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Synthesis, molecular docking studies and *in vitro* anthelmintic activities of novel substituted pyrazole bearing benzimidazole derivatives

Neetu Soni, Azad Gangwar and Namrta Soni

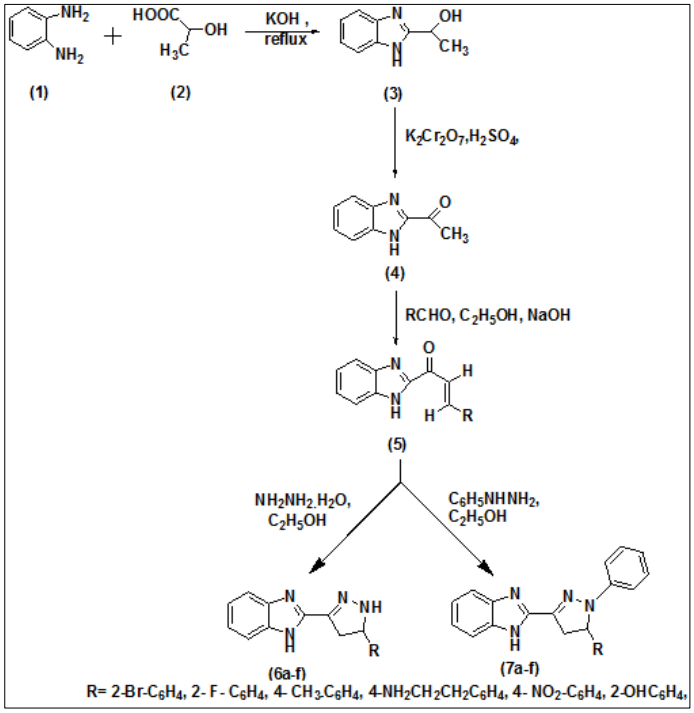
Abstract
Nitrogen containing heterocycles have got special attention in pharmaceutical chemistry due to their excellent medicinal potential. A novel series of substituted pyrazoline derivatives of benzimidazole have been synthesized by the reaction of substituted chalcones with hydrazine hydrate and phenyl hydrazine hydrate. In correlation to anthelmintic activity, compounds are subjected to molecular docking studies for the binding to b-Tubulin, target protein elite to the parasites. All the products were assayed for anthelmintic activity against *Pheretima posthuma* using albendazole as reference compounds. Out of the twelve synthesized derivatives, five compounds (6d, 6c, 7a, 7c, 7d) showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis and death with different concentrations of the derivatives. Among these two derivatives, 6c and 6d showed superior activity. In molecular docking study also, compounds showed minimum binding energy and have good affinity toward the active pocket thus, they may be considered as good inhibitor of b-Tubulin.

Keywords: Chalcone, benzimidazole, anthelmintic activity, *pheretima posthuma*

1. Introduction
Benzimidazole and pyrazole were reviewed for biological activity and found that both hetero systems possess a broad spectrum of biological activities viz antimicrobial [1, 4], ant amoebic [5, 6], antiprotozoal [7, 8], cysticidal [9], anticancer [10, 11].
The chemistry of fused ring heterocyclic compound has been the fascinating field of investigation in medicinal chemistry, as it has been found to exhibit enhanced biological profile. The present work has been directed to synthesize various substituted benzimidazole containing pyrazole ring through mild and facile synthetic route and also the study has focused on the influence of the various substituents on anthelmintic activity of benzimidazole. The presence of pyrazoline ring reports potent pharmacological activities like antifungal, antibacterial. In view of the above observation, it was thought worth-while to synthesize a new benzimidazole molecule in which it is linked with the pyrazole ring and further investigate these compounds for their anthelmintic activities. Anthelmintic infections are now being recognized as cause of much chronic ill health amongst the tropical people. More than half of population in the world suffers from worm infection of one or the other. Hence, newer benzimidazole derivatives were synthesized and their anthelmintic activities were studied. The anthelmintic drugs derived from benzimidazole 2-carbamates, such as albendazole and mebendazole, are used mainly to treat endoparasitic diseases in domestic animals and humans. These types of compounds are characterized by a high therapeutic index and low toxicity; however, they find little use in tissue-dwelling parasites mainly due to poor solubility and absorption problems.

2 Result and Discussion
2.1 Chemistry
The reaction sequence for the titled compound is outlined in Scheme 1. ortho-phenylenediamine (1) and lactic acid (2) were used as starting material for the formation of 2-hydroxy ethyl benzimidazole (3). The compound (3)on treatment with $K_2Cr_2O_7$ and H_2SO_4 gave 2-acetyl benzimidazole (4), which on treatment with substituted aromatic aldehyde resulted in the formation of substituted chalcone (5), [12] Final intermediate (5) on condensation with various hydrazine hydrates in the presence of absolute alcohol at a temperature of 80-90^{0}C for 10 hrs.

Resulted in the formation of pyrazoline derivatives of Benzimidazole (6a-f) and phenyl pyrazoline derivatives of benzimidazole (7a-f).



Scheme 1: Synthetic route for pyrazoline substituted benzimidazole derivatives

Table 1: Characterization data (6a-f and 7a-f)

Compound no.	R	% Yield	MP (^{0}C)
6a	2-BrC_6H_4	35	327 - 329
6b	2-FC_6H_4	41	266 - 268
6c	4-$CH_3C_6H_4$	42	272- 274
6d	4-$NO_2C_6H_4$	50	281 - 283
6e	4-$NH_2CH_2CH_2C_6H_4$	45	295 - 297
6f	2-OHC_6H_4	51	245 - 247
7a	2-BrC_6H_4	46	350 - 352
7b	2-FC_6H_4	42	305 - 307
7c	4-$CH_3C_6H_4$	46	302 - 304
7d	4-$NO_2C_6H_4$	43	313 - 315
7e	4-$NH_2CH_2CH_2C_6H_4$	51	310 - 312
7f	2-OHC_6H_4	51	280 - 282

2.2 Methodology for *in vitro* anthelmintic activity [13]

In vitro anthelmintic activity for the synthesized compounds Anthelmintic activity was studied with minor modifications to the standard Albendazole (Bandy Mankind Pharma Ltd., New Delhi) and Mebendazole (Mansukhlal Tribhovandas & Company, Mumbai). A group of six earthworms were released in to each of 15 ml of control drugs and the test suspensions (1, 2.5 and 5% w/v each). Observations were made for the time taken to paralysis and death of individual worms up to 1 h of the test period. Each Petri dish was placed with 6 worms and observed for paralysis (or) death. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously. The death time of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. Death was concluded when the worms lost their motility followed with fading away of their body colors.

Table 2: Anthelmintic activities of substituted benzimidazole

Compound Code	Concentration (% W/V)	Time taken in minutes (+-SD)	
		Paralysis Time	Death Time
6a	1.0	13.12+-0.25	40.12+-0.46
	2.5	11.13+-0.29	35.24+-0.33
	5.0	10.23+-0.30	20.22+-0.38
6b	1.0	25.35+-0.14	45.23+-0.16
	2.5	20.34+-0.14	40.35+-0.23
	5.0	12.20+0.38	25.23+-0.12
6c	1.0	11.12+-0.23	60.45+-0.42
	2.5	9.23+-0.12	50.36+-0.45
	5.0	8.29+-0.24	40.40+-0.38
6d	1.0	8.25+-0.40	38.39+-0.24
	2.5	6.23+-0.46	31.15+-0.16
	5.0	5.17+-0.35	25.46+-0.27
6e	1.0	15.32+-0.14	30.24+-0.32
	2.5	12.36+-0.65	27.14+-0.29
	5.0	10.32+-0.14	22.15+-0.24

6f	1.0	11.15+-0.16	35.32+-0.12
	2.5	9.65+-0.25	32.12+-0.36
	5.0	8.23+-0.39	19.38+-040
7a	1.0	10.20+-0.21	38.15+-0.25
	2.5	9.39+-0.17	36.17+-0.38
	5.0	5.40+-0.50	30.14+-0.39
7b	1.0	14.39+-0.32	42.32+-0.14
	2.5	10.26+-0.16	45.33+-0.25
	5.0	8.17+-0.28	32.15+-0.63
7c	1.0	9.36+-0.45	30.29+-0.36
	2.5	9.44+-0.34	25.39+-0.47
	5.0	8.54+-0.17	20.56+-0.21
7d	1.0	11.32+-0.30	12.14+-0.25
	2.5	9.41+-0.26	18.12+-0.47
	5.0	8.12+-0.38	25.44+-0.16
7e	1.0	16.21+-0.14	36.32+-0.16
	2.5	13.36+-0.39	30.54+-0.12
	5.0	10.25+-0.39	21.12+-0.36
7f	1.0	12.14+-0.32	30.21+-0.23
	2.5	10.25+-0.29	29.23+-0.44
	5.0	9.54+-0.12	21.14+-0.27
Albendazole	2.5	8.14+-0.38	15.38+-0.18
Normal saline	--	--	--

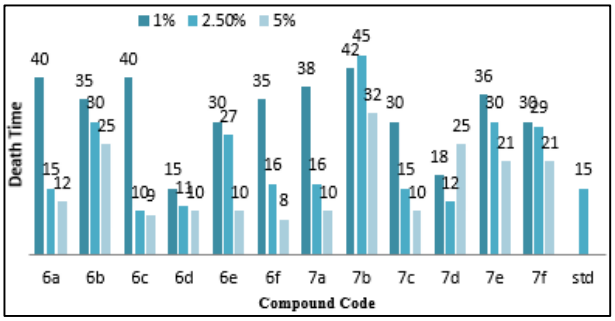


Fig 1: Graph for paralysis time in Minuits

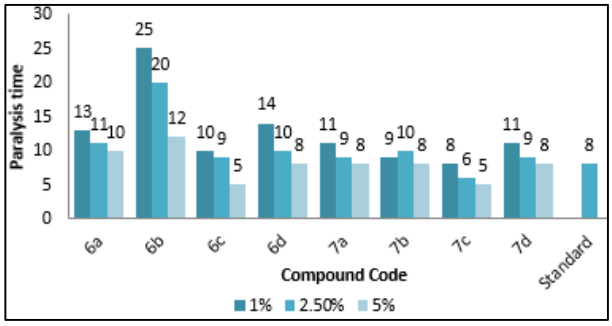


Fig 2: Graph for death time in Minuits

2.3 Molecular Docking Studies

In order to validate the selectivity of these compounds for anthelminetic activity as compared to albendazole a reference ligand from this class and the comparative docking was carried out. In this experiment the all designed compounds showed good binding affinity or selectivity towards tubulin protein as compared to albendazole. In the docking experiments all the compounds showed good hydrogen binding interactions with Gln134, His6, Thr237 and Val236 as compared to albendazole as a reference compound. The molecules showed good hydrophobic interactions with Tyr50, Gln134, Ser 165. The scoring functions were also compared with the albendazole. The compounds 7b and 7c showed good scores as compared to albendazole as they showed good hydrogen bond, hydrophobic and steric interactions as compared to albendazole. They showed promising biological activity in the in-vitro as well as in-vivo experiments. The top scoring compounds 6c and 6d from this series showed excellent docking scores as well as biological significance as found five compounds (6d, 6c, 7a, 7c, 7d) showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis and death with different concentrations of the derivatives.

Table 3: Scoring functions from docking experiments.

Ligand	MolDock Score	Re rank Score
6a	-137.191	-53.9622
6b	-115.045	-63.6142
6c	-142.309	-89.1849
6d	-133.793	-97.73931
6e	-139.217	-16.2808
6f	-138.703	-43.4587
7a	-139.965	-71.692
7b	-143.37	-79.7063
7c	-150.611	-76.5962
7d	-165.356	-74.94293
7e	-148.863	-81.4028
7f	-138.122	-51.737
Alz	-147.588	-121.701

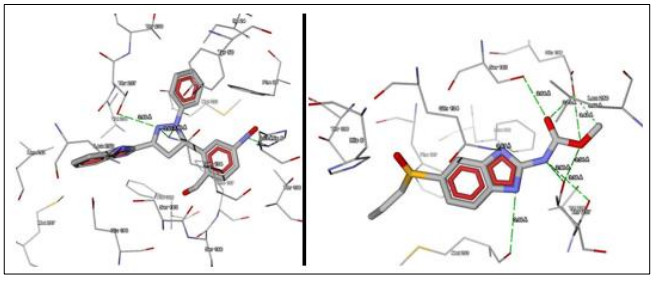


Fig 3: Molecular docking interctions of compound 7e and albendazole with tubulin proteins.

3 Experimental Section

3.1 Chemistry

Chemicals were procured from Loba Chemie. Pvt, New Delhi (LR grade). Reactions were monitored and purity of the products was checked by thin layer chromatography (TLC) (Benzene: Toluene, 7:3). TLC was performed on Merck60 F-

254 silica gel plates with visualization by UV-light. Melting points were determined on a Buchi Melting Point B-545 apparatus. IR spectra (KBr pellets) were recorded on a Nicolet 6700 FT-IR spectrometer. 1H NMR spectra were recorded on Bruker at either (200, 300, 400 MHz) and 50, 75, 100 MHz (^{13}C NMR), spectrometer instruments, in DMSO-d_6. Chemical shifts were recorded in parts per million downfield from tetramethylsilane. Mass spectra were recorded on time-of-flight (TOF) mass spectrometers.

3.2 Synthesis of pyrazoline derivatives from substituted chalcone (6a-f)

Substituted chalcone (2.08 gm, 0.01 mole) was mixed in 30 ml of absolute alcohol, and hydrazine hydrate (0.5 gm, 0.01 mole) was added, the reaction mixture was refluxed in water bath at temp. 80-90^{0}C for 10 hrs. The reaction mixture was poured into ice cold water. The product was filtered, dried and crystallized from ethanol.

3.2.1 Synthesis of 2-[5-(1H-Benzoimidazol-2-yl)-4H-pyrazol-3-yl]-3-bromobenzaldehyde (6a)

Percentage yield 35% m.p. 327 °C IR (KBr) cm^{-1}: 3426 (N-H), 316 9(C-H), 1555 (C=N), 683 (C-Br), 1494(C=C), 1296 (C-N), 960 (C-C), 1724(C=O), 1082 (C-H), 792 (C-H), 825(C-H),^{1}H-NMR (DMSO-d_6) δ ppm: 1.2(s, 2H, CH_2), 5.56 (s, 1H, NH) 7.26 (m, 2H, Ar-H), 7.34(m, 2H, Ar-H), 7.35(m, 2H, Ar-H), 7.38 (m, 2H, Ar-H), 8.00 (m, 2H, Ar-H), 8.23 (m, 2H, Ar-CHO);^{13}C-NMR (500 Hz, DMSO, δ in ppm) 164.03(C_5 pyrazole), 164.03 (C_3 pyrazole), 136.91(C_4 pyrazole), 137.85 (C_8 benzimidazole), 137.85(C_9 benzimidazole), 137.85(C_2 benzimidazole), 137.85(C_1 benzene) 136.9(C_4 benzene),123.3(C_3 benzene), 122.22 (C_5 benzimidazole), 122.22(C_6 benzimidazole), TOF-MS ES+1.55e3, (M^+); 3362.4(6.8%); Anal calcd for $C_{17}H_{11}BrN_4O$: C, 55.56; H, 2.99; N, 15.25; O, 4.35; Br, 21.75. Found: C, 55.57; H, 2.89; N, 15.26; O, 4.38; Br, 21.65.

3.2.2 Synthesis of 2-[5-(1H-Benzoimidazol-2-yl)-4H-pyrazol-3-yl]-2-fluro-benzaldehyde (6b)

Percentage yield 41% m.p. 266, °C IR (KBr) cm^{-1}: 3071 (N-H), 1583 (C=N), 2847 (C-H), 1326 (C-F), 1291(C-N), 1789 (C=O), 1127 (C-C), 1602 (C=C) 1072 (C-H $_{bend}$), 763 (C-H), 804 (C-H), 2847 (C-H).

^{1}H-NMR (DMSO-d_6) δ ppm: 8.97 (s, 1H, Ar-H),7.6487 (d, 2H,J=7.12 Ar-H), 7.35 (m, 2H, CH), 6.9773(s, 1H, CH), 6.9784 (s 1H, NH);^{13}C-NMR (500 Hz, DMSO, δ in ppm) 160.31(C_3 pyrazole), 160.31(C_5 pyrazole), 135.81(C_6 benzaldehyde), 130.35(C_4 benzaldehyde), 78.94(C_2 benzaldehyde), 127.39(C_4 benzaldehyde), 127.30 (C_5benzaldehyde), 122.20(C_5&C_6 benzimidazole), 135.81(C_8&C_9 benzimidazole), 114.98(C_4 benzimidazole), 114.98(C_7 benzimidazole) 38.9(C_3 pyrazole). TOF-MS ES+1.55e3, (M^+); 305 (11 %), anal calcd for $C_{17}H_{13}FN_4O$: C, 66.23; H, 4.22; N, 18.18; O, 5.19; F, 6.16. Found: C, 66.26; H, 4.32; N, 18.88; O, 5.39; F, 6.66.

3.2.3 Synthesis of 2-[5-(1H-Benzoimidazol-2-yl)-4H-pyrazol-3-yl]-4-hydroxy-benzaldehyde. (6c)

Percentage yield 51% m.p. 245 °C, IR (KBr) cm^{-1}; 3058 (N-H), 1603 (C=N), 1494 (C=C), 1279(C-N), 1699 (C=O), 3356 (O-H), 1177(C-C), 1103(C-H), 781(C-H), 870(C-H),701(C-H).^{1}H-NMR (DMSO-d_6) δ ppm1.24(s, 1H, CH_2), 5.3(s, 1H, NH),5.34(s, 1H, Ar-OH), 6.49 (d, 1H,J=8.6, CH), 7.98 (m, 1H, CH), 7.511 (m, 1H, CH), 7.24 (m, 2H, A-H);

^{13}C-NMR (500 Hz, DMSO, δ in ppm); 162.86(C_3 & C_5 pyrazole), 168.24(C_1 OH--benzaldehyde), 154.78(C_2 benzimidazole), 133.2(C_2 benzaldehyde), 160.35(C_3 benzaldehyde), 115.95(C_4 benzaldehyde), 121.02(C_5 benzaldehyde), 133.13(C_6 benzaldehyde), 127.43(C_1 benzaldehyde), TOF-MS ES+1.55e3, (M^+); 304 (11 %), Anal calcd for: $C_{17}H_{12}N_4O_2$; C, 66.23; H, 4.22; N, 18.18; O, 5.19; F, 6.16. Found: C, 67.11; H, 3.95; N, 18.42; O, 10.52.

3.2.4 Synthesis of 4-(2-Amino-ethyl)-2-[5-(1H-benzoimidazol-2-yl)-4H-pyrazol-3-yl]-benzaldehyde. 6d

Percentage yield 45% m.p. 295 °C, IR (KBr) cm^{-1}; 3167 (N-H), 1466 (C=C), 2949 (C-H), 1589 (N-H), 1625 (C=N), 1424 (C-N), 1776 (C=O), 1162 (C-C), 1084 (C-C), 1261 (C-H), 954 (C-H), 855 (C-H), 839 (C-H). ^{1}H-NMR (DMSO-d_6) δ ppm 1.24(s, 2H,CH_2), 2.11 (s, 2H, NH_2), 2.54 (m, 2H, CH_2), 7.982 (m, 2H, Ar-H), 7.52 (m, 2H, Ar-H), 7.39(m, 1H, Ar-H), 5.346(s, 1H, CH), 7.25(m, 2H, Ar-H). ^{13}C-NMR (500 Hz, DMSO, δ in ppm); 195.68((C=O benzaldehyde), 158.47(C_2 benzimidazole) 162.21(C_3&C_5 pyrazole), 135.11(C_8&C_9 benzimidazole), 134.78(C_1 benzaldehyde), 132.78(C_2 benzaldehyde),, 130.66(C_5 benzaldehyde),, 129.93(C_6 benzaldehyde),, 128.65(C_3 benzaldehyde), 126.57(C_5&C_6 benzimidazole), 115.10 (C_4&C_7 benzimidazole), TOF-MS ES+1.55e3, (M^+); 331(33%)., Anal calcd for: $C_{19}H_{17}N_5O$; C, 68.89; H; 5.14, N; 21.15; O; 4.83; Found: C; 68.89, H; 5.14, N; 21.15, O; 4.83.

3.2.5 Synthesis of 2-[5-(1H-Benzoimidazol-2-yl)-4H-pyrazol-3-yl]-2-nitro-benzaldehyde.6e

Percentage yield 56% m.p. 345 °C, IR (KBr) cm^{-1}; 3069 (N-H), 1612 (C=N), 1486 (C=C), 1282(C-N), 1656 (C=O), 3375 (O-H), 1165(C-C), 1112(C-H), 780(C-H), 868(C-H),797(C-H).^{1}H-NMR (DMSO-d_6) δ ppm 1.26 (s, 1H, CH_2), 5.22(s, 1H, NH),5.33(s, 1H, Ar-OH), 6.54 (d, 1H,J=8.6, CH), 7.23 (m, 1H, CH), 7.55 (m, 1H, CH), 7.34 (m, 2H, A-H); ^{13}C-NMR (500 Hz, DMSO, δ in ppm); 162.86(C_3 & C_5 pyrazole), 168.24(C_1 OH--benzaldehyde), 154.78(C_2 benzimidazole), 133.2(C_2 benzaldehyde), 160.35(C_3 benzaldehyde), 115.95(C_4 benzaldehyde), 121.02(C_5 benzaldehyde), 133.13(C_6 benzaldehyde), 127.43(C_1 benzaldehyde), TOF-MS ES+1.55e3, (M^+); 336 (14 %), Anal calcd for $C_{17}H_{13}N_5O_3$; C, 60.89, H; 3.88; N; 20.89, O; 14.32. Found: C, 60.99, H; 3.28; N; 20.76, O; 14.36.

3.2.6 Synthesis of 2-[5-(1H-Benzoimidazol-2-yl)-2-phenyl-3, 4-dihydro-2H-pyrazol-3-yl]-2-hydroxy-benzaldehyde. 6f

Percentage yield 35% m.p. 320 °C, IR (KBr) cm^{-1}; 3166 (N-H), 1467 (C=C), 2939 (C-H), 1549 (N-H), 1675 (C=N), 1474 (C-N), 1736 (C=O), 1132 (C-C), 1074 (C-C), 1265 (C-H), 964 (C-H), 865 (C-H), 839 (C-H). ^{1}H-NMR (DMSO-d_6) δ ppm 1.44(s, 2H,CH_2), 2.71 (s, 2H, NH_2), 2.74 (m, 2H, CH_2), 7.56 (m, 2H, Ar-H), 7.22 (m, 2H, Ar-H), 7.45(m, 1H, Ar-H), 5.36(s, 1H, CH), 7.75(m, 2H, Ar-H). ^{13}C-NMR (500 Hz, DMSO, δ in ppm); 192.78((C=O benzaldehyde), 156.57(C_2 benzimidazole) 161.21(C_3&C_5 pyrazole), 136.1(C_8&C_9 benzimidazole), 136.68(C_1 benzaldehyde), 133.78(C_2 benzaldehyde),, 129.66(C_5 benzaldehyde),, 129.93(C_6 benzaldehyde),, 128.78(C_3 benzaldehyde), 126.94(C_5&C_6 benzimidazole), 115.09 (C_4&C_7 benzimidazole), TOF-MS ES+1.55e3, (M^+); 386(12%)., Anal calcd for: $C_{23}H_{20}N_4O_2$; C, 71.87; H; 5.20, N; 14.58; O; 8.34; Found: C, 71.89; H; 5.23, N; 14.48; O; 8.26;

3.2.7 Synthesis of 2-[5-(1H-Benzoimidazol-2-yl)-2-phenyl-3, 4-dihydro-2H-pyrazol-3-yl]-3-bromo-benzaldehyde. 7a

Percentage yield 46% m.p. 350 °C, IR (KBr) cm^{-1}; 3167(N-H), 1650 (C=N), 3001 (C-H), 1261(C-N), 1756(C=O), 536

(C-B), 1560 (C=C), 1573(C=C), 1162 (C-C), 1084 (C-C),1065 (C-H), 778 (C-H), 696 (C-H), 677 (C-H).^{1}H-NMR (DMSO-d_6) δ ppm 7.3890 (s, 1H, CH), 7.266-7.251 (*d*, 1H,J=5.8 CH), 7.66-7.70 (m, 3H, *Ar-H*, Benzene), 5.5618(s, 1H, NH), 1.239 (s, 1H, CH). ^{13}C-NMR (500 Hz, DMSO, δ in ppm); 163.13(C_5 pyrazole), 137.85(C_1 benzaldehyde), 137.85(C_8&C_9 benzimidazole),137.85(C_2 benzaldehyde), 136.91(C_4 benzaldehyde), 129.16(C_3&C_5 benzene), 129.28(C_3 benzaldehyde)122.0(C_4 benzene), 128.10(C_6 benzaldehyde), TOF-MS ES+1.55e3, (M^+); 444(15%)., Anal calcd for: $C_{23}H_{17}BrN_4O$; C; 61.95, H; 3.81, N; 12.57; O; 3.59; Found: C; 61.55, H; 3.92, N; 12.49; O; 3.64, Br; 17.93.

3.2.8 Synthesis of 6-[5-(1H-Benzoimidazol-2-yl)-2-phenyl-3, 4-dihydro-2H-pyrazol-3-yl]-6-fluoro-cyclohexa-1, 3-dienecarbaldehyde (7b)

Percentage yield 42% m.p. 305 °C, IR (KBr) cm^{-1}; 3408 (N-H), 2934 (C-H) 1626 (C=N), 1296 (C-F), 2934(C-H), 1281(C-N), 1611 (C=C), 1725(C=O), 1094 (C-H), 813 (C-H), 863 (C-H), 1205 (C-C). ^{1}H-NMR (DMSO-d_6) δ ppm 2.53(s, 1H, J=1.52, Ar-H), 5.63(s, 1H, NH), 6.97 (m, 2H, Ar-H), 6.93(m, 2H, Ar-H), 7.38 (m, 2H, Ar-H), 7.6487-7.6309(d, 1H, *J* = 7.12 Hz). (s,H, CH). ^{13}C-NMR (500 Hz, DMSO, δ in ppm); 160.20 (C_5 pyrazole), 160.17(C_1 benzaldehyde), 130.37(C_2 benzaldehyde),130.37(C_4 benzaldehyde),128.03(C_3 benzaldehyde), 129.30(C3&C5 benzene), 115.69(C_6 benzene), 114.96(C_4&C_7 benzimidazole), 124.97 (C_5&C_6 benzimidazole), 114.96 (C_2&C_6 benene), 52.98(C_5 benzaldehyde),78.92(C_6 benzaldehyde), 38.87(C_3 pyrazole). 25.99(C_4 pyrazole). TOF-MS ES+1.55e3, 386 (46 %), Anal calcd for: $C_{23}H_{17}FN_4O$; C; 71.42, H; 4.39, N; 14.49; O; 4.14, F; 4.91 Found: C; 71.55, H; 4.92, N; 14.49; O; 4.64, Br; 4.89.

3.2.9 Synthesis of2-[5-(1H-Benzoimidazol-2-yl)-2-phenyl-3,-dihydro-2H-pyrazol-3-yl]-4-hydroxy-benzaldehyde. (7c)

Percentage yield 51% m.p. 280ºC, IR (KBr) cm^{-1}3184 (N-H), 1444 (C=C), 2805 (C-H), 1227 (C-C), 1362 (C-N), 1606 (C=N), 3445 (O-H), 1690 (C=O), 1106 (C-H), 968 (C-H), 870 (C-H), 837 (C-H). ^{1}H-NMR (DMSO-d_6) 1.48(s, 1H, Ar-H), 5.52 (s, 1H, Ar-OH), 5.5294-5.5230 (d, 1H, *J* = 2.5 Hz, NH), 7.9334 (m1H, Ar-H), 7.3958 (m, 1H, Ar-H), 7.26 (m, 2H, Ar-H), 7.20(m, 2H, Ar-H). (s,H, CH). ^{13}C-NMR (500 Hz, DMSO, δ in ppm); 154.82(C_5 pyrazole), 162.86(C_4 benzaldehyde), 154.78(C_1 benzene), 154.76(C_2 benzimidazole), 133.22 (C_8 & C_9 benzimidazole), 133.13 (C_6 benzaldehyde), 133.23(C_3& C_5 benzene), 115.95(C_4 benzene), 115.74(C_4 & C_7 benzimidazole), 127.42(C_1 benzaldehyde), 121.02(C_5 & C_6 benzimidazole), 115.95(C_2&C_6 benzene), 115.74 (C_3& C_5 benzaldehyde), 45.47(C_3 pyrazole), 45.45(C_2 benzaldehyde), 39.76(C_4 pyrazole). TOF-MS ES+1.55e3, 390(11%), Anal calcd for: $C_{23}H_{18}N_4O_2$; C; 69.08, H; 4.71, N; 14.65; O; 8.37. Found: C; 69.18, H; 4.77, N; 14.82; O; 8.43.

3.2.10 Synthesis of 4-(2-Amino-ethyl)-2-[5-(1H-benzoimidazol-2-yl)-2-phenyl-3, 4-dihydro-2H-pyrazol-3-yl]-benzaldehyde (7d)

Percentage yield 52% m.p. 310ºC, IR (KBr) cm^{-}3067 (N-H), 1465 (C=C), 2938 (C-H), 1564 (N-H), 1730 (C=O), 1433 (C-N), 1209 (C-C), 1615 (C=N), 1270 (C-C), 1047 (C-H), 960(C-H), 858 (C-H), 840 (N-H). ^{1}H-NMR (DMSO-d_6) 1.4840 (s, 1H, NH), 2.11 (s, 2H, NH_2), 5.52 (d, 1H, *J* = 2.5 Hz, NH), 7.93 (m, 2H, Ar-H), 7.39 (m, 2H, Ar-H), 7.26 (m, 2H, Ar-H), 7.23(m, 1H, Ar-H), 7.22(m, 2H, Ar-H), 7.20(m, 2H, Ar-H). ^{13}C-NMR (500 Hz, DMSO, δ in ppm); 195.68((C=O benzaldehyde), 158.17(C_5 pyrazole), 135.11(C_8&C_9 benzimidazole), 127.15(C_3 benzaldehyde), 132.78(C_1 benzaldehyde), 158.17(C_4 benzaldehyde),, 126.57(C_5 benzaldehyde), 129.93(C_3&C_5 benzene), 128.65(C_2 benzaldehyde),115.10 (C_2&C_6 benzene), 126.57(C_5&C_6 benzimidazole), 129.9(C_6 benzaldehyde), 115.10(C_4 benzene), 115.10 (C_4&C_7 benzimidazole),45.23(C_3 pyrazole) 45.23 (C_3 amino benzaldehyde), 39.95(C_2 amino benzaldehyde), 39.74(C_4 pyrazole),

TOF-MS ES+1.55e3, 410(6.8%), anal calcd for: $C_{25}H_{23}N_5O$; C; 73.34, H; 5.62, N; 17.11; O; 3.9. Found: C; 73.34, H; 5.62, N; 17.11; O; 3.9.

3.2.11 Synthesis of 6-[5-(1H-Benzoimidazol-2-yl)-2-phenyl-3, 4-dihydro-2H-pyrazol-3-yl]-6-nitro-cyclohexa-1, 3-dienecarbaldehyde (7e)

Percentage yield 56% m.p. 300ºC, IR (KBr) cm^{-} 3167 (N-H), 1485 (C=C), 3038 (C-H), 1504 (N-H), 1728 (C=O), 1439 (C-N), 1212(C-C), 1613 (C=N), 1295(C-C), 1043 (C-H), 958(C-H), 860 (C-H), 834 (N-H). ^{1}H-NMR (DMSO-d_6) 1.4840 (s, 1H, NH), 2.11 (s, 2H, NH_2), 5.52 (d, 1H, *J* = 2.5 Hz, NH), 7.93 (m, 2H, Ar-H), 7.39 (m, 2H, Ar-H), 7.26 (m, 2H, Ar-H), 7.23(m, 1H, Ar-H), 7.22(m, 2H, Ar-H), 7.20(m, 2H, Ar-H). ^{13}C-NMR (500 Hz, DMSO, δ in ppm); 195.68((C=O benzaldehyde), 158.17(C_5 pyrazole), 135.11(C_8&C_9 benzimidazole), 127.15(C_3 benzaldehyde), 132.78(C_1 benzaldehyde), 158.17(C_4 benzaldehyde),, 126.57(C_5 benzaldehyde), 129.93(C_3&C_5 benzene), 128.65(C_2 benzaldehyde),115.10 (C_2&C_6 benzene), 126.57(C_5&C_6 benzimidazole), 129.9(C_6 benzaldehyde), 115.10(C_4 benzene), 115.10 (C_4&C_7 benzimidazole),45.23(C_3 pyrazole) 45.23 (C_3 amino benzaldehyde), 39.95(C_2 amino benzaldehyde), 39.74(C_4 pyrazole), TOF-MS ES+1.55e3, 412(12%), Anal calcd for: : $C_{23}H_{19}N_5O_3$; C; 66.81, H; 4.59, N; 16.93; O; 11.61 Found: C; 66.62, H; 4.63, N; 16.84; O; 11.72.

3.2.12 Synthesis of 6-[5-(1H-Benzoimidazol-2-yl)-2-phenyl-3, 4-dihydro-2H-pyrazol-3-yl]-6-hydroxy-cyclohexa-1, 3-dienecarbaldehyde (7f)

Percentage yield 35% m.p. 320 °C, IR (KBr) cm^{-1}; 3256 (N-H), 1467 (C=C), 2967 (C-H), 1549 (N-H), 1675 (C=N), 1468 (C-N), 1727 (C=O), 1132 (C-C), 1074 (C-C), 1265 (C-H), 964 (C-H), 865 (C-H), 839 (C-H).

^{1}H-NMR (DMSO-d_6) δ ppm 1.46(s, 2H,CH_2), 2.81 (s, 2H, NH_2), 2.45 (m, 2H, CH_2), 7.56 (m, 2H, Ar-H), 7.43 (m, 2H, Ar-H), 7.45(m, 1H, Ar-H), 5.36(s, 1H, CH), 7.75(m, 2H, Ar-H). ^{13}C-NMR (500 Hz, DMSO, δ in ppm); 192.78((C=O benzaldehyde), 156.57(C_2 benzimidazole) 162.21(C_3&C_5 pyrazole), 136.1(C_8&C_9 benzimidazole), 136.68(C_1 benzaldehyde), 123.78(C_2 benzaldehyde),, 129.66(C_5 benzaldehyde),, 129.93(C_6 benzaldehyde),, 128.78(C_3 benzaldehyde), 124.94(C_5&C_6 benzimidazole), 115.09 (C_4&C_7 benzimidazole), TOF-MS ES+1.55e3, 309(21%), Anal calcd for: : $C_{17}H_{16}N_4O_2$; C; 66.20, H; 5.19, N; 18.17; O; 10.38Found: C; 66.22, H; 5.21, N; 18.19; O; 10.34.

3.3 Molecular modeling and docking studies

MolDock is a docking module of Molegro Virtual Docker (MVD) software [14] it is based on a new hybrid search algorithm, called guided differential evolution (DE). The guided DE algorithm combines the DE optimization techniques with a cavity prediction algorithm. DE was introduced by Storn and Price in 1995 and has previously been successfully applied to molecular docking [15]. The use

of predicted cavities during the search process allows for a fast and accurate identification of potential binding modes (poses). The docking scoring function of MolDock is based on a piecewise linear potential (PLP) introduced by Gehlhaar et al [16]. In MolDock, the docking scoring function is extended with a new term, taking hydrogen bond directionality into account. Moreover, a re-ranking procedure was applied to the highest ranked poses to further increase docking accuracy. Initially, the protein was considered without ligand and water molecules. The backbone was fixed, the Charm M force field and minimization using steep descent algorithm was applied for homology modeled protein and all the compounds were prepared using the Charm M forces field and minimized up to a gradient of 0.01 kcal/ (mol Å) with the help of Discovery Studio 2.0 software (Telesis Court, San Diego, CA) as reported previously [17]. Template docking is based on extracting the chemical properties like the pharmacophore elements of a ligand bound in the active site. This information is utilized in the docking of the structurally similar analogs. The Albendazole bound protein structure PDB ID 1OJ0 was used as the template with the default settings was used for docking studies, including a grid resolution of 0.30, for grid generation and a 11 Å radius from the template as the binding site as reported previously by our group [18]. Mol Dock SE was used as a search algorithm and the number of runs was set to 10. A population size of 50 and a maximum iteration of 1500 was used for parameter settings. The maximum number of poses generated was 10. Since Molegro virtual docker works by an evolutionary algorithm, consecutive docking runs do not give exactly the same pose and interactions as reported previously by our group [19, 20]. To address this inherent randomness, three consecutive runs were done and the top three poses were used to visualize the interactions of all inhibitors. The homology modeled protein structure as reported in the section above was used for docking experiments and the same protocol was used to validate the interactions and scoring functions in the docking experiments.

4. Conclusion

Newer substituted pyrazoline and phenyl pyrazoline derivatives benzimidazole were synthesized. All the synthesized compounds were tested for anthelmintic activity against adult earth worms (P. posthuma) due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human being. Albendazole, one of the reference compound in the present study is effective in a broad range of helminth infections, including round worms, hookworms, whipworms, pinworms and its mechanism of action involves inhibition of the glucose uptake system leading to a lethal depletion of energy reserves in the helminthes

From the observations made in the present study, lower concentration of the synthesized derivatives exhibited paralytic effect much earlier and the time to death was shorter for worms. Out of the twelve synthesized derivatives, five compounds (6d, 6c, 7a, 7c, 7d) showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with all three concentrations of the derivatives.

Best anthelmintic activity was reported for p-amino ethyl and p-hydroxyl group substituted pyrazoline containing benzimidazole as 6c, 6d.Compound substituted by phenyl ring (R_1=C_6H_5) showed more activity than the compound containing (R_1=H) as in7c,7d. Among these derivatives (6c, 6d) showed superior activity and derivatives 7a, 7b, 7c, 7d showed similar activity to standard drug at the same concentration.

5. References

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