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Modulation of thermo-transient receptor potential (thermo-TRP) channels by thymol-based compounds

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ABSTRACT

A series of thirty-three thymol, *p*-cymene-3-carboxylic acid, and 3-amino-*p*-cymene derivatives was synthesized and tested on TRPA1, TRPM8, and TRPV3 channels. Most of them acted as strong modulators of TRPA1, TRPM8, and TRPV3 channels with EC_{50} and/or IC_{50} values distinctly lower than those of thymol and related monoterpenoids. Some of the compounds examined, that is, **3c**, **4e**, **f**, **6b**, and **8b** exhibited an appreciable subtype-selectivity.

The transient receptor potential (TRP) family of ion channels are cation-permeable ion channels divided into six mammalian subfamilies based on sequence homology and denoted TRPA (Ankyrin), TRPC (Canonical), TRPM (Melastatin), TRPML (Mucolipin), TRPP (Polycystin), and TRPV (Vanilloid).[1] They are involved in a wide array of physiological functions including vision, taste, olfaction, hearing, touch, and thermo- and osmosensation and have emerged as important drug targets in pathological conditions such as pain, cancer, and bone development, kidney, and respiratory disorders.[2] Several TRP channels act as polymodal sensors that are activated by physical stimuli such as temperature as well as chemical stimuli, a property that is exploited by several natural product derived ligands.[3] Indeed, naturally occurring modulators, in particular monoterpenoids, have now been reported for most thermo-TRP channels, that is, TRP channels activated by distinct temperature thresholds,[2c,4] specifically TRPA1, TRPM8, and TRPV1-4 (Fig. 1). Thermo-TRPs are however generally not highly specific for one given natural ligand, but are activated by a number of chemically related monoterpenoid agonists and overlapping features have been identified in agonist profiles of thermo-TRPs, such as TRPA1, TRPM8, TRPV1, and TRPV3.

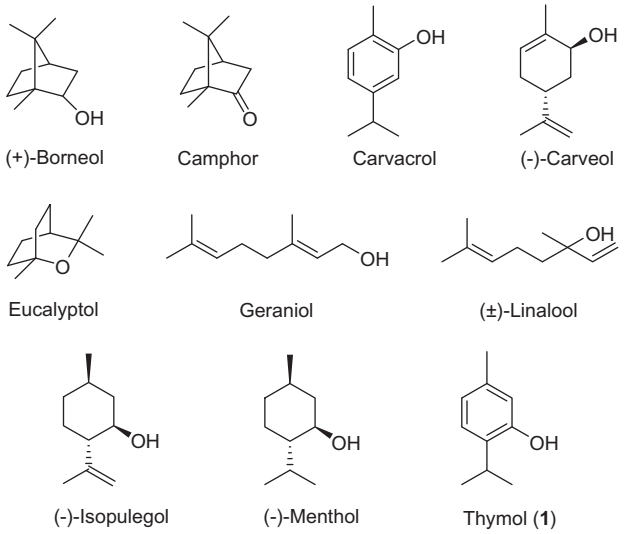

Figure 1. Structures of some monoterpenoid modulators of thermo-TRPs.

In this respect, thymol (**1**), an important phenolic monoterpene component of oils derived from thyme (*Thymus vulgaris*) and oreg-

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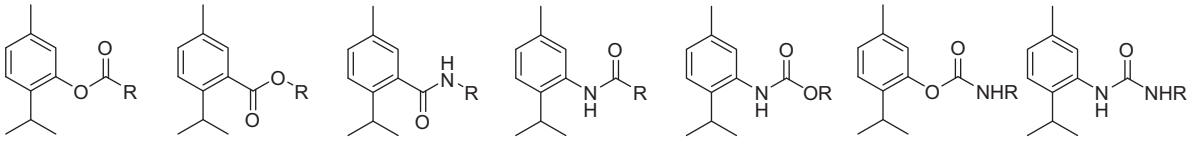
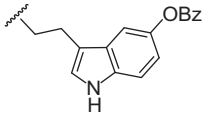
Table 1
Results of TRPA1, TRPM8, and TRPV3 assays of thymol-based compounds **2**–**8**, thymol (**1**), and 3-amino-*p*-cymene hydrochloride (**10**)[a]



2 **3** **4** **5** **6** **7** **8**

Compd	*R*	TRPA1 (efficacy)[b]	TRPA1 (EC_{50}, μM)	TRPA1 (IC_{50}, μM)[c]	TRPM8 (efficacy)[d]	TRPM8 (EC_{50}, μM)	TRPM8 (IC_{50}, μM)[e]	TRPM8 (IC_{50}, μM)[f]	TRPV3 (efficacy)[d]	TRPV3 (EC_{50}, μM)	TRPV3 (IC_{50}, μM)[g]
2a	Ph-4-Me	205.7 ± 17.9	3.1 ± 1.3	8.1 ± 1.0	<10	ND	5.2 ± 0.7	3.6 ± 0.4	55.5 ± 1.2	5.7 ± 0.6	6.9 ± 2.2
2b	Ph-4-*t*-Bu	194.1 ± 3.9	12.7 ± 1.3	11.9 ± 1.1	<10	ND	42.7 ± 4.3	57.8 ± 0.6	17.7 ± 0.3	21.4 ± 0.9	27.8 ± 2.2
2c	Ph-4-Ph	204.4 ± 10.7	3.3 ± 0.7	5.5 ± 0.3	<10	ND	21.2 ± 1.0	18.6 ± 0.7	33.8 ± 2.6	7.7 ± 1.5	6.5 ± 0.8
2d	Ph-4-OMe	149.3 ± 1.1	1.0 ± 0.2	2.7 ± 0.3	<10	ND	6.5 ± 0.3	5.0 ± 0.3	23.1 ± 0.4	4.9 ± 0.3	9.7 ± 1.5
2e	CH_2Ph-4-Ph	225.7 ± 23.8	1.8 ± 1.0	20.9 ± 6.0	<10	ND	>100	>100	52.7 ± 3.7	3.8 ± 1.3	1.5 ± 0.3
3a	Ph-4-*t*-Bu	244.7 ± 42.6	14.1 ± 10.4	>100	<10	ND	>100	>100	25.3 ± 0.4	12.2 ± 0.6	>100
3b	Ph-4-Ph	195.2 ± 27.2	7.4 ± 2.8	12.7 ± 0.7	<10	ND	>100	>100	64.3 ± 6.4	14.5 ± 5.9	2.9 ± 0.5
3c	Ph-4-Cl	259.6 ± 46.1	4.4 ± 3.1	9.2 ± 4.2	<10	ND	>100	>100	42.3 ± 0.5	20.9 ± 0.8	16.6 ± 4.3
3d	Ph-4-OMe	194.2 ± 7.4	3.6 ± 0.5	3.6 ± 1.2	<10	ND	>100	77.4 ± 1.6	27.1 ± 1.2	4.0 ± 1.0	5.0 ± 1.2
4a	Ph-4-*t*-Bu	97.9 ± 5.4	0.6 ± 0.1	0.84 ± 0.07	40.0 ± 2.5	0.6 ± 0.3	1.9 ± 0.2	1.2 ± 0.1	36.5 ± 3.6	1.2 ± 0.4	1.1 ± 0.2
4b	Ph-4-CF_3	65.4 ± 8.7	3.9 ± 2.4	3.6 ± 0.8	13.9 ± 1.3	45.8 ± 23.4	3.2 ± 0.1	4.4 ± 0.1	22.1 ± 0.6	59.7 ± 10.9	75.1 ± 4.4
4c	Ph-4-Ph	194.1 ± 3.9	11.1 ± 0.9	3.4 ± 0.8	<10	ND	12.3 ± 1.4	3.4 ± 0.3	40.9 ± 1.0	3.6 ± 0.4	4.0 ± 0.8
4d	Ph-4-Cl	<10	ND	26.15 ± 4.0	43.2 ± 1.0	0.85 ± 0.2	4.8 ± 0.4	5.0 ± 0.15	43.4 ± 2.2	11.8 ± 2.8	4.9 ± 2.2
4e	Ph-4-OMe	35.3 ± 0.1	12.4 ± 0.1	60.0 ± 0.5	65.3 ± 1.5	1.7 ± 0.2	3.4 ± 0.2	2.8 ± 0.4	<10	ND	59.5 ± 1.1
4f	CH_2CH_2Ph	<10	ND	>100	64.1 ± 0.5	0.27 ± 0.01	0.6 ± 0.01	0.32 ± 0.01	<10	ND	75.4 ± 1.6
4g		241.1 ± 5.3	25.9 ± 3.2	70.2 ± 5.6	<10	ND	69.4 ± 1.9	61.4 ± 0.1	<10	ND	41.9 ± 6.5
5a	Ph-4-*t*-Bu	204.4 ± 10.7	6.4 ± 1.6	8.8 ± 0.6	24.5 ± 3.1	0.44 ± 0.14	1.0 ± 0.1	1.0 ± 0.1	34.8 ± 1.8	10.6 ± 2.8	6.2 ± 0.7
5b	Ph-4-Ph	59.2 ± 6.3	11.3 ± 4.7	17.9 ± 5.9	<10	ND	0.9 ± 0.05	4.1 ± 0.8	43.2 ± 2.6	0.8 ± 0.3	2.5 ± 0.6
5c	Ph-4-OMe	149.3 ± 1.1	45.9 ± 0.6	>100	58.1 ± 1.2	4.8 ± 0.4	14.7 ± 0.8	15.2 ± 0.4	<10	ND	>100
5d	CH_2Ph-4-Ph	212.3 ± 4.5	6.5 ± 0.5	3.9 ± 0.3	<10	ND	31.2 ± 4.0	78.3 ± 8.4	39.3 ± 0.1	6.3 ± 0.1	29.7 ± 3.9
5e	CH_2Ph-3-I	154.3 ± 29.8	20.5 ± 11.3	43.2 ± 4.0	<10	ND	11.6 ± 1.4	14.1 ± 1.9	15.0 ± 0.1	15.9 ± 0.1	20.4 ± 1.7
5f	$(CH_2)_7$Ph	93.4 ± 7.3	0.8 ± 0.3	1.4 ± 0.3	<10	ND	0.3 ± 0.03	0.9 ± 0.3	<10	ND	39.5 ± 11.2
6a	Ph-4-*t*-Bu	132.1 ± 3.1	1.3 ± 0.2	0.78 ± 0.04	<10	ND	0.7 ± 0.1	0.85 ± 0.04	62.5 ± 1.0	1.4 ± 0.2	1.9 ± 0.3
6b	Ph-4-CF_3	178.3 ± 17.7	41 ± 16	4.1 ± 1.3	<10	ND	23.1 ± 4.0	73.7 ± 6.4	<10	ND	50.3 ± 4.2
6c	Ph-3-Ph	162.8 ± 2.7	4.7 ± 0.4	7.2 ± 0.6	<10	ND	5.8 ± 1.0	13.9 ± 1.6	32.2 ± 1.3	34.3 ± 4.9	40.9 ± 0.6
6d	Ph-4-Ph	175.9 ± 4.6	12.7 ± 1.2	4.3 ± 0.3	<10	ND	6.0 ± 0.9	16.2 ± 0.9	64.8 ± 0.2	5.2 ± 0.1	9.6 ± 0.8
6e	Ph-4-OMe	159.5 ± 5.3	34.5 ± 4.2	25.7 ± 0.2	<10	ND	9.8 ± 0.2	10.4 ± 0.4	<10	ND	59.7 ± 8.7
7a	Ph-4-*t*-Bu	145.2 ± 12.9	4.1 ± 1.8	24.4 ± 3.8	<10	ND	11.8 ± 1.8	6.1 ± 0.2	53.4 ± 0.9	2.5 ± 0.3	2.8 ± 0.8
7b	Ph-4-Ph	133.6 ± 13.1	4.8 ± 2.0	16.8 ± 2.2	<10	ND	31.7 ± 2.7	11.7 ± 3.1	27.6 ± 1.5	15.1 ± 2.6	92.5 ± 30.9
7c	Ph-4-OMe	263.6 ± 12.2	7.4 ± 1.5	4.5 ± 0.2	30.3 ± 0.2	5.1 ± 0.5	2.5 ± 0.4	5.2 ± 0.3	<10	ND	64.3 ± 14.3
8a	Ph-4-*t*-Bu	234.7 ± 18.6	4.3 ± 2.1	25.0 ± 5.8	<10	ND	>100	26.7 ± 1.3	31.6 ± 5.0	7.6 ± 5.2	3.3 ± 0.9
8b	Ph-4-Ph	223.9 ± 54.2	32.2 ± 26.3	27.9 ± 5.1	<10	ND	37.4 ± 0.4	25.4 ± 1.3	16.9 ± 0.1	5.9 ± 0.1	12.8 ± 2.4
8c	Ph-4-OMe	114.1 ± 2.3	14.1 ± 0.9	34.4 ± 1.6	<10	ND	38.5 ± 3.4	77.5 ± 1.8	<10	ND	85.4 ± 8.2
1		118.7 ± 9.6	286.6 ± 86.3	125.4 ± 0.5	31.15	52.2	314.6 ± 8.9	169.1 ± 12.5	34.7 ± 0.2	84.1 ± 1.6	—
10		57.4 ± 0.6	64.2 ± 1.4	212.3 ± 8.0	33.8 ± 2.3	43.4 ± 6.6	238.0 ± 1.3	113.4 ± 2.8	<10	ND	>100

[a] Data are means ± SEM of $N = 3$ determinations.
[b] As percent of allyl isothiocyanate (100 μM).
[c] Determined against the effect of allyl isothiocyanate (100 μM).
[d] As percent of ionomycin (4 μM).
[e] Determined against the effect of icilin (0.25 μM).
[f] Determined against the effect of menthol (20 μM).
[g] Determined against the effect of thymol (100 μM). ND, not determined when efficacy is lower than 10%.

ano (*Origanum vulgare*), behaves as a promiscuous TRP agonist, activating TRPA1, TRPM8, and TRPV3 channels.[2c,5] The evaluation of the ability of eight thymol-related alkyl-substituted phenols to activate hTRPA1 revealed that the potency of these compounds generally diminished with decreasing log*P* values, while the presence of sterically hindered and branched alkyl groups at the ortho-position of the seemingly essential hydroxyl group increased potency.[5a] Taken together, the SAR data suggest that thymol and related alkylphenols might bind in a hydrophobic pocket to activate TRPA1 via a non covalent mechanism.

In a recent work we have disclosed a series of (−)-menthylamine derivatives acting as potent TRPM8 antagonists with IC_{50} values similar or lower than those of previously reported unselective antagonists.[6] Since identification of monoterpenoid pharmacophore through systematic SAR approaches can be expected to lead to the individuation of TRP subtype-selective modulators and, perhaps, of novel potential treatment options for TRP 'channelopathies', we aimed at further investigating the pharmacological profile of some thermo-TRPs by synthesizing and testing on TRPA1, TRPM8, and TRPV3 channels a series of compounds based on the