Synthesis, Characterization and Antimicrobial Properties of Schiff Bases Derived from Condensation of 8-Formyl-7-hydroxy-4-methylcoumarin and Substituted Triazole Derivatives

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Abstract: Two biologically active classes of compounds coumarins and triazoles were employed to form the Schiff bases. The synthesized Schiff base viz., 3-aryl-[(1-isocyano-4-methyl-7-hydroxycoumarin)]-5-methyl-1,3,4-triazoline-2-one and its substituents were obtained by the condensation of amino group of mono and disubstituted derivatives of 3-methyl-5-oxo-1,2, 4-triazoles with 8-formyl-7-hydroxy-4-methylcoumarin in alcohol. The characterization were studied by elemental analysis, IR, ¹H NMR and mass spectra. The biological activities of the compounds were assayed against two bacterial via, *B. subtilis*, *E.coli* and two fungal strains *A. niger*, *C.albican*. The results indicate that the present compounds may serve as better fungicides when compared to bactericides. The synthesized compounds have turned to be wonder compounds possessing antimicrobial properties.

Keywords: Coumarin, Sydnone, Schiff base, Antimicrobial.

Introduction

The structural features of *meso*-ionic compounds have been of interest to medicinal chemist. A wide spectrum of biological activity has been claimed for a variety of mesoionic compounds¹. Their potential value as biological active substances can be explained by their dipolar structure. These rings systems are composed of delocalized electrons, which have been

appreciably perturbed. As a result of perturbation of electrons in mesoionic system there is an oppositely charged dipolar segment at the extreme of a four atom chain. The presence of oppositely charged dipolar segment in mesoionic systems is of tremendous value to the medicinal chemist². Its significance perhaps lies in its ability to electrostatically interact with two complementary partially charged positions on receptor macromolecule, such as a protein helix. Another structural feature of the mesoionic system is their highly charged, yet net neutral electrical character. Therefore, they are soluble to a much greater extent in non polar or lipoid solvents³. Thus *in vivo*, the mesoionics can cross lipid barrier even though they are internally appreciably ionic. On the contrary some quaternary salts and molecules rich in polar groups such as carbohydrates cannot pass this lipid barrier. The comparatively small size of mesoionic ring, their planar aromatic character and variation of electron density around the rings eliminates conformational problems and permitting the relatively close approach of all ring atoms to a receptor surface. Mesoionic compounds have been screened for various biological activities. In continuation of our work on synthetic biologically active sydnones⁴⁻⁵ and oxadiazolines⁶. Novel 4-amino-2-aryl-5-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-ones, by one pot ring conversions of 3-aryl-5-methyl-1,3,4-oxadiazoline-2-ones with hydrazine hydrate which were difficult to synthesize and were inaccessible by other methods have reported recently from our laboratory⁷. The prime synthetic evidence, spectral and antimicrobial studies reported from our laboratory have triggered the new routes in triazole synthetic chemistry. Schiff bases play an important role in many biochemical reactions, because of the imine linkage. Imines are to possess antibacterial and more antifungal properties.

In continuation of work, in the present investigation we focused our attention on the synthesis of 3-aryl-[(1-isocyano-4-methyl-7-hydroxy coumarin)]-5-methyl-1,3,4-triazoline-2-one from 1,2,4-triazoles-3-ones and 8-formyl-7-hydroxy-4-methyl-coumarin Schiff base (Scheme 3). The aim of this work came from the observation that triazoles contain a potential free primary amino group, which could be used for variety of synthetic studies. It was interesting to study the influencing biological behaviors with various substituted triazoles. Therefore, we felt it of interest to study the chemical reactivity of these heterocyclic coumarin moieties.

Experimental

The melting points were determined by open capillaries on a Buchi-apparatus and were uncorrected. The IR spectra were recorded on a Nicolet-Impact-410 FT-IR spectrometer, using KBr pellets. ¹HNMR and ¹³CNMR spectra were recorded on a Bruker AC-300F, 300MHz, spectrometer in CDCl₃ and DMSO-d₆ using TMS as an internal standard and the values are expressed in δ ppm. The mass spectra were recorded using EI-MS (at CRDI, Lucknow). The elemental analysis was carried out using Heraus CHN rapid analyzer. All the new compounds have given CHN analysis within $\pm 0.4\%$ of the theoretical values. The homogeneity of the compounds was described by Thin Layer Chromatography on aluminum silica gel 60F₂₅₄ (merck) detected by UV light (254 nm) and iodine vapors. All the chemicals purchased were of analytical reagent grade, and were used without further purification unless otherwise stated.

*Synthesis of 3-aryl sydnones, oxadiazolines and triazoles derivatives
(With slight modification in our earlier methods⁶)*

The scheme is represented in Figure 1.

Figure 1. Systematic scheme for preparation of 3-aryl sydnone, oxadiazolines and triazoles.

Synthesis of 7-hydroxy-4-methyl coumarin

A mixture of resorcinol (0.1 mol) and ethylacetoacetate (0.1 mol) in 50 mL sulphuric acid solution (85%) was heated on a water bath for 3 h. The resulting reddish brown colored solution was decomposed with 500 g of crushed ice. The separated bright yellow colored solid was washed with excess of cold water, dried and crystallized from methanol to get the pure product.

Dark brown crystalline solid (methanol); Yield: 80%; m.p. 180-182 °C; IR (KBr, cm^{-1}): 3423 (ν_{OH}), 1733 ($\nu_{\text{C=O}}$), 1555 ($\nu_{\text{C=C}}$); ^1H NMR (300MHz, CDCl_3 + TFA, δ ppm): 2.49 (s, 3H, C4- CH_3), 6.31 (s, 1H, C3-H), 6.92-6.95 (d, 1H, C6-H, $J=9$ Hz), 6.94 (s, 1H, C8-H), 7.57-7.60 (d, 1H, C5-H, $J=9$ Hz);

Synthesis of 8-formyl-7-hydroxy-4-methyl coumarin (I)

A mixture of 7-hydroxy-4-methyl-coumarin (5 g) and Hexamethylene tetramine (10 g) in glacial acetic acid (40 mL) was heated on a water bath for 6 h. The hexamine adduct so formed was hydrolyzed with 20% HCl (75 mL) and the mixture was heated for another 30 min. After cooling, the reaction mixture was extracted with diethyl ether, the ether layer was evaporated and the pale yellow colored solution was poured to crushed ice to get the pale yellow solid of 8-formyl-7-hydroxy-4-methyl coumarin which was crystallized from ethanol and dioxan mixture. The Scheme of the work is represented in Figure 2

Figure 2. Systematic scheme for preparation of coumarin derivative.

Pale yellow crystalline solid (Ethanol + dioxan); Yield: 22%; m.p. 176-178 °C; IR (KBr, cm^{-1}): 3446 (ν_{OH}), 1742 ($\nu_{\text{C=O}}$, lactone), 1644 ($\nu_{\text{C=O}}$, aldehyde), 1594 ($\nu_{\text{C=C}}$); ^1H NMR (300MHz, CDCl_3 , δ ppm): 2.44 (s, 3H, C4- CH_3), 6.22 (s, 1H, C3-H), 6.90-6.93 (d, 1H, C6-H, $J=9$ Hz), 7.73-7.76 (d, 1H, C5-H, $J=9$ Hz), 10.63 (s, 1H, HCO), 12.28 (s, 1H, OH);

Synthesis of 3-aryl-[(1-isocyano-4-methyl-7-hydroxy coumarin)]-5-methyl-1,3,4-triazoline-2-one from 1,2,4-triazoles-3-ones (General procedure)

Equimolar concentrations of substituted 4-amino-2-aryl-5-methyl-2,4-dihydro-3H-1,2,4-triazoles-3-ones (0.01 mol) and 8-formyl-7-hydroxy-4-methyl coumarin (0.01 mol) were stirred for 4 h at room temperature using alcohol as a solvent by adding a few drops of acetic acid. The solid obtained was filtered, washed with water and crystallized from ethanol. The scheme of the work is represented in Figure 3.

Figure 3. Systematic scheme for preparation of Schiff bases.

Results and Discussion

Physicochemical methods

The molecular formula of the synthesized compounds, their structural substitutions, yield in percentage, solvent used for recrystallization, melting point and the C, H and N analysis with the calculated and found values are represented in Table 1.

Table 1. Elemental analysis of the compounds.

Compd	R	R'	M.P °C	Yield, %	Mol. formula	Elemental analysis		
						Found (Calc), %	C	H N
I	H	H	178-79	71	$\text{C}_{11}\text{H}_8\text{O}_4$	64.70(64.67)	3.92(3.95)	31.37(31.35)
II	<i>m</i> -Cl	H	268-69	65	$\text{C}_{20}\text{H}_{15}\text{N}_4\text{O}_4\text{Cl}$	58.47(58.30)	3.65(3.68)	13.6(13.4)
III	<i>P</i> -Cl	H	265-66	67	$\text{C}_{20}\text{H}_{15}\text{N}_4\text{O}_4\text{Cl}$	58.47(59.02)	3.65(3.04)	13.6(13.4)
IV	<i>P</i> -OCH ₃	H	270-71	66	$\text{C}_{21}\text{H}_{18}\text{N}_4\text{O}_5$	62.06 (62.96)	4.43(4.23)	13.8(13.7)
V	<i>P</i> -CH ₃	H	256-57	69	$\text{C}_{21}\text{H}_{18}\text{N}_4\text{O}_4$	64.61(64.02)	4.61(4.20)	14.3(14.1)
VI	<i>P</i> -COOH	H	276-77	71	$\text{C}_{21}\text{H}_{16}\text{N}_4\text{O}_6$	60.00(60.23)	3.80(3.28)	13.3(13.5)
VII	3-Cl	4-CH ₃	282-83	82	$\text{C}_{21}\text{H}_{17}\text{N}_4\text{O}_4\text{Cl}$	59.37(58.84)	4.00(3.82)	13.2(13.1)
VIII	3-NO ₂	4-Cl	275-76	68	$\text{C}_{21}\text{H}_{14}\text{N}_4\text{O}_6\text{Cl}$	52.69(51.92)	3.07(3.24)	12.3(12.2)

Infrared spectra

The IR spectra of the compounds were recorded in 4000-400 cm^{-1} range⁸. The compounds show⁹ the characteristic band of $\nu_{(\text{O-H})}$ in the range 3410-3440 cm^{-1} . The compounds exhibited bands at casually 1745 cm^{-1} and 1640 cm^{-1} for coumarin lactone carbonyl and aldehyde respectively¹⁰ and 1727 cm^{-1} for triazolinone. The band at 1580-1600 cm^{-1} are assigned to $\nu_{(\text{C=C})}$ stretching¹¹. The azomethine group being exhibited in the range 1617-1625 cm^{-1} .

¹H NMR spectra

The ¹H NMR spectrum of the ligand was recorded in chloroform using TMS as an internal reference. The data is given in Table 2.

Table 2. Spectral data of the compounds.

Compd	Structure	Spectral data NMR Characterization
I		IR (KBr, cm^{-1}): 3446 (ν_{OH}), 1742 ($\nu_{\text{C=O}}$, lactone), 1644 ($\nu_{\text{C=O}}$, aldehyde), 1594 ($\nu_{\text{C=C}}$); ¹ H NMR (300MHz, CDCl_3 , δ ppm): 2.44 (s, 3H, C4-CH ₃), 6.22 (s, 1H, C3-H), 6.90-6.93 (d, 1H, C6-H, $J=9$ Hz), 7.73-7.76 (d, 1H, C5-H, $J=9$ Hz), 10.63 (s, 1H, HCO), 12.28 (s, 1H, OH);
II		IR (KBR) cm^{-1} : 3410 (ν_{OH}), 1745 cm^{-1} (Coumarin lactone carbonyl), 1727 cm^{-1} ($\nu_{\text{C=O}}$ of triazolinone), 1585 cm^{-1} ($\nu_{\text{C=C}}$), 1617 cm^{-1} ($\nu_{\text{C=N}}$). ¹ H NMR (300 MHz, CDCl_3): d (ppm) 2.5 (t, 1H, CH), 2.6 (d, 2H, CH ₂), 6.8e7.6 (m, 7H, ArH), δ 2.37 (3H, s, CH ₃), Coumarin (C4 7.80 δ ppm $J=9.8$ Hz.), δ 10.0 (s br 1H) OH Proton (D_2O exchanged).
III		IR (KBR) cm^{-1} : 3430 (ν_{OH}), 1735 cm^{-1} (Coumarin lactone carbonyl), 1725 cm^{-1} ($\nu_{\text{C=O}}$ of triazolinone), 1590 cm^{-1} ($\nu_{\text{C=C}}$), 1620 cm^{-1} ($\nu_{\text{C=N}}$). ¹ H NMR (300 MHz, CDCl_3): d (ppm) 2.5 (t, 1H, CH), 2.6 (d, 2H, CH ₂), 6.8e7.6 (m, 7H, ArH), δ 2.37 (3H, s, CH ₃), Coumarin (C3 6.45 δ ppm, C4 7.80 δ ppm $J=9.8$ Hz.), δ 10.0 (s br 1H) OH Proton (D_2O exchanged).
IV		IR (KBR) cm^{-1} : 3410 (ν_{OH}), 1715 cm^{-1} (Coumarin lactone carbonyl), 1727 cm^{-1} ($\nu_{\text{C=O}}$ of triazolinone), 1587 cm^{-1} ($\nu_{\text{C=C}}$), 1618 cm^{-1} ($\nu_{\text{C=N}}$). ¹ H NMR (300 MHz, CDCl_3): d (ppm) 2.5 (t, 1H, CH), 2.6 (d, 2H, CH ₂), 6.8e7.6 (m, 7H, ArH), δ 2.20 (3H, s, COCH ₃), Coumarin (C3 6.45 δ ppm, C4 7.80 δ ppm $J=9.8$ Hz.), δ 10.0 (s br 1H) OH Proton (D_2O exchanged).
V		IR (KBR) cm^{-1} : 3440 (ν_{OH}), 1732 cm^{-1} (Coumarin lactone carbonyl), 1724 cm^{-1} ($\nu_{\text{C=O}}$ of triazolinone), 1600 cm^{-1} ($\nu_{\text{C=C}}$), 1619 cm^{-1} ($\nu_{\text{C=N}}$). ¹ H NMR (300 MHz, CDCl_3): d (ppm) 2.5 (t, 1H, CH), 2.6 (d, 2H, CH ₂), 6.8e7.6 (m, 7H, ArH), δ 2.37 (3H, s, CH ₃), Coumarin (C3 6.45 δ ppm, C4 7.80 δ ppm $J=9.8$ Hz.), δ 10.0 (s br 1H) OH Proton (D_2O exchanged).

VI		IR (KBR) cm^{-1} : 3418 (Coumarin ν_{OH}), 3346 (Arm ν_{OH}), 1745 cm^{-1} (Coumarin lactone carbonyl), 1727 cm^{-1} ; 1724 cm^{-1} ($\nu_{\text{C=O}}$ of Carboxylic), ($\nu_{\text{C=O}}$ of triazolinone), 1585 cm^{-1} ($\nu_{\text{C=C}}$), 1617 cm^{-1} ($\nu_{\text{C=N}}$). ^1H NMR (300 MHz, CDCl_3): d (ppm) 2.5 (t, 1H, CH), 2.6 (d, 2H, CH ₂), 6.8e7.6 (m, 7H, ArH), δ 2.37 (3H, s, CH ₃), Coumarin (C3 6.45 δ ppm, C4 7.80 δ ppm J = 9.8 Hz.), 11.9 (1H, s, COOH), δ 10.0 (s br 1H) OH Proton (D_2O exchanged).
VII		IR (KBR) cm^{-1} : 3430 (ν_{OH}), 1736 cm^{-1} (Coumarin lactone carbonyl), 1724 cm^{-1} ($\nu_{\text{C=O}}$ of triazolinone), 1589 cm^{-1} ($\nu_{\text{C=C}}$), 1623 cm^{-1} ($\nu_{\text{C=N}}$). ^1H NMR (300 MHz, CDCl_3): d (ppm) 2.5 (t, 1H, CH), 2.6 (d, 2H, CH ₂), 6.8e7.6 (m, 7H, ArH), δ 2.37 (3H, s, CH ₃), Coumarin (C3 6.45 δ ppm, C4 7.80 δ ppm J = 9.8 Hz.), 7.86-7.88 (2H, d, j =8.7, Ar-H), δ 10.0 (s br 1H) OH Proton (D_2O exchanged).
VIII		IR (KBR) cm^{-1} : 3417 (ν_{OH}), 1738 cm^{-1} (Coumarin lactone carbonyl), 1724 cm^{-1} ($\nu_{\text{C=O}}$ of triazolinone), 1589 cm^{-1} ($\nu_{\text{C=C}}$), 1625 cm^{-1} ($\nu_{\text{C=N}}$). ^1H NMR (300 MHz, CDCl_3): d (ppm) 2.5 (t, 1H, CH), 2.6 (d, 2H, CH ₂), 6.8e7.6 (m, 7H, ArH), δ 2.37 (3H, s, CH ₃), Coumarin (C3 6.45 δ ppm, C4 7.80 δ ppm J = 9.8 Hz.), δ 10.0 (s br 1H) OH Proton (D_2O exchanged).

Biological studies

All the compounds were screened for their antimicrobial activity by cup plate method at 100 $\mu\text{g/mL}$ concentration in DMF against the *Escherichia coli* and *B. Subtilis* and also against *A niger* and *A. sereus* fungi, using Norfloxacin and Griseofulvin as the reference drugs respectively. All these compounds were less active against the bacterial strains, but some of them showed selective fungal inhibitory activity (Table 3).

Table 3. Anti-microbial activities of compounds.

Compound Code	Antibacterial		Antifungal	
	<i>E.coli</i>	<i>B. subtilis</i>	<i>A. niger</i>	<i>C. Albicans</i>
I	15	16	17	16
II	19	18	19	23
III	17	16	14	18
IV	15	14	22	21
V	16	14	24	22
VI	15	13	18	17
VII	14	14	17	18
VIII	19	18	19	21
Norf	22	22	--	--
Gris	--	--	26	26
DMF	04	04	04	04

Conclusions

The work has approached towards the synthetic and biological approach of these wonder molecules. Anti-bacterial property of the synthesized compounds has exhibited average inhibition, but the systematic substitution at various position and other derived compounds have shown remarkable antifungal properties. The compounds **2**, **3** and **5** have exhibited outstanding activity towards *A.niger* and *C. albicans* the remaining compounds have shown poor antifungal activity indicating less biological importance for a synthetic chemist.

Figure 4.Antibacterial activity of Schiff bases.

Figure 5. Antifungal activity of Schiff bases.

Efforts are under progress in evaluation of these synthesized compounds for *in vivo* studies especially the acute toxicity, analgesic and anti-inflammatory agents and the results will be published in later communications.

This class of compounds has a great scope compared to other organic moieties because of their mesoionic nature, solubility and high sensitiveness towards the biological behaviors.

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