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Search for anticonvulsant and analgesic active derivatives of dihydrofuran-2(3*H*)-one

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ABSTRACT

A series of derivatives of dihydrofuran-2(3*H*)-one (γ-butyrolactone, GBL) was synthesized and tested for anticonvulsant, neurotoxic and analgesic activity. In the anticonvulsant screening 10 lactones were effective in the maximal electroshock test (MES) at the highest doses (300 and 100 mg/kg, 0.5 h, ip, mice). Statistical analysis showed correlation between the anticonvulsant activity and relative lipophilicity parameters determined by experimental and computational methods (R_{M0}, *C*log*P* and *M*log*P*). Preliminary antinociceptive evaluation of selected derivatives revealed strong analgesic activity. The majority of the tested compounds showed high efficacy in animal models of acute pain (hot plate and writhing tests) and strong local anesthetic activity (modified tail immersion test). The obtained ED_{50} values were comparable with such analgesics as acetylsalicylic acid and morphine.

1. Introduction

Epilepsy is a serious neurological condition affecting up to 1% of the world's population.[1,2] This disorder is defined by recurrent seizures caused by synchronized discharges of neurons in the brain leading to an altered feeling or behavior of the affected patient. Worldwide, over 40 antiepileptic drugs (AEDs) are in clinical use, however these drugs are only effective in about one third of patients, the remainder being resistant to the available first-line AEDs. Many of the clinically used drugs have not been definitively linked with a specific site of the brain nor have the exact mechanisms of action been fully elucidated.[3–6] Most AEDs possess more than one mechanism of action which may account for their efficacy in different seizure types, patterns and individual patients. Due to the lack of understanding and complexity of epileptogenic processes drug candidates are typically being discovered through conventional screening and structural modifications rather than by mechanism-driven designs.[7] Therefore, the identification of anticonvulsants is usually conducted through multiple in vivo screening tests that are frequently based on seizure type or non-specific mechanisms rather than well-defined etiology.

Due to the shared pathomechanism of many central and peripheral nervous system disorders, several AEDs have found application in the treatment of non-epileptic conditions associated with neuronal irregularities. Among them chronic pain of peripheral or central origin and various psychiatric disorders (anxiety, bipolar affective disorder) are the most important. AEDs such as valproic acid, carbamazepine, gabapentin, pregabalin or lamotrigine are clinically used in neuropathic pain as the first-line treatment.[8–10] Numerous studies have also demonstrated analgesic activity of other anticonvulsant agents in animal models of pain and some of them are being currently evaluated in clinical studies (Fig. 1).[11]

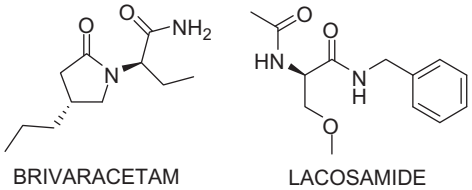

Figure 1. Examples of analgesic-active anticonvulsants.

Abbreviations: AEDs, antiepileptic drugs; ASP, Anticonvulsant Screening Program; CNS, central nervous system; GBL, γ-butyrolactone, dihydrofuran-2(3*H*)-one; GHB, γ-hydroxybutyric acid, 4-hydroxybutyric acid; DCM, dichloromethane; DMF, dimethylformamide; HP, hot plate test; ip, intraperitioneal; MIT, modified tail immersion test; po, per os; RP-TLC, reversed-phase thin-layer chromatography; TBAB, tetrabutylammonium bromide; WT, writhing test.

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In the recent years these facts have directed the search for novel antiepileptic compounds towards identification and development of potent agents endowed with both anticonvulsant and analgesic activity. In a highly saturated epilepsy market this approach has proven successful by providing expanded financial and marketing incentives that do not independently exist in the field of epilepsy.

Presented work is a part of our search for potential anticonvulsant agents among derivatives of 4-hydroxybutyric acid (γ-hydroxybutyric acid, GHB). In this paper we describe studies in a group of derivatives of dihydrofuran-2(3*H*)-one, (oxolan-2-one, γ-butyrolactone, GBL), a cyclic analogue and precursor of GHB. GBL-derived structures and the lactone itself are well known for their central nervous system (CNS) pharmacological potential and were found to possess anticonvulsant activity. GBL ring is present in the structures of different $GABA_A$ receptor ligands: antagonists (e.g., bicuculline, picrotoxin) as well as positive allosteric modulators bearing anticonvulsant properties (I-III) (Fig. 2).[12–16]

Our pilot screen of a small group of 3-substituted GBL derivatives revealed not only anticonvulsant but also potent analgesic and local anesthetic properties in several tests in mice.[17] In that study two compounds (**1**, **2**, Fig. 3) demonstrated high analgesic activity, comparable with or even higher than reference drugs: acetylsalicylic acid, morphine, lidocaine and mepivacaine.

Pharmacological evaluation of the most potent compound **2** was further developed and showed high efficacy in other models of pain, anticonvulsant activity in mice and antioxidant activity in vitro.[18] These results encouraged us to follow the GBL path of the project and to synthesize more analogs thereof and for selected compounds to extend the pharmacological screening to include analgesic activity tests.

Considering the above, we designed a series of γ-butyrolactone derivatives, synthesized and tested them for anticonvulsant and analgesic activity. This study was focused on exploration of the substitution at position 3 by introduction of various alkyl- and arylakyl-substituents linked by nitrogen atom, oxygen atom or aminomethylene group. As a natural consequence of the results from the previous studies several modifications of the phenylpiperazine moiety were examined (**3**–**7**, **10**). This conception is also supported by the fact that arylpiperazines are present in various CNS-active compounds and their anticonvulsant and analgesic activities have been reported in numerous publications, including our studies.[17–20] In order to verify the pharmacological significance of the size and aromaticity of the substituent at the α-position different substituents were introduced (**11**–**13**, **15**–**24**), among them 1,2,3,4-tetrahydroisoquinolines which have been also reported to possess anticonvulsant properties.[21,22] As a preliminary attempt to investigate the substitution at other positions of GBL ring 5-methyl analogs of **1** and **11** (**8** and **14**) and a positional isomer of compound **2**, 4-phenylpiperazine-GBL (**9**) were also synthesized.

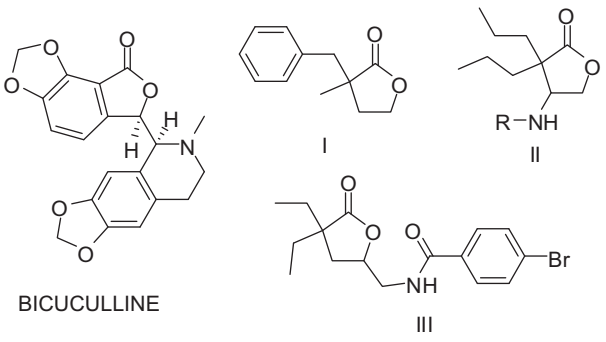


Figure 2. Structures of bicuculline and anticonvulsant-active derivatives of γ-butyrolactone.

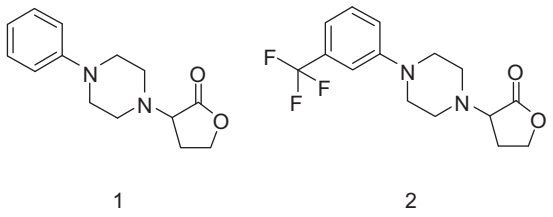


Figure 3. Analgesic-active 3-substituted GBLs.

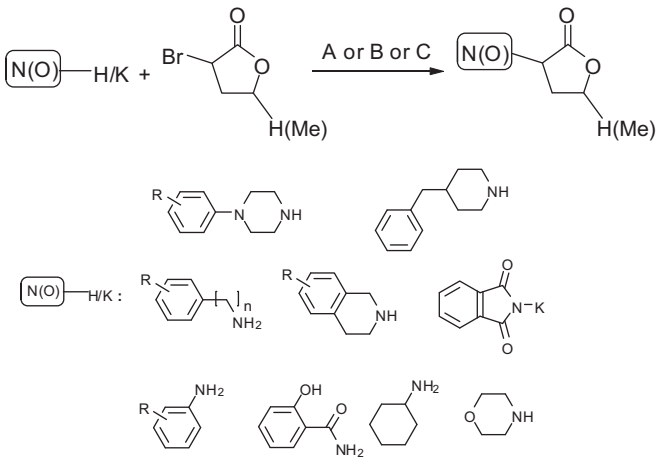
The obtained derivatives were evaluated for their anticonvulsant activity and neurotoxicity within the Anticonvulsant Screening Program at the National Institute of Neurological Disorders and Stroke, National Institutes of Health, USA formally directed by J. Stables. Selected compounds were then tested in rodent models of pain. For the purpose of properties–activity relationship analysis, lipophilicity parameters were determined by experimental and computational methods.

2. Chemistry

The majority of the derivatives were prepared by *N*- or *O*-alkylation of appropriate reagent by 3-bromodihydrofuran-2(3*H*)-one (**1**–**7**, **10**–**13**, **15**–**17**, **21**–**24**) or 3-bromo-5-methyl-dihydrofuran-2(3*H*)-one (**8**, **14**) (Fig. 4, Table 1). The reactions were carried out in dichloromethane (DCM) or acetonitrile (MeCN) in the presence of anhydrous potassium carbonate and in some cases tetrabutylammonium bromide (TBAB).[17,23–26] N-alkylation of 2, 6-xylidine (**21**) required maintenance of relatively low basic pH (around 8) which was achieved by slow addition of potassium carbonate in aqueous solution over the course of the reaction.[27] The phthalimide derivative **17** was obtained by heating dimethylformamide (DMF) solution of α-bromo-GBL with phthalimide in the form of potassium salt, thus did not required addition of a base.[28,29]

The 4-substituted dihydrofuran-2(3*H*)-one (**9**) was prepared by Michael addition of amines to 5-*H*-furan-2-one (γ-crotonolactone) (Fig. 5, Table 1).[16,30]

Derivatives of 1-amino ethylidene dihydrofuran-2(3*H*)-one (**18**–**20**) were obtained by condensation of 3-acetyl-dihydrofuran-2(3*H*)-one with primary amines (Fig. 6, Table 1).[31–33] The reaction was accomplished by refluxing toluene solution of the reagents



A: K_2CO_3, MeCN or DCM, (TBAB), r. t. 5-20 h;
B: K_2CO_3, H_2O/toluene 80°C, 5 h; C: DMF, 80°C, 8 h

Figure 4. Synthesis of 3- and 3,5-substituted derivatives of GBL.

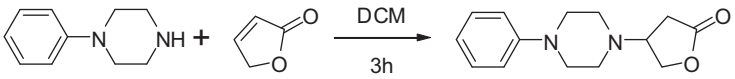


Figure 5. Synthesis of 4-substituted derivative of GBL.

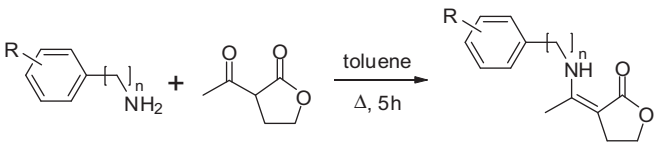


Figure 6. Synthesis of 3-arylalkylaminoetylidene derivatives of GBL.

with azeotropic removal of water by the use of Dean–Stark apparatus. All the obtained compounds were tested as racemates or mixtures of diastereoisomers (**8** and **14**).

3. Lipophilicity determination

Physicochemical properties of bioactive compounds influence both, their pharmacological activity and pharmacokinetic profile. Lipophilicity is one of the crucial physicochemical properties that comprise influence of different molecular parameters and has a strong impact on biological activity of compounds, especially considering the absorption and penetration to CNS.[34,35] The lipophilicity of the obtained dihydrofuran-2(3*H*)-one derivatives was determined using experimental and computational methods. Relative lipophilicity (R_{M0}) was estimated by reversed-phase thin-layer chromatography (RP-TLC) with mixtures of MeCN and Tris buffer (pH 7.4) as the mobile phase. Theoretical parameters were obtained from two programs: ChemOffice (*C*log*P*) and Marvin (*M*log*P*). Relative lipophilicity and the partition coefficients calculated for all the compounds are presented in Figure 7 and in Supplementary data.

The lipophilicity parameters determined by RP-TLC and expressed as R_{M0} values were in the 0.47–2.40 range which matches the requirements for CNS-active compounds and augurs well for the penetration to the brain.[34,35] Good correlation was found between relative lipophilicity R_{M0} and its partition coefficients calculated using ChemOffice and Marvin software, proving their usefulness for the prediction of the lipophilicity at early stages of studies. Linear relationship of R_{M0} values with *C*log*P* and *M*log*P* was characterized by correlation coefficients (R^2) of 0.7712 and 0.7233, respectively.

4. Pharmacology

4.1. Anticonvulsant activity

Preliminary anticonvulsant evaluation (Test 1 ASP) of all synthesized compounds was performed according to procedures described before.[36–38] These initial studies involved three in vivo tests: maximal electroshock seizure (MES), subcutaneous metrazole (scMet), and minimal locomotor impairment as an assessment of neurotoxicity (TOX). These initial screens are qualitative assays involving a relatively small number of animals (16 male albino

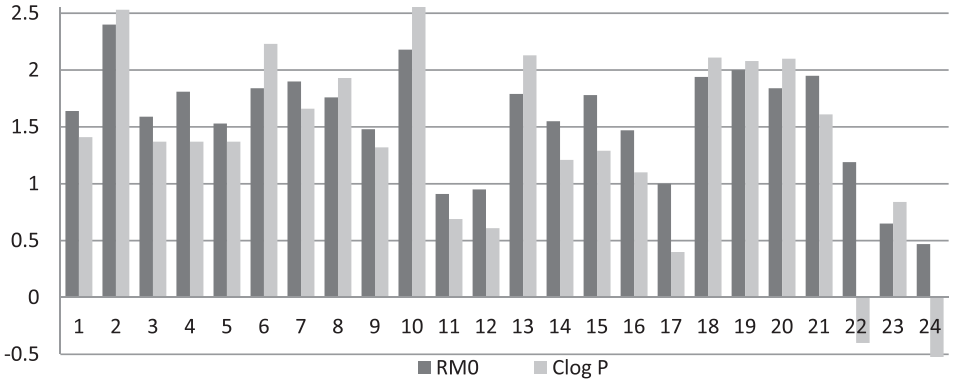


Figure 7. Relative lipophilicity of compounds **1–24**: R_{M0} and calculated log*P* values.

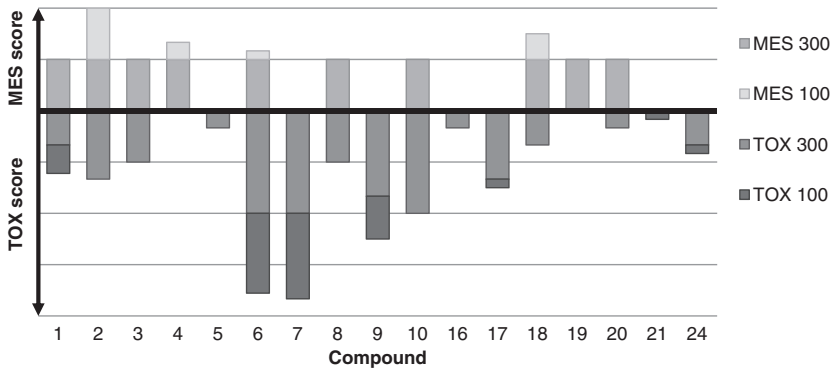


Figure 8. Test 1 ASP scores for active compounds (mice, 100 or 300 mg/kg, ip, 0.25–4 h).

mice of comparable age and weight). The tested compound is administered to one, three and one additional animal at three respective doses (30, 100, and 300 mg/kg ip), 0.5, 1, 2 and 4 h prior to the test. Identical dosage and timing is used in MES, scMet and toxicity testing. Compounds found to be effective in these seizure challenges are regarded as potentially useful candidates for new anticonvulsants. The MES test is a proven method of inducing generalized tonic-clonic seizures and identifying clinical candidates that prevent partial and generalized seizure while the scMet-active compounds are effective in the treatment of absence seizures. Neurotoxicity of the tested compounds was determined using the rotarod test that measures locomotor impairment.

Compounds selected on the basis of the data obtained from intraperitoneal (ip) screening are rolled into advanced evaluation including MES test in Sprague dawley rats and 6-Hz test.[37,38] The minimal clonic seizure test, otherwise known as the 6-Hz model, is used in much the same way as the MES test to assess a compound's efficacy against electrically-induced seizures. It is a random screening test for therapy-resistant epilepsy and can help identify novel anti-seizure agents.[39,40] Classical sodium channel antiepileptic drugs like phenytoin and carbamazepine are not active in this model at non-toxic doses. Conversely, certain newer anticonvulsant agents such as levetiracetam, which is not traditionally recognized to be effective in the conventional MES test, indeed does show protection in the minimal clonic seizure (6-Hz) model. The 6-Hz model was found to be highly sensitive to the antiepileptic drugs that positively modulate $GABA_A$ receptors and thus may be valuable as a tool for the identification of such compounds.

4.1.1. Test 1 ASP

Among 24 compounds subjected to the ASP screen, 10 lactones showed protection against MES seizures at doses of 100 and 300 mg/kg 0.5 h following administration, however only two of them did not produce neurotoxic effects (**4**, **19**). Only lactones **6** and **18** showed anticonvulsant activity 15 min. after the administration and only lactone **2** showed longer protection, being active after 4 h. Isolated neurotoxic effects at doses 100 and 300 mg/kg were observed in seven compounds (**5**, **7**, **9**, **16**, **17**, **21**, **24**). The results of the ASP screen are presented in Figure 8 in a form of scores gained in the Test 1 ASP in mice, at doses of 100 or 300 mg/kg. In the graph the columns above axis represent fraction of animals that was protected in the MES test while the columns below the axis correspond to the fraction of animals that failed in the TOX test (100 and 300 mg/kg, 0.25–4 h). Detailed results of these tests are given in Supplementary data.

In the scMet test neither of the compounds showed protective effect at the indicated doses and time points (data not shown), whereas lactone **15** displayed proconvulsive properties at all doses (30–300 mg/kg), at 0.5 and 4 h. It is interesting to note that compound **15** is a derivative of 1,2,3,4-tetrahydroisoquinoline (TIQ), a moiety present in various anticonvulsant and neuroprotective compounds.[21,22] On the other hand, TIQ derivatives are also known for their neurotoxicity, and some of them are considered to be endogenous neurotoxins.[41]

4.1.2. Advanced ASP studies

According to ASP procedures[37,38] basing on the data obtained from the ip screening compounds **2** and **18** were selected for further evaluation of their anticonvulsant (MES) and neurotoxic (TOX) properties in rats after oral administration at dose of 30 mg/kg (Fig. 9). Compound **18** was inactive while lactone **2** was effective in this model showing also some neurotoxicity, however its protective activity in MES increased while neurotoxicity decreased as a function of time. Even more important outcome from this test concerns the bioavailability and pharmacokinetic

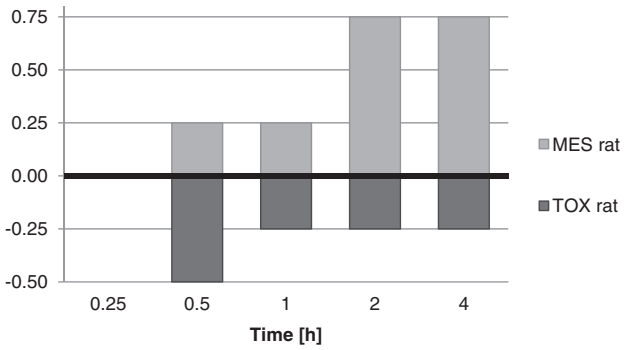


Figure 9. ASP test results for compound **2** (rats, 30 mg/kg, po).

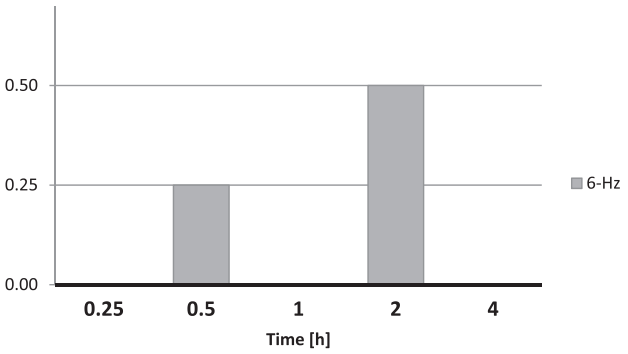


Figure 10. 6-Hz test in mice for compound **23** (mice, 100 mg/kg, ip).

properties. The results proved that the compound **2** was absorbed after po administration and penetrated to CNS. In our extended study of compound **2**, its ED_{50} value in the MES test in mice was determined to be 112 mg/kg.[18]

Two compounds (**22** and **23**) were selected for the evaluation of anticonvulsant activity in the 6-Hz test in mice (Fig. 10). Compound **23** administered ip at a dose of 100 mg/kg showed moderate protection after 1 and 2 h. Compound **22** was inactive in this model at the dose and times.

4.1.3. Structure–anticonvulsant activity relationship

The presented results show that the CNS activity of these derivatives of dihydrofuran-2(3*H*)-one hinges upon the presence of a phenylpiperazine moiety. Among eight phenylpiperazine-containing structures, seven compounds were active in the MES test and exhibited TOX effects, indicating their penetration to CNS and inhibitory influence thereon. Using unsubstituted derivative of *N*-phenylpiperazine (**1**) as a reference compound, neurotoxicity was found to exceed anticonvulsant activity (100 vs 300 mg/kg). Introduction of a methoxy substituent at the position 2 of the phenyl ring (**3**) decreased the toxicity while further introduction of a chlorine atom at the position 5 (**6**) intensified both effects. Substitution at the position 3 of the phenyl ring seems to give a beneficial effect. The MES activity of the 3-methoxy derivative (**4**) was slightly improved while the presence of an electron-attracting trifluoromethyl group (**2**) further increased anticonvulsant activity and prolonged duration of action to 4 h. Derivatives substituted at position 4 of the phenyl ring, 4-methoxy (**5**) and 4-fluoro (**7**) produced only neurotoxic effects. Substituting the *N*-phenylpiperazine fragment with a 4-benzylpiperidine group (**10**) or introducing a methyl substituent at position 5 of dihydrofuran-2(3*H*)-one (**8**) did not improve the activity profile. Transfer of the phenylpiperazine moiety to position 4 of GBL yielded inactive and more neurotoxic compounds (**9**) when compared with reference-structure **1**. Apart from

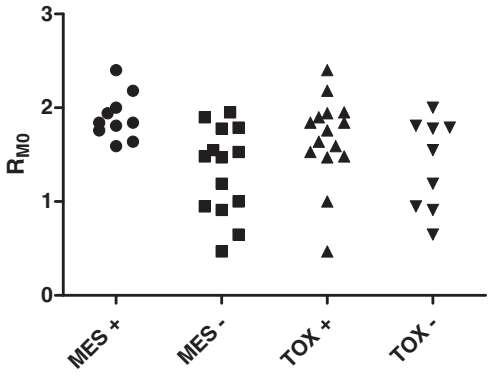


Figure 11. Correlation between lipophilicity parameters R_{M0} and activity in MES and TOX tests.

these examples only the 1-amino ethylidene derivatives of dihydrofuran-2(3*H*)-one (**18**–**20**) showed some protective effect in MES and low or no neurotoxicity. The *N*-phenyl derivative (**18**) displayed moderate activity in the MES test (100 mg/kg), along with TOX properties (300 mg/kg). Increasing the distance between the aromatic ring and the nitrogen atom by exchanging phenylamine for a benzylamine fragment (**19**) yielded decrease in anticonvulsant and TOX activity. Moreover, introducing a homoveratrylamine fragment at the same position (**20**) resulted in detectable activity in both tests (300 mg/kg). Compounds without aromatic substituents (**23**, **24**) were inactive in the MES test and TOX effects were observed only for the morpholine derivative **24**. Introduction of benzylamine substituent, its rigid or amide analogs (compounds **11**–**17**), as well as arylamino- or aryloxy- substituents (**21**, **22**) gave derivatives inactive in ASP tests.

Summing up, results of the preliminary anticonvulsant screening suggests that the substituent at position 3 determines both the activity and neurotoxicity of the derivatives of dihydrofuran-2(3*H*)-ones. 4-Phelypiperazin-1-yl fragment, along with it's 3′-trifloromethyl analogue, are preferable in terms of activity (**1**–**9**), however others substituents are able to elicit the activity as well. Anticonvulsant effect in the MES test was also observed for structures with a phenyl substituent connected to dihydrofuran-2(3*H*)-one by a suitable alkylamino linker (compound **10** but not **11**), or an aminoethylidene spacer (**18**–**20**).

4.1.4. Lipophilicity–anticonvulsant activity relationship

In search for relations between molecular properties and pharmacological activity of the presented set of dihydrofuran-2(3*H*)-one derivatives an interesting relationship has been found. Statistical analysis revealed significant differences between mean values of lipophilicity descriptors (R_{M0} as well as *C*log*P*) and activity in the MES and TOX (at any doses) tests. More specifically, active compounds (doses 30, 100 or 300 mg/kg) displayed higher R_{M0} values than inactive compounds (1.90 vs 1.33, Fig. 11, Table 2). A similar tendency was observed for calculated log *P* values (*C*log*P* and *M*log*P*) but in this case mean log*P* values for active compounds were two times higher than for the inactive. Table 2 present the results of this correlation study for *C*log*P* values. Obtained results show that inactive compounds are characterized by lower log *P* values. These findings can be helpful in elimination of potentially inactive compounds at early stages of the project.

4.2. Analgesic activity

Twelve compounds (**1**–**6**, **11**, **15**–**17**, **22**, **24**) were selected for further pharmacological evaluation focused on their antinociceptive activity in acute pain and local anesthesia tests in mice. The selection was based on the anticonvulsant activity, low toxicity and diversity of the structures.

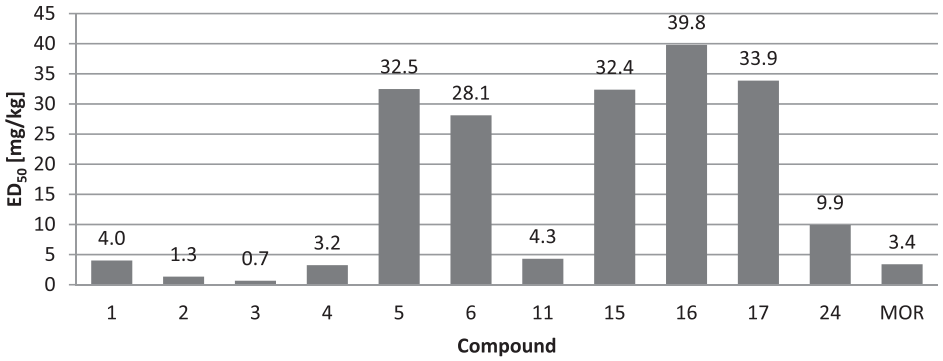


Figure 12. Analgesic activity of the selected compounds and morphine in the hot plate test in mice [ED_{50}].

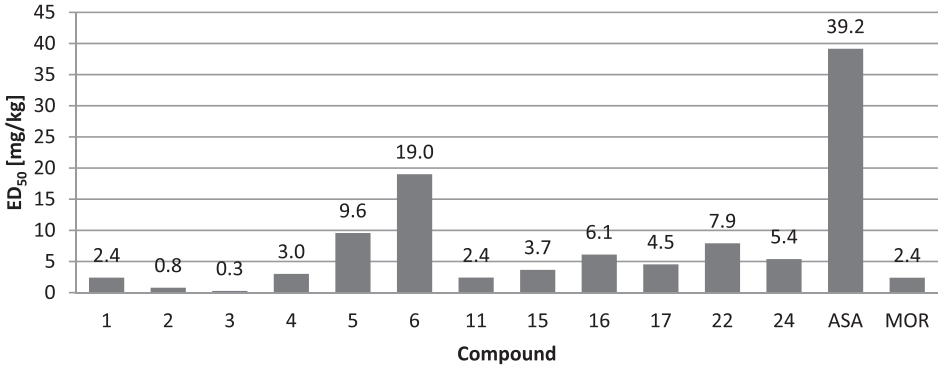


Figure 13. Analgesic activity of selected compounds, and reference drugs in the writhing test in mice [ED_{50}].

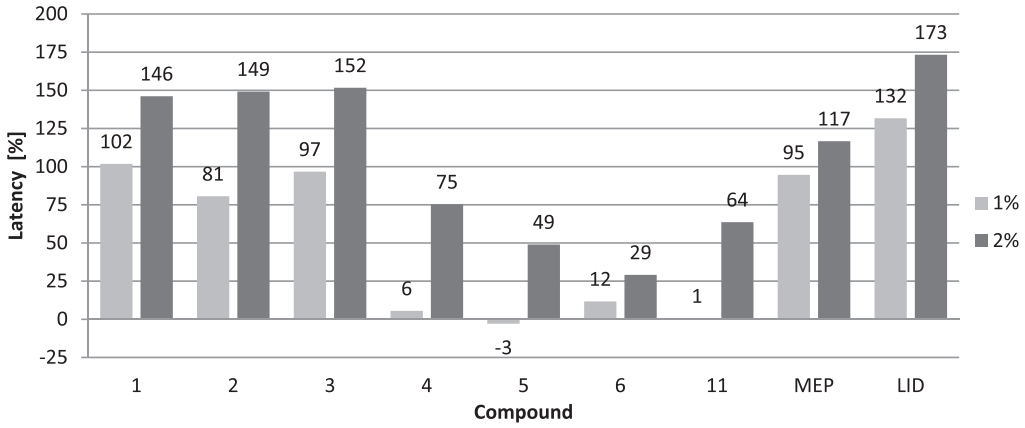


Figure 14. Local anesthetic activity of the tested compounds and the reference drugs: latency of reaction in the MTI test in mice (% of difference in comparison with control group).

Table 1
Structures of the synthesized compounds

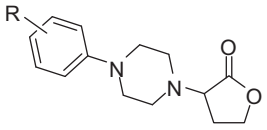
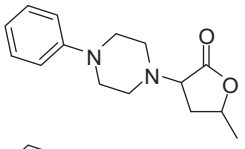
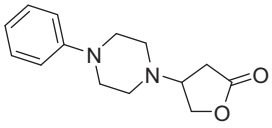
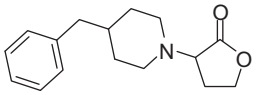
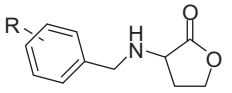
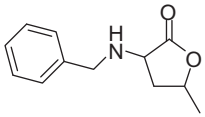
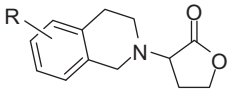
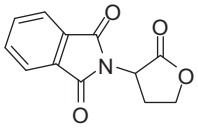
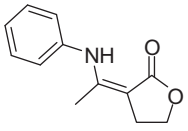
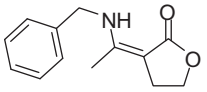
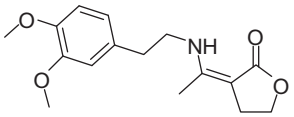
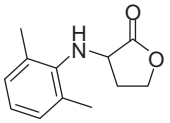
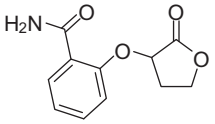
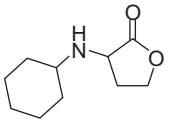
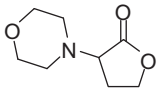
Symbol	Structure	R
1		H
2		3-CF_3
3		2-MeO
4		3-MeO
5		4-MeO
6		2-MeO-5-Cl
7		4-F
8		
9		
10		
11		x HCl
12		4-MeO x HCl
13		2-CF_3
14		
15		H
16		6,7-$(MeO)_2$
17		
18		

Table 1 (*continued*)

Symbol	Structure	R
19		
20		
21		
22		
23		x HCl
24		x HCl

4.2.1. Hot plate test

The hot plate assay (HP) is a rodent model of acute pain. In this test pain is induced by perceptible but not harmful thermal stimuli. The characteristic response that occurs (jumping, licking of paws) is of central origin and it is thought that drugs with antinociceptive properties in the hot plate test act primarily in the spinal medulla and/or higher layers of the central nervous system.[42,43] Since peripherally acting analgesic drugs are generally inactive in this pain model,[42] morphine was chosen as a reference drug.

As shown in Figure 12 almost all the tested compounds showed analgesic activity in the HP test (p <0.001 or 0.01 compared to vehicle-treated mice, Student's test). The lowest ED_{50} values were assigned for compounds **3**, **4**, **11** and **24** along with the previously published **1** and **2** and were comparable with morphine (MOR).[17] As it was already published, derivative **22** was inactive in this test.[17]

4.2.2. Writhing test

The writhing test (WT) is an inflammatory pain model widely used for detection of compounds that exhibit peripheral antinociceptive activity.[44] In this assay the phenylbenzoquinone-induced pain phenomenon is associated with the synthesis and release of arachidonic acid metabolites via cyclooxygenase and lipooxygenase pathways.

Each of the tested compounds showed strong analgesic activity in this test reducing the mean number of writhing responses in a dosage-dependent and statistically significant manner (p <0.02 compared to the control group, Student's test). All the ED_{50} values were lower than for the reference drug, acetylsalicylic acid (ASA) and in many cases comparable with morphine (Fig. 13).

4.2.3. Local anesthetic activity (modification of tail immersion test, MTI)

The tail immersion test is an assay evaluating systemic analgesic activity, although with slight modifications can be used to determine local anesthetic properties as well. In this test the latency of animal's response to thermal stimuli was measured for two concentrations of the investigated compound (1% and 2%).[45]

The MTI test confirmed local anesthetic potency of five selected compounds, however among them only compound **3** showed the efficacy similar to the previous results (**1** and **2**) and the reference compounds, mepivacaine (MEP) or lidocaine (LID). Compounds **4**, **5** and **11** were inactive in the lower concentration. The differences in latency of the response in comparison to the vehicle-treated mice is presented in Figure 14.

4.2.4. Structure–analgesic activity relationship

The antinociceptive screening revealed mostly high analgesic activity of the investigated compounds. In the group of phenylpiperazine derivatives, comparing with unsubstituted **1**, HP activity improves with introduction of a methoxy group at position 2 giving compound **3**, the most active in this test but lowers slightly with transfer thereof to the *meta*-position (**4**), and clearly drops down with the *para*-substitution (**5**). Introduction of a chlorine atom at position 5′ of compound **3** gave also distinct decrease in the activity (**6**). Benzylamino-GBL **11** showed stronger analgesic properties than its rigid or amido- analogs **15**–**17**. Similarly to the HP, in the WT test the methoxy substitution at the phenypiperazine gave the highest efficacy for the *ortho*-position yielding the lowest ED_{50} value among the tested compounds while for *meta*- and *para*-isomers and 5-chloro analogue gradual decrease in the activity was observed. The most potent non-phenylpiperazine derivative was lactone **11** (as in HP) with ED_{50} value lower than for morphine. No significant differences between ED_{50} values were observed in this group, proving that aromatic substituent is not necessary for the activity in this test. Interestingly, compound **22** was inactive in HP test implies that the presence of the lactone ring is insufficient to achieve the analgesic activity. On the other hand **22** showed definite activity in the WT test. This fact may be explained by anticipated anti-inflammatory properties of the salicylamide moiety present in the structure. In the MTI test the influence of the substibution at the phenyl ring on the activity was similar to that observed for the activity in acute pain tests. No correlation between the analgesic and anticonvulsant activity or lipophilicity has been found.

Table 2
Correlation between calculated lipophilicity parameter (*C*log*P*) and anticonvulsant activity (MES) and neurotoxicity (TOX)—statistical analysis

	ASP test	Activity	Mean	SEM	p
R_{M0}	MES	+	1.90	0.08	0002
		–	1.33	0.13	
	TOX	+	1.67	0.12	0.203
		–	1.40	0.16	
*C*log*P*	MES	+	1.97	0.14	0.001
		–	0.95	0.20	
	TOX	+	1.54	0.21	0.198
		–	1.09	0.26	

5. Conclusion

As a part of our search for potential antiepileptic drugs, a series of 3-, 4- or 5-substituted derivatives of dihydrofuran-2(3*H*)-one was synthesized and tested in maximal electroshock, subcutaneous metrazole and neurotoxicity screens. Ten lactones were found to be effective in the MES screen at a dose of 300 mg/kg and four of them showed also protective effect at doses of 100 mg/g 0.5 h after the administration. The results have shown that the CNS activity of these derivatives of dihydrofuran-2(3*H*)-one hinges upon the presence of a phenylpiperazine moiety. Among eight phenylpiperazine-containing structures, seven compounds were active in the MES test and exhibited TOX effects, indicating their penetration to CNS and inhibitory influence thereon.

Comparison of relative lipophilicity parameters determined by chromatographic or computational methods with anticonvulsant activity in MES test showed statistically significant differences between the mean values of R_{M0} and *C*log*P* for active and inactive compounds. These results along with strong correlation between R_{M0} and *C*log*P* parameters suggest that both computational methods may be used for prediction the lipophilicity of target compounds, rendering useful assistance at early stages of design of new potential anticonvulsant derivatives of 3-substituted dihydrofuran-2(3*H*)-one or closely related structures.

Due to proven efficacy of various anticonvulsant drugs in the treatment of certain kinds of pain the pharmacological screening was extended to antinociceptive activity evaluation in mice. Our studies revealed strong analgesic activity of the tested derivatives, comparable or even higher than of the reference compounds, morphine, acetylsalicylic acid or mepivacaine. Derivative **3** was the most potent among the tested lactones with ED_{50} of 0.66 mg/kg in the HP and 0.28 mg/kg in the WT tests. Low ED_{50} values obtained in these screens in combination with the lack of neurotoxic effect at low doses (TOX test 1 ASP in mice, 30 mg/kg) have positively verified the initial concept to broaden our studies giving good prospects for the development of this path of the project.

The specific mechanism which accounts for the pharmacological activity of the presented compounds remains unknown. Some conjectures concerning the activity of the most studied compound **2** have already been presented[18] although, further investigation is certainly necessary.

6. Experimental

6.1. Synthesis

The methods used for the synthesis of the desired compounds are shown in Figures 4–6 and Table 1. Melting points were determined on an Electrothermal 9300 Melting-Point Apparatus and are uncorrected. Reactions were monitored by TLC on silica gel plates (5 × 8 cm, 0.25 mm; Kiselgel 60 F_{254}, Merck) using the following solvent systems: S_1 chloroform/acetone (1:1, v/v) and S_2 $CHCl_3$/MeOH/AcOH (60:10:5, v/v/v) with visualization of spots under UV light. All reagents were purchased from Sigma–Aldrich Co. ^{1}H NMR spectra were recorded with Varian Mercury 300 spectrometer at 300 MHz with tetramethylsilane as an internal standard. The following abbreviation are used: Ph, phenyl ring; 2-, 3-, 4-, 5- or 6-Ph, indicating position of the proton at the phenyl ring; PIP, piperazine ring; α-, β- or γ-GBL, indicating 3-, 4- or 5-position

in the lactone ring; s, singlet; d, doublet; t, triplet; m, multiplet. Elemental analyses of C, H, and N were carried out on a Varian EL III Model Elemental Analyzer. Mass spectra were recorded on MDX SCILEX API 2000 (Concord, ON, Canada) using the ESI method.

The synthesis of several compounds has already been published in our publications (**1**,[23] **2**,[17] **10**,[24] **11**,[25] **15**,[26] **19**,[31] **22**,[17] **24**[46]) or elsewhere (**17**,[28,29] **18**,[32] **20**[32]).

6.1.1. General procedures for the synthesis of derivatives of dihydrofuran-2(3*H*)-one

6.1.1.1. Procedure P1 (3–9, 13, 16). A mixture of the appropriate amine (1 equiv) or its hydrochloride salt and anhydrous K_2CO_3 (1–1.2 equiv) in dry solvent (MeCN, DCM or toluene) was stirred at room temperature for 30 min. A solution of 3-bromo-dihydrofuran-2(3*H*)-one (1 equiv) in dry solvent (MeCN, DCM) was then added dropwise over 15 min and stirring continued for 5–48 h at ambient temperature. The mixture was then filtered and the filtrate was evaporated to obtain a crude oily residue which was purified by recrystallization from 2-propanol (*i*-PrOH) or EtOAc.

6.1.1.2. Procedure P2 (12, 14, 23, 24). Starting with a solution of the appropriate amine (2.5 equiv) in dry MeCN or DCM, anhydrous K_2CO_3 (1 or 2 equiv) and tetrabutylammonium bromide (TBAB, 0.1 equiv) were added and the reaction mixture was stirred at room temp for 30 min. Subsequently, the reaction mixture was cooled down to 0 °C and a solution of 3-bromo-dihydrofuran-2(3*H*)-one (2.5 equiv) in dry solvent (MeCN or DCM) was added. Stirring was continued at 0 °C for 1 h and then at room temp for 3–50 h. The resultant mixture was filtered and the solvent evaporated. The oily residue was dissolved in solution of EtOH or MeOH with Et_2O and acidified to pH 2–3 with saturated HCl solution in EtOH. After 2–7 days of at 5 °C a hydrochloride salt precipitated. The salt was then purified by recrystallization from the appropriate solvent (EtOH or MeOH).

6.1.1.3. Procedure P3 (18, 20). To a solution of 3-acetyl-dihydrofuran-2(3*H*)-one (1 equiv) in toluene, an appropriate amine (1 equiv) was added. The mixture was refluxed and the water azeotropically removed (Dean–Stark apparatus). The solvent was evaporated and a solid residue was recrystallized from *i*-PrOH.

6.1.2. 3-[4-(2-Methoxyphenyl)-piperazin-1-yl]-dihydrofuran-2(3*H*)-one (3)

Procedure P1. *Reagents*: 1-(2-methoxyphenyl)piperazine (1.92 g, 10 mmol), 3-bromo-dihydrofuran-2(3*H*)-one (1.65 g, 10 mmol), K_2CO_3 (1.66 g, 12 mmol), toluene (30 ml); reaction time 7 h; crystallization from *i*-PrOH; yield: 1.25 g (45 %); mp 127–128 °C; TLC: R_f (S_1) = 0.63, R_f (S_2) = 0.85; ^{1}H NMR ($CDCl_3$, δ ppm): 7.05–6.82 (m, 4H, *Ph*), 4.45–4.27 (m, 2H, CH_2-γ), 3.84 (s, 3H, CH_3O), 3.66 (m, 1H, C*H*-α), 3.06 (t, *J* = 4.5 Hz, 4H, CH_2-3,5-PIP), 3.05 (m, 2H, CH_2-2,6-PIP), 2.82–2.71 (m, 2H, CH_2-2,6-PIP), 2.47–2.26 (m, 2H, CH_2-β). ESI-MS (*m*/*z*) 277.4 $[M+H]^+$. Anal. calcd for $C_{15}H_{20}N_2O_3$: C, 65.20; H, 7.30; N, 10.14. Found: C, 64.91; H, 7.32; N, 9.94.

6.1.3. 3-[4-(3-Methoxyphenyl)-piperazin-1-yl]-dihydrofuran-2(3*H*)-one (4)

Procedure P1. *Reagents*: 1-(3-methoxyphenyl)piperazine (1.92 g, 10 mmol), 3-bromo-dihydrofuran-2(3*H*)-one (1.65 g, 10 mmol), K_2CO_3 (1.66 g, 12 mmol), MeCN (30 ml); reaction time 5 h; crystallization from *i*-PrOH; yield: 1.62 g (59 %); mp 79–81 °C; TLC: R_f (S_1) = 0.68, R_f (S_2) = 0.85; ^{1}H NMR ($CDCl_3$, δ ppm): 7.17 (t, *J* = 8.1 Hz, 1H, 5-*Ph*), 6.54–6.39 (m, 3H, 2,4,6-*Ph*), 4.40 - 4.22 (m, 2H, CH_2-γ), 3.79 (s, 3H, CH_3O), 3.59 (m, 1H, C*H*-α), 3.24 (t, *J* = 5.0 Hz, 4H, CH_2-3,5-PIP), 2.99 (m, 2H, CH_2-2,6-PIP), 2.72 (m, 2H, CH_2-2,6-PIP), 2.35 (m, 2H, CH_2-β). ESI-MS (*m*/*z*) 277.5 $[M+H]^+$. Anal. calcd for $C_{15}H_{20}N_2O_3$: C, 65.20; H, 7.30; N, 10.14. Found: C, 64.80; H, 7.69; N, 10.15.

6.1.4. 3-[4-(4-Methoxyphenyl)-piperazin-1-yl]-dihydrofuran-2(3*H*)-one (5)

Procedure P1. *Reagents*: 1-(4-methoxyphenyl)piperazine (4.81 g, 25 mmol), 3-bromo-dihydrofuran-2(3*H*)-one (4.12 g, 25 mmol), K_2CO_3 (3.46 g, 25 mmol), MeCN (80 ml); reaction time 5 h; crystallization from *i*-PrOH; yield: 4.6 g (67 %); mp 115–116 °C; TLC: R_f (S_1) = 0.63, R_f (S_2) = 0.84; ^{1}H NMR ($CDCl_3$, δ ppm): 6.87 (m, 4H, *Ph*), 4.40 - 4.22 (m, 2H, CH_2-γ), 3.76 (s, 3H, CH_3O), 3.59 (t, *J* = 9.5 Hz, 1H, C*H*-α), 3.14 (t, *J* = 4.9 Hz, 4H, CH_2-3,5-PIP), 3.00 (m, 2H, CH_2-2,6-PIP), 2.73 (m, 2H, CH_2-2,6-PIP), 2.35 (m, 2H, CH_2-β). ESI-MS (*m*/*z*) 277.5 $[M+H]^+$. Anal. calcd for $C_{15}H_{20}N_2O_3$: C, 65.20; H, 7.30; N, 10.14. Found: C, 65.44; H, 7.54; N, 10.09.

6.1.5. 3-[4-(5-Chloro-2-methoxyphenyl)-piperazin-1-yl]-dihydrofuran-2(3*H*)-one (6)

Procedure P1. *Reagents*: 1-(5-chloro-2-methoxyphenyl) piperazine (2.27 g, 10 mmol), 3-bromo-dihydrofuran-2(3*H*)-one (1.65 g, 10 mmol), K_2CO_3 (1.38 g, 10 mmol), DCM (30 ml); reaction time 7 h; crystallization from *i*-PrOH; yield: 2.07 g (67 %); mp 91–92 °C; TLC: R_f (S_1) = 0.72, R_f (S_2) = 0.87; ^{1}H NMR ($CDCl_3$, δ ppm): 6.91 (dd, *J* = 2.5, 8.6 Hz, 1H, 3-*Ph*), 6.89 (d, *J* = 2.5 Hz, 1H, 4-*Ph*), 6.79 (d, *J* = 8.6 Hz, 1H, 6-*Ph*), 4.40 - 4.28 (m, 2H, CH_2-γ), 3.89 (s, 3H, CH_3O), 3.62 (t, *J* = 9.5 Hz, 1H, C*H*-α), 3.21–3.06 (m, 4H, CH_2-3,5-PIP), 3.01 (m, 2H, CH_2-2,6-PIP), 2.76 (m, 2H, CH_2-2,6-PIP), 2.46–2.28 (m, 2H, CH_2-β). ESI-MS (*m*/*z*) 311.5 $[M+H]^+$. Anal. calcd for $C_{15}H_{19}N_2O_3Cl$: C, 57.97; H, 6.16; N, 9.01. Found: C, 57.41; H, 6.49; N, 8.91.

6.1.6. 3-[4-(4-Fluorophenyl)-piperazin-1-yl]-dihydrofuran-2(3*H*)-one (7)

Procedure P1. *Reagents*: 1-(4-fluorophenyl)piperazine (7.21 g, 40 mmol), 3-bromo-dihydrofuran-2(3*H*)-one (6.60 g, 40 mmol), K_2CO_3 (6.91 g, 50 mmol), MeCN (65 ml); reaction time 5 h; crystallization from *i*-PrOH; yield: 7.97 g (75 %); mp 95–97 °C; TLC: R_f (S_1) = 0.56, R_f (S_2) = 0.75; ^{1}H NMR ($CDCl_3$, δ ppm): 6.95 (m, 2H, 3,5-*Ph*), 6.87 (m, 2H, 2,6-*Ph*), 4.41 - 4.22 (m, 2H, CH_2-γ), 3.59 (t, *J* = 9.5 Hz, 1H, C*H*-α), 3.16 (m, 4H, CH_2-3,5-PIP), 3.00 (m, 2H, CH_2-2,6-PIP), 2.73 (m, 2H, CH_2-2,6-PIP), 2.40–2.29 (m, 2H, CH_2-β). ESI-MS (*m*/*z*) 265.6 $[M+H]^+$. Anal. calcd for $C_{14}H_{17}N_2OF$: C, 63.62; H, 6.48; N, 10.60. Found: C, 63.30; H, 6.33; N, 10.47.

6.1.7. 5-Methyl-3-(4-phenylpiperazin-1-yl]-dihydrofuran-2(3*H*)-one (8)

Procedure P1. *Reagents*: 1-phenylpiperazine (1.62 g, 10 mmol), 3-bromo-5-methyl-dihydrofuran-2(3*H*)-one (1.79 g, 10 mmol), K_2CO_3 (1.38 g, 10 mmol), MeCN (50 ml); reaction time 5 h; crystallization from i-PrOH; yield: 1.3 g (50 %); mp 105–107 °C; TLC: R_f (S_1) = 0.75, R_f (S_2) = 0.89; ^{1}H NMR ($CDCl_3$, δ ppm): 7.27 (m, 2H, 3,5-Ph), 7.00–6.79 (m, 3H, 2,4,6-Ph), 4.55–4.41 (m, 1H, CH-γ), 3.72 (m, 1H, CH-α), 3.29 (m, Hz, 2H, CH_2-3,5-PIP), 3.08 (m, 2H, CH_2-3,5-PP), 2.81 (m, 4H, CH_2-2,6-PIP), 2.50 (m, 1H, CH_2-β), 1.97 (m, 1H, CH_2-β), 1.52 (m, 3H, CH_3). ESI-MS (*m*/*z*) 261.5 $[M+H]^+$. Anal. calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N,10.76. Found: C, 69.53; H, 8.12; N, 10.92.

6.1.8. 4-(4-Phenylpiperazin-1-yl]-dihydrofuran-2(3*H*)-one (9)

Procedure. To a solution of 1.43 g (17 mmol) of 5-H-furan-2-one in 2 ml of DCM a solution of 2.76 g (17 mmol) of 1-phenylpiperazine in 2 ml of DCM was added dropwise over 30 minutes. The

mixture was then stirred for 3 h. Evaporation of the solvent and recrystallization from EtOAc yielded 1.87 g (45 %) of compound **28**; mp 127–129 °C; TLC: R_f (S_1) = 0.55, R_f (S_2) = 0.88; ^{1}H NMR ($CDCl_3$, δ ppm): 7.35–7.20 (m, 2H, 3,5-Ph), 6.99–6.81 (m, 3H, 2,4,6-Ph), 4.46 (dd, J = 7.0, 9.2 Hz, 1H, CH_2-γ), 4.24 (dd, J = 6.5, 9.2 Hz, 1H, CH_2-γ), 3.38 (t, J = 6.8 Hz, 1H, CH-β), 3.21 (m, 4H, CH_2-3,5-PIP), 2.72–2.51 (m, 6H, CH_2-2,6-PIP, CH_2-α). ESI-MS (m/z) 247.5 $[M+H]^+$. Anal. calcd for $C_{14}H_{18}N_2O_2$: C, 68.27; H, 7.37; N,11.37. Found: C, 67.91; H, 7.41; N, 11.27.

6.1.9. 3-(4-Methoxybenzylamine)-dihydrofuran-2(3*H*)-one x HCl (12)

Procedure P2. *Reagents*: 4-methoxybenzylamine (3.43 g, 25 mmol), 3-bromo-dihydrofuran-2(3*H*)-one (4.12 g, 25 mmol), K_2CO_3 (6.91 g, 50 mmol), MeCN (70 ml); reaction time 50 h; crystallization from EtOH; yield: 5.4 g (84 %); mp 192–195 °C; TLC: R_f (S_1) = 0.63, R_f (S_2) = 0.59; ^{1}H NMR ($CDCl_3$, δ ppm): 10.13 (s, 2H, NH_2^+), 7.48 (d, J = 8.8 Hz, 2H, 2,6-*Ph*), 6.97 (d, J = 8.8 Hz, 2H, 3,5-*Ph*), 4.46 (m, 1H, C*H*-α), 4.37–4.09 (m, 4H, CH_2-γ, CH_2-BA), 3.75 (s, 3H, CH_3O), 2.64–2.33 (m, 2H, CH_2-β). -β). ESI-MS (m/z) 222.3 $[M+H]^+$. Anal. calcd for $C_{12}H_{16}NO_3Cl$: C, 55.93; H, 6.26; N, 5.43. Found: C, 55.74; H, 6.44; N, 5.41.

6.1.10. 3-[2-Trifluomethylbenzylamine)-dihydrofuran-2(3*H*)-one (13)

Procedure P1. *Reagents*: 2-trifluomethylbenzylamine (5.25 g, 30 mmol), 3-bromo-dihydrofuran-2(3*H*)-one (4.95 g, 30 mmol), K_2CO_3 (4.15 g, 30 mmol), MeCN (30 ml); reaction time 40 h; crystallization from isopropanol; yield: 3.3 g (42 %); mp 51–52 °C; TLC: R_f (S_1) = 0.80, R_f (S_2) = 0.89; ^{1}H NMR ($CDCl_3$, δ ppm): 7.66 (m, 2H, 4,6-*Ph*), 7.59 (t, J = 7.5 Hz, 1H, 5-*Ph*), 7.42 (t, J = 7.6 Hz, 1H, 3-*Ph*), 4.41 (m, 1H, CH_2-γ), 4.25 (m, 1H, CH_2-γ), 4.07 (m, 2H, CH_2-Bz), 3.60 (m, 1H, C*H*-α), 2.49–2.21 (m, 2H, CH_2-β), N*H* proton not detected. ESI-MS (m/z) 260.4 $[M+H]^+$. Anal. calcd for $C_{12}H_{12}NO_2F_3$: C, 55.60; H, 4.67; N, 5.40. Found: C, 55.70; H, 4.88; N, 5.50.

6.1.11. 3-Benzylamine-5-methyl- dihydrofuran-2(3*H*)-one x HCl (14)

Procedure P2. *Reagents*: benzylamine (1.07 g, 10 mmol), 3-bromo-5-methyl-dihydrofuran-2(3*H*)-one (1.79 g, 10 mmol), K_2CO_3 (1.38 g, 10 mmol), MeCN (35 ml); reaction time 20 h; crystallization from acetone; yield: 1.43 g (59 %); mp 215–217 °C; TLC: R_f (S_1) = 0.74, R_f (S_2) = 0.85; ^{1}H NMR ($CDCl_3$, δ ppm): 10.31 (s, 2H, NH^+), 7.58 (d, J = 4.1 Hz, 2H, 3,5-Ph), 7.44 (d, J = 4.8 Hz, 3H, 2,4,6-Ph), 4.65 (m, 1H, CH_2-γ), 4.57–4.14 (m, 3H, CH_2-Bz, CH-α), 2.82 - 2.57 (m, 1H, CH_2-β), 2.21–2.01 (m, 1H, CH_2-β), 1.36 (m, 3H, CH_3). ESI-MS (m/z) 206.2 $[M+H]^+$. Anal. calcd for $C_{12}H_{16}NO_2Cl$: C, 59.63; H, 6.67; N,5.79. Found: C, 59.33; H, 6.75; N, 5.76.

6.1.12. 3-(6,7-Dimethoxy-3,4,dihydro-1H-isoquinolin-2-yl)-dihydrofuran-2(3*H*)-one (16)

Procedure P1. *Reagents*: 6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline (2.71 g, 14 mmol), 3-bromo-dihydrofuran-2(3*H*)-one (2.47 g, 15 mmol), K_2CO_3 (6.22 g, 45 mmol), MeCN (160 ml); reaction time 5 h; crystallization from *i*-PrOH; yield: 0.88 g (23 %); mp 100–101 °C; TLC: R_f (S_1) = 0.65, R_f (S_2) = 0.87; ^{1}H NMR ($CDCl_3$, δ ppm): 6.59 (s, 1H, 8-TQ), 6.51 (s, 1H, 5-TQ), 4.42 - 4.24 (m, 2H, CH_2-γ), 4.01 (d, J = 14.1 Hz, 1H, CH_2-1-TQ), 3.85 (s, 3H, CH_3O), 3.81 (s, 3H, CH_3O), 3.80–3.66 (m, 2H, CH_2-1-TQ , C*H*-α), 3.16–3.03 (m, 1H, CH_2-3-TQ), 2.92–2.79 (m, 3H, CH_2-3-THIQ, CH_2-4-TQ), 2.50–2.30 (m, 2H, CH_2-β). ESI-MS (m/z) 278.4 $[M+H]^+$. Anal. calcd for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05. Found: C, 64.84; H, 7.22; N, 5.09.

6.1.13. 2-(2-Oxotetrahydrofuran-3-yl)-isoindolin-1,3,dion (17)

Procedure. A mixture of 4.08 g (22 mmol) of potassium phthalimide, 3.63 g (22 mmol) of 3-bromo-dihydrofuran-2(3*H*)-one and 30 ml of DMF was stirred and heated at 100 °C for 8 h. The mixture was then poured on crumbled ice (100 g). The precipitated product was filtered off, washed with water and recrystallized from *i*-PrOH; yield 4.42 g (87 %); mp 167–169 °C; TLC: R_f (S_1) = 0.76, R_f (S_2) = 0.85; ^{1}H NMR ($CDCl_3$, δ ppm): 7.93–7.83 (m, 2H, 4,7-PHT), 7.79–7.73 (m, 2H, 5,6-PHT), 5.10 (m, 1H, C*H*-α -), 4.66 (m, 1H, CH_2-γ), 4.40 (m, 1H, CH_2-γ), 2.79 (m, 1H, CH_2-β), 2.60 (m, 1H, CH_2-β). ESI-MS (m/z) 232.4 $[M+H]^+$. Anal. calcd for $C_{12}H_9NO_4$: C, 62.34; H, 3.92; N, 6.06. Found: C, 61.69; H, 4.06; N, 5.88. According to[29] mp 173–176 °C.

6.1.14. 3-[1-(Phenylamino)-ethylidene]-dihydrofuran-2(3*H*)-one (18)

Procedure P3. *Reagents*: phenylamine (1.16 g, 12.5 mmol), 3-acetyl-dihydrofuran-2(3*H*)-one (1.60 g, 12.5 mmol), toluene (30 ml); reaction time 1 h; crystallization from *i*-PrOH; yield: 1.5 g (59 %); mp 85–87 °C; TLC: R_f (S_1) = 0.84, R_f (S_2) = 0.90; ^{1}H NMR ($CDCl_3$, δ ppm) 9.98 (s, 1H, N*H*), 7.32 (t, J = 7.8 Hz, 2H, 3,5-Ph), 7.10 (t, J = 7.4 Hz, 1H, 4-Ph), 7.09 (d, J = 7.4 Hz, 2H, 2,6-Ph), 4.35 (m, 2H, CH_2-γ), 2.91 (t, J = 7.9 Hz, 2H, CH_2-β), 2.02 (s, 3H, CH_3). ESI-MS (m/z) 204.3 $[M+H]^+$. Anal. calcd for $C_{12}H_{13}NO_2$: C, 70.92; H, 6.45; N, 6.89. Found: C, 70.50; H, 6.60; N, 6.77. According to[32] mp 75–77 °C.

6.1.15. 3-[1-[2-(3,4-Dimethoxyphenyl)ethylamino]-ethylidene]-dihydrofuran-2(3*H*)-one (20)

Procedure P3. *Reagents*: 3,4-dimethoxyphenylethylamine (3.62 g, 20 mmol), 3-acetyl-dihydrofuran-2(3*H*)-one (2.56 g, 20 mmol), toluene (30 ml); reaction time 1 h; crystallization from *i*-PrOH; yield: 3.5 g (60 %); mp 114–115 °C; TLC: R_f (S_1) = 0.77, R_f (S_2) = 0.92; ^{1}H NMR ($CDCl_3$, δ ppm) 8.34 (s, 1H, N*H*), 6.88–6.66 (m, 3H, Ph), 4.29–4.21 (m, 2H, CH_2-γ), 3.96 (s, 3H, CH_3O), 3.92 (s, 3H, CH_3O), 3.42 (m, 2H, CH_2-1-PEA), 2.87–2.70 (m, 4H, CH_2-2-PEA, CH_2-β), 1.85 (s, 3H, CH_3).). ESI-MS (m/z) 292.6 $[M+H]^+$. Anal. calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81. Found: C, 65.85; H, 7.43; N, 4.80. According to[32] mp 112–113 °C.

6.1.16. 3-(2,6-Dimethylphenylamine)-dihydrofuran-2(3*H*)-one (21)

Procedure. To a solution of 8.5 ml toluene, 3.03 g (25 mmol) of 2,6-dimethylphenylamine and 4.12 (25 mmol) of 3-bromo-dihydrofuran-2(3*H*)-one, heated to 80 °C, a solution of 1.73 g (12.5 mmol) of K_2CO_3 in 5 ml of water was added dropwise over 5 h. After cooling, the mixture was washed with 5% solution of $KHCO_3$ (10 ml) and water (3 × 25 ml). The organic layer was dried over Na_2SO_4, filtered off and the solvent removed in vacuo. The oily residue was dissolved in Et_2O which crystallized slowly after a few days at 5 °C; yield 3.2 g (62 %); mp 83–84 °C; TLC: R_f (S_1) = 0.79, R_f (S_2) = 0.94; ^{1}H NMR ($CDCl_3$, δ ppm) 7.02 (d, J = 7.4 Hz, 2H, 3,5-Ph), 6.93 (m, 1H, 4-Ph), 4.46–4.23 (m, 2H, CH_2-γ), 4.01 (m, 1H, C*H*-α), 2.68 (m, 2H, CH_2-β), 2.40 (m, 6H, 2CH_3, CH_2-β). ESI-MS (m/z) 206.0 $[M+H]^+$. Anal. calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 69.69; H, 7.71; N, 6.84. According to[48] mp 85–86 °C.

6.1.17. 3-Cyclohexylamine-dihydrofuran-2(3*H*)-one x HCl (23)

Procedure: P2. *Reagents*: cyclohexylamine (2.48 g, 25 mmol), 3-bromo-dihydrofuran-2(3*H*)-one (4.13 g, 25 mmol), K_2CO_3 (3.45 g, 25 mmol), DCM (40 ml); reaction time 20 h; crystallization from EtOH; yield: 1.65 g (30 %); mp 244–245 °C; TLC: R_f (S_1) = 0.69, R_f (S_2) = 0.73; ^{1}H NMR ($CDCl_3$, δ ppm) 10.05 (s, 1H, NH_2^+), 9.49 (s, 1H, NH_2^+), 4.66–4.43 (m, 2H, C*H*-α , CH_2-γ), 4.36–4.17 (m, 1H, CH_2-γ), 3.20 (t, J = 10.8 Hz, 1H, C*H*-1-CYC), 2.63–2.45 (m, 2H,

CH_2-β), 2.16–1.94 (m, 2H, CH_2-2,6-CYC), 1.77 (m, 2H, CH_2-2,6-CYC), 1.66–0.97 (m, 6H, CH_2-3,4,5-CYC). ESI-MS (*m*/*z*) 184.6 $[M+H]^+$. Anal. calcd for $C_{10}H_{18}NO_2Cl$: C, 54.67; H, 8.26; N, 6.37. Found: C, 54.03; H, 8.63; N, 6.34. Compound **20** has been described as a free base.[47]

6.2. Determination of lipophilicity

6.2.1. Reversed-phase thin-layer chromatography

Silica gel RP-18 F_{254s} plates, 10 × 10 cm (Merck, Darmstadt, Germany), were used for measurement by planar chromatography. Mixtures of MeCN (Merck) and Tris buffer (pH 7.4) containing 10–90% (v/v) of organic modifier decreasing in 5% (v/v) steps were used as mobile phases. The Tris buffer was prepared by dissolving 24.35 g of Tris (Merck) in 250 mL distilled water and adjusting this solution to pH 7.4 with 0.1 mol L^{-1} hydrochloric acid (POCH, Gliwice, Poland). Analytical-grade chemicals were used unless indicated otherwise. Solutions of the tested compounds in MeOH were prepared at concentrations of 1 mg/mL. 10 μL of each sample was applied onto plates which were then placed in TLC chambers (5 × 15 × 15), previously saturated with the solvents for 2 h. Following development, the plates were dried and the spots observed under UV light at λ = 254 nm. All measurements were conducted at ambient temperature. Mean retention factor (R_f) values were calculated for three independent determinations and converted to retention parameters (R_M) using the following equation:

$$R_M = log(1/R_f - 1) \quad (1)$$

The R_M values for each compound, obtained with different solvent systems, were then extrapolated to zero concentration of MeCN in the mobile phase to estimate the relative retention parameters (R_{M0}) according to the equation:

$$R_M = R_{M0} + aC \quad (2)$$

where *C* is the concentration of MeCN in the mobile phase (% v/v).

6.2.2. Calculation of partition coefficients

Theoretical partition coefficients of the tested compounds were calculated using two computer programs: ChemOffice Ver. 8,0; 1985–2003 CambridgeSoft Corporation, (*C*log*P*) and MarvinView 5.2.0 (MlogP).

6.3. Pharmacology

6.3.1. ASP - anticonvulsant assays

Preliminary anticonvulsant evaluation (Test I) of all the synthesized lactones was performed by applying test protocols[49] designed and sponsored by the National Institute of Neurological Disorders and Stroke, NIH, Rockville, MD, USA, for the Anticonvulsant Screening Program (ASP). Test I evaluations consisted of three tests performed in mice (ip): maximal electroshock, subcutaneous pentetrazole, and rotarod test for neurological toxicity. Advanced studies included MES and TOX tests in rats (po) and 6-Hz test in mice.

6.3.1.1. Animals. Male albino, CF No. 1 mice (18–25 g, Charles River, Willimington, MA, USA) and male albino, Sprague-Dawley rats (100–150 g, Charles River, Willimington, MA, USA) were used in the experiment. Compounds were either dissolved in saline or suspended in 0.5% methylcellulose. The compound was administered to mice by ip injection at three dosage levels (30, 100 and 300 mg/kg) with anticonvulsant activity and neurotoxicity noted 15, 30, 60 and 240 minutes after administration.

6.3.1.2. Maximal electroshock seizure (MES) test[49]. Maximal electroshock seizures were elicited with a 60-cycle alternating current of 50 mA intensity (5–7 times the levels necessary to elicit minimal electroshock seizures) delivered over 0.2s via corneal electrodes. A drop of 0.5% tetracaine hydrochloride in 0.9% saline was instilled in the eye prior to application of the electrodes. Protective endpoints were defined as abolition of the hind limb tonic extension component of the seizure. Results are expressed as a ratio of the number of animals protected/number of animals tested.

6.3.1.3. Pentetrazole inducted seizure (scMet). A dose of 85 mg/kg of pentetrazole which produces seizures in more than 95% of mice, was administered as a 0.5% solution subcutaneously in the posterior midline. Animals were observed for 30 minutes. Failure to observe even a threshold seizure (a single episode of clonic spasms with a minimum duration of 5 seconds) was defined as protection and the results were expressed as the number of animals protected/number of animals tested.

6.3.1.4. Neurotoxicity (TOX)[50,51]. The rotorod test was used to evaluate neurotoxicity in mice. Animals were placed on a 1-inch diameter knurled plastic rod rotating at 6 rpm. Non-toxic (normal) mice can remain on a rod rotating at this speed almost indefinitely. Neurological toxicity is defined as the failure of the animal to remain on the rod for 1 min and is expressed as the number of animals exhibiting toxicity/number of animals tested. Animals are considered toxic if they fail this test on three successive attempts. Rat toxicity was determined using overt evidence of ataxia, abnormal gait or the positional sense test.

6.3.1.5. Minimal clonic seizure (6-Hz) model[37–39]. The 6-Hz test is used to assess a compound's efficacy against electrically-induced seizures but uses a lower frequency (6 Hz) and onger duration of stimulation (3 s). Test compounds are pre-administered to mice via ip injection. Corneal stimulation (0.2 ms monopolar rectangular pulses at 6 Hz over 3 s) was delivered by a constant-current device. During the stimulation, mice were manually restrained and released into the observation cage immediately following the application of the current. Untreated mice will display seizures characterized by a minimal clonic phase followed by stereotyped, automatistic behaviors described originally as being similar to the aura of human patients with partial seizures. The duration of the seizure activity ranged from 60 to 120 s in untreated animals. Upon resolution of seizures animals resumed their normal exploratory behavior. The experimental endpoint was protection against seizure. The animal was considered to be protected if it resumed its normal exploratory behavior within 10 s of stimulation.

6.3.2. Analgesic activity tests

Analgesic activity studies were carried out in collaboration with the Department of Pharmacodynamics, Jagiellonian University, Medical College. Preliminary antinociceptive activity evaluation consisted of three tests performed in mice: hot plate test, writhing test and modified tail immersion test.

6.3.2.1. Chemicals. Except for the local anesthetic activity test, the investigated compounds were administered intraperitoneally (ip) in the form of a suspension in 0.5% methylcellulose (MC) (Loba Chemie) solution 30 min before each assay. Phenylbenzoquinone (INC Pharmaceuticals, Inc. N.Y.) was prepared as a 0.02% solution. Morphine (Morphinum hydrochloricum, Polfa Kutno), acetylsalicylic acid (Polpharma), mepivacaine (Meaverin 2%, Rhone-Poulenc Rorer) and lidocaine (Lignocainum hydrochloricum 2%, WZF) were used as reference drugs. Control animals were given appropriate amounts of vehicle (MC).

6.3.2.2. Animals. For the behavioral experiments, adult male Albino Swiss (CD-1) mice weighing 18–30 g were used. The animals were kept in groups of 15 mice in cages at a temperature of 22 ± 2 °C, under a light/dark (12/12) cycle and had free access to food and water before the experiments. Each experimental group consisted of 6–8 animals/dose and all the animals were used only once. In all experiments the mice were habituated to the vivarium for a minimum of 72 h prior to experimentation. The experiments were performed between 08:00–15:00. The procedures were approved by the Local Ethics Committee of the Jagiellonian University in Cracow (ZI/329/2006).

6.3.2.3. Hot plate test. In this test the mice were pre-treated ip either with the test compound or the vehicle 30 min before being placed on a hot plate apparatus (Hot Plate 2A Type Omega, Poland) with the temperature maintained at 55–56 °C. The time until the animal licked its back paws or jumped (i.e. the latency time of the nociceptive reaction) was recorded by means of a chronometer.[52]

6.3.2.4. Writhing test. In this test the mice were injected 0.25 ml 0.02% phenylbenzoquinone solution 30 min following ip administration of the investigated compound or the vehicle (MC). The mice were then placed individually into glass beakers and were allowed to elapse for 5 min. Subsequently a 10-min observation was conducted for each animal the number of characteristic writhes (lengthwise constrictions of the torso with a concomitant concave arching of the back) was counted. The percentage fraction of the analgesic effect was calculated as [(methylcellulose-treated mean writhes–drug-treated mean writhes)/methylcellulose-treated mean writhes] × 100.[44]

6.3.2.5. Local anesthetic activity (modification of the tail immersion test). The heat method, used for evaluating systemic analgesic activity, can also be applied (with slight modifications) to determine whether the compound possesses local anesthetic activity. The experiment was conducted by injecting the investigated substance solution subcutaneously (*s.c.*) in a constant volume of 0.2 ml about 1 cm from the root of the mouse tail. 15 min later the distal 3 cm section of the tail was immersed in water with temperature maintained at 50 ± 0.5° C. The nociceptive reaction time (i.e. time before the tail is pulled away) was measured. The maximal observation time was limited to 20 s to avoid tissue damage (cutoff time).[45]

6.3.2.6. Statistical analysis. The data obtained in the experiments is expressed as the mean ± SEM (standard error of the mean). Student's t-test was applied to compare results between two different groups of animals (the investigated compound group vs the control group). The difference of means was taken as statistically significant if $p < 0.05$. The log-probit method (Litchfield and Wilcoxon method) was applied to establish the ED_{50} values within their respective 95% confidence limits.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.08.037. These data include MOL files and InChiKeys of the most important compounds described in this article.

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