Antimalarial and insecticidal activities of newly synthesized derivatives of Benzimidazole

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Abstract: The bioactive benzimidazole and corresponding substituted phenacyl halides has been synthesized (11) new derivatives out of three compounds 8, 10 and 11 were found to inhibit the *Plasmodium falciparum* moderately after 72 hours of incubation hence acting as antimalarial agents. While these derivatives were exhibited negligible insecticidal activity too when analyzed by impregnated filter paper method.

Keywords: Benzimidazole, *Plasmodium falciparum*, Antimalarial agents and insecticidal activity.

INTRODUCTION

Benzimidazole class of compounds has specialized biological and clinical applications, this ring system is found in many bioactive heterocyclic constituents. Furthermore, benzimidazole derivatives are structural isosters of naturally occurring nucleotides, thus they can interact with biological macromolecules such as proteins, enzymes and receptors (Barker HA *et al*., 1960).

Benzimidazole and their derivatives are active for many pharmacological activities especially its ring system was proved to be responsible for plentiful activities such as antimicrobial, antiviral, antidiabetic and anticancer activity (Boiani, M., *et al*., 2005, Garuti, L., *et al*., 1999). This class of molecules is found as variety of antioxidant and antiallergic agents (Kus, C., *et al*., 2004, Nakano, H., *et al*., 1999). According to recent years report benzimidazole derivatives to the fore much interest regarding anticancer activity and in vitro anti-HIV potential. (Akbay, A., *et al*., 2003, Casse, C., *et al*., 1999).

In present research the new benzimidazole derivatives were synthesized and evaluated their insecticidal and antimalarial activities.

MATERIALS AND METHOD

Reagents were purchased from Aldrich Chemical Company. All the solvents were of analytical grade from E. Merck and were purified by distillation proceeding to use. Thin layer chromatography was monitored using pre-coated silica gel, GF-254. Spots were visualized under ultraviolet light at (254nm and 366nm) using HP-UV/Visible lamp (Dessaga Heidelberg). Melting points were determined on Gallen Kamp melting point apparatus.

General method of synthesis

2-(2´-pyridyl) benzimidazole (Ia) and corresponding substituted phenacyl halides in equimolar quantities (0.01mole) were dissolved in 15-20mL acetone separately in conical flask and mixed all together in a round bottom flask. The reaction mixture was stirred on magnetic plate for 4 hours and then refluxed on water bath for about 5 to 6 hours. Precipitates developed either on mixing the reactants at once or after some hours on refluxing. Completion of reaction was monitored by TLC with solvent system $CHCl_3$: MeOH (in varying proportions) up till end of the reaction.

TLC plates were visualized under ultraviolet light at two different wave lengths 254 nm and 366 nm for fluorescence quenching and fluorescent spot respectively on HP-UV/Visible Dessaga (Heidelberg). Iodine vapors were also employed for the detection and confirmation of spots.

The resulting precipitates of synthesized compounds were filtered and washed with warm acetone in order to remove the unreacted starting material. The precipitates of each constituent were purified by followed re-crystallization of material repeatedly to ensure purity of compounds as well as to improve color and shape of the crystals. The pure compounds of each derivative were dried in vacuum desiccators over anhydrous calcium sulphate. The procedure adopted for the preparation of derivatives of 2-(2′-pyridyl)-benzimidazole (Ia) with phenacyl halides was shown in the following fig.

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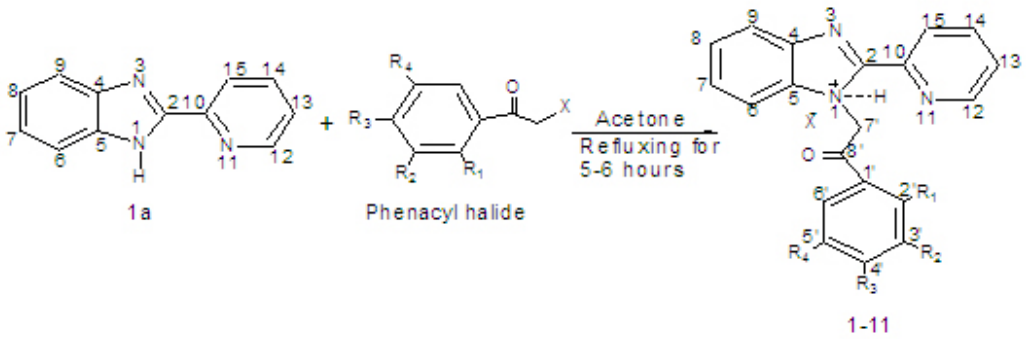


Fig. 1: Synthetic route for compounds 1 – 11

*Comp #	R_1	R_2	R3	R_4	X
1	H	NO_2	H	H	Br
2	H	OH	OH	H	Br
3	H	H	Cl	H	Br
4	NO_2	H	H	H	Br
5	H	H	NO_2	H	Br
6	H	H	F	H	Br
7	F	H	F	H	Br
8	H	H	H	H	Br
9	H	H	C_6H_5	H	Br
10	H	H	OCH_3	H	Br
11	OCH_3	H	H	OCH_3	Br

* Compound Number

Table 1: Insecticidal activity of 2-(2´-pyridyl) benzimidazole and its derivatives by Impregnated filter paper method

Compound #	Name of Organisms		
	Tribolium castaneum	*Ryzopertha dorminica*	*Callosbruchus ganarium*
Ia	0	0	0
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0
8	0	0	0
9	0	0	0
10	0	0	0
11	0	0	0

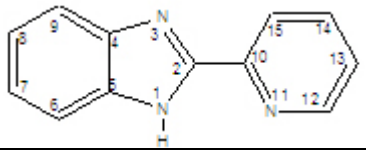
Concentration of test sample = 1.019 µg/cm^2 Concentration of std. drug = 239.5 µg/cm^2

Table 2: Antimalarial activities of 2-(2´-pyridyl) benzimidazole (Ia) and its derivatives against *plasmodium falciperum*.

S. No	IUPAC Name's	IC_{50} (µg/L)
Parent Ia	2-(2´-pyridyl) benzimidazole (Ia)	>25
2	1-[2-(3',4'-dihydroxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium chloride (2)	>25
3	1-[2-(4'-chlorophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (3)	>25
8	1-(2-oxo-2-phenylethyl)-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (8)	5.96±0.09
10	1-[2-(4'-methoxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (10)	11.96±0.68
11	1-[2-(2',5'-dimethoxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide (11)	9.40±0.760
Standard	Chloroquine diphosphate	0.025±0.01

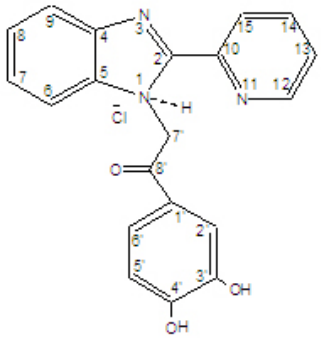
The compounds that showed activity

Parent molecule (1a)



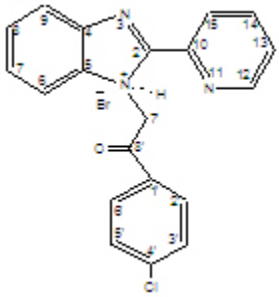
	IUPAC Name: 2-(2´-Pyridyl) Benzimidazole or 1*H*-benzimidazole-2-(2´-pyridinyl)
	Structure:-
	Physical State: - Light Yellow Powder
	Melting Point: - 218 – 220^{o}C
	Molecular Formula: - $C_{12}H_9N_3$
	Solubility: - Acetone, DMSO, Methanol
	CHN Analysis: Found C=73.83%, H=4.65%, N=21.52% Calculated C=73.76 %, H=4.61%, N=21.55%

Compound 2



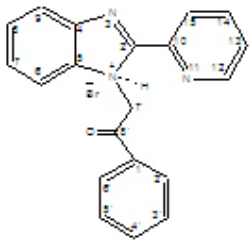
	IUPAC Name: 1-[2-(3',4'-dihydroxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*- benzimidazol-1-ium chloride
	Structure:-
	Yield: - 30 %
	Physical State: - Grey Powder
	Solubility: - 20 % DMSO, Methanol
	M. P: - 296^{o}C decompose
	Molecular Formula: - $C_{20}H_{15}N_3O_3$
	CHN Analysis: Found C=58.18%, H=4.23%, N=10.23% Calculated C=58.20%, H=4.25%, N=10.24%

Compound 3



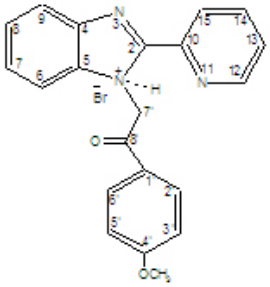
	IUPAC Name:- 1-[2-(4'-chlorophenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide
	Structure:-
	Yield: - 52 %
	Physical State: - Brown Crystals
	Solubility: - DMSO, Methanol, Ethanol
	M. P: - 276 – 280^{o}C decompose
	Molecular Formula: - $C_{20}H_{14}N_3Ocl$
	CHN Analysis: Found C=54.96%, H=3.28%, N=9.51% Calculated C=54.98%, H=3.30%,N=9.52%

Compound 8



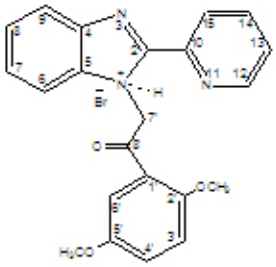
	IUPAC Name: -1-(2-oxo-2-phenylethyl)-2-(2´-pyridinyl)-1*H*-benzimidazol-1- ium bromide
	Structure:-
	Yield: - 37 %
	Physical State: - Light off white powder
	Solubility: - Ethanol, Methanol
	M. P: - 205 – 210°C
	Molecular Formula: - $C_{20}H_{15}N_3O$
	CHN Analysis: Found C=58.03%, H=3.86%, N=16.83% Calculated C = 58.05%, H=3.88%, N=16.85%

Compound 10



	IUPAC Name: - 1-[2-(4'-methoxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*- benzimidazol-1-ium bromide
	Structure:-
	Yield: - 56 %
	Physical State: - Light grey powder
	Solubility: - DMSO, Ethanol, Methanol
	M. P: - 228 – 234°C
	Molecular Formula: - $C_{21}H_{17}N_3O_2$
	CHN Analysis: Found C=57.23%, H=4.35%, N=10.24% Calculated C=57.26%, H=4.38%, N=10.26%

Compound 11



	IUPAC Name: 1-[2-(2',5'-dimethoxyphenyl)-2-oxo-ethyl]-2-(2´-pyridinyl)-1*H*-benzimidazol-1-ium bromide
	Structure:
	Yield: 22 %
	Physical State: Off white powder
	Solubility: Ethanol, Methanol
	M. P: 202 – 205°C
	Molecular Formula: $C_{22}H_{19}N_3O_3$
	CHN Analysis: Found C=56.12%, H=4.28%, N=9.41% Calculated C=56.14%, H=4.29%, N=9.37%

Insecticidal activity

All synthesized compounds of benzimidazole class were tested against different stored grain insects viz., *Tribolium castaneum*, C*allosbruchus analis* and *Rhyzopertha dominica*. The synthesized compounds were prepared by dissolving 20 mg of purified compounds in 3 ml volatile solvent and load up in Petri dishes cover up with the filter papers. After 24 hours, 10 stored grain pests were reared in the laboratory under controlled conditions (temperature and humidity) in plastic bottles containing sterile breeding media. Insects of uniform age and size were used for the experiment placed in each plate and incubated at 27^{o}C for 24 hours with 50% relative humidity in growth chamber. The results were analyzed as percentage mortality, calculated with reference to the positive Permethrin was utilized as a standard drug and negative controls as volatile solvent. Percentage inhibition or percentage mortality was calculated (Maryam *et al*., 2013) with the help of the following formula:

$$\% \text{ Mortality} = 100 - \frac{\text{No. of insects alive in test}}{\text{No. of insects alive in control}} \times 100$$

Antimalarial activity by parasite lactate dehydrogenase assay

In *vitro* antimalarial activity was performed by Parasite Lactate Dehydrogenase assay (PtLDH) using *plasmodium falciperum* 3D7 strain. LDH of *P. falciperum* rapidly used 3-acetyl pyridine adenine di-nucleotide (APAD) as a co-enzyme in the reaction leading to the formation of pyruvate from lactate. The conversion of NAD^+ to NADH + H^+ reduced tetrazolium to formazan, observation of purple color was detected on spectrophotometer. The amount of color produced was proportional to the number of parasites present (Makler and Hinrichs, *et al*., 1993; Trager, *et al.*, 1976). Synthesized compounds and standard drugs were serially diluted (two fold) with complete culture medium in 96 well plates. Infected R.B.Cs solution with 2% parasitemia and 1% hematocrit was added making the total volume 200µl in each well. Plates were incubated in candle jar with 5% CO_2, 5% O_2 and 90% N_2 at 37^{o}C for 72 hours. For malstat reaction, 20 µl solution from 100µl of malstat solution to each well of plate 1 respectively. All plates were positioned in shaking water bath at 37^{o}C for 30 minutes. 25µl solution (1:1 solution) NBT (2mg/mL) and PES (0.1mg/mL) were added in each well and the plates were read at 650 nm and the OD was recorded. The % inhibition was calculated by the formula (Microsoft Excel) and IC_{50} was calculated with the help of Ezfit computer program.

$$\% \text{ Inhibition} = 100 \text{ (test RBCs / infected RBCs)} \times 100$$

RESULTS

The bioactive benzimidazole along with corresponding 11 new synthesized derivatives were analyzed in the study. Their physical data comprise the solubility, melting point and CNH analysis whereas spectral analyses were already performed of UV, IR, Mass and NMR for structure elucidation of all these compounds. (Arfa *et al*., 2013)

Insecticidal activity

These novel derivatives of benzimidazole along with their synthesized compounds were tested for their insecticidal properties by impregnated filter paper method (Tabassum, *et al.*, 1997). This test was performed for observing the toxicity towards killing or paralyzing of insects. The compounds were tested against three common stored grain pests. *Tribolium castaneum*, *Rhyzopertha dominica* and *Callosobruchus analis* along with permethrin as standard drug their results of insecticidal bioassays of all these compounds were presented in table 1.

Antimalarial bioassay

In vitro antiplasmodial activity was performed against compounds Ia, 2, 3,8,10 and 11 on the *Plasmodium falciperum* 3D7 strains and the results of antiplasmodial activity were reported in table 2.

DISCUSSION

Effective methods of pest control are needed to solve the urgent problems of producing enough food for the world's increasing population. These methods involve the utilization of chemical or biological control agents. The biological methods of pest control are perceived to be more benign than chemical control; the use of insecticidal compounds will remain a major contribution for the foreseeable future (Stetter, *et al.*, 1993). The parent molecule and its derivatives showed no significant activity in comparison to standard drug hence found inactive as insecticidal agents.

It was evident from the results that the parent molecule (Ia) showed no activity against 3D7 strain of *Plasmodium falciperum* after 72 hours of incubation. While its derivatives, the compound 8, 10, 11 were found to exhibit moderate *in vitro* antiplasmodial activity with IC_{50} values of 5.96±0.09, 11.96±0.68 and 9.40±0.76 (µg/L) whereas, compounds 2 and 3 were found inactive against the parasite.

Structure characterization of the active compounds (8, 10, 11), showed that they contained phenyl, monomethoxy and dimethoxy groups respectively which might be responsible to impart moderately the antimalarial activity to the parent molecule (Ia).

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