Computer-Aided Selection of Potential Antihypertensive Compounds with Dual Mechanism of Action

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The prediction of biological activity spectra for substances as an approach for searching compounds with complex mechanisms of action was studied. New compounds with dual mechanisms of antihypertensive action were found by this approach. Biological activity spectra for substances were predicted on the basis of their structural formulas by the computer program PASS. Thirty molecular mechanisms of action of compounds from the MDDR 99.2 database, which cause the antihypertensive effect and can be predicted by PASS, have been identified. The analysis of predictions for compounds with 15 dual antihypertensive mechanisms of action from the MDDR 99.2 database has confirmed high accuracy of prediction. This approach was applied to databases of commercially available compounds (AsInEx and ChemBridge) and allowed us to select four substances that are potential inhibitors of angiotensin converting enzyme (ACE) and of neutral endopeptidase (NEP). At a later time, all these compounds were found to be the inhibitors of both ACE and NEP. The most potent compounds had IC_{50} of 10^{-7}−10^{-9} M for ACE and 10^{-5} M for NEP. New combinations of dual mechanisms of action never before found for antihypertensive compounds were predicted.

Introduction

The discovery of new effective drugs for treatment of hypertension still remains a top priority. In practice, patients with hypertension are often prescribed several medicines. Each of these medicines acts on a single target. It may be expected that medicines acting simultaneously on different targets will allow physicians to treat patients more flexibly and to decrease the intake of medicines, hence decreasing the side effects.Since the mid-90s, antihypertensive compounds, which act simultaneously on several targets, have been the subject of intensive research, and this led to the discovery of several agents with different dual antihypertensive mechanisms of action. These agents include calcium channel and β_1 adrenergic blockers,[1] calcium channel and α_1 adrenergic blockers,[2] calcium channel blocker and 5HT 2A antagonist,[3] endothelin and angiotensin receptor antagonists,[4] endothelin converting enzyme (ECE) and angiotensin-converting enzyme (ACE) inhibitors,[5] and NEP and ECE inhibitors.[6,7] Dual inhibitors of angiotensin-converting enzyme and neutral endopeptidase have proved to be promising compounds for the treatment of cardiovascular diseases.[8,9] The clinical study of omapatrilat[10] and sampatrilat[11] shows that these dual ACE/NEP inhibitors are more potent in the treatment of hypertension and chronic heart failure than ACE inhibitor monotherapy like lisinopril.

Research and development of new pharmaceuticals acting on single molecular targets is time-consuming and expensive. Furthermore, the cost of experimental studies of pharmaceutical agents acting on several molecular targets increases multiplicatively. It is known that traditional QSAR and 3D molecular modeling for predicting biological activity of chemical substances operate with a small number of activities and is usually for the same chemical series.[12,13] These methods do not solve the problem. In contrast to these approaches, the computer program PASS[14−17] (prediction of activity spectra for substances) simultaneously predicts more than 700 types of biological activity (mechanisms of action, pharmacological effects, mutagenicity, carcinogenicity, teratogenicity and embryotoxicity) on the basis of the structural formulas of a substance. The availability of several hundred types of biological activity already predicted and the data on the relationships between mechanisms of action and pharmacological effects allowed us to select compounds that act on different molecular targets and cause the same pharmacological effect. The present investigation is aimed at evaluating (a) the potential of this approach in finding antihypertensive compounds with a complex mechanism of antihypertensive action and (b) the possibilities of revealing both existing and new complex mechanisms of action. The search for new dual inhibitors for the two proteolytic enzymes, ACE and NEP, is described here as an example of our approach for finding new compounds with a complex mechanism of action.

Angiotensin-converting enzyme (ACE, EC 3.4.15.1) is a key factor in the renin−angiotensin and the kallikrein−kinin systems, both of which are involved in the regulation of hemodynamics, saltwater homeostasis, and activities of major hormonal systems. The enzyme participates in blood pressure control by releasing the potent vasopressor peptide angiotensin II. In addition, the enzyme also inactivates the vasodilator peptide bradykinin.[18] ACE inhibitors constitute a class of highly effective antihypertensive drugs and are considered as first-line therapy for the treatment of hypertension and

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Table 1. Names of Molecular Mechanisms of Action Inducing the Antihypertensive Effect and Contained in Both the Activity Lists of PASS and MDDR 99.2

no.	number[a]	MPA,[b] %	types of activity from the PASS training set	types of activity from the MDDR 99.2 database
1	9	86.1	5 hydroxytryptamine 1B agonist	5 HT1B agonist
2	12	81.9	5 hydroxytryptamine 2A antagonist	5 HT2A antagonist
3	35	89.3	adenosine A1 receptor antagonist	adenosine (A1) antagonist
4	21	97.5	adenosine A2 receptor agonist	adenosine (A2) agonist
5	14	84.5	aldosterone antagonist	aldosterone antagonist
6	102	89.0	α1 adrenoreceptor antagonist	adrenergic (α1) blocker
7	55	78.0	α2 adrenoreceptor antagonist	adrenergic (α2) blocker
8	290	85.6	α adrenoreceptor antagonist	adrenergic (α) blocker
9	18	91.5	angiotensin AT2 receptor antagonist	angiotensin II AT2 antagonist
10	31	95.0	angiotensin AT1 receptor antagonist	angiotensin II AT1 antagonist
11	470	97.2	angiotensin II receptor antagonist	angiotensin II blocker
12	132	94.7	angiotensin converting enzyme inhibitor	ACE inhibitor
13	42	93.1	antidiuretic hormone antagonist	vasopressin antagonist
14	5	90.0	atrial natriuretic polypeptide agonist	atrial natriuretic polypeptide
15	22	92.9	β1 adrenoreceptor antagonist	adrenergic (β1) blocker
16	335	87.9	calcium channel antagonist	calcium channel blocker
17	24	94.3	dopamine D1 agonist	dopamine (D1) agonist
18	51	86.6	endothelin a receptor antagonist	endothelin ETA antagonist
19	27	85.0	endothelin B receptor antagonist	endothelin ETB antagonist
20	26	84.1	endothelin-converting enzyme inhibitor	endothelin formation inhibitor
21	157	90.7	endothelin receptor antagonist	endothelin antagonist
22	11	71.7	neuropeptide Y antagonist	neuropeptide Y antagonist
23	63	96.1	neutral endopeptidase inhibitor	neutral endopeptidase inhibitor
24	19	92.3	nitric oxide donor	nitric oxide donor
25	4	78.0	phosphodiesterase I inhibitor	phosphodiesterase I inhibitor
26	130	91.8	phosphodiesterase IV inhibitor	phosphodiesterase IV inhibitor
27	160	94.8	potassium channel activator	potassium channel activator
28	223	97.8	renin inhibitor	renin inhibitor
29	17	88.6	vasopressin 1 antagonist	vasopressin V1 antagonist
30	14	91.7	vasopressin 2 antagonist	vasopressin V2 antagonist

[a] Number is the number of compounds from the PASS training set exhibiting a particular activity type. [b] MPA is the minimal prediction accuracy (calculated by leave-one-out procedure) for every type of activity from the PASS training set.

congestive heart failure.[19] These inhibitors are also effective in the treatment of some other cardiovascular disorders including myocardial infarction and atherosclerosis.[20,21]

Neutral endopeptidase (NEP, EC 3.4.24.11) is a zinc metallopeptidase whose active site organization is similar to the active site organization of ACE and other zinc-containing enzymes.[22,23] The enzyme has a broad selectivity and can hydrolyze many short peptides such as atrial natriuretic peptide, bradykinin, angiotensins, enkephalins, and others. The inhibitors of NEP demonstrate a potent antihypertensive effect due to the inhibition of the degradation of atrial natriuretic peptide, whose major action is associated with increasing water and sodium excretion.[23−28]

Dual ACE/NEP inhibitors may become drugs for treatment of cardiovascular diseases in addition to existing inhibitors. Owing to the ACE inhibition, these dual inhibitors are potent antihypertensive agents resulting from a simultaneous decrease of the circulating levels of angiotensin II. The NEP inhibition increases the circulating level of atrial natriuretic peptide. The main hemodynamic consequence of this double inhibition is a decrease of peripheral vascular resistance and blood volume. The concurrent block of both systems (dual inhibition) is more effective than the isolated block of a single enzyme.

Experimental Section

PASS Method. PASS predicts biological activity spectra for druglike compounds on the basis of their structural formulas. The spectrum of biological activity of a compound is presented by a list of activity names and probability values for the appropriate activity to be either active (Pa) or inactive (Pi), respectively.[14−17] Activity names reflect the result of the interaction of a chemical substance with different biological entities. The total list of the PASS predicted activity types is presented on the Web site.[18] PASS uses MNA descriptors ("multilevel neighborhoods of atoms") for representation of a structural formula of compound.[29] Any compound is represented as a set of MNA descriptors. The calculation of the biological activity spectrum is based on structure−activity relationships that are stored in the SAR knowledge base. The training set of PASS (version 1.603) contains 45 466 substances, which are represented by 41 576 different MNA descriptors.

Biological Activities. The list of biological activity types predicted by PASS includes the names of pharmacological effects and molecular mechanisms of action of compounds. We selected the molecular mechanisms of action of compounds that cause antihypertensive effects and can be predicted by PASS and reported in the MDDR 99.2 database. The names of such molecular mechanisms of actions of antihypertensive compounds are given in Table 1. Altogether, there are 30 types of activity. The number of compounds in the PASS training set for these activities varies from 4 (phosphodiesterase I inhibitors) to 335 (calcium channel blockers). The accuracy of prediction calculated by the leave-one-out procedure is different at the activities from 71.7 (neuropeptide Y antagonist) to 97.8 (renin inhibitor). The mean accuracy of prediction of these activities is 89.3. It proves the validity of using PASS for prediction of these activities. These types of activity were used for searching compounds with dual mechanisms of action in the AsInEx and ChemBridge databases.

Databases. The MDDR 99.2 database[30] (MDL Information Systems, Inc.) was used for testing the ability of PASS to search for compounds with dual mechanisms of action. The MDDR 99.2 (MDL drug data report) database contains 98 184 unique chemical structures with information about their biological activity. Every compound in the MDDR database has one or several records in the field "activity class" indicating biological activity. The substances, whose biological activity was studied in detail, have a record in the field "Action", e.g.,

experimental data on activity, LD_{50}, IC_{50}, K_i, etc. Since the information in the MDDR database is mainly from patent sources, the biological activity of many compounds is not confirmed by experiments. Keeping this in mind, we used in our experiments only so-called principal compounds. These were certainly tested experimentally and have detailed descriptions in the field "Action". Thus, we have made a subset that includes 23 770 principal compounds from the MDDR database. The method used in PASS is statistically robust[14] to compensate some errors in the data.

The AsInEx 99.7 and ChemBridge (Express Pick 99.2) databases[31,32] of commercially available compounds were used for the search of potential antihypertensive compounds with dual molecular mechanisms of action. The databases contain 183 462 unique structures.

Inhibition of Purified Bovine Kidney Cortex ACE. ACE activity was measured by the fluorometric method using 50 μM *Z*-Phe-His-Leu ("Serva", Germany) as a substrate, in triplicate in 25 mM barbital buffer, pH 7.4, containing 200 mM NaCl in a final volume of 2.0 mL.[33,34] After a 15 min preincubation of the enzyme with compounds under investigation, the substrate was added to initiate the reaction. The ACE concentration for experiments was chosen to remain between 12% and 15% of substrate utilization. The concentrations of the tested compounds varied from 10^{-3} to 10^{-11} M depending on their inhibitor activity. The mixture was incubated for 30 min at 37 °C, and the reaction was stopped by addition of 0.4 mL of 2 N NaOH. Control samples (with buffer instead of compounds) were taken to have 100% enzyme activity. The product of the reaction (dipeptide His-Leu) was analyzed by fluorometric assay with o-phthaldialdehyde.

Inhibition of Human Serum and Rat Brain Membrane ACE. Experiments were performed[35] in 250 μL of 70 mM potassium phosphate buffer, pH 8.3, containing 300 mM NaCl, 20 μL of normal human serum (diluted 1:5) or brain membrane suspension, and compounds to be tested. The concentrations of the tested compounds varied from 10^{-3} to 10^{-8} M depending on their inhibitor activity. Membrane fraction of rat brain cortex was prepared utilizing the method of Belyayev et al.[36] After a 20 min preincubation of the enzyme with the compound at 37 °C, 10 μL (25 μM) of the substrate Hip-His-Leu (Sigma) was added. This reaction was stopped after a 120 min incubation by addition of 1.45 mL of 0.28 N NaOH and 1.0 mL of the same buffer. The cleaved His-Leu was detected by a fluorometric method with *o*-phthaldialdehyde and measured at 370 nm (excitation, Ex) and 500 nm (emission, Em). The activity of the control samples (without the compounds) was considered as 100% enzyme activity. IC_{50} values (in M) were determined. The concentrations of lisinopril varied from 10^{-5} to 10^{-11} M depending on its inhibitor activity.

Inhibition of Human Serum and Rat Brain Membrane NEP. The degree of inhibition was estimated by hydrolysis of the fluorogenic substrate Suc-Ala-Ala-Phe-MCA.[35,37,38] The incubation mixture (600 μL) contained 5 μL of human serum (diluted 1:5) or 20 μL of a suspension of rat brain membranes, 6 or 60 μL of inhibitor solution at six to eight different concentrations, and the corresponding amount of 50 mM Tris-HCl buffer, pH 7.5. The concentrations of the tested compounds varied from 10^{-2} to 10^{-6} M depending on their inhibitor activity. Preincubation in the presence or absence (control) of inhibitors at 37 °C for 20 min was carried out, then 10 μL of substrate solution was added (final concentration of 20 μM), and the mixture was incubated at 37 °C for 120−180 min. The reaction was stopped by addition of 20 mM sodium acetate buffer, pH 4.0. The liberated 4-methylcoumarinyl-7-amine was measured at 383 nm (Ex) and 455 nm (Em). The activity of the control samples was considered as 100%, and the percentage of inhibition was calculated. IC_{50} values (in M) were determined. The concentrations of phosphoramidon varied from 10^{-5} to 10^{-8} M depending on its inhibitor activity.

Results and Discussion

Testing of the Approach on the MDDR 99.2 Database. The database was analyzed to identify the compounds with dual mechanisms of antihypertensive action. It appears that the MDDR 99.2 database contains 516 compounds with 15 different dual mechanisms of antihypertensive action from 435 theoretically possible combinations ($30 \times (30 - 1)/2$). PASS was used to predict biological activity spectra for 23 770 principal compounds by ordinary PC (Pentium 4, 1500 GHz). The results of a 10 min prediction were analyzed to identify the probable compounds with dual mechanism of action. The dual mechanism of action is considered to be correctly predicted for a given compound if both mechanisms of action are presented in the prediction and in the description of the compound in the MDDR 99.2 database. The compounds with the maximum probability of dual mechanisms of action were selected. The results of the analysis of these data are presented in Figure 1. The number of different complex mechanisms of action at the set with different cutting points is displayed.

It can be seen that 422 different types of complex mechanisms of action with Pa > Pi including "all known" mechanisms (15) were predicted for compounds from the MDDR 99.2 database. When the cutting point is increased, both the "known" and the "general" number of the predicted complex mechanisms of action are decreased. However, the number of the predicted compounds with complex mechanisms of action decreases more dramatically. This happens because an increase of the cutting point leads to a decrease of type I error and an increase of type II error of the prediction. Thus, with Pa > 40%, it was found that there are compounds for only 60 dual mechanisms of action including 12 of which are known. One may decide which of the dual mechanisms of action is to be selected for testing: the one with high probability or the one with moderate probability. In the former case, one may be sure that most of the selected dual mechanisms of action do exist, but their number is much less than the number of actually known dual mechanisms of action. For instance, if we use Pa > 80% as a cutoff point for selection, we select four complex mechanisms of action and all of them are known. In the latter case, we were able to find a larger number of complex mechanisms of action than actually exist and some complex mechanisms that are unknown. Nevertheless, we get a chance to discover compounds with a combination of mechanisms of action, which are still unknown. For instance, if we use Pa > 60% as a threshold, we select 11 dual mechanisms of action including 7 known and 4 unknown. Interesting combinations are found among the selected unknown complex mechanisms of action, e.g., those involving calcium channel blocker AND endothelin receptor antagonist (56 compounds) or antagonism to α1 adrenoreceptors AND endothelin A receptors (1 compound).

There is a relationship between endothelin receptors and calcium channels. Activation of endothelin receptors causes contraction of vascular smooth muscle. It does so by increasing intracellular calcium concentration via calcium channels and through stimulation of phospholipase C and the formation of inositol trisphosphate (IP) and diacylglycerol (DAG).[39] The formation of IP and DAG results in the activation of both voltage-dependent and -independent Ca^{2+} channels and in the release of calcium from endoplasmic reticulum. Therefore, block-

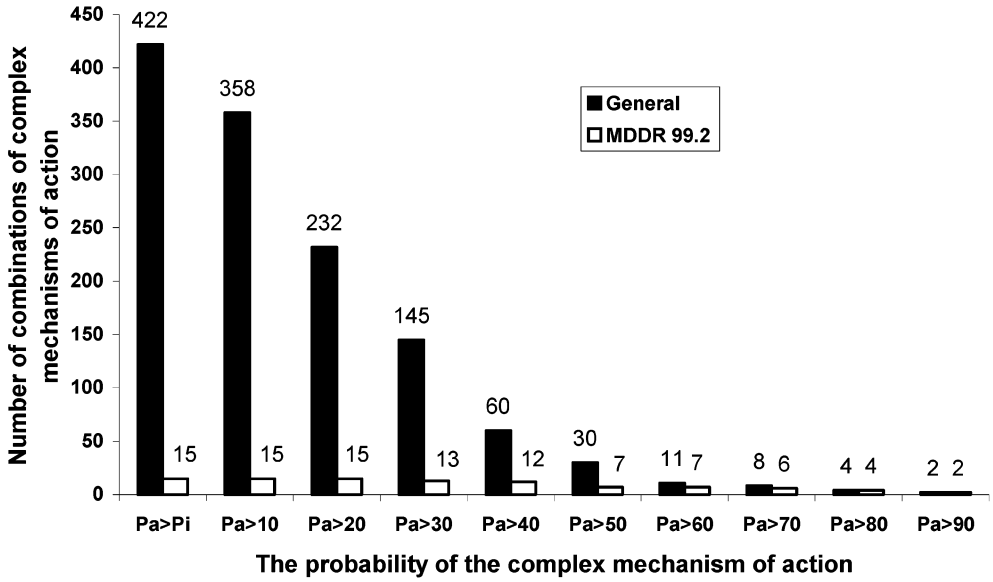


Figure 1. Dependence of the number of predicted complex mechanisms of action on the probability of the complex mechanism of action. The black bars are the number of all combinations of the mechanisms of action with the corresponding values of the prediction probabilities. The white bars are the numbers of the complex mechanism of action mentioned in MDDR 99.2 and predicted by PASS.

ing of both calcium channels and endothelin receptors may be more effective than their separate blocking. Action on α 1 adrenoreceptors causes contraction of vascular smooth muscle, such as the action on endothelin receptors via stimulation of phospholipase C and formation of IP and DAG.[40] Thus, it is possible that α 1 adreno-/endothelin A receptor antagonists stimulate phospholipase C and formation of IP and DAG in two different ways, and hence, blocking both types of receptors may be more effective than blocking only one of them.

Identification of possible molecular targets for a new medicine with dual mechanisms of action is important at the first step of R&D of new pharmaceutical agents.

Search for Compounds with Dual Mechanisms of Action in Databases of the Available Chemical Samples. In the previous study, we have shown that compounds from several databases of the available chemical samples demonstrate the complex mechanisms of antihypertensive action like those from the MDDR 99.2 database.[41] In this investigation, the AsInEx 99.7 and ChemBridge (Express Pick 99.2) databases[31,32] including the data on 183 462 chemical compounds were used. It takes about 2 h to predict the biological activity spectra for these compounds by ordinary PC (Pentium 4, 1500 GHz). We have found about 60 compounds with high probability of different complex mechanisms of action[41] based on the results the PASS method prediction, including compounds with unknown complex mechanisms of action, e.g., antagonism to α1 adrenoreceptors AND endothelin A receptors (two compounds, Pa > 40%), or calcium channel blocker AND endothelin receptor antagonist (15 compounds, Pa > 40%).

ACE and NEP Inhibitors. We have analyzed the prospects of prediction of dual ACE/NEP inhibitors from the MDDR 99.2 database. ACE and NEP were selected as a case study because of the key role of these enzymes in blood pressure regulation. ACE catalyzes the conversion of angiotensin I to angiotensin II, which binds to angiotensin receptors and causes a contraction of blood vessels. Inhibition of ACE lowers the angiotensin II concentration in blood and tissue, thereby reducing the contraction of the blood vessels. Moreover, ACE is involved in the degradation of bradykinin, which causes vasodilatation. Degradation of atrial natriuretic peptide (ANP) is one of the NEP functions.[42] The release of ANP occurs in response to the increase of central venous pressure at coronary deficiency. The main effects of ANP are decrease of water and sodium excretion, dilatation of smooth muscle of venous vessels, and inhibition of release and action of aldosterone, angiotensin II, endothelin, and antidiuretic hormones.[43,44] Thus, the substance inhibits both NEP and ACE, causing a more potent therapeutical effect upon hypertension treatment in comparison with the action of single ACE or NEP inhibitors.

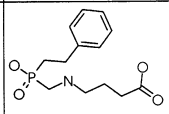
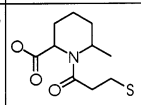
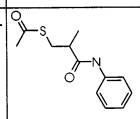
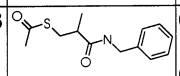
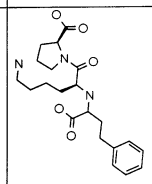
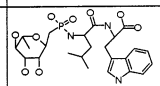
To study the PASS potential for finding ACE/NEP inhibitors, we compared the results of ACE/NEP inhibi-

Table 2. Comparison of the PASS-Predicted ACE/NEP Inhibitory Activity with the MDDR-Reported Experimental Data on the Principal Compounds

P_a threshold,[a] %	predicted as active[b]	predicted as inactive[c]	predicted at all[d]	predictivity,[e] %	enrichment,[f] %
Pa > Pi	56	856	912	6.14	25.58
Pa > 10	53	377	430	12.33	51.36
Pa > 20	46	136	182	25.27	105.31
Pa > 30	41	41	82	50.00	208.33
Pa > 40	32	20	52	61.54	256.41
Pa > 50	28	10	38	73.68	307.02
Pa > 60	25	6	31	80.65	336.02
Pa > 70	16	4	20	80.00	333.33
Pa > 80	11	2	13	84.62	352.56
Pa > 90	5	1	6	83.33	347.22

[a] Allowed range [$P_{a\ cutoff}$, 1.0] of predicted probability. [b] Compounds predicted as active for the principal compounds and found to be ACE/NEP inhibitors. [c] Compounds predicted as active for the principal compounds and not found to be ACE/NEP inhibitors. [d] Compounds predicted as active for the principal compounds (sum of columns 3 and 4). [e] Ratio (in percent) of column 2 to column 4. [f] Ratio of column 5 to percentage of actives ($^{predictivity}/_{0.24}$).

Table 3. Prediction and Experimental Results for Four Selected Compounds from ChemBridge Database[a]

No	ID	Structure	Prediction	Experiment				
				IC_{50} ACE (M)			IC_{50} NEP (M)	
			Pa Pi Activity	purified ACE	blood serum	brain membr.	blood serum	brain membr.
I	587082		0.855 0.002 NEP inhibitor 0.805 0.003 ACE inhibitor	$1 \cdot 10^{-4}$ * 18%	$1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	-	$2 \cdot 10^{-3}$
II	219863		0.440 0.005 NEP inhibitor 0.773 0.003 ACE inhibitor	$1 \cdot 10^{-9}$	$2 \cdot 10^{-8}$	$1 \cdot 10^{-7}$	-	$2 \cdot 10^{-3}$
III	119354		0.684 0.003 NEP inhibitor 0.569 0.004 ACE inhibitor	$3 \cdot 10^{-7}$	$2 \cdot 10^{-6}$	$1 \cdot 10^{-6}$	$2 \cdot 10^{-5}$	$2 \cdot 10^{-4}$
IV	119363		0.618 0.004 NEP inhibitor 0.511 0.005 ACE inhibitor	$5 \cdot 10^{-4}$	$2 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$2 \cdot 10^{-5}$	$2 \cdot 10^{-4}$
Lisinopril			0.115 0.012 NEP inhibitor 0.607 0.004 ACE inhibitor		10^{-10}			
Phosphora -midon			0.203 0.006 NEP inhibitor 0.151 0.017 ACE inhibitor				10^{-7}	$2 \cdot 10^{-7}$

[a] ID: identifier of compounds in the database. Sources of the enzyme: blood serum, human blood serum; brain membr., rat cortex brain membranes; purified ACE, highly purified bovine kidney ACE. The asterisk (*) is the % inhibition ACE by 1×10^{-4} M compound **I**.

tor activity prediction for the set of principal compounds with the description of their biological activity in the MDDR 99.2 database. There are 56 ACE/NEP inhibitors in the set. In order to discover all these inhibitors by experimental screening, each of the 23 770 compounds should be tested. The percentage of actives in the subset of principal compounds is about 0.24% ($^{56}/_{23714}$). This ratio may be considered to be preserved in a random selection for active compounds in databases of pharmacological agents. Table 2 displays the size of compound sets with the PASS prediction of ACE/NEP inhibitor activity for the principal compounds and their intersections with the active and inactive compounds as a function of the lower boundary of the probability interval [$P_{a\ cutoff}$, 1.0]. The predictivity ratio was calculated as

$$\frac{\text{size of } \{\{\text{predicted}\} \cap \{\text{actives}\}\}}{\text{size of } \{\{\text{predicted}\} \cap \{\{\text{actives}\} + \{\text{inactives}\}\}\}} \times 100$$

indicating that a very substantial enrichment is achieved even at the very lowest P_a threshold values. At the highest P_a threshold values, the predictivity ratio approaches 83%; i.e., the enrichment (predictivity/random ratio) reaches a value of 347.2 that is close to the theoretical maximum ($^{100}/_{0.24} = 416.7$).

We selected four compounds from the ChemBridge database (Table 3) with a high estimated probability of revealing the inhibitor activity toward the angiotensin-converting enzyme (EC 3.4.15.1) and toward the neutral endopetidase (EC 3.4.24.11). These compounds were tested for their interaction with the two enzymes. Experimental studies show that all selected compounds are inhibitors of ACE and NEP. The inhibitory effect was estimated by a 50% decrease of the initial enzyme activity (IC_{50}) (Table 3). Inhibitory capacity for these compounds was investigated using the highly purified ACE from bovine kidney (bk), human blood serum (hbs), and rat brain membranes (rbm). The IC_{50} values of the tested compounds with respect to ACE are the follow-

ing: 1×10^{-4} M (bk, IC_{18}), 1×10^{-4} M (hbs), 1×10^{-4} M (rbm) for compound **I**; 1×10^{-9} M (bk), 2×10^{-8} M (hbs), 1×10^{-7}M (rbm) for compound **II**; 3×10^{-7} M (bk), 2×10^{-6} M (hbs), 1×10^{-6} M (rbm) for compound **III**; 5×10^{-9} M (bk), 2×10^{-4} M (hbs), 1×10^{-4}M (rbm) for compound **IV**. The NEP used in the experimental testing was extracted from human blood serum and rat brain membranes. The IC_{50} values of the tested compounds with respect to NEP are the following: 2×10^{-3} M (rbm) for compounds **I** and **II**; 2×10^{-5} M (hbs) and 2×10^{-4} M (rbm) for compounds **III** and **IV**.

The most potent inhibitory effects for ACE from bovine kidney, human serum, and rat brain membranes were exhibited by compound **II** (with $IC_{50} = 10^{-9}$, 2×10^{-8}, and 2×10^{-7} M, respectively) and by compound **III** (with $IC_{50} = 3 \times 10^{-7}$, 2×10^{-6}, and 1×10^{-6} M, respectively). The most effective inhibitors for NEP were compounds **III** and **IV** with $IC_{50} = 2 \times 10^{-5}$ and 2×10^{-4} M for the human serum and the rat brain membrane enzymes, respectively (Table 3). The results obtained indicate that one of the four selected compounds, namely, compound **III**, exhibited the most potent dual inhibitory effect. Compound **I** has a higher probability value for inhibiting ACE and NEP. Nevertheless, the biological testing has shown that this compound has a weak inhibitory activity toward the enzymes studied. The possible explanation of this fact is that the calculated Pa value is not proportional to the potency of the compound. In fact, it is rather the probability of belonging to the class of "actives".

The inhibitory effects of the four selected compounds were compared with those of the reference ACE and NEP inhibitors—lisinopril and phosphoramidone.[22,45] It was shown that lisinopril inhibited the blood serum ACE ($IC_{50} = 10^{-10}$ M) comparably to compound **II** inhibiting the purified ACE (Table 3). Phosphoramidone was only able to inhibit NEP from human serum and rat membrane, with an IC_{50} of 10^{-7} M, i.e., 2−3 orders better than upon the action of compounds **III** and **IV** (Table 3). Nevertheless, the data obtained allow us to suggest that compound **III** or its structural analogues are prospective basic tools for the dual inhibition of ACE and NEP.

We have compared these compounds with the known ACE and NEP inhibitors from the MDDR 99.2 database. The similarity procedure of ISIS/Base 2.1.1[30] was used for the search of similar compounds. The search showed the following: for compound **I** (ID 587082), one NEP inhibitor with 60% similarity, three NEP inhibitors with 50% similarity, and two ACE/NEP inhibitors with 50% similarity; for compound **II** (ID 219863), one ACE inhibitor with 70% similarity, one ACE inhibitor with 60% similarity, eight ACE inhibitors, one NEP inhibitor, and one ACE/NEP inhibitor with 50% similarity; for compound **III** (ID 119354), three NEP inhibitors and one ACE/NEP inhibitor with 50% similarity; and last, for compound **IV** (ID 119363), one ACE/NEP inhibitor and two NEP inhibitors with 60% similarity, one ACE/NEP inhibitor and two NEP inhibitors with 50% similarity. It was assumed that compounds will likely exhibit biological activity if their similarity is above 70%. Thus, the discovered compounds are not close analogues of the known ACE/NEP inhibitors. By searching in the CAS registry database, we found that compound **II** is a metabolite of known ACE inhibitor methiopril[46] that corresponds to its 70% similarity with the known ACE inhibitors.

Conclusions

We demonstrated that biological activity spectra prediction can be used for the selection of compounds with dual mechanisms of antihypertensive action. This approach offers an ability to identify probable combinations of mechanisms of action for the antihypertensive effect. The approach was used for the search of new ACE/NEP inhibitors in the databases of available chemical samples. Four compounds with a higher probability of inhibiting ACE and NEP were selected on the basis of the data of the prediction by PASS. Compounds predicted as dual inhibitors for ACE and NEP were tested for their interaction with the two enzymes. The experimental tests have confirmed that all these compounds are inhibitors of both ACE and NEP. The most effective compounds have $IC_{50} = 10^{-7}-10^{-9}$ M for ACE (**II** and **III**) and 10^{-5} M for NEP (**III** and **IV**). Compound **III** can be considered as a prospective basic tool for the dual inhibition of ACE and NEP.

It appears that there is a high probability of finding compounds with new combinations of mechanisms of antihypertensive action such as antagonism to α1 adrenoreceptors and endothelin A receptors or calcium channel blocker and endothelin A receptor antagonist. Owing to the speed in predicting biological activity spectra and diversity of predicted biological activities, a similar approach can be applied not only in the search of new medicines with dual mechanisms of antihypertensive action but also for medicines used for treating other diseases with complex mechanisms of regulation, such as neoplastic growth, viral and bacterial infections, and others.

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