

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 multiple. **Result:** The newly produced composite was found to hold weighty anthelmintic property when we compared the same with Albendazole (Standard drug). When the concentration of the desired compound increases paralysis as well as death time found to decrease respectively. 50 µg/ml of the synthesized drug gave a time for paralysis is 30.43 ± 5.33 min & time for death is 0.56 ± 5.32 min. **Conclusion:** The synthesized final compound showed to have anthelmintic activity and its orally active property was also predicted from the Molinspiration software using physicochemical (RO5) as well as bioactive limits. It can be used for anthelmintic drugs as an oral formulation.

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

INTRODUCTION:

The structural variation of benzimidazole derivatives is so convenient for the expansion of molecules of therapeutic or biological interest. 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 improved benzimidazole form has been produced for utilization as improved drugs. Presently available some important drugs, are Omeprazole, Pimobendan, Mebendazole, etc. Various new schemes for the preparation of benzimidazole derivatives have been reported and synthesized with an intention of searching for its enhanced therapeutic values.

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 produce an innovative derivative of benzimidazole likely to have upright anthelmintic property 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 :

o-phenylenediamine (OPD) and lactic acid (Equi-molar fraction) were refluxed till the reactin is completed in the presence of 4N HCl. Thin layer chromatography was used to find out the end of reaction. Recrystallization of the resulting compound was carried out through use of methanol.

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

The developed product from step-1 was weighed and taken in a RBF. Then it was oxidized in presence of $K_2Cr_2O_7$ (Potassium dichromate) and refluxed with glacial acetic acid till end of the reaction. Thin layer chromatography was used to determine the completion of the

reaction. The residue was collected and kept in room temperature. Then it was neutralized with the help of adding ammonia solution dropwise. The resulting product was collected safely and recrystallized.

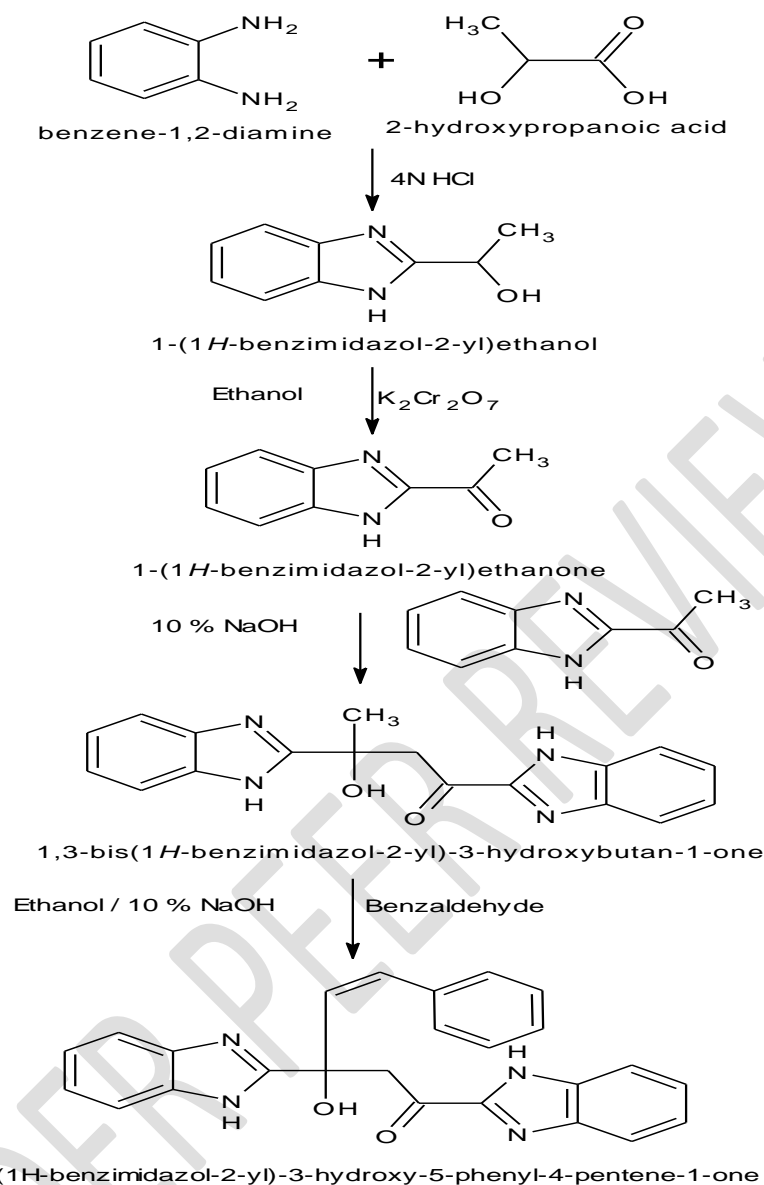
iii. Preparation of 1,3-bis(1*H*-benzimidazol-2-yl)-3-hydroxybutan-1-one:

The product obtained from step-2 along with 10% NaOH was added in a RBF. The reaction mixture was refluxed till the reaction was complete, on to it glacial acetic acid was added, Finally the content of RBF was filtered by filtering apparatus, the solid residue over the filter paper was dried, and then recrystallized ^[1]

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

Resulting residue obtained from 3rd step was allowed to condensed through aryl aldehyde. It was neutralized by means of dil. CH₃COOH subsequently recrystallization, which gave the ultimate composite with an yields value of 70%. The respective aryl aldehyde (10 mmol) selected was added to 2-acetylbenzimidazole (1.5 g, 10 mmol) in an ethanolic solution of NaOH. It was obtained by adding 40 ml ethanol in 75 mmol sodium. It was then stirred for 5 hours. It was neutralized with 30% CH₃COOH leading to formation of precipitate. Finally the product was separated out by filtration, and then dried afterward recrystallized with the help of C₇H₈ (toluene)

Scheme:



(Fig-1: Synthetic Scheme)

Screening of Anthelmintic Activity: ^[2-7].

The synthesized compound i.e. 1,3-bis(1H-benzimidazol-2-yl)-3-hydroxy-5-phenyl-4-pentene-1-one was attempted for anthelmintic potency. The anthelmintic screening was tested on Indian earthworm (*Pheretima posthuma*) which have nearly equal size. The worms became accustomed to conditions of the laboratory before performing experiment. *Pheretima posthuma* were divided into three (03) separate groups and each group contains six earthworms (06). Albendazole (Standard drug) was diluted with normal saline to obtain a medium of 20 μ g/ml. it

was considered as the standard and kept in a petri dishes. The compound going to be screened was diluted to prepare a medium of 20 µg/ml concentration. Normal saline served as a control. The time taken for paralysis and lethal time were calculated. When the earthworms found to immobile that time is paralysis time which was noted down for the same. 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. Adult Indian earthworms were used because physiologically as well as anatomically it is similar to the intestinal worms of humans. Paralysis and death time of individual worms was measured (Table-2). Paralysis occurred when the worms did not revive even in normal saline. Earthworms were mentioned death when they lost their movement and fading of body color.

Approach of "Rule of 5" for orally active drugs^[8-9]

By utilizing this rule one can predict orally active drugs from their physicochemical properties such as Molecular weight, Partition co-efficient, Hydrogen bond acceptors, Hydrogen bond donors. 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	Hydrogen bonds acceptors	Less than or equal to ten (10)
4	Hydrogen bonds donors	Less than or equal to five (5)

Approach for Bioactive score: ^[9-10]

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^[10]

Parameter	Value	Result
Bioactive score	Less than -5.0	Not-active
	Between -5.0 and 0.0	Moderately active
	More than 0.0	Active

RESULTS & DISCUSSION:

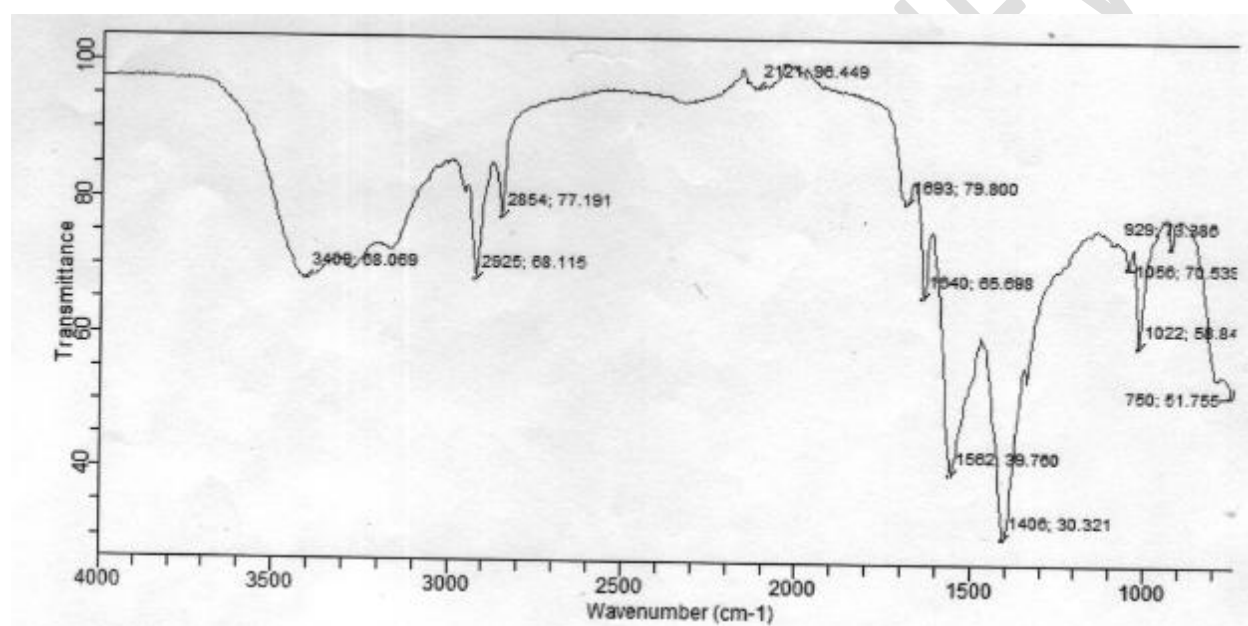
A unique derivative of benzimidazole was designed and prepared with a good yielding value using a simple laboratory conditions. The general properties of the final composite were as follows. (Table-3)

Table-3. General properties

S.N	Molecular Formula	C ₂₅ H ₂₀ N ₄ O ₂
1	Mol. Weight	408.4519
2	Mol. Formula	C ₂₅ H ₂₀ N ₄ O ₂
3	Elements present	Hydrogen (4.94%), Nitrogen (13.72%), Carbon (73.51%) and Oxygen (7.83%)
4	Molar Volume	293.2 ± 3.0 cm ³
5	Molar Refractivity	122.70 ± 0.3 cm ³
6	Index of Refraction	1.777 ± 0.02
7	Density	1.392 ± 0.06 g/cm ³
8	Surface Tension	76.2 ± 3.0 dyne/cm
9	Parachor	866.4 ± 4.0 cm ³
10	Polarizability	48.64 ± 0.5 10 ⁻²⁴ cm ³
11	Monoisotopic Mass	408.158626 Da

12	Average Mass	408.4519 Da
13	Nominal Mass	408 Da

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



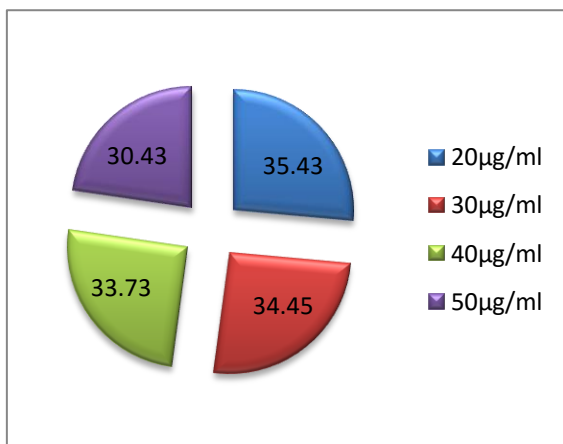
(Fig 2. FTIR Spectrum of 1,3-bis(1H-benzimidazol-2-yl)-3-hydroxy-5-phenyl-4-pentene-1-one)

The final product obtained was compared with one standard drugs Albendazole (Table-4). When 20µg/ml solution of the standard Albendazole. 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.

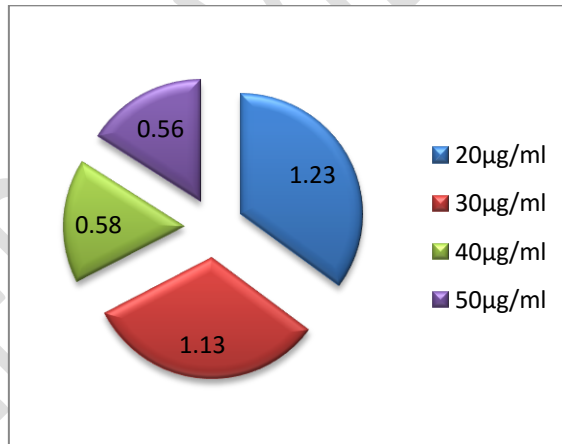
Table 4. Evaluation anthelmintic activity

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 <i>H</i> -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	Kinase inhibitor	-0.02

2	Ion channel modulator	-0.02
3	GPCR ligand	.16
4	Enzyme inhibitor	.23
5	Nuclear receptor ligand	.05
6	Protease inhibitor	-0.04

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	Hydrogen bonds acceptor (nON)	6
4	Hydrogen bonds donors (nOHNH)	3
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.^[11-12] 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.^[13] 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:

Heterocyclic compound, derivatives of benzimidazole have a vital place in the arena of synthetic medicinal chemistry. The attachment of a benzimidazole nucleus in the desired moiety have a significant role for beneficial pharmacologic activity. We designed a original and modest procedure for synthesis of a 1,3-Bis-benzimidazole derivative which exhibited noteworthy anthelmintic property. It was also confirmed that the synthesized compound is orally active with good bioavailability and has an effect on CNS.

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.

REFERENCES:

1. Mahama Ouattara, Tropical Journal of Pharmaceutical Research. 2011; 10 (6): 767-775.
2. S. Dutta, Synthesis and anthelmintic activity of some novel 2-substituted-4,5-diphenyl imidazoles; Acta Pharm. 2010; 60: 229–235.
3. Miriam Tagoe, Yaw Duah Boakye , Theresah Appiah Agana, Vivian Etsiapa Boamah, and Christian Agyare, In Vitro Anthelmintic Activity of Ethanol Stem Bark Extract of Albizia

ferruginea (Guill. & Perr.) Benth, Hindawi, Journal of Parasitology Research Volume 2021, Article ID 6690869, <https://doi.org/10.1155/2021/6690869>.

4. Neeraj Choudhary, Gopal L. Khatik, Sunanda Choudhary, Gural Singh, Ashish Sutte, In vitro anthelmintic activity of *Chenopodium album* and in-silico prediction of mechanistic role on *Eisenia foetida*, *Heliyon*, Volume 7, Issue 1, 2021, ISSN 2405-8440, <https://doi.org/10.1016/j.heliyon.2021.e05917>.
5. Muda I, Prastowo J, Nurcahyo W, Sarmin S (2021) Anthelmintic effect of *Indigofera tinctoria* L on *Haemonchus contortus* obtained from sheep in Indonesia, *Veterinary World*, 14(5): 1272-1278. : www.doi.org/10.14202/vetworld.2021
6. Olukemi Osukoya, Adewale Fadaka, Olusola Adewale, Oluwatobi Oluloye, Oluwafemi Ojo, Basiru Ajiboye, Deborah Adewumi, Adenike Kuku. *In vitro* anthelmintic and antioxidant activities of the leaf extracts of *Theobroma cacao* L.[J]. *AIMS Agriculture and Food*, 2019, 4(3): 568-577. doi: 10.3934/agrfood.2019.3.568
7. Iqbal A.M.A, Rajkhowa A, Singh P.C, Choudhury P.D, Sahu R.K, Assessment of Phytochemical and Anthelmintic Activity of Some Selected Ethnomedicinal Plants from Barak Valley Region of Assam. *Biomed Pharmacol J* 2020;13(4)
8. C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug. Delivery Rev.* 23, 4-25 (1997).
9. www.molinspiration.com
10. Tahmeena Khan & Shalini Dixit, Rumana Ahmad, Saman Raza, Iqbal Azad, Seema Joshi, Abdul Rahman Khan; Molecular docking, PASS analysis, bioactivity score prediction, synthesis, characterization and biological activity evaluation of a functionalized 2-butanone thiosemicarbazone ligand and its complexes; *J Chem Biol* (2017) 10:91–104.
11. Pajouhesh H, Lenz GR (Oct 2005). "Medicinal Chemical Properties of Successful Central Nervous System Drugs". *NeuroRx*. 2 (4): 541–553. doi:10.1602/neurorx.2.4.541. PMC 1201314. PMID 16489364.
12. Hitchcock SA, Pennington LD (May 2006). "Structure - Brain Exposure Relationships". *J. Med. Chem.* 49 (26): 7559–7583. doi:10.1021/jm060642i.

13. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. *J Med Chem.* 2002 Jun 6;45(12):2615-23. doi: 10.1021/jm020017n. PMID: 12036371.

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