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Synthesis of benzamide derivatives as TRPV1 antagonists

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Abstract—From hit compounds identified by high throughput screening (HTS), we have found compound **1** as a lead TRPV1 antagonist and confirmed its potential as a treatment for pain. Compound **1** has led to potent TRPV1 antagonistic benzamide derivatives ((±)-**2**: human IC_{50} = 23 nM, (±)-**3**: human IC_{50} = 14 nM in the capsaicin-induced calcium influx assay) containing indole and naphthyl moieties, obtained by elaboration of the tryptamine scaffold or via bioisosteric replacements.
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TRPV1 (transient receptor potential vanilloid subfamily member 1,[1] also known as VR1) is a non-selective cation channel, predominantly expressed by neurons of the peripheral and central nervous system,[2] which plays a key role in the detection of noxious painful stimuli such as acid (low pH),[3] noxious heat, and vanilloids such as capsaicin[4] (the pungent component of hot chili peppers). TRPV1 has been associated with various biological disorders such as hyperalgesia, neuropathic pain, migraine, asthma, visceral pain, chronic obstructive pulmonary disease (COPD), inflammatory bowl disease (IBD), irritable bowel syndrome (IBS), interstitial cystitis/incontinence, and osteoarthritis. Disruption of the TRPV1 gene in mice causes reduction in both thermal nociception and inflammation-induced hyperalgesia.[5] Recently, substantial advances by several pharmaceutical companies have resulted in the progress to clinical trials of TRPV1 antagonist clinical candidates such as SB-705498 (GSK, phase II for migraine and IBS), GRC6211 (Glenmark/Eli lilly, Phase II for acute pain), and NGD-8243/MK-2295 (Neurogen/Merck, phase II for acute pain).

Having identified HTS hit compounds by high throughput screening (HTS), our group has profiled compound **1** as a TRPV1 antagonist lead and confirmed its potential for the treatment of pain (Fig. 1). Elaboration of compound **1** has led to the progression to the early development stage of potent TRPV1 antagonistic benzamide compounds (±)-**2** and (±)-**3**, containing indole and naphthyl moieties, respectively. This report summarizes our SAR pursuit from the original hit compound **4** to the more advanced compounds (±)-**2** and (±)-**3** focusing on the structural novelty, the avoidance of an aniline moiety (Tables 2–5), and the bioisosteric replacement of the obtained tryptamine scaffold (Fig. 5).[6]

The TRPV1 program at Pfizer was initiated with two hit compounds (**4** and **5**) discovered through a HTS campaign in both the capsaicin(CAP)-induced calcium influx and the carbachol-induced calcium influx[7] on HEK-293 cells as shown in Table 1. The TRPV1-selective small molecule **4** was selected and modified, focusing on inhibitory activity in the capsaicin-induced calcium influx assay. At first, modifications of the trifluoroacetyl moiety were investigated, followed by replacements of the iodide. The trifluoroacetyl group could be converted to trifluoromethyl, trifluoromethoxy, and 2,2,1,1-tetrafluoroethoxy groups as shown in compounds **6c**, **6d**, and **1**. Having set R^1 as trifluoromethoxy or 2,2,1,1-tetrafluoroethoxy, the iodide was replaced by a *tert*-butyl group, thus lowering the molecular weight as shown in compounds **7a** and **1**. In the preliminary SAR around HTS hit compound **4**, compound **1** demonstrated a human IC_{50} value of 70 nM, an increase of antagonistic activity of more than 10-fold over that of **4**.

Around the same time that the first potent TRPV1 antagonist was reported,[8] the newly discovered

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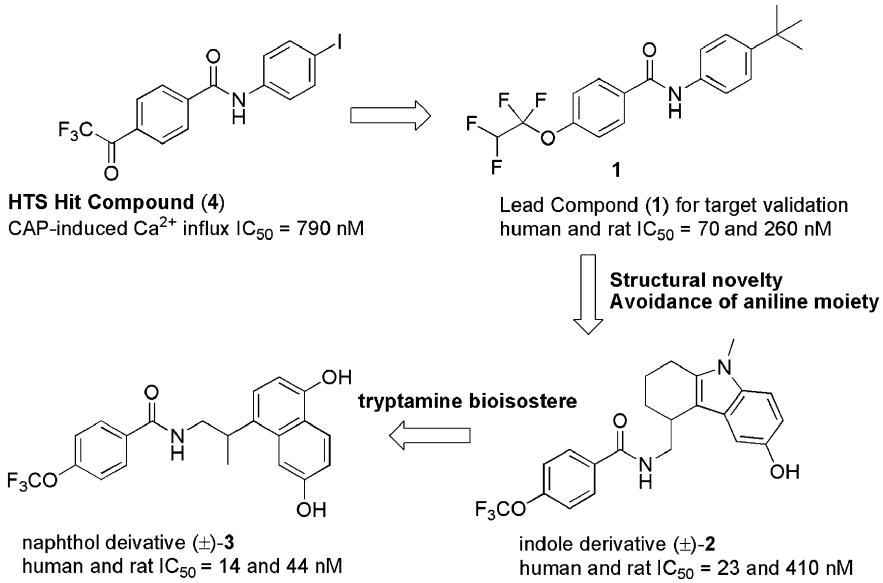
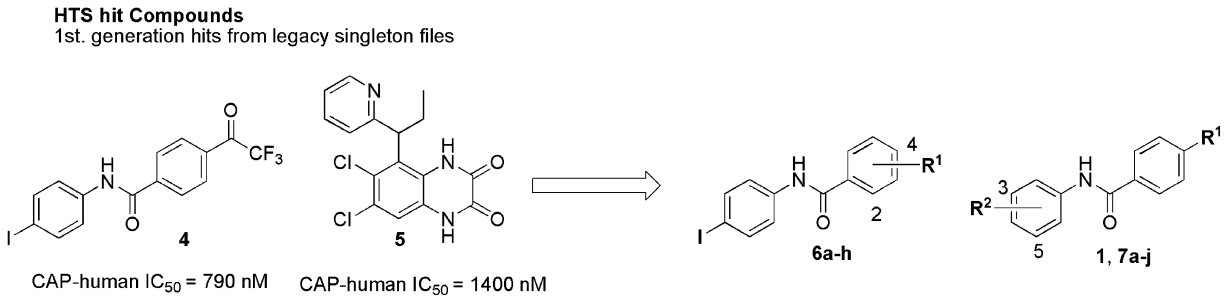


Figure 1. Advanced lead compound and potent TRPV1 antagonistic benzamide derivatives.

Table 1. Preliminary SAR from HTS Hit Compound **4**



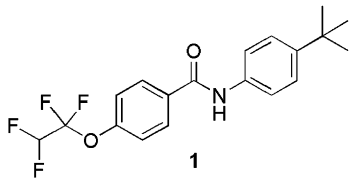
Compound	R^1	IC_{50} (nM)[a]	Compound	R^1	R^2	IC_{50} (nM)[a]
6a	4-$COCF_3$	790	**7a**	4-OCF_3	4-*t*-Bu	120
6b	4-COMe	>10,000	**7b**	4-CF_3	4-*t*-Bu	220
6c	4-OCF_3	280	**7c**	4-OCH_2CF_3	4-*t*-Bu	>10,000
6d	4-CF_3	330	**7d**	4-OCF_3	4-CF_3	220
6e	3-CF_3	>10,000	**7e**	4-OCF_3	3, 4-cyclopentyl	210
6f	2-CF_3	>10,000	**7f**	4-OCF_3	3, 5-*di*-*t*-Bu	>10,000
6g	4-SO_2Me	>10,000	**7g**	4-OCF_3	4-cyclohexyl	>10,000
6h	4-OCF_2CF_2H	N.T.	**7h**	4-OCF_3	4-phenyl	>10,000
			7i	4-SCF_3	4-*t*-Bu	253
			1	4-OCF_2CF_2H	4-*t*-Bu	70

N.T., not tested.
[a] The value of IC_{50} means 50% inhibitory concentration in CAP-induced Ca^{2+} influx on human HEK-293 cells.

compound **1** was evaluated in various models and demonstrated inhibitory activities[9] in both CCI (MED: 59% inhibition, @100 mg/kg) and carragenan-induced thermal hyperalgesia (52% inhibition, @30 mg/kg) by systemic administration (ip) as shown in Figure 2. Thus, the benzanilide derivative **1** proved to be a useful probe for evaluating the treatment of painful diseases by systemic administration, and a key scaffold for further modification.

In further SAR studies based on the benzanilide scaffold, our efforts focused on increasing the structural novelty and on avoiding the aniline substructure by the modification of the linker moiety or cyclization to benzimidazole and indole derivatives,[10] as shown in Figure 3.

Replacements of the anilide with various amide-equivalent linkers caused a significant decrease of activity



in vitro (Capsaicin-induced calcium influx assay)

human VR1 IC_{50} = 70 nM
Rat VR1 IC_{50} = 260 nM

in vivo
Capsaicin licking (mouse): ED_{50} = 3.8 mg/kg
Capsaicin hypothermia (mouse): ED_{50} = 4.5 mg/kg
Capsaicin hypothermia (rat): ED_{50} = 4.2 mg/kg
Carrgenan-thermal hyperalgesia: 52% inh.@ 30 mg/kg, ip
CCI model : 59 % inh.@100 mg/kg, ip

PK
Plasma concentration 837 ng/ml @ 100 mg/kg, ip
Brain/Plasma ratio: 2.3 (30 min. after 30 mg/kg, ip)

Figure 2. In vitro, in vivo, and PK data of compound **1**.

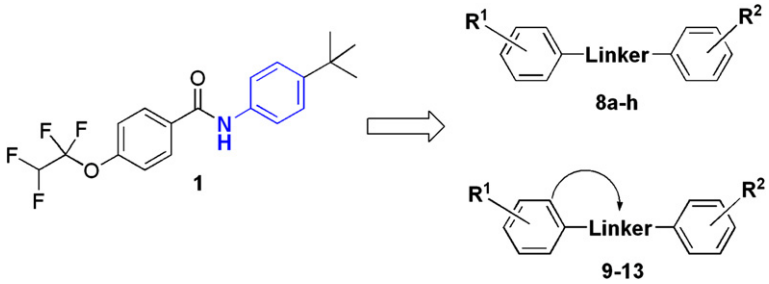


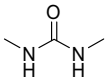
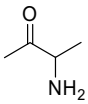
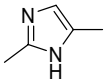
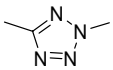
Figure 3. SAR strategies to avoid the anilide moiety.

and only derivative **8g**, a compound containing an imidazole linker and a free NH, showed weak activity with a human IC_{50} value of 6000 nM in the in vitro assay, as shown in Table 2. In subsequent reports,[11] it was found that the NH group of the linker plays an important role in the inhibitory activity against TRPV1 receptor. Furthermore, the fused benzimidazole derivative **10**, also equipped with the free NH, was 15-fold more potent than **8g** with a human IC_{50} value of 380 nM, a comparable value to the IC_{50} of the positive control capsazepin (Table 3).[12] However, the urea and reversed amide derivatives (**8a** and **8f**) demonstrated a drastic decrease of activity despite the presence of the NH moiety.

The compounds of Table 4 were prepared by an efficient parallel synthesis protocol using 4-trifluoromethyl- or 4-trifluoromethoxybenzoyl chloride. As a result of screening non-aniline amine monomers, substituted tryptamine and phenethylamine monomers were found to lead to compounds in the desired activity range with human IC_{50}s of 200–2000 nM such as compounds **14e**–**h** and **14l**–**n**. The phenethylamine SAR revealed that the 2,6-dichloro derivative **14l** had comparable activity to capsazepine (human IC_{50} of 250–300 nM). However, after extensive screening of liquid samples made by parallel synthesis, as well as powder samples obtained by conventional methods, no further improvement over the potency of **14l** could be achieved. While the tryptamine derivatives showed antagonistic activity at the sub-micromolar level, our focus on the structural novelty led us to investigate substituents at the 5-position of the indole ring. Thus we soon discovered that modifications at the 5-position are important for the mainte-

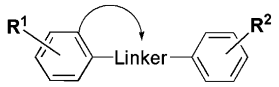
Table 2. Linkage modifications based on the benzanilide scaffold

8a-h

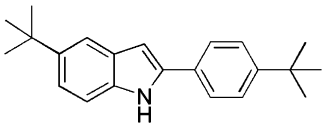
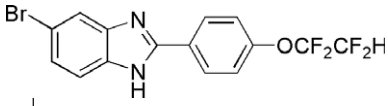
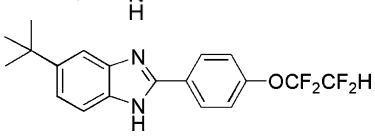
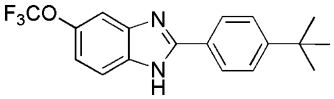
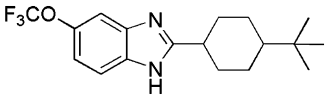
Compound	R^1	R^1-linker-R^2	R^2	IC_{50}[a] (nM)
8a	CF_3		I	>10,000
8b	CF_3		*t*-Bu	>10,000
8c	OCF_3		*t*-Bu	>10,000
8d	CF_3		*t*-Bu	>10,000
8e	OCF_3		*t*-Bu	>10,000
8f	CF_3		I	>10,000
8g	CF_3		Br	6000
8h	CF_3		*t*-Bu	>10,000

[a] The value of IC_{50} means 50% inhibitory concentration in CAP-induced Ca^{2+} influx assay on human HEK-293 cells.

Table 3. Conversion of benzanilide derivatives to benzimidazole derivatives



9-13

Compound	Cyclic compound	IC_{50}[a] (nM)
9		>10,000
10		380
11		477
12		1420
13		4970

[a] The value of IC_{50} means 50% inhibitory concentration in CAP-induced Ca^{2+} influx assay on human HEK-293 cells.

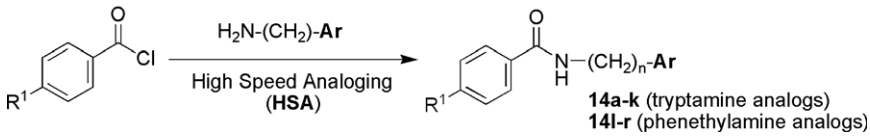
nance of activity and that 5-methoxy and 5-hydroxy compounds (**14e** and **h**) showed a 5-fold boost in activity compared with the parent **14a**.

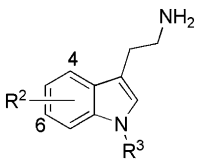
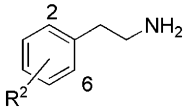
In addition to amine screening, variations of the benzoyl substituent R^1 were also investigated when coupled with 5-methoxytryptamine, as shown in Table 5. While the phenyl derivative **15b** showed a 3-fold improvement over the trifluoromethoxy group (**15a**), this result was assumed to be due to the increase in lipophilicity (and decrease in solubility) in the narrow benzamide SAR. In order to avoid increasing the lipophilicity, the pyridine derivatives (**15h–j**), as well as derivatives containing a basic R^1 group (**15f** and **g**), were synthesized, but the results showed a 3-fold decrease in potency for **15h** and **15i** compared to **15a**, and a drastic decrease of activity for **15f**, **15j**, and **15g**.

The follow-up SAR on tryptamine derivatives is summarized in Figure 4. The introduction of a methoxy group at the 5-position (**14e**) followed by mono-methylation at the benzylic position gave a compound (**16**) with comparable antagonistic activity to capsazepine. In the next step, the mono-benzyl methyl group was linked to the 2-position of the indole ring to give tetrahydrocarbazole derivative (**20**). Removal of the *O*-methyl group of **20** resulted in compound (±)-**2** (human IC_{50} = 23 nM) with 60-fold improvement in inhibitory activity over the open-chain compound **14e**. It is clear that the conformational restriction and presence of the free hydroxyl group at the appropriate position result in a dramatic increase of antagonistic activity. Compound (±)-**16** was determined to be TRPV1-selective and a competitive, full antagonist (pA_2 = 6.84; 143 nM) against capsaicin, after confirmation of other signals with various *kinase*, *GPCR/Channel* receptor, and *enzyme* assays.

Because these benzamide derivatives based on the tryptamine scaffold are similar to melatonin derivatives con-

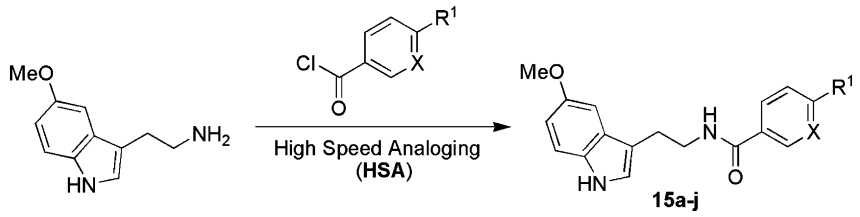
Table 4. Amidation of tryptamine and phenethylamine monomers with 4-substituted benzoyl chloride



Arylamines	**14a–r**	R^1	R^2	R^3	IC_{50}[a] (nM)
	**a**	OCF_3	H	H	7350
	b	OCF_2CF_2H	H	H	N.T.
	c	CF_3	H	H	4230
	d	CF_3	H	Me	>10,000
	e	OCF_3	5-Methoxy	H	1310
	f	CF_3	5-Methoxy	H	2090
	g	OCF_2CF_2H	5-Methoxy	H	1640
	h	OCF_3	5-OH	H	1730
	i	OCF_3	5-Cl	H	3720
	j	OCF_3	5-*t*-Bu	H	>10,000
	k	OCF_3	5-F	H	2620
	**l**	OCF_3	2,6-Cl		205
	m	OCF_3	2-Cl		776
	n	OCF_3	3-Cl		1930
	o	OCF_3	2,4-Cl		2560
	p	OCF_3	3,4-Cl		3700
	q	OCF_3	3-Cl		>10,000
	r	OCF_3	2-F		3490

[a] The value of IC_{50} means 50% inhibitory concentration in CAP-induced Ca^{2+} influx assay on human HEK-293 cells.

Table 5. Screening of benzoic moiety based on 5-methoxytryptamine



Compound	R_1	X	IC_{50}[a] (nM)
15a	Trifluoromethoxy	C	1310
15b	Phenyl	C	370
15c	Phenoxy	C	1890
15d	Cyclohexyl	C	1160
15e	Isobutyl	C	3430
15f	4-Pyridyl	C	>10,000
15g	*N*,*N*-Dimethylaminoethoxy	C	>10,000
15h	Phenyl	N	3940
15i	2,2,2-Trifluoroethoxy	N	3400
15j	Trifluoromethyl	N	>10,000

[a] The value of IC_{50} means 50% inhibitory concentration in CAP-induced Ca^{2+} influx assay on human HEK-293 cells.

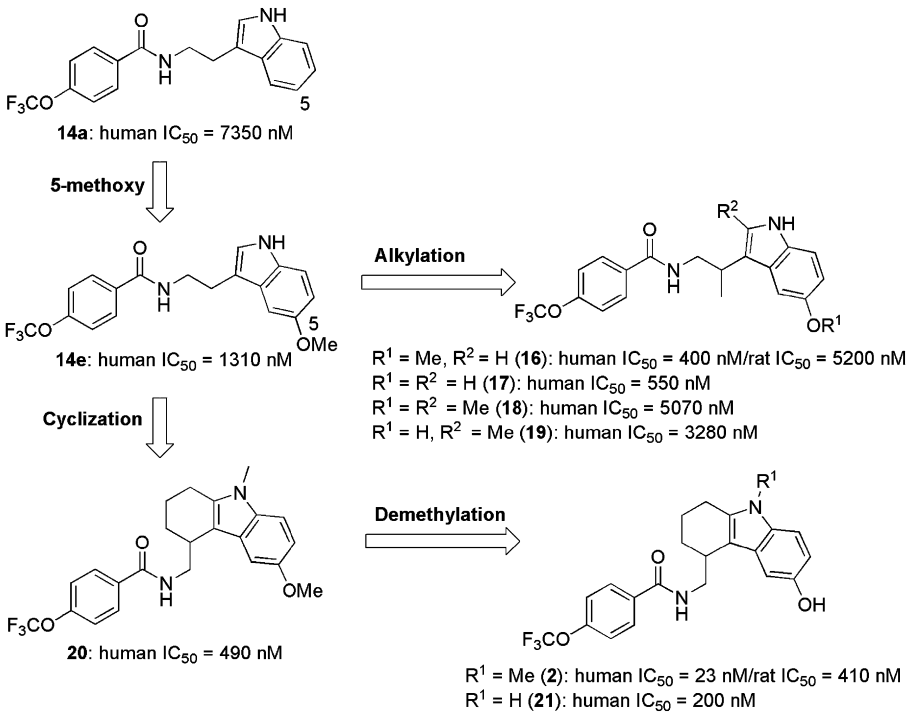
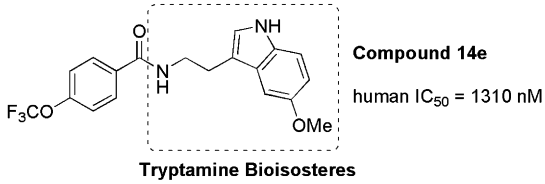


Figure 4. Conformational approach based on tryptamine derivative (**14e**).

taining the same acetamide moiety, we hypothesized that the bioisosteric replacements[13] which were successful for the melatonin receptor SAR would also be useful in the TRPV1 antagonists program as shown in Figure 5. In fact, the tetrahydrocarbazole moiety[14] of (±)-**2, 20**, and **21** was known to be a bioisostere of the tryptamine skeleton. As an example of conversion to another known bioisostere, the non-substituted naphthyl derivative (**22a**) showed moderate activity with an IC_{50} value of 2600 nM, as shown in Figure 5. In most cases, the introduction of a hydroxyl group at the 7-position of the bicyclic system increased the antagonistic activity over the unsubstituted compounds (**22a**–**g**). In particular, the naphthol derivative (**24a**) demonstrated a human IC_{50} of 150 nM, a 9-fold improvement in antagonistic activity compared to the human IC_{50} = 1310 nM for tryptamine derivative (**14e**). Furthermore, the introduction of a methyl group at the benzylic position also caused a 2- to 5-fold improvement in potency, compared to the non-methyl analogs (**24a**–**c**). As a result, unsubstituted naphthyl derivative (**22a**) could be transformed into the optimized hydroxyl-substituted compound ((±)-**3**) with human and rat IC_{50} values of 14 nM and 44 nM, respectively.

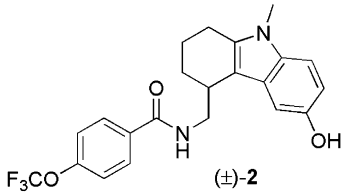


Entry	Non-Substituted analogs 22a-g	Alkoxy- Substituted analogs 23a-g (R = Me), 24a-g (R = H)	Benzyl-Me analogs 25a, c (R = Me), 26a, c (R = H), (±)-3
a	2600 (human IC_{50}, nM)	R = Me (1400) R = H (150) (human IC_{50}, nM)	R = Me (3100) R = H (81) (human IC_{50}, nM)
b		R = Me (> 10000) R = H (76)	(±)-**3**: R = H (14)
c	> 10000	R = Me (> 10000) R = H (1230)	R = Me (> 10000) R = H (740)
d	6700		
e	4600	R = Me (> 10000) R = H (960)	
f	5400	R = Me (4650) R = H (1350)	
g	7300	R = Me (3550)	

[a] The value of IC_{50} means 50 % inhibitory concentration in CAP-induced Ca^{2+} influx assay on human HEK-293 cells.

Figure 5. Approach of tryptamine moiety to bioisosteric replacements.

Table 6. Bioavailability of compound (±)-**2** using various vehicles



(±)-**2**

Capsaicin-induced human IC50 = 23 nM
Caco-2 (Permeability)(rapp x 10^{-6}): 13.8

iv Administration (rat)	po Administration (rat)	po Administration (rat)
AUC (0–t_{max}) 662 $T_{1/2}$: 2.1 h	F = 0.7 ± 0.4% $T_{1/2}$: 1.2 h	F = 30.2 ± 10.9% $T_{1/2}$: 2.1 h
Vehicle: 5% DMSO/PEG400	Vehicle: 0.1% MC solution	Vehicle: PEG400

In summary, structure–activity relationship studies starting from hit compound **4** have led to advanced compound (±)-**2** with human and rat IC_{50} values of 23 nM and 410 nM, respectively, in the capsaicin-induced calcium influx assay. Moreover, the naphthyl derivative (±)-**3** was obtained by bioisosteric replacement of the tryptamine scaffold, and showed solid activity with human and rat IC_{50} values of 14 nM and 44 nM, respectively. However, the major issue with compound (±)-**2** was its low bioavailability (F_{oral} = 0.7%, vehicle: 0.1% MC solution), possibly due to low solubility. As shown in Table 6, the PK study of compound (±)-**2** using PEG400 showed moderate bioavailability (F_{oral} = 30.2 ± 10.2%) and this compound is expected to have improved bioavailability by spray dryer dispersion (SDD) (or solid dispersion) formulation on the free base.

Acknowledgments

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References and notes

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