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CONCISE ARTICLE

Design, synthesis and biological activity of 4′-[(benzimidazol-1-yl)methyl] biphenyl-2-sulphonamides as dual angiotensin II and endothelin A receptor antagonists

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A series of novel 4′-[(benzimidazol-1-yl)methyl]biphenyl-2-sulphonamides was designed, and their molecular model simulation fitting to a new HipHop 3D pharmacophore model was examined. Several compounds showed significantly high simulation fit values. The designed compounds were synthesised, 22 of which were biologically evaluated *in vitro* using the dual receptor binding assay. Compound 11 showed potent antagonistic activity against both angiotensin II AT_1 and endothelin ET_A receptors. Obtaining a highly active compound from a candidate set of only 22 compounds illustrates the power and utility of our pharmacophore model.

Introduction

Octapeptide angiotensin II (AngII) is a potent vasoconstrictor that serves important functions in blood pressure regulation.[1] Several selective, potent and orally available AngII receptor (AT_1) antagonists have been developed and used to treat hypertension and damage associated with diseases such as atherosclerosis and diabetes.[2] Endothelin subtype A (ET_A) receptor antagonists show promise in the treatment of similar illness.[3] Preclinical studies in animals demonstrate that the simultaneous antagonism of both AT_1 and ET_A receptors results in lowered blood pressure and greater therapeutic benefit than antagonising either AT_1 or ET_A receptors alone.[4] Experimental evidence indicated that the dual AT_1 and ET_A receptor antagonists (DARAs) in humans can be more effective than the current standard therapies for treating hypertension and other cardiovascular diseases. The development of non-peptide DARAs was initiated by Walsh et al.[5] at Merck in 1995, who revealed a series of α-phenoxyphenylacetic acid derivatives. Further research by Murugesan et al.[6-8] led to the discovery of *N*-isoxazolyl biphenylsulphonamides, which are potent DARAs.

Murugesan et al.[9] proposed a structure-activity relationship (SAR) of DARAs and suggested that activity is improved by the presence of (i) a biphenyl group (the presence of a linker chain between the two phenyl moieties reduces the activity), (ii) a isoxazole sulphonamide group at the ortho position of the biphenyl group and (iii) a heterocyclic ring, to act as an acceptor in a hydrogen-bonding interaction with the receptor. The compuound BMS-346567 was discovered and named PS-433540. This compound is used to treat hypertension and diabetic nephropathy. In phase I and phase II, BMS-346567(PS433540) is safe and well tolerated; in hypertensive patients, it significantly lowers blood pressure than placebo treatment.[10] In our recent study,[11] we have generated pharmacophore models of selective AT_1 and ET_A antagonists using the programme CATALYST/HipHop. The results of the comparison of two pharmacophores agreed with the SAR of DARAs.[12]

In this communication, molecular modeling simulation studies were performed to predict the biological activities of the proposed compounds. The pharmacophore hypothesis generation of DARAs was conducted by using the HipHop module of the CATALYST software.[13] On the base of the DARAs training set (Figure 1), 10 hypotheses were selected according to their ranking scores in the HipHop program. All 10 hypotheses contained the same features: one aromatic ring (R), one negative ionisable (N), one hydrophobic aromatic (Y), two hydrophobic aliphatic (Z) and two hydrogen bond acceptors (A). The selection of the ideal hypothesis was based on the hypothesis presenting full mapping of all its features without steric clashes in the lead compound and showing high fit values with the rest of the training set. Hypo-DARA-4 was deemed the best hypothesis in this study.

Results and discussion

Figure 2 shows Hypo-DARA-4 aligned with the highest active compound (DARA-3) of the training set molecules. As predicted, Hypo-DARA-4 mapped well with DARA-3.

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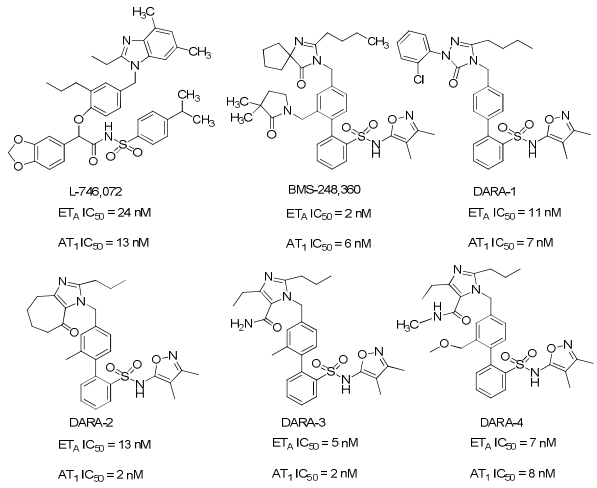


Figure 1. Structures and observed activities of DARAs for the HipHop training set

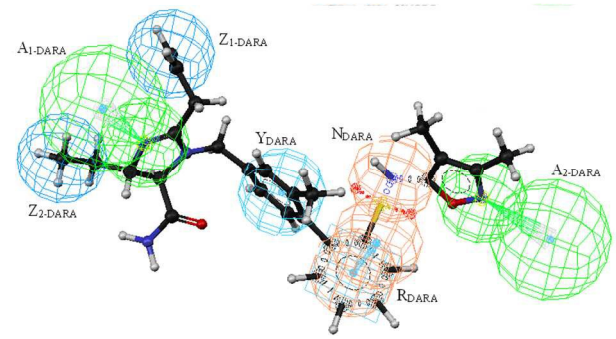


Figure 2. Mapping of the compound DARA-3 of the DARAs training set into the hypothesis Hypo-DARA-4 (R: aromatic ring; N: negative ionizable; Y: hydrophobic aromatic; Z: hydrophobic aliphatic; A: hydrogen bond acceptor)

To validate this hypothesis, a test set containing five DARAs were analysed (Figure 3). All test set molecules were built and minimised and then utilised in conformational analysis like all training set compounds. Hypo-DARA-4 showed full mapping of all its features with the compounds in the test set (Figure 4). The obtained hypothesis agreed with the 'DARA Roadmap' reported in 2002.[9] Therefore, Hypo-DARA-4 is likely to be a representative pharmacophore for DARAs.

The generated DARA pharmacophore hypothesis Hypo-DARA-4 was subjected to simulation compare/fit studies to predict its antagonistic activity. The structures of the test set of the target sulphonamides(compounds **1** to **22** in Scheme 1) were built using the CATALYST software,[13] and their conformational models were generated (in the energy range of 20 kcal/mol above the estimated global energy minimum). The fitting of the tested compounds was determined on the basis of the selected hypothes -is of DARAs for predicting their activies. The fitting was performed using Best Fit during the compare/fit process. different mappings for all the conformers of each compound in the test set

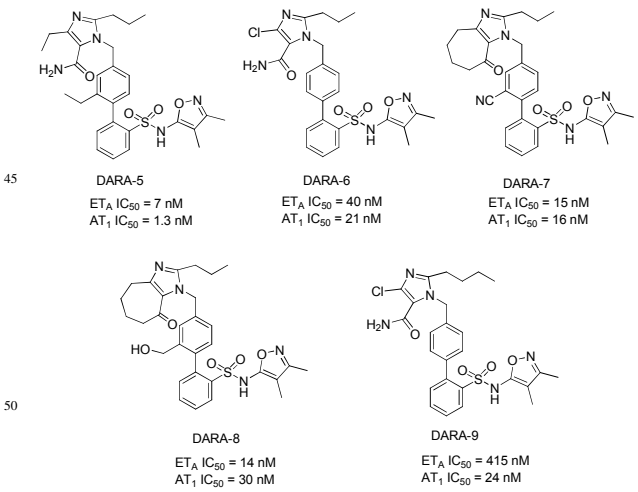


Figure 3. Structures and observed activities of DARAs for the test set

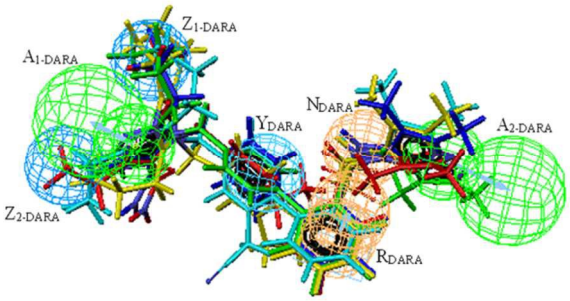


Figure 4. The hypothesis Hypo-DARA-4 aligned to the test set compounds (R: aromatic ring; N: negative ionizable; Y: hydrophobic aromatic; Z: hydrophobic aliphatic; A: hydrogen bond acceptor)

using the selected hypothesis were visualized, and the fit values of the best-fitting conformers were determined (Table 1). This molecular modelling simulation study revealed that compounds **11, 16** and **21** could be considered as promising candidates because of their high fit values to the hypothesis.

Target compounds were prepared by using the synthetic route described for compounds 1 to 22 (Scheme 1). At a minimum, the compounds were characterised by ^{1}H NMR, ^{13}C NMR and MS analyses.The preparation of biphenyl intermediate A has been described.[9] All benzimidazole precursors B were prepared according to literature procedures.[14] The alkylation of benzimidaz -oles with intermediate A led to the final compounds 1 to 22. Fur -thermore compound 11 was confirmed by X-ray crystallography (Figure. 5). [15]

AT_1 receptor antagonism was enhanced by selecting benzimidazole substituents with a biphenyl group to optimise the binding affinity.[16] Heterocyclic ring-substituted biphenyl-2-sulphonami – des should improve ET_A receptor antagonism but not reduce the AT_1 receptor affinity.

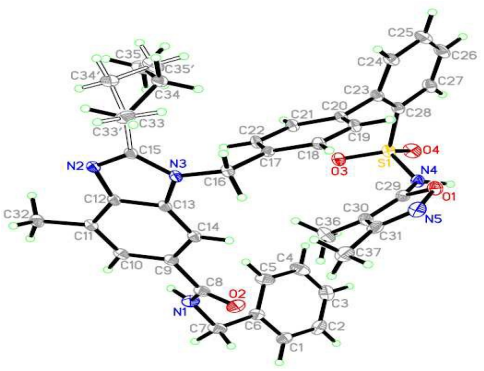


Figure 5. Crystal structure of compound **11**

Table 1. Predicted fit values of novel DARAs from the hypothesis Hypo-DARA-4.

Compounds	Predicted fit values
1	5.618
2	5.529
3	5.357
4	5.207
5	5.322
6	5.452
7	5.115
8	5.224
9	5.345
10	5.426
11	5.732
12	5.323
13	5.439
14	5.367
15	5.143
16	5,591
17	5.281
18	5.148
19	5.356
20	5.219
21	5.768
22	5.376

Table 2. Screening for dual antagonism to human AT_1 and ET_A receptors.

Compounds	R_1	AT_1 IC_{50} (nM)	ET_A IC_{50} (nM)
1	2-morpholinoethyl	31	1400
2	2-(4-methylpiperazin-1-yl)ethyl	47	1800
3	2-(piperidin-1-yl)ethyl	94	5800
4	2-(pyrrolidin-1-yl)ethyl	95	5500
5	propyl	373	35000
6	isopropyl	341	277
7	butyl	465	314
8	tert-butyl	385	140000
9	phenyl	201	27000
10	*o*-methoxy-phenyl	419	137
11	benzyl	28	10
12	*o*- methoxy-benzyl	217	310
13	*m*- methoxy-benzyl	50	898
14	*p*- methoxy-benzyl	81	388
15	*m,p*-dimethoxy-benzyl	56	28000
16	β-phenylethyl	8	956
17	*m*- methoxy-phenylethyl	42	24000
18	*p*- methoxy-phenylethyl	76	200000
19	*o,m*- dimethoxy-phenylethyl	37	3000
20	*m,p*-dimethoxy-phenylethyl	92	55000
21	*o*-F-phenylethyl	160	262
22	*p*-F-phenylethyl	152	56000

Several researchers have successfully substituted amides for the carboxylate of EXP-3174, which is a highly selective AT_1 receptor antagonist. The 7-carboxamides became our starting point for the benzimidazole substituent SAR.[17] The carboxamide analogue (Table 2) showed good activity at the AT_1 receptor, but its ET_A receptor binding affinity was moderate. Heterocyclic amine showed better activity at the AT_1 receptor than Aliphatic amines (compound **1** vs. **5**), The phenylethylamides (compounds **16** to **22**) showed diminished ET_A receptor binding, whereas the benzylamides demonstrated high ET_A receptor affinity and good AT_1 receptor binding. The methoxy substituents of amides reduced the ET_A receptor antagonism. A possible cause could be the steric congestion, which affects the compounds binding with ET_A receptor. Replacement of the methoxy group by fluoride did not improve the activity (compound **18** vs. **22**).

Compound **11** showed potent antagonistic activity against both angiotensin II AT_1 and endothelin ET_A receptors. When evaluated orally in conscious SHR, compound **11** at dose of 20 mg/kg po lowered the blood pressure, and Its hypotensive effect is similar to that of Irbesartan (Figure 6).

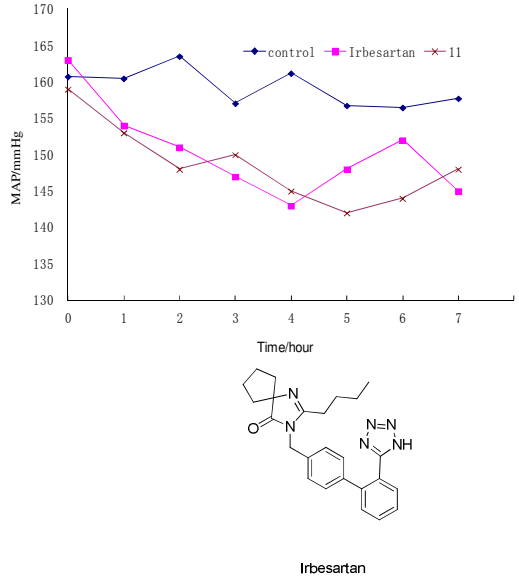
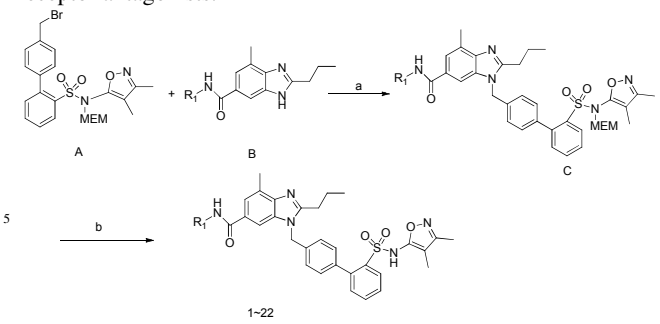


Figure 6. Effects of 11 and Irbersartan (20 mg/Kg po) on mean arterial pressure in conscious SHR after oral administration.

Conclusions

In conclusion we have designed new ligands with dual AT_1 and ET_A receptor antagonistic activities by using the CATALYST software. The ideal pharmacophore model (hypothesis) was created by the common feature hypothesis generation using a training set (Figure 1). Compounds **11**, **16** and **21** showed high fit values to the generated DARAs hypothesis. Twenty-two of the synthesised compounds were biologically evaluated *in vitro* using the dual receptor binding assay. Compound **11** showed significant dual receptor antagonism. As demonstrated by in vivo results, compound **11** showed a similar anti-hypertensive effect to Irbesartan. We obtained highly active compound **11** from a set of only 22 candidate molecules. Obtaining compound **11** from only 22 candidates demonstrated the capability and potential of our pharmacophore model in the design of novel dual AT_1 and ET_A

receptor antagonists.



Scheme 1. Reagents and conditions (a) NaH, DMF, 0 ℃ to rt, 24 h, 39-47%; (b) HCl (6N), EtOH, reflux, 1 h.

Acknowledgments

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15. Full crystallographic details of compound 11 have been deposited with the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 1015172. Copies of the data can be obtai -ned, free of charge, on application to The Director, CCDC, 12 Unio -n Road, Cambridge CB21EZ, UK (Fax: t44 1223336 033; e-mail: deposit@ccdc. cam.ac.uk or www:http://www.ccdc.cam.ac.uk)
16. AT_1 receptor binding assay was carried out by competitive displacementof the binding of 0.2 nM ^{125}I-labeled Sar^1-Ile^8-angiotensin II with human angiotensin AT_1 receptor according to ref 8.Binding to the human ET_A receptor was evaluated by incubating test compounds with CHO-K1 cells expressing the human ET_A receptor in the presence of 0.05 nM ^{125}I-labelled endothelin 1 according to ref 9. IC_{50} value was calculated by Sars 2.0 software. Each reported IC_{50} value is the mean of at least three individual experiments.
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