Linear Synthesis of {4-[(2-Alkoxy/aralkyloxy-3,5-dichlorophenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanones as Possible Therapeutic Agents

[1] Muhammad Athar Abbasi*, [1] Wajiha Khan, [1] Aziz-ur-Rehman, [1] Sabahat Zahra Siddiqui, [1] Ghulam Hussain, [2] Syed Adnan Ali Shah, [3] Muhammad Shahid and [4,5]Khalid Mohammed Khan
[1]*Department of Chemistry, Government College University, Lahore-54000, Pakistan.*
[2]*Faculty of Pharmacy and Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Level 9, FF*
[3]*Universiti Teknologi MARA, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia.*
[3]*Department of Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan.*
[4]*H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.*
[5]*Department of Clinical Pharmacy, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, P.O. Box 31441, Dammam, Saudi Arabia*
atrabbasi@yahoo.com; abbasi@gcu.edu.pk

(Received on 14th February 2018, accepted in revised form 10th December 2018)

Summary: In the present work, a series of {4-[(2-alkyloxy/aralkyloxy-3,5-dichlorophenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanones was synthesized through a linear bi-step approach. The synthesis was initiated by the coupling of 2-furyl(1-piperazinyl)methanone (**1**) with 3,5-dichloro-2-hydroxybenzenesulfonyl chloride (**2**) under dynamic pH control in aqueous alkaline medium to form parent compound, {4-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanone (**3**). Then, the *O*-substitution reaction on nucleophile **3** was carried out by treating it with different alkyl/aralkyl halides (**4a-l**) in the presence of lithium hydride (LiH) and *N,N*-dimethylformamide (DMF) to obtained the designed {4-[(2-alkoxy/aralkyloxy-3,5-dichlorophenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanones (**5a-l**). These synthesized compounds were screened against α-glucosidase enzyme and some of them exhibited considerable inhibitory activity. Additionally, compounds were also evaluated for hemolytic and cytotoxic profile.

Keywords: 2-Furyl(1-piperazinyl)methanone, 3,5-dichloro-2-hydroxybenzenesulfonyl chloride, α-glucosidase inhibition, hemolytic activity.

Introduction

The 2-furoyl-1-piperazine bearing compounds have different therapeutic applications e.g. Prazosin hydrochloride;1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furoyl)piperazine (Fig. 1) is a sympatholytic alpha-adrenergic blocker. It is used in the treatment of refractory pulmonary oedema, anxiety, hypertension and panic disorders. It also reduces peripheral fighting and blood pressure while not increasing the heart rate or considerably impairing sympathetic function [1]. Prazosin has positive effects on the plasma lipids, with reductions in low density lipoprotein cholesterol, total serum cholesterol [2,3]. It is used to cure hypertension and refractory pulmonary oedema [4]. It is also practical in treating urinary hesitancy associated with prostatic hyperplasia by preventing alpha-1 receptors, which controls the constriction the prostate and uterus. Although, it is not a superior choice for hypertension, yet it is a selection for patients who face both problems concomitantly [5]. Prazosin is also used in the treatment of trauma nightmares, sleep disorder and to improve sleep quality [6].

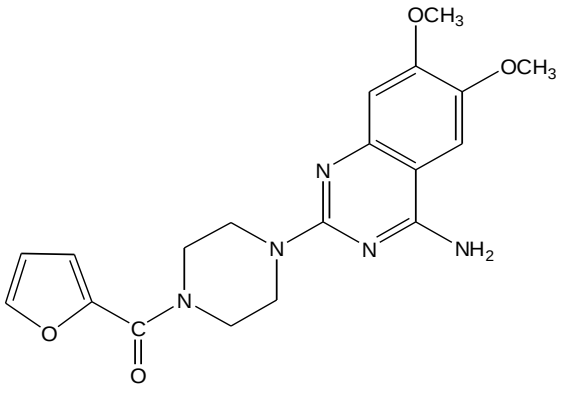


Fig. 1: Structure of Prazosin.

Sulfonamides are primarily antibacterial agents, but their uses are also proven to treat some other diseases. These are also outstanding for enzyme inhibition such as carbonic anhydrase, cysteine protease, HIV protease and cyclooxygenase [7]. In

***To whom all correspondence should be addressed.**

addition, they have broad therapeutic potential to treat other ailments such as in cancer chemotherapy, hypoglycemia and diuretics [8].

α-Glucosidase (*α*-D-Glucosideglucohydrolase, EC 3.2.1.20), a family of hydrolase enzymes [9], which hydrolyzes the 1,4-glycosidic linkage to produce *α*-D-glucose and other monosaccharides that are utilized as carbon and energy source [10]. The inhibitors of this enzyme delay the liberation of D-glucose and hence reduce postprandial hyperglycemia and type-2 diabetes [11]. Organic chemists and pharmacists have keen interest to design new therapeutically important drug constituents to cure different disorders and malfunctions [12]. Scientists' community because of increasing microbe resistance against the existing drugs, are trying to develop new multi-functionalized compounds with excellent therapeutic activity [13]. So, in the present research, a linear synthesis was designed by the amalgamation 2-furoyl-1-piperazine, sulfonamide and ether functionalities to explore their possible α-glucosidase inhibitory potential. Moreover, their hemolytic activity was also ascertained to find out their utility as less cytotoxic agents.

Experimental

General

Chemicals were purchased from Sigma Aldrich & Alfa Aesar (Germany) and solvents of analytical grades were supplied by local suppliers. By using open capillary tube method, melting points were taken on Griffin and George apparatus and were uncorrected. By using thin layer chromatography (with ethyl acetate and *n*-hexane (30:70) as mobile phase), initial purity of compounds was detected at 254 nm. Elemental analyses were performed on a Foss Heraeus CHN-O-Rapid instrument and were within ± 0.4% of the theoretical values. IR peaks were recorded on a Jasco-320-A spectrometer by using KBr pellet method. ^{1}H-NMR spectra (δ, ppm) were recorded at 600 MHz (^{13}C-NMR spectra, at 150 MHz) in $CDCl_3$ using the Bruker Advance III 600 As- cend spectrometer using BBO probe. The abbreviations used in interpretation of ^{1}H NMR spectra are as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br.t, broad triplet; q, quartet; quint, quintet; m, multiplet; dist. distorted.

Synthesis of 2{4-[(3,5-Dichloro-2-hydroxyphenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanone **(3)**

2-Furyl(1-piperazinyl)methanone (12.8 mmol; 2.31 g, **1**) was suspended in 100 mL distilled water and the pH was maintained at 9.0 to 10.0 by adding an aqueous solution of 10% Na_2CO_3 followed by slow addition of 3,5-dichloro-2-hydroxybenzenesulfonyl chloride (12.8 mmol; 3.55g, **2**) to the reaction mixture over 10-15 min. The reaction mixture was stirred at RT for 3 hrs and monitored with TLC till completion of the reaction. Conc. HCl (around 4 mL) was then added slowly to adjust the pH to 2.0 and reaction medium was rested at RT for 15 minutes, light brown amorphous solid was filtered and washed with distilled water to afford {4-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanone **(3)** after drying.

Synthesis of {4-[(2-Alkoxy/aralkyloxy-3,5-dichlorophenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanones **(5a-l)**

The parent compound, {4-[(3,5-Dichloro-2-hydroxyphenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanone (1.66 mmol;0.2 g, **3**), in *N,N*-dimethylformamide (5 mL) and catalytic amount of lithium hydride (0.42 mmol, 0.01 g) was added in a 25 mL round bottomed flask and stirred for 15 min at room temperature after which corresponding alkyl/aralkyl halides (1.66 mmol; **4a-l**) were added in the reaction mixture which was further stirred for 4-5 hrs. After reaction completion, reaction mixture was quenched with ice cold water (200 mL). The obtained solid was filtered, washed with distilled water and dried to yield the corresponding {4-[(2-alkoxy/aralkyloxy-3,5-dichlorophenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanones (**5a–l**). In some cases, the compound was isolated via solvent extraction using chloroform/ethyl acetate as organic solvent.

{4-[(3,5-Dichloro-2-ethoxyphenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanone **(5a)**

White amorphous solid; Yield: 87 %; m.p: 124-126 ºC; Mol. F.: $C_{17}H_{18}Cl_2N_2O_5S$; Mol. Mass: 432 g/mol. IR (KBr, υ, cm^{-1}): 3085 (C-H str. of aromatic ring), 2876 (C-H str. of aliphatic), 1658 (C=O str.), 1580 (C=C aromatic str.), 1432 (S=O), 1189 (C-O-C bond str.), 1115 (C-N-C bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.78 (d, J = 2.5 Hz, 1H, H-6"), 7.59 (d, J = 2.5 Hz, 1H, H-4"), 7.47 (dist. d, J = 0.9 Hz, 1H, H-5), 7.03 (d, J = 2.6 Hz, 1H, H-3), 6.48 (dd, J = 1.7, 3.4 Hz, 1H, H-4), 4.21 (q, J = 6.9 Hz, 2H, CH_2-1'"), 3.87 (br.s, 4H, CH_2-3′, CH_2-5′), 3.30 (t, J = 5.1 Hz, 4H, CH_2-2′, CH_2-6′), 1.47 (t, J = 7.0 Hz, 3H, CH_3-2'"); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 159.01 (C-2"), 152.06 (C-6), 147.52 (C-2), 143.93 (C-5), 134.99 (C-4"), 134.33 (C-5"), 131.33 (C-6"), 129.55 (C-3"), 129.52 (C-1"),

117.36 (C-3), 111.53 (C-4), 71.29 (C-1‴), 46.32 (C-2', C-3', C-5', C-6'), 15.37 (C-2‴); Anal. Calc. for $C_{17}H_{18}Cl_2N_2O_5S$ (432.03): C, 47.12; H, 4.19; N, 6.47. Found: C, 47.17; H, 4.12; N, 6.44.

(4-{[3,5-Dichloro-2-(2-chloroethoxy)phenyl]sulfonyl}-1-piperazinyl)(2-furyl)methanone **(5b)**

Light brown amorphous solid; Yield: 92 %; m.p: 129-131 °C; Mol. F.: $C_{17}H_{17}Cl_3N_2O_5S$; Mol. Mass: 466 g/mol. IR (KBr, υ, cm^{-1}): 3088 (C-H str. of aromatic ring), 2885 (C-H str. of aliphatic), 1659 (C=O str.), 1575 (C=C aromatic str.), 1429 (S=O), 1188 (C-O-C bond str.), 1117 (C-N-C bond str.), 660 (C-Cl bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.70 (d, *J* = 2.3 Hz, 1H, H-6"), 7.68 (d, *J* = 2.3 Hz, 1H, H-4"), 7.48 (d, *J* = 1.2 Hz, 1H, H-5), 7.00 (d, *J* = 3.2 Hz, 1H, H-3), 6.47 (dd, *J* = 1.3, 3.2 Hz, 1H, H-4), 3.97 (br.s, 2H, CH_2-1‴), 3.96 (br.s, 4H, CH_2-3′, CH_2-5′), 3.52 (t, *J* = 6.5, 2H, CH_2-2‴), 3.20 (br.t, *J* = 4.9 Hz, 4H, CH_2-2′, CH_2-6′); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 159.07 (C-2"), 152.45 (C-6), 147.50 (C-2), 143.93 (C-5), 134.76 (C-4"), 134.31 (C-5"), 131.13 (C-6"), 129.57 (C-1"), 129.12 (C-3"), 117.32 (C-3), 111.51 (C-4), 71.20 (C-1‴), 45.36 (C-2', C-3', C-5', C-6'), 35.04 (C-2‴); Anal. Calc. for $C_{17}H_{17}Cl_3N_2O_5S$ (465.99): C, 43.65; H, 3.66; N, 5.99. Found: C, 43.69; H, 3.61; N, 5.92.

(4-{[(2-(2-Bromoethoxy)-3,5-dichlorophenyl]sulfonyl}-1-piperazinyl)(2-furyl)methanone **(5c)**

Brown amorphous solid; Yield: 80 %; m.p: 128-130 °C; Mol. F.: $C_{17}H_{17}BrCl_2N_2O_5S$; Mol. Mass: 510 g/mol. IR (KBr, υ, cm^{-1}): 3089 (C-H str. of aromatic ring), 2887 (C-H str. of aliphatic), 1663 (C=O str.), 1580 (C=C aromatic str.), 1431 (S=O), 1198 (C-O-C bond str.), 1119 (C-N-C bond str.), 621 (C-Br bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.78 (d, *J* = 2.4 Hz, 1H, H-6"), 7.65 (d, *J* = 2.4 Hz, 1H, H-4"), 7.47 (d, *J* = 1.5 Hz, 1H, H-5), 7.07 (d, *J* = 3.3 Hz, 1H, H-3), 6.48 (dd, *J* = 1.5, 3.2 Hz, 1H, H-4), 3.88 (br.s, 2H, CH_2-1‴), 3.80 (br.s, 4H, CH_2-3′, CH_2-5′), 3.33 (t, *J* = 6.4, 2H, CH_2-2‴), 3.22 (br.t, *J* = 4.5 Hz 4H, CH_2-2′, CH_2-6′); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 159.02 (C-2"), 152.98 (C-6), 147.77 (C-2), 143.01 (C-5), 134.90 (C-4"), 134.37 (C-5"), 131.00 (C-6"), 129.17 (C-3"), 128.12 (C-1"), 117.44 (C-3), 111.32 (C-4), 67.20 (C-1‴), 46.31 (C-2', C-3', C-5', C-6'), 27.09 (C-2‴); Anal. Calc. for $C_{17}H_{17}BrCl_2N_2O_5S$ (509.94): C, 39.86; H, 3.35; N, 5.47. Found: C, 39.82; H, 3.38; N, 5.43.

(4-{[3,5-dichloro-2-(3-iodopropoxy)phenyl]sulfonyl}-1-piperazinyl)(2-furyl)methanone **(5d)**

Light brown amorphous solid; Yield: 80 %; m.p: 126-128 °C; Mol. F.: $C_{18}H_{19}Cl_2IN_2O_5S$; Mol. Mass: 572 g/mol. IR (KBr, υ, cm^{-1}): 3082 (C-H str. of aromatic ring), 2890 (C-H str. of aliphatic), 1652 (C=O str.), 1572 (C=C aromatic str.), 1430 (S=O), 1199 (C-O-C bond str.), 1112 (C-N-C bond str.), 496 (C-I bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.82 (d, *J* = 2.5 Hz, 1H, H-6"), 7.60 (d, *J* = 2.5 Hz, 1H, H-4"), 7.49 (br.s, 1H, H-5), 7.03 (d, *J* = 3.2 Hz, 1H, H-3), 6.52 (dd, *J* = 1.5, 3.3 Hz, 1H, H-4), 3.87 (br.s, 4H, CH_2-3′, CH_2-5′), 3.66 (br.s, 2H, CH_2-1‴), 3.32 (t, *J* = 6.4, 2H, CH_2-3‴), 3.30 (br.t, *J* = 4.9 Hz, 4H, CH_2-2′, CH_2-6′) 2.42 (quint, *J* = 6.8, 2H, CH_2-2‴); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 158.38 (C-2"), 152.52 (C-6), 147.47 (C-2), 143.91 (C-5), 134.97 (C-4"), 134.33 (C-5"), 131.38 (C-6"), 129.90 (C-1"), 129.51 (C-3"), 117.65 (C-3), 111.52 (C-4), 65.75 (C-1‴), 46.00 (C-2', C-3', C-5', C-6'), 32.90 (C-2‴), 4.46 (C-3‴); Anal. Calc. for $C_{18}H_{19}Cl_2IN_2O_5S$ (571.94): C, 37.71; H, 3.34; N, 4.89. Found: C, 37.77; H, 3.31; N, 4.81.

(4-{[(3,5-Dichloro-2-(2-propoxy)phenyl])sulfonyl}-1-piperazinyl)(2-furyl)methanone **(5e)**

Brown amorphous solid; Yield: 89 %; m.p: 125-127 °C; Mol. F.: $C_{18}H_{20}Cl_2N_2O_5S$; Mol. Mass: 446 g/mol. IR (KBr, υ, cm^{-1}): 3087 (C-H str. of aromatic ring), 2882 (C-H str. of aliphatic), 1649 (C=O str.), 1581 (C=C aromatic str.), 1430 (S=O), 1195 (C-O-C bond str.), 1114 (C-N-C bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.84 (d, *J* = 2.4 Hz, 1H, H-6"), 7.64 (d, *J* = 2.5 Hz, 1H, H-4"), 7.49 (d, *J* = 1.5 Hz, 1H, H-5), 7.02 (d, *J* = 3.5 Hz, 1H, H-3), 6.56 (dd, *J* = 1.4, 3.6 Hz, 1H, H-4), 4.55 (m, 1H, H-2‴), 3.90 (br.s, 4H, CH_2-3′, CH_2-5′), 3.34 (br.t, *J* = 4.9 Hz, 4H, CH_2-2′, CH_2-6′) 1.05 (d, *J* = 6.7 Hz, 6H, CH_3-1‴, CH_3-3‴); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 159.41 (C-2"), 152.65 (C-6), 147.16 (C-2), 143.53 (C-5), 134.96 (C-4"), 134.37 (C-5"), 131.36 (C-6"), 129.53 (C-1"), 126.57 (C-3"), 116.56 (C-3), 111.65 (C-4), 67.04 (C-1‴), 46.77 (C-2', C-3', C-5', C-6'), 20.53 (C-2‴, C-3‴); Anal. Calc. for $C_{18}H_{20}Cl_2N_2O_5S$ (446.05): C, 48.33; H, 4.51; N, 6.26. Found: C, 48.38; H, 4.59; N, 6.22.

{4-[(2-Butoxy-3,5-dichlorophenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanone **(5f)**

White amorphous solid; Yield: 87 %; m.p: 98-100 °C; Mol. F.: $C_{19}H_{22}Cl_2N_2O_5S$; Mol. Mass: 460g. IR (KBr, υ, cm^{-1}): 3088 (C-H str. of aromatic

ring), 2883 (C-H str. of aliphatic), 1659 (C=O str.), 1585 (C=C aromatic str.), 1439 (S=O), 1193 (C-O-C bond str.), 1119 (C-N-C bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.77 (d, J = 2.6 Hz, 1H, H-6"), 7.59 (d, J = 2.6 Hz, 1H, H-4"), 7.47 (d, J = 0.9 Hz, 1H, H-5), 7.03 (d, J = 3.4 Hz, 1H, H-3), 6.48 (dd, J = 1.7, 3.4 Hz, 1H, H-4), 4.14 (t, J = 6.8 Hz, 2H, CH_2-1'"), 3.86 (br.s, 4H, CH_2-3′, CH_2-5′), 3.29 (t, J = 5.1 Hz, 4H, CH_2-2′, CH_2-6′), 1.86 (quint, J = 3.4 Hz, 2H, CH_2-2'") 1.49 (sext, J = 4.1 Hz, 2H, CH_2-3'"), 1.00 (t, J = 7.3 Hz, 3H, CH_3-4'"); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 158.99 (C-2"), 152.19 (C-6), 147.50 (C-2), 143.92 (C-5), 135.03 (C-4"), 134.19 (C-5"), 131.36 (C-6"), 129.52 (C-3"), 129.48 (C-1"), 117.34 (C-3), 111.53 (C-4), 75.32 (C-1'"), 46.27 (C-2', C-3', C-5', C-6'), 31.93 (C-2'"), 18.90 (C-3'"), 13.89 (C-4'"); Anal. Calc. for $C_{19}H_{22}Cl_2N_2O_5S$ (460.06): C, 49.46; H, 4.81; N, 6.07. Found: C, 49.41; H, 4.88; N, 6.03.

(4-{[3,5-Dichloro-2-(2-pentoxy)phenyl]sulfonyl}-1-piperazinyl)(2-furyl)methanone **(5g)**

Colorless liquid; Yield: 80 %; m.p: 120-122 °C; Mol. F.: $C_{20}H_{24}Cl_2N_2O_5S$; Mol. Mass: 474 g/mol. IR (KBr, υ, cm^{-1}): 3093 (C-H str. of aromatic ring), 2881 (C-H str. of aliphatic), 1660 (C=O str.), 1586 (C=C aromatic str.), 1430 (S=O), 1195 (C-O-C bond str.), 1113 (C-N-C bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.89 (d, J = 2.5 Hz, 1H, H-6"), 7.61 (d, J = 2.5 Hz, 1H, H-4"), 7.49 (d, J = 1.6 Hz, 1H, H-5), 7.06 (d, J = 3.4 Hz, 1H, H-3), 6.48 (dd, J = 1.7, 3.5 Hz, 1H, H-4), 4.16 (m, 1H, H-2'"), 3.97 (br.s, 4H, CH_2-3′, CH_2-5′), 3.18 (br.t, J = 4.2 Hz, 4H, CH_2-2′, CH_2-6′), 1.87 (m, 2H, CH_2-3'"), 1.81 (sext, J = 7.2 Hz, 2H, CH_2-4'"), 1.20 (d, J = 7.3 Hz, 3H, CH_3-1'"), 0.98 (t, J = 7.1 Hz, 3H, CH_3-5'"); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 159.54 (C-2"), 152.09 (C-6), 147.56 (C-2), 143.98 (C-5), 134.63 (C-5"), 134.09 (C-4"), 131.86 (C-6"), 129.27 (C-1"), 129.22 (C-3"), 117.25 (C-3), 111.68 (C-4), 71.23 (C-2'"), 46.90 (C-2', C-3', C-5', C-6'), 41.88 (C-3'"), 19.14 (C-1'"), 17.96 (C-4'"), 14.63 (C-5'"); Anal. Calc. for $C_{20}H_{24}Cl_2N_2O_5S$ (474.08): C, 50.53; H, 5.09; N, 5.89. Found: C, 50.57; H, 5.02; N, 5.82.

(4-{[2-(Benzyloxy)-3,5-dichlorophenyl]sulfonyl}-1-piperazinyl)(2-furyl)methanone **(5h)**

Off-white amorphous solid; Yield: 87 %; m.p: 124-126 °C; Mol. F.: $C_{22}H_{20}Cl_2N_2O_5S$; Mol. Mass: 494 g/mol. IR (KBr, υ, cm^{-1}): 3082 (C-H str. of aromatic ring), 2888 (C-H str. of aliphatic), 1659 (C=O str.), 1587 (C=C aromatic str.), 1435 (S=O), 1198 (C-O-C bond str.), 1100 (C-N-C bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.83 (d, J = 2.6 Hz, 1H, H-6"), 7.65 (d, J = 2.5 Hz, 1H, H-4"), 7.60 (d, J = 6.7 Hz, 2H, H-2'", H-6'"), 7.42 (dist. d, J = 0.9 Hz, 1H, H-5), 7.41-7.38 (m, 3H, H-3'" to H-5'"), 6.96 (d, J = 2.6 Hz, 1H, H-3), 6.46 (dd, J = 1.7, 3.4 Hz, 1H, H-4), 5.15 (s, 2H, CH_2-7'"), 3.72 (br.s, 4H, CH_2-3′, CH_2-5′), 3.18 (br.t, J = 5.1 Hz, 4H, CH_2-2′, CH_2-6′); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 158.96 (C-2"), 151.30 (C-6), 147.42 (C-2), 143.88 (C-5), 135.74 (C-1'"), 135.10 (C-4"), 134.90 (C-3'", C-5'"), 131.53 (C-5"), 130.10 (C-4'"), 129.68 (C-2'", C-6'"), 129.05 (C-6"), 128.78 (C-3"), 128.61 (C-1"), 117.23 (C-3), 111.47 (C-4), 76.51 (C-7'"), 46.26 (C-2', C-3', C-5', C-6'); Anal. Calc. for $C_{22}H_{20}Cl_2N_2O_5S$ (494.05): C, 53.34; H, 4.07; N, 5.65. Found: C, 53.39; H, 4.01; N, 5.61.

(4-{[2-(2-Chlorobenzyloxy)-3,5-dichlorophenyl]sulfonyl}-1-piperazinyl)(2-furyl)methanone **(5i)**

Dark brown amorphous solid; Yield: 89 %; m.p: 125-127 °C; Mol. F.: $C_{22}H_{19}Cl_3N_2O_5S$; Mol. Mass: 528 g/mol. IR (KBr, υ, cm^{-1}): 3085 (C-H str. of aromatic ring), 2880 (C-H str. of aliphatic), 1649 (C=O str.), 1579 (C=C aromatic str.), 1432 (S=O), 1190 (C-O-C bond str.), 1108 (C-N-C bond str.) 680 (C-Cl bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.86 (d, J = 2.4 Hz, 1H, H-6"), 7.60 (d, J = 2.5 Hz, 1H, H-4"), 7.49 (d, J = 1.4 Hz, 1H, H-5), 7.34 (dd, J = 2.6, 8.2 Hz, 1H, H-3'"), 7.18 (dd, J = 3.0, 8.6 Hz, 1H, H-6'"), 7.10-7.07 (m, 2H, H-4'", H-5'"), 7.05 (d, J = 3.6 Hz, 1H, H-3), 6.42 (dd, J = 1.5, 3.5 Hz, 1H, H-4), 5.19 (s, 2H, CH_2-7'"), 3.88 (br.s, 4H, CH_2-3′, CH_2-5′), 3.15 (br.t, J = 4.8 Hz, 4H, CH_2-2′, CH_2-6′); ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 157.01 (C-2"), 156.02 (C-6), 145.69 (C-2), 143.75 (C-5), 134.61 (C-4"), 133.61 (C-5"), 133.58 (C-1'"), 131.46 (C-6"), 130.45 (C-2'"), 129.83 (C-3"), 128.80 (C-3'", C-4'"), 128.26 (C-1"), 128.12 (C-6'"), 127.96 (C-5'"), 117.34 (C-3), 111.80 (C-4), 72.85 (C-7'"), 46.09 (C-2', C-3', C-5', C-6'); Anal. Calc. for $C_{22}H_{19}Cl_3N_2O_5S$ (528.01): C, 49.47; H, 3.61; N, 5.29. Found: C, 49.44; H, 3.68; N, 5.22.

(4-{[2-(4-Chlorobenzyloxy)-3,5-dichlorophenyl]sulfonyl}-1-piperazinyl)(2-furyl)methanone **(5j)**

Off white amorphous solid; Yield: 88 %; m.p: 126-128 °C; Mol. F.: $C_{22}H_{19}Cl_3N_2O_5S$; Mol. Mass: 528 g/mol. IR (KBr, υ, cm^{-1}): 3081 (C-H str. of aromatic ring), 2888 (C-H str. of aliphatic), 1659 (C=O str.), 1588 (C=C aromatic str.), 1438 (S=O), 1199 (C-O-C bond str.), 1102 (C-N-C bond str.), 682 (C-Cl bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, δ, ppm): 7.82 (d, J = 2.5 Hz, 1H, H-6"), 7.65 (d, J = 2.5

Hz, 1H, H-4''), 7.54 (d, *J* = 6.3 Hz, 2H, H-2''', H-6'''), 7.40 (d, *J* = 6.3 Hz, 2H, H-3''', H-5'''), 7.43 (dist. d, *J* = 0.9 Hz,, 1H, H-5), 6.98 (d, *J* = 2.5 Hz, 1H, H-3), 6.46 (dd, *J* = 1.8, 3.4 Hz, 1H, H-4), 5.10 (s, 2H, CH_2-7'''), 3.75 (br.s, 4H, CH_2-3′, CH_2-5′), 3.16 (t, *J* = 5.1 Hz, 4H, CH_2-2′, CH_2-6′); ^{13}C-NMR (150 MHz, $CDCl_3$, *δ*, ppm): 158.92 (C-2''), 151.01 (C-6), 147.39 (C-2), 143.94 (C-5), 135.12 (C-1'''), 134.72 (C-4''), 134.69 (C-4'''),134.19 (C-5''), 131.47 (C-6''), 130.42 (C-2''', C-6'''), 130.33 (C-1''), 129.71 (C-3''), 128.83 (C-3''', C-5'''), 117.34 (C-3), 111.50 (C-4), 75.56 (C-7'''), 46.26 (C-2', C-3', C-5', C-6'); Anal. Calc. for $C_{22}H_{19}Cl_3N_2O_5S$ (528.01): C, 49.47; H, 3.61; N, 5.29. Found: C, 49.42; H, 3.65; N, 5.23.

(4-{[2-(4-Bromobenzyloxy)-3,5-dichlorophenyl]sulfonyl}-1-piperazinyl)(2-furyl)methanone **(5k)**

White amorphous solid; Yield: 90 %; m.p: 140-142 ºC; Mol. F.: $C_{22}H_{19}BrCl_2N_2O_5$; Mol. Mass: 572 g/mol. IR (KBr, *υ*, cm^{-1}): 3078 (C-H str. of aromatic ring), 2891 (C-H str. of aliphatic), 1668 (C=O str.), 1577 (C=C aromatic str.), 1439 (S=O), 1198 (C-O-C bond str.), 1110 (C-N-C bond str.), 630 (C-Br bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, *δ*, ppm): 7.82 (d, *J* = 2.5 Hz, 1H, H-6''), 7.65 (d, *J* = 2.6 Hz, 1H, H-4''), 7.55 (d, *J* = 6.5 Hz, 2H, H-3''', H-5'''), 7.47 (d, *J* = 6.9 Hz, 2H, H-2''', H-6'''), 7.44 (dist. d, *J* = 1.0 Hz, 1H, H-5), 6.99 (d, *J* = 3.4 Hz, 1H, H-3), 6.46 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4), 5.09 (s, 2H, CH_2-7'''), 3.75 (br.s, 4H, CH_2-3′, CH_2-5′), 3.15 (br.t, *J* = 5.1 Hz, 4H, CH_2-2′, CH_2-6′); ^{13}C-NMR (150 MHz, $CDCl_3$, *δ*, ppm): 157.65 (C-2''), 151.95 (C-6), 147.35 (C-2), 143.75 (C-5), 135.56 (C-1'''), 134.71 (C-4''), 134.14 (C-5''), 131.45 (C-6''), 130.40 (C-2''', C-6'''), 129.78 (C-3''), 128.83 (C-1''), 128.53 (C-3''', C-5'''), 124.69 (C-4'''), 117.03 (C-3), 111.19 (C-4), 75.30 (C-7'''), 46.66 (C-2', C-3', C-5', C-6'); Anal. Calc. for $C_{22}H_{19}BrCl_2N_2O_5S$ (571.96): C, 46.01; H, 3.33; N, 4.88. Found: C, 46.08; H, 3.35; N, 4.81.

(4-{[3,5-Dichloro-2-(phenethyloxy)phenyl]sulfonyl}-1-piperazinyl)(2-furyl)methanone **(5l)**

White amorphous solid; Yield: 85 %; m.p: 132-134 ºC; Mol. F.: $C_{23}H_{22}Cl_2N_2O_5S$; Mol. Mass: 508 g/mol. IR (KBr, *υ*, cm^{-1}): 3078 (C-H str. of aromatic ring), 2876 (C-H str. of aliphatic), 1652 (C=O str.), 1587 (C=C aromatic str.), 1431 (S=O), 1196 (C-O-C bond str.), 1112 (C-N-C bond str.); ^{1}H-NMR (600 MHz, $CDCl_3$, *δ*, ppm): 7.75 (d, *J* = 2.5 Hz, 1H, H-6''), 7.59 (d, *J* = 2.5 Hz, 1H, H-4''), 7.49 (br.s, 1H, H-5), 7.31-7.18 (m, 5H, H-2''' to H-6'''), 7.01 (d, *J* = 3.4 Hz, 1H, H-3), 6.50 (dd, *J* = 1.7, 3.4 Hz, 1H, H-4), 4.38 (t, *J* = 6.7 Hz, 2H, CH_2-8'''), 3.69 (br.s, 4H, CH_2-3′, CH_2-5′), 3.17 (t, *J* = 6.6 Hz, 2H, CH_2-7'''), 2.99 (br.t, *J* = 4.2 Hz, 4H, CH_2-2′, CH_2-6′); ^{13}C-NMR (150 MHz, $CDCl_3$, *δ*, ppm): 158.09 (C-2''), 152.56 (C-6), 147.43 (C-2), 143.04(C-5), 137.64 (C-1'''), 134.72 (C-4''), 134.48 (C-5''), 131.40 (C-6''), 130.40 (C-3''', C-5'''), 129.88 (C-3''), 128.74 (C-2''', C-6'''), 128.03 (C-1''), 124.37 (C-4'''), 117.31 (C-3), 112.304 (C-4), 65.05 (C-8'''), 46.16 (C-2', C-3', C-5', C-6'), 35.14 (C-7'''); Anal. Calc. for $C_{23}H_{22}Cl_2N_2O_5S$ (508.06): C, 54.23; H, 4.35; N, 5.50. Found: C, 54.26; H, 4.31; N, 5.45.

α-Glucosidase Inhibition Assay

The *α*-glucosidase inhibitory activity was performed according to the established method [14]. The percent inhibition was calculated by the following equation:

$$\textbf{Inhibition}\ (\%) = \frac{Control - Test}{Control} \times 100$$

IC_{50} values (concentration at which there is 50% in enzyme catalyzed reaction) compounds were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

Statistical Analysis

The results are written as mean ± SEM after performance in three-folds and statistical analysis by Microsoft Excel 2010.

Hemolytic Activity

Hemolytic activity was done by the reported method [15,16]. Bovine blood was obtained from Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. After centrifugation, separation and washing, the % RBCs lysis was computed by noting the absorbance.

Molecular Docking Methodology

In order to predict the bioactive conformations, various ligands were docked into the binding pockets of the following enzymes by using the default parameters of MOE-Dock program. Prior to dock the ligands in protein molecules, MOE 2009-10 Build program was used to sketch the synthesized compounds. Energy minimization was carried out up to 0.05 gradients by using MMFF94x force field through the default parameter of MOE energy minimization algorithm. Database was created in which all the ligands were saved in their 3D structures in the mdb file format. The protein

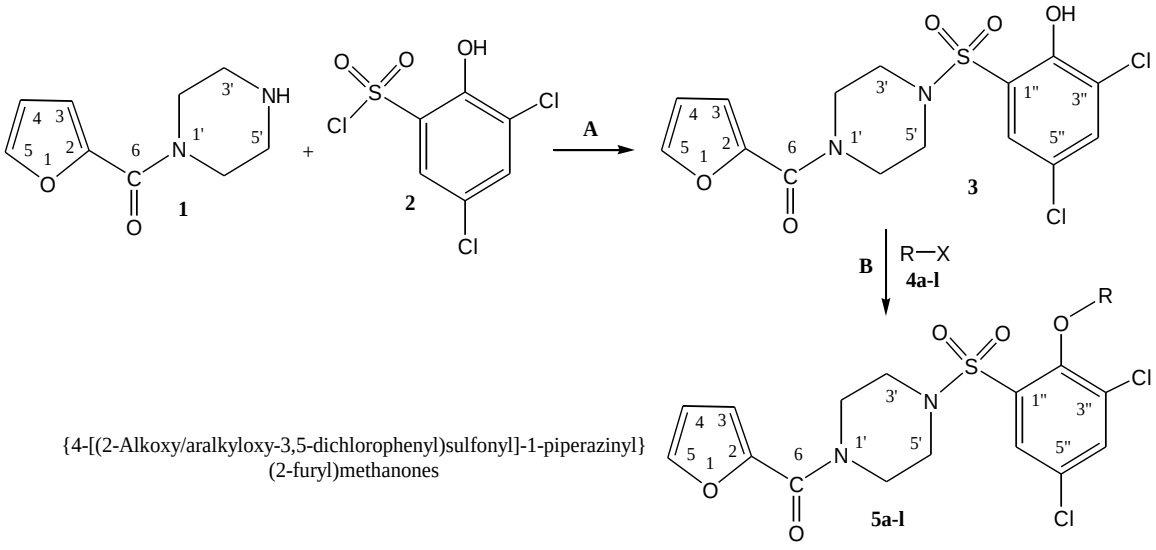
molecules of α-glucosidase (PDB ID code: 3NO4; resolution: 2.02 Å) and butyrylcholinesterase (PDB ID code: 1POP; resolution: 2.30 Å) were obtained from Protein Data Bank. All water molecules were removed from the receptor enzymes and 3D protonation was carried out by using Protonate 3D Option. The energies of protein molecules were also minimized by using the default parameters of MOE 2009-10 energy minimization algorithm (gradient: 0.05, Force Field: MMFF94X). Then all the ligands were docked into the binding pockets (selective residues/amino acids) of the above proteins using default parameters of MOE-Dock Program. A maximum of 10 conformations were acceptable to be saved for each compound in order to use the default parameters of MOE (Placement: Triangle Matcher, Rescoring 1: London dG, Refinement: Force field, Rescoring 2: GBVI/WSA dG). Re-docking was also carried to validate the accuracy of docking protocol [17]. At the end of docking, all the 2D and 3D images were analyzed for the types of interactions and bond distances.

Results and Discussion

The aim of the present research work was to synthesize some new hybrid molecules bearing 2-furoyl-1-piperazine, sulfonamide and ether functionalities to explore their anti-diabetic potential and toxicity level. It is always needed to introduce some new lead compounds as pharmacologically active drug candidates for drug designing studies.

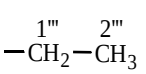
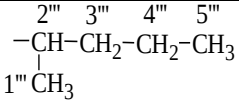
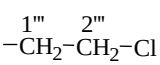
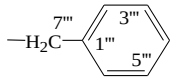
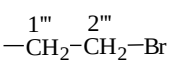
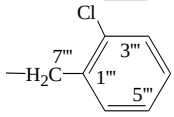
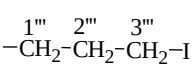
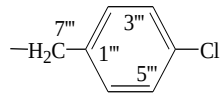
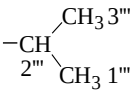
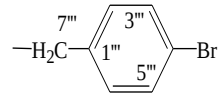
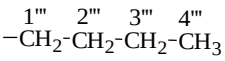
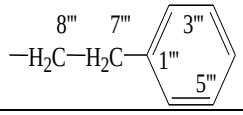
Chemistry

The linear bi-step synthesis of designed hybrid molecules was initiated by the coupling of 2-furyl(1-piperazinyl)methanone (2-fuoroyl-1-piperazine; **1**) with 3,5-dichloro-2-hydroxybenzenesulfonyl chloride (**2**) under dynamic pH control in aqueous medium [18] to obtained the parent compound, {4-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanone (**3**). In the second step, an *O*-substitution reaction was carried out on nucleophile **3** using various alkyl/aralkyl halides (**4a-l**) to acquire a series of {4-[(2-alkoxy/aralkyloxy-3,5-dichlorophenyl)sulfonyl] -1-piperazinyl}(2-furyl)methanones (**5a-l**) as outlined in Scheme-1. The varying groups are listed in Table-1. The synthesis of all these hybrid derivatives **5a-l** was performed in polar aprotic medium of DMF (*N*,*N*-dimethylformamide) using lithium hydride (LiH) as a base. The products were isolated by adding ice cold water in the reaction mixture and then filtering off the precipitated solid. In some cases, compound was taken out through solvent extraction method using chloroform as organic phase. The structure of the parent compound and its derivatives were confirmed by spectral data as described in experimental section.



Scheme 1: Linear synthesis of {4-[(2-alkoxy/aralkyloxy-3,5-dichlorophenyl)sulfonyl]-1-piperazinyl} (2-furyl)methanones (**5a-l**). Reagents & Conditions: (A) Aq. 10% Na_2CO_3 soln./pH 9-10/stirring at RT for 3 hrs. (B) DMF/LiH/stirring for 4-5 hrs.

Table-1: Different –R Groups involved in scheme 1.

Compd. No.	-R	Compd. No.	-R
4a, 5a		**4g, 5g**	
4b, 5b		**4h, 5h**	
4c, 5c		**4i, 5i**	
4d, 5d		**4j, 5j**	
4e, 5e		**4k, 5k**	
4f, 5f		**4l, 5l**	

For the benefit of the reader, the structural characterization of one compound is elaborated hereby. The molecule **5h** was obtained as off-white amorphous solid with melting point of 124-126 °C. Its molecular formula, $C_{22}H_{20}Cl_2N_2O_5S$, was established through CHN analysis data and by counting the number of protons in its ^{1}H-NMR spectrum (Fig. 2), in addition to the counting the number of carbon resonances in its ^{13}C-NMR spectrum (Fig. 3). In IR spectrum, characteristic peaks appearing at υ 3082 (C-H str. of aromatic ring), 2888 (C-H str. of aliphatic), 1659 (C=O str.), 1587 (C=C aromatic str.), 1435 (S=O), 1198 (C-O-C bond str.), and 1100 cm^{-1} (C-N-C bond str.), indicated the presence of sulfonamide, 2-furyl(1-piperazinyl) methanone and ether groups. In its ^{1}H-NMR spectrum, a *tetra*-substituted benzenesulfonyl moiety was ascertained by two *meta*-coupled doublets at δ 7.83 (d, J = 2.6 Hz, 1H, H-6"), and δ 7.65 (d, J = 2.5 Hz, 1H, H-4") while the presence of a benzyl moiety was evident by its characteristic signals at δ 7.60 (d, J = 6.7 Hz, 2H, H-2''' & H-6'''), 7.41-7.38 (m, 3H, H-3''' to H-5''') and δ 5.15 (s, 2H, CH_2-7'''). Moreover, the signals at δ 7.42 (dist. d, J = 0.9 Hz, 1H, H-5), 6.96 (d, J = 2.6 Hz, 1H, H-3), 6.46 (dd, J = 1.7, 3.4 Hz, 1H, H-4) were assignable to a 2-furoyl entity while the two signals in aliphatic region at δ 3.72 (br.s, 4H, CH_2-3′, CH_2-5′) and 3.18 (br.t, J = 5.1 Hz, 4H, CH_2-2′, CH_2-6′) were symbolic for a 1,4-di-substituted piperazine core in the molecule. The ^{13}C-NMR spectrum demonstrated overall seventeen carbon resonances due to some symmetrical carbons appearing at the same chemical shifts. Out of these, seven were quaternary carbons, eight were methines and two were assignable to methylene carbons. The carbons of 2-furoyl resonated at δ 151.30 (C-6), 147.42 (C-2), 143.88 (C-5), 117.23 (C-3), 111.47 (C-4). The carbons of *tetra*-substituted benzenesulfonyl moiety were observed at δ 158.96 (C-2"), 135.10 (C-4"), 131.53 (C-5"), 129.05 (C-6"), 128.78 (C-3"), 128.61 (C-1"), while the carbons of benzyl moiety appeared at δ 135.74 (C-1'''), 134.90 (C-3''', C-5'''), 130.10 (C-4'''), 129.68 (C-2''', C-6'''), 76.51 (C-7'''). The carbons of piperazine ring were not clearly observed most probably due to ring flipping, however one broad signal appeared at δ 46.26 (C-2', C-3', C-5' and C-6'). On the basis of aforementioned cumulative evidences, the structure of **5h** was deduced and it was given the name as (4-{[2-(benzyloxy)-3,5-dichlorophenyl]sulfonyl}-1-piperazinyl)(2-furyl)methanone. In a similar way, all other synthesized derivatives were characterized by their IR, ^{1}H-NMR, and ^{13}C-NMR spectral analysis.

α-Glucosidase Inhibition and Hemolytic Activity (in vitro)

The synthesized derivatives were screened against *α*-glucosidase enzyme. The percentage inhibition and IC_{50} values against *α*-glucosidase are given in Table-2 for the active compounds only. From the results, it was evident that the molecule, **5f** with IC_{50} value of 11.71 ± 0.02 µM was even more active than the standard Acarbose (IC_{50} = 38.25 ±

0.12 μM). The potent therapeutic potential of this molecule might be attributed to the presence of straight chain butoxy group. All the synthesized molecules were also evaluated for their cytotoxicity profile through hemolytic study and % lysis of RBCs is given in Table-3. Most of the molecules showed very modest cytotoxicity except **5d** which showed little bit high hemolytic activity (44.76 ± 0.22%) among the series, yet, it was much lower than the positive control, Triton-X, (87.26 ± 0.01%). The lowest hemolytic activity was exposed by **5j** (2.08 ± 0.11%) but little bit higher than the negative control (PBS). On the basis of these results, it was rational that the compound **5f** might be utilized as promising anti-diabetic agent with modest toxicity.

Table-2: Percent inhibition at 0.5 mM and IC_{50} values of active derivatives for α-glucosidase.

Compounds	Inhibition (%)	IC_{50} (μM)
5f	**92.87 ± 0.17**	**11.71 ± 0.02**
5h	**58.37 ± 0.32**	**328.41 ± 0.4**
5j	**65.25 ± 0.18**	**51.42 ± 0.05**
5l	**98.51 ± 0.19**	**93.21 ± 0.08**
Acarbose	**92.23 ± 0.14**	**38.25 ± 0.12**

NOTE: All compounds were dissolved in methanol and experiments were performed in triplicate (mean ± SEM, n = 3). Remaining compounds were not active.

Table-3: Hemolytic activity of synthesized derivatives (**5a–l**).

Comp	Hemolytic activity (%)	Compounds	Hemolytic activity (%)
5a	**17.99 ± 0.14**	**5g**	**25.99 ± 0.16**
5b	**29.28 ± 0.21**	**5h**	**28.45 ± 0.12**
5c	**29.34 ± 0.25**	**5i**	**15.48 ± 0.19**
5d	**44.76 ± 0.22**	**5j**	**2.08 ± 0.11**
5e	**12.55 ± 0.18**	**5k**	**31.38 ± 0.23**
5f	**27.61 ± 0.13**	**5l**	**15.89 ± 0.12**
PBS	**0.09 ± 0.01**	**Triton-X**	**87.26 ± 0.01**

Computational Docking

The enzyme inhibition data was supported by the molecular docking study of the active molecules. It is clear from the docking results of α-glucosidase that compound **5f** was strongly bound in the active site of enzyme by making two strong interactions with Arg404 amino acid residues as illustrated in Fig. 4 (2D & 3D). Both of the side chain donor interactions were found between Arg404 and two sulfonyl oxygen of the ligand **5f** showing bond distances of 1.65 Å and 2.68 Å angstroms. Some other residues like Asp73, Asp420, Trp271, Trp417, Ala480 and Lys422 were also present in the nearby vicinity.

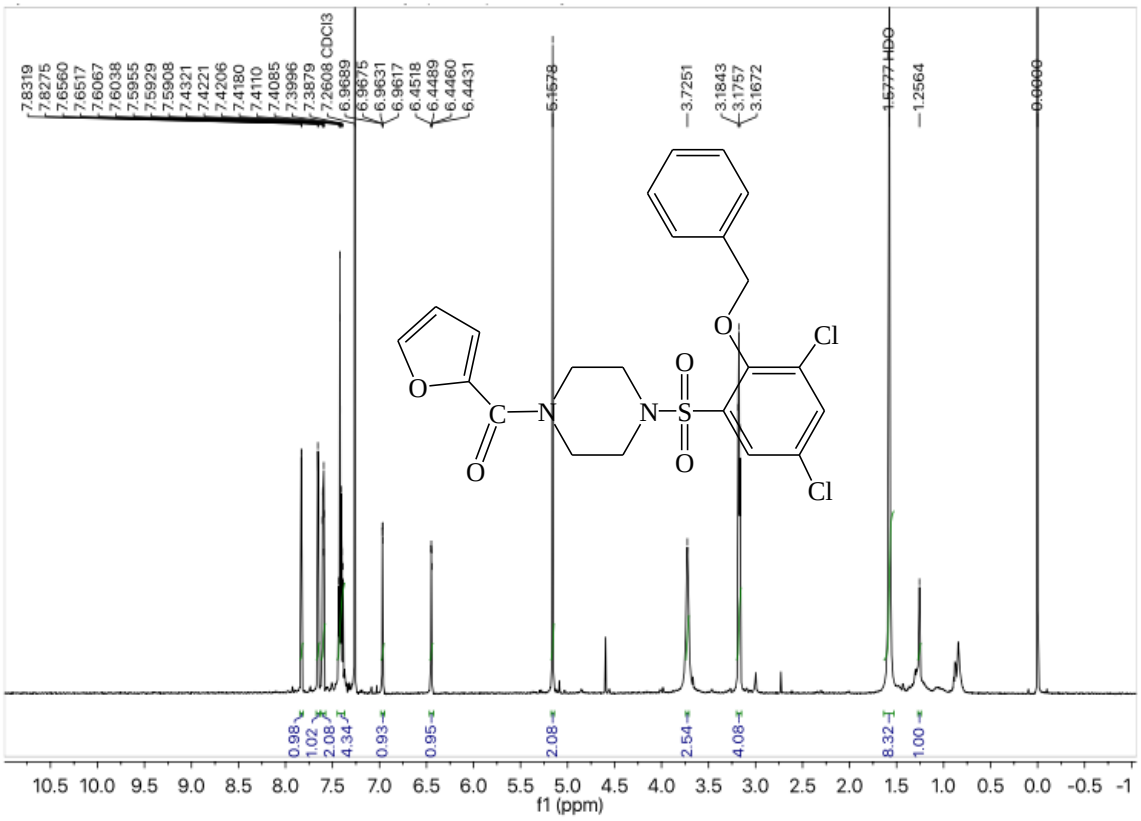


Fig. 2: ^{1}H-NMR spectrum of **5h**.

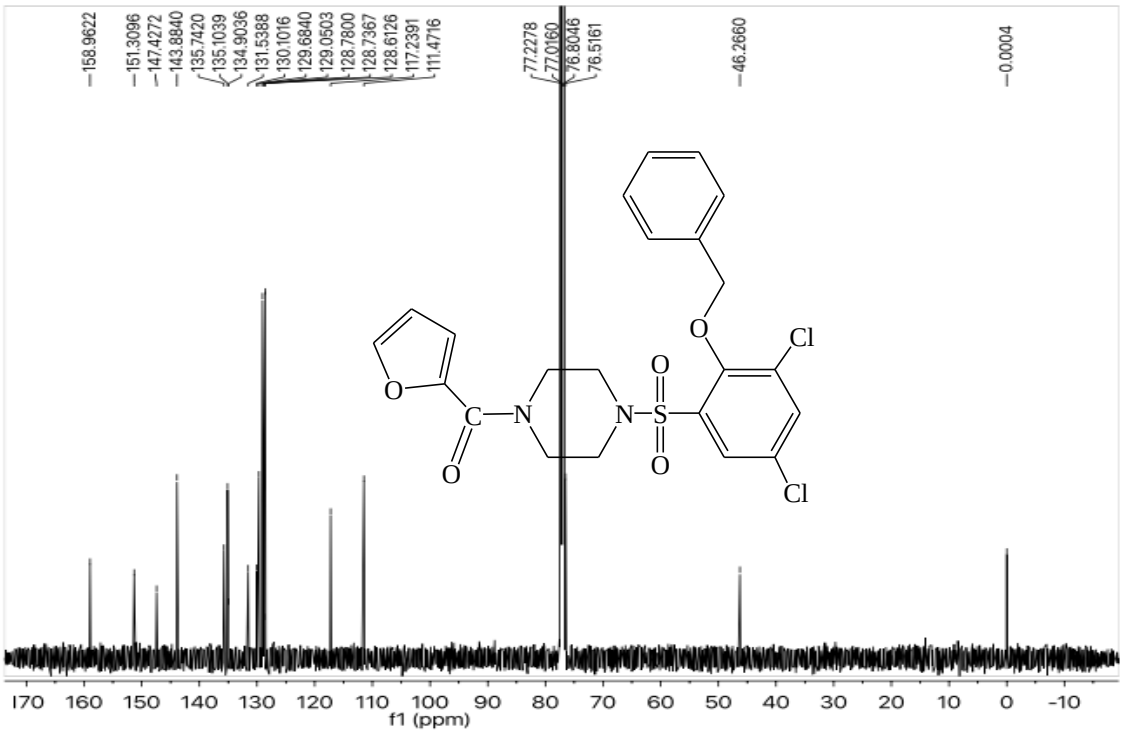


Fig. 3: ^{13}C-NMR spectrum of **5h**.

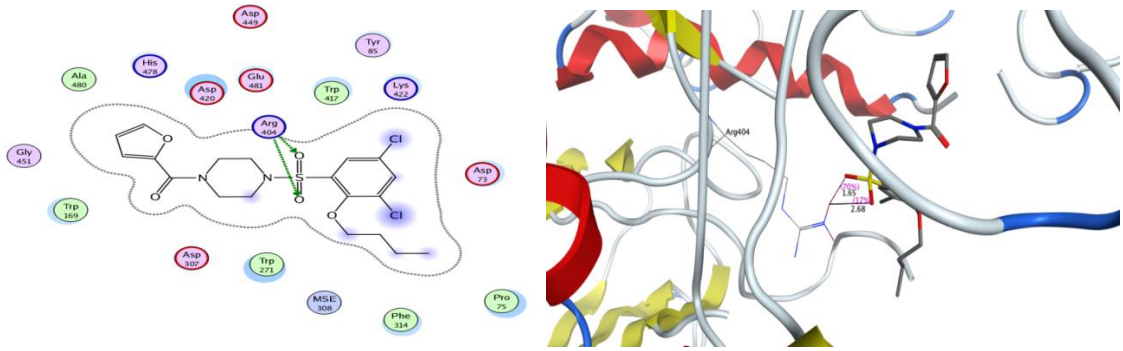


Fig. 4: (2D & 3D): The interaction analysis of compound **5f** against *α*-glucosidase enzyme.

Conclusion

The presented series of multi-functionalized compounds was synthesized in good yields through a linear bi-step approach. Among the synthesized compounds, **5f** exhibited very potent inhibitory potential relative to the standard used against *α*-glucosidase enzyme, and hence it might be considered as an interesting and lead compound for *α*-glucosidase related ailments, particularly type-II diabetes.

Acknowledgement

The authors acknowledge the Higher Education Commission (HEC) of Pakistan for the financial assistance regarding spectral analysis of the synthesized molecules.

References

1. S. C. Sweetman, *Martindale, The complete drug reference*, Pharmaceutical Press, 33rd edition, London & Chicago (2002).
2. M. Economics, *Physician's Desk Reference, Medicinal company inc*, 50th edition, New Jersey, p. 1937 (1996).
3. R. M. Graham, Selective alpha 1-adrenergic blockade, lipids, and coronary heart disease risk, *Am. J. Med.*, **87**, 53S (1989).

4. B. Adhisivam, Of prazosin and purses, *Lancet.*, **368**, 1870 (2006).
5. H. Shen, *Illustrated Pharmacology Memory Cards: PharMnemonics*, 2nd edition, Minireviews LLC (2015).
6. A. D. Krystal and J. R. Davidson, The use of prazosin for the treatment of trauma nightmares and sleep disturbances in combat veterans with PTSD, *Biol. Psychiatry.*, **61**, 925 (2007).
7. C. T. Supuran, A. Casini and A. Scozzafava, Protease inhibitors of the sulfonamide type: anticancer, antiinflammatory, and antiviral agents, *Med. Res. Rev.*, **23**, 535 (2003).
8. C. T. Supuran, A. Innocenti, A. Mastrolorenzo and A. Scozzafava, Antiviral sulfonamide derivatives, *Mini Rev. Med. Chem.*, **4**, 189 (2004).
9. A. J. Hirsh, S. Y. Yao, J. D. Young and C. I. Cheeseman, Inhibition of glucose absorption in the rat jejunum: a novel action of alpha-D-glucosidase inhibitors, *Gastroenterology*, **113**, 205 (1997).
10. S. Chiba, Molecular mechanism in alpha-glucosidase and glucoamylase, *Biosci. Biotechnol. Biochem.*, **61**, 1233 (1997).
11. H. E. Lebovitz, Alpha-glucosidase inhibitors, *Endocrinol. Metab. Clin. North. Am.*, **26**, 539 (1997).
12. R. R. Somani and P. Y. Shirodkar, Oxadiazole: A biologically important heterocycle, *Der. Pharma. Chemica*., **1**, 130 (2009).
13. R. R. Gupta, M. Kumar and V. Gupta, *Heterocyclic Chemistry: Five membered Heterocycles*, ed. 1, Springer, India (2005).
14. P. Chapdelaine, R. R. Tremblay and J. Y. Dube, *p*-Nitrophenol-alpha-D-glucopyranoside as substrate for measurement of maltase activity in human semen, *Clin. Chem.*, **24**, 208 (1978).
15. P. Sharma and J. D. Sharma, In vitro hemolysis of human erythrocytes by plant extracts with antiplasmodial activity, *J. Ethnopharmacol.*, **74**, 239 (2001).
16. W. A. Powell, C. M. Catranis and C. A. Maynard, Design of self-processing antimicrobial peptide for plant protection, *Lett. Appl. Microbiol.*, **31**, 163 (2000).
17. M. J. Bostro, J. R. Greenwood and J. Gottfries, Assessing the performance of OMEGA with respect to retrieving bioactive conformations, *J. Mol. Graph. Model.*, **21**, 449 (2003).
18. X. Deng and N. S. Mani, A facile, environmentally benign sulfonamide synthesis in water, *Green Chem.*, **8**, 835 (2006).