Rahul P. Modh*, Amit C. Patel and Kishor H. Chikhalia*

Design, synthesis, antibacterial, and antifungal studies of novel 3-substituted coumarinyl-triazine derivatives

Abstract: A series of novel 3-substituted coumarinyl-triazine derivatives were designed and synthesized from 3-carboxycoumarins via consecutive Knoevenagel and Pinner reactions in water. All synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis. Further, *in vitro* screening was carried out against four bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella typhi*) and two fungal strains (*Aspergillus niger* and *Aspergillus clavatus*).

Keywords: antibacterial activity; antifungal activity; coumarins; oxadiazoles; triazines.

***Corresponding authors: Rahul P. Modh and Kishor H. Chikhalia,** Department of Chemistry, School of Sciences, Gujarat University, Navrangpura, Ahmedabad 380 009, Gujarat, India, e-mail: rahulmodh@gmail.com; chikhalia_kh@yahoo.com
Amit C. Patel: Department of Chemistry, Veer Narmad South Gujarat University, Surat 395 007, Gujarat, India

Introduction

The cure for expedient microbial infections has become essential and exigent due to the urgent multiplication of multiple drug-resistant organisms [1, 2]. Therefore, there is an imperative need to develop new classes of derivatives which can remain unaffected by the pathogen-resistance mechanism. Many coumarin-containing compounds are known to exhibit medicinally useful properties, including anti-HIV, anticancer, antifungal, antibacterial, and anticoagulant activities [3–8]. Similarly, *s*-triazine derivatives play an important role in the field of medicinal chemistry as they display various pharmacological properties such as antimicrobial [9–11], antimycobacterial [12], anticancer [13, 14], and antiviral properties [15]. Moreover, the 1,3,4-oxadiazole system has been the focus of considerable attention because of its pharmacological importance in anti-bacterial [16–22] and anti-fungal agents [23–25].

In consideration of the above rationale and in continuation with our ongoing program [26–28] to find new leads with potential biological activities, a series of compounds **6a–m** have been synthesized and screened for antimicrobial activities. This novel series of 3-substituted coumarin derivatives is structurally related to ritonavir analogs [29] and diaryl triazines (DATA) (Figure 1) [30]. In the present study, we designed and synthesized 3-substituted coumarinyl-triazine derivatives and studied the effect of various substituents. The aim was to find an antimicrobial agent with enhanced activity.

Results and discussion

Chemistry

Scheme 1 outlines the synthetic pathway used to obtain compounds **6a–m**. The starting material 2-oxo-2*H*-chromene-3-carboxylic acid (**1**) was prepared from 2-hydroxybenzaldehyde and malononitrile via consecutive Knoevenagel and Pinner reactions in water [31]. Chlorination of compound **1** with thionyl chloride followed by condensation with hydrazine hydrate of the resultant acid chloride yielded the 2-oxo-2*H*-chromene-3-carbohydrazide (**2**). The resulting carbohydrazide was cyclized using potassium hydroxide in the presence of carbon disulfide to form the oxadiazole derivative **3** [32]. Compound **4** was obtained in a good yield by the reaction of the oxadiazole intermediate product **3** with 2,4,6-trichloro-1,3,5-triazine in the presence of 10% sodium bicarbonate solution at 0–5°C in acetone. Compound **4** was then allowed to react with morpholine in acetone at 45–50°C to give the precursor **5** to the final desired products **6a–m**. The synthesis of compounds **6a–m** was confirmed by IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis.

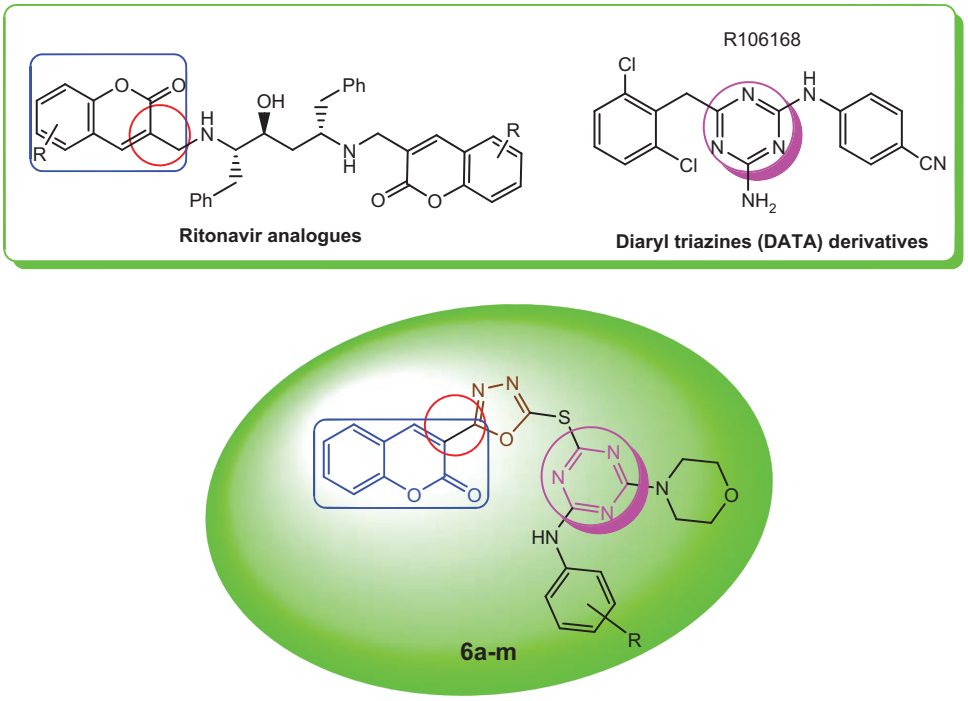
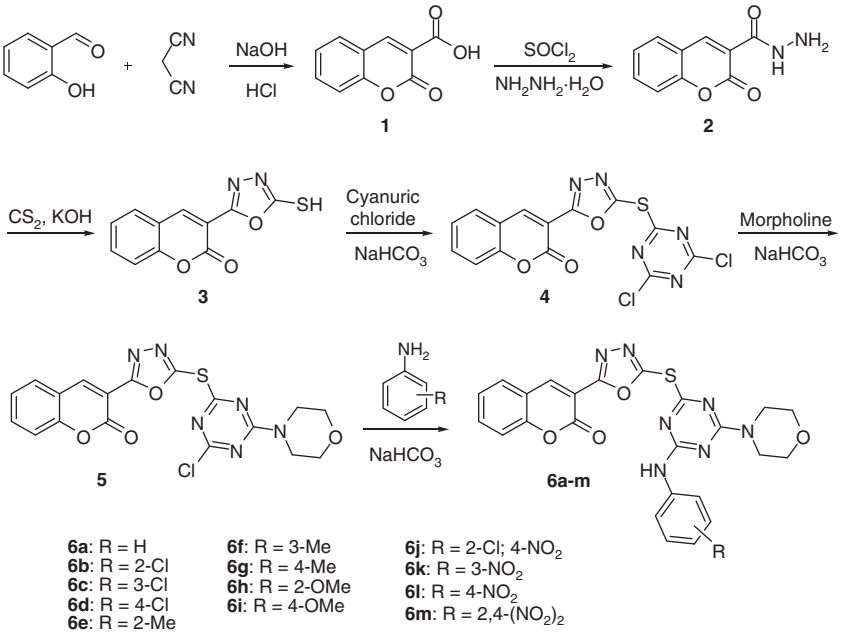


Figure 1 Analog-based design of coumarinyl-triazine derivatives.

Antimicrobial activity

The results of the antibacterial and antifungal activities of compounds **6a–m** are presented in Table 1. These results reveal that compounds **6b**, **6c**, **6i**, **6j**, and **6m** display significant activity against Gram +ve bacteria, *Staphylococcus aureus* and *Bacillus subtilis*. Compounds **6d**, **6e**, **6h**, **6k**, and **6l** show moderate to good inhibition, whereas



Scheme 1

Table 1 *In vitro* antibacterial and antifungal activities of coumarinyl-triazine derivatives **6a–m**.

***In vitro* activity zone of inhibition[a] in mm (MIC in μg/mL)[b]**												
Compound no.	**R**	**Gram +ve**				**Gram -ve**				**Fungus**		
		S. aureus		***B. subtilis***		***E. coli***		***S. typhi***		***A. niger***		***A. clavatus***
		MTCC 96		**MTCC 2388**		**MTCC 739**		**MTCC 733**		**MTCC 282**		**MTCC 1323**
6a	H	18	(400)	20	(400)	16	(200)	15	(200)	15	(400)	10	(200)
6b	2-Cl	28	(25)	26	(25)	25	(50)	20	(400)	18	(200)	20	(200)
6c	3-Cl	27	(50)	27	(100)	19	(200)	21	(400)	21	(100)	19	(200)
6d	4-Cl	22	(200)	22	(200)	23	(200)	16	(200)	22	(200)	15	(200)
6e	2-CH_3	23	(400)	–		17	(100)	17	(200)	15	(400)	13	(200)
6f	3-CH_3	19	(400)	21	(400)	15	(200)	12	(200)	10	(200)	11	(200)
6g	4-CH_3	10	(400)	12	(400)	22	(100)	18	(100)	12	(400)	09	(200)
6h	2-OCH_3	25	(200)	22	(100)	20	(25)	18	(100)	21	(100)	16	(200)
6i	4-OCH_3	26	(50)	26	(50)	25	(12.5)	20	(25)	21	(200)	20	(200)
6j	2-Cl,4-NO_2	28	(25)	28	(25)	25	(50)	22	(12.5)	23	(100)	21	(100)
6k	3-NO_2	24	(400)	23	(200)	18	(200)	20	(100)	22	(200)	15	(200)
6l	4-NO_2	22	(200)	21	(200)	23	(200)	23	(200)	19	(200)	14	(200)
6m	2,4-$(NO_2)_2$	27	(50)	26	(100)	25	(12.5)	18	(200)	22	(100)	22	(100)
Penicillin[c]	–	30	(3.12)	28	(1.56)	26	(6.25)	23	(12.5)		–		–
Streptomycin[c]	–	28	(6.25)	24	(6.25)	23	(3.12)	26	(3.12)		–		–
Griseofulvin[c]	–		–		–		–		–	24	(100)	23	(100)

[a]Zone of inhibition for coumarin derivatives (conc. 100 μg/disc).
[b]MIC values are given in parentheses. MIC (μg/mL) = minimum inhibitory concentration.
[c]Standard.

compounds **6a**, **6f**, and **6g** are definitely less active. Compounds **6b**, **6h**, **6i**, **6j**, and **6m** display good activity against Gram -ve bacteria, *Escherichia coli* and *Salmonella typhi*, and compounds **6c**, **6e**, **6g**, **6k**, and **6l** show moderate to good inhibition.

Compounds **6c**, **6h**, **6j**, and **6m** display excellent inhibition against fungi *Aspergillus niger*. Compounds **6b**, **6d**, **6i**, **6k**, and **6l** show moderate to good inhibition. Compounds **6a**, **6e**, **6f**, and **6g** show weaker activity towards tested fungi. Among the synthesized compounds, **6j** and **6m** exhibit good activity against fungi *Aspergillus clavatus*. Compounds **6b**, **6c**, **6d**, and **6i** show moderate to good activity against fungi.

Conclusion

New coumarinyl-triazine derivatives were synthesized. *In vitro* antibacterial and antifungal activities against two Gram -ve (*E. coli*, *S. typhi*), two Gram +ve (*S. aureus* and *B. subtilis*) bacterial and two fungal strains (*A. niger* and *A. clavatus*) were assayed. Compounds **6b** and **6j** exhibit good activity against both Gram +ve and -ve strains. Compounds **6j** and **6m** exhibit good activity against fungi.

Experimental

General

Melting points were determined on an electrothermal apparatus (capillary method) and are uncorrected. The IR spectra (4000–400 cm^{-1}) were recorded on a Shimadzu 8400-S FT-IR spectrophotometer with KBr pellets. ^1H NMR spectra were recorded in DMSO-d_6 on a Bruker Avance II 400 MHz and ^{13}C NMR spectra were recorded in DMSO-d_6 at 100 MHz. Mass spectra were recorded on a Finnegan Mat SSQ 7000 mode EI 70 eV. Elemental analyses were carried out on 'Haraeus Rapid Analyzer'. To monitor the reactions, thin layer chromatography (TLC) was performed on silica gel coated (Merck Kiesel 60 GF-254, 0.2 mm thickness) sheets.

Preparation of 2-oxo-2*H*-chromene-3-carboxylic acid (1)

A mixture of salicylaldehyde (12.2 g, 0.1 mol), malononitrile (6.6 g, 0.1 mol), and 0.025 M aqueous NaOH solution (pH 12.4, 50.0 mL) was vigorously stirred at room temperature and then heated under reflux for 2 h. 3-Cyano-2-iminocoumarin was separated. Then, the solution was treated with concentrated HCl (1.25–2.0 mL), and the resultant heterogeneous mixture was heated at 90°C with stirring for 1.5 to 2.5 h. After cooling, the mixture was treated with aqueous NaOH solution (1.5 M, 20 mL). After removal of a solid precipitate of 3-cyanocoumarin, the solution was heated at 90°C and stirred for 2 h.

The final solution was cooled to room temperature and acidified with concentrated HCl to pH ≤ 2. The resultant product was separated. The progress of the reaction was monitored by TLC using acetone/toluene (8:2) as eluent. The crude product was purified by crystallization from absolute ethanol: yield 85%; mp 184–186°C (lit. mp 189–192°C, [31]).

Preparation of 2-oxo-2*H*-chromene-3-carbohydrazide (2)

A mixture of 2-oxo-2*H*-chromene-3-carboxylic acid (**1**, 19.0 g, 0.1 mol) and thionyl chloride (17.8 g, 0.15 mol) was heated under reflux for 1–2 h. Excess of thionyl chloride was distilled off and the residue was cooled in an ice-bath. The resulting precipitate was dissolved in methanol and the solution was treated immediately and slowly with hydrazine hydrate (4.8 g, 0.1 mol) with constant stirring. The mixture was refluxed for 4 to 5 h. Excess solvent was removed by distillation under reduced pressure and the residue was poured into ice-cold water. The resultant solid was filtered, dried, and crystallized from absolute ethanol: yield 89%; mp 140–142°C (lit. mp 137–140°C, [25]); ^{1}H NMR: δ 8.24 (1H, s, H-4, coumarin), 8.12 (m, 1H, CONHNH$_2$), 7.62–7.39 (4H, m, Ar-H), 4.78 (s, 2H, NH$_2$, D$_2$O exchangeable).

Preparation of 3-(5-mercapto-1,3,4-oxadiazole-2-yl)-2*H*-chromene-2-one (3)

The mixture of 2-oxo-2*H*-chromene-3-carbohydrazide (**2**, 20.4 g, 0.1 mol), CS$_2$ (7.6 mL, 0.1 mol), and KOH solution (10 mL, 0.05 mol) in methanol (82 mL) was heated under reflux for 6 to 8 h. Then, the mixture was poured into crushed ice. The product was filtered, washed with water, and crystallized from ethanol. The progress of the reaction was monitored by TLC using acetone/toluene (8:2) as eluent. Product **3** was obtained in a 73% yield; mp 163–166°C (lit. mp 166–168°C, [25]); IR (cm^{-1}): 2591(SH), 1726 (CO, coumarin), 1629, 1531 (C=N, oxadiazole), 1043 (C-O-C, oxadiazole); ^{1}H NMR: δ 14.51 (s, 1H, SH), 8.21 (1H, s, H-4, coumarin), 7.58–7.37 (m, 4H, Ar-H).

Preparation of 3-(5-(4,6-dichloro-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-2*H*-chromene-2-one (4)

To a stirred solution of cyanuric chloride (18.4 g, 0.1 mol) in acetone (92 mL) at 0–5°C, the solution of 3-(5-mercapto-1,3,4-oxadiazole-2-yl)-2*H*-chromene-2-one (**3**, 24.6 g, 0.1 mol) in acetone (112 mL) was added and pH was maintained neutral by addition of 10% sodium bicarbonate solution. The stirring was continued at 0–5°C for 4 h. After completion of the reaction, the stirring was stopped and the solution was treated with crushed ice. The solid product obtained was filtered and dried. The progress of the reaction was monitored by TLC using ethyl acetate/hexane (6:4) as eluent. The crude product was purified by crystallization from absolute ethanol. Product **4** was obtained in an 80% yield; mp 179–182°C (lit. mp 178–180°C, [25]); IR (cm^{-1}): 1725 (C=O), 1624, 1527 (C=N, oxadiazole), 1040 (C-O-C, oxadiazole), 696 (C-S-C), 833 (s-triazine), 752 (C-Cl.); ^{1}H NMR: δ 8.30 (1H, s, H-4, coumarin), 7.51–7.38 (m, 4H, Ar–H).

Preparation of 3-(5-(4-chloro-6-morpholino-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-2*H*-chromene-2-one (5)

The solution of morpholine (8.7 g, 0.1 mol) in acetone was added dropwise to well-stirred suspension of 3-(5-(4,6-dichloro-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-2*H*-chromene-2-one (**4**, 39.4 g, 0.1 mol) in acetone (90.0 mL) maintaining the temperature below 40–45°C. The pH was kept neutral by the addition of 10% NaHCO$_3$ solution. The temperature was gradually raised to 50°C during 2 h and further maintained for 2 h. After completion of the reaction, the solution was poured into ice-cold water. The solid product was filtered, dried, and crystallized from absolute ethanol. Compound **5** was obtained in an 81% yield; mp 212–214°C; IR (cm^{-1}): 1741 (C=O, coumarin), 1619, 1540 (C=N, oxadiazole), 1256 (C-O-C), 1041 (C-O-C, oxadiazole), 829 (s-triazine), 749 (C-Cl), 701 (C-S-C); ^{1}H NMR: δ 8.20 (s, 1H, NH), 8.38 (s, 1H, Ar-H), 6.6–8.04 (m, 4H, Ar-H), 3.17 (t, 4H, *J* = 7.1 Hz, -CH$_2$), 3.33 (t, 4H, *J* = 7.1 Hz, CH$_2$); MS: m/z 445 (M$^+$). Anal. Calcd for $C_{18}H_{13}ClN_6O_4S$ (444.85): C, 48.60; H, 2.95; N, 18.89. Found: C, 48.72; H, 2.91; N, 18.93.

General procedure for the preparation of compounds 6a–m

The mixture of 3-(5-(4-chloro-6-morpholino-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-2*H*-chromene-2-one (**5**, 5 mmol) and the respective substituted arylamine (5 mmol) in 1,4-dioxane (50.0 mL) was heated under reflux with stirring at 80–100°C for 4 h. After completion of the reaction, the pH was adjusted to neutral by the addition of 10% NaHCO$_3$ solution and the mixture was poured into ice-cold water. The solid product **6a–m** was filtered, dried, and crystallized from absolute ethanol.

3-(5-(4-Morpholino-6-anilino-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-2*H*-chromene-2-one (6a) Yield 68%; mp 118–120°C (from ethanol); IR (cm^{-1}): 1725 (C=O), 1540 (C=N), 825 (C=N), 1210 (C-O-C, oxadiazole), 3410, 1675 (NH), 1032 (C-O-C, ether), 2955 (CH), 1470 (CH); ^{1}H NMR: δ 9.18 (s, 1H, NH), 8.38 (s, 1H, Ar-H), 7.40–7.82 (m, 4H, Ar-H), 7.63 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.20 (d, 2H, *J* = 7.5 Hz, Ar-H), 6.81 (s, 1H, Ar-H), 3.12 (t, 4H, *J* = 7.1 Hz, CH$_2$), 3.32 (t, 4H, *J* = 7.1 Hz, CH$_2$); ^{13}C NMR: δ 161.2 (C=O, coumarin), 146 (CH=C, coumarin), 116, 124.8, 126, 127, 128.3, 129.4, 153.6 (7C, coumarin), 161.4, 163.2 (2C, C-O-C, oxadiazole), 178.4 (C-N, morpholine), 166.7 (C-NH, amino linkage), 182.2 (C-S), 116.4, 117.6, 122.3, 126.4, 126.5, 142.6 (6C, aromatic amine), 48, 52, 57, 63 (4C, morpholine); MS: m/z 501 (M$^+$). Anal. Calcd for $C_{24}H_{19}N_7O_4S$ (501.12): C, 57.48; H, 3.82; N, 19.55. Found: C, 57.45; H, 3.85; N, 19.57.

3-(5-(4-(2-Chloroanilino)-6-morpholino-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-2*H*-chromene-2-one (6b) Yield 60%; mp 141–143°C (from ethanol); IR (cm^{-1}): 1700 (C=O), 1510 (C=N), 830 (C=N), 1212 (C-O-C, oxadiazole), 3410, 1675 (NH), 1032 (C-O-C, ether), 2955 (CH), 1470 (CH); ^{1}H NMR: δ 9.10 (s, 1H, NH), 8.42 (s, 1H, Ar-H), 6.75–8.04 (m, 8H, Ar-H), 3.12 (t, 4H, *J* = 7.1 Hz, -CH$_2$), 3.33 (t, 4H, *J* = 7.1 Hz, CH$_2$); ^{13}C NMR: δ 161.2 (1C of -C=O coumarin ring), 146 (1C -CH=C coumarin ring), 116, 125, 126, 127, 128, 129.2, 153.6 (7C, coumarin ring), 161.4, 163.2 (2C C-O-C oxadiazole ring), 178.4 (1C, s-triazine C-N of morpholine), 167.8 (1C, s-triazine C-NH of amino linkage), 181.6

(1C, s-triazine C-S mercapto oxadiazole), 116.4, 116.5, 128.4, 129.2, 135.5, 143.6 (6C, aromatic amine), 48.2, 54.3, 62.6, 63.4 (4C, morpholine); MS: m/z 535 (M^+); mp 141–143°C, Anal. Calcd for $C_{24}H_{18}N_7O_4SCl$ (535.96): C, 53.78; H, 3.39; N, 18.29. Found: C, 53.76; H, 3.37; N, 18.28.

3-(5-(4-(3-Chloroanilino)-6-morpholino-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-*2H*-chromene-2-one (6c) Yield 55%; mp 130–134°C (from ethanol); IR (cm^{-1}): 1705 (C=O), 1520 (C=N), 825 (C=N), 1215 (C-O-C, oxadiazole), 3410, 1675 (NH), 1002 (C-O-C, ether), 2955 (CH), 1470 (CH); ^{1}H NMR: δ 9.18 (s, 1H, NH), 8.42 (s, 1H, Ar-H), 6.81–7.90 (m, 8H, Ar-H), 3.11 (t, 4H, *J* = 7.1 Hz, CH_2), 3.32 (t, 4H, *J* = 7.1 Hz, CH_2); ^{13}C NMR: δ 161.2 (C=O, coumarin), 146 (CH=C, coumarin), 116, 125, 126, 127, 128.2, 129.1, 152.6 (7C, coumarin), 161.4, 164.2 (2C, C-O-C, oxadiazole), 178.4 (C-N, morpholine), 166.7 (C-NH, amino linkage), 182.2 (C-S), 117.4, 117.6, 129.4, 130.3, 135.5, 143.6 (6C, aromatic amine), 48.4, 54.5, 62.2, 63.7 (4C, morpholine); MS: m/z 535 (M^+). Anal. Calcd for $C_{24}H_{18}N_7O_4SCl$ (535.96): C, 53.78; H, 3.39; N, 18.29. Found: C, 53.76; H, 3.37; N, 18.27.

3-(5-(4-(4-Chloroanilino)-6-morpholino-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-*2H*-chromene-2-one (6d) Yield 64%; mp 160–164°C (from ethanol); IR (cm^{-1}): 1700 (C=O), 1500 (C=N), 825 (C=N), 1210 (C-O-C oxadiazole), 3406 (NH), 1671 (NH), 1032 (C-O-C, ether), 2960 (CH), 1475 (CH); ^{1}H NMR: δ 9.10 (s, 1H, NH), 8.20 (s, 1H, Ar-H), 7.45–8.04 (m, 4H, Ar-H), 7.42 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.24 (d, 2H, *J* = 7.5 Hz, Ar-H), 3.32 (t, 4H, *J* = 7.1 Hz, -CH_2), 3.11 (t, 4H, *J* = 7.1 Hz, CH_2); ^{13}C NMR: δ 161.2 (C=O), 146 (CH=C, coumarin), 116, 124.9, 126, 127, 128.1, 129.4, 153.6 (7C, coumarin), 161.4, 163.2 (2C, C-O-C oxadiazole), 178.4 (C-N, morpholine), 166.5 (C-NH, amino linkage), 182.2 (C-S), 117.4, 117.6, 122.3, 127.5, 127.4, 139.6 (6C, aromatic amine), 47, 48.4, 59.4, 63.5 (4C, morpholine); MS: m/z 535 (M^+). Anal. Calcd for $C_{24}H_{18}ClN_7O_4S$ (535.96): C, 53.78; H, 3.39; N, 18.29. Found: C, 53.77; H, 3.41; N, 18.26.

3-(5-(4-Morpholino-6-(*o*-tolylamino)-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-*2H*-chromene-2-one (6e) Yield 62%; mp 181–185°C (from ethanol); IR (cm^{-1}): 1725 (C=O), 1500 (C=N), 825 (C=N), 1210 (C-O-C), 3410, 1675 (NH), 1122 (C-O-C), 2955 (CH), 1470 (CH); ^{1}H NMR: δ 9.05 (s, 1H, NH), 8.42 (s, 1H, Ar-H), 6.70–7.79 (m, 8H, Ar-H), 3.11 (t, 4H, *J* = 7.1 Hz, CH_2), 3.29 (t, 4H, *J* = 7.1 Hz, CH_2), 1.93 (s, 3H, CH_3); ^{13}C NMR: δ 161.2 (C=O), 146 (CH=C), 116, 125.3, 126, 127, 128.6, 129.8, 153.6 (7C, coumarin), 161.4, 163.2 (2C, C-O-C oxadiazole), 178.4 (C-N, morpholine), 166.1 (C-NH, amino linkage), 182.2 (C-S),114.4, 114.3, 122.3, 126.4, 126.5, 139.3 (6C, aromatic amine), 46.4, 49.3, 58.4, 63.3 (4C, morpholine), 23.2 (CH_3); MS: m/z 515 (M^+). Anal. Calcd for $C_{25}H_{21}N_7O_4S$ (515.54): C, 58.24; H, 4.11; N, 19.02. Found: C, 58.21; H, 4.09; N, 19.04.

3-(5-(4-Morpholino-6-(*m*-tolylamino)-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-*2H*-chromene-2-one (6f) Yield 68%; mp 142–144°C (from ethanol); IR (cm^{-1}): 1725 (C=O), 1540 (C=N), 825 (C=N), 1210 (C-O-C), 3410, 1675 (NH), 1032 (C-O-C), 2955 (CH), 1470 (CH); ^{1}H NMR: δ 9.18 (s, 1H, NH), 8.42 (s, 1H, Ar-H), 6.59–7.85 (m, 8H, Ar-H), 3.12 (t, 4H, *J* = 7.1 Hz, CH_2), 3.32 (t, 4H, *J* = 7.1 Hz, CH_2), 1.90 (s, 3H, CH_3); ^{13}C NMR: δ 161.2 (C=O), 146 (CH=C), 116, 125.2, 126, 127, 128.4, 129.1, 153.6 (7C, coumarin), 161.4, 163.2 (2C, C-O-C), 178.4 (C-N, morpholine), 166.4 (C-NH, amino linkage), 182.2 (C-S), 117.4, 117.6, 122.3, 126.4, 126.5, 138.6 (6C, aromatic amine), 48.3, 54.3, 58.5, 64.2 (4C, morpholine), 23.4 (CH_3); MS: m/z 515 (M^+). Anal. Calcd for $C_{25}H_{21}N_7O_4S$ (515.54): C, 58.24; H, 4.11; N, 19.02. Found: C, 58.26; H, 4.13; N, 19.1.

3-(5-(4-Morpholino-6-(*p*-tolylamino)-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-*2H*-chromene-2-one (6g) Yield 59%; mp 131–133°C (from ethanol); IR (cm^{-1}): 1705 (C=O), 1559 (C=N-), 825 (C=N), 1210 (C-O-C), 3410, 1675 (NH), 1032 (C-O-C), 2955 (CH), 1470 (CH); ^{1}H NMR: δ 9.18 (s, 1H, NH), 8.42 (s, 1H, Ar-H), 6.8–8.04 (m, 4H, Ar-H), 7.24 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.09 (d, 2H, *J* = 7.5 Hz, Ar-H), 3.32 (t, 4H, *J* = 7.1 Hz, -CH_2), 3.10 (t, 4H, *J* = 7.1 Hz, CH_2), 1.90 (s, 3H, CH_3); ^{13}C NMR: δ 161.2 (C=O), 146 (CH=C, coumarin), 116, 124.9, 126, 127, 128, 129.2, 153.6 (7C, coumarin), 161.4, 163.2 (2C, C-O-C), 178.4 (C-N, morpholine), 166.8 (C-NH, amino linkage), 182.2 (C-S), 117.4, 117.6, 122.3, 126.4, 126.5, 138.6 (6C, aromatic amine), 47.2, 52.4, 60.6, 62.1 (4C, morpholine), 23.4 (CH_3); MS: m/z 515 (M^+). Anal. Calcd for $C_{25}H_{21}N_7O_4S$ (515.94): C, 58.24; H, 4.11; N, 19.02. Found: C, 58.24; H, 4.10; N, 18.89.

3-(5-(4-(2-Methoxyanilino)-6-morpholino-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-*2H*-chromene-2-one (6h) Yield 59%; mp 132–134°C (from ethanol); IR (cm^{-1}): 1710 (C=O), 1540 (C=N), 825 (C=N), 1210 (C-O-C), 3410, 1675 (NH), 1032 (C-O-C), 2955 (CH), 1470 (CH); ^{1}H NMR: δ 9.18 (s, 1H, NH), 8.42 (s, 1H, Ar-H), 7.42–7.84 (m, 4H, Ar-H), 6.52–6.85 (m, 4H, Ar-H), 3.34 (s, 3H of OCH_3), 3.32 (t, 4H, *J* = 7.1 Hz, CH_2), 3.12 (t, 4H, *J* = 7.1 Hz, CH_2); ^{13}C NMR: δ 161.2 (C=O, coumarin), 146 (CH=C, coumarin), 116, 125.2, 126, 127, 128.6, 129.5, 153.6 (7C, coumarin), 161.4, 163.2 (2C, C-O-C oxadiazole), 178.4 (C-N, morpholine), 167 (C-NH, amino linkage), 182.2 (C-S), 117.4, 117.6, 122.3, 126.4, 126.5, 138.6 (6C, aromatic amine) 45.3, 50.2, 54.8, 64.7 (4C, morpholine), 58.2 (OCH_3); MS: m/z 531 (M^+). Anal. Calcd for $C_{25}H_{21}N_7O_4S$ (515.94): C, 58.24; H, 4.11; N, 19.02; S, 6.03. Found: C, 58.24; H, 4.10; N, 18.99; S, 6.06.

3-(5-(4-(4-Methoxyanilino)-6-morpholino-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-*2H*-chromene-2-one (6i) Yield 62%; mp 154–157°C (from ethanol); IR (cm^{-1}): 1725 (C=O), 1540 (C=N), 825 (C=N), 1210 (C-O-C), 3410, 1675 (NH), 1032 (C-O-C), 2955 (CH), 1470 (CH); ^{1}H NMR: δ 9.21 (s, 1H, NH), 8.39 (s, 1H, Ar-H), 7.42–8.04 (m, 4H, Ar-H), 7.24 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.09 (d, 2H, *J* = 7.5 Hz, Ar-H), 3.09 (t, 4H, *J* = 7.1 Hz, -CH_2), 3.30 (t, 4H, *J* = 7.1 Hz, CH_2), 3.83 (s, 3H, OCH_3); ^{13}C NMR: δ 161.2 (C=O, coumarin), 146 (CH=C), 116, 124.8, 126, 127, 128.6, 129.4, 153.6 (7C, coumarin), 161.4, 163.2 (2C, C-O-C), 178.4 (C-N, morpholine), 166.6 (C-NH, amino linkage), 182.2 (C-S), 117.4, 117.6, 122.3, 126.4, 126.5, 138.6 (6C, aromatic amine), 45.6, 50.6, 54.2, 64.4 (4C, morpholine), 58.23 (OCH_3); MS: m/z 501 (M^+). Anal. Calcd for $C_{25}H_{21}N_7O_5S$: C, 56.49; H, 3.98; N, 18.45; S, 6.03. Found: C, 56.44; H, 3.99; N, 18.40; S, 6.00.

3-(5-(4-(2-Chloro-4-nitroanilino)-6-morpholino-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-*2H*-chromene-2-one (6j) Yield 43%; mp 152–156°C (from ethanol); IR (cm^{-1}): 1700 (C=O), 1549 (C=N), 820 (C=N), 1210 (C-O-C), 3400, 1670 (NH), 1232 (C-O-C), 2895 (CH), 1470 (CH); ^{1}H NMR: δ 9.05 (s, 1H, NH), 6.83 (s, 1H, Ar-H), 7.2–8.05 (m, 5H, Ar-H), 8.10 (d, 2H, *J* = 7.1 Hz, Ar-H), 3.20 (t, 4H, *J* = 7.1 Hz, CH_2), 3.32 (t, 4H, *J* = 7.1 Hz, CH_2); ^{13}C NMR: δ 161.2 (C=O, coumarin), 146 (CH=C, coumarin), 116, 125.2, 126, 127, 128.1, 129.6, 153.6 (7C, coumarin), 161.4, 163.2 (2C, C-O-C), 178.4 (C-N, morpholine), 166.6 (C-NH, amino linkage), 182.2 (C-S), 117.4, 120.6, 122.3, 126.4, 126.5, 138.6 (6C, aromatic amine), 48.3, 50.2, 60.2, 63.4 (4C, morpholine); MS: m/z 580 (M^+). Anal. Calcd for $C_{24}H_{17}ClN_8O_6S$ (580.96): C, 49.62; H, 2.95; N, 19.29. Found: C, 49.65; H, 2.93; N, 19.32.

3-(5-(4-Morpholino-6-(3-nitroanilino)-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-*2H*-chromene-2-one (6k) Yield 61%; mp 152–154°C (from ethanol); IR (cm^{-1}): 1705 (C=O), 1510 (C=N-), 820

(C=N), 1190 (C-O-C), 3400 (NH), 1675 (NH), 1120 (C-O-C), 2955 (CH), 1470 (CH); ^{1}H NMR: δ 9.18 (s, 1H, NH), 8.42 (s, 1H, Ar-H), 6.8–8.04 (m, 8H, Ar-H), 3.12 (t, 4H, *J* = 7.1 Hz, CH_2), 3.32 (t, 4H, *J* = 7.1 Hz, CH_2); ^{13}C NMR: δ 161.2 (C=O, coumarin), 146 (CH=C, coumarin), 115, 125.7, 126.4, 127, 128.5, 129.6, 153.6 (7C, coumarin), 161.4, 163.2 (2C, C-O-C, oxadiazole), 178.4 (C-N, morpholine), 166.3 (C-NH, amino linkage), 182.2 (C-S), 117.4, 122.3, 126.4, 126.5, 143.3, 148.4 (6C, aromatic amine), 47.2, 48.4, 57.5, 63.3 (4C, morpholine); MS: m/z 546 (M^+). Anal. Calcd for $C_{24}H_{18}N_8O_6S$ (546.51): C, 52.74; H, 3.32; N, 20.50; S, 5.87. Found: C, 52.69; H, 3.30; N, 20.48; S, 5.89.

3-(5-(4-Morpholino-6-(4-nitroanilino)-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-*2H*-chromene-2-one (6l) Yield 68%; mp 148–150°C (from ethanol); IR (cm^{-1}): 1735 (C=O-), 1540 (C=N), 825 (C=N), 1225 (C-O-C), 3410, 1675 (NH), 1032 (C-O-C), 2955, 1470 (CH); ^{1}H NMR: δ 9.18 (s, 1H, NH), 8.42 (s, 1H, Ar-H), 6.8–8.04 (m, 4H, Ar-H), 7.24 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.09 (d, 2H, *J* = 7.5 Hz, Ar-H), 3.14 (t, 4H, *J* = 7.1 Hz, CH_2), 3.33 (t, 4H, *J* = 7.1 Hz, CH_2); ^{13}C NMR: δ 161.2 (C=O, coumarin), 146 (CH=C, coumarin), 116.2, 125, 126, 127.2, 128.1, 129.3, 153.6 (7C, coumarin), 161.4, 163.2 (2C, C-O-C), 178.4 (C-N, morpholine), 166.5 (C-NH, amino linkage), 182.2 (C-S), 117.4, 122.3, 126.4, 126.5, 143.3, 148.4 (6C, aromatic amine), 47.8, 49.4, 59.3, 63.3 (4C, morpholine); MS: m/z 546 (M^+). Anal. Calcd for $C_{24}H_{18}N_8O_6S$ (546.51): C, 52.74; H, 3.32; N, 20.50; S, 5.87. Found: C, 52.68; H, 3.30; N, 20.46; S, 5.89.

3-(5-(4-Morpholino-6-(2,4-dinitroanilino)-1,3,5-triazin-2-ylthio)-1,3,4-oxadiazole-2-yl)-*2H*-chromene-2-one (6m) Yield 58%; mp 168–170°C (from ethanol); IR (cm^{-1}): 1712 (C=O), 1520 (C=N), 830 (C=N), 1210 (C-O-C), 3410, 1675 (NH), 1130 (C-O-C), 2905, 1470; ^{1}H NMR: δ 9.05 (s, 1H, NH), 8.85 (s, 1H, Ar-H), 7.1–8.04 (m, 5H, Ar-H), 8.40 (d, 1H, *J* = 1.5 Hz, Ar-H), 7.19 (m, 1H, Ar-H), 3.15 (t, 4H, *J* = 7.1 Hz, CH_2), 3.34 (t, 4H, *J* = 7.1 Hz, CH_2); ^{13}C NMR: δ 161.2 (C=O, coumarin), 146 (CH=C, coumarin), 114.9, 125.7, 126.2, 127.0, 128.3, 129.1, 153.6 (7C, coumarin), 161.4, 163.2 (2C, C-O-C), 178.4 (C-N, morpholine), 166.5 (C-NH, amino linkage), 182.2 (C-S), 117.4, 120.7, 126.4, 138.5, 138.6, 149.3 (6C, aromatic amine), 47.3, 51.2, 57.3, 62.5 (4C, morpholine); MS: m/z 591 (M^+). Anal. Calcd for $C_{24}H_{17}N_9O_8S$ (591.51): C, 48.73; H, 2.90; N, 21.31; S, 5.42. Found: C, 48.69; H, 2.88; N, 21.36; S, 5.40.

In vitro evaluation of antimicrobial activity

The synthesized coumarin derivatives **6a–m** were evaluated for antimicrobial activity against several bacteria (*S. aureus* MTCC 96, *Bacillus cereus* MTCC 619, *Escherichia coli* MTCC 739, *S. typhi* MTCC 733) and fungi (*A. niger* MTCC 282, *A. clavatus* MTCC 1323) species using the paper disc diffusion technique [33]. The Mueller-Hinton agar media were sterilized (autoclaved at 120°C for 30 min), poured at uniform depth of 5 mm, and allowed to solidify. The microbial suspension (105 CFU/mL) (0.5 McFarland Nephelometry Standards) was streaked over the surface of the media using a sterile cotton swab to ensure even growth of the organisms. The tested compounds were dissolved in dimethyl sulfoxide to give solutions of 3.12–100 μg/mL. Sterile filter paper discs measuring 6.25 mm in diameter (Whatman no. 1 filter paper), previously soaked in a known concentration of the respective test compound in dimethyl sulfoxide, were placed on the solidified nutrient agar medium that had been inoculated with the respective microorganism and the plates were incubated for 24 h at 37 ± 1°C. A control disc impregnated with an equivalent amount of dimethyl sulfoxide without any sample was also used and did not produce any inhibition. Penicillin and streptomycin (100 μg/disc) were used as control drugs for antibacterial and griseofulvin (100 μg/disc) for antifungal activity, respectively. Minimum inhibitory concentrations (MICs) of the compounds were determined by the agar streak dilution method [34]. A stock solution of the synthesized compound (100 μg/mL) in dimethyl sulfoxide was prepared and graded quantities of the test compounds were incorporated in a specified quantity of molten sterile agar, that is, nutrient agar for evaluation of antibacterial and sabouraud dextrose agar for antifungal activity, respectively. The medium containing the test compound was poured into a Petri dish at a depth of 4–5 mm and was allowed to solidify under aseptic conditions. A suspension of the respective microorganism of approximately 105 CFU/mL was prepared and applied to plates with serially diluted compounds with concentrations in the range of 3.12–100 μg/mL in dimethyl sulfoxide and incubated at 37 ± 1°C for 24 h (bacteria) or 48 h (fungi). The lowest concentration of the substance that prevents the development of visible growth was reported as the MIC value.

Acknowledgments: The authors are very thankful to Prof. Nisha K. Shah, Head, Department of Chemistry, School of Sciences, Gujarat University, Ahmedabad, India for her kind cooperation for providing support and research facilities and Prof. P.V. Desai, Microbiology Department, Veer Narmad South Gujarat University, Surat, India for antimicrobial screening. Mr. Rahul Modh would like to acknowledge UGC New Delhi for a Junior Research Fellowship.

Received July 3, 2013; accepted August 18, 2013

References

[1] Nathan, C. Antibiotics at the crossroads. *Nature* **2004**, *431*, 899–902.

[2] Spellberg, B.; Bartlett, J. G.; Gilbert, D. N. The future of antibiotics and resistance. *N. Engl. J. Med*. **2013**, *368*, 299–302.

[3] Murakami, A.; Gao, G.; Omura, M.; Yano, M.; Ito, C.; Furukawa, H.; Takahashi, D.; Koshimizu, K.; Ohigashi, H. 1,1-Dimethylallylcoumarins potently suppress both lipopolysaccharide- and interferon-γ-induced nitric oxide generation in mouse macrophage RAW 264.7 cells. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 59–62.

[4] Spino, C.; Dodier, M.; Sotheeswaran, S. Anti-HIV coumarins from calophyllum seed oil. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 3475–3478.

[5] Xia, Y.; Yang, Z. Y.; Xia, P.; Hackl, T.; Hamel, E.; Mauger, A.; Wu, J.-H.; Lee, K.-H. Antitumor agents. 211. Fluorinated

2-phenyl-4-quinolone derivatives as antimitotic antitumor agents 1. *J. Med. Chem*. **2001**, *44*, 3932–3936.

[6] Itoigawa, M.; Ito, C.; Tan, H. T. W.; Kuchide, M.; Tokuda, H.; Nishino, H.; Furukawa, H. Cancer chemopreventive agents, 4-phenylcoumarins from *Calophyllum inophyllum*. *Cancer Lett*. **2001**, *169*, 15–19.

[7] Yamaguchi, T.; Fukuda, T.; Ishibashi, F.; Iwao, M. The first total synthesis of lamellarin α 20-sulfate, a selective inhibitor of HIV-1 integrase. *Tetrahedron Lett*. **2006**, *47*, 3755–3757.

[8] Yamamoto, Y.; Kurazono, M. A new class of anti-MRSA and anti-VRE agents: preparation and antibacterial activities of indole-containing compounds. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 1626–1628.

[9] Singh, U.; Singh, R.; Bhat, H.; Subhashchandra, Y.; Kumar, V.; Kumawat, M.; Gahtori, P. Synthesis and antibacterial evaluation of series of novel tri-substituted *s*-triazine derivatives. *Med. Chem. Res*. **2011**, *20*, 1603–1610.

[10] Zhou, C.; Min, J.; Liu, Z.; Young, A.; Deshazer, H.; Gao, T.; Chang, Y.-T.; Kallenbach, N. R. Synthesis and biological evaluation of novel 1,3,5-triazine derivatives as antimicrobial agents. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 1308–1311.

[11] Srinivas, K.; Srinivas, U.; Bhanuprakash, K.; Harakishore, K.; Murthy, U. S. N.; Jayathirtha Rao, V. Synthesis and antibacterial activity of various substituted *s*-triazines. *Eur. J. Med. Chem*. **2006**, *41*, 1240–1246.

[12] Raval, J.; Patel, N.; Patel, H.; Patel, P. *In vitro* antimycobacterial activity of novel N′-(4-(substituted phenyl amino)-6-(pyridin-2-ylamino)-1,3,5-triazin-2-yl)isonicotinohydrazide. *Med. Chem. Res*. **2011**, *20*, 274–279.

[13] Menicagli, R.; Samaritani, S.; Signore, G.; Vaglini, F.; Dalla Via, L. *In vitro* cytotoxic activities of 2-alkyl-4,6-diheteroalkyl-1,3,5-triazines: new molecules in anticancer research. *J. Med. Chem*. **2004**, *47*, 4649–4652.

[14] Bakharev, V.; Gidaspov, A.; Yakunina, N.; Bulychev, Y. Synthesis and cytotoxic activity of trinitromethyl derivatives of 1,3,5-triazine. *Pharm. Chem. J*. **2008**, *42*, 241–244.

[15] Xiong, Y. Z.; Chen, F. E.; Balzarini, J.; De Clercq, E.; Pannecouque, C. Non-nucleoside HIV-1 reverse transcriptase inhibitors. Part 11: structural modulations of diaryltriazines with potent anti-HIV activity. *Eur. J. Med. Chem*. **2008**, *43*, 1230–1236.

[16] Desai, N.; Dodiya, A. Conventional and microwave techniques for synthesis and antimicrobial studies of novel 1-[2-(2-chloro(3-quinolyl))-5-(4-nitrophenyl)-(1,3,4-oxadiazolin-3-yl)]-3-(aryl)prop-2-en-1-ones. *Med. Chem. Res*. **2011**, *21*, 1480–1490.

[17] Bakht, M. A.; Yar, M. S.; Abdel-Hamid, S. G.; Al Qasoumi, S. I.; Samad, A. Molecular properties prediction, synthesis and antimicrobial activity of some newer oxadiazole derivatives. *Eur. J. Med. Chem*. **2010**, *45*, 5862–5869.

[18] Gupta, V.; Kashaw, S.; Jatav, V.; Mishra, P. Synthesis and antimicrobial activity of some new 3–[5-(4-substituted) phenyl-1,3,4-oxadiazole-2yl]-2-styrylquinazoline-4(3H)-ones. *Med. Chem. Res*. **2008**, *17*, 205–211.

[19] Chawla, R.; Arora, A.; Parameswaran, M.; Sharma, P. C.; Michael, S.; Ravi, T. K. Synthesis of novel 1,3,4-oxadiazole derivatives as potential antimicrobial agents. *Acta Pol. Pharm*. **2010**, *67*, 247–253.

[20] Jha, K. K.; Samad, A.; Kumar, Y.; Shaharyar, M.; Khosa, R. L.; Jain, J.; Kumar, V.; Singh, P. Design, synthesis and biological evaluation of 1,3,4-oxadiazole derivatives. *Eur. J. Med. Chem*. **2010**, *45*, 4963–4967.

[21] Şahin, G.; Palaska, E.; Ekizoğlu, M.; Özalp, M. Synthesis and antimicrobial activity of some 1,3,4-oxadiazole derivatives. *Il Farmaco* **2002**, *57*, 539–542.

[22] Cacic, M.; Trkovnik, M.; Cacic, F.; Has-Schon, E. Synthesis and antimicrobial activity of some derivatives on the basis (7-hydroxy-2-oxo-2H-chromene-4-yl)-acetic acid hydrazide. *Molecules* **2006**, *11*, 134–147.

[23] Chen, C.-J.; Song, B.-A.; Yang, S.; Xu, G.-F.; Bhadury, P. S.; Jin, L.-H.; Hu, D.-Y.; Li, Q.-Z.; Liu, F.; Xue, W.; Lu, P.; Chen, Z. Synthesis and antifungal activities of 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-thiadiazole and 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-oxadiazole derivatives. *Bioorg. Med. Chem*. **2007**, *15*, 3981–3989.

[24] Liu, F.; Luo, X.-Q.; Song, B.-A.; Bhadury, P. S.; Yang, S.; Jin, L.-H.; Xue, W.; Hu, D.-Y. Synthesis and antifungal activity of novel sulfoxide derivatives containing trimethoxyphenyl substituted 1,3,4-thiadiazole and 1,3,4-oxadiazole moiety. *Bioorg. Med. Chem*. **2008**, *16*, 3632–3640.

[25] Patel, R. V.; Patel, A. B.; Kumari, P.; Chikhalia, K. H. Synthesis of novel 3-(5-sulfanyl-1,3,4-oxadiazole-2-yl)-2*H*-chromene-2-one condensed *s*-triazinyl piperazines and piperidines as antimicrobial agents. *Med. Chem. Res*. **2012**, *21*, 3119–3132.

[26] Modh, R. P.; Clercq, E. D.; Pannecouque, C.; Chikhalia, K. H. Design, synthesis, antimicrobial activity and anti-HIV activity evaluation of novel hybrid quinazoline-triazine derivatives. *J. Enzyme Inhib. Med. Chem*. **2013**, [Epub ahead of print]; DOI: 10.3109/14756366.2012.755622.

[27] Modh, R. P.; Patel, A. C.; Chikhalia, K. H. Design, synthesis biological evaluation of some novel quinazolinone scaffolds. *Med. Chem*. **2012**, *8*, 182–192.

[28] Modh, R. P.; Patel, A. C.; Mahajan, D. H.; Pannecouque, C.; De Clercq, E.; Chikhalia, K. H. Synthesis and evaluation of novel 4-substituted styryl quinazolines as potential antimicrobial agents. *Arch. Pharm*. **2012**, *345*, 964–972.

[29] Kaye, P. T.; Musa, M. A.; Nchinda, A. T.; Nocanda, X. W. Novel heterocyclic analogues of the HIV-1 protease inhibitor, ritonavir. *Synth. Commun*. **2004**, *34*, 2575–2589.

[30] Ludovici, D. W.; Kavash, R. W.; Kukla, M. J.; Ho, C. Y.; Ye, H.; De Corte, B. L.; Andries, K.; de Béthune, M.-P.; Azijn, H.; Pauwels, R.; Moereels, H. E. L.; Heeres, J.; Koymans, L. M. H.; de Jonge, M. R.; Van Aken, K. J. A.; Daeyaert, F. F. D.; Lewi, P. J.; Das, K.; Arnold, E.; Janssen, P. A. J. Evolution of anti-HIV drug candidates part 2: diaryltriazine (DATA) analogues. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 2229–2234.

[31] Fringuelli, F.; Piermatti, O.; Pizzo, F. One-pot synthesis of 3-carboxycoumarins via consecutive Knoevenagel and Pinner reactions in water. *Synthesis* **2003**, *15*, 2331–2334.

[32] Patel, M. B.; Modi, N. R.; Raval, J. P.; Menon, S. K. Calix[4]arene based 1,3,4-oxadiazole and thiadiazole derivatives: design, synthesis, and biological evaluation. *Org. Biomol. Chem*. **2012**, *10*, 1785–1794.

[33] Gillespie, S. H. *Medical Microbiology Illustrated*; Butterworth Heinemann Ltd.: Oxford, **1994**.

[34] Hawkey, P.; Lewis, D. A. *Medical Bacteriology: A Practical Approach*; Oxford University Press: Oxford, **2004**.