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DOPAMINE D_2 RECEPTOR ANTAGONIST ACTIVITY AND MOLECULAR MODELING OF CERTAIN NEW CYCLOHEXANE DERIVED ARYLCARBOXAMIDES STRUCTURALLY RELATED TO METOCLOPRAMIDE

MOHAMED N. ABOUL-ENEIN[a*], AIDA A. EL-AZZOUNY[a], MOHAMED I. ATTIA[a,b], YOUSREYA A. MAKLAD[c], MOHAMED ABD EL-HAMID ISMAIL[d], NASSER M.S. ISMAIL[d], WALAA H.A. ABDEL-HAMID[e]

[a]*Medicinal and Pharmaceutical Chemistry Department (Medicinal Chemistry group), Pharmaceutical and Drug Industries Research Division, National Research Centre, 12622, Dokki, Giza, Egypt*

[b]*Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*

[c]*Medicinal and Pharmaceutical Chemistry Department (Pharmacology group), Pharmaceutical and Drug Industries Research Division, National Research Centre, 12622, Dokki, Giza, Egypt*

[d]*Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt*

[e]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Misr University for Science & Technology, 6th of October City, Egypt*

A series of certain new *N*-{[1-(4-aralkyl/ethylpiperazine-1-yl)cyclohexyl]methyl}arylcarbox- amides **1a-t** structurally related to the antiemetic Metoclopramide (**I**) was synthesized starting from cyclohexanone, *N*-aralkyl and/or ethylpiperazine, and KCN in the presence of conc. HCl to furnish the carbonitrile derivatives **3a-d**. Subsequent reduction of **3a-d** produced the respective amines **4a-d** which were elaborated to the desired arylcarboxamides **1a-t** through amide coupling reactions. The target compounds **1a-t** were evaluated for their dopamine D_2 receptor antagonistic activity *in vivo* by measuring their ability to inhibit apomorphine-induced chewing "Zwansgnagen" in rats. Compound **1h** (ED_{50} = 5.94 μmol/kg) is the most active congener being nearly 2-fold more potent than the previously reported cyclohexane-based dopamine D_2 receptor antagonist **II** (ED_{50} = 11.66 μmol/kg). Molecular simulation study including fitting to dopamine D_2 receptor antagonists 3D-pharmacophore model using Discovery Studio 2.5 programs showed high-fit values. The experimental dopamine D_2 receptor antagonistic activity of compounds **1a-t** was consistent with the molecular modeling study.

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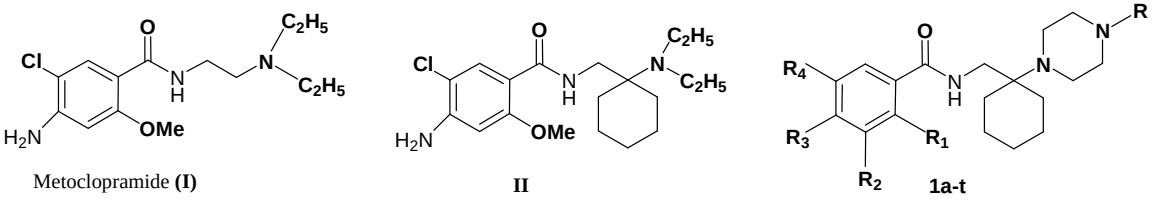
1. Introduction

Metoclopramide (**I**), the parent arylcarboxamide in the orthopramides family, is clinically used as a gastroprokinetic agent (stimulant of upper gastrointestinal motility) as well as an antiemetic.[1] This gastroprokinetic activity is ascribed to the release of acetylcholine upon stimulation of 5-HT_4 receptors whereas the antiemetic activity is attributed to the antagonistic

*Corresponding author: mnaboulenein@yahoo.com

activity at both 5-HT_3 serotoninergic and D_2 dopaminergic receptors in the chemoreceptor trigger zone (CTZ) in the central nervous system (CNS). Orthopramides possess three common structural elements required for binding to the receptor site: an aromatic moiety, carbonyl function or its bioisosteric group, and a basic nitrogen atom.[2-4] Amongst benzamide derivatives, the cyclohexane derivative **II** was originally reported as Metoclopramide analogue.[5] The weak affinity and lack of selectivity of Metoclopramide for dopaminergic and serotoninergic receptors can be explained by the large number of permissible conformers due to the flexibility of its amino chain.[6] Accordingly, the intense interest for studying certain molecular modifications of Metoclopramide implies; change in the substituents of the aromatic ring, structural variations in the amine moiety to obtain a conformationally restricted amino side chain, and increasing the lipophilicity *via* inclusion of the vicinal carbon atom of the basic nitrogen atom into a cyclohexane ring. This concept will be addressed through the synthesis and biological evaluation of new cyclohexane derived arylcarboxamides **1a-t** as potential dopamine D_2 receptor antagonists structurally related to Metoclopramide (**I**).



Metoclopramide **(I)** **II** **1a-t**

Fig. 1. Structures of Metoclopramide (I), compound II, and target compounds 1a-t.

2. Experimental

2.1. Chemistry

All melting points were determined using Electrothermal Capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded as thin film (for oils) in NaCl discs or as KBr pellets (for solids) with JASCO FT/IR-6100 spectrometer and values are represented in cm^{-1}. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were carried out on Jeol ECA 500 MHz spectrometer using TMS as internal standard and chemical shift values were recorded in ppm on δ scale. The ^1H NMR data were represented as follows: chemical shifts, multiplicity (s. singlet, d. doublet, dd. doublet of doublet, t. triplet, m. multiplet, br. broad), number of protons, and type of protons. The ^{13}C NMR data were represented as chemical shifts and type of carbons. Mass spectral data were obtained with electron impact (EI) ionization technique at 70 eV and chemical ionization (CI/CH_4) from a Finnigan Mat SSQ-7000 Spectrometer. Elemental analyses were carried out in Microanalytical Unit, National Research Centre and Cairo University. Silica gel TLC (thin layer chromatography) cards from Merck (silica gel precoated aluminum cards with fluorescent indicator at 254 nm) were used for thin layer chromatography. Visualization was performed by illumination with UV light source (254 nm). Column chromatography was carried out on silica gel 60 (0.063-0.200 mm) obtained from Merck.

2.1.1. General procedure for the synthesis of *N*-aralkylpiperazines 2b-d

Piperazine dihydrochloride monohydrate (3.54 g, 20.0 mmol) was added to a stirred warmed (65°C) solution of piperazine hexahydrate (3.89 g, 20.0 mmol) in absolute ethanol (9.87 mL). Appropriate aralkyl chloride (20.0 mmol) was added to the reaction mixture dropwise during 5 min with vigorous stirring. Separation of white needles was observed immediately and stirring was further continued for 25 min at 65 °C, then cooling at 0 °C for 30 min. The precipitated piperzine dihydrochloride was filtered off and washed with cold absolute ethanol (10 mL). The combined filtrate and washings were dried (Na_2SO_4), filtered and evaporated under reduced pressure to afford the respective piperazine derivatives **2b-d** as monohydrochloride salts in 66-95% yields.

2.1.1.1. 1-Benzylpiperazine (2b)[7]

White solid, m.p. 164 °C of monohydrochloride salt**,** yield 4.1g (95%).

2.1.1.2. 1-(3,4,5-Trimethoxybenzyl)piperazine (2c)

Buff solid, m.p. 230 °C of dihydrochloride salt (Lit.[8] 210-211), yield 4.8 g (80%).

2.1.1.3. 1-Benzhydrylpiperazine (2d)

Yellowish white solid, m.p. 70-72 °C of base (Lit. [9] 70-72), yield 3.8 g (66%).

2.1.2. General procedure for the Synthesis of [1-(4-aralkyl/ethylpiperazin-1-yl)cyclo-hexyl]acetonitriles 3a-d

Ethylpiperazine **2a** and/or the appropriate *N*-aralkylpiperazine **2b-d** as monohydrochloride (100 mmol) were mixed carefully with conc. HCl (0.96 mL, 26.0 mmol) and pH of the reaction mixture was adjusted to 3-4. Cyclohexanone (0.81 mL, 100.0 mmol) was added to the resulting solution followed by addition of potassium cyanide (0.65 g, 100.0 mmol) in H_2O (1.72 mL). The reaction mixture was stirred for 2 h at room temperature then was allowed to stand overnight. The reaction mixture was basified (10% NaOH) and the formed precipitate was filtered off and washed with water (10 mL) to afford the corresponding carbonitrile derivatives **3a-d** in 50-82% yields. The crude **3a-d** were used in the next step without further purification. Analytical samples of **3a**, **3c**, and **3d** were obtained after recrystallisation from isopropanol.

2.1.2.1. [1-(4-Ethylpiperazin-1-yl)cyclohexyl]acetonitrile (3a)

Yellow solid, m.p. 76-78 °C, yield 5.4 g (50%). IR (KBr, cm^{-1}) exhibited bands at 2217 (CN), 3742, 643. ^{1}H NMR ($CDCl_3$) δ: 1.08-1.84 (m, 15H, 5 x C*H*$_2$) cyclohexyl, C*H*$_2$-CH_3, CH_2-*CH*$_3$), 2.09-2.86 (m, 8H, (4 x CH_2) piperazine). ^{13}C NMR ($CDCl_3$) δ: 11.8 (*C*H_3), 21.9, 24.7, 33.6, 46.2, 51.8, 52.7, 60.5 (6 x CH_2), C_q), 116.6 (CN). MS (EI) *m/z* (%): 221.2 (13.7, M^+), 114 (25), 109.1 (10.3), 71 (100). Anal. Calcd. for $C_{14}H_{25}N_3$: C, 71.44; H, 10.71; N, 17.85. Found: C, 71.64; H, 10.53; N, 17.75.

2.1.2.2. [1-(4-Benzylpiperazin-1-yl)cyclohexyl]acetonitrile (3b)[10]

White solid, m.p. 94 °C, yield 9.2 g (81.4%).

2.1.2.3. {1-[4-(3,4,5-Trimethoxybenzyl)piperazin-1-yl]cyclohexyl}acetonitrile (3c)

Yellowish white solid, m.p. 95 °C, yield 11 g (75%). IR (KBr, cm^{-1}) exhibited bands at 2221(CN), 2931, 2826, 1004.^{1}H NMR ($CDCl_3$) δ: 1.49-1.73 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.46-2.66 (m, 8H, (4 x C*H*$_2$) piperazine), 3.50 (s, 2H, C*H*$_2$-C_6H_5), 3.81 (s, 3H, OC*H*$_3$) , 3.82 (s, 6H, 2 OC*H*$_3$), 6.54 (s, 2H, $H_{ar.}$).^{13}C NMR ($CDCl_3$) δ: 22.0, 22.8, 24.9, 33.9, 46.6, 46.7, 60.8 (6 x CH_2), C_q), 53.2, 56.1 (O*C*H$_3$), 105.7 (*C*H$_{ar.}$) 122.6 (CN), 129.2, 135.9, 153.1 ($C_{ar.}$). MS (EI) *m/z* (%): 373 (8.9, M^+), 265 (21), 182.2 (38), 181 (100). Anal. Calcd. for $C_{22}H_{33}N_3O_3$: C, 68.19; H, 8.58; N, 10.84. Found: C, 67.94; H, 8.82; N, 10.66.

2.1.2.4. [1-(4-Benzhydrylpiperazin-1-yl)cyclohexyl]acetonitrile (3d)

Yellow solid, m.p. 134-136 °C, yield 11.7 g (82%). IR (KBr, cm^{-1}) exhibited bands at 2096 (CN), 3425, 1443, 703. ^{1}H NMR ($CDCl_3$) δ: 1.62-2.14 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.25-2.33 (m, 8H, (4 x C*H*$_2$), piperazine), 4.20 (s, 1H, C*H*), 7.23-7.42 (m, 10H, $H_{ar.}$). ^{13}C NMR ($CDCl_3$) δ: 22.2, 25.0, 27.0, 46.9, 52.0, 53.4 (5 x C*H*$_2$), C_q), 126.9 (CN), 128.0, 128.09, 128.5 ($CH_{ar.}$), 142.8 ($C_{ar.}$). MS (EI) *m/z* (%): 354 (0.2, M^+), 167 (100), 152 (26.5). Anal. Calcd. for $C_{25}H_{31}N_3$: C, 80.39; H, 8.37; N, 11.25. Found: C, 80.22; H, 8.64; N, 11.46.

2.1.3. General procedure for the synthesis of *N*-{[1-(4-aralkyl/ethylpiperazin-1-yl)cyclohexyl]- methyl}arylcarboxamides 1a-l

A solution of anhydrous aluminum chloride (2.1 g, 16.0 mmol) in dry THF (5 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (1.9 g, 49.0 mmol) in dry THF (100 mL) at 0 °C. A solution of the appropriate carbonitrile **3a-d** (11.0 mmol) in dry THF (15 mL) was added dropwise to the cooled (0 °C) reaction mixture and stirring was continued for 24 h at room temperature. The reaction was quenched by a slow addition of saturated sodium sulfate solution at 0-5 °C. The formed precipitate was filtered off and washed with THF (10 mL) and ethyl acetate (25 mL). The combined filtrate and washings were dried (Na_2SO_4), filtered and evaporated under reduced pressure to afford 1-[1-(4-aralkyl/ethylpiperazin-1-yl)cyclohexyl] methanamines **4a-d** in 75-85% yields as pale yellow viscous oils which were solidified upon storage. The crude **4a-d** were pure enough to be used in the next step without further purification. A solution of the appropriate acyl chloride **5a-c** (4.86 mmol) in benzene (20 mL) was added dropwise to a stirred solution of **4a-d** (4.42 mmol) and triethylamine (0.04 mL) in benzene (60 mL). The reaction

mixture was refluxed for 5 h, cooled to room temperature, the formed precipitate was filtered off and washed with benzene (10 mL). The combined filtrate and washings were dried (Na_2SO_4), filtered and evaporated under reduced pressure to afford the respective arylcarboxamides **1a-l** as brown viscous oils. The obtained amides were purified through their dihydrochloride salts which were recrystallised from isopropanol to furnish pure **1a-l**.

2.1.3.1. 1-[1-(4-Ethylpiperazin-1-yl)cyclohexyl]methanamine (4a)

Pale yellow viscous oil, yield 2.1 g (87.5%). IR (KBr, cm^{-1}) exhibited bands at 3745, 3677 (NH_2), 1458, 612. ^{1}H NMR (DMSO-d_6) δ: 1.18 (t, 3H, J = 6.9, CH_3), 1.21-1.58 (m, 10H, (5 x CH_2) cyclohexyl), 3.05 (s, 4H, CH_2-CH_3 and CH_2-NH_2), 3.11-3.46 (m, 8H, (4 x CH_2) piperazine) 8.09 (br. s, 2H, NH_2). ^{13}C NMR (DMSO-d_6) δ: 9.2 (*C*H_3), 21.9, 26.3, 28.5, 42.3, 45.1, 47.8, 50.9, 50.0 (7 x *C*H_2), C_q). MS (EI) m/z (%): 225.2(0.9, M^+), 195.2 (100), 113.1 (6), 83.1 (10.1).

2.1.3.2. **1-[1-(4-Benzylpiperazin-1-yl)cyclohexyl]methanamine (4b)**

Pale yellow viscous oil, yield 2.6 g (86.6%). IR (KBr, cm^{-1}) exhibited bands at 3061, 3027 (NH_2), 14531, 741. ^{1}H NMR ($CDCl_3$) δ: 1.19-1.93 (m, 10H, (5 x CH_2) cyclohexyl), 2.06-2.08 (m, 4H, (2 x CH_2) piperazine), 2.47-2.93 (m, 6H, (2 x CH_2) piperazine, CH_2-C_6H_5), 3.47 (d, 2H, J = 5 Hz, CH_2-NH) 7.26-7.27 (m, 5H, H_{ar}.). ^{13}C NMR ($CDCl_3$) δ: 26.2, 28.4, 29.8, 37.7, 52.4, 52.7, 56.1, 66.7 (7 x CH_2), C_q), 123.0, 131.2, 132.2, (CH_{ar}.), 133.3 (C_{ar}.).

2.1.3.3. **1-{1-[4-(3,4,5-Trimethoxybenzyl)piperazin-1-yl]cyclohexylmethanamine (4c)**

Pale yellow viscous oil, yield 3.8 g (91%). IR (KBr, cm^{-1}) exhibited bands at 3060, 3029 (NH_2), 750. ^{1}H NMR ($CDCl_3$) δ: 1.16-1.43 (m, 10H, (5 x CH_2) cyclohexyl), 2.33-2.74 (m, 8H, (4 x CH_2) piperazine), 3.52 (s, 2H, CH_2-NH_2), 3.73 (s, 2H, CH_2-C_6H_5), 3.76 (s, 3H, OCH_3), 3.76 (s, 2H, 2 OCH_3), 6.48 (s, 2H, H_{ar}.). ^{13}C NMR ($CDCl_3$) δ: 25.8, 26.2, 29.4, 29.5, 35.6, 45.7, 53.4, 54.5 (7 x CH_2), C_q), 58.0, 60.8 (O*C*H_3), 106.0 (CH_{ar}.), 133.8, 136.8, 153.0 (C_{ar}.). MS (EI*) m/z* (%): 378.4 (1.8, M^+ +1), 167 (100), 181 (99), 196 (7).

2.1.3.4. **1-[1-(4-Benzhydrylpiperazin-1-yl)cyclohexyl]methanamine (4d)**

Pale viscous oil, yield 2.7 g (67.5%). IR (KBr, cm^{-1}) exhibited bands at 3060, 3029 (NH_2). ^{1}H NMR ($CDCl_3$) δ: 1.31-1.36 (m, 10H, (5 x CH_2) cyclohexyl), 2.08 (br.s, 4H, and (2x CH_2) piperazine), 3.57-3.65 (m, 6H, CH_2-NH_2, (2x CH_2) piperazine), 5.50 (s, 1H, C*H*-(C_6H_5)$_2$), 7.37-7.45 (m, 10H, H_{ar}.). ^{13}C NMR ($CDCl_3$) δ: 24.3, 25.6, 30.1, 35.6, 48.8, 51.8, 64.6 (6 x *C*H_2), C_q), 127.3, 127.5, 128.6 (*C*H_{ar}.), 142.7 (C_{ar}.). MS (EI) *m/z* (%): 363.3 (0.23, M^+), 333.2 (100), 126.2 (81), 112.1 (47.99).

2.1.3.5. ***N*-{[1-(4-Ethylpiperazin-1-yl)cyclohexyl]methyl}benzamide (1a)**

White solid, m.p. 218 °C (dihydrochloride salt), yield 0.58 g (39.9%). IR (KBr, cm^{-1}) exhibited bands at 3064 (NH), 2813, 1644 (C=O). ^{1}H NMR ($CDCl_3$) δ: 1.04 (t, 3H, J = 6.9 Hz, *C*H_2-CH_3), 1.39-1.59 (m, 10H, (5 x CH_2) cyclohexyl), 2.37 (q, 2H, J = 6.9 Hz, CH_2-CH_3), 2.40-2.67 (m, 8H, (4 x CH_2) piperazine), 3.49 (d, 2H, J = 9.5 Hz, CH_2-NH), 7.39-7.74 (m, 5H, H_{ar}.), 7.04 (br. s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 12.0 (*C*H_3-CH_2), 22.31, 25.98, 29.56, 40.7, 44.35, 52.36, 54.38, 58.05 (7 x CH_2), C_q), 126.8, 128.6, 131.3 (CH_{ar}.), 134.8 (C_{ar}.), 167.1 (C=O). MS (EI) *m/z* (%): 329.2 (0.05, M^+), 195.1 (100), 105.05 (13.74), 77.05 (9.87).

2.1.3.6. ***N*-{[1-(4-Ethylpiperazin-1-yl)cyclohexyl]methyl}-4-chlorobenzamide (1b)**

White solid, m.p. 230 °C (dihydrochloride salt), yield 0.48 g (30%). IR (KBr, cm^{-1}) exhibited bands at 3254 (NH), 2930, 1641 (C=O). ^{1}H NMR ($CDCl_3$) δ: 1.08 (t, 3H, J = 6.9 Hz, -CH_2-CH_3), 1.41-1.61 (m, 10H, 5 x CH_2) cyclohexyl), 1.88 (br. s, 4H, (2 x CH_2) piperazine), 2.38 (q, 2H, J = 6.9 Hz, CH_2-CH_3), 2.69 (br. s, 4H, (2 x CH_2) piperazine), 3.51 (d, 2H, J = 5 Hz, CH_2-NH) 7.06 (br. s,1H, NH), 7.40 (d, 2H, J = 10 Hz, H_{ar}.), 7.70 (d, 2H, J = 10 Hz, H_{ar}.). ^{13}C NMR ($CDCl_3$) δ: 12.0 (CH_2-*C*H_3), 22.4, 25.9, 29.6, 40.8, 44.3, 52.4, 54.4, 58.1 (7 x CH_2), C_q), 128.3, 128.9 (CH_{ar}.), 133.2, 137.5 (C_{ar}.), 166.2 (C=O). MS (EI) *m/z* (%): 364.2 (0.04, M^+ + 1), 195.2 (100), 111.05 (10.59), 84.1 (21.52). Anal. Calcd. for $C_{20}H_{30}ClN_3O.2HCl$: C, 54.99; H, 7.38; N, 9.62. Found: C, 54.57; H, 7.76; N, 9.65.

2.1.3.7. ***N*-{[1-(4-Ethylpiperazin-1-yl)cyclohexyl]methyl}-4-nitrobenzamide (1c)**

Buff solid, m.p. 210 °C (dihydrochloride salt), yield 0.49 g (30%). IR (KBr, cm^{-1}) exhibited bands at 3393 (NH), 2696, 1650 (C=O). ^{1}H NMR ($CDCl_3$) δ: 1.07 (t, 3H, J = 6.9, CH_2-CH_3), 1.40-1.96 (m, 10H, (5 x CH_2) cyclohexyl), 2.38 (q, 2H, J = 6.9, CH_2-CH_3), 2.68 (br. s, 8H, (4 x CH_2) piperazine), 7.22 (br. s, 1H, NH), 7.91 (d, 2H, J = 7.7 Hz, H_{ar}.), 8.27 (d, 2H, J = 7.7 Hz, H_{ar}.). ^{13}C NMR ($CDCl_3$) δ: 12.0 (*C*H_3-CH_2), 22.3, 25.8, 29.5, 44.3, 48.9, 52.3, 54.5, 58.0 (7 x CH_2),

C_q), 123.9, 128.0 ($CH_{ar.}$), 140.4, 149.5 ($C_{ar.}$), 165.0 (C=O). MS (EI) *m/z* (%): 374.2 (0.02, M^+), 195.2 (100), 104.05 (4.59), 84.1 (14.58). Anal. Calcd. for $C_{20}H_{30}N_4O_3.2HCl$: C, 53.69; H, 7.21; N, 12.52. Found: C, 54.11; H, 7.59; N, 12.43.

2.1.3.8. ***N*-{[1-(4-Benzylpiperazin-1-yl)cyclohexyl]methyl}benzamide (1d)**

Buff solid, m.p. 224 °C (dihydrochloride salt), yield 0.64 g (37.1%). IR (KBr, cm^{-1}) exhibited bands at 3265 (NH), 2447, 1673 (C=O), 719. ^{1}H NMR ($CDCL_3$) δ: 1.14-1.62 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.49 (br. s, 4H, (2 x C*H*$_2$) piperazine), 2.67 (br. s, 4H, (2 x C*H*$_2$) piperazine), 3.50 (s, 2H, C*H*$_2$-C_6H_5), 3.53 (d, 2H, *J* = 5 Hz, C*H*$_2$-NH), 7.23-7.51 (m, 10H, $H_{ar.}$), 7.6 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ: 21.4, 25.9, 28.9, 33.9, 44.2, 48.9, 54.6, 63.2 (7 x CH_2), C_q), 126.9, 128.2, 128.3, 129.1, ($CH_{ar.}$, $C_{ar.}$), 167.1 (C=O). MS (EI) *m/z* (%): 391.9 (0.52, M^+), 168 (63), 167 (43), 114 (100). Anal. Calcd. for $C_{25}H_{33}N_3O.2HCl$: C, 64.65; H, 7.60; N, 9.05. Found: C, 64.35; H, 7.45; N, 8.98.

2.1.3.9. ***N*-{[1-(4-Benzylpiperazin-1-yl)cyclohexyl]methyl}-4-chlorobenzamide (1e)**

White solid, m.p. 226 °C (dihydrochloride salt), yield 1.27 g (67.8%). IR (KBr, cm^{-1}) exhibited bands at 3296 (NH), 2872, 929, 1664 (C=O). ^{1}H NMR ($CDCl_3$ δ: 1.39-1.60 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.46 (br. s, 4H, (2 x C*H*$_2$) piperazine), 2.67 (br. s, 4H, (2 x C*H*$_2$) piperazine), 3.51 (s, 4H, C*H*$_2$-NH, C*H*$_2$-C_6H_5), 7.07 (s, 1H, N*H*), 7.25-7.29 (m, 5H, $H_{ar.}$), 7.4 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.7 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$). ^{13}C NMR ($CDCl_3$) δ: 22.3, 25.9, 29.5, 44.4, 53.1, 54.6, 58.0, 63.14 (7 x CH_2), C_q), 127.0, 127.2, 128.2, 128.8, 129.3 ($CH_{ar.}$), 133.2, 137.5, 137.8 ($C_{ar.}$) , 166.0 (C=O). MS (EI) *m/z* (%): 423 (4.3, M^+-2), 257.7 (100), 111 (25), 91 (43.3). Anal. Calcd. for $C_{25}H_{32}ClN_4O.2HCl$: C, 60.18; H, 6.87; N, 8.42. Found: C, 60.49; H, 6.65; N, 8.45.

2.1.3.10. ***N*-{[1-(4-Benzylpiperazin-1-yl)cyclohexyl]methyl}-4-nitrobenzamide (1f)**

White solid, m.p. 245 °C (dihydrochloride salt), yield 0.51 g (22.6%). IR (KBr, cm^{-1}) exhibited bands at 3250 (NH), 2948, 751, 1658 (C=O). ^{1}H NMR ($CDCl_3$) δ: 1.22-1.60 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.00 (s, 2H,C*H*$_2$ piperazine), 2.45 (br. s, 2H, C*H*$_2$ piperazine) 2.66 (s, 4H, (2 x C*H*$_2$) piperazine), 3.52 (d, 2H, *J* = 4 Hz, C*H*$_2$-NH), 7.26 (s, 6H, $H_{ar.}$, N*H*), 7.9 (d, 2H, *J* = 8 Hz, $H_{ar.}$), 8.26 (d, 2H, *J* = 8 Hz, $H_{ar.}$). ^{13}C NMR ($CDCl_3$) δ: 25.9, 28.7, 29.6, 40.8, 44.3, 54.5, 58.0, 60.4 (7 x CH_2), C_q), 123.0, 128.0, 128.3, 129.3, 137.5, ($CH_{ar.}$), 140.4 ,149.5, 165.0 ($C_{ar.}$), 171.1 (C=O). MS (EI) *m/z* (%): 434 (5, M^+-2), 141.8 (39), 139.9 (46), 91 (100). Anal. Calcd. for $C_{25}H_{32}N_4O.2HCl$: C, 58.94; H, 6.73; N, 11.00. Found: C, 58.67; H, 7.01; N, 10.85.

2.1.3.11. ***N*-({1-[4-(3,4,5-Trimethoxybenzyl)piperazin-1-yl]cyclohexyl}methyl)benzamide (1g)**

White solid, m.p. 200 °C (dihydrochloride salt), yield 1.62 g (76.1%). IR (KBr, cm^{-1}) exhibited bands at 3297 (NH), 2498, 1668 (C=O), 754. ^{1}H NMR ($CDCl_3$) δ: 1.16-1.93 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.65-2.79 (br. s, 8H, (4 x C*H*$_2$) piperazine), 3.39 (d, 2H, *J* = 5 Hz, C*H*$_2$-NH), 3.48 (s, 2H, C*H*$_2$-C_6H_5), 3.79 (s, 3H, OC*H*$_3$), 3.81 (s, 6H, 2OC*H*$_3$), 6.50 (s, 2H, $H_{ar.}$), 7.35-7.73 (m, 5H, $H_{ar.}$), 7.97 (s, 1H, *J* = 8 Hz, NH). ^{13}C NMR ($CDCl_3$) δ: 22.2, 25.6, 29.6, 40.8, 44.3, 53.0, 54.6, 60.8 (7 x C*H*$_2$, C_q), 56.13, 58.0 (O*C*H$_3$), 105.7, 127.0, 128.4, 129.5 (*C*H$_{ar.}$), 132.9, 133.0, 133.8, 153.1 ($C_{ar.}$), 167.1 (C=O). MS (EI) *m/z* (%): 482 (0.16, M^+), 209 (61), 104 (100), 76 (35). Anal. Calcd. for $C_{28}H_{39}N_3O_4.2HCl$: C, 60.64; H, 7.45; N, 7.58. Found: C, 60.93; H, 7.58; N, 7.47.

2.1.3.12. *N*-({1-[4-(3,4,5-Trimethoxybenzyl)piperazin-1-yl]cyclohexyl}methyl)-4-chloro- benzamide (1h)

White solid, m.p. 200 °C (dihydrochloride salt), yield 0.85 g (37.1%). IR (KBr, cm^{-1}) exhibited bands at 3027 (NH), 2809, 1627 (C=O), 750. ^{1}H NMR ($CDCl_3$) δ: 1.10-1.66 (m, 10H, (5 x CH_2) cyclohexyl), 2.04-2.58 (m, 8H, (4 x C*H*$_2$) piperazine), 3.34 (d, 2H, *J* = 10 Hz, C*H*$_2$-NH), 3.68 (s, 3H, OC*H*$_3$), 3.72 (s, 8H, 2OC*H*$_3$, C*H*$_2$-C_6H_5), 6.46 (s, 2H, $H_{ar.}$), 7.06 (br.s, 1H, NH), 7.29 (d, 2H, *J* = 5 Hz, $H_{ar.}$), 7.62 (d, 2H, *J* = 5 Hz, $H_{ar.}$). ^{13}C NMR ($CDCl_3$) δ: 22.1, 25.8, 28.8, 30.3, 44.2, 48.8, 53.3, 60.8 (7 x CH_2, C_q), 56.4, 58.0 (OCH_3), 105.8, 128.6, 128.7 ($CH_{ar.}$), 133.4, 134.1, 135.6, 136.9, 153.1 ($C_{ar.}$), 169.1 (C=O). MS (EI) *m/z* (%): 518 (0.34, M^+ + 2), 348 (29.9), 181 (100), 139 (26.5). Anal. Calcd. for $C_{28}H_{38}ClN_3O_4.2HCl$: C, 57.10; H, 6.85; N, 7.13. Found: C, 56.88; H, 7.08; N, 6.91.

2.1.3.13. *N*-({1-[4-(3,4,5-Trimethoxybenzyl)piperazin-1-yl]cyclohexyl}methyl)-4-nitro- benzamide (1i)

Buff solid, m.p. 100 °C (dihydrochloride salt), yield 1.39 g (52.6%). IR (KBr, cm^{-1}) exhibited bands at 3369, 3225 (NH_2), 2442, 1597 (C=O), 623. ^{1}H NMR ($CDCl_3$) δ: 1.23-1.48 (m,

10H, (5 x CH_2) cyclohexyl), 1.93-2.26 (m, 8H, (4 x CH_2) piperazine), 3.84 (s, 2H, CH_2-C_6H_5), 3.89 (d, 2H, *J* = 4 Hz, CH_2-NH), 3.96 (s, 3H, OCH_3), 3.96 (s, 6H, 2OCH_3) 6.94 (br.s, 1H, N*H*), 7.24 (d, 2H, *J* = 5 Hz, $H_{ar.}$), 8.19 (d, 2H, *J* = 5 Hz, $H_{ar.}$), 8.27-8.29 (m, 2H, $H_{ar.}$). ^{13}C NMR ($CDCl_3$) δ: 25.9, 26.3, 29.0, 48.9, 51.6, 60.8, 63.4, 63.5 (7 x CH_2, C_q), 53.6, 56.1, (OCH_3), 105.9, 113.7, 119.4 ($CH_{ar.}$), 131.6, 134.0, 136.8, 151.1, 153.0 ($C_{ar.}$), 167.2 (C=O). MS (EI) *m/z* (%): 526 (0.01, M^+), 123 (57), 84 (100), 78 (47). Anal. Calcd. for $C_{28}H_{38}N_4O_6$.2HCl: C, 56.09; H, 6.72; N, 9.34. Found: C, 56.29; H, 6.62; N, 9.56.

2.1.3.14. *N*-{[1-(4-Benzhydrylpiperazin-1-yl)cyclohexyl]methyl}benzamide (1j)

White solid, m.p. 222 °C (dihydrochloride salt), yield 0.83 g (40%). IR (KBr, cm^{-1}) exhibited bands at 3058.55 (NH), 1663 (C=O), 699. ^{1}H NMR ($CDCl_3$) δ: 1.28-1.63 (m, 10H, (5 x CH_2) cyclohexyl), 2.44-2.69 (m, 8H, (4 x CH_2) piperazine), 3.55 (s, 2H, CH_2-NH), 4.22 (s, 1H, C*H*), 7.18-7.46 (m, 15H, $H_{ar.}$), 7.79 (br.s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ: 22.4, 26.0, 29.4, 40.6, 44.6, 48.9, 53.4, (6 x CH_2, C_q), 126.9, 127.0, 128.0, 128.5, 128.6, 128.7 ($CH_{ar.}$), 142.4, 142.7 ($C_{ar.}$), 167.1 (C=O). MS (EI) *m/z* (%): 467.64 (0.42, M^+), 333 (93.2), 167 (100), 105 (51.5). Anal. Calcd. for $C_{31}H_{37}N_3O$.2HCl: C, 68.88; H, 7.27; N, 7.77. Found: C, 68.54; H, 7.47; N, 8.01.

2.1.3.15. ***N*-{[1-(4-Benzhydrylpiperazin-1-yl)cyclohexyl]methyl}-4-chlorobenzamide (1k)**

White solid, m.p. 218 °C (dihydrochloride salt), yield 1.27 g (56.9%). IR (KBr, cm^{-1}) exhibited bands at 3409 (NH), 1649.8 (C=O), 1018, 954. ^{1}H NMR ($CDCl_3$) δ: 1.12-1.97 (m, 10H, (5 x CH_2) cyclohexyl), 2.23-2.87 (m, 8H, (4 x CH_2) piperazine), 3.68 (d, 2H, *J* = 10 Hz, CH_2-NH), 4.24 (s, 1H, C*H*), 7.16-7.19 (m, 11H, $H_{ar.}$, N*H*), 7.27 (d, 2H, *J* = 5 Hz, $H_{ar.}$), 7.44 (d, 2H, *J* = 5 Hz, $H_{ar.}$). ^{13}C NMR ($CDCl_3$) δ: 26.4, 29.1, 30.4, 49.3, 52.4, 53.5, 63.4 (6 x CH_2, C_q), 126.9, 127.0, 128.0, 128.5, 128.6 ($CH_{ar.}$), 128.7, 142.4, 142.7 ($C_{ar.}$), 167.1 (C=O). MS (EI) *m/z* (%): 502.35 (0.04, M^+), 334 (100), 167 (33.26). Anal. Calcd. for $C_{31}H_{36}ClN_3O$.2HCl: C, 64.75; H, 6.66; N, 7.31. Found: C, 64.85; H, 6.91; N, 7.64.

2.1.3.16. ***N*-{[1-(4-Benzhydrylpiperazin-1-yl)cyclohexyl]methyl}-4-nitrobenzamide (1l)**

Buff solid, m.p. 222 °C (dihydrochloride salt), yield 2.12 g (93.3%). IR (KBr, cm^{-1}) exhibited bands at 3037 (NH), 1650 (C=O), 698. ^{1}H NMR ($CDCl_3$) δ: 1.09-1.94 (m, 10H, (5 x CH_2) cyclohexyl), 2.26-2.42 (m, 8H, (4 x CH_2) piperazine), 3.60 (br.s, 2H, CH_2-NH), 4.27 (s, 1H, C*H*), 7.16-7.48 (m, 14H, $H_{ar.}$), 8.20 (br.s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ: 25.7, 25.9, 34.0, 45.5, 49.1, 51.5, 52.1 (6 x CH_2, C_q), 63.5 (C*H*), 126.9, 127.0, 128.0, 128.5, 128.6, ($CH_{ar.}$) 128.7, 42.4, 142.7 ($C_{ar.}$), 167.8 (C=O). MS (EI) *m/z* (%): 512.5 (0.6, M^+), 167 (100), 150 (35.4). Anal. Calcd. for $C_{31}H_{36}N_4O_3$.2HCl: C, 63.59; H, 6.54; N, 9.54. Found: C, 63.25; H, 6.47; N, 9.84.

2.1.4. General procedure for synthesis of 4-amino-*N*-{[1-(4-aralkyl/ethylpiperazin-1-yl)cyclo- hexyl]methyl}benzamides 1m-p

A solution of the appropriate arylcarboxamide **1c**, **1f**, **1i**, and **1l** (2.62 mmol) in 250 mL ethanol (95%) was hydrogenated at room temp and normal pressure for 48 h, using (120 mg) of 10% Pd/C for **1c**.HCl and Raney nickel for **1f**, **1i**, and **1l**. The catalyst was filtered off, and ethanol was evaporated under vacuum to afford the corresponding amines **1m-p** as viscous oils in 33-78.7% yields.

2.1.4.1. *N*-{[1-(4-Ethylpiperazin-1-yl)cyclohexyl]methyl}-4-aminobenzamide (1m)

Yellow viscous oil, yield 0.30 g (33.3%). IR (KBr, cm^{-1}) exhibited bands at 3751 and 3422 (NH_2), 2927, 1636 (C=O), 1604 (NH bending). ^{1}H NMR ($CDCl_3$) δ: 1.07 (t, 3H, *J* = 6.9 Hz, CH_2-CH_3), 1.42-1.58 (m, 10H, (5 x CH_2) cyclohexyl), 2.39 (q, 2H, *J* = 6.9 Hz, CH_2-CH_3), 2.42-2.69 (m, 8H, (4 x CH_2) piperazine), 3.47 (d, 2H, *J* = 3.4, CH_2-NH), 4.01 (s, 1H, NH_2), 6.63 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 6.84 (br. s, 1H, NH), 7.58 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$). ^{13}C NMR ($CDCl_3$) δ: 11.9 (*C*H_3-CH_2), 22.9, 26.01, 29.6, 40.6, 44.2, 52.3, 54.3, 58.1 (7 x CH_2), C_q), 114.2, 124.3 ($CH_{ar.}$), 128.6, 149.5 ($C_{ar.}$), 167.0 (C=O). MS (EI) *m/z* (%): 344.25 (0.35, M^+), 265.1 (35), 181.05 (100). Anal. Calcd. for $C_{20}H_{32}N_4O$: C, 69.73; H, 9.36; N, 16.26. Found: C, 69.84; H, 9.49; N, 15.99.

2.1.4.2. *N*-{[1-(4-Benzylpiperazin-1-yl)cyclohexyl]methyl}-4-aminobenzamide (1n)

Colourless viscous oil, yield 0.84 g (78.7%). IR (KBr, cm^{-1}) exhibited bands at 3342 and 3219 (NH_2), 2927, 1636 (C=O), 1604 (NH bending), 810. ^{1}H NMR ($CDCl_3$) δ: 1.42-1.59 (m, 10H, (5 x CH_2) cyclohexyl), 2.46 (s, 4H, (2 x CH_2) piperazine), 2.67 (s, 4H, 2 x CH_2) piperazine), 3.50 (d, 2H, *J* = 8.4 Hz, CH_2-N*H*) 3.97 (br. s, 2H, , CH_2-C_6H_5), 6.7 (d, 2H, *J* = 8.4 Hz, H_{ar}), 7.25-7.30 (m, 6H, $H_{ar.}$, NH), 7.6 (d, 2H, *J* = 8.4 Hz, H_{ar}). ^{13}C NMR ($CDCl_3$) δ: 22.36, 25.85, 26.03, 29.61,

44.41, 48.87, 54.71, 63.21 (7 x CH_2), C_q), 114.2, 124.5, 125.6, 127.1, 128.3 ($CH_{ar.}$), 128.6, 137.9, 149.4 ($C_{ar.}$) 167.0 (C=O). MS (EI) *m/z* (%): 406.1 (0.08, M^+), 257 (100), 114.05 (58). Anal. Calcd. for $C_{25}H_{34}N_4O$: C, 73.85; H, 8.43; N, 13.78. Found: C, 73.65; H, 8.75; N, 13.98.

2.1.4.3. *N*-({1-[4-(3,4,5-Trimethoxybenzyl)piperazin-1-yl]cyclohexyl}methyl)-4-amino- benzamide (1o)

Yellow viscous oil, yield 0.52 g (40%). IR (KBr, cm^{-1}) exhibited bands at 3369 and 3225 (NH_2), 2850, 1636 (C=O), 1604 (NH bending), 847. ^1H NMR ($CDCl_3$) δ: 0.79-.1.51 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 1.97-2.93 (m, 8H, (4 x C*H*$_2$) piperazine), 3.38 (s, 4H, C*H*$_2$-NH and C*H*$_2$-C_6H_5), 3.78 (s, 3H, OC*H*$_3$), 3.81 (s, 6H, 2 OC*H*$_3$), 6.49 (s, 2H, $H_{ar.}$), 6.55 (d, 2H, *J* = 10 , $H_{ar.}$), 7.16 (d, 2H, *J* = 10 , $H_{ar.}$), 7.51 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ: 22.2, 22.8, 26.0, 31.9, 44.2, 51.2, 53.0, 60.8 (7 x CH_2), C_q), 54.5, 56.1 (OC*H*$_3$), 105.8, 113.7, 124.6 ($CH_{ar.}$), 129.3, 131.5, 133.5, 148.6, 153.1 ($C_{ar.}$), 170.9 (C=O). MS (EI) *m/z* (%): 495.5 (0.22, M^+ -1), 181 (22.84), 114 (100). Anal. Calcd. for $C_{28}H_{40}N_4O_4$: C, 67.71; H, 8.12; N, 11.28. Found: C, 67.91; H, 8.46; N, 11.68.

2.1.4.4. *N*-{[1-(4-Benzhydrylpiperazin-1-yl)cyclohexyl]methyl}-4-aminobenzamide (1p)

Colourless viscous oil, yield 0.56 g (52.6%). IR (KBr, cm^{-1}) exhibited bands at 3059 and 3027 (NH_2), 3223 (NH), 1605 (C=O), 920. ^1H NMR ($CDCl_3$) δ: 1.20-.1.45 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.30-2.88 (m, 8H, (4 x C*H*$_2$) piperazine), 3.56 (s, 2H, C*H*$_2$-NH), 4.21 (s, 1H, C*H*), 6.53 (d, 2H, *J* = 10 , $H_{ar.}$), 6.94 (br.s, 1H, N*H*) 7.24-7.43 (m, 10H, $H_{ar.}$), 7.60 (d, 2H, *J* = 10 , H_{ar}). ^{13}C NMR ($CDCl_3$) δ: 22.8, 29.0, 44.6, 52.4, 53.6, 57.9 (6 x CH_2), C_q),128.01, 128.09, 128.5, 128.6, 138.1 ($CH_{ar.}$), 142.3, 148.7, 150.0 ($C_{ar.}$), 170.9 (C=O). MS (EI) *m/z* (%): 482.45 (0.05, M^+), 334 (41.7), 167 (100). Anal. Calcd. for $C_{31}H_{38}N_4O$: C, 77.14; H, 7.94; N, 11.61. Found: C, 77.54; H, 7.65; N, 11.45.

2.1.5. Synthesis of methyl 2-methoxy-4-[(phenylcarbonyl)amino]benzoate (7)

Anhydrous K_2CO_3 (29.14 g, 211.0 mmol) was added to a stirred solution of 4-benzamido-2-hyroxybenzoic acid **6** (20.3 g, 79.0 mmol) in acetone (150 mL) and stirring was continued for 5 min at RT, then dimethyl sulphate (19.93 mL, 210.0 mmol) was added dropwise. The reaction mixture was stirred for 1 h at RT then stirring was continued for 18 h at 45 °C. The reaction mixture was filtered, 80% of the filtrate was evaporated under normal pressure, the evaporated solvents were replaced with water, evaporate 40% of this water, cooling (0-5 °C), then add ammonia to adjust pH 9.5-10 and the reaction mixture was stirred for 2 h at 0-5 °C. The precipitated solid was filtered off to afford 13.5 g (60%) of **7** m.p. 148 °C.

2.1.6. Synthesis of methyl 5-chloro-2-methoxy-4-[(phenylcarbonyl)amino]benzoate (8)

Compound **7** (6 g, 20.0 mmol) was mixed with glacial acetic acid (10.6 mL, 175 mmol), conc. HCl (2.84 mL, 20.0 mmol) and distilled H_2O (3.16 mL). The reaction mixture was cooled to 20 °C, and then potassium chlorate was added in four successive portions at 10 min intervals. During addition, The white color of the reaction mixture changed to canary yellow with evolution of chlorine gas. The temperature of the reaction mixture must be kept between 30-35 °C to avoid loss of chlorine gas. After complete addition, the reaction mixture was stirred at RT for 7 h, then water was added (13.2 mL) and stirring was continued for another 1 h at RT to help complete precipitation of the chlorinated compound **7**. The precipitate was filtered off, washed with water several times till neutral to afford 4.9 g (71%) of **8** as buff solid, m.p. 106 °C.

2.1.7. Synthesis of 5-chloro-2-methoxy-4-[(phenylcarbonyl)amino]benzoic acid (9)

To a stirred solution of **8** (4.0 g, 12 mmol) a solution of 1.7 N lithium hydroxide (13 mL) in THF (13.4 mL) was added. The reaction mixture was stirred overnight at RT, then was evaporated under reduced pressure. The residue was dissolved in H_2O (20 mL) and extracted with diethyl ether (2 x 15 mL). The aqueous layer was acidified with conc. HCl under cooling, extracted with ethyl acetate (3 x 15 mL), the organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to afford 3.20 g (84.2%) of **9** as yellowish white solid m.p. 170 °C.

2.1.8. General procedure for synthesis of 4-amino-*N*-{[1-(4-aralkyl/ethylpiperazin-1-yl)- cyclohexyl]methyl}-5-chloro-2-methoxybenzamides 1q-t

To a stirred solution of **9** (1.31 g, 4.30 mmol) in CH_2Cl_2 (10 m), EDCI.HCl (1.3g, 6.79 mmol) was added, then a solution of the appropriate amine **4a-d** (4.30 mmol) in CH_2Cl_2 (5 mL) was added to the reaction mixture. The reaction mixture was stirred overnight at RT, washed with water (2 x 20 mL) then with 10% $NaHCO_3$ (2 x 15 mL). The organic layer was separated, dried

(Na_2SO_4) and evaporated under vacuum to afford the corresponding benzamides *N*-{[1-(4-aralkyl/ethylpiperazin-1-yl)cyclohexyl]methyl}-5-chloro-2-methoxy-4-[(phenylcarbonyl)-amino]benzamides **10a-d** in 44-91% yields which were used in the subsequent hydrolysis step without further purification. A suspension of the appropriate benzamide **10a-d** (20.0 mmol) with 10% NaOH (40 mL) was heated to reflux for 24 h. The reaction mixture was cooled, extracted with CH_2Cl_2 (2 x 25 mL). The organic layer was separated, dried (Na_2SO_4) and evaporated under reduced pressure to give crude **1q-t** as brown viscous oils which were purified through column chromatography to afford the respective pure target compounds **1q-t** in 50-62.5% yields.

2.1.8.1. 5-Chloro-*N*-{[1-(4-ethylpiperazin-1-yl)cyclohexyl]methyl}-2-methoxy-4-[(phenyl- carbonyl)amino]benzamide (10a)

Pale yellow viscous oil, yield 1.98 g (91%). IR (KBr, cm^{-1}) exhibited bands at 3205 (NH), 1644 (C=O), 1532 (C=O), 678. ^{1}H NMR ($CDCl_3$) δ: 1.04-1.55 (m, 13H, C*H*$_3$ and (5 x C*H*$_2$) cyclohexyl), 2.15-2.39 (m, 10H, C*H*$_2$-CH_3 and (4 x C*H*$_2$) piperazine), 3,84 (s, 3H, OCH_3,), 7.26-7.88 (m, 7H, $H_{ar.}$), 8.54 (s, 1H, CH_2-N*H*), 9.29 (s, 1H, N*H*-C=O). ^{13}C NMR ($CDCl_3$) δ: 14.8 (*C*H_3-CH_2), 22.1, 27.0, 35.5, 35.8, 44.6, 45.3, 48.0, 54.2 (7 x CH_2, C_q), 56.1 (OCH_3), 103.9, 114.0, 126.9, 129.1, 132.7 ($CH_{ar.}$), 134.2, 136.9, 154.7 ($C_{ar.}$), 164.8 (C=O), 165.5 (C=O). MS (EI) *m/z* (%): 512.7 (0.05, M^+), 195 (34.5), 105 (50.7), 58 (100).

2.1.8.2. *N*-{[1-(4-Benzylpiperazin-1-yl)cyclohexyl]methyl}-5-chloro-2-methoxy-4-[(phenylß carbonyl)amino]benzamide (10b)

Pale yellow viscous oil, yield 1.6 g (65%). IR (KBr, cm^{-1}) exhibited bands at 3391.21 (NH), 1686 (C=O), 1645 (C=O), 699. ^{1}H NMR ($CDCl_3$) δ: 1.42-1.61 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.48 (br.s, 4H, (2 x C*H*$_2$) piperazine), 2.69 (br. s, 4H, (2 x C*H*$_2$) piperazine), 3.55 (s, 2H, C*H*$_2$-C_6H_5), 3.57 (d, 2H, *J* = 5, C*H*$_2$-NH), 3.92 (s, 3H, OCH_3), 7.25-7.30 (m, 10H, $H_{ar.}$,), 7.53-7.62 (m, 2H, $H_{ar.}$), 8.48 (s, 1H, CH_2-N*H*), 8.63 (s, 1H, N*H*-C_6H_5). ^{13}C NMR ($CDCl_3$) δ: 25.9, 29.0, 29.7, 44.4, 53.1, 56.5, 57.8, 63.1 (7 x CH_2), C_q), 54.1(OCH_3), 103.8, 114.0, 127.0, 127.1, 132.2, 157.1, 128.2, 128.4 ($CH_{ar.}$), 129.2, 129.3, 129.4, 129.5, 132.6, 132.7($C_{ar.}$), 165.5 (C=O), 165.6 (C=O). MS (EI) *m/z* (%): 579 (0.71, M^+ + 4), 175 (4), 91 (15), 63 (100).

2.1.8.3. 5-Chloro-2-methoxy-4-[(phenylcarbonyl)amino]-*N*-({1-[4-(3,4,5-trimethoxy-benzyl)piperazin-1-yl]cyclohexyl}methyl)benzamide (10c)

Brown viscous oil, yield 1.1 g (44%). IR (KBr, cm^{-1}) exhibited bands at 3409 (NH), 1628 (C=O), 1592 (C=O), 843. ^{1}H NMR ($CDCl_3$) δ: 1.16-1.89 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.48-2.60 (m, 8H, (4 x C*H*$_2$) piperazine), 3.42 (s, 4H, C*H*$_2$-NH, C*H*$_2$-C_6H_5), 3.81 (s, 3H, OC*H*$_3$), 3.81 (s, 9H, 3OC*H*$_3$), 6.54 (s, 2H, H_{ar}), 7.25-8.35 (m, 7H, $H_{ar.}$,), 8.50 (s, 1H, N*H*-CH_2), 8.64 (br. s, 1H, N*H*-C_6H_5). ^{13}C NMR ($CDCl_3$) δ: 25.96, 26.1, 29.4, 48.9, 53.2, 53.3, 60.9, 63.4 (7 x CH_2), C_q), 56.1 (4 x OCH_3), 105.7, 127.1, 114.7, 128.5, 129.1, 132.2, ($CH_{ar.}$), 105.7, 153.2, 132.7, 133.9 134.0, 153.1, 136.9, 157.7 ($C_{ar.}$), 163.6 (C=O), 166.8 (C=O). MS (EI) *m/z* (%): 663.5 (0.95, M^+ -2), 181.1 (37.6), 167.1 (34.3), 57 (100).

2.1.8.4. *N*-{[1-(4-Benzhydrylpiperazin-1-yl)cyclohexyl]methyl}-5-chloro-2-methoxy-4-[(phenyl- carbonyl)amino]benzamide (10d)

Pale yellow viscous oil, yield 2.15 g (77%). IR (KBr,cm^{-1}) exhibited bands at 3060 (NH), 1600 (C=O), 700. ^{1}H NMR ($CDCl_3$) δ: 1.14-1.84 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.14-2.53 (m, 8H, (4 x CH_2) piperazine), 2.79 (s, 2H, C*H*$_2$-NH), 3.6 (s, 3H, OCH_3), 4.29 (s, 1H, CH), 7.31-7.40 (m, 15H, $H_{ar.}$), 7.73 (br.s, 2H, $H_{ar.}$), 7.97 (br.s, 1H, CH_2-N*H*), 8.04 (br.s, 1H, N*H*-C_6H_5). ^{13}C NMR ($CDCl_3$) δ: 22.3, 25.3, 25.8, 27.0, 44.8, 48.3, 49.2 (6 x CH_2, C_q), 52.5 (OCH_3), 127.1, 127.7, 127.9, 128.0, 128.6, 128.7, 128.8, 129.4, ($CH_{ar.}$), 131.0, 134.9, 131.9 136.7, 137.5, 142.3 ($C_{ar.}$), 161.3 (C=O), 166.4 (C=O). MS (EI) *m/z* (%): 649.2 (2.1, M^+ -2), 167 (100), 152 (43.5).

2.1.8.5. 4-Amino-5-chloro-*N*-{[1-(4-ethylpiperazin-1-yl)cyclohexyl]methyl}-2-methoxybenz- amide (1q)

Colourless viscous oil, yield 5.0 g (62.5%). IR (KBr, cm^{-1}) exhibited bands at 3785 and 3657 (NH_2), 2929, 1634 (C=O). ^{1}H NMR ($CDCl_3$) δ: 1.04 (t, 3H, *J* = 6.9 Hz, CH_2-C*H*$_3$), 1.15-1.54 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.36 (q, 2H, *J* = 6.9 Hz, C*H*$_2$-CH_3), 2.65 (br.s, 8H, (4 x C*H*$_2$) piperazine), 3.47 (d, 2H, *J* = 5, C*H*$_2$-NH), 3.82 (s, 3H, OCH_3), 4.35 (s, 2H, N*H*$_2$), 6.28 (s, 1H, $H_{ar.}$), 7.94 (s, 1H, $H_{ar.}$), 8.09 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ: 11.83 (*CH*$_3$-CH_2), 22.1, 26.0, 29.6, 44.1, 48.5, 52.3, 56.2, 57.8 (7 x CH_2) C_q), 53.9 (OCH_3), 97.9, 111.3, 112.4, 132.9, 146.8, 157.6 ($CH_{ar.}$,

$C_{ar.}$) 164.6 (C=O). MS (EI) *m/z* (%): 408.45 (0.29, M^+), 195 (100), 58 (67.95). Anal. Calcd. for $C_{21}H_{33}ClN_4O_2$: C, 61.67; H, 8.13; N, 13.70. Found: C, 61.29; H, 7.89; N, 13.45.

2.1.8.6. 4-Amino-*N*-{[1-(4-benzylpiperazin-1-yl)cyclohexyl]methyl}-5-chloro-2-methoxy benzamide (1r)

Colourless viscous oil, yield 4.70 g (50%). IR (KBr, cm^{-1}) exhibited bands at 3059 and 3026 (NH_2), 2854, 1644 (C=O), 848. ^{1}H NMR ($CDCl_3$) δ: 1.07-1.88 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.19-2.67 (m, 8H, (4 x C*H*$_2$) piperazine), 3.53 (s, 4H, C*H*$_2$-NH and C*H*$_2$-C_6H_5), 3.72 (s, 3H, OC*H*$_3$), 4.21 (s, 2H, N*H*$_2$), 7.25-7.34 (m, 8H, $H_{ar.}$, N*H*). ^{13}C NMR ($CDCl_3$) δ: 22.8, 26.0, 29.0, 29.4, 32.0, 48.9, 53.9, 63.9 (7 x *C*H_2), C_q), 55.7 (O*C*H_3), 98.2, 110.7, 116.5, 128.4, 129.2, 137.7, 138.1, 142.6, 144.8, 153.3 (*C*$H_{ar.}$, $C_{ar.}$), 166.7 (C=O). MS (EI) *m/z* (%): 472 (0.6, M^++1), 153 (47.3), 84 (43.9). Anal. Calcd. for $C_{26}H_{35}ClN_4O_2$: C, 66.30; H, 7.49; N, 11.89. Found: C, 65.95; H, 7.75; N, 11.64.

2.1.8.7. 4-Amino-5-chloro-2-methoxy-*N*-({1-[4-(3,4,5-trimethoxybenzyl)piperazin-1-yl]cyclo- hexyl}methyl)benzamide (1s)

Colourless viscous oil, yield 3.88 g (40%). IR (KBr, cm^{-1}) exhibited bands at 3854 and 3746 (NH_2), 2851, 1629 (C=O), 891.^{1}H NMR ($CDCl_3$) δ: 0.84-2.01 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.40 (br.s, 8H, (4 x C*H*$_2$) piperazine), 3.42 (s, 4H, C*H*$_2$-NH and C*H*$_2$-C_6H_5), 3.70 (s, 3H, OC*H*$_3$), 3.70 (s, 9H, OC*H*$_3$) 6.23 (s, 1H, $H_{ar.}$), 6.52 (br. s, 3H, $H_{ar.}$), 7.10 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ: 14.2, 22.7, 29.7, 32.0, 41.9, 47.1, 52.9 (7 x CH_2), 53.3 (C_q), 55.7, 56.1,60.9, 63.2 (4 x OCH_3), 98.2, 105.7, 110.7, 116.3, 129.1, 133.6, 137.0, 144.9, 153.1, 155.3 ($CH_{ar.}$, $C_{ar.}$), 166.9 (C=O). MS (EI) *m/z* (%): 562 (0.2, M^++1), 399 (27.2), 181 (56.5). Anal. Calcd. for $C_{29}H_{41}ClN_3O_5$: C, 62.07; H, 7.36; N, 9.98. Found: C, 61.87; H, 7.76; N, 10.08.

2.1.8.8. 4-Amino-*N*-{[1-(4-benzhydrylpiperazin-1-yl)cyclohexyl]methyl}-5-chloro-2-methoxy- benzamide (1t)

Colourless viscous oil, yield 7.60 g (70%). IR (KBr, cm^{-1}) exhibited bands at 3333, 3203 (NH_2), 1619 (C=O), 1249, 752. ^{1}H NMR (DMSO-*d*$_6$**)** δ: 1.10-1.96 (m, 10H, (5 x C*H*$_2$) cyclohexyl), 2.24-2.72 (m, 8H, (4 x C*H*$_2$) piperazine), 3.29 (br.s, 2H, C*H*$_2$-NH), 3.75 (s, 3H, OCH_3), 4.21-4.42 (m, 3H, C*H*, N*H*$_2$), 6.23 (s, 2H, $H_{ar.}$), 7.17-7.41 (m, 10H, $H_{ar.}$), 8.09 (br.s, 1H,N*H*). ^{13}C NMR (DMSO-*d*$_6$**)** δ: 26.0, 28.4, 30.4, 44.7, 47.3, 49.1, 52.2 (6 x CH_2, C_q), 55.7 (OCH_3), 98.2, 110.7, 116.4, 127.2, 127.9, 128.6, 128.7, 142.3, 144.9, 155.3 ($CH_{ar.}$, $C_{ar.}$), 166.8 (C=O). MS (EI) *m/z* (%): 549 (0.34, M^++2), 251.2 (4.6), 181 (100), 167 (84.5). Anal. Calcd. for $C_{32}H_{39}ClN_4O_2$: C, 70.25; H, 7.18; N, 10.24. Found: C, 69.92; H, 7.58; N, 10.54.

2.2. Biological evaluation

Adult male albino rats weighing 200-300 g were used in this study. The animals were purchased from Animal House colony of National Research Centre, Cairo, Egypt and were housed under standardized conditions (room temperature 23±2°C, relative humidity 55±5%, 12h-light/12h-dark cycle) .They had free access to tap water and were feeded with commercially available standard rat chow throughout the whole experimental period. All animal procedures were performed after the Ethics Committee of the National Research Centre and in accordance with the recommendations for the proper care and use of laboratory animals "Canadian Council on Animal Care Guidelines, 1984." Tween-80 (Polyoxyethylene-sorbitan monooleate, Sigma USA), apomorphine hydrochloride (Research Biochemicals Inc., Wayland, USA), and Metoclopramide hydrochloride (CID Company, Giza, Egypt).

2.2.1. Dopamine D_2 receptor antagonistic activity (Zwangsnagen test)

Groups of 6 rats each were placed individually in cages having shavings of wood on the floor and an observation window and allowed to habituate for 15 minutes before injection of drugs. A series of doses ranging from 1.5 to 20 mg/kg of the test compounds **1a-t** was investigated**.** Each dose was suspended in tween-80 (7% aqueous solution) as vehicle and administered subcutaneously. A minimum of 4 dose levels per compound and 6 rats per dose were used. One hour later, 0.5% solution of apomorphine hydrochloride (1.25 mg/kg) in saline was injected intravenously, such that the injected solution does not exceed 2 mL/kg. After 5, 10, 20 min., the animals were observed for 1 min. The presence or absence of chewing movement (Zwangsnagen) or compulsory gnawing as well as severity of chewing were noted. The absence of chewing movement 5, 10 or 20 minutes after apomorphine hydrochloride injection is indicative of

dopamine D_2 receptor antagonistic activity and hence antiemetic activity.[11] The studied biological activity of the tested compounds was compared with that of Metoclopramide hydrochloride used as reference standard. The ED_{50} of the most potent compounds were calculated according to the method of Litchfield Wilcoxon.[12]

2.3. Molecular modeling

Pharmacophore was produced using the Discovery Studio 2.5 software. (Accelrys Inc., San Diego, CA, USA).

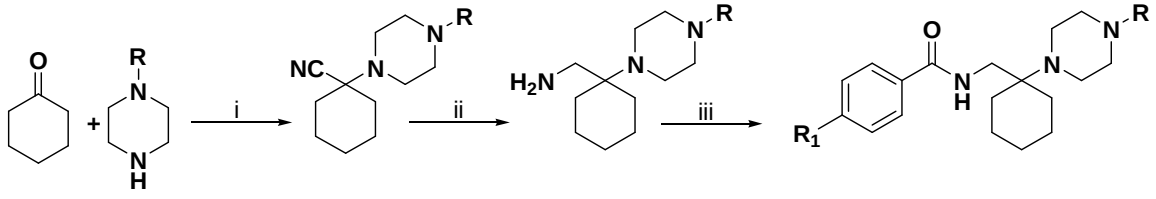
2.3.1. Generation of dopamine D_2 receptor antagonists pharmacophore

The pharmacophore modeling method has been widely used in lead discovery and optimization as a key tool of computer aided drug design. A hypothesis was formulated using generation common feature pharmacophore model protocol in Discovery studio 2.5. The lead compounds (**I-X**), which were reported to have dopamine D_2 receptor antagonistic activity (Figure 2), were used to generate common feature pharmacophore for the dopamine D_2 receptor antagonists.[13] A set of conformational models of each structure of the lead compounds was performed and used to generate the common feature hypotheses, where ten hypotheses were generated.[14]

3. Results and discussion

3.1. Chemistry

The target compounds **1a-l** were synthesized as outlined in Scheme 1. Thus, cyclohexanone was allowed to react *via* Strecker synthesis with *N*-ethyl and/or aralkylpiperzine **2a-d** and KCN in the presence of concentrated HCl to produce the carbonitrile derivatives **3a-d**. Subsequently, the nitrile functionality of **3a-d** was subjected to reduction using $LiAlH_4/AlCl_3$ reducing mixture[15] in dry THF to yield the corresponding amines **4a-d**. Compounds **4a-d** were then reacted with the appropriate acyl chloride **5a-c** in the presence of triethylamine to yield the respective target compounds **1a-l** in moderate yields.



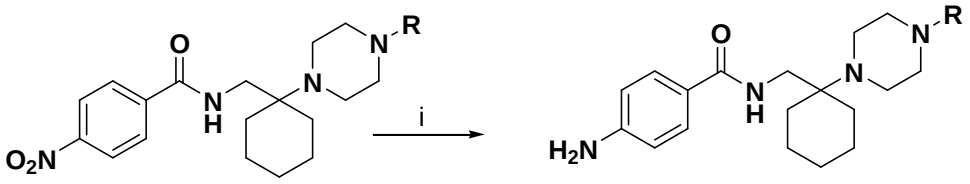
a: R = C_2H_5
b: R = CH_2-C_6H_5
c: R = CH_2-$C_6H_2(OCH_3)_3$
d: R = $CH(C_6H_5)_2$

Compound Nr.	R	R_1
1a	C_2H_5	H
1b	C_2H_5	Cl
1c	C_2H_5	NO_2
1d	CH_2-C_6H_5	H
1e	CH_2-C_6H_5	Cl
1f	CH_2-C_6H_5	NO_2
1g	CH_2-$C_6H_2(OCH_3)_3$	H
1h	CH_2-$C_6H_2(OCH_3)_3$	Cl
1i	CH_2-$C_6H_2(OCH_3)_3$	NO_2
1j	$CH(C_6H_5)_2$	H
1k	$CH(C_6H_5)_2$	Cl
1l	$CH(C_6H_5)_2$	NO_2

Scheme 1: Synthesis of the target compounds 1a-l.

Reagents and conditions: i) KCN, conc. HCl, water, RT, 18 h; ii) $LiAlH_4/AlCl_3$, THF, RT, 18 h; iii) appropriate **5a-c**, triethylamine, benzene, reflux, 18 h.

Nitro functionality of compounds **1c**, **1f**, **1i**, and **1l** was reduced using 10% Pd/C (for **1c**) or Raney nickel (for **1f**, **1i**, and **1l**) and molecular hydrogen under normal pressure and room temperature to give the respective amines **1m-p** (Scheme 2).



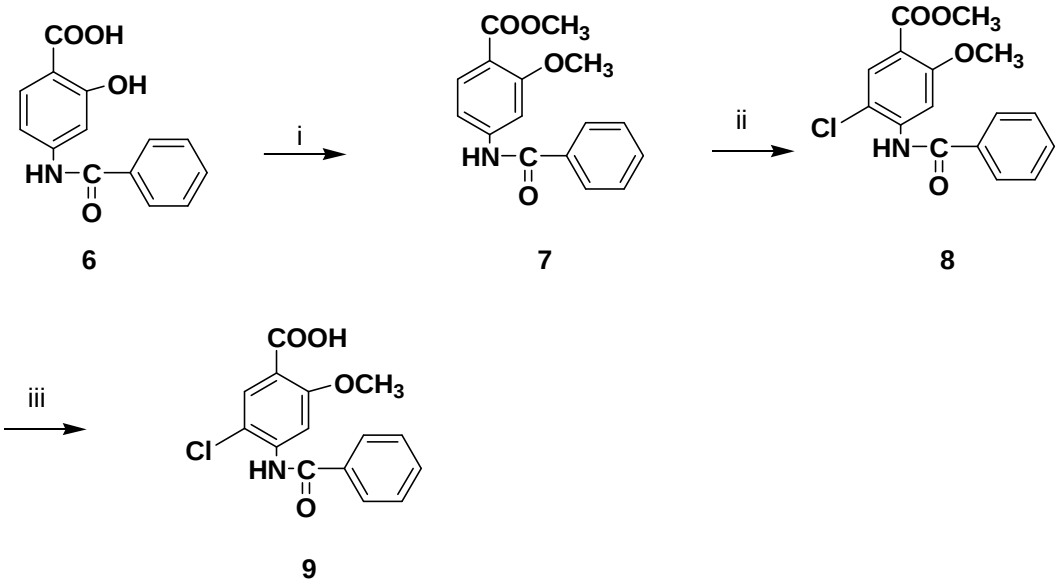
1c, 1f, and 1l **1m-p**

1c, 1m: R = C_2H_5
1f, 1n: R = CH_2-C_6H_5
1i, 1o: R = CH_2.$C_6H_2(OCH_3)_3$
1l, 1p: R = $CH(C_6H_5)_2$

Scheme 2: Synthesis of the target compounds 1m-p.

Reagents and conditions: i) 10% Pd/C (for compound **1c)** or Raney nickel (for compounds **1f, 1i,** and **1l)**, H_2, RT, 18 h.

The acid **9,** which was required to prepare the Metoclopramide analogues **1q-t**, was prepared as depicted in Scheme 3. Thus, *N*-benzoyl-4-aminosalicylic acid **6** was methylated at both phenolic OH and carboxylic acid functionalities using dimethyl sulfate in acetone at 45 °C for 24 hours to give the corresponding methoxybenzoic acid methyl ester **7**. Subsequent chlorination of **7** using $KClO_3$ in the presence of concentrated hydrochloric acid at room temperature for 24 h furnished the chlorinated compound **8**. The ester functionality of **8** was hydrolyzed by LiOH in THF at room temperature to furnish *N*-benzoyl-4-aminobenzoic acid derivative **9**.



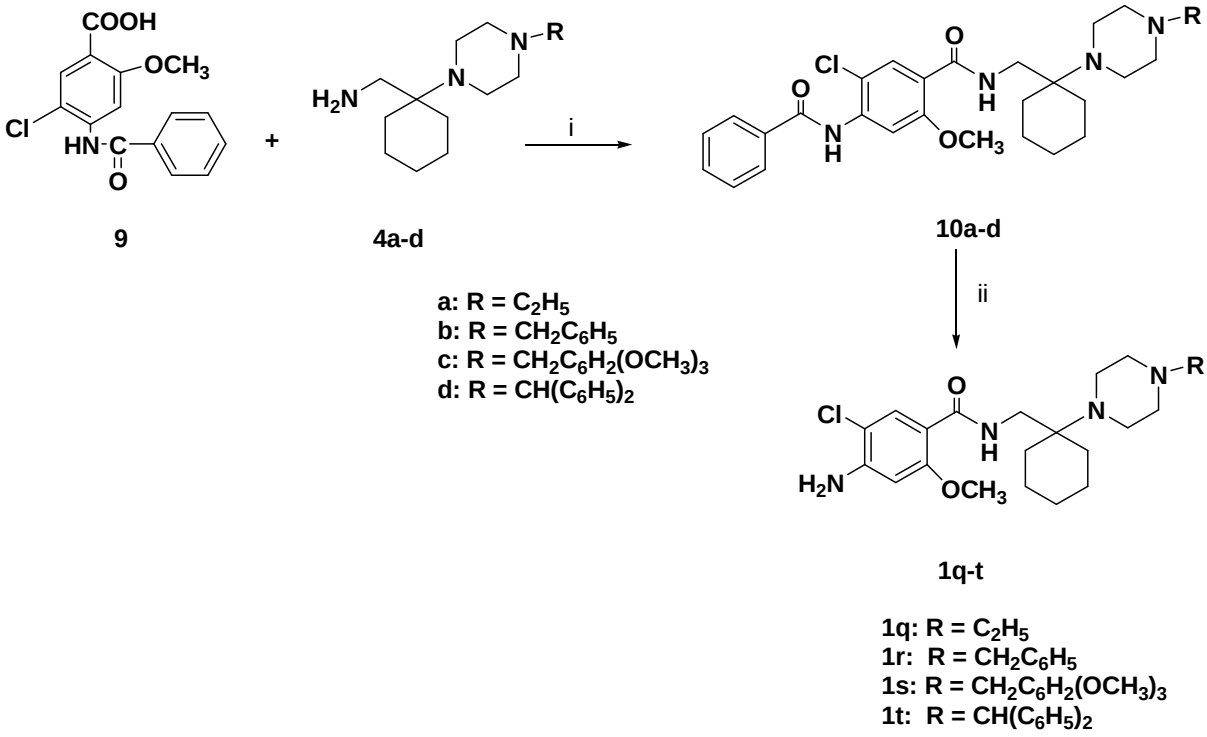
6 **7** **8**

9

***Scheme 3**: Synthesis of compound **9**.*
Reagents and conditions: i) $(CH_3)_2SO_4$, anhyd. K_2CO_3, 45 °C, 24 h; ii) $KClO_3$/HCl, RT, 24 h; iii) LiOH/THF, RT, 24 h.

Metoclopramide analogues **1q-t** were synthesized as illustrated in Scheme 4. Thus, the benzoic acid derivative **9** was coupled with the appropriate amine **4a-d** using ethyl-3-(3-

dimethylaminopropyl)carbodiimide hydrochloride (EDCI.HCl) in DCM at room temperature to furnish the respective amides **9a-d**. Subsequently, the target compounds **1q-t** were obtained *via* refluxing the *N*-benzoyl derivatives **9a-d** in 10% aqueous NaOH solution (Scheme 4).



Scheme 4*: Synthesis of the target compounds* ***1q-t****.*
Reagents and conditions: i) EDCI.HCl, DCM, RT, 18 h; ii) NaOH, H_2O, reflux, 18 h.

The spectral data of the newly synthesized compounds in the present investigation were in accordance with their assigned structures.

3.2. *In vivo* dopamine D_2 receptor antagonistic activity

The newly synthesized compounds **1a-t** were evaluated for their dopamine D_2 receptor antagonistic activity *in vivo* by measuring their ability to inhibit apomorphine-induced chewing "Zwangsnagen" in rats.[11] This test measures the inhibition of compulsive stereotyped hyperactivity behavior induced by apomorphine through its stimulation of central dopamine D_2 receptors in rats.[16] The dopamine D_2 receptor antagonistic activity of **1a-t** and ED_{50} values of the selected candidates with potent activity are displayed in Table 1.

Metoclopramide (**I**) is a relatively weak serotonin-3 (5-HT_3) as well as dopamine D_2 receptor antagonist. Metoclopramide is one of the most effective agents used intravenously in a high dose to alleviate cisplatin-induced nausea and vomiting.[17] Nevertheless, its clinical usefulness is restricted due to its extrapyramidal side effects. Accordingly, Metoclopramide is a ready target for extensive molecular modification to enhance some of its desirable effects and attenuate or abolish side effects. Certain molecular modifications were examined previously in our research group which implied structural variation in the amide side chain through incorporating cyclohexyl moiety in the β-position to the amidic nitrogen to give compound

II.[5] Insertion of cyclohexyl moiety increased the lipophilicity of compound **II** which plays a remarkable role in distribution and binding of drugs to their targets *in vivo*. Further molecular modifications of compound **II** were achieved, to improve its dopamine D_2 receptor antagonistic profile, through the synthesis of the target compounds **1a-t**.

Table 1: Fit values and dopamine D_2 receptor antagonistic activity of compounds 1a-t.

Compd. No.	Dose mg/kg (μmol)*	% Inhibition	ED_{50} mg/kg (95% confidence limit)	Fit value
1a	20 (49.70)	66.6	ND	3.00
1b	20 (45.78)	100	7.0 (13.4-3.82)	4.34
1c	10 (22.35)	100	4.0 (7.49-2.69)	4.67
1d	20 (43.06)	16.6	ND	3.21
1e	20 (40.09)	50	ND	3.90
1f	20 (39.26)	66.6	ND	3.39
1g	10 (18.03)	100	4.5 (7.72-2.79)	3.97
1h	10 (16.98)	100	3.5 (6.99-1.89)	4.86
1i	10 (16.68)	50	ND	4.14
1j	10 (18.49)	83.3	ND	4.81
1k	20 (34.78)	83.3	ND	4.81
1l	10 (17.08)	66.6	ND	3.89
1m	10 (29.03)	100	4.5 (8.42-2.77)	4.46
1n	20 (49.19)	50	ND	2.98
1o	20 (40.29)	50	ND	3.11
1p	20 (49.19)	66.6	ND	3.19
1q	10 (24.45)	100	4.0 (6.57-2.44)	4.69
1r	20 (42.46)	83.3	10.0 (17.09-5.35)	4.10
1s	10 (17.82)	100	4.0 (7.10-3.52)	4.80
1t	10 (18.28)	83.3	5.50 (10.85-2.93)	4.19
II	-	-	4.9 (6.03-3.99)**	4.01
Metoclopramide hydrochloride	5.5 (16.36)	100	1.0 (1.87-0.69)	4.78

***** The smallest dose which gives the best % inhibition. ** Data from Reference 5. ND: Not determined

The Beecham group[18] documented that selectivity of action of Metoclopramide could be achieved through restriction of the conformational freedom of its basic (diethylamino)ethyl side chain. Accordingly, in the present study this basic moiety was replaced with a substituted heteroalicyclic piperazine ring bearing ethyl (compounds **1a-c**, **1m**, and **1q**), benzyl (**1d-f**, **1n**, and **1r**), trimethoxybenzyl (**1g-i**, **1o**, and **1s**), or benzhydryl (**1j-l**, **1p**, and **1t**) substituents. Furthermore, the influence of benzoyl group substituents in **1a-t** on their dopamine D_2 receptor antagonistic activity was examined while retaining the *N*-substituted piperazine moiety.

The benzamide derivative **1a** showed 66.6% inhibition against apomorphine-induced chewing in rats at a dose level of 49.70 μmol/kg. Furthermore, substitution of the benzoyl moiety of **1a** with an electron withdrawing group like chlorine improves its inhibition activity as in **1b** which displayed 100% inhibition at a dose level of 45.78 μmol/kg. Whereas, the 4-nitro analogue **1c** showed the best activity of the *N*-ethylpiperazine congeners with 100% inhibition at a dose level of 22.35 μmol/kg. Reduction of the nitro group of **1c** yielded **1m** which produced 100% inhibition at a dose level of 29.03 μmol/kg. The *N*-ethylpiperazine analogue of Metoclopramide, compound **1q**, exhibited 100% inhibition at a dose level of 24.45 μmol/kg.

On the other hand, replacing the *N*-ethyl group by *N*-benzyl group furnished compounds **1d-f**, **1n**, and **1r**. *N*-Benzyl analogue of **1a**, compound **1d**, exhibited only 16.6% inhibition at a dose level of 43.06 μmol/kg, while the 4-chloro congener, compound **1e**, showed 50% inhibition at a dose level of 40.09 μmol/kg. Similarly, the nitro derivative **1f** displayed 66.6% inhibition at a dose level of 39.26 μmol/kg. Whereas, its respective amino candidate, compound **1n**, exhibited slightly weaker dopamine D_2 receptor antagonistic activity (50% inhibition) at a dose level of 49.19 μmol/kg. Compound **1r** showed the highest activity in the *N*-benzyl derivatives with 83.3% inhibition at a dose level of 42.46 μmol/kg.

A sizable number of antiemetics incorporate trimethoxybenzyl moiety in their structures.[12] Therfore, *N*-trimethoxybenzyl derivatives **1g-i**, **1o**, and **1s** were synthesized and screened for their dopamine D_2 receptor antagonistic potential. Trimethoxybenzyl analogue of **1a**, compound **1g**, displayed 100 % inhibition of apomorphine-induced chewing in rats at a dose level of 18.03 μmol/kg. Moreover, the 4-chloro derivative **1h** emerged as the most potent dopamine D_2 receptor antagonist of all the synthesized compounds **1a-t**. It exhibited 100% inhibition at a dose level of 16.98 μmol/kg being nearly equipotent with the reference standard, Metoclopramide hydrochloride, that displayed 100 % inhibition at a dose level of 16.36 μmol/kg. Surprisingly, the nitro derivative **1i** and its respective amino derivative **1o** showed only 50% inhibition at dose levels of 16.68 and 40.29 μmol/kg, respectively. Furthermore, the *N*-trimethoxybenzylpiperazine analogue of Metoclopramide, compound **1s**, exhibited 100% inhibition at a dose level of 17.82 μmol/kg.

In the *N*-benzhydrylpiperazine derivatives **1j-l**, **1p**, and **1t**, compounds **1j**, **1k**, and **1t** displayed 83.3% inhibition of apomorphine-induced chewing in rats at dose levels of 18.49, 34.78, and 18.28 μmol/kg, respectively. Whereas, both **1l** and **1p** exhibited only 66.6% inhibition at dose levels of 17.08 and 49.19 μmol/kg, respectively.

The most active candidates in the synthesized compounds bearing *N*-ethyl, benzyl, trimethoxybenzyl, and benzhydrylpiperazine moieties were subjected to quantitative estimation (median effective dose, ED_{50}) and the results are depicted in Table 1. Variation of the substituents at both piperazine and benzoyl moieties influence their D_2-receptor antagonistic activity as indicated by their inhibition of apomorphine-induced chewing in rats in the following decreasing order: **1h** > **1s** > **1g** > **1c** > **1q** > **1t** > **1m** > **1b** > **1r**.

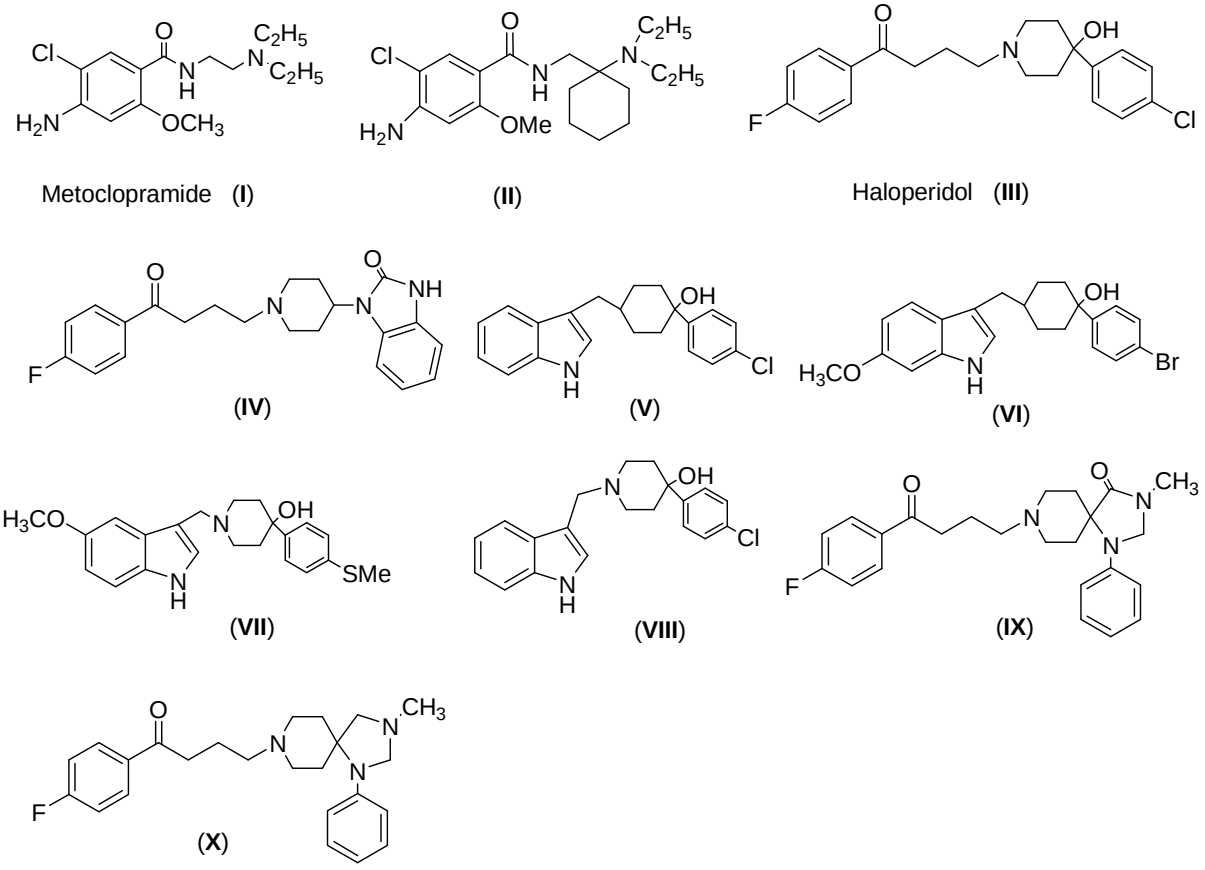
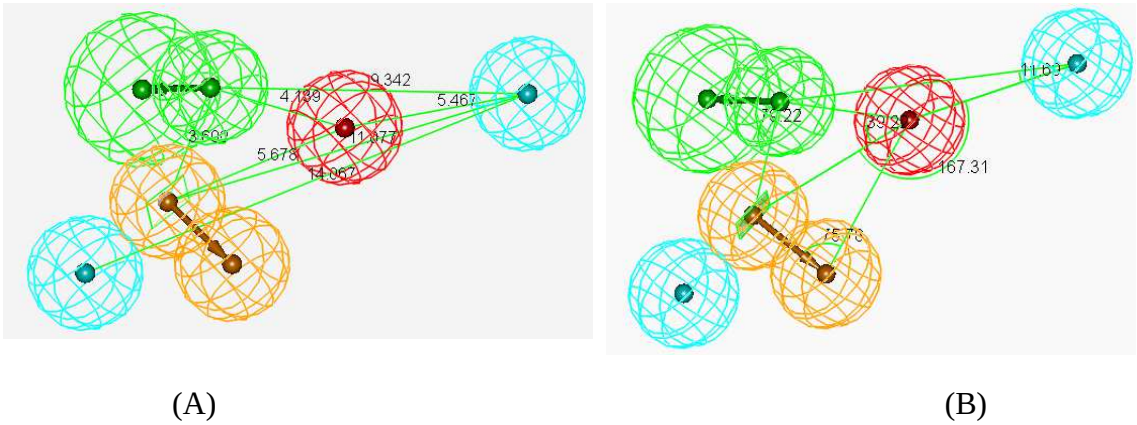


Fig. 2: Chemical structures of potent dopamine D_2 receptor antagonists.

3.3. Validation of the generated pharmacophore

Ten hypotheses were generated and the one ranked number 4 was chosen as the valid ideal hypothesis (Figure 3 of the supplementary material) based on the following: (a) the hypothesis

showed full mapping of all its features without any steric clashes together with high fit values with the training set (compounds **I-X**, e.g., see Figure 4 of the supplementary material), (b) retrospectively, the simulated fit values of test set compounds (**1a-t**) with hypothesis 4 were more consistent with the experimental results than other hypotheses, (c) the database search study for examining the affinity of such hypothesis with the molecular structures of MiniMaybridge databases revealed that only 17 hits have been retrieved from the databases (2000 compounds).[19] Such a low number of the recognized database molecules by generated hypothesis may give an additional advantage and selectivity to our hypothesis.[14] Such an ideal hypothesis encompassed five features namely; positive ionizable (PI), hydrogen bonding acceptor (HBA), ring aromatic (RA) and two hydrophobic features (HY1 and HY2). Herein, the constraint distances and angles between the essential features existed in the generated hypothesis is reported as shown Table 2 and in Fig. 3 of the supplementary material.



(A) (B)

Fig. 3: (A and B) Constraint distances and angles of dopamine D_2 receptor antagonists. The chemical features coloured light blue, green, red and orange represent hydrophobic features (HY), hydrogen bonding acceptor (HBA), positive ionisable (PI), and ring aromatic (RA), respectively.

Table 2: The constraint distances and angles between the features of the generated dopamine D_2 receptor antagonists pharmacophore model.

Dimensions	Features of D_2-receptor antagonists
Constraint distances (Å) between features	HY2-HBA, 9.34; PI-HBA, 4.18; HY1-HY2, 14.06; HY2-PI,5.46; HY2-RA, 11.07; PI-RA, 5.67; RA-HBA, 3.60
Constraint angles between features	HY2-PI-HBA, 11.69; PI-HBA-RA, 39.22; PI-RA vector, 75.78; RA-HBA vector, 79.22; HY2-PI-RA, 167.31

Structures of the test set of arylcarboxamides **1a-t** were built using the Discovery Studio software, and their conformational models were generated in the energy range of 20 kcal/mol above the estimated global energy minima. Fitting of each tested compound was performed using best fit during the compare/fit process. Different mappings for all the conformers of each compound of the test set to the hypothesis were visualized and the fit values of the best-fitting conformers are shown in Table 1 and in Fig. 4 of the supplementary material.

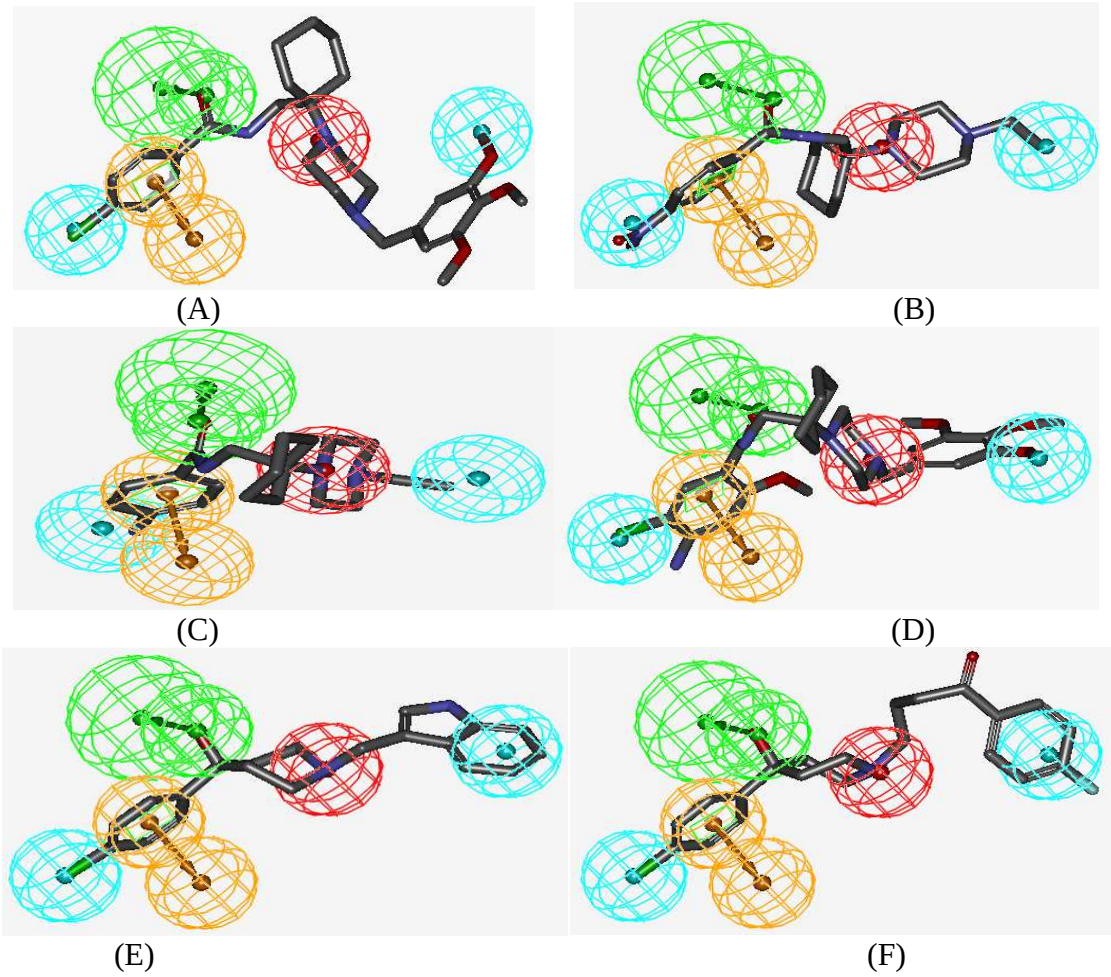


Fig. 4: (A–F) Mapping of dopamine D_2 receptor antagonist pharmacophore with lead compound VIII (E), III (F) and test set compounds 1c (A), 1h (B), 1s (C) and 1m (D), respectively.

4. Conclusion

The synthesis of certain new *N*-{[1-(4-aralkyl/ethylpiperazine-1-yl)cyclohexyl]methyl}aryl- carboxamides **1a-t** is reported. Dopamine D_2-receptor antagonistic activity of **1a-t** was determined using apomorphine-induced chewing "Zwangsnagen" test in rats. Compound **1h** is the most active congener in all the synthesized compounds **1a-t** displaying ED_{50} of 3.5 mg/kg (5.94 μmol/kg) being nearly 2-fold more potent than the previously reported cyclohexane-containing dopamine D_2 receptor antagonist **II** (ED_{50} = 4.90 mg/kg, 11.66 μmol/kg). Whereas, **1h** exhibited 100% inhibition at a dose level of 16.98 μmol/kg being nearly equipotent with the reference standard, Metoclopramide hydrochloride, that showed 100 % inhibition at a dose level of 16.36 μmol/kg. Additionally, Molecular simulation study including fitting to dopamine D_2 receptor antagonists 3D-pharmacophore model using Discovery Studio 2.5 programs showed high-fit values. The experimental dopamine D_2 receptor antagonistic activity of compounds **1a-t** was consistent with the molecular modeling study.

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