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Design and Synthesis of Novel Pyrrolidine-Containing Bradykinin Antagonists

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Abstract—The design and synthesis of novel pyrrolidine-containing bradykinin antagonists, **II**, are described. Conformational analysis suggested that a pyrrolidine moiety could substitute for the *N*-methyl *cis*-amide moiety of FR 173657. The in vitro binding data showed that the (*S*)-isomer of **II** was potent in the bradykinin B_2 receptor-binding assay with a K_i of 33 nM. The opposite isomer, (*R*)-**II**, had a K_i of 46 nM. The in vitro binding data confirmed our conformational hypothesis.

Bradykinin is an endogenous nonapeptide that has been implicated in a variety of physiological and pathophysiological responses such as pain, rhinitis, inflammation, asthma, allergy, and tissue injury.[1–6] When the nonapeptide is introduced to skin in vivo, it causes inflammation, pain, swelling, and heat.[7] Therefore, it was thought that an antagonist to the bradykinin receptor would have potential therapeutic values in such physiological conditions.

Two subtypes (B_1 and B_2) of bradykinin receptors have been identified. Although both human B_1 and B_2 subtypes have been cloned and extensively characterized,[8] the B_2 receptor seems to mediate the most pathophysiological actions of bradykinin. There have been numerous B_2 antagonists reported in literature, but most have been peptides and peptide mimetics. It was only recently that the first orally bioavailable antagonists were reported by Abe and co-workers from Fujisawa, as shown in Figure 1.[9] Since then, numerous other antagonists have been reported.[10]

A computer model of FR 173657 suggested that the compound could bind to the receptor as the *cis*-amide conformer and that the 2,6-dichloro-3-*N*-methylanilide moiety would play a key role in stabilizing the active conformation.[9a] This conformation could be mimicking a natural β-turn that is frequently provided by a proline-amino acid in natural peptides. If such a hypothesis were true, then a replacement of the *N*-methyl *cis*-amide compound with a pyrrolidine-containing compound, such as the structure **II** shown in Figure 1, would provide a similar binding affinity toward the receptor as the one observed with FR 173657. Our targeted compound also possesses an atropic isomerism like FR 173657.

Since **II** exists as two enantiomers, we carried out a conformational analysis to see which one of the isomers would match more closely with FR 173657.

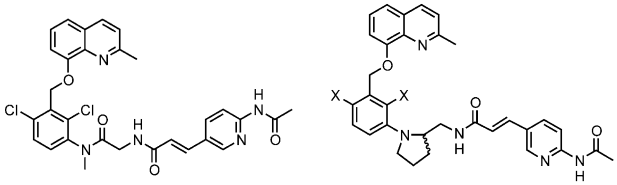

Figure 1. Bradykinin B_2 receptor antagonists.

Conformational Analysis

The structures of FR 173657 and **II** were calculated using OPLS[11] as implemented in the Maestro[12] computer program. Conformers for these two structures were

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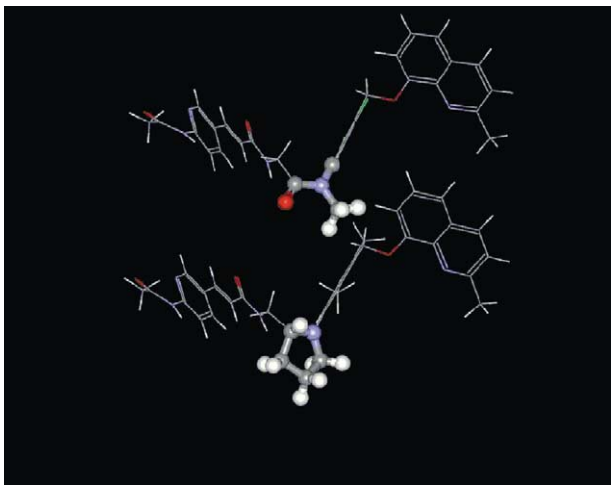


Figure 2. Amide and pyrrolidine shown as ball and stick.

subsequently generated using Catalyst.[13] Comparison of the lowest energy structures does show that the pyrrolidine moiety is a reasonable replacement for the *cis*-conformation of the amide in FR 173657 (Fig. 2).

The shape and spatial relationship between the head and tail of the molecule are similar. One aspect in which the pyrrolidine is not equivalent is the loss of a carbonyl moiety that could be available for hydrogen bonding. The structures of FR 173657 and the (*R*)-isomer of **II** were compared systematically using the Catalyst program. This analysis produced several conformations of both FR 173657 and (*R*)-**II** that overlap very well with respect to their many common structural features. One representative mapping is shown in Figure 3. The (*R*)-isomer is shown in gray. The structure of FR 173657 is not shown for simplicity.

We then inverted the chiral center to give the (*S*)-isomer of **II** and compared it to (*R*)-**II**. This inversion leads to an apparently large change in **II** as shown in Figure 3. The (*S*)-isomer is shown in cyan. Of course, it is possible to move the long, fairly rigid, side chain in (*S*)-**II** back to the same side as was found for the (*R*)-**II** mapping to FR 173657 by rotating bonds, but the fit was expected to be relatively poor. Therefore, our initial analysis suggested that if our targeted molecules would bind to the receptor similar to FR 173657, the (*R*)-isomer would fit to the receptor better than the (*S*)-isomer. This conclusion is based on the modeling of only one atropic isomer of FR 173657. The (*S*)- and (*R*)-isomers were synthesized to test this hypothesis.

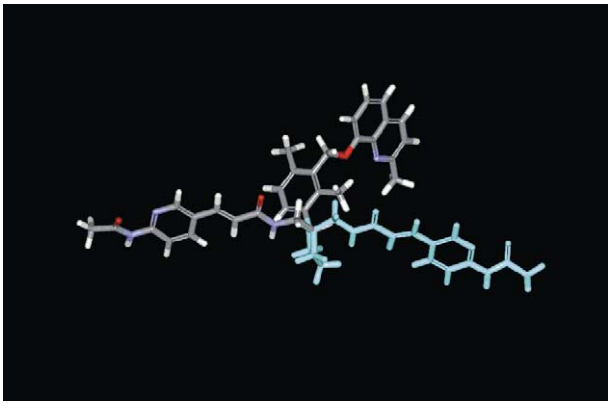
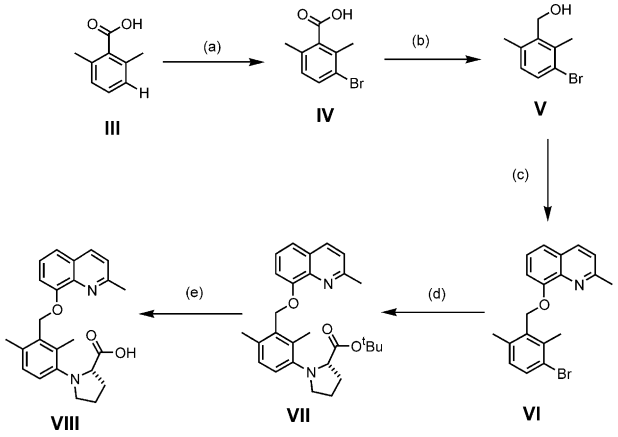


Figure 3. *R*-Isomer colored by atom type. *S*-isomer colored cyan.

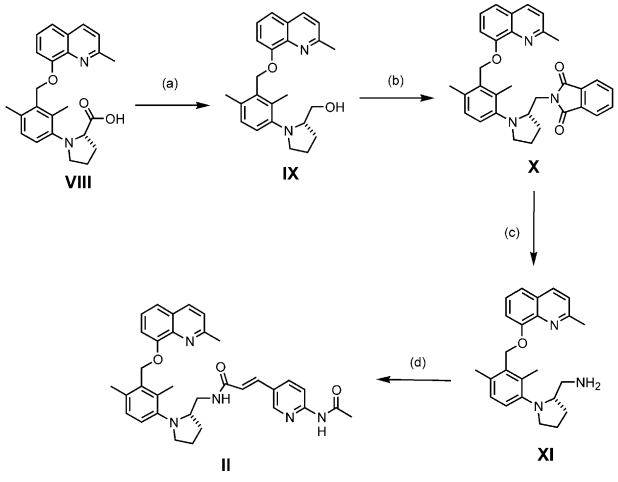
Chemistry

The synthetic route to the desired target is shown in Scheme 1. 2,6-Dimethylbenzoic acid **III** was treated with bromine in acetic acid to obtain 3-bromo-2,6-dimethylbenzoic acid. After converting the acid **IV** to the corresponding alcohol **V**, 8-hydroxyquinaldine was coupled with **V** via Mitsunobu coupling to obtain the benzyl ether **VI**. Buchwald–Hartwig coupling between **VI** and (*S*)-proline *t*-butyl ester provided the desired aniline **VII**.[14] Removal of the *t*-butyl ester was accomplished by treating of the ester a solution of trifluoroacetic acid and methylene chloride (1:1) to obtain **VIII**. The right side of the targeted molecule was built as shown in Scheme 2.

The carboxylic acid **VIII** was converted to the corresponding alcohol **IX** via a mixed anhydride method.



Scheme 1. (a) Br_2, CH_3CO_2H; (b) BH_3–SMe_2; (c) 8-hydroxyquinaldine, TMAD, $(n\text{-}Bu)_3P$; (d) (*S*)-proline *t*-butyl ester, $Pd_2(dba)_3$, $(t\text{-}Bu)_3P$, NaOtBu, toluene; (e) TFA–CH_2Cl_2 (1:1).



Scheme 2. (a) (i) $ClCO_2CH_2CH(CH_3)_2$, Et_3N; (ii) $NaBH_4$, H_2O; (b) phthalimide, $(n\text{-}Bu)_3P$, TMAD; (c) hydrazine, EtOH; (d) (i) EDC, 3-(6-acetylamino-pyridin-3-yl)-acrylic acid; (ii) HCl, Et_2O.

Mitsunobu coupling reaction between **IX** and a phthalimide, and deprotection of the resulting phthalimide **X** using hydrazine provided the aminomethyl proline intermediate **XI**. The amide coupling between 3-(6-acetylamino-pyridin-3-yl)-acrylic acid and **XI** using EDCI provided the desired target (*S*)-**II**.[15] The corresponding enantiomer was prepared using (*R*)-proline *tert*-butyl ester.

Result and Discussion

The two enantiomers, (*S*)-**II** and (*R*)-**II**, along with the intermediates **VIII**, **IX**, and **X** were assayed in both B_1 and B_2 receptor binding assays.[16] As shown in Table 1, the two enantiomers, (*S*)- and (*R*)-**II**, showed potent binding affinities toward the B_2 receptor, but the compounds did not show any affinities toward the B_1 receptor. (*S*)-**II** had a K_i of 33 nM and (*R*)-**II** had a K_i of 46 nM toward the B_2 receptor. None of the intermediates showed any binding affinity to either of the B_1 and B_2 receptor, suggesting that the right side of the molecules was essential for the binding activity. The two compounds [(*R*)-, (*S*)-**II**] were also evaluated for their functional activity using GTPγS assay[17] and shown to be functional antagonist to the B_2 receptor (data not shown). (*S*)-**II** was further evaluated in the Graded Abdominal Irritant Test (GrAIT).[18] Upon oral administration of the compound in mouse at 160 μmol/kg, it showed 59% inhibition.

As discussed before, our initial conformational analysis suggested that the (*R*)-isomer would be more potent than the (*S*)-isomer, but the binding data indicated that the (*S*)-isomer was actually a little more potent than the (*R*)-isomer. Since the experimental results were opposite of what we expected from the conformational analysis, we went back to the analysis. Upon further study, we were able to find a more optimal conformation for the (*S*)-isomer by inverting the nitrogen of the pyrrolidine ring. When the ring is inverted, the (*S*)-isomer fits as well to the estimated conformation of FR 173657 as the (*R*)-isomer. In fact the fit for the (*S*)-isomer is slightly better, as shown in Figure 4. The reason that both isomers show similar potency is probably because each isomer can adopt a different conformation with respect to inverting the nitrogen. The (*S*)-isomer would bind to the receptor in an inverted position, and the (*R*)-isomer would bind to the receptor in the non-inverted way as shown in Figure 4.

Table 1. In vitro binding affinity against bradykinin B_1 and B_2 receptors

Compd	B_1% Inhibition @ 1 μM	B_2% Inhibition @ 1 μM	Inhibition Constant K_i, (nM)
(*R*)-**VIII**	1	11	—
(*R*)-**IX**	9	12	—
(*R*)-**X**	1	8	—
(*R*)-**II**	1	76	46±3
(*S*)-**VIII**	6	19	—
(*S*)-**IX**	1	19	—
(*S*)-**X**	5	8	—
(*S*)-**II**	1	84	33±1

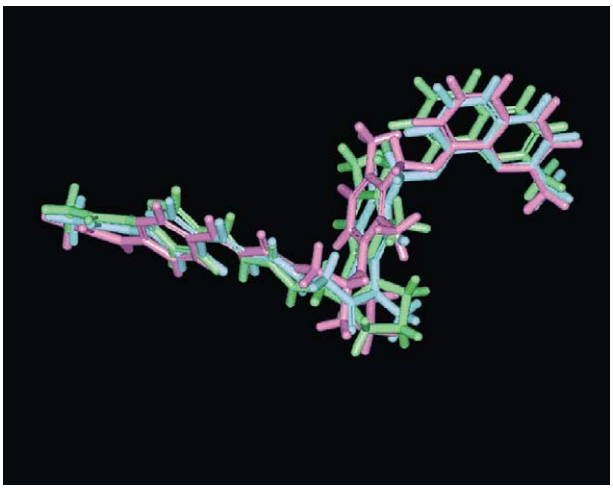


Figure 4. FR 173675 cyan, *R*-isomer magenta, *S*-isomer green.

In summary, we designed β-turn containing surrogates of FR 173657 based on our initial molecular modeling studies. The pyrrolidine containing compounds **II** were prepared as first targets and the compounds were potent binders to the bradykinin B_2 receptor. Furthermore, the more potent isomer (*S*)-**II** was orally active in GrAIT, suggesting that the novel compounds could be useful in antinociception. Finally, it would be interesting to see if one can apply the result we obtained to other bioactive molecules that contain the cis-amide conformational moiety.

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15. The (*R*)-**II** isomer; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (1H, d, $J = 2.1$ Hz), 8.15 (1H, d, $J = 8.5$ Hz), 7.89 (1H, dd, $J = 2.4$, 8.8 Hz), 7.33–7.51 (5H, m), 7.18 (1H, d, $J = 8.2$ Hz), 7.02 (1H, d, $J = 8.2$ Hz), 6.53 (1H, d, $J = 15.8$ Hz), 5.27 (2H, s), 3.82 (1H, m), 3.11–3.51 (4H, m), 2.71 (1H, m), 2.59 (3H, s), 2.42 (3H, s), 2.30 (3H, s), 2.15 (3H, s), 1.67–2.22 (4H, m); Mass spectrum (ESI) m/z 564 ($M + H^+$). The (*S*)-**II** isomer; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (1H, d, $J = 2.1$ Hz), 8.18 (1H, d, $J = 8.4$ Hz), 8.02 (1H, d, $J = 8.4$ Hz), 7.93 (1H, dd, $J = 2.3$, 8.7 Hz), 7.37–7.53 (5H, m), 7.22 (1H, d, $J = 8.2$ Hz), 7.06 (1H, d, $J = 8,2$ Hz), 6.55 (1H, d, $J = 15.8$ Hz), 5.31 (2H, s), 3.86 (1H, m), 3.16–3.51 (4H, m), 2.75 (1H, m), 2.62 (3H, s), 2.46 (3H, s), 2.34 (3H, s), 2.18 (3H, s), 1.74–2.22 (4H, m); Mass spectrum (ESI) m/z 564 ($M + H^+$). The enantiomeric purities of the isomers were checked on a Chiracel AD column (0.46 × 25 cm, 1 mL/min) with a 20-min run on an isocratic solution containing hexane/isopropanol/triethylamine, 65/35/0.05). The (*S*)-**II** isomer had a rentention time of 7.55 min and the (*R*)-**II** isomer had a retention time of 10.14 min. Each isomer had >90% ees.

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