doi.org/10.36721/PJPS.2020.33.2.SUP.847-854.1

Synthesis, molecular docking and anti-diabetic studies of novel benzimidazole-pyrazoline hybrid molecules

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Abstract: Pyrazoline and benzimidazoles derivatives have been widely studied due to their potential applications in the medicinal field. In this research project, we have hybridized these two heterocyclic systems in the same molecule. A new series of compounds, 2-((3,5-diaryl-4,5-dihydro-*1H*-pyrazol-1-yl)methyl)-*1H*-benzo[d]imidazole (5a-i) were synthesized through a multistep reaction. In the first step, chalcones 3a-i were prepared by coupling of various acetophenones and benzaldehydes under alkaline conditions. These chalcones were cyclized with hydrazine hydrate to form a series of pyrazolines which were finally coupled with 2-chloromethyl-1*H*-benzimidazole to get a new series of titled hybrid molecules. The structures of these compounds were elucidated by spectral (^{1}H NMR and ^{13}C NMR) analysis. The anti-diabetic potential of these compounds was studied by screening them for their α-glucosidase inhibition activity. The SAR was established through molecular docking analysis. Compound 5d appeared as effective inhibitor with IC_{50} = 50.06µM as compared to reference drug (acarbose) having IC_{50} = 58.8µM.

Keywords: Pyrazoline-benzimidazole hybrids, anti-diabetic activity, α-glucosidase inhibition activity.

INTRODUCTION

Benzimidazole ring system is an important pharmacophore in medicinal chemistry as being a part of a number of medicinal compounds (Milen *et al*., 2019). It is acting as a template for various drugs like albendazole (1), mebendzole (2) and thiabendazole (3) which are used to treat ulcer disease andparasitic worm infestations respectively (Horvat, *et al*., 2012) while, carbendazim (4), phenzidole (5) and maribavir (6) are fungicidal, anti-parasitic and antiviral drugs respectively (Figure 1) (Anand & Wakode, 2017).

Benzimidazole derivatives have been reported as anti-inflammatory (Rathore *et al*., 2017), anticancer (single *et al*., 2015), anti-antitubercular (Shaharyar *et al*., 2017), antimicrobial activity (Alasmary *et al*., 2015), anti-tumor (El-gohary *et al.*, 2017), anthelmintic (Ajani *et al*., 2017), anti-oxidant (Rodriguez *et al.*, 2020) and anti-leishmanial (Nieto-Meneses *et al.*, 2018).

On the other hand, pyrazolines and pyrazoles are known for their anti-microbial (Ahmad *et al.*, 2016), anti-malarial (Rnghuvanshi *et al.*, 2019), anti-tumor (Chen *et al.*, 2018), anti-convulsant (Beyhan *et al.*, 2017) and anti-cancer (Lu *et al.*, 2017) activities. Pyrazoline-benzimidazole hybrids were reported for their antimicrobial activities (Desai *et al*., 2014).

In this research work, we have hybridized the two ring systems with the hope to get potent biologically active molecules. The compounds were screened for their anti-diabetic potential and compound 5d was observed as a potent α-glucosidase inhibitor.

MATERIALS AND METHODS

Chemistry

All the required chemicals were purchased from Sigma Aldrich & Alfa Aesar and were consumed without further purification. ^{1}H NMR and ^{13}C NMR spectra were taken on Bruker DPX 400 instrument at 600 MHz. The precursor compounds, 4a-i were synthesized according to the reported methods and were used for the synthesis of new compounds 5a-i.

General procedure for the synthesis of chalcones, 3a-i

Chalcones 3a-i were synthesized by Claisen-Schmidt base catalyzed condensation reaction of acetophenones and benzaldehydes by known literature methods (Choudhary *et al*., 2011). Equimolar quantities of acetophenone and benzaldehyde were dissolved in methanol, then 5% aqueous solution of KOH was added dropwise. The mixture was stirred at room temperature until the ppts of respective chalcone were formed. These ppts. were filtered, washed with water and methanol respectively and were dried.

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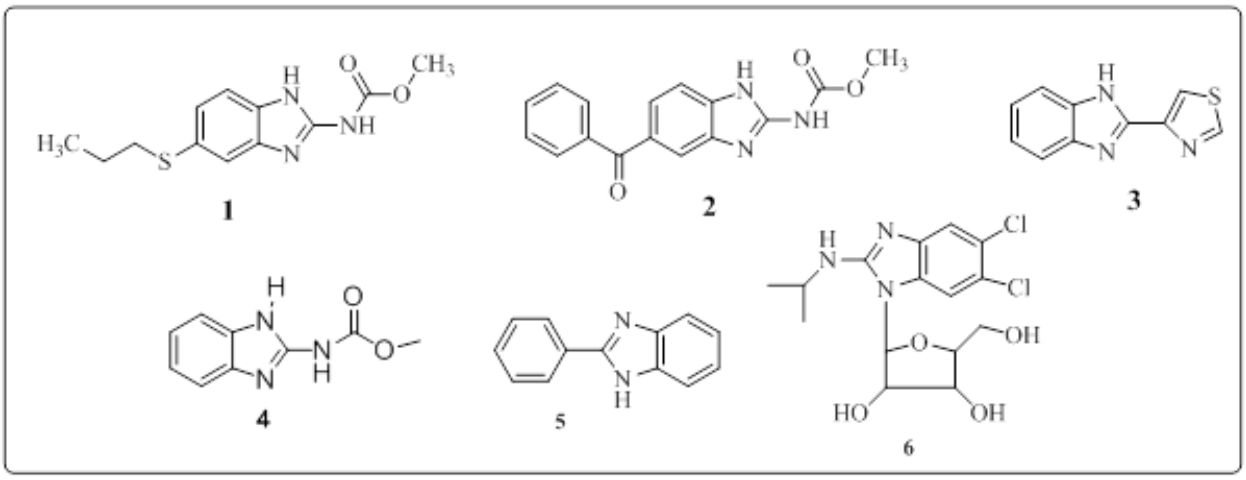
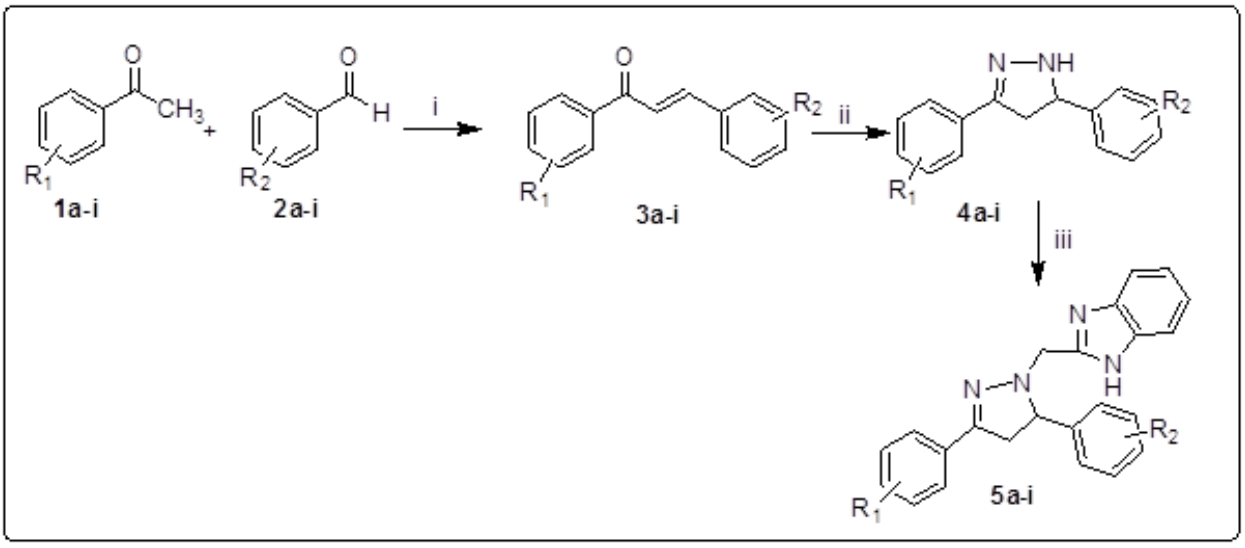


Fig. 1: Chemical structures of benzimidazole based drugs



Reagents and Conditions. i: Methanol, aq. NaOH (20%), rt. ii: Methanol, hydrazine, reflux. iii: Acetonitrile, K_2CO_3, 2-Chloromethylbenzimidazole, reflux.

Scheme 1: Synthetic layout for the titled hybrids.

General procedure for the synthesis of pyrazoline derivatives, 4a-i

Pyrazoline derivatives 4a-i were synthesized according to reported procedures (Lévai *et al*., 2005). Chalcone and hydrazine hydrate were mixed in methanol in 1:7 mole ratio and the mixture was refluxed until the completion of reaction. On completion of reaction, the contents of flask were poured in cold 5% solution of HCl and the ppts were obtained which were purified by column chromatography. The compounds were characterized by comparing the melting points with literature values (table 2).

General procedure for the synthesis of new benzimidazoles and pyrazoline hybrids, 5a-i

A mixture of pyrazoline (3 mmoles), 2-chloromethyl-1*H*-benzimidazole (3mmoles) and K_2CO_3 (6mmoles) in acetonitrile (15ml) was refluxed for 4 hours. TLC was performed to monitor the reaction. After completion of reaction, the precipitates of benzimidazole-pyrazoline hybrids were generated by adding ice cold water which were filtered, washed with cold water and dried.

Alpha-glucosidase inhibition activity

In vitro enzyme inhibition activity of all the compounds was evaluated spectrophotometrically by using the previously reported assay with slight modifications (Wang *et al*., 2017a). DMSO was used as solvent to prepare solutions of titled compounds, while solution of α-glucosidase enzyme (*Saccharomyces cerevisiae*, Sigma-Aldrich) was prepared in phosphate buffer (pH 6.8, 100mM). Test compounds (12.5µL), 0.5U/mL enzyme (40µL) and 100 mM phosphate buffer (120µL) were added in 96-well microliter plates. Incubation at 37°C for 5min was executed and then 5mM of PNPG (*p*-

nitrophenyl-α-D-glucopyranoside, Sigma Aldrich) was dropped in each well. After incubation for further 30min, 1000mM sodium carbonate solution (30µL) was added and absorbance was found at 405 nm and 37°C. Acarbose (Sigma-Aldrich) and DMSO were used as standard inhibitor and negative control respectively. Experiment was performed in triplicate and finally IC_{50} values were determined.

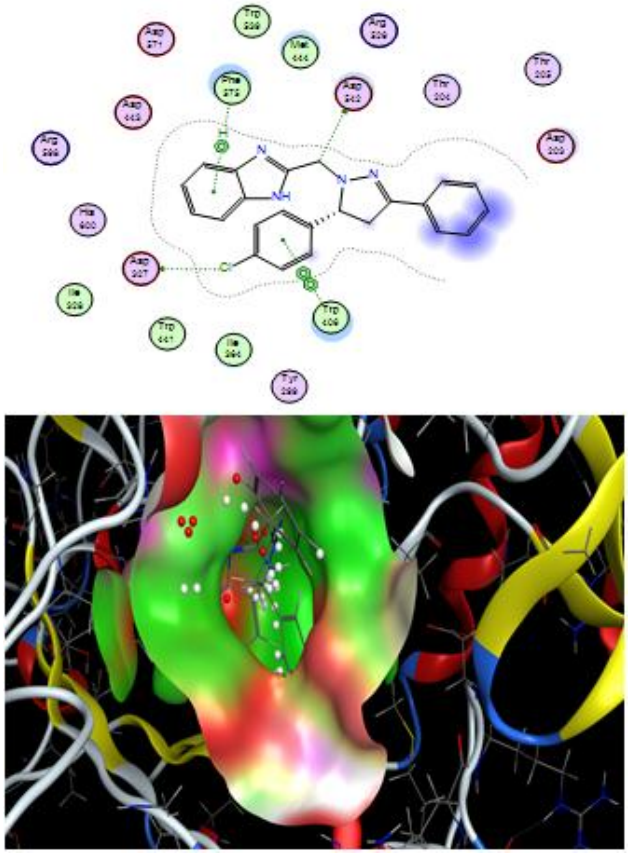


Fig. 2: Binding interactions of compound **5d** inside the binding pocket of α-glucosidase.

Molecular docking studies

The 3D structure of alpha-glucosidase was retrieved from protein data bank (PDB) using PDB ID: 2QMJ. The retrieved structure was optimized by removing ligand and solvent residues, 3D protonation and energy minimization using Molecular Operating Environment (MOE) (Vilar, Cozza *et al*. 2008). Active site containing Asp (A203), Asp (A542), Asp (A327), His (A600), and Arg (A526) were selected. Compounds were docked with interacting residues of α- glucosidase through docking algorithm of MOE software. The MOE program validates accurate conformation of ligand to get minimum energy structure.

STATISTICAL ANALYSIS

All experiments were conducted in replicate. *Mean of experiments are presented, n=3 and $p<0.05$ were considered statistically significant. All statistical analysis were performed through SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Chemistry

The structures of the synthesized compounds 5a-i were elucidated with the help of NMR analysis and the results are described in table 3. While, the intermediates 3a-i and 4a-i were confirmed with the help of literature (table 2).

Anti-diabetic activity

The pyrazoline-benzimidazole hybrids 5a-i were evaluated for their anti-diabetic activity via α-glucosidase enzyme inhibition assay (table 4). The compounds 5d, 5f and 5h showed 85.05%, 81.94%, 66.44% and 89.48% inhibition with IC_{50} values of 50.06µM, 149.5µM and 78.01µM, respectively. The results showed that the compound 5d exhibited better enzyme inhibition activity with IC_{50} of 50.06µM as comparison to acarbose (IC_{50} =58.88 µM) as a positive control.

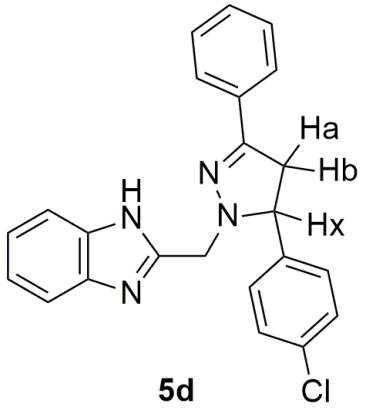


Fig. 3: Structure of compound 5d

Table 1: Structural parameters of the compounds 5a-i

Compound	R_1	R_2
5a	4-NO_2	4-$N(CH_3)_2$
5b	H	4-$N(CH_3)_2$
5c	4-Br	4-$N(CH_3)_2$
5d	H	4-Cl
5e	4-NO_2	4-Cl
5f	H	3,4-(OCH_3)
5g	4-Br	3,4-(OCH_3)
5h	2-NO_2	4-$N(CH_3)_2$
5i	4-Br	4-Cl

In-silico screening

Compound with best and top conformation were determined on the basis of S-score and RMSD value. Compound 5d exhibited minimum docking score -10.26 as shown in table 5 and fig. 2.

Table 2: Literature dealing the synthesis of chalcones 3a-i and pyrazolines 4a-i

Chalcones	M.pt ^{o}C	References	Pyrazolines	M.pt ^{o}C	References
3a	-	(Niu *et al.,* 2017)	4a	108-110	(Gupta *et al.,* 2013)
3b	113-114	(Alam *et al.*,2015)	4b	-	(Havrylyuk *et al.,* 2012)
3c	120-122	(Asiri *et al.,* 2016)	4c	-	(El-Hashash *et al.,* 2019)
3d	-	(Xu *et al.,* 2004)	4d	-	(Raghav *et al.,* 2014)
3e	176-178	(Sebti *et al.,* 2001)	4e	162-164	(Gupta *et al.,* 2013)
3f	86.5-88.5	(Sahin *et al.,* 2011)	4f	85-88	(Plaska *et al.,* 2001)
3g	-	(Zheng *et al.,* 2015)	4g	75-79	(Plaska *et al.,* 2001)
3h	-	(Bunce *et al.,* 2011)	4h	-	-
3i	164-168	(Sivamani *et al.,* 2014)	4i	-	(Applequist *et al.,* 1981)

Table 3: Spectral data of compounds 5a-i

Compound	M.p. (^{o}C)	Yield (%)	Characterization Data
5a	110	80	^{1}H NMR (DMSO-d6, ppm, 600 MHz) δ 2.93 (s, 6H, $N(CH_3)_2$), 3.04 (dd, 1H, J_1=30.5 Hz, J_2=16.29 Hz, pyrazoline), 3.57 (dd, 1H, J_1=16.24 Hz, J_2=10.60 Hz, pyrazoline), 4.25 (d, 1H, J = 15.15 Hz), 4.55 (d, 1H, J = 15.17 Hz), 4.67 (t, 1H, pyrazoline), 6. 78 (d, 2H, J = 7.9 Hz, Ar-H), 7.18 (s, 2H, Ar-H), 7.41 (d, 2H, J=7.9 Hz, Ar-H,), 7.55 (s, 2H, Ar-H), 7.89 (d, 2H, J=8.08 Hz, Ar-H,), 8.26 (d, 2H, J =8.1 Hz, Ar-H,). C^{13} NMR (DMSO-d_6) δ (ppm): 39.0 (C), 49.4 (2C), 69.2 (2C), 112.4 (4C), 123.8 (2C), 126.0 (2C), 126.3 (2C), 128.4 (2C), 139.0 (2C), 146.6 (2C), 147.6 (C),150.3 (C), 150.6 (2C).
5b	139	87	^{1}H NMR (DMSO-d6, ppm, 600 MHz) δ 2.9 (s, 6H, $N(CH_3)_2$), 3.0 (dd, 1H, J_1=30.5 Hz, J_2=16.29 Hz, pyrazoline), 3.5 (dd, 1H, J_1=16.24 Hz, J_2=10.60 Hz, pyrazoline), 4.10 (d, 1H, J=15 Hz), 4.4 (d, 1H, J=15Hz), 4.5 (dd, 1H, J_1=14.05 Hz, J_2=10.06 Hz, pyrazoline), 6.7 (d, 2H , J= 8.72 Hz, Ar-H), 7.11 (dd, 2H, J_1=5.97 Hz, J_2=3.13 Hz, Ar-H), 7.32-7.39 (m, Ar-H), 7.5 (dd, 2H, J_1=5.85 Hz, J_2=3.22 Hz, Ar- H),7.64 (d, 2H, J=7.12 Hz, Ar-H). C^{13} NMR (DMSO-d_6) δ (ppm): 40.0 (C), 41.4 (2C), 69.2 (2C), 112.4 (4C), 121.8 (2C), 125.0 (2C), 126.3 (2C), 128.4 (2C), 128.6 (2C), 132.6 (2C), 150.2 (4C).
5c	120	82	^{1}H NMR (DMSO-d6, ppm, 600 MHz) δ 2.9 (s, 6H, $N(CH_3)_2$), 3.0 (dd, 1H, J_1=30.5 Hz, J_2=16.29 Hz, pyrazoline), 3.5 (dd, 1H, J_1=16.24 Hz, J_2=10.60 Hz, pyrazoline), 4.11 (d, 1H, J=15 Hz), 4.36 (d, 1H, J=15 Hz), 4.48 (dd, 1H, J_1=13.8 Hz, J_2=10.3 Hz, pyrazoline), 6.73 (d, 2H, J=8.6 Hz, Ar-H), 7.11-7.13 (m, 2H, Ar-H), 7.38 (d, 2H, J=8.56 Hz), 7.49 (dd, 2H, J_1=5.4 Hz, J_2=3.36 Hz, Ar-H), 7.6 (q, 2H, J=4.9 Hz, J=14 Hz, Ar-H). C^{13} NMR (DMSO-d_6) δ (ppm): 39.0 (C), 40.1 (2C), 69.4 (2C), 112.4 (4C), 121.3 (2C), 126.3 (2C), 127.7 (2C), 128.4 (2C), 131.4 (2C), 131.9 (2C), 149.2 (C), 150.2 (C), 151.0 (2C).
5d	178	77	^{1}H NMR (DMSO-d6, ppm, 600 MHz) δ 2.9 (s, 6H, $N(CH_3)_2$), 3.0 (dd, 1H, J_1 = 30.5 Hz, J_2 = 16.29 Hz, pyrazoline), 3.5 (dd, 1H, J_1 = 16.24 Hz, J_2 = 10.60 Hz, pyrazoline), 4.11 (d, 1H, J = 15 Hz), 4.36 (d, 1H, J = 15 Hz), 4.48 (dd, 1H, J_1 = 13.8 Hz, J_2 = 10.3 Hz, pyrazoline), 7.21 (s, 2H, Ar-H), 7.37-7.48 (m, 7H, Ar-H), 7.84 (m, 8H, Ar-H). C^{13} NMR (DMSO-d_6) δ (ppm): 39.8 (C), 49.9 (2C), 68.2 (2C), 120.4 (2C), 122.8 (2C), 125.0 (2C), 125.3 (2C), 127.4 (2C), 128.0 (2C), 145.6 (2C), 146.7 (C),149.3 (C), 150.7 (2C).
5e	140	85	^{1}H NMR (DMSO-d6, ppm, 600 MHz) δ 3.02 (dd, 1H, J_1 = 16.5 Hz, J_2 = 14.05 Hz, pyrazoline), 3.66 (dd, 1H, J_1 = 16.5 Hz, J_2 = 10.6 Hz, pyrazoline), 4.27 (d, 1H, J = 15 Hz), 4.6 (d, 1H, J = 15 Hz), 4.77 (dd, 1H, J_1 = 13.8 Hz, J_2 = 10.8 Hz, pyrazoline), 7.13-7.16 (m, 2H, Ar-H), 7.44 (t, 2H, Ar-H), 7.50-7.52 (m, 2H, Ar-H), 7.58 (d, 2H, J = 8.5 Hz, Ar-H), 7.85 (d, 2H, J = 9 Hz, Ar-H), 8.22 (d, 2H, J = 9 Hz, Ar-H). C^{13} NMR (DMSO-d_6) δ (ppm): 39.9 (C), 49.9 (C), 68.6 (C), 121.5 (2C), 123.8 (2C), 126.5 (2C), 128.9 (2C), 130.5 (2C), 132.4 (2C), 138.6 (2C), 146.8 (2C), 147.8 (2C), 150.1 (2C).
5f	165	80	^{1}H NMR (DMSO-d6, ppm, 600 MHz) δ 2.99(t, 1H, pyrazoline), 3.52 (dd, 1H, J_1=16.37 Hz, J_2= 10.04 Hz, pyrazoline), 3.74 (s, 3H, OCH_3), 3.8(s, 3H, OCH_3), 4.25(d, 1H, J=15 Hz), 4.40 (d, 1H, J=15 Hz), 4.51 (dd, 1H, J_1=14.0 Hz, J_2=10.2 Hz, pyrazoline), 6.92 (d, 1H, J=8.20 Hz, Ar-H,), 7.06 (dd, 1H, J_1=8.2 Hz, J_2=1.71 Hz, Ar-H), 7.13-7.16 (m, 2H, Ar-H), 7.22 (d, 1H, J=1.20 Hz, Ar-H), 7.33-7.39 (m, 3H, Ar-H),7.52 (dd, 2H, J_1=5.7 Hz, J_2=3.28 Hz, Ar-H), 7.66 (d, 2H, J =7. 32 Hz, Ar-H). C^{13} NMR (DMSO-d_6) δ (ppm): 40.0 (C), 50.4 (C), 55.5 (2C), 69.6 (2C), 111.0 (2C), 111.8 (C), 120.1(C), 121.4 (2C), 127.7 (C), 131.4 (2C), 131.8 (2C), 131.8 (2C), 148.5 (2C), 148.9 (C), 149.3 (2C), 150.9 (C).

Continue…

Compound	M.p. (^oC)	Yield (%)	Characterization Data
5g	132	79	1H NMR (DMSO-d6, ppm, 600 MHz) δ 2.98 (dd, 1H, J_1=16Hz, J_2=14.5 Hz, pyrazoline), 3.52 (dd, 1H, J_1 = 16 Hz, J_2 = 10.2 Hz, pyrazoline), 3.74 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.22 (d, 1H, J= 15 Hz), 4.4(d, 1H, J = 15Hz), 4.53 (dd, 1H, J_1=14.1 Hz, J_2 = 10.2 Hz, pyrazoline), 6.93 (d, 1H, J = 8.21 Hz,Ar-H), 7.05 (dd, 1H, J_1 = 8.19 Hz, J_2 = 1.79 Hz, Ar-H), 7.12-7.15 (m, 2H, Ar-H), 7.2 (d, 1H, J =1.19 Hz, Ar-H), 7.50 (dd, 2H, J_1 = 5.44 Hz, J_2 = 3.32 Hz, Ar-H), 7.57-7.60 (m, 4H, Ar-H). C^{13} NMR (DMSO-d_6) δ (ppm): 40.0 (C), 50.4 (C), 55.5 (2C), 69.6 (2C), 111.0 (2C), 111.8 (C), 120.1 (C), 121.4 (2C), 127.7 (C), 131.4 (2C), 131.8 (2C), 131.8 (2C), 148.5 (2C), 148.9 (C), 149.3 (2C), 150.9 (C).
5h	118	70	1H NMR (DMSO-d6, ppm, 600 MHz) δ 3.17 (s, 6H, $N(CH_3)_2$), 4.23 (s, 3H, pyrazole), 6.28-6.34 (m, 2H, Ar-H), 6.49 (d, 1H, J=1.97 Hz, Ar-H), 6.98 (d, 1H, J= 7.16 Hz, Ar-H), 7.44-7.49 (m, 2H, Ar-H), 7.75 (d, 1H, J=7.41 Hz, Ar-H). C^{13} NMR (DMSO-d_6) δ (ppm): 39.0 (C), 40.4 (2C), 69.2 (2C), 112.4 (4C), 123.8 (2C), 126.0 (2C), 126.3 (2C), 128.4 (2C), 139.0 (2C), 146.6 (2C), 147.7 (C), 150.3 (C), 150.6 (2C).
5i	167	78	^{1}H NMR (DMSO-d6, ppm, 600 MHz) δ 2.91(t, 6H, pyrazoline), 3.34 (dd, 1H, J_1=16Hz, J_2 =14.58 Hz, pyrazoline), 4.11 (d, 1H, J=14.9 Hz), 4.36 (d,1H, J=15Hz), 4.48 (dd, 1H, J_1= 14 Hz, J_2=10.1 Hz, pyrazoline), 6.73 (d, 2H, J=8.58 Hz, Ar-H), 7.11-7.14 (m, 2H, Ar-H), 7.37 (d, 2H, J=8.56 Hz, Ar-H), 7.49 (dd, 2H, J_1=5.28 Hz, J_2 = 3.41 Hz, Ar-H), 7.57 (q, 4H, J=5.1 Hz, Ar-H). C^{13} NMR (DMSO-d_6) δ (ppm): 39.4 (C), 49.9 (2C), 69.1 (2C), 121.5 (2C), 123.7 (2C), 126.2 (2C), 125.3 (2C), 127.0 (2C), 129.0 (2C), 145.5 (2C), 146.6 (C),150.2 (2C), 151.5 (C).

Table 4: α-Glucosidase inhibition activity of compounds 5a-i

Compounds	% Inhibition	IC_{50} Value (µM)
5a	27.26%	ND
5b	39.513%	ND
5c	85.056%	251.90 µM
5d	81.947%	50.06 µM
5e	17.05%	ND
5f	66.44%	149.5 µM
5g	27.8%	ND
5h	89.48%	78.02 µM
Acarbose	98.4%	58.8 µM

*ND = Not Determined

Table 5: Molecular Docking of compound 5d and acarbose

Sr. No.	Compound	Score	Rmsd	Residues
1	Control (Acarbose)	-17.1746	1.421	Asp(A203), Asp(542), Asp(A327), His (600), Thr (205)
2	5d	-10.260	1.971	Asp327, asp542, TRP406, PHE575

DISCUSSION

Chemistry

The structural linkage of pyrazolines with benzimidazoles was carried out through a multi-step reaction. In the first step, the synthesis of chalcones was carried out according to previously reported methodology (table 2), in the next step, a series of pyrazolines was prepared by refluxing chalcones with hydrazine hydrate.

In the final step, these pyrazolines were coupled with 2-chloromethylbenzimidazole. The NMR analyses were carried out for characterization of these compounds. The aromatic hydrogens were observed in the 6-8 ppm range while the methylene group (a linker between pyrazoline and benzimidazole) was observed as two doublets due to enantiotropic protons at around shift values of 4.25 and 4.40ppm. The three protons of pyrazoline ring system were confirmed as three individual signals of doublet of doublets (dd) around 2.91, 3.34 and 4.48 ppm, each for one proton. These dd signals were attributed to the coupling of Hx, Ha and Hb protons (fig. 3). The literature was reviewed for the examples of appearance of these dd and many references were found for such cases in pyrazolines. The CH_2 and CH protons of pyrazoline were reported as doublets and multiplets around 3.49-3.63 ppm and 5.47-5.57ppm respectively. The CH_2 protons due to chiral CH in vicinity showed doublet of doublets at 3.11-3.63ppm (Jois *et al*., 2014). In many other cases, the pyrazoline protons showed doublet of doublets. Methine

protons of pyrazoline showed the doublet of doublet at 6.01-6.12 ppm while the two methylene protons displayed the doublet of doublets at 3.41-3.52 ppm and 3.90-3.96 ppm (Desai *et al*., 2014). The three distinct types of protons in pyrazoline i.e., Hx, Ha and Hb protons appeared as three doublet of doublets in ^{1}HNMR spectra. These dd were reported at 5.94 ppm (Hx), 4.46 ppm and 3.41 ppm (Ha & Hb) (Asiri *et al*., 2019). Salian *et al*. explained another case of pyrazoline ring system. Similar observations of germinal and vicinal coupling were carried out by other researchers (Salian *et al*., 2019), (Kumar *et al*., 2012), (Viveka *et al*., 2015) (Abdul-halim *et al.,* 2013). The other substituents on aromatic rings were observed at usual positions such as, $N(CH_3)_2$ moiety was observed as singlet at 3.18 (5a), OCH_3 moiety at 3.6 (5f) etc.

In vitro anti-diabetic activity and in silico screening

Heterocyclic compounds are frequently reported in literature for their potential to inhibit α-glucosidase. The *N*-containing heterocycles, such as piperidines and triazoles, are recently reported for the inhibition of α-glucosidase enzyme (Rafiq *et al.,* 2018). Various triazole containing sulphonamides In ourrecent work, we have reported 1,2-benzothiazine derivatives as anti-diabetic agents (Saddique *et al.,* 2019). Pyrazolines are reported in literature as excellent templates for alpha-gulcoside inhibitors (Emayavaramban *et al.,* 2013) and these observations led us for designing pyrazoline derivatives. The compounds 5d, 5f and 5h showed 85.05%, 81.94%, 66.44% and 89.48% inhibition with an IC_{50} values of 50.06µM, 149.5µM and 78.01µM, respectively. The insight in the chemical structures of active compounds reveals interesting information about the structure-activity relationship. The active compounds, 5d and 5h bear 4-Cl and 4-$N(CH_3)$ substitutions as R_2 respectively. Overall, these groups possess electron donating influence by resonance effect. Similar case is observed for 3,4-dimethoxy substitution for 5f.

The *in silico* approach was used for the further insight in order to establish SAR. The docking studies of various benzimidazole based α-glucosidase inhibitors is reported to bind with the active site residues *i.e.* Asp68, Asp214, Asp349 and Arg439 of target protein in alpha-glucosidase (Zawawi *et al.,* 2016). In another study of benzimidazole-quinoline hybrids, the active compounds were found to strongly bind to enzyme forming π−π interaction with the amino group of Lys513 amino acid residue of target site (Bharadwaj *et al.,* 2018).

In the current study, the molecular docking of active molecule 5d was performed. It was observed that the compound 5d showed a good docking score and binding interactions with the targeted site residues Asp(A203, Asp(542), Asp(A327), His (600, Thr(205). The minimum docking score of compound 5d is -10.26 with respect to the binding energy of acarbose (-17.17).

CONCLUSION

In short, we herein reported the synthetic methods for the structural hybridization of benzimidazole and pyrazoline heterocyclic ring systems. The structures of final compounds are well elucidated by using advanced spectroscopic techniques. In this study, three compounds, 5d, 5f and 5h appeared as good inhibitors of alpha-glucosidase. Compound 5d exhibited potent activity comparable to the reference (acarbose). The SAR was established by using molecular docking.

ACKNOWLEDGEMENTS

The authors are grateful to Government College University, Faisalabad for financial support through GCUF-RSP research grant.

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