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# TRPA1 channels as targets for resveratrol and related stilbenoids

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ABSTRACT

A series of twenty resveratrol analogues was synthesized and tested on TRPA1 and TRPV1 channels. None was able to significantly modulate TRPV1 channels. Conversely, most of them exhibited remarkably higher TRPA1 modulating activity than resveratrol. Optimal potency was observed with ortho monoxygenated stilbenes **6** and **17**.



The polyphenol resveratrol [(*E*)-3,4′,5-trihydroxystilbene] (Fig. 1) is a phytoalexin produced in a wide range of plants in response to environmental stress or pathogenic attack and has been detected in various food sources such as grapes, berries, chocolate, and peanuts.[1] Over the last decade, about 3000 articles (PubMed, November 2015) concerning health benefits in humans of resveratrol and related stilbenoids (in particular, pinostilbene and pterostilbene) have been published. The chemopreventive properties of resveratrol include the cardiovascular system, inflammation, cancer development, ageing processes, diabetes, and cerebral functions[2] and are ascribed to its antioxidant activity and ability to interfere with multiple signalling pathways (inflammatory mediators, transcription factors, cell cycle regulatory genes, angiogenic and apoptotic genes, antioxidant enzymes, protein kinases).[3] The detailed mechanisms by which resveratrol exerts its effects are however still unclear.

The transient receptor potential (TRP) channels ankyrin type-1 (TRPA1) and vanilloid type-1 (TRPV1) are two prominent members of the TRP family of structurally related non-selective cation channels[4] that play numerous roles in many physiological and pathophysiological processes.[5] In particular, TRPA1 and TRPV1 are involved in the perception of nociceptive and inflammatory pain via sensory nerve activation.[5c,d,k] Accordingly, the possibility that antinociceptive, antioxidant, and anti-inflammatory properties of resveratrol could derive, at least in part, from a TRPA1 and/or TRPV1 inhibitory activity has been recently investigated by Yu et al.[6] Resveratrol was found indeed to suppress the allyl isothiocyanate (AITC)-induced currents in mTRPA1-transfected HEK293 cells with an $IC_{50}$ value of approximately 0.75 μM. AITC, the pungent component of wasabi, horseradish, and mustard oil, is probably the best recognized TRPA1 agonist.[5a–c,h] It covalently binds to cysteine residues in the cytoplasmatic N-terminal domain of TRPA1 and opens the channel, increasing intracellular $Ca^{2+}$ concentrations and ultimately leading to a noxious or nociceptive response. In contrast to TRPA1, the TRPV1 channel was not inhibited significantly by resveratrol. Interestingly, and rather surprisingly, an opposite behaviour was exhibited by pinosylvin methyl ether.

Since previous SAR studies have shown that some resveratrol analogues exhibited enhanced antimicrobial, antioxidant, and cytotoxic activities,[7] we have now prepared 20 resveratrol analogues (compounds **1**–**20**) and examined their functional activity at TRPA1 and TRPV1 channels, with the aim of identifying more potent TRPA1 and/or TRPV1 modulators based on the resveratrol structure. The compounds differed by the number and position of hydroxy and/or methoxy or acetyloxy groups and were synthesized using standard chemical methodologies[7b,8,9] (resveratrol was obtained from a commercial supplier and was of analytical grade). In detail, stilbenoids **4**, **6**, **7**, **9**–**17** were synthesized by a Mizoroki–Heck coupling reaction between appropriate iodoarenes **21** and styrenes **22** (Scheme 1).[7c,8c] Non-commercially

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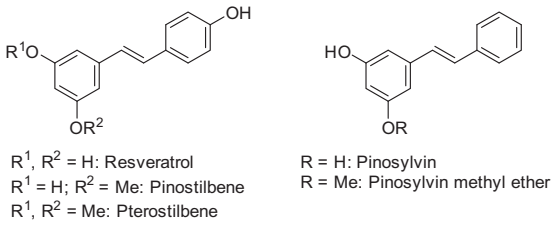



**Figure 1.** Structures of resveratrol and some related stilbenoids.

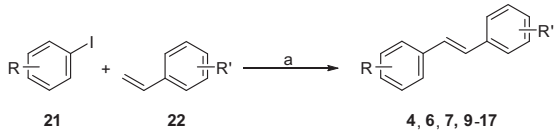
available 3,5-dimethoxystyrene was obtained by a Wittig reaction of 3,5-dimethoxybenzaldehyde and methyltriphenylphosphonium iodide and *t*-BuOK.[9] Preparation of hydroxystilbenes **1**, **2**, **3**, **5**, **8** was accomplished by $BBr_3$-promoted demethylation of the corresponding methoxystilbenes **12**, **9**, **14**, **16**, **12**, respectively (Scheme 2).[8e] Finally, acetylation of resveratrol and of hydroxystilbenes **4**, **6** with acetyl chloride afforded acetyloxystilbenes **18**, **19**, **20** (Scheme 3).[8a,b,d] IR and NMR data of compounds **1**–**20** were consistent with those reported in the literature.[10]

Resveratrol and the 20 stilbenoids synthesized here were tested for their ability to induce intracellular $Ca^{2+}$ elevation in HEK293 cells stably transfected with the rat TRPA1, or the human TRPV1 cDNAs.[11] The antagonist or desensitizing activity was assessed by adding the test compounds 5 min before stimulation of cells with reference agonists AITC and capsaicin.[11]

As concerns TRP assays on TRPV1-HEK293 cells, neither resveratrol nor any of 20 analogues **1**–**20** were able to significantly modulate this channel (efficacies and $IC_{50}$ values were invariably <10% and >10 μM, respectively). Pinosylvin methyl ether (**8**) was reported by Yu et al.[6] to significantly suppress TRPV1 activity both in heterologous HEK293 cells and DRG neurons. Actually, however, an $IC_{50}$ in the range 15 and 30 μM could be inferred from their bars graph showing the suppressive effect of pinosylvin methyl ether on 20 nM capsaicin-induced currents in TRPV1-HEK293 cells, a value comparable with that observed by us (23.7 μM, Fig. 2).

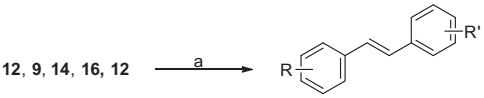
On the contrary, rat TRPA1-HEK293 cells exhibited a sharp increase in intracellular $Ca^{2+}$ upon application of 19 out of the 21 compounds examined (Table 1). Only resveratrol and pinosylvin (compound **1**) were devoid of rat TRPA1 activating ability. Seven of the stilbenoids **2**–**20**, that is, **5**, **6**, **11**–**13**, **16**, **17** produced a robust activation of TRPA1 in transfected cells with $EC_{50}$ values <2 μM.

Five minute preincubation of TRPA1-HEK293 cells with resveratrol and 20 analogues, and then continued incubation with AITC caused inhibition of TRPA1 response to this agonist with $IC_{50}$ values between 1.7 μM and 29.4 μM (Table 1). The $EC_{50}$ and $IC_{50}$ values were, with the exception of resveratrol, pinosylvin (**1**) and, partially, **15**, comparable and thus, only resveratrol and pinosylvin acted as 'true' antagonists (that is, inhibition without agonism per



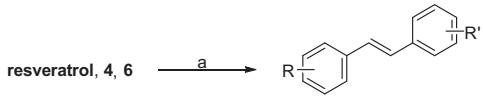

4: R = 4-OH; R' = H
6: R = 2-OH; R' = H
7: R = 4-OH; R' = 3,5-OMe
9: R = 4-OH; R' = 3-OMe
10: R = 4-OH; R' = 4-OMe
11: R = 4-OMe; R' = 3,5-OMe
12: R = H; R' = 3,5-OMe
13: R = 4-OMe; R' = 3-OMe
14: R = 4-OMe; R' = 4-OMe
15: R = 4-OMe; R' = H
16: R = H; R' = 3-OMe
17: R = 2-OMe; R' = H

**Scheme 1.** Synthesis of compounds **4**, **6**, **7**, **9**–**17**. Reagents and conditions: (a) $Pd(OAc)_2$, $N(CH_2CH_2OH)_3$, DMF, 100 °C, 1 h (42–67%).




**1**: R = H; R' = 3,5-OH
**2**: R = 4-OH; R' = 3-OH
**3**: R = 4-OH; R' = 4-OH
**5**: R = H; R' = 3-OH
**8**: R = H; R' = 3-OH, 5-OMe

**Scheme 2.** Synthesis of compounds **1**, **2**, **3**, **5**, **8**. Reagents and conditions: (a) $BBr_3$, $CH_2Cl_2$, rt, 1 h (26–37%).




**18**: R = 3,5-OAc; R' = 4-OAc
**19**: R = H; R' = 4-OAc
**20**: R = 2-OAc; R' = H

**Scheme 3.** Synthesis of compounds **18**–**20**. Reagents and conditions: (a) AcCl, pyridine, $CH_2Cl_2$, rt, 1 h (64–73%).

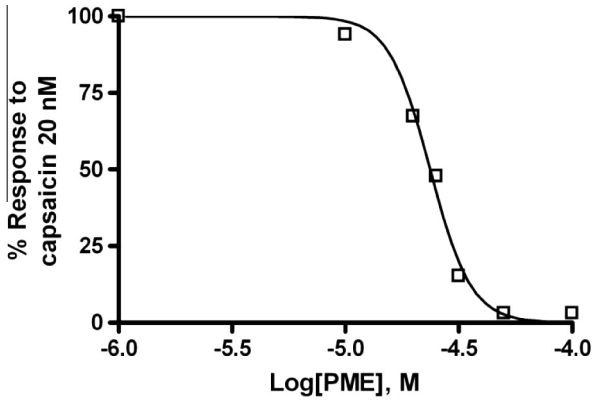



**Figure 2.** Dose–response curve showing inhibition of capsaicin-induced hTRPV1 intracellular $Ca^{2+}$ elevation by pinosylvin methyl ether (PME).

se, and hence not due to desensitization), while **15** behaved as a weakly desensitizing agonist. During preparation of this manuscript, a short communication highlighting the ability of pinosylvin to inhibit TRPA1-induced calcium influx in hTRPA1-HEK293 cells with an $IC_{50}$ value slightly higher than that presented here (26.5 vs. 12.1 μM) appeared online.[12]

As mentioned initially, the inhibition of AITC-induced currents by resveratrol was reported by Yu et al. to appear from low concentration with an $IC_{50}$ value of approximately 0.75 μM, a distinctly lower value than ours (19.9 μM, Fig. 3). Yu et al. measured however an $EC_{50}$ of 61.2 and 61.7 μM for AITC and AITC with resveratrol, respectively, while the corresponding values recorded by us were 1.40 and 2.74 μM, respectively (Fig. 4). Clearly, under experimental conditions in which agonists exhibit lower potency, stronger inhibitory effects with antagonists and/or desensitizing agents will be observed.

The TRPA1 modulating activity of resveratrol and hydroxystilbenes **1**–**6** exhibited an inverse relationship with the number of hydroxyl groups, being the monohydroxystilbenes **4**–**6** the most active ones. The position of the OH group was also important for activity, with the ortho isomer **6** endowed with a submicromolar activating potency. By contrast, the number of MeO groups did not influence appreciably the activity of methoxystilbenes **11**–**17**, but, again, the ortho isomer (compound **17**) was the most active member of the series.