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The design and synthesis of potent, selective benzodiazepine sulfonamide bombesin receptor subtype 3 (BRS-3) agonists with an increased barrier of atropisomerization

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ABSTRACT

Bombesin receptor subtype 3 (BRS-3) is an orphan G-protein coupled receptor expressed primarily in the hypothalamus which plays a role in the onset of both diabetes and obesity. We report herein our progress made towards identifying a potent, selective bombesin receptor subtype-3 (BRS-3) agonist related to the previously described MK-7725[1] Chobanian et al. (2012) that would prevent atropisomerization through the increase of steric bulk at the C-2 position. This would thereby make clinical development of this class of compounds more cost effective by inhibiting racemization which can occur over long periods of time at room/elevated temperature.

1. Introduction

Obesity is a serious and chronic medical condition that has become a major global health issue. In the United States alone, obesity rates for adults have doubled and rates for children have tripled over the last 30 years. Currently, around one third of U.S. adults and 16% of U.S. children are considered obese.[2] In many cases, the excessive body weight is the root cause of subsequent co-morbidities, including type 2 diabetes, hypertension, cardiovascular disease, cancer, and arthritis. Our laboratories became interested in the bombesin receptor subtype-3 (BRS-3) receptor because of its implication in energy homeostasis and weight loss. Mice lacking functional BRS-3 develop metabolic defects and obesity.[3,4]

Recently, we disclosed studies which focused on a novel class of benzodiazepine sulfonamides BRS-3 agonists. This class of molecules was shown to be potent and selective but lacked the pharmacokinetic profile necessary for further development. In addition, pregnane X receptor (PXR) activation also was a major liability that ultimately needed to be addressed as well.[5] In turn, our laboratories discovered MK-7725[1] which provided the preclinical pharmacokinetic properties desired for a development compound. The last major hurdle for development of MK-7725 and its related compounds is the atropisomerism that plagues this particular class due to its unusual planar chirality. Any appreciable isomerization to the inactive enantiomer can be a major roadblock by lowering the desired exposure of MK-7725 and thus affecting the clinically efficacious dose.

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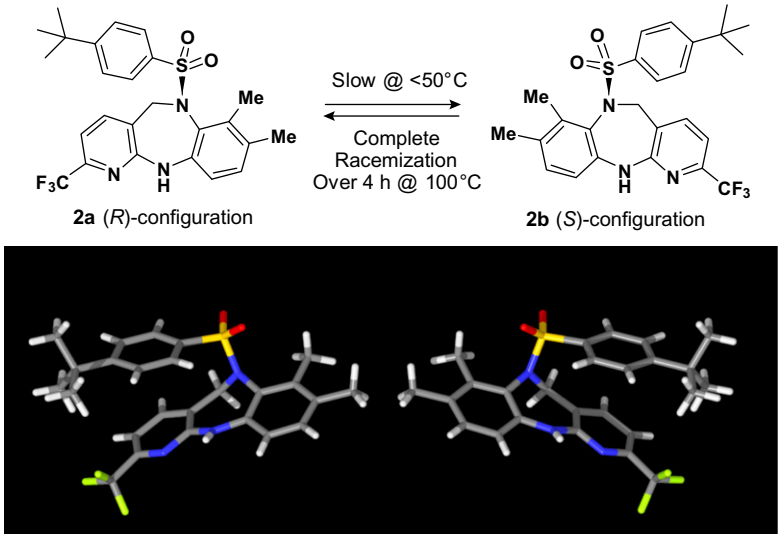


Figure 1. X-ray structure showing atropisomerism of benzodiazepine sulfonamides.

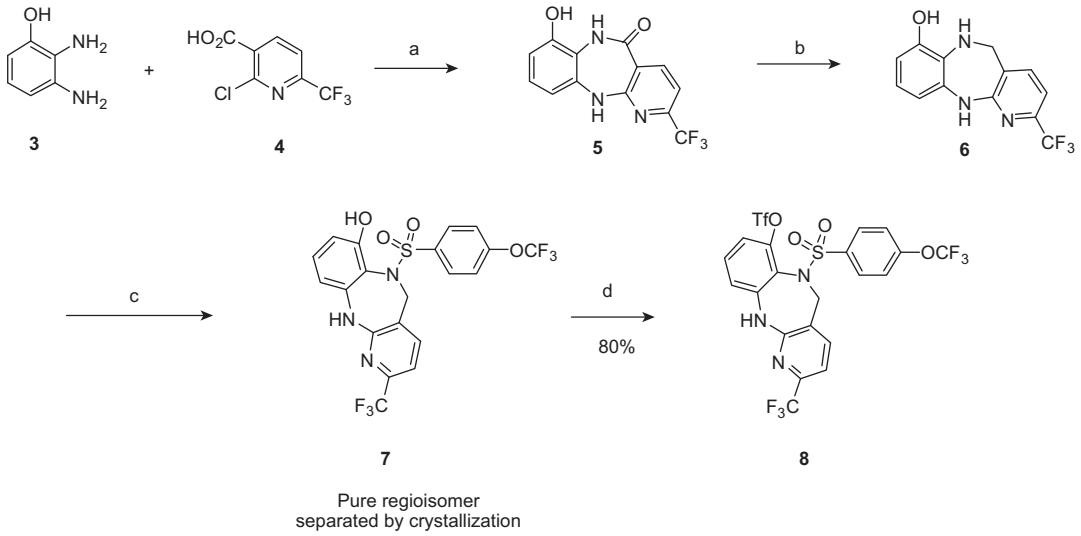
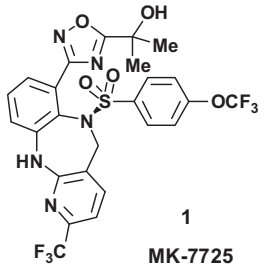


Figure 2. Synthesis of triflate intermediate. Reagents and conditions: (a) Sulfolane, 150 °C (87%); (b) BH_3·THF, THF (61%); (c) 4-trifluoromethoxyphenylsulfonyl chloride, pyridine (54%); (d) Tf_2O, DCM, TEA (80%).



1

MK-7725

hBRS-3 IC_{50} 3 nM (100% act)
mBRS-3 EC_{50} 22 nM (105% act)

The atropisomerism present with this class of molecules is specific to the conformationally restrained seven-membered diazepine central ring system. Molecular modeling on similar systems has suggested a barrier of inversion of ~33 kcal/mol (Fig. 1). Typically complete interconversion can be seen after 4 h at 100 °C.[5] While this can be dealt with in preclinical development, it can pose a problem for long term thermal stability. The major issue posed is that the *S*-enantiomer is the active enantiomer while the *R*-enantiomer is significantly less active on the BRS-3 receptor. We decided to embark on the synthesis on novel analogs which could significantly raise the barrier to atropisomerism.

In collaboration with our colleagues in the analytical chemistry group, we had previously shown that the use of CD spectroscopy as a tool for measuring the $t_{1/2}$ for this class of compounds could be done in an effective manner. The effect of structure of the benzodiazepine sulfonamides could be ascertained very quickly and would lead us to understand exactly what needed to be done to further increase this barrier. Ideally, we would like to increase this barrier substantially while further modifying a very sterically hindered position of the benzodiazepine sulfonamide. We began to synthesize a series of new analogs that would be further evaluated by CD spectroscopy.[6]

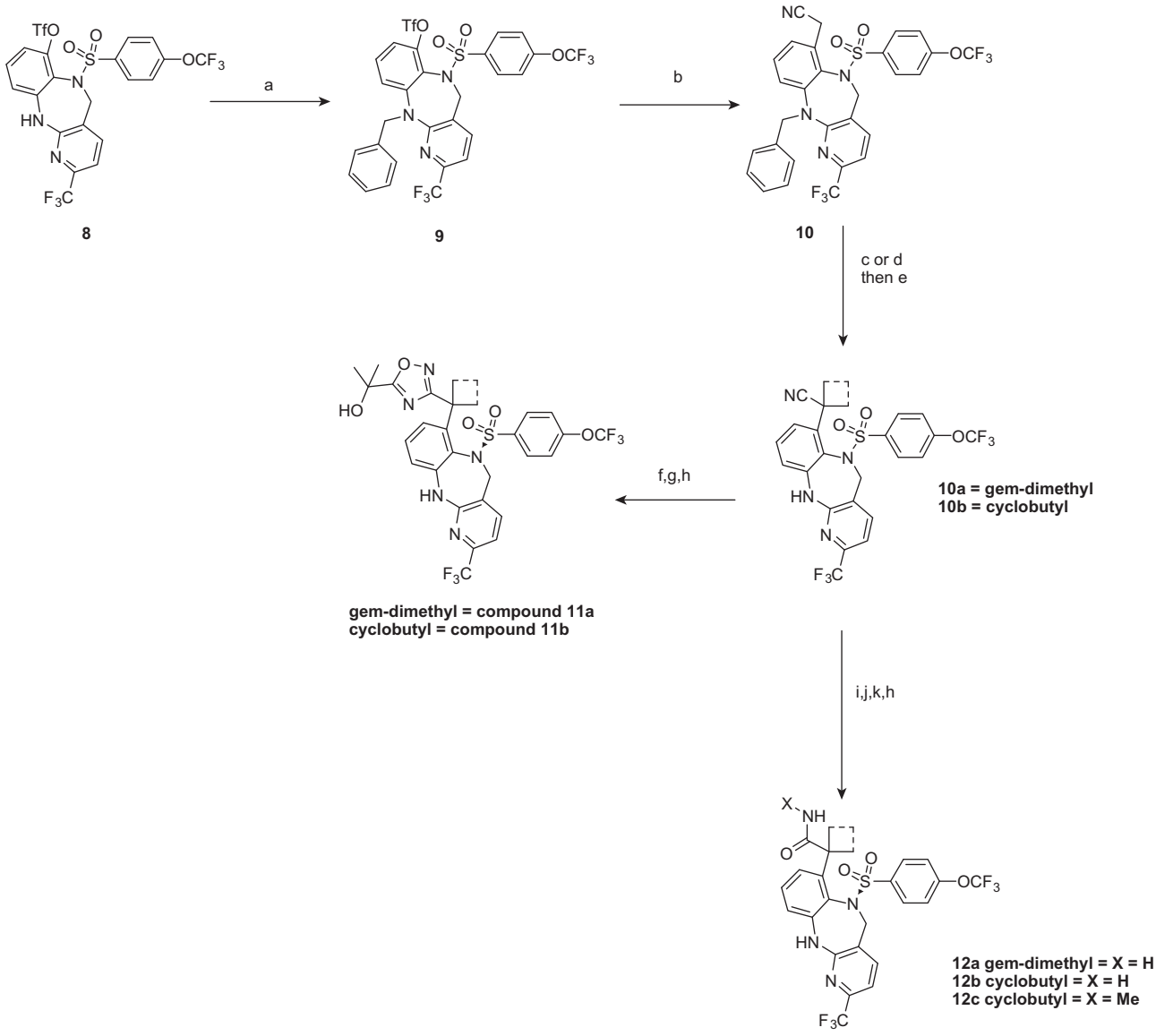


Figure 3. Synthesis of oxadiazole analogs. Reagents and conditions: (a) BnBr, NaOH (50% v/v), benzyltriethylammonium chloride, toluene, 120 °C (75%); (b) $TMSCH_2CN$, ZnF_2, Pd_2dba_3, S-Phos, DMF, 180 °C (68%); (c) MeI, NaH, THF (91%); (d) diiodopropane, NaH, THF (66%); (e) TMSI, ACN (70%); (f) NH_2OH, K_2CO_3, EtOH (100%); (g) acetoxyisobutyryl chloride, pyridine, 80 °C, then K_2CO_3, MeOH (30–52% overall); (h) Chiral separation (Chiralpak OD, EtOH/hexane) (i) DIBAl-H, THF, (72%); (j) $NaClO_2$, NaH_2PO_4, 2-Me-2-butene, *t*BuOH/H_2O (64–93%); (k) X-NH_2 (X = H or Me), EDC, HOBt, DCM (80–92%).

Table 1
Binding and functional activity at human and mouse BRS-3

Compound	Human BRS-3 binding[12] (IC_{50}, nM)	Human BRS-3 agonism[a13] (EC_{50}, nM) (activity %)	Mouse BRS-3 agonism[a] (EC_{50}, nM) (activity %)
MK-7725	3.6	73 (106)	130 ± 89 (105)
11a	1.4 ± 0.3	64 ± 12 (97)	67 ± 9 (105)
11b	0.6 ± 0.3	82 ± 32 (97)	76 ± 13 (99)
12a	0.5 ± 0.1	26 ± 8 (90)	27 ± 3 (98)
12b	0.5 ± 0.3	21 ± 7 (95)	22 ± 7 (100)
12c	2.1 ± 0.6	15 ± 5 (106)	22 ± 5 (112)
13	10	31 (96)	11 ± (87)

[a] % Act represents the maximum activation of tested compound relative to that of the dY peptide.

2. Chemistry

The synthesis of the key triflate intermediate of interest commenced with a condensation of 2-chloro-6-trifluoromethyl nicotinic acid (**4**) and 2,3-diaminophenol (**3**) under thermal conditions giving rise to a regioisomeric ratio of the phenol (1:1) which could be separated out by crystallization following subsequent borane reduction and sulfonylation. The pure regioisomer **7**, isolated via crystallization was then treated with trifluoromethanesulfonic anhydride giving rise to the corresponding triflate **8**.[6]

Table 2
Rat PK data for BRS-3 agonists[14]

Compound	Clp (mL/min/kg)	$T_{1/2}$ (h)	PO AUC_N (μM h kg/mg)	F_{oral} (%)
MK-7725	16	11	1.60	93
11a	12	3.1	2.60	100
11b	21	2.9	0.60	49
12a	33	1.6	0.30	31
12b	31	0.8	0.30	32
12c	25	1.7	0.69	60

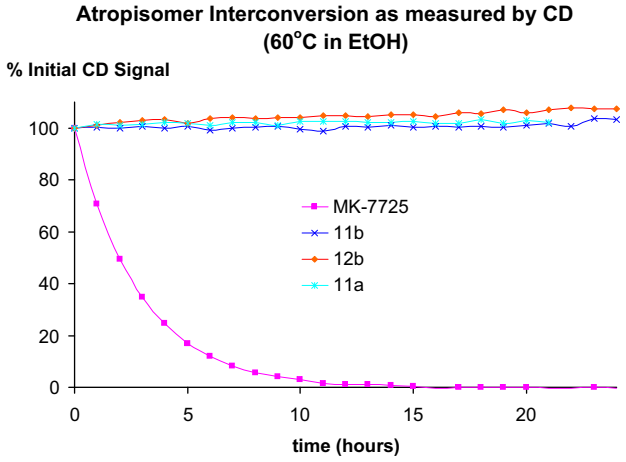


Figure 4. CD spectra for specific analogs of interest.

At this point, our goal was to introduce a bulky substituent alpha to the oxadiazole ring present in MK-7725 (Fig. 2). With the desired triflate **8** in hand, we sought to protect the free amine present. To accomplish this, we chose phase transfer conditions using benzyl bromide and benzyltriethylammonium chloride with 50% sodium hydroxide in toluene. Upon heating to reflux, the desired benzyl protected intermediate was isolated. Triflate **9** was subjected to a modified palladium catalyzed coupling of trimethylsilylacetonitrile using the S-Phos ligand.[7,8] The resulting acetonitrile derivative **10** was treated with NaH followed by either MeI or diiodopropane giving rise to the respective gem-dimethyl nitrile (**10a**) or the cyclobutylnitrile (**10b**) derivative. Upon surveying a host of debenzylation conditions, the most reproducible conditions were using TMS-I in acetonitrile. The debenzylated acetonitrile derivative was converted to the subsequent amide oxime, acylated with acetoxyisobutyryl chloride, and cyclized thermally to furnish the desired 1,2,4-oxadiazole. Compounds **11a** and **11b** were obtained in enantiopure form after deacetylation with K_2CO_3/MeOH and subsequent chiral HPLC resolution (Fig. 3).[9,10]

Primary and secondary amide derivatives were prepared via a three-step sequence consisting of DIBAl-H reduction, Pinnick oxidation,[11] and EDC coupling. Enantiomerically pure compounds **12a**, **12b**, and **12c** were then obtained by chiral HPLC resolution.

Compounds **11a** and **11b** both possessed excellent binding potency (hIC_{50} = 1.4 and 0.6 nM) and both had very good mouse functional activity (mEC_{50} = 67 and 76 nM) (Table 1). In comparison to **MK-7725**, both compounds show a several fold improvement in human binding potency in the in vitro assay. Simple amides such as compounds **12b** and **12c** showed exquisite potency (hIC_{50} = 0.5 and 2.1 nM). In comparison to its truncated primary amide cousin, compound **13**, the intrinsic binding potency was improved by 5- to 10-fold. This would indicate not only was the space of importance as a possible means to increase the barrier of inversion for the atropisomerism that exists with MK-7725, but is also a source of a potency boost. The secondary amide **12a** also showed excellent in vitro potency across species making it a viable candidate for further profiling.

These exciting in vitro results prompted a comparative PK study of **11a**, **11b**, **12a–c**, and **MK-7725** (Table 2). One can surmise that the linker in compounds **11a** and **11b** seems to cut the half-life by ~fourfold, however the exposure and bioavailability remain excellent in only the case of compound **11a**. Compound **11b** has lower oral exposure (0.60 μM h kg/mg) and comparatively worse bioavailability (49%) than oxadiazole **11a**. This could be due to the decreased measured aqueous solubility of compound **11b** versus **11a**. Of the amides **12a–c**, each had comparable PK in the rat. With all this information at hand, we now set out on our ultimate goal to determine whether any of these compounds would be improved inherently with regards to the atropisomerism issue.

CD spectroscopy is a useful tool for monitoring atropisomer equilibration particularly for compounds which have chromophores. In order to achieve this, a timecourse CD spectrum of pure MK-7725 and its respective enantiomer are subjected to a CD scan. Once the basal levels of absorption are realized, a time course experiment can be performed whereby MK-7725 is heated to 60 °C in EtOH over a 3 day period. The respective data obtained at the 260 nm wavelength is plotted versus time in order to arrive at a half-life calculation for the interconversion. In the case of MK-7725, the $t_{1/2}$ was calculated to 2.2 h. However, once we began to examine novel derivatives such as **11a**, **11b**, and **12b** an interesting trend developed (Fig. 4). All three compounds possessed a half-life greater than 250 h. This represented a major improvement and makes subsequent development of these particular chemical entities significantly less complex and more cost-effective.

3. Conclusions

In summary, we were able to solve a long standing atropisomerism problem with the benzodiazepine sulfonamides. This increase in the barrier of atropisomerism would allow for more rapid development of a solid, stable crystalline form of these compounds. In addition, any racemization at room temperature could be avoided by building in a linker at the alpha position. The synthetic complexity was increased but with the added benefit of a >25-fold increase in the barrier of inversion. In addition, to our surprise, these analogs generally maintained or improved upon existing potency values without a significant change in rat PK. Follow up studies will be reported in due course.

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12. For human BRS-3 binding assays, 1–4 μg of membrane protein obtained from NFAT-CHO cells expressing the receptor were incubated with 0.3 pM [^{125}I]-[D-Tyr^{6}, β-Ala^{11}, Phe^{13}, Nle^{14}]-bombesin (6–14) (^{125}I-dY-peptide) and various concentrations of test compounds in 200 μL of binding buffer (50 mM Tris, pH 7.2, 5 mM $MgCl_2$, 0.1% BSA). After 2 h incubation at room temperature, the binding reaction was terminated by filtering through a GF/c filter and washing the filter with PBS using a Packard 96-well Harvester. The amount of radioligand bound to the receptor was measured by liquid scintillation counting of the radioactivity on the filter. The nonspecific binding was defined as the binding in the presence of 100 nM unlabeled dY-bombesin. The data, as % inhibition of binding, was plotted vs. the log molar concentration of receptor ligand (compound). The IC_{50} was reported as the inflection point of the resulting sigmoidal curve.
13. The functional assay is an aequorin bioluminescence assay. It was performed in a 96-well format using a Wallac Microbeta luminometer equipped with microinjector module. Compounds in DMSO (0.5% final concentration) were titrated in the plates at 2 × concentration in a volume of 0.1 mL ECB buffer (20 mM HEPES, pH 7.4, 140 mM NaCl, 20 mM KCl, 1 mM $MgCl_2$, 1 mM CaCl, 5 mM glucose, 0.1 mg/ml BSA). The HEK293AEQ cells from lines expressing either human, rat or mouse BRS3 (20,000 per well) were charged with coelenterazine (Molecular Probes) and then injected in 0.1 mL ECB buffer into the compound containing wells. The bioluminescence was monitored for 30 s, or alternatively, total bioluminescence was determined over 10 min. The bioluminescent readings were plotted versus the log molar concentration of receptor ligands (compounds). The EC_{50} for activation was reported as the inflection point of the resulting sigmoidal curve. The percentages of activation are the maxim activations of tested compounds relative to that of dY-peptide.
14. Sprague Dawley (SD) rat was used. Plasma clearance (Clp) and half life ($T_{1/2}$) calculated following 0.5 mg/kg iv dose. Normalized oral exposure (PO AUCN) and oral bioavailability (F%) calculated following 1 mg/kg po dose.