

Synthesis, Analgesic and Antimicrobial Activities of Some Novel Isoxazole Derivatives

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ABSTRACT: Substituted aryl-N-chalconyl aminophenols **1a-f** were synthesized by base catalysed condensation of equimolar mixture of N-(4-hydroxyphenyl)-acetamide and appropriate araldehydes. Treatment of compounds **1a-f** with hydroxylamine hydrochloride in ethanol afforded a series of novel 4-(5'-substituted-aryl-4',5'-dihydro-isoxazole-3'-yl-amino) phenols have been synthesized by treating substituted aryl-N-chalconyl aminophenol with hydroxylamine hydrochloride. Structures of newly synthesized compounds **2a-f** were confirmed by IR, ¹H-NMR and elemental analysis data. The synthesized compounds were investigated for their analgesic and antimicrobial activities. Compounds **2e** and **2f** exhibited significant analgesic activity in comparison to the reference drug paracetamol. In *in vitro* anti-microbial screening, compounds **2c** and **2f** showed higher antibacterial and antifungal activity in comparison to the reference standard ciprofloxacin and clotrimazole, respectively. Compound **2f** bearing 4-Cl phenyl substitution at 5 position of Ioxazoline ring was found to be the most potent compound of the series.

Key words: Isoxazole, Analgesic, Antibacterial activity.

INTRODUCTION

The prevalence of isoxazole cores in natural and biologically active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic lead. The treatment of pain continues to be the subject of considerable pharmaceutical and clinical research, recent years; isoxazoles are of great interest due to their exceptional biological activities. A systematic investigation of this class of heterocycle revealed that isoxazole containing pharmacoactive agents play important role in medicinal chemistry. Microbial infections often produce pain and inflammation. Chemotherapeutic, analgesic and anti-

inflammatory drugs are prescribed simultaneously in normal practice. The compound possessing all activities is not common. It has been reported that isoxazolines possess analgesic, anti-inflammatory¹⁻⁴ and antimicrobial⁵⁻¹¹ activities. In view of these above fact, an attempt has been made for the synthesis of novel 4-(5'-substituted-aryl-4',5'-dihydro-isoxazole-3'-yl-amino) phenols possessing potent biological activities. The synthesized compounds were tested for their possible analgesic and anti-microbial activities.

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MATERIALS AND METHODS

Melting points were determined in open capillaries and were uncorrected. Purity of the

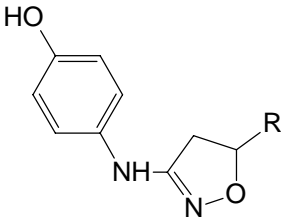
compounds was checked by TLC. IR spectra (KBr) were recorded on a JASCO FT/IR 410 spectrophotometer (ν_{\max} in cm^{-1}). ^1H NMR (CDCl_3) on a Bruker DPX 300-MHz spectrometer using TMS as internal reference (chemical shifts in δ ppm). C, H and N analyses were carried out on a Euro EA (Italy) analyzer. N-(4'-hydroxy phenyl)-acetamide (99 %, Cipla Ltd.), Benzaldehyde (99 %, Merck), Furfuraldehyde (99 %, Burgoyne Burbidges and co.), Salicylaldehyde (99 %, CDH Lab), anisaldehyde (99 %, SD-fine chemicals), p-cholorobenzaldehyde (99 %, Hi-media), p-nitrobenzaldehyde (99 %, Hi-media), NaOH (99 %, SD-fine chemicals), Ethanol (97 %, LOBA chemicals), Hydroxylamine hydrochloride (98 %, SD-fine chemicals), Mueller Hinton agar (99 %, Hi-media), Sabouraud dextrose agar (99 %, Hi-

media). Carboxymethylcellulose (Burgoyne Burbidges and co.).

Chemistry

Preparation of N-(4'-hydroxyphenyl)-3-phenyl-acrylamide 1a: To a mixture of N-(4'-hydroxy phenyl)-acetamide (0.01 mol) and benzaldehyde (0.01 mol) in ethanol (50 ml), 2 % NaOH solution (1 ml) was added and stirred for 10 h at room temperature and then refluxed for 6 h on a water bath. The excess solvent was distilled off under vacuum and poured into ice-cold water. The solid **1a** thus separated was filtered, dried and recrystallized from ethanol. Compounds **1b-f** were prepared similarly by using appropriate araldehydes. Melting points, yields and molecular formula are summarized in Table 1.

Table 1. Characterization of synthesized compounds **1a-f** and **2a-f**:



Compound	(R)	Formula	MP ($^{\circ}\text{C}$)*	Yield (%)
1a	-C ₆ H ₅	C ₁₅ H ₁₃ O ₂ N	150	72
1b	-Furyl	C ₁₃ H ₁₁ O ₃ N	160	63
1c	-4-NO ₂ -C ₆ H ₄	C ₁₅ H ₁₂ O ₄ N ₂	108	81
1d	-4-OCH ₃ -C ₆ H ₄	C ₁₆ H ₁₅ O ₃ N	152	74
1e	-2-OH-C ₆ H ₄	C ₁₅ H ₁₃ O ₃ N	142	72
1f	-4-Cl-C ₆ H ₄	C ₁₅ H ₁₂ O ₂ NCl	150	75
2a	-C ₆ H ₅	C ₁₅ H ₁₄ N ₂ O ₂	210	73
2b	-C ₆ H ₅	C ₁₃ H ₁₂ N ₂ O ₃	176	66
2c	-4-NO ₂ -C ₆ H ₄	C ₁₅ H ₁₃ N ₃ O ₄	120	78
2d	-4-OCH ₃ -C ₆ H ₄	C ₁₆ H ₁₆ N ₂ O ₃	190	73
2e	-2-OH-C ₆ H ₄	C ₁₅ H ₁₄ N ₂ O ₃	164	71
2f	-4-Cl-C ₆ H ₄	C ₁₅ H ₁₃ N ₂ O ₂ Cl	152	77

*= $\pm 2^{\circ}\text{C}$.

1a (R = -C₆H₅):-IR (KBr, cm^{-1}): 3452 (Ar-OH str.), 3301 (NH str.), 3016 (C-H str.), 1650 (C=O), 1610 (C=C str.); ^1H NMR (δ ppm) (CDCl_3 + DMSO- d_6): 5.35 (1H, s, Ar-OH), 6.11 (1H, s, N-H), 6.76 (2H, dd, CH), 7.10-8.00 (m, 9H, Ar-H); Analysis (C₁₅H₁₃O₂N) cal. (found) %: C; 75.30 (75.52), H; 5.48 (4.98) N; 5.88 (6.21); MS (m/z) : 239 (M⁺).

1b (R = -Furyl):- IR (KBr, cm^{-1}): 3300 (Ar-OH str.), 3253 (NH str.), 2922 (CH₂ str.), 1665 (C=O), 1476 (C=C str.), 1137 (C-O-C str.); ^1H NMR (δ ppm) (CDCl_3 + DMSO- d_6): 4.45 (m, 3H, CH furyl), 5.65 (1H, s, Ar-OH), 6.21 (1H, s, N-H), 6.76 (2H, dd, CH), 7.13-8.00 (m, 4H, Ar-H); Analysis (C₁₃H₁₁O₃N) cal. (found) %: C; 68.11 (68.43), H; 4.84 (4.49), N;

6.11 (5.89); MS (m/z): 229. **1c** (R=p-NO₂-C₆H₄):- IR (KBr, cm⁻¹): 3490 (Ar-OH str.), 3291 (NH str.), 3099 (C-H str.), 2851(CH₂ str.), 1560 (C-NO₂ asym. str.), 1485(C=C str.), ¹HNMR (δ ppm) (CDCl₃ + DMSO-d₆), 6.76 (2H, dd, CH), 6.13 (1H, s, N-H), 5.35 (1H, s, Ar-OH), 7.10-8.02 (m, 8H, Ar-H); Analysis (C₁₅H₁₂O₄N₂) cal. (found) %: C; 63.38 (63.42), H; 4.25 (4.52), N; 9.85(9.53). MS: (m/z) 284 (M⁺). **1d** (R = p- OCH₃-C₆H₄):- IR (KBr, cm⁻¹): 3431(Ar-OH str.), 3211 (NH str.), 2831 (CH₂ str), 1493(C=C str), 1101(C-O-C str); ¹HNMR (δ ppm) (CDCl₃ + DMSO-d₆), 6.76 (2H, dd, CH), 6.14 (1H, s, N-H), 5.35 (1H, s, Ar-OH), 7.10 -8.01(m, Ar-H); Analysis (C₁₆H₁₅ON₃) cal. (found) %: C; 71.36 (71.57), H; 5.61(6.02), N; 5.26 (4.99); MS (m/z): 269 (M⁺). **1e** (R= 2-OH-C₆H₄):- IR (KBr, cm⁻¹): 3312 (Ar-OH str.), 3208(NH str.), 2834(CH₂ str.), 1505(C=C str.); ¹HNMR (δ ppm) (CDCl₃ + DMSO-d₆): 5.18 (1H, s, Ar-OH), 5.35 (1H, s, Ar-OH), 6.56 (2H, dd, CH), 6.23 (1H, s, N-H), 7.10-8.00 (m, 8H, Ar-H); Analysis (C₁₅H₁₃O₃N) cal. (found) %: C; 70.58 (70.82), H; 5.13(5.34), N; 5.49 (5.26); MS (m/z): 255 (M⁺). **1f** (R = p-Cl-C₆H₄):- IR (KBr, cm⁻¹): 3417 (Ar-OH str.), 3278 (NH str.), 2932 (C-H str.), 2836 (CH₂ str.), 742 (C-Cl), ¹HNMR (δ ppm) (CDCl₃ + DMSO-d₆), 6.76 (2H, dd, CH), 6.21(1H, s, N-H), 5.35 (1H, s, Ar-OH), 7.19-8.00 (m, 8H, Ar-H). Analysis (C₁₅H₁₂O₂NCl) cal. (found) %: C; 65.82 (66.12), H; 4.42 (4.63), N; 5.12 (5.43); MS (m/z): 273(M⁺).

Preparation of 4-[(5'-phenyl-4',5'-dihydroisoxazol-3'-yl-amino) phenol 2a. A mixture of compound **1a** (0.01mol) and hydroxylamine hydrochloride (0.01 mol) in ethanol (30ml) was refluxed on a water bath for 6 h. The reaction mixture was concentrated under vacuum, cooled and poured into ice-cold water. The solid **2a** thus separated was filtered, dried and recrystallized from ethanol. Compounds **2b-f** was prepared similarly. Melting points, yields and molecular formulas are given in Table 1. **2a** (R = -C₆H₅):- IR (KBr, cm⁻¹): 3460 (Ar-OH str.), 3295 (NH str.), 3028 (C-H str.), 1630 (C=N str.), 1255 (C-O str.) ¹HNMR (δ ppm) (CDCl₃ + DMSO-d₆), 3.20 (1H, t, CH), 6.11 (1H, s, N-H), 4.30

(2H, d, CH₂), 5.10 (1H, s, Ar-OH), 6.79 – 8.01 (m, 9H, Ar-H); Analysis (C₁₅H₁₄N₂O₂) cal. (found) %: C; 70.85(70.45), H; 5.55(5.76), N; 11.02(11.29). MS:(m/z): 254(M⁺). **2b** (R=Furyl):- IR (KBr, cm⁻¹): 3309 (Ar-OH str.), 3260 (NH str.), 3065 (O-H str.), 2927 (CH₂ str.), 1630 (C=N str.), 1479 (C=C str.), 1229 (C-O str.), 1137 (C-O-C str.); ¹HNMR (δ ppm) (CDCl₃ + DMSO-d₆), 3.13 (3H, m, CH furyl), 4.41 (d, 2H, CH₂ ioxazoline), 5.32 (1H, s, Ar-OH), 6.24 (1H, d, N-H), 6.62 (1H, t, CH ioxazoline), 6.82-7.88 (m, 4H, Ar-H); Analysis (C₁₃H₁₂N₂O₃) cal. (found) %: C; 63.93 (64.23), H; 4.95 (4.56), N; 11.47 (11.63). MS (m/z): 244 (M⁺); **2c** (R = p-NO₂-C₆H₄):-IR(KBr, cm⁻¹): 3499 (Ar-OH str.), 3298 (NH str.), 3091 (C-H str.), 2841 (CH₂ str.), 1630 (C = N str.), 1560 (C-NO₂ asym. str.), 1489 (C=C str.), 1235 (C-O str.); ¹HNMR (δ ppm) (CDCl₃ + DMSO-d₆), 6.23 (1H, s, N-H), 4.01- 4.14 (2H, d, CH₂), 5.26 (1H, s, Ar-OH), 6.73 (1H, t, CH), 7.22-8.21 (m, 4H, Ar-H); Analysis (C₁₅H₁₃N₃O₄) cal. (found) % : C; 60.20 (60.43), H; 4.38 (4.87), N; 14.04 (14.43). MS (m/z): 299 (M⁺). **2d** (R = p- OCH₃-C₆H₄):- IR(KBr, cm⁻¹): 3436 (Ar-OH str.), 3207 (NH str.), 3045 (C-H str), 2831 (CH₂ str.), 1617 (C=N str), 1499 (C=C str), 1223 (C-O str), 1093 (C-O-C str); ¹HNMR (δ ppm) (CDCl₃ + DMSO-d₆): 3.83 (s, 3H, OCH₃), 4.38 (2H, d, CH₂), 5.43 (1H, s, Ar-OH), 6.15 (1H, s, N-H), 6.73 (1H, t, CH), 7.10-8.05 (m, 8H, Ar-H); Analysis (C₁₆H₁₆N₂O₃) cal. (found) % : C; 67.59 (68.01), H ; 5.67 (5.33), N; 9.85 (10.12). MS: (m/z) 284 (M⁺). **2e** (R=2-OH-C₆H₄):- IR (KBr, cm⁻¹): 3309 (Ar-OH str.), 3201 (NH str.), 2841 (CH₂ str.), 1630 (C=N str.), 1505 (C=C str.), 1235 (C-O str.); ¹HNMR (δ ppm) (CDCl₃ + DMSO-d₆): 4.21 (2H, d, CH₂), 5.42 (1H, s, Ar-OH), 5.48 (1H, s, Ar-OH), 6.21(1H, s, N-H), 6.59 (1H, t, CH), 7.12-8.11 (m, 8H, Ar-H). Analysis (C₁₅H₁₄N₂O₃) cal.(found) % : C; 66.66 (66.24), H; 5.22 (5.47), N; 10.36 (10.01). MS (m/z): 270 (M⁺); **2f** (R=p-Cl-C₆H₄):- IR (KBr, cm⁻¹): 3412 (Ar-OH str.), 3276 (NH str.), 2921 (C-H str.), 2826 (CH₂ str.), 1024 (C=N str.), 742 (C-Cl str.); ¹HNMR (δ ppm) (CDCl₃ + DMSO-d₆): 4.31 (2H, d, CH₂), 5.37 (1H, s, Ar-OH), 6.25 (1H, s, N-H), 6.81(1H, t, CH), 7.24-8.14 (m, 8H, Ar-H); Analysis (C₁₅H₁₃N₂O₂Cl)

cal.(found) % : C; 62.40 (62.78), H; 4.54 (4.02), N; 9.70 (10.09); MS:(m/z): 288 (M⁺).

Biological evaluation

Analgesic activity: The analgesic activity was determined by tail immersion method.¹² Wistar albino mice (n = 6) of either sex selected by random sampling technique were used for the study. Paracetamol at (100 mg/kg) was administered as standard drug for comparison. The test compounds (100 mg/kg) were administered orally by intragastric tube. The animals were held in position by a suitable restrained with the tail extending out and the tail (up to 5 cm) was then dipped in a beaker of water maintained at 55 ± 5 °C. The time in seconds taken to withdraw the tail clearly out of water was taken as the reaction time. The reading was recorded at 30, 60, 120 and 180 min after administration of compounds. A cut off point of 10 sec was observed to prevent the tail damage. The results are presented in Table 2.

Antimicrobial activity: *In vitro* antimicrobial study was carried on Muller Hinton agar (Hi-media) plates (37°C, 24 h) by agar diffusion cup plate method (13). The test microorganisms were obtained from Department of Microbiology, OUAT, Orissa, India. All the compounds were screened for antimicrobial activity at 100 µg/ml concentration level against the following bacterial Strains: *Staphylococcus aureus*, *Escherichia coli*, and

Salmonella typhi. Antifungal activity was tested on Sabouraud dextrose agar (Himedia) plates (26°C, 48-72 h) by cup plate method against *Candida albicans* and *Aspergillus niger* at a concentration level of 100 µg/ml. Ciprofloxacin and clotrimazole were used as reference standard for comparison of antibacterial and antifungal activity. DMSO was used as a solvent control for both antibacterial and antifungal activities. The results are presented in Table 3.

RESULTS AND DISCUSSION

Biological results are reported in Tables 2 and 3, which also records the effects of standard drugs included for comparison. Series of compounds were prepared in this study, exhibited significant pharmacological properties in different biological models. The general pattern of pharmacological activity encountered with these synthesized compounds were seen mainly for their effect on pain perception. However, there was a moderate, well defined antimicrobial activity associated with many of these compounds. Considerable variation of these effects were seen with each structural changes, varying from agents that had less activity to those with high potency, and significant changes in potency resulted even from minor change in chemical structure as shown in Table 2 and 3.

Table 2. Screening of analgesic activity of compounds 2a-f by tail immersion method

Compound Code	Dose (mg/kg)	Percentage of analgesic activity			
		30 min.	1 hour	2 hour	3 hour
2a	100	30 ± 0.38*	32 ± 0.72*	37 ± 0.47*	28 ± 0.91*
2b	100	36 ± 0.28*	41 ± 0.45**	44 ± 0.49*	36 ± 0.26*
2c	100	40 ± 0.52**	44 ± 0.23**	49 ± 0.21**	36 ± 0.29*
2d	100	45 ± 0.22*	50 ± 0.22**	57 ± 0.43*	47 ± 0.27*
2e	100	39 ± 0.26*	43 ± 0.31**	47 ± 0.32**	36 ± 0.44**
2f	100	41 ± 0.46**	45 ± 0.61**	51 ± 0.52**	37 ± 0.22**
Paracetamol	100	38 ± 0.42**	47 ± 0.82**	52 ± 0.71**	33 ± 0.31**

Results are expressed in mean ± SEM (n=6) significance levels * P<0.05, ** P < 0.01 and *** P < 0.001 as compared with the respective control.

Table 3. Antibacterial and antifungal activity of compounds 2a-f

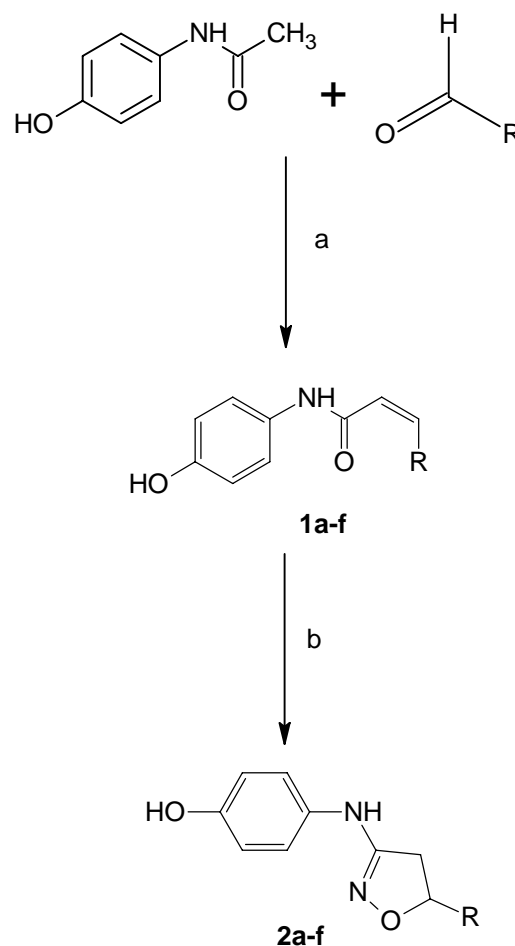
Compound	Conc. ($\mu\text{g/ml}$)	Zone of inhibition (mm)					
		<i>S. a</i>	<i>S. f</i>	<i>E. c</i>	<i>S. t</i>	<i>C. a</i>	<i>A. n</i>
2a	100	12	16	11	14	08	12
2b	100	14	13	15	12	14	13
2c	100	22	20	21	19	17	19
2d	100	13	13	12	14	12	11
2e	100	19	17	16	19	14	17
2f	100	21	22	21	18	21	24
Ciprofloxacin	10	29	31	32	26	-	-
Clotrimazole	20	-	-	-	-	28	27

*Average of three readings

S. a: *Staphylococcus aureus*; *S. f*: *Staphylococcus fecalis*; *E. c*: *Escherichia coli*; *S. t*: *Salmonella typhi*; *C. a*: *Candida albicans*; *A. n*: *Aspergillus niger*

Analgesic activity. Few of the compounds among the tested compounds **2a-2f** exhibited significant activity in experimental model used. Particularly interests are the results obtained in the Glassman's procedure, which utilizes selective inhibition of inflammatory pain as a model for screening anti-inflammatory drugs. On comparing the structure of synthesized compounds, it appeared that a p-nitrophenyl, p-methoxyphenyl or p-chlorophenyl **2c**, **2e** and **2f** substituents at 5 position of isoxazole ring resulted in remarkable increase in analgesic activity.

Antimicrobial activity. The in vitro antimicrobial activity of compounds **2a-2f** were determined by agar cup plate method, the results of which are summarized in Table 3. The antimicrobial data in Table 3 clearly indicated that the halogen, nitro and hydroxyphenyl substituents at 5 position of isoxazole ring were by far the most active substituents. The methoxy group generally conferred weak antimicrobial activity. Phenyl and furyl substitutions are weakly active to inactive among the synthesized compounds. The compounds **2c**, **2e** and **2f** showed significant activity against *S. aureus* and *S. typhi*; the compounds **2c** and **2f** exhibited promising activity against *C. albicans* and *A. niger*. However, the entire tested compounds were found to be less active as antibacterial and antifungal agents in comparison to ciprofloxacin and clotrimazole, respectively.



R = -C₆H₅, -2-Furyl, -4-NO₂-C₆H₄, -4-OCH₃-C₆H₄, -2-OH-C₆H₄, -4-Cl-C₆H₄

Scheme 1. a) Ethanol, 2 % NaOH, 50-60 °C; b) Ethanol, Hydroxylamine hydrochloride

CONCLUSION

The purpose of the present study was to examine whether molecular modification might result in detection of new potential antimicrobial and analgesic drugs. A series of compounds were prepared and assayed in a variety of biological test for analgesic and antimicrobial activity. The data reported in Tables 2 and 3 showed that effect of variation in chemical structure on activity was rather unpredictable. Seldom a particular structural modification led to uniform alteration in activity in all tests. However, some points of interest did emerge and a few generalizations can be made. The substitution which appeared to be most important for high order of activity in the greatest number of test was the p-choloroaryl group. The substitution of p-nitrophenyl and p-hydroxyphenyl group at 5 position of isoxazole ring resulted in compounds **2c** and **2e**, respectively with potent analgesic and antimicrobial activities. Obviously, the comparative evaluation of the active compounds will require further studies. The data reported in this article may be a helpful guide for the medicinal chemists working in the area.

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REFERENCES

- Rani, P., Srivastava, V.K. and Kumar, A. 2003. Isoxazoliny Derivatives of Anthranilic Acid as Anti-Inflammatory Agents. *Ind. J. Chem.* **42B**, 1729-1733.
- Habeeb, G.A., Rao, P.N.P. and Kanus, E.E. 2001. Design and Synthesis of 4,5-Diphnyl-4-isoxazoline: Novel Inhibitors of Cyclooxygenase-2 with Analgesic and Anti-inflammatory Activity. *J. Med. Chem.* **44**, 2921-2927.
- Habeeb, G.A., Rao, P.N.P. and Kanus, E.E. 2000. Design and Synthesis of 4,5-Diarylisoaxazoles: Novel Inhibitors of Cyclooxygenase-2 (cox-2) with Analgesic and Anti-inflammatory Activity. *Drug Dev. Res.* **51**, 273-286.
- Safak, C., Erdojjan, I.H., Palaska, T.E., Sunal, T.R. and Durd. 1992. Synthesis of 3-(Pyridylethyl) Benzoxazolinone Derivatives: Potent Analgesic and Anti-inflammatory Compounds Inhibiting Prostaglandin E2. *J. Med. Chem.* **5**, 1296-1299.
- Goda, F.E., Maarouf, A.R, and EL-Bendary, E.R. 2003. "Synthesis and Antimicrobial Evaluation of New Isoxazole and Pyrazole Derivatives." *S. Pharm. Journal.* **11**, 111-117.
- Diadone, G., Raffa, D., Maggio, B., Plescia, F., Curuli, VMC., Mangano, N.G. and Caruso, A. 2007. Synthesis and Pharmacological Activities of Novel-3-(Isoxazol-3-yl)-Quinazolin-4(3H)-one. *Archive Pharmazie.* **340A**, 163-164.
- Imran, M. and Khan, S.A. 2004. Synthesis of 3,5-Disubstituted Isoxazoles as Antibacterial and Antifungal agents. *Indian J. Heterocycl. Chem.* **13**, 213-216.
- Naik, S.M. and Naik, HB. 1998. Synthesis and antibacterial activity of some chalcones and isoxazoles. *Oriental J. Chem.* **14**, 167-1671.
- Yasuda, N., Iwagami, H. and Sasaki, Y. 1983. Synthesis and Antibacterial Activity of Triazole and Isoxazoles Derivatives of Ampicillin. *J. Antibiot (Tokyo).* **36**(11), 1516-24.
- Merja, B.C., Joshi, A.M., Parikh, K.A. and Parikh, A.R. 2004. Synthesis and Biological Evaluation of Pyrido (1,2-a) Pyrimidine and Isoxazoline Derivatives. *Ind. J. Chem.* **43B**, 909-912.
- Sahu, S.K., Mishra, S.K., Banerjee, M., Panda, P.K. and Mishro, P.K. 2006. Synthesis, Partition Coefficient and Antibacterial Activity of 3'aryl-6-phenyl (substituted) -cis-5'a,6'dihydrospiro [3H-indole-3,4'thiazolo (5'1'c)-isoxazolo-2(1H)-ones]. *J. Indian Chem. Soc.* **83**, 725-727.
- Glassman, J.M. 1971. Agents with Analgesic Activity and Dependences Liability. In: Turner R. A. and Hebborn P. (Ed) Screening Methods in Pharmacology, Academic Press, New York and London. pp. 231-232.
- Berry, A. (Eds) 1991. In: Antibiotics In Laboratory Medicine, 5th edn, William and Willkisks, Baltimore, MD., 1.