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Synthesis and biological evaluation of 5-substituted and 4,5-disubstituted-2-arylamino oxazole TRPV1 antagonists

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ABSTRACT

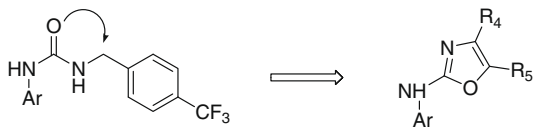
The synthesis and structure–activity relationships of a series of 5-monosubstituted and 4,5-disubstituted 2-arylaminooxazoles as novel antagonists of the transient receptor potential vanilloid 1 (TRPV1) receptor are described. The 7-hydroxy group of the tetrahydronaphthyl moiety on the 2-amino substituent of the oxazole ring was important for obtaining excellent in vitro potency at the human TRPV1 receptor, while a variety of alkyl and phenyl substituents at the 4- and 5-positions of the oxazole ring were well tolerated and yielded potent TRPV1 antagonists. Despite excellent in vitro potency, the 5-monosubstituted compounds suffered from poor pharmacokinetics. It was found that 4,5-disubstitution on the oxazole ring was critical to the improvement of the overall pharmacokinetic profile of these analogues, which led to the discovery of compound **(*R*)-27**, a novel TRPV1 antagonist with good oral activity in preclinical animal models of pain.

1. Introduction

The TRPV1 (transient receptor potential vanilloid 1) receptor is a well-characterized member of the transient receptor potential (TRP) superfamily of ion channels.[1–3] The role of TRPV1 in the transmission of pain signaling has been extensively discussed.[4,5] The TRPV1 receptor is termed a polymodal receptor, since it can be activated not only by endogenous lipid agonists, such as anandamide and arachidonic acid metabolites, but also by heat and acidic extracellular media, as well as the exogenous vanilloid capsaicin.[6] In addition, TRPV1 activation can be potentiated by pro-nociceptive mediators such as bradykinin, ATP, NGF, and others.[7] Taken together, these observations demonstrate the significant role this receptor plays as a principle integrator of multiple pain-producing stimuli and, thus, the role of TRPV1 in pain signaling pathways.

For this reason, the discovery of small molecule antagonists of TRPV1 has been the subject of intense investigations among many pain research groups. It is well documented that TRPV1 antagonists can effectively reduce inflammatory hyperalgesia in animal models,[8] and more recently, it was demonstrated that the analgesic profile of TRPV1 antagonists can be significantly broadened if the receptor blockade occurs both in the peripheral as well as in the central nervous system.[9] Therefore, development of selective TRPV1 receptor antagonists with good CNS penetration presents an important opportunity to treat a variety of pathological pain states.

Within our TRPV1 antagonist discovery program we have extensively investigated a series of 1-benzyl-3-(isoquinolin-5-yl)ureas, exemplified by the early lead **1** (Fig. 1), as well as 1-benzyl-3-(1*H*-indazol-4-yl)ureas such as **2**.[10–13] The structure–activity relationship studies surrounding this class of compounds have been very fruitful and have provided a number of selective TRPV1 antagonists for further study, including our first clinical candidates



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Ar	hTRPV1 IC_{50} (nM)
1: isoquinolin-5-yl	4 ± 3
2: 1*H*-indazol-4-yl	9 ± 1

Figure 1. General structures of urea TRPV1 antagonists.

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ABT-102 and ABT-116.[14] As these promising candidates progressed into clinical development, we sought to identify second-generation TRPV1 antagonists from a different structural class compared to ABT-102, with improved CNS penetration while maintaining the favorable analgesic and pharmacokinetic properties. In implementing this strategy we sought to replace the urea moiety with a suitable group that allowed for a favorable interaction with the TRPV1 receptor. The 2-aminobenzoxazole fragment has been reported to be a bioisostere for the urea in a series of acyl CoA: cholesterol acyltransferase (ACAT) inhibitors.[15] While there are several different ways to cyclize the urea in our molecules, herein we describe the SAR studies on the chemotype resulting from the cyclization of the oxygen atom with the benzylic carbon of the urea nitrogen substituent to form the 2-arylaminooxazoles **3**.

2. Chemistry

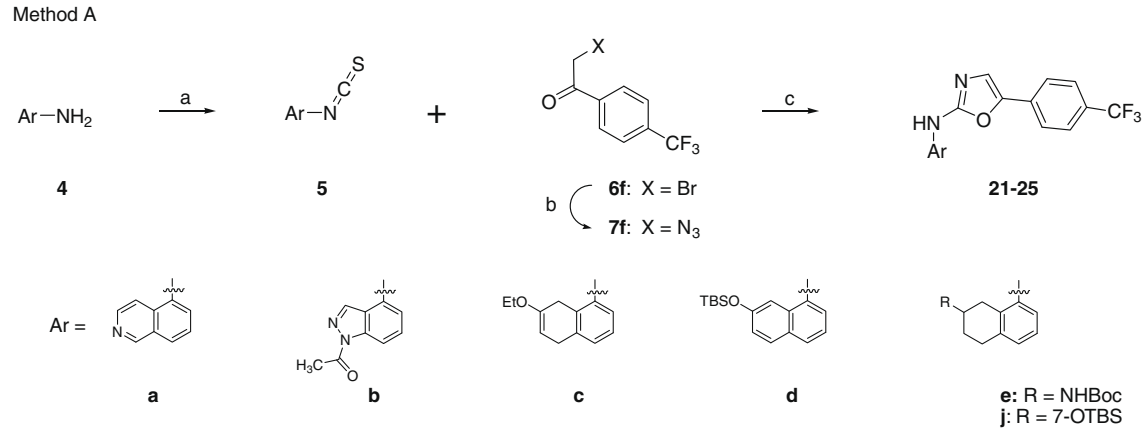
The synthesis of 4- and 5-monosubstituted and 4,5-disubstituted 2-arylaminooxazoles **3** was accomplished using two general synthetic routes. In the first route (method A) shown in Scheme 1, the oxazole ring was constructed in the final step through reaction of aryl isothiocyanates **5** with α-azidoketones **7** in the presence of triphenylphosphine in 1,4-dioxane at 85 °C according to Dhar et al.[16] The requisite isothiocyanates **5** were prepared by standard methods through treatment of anilines **4** with either thiocarbonyldiimidazole or di-2-pyridyl thionocarbonate in methylene chloride, while α-azidoketones **7** were obtained by reacting the corresponding α-bromoketones **6** with sodium azide in acetone following the procedure of Patonay et al.[17] In this manner, **23** and **25** were synthesized from commercially available anilines **4a** and **4f** (after conversion to the silyl ether **4d**), respectively, while **24** was synthesized from **4b**[18] (Table 1).

The synthesis of the tetrahydronaphthalene analogues **9–11** is shown in Scheme 2. Ethyl enol ether **4c**, prepared from the Birch reduction of 7-ethoxynaphthalen-1-amine,[19] was converted to isocyanate **5c** and reacted with azidoketones **7a–j** in the presence of triphenylphosphine to give the oxazoles **8** as described for method A. The enol ethers **8a–j** were hydrolyzed with hydrochloric acid to yield the β-tetralones **9a–j,** which were reduced with sodium borohydride to afford the desired 7-hydroxytetralin analogues **10a–j** (Table 2). Ketone **9f** was also reacted with methylmagnesium bromide and titanium tetrachloride to afford the tertiary alcohol **11**.

The tetrahydronaphthalene amine fragments **4j** and **4e** necessary for the synthesis of analogues **21** and **22**, respectively, were synthesized as shown in Scheme 3. It was found the 7-hydroxy (**4f**) and 6-hydroxy (**4g**) 1-aminonaphthalenes could be selectively hydrogenated in the presence of Raney nickel to yield the tetrahydronaphthalenes **4h** and **4i**, respectively, and the hydroxyl groups could be selectively protected as the *t*-butyldimethylsilyl ethers **4j** and **4k**. This result circumvented the Birch reduction and allowed for a more streamlined approach to the preparation of tetrahydronaphthalene analogues using method A by reducing the overall number of steps from eight to five. Compound **4j** was converted to **5j** and reacted with **7f** according to Scheme 1 to afford the silyl-protected oxazole, which was N-methylated and then treated with tetrabutylammonium fluoride to yield **21**. For the synthesis of **22**, reaction of the aniline function of **4h** with benzyl chloroformate gave **12**, which was converted to the tetrahydronaphthalene **4e** through a multi-step process of activation of the hydroxyl group with methanesulfonyl chloride and displacement of the resulting mesylate with sodium azide to give **13**. The azide was reduced under Staudinger conditions and the resulting alkyl amine differentially protected with a *t*-butoxycarbonyl group to yield **14**. The aniline of **14** was then deprotected to afford **4e** for elaboration to the desired oxazole **22** according to Scheme 1.

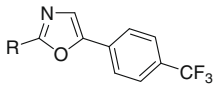
Scheme 4 illustrates an alternate route (method B) for the synthesis of 4- and 5-monosubstituted and 4,5-disubstituted 2-arylaminooxazoles **3**. This method was much better suited to the synthesis of analogues wherein an electron-withdrawing group was present on the aromatic ring in the 5-position of the oxazole. Such analogues (e.g., $R_5 = p\text{-}CF_3Ph$) could only be prepared in very low yield using method A, which inhibited the ability to scale up these compounds for in vivo evaluation. In this route, the oxazole ring was constructed in the first step through reaction of aldehyde **15** with a variety of *p*-toluenesulfonylmethyl isocyanide (TosMIC) derivatives in methanol at reflux to give oxazoles **16a**, **16c**, and **16d** in very high yield.[20] Chlorination of **16** by treatment with lithium bis(trimethylsilyl)amide in tetrahydrofuran at low temperature, followed by reaction with hexachloroethane afforded chlorides **17a**, **17c**, and **17d** in nearly quantitative yield using the method of Atkins and Vedejs.[21] The desired analogues were then furnished in very good yield by heating **17** with **4**, most effectively in an alcohol solvent with catalytic *p*-toluenesulfonic acid. In this manner, compounds **10f**, **19**, **20**, **27**, and **28** were synthesized.

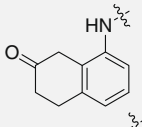
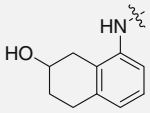
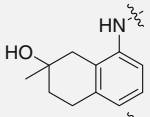
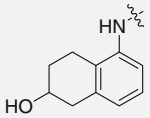
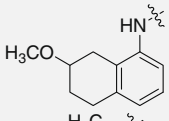
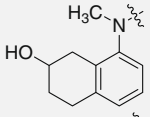
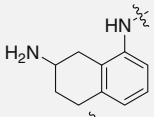
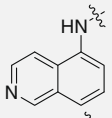
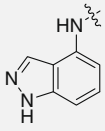
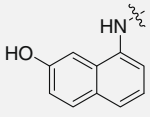
Tetrahydronaphthalenes **4j–k** or **4h–i** shown in Scheme 3 could be utilized directly for the synthesis of **19** and **27–32** according to Scheme 4. It was found during the course of this investigation that protection of the hydroxyl group was not necessary, thus further optimizing this synthetic route. For the synthesis of **20**, **4h** was converted to **4l** via protection of the aniline group of **4h** with benzyl chloroformate to afford **12**, which was O-methylated with methyl iodide and silver oxide and then N-deprotected to yield **4l** (Scheme 3).



Scheme 1. Reagents and conditions: (a) thiocarbonyldiimidazole or di-2-pyridyl thionocarbonate, CH_2Cl_2, rt; (b) NaN_3, acetone, rt; (c) PPh_3, 1,4-dioxane, 85 °C.

Table 1
In vitro biological activity of 2-amino-5-(*p*-trifluoromethyl)phenyloxazole analogues in the human TRPV1 Ca^{2+} influx assay[a]



Compound	R	IC_{50} (nM)
9f		374 ± 73
10f		8.3 ± 1.6
11		840 ± 182
19		120 ± 25
20		150 ± 28
21		277 ± 48
22		327 ± 32
23		229 ± 27
24		101 ± 5
25		10 ± 1

[a] All values were derived from a 1321 stable cell line expressing hTRPV1, and are the mean ± SEM of at least three separate experiments run in triplicate.

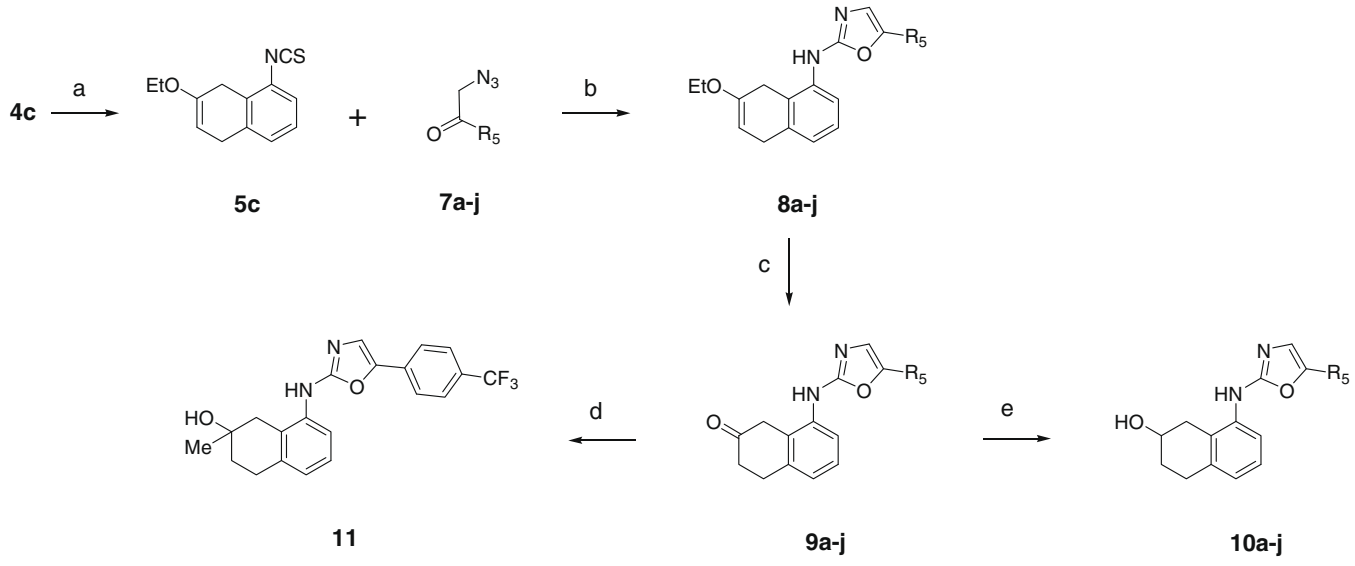
For analogues wherein R_4 was a substituent other than hydrogen, methyl, or ethyl, an alternate synthesis of **17** was employed as shown in Scheme 4. TosMIC was reacted with **15** in methanol at reflux to yield **16a** (R_4 = H), which was subsequently brominated by treatment with lithium bis(trimethylsilyl)amide in a mixed solvent of THF/DMPU at low temperature, followed by reaction with a solution of bromine (1 equiv) in tetrahydrofuran to afford **16b** in good overall yield.[22] Suzuki coupling reactions on **16b** then gave the desired 4-alkyl and 4-aryl oxazoles **16e–g**.[23] Chlorination as discussed above for Scheme 4 furnished the chlorooxazoles **17e–g** in very high yield, which were coupled with **4j** to afford **29**, **31**, and **32**, respectively.

For 4-monosubstituted analogues such as **26** (R_4 = Ph and R_5 = H), a different route was required for the synthesis of oxazole **16h** (Scheme 5).[24] Bromoketone **6a** was first treated with sodium formate to yield 2-oxo-2-phenylethyl formate, which was then reacted with ammonium acetate in acetic acid at reflux to give **16h** in moderate yield. Compound **16h** was converted to **17h,** then reacted with **4j** to afford **26** as described for method B.

Hydroxytetrahydronaphthalene **27** was successfully resolved into the constituent enantiomers by preparative chiral chromatography. While this method provided for the separation of the enantiomers, information about the absolute configuration of the early and late eluting compounds could not be obtained. To aid in assigning the absolute configuration to each enantiomer, bromo analogue **30** was synthesized as shown in Scheme 6. The requisite 2,4-dibromooxazole **18** was prepared from **16a** by treatment with lithium bis(trimethylsilyl)amide in tetrahydrofuran at low temperature, followed by reaction with 2 equiv of a solution of bromine in tetrahydrofuran. **18** was subsequently reacted with **4j** to afford **30** as described for method B. Interestingly, the 4-bromo-2-chlorooxazole **17b** (Scheme 4) was not reactive enough to yield any of the desired product, necessitating the use of **18**. Compound **30** was successfully resolved by chiral chromatography, and the crystal structure of the early eluting enantiomer was solved which provided the absolute configuration (Fig. 2). This enantiomer, **(*R*)-30**, was converted to the corresponding enantiomer of **27** via Negishi coupling reaction using dimethylzinc and bis(diphenylphosphino)ferrocene palladium(II) chloride.[25] Through chiral HPLC experiments using this material and the racemate, the configurations of the early and late eluting enantiomers of **27** were determined.

3. Results and discussion

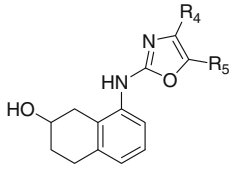
Patterned after our early urea leads, the isoquinoline and indazole analogues **23** and **24** (Table 1) were the prototype molecules in this series. While these two heterocycles yielded many potent analogues in the urea series, this was not the case in the oxazole series. **23** and **24** were on the order of 50-fold and 10-fold less potent, respectively, than the corresponding urea analogues at inhibiting capsaicin activation of the recombinant human TRPV1 receptor in a 1321 stable cell line in the calcium influx functional assay. The breakthrough came when the naphthol moiety, present in our original TRPV1 high-throughput screening hit,[10] was employed (compound **25**). This analogue had very good in vitro potency with an IC_{50} value of 10 nM, which demonstrated the viability of this chemotype. However, there was concern going forward with this fragment due to the fact it would likely impart poor pharmacokinetic properties for this class of molecules through fast metabolism of the phenol group. Thus, we investigated the partially saturated tetrahydronaphthalene ring system, which had been disclosed as part of a series of urea TRPV1 antagonists.[19] Indeed, the 7-hydroxytetrahydronaphthalene analogue **10f** retained the in vitro potency of the naphthol analogue with an IC_{50} of 8.3 nM, while at the same time hinting at improved aqueous solubility properties of these molecules over the completely aromatic system. The importance of the 7-hydroxy substituent was illustrated through various modifications. For example, ketone **9f** was 45-fold less potent than **10f**, while shifting the hydroxyl group to the 6-position led to a 14-fold loss in potency (compound **19**). Conversion to the tertiary alcohol (compound **11**) or methyl ether (compound **20**) also resulted in a reduction in TRPV1 antagonist potency with IC_{50} values of 840 nM and 150 nM, respectively. Finally, methylation of the nitrogen atom linking the oxazole (compound **21**) or replacement of the hydroxyl group with an amino group as in **22** resulted in a 33-fold and 39-fold loss of TRPV1 activity, respectively.



6-10	R_5	6-10	R_5
a	Ph	**f**	4-CF_3-Ph
b	2-CH_3-Ph	**g**	4-Cl-Ph
c	3-CH_3-Ph	**h**	4-OCH_3-Ph
d	4-CH_3-Ph	**i**	4-pyrrolidinyl-Ph
e	4-tBu-Ph	**j**	benzyl

Scheme 2. Reagents and conditions: (a) di-2-pyridyl thionocarbonate, CH_2Cl_2, rt; (b) PPh_3, 1,4-dioxane, 85 °C; (c) 2 M aq HCl, THF, 40 °C; (d) $TiCl_4$, MeMgCl, THF–CH_2Cl_2, −40 °C to 0 °C; (e) $NaBH_4$, EtOH, 0 °C.

Table 2
In vitro biological activity of 8-(oxazol-2-ylamino)-1,2,3,4-tetrahydronaphthalen-2-ol analogues in the human TRPV1 Ca^{2+} influx assay[a]



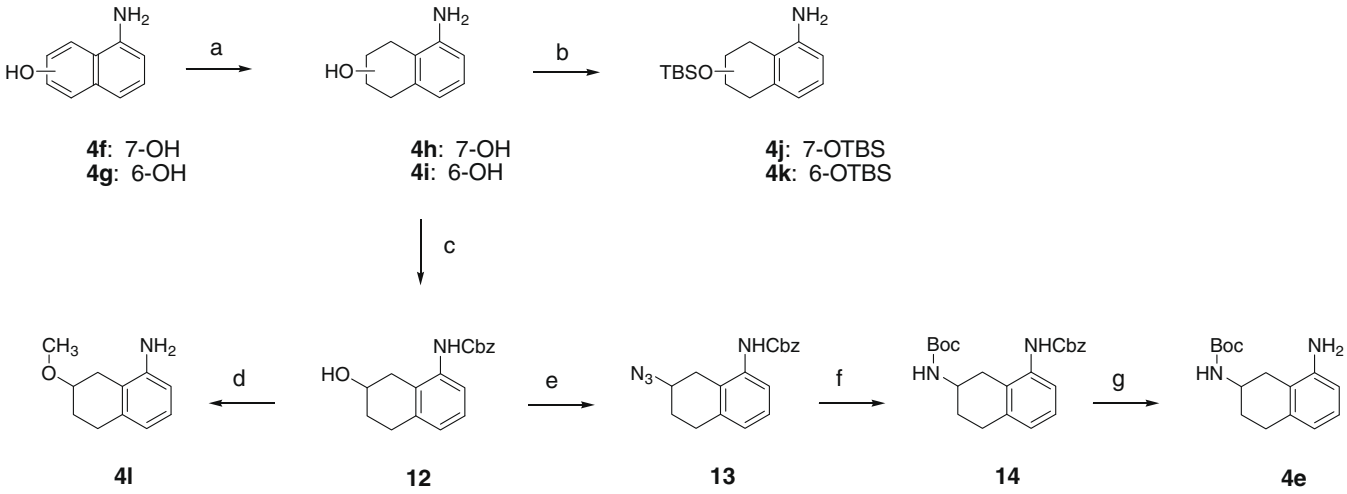
Compound	R_4	R_5	IC_{50} (nM)
10a	H	Ph	187 ± 27
10b	H	2-CH_3-Ph	416 ± 78
10c	H	3-CH_3-Ph	157 ± 20
10d	H	4-CH_3-Ph	62 ± 11
10e	H	4-*t*-Bu-Ph	26 ± 4
10f	H	4-CF_3-Ph	8.3 ± 1.6
10g	H	4-Cl-Ph	15 ± 2
10h	H	4-OCH_3-Ph	81 ± 16
10i	H	4-Pyrrolidinyl-Ph	101 ± 23
10j	H	Benzyl	1820 ± 171
26	Ph	H	864 ± 212

[a] All values were derived from a 1321 stable cell line expressing hTRPV1, and are the mean ± SEM of at least three separate experiments run in triplicate.

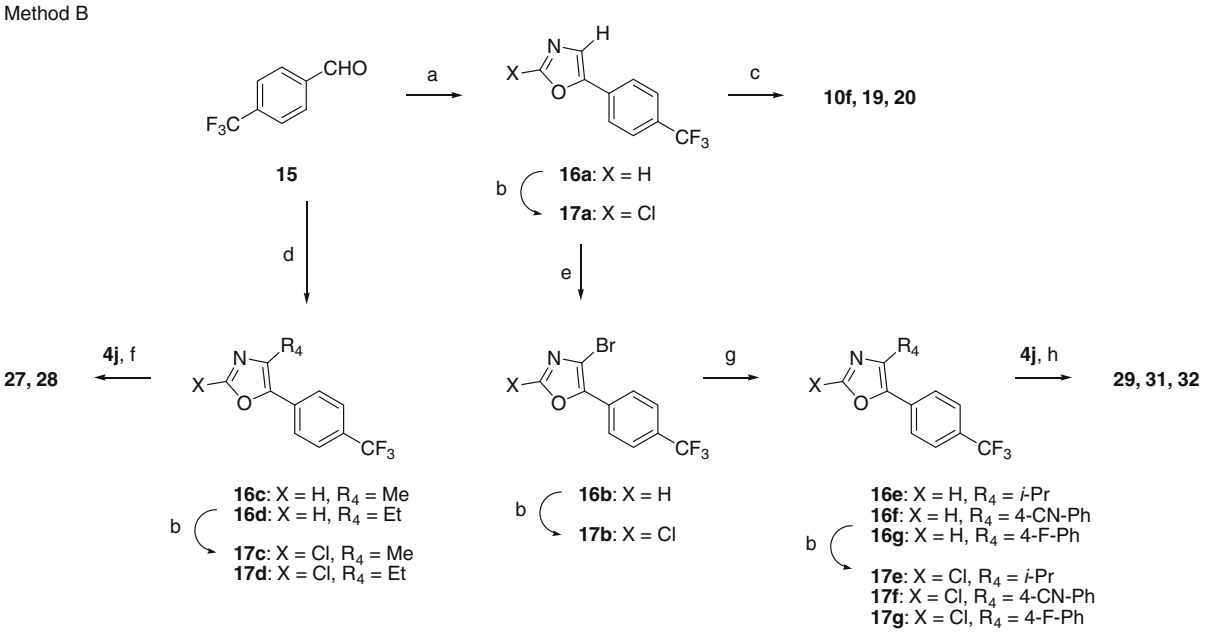
Satisfied that the 7-hydroxytetrahydronaphthalene moiety was the optimal group for the left-side of the molecule, we turned our investigation to the 4- and 5-positions of the oxazole ring and the results are summarized in Table 2. Monosubstitution between these two positions seemed to favor the 5-position (e.g., cf. compounds **10a** and **26**). In addition, much of the SAR observed for the urea series was conserved in the oxazole series. For example, *para* substitution on the aromatic ring in the 5-position was preferred (**10d**), and electronegative substituents yielded the most potent analogues (e.g., **10f**). One significant difference was that larger *para* substituents such as pyrrolidinyl (compound **10i**) led to less potent analogues, while in the urea series this type of amino substituent often yielded single-digit nanomolar compounds.[12] Finally, alkyl and aralkyl substitution at the 5-position also led to a drastic reduction in TRPV1 antagonist potency. For example, the benzyl analogue **10j** had an IC_{50} value of 1820 nM.

Despite the excellent in vitro potency, compound **10f** was precluded from in vivo evaluation due to a short half-life, high clearance, and low (5%) oral bioavailability (Table 4). However, from our investigation of the pharmacokinetic properties of a related benzimidazole series (data not shown), we reasoned that a 4,5-disubstituted oxazole would possess improved pharmacokinetics over the monosubstituted derivatives. Gratifyingly, this proved to be the case and the results are shown in Table 4. Not only did the 4-methyl analogue **27** have a better pharmacokinetic profile than **10f**, the excellent in vitro potency was retained with an IC_{50} value of 3.2 nM. Moreover, the compound had very good CNS penetration, a characteristic we considered to be important for maximal broad spectrum analgesic activity for TRPV1 antagonists, with a brain tissue to plasma ratio of 3.[9] Structure–activity relationship studies around the 4-position revealed small, straight-chain alkyl groups were well tolerated, however, branching such as the isopropyl analogue **29** resulted in reduced potency (Table 3). Surprisingly, phenyl substituents at the 4-position were also well tolerated, often resulting in very potent single-digit nanomolar analogues (compounds **31** and **32**). The poor physicochemical properties and low CNS penetration of **31** and **32** made these compounds less interesting from a drug development perspective.

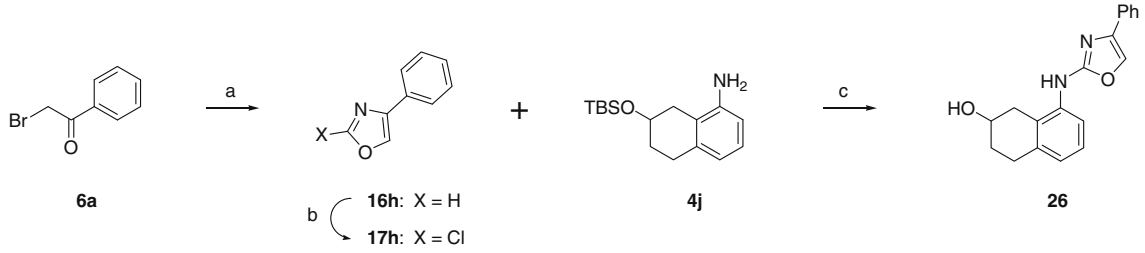
Selected compounds were screened for analgesic activity in preclinical animal pain models and the most interesting analogues, such as **27** and **28** with potent TRPV1 antagonist activity and high brain levels, were resolved and the individual enantiomers were evaluated. For example, in the case of **27**, the *R*-enantiomer



Scheme 3. Reagents and conditions: (a) Raney Ni, aq NaOH, EtOH, H_2, 1300 psi, 85 °C; (b) TBSCl, imidazole, CH_2Cl_2, rt; (c) (i) benzyl chloroformate, Hunig's base, CH_2Cl_2, rt; (ii) 1 M aq NaOH, rt; (d) (i) Ag_2O, MeI, CH_3CN, rt; (ii) 10% Pd/C, MeOH, H_2, 1 atm, rt; (e) (i) MsCl, Hunig's base, CH_2Cl_2, 0 °C; (ii) NaN_3, DMF, 75 °C; (f) (i) PPh_3, THF–H_2O, rt; (ii) Boc_2O, Hunig's base, CH_2Cl_2, rt; (g) 20% $Pd(OH)_2$, MeOH, H_2, 60 psi, rt.

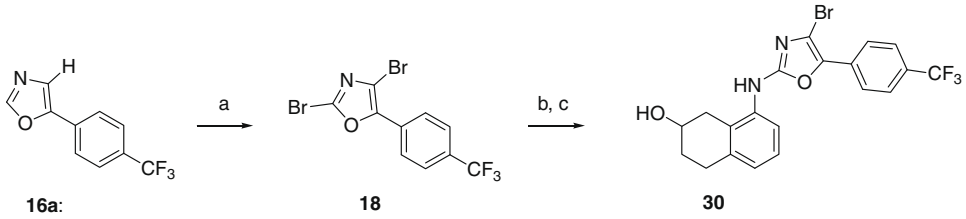


Scheme 4. Reagents and conditions: (a) TosMIC, K_2CO_3, MeOH, reflux; (b) *n*-BuLi or LiHMDS, hexachloroethane, THF, −78 °C; (c) **4j**, CH_3CN, microwave, 150 °C or **4k**, *i*-PrOH, 80 °C or **4l**, *i*-PrOH, microwave, 150 °C; (d) TosCH(R_4)NC, K_2CO_3, MeOH, reflux; (e) LiHMDS, Br_2 (1 equiv), THF–DMPU, −78 °C; (f) *n*-BuOH, reflux or, CH_3CN, microwave, 150 °C; (g) (i) 2-propenylboronic acid pinacol ester, K_2CO_3, $Pd[P(Ph)_3]_4$, DME–H_2O, reflux; (ii) 5% Pd/C, MeOH, H_2, 30 psi, rt; or 4-cyanophenylboronic acid or 4-fluorophenylboronic acid, aq Na_2CO_3, $[P(Ph)_3]_2PdCl_2$, toluene–EtOH, reflux; (h) CH_3CN, microwave, 150 °C or *n*-BuOH, reflux.



Scheme 5. Reagents and conditions: (a) (i) HCO_2Na, DMF, rt; (ii) NH_4OAc, HOAc, reflux; (b) LiHMDS, hexachloroethane, THF, −78 °C; (c) CH_3CN, microwave, 150 °C.

(*R*)-27 was slightly less potent in vitro than the *S*-enantiomer **(*S*)-27** (15 nM vs 5 nM). However, **(*R*)-27** had superior half-life, clearance, and oral bioavailability values compared to **(*S*)-27** (Table 4). **(*R*)-27** was orally active in animal models of pain, exhibiting a statistically significant (p <0.01) 52% increase in the paw withdrawal latency in the rat carrageenan hotbox model of thermal



Scheme 6. Reagents and conditions: (a) *n*-BuLi, Br_2 (2 equiv), THF, −78 °C; (b) **4j**, *i*-PrOH, reflux; (c) $Et_3N{\cdot}3HF$, THF, rt.

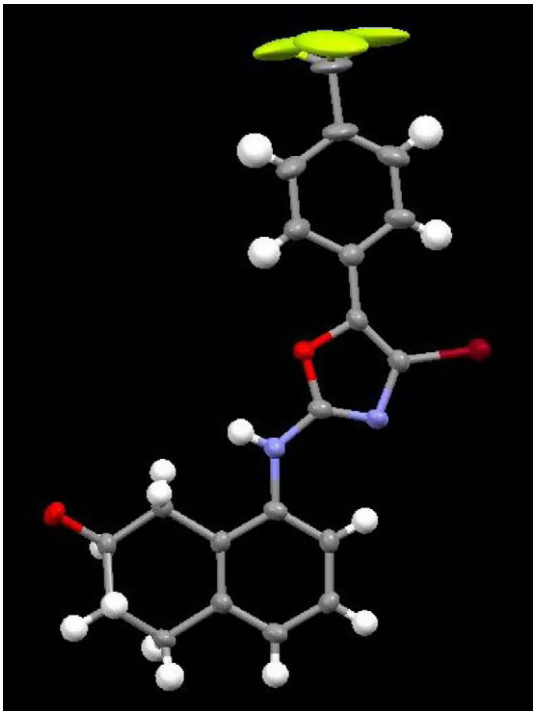
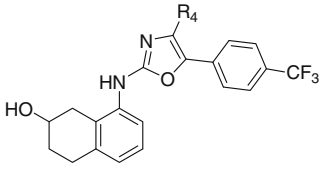


Figure 2. X-ray crystal structure of (***R***)-**30**.

Table 3
In vitro human TRPV1 Ca^{2+} influx assay biological activity [a] and brain to plasma ratios of 8-(oxazol-2-ylamino)-1,2,3,4-tetrahydronaphthalen-2-ol analogues



Compound	R_4	IC_{50} (nM)	Brain/plasma
10f	H	8.3 ± 1.6	0
27	Me	3.2 ± 0.5	3
(***R***)-**27**	Me	15 ± 3	3
(***S***)-**27**	Me	4.8 ± 1.0	3
28	Et	2.1 ± 0.4	3.7
29	*i*-Pr	114 ± 38	nd[c]
30[b]	Br	9	nd
31	4-CN-Ph	2.0 ± 0.4	0.13
32	4-F-Ph	1.3 ± 0.2	0.21

[a] All values were derived from a 1321 stable cell line expressing hTRPV1, and are the mean ± SEM of at least three separate experiments run in triplicate.
[b] Value is from one determination run in triplicate.
[c] nd = not determined.

hyperalgesia at 10 μmol/kg po, and an ED_{50} of 14 μmol/kg (95% CI, 11–20 μmol/kg po) in the rat osteoarthritis model of chronic pain[26]

Table 4
Pharmacokinetic profile[a] of compounds **10f**, **27**, (***R***)-**27**, and (***S***)-**27**

Parameter	**10f**	**27**	(***R***)-**27**	(***S***)-**27**
hTRPV1 IC_{50} (nM)	8.3 ± 1.6	3.2 ± 0.5	15 ± 3	4.8 ± 1.0
V_β iv (L/kg)	5.4	10	6.8	12
CL_p (L/h/kg)	5.1	2.4	1.2	4.4
$t_{1/2}$ iv (h)	0.7	2.8	3.8	1.9
C_{max} po (μg/mL)	0.023	0.096	0.13	0.02
F po: rat (%)	5	33	52	7

[a] Pharmacokinetic analysis determined in rat (three animals per group each iv and oral) following administration of 10 μmol/kg.

Table 5
TRP channel antagonist selectivity and in vivo analgesic activity of (***R***)-**27**

hTRPV1 (IC_{50})	0.015 ± 0.003 μM
rTRPV2 (IC_{50})	37 μM
hTRPV3 (IC_{50})	>100 μM
hTRPV4 (IC_{50})	>100 μM
hTRPA1 (IC_{50})	>100 μM
hTRPA1 (agonist) (EC_{50})	>100 μM
hTRPM8 (IC_{50})	44 μM
Carrageenan (thermal hyperalgesia)	52% increase in paw withdrawal latency @ 10 μmol/kg versus vehicle[b]
Osteoarthritis[a] (inflammatory pain)	ED_{50} = 14 μmol/kg[c]

[a] Values were determined from three-part dose response experiments, with 6–12 animals per dose group.
[b] p <0.01.
[c] 95% confidence interval: 11–20 μmol/kg.

(Table 5). In terms of off-target effects, (***R***)-**27** was found to have a benign cardiovascular safety profile, with no hemodynamic changes observed up to 57× the estimated clinical C_{max} in the anesthetized dog. (***R***)-**27** had a clean hERG profile and was not an inhibitor of five major human CYP enzymes. In addition, (***R***)-**27** was negative in Ames and micronucleus assays. In assays to assess the in vitro selectivity, (***R***)-**27** was found to be highly selective for the TRPV1 receptor versus other TRP channels (Table 5), and exhibited only very weak binding (<40% @ 10 μM) in a screen of a diverse array of receptors, ion channels, reuptake sites, and enzymes (CEREP, Poitiers, France).

4. Conclusion

In conclusion, a novel series of oxazole TRPV1 antagonists were synthesized and evaluated. These compounds satisfied our goal of identifying potent non-urea TRPV1 antagonists with good CNS penetration. Many of the analogues had excellent in vitro potency at the human TRPV1 receptor. The key to the excellent potency was the hydroxyl group at the 7-position of the tetrahydronaphthalene ring on the amino group of the oxazole ring. *para*-Substitution on the phenyl group at the 5-position of the oxazole was preferred, and electronegative substituents led to the most potent analogues. While these analogues had good vitro activity, their oral bioavailability was very poor. The 4,5-disubstituted analogues were

pursued in an effort to improve the pharmacokinetic properties. It was discovered a variety of 4-alkyl and 4-aryl substituents were tolerated in the presence of the 5-phenyl substituent, and resulted in compounds with excellent in vitro potency and good oral bioavailability. One analogue, **27**, was resolved and the enantiomer with the better pharmacokinetic profile, (***R***)-**27**, was fully characterized. (***R***)-**27** showed many attributes desired in a drug development candidate such as excellent in vitro potency, good activity in preclinical animal pain models, cardiovascular safety, selectivity for the target, as well as good ADME properties.

5. Experimental section

^{1}H NMR spectra were obtained on Varian Mercury, Varian Mercury plus, Varian UNITY plus (300 MHz), and Varian Inova (500 MHz) spectrometers using tetramethylsilane as internal standard. The mass spectra (electron spray ionization (ESI) and dissolvable chemical ionization (DCI)) were recorded on Finnigin-4000 instruments. Elemental combustion analyses were obtained from Robertson Microlit Laboratories or Quantitative Technologies, Inc. and were within ±0.4% of theoretical values. Purification via column chromatography was carried out on EM Science Silica Gel 60 (230–400 mesh) in glass flash columns or using an Analogix Intelliflash 280 flash chromatograph outfitted with Analogix Super Flash columns. Reactions were routinely conducted under inert atmosphere (N_2) using commercial high purity solvents as received. Isoquinolin-5-amine (**4a**), 8-aminonaphthalen-2-ol (**4f**), and 5-aminonaphthalen-2-ol (**4g**) were obtained from commercial sources. The syntheses of 1-(4-amino-1*H*-indazol-1-yl)ethanone (**4b**),[18] 7-ethoxynaphthalen-1-amine (**4c**),[19] and 4-phenyloxazole (**16h**)[23] have been described. Bromoketones 6a–**d** and **6f–i** were purchased from commercial sources, as were 1-(isocyanomethylsulfonyl)-4-methylbenzene, 1-(1-isocyanoethylsulfonyl)-4-methylbenzene, and 1-(1-isocyanopropylsulfonyl)-4-methylbenzene.

The following is a description of the synthesis of **10f** using method A, and is typical for the syntheses of **9f**, **10a–j**, **11**, and **21–25**.

5.1. 2-Ethoxy-8-isothiocyanato-1,4-dihydronaphthalene (5c)

A solution of **4c**[19] (200 mg, 1.06 mmol) in CH_2Cl_2 (2.5 mL) was added to a solution of di-(2-pyridyl)thionocarbonate (246 mg, 1.06 mmol) in CH_2Cl_2 (5 mL) at room temperature. After stirring at room temperature for 18 h, the mixture was concentrated, then filtered through silica gel, eluting with 5% EtOAc/hexane. Evaporation of the filtrate in vacuo afforded 225 mg (92%) of the title compound as a pale pink solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.30–7.17 (m, 3H), 4.89–4.83 (m, 1H), 3.81 (q, J = 7.0 Hz, 2H), 3.52–3.45 (m, 2H), 3.39–3.33 (m, 2H), 1.27 (t, J = 7.0 Hz, 3H); MS (DCI$^+$) m/z 232 (M+H).

5.2. 2-Azido-1-[4-(trifluoromethyl)phenyl]ethanone (7f)

To a solution of 2-bromo-1-[4-(trifluoromethyl)phenyl]ethanone (**6f**) (1.22 g, 4.57 mmol) in acetone (75 mL) was added sodium azide (0.59 g, 9.1 mmol) all in one portion. The reaction mixture was stirred at ambient temperature for 18 h and then concentrated in vacuo. The residue was partitioned between CH_2Cl_2 and H_2O. The separated aqueous phase was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The result was 0.82 g (78%) of the title compound as an orange oil, which solidified on standing overnight. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.13 (d, J = 8.1 Hz, 2H), 7.94 (d, J = 8.3 Hz, 2H), 4.96 (s, 2H).

5.3. *N*-(7-Ethoxy-5,8-dihydronaphthalen-1-yl)-5-[4-(trifluoromethyl)phenyl]oxazol-2-amine (8f)

A solution of **5c** (398 mg, 1.72 mmol), **7f** (473 mg, 2.07 mmol), and triphenyl phosphine (542 mg, 2.07 mmol) in 1,4-dioxane (9 mL) was heated at 85 °C for 30 min. The solution was cooled to room temperature and evaporated in vacuo. The residue was chromatographed on silica gel, eluting with 25% EtOAc/hexane to afford 150 mg (22%) of the title compound as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.38 (s, 1H), 7.81–7.70 (m, 4H), 7.65 (d, J = 8.5 Hz, 1H), 7.62 (s, 1H), 7.18 (t, J = 8.2 Hz, 1H), 6.97 (d, J = 8.4 Hz, 1H), 4.88–4.81 (m, 1H), 3.79 (q, J = 6.7 Hz, 2H), 3.52–3.43 (m, 2H), 3.70–3.30 (m, 2H), 1.26 (t, J = 6.8 Hz, 3H); MS (ESI$^+$) m/z 401 (M+H).

5.4. 8-{5-[4-(Trifluoromethyl)phenyl]oxazol-2-ylamino}-3,4-dihydronaphthalen-2(1*H*)-one (9f)

A solution of **8f** (150 mg, 0.375 mmol) in THF (2.3 mL) was treated with 2 M aq HCl (0.76 mL, 1.5 mmol), and the mixture was heated at 40 °C for 1 h. After cooling to room temperature, the solution was brought to pH 8 with saturated $NaHCO_3$ solution, and extracted with EtOAc. The organic extracts were washed with H_2O, dried over Na_2SO_4, filtered and evaporated in vacuo to yield 140 mg (100%) of the title compound as a brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.56 (s, 1H), 7.80–7.70 (m, 4H), 7.67 (d, J = 7.8 Hz, 1H), 7.62 (s, 1H), 7.22 (t, J = 7.8 Hz, 1H), 7.06 (d, J = 7.4 Hz, 1H), 3.58 (s, 2H), 3.06 (t, J = 6.8 Hz, 2H), 2.51–2.45 (m, 2H). MS (ESI$^+$) m/z 373 (M+H). Anal. Calcd for $C_{20}H_{15}F_3N_2O_2$: C, 64.51; H, 4.06; N, 7.52. Found: C, 64.53; H, 3.73; N, 7.52.

5.5. 8-{5-[4-(Trifluoromethyl)phenyl]oxazol-2-ylamino}-1,2,3,4-tetrahydronaphthalen-2-ol (10f)

A solution of **9f** (60.0 mg, 0.161 mmol) in ethanol (6 mL) at 0 °C was treated with $NaBH_4$ (7.0 mg, 0.18 mmol). The reaction was stirred at 0 °C for 1 h, then poured into H_2O and extracted with EtOAc. The extracts were dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with 70% to 85% EtOAc/hexane to afford 34 mg (56%) of the title compound as a tan solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.33 (s, 1H), 7.80–7.69 (m, 4H), 7.62 (s, 1H), 7.55 (d, J = 7.4 Hz, 1H), 7.14–7.06 (m, 1H), 6.88 (d, J = 7.2 Hz, 1H), 4.83 (d, J = 4.1 Hz, 1H), 3.99–3.85 (m, 1H), 2.98–2.69 (m, 4H), 1.94–1.80 (m, 1H), 1.69–1.53 (m, 1H). MS (ESI$^+$) m/z 375 (M+H). Anal. Calcd for $C_{20}H_{17}F_3N_2O_2$: C, 64.17; H, 4.58; N, 7.48. Found: C, 64.44; H, 4.26; N, 7.33.

The following is a description of the synthesis of **27** using method B, and is typical for the syntheses of **19**, **20**, and **26–32**.

5.6. 8-Amino-1,2,3,4-tetrahydronaphthalen-2-ol (4h)

A hydrogenation reaction vessel was charged with 100 mL ethanol, 8-aminonaphthalen-2-ol (**4f**) (5.00 g, 31.4 mmol), 0.2 g of 50% w/w NaOH solution, and 2 g of Raney Ni (wet 40 wt % load). The vessel was vacuum purged with hydrogen several times before heating to 85 °C and maintaining a hydrogen pressure of 1300 psi. The mixture was filtered after 6 h, and the filtrate was concentrated to yield 4.97 g (97%) of the title compound as a brown solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.78 (t, J = 7.6 Hz, 1H), 6.44 (d, J = 7.8 Hz, 1H), 6.30 (d, J = 7.5 Hz, 1H), 4.75 (d, J = 4.1 Hz, 1H), 4.63 (s, 2H), 3.99–3.85 (m, 1H), 2.85–2.56 (m, 3H), 2.20 (dd, J = 16.5, 7.6 Hz, 1H), 1.94–1.79 (m, 1H), 1.68–1.44 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.35, 31.41, 33.36, 65.81, 111.35, 116.48, 119.13, 125.53, 136.00, 146.12.

5.7. 7-(*tert*-Butyldimethylsilyloxy)-5,6,7,8-tetrahydronaphthalen-1-amine (4j)

A mixture of **4h** (2.33 g, 14.3 mmol), *tert*-butylchlorodimethylsilane (2.60 g, 17.2 mmol), and imidazole (2.90 g, 42.3 mmol) was stirred in CH_2Cl_2 (40 mL) at room temperature overnight. The mixture was washed several times with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to afford 2.6 g (65%) of the title compound as a dark purple oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.77 (dd, J = 7.8, 7.4 Hz, 1H), 6.42 (d, J = 7.8 Hz, 1H), 6.28 (d, J = 7.4 Hz, 1H), 4.70 (br s, 2H), 4.18–4.08 (m, 1H), 2.78 (dt, J = 16.5, 5.4 Hz, 1H), 2.73–2.61 (m, 2H), 2.26 (dd, J = 16.6, 7.3 Hz, 1H), 1.90–1.81 (m, 1H), 1.71–1.60 (m, 1H), 0.88 (s, 9H), 0.09 (s, 6H); MS (ESI$^+$) m/z 278 (M+H).

5.8. 4-Methyl-5-[4-(trifluoromethyl)phenyl]-1,3-oxazole (16c)

A mixture of 4-(trifluoromethyl)benzaldehyde (3.45 mL, 25.8 mmol), 1-(1-isocyanoethylsulfonyl)-4-methylbenzene (5.40 g, 25.8 mmol), and K_2CO_3 (4.28 g, 31.0 mmol) in methanol (125 mL) was heated to reflux. After 2.5 h the volatiles were evaporated, and the residue was partitioned between Et_2O and H_2O. The aqueous phase was extracted with Et_2O, and the combined organic extract was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by chromatography on silica gel (Analogix Intelliflash 280; 10–30% ethyl acetate/hexanes eluant; SF65–400 g column), which yielded 4.01 g (68%) of the title compound as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.44 (s, 1H), 7.90–7.81 (m, 4H), 2.42 (s, 3H). MS (DCI$^+$) m/z 228 (M+H).

5.9. 2-Chloro-4-methyl-5-[4-(trifluoromethyl)phenyl]-1,3-oxazole (17c)

A solution of lithium bis(trimethylsilyl)amide (1.0 M in THF, 19 mL, 19 mmol) was added dropwise to a solution of **16c** (4.0 g, 18 mmol) in THF (80 mL) at −78 °C. After stirring 30 min, solid hexachloroethane (8.34 g, 35.2 mmol) was added in one portion, and the reaction was allowed to gradually warm to ambient temperature. After 16 h the reaction was quenched with half-saturated NH_4Cl solution. The product was extracted with Et_2O, and the combined organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (Analogix Intelliflash 280; 0–25% EtOAc/hexanes; SF65-600 g column; loading with hexane/CH_2Cl_2) to yield 4.69 g (100%) of the title compound as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.89–7.84 (m, 2H), 7.84–7.79 (m, 2H), 2.40 (s, 3H). MS (DCI$^+$) m/z 262 (M+H).

5.10. 8-{5-[3-Methyl-4-(trifluoromethyl)phenyl]oxazol-2-ylamino}-1,2,3,4-tetrahydronaphthalen-2-ol (27)

A solution of **4j** (1.08 g, 3.89 mmol) and **17c** (1.02 g, 3.89 mmol) in 15 mL *n*-butanol was heated to reflux for 1.5 h. The reaction mixture was cooled to ambient temperature and partitioned between ethyl acetate and saturated aqueous $NaHCO_3$ solution. The separated organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated at the rotary evaporator to give a dark red oil. Hexane was added to the residue, and upon mixing a solid formed which was collected by suction filtration, washed with hexanes and dried under vacuum. The result was 0.89 g (59%) of the title compound as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.20 (s, 1H), 7.81–7.74 (m, 2H), 7.70–7.62 (m, 2H), 7.58–7.51 (m, 1H), 7.15–7.06 (m, 1H), 6.90–6.82 (m, 1H), 4.81 (d, J = 4.0 Hz, 1H), 3.98–3.85 (m, 1H), 3.00–2.81 (m, 2H), 2.80–2.66 (m, 1H), 2.56–2.42 (m, 1H), 2.33 (s, 3H), 1.93–1.80 (m, 1H), 1.69–1.52 (m, 1H). MS (ESI$^+$) m/z 389 (M+H). Anal. Calcd for $C_{21}H_{19}F_3N_2O_2$: C, 64.94; H, 4.93; N, 7.21. Found: C, 64.54; H, 4.55; N, 6.96.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC766241. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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Supplementary data

Supplementary data (experimental and characterization data for all compounds, and the protocols for IC_{50} determination and the carrageenan experiment) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.099.

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