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Synthesis and biological evaluation of [6]-gingerol analogues as transient receptor potential channel TRPV1 and TRPA1 modulators

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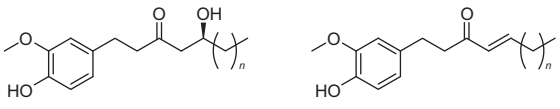
ABSTRACT

In order to explore the structural determinants for the TRPV1 and TRPA1 agonist properties of gingerols, a series of nineteen analogues (**1b**–**5**) of racemic [6]-gingerol (**1a**) was synthesized and tested on TRPV1 and TRPA1 channels. The exploration of the structure–activity relationships, by modulating the three pharmacophoric regions of [6]-gingerol, led to the identification of some selective TRPV1 agonists/desensitizers of TRPV1 channels (**3a**, **3f,** and **4**) and of some full TRPA1 antagonists (**2c**, **2d**, **3b**, and **3d**).

Ginger (*Zingiber officinale* Roscoe), one of the world's favourite spices in culinary preparations, is also widely used in traditional oriental herbal medicine to treat various kinds of diseases such as asthma, catarrh, muscle ache, rheumatism, nervous diseases, headache, bacterial and fungal infections, diabetes, and other ailments.[1] Recent research confirmed the pharmacological potential of ginger, in particular its antioxidant, anticancer, antimicrobial, and anti-inflammatory properties.[2] The main active and pungent constituents of this plant, identified in the nonvolatile oleoresin fraction of processed ginger, are gingerols and shogaols (Fig. 1). Molecular targets involved in their therapeutic properties encompass matrix metalloproteinases MMP-2 and MMP-9,[3] nuclear-factor-*k*B (NF-*k*B),[4] cyclooxygenase (COX) and lipooxygenase (LOX),[5] and peroxisome proliferator-activated receptor (PPARγ).[6] In addition, gingerols and shogaols have been reported to activate the transient receptor potential channels, TRPV1[7] and TRPA1,[8] which belong respectively to the TRPV (vanilloid) and TRPA (ankyrin) subfamilies of the large transient receptor potential (TRP) cation channel family.[9] TRPV1 and TRPA1 channels display a wide diversity of activation and regulation by temperature, flavours, mechanical and osmotic input, and noxious chemical stimuli and play a fundamental role in the pathophysiology of many illnesses like pain, cancer, and bladder, skin, and respiratory disorders.[10]

The best known of the exogenous chemical stimuli for TRPV1, capsaicin (Fig. 2), that is, the pungent component of hot chilli peppers, shares the vanillyl moiety with gingerols and shoagols.[9e,10d,i] In the case of TRPA1 agonists, in addition to several exogenous electrophiles that activate the channel through covalent binding to critical cysteine residues at the intra-cellular domain, there is a number of TRPA1 agonists that specifically bind to and activate the channel via non-covalent ligand–receptor interactions.[10b] Several phenolic derivatives (Fig. 2) belong to this latter group and, on the basis of SAR analyses, an increase of potency correlated with high log*P* values and an enhancement of steric hindrance of branched alkyl substituents in the *ortho* position of the phenolic hydroxyl group was observed.[11]

Despite remarkable interest for the major gingerol, [6]-gingerol, which is deemed of prime importance for the pharmacological



n = 4, (+)-(*S*)-[6]-Gingerol
n = 6, (+)-(*S*)-[8]-Gingerol
n = 8, (+)-(*S*)-[10]-Gingerol

n = 4, [6]-Shogaol
n = 6, [8]-Shogaol
n = 8, [10]-Shogaol

Figure 1. Structures of gingerols and shogaols.

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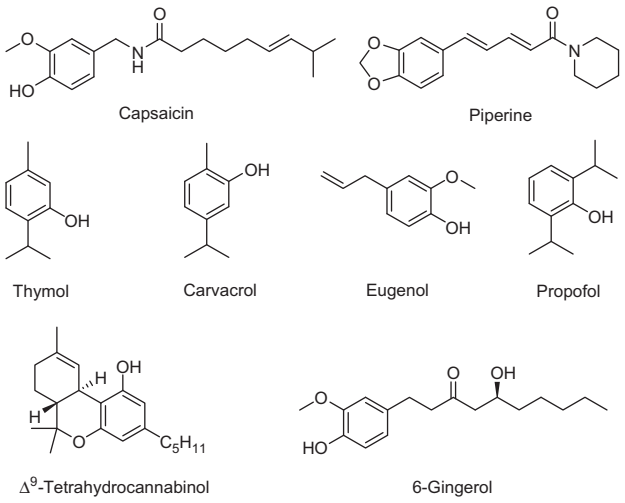


Figure 2. Structures of TRPV1 agonists capsaicin and piperine and of some phenolic modulators of TRPA1.

activities of ginger,[12] little is known on its structure–activity relationships, in particular with respect to the activity on TRPV1 and TRPA1 channels.[7a,13] Conceivably, the dual action of [6]-gingerol at TRPV1 and TRPA1 channels seems well related to its structural analogy with capsaicin and its phenolic nature, respectively. The [6]-gingerol structure could be ideally divided into three regions, as described for capsaicin by Walpole et al.[14] that is, the aromatic region, the β-hydroxy ketone polar region, and the hydrophobic side chain (Fig. 3). To investigate the possibility to identify new potent and selective modulators of TRPV1 and TRPA1 channels through structure modifications of [6]-gingerol, we synthesized a series of racemic gingerol (**1a**) analogues (compounds **1b–5**), by modifying the three aforementioned regions. Firstly, we assessed the importance of the phenolic hydroxyl group of gingerol and its steric requirements, by selecting, inter alia, aromatic moieties that are either correlated to thymol and propofol, two alkyl phenols active on TRPA1, or lacking the hydroxyl functionality, like the TRPV1 agonist piperine, the major pungent compound from pepper (*Piper nigrum* L.).[15] In the second place, since it was reported that the potency of gingerols as TRPV1 agonists increased with increasing lipophilicity of the side chain,[7b,13] we explored the effect on activity of the introduction of phenylalkyl side chains of different length. Finally, with the aim of evaluating the influence of the hydroxyl group in the β-hydroxy ketone polar linker, we synthesized [6]-shogaol (**4**), and its analogue **5**.

The synthesis of racemic gingerol (**1a**) and of gingerol analogues **1b–3** was performed by a regioselective aldol condensation between appropriate 4-aryl-2-butanones **6** and appropriate aldehydes **7**.[16] In the case of aryl moieties carrying a hydroxyl group, the phenolic functionalities were protected as trimethysilyl ethers before the deprotonation step,[17] and the protecting group was removed at the end of the coupling reaction by a brief treatment with tetrabutylammonium fluoride (TBAF) (Scheme 1). An acid catalyzed dehydration was exploited for the synthesis of shogaol **4** and its analogue **5**.[18] The twenty compounds synthesized here were tested for their ability to induce intracellular Ca^{2+} elevation in HEK293 cells stably transfected with either the human TRPV1 or the rat TRPA1 cDNAs (Table 1).[19] Control experiments were carried out using non-transfected HEK293 cells.

The biological assays underlined the importance of a phenolic hydroxyl group for the activity at TRPV1 channels. In particular, the substitution of a methylenedioxy group for the vanillyl moiety reduced or abolished the activity (compare compounds **1a**, **2a**, and **3a** with **1b**, **2b**, and **3b**, respectively). An analogous result had been previously observed with capsaicin, whereas the opening of the methylenedioxy group of piperine proved to be detrimental to its activity at hTRPV1.[20] Among the phenol derivatives, compounds **1e**, **2e**, **3a**, and **3f** were more potent than [6]-gingerol (**1a**) at activating TRPV1 channels, while **1c**, **2c**, and **3c** were inactive, thus highlighting the negative impact on TRPV1 activity of high steric hindrance around the phenolic hydroxylic group. With regard to the hydrophobic side chain, the potency increased with lipophilicity, with the 6-phenylhexyl derivatives **3a** and **3f** being the most potent and selective TRPV1 activators (EC_{50} = 0.11 ± 0.02 μM and EC_{50} = 0.5 ± 0.2 μM, respectively). An opposite trend was observed for TRPA1 activation, where the

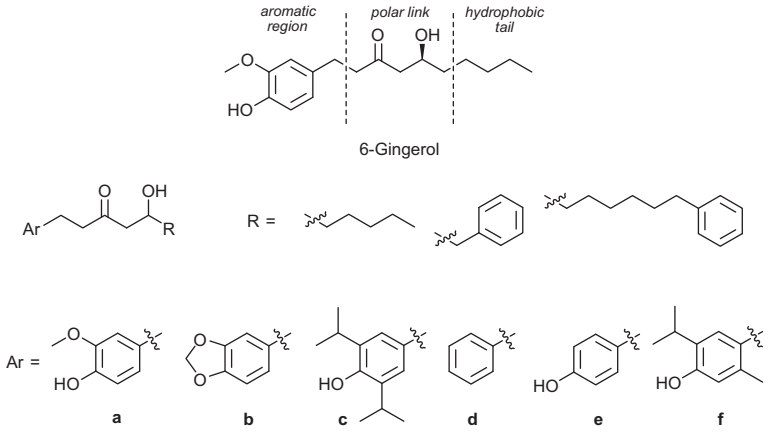
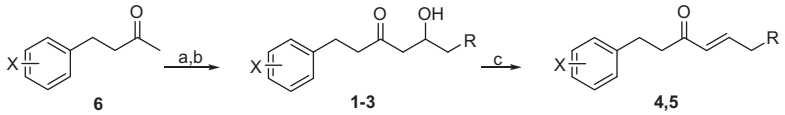
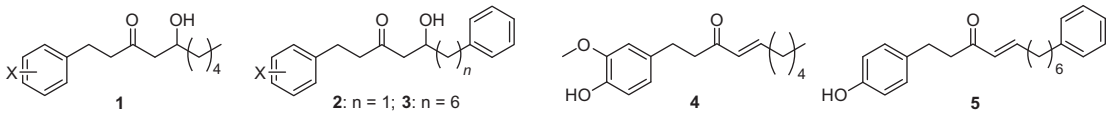


Figure 3. General structure of gingerol analogues synthesized.



Scheme 1. Synthesis of compounds **1–5**. Reagents and conditions: (a) LDA, THF, N_2, −78 °C, 30 min, then RCH_2CHO (**7**), THF, −78 °C, 2 h. (b) TBAF, H_2O, 0 °C, 30 min (in case of Me_3Si-protected phenol derivatives). (c) *p*-TsOH, C_6H_6, reflux, 3 h.

Table 1
Results of TRPV1 and TRPA1 assays of racemic gingerol (**1a**), shogaol (**4**), gingerol (**1b**–**3**), and shogaol (**5**) analogues[a]



Compd	X	TRPV1[b] (efficacy)	TRPV1 (EC_{50}, μM)	TRPV1[c] (IC_{50}, μM)	TRPA1[d] (efficacy)	TRPA1 (EC_{50}, μM)	TRPA1[e] (IC_{50}, μM)
1a (Racemic [6]-gingerol)	3-OMe, 4-OH	51.4 ± 1.7	3.3 ± 0.5	5.0 ± 0.2	11.4 ± 0.1	10.4 ± 0.03	>100
1b	3,4-OCH_2O	<10	ND	>100	<10	ND	>100
1c	3,5-bis *i*-Pr, 4-OH	<10	ND	>50	63.6 ± 3.3	2.1 ± 0.9	2.8 ± 0.3
1d	H	<10	ND	>50	<10	ND	56.1 ± 0.4
1e	4-OH	23.6 ± 0.1	1.2 ± 0.1	10.5 ± 0.2	114.3 ± 1.5	8.4 ± 0.4	22.6 ± 2.4
1f	2-Me, 4-OH, 5-*i*-Pr	<10	ND	4.8 ± 0.1	78.2 ± 1.1	1.1 ± 0.1	3. 9 ± 0.6
2a	3-OMe, 4-OH	56.9 ± 1.6	14.3 ± 2.6	13.2 ± 0.8	<10	ND	97.85 ± 0.02
2b	3,4-OCH_2O	<10	ND	>50	16.5 ± 1.0	ND	14.4 ± 0.6
2c	3,5-bis *i*-Pr, 4-OH	<10	ND	>50	<10	ND	3.3 ± 0.3
2d	H	<10	ND	>50	17.5 ± 1.0	ND	4.5 ± 0.7
2e	4-OH	10.8 ± 0.1	1.0 ± 0.1	33.5 ± 5.8	130.9 ± 2.9	10.7 ± 0.1	35.1 ± 3.4
2f	2-Me, 4-OH, 5-*i*-Pr	<10	ND	35.3 ± 2.3	51.3 ± 0.1	3.5 ± 0.1	39.7 ± 1.5
3a	3-OMe, 4-OH	60.0 ± 1.4	0.11 ± 0.02	0.11 ± 0.02	<10	ND	38.2 ± 4.5
3b	3,4-OCH_2O	<10	ND	>10	<10	ND	2.8 ± 0.6
3c	3,5-bis *i*-Pr, 4-OH	<10	ND	>10	<10	ND	11.1 ± 2.0
3d	H	<10	ND	>10	<10	ND	3.6 ± 0.9
3e	4-OH	43.0 ± 0.3	14.6 ± 0.1	9.4 ± 0.1	<10	ND	15.8 ± 1.6
3f	2-Me, 4-OH, 5-*i*-Pr	33.5 ± 0.8	0.5 ± 0.2	1.3 ± 0.3	163.2 ± 7.3	22.1 ± 4.5	14.3 ± 1.0
4 ([6]-Shogaol)		79.2 ± 0.9	0.32 ± 0.02	0.29 ± 0.02	91.1 ± 2.5	16.0 ± 2.3	16.7 ± 0.4
5		19.7 ± 0.5	0.10 ± 0.01	1.2 ± 0.2	89.2 ± 5.1	2.2 ± 0.8	2.3 ± 0.4
Capsaicin		78.6 ± 2.4	0.0039 ± 0.0004				
Allyl isothiocyanate (AITC)					100		
					41.8 ± 3.8[b]	2.5 ± 0.7	

[a] Data are means ± SEM of $N = 3$ determinations.
[b] As percent of ionomycin (4 μM).
[c] Determined against the effect of capsaicin (0.1 μM).
[d] As percent of allyl isothiocyanate (100 μM).
[e] Determined against the effect of allyl isothiocyanate (100 μM). ND, not determined when efficacy is lower than 10%.

highest activity was exhibited by analogues featuring *n*-hexyl (**1c**, **1e**, **1f**) or benzyl (**2e**, **2f**) side chains. With respect to the aryl substitution, again the phenolic functionality was a structural requirement for efficient activation of TRPA1 but, in contrast with TRPV1, branched alkyl substituents at the *ortho* position of the phenolic hydroxyl group were beneficial for the activity, thus rendering **1c** and **1f** the most potent and selective TRPA1 activators ($EC_{50} = 2.1 \pm 0.9$ μM and 1.1 ± 0.1, respectively). Data obtained with shogaol (**4**) and its analogue **5**, which were shown to potently activate both TRPV1 ($EC_{50} = 0.32 \pm 0.02$ μM and $EC_{50} = 0.10 \pm 0.01$ μM, respectively) and TRPA1 channels ($EC_{50} = 16.0 \pm 2.3$ μM and $EC_{50} = 2.2 \pm 0.8$ μM, respectively), highlighted the marginal importance of the β-hydroxylic group for the activity.

Five-min preincubation of TRPV1–HEK293 cells with compounds **1a**, **1f**, **3a**, **3f**, **4**, and **5**, before stimulation with capsaicin induced desensitization of TRPV1 channel with IC_{50} values <5 μM. An analogous pre-exposure of TRPA1–HEK293 cells, and then continued incubation with allyl isothiocyanate caused inhibition of TRPA1 response to this agonist with IC_{50} values <5 μM for compounds **1c**, **1f**, **2c**, **2d**, **3b**, **3d**, and **5**. In particular, compounds **2c**, **2d, 3b**, and **3d** behaved as rather selective 'true' TRPA1 antagonists, in as much as they inhibited the response to allyl isothiocyanate even though they were devoid of stimulatory activity per se.

In conclusion, in this study, the relationship between activity and structural features of [6]-gingerol was explored, allowing for the identification of some novel potent and selective TRPV1 or TRPA1 modulators. Compounds **3a**, **3f**, and **4**, which act as potent and selective TRPV1 agonists/desensitizers, and compounds **2c**, **2d**, **3b**, and **3d**, which behave as novel selective TRPA1 antagonists, deserve a special mention as they might find potential therapeutic applications against pain, inflammation, and migraine.

Supplementary data

Supplementary data (detailed experimental procedures and characterization data for all products and further details of biochemical assays) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.12.113.

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16. Synthesis of [6]-gingerol (**1a**) as representative procedure for the preparation of compounds **1**–**3**. A solution of 4-(3-methoxy-4-trimethylsilyloxyphenyl)butan-2-one (266 mg, 1.0 mmol) in 1.0 mL of dry THF was added dropwise, over 30 min, to a stirred 1.0 M solution of lithium diisopropylamide in THF (1.5 mL, 1.5 mmol) cooled at −78 °C, under a nitrogen atmosphere. After a further 30 min at −78 °C, hexanal (150 μL, 1.3 mmol) was added, and the mixture was stirred for 2 h at −78 °C. The reaction mixture was warmed to 0 °C, diluted with Et_2O (20 mL), and then treated with a 75% solution of TBAF in H_2O (0.4 mL) for 30 min. The ethereal solution was washed with water (20 mL), brine (20 mL), dried (Na_2SO_4), and evaporated under vacuum. The residue (313 mg) was purified by chromatography on silica gel (31 g) using CH_2Cl_2/AcOEt = 9:1 as eluent to give 150 mg (51%) of **1a** as an.
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18. Synthesis of [6]-shogaol **4** as representative procedure for the preparation of compounds **4** and **5**. A solution of [6]-gingerol (**1a**) (198 mg, 0.67 mmol) in benzene (50 mL) was refluxed in the presence of *p*-toluenesulfonic acid monohydrate (54 mg) for 3 h. After cooling to room temperature, H_2O was added and the mixture was exctracted with AcOEt (50 mL). The organic phase was washed with water (20 mL), saturated aqueous $NaHCO_3$ (20 mL), brine (20 mL), dried (Na_2SO_4), and evaporated under vacuum. The residue (177 mg) was purified by chromatography on silica gel (7 g) using *n*-hexane/AcOEt = 8:2 as eluent to give 108 mg (58%) of **4** as an oil.
19. Cells transfected with either human TRPV1 or rat TRPA1 were loaded with the fluorescent probe 4 μM Fluo-4-AM in EMEM, then were washed twice in Tyrode's buffer and transferred to the quartz cuvette of a spectrofluorimeter. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined before and after the addition of various concentrations of test compounds by measuring cell fluorescence (λ_{EX} = 488 nm, λ_{EM} = 516 nm). Potency was expressed as the concentration of test substances exerting a half-maximal agonist effect (i.e. half-maximal increases in $[Ca^{2+}]_i$) (EC_{50}). The efficacy of TRPV1 agonists was determined by normalizing their effect to the maximum Ca^{2+} influx effect on $[Ca^{2+}]_i$ observed with application of 4 μM ionomycin, while the effects of TRPA1 agonists are expressed as a percentage of the effect obtained with 100 μM allyl isothiocyanate. For both TRPV1 and TRPA1 agonism, the values of the effect on $[Ca^{2+}]_i$ in wild type HEK293 (i.e., not transfected with any TRP construct) were taken as baselines and subtracted from the values obtained from transfected cells. Antagonist/desensitizing behaviour was evaluated against capsaicin (0.1 μM) for TRPV1 and AITC (100 μM) for TRPA1.
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