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3-Acyloxy-2-phenalkylpropyl Amides and Esters of Homovanillic Acid as Novel Vanilloid Receptor Agonists

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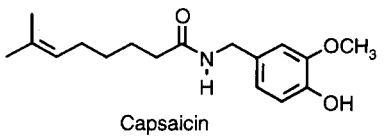
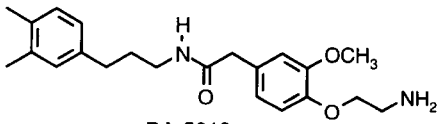
Abstract: A series of 3-acyloxy-2-phenalkylpropyl amides and esters of homovanillic acid were designed and synthesized as vanilloid receptor agonists containing the three principal pharmacophores of resiniferatoxin. Amide analogues **23**, **5** and **11** were found to be potent agonists in vanilloid receptor assay both for ligand binding and for activation.

The vanilloid receptor (VR) is a specific neuronal membrane recognition site for capsaicin (CAP) and related irritant compounds. It is expressed almost exclusively by primary sensory neurons involved in nociception and neurogenic inflammation.[1] The functional VR subtype, VR1, activated by both capsaicin and noxious heat has recently been cloned.[2] This receptor functions as a cation-selective ion channel with a preference for calcium. Its desensitization by ligands has been recognized as a promising therapeutic approach to mitigate neuropathic pain and other pathological conditions in which neuropeptides released from primary sensory neurons play a crucial role.[3]

Most exogenous VR agonists which are being developed or used as analgesics are structually related to capsaicin (i.e., Olvanil, SDZ-249482, and DA-5018) and resiniferatoxin (RTX).[4] All the structures include a common vanilloid ring which appears to be important for agonist activity. However, in a recent report, it was demonstrated that compounds lacking the vanilloid moiety, such as sesquiterpenoid unsaturated dialdehydes or triprenyl phenol may also activate the receptor.[5] Although receptor antagonists are fewer, capsazepine, which acts competitively at the capsaicin binding site,[6] the channel blocker ruthenium red,[7] and capsazocaine[8] have been reported.



Capsaicin



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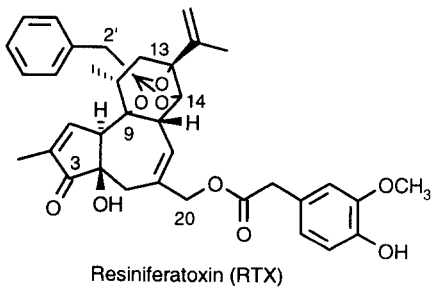

Resiniferatoxin (RTX)

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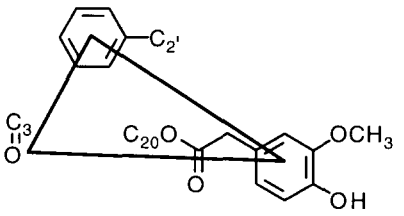
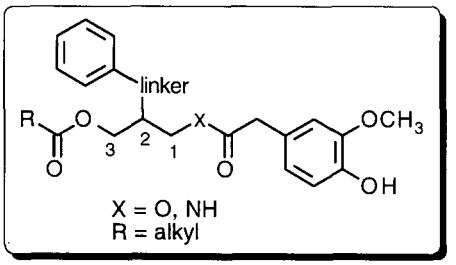
Resiniferatoxin (RTX), a tricyclic diterpene isolated from *Euphorbia resinifera*, has been regarded as an ultrapotent capsaicin analogue. Indeed, the specific binding of RTX to the capsaicin binding site in dorsal root ganglia has been demonstrated with use of [^{3}H]RTX.[9] Recently, a totally enantiocontrolled synthesis[10] and conformational analysis of RTX[11] have been reported. However, the pharmacophores of RTX have not yet been clearly defined, although structural-activity studies have suggested that a plausible pharmacophore model involves the C_{20}-homovanillic moiety, the C_3-keto group, and the orthoester phenyl group on ring C as crucial structural elements responsible for the extremely high potency of RTX.[12] The lower potency of capsaicin, on the other hand, may be rationalized by the lack of some of these critical pharmacophores, especially C_3-keto group. Although a number of vanilloid agonist based on the structures of capsaicin and RTX have been reported as potental analgesics,[4] capsaicin-like analogues are limited by their intrinsic lower potency and narrow therapeutic index, and RTX analogues are either of limited availability from natural sources or difficult to obtain synthetically due to their structural complexity.



Proposed Pharmacophore Model of RTX



Templates as Simplified RTX Analogues

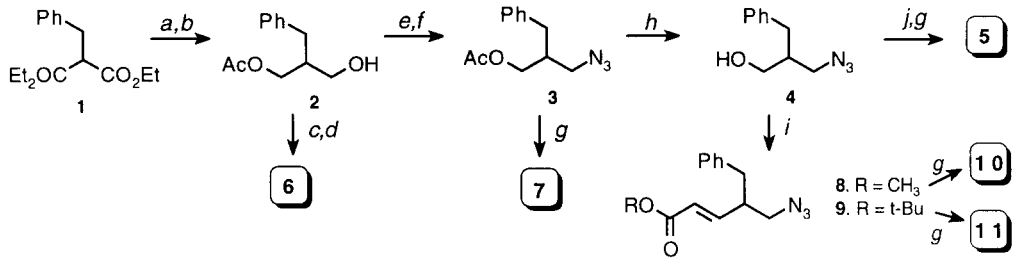
As part of an ongoing program directed towards the discovery of novel analgesic agents based on the vanilloid receptor, we set out to investigate simplified analogues of RTX according to the proposed three-pharmacophores model. The strategy consisted in placing surrogates of two of the essential pharmacophores (the C_{20}-homovanillic and C_3-keto moieties) at the ends of a propane chain (templates I and II, Table 1) or a 1-butene chain (template III, Table 1), which are then connected at or near the middle to the third pharmacophore (the orthoester phenyl) through a variable linker. The homvanillic moiety was coupled to these linear templates by either ester or amide linkages, and based on the calculated distance between the C_3-keto and the carbonyl homovanillate ester in RTX, the two carbonyl moieties on the target compounds appear separated by five intervening atoms. Because the location of the orthoester phenyl group in the active conformation of RTX is not well defined, the phenyl rings in the templates were connected to the propane and 1-butene chains by a variable tether one to four atoms in length.

This paper describes the syntheses and biological activities of a series of simple mimics of RTX, which were designed according to the principles discussed above.

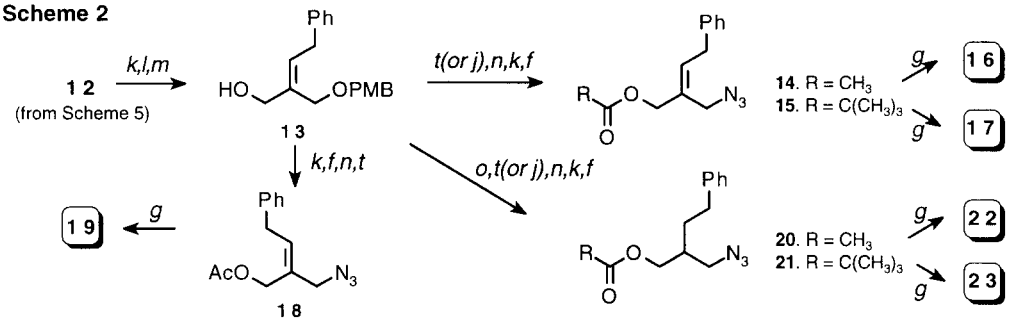
Chemistry

The synthetic approaches to the various racemic target compounds were divided according to the type of linker to the propane or 1-butene scaffolds: compounds with a one-atom linker (phenylmethyl group) attached to a propane or 1-butene chain (Scheme 1); compounds with a two-atoms linker (phenylethyl or phenylethylidene group) attached to a propane chain (Scheme 2); compounds with a two-atoms linker (benzyloxy group) attached

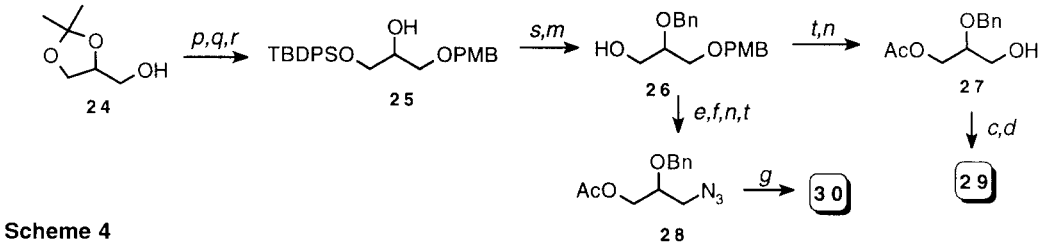
Scheme 1



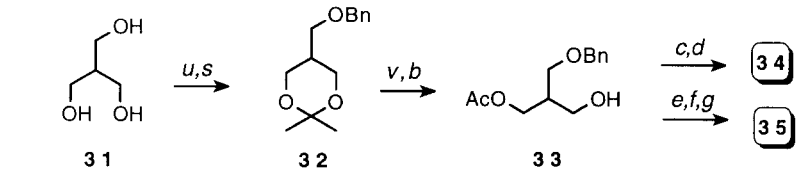
Scheme 2



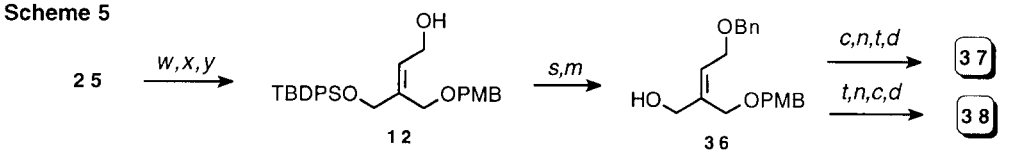
Scheme 3



Scheme 4



Scheme 5



Reagents and Conditions: (a) $LiAlH_4$, Et_2O (b) $CH(OMe_3)_3$, TsOH, CH_2Cl_2; H_2O (c) *O*-methoxymethyl homovanillic acid, DCC, DMAP, CH_2Cl_2 (d) CF_3COOH, CH_2Cl_2 (e) MsCl, NEt_3, CH_2Cl_2 (f) NaN_3, DMF (g) homovanillic pentafluorophenol ester, H_2, Lindler's cat., EtOAc (h) K_2CO_3, MeOH (i) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2; $Ph_3P{=}CHCO_2CH_3$ (or $Ph_3P{=}CHCO_2CMe_3$) (j) Me_3CCOCl, NEt_3, CH_2Cl_2 (k) MsCl, LiCl, 2,6-lutidine, DMF (l) PhMgBr, ether (m) Bu_4NF, THF (n) DDQ, CH_2Cl_2-H_2O (o) H_2, Pd-C, EtOAc (p) p-methoxybenzyl chloride (PMBCl), NaH, THF (q) 1N HCl (r) TBDPSCl, NEt_3, CH_2Cl_2 (s) BnBr, NaH, THF (t) Ac_2O, pyridine, CH_2Cl_2 (u) H_2SO_4, $CuSO_4$, acetone (v) TsOH, MeOH (w) PCC, CH_2Cl_2 (x) $Ph_3P{=}CHCO_2CH_3$, CH_2Cl_2 (y) DABAL, CH_2Cl_2

to a propane chain (Scheme 3); compounds with a three-atoms linker (benzyloxymethyl group) attached to a propane chain (Scheme 4); and compounds with a four (benzyloxyethylidene group) linker attached to a propane chain (Scheme 5). The synthesis of ester analogues was accomplished by DCC-mediated coupling of *O*-methoxymethyl homovanillic acid with the corresponding 3-acyloxy-2-substituted propanols followed by acid hydrolysis with trifluoroacetic acid. The syntheses of amide analogues was achieved after catalytic reduction (Lindler's catalyst) of the corresponding 3-acyloxy-2-substituted propyl azides and 5-azapent-2-enoate esters performed in the presence of homovanillic pentafluorophenol ester.

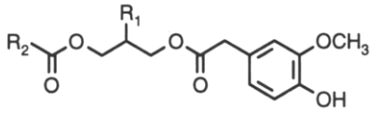
Results and Discussion

The capsaicin-like activity of the target compounds was determined by an *in vitro* receptor binding assay[12e] and CAP-activated single channel activation.[13] In the receptor binding assay, the compounds were evaluated for their ability to displace bound [^{3}H]RTX from the receptor. The results are expressed in terms of K_i values (mean ± SEM, 3 experiments) which represent the concentration of the non-radioactive ligand which displaces half of the bound labeled RTX (Table 1). In the CAP-activated single channel activation, the increase of inward currents resulting from the nonselective cation influx following extracellular application of the compounds to cultured neonatal rat dorsal root ganglion (DRG) neurons was measured. Activities of the compounds are expressed in terms of the relative difference in ion conductance compared to CAP as a control.

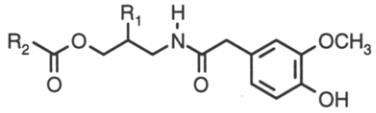
None of ester analogues (template I) showed any agonistic response in either assay at the concentrations examined regardless of nature of the linker attached to the propane chain. However, a switch to an amide functionality (compare **34** to **35** and **6** to **7**) led to measurable CAP-like responses. This represents an important distinction from the CAP and RTX analogues, where the ester analogues are comparable or more potent, respectively, than the corresponding amide surrogates.[12e,14] The potency of the amide analogues (template II), was sensitive to the length of linkers and the nature of the acyl substituents at C_3. Conformational studies on RTX have demonstrated that the spatial disposition of the phenyl orthoester may be critical for activity due to its specific hydrophobic interaction with the receptor.[11] The fact that both phenethyl **23** and benzyl amide **5** analogues showed the best binding affinities with K_i = 0.295 and 0.404 μM indicated that for this series an one or two atom linker is optimal to reach the same hydrophobic pocket on the receptor. Conformationally restricted analogues (**16**, **17**, and **19**) of the phenethyl analogue (**23**) had reduced its binding affinity suggesting the phenyl group must be involved in a very specific hydrophobic interaction with the receptor. Regarding the nature of the acyl group at C_3, change from methyl to pivaloyl group (**17**, **23**, and **5**) enhanced binding affinity and cation influx. Further structure-activity relationship studies at this position may reveal the possibility of exploiting another hydrophobic interaction with the receptor. Transposed ester analogues (template III, **10** and **11**) of benzyl amide analogues (**7** and **5**) retained the activities of the parent compounds and will be investigated further as chemically or metabolically stable surrogates.

In summary, 3-acyloxy-2-phenalkylpropyl and 5-aminopent-2-enoate esters and amides of homovanillic acid were constructed as novel agonists of the vanilloid receptor with the intent to mimic the positions of the C_{20}-homovanillate, the C_3-carbonyl, and phenyl orthoester side chain of RTX. Two compounds, **23** and **5**, showed potent CAP-like activities in terms of the binding assay and CAP-activated single channels assay. These compounds will serve as lead compounds for the development of VR ligands as analgesics. Additional structure-activity studies encompassing *in vivo* studies will be reported elsewhere.

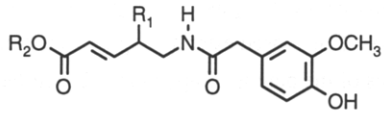
Table 1. Affinities and Channel Activation of Homovanillic Esters and Amides to Vanilloid Receptor



Template I	R_1	R_2	K_i (μM)	Cation Influx[a]
Capsaicin			5.31 (± 0.37)	+
DA-5018			1.40 (± 0.03)	+
RTX			0.000023	+++
29	OCH_2Ph	CH_3	>100	NR
34	CH_2OCH_2Ph	CH_3	>100	NR
37	(E) $=CHCH_2OCH_2Ph$	CH_3	>100	NR
38	(Z) $=CHCH_2OCH_2Ph$	CH_3	>100	NR
6	CH_2Ph	CH_3	>100	NR



Template II	R_1	R_2	K_i (μM)	Cation Influx
30	OCH_2Ph	CH_3	>100	NR
35	CH_2OCH_2Ph	CH_3	78 (± 29)	NR
16	(E) $=CHCH_2Ph$	CH_3	12.9 (± 3.7)	W
17	(E) $=CHCH_2Ph$	$C(CH_3)_3$	1.22 (± 0.28)	++
19	(Z) $=CHCH_2Ph$	CH_3	29.2 (± 4.8)	W
22	CH_2CH_2Ph	CH_3	45.2 (± 11)	W
23	CH_2CH_2Ph	$C(CH_3)_3$	0.295 (± 0.037)	++
7	CH_2Ph	CH_3	19.3 (± 6.8)	W
5	CH_2Ph	$C(CH_3)_3$	0.404 (± 0.037)	++



Template III	R_1	R_2	K_i (μM)	Cation Influx
10	CH_2Ph	CH_3	13.5 (± 3.1)	W
11	CH_2Ph	$C(CH_3)_3$	0.48 (± 0.05)	++

[a] NR: no response, W: weaker than CAP, +: same as CAP, ++: 10 times stronger than, +++: 100 times stronger than CAP

Acknowledgement

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