Synthesis, characterization and antibacterial activity of various substituted oxadiazolylpyrazolinyl/isoxazolinylcoumarin derivatives.

Hemlata Kaur, Sunil Kumar, Indu Singh and Ashok Kumar*

ABSTRACT

A series of 7-un/substituted-2-spiro-[5-((1-acetyl-5-(substitutedphenyl)amino)-3-(1-acetyl-5-(substitutedphenyl)) pyazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarins **(4a-4h)** and 7-un/substituted-2-spiro-2-[(-5-(substitutedphenyl)amino-4-(5-(substitutedphenyl))pyazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarins **(5a-5h)** by the reaction of 7-un/substituted-2-spiro-(3-substitutedarylidinyl chalconyl)-5-(substitutedarylidinylaminochalconyl)-oxadiazol-2-yl)coumarins **(3a-3h)** with hydrazine hydrate and hydroxyl amine respectively. All the newly synthesized compounds were screened for their antibacterial activity against K. pneumoniae, S. aureus, E. coli and B. sublitis and were compared with the standard drug ciprofloxacin. The most potent antibacterial compound of this series was **4g**. Structure of all the compounds were established by the elemental (C, H, N) and spectral (IR, ^{1}H NMR and mass) analysis.

Keywords: Substitutedcoumarin, Oxadiazolylcoumarins, Pyrazolinylcoumarins, Isoxazolinylcoumarins, Antibacterial activity, Acute toxicity.

INTRODUCTION

Coumarin is versatile pharmacophore which exhibits a wide variety of biological activities like antibacterial [1-3] and antimicrobial [4]. Moreover, various organic compounds containing a five membered heterocyclic ring i.e. oxadiazole make up a broad class that attracted attention in the past few years owing to its wide range of biological activities especially antimicrobial [5,6], antibacterial [7], antifungal [7], and anticonvulsant [8] and anti-inflammatory [9] activities. It is interesting to note from chemical literature that various pyrazoline and isoxazoline derivatives were also found to possess wide spectrum of bactericidal, fungicidal, and antimicrobial activities [10-13]. In the light of above observations we report herein the synthesis of compounds **(4a-4h)** and **(5a-5h)** with the hope to get better antibacterial agents.

MATERIAL AND METHODS

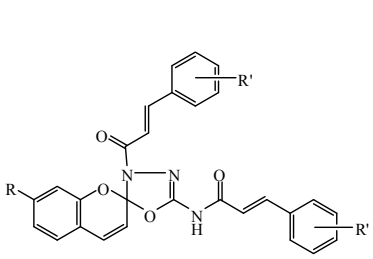
Antibacterial activity: All the newly synthesized compounds **3a-3h**, **4a-4h** and **5a-5h** were tested for their antibacterial activity. Antibacterial activity was determined by agar cup plate method [14] at a concentration of 100mg/ml using DMF as a solvent against the following organism- Escherichia coli, Staph. Aureus, Klebsiella pneumoniae and B. sublitis. The zone of inhibition of each strain was recorded. The activity has been compared with known standard drug ciprofloxacin at 10 µg/ml concentration. The biological results were analysed statistically by student't' test. Propylene glycol treated group served as control.

Approximate lethal dose (LD_{50}): The LD_{50} was determined by the method of Smith [15].

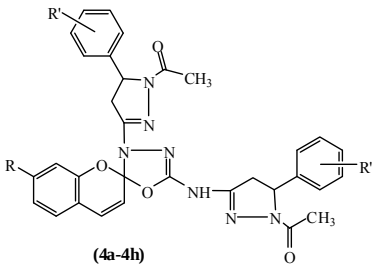
RESULTS AND DISCUSSION

All the newly synthesized compounds **3a-3h, 4a-4h** and **5a-5h** were screened for their antibacterial activity. The pharmacogical data of all the compounds have been reported in table 1.

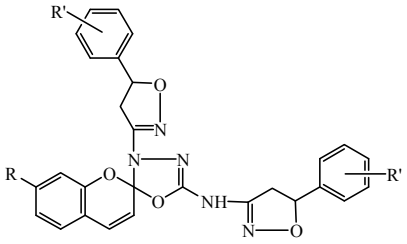
Table 1: Antibacterial activity of compounds 3a-3h, 4a-4h and 5a-5h.



(3a-3h)



(4a-4h)



(5a-5h)

Com. No.	R	R'	Zone of inhibition (diameter in mm)				LD_{50} mg/kg i.p.
			K. Pneumoniae	S. aureus	E. coli	B. sublitis	
Control	-	-	Nil	Nil	Nil	Nil	-
Ciprofloxacin	-	-	26	23	25	22	-
3a.	H	2-OH	-	-	8	4	>1000
3b.	H	4-OCH_3	7	10	12	10	>1000
3c.	H	4-OH,3-OCH_3	10	12	17	12	>1000
3d.	H	4-$N(CH_3)_2$	11	-	12	-	>1000
3e.	OCH_3	2-OH	13	12	-	-	>1000
3f.	OCH_3	4-OCH_3	8	15	16	10	>1000
3g.	OCH_3	4-OH,3-OCH_3	-	12	20	9	>1000
3h	OCH_3	4-$N(CH_3)_2$	9	20	-	12	>1000
4a.	H	2-OH	20	18	-	-	>1000
4b.	H	4-OCH_3	15	14	15	14	>1000
4c.	H	4-OH,3-OCH_3	20	18	22	20	>1000
4d.	H	4-$N(CH_3)_2$	18	17	-	-	>1000
4e.	OCH_3	2-OH	18	-	10	8	>1000
4f.	OCH_3	4-OCH_3	21	14	24	19	>1000
4g.	OCH_3	4-OH,3-OCH_3	28	24	27	22	>2000
4h.	OCH_3	4-$N(CH_3)_2$	24	18	22	20	>1000
5a.	H	2-OH	16	12	12	-	>1000
5b.	H	4-OCH_3	-	12	20	12	>1000
5c.	H	4-OH, 3-OCH_3	16	-	20	14	>1000
5d.	H	4-$N(CH_3)_2$	20	21	12	12	>1000
5e.	OCH_3	2-OH	17	16	-	6	>1000
5f.	OCH_3	4-OCH_3	20	19	14	15	>1000
5g.	OCH_3	4-OH,3-OCH_3	26	23	26	21	>1000
5h.	OCH_3	4-$N(CH_3)_2$	22	17	16	17	>1000

The characteristic feature of this series is the presence of different heterocyclic moieties at 2nd position of substituted coumarin ring. Compound **3a** showed mild antibacterial activity against E. coli and B. Sublitis. This compound was devoid of antibacterial activity against K. pneumoniae and S. aureus. Compounds **3b, 3c** and **3f** exhibited moderate

activity against S. aureus, E. coli. and B. sublitis. Moreover, compound **3g** (having 4-hydroxy-3-methoxyphenyl moiety) showed good activity against E. coli, whereas compound **3h** (having NN'-dimethylaminophenyl moiety) showed good activity against S. aureus (20 mm zone of inhibition). Furthermore, in the next step compounds (various substituted pyrazoline derivatives), an increase in antibacterial activity was noticed. Compounds **4a** showed significant antibacterial activity against K. pneumoniae. Moreover, compounds **4c, 4f** and **4h** elicited good antibacterial response against K. pneumoniae, E. coli and B. sublitis (i.e. 19-24 mm zone of inhibition) and moderate activity against S. aureus. Furthermore, compound **4g** namely 7-methoxy-3-spiro-[2-(1-acetyl-5-(4-hydroxy-3-methoxyphenyl)amino-4-(1-acetyl-5-(4-hydroxy-3-methoxyphenyl))pyazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin has shown higher antibacterial activity (i.e. zone of inhibition 28, 24, 27, and 22mm against K. pneumoniae, S. aureus, E. coli, and B. sublitis respectively) than standard drug ciprofloxacin. On the other side, i. e. compounds **5a-5h** (having various substituted isoxazoline ring) exhibited different antibacterial responses against different bacterial strain. In these compounds, compound **5d** and **5f** showed good activity against K. pneumoniae, S. aureus and moderate activity against E. coli and B. sublitis. However, compound **5h** exhibited good response against K. pneumoniae. Moreover, compound **5g** exhibited equipotent activity against K. pneumoniae, S. aureus and higher activity against E. coli than standard drug ciprofloxacin. The latter compound showed less potent activity against B. sublitis than standard drug ciprofloxacin.

5. Conclusion

From this study, we may concluded that :

1. Compounds having 5-membered pyrazoline ring exhibited better activity than their corresponding isoxazoline derivatives.
2. Compounds with 4-hydroxy-3-methoxyphenyl moiety (i.e. compound **4g** and **5g**) at 5th position of pyrazoline and isoxazoline ring showed more promising results than the other substituted derivatives against all the bacterial strains.

All reagents and solvents were of analytical grade and used directly. All reagents and solvents were generally used as received from the analytical grade. Reactions were routinely performed in oven-dried borosil glassware. The melting points of compounds were determined in open capillaries with the help of thermonic melting point apparatus and were uncorrected. Homogeneity of all the newly synthesized compounds was routinely checked by thin layer chromatography (TLC) on silica gel G coated plate of 0.5 mm thickness. The eluent was a mixture of different polar and nonpolar solvents in different proportions, and spots were visualized under iodine chamber. Elemental analysis (C, H, N) of all the compounds were performed on CHN analyzer, Carlo Erba 1108 analyzer at the Central Drug Research Institute (Lucknow, India). The IR spectra were recorded on Perkin Elmer 881 FTIR spectrophotometer (n_{max} in cm^{-1}). The ^{1}H-NMR spectra were recorded in $CDCl_3$ and $DMSOd_6$ on Brucker DRX-300 FTNMR instrument. Mass spectra were determined on JEOL D-300 instrument.

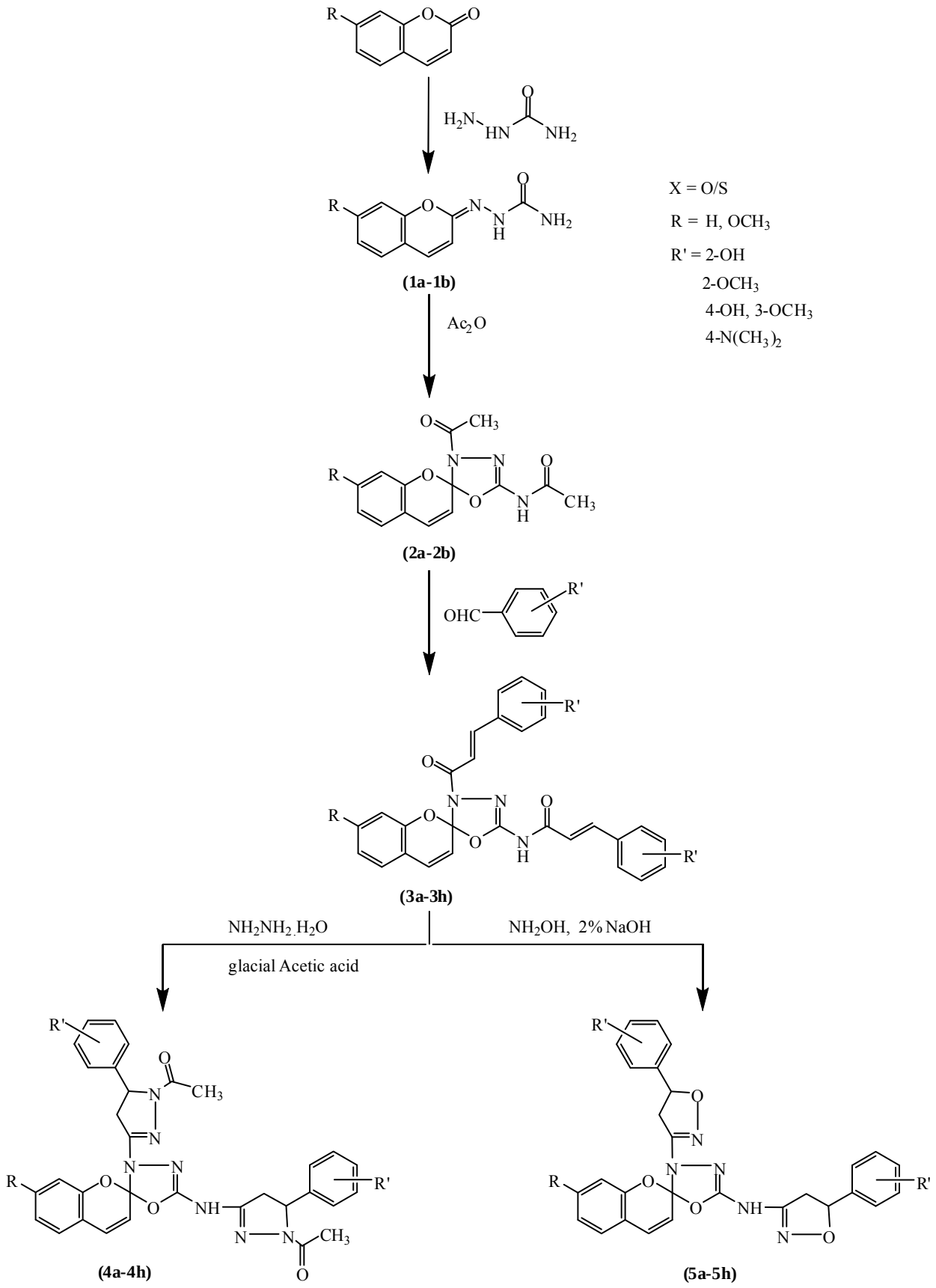
EXPERIMENTAL PROCEDURE

General procedure for the synthesis of 7-un/substituted-coumarin-2-semicarbazone 1a-1b

A solution of semicarbazide and thiosemicarbazide (2.0 mole) in glacial acetic acid (26 ml) and isatin or indole 2, 3 dione (2.0 mole) and the mixture was refluxed for 3 h. After cooling the solid mass was collected by filtration, washed well with water, dried and recrystallized from appropriate solvent to give compounds **1a** and **1b** respectively.

Coumarin-2-semicarbazone 1a: Yield 85% (Methanol); m.p. 84 ^{0}C. IR (KBr, □max in cm^{-1}): 3330 (NH sym.), 3000 (Aromatic C-H str.), 2170 ((-CN str.), 1690 (C=O str.), 1292 (N-N), 1040 (C-O-C); ^{1}H-NMR ($CDCl_3$) □in ppm 6.82-7.80 (m, 6H, Ar-H), 4.32 (s, 2H, NH_2), 6.60 (s, 1H, NH). Anal. calcd. for $C_{10}H_9N_3O_2$: C, 59.11; H, 4.46; N, 20.68: Found : C, 59.10; H, 4.48; N, 20.66%

7-methoxy-coumarin-2-semicarbazone 1b: Yield 75% (ethanol); m.p. 90^{0}C. IR (KBr, □max in cm^{-1}): 3300 (NH sym.), 3010 (Aromatic C-H str.), 2177 ((-CN str.), 1685 (C=O str.), 1294 (N-N), 1035 (C-O-C); ^{1}H-NMR ($CDCl_3$) □in ppm 3.81 (s, 3H, OCH_3), 6.92-7.83 (m, 5H, Ar-H), 4.32 (s, 2H, NH_2), 6.63 (s, 1H, NH). Anal. calcd. for $C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.75; N, 18.02: Found : C, 56.63; H, 4.70; N, 18.03%



Scheme 1: Synthetic route of coumarin derivatives

General procedure for the synthesis of 7-un/substituted-2-[5-acetylamino-3-(acetyl)-1, 3, 4-oxadiazolyl]-coumarin 2a-2b

A mixture of compounds **1a** and **1b** (1.0 mole) and freshly distilled acetic anhydride (40 ml) was heated to 110-120 ^{0}C for 4 h and after removal of acetic anhydride from the reaction mixture with the help of rotary vaccum evaporator, a solid mass was obtained which was recrystallized from suitable solvents to give compounds **2a** and **2b.**

2-[5-acetylamino-3-(acetyl)-1, 3, 4-oxadiazolyl]-coumarin 2a: Yield 76% (Acetone); m.p. 111 ^{0}C. IR (KBr, max in cm^{-1}): 3330 (NH sym.), 3008 (Aromatic C-H str.), 2178 (-CN str.), 1684 (C=O str.), 1290 (N-N), 1038 (C-O-C); ^{1}H-NMR ($CDCl_3$) in ppm 3.47 (s, 6H, 2 x $COCH_3$), 6.98-7.81 (m, 6H, Ar-H), 8.63 (s, 1H, NH). Anal. calcd. for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63: Found : C, 58.50; H, 4.54; N, 14.66%

7-Methoxy-2-[5-acetylamino-3-(acetyl)-1, 3, 4-oxadiazolyl]-coumarin 2b: Yield 73% (Methanol); m.p. 120 ^{0}C. IR (KBr, max in cm^{-1}): 3430 (OH), 3420 (NH sym.), 3000 (Aromatic C-H str.), 2180 (-CN str.), 1689 (C=O str.), 1295 (N-N), 1037 (C-O-C); ^{1}H-NMR ($CDCl_3$+$DMSOd_6$) in ppm 2.70 (s, 6H, 2 x $COCH_3$), 3.52 (s, 3H, OCH_3), 6.88-7.71 (m, 5H, Ar-H), 8.60 (s, 1H, NH). Anal. calcd. for $C_{15}H_{15}N_3O_5$: C, 56.78; H, 4.76; N, 13.24: Found : C, 56.78; H, 4.76; N, 13.24%

General procedure for the synthesis of 7-un/substituted-2-spiro-(3-substitutedarylidinyl chalconyl)-5-(substituted arylidinylaminochalconyl)-oxadiazol-2-yl)coumarins 3a-3h

A solution of compound **2a-2b** (0.5 mol) in absolute ethanol (50 ml) in 2% NaOH and various substituted aromatic aldehydes (0.1 mole) was refluxed for 8-12 h, concentrated, cooled and poured onto ice. The solid thus obtained was filtered, washed with water and recrystallised from appropriate solvent to obtained compounds **3a-3d**.

2-spiro-(3-(2-hydroxyarylidinylchalconyl)-5-(2-hydroxyarylidinylaminoxhalconyl)-oxadiazol-2-yl)coumarin 3a: Yield 80% (ethanol); m.p. 138 ^{0}C. IR (KBr, max in cm^{-1}): 3440 (OH), 3368 (NH sym.), 3010 (Aromatic C-H str.), 2195 (-CN str.), 1698 (C=O str.), 1570 (CH=CH), 1292 (N-N), 1045 (C-O-C); ^{1}H-NMR ($CDCl_3$) in ppm 6.60 (d, 2H, 2 x COCH), 6.88-7.79 (m, 14H, Ar-H), 8.87 (s, 1H, NH), 8.82 (d, 2H, 2 x =CHAr), 11.21 (s, 2H, 2 x OH). Anal. calcd. for $C_{28}H_{21}N_3O_6$: C, 67.87; H, 4.27; N, 8.48: Found : C, 67.89; H, 4.24; N, 8.46%

2-spiro-(3-(2-methoxyarylidinylchalconyl)-5-(2-methoxyarylidinylaminoxhalconyl)-oxadiazol-2-yl)coumarin 3b: Yield 70% (Methanol); m.p. 142 ^{0}C. IR (KBr, max in cm^{-1}): 3347 (NH sym.), 3000 (Aromatic C-H str.), 2180 (-CN str.), 1687 (C=O str.), 1577 (CH=CH), 1290 (N-N), 1038 (C-O-C); ^{1}H-NMR ($CDCl_3$+$DMSOd_6$) in ppm 6.60 (d, 2H, 2 x COCH), 6.78-7.89 (m, 14H, Ar-H), 8.80 (s, 1H, NH), 8.87 (d, 2H, 2 x =CHAr), 3.50 (s, 6H, OCH_3). Anal. calcd. for $C_{30}H_{25}N_3O_6$: C, 68.82; H, 4.81; N, 8.03: Found : C, 68.80; H, 4.86; N, 8.02%

2-spiro-(3-(4-hydroxy-3-methoxyarylidinylchalconyl)-5-(4-hydroxy-3-methoxyaryli dinylamino chalconyl)-oxadiazol-2-yl)coumarin 3c: Yield 65% (ethanol); m.p. 154 ^{0}C. IR (KBr, max in cm^{-1}): 3447 (OH), 3012 (Aromatic C-H str.), 3337 (NH sym.), 2190 (-CN str.), 1682 (C=O str.), 1580 (CH=CH), 1292 (N-N), 1039 (C-O-C); ^{1}H-NMR ($DMSOd_6$) in ppm 6.63 (d, 2H, 2 x COCH), 6.79-7.80 (m, 12H, Ar-H), 8.79 (s, 1H, NH), 8.89 (d, 2H, 2 x =CHAr), 3.45 (s, 6H, OCH_3), 11.20 (s, 2H, 2 x OH). Anal. calcd. for $C_{30}H_{25}N_3O_8$: C, 64.86; H, 4.54; N, 7.56: Found : C, 64.83; H, 4.52; N, 7.59%

2-spiro-(3-(4-NN'-dimethylarylidinylchalconyl)-5-(4-NN'-dimethylarylidinylaminochalconyl)-oxadiazol-2-yl)coumarin 3d: Yield 75% (Petroleum ether); m.p. 149 ^{0}C. IR (KBr, max in cm^{-1}): 3341 (NH sym.), 3000 (Aromatic C-H str.), 2180 (-CN str.), 1683 (C=O str.), 1571 (CH=CH), 1292 (N-N), 1037 (C-O-C); ^{1}H-NMR ($CDCl_3$) in ppm 1.30 (s, 12H, 2 x N $(CH_3)_2$), 6.61 (d, 2H, 2 x COCH), 6.76-7.84 (m, 14H, Ar-H), 8.70 (s, 1H, NH), 8.80 (d, 2H, 2 x =CHAr). Anal. calcd. for $C_{32}H_{31}N_5O_4$: C, 69.93; H, 5.69; N, 12.74: Found : C, 69.90; H, 5.66; N, 12.78%

7-Methoxy-2-spiro-(3-(2-hydroxyarylidinylchalconyl)-5-(2-hydroxyarylidinylamino chalconyl)-oxadiazol-2-yl)coumarin 3e: Yield 74% (ethanol); m.p. 162 ^{0}C. IR (KBr, max in cm^{-1}): 3443 (OH), 3350 (NH sym.), 3010 (Aromatic C-H str.), 2194 (-CN str.), 1680 (C=O str.), 1578 (CH=CH), 1292 (N-N), 1038 (C-O-C); ^{1}H-NMR ($CDCl_3$) in ppm 3.48 (s, 3H, OCH_3), 6.64 (d, 2H, 2 x COCH), 6.89-7.79 (m, 13H, Ar-H), 8.87 (s, 1H, NH), 8.82 (d, 2H, 2 x =CHAr), 11.21 (s, 2H, OH). Anal. calcd. for $C_{29}H_{23}N_3O_7$: C, 66.28; H, 4.41; N, 8.00: Found : C, 66.30; H, 4.40; N, 8.03%

7-Methoxy-2-spiro-(3-(2-methoxyarylidinylchalconyl)-5-(2-methoxyarylidinylamino chal- conyl)-oxadiazol-2-yl)coumarin 3f: Yield 78% (Methanol); m.p. 171 ^{0}C. IR (KBr, max in cm^{-1}): 3335 (NH sym.), 3000 (Aromatic C-H str.), 2189 (-CN str.), 1681 (C=O str.), 1578 (CH=CH), 1292 (N-N), 1065 (C-O-C); ^{1}H-NMR ($CDCl_3$) in ppm

6.61 (d, 2H, 2 x COCH), 6.77-7.83 (m, 13H, Ar-H), 8.83 (s, 1H, NH), 8.89 (d, 2H, 2 x =CHAr), 3.53 (s, 9H, 3 x OCH_3). Anal. calcd. for $C_{31}H_{27}N_3O_7$: C, 67.27; H, 4.92; N, 7.59: Found : C, 67.25; H, 4.94; N, 7.60%

7-Methoxy-2-spiro-(3-(4-hydroxy-3-methoxyarylidinylchalconyl)-5-(4-hydroxy-3-methoxyarylidinylaminochalconyl)-oxadiazol-2-yl)coumarin 3g: Yield 72% (DMF-water); m.p. 179 ^{0}C. IR (KBr, □max in cm^{-1}): 3452 (OH), 3352 (NH sym.), 3000 (Aromatic C-H str.), 2196 (-CN str.), 1689 (C=O str.), 1582 (CH=CH), 1294 (N-N), 1064 (C-O-C); ^{1}H-NMR ($CDCl_3$) □in ppm 6.64 (d, 2H, 2 x COCH), 6.74-7.80 (m, 11H, Ar-H), 8.79 (s, 1H, NH), 8.89 (d, 2H, 2 x =CHAr), 3.46 (s, 9H, 3 x OCH_3), 11.18 (s, 2H, 2 x OH). Anal. calcd. for $C_{31}H_{27}N_3O_9$: C, 63.59; H, 4.65; N, 7.18: Found : C, 63.59; H, 4.65; N, 7.20%

7-Methoxy-2-spiro-(3-(4-NN'-dimethylarylidinylchalconyl)-5-(4-NN'-dimethylaryli-dinylamino chalconyl)-oxadiazol-2-yl)coumarin 3h: Yield 76% (Methanol); m.p. 173 ^{0}C. IR (KBr, □max in cm^{-1}): 3342 (NH sym.), 3015 (Aromatic C-H str.), 2200 (-CN str.), 1698 (C=O str.), 1579 (CH=CH), 1292 (N-N), 1067 (C-O-C); ^{1}H-NMR ($CDCl_3$) □ in ppm: 1.32 (s, 12H, 2 x N $(CH_3)_2$), 3.55 (s, 3H, OCH_3), 6.65 (d, 2H, 2 x COCH), 6.75-7.86 (m, 13H, Ar-H), 8.70 (s, 1H, NH), 8.80 (d, 2H, 2 x =CHAr), 11.22 (s, 1H, OH). Anal. calcd. for $C_{33}H_{33}N_5O_5$: C, 68.38; H, 5.74; N, 12.08: Found : C, 68.30; H, 5.76; N, 12.05%

General procedure for the synthesis of 7-un/substituted-2-spiro-[5-(1-acetyl-5-(substitutedphenyl)amino-3-(1-acetyl-5-(substitutedphenyl))pyazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarins 4a-4h

To a solution of **3a-3h** (0.03 mole) in methanol, hydrazine hydrate (99%) (0.03 mole) and few drops of glacial acetic acid were added. The reaction mixture were refluxed for 10 h, distilled and cooled. The separated solid was filtered, washed with water and recrystallised from suitable solvent to furnish compound **4a-4h.**

2-spiro--[5-(1-acetyl-5-(2-hydroxyphenyl)amino-3-(1-acetyl-5-(2-hydroxyphenyl) pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4a: Yield 75% (ethanol); m.p. 197 ^{0}C. IR (KBr, □max in cm^{-1}): 3441 (OH), 3356 (NH sym.), 2980 (Aromatic C-H str.), 2210 (-CN str.), 1720 (C=O str.), 1290 (N-N), 1067 (C-O-C); ^{1}H-NMR ($CDCl_3$) □in ppm: 3.57 (s, 6H, 2 x $COCH_3$), 3.75 (d, 4H, 2 x CH_2 of pyrazoline ring), 5.74 (d, 2H, 2 x CH of pyrazole ring), 6.76-7.79 (m, 14H, Ar-H), 8.88 (s, 1H, NH), 11.25 (s, 2H, OH). Anal. calcd. for $C_{32}H_{29}N_7O_6$: C, 63.25; H, 4.81; N, 16.14: Found : C, 63.23; H, 4.86; N, 16.13%

2-spiro-[5-(1-acetyl-5-(2-methoxyphenyl)amino-3-(1-acetyl-5-(2-methoxyphenyl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4b:

Yield 70% (ethanol); m.p. 208 ^{0}C. IR (KBr, □max in cm^{-1}): 3337 (NH sym.), 2982 (Aromatic C-H str.), 2212 (-CN str.), 1712 (C=O str.), 1292 (N-N), 1065 (C-O-C); ^{1}H-NMR ($CDCl_3$) □in ppm: 3.53 (s, 6H, 2 x $COCH_3$), 3.73 (d, 4H, 2 x CH_2 of pyrazoline ring), 5.75 (d, 2H, 2 x CH of pyrazoline ring), 6.97-7.87 (m, 14H, Ar-H), 8.85 (s, 1H, NH), 3.52 (s, 6H, 2 x OCH_3). Anal. calcd. for $C_{34}H_{33}N_7O_6$: C, 64.24; H, 5.23; N, 15.42: Found : C, 64.24; H, 5.23; N, 15.42%

2-spiro-[5-(1-acetyl-5-(4-hydroxy-3-methoxyphenyl)amino-3-(1-acetyl-5-(4-hydroxy-3-methoxyphenyl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4c: Yield 71% (Acetone); m.p. 228 ^{0}C. IR (KBr, □max in cm^{-1}): 3450 (OH), 3351 (NH sym.), 2985 (Aromatic C-H str.), 2200 (-CN str.), 1719 (C=O str.), 1291 (N-N), 1061 (C-O-C); ^{1}H-NMR ($CDCl_3$) □in ppm: 3.50 (s, 6H, 2 x $COCH_3$), 3.74 (d, 4H, 2 x CH_2 of pyrazoline ring), 5.76 (d, 2H, 2 x CH of pyrazoline ring), 6.74-7.80 (m, 12H, Ar-H), 8.79 (s, 1H, NH), 3.46 (s, 6H, 3 x OCH_3), 11.18 (s, 2H, 2 x OH). Anal. calcd. for $C_{34}H_{33}N_7O_8$: C, 61.16; H, 4.98; N, 14.68: Found : C, 61.17; H, 4.97; N, 14.69%

2-spiro--[5-(1-acetyl-5-(4-NN'-dimethylaminophenyl)amino-3-(1-acetyl-5-(4-4-NN'-dimethylaminophenyl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4d: Yield 70% (Ethanol); m.p. 212 ^{0}C. IR (KBr, □max in cm^{-1}): 3344 (NH sym.), 2990 (Aromatic C-H str.), 2210 (-CN str.), 1715 (C=O str.), 1294 (N-N), 1048 (C-O-C); ^{1}H-NMR ($CDCl_3$) □ in ppm: 1.32 (s, 12H, 2 x $N(CH_3)_2$), 3.53 (s, 6H, 2 x $COCH_3$), 3.70 (d, 4H, 2 x CH_2 of pyrazoline ring), 5.72 (d, 2H, 2 x CH of pyrazoline ring), 6.75-7.86 (m, 14H, Ar-H), 8.70 (s, 1H, NH). Anal. calcd. for $C_{36}H_{39}N_9O_4$: C, 65.34; H, 5.94; N, 19.05: Found : C, 65.37; H, 5.92; N, 19.05%

7-Methoxy-2-spiro--[5-(1-acetyl-5-(2-hydroxyphenyl)amino-3-(1-acetyl-5-(2-hydroxyphenyl) pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4e: Yield 73% (Methanol); m.p. 218 ^{0}C. IR (KBr, □max in cm^{-1}): 3453 (OH), 3356 (NH sym.), 2992 (Aromatic C-H str.), 2190 (-CN str.), 1719 (C=O str.), 1290 (N-N), 1070, (C-O-C); ^{1}H-NMR ($DMSOd_6$) □in ppm: 3.39 (s, 3H, OCH_3), 3.61 (s, 6H, 2 x $COCH_3$), 3.70 (d, 4H, 2 x CH_2 of pyrazoline ring), 5.74 (d, 2H, 2 x CH of pyrazoline ring), 6.89-7.88 (m, 13H, Ar-H), 8.80 (s, 1H, NH), 11.18 (s, 2H, 2 x OH). Anal. calcd. for $C_{33}H_{31}N_7O_7$: C, 62.16; H, 4.90; N, 15.38: Found : C, 62.17 H, 4.90; N, 15.36%

7-Methoxy-2-spiro--[5-(1-acetyl-5-(2-methoxyphenyl)amino-3-(1-acetyl-5-(2-methoxyphenyl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4f: Yield 70% (ethanol); m.p. 228 ^{0}C. IR (KBr, max in cm^{-1}): 3345 (NH sym.), 2990 (Aromatic C-H str.), 2215 (-CN str.), 1710 (C=O str), 1290 (N-N), 1046 (C-O-C); ^{1}H-NMR ($CDCl_3$) in ppm: 3.56 (s, 6H, 2 x $COCH_3$), 3.74 (d, 4H, 2 x CH_2 of pyrazoline ring), 5.76 (d, 2H, 2 x CH of pyrazoline ring), 6.74-7.80 (m, 13H, Ar-H), 8.79 (s, 1H, NH), 3.46 (s, 9H, 3 x OCH_3). Anal. calcd. for $C_{35}H_{35}N_7O_7$: C, 63.15; H, 5.30; N, 14.73: Found : C, 63.12; H, 5.29; N, 14.76%

7-Methoxy-2-spiro--[5-(1-acetyl-5-(4-hydroxy-3-methoxyphenyl)amino-3-(1-acetyl-5-(4-hydroxy-3-methoxyphenyl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4g: Yield 69% (DMF-water); m.p. 240 ^{0}C. IR (KBr, max in cm^{-1}): 3440 (OH), 3348 (NH sym.), 2987 (Aromatic C-H str.), 2220 (-CN str.), 1713 (C=O str.), 1292 (N-N), 1048 (C-O-C); ^{1}H-NMR ($CDCl_3$) in ppm: 3.49 (s, 6H, 2 x $COCH_3$), 3.74 (d, 4H, 2 x CH_2 of pyrazoline ring), 5.75 (d, 2H, 2 x CH of pyrazoling ring), 6.74-7.80 (m, 11H, Ar-H), 8.79 (s, 1H, NH), 3.46 (s, 9H, 3 x OCH_3), 11.18 (s, 2H, 2 x OH). Anal. calcd. for $C_{35}H_{35}N_7O_9$: C, 60.25; H, 5.06; N, 14.05: Found : C, 60.23; H, 5.07; N, 14.07%

7-Methoxy-2-spiro-[5-(1-acetyl-5-(4-NN'-dimethylaminophenyl)amino-3-(1-acetyl-5-(4-4-NN'-dimethylaminophenyl)pyrazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 4h: Yield 71% (Methanol); m.p. 231 ^{0}C. IR (KBr, max in cm^{-1}): 3347 (NH sym.), 2992 (Aromatic C-H str.), 2200 (-CN str.), 1714 (C=O str.), 1291 (N-N), 1062 (C-O-C); ^{1}H-NMR ($CDCl_3$) in ppm: 1.34 (s, 12H, 2 x $N(CH_3)_2$), 3.40 (s, 3H, OCH_3), 3.50 (s, 6H, 2 X $COCH_3$), 3.83 (d, 4H, 2 x CH_2 of pyrazoline ring), 5.77 (d, 2H, 2 x CH of pyrazoline ring), 6.78-7.89 (m, 13H, Ar-H), 8.73 (s, 1H, NH), 11.22 (s, 1H, OH). Anal. calcd. for $C_{37}H_{41}N_9O_5$: C, 64.24; H, 5.97; N, 18.22: Found : C, 64.27; H, 5.93; N, 18.29%

General procedure for the synthesis of 7-un/substituted-3-spiro-[2-(-5-(substitutedphenyl)amino-4-(-5-(substitutedphenyl))isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarins 5a-5h

To a solution of **3a-3h** (0.03 mole) in methanol (50 ml), hydroxyl amine (0.03 mole) was added. The reaction mixture was refluxed for 10 h in presence of 2% NaOH solution. The resulting mixtures were concentrated and poured onto ice. The completion of reaction was monitored by TLC. The solid thus obtained were filtered, washed and recrystallized with appropriate solvents to furnish compounds **5a-5h**.

2-spiro-[5-(5-(2-hydroxyphenyl)amino-3-(5-(2-hydroxyphenyl)isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5a: Yield 72% (Ethanol); m.p. 250 ^{0}C. IR (KBr, max in cm^{-1}): 3435 (OH), 3339 (NH sym.), 2995 (Aromatic C-H str.), 2230 (-CN str.), 1722 (C=O str.), 1294 (N-N), 1060 (C-O-C); ^{1}H-NMR ($DMSOd_6$) in ppm: 3.80 (d, 4H, 2 x CH_2 of isoxazoline ring), 5.87 (d, 2H, 2 x CH of isoxazoline ring), 6.76-7.79 (m, 14H, Ar-H), 8.88 (s, 1H, NH), 11.25 (s, 2H, 2 x OH). Anal. calcd. for $C_{28}H_{23}N_5O_6$: C, 63.99; H, 4.41; N, 13.33: Found : C, 63.96; H, 4.43; N, 13.36%

2-spiro-[5-(5-(2-methoxyphenyl)amino-3-(5-(2-methoxyphenyl)isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5b: Yield 71% (Acetone); m.p. 261 ^{0}C. IR (KBr, max in cm^{-1}): 3420 (NH sym.), 2998 (Aromatic C-H str.), 2225 (-CN str.), 1715 (C=O str.), 1293 (N-N), 1065 (C-O-C); ^{1}H-NMR ($CDCl_3$) in ppm: 3.83 (d, 4H, 2 x CH_2 of isoxazoline ring), 5.85 (d, 2H, 2 x CH of isoxazoline ring), 6.97-7.87 (m, 14H, Ar-H), 8.85 (s, 1H, NH), 3.50 (s, 6H, 2 x OCH_3). Anal. calcd. for $C_{30}H_{27}N_5O_6$: C, 65.09; H, 4.92; N, 12.65: Found : C, 65.04; H, 4.95; N, 12.63%

2-spiro-[5-(5-(4-hydroxy-3-methoxyphenyl)amino-3-(5-(4-hydroxy-3-methoxy phenyl)isoxazolin -3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5c: Yield 68% (Methanol); m.p. 274 ^{0}C. IR (KBr, max in cm^{-1}): 3444 (OH), 3420 (NH sym.), 3000 (Aromatic C-H str.), 2235 (-CN str.), 1730 (C=O str.), 1292 (N-N), 1060 (C-O-C); ^{1}H-NMR ($CDCl_3$) in ppm: 3.87 (d, 4H, 2 x CH_2 of isoxazoline ring), 5.86 (d, 2H, 2 x CH of isoxazoline ring), 6.74-7.80 (m, 12H, Ar-H), 8.79 (s, 1H, NH), 3.46 (s, 6H, 3 x OCH_3), 11.18 (s, 2H, 2 x OH). Anal. calcd. for $C_{30}H_{27}N_5O_8$: C, 61.53; H, 4.65; N, 11.96: Found : C, 61.52; H, 4.65; N, 11.94%

2-spiro--[5-(5-(4-NN'-dimethylaminophenyl)amino-3-(5-(4-4-NN'-dimethylaminophenyl) isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5d: Yield 64% (ethanol); m.p. 264 ^{0}C. IR (KBr, max in cm^{-1}): 3420 (NH sym.), 2994 (Aromatic C-H str.), 2200 (-CN str.), 1709 (C=O str.), 2985 (Aromatic C-H str.), 1294 (N-N), 1071 (C-O-C); ^{1}H-NMR ($CDCl_3$) in ppm: 1.32 (s, 12H, 2 x $N(CH_3)_2$), 3.86 (d, 4H, 2 x CH_2 of isoxazoline ring), 5.82 (d, 2H, 2 x CH of isoxazoline ring), 6.75-7.86 (m, 14H, Ar-H), 8.73 (s, 1H, NH). Anal. calcd. for $C_{32}H_{33}N_7O_4$: C, 66.31; H, 5.74; N, 16.91: Found : C, 66.30; H, 5.72; N, 16.96%

7-Methoxy-2-spiro-[5-(5-(2-hydroxyphenyl)amino-3-(5-(2-hydroxyphenyl)isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5e: Yield 68% (petroleum ether); m.p. 271 ^{0}C. IR (KBr, max in cm^{-1}): 3454 (OH), 3351 (NH sym.),

2995 (Aromatic C-H str.), 2240 (-CN str.), 1689 (C=O str.), 1290 (N-N), 1070 (C-O-C); ^{1}H-NMR ($CDCl_3$+$DMSOd_6$) □in ppm: 3.57 (s, 3H, OCH_3), 3.80 (d, 4H, 2 x CH_2 of isoxazoline ring), 5.84 (d, 2H, 2 x CH of isoxazoline ring), 6.89-7.88 (m, 13H, Ar-H), 8.80 (s, 1H, NH), 11.18 (s, 2H, 2 x OH). Anal. calcd. for $C_{29}H_{25}N_5O_7$: C, 62.70; H, 4.54; N, 12.61: Found : C, 62.68; H, 4.51; N, 12.64%

7-Methoxy-2-spiro-[5-(5-(2-methoxyphenyl)amino-3-(5-(2-methoxyphenyl) isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5f: Yield 69% (Acetone); m.p. 284 ^{0}C. IR (KBr, □max in cm^{-1}): 3337 (NH sym.), 2220 (-CN str.), 1715 (C=O str.), 1290 (N-N), 1042 (C-O-C); ^{1}H-NMR ($DMSOd_6$) □ in ppm: 3.74 (d, 4H, 2 x CH_2 of isoxazoline ring), 5.76 (d, 2H, 2 x CH of isoxazoline ring), 6.74-7.80 (m, 13H, Ar-H), 8.79 (s, 1H, NH), 3.46 (s, 9H, 3 x OCH_3). Anal. calcd. for $C_{31}H_{29}N_5O_7$: C, 63.80; H, 5.01; N, 12.00: Found : C, 63.86; H, 5.00; N, 12.03%

7-Methoxy-2-spiro-[5-(5-(4-hydroxy-3-methoxyphenyl)amino-3-(5-(4-hydroxy-3-methoxyphenyl) isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5g: Yield 70% (Methanol); m.p. 294 ^{0}C. IR (KBr, □max in cm^{-1}): 3455 (OH), 3351 (NH sym.), 2225 (-CN str.) 1690 (C=O str), , 1292 (N-N), 1060 (C-O-C); ^{1}H-NMR ($CDCl_3$) □ in ppm: 3.81 (d, 4H, 2 x CH_2 of isoxazoline ring), 5.87 (d, 2H, 2 x CH of isoxazoline ring), 6.77-7.80 (m, 11H, Ar-H), 8.79 (s, 1H, NH), 3.46 (s, 9H, 3 x OCH_3), 11.18 (s, 2H, 2 x OH). Anal. calcd. for $C_{31}H_{29}N_5O_9$: C, 60.48; H, 4.75; N, 11.38: Found : C, 60.44; H, 4.76; N, 11.34%

7-Methoxy-2-spiro-[5-(5-(4-NN'-dimethylaminophenyl)amino-3-(5-(4-4-NN'-dimethyl amino phenyl) isoxazolin-3-yl)-1,3,4-oxadiazol-2-yl]coumarin 5h:

Yield 73% (ethanol); m.p. 280 ^{0}C. IR (KBr, □max in cm^{-1}): 3339 (NH sym.), 2210 (-CN str.), 1680 (C=O str.), 1290 (N-N), 1044 (C-O-C); ^{1}H-NMR ($CDCl_3$) □ in ppm: 1.50 (s, 12H, 2 x $N(CH_3)_2$), 3.55 (s, 3H, OCH_3), 3.82 (d, 4H, 2 x CH_2 of isoxazoline ring), 5.88 (d, 2H, 2 x CH of isoxazoline ring), 6.68-7.88 (m, 13H, Ar-H), 8.73 (s, 1H, NH). Anal. calcd. for $C_{33}H_{35}N_7O_5$: C, 65.01; H, 5.79; N, 16.08: Found : C, 65.06; H, 5.75; N, 16.09%.

Acknowledgement

We are thankful to SAIF Punjab University, Chandigarh India for spectral and analytical analysis of newly synthesized compounds. One of the author, Hemlata Kaur is also thankful to UGC New Delhi, India for the award of J.RF.- Rajiv Gandhi National Junior Research Fellowship and financial support for this work.

References:

[1] S. Shah and R.H. Mehta, el al. Synthesis of some New 8-Methoxy-5-substituted-amino-methyl coumarins as Possible Antibacterial Agents; J. Indian Chem. Soc. 1989, 66: 802-803.
[2] A. K. Sengupta, S. Sen, V. Srivastava ; Synthesis of Coumarin Derivatives as Possible Antifungal and Antibacterial Agents. J. Indian Chem. Soc. 1989, 66: 710-716.
[3] R. Shridhar, Sastry, C.V. Reddy, N. K Vaidya, S. R. Moorty, G. S. Reddy, G. S. Thapar, et al. Antimicrobial agents: Synthesis and antibacterial activity of some new 4-[2-(heteroaryl) vinyl]coumarins, Indian J. Chem 1978, 16B: 704-708.
[4] Gadaginmath, S. Guru, Joshi, G. Raghvendra, et al. Synthesis and antimicrobial activity of benimidazolyl/coumarinyl methoxyindoles. Indian J. Chem. 1995, 34B: 120-124.
[5] N. G. Gawande, M. S. Shingare, et al. Synthesis of some Thiazolyl-thiosemicarbazides. Triazoles, Oxadiazoles, Thiadiazoles & Their Microbial Activity ; Indian J. Chem. 1987, 26B: 387-389.
[6] K. P. Roda, Vansdadia, H. Parekh, et al. Studies on 1,3,4-oxadiazoles, Part-II. Preparation and Antimicrobial Activity of 2-Benzoyl-amino-5-(2'-isopropyl-5'-methylphenoxy-methyl)-q,3,4-oxadiazoles ; J. Indian Chem. Society 1988, LXV: 807-811.
[7] A. O. Maslat, M. Abussaud, H. Tashtoush, M.Al-Talib, et al. Synthesis, antibacterial, antifungal and genotoxic activity of Bis-1,3,4-oxadiazole derivatives. Pol. J. Pharmacol. 2002, 54 (1): 55-59.
[8] A. Almasirad, S. A. Tabatabai, M. Faizi, A. Kebriacezadch, N. Mehrabi, A. Dalvandi, A.Shafiee, et al. Synthesis and anticonvulsant activity of new 2-Substituted-5-[2- (2-fluorophenoxy) phenyl]-1,3,4-oxadiazoles 1,2,4-triazoles, Bio. Med. Chem. 2004, 14: 6057-6059.
[9] A. A. Kadi, N. R. El-Brollosy, O. A. Al-Deep, E. E. Habib, T. M. Ibrahim, A. A. El-Emam, et al. Synthesis, antimicrobial and anti-inflammatory activities of novel 2-(1-adamantyl)-5-substituted- 1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles. Eur. J. Med. Chem. 2007, 42(2):235–242
[10] V. K. Ahluwalia, U. Uttara, H. R. Sharma, et al. Synthesis of some 1-substituted-3-(4'- methylphenyl)-4-N'-substituted p-sulphamyl benzeneazo Pyrazolin-5-ones as Potential Fungicides and Bactericides. Indian J. Pharm. Sci. 1986, 48 (6): 176-180.
[11] H. Kumar, V. Patel, P. S. Fernandes, et al. Synthesis of 5-Aryl-1,5-dihydro-6-nitropyrazolo[4,3-c]pyrazoles & Their Biological Activity. Indian J. Chem. 1989, 28B: 56-60.
[12] K. A. Thakar and A. M. Padhye, et al. Synthesis and antifungal Activity of 7-Benzodiazepinyl-7-Pyrazolyl and 7-Isoxazolyl-1,2-benzisoxazoles. J. Indian Chem. Soc. 1985, LXII: 465-467.
[13] K. C. Joshi, R. Jain, K. Sharma, et al. Studies in spiro-heterocycles. Part XIV. Synthesis and Antimicrobial Activities of Certain Fluorine containing spiro[3H-indole-3, 5'-isoxazolin]-2-[1H]–ones. J. Indian Chem. Soc. 1988, LXV: 640-642.
[14] D. Greenwood, R. C. B. Slack, J. F. Peutherer, et al. In; Medical Microbiology, 14th Edition., London, 1992, 1.Smith Q.E., In : Pharmacological screening tests progress in medicinal chemistry, Butterworths, London, 1960, 1: 1-33.