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Synthesis and structure–antibacterial activity relationship investigation of isomeric 2,3,5-substituted perhydropyrrolo[3,4-*d*]isoxazole-4,6-diones

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Abstract—The synthesis of 2,3,5-substituted perhydropyrrolo[3,4-*d*]isoxazole-4,6-diones (44 compounds) has been accomplished by the cycloaddition reaction of N-methyl-C-arylnitrones with N-substituted maleimides. The compounds were screened for their antibacterial activities and most of them exhibited activity against *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 25923). ***cis*-3a** and ***cis*-3d** were found fairly effective against *E. faecalis* (ATCC 29212) and *S. aureus* (ATCC 25923) with MIC values of 25 and 50 μg/ml. With the changes of *cis* isomers of the compounds to *trans*, their antibacterial activities also changed against the bacteria studied. First, pharmacophoric fragments had been calculated in accordance with the rules of the electronic-topological method (ETM). Next, both active compounds and pharmacophores had been projected to the nodes of Kohonen's self-organizing maps (SOM) to obtain the weights of pharmacophore fragments as numerical descriptors, that were used after this for the associative neural networks (ASNN) training. A model for the activity prediction was developed as the result of training the ASNNs.

1. Introduction

Over the past decades, the frequency of resistance in antimicrobial agents has increased dramatically.[1] Therefore, this places new emphasis on the search for alternative substances which are effective against organisms resistant to currently available drugs. Cycloaddition of nitrones to a variety of unsaturated systems has been exploited to synthesize isoxazoline/isoxazolidine ring.[2–4] Recently, useful antiinflammatory,[5] immunosuppressive[6] and antibacterial[7–12] properties have been ascribed to molecules possessing such heterocyclic functionalities.

For structural characterization, we have recently prepared isomeric 2,3,5-substituted perhydropyrrolo [3,4-*d*]isoxazole-4,6-diones by the cycloaddition reactions of N-methyl-C-arylnitrones to N-substituted maleimides.[13,14] In view of the biological interest shown in these compounds, we have decided to synthesize a wide range of isoxazole derivatives to screen the antibacterial activities against various bacteria.

The other goal of the present study was the search of the most potent and selective antibacterial preparations against *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 25923) by applying the combined approach that is the known electronic-topological method (ETM) followed by the artificial neural networks application (in short, ETM–ANNs approach) to the ETM results. Both ETM and ANN approaches have already been successfully applied but separately to a wide enough variety of tasks related to

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the structure–activity relationship (SAR) investigation.[15–21]

ANNs are of significant interest for QSAR studies.[22] If the dependencies between analysed descriptors and molecular parameters are non-linear, the neural networks can produce more accurate models than linear regression methods do. As an example, the feed-forward neural networks (FFNN) trained with the back propagation algorithm[23,24] are widely used to perform different chemical calculations. This can be also very important for such practical applications as design of new compounds possessing desirable properties. In our case, ANNs provide more fast and easy implementation of the activity prediction for new potential drugs, because this prediction is not fully automated in the ETM approach. Meantime, ANNs are capable of providing estimation of a compound's activity immediately from the structural description of the compound.

2. Results and discussion

2.1. Chemistry

1,3-Dipolar cycloaddition reactions of alkenes to nitrones often give a pair of diastereomeric isoxazolidines.[25] In these reactions, a small structural change in the nitrone can lead to a significant change in the stereoselectivity of the cycloaddition.[26] Regiochemical and stereochemical courses of 1,3-dipolar cycloaddition of N-alkyl-C-phenylnitrones to alkenes have been described in the literature.[27–41]

In this work, a wide range of substituents have been used for the cycloaddition of *N*-methyl-C-substituted phenylnitrones to *N*-methyl and *N*-phenylmaleimides (see Scheme 1). The stereochemical assignment of the *cis*- and *trans*-isomers is made on the basis of the magnitude of the H_b–H_c coupling constants. The *cis*-isomers of isoxazolidine give rise to larger H_b–H_c coupling constants ($J \approx 6$–8 Hz) than those observed for the *trans*-isomers ($J \approx 2$–5 Hz).[28,35,42] In the NMR spectra, the H_c protons of the *cis*-isomers have been observed as multiplets together with H_b protons at about 3.8–4.00 ppm, whereas the H_c protons of the *trans*-isomers appeared as very broad peaks at about 4.3–4.5 ppm.

From the cycloaddition of *N*-methyl-C-substituted nitrones (which carry electron-releasing substituents) to *N*-methylmaleimide, we obtained major *cis*-isomers along with minor *trans*-isomers. The same reaction of cycloaddition but for nitrones (which carry electron-withdrawing substituents) gave about 50:50 mixtures of *cis*/*trans* isomers. On the other hand, the cycloaddition of all nitrones with *N*-phenylmaleimide gave both isomers with 50:50 mixtures. The structures of all newly synthesized compounds were identified by ^{1}H NMR, IR and micro analyses. The data are given in the experimental section. The data on the antibacterial activity (MIC values) are given in Table 1, which also contains ampicillin results for the microorganisms used in this work to be compared with the results of the method used and to control its reliability.

2.2. Antibacterial activity investigation

The antibacterial activities of 44 compounds were determined by using broth microdilution susceptibility test outlined by the National Committee for Clinical Laboratory Standards.[43] Minimal inhibitory concentrations for each compound were investigated against *E. faecalis* (ATCC 29212) and *S. aureus* (ATCC 25923). For broth microdilution procedures, sterile, disposable, multiwell microdilution plates (96 U-shaped wells) were used. The stock solutions were prepared in dimethylsulfoxide (DMSO; Sigma), and DMSO had no effect on the microorganisms in the concentrations studied.

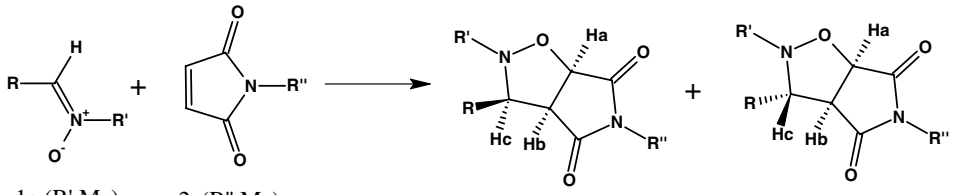
2.3. Dilutions of the compounds

All of the dilutions were done with Mueller–Hinton Broth (Oxoid) in the wells of microdilution plates. The concentrations of 30 compounds tested were 1600, 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 μg/ml (the concentrations are given as μmol/ml in Table 1). The highest concentration of eleven compounds (***cis*-3l**, ***cis*-3p**, ***cis*-3r**, ***cis*-3s**, ***cis*-3t**, ***cis*-3u**, ***trans*-3f**, ***trans*-3d**, ***trans*-3m**, ***trans*-3o**, ***trans*-3q**) was 1000 μg/ml, therefore their tested concentrations were prepared as follows: 1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, 1.95 and 0.975 μg/ml. Ampicillin (Fako, Istanbul, Turkey) was used as a reference compound.

2.4. Inoculum preparation

After diluting the compounds, standardized inoculum of each bacterium (0.5 Mc Farland standard unit, 1×10^8 CFU/ml; colony forming unit/ml) was prepared. Then, the compounds were diluted once more (1/10), and final concentrations became 1×10^7 CFU/ml. Five microliters from each dilution was placed into each well containing 100 μl of dilutions of compounds so that each well contained 5×10^5 CFU/ml of inoculum. All the inoculated plates were incubated at 35 °C for 16–20 h. The lowest concentration of compounds that prevents visible growth was considered to be the minimal inhibitory concentration (MIC). MIC values are given in Table 1. Ampicillin was used as reference antimicrobial reagent to compare its parameters with the data that result from the method applied in this work and to control the reliability of the latter. Data on the antibacterial activity of the compounds demonstrate that structural modifications can affect the antibacterial activity of the tetrahydropyrroloisoxazole skeleton.

All tested compounds exhibited expressive antibacterial activity against *E. faecalis* and *S. aureus*. However, these activities were lesser than that observed with Ampicillin. ***cis*-3a** and ***cis*-3d** were found fairly effective against *E. faecalis* and *S. aureus* with MIC values of 25 and 50 μg/ml, respectively. It was observed that if *cis*-isomers change to *trans*-isomers, the corresponding activities also change against *E. faecalis* and *S. aureus*. In general, ***trans*-3a, d, f, g, j, m, o, q, r, t, u** and ***cis*-3a, d, f, h, l, n, r,**



Comp. 3	R	R'	R''	Cis/Trans ratio
a	*p*-$Et_2NC_6H_4$	Me	Me	2.03
b	*p*-$Me_2NC_6H_4$	Me	Me	1.96
c	*p*-$MeOC_6H_4$	Me	Me	2.82
d	4-BzO-3-$MeOC_6H_3$	Me	Me	2.48
e	*p*-MeC_6H_4	Me	Me	1.24
f	C_6H_5	Me	Me	0.79
g	*m*-ClC_6H_4	Me	Me	1.00
h	*m*-BrC_6H_4	Me	Me	1.07
i	*p*-ClC_6H_4	Me	Me	1.09
j	*p*-BrC_6H_4	Me	Me	1.12
k	*p*-$NO_2C_6H_4$	Me	Me	1.07
l	*p*-$Et_2NC_6H_4$	Me	Ph	1.11
m	*p*-$Me_2NC_6H_4$	Me	Ph	0.92
n	*p*-$MeOC_6H_4$	Me	Ph	1.11
o	4-BzO-3-$MeOC_6H_3$	Me	Ph	1.23
p	*p*-MeC_6H_4	Me	Ph	0.89
q	C_6H_5	Me	Ph	0.85
r	*m*-BrC_6H_4	Me	Ph	0.93
s	*p*-ClC_6H_4	Me	Ph	0.97
t	*p*-BrC_6H_4	Me	Ph	1.00
u	*p*-$NO_2C_6H_4$	Me	Ph	0.92
v	*p*-$Me_2NC_6H_4$	Ph	Me	1.09

Scheme 1. The synthesis of compounds **3a**–**3v**.

s, t, u and **v** were found effective to inhibit the growth of the bacteria with the MIC values between 25 and 250 μg/ml.

To complete the study, the structure–activity relationship study for new compounds as antibacterial agents is done as well. This gave rise to the identification of several potent broad-spectrum antibacterial compounds.

2.5. Brief review of the combined ETM–ANNs approach

The aim of the ETM approach is to find molecular fragments (pharmacophores) common for the structures of all active compounds (for a fixed activity) and absent in all inactive compounds possessing similar structures. After this, the fragments found are used to predict the activity for newly synthesized compounds. However, the procedure of the activity prediction is difficult enough and needs the expert's participation. To make the procedure easier and faster, the ETM application was followed by the ANNs application (with unsupervized and supervized learning algorithms), and, as a whole, the calculations were named the combined ETM–ANNs approach.

Since details of the ETM can be found in the literature,[44–47] we only give here the most distinguished properties of the ETM relative to other methods used in diverse SAR studies. ETM belongs to the so-called structural methods. So, the main part of the method is a language for the compound structure description. The language reflects the discrete nature of compounds that are viewed as consisting of atoms some of which are chemically bonded. Labelled graphs appeared to be the most appropriate mathematical counterparts of chemical structures and relationships on their atoms and bonds. As known, a graph's representative is a matrix of the order $n \times n$, where n is the number of the graph's vertices. Therefore, the ETM proposes electronic-topological matrices of contiguity (ETMC) to be its own, very special language, for chemical compounds' description. Bonds have no orientation, thus the matrices are symmetrical relative to their left diagonal, and it is enough to have only the right upper triangle of any such matrix along with its diagonal.

To begin the ETM-study, one must have a representative series of compounds (a few tens of compounds, at least, but the more is the better). The measured activity can be either qualitative (i.e., active/inactive, in which case there are two classes for comparison) or quantitative (in which case there can be more than two classes). Ideally, a half of compounds possessing similar structures should be inactive. The main steps of the ETM-study are given below:

1. Calculate the values of spatial and electron characteristics for all atoms and bonds of each compound from the initial selection.
2. Form the corresponding ETMC (see Fig. 1) for each molecular structure by choosing appropriate values from the data calculated; usually, they are *charges*

Table 1. Minimal antibabacterial inhibitory concentration (μmol/ml) and data on the experimental versus theoretical activities of compounds under study

Compound	*E. faecalis* ATCC 29212	Exp.	Theor.	*S. aureus* ATCC 25923	Exp.	Theor.
Ampicillin	0.002	+	+	0.002	+	+
***cis*-3a**	0.08	+	–	0.08	+	+
***trans*-3a**[a]	0.63	+	–	0.63	+	+
***cis*-3b**	1.38	–	–	1.38	–	+
***trans*-3b**	5.53	–	–	1.38	–	–
***cis*-3c**	>5.79	–	–	5.79	–	–
***trans*-3c**[a]	>5.79	–	–	1.45	–	–
***cis*-3d**	0.13	+	+	0.13	+	+
***trans*-3d**	0.65	+	–	0.33	+	–
***cis*-3e**	>6.15	–	–	6.15	–	–
***trans*-3e**[a]	6.15	–	–	3.07	–	–
***cis*-3f**[a]	1.62	–	–	0.81	+	–
***trans*-3f**	1.02	+	+	0.51	+	+
***cis*-3g**	2.85	–	+	1.43	–	–
***trans*-3g**	1.43	–	–	0.71	+	+
***cis*-3h**[a]	0.62	+	+	1.23	–	–
***trans*-3h**	1.23	–	–	0.62	+	+
***cis*-3i**	1.43	–	–	1.43	–	–
***trans*-3i**	1.43	–	–	1.43	–	–
***cis*-3j**[a]	1.23	–	+	2.46	–	–
***trans*-3j**	0.62	+	+	0.31	+	+
***cis*-3k**	0.69	+	+	1.37	–	–
***trans*-3k**	5.49	–	–	2.75	–	–
***cis*-3l**[a]	0.66	+	+	0.33	+	+
***trans*-3l**	4.22	–	–	4.22	–	–
***cis*-3m**[a]	>4.55	–	–	4.55	–	–
***trans*-3m**	0.71	+	–	0.36	+	–
***cis*-3n**	1.18	–	–	0.59	+	+
***trans*-3n**	1.18	–	–	1.18	–	–
***cis*-3o**	>3.6	–	–	3.60	–	–
***trans*-3o**	1.13	–	–	0.28	+	+
***cis*-3p**	3.10	–	–	3.10	–	–
***trans*-3p**[a]	1.24	–	–	0.62	+	+
***cis*-3q**	5.19	–	–	>5.19	–	–
***trans*-3q**	1.62	–	–	0.41	+	+
***cis*-3r**[a]	0.65	+	+	0.32	+	+
***trans*-3r**	0.52	+	+	0.13	+	+
***cis*-3s**[a]	0.73	+	+	0.36	+	+
***trans*-3s**	1.17	–	–	1.17	–	–
***cis*-3t**	0.65	+	+	0.32	+	–
***trans*-3t**[a]	0.52	+	+	0.13	+	+
***cis*-3u**	0.71	+	+	0.35	+	+
***trans*-3u**	0.28	+	+	0.57	+	+
***cis*-3v**	0.28	+	+	0.57	+	+
***trans*-3v**	1.14	–	–	0.28	+	+

[a] Test set compounds.

for diagonal elements (q_i) and the Wiberg's indices for bonds (W_{ij}); otherwise, distances (R_{ij}) are taken for non-bonded atoms.

3. Set some desirable level of probability (P_a) for the fragment selection and some precision values for diagonal (Δ_1) and out-diagonal (Δ_2) elements of ETMCs to have ability to compare the values of corresponding atomic and bond characteristics (i.e., to take into account the molecule's flexibility).
4. By comparing all ETMCs with the ETMC of the most active compound (taken as a template), select those structural fragments that are common for all active compounds only (i.e., pharmacophores, Ph_i). Ph_i are represented by submatrices of ETMCs, or, in short, by ETSCs.
5. Estimate the fragments selected (Ph_i) in accordance with probabilistic criterion P_a and choose those of them that correspond to the desired level of P_a that has been set before calculations. If the fragments found are not informative enough, change some initial settings (or all of them) and repeat steps 3–5.

Criterion that is commonly used in structural methods for the probability of each structural feature occurrence in active compounds under view is given by the equation

$$P_a = (n_1 + 1)/(n_1 + n_2 + 2),$$

where n_1 and n_2 are numbers of molecules, which possess the feature of activity in the class of active and inactive compounds, respectively.

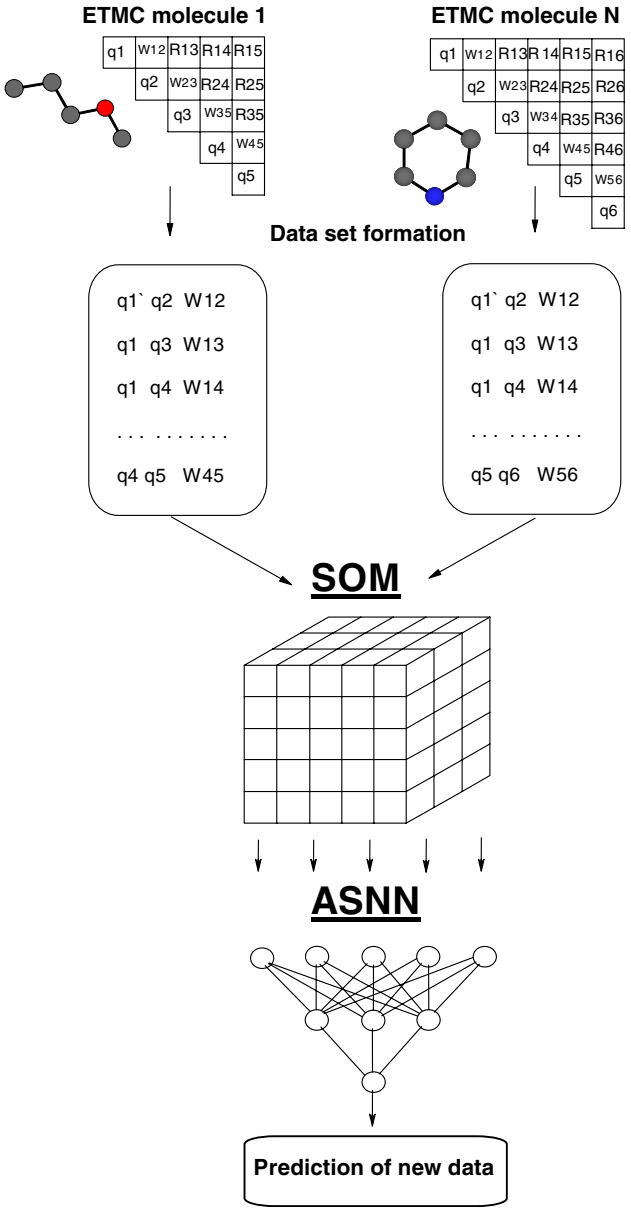


Figure 1. General scheme of the data analysis.

Analogously, the features of inactivity, or anti-pharmacophores (APh_j), can be calculated and estimated with the help of the same procedure, but relative to an inactive template compound. However, the further fragments' analysis cannot be done by feed forward neural networks (FFNNs) in a straightforward manner. To overcome this problem, a special algorithm being a combination of FFNNs and the Kohonen's self-organizing map (SOM) has been proposed.[48] The principal idea of the combined approach is to determine the weights of fragments represented by ETSCs and, afterwards, to use these weights as descriptors (WDs) for the FFNNs training. To do this, the ETMCs of molecules and then the fragments found by the ETM software and presented as ETSCs are being projected on the Kohonen's maps. In such way, the degree of each fragment's presence in the molecule (or its weight, WD) can be determined.

The supervised learning was performed using a variant of FFNNs known as the Associative Neural Network.[49] This type of networks improves the prediction ability of the FFNNs by explicit correction of biases. The training of the Kohonen's SOM is carried out in such a manner that input vectors from the n-dimensional space ($n \gg 2$), which possess similar properties, are mapped to the same or nearby neurons in the two-dimensional space. Therefore, it is possible to determine clusters of vectors that have similar properties in the n-dimensional space by considering all projections of input vectors to the same SOM neurons.

The general block-scheme of the ETM–ANNs data analysis is presented in Figure 2.

According to this algorithm, the process of the ETM-analysis is as follows:

(1) Form the matrices called ETMCs calculate their common submatrices being molecular fragments (called ETSCs) by applying ETM procedure and form the input data set to be further projected to the SOM's nodes. A new table containing k input samples X_i is being formed from the ETMCs (the value of k depends on the matrix order for each molecule and the total number N_{mol} of molecules in the initial set). Each data sample X_i is a triple (x_{1i}, x_{2i}, x_{3i}), where x_{1i} and x_{2i} are charges for a pair of atoms (q1 and q2) and x_{3i} is a value that characterises the connection between them, bond or distance (see Fig. 1). Analogous procedure is used for the input data

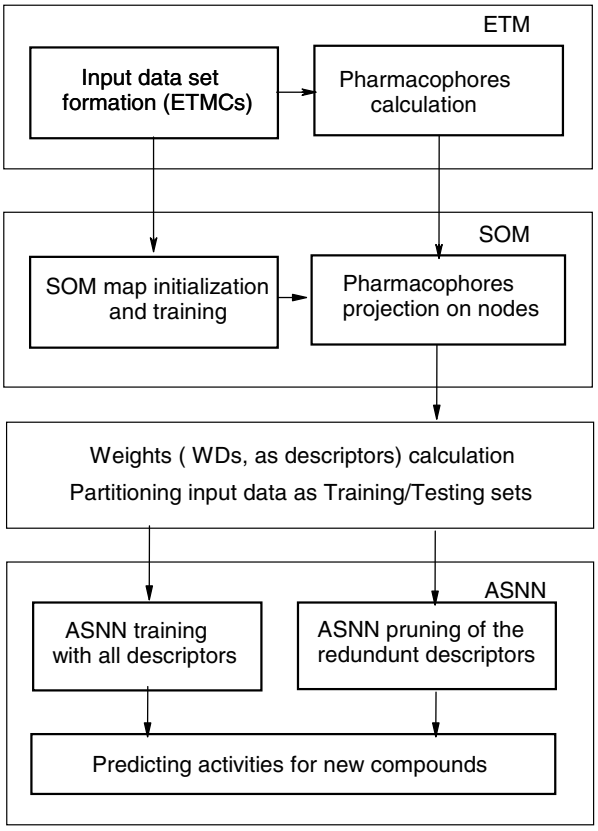


Figure 2. Block-scheme of the ETM–ANNs calculations.

formation based on the submatrices corresponding to the fragments, or ETSCs. A new table containing input samples Y_l for all ETSCs is being formed by means of the procedure described at the first step for the ETMCs (see Fig. 1).

(2) Initialize the Kohonen's network parameters; calculate projections of the ETMCs of all compounds on the nodes of the SOM. The approximate number of elements in our Kohonen's map is evaluated as $S = k^*S_{\mathrm{ETM}}$, where k belongs to the interval $[1.0, 2.0]$ and S_{ETM} is the size of the largest ETM matrix. The value of k was found by varying it in the range of $[1, N_{\mathrm{mol}}]$. The researches have shown that the values of k that are higher than 2 practically do not improve the quality of the models obtained. The Kohonen's networks realize a nonlinear projection of high-dimensional data set onto a low-dimensional domain. Detailed description of Kohonen's networks can be found in Refs. 50,51.

(3) For each fragment, that is either a pharmacophore or antipharmacophore, calculate its projection on the units of the Kohonen's SOM and the projection error (E_{ij}). Then take the weight WD_{ij} of each fragment equal to the inverse of its error E_{ij}. Here i is the molecule's number and j is the fragment's number.

The way for the weights calculation is shown at the example of one definite submatrix, or ETSC. For the jth ETSC, the minimal distance d_l between an input vector Y_l and vectors U_m is to be searched for, where $U_m(u_{1m},u_{2m},u_{3m})$ is the reference vector of a map unit that corresponds to the mth node of SOM.

It should be noted that only nodes being projections of the X-vectors of the ETMC for the ith molecule are used to calculate d_l. In other words, we are seeking for a molecular fragment projection to a node related to the ETMC matrix analysed.

The projection error e_l for the element Y_l is calculated as the difference between Y_i and X_r, where X_r is a vector for which the error is minimal. That is, many vectors belonging to the same matrix can be projected to the same node, but the closest of them is selected, which is just the projection error for this element.

The total error E_{ij} of the ETSC projection is

$$E_{ij} = \frac{1}{k}\sum_{l=1}^{k} e_l;$$

here k is the number of samples in the jth ETSC.

Then the weight of ETSC is taken as the inverse of its error E_{ij}: $WD_{ij} = 1 - E_{ij}/E_{\max,j}$. Here $E_{\max,j}$ is the maximal error, for all j, that is, $E_{\max,j}$ is the maximal error value relative to the jth column. As the result, a table is being formed, which contains weights of all ETSCs relative to each molecule of the initial series. Then the data are divided into training set and test set and used for the model development.

(4) After training the ASNN, select the most informative ETMC fragments by using special pruning methods.[52,53]

The table containing the calculated fragment weights WDs is being formed for the further ASNNs training. The number of neurons in the input layer of the network corresponds to the number of descriptors. The hidden layer contains five neurons. The bias neuron is presented both on the input and hidden layers. An ensemble of $M = 100$ neural networks was trained. By this, the activity value for each compound was calculated for each ASNN and averaged over all M networks. The resulting value was used to calculate statistical coefficients.[54] Finally, the pruning methods[52,53] were applied to select the best of the ETMC fragments and obtain the most appropriate model for the activity prediction.

After finding the best model, parameters and weight matrices that have been found for each ASNN are to be saved. They are used to predict activities of new potential drugs (for details, see Ref. 54).

2.6. Analysis of pharmacophores and anti-pharmacophores

Optimized geometry data and calculated electronic characteristics were used in ETMCs that had been formed for all compounds (44 molecules), in accordance with the main steps of the ETM-study. Conformational analysis was done for all compounds, that is, their electronic structures were calculated from the semi-empirical AM1 method.[55] Effective charges on atoms (q_i) were chosen as diagonal elements (local atomic characteristics); at place of off-diagonal elements, either values of a bond characteristic (here, Wiberg's indices, W_{ij}) or optimized distances (R_{ij}, in) were used, respectively.

For *E. faecalis* (ATCC 29212), all compounds fall into classes of active molecules (18 mol, MIC $\leqslant$ 0.6538 μϖ mol/ml) and inactive ones (26 mol, $\mathrm{IC}_{50} >$ 0.6538 μϖ mol/ml), in a natural way. Analogously, the classes of active and inactive compounds relative to *S. aureus* (ATCC 25923) include 24 and 20 molecules, respectively. To have more stable activity features, every active compound was used as a template for comparison with the rest of ETMCs in the course of the ETM study.

After processing all ETMCs, a set of pharmacophores and anti-pharmacophores was obtained for each type of activity. These features form a basis for a system capable of carrying out computer screening of new drug prototypes and forecasting their activities. Optimal values of variations that are allowable in the process of the matrices comparison (when testing if atoms and bonds match) were found as $\Delta_1 = \pm 0.07$ for diagonal elements (q_i) and $\Delta_2 = \pm 0.20$ for off-diagonal values (W_{ij} and R_{ij}). To determine the most informative activity features, the lowest level of probabilistic estimations, P_{a}, was taken as 0.80.

To form the basis of a system for the antibacterial activity prediction, the ***cis*-3a** compound possessing the

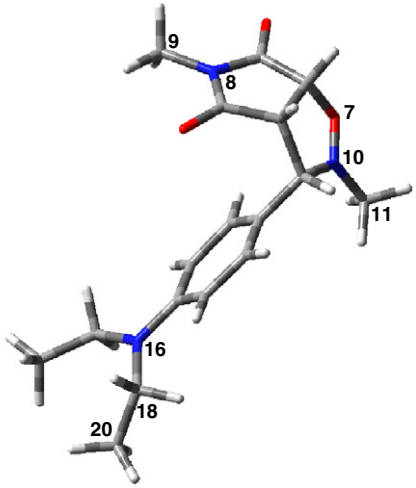
highest activity was taken first of all as a template for the comparison. In Figure 3, a submatrix of this template ETMC (or, in short, its ETSC) is given, which corresponds to one of the pharmacophores revealed (namely, to Ph_1).

The given Ph_1 pharmacophore consists of 8 atoms located in different parts of the template. The nitrogen atom N_8 has a high negative charge $q = -0.34\bar{e}$; negative charges are concentrated on the atoms N_{16}, O_7, C_{20} and C_{11}, as well. The charges on the C_9 and C_{18} carbon atoms are close to zero. The rest of pharmacophores were found analogously, and the probabilities of their realization in the class of active compounds varied in the limits of 0.86–0.95.

To determine anti-pharmacophores, ETMCs of several inactive compounds were taken as templates for comparison with the rest of compounds. ETSC that corresponds to the APh_1 anti-pharmacophore is given in Figure 4 along with the structure of the corresponding template from which all anti-pharmacophores have been found.

As seen from Figure 4, APh_1 (found from the ***cis*-3e** inactive compound used as a template) consists of atoms C_4, N_8, C_{16}, C_{17} and O_{18}.

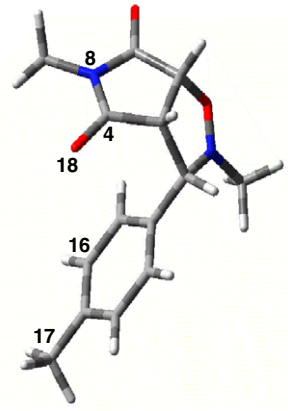
So, a set of pharmacophores and anti-pharmacophores was calculated (relative to the series under study) by the ETM. Both types of molecular fragments formed the basis of a system for the antibacterial activities against *E. faecalis* (ATCC 29212) and *S. aureus* (ATCC 25923) prediction. In Table 2, statistical characteristics of six pharmacophores (P) and six anti-pharmacophores (AP) entering the system are given.



***cis*-3a** (MIC=25)

O7	N8	C9	N10	C11	N16	C18	C20
-0.20	3.33	4.61	0.96	2.29	8.70	7.91	9.69
	-0.34	0.92	3.60	5.06	7.20	7.40	8.71
		0.07	4.82	6.25	7.75	7.45	8.60
			-0.07	0.99	6.53	8.01	9.41
				-0.16	6.82	8.33	9.65
					-0.29	0.94	2.60
						-0.02	0.98
							-0.22

Figure 3. Submatrix (ETSC) and corresponding structure of the Ph_1 pharmacophore (template active compound ***cis*-3a**).



Cis-3e (MIC=1600)

C4	N8	C16	C17	O18
0.32	0.99	6.42	4.91	1.84
	-0.34	7.58	6.23	2.33
		-0.18	2.49	5.71
			-0.13	4.46
				-0.29

Figure 4. Submatrix (ETSC) and corresponding structure of the APh_2 antipharmacophore (the template compound ***cis*-3e**).

Figure 5 shows the dependences between frequencies of the Ph_1–Ph_6 occurrence in the compounds studied and corresponding values of their activities. As seen from Figure 5, pharmacophores appear with high frequencies in the class of active compounds. They are practically absent in the class of inactive compounds. In a similar way, maximal values are observed for the frequencies of APh_1–APh_2 appearance in the class of inactive compounds while for Ph_1–Ph_6 the frequencies are close to zero.

Table 2. Statistical characteristics for some of pharmacophores (Ph_i) and anti-pharmacophores (APh_i) calculated by ETM

Type of pharmacophore/ antipharmacophore (*template compound*)	*E. faecalis*		*S. aureus*	
	P_a	P_{in}[a]	P_a	P_{in}[a]
Ph_1(***cis*-3a**)	0.92	0.08	0.91	0.09
Ph_2(***cis*-3a**)	0.92	0.08	0.90	0.06
Ph_3(***cis*-3d**)	0.86	0.14	0.87	0.13
Ph_4(***cis*-3d**)	0.90	0.10	0.90	0.10
Ph_5(***trans*-3u**)	0.91	0.09	0.91	0.09
Ph_6(***cis*-3v**)	0.88	0.12	0.86	0.14
APh_1(***cis*-3e**)	0.19	0.81	0.18	0.82
APh_2(***cis*-3e**)	0.16	0.84	0.14	0.86
APh_3(***trans*-3c**)	0.16	0.84	0.15	0.85
APh_4(***trans*-3c**)	0.08	0.92	0.08	0.92
APh_5(***trans*-3k**)	0.12	0.88	0.11	0.89
APh_6(***cis*-3q**)	0.14	0.86	0.13	0.87

[a] Probability estimates for inactive compounds.

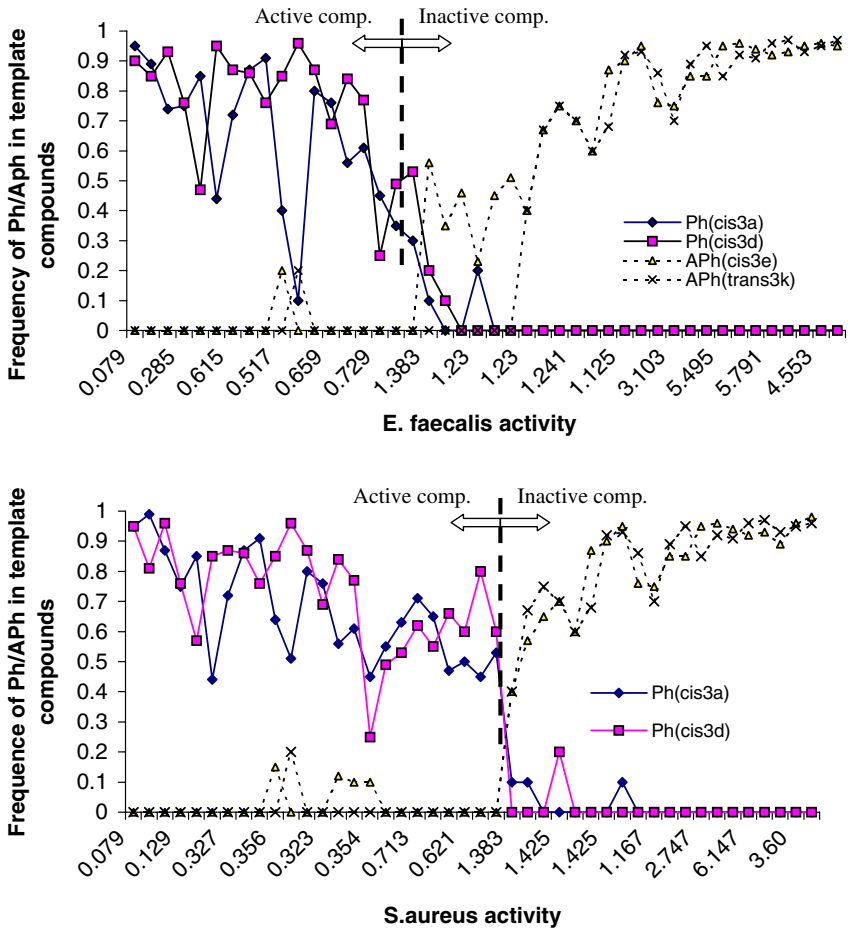


Figure 5. Frequency of the pharmacophores' (antipharmacophore's) occurrences in the compounds studied.

When comparing the structures of the pharmacophores and anti-pharmacophores, one can pay attention to the differences in their spatial and electron characteristics. Thus, both pharmacophores and anti-pharmacophores used together definitely play an important role for the activity prediction in the process of searching for a new drug. Thus, the set of activity/inactivity fragments found as the result of this study forms a basis for a system of the antibacterial activity prediction.

2.7. Results of the ETM–ANNs approach application

A data set containing 44 molecules was used. Thirty-two of the compounds were used for the model development, and 12 randomly selected compounds (**2, 6, 10, 11, 15, 19, 23, 25, 32, 35, 37, 40**) were used for the model validation (see Table 1) for both *E. faecalis* and *S. aureus*. As seen from the table, classes of active and inactive compounds against these two types of activity are very similar to each other.

For the *E. faecalis*, 170 fragments were selected from the ETM study. The ASNNs recognized correctly 71.9%, or 23 from 32 compounds, for the training set and 50% for the test set (6 compounds from 12) (Table 3).

The importance of the detected fragments for the observed activity was evaluated as the result of using

Table 3. The results based on data obtained from the pharmacophores set

Sets	All pharmacophores			Pharmacophores selected by pruning methods		
	WDs[a] number	Molecule		WDs number	Molecule	
		Amount	Predicted (%)		Amount	Predicted (%)
Data set 1 (*Enterococcus faecalis*)						
Training set	170	32	23 (71.9%)	3	32	28 (87.5%)
Test set	170	12	6 (50.0%)	3	12	10 (83.3%)
Data set 2 (*Staphylococcus aureus*)						
Training set	119	32	25 (78.1%)	5	32	28 (87.5%)
Test set	119	12	11 (91.6%)	5	12	11 (91.6%)

[a] WDs, weight descriptors.

pruning methods at the last step of the algorithm. The most part of the ETMC descriptors were found to be non-significant for the system of prognosis and eliminated by the pruning algorithms. As the result, only three ETMC fragments from 170 were selected as the most important ones. By this, ASNNs classified correctly 87.5%, or 28 compounds, from 32 for the training set and 83.3%, or 10 compounds, from 12 for the test set (Table 3).

For the *S. aureus* 119 fragments were selected. The results were correspondingly 78.1% (25 from 32 compounds) for the training set, and 91.6% (11 compounds from 12) for the test set. After applying the pruning methods, only five fragments from 119 were selected for further use (Table 3). After this, ASNNs classified correctly 87.5%, or 28 compounds from 32, for the training set and 91.6%, or 11 compounds from 12, for the test set (Table 3).

3. Conclusion

Peculiarities of conformational and electronic structures of compounds belonging to a large series of 2,3,5-substituted perhydropyrrolo[3,4-*d*]isoxazole-4,6-diones which possess antibacterial activity against *E. faecalis* (ATCC 29212) and *S.aureus* (ATCC 25923) have been studied. The results of the study agree satisfactorily with data obtained by other researchers relative to the same classes of compounds. A system for the antibacterial activity prediction was developed on the base of the pharmacophores and anti-pharmacophores revealed. The obtained system allows for the screening of active compounds and design of potent drugs. The results of the ETM–ANNs application to the test sample have shown that 75% of the compounds are classified correctly (Table 3). Correspondingly, the prediction made immediately from the results of the ETM can give much higher percentage, but this work is very tedious and needs the experts' participation, while ANNs can be used even by non-experts.

4. Experimental

All preparative experiments were carried out in benzene, which had been dried with the standard methods.[56] Melting points were determined on Electrothermal 9200 apparatus and uncorrected. IR spectra were recorded on Shimadzu FTIR-821 PC Fourier Transform IR Spectrometer; they are given in wavenumbers (cm^{-1}). ^1H NMR spectra were recorded on Bruker DPX-400 (400 MHz) High Performance Digital FT NMR Spectrometer using $CDCl_3$ with Me_4Si as an internal standard. Elemental analyses were performed on Carlo Erba-1106 instrument. Preparative chromatography was performed using Silica gel (Merck). *N*-Alkyl(aryl)-C-substituted phenylnitrones were prepared by the literature method.[57]

4.1. Synthesis of 3-(4-diethylaminophenyl)-2,5-dimethylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *cis*-3a and *trans*-3a (General procedure)

A mixture of *N*-methyl-*C*-(4-diethylaminophenyl)nitrone **1a** (3 mmol, 0.630 g) and *N*-methylmaleimide **2a** (3.3 mmol, 0.370 g) was dissolved in 50 ml benzene. The reacting mixture was refluxed for 6–12 h. During this time, the reaction was monitored by TLC. The products were separated by dry-column flash chromatography.[34] The mixture of ethylacetate and petroleum ether was used as an eluent (the polarity of eluent was increased to $R_f = 0.5$). The ***cis*-3a** and ***trans*-3a** isomers were recrystallized separately from THF/*n*-hexane mixture (1:6).

Spectroscopic and analytical data of 30 new compounds are given below.

4.1.1. 3-(4-Diethylaminophenyl)-2,5-dimethylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *cis*-3a and *trans*-3a. ***cis*-3a**: Yield: 61%, mp 129 °C. ^1H NMR: δ (ppm); 1.08 (t, 6H, $2CH_3$, $J \approx 7.0$ Hz), 2.53 (s, 3H, CH_3), 2.95 (s, 3H, CH_3), 3.19–3.31 (m, 4H, $2CH_2$), 3.55 (dd,1H, H_b, $J \approx 8.2/7.5$ Hz), 3.63 (d, 1H, H_c, $J \approx 8.59$ Hz), 4.78 (d, 1H, H_a, $J \approx 7.16$ Hz), 6.54 (d, 2H Ar-H, $J \approx 8.7$ Hz), 6.87(d, 2H Ar-H, $J \approx 8.6$ Hz). IR (KBr): ν (cm^{-1}) 1708.8 (C═O). Anal. Calcd For $C_{17}H_{23}N_3O_3$: C, 64.33; H, 7.30; N, 13.24. Found: C, 64.46; H, 7.58; N, 13.83.

***trans*-3a**. Yield: 30%, mp 117 °C. ^1H NMR: δ (ppm); 1.10 (t, 6H, $2CH_3$, $J \approx 7.0$ Hz), 2.34 (br, 3H, CH_3), 2.98 (s, 3H, CH_3), 3.26–3.31 (m, 4H, $2CH_2$), 3.60 (dd, 1H, H_b, $J \approx 3.7/3.7$ Hz), 4.39 (very broad, 1H, H_c), 3.84 (d, 1H, H_a, $J \approx 6.97$ Hz), 6.58 (d, 2H, Ar-H, $J \approx 8.50$ Hz), 7.02 (br, 2H, Ar-H). **IR** (KBr): ν (cm^{-1}); 1705.0 (C═O). Anal. Calcd For $C_{17}H_{23}N_3O_3$: C, 64.33; H, 7.30; N, 13.24. Found: C, 63.99; H, 7.30; N, 13.08.

4.1.2. 3-(4-Dimethylaminophenyl)-2,5-dimethylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *trans*-3b. Yield: 25%, mp 140 °C. **^1H NMR:**δ(ppm); 2.42 (br, 3H, CH_3), 2.90 (s, 6H, $2CH_3$), 2.98 (s, 3H, CH_3), 3.58 (dd, 1H, H_b, $J \approx 4.0/4.6$ Hz), 4.30 (very broad, 1H, H_c), 4.84 (d, 1H, H_a, $J \approx 7.21$ Hz), 6.81–7.19 (m, 4H, Ar-H). IR (KBr): ν (cm^{-1}); 1705.0 (C═O). Anal. Calcd For $C_{15}H_{19}N_3O_3$: C, 62.27; H, 6.62; N, 14.52. Found: C, 62.31; H, 6.93; N, 14.92.

4.1.3. 3-(4-Methoxyphenyl)-2,5-dimethylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *cis*-3c and *trans*-3c. ***cis*-3c**. Yield: 65%, mp 135 °C. ^1H NMR: δ (ppm); 2.54 (s, 3H, CH_3), 2.93 (s, 3H, CH_3), 3.57 (dd, 1H, H_b, $J \approx 8.2/8.8$ Hz), 3.71 (d, 1H, H_c, $J \approx 8.84$ Hz) 3.73 (s, 3H, CH_3), 4.80 (d, 1H, H_a, $J \approx 7.25$ Hz), 6.83 (d, 2H, Ar-H, $J \approx 8.0$ Hz), 7.03 (d, 2H, Ar-H, $J \approx 14.2$ Hz). IR (KBr): ν (cm^{-1}); 1712.7 (C═O). Anal. Calcd for $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.94; H, 6.09; N, 10.12.

***trans*-3c**. Yield: 23%, mp 193 °C. ^1H NMR: δ (ppm); 2.37 (br, 3H, CH_3), 2.99 (s, 3H, CH_3), 3.59 (dd, 1H,

H_b, $J \approx 3.4/3.7$ Hz), 3.75 (s, 3H, CH_3), ~4.30 (very broad, 1H, H_c), 4.85 (d, 1H, H_a, $J \approx 7.24$ Hz), 6.85 (d, 2H, Ar-H, $J \approx 8.52$ Hz), 7.15 (br, 2H, Ar-H). IR (KBr): ν (cm^{-1}); 1701.1 (C=O). Anal. Calcd For $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.56; H, 6.27; N, 10.11.

4.1.4. 3-(4-Benzyloxy-3-methoxyphenyl)-2,5-dimethylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *trans*-3d. Yield: 25%, mp 100 °C. ^{1}H NMR: δ (ppm); 2.42 (br, 3H, CH_3), 2.98 (s, 3H, CH_3), 3.60 (dd, 1H, H_b, $J \approx 4.0/4.2$ Hz), 3.83 (s, 3H, CH_3), ~4.50 (very broad, 1H, H_c), 4.84 (d, 1H, H_a, $J \approx 7.21$ Hz),5.08 (s, 2H, CH_2), 6.73-6.81 (m, 3H Ar-H), 7.20–7.35 (m, 5H, Ar-H). IR (KBr): ν (cm^{-1}); 1705.0 (C=O); Anal. Calcd For $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.33. Found: C, 64.71; H, 5.57; N, 6.92.

4.1.5. 2,5-Dimethyl-3-(4-methylphenyl)perhydropyrrolo-[3,4-*d*]isoxazole-4,6-dione, *cis*-3e and *trans*-3e. *cis*-3e. Yield: 46%, mp 161 °C. ^{1}H NMR: δ (ppm); 2.26 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.92 (s, 3H, CH_3), 3.61 (dd, 1H, H_b, $J \approx 8.2/7.6$ Hz), 3.72 (d, 1H, H_c, $J \approx 8.58$ Hz), 4.78 (d, 1H, H_a, $J \approx 7.22$ Hz), 6.99 (d, 2H, Ar-H, $J \approx 7.8$ Hz), 7.07 (d, 2H, Ar-H, $J \approx 7.8$ Hz). **IR** (KBr): ν (cm^{-1}); 1705.0 (C=O). Anal. Calcd for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.13; H, 6.00; N, 10.75.

***trans*-3e**. Yield: 37%, mp 131 °C. ^{1}H NMR: δ (ppm); 2.38 (s, 3H, CH_3), 2.45 (br, 3H, CH_3), 3.08 (s, 3H, CH_3), 3.71 (dd, 1H, H_b, $J \approx 6.6/6.7$ Hz), ~4.30 (very broad, 1H, H_c) 4.94 (d, 1H, H_a, $J \approx 7.20$ Hz), 7.20 (br, 4H, Ar-H). **IR** (KBr): ν (cm^{-1}); 1705.0 (C=O). Anal. Calcd For $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.72; H, 5.68; N, 10.15.

4.1.6. 2,5-Dimethyl-3-phenylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *cis*-3f and *trans*-3f. *cis*-3f. Yield: 34%, mp 127 °C. ^{1}H NMR: δ (ppm); 2.57 (s, 3H, CH_3), 2.92 (s, 3H, CH_3), 3.64 (dd, 1H, H_b, $J \approx 8.4/7.5$ Hz), 3.75 (d, 1H, H_c, $J \approx 8.62$ Hz), 4.81 (d, 1H, H_a, $J \approx 7.25$ Hz), 7.10–7.12 (m, 2H, Ar-H), 7.20-7.33 (m, 3H, Ar-H). IR (KBr): ν (cm^{-1}); 1705.0 (C=O). Anal. Calcd For $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.60; H, 5.66; N, 11.31.

***trans*-3f**. Yield: 43%, mp 140 °C. ^{1}H NMR: δ (ppm); 2.39 (br, 3H, CH_3), 3.00 (s, 3H, CH_3), 3.65 (dd, 1H, H_b, $J \approx 3.6/3.4$ Hz), ~4.20 (very broad, 1H, H_c) 4.87 (d, 1H, H_a, $J \approx 7.28$ Hz), 7.24–7.34 (m, 5H, Ar-H). IR (KBr): ν (cm^{-1}); 1703.0 (C=O). Anal. Calcd For $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.69; H, 5.86; N, 11.49.

4.1.7. 3-(3-Chlorophenyl)-2,5-dimethylperhydropyrrolo-[3,4-*d*]isoxazole-4,6-dione, *cis*-3g and *trans*-3g. *cis*-3g. Yield: 38%, mp 184 °C. ^{1}H NMR: δ (ppm); 2.58 (s, 3H, CH_3), 2.93 (s, 3H, CH_3), 3.64 (dd, 1H, H_b, $J \approx 8.3/7.5$ Hz), 3.72 (d, 1H, H_c, $J \approx 8.63$ Hz), 4.81 (d, 1H, H_a, $J \approx 7.18$ Hz), 7.02 (d, 2H, Ar-H, $J \approx 17.3$ Hz), 7.12 (s, 1H, Ar-H), 7.22 (dd, 1H Ar-H, $J \approx 9.6/7.6$ Hz). **IR** (KBr): ν (cm^{-1}); 1708.8 (C=O). Anal. Calcd for $C_{13}H_{13}ClN_2O_3$: C, 55.62; H, 4.67; N, 9.98. Found: C, 55.55; H, 4.73; N, 10.46.

***trans*-3g**. Yield: 38%, mp 126 °C. ^{1}H NMR: δ (ppm); 2.42 (br, 3H, CH_3), 3.00 (s, 3H, CH_3), 3.61 (dd, 1H, H_b, $J \approx 3.7/3.7$ Hz), ~4.0 (very broad,1H, H_c) 4.86 (d, 1H, H_a, $J \approx 7.30$ Hz), 7.05–7.27 (m, 4H, Ar-H). IR (KBr): ν (cm^{-1}); 1701.1 (C=O). Anal. Calcd for $C_{13}H_{13}ClN_2O_3$: C, 55.62; H, 4.67; N, 9.98. Found: C, 55.17; H, 4.67; N, 10.02.

4.1.8. 3-(3-Bromophenyl)-2,5-dimethylperhydropyrrolo-[3,4-*d*]isoxazole-4,6-dione, *cis*-3h and *trans*-3h. *cis*-3h. Yield: 42%, mp 186 °C. ^{1}H NMR: δ (ppm); 2.57 (s, 3H, CH_3), 2.93 (s, 3H, CH_3), 3.66 (dd, 1H, H_b $J \approx 8.6/8.3$ Hz), 3.72 (d, 1H, H_c, $J \approx 8.67$ Hz), 4.82 (d, 1H, H_a, $J \approx 7.14$ Hz), 7.05 (d, 1H, Ar-H, $J \approx 7.6$ Hz), 7.18 (dd, 1H, Ar-H, $J \approx 7.8/6.5$ Hz), 7.28 (s, 1H, Ar-H), 7.39 (d, 1H, Ar-H, $J \approx 7.8$ Hz). **IR** (KBr): ν (cm^{-1}); 1705.0 (C=O). Anal. Calcd for $C_{13}H_{13}BrN_2O_3$: C, 48.02; H, 4.03; N, 8.62. Found: C, 47.80; H, 4.05; N, 8.56.

***trans*-3h**. Yield: 39%, mp 129. ^{1}H NMR: δ (ppm); 2.42 (br, 3H, CH_3), 2.99 (s, 3H, CH_3), 3.61 (dd, 1H, H_b, $J \approx 3.8/3.8$ Hz), ~4.0 (very broad,1H, H_c) 4.87 (d, 1H, H_a, $J \approx 7.30$ Hz), 7.20–7.41 (m, 4H, Ar-H). IR (KBr): ν (cm^{-1}); 1705.0 (C=O). Anal. Calcd for $C_{13}H_{13}BrN_2O_3$: C, 48.02; H, 4.03; N, 8.62. Found: C, 48.14; H, 4.22; N, 8.87.

4.1.9. 3-(4-Bromophenyl)-2,5-dimethylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *cis*-3j and *trans*-3j. *cis*-3j. Yield: 45%, mp 195 °C. ^{1}H NMR: δ (ppm); 2.55 (s, 3H, CH_3), 2.92 (s, 3H, CH_3), 3.63 (dd, 1H, H_b, $J \approx 8.3/7.5$ Hz), 3.71 (d, 1H, H_c, $J \approx 8.57$ Hz), 4.81 (d, 1H, H_a, $J \approx 7.22$ Hz), 7.00 (d, 2H, Ar-H, $J \approx 8.4$ Hz), 7.41 (d, 2H, Ar-H, $J \approx 8.4$ Hz). IR (KBr): ν (cm^{-1}); 1708.8 (C=O). Anal. Calcd for $C_{13}H_{13}BrN_2O_3$: C, 48.02; H, 4.03; N, 8.62. Found: C, 48.02; H, 3.88; N, 8.71.

***trans*-3j**. Yield: 40%, mp 163 °C. ^{1}H NMR: δ (ppm); 2.40 (br, 3H, CH_3), 2.99 (s, 3H, CH_3), 3.58 (dd, 1H, H_b, $J \approx 3.7/3.6$ Hz), ~4.10 (very broad, 1H, H_c) 4.82 (d, 1H, H_a, $J \approx 7.30$ Hz), 7.13 (br, 2H, Ar-H), 7.45 (d, 2H, Ar-H, $J \approx 8.4$ Hz). IR (KBr): ν (cm^{-1}); 1701.1 (C=O). Anal. Calcd for $C_{13}H_{13}BrN_2O_3$: C, 48.02; H, 4.03; N, 8.62. Found: C, 48.05; H, 4.07; N, 8.78.

4.1.10. 3-(4-Diethylaminophenyl)-2-methyl-5-phenylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *cis*-3l and *trans*-3l. *cis*-3l. Yield: 48%, mp 142 °C. ^{1}H NMR: δ (ppm); 1.08 (t, 6H, $2CH_3$, $J \approx 7.0$ Hz), 2.60 (s, 3H, CH_3), 3.18–3.28 (m, 4H, $2CH_2$), 3.70 (dd, 1H, H_b, $J \approx 8.4/7.5$ Hz), 3.77 (d, 1H, H_c, $J \approx 8.70$ Hz), 4.93 (d, 1H, H_a, $J \approx 7.22$ Hz), 6.53 (d, 2H, Ar-H, $J \approx 8.5$ Hz), 7.01 (d, 2H, Ar-H, $J \approx 8.5$ Hz), 7.20–7.37 (m, 5H, Ar-H). IR (KBr): ν (cm^{-1}); 1708.8 (C=O). Anal. Calcd for $C_{22}H_{25}N_3O_3$: C, 69.64; H, 6.64; N, 11.07. Found: C, 69.59; H, 6.70; N, 11.39.

***trans*-3l**. Yield: 43%, 143 °C. ^{1}H NMR: δ (ppm); 1.10 (t, 6H, $2CH_3$), 2.41 (br, 3H, CH_3), 3.26–3.31 (m, 4H, $2CH_2$), 3.77 (dd, 1H, H_b, $J \approx 3.6/3.6$ Hz), ~4.39 (very

broad, 1H, H_c), 4.98 (d, 1H, H_a, $J \approx 7.28$ Hz), 6.60 (d, 2H, Ar-H, $J \approx 7.8$ Hz), 7.06 (br, 2H, Ar-H), 7.28–7.41 (m, 5H, Ar-H). IR (KBr): ν (cm^{-1}); 1716.5 (C=O). Anal. Calcd for $C_{22}H_{25}N_3O_3$: C, 69.64; H, 6.64; N, 11.07. Found: C, 69.31; H, 6.75; N, 10.96.

4.1.11. 3-(4-Methoxyphenyl)-2-methyl-5-phenylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *cis*-3n and *trans*-3n. *cis*-3n. Yield: 40%, mp 165 °C. ^{1}H NMR: δ (ppm); 2.61 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 3.75 (dd, 1H, H_b, $J \approx 7.3/7.4$ Hz), 3.83 (d, 1H, H_c, $J \approx 8.74$ Hz), 4.94 (d, 1H, H_a, $J \approx 7.38$ Hz), 6.85 (d, 2H, Ar-H, $J \approx 8.7$ Hz), 7.13–7.19 (m, 5H, Ar-H), 7.27–7.39 (m, 2H, Ar-H). **IR** (KBr): ν (cm^{-1}); 1712.7 (C=O). Anal. Calcd for $C_{19}H_{18}N_2O_4$: C, 67.44; H,5.36; N,8.28. Found: C, 67.38; H, 5.44; N, 8.21.

***trans*-3n**. Yield: 36%, mp 166 °C. ^{1}H NMR: δ (ppm); 2.42 (br, 3H, CH_3), 3.75 (s, 4H, H_b and CH_3), ~4.5 (very broad, 1H), 4.98 (d,1H, H_a, $J \approx 7.38$ Hz), 6.85 (d, 2H, Ar-H, $J \approx 8.6$ Hz), 7.28 (d, 2H, Ar-H, $J \approx 7.4$ Hz), 7.39–7.43 (m, 2H, Ar-H). IR (KBr): ν (cm^{-1}); 1712.7(C=O). Anal. Calcd for $C_{19}H_{18}N_2O_4$: C, 67.44; H, 5.36; N, 8.28. Found: C, 67.35; H, 5.34; N, 8.43.

4.1.12. 2-Methyl-3-(4-methylphenyl)-5-phenylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *cis*-3p and *trans*-3p. *cis*-3p. Yield: 44%, mp 138 °C. ^{1}H NMR: δ (ppm); 2.25 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 3.73 (dd, 1H, H_b, $J \approx 7.7.6/7.7$ Hz) ,3.81 (d, 1H, H_c, $J \approx 8.74$ Hz), 4.92 (d, 1H, H_a, $J \approx 7.35$ Hz), 7.10–7.30 (m, 9H, Ar-H). **IR** (KBr): ν (cm^{-1}); 1716.5 (C=O). Anal. Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.80; H, 5.65; N, 8.81.

***trans*-3p**. Yield: 49%, mp 149 °C. ^{1}H NMR: δ (ppm); 2.29(s, 3H, CH_3), 2.43 (br, 3H, CH_3), 3.76 (dd, 1H, H_b, $J \approx 3.6/3.5$ Hz), ~4.50 (very broad, 1H, H_c,), 4.97 (d, 1H, H_a, $J \approx 7.41$ Hz), 7.13–7.33 (m, 9H, Ar-H). IR (KBr): ν (cm^{-1}); 1716.5 (C=O). Anal. Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 71.11; H, 5.68; N, 8.97.

4.1.13. 2-Methyl-3,5-diphenylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *cis*-3q and *trans*-3q. *cis*-3q. Yield: 41%, mp 146 °C. ^{1}H NMR: δ (ppm); 2.64 (s, 3H, CH_3), 3.79(dd, 1H, H_b, $J \approx 8.6/7.5$ Hz), 3.89 (d, 1H, H_c, $J \approx 8.77$ Hz), 4.98 (d, 1H, H_a, $J \approx 7.39$ Hz), 7.09–7.47 (m, 10H, Ar-H). IR (KBr): ν (cm^{-1}); 1720.4 (C=O). Anal. Calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09. Found: C, 69.93; H, 5.54; N, 9.07.

***trans*-3q**. Yield: 48%, mp 182 °C. ^{1}H NMR: δ (ppm); 2.47 (br, 3H, CH_3), 3.80 (dd, 1H, H_b, $J \approx 3.8/3.6$ Hz), ~4.25 (very broad, 1H, H_c), 5.01 (d, 1H, H_a, $J \approx 7.42$ Hz), 7.19-7.44 (m, 10H, Ar-H). **IR** (KBr): ν (cm^{-1}); 1712.7 (C=O). Anal. Calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09. Found: C, 70.48; H, 5.55; N, 9.53.

4.1.14. 3-(3-Bromophenyl)-2-methyl-5-phenylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *cis*-3r and *trans*-3r. *cis*-3r. Yield: 43%, mp 197 °C. ^{1}H NMR: δ (ppm); 2.65 (s, 3H, CH_3), 3.78 (dd, 1H, H_b, $J \approx 7.3/8.8$ Hz), 3.85 (d, 1H, H_c, $J \approx 8.84$ Hz), 4.98 (d, 1H, H_a, $J \approx 7.25$ Hz), 7.19–7.25 (m, 4H, Ar-H), 7.36–7.44 (m, 5H, Ar-H). IR (KBr): ν (cm^{-1}); 1716.5 (C=O). Anal. Calcd For $C_{18}H_{15}BrN_2O_3$: C, 55.83; H, 3.90; N, 7.23. Found: C, 55.84; H, 3.69; N, 6.93.

***trans*-3r**. Yield: 46%, mp 149 °C. ^{1}H NMR: δ (ppm); 2.49 (s, 3H, CH_3), 3.75 (dd, 1H, H_b, $J \approx 3.7/3.6$ Hz), ~4.20 (very broad, 1H, H_c), 4.98 (d, 1H, H_a, $J \approx 7.46$ Hz), 7.18-7.27 (m, 4H, Ar-H), 7.33–7.44 (m, 5H, Ar-H). IR (KBr): ν (cm^{-1}); 1716.5 (C=O). Anal. Calcd for $C_{18}H_{15}BrN_2O_3$: C, 55.83; H, 3.90; N, 7.23. Found: C, 55.81; H, 3.98; N, 7.15.

4.1.15. 3-(4-Bromophenyl)-2-methyl-5-phenylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *cis*-3t and *trans*-3t. *cis*-3t. Yield: 42%, mp 136 °C. ^{1}H NMR: δ (ppm); 2.62 (s, 3H, CH_3), 3.78 (dd, 1H, H_b, $J \approx 7.86/8.0$ Hz), 3.84 (d, 1H, H_c, $J \approx 8.71$ Hz), 4.98 (d, 1H, H_a, $J \approx 7.23$ Hz), 7.13-7.40 (m, 9H, Ar-H). IR (KBr): ν (cm^{-1}); 1716.5 (C=O). Anal. Calcd for $C_{18}H_{15}BrN_2O_3$: C, 55.83; H, 3.90; N, 7.23. Found: C, 55.77; H, 3.75; N, 7.13.

***trans*-3t**. Yield: 42%, mp 182 °C. ^{1}H NMR: δ (ppm); 2.48 (br, 3H, CH_3), 3.74 (dd, 1H, H_b, $J \approx 3.6/3.7$ Hz), ~4.20 (very broad, 1H, H_c) 4.99 (d, 1H, H_a, $J \approx 7.44$ Hz), 7.20–7.43 (m, 9H, Ar-H). **IR** (KBr): ν (cm^{-1}); 1716.5 (C=O). Anal. Calcd for $C_{18}H_{15}BrN_2O_3$: C, 55.83; H, 3.90; N, 7.23. Found: C, 55.93; H, 3.74; N, 7.12.

4.1.16. 3-(4-Dimethylaminophenyl)-5-methyl-2-phenylperhydropyrrolo[3,4-*d*]isoxazole-4,6-dione, *cis*-3v and *trans*-3v. *cis*-3v. Yield: 45%, mp 186 °C. ^{1}H NMR: δ (ppm); 2.87 (s, 6H, $2CH_3$), 2.89 (s, 3H, CH_3), 3.78 (dd, 1H, H_b, $J \approx 7.9/8.6$ Hz), 4.55 (d, 1H, H_c, $J \approx 9.02$ Hz), 4.99 (d, 1H, H_a, $J \approx 7.51$ Hz), 6.58 (d, 2H, Ar-H, $J \approx 8.5$ Hz), 6.93-7.19 (m, 7H, Ar-H). IR (KBr): ν (cm^{-1}); 1708.8 (C=O). Anal. Calcd for $C_{20}H_{21}N_3O_3$: C, 68.36; H, 6.02; N, 11.96. Found: C, 68.15; H, 5.97; N, 11.89.

***trans*-3v**. Yield: 41%, mp 178 °C. ^{1}H NMR: δ (ppm); 2.64 (s, 3H, CH_3), 2.86 (s, 6H, $2CH_3$), 3.72 (d, 1H, H_b, $J \approx 7.15$ Hz), 4.92 (d, 1H, H_a, $J \approx 7.21$ Hz), 5.32 (s, 1H, H_c), 6.62 (d, 2H, Ar-H, $J \approx 7.5$ Hz), 6.80–6.83 (m, 1H, Ar-H), 6.93 (d, 2H, Ar-H, $J \approx 7.5$ Hz), 7.11 (t, 2H, Ar-H, $J \approx 8.0$ Hz), 7.21 (t, 2H, Ar-H, $J \approx 8.50$ Hz). IR (KBr): ν (cm^{-1}); 1705 (C=O). Anal. Calcd for $C_{20}H_{21}N_3O_3$: C, 68.36; H, 6.02; N, 11.96. Found: C, 68.26; H, 6.32; N, 12.28.

Spectroscopic and analytical data of ***cis*-3b**, ***cis*-3d**, ***cis*-3i**, ***Trans*-3i**, ***cis*-3k**, ***trans*-3k**, ***cis*-3m**, ***trans*-3m**, ***cis*-3o**, ***trans*-3o**, ***cis*-3s**, ***trans*-3s**, ***cis*-3u**, ***trans*-3u** were reported previously.[13,14]

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