Original Research Article

Screening of a Benzimidazole derivative for anthelmintic activity with "Rule of 5" approach

ABSTRACT:

Purpose: Synthetic chemistry is an important route to develop new therapeutics for every disease. The presented research is focusing on the design, synthesis, evaluation, and Lipinski's rule approach for a new biologically active benzimidazole derivative. **Methods:** The synthesis comprises the reaction between lactic acid and o-phenylenediamine (OPD) to produce a corresponding product followed by self-condensation for obtaining a complex compound. **Result**: The newly synthesized compound was found to possess significant anthelmintic activity as compared to the standard drug (Albendazole). When the concentration of the desired compound increases the paralysis time as well as the death time also decreases. 50µg/ml of the synthesized drug gave a paralysis time of 30.43 ± 5.33 min & a death time of 0.56 ± 5.32 min. **Conclusion:** It was concluded that the synthesized compound found to possess the anthelmintic activity and its orally active property was also predicted from the Molinspiration software using physicochemical (RO5) & bioactive parameters. It can be used for anthelmintic drugs as an oral formulation.

KEY WORDS: Benzimidazole, Self-condensation, Anthelmintic activity, RO5

INTRODUCTION:

The structural alteration of benzimidazole is so convenient for the development of molecules of pharmaceutical or biological interest. Applicably benzimidazole derivatives with different substitutions on different positions have found to be sources of much pharmacological utility. They are used in the treatment of ulcer, hypertension, viral & fungal infection, cancer, and antihistaminics, etc.[1] The modified form of benzimidazole has been produce various drugs. Currently available some important drugs, are Omeprazole Pimobendan, Mebendazole, etc. Various new scheme for the preparation of benzimidazole derivatives have been discovered and reported due to its wide range of pharmacological application. Anthelmintics are the drugs that

kill or removed out parasitic worm from the living body. They are also sometimes called vermicides or vermifuges.

The aim & objective of this study is to synthesize a novel Benzimidazole derivative expected to have good anthelmintic activity and to explore its physicochemical properties for RO5 approach & to predict its bioactive score.

MATERIALS:

The chemicals used for this research work were of analytical grade (S.D. Fine Chem. Ltd. Mumbai, India). The software used was ChemSketch (Drawing the scheme & for obtaining general properties) & Molinspiration (Bioactive score & Physicochemical properties). The instruments used are the melting point apparatus (Secor India). Electronic balance (Darvin, India), Infrared spectrophotometer (Shimadzu).

METHODS

Procedure:[1]

i. **Preparation of 1-(1H-benzimidazol-2-yl) ethanol ; 2-hydroxy ethyl benzimidazole :**

Equi-molar fraction of o-phenylenediamine and lactic acid were refluxed for 7 hours in the presence of 4N HCl and the completion of the reaction was determined by performing TLC. Recrystallization of the product was carried out with the help of methanol.

ii. **Preparation of 1-(1H-benzimidazol-2-yl) ethanone ; 2- acetyl benzimidazole:**

2-hydroxy ethyl benzimidazole was taken and then it was oxidized in presence of potassium dichromate and refluxed for 4 hours with glacial acetic acid. Thin layer chromatography was used to determine the completion of the reaction. After refluxing the

product it was cooled and neutralized by adding ammonia solution and then it was dried and recrystallized

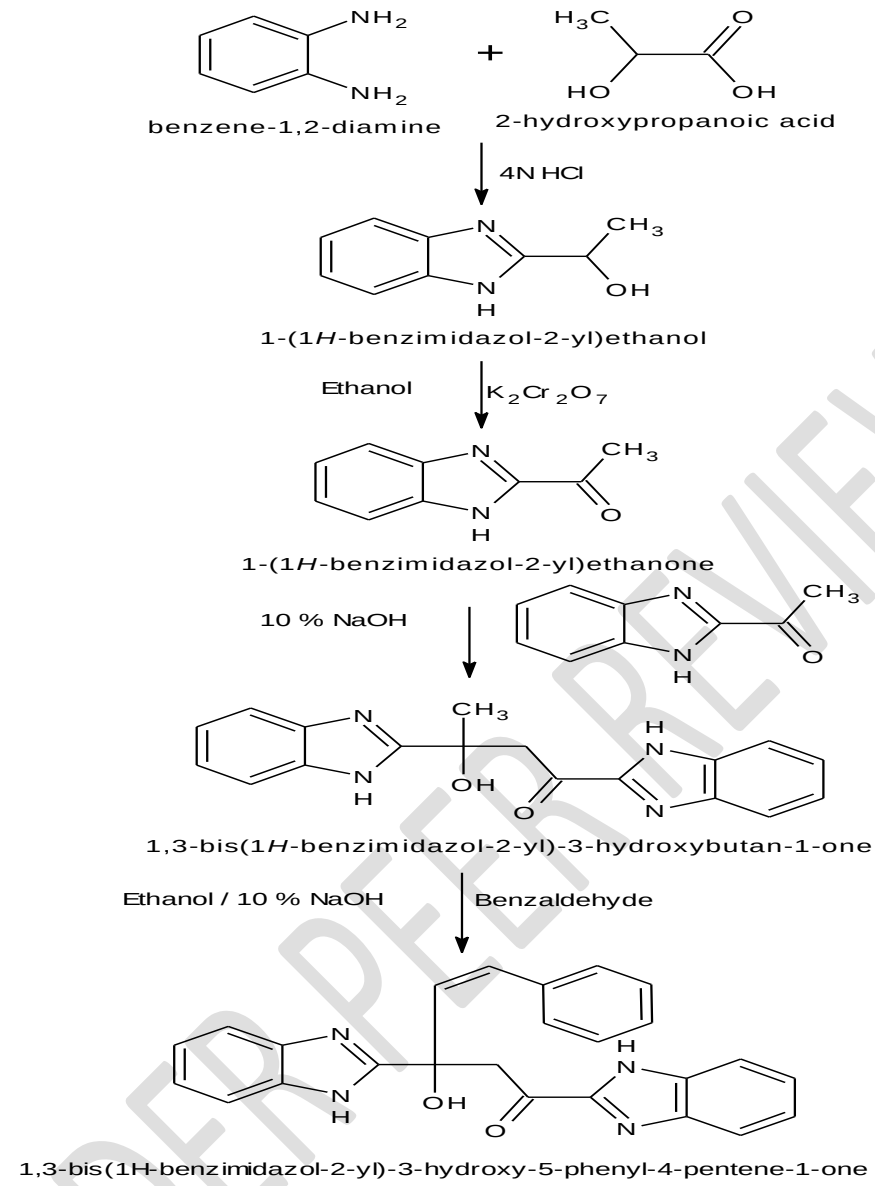
iii. Preparation of 1,3-bis(1*H*-benzimidazol-2-yl)-3-hydroxybutan-1-one:

Acetyl benzimidazole was taken and 10% NaOH was added and then refluxed for 4 hours after refluxing, glacial acetic acid was added, Then it was filtered, dried, and recrystallized [1]

iv. Preparation of 1,3-bis(1*H*-benzimidazol-2-yl)-3-hydroxy-5-phenyl-4-pentene-1-one:

This ketone obtained from 3rd step was condensed with aryl aldehyde. It was neutralized using dilute acetic acid followed by recrystallization, which gave the final compounds yields of 70%. The aryl aldehyde (10 mmol) selected was added to 2-acetylbenzimidazole (1.5 g, 10 mmol) in an ethanolic solution of sodium hydroxide. It was obtained by adding 40 ml ethanol in 75 mmol sodium. It was then stirred for 5 hours. It was neutralized with 30% acetic acid leading to a precipitate. Finally the product was separated out, dried, and recrystallized in toluene.

Scheme:



(Fig-1: Synthetic Scheme)

Screening of Anthelmintic Activity:

The title compounds were screened for anthelmintic property based on the method explained by Dutta et al[2]. Indian earthworm (*Pheretima posthuma)* of approximately equal size was selected randomly for the present study. The worms became accustomed to laboratory conditions before experimentation. The earthworms were divided into three (03) separate groups and each group contains six earthworms (06). Albendazole diluted with normal saline to obtain 20 µg/ml served as the standard and was poured into Petri dishes. The title compounds were

dissolved in a minimum quantity of ethanol and diluted to prepare 20 µg/ml. Normal saline served as a control. The paralysis time and lethal time were calculated for the benzimidazole derivative. When the worms become immobile that time was noted as paralysis time. To determine the death condition of every single worm they were exposed to external stimuli frequently that stimulate and induce movement in earthworms if were they alive. To evaluate the anthelmintic activity, adult Indian earthworms were used because it is anatomically and physiologically similar to the intestinal worms of humans. The time taken to paralysis and death of individual worms was measured (Table-2). Paralysis occurred when the worms did not revive even in normal saline. When the worms lost motility, followed by fading away of their body colors then they were declared as death.

Approach of "Rule of 5" for orally active drugs[3-4]

By utilizing this rule one can predict orally active drugs from their physicochemical properties such as Molecular weight, Partition co-efficient, Hydrogen bond donors, Hydrogen bond acceptors. All the values of these properties can be obtained by the software Molinspiration cheminformatics. Lipinski's rule says that, if the values of all the properties obey the range as given in table-1 then the compound must be active orally.

Table:1 Standard values of "Rule of 5"

S.N	Physicochemical Properties	Limiting value
1	Partition co-efficient	Less than or equal to five (5)
2	Molecular weight	Less than or equal to five hundred (500)
3	Number of hydrogen bonds donors	Less than or equal to five (5)
4	Number of hydrogen bonds acceptors	Less than or equal to ten (10)

Approach for Bioactive score: [4-5]

The bioactive score can also obtain from the software Molinspiration cheminformatics. It gives the score for GPCR, Ion channel, Nuclear receptor, Protease inhibitor, Kinase inhibitor, Enzyme inhibitor, etc. The score will determine whether the drug is active or inactive. The score can be interpreted using the table-2.

Table:2 Standard values of the bioactive score[5]

Parameter	Value	Result
Bioactive score	More than 0.0	Active
	Between −5.0 and 0.0	Moderately active
	Less than −5.0	Inactive

RESULTS & DISCUSSION:

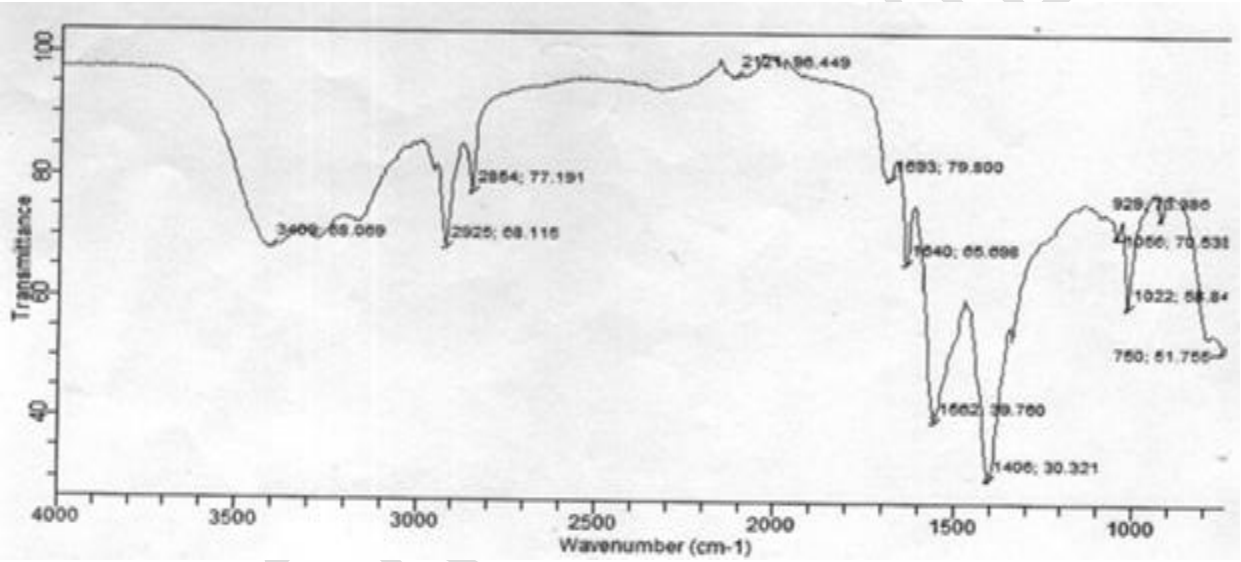
A novel Benzimidazole derivative was synthesized with good yields using ambient reaction conditions in a simple work-up procedure. The general properties of the title compounds were shown in Table-3.

Table-3. General properties of 1,3-bis(1*H*-benzimidazol-2-yl)-3-hydroxy-5-phenyl-4-pentene-1-one.

S.N		
1	**Molecular Formula**	$C_{25}H_{20}N_4O_2$
2	**Formula Weight**	408.4519
3	**Composition**	C(73.51%)H(4.94%) N(13.72%) O(7.83%)
4	**Molar Refractivity**	122.70 ± 0.3 cm^3
5	**Molar Volume**	293.2 ± 3.0 cm^3
6	**Parachor**	866.4 ± 4.0 cm^3
7	**Index of Refraction**	1.777 ± 0.02
8	**Surface Tension**	76.2 ± 3.0 dyne/cm
9	**Density**	1.392 ± 0.06 g/cm^3
10	**Polarizability**	48.64 ± 0.5 $10^{-24}cm^3$

10	**Monoisotopic Mass**	408.158626 Da
11	**Nominal Mass**	408 Da
12	**Average Mass**	408.4519 Da

The structure of the title compound was confirmed on the basis of FTIR (Fig-2). The data obtained from Fig-2 was as follows IR(KBr,cm^{-1})1056(O-H bending),1022(C-N vibration),2925(C-H stretching), 2854(C-H stretching), 1640(C=N stretching) ,1582(C=C stretching) ,1408 (C-O stretching), 3409 (N-H stretching), 750 (C-H bending).



(Fig 2. FTIR Spectrum of 1,3-bis(1*H*-benzimidazol-2-yl)-3-hydroxy-5-phenyl-4-pentene-1-one)

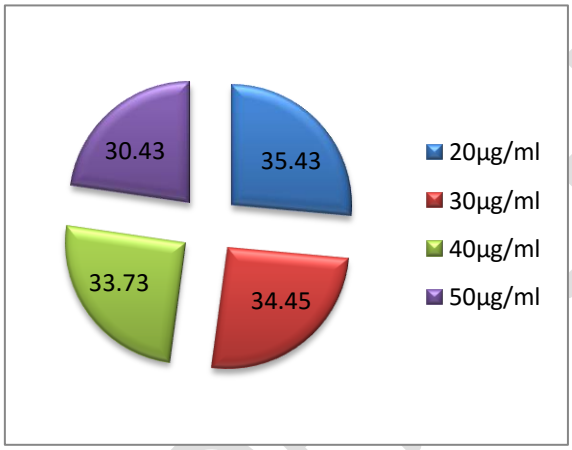
The synthesized drug was compared with one of the effective anthelmintic standard drugs Albendazole with different concentrations (Table-4). When 20µg/ml solution of the standard Albendazole & our sample was tested then it was found that Albendazole takes 25.43± 1.16 min for paralysis whereas synthesized drug take 35.43± 3.22 min for paralysis & death time for both are found to be 1.10± 1.65 and 1.23 ± 2.26 respectively. Hence, the desired compound has

definitely a significant anthelmintic activity. When we increase the concentration of the desired compound the paralysis time and death time also decrease accordingly.

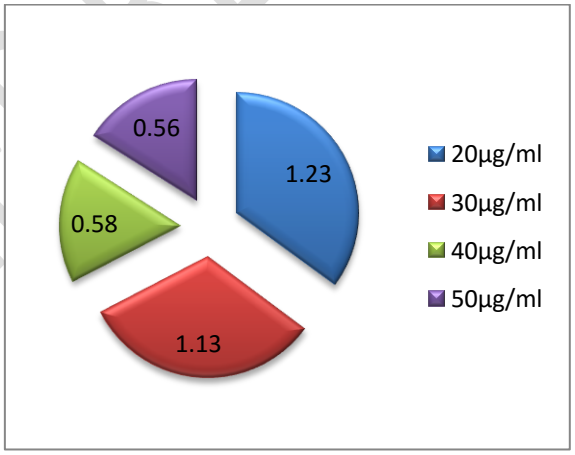
Table 4. Evaluation anthelmintic activity

S.N	Compound name	Concentration	Time taken for paralysis (Min.)	Time taken for death (Hrs)
1	Albendazole (Std.)	20µg/ml	25.43 ± 1.16	01.10 ± 1.65
2	1,3-bis(1*H*-benzimidazol-2-yl)-3-hydroxy-5-phenyl-4-pentene-1-one	20µg/ml	35.43 ± 3.22	01.23 ± 2.16
3		30µg/ml	34.45 ± 1.17	01.13 ± 1.21
4		40µg/ml	33.73 ± 4.01	0.58 ± 3.54
5		50µg/ml	30.43 ± 5.33	0.56 ± 5.32

(Concentration were prepared normal saline, Values are expressed as mean ± SEM, n = 6)



(Fig-3: Time Vs Paralysis time)



(Fig-4: Time Vs Death time)

Bioactive score evaluation:

The bioactive score of the title compound as tabulated below (table-5). It was observed that GPCR ligand, Enzyme inhibitor & nuclear receptor ligand score is more than 0.0, hence the compound is the biologically active compound for the respective receptor. Whereas Ion channel modulator, Kinase inhibitor, Protease inhibitor score is in between 0.0 to -5.0, hence the title compound has moderately active on respective cases.

Table-5 (Bioactive score):

S.N	Receptor	Bioactive score
1	GPCR ligand	0.16
2	Ion channel modulator	-0.02
3	Kinase inhibitor	-0.02
4	Protease inhibitor	-0.04
5	Enzyme inhibitor	0.23
6	Nuclear receptor ligand	0.05

Physicochemical properties evaluation:

According to Lipinski's rule of five, all the parameters of the synthesized title compound are in the range (Mentioned in table-1). The values obtained were tabulated (table-6). It can be concluded that the title compound will be an orally active anthelmintic drug.

Table 6: Physicochemical properties

S.N	Parameter	Obtained value
1	Molecular weight	408.46
2	Log P	4.30
3	Number of hydrogen bonds donors (nOHNH)	3
4	Number of hydrogen bonds acceptor (nON)	6
5	Topological polar surface area	94.67
6	Molecules violation	0
7	Number of Rotatable Bonds	6

The topological polar surface area (TPSA) value was found to be 94.67. A value more than 140, usually found to have less permeability in the cell membrane and value below 90, can cross BBB or effective for CNS. Hence the title compound of this research has said to appreciable activity on CNS. [6-7] Molecules violation was found to Zero (0). If the resulted values are more than 1 then it may interfere with the bioavailability. Hence the title compound

will better on the aspect of bioavailability. If the rotatable bonds number is 10 or less it may results in an efficient orally active drug.[8] Here the value of the title compound found to be six.

So finally we conclude that the synthesized compound, 1,3-bis(1*H*-benzimidazol-2-yl)-3-hydroxy-5-phenyl-4-pentene-1-one has definitely significant anthelmintic activity & also it is orally active.

CONCLUSION:

Benzimidazole derivatives have an important place in the field of synthetic chemistry. The presence of the benzimidazole nucleus in the ultimate compound plays an important role in its therapeutic activity. I tried for a new, simple method for the synthesis of 1,3-Bis-benzimidazole derivative which showed significant anthelmintic activity. It was also confirmed that the synthesized compound is orally active with good bioavailability and has an effect on CNS. In the future, it may be effectively used for the formulation of anthelmintic drugs.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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