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Naphthol derivatives as TRPV1 inhibitors for the treatment of urinary incontinence

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ABSTRACT

We have identified naphthol derivatives as inhibitors of the vanilloid receptor TRPV1 by high throughput screening. The initial lead showed high clearance in rats and has been optimized by enhancing the acidity of the phenol group. Compound **6b** has reduced clearance, improved potency and is active in rat cystometry models of urinary incontinence after intravenous administration.

TRPV1 (Transient receptor potential vanilloid 1, vanilloid receptor 1 or VR1) is an ion channel that gates after a variety of stimuli such as heat, acid or chemical agents like capsaicin (**1a**) or resiniferatoxin (**1b**). It has been suggested that inhibiting TRPV1 could

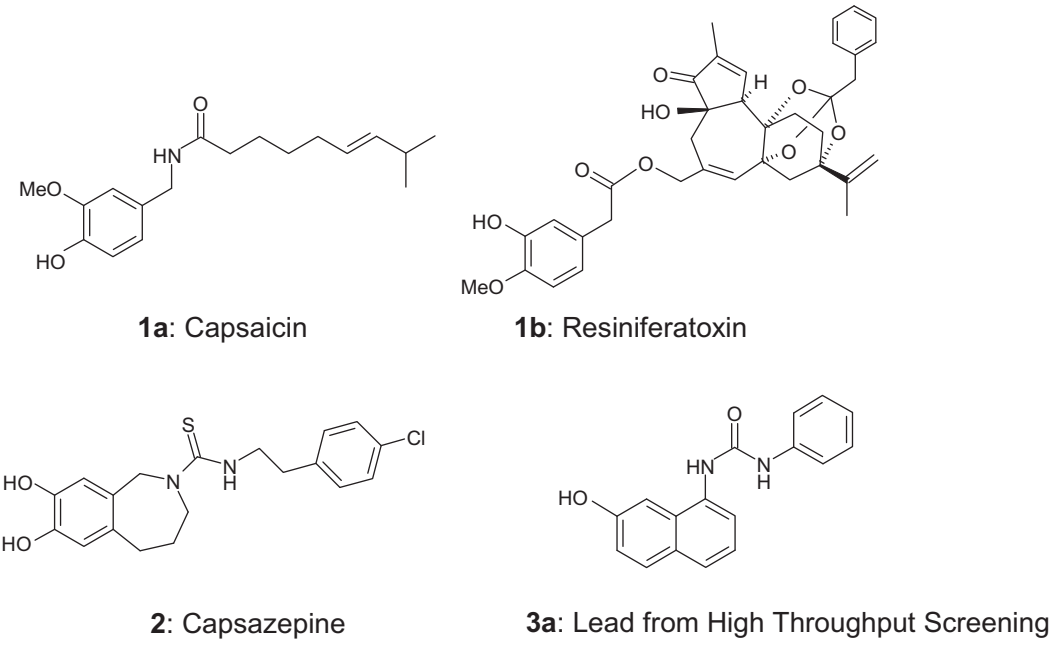

Figure 1. Structural comparison of HTS hit **3a** with the agonists **1a/b** and the known antagonist **2**.

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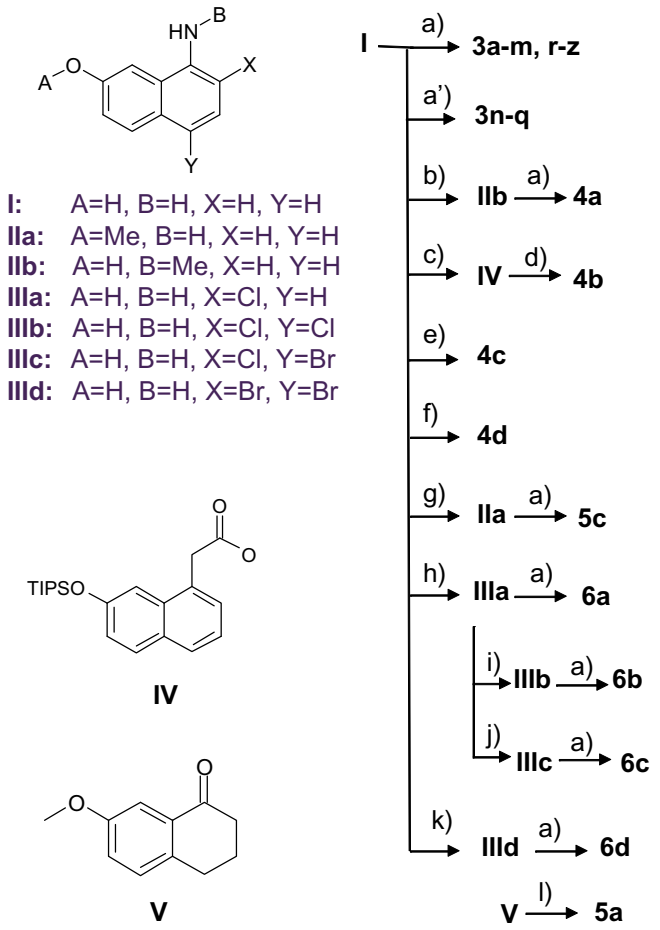
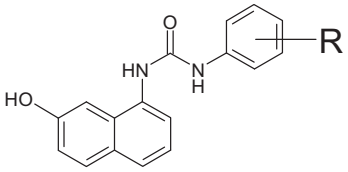


Figure 2. Synthesis of TRPV1 inhibitors. Reactions and conditions: (a) ArNCO, dioxane, reflux, 3 h, 20–95%. (a') 1,1'-carbonyldi-(1,2,4 triazole) (1 equiv) THF, rt 1 h, then $ArNH_2$, 50 °C, 15 h, (40–80%). (b) Pyridine (2 equiv), $(CF_3CO)_2O$ (1.5 equiv), THF, rt, 1.5 h 30%; (2) MeI (1.2 equiv), K_2CO_3 (5 equiv), Bu_4NI, acetone, rt, 2.5 h (63%). (3): $NaBH_4$ (0.95 equiv) EtOH, rt, 87%. (c) (1) $NaNO_2$ (1.1 equiv), KI (1.1 equiv), THF/3 N HCl (1:2), 1 h, 0 °C, 26%; (2). $Bu_3SnCHCH_2$ (1.2 equiv), $Pd(PPh_3)_4$ (0.2 equiv), toluene, 90 °C, 16 h, 100%; (3) Imidazole (1.5 equiv), $(i\text{Pr})_3SiCl$ (1.1 equiv), DMF, 50 °C, 16 h, 63%; (4) 9-BBN (1.1 equiv), THF, rt, 5 h, then aq NaOH (3 N), aq H_2O_2 (30%), rt 6 h, 56%. (5) HIO_4 (1 equiv), CrO_3 (1 equiv) CH_3CN/H_2O (3:1), 0 °C, 30 min, 24%. (d) (1) DMAP (0.2 equiv), 1-(3-diethylaminopropyl)3-ethylcarbodiimide (1.2 equiv), CH_2Cl_2, rt, 16 h, 89%; (2) Bu_4NF (3 equiv), THF, rt, 30 min, 65%. (e) $(EtS)_2NCN$ (2 equiv), $(n\text{Bu})_2O$, reflux, 3 h, then 4Cl-3CF_3ArNH_2 (3 equiv) reflux, 12 h, 30%. (f) 3-F-$C_6H_4CH_2COOH$, DMAP (0.2 equiv), 1-(3-diethylaminopropyl)3-ethylcarbodiimide (1.2 equiv), CH_2Cl_2, rt, 16 h, 62%. (g) (1) PhCHO (1.1 equiv), Na_2SO_4 (5 equiv), THF, reflux, 12 h, 98%. (2) MeI (2 equiv), NaOH (2 equiv), acetone, rt, 2 h, then 2 N HCl/THF (2:1), rt 2 h, 93%. (h) NCS (1 equiv), THF, rt, 16 h, 69%. (i) NCS (2.2 equiv), THF, rt 16 h, 70%. (j) NBS (1 equiv), THF, rt, 16 h, 41%. k) NBS (2 equiv), THF, rt, 16 h, 21%. (l) (1) NH_2OHHCl (3 equiv), K_2CO_3 (4 equiv), MeOH, reflux, 16 h, quant. (2) Pd/C (10%), AcOH (cat.), H_2 (1 atm), MeOH, rt, 16 h, quant. (3) BBr_3 (1.3 equiv), CH_2Cl_2, 0 °C, 98%. (4) 4-Cl-3-CF_3-$C_6H_3NHCOPh$, DMSO, 90 °C, 16 h, 60%.

provide potential treatments for pain, cough or urinary incontinence. First TRPV1 antagonists have now reached clinical trials with promising results for nociception, however, most compounds exhibit mechanism-based hyperthermia as a side effect. Strategies aimed at widening the therapeutic window include co-administration of anti-pyretics, shortening the compounds' half life or addressing alternate indications, such as urinary incontinence (UI).[1]

UI is characterized by involuntary leakage of urine and can have profound and distressing impact on the quality of life for affected individuals. The current treatment of UI involves muscarinic antagonists and is associated with mechanism-based side effects such as dry mouth symptoms.[2]

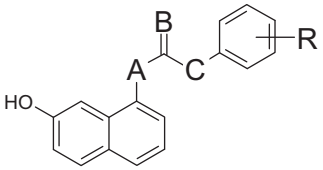
Table 1
Structure–activity relationship of naphthol-based TRPV-1 antagonists Variation of the anilino substitution R



Compd	R	r-IC_{50} [nM]	h-IC_{50} [nM]	*c* Log *P*
3a	H	53	29	4.1
3b	2-CF_3	64	87	4.1
3c	3-CF_3	4.5	3.5	4.9
3d	4-CF_3	4.9	5.4	4.9
3e	2-Cl	38	52	4.0
3f	3-Cl	3.2	6.1	4.5
3g	4-Cl	11	12	4.5
3h	3-Me	19	11	4.0
3i	3-F	21	14	4.0
3j	3-OMe	8.6	8.0	3.6
3k	3-NO_2	14	7.5	3.9
3l	3-COMe	39	28	3.5
3m	3-Br	8.0	5.7	4.7
3n	3-COOEt	1.6	1.7	4.6
3o	3-CH_2OH	520	>1000	2.5
3p	3-$CH(CH_3)OH$	670	>1000	2.8
3q	3-COOH	270	520	3.6
3r	3-SMe	7.1	3.9	4.1
3s	2,6-Cl_2	>1000	>1000	4.2
3t	2,5-Cl_2	30	24	4.8
3u	2,4-Cl_2	16	12	4.8
3v	3,4-Cl_2	4.5	4.9	5.2
3w	3,5-Cl_2	12	12	5.3
3x	2-CF_3, 4-Cl	19	31	4.9
3y	5-CF_3, 2-Cl	12	13	5.2
3z	3-CF_3, 4-Cl	1.9	2.2	5.5

IC_{50} values are medians of three dose–response curves.

Table 2
Structure–activity relationship of naphthol-based TRPV-1 antagonists Variation of the urea portion R



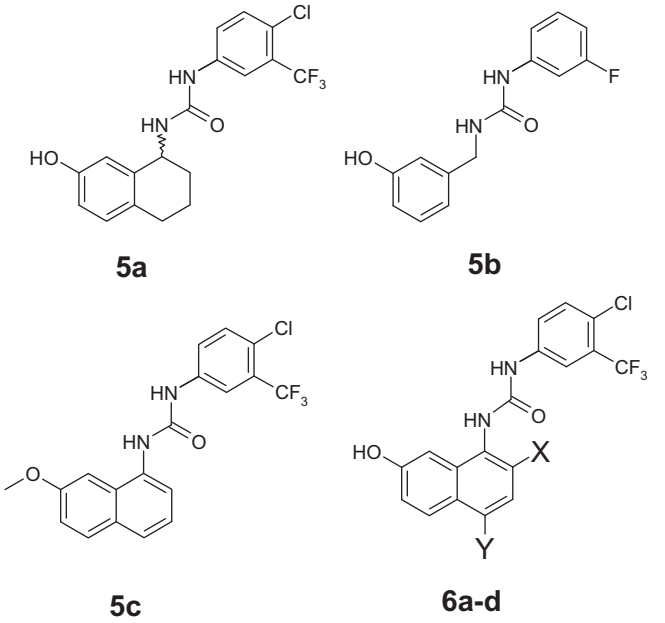
Compd	A	B	C	R	h-IC_{50} [nM]
4a	NMe	O	NH	3-CF_3, 4-Cl	44
4b	CH_2	O	NH	3-CF_3, 4-Cl	34
4c	NH	NCN	NH	3-CF_3, 4-Cl	340
4d	NH	O	CH_2	3-F	>1000

IC_{50} values are medians of three dose–response curves.

Using a Ca-flux primary assay system, we performed a high throughput screen and identified **3a** as a lead with selectivity against the P2X1 ion channel.[3] This compound is a nanomolar inhibitor of both rat and human TRPV1. Whilst its phenol moiety is structurally related to the agonists **1a** and **1b**, its bicyclic structure is reminiscent to the known antagonist Capsazepine (**2**) and offers ample opportunity for structural modification (Fig. 1). Interestingly, other groups have identified related HTS-hits, but followed optimization strategies different to ours, indicating the versatility of the lead.[4]

The majority of compounds were prepared from naphthol **I** by alkylation or halogenation to furnish intermediates **IIa–b** and **IIIa–d**. Ureas were synthesized from these intermediates or **I** by coupling directly with the corresponding aryl isocyanate.

Table 3
Structure–activity relationship of naphthol-based TRPV-1 antagonists Variation of the naphthalene system



Compd	X	Y	r-IC_{50} [nM]	h-IC_{50} [nM]	AUC [ng h ml^{-1}]
5a	—	—	nd	260	nd
5b	—	—	>1000	>1000	nd
5c	—	—	19	32	114
6a	Cl	H	4.5	3.4	3
6b	Cl	Cl	2.1	5.9	84
6c	Cl	Br	4.9	6.8	477
6d	Br	Br	16	7.5	596

The area under the curve (AUC) is obtained after oral administration to rats and relates to a 1 mg kg^{-1} dose.

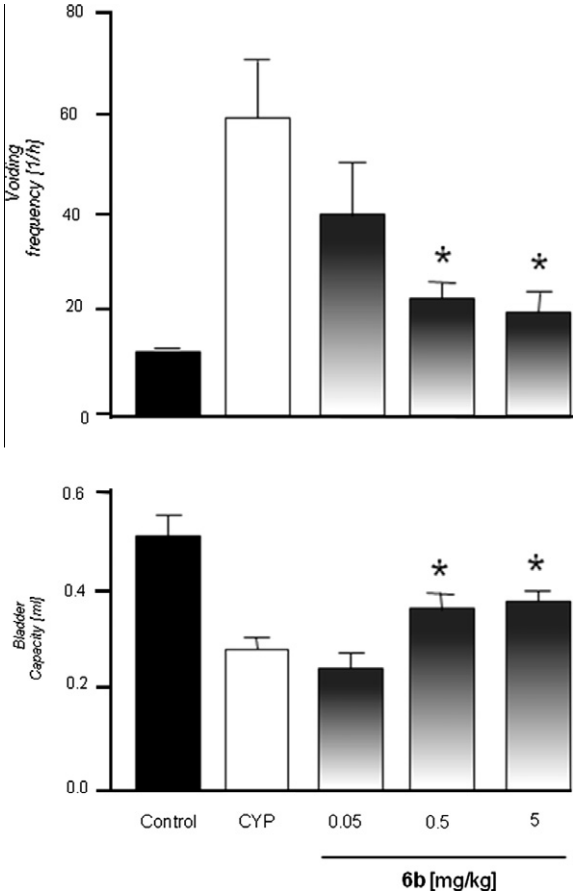


Figure 3. Cystometry evaluation in the cyclophosphamide (CYP)-induced cystitis model. Compound **6b** dose-dependently inhibits voiding frequency and bladder capacity after intravenous administration to female rats.

Table 4
Cystometry evaluation in the capsaicin-induced overactive bladder (OAB) model

Compd	Capsaicin-induced OAB	
	3 mg kg^{-1}	10 mg kg^{-1}
6b	n.d.	55%
6c	88%	n.d.
6d	11%	65%

Compounds **6b–d** were given intravenously to rats. Inhibition of micturition frequency is expressed in percent as compared to control.[7]

Compounds **3n–q** display sensitive functional groups and therefore we used the CDT coupling method[5,6] (Fig. 2). Compound **5b** is commercially available.[7]

We explored the substitution pattern of **3a**'s aniline fragment with compounds **3b–g** and the 3-position appeared to contribute most strongly to TRPV1 activity (Table 1): **3c** and **3f** offered by an order of magnitude better potency than **3a**. Therefore, we investigated the 3-position with further substituents (**3h–r**). Not surprisingly, TRPV1 activity correlated well with *c* log *P* for all derivatives **3a–z**, indicating the lipophilic nature of the binding site. The 2,6-disubstituted **3s** was inactive, probably indicating the importance of an *in-plane* urea/aniline conformation for activity. Comparing the di-substituted derivatives **3s–z**, we concluded that the 4-Cl, 3-CF_3 substitution pattern in **3z** was most potent, albeit most lipophilic. Our compounds showed no notable differences between rat and human TRPV1.

We also explored the lead's urea moiety (Table 2). **3c**'s proximal urea NH (A) seemed to be involved in a H-bond as methylation (**4a**) or isoelectronic replacement (**4b**) resulted in drop of activity by an order of magnitude.

The carbonyl group (B) appeared to be even more critically involved in binding as the corresponding cyano-guanidine **4c** was 100-fold less active. Comparison of **4d** with **3i** highlighted the importance of the distal urea NH (C) for molecular recognition.

We also undertook first variations of the naphthalene system (Table 3). Compared to **3z,** the tetrahydro-naphthalene **5a** showed a 100-fold drop in activity. Similarly, eliminating the ring altogether reduced activity markedly (**5b** vs **3i**).

Whilst **3z** was the most potent compound of the series, we didn't consider it for in vivo investigations. This naphthol was highly cleared in rats (23 l h^{-1} kg^{-1}), probably as a result of phase II metabolism. Simple alkylation of the hydroxyl group reduced clearance 10-fold (**5c:** 2.0 l h^{-1} kg^{-1}) and furnished detectable AUC's after oral administration. Unfortunately, **5c**'s TRPV1 activity was also reduced indicating the importance of the hydroxyl group for the pharmacophore.

We were speculating that enhancing the naphthol's acidity by introducing electron withdrawing substituents would reduce clearance and enhance oral exposure, whilst leaving the pharmacologically important hydroxyl group intact. Whereas potency was maintained, **6a–d** showed a good correlation of naphthol acidity with oral AUC and **6b–d** reduced capsaicin-induced micturition frequency in female rats (Table 4).[8] Due to its low clearance (0.16 l h^{-1} kg^{-1}), **6b** was selected for further characterisation in the cyclophosphamide-induced cystitis model and favourably altered voiding frequency as well as bladder capacity at intravenous doses above 0.05 mg kg^{-1} (Fig. 3).[9] Whilst useful

as a pharmacological tool for intravenous studies, **6b**'s oral bioavailability was still low (3%). This was probably due to **6b**'s limited solubility (0.03 mg l^{-1}), a feature not uncommon for urea-based leads.

Starting from an HTS lead, we have identified single-digit nanomolar TRPV1 antagonists. In preparing more acidic compounds, we have reduced rat clearance by two orders of magnitude. Compound **6b** showed intravenous activity in two rat cystometry models, highlighting the potential of TRPV1 antagonists for the treatment of UI. Further modifications of this lead towards orally active compounds will be reported shortly.

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3. *Assay descriptions*: h/r-VR1 c-DNA was transfected into a CHOluc9aeq cell line (pcDNA3) containing aequorin and CRE-luciferase reporter genes. After loading with Fluo-3AM (2 μM, Molecular Probes) and stimulation with capsaicin (10 nM), fluorescence changes were recorded for 1 min (λ_{ex} = 488 nm/ λ_{em} = 540 nm, Hamamatsu photonics) and IC_{50}values determined.
   A h-P2X1 transfected CHOluc9aeq cell line was used as a counterscreen using Fluo-3AM (1 μM). Fluorescence intensity (λ_{ex} = 410 nm, λ_{em} = 510 nm) was measured at 250 msec intervals and IC_{50} values were determined after agonist stimulation (200 nM α,β methylene ATP). All compounds showed activity >1000 nM. 2',3'-*O*-(2,4,6-trinitrophenyl)adenosine-5'-triphosphate was used as a reference.
4. The initial hit compound identified from HTS was compound **3r** (singleton), a commercially available material, which was apparently present in HTS compound collections of several competitors. Therefore, it is not surprising that related leads have been described by other groups and optimised in an independent, different way following our initial patent applications: Gomtsyan, A.; Rayburt, E.K.; Schmidt, R.G.; Zheng, G.Z., Perner, R.J.; Didomenico, S.; Koenig, J.R.; Turner, S.; Jinkerson, T.; Drizin, I.; Hannick, S.M.; Macri, B.S., McDonald, H.A.; Honore, P.; Wismer, C.T., Marsh, K.C.; Wetter, J.; Stewart, K.D.; Oie, T.; Jarvis, M.F.; Surowy, C.S.; Faltynek, C.R.; Lee, C.H.; *J. Med. Chem,* **2005**, *48*, 744. This group immediately abandons the phenolic hydroxy moiety whilst our strategy includes maintaining this structural feature. McDonnell, M.E.; Zhank, S.P.; Nasser; N. Dubin, A.E.; Dax, S.L.; *Bioorg. Med. Chem. Lett*., **2004**, *14*, 531. In contrast to the work presented here, this group describes mainly benzylic analogs of our lead.
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   Analytical data for **6a**: mp 260 decomp.; MS(ESI): 415; **6b**: mp 255, decomp.; MS(ESI): 449; **6c**: mp 209, decomp.; **6d**: mp 242–243, MS(ESI): 536.
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7. [CAS Nr. 1183505-20-5].
8. Acidities were calculated using ACD/PhysChem Suite Vers.12 (Advanced Chemistry Development, Toronto, On, Canada, 2010). p*Ka*'s: **3z**: 9.66; **6a**:9.28; **6b**: 8.83; **6c**: 8.81; **6d**: 8.79.
9. Cystometry models were performed according to Lecci, A.; Giuliani, S.; Santicioli, P.; Maggi, C.A. *Eur. J. Pharmacol*., **1994**, *259* 129.
   Cyclophosphamide-induced cystitis: cystitis was induced by cyclophosphamide administration (150 mg/kg, ip, saline) to female Sprague Dawley rats (180–250 g). After 48 h a bladder catheter was implanted and saline at room temperature infused into the bladder for 20 min (3.6 ml/min, micturition cycle). Intravesical pressure was recorded (Viggo-Spectramed PTe Ltd, DT-XXAD) and after 3 micturition cycles, **6b** was administered intravenously (ethanol, Tween 80, saline 1:1:8). The cystometry parameters were determined from the micturition interval and the volume of infused saline and evaluated using unpaired Student's *t*-test. Probability levels of less than 5% were accepted as statistically significant. Data shown are mean ± SEM.
   Capsaicin-induced micturition: following the protocol above, instead of cyclophosphamide administration 48 h prior to the experiment, Capsaicin (30 μM in saline) was infused at room temperature 2 min after compound administration (**6b**, **6c**, **6d**, iv in ethanol, Tween 80, saline 1:1:8). Micturition frequency is expressed as percent of control.