



CB₂ selective sulfamoyl benzamides: Optimization of the amide functionality

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ABSTRACT

Previous research within our laboratories identified sulfamoyl benzamides as novel cannabinoid receptor ligands. Optimization of the amide linkage led to the reverse amide **40**. The compound exhibited robust antiallodynic activity in a rodent pain model when administered intraperitoneally. Efficacy after oral administration was observed only when ABT, a cytochrome P450 suicide inhibitor, was coadministered.

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Activation of the endogenous cannabinoid receptors by compounds such as Δ^9 -tetrahydrocannabinol (THC) (**1**) (Fig. 1), the active component of *Cannabis sativa*, has long been used in a variety of medical applications. These include appetite stimulation as well as treatments for emesis, cramps, fever, rheumatism and pain.¹ Two cannabinoid receptors, designated CB₁ and CB₂, have been characterized from the superfamily of G protein-coupled receptors (GPCRs). Sharing approximately 44% amino acid sequence homology, the two receptors differ in anatomical distribution with CB₁ receptors found mainly in the CNS, while CB₂ receptor expression occurs primarily in peripheral tissues associated with immune functions, including B and T cells and macrophages.² CB₂ receptors have also been isolated from peripheral nerve endings and mast cells.³

The clinically undesired effects associated with cannabis use, such as euphoria, are believed to be predominantly centrally mediated through activation of the CB₁ receptors. The use of CB₂ selective agonists is, therefore, one approach that could be adopted for the use of cannabinoid receptor activation in the treatment of pain.

The antihyperalgesia produced by the selective CB₂ agonist AM1241 (**2**) in the rat carrageenan induced inflammatory thermal hyperalgesia assay was reversed by pretreatment with a CB₂ selec-

tive antagonist, but not by a CB₁ selective antagonist, thus demonstrating a CB₂ receptor mediated effect.⁴ Additionally, GW405833 (**3**) exhibited antihyperalgesic activity in rodent models of incisional, neuropathic and chronic inflammatory pain, but was devoid of significant activity in similar models in CB₂ knock-out mice.⁵

It is perhaps not surprising then that development of CB₂ selective agonists for the treatment of chronic and inflammatory pain has recently received a large amount of interest. From this research several structurally diverse classes of compounds have been identified. These include indoles (**2**, **3**),^{4,5} benzimidazoles (**4**),⁶ iminopyrazoles (**5**),⁷ oxadiazoles (**6**)⁸ and aryl sulfonamides (**7**) (Fig. 2).⁹

Independently, through high-throughput screening, research within our organization identified the sulfamoyl benzamide **8** displaying weak affinity for the CB₂ receptor (Fig. 3).¹⁰

Optimization of the scaffold led to compound **9** which exhibited vastly improved affinity and selectivity for the CB₂ receptor. To further explore the SAR around the phenylsulfonamide core we first

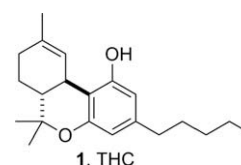


Figure 1. Structure of THC.

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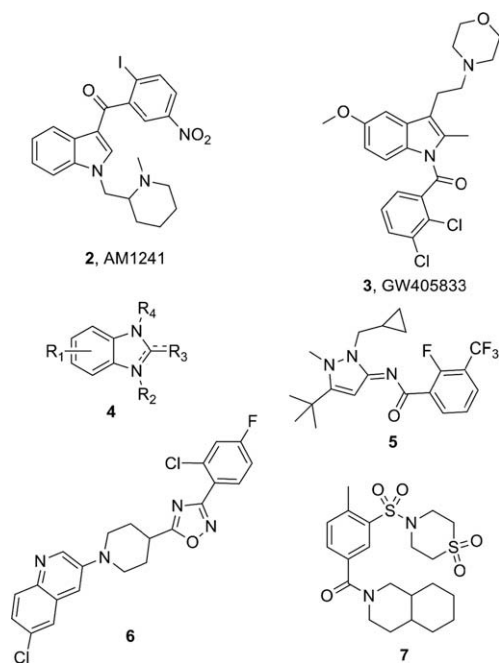
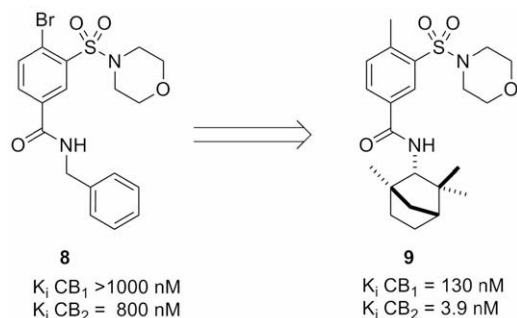
Figure 2. Structures of CB₂ selective agonists.

Figure 3. Optimization of high-throughput screening hit.

looked at the isosteric replacement of the amide functionality of **9** with various heterocycles.¹¹ These included thiazoles, oxadiazoles, pyrazoles, imidazoles, triazoles and tetrazoles. Examples are shown in Table 1.¹² Synthesis of compounds **10–27** is described in Scheme 1. Coupling of the previously described sulfonamide benzoic acid **28**¹⁰ with ammonium chloride gave the primary amide **29**. Thiazoles **10–12** were then prepared from **29** in a two step process, that is, thioamide formation using Lawesson's reagent followed by cyclization with the respective bromoacetate. Dehydration of intermediate **29** with phosphorus oxychloride gave the nitrile **30** which was then used as a common intermediate for the synthesis of the tetrazoles **13–15**, the triazoles **16–18**, the

Table 1
Bioisosteric replacement of amide functionality—in vitro results^{a,b,c,d}

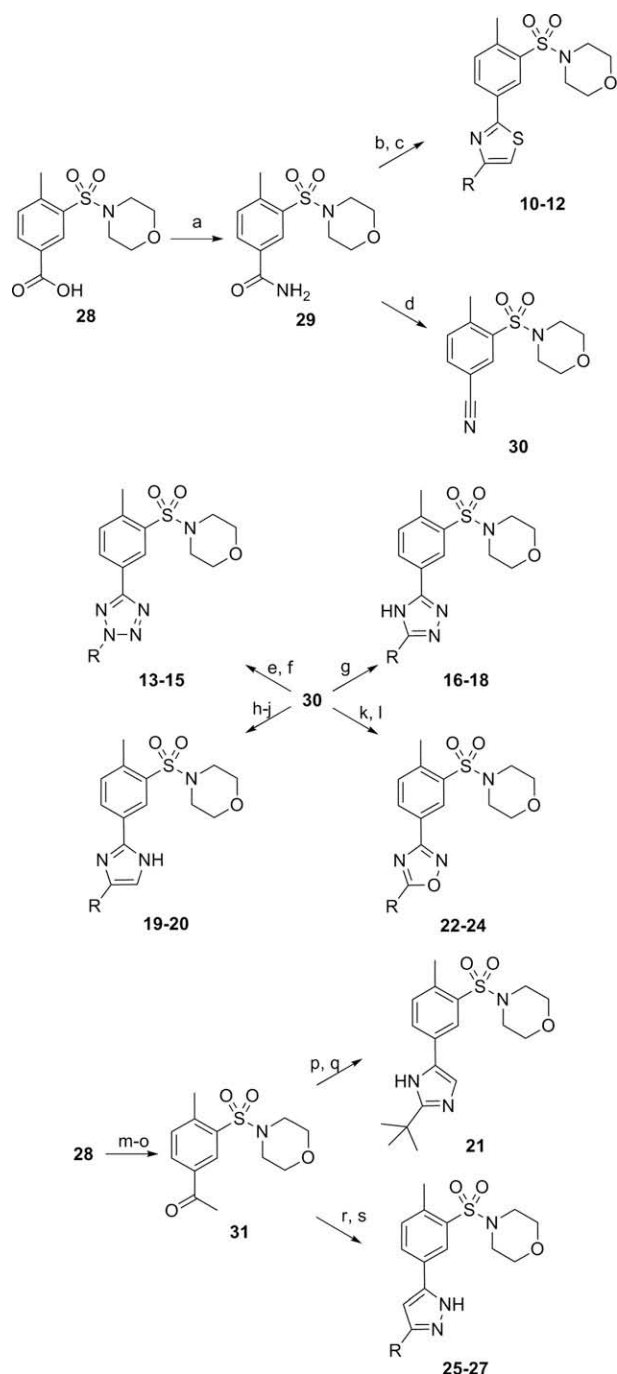
Compound	R	CB ₁ K _i ^{a,b} (nM)	CB ₂ K _i ^{a,b} (nM)	Ratio CB ₁ /CB ₂	CB ₂ EC ₅₀ ^{a,c} (nM)	Compound	R	CB ₁ K _i ^{a,b} (nM)	CB ₂ K _i ^{a,b} (nM)	Ratio CB ₁ /CB ₂	CB ₂ EC ₅₀ ^{a,c} (nM)
10		200	5.2	38	23	19		1100	36	31	47
11		390	23	17	120	20		1100	23	48	58
12		260	5.7	47	21	21		770	81	9.4	130
13		130	17	7.6	49	22		270	59	4.5	70
14		560	82	6.9	78	23		740	130	5.9	110
15		>5000	>5000	n.d. ^d	n.d. ^d	24		510	75	6.8	77
16		140	85	1.6	64	25		750	75	10	120
17		310	27	11	57	26		1400	87	16	190
18		530	100	5.3	130	27		6990	160	6.1	130

^a Values are the geometric means computed from at least three separate determinations.

^b For assay description see Ref. 13a.

^c EC₅₀ values determined through [³⁵S]GTPγS stimulation. For assay description see Ref. 13b.

^d Not determined.



Scheme 1. Reagents and conditions¹²: (a) NH_4Cl , TBTU, DIEA, CH_3CN , 89%; (b) Lawesson's Reagent, toluene, 66%; (c) RCOCH_2Br , DBU, EtOH, 11–70%; (d) POCl_3 , DMF, 75%; (e) NaN_3 , ZnBr , H_2O , 52%; (f) RX , Et_3N , DMF, 51–81%; (g) RCONHNH_2 , K_2CO_3 , $\text{CH}_3(\text{CH}_2)_3\text{OH}$, 10–74%; (h) $\text{NH}_2\text{OH}\cdot\text{HCl}$, K_2CO_3 , EtOH, 32–65%; (i) Raney Nickel, CH_3COOH , MeOH, H_2 , 28–91%; (j) RCOCH_2Br , DBU, EtOH, 11–14%; (k) $\text{NH}_2\text{OH}\cdot\text{HCl}$, K_2CO_3 , EtOH, 32%; (l) RCOCl , pyridine, 2–22%; (m) SOCl_2 , DMF, 94%; (n) $\text{CH}_3\text{NHOCH}_3$, Et_3N , CH_2Cl_2 , 76%; (o) CH_3MgBr , Et_2O , THF, 92%; (p) $\text{C}_6\text{H}_5\text{CH}_2\text{N}(\text{CH}_3)_3^+\text{Br}_3^-$, CH_2Cl_2 , MeOH, 47%; (q) $(\text{CH}_3)_3\text{CC}=(\text{NH})\text{NH}_2\cdot\text{HCl}$, K_2CO_3 , DMF, 83%; (r) CDI, RCOOH , LAH, THF, 56–89%; (s) NH_2NH_2 , H_2O , EtOH, 8–21%.

imidazoles **19–20** and the oxadiazoles **22–24**. Compounds **21** and **25–27** were generated from the ketone intermediate **31**. Compound **31** was prepared in a three step procedure from the acid **28**, i.e. acid chloride formation using thionyl chloride, Weinreb amide synthesis followed by reaction with methylmagnesium bromide.

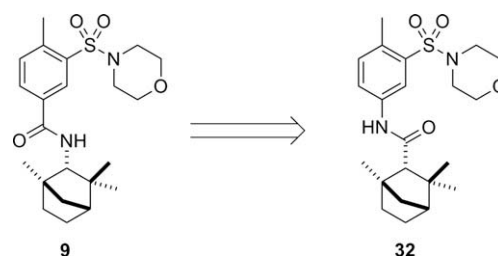


Figure 4. Reversal of amide linkage.

Table 2

In vitro binding of 'reverse amides' for select compounds^{a,b,c}

Compound	R	X	Y	$\text{CB}_1 K_i^{\text{a,b}}$ (nM)	$\text{CB}_2 K_i^{\text{a,b}}$ (nM)	Ratio CB_1/CB_2	$\text{CB}_2 \text{EC}_{50}^{\text{a,c}}$ (nM)
9		C=O	NH	130	3.9	31	4.6
32		NH	C=O	640	1.7	380	9.6
33		C–O	NH	540	48	11	25
34		NH	C=O	1200	9.4	130	49
35		C=O	NH	310	34	9.0	21
36		NH	C=O	1200	11	110	31

^a Values are the geometric means computed from at least three separate determinations.

^b For assay description see Ref. 13a.

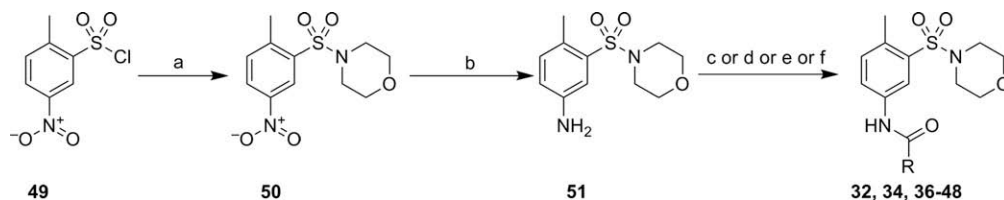
^c EC_{50} values determined through [^{35}S]GTP γS stimulation. For assay description see Ref. 13b.

From this research the thiazole substituted compounds (**10, 12**) showed the best overall profile for the CB_2 receptor. However, as can be seen from Table 1, replacement of the amide moiety with various heterocycles did not significantly improve affinity or selectivity for the CB_2 receptor in comparison to the lead compound **9**. Continuing our focus on optimization of the amide moiety we then turned our attention to reversal of the amide linkage (Fig. 4).¹⁴ Three compounds from our original study were initially selected for comparison and their reverse amide analogs prepared (Table 2).

Reversal of the amide linkage of compound **9**, to give **32**, led to a compound with superior selectivity over the CB_1 receptor (CB_1/CB_2 **9** = 30-fold; CB_1/CB_2 **32** = 380-fold) while retaining good affinity for CB_2 . Similarly, compounds **34** and **36**, when compared to their respective analogs **33, 35**, exhibited superior affinities and selectivities for the CB_2 receptor in in vitro testing. Following the identification of these novel and selective CB_2 agonists, we decided to extend the SAR around the amide functionality. Compounds **37–48** were prepared as shown in Scheme 2.

The synthesis of the morpholinylsulfonamide intermediate **50** was achieved by addition of morpholine to the commercially available nitrophenylsulfonyl chloride **49**. After reduction of the nitro group to give **51**, the target compounds (**32, 34, 36–48**) were generated using a variety of coupling methods.

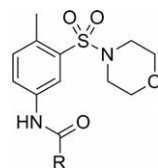
In vitro binding data for compounds **37–48** at the CB_1 and CB_2 receptors is shown in Table 3.



Scheme 2. Reagents and conditions: ¹² (a) Morpholine, EtOAc, 100%; (b) Fe, NH₄Cl, EtOH, H₂O, 100%; (c) RCOOH, Bop-Cl, Et₃N, THF, 20–81%; (d) RCOOH, TBUTU, DIEA, CH₃CN, 3–18%; (e) RCOOH, BEP, DIEA, CH₂Cl₂, 20%; (f) RCOCl, Et₃N, THF, 20–28%.

Table 3

In vitro binding results for reverse amides at the CB₁ and CB₂ receptors^{a,b,c,d}



Compound	R	CB ₁ K _i ^{a,b} (nM)	CB ₂ K _i ^{a,b} (nM)	RatioCB ₁ / CB ₂	CB ₂ EC ₅₀ ^{a,c} (nM)	Compound	R	CB ₁ K _i or % inh. @ 10 μM ^{a,b} (nM)	CB ₂ K _i ^{a,b} (nM)	RatioCB ₁ / CB ₂	CB ₂ EC ₅₀ ^{a,c} (nM)
37		790	11	72	8400	43		4.7%	540	n.d. ^d	n.d. ^d
38		1800	14	130	42	44		270	120	2.3	220
39		470	23	20	29	45		1100	170	6.5	390
40		3400	23	150	11	46		25%	2900	n.d. ^d	n.d. ^d
41		1200	35	34	33	47		2900	660	4.4	n.d. ^d
42		1900	120	16	n.d. ^d	48		44%	2000	n.d. ^d	n.d. ^d

^a Values are the geometric means computed from at least three separate determinations.

^b For assay description see Ref. 13a.

^c EC₅₀ values determined through [³⁵S]GTPγS stimulation. For assay description see Ref. 13b.

^d Not determined.

Attempts to replace the lipophilic amide group of **32** generally led to a decrease in affinity for the CB₂ receptor. For example, in the aryl chloride series of compounds, **44–46**, the affinity for the receptor was decreased by ~100- to 2000-fold compared to compound **32**. Likewise, incorporation of heteroatoms into the amide substituent (**47, 48**) was not tolerated, leading to substantial loss in receptor affinity. However, other lipophilic substituents (**37–41**) were well tolerated and retained good activity for the CB₂ receptor. From this subset of ligands, the tetramethylcyclopropyl compound (**40**) (EC₅₀ = 11 nM, E_{max} = 84%) was determined to possess the best overall profile and was selected for in vivo evaluation in the rodent hindpaw incisional model (Fig. 5).¹⁵

As can be seen from Figure 5, compound **40** exhibited robust antiallodynic activity when administered at a dose of 30 mg/kg ip. However, the compound did not produce a significant antiallodynic effect following oral administration.

To determine whether this lack of efficacy after oral dosing was due to poor absorption or rapid metabolism, a second study was performed. In this study, rats were pretreated with the cytochrome P450 suicide inhibitor aminobenzotriazole (ABT) before administration of compound **40** (300 mg/kg po) (Fig. 6).

The robust activity of the ABT pretreated animals with compound **40** supported our assertion that the compound was rapidly metabolized. In vitro metabolism studies of compound **40** in the presence of human and rat liver microsomes confirmed these findings (RLM = 2%; HLM = 1% remaining at 30 min).¹⁶

In summary, the SAR of the phenylsulfonamide series of CB₂ ligands was expanded. Replacement of the amide substituent with various heterocycles offered no improvement in potency or selectivity for the CB₂ receptor over the CB₁ receptor. However, reversal of the amide linkage led to a series of sulfamoyl benzamides with improved affinity and selectivity for the CB₂ receptor. Optimization

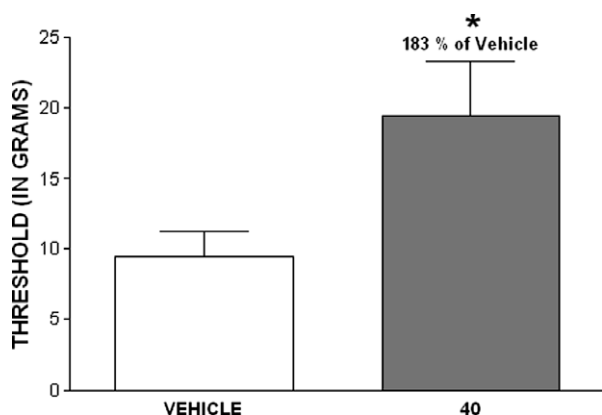


Figure 5. In vivo activity of compound **40** in the hindpaw incision model. Mechanical paw-withdrawal thresholds for the left hindpaw of the hindpaw incision group and compound **40** treated group. Data are plotted as the mean ($\pm$ SEM) paw-withdrawal threshold of the left paw for each group. $p < 0.05$ compared to vehicle-treated group. $N = 8$ /group. Vehicle response 9.4 g. Drugs were administered 30 min before testing.

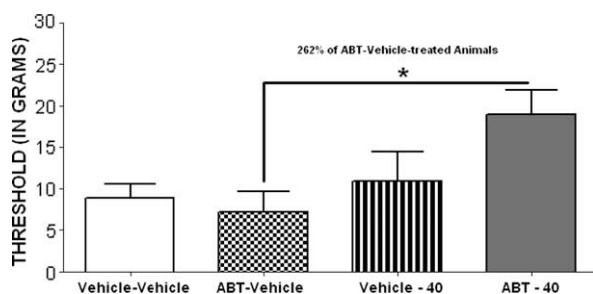


Figure 6. In vivo activity of orally administered compound **40** in the presence and absence of ABT in the hindpaw incision model. Mechanical paw-withdrawal thresholds for the left hindpaw of the hindpaw incision group, ABT, compound **40** and ABT/compound **40** treated groups. Data are plotted as the mean ($\pm$ SEM) paw-withdrawal threshold of the left paw for each group. All statistical analyses were performed with one-way ANOVA followed by post-hoc comparisons (protected t -test) among groups. $p < 0.01$ compared to ABT-vehicle treated hindpaw incised animals. $N = 8$ /group. Vehicle response 8.9 g. Drugs were administered 120 min before testing.

of this scaffold afforded compound **40**. In vivo efficacy of **40** was demonstrated in the rodent hindpaw incisional model after intra-peritoneal administration. Lack of activity after oral dosing of **40** was attributable to the rapid metabolism of the compound. Attempts to improve the metabolic stability of these CB₂ selective compounds will be reported in due course.

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- (a) Abbreviations: TBUT, O-(Benzotriazol-1-yl)-N, N, N', N'-tetramethyluronium tetrafluoroborate; BEP, 2-Bromo-1-ethylpyridinium tetrafluoroborate; Bop-Cl, Bis(2-oxo-3-oxazolidinyl)phosphonic chloride; DIEA, diisopropylethylamine; DBU, 1, 8-diazabicyclo[5.4.0]undec-7-ene; (b) compounds were fully characterized by ¹H NMR and LC/MS.
- (a) Binding assays were performed by modification of the method of Pinto, J. C.; Potie, F.; Rice, K. C.; Boring, D.; Johnson, M. R.; Evans, D. M.; Wilken, G. H.; Cantrell, C. H.; Howlett, A. *Mol. Pharmacol.* **1994**, *46*, 516–522: Receptor binding assays were performed by incubating 0.2–0.6 nM [³H]CP55940 with membranes prepared from cells expressing cloned human CB₁ or CB₂ receptors in buffer consisting of 50 mM Tris-HCl, pH 7.0, 5.0 mM MgCl₂, 1.0 mM ethylene glycol-bis(2-aminoethyl)ether-N,N,N',N'-tetraacetic acid (EGTA), and 1.0 mg/ml fatty acid free bovine serum albumin. After incubation for 60 min at room temperature for CB₂ binding or 120 min at 30 °C for CB₁ binding, the assay mixtures were filtered through GF/C filters that had been pre-soaked overnight in 0.5% (w/v) poly(ethyleneimine) and 0.1% BSA in water. The filters were rinsed 6 times with one mL each of cold assay buffer, 30 μ L of MicroScint 20 (Perkin-Elmer) was added to each filter and the radioactivity on the filters was determined by scintillation spectroscopy in a TopCount (Perkin-Elmer). Nonspecific binding was determined in the presence of 10 μ M WIN55212-2. (b) The [³⁵S]GTP γ S binding method is a major modification of the method by Selley, D. E.; Stark, S.; Sim, L. J.; Childers, S. R. *Life Sci.* **1996**, *59*, 659–668: CB₂-mediated stimulation of [³⁵S]GTP γ S binding was measured in a mixture containing 100–150 pM [³⁵S]GTP γ S, 150 mM NaCl, 45 mM MgCl₂, 3 μ M GDP, 0.4 mM dithiothreitol, 1.0 mM EGTA, 1.0 mg/mL fatty acid free bovine serum albumin, 25 μ g of membrane protein, and agonist in a total volume of 250 μ L of 50 mM Tris-HCl buffer, pH = 7.0 in 96-well Basic FlashPlates (PerkinElmer). After incubation at room temperature for 6 h, the plates were centrifuged at 800g at 4 °C for 5 min and the radioactivity bound to the membranes was determined by scintillation spectrometry using a TopCount (PerkinElmer). The extent of stimulation over basal [³⁵S]GTP γ S binding was calculated as a percentage of the stimulation by 10 μ M WIN55212-2. Basal [³⁵S]GTP γ S binding was determined in the absence of agonist. Generally, the stimulation by 10 μ M WIN55212-2 was between 50% and 100% over basal binding. Full agonists stimulate binding to the same maximal extent as WIN55212-2.
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