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Synthesis and Preliminary Pharmacological Evaluation of 4′-Arylalkyl Analogues of Clozapine. IV.* The Effects of Aromaticity and Isosteric Replacement

Ben Capuano,[A,B] *Ian T. Crosby,*[A] *Edward J. Lloyd,*[A] *Anna Podloucka,*[A] *and David A. Taylor*[A]

[A]Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Vic. 3052, Australia.

[B]Corresponding author. Email: ben.capuano@pharm.monash.edu.au

We report the synthesis and preliminary pharmacological activity of a new series of tricyclic analogues of clozapine as potential antipsychotic agents for the treatment of schizophrenia. These compounds were designed based on a revised structural model, and investigate the length and nature of a designated linker (alkyl and alkyloxy) and the nature of the introduced aryl group (aromatic and heteroaromatic). The chemistry and structural characterization of this series of 4′-arylalkyl(oxy) analogues of clozapine are described. Preliminary results on the pharmacological effects of the selected linkers and introduced aryl groups on affinity for dopamine D_4 and serotonin 5-HT_{2A} receptors are discussed. Psychosis-related animal behavioural data for promising compounds identified from the receptor binding screen are also presented.

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Introduction

Schizophrenia is a debilitating mental illness that severely distorts a patient's perception of reality, and significantly impairs various emotional, behavioural, and cognitive functions. This most common form of psychosis boasts a devastating and overwhelming worldwide incidence of ∼1%.[1] Clozapine **2** is heralded as the prototypical atypical antipsychotic and is uniquely superior in efficacy against refractory schizophrenia. Clozapine effectively treats both the positive (delusions, hallucinations, and disorganized behaviour) and negative (social and emotional withdrawal, affective flattening) symptoms of schizophrenia, in addition to associated neurocognitive deficits, and is virtually free of movement-related side-effects.[2] The adverse effects profile, however, requires improvement owing to clozapine's propensity to induce a potentially fatal blood disorder.[3,4]

Previous analogues of clozapine were designed by the structural hybridization of haloperidol (**1**) and clozapine **2**.[5,6] The preliminary pharmacological investigation of the previous chain-extended series of clozapine analogues[6] revealed that the optimal length of the hydrocarbon linker between N4′ and the introduced aryl system (phenyl) was three to five atoms. This observation afforded valuable insight into the spatial requirements in the region of clozapine's distal nitrogen atom and provided the impetus for the ensuing study. The series of analogues described herein investigates the pharmacological effects of introducing an alkyl and alkyloxy linker of three to four atoms in length, attached to a distal aromatic and heteroaromatic ring at the N4′ position of clozapine. Preliminary pharmacological evaluations include in vitro receptor binding assays to ascertain their affinity for dopamine D_4 and serotonin 5-HT_{2A} receptors, with particular reference to the effects of isosteric replacement of CH_2 for O in the linker and CH for N in the aryl system. The clozapine analogues were also evaluated for their potential to antagonize apomorphine-induced climbing in mice, a psychosis-related behavioural model predictive of mesolimbic dopaminergic activity and therefore potential antipsychotic activity.

To develop a new structural model for antipsychotic activity, the chemical structures of some current therapeutic antipsychotics were analyzed initially (Fig. 1), namely haloperidol **1**, clozapine **2**, risperidone **3**, and olanzapine **4**. Depicted in bold is the halogenated ring of haloperidol **1**, the nitrogen atom that is ionized at physiological pH and a distant aromatic moiety. Clozapine **2** also displays a halogenated aromatic ring and a nitrogen atom that is ionized at physiological pH, but lacks an aryl-containing moiety attached to the ionizable or distal nitrogen atom. Risperidone displays similar structural characteristics to haloperidol, exhibiting a halogenated aromatic ring, a nitrogen atom that is ionized at physiological pH and a distant conjugated π-system. Olanzapine shows structural similarities to clozapine, differing only in the absence of a halogen substituent, and contains a substituted thiophene ring in place of clozapine's benzene ring.

The X-ray crystal structures of the aforementioned antipsychotic agents were superimposed (Fig. 2) using the six ring

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atoms of a piperazine or piperidine ring in the chair form as the reference point, while maintaining a common location for the nitrogen atom that is ionized at physiological pH. The structural model depicted in Fig. 3 conceptualizes the superimposition, highlighting a collection of key structural features. The envisaged assembly of a halogenated aromatic ring (monocyclic or polycyclic nucleus), a nitrogen atom that is ionized at physiological pH and a secondary aromatic or π-system separated by an aliphatic linker of designated length and nature from the nitrogen atom may provide comparatively potent and superior antipsychotic activity. Based on consideration of the superimposition of crystal structures and the development of the structural model, we embarked on an investigation of the synthesis, structural characterization and biochemical effects of a series of compounds related to the structural template shown in Fig. 4. The target compounds contain the tricyclic nucleus of clozapine connected to a piperazine ring system, from which a series of chain-extended fragments are anchored. Substitution of arylalkyl and arylalkoxy substituents at the N4′ position were designed to generate compounds with an 'extended' appearance similar to that of haloperidol and risperidone, with the view of encapsulating biochemical attributes of these commercial therapeutics within the novel series described herein.

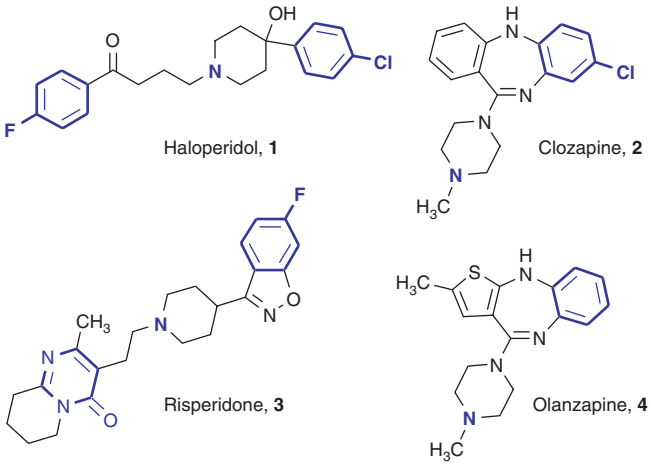


Fig. 1. The chemical structures of clinically prescribed drugs to treat schizophrenia (clozapine, **1**; haloperidol, **2**; risperidone, **3**; and olanzapine, **4**) indicating important structural features coloured in bold, used in the development of a general structural model.

Chemistry

All of the target amidines (**5a**–**g**) were synthesized using one of three synthetic pathways, namely: (a) Lewis acid-mediated condensation of a tricyclic lactam **24** and a monosubstituted piperazine for compounds **5a**–**d**; (b) activation of the tricyclic lactam to the corresponding imino chloride followed by treatment with a monosubstituted piperazine for compounds **5e**–**f**; and (c) a reductive amination of desmethylclozapine **27** and a suitable phenylalkanal for compound **5g**. 4-(4-Pyridinyl)but-3-yn-1-ol

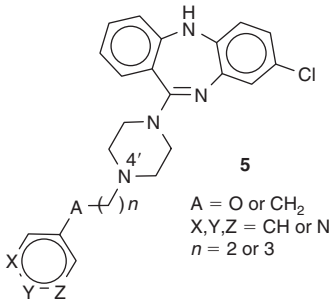


Fig. 4. New structural template selected for investigation.

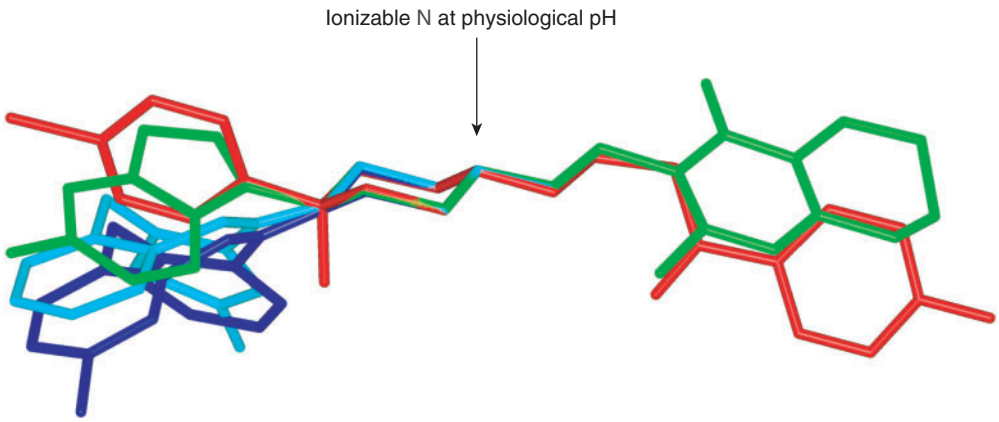


Fig. 2. Superimposition of the X-ray crystal structures of currently prescribed antipsychotics: haloperidol (**1**, red), clozapine (**2**, dark blue), risperidone (**3**, green), and olanzapine (**4**, light blue). Hydrogen atoms have been omitted for clarity. (The X-ray crystal structures were sourced from the Cambridge Structural Database; molecular modelling was performed on a Silicon Graphics Iris Indy workstation using the commercial software package *Insight II.*)

Fig. 3. Structural model detailing generic structural features that may afford potent antipsychotic activity based on the superimposition of known antipsychotic agents (X = C or N).

9 and 4-(5-pyrimidinyl)but-3-yn-1-ol **10** were prepared according to Scheme 1. The first step of the syntheses used a Sonogashira coupling of but-3-yn-1-ol **8** with commercially available 4-bromopyridine hydrochloride **6** and 5-bromopyrimidine **7** to afford the corresponding alkynols **9** and **10**, respectively. Subsequent reduction of the alkynols under standard hydrogenation conditions produced the desired alkanols **11** and **12** in 76 and 97% overall yields, respectively.

The pyridine- and pyrimidine-containing monosubstituted piperazines **16**–**19** were furnished from the aforementioned alcohols **11** and **12**, and from commercially available starting materials **13** and **14** (Scheme 2) via activation as mesylates and subsequent treatment with piperazine **15** in 53–63% yield. These monosubstituted piperazines represent the precursors to the target nitrogen-containing heterocycles with a three- and four-atom hydrocarbon linker (**5c**–**f**), respectively.

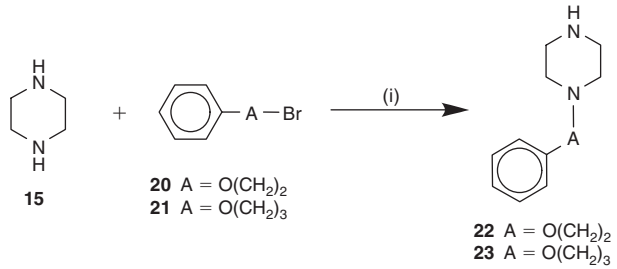
The phenoxyalkylpiperazines **22** and **23** were synthesized by alkylation of piperazine with the corresponding phenoxyalkyl bromides (Scheme 3) and purified by distillation to afford colourless liquids in 76 and 81% yield, respectively. These compounds represent the precursors to the target phenoxyalkyl analogues **5a** and **5b** displaying a four-atom linker that investigate the effects of isosteric replacement of CH_2 for O.

The target amidines **5a**–**d** were synthesized according to Scheme 4 in 42–81% yield, whereby the corresponding monosubstituted piperazines were coupled to the tricyclic lactam **24** using the Lewis acid titanium tetrachloride. A drawback associated with this method is the uneconomical use of ~five equivalents of monosubstituted piperazine for the formation of the reactive titanium–amine complex and presence of an 'acid-binding agent'.[7] The synthesis of compounds **5e**,**f** is outlined in Scheme 5 whereby the lactam was converted to the activated imino chloride intermediate by phosphorus pentachloride, and subsequently treated with approximately two equivalents of the monosubstituted piperazine. The yields for compounds **5e**,**f** were unfortunately significantly lower (14 and 24%, respectively) by this method compared with the titanium–amine method.

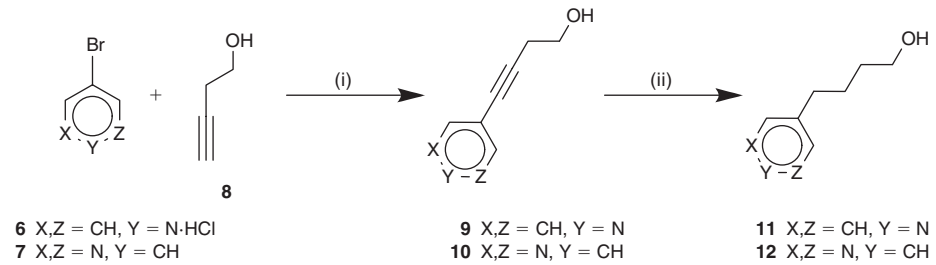
The phenylbutyl analogue of clozapine **5g** was synthesized according to the method detailed in Scheme 6. The alcohol **25** was oxidized to the corresponding aldehyde **26** (77%) using pyridinium chlorochromate (PCC), then reacted under reductive amination conditions with desmethylclozapine **27** in the presence of sodium triacetoxyborohydride to yield the desired analogue **5g** in 66% yield.

Receptor Binding Studies

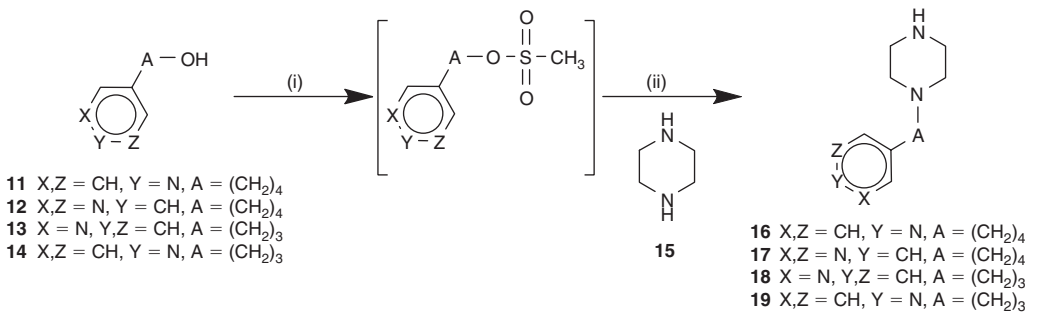
Details of the in vitro assays for the synthesized compounds are described in the Experimental section. Table 1 lists preliminary percentage inhibition (% I) of radioligand binding at a compound concentration of 10^{-6} M for the dopamine $D_{4.4}$ (the most common polymorphic variant of the D_4 receptor)[8] and serotonin 5-HT_{2A} receptors for all test compounds. Data for the compound corresponding to the 4′-phenylpropyl analogue of clozapine (A = CH_2; X,Y,Z = CH; $n = 2$) were also included for comparative purposes, and provided a firmer basis for structure–activity relationships pertaining to the length and nature of the



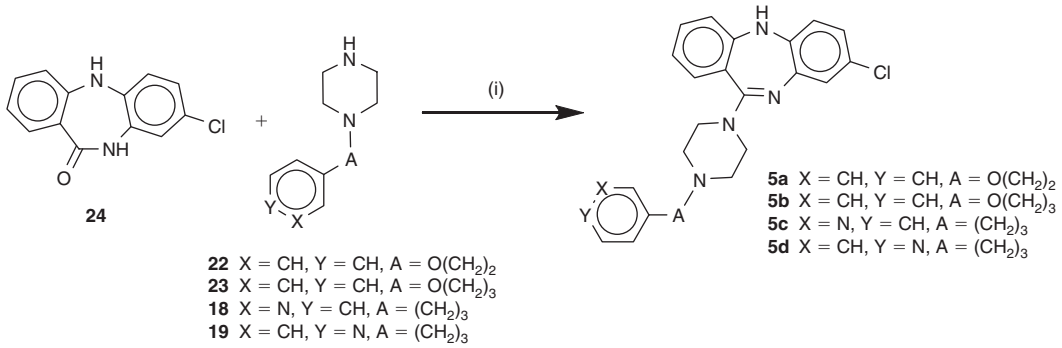
Scheme 3. Reagents and conditions: (i) toluene, 85°C.



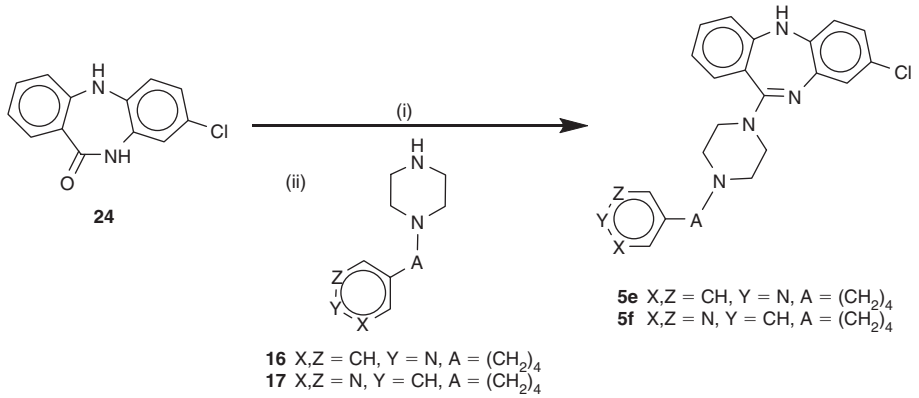
Scheme 1. Reagents and conditions: (i) LiCl, CuI, $Pd(Ph_3P)_4$, Et_3N, H_2O, reflux, 2.5 h; (ii) 10% Pd/C, $H_2(g)$, EtOH, room temp.



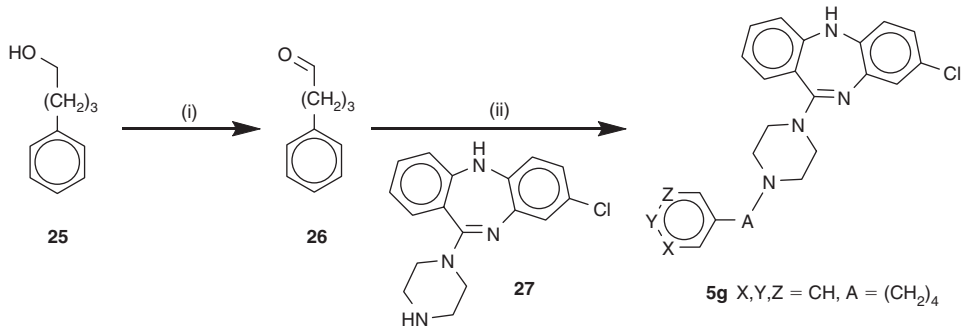
Scheme 2. Reagents and conditions: (i) methanesulfonyl chloride, Et_3N, CH_2Cl_2, −50°C; (ii) tetrahydrofuran or 1,2-dimethoxyethane, reflux.



Scheme 4. Reagents and conditions: (i) $TiCl_4$, anisole, 25–55°C, reflux.



Scheme 5. Reagents and conditions: (i) PCl_5, dichloromethane, reflux, 1 h; (ii) 1,4-dioxan, reflux.

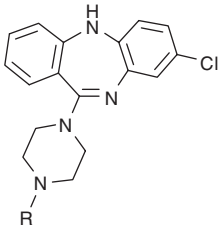


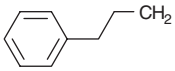
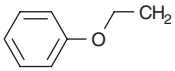
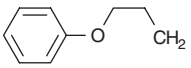
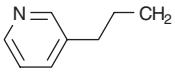
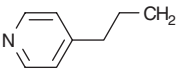
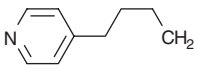
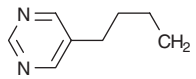
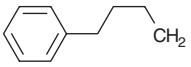
Scheme 6. Reagents and conditions: (i) pyridinium chlorochromate (PCC), SiO_2, dichloromethane, room temp.; (ii) $NaBH(OAc)_3$, 1,2-dichloroethane, room temp.

linker between the distal nitrogen atom N4′ and the introduced aryl ring.

In general, all test compounds showed comparable or better affinity for the dopamine $D_{4.4}$ receptor than clozapine (72% I) except for the pyridin-3-ylpropyl (**5c**) and pyrimidin-5-ylbutyl (**5f**) analogues, which were only marginally reduced (61% I and 64% I, respectively). Equally, their affinity for the serotonin 5-HT_{2A} receptor was comparable with clozapine (85% I), with the pyridin-4-ylpropyl analogue **5d** demonstrating the least inhibition of radioligand (72% I). The analogues containing a three-atom linker, namely the phenylpropyl and pyridin-3-ylpropyl **5c** compounds, showed reduced affinity for the dopamine $D_{4.4}$ receptor compared with clozapine, whereas the phenoxypropyl (**5a**) and pyridin-4-yl (**5d**) analogues displayed enhanced affinity. It was also evident within this series that isosteric replacement of CH_2 (phenylpropyl analogue) for O (**5a**) improved the affinity for the $D_{4.4}$ receptor relative to clozapine while maintaining a somewhat comparable affinity for the 5-HT_{2A} receptor. Additionally, replacement of the phenyl ring (phenylpropyl analogue) with a pyridin-4-yl ring (**5d**) improved the $D_{4.4}$ affinity but reduced the 5-HT_{2A} affinity compared with clozapine, whereas the isomeric pyridin-3-yl analogue **5c** displayed essentially the opposite effect. The analogues containing a four-atom linker displayed excellent affinity for both receptor systems compared with clozapine except for the pyrimidin-5-yl analogue **5f**, which displayed somewhat reduced $D_{4.4}$ affinity. Isosteric replacement of CH_2 (**5g**) for O (**5b**) within this set of compounds displayed no discernible difference in overall

Table 1. Preliminary binding studies



Compound	R	Binding affinity percentage inhibition (% I) at 10^{-6} M	
		$D_{4.4}$[A] [^{3}H]spiperone	5-HT_{2A}[A] [^{3}H]ketanserin
Clozapine	CH_3	72 ± 1	85 ± 1
4′-Phenylpropyl analogue		40[B]	87[B]
5a		85 ± 2	77 ± 1
5b		81 ± 3	83 ± 1
5c		61 ± 1	88 ± 2
5d		85 ± 1	72 ± 2
5e		78 ± 2	87 ± 1
5f		64 ± 7	85 ± 2
5g		82 ± 3	77 ± 1

[A]Determined in duplicate by MDS Pharma Services (Taiwan).
[B]Determined in duplicate by PANLABS (Taiwan) at a concentration of 10^{-6} M for the tested compound and representing the mean of duplicate tubes with a maximum standard error in the mean of ±5; reference values for clozapine (10^{-6} M) were 54% I and 90% I for the dopamine $D_{4.4}$ and serotonin 5-HT_{2A} receptors, respectively.

receptor affinity as did replacement of the phenyl ring (**5g**) with a pyridin-4-yl ring (**5e**). In general, the receptor binding data suggest that substitution for methyl at the N4′ position with the designated substituents essentially maintains dopamine $D_{4.4}$ and serotonin 5-HT_{2A} affinity relative to clozapine. Based on the affinity data from this initial receptor binding screen, all compounds were selected for further investigation in a preliminary in vivo behavioural assay that is predictive of potential antipsychotic efficacy.

In Vivo Behavioural Studies

All the target compounds in the present study were evaluated in vivo for their ability to antagonize apomorphine-induced climbing in mice, a psychosis-related behavioural model predictive of mesolimbic, dopaminergic activity, and potential

Table 2. Antagonism of apomorphine-induced climbing in mice
*Compounds with statistically significant ($P < 0.05$) activity

Compound[A]	Climbing index[B]	% Inhibition of climbing
Apomorphine	18.2 ± 1.5	—
Vehicle	0.4 ± 0.4	—
Clozapine	6.8 ± 2.6*	63
4′-Phenylpropyl analogue	18.8 ± 0.5[C]	0[C]
5a	11.5 ± 2.6	37
5b	5.2 ± 3.2*	71
5c	17.0 ± 2.0	7
5d	9.6 ± 2.0*	47
5e	5.6 ± 3.9*	69
5f	12.4 ± 3.4	32
5g	2.0 ± 1.1*	89

[A]All compounds were tested as their hydrochloride salts at a dose of 10 mg kg^{-1} intraperitoneal (ip).
[B]Values represent the mean ± s.e.m. climbing index.
[C]Determined previously;[6] reference climbing index value for clozapine hydrochloride was 12.8 ± 1.3 at a dose of 10 mg kg^{-1} ip; climbing indices for apomorphine hydrochloride (3 mg kg^{-1} ip) and vehicle were 18.2 ± 1.5 and 0.4 ± 0.4, respectively. Values represent the mean ± s.e.m.

antipsychotic efficacy.[9–12] The mice were pretreated with clozapine (10 mg kg^{-1} intraperitoneal (ip)) and test compounds (10 mg kg^{-1} ip), as their hydrochloride salts, 30 min before an injection of apomorphine hydrochloride (3.0 mg kg^{-1} ip) and their climbing behaviour assessed. The results of this behavioural assay are displayed in Table 2. Clozapine effectively diminished apomorphine-induced climbing in mice at 10 mg kg^{-1} ip, displaying 63% inhibition of climbing. The analogues **5b** (phenoxypropyl), **5e** (pyridin-4-ylbutyl), and **5g** (phenylbutyl) were the standout compounds and significantly antagonized the apomorphine-induced climbing behaviour in mice, with the phenylbutyl analogue **5g** displaying 89% inhibition of climbing. These compounds contain a four-atom spacer between the ionizable nitrogen atom and the introduced aryl ring and exhibited an appreciably greater potency compared with clozapine. Isosteric replacement (**5b**) and the inclusion of a heterocycle (**5e**) appeared to maintain in vivo activity compared with the saturated hydrocarbon linker and phenyl ring of the phenylbutyl analogue. Of the analogues containing a three-atom spacer, only the pyridin-4-ylpropyl compound **5d** displayed statistically significant antagonism of apomorphine-induced climbing (47% inhibition) that was comparable with clozapine and greatly enhanced compared with the benchmark phenylpropyl analogue. The two compounds showing the worst anticlimbing activity correspond to the pyridin-3-ylpropyl (**5c**) and pyrimidin-5-ylbutyl (**5f**), which also happen to display the least affinity for the dopamine $D_{4.4}$ receptor. Although exhibiting a favourable receptor binding profile, the phenoxypropyl analogue **5a** showed only a modest reduction in climbing (37% inhibition), which failed to be statistically significant; however, greater significant anticlimbing activity was observed at higher doses (climbing index = 8.6 ± 1.1 at 30 mg kg^{-1} ip). Overall, the in vivo data suggest that substitution for methyl at the N4′ position of clozapine with the designated substituents has quite a variable effect on behavioural activity with no definitive structure–activity correlation. This may be attributed to insufficient receptor binding data at the target and other receptor systems implicated in this complex behavioural model. Pharmacokinetic factors and physicochemical properties such as log P, molecular volume and

polar surface area may also, in part, influence the variability in activity.

Conclusions

A series of 4′-arylalkyl analogues of clozapine were synthesized based on a new structural model derived from the superimposition of X-ray crystal structures of clinically prescribed antipsychotics. These compounds were designed to investigate the biochemical effects of isosteric replacement (CH_2 for O) and varying aromaticity (phenyl for heterocycle) compared with previously published clozapine analogues. Preliminary in vitro binding data revealed that all target analogues exhibited desirable affinity for the dopamine $D_{4.4}$ and serotonin 5-HT_{2A} receptors compared with clozapine, with the pyridin-3-ylpropyl (**5c**) and the pyrimidin-5-ylbutyl (**5f**) analogues showing slightly reduced $D_{4.4}$ affinity. The binding data suggest that both receptor systems favoured a linker of three to four atoms in length, and are tolerant to isosteric replacement and substitution of a phenyl ring for a heterocycle such as a pyridinyl.

An in vivo investigation of this series showed that three of the target compounds (**5b**, **5e**, and **5g**), corresponding to the phenoxypropyl, pyridin-4-ylbutyl, and phenylbutyl analogues, respectively, dramatically diminished apomorphine-induced climbing in mice. These results compared favourably with clozapine's anti-climbing properties. The behavioural data have significantly contributed to refining the structural model and strongly suggest that analogues containing a four-atom linker (saturated hydrocarbon and isosteric replacement of CH_2 for O) between the ionizable nitrogen atom and the introduced aryl system (phenyl and pyridinyl) afford potential antipsychotic activity. Therefore, the aforementioned three lead compounds will form the basis for further structural investigation and pharmacological evaluation in our research group, in pursuit of the elusive 'ideal' antipsychotic drug.

Experimental

General

Melting points were determined on a Reichert Micro-melting point apparatus and are uncorrected. Elemental analyses were carried out on samples dried under vacuum by Microanalytical Service, Chemistry Department, University of Queensland, Brisbane, or Chemical & Micro Analytical Services Pty Ltd, Belmont, Victoria, Australia. Analytical reverse-phase high-performance liquid chromatography (HPLC) was performed on target compounds converted to hydrochloride salts by treatment with 1 M hydrogen chloride in diethyl ether and evaporated to dryness, using a Waters HPLC system fitted with a Waters Nova-Pak C_{18} Radial-Pak cartridge (8 mm × 100 mm, 6 μm, 60 Å) inside a Waters 8 × 10 compression module using a binary system (solvent A: 0.1% TFA/H_2O and solvent B: 0.1% TFA/90% CH_3CN/H_2O). Analyses were conducted using isocratic (60% A/40% B) and gradient elution mode (100% A to 100% B over 30 min) at 1.5 mL min^{-1} flow rate, UV detection at 254 nm. IR spectra were recorded on Bio-Rad FTS 3500GX utilising *Merlin* software for solids with KBr powder as background and on a Hitachi 270–30 infrared spectrophotometer with NaCl discs for oils or as a neat compound on a Scimitar Series Varian 800 Fourier-transform (FT) IR spectrophotometer with a PIKE Technologies MIRacle ATR. UV-Visible spectra were recorded as ethanolic solutions on a Pharmacia Biotech Ultraspec 2000 UV-visible spectrometer utilising *Swift II* software. Wavelengths of maximum absorbance (λ_{max}) are quoted with respective molar absorptivities (ε). ^1H and ^{13}C NMR (nuclear magnetic resonance) spectra were recorded at 300.13 and 75.47 MHz respectively in $CDCl_3$ using a Brüker Avance DPX 300 spectrometer equipped with a Silicon Graphics work station. Chemical shifts (δ) for all ^1H and ^{13}C spectra are reported in parts per million (ppm) using tetramethylsilane (TMS, 0 ppm) and deuterated chloroform ($CDCl_3$, 77.16 ppm or as specified) as the reference, respectively, unless otherwise stated. In reporting spectral data, the following abbreviations have been used: s, singlet; d, doublet; t, triplet; q, quartet; p, quintet; m, multiplet; br, broad; app, apparent; *J*, coupling constant in Hz; C, quaternary carbon; CH, methine carbon; CO, carbonyl carbon; CH_2, methylene carbon; CH_3, methyl carbon. Electrospray ionization (ESI) mass spectra (MS) were determined in positive ion mode using a Micromass Platform II mass spectrometer. High-resolution MS were determined using a Brüker BioApex II FTICR mass spectrometer. TLC was carried out routinely on silica gel $60F_{254}$ precoated plates (0.25 mm, Merck). Flash column chromatography was carried out using Merck silica gel 60, 230–400 mesh ASTM. All solvents were distilled before use. Tetrahydrofuran (THF) was distilled from sodium metal and benzophenone ketyl under nitrogen immediately before use. Distilled dichloromethane was stored over Type 4 Å molecular sieves. 1,4-Dioxan was distilled from phosphorus pentoxide under nitrogen and stored over sodium metal. All chemicals used were purchased from Sigma–Aldrich Pty Ltd.

4-(4-Pyridinyl)but-3-yn-1-ol **9**

To a stirred solution of 4-bromopyridine hydrochloride (**6**, 2.99 g, 15.4 mmol) in triethylamine (30 mL) and water (6 mL) was added lithium chloride (69.8 mg, 1.54 mmol), copper(I) iodide (81.4 mg, 0.462 mmol) and but-3-yn-1-ol (**8**, 1.30 g, 18.5 mmol). The reaction mixture was stirred for 15 min under nitrogen, after which *tetrakis*(triphenylphosphine)palladium (160 mg) was added and the reaction mixture was heated at reflux for 2.5 h. Then the mixture was cooled, concentrated under vacuum, treated with sodium hydroxide solution (1 M, 40 mL) and the product extracted with chloroform (3 × 30 mL). The solution was concentrated under vacuum and the product was purified via flash chromatography (ethyl acetate), to afford a pale brown solid (2.25 g, 99%). δ_H[13] 8.48 (2H, dd, *J* 4.5, 1.5, H2′, H6′), 7.23 (2H, dd, *J* 4.5, 1.5, H3′, H5′), 4.47 (1H, br s, OH), 3.84 (2H, t, *J* 6.5, H1), 2.70 (2H, t, *J* 6.5, H2). δ_C 149.2 (CH), 132.3 (C), 126.0 (CH), 93.3 (C), 79.4 (C), 60.5 (CH_2), 23.9 (CH_2).

4-(5-Pyrimidinyl)but-3-yn-1-ol ***10***

To a stirred solution of 5-bromopyrimidine (**7**, 3.01 g, 18.9 mmol) in triethylamine (30 mL) and water (6 mL) was added lithium chloride (62.5 mg, 1.38 mmol), copper(I) iodide (73.8 mg, 0.419 mmol), and but-3-yn-1-ol (**8**, 1.16 g, 16.5 mmol). The reaction mixture was stirred for 15 min under nitrogen, after which *tetrakis*(triphenylphosphine)palladium (160 mg) was added and the reaction was worked up as for the preparation of 4-(4-pyridinyl)but-3-yn-1-ol (**9**) to afford a pale brown solid (2.02 g, 83%). δ_H[13] 9.08 (1H, s, H2′), 8.73 (2H, s, H4′, H6′), 4.29 (1H, br s, OH), 3.86 (2H, t, *J* 6.5, H1), 2.74 (2H, t, *J* 6.5, H2). δ_C 158.6 (CH), 155.9 (CH), 120.0 (C), 95.3 (C), 74.8 (C), 60.2 (CH_2), 23.7 (CH_2).

4-(4-Pyridinyl)butan-1-ol **11**

To a stirred solution of 4-(4-pyridinyl)but-3-yn-1-ol (**9**, 2.25 g, 15.3 mmol) in ethanol (50 mL) was added palladium-on-carbon (10%, 240 mg). The mixture was hydrogenated at atmospheric pressure for 4 h, after which further 10% Pd/C (100 mg) was added and hydrogenation continued for an additional 2 h. The catalyst was removed by filtration through a Celite pad, washed with methanol and the filtrate concentrated under vacuum to afford the product as an orange-brown liquid (2.26 g, 98%). δ_H[14] 8.41 (2H, d, *J* 4.5, H2′, H6′), 7.10 (2H, d, *J* 5.5, H3′, H5′), 3.96 (1H, br s, OH), 3.67 (2H, t, *J* 7.5, H1), 2.63 (2H, t, *J* 7.5, H4), 1.72 (2H, m, H2), 1.61 (2H, m, H3). δ_C 151.8 (C), 149.2 (CH), 124.0 (CH), 61.8 (CH_2), 34.9 (CH_2), 32.2 (CH_2), 26.5 (CH_2).

4-(5-Pyrimidinyl)butan-1-ol **12**

To a stirred solution of 4-(5-pyrimidinyl)but-3-yn-1-ol (**10**, 2.02 g, 13.6 mmol) in ethanol (50 mL) was added Pd/C (10%, 300 mg). The mixture was hydrogenated at atmospheric pressure overnight, after which further 10% Pd/C (100 mg) was added and hydrogenation continued for another hour. The catalyst was removed by filtration through a Celite pad, washed with methanol, and the filtrate concentrated under vacuum to afford the product[15] as an orange-brown liquid (1.88 g, 91%). δ_H 9.04 (1H, s, H2′), 8.59 (2H, s, H4′, H6′), 3.68 (2H, t, *J* 6, H1), 3.44 (1H, br s, OH), 2.67 (2H, t, *J* 7.5, H4), 1.75 (2H, m, H2), 1.63 (2H, m, H3). δ_C 156.8 (CH), 156.7 (CH), 135.3 (C), 62.2 (CH_2), 32.1 (CH_2), 30.3 (CH_2), 27.1 (CH_2).

1-[4-(4-Pyridinyl)butyl]piperazine **16**

To a stirred solution of the 4-(4-pyridinyl)butan-1-ol (**11**, 1.97 g, 13.4 mmol) and triethylamine (3.10 g, 30.6 mmol) in dry dichloromethane (20 mL) at −50°C under nitrogen was added methanesulfonyl chloride (1.80 mL, 22.4 mmol) dropwise. The reaction mixture was slowly warmed up to 0°C when the mixture became orange, after which the mixture was promptly transferred to a separating funnel, washed with cold water and the product was extracted with cold dichloromethane (3 × 20 mL). The organic fractions were combined, washed with water, dried over magnesium sulfate and the majority of dichloromethane was removed under vacuum. The red mesylate solution (∼5 mL) was diluted with dimethoxyethane (20 mL) and solid piperazine (**15**, 5.16 g, 59.8 mmol) was added. The reaction mixture was heated at reflux for 3 h, after which the mixture was evaporated to dryness and the residue partitioned between aqueous hydrochloric acid solution (1 M, 30 mL) and dichloromethane (30 mL). The organic layer was removed and the aqueous layer was washed with dichloromethane (20 mL) and the pH adjusted to 14 with potassium hydroxide pellets. The product was extracted with dichloromethane (3 × 40 mL), washed with water, dried over magnesium sulfate and evaporated to dryness. The product was purified using flash chromatography (100:30:3 ethyl acetate/methanol/25% w/v aqueous ammonia) affording red-brown oil. The product was further purified by distillation affording pale yellow oil (1.80 g, 62%), bp 235–240°C/0.5 mm Hg. δ_H 8.48 (2H, dd, *J* 4.5, 1.5, H2″, H6″), 7.01 (2H, d, *J* 6, H3″, H5″), 2.88 (4H, m, H3, H5), 2.62 (2H, t, *J* 7.5, H1′), 2.34 (6H, m, H2, H6, H4′), 1.91 (1H, s, H4), 1.66 (2H, m, H2′), 1.52 (2H, m, H3′). δ_C 151.4 (C), 149.8 (CH), 124.0 (CH), 59.2 (CH_2), 55.3 (CH_2), 46.2 (CH_2), 35.2 (CH_2), 28.3 (CH_2), 26.3 (CH_2). *m/z* (ESI, 70 V) 220 (MH^+, 20%), 134 (38), 106 (100), 91 (23).

1-[4-(5-Pyrimidinyl)butyl]piperazine **17**

To a stirred solution of the 4-(5-pyrimidinyl)butan-1-ol (**12**, 2.06 g, 13.9 mmol) and triethylamine (4.50 g, 44.5 mmol) in dry dichloromethane (20 mL) at −50°C under nitrogen was added methanesulfonyl chloride (1.13 mL, 14.6 mmol) dropwise and the mesylate was worked up as described for the preparation of **16**. The crude mesylate was taken up in dimethoxyethane (20 mL) to which solid piperazine (**15**, 3.05 g, 35.6 mmol) was added. The reaction mixture was heated at reflux for 6 h and worked up as described for the preparation of **16**. The resulting residue was purified using flash chromatography (100:30:3 ethyl acetate/methanol/25% w/v aqueous ammonia) affording, on evaporation, the *title compound* as a yellow oil, which crystallized as pale yellow waxy needles on standing (1.17 g, 39%), mp 50–53°C. δ_H 9.06 (1H, s, H4″), 8.58 (2H, s, H2″, H6″), 2.90 (4H, br s, H3, H5), 2.64 (2H, t, *J* 7.5, H1′), 2.36 (6H, m, H2, H6, H4′), 1.68 (3H, m, H4, H2′), 1.55 (2H, m, H3′). δ_C 156.7 (CH), 156.6 (CH), 135.2 (C), 58.7 (CH_2), 54.5 (CH_2), 46.0 (CH_2), 30.3 (CH_2), 28.5 (CH_2), 26.1 (CH_2). *m/z* (ESI, 70 V) 221 (MH^+, 28%), 177 (39), 135 (39), 93 (100), 84 (54), 66 (30).

1-[3-(3-Pyridinyl)propyl]piperazine **18**

To a stirred solution of 3-(3-pyridinyl)propan-1-ol (**13**, 2.0 g, 14.7 mmol) and triethylamine (2.95 g, 29.1 mmol) in dry dichloromethane (20 mL) at −50°C was added methanesulfonyl chloride (1.92 mL, 24.7 mmol) dropwise and the mesylate was worked up as described for the preparation of **16**. The crude mesylate was taken up in THF (20 mL) to which solid piperazine (**15**, 5.00 g, 61.0 mmol) was added. The reaction mixture was heated at reflux overnight and extracted as described for the preparation of **16**. The organic extract was washed with water and dried over dry magnesium sulfate. The solution was evaporated to dryness and the residue purified by vacuum distillation affording a very pale yellow oil (1.69 g, 56%), bp 185–195°C/0.1 mm Hg. δ_H[16] 8.45 (1H, d, *J* 2, H2″), 8.43 (1H, dd, *J* 5, 1.5, H6″), 7.50 (1H, m, H5″), 7.20 (1H, m, H4″), 2.89 (4H, m, H3, H5), 2.65 (2H, m, H3′), 2.40 (4H, m, H2, H6), 2.34 (2H, m, H1′), 1.97 (1H, s, H4), 1.82 (2H, app p, *J* 7.5, H2′). δ_C 150.2 (CH), 147.5 (CH), 137.5 (C), 135.9 (CH), 123.4 (CH), 58.3 (CH_2), 54.7 (CH_2), 46.3 (CH_2), 30.9 (CH_2), 28.1 (CH_2). *m/z* (ESI, 30 V) 207 (MH^+, 15%) 206 (100), 113 (40), 94 (29).

1-[3-(4-Pyridinyl)propyl]piperazine **19**

To a stirred solution of the 3-(4-pyridinyl)propan-1-ol (**14**, 5.00 g, 36.4 mmol) and triethylamine (7.38 g, 72.8 mmol) in dry dichloromethane (25 mL) at −50°C under nitrogen was dropwise added methanesulfonyl chloride (4.80 mL, 62.0 mmol) and the mesylate was worked up as described for the preparation of **16**. The crude mesylate was taken up in anhydrous THF (25 mL) to which solid piperazine (**15**, 12.5 g, 146 mmol) was added. The reaction mixture was heated at reflux for 4 h and worked up as described for the preparation of **16**, affording the product, following vacuum distillation (3.95 g, 53%), as a pale yellow liquid, bp 150–155°C/0.3 mm Hg. δ_H 8.48 (2H, dd, *J* 4.5, 1.5, H2″, H6″) 7.11 (2H, dd, *J* 4.5, 1.5, H3″, H5″), 2.89 (4H, m, H3, H5), 2.64 (2H, t, *J* 7.5, H3′), 2.40 (4H, m, H2, H6), 2.33 (2H, t, *J* 7.5, H1′), 1.91 (1H, s, H4), 1.82 (2H, app p, *J* 7.5, H2′). δ_C 150.9 (C), 149.4 (CH), 123.6 (CH), 57.9 (CH_2), 54.2 (CH_2), 45.8 (CH_2), 32.6 (CH_2), 26.9 (CH_2). *m/z* (+ESI, 100 V) 206 (MH^+, 100%), 120 (61).

1-(2-Phenoxyethyl)piperazine ***22***

A mixture of piperazine (**15**, 17.1 g, 199 mmol), β-bromophenetole (**20**, 10.0 g, 49.7 mmol), and toluene (250 mL) was stirred at 85°C for 2 h, after which it was cooled, filtered, and the filtrate concentrated under vacuum. The resulting residue was partitioned between aqueous hydrochloric acid (2 M, 70 mL) and dichloromethane and the aqueous phase washed with dichloromethane (100 mL). The aqueous phase was adjusted to pH 14 with solid sodium hydroxide and extracted with dichloromethane (100 mL, 2 × 50 mL). The organic fractions were combined, washed with water (50 mL), dried over anhydrous sodium sulfate, filtered and then concentrated under vacuum. The product was purified by vacuum distillation to yield a colourless oil (7.78 g, 76%), bp 175–180°C/1.5 mm Hg (lit.[17] 143–144°C/0.53 mm Hg). ν_{max}/cm^{-1} 3274, 3064, 2938, 2818, 1599. δ_H 7.23–7.29 (2H, m, H3″, H5″), 6.88–6.95 (3H, m, H2″, H4″, H6″), 4.09 (2H, t, *J* 6, H2′), 2.89 (4H, m, H3, H5), 2.77 (2H, t, *J* 6, H1′), 2.52 (4H, m, H2, H6), 1.65 (1H, s, H4). δ_C 158.8 (C), 129.4 (CH), 120.5 (CH), 114.6 (CH), 65.7 (CH_2), 58.8 (CH_2), 55.0 (CH_2), 46.1 (CH_2). *m/z* (+ESI, 20 V) 207 (MH^+, 100%).

1-(3-Phenoxypropyl)piperazine ***23***

A mixture of piperazine (**15**, 16.0 g, 186 mmol), 1-(3-phenoxy)propyl bromide (**21**, 10.0 g, 46.5 mmol) and toluene (250 mL) was stirred at 85°C for 3 h and worked up as described in the preparation of **22**. Kugelrohr distillation afforded the product as a pale yellow oil (8.30 g, 81%), bp 225°C/2.5 mm Hg (lit.[18,19] 115°C/0.05 mm Hg), which formed a waxy solid on standing, mp 48°C. δ_H 7.25 (2H, m, H3″, H5″), 6.89 (3H, m, H2″, H4″, H6″), 3.99 (2H, t, *J* 6.5, H3′), 2.87 (4H, m, H3, H5), 2.49 (2H, m, H1′), 2.42 (4H, m, H2, H6), 1.95 (2H, app p, *J* 7, H2′), 1.86 (1H, s, H4). δ_C 159.2 (C), 129.5 (CH), 120.7 (CH), 114.7 (CH), 66.3 (CH_2), 55.9 (CH_2), 54.8 (CH_2), 46.3 (CH_2), 26.7 (CH_2). *m/z* (+ESI, 70 V) 221 (MH^+, 64%), 147 (31), 107 (43), 99 (100).

4-Phenylbutanal ***26***

Pyridinium chlorochromate (21.5 g, 66.6 mmol) was ground with silica gel (21.5 g) using a mortar and pestle and the resulting free-running light orange solid was suspended in dichloromethane (200 mL) at room temperature. To the stirred suspension was added a solution of 4-phenyl-1-butanol (**25**, 1.97 g, 10.2 mmol) in dichloromethane (10 mL) and stirring maintained for 3 h. The mixture was filtered (Celite) and the brown granular residue washed with dichloromethane (2 × 500 mL). The resulting filtrate was concentrated under vacuum, the residue taken up in dichloromethane (10 mL), filtered through a silica gel plug and the residue washed with dichloromethane. The combined filtrate was concentrated under vacuum and the resulting oil distilled to give the product aldehyde[20] (1.50 g, 77%) as a colourless liquid, bp 83–84°C/0.4 mm Hg (lit.[21] bp 120–122°C/16 mm Hg). δ_H 9.68 (1H, t, *J* 1.5, H1), 7.21–7.30 (2H, m, H3′, H5′), 7.11–7.20 (3H, m, H2′, H4′, H6′), 2.62 (2H, t, *J* 7.5, H4), 2.38 (2H, dt, *J* 7.5, 1.5, H2), 1.92 (2H, app p, *J* 7.5, H3). δ_C ([D_3]acetonitrile) 203.7 (CH), 142.9 (C), 129.5 (2 × CH), 127.0 (CH), 43.8 (CH_2), 35.7 (CH_2), 24.7 (CH_2). *m/z* (+ESI, 30 V) 467 ($3M + Na^+$, 28%), 209 (100), 91 (100).

8-Chloro-11-[4-(2-phenoxyethyl)piperazino]-5H-dibenzo[b,e][1,4]diazepine ***5a***

To a solution of 1-(phenoxyethyl)piperazine (**22**, 1.27 g, 6.14 mmol) in anhydrous anisole (5 mL) under nitrogen was added a solution of titanium tetrachloride in toluene (1 M, 1.4 mL, 1.4 mmol), which resulted in an immediate deep green colour. The mixture was then warmed to 50–55°C and a hot solution of 8-chloro-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepine-11-one (**24**, 300 mg, 1.23 mmol) in dry anisole (15 mL) was added. The mixture was heated at reflux overnight, was cooled and evaporated to dryness under vacuum. The residue was partitioned between ethyl acetate (80 mL) and aqueous sodium hydroxide (1 M, 30 mL) and filtered. The organic layer was separated and the aqueous phase extracted with ethyl acetate (2 × 50 mL). The organic fractions were combined, washed with water (2 × 30 mL), dried over anhydrous sodium sulfate and evaporated to dryness. The resulting oily residue was purified using flash chromatography (1:1 ethyl acetate/hexane) and the major product evaporated to dryness. The product was recrystallized from methanol/water to afford the *title compound* as bright yellow crystals (430 mg, 81%), mp 79–81°C. (Found: C 67.1, H 6.2, N 11.9. $C_{25}H_{25}ClN_4O{\cdot}CH_3OH$ requires C 67.2, H 6.3, N 12.1%). ν_{max} (KBr)/cm^{-1} 3352, 3060, 2932, 2840, 1600. λ_{max}/nm ($\varepsilon/M^{-1}\,cm^{-1}$) 216 (34670), 262 (17380), 298 (10720). δ_H ([D_6]acetone) 7.33–7.36 (1H, m, H3), 7.24–7.32 (3H, m, H1, H3‴, H5‴), 7.00–7.09 (2H, m, H2, H4), 6.91 (5H, m, H6, H9, H2‴, H4‴, H6‴), 6.88 (1H, dd, *J* 8, 2, H7), 6.53 (1H, s, H5), 4.15 (2H, t, *J* 6, H2″), 3.43 (4H, m, H2′, H6′), 2.82 (2H, t, *J* 6, H1″), 2.67 (4H, m, H3′, H5′). δ_C ([D_6]acetone) 164.1 (C), 160.0 (C), 155.1 (C), 150.9 (CH), 143.5 (C), 143.0 (C), 132.9 (CH), 131.0 (CH), 130.3 (CH), 128.6 (C), 127.0 (CH), 124.7 (C), 123.5 (CH), 121.4 (CH), 121.2 (CH), 115.5 (CH), 66.9 (CH_2), 58.1 (CH_2), 54.3 (CH_2), 48.4 (CH_2), 29.7 (CH_2). *m/z* (+ESI, 70 V) 435 ($M[^{37}Cl]H^+$, 10%), 433 (MH^+, 25%), 296 (36), 273 (33), 270 (100).

8-Chloro-11-[4-(3-phenoxypropyl)piperazino]-5H-dibenzo[b,e][1,4]diazepine ***5b***

To a solution of 1-(3-phenoxypropyl)piperazine (**23**, 0.984 g, 4.47 mmol) in anhydrous anisole (5 mL) under nitrogen was added a solution of titanium tetrachloride in toluene (1.0 M, 0.90 mL, 0.90 mmol), which resulted in an immediate deep green colouration. The mixture was then warmed to 55°C and a hot solution of 8-chloro-10,11-dihydro-5*H*-dibenzo[*b*,*e*][1,4]diazepine-11-one (**24**, 204 mg, 0.834 mmol) in dry anisole (10 mL) was added. The mixture was heated at reflux overnight after which time it was cooled and then evaporated to dryness under vacuum. The reaction mixture was partitioned between aqueous hydrochloric acid solution (1 M, 50 mL) and ethyl acetate (75 mL). The aqueous layer was washed with ethyl acetate and the pH was adjusted to 14 by potassium hydroxide pellets. The product was extracted with ethyl acetate (3 × 50 mL) and washed with water, dried over magnesium sulfate, then evaporated to dryness. The resulting oily residue was purified using flash chromatography (4:1 ethyl acetate/hexane) and the major product evaporated to dryness to afford the product as yellow oil, which recrystallized from dichloromethane/hexane to afford the *title compound* as yellow platelet crystals (183 mg, 49%), mp 139–140°C. (Found: C 69.9, H 6.1, N 12.4. $C_{26}H_{27}ClN_4O$ requires C 69.9, H 6.1, N 12.5%). ν_{max} (KBr)/cm^{-1} 3353, 1602, 1563. λ_{max}/nm ($\varepsilon/M^{-1}\,cm^{-1}$) 217 (37150), 262 (18620), 296 (11480). δ_H 7.23–7.36 (4H, m, H1, H3, H3‴, H5‴), 7.07 (1H,

m, H9), 7.02 (1H, m, H2), 6.91 (5H, m, H4, H7, H2‴, H4‴, H6‴), 6.81 (1H, dd, *J* 8.5, 2.5, H6), 6.52 (1H, s, H5), 4.07 (2H, t, *J* 6.5, H3″), 3.36 (4H, m, H2′, H6′), 2.55 (6H, m, H3′, H5′, H1″), 1.96 (2H, app p, *J* 6.5, H2″). δ_C 164.1 (C), 160.3 (C), 155.1 (C), 143.5 (C), 142.9 (C), 132.8 (CH), 131.0 (CH), 130.3 (CH), 128.6 (C), 127.0 (CH), 124.7 (C), 123.5 (CH), 123.4 (CH), 121.4 (CH), 121.3 (CH), 121.2 (CH), 115.4 (CH), 66.7 (CH_2), 55.7 (CH_2), 54.0 (CH_2), 48.4 (CH_2), 27.7 (CH_2). *m/z* (+ESI, 70 V) 447 (MH^+, 14%), 296 (30), 272 (33), 270 (100).

*8-Chloro-11-{4-[3-(3-pyridinyl)propyl]piperazino}-5*H*-dibenzo[*b,e*][1,4]diazepine* ***5c***

1-[3-(3-Pyridinyl)propyl]piperazine (**18**, 1.05 g, 5.10 mmol) in anhydrous anisole (5 mL) was treated with a solution of titanium tetrachloride in toluene (1.0 M, 1.12 mL, 1.12 mmol), followed by a hot solution of 8-chloro-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one (**24**, 250 mg, 1.02 mmol) in dry anisole (10 mL) and worked up as described in the preparation of **5b**. The product was purified via flash chromatography (2:1 ethyl acetate/methanol) and the resulting yellow oil was recrystallized from methanol/water to yield the *title compound* as yellow prisms (184 mg, 42%), mp 83–85°C. (Found: C 64.4, H 6.6, N 14.2. $C_{25}H_{26}ClN_5 \cdot CH_3OH \cdot H_2O$ requires C 64.8, H 6.7, N 14.5%). ν_{max} (KBr)/cm^{-1} 3255, 1605, 1554. λ_{max}/nm (ε/M^{-1} cm^{-1}) 228 (29510), 262 (21880), 295 (12020). δ_H 8.44 (2H, m, H2‴, H6‴), 7.50 (1H, d, *J* 7.5, H5‴), 7.18–7.30 (3H, m, H1, H3, H4‴), 7.06 (1H, d, *J* 2.5, H9), 6.99 (1H, t, *J* 7.5, H2), 6.80 (2H, dd, *J* 8.5, 2.5, H4, H7), 6.60 (1H, d, *J* 8.5, H6), 4.96 (1H, s, H5), 3.47 (4H, s, H2′, H6′), 2.66 (2H, t, *J* 7.5, H1″), 2.51 (4H, s, H3′, H5′), 2.41 (2H, t, *J* 7.5, H3″), 1.84 (2H, app p, *J* 7.5, H2″). δ_C 162.9 (C), 152.9 (C), 150.0 (CH), 147.4 (CH), 142.0 (C), 140.6 (C), 137.4 (C), 136.0 (CH), 132.0 (CH), 130.4 (CH), 129.1 (C), 126.9 (CH), 123.6 (C), 123.4 (CH), 123.2 (CH), 123.1 (CH), 120.2 (CH), 120.1 (CH), 57.7 (CH_2), 53.2 (CH_2), 47.4 (CH_2), 30.8 (CH_2), 28.2 (CH_2). *m/z* (+ESI, 30 V) 432 (MH^+, 32%), 432 (100), 430 (25).

*8-Chloro-11-{4-[3-(4-pyridinyl)propyl]piperazino}-5*H*-dibenzo[*b,e*][1,4]diazepine* ***5d***

1-[3-(4-Pyridinyl)propyl]piperazine (**19**, 1.05 g, 5.11 mmol) in anhydrous anisole (5 mL) was treated with a solution of titanium tetrachloride in toluene (1.0 M, 1.13 mL, 1.13 mmol), followed by a hot solution of 8-chloro-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one (**24**, 250 mg, 1.02 mmol) in dry anisole (10 mL) and worked up as for the preparation of **5b**. The product was purified via flash chromatography (3:1 ethyl acetate/methanol), redissolved in dichloromethane (10 mL), filtered, concentrated under vacuum and then recrystallized from methanol/water to give the *title compound* as bright yellow prisms (211 mg, 48%), mp 92–96°C. (Found: C 67.1, H 6.4, N 15.5. $C_{25}H_{26}ClN_5 \cdot CH_3OH$ requires C 67.3, H 6.5, N 15.1%). ν_{max} (KBr)/cm^{-1} 3276, 1602, 1560. λ_{max}/nm (ε/M^{-1} cm^{-1}) 228 (29510), 258 (20420), 296 (11750). δ_H ([D_6]acetone) 8.45 (2H, dd, *J* 4.5, 1.5, H2‴, H6‴), 7.33 (1H, m, H3), 7.29 (1H, m, H1), 7.23 (2H, dd, *J* 4.5, 1.5, H3‴, H5‴), 7.07 (1H, dd, *J* 8, 1, H4), 7.02 (1H, app td, *J* 7.5, 1, H2), 6.95 (1H, d, *J* 2.5, H9), 6.88 (1H, d, *J* 8.5, H6), 6.81 (1H, dd, *J* 8.5, 2.5, H7), 6.58 (1H, s, H5), 3.41 (4H, m, H2′, H6′), 2.70 (2H, t, *J* 7.5, H3″), 2.50 (4H, m, H3′, H5′), 2.38 (2H, t, *J* 7.5, H1″), 1.84 (2H, app p, *J* 7.5, H2″). δ_C ([D_6]acetone) 164.1 (C), 155.0 (C), 152.1 (C), 150.5 (CH), 143.4 (C), 142.9 (C), 132.8 (CH), 131.0 (CH), 128.5 (C), 127.0 (CH), 124.9 (CH), 124.7 (C), 123.5 (CH), 123.4 (CH), 121.4 (CH), 121.2 (CH), 58.1 (CH_2), 53.8 (CH_2), 48.3 (CH_2), 33.3 (CH_2), 28.3 (CH_2). *m/z* (+ESI, 70 V) 434 (M[^{37}Cl]H^+, 11%), 432 (MH^+, 30%), 272 (31), 270 (90), 192 (100), 120 (87).

*8-Chloro-11-{4-[4-(4-pyridinyl)butyl]piperazino}-5*H*-dibenzo[*b,e*][1,4]diazepine* ***5e***

A mixture of phosphorus pentachloride (283 mg, 0.924 mmol) and 8-chloro-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one (**24**, 205 mg, 0.840 mmol) in dry dichloromethane (20 mL) was heated at reflux for 1 h. The solvent was removed under vacuum and the residue was azeotroped twice with benzene and evaporated to dryness. The crude iminochloride was dissolved in anhydrous 1,4-dioxan (15 mL) and treated with 1-[4-(4-pyridinyl)butyl]piperazine (**16**, 356 mg, 1.62 mmol) in anhydrous 1,4-dioxan (5 mL). The reaction mixture was heated at reflux overnight, and worked up as described in the preparation of **5b**. The product was purified via flash chromatography (2:1 ethyl acetate/methanol) and the resulting oil was recrystallized from dichloromethane/hexane to give the *title compound* as yellow platelets (51 mg, 14%), mp 94–95°C. (Found: (MH^+) 446.210. $C_{26}H_{29}ClN_5$ requires (MH^+) 446.211). HPLC ($\lambda = 254$ nm) retention time (t_R) 3.40 min (isocratic), t_R 20.74 min (gradient). ν_{max} (KBr)/cm^{-1} 3259, 1608, 1560. λ_{max}/nm (ε/M^{-1} cm^{-1}) 229 (27540), 257 (19050), 296 (10960). δ_H 8.48 (2H, m, H2‴, H6‴), 7.26 (2H, m, H1, H3), 7.06 (1H, m, H9), 6.97–7.11 (2H, m, H3‴, H5‴), 7.00 (1H, m, H2), 6.81 (2H, m, H4, H7), 6.60 (1H, d, *J* 8.5, H6), 4.90 (1H, s, H5), 3.46 (4H, br s, H2′, H6′), 2.63 (2H, m, H1″), 2.50 (4H, m, H3′, H5′), 2.40 (2H, m, H4″), 1.68 (2H, m, H2″), 1.55 (2H, m, H3″). δ_C 162.9 (C), 152.9 (C), 151.4 (C), 149.9 (CH), 142.0 (C), 140.5 (C), 132.0 (CH), 130.4 (CH), 129.2 (C), 126.9 (CH), 124.0 (CH), 123.6 (C), 123.2 (CH), 123.1 (CH), 120.2 (CH), 120.1 (CH), 58.5 (CH_2), 53.3 (CH_2), 47.4 (CH_2), 35.2 (CH_2), 28.3 (CH_2), 26.5 (CH_2). *m/z* (+ESI, 70 V) 446 (MH^+, 10%), 296 (23), 272 (32), 270 (100).

*8-Chloro-11-{4-[4-(5-pyrimidinyl)butyl]piperazino}-5*H*-dibenzo[*b,e*][1,4]diazepine* ***5f***

A mixture of phosphorus pentachloride (187 mg, 0.920 mmol) and 8-chloro-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one (**24**, 204 mg, 0.836 mmol) in dry dichloromethane (20 mL) was heated at reflux for 1 h. The solvent was removed under vacuum and the residue was azeotroped twice with benzene and evaporated to dryness. The crude iminochloride was dissolved in anhydrous 1,4-dioxan (15 mL) and treated with the 1-[4-(5-pyrimidinyl)butyl]piperazine (**17**, 370 mg, 1.68 mmol) in anhydrous 1,4-dioxan (5 mL). The reaction mixture was heated at reflux for 19 h, and worked up as described in the preparation of **5b**. The product was purified via flash chromatography (10:3:0.03 ethyl acetate/methanol/25% w/v ammonia solution) and recrystallized from methanol/water to afford the *title compound* as yellow microcrystals (89 mg, 24%), mp 91–92°C. (Found: C 65.5, H 6.2, N 17.8. $C_{25}H_{27}ClN_6 \cdot CH_3OH$ requires C 65.2, H 6.5, N 17.5%). ν_{max} (KBr)/cm^{-1} 3270, 1607, 1561. λ_{max}/nm (ε/M^{-1} cm^{-1}) 229 (25700), 259 (18200), 293 (10720). δ_H 9.07 (1H, s, H2‴), 8.58 (2H, s, H4‴, H6‴), 7.27 (2H, m, H1, H3), 7.06 (1H, d, *J* 2.5, H9), 7.00 (1H, m, H2), 6.81 (2H, m, H4, H7), 6.60 (1H, d, *J* 8.5, H6), 4.90 (1H, s, H5), 3.46 (4H, m, H2′, H6′), 2.64 (2H, m, H1″), 2.50 (4H, m, H3′, H5′), 2.42 (2H, m, H4″), 1.70 (2H, m, H2″), 1.57 (2H, m, H3″). δ_C 162.8

(C), 156.9 (CH), 156.8 (2 × CH), 152.99 (C), 142.0 (C), 140.5 (C), 135.3 (C), 132.0 (CH), 130.4 (CH), 129.2 (C), 126.9 (CH), 123.6 (C), 123.2 (CH), 123.1 (CH), 120.2 (CH), 120.1 (CH), 58.3 (CH_2), 53.3 (CH_2), 47.4 (CH_2), 30.4 (CH_2), 28.7 (CH_2), 26.4 (CH_2). *m/z* (+ESI, 70 V) 447 (MH^+, 10%), 272 (32), 270 (100), 91 (75).

8-Chloro-11-[4-(4-phenylbutyl)piperazino]-5H-dibenzo[b,e][1,4]diazepine ***5g***

A stirred solution of 8-chloro-11-piperazino-5*H*-dibenzo[*b*,*e*][1,4]diazepine (**27**, 250 mg, 0.799 mmol) in dry 1,2-dichloroethane (20 mL) under nitrogen was treated with 4-phenylbutanal (**26**, 124 mg, 0.839 mmol) in 1,2-dichloroethane (5 mL) followed by sodium triacetoxyborohydride (237 mg, 1.12 mmol). Stirring was maintained for 2 h, after which aqueous hydrochloric acid (2 M, 30 mL) was added to produce a bright yellow-orange precipitate (gum-like, sparingly soluble in water). The organic layer was separated and discarded and the aqueous layer washed with dichloromethane (2 × 25 mL). The aqueous layer and precipitate were combined, adjusted to pH 14 by the addition of solid potassium hydroxide, then extracted with dichloromethane (3 × 50 mL). The combined organic extracts were combined, washed with water (30 mL), dried over dried magnesium sulfate and then concentrated under vacuum. The resulting oil was purified using flash chromatography (2:3 ethyl acetate/hexane) and the product recrystallized from dichloromethane/hexane as bright yellow needles (235 mg, 66%), mp 169–171°C. (Found: C 72.7, H 6.6, N 12.6. $C_{27}H_{29}ClN_4$ requires C 72.9, H 6.6, N 12.6%). ν_{max} (KBr)/cm^{-1} 3280, 2940, 1600, 1562. λ_{max}/nm (ε/M^{-1} cm^{-1}) 229 (25700), 261 (16980), 296 (10720). δ_H ([D_6]acetone) 7.10–7.36 (7H, m, H1, H3, H2‴, H3‴, H4‴, H5‴, H6‴), 7.06 (1H, app d, *J* 8, H4), 7.00 (1H, app td, *J* 7.5, 1, H2), 6.95 (1H, d, *J* 2.5, H9), 6.86 (1H, d, *J* 8.5, H6), 6.79 (1H, dd, *J* 8.5, 2.5, H7), 6.46 (1H, s, H5), 3.39 (4H, m, H2′, H6′), 2.63 (2H, t, *J* 7.5, H4″), 2.46 (4H, m, H3′, H5′), 2.37 (2H, t, *J* 7, H1″), 1.67 (2H, m, H3″), 1.52 (2H, m, H2″). δ_C ([D_6]acetone) 164.1 (C), 155.1 (C), 143.63 (C), 143.56 (C), 142.9 (C), 132.8 (CH), 131.1 (CH), 129.3 (CH), 129.2 (CH), 128.7 (C), 127.1 (CH), 126.6 (CH), 124.8 (C), 123.5 (2 × CH), 121.4 (CH), 121.2 (CH), 59.0 (CH_2), 54.0 (CH_2), 48.4 (CH_2), 36.5 (CH_2), 30.1 (CH_2), 27.3 (CH_2). *m/z* (+ESI, 70 V) 447 ($M[^{37}Cl]H^+$, 9%), 445 (MH^+, 27%), 272 (33), 270 (100), 192 (50), 91 (25).

Pharmacology Procedures

Receptor Binding Assays

Receptor affinities were determined by MDS Pharma Services (Taiwan), by the ability of the tested compounds to displace selective radioligands. All assayed compounds were dissolved in dimethylsulfoxide (DMSO) to a stock concentration of 10^{-2} M, then diluted with assay buffer to a final concentration of 10^{-6} M. The assays were carried out using the following: (i) for dopamine $D_{4.4}$ receptors: human recombinant (CHO-K_1 cells), [^{3}H]spiperone (0.3 nM) as radioligand and haloperidol (10 μM) as reference compound for non-specific binding; (ii) for serotonin 5-HT_{2A} receptors: rat cortex, [^{3}H]ketanserin (0.5 nM) as radioligand, and ketanserin (1 μM) as reference compound for non-specific binding. The binding results are expressed as the percentage inhibition of specific binding at a concentration of 10^{-6} M for the tested compound and represent the mean of duplicate tubes ± the maximum standard error in the mean.

Antagonism of Apomorphine-Induced Climbing in Mice

Adult female mice (20–33 g) were used for all the experiments and housed five per cage under controlled conditions of temperature (20 ± 1°C) and 12-h light/dark cycle (lights on 0600–1800 hours), with free access to food and tap water. The animals were brought into the laboratory and weighed 1 h before the commencement of the experiments to allow for acclimatization. All compounds assayed were prepared as their hydrochloride salts and dissolved in water for injections BP (British Pharmacopoeia) before ip injection. Mice (five per dose) were pretreated with test compound (10 mg kg^{-1} ip; 0.1 mL per 10 g bodyweight) 30 min before an injection of apomorphine hydrochloride (3.0 mg kg^{-1} ip). Climbing behaviour was assessed[22] at 5-min intervals over 25 min, commencing 5 min after apomorphine administration, using the following scoring system: 0 – no paws on the cage; 1 – one paw on the cage; 2 – two paws on the cage; 3 – three paws on the cage; 4 – four paws on the cage. The score recorded for each animal was based on the number of paws on the cage at a particular time-point, at the moment the animal was first observed. The mean climbing score (m.c.s.) was calculated by summation of the total score per mouse for all mice per dose over the course of the experiment, and dividing that figure by the number of mice per dose. The results of the experiment were expressed as a mean value per mouse ± s.e.m. (standard error of mean calculated by $s/\sqrt{n}$). The % inhibition climbing value was calculated using the following formula:

$$\%\ \text{Inhibition climbing} = \frac{[\text{m.c.s.(apomorphine)} - \text{m.c.s.(drug)}]}{\text{m.c.s.(apomorphine)}} \times 100$$

Biological Methods

The experimental protocols for the use of animals were approved by the Victorian College of Pharmacy, Monash University Animal Ethics Committee, and are in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (Australian Government, National Health and Medical Research Council, Canberra, 2004) and all government regulations.

Statistical Analysis

The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test for pair-wise comparison of drug-treated groups and apomorphine-treated control, using *GraphPad Prism 4* (2003), with $P < 0.05$ being considered as statistically significant.

Accessory Publication

Analytical HPLC conditions used in the present study are available from the journal's website.

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