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Title: Design, synthesis, and evaluation of isoquinoline ureas as TRPV1 antagonists

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Abstract: ***Background*:** Inhibition of transient receptor potential vanilloid receptor 1 (TRPV1) has emerged as a novel approach in the treatment of various pain states. Pyrrolidinyl urea, SB 705498 with pK_b = 7.3 in guinea pig TRPV1 receptor has been investigated in Phase II clinical trials for pain and chronic cough. Another heteroaryl urea derivative, A-425619 **1**, has been reported to be a potent and selective TRPV1 antagonist of capsaicin-evoked receptor activation with an IC_{50} value of 4 nM in hTRPV1.

Objective: A series of thirteen A-425619 1 analogues with modifications centered around the C-region was synthesized to understand the binding site characteristics of TRPV1 receptors.

Method: We synthesized a series of isoquinoline ureas and evaluated their antagonist potency using smooth muscle assay using guniea pig trachea along with evaluation of the molecular properties and molecular modelling using CoMFA studies.

Results: *p*-Chloro **4**, *p*-bromo **5**, *m*-isothiocyanate **15**, and *p*-isothiocyanate **16** derivatives were found to be the most potent members of the series with pK_b values in the range of 7.3-7.4 in the functional assay using guinea pig trachea. The lead compound A-425619 1 exhibited a pK_b value of 8.1 in this assay.

Conclusion: The *para*-substituted analogues were found to be more potent than the *ortho*- and *meta*- analogues in biological assay. This observation was further supported by molecular modeling studies using CoMFA.

Keywords: TRPV1, Pain, Isoquinoline ureas, A-425619, SB 705498, Smooth Muscle Assay

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1. INTRODUCTION

The TRPV subfamily comprises of six mammalian members, TRPV1-6. Transient Receptor Potential Vanilloid 1 (TRPV1), a member of the TRP superfamily of cation channels, was originally named vanilloid receptor 1 (VR1) and is commonly referred to as the capsaicin receptor [1,2]. It is a multiple signal integrator capable of transducing signals evoked by several noxious stimuli including capsaicin, resiniferatoxin, heat (≥43 °C), acidic pH, and the products of lipid bilayer metabolism such as anandamide, eicosanoid metabolites, and leukotriene LTB4 [3]. TRPV1 is widely expressed in trigeminal ganglion (TG), dorsal root ganglion (DRG) and nodose ganglion (NG) neurons, keratinocytes, and urinary bladder [4]. TRPV1 was the first mammalian TRPV receptor isolated by expression cloning from a rat dorsal-root ganglion library using the hot pepper compound capsaicin, which gives spicy foods their characteristic hot taste. The TRPV1 receptor was first cloned and characterized by Caterina and colleagues in 1997 [5]. TRPV1 is a 95-kDa, 838-amino-acid protein consisting of six transmembrane (TM) domains with a short pore forming region between the fifth and sixth TM domains. The vanilloid binding domain is located between the second and third TM domains.

TRPV1 serves a dual function, firstly as a rapid and direct detector of potentially damaging thermal and chemical stimuli, which are perceived as painful, and secondly as a major contributor to the establishment of persistent peripheral inflammation and central sensitization [6]. Diseases with a prominent neurogenic inflammatory component include migraine, rhinitis, asthma, and inflammatory bowel disease, and as such are candidates that can be affected by TRPV1 therapy. TRPV1 receptors have also been targeted for the development of the treatments for advanced osteoarthritis, tendonitis and post-surgical pain [7]. The clinical potential of TRPV1 beyond the chronic pain includes cough, overactive bladder, obesity, and metabolic disorders including diabetes [8].

Vanilloids are the best known kinds of TRPV1 agonists and capsaicin is a representative of this family. Major obstacles in the development of the TRPV1 agonists as systemic analgesics are related to their excitatory effects and include initial irritation, hypothermia, bronchoconstriction, and hypertension. The TRPV1 antagonists produce rapid onset of action by blocking the TRPV1 mediated signaling, avoiding the initial painful stimuli, and desensitization and can be used in combination with existing therapies targeted at other mechanisms. This advantage over the conventional TRPV1 agonists has led to the search for novel TRPV1 antagonists as potential therapeutics for pain.

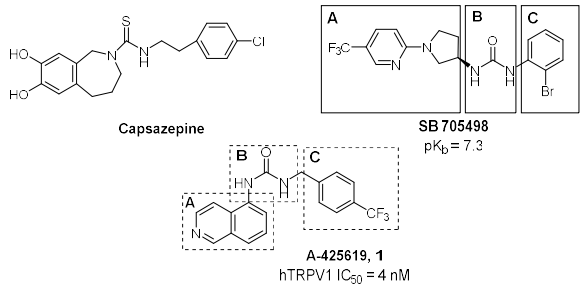


Figure 1. Representative TRPV1 antagonists.

Capsazapine, a synthetic analogue of capsaicin, was identified as the first competitive small molecule antagonist of TRPV1 [9]. SAR of small molecule TRPV1 antagonists can be generalized based on capsazepine. The antagonist molecules can be divided into three important regions, A, B and C (Figure **1** [10, 11]. The region A typically consists of heterocyclic rings, the linker region B consists of groups with potential for hydrogen bond interactions such as urea, thiourea, amide or reverse amide and plays a role in proper positioning/spacing of regions A and C. Region C consisting of aryl rings with various lipophilic substituents is crucial for optimal TRPV1 potency [10].

Various classes of TRPV1 antagonists have been discovered including capsazepine based thioureas, pyridylpiperazinyl ureas, cinnamides, carboxamides, pyrimidines, urea derivatives, and many others [12-15]. Among the urea analogues, SB 705498 (pK_b = 7.3, antagonist activity in FLIPR assay versus capsaicin in guinea pig TRPV1 receptor) is a rapid, potent and reversible inhibitor of capsaicin-mediated activation of human TRPV1 and has been investigated in a number of Phase I and Phase II clinical trials for conditions related to migraine, rhinitis, chronic cough and dermatitis [11,16-19]. A-425619 **1**, an isoquinazolinyl urea containing regions A, B and C that are important for the TRPV1 activity was optimized from a HTS hit. It is a competitive antagonist of capsaicin with an inhibitory potency of 4 nM in hTRPV1 and was found to be active in animal models of visceral and chronic inflammatory pain [20].

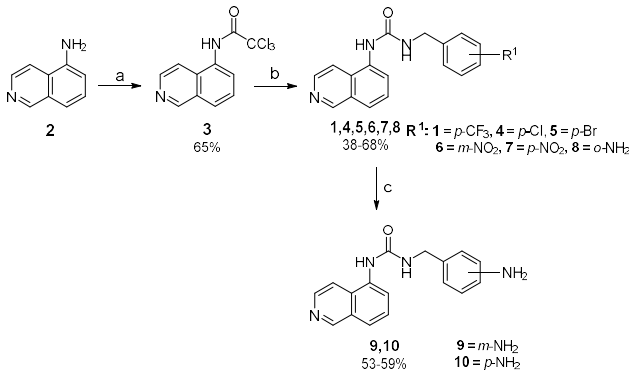
The 5-isoquinoline urea **1** was chosen as the lead compound to design and synthesize a series of TRPV1 antagonists. Modifications involved substitutions at *ortho*, *meta*, and *para*- positions of the phenyl ring (region C) of A-425619 with functional groups that will impart various steric, electronic, and solubility characteristics. The functional groups were chosen to help further delineate the SAR of the isoquinoline urea lead **1**. The *p*-chloro analogue **4** and *p*-bromo analogue **5** were designed to serve as the bioisosteric analogues of the lead molecule **1** which contains a trifluoromethyl group at the *para* position. Nitro analogues **6**-**7**, amino analogues **8**-**10**, and acetamide analogues **11**-**13** were designed to evaluate the effect of electron donating and withdrawing groups with varying polar and steric characteristics. In addition, isothiocyanate analogues **14**-**16** were designed to serve as potential electrophilic affinity labels due to their reactivity towards various nucleophiles that may be present at the receptor binding site.

2. RESULTS AND DISCUSSION

2.1 Chemistry

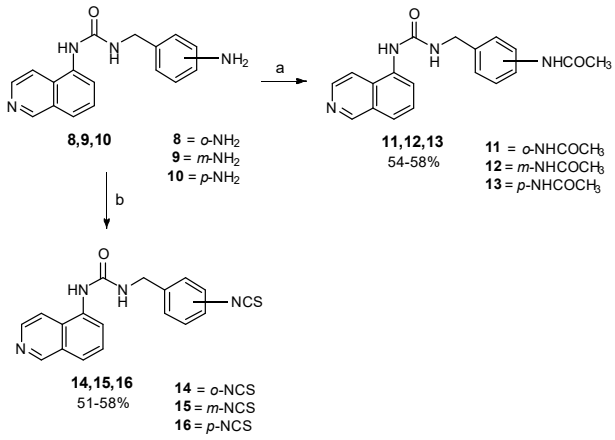
Synthesis of compounds **1**, **4**, **5**, **6**, **7**, and **8** involved the common intermediate 2,2,2-trichloro-*N*-isoquinolin-5-yl-acetamide **3** which was obtained by acylating 5-aminoisoquinoline **2** with trichloroacetyl chloride according to the literature procedure [20]. The reaction was observed to have gone to completion in 4 h as compared to 14 h reported in the literature (Scheme **1**) [20]. Various substituted benzyl amines were reacted with the trichloroacetamide intermediate **3** to yield the desired 5-isoquinoline urea derivatives (Scheme **1**). *Para*-trifluoromethyl derivative **1**, *para*- chloro and bromo derivatives **4** and **5,** *meta*- and *para*- nitro derivatives **6** and

7, and *ortho*- amino derivative **8** were synthesized by reacting the trichloroacetamide **3** with corresponding substituted benzylamines in yields ranging from 38% - 68%. Reduction of nitro analogues **6** and **7** using stannous chloride in non-aqueous solvent which resulted in 53% and 59% yield, respectively, of amino analogues **9** and **10** was adopted as a method of choice (Scheme **1**) over hydrogenation and zinc/hydrazinium monoformate [21].



Scheme 1. (a) Cl_3CCOCl, Et_3N, CH_2Cl_2, 0 °C to rt. (b) substituted benzylamines, Et_3N, DBU, MeCN, 4 h, reflux. (c) $SnCl_2.2H_2O$, EtOAc, reflux.

The amino compounds **8-10** were acetylated using acetic anhydride in dichloromethane at room temperature to obtain *o*-, *m*-, and *p*- acetamide analogues **11-13** in yields ranging from 54-58% (Scheme **3**) [22]. *o*-, *m*-, and *p*- Isothiocyanate analogues **14-16** were synthesized by reacting the amino analogues **8-10** with di-2-pyridyl thionocarbonate in chloroform at room temperature which resulted in the formation of the corresponding isothiocyanates **14-16** in greater than 50% yields (Scheme 2) [23].

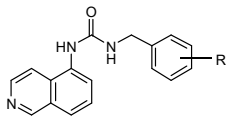


Scheme 2. (a) Acetic anhydride, $NaHCO_3$, DCM, rt. (b) Di-2-pyridyl thionocarbonate, DCM, rt.

2.2 Biological activity

The target compounds were tested for their antagonist potency using the smooth muscle preparation and the results are presented in Table **1**. Lead compound **1** was also tested in this assay to determine its pK_b value which has not been previously reported. The smooth muscle assays were conducted using the guinea pig trachea according to the previously reported experimental protocols [24]. Guinea pig trachea was activated by the TRPV1 selective agonist capsaicin, and the antagonist potencies of the test compounds are expressed as pK_b values.

Table 1. TRPV1 antagonist potencies of the target compounds



No.	-R	[a],[b]pK_b	miLog *P*[c]	Molecular Volume (Å)[c]
1	4-CF_3	8.1	4.10	270.37
4	4-Cl	7.3	3.88	252.61
5	4-Br	7.4	4.02	256.96
6	3-NO_2	6.3	3.17	262.41
7	4- NO_2	6.8	3.17	262.41
8	2- NH_2	*	2.28	250.36
9	3- NH_2	*	2.28	250.36
10	4- NH_2	*	2.28	250.36
11	2- $NHCOCH_3$	*	2.43	287.02
12	3- $NHCOCH_3$	*	2.43	287.02
13	4- $NHCOCH_3$	*	2.43	287.02
14	2-NCS	*	4.57	273.85
15	3-NCS	7.4	4.57	273.85
16	4-NCS	7.3	4.57	273.85

*pK_b values <6.0. [a]pK_b are determined against capsaicin in isolated guinea pig trachea, where n = 4 for compounds with pK_b value >6.0 and n = 2 for compounds with pK_b values <6.0. [b]pK_b is negative log of K_b where K_b = [antagonist]/(dose ratio - 1). [c]Calculated octanol/water partition coefficient logP and molecular volumes were obtained by using Molinspiration software (http://www.molinspiration.com).

The lead compound **1** was found to exhibit the most potent TRPV1 antagonist activity with a pK_b value of 8.1 in this assay (Table **1**). The halogenated derivatives **4** and **5** with lipophilic chloro and bromo groups at the *para* position also exhibited good antagonist potency with pK_b values of 7.3 and 7.4, respectively. The *m*-nitro analogue **6** and *p*-nitro analogue **7** exhibited lower TRPV1 antagonist potencies with pK_b values of 6.3 and 6.8, respectively. Both *m*-isothiocyanate **15** and *p*-isothiocyanate **16** analogues exhibited better antagonist potencies than the corresponding nitro analogues with pK_b values of 7.4 and 7.3, respectively. Wash experiments led to full dose response curves with a leftward shift, suggesting that these isothiocyanate derivatives were reversible inhibitors of TRPV1 [24]. These results suggest the absence of a nucleophilic group in the TRPV1 receptor in the vicinity of where the isothiocyanate groups of **15** and **16** bind. The remaining molecules in the series exhibited TRPV1 antagonist activity with pK_b values <6.0. Relatively high hydrophilicity in case of amino analogues **8-10**, and polar and possibly steric factors in case of the acetamides **11-13** may explain the reduced activity of these molecules. Analogues substituted at *ortho*- position

were in general weaker than the *para*- substituted analogues which might be an indication that the *ortho*- substitution is not favorable for TRPV1 antagonist activity. Although a direct correlation between the miLogP and the pK_b values was not found, the most potent compounds had miLogP values in the range of 3.88-4.57, whereas miLogP values of the inactive compounds were in the range of 2.28-2.43

2.3 Molecular Modelling

Contour map generated from the CoMFA studies shows the region where bulky substituent would increase the trpv1 inhibitory activity (green polyhedra), the regions where the steric bulk would not be well tolerated (yellow polyhedra), and the region where the H-bond donor is favorable for the activity (blue polyhedra) (Figure 2). COMFA contours maps reflect that the biological data is supported by the COMFA studies where *para*- substitution is well tolerated on the aromatic ring in region C [25].

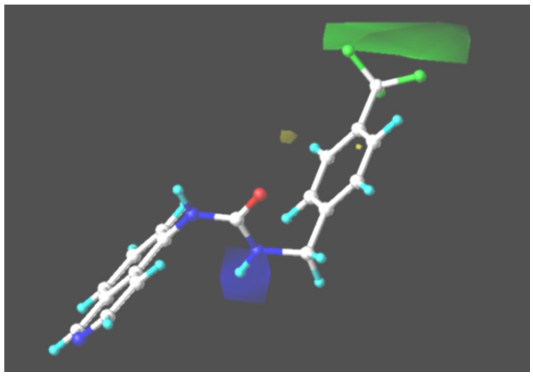


Figure 2. Contour map generated by CoMFA. Green polyhedra indicate that steric bulk is favored, blue polyhedra indicate H-bond donors and positive charge is favored and H-bond acceptor and negative charge is disfavored and yellow polyhedra indicate steric bulk is disfavored [25].

CONCLUSION

A series of analogues **4-16** derived from the modification of the phenyl moiety of the isoquinoline urea lead compound A-425619 **1** has been successfully synthesized. The lead compound and the target compounds were evaluated for their TRPV1 antagonist activity in the guinea pig trachea assay. The smooth muscle data indicates that the lead compound **1** was the most potent analogue with a pK_b value of 8.1 in this assay. The *p*-substituted analogues generally exhibited potent TRPV1 antagonist activity with pK_b values in the range of 6.8-7.4, with the exception of the *p*-amino and *p*-acetamido analogues **10** and **13**. Polar and possibly steric factors might be attributed to their weak antagonist activities. The *meta*-substituted nitro and isothiocyanate analogues **6** and **15** were also found to show reasonable antagonist potencies with pK_b values of 6.3 and 7.4, respectively. The *ortho*-substituted analogues on the other hand, exhibited weak antagonist potency with pK_b values <6.0. The amino derivatives **8-10** exhibited weak antagonist activity which might be attributed to their high hydrophilicity. Polar and steric factors might be playing a role in the weak antagonist activity of acetamide derivatives **11-13**. Wash experiments in case of the isothiocyanate derivatives **15** and **16** suggested that while these analogues exhibited good potency, they were reversible antagonists of the TRPV1 receptor. CoMFA studies using Sybyl 7.2 were conducted and a model was generated which was helpful in understanding the observed biological data.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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None.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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[25] All computational studies were performed using SYBYL version 7.2 on a Dell Precision 470n workstation with the RHEL 4.0 operating system. The 3D structures of the compounds in the training and test sets were constructed using the Sketch Molecule function in SYBYL. Energy minimizations were performed with the conjugate gradient method using the Tripos force field and Gasteiger-Marsili charges with a convergence criterion of 0.001 kcal/mol Å. Each structure was then subjected to simulated annealing as it enables the rapid identification of the good solutions, ideally the global minimum. The system was heated at 1000 K for 1 ps and then cooled at 200 K for 1 ps. The exponential annealing function was used and 10 such cycles were run.

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