione **(10b)**.* To a solution of 6-methoxy-1-tosyl-1H-indole-4,7-dione **9** (0.700 g, 2.16 mmol) in MeOH and THF (1 : 1, 100 mL) at room temperature, a solution of 4-nitrobenzylamine hydrochloride **6b** (0.540 g, 3.24 mmol) in MeOH (4 mL) and Et_3N (0.330 g, 3.24 mmol) was added and stirred for 20 hours. TLC analysis (EtOAc/$CHCl_3$, 1 : 1) revealed that the reaction was complete. The solvent was removed under reduced pressure, and the reddish yellow residue was purified by column chromatography over silica gel using EtOAc/$CHCl_3$ (1 : 10) as eluent to furnish pure compound **10b** (0.700 g, 70% yield); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.45 (s, 3H), 4.42 (d, 2H, J = 6.0 Hz), 5.23 (s, 1H), 6.19 (bt, 1H, J = 6.0 Hz), 6.72 (d, 1H, J = 3.0 Hz), 7.37 (d, 2H, J = 8.4 Hz), 7.42 (d, 2H, J = 8.7 Hz), 7.82 (d, 1H, J = 3.0 Hz), 8.01 (d, 2H, J = 8.4 Hz), and 8.21 (d, 2H, J = 8.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.8, 46.4, 98.5, 108.7, 124.2 (2C), 126.9, 128.0, 129.0 (2C), 129.8 (2C), 131.5 (2C), 133.9, 134.1, 143.2, 146.4, 147.6, 147.8, 170.1, and 181.4; MS (ES+) m/z 451 [M + H].

*4.1.18. 6-(4-Methoxybenzylamino)-1-tosyl-1H-indole-4,7-dione **(10c)**.* To a solution of 6-methoxy-1-tosyl-1H-indole-4,7-dione **9** (1.13 g, 3.41 mmol) in a mixture of MeOH and THF (1 : 1, 100 mL) at room temperature, a solution of 4-methoxybenzylamine **6c** (0.70 g, 5.1 mmol) in MeOH (4 mL) was added, and the reaction mixture was stirred at room temperature for 20 hours. TLC analysis (EtOAc/$CHCl_3$, 1 : 1) revealed that the reaction was complete. The solvent was removed under reduced pressure, and the reddish yellow residue obtained was purified by column chromatography over silica gel using EtOAc/$CHCl_3$ (1 : 10) as eluent to furnish pure compound **10c** (1.40 g, 94%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.43 (s, 3H), 3.80 (s, 3H), 4.16 (d, 2H, J = 6.0 Hz), 5.35 (s, 1H), 5.99 (bt, 1H, J = 6.0 Hz), 6.72 (d, 1H, J = 3.0 Hz), 6.87 (d, 2H, J = 8.7 Hz), 7.18 (d, 2H, J = 8.7 Hz), 7.34 (d, 2H, J = 8.4 Hz), 7.80 (d, 1H, J = 3.0 Hz), and 7.98 (d, 2H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.8, 46.8, 55.3, 97.3, 108.6, 114.4 (2C), 126.9, 127.7, 128.9 (2C), 129.2 (2C), 129.8 (2C), 131.3, 134.0, 134.4, 146.2, 147.8, 159.5, 170.2, and 181.4; MS (ES+) m/z 434 [M + H].

4.2. CAN Mediated Oxidative Cyclization for 16–19: General Procedure. To a solution of bicyclic quinone **10a–c** (1 equiv) and β-dicarbonyl compound (4 equiv) in MeOH and CH_2Cl_2 (5 : 1), CAN (4 equiv) was added in four equal portions at 10 min intervals. The reaction mixture was stirred for another 10 min at room temperature. TLC analysis (EtOAc/hexanes, 1 : 1) revealed completion of the reaction. Solvent was completely removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (75 mL), washed with water (3 × 50 mL), brine (1 × 50 mL) and dried over anhydrous Na_2SO_4. The drying agent was filtered off, and the solvent was evaporated under reduced pressure. The crude product obtained was purified by column chromatography over silica gel using EtOAc/hexanes (1 : 10) as eluent to obtain the pure bispyrroloquinones **5a-l** in 52–71% yield.

*4.2.1. 1-Benzyl-2-methyl-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydropyrrolo[3,2-f]indole-3-carboxylic Acid Ethyl Ester **(5a)**.* Following the general procedure, compound **10a** (0.094 g, 0.23 mmol) was treated with ethyl acetoacetate (0.12 g, 0.92 mmol) and CAN (0.51 g, 0.92 mmol) in anhydrous MeOH and CH_2Cl_2 (5 : 1, 12 mL) to furnish compound **5a** (0.081 g, 68%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.39 (t, 3H, J = 7.2 Hz), 2.32 (s, 3H), 2.39 (s, 3H), 4.37 (q, 2H, J = 7.2 Hz), 5.66 (s, 2H), 6.73 (d, 1H, J = 3.2 Hz), 6.95–7.05 (m, 2H), 7.20 (d, 2H, J = 8.4 Hz), 7.25–7.30 (m, 3H), 7.71 (d, 1H, J = 3.2 Hz), and 7.90 (d, 2H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.9, 14.1, 21.7, 48.5, 61.1, 108.3, 113.9, 124.4, 126.5 (2C), 127.6, 128.7 (2C), 128.9 (2C), 129.4 (3C), 129.6, 130.1, 132.6, 134.0, 135.6, 141.2, 145.7, 164.5, 167.1, and 177.0; MS (ES+) m/z 515 [M + H].

*4.2.2. 1-(4-Nitrobenzyl)-2-methyl-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydropyrrolo[3,2-f]indole-3-carboxylic Acid Ethyl Ester **(5b)**.* Following the general procedure, compound **10b** (0.050 g, 0.11 mmol) was treated with ethyl acetoacetate (0.058 g, 0.44 mmol) and CAN (0.24 g, 0.44 mmol) in anhydrous MeOH and CH_2Cl_2 (5 : 1, 12 mL) to furnish compound **5b** (0.037 g, 60%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.40 (t, 3H, J = 7.2 Hz), 2.35 (s, 3H), 2.37 (s, 3H), 4.39 (q, 2H, J = 7.2 Hz), 5.72 (s, 2H), 6.76 (d, 1H, J = 3.3 Hz), 7.12 (d, 2H, J = 8.7 Hz), 7.17 (d, 2H, J = 8.3 Hz), 7.72 (d, 1H, J = 3.3 Hz), 7.86 (d, 2H, J = 8.3 Hz), and 8.13 (d, 2H, J = 8.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.8, 14.2, 21.6, 48.1, 61.2, 108.5, 114.4, 124.0 (2C), 124.7, 127.2 (2C), 128.9 (2C), 129.4 (3C), 129.7, 129.9, 132.9, 134.0, 140.8, 143.1, 146.1, 147.4, 164.2, 166.9, and 176.7; MS (ES+) m/z 562 [M + H].

*4.2.3. 1-(4-Methoxybenzyl)-2-methyl-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydro-pyrrolo[3,2-f]indole-3-carboxylic Acid Ethyl Ester **(5c)**.* Following the general procedure, compound **10c** (0.054 g, 0.12 mmol) was treated with ethyl acetoacetate

(0.064 g, 0.50 mmol) and CAN (0.27 g, 0.50 mmol) in anhydrous MeOH and CH_2Cl_2 (5 : 1, 12 mL) to furnish compound **5c** (0.047 g, 71%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.38 (t, 3H, J = 7.2 Hz), 2.33 (s, 3H), 2.41 (s, 3H), 3.78 (s, 3H), 4.35 (q, 2H, J = 7.2 Hz), 5.58 (s, 2H), 6.74 (d, 1H, J = 3.2 Hz), 6.77 (d, 2H, J = 8.7 Hz), 6.91 (d, 2H, J = 8.7 Hz), 7.24 (d, 2H, J = 8.1 Hz), 7.71 (d, 1H, J = 3.2 Hz), and 7.93 (d, 2H, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.0, 14.2, 21.7, 48.1, 55.3, 61.0, 108.3, 114.0, 114.1 (2C), 124.5, 127.8, 128.1 (2C), 129.0 (2C), 129.4, 129.5 (2C), 129.6, 130.2, 132.6, 134.2, 141.1, 145.8, 159.1, 164.5, 167.1, and 176.9; MS (ES+) m/z 546 [M + H].

*4.2.4. 3-Acetyl-1-benzyl-2-methyl-7-tosyl-1H,7H-pyrrolo[3,2-f]indole-4,8-dione **(5d)**.* Following the general procedure, compound **10a** (0.050 g, 0.12 mmol) was treated with acetyl acetone (0.049 g, 0.48 mmol) and CAN (0.27 g, 0.48 mmol) in anhydrous MeOH and CH_2Cl_2 (5 : 1, 12 mL) to furnish compound **5d** (0.040 g, 67%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.26 (s, 3H), 2.40 (s, 3H), 2.65 (s, 3H), 5.66 (s, 2H), 6.74 (d, 1H, J = 2.4 Hz), 6.90–7.00 (m, 2H), 7.21 (d, 2H, J = 8.0 Hz), 7.20–7.40 (m, 3H), 7.73 (d, 1H, J = 2.4 Hz), and 7.93 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.9, 21.8, 31.6, 48.5, 108.2, 122.6, 123.7, 126.5 (2C), 127.6, 128.7 (2C), 129.0 (2C), 129.5 (3C), 129.6, 130.1, 132.3, 133.9, 135.6, 140.8, 145.9, 167.0, 178.3, and 199.1; MS (ES+) m/z 487 [M + H].

*4.2.5. 3-Acetyl-2-methyl-1-(4-nitrobenzyl)-7-tosyl-1H,7H-pyrrolo[3,2-f]indole-4,8-dione **(5e)**.* Following the general procedure, compound **10b** (0.045 g, 0.10 mmol) was treated with acetyl acetone (0.040 g, 0.40 mmol) and CAN (0.22 g, 0.40 mmol) in anhydrous MeOH and CH_2Cl_2 (5 : 1, 12 mL) to furnish compound **5e** (0.027 g, 58%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.30 (s, 3H), 2.37 (s, 3H), 2.67 (s, 3H), 5.72 (d, 2H), 6.75 (d, 1H, J = 3.3 Hz), 7.14 (d, 2H, J = 8.7 Hz), 7.19 (d, 2H, J = 8.1 Hz), 7.74 (d, 1H, J = 3.3 Hz), 7.88 (d, 2H, J = 8.1 Hz), and 8.14 (d, 2H, J = 8.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.8, 21.6, 31.7, 48.0, 108.5, 122.9, 124.0, 124.1, 127.2 (2C), 128.9, 129.0 (2C), 129.4 (2C), 129.7 (2C), 129.9, 132.5, 133.7, 140.4, 143.0, 146.2, 147.4, 166.9, 178.1, and 198.8; MS (ES+) m/z 532 [M + H].

*4.2.6. 3-Acetyl-1-(4-methoxybenzyl)-2-methyl-7-tosyl-1H,7H-pyrrolo[3,2-f]indole-4,8-dione **(5f)**.* Following the general procedure, compound **10c** (0.070 g, 0.16 mmol) was treated with acetyl acetone (0.064 g, 0.64 mmol) and CAN (0.35 g, 0.64 mmol) in anhydrous MeOH and CH_2Cl_2 (5 : 1, 12 mL) to furnish compound **5f** (0.056 g, 68%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.28 (s, 3H), 2.41 (s, 3H), 2.63 (s, 3H), 3.78 (s, 3H), 5.58 (s, 2H), 6.73 (d, 1H, J = 3.0 Hz), 6.78 (d, 2H, J = 8.0 Hz), 6.93 (d, 2H, J = 8.5 Hz), 7.25 (d, 2H, J = 8.0 Hz), 7.73 (d, 1H, J = 3.0 Hz), and 7.95 (d, 2H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.9, 21.7, 31.6, 48.0, 55.3, 108.2, 114.1 (2C), 122.7, 123.8, 127.7, 128.1 (2C), 129.0, 129.1 (2C), 129.5 (2C), 129.6, 130.3, 132.3, 134.1, 140.7, 145.9, 159.1, 167.1, 178.3, and 199.0; MS (ES+) m/z 517 [M + H].

*4.2.7. 3-Benzoyl-1-benzyl-2-methyl-7-tosyl-1H,7H-pyrrolo[3,2-f]indole-4,8-dione **(5 g)**.* Following the general procedure, compound **10a** (0.10 g, 0.25 mmol), 1-phenyl-2-propanone (0.16 g, 0.99 mmol) and CAN (0.47 g, 0.99 mmol) in anhydrous MeOH and CH_2Cl_2 (5 : 1, 12 mL) to furnish compound **5 g** (0.090 g, 67%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.19 (s, 3H), 2.41 (s, 3H), 5.69 (s, 2H), 6.59 (d, 1H, J = 2.4 Hz), 6.90–7.10 (m, 2H), 7.20–7.35 (m, 5H), 7.40 (t, 2H, J = 7.2 Hz), 7.54 (t, 1H, J = 7.2 Hz), 7.68 (d, 1H, J = 2.4 Hz), 7.82 (d, 2H, J = 7.5 Hz), and 7.95 (d, 2H, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.7, 21.8, 48.7, 108.2, 120.9, 125.1, 126.6 (2C), 127.7, 128.4 (2C), 128.7, 128.8 (2C), 128.9, 129.0 (2C), 129.2 (2C), 129.5 (2C), 130.6, 132.0, 133.1, 134.1, 135.7, 138.4, 139.7, 145.8, 167.0, 177.3, and 192.9; MS (ES+) m/z 549 [M + H].

*4.2.8. 3-Benzoyl-2-methyl-1-(4-nitrobenzyl)-7-tosyl-1H,7H-pyrrolo[3,2-f]indole-4,8-dione **(5h)**.* Following the general procedure, compound **10b** (0.050 g, 0.11 mmol) was treated with 1-phenyl-2-propanone (0.072 g, 0.44 mmol) and CAN (0.24 g, 0.44 mmol) in anhydrous MeOH and CH_2Cl_2 (5 : 1, 12 mL) to furnish compound **5h** (0.034 g, 52%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.23 (s, 3H), 2.39 (s, 3H), 5.76 (s, 2H), 6.61 (d, 1H, J = 2.4 Hz), 7.15–7.25 (m, 4H), 7.43 (t, 2H, J = 8.0 Hz), 7.57 (t, 1H, J = 7.6 Hz), 7.69 (d, 1H, J = 2.4 Hz), 7.82 (d, 2H, J = 7.6 Hz), 7.90 (d, 2H, J = 8.0 Hz), and 8.18 (d, 2H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.6, 21.6, 48.1, 108.4, 121.1, 124.1 (2C), 125.3, 127.3 (2C), 128.4, 128.6 (2C), 129.0 (2C), 129.2 (2C), 129.4 (2C), 129.8, 130.2, 132.2, 133.3, 133.9, 138.1, 139.2, 143.1, 146.1, 147.5, 166.9, 177.0, and 192.5; MS (ES+) m/z 594 [M + H].

*4.2.9. 3-Benzoyl-1-(4-methoxybenzyl)-2-methyl-7-tosyl-1H, 7H-pyrrolo[3,2-f]indole-4,8-dione **(5i)**.* Following the general procedure, compound **10c** (0.080 g, 0.18 mmol) was treated with 1-phenyl-2-propanone (0.090 g, 0.72 mmol) and CAN (0.36 g, 0.22 mmol) anhydrous MeOH and CH_2Cl_2 (5 : 1, 12 mL) to furnish compound **5i** (0.074 g, 70%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.21 (s, 3H), 2.43 (s, 3H), 3.8 (s, 3H), 5.61 (s, 2H), 6.59 (d, 1H, J = 3.3 Hz), 6.81 (d, 2H, J = 8.4 Hz), 7.01 (d, 2H, J = 8.4 Hz), 7.25–7.30 (m, 2H), 7.40 (t, 2H, J = 8.0 Hz), 7.54 (t, 1H, J = 8.0 Hz), 7.68 (d, 1H, J = 3.3 Hz), 7.81 (d, 2H, J = 8.0 Hz), and 7.97 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.8, 21.8, 48.1, 55.3, 108.2, 114.1 (2C), 120.8, 125.1, 127.8, 128.2 (2C), 128.3 (2C), 128.6, 129.1 (2C), 129.2 (2C), 129.4, 129.5 (2C), 130.6, 131.9, 133.1, 134.1, 138.3, 139.7, 146.0, 159.1, 167.1, 177.3, and 192.9; MS (ES+) m/z 579 [M + H].

*4.2.10. 1-Benzyl-2-methyl-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydropyrrolo[3,2-f]indole-3-carboxylic Acid Dimethylamide **(5j)**.* Following the general procedure, compound **10a** (0.050 g, 0.12 mmol) was treated with N,N-dimethylacetoacetamide (0.064 g, 0.49 mmol) and CAN (0.27 g, 0.49 mmol) in anhydrous MeOH and CH_2Cl_2 (5 : 1, 12 mL) to furnish compound **5j** (0.043 g, 68%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.15 (s, 3H), 2.41 (s, 3H), 2.88 (s, 3H), 3.13 (s, 3H), 5.55–5.75 (m, 2H), 6.70 (d, 1H, J = 3.0 Hz), 6.90–7.10 (m, 2H), 7.20–7.30 (m, 5H), 7.71 (d, 1H, J = 3.0 Hz), and 7.93 (d, 2H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.6, 21.8, 34.8, 38.0, 48.6, 108.0, 117.5, 123.3, 126.7 (2C), 127.6, 128.5, 128.7 (2C), 129.0 (2C), 129.4 (3C),

131.0, 131.9, 134.0, 135.8, 137.1, 145.8, 165.5, 167.0, and 178.0; MS (ES+) *m/z* 516 [M + H].

*4.2.11. 2-Methyl-1-(4-nitrobenzyl)-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydropyrrolo[3,2-f]indole-3-carboxylic Acid Dimethylamide **(5k)**.* Following the typical procedure, compound **10b** (0.045 g, 0.10 mmol) was treated with N,N-dimethylacetoacetamide (0.051 g, 0.40 mmol) and CAN (0.22 g, 0.40 mmol) in anhydrous MeOH and CH_2Cl_2 (5 : 1, 12 mL) to furnish compound **5k** (0.031 g, 58%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.20 (s, 3H), 2.38 (s, 3H), 2.90 (s, 3H), 3.15 (s, 3H), 5.60–5.80 (m, 2H), 6.71 (d, 1H, J = 3.3 Hz), 7.15–7.25 (m, 4H), 7.72 (d, 1H, J = 3.3 Hz), 7.88 (d, 2H, J = 8.4 Hz), and 8.14 (d, 2H, J = 8.7 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.5, 21.6, 34.9, 38.1, 48.1, 108.1, 117.9, 123.6, 124.0 (2C), 127.4, 128.4 (2C), 129.0, 129.4 (2C), 129.7 (2C), 130.4, 132.1, 133.9, 136.8, 143.1, 146.1, 147.4, 165.1, 166.7, and 177.7; MS (ES+) *m/z* 561 [M + H].

*4.2.12. 1-(4-Methoxybenzyl)-2-methyl-4,8-dioxo-7-tosyl-1,4,7,8-tetrahydro-pyrrolo[3,2-f]indole-3-carboxylic Acid Dimethylamide **(5l)**.* Following the typical procedure, compound **10c** (0.070 g, 0.16 mmol) was treated with N,N-dimethylacetoacetamide (0.083 g, 0.64 mmol) and CAN (0.35 g, 0.64 mmol) in anhydrous MeOH and CH_2Cl_2 (5 : 1, 12 mL) to furnish compound **5l** (0.063 g, 68%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.17 (s, 3H), 2.42 (s, 3H), 2.86 (s, 3H), 3.13 (s, 3H), 3.79 (s, 3H), 5.40–5.60 (m, 2H), 6.69 (d, 1H, J = 3.3 Hz), 6.78 (d, 2H, J = 7.6 Hz), 6.96 (d, 2H, J = 8.5 Hz), 7.2-7.3 (m, 2H), 7.71 (d, 1H, J = 3.3 Hz), and 7.95 (d, 2H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.7, 21.7, 34.8, 38.0, 48.1, 55.3, 107.9, 114.1 (2C), 117.6, 123.3, 127.9 (2C), 128.3 (2C), 128.4, 129.0, 129.4 (3C), 131.0, 131.9, 134.2, 137.0, 145.8, 159.1, 165.6, 167.0, and 178.0; MS (ES+) *m/z* 546 [M + H].

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References

[1] B. R. Copp, C. M. Ireland, and L. R. Barrows, "Wakayin: a novel cytotoxic pyrroloiminoquinone alkaloid from the ascidian Clavelina species," *The Journal of Organic Chemistry*, vol. 56, no. 15, pp. 4596–4597, 1991.

[2] N. K. Utkina, A. E. Makarchenko, and V. A. Denisenko, "Zyzzyanones B-D, dipyrroloquinones from the marine sponge Zyzzya fuliginosa," *Journal of Natural Products*, vol. 68, no. 9, pp. 1424–1427, 2005.

[3] N. K. Utkina, A. E. Makarchenko, V. A. Denisenko, and P. S. Dmitrenok, "Zyzzyanone A, a novel pyrrolo[3,2-f]indole alkaloid from the Australian marine sponge Zyzzya fuliginosa," *Tetrahedron Letters*, vol. 45, no. 40, pp. 7491–7494, 2004.

[4] G. J. Hooper, M. T. Davies-Coleman, M. Kelly-Borges, and P. S. Coetzee, "New alkaloids from a South African latrunculid sponge," *Tetrahedron Letters*, vol. 37, no. 39, pp. 7135–7138, 1996.

[5] E. M. Antunes, B. R. Copp, M. T. Davies-Coleman, and T. Samaai, "Pyrroloiminoquinone and related metabolites from marine sponges," *Natural Product Reports*, vol. 22, no. 1, pp. 62–72, 2005.

[6] D. J. Faulkner, "Marine natural products," *Natural Product Reports*, vol. 19, no. 1, pp. 1–48, 2002.

[7] E. Dolusic, P. Larrieu, C. Meinguet et al., "Indoleamine 2, 3-dioxygenase inhibitory activity of derivatives of marine alkaloid tsitsikammamine A," *Bioorganic & Medicinal Chemistry Letters*, vol. 23, no. 1, pp. 47–54, 2013.

[8] A. Rives, T. Delaine, L. Legentil, and E. Delfourne, "Total synthesis of the marine pyrroloiminoquinone alkaloid tsitsikammamine A," *Tetrahedron Letters*, vol. 50, no. 10, pp. 1128–1130, 2009.

[9] S. M. Lee, X. F. Li, H. Jiang et al., "Terreusinone, a novel UV-A protecting dipyrroloquinone from the marine algicolous fungus Aspergillus terreus," *Tetrahedron Letters*, vol. 44, no. 42, pp. 7707–7710, 2003.

[10] C. Wang and J. Sperry, "Total synthesis of the photoprotecting dipyrrolobenzoquinone (+)-terreusinone," *Organic Letters*, vol. 13, no. 24, pp. 6444–6447, 2011.

[11] V. Bénéteau and T. Besson, "Synthesis of novel pentacyclic pyrrolothiazolobenzoquinolinones, analogs of natural marine alkaloids," *Tetrahedron Letters*, vol. 42, no. 14, pp. 2673–2676, 2001.

[12] V. Bénéteau, A. Pierré, B. Pfeiffer, P. Renard, and T. Besson, "Synthesis and antiproliferative evaluation of 7-amino-substituted pyrroloiminoquinone derivatives," *Bioorganic and Medicinal Chemistry Letters*, vol. 10, no. 19, pp. 2231–2234, 2000.

[13] J. M. Kokoshka, T. L. Capson, J. A. Holden, C. M. Ireland, and L. R. Barrows, "Differences in the topoisomerase I cleavage complexes formed by camptothecin and wakayin, a DNA-intercalating marine natural product," *Anti-Cancer Drugs*, vol. 7, no. 7, pp. 758–765, 1996.

[14] R. Zhao, B. Oreski, and J. W. Lown, "Synthesis and biological evaluation of hybrid molecules containing the pyrroloquinoline nucleus and DNA-minor groove binders," *Bioorganic and Medicinal Chemistry Letters*, vol. 6, no. 18, pp. 2169–2172, 1996.

[15] L. Legentil, L. Benel, V. Bertrand, B. Lesur, and E. Delfourne, "Synthesis and antitumor characterization of pyrazolic analogues of the marine pyrroloquinoline alkaloids: wakayin and tsitsikammamines," *Journal of Medicinal Chemistry*, vol. 49, no. 10, pp. 2979–2988, 2006.

[16] L. Legentil, B. Lesur, and E. Delfourne, "Aza-analogues of the marine pyrroloquinoline alkaloids wakayin and tsitsikammamines: synthesis and topoisomerase inhibition," *Bioorganic and Medicinal Chemistry Letters*, vol. 16, no. 2, pp. 427–429, 2006.

[17] C. Wang and J. Sperry, "Total syntheses of the dipyrrolobenzoquinone (+)-terreusinone enabled by an evaluation of 4-methylpent-1-yn-3-ols in the Larock indole synthesis," *Tetrahedron*, vol. 69, no. 23, pp. 4563–4577, 2013.

[18] B. A. Moharam, I. Jantan, J. Jalil, and F. Ahmad, "Inhibitory effect of compounds from Goniothalamus tapis Miq. and Goniothalamus uvaroides King on platelet-activating factor receptor binding," *Phytotherapy Research*, vol. 26, no. 5, pp. 687–691, 2012.

[19] D. H. Nadkarni, S. Murugesan, and S. E. Velu, "Total synthesis of zyzzyanones A-D," *Tetrahedron*, vol. 69, no. 20, pp. 4105–4113, 2013.

[20] S. Murugesan, D. H. Nadkarni, and S. E. Velu, "A facile synthesis of bispyrroloquinone and bispyrroloiminoquinone ring system of marine alkaloids," *Tetrahedron Letters*, vol. 50, no. 25, pp. 3074–3076, 2009.

[21] J. Iqbal, B. Bhatia, and N. K. Nayyar, "Transition metal-promoted free-radical reactions in organic synthesis: the formation of carbon-carbon bonds," *Chemical Reviews*, vol. 94, no. 2, pp. 519–564, 1994.

[22] C.-C. Tseng, Y.-L. Wu, and C.-P. Chuang, "Cerium salts in the oxidative free radical reactions between 2-amino-1,4-naphthoquinones and β-dicarbonyl compounds," *Tetrahedron*, vol. 58, no. 38, pp. 7625–7633, 2002.

[23] C.-P. Chuang and Y.-L. Wu, "A novel oxidative free radical reaction between 2-hydroxy-1,4-naphthoquinone and β-enamino carbonyl compounds," *Tetrahedron Letters*, vol. 42, no. 9, pp. 1717–1719, 2001.

[24] C.-P. Chuang and A.-I. Tsai, "Oxidative free radical reactions between 2-amino-1,4-benzoquinones and carbonyl compounds," *Tetrahedron*, vol. 63, no. 48, pp. 11911–11919, 2007.

[25] C.-M. Tseng, Y.-L. Wu, and C.-P. Chuang, "Solvent effects on the oxidative free radical reactions of 2-amino-1,4-naphthoquinones," *Tetrahedron*, vol. 60, no. 52, pp. 12249–12260, 2004.

[26] Y.-L. Wu, C.-P. Chuang, and P.-Y. Lin, "Oxidative free radical reactions between 2-amino-1,4-naphthoquinones and carbonyl compounds," *Tetrahedron*, vol. 57, no. 26, pp. 5543–5549, 2001.

[27] R. Mohan, S. A. Kates, M. A. Dombroski, and B. B. Snider, "Manganese (III) based oxidative free-radical cyclizations. 3. Polycyclization reactions proceeding through secondary radicals," *Tetrahedron Letters*, vol. 28, no. 8, pp. 845–848, 1987.

[28] B. B. Snider and J. J. Patricia, "Manganese(III)-based oxidative free-radical cyclizations. Oxidative cyclization and aromatization of 3-oxo-6-heptenoate esters," *The Journal of Organic Chemistry*, vol. 54, no. 1, pp. 38–46, 1989.

[29] S. Patai and Z. Rappoport, *The Chemistry of Functional Groups: The Chemistry of the Quinoid Compounds*, Wiley, New York, NY, USA, 1988.

[30] R. H. Thomson, *Natural Occurring Quinones IV: Recent Advances*, Chapman & Hall, London, UK, 1997.

[31] E. V. Sadanandan, S. K. Pillai, M. V. Lakshmikantham, A. D. Billimoria, J. S. Culpepper, and M. P. Cava, "Efficient syntheses of the marine alkaloids makaluvamine D and discorhabdin C: the 4,6,7-trimethoxyindole approach," *The Journal of Organic Chemistry*, vol. 60, no. 6, pp. 1800–1805, 1995.

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Research Article

Clauson Kaas Pyrrole Synthesis Catalyzed by Acidic Ionic Liquid under Microwave Irradiation

Feray Aydogan and Cigdem Yolacan

Department of Chemistry, Yildiz Technical University, Davutpasa Campus, Esenler, 34010 Istanbul, Turkey

Correspondence should be addressed to Feray Aydogan; feray_aydogan@yahoo.com

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A new procedure to synthesize the N-substituted pyrrole derivatives by Clauson Kaas reaction catalyzed by acidic ionic liquid under microwave irradiation was developed. This procedure provides several advantages such as high yield, clean product formation, and short reaction time.

Dedicated to the memory of Professor Ayhan S. Demir

1. Introduction

Pyrrole derivatives have great importance in organic chemistry, because they are present in many compounds which were natural, medicinal, and agricultural products and semiconductor polymers [1–3]. They are also very useful starting materials for biologically important compounds such as indolizidine alkaloids, bicyclic lactams, and unsaturated γ-lactams [4–7]. Various pyrrole syntheses have been reported in the literature [8–15]. One of these methods is Clauson Kaas pyrrole synthesis which uses 2,5-dimethoxytetrahydrofuran as four-carbon source. Although this reaction provides an important and useful way for synthesis of N-substituted pyrroles, the necessity of high temperature and acidic conditions is resulted by the decomposition of product so low yields and difficult product separation are the main problems with this method [16, 17]. To overcome the problems with classical Clauson Kaas reaction, new synthetic methods such as using two phase system [16], initial aqueous hydrolysis of 2,5-dimethoxytetrahydrofuran [17], and using acetic acid, water, or sodium acetate buffer under microwave irradiation [18] and new catalysts [19–21] have been still developed successfully.

Using microwaves for carrying out reactions is advantageous for the synthesis of numerous types of compound. The most important improvements with this technique are reduced reaction time, cleaner reactions due to fewer side reactions, and the use of minimal quantities of solvent [22, 23]. So, microwave-assisted synthesis is more economical and environmentally friendly method.

Ionic liquids (ILs) have been widely used in organic reactions as solvent due to their advantages such as negligible vapor pressure, variable polarity, and good solvating ability [24–26]. Recently, they have also attracted the researchers' attention due to their significant role in organic reactions as catalyst [27–32]. After the first use of imidazolium chloroaluminate as catalyst in Friedel-Crafts acylations [33], various ionic liquids have been developed and used in many types of organic reactions as catalyst. Many Lewis acidic and Bronsted acidic ionic liquids have been successfully used as acid catalysts in organic synthesis with advantages such as solvent-free reaction conditions, easy product separation, and recycling [34–39]. As far as we know, acidic ionic liquids have not been used to promote Clauson Kaas reaction, and only a few methods have been developed for this reaction under microwave irradiation [18, 40, 41]. So, we wish to report here the clean, short time synthesis of N-substituted pyrrole derivatives by the Clauson Kaas reaction catalyzed by acidic ionic liquid, 1-hexyl-3-methylimidazolium hydrogen sulfate ([hmim][HSO_4]), under microwave irradiation.

Table 1: Comparison of the catalytic activity of the catalysts.

Catalyst	Time (min)	Yield (%)
CH_3CO_2H	80	52
[bmim][BF_4]	30	56
[hmim][H_2PO_4]	30	61
[hmim][HSO_4][a]	60	73
[hmim][HSO_4]	4	85

[a] Without MW.

2. Experimental

All reagents were of commercial quality, and reagent quality solvents were used without further purification. IR spectra were determined on a Perkin Elmer, Spectrum One FT-IR spectrometer. NMR spectra were recorded on Mercury VX-400 MHz and Bruker Avance III 500 MHz spectrometer. Chemical shifts δ are reported in ppm relative to $CDCl_3$ (^{1}H: δ = 7.27) and TMS as internal standard. Column chromatography was conducted on silica gel 60 (40–63 μM). TLC was carried out on aluminum sheets precoated with silica gel $60F_{254}$ (Merck). Elemental analysis was carried out on Thermo Flash EA 1112 series apparatus. Optical rotations were measured with Bellingham Stanley ADP-410 Polarimeter. Microwave-assisted reactions were carried out on an Arcelik MD 554 household oven.

2.1. General Procedure for Clauson Kaas Reaction. Amine (1 mmol) was added to 2,5-dimethoxytetrahydrofuran (1 mmol or 2 mmol for amine 2b) in [hmim][HSO_4] (1 mmol) and mixed thoroughly. The mixture was then exposed to microwave irradiation (90 W) for a period of time enough to complete the reaction. The reaction mixture was dissolved in water, and the product was extracted with diethyl ether (3 × 5 mL), and the combined organic phases were dried over $MgSO_4$. The crude product obtained after evaporation of the solvent was purified by column chromatography over silica gel (EtOAc : hexane 1 : 2, 1 : 6, or 1 : 10).

*2.1.1. (S)-1-(1-Phenylethyl)-1H-pyrrole (S)-**3a**.* Colorless liquid (85% yield). $[\alpha]_D^{20}$ = +6.6; Lit. +6.8 [42] (*c* 2.7, $CHCl_3$); IR (neat): ν 3027, 2965, 1603, and 1377 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.75 (d, J = 7.02 Hz, 3H, CH_3), 5.20 (q, J = 7.02 Hz, 1H, N–CH), 6.11 (brs, 2H, =CH), 6.68 (brs, 2H, =CH), 7.01 (d, J = 7.80 Hz, 2H, ArH), and 7.14 (m, 3H, ArH). Anal. Calcd for $C_{12}H_{13}N$ (171.24): C, 84.17; H, 7.65; N, 8.18. Found: C, 84.22; H, 7.61; N, 8.15.

*2.1.2. 1-(4-(1H-Pyrrol-1-yl)butyl)-1H-pyrrole **3b**.* Colorless oil (91% yield). IR (neat): ν 3098, 2929, 2872, 1498, 1279, and 1087 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.75 (m, 4H, CH_2), 3.85 (t, J = 6.64 Hz, 4H, CH_2), 6.15 (apparent t, J = 1.95, 1.95 Hz, 4H, =CH), and 6.63 (apparent t, J = 2.34, 1.95 Hz, 4H, =CH). Anal. Calcd for $C_{12}H_{16}N_2$ (188.13): C, 76.55; H, 8.57; N, 14.88. Found: C, 76.49; H, 8.58; N, 14.86.

*2.1.3. (R)-Methyl 2-(1H-Pyrrol-1-yl)propanoate (R)-**3c**.* Colorless oil (73% yield). $[\alpha]_D^{20}$ = +12.7; Lit. +12.83 [42] (*c* 1.1, $CHCl_3$); IR (neat): ν 3102, 2992, 2954, 1747, 1378, and 1282 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.65 (d, J = 7.33 Hz, 3H, CH_3), 3.64 (s, 3H, OCH_3), 4.69 (q, J = 7.33 Hz, 1H, N–CH), 6.11 (apparent t, J = 2.44, 1.96 Hz, 2H, =CH), and 6.67 (apparent t, J = 2.44, 1.95 Hz, 2H, =CH). Anal. Calcd for $C_8H_{11}NO_2$ (153.18): C, 62.73; H, 7.24; N, 9.14. Found: C, 62.76; H, 7.21; N, 9.15.

*2.1.4. (S)-Methyl 3-Methyl-2-(1H-pyrrol-1-yl)butanoate (S)-**3d**.* Colorless oil (69% yield). $[\alpha]_D^{20}$ = −2.7; Lit. −2.8 [42] (*c* 4.2, $CHCl_3$); IR (neat): ν 3055, 2986, 1727, 1372, and 1285 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (d, J = 6.65 Hz, 3H, CH_3), 0.91 (d, J = 6.65 Hz, 3H, CH_3), 2.32 (m, 1H, CH), 3.63 (s, 3H, CH_3), 4.00 (d, J = 10.23 Hz, 1H, N–CH), 6.02 (brs, 2H, =CH), and 6.63 (brs, 2H, =CH). Anal. Calcd for $C_{10}H_{15}NO_2$ (181.23): C, 66.27; H, 8.34; N, 7.73. Found: C, 66.28; H, 8.40; N, 7.69.

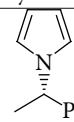
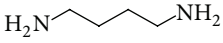
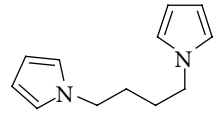
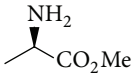
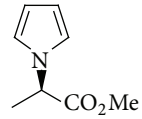
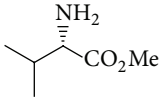
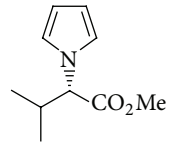
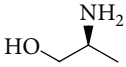
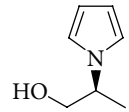
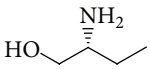
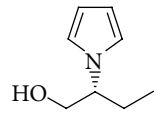
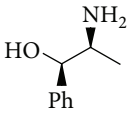
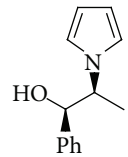
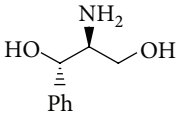
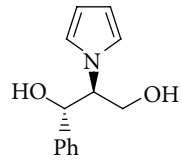
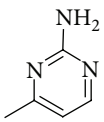
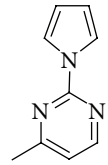
*2.1.5. (S)-2-(1H-Pyrrol-1-yl)propan-1-ol (S)-**3e**.* Colorless oil (89% yield). $[\alpha]_D^{20}$ = +8.3; Lit. +8.25 [42] (*c* 2.2, $CHCl_3$); IR (neat): ν 3524, 3098, 2982, 1558, 1378, and 1280 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (d, J = 7.02 Hz, 3H, CH_3), 1.63 (brs, 1H, OH), 3.59 (m, 2H, CH_2), 4.09 (m, 1H, N–CH), 6.10 (brs, 2H, =CH), and 6.67 (brs, 2H, =CH). Anal. Calcd for $C_7H_{11}NO$ (125.17): C, 67.17; H, 8.86; N, 11.19. Found: C, 67.21; H, 8.84; N, 11.16.

*2.1.6. (R)-2-(1H-Pyrrol-1-yl)butan-1-ol (R)-**3f**.* Colorless oil (91% yield). $[\alpha]_D^{20}$ = +14.3; Lit. +14.3 [42] (*c* 0.4, $CHCl_3$); IR (neat): ν 3517, 3048, 2998, 1602, 1376, and 1282 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 7.41 Hz, 3H, CH_3), 1.68 (brs, 1H, OH), 1.77 (m, 2H, CH_2), 3.73 (m, 2H, CH_2), 3.85 (m, 1H, N–CH), 6.18 (apparent t, J = 2.34, 1.95 Hz, 2H, =CH), and 6.70 (apparent t, J = 2.34, 1.95 Hz, 2H, =CH). Anal. Calcd for $C_8H_{13}NO$ (139.19): C, 69.03; H, 9.41; N, 10.06. Found: C, 69.01; H, 9.42; N, 9.96.

*2.1.7. (1R, 2S)-1-Phenyl-2-(1H-pyrrol-1-yl)propan-1-ol (1R, 2S)-**3g**.* Yellow oil (83% yield). $[\alpha]_D^{20}$ = +26.6; Lit. +26.8 [42] (*c* 3.6, $CHCl_3$); IR (neat): ν 3523, 3054, 2994, 1605, 1376, and 1283 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.33 (d, J = 7.00 Hz, 3H, CH_3), 2.11 (brs, 1H, OH), 4.08 (m, 1H, N–CH), 4.61 (d, J = 4.68 Hz, 1H, O–CH), 5.94 (brs, 2H, =CH), 6.47 (brs, 2H, =CH), 7.04 (d, J = 7.60 Hz, 2H, ArH), and 7.16 (m, 3H, ArH). Anal. Calcd for $C_{13}H_{15}NO$ (201.26): C, 77.58; H, 7.51; N, 6.96. Found: C, 77.51; H, 7.60; N, 7.01.

*2.1.8. (1S, 2S)-1-Phenyl-2-(1H-pyrrol-1-yl)propane-1,3-diol (1S, 2S)-**3h**.* Yellow oil (81% yield). $[\alpha]_D^{20}$ = +93.2; Lit. +93.5 [42] (*c* 0.5, $CHCl_3$); IR (neat): ν 3525, 3050, 1605, 1375, and 1284 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.00 (brs, 1H, OH), 2.45 (brs, 1H, OH), 3.72 (m, 2H, CH_2), 4.06 (m, 1H, N–CH), 4.92 (d, J = 6.6 Hz, 1H, O–CH), 6.15 (apparent t, J = 1.95, 1.95 Hz, 2H, =CH), 6.70 (apparent t, J = 2.34, 1.95 Hz, 2H, =CH), 7.19 (m, 2H, ArH), and 7.31 (m, 3H, ArH). Anal. Calcd

Table 2: The synthesized pyrrole derivatives.

Amine (**2**)	Pyrrole (**3**)	Reaction time (min)	Yield (%)[a]
 (*S*)-**2a**	 (*S*)-**3a** [42]	4	85
 **2b**	 **3b** [43]	4	91
 (*R*)-**2c**	 (*R*)-**3c** [42]	3	73
 (*S*)-**2d**	 (*S*)-**3d** [42]	3	69
 (*S*)-**2e**	 (*S*)-**3e** [42]	5	89
 (*R*)-**2f**	 (*R*)-**3f** [42]	5	91
 (1*R*, 2*S*)-**2g**	 (1*R*, 2*S*)-**3g** [42]	5	83
 (1*S*, 2*S*)-**2h**	 (1*S*, 2*S*)-**3h** [42]	5	81
 **2i**	 **3i** [44]	25	72

[a] Isolated yield.

for $C_{13}H_{15}NO_2$ (217.26): C, 71.87; H, 6.96; N, 6.45. Found: C, 71.82; H, 6.97; N, 6.41.

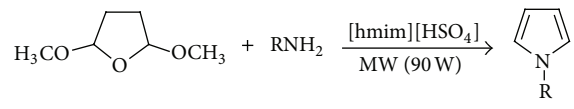
2.1.9. 1-(4-Methyl-2-pyrimidinyl)-1H-pyrrole ***3i***. White solid, mp. 44-45°C; Lit. 44.5–46°C [44] (72% yield). IR (neat): ν 2917, 1583, 1475, 1434, and 1384 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.51 (s, 3H, CH_3), 6.32 (apparent t, J = 2.25 Hz, 2H, =CH), 6.90 (d, J = 5.00 Hz, 1H, ArH), 7.79 (apparent t, J = 2.25 Hz, 2H, =CH), and 8.44 (d, J = 5.05 Hz, 1H, ArH). Anal. Calcd for $C_9H_9N_3$ (159.19): C, 67.90; H, 5.70; N, 26.40. Found: C, 67.86; H, 5.72; N, 26.41.

3. Results and Discussion

The acidic ionic liquid [hmim][HSO_4] was prepared under microwave irradiation starting from [hmim][Br] according to the literature procedure [45], and the spectral data of the compound were in accordance with the reported data [46]. We started the research with (*S*)-phenylethylamine as a representative reactant to see if the acidic IL would catalyze the Clauson Kaas reaction and to achieve optimum reaction conditions such as microwave irradiation power, IL amount, and the necessity of cosolvent such as diethyl ether, chloroform. It was found that the reaction was completed within 4 min in the presence of equimolar amount of acidic ionic liquid under solvent-free conditions without any decomposition of product. The reaction was also carried out without microwave irradiation by stirring the reaction mixture at room temperature. It was found that the reaction completed in 1 hour with the decomposition of small amount product.

The catalytic activity of [hmim][HSO_4] was compared with those of acetic acid, [bmim][BF_4], and [hmim][H_2PO_4], which was prepared by the same procedure with [hmim][HSO_4], under the optimum conditions. As one can see from the results shown in the Table 1, [hmim][HSO_4] was found to be best catalyst providing 85% yield of product.

In a typical reaction procedure, 2,5-dimethoxytetrahydrofuran (1 mmol), equimolar amount of amine, and [hmim][HSO_4] were mixed, and the mixture was exposed to microwave irradiation (90 W) for a period of time enough to complete the reaction. The reaction mixture was dissolved in water, and the product was extracted with diethyl ether and purified by column chromatography. To check the reusability of the ionic liquid, water was removed from the aqueous layer under vacuum, and the residue was washed with diethyl ether and dried under vacuum. Decreasing in the yield of pyrrole was seen after recycling of ionic liquid. The main problem with the Clauson Kaas reaction is the decomposition of acid sensitive derivatives, especially derived from amino acids. Using this procedure, various amine compounds such as aliphatic amines, amino acid esters, amino alcohols, and heteroaromatic amine were converted to their pyrrole derivatives without any significant decomposition (Scheme 1) in 69–91% yield, as summarized in Table 2. Some of the amines were chiral, and any racemization was not observed with these amines. All of the pyrrole derivatives are known in the literature, and their spectroscopic data are in full agreement with their structures.



(**1**) (**2**) (**3**)

Scheme 1: The synthesis of N-substituted pyrroles catalyzed by [hmim][HSO_4].

4. Conclusion

In conclusion, efficient synthesis of N-substituted pyrrole derivatives has been achieved by Clauson Kaas reaction catalyzed by acidic ionic liquid under microwave irradiation. This new method provides a clean, fast, high yielded, environmentally friendly, and effective way to pyrroles without any significant decomposition of acid sensitive derivatives.

References

[1] R. Ragno, G. R. Marshall, R. Di Santo et al., "Antimycobacterial pyrroles: synthesis, anti-*Mycobacterium tuberculosis* activity and QSAR studies," *Bioorganic and Medicinal Chemistry*, vol. 8, no. 6, pp. 1423–1432, 2000.

[2] R. K. Dieter and H. Yu, "A facile synthesis of polysubstituted pyrroles," *Organic Letters*, vol. 2, no. 15, pp. 2283–2286, 2000.

[3] C. Franc, F. Denonne, C. Cuisinier, and L. Ghosez, "A general synthesis of 2-formyl-3-arylpyrroles," *Tetrahedron Letters*, vol. 40, no. 24, pp. 4555–4558, 1999.

[4] J. Liu, Q. Yang, T. C. W. Mak, and H. N. C. Wong, "Highly regioselective synthesis of 2,3,4-trisubstituted 1*H*-pyrroles: a formal total synthesis of lukianol A," *Journal of Organic Chemistry*, vol. 65, no. 12, pp. 3587–3595, 2000.

[5] C. W. Jefford, K. Sienkiewicz, and S. R. Thornton, "Short, enantiospecific synthesese of indolizidines 209B and 209D, and piclavine A from diethyl-L-glutamate," *Helvetica Chimica Acta*, vol. 78, no. 6, pp. 1511–1524, 1995.

[6] R. I. J. Amos, B. S. Gourlay, P. P. Molesworth, J. A. Smith, and O. R. Sprod, "Annulation of pyrrole: application to the synthesis of indolizidine alkaloids," *Tetrahedron*, vol. 61, no. 34, pp. 8226–8230, 2005.

[7] C. W. Jefford, Q. Tang, and A. Zaslona, "Short, enantiogenic syntheses of (-)-indolizidine 167b and (+)-monomorine," *Journal of the American Chemical Society*, vol. 113, no. 9, pp. 3513–3518, 1991.

[8] V. Estevez, M. Villacampa, and J. C. Menedez, "Multicomponent reactions for the synthesis of pyrroles," *Chemical Society Reviews*, vol. 39, no. 11, pp. 4402–4421, 2010.

[9] H. Valizadeh, M. M. Heravi, and M. Amiri, "Conventional and microwave-assisted facile one-pot synthesis of *N*-substituted pyrrole-2,3,4,5-tetracarboxylates under neat conditions," *Synthetic Communications*, vol. 40, no. 24, pp. 3699–3706, 2010.

[10] O. A. Attanasi, G. Favi, F. Mantellini, G. Moscatelli, and S. Santeusanio, "Synthesis of functionalized pyrroles via catalyst- and solvent-free sequential three-component enamine-azenone annulation," *Journal of Organic Chemistry*, vol. 76, no. 8, pp. 2860–2866, 2011.

[11] F. J. Leeper and J. M. Kelly, "Synthesis of 3,4-disubstituted pyrroles. A review," *Organic Preparations and Procedures International*, vol. 45, no. 3, pp. 171–210, 2013.

[12] B. Li, N. Wang, Y. Liang, S. Xu, and B. Wang, "Ruteniumcatalyzed pyrrole synthesis via oxidative annulation of enamides and alkynes," *Organic Letters*, vol. 15, no. 1, pp. 136–139, 2013.

[13] R. J. Billedeau, K. R. Klein, D. Kaplan, and Y. Lou, "A new pyrrole synthesis via silver(I)-catalyzed cycloaddition of vinylogous dazoester and nitrile," *Organic Letters*, vol. 15, no. 7, pp. 1421–1423, 2013.

[14] D. G. Dunford, D. W. Knight, and R. C. Wheeler, "An efficient copper-ctalysed pyrrole synthesis," *ARKIVOC*, vol. 2012, no. 7, pp. 253–273, 2012.

[15] F. Aydogan and A. S. Demir, "Clean and efficient microwave-solvent-free conversion of homochiral amines, α-amino alcohols and α-amino acids to their chiral 2-substituted pyrrole derivatives," *Tetrahedron*, vol. 61, no. 12, pp. 3019–3023, 2005.

[16] C. W. Jefford, F. De Villedon De Naide, and K. Sienkiewicz, "The synthesis of chiral 1-(1*H*-pyrrole) derivatives," *Tetrahedron Asymmetry*, vol. 7, no. 4, pp. 1069–1076, 1996.

[17] B. S. Gourlay, P. P. Molesworth, J. H. Ryan, and J. A. Smith, "A new and high yielding synthesis of unstable pyrroles via a modified Clauson-Kaas reaction," *Tetrahedron Letters*, vol. 47, no. 5, pp. 799–801, 2006.

[18] K. C. Miles, S. M. Mays, B. K. Southerland, T. J. Auvil, and D. M. Ketcha, "The Clauson-Kaas pyrrole synthesis under microwave irradiation," *Arkivoc*, vol. 2009, no. 14, pp. 181–190, 2009.

[19] F. P. Ma, P. H. Li, B. L. Li et al., "A recyclable magnetic nanoparticles supported antimony catalyst for the synthesis of *N*-substituted pyrroles in water," *Applied Catalysis A*, vol. 457, pp. 34–41, 2013.

[20] X. Zhang and J. Shi, "Unique chemoselective Clauson-Kass reaction of substituted aniline catalyzed by MgI_2 etherate," *Tetrahedron*, vol. 67, no. 5, pp. 898–903, 2011.

[21] V. Polshettiwar, B. Baruwati, and R. S. Varma, "Magnetic nanoparticle-supported glutathione: a conceptually sustainable organocatalyst," *Chemical Communications*, no. 14, pp. 1837–1839, 2009.

[22] L. Perreux and A. Loupy, "A tentative rationalization of microwave effects in organic synthesis according to the reaction medium, and mechanistic considerations," *Tetrahedron*, vol. 57, no. 45, pp. 9199–9223, 2001.

[23] P. Lidström, J. Tierney, B. Wathey, and J. Westman, "Microwave assisted organic synthesis-a review," *Tetrahedron*, vol. 57, no. 45, pp. 9225–9283, 2001.

[24] S. Suresh and J. S. Sandhu, "Recent advances in ionic liquids: green unconventional solvents of this century: part I," *Green Chemistry Letters and Reviews*, vol. 4, no. 4, pp. 289–310, 2011.

[25] T. Welton, "Room-temperature ionic liquids. Solvents for synthesis and catalysis," *Chemical Reviews*, vol. 99, no. 8, pp. 2071–2083, 1999.

[26] H. Olivier-Bourbigou and L. Magna, "Ionic liquids: perspectives for organic and catalytic reactions," *Journal of Molecular Catalysis A*, vol. 182-183, pp. 419–437, 2002.

[27] S. Toma, M. Meciarova, and R. Sebesta, "Are ionic liquids suitable media for organocatalytic reactions?" *European Journal of Organic Chemistry*, no. 3, pp. 321–327, 2009.

[28] A. D. Sawant, D. G. Raut, N. B. Darvatkar, and M. M. Salunkhe, "Recent developments of task-specific ionic liquids in organic synthesis," *Green Chemistry Letters and Reviews*, vol. 4, no. 1, pp. 41–54, 2011.

[29] A. G. Ying, L. Liu, G. F. Wu, G. Chen, X. Z. Chen, and W. D. Ye, "Aza-Michael addition of aliphatic or aromatic amines to α,β-unsaturated compounds catalyzed by a DBU-derived ionic liquid under solvent-free conditions," *Tetrahedron Letters*, vol. 50, no. 14, pp. 1653–1657, 2009.

[30] D. Chaturvedi, "Recent developments on task specific ionic liquids," *Current Organic Chemistry*, vol. 15, no. 8, pp. 1239–1248, 2011.

[31] S. Narayanaperumal, R. Cesar da Silva, K. S. Feu, A. Fernández de la Torre, A. G. Correa, and M. W. Paixao, "Basic-functionalized recyclable ionic liquid catalyst: a solvent-free approach for Michael addition of 1,3-dicarbonyl compounds to nitroalkenes under ultrasound irradiation," *Ultrasonics Sonochemistry*, vol. 20, no. 3, pp. 793–798, 2013.

[32] A. Mulik, D. Chandam, P. Patil, D. Patil, S. Jagdale, and M. Deshmukh, "Proficient synthesis of quinoxaline and phthalazinetrione derivatives using $[C_8$dabco$]$Br ionic liquid as catalyst in aqueous media," *Journal of Molecular Liquids*, vol. 179, pp. 104–109, 2013.

[33] J. A. Boon, J. A. Levisky, J. L. Pflug, and J. S. Wilkes, "Friedel-Crafts reactions in ambient-temperature molten salts," *Journal of Organic Chemistry*, vol. 51, no. 4, pp. 480–483, 1986.

[34] A. R. Hajipour and F. Rafiee, "Acidic bronsted ionic liquids," *Organic Preparations and Procedures International*, vol. 42, no. 4, pp. 285–362, 2010.

[35] R. Srivastava, "Assessment of the catalytic activities of novel brönsted acidic ionic liquid catalysts," *Catalysis Letters*, vol. 139, no. 1-2, pp. 17–25, 2010.

[36] S. Sajjadifar, E. R. Nezhad, and G. Darvishi, "1-Methyl-3-(2-(sulfooxy)ethyl)-1*H*-imidazol-3-ium chloride as a new and green ionic liquid catalyst for one-pot synthesis of dihydropyrimidinones under solvent-free conditions," *Journal of Chemistry*, vol. 2013, Article ID 834656, 6 pages, 2013.

[37] Z. S. Qureshi, K. M. Deshmukh, K. P. Dhake, and B. M. Bhanage, "Brønsted acidic ionic liquid: a simple, efficient and recyclable catalyst for regioselective alkylation of phenols and anti-Markovnikov addition of thiols to alkenes," *RSC Advances*, vol. 1, no. 6, pp. 1106–1112, 2011.

[38] X. Q. Chu, R. Jiang, Y. Fang et al., "Acidic-functionalized ionic liquid as an efficient, green, and metal-free catalyst for benzylation of sulfur, nitrogen, and carbon nucleophiles to benzylic alcohols," *Tetrahedron*, vol. 69, no. 3, pp. 1166–1174, 2013.

[39] H. R. Shaterian and K. Azizi, "Acidic ionic liquids catalyzed one-pot, pseudo five-component, and diastereoselective synthesis of highly functionalized piperidine derivatives," *Journal of Molecular Liquids*, vol. 180, pp. 187–191, 2013.

[40] M. Abid, S. M. Landge, and B. Török, "An efficient and rapid synthesis of *N*-substituted pyrroles by microwave assisted solid acid catalysis," *Organic Preparations and Procedures International*, vol. 38, no. 5, pp. 495–500, 2006.

[41] C. C. Silveira, M. P. Fortes, and S. R. Mendes, "Cerium (III) chloride as a catalyst in the Clauson-Kaas reaction: synthesis and reactivity of N-Aryl-2-thiocyanatopyrroles," *Current Organic Chemistry*, vol. 16, no. 12, pp. 1540–1548, 2012.

[42] F. Aydogan, M. Basarir, C. Yolacan, and A. S. Demir, "New and clean synthesis of *N*-substituted pyrroles under microwave irradiation," *Tetrahedron*, vol. 63, no. 39, pp. 9746–9750, 2007.

[43] N. D. Scott, "Production of N-alkyl pyrroles," 1949, US 2488336 19491115.

[44] I. Becker, "Preparation of pyrrole and pyrrolidine derivatives of pyrimidine. 1-(2-Pyrimidinyl)pyrrole-an inhibitor of *X. Phaseoli* and *X. Malvacearum*," *Journal of Heterocyclic Chemistry*, vol. 41, no. 3, pp. 343–348, 2004.

[45] V. Singh, S. Kaur, V. Sapehiyia, J. Singh, and G. L. Kad, "Microwave accelerated preparation of [bmim][HSO_4] ionic liquid: an acid catalyst for improved synthesis of coumarins," *Catalysis Communications*, vol. 6, no. 1, pp. 57–60, 2005.

[46] J. Fraga-Dubreuil, K. Bourahla, M. Rahmouni, J. P. Bazureau, and J. Hamelin, "Catalysed esterifications in room temperature ionic liquids with acidic counteranion as recyclable reaction media," *Catalysis Communications*, vol. 3, no. 5, pp. 185–190, 2002.

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Research Article

Convenient Synthesis of 1,4-Dideoxy-1,4-imino-D-ribitol from D-Ribose

Makoto Oba, Shoi Kawaji, Hironobu Kushima, Takanori Sano, and Kozaburo Nishiyama

Department of Materials Chemistry, Tokai University, 317 Nishino, Numazu, Shizuoka 410-0395, Japan

Correspondence should be addressed to Makoto Oba; moba@tokai-u.jp

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This paper describes a convenient synthesis of 1,4-dideoxy-1,4-imino-D-ribitol (DRB) from D-ribose. L-Lyxonolactone, a key intermediate in this synthesis, was prepared by base-promoted hydrolysis of a 5-chlorinated D-ribonolactone derivative with inversion of configuration at the C-4 position. Cyclization of the generated dimesylated L-lyxitol with benzylamine proceeded with another configurational inversion at C-4 to afford the D-*ribo*-configured pyrrolidine system, which upon deprotection gave DRB.

1. Introduction

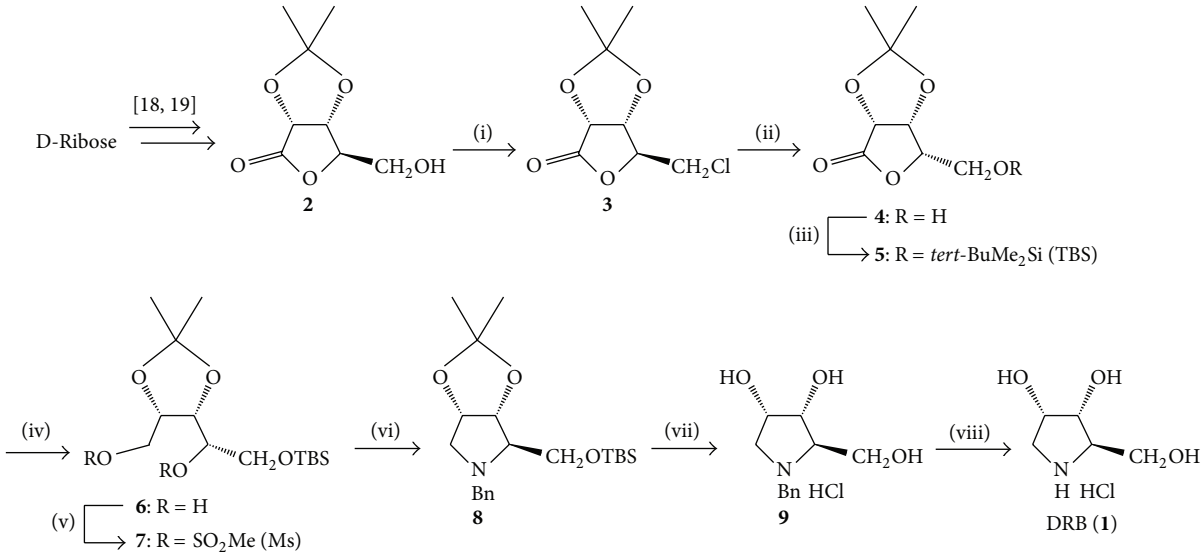
1,4-Dideoxy-1,4-imino-D-ribitol (DRB, **1**) is a polyhydroxylated pyrrolidine alkaloid isolated from the roots of mulberry trees (*Morus alba*) [1] and from the bark and pods of leguminous plants (*Angylocalyx pynaertii*) [2, 3]. Owing to its structural [4-aza]ribofuranose feature, DRB and its derivatives have attracted considerable attention as enzyme inhibitors that mimic glycoside and nucleoside substrates. In fact, DRB was found to be a potent inhibitor of lysosomal β-mannosidase [3] and eukaryotic DNA polymerases [4] and was also employed as a synthetic precursor of some enzyme inhibitors containing the [4-aza]ribosyl group [5–8]. Therefore, there is a need to develop a simple method for the preparation of DRB derivatives.

Two major approaches have been used to construct the DRB framework. One is the stereoselective dihydroxylation of optically active 2-substituted 3-pyrroline derivatives, in which the oxidation is usually carried out using a highly toxic osmium catalyst [9–13]; the other is a sugar-based approach. The D- and L-forms of 1,4-dideoxy-1,4-iminoribitol were prepared from D-gulonolactone (29% overall yield over 9 steps) and D-mannose (28% overall yield over 9 steps), respectively [14–16]. From the viewpoint of atom economy, pentose as a starting material is more favorable. Recently, a related study was reported by Mercer and coworkers [17], in which both enantiomers of 1,4-dideoxy-1,4-iminolyxitol were efficiently synthesized from D- and L-ribonolactone. Since the process involves configurational inversion at the C-4 position, a straightforward precursor to DRB is considered to be L-lyxose, which is an expensive unnatural pentose. Herein, we describe a convenient synthesis of DRB starting from D-ribose via L-lyxonolactone , in which the D-*ribo*-configured pyrrolidine ring is constructed with overall retention of the stereochemistry at C-4 by a double inversion.

2. Results and Discussion

The synthetic route to DRB is illustrated in Scheme 1. 2,3-*O*-Isopropylidene-D-ribono-1,4-lactone (**2**) is easily obtained from inexpensive D-ribose using a well-established procedure [18, 19] or is commercially available. At the beginning of the synthesis, we examined the conversion of D-ribonolactone **2** to L-lyxonolactone **4** with inversion of stereochemistry at C-4. A production-scale synthesis of **4** from **2** via a 5-*O*-methanesulfonyl derivative was reported (59% yield at a 200 kg scale) [18]; however, we experienced variable yields at a laboratory scale. In this study, therefore, we adopted an alternative route via the corresponding chloride **3**.

Chlorination of the hydroxyl group at C-5 of **2** was performed using a Vilsmeier reagent prepared in situ from DMF



Scheme 1: Synthesis of DRB (**1**). Reagents and conditions: (i) $(COCl)_2$, DMF, CH_2Cl_2, 97%; (ii) KOH, H_2O, then, 3 M HCl, quant; (iii) TBSCl, imidazole, CH_2Cl_2, 91%; (iv) $NaBH_4$, MeOH, 95%; (v) MsCl, pyridine, 85%; (vi) $PhCH_2NH_2$ ($BnNH_2$), toluene, 86%; (vii) 1 M HCl, quant; and (viii) H_2, 10% Pd/C, H_2O, quant.

and oxalyl chloride to afford 5-chloro-5-deoxy derivative **3** in 97% yield [20]. Treatment of chloride **3** with an aqueous KOH solution followed by acidification gave 2,3-*O*-isopropylidene-L-lyxono-1,4-lactone (**4**) in quantitative yield. It is believed that configurational inversion at the C-4 position occurred as reported for the mesylate reaction [21]. Namely, a base-promoted ring opening of the chlorinated ribonolactone **3** followed by intramolecular S_N2 reaction gave epoxide **10** (Scheme 2). Subsequent 5-*exo*-tet [22] ring closure between the carboxylate and epoxide proceeded with inversion of configuration at C-4 to furnish the lactone, which was then hydrolyzed to the open-chain derivative **11** under strongly basic conditions. Upon acidification, carboxylate **11** immediately cyclized to lyxonolactone **4**.

After protection of the primary hydroxyl group of **4** as a *tert*-butyldimethylsilyl (TBS) ether in 91% yield, the fully protected lactone **5** was subjected to reductive ring opening by $NaBH_4$ in MeOH to afford partially protected L-lyxitol derivative **6** in 95% yield. Diol **6** was then treated with methanesulfonyl chloride in pyridine to give the corresponding dimesylate **7** in 85% yield. Cyclization of **7** with benzylamine involving inversion at C-4 was performed in refluxing toluene for 3 days to give fully protected DRB **8** in 86% yield. Acidic hydrolysis of both the acetonide and TBS protective groups in 1 M HCl gave *N*-benzyl DRB derivative **9** in quantitative yield. Finally, DRB was quantitatively obtained as its hydrochloride salt by catalytic hydrogenolysis of the *N*-benzyl group. Comparison of the physical and spectral data of DRB with the literature data completely confirmed its identity.

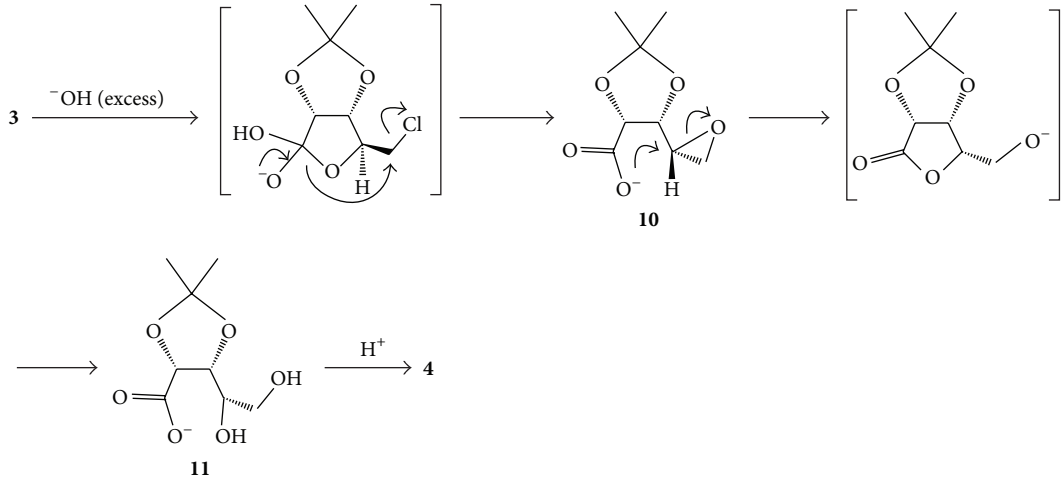
In conclusion, we have achieved a convenient synthesis of DRB in 61% overall yield from D-ribonolactone **2** over eight steps. The D-*ribo*-configured pyrrolidine system was constructed with overall retention of the stereochemistry at C-4 by a double S_N2 inversion.

3. Experimental

3.1. General. Melting points were determined using a Yamato MP-21 melting point apparatus in open capillaries and are uncorrected. ^1H and ^{13}C-nuclear magnetic resonance (NMR) spectra were measured on a Varian Mercury plus 400 spectrometer at 400 and 100 MHz, respectively. All chemical shifts are reported as δ values (ppm) relative to residual chloroform (δ_H 7.26), HDO (δ_H 4.79), the central peak of deuteriochloroform (δ_C 77.0), or dioxane (δ_C 67.2); J values are expressed in Hz. Optical rotations were measured on a HORIBA SEPA-200 polarimeter. Elemental analyses were performed using a PerkinElmer 2400 Series II analyzer.

All reagents and solvents were of commercial grade and used according to supplier instructions unless otherwise mentioned.

*3.2. 5-Chloro-5-deoxy-2,3-O-isopropylidene-D-ribono-1,4-lactone (**3**) [20, 23].* DMF was added (117 μL, 110 mg, 1.51 mmol) to a solution of oxalyl chloride (129 μL, 194 mg, 1.52 mmol) in CH_2Cl_2 (4 mL) at 0°C, and the mixture was stirred for 12 min. To the resultant cloudy suspension, a solution of compound **2** (188 mg, 0.999 mmol) in CH_2Cl_2 (2 mL) was added dropwise at the same temperature, and the mixture was refluxed for 90 min. The cooled reaction mixture was diluted with $CHCl_3$, washed with brine, and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on SiO_2. Elution with a mixture of hexane and AcOEt (7/3) gave compound **3** (200 mg, 0.968 mmol, 97%) as a white solid. An analytical sample was obtained by recrystallization from a mixture of EtOH and acetone. Colorless powder, mp 97.5–98.5°C. $[\alpha]_D^{23}$ −60.8 (c 1.00, $CHCl_3$). ^1H-NMR ($CDCl_3$) δ 1.40 (s, 3H), 1.49 (s, 3H), 3.79 (dd, J = 12 and 2 Hz, 1H), 3.85 (dd, J = 12 and 3 Hz, 1H), 4.74 (d, J = 6 Hz, 1H), 4.87 (dd, J = 3



Scheme 2: Plausible reaction pathway for the configurational inversion at C-4 by base-promoted hydrolysis of lactone **3**.

and 2 Hz, 1H), 4.89 (d, J = 6 Hz, 1H). ^{13}C-NMR ($CDCl_3$) δ 25.4, 26.5, 44.7, 75.2, 78.2, 80.8, 113.7, 173.3.

*3.3. 2,3-O-Isopropylidene-L-lyxono-1,4-lactone (**4**) [18].* Compound **3** (207 mg, 1.00 mmol) was added to a 2.5 M aqueous solution of KOH (1.00 mL, 2.50 mmol), and the resulting mixture was stirred at room temperature overnight. The solution was acidified with 3 M HCl to pH 3 and concentrated. The residue was triturated with acetone (6 mL) and heated to reflux. After removal of the insoluble materials by filtration, the filtrate was dried over $MgSO_4$ and concentrated under reduced pressure to give compound **4** (193 mg) in quantitative yield as a white solid, mp 94-95°C (lit [18], mp 98-99°C). $[\alpha]_D^{25}$ −88.0 (c 0.50, acetone) (lit [18], $[\alpha]_D^{25}$ −89.0 (c 1.00, acetone)). ^{1}H-NMR ($CDCl_3$) δ 1.40 (s, 3H), 1.49 (s, 3H), 2.10 (br s, 1H), 3.97 (dd, J = 12 and 5 Hz, 1H), 4.04 (dd, J = 12 and 7 Hz, 1H), 4.60 (ddd, J = 7, 5, and 4 Hz, 1H), 4.87 (d, J = 6 Hz, 1H), 4.89 (dd, J = 6 and 4 Hz, 1H). ^{13}C-NMR ($CDCl_3$) δ 25.7, 26.6, 60.8, 76.1, 76.2, 79.1, 114.5, 173.5.

*3.4. 5-O-tert-butyldimethylsilyl-2,3-O-isopropylidene-L-lyxono-1,4-lactone (**5**) [24].* A solution of compound **4** (193 mg), *tert*-$BuMe_2SiCl$ (166 mg, 1.10 mmol), and imidazole (102 mg, 1.50 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature for 1 h. The reaction mixture was then diluted with $CHCl_3$, washed with brine, and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on SiO_2. Elution with a mixture of hexane and AcOEt (7/3) gave compound **5** (276 mg, 0.913 mmol, 91%) as a white solid. An analytical sample was obtained by recrystallization from hexane. Colorless powder, mp 87-88°C (data for enantiomer [25]: mp 90–91°C). $[\alpha]_D^{27}$ −52.2 (c 1.00, $CHCl_3$) (data for enantiomer [25]: $[\alpha]_D^{22}$ +54.9 (c 1.03, $CHCl_3$)). ^{1}H-NMR ($CDCl_3$) δ 0.09 (s, 6H), 0.90 (s, 9H), 1.38 (s, 3H), 1.45 (s, 3H), 3.93 (dd, J = 11 and 7 Hz, 1H), 3.97 (dd, J = 11 and 6 Hz, 1H), 4.52 (ddd, J = 7 and 6 and 2 Hz, 1H), 4.79–4.82 (m, 2H). ^{13}C-NMR ($CDCl_3$) δ −5.6, −5.4, 18.3, 25.7, 25.8, 26.7, 60.8, 75.7, 76.0, 79.4, 114.0, 173.8.

*3.5. 5-O-tert-butyldimethylsilyl-2,3-O-isopropylidene-L-lyxitol (**6**) [24].* $NaBH_4$ (351 mg, 9.28 mmol) was added to a solution of compound **5** (561 mg, 1.85 mmol) in MeOH (19 mL) at 0°C, and the resulting mixture was stirred at room temperature for 1 h. After removal of the solvent, the residue was diluted with $CHCl_3$, washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated under reduced pressure to give compound **6** (543 mg, 1.77 mmol, 96%) as a white solid. An analytical sample was obtained by recrystallization from hexane. Colorless powder, mp 64-65°C (data for enantiomer [25]: mp 67–68°C). $[\alpha]_D^{26}$ +9.3 (c 1.02, $CHCl_3$) (data for enantiomer [25]: $[\alpha]_D^{23}$ −9.2 (c 0.08, $CHCl_3$)). ^{1}H-NMR ($CDCl_3$) δ 0.08 (s, 6H), 0.90 (s, 9H), 1.38 (s, 3H), 1.51 (s, 3H), 2.83 (dd, J = 7 and 5 Hz, 1H), 2.90 (d, J = 5 Hz, 1H), 3.63 (dd, J = 10 and 7 Hz, 1H), 3.72 (dd, J = 10 and 6 Hz, 1H), 3.77–3.85 (m, 3H), 4.23–4.25 (m, 2H). ^{13}C-NMR (CDCl3) δ −5.5, −5.4, 18.2, 25.0, 25.8, 27.1, 61.3, 64.5, 69.1, 75.7, 77.3, 108.2.

*3.6. 5-O-tert-butyldimethylsilyl-1,4-di-O-methanesulfonyl-2,3-O-isopropylidene-L-lyxitol (**7**) [24].* Methanesulfonyl chloride (0.411 mL, 608 mg, 5.31 mmol) was added to a solution of compound **6** (543 mg, 1.77 mmol) in pyridine (10 mL) at 0°C, and the resulting mixture was stirred at room temperature overnight. After removal of the solvent, the residue was diluted with AcOEt, successively washed with 1 M HCl and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated under reduced pressure to give compound **7** (695 mg, 1.50 mmol, 85%) as a colorless oil. $[\alpha]_D^{26}$ −5.5 (c 1.02, $CHCl_3$) (data for enantiomer [25]: $[\alpha]_D^{24}$ +5.0 (c 0.14, $CHCl_3$)). ^{1}H-NMR ($CDCl_3$) δ 0.097 (s, 3H), 0.103 (s, 3H), 0.90 (s, 9H), 1.38 (s, 3H), 1.51 (s, 3H), 3.08 (s, 3H), 3.11 (s, 3H), 3.83 (dd, J = 11 and 6 Hz, 1H), 3.96 (dd, J = 11 and 5 Hz, 1H), 4.37–4.45 (m, 4H), 4.74 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ−5.6 (2C overlapped), 18.2, 25.4, 25.8, 27.2, 37.6, 38.9, 63.1, 67.9, 74.4, 75.3, 78.9, 109.6.

*3.7. N-Benzyl-5-O-tert-butyldimethylsilyl-2,3-O-isopropylidene-1,4-dideoxy-1,4-imino-D-ribitol hydrochloride (**8**) [5, 8].*

A mixture of compound **7** (695 mg, 1.50 mol) and benzylamine (891 μL, 874 mg, 8.16 mmol) in toluene (8 mL) was heated to reflux for 3 days. The reaction mixture was then diluted with $CHCl_3$, successively washed with water and saturated aqueous $NaHCO_3$, and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on SiO_2. Elution with a mixture of hexane and AcOEt (9/1) gave compound **8** (487 mg, 1.29 mmol, 86%) as a colorless oil. $[\alpha]_D^{27}$ −28.0 (*c* 1.01, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ 0.07 (s, 3H), 0.09 (s, 3H), 0.92 (s, 9H), 1.35 (s, 3H), 1.58 (s, 3H), 2.74 (dd, *J* = 10 and 3 Hz, 1H), 3.03 (ddd, *J* = 4, 4, and 2 Hz, 1H), 3.12 (dd, *J* = 10 and 6 Hz, 1H), 3.66 (dd, *J* = 11 and 4 Hz, 1H), 3.74 (d, *J* = 13 Hz, 1H), 3.79 (dd, *J* = 11 and 4 Hz, 1H), 4.04 (d, *J* = 13 Hz, 1H), 4.58 (dd, *J* = 7 and 2 Hz, 1H), 4.67 (ddd, *J* = 7, 6, and 3 Hz, 1H), 7.15–7.38 (m, 5H). ^{13}C-NMR ($CDCl_3$) δ −5.6, −5.5, 18.2, 25.1, 25.9, 27.1, 56.9, 59.2, 63.1, 68.8, 79.4, 83.2, 111.8, 126.8, 128.2, 128.5, 139.2.

*3.8. N-Benzyl-1,4-dideoxy-1,4-imino-D-ribitol hydrochloride (**9**)* . A mixture of compound **8** (354 mg, 0.938 mmol) and 1 M HCl (10 mL) was refluxed for 1 h. The cooled solution was washed with $CHCl_3$ and concentrated to give a quantitative yield of compound **9** (250 mg) as a brown solid. An analytical sample was obtained by recrystallization from a mixture of EtOH and acetone. Colorless powder, mp 190-191°C. $[\alpha]_D^{23}$ +16.9 (*c* 1.00, H_2O). ^{1}H-NMR (D_2O) δ 3.43 (dd, *J* = 13 and 3 Hz, 1H), 3.56 (dd, *J* = 13 and 4 Hz, 1H), 3.62 (dd, *J* = 13 and 4 Hz, 1H), 3.68 (ddd, *J* = 8, 4, and 3 Hz, 1H), 3.75 (dd, *J* = 13 and 4 Hz, 1H), 4.21 (dd, *J* = 8 and 4 Hz, 1H), 4.37 (ddd, *J* = 4, 4, and 4 Hz, 1H), 4.48 (d, *J* = 13 Hz, 1H), 4.62 (d, *J* = 13 Hz, 1H), 7.50–7.57 (m, 5H). ^{13}C-NMR (D_2O) δ 57.6, 57.7, 62.0, 69.3, 70.7, 71.5, 130.0, 131.0, 131.5 (2C overlapped). Anal. Calcd for $C_{12}H_{18}NO_3Cl$: C, 55.49; H, 6.99; N, 5.39. Found: C, 55.59; H, 7.22; N, 5.33.

*3.9. 1,4-Dideoxy-1,4-imino-D-ribitol hydrochloride (DRB, **1**) [14].* A mixture of compound **9** (250 mg) and 10% Pd/C (63 mg) in H_2O (20 mL) was stirred at room temperature overnight under an atmospheric pressure of hydrogen. After removal of the catalyst with the use of Hyflo Super-Cel, the mixture was washed with $CHCl_3$ and concentrated to give a quantitative yield of the compound **1** (162 mg) as a brown solid. An analytical sample was obtained by recrystallization from a mixture of EtOH and acetone. Colorless powder, mp 124–126°C (lit [14], mp 128–132°C). $[\alpha]_D^{25}$ +57.7 (*c* 0.14, H_2O) (lit [14], $[\alpha]_D^{25}$ +57.6 (*c* 0.59, H_2O)). ^{1}H-NMR (D_2O) δ 3.37 (dd, *J* = 13 and 2 Hz, 1H), 3.49 (dd, *J* = 13 and 4 Hz, 2H), 3.63 (ddd, *J* = 9, 6, and 3 Hz, 1H), 3.83 (dd, *J* = 13 and 6 Hz, 1H), 3.97 (dd, *J* = 13 and 3 Hz, 1H), 4.21 (dd, *J* = 9 and 4 Hz, 1H), 4.38 (ddd, *J* = 4, 4, and 2 Hz, 1H). ^{13}C-NMR (D_2O) δ 50.2, 58.6, 62.4, 70.0, 71.8.

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References

[1] N. Asano, K. Oseki, E. Tomioka, H. Kizu, and K. Matsui, "*N*-containing sugars from *Morus alba* and their glycosidase inhibitory activities," *Carbohydrate Research*, vol. 259, no. 2, pp. 243–255, 1994.

[2] N. Asano, K. Yasuda, H. Kizu et al., "Novel α-L-fucosidase inhibitors from the bark of *Angylocalyx pynaertii* (Leguminosae)," *European Journal of Biochemistry*, vol. 268, no. 1, pp. 35–41, 2001.

[3] K. Yasuda, H. Kizu, T. Yamashita et al., "New sugar-mimic alkaloids from the pods of *Angylocalyx pynaertii*," *Journal of Natural Products*, vol. 65, no. 2, pp. 198–202, 2002.

[4] Y. Mizushina, X. Xu, N. Asano et al., "The inhibitory action of pyrrolidine alkaloid, 1,4-dideoxy-1,4-imino-D-ribitol, on eukaryotic DNA polymerases," *Biochemical and Biophysical Research Communications*, vol. 304, no. 1, pp. 78–85, 2003.

[5] V. L. A. Malladi, A. J. Sobczak, T. M. Meyer, D. Pei, and S. F. Wnuk, "Inhibition of LuxS by *S*-ribosylhomocysteine analogues containing a [4-aza]ribose ring," *Bioorganic & Medicinal Chemistry*, vol. 19, no. 18, pp. 5507–5519, 2011.

[6] A. Goeminne, M. Berg, M. McNaughton et al., "*N*-arylmethyl substituted iminoribitol derivatives as inhibitors of a purine specific nucleoside hydrolase," *Bioorganic & Medicinal Chemistry*, vol. 16, no. 14, pp. 6752–6763, 2008.

[7] A. Goeminne, M. McNaughton, G. Bal et al., "Synthesis and biochemical evaluation of guanidino-alkyl-ribitol derivatives as nucleoside hydrolase inhibitors," *European Journal of Medicinal Chemistry*, vol. 43, no. 2, pp. 315–326, 2008.

[8] B. A. Horenstein, R. F. Zabinski, and V. L. Schramm, "A new class of C-nucleoside analogues. 1-(*S*)-aryl-1,4-dideoxy-1,4-imino-D-ribitols, transition state analogue inhibitors of nucleoside hydrolase," *Tetrahedron Letters*, vol. 34, no. 45, pp. 7213–7216, 1993.

[9] C. Murruzzu and A. Riera, "Enantioselective synthesis of hydroxylated pyrrolidines via Sharpless epoxidation and olefin metathesis," *Tetrahedron Asymmetry*, vol. 18, no. 1, pp. 149–154, 2007.

[10] F. A. Davis, T. Ramachandar, J. Chai, and E. Skucas, "Asymmetric synthesis of α-amino aldehydes from sulfinimine (*N*-sulfinyl imine)-derived α-amino 1,3-dithianes. Formal synthesis of (−)-2,3-*trans*-3,4-*cis*-dihydroxyproline," *Tetrahedron Letters*, vol. 47, no. 16, pp. 2743–2746, 2006.

[11] T. S. Cooper, A. S. Larigo, P. Laurent, C. J. Moody, and A. K. Takle, "*O*-(1-phenylbutyl)benzyloxyacetaldoxime, a versatile reagent for the asymmetric synthesis of protected 1,2-aminoalcohols and 2-hydroxymethyl nitrogen heterocycles," *Synlett*, no. 10, pp. 1730–1732, 2002.

[12] R. Kumareswaran and A. Hassner, "Asymmetric synthesis of 1,4-dideoxy-1,4-imino-D-ribitol via stereoselective addition of allylphenylsulfone to an aryl *N*-sulfinylimine," *Tetrahedron Asymmetry*, vol. 12, no. 24, pp. 3409–3415, 2002.

[13] D. M. Goli, B. V. Cheesman, M. E. Hassan, R. Lodaya, and J. T. Slama, "Synthesis of (2*R*,3*R*,4*S*)-2-hydroxymethylpyrrolidine-3,4-diol from (2*S*)-3,4-dehydroproline derivatives," *Carbohydrate Research*, vol. 259, no. 2, pp. 219–241, 1994.

[14] G. W. J. Fleet and J. C. Son, "Polyhydroxylated pyrrolidines from sugar lactomes: synthesis of 1,4-dideoxy-1,4-imino-d-glucitol from D-galactonolactone and syntheses of 1,4-dideoxy-1,4-imino-D-allitol, 1,4-dideoxy-1,4-imino-D-ribitol, and (2*S*,3*R*,4*S*)-3,4-dihydroxyproline from d-gulonolactone," *Tetrahedron*, vol. 44, no. 9, pp. 2637–2647, 1988.

[15] G. W. J. Fleet, J. C. Son, D. S. C. Green, I. C. di Bello, and B. Winchester, "Synthesis from D-mannose of 1,4-dideoxy-1,4-imino-L-ribitol and of the α-mannosidase inhibitor 1,4-dideoxy-1,4-imino-D-talitol," *Tetrahedron*, vol. 44, no. 9, pp. 2649–2655, 1988.

[16] H. Setoi, H. Kayakiri, H. Takeno, and M. Hashimoto, "Synthesis of some polyhydroxylated pyrrolidine derivatives," *Chemical and Pharmaceutical Bulletin*, vol. 35, no. 10, pp. 3995–3999, 1987.

[17] T. B. Mercer, S. F. Jenkinson, B. Bartholomew et al., "Looking glass inhibitors: both enantiomeric *N*-benzyl derivatives of 1,4-dideoxy-1,4-imino-D-lyxitol [a potent competitive inhibitor of α-D-galactosidase] and of 1,4-dideoxy-1,4-imino-L-lyxitol [a weak competitive inhibitor of α-D-galactosidase] inhibit naringinase, an α-L-rhamnosidase competitively," *Tetrahedron Asymmetry*, vol. 20, no. 20, pp. 2368–2373, 2009.

[18] H. Batra, R. M. Moriarty, R. Penmasta et al., "A concise, efficient and production-scale synthesis of a protected L-lyxonolactone derivative: an important aldonolactone core," *Organic Process Research & Development*, vol. 10, no. 3, pp. 484–486, 2006.

[19] J. D. Williams, V. P. Kamath, P. E. Morris, and L. B. Townsend, "D-ribonolactone and 2,3-isopropylidene-(D-ribonolactone)," *Organic Syntheses*, vol. 82, pp. 75–79, 2005.

[20] H. Suh and C. S. Wilcox, "Chemistry of F_1F_0-ATPase inhibitors. Stereoselective total syntheses of (+)-citreoviral and (−)-citreoviridin," *Journal of the American Chemical Society*, vol. 110, no. 2, pp. 470–481, 1988.

[21] H. Kold, I. Lundt, and C. Pedersen, "Synthesis of L-ribono- and L-lyxino-lactone," *Acta Chemica Scandinavica*, vol. 48, no. 8, pp. 675–678, 1994.

[22] J. E. Baldwin, "Rules for ring closure," *Journal of the Chemical Society, Chemical Communications*, no. 18, pp. 734–736, 1976.

[23] E. C. Bigham, C. E. Gragg, W. R. Hall et al., "Inhibition of arabinose 5-phosphate isomerase. An approach to the inhibition of bacterial lipopolysaccharide biosynthesis," *Journal of Medicinal Chemistry*, vol. 27, no. 6, pp. 717–726, 1984.

[24] K. Jayakanthan, B. D. Johnston, and B. M. Pinto, "Stereoselective synthesis of 4′-selenonucleosides using the Pummerer glycosylation reaction," *Carbohydrate Research*, vol. 343, no. 10-11, pp. 1790–1800, 2008.

[25] K. Clinch, G. B. Evans, G. W. J. Fleet et al., "Syntheses and bio-activities of the L-enantiomers of two potent transition state analogue inhibitors of purine nucleoside phosphorylases," *Organic & Biomolecular Chemistry*, vol. 4, no. 6, pp. 1131–1139, 2006.