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Discovery of TD-8954, a clinical stage 5-HT$_4$ receptor agonist with gastrointestinal prokinetic properties

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ABSTRACT

The discovery of a series of 5-HT$_4$ receptor agonists based on a novel 2-alkylbenzimidazole aromatic core is described. Optimization of the 2-substituent of the benzimidazole ring led to a series of agonists with subnanomolar binding affinity and moderate-to-high intrinsic activity relative to that of 5-HT. Consistent with our previously described multivalent design approach to this target, subsequent optimization of the linker and secondary binding group regions of the series afforded compound **18** (**TD-8954**), a potent and selective 5-HT$_4$ receptor agonist in vitro with demonstrated prokinetic activity in multiple species.

The 5-HT$_4$ receptor belongs to the superfamily of seven transmembrane G protein-coupled receptors (GPCRs) and its expression has been demonstrated in a range of human tissues, including the brain,[1] gastrointestinal (GI) tract,[2] heart,[3] adrenal cortex,[4] and bladder.[5] Its potential role in many central and peripherally mediated disorders (irritable bowel syndrome,[6] gastroparesis,[7] Alzheimer's disease,[8] arrhythmia[9]), has made it an attractive target for drug discovery. In particular, the gastrointestinal (GI) prokinetic activity of 5-HT$_4$ receptor agonists (Fig. 1) such as cisapride (Propulsid™) and tegaserod (Zelnorm™) led to their widespread use in the treatment of GI disorders of reduced motility.[10] However, the clinical use of these agents is now restricted on the basis of cardiovascular safety concerns potentially resulting from their lack of selectivity for the 5-HT$_4$ receptor.[11] More recently, the 5-HT$_4$ agonist prucalopride (Resolor™) has been approved in Europe for the symptomatic treatment of women with chronic idiopathic constipation who have not responded adequately to laxatives. In contrast to cisapride and tegaserod, prucalopride has a high degree of selectivity for the 5-HT$_4$ receptor over other 5-HT or non-5-HT receptors.[12]

In previous publications, we described the application of a multivalent approach towards the discovery of novel, selective 5-HT$_4$ receptor agonists, leading to the identification of clinical candidates THRX-194556, TD-2749 and velusetrag (TD-5108).[13] The initial focus of that research was the discovery and optimization of novel 'linker' and 'secondary binding group' motifs according to our proposed 5-HT$_4$ receptor agonist pharmacophore depicted in Figure 2. The 'primary binding group' of 5-HT$_4$ agonists, based on this model, consists of an aromatic core and a heterocyclic amine, usually connected through an amide bond or amide bioisostere. From our previous work, the primary binding group is typically a critical determinant of the ligand's binding affinity to the 5-HT$_4$ receptor and largely controls its functional profile (agonist or antagonist).

In an extension of our previous work, we utilized our preferred linker and secondary binding group fragments as starting points to screen novel primary binding groups that could provide potent 5-HT$_4$ agonism, selectivity over other serotonergic receptors and structural differentiation over existing agonists. Of particular interest to us was the 4-carboxamido-benzimidazole system (Fig. 2). This primary binding group had previously been described in both 5-HT$_3$ and 5-HT$_4$ antagonists.[14] Herein, we examine the previously unexplored 2-position of the benzimidazole ring and its effect on the functional profile of this head group at the 5-HT$_4$ receptor, and the subsequent identification of **TD-8954** as a novel, potent and highly selective 5-HT$_4$ agonist.

In order to synthesize and screen the novel benzimidazole derivatives efficiently it was necessary to first assemble the linker and secondary binding group components of the 5-HT$_4$ pharmacophore and then attach each new benzimidazole core in the last synthetic step. In our previous work, we discovered that the 2-hydroxypropyl linker coupled to the piperazine-methylsulfonamide secondary binding group imbued 5-HT$_4$ agonists with an attractive set of drug-like properties such as oral bioavailability

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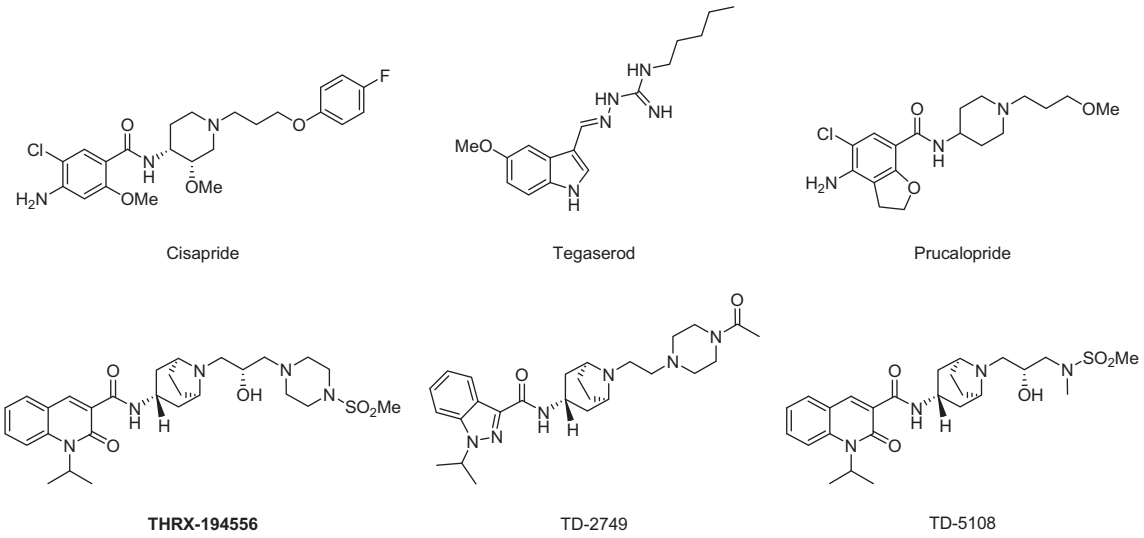


Figure 1. 5-HT_4 receptor agonists.

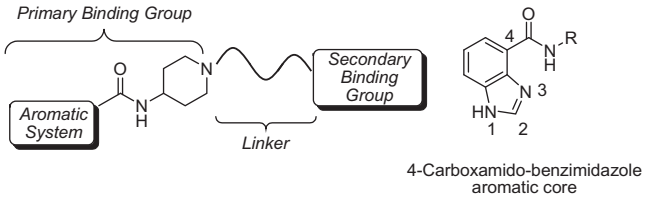


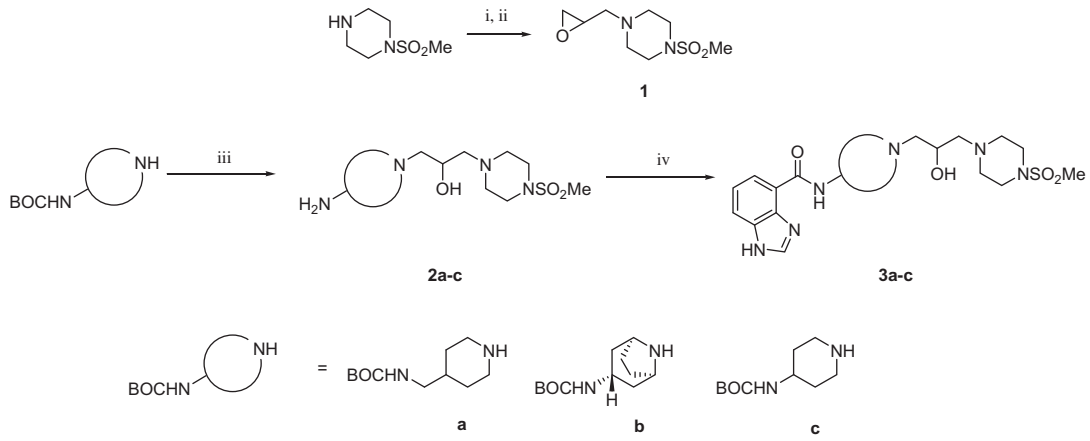
Figure 2. Major components of selective 5-HT_4 receptor agonist pharmacophore.

and selectivity for the 5-HT_4 receptor. As such, these fragments were combined and then connected to three heterocyclic amines *t*-butyl *N*-(4-piperidinylmethyl)carbamate (**a**), *t*-butyl 8-azabicyclo[3.2.1]oct-3-yl carbamate (**b**), and *t*-butyl *N*-(4-piperidyl)carbamate (**c**) that would provide variable positioning of the basic nitrogen atom relative to the benzimidazole core (Scheme 1). The synthesis proceeded via alkylation of each amine with intermediate epoxide **1** followed by deprotection of the primary amine to generate fragments **2a**–**c**. The benzimidazole cores were then attached to **2a**–**c** using standard amide bond-forming conditions. The binding affinity (pK_i), functional potency (pEC_{50}), and intrinsic activity relative to the maximum response achieved by 5-HT in a cAMP accumulation assay (IA) of **3a**–**c** at the human recombinant 5-HT_4 receptor was determined and is shown in Table 1. Since 5-HT_3 antagonism is known to reduce gastrointestinal motility in man and may partially attenuate the clinical benefit of 5-HT_4 agonists,[15] selectivity over this receptor was also determined.

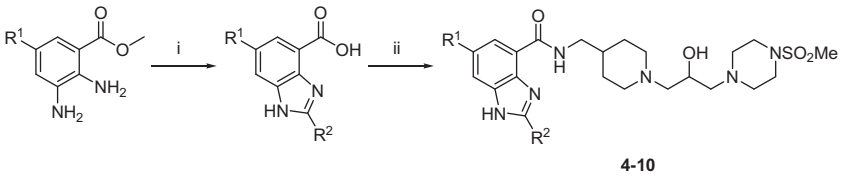
Table 1

Compounds	5-HT_4 (pK_i)	5-HT_4 (pEC_{50})	5-HT_4 IA (%)	5-HT_3 (pK_i)
3a	8.2	8	6	4.3
3b	6.5	6.6	54	4.7
3c	6.6	6.2	7	4.2

The data on **3a**–**c** illustrate that the benzimidazole core lacking a substituent at the 2-position is unsuitable for use as a 5-HT_4 agonist. Whilst the methylpiperidine derivative **3a** afforded nanomolar binding affinity at the human recombinant 5-HT_4 receptor (pK_i = 8.2), its IA was only 6% of the maximum response achieved by 5-HT in a cAMP accumulation assay. The 8-azabicyclo[3.2.1]octane derivative **3b** and piperidine analog **3c** had poor binding affinity (pK_i <7), suggesting that the position of the basic nitrogen atom relative to the benzimidazole core in these systems was not optimal for the receptor binding site. Hence, compound **3a** was selected for further optimization.



Scheme 1. Reagents and conditions: (i) 3-Chloro-1,2-propylene oxide, EtOH; (ii) NaOH, THF/H_2O (4:1); (iii) (a) **1**, EtOH, reflux; (b) TFA, DCM; (iv) EDCI, HOBt, *i*Pr_2NEt, DMF.



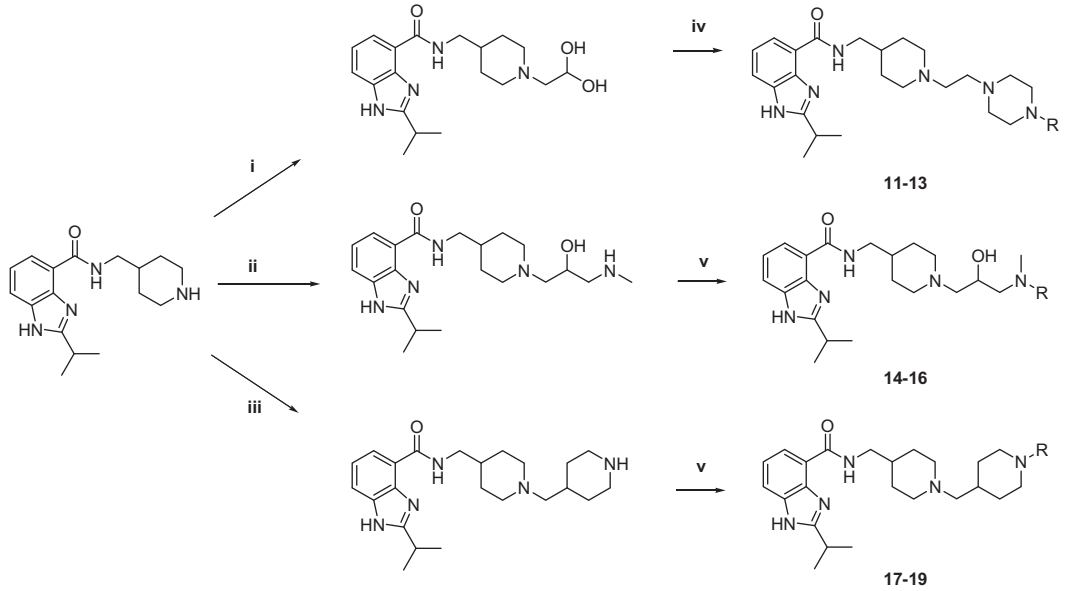
Scheme 2. Reagents and conditions: (i) R_2CO_2H, 4 M HCl, reflux; (ii) **2a**, EDCI, HOBt, iPr$_2$NEt, DMF.

Table 2

Compounds	R^1	R^2	5-HT$_4$ (pK_i)	5-HT$_4$ (pEC$_{50}$)	5-HT$_4$ IA(%)	5-HT$_3$ (pK_i)	RLM $t_{1/2}$ (min)	Caco-2 K_p (1×10^{-6} cm/s)	hERG Inhibition (%)
Cisapride	––	––	7.0	7.1	71	5.7	47	––	100
Tegaserod	––	––	8.4	8.6	92	5.6	>90	11	33
3a	H	H	8.2	8.0	7	4.3	––	––	––
4	H	Et	9.3	9.0	22	4.1	––	––	––
5	H	*i*-Pr	9.3	9.2	61	4.1	>90	0.2	5
6	H	Cyclopropyl	9.3	9.2	64	4.3	>90	2.1	––
7	H	*t*-Bu	7.9	8.5	94	4.4	>90	0.5	––
8	H	CF_2CH_3	8.7	9.1	59	5.1	––	––	––
9	H	Ph	8.9	8.8	41	4.8	––	––	––
10	Cl	*i*-Pr	9.5	––	0	5.9	––	––	––

In an effort to improve the intrinsic activity of **3a**, the 2-position of benzimidazole was explored. Preparation of these analogs was accomplished by a one-pot condensation/ester hydrolysis reaction between methyl 2,3-diaminobenzoate and the requisite carboxylic acid in refluxing 4 M hydrochloric acid.[16] The resulting acids were then coupled to fragment **2a** to provide compounds **4**–**10** (Scheme 2).

Encouragingly, introduction of an ethyl group to the 2-position of the benzimidazole ring (compound **4**, Table 2) produced a moderate gain in intrinsic activity (IA = 22% vs 6% for **3a**), coupled with a 10-fold improvement in binding affinity (pK_i = 9.3) and functional potency (pEC$_{50}$ = 9.0) at the 5-HT$_4$ receptor. This structural modification had no effect on 5-HT$_3$ receptor affinity, leading to very high selectivity for the 5-HT$_4$ receptor over this target. Increasing the size of the 2-substituent (compounds **5** and **6**) further increased the intrinsic activity to approximately 60% of the maximum response achieved by 5-HT, without compromising other key binding parameters. Consistent with this trend, introduction of a tertiary butyl group to the 2-position yielded a full agonist (compound **7**, IA = 94%), but now was accompanied by significant reduction in binding affinity and functional potency at the 5-HT$_4$ receptor. Fluorination of the alkyl substituent (compounds **8**) also led to an improvement in intrinsic activity compared to compound **4** (59% vs 22%), but also increased affinity for the 5-HT$_3$ receptor by 10-fold. A phenyl substituent in the 2-position afforded a weak agonist (compound **9**, IA = 41%). Introduction of a chloro substituent to the 6-position of the benzimidazole ring (compound **10**) led to an inversion in functional profile (from agonist to antagonist) without impacting the binding affinity for the 5-HT$_4$ receptor.



Scheme 3. Reagents and conditions: (i) (a) $CH(OMe)_2CHO$, AcOH, DMF; (b) $NaBH(OAc)_3$, DMF; (c) 6 M HCl; (ii) (a) 3-bromo-1,2-propylene oxide, EtOH; (b) $MeNH_2$, EtOH, reflux; (iii) (a) 1-BOC-4-piperidinecarboxaldehyde, $NaBH(OAc)_3$, DCM/DMF (5:1); (b) TFA, DCM; (iv) $RN(CH_2CH_2)_2NH$, $NaBH(OAc)_3$, iPr$_2$NEt, DCM/DMF (5:1); (v) $MeSO_2Cl$, iPr$_2$NEt, DMF (R = SO_2Me); or $ClCO_2Me$, iPr$_2$NEt, DMF (R = CO_2Me); or AcCl, iPr$_2$NEt, DMF (R = Ac).

Table 3

Compounds	R	5-HT$_4$ (pK_i)	5-HT$_4$ (pEC$_{50}$)	5-HT$_4$ IA (%)	5-HT$_3$ (pK_i)	RLM $t_{1/2}$ (min)	Caco-2 K_p (1×10^{-6} cm/s)	hERG Inhibition (%)
Cisapride	––	7.0	7.1	71	5.7	47	––	100
Tegaserod	––	8.4	8.6	92	5.6	>90	11	33
5	––	9.3	9.2	61	4.1	>90	0.2	5
11	SO_2Me	9.1	9.1	64	4	>90	4	––
12	CO_2Me	8.8	8.8	72	4.1	>90	9	––
13	$COCH_3$	8.3	8.5	76	4.4	>90	0	––
14	SO_2Me	8.9	9.4	83	4.1	>90	1	––
15	CO_2Me	8.4	8.7	64	4.3	79	2	––
16	$COCH_3$	8.4	8.8	64	4.2	>90	0	––
17	SO_2Me	9.3	9	63	4	88	5	8
18	CO_2Me	9.4	9.3	83	4	86	54	11
19	$COCH_3$	9	8.8	87	4	>90	6	22

Table 4

Compound	Rat				Dog			
	C_{max}[a] (μg/mL)	AUC$_{(0-t)}$ (μg h/mL)	$t_{1/2}$ (h)	F[b] (%)	C_{max}[a] (μg/mL)	AUC$_{(0-t)}$ (μg h/mL)	$t_{1/2}$ (h)	F[b] (%)
18	0.818	1.24	1.6	77	0.464	2.28	3.5	63

[a] Pharmacokinetic properties were evaluated in male Sprague–Dawley rats (5 mg/kg) or male beagle dogs (2 mg/kg) dosed with test compounds via oral gavage (PO).
[b] F = oral bioavailability.

Whilst compounds **5**–**7** had attractive 5-HT$_4$ activity profiles and had negligible inhibition of the hERG potassium ion channel relative to cisapride and tegaserod (each compound tested at 3 μM), they were limited by poor permeability (as measured by the Caco-2 assay), which compromised their potential as orally bioavailable therapeutics.

In an effort to improve the permeability of this novel series of agonists, our attention focused on re-optimization of the linker and secondary binding group fragments of the molecule. Our previous work suggested that these fragments had relatively limited impact on the functional profile of 5-HT$_4$ receptor agonists, but strongly influenced permeability, metabolic stability and selectivity over other targets.[13] Using the 2-*i*-Pr-benzimidazole aromatic core (as in compound **5**) as a start point, we explored a small matrix of alternative linker and secondary binding group combinations (Scheme 3). In each case, the amine of the secondary binding group was capped to form sulfonamides, carbamates and amides.

As expected, compounds **11**–**19** preserved the intrinsic activity of **5** as well as high affinity for the 5-HT$_4$ receptor and high selectivity over the 5-HT$_3$ receptor (Table 3). Metabolic stability in rat microsomes was also maintained. With respect to permeability, the methylcarbamate-capped compounds (**12**, **15**, and **18**) were generally more permeable than methylsulfonamides or acetamides. Compounds containing the 2-propanol linker (**14**–**16**) had the lowest permeability ($K_p \leqslant 2$), whilst the methylene-piperidine derivatives (**17**–**19**) were the most permeable ($K_p \geqslant 5$). In the case of **18**, the methylene-piperidine-methylcarbamate combination afforded a remarkable improvement in permeability ($K_p = 54$) over compound **5**. This compound also had negligible inhibition of the hERG potassium ion channel relative to cisapride (11% at a concentration of 3 μM).

As a result of its enhanced profile over other close analogs, compound **18** was selected for in vivo pharmacokinetic assessment in rats and dogs. In both species, **18** displayed high oral bioavailability (Table 4), in agreement with the measured in vitro permeability and microsomal stability data. Hence, **18** was selected for advanced characterization.

The chemical structure of **18** was unambiguously assigned by X-ray crystallographic analysis of the freebase (Fig. 3). The coplanar orientation of the benzimidazole ring and amide group, together with the relevant bond angles and interatomic distances ($d_{N\cdots N} = 2.74$ Å, $d_{H\cdots N} = 1.98$ Å, $\theta_{N-H\cdots N} = 143.8°$) is strongly suggestive of an intramolecular hydrogen bond involving the amide hydrogen atom and the proximal nitrogen atom of the benzimidazole ring (indicated).

With respect to selectivity over other 5-HT receptors, compound **18** 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$_7$ when tested at a concentration of 1 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 16-fold selective for the 5-HT$_4$ receptor relative to the 5-HT$_3$ receptor (pK_i values of 6.9 and 5.7, respectively). Cisapride has moderate or high binding affinity for human 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_7$ receptors (pK_i values of 6.1, 8.2, 7.4 and 6.0, respectively).[17] Compound **18** was also >2000 fold selective for a panel of non-5-HT receptors, transporters, ion channels and enzymes tested (data not shown). Although it has been proposed that coronary artery constriction may be responsible for the potential association of tegaserod with ischemic episodes, contractile activity generally only occurs at high, supratherapeutic concentrations and so a causal link seems unlikely.[18] Nonetheless, the influence of compound **18**, and tegaserod for comparison, on human, porcine and canine in vitro coronary artery tone was stud-

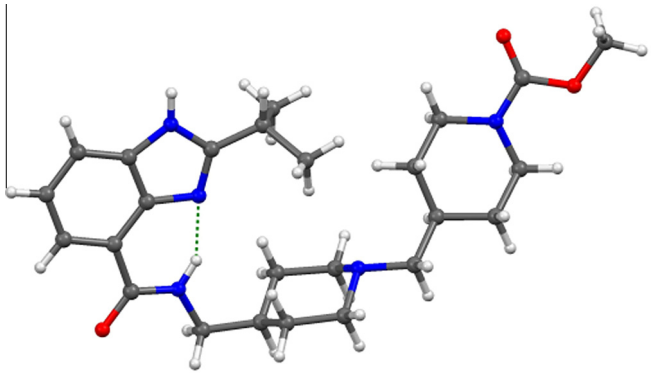


Figure 3. X-ray crystal structure of the freebase crystal of compound **18**.

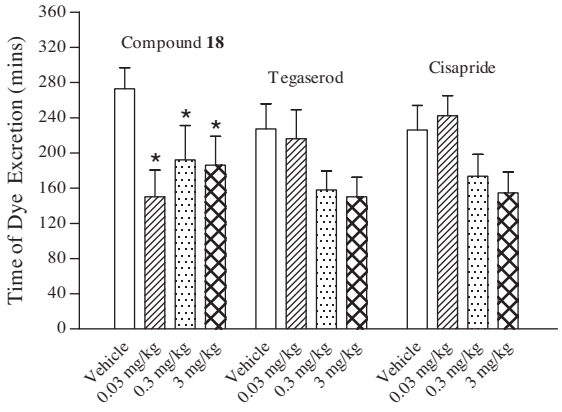


Figure 4. The effects of **18**, tegaserod, cisapride (each at 0.03, 0.3 and 3 mg/kg), and vehicle (2 mL/kg), administered sc, on the colonic transit of dye in conscious guinea pigs (n = 9–22 for each group; p <0.05 (ANOVA followed by Dunnett's post-hoc test vs vehicle).

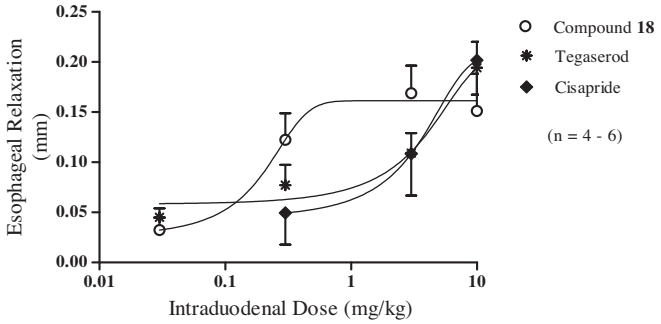


Figure 5. Dose-dependent relaxation mean (±SEM) change in inter-crystal distance (in mm) of the rat esophagus following ID administration of **18**, tegaserod or cisapride.

ied. Compound **18** was inactive, while tegaserod was associated with a small constriction of the canine coronary artery at high concentrations.[17]

In an ex vivo tissue assay, compound **18** produced a concentration-dependent contraction of the guinea pig colonic longitudinal muscle/myenteric plexus preparation. The potency of **18** (pEC_{50} = 8.6) was greater than tegaserod and cisapride (pEC_{50} = 7.9 and 7.0, respectively). Compound **18** had an intrinsic activity less than that of cisapride (55% vs 75% of the 5-HT maximum, respectively) but greater than that of tegaserod (45%). Incubation of tissues with piboserod (a selective 5-HT4 receptor antagonist) resulted in a 614-fold shift (apparent pK_b value = 9.3) of the **18** concentration–response curve, confirming that the observed activity of **18** was due to 5-HT_4 activation.

To characterize the in vivo prokinetic activity of **18**, it was tested in the guinea pig colonic transit model. Prokinetic activity is evident when the time for excretion of the first fecal pellet containing the carmine red dye marker is recorded (Fig. 4). Compound **18** produced a statistically significant decrease in the mean time taken for excretion of the first fecal pellet containing red dye relative to vehicle treated animals. Unlike tegaserod and cisapride, **18** was associated with a statistically significant reduction in transit time at doses as low as 0.03 mg/kg. The magnitude of the prokinetic responses evoked by **18** amounted to approximately a 40–50% reduction in transit time compared to that in vehicle-treated animals. At 0.03 mg/kg, **18** had already achieved its maximum effect.

Digital sonomicrometry was then used to monitor 5-HT_4 receptor-mediated esophageal relaxation in the anesthetized rat following intraduodenal administration of compound **18**.[19] This method provided a novel and sensitive means to demonstrate 5-HT_4 receptor agonist-mediated changes in endogenous esophageal tone. Compound **18** produced a dose-dependent, 5-HT_4 receptor-mediated relaxation of the esophagus (Fig. 5). The mean ED_{50} value for the relaxation response mediated by **18** was 0.15 mg/kg. Accurate ED_{50} values could not be calculated for tegaserod and cisapride, as solubility limitations precluded verification that their maximum relaxations had been achieved. To compare the potencies of each compound, the doses of **18**, tegaserod and cisapride associated with a relaxation response of 0.1 mm were calculated (i.e., 0.23, 2.43, 2.66 mg/kg, respectively). Compound **18** was therefore greater than 10-fold more potent than tegaserod and cisapride in this model.

Based on its encouraging preclinical safety and efficacy profile, compound **18** was nominated as a development candidate (**TD-8954**) and was subsequently advanced into clinical trials, where its prokinetic effect in healthy human subjects was determined.[20] The pharmacokinetics of **TD-8954** over the single dose-range demonstrated dose-dependent increases in systemic exposure and was supportive of once daily dosing (elimination half-life of 12–14 h). Compared to placebo, the number of bowel movements from 0 to 24 h after dosing increased significantly (p <0.03) at all doses of **TD-8954** tested (0.1–20 mg). Each dose of **TD-8954** was also associated with a significant reduction in the time to first bowel movement (p <0.05).

In an effort to discover novel and selective 5-HT_4 agonists, substitution on the 4-carboxamido-benzimidazole system was explored as an approach to novel aromatic core. Substitution of the 2-position was found to control the intrinsic activity of this moiety such that partial or full agonists of the 5-HT_4 receptor could be obtained. The 2-isopropyl group was found to provide the optimum balance of intrinsic activity and binding affinity. Subsequent optimization of the linker and secondary binding group regions of the molecule led to the discovery of **TD-8954**, a highly potent and selective 5-HT_4 receptor agonist with an attractive oral pharmacokinetic profile. **TD-8954** has demonstrated prokinetic activity in healthy human subjects, and may have value in the treatment of patients with disorders related to reduced GI motility.

Acknowledgments

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