

ASSESSMENT OF HUMAN AT₁ BINDING AFFINITY OF SOME NOVEL 2-ALKYLTHIO-1-[4-(N- α -ETHOXYCARBONYLBENZYL)AMINO BENZYL]-5-HYDROXYMETHYLIMIDAZOLES

SETAREH BADA KHSHANNOORY, FARHAD.HADIZADEH AND ABBAS SHAFIEE

Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Research Centre, Tehran University of Medical Sciences, Tehran, Iran.

ABSTRACT

Antagonists of various components of the renin-angiotensin system have been the subject of many studies for the control of blood pressure. Compounds with a phenoxyphenylacetic acid moiety that mimic the structure of losartan which is a powerful competitive antagonist of angiotensin receptor, have shown to be effective. In this study, the affinity of some 2-alkylthio-1-[4-(N- α -ethoxycarbonylbenzyl)aminobenzyl]-5-hydroxymethyl imidazoles for the human AT₁ receptor was assessed in a radioligand binding assay. It was found that an alkyl chain of appropriate length would be most suitable if situated on the imidazole ring. Furthermore, variations of the lower phenyl rings demonstrated that introduction of a methyl group in this position will account for the most desired effect.

Key words: Renin-angiotensin system, AngiotensinII, Losartan, Phenoxyphenylacetic acid, Radioligand binding assay

INTRODUCTION

Since its discovery over 30 years ago, angiotensin II (Ang II) has been the subject of extensive studies because of its key role in regulation of the cardiovascular function. Over the course of this period, components of the renin-angiotensin system (RAS) have been avidly pursued as therapeutic targets (1,2). Indeed, highly successful marketing of angiotensin converting enzyme (ACE) inhibitors in the early 1980s, demonstrated the widespread benefit of drugs targeted at the RAS, especially for the treatment of hypertension (2,3). However, ACE inhibitors, in addition to their effects on the RAS, inhibit bradykinin metabolism, and as a result may produce cough and angioedema. An alternative and perhaps the more selective approach to interfere with RAS, is to inhibit binding of AngII to its receptor. Such an antagonist would be expected to exhibit similar therapeutic effects as the ACE inhibitors but may lack undesirable side effects related to bradykinin potentiation (4).

In spite of attractiveness of directly blocking Ang II receptor (5), early progress resulting in identification of potent and specific peptide antagonists of Ang II was not rewarding (6,7). The presumed concept that nonpeptide Ang II

receptor antagonists would lack disadvantages associated with peptide Ang II antagonists, and thereby provide better therapeutic agents, proved to be an elusive strategy until 1982, that some polysubstituted imidazole-acetic acid derivatives were discovered (8,9), and subsequently led to the development of Dup753 (Losartan).

Losartan has been a prototype for numerous drug discovery programs (10). Replacing the imidazole of this compound with other heterocycles, or refinement the substituents on this ring has been a popular direction for investigation. Conversely, few studies have focused on structural modifications on the biphenyl moiety of losartan lead (7). However, it is known that the linkage between the two phenyl rings could vary up to three atoms in length and still preserves the desired effect (11).

Based on this supposition, and the observation that modelling a phenoxyphenylacetic acid moiety into the biphenyl of losartan results in an almost exact overlay (12), a series of 2-alkylthio-1-[4-(N- α -ethoxycarbonylbenzyl) aminobenzyl] -5-hydroxymethyl imidazoles were prepared (13) and the affinity of these compounds for the human AT₁ receptor was assessed in a radioligand binding assay.

MATERIALS AND METHODS

Chemistry:

The desired compounds were synthesized by the reported method (13) according to the procedure depicted in Scheme 1.

Pharmacology:

The Ang II binding assay was an adaptation of the procedure developed by Bergsma et al. (14). The title compounds were tested for their affinity toward the human AT₁ receptor as measured by their ability to displace the labeled ligand from its specific binding sites in human recombinant (CHO) cells. The assays were performed for 60 min in 1.0 ml of DPBS⁺⁺ (10 mM MgCl₂, 0.1% glucose and 0.2% BSA) at 37°C with 40-50 pM of [¹²⁵I][sar¹,Ile⁸]-AII in the absence or presence of the appropriate concentrations of unlabeled competitors. Following incubation, the membranes were rapidly filtered under vacuum through glass fiber filters (GF/B, Packard). The filters were then washed several times with an ice-cold buffer using a cell harvester (Packard). Bound radioactivity was measured with a scintillation counter (Topcount, Packard) using a liquid scintillation cocktail (Microscinto, Packard). By the same process the reference compound (saralasin) was tested at nine concentrations in duplicate to obtain a competitive curve for validation of this experiment.

IC₅₀ value (concentration causing a half maximal inhibition of control specific binding) and Hill coefficient (nH) were determined for the reference compound by nonlinear regression analysis of its competition curve. These parameters were obtained by Hill equation curve fitting. The IC₅₀ value obtained for the reference compound passed the required inspection and was within accepted limits of the historic average obtained ±0.5 log unit. The specific radioligand binding to the receptor is defined as the difference between total binding determined in the presence of an excess of unlabelled ligand. Individual results are reported in table 2. The results expressed in tables 1 and 2 were analyzed using one way ANOVA and both groups demonstrated significant differences (*p*<0.0001). Further analysis by Student-Newman-Keuls revealed an order of potency consistent with previous studies: **7e>7f>7d>7h=7a>7i=7b>7g>7c**.

RESULTS AND DISCUSSION

Tables 1 and 2 summarize effects of compounds **7a-i** which were tested at 1μM and 10μM on the specific radioligand binding to the human AT₁ receptor. Results are expressed as a percent of control specific binding and as a percent of

inhibition of control specific binding obtained in the presence of the test compounds.

The investigation of these compounds began with variations of the R group (R= methyl, ethyl, propyl) and substitutions on the lower phenyl ring. Comparison of the activity of compounds **7a-i** indicate that the order of potency for each X group (X= CH₃,Cl) was ethyl>propyl>methyl. This could serve as a reliable premise to support the postulation that there is a need for an alkyl chain with an appropriate length in position 2. These results are in agreement with previous studies (7) that an alkyl chain with 2-4 atoms is suitable for this area. However a flaw to this rule could be observed in the case of X=H, where the order of potency was changed to methyl>ethyl>propyl. This observation opens a basis for further investigation.

Table 1 Effects of the test compounds on the specific radioligand binding to the human AT₁ receptor

| Test compound | Concentration | |
|---------------|---------------|------|
| | 1μM | 10μM |
| 7a | - | 43 |
| 7b | - | 34 |
| 7c | - | 18 |
| 7d | - | 57 |
| 7e | 29 | 79 |
| 7f | 13 | 65 |
| 7g | - | 23 |
| 7h | - | 46 |
| 7i | - | 35 |

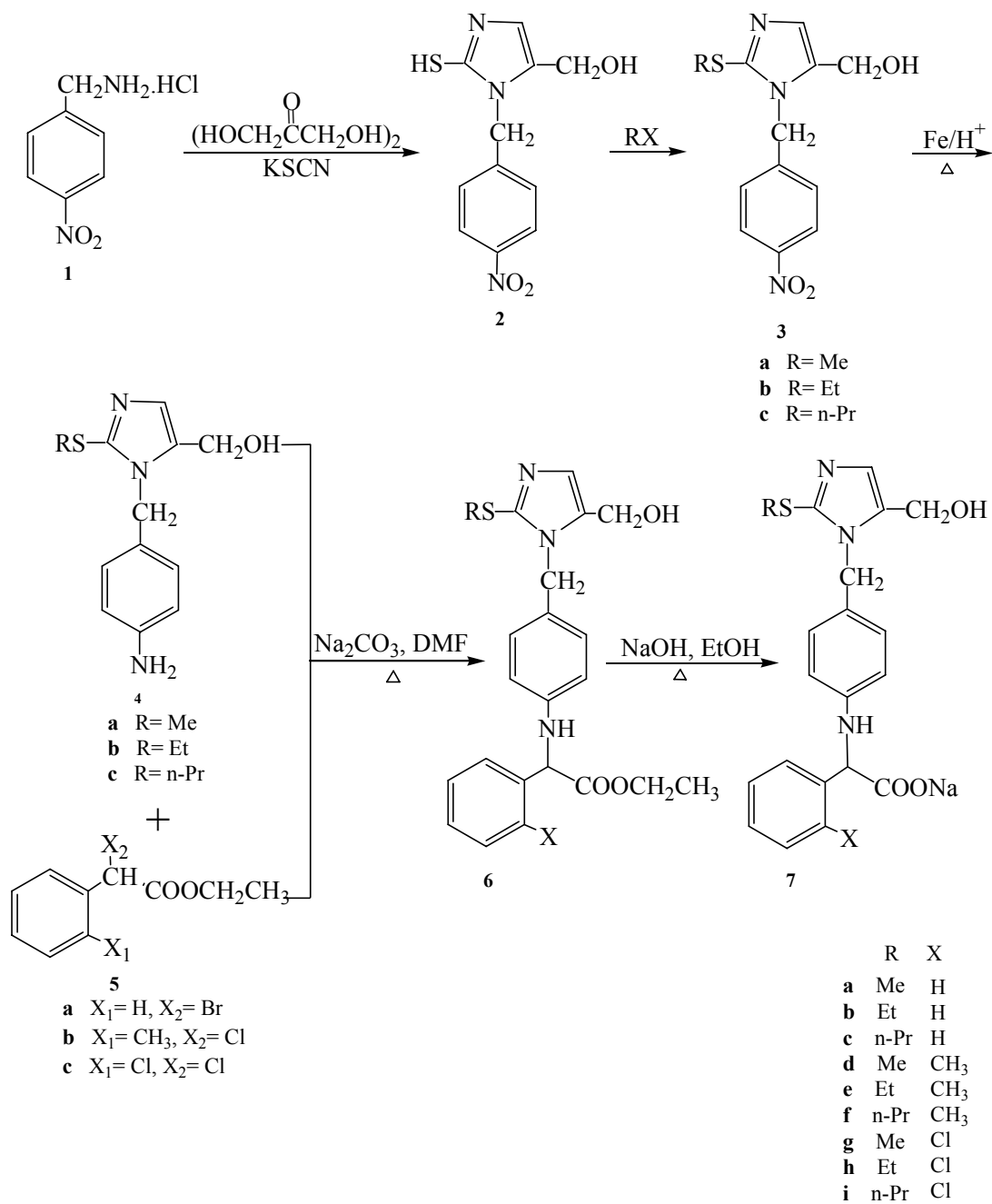
For the test compounds, the results are expressed as a percent inhibition of control specific binding (mean values; n=2)

The symbol “-” indicates an inhibition of less than 10%. The results are compared to saralasin (IC₅₀=0.81 nM)

Focusing next on the lower phenyl ring, it appears that analogues **7d**, **7e** and **7f** have improved binding affinity, and in these series the introduction of a methyl group in the lower phenyl ring is preferred, and that substitution ranking is CH₃>Cl>H.

The results of radioligand binding affinity tests revealed that these compounds exhibit moderate activity in comparison to saralasin. The most active compound in these series was **7e**. The high potency of this compound is consistent with the previous reasoning since the compound consists of both an ethyl chain on the imidazole ring and a methyl substitute in the lower ring. Further investigation on the lead compound in these series, and SAR studies could be bases for future surveys.

In summary, our goal to develop an AT₁ receptor antagonist with properties comparable to losartan led to new derivatives, in which the biphenyltetrazole moiety of losartan is replaced with phenoxyphenylacetic acid. We tried to



Scheme 1. Preparation of 2-Alkylthio-1-[4-(N- α -ethoxycarbonyl-benzyl)aminobenzyl]-5-hydroxymethylimidazoles.

Table 2 Individual results for each test compound as the percent specific binding of the labeled ligand to human AT₁ receptor

| Compound | Concentration [μM] | %Specific binding | | Mean |
|----------|--------------------|-----------------------|-----------------------|-----------|
| | | 1 st value | 2 nd value | |
| 7a | 1 | 95.3 | 92.7 | 94.0±1.3 |
| | 10 | 54.1 | 59.6 | 56.9±2.75 |
| 7b | 1 | 100.4 | 97.0 | 98.7±1.7 |
| | 10 | 65.7 | 66.1 | 65.9±0.2 |
| 7c | 1 | 101.4 | 01.6 | 101.5±0.1 |
| | 10 | 81.2 | 82.7 | 81.9±0.75 |
| 7d | 1 | 90.3 | 93.4 | 91.9±1.55 |
| | 10 | 43.7 | 41.7 | 42.7±1.0 |
| 7e | 1 | 69.7 | 72.3 | 71.0±1.3 |
| | 10 | 20.2 | 20.9 | 20.5±0.35 |
| 7f | 1 | 86.1 | 88.4 | 87.2±1.15 |
| | 10 | 35.2 | 34.8 | 35.0±0.2 |
| 7g | 1 | 94.7 | 94.2 | 94.4±0.25 |
| | 10 | 77.2 | 77.0 | 77.1±0.1 |
| 7h | 1 | 86.3 | 95.4 | 90.9±4.55 |
| | 10 | 54.6 | 53.4 | 54.0±0.6 |
| 7i | 1 | 97.9 | 89.9 | 93.9±4.0 |
| | 10 | 66.1 | 63.3 | 64.7±1.4 |

The results are expressed as the percent specific binding of the labeled ligand in the presence of the test compounds "mean" values are arithmetic means of the results obtained in two sets of concentrations (1, 10 μM; n = 2)
The results are compared to saralasin (IC₅₀=0.81 nM)

preserve the key characteristics essential for a perfect interaction between the receptor and the ligand. Despite the unexpected decline in affinity toward the receptor in comparison to losartan which had served as a standard compound, the order of potency amongst the compounds followed our previous assumptions.

ACKNOWLEDGMENT

The authors acknowledge the assistance of Fournier Laboratories (CEREP, Le Bois l'Eveque- B.P. 1- 86600 Celle l'Evescault) for AT₁ receptor binding assay. Also, the authors thank the research council of Tehran University of Medical Sciences for their supportive grant.

REFERENCES

- Hollenberg, N.K. (1979) Pharmacologic interruption of the renin-angiotensin system. *Ann. Rev. Pharmacol. Toxicol.* 19:559-582.
- Streeten, D.H.P., Anderson, Jr. G.H. (1984) Angiotensin-receptor blocking drugs. In: Doyle, A. (ed) *Handbook of Hypertension, Vol.5. Clinical Pharmacology of Antihypertensive Drugs*, Elsevier, Amsterdam, pp: 246-271.
- Wyvratt, M.J., Patchett, A.A. (1985) Recent Developments in the design of angiotensin-converting inhibitors. *Med. Res. Rev.* 5:483-531.
- Mais, D.E., Bowling, N.L., True, T.A., Naka, M., Morinelli, T.A., Oatis, Jr. J.E., Hamanaka, N., Halushka, P.V. (1991) 1-(Carboxybenzyl)imidazole-5-acrylic acids: potent and selective angiotensin II receptor antagonists. *J. Med. Chem.* 34: 1514-1517.
- Soffer, R.L., Sen, I. (1985) Angiotensin receptor is a desirable locus for physiologically specific inhibition of the renin-angiotensin system. *J. Cardiovasc. Pharmacol.* 7(Suppl. 4):S69-72.
- Duncia, J.V., Chiu, A.T., Carini, D.J., Gregory, G.B., Johnson, A.L., Price, W.A., Wells. G.J., Wong, P.C., Calabrese, J.C., Timmermans, P.B.M.W.M. (1990) The discovery of potent nonpeptide angiotensin II receptor antagonists: a new class of potent antihypertensives. *J. Med. Chem.* 33:1312-1329.
- Hodges, J.C., Hamby, J.M., Blankley, C.J. (1992) Angiotensin II receptor binding inhibitors. *Drugs Fut.* 17:575-593.
- Furukawa, Y., Kishimoto, S., Nishikawa, K. (1984) 4-Chloro-2-phenylimidazole-5-acetic acid derivatives. *Eur. Pat. Appl.* 103,647.
- Furukawa, Y., Kishimoto, S., Nishikawa, K. (1982) Hypotensive imidazole derivatives. U.S. Patent 4,340,598 and Hypotensive imidazole-5-acetic acid derivatives. U.S. Patent 4,355,040.

10. Le Bourdonnec, B., Meulon, E., Yous, S., Goossens, J., Houssin, R., Hénishart, J. (2000) Synthesis and pharmacological evaluation of new pyrazolidine-3,5-diones as AT₁ angiotensin II receptor antagonists. *J. Med. Chem.* 43:2685-2697.
11. Carini, D.J., Duncia, J.V., Aldrich, P.E., Chiu, A.L., Johnson, A.L., Pierce, M.E., Price, W.A., Santella III, J.B., Wells, G.J., Wexler, R.R., Wong, P.C., Yoo, S., Timmermans, P.B.M.W.M. (1991) Nonpeptide angiotensin II receptor antagonists: the discovery of a series of N-(biphenylmethyl)imidazoles as potent, orally active antihypertensives. *J. Med. Chem.* 34:2525-2547.
12. Fitch, K.J., Walsh, T.F., Patchett, A.A., Chang, R.S.L., Siegl, P.K.S., Faust, K.A., Chen, T.B., Lotti, V.J., Kivlighn, S.D., Zingam G.J., Greenlee, W.J. (1995) AT₁ selective angiotensin II antagonists with phenoxyphenylacetic acid as a biphenyl replacement part 1. *Bioorganic Med. Chem. Lett.* 5:155-158.
13. Shafiee, A., Hadizadeh, F., Noori, S.B. (2000) Syntheses of 2-alkylthio-1-[4-(N- α -ethoxycarbonylbenzyl)aminobenzyl]-5-hydroxymethylimidazoles. *J. Heterocyclic Chem.* 37:1015-1018.
14. Bergsma, D.J., Ellis, C., Kumar, C., Nuthulaganti, P., Kersten, H., Elshourbagy, N., Griffin, E., Stadel, J.M., Aiyar, N. (1992) Cloning and characterization of a human angiotensin II type 1 receptor. *Biochem. Biophys. Res. Commun.* 183:989-995.