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A theoretical study of angiotensin-converting enzyme inhibitors

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SUMMARY

INDO molecular orbital calculations are reported for 35 selected angiotensin-converting enzyme inhibitors. QSARs are developed between pI_{50} data and molecular electronic indices. The QSARs obtained reflect the importance of both charge–charge interactions between inhibitor and receptor and of specific interactions between groups on the inhibitor with points around the molecule which are postulated to correspond to binding sites at the receptor.

INTRODUCTION

Angiotensin-converting enzyme (ACE) is a zinc-containing peptidase which hydrolytically cleaves a carboxy terminal dipeptide from a wide range of peptide substrates. By hydrolysing angiotensin I to the pressor peptide angiotensin II, and by breaking down the potent vasodilator bradykinin, ACE plays a key role in the renin-angiotensin system controlling blood pressure. It is known that hypertensive disease may be controlled by inhibition of ACE, and consequently a wide variety of compounds have been synthesised and tested.

In order to produce more effective ACE inhibitors a detailed description of the active site of ACE would be useful. The first orally active ACE inhibitor to be used clinically was captopril [1], and a large number of similar compounds have been investigated by means of in vitro testing with a view to providing a reasonable description of the ACE active site.

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The main aim of the present work is to attempt to quantify some of the known structure-activity relationships of ACE inhibitors in terms of molecular electronic structure, the electronic indices being obtained from the INDO [2] molecular orbital procedure.

In general, ACE inhibitors appear to contain one or more of the following molecular functionalities: a zinc binding ligand, a hydrogen bond acceptor, and a carboxy terminal group. As shown in Table 1, the inhibitors chosen in the current study include some which lack one or more of these vital groupings. The reason for this is that if only compounds containing all of these groups were chosen then the resulting regression equations would be biased and the importance of the interactions of these groups would be included in the constant term.

A suitable model receptor for predictive purposes should give a quantitative account of all the important enzyme-inhibitor interactions rather than attempt to explain away small potency differences between closely related members of a known active series.

As shown in Table 1 the compounds chosen for study exhibit a range of pI_{50} values of about 5.3 log units. As pI_{50} is defined as $-\log_{10} IC_{50}$ where IC_{50} is the molar concentration required to give 50% inhibition of the rabbit lung enzyme under standard conditions, this represents a range of about 2×10^5 for the potencies. It was felt that this was a sufficiently broad spread to allow all of the relevant interactions to be considered in the regression equations and provide a reasonable basis for distinguishing between active and inactive compounds.

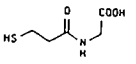
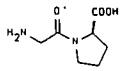
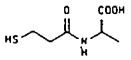
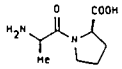
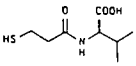
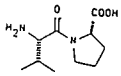
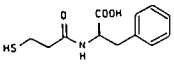
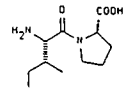
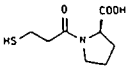
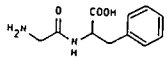
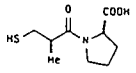
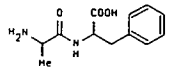
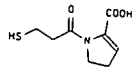
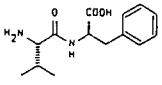
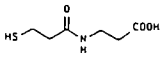
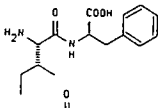
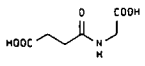
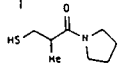
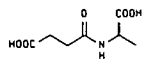
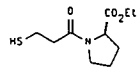
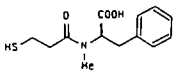
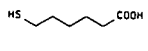
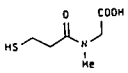
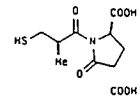
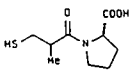
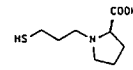
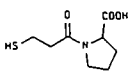
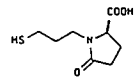
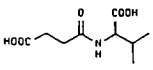
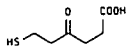
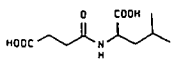
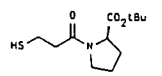
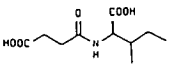
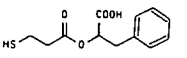
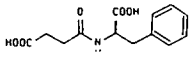
The compounds in Table 1 include some imino-acid derivatives which are capable of exhibiting cis-trans isomerism. NMR studies suggest that the conformers are of roughly equal stability such that a given solution contains equal amounts of each. Since it is unlikely that both conformers are active, the effect of the conformational equilibrium is to effectively reduce the inhibitor concentration by one half. Thus the pI_{50} data given in Table 1 for compounds capable of exhibiting such conformational equilibria were adjusted to take account of this before being employed in the regression equations. Otherwise, pI_{50} values were unchanged from those obtained from the references in Table 1.

METHODS

In order to derive QSARs for the 35 selected inhibitors in Table 1, a modified version of QCPE 261 was employed [8] together with an implicit model of the receptor. This model receptor site was obtained from a consideration of the conformational properties of a model substrate of the enzyme and analogy with the well-characterised crystal structure of the closely related zinc-containing peptidase carboxypeptidase A [9]. The global minimum energy conformation of the model substrate acetyl-L-Ala-L-Pro was found by means of molecular mechanics. The binding groups were placed around this conformation in analogous positions to those occupied by Arg-145 and the zinc atom in the Gly-Tyr complex of carboxypeptidase A, and in a position suitable for hydrogen bonding to the carbonyl of the alanyl residue.

The starting point for consideration of the ACE binding reaction is taken to be the global minimum energy conformation of the inhibitor obtained from molecular mechanics procedures. In all cases, an initial INDO calculation is performed on the molecule to obtain a set of charges for inclusion in the geometry optimisation. In all cases the sulphydryl and carboxyl groups are considered to be ionised: thus calculations are performed variously on mono- or di-anions as necessary.

TABLE I
SELECTED INHIBITORS

Compound		Activity (pI_{50})	Ref.	Compound		Activity (pI_{50})	Ref.
1		5.5528	4	19		3.3468	5
2		6.0706	4	20		3.6383	5
3		5.7959	4	21		3.3768	5
4		6.3665	4	22		3.8861	5
5		6.6990	4	23		3.2207	5
6		7.6383	4	24		3.7212	5
7		6.1871	1	25		4.2757	5
8		3.3098	4	26		3.0315	5
9		2.7011	4	27		3.6198	6
10		2.8729	4	28		4.7696	7
11		4.5086	3	29		2.9586	3
12		5.5229	1	30		8.0458	3
13		4.9586	4	31		3.6198	3
14		2.7447	1	32		3.1938	3
15		2.9586	4	33		5.6198	3
16		3.2147	4	34		4.4089	3
17		2.9830	4	35		6.1549	3
18		3.2596	4				

The bound conformation of the inhibitor is taken to be that in which the three essential groupings of the inhibitor are able to interact as effectively as possible with the constituents of the postulated binding site, shown in Fig. 1 for captopril. This conformation is obtained by twisting the inhibitor about its single bonds to give a suitable conformation. No changes were made in bond lengths or bond angles.

For simplicity of calculation, the implicit receptor site model is represented by four points around the molecule, three of them situated at the postulated positions of the three principal binding sites and one some distance beyond the carboxy terminus which is considered to be a control point.

The axis system used, of relevance for the interpretation of calculated vector properties, is illustrated in Fig. 2 for captopril, and the cartesian coordinates of the four points which constitute the model receptor in this axis system are given in Table 2.

An estimate of the energy change involved in twisting the molecule from its global minimum into the postulated bound conformation is obtained from INDO calculations on the unbound and bound structures.

67 indices (51 independent and 16 derived quantities) were recorded for each molecule. These were predominantly electronic indices calculated from the INDO wave functions of the inhibitors, but also included the mean molecular van der Waals radius, and the van der Waals surface area and volume (calculated empirically), and the number of freely rotatable bonds (supplied manually), as it was felt that one or more of these empirical quantities might be of use in representing the entropy changes taking place on binding of inhibitor to enzyme. We have calculated the linear regressions of pI_{50} on each of the recorded variables, on each possible pair of variables, and on each possible set of three variables, the variance ratio F being used to determine the level of significance of the various regressions. We have also calculated regressions of pI_{50} on certain sets of more than three variables.

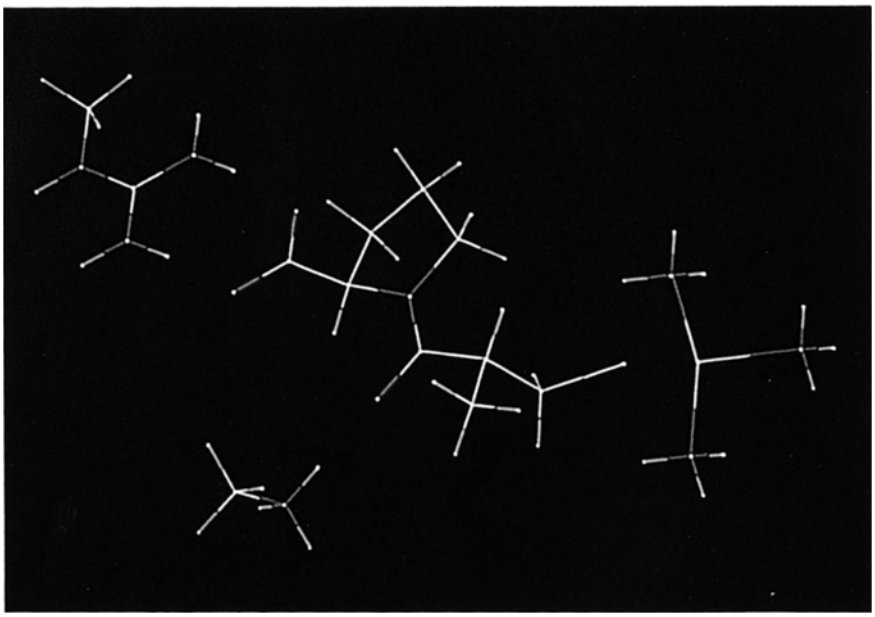


Fig. 1. Diagram of captopril in postulated receptor site.

TABLE 2
POSTULATED 'RECEPTOR POINTS'

Point	Description	Coordinates		
1	Zinc site	4.629,	−4.483,	−0.903
2	Carboxyl binding site	0.277,	3.399,	−3.307
3	H-bonding site	−1.800,	0.000,	0.000
4	'Control'	2.500,	7.000,	0.000

RESULTS AND DISCUSSION

Since ACE inhibition is thought to involve interaction with at least three binding sites, it is unlikely that any single or two variable regressions would give very good r values. In all cases for such regressions, the r values are calculated to be less than 0.73. Thus one and two variable regressions are given no serious consideration in the present analysis. Appendix 1 gives the 10 best three variable regressions, and the occurrence of variables is summarised in column 1 of Table 3.

Perhaps the most striking feature of the three variable regression equations is the frequency of occurrence of μ_z. This quantity is the z component of the calculated INDO dipole molecule for the inhibitor, but it should be borne in mind that the INDO calculations were performed on charged species, and in this circumstance, the calculated dipole moment is dependent both on the choice of axes and on the total charge on the system. Thus μ_z can be thought of as having a major contribution from the z displacement of the molecular centre of charge and the magnitude of that charge, and a minor contribution from the molecular dipole moment. The quantity is thus largely a reflection of the importance of the electrostatic interaction between the binding site and the inhibitor rather than of any dipole–dipole interaction.

The uncertain nature of the dipole moment for charged species makes it a candidate for exclusion from the regression equations if we wish to interpret the interactions of the inhibitors with the model receptor site in a very specific fashion. It would also be hoped that other properties calculated around the molecule (electrostatic potentials, electric field gradients and second order energies of polarisation) should prove capable of reflecting the interactions which are represented by the occurrence of dipole moment in the regressions. This is indeed seen to be the case, and exclu-

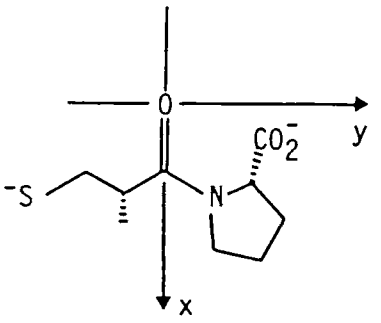


Fig. 2. Axis system used to define the receptor site.

TABLE 3
FREQUENCY OF OCCURRENCE OF VARIABLES IN REGRESSIONS WITH THREE, FOUR OR FIVE VARIABLES

Variable	Occurrences in Appendix			Variable	Occurrences in Appendix		
	1	2	3		1	2	3
$V_1^2(2)$	2	9	10	$E_x(4)$	0	0	1
$V_2(3)$	3	5	10	$E^2(4)$	0	0	1
$E_y(2)$	4	5	4	$V_2(4)$	0	0	1
$E_x(1)$	2	7	6	$E^2(3)$	0	2	0
E_{st}	1	1	4	α*ip	3	2	0
r	1	1	4	E(3)	0	1	0
$V_1(3)$	0	1	1	$V_1(2)$	0	1	0
$E_x(3)$	0	1	1	$E_y(3)$	0	1	0
$V_1^2(3)$	0	1	1	$E_z(2)$	0	1	0
n_{rot}	0	0	1	$E_x(3)$	2	1	0
$V_1^2(1)$	0	0	1	μ_z	8	0	0
$V_1(1)$	0	0	1	$E_x(1)$	2	0	0
$E_y(4)$	0	0	1	ε_{homo}	2	0	0

sion of regressions involving dipole moment indices still allows many three variable regressions which are highly significant to be obtained.

Selecting the best three variable regressions from this subset of the data, it is possible to proceed in a stepwise fashion to equations with greater numbers of independent variables. Appendix 2 gives the 10 best four variable regressions using the 20 best three variable regressions as a starting point, and Appendix 3 gives the 10 best five variable regressions using the 20 best four variable regressions as starting point. Rather than select any one particular equation, it is probably more instructive to examine the broad pattern of occurrence of the various variables. These are summarised in columns 2 and 3 of Table 3.

The most frequently occurring variables in Appendix 3, both of which appear in all of the equations, are the square of the electrostatic potential at the postulated carboxyl binding site and the second order polarisation energy at the postulated hydrogen bonding site. The second of these quantities represents the stabilisation of the inhibitor by the presence of a point positive charge at that point, and can be thought of as a measure of the likelihood of forming a hydrogen bond to a proton situated there. The relationship of the first quantity to the ability of the inhibitor to interact with a positively-charged species needs no explanation. Coefficients of $V_1^2(2)$ are typically 0.03 while the quantity has values in the range of 0–100, typically 70 for a reasonably good inhibitor. Thus we can estimate the contribution of this quantitiy to pI_{50} for a typical inhibitor at about 2-3 (in log units of pI_{50}). Similarly, the contribution from the polarisation energy is of the order of 3.5.

After these two quantities, the next most frequent occurrences are of the z-component of the electric field at the postulated zinc position and the y-component of the electric field at the carboxyl binding site. For reasonably good inhibitors, their contributions to pI_{50} are around 1.2 and -0.5 respectively.

The next most frequently occurring variables (which always occur together) are the total electrostatic energy of the system E_{st} and the mean van der Waals radius r. Their respective contribu-

tions to a typical pI_{50} are 10 and -10, strongly suggesting that this may be a chance correlation between a linear combination of two of the variables and the residuals obtained from the corresponding three variable regression. Alternatively, it can be postulated that the interaction between the negatively-charged inhibitor and a positively-charged receptor is of overwhelming importance to the interaction, with steric interactions also playing an important role, and other more specific interactions at the model receptor site playing a secondary role. In general terms, however, we would expect to see pI_{50} made up of a sum of small terms rather than a difference of very large ones.

All other variables in the table occur only once, and make extremely small contributions to pI_{50}, suggesting that they are in general quite unimportant. Constant terms usually have quite large standard errors, and can thus also be thought of as being quite unimportant. We can postulate that the constant should be a small value close to (and probably less than) zero. For regressions involving the electrostatic energy and radius the constant is between 2 and 4, once again suggesting that these are chance correlations.

From the above discussion we can conclude that the hydrogen bonding site appears to provide the bulk of the binding energy of inhibitor to receptor. The gross electrostatic interaction between receptor and inhibitor is clearly of importance, and the binding to the zinc site and carboxyl site appear to make lesser contributions. These findings are not inconsistent with the known structure-activity relationships of ACE inhibitors.

CONCLUSIONS

A consideration of the molecular wave functions of a series of enzyme inhibitors is capable of giving rise to simple models which quantify the various interactions with a receptor. Such models are of value in assessment of the likelihood that a particular compound will be a good or a poor inhibitor of the enzyme. In the absence of a known active site structure, the postulation of a reasonable model for binding can give rise to quite acceptable agreement between calculated and observed activities.

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APPENDIX I

Regressions of pI_{50} on three variables

	r	s	F(3,31)
$pI_{50} = (0.1208 \pm 0.0285)\,\mu_z - (0.0187 \pm 0.0023)\,\alpha^*ip - (8.8114 \pm 1.9635)\,V_2(3) - (0.8080 \pm 1.0280)$	0.840	0.880	24.763
$pI_{50} = (0.1307 \pm 0.0316)\,\mu_z - (2.7159 \pm 0.3673)\,E_x(1) - (6.9584 \pm 2.0634)\,V_2(3) - (1.6900 \pm 1.1740)$	0.810	0.951	19.678
$pI_{50} = (0.3960 \pm 0.0536)\,\varepsilon_{homo} + (0.1166 \pm 0.0308)\,\mu_z - (8.2749 \pm 2.1100)\,V_2(3) - (0.5732 \pm 1.1060)$	0.810	0.952	19.660
$pI_{50} = (0.4306 \pm 0.0582)\,\varepsilon_{homo} + (0.1976 \pm 0.0382)\,\mu_z + (0.6275 \pm 0.1688)\,E_y(2) + (3.8730 \pm 0.2151)$	0.802	0.968	18.650
$pI_{50} = (0.1956 \pm 0.0384)\,\mu_z - (2.8823 \pm 0.3941)\,E_x(1) + (0.4985 \pm 0.1629)\,E_y(2) + (1.9330 \pm 0.4060)$	0.799	0.975	18.253
$pI_{50} = -(4.2665 \pm 0.5994)\,E_z(1) + (0.6139 \pm 0.1766)\,E_y(2) + (0.0337 \pm 0.0072)\,V_1^2(2) + (2.2400 \pm 0.4848)$	0.796	0.981	17.886
$pI_{50} = -(3.6872 \pm 0.5649)\,E_z(1) + (0.0167 \pm 0.0057)\,V_1^2(2) + (0.9448 \pm 0.2815)\,E_x(3) + (0.3770 \pm 0.8761)$	0.792	0.990	17.352
$pI_{50} = (0.1356 \pm 0.0326)\,\mu_z - (0.0154 \pm 0.0024)\,\alpha^*ip + (0.8583 \pm 0.2839)\,E_x(3) + (1.3270 \pm 0.8215)$	0.790	0.993	17.217
$pI_{50} = (0.1808 \pm 0.0381)\,\mu_z - (0.0184 \pm 0.0026)\,\alpha^*ip + (0.5028 \pm 0.1665)\,E_y(2) + (3.9200 \pm 0.2186)$	0.790	0.993	17.202
$pI_{50} = -(1.7^*10^{-4} \pm 2.4^*10^{-5})\,E_{st} + (0.1186 \pm 0.0317)\,\mu_z - (2.3632 \pm 0.6007)\,r + (4.9420 \pm 2.0690)$	0.790	0.993	17.197

APPENDIX 2

Regressions of pI_{50} on four variables

	r	s	F(4,30)
$pI_{50} = -(4.3871 \pm 0.5315)\,E_z(1) + (0.0369 \pm 0.0064)\,V_1^2(2) - (5.9432 \pm 1.9134)\,V_2(3) + (0.5531 \pm 0.1574)\,E_y(2) - (1.0090 \pm 1.1310)$	0.850	0.868	19.569
$pI_{50} = -(4.1800 \pm 0.5630)\,E_z(1) + (0.4476 \pm 0.1806)\,E_y(2) + (0.0285 \pm 0.0071)\,V_1^2(2) + (0.7070 \pm 0.3072)\,E(3) - (0.4727 \pm 0.8923)$	0.830	0.919	16.598
$pI_{50} = -(4.0869 \pm 0.5710)\,E_z(1) + (0.4429 \pm 0.1836)\,E_y(2) + (0.0275 \pm 0.0073)\,V_1^2(2) + (0.1654 \pm 0.0749)\,E^2(3) + (1.1710 \pm 0.6660)$	0.828	0.925	16.310
$pI_{50} = -(4.1725 \pm 0.5674)\,E_z(1) + (0.0281 \pm 0.0073)\,V_1^2(2) + (0.6423 \pm 0.2932)\,E_x(3) + (0.4344 \pm 0.1858)\,E_y(2) + (0.7181 \pm 0.8320)$	0.827	0.926	16.258
$pI_{50} = -(0.0229 \pm 0.0035)\,\alpha^*ip + (0.0282 \pm 0.0080)\,V_1^2(2) - (14.9894 \pm 2.7657)\,V_2(3) + (0.7124 \pm 0.2291)\,V_1(3) - (0.7182 \pm 1.2830)$	0.822	0.939	15.590
$pI_{50} = -(3.7723 \pm 0.5394)\,E_z(1) + (0.0302 \pm 0.0063)\,V_1^2(2) - (7.8945 \pm 2.1127)\,V_2(3) + (2.1555 \pm 0.8879)\,E_z(3) - (1.4810 \pm 1.2520)$	0.820	0.942	15.443
$pI_{50} = -(0.0230 \pm 0.0035)\,\alpha^*ip + (0.0269 \pm 0.0078)\,V_1^2(2) - (15.0689 \pm 2.8152)\,V_2(3) - (0.0499 \pm 0.0164)\,V_1^2(3) - (3.1210 \pm 1.3660)$	0.820	0.944	15.350
$pI_{50} = -(2*10^{-4} \pm 2*10^{-5})\,E_{st} - (2.4806 \pm 0.5811)\,r - (8.7588 \pm 2.1868)\,V_2(3) + (0.0158 \pm 0.0055)\,V_1^2(2) + (0.8987 \pm 2.1110)$	0.819	0.946	15.244
$pI_{50} = -(4.4635 \pm 0.6589)\,E_z(1) - (0.1496 \pm 0.0568)\,V_1(2) + (0.2223 \pm 0.0703)\,E^2(3) + (0.4067 \pm 0.1478)\,E_z(2) + (0.0211 \pm 0.8266)$	0.817	0.949	15.104
$pI_{50} = -(4.4656 \pm 0.5936)\,E_z(1) - (0.8115 \pm 0.2071)\,E_y(2) + (0.0384 \pm 0.0075)\,V_1^2(2) + (1.1896 \pm 0.6995)\,E_y(3) + (1.5900 \pm 0.6062)$	0.816	0.952	14.957

APPENDIX 3

Regressions of pI_{50} on five variables

	r	s	F(5,29)
$pI_{50} = -(3.968 \pm 0.536)\,E_x(1) + (0.635 \pm 0.153)\,E_y(2) + (0.040 \pm 0.006)\,V_1^2(2) - (6.918 \pm 1.857)\,V_2(3) - (0.187 \pm 0.085)\,n_{rot} - (0.537 \pm 1.087)$	0.873	0.818	18.584
$pI_{50} = -(2*10^{-4} \pm 2*10^{-5})\,E_{st} - (3.837 \pm 0.686)\,r + (0.032 \pm 0.007)\,V_1^2(2) - (12.947 \pm 2.391)\,V_2(3) - (0.040 \pm 0.013)\,V_1^2(3) + (2.320 \pm 1.934)$	0.865	0.841	17.260
$pI_{50} = -(2*10^{-4} \pm 2*10^{-5})\,E_{st} - (3.752 \pm 0.671)\,r + (0.033 \pm 0.008)\,V_1^2(2) - (12.71 \pm 2.358)\,V_2(3) + (0.557 \pm 0.188)\,V_1(3) + (4.043 \pm 2.157)$	0.864	0.843	17.147
$pI_{50} = -(2*10^{-4} \pm 2*10^{-5})\,E_{st} - (3.085 \pm 0.571)\,r + (0.027 \pm 0.006)\,V_1^2(2) - (7.316 \pm 2.052)\,V_2(3) - (0.034 \pm 0.013)\,V_1^2(1) + (2.529 \pm 2.005)$	0.859	0.858	16.324
$pI_{50} = -(2*10^{-4} \pm 2*10^{-5})\,E_{st} - (3.200 \pm 0.589)\,r + (0.026 \pm 0.006)\,V_1^2(2) - (7.290 \pm 2.055)\,V_2(3) + (0.307 \pm 0.112)\,V_1(1) + (3.223 \pm 2.096)$	0.859	0.859	16.311
$pI_{50} = -(4.302 \pm 0.532)\,E_x(1) + (0.459 \pm 0.174)\,E_y(2) + (0.038 \pm 0.007)\,V_1^2(2) - (6.654 \pm 1.986)\,V_2(3) + (1.100 \pm 0.904)\,E_x(3) - (1.293 \pm 1.146)$	0.858	0.861	16.201
$pI_{50} = -(4.610 \pm 0.561)\,E_x(1) + (0.567 \pm 0.157)\,E_y(2) + (0.037 \pm 0.006)\,V_1^2(2) - (6.039 \pm 1.903)\,V_2(3) - (0.007 \pm 0.006)\,E_y(4) - (1.134 \pm 1.128)$	0.858	0.862	16.141
$pI_{50} = -(4.610 \pm 0.561)\,E_x(1) + (0.566 \pm 0.157)\,E_y(2) + (0.037 \pm 0.006)\,V_1^2(2) - (6.041 \pm 1.903)\,V_2(3) + (0.007 \pm 0.006)\,E_x(4) - (1.133 \pm 1.128)$	0.858	0.862	16.131
$pI_{50} = -(4.606 \pm 0.561)\,E_x(1) + (0.566 \pm 0.157)\,E_y(2) + (0.037 \pm 0.006)\,V_1^2(2) - (6.042 \pm 1.904)\,V_2(3) + (3*10^{-4} \pm 3*10^{-4})\,V_2(4) - (1.133 \pm 1.129)$	0.858	0.862	16.117
$pI_{50} = -(4.606 \pm 0.561)\,E_x(1) + (0.566 \pm 0.157)\,E_y(2) + (0.037 \pm 0.006)\,V_1^2(2) - (6.037 \pm 1.904)\,V_2(3) + (1*10^{-5} \pm 1*10^{-5})\,E^2(4) - (1.128 \pm 1.128)$	0.858	0.862	16.113