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Structure–activity relationships of the ultrapotent vanilloid resiniferatoxin (RTX): The side chain benzylic methylene

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Dedicated to Professor Aldo Martelli on the occasion of his retirement as a Dean of the Faculty of Pharmacy in Novara

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ABSTRACT

The side chain benzylic methylene is a critical element for the vanilloid activity of resiniferatoxin (**2a**, RTX), and introduction of branching, oxygen functions, or isosteric substitution at this center proved detrimental, with a decrease of potency of 2–3 orders of magnitude compared to the natural product. Conversely, only a modest erosion of activity was observed upon α-methylation and α-methylenation of the side chain. Surprisingly, introduction of an iodine atom in the guaiacyl moiety of the oxygen isoster **2h** led to an unexpected and remarkable (>1000-fold) increase of potency, affording **2i**, a compound that outperforms RTX in terms of vanilloid agonism and represents the first one-digit picomolar ligand of a TRP channel discovered to date.

Ever since its isolation in 1975,[1] and its identification as a capsaicin (**1a**) analogue in 1989,[2] the daphnane diterpenoid resiniferatoxin (**2a**) has amazed life scientists for its extraordinary and still unmatched potency in animal models of inflammation and in cellular assays of vanilloid activity.[1,2] While the clinical development of RTX seems to have come to a standstill,[3] this compound remains an indispensable tool to investigate the capsaicin receptor (TRPV1),[4] where its role as a privileged structure was further validated by the serendipitous discovery that the powerful agonistic activity of the natural product (EC_{50} ca. 19 pM on hTRPV1) could be reversed by aromatic iodination of the acyl moiety, generating the potent (IC_{50} = 4.0 nM) vanilloid antagonist iodoresiniferatoxin (I-RTX, **2b**).[5]

Capitalizing on the development of an expeditious protocol to obtain the terpenoid core of RTX (resiniferonol orthophenylacetate, ROPA, **3**), from the household ornamental *Euphorbia resinifera* Berg,[6] we have adventured in the still substantially uncharted field of the structure–activity relationships of this remarkable natural product. The homovanillyl moiety has long been known to be essential for binding of RTX to TRPV1.[7] Within this element, the benzylic methylene is critical,[8] since ultrapotency was only observed in analogues of the natural products where only one carbon links the ester carbonyl and the aryl moiety, with homologation and deletion of this methylene leading to a dramatic (ca. three orders of magnitude) decrease of potency.[8] By comparison, manipulation of the substitution pattern of the aryl moiety was better tolerated.[8,9]

With the aim of obtaining information on the molecular bases of this observation, we have investigated the effect of inserting a branching or an oxygen function on the critical side chain methylene, and its isosteric replacement with an oxygen atom. These changes are expected to perturb the spatial relationship between the carbonyl oxygen and the aromatic ring, providing information useful to map the vanilloid recognition site of TRPV1.[10] Furthermore, we reasoned that by identifying alternative side chain templates for iodination, data useful to rationalize in molecular terms the amazing reversal of activity observed upon iodination of the natural product might also emerge.

The esters **2c–g** were prepared by Mitsunobu esterification of ROPA with the corresponding carboxylic acids.[11] These were in turn prepared from vanillin (**4a**) by straightforward chemistry, as

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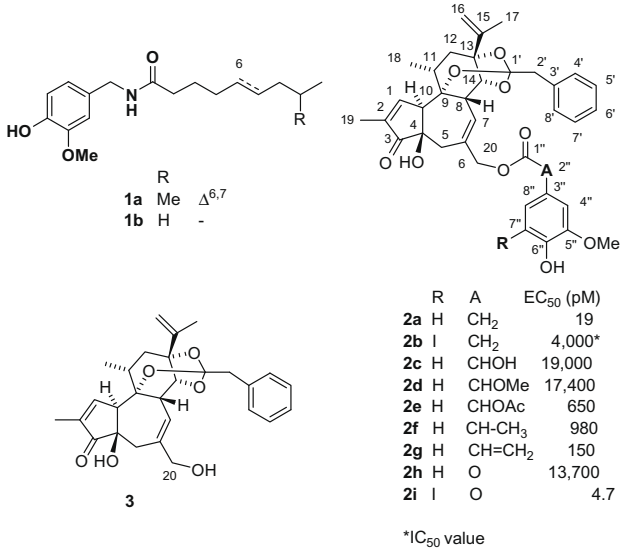
outlined in Scheme 2. Thus, after protection of the phenolic hydroxyl as a pivalate, addition of trimethylsilyl cyanide (TMSCN) afforded the cyanidrine **5**, next converted by selective hydrolysis of its corresponding diacetate to the vanillomandelate **6**, and, by hydrolysis of the corresponding (2-(*O*)methyl) ester, to the vanillomandelate **7**. Alternatively, **5** was esterified to **8**, and next oxidized, affording, after hydrolysis, the phenylglyoxylate **9**.[1] Wittig methylenation of **8** could only be achieved after protection of the phenolic hydroxyl as a TIPS (triisopropylsilyl) ether, providing, after global deprotection, the phenylacrylate **10b**. Alternatively, **10a** was hydrogenated and next hydrolyzed to the α-phenylpropionate **11b**.

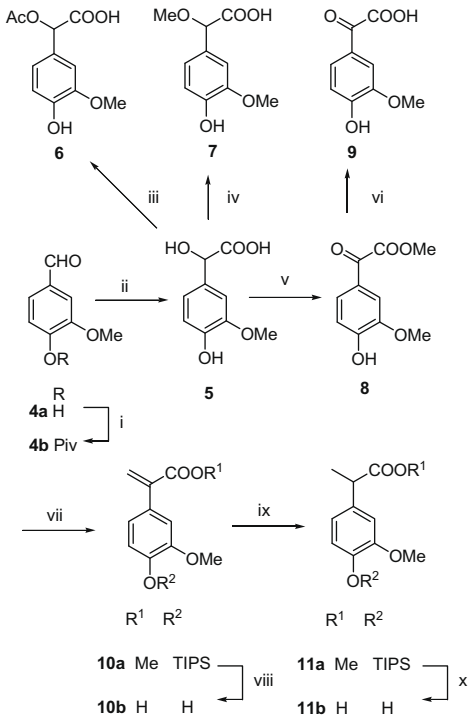
After esterification with ROPA and deprotection, all crude final products were further purified to >95% purity by preparative HPLC on silica gel. Little diastereomeric resolution was observed by esterification of the racemic acids **5–7**, **11b** with chiral and enantiopure ROPA (**3**), a primary alcohol, and the HPLC profile of **2c–f** showed a narrowly and almost symmetrically split peak in all cases. A similar splitting was observed in the signal of a few side chain signal of the ^{1}H and the ^{13}C NMR signals. No attempt was done to resolve the diastereomeric mixtures **2c–f** and assess the configuration of their individual components.

All final products were assayed in human embryonic kidney (HEK)-293 cells over-expressing the human TRPV1 (Scheme 1).[12] Despite the limitation that **2c–f** were diastereomeric pairs, the biological investigation of this set of 2″-modified analogues afforded interesting results. Thus, introduction of a hydroxyl (**2c**) or a methoxyl (**2d**) on the side chain benzylic methylene caused a decrease of activity of three orders of magnitude, while the decrease of potency was more modest (ca. 30-fold) for an acetoxyl. On the other hand, a certain tolerance was observed with the introduction of an alkyl group, and only a modest (ca. sevenfold) erosion of potency was observed in the methylene analogue **2g**. These data, while confirming the critical role of the side chain benzylic position, also showed that polar groups are poorly accommodated at this site, presumably because of the lipophilicity of the complementary surface of the ligand-binding pocket of TRPV1. The better tolerance to the introduction of a 2″-methylene or a 2″-methyl supports this view.

The presence of diastereomeric mixtures precluded any more refined speculation on nature of the active conformation of RTX, that is critically dependant on the spatial orientation of the substituents around the side chain C1″–C2″ bond. To overcome this limitation, isosteric substitution of the benzylic methylene was investigated,

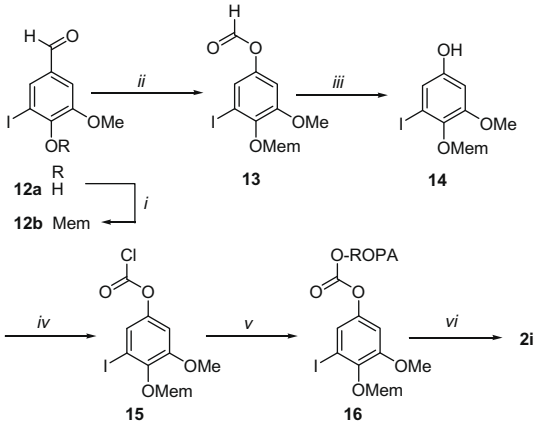


Scheme 1.



Scheme 2. Synthesis of the modified homovanillic acids **5–7**, **9**, **10b**, and **11b**. Reagents and conditions: (i) pivaloyl chloride, pyridine (86%); (ii) (a) TMSCN, cat. CuOTf, THF; (b) KOH, MeOH (overall 61%); (iii) (a) Ac_2O (b) pyrrolidine (3 equiv), THF (overall 31%); (iv) (a) $SOCl_2$, MeOH, 72 h (26%); (b) LiOH (5 equiv), THF–MeOH; (v) Jones reagent (76%); (vi) LiOH (3 equiv), THF–MeOH (72%); (vii) (a) TIPS-Cl (4 equiv), imidazole, DMF; (b) $CH_2{=}PPh_3$, THF, −78% (overall 71%); (viii) LiOH (6 equiv), THF–MeOH (71%); (ix) H_2, Pd(C); (b) LiOH (6 equiv), THF–MeOH (overall 70%).

and **2h**, the carbonate analogue of RTX, was prepared by esterification of ROPA with the protected and complementary guaiacylchloroformate. After deprotection, the carbonate **2h** was assayed for vanilloid activity, showing a decrease of activity similar to that observed for the polar analogues **2c** and **2d**. Overall, **2h** was still a good TRPV1 agonist, ca. threefold more potent than capsaicin (EC_{50} = 13.7 nM vs 40 nM), and was therefore chosen as a platform for iodination because of its substantial structural difference with the side chain of RTX. We had previously observed that 5′-iodination potentiates (ca. fivefold), rather than reverting, the activity of ROPA vanillate, the lower, and considerably less potent (EC_{50} 9400 pM vs 19 pM), homologue of RTX.[8] Since carbon–oxygen bonds are considerable shorter than carbon–carbon bonds (1.34 Å for $C(sp^2)$–O bonds vs 1.51 Å for $C(sp^3)$–$C(sp^2)$ bonds),[13] we expected a somewhat similar situation, an important hint to suggest that the reversal effect critically depends on the carbonyl-to-aryl distance in the side chain. The iodinated chloroformate (**16**) requested for the synthesis of **2i** was prepared from commercial 5-iodovanillin (**12a**) as outlined in Scheme 3. After protection of the phenolic hydroxyl as a Mem (methyoxyethoxymethyl)ether, Dakin oxidation introduced the missing aryl hydroxyl, then reacted with oxalyl chloride to install the activated carbonyl function. After coupling with ROPA, removal of the Mem ether could only be achieved by using the $SnCl_4$–THF adduct,[14] since more conventional method (Brønsted acids and $SnCl_4$ in CH_2Cl_2) led to complete degradation. Surprisingly, evaluation of the vanilloid activity of **2i** showed that the introduction of the iodine atom had caused a >1000-fold increase of activity, affording an agonist that outperformed RTX in a comparative assay of vanilloid potency (EC_{50} = 5.4 vs 19 pM).[12] Indeed, with a one-digit picomolar EC_{50} value, **2i** is the most potent ligand ever reported for a TRP channel1.[10,15] Given the intracellular localization of the vanilloid-binding site on TRPV1,[10] it is unclear if the dramatic increase of activity



Scheme 3. Synthesis of the modified homovanillic acids **4–9**. Reagents and conditions: (i) MemCl, DIPEA, toluene (91%); (ii) MCPBA, DCM, 90%; (iii) K_2CO_3 (60%); (iv) $COCl_2$, 79%; (v) (a) **3**, DMAP, pyridine (41%); (b) $SnCl_4$, THF (56%) ROPA = **3**; MCPBA = *m*-chloroperoxybenzoic acid; Mem = methoxyethoxymethyl; DIPEA = diisopropyletylamine; DMAP = 4-dimethylaminopyridine.

associated to iodination of the carbonate **2h** is due to higher binding, better membrane penetration, or to a combination of both.

Our limited knowledge on the vanilloid-binding site of TRPV1[10] makes it difficult to translate these observations into new molecular insights. Nevertheless, it seems reasonable to assume that iodine plays a directing role in the interaction between TRPV1 and halogenated vanilloids, as suggested by a study on the introduction of various substituents on the structurally simple capsaicinoid agonist nonivamide (**1b**).[16] Thus, reversal of activity, though observed with all halogens, followed the strength order of halogen bonding (I > Br > Cl),[17] while the replacement of iodine with alkyl, alkenyl or alkynyl groups led to a dramatic decrease of affinity. Iodine is easily engaged in halogen bonding with tyrosine residues,[17] and the vanilloid-binding site of TRPV1 contains tyrosine residues that are essential for ligand binding.[10] It is therefore tempting to assume that a switch from hydrogen-bonding to iodine-bonding underlies both the reversal of activity of RTX (**2a**) and the amplification of activity of its oxo-isoster **2h**, caused by the introduction of a iodine atom *ortho* to the phenolic hydroxyl. Though speculative, these considerations have a chemical precedent, since competition between H- and I-bonding is well documented in supramolecular crystal assemblies,[18] thus qualifying the RTX–TRPV1 interaction as a critical probe to investigate the biological relevance of halogen bonding.

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