



Original article

Synthesis and antimicrobial studies on novel sulfonamides containing 4-azidomethyl coumarin

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ABSTRACT

A series of new and novel coumarin-6-sulfonamides with a free C4-azidomethyl group have been synthesized as antimicrobials in three steps starting from 7-methyl-4-bromomethylcoumarin **1**. The reaction of **1** with chlorosulfonic acid was found to yield the corresponding 6-sulfonylchloride **2**, which when treated with sodium azide led to intermediate **3**. The title sulfonamides **5a–y** were obtained from the reaction of **3** with various aromatic amines **4** in refluxing benzene. The chemical structures of the compounds were elucidated by IR, NMR and LC-MS spectral data. All the synthesized compounds have been screened for their *in vitro* anti-bacterial and anti-fungal activities. Some of the compounds have been found to be active against both bacterial species at a concentration of 1 µg/mL.

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1. Introduction

Coumarins are a class of lactones which are found widely in nature and possess antiinflammatory [1,2], antiviral [3], antiHIV [4], anti-fungal [5] and cytotoxic activities [6,7], which have been reviewed [8]. In the development of newer antimicrobials, coumarins have been identified as target specific plant anti-bacterial agents with growth inhibitory potential particularly against Gram-positive species [9]. This is based on the related antibiotic novobiocin which targets the DNA gyrase [10]. Synthetic coumarins with chloramphenicol [11] side chain and cephalosporin amides [12] at C-3 position, have been screened against a number of bacterial species. One of the earliest attempts to enhance their antimicrobial potency led to 6-sulphonamido coumarins [13–16], as anti-tubercular agents. Introduction of a basic side chain at the allylic position with respect to the biogenetically important C3–C4 double bond [17] was attempted to reduce the acidic character of coumarin antibiotics. Amongst the various alkyl amines and sulfides synthesized, the 4-piperazinomethyl and imidazomethyl sulfides [18] were found to be the most active compounds. We have been studying structurally analogous, 4-arylaminoethyl [19] 4-sulphonamidomethyl [20] and 4-

dichloroacetamidomethyl coumarins [21] for their anti-bacterial activity. Recent reports on the cytotoxic effects of coumarin sulfonamides [22,23], on leukemia cell lines have generated a renewed interest in such class of compounds. Azido group has been recognized as a bioorthogonal chemical reporter which is stable in physiological conditions [24] and has been employed in the study of biosynthetic pathways of biomolecules [25,26]. It is also expected that the polar nature of the azide would play an important role in penetrating the cell wall thus enhancing the bacterial potency of the compounds. In continuation of our interest in designing oxygen heterocycle based anti-bacterials [27], we have synthesized new sulfonamides with an azido group at the allylic position in the coumarin ring and the results of anti-bacterial and anti-fungal activity have been discussed in this paper.

2. Chemistry

4-Bromoethylacetoacetate obtained from the bromination of ethylacetoacetate, was treated with *m*-cresol under Pechmann cyclization conditions using neat sulphuric acid as the condensing agent. The reaction resulted in the formation of 4-bromomethyl-7-methylcoumarin **1**. The reactivity of this compound has been explored in a cascaded manner via a regioselective electrophilic substitution, allylic S_N reaction and a nucleophilic displacement at the oxidized sulfur. Compound **1** was refluxed with excess of

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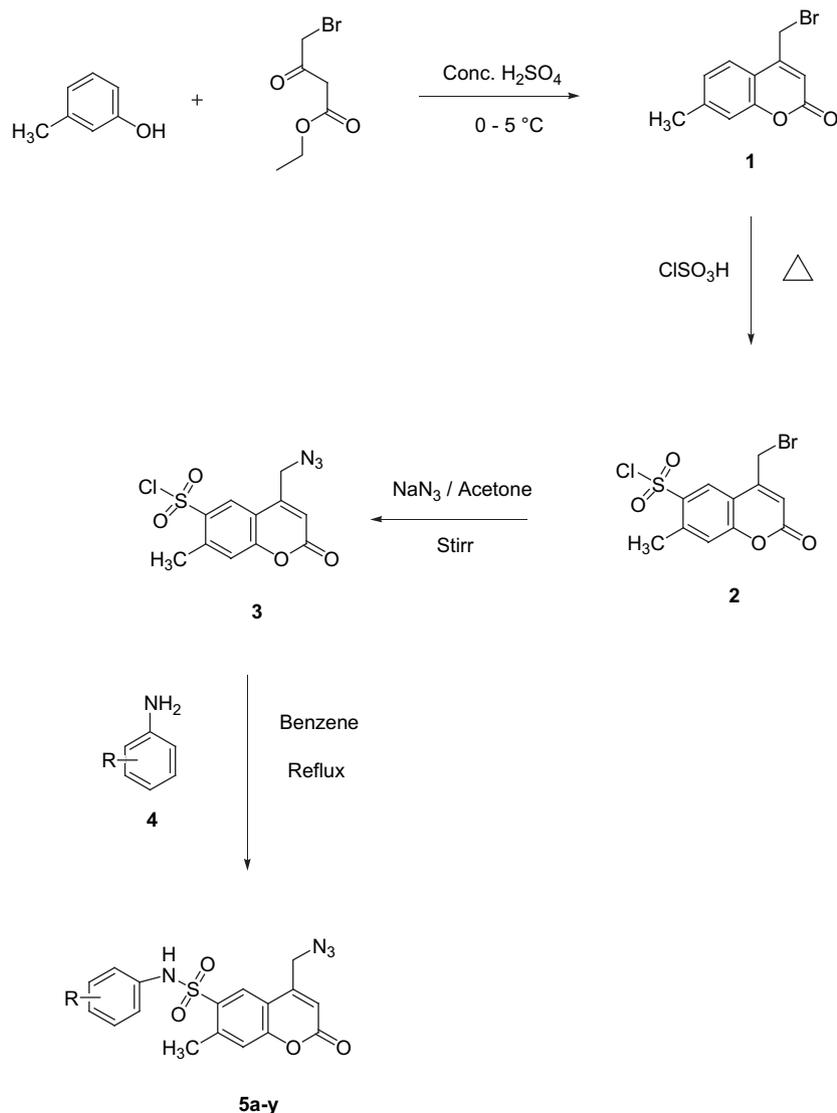
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chlorosulfonic acid to give 4-bromomethyl-7-methyl-coumarin-6-sulfonylchloride **2**. The regioselective electrophilic substitution at C-6 is probably because of the electron donating C7-CH₃ group and the spatial proximity of C-6 with the ring oxygen, which resembles the *para*-position of phenols.

Treatment **2** with sodium azide in aqueous acetone at room temperature resulted in the formation of 4-azidomethyl-7-methylcoumarin-6-sulfonyl chloride **3**, the structure of which was supported by spectral data. Preferential attack of the linear azide nucleophile at the allylic carbon is favorably due to the better leaving group ability of bromine and slight steric hindrance caused by the ortho-methyl group at C-7. However use of two equivalents of sodium azide at room temperature leads to the formation of bis-azide expected because of the higher reactivity of sulfonyl chloride which has been recently reported in the reactions of 4-bromo-methyl-benzenesulfonyl chloride [28]. Sulfonamides **5a–y** were obtained by refluxing aromatic amines and sulfonyl chloride derivative **3** in dry benzene. Removal of solvent under reduced pressure afforded the title compounds as solids which were purified by routine methods (Scheme 1).

3. Results and discussion

Structure of C-6 sulfonyl chloride **2** was supported by the appearance of two singlets in the ¹H NMR at 7.40 and 8.45 ppm due to C5-H and C8-H respectively. The C₄-CH₂ protons appeared as singlet at 4.52 ppm where as C₃-H observed as singlet at 6.63 ppm. Formation of azide **3** was indicated by a strong band at 2135 cm⁻¹. It was the change in the chemical shift of methylene protons to 5.00 ppm which supported the observed regiochemistry of the nucleophilic displacement at the allylic carbon under the present experimental conditions. Expectedly there was no significant change in the aromatic region with singlets appearing at 7.69 and 8.24 ppm. Generation of sulfonamides **5a–y** was indicated by a sharp band around 3360 cm⁻¹ and the azide group remained in-tact during the nucleophilic displacement at the sulfonyl chloride **3**. In the PMR spectrum of 4-azidomethyl-7-methylcoumarin-6-sulfonic acid *p*-tolylamide (**5c**) (R = -C₆H₄-CH₃-*p*) showed two singlets at 2.29 and 2.66 δ ppm due to C7-CH₃ and CH₃ of phenyl ring, respectively. The NH proton was observed as singlet at 4.49 ppm. The methylene protons linked to azido group were observed as



Scheme 1. Synthesis of 4-azidomethyl-7-methylcoumarin-6-sulfonamide **5**.

singlet in the down field at 4.78 ppm. The C3-H of coumarin was observed as singlet at 6.47 ppm. The aromatic protons resonated in the region of 6.55–8.30 ppm. Further evidence for the formation of sulfonamides was obtained from the mass spectral data. The Mass spectrum of (**5c**) ($R = -C_6H_4-CH_3-p$) shows M + H peak at m/z 385.

4. Biological screening

4.1. Anti-bacterial screening

All the newly synthesized compounds were evaluated for their *in vitro* anti-bacterial activity against (i) Gram-positive bacteria: *Streptococcus faecalis* (MTCC 3382), *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 297) and (ii) Gram-negative bacteria: *Pseudomonas aeruginosa* (MTCC 1034), *Klebsiella pneumoniae* (MTCC 3384) and *Escherichia coli* (MTCC 1089) by broth microdilution method [29]. The anti-bacterial data (Table 1) revealed that all the sulfonamides exhibited moderate activity a few of them highly active, but majority of them inactive against *E. coli*. Compounds with methoxy group **5g**, **5h**, **5i**, **5j**, **5k** were highly active against *S. faecalis* with MIC of 1 $\mu\text{g/mL}$. The *m*-methoxy compound **5h** was found to equally effective against *P. aeruginosa* and *K. pneumoniae* with MIC of 1 and 2 $\mu\text{g/mL}$, respectively. The 2-chloro compound **5n** and 4-bromo compound **5s** were found to be very active against *S. faecalis*, *P. aeruginosa* and *K. pneumoniae*. Many compounds exhibited MIC similar to ciprofloxacin against three bacterial species except *E. coli*. Highest activity was associated with mono-nitro, chloro, bromo and methoxy substituents.

4.2. Anti-fungal screening

All the sulfonamides were screened for their anti-fungal activity against *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium chrysogenum*, *Mucor fuscus* and *Fusarium oxysporum* in DMSO by broth micro dilution method [30].

The screening data (Table 2) gives a spectrum of their activity and all of them were most active against *F. oxysporum*. The most

active compounds against *C. albicans* were **5c**, **5b** and **5u** with 4-methyl, 3-methyl and 2-chloro-5-nitro substituents exhibiting MICs of 1, 4 and 4 $\mu\text{g/mL}$, respectively. The 2,6-dimethyl, 4-methoxy, 2-chloro-5-nitro and naphthyl exhibited 2 $\mu\text{g/mL}$ MIC against *A. fumigatus*. A broad spectrum of anti-fungal activity was observed in **5b**, **5h**, **5i**, **5y** for *A. niger*. Other compounds those were effective at 2 $\mu\text{g/mL}$ against *P. chrysogenum* were **5a**, **5d**, **5l**, **5o**, **5r** and **5x**. The compounds **5a**, **5d**, **5h**, **5l** and **5x** showed significant activity against *M. fuscus*. It is interesting to note that all these compounds exhibited a better activity than fluconazole.

5. Experimental

5.1. Chemistry

The melting points were determined by open capillary method and are uncorrected. The IR spectra (KBr disc) were recorded on a Nicolet-5700 FT-IR spectrophotometer. ^1H NMR spectra were recorded on Bruker 300 MHz spectrometer using CDCl_3 and $\text{DMSO-}d_6$ as solvents and TMS as an internal standard. The chemical shifts are expressed in δ ppm. The mass spectra were recorded using Agilent-single Quartz LC-MS. The elemental analysis was carried out using Heraeus CHN rapid analyzer. The purity of the compound was checked by T.L.C. All the chemicals purchased were of analytical grade, and were used without further purification unless otherwise stated.

5.1.1. Synthesis of 4-bromomethyl-7-methylcoumarin (**1**)

The required 4-bromomethyl-7-methylcoumarin [19] have been synthesized by the Pechmann cyclization of *m*-cresol with 4-bromoethylacetoacetate [31].

5.1.2. Synthesis of 4-bromomethyl-7-methyl-coumarin-6-sulfonyl chloride (**2**)

4-Bromomethyl-7-methylcoumarin **1** (2.53 g, 0.01 mol) was taken in a two-necked R. B. flask with a dropping funnel and reflux

Table 1
Results of anti-bacterial activities of compounds **2**, **3** and **5a–y** MICs ($\mu\text{g/mL}$).

Compound	R	Gram-positive			Gram-negative		
		<i>S. faecalis</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
2	–	16	16	16	125	125	125
3	–	31.25	4	31.25	125	125	125
5a	H	31.25	4	31.25	125	125	125
5b	3-CH ₃	250	16	16	2	4	>500
5c	4-CH ₃	16	16	8	125	1	>500
5d	3,4-CH ₃	8	4	31.25	125	125	125
5e	2,4-CH ₃	62.5	16	125	4	62.5	>500
5f	2,6-CH ₃	>500	125	>500	2	1	>500
5g	2-OCH ₃	1	31.25	>500	125	62.5	250
5h	3-OCH ₃	1	16	>500	1	2	>500
5i	4-OCH ₃	1	4	>500	8	1	250
5j	4-OC ₂ H ₅	1	2	>500	62.5	16	62.5
5k	2-NO ₂	1	1	62.5	4	16	>500
5l	3-NO ₂	8	4	8	125	125	125
5m	4-NO ₂	4	2	125	1	2	250
5n	2-Cl	1	1	125	31.25	1	>500
5o	3-Cl	8	1	16	1	4	>500
5p	4-Cl	4	8	16	1	1	>500
5q	2,4-Cl	1	31.25	250	2	2	>500
5r	3,4-Cl	16	4	2	8	1	>500
5s	4-Br	1	16	>500	1	1	>500
5t	3-Cl, 4-CH ₃	8	8	4	1	8	>500
5u	2-Cl, 5-NO ₂	2	2	125	2	62.5	>500
5v	2-NO ₂ , 4-Cl	4	4	250	8	16	>500
5w	2-NO ₂ , 4-CH ₃	1	16	250	16	4	250
5x	2-CH ₃ , 4-NO ₂	4	16	4	125	31.25	125
5y	Naphthylamine	2	16	>500	8	1	>500
Ciprofloxacin	–	1	1	1	1	1	1

Table 2
Results of anti-fungal activities of compounds **2**, **3** and **5a–y** MICs ($\mu\text{g/mL}$).

Compound	R	<i>C. albicans</i>	<i>A. fumigatus</i>	<i>A. niger</i>	<i>P. chrysogenum</i>	<i>M. fuscus</i>	<i>F. oxysporum</i>
2	–	8	4	4	1	1	1
3	–	8	8	8	4	2	2
5a	H	16	8	8	2	4	4
5b	3-CH ₃	4	8	1	8	62.5	125
5c	4-CH ₃	1	16	4	16	62.5	1
5d	3,4-CH ₃	8	8	4	2	1	1
5e	2,4-CH ₃	8	16	4	31.25	16	1
5f	2,6-CH ₃	8	2	8	31.25	16	1
5g	2-OCH ₃	31.25	62.5	16	>500	31.25	1
5h	3-OCH ₃	8	31.25	2	125	4	1
5i	4-OCH ₃	8	2	2	250	250	1
5j	4-OC ₂ H ₅	16	>500	250	>500	62.5	1
5k	2-NO ₂	250	8	8	4	8	1
5l	3-NO ₂	16	8	8	2	4	2
5m	4-NO ₂	62.5	250	62.5	62.5	8	1
5n	2-Cl	62.5	31.25	62.5	31.25	16	4
5o	3-Cl	>500	16	31.25	2	>500	1
5p	4-Cl	8	125	31.25	16	16	1
5q	2,4-Cl	8	250	250	62.5	16	1
5r	3,4-Cl	16	31.25	16	2	31.25	1
5s	4-Br	125	500	500	16	250	1
5t	3-Cl, 4-CH ₃	31.25	16	16	4	16	1
5u	2-Cl, 5-NO ₂	4	2	8	125	62.5	1
5v	2-NO ₂ , 4-Cl	16	500	62.5	125	8	1
5w	2-NO ₂ , 4-CH ₃	16	125	31.25	31.25	62.5	1
5x	2-CH ₃ , 4-NO ₂	16	8	4	2	2	2
5y	Naphthylamine	8	2	1	16	31.25	1
Fluconazole	–	8	8	8	8	8	8

condenser containing a CaCl₂ guard tube. Chlorosulfonic acid (4 ml, 0.06 mol) was added through a dropping funnel in small portion with occasional shaking. The reaction mixture was heated on a water bath for 1 h (monitored by TLC), cooled and poured on to crushed ice (100 g). The separated solid was filtered off, washed with water, dried and recrystallized from chloroform to obtain a colorless solid in 55% yield. m.p. 170–171 °C; IR (KBr) cm⁻¹ 1716 (C=O); ¹H NMR (CDCl₃, 300 MHz, TMS): δ 2.89 (s, 3H, C7-CH₃), 4.52 (s, 2H, C4-CH₂), 6.63 (s, 1H, C3-H), 7.40 (s, 1H, C8-H), 8.45 (s, 1H, C5-H); LC-MS: 353 (M + 2). Anal. Calcd for C₁₁H₈BrClO₄S (%): Calcd. C, 37.58; H, 2.29. Found: C, 37.21; H, 2.02.

5.1.3. Synthesis of 4-azidomethyl-7-methyl-coumarin-6-sulfonyl chloride (**3**)

4-Bromomethyl-7-methyl-coumarin-6-sulfonyl chloride **2** (3.51 g, 0.01 mol) was taken in acetone (20 ml) in R. B. flask. To this, solution of sodium azide (0.78 g, 0.012 mol) in water (3 ml) was added drop wise with stirring. The stirring was continued for 10 h. Then, the reaction mixture was poured on to crushed ice (100 g). The separated solid was filtered and recrystallized from benzene to obtain a colorless solid in 65% yield. m.p. 120–121 °C; IR (KBr) cm⁻¹ 1728 (C=O), 2135 (N₃, azido); ¹H NMR (CDCl₃, 300 MHz, TMS): δ 2.77 (s, 3H, C7-CH₃), 4.66 (s, 2H, C4-CH₂), 6.67 (s, 1H, C3-H), 7.39 (s, 1H, C8-H), 8.35 (s, 1H, C5-H); Anal. Calcd for C₁₁H₈ClN₃O₄S (%): Calcd. C, 42.11; H, 2.57; N, 13.39. Found: C, 42.01; H, 2.31; N, 13.11.

5.1.4. Synthesis of 4-azidomethyl-7-methyl-coumarin-6-sulfonamides (**5**): general procedure

To a mixture of 4-azidomethyl-7-methyl-coumarin-6-sulfonyl chloride **3** (3.13 g, 0.01 mol) in dry benzene (30 ml) the appropriate aromatic amines **4** (0.015 mol) were added and heated under reflux for 4 h (18–20 h for nitro amines) (monitored by TLC). The solvent was removed under reduced pressure. The residue obtained was filtered off and crystallized from benzene.

5.1.4.1. Synthesis of 4-azidomethyl-7-methyl-coumarin-6-sulfonic acid phenylamide (**5a**). Colorless solid; yield 70%; m.p. 168 °C; IR

(KBr) cm⁻¹ 1716 (C=O), 2124 (N₃), 3367 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.67 (s, 3H, C7-CH₃), 5.12 (s, 2H, C4-CH₂), 6.37 (s, 1H, C3-H), 6.63–8.39 (m, 8H, Ar-H and NH); Anal. Calcd for C₁₇H₁₄N₄O₄S (%): Calcd. C, 55.13; H, 3.81; N, 15.13. Found: C, 55.02; H, 3.57; N, 15.01.

5.1.4.2. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid *m*-tolylamide (**5b**). Yellow solid; yield 75%; m.p. 110 °C; IR (KBr) cm⁻¹ 1714 (C=O), 2120 (N₃), 3345 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.66 (s, 3H, C7-CH₃), 3.51 (s, 3H, 3-CH₃ of phenyl), 4.59 (s, 1H, NH, D₂O exchangeable), 4.93 (s, 2H, C4-CH₂), 6.34 (s, 1H, C3-H), 6.55–8.37 (m, 6H, Ar-H); LC-MS 385 (M + H). Anal. Calcd for C₁₈H₁₆N₄O₄S (%): Calcd. C, 56.24; H, 4.20; N, 14.57. Found: C, 56.14; H, 4.13; N, 14.40.

5.1.4.3. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid *p*-tolylamide (**5c**). Brown solid; yield 72%; m.p. 188 °C; IR (KBr) cm⁻¹ 1710 (C=O), 2115 (N₃), 3341 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.29 (s, 3H, 4-CH₃ of phenyl), 2.66 (s, 3H, C7-CH₃), 4.49 (s, 1H, NH, D₂O exchangeable), 4.78 (s, 2H, C4-CH₂), 6.47 (s, 1H, C3-H), 6.55–8.30 (m, 6H, Ar-H); LC-MS 385 (M + H). Anal. Calcd for C₁₈H₁₆N₄O₄S (%): Calcd. C, 56.24; H, 4.20; N, 14.57. Found: C, 56.04; H, 4.04; N, 14.39.

5.1.4.4. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (3,4-dimethyl-phenyl)-amide (**5d**). Colorless solid; yield 70%; m.p. 190 °C; IR (KBr) cm⁻¹ 1712 (C=O), 2115 (N₃), 3352 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.00 (s, 3H, 4-CH₃ of phenyl), 2.66 (s, 3H, C7-CH₃), 3.90 (s, 3H, 3-CH₃ of phenyl), 5.00 (s, 2H, C4-CH₂), 6.60 (s, 1H, C3-H), 6.70–8.40 (m, 5H, Ar-H), 10.0 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₁₉H₁₈N₄O₄S (%): Calcd. C, 57.27; H, 4.55; N, 14.06. Found: C, 57.03; H, 4.32; N, 13.92.

5.1.4.5. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (2,4-dimethyl-phenyl)-amide (**5e**). Brown solid; yield 69%; m.p. 161 °C; IR (KBr) cm⁻¹ 1730 (C=O), 2134 (N₃), 3440 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.13 (s, 6H, 2-CH₃ and 4-CH₃ of phenyl), 2.69 (s,

3H, C7-CH₃), 5.16 (s, 2H, C4-CH₂), 6.35 (s, 1H, C3-H), 6.70–8.25 (m, 5H, Ar-H), 8.51 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₁₉H₁₈N₄O₄S (%): Calcd. C, 57.27; H, 4.55; N, 14.06. Found: C, 57.12; H, 4.48; N, 14.01.

5.1.4.6. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (2,6-dimethyl-phenyl)-amide (5f). Green solid; yield 65%; m.p. 185 °C; IR (KBr) cm⁻¹ 1730 (C=O), 2134 (N₃), 3440 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.13 (s, 6H, 2-CH₃ and 4-CH₃ of phenyl), 2.69 (s, 3H, C7-CH₃), 5.16 (s, 2H, C4-CH₂), 6.35 (s, 1H, C3-H), 6.70–8.25 (m, 5H, Ar-H), 8.51 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₁₉H₁₈N₄O₄S (%): Calcd. C, 57.27; H, 4.55; N, 14.06. Found: C, 57.12; H, 4.48; N, 13.97.

5.1.4.7. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (2-methoxy-phenyl)-amide (5g). Black solid; yield 75%; m.p. 206 °C; IR (KBr) cm⁻¹ 1739 (C=O), 2143 (N₃), 3433 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.68 (s, 3H, C7-CH₃), 3.82 (s, 3H, 2-OCH₃ of phenyl), 4.66 (s, 2H, C4-CH₂), 6.27 (s, 1H, C3-H), 6.47–8.28 (m, 6H, Ar-H), 8.41 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₁₈H₁₆N₄O₅S (%): Calcd. C, 53.99; H, 4.03; N, 13.99. Found: C, 53.90; H, 3.92; N, 13.94.

5.1.4.8. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (3-methoxy-phenyl)-amide (5h). Green solid; yield 77%; m.p. 119 °C; IR (KBr) cm⁻¹ 1725 (C=O), 2123 (N₃), 3372 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.66 (s, 3H, C7-CH₃), 3.58 (s, 3H, 3-OCH₃ of phenyl), 4.55 (s, 1H, NH, D₂O exchangeable), 4.93 (s, 2H, C4-CH₂), 6.38 (s, 1H, C3-H), 7.65 (s, 1H, C8-H), 6.61–8.21 (m, 4H, Ar-H), 8.38 (s, 1H, C5-H); LC-MS 401 (M + H). Anal. Calcd for C₁₈H₁₆N₄O₅S (%): Calcd. C, 53.99; H, 4.03; N, 13.99. Found: C, 53.90; H, 3.95; N, 13.92.

5.1.4.9. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (4-methoxy-phenyl)-amide (5i). Green solid; yield 74%; m.p. 200 °C; IR (KBr) cm⁻¹ 1714 (C=O), 2123 (N₃), 3356 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.67 (s, 3H, C7-CH₃), 3.39 (s, 3H, 4-OCH₃ of phenyl), 4.83 (s, 2H, C4-CH₂), 6.58 (s, 1H, C3-H), 7.05–7.48 (m, 5H, Ar-H), 7.57 (s, 1H, NH, D₂O exchangeable), 8.14 (s, 1H, C5-H); LC-MS 401 (M + H). Anal. Calcd for C₁₈H₁₆N₄O₅S (%): Calcd. C, 53.99; H, 4.03; N, 13.99. Found: C, 53.94; H, 3.99; N, 13.94.

5.1.4.10. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (4-ethoxy-phenyl)-amide (5j). Green solid; yield 70%; m.p. 191 °C; IR (KBr) cm⁻¹ 1705 (C=O), 2115 (N₃), 3325 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 1.31 (t, 3H, CH₃ of C₂H₅), 2.64 (s, 3H, C7-CH₃), 3.46 (q, 2H, CH₂ of C₂H₅), 4.51 (s, 2H, C4-CH₂), 6.37 (s, 1H, C3-H), 6.51–7.34 (m, 5H, Ar-H), 7.57 (s, 1H, NH, D₂O exchangeable), 8.35 (s, 1H, C5-H); Anal. Calcd for C₁₉H₁₈N₄O₅S (%): Calcd. C, 55.06; H, 4.38; N, 13.52. Found: C, 55.00; H, 4.29; N, 13.48.

5.1.4.11. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (2-nitro-phenyl)-amide (5k). Yellow solid; yield 64%; m.p. 101 °C; IR (KBr) cm⁻¹ 1725 (C=O), 2122 (N₃), 3351 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.68 (s, 3H, C7-CH₃), 4.98 (s, 2H, C4-CH₂), 6.63 (s, 1H, C3-H), 6.91–8.09 (m, 5H, Ar-H), 8.24 (s, 1H, C5-H), 8.40 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₁₇H₁₃N₅O₆S (%): Calcd. C, 49.16; H, 3.15; N, 16.86. Found: C, 49.09; H, 3.08; N, 16.73.

5.1.4.12. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (3-nitro-phenyl)-amide (5l). Yellow solid; yield 62%; m.p. 101 °C; IR (KBr) cm⁻¹ 1727 (C=O), 2099 (N₃), 3344 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.50 (s, 3H, C7-CH₃), 4.80 (s, 2H, C4-CH₂), 6.20 (s, 1H, C3-H), 6.30–9.17 (m, 7H, Ar-H and NH). Anal. Calcd for C₁₇H₁₃N₅O₆S (%): Calcd. C, 49.16; H, 3.15; N, 16.86. Found: C, 49.02; H, 3.01; N, 16.81.

5.1.4.13. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (4-nitro-phenyl)-amide (5m). Yellow solid; yield 61%; m.p. 165 °C; IR (KBr) cm⁻¹ 1726 (C=O), 2123 (N₃), 3431 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.68 (s, 3H, C7-CH₃), 4.98 (s, 2H, C4-CH₂), 6.58 (s, 1H, C3-H), 6.81–7.95 (m, 5H, Ar-H), 8.24 (s, 1H, C5-H), 8.40 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₁₇H₁₃N₅O₆S (%): Calcd. C, 49.16; H, 3.15; N, 16.86. Found: C, 49.10; H, 3.08; N, 16.78.

5.1.4.14. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (2-chloro-phenyl)-amide (5n). Brown solid; yield 65%; m.p. 184 °C; IR (KBr) cm⁻¹ 1728 (C=O), 2125 (N₃), 3409 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.67 (s, 3H, C7-CH₃), 4.97 (s, 2H, C4-CH₂), 6.63 (s, 1H, C3-H), 6.80–7.68 (m, 5H, Ar-H), 8.23 (s, 1H, C5-H), 8.39 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₁₇H₁₃ClN₄O₄S (%): Calcd. C, 50.44; H, 3.24; N, 13.84. Found: C, 50.32; H, 3.16; N, 13.78.

5.1.4.15. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (3-chloro-phenyl)-amide (5o). Grey solid; yield 61%; m.p. 196 °C; IR (KBr) cm⁻¹ 1719 (C=O), 2128 (N₃), 3395 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.68 (s, 3H, C7-CH₃), 4.98 (s, 2H, C4-CH₂), 6.34 (s, 1H, C3-H), 6.58–7.69 (m, 5H, Ar-H), 8.24 (s, 1H, C5-H), 8.38 (s, 1H, NH, D₂O exchangeable); LC-MS 405 (M + H). Anal. Calcd for C₁₇H₁₃ClN₄O₄S (%): Calcd. C, 50.44; H, 3.24; N, 13.84. Found: C, 50.38; H, 3.20; N, 13.80.

5.1.4.16. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (4-chloro-phenyl)-amide (5p). Colorless solid; yield 63%; m.p. 140 °C; IR (KBr) cm⁻¹ 1725 (C=O), 2122 (N₃), 3395 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.66 (s, 3H, C7-CH₃), 4.93 (s, 2H, C4-CH₂), 6.62 (s, 1H, C3-H), 7.10–7.65 (m, 5H, Ar-H), 8.22 (s, 1H, C5-H), 8.36 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₁₇H₁₃ClN₄O₄S (%): Calcd. C, 50.44; H, 3.24; N, 13.84. Found: C, 50.39; H, 3.20; N, 13.76.

5.1.4.17. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (2,4-dichloro-phenyl)-amide (5q). Black solid; yield 67%; m.p. 165 °C; IR (KBr) cm⁻¹ 1726 (C=O), 2121 (N₃), 3429 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.68 (s, 3H, C7-CH₃), 4.98 (s, 2H, C4-CH₂), 6.63 (s, 1H, C3-H), 6.81–7.71 (m, 4H, Ar-H), 8.24 (s, 1H, C5-H), 8.40 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₁₇H₁₂Cl₂N₄O₄S (%): Calcd. C, 46.48; H, 2.75; N, 12.75. Found: C, 46.41; H, 2.71; N, 12.70.

5.1.4.18. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (3,4-dichloro-phenyl)-amide (5r). Grey solid; yield 62%; m.p. 196 °C; IR (KBr) cm⁻¹ 1726 (C=O), 2123 (N₃), 3374 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.68 (s, 3H, C7-CH₃), 4.98 (s, 2H, C4-CH₂), 6.63 (s, 1H, C3-H), 6.81–7.68 (m, 4H, Ar-H), 8.23 (s, 1H, C5-H), 8.37 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₁₇H₁₂Cl₂N₄O₄S (%): Calcd. C, 46.48; H, 2.75; N, 12.75. Found: C, 46.45; H, 2.69; N, 12.67.

5.1.4.19. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (4-bromo-phenyl)-amide (5s). Brown solid; yield 58%; m.p. 175 °C; IR (KBr) cm⁻¹ 1709 (C=O), 2112 (N₃), 3354 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.65 (s, 3H, C7-CH₃), 4.91 (s, 2H, C4-CH₂), 6.38 (s, 1H, C3-H), 6.61–8.38 (m, 7H, Ar-H and NH). Anal. Calcd for C₁₇H₁₃BrN₄O₄S (%): Calcd. C, 45.45; H, 2.92; N, 12.47. Found: C, 45.37; H, 2.88; N, 12.37.

5.1.4.20. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (3-chloro-4-methyl-phenyl)-amide (5t). Yellow solid; yield 60%; m.p. 154 °C; IR (KBr) cm⁻¹ 1726 (C=O), 2135 (N₃), 3374 (NH); ¹H NMR (DMSO, 300 MHz, TMS): δ 2.15 (s, 3H, 4-CH₃ of phenyl), 2.68 (s, 3H, C7-CH₃), 4.98 (s, 2H, C4-CH₂), 6.34 (s, 1H, C3-H), 6.56–7.75 (m, 4H, Ar-H), 8.24 (s, 1H, C5-H), 8.39 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₁₈H₁₅ClN₄O₄S (%): Calcd. C, 51.62; H, 3.61; N, 13.38. Found: C, 51.58; H, 3.55; N, 13.29.

5.1.4.21. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (2-chloro-5-nitro-phenyl)-amide (**5u**). Yellow solid; yield 58%; m.p. 153 °C; IR (KBr) cm^{-1} 1729 (C=O), 2122 (N_3), 3429 (NH); ^1H NMR (DMSO, 300 MHz, TMS): δ 2.68 (s, 3H, C7-CH₃), 4.99 (s, 2H, C4-CH₂), 6.06 (s, 1H, C3-H), 6.65–7.62 (m, 4H, Ar-H), 8.23 (s, 1H, C5-H), 8.41 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₁₇H₁₂ClN₅O₆S (%): Calcd. C, 45.39; H, 2.69; N, 15.57. Found: C, 45.32; H, 2.61; N, 15.50.

5.1.4.22. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (4-chloro-2-nitro-phenyl)-amide (**5v**). Yellow solid; yield 56%; m.p. 189 °C; IR (KBr) cm^{-1} 1726 (C=O), 2122 (N_3), 3430 (NH); ^1H NMR (DMSO, 300 MHz, TMS): δ 2.68 (s, 3H, C7-CH₃), 4.98 (s, 2H, C4-CH₂), 6.63 (s, 1H, C3-H), 6.81–7.69 (m, 4H, Ar-H), 7.93 (s, 1H, NH, D₂O exchangeable), 8.23 (s, 1H, C5-H). Anal. Calcd for C₁₇H₁₂ClN₅O₆S (%): Calcd. C, 45.39; H, 2.69; N, 15.57. Found: C, 45.35; H, 2.64; N, 15.54.

5.1.4.23. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (4-methyl-2-nitro-phenyl)-amide (**5w**). Yellow solid; yield 59%; m.p. 179 °C; IR (KBr) cm^{-1} 1725 (C=O), 2122 (N_3), 3347 (NH); ^1H NMR (DMSO, 300 MHz, TMS): δ 2.18 (s, 3H, 4-CH₃ of phenyl), 2.67 (s, 3H, C7-CH₃), 4.97 (s, 2H, C4-CH₂), 6.63 (s, 1H, C3-H), 6.80–7.68 (m, 4H, Ar-H), 7.74 (s, 1H, NH, D₂O exchangeable), 8.23 (s, 1H, C5-H). Anal. Calcd for C₁₈H₁₅N₅O₆S (%): Calcd. C, 50.35; H, 3.52; N, 16.31. Found: C, 50.29; H, 3.41; N, 16.27.

5.1.4.24. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid (2-methyl-4-nitro-phenyl)-amide (**5x**). Yellow solid; yield 60%; m.p. 110 °C; IR (KBr) cm^{-1} 1720 (C=O), 2121 (N_3), 3360 (NH); ^1H NMR (DMSO, 300 MHz, TMS): δ 2.08 (s, 3H, 2-CH₃ of phenyl), 2.65 (s, 3H, C7-CH₃), 5.39 (s, 2H, C4-CH₂), 6.57 (s, 1H, C3-H), 6.72–8.27 (m, 6H, Ar-H and NH). Anal. Calcd for C₁₈H₁₅N₅O₆S (%): Calcd. C, 50.35; H, 3.52; N, 16.31. Found: C, 50.11; H, 3.29; N, 16.12.

5.1.4.25. 4-Azidomethyl-7-methyl-coumarin-6-sulfonic acid naphthalen-1-ylamide (**5y**). Grey solid; yield 64%; m.p. 199 °C; IR (KBr) cm^{-1} 1729 (C=O), 2128 (N_3), 3410 (NH); ^1H NMR (DMSO, 300 MHz, TMS): δ 2.69 (s, 3H, C7-CH₃), 4.98 (s, 2H, C4-CH₂), 6.39–8.05 (m, 9H, Ar-H), 8.24 (s, 1H, C5-H), 8.55 (s, 1H, NH, D₂O exchangeable). Anal. Calcd for C₂₁H₁₆N₄O₄S (%): Calcd. C, 59.99; H, 3.84; N, 13.33. Found: C, 59.85; H, 3.78; N, 13.27.

5.2. Anti-bacterial screening

Standard strains were procured from the microbial type culture collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. The anti-bacterial activity of the synthesized compounds **2**, **3**, **5a–y** were performed *in vitro* against (i) Gram-positive bacteria: *S. faecalis* (MTCC 3382), *S. aureus* (MTCC 3160), *B. subtilis* (MTCC 297) and (ii) Gram-negative bacteria: *P. aeruginosa* (MTCC 1034), *K. pneumoniae* (MTCC 3384) and *E. coli* (MTCC 1089) by broth micro dilution method [29].

The MIC determination of the tested compounds was investigated in comparison with Ciprofloxacin. Double dilutions of the test compounds and reference drugs were prepared in Muller-Hinton agar. 10 mg of each test compounds were dissolved in 1 ml of dimethyl sulfoxide (DMSO) separately to prepare stock solution. Further, progressive dilutions with melted Mueller-Hinton agar were performed to obtain the required concentrations of 500, 250, 125, 62.5, 31.25, 16, 8, 4, 2, 1 $\mu\text{g}/\text{ml}$. The Petri dishes were inoculated with $1\text{--}5 \times 10^4$ colony forming units (cfu/ml) and incubated at 37 °C for 18 hours. The minimum inhibitory concentration (MIC) was the lowest concentration of the tested compounds that yield no visible growth on the plate was recorded in Table 1. To ensure that the solvent had no effect on the bacterial growth, a control was

performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments.

5.3. Anti-fungal screening

The anti-fungal activity was performed against the following standard fungal strains: *C. albicans*, *A. fumigatus*, *A. niger*, *P. chrysogenum*, *M. fuscus* and *F. oxysporum*.

The MIC determination of the tested compounds was investigated in comparison with Fluconazole by broth micro dilution method [30]. Double dilutions of the test compounds and reference drugs were prepared in Sabouraud's dextrose broth. 10 mg of each test compounds were dissolved in 1 ml of dimethyl sulfoxide (DMSO) separately to prepare stock solution. Further progressive dilutions with Sabouraud's dextrose broth were performed to obtain the required concentrations of 500, 250, 125, 62.5, 31.25, 16, 8, 4, 2, 1 $\mu\text{g}/\text{ml}$. The Petri dishes were inoculated with $1\text{--}5 \times 10^4$ colony forming units (cfu/ml) and incubated at 25 °C for 48–72 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the tested compound that yield no visible growth on the plates. To ensure that the solvent had no effect on the fungal growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments. The results are incorporated in Table 2.

6. Conclusions

We have synthesized a series of coumarin sulfonamides bearing an azido group. The investigation of antimicrobial screening data reveals that the synthesized compounds showed significant antibacterial activity to both Gram-positive and Gram-negative species. In many cases these showed equivalent to Ciprofloxacin. On the other hand these compounds showed fungicidal activity which was higher than Fluconazole. As a result, we can say that these are better fungicidal when their antimicrobial activity is compared. Hence, it is concluded that there is ample scope for further study in developing these as good lead compounds in developing new antimicrobials.

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