Synthesis of Novel Quinolone and Coumarin Based 1,3,4-Thiadiazolyl and 1,3,4-Oxadiazolyl *N*-Mannich Bases as Potential Antimicrobials

Rahul V. Patel[a*], Jigar K. Patel[a], Premlata Kumari[a] and Kishor H. Chikhalia[b]

[a]*Department of Applied Chemistry, S. V. National Institute of Technology, Surat- 395 007, Gujarat, India*

[b]*Department of Chemistry, School of Science, Gujarat University, Ahmedabad-380 009, Gujarat, India*

Received February 29, 2012: Revised April 20, 2012: Accepted May 02, 2012

Abstract: Two series of 1,3,4-thiadiazole and 1,3,4-oxadiazole derivatives have been synthesized and characterized by elemental and spectral (IR, ^{1}H-NMR) data. The hydrazide derivatives of 4-hydroxy quinolone and coumarin moiety were refluxed in carbon disulfide in ethanolic potassium hydroxide to obtain the corresponding hydrazinecarbodithioate salts which were then treated in two ways with (i) sulfuric acid and (ii) hydrochloric acid at cooled temperature to furnish the corresponding 1,3,4-thiadiazole and 1,3,4-oxadiazole intermediates, respectively, which were then treated with piperazine bases in the presence of formalin in methanol to furnish the final *N*-Mannich products **7i-10vi**. The newer analogs were examined for their antimicrobial activity against five bacteria (*S. aureus* and *B. cereus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*) and two fungi (*A. niger* and *C. albicans*) and the results revealed that compounds demonstrated excellent activity (MICs: 3.12-25 μg/mL) as compared with the standards (MICs: 6.25-25 μg/mL).

Keywords: 1,3,4-thiadiazole, 1,3,4-oxadiazole, antimicrobial activity, coumarin, piperazine, quinolone.

INTRODUCTION

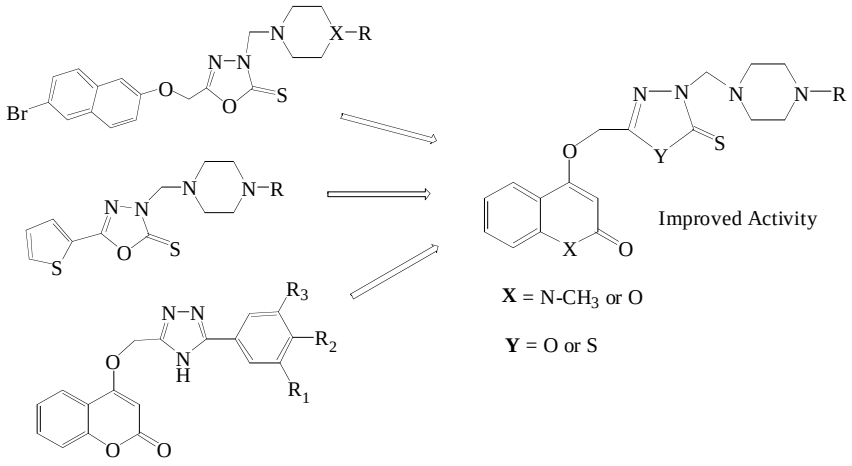
Nowadays, many Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus* and fungi like Candida species are found to withstand the attack of currently available chemotherapeutic agents, the so-called antimicrobial resistance [1-3]. The issue has raised fears that such opportunistic microbial infections may once again become major cause of death in developing/developed countries immerging with the steady increase of immunocompromised individuals, patients with malignancies and transplant recipients [4]. The mechanism of resistantce is continuously evolving in pathogenic bacteria to currently used antimicrobial regimen, thereby jeopardizing the medical successes achieved at healthcare systems. To meet this crisis successfully, many researchers across the globe are working to unearth new compounds which can selectively attack novel targets in different microorganisms. One possible way to reach such results is the modification of the structure of pre-established molecules active against the said targets. In this context, framing of two or more bioactive pharmacpohore in a single molecular scaffold may furnish better results.

During last few decades, 1,3,4-oxadiazole derivatives are implicated in a variety of biological applications as antimicrobial [5-10] and antituberculosis [11-13] agents, whereas, 1,3,4-thiadiazole nucleus is established as a core scaffold having important bioprofiles as antimicrobial [14-16], antituberculosis [17] and anticancer [18,19] agents. In addition, extensive studies have been conducted to examine the antimicrobial efficacy of 1,3,4-oxa/thiadiazole derivatives involving substitution of different aromatic or heterocyclic moieties *via* substitution to the sulphur atom [20, 21]. In connection with these studies, we planned to modify the said derivatives *via* structural modification to the nitrogen atom of the oxa/thiadiazole ring rather than the said sulphur linkage to develop new antimicrobial agents with novel mechanism of action. Furthermore, the type of 1,3,4-oxadiazole scaffolds we plan to design have found intrinsic biological applications as anti-inflammatory [22, 23] antitubercular [24], antifungal [25] and anticancer activities [26], where as Aggarwal *et al.* recently synthesized potential active antimicrobial 1,3,4-oxa/thiadiazoles [27] based on similar structural features as with quinoline moiety as a core molecules in the form of nalidixic acid. In a view of these findings, we are directed to furnish similar scaffolds with quinolone and coumarin nucleus. It is well known that many currently available first line antimicrobial drugs are quinolones as ciprofloxacin, norfloxacin etc, whereas, the presence of 4-hydroxy-chromen-2-one is also found to enhance the various biological activities [28-30]. In addition, fluoropiperazines are found to play an important role by enhancing the antimicrobial and antituberculosis properties [31] of the resultant molecule and the results are recently published by us in continuation of our research towards novel biologically active agents [32-34]. Furthermore, thiadiazole condensed with the moieties having methoxy functional group is reported to exhibit antituberculosis activity against isoniazid resistant clinical strain [35].

Literature survey ascribed that oxadiazolo condensed piperazine derivatives based on aromatic or heterocyclic core displayed good antimicrobial activity against various human

*Address correspondence to this author at the Department of Applied Chemistry, S. V. National Institute of Technology, Surat–395007, Gujarat, India; Tel: +91–9712755525; E-mail: rahul.svnit11@gmail.com

1875-6255/12 $58.00+.00



Scheme 1. Rationalization of the scaffold synthesized.

pathogenic microorganisms [36, 37]. Couramain nucleus is recently employed to produce potential antimicrobial actitivities as presented in the present study (Scheme **I**) [38]. Prompted by these observations and in continuation of our interest in 1,3,4-oxadiazole derivatives [39, 40], it was contemplated to synthesize a novel series of fluoro/methoxy piperazino-1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives involving quinolone and coumarin moieties in order to establish newer molecules in a recent drug discovery process and the results indicated that the biological activity is increased significantly.

EXPERIMENTAL

Chemistry

Solvents of HPLC grade were purchased from Rankem, Surat, India. The TLC plates (silica gel 60 F254) were obtained from Merck, Germany. Melting points were determined in open capillaries on a Veego electronic apparatus VMP-D (Veego Instrument Corporation, Mumbai, India) and are uncorrected. IR spectra (4000-400 cm^{-1}) of synthesized compounds were recorded on a Shimadzu 8400-S FT-IR spectrophotometer (Shimadzu India Pvt. Ltd., Mumbai, India) using KBr pellets. Thin layer chromatography was performed on object glass slides (2 x 7.5 cm) coated with silica gel-G and spots were visualized under UV irradiation. ^1H NMR spectra were recorded on a Varian 400 MHz model spectrometer (Varian India Pvt. Ltd., Mumbai, India) using DMSO as a solvent and TMS as internal standard with ^1H resonant frequency of 400 MHz. The ^1H NMR Chemical Shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si) and were performed at center for excellence, Vapi, India. The splitting patterns are designated as follows; s, singlet; d, doublet; dd, doublet of doublets; q, quartet, m, multiplet. Elemental analyses (C, H, N) were performed using a Heraeus Carlo Erba 1180 CHN analyzer (Hanau, Germany).

General procedure for the preparation of ester derivatives (2a, 2b)

4-hydroxy-1-methylquinolin-2(1H)-one (**1a**) and 4-hydroxy-2H-chromen-2-one (**1b**) (0.05 mol) was dissolved in 250 ml dry acetone and mixed with anhydrous potassium carbonate (0.09 mol). This was treated with ethylchloroacetate (0.05 mol) and the mixture was refluxed for 7 h (for **1a**) and 9 h (for **1b**). After the completion of reaction (monitored by TLC), the reaction mixture was filtered and the filtrate was distilled under reduced pressure. The solid thus obtained was subjected to column chromatography using 5% ethyl acetate: petroleum ether to furnish the final products **2a** and **2b** [41].

Ethyl 2-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)acetate (2a)

Yield 88 %, m.p. 130-132°C. IR (KBr, cm^{-1}): 1732 (ester, C=O), ^1H NMR (400 MHz, Me_2SO- d_6): δ 8.03 (d, J = 7.4 Hz, 1H, C_8 proton of quinoline), 7.70 (t, J = 7.5 Hz, 1H, C_7 proton of quinoline), 7.57 (d, J = 7.9 Hz, 1H, C_5 proton of quinoline), 7.42 (t, J = 7.6 Hz, 1H, C_6 proton of quinoline), 7.41 (s, 1H, C_3 proton of quinoline), 5.02(2H, s, O-CH_2), 4.14 (2H, q, J = 5.9 Hz, OCH_2CH_3), 3.61 (s, 3H, N-C<u>H</u>$_3$), 1.33 (3H, t, J = 6.0 Hz, CH_2CH_3).

Ethyl 2-(2-oxo-2H-chromen-4-yloxy)acetate (2b)

Yield 82 %, m.p. 98-99 °C (Lit. m.p. 97 °C). IR (KBr, cm^{-1}): 1728 (ester, C=O), ^1H NMR (400 MHz, Me_2SO- d_6): δ 7.96 (dd, J = 1.6, 1.3 Hz, 1H, C_5 proton of coumarin), 7.55-7.62 (m, 1H, coumarin), 7.50 (t, J = 8.7 Hz, 1H, C_6 proton of coumarin), 7.33-7.48 (m, 1H, coumarin), 5.02(2H, s, O-CH_2), 4.14 (2H, q, J = 5.9 Hz, OCH_2CH_3), 1.33 (3H, t, J = 6.0 Hz, CH_2CH_3).

General procedure for the preparation of hydrazide derivatives (3a, 3b)

A mixture of ethyl 2-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)acetate (**2a**) or ethyl 2-(2-oxo-2H-chromen-4-yloxy)acetate (**2b**) (0.03 mol) and hydrazine hydrate (0.03 mol) in ethanol (60 ml) was heated under reflux for 8 h (for **2a**) and 6 h (for **2b**). The reaction mixture was cooled to room temperature and the solid separated was collected by filtration. It was washed with ethanol and recrystallized in methanol. The products **3a** and **3b** were obtained as light yellow solid [38].

2-(1-Methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)acetohydrazide (3a)

Yield 86 %, m.p. 173-175 °C. IR (KBr, cm^{-1}): 1653 (amide, C=O), 3310, 3203 (NH-NH_2), ^{1}H NMR (400 MHz, Me_2SO- d_6): δ 9.83 (1H, br s, -CO-NH), 8.03 (d, *J* = 7.4 Hz, 1H, C_8 proton of quinoline), 7.70 (t, *J* = 7.5 Hz, 1H, C_7 proton of quinoline), 7.57 (d, *J* = 7.9 Hz, 1H, C_5 proton of quinoline), 7.42 (t, *J* = 7.6 Hz, 1H, C_6 proton of quinoline), 7.41 (s, 1H, C_3 proton of quinoline), 5.02(2H, s, O-CH_2), 4.58 (2H, br s,–NH_2), 3.67 (s, 3H, N-C$\underline{H}_3$).

2-(2-Oxo-2H-chromen-4-yloxy)acetohydrazide (3b)

Yield 80 %, m.p. 194-195 °C (Lit. m.p. 192 °C). IR (KBr, cm^{-1}): 1657 (amide, C=O), 3303, 3221 (NH-NH_2), ^{1}H NMR (400 MHz, Me_2SO- d_6): δ 9.83 (1H, br s, -CO-NH), 7.96 (dd, *J* = 1.6, 1.3 Hz, 1H, C_5 proton of coumarin), 7.55-7.62 (m, 1H, coumarin), 7.50 (t, *J* = 8.7 Hz, 1H, C_6 proton of coumarin), 7.33-7.48 (m, 1H, coumarin), 5.02 (2H, s, O-CH_2), 4.58 (2H, br s,–NH_2).

General procedure for the preparation of hydrazinecarbodithioate salts (4a, 4b)

2-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)acetohydrazide (**3a**) and 2-(2-oxo-2H-chromen-4-yloxy)acetohydrazide (**3b**) (0.01 mol) were added to a solution of potassium hydroxide (0.01 mol) in absolute ethanol (70 mL) placed in a 100 mL round bottomed flask mounted over a magnetic stirrer. The reaction mixture was stirred for 1 h in an ice bath. Carbon disulfide (0.015 mol) was added drop wise to the above pre-cooled reaction mixture, which resulted in the formation of a pale yellow precipitate. The pale yellow precipitate was filtered and repeatedly washed with cold acetone (2 x 10 mL) and dried in vacuum oven to obtain **4a** and **4b**.

General procedure for the preparation of 1,3,4-oxadiazoles derivatives (5a, 5b)

The above dried potassium 2-(2-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)acetyl)hydrazinecarbodithioate (**4a**) and potassium 2-(2-(2-oxo-2H-chromen-4-yloxy)acetyl) hydrazinecarbodithioate (**4b**) were added in very small portions to a cooled hydrochloric acid (10 mL, 0-5 ^{o}C) taken in a round-bottomed flask and stirred till the solution became homogenous. The homogenous mixture was poured in crushed ice. The separated precipitates were filtered at pump, washed with water, and dried in vacuum oven to obtain **5a** and **5b**.

Methyl-4-((5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methoxy)quinolin-2(1H)-one (5a)

78 % yield, m.p. 233-235 °C. IR (KBr, cm^{-1}): 3054 (-CH Str.), 1651 (C=O, quinoline), 1632 (C=N of oxadiazole), 1232 (C=S), 1140 (N-N, oxadiazole), 1074 (Ph-O-CH_2), 1055 (C-O-C-, oxadiazole). ^{1}H NMR (400 MHz, Me_2SO- d_6): δ10.52 (bs, 1H, NH), 7.98 (d, *J* = 7.1 Hz, 1H, C_8 proton of quinoline), 7.68 (t, *J* = 7.5 Hz, 1H, C_7 proton of quinoline), 7.59 (d, *J* = 8.5 Hz, 1H, C_5 proton of quinoline), 7.47 (t, *J* = 7.8 Hz, 1H, C_6 proton of quinoline), 7.42 (s, 1H, C_3 proton of quinoline), 7.31-6.84 (4H, m, Ar-H), 4.89 (2H, s, O-CH_2), 3.73 (s, 3H, N-C$\underline{H}_3$).

4-((5-Thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methoxy)-2H-chromen-2-one (5b)

73 % yield, m.p. 225-226 °C. IR (KBr, cm^{-1}): *v* 3078 (-CH Str.), 1680 (C=O, coumarin), 1635 (C=N of oxadiazole), 1218 (C=S), 1141 (N-N, oxadiazole), 1070 (Ph-O-CH_2), 1041 (C-O-C-, oxadiazole) ^{1}H NMR (400 MHz, Me_2SO- d_6): δ 10.55 (bs, 1H, NH), 8.07 (dd, *J* = 1.2, 1.3 Hz, 1H, C_5 proton of coumarin), 7.61-7.69 (m, 1H, coumarin), 7.50 (t, *J* = 8.4 Hz, 1H, C_6 proton of coumarin), 7.35-7.42 (m, 1H, coumarin), 7.23-6.84 (5H, m, Ar-H), 4.85 (2H, s, O-CH_2).

General procedure for the preparation of 1,3,4-thiadiazole derivatives (6a, 6b)

The above dried potassium 2-(2-(1-methyl-2-oxo-1,2-dihydroquinolin-4-yloxy)acetyl)hydrazinecarbodithioate (**4a**) and potassium 2-(2-(2-oxo-2H-chromen-4-yloxy)acetyl) hydrazinecarbodithioate (**4b**) was added in very small portions to a cooled sulfuric acid (10 mL, 0-5 ^{o}C) and stirred till the solution became homogenous. The homogenous mixture was poured in crushed ice. The separated precipitates were filtered, washed with water, and dried in vacuum oven to obtain **6a** and **6b**.

1-Methyl-4-((5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)methoxy)quinolin-2(1H)-one (6a)

Yield 72 %, m.p. 209-211 °C. IR (KBr, cm^{-1}): 3061 (-CH Str.), 1637 (C=O, quinoline), 1611 (C=N of thiadiazole), 1232 (C=S), 1151 (N-N, thiadiazole), 1076 (Ph-O-CH_2), 697 (C-S-C, thiadiazole). ^{1}H NMR (400 MHz, Me_2SO- d_6): δ 10.54 (bs, 1H, NH), 8.09 (d, *J* = 7.4 Hz, 1H, C_8 proton of quinoline), 7.60 (d, *J* = 8.2 Hz, 1H, C_5 proton of quinoline), 7.54 (t, *J* = 7.5 Hz, 1H, C_7 proton of quinoline), 7.47 (t, *J* = 7.6 Hz, 1H, C_6 proton of quinoline), 7.38 (s, 1H, C_3 proton of quinoline), 7.31-6.89 (4H, m, Ar-H), 4.86 (2H, s, O-CH_2), 3.71 (s, 3H, N-C$\underline{H}_3$).

4-((5-Thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)methoxy)-2H-chromen-2-one (6b)

Yield 69 %, m.p. 218-219 °C. IR (KBr, cm^{-1}): 3073 (-CH Str.), 1681 (C=O, coumarin), 1624 (C=N of thiadiazole), 1238 (C=S), 1161 (N-N, thiadiazole), 1087 (Ph-O-CH_2), 702 (C-S-C, thiadiazole). ^{1}H NMR (400 MHz, Me_2SO- d_6): δ 10.47 (bs, 1H, NH), 8.03 (dd, *J* = 1.3, 1.3 Hz, 1H, C_5 proton of coumarin), 7.49-7.64 (m, 1H, coumarin), 7.46 (t, *J* = 8.3 Hz, 1H, C_6 proton of coumarin), 7.30-7.39 (m, 1H, coumarin), 7.27-6.77 (5H, m, Ar-H), 4.88 (2H, s, O-CH_2).

General procedure for the preparation of final derivatives (7i-10vi)

To a 0.005 mol stirred solution of 1-methyl-4-((5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)methoxy)quinolin-2(1H)-one (**5a**), 4-((5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)methoxy)-2H-chromen-2-one (**5b**), 1-methyl-4-((5-

thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methoxy)quinolin-2(1H)-one (**6a**) and 4-((5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methoxy)-2H-chromen-2-one (**6b)** in ethanol, 0.006 mol of 40% formalin was added and the reaction mixture was allowed to stir for half an hour. After that, appropriate piperazine bases were dissolved in ethanol and added drop wise to the above stirred mixture and allowed to reflux for 3-8 h. The progress of the reaction was monitored by Thin Layer Chromatography using ethyl acetate: n-hexane (7: 3) as the mobile phase. After the completion of the reaction, mixture was allowed to cool and poured onto crushed ice. The precipitate formed was collected by filtration, washed with water and dried in vacuum oven to furnish **7i-10vi** [27].

4-((4-((4-(2-Fluorophenyl)piperazin-1-yl)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methoxy)-1-methylquinolin-2(1H)-one (7i)

Yield 66%, m.p. 248-250 ^{o}C. IR (KBr, cm^{-1}): 3068 (-CH Str.), 1650 (C=O, quinoline), 1620 (C=N of oxadiazole), 1241 (C=S), 1135 (N-N, oxadiazole), 1085 (Ph-O-CH_2), 1048 (C-O-C-, oxadiazole), 753 (C-F). ^{1}H NMR (400 MHz, Me_2SO- d_6): δ 8.02 (d, J = 7.0 Hz, 1H, C_8 proton of quinoline), 7.70 (t, J = 7.7 Hz, 1H, C_7 proton of quinoline), 7.62 (d, J = 8.3 Hz, 1H, C_5 proton of quinoline), 7.49 (t, J = 7.7 Hz, 1H, C_6 proton of quinoline), 7.39 (s, 1H, C_3 proton of quinoline), 7.28-6.80 (4H, m, Ar-H), 5.19 (s, 2H, NCH_2N), 4.95 (2H, s, O-CH_2), 3.87 (4H, br s, piperazine), 3.75 (s, 3H, N-C<u>H</u>$_3$), 3.47 (4H, br s, piperazine). Anal Calcd for $C_{24}H_{24}FN_5O_3S$: C, 59.86; H, 5.02; N, 14.54. Found: C, 59.74; H, 5.06; N, 14.57.

4-((4-((4-(2-Fluorophenyl)piperazin-1-yl)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methoxy)-2H-chromen-2-one (8i)

Yield 73%, m.p. 264-266 ^{o}C. IR (KBr, cm^{-1}): 3075 (-CH Str.), 1692 (C=O, coumarin), 1628 (C=N of oxadiazole), 1228 (C=S), 1146 (N-N, oxadiazole), 1084 (Ph-O-CH_2), 1048 (C-O-C-, oxadiazole), 753 (C-F). ^{1}H NMR (400 MHz, Me_2SO- d_6): δ 8.06 (dd, J = 1.3, 1.3 Hz, 1H, C_5 proton of coumarin), 7.58-7.61 (m, 1H, coumarin), 7.45 (t, J = 8.6 Hz, 1H, C_6 proton of coumarin), 7.32-7.39 (m, 1H, coumarin), 7.25-6.87 (5H, m, Ar-H), 5.20 (s, 2H, NCH_2N), 4.89 (2H, s, O-CH_2), 3.83 (4H, br s, piperazine), 3.43 (4H, br s, piperazine). Anal Calcd for $C_{23}H_{21}FN_4O_4S$: C, 58.96; H, 4.52; N, 11.96. Found: C, 58.85; H, 4.58; N, 11.93.

4-((4-((4-(2-Fluorophenyl)piperazin-1-yl)methyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)methoxy)-1-methylquinolin-2(1H)-one (9i)

Yield 69%, m.p. 285-287 ^{o}C. IR (KBr, cm^{-1}): 3068 (-CH Str.), 1647 (C=O, quinoline), 1614 (C=N of thiadiazole), 1229 (C=S), 1149 (N-N, thiadiazole), 1085 (Ph-O-CH_2), 750 (C-F), 685 (C-S-C, thiadiazole). ^{1}H NMR (400 MHz, Me_2SO- d_6): δ 8.04 (d, J = 7.2 Hz, 1H, C_8 proton of quinoline), 7.66 (t, J = 7.7 Hz, 1H, C_7 proton of quinoline), 7.62 (d, J = 8.0 Hz, 1H, C_5 proton of quinoline), 7.53 (t, J = 7.8 Hz, 1H, C_6 proton of quinoline), 7.41 (s, 1H, C_3 proton of quinoline), 7.28-6.86 (4H, m, Ar-H), 5.19 (s, 2H, NCH_2N), 4.92 (2H, s, O-CH_2), 3.88 (4H, br s, piperazine), 3.72 (s, 3H, N-C<u>H</u>$_3$), 3.48 (4H, br s, piperazine). Anal Calcd for $C_{24}H_{24}FN_5O_2S_2$: C, 57.93; H, 4.86; N, 14.07. Found: C, 57.99; H, 4.80; N, 14.14.

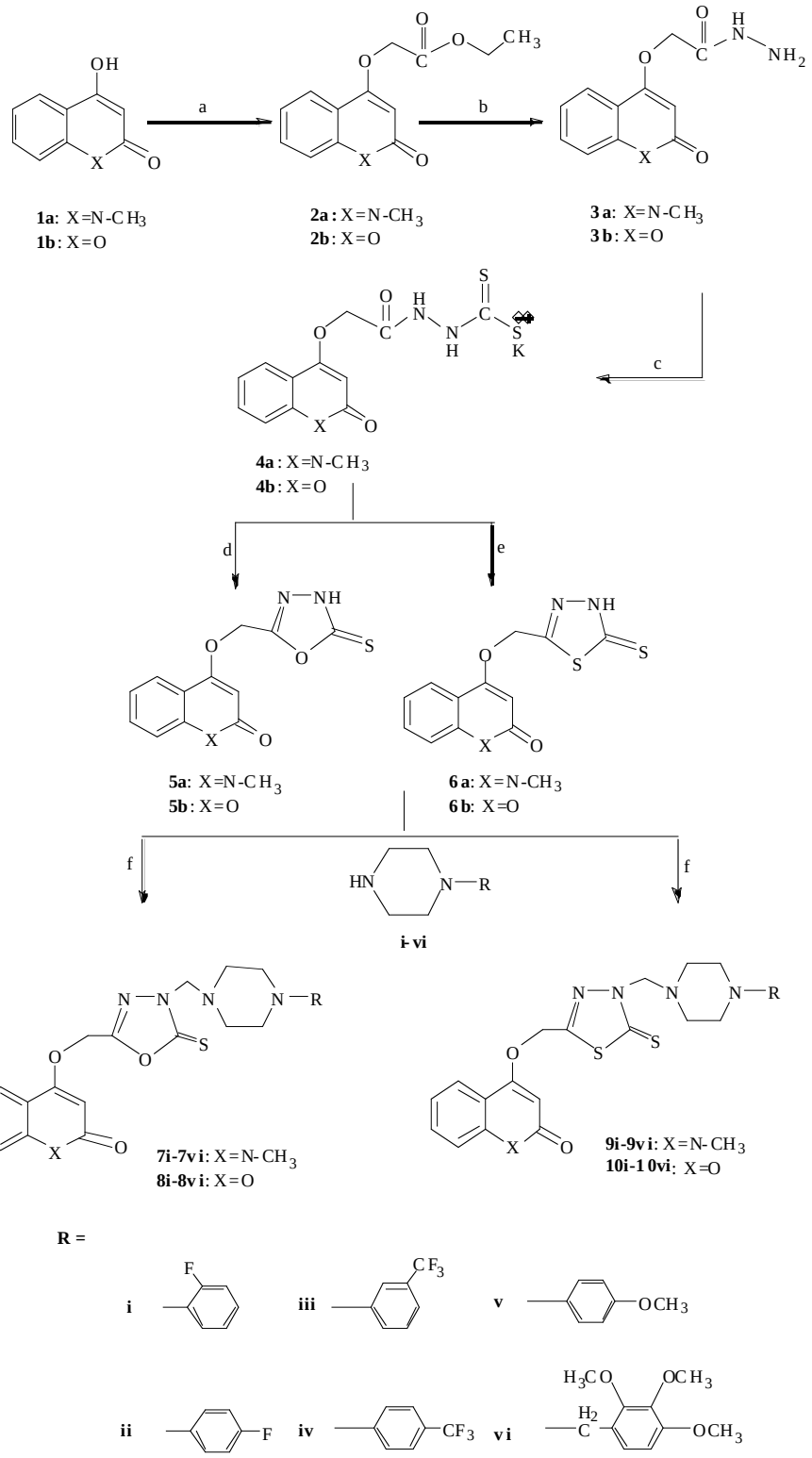
4-((4-((4-(2-Fluorophenyl)piperazin-1-yl)methyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)methoxy)-2H-chromen-2-one (10i)

Yield 71%, m.p. 280-282 ^{o}C. IR (KBr, cm^{-1}): 3068 (-CH Str.), 1679 (C=O, coumarin), 1618 (C=N of thiadiazole), 1240 (C=S), 1152 (N-N, thiadiazole), 1086 (Ph-O-CH_2), 753 (C-F), 687 (C-S-C, thiadiazole). ^{1}H NMR (400 MHz, Me_2SO- d_6): δ 8.07 (dd, J = 1.5, 1.3 Hz, 1H, C_5 proton of coumarin), 7.54-7.61 (m, 1H, coumarin), 7.49 (t, J = 8.3 Hz, 1H, C_6 proton of coumarin), 7.32-7.37 (m, 1H, coumarin), 7.30-6.85 (5H, m, Ar-H), 5.26 (s, 2H, NCH_2N), 4.94 (2H, s, O-CH_2), 3.82 (4H, br s, piperazine), 3.53 (4H, br s, piperazine). Anal Calcd for $C_{23}H_{21}FN_4O_3S_2$: C, 57.01; H, 4.37; N, 11.56. Found: C, 57.07; H, 4.42; N, 11.59.

Biological Assay

In vitro Evaluation of Antimicrobial Activity

Antimicrobial activity for the final analogs carried out as described by Clause [42] with minor modifications against two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 430), three Gram-negative bacteria (*Escherichia coli* MTCC 739, *Pseudomonas aeruginosa* MTCC 741, *Klebsiella pneumoniae* MTCC 109) and against two fungal species (*Aspergillus niger* MTCC 282, *Candida albicans* MTCC 183). Ampicillin and Gentamicina as well as Fluconazole were used as standard antibacterial and antifungal agents, respectively. Solutions of the test compounds and reference drugs were dissolved in DMSO at a concentration of 500 µg/mL. The dilution of the compounds and reference drugs was prepared (500, 250, 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.13) µg/mL. Antibacterial activities of the bacterial strains were carried out in Muller–Hinton broth (Difco) medium, at pH 6.9, with an inoculum of (1–2) x 103 cells/mL by the spectrophotometric method and an aliquot of 100 µL was added to each tube of the serial dilution. The chemical compounds-broth medium serial tube dilutions inoculated with each bacterium were incubated on a rotary shaker at 37 ^{o}C for 24 h at 150 rpm. The minimum inhibitory concentrations of the chemical compounds were recorded as the lowest concentration of each chemical compounds in the tubes with no growth (i.e., no turbidity) of inoculated bacteria. All fungi were cultivated in Sabouraud Dextrose Agar (Merck). The fungi inoculums were prepared in Sabouraud liquid medium (Oxoid) which had been kept at 36 ^{o}C overnight and diluted with RPMI-1640 medium with L-glutamine buffered with 3-[*N*-morpholino]-propansulfonic acid (MOPS) at pH 7 to give a final concentration of 2.5 x 103 cfu/mL. The microplates were incubated at 36 ^{o}C and read visually after 24 h, except for Candida species when it was at 48 h. The incubation chamber was kept humid. At the end of the incubation period, MIC values were recorded as the lowest concentrations of the substances that gave no visible turbidity.



Scheme 2. Synthetic protocol for the final analogs **7i-10vi**.
Reagents and conditions: (a) $ClCH_2COOC_2H_5$, Anhyd. K_2CO_3, Acetone, Reflux; (b) 90% $NHNH_2.H_2O$, EtOH, Reflux; (c) CS_2/KOH, EtOH, Reflux; (d/e) H_2SO_4/HCl, 0-5 ^oC; (f) HCHO, piperazines, EtOH, Reflux.

RESULTS AND DISCUSSION

Chemistry

Scheme **2** outlines the synthetic pathway followed for the synthesis of the title compounds **7i-10vi**. The solvents and reagents were used as received or were dried prior to use as needed. 4-hydroxy-1-methylquinolin-2(1H)-one (**1a**) and 4-hydroxy-2H-chromen-2-one (**1b**) was treated with ethyl-chloroacetate to yield the corresponding ester derivatives (**2a**, **2b**). IR spectra of the ester compounds revealed an ab-

sorption band of an ester functional group at 1728-1732 cm^{-1}. The resulting ester derivative was then treated with 99% hydrazine hydrate in ethanol to give the corresponding hydrazide derivatives (**3a**, **3b**) in good yield and its correct synthesis was confirmed by a broad band at 1653-1657 cm^{-1} due to –C=O of amide and disappearance of an ester frequency (1728-1732 cm^{-1}) in the IR spectra. The resulted hydrazide derivatives (**3a**, **3b**) were then cyclized using carbon disulfide in ethanolic potassium hydroxide to furnish the hydrazinecarbodithioate salts (**4a**, **4b**). The said hydrazinecarbodithioate salts (**4a**, **4b**) were further treated with sulfuric acid and hydrochloric acid at cooled temperature to furnish the corresponding 1,3,4-thiadiazole derivatives **(5a**, **5b)** and 1,3,4-oxadiazoles derivatives **(6a**, **6b)**, respectively. IR spectra of the thiadiazole derivatives revealed a band at 697-702 cm^{-1} due to C-S-C group and IR spectra of the oxadiazoles derivatives revealed a band at 1041-1057 cm^{-1} due to C-O-C- group. ^{1}H NMR spectra clearly evidenced the presence of an –NH group in the ring of cyclized derivatives appearing broad as singlet peak at around 10.50 ppm. The said 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives were then treated with different fluoro or methoxy substituted piperazine moieties in the presence of formalin in methanol to furnish the final Mannich bases **7i-10vi**. All the IR, ^{1}H NMR spectral data of compounds **7i-10vi** were in accordance with assumed structures. The purity of the synthesized compounds was monitored by TLC and ascertained by elemental analysis.

Pharmacology

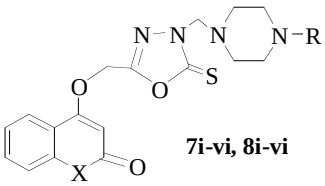
Several novel heterocyclic scaffolds were synthesized and examined for their *in vitro* antimicrobial activities. The bioassay results presented in Table **1** demonstrated that the majority of the final analogs succeeded to indicate remarkable activity against the mentioned microorganisms. From the activity results it can be stated that 1,3,4-thiadiazole derivatives were more active against majority of the microorganism studied when compared to those of 1,3,4-oxadiazole derivatives. Final analogs with trimethoxy benzyl piperazine substituents either linked through quinolone (**9vi**) or coumarin (**10vi**) nucleus displayed excellent activity against Gram-positive *S. aureus* at 3.12 μg/mL of MIC. These analogs were found more potent against the said bacteria than the standard drugs ampicillin and gentamicina. In addition, quinolone based 1,3,4-oxadiaazole analogs with highly electronegative terifluoromehtyl functional group linked to the meta (**7iii**) or *para*-position (**7iv**) of the phenyl ring of piperazine substituent indicated higher anti Gram-positive activity against *B. cereus* at 6.25 μg/mL of MIC, i.e. equipotent to the standard drugs. 1,3,4-Oxadiazole (**8i**, **8ii**) and 1,3,4-thiadizole (**10i**, **10ii**) derivatives involving coumarin nucleus and substitution of single fluorine atom at *ortho* and *para*-position showed good inhibitory effects against Gram-negative *E. coli* strain at 12.5 μg/mL of MIC, the concentration was comparable to the standards. Final 1,3,4-thiadiazole derivatives with trifluoromethyl functionality either at quinolone (**9ii**, **9iv**) or coumarin (**10iii**) ring system displayed higher antibacterial activity (6.25 μg/mL) than the standard drugs (12.5-25 μg/mL) against Gram-negative *P. aeruginosa*, while similar thiadiazoles with quinolone ring system and either trifluoro (**9iii**) or trimethoxy (**9vi**) functional groups exerted higher potency at 6.25 μg/mL of MIC than the standards (25 μg/mL). All the final 1,3,4-oxa/thiadizoles having substitution of *para*-methoxy group at the phenyl ring of piperazine base indicated good activity at 12.5 μg/mL of MIC against *A. niger* fungi; these derivative were found half fold active against the mentioned fungi when compared to standard drug fluconazole (6.25 μg/mL). 1,3,4-Oxadiazole derivative with *meta*-trimethoxy functional group at quinolone (**7vi**) and coumarin (**8vi**) ring systems indicated good antifungal properties at 25 μg/mL of MIC, i.e., half fold active than the standard drug fluconazole. Majority of the remaining derivatives were found to exhibit good antimicrobial profiles either similarly active or half fold active to the standard drug, while some of them were moderately active.

Hence, from the studied bioassay it can be stated that 1,3,4-thiadiazole derivatives were more active than the derivative involving 1,3,4-oxadiazole ring system. In addition, all the final derivatives with quinolone ring system as a core scaffold showed good inhibition of all the microorganisms studied when compared to those with coumarin as a core scaffold. However, with an exception, it will suffice to mention here that coumarin derivatives with 1,3,4-thiadiazole ring system were more or equally active than the quinolone derivatives having 1,3,4-oxadiazole ring. Analogs with single fluorine or trifluoromehthyl functional group were found to be good antibacterial agents, while those involving methoxy or trimethoxy functionality indicated good antifungal properties.

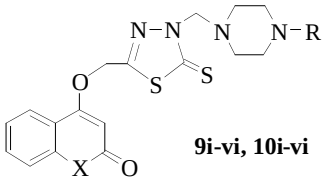
CONCLUSION

Novel 1,3,4-thiadiazole and 1,3,4-oxadiazole derivatives involving condensation of different fluoro/methoxy piperazines based on quinolone or coumarin nucleus have been synthesized. Investigation of antimicrobial properties showed that many compounds exhibited higher potency than the standard drugs. In fact, some analogs were more active against Gram-positive *S. aureus* and Gram-negative *P. aeruginosa* at excellent MICs of 3.12-6.25 μg/mL than the standard drugs ampicillin and gentamicina (6.25-25 μg/mL), however, many derivatives demonstrated equipotency to the standard drugs. 1,3,4-thiadiazole analogs were found remarkably potent when compared to 1,3,4-oxadiazoles, and the activity may be due to the presence of sulphur atom in the ring system. Many final analogs showed half fold activity (MIC: 12.5-25 μg/mL) in the antifungal bioassay. From the results it can be stated that a combination of quinolone ring with 1,3,4-thiadiazole ring system may lead to the improved antimicrobial agents (variety of human pathogenic microorganisms), however, other derivatives may also be optimized to derive fruitful results. Hence, the mentioned scaffolds provide a good starting point for further antimicrobial drug discovery process. Other experiments on the optimization of structural features of the presented analogs are underway in our laboratory and the results will be derived soon.

Table 1. ***In–vitro*** **Antimicrobial Activity of 7i-10vi**



7i-vi, 8i-vi



9i-vi, 10i-vi

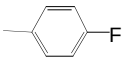
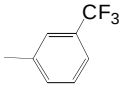
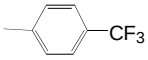
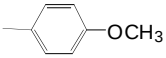
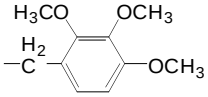
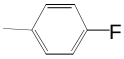
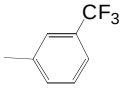
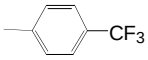
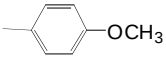
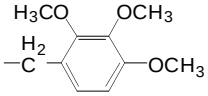
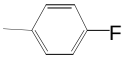
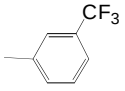
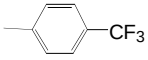
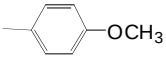
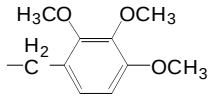
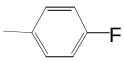
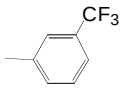
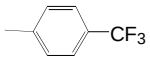
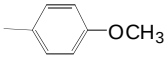
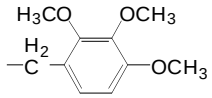
Entry	X	R	MIC in µg/mL						
			S.a	***B.c***	***E.c***	***P.a***	***K.p***	***A.n***	***C.a***
7i	N-CH_3		100	62.5	62.5	**50**	50	200	100
7ii	N-CH_3		100	50	50	62.5	62.5	250	200
7iii	N-CH_3		62.5	**6.25**	50	25	12.5	62.5	100
7iv	N-CH_3		25	**6.25**	25	12.5	25	50	50
7v	N-CH_3		50	100	100	100	250	**12.5**	62.5
7vi	N-CH_3		25	50	62.5	100	100	25	**25**
8i	O		200	200	**12.5**	50	100	250	250
8ii	O		100	100	**12.5**	25	62.5	200	250
8iii	O		50	62.5	62.5	50	100	100	500
8iv	O		50	50	100	50	50	200	62.5
8v	O		100	200	100	250	100	**12.5**	100
8vi	O		12.5	50	100	200	50	50	**25**
9i	N-CH_3		100	62.5	25	25	25	100	200
9ii	N-CH_3		62.5	100	50	**6.25**	50	200	100
9iii	N-CH_3		12.5	12..5	62.5	12.5	**6.25**	50	100
9iv	N-CH_3		6.25	25	50	**6.25**	**6.25**	**50**	62.5
9v	N-CH_3		25	100	50	50	100	**12.5**	**25**

Table 1. contd….

Entry	X	R	MIC in µg/mL						
			S.a	***B.c***	***E.c***	***P.a***	***K.p***	***A.n***	***C.a***
9vi	N-CH_3		**3.12**	25	100	50	62.5	25	50
10i	O		50	200	**12.5**	25	62.5	200	100
10ii	O		50	200	**12.5**	25	62.5	250	100
10iii	O		12.5	25	50	**6.25**	25	100	50
10iv	O		25	50	62.5	12.5	25	62.5	62.5
10v	O		62.5	100	200	62.5	200	**12.5**	50
10vi	O		**3.12**	62.5	100	50	100	25	50
Amp.			12.5	12.5	6.25	25	25	-	-
Gen.			6.25	6.25	12.5	12.5	25	-	-
Flu.								6.25	12.5
DMSO			-	-	-	-	-	-	-

Amp.: Ampicillin, Gen.:- Gentamicina, Flu.: Fluconazole

S.a - Staphylococcu aureus, B.c - Bacillus cereus, E.c - Escherichia coli, P.a - Pseudomonas aeruginosa, K.p - Klebsiella pneumoniae, A.n - Aspergillus niger, C.a - Candida albicans.

ACKNOWLEDGEMENTS

The authors are thankful to the Applied Chemistry Department of S. V. National Institute of Technology, Surat for the scholarship, encouragement and facilities. The authors wish to offer their deep gratitude to Microcare Laboratory, Surat, India for carrying out the biological screenings. We are also thankful to the Center of Excellence, Vapi, India for recording ^{1}H NMR spectra.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

SUPPLEMENTARY INFORMATION

Supplementary material is available on the publishers Web site along with the published article.

REFERENCES

[1] Okuma, K.; Iwakawa, K.; Turnidge, J.D.; Grubb,W.B.; Bell,J.M.; O'Brien, F.G.; Coombs, G.W.; Pearman, J.W.; Tenover, F.C.; Kapi, M.; Tiensasitorn, C.; Ito, T.; Hiramatsu, K. Dissemination of new methicillin-resistant Staphylococcus aureus clones in the community. *J. Clin. Microbiol.*, **2002**, *40*, 4289–4294.

[2] Klein, E.; Smith, D.L.; Laxminarayan, R. Hospitalizations and Deaths Caused by Methicillin-Resistant Staphylococcus aureus, United States, 1999–2005. *Emerg. Infect. Dis.*, **2007**, *13*(*12*), 1840–1846.

[3] Johnson, E.M.; Warnock, D.W.; Luker, J.; Portor, S.R.; Crispian, S.J. Emergence of azole drug resistance in Candida species from HIV-infected patients receiving prolonged fluconazole therapy for oral candidosis. *Antimicrob. Chemother.*, **1995**, *35*(*1*), 103–114.

[4] World Health Organization, Global tuberculosis control: a short update to the 2009 report. Available at http://www.who.int/tb/publications/global_report/2009/update/en/index.htm.

[5] 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*(*12*), 5862–5869.

[6] 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.

[7] 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*(*11*), 4963–4967.

[8] Khanum, S.A.; Shashikanth, S.; Umesh, S.; Kavitha, R. Synthesis and antimicrobial study of novel heterocyclic compounds from hydroxybenzophenones. *Eur. J. Med. Chem.*, **2005**, *40*(*11*), 1156–1162.

[9] Naveena, C.S.; Boja, P.; Kumari, N.S. Synthesis, characterization and antimicrobial activity of some disubstituted 1,3,4-oxadiazoles carrying 2-(aryloxymethyl)phenyl moiety. *Eur. J. Med. Chem.*, **2010**, *45*(*11*), 4708–4719.

[10] 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*(*11*), 3981–3989.

[11] Mamolo, M.G.; Zampieri, D.; Vio, L.; Fermeglia, M.; Ferrone, M.; Pricl, S.; Scialinoc, G.; Banfi, E. Antimycobacterial activity of new 3-substituted 5-(pyridin-4-yl)-3H-1,3,4-oxadiazol-2-one and 2-thione derivatives. Preliminary molecular modeling investigations. *Bioorg. Med. Chem.*, **2005**, *13*(*11*), 3797–3809.

[12] Kucukguzel, S.G.; Oruc, E.E.; Rollas, S.; Sahin, F.; Ozbek, A. Synthesis, characterisation and biological activity of novel 4-thiazolidinones, 1,3,4-oxadiazoles and some related compounds. *Eur. J. Med. Chem.*, **2002**, *37*(*3*), 197–206.

[13] Ali, M.A.; Shaharyar, M. Oxadiazole mannich bases: Synthesis and antimycobacterial activity *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 3314–3316.

[14] Desai, K.; Baxi, A.J. Studies on 2-azetidinone: part vi synthesis and antimicrobial activity of 5-(2',4'-dichlorophenoxymethyl)-2-(4"-aryl-3"-chloro-2"-azetidinone-1"-yl)-1,3,4-thiadiazole. *Indian J. Pharm. Sci.*, **1992**, *54*, 183-188.

[15] Gawande, N.G.; Shingare, M.S. Synthesis of some thiazolyl-thiosemicarbazides. triazoles, oxadiazoles, thiadiazoles & their microbial activity. *Indian J. Chem.*, **1987**, *26*, 387-389.

[16] Mamolo, M.G.; Vio, L.; Banfi, E. Synthesis and antimicrobial activity of some 2,5-disubstituted 1,3,4-thiadiazole derivatives. *Farmaco.*, **1996**, *51*, 71-74.

[17] Shucla, H.K.; Desai, N.C.; Astik, R.R.; Thaker, K.A. Studies on some thiosemicarbazones and 1,3,4-thiadiazoles as potential antitubercular and antibacterial agents. *J. Indian Chem. Soc.*, **1984**, *61*, 168-171.

[18] Miyamoto, K.; Koshiura, R.; Mori, M.; Yokoi, H.; Mori, C.; Hasegawa, T.; Takatori, K. Antitumour activity 5-substituted 2-acylamino-1,3,4thiadiazole against transplantable rodent tumours. *Chem. Pharm. Bull.*, **1985**, *33*, 5126-5129.

[19] Chou, J.Y.; Lai, S.Y.; Pan, S.L., Jow, G.M., Chern, J.W., Guh, J.H. Investigation of anticancer mechanism of thiadiazole based compound in human nonsmall cell lung cancer A549 cells. *Biochem. Pharmacol.*, **2003,** *66*, 115-124.

[20] Singh, A.K.; Mishra G.; Jyoti, K. Review on Biological Activities of 1,3,4-Thiadiazole Derivatives. *J. Appl. Pharm. Sci.*, **2011,** *01*, 44-49.

[21] Nagaraj; Chaluvaraju, K.C.; Niranjan, M.S.; Kiran S. 1, 1, 3, 4 oxadiazole: a potent drug candidate with various pharmacological Activities. *Int. J. Pharm. Pharm. Sci.*, **2011**, *3*, 9-16.

[22] Boschelli, D.H.; Connor, D.T.; Bornemeier, D.A.; Dyer, R.D.; Kennedy, J.A.; Kuipers, P.J.; Okonkwo, G.C.; Schrier, D.J.; Wright, C.D. 1,3,4-Oxadiazole, 1,3,4-thiadiazole, and 1,2,4-triazole analogs of the fenamates: in vitro inhibition of cyclooxygenase and 5-lipoxygenase activities. *J. Med. Chem.*, **1993,** *36*, 1802-1810.

[23] Nargund, L.V.; Reddy, G.R.; Hariprasad, V. Anti-inflammatory activity of substituted 1,3,4-oxadiazoles.J Pharm Sci., **1994**, *83*(*2*), 246-248.

[24] Ksepko, D.P.; Foks, H.; Janowiec, H.; Kwiek, Z.Z. Synthesis and tuberculostatic activity of reaction products 5-(2-,3- and 4-pyridil)-1,3,4-oxadiazol-2-thione with amines. *Acta. Pol. Pharm.*, **1993**, *50*, 259-267.

[25] Mishra, A.R.; Singh, S. Antifungal Activity of New 1,3,4-oxadiazolo[3,2-a]-s-triazine-5,7-diones and Their 5-thioxo-7-ones. J. Agric. Food Chem., **2000**, *48* (*11*), 5465–5468.

[26] Mazonne, G.; Bonina F. Synthesis and antimycotic activity of 3-methylamino derivatives of various 2- mercapto-5-aryl-1,3,4-oxadiazoles. *Farmaco. Ed. Sci.*, **1979**, *34*, 390-342.

[27] Aggarwal, N.; Kumar, R; Dureja, P.; Khurana, J. M. Synthesis of novel nalidixic acid based 1,3,4-thiadiazole and 1,3,4-oxadiazole derivatives as potent antibacterial agents, Chemical biology & Drug Design. *Chem. Bio. & Drug Design*, DOI: 10.1111/j.1747-0285.2011.01316.x.

[28] Vukovic, N.; Sukdolak, S.; Solujic, S.; Niciforovic, N. Substituted imino and amino derivatives of 4-hydroxycoumarins as novel antioxidant, antibacterial and antifungal agents: Synthesis and in vitro assessments. *Food Chem.*, **2010**, *120*(*4*), 1011-1018.

[29] Mladenovic, M.; Vukovic, N.; Niciforovic, N.; Sukdolak, S.; Solujic, S. Synthesis and molecular descriptor characterization of novel 4-hydroxy-chromene-2-one derivatives as antimicrobial agents. *Molecules*, **2009**, *14*(*4*), 1495-1512.

[30] Vukovic, N.; Sukdolak, S.; Solujic, S.; Milosevic, T. Synthesis and Antimicrobial Evaluation of Some Novel 2-Aminothiazole Derivatives of 4-Hydroxy-chromene-2-one. *Arch. Pharm. Chem. Life Sci.*, **2008**, *341*(*8*), 491-496.

[31] Patel, R.V.; Kumari, P.; Rajani, D.P.; Chikhalia, K.H. Synthesis, characterization and pharmacological activities of 2-[4-cyano-(3-trifluoromethyl)phenyl amino)]-4-(4-quinoline/coumarin-4-yloxy)-6-(fluoropiperazinyl)-s triazines. *J. Fluorine Chem.*, **2011**, *132*, 617–627.

[32] Patel, R.V.; Kumari, P.; Rajani, D.P.; Chikhalia, K.H. Fluorinated *s*-triazinyl piperazines as antimicrobial agents. Z Naturforsch C. *J. Biosci.*, **2011**, *66*, 341-345.

[33] Patel, R.V.; Kumari, P.; Rajani, D.P.; Chikhalia, K.H. A new class of 2-(4-cyanophenyl amino)-4-(6-bromo-4-quinolinyloxy)-6-piperazinyl (piperidinyl)-1,3,5-triazine analogues with antimicrobial/antimycobacterial activity. *J. Enzyme Inhib. Med. Chem.*, **2012**, *27*, 370-379.

[34] Patel, R.V.; Kumari, P.; Rajani, D.P.; Chikhalia, K.H. Synthesis and studies of novel 2-(4-cyano-3-trifluoromethylphenyl amino)-4-(quinoline-4-yloxy)-6-(piperazinyl/piperidinyl)-*s*-triazines as potential antimicrobial, antimycobacterial and anticancer agents. Eur. *J. Med. Chem.*, **2011**, *46*, 4354-4365.

[35] Chitre, T.S.; Panda, S.; Patil, S.M.; Chothe, A.S.; Vignesh, G.; Salake, A.B.; Kathiravan, M.K. Novel 1,3,4-(thiadiazol-2-ylamino) methyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-thiones: synthesis, docking and antimycobacterial testing. *Adv. in Bio. Chem.*, **2011**, *1*, 7-14.

[36] Al-Omar, M.A. Synthesis and Antimicrobial Activity of New 5-(2-Thienyl)-1,2,4-triazoles and 5-(2-Thienyl)-1,3,4-oxadiazoles and Related Derivatives. *Molecules*, **2010**, *15*, 502-514.

[37] Mayekar, A.N. Synthesis and Antimicrobial Studies on New Substituted 1,3,4-Oxadiazole Derivatives Bearing 6-Bromonaphthalene Moiety. *Int. J. Chem.*, **2010**, *2*, 38-54.

[38] Panda, S.S.; Malik, R.; Chand, M; Jain, S.C. Synthesis and antimicrobial activity of some new 4-triazolylmethoxy-2H-chromen-2-one derivatives. *Med. Chem. Res.* DOI 10.1007/s00044-011-9881-0.

[39] Patel, R.V.; Kumari, P.; Rajani, D.P.; Chikhalia, K.H. Synthesis of coumarin-based 1,3,4-oxadiazol-2ylthio-*N*-phenyl/benzothiazolyl acetamides as antimicrobial and antituberculosis agents. *Med. Chem. Res.*, DOI 10.1007/s00044-012-0026-x.

[40] Patel, R.V.; Patel, P.K.; Kumari, P.; Rajani, D.P.; Chikhalia, K.H. Synthesis of benzimidazolyl-1,3,4-oxadiazol-2ylthio-N-phenyl (benzothiazolyl)acetamides as antibacterial, antifungal and antituberculosis agents. *Eur. J. Med. Chem.*, **2012**, *53*, 41-51.

[41] Chimichi, S.; Boccalini, M.; Cosimelli, B. A new convenient route to 2-oxoethoxycoumarins: key intermediates in the synthesis of natural products. *Tetrahedron*, **2002**, *58*, 4851-4858.

[42] Clause, G.W. *Understanding microbes: A laboratory textbook for microbiology*; W.H. Freeman and Company: New York, USA, **1989**.