Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 17–22

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

2. ENDOTHELIN ANTAGONISTS: EVALUATION OF 2,1,3-BENZOTHIADIAZOLE AS A METHYLENDIOXYPHENYL BIOISOSTER

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Received 20 August 1997; accepted 3 November 1997

Abstract: The methylendioxyphenyl group is present in a number of endothelin receptor antagonists thus far reported. By means of a Kohonen neural network we discovered with a benzothiadiazole a bioisosteric replacement instead. This group should be devoid of the negative metabolic interactions with cytochrome P450 ascribed to methylendioxyphenyl *in vivo*. The synthesis of a potent benzothiadiazole analogue EMD 122801 together with *in vitro* studies of different methylendioxyphenyl, benzothiadiazole and benzofurazan derivatives is described.

Introduction:

The endothelins are the most potent endogenous peptide vasoconstrictors known to date and are also potent mitogens. There has been a great effort to discover endothelin receptor antagonists which may be of benefit in diseases with a significant vasoconstrictive or proliferative component. In recent years this therapeutic potential for endothelin receptor antagonists has led to numerous reports in the literature of structurally diverse antagonists with varying potency and subtype selectivity. Several non-peptide ET_A selective antagonists were disclosed including BMS-182874,[1] PD156707,[2] and A-127722,[3] as well as the non-selective ET_A / ET_B antagonists Ro 47-0203 (Bosentan),[4] SB 209670,[5] and L-749,329.[6] With the exception of BMS-182874 and Bosentan, both series of antagonists contain a methylendioxyphenyl group. The methylendioxy group is very common in natural and synthetic medicinal compounds and provides an electronegative function that is relatively unreactive and non polar. The function can be oxidized by cytochrome P450, an ubiquitious monooxygenase, to form a catechole and formate or carbon monoxide or alternatively, forms a complex with the heme iron of cytochrome P450. This complex, characterized by its absorption in the 455 nm range, can be very stable and inhibits the catalytic cycle of the enzyme. Thus, this type of metabolism may lead to drug-drug interactions or nonlinear pharmacokinetics.[7]

In a previous paper, we described our efforts for the search of bioisosteric candidates of the methylendioxyphenyl group through comparison of physicochemical properties by means of the Kohonen neural network approach.[8] In this respect, we discovered the benzothiadiazole group as a suitable bioisoster. With this knowledge we tried to develop compounds which bear a benzothiadiazole functionality instead of methylendioxyphenyl.

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0960-894X/98/$19.00
PII: S0960-894X(97)10151-2

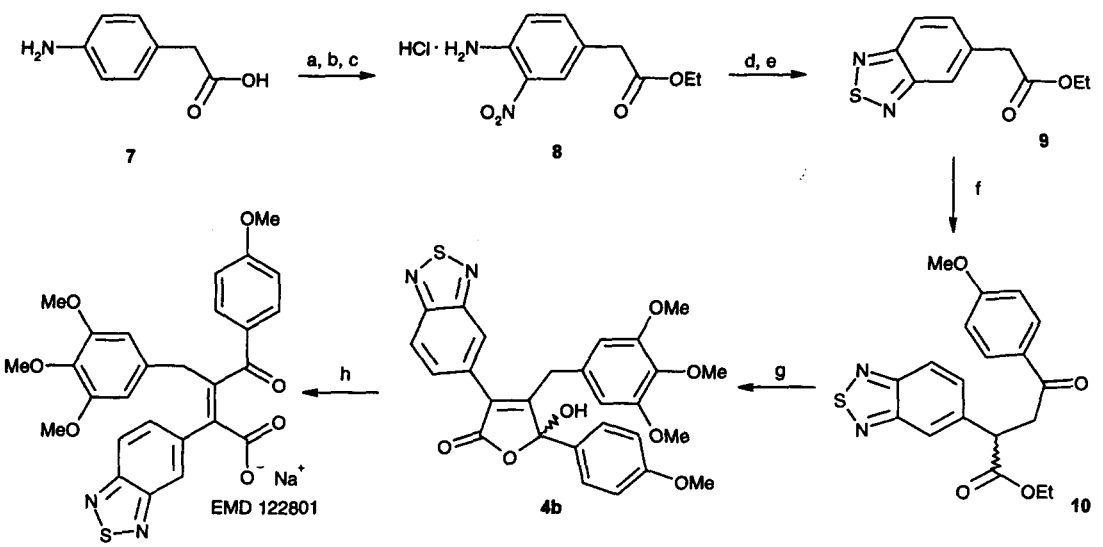
In this publication, we report on the synthesis and biological properties of endothelin receptor antagonists containing a benzothiadiazole or benzofurazan moiety together with their methylendioxyphenyl analogues.

Chemistry:

Naphthalenesulfonamides **1a** and **1b**, benzofuro[3,2-b]pyridine **2a**, 2-carboxyindole **3a** and **5a** and γ-hydroxybutenolide **4a** in table 1 and 2 were prepared as previously described.[9,10,11,2] The synthesis of benzothiadiazole derivatives **2b**, **3b**, **5b**, **6a** and **6b** and benzofurazan **5c** was performed in analogy to the synthesis of **2a** and **3a**, respectively.

However, benzothiadiazole derivative **4b** has been synthesized via a new route shown in Scheme 1. Acetylation of 4-aminophenyl acetic acid **7** with acetyl chloride, nitration of the acetamido intermediate and esterification with saturated hydrochloric acid in refluxing ethanol afforded nitro ester **8** in good overall yield. Compound **8** was then hydrogenated over palladium on charcoal and the resulting diamine was treated with thionyl chloride to give the benzothiadiazole **9** in high yield. Condensation of **9** with the requisite 2-bromo-4′-methoxyacetophenone provided γ-keto ester **10** in 75% yield. The resulting keto ester **10** was reacted with trimethoxybenzaldehyde and sodium methoxide in hot methanol to give the cyclized benzothiadiazole butenolide **4b** in excellent yield. With sodium hydroxide as a base butenolide **4b** can be converted to the open chain keto acid form EMD 122801 which appears as a stable and water soluble salt.

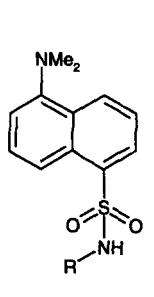
Scheme 1 Synthesis of EMD 122801



a. Ac_2O; b. HNO_3 / AcOH; c. EtOH / HCl (68% for 3 Steps); d. H_2, Pd/C, EtOH (90%); e. $SOCl_2$, DMF (cat.) (75%); f. 2-Bromo-4′-methoxyacetophenone, KO*t*Bu, NMP (75%); g. Na / MeOH, 3,4,5-Trimethoxybenzaldehyde, AcOH (86%); h. 1N NaOH, MeOH (100%).

Biological results and discussion:

Table 1 Biological data of benzodioxole and benzothiadiazole compounds



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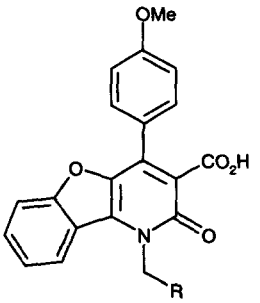

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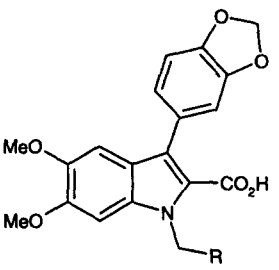

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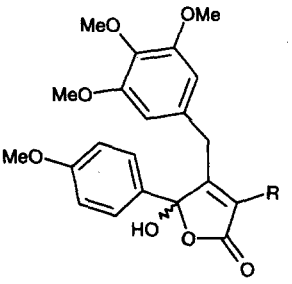
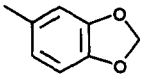
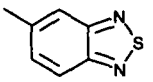
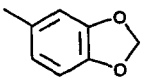
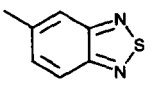
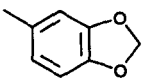
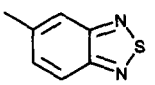
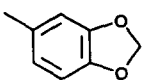
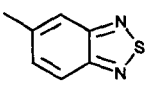


4

Cpd.	R	ET_A [binding, IC_{50} (nM)]	ET_B [binding, IC_{50} (nM)]
1a		370.0	>10,000.0
1b[1]		22.0	>10,000.0
2a		1,300.0	440.0
2b		390.0	300.0
3a		150.0	1,200.0
3b		33.0	1,600.0
4a[2]		0.44	300.0
4b[3]		0.30	340.0

Functional ET_A antagonism: [1] EMD 94246 (potassium salt of **1b**) $pA_2 = 7.5$. [2] PD156707 (sodium salt of **4a**) $pA_2 = 7.6$. [3] EMD 122801 (sodium salt of **4b**) $pA_2 = 8.5$.

The compounds were screened for their ability to inhibit specific [^{125}I]-ET-1 binding to rat aorta membranes (ET_A) and porcine kidney (inner medulla) membranes (ET_B).[12] *In vitro* functional ET_A antagonism was determined by generating an ET-1 concentration-response curve in isolated rat aortic rings without endothelium.[13] The receptor binding affinities of compounds **1** - **6** as well as functional ET_A antagonism of selected compounds are summarized in table 1 or 2, respectively.

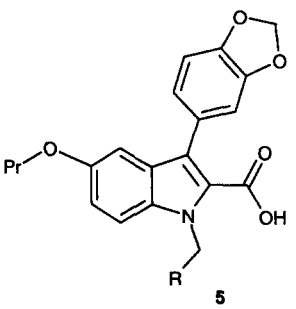
At the outset of our studies we discovered 5-(4-aminobenzenesulfonamido)-2,1,3-benzothiadiazole as a lead structure independently from the Kohonen map approach. This derivative was identified by screening of our compound library. SAR studies of such sulfonamides revealed EMD 94246 (potassium salt of **1b**) which is currently under preclinical development as an ET_A selective endothelin antagonist. In order to verify our hypothesis concerning bioisosterism methylendioxyphenyl analogue **1a** was synthesized. This compound was an order of magnitude less potent in ET_A binding affinity in comparison with **1b**. It is well known that sulfonamide antagonists such as BMS-182874 and Bosentan are weak acids due to their arylsulfonamide functional groups.[14] In our case it can be argued that the benzothiadiazole has a more pronounced electron-withdrawing character than methylendioxyphenyl which is able to contribute to the differences in binding affinity.

In search of non-selective antagonists we discovered benzofuro[3,2-b]pyridine-3-carboxylic acid and the more potent 2-carboxyindole core structures which are substituted by different aryl groups in 1, 4 or 1, 3 position, respectively. To further verify our bioisosterism hypothesis we introduced a 1,3-benzodioxol-5-ylmethyl or a 2,1,3-benzothiadiazole-5-ylmethyl in position one of these heterocycles. In both cases the benzothiadiazole analogues **2b** and **3b** exhibited improved ET_A binding affinities compared to methylendioxyphenyl derivatives **2a** and **3a**, respectively. However, both pairs displayed no significant difference concerning ET_B binding affinity.

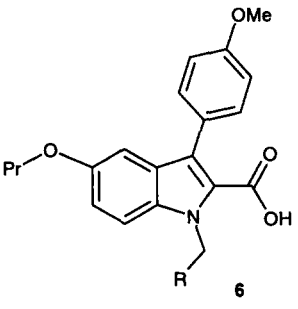
To firmly establish our approach, we focused on the potent and easy to synthesize reference compound PD156707 (sodium salt of **4a**), an orally active, highly ET_A selective antagonist[2] and its benzothiadiazole analogue **4b**. Both compounds **4a** and **4b** showed subnanomolar ET_A binding affinities together with low micromolar affinities for the ET_B receptor. However, benzothiadiazole EMD 122801 (sodium salt of **4b**) displayed clearly superior functional activity compared to PD156707 with a pA_2 value of about one order of magnitude higher (8.5 *versus* 7.6).

In the preceding paper it was explained by means of the Kohonen map approach that a benzofurazan or a triazolo[4,5-b]pyridine was less similar to the benzodioxole group.[8] To further manifest our bioisosterism hypothesis, a series of indole derivatives **5a** - **5c**, **6a** and **6b** was synthesized which contain either a benzodioxole, a benzothiadiazole or a benzofurazan moiety. As expected from our earlier studies in the indole series (*cf* compound **3a** and **3b**) benzodioxole **5a** and benzothiadiazole **5b** showed comparable IC_{50} values at the ET_A as well as the ET_B receptor. In contrast to benzothiadiazole **5b** benzofurazan **5c** demonstrated diminished affinity for both the ET_A and ET_B receptor. The small exchange, replacement of a sulfur atom through an oxygen, caused a significant alteration in binding affinity. Replacement of the benzodioxole proup in position three of the indole nucleus (compound **5b**) by a 4-methoxyphenyl (compound **6a**) has no negative effect on both receptor subtypes. However, introducing an additional methyl group in position six of the benzothiadiazole subunit (compound **6b**) enhanced the binding affinity to the ET_B receptor significantly.

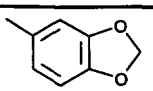
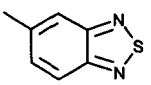
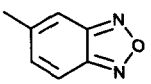
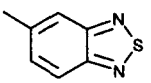
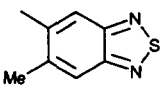
Table 2 Biological data of benzodioxole, benzothiadiazole and benzofurazan compounds



5



6

Cpd.	R	ET_A [binding, IC_{50} (nM)]	ET_B [binding, IC_{50} (nM)]
5a		250.0	1,600.0
5b		97.0	1,600.0
5c		2,500.0	5,300.0
6a		210.0	1,200.0
6b		160.0	330.0

In conclusion, we confirmed our hypothesis that a benzothiadiazole group might be a bioisoster of methylendioxyphenyl in the field of endothelin receptor antagonists. In addition, we discovered with EMD 122801 a compound which might prove useful for evaluation of any benefit of ET_A antagonism in endothelin related diseases.

Acknowledgement.

We would like to thank Gabriele Czmok, Herbert Glas, Dieter Kux, Corinna Rettig and Angela Rittersberger for preparing the compounds mentioned in this communication and Christina Heiner, Patric Kriesch, Gabriele Mahr, Rolf Löffler and Lydia Schulze for determining the biological data.

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