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Synthesis of Some New Isoxazoline Derivatives of Chalconised Indoline 2-one as a Potential Analgesic, Antibacterial and Anthelmimtic Agents

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ABSTRACT

A series of novel 1[5′′-(2′′′-substituted phenyl)-4′′,5′′′-dihydro isoxazole-3′′-yl]-3-[(4 substituted phenyl)imino]1-3-dihydro-2*H*-indole-2-one were synthesized from different substituted chalconised indole-2,3-dione was prepared from the different chalconised Isatin. The structures of the compounds were elucidated by elemental and spectral (IR, ^{1}H NMR, and MS) analysis. The synthesized compounds were screened for their analgesic activity by the acetic acid induced Writhing method and *in vitro* antimicrobial activity against the Gram-positive bacteria—*Staphylococcus aureus* and the Gram-negative bacteria—*Pseudomonas auroginosa*, *Pseudomonas mirabilis*, and *E. coli* by the cup plate agar diffusion method. Compounds $6a_1$, $6a_3$, $6b_3$, and $6b_2$ were found to be active against bacteria. The compounds $6a_1$, $6b_3$, and $6a_3$ show a significant analgesic activity. Synthesized compounds also screened for anthelmintic activity against *Pheretima posthuma*. Compounds $6a_1$, $6b_1$, and $6b_3$ show significant anthelmintic activity.

Key words: Analgesic, anthelmintic, antibacterial, *in vitro,* isatin, isoxazoline

INTRODUCTION

Isatin and isoxazoline are biologically active, synthetically useful, and important heterocycles having a wide role in medicinal chemistry. Isatin is an endogenous compounds[1] widely used as an antibacterial,[2,3] anti-inflammatory,[4] antifungal agent. Isoxazolines are also reported[5] to possess good antimicrobial, analgesic, anti-inflammatory activity. In view of the biological activities, some isatino isoxazoline derivatives and in continuation of our research work on synthesis of biologically active heterocyclic compounds, we investigated that the synthesis of some novel isoxazoline derivatives from chalconised isatin derivatives is synthesized and screened for their antibacterial activities against gm (+ve) and gm (−ve) bacteria, analgesic activity, and also anthelmintic activity. The structure of all compounds was established on the basis of spectral and elemental analysis. Almost all the isatino isoxazoline derivatives show very good antibacterial activities at 100 μm/ml and analgesic activity [Scheme 1].

Access this article online	
Quick Response Code:	Website: www.jyoungpharm.in
	DOI: 10.4103/0975-1483.93574

MATERIALS AND METHODS

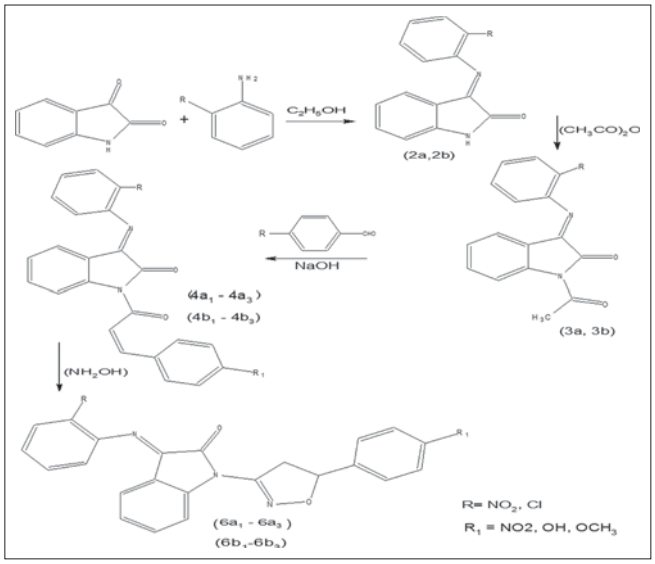
Melting points were recorded in open capillary tubes and are uncorrected. The IR spectra are recorded on a SHIMADZU-FT-IR spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker-400 MHz spectrometer. Mass spectra were recorded in Maldi MS which shows a *m/z* peak at 465. Purity of the compounds was checked by the TLC. The characterizations of the synthesized compounds were given in Table 1. For antibacterial activity, the microbial strains are taken from Utkal University, Vani Vihar, Bhubneswer. The title compounds were prepared using the general synthetic strategy for the preparation of isoxazolin derivatives from the chalcones described as follows.

Preparation of 1[5′′-(2′′′-substituedphenyl)-4′′,5′′′-dihydro isoxazole-3′′-yl]-3-[(4 substituted phenyl) imino]1-3 dihydro-2H-indole-2-one ($6a_1$–$6a_3$), ($6b_1$–$6b_3$)

Compounds (isatinoid chalcones) of 0.1 m were dissolved in ethanol (Sol^n – 1). Hydroxyl amine of 0.1 m was dissolved in ethanol (Sol^n – 2) and 1 g of anhydrous sodium acetate was dissolved in 20 ml of glacial acetic acid (Sol^n – 3). Then Sol^n 1, 2, and 3 were taken in a RBF, and reflux the reaction mixture for about 6–8 h.[6] The content was kept overnight in room temperature. Collected and recrystallized from the ethanol–chloroform mixture (1:1).

Antibacterial activity

In vitro antibacterial activity[7] was carried out against 24-h old cultures of four bacteria by the cup plate method. The compounds ($6a_1$–$6a_3$) ($6b_1$–$6b_3$) were tested against *Pseudomonas mirabilis* (ATCC-224), *Pseudomonas auroginosa* (ATCC-32), *E. coli* (ATCC-3), and *Staphylococcus aureus* (ATCC-44). For antibacterial studies, incubation was carried out at 37°C for 48 h. Ampicillin was used as a standard drug for antibacterial activity. The compounds were tested at a concentration of 100 µg/ml in dimethyl formamide against all organisms. The zone of inhibition was calculated in millimeters and compared with the standard. The results were reported in Table 2.



Scheme 1: Synthesis of new isoxazoline derivatives

Analgesic activity

The analgesic activity[8] was evaluated by the acetic acid induced Writhing test. Adult Swiss albino mice of either sex were used. Mice are made to writhe by a simple intraperitonial injection of 0.6% v/v aqueous acetic acid (0.1 ml/kg). Test substances were administered 30 min before the injection of acetic acid. Nimesulide was taken as a standard. The numbers of writhes (full extension of hind paws) were recorded. Results are given in Table 3.

Anthelmintic activity

The anthelmintic activity was evaluated on adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal earthworm parasite of human beings.[9] The earthworms were collected from moist soil and washed to remove all fecal materials. The earthworms in 3–5 cm. in length and 0.1 to 0.1–2 cm in width were used for all experimental protocol. The newly synthesized compounds were tested for anthelmintic

Table 1: Characterization data of the synthesized compounds

Compounds	R	R_1	Melting point (°C)	% of Yield	R_f value
$6a_1$	$-NO_2$	$-NO_2$	190–191	76	0.53
$6a_2$	$-NO_2$	–OH	100–102	69	0.49
$6a_3$	$-NO_2$	$-OCH_3$	182–183	83	0.58
$6b_1$	–Cl	$-NO_2$	188–189	74	0.72
$6b_2$	–Cl	–OH	107–108	67	0.69
$6b_3$	–Cl	$-OCH_3$	218–220	78	0.63

Table 2: Results of antibacterial activity

Compounds	Zone of inhibition (mm) of compounds at 100 µg/ml			
	P. mirebelis (ATCC-224)	*P. auroginosa* (ATCC-32)	*E. coli* (ATCC-3)	*S. aureus* (ATCC-44)
$6a_1$	11	09	10.5	10.5
$6a_2$	13	14	13	8.6
$6a_3$	10.5	07	10.8	12
$6b_1$	08	9.8	8.5	08
$6b_2$	09	11	10.5	09
$6b_3$	11.5	09	11	11.7
Ampicillin	22	21	23	26
DMF	00	00	00	00

Average of three readings, DMF: Di Methyl Formamide

activity.[10] *Pheretima posthuma* of nearly equal size (6 cm ± 1) were selected randomly for the present study.[9,11,12] The worms were acclimatized to the laboratory condition before experimentation. The earthworms were divided into four groups of six earthworms in each. Albendazole diluted to with normal saline solution to obtained 0.1% w/v, 0.2% w/v, 0.5% w/v, and 1% w/v served as standard and poured into petridishes. The synthesized compounds were prepared in a minimal quantity of DMSO and diluted to prepare four concentrations, i.e. 0.1% w/v, 0.2% w/v, 0.5% w/v, and 1% w/v for each compound. Normal saline serves as control. Six earthworms nearly equal size (6 cm ± 1) are taken for each concentration and placed in petridishes at room temperature.[10] The time taken for complete paralysis and death is recorded. The mean paralysis time and mean lethal time for each sample were calculated (each reading taken in triplicate). The time taken for worms to become motionless was noted as the paralysis time and to ascertain death, each worm was frequently applied with external stimuli which stimulates and induces movement in the earthworms, if alive.[11] All the results were shown in Table 4 and expressed as a mean±SEM of six worms in each group.

RESULTS

The structures of newly synthesized compounds were elucidated by IR, ^{1}H NMR, and mass spectroscopic analysis and reported as follows.

Table 3: Analgesic activity of the compounds

Coumpounds	Dose (100mg/kg)	No. of writhing movements	% of protection
0.5% CMC	2ml/kg	58.8±0.95	-
Nimesulide	10ml/kg	11.2±4.32**	79.56
$6a_1$	100	27.5±2.85**	49.81
$6a_2$	100	30.8±2.23*	43.79
$6a_3$	100	17.8±2.19**	67.51
$6b_1$	100	37.2±3.21*	32.11
$6b_2$	100	36.6±2.97*	33.21
$6b_3$	100	19.3±2.98**	64.78

Results expressed as mean±sem from six observations. Significant differences by student "t" test *P<0.01, **P<0.001 as compared to control n=5.

$6a_1$: 1-(5-(4-nitroyphenyl)-4,5-dihydroisoxazol-3-yl)-3-(2-nitrophenylimino)indoline-2-one

IR (KBr, cm^{-1}): 1328(Ar–NO_2), 1630 (C=N), 1730 (C=O), 1044 (C–O), 841 (CH=CH of aroma) ^{1}H NMR (DMSO-d_6): 2.5–5.4 (dd H isoxazoline ring), 6.5–7.9 (8H, m, 2Ar–H); MS: m/z 457.10 (M^+).

$6a_2$: 1-(5-(4-hydroxyphenyl)-4,5 dihydroisoxazol-3-yl)-3-(2-nitrophenylimino) indoline-2-one

IR (KBr, cm^{-1}): 1335 (Ar–NO_2), 1634 (C=N), 1709 (C=O), 1014 (C–O), 3698 (Ar–OH). ^{1}H NMR: (DMSO-d_6): 4.8–5.1 (dd H isoxazoline ring), 11.0 (s, 1H, OH), 6.8–7.9 (8H, m, 2Ar–H); MS: m/z 428.11 (M^+).

$6a_3$: 1-(5-(4-methoxyphenyl)-4,5 dihydroisoxazol-3-yl)-3-(2-nitrophenylimino)indoline-2-one IR (KBr, cm^{-1})

2935 (Ar–CH_3), 1402 (Ar–NO_2), 1638 (C=N), 1709 (C=O), 1034 (C–O), ^{1}H NMR (DMSO-d_6): 4.7–4.9 (dd H isoxazoline ring), 6.5–7.37 (8H, m, 2Ar–H), 2.70 (3H, s, –OCH_3); MS: m/z 442.13 (M^+).

$6b_1$: 3-(2 chlorophenylimino)-1-(5-(4-nitrophenyl)-4,5-dihydroisoxazole-3-yl)indoline-2-one

IR (KBr, cm^{-1}): 1334 (Ar–NO_2), 1564 (C=N), 1013 (C–O), 896 (CH=CH of aroma), 648 (C–Cl) ^{1}H NMR (DMSO-d_6): 2.4, 5.4 (dd H isoxazoline ring), 6.3–7.9 (8H, m, 2Ar–H). MS: m/z 446.08 (M^+).

$6b_2$: 3-(2-chlorophenylimino)-1-(5-(4-hydroxyphenyl)-4,5-dihydroisoxazole-3-yl) indoline-2-one

IR (KBr, cm^{-1}): 3482 (Ar–OH), 1631 (C=N), 999 (C–O), 841 (CH=CH of aroma), 698 (C–Cl). ^{1}H NMR (DMSO-d_6): 4.8–5.0 (dd H isoxazoline ring), 11.0 (s, 1H, OH), 6.8–7.9 (8H, m, 2Ar–H). MS: m/z 417.09 (M^+).

Table 4: Anthelmintic activity of the synthesized compounds

Compounds	Time in min. (mean±SEM) for paralysis concentration (%)				Time in min. (mean±SEM) for death concentration (%)			
	0.1	0.2	0.5	1	0.1	0.2	0.5	1
$6a_1$	3.133 ± 0.027	2.418 ± 0.015	1.713 ± 0.136	1.243 ± 0.016	3.432 ± 0.123	2.418 ± 0.172	2.415 ± 0.107	1.193 ± 0.019
$6a_2$	5.123 ± 0.118	4.145 ± 0.171	3.812 ± 0.177	2.316 ± 0.101	5.150 ± 0.122	4.142 ± 0.329	4.125 ± 0.321	2.120 ± 0.138
$6a_3$	4.727 ± 0.120	3.212 ± 0.042	2.512 ± 0.012	2.120 ± 0.121	5.923 ± 0.143	4.112 ± 0.312	3.110 ± 0.023	2.812 ± 0.102
$6b_1$	3.812 ± 0.177	3.133 ± 0.027	2.418 ± 0.015	1.713 ± 0.137	4.417 ± 0.014	3.420 ± 0.240	3.120 ± 0.027	2.127 ± 0.167
$6b_2$	5.318 ± 0.015	4.222 ± 0.139	3.212 ± 0.042	2.512 ± 0.011	6.912 ± 0.126	6.122 ± 0.015	4.727 ± 0.120	3.298 ± 0.120
$6b_3$	4.112 ± 0.129	3.212 ± 0.042	2.412 ± 0.021	1.912 ± 0.106	6.022 ± 0.025	4.252 ± 0.139	3.389 ± 0.120	2.320 ± 0.017
Albendazole	3.120 ± 0.115	2.728 ± 0.148	2.210 ± 0.135	1.537 ± 0.236	4.417 ± 0.139	3.110 ± 0.241	3.451 ± 0.193	1.126 ± 0.024

Results expressed in mean±SEM

$6b_3$: 3-(2 chlorophenylimino)-1-(5-(4-methoxyphenyl)-4,5-dihydroisoxazole-3-yl) indoline-2-one

IR (KBr, cm^{-1}): 3482 (Ar–CH_3), 1639 (C=N), 1064 (C–O), 1703 (C=O), 696 (C–Cl); ^{1}H NMR (DMSO-d_6): 4.7–4.9 (dd H isoxazoline ring), 6.5–7.37 (8H, m, 2Ar–H), 2.72 (3H, s, –OCH_3). MS: m/z 431.10 (M^+).

The characterization of synthesized compounds is given in Table 1, the results of the antibacterial activity were reported in Table 2, and also the results of the analgesic and anthelmintic activity were reported in Tables 3 and 4, respectively.

DISCUSSION

Analytical data of the compounds support the proposed structures. All six compounds have shown good antibacterial activity. Among them, compounds $6a_2$, $6b_1$, and $6b_2$ show very good activity only against gm (−ve) bacteria, but not against gm (+ve), these are weak. However, compounds $6a_3$, $6b_3$, and $6a_1$ show good activity against both gm (+ve) and gm (−ve) bacteria. All the compounds also show good analgesic effects, among them $6a_1$, $6a_3$, and $6b_3$ exhibit significant analgesic activity (P<0.001), % of inhibition of 49.81, 67.51, and 64.78, respectively, as compared with standard Nimesulide (79.56). All the compounds also show good anthelmintic activity against *Pheretima posthuma*. However, compounds $6a_1$, $6b_1$, and $6b_3$ shows significant activity both in the case of paralysis and death time when compared with the standard albendazole given in Table 4.

CONCLUSION

On a critical overview of synthesized compounds, it has been found that compounds with methoxy (–OCH_3) substitution on a phenyl ring at the *para* position and the hydroxy (–OH) group at the *para*-position found potent and chloro (–Cl) substitution also makes the compound potent antibacterial, analgesic, and anthelmintic agents including nitro (–NO_2) substitution which also makes the compound potent anthelmintic agent. In conclusion, a novel series of isoxazolin analogous possessing indole nucleus were synthesized for their potential analgesic and antibacterial and anthelmintic activity. The presence of considerable analgesic, antibacterial, and anthelmintic activity in the test compounds may be endorsed to the presence of isoxazolin, indole, and phenyl ring, all of which together might have contributed for the increase in analgesic and lipophilic character responsible for penetration of the compound inside the bacterial strain.

ACKNOWLEDGEMENTS

The authors are thankful to the management of the Institute of Pharmacy and Technology, Salipur, for providing the experimental facilities. The authors also extend their thanks to Dr. Ranjan Mukherjee from IICB Kolkata and Dr. S. N. Meyyanathan of J.S.S College of Pharmacy, Ooty, for carrying out ^{1}H NMR, Mass and IR spectral analysis of the compounds. The authors are also thankful to the Principal and management of Vaageswari College of Pharmacy, Karimnagar, AP, for providing the facility of to carry out the part of the experiment.

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How to cite this article: Mondal P, Jana S, Balaji A, Ramakrishna R, Kanthal LK. Synthesis of some new isoxazoline derivatives of chalconised indoline 2-one as a potential analgesic, antibacterial and anthelmimtic agents. J Young Pharmacists 2012;4:38-41.

Source of Support: Nil, **Conflict of Interest:** None declared.