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SYNTHESIS, CHARACTERIZATION AND ANTHELMINTIC ACTIVITY OF NOVEL 3-BENZYL-2-(4'-SUBSTITUTED PHENYL)-4(5*H*)-(4''-NITROPHENYL AMINO)-1, 3-OXAZOLIDINES DERIVATIVES

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ABSTRACT
The study aimed at screening synthetic compounds for pharmacological activity. The anthelmintic activity of 3-benzyl-2-(4'-substituted phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1, 3-oxazolidines **6a-e** compounds was evaluated by Passive avoidance test. The purity of the synthesized compounds was characterized by means of IR, ^{1}H-NMR, mass spectral and elemental analysis.

Key words: Oxazolidine; Anthelmintic activity.

INTRODUCTION

Parasitic nematodes cause significant problems to the health and life of many plants and animals, and also of humans. Gastrointestinal parasites create a serious threat to the production of livestock in developing nations[1]. Despite the fact of development of anthelmintic resistance in parasites of high economic significance, chemotherapy is still the most widely used option for the control of helminthes. Helminthes parasite infections are global problems with serious social and economic repercussions in the Third World countries[2]. The diseases affect the health status of a large fraction of the human population as well as animals. Some type of dangerous helminthes infections like filariasis has only a few therapeutic modalities at present[3]. The continuous and long-term reliance on a small range of compounds has led to the development of drug resistance in many helminthic strains. In addition, after treatment with albendazole ormebendazole, several side effects have been reported in hosts such as gastrointestinal symptoms (epigastric pain, diarrhea, nausea, vomiting), nervous system symptoms (headache, dizziness), and allergic phenomena (edema, rashes, urticaria). Some anthelmintic drugs, such as praziquantel and albendazole, are contraindicated for certain groups of patients like pregnant and lactating woman. These drugs have also to be used with caution in hepatitis patients and in children below 2 years of age[4]. Following the discovery of oxazolidine derivatives, numerous structural modifications have been made to the oxazolidine nucleus to increase the anthelmintic potency.

MATERIALS AND METHODS

Materials

Synthetic starting material, reagents and solvents were of analytical reagent grade or of the highest quality commercially available and were purchased from Aldrich Chemical Co., Merck Chemical Co. and were dried when necessary.

The melting points were taken in open capillary tube and are uncorrected. IR spectra were recorded with KBr pellets (ABB Bomem FT-IR spectrometer MB 104 ABB Limited Bangaluru, India). Proton (^{1}H) NMR spectra (Bruker 400 NMR spectrometer Mumbai, India) were recorded with TMS as internal references. Mass spectral data were recorded with a quadrupol mass spectrometer (Shimadzu GC MS QP 5000, Chennai, India), and microanalyses were performed using a *vario EL* V300 *elemental* analyzer (*Elemental* Analysensysteme *GmbH* Chennai, India). The purity of the compounds was checked by TLC on pre-coated SiO_2 gel (HF_{254}, 200 mesh) aluminium plates (E.Merck) using ethyl acetate: benzene (1:3) and visualized in UV chamber. IR, ^{1}H-NMR, mass spectral datas and elemental analyses were consistent with the assigned structures.

General Procedures. The target novel oxazolidine derivatives were synthesized by previously reported method[5] (Zarghi et al., 2007). Accordingly, benzylamine **1** was treated with an equimolar amount of substituted benzaldehyde **2** and an hydroxy acetic acid **3** in dry toluene under reflux 24- 48 h to give 3-benzyl-2-(4'-substituted phenyl)-1,3-oxazolidine-4(5*H*)-one **4**, further its treat with thionyl chloride and DMF to get chloro derivative **5** 3-benzyl-2-(4'-substituted phenyl)-4(5H)-chloro-1,3-oxazolidine and then coupled with *p*-nitro anilines in DMF at 80^0C and quenched in ice-water to get the product were separated by filtration, vaccum dried and recrystallized from warm ethanol to yields 3-benzyl-2-(4'-substituted phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1,3-oxazolidines **6a-e**.

3-benzyl-2-(4'-hydroxy phenyl)-1,3-oxazolidine-4(5*H*)-one (4)

Yellow solid; Yield: 78%; mp. 183-185°C, IR : 3476 (O-H), 3096 (Ar-CH), 1728 (C=O), 1468 (C=C) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 9.84 (s, 1H, Ar-OH), 6.96-7.54 (m, 9H, Ar-H), 6.67 (s, 1H, -CH), 4.12-4.62 (m, 4H, 2 × CH_2); EI-MS (m/z, %): 269 [M]+; (Calcd for $C_{16}H_{15}NO_3$; 269.3). Anal. Calcd for $C_{16}H_{15}NO_3$, C, 71.36; H, 5.61; N, 5.20; Found: C, 71.41; H, 5.69; N, 5.27.

3-benzyl-2-(4'-hydroxy phenyl)-4(5*H*)-(4''-nitrophenyl amino)-1,3-oxazolidines (6a)

Pale yellow solid; Yield: 76%; mp. 156-158°C, IR : 3464 (O-H), 3027 (Ar-CH), 1494 (C=C), 1564 (N=O), 1306 (N-H bending), 3396 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 9.87 (s, 1H, Ar-OH), 6.76-7.27 (m, 13H, Ar-H), 6.31 (s, 2H, -CH), 7.21 (s, 1H, N-H), 3.44-3.67 (m, 4H, 2 × CH_2); EI-MS (m/z, %): [M]+ 391; (Calcd for $C_{22}H_{21}N_3O_4$; 391.42). Anal. Calcd for $C_{22}H_{21}N_3O_4$; C, 67.51; H, 5.41; N, 10.74; Found: C, 67.57; H, 5.44; N, 10.79.

3-benzyl-2-(4'-methoxy phenyl)-4(5*H*)-(4''-nitrophenyl amino)-1,3-oxazolidines (6b)

White solid; Yield: 89%; mp. 184-186°C, IR : 3026 (Ar-CH), 1524 (C=C), 1567 (N=O), 1316 (N-H bending), 3319 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.72-7.23 (m, 13H, Ar-H), 6.36 (s, 2H, -CH), 3.78 (s, 3H –OCH_3), 7.15 (s, 1H, N-H), 3.54-3.72 (m, 4H, 2 × CH_2); EI-MS (m/z, %): [M]+ 405; (Calcd for $C_{23}H_{23}N_3O_4$; 405.45). Anal. Calcd for $C_{23}H_{23}N_3O_4$; C, 68.13; H, 5.72; N, 10.36; Found: C, 68.19; H, 5.76; N, 10.31.

3-benzyl-2-(4'-methyl phenyl)-4(5*H*)-(4''-nitrophenyl amino)-1,3-oxazolidines (6c)

Paleyellow solid; Yield: 77%; mp. 170-123°C, IR : 3027 (Ar-CH), 1413 (C=C), 1570 (N=O), 1334 (N-H bending), 3313 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.62-7.18 (m, 13H, Ar-H), 6.29 (s, 2H, -CH), 3.69 (s, 3H –CH_3), 7.21 (s, 1H, N-H), 3.49-3.63 (m, 4H, 2 × CH_2); EI-MS (m/z, %): [M]+ 389; (Calcd for $C_{23}H_{23}N_3O_3$; 389.45). Anal. Calcd for $C_{23}H_{23}N_3O_3$; C, 70.93; H, 5.95; N, 10.79; Found: C, 70.95; H, 5.91; N, 10.83.

3-benzyl-2-(4'-nitro phenyl)-4(5*H*)-(4''-nitrophenyl amino)-1,3-oxazolidines (6d)

Pale solid; Yield: 71%; mp. 181-183°C, IR : 3027 (Ar-CH), 1413 (C=C), 1546 (N=O), 1334 (N-H bending), 3313 (N-H stretching) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.79-7.33 (m, 13H, Ar-H), 6.21 (s, 2H, -CH), 7.27 (s, 1H, N-H), 3.46-3.78 (m, 4H, 2 × CH_2); EI-MS (m/z, %): [M]+ 420; (Calcd for $C_{22}H_{20}N_4O_5$; 420.42). Anal. Calcd for $C_{22}H_{20}N_4O_5$; C, 62.85; H, 4,79; N, 13.33; Found: C, 62.87; H, 4,75; N, 13.37.

3-benzyl-2-(4'-chloro phenyl)-4(5*H*)-(4''-nitrophenyl amino)-1,3-oxazolidines (6e)

Brown solid; Yield: 81%; mp. 184-186°C, IR : 3026 (Ar-CH), 1524 (C=C), 1532 (N=O), 1316 (N-H bending), 3319 (N-H stretching), 749 (C-Cl) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.71-7.37 (m, 13H, Ar-H), 6.34 (s, 2H, -CH), 7.31 (s, 1H, N-H), 3.48-3.81 (m, 4H, 2 × CH_2); EI-MS (m/z, %): [M]+ 409; (Calcd for $C_{22}H_{20}ClN_3O_3$; 409.87). Anal. Calcd for $C_{22}H_{20}ClN_3O_3$; C, 64.47; H, 4.92; N, 10.25; Found: C, 64.43; H, 4.99; N, 10.29.

Animals

Indian adult ethworms (pheretima posthuma) were used to study anthelmintic activity. The earthworms were collected from moist soil and washed to remove all fecal materials. The earthworms in 3-5 cm. in length and 0.1-0.1-2 cm in width were used for all experimental protocol. The earthworm resembles both anatomically and physiologically to the intestinal roundworms parasites of human beings, hence can be used to study anthelmintic activity[6].

Antihelmintic activity

The newly synthesized compounds were tested for anthelmintic activity [1]. Pheretima posthuma (earthworm obtained from Lalbagh Botanical Garden, Bangalore) of nearly equal size (6cm ± 1) were selected randomly for present study[7-9]. 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 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 serve as control. Six earthworms nearly equal size (6cm ± 1) are taken for each concentration and placed in petridishes at room temperature[10]. The time taken for complete paralysis and death are recorded. The mean paralysis time and mean lethal time for each sample was calculated (each reading taken in triplicate). The time taken for worms to become motionless was noted as paralysis time and to ascertain death, each worm was frequently applied with external stimuli which stimulates +and induce movement in the earthworms, if alive[11].

RESULTS AND DISCUSSION

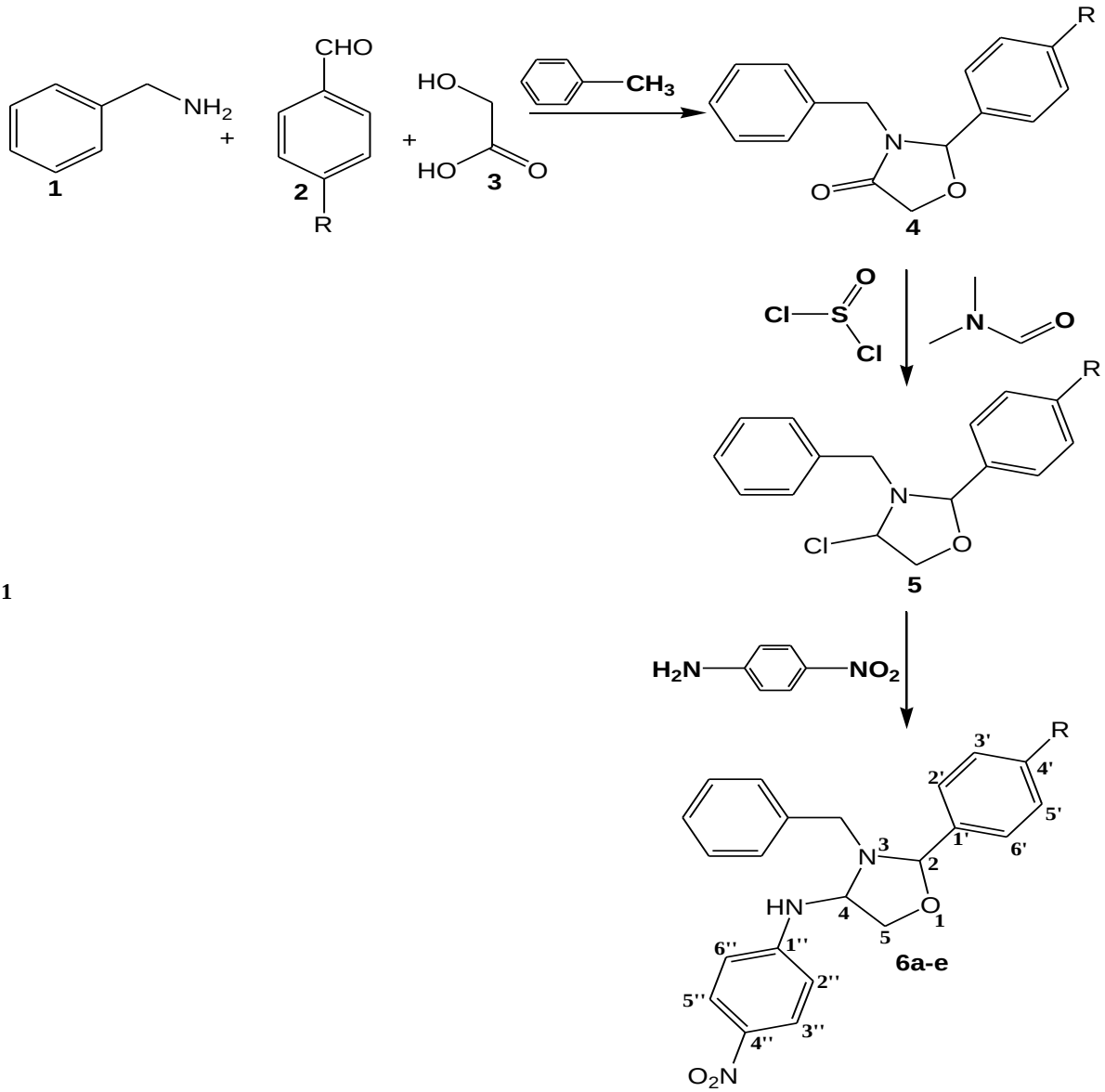
Chemistry

The synthesized series of heterocycles, **6a-e** by the reaction of **5** with appropriate *p* –nitro aniline in the presence of DMF as presented in **Scheme 1**. The IR, ^{1}H-NMR, mass spectroscopy and elemental analysis for the new compound is in accordance with the assigned structures. The IR spectra of compounds **4** showed stretching bands of keto group at 1728 cm^{-1}. In **5**, stretching bands of chloro group at 749 cm^{-1} is evidence to conversion of oxazolidinone. The title compounds **6a-e** stretching and bending NH bands appear at 3300-3400 cm^{-1}, 1300-1350 cm^{-1} respectively. The observed data on the anthelmintic activity of the synthesized compounds and standard drugs are given in **Table 1**.

The recorded IR spectrum of representative compounds **6a-e** showed missing of chloro group bands. This clearly envisages that the chloro group of **5** is converted into secondary NH. The proton magnetic resonance spectra of oxazolidine and their corresponding derivatives have been recorded in $CDCl_3$. In this **6a-e** NH signal of 3-benzyl-2-(4'-substituted phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1,3-oxazolidines moiety appear at 7.26 (s), 7.15 (s), 7.21 (s), 7.27 (s), 7.34 (s), ppm respectively. The position and presence of NH signal in the ^{1}H-NMR spectra of final compounds conforms the secondary NH proton in oxazolidine moiety. This clearly envisages that oxazolidine-4(5*H*)-one moiety involve in 4(5*H*)-chloro-1,3-oxazolidine and further (4"-nitrophenyl amino)-1,3-oxazolidines formation. All these observed facts clearly demonstrate that the 4th position of keto group in oxazolidine ring is converted into secondary amino group as indicated in **scheme 1** and conforms the proposed structure (**6a -e**).

Antihelmintic activity

The anthelmintic screening of the compounds 3-benzyl-2-(4'-substituted phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1, 3-oxazolidines **6a-e** showed an excellent activity than standard albendazole. A closer inspection of data from this table indicates that compounds hydroxy, nitro and chloro substitution of oxazolidine having activity than other compounds compared with standard. Compounds methoxy and methyl substitution showed very less activity. The transformation order of screened compounds is **6a** >**6d** >**6e** >**6c** >**6b** and albendazole were used as standard anthelmintic drug.


NH2
CHO
HO
HO
O
CH3
R
N
O
O
1
2
3
4
Cl
S
O
Cl
N
O
5
H2N
NO2
HN
6a-e
O2N
R = OH, OCH3, CH3, NO2, Cl

Scheme 1

Compounds	Time in min. (mean ± SEM) for paralysis				Time in min. (mean ± SEM) for death			
	Concentration (%)				Concentration (%)			
	0.1	0.2	0.5	1	0.1	0.2	0.5	1
6a	2.120± 0.107	2.526±0.126	2.130±0.016	1.217±0.121	4.417±0.014	3.120±0.240	3.123±0.014	1.425±0.214
6b	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
6c	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	3.289± 0.120
6d	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
6e	2.127±0.167	2.027±0.192	1.522±0.031	0.831±0.141	3.110± 0.047	2.518±0.092	1.810±0.135	1.125± 0.046
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

Table1. Anthelmintic activities of 3-benzyl-2-(4'-substituted phenyl)-4(5*H*)-(4"-nitrophenyl amino)-1, 3-oxazolidines. Values are expressed in mean ±SEM

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