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# IN VITRO ANTHELMINTIC ACTIVITY OF NOVEL BENZIMIDAZOLE DERIVATIVES FROM O-PHENYLENE DIAMINE

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## ABSTRACT

The present study is an attempt to explore the anthelmintic activity of Benzimidazole by molecular docking with tubulin and in vitro activity against adult Indian earthworms, Pheretima posthuma. The time taken for each worm for paralysis and death were determined. A benzimidazole derivative binds very efficiently within the active pocket of tubulin which is better when compared to orientation of standard drug, Piperazine citrate. In vitro results correlated with the in silico studies. The time taken for paralysis and death with benzimidazole 2 at 80 mg/ml was comparable to standard Piperazine citrate.

**KEYWORDS:** Anthelmintic activity, benzimidazole, O-Phenylene diamine docking, tubulin.

## INTRODUCTION

The benzo derivative of imidazole is referred to as benzimidazole (Bansal, 2002). Although benzimidazole is the commonest name of the parent compound of the series, other names such as benzimidazole and 1,3-benzodiazole (1) are often used Mono acyl derivative of o-phenylenediamine is readily converted into the corresponding benzimidazole by the action of heat alone. These conversions are generally carried out at a temperature somewhat above the melting point of the starting compounds. This is a convenient method for preparing benzimidazoles when monoacyl derivatives are easily obtainable. The procedure may be improved by heating the monoacyl derivative of diamine in an atmosphere of nitrogen to prevent oxidation (Kelly, 1945). The diacyl derivatives of o-phenylenediamines are also converted into benzimidazoles but higher temperatures are required (Bistrzycki, 1890). Mono basic acid in 4 N hydrochloric acid. The benzimidazole is then precipitated by neutralizing the solution with ammonium hydroxide. Benzoic acid gives only traces of 2-phenylbenzimidazole. Apparently this method is not applicable to the aromatic monobasic acid. Benzimidazole derivative are associated with various types of pharmacokinetic and pharmacodynamic properties. Benzimidazole nucleus is one of the bioactive heterocyclic compounds that exhibit a range of biological activities. Specifically, this nucleus is a constituent of vitamin B12 (O'Neil et al., 2001).The pharmacological activities of the benzimidazole containing moiety have been well documented (Amari et al., 2002). Albendazole, Mebendazole and Thiabendazole are widely used as anthelmintic drugs (Kohler, 2001). Literature survey reveals that the various derivatives of benzimidazole have been synthesized for their pharmacological activities. Some of the already synthesized compounds from the above mentioned field have found very strong application in medicine praxis (Mavrova et al., 2006). The activity against bacteria, fungi and helminthes resulted their mode of action, which resulted in the blockage of microtubule in various nematode, trematode and cystode. (Campbell and Denham, 1983).

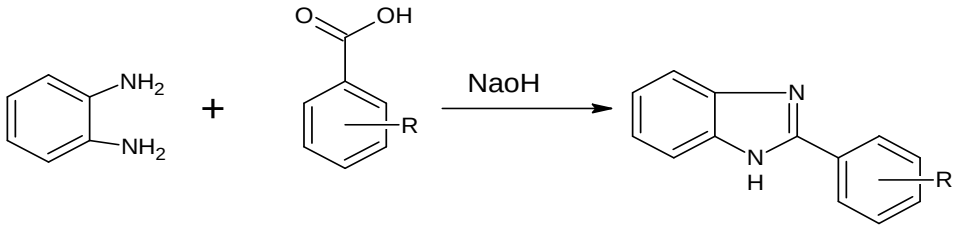
Tubulin is a known anticancer and anthelmintic drug target. The investigation of tubulin inhibitors could lead to the development of new anthelminthic drugs. Inhibitors bind selectively to β-Tubulin of nematodes, cestodes and fluke, a protein subunit of microtubule and thereby disrupting microtubule structure and function.[13,14,15] Microtubules are highly dynamic, ubiquitous cellular organelles serving a variety of vital functions including mitosis, motility and transport, in all eukaryotes. Many of these structures exist in a dynamic equilibrium in which assembly and disassembly of the soluble subunits are balanced. In such systems, the drug–tubulin interaction results in a shift of this equilibrium with a net loss of microtubules and accumulation of free tubulin. In view of the crucial roles, that microtubules play in many cellular processes, their drug-induced destruction eventually leads to the death of the organism.[15] Some anthelmintic drugs act rapidly and selectively on neuromuscular transmission of nematodes. Levamisole, pyrantel and morantel are agonists at nicotinic acetylcholine receptors of nematode muscle and cause spastic paralysis. Dichlorvos and haloxon are

organophosphorus cholinesterase antagonists.[16] Diethylcarbamazine blocks host, and possibly parasite, enzymes involved in arachidonic acid metabolism, and enhances the innate, nonspecific immune system. Some drugs are known to affect the fatty acid oxidation pathway in mammals, caused a reduction in oxygen consumption rates in C. elegans and genome-wide gene expression profiles provided an additional confirmation of its mode of action.[17] In silico molecular docking technique play an important role in the drug design and discovery to predict the conformations of each ligand molecule at the active site, hence the molecular docking study was carried out to predict the β-Tubulin inhibitory activity and results are reported. Even though Benzyl derived compounds are known to have antiparasitic effect, it is now banned in many countries. Piperzine has broad spectrum activities like anthelmintic, antiallergenic, antibacterial, antihistamic antiemetic and antimigraine agents. It is used as an anthelmintic for humans and farm animals against intestinal roundworms and pinworms infection; administered orally. Because of its broad spectrum usage it is used as a standard drug in our study and there are various research articles available which support our study. Since in our previous study,[11] we used piperazine citrate as reference standard.

## MATERIALS AND METHODS

O-Pheylene Diamine, benzoic acid derivatives, acetate, ethyl acetate, chloroform, DMF saline water of the LR grade was to be purchased.

**Reaction**




R= H,2,3 di -$CH_3$,3 $NO_2$ 4 Cl,4 $NH_2$,5 $NO_2$ ,6 Cl

**Procedure**[12]

A mixture of 4gm orthophenylene diamine, 36 ml of 4 N HCL and 3.4 ml of benzoic acid derivatives were taken in a round bottom flask and the solution was boiled under reflux for 3 hours until reaction completes which is checked by T.L.C analysis.[3] Further the solution was cooled on ice and made alkaline by the addition of 30% NH3 solution. The precipitate formed was filtered, dried and recrystallized from suitable solvents.

**In vitro Anthelmintic activity**[13]

**Earthworm collection**[14]

Earth-worms in moist soil were washed with normal saline and used for the study. The earthworms 3 -5 cm in length and 0.1-0.2 cm width were used due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.[18,19]

**Preparation of solutions**

Here the synthesised compounds were prepared by using the 5% DMF and saline solutions.

**In vitro Anthelmintic activity**[15-18]

The anthelmintic assay was carried as per the method of Ajaiyeoba et. al.[9] with minor modifications. All the test solutions and standard drug solutions were prepared freshly before starting the experiment. Six groups of earthworms of approximately equal size were released in to 25 ml solutions of three different concentrations (20,40,80 mg/ml) in petri dishes containing 5 % of DMF solution. Piperazine citratae was used as reference standard and saline as control. Determination of time of paralysis and time of death of the worm were done. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50$^{o}$C) followed with fading away of their body colours.

## RESULTS AND DISCUSSION

**Physical properties of compounds**

| S. No. | Name of the compound | Molecular formula | Relative Mass | M.P | % yield |
|---|---|---|---|---|---|
| 1 | BI-01 | $C_{13}H_{10}N2$ | 194.23 | 166 | 94 |
| 2 | BI-02 | $C_{15}H_{14}N_2$ | 222.24 | 174 | 96 |
| 3 | BI-03 | $C_{13}H_8ClN_3O_2$ | 273.25 | 180 | 84 |
| 4 | BI-04 | $C_{13}H_{11}N_3$ | 209.25 | 184 | 76 |
| 5 | BI-05 | $C_{13}H_9N_3O_2$ | 239.25 | 186 | 84 |
| 6 | BI-06 | $C_{13}H_9ClN_2$ | 227.25 | 182 | 86 |

**Elemental analysis**

| S. No. | Calculated | | | | Observed | | | |
|---|---|---|---|---|---|---|---|---|
| | C | H | N | O | C | H | N | O |
| BI-01 | 80.39 | 5.19 | 14.42 | _ | 80.19 | 5.12 | 14.40 | _ |
| BI-02 | 81.05 | 6.35 | 12.60 | _ | 81.02 | 6.30 | 12.55 | _ |
| BI-03 | 57.05 | 2.95 | 15.35 | 11.69 | 57.03 | 2.92 | 15.32 | 11.62 |
| BI-04 | 74.62 | 5.30 | 20.08 | _ | 74.58 | 5.24 | 20.02 | _ |
| BI-05 | 65.27 | 3.79 | 17.56 | 13.38 | 65.22 | 3.72 | 17.52 | 13.32 |
| BI-06 | 68.28 | 3.97 | 12.25 | _ | 68.21 | 3.95 | 12.22 | _ |

**Spectral data**

| S. No. | Name of the compound | Spectral data |
|---|---|---|
| 1 | BI-01 | Pale yellow crystals, IR (KBr): 1626 (C=N), 3436 (NH) cm-1. 1H NMR (600 MHz, DMSO-d6) δ 12.96 (s, 1H), 8.21-8.20 (t, J = 9.0 Hz, 2H), 7.62-7.49 (m, 5H), 7.23-7.20 (m, 2H). 13C NMR (150 MHz, DMSO-d6) δ 151.70, 130.65, 130.31, 129.42, 126.91, 122.58. HRMS (ESI) Calc. for [M+H]+: 195.0917, found: 195.0916. |
| 2 | BI-02 | Yellow crystals, IR (KBr): 1623 (C=N), 2965 (CH3), 3449 (NH) cm-1. 1H NMR (600 MHz, DMSO-d6) δ 12.83 (s, 1H), 8.08 (d, J = 8.4 Hz, 2H), 7.58 (s, 2H), 7.37 (d, J = 7.8 Hz, 2H), 7.20 (dd, J1=6.0 Hz, J2 = 3.0 Hz, 2H), 2.39 (s, 3H). 13C NMR (150 MHz, DMSO-d6) δ 151.84, 140.04, 129.98, 127.90, 126.87, 122.43, 21.44. HRMS (ESI) Calc. [M+H]+: 209.1073, found: 209.1072. |
| 3 | BI-03 | Pale yellow crystals, IR (KBr): 1623 (C=N), 3439 (NH) cm-1. 1H NMR (600 MHz, DMSO-d6) δ 13.05 (s, 1H), 8.24 (s, 1H), 8.16-8.15 (m, 1H), 7.61-7.56 (m, 4H), 7.24 (s, 2H). 13C NMR (150 MHz, DMSO-d6) δ 150.20, 134.24, 132.68, 131.41, 130.01, 126.49, 125.48. HRMS (ESI) Calc. [M+H]+: 229.0527, found: 229.0523. |
| 4 | BI-04 | Pale yellow crystals, IR (KBr) cm:3327.94 (N-H), 3461.08 *f* (NH), 1530.41(C=C), 1621.06 (C=N), 1193.85 (C-N), 894.91 2 (Ar-H). |
| 5 | BI-05 | Yellow crystals, IR (KBr): 1334, 1512 (NO2), 1624 (C=N), 3436 (NH). 1H NMR (600 MHz, DMSO-d6) δ 13.64 (s, 1H), 8.51 (s, 1H), 8.23-8.14 (m, 3H), 7.78 (s, 1H), 7.62 (d, J=6.6 Hz, 3H). 13C NMR (150 MHz, DMSO-d6) δ 143.18, 131.43, 129.62, 129.51, 127.46, 118.44. HRMS (ESI) Calc. for [M+H]+: 240.0768, found: 240.0763. |
| 6 | BI-06 | White crystals, IR (KBr): 1623 (C=N), 3433 (NH) cm-1. 1H NMR (600 MHz, DMSO-d6) δ 12.73 (s, 1H), 7.92 (dd, J1 = 7.8 Hz, J2 = 1.8 Hz, 1H), 7.67 (dd, J1 = 8.4 Hz, J2 = 1.2 Hz, 2H), 7.57-7.52 (m, 3H), 7.25 (s, 2H). 13C NMR (150 MHz, DMSO-d6) δ 149.57, 132.56, 132.11, 131.67, 130.82, 130.45, 127.91. HRMS (ESI) [M+H]+: 229.0527, found: 229.0523 |

**Anthelmintic activity of Benzimidazole Derivatives**

| S. No. | Parameter | Concentration (mg/ml) | BI-01 | BI-02 | BI-03 | BI-04 | BI-05 | BI-06 | Piperazine citrate 15 (mg/ml) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Time taken for paralysis | 80 | 2.55 ± 0.18 | 1.87+0.291 | 2.01± 0.11 | 2.64 ± 0.17 | 2.33 ± 0.14 | 2.24 ± 0.15 | 41.53 ± 0.13 |
| 2 | | 40 | 3.28 ± 0.22 | 2.01± 0.31 | 4.27 ± 0.12 | 3.77 ± 0.13 | 4.51± 0.28 | 4.09 ± 0.22 | |
| 3 | | 20 | 5.11 ± 0.23 | 3.11 ±0.14 | 5.87 ± 0.23 | 4.25± 0.22 | 6.52 ± 0.32 | 6.12 ± 0.21 | |
| 4 | Time taken for Death | 80 | 3.44 ± 0.22 | 2.02 ± 0.22 | 3.12± 0.32 | 3.65 ± 0.31 | 3.25 ± 0.22 | 3.25 ± 0.22 | 45.23 ± 0.22 |
| 5 | | 40 | 5.15 ± 0.12 | 4.12 ± 0.22 | 5.01 ± 0.55 | 5.65±0.24 | 5.75 ± 0.15 | 5.85±0.14 | |
| 6 | | 20 | 6.15 ± 0.23 | 4.95 ± 0.22 | 6.12 ± 0.18 | 6.25 ± 0.15 | 6.65 ± 0.25 | 6.25 ±0.17 | |

## DISCUSSION

Based on invitro the benzimidazole molecules show better Anthelmintic activity. Here the compound benzimidazole-2 (2-(3,5-dimethylphenyl)-1*H*-benzimidazole) shows better activity than other molecules.

## CONCLUSION

The compound benzimidazole-2 (2-(3,5-dimethylphenyl)-1*H*-benzimidazole) shows better activity than other molecules.

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