ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Multicomponent Synthesis and Biological Evaluation of a Piperazine-Based Dopamine Receptor Ligand Library

Mattia Stucchi,[†] Peter Gmeiner,[‡] Harald Huebner,*[,‡] Giulia Rainoldi,[†] Alessandro Sacchetti,*[,§] Alessandra Silvani,*[,†] and Giordano Lesma[†]

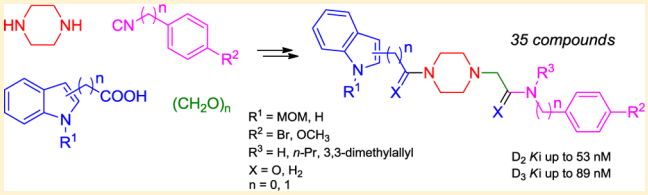
[†]Dipartimento di Chimica, Universit degli Studi di Milano, via Golgi 19, 20133 Milano, Italy

[‡]Department of Chemistry and Pharmacy Emil Fischer Center, Friedrich Alexander University, Schuhstraße 19, D-91052 Erlangen, Germany

[§]Dipartimento di Chimica, Materiali ed Ing. Chimica "Giulio Natta", Politecnico di Milano, p.zza Leonardo da Vinci 32, 20133 Milano, Italy

Supporting Information

ABSTRACT: A series of 1,4-disubstituted piperazine-based compounds were designed, synthesized, and evaluated as dopamine D2/D3 receptor ligands. The synthesis relies on the key multicomponent split-Ugi reaction, assessing its great potential in generating chemical diversity around the piperazine core. With the aim of evaluating the effect of such diversity on the dopamine receptor affinity, a small library of compounds was prepared, applying post-Ugi transformations. Ligand stimulated binding assays indicated that some compounds show a significant affinity, with K_i values up to 53 nM for the D2 receptor. Molecular docking studies with the D2 and D3 receptor homology models were also performed on selected compounds. They highlighted key interactions at the indole head and at the piperazine moiety, which resulted in good agreement with the known pharmacophore models, thus helping to explain the observed structure−activity relationship data. Molecular insights from this study could enable a rational improvement of the split-Ugi primary scaffold, toward more selective ligands.



KEYWORDS: *Multicomponent reaction, split-Ugi, D2/D3 dopamine receptor, molecular docking*

Dopamine receptors have been targeted in pharmacotherapeutic treatments of numerous disorders, including schizophrenia, depression, abuse of drugs, and Parkinson's disease.[1] They are members of the G-protein-coupled receptor (GPCR) family of proteins and are classified into two types, the D1-like and D2-like receptors, based on their sequence and similarity in signal transduction. Among the D2-like, the most abundant D2 and D3 receptor subtypes share about 50% overall sequence homology and 78% in their agonist binding sites. We are sure that even moderately D3/D2 selective compounds might offer a favorable profile compared to currently available drugs. Therefore, the design of selective ligands is a notable and challenging task.[2,3] D2-like receptor antagonists are of great interest for the treatment of schizophrenia. Starting from the privileged ligand family of 1,4-disubstituted aromatic piperazines and piperidines (1,4-DAPs) including the traditional antipsychotic agent haloperidol, both subtype-selective and balanced antagonists showing polypharmacological affinity patterns were developed.[4]

In particular, an intensive effort has been directed toward the development of selective ligands for the dopamine D3 receptor, affording a large number of compounds with various selectivities, acting as full or partial agonists and antagonists.[5−12] Recently, D3 receptor bound to an antagonist was also crystallized,[13] leading to additional ways to substantiate a reliable pharmacophore model for the rationalization of the observed binding affinities and selectivities.[14]

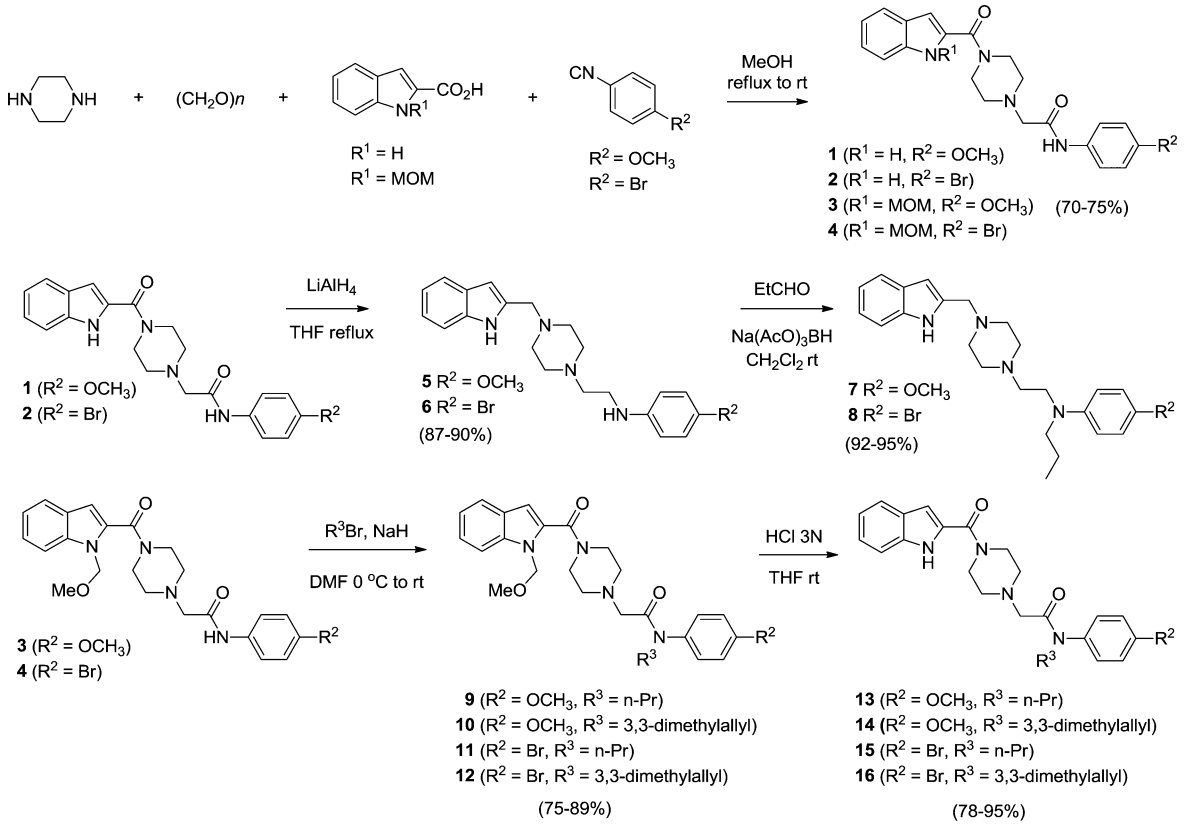
Most of the D3 receptor ligands contain a piperazine ring, connected to suitable lipophilic moieties via linkers of variable size and an aromatic head residue. Structure−activity relationship studies show that the lipophilic appendage controls the affinity, the linking unit is involved with the subtype selectivity among dopamine receptors, while the aromatic group ensures the overall activity.

Being aware that even minor structural modifications of these three key elements would be able to deeply affect the biological action, we decided to exploit the great potential of multicomponent reactions (MCR) in order to generate molecular diversity around a well-defined array of assessed recognition elements. MCRs enable the synthesis of target compounds in a single step from three or more reactants with greater efficiency and atom economy. Nowadays, MCR chemistry represent a successful approach both to combinatorial and diversity-oriented synthesis playing a central role in the development of efficient synthetic methodologies for pharmaceutical and drug discovery research.

Received: March 30, 2015
Accepted: June 23, 2015
Published: June 23, 2015

DOI: 10.1021/acsmedchemlett.5b00131
ACS Med. Chem. Lett. 2015, 6, 882−887

Scheme 1. General Scheme for the Synthesis of Compounds 1−16



Relying on our previous experience with isocyanide-based Ugi reactions,[15−18] we investigated into the split-Ugi methodology, a modified four-component protocol suitable for bis-secondary diamines, developed by Giovenzana et al. in 2006.[19,20] When applied to the piperazine heterocycle, it allows to generate a molecular scaffold in which one nitrogen atom is acylated and the other is alkylated, achieving in this way the regiochemical desymmetrization of the amine core in only one step, without the necessity of protecting groups. This is a most relevant advantage, in view of the preparation of a large array of compounds for drug discovery and lead optimization programs.

With the aim of first evaluating the applicability and limitations of the split-Ugi methodology to the dopamine receptor ligands chemistry, we focused our attention on some of the most D3 selective agonists known to date. Starting from common elements of their molecular framework, we carried out the selection of promising components for the MCR reaction.

We report here the synthesis of a small library of piperazine-based potential ligands (**1**−**35**), the evaluation on their binding affinity on D2 and D3 dopamine receptors, and a molecular docking study on most active compounds **22** and **24**.

Starting from 1*H*-indole-2-carboxylic acid or the corresponding *N*-(methoxymethyl)-derivative[21] as acid components, piperazine, formaldehyde as carbonyl component, and two different aromatic isocyanides,[22] namely, 1-isocyano-4-methoxybenzene or 1-isocyano-4-bromobenzene, reaction in methanol at reflux allowed to obtain cleanly the split-Ugi products **1**−**4** in high yields (Scheme 1). In order to evaluate the effect on biological activity of a higher flexibility and more basicity of the original scaffold, we considered to reduce the amide carbonyl groups in selected compounds. From **1** and **2**, double reduction using $LiAlH_4$ afforded quantitatively the corresponding triamines **5** and **6**, from which the more lipophilic compounds **7** and **8** could be easily obtained by reductive amination with propionaldehyde. From **3** and **4**, alkylation with 1-bromopropane and with 3,3-dimethylallyl bromide afforded overall compounds **9**−**12**, from which the corresponding NH-indole derivatives **13**−**16** could be achieved, by reaction with aq. HCl 3 N in THF.

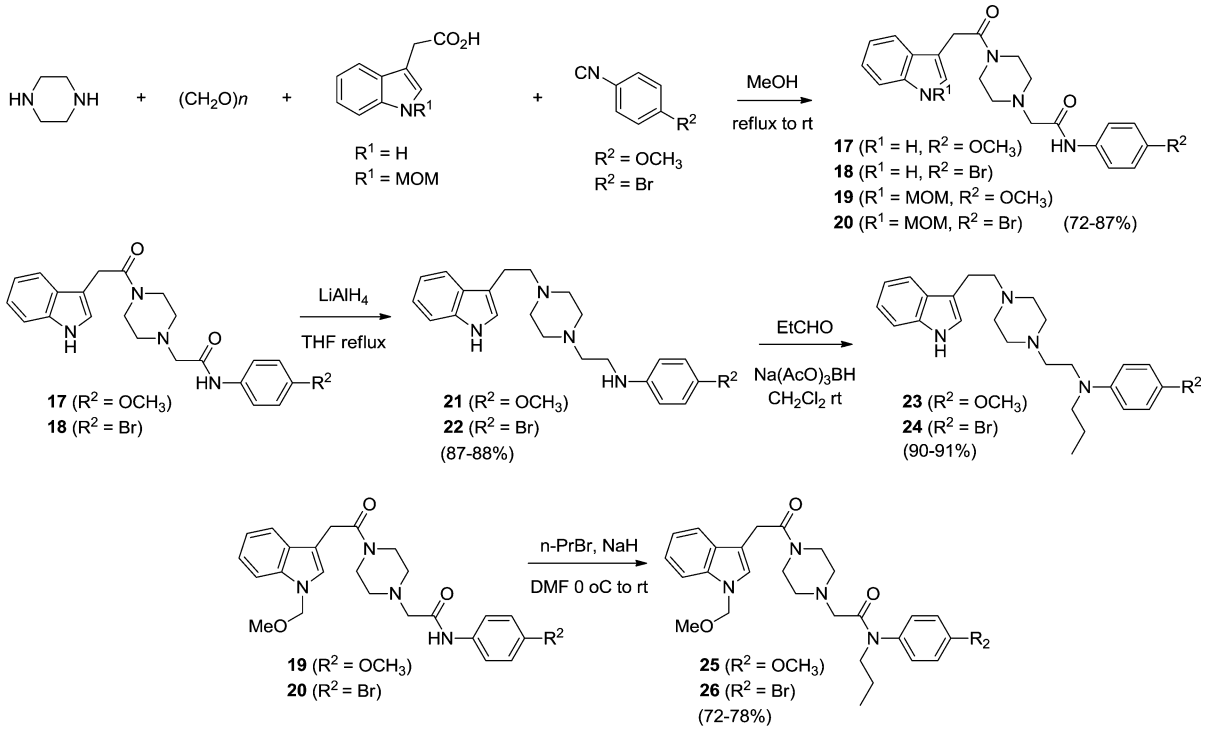
The split-Ugi reaction showed to perform well also using 2-(1*H*-indol-3-yl)acetic acid and the corresponding *N*-(methoxymethyl)-derivative as acid components, affording in comparable yields compounds **17**−**20** (Scheme 2). Reactions conducted on **17**−**18** allowed to obtain triamines **21**−**22** and the corresponding *N*-propyl derivatives **23**−**24**. From direct alkylation of **19**−**20**, amides **25**−**26** have been achieved.

Finally, aiming to consider the linker's length as valuable element for biological activity, we employed 1-(isocyanomethyl)-4-methoxybenzene and 1-(isocyanomethyl)-4-bromobenzene[23] as isocyanide component for the split-Ugi reaction (Scheme 3). The multicomponent step proceeded well to afford amides **27**−**29**, from which final compounds **30**−**35** have been easily synthesized.

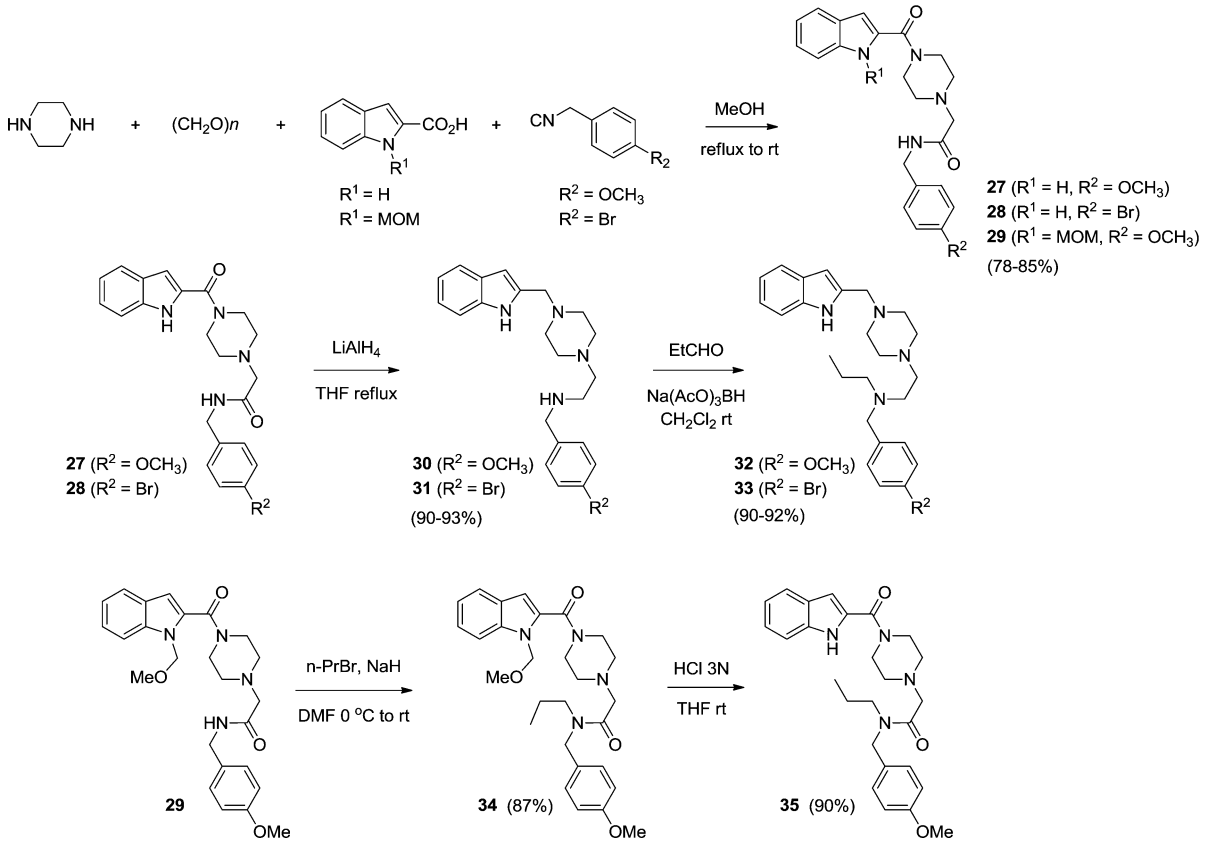
Compounds **1**−**35** have been evaluated for their affinity and selectivity through competitive binding assay for the dopamine D_2 and D_3 receptors. Selected data are summarized in Table 1. More active compounds are among these characterized by the triamine scaffold, with *p*-Br-substituted compounds more active than the correspondent *p*-OMe-substituted ones, with **22** and **24** showing the best D2 affinities of 53 and 58 nM, respectively. From comparison between compounds **8**, **24**, and **33**, the beneficial effect of elongation from the indolyl substituent side could be highlighted. For the most promising compounds **22** and **24** binding selectivity within the dopamine receptor family was

DOI: 10.1021/acsmedchemlett.5b00131
ACS Med. Chem. Lett. 2015, 6, 882−887

Scheme 2. General Scheme for the Synthesis of Compounds 17−26



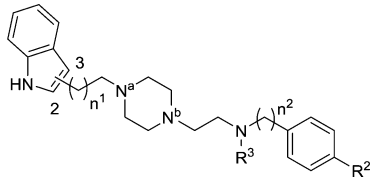
Scheme 3. General Scheme for the Synthesis of Compounds 27−35



determined. While both ligands display D_{2S} affinities (48 nM, 51 nM), similar to D_{2L}, reduced binding data was observed for D_1 (480 nM, 250 nM) and D_5 (3600 nM, 1300 nM). Surprisingly, best affinities within the dopamine receptor family could be determined for D4 with 20 nM for **22** and even 0.72 nM for **24**. Furthermore, **22** and **24** were tested on D_{2S} receptor activation properties showing antagonist effects in a cAMP accumulation assay.

DOI: 10.1021/acsmedchemlett.5b00131
ACS Med. Chem. Lett. 2015, 6, 882−887

Table 1. Radioligand Binding Data for the Most Promising Test Compounds 5−8, 21−24, and 30−33 Employing the Human D_{2L} and D_3 Receptors



						$K_i \pm$ (SEM) [nM][a] of [^{3}H]spiperone	
compd	pos.	n^1	n^2	R^2	R^3	hD_{2L}	hD_3
5	2	0	0	OCH_3	H	7500 ± 990	18000 ± 0
6	2	0	0	Br	H	920 ± 49	2300 ± 730
7	2	0	0	OCH_3	*n*-Pr	520 ± 150	1300 ± 0
8	2	0	0	Br	*n*-Pr	220 ± 32	600 ± 100
21	3	1	0	OCH_3	H	430 ± 160	620 ± 65
22	3	1	0	Br	H	53 ± 11	200 ± 18
23	3	1	0	OCH_3	*n*-Pr	120 ± 15	280 ± 38
24	3	1	0	Br	*n*-Pr	58 ± 6.7	89 ± 3.8
30	2	0	1	OCH_3	H	4000 ± 2500	4300 ± 1600
31	2	0	1	Br	H	5200 ± 1800	1800 ± 640
32	2	0	1	OCH_3	*n*-Pr	4100 ± 990	4600 ± 570
33	2	0	1	Br	*n*-Pr	920 ± 680	930 ± 28
haloperidol						0.96 ± 0.15	6.8 ± 1.3

[a] K_i values in nM ± standard error of mean (SEM) derived from 3 to 4 individual experiments each performed in triplicate.

Aimed to guide the future structure optimization of the split-Ugi primary scaffold, the ligand receptor interactions were investigated for selected most active compounds **22** and **24**, by molecular docking with Autodock 4.2[24] on D_2 and D_3 homology models (prepared with the YASARA[25] software, starting from the X-ray structure of human D_3 receptor (PDB code: 3BPL)). The docking results were then submitted to a molecular dynamics optimization in a membrane model with YASARA. As most piperazine-based reference structures, at physiological pH both the two piperazine nitrogens are protonated, while the less basic aniline nitrogen is present as free base. Referring to the D_3 receptor, for both compounds, a piperazine N_a^+H hydrogen is involved in a strong hydrogen bond with the key residue Asp $110^{3.32}$ thus anchoring the ligand to the receptor binding site.[26,27] Indole ring as headgroup is disposed in a more internal region promoting $\pi-\pi$ interactions with Phe345, Phe346, and His349. Cation$-\pi$ interactions are also present between the protonated nitrogens and Phe345 and Tyr365, in particular for compound **24**. Compound **24** exhibits additional strong lipophilic interactions between the *n*-propyl residue and Val86, Leu89, and Glu90 and a stronger $\pi-\pi$ contact between the bromoaryl group and Tyr 365. Notably, the presence of the *n*-propyl group enforces the indole ring to go deeper in the receptor pocket thus enabling a further H-bond with Ser-192. This different orientation of both bromoaryl and indole terminal groups could be related to the slightly higher activity of **24**. The relevant contribution of the cited above individual amino acid residues to the binding of small molecules in the dopamine receptor D_3 has been recently highlighted, after accurate QM/MM calculations (Figure 1).[28]

Docking of **22** and **24** on the D_2 model receptor yielded similar results, even if in this case no practical difference has been observed in the biological activity of the two compounds (Figure 2). The H-bond interaction of N_a^+H with Asp 114 is present for both **22** and **24**. In **24** an additional N_b^+H-Tyr 408 H-bond is observed, whereas **22** shows an indole−Ser 193 H-bond. Other lipophilic contacts are established with Val 91, Leu 94, and Thr 412. Residues Trp 386, Phe 389, Phe 390,

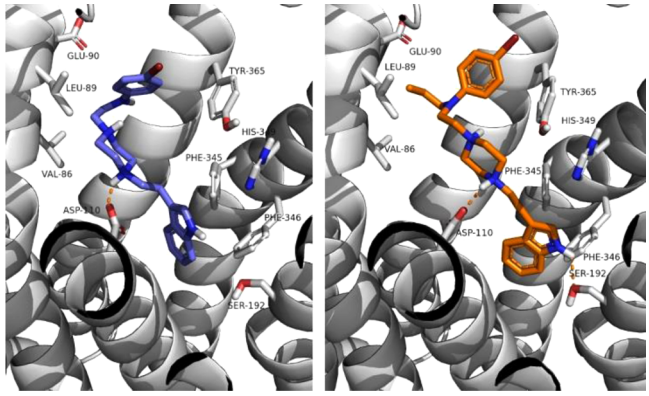


Figure 1. Docking poses for **22** (cyan) and **24** (orange) on the D_3 receptor model.

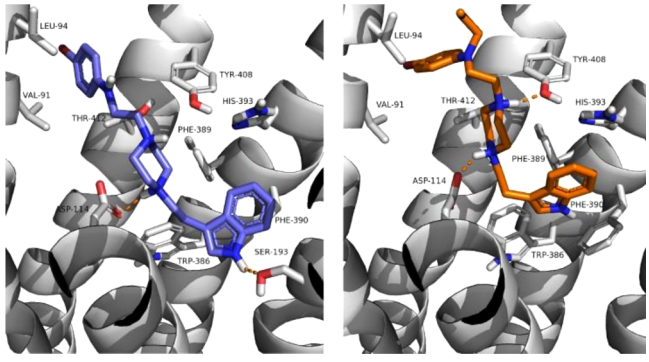


Figure 2. Docking poses for **22** (cyan) and **24** (orange) on the D_2 receptor model.

DOI: 10.1021/acsmedchemlett.5b00131
ACS Med. Chem. Lett. 2015, 6, 882−887

and His 393 are involved in $\pi-\pi$ interactions with the indole ring of both the compounds.

However, the lower activity of compounds **21** and **23** has to be ascribed to a different position of the phenyl ring in the binding site, due to the presence of the more hindered MeO group, which causes a reorganization of the entire molecule thus resulting in a less efficient interaction with both the D2 and D3 receptors.

In summary, we developed an original approach to the synthesis of piperazine-based dopamine receptor ligands, relying on the key split-Ugi multicomponent reaction and successive modifications. This strategy is suitable for the modification and distancing of all key pharmacophoric elements, such as the indole head, the piperazine core, and the linker to the aromatic moiety, resulting also in tuning flexibility and basicity of the whole molecule.

Biological evaluation on D_2 and D_3 receptors highlighted few active compounds, for which docking studies allowed identification of key intramolecular interactions, thus confirming the ability of such compounds to assume a biologically active correct conformation. Further work is currently underway in order to extend the split-Ugi library with the aim of improving the current poor D_2/D_3 selectivity.

■ ASSOCIATED CONTENT

Supporting Information

Complete experimental procedures and supporting data. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00131.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: alessandra.silvani@unimi.it.
*E-mail: harald.huebner@fau.de.
*E-mail: alessandro.sacchetti@polimi.it.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Missale, C.; Nash, S. R.; Robinson, S. W.; Jaber, M.; Caron, M. G. Dopamine Receptors: from Structure to Function. *Physiol. Rev.* **1998**, *78*, 189−25.

(2) Micheli, F.; Heidbreder, C. Dopamine D3 Receptor Antagonists: a Patent Review (2007−2012). *Expert Opin. Ther. Pat.* **2013**, *23*, 363−381.

(3) Ye, N.; Neumeyer, J. L.; Baldessarini, R. J.; Zhen, X.; Zhang, A. Recent Progress in Development of Dopamine Receptor Subtype-Selective Agents: Potential Therapeutics for Neurological and Psychiatric Disorders. *Chem. Rev.* **2013**, *113*, 123−178.

(4) Lober, S.; Hubner, H.; Tschammer, N.; Gmeiner, P. Recent Advances in the Search for D3- and D4-Selective Drugs: Probes, Models and Candidates. *Trends Pharmacol. Sci.* **2011**, *32*, 148−157.

(5) Modi, G.; Antonio, T.; Reith, M.; Dutta, A. Structural Modifications of Neuroprotective Anti-Parkinsonian (−)-N6-(2-(4-(Biphenyl-4-yl)piperazin-1-yl)-ethyl)-N6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (D-264): An Effort toward the Improvement of in Vivo Efficacy of the Parent Molecule. *J. Med. Chem.* **2014**, *57*, 1557−1572.

(6) Gopishetty, B.; Zhang, S.; Kharkar, P. S.; Antonio, T.; Reith, M.; Dutta, A. K. Modification of Agonist Binding Moiety in Hybrid Derivative 5/7-{[2-(4-Aryl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthalen-1-ol/-2-amino Versions: Impact on Functional Activity and Selectivity for Dopamine D2/D3 Receptors. *Bioorg. Med. Chem.* **2013**, *21*, 3164−3174.

(7) Du, P.; Xu, L.; Huang, J.; Yu, K.; Zhao, R.; Gao, B.; Jiang, H.; Zhao, W.; Zhen, X.; Fu, W. Design, Synthesis, and Evaluation of Indolebutylamines as a Novel Class of Selective Dopamine D3 Receptor Ligands. *Chem. Biol. Drug Des.* **2013**, *82*, 326−335.

(8) Sati, N.; Kumar, S.; Rawat, M. S. M. Rational Design, synthesis, SAR study of N-Naphthalen-1-yl-2-[4-(substituted phenyl)piperazin-1-yl]-acetamides as Atypical Antipsychotic Agents. *Indian J. Chem. B* **2012**, *51B*, 318−322.

(9) Johnson, M.; Antonio, T.; Reith, M. E. A.; Dutta, A. K. Structure-Activity Relationship Study of N6-(2-(4-(1H-Indol-5-yl)piperazin-1-yl)ethyl)-N6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine Analogues: Development of Highly Selective D3 Dopamine Receptor Agonists along with a Highly Potent D2/D3 Agonist and Their Pharmacological Characterization. *J. Med. Chem.* **2012**, *55*, 5826−5840.

(10) Bettinetti, L.; Schlotter, K.; Hübner, H.; Gmeiner, P. Interactive SAR Studies: Rational Discovery of Super-Potent and Highly Selective Dopamine D_3 Receptor Antagonists and Partial Agonists. *J. Med. Chem.* **2002**, *45*, 4594−4597.

(11) Leopoldo, M.; Berardi, F.; Colabufo, N. A.; De Giorgio, P.; Lacivita, E.; Perrone, R.; Tortorella, V. Structure-Affinity Relationship Study on N-[4-(4-Arylpiperazin-1-yl)butyl]arylcarboxamides as Potent and Selective Dopamine D_3 Receptor Ligands. *J. Med. Chem.* **2002**, *45*, 5727−5735.

(12) Hackling, A.; Ghosh, R.; Perachon, S.; Mann, A.; Höltje, H.-D.; Wermuth, C. G.; Schwartz, J.-C.; Sippl, W.; Sokoloff, P.; Stark, H. N-(omega-(4-(2-Methoxyphenyl)piperazin-1- yl)alkyl)carboxamides as Dopamine D_2 and D_3 Receptor Ligands. *J. Med. Chem.* **2003**, *46*, 3883−3899.

(13) Chien, E. Y. T.; Liu, W.; Zhao, Q.; Katritch, V.; Won Han, G.; Hanson, M. A.; Shi, L.; Newman, A. H.; Javitch, J. A.; Cherezov, V.; Stevens, R. C. Structure of the Human Dopamine D3 Receptor in Complex with a D2/D3 Selective Antagonist. *Science* **2010**, *330*, 1091−1095.

(14) Ehrlich, K.; Gotz, A.; Bollinger, S.; Tschammer, N.; Bettinetti, L.; Harterich, S.; Hubner, H.; Lanig, H.; Gmeiner, P. Dopamine D2, D3, and D4 Selective Phenylpiperazines as Molecular Probes To Explore the Origins of Subtype Specific Receptor Binding. *J. Med. Chem.* **2009**, *52*, 4923−4935.

(15) Stucchi, M.; Cairati, S.; Cetin-Atalay, R.; Christodoulou, M. S.; Grazioso, G.; Pescitelli, G.; Silvani, A.; Yildirim, D. C.; Lesma, G. Application of the Ugi Reaction with Multiple Amino Acid-Derived Components: Synthesis and Conformational Evaluation of Piperazine-Based Minimalist Peptidomimetics. *Org. Biomol. Chem.* **2015**, *13*, 4993−5005.

(16) Lesma, G.; Meneghetti, F.; Sacchetti, A.; Stucchi, M.; Silvani, A. Asymmetric Ugi 3CR on Isatin-Derived Ketimine: Synthesis of Chiral 3,3-Disubstituted 3-Aminooxindole Derivatives. *Beilstein J. Org. Chem.* **2014**, *10*, 1383−1389.

(17) Silvani, A.; Lesma, G.; Crippa, S.; Vece, V. Multicomponent Access to Novel Dihydroimidazo[1′,5′:1,2]pyrido[3,4-b]indol-2-ium Salts and Indoles by Means of Ugi/Bischler-Napieralski/heterocyclization Two Step Strategy. *Tetrahedron* **2014**, *70*, 3994−4001.

(18) Lesma, G.; Cecchi, R.; Crippa, S.; Giovanelli, P.; Meneghetti, F.; Musolino, M.; Sacchetti, A.; Silvani, A. Ugi 4-CR/Pictet-Spengler Reaction as a Short Route to Tryptophan-Derived Peptidomimetics. *Org. Biomol. Chem.* **2012**, *10*, 9004−9012.

(19) La Spisa, F.; Feo, A.; Mossetti, R.; Tron, G. C. An Efficient Synthesis of Symmetric and Unsymmetric Bis-(β-aminoamides) via Ugi Multicomponent Reaction. *Org. Lett.* **2012**, *14*, 6044−6047.

(20) Giovenzana, G. B.; Tron, G. C.; Di Paola, S.; Menegotto, I. G.; Pirali, T. A Mimicry of Primary Amines by Bis-Secondary Diamines as Components in the Ugi Four-Component Reaction. *Angew. Chem., Int. Ed.* **2006**, *45*, 1099−1102.

(21) Sundberg, R. J.; Russell, H. F. Syntheses with N-Protected 2-Lithioindoles. *J. Org. Chem.* **1973**, *38*, 3324−30.

(22) Polisar, J. G.; Norton, J. R. On the Reaction of Carboxylic Acids and Isonitriles with Conventional Heating. *Tetrahedron* **2012**, *68*, 10236−10240.

(23) Yu, S.-J.; Zhu, C.; Bian, Q.; Cui, C.; Du, X.-J.; Li, Z.-M.; Zhao, W.-G. Novel Ultrasound-Promoted Parallel Synthesis of Trifluoroatrolac-

DOI: 10.1021/acsmedchemlett.5b00131
ACS Med. Chem. Lett. 2015, 6, 882−887

tamide Library via a One-Pot Passerini/Hydrolysis Reaction Sequence and Their Fungicidal Activities. *ACS Comb. Sci.* **2014**, *16*, 17−23.
(24) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *J. Comput. Chem.* **2009**, *16*, 2785−2791.
(25) www.yasara.org.
(26) Malmberg, A.; Nordvall, G.; Johansson, A. M.; Mohell, N.; Hacksell, U. Molecular Basis for the Binding of 2-Aminotetralins to Human Dopamine D_{2A} and D3 Receptor. *Mol. Pharmacol.* **1994**, *46*, 299−312.
(27) Trumpp-Kallmeyer, S.; Hoflack, J.; Bruinvels, A.; Hibert, M. Modeling of G-Protein-Coupled Receptors: Application to Dopamine, Adrenaline, Serotonin, Acetylcholine, and Mammalian Opsin Receptors. *J. Med. Chem.* **1992**, *35*, 3448−3462.
(28) Zanatta, G.; Nunes, G.; Bezerra, E. M.; da Costa, R. F.; Martins, A.; Caetano, E. W. S.; Freire, V. N.; Gottfried, C. Antipsychotic Haloperidol Binding to the Human Dopamine D3 Receptor: Beyond Docking Through QM/MM Refinement Toward the Design of Improved Schizophrenia Medicines. *ACS Chem. Neurosci.* **2014**, *5*, 1041−1054.

DOI: 10.1021/acsmedchemlett.5b00131
ACS Med. Chem. Lett. 2015, 6, 882−887