http://www.e-journals.net

ISSN: 0973-4945; CODEN ECJHAO
E-Journal of Chemistry
Vol. 5, No.4, pp. 963-968, October 2008

Synthesis and Antimicrobial Activities of 1,2,4-Triazole and 1,3,4-Thiadiazole Derivatives of 5-Amino-2-Hydroxybenzoic Acid

SABIR HUSSAIN[§*], JYOTI SHARMA[#] and MOHD.AMIR[§]

[§]Department of Chemistry, R. R. College, Alwar, Rajasthan.
[#]Department of Pharmaceutical Chemistry,
Jamia Hamdard, New Delhi, India.

03shussain@gmail.com

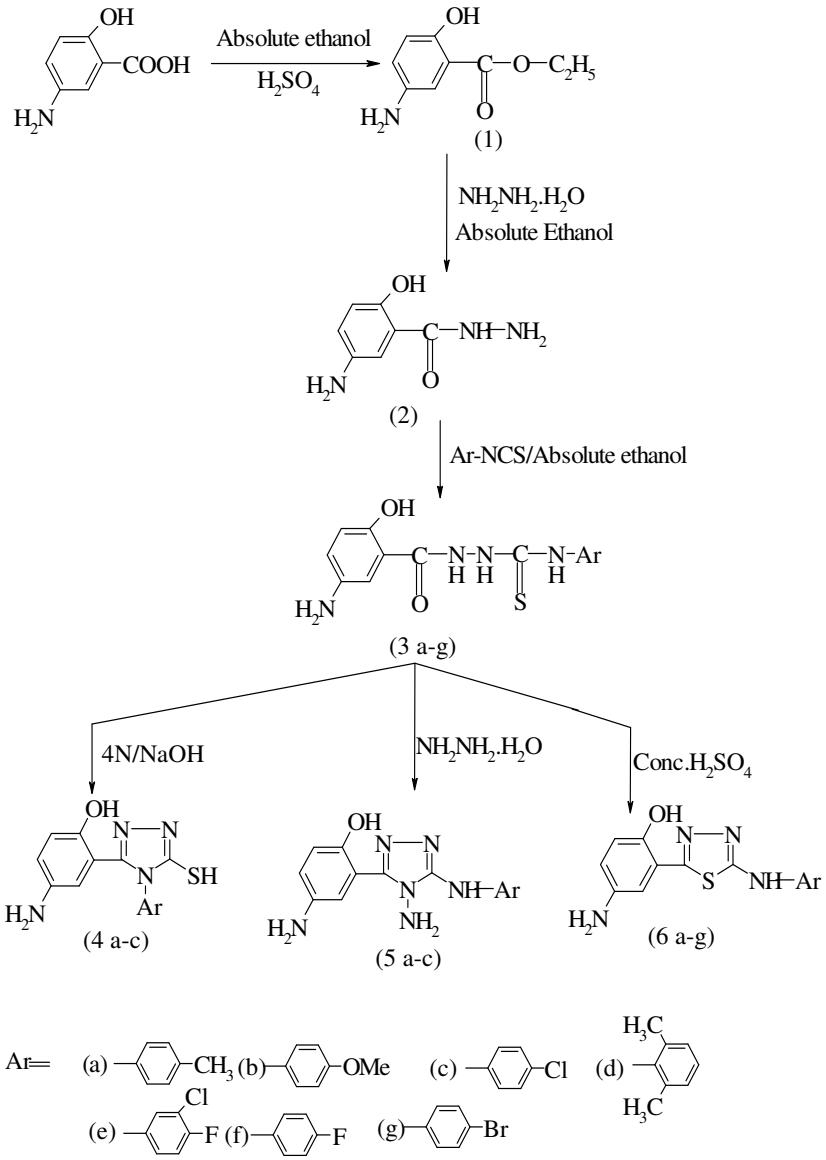
Received 8 May 2008; Revised 19 June 2008; Accepted 23 June 2008

Abstract: Various 4-amino-2-[4-(4-substituted phenyl)-5-sulfanyl-4H-1,2,4-triazol-3-yl] phenol (4a-c), 4-amino-2-{4-amino-5-[(4-substituted phenyl)amino]-4H-1,2,4-triazol-3-yl} phenol (5a-c) and 4-amino-2-{5-[(4-substituted phenyl)amino]-1,3,4-thiadiazole-2-yl} phenol (6a-g) were synthesized and evaluated for their antibacterial and antifungal activity. The compounds showed significant antibacterial activity against *S. aureus* (gram-positive) and *E.coli* (gram-negative) bacteria and antifungal activity against *A. niger* fungi using cup plate technique

Keywords: 1,2,4-Triazole, 1,3,4-Thiadiazole, Antibacterial, Antifungal.

Introduction

Several five membered aromatic systems having three hetero atoms at symmetrical position have been studied because of their interesting physiological properties[1,2]. It is also well established that various derivatives of 1,2,4-triazole, 1,3,4-thiadiazole exhibit broad spectrum of pharmacological properties such as antibacterial and antifungal activities[3,4]. The available therapeutically important medicines are terconazole, itraconazole, fluconazole, cefazoline and ribavirin *etc*. are some of the examples which contain one of these heterocyclic nucleus. In view of the above mentioned facts and in continuation of our work on the synthesis of biologically important heterocyclic compounds[5-7], we describe herein the synthesis of some triazole, thiadiazole derivatives and evaluation of their antimicrobial activities. The reaction sequence leading to the formation of desired heterocyclic compounds are outlined in Scheme 1. The structures of the compounds were assigned on the basis of IR, ^{1}H NMR spectral data.

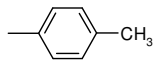
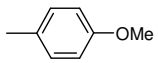
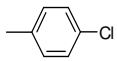
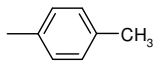
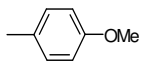
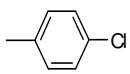
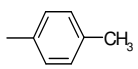
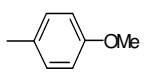
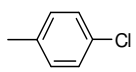
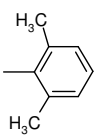
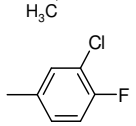
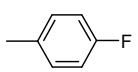
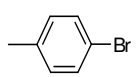


Scheme 1

Experimental

Melting points were determined in open capillary tubes. IR spectra were recorded on a Perkin-Elmer 157 spectrometer and ^{1}HNMR spectra on a Bucker WM-400 (400 MHZ FT NMR) spectrophotometer using TMS (Tetramethyl Silane) as internal reference (chemical shift in δ ppm). Purity of the compounds was checked by TLC (Thin Layer Chromatography) on silica gel plates and spot were visualized by exposure to iodine vapours. The physical data of the compounds prepared are presented in Table 1.

Table 1. Characterization data of the compounds

Compound	Ar	M.P, °C	Yield %	Mol. Formula	N, % Found	Calcd.
4a		244	63	$C_{15}H_{14}N_4OS$	18.80	18.78
4b		247	67	$C_{15}H_{14}N_4O_2S$	17.95	17.82
4c		253	70	$C_{14}H_{11}ClN_4OS$	17.75	17.58
5a		240	57	$C_{15}H_{16}N_6O$	28.45	28.36
5b		242	61	$C_{15}H_{16}N_6O_2$	26.88	26.91
5c		238	65	$C_{14}H_{13}ClN_6O$	26.65	26.53
6a		282	68	$C_{15}H_{14}N_4OS$	18.91	18.78
6b		287	61	$C_{15}H_{14}N_4O_2S$	17.88	17.82
6c		278	58	$C_{14}H_{11}ClN_4OS$	17.80	17.58
6d		281	65	$C_{16}H_{16}N_4OS$	18.10	17.93
6e		268	67	$C_{14}H_{10}ClFN_4OS$	16.90	16.64
6f		265	62	$C_{14}H_{11}FN_4OS$	18.83	18.53
6g		264	59	$C_{14}H_{11}BrN_4OS$	15.42	15.42

*Synthesis of ethyl 5-amino-2-hydroxybenzoate (**1**)*

To a 100 mL RB flask, a mixture of 5-amino sylicilic acid (0.001 mol) and absolute alcohol (50 mL) were taken. Few drop of conc. H_2SO_4 along with a small porcelain chip were added. A condenser was attached to the RB flask fitted with a calcium chloride guard tube to maintain anhydrous condition. The reaction mixture was refluxed for 40 h on water bath, concentrated under reduced pressure to give the ester. ^{1}HNMR ($CDCl_3$): δ 1.41 (t, 3H, CH_3), δ 4.38 (q, 2H, OCH_2), δ 7.48-7.64 (m, 3H, Ar-H).

*Synthesis of 5-amino-2-hydroxybenzohydrazide (**2**)*

To a RB flask, compound **1** (0.01 mol), hydrazine hydrate (0.2 mol) and absolute alcohol (50 mL) were taken. A condenser with calcium guard tube was attached to the flask and mixture was refluxed for 60h on water bath. The mixture was concentrated, cooled and poured in to crushed ice. It was kept for 3-4 h at room temperature and solid mass separated out was filtered and dried. ^{1}HNMR ($CDCl_3$): δ 7.50-7.78 (m, 3H, Ar-H), δ 7.87-7.97 (m, 3H, $CONHNH_2$)

*Synthesis of 2-[(5-amino-2-hydroxyphenyl) carbonyl]-N- (4-methyl) hydrazine carbothioamide (**3a**)*

A mixture of compound 2 (0.001 mol) and 4-methylphenyl isothiocyanate (0.001 mol) in ethanol (25.0 mL) was refluxed on a water bath for 2 h. The solvent was concentrated and the precipitated product was filtered, dried and recrystalized from methanol. **3a** IR (KBr): 3390 (N-H), 1620 (CONH), 600 (ArH), 1040 (C=S). **3a** ^{1}HNMR ($CDCl_3$): δ 7.12-7.60 (m, 3H, Ar-H), δ 7.72-7.83 (m, 3H, CONHNHCSNH).Other compounds **3b-g** were prepared similarly and their characterization data are recorded in Table 1

*Synthesis of 4-amino-2-[4-(4-methylphenyl)-5-sulfanyl-4H-1,2,4-triazol-3-yl]phenol (**4a**)*

Compound **3a** (0.002 mole) was added to ethanol (20 mL). To this NaOH (4 N, 2 mL) was added which resulted in clear solution. It was refluxed for 1h and treated with decolorizing charcoal and filtered. The filtrate was cooled and pH was adjusted to 4-6 with dilute glacial acetic acid. The mixture was kept aside for 1h and the crystals produced were filtered, dried and recrystallised from methanol. Other compounds **4b,c** were prepared similarly and their characterization data are recorded in Table 1 **4a** IR (KBr): 3375 (N-H), 1591 (C=N), 1208 (C=S) 2935 (C-H). **4a** ^{1}HNMR ($CDCl_3$): δ 1.82 (s, 3H, CH_3), δ 6.28-7.47 (complex m, 7 Ar-H), 8.03 (s, 1H, SH). **4b** IR (KBr): 3370 (N-H), 1577 (C=N), 1216 (C=S) 2935 (C-H). **4b** ^{1}HNMR ($CDCl_3$): δ 3.48 (s, 3H, OCH_3), δ 6.32-7.52 (complex m, 7 Ar-H), 8.08 (s, 1H, SH).

*4-Amino-2-{4-amino-5-[(4-methylphenyl)amino]-4H-1,2,4-triazole-3-yl}phenol (**5a**)*

Compound **3a** (0.025 mole) and hydrazine hydrate (0.025 mole) was refluxed in methanol for 2h at a temperature between 50-60°C, reaction mixture was cooled and poured over crushed ice. Solid was filtered and recrystallised from methanol. Other compounds **5b,c** were prepared similarly and their characterization data are recorded in Table 1. 5a IR (KBr): 3298 (N-H), 1621 (C=N), 2923 (C-H).

5a ^{1}HNMR ($CDCl_3$): δ 2.23 (s, 3H, CH_3), 8.21 (bs, 1H, NH), δ 7.05-7.87 (complex m, 7 Ar-H). 5b IR (KBr): 3294 (N-H), 1628 (C=N), 2928 (C-H). **5b** ^{1}HNMR ($CDCl_3$): δ 2.34 (s, 3H, OCH_3), 8.26 (bs, 1H, NH), δ 7.05-7.68 (complex m, 7 Ar-H).

*Synthesis of 4-amino-2-{5-[(4-methylphenyl) amino]-1,3,4-thiadiazole-2-yl} phenol (**6a**)*

Compound **3a** (0.002 mole) was added portion wise in 5.0 mL conc. H_2SO_4 and stirred with cooling for 2h. The mixture was poured over crushed ice and the precipitated solid was filtered, washed with water, dried and recrystallised from methanol. Other compounds 6b-g were prepared similarly and their characterization data are recorded in Table 1 **6a** IR (KBr): 3412 (N-H), 1615 (C=N), 2920 (C-H). **6a** ^{1}HNMR ($CDCl_3$): δ 3.84 (s, 3H, CH_3), δ 6.58-7.53 (complex m, 7 Ar-H and 1NH). 6b IR (KBr): 3436 (N-H), 1624 (C=N), 2938 (C-H). **6b** ^{1}HNMR ($CDCl_3$): δ 3.86 (s, 3H, OCH_3), δ 6.59-7.55 (complex m, 7 Ar-H and 1NH).

Result and Discussion

Spectral characterization of the compounds

The IR spectrum of the compounds (**4a-c**) showed peaks at 3375-3361 cm^{-1}, N─H stretching; 2935-2932 cm^{-1}, CH stretching; 1591-1577 cm^{-1}, C=N stretching and 1216-1208 cm^{-1}, C=S stretching. The NMR spectrum of the compound **4a** showed a singlet at δ 1.82 indicating the presence of CH_3 protons. In the aromatic region complex multiplet at δ 6.28-7.47 was observed indicating the presence of seven aromatic protons. Furthermore a singlet at δ 8.03 was observed for one SH protons. The NMR spectrum of the compound **4b** showed a singlet at δ 3.48 indicating the presence of OCH_3 protons. In the aromatic region complex multiplet at δ 6.32-7.52 was observed indicating the presence of seven aromatic protons. A singlet at δ 8.08 was observed for one SH protons. The IR spectrum of the compounds (**5a-c**) showed peaks at 3298-3249 cm^{-1}, N─H stretching; 2934-2923 cm^{-1}, CH stretching; 1628-1621 cm^{-1}, C=N stretching. The NMR spectrum of the compound **5a** showed a singlet at δ 2.23 indicating the presence of CH_3 protons. In the aromatic region complex multiplet at δ 7.05-7.87 was observed indicating the presence of seven aromatic protons. A broad singlet at δ 8.21 was observed for one NH protons. The NMR spectrum of the compound **5b** showed a singlet at δ 2.34 indicating the presence of OCH_3 protons. In the aromatic region complex multiplet at δ 7.05-7.68 was observed indicating the presence of seven aromatic protons. Furthermore a broad singlet at δ 8.26 was observed for one NH protons. The IR spectrum of the compounds (**6a-g**) showed peaks at 3436-3410 cm^{-1}, N─H stretching; 2938-2920 cm^{-1}, CH stretching; 1624-1609 cm^{-1}, C=N stretching. The NMR spectrum of the compound **6a** showed a singlet at δ 3.84 indicating the presence of CH_3 protons. In the aromatic region complex multiplet at δ 6.58-7.53 was observed indicating the presence of seven aromatic protons and one NH protons. The NMR spectrum of the compound **6b** showed a singlet at δ 3.86 indicating the presence of OCH_3 protons. In the aromatic region complex multiplet at δ 6.59-7.55 was observed indicating the presence of seven aromatic protons and one NH protons.

Antimicrobial activity

The synthesized compounds were evaluated for their antimicrobial activity against bacterial strain *Staphylococcus aureus* (*S. aureus*) (gram-posative), *Escherchia coli* (*E. coli*) (gram-negative) and fungal strain *A niger* by cup plate method[8] at 200, 100, 50 and 25 µg/mL concentration. Ofloxacin and ketoconazole were used as standard drugs for antibacterial and antifungal activity respectively. The minimal inhibitory concentration (MICs, μgmL^{-1}) of the tested compounds are recorded in Table 2

The 1,2,4-triazole derivative **4c** having chloro group at *para* position of phenyl ring exhibited a MIC of 25 µg/mL against *A. niger*, whereas **4a** exhibited significant antimicrobial activity (MIC 50 µg/mL) against *S. aureus*, *E. coli*. The compound **5a** exhibited promising antibacterial activity (MIC 25 µg/mL) against *A.niger* strains, whereas **5c** exhibited significant antimicrobial activity (MIC 50 µg/mL) against *S.aureus*, *E. coli* and *A. niger***.** The 5-amino-2-hydroxybenzohydrazide derivative 6e having 3-chloro-4-fluorophenyl amino group at 2nd position of thiadiazole ring was found to have MIC 25 µg/mL against *S. aureus*, *E.coli* and *A. niger* 1,3,4-thiadiazole derivatives **6c, 6f** also exhibited promising antibacterial activity (MIC 25 µg/mL) against *S. aureus* and *A. niger*.

Table 2 . Antimicrobial activities of the compounds.

Compounds	MIC, µg/mL		
	S. aureus	*E. coli*	*A. niger*
Ofloxacin	10	12.5	--
Ketoconazole	--	--	12.5
4a	50	50	50
4b	100	100	100
4c	100	50	25
5a	50	100	25
5b	100	100	50
5c	50	50	50
6a	50	100	50
6b	100	100	50
6c	25	50	25
6d	100	100	100
6e	25	25	25
6f	25	50	25
6g	100	100	50

-- Not tested

Conclusion

A total of 13 compounds (06 1,2,4-triazoles and 07 1,3,4-thiadiazoles) were synthesized and screened for their antibacterial activity against *S. aureus* (gram positive) and *E. coli* (gram negative) bacteria and antifungal activity against *A. niger*. The minimal inhibitory concentrations (MIC) of all the compounds were determined by observing the zones of inhibition formed around the cup after 24h of incubation for antibacterial and 48h for antifungal activities. Compounds were found to have moderate antimicrobial activity.

Acknowledgement

The authors are thankful to the Head, Department of chemistry, for providing laboratory facilities and to the Head, RSIC-CDRI, Lucknow, for spectral analysis.

References

1. Hetzhein A and Mockel K, *Adv Heterocycle Chem*., 1996, **7**, 183.
2. Sandstrom J, *Adv. Heterocycle Chem*., 1968, 9, 165.
3. Hosam Saad, *Indian J Chem*., 1996, 35B, 980.
4. Hui XP, Chu C H, Zang ZY, Wang Q and Zhang Q, *Indian J Chem.*, 2002, **41B**, 2176.
5. Amir M, Dhar N & Tiwari S K, *Indian J Chem.*, 1997, **36B**, 96.
6. Amir M and Shahani S, *Indian J Chem.*, 1998, **37B**, 502.
7. Amir M, Oberoi A and Alam S, *Indian J Chem.*, 1999, **38B**, 237.
8. Barry AL, The antimicrobial susceptibility test: principle and practices, Edited by Illuslea and febiger, (Philadelphia, USA) 1976, 180; *Biol Abstr*, 1977, **64**, 25183.