Bioorganic & Medicinal Chemistry Letters 20 (2010) 7011–7014

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Indazole derivatives as novel bradykinin B_1 receptor antagonists

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ARTICLE INFO

Article history:
Received 20 August 2010
Revised 22 September 2010
Accepted 23 September 2010
Available online 29 September 2010

Keywords:
Bradykinin
Antagonist
Indazole
P-Glycoprotein

ABSTRACT

A new class of indazole-derived bradykinin B_1 antagonists and their structure–activity relationships (SAR) is reported. A number of compounds were found to have low-nanomolar affinity for the human B_1 receptor and possess acceptable P-gp and pharmacokinetics properties.

The kinin peptides exhibit a wide range of biological activities including pain, inflammation, vasodilatation, and smooth muscle contraction. Their biological response is mediated by the bradykinin B_1 and B_2 receptors.[1] While the B_2 receptor is widely expressed in many cell types, the B_1 receptor was shown to be generally absent from normal tissues. However, it can be rapidly induced following injury.[2] Data from transgenic bradykinin B_1 receptor knockout mice has implied a role for the receptor in pain,[3] and the need for novel pain modulators exempt of tolerance or abuse issues related with opiates has since prompted the development of selective bradykinin B_1 receptor antagonists.

While peptide antagonists for the B_1 receptor were discovered over 2 decades ago,[4] a number of non-peptidic antagonists have been disclosed to date.[5–8] In parallel to investigating the previously reported biphenyl motif,[6c–8] a 1-phenyl-1*H*-indazole (**1**, Fig. 1) was identified. Accordingly, an SAR examination of the structural requirements of both the indazole moiety and the cyclopropylcarboxamide part were performed en route for an optimal bradykinin B_1 antagonist, and are described herein.

The featured compounds were prepared according to Scheme 1. Copper-catalyzed N-arylation of commercially available indazoles **3a–d** and bromide **2**[8] using the *trans*-diaminocyclohexane/CuI catalytic system in the presence of potassium phosphate[9] provided amines **4a–d** after subsequent hydrolysis of the acetamide groups in acidic conditions. EDC–promoted coupling of **4a–d** with cyclopropanecarboxylic acid derivative **5** delivered amines **6a–d** after cleavage of the *tert*-butyl carbamate in acidic conditions. The biological study targets **7a–v** were prepared employing standard amide bond forming procedures.

Acetamide **8**, obtained after N-arylation of 3-bromo-1*H*-indazole **3c** with **2**, was subjected to Suzuki coupling conditions[10] with the appropriate boronic acids to deliver acetamides **9a–d** (Scheme 2), which subsequently underwent acidic hydrolyses and EDC-coupling reactions with **10** to provide targets **7w–z**. Compound **11** was

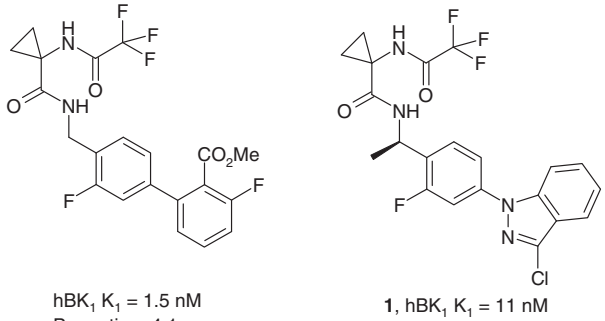

Figure 1. Lead indazole bradykinin B_1 antagonist **1**.

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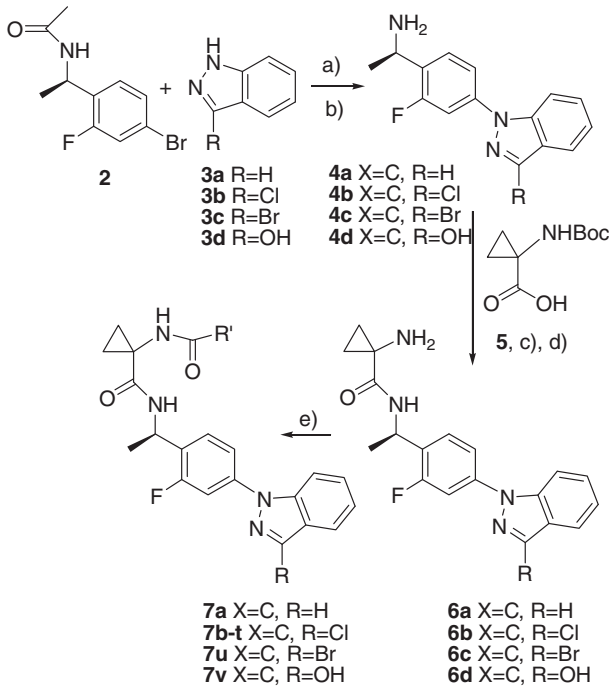
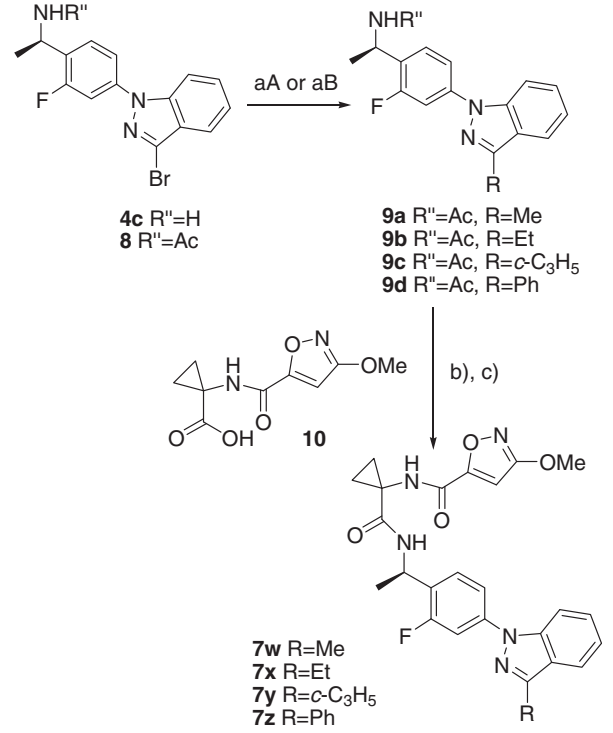
doi:10.1016/j.bmcl.2010.09.121



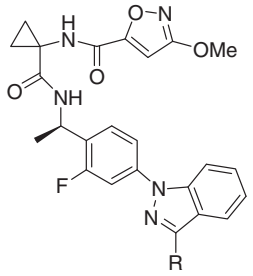
Scheme 1. Reagents and conditions: (a) CuI, *trans*-1,2-diaminocyclohexane, K_3PO_4, dioxane; (b) anhyd HCl, MeOH, 70 °C; (c) **5**, 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt), Et_3N, CH_2Cl_2; (d) anhyd HCl, EtOAc, 0 °C; (e) R′CO_2H, EDC, HOBt, Et_3N, CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) method A: $RB(OH)_2$ $Pd(PPh_3)_2Cl_2$, Cs_2CO_3, 7:3:1 dioxane/EtOH/H_2O, 80 °C; method B: $RB(OH)_2$ $Pd(dba)_2$, $Ph_5FcP(t\text{-}Bu)_2$, K_3PO_4, toluene, 80 °C; (b) anhyd HCl, MeOH, 70 °C, quant; (c) **10**, 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt), Et_3N, CH_2Cl_2.

prepared starting from 3-methoxy-1*H*-indazole.[11] The cyano-derivative **12** was obtained after palladium-catalyzed cyanation of bromide **8**,[12] in a similar fashion.

Table 1
Structural modifications of the indazole ring to improve bradykinin B1 receptor affinities



7a-z, 11, 12

Compd[a]	R	hBK$_1$ K_i (nM)[b]
7a	H	11
7w	Me	1.3
7x	Et	19
7y	*c*-C_3H_5	33
7z	Ph	82
7b	Cl	0.67
7u	Br	0.81
7v	OH	335
11	OMe	21
12	CN	2.4

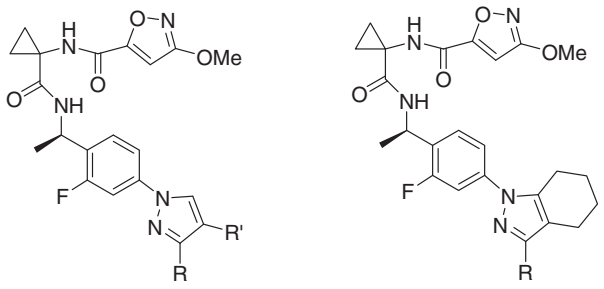
[a] All compounds are >95% pure by LC/MS at 215 nm and characterized by ^{1}H NMR and HRMS.
[b] Values represent the numerical average of at least two experiments. Interassay variability was ±25% for the binding assays.

K_i values (nM) were determined radiometrically using the appropriate radioligand and Chinese hamster ovary (CHO) cells stably expressing the human B_1 and the human B_2 receptors.

The un-substituted indazole (**7a**, Table 1) provided a hBK$_1$ K_i value of 11 nM. Optimization initially involved systematic substitution of each indazole hydrogen (data not shown); however, it was rapidly concluded that the most promising compounds derived from substitution at the 3-position (Table 1). All compounds were B_1 selective (K_i >10 μM vs the B_2 receptor).

Among the alkyl and cycloalkyl derivatives examined (**7w–z**), the 3-methyl indazole **7w** was moderately potent (hBK$_1$ K_i = 1.3). A substantial drop in binding affinity was observed with oxophilic substituents (compounds **7v** and **11**). The cyano-derivative **12** exhibited a 4.5-fold increase in affinity relative to **7a**; however, sub-nanomolar activity was achieved only with a 3-halogen substituent (**7b** and **7u**).

An additional phenyl group decreases the compound's binding ~7 fold (compound **7z** versus **7a**). Removal of the fused aryl ring of the indazole system leading to a pyrazole (**13a**, Fig. 2) induced



13a, R=H, R_1=H, hBK$_1$ K_i = 220 nM
13b, R=Ph, R_1=H, hBK$_1$ K_i = 610 nM
13c, R=H, R_1=Ph, hBK$_1$ K_i = 510 nM
14a, R=H, hBK$_1$ K_i = 27 nM
14b, R=Me, hBK$_1$ K_i = 10 nM

Figure 2. Pyrazole and 4,5,6,7-tetrahydroindazole containing analogs.

a 20-fold decrease in binding affinity to the human B_1 receptor. The 3 or 4-phenyl substituted pyrazoles **13b** and **13c** were found to be similarly less active. Furthermore, the 4,5,6,7-tetrahydroindazole derivatives **14a** and **14b** showed hBK_1 K_i of 27 and 10 nM, respectively, indicating that the fused aromatic heterocyclic indazole system is required to achieve sub-nanomolar binding values.

Having identified the 3-chloro-indazole as a potent modification, our attention turned to examining the role that the differently substituted cyclopropylamino acid amides have within this series. Data for selected compounds is shown in Table 2.

The methoxyisoxazole derivative **10b** proved fairly potent with a K_i of 0.67 nM. In the 1,3-thiazole series (compounds **10c**–**e**) the 2-methoxy derivative **10d** was the most active with a K_i of 0.29 nM. The un-substituted thiazole derivative **10c** showed comparable potency, however, introducing a methyl function in the 4-position lowered the binding affinity to 8.2 nM (**10e**). Incorporation of an additional heteroatom at the 2-position of the thiazole (**10f**) brings the affinity back to a sub-nanomolar value; however, the 4-methyl-1,2,3-thiadiazole ring proved extremely vulnerable in vitro in human liver microsomes (data not shown). The 3-pyrrolo amide **10g** is a potent compound (K_i = 0.38 nM) with increased stability in human liver microsomes.

Pyridyl cyclopropylcarboxamides (for example **10h**) proved most potent at the 3-position. Substituting the 2- or 4-positions led to significant decrease in affinity (data not shown), while the 5- and 6-positions proved tolerant to substitution. For example, the 6-methylnicotinamide (compound **10i**) gave comparable binding to **10h** (hBK_1 K_i = 1.4 nM), while the 6-bromonicotinamide carried a threefold increase in affinity (**10j**). Substitution at the 5-position provided several subnanomolar activity compounds (**10k**–**p**), with 5-bromopyridine **10p** of note (hBK_1 K_i of 0.12 nM).

A key issue with these compounds was susceptibility as substrates for the efflux transporter, P-glycoprotein (P-gp). We have previously observed that pyridine derivatives in the biphenyl series[6g] proved to be good substrates for human P-gp, so select indazole compounds were consequently evaluated in a P-gp transport assay[13] (Table 2).

Compounds with P-gp directional transport ratios less than 2.5 are generally not considered substrates. Optimal passive cell permeability (P_{app}) should usually exceed 15. Analogs **10b–f** showed good permeability and were not substrates for human P-gp. However, the otherwise promising pyrazole derivative **10g** showed a significant P-gp transport ratio of 55.6.

Pyridine derivatives **10k**, **10o**, and **10p** were not substrates for human P-gp, and possessed sufficient (>15) cell permeability.

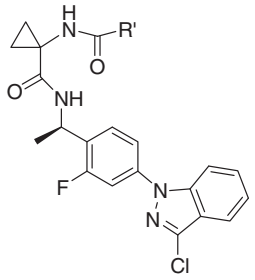
Overall, the P-gp profile of indazole containing compounds can possess adequate P-gp profiles with the appropriate amide modification.

In the diazine series, going from pyridazine **10q** to pyrimidine **10r** and pyrazine **10s** reduced the undesired P-gp efflux but the desired binding affinity decreased as well. Of note, chloropyrazine analog **10t** was reasonably potent with a low P-gp ratio of 1.3.

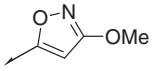
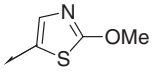
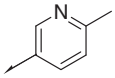
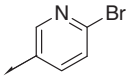
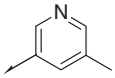
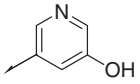
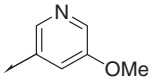
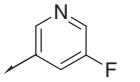
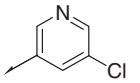
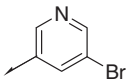
As far as the stability of the 3-halo-indazoles is concerned, while we have not looked for displacement with biological nucleophiles, based upon our experience with these systems, direct displacement would not be expected.

Indazoles with acceptable binding and P-gp profiles were evaluated for their rat and dog pharmacokinetic properties (Table 3).[8] In the thiazole series, the 2-methoxy-1,3-thiazole **10d** displayed high clearance and a low bioavailability in rat. The 5-methoxynicotinamide analog **10m** showed a significant drop in rat half-life and a poor bioavailability. These parameters could be notably improved with the 5-bromonicotinamide derivative **10p**: in rat, which displayed fair oral bioavailability, a half-life of 3 h and a low clearance of 2.5 mL/min/kg. In dog the compound retained low clearance with a half-life as high as 7.6 h. Another promising

Table 2
Effect of the variation of the cyclopropylamino acid amide on the binding affinity and on the P-gp efflux of novel indazole BK_1 antagonists

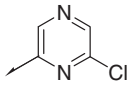


10b-t

Compd[a]	R	hBK_1 K_i (nM)[b]	P-gp[c]	P_{app}
10b		0.67	1.6	26
10c		0.54	2.8	19
10d		0.29	1.8	14
10e		8.2	—	—
10f		0.71	2.3	21
10g		0.38	55.6	19
10h		1.5	—	—
10i		1.4	—	—
10j		0.49	2.2	8.2
10k		0.47	1.9	22
10l		0.30	89.0	18
10m		0.52	5.3	21
10n		0.37	7.5	24
10o		0.20	2.5	15
10p		0.12	2.3	13
10q		0.65	29.4	25
10r		0.18	5.9	24

(*continued on next page*)

Table 2 (*continued*)

Compd[a]	R	hBK$_1$ K_i (nM)[b]	P-gp[c]	P_{app}
10s		16.5	1.6	30
10t		1.2	1.8	24

[a] All compounds are >95% pure by LC/MS at 215 nm and characterized by ^{1}H NMR and HRMS.
[b] Values represent the numerical average of at least two experiments. Interassay variability was ±25% for the binding assays.
[c] Values represent the numerical average of three experiments.

Table 3
Pharmacokinetic properties of selected indazole derivatives

Compd	Rat PK[a]			Dog PK[b]	
	F%	$t_{1/2}$	CL	$t_{1/2}$	CL
10b	49	3.1	5.3	20.8	1.3
10c	—			4.3	1.1
10d	13	8.3	12.7	8.3	1.7
10f	—			2.4	9.6
10j	—			7.1	2.6
10k	—			2.6	4.3
10m	6	0.7	7.8	2.3	5.5
10o	—			6.0	3.1
10p	24	3.1	2.5	7.6	3.8
10t	—	1.6	105	15.1	10.5

[a] *F*% oral bioavailability, half-life is represented in hours, CL in mL/min/kg. Sprague-Dawley rats (n = 3). Oral dose = 10 mg/kg, iv dose = 2 mg/kg in DMSO. Interanimal variability was less than 20%.
[b] Mongrel dogs (n = 2). Oral dose = 3 mg/kg, iv dose = 1 mg/kg in DMSO. Interanimal variability was less than 20% for all values.

pharmacokinetic profile in both rat and dog was observed for the isoxazole derivative **10b**.

In conclusion, SAR evaluation of a novel class of human BK$_1$ receptor antagonists bearing a heteroaromatic ring system was undertaken. The indazole portion of the molecule was identified as a sensitive feature for the optimal binding to the receptor with a chlorine at the 3-position of the indazole optimal for high receptor affinity. SAR effects on P-gp efflux potential was examined for these indazoles on the cyclopropylcarboxamide region identifying 5-bromonicotinamide **10p** as a potent bradykinin B$_1$ antagonist possessing good pharmacokinetics properties. The result of this study yielded a combination of structural features allowing for the preparation of further compounds with novel properties as improved bradykinin B$_1$ antagonists.

Acknowledgments

We would like to acknowledge Sandor L. Varga and Joan S. Murphy for analytical support.

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