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Antipsychotic Benzamides Amisulpride and LB-102 Display Polypharmacy as Racemates, *S* Enantiomers Engage Receptors D_2 and D_3, while *R* Enantiomers Engage 5-HT_7

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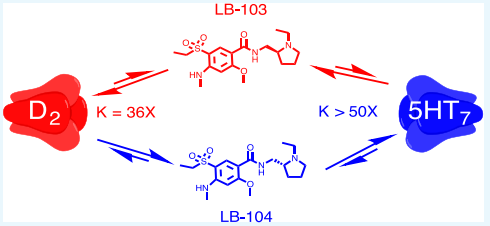
Supporting Information

ABSTRACT: Benzamide antipsychotics such as amisulpride are dosed as racemates though efficacy is assumed to be mediated through *S* enantiomer binding to D_2 receptors. At prescribed doses, the benzamides likely display polypharmacy since brain exposure should be sufficient to engage the 5-HT_7 receptors, as well. Curiously, the studies herein reveal that racemic dosing is required to engage both targets since the D_2 receptor has an almost 40-fold selectivity for the *S* enantiomer, while the 5-HT_7 receptor has greater than 50-fold preference for the *R* enantiomer.



INTRODUCTION

There is a strong preference for new drugs to be developed as single enantiomers to avoid risks associated with dosing a distomer (inactive enantiomer) along with its eutomer (active enantiomer) unless there is a compelling reason to use the racemic mixture.[1,2] As such, when developing a new drug with a chiral center, it has become a common practice to identify the active enantiomer and to develop it as a chirally pure compound. The regulatory environment of the past was less stringent, leading to a number of racemic drugs on the market in the US and Europe.[1] For example, many members of the benzamide class of antipsychotics exemplified by the compounds listed in Figure 1 are dosed as racemates.

Benzamide antipsychotics were developed to engage the brain dopamine D_2 and D_3 receptors (referred to collectively as $D_{2/3}$ in the text) and fall into the category of atypical antipsychotics.[3] Though never submitted for regulatory approval in the United States, numerous clinical trials demonstrate that the benzamide amisulpride is one of the most effective antipsychotics on the market in Europe.[4] In addition to the antipsychotic activity of amisulpride, there are many reports of its antidepressant activity in schizophrenia and other indications.[5−7] The work of Abbas et al., consisting of rodent in vivo studies, as well as the examination of a panel of off-target receptors, identified the serotonin 5-HT_7 receptor as the likely mediator of amisulpride's antidepressant activity.[8]

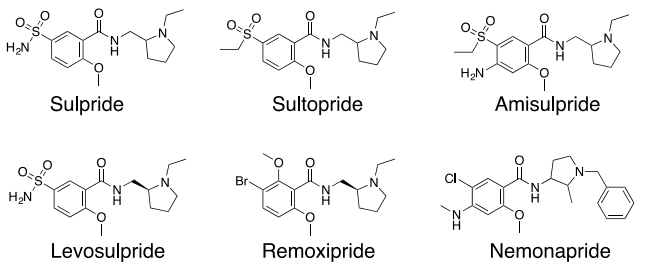


Figure 1. Representative benzamide antipsychotics. Top panels: plots of D_2 apparent affinity for dopamine in the presence of varied amisulpride concentration (left) or varied LB-102, LB-103, or LB-104 concentration (right). Bottom panels: plots of 5-HT_7 apparent affinity for serotonin in the presence of varied (*S*), (*R*), or (rac) amisulpride concentration (left) or varied LB-102, LB-103, or LB-104 concentration (right).

Polypharmacology occurs when one drug engages multiple targets that each contribute to a disease and is distinct from polypharmacy where multiple drugs are used to engage these targets.[9] As is the case with the benzamides, often, polypharmacology is a serendipitous outcome. Nevertheless, when discovered, polypharmacology is frequently invoked to explain the unusually high efficacy observed with such drugs. Adding to the complexity surrounding amisulpride's efficacy is the racemic nature of the drug. The chiral preference of the $D_{2/3}$ receptors was determined in 2001 when Castelli et al. reported that the *S* enantiomer of amisulpride bound $D_{2/3}$ nearly 40-fold more potently than the *R* enantiomer.[10] The enantiomeric preference of the 5-HT_7 receptor for amisulpride is reported in this work.

LB Pharmaceuticals is developing a new generation of N-alkylated benzamide antipsychotics. Promising preclinical results were obtained with LB-102, an N-methylated analogue of amisulpride, and with LB-103, the N-methylated *S* enantiomer of amisulpride[11,12] (Figure 2). The 5-HT_7 receptor stereo preference played a key role in deciding which asset to advance. If the 5-HT_7 receptor, like the $D_{2/3}$ receptors,

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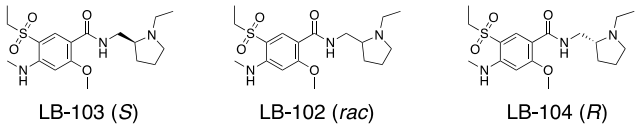

Figure 2. N-Methylated benzamides under development.

displayed a preference for the *S* enantiomer, then the logical choice would be to advance chirally pure LB-103. Conversely, if the 5-HT$_7$ receptor was selective for the *R* enantiomer (LB-104), then to harness the benzamide polypharmacology, we will advance the racemic compound LB-102, which being two distinct compounds would define the effect as polypharmacy rather than polypharmacology.

RESULTS AND DISCUSSION

Receptor binding studies were conducted in a cell-based assay containing overexpressed target receptors and a modified cyclic nucleotide-gated ion channel.[13]

Receptor engagement by the antagonists was determined from an analysis of observed response to a concentration matrix of natural ligands, dopamine or serotonin, versus a gradient of concentrations of antagonists. The apparent affinity Kd$_{(apparent)}$ of the natural ligand was calculated at fixed concentrations of the antagonist and then replotted as a function of antagonist concentration (Figure 3). These studies are ligand displacement assays and conform to the Cheng–Prusoff relationship for competitive inhibition.[14] When (*R*), (*S*), and (rac) compounds were examined against the receptor, eqs 1–3 were fit globally to the results. Kd refers to the binding affinity of the natural ligand and K_I refers to the binding affinity of the antagonist

$$\mathrm{Kd}_{\mathrm{apparent}} = \mathrm{Kd}\left(1 + \frac{[(S)]}{K_{I(S)}}\right) \quad (1)$$

$$\mathrm{Kd}_{\mathrm{apparent}} = \mathrm{Kd}\left(1 + \frac{[(R)]}{K_{I(R)}}\right) \quad (2)$$

$$\mathrm{Kd}_{\mathrm{apparent}} = \mathrm{Kd}\left(1 + \frac{0.5[(\mathrm{rac})]}{K_{I(S)}} + \frac{0.5[(\mathrm{rac})]}{K_{I(R)}}\right) \quad (3)$$

As seen in Figure 3, the displacement assays are consistent with a competitive Cheng–Prusoff relationship since they produce the expected linear relationships between apparent ligand affinities and antagonist concentrations. The antagonist binding affinities are presented in Table 1. The D$_2$ receptor prefers the *S* benzamide enantiomer having an almost 40-fold preference for LB-103 v LB-104, comparable to the amisulpride preferences reported by Castelli et al.[10] As well, within experimental uncertainty, LB-102 and amisulpride bind to the D$_2$ receptor with a Kd of 1 nM. In contrast, the 5-HT$_7$ receptor has a striking 50-fold preference for the *R* enantiomers of the benzamides. The racemic benzamide affinity for the 5-HT$_7$ receptor is approximately 30-fold weaker than its D$_2$ receptor affinity though potentially still pharmacologically relevant.

Table 1. Receptor Binding Affinity[a]

antagonist	D$_2$ K_I (nM)	5-HT$_7$ K_I (nM)
amisulpride (*S*)	n.d.	900 ± 1300
amisulpride (rac)	1.1 ± 0.12	44 ± 3
amisulpride (*R*)	n.d.	22 ± 1.5
LB-102	0.82 ± 0.02	31 ± 1
LB-103	0.4 ± 0.04	>1000
LB-104	14.4 ± 2.2	15.6 ± 0.9

[a]n.d. not determined.

Amisulpride effectively moderates schizophrenia's negative symptoms at moderate daily doses (50–300 mg) and the positive symptoms at higher daily doses (800–1600 mg).[15] The human pharmacokinetics of amisulpride are dose-linear

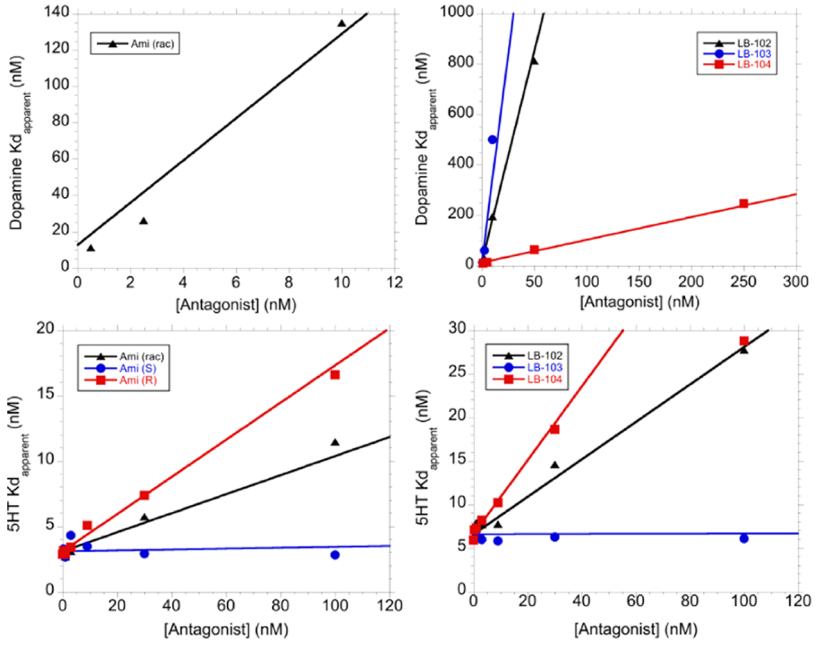

Figure 3. Natural ligand displacement by antagonists.

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over its therapeutic dose range, and its plasma PK and brain $D_{2/3}$ occupancies are reported.[15,16] PK−$D_{2/3}$ occupancy models of amisulpride and LB-102 were generated[12] by combining reports on amisulpride human PK,[15,16] $D_{2/3}$ receptor occupancy,[16] rat dose−$D_{2/3}$ receptor occupancy and behavioral studies,[17] and in-house rat PK studies. The absence of in vivo 5-HT_7 receptor occupancy studies in animals or humans limits our ability to reliably model 5-HT_7 in vivo occupancy since in vitro binding affinities rarely translate well to in vivo occupancies. Our previous work predicts that an 800 mg dose of amisulpride will produce a peak brain concentration of 20 ng/g, 10 h post dose, which provides a 90% $D_{2/3}$ occupancy. Our work determined an effective human brain $D_{2/3}$ EC_{50} of 1.6 ng/g. Extrapolating the in vitro differences in potency between D_2 and 5-HT_7 to approximate in vivo 5-HT_7 EC_{50} predicts a 5-HT_7 occupancy of around 30% at 800 mg. Abbas reports an amisulpride K_I of 11.5 nM v LSD, which would translate to a 50% occupancy at an 800 mg dose. Reliably modeling 5-HT_7 occupancy in humans will require a competing PET ligand study, as has been done with the $D_{2/3}$ receptors.

CONCLUSIONS

In the development of new pharmaceuticals, racemates are avoided when possible and new drugs are preferentially developed in enantiomerically pure form. As described in this work, the benzamide antipsychotics present an interesting challenge to the developmental process. The benzamides display polypharmacy if dosed as a racemate: antipsychotic activity is provided by targeting $D_{2/3}$ receptors with the *S* enantiomer, while antidepressant effects are provided by targeting the 5-HT_7 receptor with the *R* enantiomer. Based on the findings in this report, racemic LB-102 will be advanced to the clinic rather than the enantiomerically pure *S* LB-103.

EXPERIMENTAL SECTION

Compounds: amisulpride (rac) and (*S*) enantiomers were purchased from Toronto Research Chemicals (Toronto, ON); amisulpride (*R*), LB-102 (rac), LB-103 (*S*), and LB-104 (*R*) were prepared by Kalexsyn (Kalamazoo, MI): briefly, racemic amisulpride (and individual enantiomers) was suspended in *N*,*N*-dimethylformamide dimethyl acetal and stirred at 90 °C for 2 h; the mixture was cooled to ambient temperature, $NaBH_4$ was added portionwise, and the mixture was stirred for 1 h. The organic phase was removed under reduced pressure, and the residue was extracted with dichloromethane, washed with brine, dried, and filtered, and the solvent was removed under reduced pressure. Purification was achieved by column chromatography followed by recrystallization from acetone.

Compound structural integrities were determined by proton NMR. The purities were assessed using HPLC. All compounds possess a purity of at least 95%. Details are available in the Supporting Information.

Receptor binding studies were conducted by CODEX BIOSOLUTIONS using their ACTOne system assay procedures, and details are provided on their website.[13] In brief, ACTOne cell system contains a modified cyclic nucleotide-gated ion channel as a biosensor of cAMP activity in live cells. The channel responds to increases or decreases in intracellular cAMP levels by coordinately altering calcium flux, which is measured with a calcium-sensitive dye.[13] Cell lines were developed containing overexpressed D_2 dopamine receptors or 5-HT_7 serotonin receptors.

Assays were conducted in duplicate in 384-well plates. The concentration matrix consisted of five concentrations of the antagonist at 3-fold dilutions (and a 0 concentration) versus six concentrations of natural ligand also at 3-fold dilutions. Natural ligand binding (dopamine for D_2 and 5-HT for 5-HT_7) was determined from the response curves of natural ligand at a fixed antagonist concentration using a four-parameter binding equation

$$\text{response} = \frac{\max - \min}{1 + \left(\frac{\text{Kd}_{\text{apparent}}}{[\text{ligand}]}\right)^{n}} + \min$$

where max = response at a saturating ligand concentration, min = response in the absence of ligand, $Kd_{apparent}$ = ligand concentration producing half the maximum signal, and n = the Hill coefficient. Binding affinities for the antagonists were determined from fits of $Kd_{apparent}$ versus antagonist concentration using eqs 1−3.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02144.

Purity and characterization of (*R*)-amisulpride, rac *N*-methyl amisulpride, (*S*)-*N*-methyl amisulpride, and (*R*)-*N*-methyl amisulpride; racemic amisulpride, along with *R* and *S* enantiomers, was purchased; ^{1}H NMR spectra were recorded at 400 MHz on a Bruker 400 Avance spectrometer or at 300 MHz on a Bruker Fourier 300 (PDF)

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Author Contributions

V.G., A.R.V., Z.P., and M.S.H. designed the studies; M.S.H. analyzed the results; M.S.H. and A.R.V. wrote the manuscript; and V.G., A.R.V., Z.P., and M.S.H. reviewed the manuscript.

Notes

The authors declare the following competing financial interest(s): ARV and ZP are employees of LB Pharmaceuticals MSH and VG are consultants to LB Pharmaceuticals.

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ABBREVIATIONS

5-HT, 5-hydroxytrypamine (serotonin); cAMP, cyclic adenosinemonophosphate; EC_{50}, effective concentration 50; EMA, European Medical Agency; FDA, Food and Drug Administration; K_d, dissociation constant; K_I, inhibition constant; LSD, lysergic acid diethylamide; ng, 10^{-9} gram; nM, 10^{-9}

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molar; PD, pharmacodynamics; PET, positron emission tomography; PK, pharmacokinetics

REFERENCES

(1) Department of Health and Human Services, Food and Drug Administration. FDA's policy statement for the development of new stereoisomeric drugs. *Fed. Regist.* **1992**, *57*, No. 22249.

(2) Srinivas, N. R.; Barbhaiya, R. H.; Midha, K. K. Enantiomeric Drug Development: Issues, Considerations and Regulatory Requirements. *J. Pharm. Sci.* **2001**, *90*, 1205−1215.

(3) Perrault, G.; Depoortere, R.; Morel, E.; Sanger, D. J.; Scatton, B. Psychopharmacological profile of amisulpride: an antipsychotic drug with presynaptic D2/D3 dopamine receptor antagonist activity and limbic selectivity. *J. Pharmacol. Exp. Ther.* **1997**, *280*, 73−82.

(4) Racagni, G.; Canonico, P. L.; Ravizza, L.; Pani, L.; Amore, M. Consensus on the use of substituted benzamides in psychiatric patients. *Neuropsychobiology* **2004**, *50*, 134−143.

(5) Kim, S. W.; Shin, I. S.; Kim, J. M.; Lee, S. H.; Lee, J. H.; Yoon, B. H.; Yang, S. J.; Hwang, M. Y.; Yoon, J. S. Amisulpride versus risperidone in the treatment of depression in patients with schizophrenia: a randomized, open-label, controlled trial. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2007**, *31*, 1504−1509.

(6) Montgomery, S. A. Dopaminergic deficit and the role of amisulpride in the treatment of mood disorders. *Int. Clin. Psychopharmacol.* **2002**, *17*, S9−S15 discussion S16−S17 .

(7) Pani, L.; Gessa, G. L. The substituted benzamides and their clinical potential on dysthymia and on the negative symptoms of schizophrenia. *Mol. Psychiatry* **2002**, *7*, 247−253.

(8) Abbas, A. I.; Hedlund, P. B.; Huang, X.-P.; Tran, T. B.; Meltzer, H. Y.; Roth, B. L. Amisulpride is a potent 5-HT_7 antagonist: relevance for antidepressant actions in vivo. *Psychopharmacology* **2009**, *205*, 119−128.

(9) Anighoro, A.; Bajorath, J.; Rastelli, G. Polypharmacology: Challenges and Opportunities in Drug Discovery. *J. Med. Chem.* **2014**, *57*, 7874−7887.

(10) Castelli, M. P.; Mocci, I.; Sanna, A. M.; Gessa, L.; Pani, L. (-)S amisulpride binds with high affinity to cloned dopamine D_3 and D_2 receptors. *Eur. J. Pharmacol.* **2001**, *432*, 143−147.

(11) Neill, J.; Grayson, B.; Podda, J.; Cadinu, D.; Beconi, M.; Grattan, V.; Hixon, M. S.; Prensky, Z.; Vaino, A. Pre-clinical evaluation of two novel benzamides, LB-102 and LB-103, for the treatment of schizophrenia. 30th ECNP Congress, Paris, France (9/2017) (Poster online at http://lbpharma.us/wp-content/uploads/2018/05/ECNP-LB_poster_FINAL.09.03.17-1.pdf).

(12) Hixon, M. S.; Vaino, A. R.; Prensky, Z.; Grattan, V. Establishing a PK-PD-E Relationship for Clinical Candidate LB-102, a Next-Generation Benzamide Antipsychotic. Society of Biological Psychiatry 73rd meeting New York, NY (05/2018) (Poster online at http://lbpharma.us/wp-content/uploads/2018/05/LB-102-PK-PD-E-final-poster-SOBP-May-2018.compressed.pdf).

(13) CODEX BIOSOLUTIONS 401 Professional Drive | Suite 160 | Gaithersburg, MD 20879. http://www.codexbiosolutions.com/.

(14) Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibition constant (K_I) and the concentration of inhibitor which cause 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

(15) la Fougère, C.; Meisenzahl, E.; Schmitt, G.; Stauss, J.; Frodl, T.; Klaus, T.; Klaus, H.; Möller, H.-J.; Dresel, S. D_2 Receptor Occupancy During High- and Low- Dose Therapy with the Atypical Antipsychotic Amisulpride: A ^{123}I-Iodobenzamide SPECT Study. *J. Nucl. Med.* **2005**, *46*, 1028−1033.

(16) Rosenzweig, P.; Canal, M.; Patat, A.; Bergougan, L.; Zieleniuk, I.; Bianchetti, G. A review of the pharmacokinetics, tolerability and pharmacodynamics of amisulpride in healthy volunteers. *Hum. Psychopharmacol.* **2002**, *17*, 1−13.

(17) Natesan, S.; Reckless, G. E.; Barlow, K. B. L.; Nobrega, J. N.; Kapur, S. Amisulpride the "atypical" atypical antipsychotic − Comparison to haloperidol, risperidone and clozapine. *Schizophr. Res.* **2008**, *105*, 224−235.

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