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Synthesis and evaluation of anti-inflammatory and antimicrobial activity of 2,5-disubstituted-1,3,4-oxadiazoles

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Abstract

In the present study two new series of [5-(4-substituted-phenyl)-[1,3,4]oxadiazol-2-yl]-pyridine (1a-1d) and 2-(4-substituted phenyl)-5-(2-aminophenyl)-1,3,4-oxadiazole (2a-2e) were synthesized from esters and hydrazine hydrate in the presence of ethanol through isonicotinic acid hydrazide and 2-aminobenzohydrazide respectively followed by reaction with phosphorus oxychloride and various aromatic acids. Synthesized compounds were subjected to anti-inflammatory and antimicrobial activity. A fair number of compounds were found to have good anti-inflammatory activity in carrageenan-induced rat paw oedema test, while all the compounds showed significant antibacterial activity.

Key words: 2,5-Disubstituted-1,3,4-oxadiazole, anti-inflammatory activity, antimicrobial agents.

INTRODUCTION

A diversity of useful biological effects is possessed by heterocyclic compounds containing oxadiazole nucleus [1]. In particular, compounds bearing 1,3,4-oxadiazole nucleus are known to exhibit unique antiedema and anti-inflammatory activity [2-5] Differently substituted oxadiazole moiety has been found to have other interesting activities such as analgesic [3,4], antimicrobial [6,7], antitubercular [8], anticonvulsant [9] and antitumour [10,11]. In the present work, an attempt has been made to synthesize analogues of 2,5-disubstituted 1,3,4-oxadiazole anticipating to possess anti-inflammatory and antimicrobial activity.

MATERIALS AND METHODS

Preparation of hydrazide

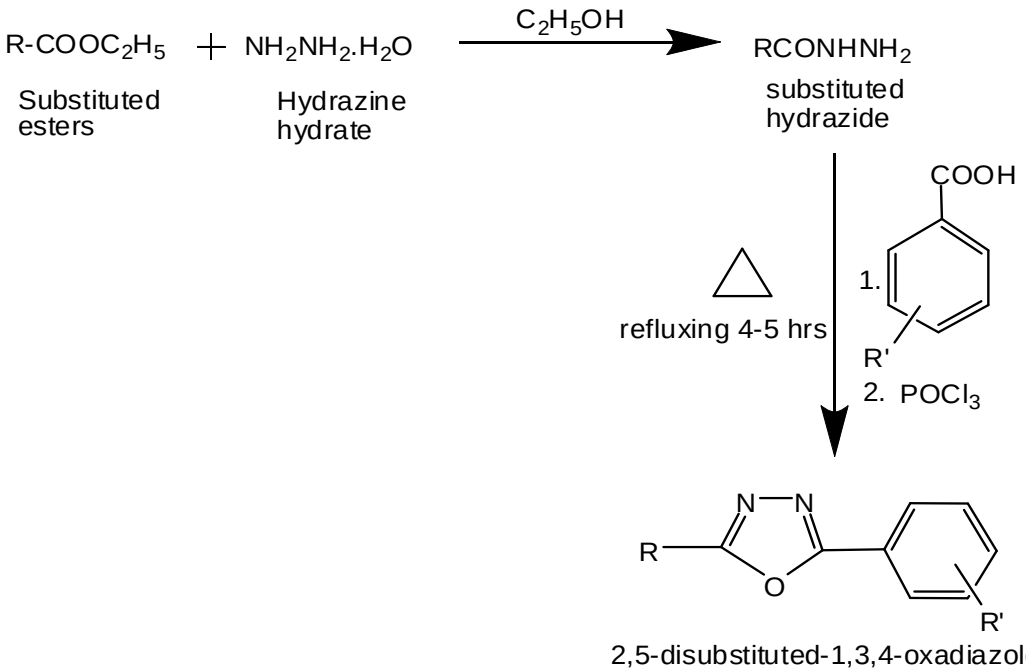
A mixture of substituted ester (0.1mole), excess of hydrazine hydrate (20ml, 0.4 mole) and absolute alcohol (50ml) was refluxed for 5 hours. Excess of solvent was distilled off. The

reaction mixture was cooled to 4-5°C and separated solid crystals were filtered, washed with cold water, dried and recrystallised from ethanol [12].

Synthesis of 1,3,4-oxadiazoles from respective hydrazides

A mixture of substituted aromatic acid (0.01mole) with hydrazide (0.01mole) dissolved in phosphorusoxytrichloride (15ml) was refluxed over a water bath for 4-5 hours. The progress of reaction was monitored by TLC using ethylacetate: acetone (9:1) as eluent. The reaction mixture was cooled and poured on to crushed ice drop wise with continuous stirring. The separated solid mass was neutralised with ammonia solution. The mixture was left overnight in refrigerator. The resulting solid thus obtained was collected by filtration, washed well with cold water, dried and recrystallized from absolute ethanol [13].

Scheme



2,5-disubstituted-1,3,4-oxadiazole

R=2-$NH_2C_6H_4$, C_6H_4N; R'= 4-ClC_6H_4, 4-$NH_2C_6H_4$, 4-$NO_2C_6H_4$, 3-$NO_2C_6H_4$, 2-OH-3-$CH_3C_6H_3$, 4-NH_2-2-OHC_6H_3

Physical and Spectral Characterisation [20,21]

Preparation of 2-(4-chlorophenyl)-5-(pyridine-4-yl)-1,3,4 oxadiazole 1(a)

$C_{13}H_8N_3OCl$, yield: 85.6%, Mp: 110-117˚C. TLC ethylacetate: acetone (9:1) R_f: 0.71. IR cm^{-1} ($KBr_{):}$ ν 3095 (aromatic C-H), 1598, 1481(aromatic C=C), 1722.7 (C=N), 1227 (asymmetric C-O-C), 1087 (symmetric C-O-C), 1050 (Ar-Cl), 738.4 (C-H Para subst); ^{1}HNMR (DMSO-d_6, δ ppm): 7.38-8.59 (m, 4H, C-H Pyr), 7.30-7.32 (m, 4H); MS (FAB) m/z: 257 (M^+), 258 (M^++1, 100%).

Preparation of 4-(5-(pyridine-4-yl)-1,3,4 oxadiazol-2-yl) aniline 1(b)

$C_{13}H_{10}N_4O$, yield: 82.5%, Mp: 100-105˚C. TLC ethylacetate: acetone (9:1) R_f: 0.72. IR cm^{-1} ($KBr_{):}$ ν 3443 (stretch NH_2), 1600 (bend NH_2), 3100 (aromatic C-H), 1654, 1405 (aromatic C=C), 1654 (C=N), 1256 (asymmetric C-O-C), 1182 (symmetric C-O-C), 846 (C-H Para subst); ^{1}HNMR (DMSO-d_6, δ ppm): 7.41-8.59 (m, 4H, C-H Pyr), 7.30-7.35 (m, 4H Arom.), 4.13 (s, NH_2); MS (FAB) m/z: 238 (M^+), 239 (M^++1, 100%).

Preparation 2-(4-nitrophenyl)-5-(pyridine-4-yl)-1,3,4-oxadiazole 1(c)
$C_{13}H_8N_4O_3$, yield: 60.2%, Mp: 160-166˚C. TLC ethylacetate: acetone (9:1) R_f: 0.48. IR cm^{-1} ($KBr_{):}$ ν 3091 (aromatic C-H), 1606, 1485 (aromatic C=C), 1664 (C=N), 1220 (asymmetric C-O-C), 1066 (symmetric C-O-C), 858 (C-H Para subst); 1549 (asymm. Ar-NO_2),1341(symm. Ar-NO_2); ^{1}HNMR (DMSO-d_6, δ ppm): 7.40-8.62 (m, 4H, C-H Pyr), 7.28-7.35 (m, 4H Arom.); MS (FAB) m/z: 268 (M^+), 269 (M^++1, 100%).

Preparation of 2-(3-nitrophenyl)-5-(pyridine-4-yl)-1,3,4 oxadiazole 1(d)
$C_{13}H_8N_4O_3$, yield: 79.8%, Mp: 125-130˚C. TLC ethylacetate: acetone (9:1) R_f: 0.63. IR cm^{-1} ($KBr_{):}$ ν3074 (aromatic C-H), 1596, 1475 (aromatic C=C), 1716 (C=N), 1225 (asymmetric C-O-C), 1062 (symmetric C-O-C), 725, 827 (C-H meta subst); 1528 (asymm. Ar-NO_2), 1344 (symm. Ar-NO_2); ^{1}HNMR (DMSO-d_6, δ ppm): 7.40-8.62 (m, 4H, C-H Pyr), 7.35-8.19 (m, 4H Arom.); MS (FAB) m/z: 268 (M^+), 269 (M^++1, 100%).

Preparation of 2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)aniline (2a)
$C_{14}H_{10}N_3OCl$, yield: 80.3%, Mp: 152-157˚C. TLC ethylacetate: acetone (9:1) R_f: 0.60. IR cm^{-1} ($KBr_{):}$ ν 3413 (stretch NH_2), 1560 (bend NH_2), 3074 (aromatic C-H), 1596, 1475 (aromatic C=C), 1614 (C=N), 1217 (asymmetric C-O-C), 1087 (symmetric C-O-C), 1050 (Ar-Cl), 769, 827 (C-H ortho and para subst); ^{1}HNMR (DMSO-d_6, δ ppm): 6.52-7.01 (m, 4H, C-H Arom), 7.22-7.34 (m, 4H Arom.), 4.21 (s, NH_2); MS (FAB) m/z: 281 (M^+), 282 (M^++1, 100%).

Preparation of 2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)aniline (2b)
$C_{14}H_{10}N_4O_3$, yield: 80.6%, Mp: 170-175˚C. TLC ethylacetate: acetone (9:1) R_f: 0.48. IR cm^{-1} ($KBr_{):}$ ν3440 (stretch NH_2), 1551 (bend NH_2), 3167 (aromatic C-H), 1619, 1402 (aromatic C=C), 1681 (C=N), 1258 (asymmetric C-O-C), 1086 (symmetric C-O-C), 746, 815 (C-H ortho and meta subst); 1531 (asymm. Ar-NO_2), 1350 (symm. Ar-NO_2); ^{1}HNMR (DMSO-d_6, δ ppm): 6.52-7.01 (m, 4H, C-H Arom), 7.52-8.19 (m, 4H Arom.), 4.20 (s, NH_2); MS (FAB) m/z: 282 (M^+), 283 (M^++1, 100%).

Preparation of 5-amino-2-(5-(2-aminophenyl)-1,3,4-oxadiazol-2-yl)phenol 2(c)
$C_{14}H_{12}N_4O_2$, yield: 75.1%, Mp: 163-169˚C. TLC ethylacetate: acetone (9:1) R_f: 0.71. IR cm^{-1} ($KBr_{):}$ ν 3695 (Aromatic OH), 3190 (stretch NH_2), 1565 (bend NH_2), 3167 (aromatic C-H), 1596, 1477 (aromatic C=C), 1670 (C=N), 1256 (asymmetric C-O-C), 1093 (symmetric C-O-C), 768, 831 (C-H ortho and para subst); ^{1}HNMR (DMSO-d_6, δ ppm): 4.22 (s, NH_2), 6.51-7.01 (m, 4H, C-H Arom), 6.75-7.10 (m, 4H Arom.), 5.92 (d, OH), 4.03 (d, NH_2); MS (FAB) m/z: 268 (M^+), 269 (M^++1, 100%).

Preparation of 2,2'- (1,3,4-oxadiazole-2,5-diyl)dianiline 2(d)
$C_{12}H_{12}N_4O$, yield: 88.3%, Mp: 155-160˚C. TLC ethylacetate: acetone (9:1) R_f: 0.66. IR cm^{-1} ($KBr_{):}$ ν 3213 (stretch NH_2), 1562 (bend NH_2), 3020 (aromatic C-H), 1597, 1490 (aromatic C=C), 1674 (C=N), 1216 (asymmetric C-O-C), 1094 (symmetric C-O-C), 1050 (Ar-Cl), 827 (C-H ortho subst); ^{1}HNMR (DMSO-d_6, δ ppm): 6.49-7.01 (m, 4H, C-H Arom), 6.51-7.01 (m, 4H Arom.), 4.28 (d, NH_2); MS (FAB) m/z: 258 (M^+), 260 (M^++2, 100%).

Preparation of 2-(5-(2-aminophenyl)-1,3,4-oxadiazol-2-yl)-6-methylphenol 2(e)
$C_{15}H_{13}N_3O_2$, yield: 84.4%, Mp: 141-145˚C. TLC ethylacetate: acetone (9:1) R_f: 0.56. IR cm^{-1} ($KBr_{):}$ ν 3506 (Aromatic OH), 3418 (stretch NH_2), 1601 (bend NH_2), 3157 (aromatic C-H), 1597, 1490 (aromatic C=C), 1674 (C=N), 1254 (asymmetric C-O-C), 1087 (symmetric C-O-C), 690, 770, 837 (C-H ortho and meta subst); ^{1}HNMR (DMSO-d_6, δ ppm): 6.49-7.01 (m, 4H, C-H

Arom), 6.79-7.01 (m, 4H Arom.), 4.01 (s, NH_2), 1.30 (d, CH_3), 5.82 (s, OH Phenolic); MS (FAB) m/z: 267 (M^+), 268 (M^++1, 100%).

Pharmacological activity

Anti-inflammatory activity against carrageenan-induced rat paw oedema:

All the newly synthesized compounds 1a-1d and 2a-2e were evaluated for their anti-inflammatory activity against carrageenan-induced acute paw edema in wistar rats weighing 150-200 g [14]. The animals were fed with standard pellet diet and water was given *ad libitum*. The animals were acclimatized for one week under laboratory conditions before performing the test. Carrageenan, 0.1 ml (1% w/v prepared in 0.9% saline solution) was injected in the subplanter region of right hind paw. Indomethacin, 1.5 mg/kg body weight per oral (suspended in PEG 400) was used as the standard drug. The synthesized compounds 50 mg/kg body weight were suspended in PEG 400 and administered to rats by the oral route. Before performing these experiments, ethical clearance was obtained from Institutional Animal Ethics Committee and conducted according to Indian National Science Academy guidelines for the use and care of Experimental animals (Reg. No) BBDNITM/IAEC/Clear/11/2009.

The animals were weighed and numbered into six groups each group containing six animals. A mark was made on the right hind paw, so that every time the paw was dipped in the mercury column up to the fixed mark, constant paw volume was ensured. The initial paw volume of each rat was noted by the mercury displaced method. To the first group saline was administered whereas indomethacin to the second group was administered orally. Test compounds were administered orally to the third, fourth, fifth and sixth groups. After 30 minutes, carrageenan 0.1 ml of 1% (w/v) was injected in the subplanter region of the right paw in all drug treated groups. Immediately after the injection of carrageenan, paw volumes were measured in a mercury plethysmograph. Thereafter the paw volume was measured at 1, 2, 4 and 8 hour intervals. The amount of edema in the drug treated groups was compared in relation to the control group with the corresponding time intervals. The percentage of inhibition by the drugs was calculated using the formula,

$$\% \text{ of edema inhibition} = 100 - (V_{test} / V_{control}) \times 100$$

Where $V_{control}$ = volume of paw edema in the control group
V_{test} = volume of paw edema in the drug treated group

Table 1 shows the effect of synthesized compounds on carrageenan-induced paw edema. The percentage of inhibition was compared with that of the standard drug.

Screening for antimicrobial activity:

The antimicrobial activity of all the synthesized compounds was evaluated by cup plate method against gram positive and gram negative bacterial strains and a fungal strain using ofloxacin and fluconazole as standard drugs for antibacterial and antifungal activities respectively. The bacterial strain used were *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* (MTCC 96) for gram positive and *Esherichia coli* (MTCC 739), *Pseudomonas aeruginosa* (MTCC 2453) for gram negative and for fungal strain viz., *Candida albicans* (MTCC 227). The in vitro antimicrobial activity was carried against 24 hour old cultures. The compounds were tested at a concentration of 50, 100, 200 and 400 µg/ml in 8%v/v dimethyl sulfoxide against all the organisms [15-19]. Among the compounds tested for antimicrobial activity, the compounds 1(a), 1(b), 1(d) and 2(c) showed promising activity.

Table 1. Anti-inflammatory Activity

Compound Code	Normal paw Volume	Mean paw volume ± SEM(ml) and % Inhibition			
		Time after Carrageenan injection			
		1 hr	2 hr	4 hr	8 hr
Control	1.512±0.062	3.067± 0.067	3.900± 0.106	4.850± 0.109	5.700± 0.110
Indomethacin	1.500±0.086	2.500±0.193** (70.19%)	2.417±0.140** (78.27%)	2.300±0.146** (87.27%)	2.100±0.103** (95.31%)
1(a)	1.267±0.033	2.600±0.010** (38.282%)	2.417±0.081** (37.610%)	2.717±0.162** (63.870%)	2.467±0.088** (76.831%)
1(b)	1.317±0.048	2.917±0.101 (40.738%)	3.150±0.118** (45.061%)	2.700±0.063** (68.887%)	2.383±0.060** (79.793%)
1(c)	1.450±0.022	2.950±0.099 (44.399%)	4.000±0.097 (50.639)	4.733±0.084 (63.671%)	5.633±0.088 (72.419%)
1(d)	1.267±0.033	2.483±0.048** (54.757%)	2.633±0.067** (59.069%)	2.133±0.042** (80.748%)	1.717±0.060** (91.478%)
2(a)	1.450±0.022	2.600±0.058** (58.137%)	2.800±0.058** (60.108%)	2.317±0.070** (80.552%)	1.867±0.088** (92.282%)
2(b)	1.650±0.043	2.933±0.042 (52.290%)	3.717±0.060 (48.144%)	4.150±0.076** (67.138%)	4.767±0.128* (75.756%)
2(c)	1.467±0.049	2.690±0.0710* (63.895%)	2.700±0.106** (63.358%)	2.133±0.131** (85.325%)	1.650±0.115** (94.168%)
2(d)	1.350±0.043	3.517±0.083** (20.867%)	3.750± 0.043** (28.643%)	3.167±0.236** (55.924%)	3.050±0.123** (68.849%)
2(e)	1.333±0.042	2.867±0.067 (43.144%)	3.317± 0.075** (45.699%)	3.867±0.158** (69.277%)	3.100±0.060** (80.172%)

*Values are expressed as mean ±SEM; n=6. *P<0.05, **P<0.01 compared with vehicle treated group using one way ANOVA followed by Dunnett's test.*

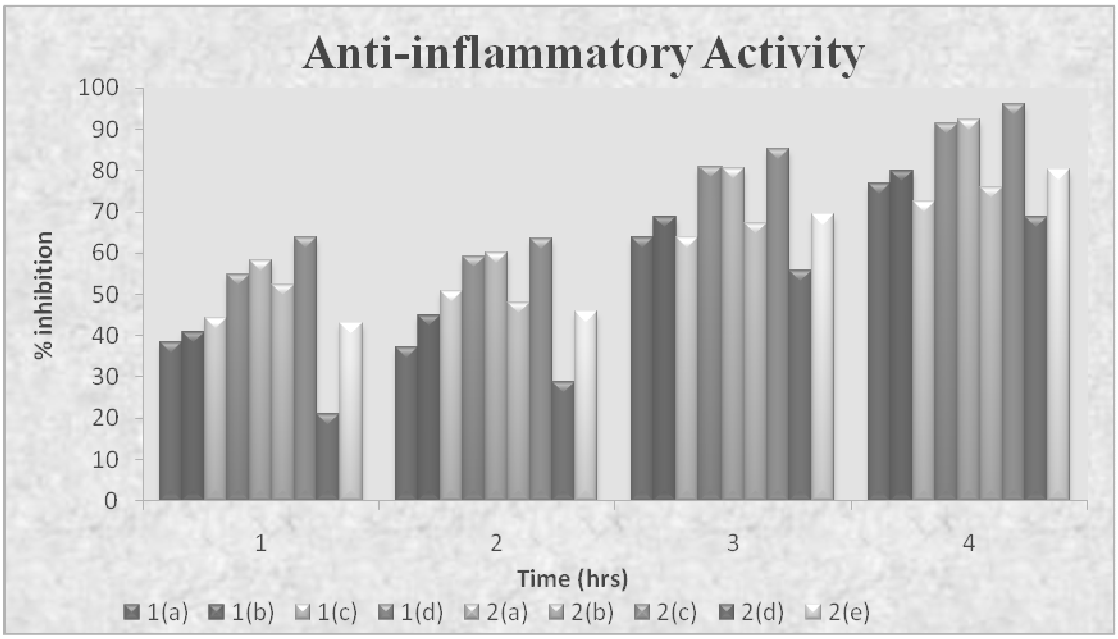


Table 2. Antimicrobial Activity

Compound code	Conc. (µg/ml)	Zone of Inhibition (mm)				
		Gram+ve bacteria		Gram-ve bacteria		Fungus
		SA	BS	EC	PA	CA
Ofloxacin	100	17	18	20	17	-
Fluconazole	100	-	-	-	-	20
1(a)	50	10	11	16	16	-
	100	09	15	18	19	-
	150	13	18	18	17	-
	200	18	18	18	16	-
1(b)	50	09	09	14	09	-
	100	19	17	15	15	-
	150	08	18	17	16	-
	200	-	18	17	17	-
1(c)	50	09	08	09	09	-
	100	09	10	08	09	-
	150	08	18	09	09	-
	200	09	17	18	18	-
1(d)	50	09	08	09	09	-
	100	09	17	16	18	-
	150	08	17	18	18	-
	200	09	18	17	19	-
2(a)	50	07	18	14	09	-
	100	08	18	14	16	-
	150	15	18	17	16	-
	200	17	18	18	18	-
2(b)	50	09	09	08	09	-
	100	09	13	09	18	-
	150	09	18	14	17	-
	200	14	16	17	18	-
2(c)	50	09	09	08	17	-
	100	18	18	14	16	-
	150	15	17	18	18	-
	200	18	17	19	18	-
2(d)	50	09	09	08	08	-
	100	09	08	14	16	-
	150	09	09	18	19	-
	200	09	14	18	17	-
2(e)	50	08	09	09	09	-
	100	08	17	18	19	-
	150	09	05	09	10	-
	200	18	17	19	11	-
Control (8%v/v DMSO)		-	-	-	-	-

SA - *Staphylococcus aureus*, BS -*Bacillus subtilis*, EC- *Escherichia coli*, PA- *Pseudomonas auruginosa*, CA- *Candida albicans*

RESULTS AND DISCUSSION

An insight into the anti-inflammatory activity with respect to the chemical structure reveals that compounds 1(d), 2(a) and 2(c) bearing a para amino, para chloro, meta nitro and ortho hydroxyphenyl moiety exhibited good anti-inflammatory activity. Compounds 1(a), 1(b), 2(b), 2(e) showed moderate anti-inflammatory activity. However compounds 2(d) did not show any activity in comparison to the standard drug.

On the other hand all the compounds showed promising antimicrobial activity against all the six organisms. However, none of the compounds showed any activity against *Candida albicans*.

CONCLUSION

All the synthesized compounds 1(a)-1(d) and 2(a)-2(e) were evaluated for their anti-inflammatory and antimicrobial activity by using standard methods. The investigation of anti-inflammatory activity revealed that compounds 1(a), 1(b), 1(d), 2(a), 2(b), 2(c) and 2(e) showed significant anti-inflammatory activity at a dose of 50 mg/kg in comparison to standard drug and all the compounds possessed remarkable antibacterial activity.

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REFERENCES

[1] R, Franski. *Asian J. Chem*., **2005**, 17, 2063
[2] F. A. Omar, N. M. Mahfouz and M. A. Rahman *Eur J. Med. Chem*., **1996**, 31, 819.
[3] B, Narayana, K. K. Vijayraj, B. V. Ashalatha and N. S. Kumari, *Arch Pharm.* **2005**, 338, 372-7
[4] R. R. Kamble and B. S. Sudha, *Indian J. Pharm Sci*. **2006**, 68**,** 249.
[5] A. A. Husain, P. Ahuja and Sarafroz, *Indian J. Pharm. Sci*. **2009**, 71, 62.
[6] P. S. Ingole, S. R. Mohane and B. N. Berad *Asian J. chem*. **2007**,19**,** 4, 2683.
[7] M. S. Karthikeyan, D. J. Prasad, M. Mahalinga, B. S. Holla and N. S. Kumari, *Eur J. Med Chem*., **2008**, 43, 25.
[8] R. R. Somani and P. Y. Shirodkar *Asian J. chem*., **2008**, 20, 8, 6189
[9] A. Zarghi, S. A. Tabatabai, M. Faizi, A. Ahadian, P. Navabi, V. Zanganeh and A. Shafiee *Bioorg. Med. Chem. Lett*. **2005**, 15, 1863.
[10] K. S. Bhat, M. S. Karthikeyan, B. S. Holla and N. S. Shetty, *Indian J. Chem*. **2004**, 43B, 1765.
[11] B. S. Holla, K. N. Poojary, K. S. Bhat, M. Ashok, and B. Poojary, *Indian J Chem*. **2005**, 44B, 1669.
[12] B.S. Furniss, A.J. Hannaford, P.W.G. Smith and A.R Tatchell., Vogel's Text Book of Practical Organic Chemistry" **1989**, 5, 1269.
[13] G.Nagalakshmi, *Indian J. Pharm. Sci*. **2008**, 70, 1, 1.
[14] S.K. Kulkari Handbook of Experimental Pharmcology Vallabh Prakashan, New Delhi, **1999**, 3, 123.
[15] M. L. Prescott, P. J. Harley and A. D. Klein, Microbiology, McGraw-Hill Companies, NewYork, **2007**, 6, 93.
[16] E. P. Abraham, E. Chain, C. M. Fletcher, A. D. Gardner, Heatley and M. A. Jennings,
[17] *Lancet*, **1941**, 2, 177.
[18] 17. F. Woodruff, *J. Bacteriology*, **1943**, 46, 187.
[19] 18. Grove, Randall, Assay methods of antibiotics. Medical Encyclopedia, Inc New York,
[20] **1955**, 171.
[21] 19. Indian Pharmacopoeia, 1996, Vol. II (P-Z), Published by the Controller of Publications Delhi, A-100
[22] 20. P. S. Kalsi pectroscopy of Organic Compounds, New Age International Ltd., **2004**, 6, 133.

[23] 21. R.M. Silverstein, F. X. Webster, Spectrometric Identification of Organic Compounds, John Wisely and Sons, Inc. **2004**, 6, 79.