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Novel, selective indole-based ECE inhibitors: Lead optimization via solid-phase and classical synthesis

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Abstract—A novel class of indole-based endothelin-converting enzyme (ECE) inhibitors was identified by high throughput screening. We report systematic optimization of this compound class by means of classical and solid-phase chemistry. Optimized compounds with a bisarylamide side chain at the 2-position of the indole skeleton exhibit low-nanomolar activity on ECE.

1. Introduction

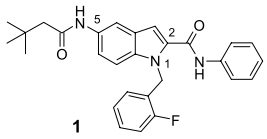
Since its discovery in 1988, endothelin (ET-1), its metabolism, and potential implications in several diseases have attracted considerable attention.[1] Blockade of the action of ET-1 by ET-receptor antagonists has been widely studied in animal models as well as in clinical trials aimed at evaluating their use in the treatment of various diseases such as hypertension, congestive heart failure, and cancer.[2] Notably, bosentan, the first marketed ET-receptor antagonist, was approved for the treatment of pulmonary arterial hypertension in 2001.[3] Since the 21-amino acid peptide ET-1 is formed by cleavage of its precursor big-ET via the action of endothelin-converting enzyme (ECE), inhibition of this metalloprotease has become an attractive target to modulate ET-1 levels. Several inhibitors of ECE have been described in recent years.[4] The majority of those investigational compounds typically contains structural motifs such as thiols or phosponates addressing the Zn-bearing catalytic center of the enzyme. Such pharmacophoric groups, however, can lead to detrimental pharmacokinetic properties. In addition, selectivity versus related metalloproteases such as neutral endopeptidase 24.11 (NEP) or angiotensin-converting enzyme (ACE) has been reported to be difficult to achieve.[4] Thus, there is still need to discover novel, selective lead structures for the inhibition of ECE.

Through screening of the Bayer compound collection, indole derivative **1** has been identified to be a potent ECE inhibitor.[5] In a standard enzyme-linked immunosorbent assay (ELISA) format,[6] **1** had an IC_{50} value of 1.8 μM. The interesting in vitro activity, the unprecedented structure as well as high selectivity observed for this compound versus the related enzymes NEP and ACE (IC_{50} values >10μM) rendered indole **1** into an attractive starting point for a medicinal chemistry optimization program.

In this article, we describe a systematic approach towards the optimization of ECE inhibitor **1** and disclose the details of the resulting structure–activity relationship (SAR).

2. Chemistry

As no obvious Zn-chelating group is present in ECE inhibitor **1**, we decided to systematically vary the structural features of the lead compound. For this, we focused on the three substituents at positions 1, 2, and 5 of the indole core (see Fig. 1) which can be subjected



1

Figure 1. Lead structure **1** from HTS.

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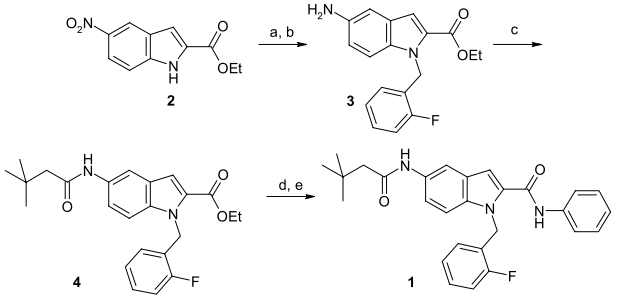
to variations independently from each other. In addition, changes in the indole scaffold itself and position shifts of the side chains were envisaged.

Synthesis of indole derivatives was achieved by starting from commercially available indole **2** through a standard sequence (Scheme 1). Alkylation of the indole nitrogen in **2** was accomplished by treatment with 2-fluorobenzylbromide under basic conditions. After reduction, the resulting aminoindole **3** was treated with pivaloyl chloride to give the corresponding amide **4**. Saponification of the ester moiety and coupling with aniline yielded the final indole-2-carboxamide **1**.

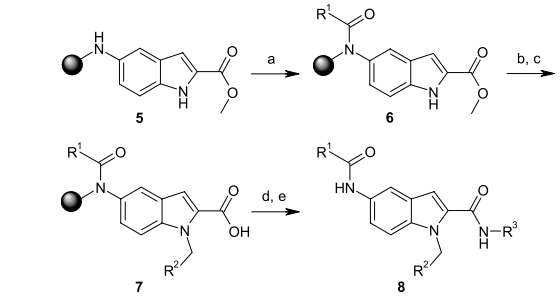
Synthetic throughput was dramatically increased by employing solid-phase organic chemistry as outlined in Scheme 2. Thus, library synthesis began with indole **5** that was obtained from reductive amination of 4-(4-formyl-3-methoxyphenoxy)butyrylamide resin with methyl-5-amino-1*H*-indole-2-carboxylate. Acylation of **5** with diverse carboxylic acid chlorides generated **6**. N-alkylation with benzyl and alkyl halides using LiHMDS followed by ester hydrolysis afforded the indole carboxylic acid **7**. Amide formation of **7** with anilines was most efficient using HATU (method A), whereas coupling of amino phenols worked best with PPh_3 and NBS (method B). Finally, **8** was released from the resin with TFA in CH_2Cl_2. The combinatorial library was synthesized using a split-and-pool IRORI Kan® method.[7] Using this synthetic route, 1332 new compounds were prepared, which gave satisfactory spectral and analytical data, and were therefore tested for their ECE inhibitory activity.

For the synthesis of the inverse amide at the 5-position (**12**, Scheme 3), commercially available 4-hydrazinobenzoic acid (**9**) was refluxed with ethylpyruvate in *i*-PrOH/AcOH, followed by coupling with neopentyl amine. The resulting phenylhydrazone was treated under Fischer-indole type reaction conditions using polyphosphoric acid[8] to yield the desired indole-5-carboxamide **10**. Alkylation of indole nitrogen and subsequent installation of a C-2 amide side chain was carried out as described in Scheme 1.

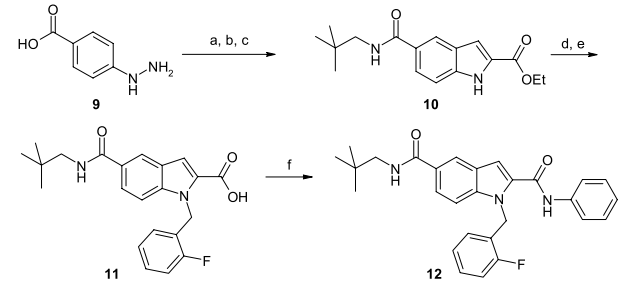
Synthesis of benzimidazole derivative **17** (Scheme 4) was achieved starting from commercially available 4-nitrobenzene-1,2-diamine (**13**) through a standard synthesis sequence.[9] After treatment with a trichloroacetimidate, the resulting intermediate **14** was converted into ester **15** under standard conditions. N-benzylation of **15** employing basic conditions resulted in a 1:1 mixture of regioisomers from which the desired 5-nitrobenzimidazole isomer was separated by flash chromatography (cyclohexane/ethyl acetate) and



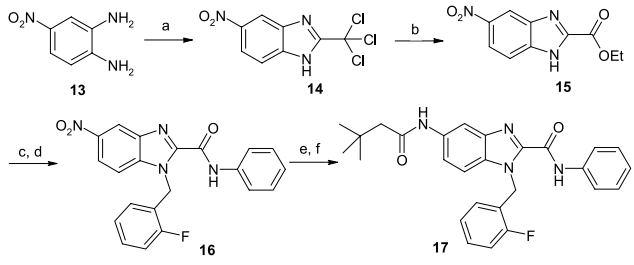
Scheme 1. Synthesis of indole-2-carboxamide **1**. Reagents and conditions: (a) KO*t*-Bu, 18-crown-6, 0 °C, 2-fluorobenzylbromide, THF, rt, 86%; (b) Pd/C, ammonium formate, EtOH/ethyl acetate, 95%; (c) $C(CH_3)_3CH_2COCl$, NEt_3, CH_2Cl_2, 0 °C to rt, 98%; (d) LiOH, MeOH/H_2O 3:1, rt, 89%; and (e) HATU, DIPEA, DMF, aniline, rt, 84%.



Scheme 2. Solid-phase synthesis of **8**. Reagents and conditions: (a) $R^1C(O)Cl$, acetone, Et_3N, rt; (b) LiHMDS, R^2CH_2Br, DMF, rt; (c) KOH, MeOH/dioxane (1:2), rt; (d) method A (anilines): HATU, DMF/Pyr (1:2), rt, or method B (amino phenols): PPh_3, NBS, CH_2Cl_2, Pyr, THF, −25 °C to rt; and (e) TFA, CH_2Cl_2, rt.



Scheme 3. Synthesis of indole-5-carboxamide **12**. Reagents and conditions: (a) ethyl pyruvate, AcOH, *i*-PrOH, reflux, 76%; (b) TBTU, DIPEA, $C(CH_3)_3CH_2NH_2$, CH_2Cl_2, 0 °C to rt, 59%; (c) PPA, 120 °C, 5 h, 34%; (d) KO*t*-Bu, 18-crown-6, 0 °C, 2-fluorobenzylbromide, THF, rt, 55%; (e) LiOH, MeOH/H_2O 3:1, rt, 98%; and (f) HATU, pyr/DMF 2:1, aniline, rt, 47%.



Scheme 4. Synthesis of benzimidazole **17**. Reagents and conditions: (a) methyl 2,2,2-trichloroacetimidate, AcOH, rt, 3 h, 92%; (b) $AgNO_3$, EtOH, reflux, 15 h, 99%; (c) KO*t*-Bu, 18-crown-6, 0 °C, *o*-fluorobenzylbromide, THF, rt, 29% desired regioisomer; (d) aniline, NaH, THF, reflux, 65%; (e) $SnCl_2$, EtOH, 32%; and (f) $C(CH_3)_3CH_2COCl$, NEt_3, CH_2Cl_2, 0 °C to rt, 20%.

readily converted into amide **16**. Reduction of the nitro group and amide formation yielded benzimidazole derivative **17**.

3. Pharmacology

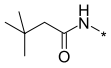
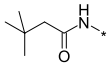
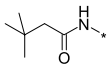
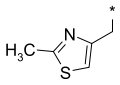
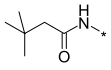
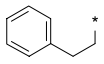
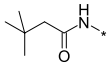
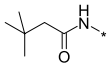
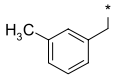
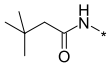
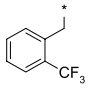
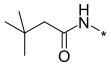
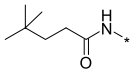
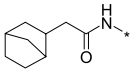
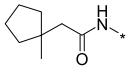
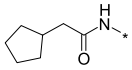
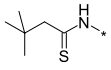
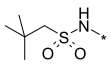
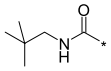
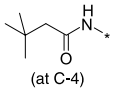
Novel compounds have been tested for their ability to inhibit ECE-1 by using a high throughput assay employing an artificial, bradykinin-derived substrate allowing for the rapid readout of a fluorescence signal.[10] Selected compounds have been assessed in a lower throughput ELISA assay using big-ET-1 as the endogenous substrate of ECE-1.[6] Since the assay employing the fluorogenic substrate turned out to be more sensitive (e.g., IC_{50} for lead compound **1** is 0.22 μM in this assay vs 1.8 μM in the ELISA), it was well suited for the ranking of compounds, and therefore it was used for the optimization program.

First, we focused on changing the nature of the benzylic group at the N-1 position of **1** (Table 1). Whereas replacement of the phenyl group by cyclohexyl leads to an only moderate drop in activity (**18**), direct attachment of a phenyl group (**19**) as well as extension of the linker length by one additional methylene unit (**21**) result in less active compounds. A small substituent like a methyl group in *m*-position of the benzyl group is not tolerated (**23**), indicating a tight binding pocket at that site, which is further underlined by the low activity of a corresponding thiazole bioisoster replacement (**20**). Thus, the only variations tolerated were the attachment of an additional fluorine atom in the second ortho position (**22**) and its replacement by a trifluoromethyl group (**24**).

We next explored the neopentyl group in the amide substituent at the 5-position. Chain elongation by one methylene unit as in **25** proved to be detrimental for activity, whereas the bulky bornane residue turned out to be the only group tolerated in this position not bearing a quaternary carbon atom (**26**). The necessity for the quaternary center is clearly highlighted through comparison of derivatives **27** and **28.** Further alterations in the C-5 side chain revealed that variations in the amide linker group are not tolerated: thioamide **29** shows some residual activity, whereas replacement of the amide by a sulfonamide (**30**) or its inverted counterpart (**12**) leads to a dramatic loss in activity. Finally, the shift of the side chain to the C-4 position of the indole core (**31**) deleted inhibitory activity.

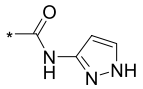
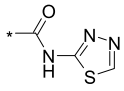
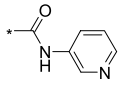
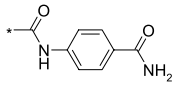
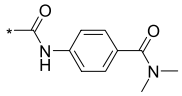
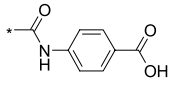
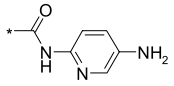
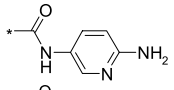
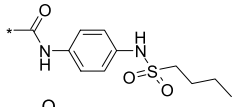
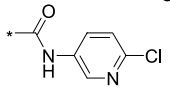
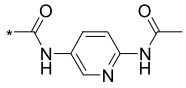
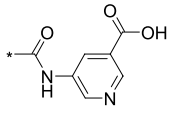
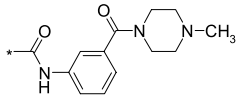
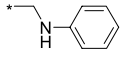
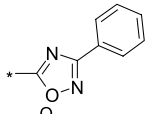
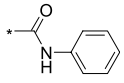
Having already observed two tightly binding groups in lead structure **1**, we hoped that the C-2 side chain would tolerate more variations allowing for increase in in vitro potency and for tuning of physicochemical properties (Table 2). Simple replacement of the phenyl group of the C-2 amide by heterocycles led to a drop in activity (**32**/**33**), with the pyridyl group (**34**) being the best replacement. More interestingly, the 4-position tolerated a range of substituents (**35**–**42**). In addition, some derivatives in which an additional group was attached to the 3-position of the phenyl ring (entries **43**/**44**) yielded highly active compounds. Several of those compounds with diverse substituents in the 3- and 4-positions of the C-

Table 1. Modification of the N-1 and C-5 side chains of the indole **1** (R^1 at N-1/R^2 at C-5 position of **1**, IC_{50} values determined via fluorescence read-out[10].)

Compound	R^1	R^2	Method	IC_{50} (μM)
1			Scheme 1	0.22
18			Scheme 2	0.86
19			Scheme 1[11]	5.9
20			Scheme 2	2.2
21			Scheme 1	5.1
22			Scheme 2	0.65
23			Scheme 2	3.0
24			Scheme 2	0.17
25			Scheme 1[a]	0.74
26			Scheme 2	0.30
27			Scheme 1[12]	0.12
28			Scheme 2	6.4
29			Scheme 1[b]	0.66
30			Scheme 1[c]	6.7
12			Scheme 3	7.0
31		 (at C-4)	Scheme 1[13]	7.4

[a] 4,4-Dimethylpent-2-ynoic acid was used in the amide formation step followed by catalytic hydrogenation.
[b] Lawesson's reagent was used for thioamide formation.
[c] 2,2-Dimethyl-propane-1-sulfonyl chloride was prepared from 2,2-dimethyl-propane-Grignard reagent and sulfuryl chloride in THF.

Table 2. Modification of the C-2 side chain of the indole **1** (IC_{50} values μM determined via fluorescence read-out[10].)

Compound	R	Method	IC_{50}
32		Scheme 2	>10
33		Scheme 2	2.3
34		Scheme 2	0.85
35		Scheme 2	0.22
36		Scheme 2	0.21
37[a]		Scheme 1	0.39
38[a]		Scheme 1	1.2
39[a]		Scheme 1	0.17
40		Scheme 2	0.20
41		Scheme 2	0.44
42		Scheme 2	0.13
43[a]		Scheme 1	(74)[e]
44[b]		Scheme 1	(90)[e]
45[c]		Scheme 1	>10
46[d]		Scheme 1	>10
47[14]	 (at C-3)	Scheme 1	>10

[a] Amino or carboxy groups introduced as part of the anilines were appropriately protected (*N*-Boc or *N*-Cbz; methyl ester) and deprotected under standard conditions.

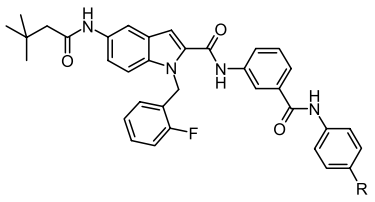
[b] Phenyl analog of **43** was coupled with methylpiperazine using HATU in DMF.

[c] 2-Hydroxymethyl indole derivative obtained from $LiAlH_4$ reduction of **4** was converted to **45** using PPh_3, NBS, and aniline in THF.

[d] Oxadiazole formation was achieved by reaction of the carboxylic acid derived from **4** and phenyl amidoxime using PyBOP, NEt_3, DME, and reflux.

[e] % inhibition at 5 μM.

Table 3. Second generation SAR (IC_{50} values determined in ELISA[6].)



Compound	R	IC_{50} (μM)
48	SO_2NH_2	0.039
49	SO_2NHCH_3	0.150
50	$SOCH_3$	0.066
51	SO_2CH_3	0.120

2 arylamide have been found through the solid-phase combinatorial approach described in Scheme 2. Thus for the first time, analogs with improved in vitro activity compared to lead compound **1** could be identified as shown by their IC_{50} values in the big-ET ELISA[6] (**1**: 1.8 μM; **41**: 0.22 μM; **43**: 0.18 μM; and **44**: 0.50 μM). On the contrary, all efforts to change the nature of the C-2 amide linker led to inactive compounds as exemplified by amine **45** and amide bioisoter **46**. As already observed for the C-5 side chain, a shift in the attachment point of the arylamide side chain from C-2 to C-3 of the indole skeleton deletes all inhibitory activity (**47**).

Finally, a variation of the scaffold itself was explored. However, benzimidazole analog **17** (Scheme 4) turned out to be less active (IC_{50} = 1.3 μM fluorescence read-out) compared to lead compound **1**. The same trend was observed for other benzimidazole derivatives that were prepared (data not shown).

Thus in summary, the systematic optimization effort revealed that major structural variations of lead structure **1** were only tolerated in the aryl part of the C-2 amide side chain. This prompted us to perform a second-generation lead structure optimization program. With extensive use of solid-phase synthesis, a comprehensive program of derivatization concentrating on this site of the molecule was carried out. Through this, several compounds with a second arylamide group attached to the 3-position of the phenyl group were found to exhibit high activity in the big-ET ELISA assay. Of particular interest were those derivatives bearing an additional sulfonamide, sulfoxide or sulfone group in para position as exemplified in Table 3, compounds **48**–**51**. The highest activity was observed for sulfonamide **48**, for which in vitro inhibitory activity on ECE was optimized by a factor of nearly 50 when compared to screening lead **1** (IC_{50} in ELISA is 1.8 μM). As observed for lead structure **1**, none of the described compounds including **48** show any appreciable activity toward inhibition of ACE or NEP at a concentration of 10 μM.

4. Conclusion

Systematic variation of indole lead structure **1** led to the discovery of highly potent and selective ECE inhibitors

such as sulfonamide **48** representing a novel type of ECE inhibitor that is structurally different from all the classes described so far. The absence of any Zn-chelating elements in its structure is a distinct feature and gives rise to the speculation that this compound class might exhibit an unexpected binding mode to the enzyme.[15] Therefore, compounds such as sulfonamide **48** and its close analogs are expected to serve as valuable tools for structural biology as well as for pharmacological studies. More details on the biological characterization and pharmacology of these compounds will be published in due course.

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15. Computational chemistry studies toward an understanding of the binding mode of compound **48** will be published soon.