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Bioorganic & Medicinal Chemistry Letters 16 (2006) 208–212
Bioorganic & Medicinal Chemistry Letters

In vitro photo-release of a TRPV1 agonist

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Received 23 August 2005; accepted 6 September 2005
Available online 5 October 2005

Abstract—Intracellular photolysis of a novel 'caged' capsaicin analogue results in in vitro activation of the capsaicin receptor TRPV1.

Capsaicin (**1**), the main pungent component of chilli peppers, has long been known to produce a painful irritant effect if injected into the skin, applied to sensitive structures such as the cornea, or tasted. Comprehensive structure–activity studies have been carried out on capsaicin and analogues, elucidating the main requirements for a compound to display capsaicin-like biological activity;[1–4] however, the molecular target of capsaicin has only recently been discovered. It is now clear that capsaicin exerts its main biological effects via activation of a receptor.

The 'capsaicin receptor', or transient receptor potential channel–vanilloid subtype 1 (TRPV1), is a cation selective ligand-gated ion channel.[5] Originally named the vanilloid receptor subtype 1 (VR1), it has now been shown that TRPV1 is a member of the TRP family of receptors[6] and is activated by capsaicin and other noxious compounds including resiniferatoxin (RTX, **2**, Fig. 1). TRPV1 is also activated by heat above ~45 °C and conditions below ~pH 5.5.[5] This receptor represents a realistic therapeutic target for the treatment of pain,[7–10] and the use of topically applied capsaicin to treat painful skin disorders has met with some success.[11] The analgesic effect of capsaicin can be rationalised by prolonged activation of TRPV1, causing the receptor to desensitise. Longer-term resistance to capsaicin can be attributed to the large influx of Ca^{2+} causing nerve terminal degeneration. Therefore, TRPV1 antagonists rather than agonists are likely to be the therapeutically most important compounds in the treatment of pain.[12–14] Recently, many potent and selective agonists and antagonists of TRPV1 have been reported.[15,9] However, pharmacological tools required to accurately study the function of a receptor are not limited to selective agonists and antagonists. The use of 'caged' or photolabile compounds, which allow temporal and spatial control over receptor activation, has frequently proved invaluable in the study of receptor function.[16,17] Very few chemical tools of this type to assist the study of TRPV1 exist. Herein we report the synthesis of two novel caged capsaicin analogues **8** and **9**, the in vitro biological activity of **8** in cultured dorsal root ganglia (DRG)

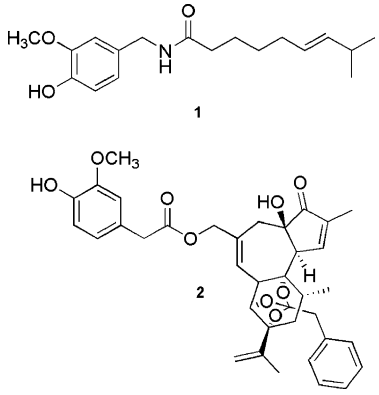

Figure 1. Capsaicin (**1**) and resiniferatoxin (RTX, **2**).

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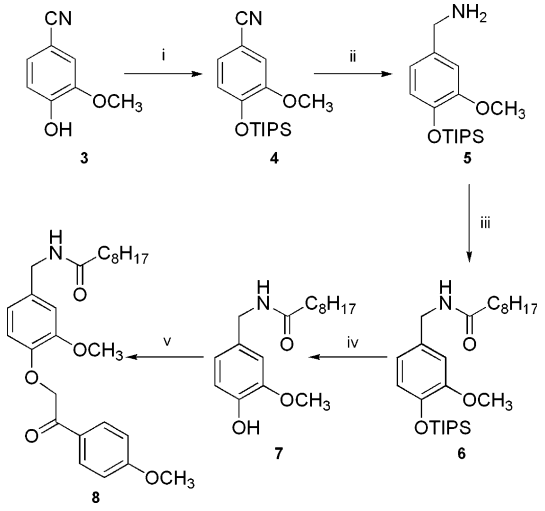
doi:10.1016/j.bmcl.2005.09.018

neurones from neonatal rats and some studies on the photolysis characteristics of both compounds.

Previous studies have shown that alkylation of the phenolic hydroxyl group of capsaicin will remove all biological activity.[4] We have alkylated this position with a 4-methoxyphenacyl group (see **8**, Scheme 1), which has been shown to undergo in vitro photolysis to reveal the desired biologically active product and two biologically inert by-products.[18] We have opted to cage the *N*-nonanoyl analogue of capsaicin (**7**), which has been shown to possess pharmacology similar to that of capsaicin itself.[3] Although capsaicin is commercially available, the use of compound **7** has allowed us to develop a robust synthesis that can also be applied to a number of capsaicin analogues. The synthesis commenced from the commercially available 4-hydroxy-3-methoxybenzonitrile (**3**, Scheme 1) and proceeded in good overall yield to give the desired compound **8**, using standard methods. All compounds displayed analytical and spectroscopic data consistent with the assigned structure (please see supplementary data for spectroscopic and analytical data for compounds **8** and **9**).

Before investigating the photodeprotection of **8**, it was necessary to determine the pharmacological response of the uncaged compound (**7**) in cultured dorsal root ganglia (DRG) neurones (see supplementary data for details). The results obtained suggest that compound **7** exerts its main biological effects through activation of TRPV1, which was expected from literature reports.[2–4]

Whole cell patch clamp recording was used to examine changes in the electrophysiological properties of cultured DRG neurones as a result of intracellular flash photolysis of compound **8** resulting in photo-release of compound **7**. Extracellular photolysis of compound **8** (0.1–1 mM **8** in saline containing 0.5–5% DMSO) produced no significant current or voltage responses in six neurones. The endocannabinoid, anandamide, is a more effective activator of this receptor when applied to the intracellular environment rather than extracellularly.[19] We therefore postulated that intracellular photolysis of compound **8** might be a more efficient way of activating TRPV1 receptors. Intracellular photolysis of compound **8** (0.5 mM caged compound in the patch pipette solution containing 2.5% DMSO) evoked sustained membrane potential depolarisations of 15 ± 2 mV in four out of five neurones (Fig. 2A) and action potentials were evoked in two out of these four neurones (Fig. 2B). Under voltage clamp, a comparison was made between inward currents activated by extracellular application of 1 μM capsaicin and intracellular photolysis of compound **8**. Consistent with the imaging data, capsaicin (1 μM) failed to evoke responses in five neurones, and subsequently intracellular photolysis of compound **8** also failed to evoke currents (Figs. 2C and D). However, DRG neurones that responded to capsaicin also produced modest (>∼10% of capsaicin-activated current) but consistent inward currents to intracellular photolysis of compound **8** (Figs. 2C and E). Repeated flash photolysis produced a cumulative inward current but the increases in amplitude may have been limited by desensitisation. Interestingly, in one DRG neurone a transient burst of action currents was evoked by intracellular photolysis of compound **8** (Figs. 2F and G). These events were not preceded by a clear inward current (Fig. 2) and are likely to be due to responses being initiated on DRG neurone processes resulting in action potential firing and the spread of this excitation into the voltage clamped cell body.



Scheme 1. Synthesis of the caged capsaicin analogue **8**. Reagents and conditions: (i) TIPSCl, imidazole, DMF, rt, 91%; (ii) $LiAlH_4$, THF, rt, 90% crude yield; (iii) nonanoyl chloride, 4-DMAP, pyridine, CH_2Cl_2, 0 °C–rt, 78%; (iv) TBAF, THF, 81%; (v) 2-bromo-4′-methoxyacetophenone, NaH, DMF, 0 °C–rt, 74%.

Apart from the final case (Figs. 2F and G), the photolysis of the phenacyl compound produced smaller responses than those observed by Zemelman et al. when uncaging a dimethoxynitrobenzyl caged compound.[20] This observation led us to investigate the amount of uncaging of **8** that was occurring under the experimental conditions. In an attempt to mimic our biological experiments as closely as possible, an aliquot (10 μl) of a 50 μM solution was exposed to a 300 V flash from an identical xenon flash lamp equipped with a 360 nm filter. The small volume of material used, however, has made it impossible to detect whether uncaging has occurred and to what degree. Attempts were made using HPLC, ^{1}H NMR and mass spectrometry (MS), but in all cases, and even when using several combined aliquots, it was impossible to detect uncaging and in the case of ^{1}H NMR it was not possible to detect even the caged material.

The photolysis of a dimethoxynitrobenzyl caged compound similar to that used by Zemelman was studied by Katritzky et al.[21] We therefore decided to investigate photolysis of compound **8** using conditions similar to those reported. Katritzky employed a 450 W immersion lamp with a 363 nm filter. We used a 125 W immersion lamp with a 375 nm filter. As we were unable to fully match Katritzky's conditions we synthesised the dimethoxynitrobenzyl caged derivative of compound **7** (compound **9**, Scheme 2) to allow direct

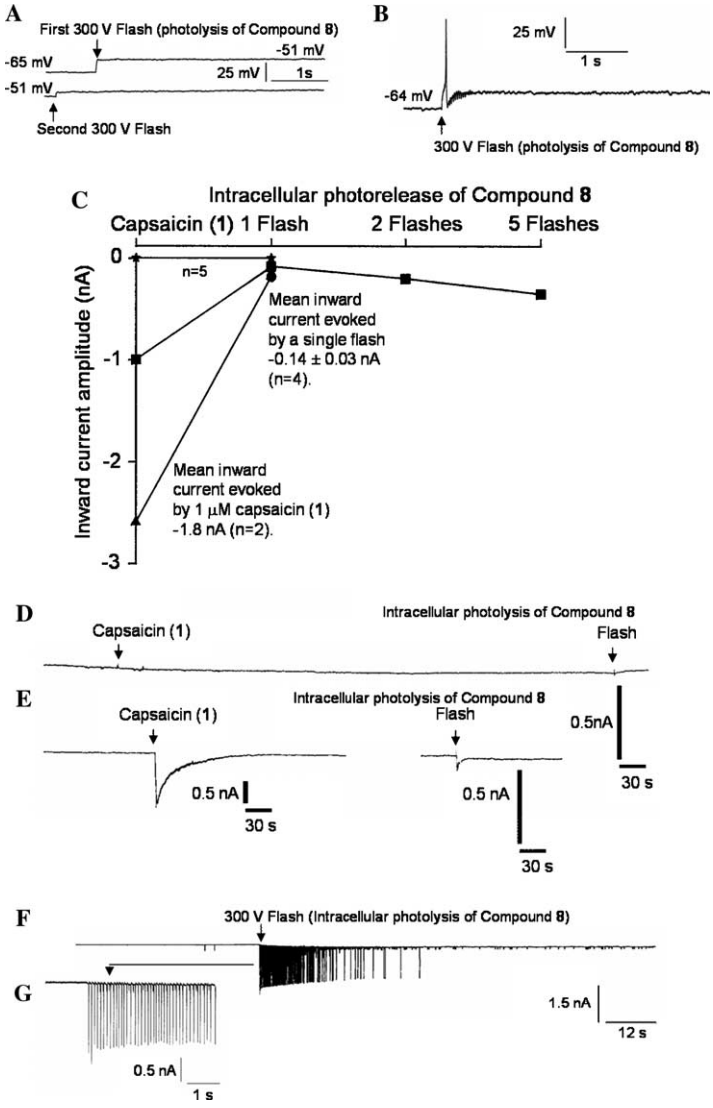
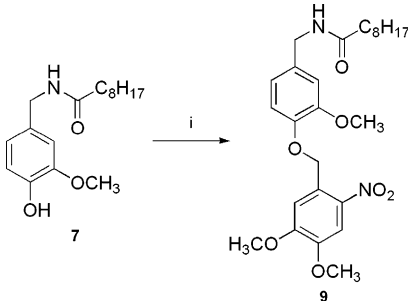


Figure 2. Intracellular actions of compound **8** photolysis on the excitability of cultured DRG neurones. The same extracellular solution was used for both the electrophysiology and the Ca^{2+} imaging experiments. The patch pipette solution contained (in mM): KCl, 140; EGTA, 5; $CaCl_2$, 0.1; $MgCl_2$, 2.0; HEPES, 10.0; ATP, 2.0; compound **8** 0.5–0.1% and 2.5% DMSO. This solution after correction with Tris and sucrose had a pH of 7.2 and osmolarity of 320 mOsm L^{-1}. An Axoclamp 2 A switching amplifier (Axon Instruments) operated at a switching frequency of 15 kHz was used. (A) Record showing two depolarising responses to intracellular photolysis of compound **8** from the same neurone. No action potentials were evoked in this neurone and no further depolarisation was obtained with additional photolysis (3rd flash not shown). (B) Example record showing a single action potential and sustained depolarisation obtained in response to intracellular photolysis of compound **8**. (C) Line chart showing the diversity of DRG neurone current responses to capsaicin and intracellular photolysis of compound **8**. Only 2 out of 7 DRG neurones responded to capsaicin but both these neurones also responded to intracellular photolysis of compound **8**. Five cells failed to respond to both drugs. (D) Example voltage clamp record showing a non-responding neurone that failed to respond to both capsaicin and intracellular photolysis of compound **8**. (E) Records showing inward currents activated by capsaicin and intracellular photolysis of compound **8**; note the difference in current scale. (F) Action currents evoked by intracellular photolysis of compound **8**; showing the burst firing behaviour that gradually declines as the neurone recovers to a resting state. This neurone was voltage clamped at a holding potential of −70 mV; the excitatory action of photoreleased compound **7** appears to have occurred in an unclamped region of the cell and a burst of action potentials has spread into the cell body to be recorded as currents. (G) The same record as (F) but on an expanded time scale to show the high frequency action potential firing and that the first action potential was not initiated by a clear inward current in the cell soma. Arrows mark the points at which 300 V flashes (175 mJ; lasting ~1 ms) from a xenon flash lamp, equipped with a 360 nm filter, were applied to the DRG neurones. Under voltage clamp all neurones were held at −70 mV.

comparison of the two caging groups using our photolysis conditions.

Aliquots (1 ml) of a 7.3 mM solution of compound **8** were irradiated using a 125 W mercury arc lamp through a 375 nm filter. ^{1}H NMR analysis of samples irradiated for 1, 15 and 30 min showed no detectable uncaging occurring. The ^{1}H NMR spectrum of the sample that was irradiated for 60 min showed a trace of the uncaged material (see supplementary data). ^{1}H NMR analysis of a sample that had been irradiated for 16 h 40 min showed that the caged material had degraded (data not shown). However, the expected peaks for compound **7** were not clearly present, indicating that this compound may be further broken down on extended irradiation. Irradiation of compound **8** using a 125 W



Scheme 2. Synthesis of the caged capsaicin analogue **9**. Reagents and conditions: (i) 4,5-dimethoxy-2-nitrobenzyl bromide, ^{t}BuOK, THF, rt, 66%.

mercury arc lamp *with no filter* showed a trace of compound **7** after 15 min.

Irradiation of aliquots (1 ml) of a 7.3 mM solution of compound **9** using a 125 W mercury arc lamp through a 375 nm filter showed more rapid uncaging (Fig. 3). After 30 min 35% uncaging had occurred. Examination of the UV/vis spectra of these compounds explains these results clearly as the absorption maximum for the carbonyl of the compound **8** is at 278 nm, whereas the absorption maximum for the nitro group of compound **9** is at 345 nm, much closer to the wavelength of irradiation (see supplementary data).

Zemelman observed a 1–2 s interval between flash photolysis and TRPV1 activation.[20] We did not observe this with our studies on compound **8**. It is not clear to us why Zemelman and co-workers observed the interval that they did; we will employ compound **9** to investigate this further.

Although it is not possible to draw any quantitative conclusions from these studies, our investigations into the photolysis of compounds **8** and **9** correlate with biological activity observed for these compounds. It can be assumed that during in vitro flash photolysis of compound **8**, using the conditions described above, only a very small amount of compound **7** is being released. The fact that a biological response is observed reflects the high potency of compound **7** for TRPV1. Flash photolysis of compound **9** releases a greater amount of compound **7** and this is reflected in the more prominent biological response observed by Zemelman et al.[20] The different amounts of photolysis observed on irradiation of these compounds will render them useful and complementary biological tools for the investigation of TRPV1. The fact that the two caging groups behave in different manners on irradiation at the same wavelength suggests that compounds **8** and **9** may be of use in investigating the concept of wavelength orthogonality as applied to biologically active compounds. This is currently under investigation and our findings will be published in due course.

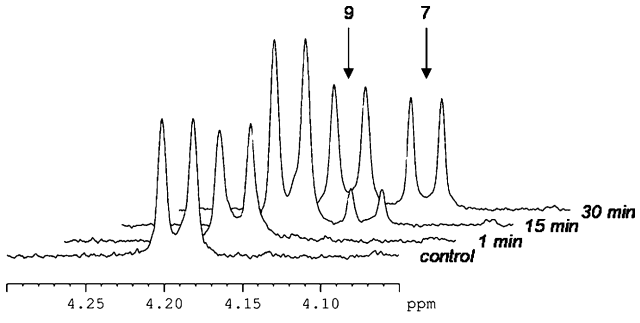


Figure 3. Partial 300 MHz ^{1}H NMR spectra of compound **9** irradiated, for the time shown, with a 125 W mercury arc lamp through a 375 nm filter. Thirty-five percentage uncaging (as adjudged by appearance of compound **7** vs total material) was observed after 30 min. The doublet at 4.19 ppm is from compound **9**; the doublet at 4.14 ppm is from compound **7**.

Acknowledgments

We thank Dr. Ruth Ross (University of Aberdeen) and Dr. Douglas Philp (University of St Andrews) for helpful discussions and critical reading of the manuscript. We acknowledge the School of Chemistry, University of St. Andrews and Pfizer Research UK (Sandwich) for financial support.

Supplementary data

The biological characterisation of compound **1**, selected partial NMR spectra for the photolysis of compound **8** and full analytical and spectroscropic data for compounds **8** and **9** are available in the supplementary material, in the online version, at doi:10.1016/j.bmcl.2005.09.018.

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