

# Design, synthesis, computational and biological evaluation of new anxiolytics

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**Abstract**—New anxiolytics have been discovered by prediction of biological activity with computer programs PASS and DEREK for a heterogeneous set of 5494 highly chemically diverse heterocyclic compounds (thiazoles, pyrazoles, isatins, a-fused imidazoles and others). The majority of tested compounds exhibit the predicted anxiolytic effect. The most potent activity was found in 2-(4-nitrophenyl)-3-(4-phenylpiperazinomethyl)imidazo[1,2-*a*]pyridine **8**, 1-[(4-bromophenyl)-2-oxoethyl]-3-(1,3-dioxolano)-2-indolinone **3**, 5-hydroxy-3-methoxycarbonyl-1-phenylpyrazole **5** and 2-(4-fluorophenyl)-3-(4-methylpiperazinomethyl)imidazo[1,2-*a*]pyridine **7**. The application of the computer-assisted approach significantly reduced the number of synthesized and tested compounds and increased the chance of finding new chemical entities (NCEs).

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## 1. Introduction

Neurotic disturbances, anxiety, neurosis-like disorders and stress-related illnesses are widespread, and many types of pharmaceuticals have been developed for their treatment. They influence different molecular targets including GABA-A-benzodiazepine receptor complex,<sup>1</sup> glutamate<sup>2</sup> and 5HT<sup>1</sup> receptors. However, all currently used anxiolytics demonstrate serious adverse effects.<sup>3</sup> Therefore, to reduce the probability of side reactions, there is an urgent need to find new anxiolytics, preferably in chemical classes in which such activity has not yet been observed.

**Keywords:** Anxiolytics; Synthesis; Thiazoles; Pyrazoles; 2-Indolinones; Fused imidazoles; PASS.

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Computer-aided structure–activity relationship analysis and molecular modelling are widely used now by pharmaceutical chemists to discover new lead compounds and optimize their structure and properties.<sup>4</sup> However, the majority of available approaches are focused on a single macromolecular target, or on the only known pharmacological/biochemical action, and/or compounds from the same chemical series. Most (Q)SAR methods are focused on a single biological activity, whereas in reality each compound has both main and side pharmacological effects.<sup>5,6</sup> Moreover, the number of existing targets is expected to increase from about 500 to about 5000–10,000 in a few years;<sup>7</sup> thus, experimental evaluation of potential biological activity is becoming more complicated.

An innovative approach to computer-aided prediction of general biological activity spectra on the basis of chemical

structure of a compound has been developed<sup>8–11</sup> and is now widely used.<sup>12–17</sup> This approach is based on a robust analysis of structure–activity relationships in a heterogeneous training set,<sup>9</sup> including many thousands of compounds from different chemical series. The approach is the basis of the computer program PASS (Prediction of Activity Spectra for Substances). PASS version 1.811 predicts 900 types of biological activity for a compound with an average accuracy of about 85% according to the leave-one-out cross-validation procedure (LOO CV).

To provide greater diversity, many potentially synthesizable compounds from different chemical classes should be investigated. In contrast to high-throughput screening programs carried out by pharmaceutical companies, the academic community has very limited resources for the execution of interdisciplinary projects for experimental studies of new pharmaceutical agents. Using PASS predictions, the number of ‘actives’ in the selected compounds can be increased by up to 17 times.<sup>15</sup> Thus, PASS-based computer pre-screening of large databases of diverse compounds can increase the probability of finding of new anxiolytic agents, and reduce the number of compounds that have to be synthesized and studied experimentally in *in vivo* tests.

## 2. Results and discussion

### 2.1. Compounds design

Potentially synthesizable compounds were designed by five groups of organic chemists from different institutions: Moscow State University (MSU), the Institute of Organic Chemistry of Urals Division of the Russian Academy of Science (IOC), the Institute of Chemistry of the Moldova Academy of Science (IC), Leuven University (LU) and the Aristotelian University of Thessaloniki (AU). In total, a virtual set of 5494 structures has been designed. The proposed compounds belonged to numerous chemical classes, including substituted isatins, pyrazoles, thiazoles and imidazoles fused with pyridine, thiazole and benzothiazole rings.

Isatins (1*H*-indole-2,3-diones) are synthetically versatile precursors of several other classes (e.g., indoles and quinolines), and are also intermediates for drug synthesis. It has recently been reported that isatin possesses anticonvulsant and proconvulsant activities<sup>18</sup> along with other pharmacological properties.<sup>19,20</sup> 3-Hydroxy-3-substituted oxindoles<sup>21</sup> derived from isatin, 3-(4-thiazolidone-2-hydrazono)-isatin,<sup>22</sup> 1-morpholino-methyl-3-(aryloxy-arylthioacetyl hydrazono)-isatin<sup>23</sup> and isatin based spiroazetidiones<sup>24</sup> have been reported to possess anticonvulsant activity. Hydrazones and Schiff and Mannich bases of isatin also exhibit significant anticonvulsant activity.<sup>25</sup> Isatin derivatives are also reported to show antibacterial, antifungal, cytotoxic and anti-HIV activities.<sup>26–28</sup>

The thiazole group is of great importance in biological systems. It has been found that alkyl/aryl-aminoacetyl derivatives of 2-amino-4-phenylthiazolyl,<sup>29</sup> 2-amino-benzothiazolyl,<sup>30</sup> 2-amino (substituted) benzothiazolyl,<sup>31</sup>

2-phenyl-amino-4-phenyl-thiazolyl,<sup>32</sup> 2-amino-4-methyl-thiazolyl<sup>33</sup> and in general 2-(*N*-substituted or *N,N*-disubstituted) acetamido derivatives<sup>34</sup> have significant local anaesthetic activity. Anti-inflammatory, analgesic and antipyretic activities for some thiazolyl and benzothiazolyl derivatives are also known.<sup>35,36</sup> Meloxicam, for example, is a new NSAID possessing a thiazole group. A number of thiazolyl-amino ketones as well as thiazolyl amides have been found to be strong anti-inflammatory agents.<sup>37,38</sup> Also, in a series of hydrazine-thiazoles and derivatives (and their ‘open chain’ thiosemicarbazide analogues) inhibitory activity to MAO rat liver mitochondria was found.<sup>39</sup>

Heterocycles of the pyrazole class are widely used in medicine. They are non-narcotic analgesics,<sup>40</sup> PDE 5 inhibitors<sup>41</sup> and factor Xa inhibitors.<sup>42</sup> Pyrazole derivatives also have antimicrobial and antitumour activity.<sup>43</sup> Although fused imidazo[1,2-*a*]pyridine-2-acetamides (alpidem and zolpidem) possess anxiolytic activity, there are no reports on such properties for 3-dialkylamino-methyl-derivatives of imidazo[1,2-*a*]pyridines and related *a*-fused imidazoles.

In spite of the lack of information about the anxiolytic effect of the above-mentioned classes of heterocyclic compounds, such an effect was predicted with the computer program PASS for some particular derivatives (see below).

### 2.2. Compound selection

Prediction of biological activity spectra was made for 5494 structures designed by the chemical synthesis teams. The computer program PASS was used to predict the biological activity spectra of designed compounds, including 900 pharmacological effects and mechanisms of actions. Since PASS is based on so-called ligand-based design approach, it cannot discover new targets and needs the training set of ligands that were earlier tested experimentally on anxiolytic action. The list of activities, which are currently predicted by PASS, includes 40 kinds of biological activity associated with the anxiolytic effect (Table 1).

On the basis of computer-aided predictions we selected potential anxiolytics (virtual hits). The following criteria were used for the hits’ selection:

1. Compounds were selected as hits if the value of probability ( $P_a$ ) of possessing anxiolytic activity exceeded 50%.
2. If, among the compounds designed by a certain chemical team, too many similar compounds satisfied criterion 1, then only several representative structures were selected.
3. If none of the designed compounds appeared as hits, then the cutoff value  $P_a$  was decreased for the compounds designed by a given chemical team.
4. If, for a compound selected as a hit, any adverse and/or toxic effects were predicted, then this compound was excluded from the subset of hits.

The structures of eight potential anxiolytics from different chemical series, selected on the basis of these criteria,

**Table 1.** Biological activities associated with anxiolytic effect in PASS

No.	Number <sup>a</sup>	MPA <sup>b</sup> , %	Activities
1	1462	82	Anxiolytic
2	390	93	5 Hydroxytryptamine 1 agonist
3	232	92	5 Hydroxytryptamine 1A agonist
4	126	91	5 Hydroxytryptamine 1A antagonist
5	131	94	5 Hydroxytryptamine 1D agonist
6	60	89	5 Hydroxytryptamine 1D antagonist
7	131	90	5 Hydroxytryptamine 2A antagonist
8	24	85	5 Hydroxytryptamine 2B antagonist
9	16	79	5 Hydroxytryptamine 2C agonist
10	60	86	5 Hydroxytryptamine 2C antagonist
11	20	83	5 Hydroxytryptamine 3 agonist
12	242	94	5 Hydroxytryptamine 3 antagonist
13	51	93	5 Hydroxytryptamine 4 antagonist
14	986	86	5 Hydroxytryptamine antagonist
15	219	87	5 Hydroxytryptamine uptake inhibitor
16	30	97	Adenosine A1 receptor agonist
17	147	92	AMPA receptor antagonist
18	146	92	Benzodiazepine agonist
19	16	91	Benzodiazepine inverse agonist
20	5	83	Benzodiazepine omega receptor agonist
21	325	91	Beta adrenoreceptor antagonist
22	40	83	Chloride channel agonist
23	88	94	Cholecystokinin B antagonist
24	7	84	Corticotropin releasing factor 1 receptor antagonist
25	10	91	DOPA decarboxylase inhibitor
26	58	81	GABA A receptor agonist
27	11	76	GABA A receptor antagonist
28	12	95	GABA B receptor antagonist
29	209	83	GABA receptor agonist
30	104	91	Glutamate receptor agonist
31	14	97	Histamine H3 receptor agonist
32	42	84	MAO A inhibitor
33	38	95	Melatonin agonist
34	47	89	Neurokinin 1 antagonist
35	79	95	Neurokinin 2 antagonist
36	161	94	Neurokinin antagonist
37	414	89	NMDA receptor antagonist
38	41	92	NMDA receptor glycine site antagonist
39	101	90	Sigma receptor antagonist
40	255	94	Substance P antagonist

<sup>a</sup> Number, is the number of compounds from the PASS training set exhibiting a particular activity.

<sup>b</sup> MPA, is the minimal prediction accuracy (calculated by leave-one-out procedure) for every type of activity from the PASS training set.

are presented in Figure 1: **1** (from IOC), **2** and **3** (from IC), **4** (from AU); **5** (from LU), **6–8** (from MSU).

As additional criteria, prediction of toxicities with the expert system DEREK<sup>44</sup> was performed for the eight selected compounds. For the most of them, carcinogenicity, mutagenicity and skin sensitization were estimated as being plausible. This level of probability of possessing these undesirable effects is not an obstacle for investigation of compounds at the stage of lead finding; however, these adverse effects should be tested for at the next stages of preclinical study. Thus, all selected hits were synthesized and experimentally tested for their anxiolytic effect.

### 2.3. Chemistry

All selected compounds predicted to be anxiolytics were successfully synthesized. Pyrazole derivatives **1** and **5** were obtained from phenylhydrazine by reaction with

fluorinated  $\beta$ -oxo-ester (in refluxing ethanol) or with dimethyl acetylenedicarboxylate (Scheme 1).

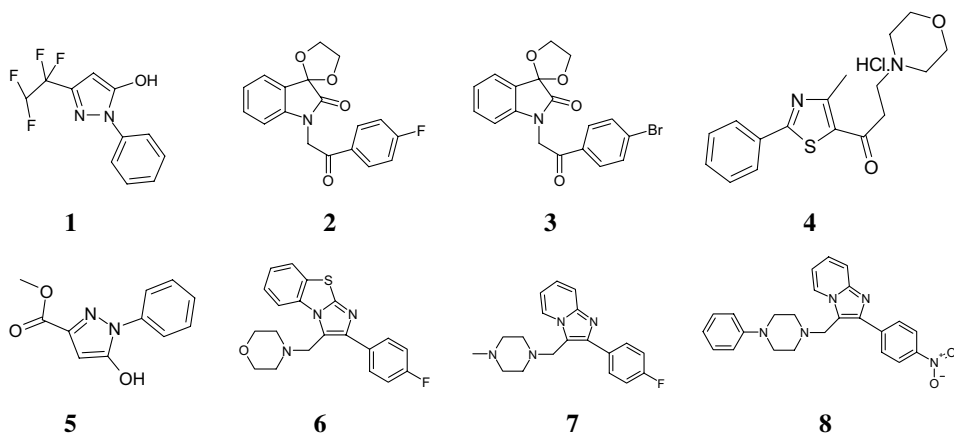
Isatin derivatives **2** and **3** were prepared by phenacylation of isatin ketal **9** with  $\omega$ -bromoacetophenones at room temperature (Scheme 2).

The thiazole derivative **4** was obtained by a modified Mannich reaction (Scheme 3) described earlier.<sup>33</sup>

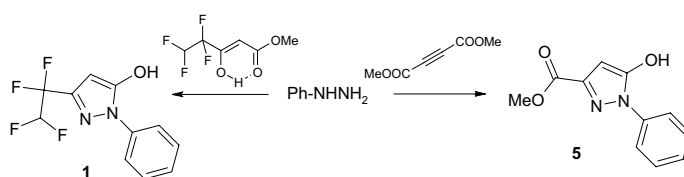
Fused dialkylaminomethylimidazoles **6**, **7** and **8** were obtained by adapting the Mannich reaction (rarely applied to this class<sup>45</sup>) to known parent imidazoles **10–12**<sup>46–48</sup> (Scheme 4).

### 2.4. Pharmacology

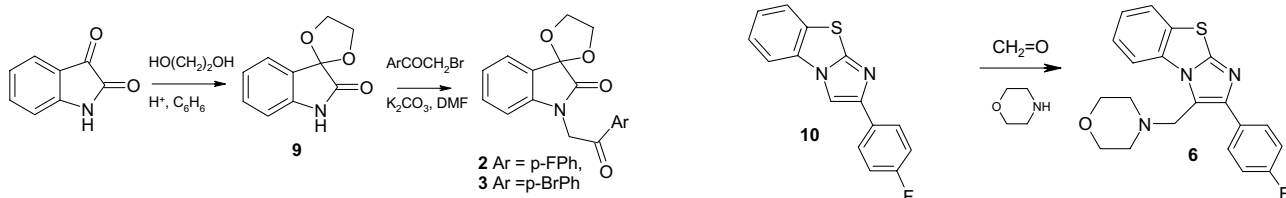
Anxiolytic activity in selected compounds was evaluated by the conflict situation test (Table 2). This showed that the majority of tested compounds produced a significant



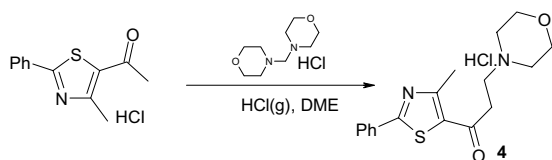
**Figure 1.** Structures of potential anxiolytics selected on the basis of PASS prediction from 5494 virtually designed compounds.



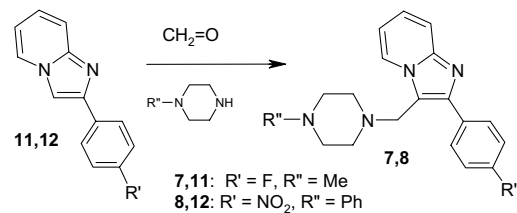
**Scheme 1.** The synthesis of pyrazoles **1** (5-hydroxy-3-(1,1,2,2-tetrafluoroethyl)-1-phenylpyrazole) and **5** (5-hydroxy-3-methoxycarbonyl-1-phenylpyrazole).



**Scheme 2.** The synthesis of compounds **2** (1-[(4-fluorophenyl)-2-oxoethyl]-3-(1,3-dioxolano)-2-indolinone) and **3** (1-[[2-(4-bromophenyl)-2-oxoethyl]-3-(1,3-dioxolano)-2-indolinone).



**Scheme 3.** The synthesis of compound **4** (1-(4-methyl-2-phenyl-1,3-thiazol-5-yl)-3-morpholin-4-yl-propan-1-one hydrochloride).



**Scheme 4.** The synthesis of compounds **6** (3-(morphinomethyl)-2-(4-fluorophenyl)imidazo[2,1-*b*][1,3]thiazole), **7** (2-(4-fluorophenyl)-3-(4-methylpiperazinomethyl)imidazo[1,2-*a*]pyridine) and **8** (2-(4-nitrophenyl)-3-(4-phenylpiperazinomethyl)imidazo[1,2-*a*]pyridine).

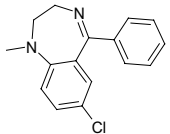
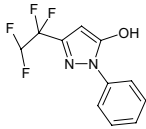
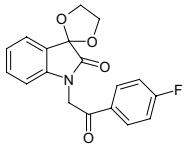
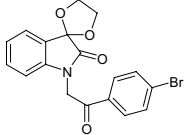
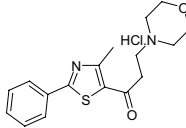
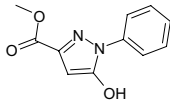
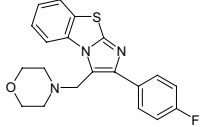
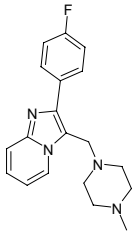
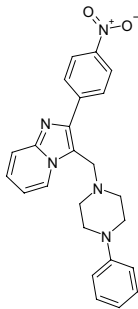
anxiolytic action displayed as a considerably increased number of punished water licks (Fig. 2).

The reference anxiolytic agent medazepam was also tested in the same series of experiments. Administration of medazepam in rats increased the number of punished water licks from 167 to 392. Medazepam is a benzodiazepine-like compound acting on the GABA-A-benzodiazepine receptor complex. Biological activities of medazepam agreed well with those predicted by PASS: calculated probabilities ( $P_a$ ) equal to 89%, 78%, 73% for ‘anxiolytic’, ‘Benzodiazepine 1 agonist’ and ‘GABA receptor agonist’ activities, respectively.

The average number of punished water licks caused by administration of compound **1** is 243; that is, less than for medazepam but more than found for the control. ‘Benzodiazepine 1 agonist’ is predicted as the most probable (36.0%) mechanism of anxiolytic action for compound **1**. Compound **2** caused on average 308 punished water licks, which is comparable to both the control and compound **1** but less than for medazepam. ‘Benzodiazepine 1 agonist’ (46.6%) and ‘5HT2A antagonist’ (33.8%) are predicted as the most probable mechanisms of anxiolytic action for compound **2**.

Compound **3** was administrated in DMSO because it is unstable in aqueous solution. In general, DMSO is

**Table 2.** The results of PASS prediction and experimental testing of selected compounds

Name	Structure	$P_a$	$P_i$	Predicted activity	Number of punished water licks
Control					167 ± 32
Medazepam		0.891 0.783 0.734	0.006 0.002 0.005	Anxiolytic Benzodiazepine 1 agonist GABA receptor agonist	398 ± 52
1		0.434 0.359	0.051 0.132	Anxiolytic Benzodiazepine 1 agonist	243 ± 71*
2		0.318 0.466 0.338	0.088 0.030 0.020	Anxiolytic Benzodiazepine 1 agonist 5 HT 2A antagonist	308 ± 53*
DMSO					172 ± 52
3 (in DMSO)		0.394 0.450	0.062 0.038	Anxiolytic Benzodiazepine 1 agonist	785 ± 38*
4		0.372 0.397	0.068 0.022	Anxiolytic GABA A agonist	463 ± 100*
5		0.714 0.506 0.392	0.008 0.017 0.023	Anxiolytic Benzodiazepine 1 agonist GABA A agonist	655 ± 78*
6		0.694 0.688 0.354	0.010 0.005 0.007	Anxiolytic GABA A agonist Benzodiazepine agonist	475 ± 59*
7		0.579 0.641 0.515 0.469 0.425	0.023 0.001 0.009 0.010 0.007	Anxiolytic Benzodiazepine omega agonist GABA A agonist 5HT2C agonist 5HT3 agonist	588 ± 66*
8		0.655 0.670 0.424 0.415 0.368	0.014 0.001 0.018 0.007 0.045	Anxiolytic Benzodiazepine omega agonist GABA A receptor agonist 5HT3 agonist 5HT2C agonist	800 ± 128*

\* Difference from control group is determined by  $P < 0.05$ .

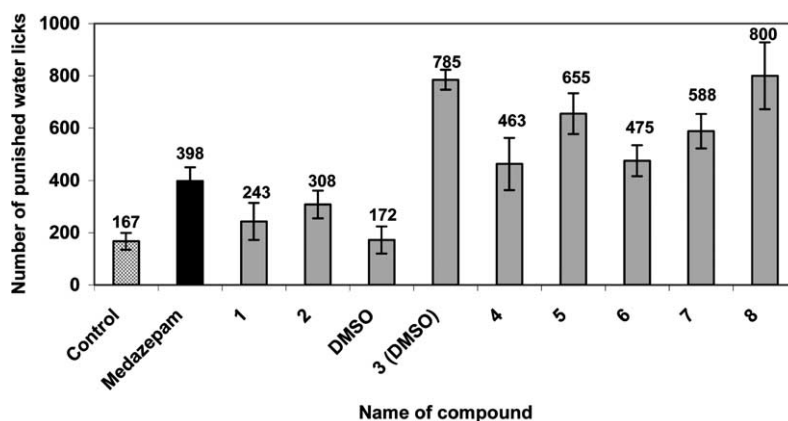


Figure 2. Anxiolytic activity of tested compounds.

widely used in biological screening as a universal solvent. However, to exclude the influence of DMSO on the anxiolytic effect shown by compound **3**, we determined the anxiolytic activity of DMSO separately. No significant anxiolytic activity of DMSO was observed. The average number of punished water licks caused by compound **3** was 785. This value is almost twice of that for medazepam. Agonistic action on benzodiazepine 1 receptors is predicted as the most probable (45%) mechanism of anxiolytic action for compound **3**.

Administration of compound **4** increased the average number of punished water licks to 463. However, its anxiolytic activity does not differ significantly from that of medazepam. ‘GABA A agonist’ (39.7%) is predicted as the most probable mechanism of anxiolytic action of compound **4**.

The average number of punished water licks caused by compound **5** was 655. This value is less than that for compound **3**, but is significantly more than the number of punished water licks observed for medazepam. ‘Benzodiazepine 1 agonist’ (50.6%) and ‘GABA A agonist’ (39.2%) are predicted as the most probable mechanisms of anxiolytic action for compound **5**.

Administration of compound **6** increased the average number of punished water licks to 475, which is approximately the same as the value for medazepam. The most probable mechanisms of anxiolytic action predicted by PASS for compound **6** are ‘GABA A agonist’ (68.8%) and ‘Benzodiazepine agonist’ (35.4%).

Administration of compound **7** increased the number of punished water licks to 588. This value exceeds the value for compound **6** and medazepam but it is less than that for compounds **5** and **3**. The most probable predicted mechanisms of anxiolytic action are ‘Benzodiazepine omega agonist’ (64.1%), ‘GABA A agonist’ (51.5%), ‘5HT2C agonist’ (46.9%) and ‘5HT3 agonist’ (42.5%).

Compound **8** revealed the most potent anxiolytic effect in comparison with the other tested substances. The number of observed punished water licks for this compound was 800. The most probable predicted mecha-

nisms of anxiolytic action for compound **8** are ‘Benzodiazepine omega agonist’ (67%), ‘GABA A agonist’ (42.4%), ‘5HT3 agonist’ (41.5%) and ‘5HT2C agonist’ (36.8%).

Thus, we found the following order of average anxiolytic potency for tested compounds: **8** > **3** > **5** > **7** > **6** > **4** > medazepam > **2** > **1** > DMSO > control. This does not completely correspond to the order of calculated probabilities for their anxiolytic effect  $P_a$ : 65.6% (compd **8**), 39.4% (compd **3**), 71.4% (compd **5**), 57.9% (compd **7**), 69.4% (compd **6**), 37.2% (compd **4**), 89.1% (medazepam), 31.8% (compd **2**), 43.4% (compd **1**). Since  $P_a$  values calculated by PASS reflect the probabilities of compounds’ belonging to the class of ‘actives’ rather than the values of their potency, the absence of such correspondence is not surprising.

In the majority of tested compounds the probability of anxiolytic effect ( $P_a$ ) is less than 70%, hence, one may suggest that they may appear to be new chemical entities (NCEs).<sup>9,10</sup> To check this suggestion, we performed a direct estimation of their similarity to known pharmacological agents.

## 2.5. Similarity assessment

We compared the selected compounds with those from the MDDR database.<sup>49</sup> The ‘similarity’ procedure of ISIS/Base 2.1.1<sup>49</sup> was used in the search for similar compounds. It is considered that compounds exhibit similar biological activity if their similarity is more than 70%.

No compound had a similarity with compound **1** greater than 65%. Forty-one compounds had about 60% similarity to compound **1**. However, most of these compounds possess antifungal (20 compounds), antihypertensive (11 compounds) and antiarthritic (6 compounds) effects, but none has an anxiolytic effect. The most similar anxiolytics (19 compounds) from the MDDR database have about 50% similarity to compound **1**. Compound **2** had about 70% similarity with nine compounds from MDDR database, but none of them is known to have anxiolytic activity. Most of them (seven compounds) have an antidiabetic effect and



aldose reductase inhibiting activity. The most similar anxiolytics (five compounds) had about 65% similarity to compound **2**. Similar results were obtained for compound **3**: 10 compounds from MDDR database with antidiabetic effect and aldose reductase inhibiting activity had about 70% similarity. The most similar anxiolytics (three compounds) had about 65% similarity to compound **3**. Eight compounds from MDDR database had about 70% similarity to compound **4**. All of them are antipsychotics; seven are dopamine D4 antagonists and one is a sigma antagonist. One anxiolytic from the MDDR database had 65% similarity to compound **4**. There are six compounds in MDDR database whose similarity to compound **5** exceeded 70%. All of them are neuronal injury inhibitors; five compounds are lipid peroxidation inhibitors and one compound is an antioxidant. Ten anxiolytics from the MDDR database had about 60% similarity to compound **5**. Thirteen compounds with 80% similarity to compound **6** were found in the MDDR database. Most of them are anxiolytics (nine compounds), anticonvulsants (nine compounds) and cognition enhancers (four compounds). Ten compounds from the MDDR database had 80% similarity to compound **7**. They are antipsychotics (six compounds), dopamine D4 antagonists (five compounds), anxiolytics (four compounds), anticonvulsants (three compounds) and three compounds with sedative/hypnotic effect. Three compounds from the MDDR database had about 80% similarity to compound **8**. All of them are antipsychotics and two are dopamine D4 antagonists. Sixteen anxiolytics were similar to compound **8**, with 70% similarity.

Thus, only 3-(Morpholinomethyl)-2-(4-fluorophenyl)-imidazo[2,1-*b*][1,3]thiazole **6**, 2-(4-fluorophenyl)-3-(4-methylpiperazinomethyl)imidazo[1,2-*a*]pyridine **7** and 2-(4-nitrophenyl)-3-(4-phenylpiperazinomethyl)imidazo[1,2-*a*]pyridine **8** from the tested compounds had considerable similarity to known anxiolytics from the MDDR database, and could have been discovered by similarity search at the 70% threshold. All other compounds can be considered as quite new anxiolytic agents, presumably NCEs.

### 3. Conclusions

To discover new anxiolytics, an innovative computer-assisted approach based on the PASS predictions has been applied. The probability of finding new chemical entities was increased by virtual combinatorial design of highly diverse chemical compounds including different types of heterocycles (thiazoles, pyrazoles, isatins, a-fused imidazoles). Eight prospective hits from 5494 structures presented in the initial database, selected on the basis of computer prediction, were synthesized and tested as potential anxiolytics. Six tested compounds **3**, **4**, **5**, **6**, **7** and **8** have equivalent or higher anxiolytic effect than the reference anxiolytic medazepam. Compounds **8** (2-(4-nitrophenyl)-3-(4-phenylpiperazinomethyl)imidazo[1,2-*a*]pyridine) and **3** (1-[(4-bromophenyl)-2-oxoethyl]-3-(1,3-dioxolano)-2-indolinone) have the most potent anxiolytic effect, exceeding that of medazepam by a fac-

tor of two. Although an anxiolytic effect of some of the tested compounds might be predicted based on their structural similarity to known anxiolytics, the finding of an anxiolytic effect in compounds **3**, **5** and **4** can be considered as the discovery of NCEs. Thus, computer prediction provides the possibility of (1) finding of new potent anxiolytic agents and (2) significantly decreasing the number of synthesized and tested compounds.

## 4. Experimental

### 4.1. PASS method

PASS software (Prediction of Activity Spectra for Substances) was used for prediction of anxiolytic effect for 5494 compounds designed in this study. PASS version 1.811 predicts 900 types of biological activity with a mean accuracy of 85%. The general list of activity types predicted by the current version of PASS is given on the Website.<sup>9</sup> For representation of the structural formula of a compound, PASS uses MNA (multilevel neighbourhoods of atoms) descriptors.<sup>50</sup> The PASS training set contains 45,660 substances, which are represented by 41644 different MNA descriptors. The calculation of biological activity spectrum is based on structure–activity relationships that are stored in a SAR knowledgebase.

A list of predicted activities, including anxiolytic effect and molecular mechanisms of action, is given in Table 1. In most cases the prediction accuracy, calculated by leave-one-out cross-validation, is better for molecular mechanisms than for anxiolytic effect. Mean prediction accuracy is about 82% for anxiolytic effect, whereas for various mechanisms of anxiolytic action it varies from 83% to 97%. Only three mechanisms have slightly less prediction accuracy than that of anxiolytic effect (5 Hydroxytryptamine 2C agonist, GABA A receptor antagonist and GABA A receptor agonist).

PASS uses MOL- or SDF-files as input of structural formula(s), and PASS output is presented as a list of activity names and probability values for the compound to be either active ( $P_a$ ) or inactive ( $P_i$ ), respectively. Interpretation of prediction results is based on consideration of  $P_a$  values.

1.  $P_a > 0.7$ : the chance of finding activity experimentally is high; in many cases the compound may be a close analogue of known pharmaceutical agents.
2.  $0.5 < P_a < 0.7$ : the chance of finding activity experimentally is less; the compound is not so similar to known pharmaceutical agents.
3.  $P_a < 0.5$ : the chance of finding activity experimentally is even less; the compound has only a low similarity to the compounds from the training set.

### 4.2. DEREK method

To estimate the toxic effects of compounds, their structures were run through DEREK,<sup>44</sup> an expert system for prediction of toxicity, developed by Lhasa Ltd (Leeds,

UK). DEREK predicts six categories, namely certain, probable, plausible, implausible, improbable or impossible, for carcinogenicity, mutagenicity and skin sensitization.

### 4.3. Chemical methods

<sup>1</sup>H NMR spectra were recorded on Aspect 3000, Bruker AC400 (400 MHz), Bruker AC-80 (80 MHz), Bruker-AW-80 spectrometers, and the chemical shifts are reported in parts per million ( $\delta$ ) downfield from tetramethylsilane as internal standard. Electron ionization mass spectra were recorded on a VG-250 spectrometer (VG Labs., Tritech England) with ionization energy maintained at 70 eV. The IR spectra were recorded with Perkin–Elmer 597 Specord M-80 spectrometer (The Perkin–Elmer Corporation Ltd, Beaconsfield, Bucks, England). Elemental analyses were obtained with an acceptable range ( $\pm 0.4\%$ ) using a Perkin–Elmer 2400B CHN analyzer. Thin-layer chromatography (TLC) was performed on silica gel analytical TLC plates (60 F<sub>254</sub>, Merck, Darmstadt, Germany) and Silufol® (Silpearl on aluminium foil Czechia). Melting points (uncorrected) were determined on a Boetius apparatus.

**4.3.1. 5-Hydroxy-3-(1,1,2,2-tetrafluoroethyl)-1-phenylpyrazole (1).** A mixture of ethyl-3-oxo-4,4,5,5-tetrafluoropentanoate (20.14 g, 0.1 mol) and phenylhydrazine (10.5 g, 0.1 mol) in ethanol (25 mL) was refluxed for 4 h. The solvent was removed under reduced pressure. The resulting residue was recrystallized from hexane to give pyrazole **1** as a white powder. Yield 13.8 g (53%), mp 156–157 °C. C<sub>11</sub>H<sub>8</sub>F<sub>4</sub>N<sub>2</sub>O 260.1. Calculated (%): C 50.77; H 3.08; N 10.77. Found (%): C 50.87; H 3.78; N 10.58. IR spectrum (cm<sup>-1</sup>): 2200 (OH); 1600, 1515 (C=C, C=N). <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>):  $\delta$  5.91 s (1H, CH), 6.44 tt (1H, H(CF<sub>2</sub>)<sub>2</sub>, *J* = 56.6, 5.2 Hz), 7.5 ws (1H, OH), 7.83 m (5H, C<sub>6</sub>H<sub>5</sub>).

**4.3.2. 3-(1,3-Dioxolano)-2-indolinone (9) (for compounds 2 and 3).** A mixture of isatin (14.7 g, 0.1 mol), of ethylene glycol 18.62 g, 0.3 mol) and KY-2-8 (2.0 g) (in acidic form) in benzene (400 mL) was refluxed for 8–10 h with water separator. The reaction was controlled by TLC (Silufol, UV 254, chloroform/acetone = 10:1, iodine vapours for peak detection). On complete reaction, KY-2-8 was filtered, the solvent was evaporated in vacuum to 100 mL and the residue was cooled. The product was filtered to give white crystals of **9**. Additional amounts of product **9** were obtained from the mother liquor.

Yield 14.95 g (78.3%), mp 134 °C (from Benzene). C<sub>10</sub>H<sub>9</sub>NO<sub>3</sub> 191.18. Calculated (%): C 62.82; H 4.74; N 7.32. Found (%): C 62.79; H 4.78; N 7.48. IR spectrum (cm<sup>-1</sup>): 3280 (N–H); 1740 (C=O); 1200 and 1600 (–C–O– of dioxolane ring).

**4.3.3. 1-[(4-Fluorophenyl)-2-oxoethyl]-3-(1,3-dioxolano)-2-indolinone (2).** 2-Bromo-1-(4-fluorophenyl)-1-ethanone (2.17 g, 0.01 mol) was added by small portions to stirred mixture of DMF (20 mL), potassium carbonate (2.07 g, 0.015 mol) and 3-(1,3-dioxolano)-2-indolinone **9**

(1.92 g, 0.01 mol) for 1 h. The mixture was stirred for 1 h, and poured into water (250 mL). The white precipitate was filtered, washed with water and dried over P<sub>2</sub>O<sub>5</sub>. Pure product **2** was prepared by crystallization from ethanol. Yield 2.56 g (78.2%), mp 190–191 °C. C<sub>18</sub>H<sub>14</sub>FNO<sub>4</sub> 327.30.

Calculated (%): C 66.04; H 4.31; F 5.81; N 4.30. Found (%): C 66.28; H 4.50; F 5.63; N 4.18. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 4.35–4.55 m (4H, dioxolane ring); 4.95 s (2H, CH<sub>2</sub>CO); 7.15–7.43 m (4H, isatin ring); 7.93–8.04 m (4H, aromatic ring).

**4.3.4. 1-[2-(4-Bromophenyl)-2-oxoethyl]-3-(1,3-dioxolano)-2-indolinone (3).** 1-[2-(4-Bromophenyl)-2-oxoethyl]-3-(1,3-dioxolano)-2-indolinone **3** was prepared by the same method as for **2**, using the following amounts: 3-(1,3-dioxolano)-2-indolinone **9** (1.91 g, 0.01 mol), potassium carbonate (2.07 g, 0.015 mol), 2-bromo-1-(4-bromophenyl)-1-ethanone (2.95 g, 0.01 mol), DMF (20 mL). Yield 3.21 g (82.7%), mp 155–156 °C (from EtOH). C<sub>18</sub>H<sub>14</sub>FNO<sub>4</sub> 327.30. Calculated (%): C 55.68; H 3.63; Br 20.58; N 3.62. Found (%): C 55.52; H 3.84; Br 20.76; N 3.47. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 4.37–4.54 m (4H, dioxolane ring); 4.93 s (2H, CH<sub>2</sub>CO); 7.14–7.32 m (4H, isatin ring); 7.43–7.63 m (4H, Ar).

**4.3.5. 1-(4-Methyl-2-phenyl-1,3-thiazol-5-yl)-3-morpholin-4-yl-propan-1-one hydrochloride (4)<sup>51</sup>.** The distilled acetylchloride (0.15 mol) was added to the 4,4'-methylene-dimorpholine (0.15 mol) in dimethoxyethane (100 mL). The product obtained was allowed to stand at room temperature for 2 h. Then 2-phenyl-4-methyl-5-acetylthiazole (0.1 mol) was added, hydrogen chloride was passed for 15 min and the mixture was refluxed for 2 h.

The solvent was evaporated and the residue was recrystallized from CH<sub>3</sub>OH/CH<sub>3</sub>COOC<sub>2</sub>H<sub>5</sub> to give the final product **4** (79%) as a white powder, mp 216–216.5. UV: 8.49 × 10<sup>-4</sup> mol/mL in abs C<sub>2</sub>H<sub>5</sub>OH gave:  $\lambda_1$  340 nm,  $\lambda_2$  227 nm,  $\lambda_3$  203 nm. IR: Hydrochloride salt **4** in liquid paraffin showed strong absorption in the region 1720–1740 cm<sup>-1</sup> ( $\nu$ C=O) and 2700–2650 cm<sup>-1</sup> ( $\nu$ N<sup>+</sup>–H). Analysis of base: C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>SO<sub>2</sub> 316.40. Calculated (%): C 64.54; H 6.37; N 8.85. Found (%): C 64.31; H 6.44; N 8.89.

<sup>1</sup>H NMR of base in CDCl<sub>3</sub>: 2.75 (s, 3H, 3-thiazole CH<sub>3</sub>), 3.2–3.8 (m, 4H, CH<sub>2</sub>C=O), 7.1–6.8  $\delta$  (m, 2H, Ar), 8.05–7.8 (m, 2H, Ar).

**4.3.6. 5-Hydroxy-3-methoxycarbonyl-1-phenylpyrazole (5).** A solution of phenylhydrazine (5.41 g, 50 mmol) in methanol (100 mL) was treated with dimethyl acetylene dicarboxylate (7.11 g, 50 mmol). The solution was allowed to stand at ambient temperature for 24 h. After evaporation of the solvent, xylenes (100 mL) were added and the mixture was heated under reflux for 2 h. After removal of the xylenes, the hydroxypyrazole **5** (71%) was crystallized from toluene, mp 192–194 °C, IR (KBr) 1735 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): 3.9



(s, 3H, OCH<sub>3</sub>), 6.3 (s, 1H, CH-4), 7.2–7.8 (m, 5H, Ph), 12.2 (br, 1H, OH); <sup>13</sup>C NMR (100 MHz): 51.5 (OCH<sub>3</sub>), 89.2 (CH-4), 122.1, 127.0, 129.0 (CHPh), 138.1 (CPh), 141.9 (C<sub>3</sub> pyrazole), 153.4 (C<sub>5</sub> pyrazole), 162.3 (CO); MS (EI, *m/z*, %) 218 (M<sup>+</sup>, 100), 187 (M–OCH<sub>3</sub>, 26), 77 (Ph, 71).

**4.3.7. Precursors of compounds 6, 7 and 8.** The following starting materials were obtained according to published methods and further used to prepare the compounds **6–8**: 2-(4-fluorophenyl)-benzo[*d*]imidazo[2,1-*b*]thiazole **10**, mp 151 °C,<sup>40</sup> 2-(4-fluorophenyl)-imidazo[1,2-*a*]pyridine **11**, mp 158–160 °C,<sup>41</sup> 2-(4-nitrophenyl)-imidazo[1,2-*a*]pyridine **12**, mp 265–267 °C.<sup>42</sup>

**4.3.8. Synthesis of compounds 6, 7 and 8 (general procedure).** A solution of fused imidazole A (2 mmol), paraform (2.5 mmol) and secondary amine (2.5 mmol) in 50 mL of acetic acid was stirred at 60 °C for 4–5 h. The reaction mixture was poured into water, neutralized and the precipitate filtered and, if necessary, additionally purified by column chromatography (silica gel, chloroform). The following compounds have been obtained by this method.

**4.3.8.1. 3-(Morpholinomethyl)-2-(4-fluorophenyl)imidazo[2,1-*b*][1,3]thiazole (6).** Yield 75%, mp 187–188 °C. C<sub>20</sub>H<sub>18</sub>FN<sub>3</sub>OS 367.45. Calculated (%): N 11.4. Found (%): N 11.3. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 3.19 (morpholine, 4H, m), 3.86 (morpholine, 4H, m), 4.06 (3-CH<sub>2</sub>, 3H, s), 7.3 (fluorophenyl, 2H, m), 7.43 (H-6, 1H, m), 7.57 (H-7, 1H, m), 7.78 (fluorophenyl, 2H, m), 8.02 (H-8, 1H, d, *J* = 7.8 Hz), 8.13 (H-5, 1H, d, *J* = 8.2 Hz).

**4.3.8.2. 2-(4-Fluorophenyl)-3-(4-methylpiperazinomethyl)imidazo[1,2-*a*]pyridine (7).** Yield 72%, mp 150–152 °C. C<sub>19</sub>H<sub>21</sub>FN<sub>4</sub> 324.40. Calculated (%): N 17.3. Found (%): N 17.4. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 2.72 (3H, N-CH<sub>3</sub>, s), 3.1 (8H, piperazine, br m), 4.07 (2H, 3-CH<sub>2</sub>, s), 6.37 (H-8, 1H, d, *J* = 9.6 Hz), 7.01 (H-6, 1H, m), 7.89 (fluorophenyl, 2H, m), 7.3 (fluorophenyl, H-7, 3H, m), 8.58 (H-5, 1H, d, *J* = 6.3 Hz).

**4.3.8.3. 2-(4-Nitrophenyl)-3-(4-phenylpiperazinomethyl)imidazo[1,2-*a*]pyridine (8).** Yield 69%, mp 176–178 °C. C<sub>24</sub>H<sub>23</sub>N<sub>5</sub>O<sub>2</sub> 413.48. Calculated (%): N 16.9. Found (%): N 16.9. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 3.1–3.2 (piperazine, 8H, m), 4.15 (3-CH<sub>2</sub>, 2H, s), 6.7–7.0 and 7.2–7.3 (H-6, H-7, nitrophenyl, phenyl, 9H, m), 7.65 (H-7, 1H, d, *J* = 7.9 Hz), 8.3 (nitrophenyl, 2H, m), 8.43 (H-5, 1H, d, *J* = 7.1 Hz).

#### 4.4. Anxiolytic test

The conflict situation test<sup>52</sup> for the experimental evaluation of anxiolytic effect was used. Experiments were carried out on 235 male rats of Wag/Rij strain in the weight range 300–350 g. Substances at dose 10 mg/kg were injected intraperitoneally in suspension in Tween-80, except for compound **3**, which was administered in dimethyl sulfoxide (DMSO). The device for carrying out experiments using a method involving a conflict sit-

uation consisted of three parts: the experimental chamber, the electronic block and the counter.

The experimental chamber, of size 275 × 275 × 450 mm, was made of an organic glass.<sup>53</sup> It was based on a standard electrode floor made from stainless steel bars of 4 mm diameter with a distance between them of 8–10 mm. A drinking bowl was attached to a lateral wall of each chamber, comprising a glass vessel with a nipple made of stainless steel. Nipples protruded on 2 cm into the chamber at a height of 5 cm from the floor. In contrast to many other devices, in our device the drinking bowl was in the chamber space, and not in the blacked out compartment. This is because animals in a new situation instinctively try to hide in a dark compartment. Thus, they are able to find a drinking bowl not due to the deliberate search for satisfaction of motivation, but accidentally. The electrode floor and nipples of the drinking bowl were connected to an electronic block.

The electronic block contained current stabilizers (one on each channel that provided an opportunity for independent adjustment of their current), drive circuits of standard signals for the counting device and delay generators for delivering punishing current to the drinking bowls on the day of experiment. The device allowed the registration of nonpunishable water licks during acquisition of the skill of water licks (training, without delivering the current to drinking bowls), and also punishing current and signals of punishable water licks during experiments.

The counter provided registration of nonpunishable water licks during training and punishable water licks during the experiments. The program recorded duration of freezing after placing the animal into the device chamber, the latent period of the first approach to a drinking bowl, locomotor activity and the number of water licks. The data saved up in these files were subjected to statistical processing by means of the statistical package 'Statistica'.

Each experiment was carried out for 3 days. On the first day the animals were completely deprived of water. Next day, that is, after 24-h deprivation, the training (acquisition of skill of water licking from a drinking bowl) was performed. For this purpose animals were placed in experimental chambers for 5 min. The animals explored the chamber, and after a while found a drinking bowl and started to drink. That day a weak current (0.05 mA) not felt by the rats was delivered to the drinking bowl and the floor of the chamber, therefore water licks were nonpunishable and their number characterized the intensity of drinking motivation. The third day the animals were placed again into the experimental chambers for 10 min, but this time a direct current of 0.25 mA was delivered to dummy drinking bowls and the electrode floor of the chambers after the first water lick for 10 s. Hence, each water lick became punishable and for satisfaction of thirst the rats had to overcome the fear developed as a result of punishment. The increase in the number of punishable water licks in animals of the treated group in comparison with the

control group served as a measure of the effect of strain and/or of drug injected.

The rats were divided into groups with equal numbers of individuals in each one. One group served as a control (animals of this group were injected with a physiological solution with several drops of the Tween-80), whilst the animals in the other group were administered a drug. In the statistical estimation of results, average and confidence intervals at  $P < 0.05$  were calculated. Analysis of variance was used for an estimation of reliability of differentiation from the control group.

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### References and notes

- Nash, L. T.; Hack, S. *Expert Opin. Pharmacother.* **2002**, *3*, 555.
- Sanger, D. J.; Joly, D. *Behav. Pharmacol.* **1991**, *2*, 57.
- Kalachnik, J. E.; Hanzel, T. E.; Sevenich, R.; Harder, S. R. *Am. J. Ment. Retard.* **2002**, *107*, 376.
- Burger's Medicinal Chemistry and Drug Discovery*; Abraham, D. J., Ed.; Wiley-VCH, 2003; Vol. 1.
- The Practice of Medicinal Chemistry*, 2nd ed.; Wermuth, C. G., Ed.; Academic Press: New York, 2003.
- Goodman & Gilman's Pharmacological Basis of Therapeutics*, 10th ed.; McGraw Hill, 2001.
- Drews, J. *Science* **2000**, *287*, 1960.
- Poroikov, V. V.; Filimonov, D. A.; Borodina, Yu. V.; Lagunin, A. A.; Kos, A. *J. Chem. Inf. Comput. Sci.* **2000**, *40*, 1349.
- Website: <http://www.ibmh.msk.su/PASS>.
- Poroikov, V. V.; Filimonov, D. A. *J. Comput. Aid. Molec. Des.* **2003**, *16*, 819.
- Stepanchikova, A. V.; Lagunin, A. A.; Filimonov, D. A.; Poroikov, V. V. *Curr. Med. Chem.* **2003**, *10*, 225.
- Geronikaki, A.; Lagunin, A.; Poroikov, V.; Filimonov, D.; Hadjipavlou-Litina, D.; Vicini, P. *SAR QSAR Environ. Res.* **2002**, *13*, 457.
- Manallack, D. T.; Pitt, W. R.; Gancia, E.; Montana, J. G.; Livingstone, D. J.; Ford, M. G.; Whitley, D. C. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 1256.
- Di Giorgio, C.; Delmas, F.; Filloux, N.; Robin, M.; Seferian, L.; Azas, N.; Gasquet, M.; Costa, M.; Timon-David, P.; Galy, J.-P. *Antimicrob. Agents Chemother.* **2003**, *47*, 174.
- Poroikov, V. V.; Filimonov, D. A.; Ihlenfeld, W.-D.; Glorizova, T. A.; Lagunin, A. A.; Borodina, Yu. V.; Stepanchikova, A. V.; Nicklaus, M. C. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 228.
- Lagunin, A. A.; Gomazkov, O. A.; Filimonov, D. A.; Gureeva, T. A.; Kugaevskaya, E. V.; Elisseeva, Y. E.; Solovyeva, N. I.; Poroikov, V. V. *J. Med. Chem.* **2003**, *46*, 3326.
- Geronikaki, A.; Dearden, J.; Filimonov, D.; Galaeva, I.; Garibova, T.; Glorizova, T.; Krajneva, V.; Lagunin, A.; Macaev, F.; Molodavkin, G.; Poroikov, V.; Pogrebnoi, S.; Shepeli, F.; Voronina, T.; Tsitlakidou, M.; Vlad, L. *J. Med. Chem.* **2004**, *47*, 2870.
- Bhattacharya, S. K.; Chakrabarti, S. *Indian J. Exp. Biol.* **1998**, *36*, 118.
- Bhattacharya, S. K.; Mitra, S. K.; Acharya, S. B. *J. Psychopharmacol.* **1991**, *5*, 202.
- Hota, D.; Acharya, S. B. *Indian J. Exp. Biol.* **1994**, *32*, 710.
- Pajaoshesh, H.; Parson, R.; Popp, F. D. *J. Pharm. Sci.* **1983**, *72*, 318.
- Karali, K.; Gursoy, A. *Il Farmaco* **1994**, *49*, 819.
- Gursoy, A.; Karali, N.; Buyuktimkin, S.; Demirayak, S.; Ekinic, A. C.; Ozer, H. *Il Farmaco* **1996**, *51*, 432.
- Singh, G. S.; Singh, T.; Lakhani, R. *Indian J. Chem.* **1997**, *36B*, 951.
- Sridhar, S. K.; Pandeya, S. N.; Stables, J. P.; Ramesh, A. *Eur. J. Pharm. Sci.* **2002**, *16*, 129.
- Raj, A. A.; Raghunathan, R.; SrideviKumari, M. R.; Raman, N. *Bioorg. Med. Chem.* **2003**, *11*, 407.
- Kalari, N. *Eur. J. Med. Chem.* **2002**, *37*, 909.
- Pandeya, S. N.; Sriram, D.; Nath, G.; De Clercq, E. *Eur. J. Med. Chem.* **2000**, *35*, 249.
- Srivastava, P. N.; Rai, S. K. *Eur. J. Med. Chem.* **1980**, *15*, 274.
- Bhargava, P. N.; Nair, M. G. R. *J. Indian Chem. Soc.* **1957**, *34*, 42.
- Srivastava, P. K.; Srivastava, P. N. *J. Med. Chem.* **1970**, *13*, 304.
- Lakhani, R.; Rai, B. J. Local anaesthetics IV. *Il Farmaco* **1986**, *41*, 788.
- Geronikaki, A.; Theophilidis, G. *Eur. J. Med. Chem.* **1992**, *27*, 709.
- Vicini, P.; Amoretti, L.; Chiavarini, M.; Impicciatore, M. *Il Farmaco* **1990**, *45*, 933.
- Klose, N.; Niedbolla, K.; Schwartz, K.; Bottcher, I. *Arch. Pharm.* **1983**, *316*, 941.
- Satsangi, R. K.; Zaidi, S. M.; Misra, V. C. *Pharmazie* **1983**, *38*, 341.
- Pignatello, R.; Mazzone, S.; Panico, A. M.; Mazzone, G.; Penissi, G.; Castano, R.; Matera, M.; Blandino, G. *Eur. J. Med. Chem.* **1991**, *26*, 929.
- Hadjipavlou-Litina, D.; Geronikaki, A.; Sotiropoulou, E. *Res. Commun. Chem. Pathol. Pharmacol.* **1993**, *79*, 355.
- Raciti, G.; Mazzone, P.; Raudino, A.; Mazzone, G.; Cambria, A. *Bioorg. Med. Chem.* **1995**, *3*(11), 1485.
- Kayser, V.; Farre, A.; Hamon, M.; Bourgoin, S. *Pain* **2003**, *104*, 169.
- Dal Piaz, V.; Castellana, M. C.; Vergelli, C.; Giovannoni, M. P.; Gavalda, A.; Segarra, V.; Beleta, J.; Ryder, H.; Palacios, J. M. *J. Enzyme Inhib. Med. Chem.* **2002**, *17*, 227.
- Quan, M. L.; Wexler, R. R. *Curr. Top. Med. Chem.* **2001**, *1*, 137.
- Baraldi, P. G.; Pavani, M. G.; Nunez Mdel, C.; Brigidi, P.; Vitali, B.; Gambari, R.; Romagnoli, R. *Bioorg. Med. Chem.* **2002**, *10*, 449.
- Website: <http://www.lhasalimited.org>.
- Lange, J.; Karolak-Wojciechowska, J.; Wejroch, K.; Rump, S. *Acta Pol. Pharm.* **2001**, *58*, 43.
- Mase, T.; Arima, H.; Tomioka, K.; Yamada, T.; Murase, K. *J. Med. Chem.* **1986**, *29*, 386.
- Xie, Y.; Chen, Z.; Zheng, Q. *Synthesis* **2002**, *11*, 1505.
- Paolini, J. P.; Lendvay, L. J. *J. Med. Chem.* **1971**, *14*, 988.
- Website: <http://www.mdli.com>.
- Filimonov, D.; Poroikov, V.; Borodina, Yu.; Glorizova, T. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 666.
- Sotiropoulou, E.; Kourounakis, P. N. *Pharmazie* **1992**, *47*, 298.
- Vogel, J. R.; Beer, B.; Clody, D. E. *Psychopharmacology* **1971**, *21*, 1.
- Molodavkin, G. M.; Voronina, T. A. *Eksp. Klin. Farmakol. (Rus.)* **1995**, *58*, 54.