EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 9
427-433
Year: 2018

# SYNTHESIS CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF SUSBSTITUTED BENZIMIDAZOLES

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**Article Received on 28/06/2018** **Article Revised on 19/07/2018** **Article Accepted on 10/08/2018**

## ABSTRACT
This research work deals with synthesis of benzimidazole and its halogen derivatives that can be potentially developed as Anthelmenthic drugs. Benzimidazole has been synthesized by cyclocondensation reaction between Orthophenylene diamine and formic acid. Considering the antibacterial, antifungal and anthelmenthic properties shown by various benzimidazole derivatives, halogen substituted benzimidazole were prepared by electrophilic substitution of halogen in presence of acetic acid.two bromine derivaties of Benzimidazole have been synthesized. The synthesized derivatives were characterized by various analytical methods and further the anthelmintic activity was performed on *Pherithima posthuma* and the derivatives shown good anthelmenthic activity. Further the above synthesized derivatives can be studied for identifying the antibacterial, antifungal activites.

**KEYWORDS:** Benzimidazole, Anthelmenthic, Antibacterial, Antifungal, and Halogen derivaties.

## INTRODUCTION
The term anthelmintic is restricted to drugs acting locally to expel parasites from gastro intestinal tract. There are several types of worms which penetrate other tissues, drugs which act on these parasitic infections are also known as anthelmintics. The worm parasites of man belong to two phyla: Nemathelminthes (round- worms) and platyhelminthes (flatworms). The roundworms include hookworm, whipworm, pinworm *Strongyloides stercoralis*, *Trichinella spiralis* and *Wuchereria bancrofti.*

Benzimidazoles of heterocyclic compounds are interesting class of organic compounds, being studied over the years and reported to possess a wide spectrum of biological activities such as antibacterial, anti tubercular, anti inflammatory, antiviral and antioxidant activity. Benzimidazoles also show some analytical applications. Although a number of anthelmintic, antibacterial and antifungal drugs are available in the market, the search for new molecules still continues because of the need for less costly drugs and drugs with minimal side effects. In view of the above mentioned facts and need to develop some novel biologically important compounds and utilizing environmental friendly methods, an attempt has been made. To synthesize Benzimidazole from orthophnylene diamne as depicted in Scheme I, to purify the new compounds by recrystallization, to synthesize Halogen substituted Benzimidazole, to purify the new compounds by recrystallization, to characterize the new compounds by physical and spectral data (TLC, IR), to screen Substiuted Benzimidazole for *in-vitro* Anthelmintic activities by standard protocol available in literature.

## EXPERIMENTAL SECTION
### Methodology
The present work is based on cyclizaion reaction between Orthophenlene damine and formic acid to give Benzimidazole. Furher substitution of halogens to Benzimidazole. Substituted Benzimidazoles were screened for *in vitro* anthelmintic activity.

**Chemicals and Reagents**

The chemicals and reagents (Table 1) used in the present project were of AR and LR grade, procured from S.D-Fine Chem. Ltd.

**Table no. 1: List of chemicals and reagents.**

| | |
|---|---|
| Ortho phenylene diamine | Formic acid |
| 3 aminobenzoic acid | Glacial acetic acid |
| Ethanol | methanol |
| Tetrahydrofuran | Silica gel G |
| Dimethylformamide | Charcoal |
| Thioglycolic acid | n-hexane |

**Analytical Techniques**

1. **Physical data:** Melting points of the synthesized compounds were recorded using melting point apparatus with open capillary tubes.
2. **Thin Layer Chromatography:** Purity of the compounds was determined by thin layer chromatography using silica gel G as stationary phase and various combinations of methanol: water: tetrahydrofuran in the ratio 2:2:1.

a. **Instrumentation:** The techniques employed for the characterization of the synthesized compounds were IR.

b. **IR spectra**: The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400-4000cm$^{-1}$ using KBr pellets and values are reported in cm$^{-1}$ and the spectra were interpreted.

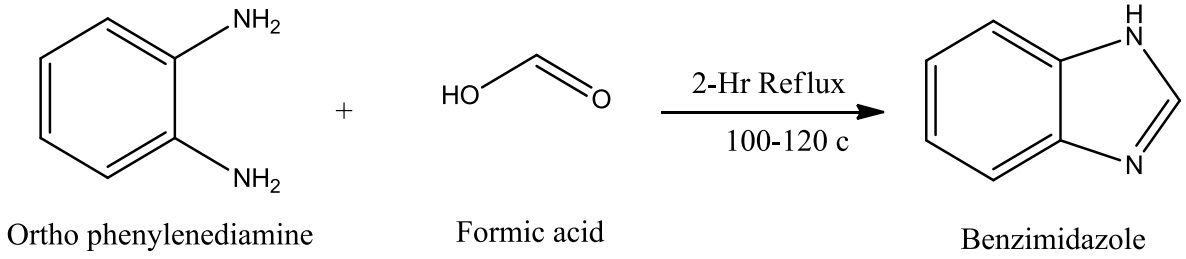
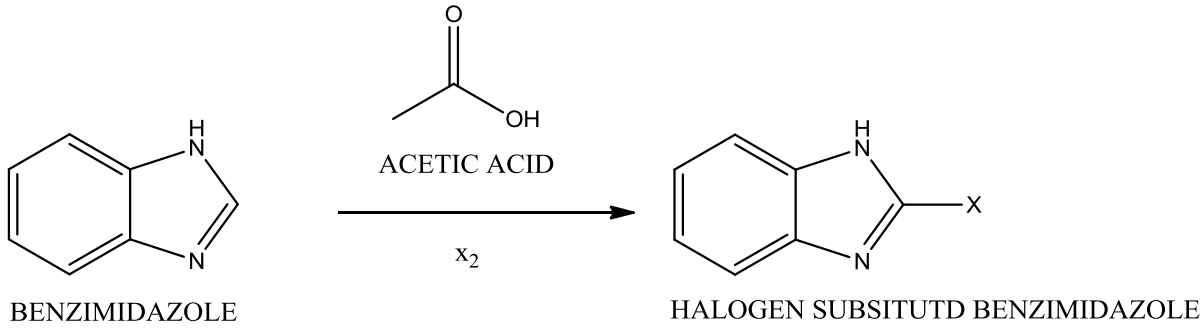



**Figure A: Synthesis of Benzimidazole from O-Phenylenediamine and Formic Acid.**

**SCHEME FOR HALOGEN SUBSTITUTED BENZIMIDAZOLES**




X= Cl , Br

**Figure B: Synthesis of Halogenated Substitued from Benzimidazole in the presence of Acetic acid.**

**GENERAL PROCEDURES**

**Procedure Derivative for Benzimidazole**

Place 2.7 gm of ortho phenyl diamine in 250 ml of round bottom flask and add 5 ml of formic acid and reflux. Condense the round bottom flask on water bath at 100 degree for 2 hours and then cool .The content and add 10% sodium hydroxide solution slowly with constant stirring till the mixture is alkaline. Filter the obtain product and wash with ice cold water and drain nill dissolve the crude product in 50 ml of boiling water and add a pinch of decolourised carbon and digest it for 15 minutes . Filter of the condenser at the pump to the pre-heated bun funnel. cool the filtrate to above 10 degree centrigate filter off the obtained Benzimidazole wash it with 25 ml of water and dry at 100 degree centrigate.

**Table No. 2: Physical data of Benzimidazole.**

| Code | Mol.F | Mol.wt (g.mol$^{-1}$) | M.P($^{o}$C) | %Yield |
|---|---|---|---|---|
| **SBEINZ-I** | $C_7H_6N_2$ | **188.14** | **163** | **86** |

**Figure C: Recrystallisation of compound.**

**PROCEDURE DERIVATIVE FOR HALOGENATION BENZIMIDAZOLE**

Glacial acetic acid into 250 ml conical flask (beaker) add dropwise by burette 3.5 ml Bromine. dissolve in glacial acetic acid with constant shaking .allow to stand at orange colour reaction . mix at room temperature for half an hour then pour the content in cool water stir well . add sufficient amount of sodium bisulphate to discharge orange colour filter the crude product wash with cold water and recrystalized with dilute ethanol to obtain with white crystalline compound. Two derivatives of bromine i.e SBEINZ-II and SBEINZ-III were synthesized the former is mono substituted while to the latter the method of halogenations is repeated to get di bromosubstituted product.

**Table No. 3: Physical data of Benzimidazole derivatives.**

| Code | Molecular Formula | Molecular weight ($g.mol^{-1}$) | Melting Point ($^oC$) | %Yield |
|---|---|---|---|---|
| SBEINZ-II | $C_7H_5BrN_2$ | 195.96 | 215 | 84 |
| SBEINZ-III | $C_7H_4Br_2N_2$ | 273.87 | 247 | 75 |

**Biological Activity**

**Anthelmintic Activity**

Helminthes infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases.

Anthelmentics are drugs that are used to treat infections due to parasitic worms. This includes both flat worms, e.g. flukes, tapeworms and roundworms, i.e. nematodes. They are of huge importance for human tropical medicine and for veterinary medicine. The World Health Organization estimates that a staggering 2 million people harbor parasitic worm infections. Despite the prevalence of parasitic worms, anthelmintic drug discovery is the poor relation of the pharmaceutical industry. The simple reason is that the nations which suffer most from these tropical diseases have little money to invest in drug discovery and therapy. It comes as no surprise therefore that the drugs available for human treatment were first developed as veterinary medicines.

**Procedure**

The synthesized Benzimidazole were screened for anthelmentic activity using *Pheretima Posthuma* (earthworms). 11 earthworms of nearly equal size were placed in control, standard and test compound's solutions at room temperature. Normal saline was used as control. The standard drug used was albendazole. The standard drug and test compound were dissolved in minimum quantity of ethanol and adjusted the volume upto 10ml with normal saline solution to get the concentrations 0.1%, 0.2% and 0.5%w/v. The compounds were evaluated by the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by earthworms to become motionless was noted as paralysis time. To ascertain the death of motionless earthworms, they were frequently applied with external stimuli, which stimulates and induces movement in earthworms, if alive. The mean lethal time and paralysis time of the earthworms for different test compounds and standard drug are tabulated.

**RESULTS AND DISCUSSION**

Benzimidazole and halogen substituted benzimidazoles show a wide spectrum of pharmacological applications. The synthesis of all compounds was carried out as depicted in Scheme – I. The resulting compounds were purified by recrystallization using ethanol and methanol. The synthesized compounds were characterized by both physical and spectral data like FT-IR. Newly synthesized compounds were evaluated for anthelmentic activities. The synthesized novel Benzimidazole were characterized from the spectral analysis, it is revealed that, IR shows presence of C=N peak at around $1600cm^{-1}$ for all the Benzimidazole.IR Qualitative methods were used to identify the presence of extra element in the above compounds.

**Physical characterization of synthesized compounds**
**Compound SBEINZ-I**

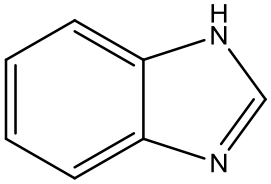
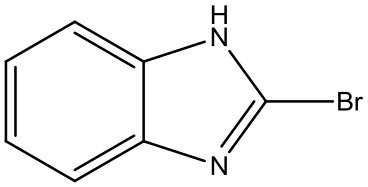


**Figure D: Structure of Benzimidazole.**

**Table No 4: Physical characterization data of benzimidazole.**

| | |
|---|---|
| **IUPAC Name** | 1H-benzo[d]imidazole |
| **Mol.F** | $C_7H_6N_2$ |
| **Mol.wt** | 188.14 g $mol^{-1}$ |
| **M.P** | 163 $^oC$ |
| **Solubility** | Methanol and ethanol |
| **TLC solvent** | Methanol: Water:Tetrahydrofuran-2:2:1 |
| **$R_f$ value** | 0.65 |
| **% Yield** | 86% |

**Compound SBEINZ-II**



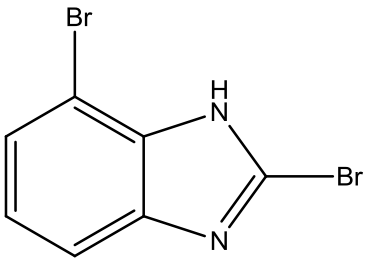
2-Bromo benzimidazole

**Figure E: Structure of 2-Bromo Benzimidazole.**

**Table No. 5: Physical characterization of Halogen substituted benzimidazole.**

| | |
|---|---|
| **IUPAC Name** | 2-bromo-1H-benzo[d]imidazole |
| **Mol.F** | $C_7H_5BrN_2$ |
| **Mol.wt** | 195.96 |
| **M.P** | 185-190 $^oC$ |
| **Solubility** | Tetrahydrofuran |
| **TLC solvent** | Methanol: Water: Tetrahydrofuran-2:2:1 |
| **$R_f$ value** | 0.56 |
| **% Yield** | 70% |

**Compound SBEINZ-III**



2,7 dibromo benzimidazole

**Figure F: Structure of 2,7 Dibromo Benzimidazole.**

**Table No. 6: Physical characterization of Halogen substituted benzimidazole.**

| | |
|---|---|
| **IUPAC Name** | 2,7-dibromo-1H-benzo[d]imidazole |
| **Mol.F** | $C_7H_4Br_2N_2$ |
| **Mol.wt** | 275 |
| **M.P** | 185-190 $^oC$ |
| **Solubility** | Tetrahydrofuran |
| **TLC solvent** | Methanol: Water: Tetrahydrofuran-2:2:1 |
| **$R_f$ value** | 0.56 |
| **% Yield** | 85% |

**Chemical characterization data of synthesized compounds**
**Compound SBEINZ-I: Benzimidazole**
**IR data**

| **Functional group** | **Frequency in $cm^{-1}$** |
|---|---|
| C=N stretching | 1603.88 |
| CH=N stretching | 1501.65 |
| Aromatic C-H stretching | 2570.26 |

**Compound SBEINZ-II: 2-Bromo Benzimidazole**
**IR data**

| **Functional group** | **Frequency in $cm^{-1}$** |
|---|---|
| C=N stretching | 1594.23 |
| CH=N stretching | 1499.72 |
| C-H stretching | 3119.03 |
| Aromatic C-H stretching | 2850.91 |

**Compound SBEINZ-III: 2,7 Dibromo Benzimidazole**
**IR data**

| **Functional group** | **Frequency in $cm^{-1}$** |
|---|---|
| C=N stretching | 1594.23 |
| CH=N stretching | 1499.72 |
| C-H stretching | 3119.03 |
| Aromatic C-H stretching | 2850.91 |


**Figure 1: FT-IR spectra of Benzimidazole (SBENZ-I).**

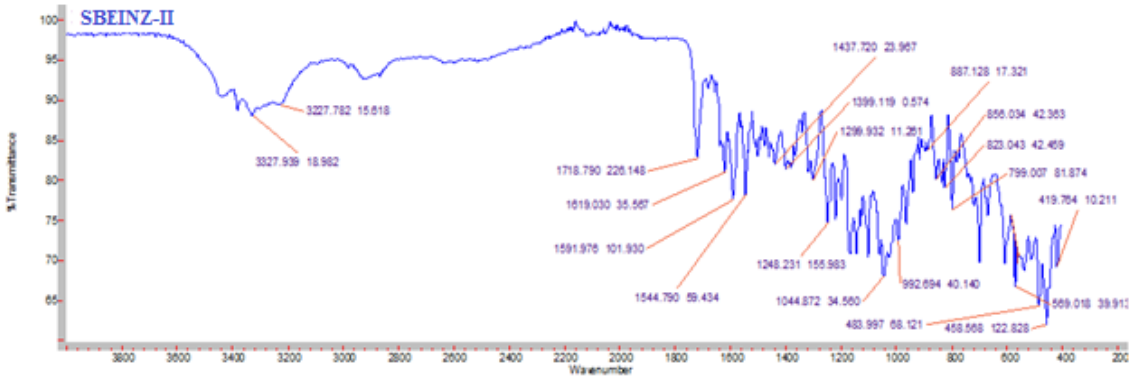



**Figure 2: FT-IR spectra of Benzimidazole (SBENZ-II).**


**Figure 3: FT-IR spectra of Benzimidazole (SBENZ-III).**

**Anthelmentic activity**

The synthesized compounds SBEINZ-I, SBEINZ-II and SBEINZ-III were evaluated for anthelmintic activity on Indian earthworms (*Pherethima posthuma)*. All compounds showed anthelmintic activity. Among the compounds tested, all the compounds showed significant paralytic time for earthworms compared to standard drug albendazole at (0.1%, 0.2% and 0.5%) concentration of compounds.

**Table No. 7: Anthelmentic activity of sunstituted Benzimidazole.**

| S. No. | Name | Time in minutes | | | | | |
|---|---|---|---|---|---|---|---|
| | | For paralysis | | | For death | | |
| | | % Drug concentraton | | | % Drug concentration | | |
| | | 0.1 | 0.2 | 0.5 | 0.1 | 0.2 | 0.5 |
| 1 | Control | - | - | - | - | - | |
| 2 | Albendazole | 14 | 12 | 9 | 42 | 32 | 25 |
| 3 | SBEINZ-I | 16 | 11 | 8 | 48 | 40 | 35 |
| 4 | SBEINZ-II | 18 | 16 | 13 | 45 | 37 | 28 |
| 5 | SBEINZ-III | 12 | 9 | 7 | 39 | 30 | 22 |

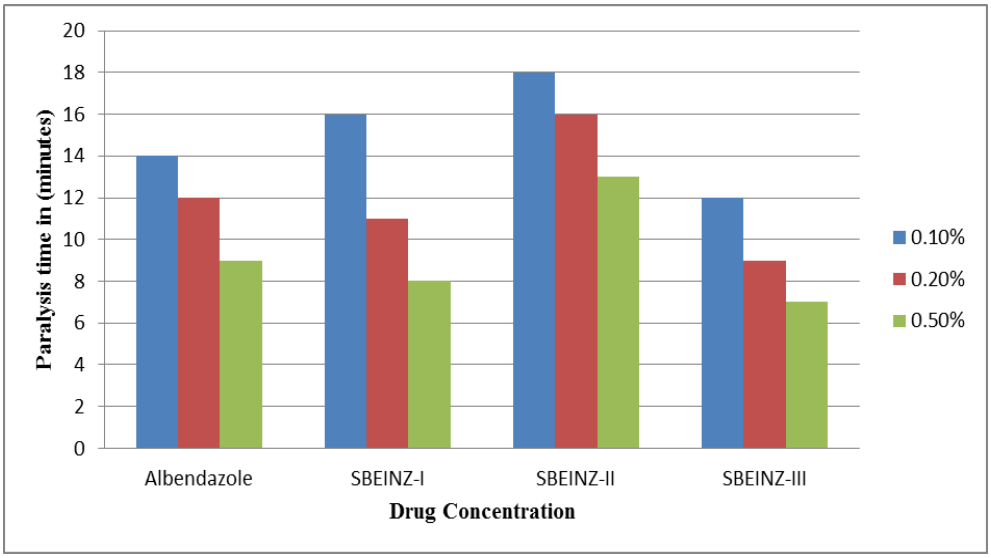



**Figure 4: Anthelmentic activity.**

Graphical representation of anthelmintic activity of compounds (**SBEINZ-I**, **SBEINZ-II SBEINZ-III)**
Where
**SBEINZ-I:-** Benzimidazole
**SBEINZ-II:-** 2-Bromo benzimidazole
**SBEINZ-III:-** 2,7- Dibromo benzimidaole

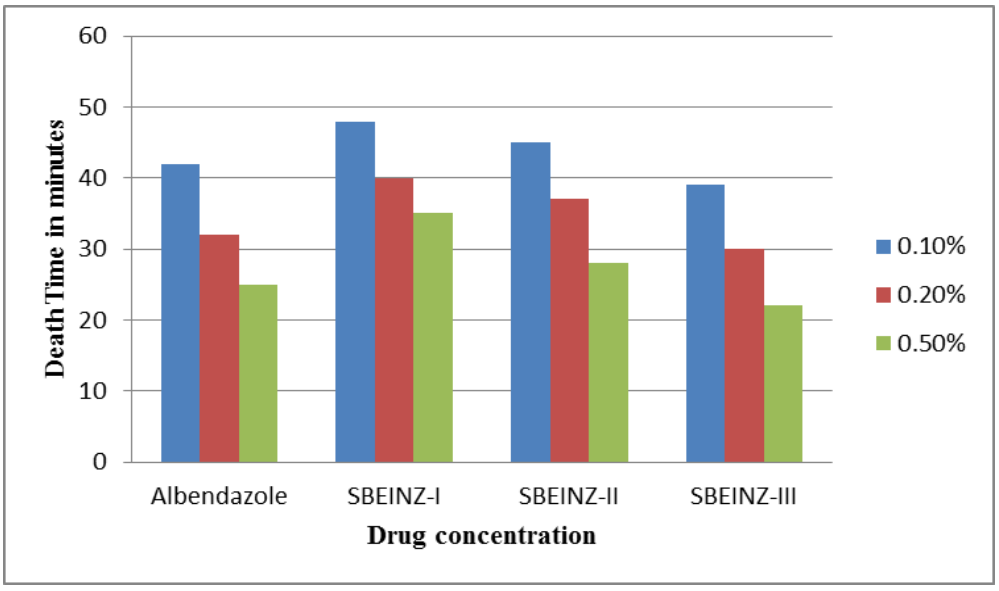



**Figure 5: Anthelmentic activity.**

Graphical representation of anthelmintic activity of compounds (**SBEINZ-I**, **SBEINZ-II SBEINZ-III)**
Where
**SBEINZ-I: -** Benzimidazole
**SBEINZ-II: -** 2-Bromo benzimidazole
**SBEINZ-III: -** 2,7- Dibromo benzimidaole

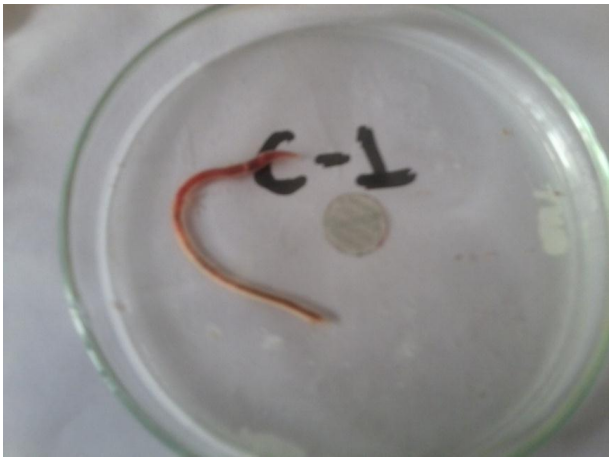



**Figure 6: Photograph of various novel Benzimidazole - Anthelmentic activity.**

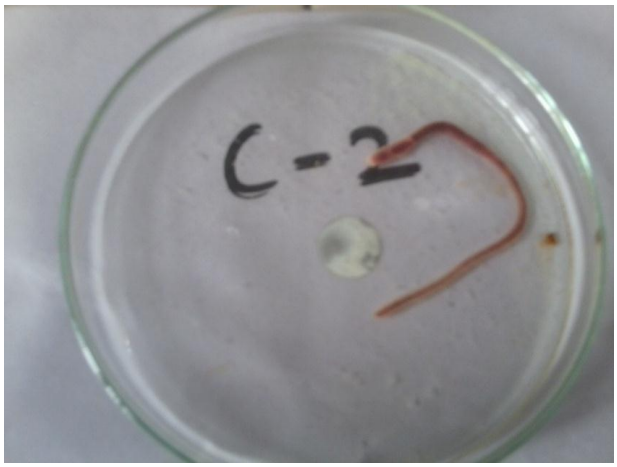



**Figure 7: Photograph of various novel Benzimidazole- Anthelmentic activity.**

The synthesized compound was characterized where the presence of functional groups is confirmed by FT-IR and presence extra elements have been identified by using qualitative analysis. The obtained derivatives were subjected for anthelmenthic activity and among the synthsised derivatives SBEINZ-III shown good anthelminthic activity.

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