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Identification of a 5-HT$_4$ receptor antagonist clinical candidate through side-chain modification

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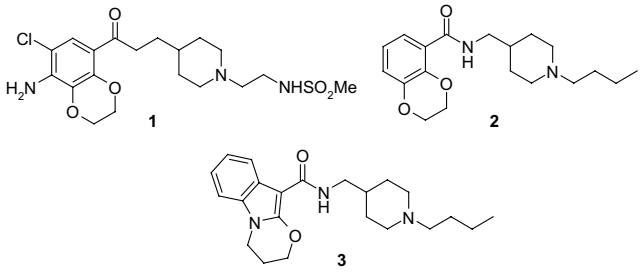
Abstract—Replacement of the *N*-butyl side-chain of lead 5-HT$_4$ receptor antagonist **2** with propanesulfonylpiperidinyl, morpholinyl, and piperazinyl groups led to higher affinity analogs **4**–**6**. In vitro drug metabolism screens and cassette pharmacokinetic studies in the dog led to identification of the *N*-methylpiperazinyl analog (**6b**), which displayed pharmacokinetic, selectivity, and safety parameters sufficient for advancement to the clinic for the treatment of urinary incontinence.

Advances in lead identification methodologies (medium and high-throughput screening, molecular modeling, etc.) have in many cases shifted the bottle neck in drug discovery to the lead-optimization stage. High-affinity leads are commonly identified early in medicinal chemistry programs; however, the achievement of acceptable pharmacokinetic parameters, in particular oral bioavailability, remains an often elusive and time-consuming goal. Herein we describe the optimization of pharmacokinetic properties through modification of side-chain structure in a series of 5-HT$_4$ receptor antagonists, which allowed clinical candidate selection in a timely manner.

The structure–activity relationships and potential clinical utility of 5-HT$_4$ receptor antagonists have been reviewed.[1] Our primary interest was in developing an agent for the treatment of urinary incontinence (unstable bladder) on the basis of the apparent relationship between 5-HT$_4$ receptor-mediated release of acetylcholine in detrusor smooth muscle and bladder contractility.[2,3]

Our initial clinical candidate, the aryl ketone sulamserod (**1**),[3] demonstrated good oral pharmacokinetics and safety in Phase 1 clinical trials in man. However, due to an unacceptable toxicity finding in the long-term safety studies in the dog, which could not be easily monitored in humans, further development of sulamserod was not pursued. The search for a 'second generation' clinical candidate was centered on a related series based on the lead benzodioxane carboxamide **2**. The related compound **3** (SB-207266, piboserod),[4] has been studied in the clinic for the treatment of irritable bowel syndrome,[5] and atrial fibrillation.[6]



Although compound **2** had high 5-HT$_4$ receptor affinity (pK_i 9.1), its generic (nonproprietary) structure, coupled with poor pharmacokinetics ($t_{1/2}$ < 1 h, 18% F in the rat)

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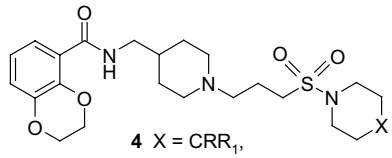
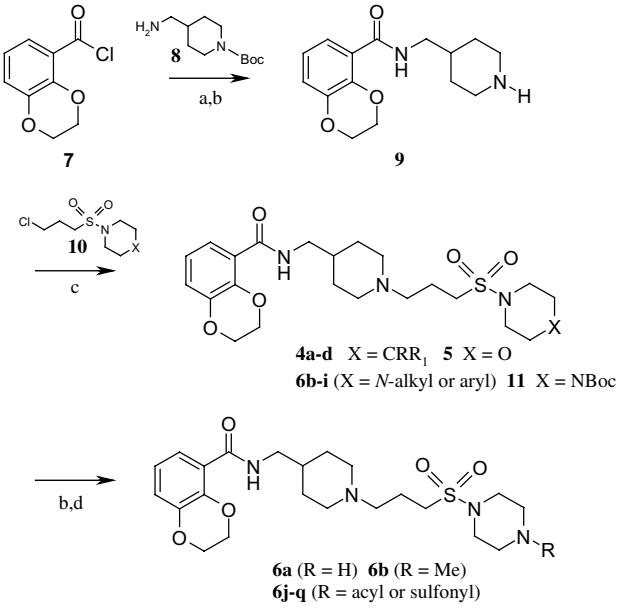
dictated structural modification. Previous work had demonstrated steric tolerance in the piperidine *N*-substituent of **1**;[3] hence, this was an attractive position for modification. A series of compounds **4–6** was selected based on novelty, synthetic accessibility, and opportunity for variation in the pendant piperidine and piperazine rings.



The series of compounds **4–6** were prepared as described in Scheme 1.[7] Treatment of 1,4-benzodioxan-5-carbonyl chloride (**7**) with amine **8**, followed by deprotection with TFA afforded intermediate **9**. Alkylation with chlorides **10** (prepared by reaction of commercially available 3-chloropropanesulfonyl chloride with the requisite piperidine, morpholine, *N*-alkylpiperazine, or Boc–piperazine) furnished final compounds **4a–d**, **5**, and **6b–i**, as well as intermediate **11**. Deprotection of the latter to **6a** followed by derivatization of the piperazine nitrogen gave analogues **6j–q**. Analog **6b** was more efficiently prepared by reductive alkylation of **6a** with formaldehyde.

Binding affinity for the human cloned 5-HT_4 receptor, dog and human liver microsomal degradation rates, and dog pharmacokinetic parameters are presented in Table 1. All analogs demonstrated high binding affinity



Scheme 1. Reagents and conditions: (a) **8**, TEA, dichloromethane; (b) TFA, dichloromethane; (c) TEA, DMF, 80 °C; (d) for **6b**: CH_2O, H_2, Pd–C, ethanol; for **6j–q**: acyl or sulfonyl halide, TEA, dichloromethane.

with a general trend toward higher binding with increased size/lipophilicity of the piperidine and piperazine 4-substituents, culminating in the cyclopentyl derivatives **4d** and **6g** with binding constants of 10.6 and 10.8, respectively. However, there was a trend toward decreased microsomal stability for these more lipophilic analogues.

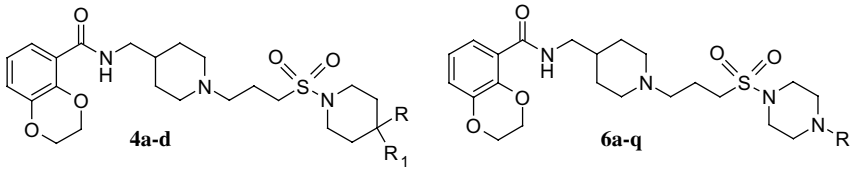
In addition to the liver microsomal data, pharmacokinetic parameters determined using cassette dosing in the dog were used to identify the most promising compounds for further evaluation. Under the cassette screening protocol, 7–12 compounds were coadministered orally or intravenously to two to four dogs per dose route in a parallel study design. Each dog received 0.25–0.5 mg/kg/compound for a combined drug load of $\leqslant$6 mg/kg/study. Serial blood samples were collected through 24 h post dose from each animal and the resulting plasma for each dose route was pooled by time point prior to quantitative analysis by LC–MS/MS. Pharmacokinetic parameters were determined using noncompartmental analysis. Selected parameters are presented in Table 1. Under this protocol, many compounds demonstrated good estimated oral bioavailability (%F >50% in 12 of 17 shown) even though a wide range of in vivo clearances were observed. The main differentiating features were related to differences in persistence and absolute systemic exposure (as shown with parameters $t_{1/2}$ and Vz).

By using the in vitro–in vivo screening cascade during the optimization process, a total of 74 compounds (not all shown and some from a related series) were rapidly screened for metabolic stability. Of these, roughly half (47) advanced to the cassette PK testing in the dog which identified nine compounds for advancement into conventional, more time-consuming pharmacokinetic studies.

On the basis of the totality of the data, the two closely related analogues **6b–c** appeared to have the most promise and of these, the *N*-methyl derivative **6b** was chosen for further development. This decision was based primarily on the lower clearance and longer half life of **6b** relative to **6c**.

When examined in a conventional pharmacokinetic study in the dog (single-compound iv/po cross-over, extended to a 72 h collection period), **6b** demonstrated a multiphasic disposition with an initial distribution phase of approximately 7 h. The inclusion of the late post-dose time points lead to the improved ability to calculate the terminal elimination $t_{1/2}$ (18 h). Mean oral bioavailability ($n = 4$) was 75 %F. When compared to the cassette dosing regimen, the plasma half life was similar if calculated within the same time intervals but the shorter collection time used in the screening protocol missed the terminal elimination phase. Additionally, oral bioavailability, though ranked as good, was underestimated. A similar underestimate of the $t_{1/2}$ was observed for the *N*-acetyl and *N*-methanesulfonyl analogs **6j** ($t_{1/2}$ 4.6 h) and **6m** ($t_{1/2}$ 3.6 h), respectively, upon oral administration to the dog under the conventional protocol. Thus

Table 1. SAR of 5-HT$_4$ receptor ligands **4–6**



	R	R_1	Mp (°C)[a]	pK_i[b]	dLM[c]	hLM[d]	DogPK				
							n[e]	CL[f]	Vz[g]	$t_{1/2}$ (h)[h]	%F
4a	H	H	165–168	9.7	160	14	12	13	0.5	0.5	87
4b	Me	Me	107–108	9.9	440	110	7	27	1.5	0.7	100
4c	H	*n*-Pr	124–125	10.2	460	101	7	33	1.4	0.5	15
4d	Spirocyclopentyl		160–165	10.6	405	250	—	—	—	—	—
5			192–195	9.4	16	2	12	7	0.6	1.0	76
6a	H		92–94	9.8	2	2	8	11	34	37	33
6b	Me		186–189	9.6	20	3	12	4	2.2	6.6	46
6c	Et		220–224	10.3	13	6	7	13	3.6	3.1	94
6d	*n*-Pr		243–245	10.5	110	0	7	23	5.7	2.8	9
6e	*i*-Pr		233–236	10.5	22	17	7	14	3.6	3.0	72
6f	*i*-Bu		271–272	10.6	65	12	—	—	—	—	—
6g	Cyclopentyl		245–256	10.8	53	22	8	37	9.3	2.9	42
6h	4-F-phenyl		220–221	10.3	210	55	7	27	1.5	0.7	44
6i	2-Pyrimidinyl		173–177[i]	9.6	90	42	7	5	0.3	0.7	65
6j	Acetyl		204–208	9.9	56	39	7	6	0.7	1.3	51
6k	2-Furoyl		148–150	10.0	50	11	8	2	0.2	1.0	64
61	3-Picolinyl		>300	9.8	3	7	—	—	—	—	—
6m	Methylsulfonyl		236–237	9.5	18	3	7	3	0.3	1.2	90
6n	*n*-Propylsulfonyl		201–202	10.0	68	34	7	12	0.7	0.7	82
6o	*i*-Propylsulfonyl		165–170	9.9	3	7	—	—	—	—	—
6p	4-F-phenylsulfonyl		185–186	10.5	51	93	—	—	—	—	—
6q	1-Pyrolidinylsulfonyl		165–168	10.1	39	36	7	15	0.8	1.0	54
3				9.6	4	5	12	8	5.0	7.1	71

[a] Melting point measured on a hot-stage apparatus of the hydrochloride (**4a–d**, **5**, **6j–q**) or dihydrochloride salt (**6a–h**) unless otherwise noted.
[b] Determined in human cloned 5-HT$_4$ receptors. Mean of two determinations.
[c] Intrinsic clearance (μL/min/mg protein) in dog liver microsomes.
[d] Intrinsic clearance (μL/min/mg protein) in human liver microsomes.
[e] Number of compounds dosed in cassette to the dog. Dose of each compound was 0.25 mg/kg iv or 0.5 mg/kg po.
[f] Plasma clearance (mL/min/kg).
[g] Volume of distribution (L/kg).
[h] Half-life upon iv administration.
[i] Free base.

our experience confirms that cassette dosing is useful for efficient ranking of related compounds rather than affording absolute pharmacokinetic data. The latter should be obtained from subsequent single-compound studies.

Compound **6b** demonstrated good exposure and prolonged $t_{1/2}$ in other species, including the mouse ($t_{1/2}$ 7 h), rat ($t_{1/2}$ 12 h) and mini-pig ($t_{1/2}$ 21 h). The result in the rat was a dramatic improvement over the original lead compound **2**, which had a $t_{1/2}$ of less than 1 h. Allometric scaling predicted a $t_{1/2}$ of 60 h in man. Metabolism of **6b** to **6a** was observed in vivo, although in higher species such as the dog this was a relatively minor phenomenon (ratio of AUC of **6a** to **6b** = 0.2 following an oral dose).[8]

In the rat isolated esophagus,[9] **6b** was devoid of 5-HT$_4$ receptor agonist activity and was an antagonist with pK_b of 8.95. 5-HT$_4$ receptor antagonist activity was further confirmed by inhibition of 5-HT-induced tachycardia in the anesthetized vagotomized mini-pig[9] (ID_{50} 1.2 μg/kg, iv and 52μg/kg, id).

Profiling against standard receptor, ion channel, and uptake system panels indicated that **6b** was extremely selective (IC_{50} > 1 μM)[10] with the exception of affinity for the central histamine H_1 receptor where an IC_{50} of 100 nM was observed. In theory an antagonist at the H_1 receptor could induce sedation if the antagonist crosses the blood–brain barrier. However, **6b** did not appear to penetrate the CNS in the rat (as determined by quantitative whole body autoradiography) and sedation was not observed in any species examined, including man. The safety profile (CYP inhibition, dog Purkinje fiber, Ames test, etc.) of **6b** was also unexceptional, as were toxicology studies in the rat and dog; hence this compound was chosen for clinical study in man.

In Phase I clinical trial, **6b** was found to have good apparent oral bioavailability with a steady state plasma $t_{1/2}$ of >100 h. Thus the general pharmacokinetic properties of the molecule targeted by the preclinical lead optimization effort (high oral bioavailability, suitable systemic persistence to allow once-daily dosing) were confirmed in the clinic.

In summary, modification of the side-chain of the initial lead compound **2** led to 5-HT_4 antagonists with increased affinity and dramatically improved pharmacokinetic properties. Cassette pharmacokinetic dosing was used in concert with microsomal stability studies to rapidly differentiate between numerous high-affinity compounds and led to the selection of **6b** as a clinical candidate. Results of a Phase IIa study for the treatment of urinary frequency and incontinence in women with overactive bladder will be reported in due course.

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8. The relatively low basicity (measured pK_a of 6.6) of the methyl substituted piperazine nitrogen in **6b** (compared to the tertiary piperidine nitrogen with a pK_a of 8.5) could be a contributing factor to the low observed rate of *N*-demethylation.
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10. Receptors included: adenosine, alpha and beta adrenergic, bradykinin, NE uptake, angiotensin-1, benzodiazepine, dopaminergic, dopamine uptake, endothelin, GABA, NMDA, muscarinic, neurokinin, NPY, opiate, sigma, other serotonergic, and serotonin uptake.