In vitro Anthelmintic, Antioxidant Activity and In silico Adme Study of Synthesized Nitro benzimidazole Derivatives

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ABSTRACT

New derivatives of benzimidazole were synthesized which were containing 1- and 2-substituted 5-nitro benzimidazole derivatives. The presence of specific functional group was confirmed by IR spectroscopic analysis. The determination of structure for the synthesized compounds was confirmed by 1H proton magnetic resonance. Anthelmintic activity of the derivatives was investigated and compared with standard FDA approved anthelmintic drug albendazole. The obtained results show that out of the investigated compositions 100mg/mL were found much active compound in paralyzing and death of the earth worm that shows the time of paralysis 20 min and the time of death stage is 24 minutes. In silico ADMET and pharmacokinetic parameters of compounds (DP-1 to DP-3) were also evaluated for drug likeliness. Calculations related to protein binding, blood–brain barrier (BBB), MDCK cell permeability, Caco-2 cell permeability and human oral absorption in the gastrointestinal tract showed that these values for the derivatives (DP-1-DP-3) fell within the standard ranges generally observed for drugs.

Keywords: Benzimidazole, Nitration, Spectroscopy, Earth worm paralysis, Anthelmintic activity, Anti-oxidant activity, In silico ADMET study.

INTRODUCTION

Number of studies has disclosed that heterocyclic compounds show medicinal activity. Number of moieties based on heterocyclic compound reported as bioactive molecules. Benzimidazole also lies on this area and its derivatives are found to have various biological and pharmaceutical activities. Benzimidazole derivatives are reported as an active ingredient against antiviral, antifungal, anticancer, anti-histaminic, anti-tubercular, anti-allergic, antioxidant antimicrobial and anti-ulcer activities etc. Substituted benzimidazole derivatives especially at 2nd and 5th position appears to be an important scaffold for anti-parasitic, anti-anthelmintic and medicine drugs.

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derivative shows better anthelmintic activity against Indian earthworm\textsuperscript{13}. It also include the inhibition of reactive oxygen species (ROS) generation, direct or indirect scavenging of free radicals, and alteration of intracellular redox potential\textsuperscript{14}. Antioxidants express its activity by inhibiting apoptosis as it is considered to be mediated by oxidative stress\textsuperscript{15}.

In the present study 2-substituted benzimidazole were synthesized by treating \textit{o}-phenylenediamine with different aromatic acids. Further nitration has been carried out at room temperature to get 2-substituted 5-nitro benzimidazole derivatives. To study anti-anthelmintic activity of all synthesized compounds are analyzed by the application on Indian earth worm. Also to evaluate Anti-oxidant activity of all the synthesized compound evaluated and compared with marketed standard anti-oxidant drug molecule. Insilco pharmacokinetic parameters of the synthesized compounds were also evaluated to study the equivalence of these molecules as drugs.

**MATERIALS AND METHODE**

\textit{o}-phenylenediamine, aromatic acids \textit{o}-Chloro benzoic acid, \textit{p}-Chloro benzoic acid and Benzoic acid were purchased from Loba Chemicals Ltd. Baroda (Gujarat). Ethanol of the LR grade was to be purchased. All the reagents were obtained commercially grade are used with further purification. Solvents used were of analytical grade.

**General procedure for the synthesis of 5-nitro benzimidazole derivative**

**Step-I**

**Benzimidazole compound synthesis**

\textit{o}-phenylenediamine (1 mole) and aromatic acids (\textit{o}-chloro benzoic acid, \textit{p}-chloro benzoic acid and benzoic acid) (1 mole) in stoichiometric proportion were taken in ethanol as solvent. The reaction mixture was constant stirred for 2 hours. at room temperature on magnetic stirrer. After the completion of reaction, the reaction mixture was poured in the ice-cold water. The granular solid was obtained and filter it and dry the product and crystallized from the alcohol. The progresses of the reaction were monitored by TLC. The melting point was determined by using melting point test apparatus.

**Step-II-modification of benzimidazole compounds**

Take above compound (1 g) and \( \text{H}_2\text{SO}_4 \) (10 mL) and \( \text{HNO}_3 \) (10 mL) take in 25 mL beaker and stirred at room temperature for 10 horus., filter it yellow color solid obtained and recrystallized by ethanol, and the yield is 85.00\%. All other compounds (DP-1, DP-2 & DP-3) were synthesized in similar manner by treatment of (1) with substituted aromatic acids [DP-a-c)] respectively as mention in Table 1. The progress of reaction was monitoring by TLC in chloroform: methanol 9:1 solvent system. The melting point was determined by using melting point test apparatus.

**Reaction**

\[
\text{N} \quad \text{N}
\]

\[
\text{O}_2 \text{N}
\]

\[
\text{5-nitro-2-phenyl-1H-benzo[d]imidazole}
\]

\[
\text{N} \quad \text{N}
\]

\[
\text{O}_2 \text{N}
\]

\[
2-(\text{4-chlorophenyl})-5-nitro-1H-benzo[d]imidazole \quad (\text{DP-1})
\]

\[
\text{N} \quad \text{N}
\]

\[
\text{O}_2 \text{N}
\]

\[
2-(\text{4-chlorophenyl})-5-nitro-1H-benzo[d]imidazole \quad (\text{DP-2})
\]

\[
\text{N}
\]

In vitro anthelmintic activity

**Earth worm collection**

Indian earthworms in humid soil were washed with the normal solution and used for the anthelmintic study. The Indian earthworms 3-5 cm in length and 0.1-0.2 cm width were used due to their physiological and anatomical similarities with the abdominal roundworm parasites of human beings.

**Preparation of test solutions**

Here the synthesized compounds were prepared by using the 5\% Methanol solutions.

**In vitro anthelmintic activity**\textsuperscript{16}

All the synthesized compounds were checked for their anthelmintic activity again Indian earthworm. All the test compounds and standard drugs were freshly prepared before the experiment. There were an equal group and equal size Indian
earthworm put into the different concentration (25 mL, 50 mL, 100 mL) 100 mL test solution in Petri plate and albendazole taken as a marketed standard drug and methanol as control solution. Time of paralysis and time of death were observed by inspecting the no movement of any kind and time for paralysis was noted except when the worms were shaken vigorously. Time for the death of worms was recorded after ascertaining those worms neither moved when shaken vigorously nor when dipped in warm water (50°C) followed with dying away of their body color.

**In vitro antioxidant activity**\(^{17}\)

The antioxidant potential of all synthesized compounds was checked by DPPH (2, 2-Di-Phenyl-1-Picryl Hydroxyl) free radical scavenging assay. 10 mg of DPPH was dissolved in 10 mL of methanol. From this stock solution, dilutions were made to obtain solutions of concentrations 10 μg/mL, 20 μg/mL, and 30 μg/mL. The absorbance values were recorded for these dilutions at 516nm. The solution was prepared in the amber reagent bottle and kept in the light-proof box. Ascorbic acid, a potential antioxidant, was used as a positive control. 10 mg of Ascorbic acid was dissolved in methanol to get a mother solution having a concentration of 10 μg/mL, 20 μg/mL, and 30 μg/mL. The test compounds (DP-1 to DP-3), each 10 mg in amount, were dissolved in methanol to prepare a stock solution whose concentration is 1000 μg/mL. The test samples were prepared from this stock solution by serial dilution with methanol to attain concentrations similar to DPPH. 1.0 mL solution of the test compounds was mixed with 1.0 mL of DPPH solution (300 μg/mL). The mixture was then shaken vigorously and allowed to stand at room temperature for 30 min in a dark place and the absorbance was measured at 516 nm by UV-Spectrophotometer against methanol as blank. % of inhibition was using equation %inhibition =\((1-A_{\text{sample}}/A_{\text{blank}})\times 100\) Where A blank is the absorbance of control reaction (containing all reagents except the test material)

**In silico pharmacokinetic study**

In order to better understand the drug-likeness properties of the compounds (DP-1 to DP-3), the parameters such as log P, PSA, molecular weight, volume, water-solubility, %HIA, BBB, %PPB etc. were calculated using ADMET and Swiss ADME software and the data are presented in Table 5 and Table 6.

**RESULTS AND DISCUSSION**

**Characterization**

| Table 1: Physical properties of compounds |
|-----------------|-----------------|--------------|----------------|-----------------|
| Sr. No | Name | -RDP-(a-c) | Yield(%) | Melting Point°C | Molecular Formula | Elemental analysis of N (%) |
|-------|------|---------|---------|----------------|------------------|--------------------------|
| 1    | (DP-1)2-(2-chlorophenyl)-5-nitro-1H-benzo[d]imidazole (DP-2)2-(4-chlorophenyl)-5-nitro-1H-benzo[d]imidazole (DP-3)(5-nitro-2-phenyl-1H-benzoimidazole) | o-Chloro Benzoic acid | 85.78 | 112°C | C<sub>13</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>2</sub> | 57.05(58.00) 2.95(3.00) 15.35(16.04) |
| 2    |  | p-Chloro benzoic acid | 80.00 | 270°C | C<sub>13</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>2</sub> | 57.05(58.00) 2.95(3.00) 15.35(16.04) |
| 3    |  | Benzoic acid | 78.08 | 210°C | C<sub>13</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub> | 64.56(65.27) 3.21(3.79) 17.00(17.56) |

**Table 2: Spectral Data**

| Sr. No | Name of compound | Spectral data |
|--------|-----------------|---------------|
| 1      | DP-1 | IR:3435 cm<sup>-1</sup> (NH), 3101 cm<sup>-1</sup> (C-H), 1635 cm<sup>-1</sup> (C=N), 1483 cm<sup>-1</sup> (C-N), 1557 cm<sup>-1</sup> (N-O).
  1H NMR: (400 MHz, DMSO) δ=12.72 (s, 1H), 7.93 (s, 1H), 7.62 (d, 5H), 7.27 (s, 2H)
| 2      | DP-2 | IR: 3435 cm<sup>-1</sup> (NH), 3101 cm<sup>-1</sup> (CH), 1635 cm<sup>-1</sup> (C=N), 1483 cm<sup>-1</sup> (C-N), 1557 cm<sup>-1</sup> (N-O).
  1H NMR: (400 MHz, DMSO) δ = 12.72 (s, 1H), 7.93 (s, 1H), 7.62 (d, 5H), 7.27 (s, 2H)
| 3      | DP-3 | 3268 cm<sup>-1</sup> (N-H Str), 3120 cm<sup>-1</sup> (C-H Str), 1628 cm<sup>-1</sup> (C≡N Str), 14330 cm<sup>-1</sup> (C-N Str), 1570 cm<sup>-1</sup> (N-O Str)
  1H NMR: (400 MHz, DMSO) δ=12.88 (s, 1H), 8.24–8.14 (m, 2H), 7.64–7.47 (m, 5H), 7.27–7.15 (m, 2H) |
From Table 1 and Table 2 it is evidenced that the adopted scheme was working well and synthesized products were confirmed by spectral data as mentioned in Table 2. The yield of the product is also appreciable. The Compound DP-1 was produced with 85.78% yield comparable to the compounds DP-2 and DP-3 at 80 and 78.08% respectively.

Evaluation of Anthelmintic activity

Based on Table 3, the benzanidazole compounds show better Anthelmintic activity. Anthelmintic activity was studied using three different concentrations of the benzanidazole compounds (25 mg/mL, 50 mg/mL and 100 mg/mL). The 100 mg/mL proved to be very active by paralyzing and killing the earthworms in a shorter time. Here the compound benzanidazole (5-nitro-2-phenyl-1H-benzimidazole) (DP-3) showed best at 100 mg/mL concentration against earthworms (20 min for paralysis stage and 24 min for death stage).

Antioxidant activity of the (5-nitro-2-phenyl-1H-benzimidazole) (DP-3) is more capable and active than other synthesized benzimidazole derivatives.

| Sr. No | Parameter   | Concentration in mg/mL | DP-1 | DP-2 | DP-3 | Standard drug albendazole |
|--------|-------------|-------------------------|------|------|------|---------------------------|
| 1      | Time taken  | 25                      | 48 ± 0.09 | 45 ± 0.15 | 39 ± 0.18 | 30 ± 0.25 |
| 2      | for Paralysis | 50                      | 36 ± 0.05 | 35 ± 0.19 | 25 ± 0.20 | 19 ± 0.30 |
| 3      | (in minute) | 100                     | 28 ± 0.12 | 25 ± 0.25 | 20 ± 0.09 | 09 ± 0.20 |
| 1      | Time taken  | 25                      | 55 ± 0.10 | 52 ± 0.17 | 45 ± 0.12 | 35 ± 0.38 |
| 2      | for Death   | 50                      | 44 ± 0.20 | 42 ± 0.10 | 32 ± 0.17 | 25 ± 0.45 |
| 3      | (in minute) | 100                     | 35 ± 0.25 | 32 ± 0.09 | 24 ± 0.20 | 14 ± 0.95 |

Antioxidant activity of benzimidazole derivatives

The result of the radical scavenging was expressed in terms of half-inhibition concentration (IC_{50}) which denotes the concentration required to scavenge 50% of DPPH radicals. Scavenging activity was measured at concentration 10, 20, 30 µg/mL and IC_{50} (µM) values were ranging from 21.29, 21.19 and 21.61 respectively. All the synthesized compounds are showing good antioxidant activity when compared with the ascorbic acid and the results are expressed in Table 4.

| Compounds | 10 µg/mL | 20 µg/mL | 30 µg/mL | IC_{50} (µM) |
|-----------|----------|----------|----------|--------------|
| DP-1      | 34.70    | 35.20    | 37.65    | 21.29        |
| DP-2      | 42.40    | 44.50    | 46.05    | 21.19        |
| DP-3      | 47.40    | 49.90    | 57.40    | 21.61        |
| Ascorbic acid | 55.20 | 57.40    | 65.20    | 22.19        |

Evaluation of pharmacokinetic parameters

From the data presented in Table 5, it was remarkable to note that all the compounds (DP-1 to DP-3) showed significant values for the various parameters analyzed and showed good drug-likeness characteristics based on Lipinski's rule of five. The data obtained for all the analogues (DP-1 to DP-3) was within the range of accepted values. None of the molecules had violated Lipinski's rule of five. The value of polar surface area (PSA) for compounds (DP-1 to DP-3) indicated good oral bioavailability.

| Compound | Mol.wt/g/mol | HBA | HBD | Rot.B | Drug linkerase LogP | Logs | PSA | Bio-availability |
|----------|--------------|-----|-----|-------|--------------------|------|-----|-----------------|
| DP-1     | 273.03       | 4   | 2   | 2     | Yes                | 3.568| -4.777 | 94.73 | 0.55 |
| DP-2     | 273.04       | 5   | 3   | 2     | Yes                | 3.338| -4.828 | 103.96| 0.55 |
| DP-3     | 239.07       | 3   | 1   | 2     | Yes                | 3.179| -3.077 | 74.50 | 0.55 |

a = Molecular Weight ≤ 500 (g/mol); b = Hydrogen Bond Acceptor ≤ 10; c = Hydrogen Bond Donor ≤ 5; d = Rotatable Bonds ≤ 10; e = C log P ≤ 5, f = Water Solubility range -0.5 to -6.5 (mol/l); g = Polar Surface Area ≤ 140°A².

Evaluation of admet parameters

**a=BBB(Blood Brain Barrier):** High absorption CNS>2.0, Middle absorption CNS 2.0-0.1, Low absorption to CNS<0.1; **b=Caco2:** High absorption CNS>2.0, Middle absorption CNS 2.0-0.1, Low absorption to CNS<0.1;
permeability >70, Middle permeability 4-70, Low permeability <4; c = % HIA (Human Intestinal Absorbance): Well absorbed compounds 70-100%, Moderately absorbed compounds 20-70%, Poorly absorbed compounds 0-20%; d = %PPB (Plasma Protein Binding): Strongly bound >90%, Weakly bound <90%, e = MDCK: Higher permeability >500, Medium Permeability 25-500, lower permeability <25.

Calculations related to protein binding, blood-brain barrier (BBB), MDCK cell permeability, Caco-2 cell permeability and human oral absorption in the gastrointestinal tract showed that these values for the derivatives (DP-1-DP-3) fell within the standard ranges generally observed for drugs (Table 6). HERG is best known for its contribution to the electrical activity of the heart that coordinates the heart’s beating. In the present investigation, all the molecules (DP-1 to DP-3) had a moderate risk suggesting that these analogues (DP-1 to DP-3) were good drug candidates.

Table 6: Evaluation of ADMET parameters of molecules

| Compound | BBBa | CaCO2b | % HIAc | %PPBd | MDCKe(nm/sec) | HERG inhibition |
|----------|------|--------|--------|--------|---------------|----------------|
| DP-1     | 0.2883 | 4.2317 | 95.952 | 92.518 | 50.40         | Medium Risk    |
| DP-2     | 0.2087 | 5.8104 | 95.954 | 93.416 | 6.29107       | Medium Risk    |
| DP-3     | 1.1106 | 1.4341 | 91.219 | 97.984 | 18.2058       | Medium Risk    |

CONCLUSION

The benzimidazole compounds were showing better anthelmintic activity. Anthelmintic activity at concentrations 100 mg/mL proved to be very active. Here the compound benzimidazole (5-nitro-2-phenyl-1h-benzimidazole) (DP-3) showed the best result at 100 mg/mL concentration against earthworms. All the synthesized compounds were showing good antioxidant activity when compared with the ascorbic acid, ic50(μm) values were 21.29, 21.19 and 21.61 respectively. Pharmacokinetic parameters for all the compounds (DP-1 to dp-3) revealed significant values for the various parameters analyzed and showed good drug-likeness characteristics based on Lipinski’s rule of five. The value of polar surface area (PSA) for compounds (DP-1 to dp-3) indicated good oral bioavailability. ADMET parameters showed that all the molecules (DP-1 to DP-3) had a moderate risk suggesting that these analogues (DP-1 to dp-3) were good drug candidates.

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