Communications

Photopharmacology

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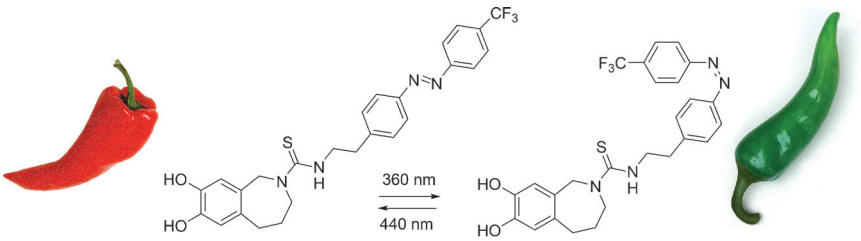
Optical Control of TRPV1 Channels



Controlling pain with light: TRPV1 channels mediate the response to noxious heat and can be activated by capsaicin, the major ingredient of chili pepper. Novel azobenzene photoswitches can be used for the optical control of TRPV1. One of these compounds antagonizes capsaicin in a light-dependent fashion, demonstrating that a photoswitchable antagonist and an agonist can be applied in concert to modulate ion channel activity.

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Optical Control of TRPV1 Channels**

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Transient receptor potential channels (TRP channels) constitute one of the largest ion channel families in the human genome and are versatile cellular sensors involved in the perception of pain, warm and cold temperatures, noxious and pungent chemicals, and pressure.[1] They are also involved in visual processing and might shape the circadian rhythm in mammals.[2] As such, members of this ion channel family play a key role in sensory physiology, the full extent of which is still unknown. In addition, they are implicated in the regulation of gastrointestinal motility, absorptive and secretory processes, blood flow, and mucosal homeostasis.[1a] In fact, several human diseases are known that are caused by mutations in TRP channel genes.[1a] All of this has recently sparked intense research activity in academia and industry, giving rise to various new probes and drug candidates that target TRP channels.

The vanilloid receptor 1 (TRPV1) is one of the most important and best understood representatives of the family.[1] It is a nonselective cation channel that is, like most TRP channels, permeable to Ca^{2+} ions but shows little discrimination between mono- and divalent cations.[3] TRPV1 is abundantly expressed in all sorts of nociceptive neurons, such as dorsal root ganglion (DRG) and trigeminal ganglion (TG) neurons, as well as spinal and peripheral nerve terminals and the cornea.[1c] Usually, the channel is located in the plasma membrane but it is occasionally also found in intracellular membranes (e.g. sarco-/endoplasmic reticulum) where it may function as an intracellular Ca^{2+} release channel.[4] Activation of TRPV1 results in a sensation of burning and pain, making this channel a promising target for the development of potential analgesics. TRPV1 is activated by several chemical and physical stimuli, such as voltage,[5] heat,[6] capsaicin,[6a] spider toxins,[7] low pH,[8] and several fatty acids such as the endocannabinoid anandamide[9] and is potentiated by phosphorylation.[10] However, despite its multimodal activation mechanisms, TRPV1 is not known to naturally respond to light.

Over the last decade, we have developed general methods to optically control receptor proteins by either covalently attaching a photoswitchable tethered ligand (PTL), or providing a freely diffusible photochromic ligand (PCL).[11] We have applied both approaches to voltage-dependent ion channels in order to control heartbeat,[12] pain sensation,[13] and visual responses[14] in a variety of animals. Moreover, we have been able to convert nicotinic acetylcholine receptors,[15] as well as ionotropic[16] and metabotropic glutamate receptors[17] into artificial photoreceptors. Most recently, we succeeded in developing light-switchable anaesthetics based on propofol,[18] which potentiate GABA-induced chloride currents.

We now report the development of photoswitchable drugs that effectively convert TRPV1 channels into photoreceptors. Our studies were enabled by the highly developed pharmacology of TRPV1, which is outlined in Figure 1. The vanillamine derivative capsaicin (CAP), the pungent ingredient in hot chili peppers, is the best known agonist of TRPV1.[6a] A much more complex diterpenoid, resiniferatoxin (RTX), isolated from the cactus *Euphorbia resinifera*,[19] is considered to be the spiciest substance known. The competitive antagonist capsazepine (CPZ) was found by Sandoz in 1994[20] in an attempt to explore the structure–activity relationship (SAR) of CAP. To date, BCTC and thio-BCTC are the most specific blockers of TRPV1 activity.[21] BCTC was discovered during SAR studies in 2003[22] and has become a popular TRPV1 antagonist. It was found to be superior to CPZ in terms of its CAP-antagonizing effects on TRPV1, but also upon activation by low pH.

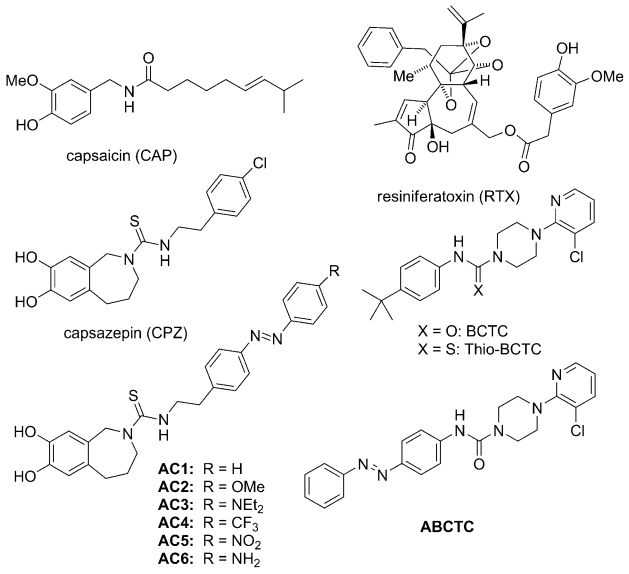

Figure 1. Agonists and antagonists of TRPV1 and their photoswitchable derivatives **ABCTC** and **AC1**–**6**.

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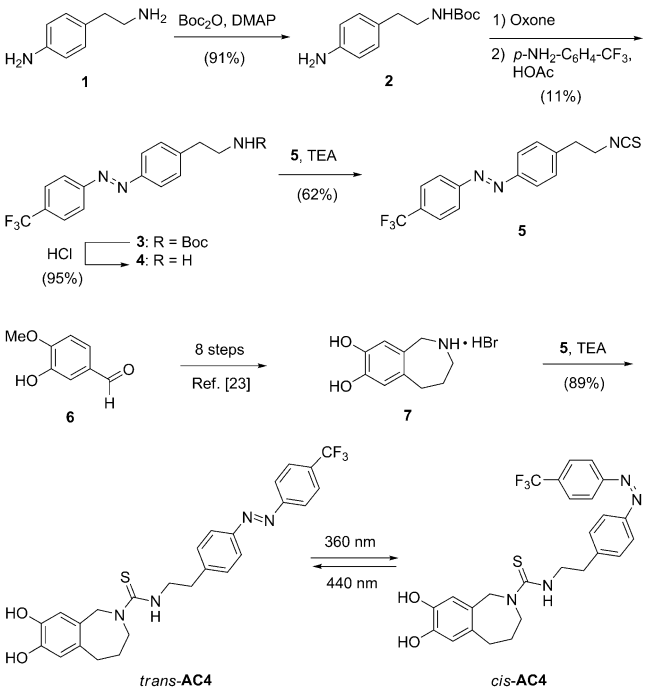
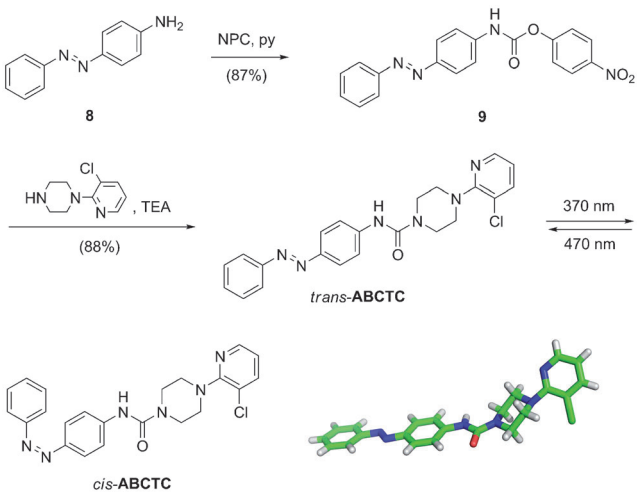
Capsazepine and BCTC contain a phenyl ring with a chloro and *tert*-butyl substituent, respectively, in *para* position with respect to the remainder of the molecule. In both cases, it was known that these substituents could be varied to a certain extent without complete loss of activity.[20b,22] We thus reasoned that the phenyl rings could be extended to azobenzenes, hoping that the *cis* and *trans* configurations of these moieties would afford antagonists with different efficacies. Based on these considerations, we designed azobenzene derivatives of CPZ and BCTC, termed Azo-Capsazepines 1–6 (**AC1–6**) and Azo-BCTC (**ABCTC**), which cover a range of photophysical and pharmacological properties.

The synthesis of **AC4**, which emerged as our most useful candidate, is depicted in Scheme 1 (for the syntheses of the other AC derivatives, see the Supporting Information). First, amine **1** was protected as a *tert*-butyl carbamate to yield aniline **2**, which was then oxidized to the corresponding nitroso derivative and subsequently coupled to 4-(trifluoromethyl)aniline in a Mills reaction, affording azobenzene **3**. Removal of the Boc group gave free amine **4**, which was converted to the corresponding isothiocyanate **5** using 1,1′-thiocarbonyldi-2(1*H*)-pyridone. Compound **5** was then coupled to azepine **7**, which was synthesized from isovanilline **6** in eight steps according to a modified literature protocol[23] (see the Supporting Information) to afford **AC4**.

The synthesis of **ABCTC** commenced with the coupling of aniline **8** and 4-nitrophenyl chloroformate to give carbamate **9**, which was then treated with 1-(3-chloropyridin-2-yl)piperazine to afford **ABCTC** (Scheme 2). The X-ray structure of



Scheme 1. Synthesis of **AC4**, which isomerizes from its *trans* form to the *cis* state when $\lambda = 360$ nm light is applied and reverts back when $\lambda = 440$ nm light is applied. Boc = *tert*-butyloxycarbonyl, DMAP = 4-dimethylaminopyridine, TCDP = 1,1′-thiocarbonyldi-2(1*H*)-pyridone, TEA = triethylamine.



Scheme 2. Synthesis of **ABCTC** and its X-ray structure in the *trans* configuration. The *trans*⇄*cis* isomerization occurs with $\lambda = 370$ nm and $\lambda = 470$ nm light, respectively. NPC = 4-nitrophenyl chloroformate, py = pyridine.

ABCTC illustrates the importance of the *o*-chloro substituent, which forces the pyridine ring out of the average plane of the piperazine ring.[24]

We next tested our compounds for their ability to act as antagonists using electrophysiology in HEK cells transiently transfected with TRPV1. First, our functional analyses took advantage of the voltage sensitivity of TRPV1.[5] We found that all six AC derivatives synthesized function as TRPV1 antagonists at concentrations of 5–100 μM. However, the trifluoromethyl derivative **AC4** emerged as the best photoswitchable antagonist among all Azo-Capsazepines. Under voltage-gating conditions, it functions as a *trans* antagonist, blocking more current at 440 nm, than in the *cis* state at 360 nm (Figure 2a,b). Channel gating by depolarization to +200 mV allows for fast and fully reversible inhibition of TRPV1 currents of > 1 nA when the respective wavelength is applied to 100 μM **AC4** containing bath solution (Figure 2c).

Conversely, the BCTC derivative **ABCTC** was found to block more TRPV1 current in its *cis* state (at 370 nm) than in its *trans* form (at 470 nm; Figure 2d–f). As such, **ABCTC** functions as a reversible *cis* antagonist. The *I*/*V* curves of **AC4** and **ABCTC** are similar, with both antagonists blocking > 20% of maximum current in their more active form (*trans*-**AC4** and *cis*-**ABCTC**, respectively).

Since the voltage protocols involve nonphysiological conditions, we next investigated the effect of our photoswitchable TRPV1 antagonists on CAP-induced TRPV1 currents in a Ca^{2+} luminescence essay. Whereas **AC1–3**, **AC5**, and **AC6** showed only low antagonistic effects upon TRPV1 activation with 1 μM CAP (IC_{50} values > 50 μM, see the Supporting Information), **AC4** and **ABCTC** exhibited higher activities. We recorded IC_{50} values of (3.1 ± 0.6) μM for **AC4** compared to (0.2 ± 0.06) μM for CPZ, and (12.8 ± 0.7) μM for **ABCTC** compared to (0.2 ± 0.6) μM for BCTC, respectively (Figure 3a,b).

We then investigated whether we could regulate CAP-induced TRPV1 currents with light in the presence of either

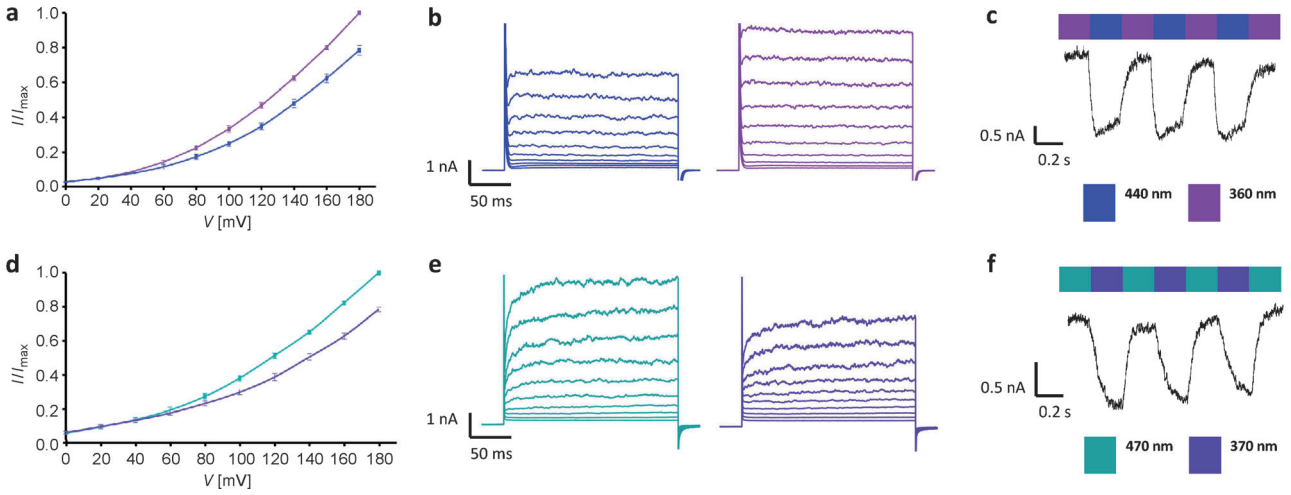


Figure 2. a) *I*/*V* curves of voltage-activated TRPV1 channels in 20 mV steps up to +180 mV upon application of $\lambda = 440$ nm light (blue) and $\lambda = 360$ nm light (magenta) to 100 μM of **AC4**, respectively (normalized to I_{max}; $n = 6$). b) Representative traces from (a). c) Photoswitching cycles by wavelength alternation at +200 mV with 100 μM **AC4**. d)–f) Analogous data for 10 μM **ABCTC** with application of $\lambda = 370$ nm light (purple) and $\lambda = 470$ nm light (turquoise).

AC4 or **ABCTC**. Whereas **ABCTC** gave no change in activity upon irradiation, the antagonistic action of **AC4** could be modulated in this fashion. Puff application of 100 nM CAP to HEK cells, which were transiently transfected with TRPV1 and immersed in a bath solution containing 1 μM **AC4**, resulted in a large current increase that could be reduced by up to 82% upon irradiation with $\lambda = 360$ nm, which isomerizes **AC4** into its *cis* configuration. By applying $\lambda = 440$ nm light, the CAP-induced current could be restored (Figure 3c). This process is fully reversible. Statistical analysis ($n = 8$, $P = 0.01$, Student's t-test) revealed a photoswitching index (determined by the ratio of current increase upon CAP application at $\lambda = 360$ nm and 440 nm, respectively) of 49% (Figure 3d). A control experiment (Figure 3e) showed that the CAP-induced current could not be modulated with light in the absence of the photoswitchable antagonist.

Remarkably, **AC4** acted as a *cis* antagonist of CAP-induced TRPV1 currents, whereas it functioned as *trans* antagonist upon voltage activation. This might appear paradox at first sight but is in good agreement with the observation that agonists interact with TRPV1 channels in a state-dependent fashion and that specific antagonists differ in their ability to block distinct agonists.[25] Thus, our study may open up new avenues for the development of modality-selective antagonists, which might help to overcome unwanted side effects of analgesics targeting TRPV1.

In conclusion, we have developed a method to optically control TRP channel activity with azobenzene derivatives of the TRPV1 antagonists capsazepine (CPZ) and BCTC. Six derivatives of CPZ, termed **AC1–6**, have been synthesized, amongst which the trifluoromethyl derivative **AC4** proved to be the most useful and interesting compound. Compound **AC4** functions as *trans* antagonist upon voltage-activation of TRPV1 and as *cis* antagonist upon stimulation with capsaicin (CAP). Another azobenzene, **ABCTC**, was

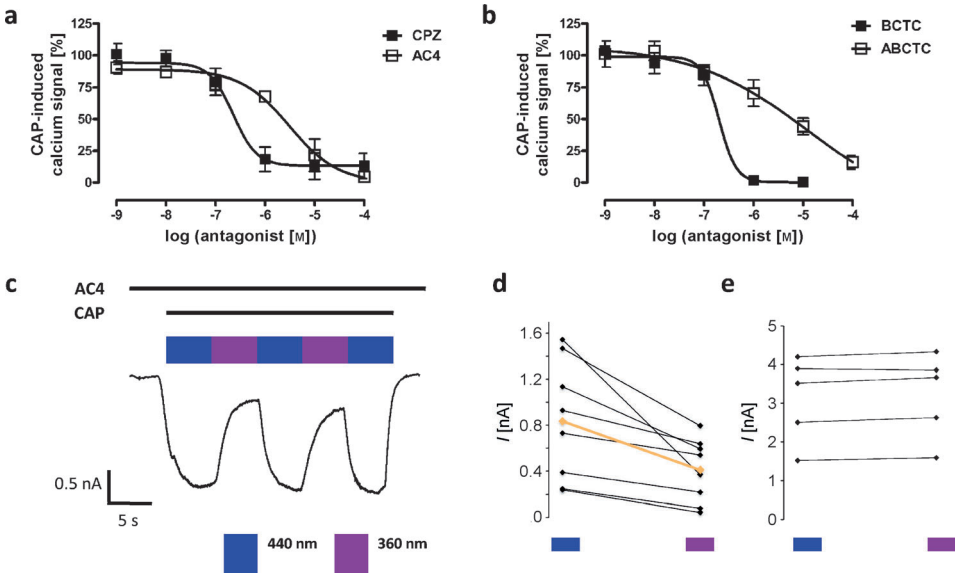


Figure 3. a) Dose–response curve of **AC4**, in comparison with that of CPZ; $n = 5$. b) Dose–response curve of **ABCTC**, in comparison with that of BCTC; $n = 5$. c) CAP-induced currents (100 nM CAP) can be modulated in the presence of **AC4** (1 μM) by switching between $\lambda = 440$ nm and $\lambda = 360$ nm light (holding potential −60 mV). d) Statistical analysis of CAP-induced TRPV1 current photoswitching by **AC4** (orange = median). The difference is significant ($P = 0.01$, Student's t-test, $n = 8$). e) No photoswitching of CAP-induced TRPV1 currents was observed the absence of **AC4** ($n = 5$).

found to act as a *cis* antagonist upon voltage-activation of TRPV1 but did not counteract CAP in a light-dependent fashion. Our photoswitchable compounds add an additional input signal to TRPV1 channels and provide a strategy to stimulate TRP channels with light. As such, they complement the recently described photochemically active molecule "optovin", which acts on TRPA1 channels and through a different mechanism.[26] Our lead compound, **AC4**, demonstrates that a photoswitchable antagonist and an agonist can be used in concert. This broadens the scope of photopharmacology and provides a new functional dimension to the optical manipulation of ion channels with synthetic photoswitches. The application of **AC4**, **ABCTC**, and related compounds in sensory physiology is under active investigation and will be reported in due course.

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[24] X-ray structure of **ABCTC**: $C_{22}H_{21}ClN_6O$, $M_r = 420.895\ g\,mol^{-1}$, yellow rod, $0.31 \times 0.04 \times 0.03\ mm^3$, monoclinic, $P2_1/c$, $a = 5.9574(2)$, $b = 21.2314(8)$, $c = 15.6580(6)$ Å; $\beta = 97.353(2)°$, $V = 1964.20(1)$ Å^3, $Z = 4$, $\rho = 1.423\ g\,cm^{-3}$, $\mu(Mo_{K\alpha}) = 0.223\ mm^{-1}$, $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å), $T = 173$ K, $2\theta_{max}$ 48.20°, 10388 reflections, 3104 independent, 2064 with $I \geq 2\sigma(I)$, $R_{int} = 0.0735$, mean $\sigma(I)/I = 0.0625$, 274 parameters, $R(F_{obs}) = 0.0446$, $R_w(F^2) = 0.1092$, $S = 1.038$, min. and max. residual electron density: -0.255, $0.199\ e\,Å^{-3}$; data collection by means of a Nonius KappaCCD diffractometer equipped with a rotating anode generator (ω-scans), structure solution by direct methods with SIR97, structure refinement with SHELXL-97. C-bound H atoms have been added geometrically treated as riding on their parent atoms and N-bound H atoms have been fixed at 0.87(1) Å with $U(H) = 1.2\ U(N)$; CCDC 923926 (**ABCTC**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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