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5-Benzyloxytryptamine as an antagonist of TRPM8

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ABSTRACT

5-Benzyloxytryptamine **19** was found to act as an antagonist of the TRPM8 ion-channel. For example, **19** had an IC_{50} of 0.34 μM when menthol was used as the stimulating agonist. Related commercially-available tryptamine derivatives showed diminished, or no antagonist activity at TRPM8. The structural similarity of 5-benzyloxytryptamine to other literature TRPM8 antagonists was noted.

Menthol **1**, a small molecule terpenoid from peppermint oil, has been known to elicit a sensation of cold in human subjects for many decades.[1] In the 1970s, structure–activity studies around menthol and other cooling agents in human volunteers, led to the concept that such molecules acted through a drug-receptor mechanism at neurons responsible for sensing cold temperatures.[2,3] This prediction was confirmed in 2002 when the research groups of Julius[4] and Patapoutian[5] identified that menthol and other cooling agents, such as icilin **2**[4] and eucalyptol **3**,[4] stimulated an ion-channel belonging to the transient receptor potential (TRP) superfamily. Julius named this ion-channel the cold menthol receptor (CMR), as cold temperature was also found to act as an activating stimulus. However, researchers at Dendreon had previously identified this same ion-channel in prostate and other tumor cell lines (naming it Trp-p8); homology to the melastatin subtype of TRP ion-channels was also recognized.[6] Thus, in 2002 the CMR/Trp-p8 was renamed TRPM8 (transient receptor potential melastatin 8).[7] Subsequent experimentation has shown that activation (agonism) of TRPM8 may be useful in the treatment of prostate cancer[8] and benign prostate hyperplasia (BPH),[9] while knockdown of TRPM8 activity with genetic ablation studies, or small molecule antagonists, has shown a role in pain, inflammation and bladder conditions.[10,11] Thus, academic and industrial research groups have been interested in identifying agonists and antagonists of TRPM8. For example, in addition to menthol **1**, icilin **2**, and eucalyptol **3**, other TRPM8 agonists include WS-12 **4**, WS-148 **5**, WS-30 **6** and menthol-derived amide **7** (Fig. 1).[12,13] Indeed, a small molecule TRPM8 agonist, known as D3263, has now advanced in to human clinical trials for the treatment of prostate cancer.[14]

Antagonists of TRPM8 include some menthane derivatives such as **8**,[15] the marketed antimycotics clotrimazole **9**[16] and econazole **10**,[17] and ligands such as BCTC **11**,[18] capsazepine **12**[19] and SB-452533 **13**,[20] that were originally described as potent antagonists of the TRPV1 ion-channel, a close molecular cousin of TRPM8. Recently, researchers from Bayer,[21] Glenmark Pharmaceuticals,[22] Janssen[23] and Amgen[24] have described alternative TRPM8 antagonists, exemplified by compounds **14** to **18** (Fig. 1).[3] In this Letter we show that 5-benzyloxytryptamine also acts as an antagonist of TRPM8, and describe some important determinants of TRPM8 activity by undertaking a limited structure–activity study with related commercially-available tryptamine derivatives.

Screening of our corporate compound collection identified a number of diverse chemotypes as antagonists of TRPM8. Among the compounds identified as a TRPM8 antagonist was 5-benzyloxytryptamine **19** (Table 1), a known ligand of 5-hydroxytryptamine receptors.[25] Since the tryptamine substructure is well-known in many substances with pharmacological activity, we decided to examine a small number of related commercially-available compounds in order to delineate some important determinants of TRPM8 activity. In total, eleven compounds were evaluated for their potential to serve as an antagonist of TRPM8.[26]

Compounds were screened against human TRPM8 (hTRPM8), stably expressed in T-REx™-293 cells, by quantifying the ability to block the influx of ^{45}Ca in response to stimulation with a

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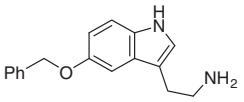
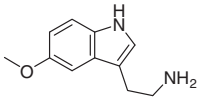
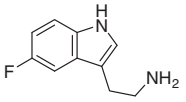
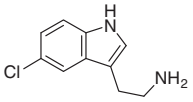
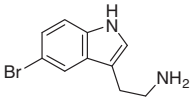
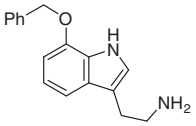
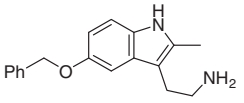
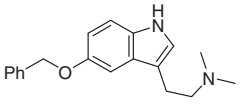
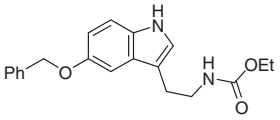
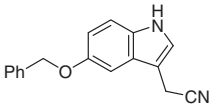
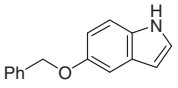
Table 1
TRPM8 antagonist activity of select commercial tryptamines

Entry	Compound	Structure	hTRPM8 IC_{50}[a,b] (μM)	hTRPM8 IC_{50}[c] (μM)
1	**19**		0.34 ± 0.05	0.82 ± 0.19
2	**20**		12.4 (*n* = 1)	11.6 (*n* = 1)
3	**21**		12.3 (*n* = 1)	13.1 (*n* = 1)
4	**22**		21.5 ± 0.4	32.2 ± 0.6
5	**23**		22.7 ± 4.7	30.4 (*n* = 1)
6	**24**		3.34 ± 0.90	8.93 ± 0.94
7	**25**		1.50 ± 0.10	2.36 ± 0.19
8	**26**		5.63 ± 1.86	5.96[d]
9	**27**		21.6 ± 0.7	28.8 (*n* = 1)
10	**28**		Not active	-
11	**29**		Not active	-

[a] Values are means of two or three experiments (standard error is given in brackets).
[b] Menthol at an EC_{50} concentration used as the TRPM8-stimulating agonist.
[c] Icilin at an EC_{50} concentration used as the TRPM8-stimulating agonist.
[d] Incomplete inhibition observed.

TRPM8-activating ligand (menthol or icilin). The results from our experiments can be seen in Table 1. Thus, 5-benzyloxytryptamine **19** (entry 1) was shown to be a potent antagonist of TRPM8, with an IC_{50} of 0.34 μM, when menthol at an EC_{50} concentration was used as a stimulating agonist.[27] Replacing the benzylether in compound **19** with a methyl ether reduced the TRPM8 antagonist activity dramatically, thus demonstrating the importance of a phenyl ring to the activity of compound **19** (compare entries 1 and 2). Other 5-substituted derivatives such as the 5-fluoro, 5-chloro or 5-bromo congeners also gave compounds with much diminished antagonist activity (entries 3–5). The importance of the substitution pattern around the indole ring was illustrated by testing 7-benzyloxytryptamine **24** (entry 6). This compound was approximately 10-fold less active than the 5-benzyloxytryptamine parent **19** (compare entry 6 to entry 1). The effect of other substituents on the indole ring was also illustrated by adding a methyl group to the 2-position. Again, activity was somewhat diminished when compared to the unsubstituted parent **19** (compare entry 7 to entry 1). Finally, the importance of the amino group in compound **19** was illustrated by testing *N*,*N*-dimethyl, ethyl carbamate, nitrile or unsubstituted analogues (entries 8–11). Again, activity was severely diminished, or abolished, when compared with the parent compound (compare entries 8–11 to entry 1). The antagonist activity of compounds when icilin **2** was used as the TRPM8-stimulating agonist is also shown in Table 1. For most compounds, antagonist activity was shifted to lower potencies when icilin was employed in the experiments. As IC_{50} values are determined using one concentration of agonist, they do not necessarily reflect the significant

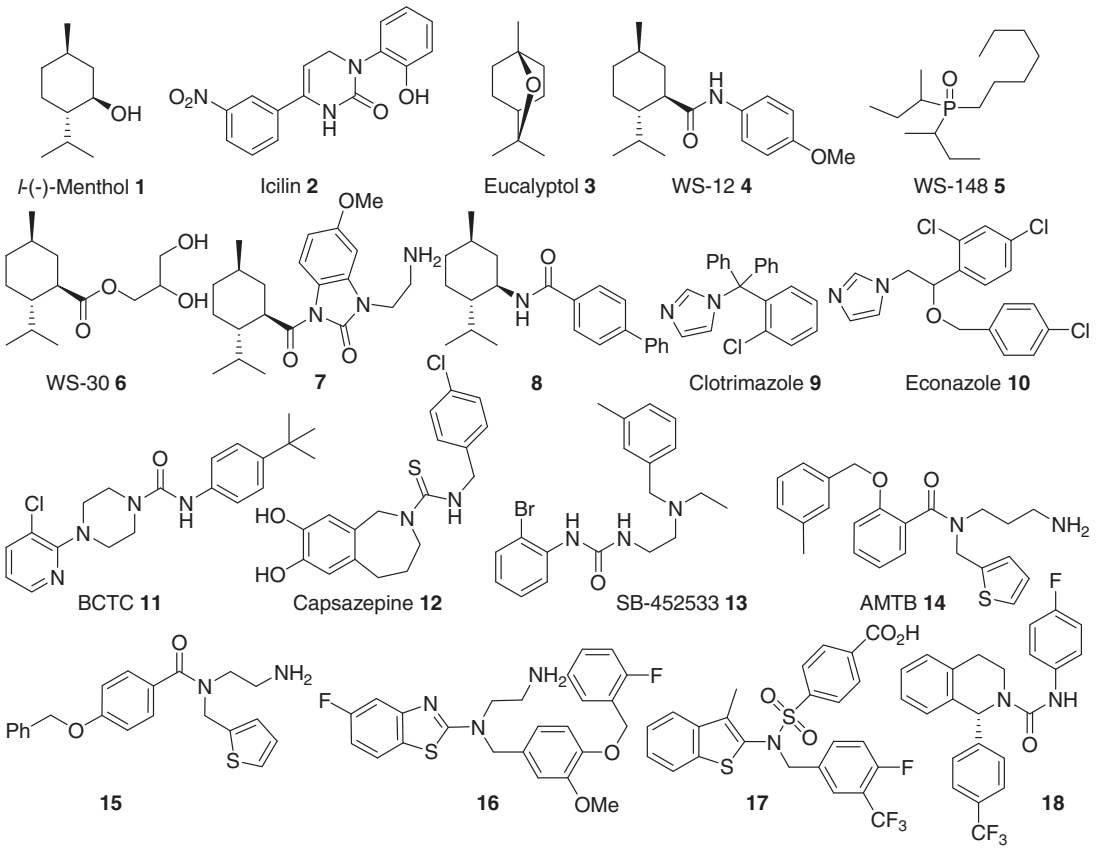
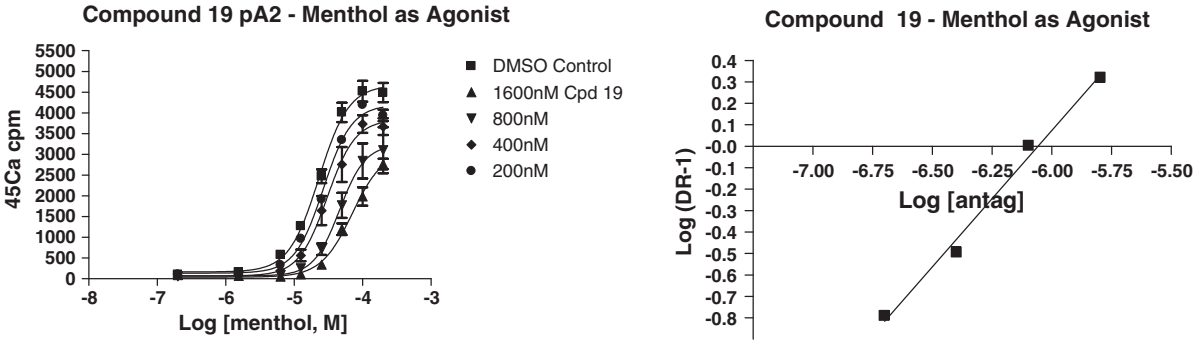


Figure 1. Representative agonists and antagonists of TRPM8.

a. pA2 for 5-benzyloxytryptamine **19** using menthol as a TRPM8-stimulating agonist (corresponding A2 value = 0.91 ± 0.11 μM)



b. pA2 for 5-benzyloxytryptamine **19** using icilin as a TRPM8-stimulating agonist (corresponding A2 value = 0.66 ± 0.05 μM)

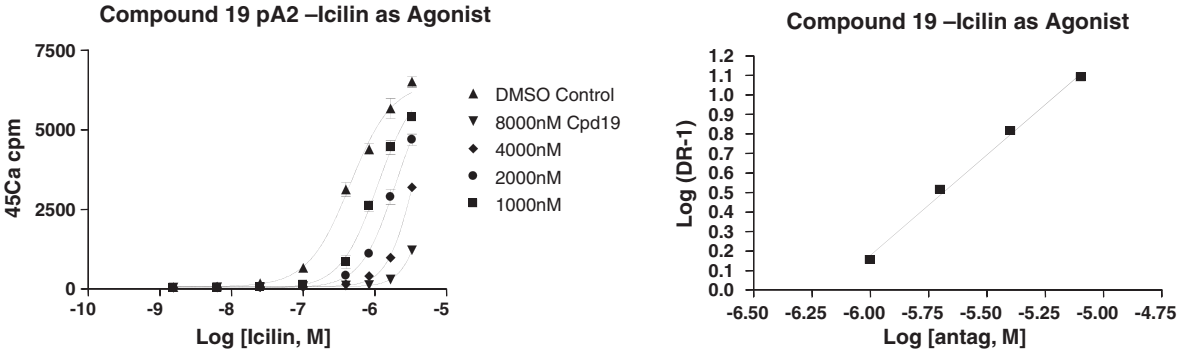


Figure 2. pA2 and A2 determination for 5-benzyloxytryptamine **19** using menthol and icilin as TRPM8-stimulating agonists.

differences between the respective effects of menthol and icilin on TRPM8 activation (icilin is 2.5-fold more efficacious and 200-fold more potent than menthol at the channel).[4]

To account for the unique dose–response curve of each agonist, the effect of multiple concentrations of 5-benzyloxytryptamine **19** were tested for their effect on multiple concentrations of each agonist. Interestingly, the subsequently calculated A2 values reveal **19** to be a slightly more potent inhibitor of icilin-activation than menthol-activation. Specifically, **19** had an A2 value of 0.66 ± 0.05 μM against icilin-stimulated TRPM8 and an A2 value of 0.91 ± 0.11 μM against menthol-stimulated TRPM8 (Fig. 2). By way of comparison, we found that AMTB **14**, a literature TRPM8 antagonist[21] that has shown activity in a rat model of urinary incontinence,[11] was somewhat more potent against TRPM8 than 5-benzyloxytryptamine **19**, with A2 values of 0.16 μM (menthol) and 0.19 μM (icilin).[28] From a chemical standpoint, it is interesting to note the structural similarities between 5-benzyloxytryptamine **19**, AMTB **14** and related structures such as compound **15**. All of these TRPM8 antagonists possess an aromatic, or heteroaromatic core, decorated with a benzyl ether and an appropriately-positioned amine side-chain. The similarities in chemical structure between compounds **19**, **14**, and **15** may indicate a similarity in the manner in which these compounds bind to TRPM8. However, detailed studies would need to be undertaken in order to substantiate this inference. Finally, we tested compound **19** against a small panel of TRP-receptors, and found no significant inhibition when **19** was tested against TRPV1, TRPV4 or TRPA1 at concentrations up to 10 μM.

In conclusion, this Letter describes the use of 5-benzyloxytryptamine **19** as an antagonist of the TRPM8 receptor. The importance of a phenyl ring and ethylamine side-chain to the antagonist activity of compound **19** was illustrated by undertaking some limited SAR studies with related commercially-available tryptamines. Similarities between the chemical structure of 5-benzyloxytryptamine **19** and that of other TRPM8 antagonists, such as literature antagonists **14** and **15** were also noted, and may imply that such compounds act at a common site on the TRPM8 receptor. However, further studies will be needed in order to confirm, or refute, this hypothesis.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.099.

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26. In addition to the compounds detailed in Table 1, a number of marketed 5-HT_{1D} tryptamine derivatives were examined as TRPM8 antagonists. All triptans tested were devoid of TRPM8 activity.
27. Electrophysiology experiments suggested that compound **19** directly inhibited the TRPM8 ion-channel. For example, no effect on the inward current was observed when tryptamine was tested alongside compound **19**. See Supplementary data for details.
28. See Supplementary data for details.