MedChemComm

Accepted Manuscript

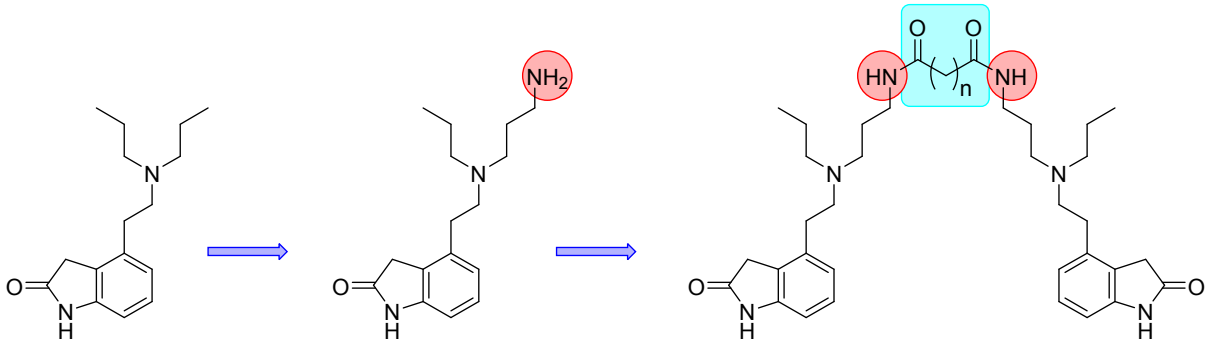
ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/medchemcomm

Investigation of novel ropinirole analogues: Synthesis, pharmacological evaluation and computational analysis of dopamine D_2 receptor functionalized congeners and homobivalent ligands

Manuela Jörg, Agnieszka A. Kaczor, Frankie S. Mak, Kiew Ching K. Lee, Antti Poso, Neil D. Miller, Peter J. Scammells, Ben Capuano

Graphical Abstract



ropinirole functionalized congener homobivalent ligands

Text

This study includes the synthesis, pharmacological evaluation and molecular modeling study of novel ropinirole-based monovalent and homobivalent ligands.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Investigation of novel ropinirole analogues: Synthesis, pharmacological evaluation and computational analysis of dopamine D_2 receptor functionalized congeners and homobivalent ligands

Manuela Jörg,[a] Agnieszka A. Kaczor,[b,c] Frankie S. Mak,[d] Kiew Ching K. Lee,[d] Antti Poso,[c] Neil D. Miller,[d] Peter J. Scammells,*[a] Ben Capuano*[a]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Herein, we report the development of novel functionalized congeners of ropinirole towards the design of pharmacological tools to probe structural requirements at the dopamine D_2 receptor. Subsequently, we have used the functionalized amine congener and synthesized and pharmacologically evaluated a series of homobivalent ligands of ropinirole with designated spacer lengths ranging from 14 to 30 atoms. The most potent homobivalent ligands (22-, 26- and 30-atom spacers) showed approximately 20- to 80-fold greater potency (EC_{50} = 3.9, 6.2 and 14 nM, respectively) than ropinirole (304 nM) in a [^{35}S]GTPγS functional assay. Molecular modeling studies suggest that the observed increase in potency of the homobivalent ligands is possibly due to a bitopic binding mode involving the orthosteric site and an allosteric interaction at the dopamine D_2 receptor protomer rather than bridging interactions at two orthosteric sites across a dopamine D_2 receptor dimer. This research has the potential to advance the development of structurally related bitopic ligands, biomarkers such as radioligands and fluorescently labeled probes, and furnish new homo- and heterobivalent ligands towards a better understanding of the dopamine D_2 receptor and potential novel treatment for Parkinson's disease.

Introduction

The dopamine receptors, are members of the G protein-coupled receptor (GPCR) super family, and implicated in many neurological processes, such as motivation, pleasure, cognition, memory, learning, and fine motor control.[1] Dopamine initiates its effects through the activation of five GPCR subtypes; namely dopamine receptors D_1 to D_5.[1] Commercially available dopaminergic drugs (pro-drugs, agonists, antagonists and enzyme inhibitors) demonstrate clinical utility in the treatment of a broad range of diseases including Parkinson's disease (PD), restless legs syndrome, sexual dysfunction, dementia, depression and schizophrenia.

More recent studies provide evidence that some of the targeted dopaminergic receptors exist not only in monomeric form, but also as homo- and heterodimers and/ or higher ordered oligomers.[2-4] Homobivalent ligands are described as molecules comprised of two identical pharmacophoric entities[5,6] covalently tethered by an appropriate spacer and have been used to investigate the properties and function of dopamine homodimers and higher ordered oligomers. To date, there are numerous published examples of dopamine D_2 receptor homobivalent ligands based on 1,4-disubstituted aromatic piperidine/piperazines,[7-10] clozapine,[11] 5-hydroxy-2-(dipropylamino)tetralin (5-OH-DPAT)[12] and apomorphine[13] pharmacophores. The most remarkable gain in binding affinity or functional potency compared to the original pharmacophore has been reported for homobivalent ligands of the dopamine D_2 antagonist clozapine[11] (~80-fold) and the dopamine D_2 agonist 5-OH-DPAT[12] (~95-fold).

This study is focused on agonism of the dopamine D_2 receptor which is linked to the treatment of motor dysfunction in PD. Currently, the dopamine pro-drug L-dopa as well as dopamine D_2 receptor agonists are used for the treatment of PD.[14,15] These drugs give some relief to patients by compensating for the progressive loss of dopaminergic neurons of substantia nigra in the basal ganglia. Nevertheless, clinically used L-dopa and dopamine D_2 receptor agonists are not able to cure patients with PD and can cause side effects such as induced dyskinesia, 'on-off' oscillation and 'wearing off' effects.[15] Consequently, a better understanding of the dopamine D_2 receptor in relation to its intrinsic structural and functional properties is desperately needed to develop novel treatment methods for patients with PD.

Ropinirole

In 1996, ropinirole (**1**) entered the market as a non-ergoline dopamine D_2 receptor agonist for the treatment of PD.[16, 17] This molecule acts as a full agonist at the D_2, D_3, and D_4 receptors and has little affinity towards the D_1 and D_5 receptors (Fig. 1).[18-20] Ropinirole (**1**) has been chosen for this study due to its low molecular weight, its simple and accessible chemical structure and the absence of any stereocentres. To our knowledge this is the first example of a series of homobivalent ligands investigating dopamine D_2 agonism using a pharmacophore of clinical relevance.

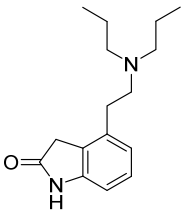


Fig. 1 Chemical structure of the dopamine D_2 receptor agonist ropinirole (**1**) and K_i values for the different dopamine receptor subtypes from experiments in human cell-lines.[18]

Design, Synthesis and Pharmacological Evaluation of Ropinirole Congeners

To rationally design and synthesize a functionalized congener - a molecule that comprises a chemical functional group (e.g. amine or carboxylic acid) that is suitably linked to a pharmacophore[21, 22] - it is crucial to locate the optimal attachment point for a linker on the pharmacophore. Not only must the activity of the functionalized pharmacophore be retained but also its chemical accessibility is essential. The functional congener approach has been successfully used to investigate GPCRs such as the adenosine, muscarinic and adrenergic receptor as well as the purinergic P2Y nucleotide receptors.[23] Adding further functionality to the correct position of an existing pharmacophore potentially enhances ligand affinity and selectivity of drug candidates. These functionalized congeners also provide a suitable starting point for the design of bitopic ligands, biomarkers such as radioligands and fluorophores as well as hetero- and homobivalent ligands. Despite the handicap of high molecular weight associated with some of these concepts, which may limit their usefulness as potential drugs, they are valuable pharmacological tools to further explore the structural features and functional properties of GPCRs.

Structure-activity relationship data from the literature were used to guide the design of our ropinirole-based functional congeners. The design of our congeners and homobivalent ligands was based on the findings published by Namil *et al.*; who successfully replaced one *n*-propyl chain with either ethyl- or methyl 4-butanoate, while maintaining binding affinity (K_i) values for the dopamine D_2 receptor similar to that of ropinirole (**1**).[24] This general approach was adopted based upon literature precedent as well as the synthetic accessibility of the tertiary amine (Scheme 1).

The commercially available hydrochloride salt of ropinirole (**2**) was transformed to the free base **1**, followed by dealkylation of a single propyl chain with 1-chloroethyl chloroformate. The hydrochloride salt of the dealkylated ropinirole **4** was formed in 43% yield as well as the doubly dealkylated compound **3** as the minor byproduct (12% yield, product/byproduct ratio 4:1). Alternatively compound **4** was synthesized from the alcohol 4-(2-hydroxyethyl)indolin-2-one (**5**), which was subsequently activated to the stable, crystalline tosylate derivative **6**, then further reacted with *n*-propylamine at reflux to give **4** in 74% yield. The second pathway was preferred due to robustness, simple purification (no column chromatography required) and higher yields. Compound **4** was converted to the free base and then further reacted with either ethyl 4-bromobutyrate or *tert*-butyl (3-bromopropyl)carbamate to afford intermediates **7** and **9** in moderate yields of 28% and 38%, respectively. It is noteworthy that for the scenario involving alkylation with the amine linker (*tert*-butyl (3-bromopropyl)carbamate), the use of a stoichiometric amount of the reagents was critical to avoid the dialkylated byproduct **10**. Alkaline hydrolysis of **7**, followed by acidic workup, potentially leads to the functionalized oxindole carboxylic acid congener (OCAC) (**8**) which can then be used to synthesize derivatives that further extend towards the extracellular space. On the other hand, removal of the Boc-group of **9** furnished the functionalized oxindole amine congener (OAC) (**11**) which could be utilized for the same purpose. It is important to note that compound **9** was stable in the solid state at 5 °C but slowly degraded in solution. Compound **11** rapidly degraded in both states therefore immediate use is recommended. Consequently, the free amine linker **11** was reacted with decanoyl chloride to afford the monovalent variant **12** in 17% yield (Scheme 2). This compound was utilized to evaluate if extensions in this position are well tolerated in view of designing more elaborate pharmacological tools.

The monovalent ligands were tested in a [^{35}S]GTPγS assay (results Table 1) using Chinese hamster ovary cells. This assay measures the agonist-stimulated activation of GPCRs close to the receptor in the signaling cascade. As a result little amplification of the signal is perceived compared to other assays downstream in the signaling cascade.[25] The results were compared to that of quinelorane, which behaved as a full agonist at the dopamine D_2 receptor with a pEC_{50} of 7.35 ± 0.14 (EC_{50} = 45 nM).

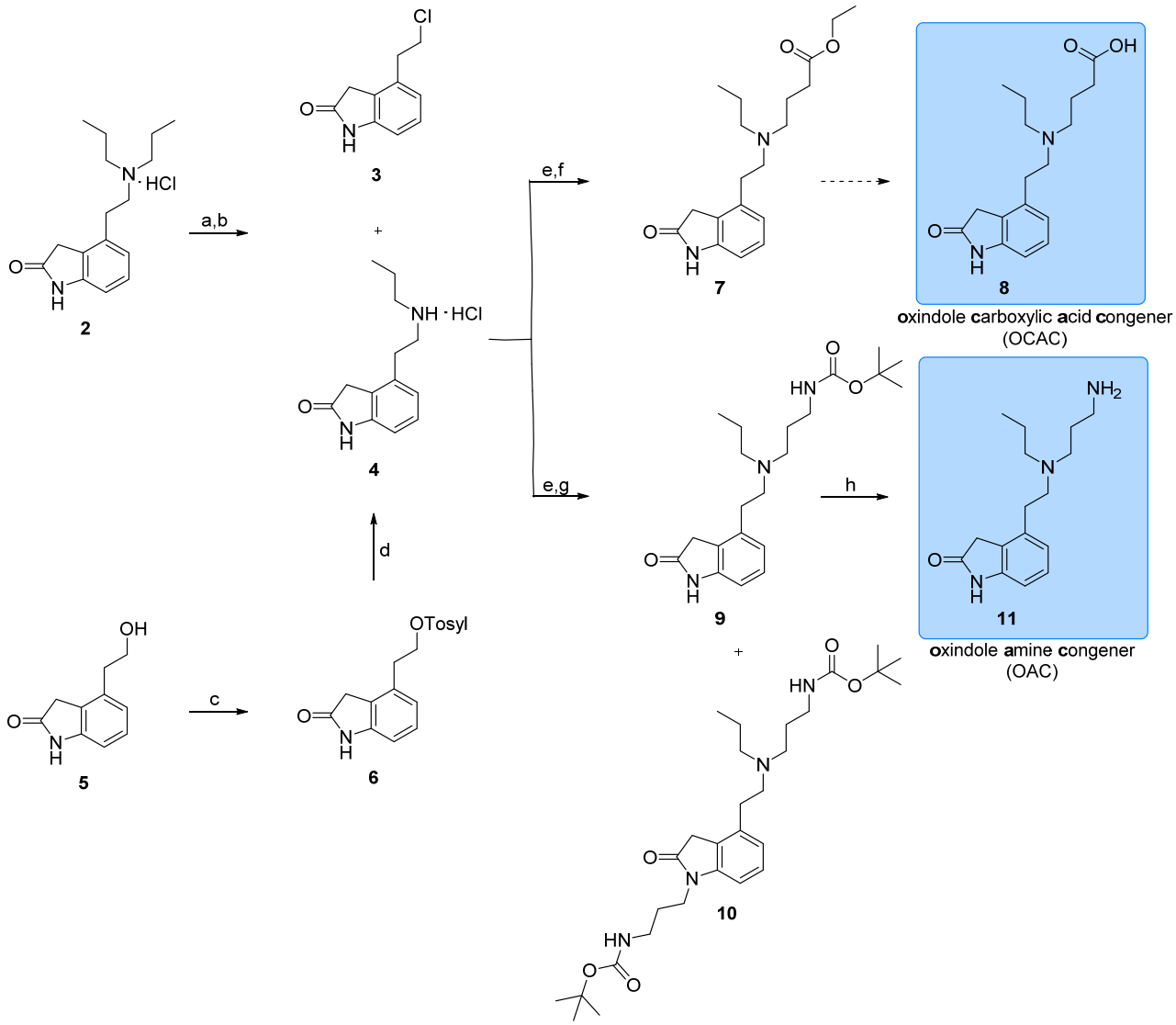
Table 1. Functional potency[a] and efficacy[a] of synthesized dopamine D_2 monovalent ligands using a [^{35}S]GTPγS assay. E_{max} values were referenced to quinelorane at 100 μM.

Compound #	pEC_{50} ± SEM	EC_{50} (nM)	E_{max} (%)
Quinelorane[b]	7.35 ± 0.14	45	100 ± 2
Ropinirole (**1**)[c]	6.52 ± 0.02	304	74 ± 1
4	7.07 ± 0.09	85	92 ± 4
7	7.26 ± 0.06	55	90 ± 2
9[d]	6.66 ± 0.02	219	69 ± 7
10	5.84 ± 0.16	1413	45 ± 11
12[b]	7.38 ± 0.15	42	78 ± 4

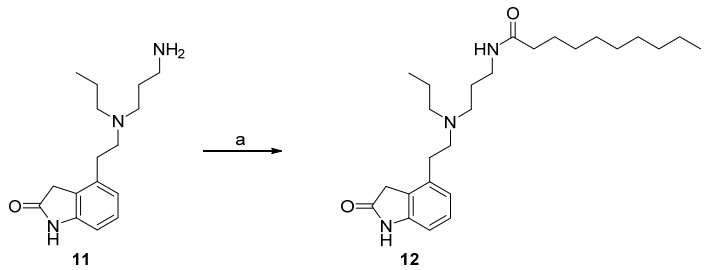
[a]Data represent the mean ± SEM of four separate experiments performed in duplicate
[b]Data represent the mean ± SEM of six separate experiments performed in duplicate
[c]Literature value (Ghosh *et al.*);[27] Ropinirole was not tested in this assay but the potency of quinelorane is consistent with the literature[26] thereby permitting subsequent comparisons of test compounds to ropinirole (**1**).
[d]Data represent the mean ± SEM of two separate experiments performed in duplicate due to instability of the stock solution

All the synthesized monovalent compounds with the exception of **10** showed higher functional potencies (EC_{50} values of 42-219 nM) compared to ropinirole (**1**, EC_{50} = 304 nM) with compound **12** as the standout. These results indicate that variations to one of

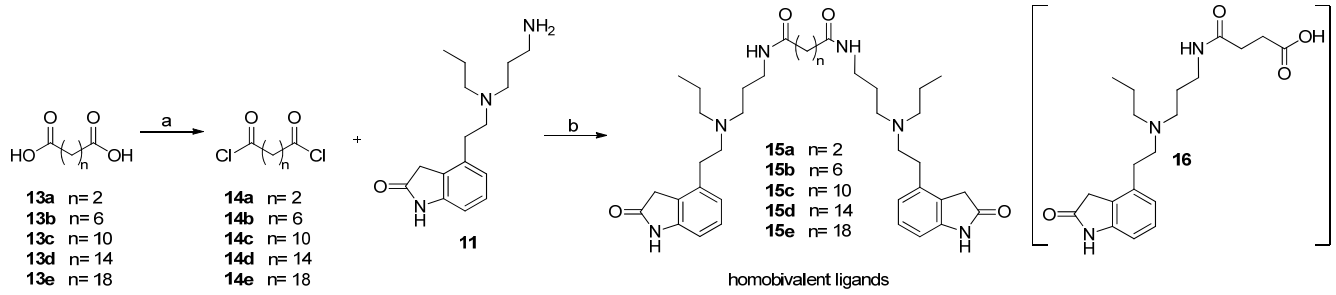
the alkyl chains are very well tolerated, maintaining functional potency at the dopamine D_2 receptor; neither the removal of one propyl chain (compound **4**) nor the attachment of a long spacer (compound **12**) diminished the potency or efficacy of the original pharmacophore. Linker attachment to the lactam nitrogen (compound **10**) on the other hand not only displayed a 5-fold decrease in potency relative to ropinirole (**1**) but also a substantial drop in the maximum response. In summary, the tertiary amine of ropinirole (**1**) has been shown to be a suitable position to extend towards the extracellular space of the dopamine D_2 receptor. As a consequence, the functionalized congeners **8** and **11** proved to be promising starting points for the synthesis of pharmacological tools to target the dopamine D_2 receptor.



Scheme 1. Reaction pathway to synthesize the functionalized oxindole based congeners **8** and **11**. Reagents and conditions: (a) 1 M NaOH, DCM, rt, 30 min, 94% (**1**); (b) 1-chloroethyl chloroformate, Na_2CO_3, DME, 85 °C, 17h followed by MeOH, reflux, 18 h, 12% (**3**), 43% (**4**); (c) *p*-toluenesulfonyl chloride, DCM, pyridine, 5-10 °C, 4 h, 80%; (d) *n*-propylamine, reflux, 1.5 h, 74%; (e) 1 M NaOH, DCM, rt, 20 min, 92%; (f) ethyl 4-bromobutyrate, K_2CO_3, acteone, reflux, 27 h, 28%; (g) *tert*-butyl (3-bromopropyl)carbamate, K_2CO_3, acetonitrile, reflux, 19 h, 38%; (h) 4 M HCl in dioxane, MeOH, rt, 1 h, 84%.



Scheme 2. Synthesis of monovalent ligand **12** as the decanamide. Reagents and conditions: (a) decanoyl chloride, DIPEA, DCM, 45 min, 17%.



Scheme 3. Synthesis of homobivalent ligands **15a-15e**. Reagents and conditions: (a) oxalyl chloride, DCM, DMF, rt , 1 h; (b) **11**, DCM, DIPEA, rt, 1 h, 4% (**15a**), 16% (**15b**), 17% (**15c**), 22% (**15d**), 31% (**15e**) and 4% (**16**).

Synthesis and Pharmacological Evaluation of Homobivalent Ligands of Ropinirole

On the basis of the results from our functionalized congeners, linking through the tertiary amine was used for the synthesis of a series of homobivalent ligands with spacer lengths (counting the atoms between the ionizable nitrogen atoms) from 14 to 30 atoms. The homobivalent ligands of ropinirole ranging from 2 to 16 methylene units in length were synthesized (Scheme 3) by adding the functionalized OAC (**11**) to the appropriate diacid chloride **14a-e**, that was formed *in situ* from the corresponding dicarboxylic acid, oxalyl chloride and catalytic dimethylformamide (Vilsmeier reaction). The reaction was generally complete within one hour at room temperature affording product yields varying from 4-31% and was strongly dependent on the degree of degradation of amine **11**. In one case, where the degradation of **11** had progressed extensively, only the monovalent carboxylic acid with the four carbon linker (n= 2) was isolated (compound **16**).

The homobivalent ligands were tested in the previously described [^{35}S]GTPγS assay to ascertain their functional potency and degree of agonism (results Table 2). The synthesized homobivalent ligands **15a-e** exhibited higher potency than the original pharmacophore **1** (Fig. 2, top), whereas the monovalent compound **16** showed a significant decrease in potency (EC_{50} > 4 μM). The analogues with a spacer length of 14 to 18 atoms (compound **15a** and **b**) showed approximately 11- to 14-fold greater functional potencies relative to ropinirole (**1**), which are comparable to the most active monovalent ligands represented in Table 1. An additional substantial gain in potency, ranging from 20- up to 80-fold compared to ropinirole (**1**) and 3- to 11-fold compared to quinelorane, was observed for the homobivalent ligands with a spacer length of 22 to 30 atoms (compounds **15c-e**). Interestingly, compounds **15a-d** maintained full agonism whereas the largest homobivalent ligand **15e** showed a substantial drop in the maximum response (Fig. 2, bottom). On the other hand, the monovalent analogue **16** exhibited a similar maximum response to ropinirole (**1**) but with significantly reduced functional potency.

Table 2. Functional potency[a] and efficacy[a] of synthesized dopamine D_2 homobivalent ligands **15a-e** and monovalent ligand **16** using a [^{35}S]GTPγS assay. E_{max} values were referenced to quinelorane at 100 μM.

Compound #	n	Spacer	pEC_{50} ± SEM	EC_{50} (nM)	E_{max} (%)
Quinelorane			7.35 ± 0.14	45	100 ± 2
Ropinirole (**1**)[b]			6.52 ± 0.02	304	74 ± 1
15a	2	14	7.55 ± 0.17	28	94 ± 3
15b	6	18	7.65 ± 0.13	22	99 ± 2
15c	10	22	8.41 ± 0.06	3.9	85 ± 4
15d	14	26	8.21 ± 0.11	6.2	85 ± 3
15e	18	30	7.85 ± 0.12	14	58 ± 4
16[c]	2	9	5.33 ± 0.06	4677	72 ± 4

[a]Data represent the mean ± SEM of six separate experiments performed in duplicate
[b]Literature value (Ghosh *et al.*);[27] Ropinirole was not tested in this assay but the potency of quinelorane is consistent with the literature[26] thereby permitting subsequent comparisons of test compounds to ropinirole (**1**).
[c]Data represent the mean ± SEM of four separate experiments performed in duplicate due to insufficient amount of sample

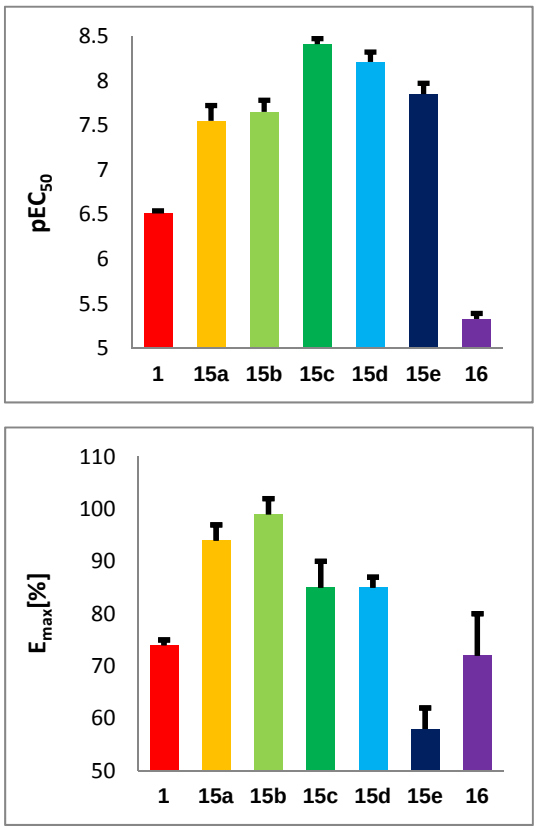


Fig. 2 Graphical representation of the functional potency (top) and efficacy (E_{max}, % maximum stimulation quinelorane) (bottom) of ropinirole (**1**), homobivalent ligands (**15a-e**) with increasing length and monovalent ligand **16**.

Docking

A dopamine D_2 receptor dimer model was built to correlate the pharmacological findings with a possible binding mode. In this study, a symmetric homodimer with a TM3TM4TM5 interface from both individual protomers was built. This particular interface was chosen since it is the commonly used approach to build GPCR dimers[28] and the same interface has been used for the dopamine D_2 homodimer[29,30] and dopamine D_2- adenosine A_{2A} heterodimer models.[31] The dimensions of the dopamine D_2 homodimer model were measured out and the approximate distances between the different binding sites are documented in Table 3.

Table 3. Distances between different binding sites in the dopamine D_2 receptor dimer model (in angstroms).

Orthosteric-orthosteric in different protomers through the membrane region	~34
Orthosteric-orthosteric in different protomers without crossing a membrane region	~50-60
Orthosteric-allosteric in one protomer	~18
Orthosteric-allosteric in different protomers across the homodimer	~30-40

Ropinirole (**1**), the monovalent ligands **4**, **7**, **9**, **10**, **12** and **16** as well as the homobivalent ligands **15a** were docked into the model of the receptor monomer, whereas the compounds **15b-15e** were manually placed in the dopamine D_2 receptor dimer and then re-docked for pose refinement. The main interactions observed for ropinirole (**1**) originate from the carbonyl group of the oxindole scaffold and the ionizable nitrogen of the side chain with Ser197(5.46) and Asp114(3.32), respectively (numbers in parenthesis indicate residue numbers according to Ballesteros-Weinstein system).[32] Linker attachment to the tertiary amine (compounds **7**, **9** and **12**) lead to a slight increase in functional potency (up to 7-fold compared to ropinirole) which is possibly due to an additional interaction between the introduced side chain carbonyl group of the analogues and Thr412(7.39) (Fig. 3). Compound **10**, which represents a molecule with a linker attached to the lactam group and the tertiary amine not surprisingly was unable to dock in the same pose as ropinirole (**1**), potentially explaining the poor biological results. The low potency ascribed to compound **16** could not be explained with the docking results and may result from unfavourable receptor interactions due to the presence of the carboxylic acid group (as the carboxylate anion) and/ or the zwitterionic state of the molecule at physiological pH.

Docking of homobivalent ligands **15a-15e** was performed methodologically to ensure the interaction of the ionizale nitrogen of the ligand with Asp114(3.32) in at least one protomer. Docking of the homobivalent ligands **15a** and **15b** showed similar interactions observed with the monovalent ligands (**7**, **9** and **12**). The docking results showed that **15b** is unable to reach the second protomer whereas the homobivalent ligands **15c** and **15d** are sufficiently extended to exhibit significant interactions at both protomers across the homodimer (Fig. 4). The docking poses of homobivalent ligands **15c** to **15e** direct towards the allosteric sites in protomer B involving the extracellular end of helices TM4 and TM5 as well as the extracellular loop ecl2. Figure 5 illustrates a binding mode where the homobivalent ligand **15e** interacts with the extracellular region of the second protomer (A) or crosses the membrane region allowing interactions with both orthosteric sites simultaneously (B). The second binding mode, although theoretically possible, is unlikely to occur in a biological system due to steric considerations. It is noteworthy that the aforementioned unfavourable binding mode for **15e** was the only docking position conserved that ensured ligand interaction with the critical Asp114(3.32) residues in both protomers. The docking results of the homobivalent ligands **15a-e** are in accordance with preliminary performed measurements of the size of the molecules (Table 4) and the comparison with the distances between the different binding sites (Table 3). The results suggest that the additional increase in potency of homobivalent ligands **15c-e** is possibly due to a bitopic mode of binding involving the orthosteric site of protomer A with an allosteric site on protomer B. It should be noted that this predicted binding mode is one of many possible binding modes; the same increase in potency could be explained by a bitopic binding mode involving the orthosteric and allosteric sites present within the same protomer. Similar conclusions were previously made by Gmeiner's group for another class of D_2 receptor bivalent ligands (whereby bivalent ligands displace just one equivalent of orthosteric ligand).[8]

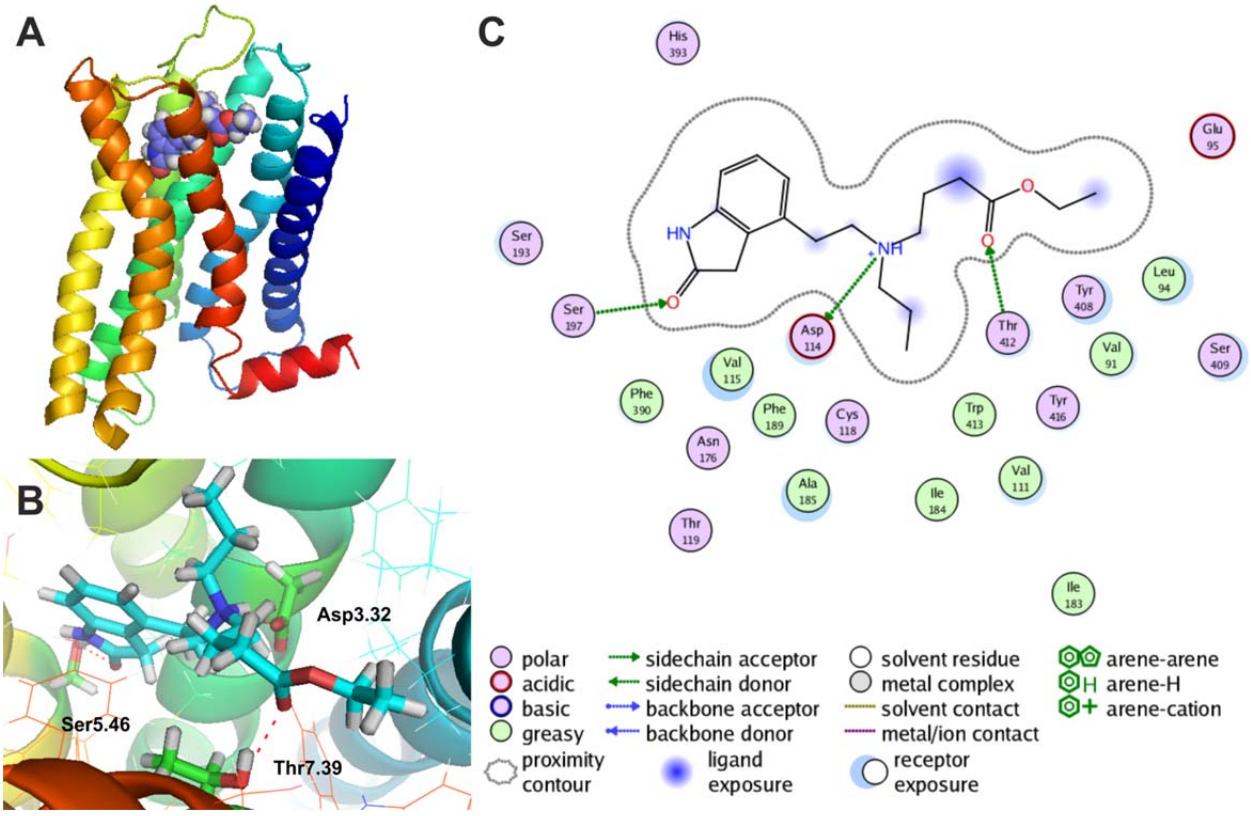


Fig. 3 Schematic representation of the binding site and the most important interactions of ligand **7** with the dopamine D_2 receptor. A – overview of the ligand-receptor complex; B - details of the binding site; C – schematic representation of the binding site. The crystal structure of the β_2 adrenergic receptor in a complex with the G_s protein (PDB ID: 3SN6)[33] was used as a template for the homology model of the dopamine D_2 receptor in active conformation. There are two disulfide bridges present in the model: one linking TM3 and e2 (Cys107-Cys182) and the other in e3 loop (Cys399-Cys401).

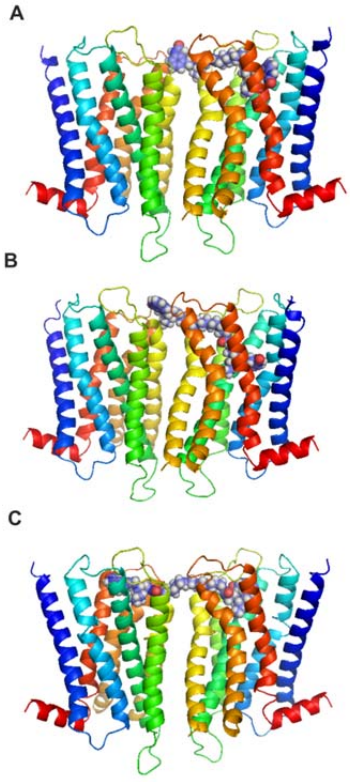


Fig. 4 Representation of dopamine D_2 receptor dimer model with homobivalent ligands docked to the binding cavity. A -compound **15b**; B - compound **15c**; C - compound **15d.**

Although the absolute binding mode of the synthesized ropinirole-based homobivalent ligands remains elusive, this study represents the first time a dopamine D_2 receptor dimer model has been used to demonstrate that the spacer length of 30 carbon atoms is probably insufficient to allow interaction of the ligand with the orthosteric sites of both protomers in a favorable manner, i.e. with the linker in the extracellular region, rather than across the membrane region.

Table 4. The calculated overall size and the distance between the ionizable nitrogen atoms of the homobivalent ligands **15a-15e** (in angstroms).

Compound #	Length of compounds	Distance between ionizable nitrogen atoms (Å)
15a	~29	14
15b	~31	19
15c	~36	24
15d	~37	28
15e	~48	33

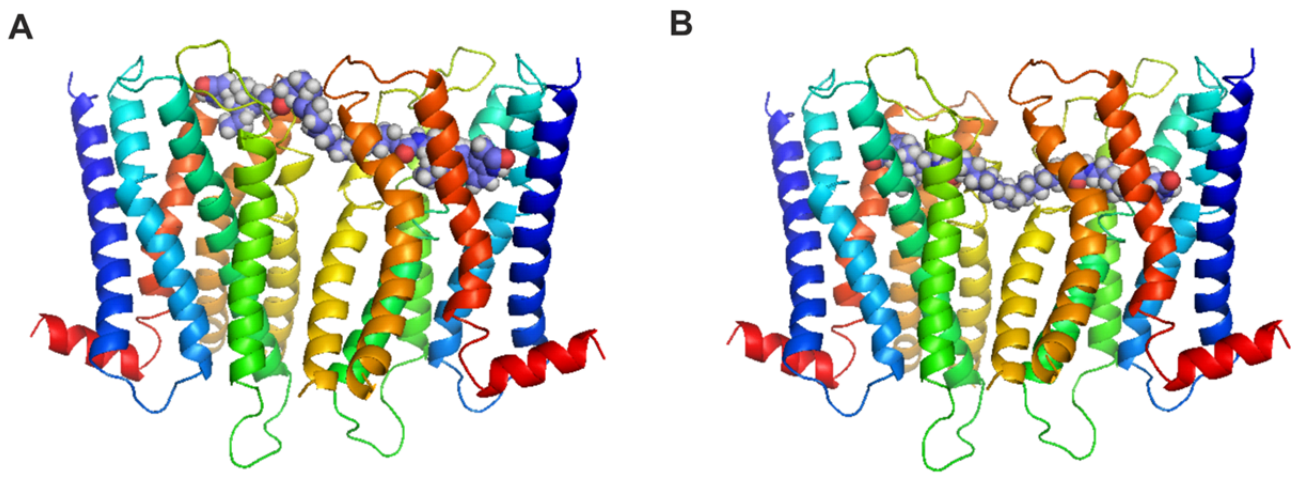


Fig. 5 Alternative poses for homobivalent ligands **15e**. Pose B is unrealistic due to steric clashes and was generated to depict how this ligand can reach orthosteric sites in two protomers.

Discussion and Conclusions

In summary, we have developed a functionalized oxindole carboxylic acid congener (OCAC) (**8**) and oxindole amine congener (OAC) (**11**) which offer excellent starting points for the design and synthesis of biological tools to further investigate the dopamine D_2 receptor topology and its role in biological processes. On the basis of our functionalized congener **11**, a series of homobivalent ligands with spacer lengths from 14 to 30 atoms were synthesized and pharmacologically evaluated in a [^{35}S]GTPγS assay to ascertain functional potency and agonism. Ropinirole analogues with the linker attached to the tertiary amine and the homobivalent ligands with a spacer length of 14 and 18 carbons atoms showed an approximate 1.5- to 14-fold increase in functional potency compared to ropinirole as well as full agonism; molecular modeling studies indicate that the gain in ligand functional potency is possibly due to an additional interaction of the introduced carbonyl group with Thr412 within the same protomer. However, the designed monovalent ligands (**7**, **9** and **12**) have a deficiency in hydrogen-bond acceptor and donor atoms whilst the homobivalent ligands **15a** and **15b** lack molecular size in order to interact with the key residues in the proximity of the allosteric region of the D_2 receptor, therefore limiting their gains in potency. The further extended homobivalent ligands with a spacer length of 22 up to 30 carbon atoms (compounds **15c-e**) showed a 20- to 80-fold increase in functional potency compared to the parent molecule and a 3- to 11-fold increase compared to the reference compound quinelorane. Interestingly, compound **15e** was the only homobivalent ligand which showed a substantial drop in efficacy. Molecular modeling studies of these molecules proposed that the substantial increase in potency is unlikely due to interactions at both orthosteric sites within a dopamine D_2 receptor homodimer. The lengths of the homobivalent ligands do not allow interactions at both orthosteric sites simultaneously due to steric considerations, however we predict that the second pharmacophore possibly interacts with an allosteric site present at the second protomer of the homodimer. The bitopic binding mode across the dimer is just one of many possible binding modes. The increased ligand potency resulting from additional interactions at an allosteric site within a single protomer cannot be excluded. Similar changes in potency have been reported with homobivalent ligands based on the dopamine D_2 receptor antagonist clozapine and the dopamine D_2 receptor agonist 5-hydroxy-2-(dipropylamino)tetralin (5-OH-DPAT).[12] The molecular modeling study presented gives supporting evidence that the ropinirole-based homobivalent ligands described herein are unlikely to interact at two orthosteric sites of a dopamine D_2 receptor homodimer simultaneously. Consequently, it is feasibly that published homobivalent ligands with similar spacer spans between the two pharmacophores (1,4-disubstituted aromatic piperidine/ piperazines,[7-10] clozapine,[11] 5-hydroxy-2-(dipropylamino)tetralin (5-OH-DPAT)[12] and apomorphine[13] based molecules) may also act in a bitopic mode rather than targeting two orthosteric sites across a homodimer.

The finding that the most potent ropinirole-based homobivalent ligands exhibited 20- to 80-fold greater potency than ropinirole is intriguing, even more so considering the reduced likelihood of these molecules interacting at two orthosteric sites across a homodimer. Consequently, further investigations should consider: (a) if dopamine D_2 receptor homobivalent ligands published in the literature are generally too short to interact at two orthosteric sites of a homodimer, would exchanging one of the orthosteric pharmacophores with an allosteric dopamine D_2 receptor fragment further improve the potency, selectivity and ligand efficiency of such pharmacological tools? (b) would the incorporation of a longer linker able to target the two orthosteric sites simultaneously further increase the potency of homobivalent ligands? and (c) if as suggested in the literature that only one protomer becomes activated and signals to a G protein, would it be possibly more beneficial to design and synthesize a molecule that incorporates a dopamine D_2 receptor agonist and a dopamine D_2 receptor antagonist? In addition, complementary mutagenesis studies should be considered to determine the amino acid residues that are involved in the interactions of the aforementioned pharmacological tools and the dopamine D_2 receptor.

The study presented provides further insight that will assist in the understanding of the complex mechanism of receptor dimers and signalling pathways associated with them.

Acknowledgments

M.J. is a recipient of the Australian Postgraduate Award Industry (APAI) and Monash University Postgraduate Publication Award. We thank GSK R&D-Singapore for financial support. Thank you to Francisco Javie Colilla-Rodriguez (GSK R&D Tres Cantos, Spain) for the dopamine D_2 receptor testing of our compounds. Thanks to the members of the Medicinal Chemistry department in GSK Singapore where some of this work was performed. A part of research was carried out during the postdoctoral fellowship of A.A.K. under Marie Curie IEF grant. Calculations were performed under a computational grant by Interdisciplinary Center for Mathematical and Computational Modelling (ICM), Warsaw, Poland, grant number G30-18 and under resources of CSC, Finland. Thanks to Dr Stephen Headey (Monash University) for his valuable support with the NMR experiments.

Notes and references

[a]*Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, 381 Royal Parade, Parkville, Victoria 3052, Australia. E-mail: ben.capuano@monash.edu; peter.scammells@monash.edu*
[b]*Department of Synthesis and Chemical Technology of Pharmaceutical Substances with Computer Modeling Lab, Faculty of Pharmacy with Division of Medical Analytics, Medical University of Lublin, 4A Chodźki St., PL-20093 Lublin, Poland*
[c]*School of Pharmacy, University of Eastern Finland, Yliopistonranta 1, P.O. Box 1627, FI-70211 Kuopio, Finland*
[d]*GSK R&D, Neural Pathways DPU, Neurosciences TAU, 11 Biopolis Way, Helios Bldg #03-01/02, Singapore 138667, Singapore*

† Electronic Supplementary Information (ESI) available: Details of chemical synthesis and characterization of all the reported compounds. Details of the [^{35}S]GTPγS assay including example curves. Details of Molecular modeling study. See DOI: 10.1039/b000000x/

1 J.-M. Beaulieu and R. R. Gainetdinov, *Pharmacol. Rev.*, 2011, **63**, 182-217.
2 R. K. Somvanshi and U. Kumar, *Pharmaceuticals*, 2012, **5**, 417-446.
3 S. Gines, J. Hillion, M. Torvinen, C. S. Le, V. Casado, E. I. Canela, S. Rondin, J. Y. Lew, S. Watson, M. Zoli, L. F. Agnati, P. Vernier, C. Lluis, S. Ferre, K. Fuxe and R. Franco, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 8606-8611.
4 M. Rocheville, D. C. Lange, U. Kumar, S. C. Patel, R. C. Patel and Y. C. Patel, *Science*, 2000, **288**, 154-157.
5 J. Shonberg, P. J. Scammells and B. Capuano, *ChemMedChem*, 2011, **6**, 963-974.
6 I. Berque-Bestel, F. Lezoulac'h and R. Jockers, *Curr. Drug Discovery Technol.*, 2008, **5**, 312.
7 D. Huber, H. Hubner and P. Gmeiner, *J. Med. Chem.*, 2009, **52**, 6860-6870.
8 D. Huber, S. Loeber, H. Huebner and P. Gmeiner, *Bioorg. Med. Chem.*, 2012, **20**, 455-466.
9 J. Kuehhorn, A. Goetz, H. Huebner, D. Thompson, J. Whistler and P. Gmeiner, *J. Med. Chem.*, 2011, **54**, 7911-7919.
10 J. Kühhorn, H. Hübner and P. Gmeiner, *J. Med. Chem.*, 2011, **54**, 4896-4903.
11 F. M. McRobb, I. T. Crosby, E. Yuriev, J. R. Lane and B. Capuano, *J. Med. Chem.*, 2012, **55**, 1622-1634.
12 S. Gogoi, S. Biswas, G. Modi, T. Antonio, M. E. A. Reith and A. K. Dutta, *ACS Med. Chem. Lett.*, 2012, **3**, 991-996.
13 J. Shonberg, J. R. Lane, P. J. Scammells and B. Capuano, *MedChemComm*, 2013, **4**, 1290-1296.
14 R. Tintner and J. Jankovic, *Expert Opin. Inv. Drug.*, 2003, **12**, 1803-1820.
15 T. Müller, *Translational Neurodegeneration*, 2012, **1**, 10.
16 US4452808A, 1984.
17 WO9200735A1, 1992.
18 P. M. Luthra and J. B. S. Kumar, *Mini-Rev. Med. Chem.*, 2012, **12**, 1556-1564.
19 R. J. Eden, B. Costall, A. M. Domeney, P. A. Gerrard, C. A. Harvey, M. E. Kelly, R. J. Naylor, D. A. A. Owen and A. Wright, *Pharmacol. Biochem. Be.*, 1991, **38**, 147-154.
20 C. Reavill, I. Boyfield, M. Coldwell and P. Nelson, *J. Pharm. Pharmacol.*, 2000, **52**, 1129-1135.
21 K. A. Jacobson, D. Ukena, W. Padgett, K. L. Kirk and J. W. Daly, *Biochem. Pharmacol.*, 1987, **36**, 1697-1707.
22 M. Jörg, J. Shonberg, F. S. Mak, N. D. Miller, E. Yuriev, P. J. Scammells and B. Capuano, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 3427-3433.
23 K. A. Jacobson, *Bioconjugate Chem.*, 2009, 20, 1816-1835.
24 A. Namil, M. Benoit-Guyod and G. Leclerc, *Eur. J. Med. Chem.*, 1995, **30**, 973-981.
25 P. G. Strange, *Br. J. Pharmacol.*, 2010, 161, 1238-1249.
26 A. Gobert, A. Dekeyne and M. J. Millan, *Neuropharmacology*, 2000, **39**, 1608-1616.
27 B. Ghosh, T. Antonio, M. E. A. Reith and A. K. Dutta, *J. Med. Chem.*, 2010, **53**, 2114-2125.
28 A. A. Kaczor and J. Selent, *Curr. Med. Chem.*, 2011, 18, 4606-4634.
29 S. P. Lee, B. F. O'Dowd, R. D. Rajaram, T. Nguyen and S. R. George, *Biochemistry*, 2003, **42**, 11023-11031.
30 Y. Liang, D. Fotiadis, S. Filipek, D. A. Saperstein, K. Palczewski and A. Engel, *J. Biol. Chem.*, 2003, **278**, 21655-21662.
31 A. Soriano, R. Ventura, A. Molero, R. Hoen, V. Casadó, A. Cortés, F. Fanelli, F. Albericio, C. Lluís, R. Franco and M. Royo, *J. Med. Chem.*, 2009, **52**, 5590-5602.
32 J. A. Ballesteros and H. Weinstein, *Methods Neurosci.*, 1995, **25**, 366-428.
33 S. G. F. Rasmussen, B. T. DeVree, Y. Zou, A. C. Kruse, K. Y. Chung, T. S. Kobilka, F. S. Thian, P. S. Chae, E. Pardon, D. Calinski, J. M. Mathiesen, S. T. A. Shah, J. A. Lyons, M. Caffrey, S. H. Gellman, J. Steyaert, G. Skiniotis, W. I. Weis, R. K. Sunahara and B. K. Kobilka, *Nature*, 2011, **477**, 549-555.