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[Research article]

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW BENZOXOZOLE DERIVATIVES

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ABSTRACT

Benzoxazoles[1] are usually prepared by heating 2-Aminophenol with formic acids in the presence of Boric acid under reflux. Condensation of these two substances under milder conditions. Being a heterocyclic compound, benzoxazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. It is found within the chemical structures of pharmaceutical drugs such as flunoxaprofen. Benzoxazole derivatives are provided a protection against noxious UV radiation.Benzoxazole derivatives are also used in cosmotic compositions, such as for examples mainly cinnamic acid, 4-aminobenzoic acid.Benzoxazole derivatives are also used in the optical brighteners.These derivatives are used as Anticonvulsant and Neurotoxicity[2] , Anti-inflammatory agents[3], "Antibacterial activity"[4], Cholesteryl ester transfer Protein inhibitors[5], Antimicrobial activity[6,7], Antifungal activity'[8], Cyclooxygenase inhibitors[9], hair treatment products and also used as a skin protectants.

Key words: Inflammatory agents, Antibacterial activity, Benzoxazole, cinnamic acid, 4-aminobenzoic acid, optical brighteners.

INTRODUCTION

Being a heterocyclic compound, benzoxazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. It is found within the chemical structures of pharmaceutical drugs such as flunoxaprofen. Benzoxazole derivatives are provided a protection against noxious UV radiation.Benzoxazole derivatives are also used in cosmotic compositions, such as for examples mainly cinnamic acid, 4-aminobenzoic acid. Cyclooxygenase (COX; prostaglandinendoperoxide synthase) metabolizes Arachidonic acid to Prostaglandin (PG) H2, which serves as the precursor for the biosynthesis of various PGs, Thromboxanes, and Prostacyclin.COX activity originates from two distinct and independently regulated isozymes, COX-1 and COX-2.COX-1 is a constitutive enzyme, whereas COX-2 is inducible and short-lived. COX-2 is the product of an immediate-early gene, and is stimulated by a host of growth factors, cytokines, and mitogens.

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Its expressionCOX-1 appears responsible for the biosynthesis of PGs in the gastric mucosa and in the kidney, whereas COX-2 appears responsible for biosynthesis in inflammatory cells and the central nervous system.Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the two isoforms to different extents, and this feature accounts for their shared therapeutic properties and side effects.The differential tissue distribution of the COX isozymes has provided a rationale for the development of COX-2-selective inhibitors as non-ulcerogenic, anti-inflammatory, and analgesic agents.Most selective COX-2 inhibitors, including the recently approved drugs celecoxib and rofecoxib belong to the diaryl heterocycle class of compounds.

MATERIALS

Glassware is of Borosilicate grade. Melting point apparatus, TLC on silica gel-G plate, KBr on a THERMONICOLATE NEXUS-670 sprectro photo meter, DMSO – d_6 with TMS as internal standard, 2-Aminophenol, Chloro acetic acid (0.1mole), methanol .

METHOD

All the chemicals were obtained from S.D. Fine chem. Limited mumbai all the glassware is of Borosilicate grade . Melting points were determined in open capillaries and are uncorrected. The purity of the compound was ascertained by TLC on silica gel-G plate. Characterization of synthesized compound was done by spectral studies. IR spectra were taken in KBr on a THERMONICOLATE NEXUS-670 sprectro photo meter. ^{1}H NMR spectra was recorded on brucker avance 300 MHZ spectrophoto meter in DMSO – d_6 with TMS as internal standard. The chemical shift values are in delta (ppm). Physical data and Analgesic activities of synthesized compound was recorded in table .

SYNTHESIS OF 3-[(BENZOOXAZOLE-2-YL METHYL) - AMINO]-2-(4-NITRO PHENYL) -THIOZOLIDINE -4-ONE

SYNTHESIS OF 2-CHLORO METHYL BENZOXAZOLE (I)

2-Aminophenol (0.1mole) is taken into a clean 250ml of RBF flask and it is fixed to the condenser and add Chloro aceticacid(0.1mole) in excess under reflux for 2 hours. The reaction mixture was cooled in ice water. The product thus separated with filtered and washed with cold water. The product was recrystallized by methanol as a solvent.(M.P – 61^0 C ; Product Yield – 72%)

SYNTHESIS OF BENZOOXAZOLE 2-YL-METHYL HYDRAZINE (II)

2-Chloro methyl benzoxazole (0.01mole) in ethanol is refluxed with Hydrazine hydrate (0.01mole) for 6 hours. After the reaction mixture was taken into a china dish and it was kept on a flame after few minutes pink, cream like solid mass was appeared after it was cooled for few minutes a light pink solid mass appeared. This is recrystallized from ethanol to get the desired compound as a solid.(M.p – 75^0 C ; Product yield – 78%)

SYNTHESIS OF N-BENZOOXAZOLE-2-YL METHYL-N-(4-NITRO-BENZYLIDENE)-HYDRAZINE (III)

Benzooxazole-2-yl-methyl hydrazine (0.01mole) in ethanol is refluxed with 4-Nitro Benzaldehyde (0.01mole) for 10 hours. After the mixture was taken into a beaker and it was kept in a ice cold water, a yellow solid mass separated. This crude product is recrystallized from ethanol to get desired product as solid. (M.p – 93^0 C ; Product yield – 69%)

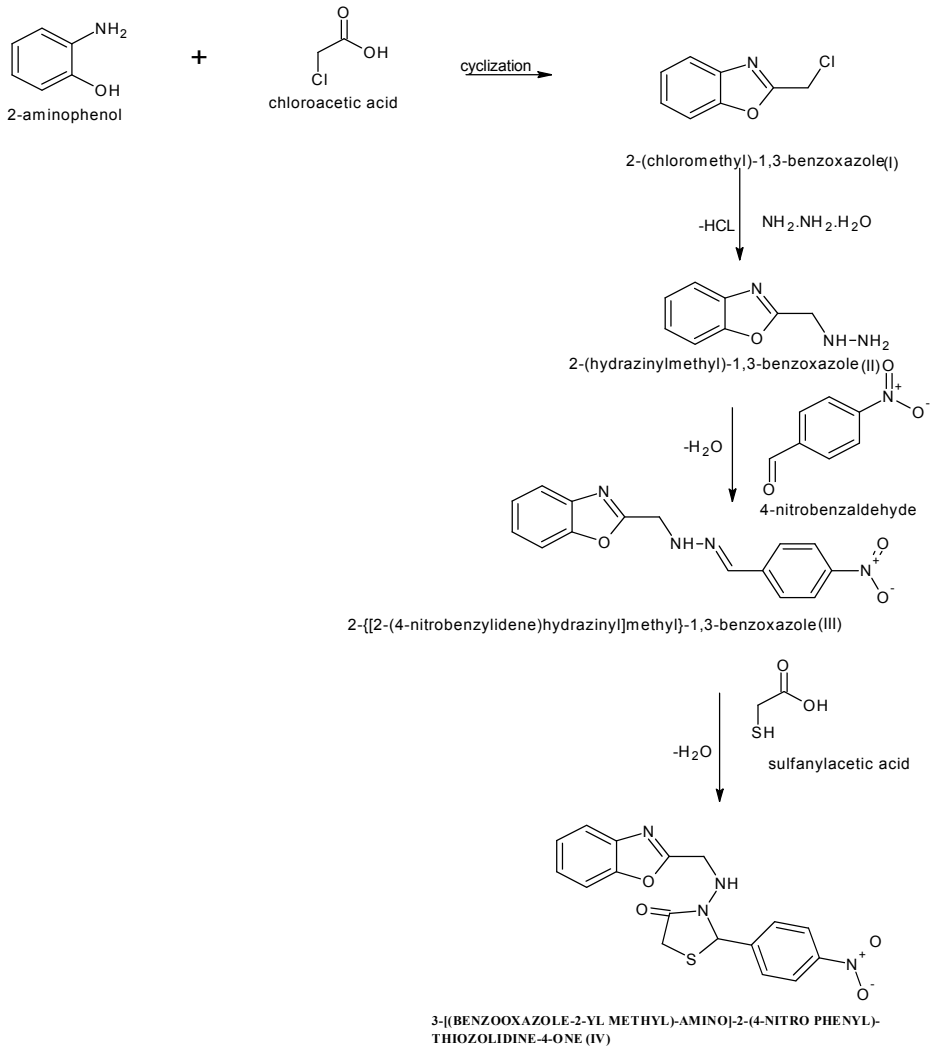
SYNTHESIS OF 3-[(BENZOOXAZOLE-2-YLMETHYL)-AMINO]-2-(4-NITRO PHENYL)-THIOZOLIDINE-4-ONE (IV)

0.01mole of N-Benzooxazole-2-yl-methyl-N-(4-nitro-benzylidene)-hydrazine is dissolved/soluble in dry benzene and to this (0.01mole) of thioglycolic acid is added. A pinch of fussed zinc chloride is added to the reaction mixture. The reaction mixture is then refluxed for 10hours. The removal of benzene is carried out using the dean stark water separator till no more water separated with benzene.i.e. The turbidity of benzene is removed. The excess of benzene removed by vacuum distillation. The above mixture is then cooled to room temperature and poured into a beaker containing ice cold water.

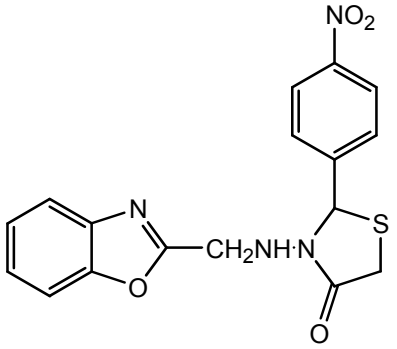
The reaction mixture is then treated with ice-cold water containing sodium bicarbonate ($NaHCO_3$) to remove the unreacted thioglycolic acid. The separated product is then recrystallized from absolute ethanol to yield.

(M.p – 103^0 C ; Product yield – 71%)

SCHEME



Physical data of Synthesized compound (IV)

Compound	Structure	M.P (^{0} C)	Yield (%)	Molecular Formula
IV	 3-[(Benzooxazol-2-ylmethyl)-amino]-2-(4-nitro-phenyl)-thiazolidin-4-one	103	71	$C_{17}H_{14}N_4O_4S$

BIOLOGICAL EVALUATION

***Invivo* biological evaluation**

Materials and methods

The various animals and materials used for the present study and their sources are as follows.

a) Swiss albino mice
b) 1% of carboxy methyl cellulose (Rolex laboratories)
c) Pentazocin (Fortwin)
d) Water for injection
e) Synthesized compound
f) Hot plate

The animals used in the present study such as swiss albino mice weighing 20-50gm was kept in colony cage at 25 ± 2^0 C, relative humidity of 45-55% under 12hrs light and dark cycle. The animal was feed with standard animal feed and water *ad libitum.* The test compound was administered orally using intragastric tube in the form of suspension using 1% CMC as suspending agent. The experimental dose was selected between the minimum effective dose and maximal non lethal dose.

ANOVA followed by dunnets "t" test was performed to ascertain the significance of the exhibited analgesic activity of the synthesized compound.

- Acute oral toxicity (Acute toxic class method in mice)
- Analgesic activity (Tail – Immersion method in mice)

Evaluation of Acute oral Toxicity

Introduction

Acute oral toxicity defines to dose adverse effects occurring following oral administration of a single dose of substances or multiple doses given within 24 hrs. The various methods used to evaluate the acute oral toxicity are as follows.

Fixed dose procedure (OECD guideline-420)
Acute toxic class methods (OECD guideline-423)
Ups and down Procedure (OECD guideline-425)

OECD guideline-423

OECD guidelines for the testing of chemicals are periodically reviewed in the light of scientific progress or changing as the second alternative to the conventional acute toxicity test, described in the test guideline 401.

Experimental protocol (Acute toxic class method in mice)

In the present study the acute oral toxicity of the synthesized compound was performed by acute toxic class method. In this methods the toxicity of the synthesized compound was tested using a step wise procedure, each step using three mice of a single sex. The mice were fasted prior to dosing (food but not water should be with held) for 3-4 hrs.

Following the period of fasting the animal should be weighed and the synthesized compound is administered orally at a dose of 2000 mg/kg body weight. Animals were observed individually after dosing atleast once during the first 30 minutes; periodically during the first 24 hrs with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days. As know mortality was observed with the above dose. So, a series of doses 50 and 100 mg/kg body weight were selected for the further pharmacological evaluation. The test procedure with a starting dose of 2000mg/kg body weight as per OECD-423 guidelines.

Evaluation of Invivo Analgesic activity

Introduction

Pain is unpleasant sensory and emotional experience associated with actual or potential tissue damage is described in terms of such damage. Analgesics are substances which relieve or decrease pain sensation by increasing threshold to pain full stimuli without causing loss of consciousness. Analgesics can be evaluated in three different ways as follows.

a) Prevention or relief of artificially induced pain in experimental animals.
b) Relief of experimental pain in human volunteers and
c) Relief of pathological or incisional pain in patients.

In human beings, the analgesics are evaluated either against experimentally induced pain (Radiant heat, ischemia induced with sphygmomanometer cuff , intra peritoneal bradikinin) or against endogenous pain (post-puerperal pain , post operative pain and pain due to malignancy).

In animals painfull reaction can be artificially induced by the any one following methods.

- Thermal method (Radiant heat as a source of pain)
- Chemical method (Irritants such as acetic acid &bradykinin)
- Physical pressure method (Tail compression).

Experimental protocol 10 (Tail immersion method in mice)

The analgesic activity was determined by tail-immersion method. Swiss mice (n=6) of either sex selected by random sampling technique was used for the study. Pentazocine at the dose of 10mg/kg (i.p.) was administered as standard drug for comparison the test compounds at dose level 50 & 100 mg/kg were administered orally. The animals were held in position by a suitable restrainer with the tail extending out and the tail (up to 5cm) was taken dipped in a beaker of water maintained at 55 ± 0.5^0 C. The time in sec taken to withdraw the tail clearly out of water was taken as reaction time. The first reading (0 min) was taken immediately after the administration of the test compound and subsequent reaction time was recorded at 30,60,120 & 180 min after the administration of compound. A cut off point of 15 sec was observed to prevent the tail damage. The percentage analgesic activity was calculated using the following the formula and results are presented in table no 7.2.

$$PAA = [(T_2-T_1)/T_2]\times100$$

Where,

T_1 is the reaction time (in sec) before treatment.
T_2 is the reaction time (in sec) after treatment.
PAA is the percentage analgesic activity.

RESULTS & DISCUSSION

Acute oral toxicity study of synthesized compound

Acute oral toxicity studies were performed according to the OECD guideline 423 method.

- This method has been designed to the evaluate the substance at the fixed doses and providing information both for hazard assessment and substance to the ranked for hazard classification purpose.
- The synthesized compounds were administered initially at a starting dose of 2000mg/kg b.w. in 1% CMC (p.o.) and observed 14 days mortality due to acute toxicity.
- Careful observation were made at least twice a day for the effect on CNS , ANS , motor activity salivation, skin coloration and other general signs of toxicity were also observed and recorded.
- Since no sign of toxicity observed at 2000mg/kg b.w. to the group of animals , the LD_{50} value of the title compounds expected to exceed 2000mg/kg b.w. and represented as class 5.

  (2000mg/kg < LD_{50}<2500mg/kg)
- From the toxicity studies the data revealed that all the synthesized compounds proved to be non toxic at tested dose levels and well tolerated by the experimental animals as their LD_{50} cut of values less than 2000mg/kg b.w.

Invivo Analgesic Activity of the Synthesized Compound

Synthesized Compound was evaluated for Analgesic activity by Tail Immersion method. The activity was studied at 50mg/kg , 100mg/kg b.w (p.o) and their effects were measured at the time interval of 30, 60,120 and 180 minutes.

Synthesized compound showed significant analgesic activity. Heighest analgesic activity was observed at 120 minutes for the compound (100mg/kg).

When compared with standard drug (Pentazocin 10mg/kg) the compound exhibited comparable analgesic activity at 100mg/kg b.w

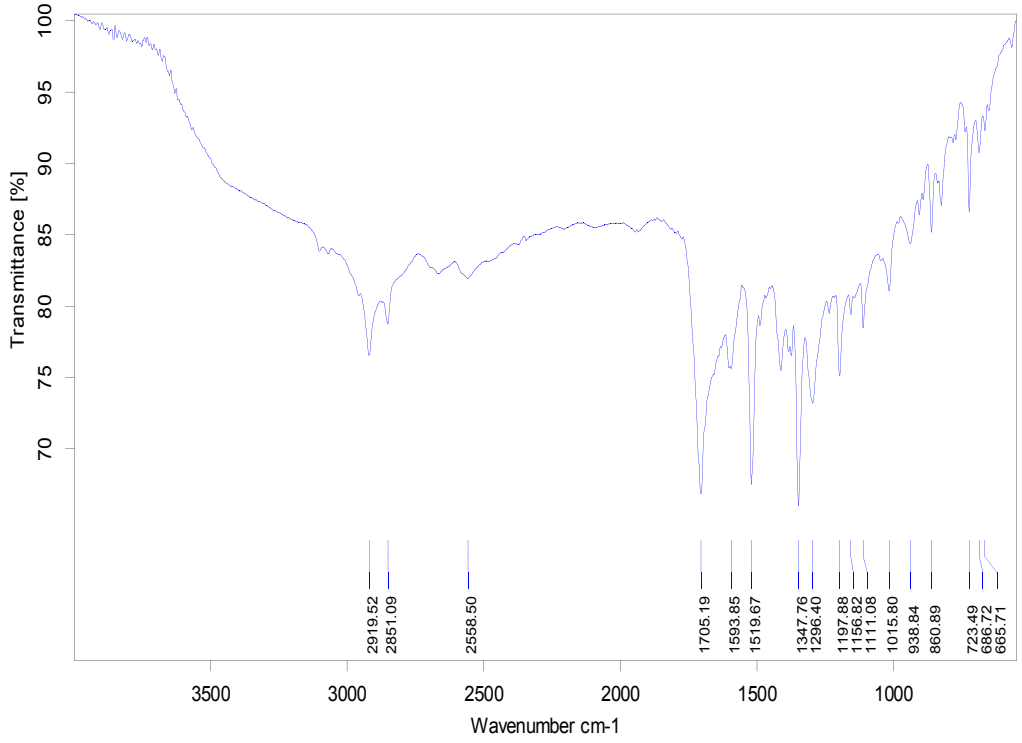
0												
Compounds	**Dose (mg/kg)**	**0 min**	**30 min**		**60 min**		**120 min**		**180 min**	
		MEAN± SEM	**MEAN± SEM**	%	**MEAN± SEM**	%	**MEAN± SEM**	%	**MEAN± SEM**	%
IV	50	3.83±0.40	5.50±0.43 NS	41.07	6.67±0.33 *	50.06	7.33±0.42 *	44.02	6.17±0.31 NS	27.92
IV	100	3.50±0.34	5.67±0.33 *	68.27	7.17±0.40 *	71.18	8.33±0.42 **	77.98	6.67±0.42 *	57.52
STD (Pentazocin)	10	3.35±0.19	8.2±0.31**	78.10	8.46±0.20 1**	80.10	8.47±0.23 5**	80	8.46±0.20 **	70.10
Control	2ml (1% CMC)	3.31±0.2	3.32±0.26	-----	3.31±0.19	-----	3.27±0.09	------	3.19±0.10	------

Analgesic activity data of the Synthesized compound

I.R. Spectra data of synthesized compound

The synthesized compound of the present study was characterized through IR spectra. The synthesized compound of the present study showed characteristic absoption bands for -NO_2-, -S-CH_2-CO, HN-CH_2-C, N-CH-S, Ar-H groups.

S.NO	Functional groups	Wave number(cm^{-1})
1.	NO_2 (1307-1610 cm^{-1})	1519.67
2.	-N-CO-CH_2 (600-700 cm^{-1})	686.72
3.	HN-CH_2-C (1600-1710cm^{-1})	1705.19
4.	N-CH-S (700-1563cm^{-1})	1347.76
5.	S-C-CO (2300-3060 cm^{-1})	2919.52



C:\Program Files\OPUS_65\MEAS\benzoxazole.0 benzoxazole Instrument type and / o 23/04/2012

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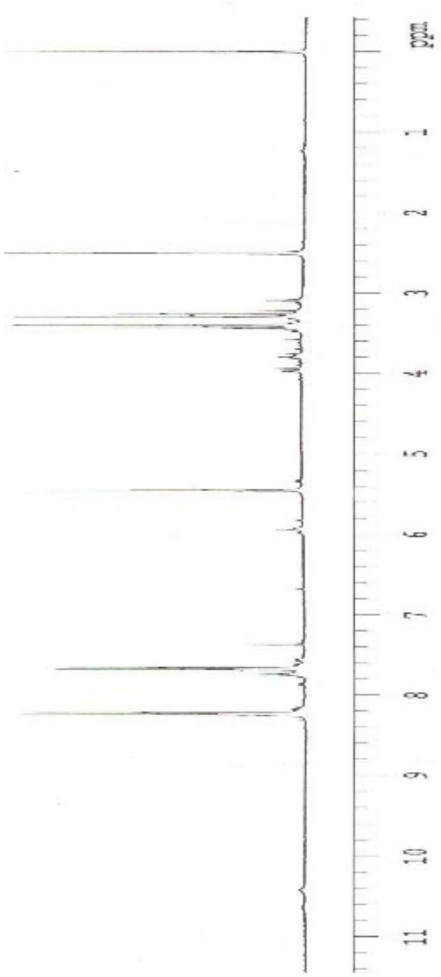
^{1}H NMR. Spectral data of synthesized compound

The synthesized compound of the present study was characterized through ^{1}H NMR spectra. The synthesized compound of the present study showed characteristic absoption bands for -NH-, -S-CH_2-CO, HN-CH_2-C, N-CH-S, Ar-H groups.

S.NO	Proton type	δ
1.	-NH-	2.5 (s)
2.	-S-CH_2-CO	3.3 (s)
3.	HN-CH_2-C	3.45 (s)
4.	N-CH-S	5.5 (s)
5.	Ar-H	7.6-7.8 (s)
6.	Ar-H	8.2-8.4 (d)



SUMMARY AND CONCLUSION

The thesis deals with the synthesis and biological evaluation of novel benzoxazole derivatives. The synthesized compound was characterized by IR & 1H NMR.

The thesis deals with the introduction of therapeutic agent based on Benzoxazole moiety, Literature survey on the investigation carried out by the earlier workers in the synthesis and biological evaluation of novel benzoxazole derivatives, Aims & objectives, Plan of work, Experimental work including detailed procedure for synthesis of title compounds and Physical data of synthesized compounds containing molecular formula, melting point, percentage of yield finally describes results of experimental investigations and discussion of results.

Synthesized compound was evaluated for analgesic activity by tail-immersion method. The activity was observed at 50 mg/kg &100mg/kg b.w. (p.o) and their effects were measured at the time interval of 30, 60, 120 and 180 minutes.

The synthesized compound **(IV)** was found at 100 mg/kg b.w. dose "t" Significant analgesic activity in all the cell lines.

Finally for the development of better therapeutic agent for clinical assessment, detailed pharmacology and toxicology studies need to be performed in order to generate data on the potential short and long – term toxicities as well as affirmed pharmacological action. The discovery and the applications of such synthetic drugs will play a role in human as well as veterinary medicine in the future.

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