Synthesis, characterization and SAR of novel Bezimidazole derivatives as Nematicidal agents

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Abstract: Six novel analogues were prepared by reacting benzimidazole molecules (BM and CMB) propiophenone and benzoyl chlorides respectively. The structures of newly synthesized compounds were determined with the help of spectroscopic techniques. The compounds were subjected to *in-vitro* screening for their activity against nematodes. It was observed that the benzimidazole (BM) derivatives possessed more nematicidal activity as compared to that of cyanomethyl-benzimidazole (CMB) for *Meloidogyne incognita*. Among them, the propiophenone substituted benzimidazole derivative B3 was found to be the most active compound and can be further studied as lead molecule for development of anthelmintic drugs.

Keywords: Benzimidazole, Nematocidal activity, GERD, *Meloidogyne incognita*.

INTRODUCTION

Among the nitrogen containing heterocyclic compounds, benzimidazole core is identified in a variety of pharmaceutical agents spanning a range of target classes and therapeutic indications. Thousands of benzimidazole derivatives have been synthesized uptill now with speckled pharmacological and biological potentials (El-Masry *et al.*, 2000; Sondhi *et al.*, 2002; Rida *et al.*, 2006). Many important drugs used as proton pump inhibitors in gastro-esophageal reflux disease (GERD) like Pneracid, Aciplex, Kapidex, Nexium and Protonix contained substituted benzimidazole as part of their pharmacophore (Li, 2013). Several of the past researches have highlighted the anthelmintic potential of benzimidazole moiety (Márquez-Navarro *et al.*, 2009; Hernández-Luis *et al.*, 2010).Two important benzimidazole derivatives, mebendazole and omeprazole are successfully prevailing in the market as potent anthelmintic drugs for the treatment of veterinary and human infections of parasitic worms.

The parasitic worms, *Meloidogyne incognita, Helicotylenchus* and *Tylenchorhynchus* are considered amongst the most devastating and widespread nematodal pests of agricultural crops. These nematodes have exceedingly wide host range and infect more than 5500 plant species including almost all cereals, vegetables, fibers, fruits and beverage crops causing substantial loss (Nickle, 1991; Stirling *et al.*, 1992). The genus *Meloidogyne* comprises more than 80 species and has a crucial role in damaging most of the crop production economically. Plant-parasitic nematodes, especially root-knot species, *Meloidogyne incognita*, infected a wide range of cultivated plants, and were responsible for crops destruction annually (Sasser and Fackman, 1987).

Due to the structural modification of these organisms, resistant strains of nematode emerge making anthelmintic drugs ineffective for them (Kearn *et al.*, 2014). Therefore, the synthesis of new drugs of this class is in perpetual demand. Keeping this in view, herein we attempted to synthesize benzimidazole derivatives and evaluated their potential to be used as anthelmintic agents.

MATERIALS AND METHODS

Acetone, ethanol and all the reagents were purchased from Aldrich chemical company. The solvents were double distilled prior to use. Reaction was observed via thin layer chromatography (TLC) plates of pre-coated silica gel, GF-254. Spots were visualized under ultraviolet light at 254nm using HP-UV/Visible lamp. Melting points of the products were determined on Gallen Kamp melting point apparatus and were uncorrected.

Electronic absorption spectra were recorded in methanol on Shimadzu UV/Visible 1601 spectrophotometer. Infrared (IR) spectra were recorded in KBr (pellet form) on a Nicolet Avatar 330 Fourier Transform spectrophotometer. Mass spectrometers MAT 312 MAT 113D MASPEC system was used for Electron Impact (EI) spectrta. Proton Nuclear Magnetic Resonance (^{1}HNMR) spectroscopy was performed on a Bruker SF 300 at 400MHz.

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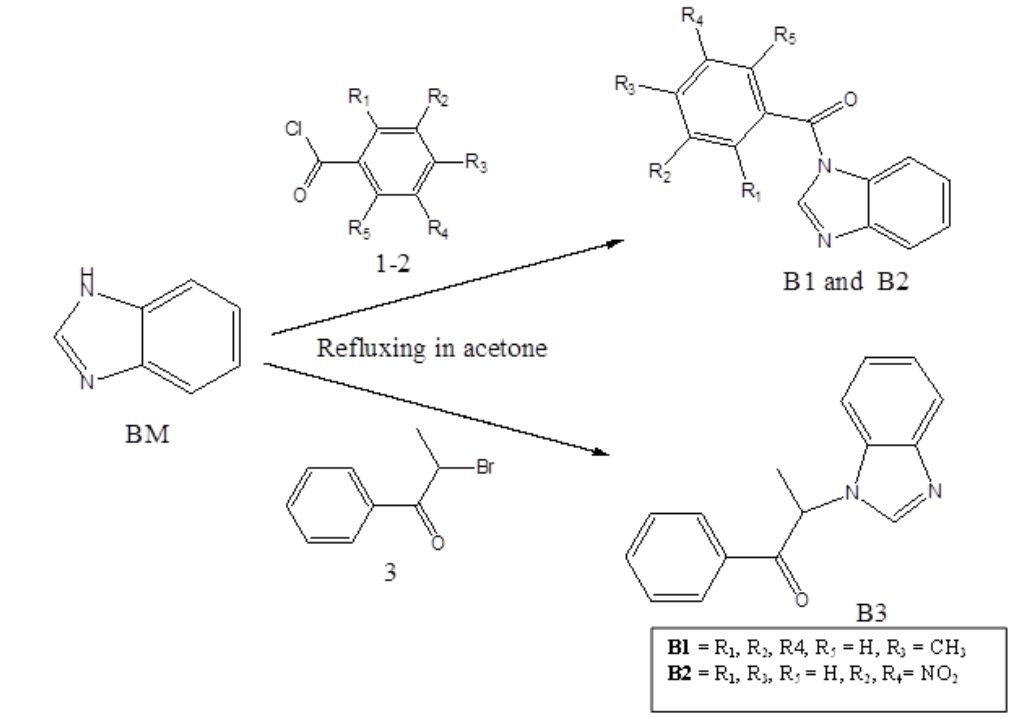
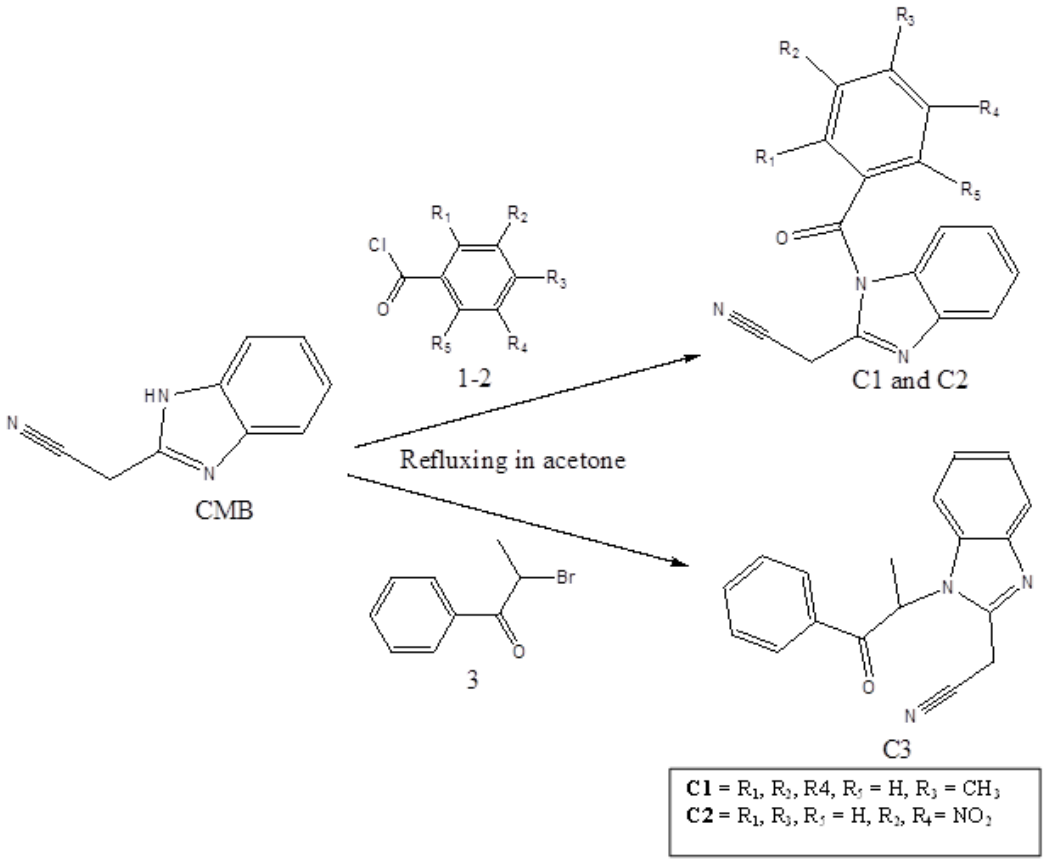


Scheme 1: Synthetic route for derivatives B1-B3



Scheme 2: Synthetic route for derivatives C1-C3

Table 1: Physical properties of derivatives B1-B3 and C1-C3

Compound	State/Color	Molecular formula	Solubility	Yield	Molecular weight (gm)	Melting point (°C)
B1	White ppt.	$C_{15}H_{13}N_2O$	Ethanol, Methanol, DMSO	50%	237	280
B2	White ppt.	$C_{14}H_{10}N_4O_5$	Ethanol, Methanol, DMSO	20%	314	decompose
B3	White ppt.	$C_{16}H_{14}N_2O$	Ethanol, methanol, DMSO	60%	250	300
C1	Dark brown ppt.	$C_{17}H_{13}N_3O$	Ethanol, Methanol, DMSO	40%	275.3	200
C2	Dark brown ppt.	$C_{16}H_9N_5O_5$	Ethanol, Methanol, DMSO	73%	352	decompose
C3	Dark brown ppt.	$C_{18}H_{15}N_3O$	Ethanol, Methanol, DMSO	50%	289	250

Table 2: Nematocidal activity of derivatives B1-B3

Compound	Dose (µg/ml)	% mortality observed							
		30 min	60 min		3hr	4hr	24hr	48hr	Total Dead (%)
BM	1	0	0	2	3	2	1	1	90.00
	0.5	0	0	2	3	1	2	2	90.00
	0.25	0	0	1	3	1	1	1	70.00
	0.13	0	0	1	3	0	2	2	70.00
B1	1	0	0	3	1	1	1	1	70.00
	0.5	0	0	2	2	1	1	1	70.00
	0.25	0	0	2	1	0	2	2	70.00
	0.13	0	0	2	1	0	1	1	50.00
B2	1	0	0	2	3	1	2	2	100.00
	0.5	0	0	3	2	1	1	1	80.00
	0.25	0	0	1	3	1	2	2	90.00
	0.13	0	0	2	2	1	1	1	70.00
B3	1	0	0	3	4	1	1	1	100.00
	0.5	0	0	2	5	2	0	0	90.00
	0.25	0	0	1	3	2	2	2	100.00
	0.13	0	0	1	2	3	2	2	100.00
Furadan	1	0	0	0	0	0	100	-	100.00
	0.5	0	0	0	0	0	100	-	100.00
	0.25	0	0	0	0	0	80	100	100.00
	0.13	0	0	0	0	0	40	100	100.00
Control 5% DMSO	1	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-
	0.25	-	-	-	-	-	-	-	-
	0.13	-	-	-	-	-	-	-	-

(-) indicates no activity

Table 3: Nematocidal activity of derivatives C1-CB3

Compound	Dose (µg/ml)	% mortality observed							
		30 min	60 min	2hr	3hr	4hr	24hr	48hr	Total Dead (%)
CMB	1	0	0	3	2	2	1	1	90.00
	0.5	0	0	3	3	0	0	0	60.00
	0.25	0	0	1	3	1	1	1	70.00
	0.13	0	0	1	2	1	2	2	80.00
C1	1	0	0	2	2	2	2	2	100.00
	0.5	0	0	1	3	1	1	1	70.00
	0.25	0	0	1	2	2	1	1	70.00
	0.13	0	0	1	2	1	1	1	60.00
C2	1	0	0	3	1	0	2	2	80.00
	0.5	0	0	2	3	1	0	0	60.00
	0.25	0	0	2	2	1	0	0	50.00
	0.13	0	0	2	2	1	0	0	50.00
C3	1	0	0	2	2	0	1	1	60.00
	0.5	0	0	3	0	0	1	1	50.00
	0.25	0	0	1	1	0	2	2	60.00
	0.13	0	0	1	1	1	1	1	50.00
Furandan	1	0	0	0	0	0	100	-	100.00
	0.5	0	0	0	0	0	100	-	100.00
	0.25	0	0	0	0	0	80	100	100.00
	0.13	0	0	0	0	0	40	100	100.00
Control 5% DMSO	1	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-
	0.25	-	-	-	-	-	-	-	-
	0.13	-	-	-	-	-	-	-	-

(-) indicates no activity

Chemical shifts were given in δ (ppm). Selected data were reported as multiplicities, and characterized by "s" (singlet), "d" (doublet), "t"(triplet), "m" (multiplet), "q" (quartet), and coupling constant (*J*-values) were reported in hertz (Hz).

General method for the synthesis of derivatives

Equimolar quantities (0.01moles) of BM and substituted benzoyl chloride/propiophenone (1-3) in acetone were mixed with initial stirring for 4-6 hrs at room temperature and then refluxed on a water bath for about 10-15 hrs (Scheme 1). The obtained precipitates were filtered, washed with acetone, recrystallized with ethanol and then dried in a vacuum desicator over silica beads. Same procedure was adopted to obtain the products of CMB (Scheme 2).

Determination of nematicidal activity of the synthesized derivatives

The *in-vitro* nematicidal activity of parent compounds and their derivatives was performed by the mixture of root knot nematode *Meloidoghyneincognita, Helicothlenchus,* and *Tylenchorrhynchus*. The root knot mixture of all these nematodes was inoculated on the respective host under maintained net house conditions which was obtained from NNRC (National Nematological Research Center), University of Karachi, Pakistan. The concentrations of 1, 0.5, 0.25, 0.125mg/ml were subjected with 100 freshly hatched second stage juveniles, for the evaluation of nematicidal activity. The concentrations were prepared in distilled water while the stock solution was prepared in 5% DMSO, so as the control 5% aq. DMSO and

conventional nematicide furandan was taken for standard comparison. The percent of mortality was evaluated timely up to 48 hours under binocular microscope. Mortality of nematodes was confirmed by their inactive response by touching them with the needle. The treatment was replicated thrice for the determination of average percentage of mortality.

RESULTS

Physical data of all the prepared compounds was reported in table 1. The compounds were characterized by UV/Visible, IR, EI-Mass and ^{1}H-NMR spectroscopy. It can be seen that all the synthesized compounds displayed activity against tested nematodes.

Spectral studies

B1: (1H-benzo[d]imidazol-1-yl)(p-tolyl)methanone

UV λ_{max}(nm)(Methonal): 237.5, IR λ_{max}(KBr) cm^{-1}: 2810(C-H aliphatic), 2655(C-H aromatic), 2193 (C≡N), 1625 (C=O), 1610 (C=C), 1668 (C=N). ^{1}HNMR (d6-DMSO, 300MHz) δ: 2.395 (s, 3H at H-1), 7.257(s, 2H, H-4, H-5), 7.278(d, 2H, H-8, H-9), 7.882 (s, 2H, H-3, H-6), 7.902 (s, 2H, H-7, H-10). EIMS m/z: 279 (M^+, $C_{17}H_{13}O_4$, -HBr).

B2: (1H-benzo[d]imidazol-1-yl)(3,5-dinitrophenyl) methanone

UV λ_{max}(nm)(Methonal): 253.0, IR (KBr) cm^{-1}: 3836-3742 (-NH aromatic), 3048 (C-H aromatic), 2970 (C-H aliphatic), 1642 (C=O), 1611 (C=C). ^{1}HNMR (d6-DMSO, 300MHz) δ: 7.205 (s, 2H, H-3, H-4), 7.785 (s, 2H, H-2, H-5), 8.031 (s, 1H, H-1), 8.888-8.909 (m, 4H, H-6, H-8), 12.523(s, 1H, H-7). EIMS m/z: 312 ($C_{14}H_8N_4O_5$)

B3: 2-(1H-benzo[d]imidazol-1-yl)-1-phenylpropan-1-one

UV λ_{max}(nm)(Methonal): 254.0. IR (KBr) cm^{-1}: 3725(-NH aromatic), 3421(O-H), 2193 (C≡N), 1683 (C=O), 1623(C=C). ^{1}HNMR (d6- DMSO, 300MHz) δ: 1.822 (d, 3H at CH3), 7.487(s, 1H, H-8, H-10), 7.506(d, 1H, H7-H-11), 7.79(t,1H, H-9), 8.02 (s, 2H,H-2), 5.554 (q, 2H, H-12),7.635(s,2H, H-3, H-6), 7.598(s,2H,H-4, H-5). EIMS m/z: 265(M^+, $C_{17}H_{17}N_2O$)

C1: 2-(1-(4- methyl phenyl) carboxyl)-H-1,3-Benzodiazol-2-yl)acetonitrile

UV λ_{max}(nm) (Methonal): 206, IR (KBr) cm^{-1}: 3725(-NH aromatic), 2200(C≡N), 1450, 1580, 1600 (skeletal vibration of benzene ring), 1623(-C=O). ^{1}HNMR (d6-DMSO, 300MHz) δ: 2.320 (s, 3H at CH_3), 2.494(t, 2H, H-2, J=1.6, J=2.4),7.258-7.275(dd, 2H, H-8, H-9), 7.385(dd, 2H, H-4, H-5), 7.53(s, 2H, H-3, H-6), 7.687-7.722(m, 2H, H-7-10). EIMS m/z: 275 (M^+, $C_{17}H_{13}N_3O$)

C2: 2-(1-(3,5-dinitrobenzoyl)-1H-benzo[d]imidazol-2-yl) acetonitrile

UV λ_{max}(nm)(Methonal): 206, IR (KBr) cm^{-1}: 3855(-NH aromatic), 3093(C-H aromatic), 2193(C≡N), 1623(-C=O), 1499.5(-C=C aromatic), 1583.1(Cyclic -C-O-C-), 1452(C=C), 1167 (C-O). ^{1}HNMR (d6- DMSO, 300MHz) δ: 3.971(s, 2H, H-1), 7.296(s, 1H, H-3, H-4), 7.415-7.457 (m, 1H, H-7), 7.582(dd, 2H, H-2, H-5), 8.879-8.929(m, 2H, H-6, H-8). EIMS m/z: (M^+, $C_{16}H_9N_5O_5$, -HCl)

C3: 2-(1-(1-oxo-1-phenylpropan-2-yl)-1H-benzo[d]imidazol-2-yl)acetonitrile

UV λ_{max}(nm)(Methonal): 237. IR λ_{max}(KBr) cm^{-1}: 2810(C-H aliphatic), 2655(C-H aromatic), 2193(C≡N), 1625(-C=O), 1610(C=C), 1668(C=N). ^{1}HNMR (d6- DMSO, 300MHz) δ: 2.395 (s, 3H at H-1), 7.257(s, 2H, H-4, H-5), 7.278(d, 2H, H-8, H-9), 7.882(s, 2H, H-3, H-6), 7.902(s, 2H, H-7, H-10). EIMS m/z: 279 (M^+, $C_{17}H_{13}O_4$, - HBr)

Nematocidal activity

The results of nematicidal activity were presented in table 2 and table 3 which revealed that the parent molecule, benzimidazole (BI) exhibited good activity at the doses of 1 and 0.5µg/ml and moderate activity at 0.25 and 0.125µg/ml dose. At low dose, the % mortality was more.

Derivatives B1 showed moderate activity at all doses but low than its parent. At the dose of 1µg/ml, the % mortality of B2 was more than that of the parent while at other doses the effects were comparable. Moreover, it was found equipotent to the standard drug, furadan at the same dose. In contrast to B1 and B2, compound B3 displayed maximum nematicidal action in the series.

Compound C1 and C2 showed mild to moderate activity against tested nematodes while C3 exhibited only a slight action.

DISCUSSION

Synthesis and characterization

The synthetic route as given in the Scheme-1 and Scheme-2 showed the chemistry part of the present work which was adopted from the previous work reported in literature (Akhtar *et al.*, 2006; Kamil *et al.*, 2013; Saify *et al.*, 2014). The required products were prepared simply by condensing BM and CMB with substituted benzoyl halides and chloropropiophenone undergoing nucleophilic substitution reaction at refluxing temperature (50-55^{o}C) in acetone. The adopted synthetic rout was found efficient and afforded the derivatives of benzimidazole in moderate to excellent yield (40-80%).

The synthesized compounds B1-B3 and C1-C3 were characterized by UV/visible, ^{1}HNMR, FT-IR, EIMS spectra and elemental analysis. The IR spectra (in KBr) of these compounds revealed the presence of amide, amine, cyano and carbonyl groups due to the appearance of absorption bands around 3742-3836, 2220-2260cm^{-1} and 1580-1638 cm^{-1} respectively. The higher frequency 1630 cm^{-1} showed the carbonyl was of amide group.

Characteristic skeleton vibrations of benzene ring were found around 1450, 1500, 1580 and 1600cm^{-1}. The IR spectrum of all the synthesized derivatives also showed strong absorption band at 1470-1575cm^{-1}. The medium to strong C-H band and C-N band appeared at 3000-3050 cm-1 and 1470-1350cm^{-1} respectively.

Typical stretching and bending vibrations for CH_3 group occurred at 2960-2870cm^{-1} and 1356-1382 cm^{-1} respectively. On the other hand, methylene (CH_2) group created band at 1450-1500cm^{-1}. Weak to strong vibrations at 1680-1640cm-1 specifically stood for the imine (C=N) group. Strong band at 1545cm^{-1} was due to the nitro group.

The ^{1}HNMR in DMSO at 300 MHz exhibited various signals at different ppm due to the presence of different types of protons in the molecules such as a singlet around δ 2.320-2.395 appeared for methyl protons of aryl ring attached to the pyrazole unit. Aromatic protons produced multiplets in the range of 7.415-8.879ppm (in C1-C3) and doublets around 7.275-7.7698 ppm. A distinct singlet at 7.902 was attributed for proton of imidazole unit (B1-B3).

Nematicidal activity and SAR

When we discuss the SAR of synthetic analogs it was found that the attachment of 4-methyl benzoyl chloride in B1 was responsible to decrease the lethal potential effect of parent compound whereas 3,5-dinitro benzoyl chloride derivative of benzimidazole (B2) possessed good nematicidal activity. On the other hand, addition of aliphatic carbon chain at nitrogen atom in B3 (substituted with propiophenone) might be responsible for enhanced activity.

Compound C1 with *p*-CH_3 substitution at benzoyl ring was found to augment the effects. C2 showing mild activity is substituted with *m*-NO_2 groups at benzoyl ring. C3 on the other hand exhibiting only a slight action contains propiophenone moiety. In comparison to B3, this observation tells us that addition of carbon chain in this case resulted in decreased activity.

Comparing the lethal effects produced by all the compounds, it was observed that the derivatives of parent BI expressed more good lethal activity against the nematodes and the most active compound, B3 might be offered to compete the existing standard drug furadan.

CONCLUSION

Substituted and un-substituted benzimidazoles behave differently in expressing the nematicidal activity as we have seen in case of B3 and C3. Both contains propiophenone moiety and Compound B3 showed 100% mortality.

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REFERENCES

Akhtar S, Saify Z, Arif M, Mushtaq N, Dar A and Ahmed A (2006). Synthesis and evaluation of novel phenacyl based carboxamide derivatives of piperidine as antihypertensive agents. *Pak. J. Physiol.*, **2**(1): 42-48.

El-Masry AH, Fahmy H and Ali Abdelwahed S (2000). Synthesis and antimicrobial activity of some new benzimidazole derivatives. *Molecules*, **5**(12): 1429-1438.

Hernández-Luis F, Hernández-Campos A, Castillo R, Navarrete-Vázquez G and Soria-Arteche O *et al.* (2010). Synthesis and biological activity of 2-(trifluoromethyl)-1H-benzimidazole derivatives against some protozoa and Trichinella spiralis. *Eur. J. Med. Chem.*, **45**(7): 3135-3141.

Kamil A, Akhtar S, Karim A, Ahmed A and Wajdi M *et al.* (2013). Benzimidazole derivatives with potential cytotoxic activity-synthesis and their structure-activity relationship. *FUUAST J. Biol.*, **3**(2): 87.

Kearn J, Ludlow E, Dillon J, O'Connor V and Holden-Dye L (2014). Fluensulfone is a nematicide with a mode of action distinct from anticholinesterases and macrocyclic lactones. *Pestic. Biochem. Physiol.*, **109**: 44-57.

Li JJ (2013). Imidazole and Benzimidazole In: Heterocyclic chemistry in drug discovery, John Wiley & Sons, New Jersey. pp.254-356.

Márquez-Navarro A, Nogueda-Torres B, Hernández-Campos A, Soria-Arteche O and Castillo R *et al.* (2009). Anthelmintic activity of benzimidazole derivatives against Toxocara canis second-stage larvae and Hymenolepis nana adults. *Acta. Tropica.*, **109**(3): 232-235.

Nickle WR (1991). Stunt nematodes: Tylenchorhynchus, Merlinius, and related genera. *In*: Manual of Agricultural Nematology, Marcel Dekker, Inc., New York. pp.359-360.

Rida SM, El-Hawash SA, Fahmy HT, Hazzaa AA and El-Meligy MM (2006). Synthesis of novel benzofuran and related benzimidazole derivatives for evaluation of *in-vitro* anti-HIV-1, anticancer and antimicrobial activities. *Arch. Pharm. Res.*, **29**(10): 826-833.

Saify ZS, Kamil A, Akhtar S, Taha M and Khan A *et al.* (2014). 2-(2′-Pyridyl) benzimidazole derivatives and their urease inhibitory activity. *Med. Chem. Res.*, **23**(10): 4447-4454.

Sasser JN and Fackman DWL FL (1987). A World perspective on Nematology: The role of the society. *In*: Vistas on nematology: A commemoration of the 25th

anniversary of the society of nematologists., Society of nematologists. pp.7-14.

Sondhi SM, Rajvanshi S, Johar M, Bharti N, Azam A and Singh AK (2002). Anti-inflammatory, analgesic and antiamoebic activity evaluation of pyrimido [1, 6-a] benzimidazole derivatives synthesized by the reaction of ketoisothiocyanates with mono and diamines. *Eur. J. Med. Chem.*, **37**(10): 835-843.

Stirling G, Stanton J and Marshall J (1992). The importance of plant-parasitic nematodes to Australian and New Zealand agriculture. *Australas. Plant Path.*, **21**(3): 104-115.