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THIOUREA ANALOGUES OF RESINIFERATOXIN AS LIGANDS FOR THE VANILLOID RECEPTOR

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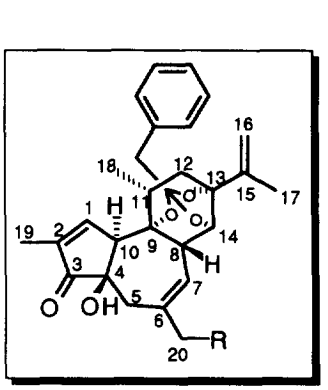
Abstract. A thiourea bridge was tested as a linker to replace the $OC(O)CH_2$ ester group that connects resiniferonol-9,13,14-orthophenylacetate to the 3-methoxy-4-hydroxyphenyl moiety in resiniferatoxin (RTX). The resulting compounds showed lower affinity for the vanilloid receptor than did the parent RTX.

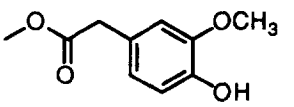
Resiniferatoxin (**1b**, RTX) is an extremely potent irritant tricyclic diterpene which is structurally related to other phorbol-related diterpenes, such as phorbol 12-myristate-13-acetate (**2**, PMA), except for its homovanillyl ester group at C-20.[1,2] In terms of irritancy, RTX is 100- to 1000-times more potent than PMA[3] but fails to induce other typical phorbol ester responses, and its affinity for protein kinase C is also much lower than that of the phorbol esters.[4] Instead, RTX appears to function pharmacologically as a "vanilloid", a group of compounds also characterized by their irritant properties that include the prototype capsaicin (**3**) and its analogues.[5,6] Capsaicin and its analogues comprise a class of potential non-narcotic, non-steroidal, analgesic-antiinflammatory agents which desensitize primary sensory afferent neurons. One of the functions of these neurons is to transmit nerve impulses to the central nervous system which are perceived as pain.[7] RTX and capsaicin share a common vanilloid pharmacophore which is essential for the extreme potency of RTX. Indeed, resiniferonol-9,13,14-orthophenylacetate (**1a**, ROPA), the 20-deacylated parent structure, fails to bind to the RTX receptor.[8,9] Both RTX and capsaicin bind to the same receptor, but RTX displays 1000- to 10000-fold greater potency than capsaicin, thus functioning as an ultrapotent capsaicin analogue.[5] Structure-activity studies on capsaicin analogues[10-13] have established that the mid-region, comprising the amide bond that connects the aromatic 3-methoxy-4-hydroxyphenyl moiety to the aliphatic side chain, can tolerate an ester function and even the reversal of the amide bond without seriously compromising activity.[11] From this study, a thiourea bridge, as in *N*-(4-hydroxy-3-methoxybenzyl)-*N'*-octylthiourea (**4**), provided the most potent compound of the series.[11] Armed with this information, we wished to modify the RTX structure with a similar thiourea bridge connecting the aromatic region and the diterpene moiety to give compounds **1c** and **1d**. These compounds were of further interest in that they would be expected to be metabolized differently from RTX. Hydrolysis of the ester in RTX yields ROPA, a relatively weak protein kinase C agonist, whereas hydrolysis of the ureas would not only be more difficult, but it would generate the amine analogue of ROPA (**12**), a compound that we have shown previously to have very poor affinity for protein kinase C.[14] Since structure-activity studies on capsaicin have also indicated the importance of the length of the mid-region, this issue was considered by attaching the thiourea bridge either directly to the aromatic ring as in **1c**, or

through a methylene spacer as in **1d**.

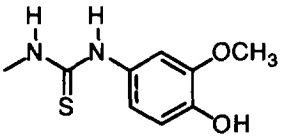
The requisite isothiocyanates were prepared according to Scheme 1. Reduction of commercially available 4-nitroguaiacol (**5**) provided the intermediate amine which upon treatment with di-2-pyridylthionocarbonate[15] afforded isothiocyanate **7**. For the higher homologue, commercially available 4-hydroxy-3-methoxybenzyl amine hydrochloride was reacted with the same reagent in the presence of



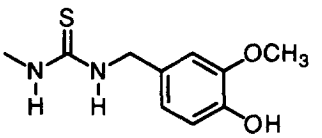
R = OH **(1a)**



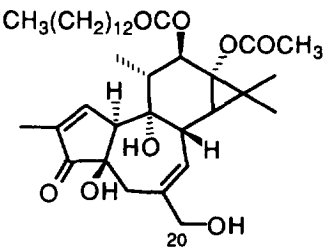
(1b)



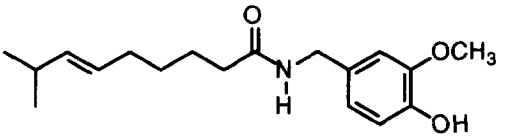
(1c)



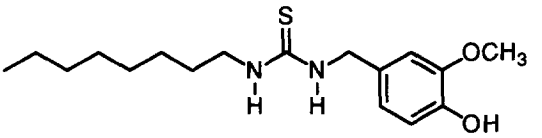
(1d)



(2)



(3)

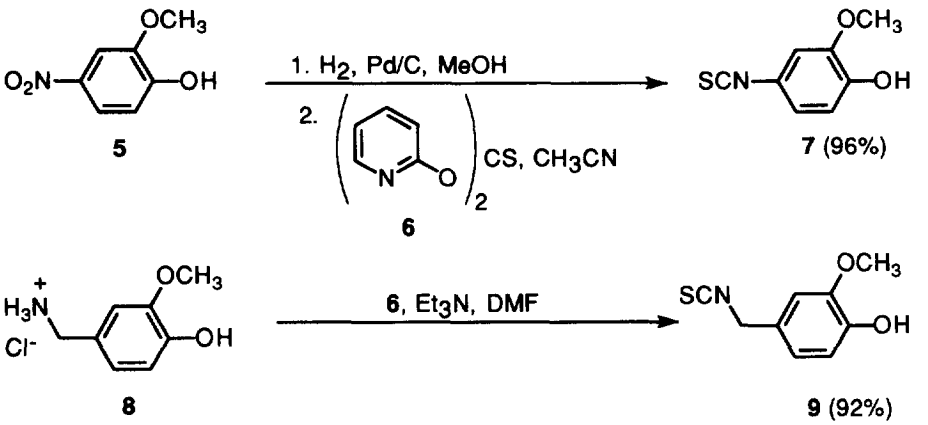


(4)

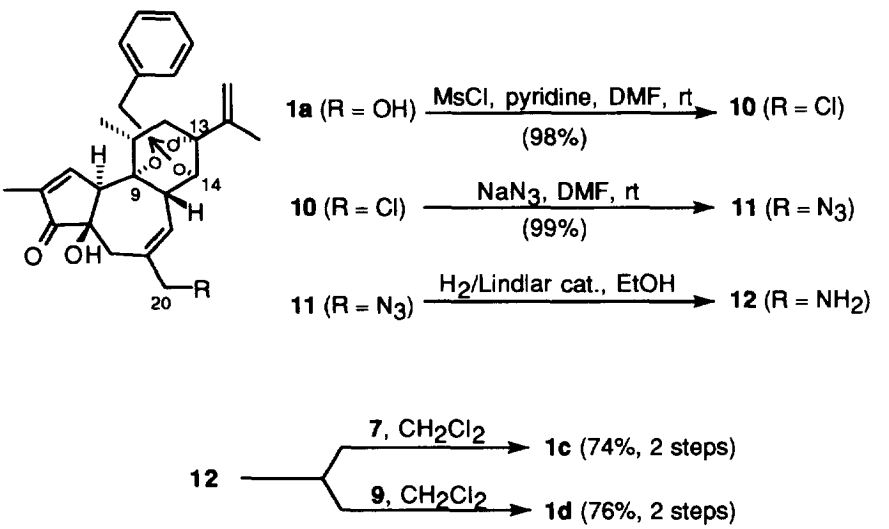
triethylamine to give isothiocyanate **9**. By using known phorbol chemistry,[16] ROPA (**1a**) was converted to the corresponding chloride **10** after treatment with methanesulfonyl chloride (Scheme 2). Displacement with NaN_3 gave the corresponding azide **11**, which was then reduced in the presence of Lindlar catalyst[17] to the amine **12**. This amine was used directly in condensation reactions with isothiocyanates **7** and **9** to give the final products (Scheme 2).[18,19]

The capsaicin-like activities of these RTX analogues were measured by *in vitro* binding experiments

Scheme 1.



Scheme 2.



in which specifically bound [^{3}H]RTX was displaced by the non-radioactive competing ligands. The specific details of this method have been published.[14] Activity is expressed in terms of a K_i value (mean ± SEM, 3 experiments) which represents the concentration of the non-radioactive ligand which displaces half of the specifically bound labelled RTX, corrected for RTX concentration and RTX affinity at the receptor. The values obtained for **1c** (K_i = 66.1 ± 0.8 nM) and for **1d** (K_i = 149 ± 11 nM) indicate that these compounds are, respectively, 3 x 10^3 and 6.5 x 10^3 less potent than RTX (K_i = 23.1 ± 3.1 pM). We can conclude that despite having a common "vanilloid" pharmacophore, a simple juxtaposition of an "optimized" capsaicin segment on the resiniferonol-9,13,14-orthophenylacetate moiety does not produce a better ligand. Changes in the homovanillyl portion of RTX, as the ones described here, presumably disrupt the alignment between the diterpene moiety and the homovanillyl ester group at C-20 that contributes to the compound's ultra-high potency.

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18. Compound **1c**: white solid, mp 148-50 °C; ^{1}H NMR ($CDCl_3$) δ 7.66 (br s, 1 H, S=CNH-Ar), 7.42 (s, 1 H, H-1), 7.10-7.40 (m, 5 H, Ph), 6.70-7.00 (m, 3 H, Ar), 5.60-6.00 (m, 2 H, OH, H-7), 4.68 (br s, 2 H, H-16), 4.05-4.50 (m, 3 H, H-14, H-20), 3.84 (s, 3 H, OCH_3), 3.00-3.23 (m, 4 H, PhC$\underline{H}_2$, H-8, H-10), 2.00-2.60 (m, 5 H, H-5, H-11, H-12), 1.78 (br s, 3 H, H-19), 1.50 (br s, 3 H, H-17), 0.92 (d, 3 H, H-18); FAB MS (*m/z*, relative intensity) 645.6 (MH^+, 23).
19. Compound **1d**: white solid, mp 144-46 °C; ^{1}H NMR ($CDCl_3$) δ 7.45 (s, 1 H, H-1), 7.15-7.38 (m, 5 H, Ph), 6.90-7.05 (m, 3 H, Ar), 5.98 (br s, 1 H, OH), 5.90 (br s, 1 H, NH), 5.42 (s, 1 H, H-7), 4.26-4.90 (m, 4 H, H-16, NHC$\underline{H}_2$Ar), 3.70-4.10 (m, 6 H, OCH_3, H-20, H-14), 2.50-3.20 (m, 6 H, PhC$\underline{H}_2$, H-8, H-10, H-5), 1.96-2.18 (m, 3 H, H-11, H-12), 1.78 (br s, 3 H, H-19), 1.56 (br s, 3 H, H-17), 0.97 (d, 3 H, H-18); FAB MS (*m/z*, relative intensity) 659 (MH^+, 24).

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