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1. ENDOTHELIN ANTAGONISTS: SEARCH FOR SURROGATES OF METHYLENDIOXYPHENYL BY MEANS OF A KOHONEN NEURAL NETWORK

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Abstract: The methylendioxyphenyl group, present in a number of potent endothelin receptor antagonists, could have undesirable metabolic interactions with cytochrome P450 *in vivo*. Using a self-organizing neural network we analysed the features of molecular electrostatic potentials of several endothelin receptor ligands. A library of small "fragments and functional groups" together with their corresponding Kohonen maps was generated. By means of this Kohonen map library we discovered the benzothiadiazole group as a surrogate for methylendioxyphenyl.

Introduction:

The shape and the electronic properties such as the electrostatic potential strongly influence many chemical and biological features of a molecule. The intuitively appealing picture of a key fitting into a lock for describing substrate-receptor interactions emphasizes the role of shape in biological activity. In the last decade, the three-dimensional comparison of molecular electrostatic fields and volumes was successfully applied in many cases to reveal effective structure-activity relationships.[1] The application of neural networks in chemistry has increased dramatically in recent years. In the Kohonen neural network (KNN) the artificial neurons self-organize in an unsupervised learning process and thus can be used to generate topological feature maps.[2] It will be shown here that this potential can be utilized to analyze the surface properties of those three-dimensional objects responsible for biological activity of molecules. This characteristic of a Kohonen neural network has already been used for the mapping of molecular surface properties such as the molecular electrostatic potentials (MEP) into two dimensions.[3] The use of a Kohonen network in SAR and drug design is also given.[4,5] We applied this innovative method to the discovery of endothelin receptor antagonists. The endothelins (ET-1, ET-2 and ET-3) are a peptide family of potent endogenous vasoconstrictor and pressor agents.[6] The ETs exert their biological effects by interacting with at least two specific G-protein coupled receptors (ET_A and ET_B) which are distinguished by their relative affinities for these peptides.[7] The ET_A subtype, which is selective for ET-1 over ET-3, is mainly found in vascular smooth muscle tissues and mediates vasoconstriction while the non-selective ET_B receptor appears to mediate either vasodilation or vasoconstriction, depending upon the tissue type.[8] Regarding the potential pathophysiological role of ET in cardiovascular disease, there is good evidence that ET regulates vascular tone and blood pressure. Studies with endothelin receptor an

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tagonists in conditions associated with chronic vasoconstriction such as hypertension and heart failure, as well as in vasospastic disorders, such as subarachnoid haemorrhage and Raynaud's disease support the import-ance of endothelin in several diseases.[9] With some exceptions most of the potent non-peptide endothelin antagonists reported to date contain a methylendioxyphenyl group. It is well known, that cytochrome P450 is essential for phase I metabolism of foreign compounds. This type of metabolism may lead to drug-drug interactions or nonlinear pharmacokinetics. We were concerned with the propensity of benzodioxole-containing compounds to undergo cytochrome P450-mediated metabolism, wherein the substrate becomes irreversibly bound to the enzyme.[10] Therefore we sought to develop compounds devoid of this functionality.

Methods:

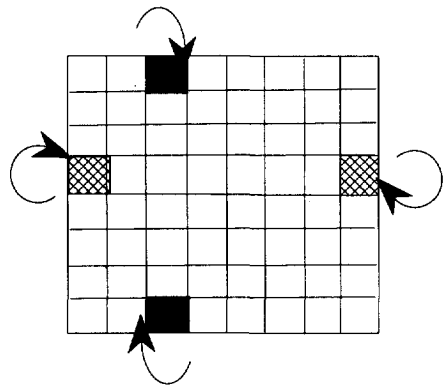
The structures were built from the connection tables using the program CORINA.[11] Charge distributions of fragments in a fragment library were calculated by the PM3 (MOPAC version 5.0). The molecular electrostatic potential (MEP) was obtained in a classical manner by moving a point charge on the molecular surface and calculating the potential according to the Coulomb's law from the partial atomic charges. A Kohonen net is trained by random-sampling of points on a van der Waals surface as input using the Kohonen neural network (KMAP) simulator.[12] Such a network can be used to map a molecular surface into a two-dimensional plane. For this mapping, points on the surface are chosen by random and their three cartesian coordinates are taken as input into a Kohonen neural network. With three input variables each neuron also has three weights. The neurons of such a Kohonen network are arranged in two-dimensions. In the projection of the molecular surfaces of the molecules studied here, an arrangement of 50 x 50 neurons was used. The surface of a molecule is continuous, without a beginning and end. Thus, it seemed desirable to also have an arrangement of neurons without beginning and without end. This can be achieved by arranging the neurons on the surface of a torus. For visualization, this torus is cut along two perpendicular lines and then the torus surface is spread into a plane. As shown in Figure 1, the neurons placed at the outskirts of the square are direct neighbours to each other. Since these cuts can be made at arbitrary lines, the maps can be shifted into any direction. Once the network has been trained, the entire dataset is sent through the network and each neuron is coloured with a property on the molecular surface that exists at that points that are mapped into the neuron considered. In our case, the trained neurons were then coloured according to the MEP values at these points.

Bioisosteric design:

The bioisosteric-database by Istvan Ujvary (BIOISOSTER version 1.3), a database for analogue design, including 1515 bioisosteric groups was analysed.

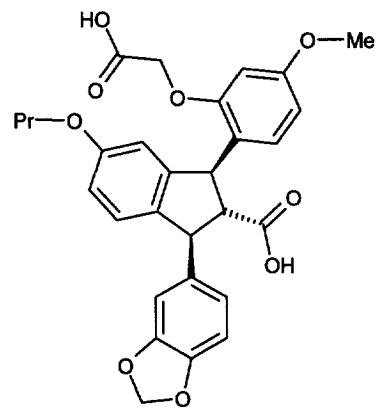
Several hundred pairs from this database were selected. The selection of these compounds was based on diverse structural fragment-pairs as far as possible. From the selected database the bioisosteric groups are then chosen. The 3D-structures of the selected fragments are calculated using the program CORINA. The MEP were calculated on the van der Waals surface. Thus, we calculated the Kohonen maps for each bioisosteric-groups with a unique colour plate. The calculated map-pairs show similarity on the electrostatic potential patterns. Thus, a coherency between the physicochemical properties and the bioisosteric effect can be deduced by looking at the calculated Kohonen maps of such groups.[13]

Figure 1 Plane obtained by making two cuts into a torus, which can be shifted into any direction. The two neurons marked by a cross and those shown as black squares are neighbours, respectively.



Several non-peptide ET_A selective antagonists as well as non-selective ET_A / ET_B antagonists described in the literature contain a methylendioxyphenyl group. This group is an important part of the interaction of the most potent non-peptide endothelin antagonists such as SB 209670 (Figure 2) with the endothelin receptors.[14]

Figure 2 Structure of SB 209670



As mentioned above it was desirable to find a bioisosteric moiety for this group. Therefore we calculated the electrostatic potential map of this group and compared it with all other calculated maps of the Kohonen map library. Such a comparison of maps of molecular surface properties offers a technique for the perception of similarities in ligands binding to the same receptor.

Figure 3 Comparison of the calculated Kohonen maps of five selected bioisosteric candidates of the methylendioxyphenyl group. A: methylendioxyphenyl, B: benzothiadiazole, C: triazolo[1,5-a]pyridine, D: imidazo[1,2-a]pyridine, E: benzofurazan and F: triazolo[4,5-b]pyridine.

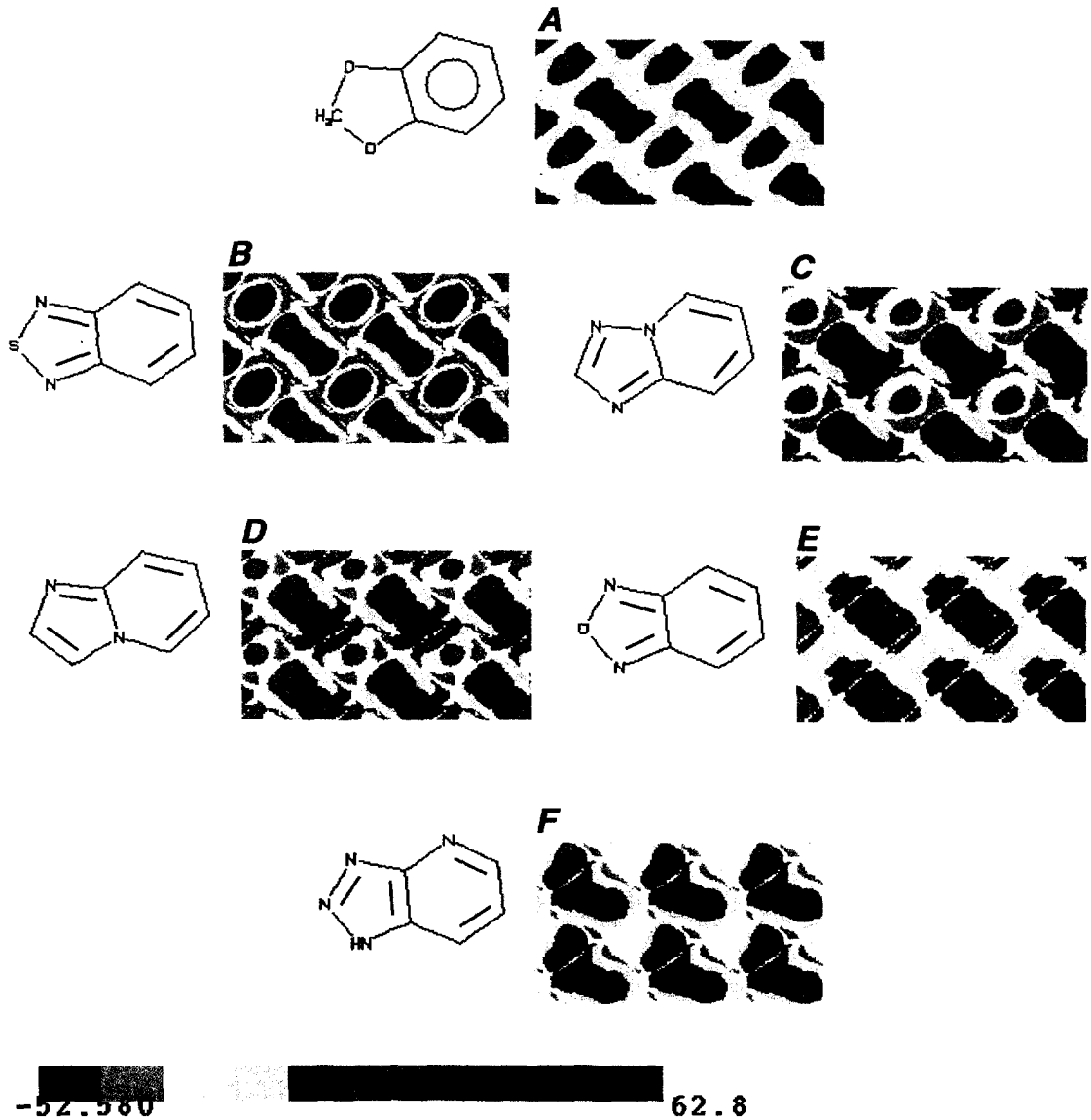


Figure 3 shows five fragments with their corresponding Kohonen maps, which are selected as bioisosteric candidates for the methylendioxyphenyl fragment and display similar map-patterns. The unique colour shows the range of the values of the electrostatic potential on the molecular surfaces. The red-yellow colour represents the most negative potential sites on the maps and the violet colour shows the positive potential sites. In order to identify the patterns of the maps for comparison without shifting the unit segment, the maps are replicated three times in horizontal and twice in vertical direction (2 x 3). The similarity of the MEP pattern on the maps represents the similarity of the MEP on the three-dimensional molecular surfaces. The colours on the maps show the pronunciation of the electrostatic potential interactions of the molecules with the binding site. Visual inspection of these maps clearly shows characteristics that are common to the map of methylendioxyphenyl group. In addition, the molecular electrostatic potential values on the surface of the benzothiadiazole are higher than from the reference molecule. Compared to methylendioxyphenyl, this group can cause stronger interactions with the receptor binding site. As can be seen in Figure 3, a small exchange from the fragment B to the fragment E (replacement of a sulfur atom through an oxygen) causes a significant alteration of the MEP patterns and colours in their maps. The MEP patterns of the most polar area in the map of benzothiadiazole is the most pronounced one whereas benzofurazan shows less similar pattern compared to the reference compound. Therefore we decided to investigate the benzothiadiazole as a first suitable bioisoster.

In conclusion, the MEP similarity to the methylendioxyphenyl map can be classified in the following order: benzothiadiazole (B) > triazolo[1,5-a]pyridine (C) > imidazo[1,2-a]pyridine (D) > benzofurazan (E) > triazolo[4,5-b]pyridine (F).

In this respect, a series of novel derivatives of endothelin antagonists with the benzothiadiazole as a bioisosteric group for the methylendioxyphenyl is developed. The synthesis and *in vitro* studies of such derivatives are reported in a subsequent article.

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