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A NEW SCAFFOLD FOR D_3 DOPAMINERGIC AFFINITY CONTAINING ARYLPIPERAZINE FRAGMENT: MOLECULAR MODELING, SYNTHESIS, *IN VITRO* AND *IN VIVO* PHARMACOLOGICAL EVALUATION

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يتناول هذا البحث تحضير سلسلة جديدة من مركبات ١٣ ف ، ١٦ ف تحتوي علي نواه غير متجانسة الحلقات ومرتبطة بنواة أريل بيبرازين خلال فواصل مختلفة. وقد تم إجراء بعض التجارب الخاصة علي الأنواع الفرعية لمستقبلات الدوبامين (د١ ، د٢ ، د٣ ، د٥) ولقد أظهر مركب ١٦ أ أحسن قابلية إرتباط بمستقبل الدوبامين (د٣). علاوة على ذلك تم دراسة تأثير بعض المركبات المشيدة علي ضغط دم القطط ذات ضغط الدم الطبيعي. وقد أظهرت النتائج فاعلية قليلة مقارنه بعقار البيرازوسين مما قد يثبت ارتباط محدود لهذه المركبات تجاه مستقبلات الألفا الأدرينالية.

*A new series of N-(6-substitutedbenzo[d]thiazol-2-yl)-2-(4-arylpiperazin-1-yl) acetamides (**3a-f**) and 2-(3-(4-arylpiprazin-1-yl)propylthio)benzo[d]thiazoles/-oxazoles/-imidazole (**6a-f**) was synthesized by connecting arylpiperazine through a semi-rigid or flexible spacer to a heterocyclic moiety, respectively. The radioligand binding experiments for the D_1, D_2, D_3 and D_5 subtypes expressed in CHO cells were examined for the target compounds **3a-f, 6a, 6b, 6d** and **6f**. Compound **6a** showed the best binding affinity for dopamine D_3 receptor and is considered as a new scaffold for D_3 dopaminergic affinity. Furthermore, molecular modeling of the best-fitted conformer of target compounds **3a**, **6b**, **6c**, **6d** and **6f** to α_1-adrenoceptor (α_1-**AR**) antagonist hypothesis was performed using CATALYST software, HipHop modules. Based on the results of simulation studies, these target compounds were evaluated for their in vivo hypotensive activity on blood pressure of normotensive cats.*

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INTRODUCTION

Arylpiperazine is a core fragment of many bioactive compounds exhibiting a variety of pharmacological effects. It has been shown that their action can be mediated by different subpopulations of serotonin (5-hydroxytryptamine, 5-HT)[1], α_1-adrenergic[1&2] and dopaminergic[1,3&4] receptors. Such a multireceptor potential implicates their frequent use as a source of new agents with different therapeutic properties.

The dopaminergic system plays important role in regulating neuronal motor control, cognition, emotion and vascular function. Neuropsychiatric diseases such as schizophrenia, Parkinson's disease, or addiction are strongly related to a disregulation of the dopaminergic signal transduction[5&6]. Therefore, dopamine receptors are attractive as therapeutic targets. There are five dopamine receptor subtypes that may be divided into two subfamilies: G_s-coupled D_1-like receptors (D_1, D_5) and G_i-coupled D_2-like receptors (D_2, D_3, D_4)[7]. The therapy of schizophrenia with typical antipsychotic drugs can implicate severe side effects, such as extrapyramidal motor effects. Higher subtype-selectivity and new binding profiles of different dopamine receptor subtypes may lead to more effective neuroleptic drugs with fewer therapy-limiting side effects. The discovery of new dopaminergic ligands[3,4,8-11] with high affinity and selectivity for D_3 receptor subtype represented a breakthrough in the pharmacology of dopamine receptors. Recently, lead compound BP 897[3&12] was designed and investigated as ligand for dopamine D_3 receptor. On the basis of the features in the lead structures BP 897 (*Ki* = 1.4 nM), ST 198 (*Ki* = 12 nM)[10] and FAUC 365 (*Ki* = 0.5 nM)[10], it was promising to prepare a potential D_3 ligands retaining the same pharmacophoric features (Aryl / Heteroaryl moiety, spacer and basic moiety), Figure 1. The plan of investigation involved the incorporation of benzo[*d*]thiazole / -oxazole/ -imidazole unit as a heteroaryl bioisostere attached to arylpiperazine (basic moiety) through modified semi-rigid or flexible spacer.

Moreover, with respect to the potential multireceptor profile of such derivatives, molecular modeling studies[13] for α_1-ARs were evaluated. The α_1-ARs are mainly involved in the cardiovascular and central nervous system[14] and they have divergent affinities for many synthetic drugs, which interact selectively as agonist or antagonists. Ligands acting as antagonists at the α_1-ARs subtypes have been used in the treatment of a variety of diseases including hypertension[15]. In view of molecular simulation results, selected compounds were evaluated for their hypotensive activity.

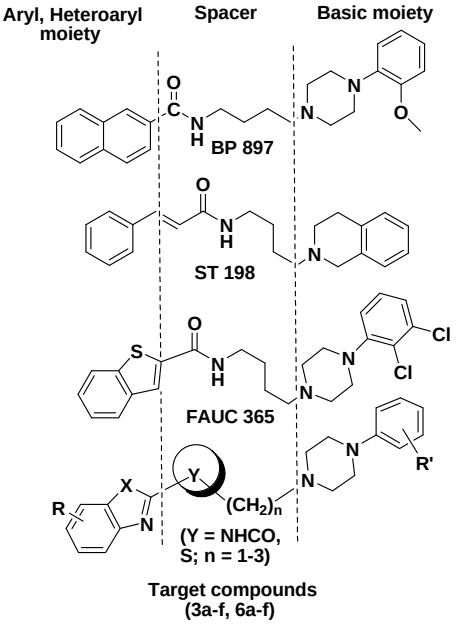


Fig. 1: Features similarities between the leads (BP 897, ST 198 and FAUC 365) and target compounds (**3a-f**, **6a-f**)

MATERIALS AND METHODS

Melting points were determined with a Stuart Scientific apparatus and are uncorrected. FT- IR spectra were recorded on a Perkin-Elmer spectrophotometer and measured by ν`cm^{-1} scale using KBr cell. ^{1}H-NMR spectra were measured in δ scale on Brucker 200, 400 and 500 MHz spectrometers. All the spectra were referred to TMS. ^{13}C-NMR spectra were measured in δ scale on Brucker 200, 400 and 500 MHz spectrometers. The electron impact (EI) mass spectra were recorded on Finnigan Mat SSQ 7000 (70 eV) mass spectrometer. Analytical thin layer chromatography (TLC) on silica gel plates containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products. All reagents and solvents were purified and dried by standard techniques. Elemental microanalyses were performed at Microanalytical Center, Cairo and Vienna Universities.

Chemistry

2-(4-arylpiperazin-1-yl)-N-(6-substitutedbenzo[*d*]thiazol-2-yl)acetamides (3a-f)

General procedure: To a hot solution of 2-chloro-*N*-(6-substituted benzo[*d*]thiazol-2-yl)acetamide (**2a-c**) (1.15 mmol) in acetonitrile (15 mL) and DMF (2 mL), a mixture of arylpiprazine (1.15 mmol) and triethylamine (0.3 mL) in acetonitrile (3 mL) was added. The reaction mixture was refluxed for 8 h. The solvent was evaporated under vacuum and the residue was triturated with water. The formed solid was recrystallized from ethanol – acetone for compound **3a** and from ethanol - water to produce the titled compounds **3b-f**.

N-(6-Chlorobenzo[*d*]thiazol-2-yl)-2-(4-phenylpiperazin-1-yl)acetamide (3a)

It was separated as white crystals (0.38 g, 0.98 mmol, 86.4%), m.p. 225-226°C. IR (KBr, cm^{-1}): 3589 (br, NH), 2849 (CH_2), 1686 (NC=O). ^{1}H-NMR**,** 400 MHz (DMSO-d_6:): δ 2.75

(m, 4H, 2 CH_2 of piperazine moiety), 3.22 (m, 4H, 2 CH_2 of piperazine moiety), 3.41 (s, 2H, $COCH_2$), 6.95 - 7.32 (m, 5H, C_6H_5), 7.42 (dd, 1H, $J_{5,4}$ = 9.5 Hz, $J_{5,7}$ = 2.7 Hz, H-5), 7.72 (d, 1H, $J_{4,5}$ = 9.5 Hz, H-4), 8.18 (d, 1H, $J_{7,5}$ = 2.7 Hz, H-7). MS (m/z, %): 386 (M^+, 44), 388 (M^++2, 15). Anal**.** Calcd for $C_{19}H_{19}ClN_4OS$: C, 58.98; H, 4.95; N, 14.48. Found: C, 58.74; H, 4.96; N, 14.23.

_N_-(6-Chlorobenzo[_d_]thiazol-2-yl)-2-(4-(3-trifluoromethyl)phenyl) piperazin-1-yl)acetamide (3b)

It was separated as beige crystals (0.18 g, 0.39 mmol, 34.6%), m.p. 156-158°C. IR (KBr, cm^{-1}): 3589 (br, NH), 2849 (CH_2), 1690 (NC=O). ^{1}H-NMR**,** 400 MHz (DMSO-d_6:): δ 2.75 (m, 4H, 2 CH_2 of piperazine moiety), 3.29 (m, 4H, 2 CH_2 of piperazine moiety), 3.42 (s, 2H, $COCH_2$), 7.08 (d, 1H, $J_{6',5'}$ = 9.4 Hz, H-6' of 3-CF_3-C_6H_4), 7.19 (s, 1H, H-2' of 3-CF_3-C_6H_4), 7.25 (d, 1H, $J_{4',5'}$ = 9.4 Hz, H-4' of 3-CF_3-C_6H_4), 7.41 (t,1H, $J_{5',4'}$ = $J_{5',6'}$ = 9.4 Hz, H-5' of 3-CF_3-C_6H_4), 7.48 (dd, 1H, $J_{5,4}$ = 9.4 Hz, $J_{5,7}$ = 2.3 Hz, H-5), 7.75 (d, 1H, $J_{4,5}$ = 9.4 Hz, H-4), 8.14 (d, 1H, $J_{7,5}$ = 2.3 Hz, H-7). ^{13}C-NMR, 400 MHz (DMSO-d_6:): δ 47.49, 52.15, 59.92, 110.80, 114.48, 118.69, 121.37, 121.66, 126.41, 127.56, 129.63, 129.89, 133.06, 147.26, 151.09, 158.28, 169.49. MS (m/z, %): 454 (M^+, 10), 456 (M^++2, 4). Anal**.** Calcd for $C_{20}H_{18}ClF_3N_4OS$. 0.4 H_2O: C, 51.93; H, 4.07; N, 12.11. Found: C, 52.35; H, 4.46; N, 11.60.

_N_-(6-Chlorobenzo[_d_]thiazol-2-yl)-2-(4-(2-methoxyphenyl)piperazin-1-yl)acetamide (3c)

It was separated as beige crystals (0.22 g, 0.53 mmol, 45.8%), m.p. 170-172°C. IR (KBr, cm^{-1}): 3581(br, NH), 2822 (CH_2),1691 (NC=O). ^{1}H-NMR**,** 400 MHz (DMSO-d_6:): δ 2.73 (br, 4H, 2 CH_2 of piperazine moiety), 3.05 (br, 4H, 2 CH_2 of piperazine moiety), 3.40 (s, 2H, $COCH_2$), 3.79 (s, 3H, OCH_3), 6.94 (m, 4H, 2-OCH_3-C_6H_4), 7.47 (d, 1H, $J_{5,4}$ = 9.5 Hz, H-5), 7.74 (d, 1H, $J_{4,5}$ = 9.5 Hz, H-4), 8.14 (s, 1H, H-7). ^{13}C-NMR, 400 MHz (DMSO-d_6:): δ 42.85, 50.21, 53.08, 55.67, 60.37, 112.22, 118.38, 121.19, 121.83, 121.91, 122.12, 122.31, 126.99, 128.04, 133.51, 141.42, 147.69, 152.33, 158.69. MS (m/z, %): 416 (M^+, 1.66), 418 (M^++2, 1). Anal**.** Calcd for $C_{20}H_{21}ClN_4O_2S$: C, 57.62; H, 5.08; N, 13.44. Found: C, 57.17; H, 5.08; N, 13.55.

_N_-(6-Chlorobenzo[_d_]thiazol-2-yl)-2-(4-(4-nitrophenyl)piperazin-1-yl)acetamide (3d)

It was separated as faint yellow crystals (0.20 g, 0.46 mmol, 40.8%), m.p. 155-160°C. IR (KBr, cm^{-1}): 3569 (br, NH), 2822 (CH_2), 1691 (NC=O)**.** ^{1}H-NMR**,** 400 MHz (DMSO-d_6:): δ 2.95 (br, 4H, 2 CH_2 of piperazine moiety), 3.25 (br, 4H, 2 CH_2 of piperazine moiety), 3.34 (s, 2H, $COCH_2$), 7.08 (m, 2H, H-2' and H-6' of 4-nitro-C_6H_4), 7.49 (d, 1H, $J_{5,4}$ = 8.7 Hz, H-5), 7.72 (m, 2H, H-3' and H-5' of 4-nitro-C_6H_4), 8.08 (d, 1H, $J_{4,5}$ = 8.9 Hz, H-4), 8.18 (s, 1H,

H-7). MS (m/z, %): 431 (M^+, 6), 433 (M^++2, 2.5). Anal. Calcd for $C_{19}H_{18}ClN_5O_3S$: C, 52.84; H, 4.20; N, 16.22. Found: C, 52.55; H, 4.13; N, 15.97.

2-(4-(2-Methoxyphenyl)piperazin-1-yl)-*N*-(6-nitrobenzo[*d*]thiazol-2-yl)acetamide (3e)

It was separated as faint yellow crystals (0.23 g, 0.54 mmol, 46.9%), mp 190-192°C. IR (KBr, cm^{-1}): 3280 (NH), 2933 and 2818 (CH_2), 1703 (NC=O). ^{1}H-NMR, 200 MHz (DMSO-d_6:) : δ 2.89 (br, 4H, 2 CH_2 of piperazine moiety), 3.06 (br, 4H, 2 CH_2 of piperazine moiety), 3.68 (s, 2H, -$COCH_2$), 3.77 (s, 3H, OCH_3), 6.93 (m, 4H, 2-OCH_3-$\underline{C_6H_4}$), 7.90 (d, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 8.29 (d, 1H, $J_{5,4}$ = 9.7 Hz, H-5), 9.07 (s, 1H, H-7). ^{13}C-NMR, 200 MHz (DMSO-d_6:): δ 49.33, 52.63, 55.33, 59.38, 111.89, 118.07, 119.12, 120.67, 120.83, 121.83, 122.69, 132.20, 140.76, 143.07, 151.95, 153.33, 163.o4, 168.55. MS (m/z, %): 427 (M^+, 39). Anal. Calcd for $C_{20}H_{21}N_5O_4S$. H_2O: C, 53.87; H, 4.75; N, 15.71. Found: C, 53.51; H, 4.75; N, 15.40.

2-(4-(2-Methoxyphenyl)piperazin-1-yl)-*N*-(6-methylbenzo[*d*]thiazol-2-yl)acetamide (3f)

It was separated as beige crystals (0.25 g, 0.63 mmol, 54.9%), mp 122-124°C. IR (KBr, cm^{-1}): 3171 (br, NH), 2964 and 2819 (CH_2), 1689 (NC=O). ^{1}H-NMR, 500 MHz (DMSO-d_6:): δ 2.40 (s, 3H, CH_3), 2.72 (br, 4H, 2 CH_2 of piperazine moiety), 3.00 (br, 4H, 2 CH_2 of piperazine moiety), 3.41 (s, 2H, $COCH_2$), 3.76 (s, 3H, OCH_3), 6.90 (m, 4H, 2-OCH_3-$\underline{C_6H_4}$), 7.24 (d, 1H, $J_{5,4}$ = 8.0 Hz, H-5), 7.63 (d, 1H, $J_{4,5}$ = 8.0 Hz, H-4), 7.75 (s, 1H, H-7). ^{13}C-NMR, 500 MHz (DMSO-d_6): δ 20.96 ($\underline{C}H_3$), 49.98 (2 C of piperazine moiety), 52.75 (2 C of piperazine moiety), 55.29 ($O\underline{C}H_3$), 60.22 ($N\underline{C}H_2$), 111.87, 117.99, 120.16 (C-4), 120.81, 121.30 (C-7), 122.44, 127.25 (C-5), 131.50 (C-7a), 132.98 (C-6), 141.15 (C-1'), 146.41 (C-3a), 151.98 (C-2'), 156.57 (C-2), 169.21 (C=O). MS (m/z, %): 396 (M^+, 6%). Anal. Calcd for $C_{21}H_{24}N_4O_2S$. 0.85 H_2O: C, 61.19; H, 6.24; N, 13.60. Found: C, 61.53; H, 6.09; N, 13.42.

2-(3-(4-arylpiperazin-1-yl)propyl-thio)benzo[*d*]thiazoles / -oxazoles /-imidazole (6a-f)

General procedure: To a hot solution of 2-mercapto-benzo[*d*] thiazoles / -oxazole or -imidazole (**4a-d**) (1.15 mmol) in acetonitrile (20 mL) and DMF (1 mL), arylpiprazine (**5a** / **5b**, 1.15 mmol) in acetonitrile (3 mL) was added. To the reaction mixture, anhydrous potassium carbonate (1.15 mmol) and few crystals of potassium iodide was added. The reaction mixture was refluxed for 7 h. After cooling, water was added. The formed solid was filtered and recrystallized from ethanol / water to produce the titled compounds **6a-f**.

2-(3-(4-Phenylpiperazin-1-yl)propylthio)benzo[*d*]thiazole (6a)

It was separated as colourless crystals (0.12 g, 0.32 mmol, 28.6%), mp 115-116°C. IR (KBr, cm^{-1}): 2931 and 2833 (CH_2), 1597 (C=C). ^{1}H-NMR, 200 MHz (DMSO-d_6:): δ 2.15 (m, 2H, $SCH_2\underline{CH_2}CH_2N$), 3.09 (br, 6H, $SCH_2CH_2\underline{CH_2}N$ and 2 CH_2 of piperazine moiety), 3.30 (br, 4H, 2 CH_2 of piperazine moiety), 3.44 (t, 2H, J = 7.5 Hz, SCH_2), 6.83 (t, 1H, $J_{4',3'} = J_{4',5'}$ = 7.4 Hz, H-4' of phenylpiperazine), 6.97 (d, 2H, J = 8.9 Hz, H-2' and H-6' of phenylpiperazine), 7.23 (t, 2H, J = 7.4 Hz, H-3' and H-5' of phenylpiperazine), 7.37 (t, 1H, $J_{5,4} = J_{5,6}$ = 7.9 Hz, H-5), 7.47 (t, 1H, $J_{6,5} = J_{6,7}$ = 7.9 Hz, H-6), 7.86 (d, 1H, $J_{7,6}$ = 7,9 Hz, H-7), 8.00 (d, 1H, $J_{4,5}$ = 7.9 Hz, H-4). ^{13}C-NMR, 200 MHz (DMSO-d_6:): δ 24.39 ($SCH_2\underline{C}H_2CH_2N$), 30.18 ($S\underline{C}H_2$), 46.49 (2 C of piperazine moiety), 51.52 (2 C of piperazine moiety), 54.93 ($\underline{C}H_2N$), 115.79 (C-4'), 119.66 (C-2' and C-6'), 121.15 (C-7), 121.86 (C-4), 124.56 (C-5), 126.45 (C-6), 129.08 (C-3' and C-5'), 134.59 (C-1'), 150.03 (C-3a), 152.70 (C-7a), 166.36 (C-2). MS (m/z, %): 369 (M^+, 6%). Anal. Calcd for $C_{20}H_{23}N_3S_2$. 0.2 H_2O: C, 64.32; H, 6.27; N, 11.25. Found: C, 64.02; H, 5.85; N, 10.94.

2-(3-(4-(3-Chlorophenyl)piperazin-1-yl)propylthio)benzo[*d*]thiazole (6b)

It was separated as colorless crystals (0.29 g, 0.72 mmol, 63.0%), mp 148-150°C. IR (KBr, cm^{-1}): 2932 and 2830 (CH_2), 1591 (C=C). ^{1}H-NMR, 500 MHz (DMSO-d_6:): δ 2.03 (m, 2H, $SCH_2\underline{CH_2}CH_2N$), 2.66 (br, 6H, $SCH_2CH_2\underline{CH_2}N$ and 2 CH_2 of piperazine moiety), 3.23 (br, 4H, 2 CH_2 of piperazine moiety), 3.41 (t, 2H, J = 6.7 Hz, SCH_2), 6.78 (d, 1H, $J_{6',5'}$ = 8.1 Hz, H-6' of 3-Cl-C_6H_4), 6.88 (d, 1H, $J_{4',5'}$ = 8.1 Hz, H-4' of 3-Cl-C_6H_4), 6.94 (s, 1H, H-2' of 3-Cl-C_6H_4), 7.20 (t, 1H, $J_{5',4'} = J_{5',6'}$ = 8.1 Hz, H-5' of 3-Cl-C_6H_4), 7.36 (t, 1H, $J_{5,4} = J_{5,6}$ = 7.5 Hz, H-5), 7.46 (t, 1H, $J_{6,5} = J_{6,7}$ = 7.5 Hz, H-6), 7.85 (d, 1H, $J_{7,6}$ = 7.5 Hz, H-7), 8.00 (d, 1H, $J_{4,5}$ = 7.5 Hz, H-4). ^{13}C-NMR, 500 MHz (DMSO-d_6:): δ 25.49 ($SCH_2\underline{C}H_2CH_2N$), 30.76 ($S\underline{C}H_2$), 47.03 (2 C of piperazine moiety), 52.02 (2 C of piperazine moiety), 55.73 ($\underline{C}H_2N$), 113.66 (C-4'), 114.59 (C-2'), 118.19 (C-6'), 121.06 (C-7), 121.73 (C-4), 124.39 (C-5), 126.33 (C-6), 130.39 (C-5'), 133.80 (C-1'), 134.50 (C-3a), 151.89 (C-3'), 152.73 (C-7a), 166.70 (C-2). MS (m/z, %): 403 (M^+, 9), 405 (M^++2, 4). Anal. Calcd for $C_{20}H_{22}ClN_3S_2$. 0.25 H_2O: C, 58.75; H, 5.51; N, 10.28. Found: C, 58.27; H, 5.82; N, 10.02.

5-Chloro-2-(3-(4-(3-chlorophenyl)piperazin-1-yl) propylthio)benzo[*d*]thiazole (6c)

It was separated as colorless crystals (0.20 g, 0.45 mmol, 40.0%), mp 50-52°C**.** IR (KBr,cm^{-1}): 2931 and 2827 (CH_2), 1590 (C=C). ^{1}H-NMR, 200 MHz (DMSO-d_6:): δ 1.97 (p, 2H, J = 7.5 Hz, $SCH_2\underline{CH_2}CH_2N$), 2.49 (m, 6H, $SCH_2CH_2\underline{CH_2}N$ and 2 CH_2 of piperazine moiety), 3.17 (m, 4H, 2 CH_2 of piperazine moiety), 3.27

(m, 2H, SCH_2), 6.52 (dd, 1H, $J_{6',5'}$ = 8.2 Hz, $J_{6',4'}$ = 1.8 Hz, H-6' of 3-Cl-C_6H_4), 6.86 (dd, 1H, $J_{4',5'}$ = 8.2 Hz, $J_{4',6'}$ = 1.8 Hz, $J_{4',6'}$ = 1.8 Hz, H-4' of 3-Cl-C_6H_4), 6.92 (s, 1H, H-2' of 3-Cl-C_6H_4), 7.19 (t, 1H, $J_{5',4'}$ = $J_{5',6'}$ = 8.2 Hz, H-5' of 3-Cl-C_6H_4), 7.40 (dd, 1H, $J_{6,7}$ = 8.9 Hz, $J_{6,4}$ = 1.8 Hz , H-6), 7.90 (d, 1H, $J_{4,6}$ = 1.8 Hz, H-4), 8.02 (d, 1H, $J_{7,6}$ = 8.9 Hz, H-7). MS (m/z, %): 438 (M^+, 2). Anal. Calcd for $C_{20}H_{21}Cl_2N_3S_2$. 1.5 H_2O: C, 51.56; H, 5.16; N, 9.02. Found: C, 51.88; H, 4.71; N, 8.96.

2-(3-(4-Phenylpiperazin-1-yl)-propylthio)benzo[*d*]oxazole (6d)

It was separated as colourless crystals (0.16 g, 0.46 mmol, 40.0%), mp 79-80°C. IR (KBr, cm^{-1}): 2942 and 2817 (CH_2), 1597 (C=C). ^{1}H-NMR, 200 MHz (DMSO-d_6:): δ 1.97 (p, 2H, J = 7.5 Hz, $SCH_2\underline{CH_2}CH_2N$), 2.50 (m, 6H, $SCH_2CH_2\underline{CH_2}N$ and 2 CH_2 of piperazine moiety), 3.10 (br, 4H, 2 CH_2 of piperazine moiety), 3.36 (t, 2H, J = 7.5 Hz, SCH_2), 6.75 (t, 1H, $J_{4',5'}$ = $J_{4',3'}$ = 7.5 Hz, H-4' of phenyl-piperazine), 6.89 (d, 2H, J = 7.5 Hz, H-2' and H-6' of phenylpiperazine), 7.18 (t, 2H, J = 7.5 Hz, H-3' and H-5' of phenylpiperazine), 7.31 (m, 2H, H-5 and H-6), 7.61-7.64 (m, 2H, H-7 and H-4). ^{13}C-NMR, 200 MHz (DMSO-d_6:): δ 26.08 ($SCH_2\underline{C}H_2CH_2N$), 29.90 ($S\underline{C}H_2$), 48.13 (2 C of piperazine moiety), 52.02 (2 C of piperazine moiety), 56.07 ($\underline{C}H_2N$), 110.10 (C-4'), 115.31 (C-2' and C-6'), 118.17 (C-7), 118.66 (C-4), 124.13 (C-5), 124.53 (C-6), 129.05 (C-3' and C-5'), 141.34 (C-1'), 150.99 (C-3a), 151.01 (C-7a), 164.62 (C-2). MS (m/z, %): 353 (M^+, 6%). Anal. Calcd for $C_{20}H_{23}N_3OS$: C, 67.96; H, 6.56; N, 11.89. Found: C, 67.44; H, 6.54; N, 11.65.

2-(3-(4-(3-Chlorophenyl)piperazin-1-yl)propylthio)benzo[*d*]oxazole (6e)

It was separated as colourless crystals (0.17 g, 0.44 mmol, 38.6%), mp 70-71°C. IR (KBr, cm^{-1}): 2941 and 2835 (CH_2), 1593 (C=C); ^{1}H-NMR, 500 MHz, (DMSO-d_6:): δ 2.03 (p, 2H, J = 7.69 Hz, $SCH_2\underline{CH_2}CH_2N$), 2.61 (t, 2H, overlapped, $SCH_2CH_2\underline{CH_2}N$), 2.64 (br, 4H, 2 CH_2 of piperazine moiety), 3.21 (br, 4H, 2 CH_2 of piperazine moiety), 3.38 (t, 2H, J = 7.69 Hz, SCH_2), 6.78 (dd, 1H, $J_{6',5'}$ = 7.57 Hz, $J_{6',4'}$ = 1.90 Hz, H-6' of 3-Cl-C_6H_4), 6.88 (dd, 1H, $J_{4',5'}$ = 7.57 Hz, $J_{4',6'}$ = 1.90 Hz, H-4' of 3-Cl-C_6H_4), 6.94 (s, 1H, H-2' of 3-Cl-C_6H_4), 7.20 (t, 1H, $J_{5',4'}$ = 7.57 Hz, H-5' of 3-Cl-C_6H_4), 7.30-7.33 (m, 2H, H-5 and H-6), 7.63-7.65 (m, 2H, H-7 and H-4). **^{13}C**-NMR, 500 MHz, (DMSO-d_6:): δ 25.59 ($SCH_2\underline{C}H_2CH_2N$), 29.71 ($S\underline{C}H_2$), 47.09 (2 C of piperazine moiety), 52.06 (2 C of piperazine moiety), 55.66 ($\underline{C}H_2N$), 110.09 (C-7), 113.63 (C-4'), 114.55 (C-2'), 118.15 (C-6' and C-4), 124.13 (C-5), 124.52 (C-6), 130.37 (C-5'), 133.78 (C-1'), 141.28 (C-3a), 151.19 (C-3'), 151.93 (C-7a), 164.48 (C-2). MS (m/z, %): 387 (M^+, 8), 389 (M^++2, 4). Anal. Calcd for $C_{20}H_{22}ClN_3OS$. 0.5 H_2O: C, 60.46; H, 5.79; N, 10.58. Found: C, 60.16; H, 5.50; N, 10.16.

2-(3-(4-(3-Chlorophenyl)piperazin-1-yl)propylthio)-1*H*-benzo-[*d*]-imidazole (6f)

It was separated as brown crystals (0.37g, 0.95 mmol, 84.1%), mp. 100°C**.** IR (KBr, cm^{-1}): 3161 (br, NH), 2948 and 2818 (CH_2), 1594 (C=C). ^{1}H-NMR, 500 MHz, (DMSO-d_6:): δ 1.98 (p, 2H, J = 7.33 Hz, $SCH_2\underline{CH_2}CH_2N$), 2.70 (m, 6H, $SCH_2CH_2\underline{CH_2}N$ and 2 CH_2 of piperazine moiety), 3.23 (br, 4H, 2 CH_2 of piperazine moiety), 3.32 (t, 2H, J = 7.33 Hz, SCH_2), 6.77 (dd, 1H, $J_{6',5'}$ = 8.10 Hz, $J_{6',4'}$ = 1.80 Hz, H-6' 3-Cl-C_6H_4), 6.87 (dd, 1H, $J_{4',5'}$ = 8.10 Hz, $J_{4',6'}$ = 1.80 Hz, H-4' of 3-Cl-C_6H_4), 6.93 (s, 1H, H-2' of 3-Cl-C_6H_4), 7.11 (m, 2H, H-5 and H-6), 7.19 (t, 1H, $J_{5',4'}$ = 8.10 Hz, H-5' of 3-Cl-C_6H_4), 7.44 (m, 2H, H-4 and H-7). ^{13}C-NMR, 500 MHz, (DMSO-d_6:): δ 25.87 ($SCH_2\underline{C}H_2CH_2N$), 29.05 ($S\underline{C}H_2$), 46.88 (2 C of piperazine moiety), 51.95 (2 C of piperazine moiety), 55.72 ($\underline{C}H_2N$), 113.78 (C-4'), 114.72 (C-2'), 118.39 (C-6'), 121.41 (C-4-7), 130.51 (C-5'), 133.91 (C-1'), 150.11 (C-3'), 151.87 (C-3a and 7a). MS (m/z, %): 386 (M^+, 3), 388 (M^++2, 1.3). Anal. Calcd for $C_{20}H_{23}ClN_4S$. 1.1 H_2O: C, 59.00; H, 6.19; N, 13.76. Found: C, 58.52; H, 5.96; N, 13.61.

Pharmacology

***In vitro* biological evaluation radioligand binding experiments**

Cell culture and receptor density

Human D_1, D_2, D_3, D_5 receptors were stably expressed in Chinese Hamster Ovary (CHO) cells. The densities of receptors measured with [^{3}H]-spiperone, Cells were grown at 37°C under a humidified atmosphere of 5% CO_2: 95% air in HAM/F12-medium (Sigma-Aldrich) for CHO cells supplemented with 10% fetal bovine serum, 1 mM *L*-glutamine and 0.2 µg/mL of G 418 (all by Sigma-Aldrich).

Preparation of Whole-Cell-Suspension[16]

Human D_1, D_2, D_3 and D_5 receptor cell lines were grown on T 175 culture dishes (Greiner bio-one, Frickenhausen) to 85% confluency, the medium was removed and the cells were incubated with 3 mL trypsine-EDTA-solution (Sigma-Aldrich) to remove the cells from the culture dish. After incubation, cells were suspended in 3-6 mL added medium in order to stop the effect of trypsine-EDTA-solution. The resulting suspension was centrifuged (1800-2400 rot/min, 4°C, 4 min.), the pellet resuspended in 10 mL PBS (ice-cooled, calcium- and magnesium-free), pelleted, and this procedure was repeated. The resulting pellet was then resuspended in 12 mL of buffer (5 mM magnesium chloride, 50 mM TRIS-HCl, pH=7.4) and the resulting suspension was directly used for the radioligand binding assay.

Radioligand binding assay

The binding studies were performed following the protocol previously described but in 96- well format[17]. The assays with the whole-cell-suspension were carried out in

triplicate in a volume of 550 µL (final concentration): TRIS-Mg^{2+}-buffer (345 µL), [^{3}H]-SCH 23390 or [^{3}H]-Spiperone (50 µL) for D_1 and D_2 family, respectively, whole-cell-suspension (100 µL) and appropriate drugs (55 µL). Non-specific binding was determined using haloperidol (10 µM). The incubation was initiated by addition of the radioligand [^{3}H]-Spiperone (Amersham Biosiences, Little Chalfont, UK). It was carried out in 96 deep well plates (Greiner bio-one, Frickenhausen) using a Thermocycler (Thermocycler comfort, Eppendorf, Wessling) at 27°C. The incubation was terminated after 90 min by rapid filtration with a PerkinElmer Mach III Harvester™ using a PerkinElmer Filtermat A, previously treated with a 0.25% polyethyleneimine-solution (Sigma-Aldrich) and washed once with water. The filtermat was dried for 3 min with 400 watt using a microwave (MW 21, Clatronic, Kempen). The dry filtermat was placed in a filter plate (Omni filter plates, PerkinElmer Life Sciences) and each field of the filtermat moistened with 50 µL Microscint 20™ scintillation cocktail. The radioactivity retained on the filters was counted using a Top Count NXT™ microplate scintillation counter (Packard, Ct., USA). For determining the K_i values at least two independent experiments each in triplicate were performed.

The competition binding data were analyzed with GraphPad Prism™ software using nonlinear least squares fit. For calculating the mean, standard deviation and standard error of the mean the software Microsoft Excel™ was used. K_i values were calculated from IC_{50} values applying the equation of Cheng and Prusoff[18].

***In vivo* biological evaluation**

In vivo biological evaluation of the tested compounds on the arterial blood pressure of normotensive adult cats was performed according to the reported method[19].

Materials

Heparin (5000 IU/mL), phenobarbitone sodium (30 mg/kg), and prazosin ((250-1000 µg/Kg)), tested compounds are **3a, 6b, 6c, 6d** and **6f** (250-1000 µg/Kg).

Method

Male cats weighing 2–3 kg were anaesthetized with phenobarbitone sodium (30 mg/kg ip) and the femoral artery of the leg was exposed and then connected to saline infusion through a cannula. Tested drugs were all dissolved in 1 mL DMSO and then diluted with water to the final volume. Tested compounds were injected gradually in increasing doses. The effects of prazosin (reference drug) and saline/DMSO (control) were compared to those of the tested compounds.

Statistical analysis

Student's *t* test was used for analysis of the biochemical parameters. The data were expressed as mean ± standard error. Statistical

analysis was done according to Snedecor and Cochron[20].

RESULTS AND DISCUSSION

Generation of α_1-AR antagonist hypothesis

The generated α_1-AR antagonist hypothesis was carried out adopting a reported method[13] by using CATALYST software and HipHop modules. Such an ideal hypothesis encompassed five features namely; positive ionizable (PI, red sphere), hydrogen bonding acceptor (HBA, green sphere) and three hydrophobic features (HY1, HY2 and HY3, blue sphere). Molecular modeling simulation studies were then conducted by measuring the compare/fit values, separately, between the conformational models of **3a, 6b, 6c, 6d, 6f** and the ideal α_1-AR antagonist hypothesis (Figs. 2&3). The results of the best fitting value, as well as the conformational energy of the best-fitted conformer with this hypothesis are given in Table I.

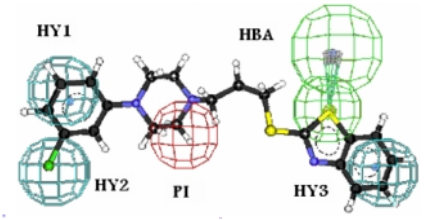


Fig. 2: mapping of α_1-AR antagonist hypothesis and **6b**.

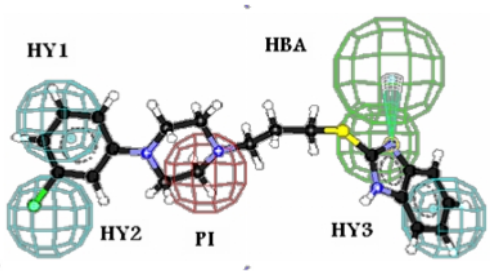


Fig. 3: mapping of α_1-AR antagonist hypothesis and **6f**.

Table I: Compare/fit and conformational energy values of the best fitted conformers of compounds **3a**, **6b**, **6c**, **6d**, **6f** and the α_1-AR antagonist hypothesis.

Compds No.	Fitting values with α_1-antagonist hypothesis	Conf. energy at the antagonist hypothesis (kcal mol^{-1})
3a	2.90	14.80
6b	3.82	4.03
6c	3.61	0.07
6d	2.99	11.61
6f	3.20	0

Compounds **3b-f**, **6a**, **6b** are not mentioned due to low fitting values.

Chemistry

The designed target compounds were depicted in schemes 1 and 2. 2-Chloro-*N*-(6-substituted benzo[*d*]thiazol-2-yl)acetamides (**2a-c)** were prepared through acylation of 2-aminobenzothiazoles **(1a-c)** with chloroacetyl chloride obeying the reported methods[21-23].

Alkylation of various arylpiperazines using the prepared chloroacetamidobenzothiazoles **2a-c** afforded the target compounds N-(6-substitutedbenzo[*d*]thiazol-2-yl)-2-(4-arylpiperazin-1-yl)acetamides (**3a-f**), Scheme 1.

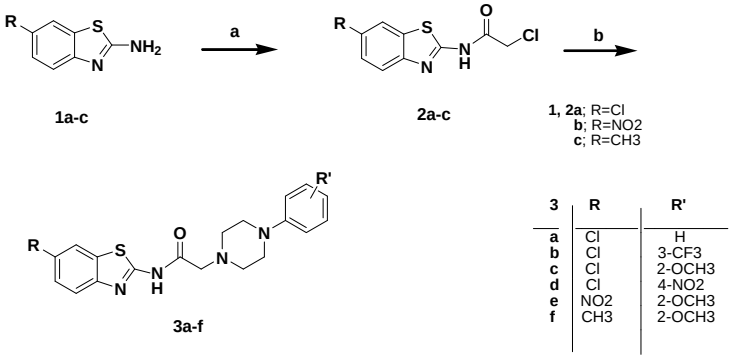
On the other hand, alkylation of the different 2-mercaptobenzothiazoles / -oxazole / -imidazole **4a-d** with 1-(3-chloropropyl)-4-arylpiperazines **5a,b** produced the target compounds 2-(3-(4-arylpiperazin-1-yl)propylthio)benzo[*d*]thiazoles **(6a-c)**, 2-(3-(4-arylpiperazin-1-yl)propylthio)benz[*d*]oxazoles (**6d**,**e)** and 2-(3-(4-(3-chlorophenyl)piperazin-1-yl)-propylthio)-1*H*-benz[*d*]imidazole (**6f**), Scheme 2.

The structures of the prepared compounds **3a-f** and **6a-f** were confirmed by elemental analysis and spectral data. ^{1}H- and ^{13}C-NMR spectra (500 MHz) as well as HH COSY, CH COSY and COLOC (long range CH-correlation) revealed the positions of protonated and quaternary carbon atoms of compounds **3a**, **6b**, **6e** and **6f**.

Pharmacology

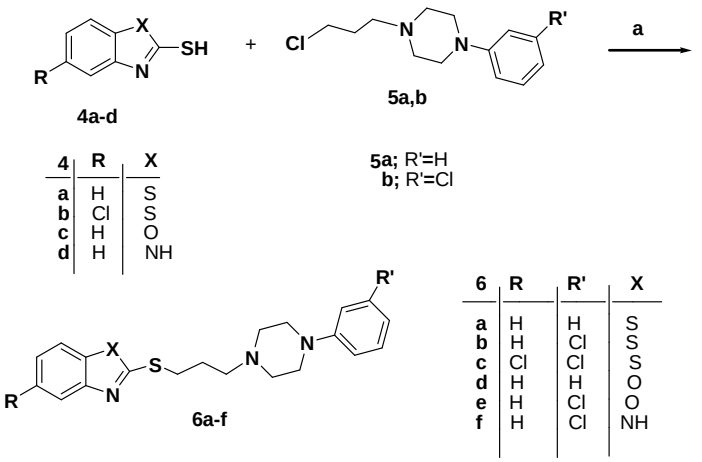
***In vitro* biological evaluation**

Benzo[*d*]thiazole-acetamides, **3a-f** and heteroarylthiopropylpiperazines, **6a-f** were subjected to an *in vitro* biological evaluation for their affinities for D_1, D_2, D_3 and D_5 receptor subtypes stably expressed in CHO cells (Table II). Heteroarylthiopropylpiprazines, **6a, 6b, 6d, 6e,** and **6f** showed certain affinity for D_3, however, the benzothiazole-acetamidopiperazines **3a-f** lacked the affinity toward all the dopamine receptor subtypes. Based on this finding, thiopropyl spacer seems



Reagents: **a**; Chloroacetyl chloride/triethylamine, **b**; Arylpiperazine/triethylamine.

Scheme 1



4	R	X
a	H	S
b	Cl	S
c	H	O
d	H	NH

6	R	R'	X
a	H	H	S
b	H	Cl	S
c	Cl	Cl	S
d	H	H	O
e	H	Cl	O
f	H	Cl	NH

Reagents: **a**; Anhydrous potassium carbonate/ potassium iodide.

Scheme 2

Table II: Results of radioligand binding studies (affinity) of compounds **3a-f**, **6a**, **6b**, **6d**, **6e** and **6f** for the human dopamine receptors stably expressed in CHO-cells.

Compds No	K_i-values [nM] Average ± SD or SEM (Number of experiments in triplicate)				Ratio of *Ki* values		
3a	D_1	D_2	D_3	D_5	D_1/D_3	D_2/D_3	D_5/D_3
	Inactive	> 10 000	inactive	> 10 000	-	-	-
3b	inactive	>10 000	inactive	> 10 000	-	-	-
3c	>10 000	>10 000	inactive	> 10 000	-	-	-
3d	> 10 000	> 10 000	>10 000	> 10 000	-	-	-
3e	Inactive	Inactive	>10 000	> 10 000	-	-	-
3f	> 10 000	> 10 000	Inactive	> 10 000	-	-	-
6a	569 ± 198 (2)	203 ± 5 (2)	14 ± 5 (2)	> 10 000	41	15	>714
6b	200 ± 50 (3)	537 ± 24 (2)	69 ± 6 (2)	460 ± 59 (2)	3	8	7
6d	718 ± 69 (2)	243 ± 9 (2)	80 ± 2 (2)	358 ± 34 (2)	9	3	4
6e	146 ± 39 (3)	411 ± 70 (2)	126 ± 7 (2)	224 ± 20 (2)	1	3	2
6f	1438 ± 115 (2)	952 ± 117 (2)	123 ± 1 (2)	522 ± 301 (4)	12	8	4

SD = Standard deviation
SEM = Standard error of the mean
The SEM was used, when the number of values was less than three.

playing an important role in binding selectively for D_3, where the sulfur atom mimics the electronegative amide moiety present in BP 897, ST 198 and FAUC 365. Moreover, the three-carbon chain (spacer) probably gives the suitable distance between the positive ionizable nitrogen of piperazine and hydrophobic portion of the heteroaryl moiety. Among the tested compounds, **6a** showed the highest binding affinity for D_3 (*Ki* = 14 nM) which was comparable to that of the lead ST 198 (*Ki* = 12 nM), where **6a** has displayed the selectivity ratios: $D_1/ D_3 = 41$, $D_2/ D_3 = 15$ and $D_5/ D_3 > 714$. On the other hand, compounds **6b, 6d, 6e, 6f** revealed moderate binding affinity (*Ki* values = 69-126 nM, Table II) for D_3 compared to that of the lead ST 198.

***In vivo* biological evaluation**

Hypotensive evaluation of the tested compounds **3a, 6b, 6c, 6d** and **6f** revealed that **6d** and **6f** elicited moderate hypotensive activity compared to that of prazosin at dose 250 μg/Kg. However, the rest of compounds lacked hypotensive activity at the same dose level (250 μg/Kg, Table III).

Conclusion

According to the data obtained from α_1-AR hypothesis, radioligand binding experiments on dopamine receptor subtypes and *in vivo* hypotensive activity, three pharmacophoric features seem to be important for binding affinity to D_3. These features are firstly, the hydrophobic moiety represented by the heteroaryl bioisostere and secondly, the positive ionizable nitrogen in the arylpiperazine. These two pharmacophoric features are connected together with spacer which is considered the third important feature; its length and electronegativity may play important role in the selectivity for D_3. Additionally, the unsubstiuted benzothiazole attached to phenylpiperazine fragment through thiopropyl spacer may be new scaffold for binding to D_3. Optimization of this spacer is the next goal for more selective D_3 ligands in the future work.

Acknowledgments

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Table III: Effects of compounds **3a**, **6b**, **6c**, **6d**, and **6f** on systolic (SBP) and diastolic blood pressure (DBP) of anaesthetized normotensive cats.

Compds No.	Dose (µg/Kg)	SBP (mmHg) ± SE	Mean decrease %	DBP (mmHg) ± SE	Mean decrease %
Control	-	100 ± 1.2	-	90 ± 1.3	-
Prazosin	250	94.8 ± 1.5*	-5.20%	86.5 ± 2.2	-3.86%
	500	89.6 ±1.9*	-10.40%	82.7 ± 0.4*	-8.11%
	1000	79.7 ± 2.3*	-20.30%	79.1 ± 2.7*	-12.14%
3a	250	100.0 ± 2.8	0.00%	90.0 ± 2.8	0.00%
	500	95.8 ± 3.1*	-4.21%	88.0 ± 1.4	-2.22%
	1000	94.7 ± 0.5*	-5.26%	87.0 ± 1.4*	-3.33%
6b	250	100.0 ± 1.3	0.00%	90.0 ± 2.3	0.00%
	500	98.9 ± 0.7	-1.10%	89.0 ±0.1	-1.11%
	1000	91.6 ± 2.2*	-8.40%	80.0 ± 0.6*	-11.11%
6c	250	100.0 ± 3.4	0.00%	90.0 ± 2.6	0.00%
	500	100.0 ± 1.2	0.00%	90.0 ± 1.9	0.00%
	1000	94.4 ± 1.2*	-5.56%	87.8 ± 1.5	-2.50%
6d	250	97.3 ± 2.4	-2.73%	88.3 ± 4.2	-1.88%
	500	97.3 ± 0.6*	-2.73%	87.3 ± 1.8	-2.99%
	1000	92.7 ± 1.7*	-7.27%	85.3 ± 2.7*	-5.21%
6f	250	98.0 ± 2.4	-2.03%	88.1 ± 2.6	-2.11%
	500	93.9 ± 1.6*	-6.12%	87.1 ± 1.6	-3.16%
	1000	91.2 ± 3.0*	-8.85%	81.5 ± 2.0*	-9.47%

*Statistically different from control with p value < 0.05.

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