1-(2-Nitrophenyl)thiosemicarbazides: A Novel Class of Potent, Orally Active Non-Peptide Antagonist for the Bradykinin B_2 Receptor

Edward K. Dziadulewicz,*[,†] Timothy J. Ritchie,[†] Allan Hallett,[†] Christopher R. Snell,[†] Soo Y. Ko,[†,#] Roger Wrigglesworth,[†,▽] Glyn A. Hughes,[†] Andrew R. Dunstan,[†,⊥] Graham C. Bloomfield,[†,⊥] Gillian S. Drake,[†] Michael C. Brown,[‡,∥] Wai Lee,[‡] Gillian M. Burgess,[‡,◇] Clare Davis,[‡] Mohammed Yaqoob,[‡] Martin N. Perkins,[§,⊗] Elizabeth A. Campbell,[§,⊥] Andrew J. Davis,[§] and Humphrey P. Rang[§]

Departments of Medicinal Chemistry, Biology, and Pharmacology, Novartis Institute for Medical Sciences, 5 Gower Place, London WC1E 6BN, England

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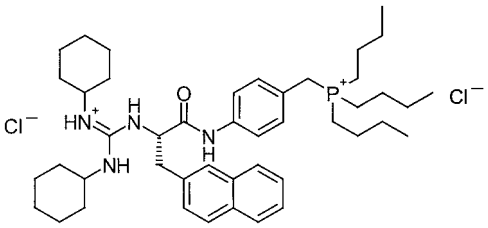
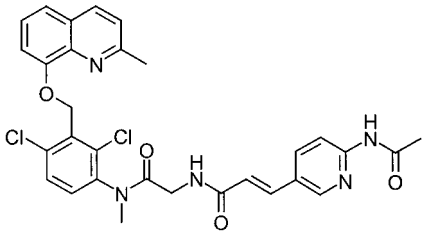
Bradykinin (BK) is an endogenous hormonal nonapeptide (RPPGFSPFR) cleaved from high-molecular-weight protein precursors (kininogens) by the action of kallikrein enzymes under conditions of tissue trauma or injury.[1] The discovery of potent peptidic BK antagonists[2] together with the availability of the cloned receptor subtypes (B_1 and B_2)[3,4] has led to an intense exploration of the pharmacology of BK. The actions of BK are mediated mainly by the B_2 class of receptors, which are constitutively expressed inter alia on nociceptive neurons suggesting a principal role for this mediator in the perception of pain.[5] Indeed, BK has been described as the most potent algogenic substance known.[5,6] Consequently, a B_2 antagonist may be of therapeutic benefit in the symptomatic treatment of chronic pain and various inflammatory disorders in which elevated levels of BK are a feature.

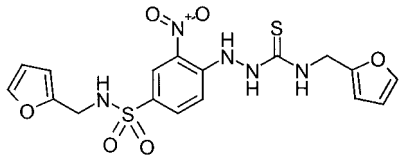
To date, only two classes of structurally different nonpeptide B_2 antagonists have been identified, the phosphonium-derived WIN 64338 (**1**)[7] and the heteroaryl benzyl ether FR 173657 (**2**)[8] being the most thoroughly studied representatives (Figure 1). Given the fundamental difficulty in projecting pharmacophoric information from a peptide agonist onto a non-peptide scaffold, we initiated a screening program which culminated in the discovery of the thiosemicarbazide (TSC) derivative **3** with a K_i of 0.54 μM at the rat B_2 receptor (expressed in NG108-15 neuroblastoma−glioma hybrid cell membranes). Compound **3** was also able to antagonize the functional response to BK with an IC_{50} of 2 μM in a $^{45}Ca^{2+}$ efflux functional assay (NG108-15 cells).



WIN 64338 (**1**)



FR 173657 (**2**)



(**3**)

Figure 1. Structures of BK B_2 receptor antagonists WIN 64338 (**1**) and FR 173657 (**2**) and the thiosemicarbazide lead **3**.

An extensive structure−activity study was implemented, part of which is summarized in Tables 1 and 2. The terminal furan rings of **3** could be replaced by phenyl without loss of binding affinity (**4**, $K_i = 0.31$ μM). By gradual deletion of functionality from each terminus in turn, the minimum pharmacophore required for binding to the rat B_2 receptor was established, micromolar affinity arising from the 1-(2-nitrophenyl)-4-benzylthiosemicarbazide half-structure (**5**). Replacement of the *N*(4)-benzyl moiety with a benzhydryl group afforded the compound **6**, resulting in a 42-fold gain in affinity. Molecular recognition of the benzhydryl motif in the field of G-protein-coupled receptor research has been previously documented[9] and supported the premise that this part of the molecule is interacting with a complementary hydrophobic binding site in the B_2 receptor, as polar functionality is not tolerated in this part of the molecule. With the benzhydryl group in place, a significant advancement in the potency of the series was observed in those analogues incorporating simple functionality in the aryl ring *para* to the TSC backbone (**7** and **8**).

There were very few changes that could be made to the TSC core which were not detrimental to binding affinity (Table 2). Neither of the thiocarbonyl-flanking nitrogen atoms, N(2) and N(4), could be replaced by methylene without a complete loss in binding (**9** and **10**), presumably due to an unacceptable increase in

* To whom correspondence should be addressed. Tel: 0044 171 333 2167. Fax: 0044 171 387 4116. E-mail: ed.dziadulewicz@pharma.novartis.com.
† Department of Medicinal Chemistry.
‡ Department of Biology.
§ Department of Pharmacology.
# Present address: Department of Chemistry, Ewha Woman's University, Seoul 120-750, South Korea.
▽ Present address: Institut de Recherche Jouveinal, Parke-Davis, 3-9 Rue de la Loge, B.P. 100, 94265 Fresnes Cedex, France.
⊥ Present address: Novartis Horsham Research Centre, Wimblehurst Rd., Horsham, West Sussex RH12 5AB, England.
∥ Present address: Packard, Brook House, 14 Station Rd., Pangbourne, Berkshire RG8 7AN, England.
◇ Present address: Pfizer Central Research, Sandwich, Kent CT13 9NJ, England.
⊗ Present address: AstraZeneca Research Center Montreal, 7171 Frederick Banting, St. Laurent, Quebec H1S 1B7, Canada.

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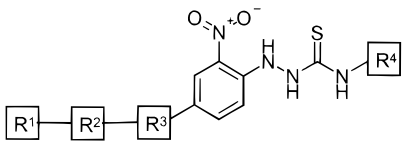
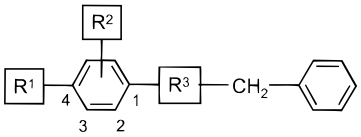
Table 1. BK B_2 Receptor Binding Affinities (K_i) of Compounds **3**−**8** and **16**−**18**[a]



compd[b]	R^1	R^2	R^3	R^4	K_i (nM)[c]
3	−	$NHCH_2$(2-furyl)	SO_2	CH_2(2-furyl)	540 ± 160
4	−	$NHCH_2Ph$	SO_2	CH_2Ph	310 ± 80
5	−	−	H	CH_2Ph	1600 ± 500
6	−	−	H	$CHPh_2$	38 ± 8
7	−	NH_2	SO_2	$CHPh_2$	4 ± 1
8	−	NH_2	CO	$CHPh_2$	1.8 ± 0.8
16[d]	−	$N[(CH_2)_3NMe_2]_2$	SO_2	$CHPh_2$	148 ± 70
17[d]	DME[e]	(*S*)-prolyl	SO_2	$CHPh_2$	0.5 ± 0.2
18[d]	DME[e]	(*R*)-prolyl	SO_2	$CHPh_2$	0.6 ± 0.12

[a] [^{3}H]BK binding assay in NG108-15 cell membranes expressing the rat B_2 receptor (see ref 13). [b] All compounds gave satisfactory ^{1}H NMR, mass spectra, and elemental microanalyses. [c] K_i values were calculated on the basis of a one-site binding model using LIGAND software (see ref 10). The values shown are the mean ± SEM ($n \geq 3$). [d] Compound isolated as amorphous TFA salt after preparative HPLC. [e] Abbreviation: DME = 2-[(2-*d*imethylaminoethyl)*m*ethylamino]ethylamino ($Me_2N(CH_2)_2N(Me)(CH_2)_2NH$−).

Table 2. Effects of TSC Core Changes on BK B_2 Receptor Binding Affinities[a]



compd[b]	R^1	R^2	R^3	K_i (μM)[c]
4	$BnNHSO_2$	2-NO_2	NHNHCSNH	0.31 ± 0.08
5	H	2-NO_2	NHNHCSNH	1.6 ± 0.5
9	$BnNHSO_2$	2-NO_2	$NHCH_2CSNH$	>100
10	$BnNHSO_2$	2-NO_2	$NHNHCSCH_2$	>100
11	H	2-NO_2	$CH_2NHCSNH$	7.1 ± 4.9
12	H	2-NO_2	$CH_2NHCONH$	>100
13	H	2-H	NHNHCSNH	~100
14	H	3-NO_2	NHNHCSNH	>100
15	H	2-CO_2H	NHNHCSNH	>100

[a−c] See corresponding footnotes in Table 1.

backbone flexibility of these compounds although loss of key hydrogen-bonding elements could not be ruled out as a contributing factor. On the other hand, replacement of N(1) by CH_2 to afford the thiourea **11** resulted in a tolerable 4-fold loss in affinity compared to **5**. The corresponding urea **12**, however, was inactive.

The presence of the *o*-nitro group in these early *N*(4)-benzyl analogues was crucial to the level of binding achieved. Deletion of the group to afford the known TSC **13**,[11] transposition to the *meta* position (**14**), or replacement with a number of other functionalities,[12] for example, CO_2H (**15**), afforded analogues which were all devoid of activity. A measure of affinity could be restored in the *N*(4)-benzhydryl series, but activities were generally in the 1−10 μM range, and although some trends could be discerned (2-F > 2-Cl ≈ 2-CO_2Me > 2-Br), the nitro group remained the best *ortho* substituent (**6**, K_i = 38 nM) and was retained for the present time.

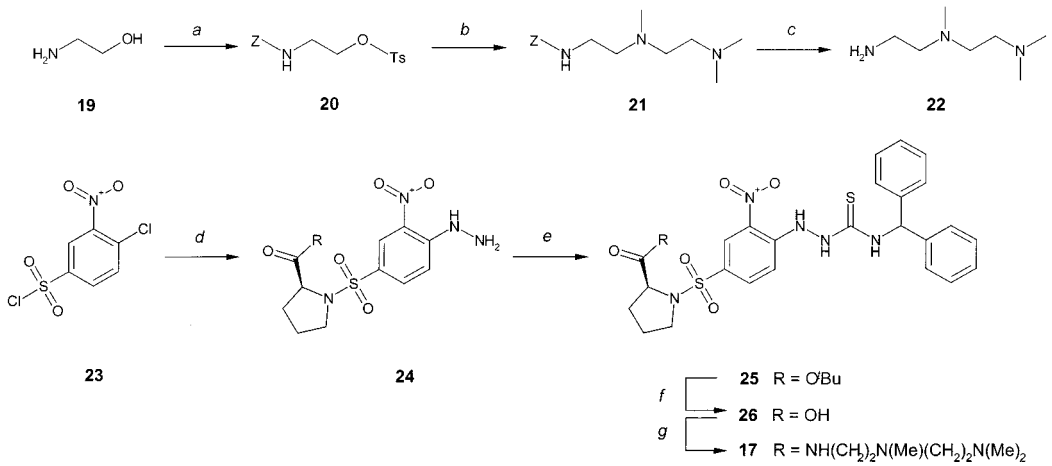
A common physicochemical property of compounds **3**−**15** was their very low solubility in physiological media, typically less than 0.0005 mg/mL at pH 7.4. To improve the in vivo transport characteristics of the TSCs, it was necessary to incorporate functionality which could facilitate dissolution without compromising receptor binding. The sulfonamide and carboxamide groups of **7** and **8,** respectively, were thus conveniently located synthetic handles, potentially allowing further structural elaboration. When the primary sulfonamide group of **7** was exchanged for a bis(3-dimethylaminopropyl)sulfonamido group, permitting salt formation (**16**, Table 1) and increasing solubility (>9.7 mg/mL at pH 7.4), a 37-fold loss in affinity was compensated by the first demonstration of oral activity (ED_{50} = 33 μmol/kg po in the Freund's adjuvant-induced mechanical hyperalgesia model in the rat knee joint). Furthermore, we found that the in vitro potency of such amphiphilic molecules could be restored by altering the proximity of the basic center(s) to the rest of the pharmacophore. This was achieved by incorporation of an α-amino acid backbone into the sulfonamide bond. Within the modest number of amino acid residues explored, the (*S*)-(−)-proline derivative **17** (Bradyzide[13]) emerged as the most potent antagonist overall (K_i = 0.5 ± 0.2 nM). Incorporation of the basic centers into a single chain, as opposed to the bifurcated arrangement of **16**, accorded **17** a better overall in vitro profile albeit with a decreased relative solubility (0.19−0.49 mg/mL at pH 7.4 in all buffers tested). The synthesis of **17** is presented in Scheme 1 and is illustrative of the approach adopted for this class.

During the course of the present study it transpired that the configuration of the proline C-2 stereocenter was not an important determinant of receptor affinity. The (*R*)-(+)-proline antipode **18** was found to be equipotent to **17**, a lack of stereochemical discrimination between the enantiomers suggesting that the proline rings do not make intimate contact with the receptor but nevertheless allow the conformationally flexible amine chains to approach a common complementary site.

The presence of nitroaromatic and thiocarbonyl functionality in this initial congeneric series made it important to assess the mutagenic potential of **17**. Incubation of **17** with rat S9-liver homogenate in V79 Chinese hamster cells did not increase the frequency of cells containing membrane-encapsulated fragments ('micronuclei') at any of the concentrations tested, irrespective of the presence or absence of metabolic activation. The compound was therefore not considered to be clastogenic and/or aneugenic.

Compound **17** exhibited competitive antagonism. It inhibited the increase in $^{45}Ca^{2+}$ efflux from NG108-15 cells caused by activation of the B_2 receptor with a pA_2 of 8.0 ± 0.16 (n = 5) and inhibited B_2 receptor-mediated contractions of rat uterus smooth muscle with a pA_2 value of 8.6 ± 0.13 (n = 4). In each case the compound caused a parallel shift in the log concentration−response curve with no decrease in the maximum response. Compound **17** also displayed in vivo functional antagonist activity against Freund's adjuvant-induced mechanical hyperalgesia in rats with an ED_{50} value of 0.84 μmol/kg by oral administration. When dosed at 5 μmol/kg po, compound **17** completely reversed the mechanical hyperalgesia with a duration of action in excess of 4 h. Preliminary pharmacokinetic studies in rats at this dose and route of administration (n = 4) revealed that **17**

Scheme 1[a]



[a] Reagents: (a) (i) ZOSu, EtOAc (85%), (ii) TsCl, py (80%); (b) *N*,*N*,*N*′-trimethylethylenediamine (93%); (c) HCO_2NH_4, Pd/C, MeOH (95%); (d) (i) H-Pro·O^{t}Bu, Et_3N, THF, (ii) $H_2NNH_2 \cdot H_2O$ (92%); (e) $Ph_2CHN{=}C{=}S$ (91%); (f) HBr, AcOH (95%); (g) ^{i}BuOCOCl, Et_3N, THF, **22** (67%).

achieved only very low plasma levels (<100 nM 30 min after dosing) suggesting rapid blood clearance. Following iv injection of 10 μmol/kg, the compound was seen to disappear rapidly from plasma, falling to a concentration of 4.4 ± 0.48 μM (n = 4) in 5 min. Its metabolic fate in vivo remains to be ascertained.

To determine the selectivity of **17**, this compound was also evaluated in binding assays for the A_1, A_2, AT_1, B_1, CCK_A, CCK_B, H_3, kainate, M_2, NK_1, NMDA, 5-HT_{1A}, and 5-HT_3 receptors. At concentrations up to 10 μM, compound **17** did not significantly affect (<20%) radioligand binding to these receptors. When the binding activity of **17** was evaluated against the human B_2 receptor expressed in Cos-7 cells, a K_i value of 772 ± 144 nM (n = 3) was obtained.[13]

In conclusion, we have developed a novel series of potent non-peptide antagonists of the rat BK B_2 receptor from a high-throughput screening lead **3**, by appending appropriate functionality onto the nitrophenylthiosemicarbazide framework. The subnanomolar-potent compound **17** is a competitive and selective B_2 antagonist and is orally efficacious in a model of inflammatory hyperalgesia. A full exploration of the SAR and optimization of this class of B_2 antagonist for the human receptor will be reported in subsequent papers.

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