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Antibacterial, antifungal and cytotoxic properties of novel N-substituted sulfonamides from 4-hydroxycoumarin

ZAHID H. CHOHAN[†,1], ALI U. SHAIKH[2], ABDUL RAUF[3], & CLAUDIU T. SUPURAN[4]

[1]*Department of Chemistry, Bahauddin Zakariya University, Multan 60800, Pakistan,* [2]*Department of Chemistry, University of Arkansas at Little Rock, Little Rock, AR, 72204, USA,* [3]*Department of Chemistry, Islamia University, Bahawalpur, Pakistan, and* [4]*Università degli Studi di Firenze, Polo Scientifico, Laboratorio di Chimica Bioinorganica, Rm, 188, Via della Lastruccia 3, 50019 Sesto Fiorentino (Florence), Italy*

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Abstract

A new series of 4-({[2, 4-dioxo-2*H*-chromen-3 (4*H*)-ylidene] methyl} amino) sulfonamides have been obtained by the condensation reaction of 4-hydroxycoumarin with various sulfonamides (sulfanilamide, sulfaguanidine, p-aminomethyl-sufanilamide, p-aminoethylsufanilamide, sulfathiazole, sulfamethoxazole, sulfamethazine and 4-[(2-amino-4-pyrimidinyl) amino] benzenesulfonamide) in the presence of an excess of ethylorthoformate. These compounds were screened for their *in-vitro* antibacterial activity against four Gram-negative (*E. coli, S. flexneri, P. aeruginosa and S. typhi*) and two Gram-positive (*B. subtilis* and *S. aureus*) bacterial strains and for *in-vitro* antifungal activity against *T. longifusus, C. albicans, A. flavus, M. canis, F. solani* and *C. glaberata*. Results revealed that a significant antibacterial activity was observed by compounds (4) and (5), (6) and (8) against two Gram-negative, *(P. aeruginosa and S. typhi)* and two Gram-positive (*B. subtilis* and *S. aureus*) species, respectively. Of these (4) was found to be the most active. Similarly, for antifungal activity compounds (3) and (8) showed significant activity against *M. canis* and, (6) and (8) against *F. solani*. The brine shrimp bioassay was also carried out to study their *in-vitro* cytotoxic properties and only two compounds, (**4**) and (**8**) possessing $LD_{50} = 2.9072 \times 10^{-4}$ and 3.2844×10^{-4} M, respectively, displayed potent cytotoxic activity against *Artemia salina*

Keywords: *4-Hydroxycoumarin, sulfonamides, antibacterial, antifungal, cytotoxicity*

Introduction

Previously, we prepared and investigated some Schiff bases of aromatic/heterocyclic sulfonamide derived chromones [1–7] as inhibitors of the zinc enzyme carbonic anhydrase. The 15 CA isozymes presently known in humans are involved in many physiological and pathological processes, and their inhibition may thus be exploited clinically for the treatment of glaucoma in which CA II and CA XII are targeted by sulfonamide or sulfamate inhibitors [8,9]. Coumarins, member of the class benzopyrones are also good inhibitors for carbonic anhydrase [10] and display a variety of pharmacological properties depending on their substitution pattern. Natural [11] and synthetic coumarins are known to possess antifungal/antibacterial properties [12–14]. The diverse biological activity of coumarin derivatives as anticoagulant is also well known [15,16]. The rapid spread of the acquired immunodeficiency syndrome (AIDS) epidemic has stimulated discovery of therapeutic agents to arrest the replication of the causative virus, human immunodeficiency virus (HIV). Recently, 3-substituted-4-hydroxycoumarin, phenoprocaumen [3-(*d*-ethyl-benzyl)-4-hydroxycoumarin] and analogous compounds have been identified as active nonpeptidic HIV protease inhibitors [17–21].

Correspondence: Professor Claudiu Supuran, Universita degli Studi di Firenze, Polo Scientifico, Laboratorio di Chimica Bioinorganica Rm, 188, Via della Lastruccia 3, Sesto Fiorentino (Florence) 50019, Italy. Tel.: 39 055 4573005. Fax: 39 055 4573385.
E-mail: claudiu.supuran@unifi.it
[†]Present address: Department of Chemistry, University of Arkansas at Little Rock, Little Rock, AR 72204, U.S.A.
E-mail: zchohan@mul.paknet.com.pk

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The coumarin ring forms a part of many heterocyclic compounds of pharmacological interest. Based on these findings and on the recent report [22] for the new synthetic routes to this class of compounds and their different important and interesting biological and pharmacological activities initiated us to synthesize a new class of sulfonamide derived coumarins and to explore their biological activities with the aim of obtaining more potent antibacterial and antifungal compounds. These synthesized compounds (1)–(8), were tested for *in-vitro* antibacterial activity against four Gram-negative (*Escherichia coli, Shigella flexneri, Pseudomonas aeruginosa and Salmonella typhi*) and two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacterial strains and for *in-vitro* antifungal activity against *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glaberata.* The coumarin-derived sulfonamide reported in this paper formulate not only a new class of antibacterial and antifungal agents but may also participate as good candidates for the globally alarming drug resistance problems in the clinic.

Material and methods

All reagents and solvents were used as obtained from the supplier or recrystallized/ redistilled as necessary. Thin-layer chromatography was performed using aluminum sheets (Merck) coated with silica gel 60 F_{254}. Infrared spectra (KBr discs) were recorded with a Hitachi Model 200–50 IR spectrophotometer. ^{1}HNMR spectra were recorded in d_6-DMSO with Bruker AM 300 and AM 400 spectrometers (Rheinstetten–Forchheim, Germany) operating at 300 and 400 MHz, respectively. Tetramethylsilane was used as an internal standard. Microanalytical data were determined using an Elemental Analyzer Flash EA 1112. Melting points were taken on a Gallenkamp apparatus and are uncorrected. *In-vitro* antibacterial and antifungal properties were studied at HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan.

General procedure for the preparation of compounds (1)–(8)

To a stirred solution of 4-hydroxycoumarin (1.62 g, 0.01 mole) and ethylorthoformate (2.25 g, 0.015 mole) in 2-butanol (30 mL) was added the respective sulfonamide (0.01 mole). The mixture was refluxed for 3 h. The precipitates formed during refluxing were cooled to room temperature and collected by suction filtration. Washing with hot ethanol, afforded TLC pure products in good yield.

4-({[2,4-dioxo-2H-chromen-3(4H)-ylidene]methyl} amino) benzenesulfonamide (1). Yield 90%; m.p. 294–295 °C; IR (KBr, cm^{-1}): 3450 (NH_2), 1740 (lactone, C=O), 1680 (ketone, C=O), 1440 (S=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.35 (d, 2H, benzene $C_{3,5}$-H), 7.42 (dd, 1H, chromen C_8-H), 7.54 (d, 2H, benzene $C_{2,6}$-H), 7.65 (ddd, 1H, chromen C_6-H), 7.70 (br s, 2H, SO_2NH_2), 7.78 (ddd, 1H, chromen C_7-H), 8.12 (dd, 1H, chromen C_5-H), 8.62 (s, 1H, =CH-N-), 9.85 (s, 1H, = C-NH-); Anal. Calcd. for $C_{16}H_{12}N_2O_5S$ (344.34): C, 55.81; H, 3.51; N, 8.14. Found: C, 55.95; H, 3.40; N, 8.25%.

3-{[4-({[amino(imino)methyl]amino}sulfonyl)anilino] methylidene}-2,4-dioxo-2H-chromene (2). Yield 96%; m.p. 276–277 °C; IR (KBr, cm^{-1}): 3440 (NH_2), 3315 (NH), 1745 (lactone, C=O), 1690 (ketone, C=O), 1580 (guanidine, C=N), 1445 (S=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.40 (d, 2H, benzene $C_{3,5}$-H), 7.43 (dd, 1H, chromene C_8-H), 7.58 (d, 2H, benzene $C_{2,6}$-H), 7.67 (ddd, 1H, chromene C_6-H), 7.72 (s, 2H, -N=C-NH_2), 7.76 (ddd, 1H, chromene C_7-H), 7.82 (s, 1H, SO_2NH-), 8.12 (s,1H, -C=NH), 8.14 (dd, 1H, chromene C_5-H), 8.65 (s, 1H, =CH-N-), 9.89 (s, 1H, =C-NH-); Anal. Calcd. for $C_{17}H_{14}N_4O_5S$ (386.38): C, 52.84; H, 3.65; N, 14.50. Found: C, 52.60; H, 3.80; N, 14.65%.

4-[({[2,4-dioxo-2H-chromen-3(4H)-ylidene]methyl} amino)methyl]benzenesulfonamide (3). Yield 78%; m.p. 218–219 °C; IR (KBr, cm^{-1}): 3430 (NH_2), 3310 (NH), 1750 (lactone, C=O), 1700 (ketone, C=O), 1445 (S=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 4.32 (d, 2H, -CH_2-), 7.36 (dd, 1H, chromen C_8-H), 7.44 (d, 2H, benzene $C_{3,5}$-H), 7.62 (ddd, 1H, chromen C_6-H), 7.65 (d, 2H, benzene $C_{2,6}$-H), 7.78 (s, 2H, SO_2NH_2), 7.75 (ddd, 1H, chromen C_7-H), 8.10 (dd, 1H, chromen C_5-H), 8.50 (s, 1H, =CH-N-), 8.62 (s, 1H, =C-NH-); Anal. Calcd. for $C_{17}H_{14}N_2O_5S$ (358.37): C, 56.98; H, 3.94; N, 7.82. Found: C, 56.75; H, 3.80; N, 7.98%.

4-[2-({[2,4-dioxo-2H-chromen-3(4H)-ylidene]methyl} amino)ethyl] benzenesulfonamide (4). Yield 88%; m.p. 256–257 °C; IR (KBr, cm^{-1}): 3435 (NH_2), 3320 (NH), 1745 (lactone, C=O), 1710 (ketone, C=O), 1450 (S=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 3.10 (t, 2H, -CH_2-aromatic ring-), 3.32 (t, 2H, -CH_2-N-C=C-),

7.36 (dd, 1H, chromen C_8-H), 7.39 (d, 2H, benzene $C_{3,5}$-H), 7.61 (ddd, 1H, chromen C_6-H), 7.52 (d, 2H, benzene $C_{2,6}$-H), 7.70 (s, 2H, SO_2NH_2), 7.74 (ddd, 1H, chromen C_7-H), 8.10 (dd, 1H, chromen C_5-H), 8.47 (s, 1H, =CH-N-), 8.56 (s, 1H, =C-NH-); Anal. Calcd. for $C_{18}H_{16}N_2O_5S$ (372.39): C, 58.05; H, 4.33; N, 7.52. Found: C, 57.80; H, 4.20; N, 7.70%.

4-({[2,4-dioxo-2H-chromen-3(4H)-ylidene]methyl}-amino)-N-(1,3-thiazol-2-yl) benzenesulfonamide (5). Yield 95%; m.p. 254 °C (decomp.); IR (KBr, cm^{-1}): 3315 (NH), 1740 (lactone, C=O), 1680 (ketone, C=O), 1615 (thiazole, C=N), 1440 (S=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 6.35 (d, 1H, thiazole C_5-H), 6.67 (d, 1H, thiazole C_4-H), 7.44 (dd, 1H, chromen C_8-H), 7.48 (d, 2H, benzene $C_{3,5}$-H), 7.62 (d, 2H, benzene $C_{2,6}$-H), 7.68 (ddd, 1H, chromen C_6-H), 7.77 (ddd, 1H, chromen C_7-H), 7.88 (s, 1H, SO_2NH-), 8.16 (dd, 1H, chromen C_5-H), 8.67 (s, 1H, =CH-N-), 9.92 (s, 1H, =C-NH-); Anal. Calcd. for $C_{19}H_{13}N_3O_5S_2$ (427.45): C, 53.39; H, 3.07; N, 9.83. Found: C, 53.56; H, 2.70; N, 9.68%.

4-({[2,4-dioxo-2H-chromen-3(4H)-ylidene]methyl}-amino)-N-(5-methyl-3-isoxazolyl) benzenesulfonamide (6). Yield 80%; m.p. 273–274 °C; IR (KBr, cm^{-1}): 3310 (NH), 1735 (lactone, C=O), 1675 (ketone, C=O), 1605 (isoxazolyl, C=N), 1435 (S=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.32 (s, 1H, -CH_3), 5.78 (s, 1H, isoxazolyl C_4-H), 7.44 (dd, 1H, chromen C_8-H), 7.50 (d, 2H, benzene $C_{3,5}$-H), 7.64 (d, 2H, benzene $C_{2,6}$-H), 7.69 (ddd, 1H, chromen C_6-H), 7.78 (ddd, 1H, chromen C_7-H), 7.90 (s, 1H, SO_2NH-), 8.16 (dd, 1H, chromen C_5-H), 8.68 (s, 1H, =CH-N-), 9.94 (s, 1H, =C-NH-); Anal. Calcd. for $C_{20}H_{15}N_3O_6S$ (425.41): C, 56.47; H, 3.55; N, 9.88. Found: C, 56.67; H, 3.62; N, 9.68%.

N-(4,6-dimethyl-2-pyrimidinyl)-4-({[2,4-dioxo-2H-chromen-3(4H)-ylidene]methyl}amino) benzenesulfonamide (7). Yield 72%; m.p. 259–260 °C; IR (KBr, cm^{-1}): 3305 (NH), 1725 (lactone, C=O), 1670 (ketone, C=O), 1590 (pyrimidinyl, C=N), 1425 (S=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 2.48 (s, 6H, -CH_3), 7.48 (dd, 1H, chromen C_8-H), 7.52 (d, 2H, benzene $C_{3,5}$-H), 7.74 (d, 2H, benzene $C_{2,6}$-H), 7.77 (ddd, 1H, chromen C_6-H), 7.84 (ddd, 1H, chromen C_7-H), 7.88 (s, 1H, pyrimidinyl C_5-H), 8.20 (dd, 1H, chromen C_5-H), 8.28 (s, 1H, SO_2NH-), 8.78 (s, 1H, =CH-N-), 10.21 (s, 1H, =C-NH-); Anal. Calcd. for $C_{22}H_{18}N_4O_5S$ (450.46): C, 58.66; H, 4.03; N, 12.44. Found: C, 58.48; H, 4.16; N, 12.55%.

4-{[2-({[2,4-dioxo-2H-chromen-3(4H)-ylidene]methyl} amino)-4-pyrimidinyl]amino} benzenesulfonamide (8). Yield 75%; m.p. 306 °C (decomp.); IR (KBr, cm^{-1}): 3300 (NH), 1720 (lactone, C=O), 1670 (ketone, C=O), 1575 (pyrimidinyl, C=N), 1420 (S=O); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.31 (d, 1H, pyrimidinyl C_6-H),7.55 (dd, 1H, chromen C_8-H), 7.62 (d, 2H, benzene $C_{3,5}$-H), 7.72 (ddd, 1H, chromen C_7-H), 7.77 (d, 2H, benzene $C_{2,6}$-H), 7.80 (ddd, 1H, chromen C_6-H), 7.84 (s, 1H, SO_2NH-),7.92 (d, 1H, pyrimidinyl C_5-H), 8.22 (dd, 1H, chromen C_5-H), 8.88 (s, 1H, =CH-N-), 9.22 (s, 1H, pyrimidinyl-NH-benzene), 10.62 (s, 1H, =C-NH-); Anal. Calcd. for $C_{20}H_{15}N_5O_5S$ (437.43): C, 54.91; H, 3.46; N, 16.01. Found: C, 54.72; H, 3.55; N, 15.84%.

*Antibacterial bioassay (*in-vitro*)*

The synthesized compounds (1)–(8) were screened *in vitro* for their antibacterial activity against four Gram-negative (*E. coli, S. flexneri, P. aeruginosa and S. typhi*) and two Gram-positive (*B. subtilis* and *S. aureus*) bacterial strains by the agar-well diffusion method [23]. The wells (6 mm in diameter) were dug in the media with the help of a sterile metallic borer with centers at least 24 mm apart. Two to eight hours old bacterial inocula containing approximately 10^4–10^6 colony-forming units (CFU/ml) were spread on the surface of the nutrient agar with the help of a sterile cotton swab. The recommended concentration of the test sample (1 mg/ml in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, imipenum, served as negative and positive controls, respectively. The plates were incubated immediately at 37 °C for 24 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial strains.

Antifungal activity (in-vitro)

Antifungal activities of all compounds were studied against six fungal cultures, *T. longifusus*, *C. albicans*, *A. flavus, M. canis, F. solani* and *C. glaberata*. Sabouraud dextrose agar (Oxoid, Hampshire, England) was seeded with 10^5 (cfu) ml^{-1} fungal spore suspensions and transferred to petri plates. Discs soaked in 20 ml (200 μg/mL in DMSO) of all

compounds were placed at different positions on the agar surface. The plates were incubated at 32 °C for seven days. The results were recorded [24] as zone of inhibition and compared with standard drugs miconazole and amphotericin B.

Cytotoxic activity

Brine shrimp (*Artemia salina leach*) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial seawater, which was prepared with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the other compartment was opened to ordinary light. After two days nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 mL of DMF. From this stock solutions 500, 50 and 5 μg/mL were transferred to 9 vials (three for each dilutions were used for each test sample and LD_{50} is the mean of three values) and one vial was kept as control having 2 mL of DMF only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 mL of sea water and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 mL per vial. After 24 h the numbers of survivors were counted [25]. Data were analyzed by a Finney computer program to determine the LD_{50} values [26].

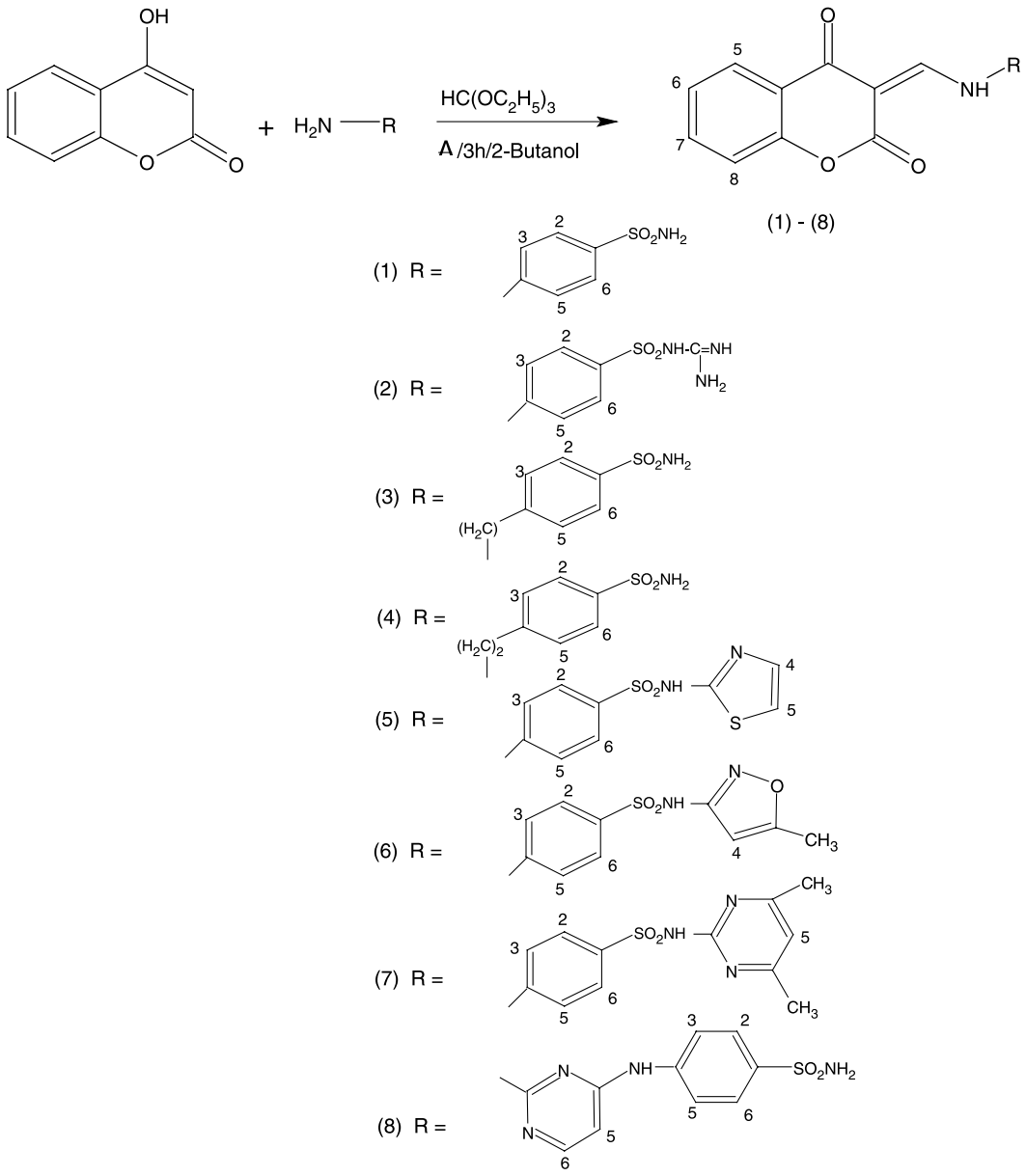
Result and discussion

Chemistry

All synthesized compounds were prepared by refluxing an equimolar ratio of 4-hydroxycoumarin and the respective sulfonamide such as sulfanilamide, sulfaguanidine, p-aminomethylsufanilamide, p-aminoethylsufanilamide, sulfathiazole, sulfamethoxazole, sulfamethazine and 4-[(2-amino-4-pyrimidinyl) amino] benzenesulfonamide in 2-butanol. The reaction was carried out in the presence of ethyl orthoformate (Scheme 1) which successfully led to a new series of corresponding 4-({[2,4-dioxo-2*H*-chromen-3 (4*H*)-ylidene] methyl} amino) sulfonamides in good yield (72–96%). The reaction was rapid, and no observation supported any kind of competition from the intermolecular condensation of 4-hydroxycoumarin [27]. All the products were obtained as solids and their purities were checked by thin layer chromatography (eluent = ethanol/chloroform/ethylacetate, 1/2/1 v/v). All the synthesized compounds were characterized by spectroscopic techniques (IR & ^{1}HNMR) and their elemental analyses.

IR Spectra. The IR spectra of (1)–(8) showed bands resulting from the S=O, ketone (C=O) and lactone (C=O) stretchings in the region at 1420–1455, 1670–1710 and 1720–1750 cm^{-1}, respectively, in all the cases. In addition, the spectra of (2), (5)–(8) showed bands resulting from the guanidine (C=NH), thiazole (C=N), isoxazolyl (C=N), -SO_2-NH-pyrimidinyl (C=N) and -NH-pyrimidinyl (C=N) stretchings at 1580, 1615, 1605, 1590 and 1575 cm^{-1}, respectively. The IR spectra of (1)–(4), exhibited the –NH_2 stretchings in the region at 3430–3450 cm^{-1}. Also the IR spectra of (2)–(8) showed a band resulting from the NH stretching in the 3305–3320 cm^{-1} region. All this evidence was supportive [28,29] of the formation of compounds (1)–(8).

^{1}H-NMR Spectra. The ^{1}H-NMR spectra of compounds (1)–(8) displayed the coumarin C_5-*H* and C_8-*H* protons at δ 8.10–8.22 and δ 7.36–7.55, respectively, as double doublets. The C_7-*H* and C_6-*H* protons of all the compounds appeared [30] as a separate doublet of double doublet at δ 7.72–7.84 and δ 7.61–7.80, respectively. The ^{1}HNMR spectra displayed singlets between δ 9.56 and δ 10.62 and between δ 8.47 and δ 8.88 attributed to the N*H* and ethenylic protons. However, benzene $C_{2,6}$-*H* and $C_{3,5}$-*H* protons appeared as separate doublets at δ 7.52–7.77 and δ 7.35–7.62, respectively. The SO_2NH_2 or SO_2N*H*- protons in all cases appeared as a singlet at (7.70–δ 8.28. The ^{1}H-NMR spectrum of compound (2) also displayed -C=N*H* and –N=C-NH_2 protons as a singlet at δ 8.12 and δ 7.72, respectively. In the case of compound (3), the ^{1}H-NMR spectrum exhibited [31] methylenic (-CH_2-) protons as a doublet at δ 4.32. Similarly, in the spectrum of compound (4), the methylenic, (=C-N*H*-CH_2) and (-N=C-CH_2-) protons appeared as a triplet at δ 3.32 and δ 3.10, respectively. The ^{1}H-NMR spectrum of compound (5) displayed thiazole C_4-*H* and C_5-*H* protons as doublets at δ 3.32 and δ 3.10. In the case of compounds (6) & (7), the isoxazolyl C_4-*H* and pyrimidinyl C_5-*H* protons appeared as a singlet at δ 5.78 and δ 7.88, respectively. The spectra also displayed the methyl protons as a singlet at δ 2.32 and δ 2.48, respectively. The ^{1}H-NMR spectrum of compound (8) showed signals between δ 7.92 and δ 7.31 as a doublet, due to pyrimidinyl C_5-*H* and C_6-*H* protons, respectively. The spectrum also displayed the pyrimidinyl-N*H*-benzene proton as a singlet at δ 9.22. The elemental analysis data of these



Scheme 1. Synthesis of compounds (1)–(8)

compounds was also found to be in good agreement with the proposed structures of the synthesized compounds.

*Antibacterial bioassay (*In-vitro*)*

All compounds were tested against four Gram-negative (*E. coli, S. flexneri, P. aeruginosa and S. typhi*) and two Gram-positive (*B. subtilis* and *S. aureus*) bacterial strains according to the literature protocol [23] The results were compared with those of the standard drug imipenum. All the synthesized compounds exhibited varying degree of inhibitory effects on the growth of different tested strains (Table I). A significant activity was observed by compounds (4) and (5), (6) and (8) against two Gram-negative *(P. aeruginosa and S. typhi)* and two gram positive (*B. subtilis* and *S. aureus*) species, respectively. Of these (4) was found to be the most active. However, compound (1) and (2) showed moderate activity against *P. aeruginosa*, *S. typhi*, *B. subtilis* and *S. aureus;* (3), against *P. aeruginosa*, *B. subtilis* and *S. aureus;* (4), (5), (6) and (8) against *E. coli* and *S. flexenari*; (7) against *S. typhi, B. subtilis* and *S. aureus*. Compounds (1), (2) and (3) were found to be inactive against *E. coli* and *S. flexneri*; in addition (3) was also found to be inactive against *S. typhi*. Compound (7) was found to be inactive against *E. coli, P. aeruginosa* and *S. flexneri*.

Table I. Antibacterial b ioassay of compounds (1)–(8) (1 mg/mL in DMSO).

Bacteria	Compound (zones of inhibition in mm)								
	1	2	3	4	5	6	7	8	SD*
Gram-negative									
E. coli	00	00	00	10	07	08	00	07	30
S. flexneri	00	00	00	09	08	08	00	07	27
P. aeruginosa	10	12	15	20	19	17	00	18	24
S. typhi	08	11	00	20	19	18	12	17	25
Gram-positive									
S. aureus	07	10	08	27	27	26	16	25	33
B. subtilis	08	10	08	27	25	24	14	25	33

10 < : weak; 10-16: moderate; > 16: Significant.
* Standard Drug (Imipenem)

*Antifungal bioassay (*In vitro*)*

The antifungal screening of all compounds was carried out against *T. longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani* and *C. glaberata* fungal strains according to the literature protocol [24]. The results were compared with those from the standard drugs miconazole and amphotericin B. These results (Table II) indicate that most of the compounds were found to be inactive against all fungal species except for compounds (3) and (8) which showed significant activity against *M. canis* and (6) and (8) against *F. solani*. However, compounds (1), (3), (4), (6), (7) and (8) exhibited low to moderate activity against at least one of the fungal strains, *F. solani, A. flavus, C. albicans, C. glaberata and T. longifusus*.

Minimum inhibitory concentration (MIC)

Preliminary screening showed that compounds (4), (5), (6) and (8) were the most active against both gram-negative and Gram-positive organisms. These four compounds were selected for minimum inhibitory concentration (MIC) studies (Table III). The MIC of all the four active compounds varied from 2.2806×10^{-8}–1.1430×10^{-7} M. Compound (4) again proved to be the most active. It inhibited the growth of *P. aeruginosa*, *S. typhi* and *S. aureus* at 2.6853×10^{-8} M. The minimum inhibitory concentration was determined using the disc diffusion technique [23] by preparing discs containing 10, 25, 50 and 100 μg/mL of the compounds and applying the literature protocol [32].

Cytotoxic bioassay

All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer *et al* [25]. From the data recorded in Table IV, it is evident that only two compounds, (**4**) and (**8**) displayed potent cytotoxic activity against *Artemia salina*, while the other compounds were almost inactive in this assay. Compound (**4**) showed maximum activity ($LD_{50} = 3.2844 \times 10^{-4}$ M) in the present series of compounds, whereas the other active compound (**8**) of the series demonstrated slightly less activity ($LD_{50} = 2.9072 \times 10^{-4}$ M) than compound (4). The relationship between cytotoxicity and activity however, reveals that cytotoxicity is approximately 100-fold greater than concentration for the activity of the most active compound against the selected bacterial strains.

Table II. Antifungal bioassay of compounds (1)–(8) (200 μg/mL in DMSO).

Organism	Compound (% Inhibition)								
	1	2	3	4	5	6	7	8	SD*
T. longifusus	15	00	10	00	00	11	10	09	A
C. albicans	00	00	00	00	00	00	10	00	B
A. flavus	00	00	00	06	00	00	00	00	C
M. canis	00	09	74	00	00	00	00	86	D
F. solani	00	00	00	00	00	69	00	82	E
C. glaberata	00	00	10	00	00	00	18	00	F

* Standard Drugs MIC μg/mL. A = Miconazole (70 μg/mL: 1.6822×10^{-7} M), B = Miconazole (110.8 μg/mL: 2.6626×10^{-7} M), C = Amphotericin B (20 μg/mL: 2.1642×10^{-8} M), D = Miconazole (98.4 μg/mL: 2.3647×10^{-7} M), E = Miconazole (73.25 μg/mL: 1.7603×10^{-7} M), F = Miconazole (110.8 μg/mL: 2.66266×10^{-7} M)

Table III. Minimum inhibitory concentration (M) for compounds (3), (4), (5), (6), (7) and (8) against selected bacteria.

No.	(4)	(5)	(6)	(8)
P. aeruginosa	2.6853×10^{-8}	2.3394×10^{-8}	2.3506×10^{-8}	5.7152×10^{-8}
S. typhi	2.6853×10^{-8}	2.3394×10^{-8}	2.3506×10^{-7}	2.2806×10^{-8}
S. aureus	2.6853×10^{-8}	5.8486×10^{-8}	2.3506×10^{-7}	1.1430×10^{-7}
B. subtilis	6.7133×10^{-8}	2.3394×10^{-7}	5.8766×10^{-8}	2.2860×10^{-7}

Table IV. Brine shrimp lethality bioassay data for compounds (1)–(8).

Compound	LD_{50} (M)
1	2.9041×10^{-3}
2	2.5881×10^{-3}
3	2.7904×10^{-3}
4	3.2844×10^{-4}
5	2.3394×10^{-3}
6	2.3506×10^{-3}
7	2.2199×10^{-3}
8	2.9072×10^{-4}

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