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In Vitro Anthelmintic Activity of Some 1-Substituted Imidazole Derivatives

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Abstract: Anthelmintic resistance creates a major hitch over the decades throughout the world. As per WHO only few drugs are frequently used in the treatment of helminth infestations in human beings. In view of this, an attempt has been made to study the anthelmintic activity of some newly synthesized 1-substituted imidazoles (1a-1d, 2a-2d) were synthesized and characterized by their spectral data. All the prototypes were examined for their anthelmintic activity against african earthworms *Eudrilus eugeniae* at various concentrations (5, 10 and 20 mg/ml). Each prototype was tested for their onset of paralysis time followed by time of death of worms. All the prototypes were found not only paralyze (vermifuge) but also to kill the earthworms (vermicidal). All prototypes at the concentration of 20 mg/ml were shown significant activity ($P<0.01$) with that of control. The bromo substituted phenacyl imidazoles (1b and 2b), phenyl substituted phenacyl imidazoles (1c and 2c) and nitro substituted phenacyl imidazoles (1d and 2d) were found to be comparable to that of the reference standards albendazole and piperazine citrate (20 mg/ml).

Key words:1-substituted Imidazole % Anthelmintic activity % *Eudrilus eugeniae* % Albendazole and Piperazine Citrate

INTRODUCTION

Anthelmintics or antihelminthics are drugs that expel helminth parasitic worms (helminths) from the body, either by stunning or killing them. They may also be called vermifuges (stunning) or vermicides (killing). However they have shown the development of resistance to some broad spectrum anthelmintics (benzimidazoles, levamisole, avermectins) and also some narrow spectrum wormers such as the salicylanilides (closantel). Anthelmintic resistance is a major problem for the control of many parasitic nematode species and has become a major constraint to livestock production in many parts of the world. Due to the prevalence of parasitic infections and the developed resistance of some anthelmintic drugs is now an enclosing area in the field of research.

Imidazole nucleus has proved to be a versatile moiety for a number of medicinal agents. The various activities associated with the imidazole nucleus are antiprotozoal, mutagenic properties, anticancer, antiviral, enzyme inhibition, H_2-Antagonism, "-adrenergic agonist and $-blocking, anticonvulsant, broad spectrum antibacterial and antifungal activities [1-11]. Metronidazole an imidazole nucleus was first reported in 1961 and used as an effective class of anthelmintic in human and animal pathogenic helminths [12]. Structural modification of metronidazole led to discovery of tinidazole, nimorazole and panidazole and their clinical trials established the value of tinidazole in the treatment of intestinal and hepatic amoebiasis in humans [13].

The aim of the present revision is to investigate the anthelmintic activity of newly synthesised 1-substituted 2-methyl imidazoles (1a-1d) and 1-substituted imidazoles (2a-2d). Imidazoles are very effective against various helminths in decades. Moreover there are certain helminths, which are found to be resistant to the major classes of anthelmintics viz benzimidazoles, imidazothiazoles and macrocyclic lactones. Therefore a search of these novel 1-substituted imidazoles and its derivatives leads to the evaluation of prototype compounds with anthelmintic activity.

MATERIAL AND METHODS

Melting points were determined in open glass capillaries using an Electro thermal IA 9000 SERIES digital melting point apparatus (Electrothermal, UK) and are uncorrected. IR spectra (KBr wafers) were confirmed by

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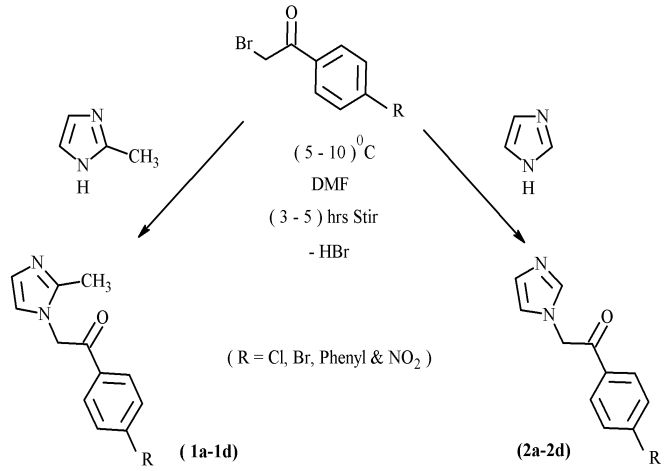

Scheme 1:

Shimadzu FT-IR Spectrophotometer, Model No.8400S (Japan). ^{1}H NMR spectra were recorded on Bruker 300 MHz NMR spectrometer (Switzerland) using CDCl3 as solvent. Mass spectra were recorded on Joel SX-102(EI/CI/DART/MS) (USA). Microanalysis was done on a Vario EL III Elementor C, H, N analyzer (Germany). Iodine vapour was used as developing agent and the solvent system used was benzene: ethanol (8:2). Imidazole, 2-methyl imidazole and phenacyl halides were procured from sigma-Aldrich, USA. All other chemicals used in the present studies were either of A.R or G.R quality. Anhydrous sodium sulphate was used as drying agent. The purity of all compounds was established by single spot on the TLC plates (Merck, Germany).

Drugs and Chemicals: 2-methyl imidazole, imidazole (Aldrich), phenacyl halides (Aldrich), dimethyl formamide (Sigma), sodium chloride (CDH), saline water (Claris life sciences Ltd, Ahmedabad) albendazole and piperazine citrate (pure drugs) and vehicle (2% v/v Tweens 80 in distilled water were used. All the prototypes were dissolved in minimum quantity of 2% v/v Tween80 and then the volume was adjusted to 10 ml with normal saline for making the concentration of 5, 10 and 20 mg/m).

Standard Drug: Albendazole and Piperazine citrate were taken as a reference standards and the concentration of the standard drugs were prepared in 2%v/v Tween80 in normal saline to give 20mg/ml.

Chemical Synthesis [14]

Synthesis of 1-(4-substitutedphenyl)-2-(2-methyl-1*h*-imidazol-1-yl) Ethanone (1a-1d): In the present scheme, N-phenacyl 2-Methyl Imidazole derivatives (1a-1d, Scheme:1) have been synthesized by treating 2.433 g (0.03mol) of 2-methyl imidazole and 0.461g (0.002 mol) of appropriate para substituted phenacyl bromides (chloro, bromo, phenyl and nitro) in presence of dry DMF (dimethyl formamide) with the cold stirring (5-10°C) for about (3-6) hrs. Next, the mixture was poured into cold water (20ml) and stirred for further one hour. The precipitate was collected and then treated with benzene and filtered. The benzene soluble portion on removal of solvent yielded a solid mass, which was recrystallized from benzene-ethanol. The purity of all compounds was established by single spot on the TLC plates.

Synthesis of 1-(4-substituted Phenyl)-2-(1*h*-imidazol-1-yl) Ethanone (2a-2d) [14]: N-phenacyl Imidazole derivatives (2a-2d, Scheme:1) have been synthesized by treating 2.0124 g (0.03mol) of imidazole and 0.461g (0.002 mol) of appropriate para substituted phenacyl bromides (chloro, bromo, phenyl and nitro) in presence of dry DMF (dimethyl formamide) with the cold stirring (5-10°C) for about 3-6 hrs. Next, the mixture was poured into cold water (20ml) and stirred for further one hour. The precipitate was collected and then treated with benzene and filtered. The benzene soluble portion on removal of solvent yielded a solid mass, which was recrystallized from benzene-ethanol. The purity of all compounds was established by single spot on the TLC plates.

Scheme: 1

Experimental Model: Adult African earthworms of the genus and species, *Eudrilus eugeniae* (family: eudrlidae), were used to study the anthelmintic activity. The earthworms were obtained from Divyayan krishi vigyan Kendra, morabadi, Ranchi, Jharkhand, India and washed

with normal saline to remove all the fecal matter and waste surrounding their body. The earth worms (*Eudrilus eugeniae*) 3 –5 cm in length and 0.1–0.2 cm in width weighing 0.8–3.04 g were used for all experiment protocols. The earthworms resembled the intestinal roundworm parasites of human beings both anatomically and physiologically and hence where used to study the anthelmintic activity [15].

Anthelmintic Investigation: African adult earth worm 4 - 5 cm in length and 0.1 - 0.2 cm in width were used for the *in vitro* anthelmintic bio assay of all newly synthesized prototypes. The worms were divided into the respective groups containing six-earth worms in each group. All the prototypes were dissolved in minimum quantity of 2%v/v Tween80 and the volume was adjusted to 10 ml with normal saline for making the concentration of 5, 10 and 20mg/ml. All the prototypes and the standard drug solution were freshly prepared before commencement of the experiments. All the earthworms were washed in normal saline solution before they were released into 10 ml of respective formulation as follows, vehicle (2% v/v Tween80 in normal saline), Albendazole and Piperazine Citrate (20 mg/ml) and prototypes (5, 10 and 20mg/ml). The anthelmintic activity was determined in six observations. Six worms of about the same size per petridish were used. They were observed for their spontaneous motility and evoked responses. Observations were made for the time taken to paralysis and death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body color.

Statistical Analysis: Results were expressed as mean±s.e.m. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test, with the level of significance at $P < 0.001$, $P < 0.01$ and $P < 0.05$.

RESULTS AND DISCUSSION

The objective of this work was to study the anthelmintic activity of 1-substituted imidazoles on *Eudrilus eugenia* (African earth worm) to explore the beneficial use of these derivatives in gastro intestinal disorders. As evident from the available literature, compounds possessing imidazole nuclei viz., metronidazole, tinidazole, nimorazole and panidazole showed significant anthelmintic activity. Anthelmintic activities of all prototypes were tested in this bioassay at various concentrations of 5, 10 and 20 mg/ml [Table 1].

All the investigational compounds 1a-1d, 2a-2d acquired the anthelmintic activity at minimal dose of 5mg/ml. Compound 2d had shown its significant activity (P<0.05) at 5mg/ml for time taken to paralysis and death when compared to the standard drugs Albendazole and Piperzine citrate used at 20mg/ml respectively.

Table 1: Anthelmintic activity of novel 1-substituted imidazole derivatives

	Time Taken for Paralysis (P) and Death (D)					
	Paralysis time (min)			Death time (min)		
Test Compounds	5mg/ml	10 mg/ml	20 mg/ml	5 mg/ml	10 mg/ml	20 mg/ml
Control	-	-	-	-	-	-
1a	41.03±1.41	34.56±1.21	11.51±0.88	45.88±0.35	37.32±0.28	14.97±0.34[a]
1b	30.91±1.29	25.01±0.88	08.94±0.91[a]	37.17±0.56	29.99±0.39	14.08±0.32[b]
1c	34.66±1.25	22.57±0.64[a]	08.70±0.83[a]	38.05±0.31	25.30±0.05[a]	13.40±0.14[b]
1d	29.50±1.15	18.02±0.88[a]	09.34±0.99[b]	35.35±0.63	21.93±0.34[a]	14.02±0.26[b]
2a	37.35±1.05	25.93±0.25	10.58±0.27	40.56±0.34	29.79±0.37	14.67±0.18[a]
2b	29.89±1.17	19.75±0.91[a]	07.40±0.83[b]	33.76±0.48	22.53±0.29[a]	11.43±0.06[b]
2c	26.07±0.80	17.97±0.87[a]	06.87±0.73[b]	32.77±0.33	23.04±0.33[a]	10.93±0.29[c]
2d	21.40±1.02[a]	15.88±0.87[b]	07.24±1.13[b]	24.01±0.24[a]	22.07±0.25[a]	10.65±0.28[c]
PZC	-	-	05.06±0.26	-	-	09.39±0.02
ALB	-	-	04.32±0.28	-	-	08.35±0.05

All determinations were done in triplicate and results are expressed as Mean±SEM.

P value was calculated by comparing with control by one-way ANOVA. Control worms were alive up to 24 hrs of observation.

[a] $P<0.05$, [b] $P<0.01$ and $P<0.001$, Significantly different when compared with reference compound, Piperazine citrate and Albendazole

Prototypes used for the study were designated as 1a, 1b, 1c, 1d (1-substituted-2-methyl imidazoles) 2a, 2b, 2c, 2d (1-substituted imidazoles) respectively and the standard drugs Piperzine Citrate (PZC) and Albendazole (ALB) were used at 20mg/ml.

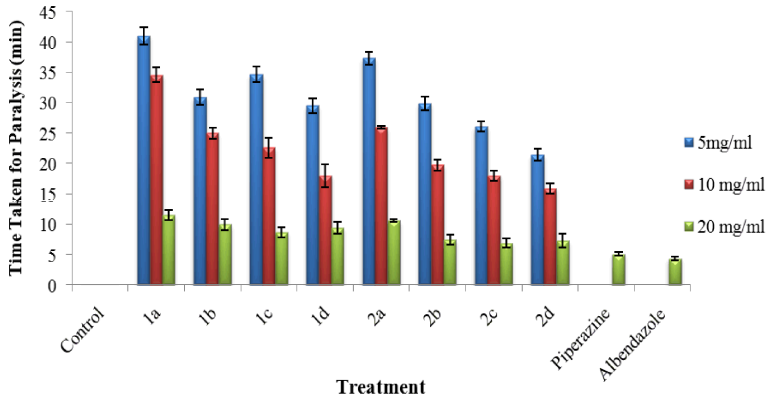

Fig: 1 Anthelmintic activity of novel 1-substituted imidazoles

At the concentration of 10mg/ml, compounds 1d, 2b, 2c and 2d exhibited their significant action ($^{a}P<0.01$) for time taken to paralysis and death. In which compounds 1band 1c showed their moderate significant action for time taken to paralysis ($^{a}P<0.05$) when compared to standards used at 20mg/ml.

While increasing the concentration (20mg/ml), compounds 1a and 2a significantly ($^{a}P<0.05$) reduced the paralysis and death time as well. In which compounds 1b, 1c, 1d and 2b showed their moderate significant action for time taken to paralysis and death ($^{b}P<0.01$) when compared to standards. Rather compounds 2c and 2d exhibited their highly significant action ($^{c}P<0.001$) for time taken to paralysis and death and which is almost equipotent action when compared to standards Albendazole and Piperzine citrate. In the higher concentration all the compounds showed their time taken to paralysis and death was drastically reduced and almost comparable to standard drugs [Fig. 1].

CONCLUSION

Amongst the various compounds synthesized, the prototypes (2a-2d) containing imidazole moiety possess greater activity compared to similar analogs containing 2-methyl imidazole (1a-1d). The probable reason for the above observation might be steric hindrance due to the presence of methyl group in second position and which is an electro positive moiety. The prototypes 1b, 1c, 1d, 2b, 2c and 2d showed significant activity at 20mg/ml compared to standards ($^{b}P<0.01$ and $^{c}P<0.001$) [Fig.1]. This might be due to the investigational compounds possessing an electron withdrawing groups (bromo, phenyl and nitro) substituted at their para position. Hence in future studies various *in vivo* models may be used to establish the effectiveness of these 1-Substituted imidazoles as anthelmintic drugs. Thus the prototypes may be further explored for their pharmacophore model using QSAR studies to develop compounds with enhanced anthelmintic activities.

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