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Discovery, oral pharmacokinetics and in vivo efficacy of a highly selective 5-HT$_4$ receptor agonist: Clinical compound TD-2749

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ABSTRACT

Further application of our multivalent approach to drug discovery directed to 5-HT$_4$ receptor agonists is described. Optimization of the linker and secondary binding amine in the indazole-tropane primary binding group series, for binding affinity and functional potency at the 5-HT$_4$ receptor, selectivity over the 5-HT$_3$ receptor, oral pharmacokinetics, and in vivo efficacy in models of GI motility, resulted in the identification of clinical compound TD-2749.

The 5-HT$_4$ receptor belongs to the superfamily of seven-transmembrane G protein-coupled receptors (GPCRs) and is an attractive target for drug discovery due to its potential role in many central and peripherally mediated disorders (irritable bowel syndrome,[1] gastroparesis,[2] Alzheimer's disease,[3] arrhythmia[4]). In particular, activation of the 5-HT$_4$ receptor has been demonstrated both preclinically and clinically to promote gastrointestinal (GI) motility. The peristaltic reflex describes the synchronized contraction and relaxation of GI smooth muscle resulting in the propulsion of luminal contents and is dependent on increases in neurotransmission due to 5-HT$_4$ receptor activation on intrinsic primary afferent neurons, interneurons and motor neurons.[5] In addition, agonism of 5-HT$_4$ receptors on colonic circular smooth muscle cells and on enterocytes promotes a direct relaxant effect and fluid secretion, respectively, further facilitating GI transit.[6]

Three 5-HT$_4$ receptor agonists have been approved for the treatment of GI functional disorders, Figure 1. Cisapride (Propulsid®) (**1**) and tegaserod (Zelnorm®) (**2**) were frequently prescribed to treat upper and lower GI disorders of reduced motility, respectively, although their clinical efficacy in many patients was possibly reduced due to significant activity at other 5-HT receptor subtypes.[7] Cisapride (**1**) is associated with an increased risk of mortality as a consequence of inhibition of the human ether-a-go-go-related gene (hERG) potassium ion channel.[8] Tegaserod (**2**) is also associated with cardiovascular concerns, potentially as a result of its interactions at non-5-HT$_4$ serotonergic receptors.[9] However, two recent epidemiological studies failed to support the purported increased risk of serious ischemic cardiovascular events with tegaserod.[10] Marketing in the US of both cisapride (**1**) and tegaserod (**2**) has been suspended although the clinical prescription of both drugs is now possible, but highly restricted.[11]

Drug discovery efforts to identify novel selective 5-HT$_4$ receptor agonists have focused on approaches to improve both clinical efficacy and patient safety.[12] Prucalopride (Resolor®) (**3**) was recently approved in Europe for the treatment of women with chronic idiopathic constipation who have not responded adequately to laxatives and represents a next generation, highly selective 5-HT$_4$ receptor agonist.[13] We recently reported our multivalent approach to the design and discovery of selective 5-HT$_4$ receptor agonists such as THRX-194556 (**4**), Figure 2.[14] Key to the strategy was the exploration of primary and secondary binding groups and the connecting linker, as represented by the proposed 5-HT$_4$ receptor agonist pharmacophore shown in Figure 2. From the initial screening study, combining the quinolone-tropane primary binding group of THRX-194556 (**4**) with amine-based secondary binding groups afforded high affinity 5-HT$_4$ receptor agonists, with high selectivity over the 5-HT$_3$ receptor, somewhat independent of linker length and composition. Additional optimization of the amine-based secondary binding group and the linker focused on 'drug-likeness' features, specifically permeability in the Caco-2 assay, rat in vivo oral pharmacokinetic data and hERG potassium ion channel

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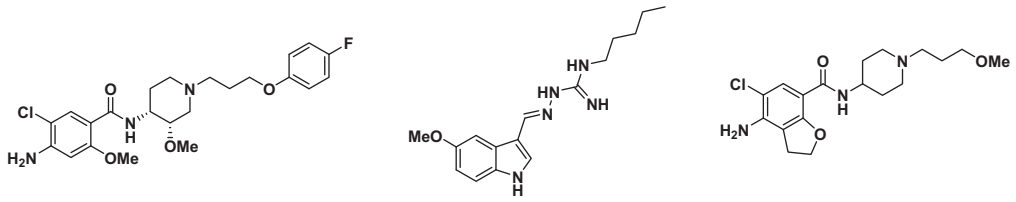


Figure 1. 5-HT_4 receptor agonists to have reached the market.

inhibition. It was found that the best balance of these properties was associated with a low pK_a of the secondary binding group resulting in identification of THRX-194556 (**4**).[15] As a further example of our multivalent approach to drug discovery[16] we herein describe the optimization and characterization of the indazole-tropane primary binding group to afford clinical compound TD-2749 (**14**).

Results and Discussion

Schemes 1–3 detail the preparation of the compounds described in this communication. From the previously reported initial screen the indazole-tropane primary binding group **5**, Scheme 1, exhibited high binding affinity (pK_i = 7.2) and functional potency (pEC_{50} = 7.4) along with high intrinsic activity (IA = 94% of the maximum response achieved by 5-HT in a cAMP accumulation assay) at the human recombinant 5-HT_4 receptor.[14] However, it also had high binding affinity for the human recombinant 5-HT_3 receptor (selectivity <1). A variety of amine derived secondary binding groups attached to the indazole-tropane core by either the previously optimized 2-propanol, or simple hexyl linkers, were first investigated, Table 1, Scheme 1.

As expected from the previously reported SAR with the quinolone-tropane primary binding group,[14] addition of the amine derived secondary binding groups to the indazole-tropane **5** increased both the binding affinity (pK_i >8.4) and the functional potency (pEC_{50} >7.9) at the 5-HT_4 receptor. The observed trend was that the binding affinity was highest for the hexyl linked compounds (**6**–**10b** vs **6**–**10a**, respectively) which correlated with the increased cpK_a1 and cpK_a2. This same trend was not apparent with regards to the functional potency of the compounds. In fact whilst all the compounds **6**–**10a**,**b** had higher binding affinity than the quinolone-tropane THRX-194556 (**4**), they all had lower functional potency. The selectivity of the compounds in this study over the 5-HT_3 receptor was very dependent on the linker. For each of the amine secondary binding groups attached via the 2-propanol linker (compounds **6**–**10a**) excellent selectivity of >3000-fold was observed. In contrast, the selectivity was <25-fold for all the hexyl-linked compounds **6**–**10b** and as a consequence these were not advanced to subsequent screening assays. Stability was high for all the compounds **6**–**10a** in the rat liver microsome (RLM) assay. The permeability (as measured by the Caco-2 assay) for the 2-propanol linked compounds **6**–**8a** and **10a** (K_p >19) compared favorably with the previously optimized THRX-194556 (**4**), (K_p = 14). However, for the primary sulfonamide substituted piperidine **9a** permeability was low (K_p = 1) possibly due to the H-bond donating character of this motif. Inhibition of the hERG potassium ion currents for compounds **6**–**8a** and **10a** was very dependent on the secondary binding amine although there did not appear to be a relationship between channel inhibition and cpK_a2. The piperidine containing compound **6a** exhibited 62% inhibition of the channel at 3 μM. The isopropyl-methylamine **7a** and 2-methoxypyrolidine **8a** compounds displayed moderate hERG inhibition (29–33%) and the piperazine sulfonamide **10a** showed encouragingly weak inhibition (4%) similar to THRX-194556 (**4**), containing this same motif.

On the basis of the promising potency, good in vitro permeability and weak hERG inhibition observed with compound **10a** its single enantiomers **11** and **12** were synthesized and profiled, Table 1, Scheme 2. Following the same trend as observed with THRX-194556 (**4**), the (*S*)-enantiomer **12** was found to have significantly higher 5-HT_4 receptor affinity and functional potency, and selectivity over the 5-HT_3 receptor, relative to the (*R*)-enantiomer **11**. The oral pharmacokinetics (PK) in rats of the (*S*)-enantiomer **12** was determined and found to be comparable to that of the previously identified THRX-194556 (**4**), Table 2. The binding affinity for the indazole-tropane compound **12** was greater than that for THRX-194556 (**4**), although the functional potency was equal.

Since it had been previously concluded that lowering the cpK_a1 and cpK_a2 of the propyl linker by introduction of the 2-hydroxy group was beneficial to the overall profile, the effect of further reducing the basicity by using an ethyl linker was explored. This was significantly deleterious to 5-HT_4 receptor binding in the previously reported case of the quinolone-tropane series.[14] The ethyl linked piperazine-sulfonamide and the less basic acetyl-piperazine analogues, **13** and **14** respectively, were both prepared, Table 1, Scheme 3.[17] Relative to the 2-(*S*)-propanol linked compound **12**, both ethyl-linked analogues had lower 5-HT_4 receptor binding affinity and functional potency although microsome stability and hERG inhibition properties remained favorable, whilst permeability was improved. Significantly, the binding selectivity over the 5-HT_3 receptor was reduced for the piperazine sulfonamide analogue **13** relative to the acetyl-piperazine **14** (110-fold and 980-fold, respectively). Both compounds **13** and **14** were independently dosed orally to rats to see if the in vitro permeability increase

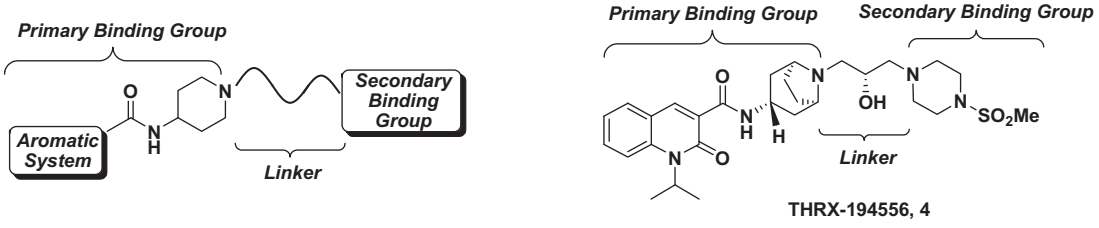
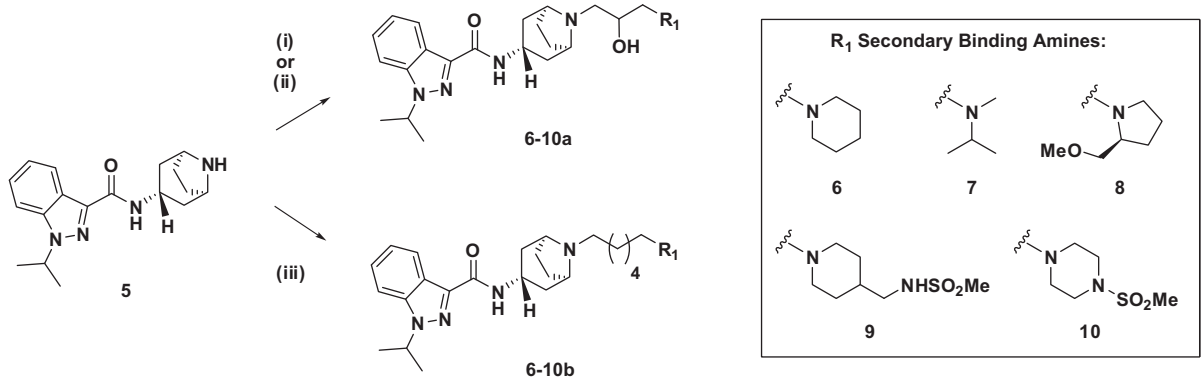
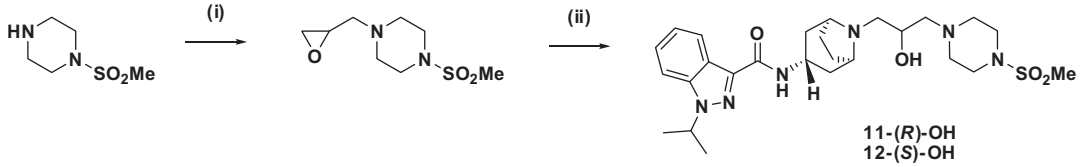


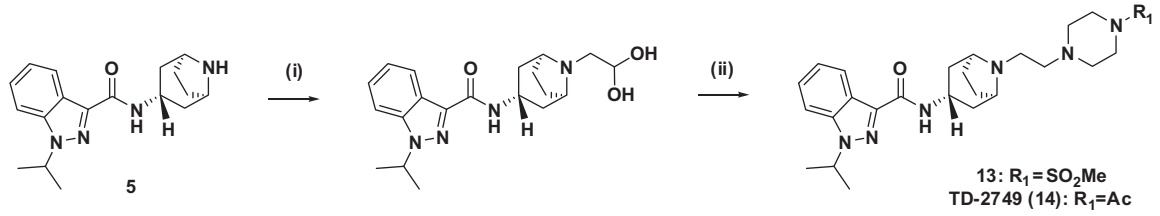
Figure 2. Proposed 5-HT_4 receptor agonist pharmacophore.



Scheme 1. Reagents and conditions: (i) Amines **6**–**8** and **10**, 1,3-dibromo-2-propanol, DIPEA, MeOH, 65 °C; (ii) (a) *rac*-epibromohydrin, DIPEA, EtOH, (b) amine **9**, EtOH, 90 °C; (iii) amines **6**–**10**, 1,6-dibromohexane, DIPEA, MeOH, 65 °C.



Scheme 2. Reagents and conditions: (i) For **11**: (a) (*R*)-epichlorohydrin, DIPEA, EtOH, (b) NaOH, THF, H_2O; for **12**: (a) (*S*)-epichlorohydrin, EtOH, (b) NaOH, THF, H_2O, 0 °C (ii) **5**, toluene, 98 °C.



Scheme 3. Reagents and conditions: (i) (a) Dimethoxyacetaldehyde in ^{t}BuOMe, DIPEA, CH_2Cl_2 then $NaBH(OAc)_3$, (b) 6M HCl, 70 °C; (ii) For **13**: 1-methanesulfonyl-piperazine, $NaBH(OAc)_3$, DIPEA, CH_2Cl_2; For **14**: 1-acetyl-piperazine, $NaBH(OAc)_3$, DIPEA, CH_2Cl_2.

translated into improved oral exposure, Table 2. Whilst piperazine–sulfonamide compound **13** had a similar rat PK profile to its 2-(*S*)-propanol analogue **12**, the acetyl piperazine compound **14** was significantly improved as reflected by increased C_{max}, AUC and overall %F.

Due to its balanced overall profile and structural diversity relative to both the quinolone-tropane and indazole-tropane 2-(*S*)-propanol-piperazine sulfonamides **4** and **12**, the ethyl linked acetyl-piperazine **14** was selected for further evaluation.

With respect to selectivity over other 5-HT receptors, compound **14** displayed less than 50% inhibition of 5-$HT_{1A,B,D}$, 5-$HT_{2A,B,C}$, 5-HT_{3A}, 5-HT_{5A}, 5-HT_6 and 5-HT_7 at a concentration of 10 μM. In contrast, tegaserod displays relatively high binding affinity (pK_i >7.0) at 5-HT_{1D}, 5-$HT_{2A,C}$ and 5-HT_7 receptors, and has particularly high binding affinity (pK_i >8.7) for the 5-HT_{2B} receptor. Cisapride is only 20-fold selective for the 5-HT_4 receptor relative to the 5-HT_3 receptor.

The potency and intrinsic activity of compound **14** in the functional cAMP accumulation assay were determined to be high (pEC_{50} = 8.6; IA = 83%) using a cell line expressing the 5-$HT_{4(c)}$ receptor at 100-fold physiological levels. However, when a cell line expressing the 5-$HT_{4(c)}$ receptor at 10-fold physiological levels was used the potency (pEC_{50} = 8.0) was similar but the intrinsic activity was more modest (IA = 53%). Partial agonist activity was also observed in a 5-$HT_{4(c)}$ receptor-mediated exchange of GDP for europium labeled GTP assay (pEC_{50} = 8.2; IA = 57%). These results, reflecting a partial agonist property of compound **14** were in contrast to tegaserod and THRX-194556 (**4**) which exhibited good potency and high intrinsic activity in these assays. [pEC_{50} = 9.0, 8.7, 7.8; IA = >100%, 99%, 71%, respectively for tegaserod; pEC_{50} = 9.4, 8.7, 8.4; IA = 94%, >100%, >100%, respectively for THRX-194556 (**4**)].

Compound **14** produced a concentration-dependent contraction of the guinea pig isolated colonic longitudinal muscle/myenteric plexus preparation. The potency of **14** (pEC_{50} = 8.0) was similar to that of 5-HT and tegaserod (**2**) (pEC_{50} = 8.0 and 8.2, respectively). However, **14** had an intrinsic activity greater than that of tegaserod (85% vs 65% of the 5-HT maximum, respectively). Considering the wealth of literature demonstrating that 5-HT_4 receptor activation results in contraction of this smooth muscle preparation,[18,19] and the presence of antagonists of 5-HT_1, 5-HT_2 and 5-HT_3 receptors in these isolated tissue experiments, the observed activity of **14** was concluded to represent 5-HT_4 receptor activation.

To characterize the in vivo activity of **14**, digital sonomicrometry was used to monitor 5-HT_4 receptor-mediated esophageal relaxation in the anesthetized rat.[20] This method provided a novel and sensitive means to demonstrate 5-HT_4 receptor agonist-mediated changes in endogenous esophageal tone. Compound **14** produced a dose-dependent, 5-HT_4 receptor-mediated relaxation of

Table 1

Compound	Linker: 2-propanol (a) hexyl (b) ethyl (c)	5-HT$_4$ pK_i[a]	Selectivity vs 5-HT$_3$[b]	5-HT$_4$ pEC$_{50}$[c]	5-HT$_4$ IA[d]	cpK_a1[e]	cpK_a2[e]	RLM $t_{1/2}$ (min)	Caco-2 *Kp* (1×10^{-6} cm/s)	hERG % inhibition[f]
Cisapride	—	7.0	20	7.7	117	—	—	47	—	100
Tegaserod	—	8.4	580	9.0	115	—	—	>90	11	33
4	(*S*)-a	7.9	7400	9.4	94	10.0	9.0	>90	14	4
5	—	7.2	<1	7.4	94	—	—	—	—	—
6a	a	8.7	13000	8.7	85	10.0	7.5	>90	30	62
6b	b	9.0	12	8.6	102	10.9	10.6	—	—	—
7a	a	8.4	6300	8.3	78	10.3	8.6	>90	26	29
7b	b	9.0	23	8.3	92	10.9	10.0	—	—	—
8a	a	8.4	3000	8.6	108	10.0	7.7	75	48	33
8b	b	9.1	14	9.1	117	10.9	10.1	—	—	—
9a	a	8.6	19000	9.2	93	10.0	8.1	>90	1	—
9b	b	8.8	4.3	8.8	91	11.2	10.9	—	—	—
10a	a	8.4	7300	9.1	95	10.0	9.0	>90	19	4
10b	b	8.6	3.6	7.9	75	11.1	10.9	—	—	—
11	(*R*)-a	8.1	240	9.1	94	10.0	9.0	>90	18	7
12	(*S*)-a	8.6	23000	9.4	86	10.0	9.0	>90	19	7
13	c	7.9	110	8.4	88	10.4	4.6	>90	31	9
14	c	8.0	980	8.6	83	10.4	7.2	>90	31	9

[a] 5-HT$_4$ receptor binding pK_i values were determined using a [^{3}H]-GR113808 radioligand binding assay with membranes prepared from HEK-293 cells stably-transfected with human recombinant 5-HT$_{4(c)}$ receptor splice variant.
[b] 5-HT$_3$ receptor binding pK_i values were determined with a [^{3}H]-GR65630 radioligand binding assay with membranes prepared from HEK-293 cells stably-transfected with human 5-HT$_{3A}$ receptor and selectivity for the 5-HT$_4$ receptor type with respect to the 5-HT$_3$ receptor type was calculated as the ratio K_i(5-HT$_{3A}$)/K_i(5-HT$_{4(c)}$).
[c] pEC$_{50}$ values were determined using whole-cell cAMP accumulation studies in HEK-293 cells stably-transfected with the human recombinant 5-HT$_{4(c)}$ receptor splice variant.
[d] Maximum compound-evoked response (minus basal) expressed as a percentage of the maximum response evoked by 5-HT.
[e] Calculated pK_a was calculated with the pKalc 3.2 prediction module of Pallas system (CompuDrug Chemistry, Ltd, Hungary).
[f] Inhibition of hERG potassium ion currents was determined at a concentration of 3 μM using Chinese Hamster Ovary (CHO) cells stably-expressing hERG potassium channels.

Table 2

Compound	Linker: 2-propanol (a) ethyl (c)	C_{max}[a] (μg/mL)	AUC$_{(0-t)}$ (μg h/mL)	$t_{1/2}$ (h)	%*F*[b]
4	(S)-a	0.031	0.20	5.2	20
12	(S)-a	0.035	0.24	7.4	36
13	c	0.099	0.26	1.8	48
14	c	0.254	0.73	3.2	>100

[a] Pharmacokinetic properties were evaluated in male Sprague–Dawley rats dosed with test compounds via oral gavage (PO) at a dose of 5 mg/kg, $n = 3$.
[b] *F* = Oral bioavailability.

the esophagus, following either intravenous (Fig. 3) or intraduodenal administration (data not shown). Following intravenous administration, the rank order of potencies was tegaserod > **14** > cisapride. The mean ED$_{50}$ values for the relaxation response mediated by intravenous tegaserod, **14** and cisapride were 10.6, 37.8 and 141.6 μg/Kg, respectively.

In the guinea pig colonic transit model, **14** (0.3 and 3.0 mg/kg sc) produced a statistically significant decrease in the colonic transit time of carmine red dye relative to vehicle treated animals.[21] Following subcutaneous administration, prokinetic activity was evident when the distance traveled by the dye was measured

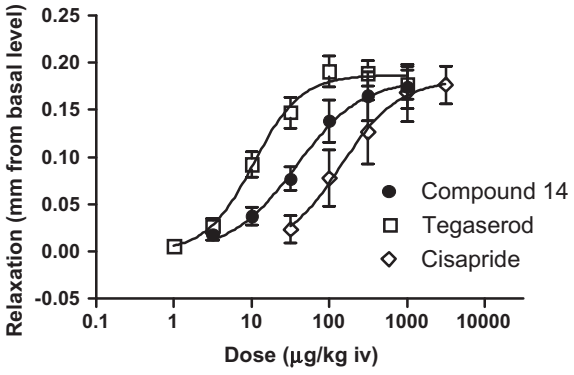


Figure 3. Relaxation of rat esophagus following intravenous dosing of compound **14**.

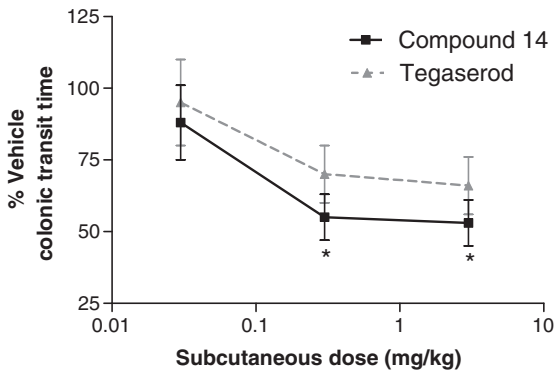


Figure 4. Guinea pig colonic transit following subcutaneous dosing of compound **14**.

60 min later (data not shown), or when the time for excretion of the first fecal pellet containing the marker was recorded, Figure 4. In this assay, **14** appeared to have superior potency to tegaserod, although tegaserod did not achieve a statistically significant increase in colonic transit at any of the doses tested.

Upon completion of the preclinical profiling including confirmation of the impressive in vivo efficacy of compound **14** in additional species it was designated as a development candidate, TD-2749 and advanced to human clinical trials.

Conclusions

Additional application of the previously reported multivalent approach to drug discovery towards 5-HT_4 receptor agonists resulted in the identification of TD-2749, which was selected as a development candidate. TD-2749 is a potent and highly selective 5-HT_4 receptor agonist in vitro with robust in vivo GI activity and an attractive oral pharmacokinetic profile.

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