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Original article

# Synthesis and biological evaluation of new derivatives of 2-substituted 4-hydroxybutanamides as GABA uptake inhibitors

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ABSTRACT

This study presents the synthesis of novel substituted 4-hydroxybutanamides and their influence on the activity of murine GABA transport proteins GAT1–GAT4. The active compounds, derivatives of *N*-arylalkyl-2-(4-diphenylmethylpiperazin-1-yl)-4-hydroxybutyramide, are characterized by $pIC_{50}$ values in range of 3.92–5.06 and by slight subtype-selectivity. Among them *N*-4-chlorobenzylamide was the most potent GAT inhibitor (mGAT3), while *N*-benzylamide was the most active in GAT1-binding assay ($pK_i$ = 4.96). The results pointed out that benzhydryl and benzylamide moieties are crucial for the activity of this class of compounds as murine GAT inhibitors.



## 1. Introduction

4-Aminobutanoic acid (γ-aminobutyric acid, GABA) is the essential inhibitory neurotransmitter in mammalian brain which influences all neurological and psychological processes in central nervous system (CNS) [1]. Since the activity of GABAergic system is so extensive, its dysfunctions can lead to various neurological disorders such as epilepsy, insomnia, spasticity, neuropathic pain, as well as anxiety and other mental disorders [2–5]. The majority of drugs effective in the treatment of disturbances mentioned above exert the pharmacological activity by direct influence on GABA receptors or increasing GABA accessibility in synaptic cleft through inhibition of its metabolism or inhibition of specific transporters. The identification of GABA transporter proteins (GATs) and their crucial role in regulation of GABA concentration in the brain opened up new possibilities in the search for GABAergic-active compounds and thereby potential CNS drugs [6]. To date, four subtypes of membrane-bound proteins transporting GABA have been identified and cloned: GAT1, GAT2, GAT3, and GAT4 [7]. The various types of GAT display different physiological activities and distribution in CNS [8]. Tiagabine (**1**) is an example of a selective GAT1 inhibitor and one of the most potent drugs used in the treatment of epilepsy [9,10]. Although the extensively investigated GAT1 seems to be the most promising one, the three latter still remain in the area of interest of current medicinal chemistry. Potent and selective inhibitors of GAT2–GAT4 are sought-after not only as potential drugs, but mainly as research tools for investigation of the physiological role and therapeutic potential of each subtype [11–17]. Among GAT inhibitors, the GABA analogs nipecotic acid (**2**), guvacine (**3**), and compounds SKF-89976-A (**4**), Cl-966 (**5**), NO711 (**6**) (Fig. 1) represent a significant and large group.

The present work is a part of an extended search for new anticonvulsant agents and potential antiepileptics in the group of derivatives of 4-hydroxybutanamide [18,19]. In this paper, we present the synthesis of substituted 4-hydroxybutanamides and biological evaluation of their influence on murine GABA uptake proteins GAT1–GAT4. 4-Hydroxybutyric acid (γ-hydroxybutyric acid, GHB), which was chosen as the core-structure of the new compounds, is an endogenous substance, a metabolite of GABA, which acts as an inhibitory neurotransmitter in mammalian CNS [20,21]. Based on structures of GAT inhibitors and derivatives of *N*-benzylamides of GHB with anticonvulsant activity, a group of new structures was designed. The carboxylic acid function of GHB was transformed into more lipophilic primary amides or arylalkylamides. In position 2 of GHB, a *N*-diphenylmethylpiperazine moiety was introduced as a part mimicking the biaryl moieties of known GAT inhibitors and a fragment corresponding to the anticonvulsant-active arylpiperazine derivatives of GHB [22]. Other modifications in position 2, including

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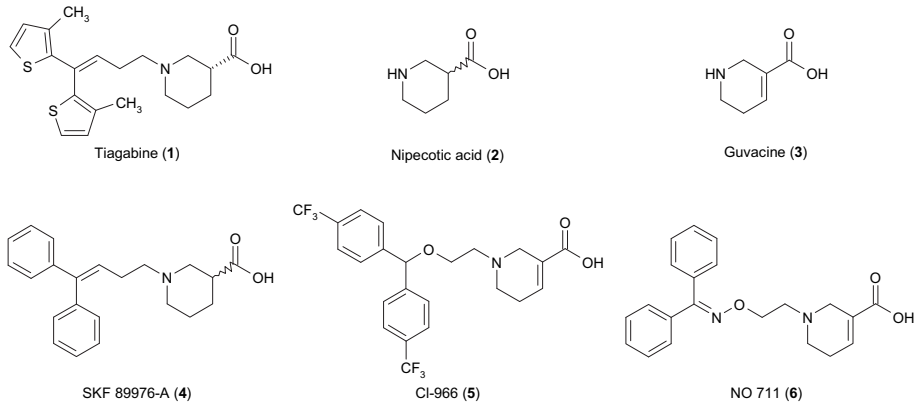



**Fig. 1.** Structures of selected GABA uptake inhibitors.

introduction of analogous arylalkyl- and alkylamine residues were also applied. Schematic structures of the designed and synthesized compounds are presented in Fig. 2. all the designed compounds were synthesized and tested for their inhibitory potency and selectivity towards cloned murine GABA transporters GAT1–GAT4 in uptake assays and their affinity to GAT1 in an MS-binding assay.

## 2. Chemistry

The herein presented derivatives of 4-hydroxybutanamide were obtained by the aminolysis of the appropriate substituted butyrolactones (Fig. 3). The lactones (**8**–**12**) were prepared by *N*-alkylation of the substituted piperazines, morpholine, or 4-benzylpiperidine by 3-bromodihydrofuran-2(3*H*)-one (**7**). The reactions were carried out in the different solvents in the presence of an anhydrous $K_2CO_3$ at room temperature for 3–20 h. Aminolysis of the obtained lactones by ammonia or primary arylalkylamines resulted in primary or secondary amides of 2-substituted-4-hydroxybutanoic acid (**13**–**33**, **37**, **38**). The aminolysis was performed in the different conditions depending on reactivity and solubility of both lactones and amines, including reactions in the solution or fusion in ambient or increased temperature, using conventional methods of heating or microwave irradiation. Compounds **34**–**36** were obtained by deprotection of carbamates **31**–**33** by trifluoroacetic acid (TFA) in DCM (Fig. 3). These methods led to 23 final compounds.

## 3. Biological evaluation

Inhibitory potency of the amides **13**–**30** and **34**–**38** was tested at four murine GABA transporter subtypes GAT1–GAT4. The study was performed as a [$^3$H] GABA uptake assay based on stably transfected HEK cells, according to the procedure recently described [17]. The affinity to GAT1 was determined by MS-binding assay with NO711 as a non-labeled marker [23]. The compounds were considered as active if GABA uptake or NO711 binding was reduced at least by 50% at a concentration of 100 μM. For the active compounds, $pIC_{50}$- or $pK_i$-values were assessed.

## 4. Results and discussion

The majority of the herein described compounds share the same 2-piperazin-1-yl-4-hydroxybutanamide scaffold that corresponds to arylpiperazine derivatives of the anticonvulsant-active *N*-benzylamides of GHB and GABA [18,19]. These compounds can be classified by the type of substituent on the piperazine ring and on nitrogen atom of the amide functionality. The present study focuses on two series of arylalkylamides bearing on the piperazine-part benzhydryl (compounds **13**–**21**) or 4,4′-difluorobenzhydryl moieties (compounds **22**–**30**) (series A and B respectively) mimicking the lipophilic groups present in the known GAT inhibitors (Fig. 1).

To verify the influence of the substituents on the inhibitory potency of the GHB derivatives, several analogs of structures mentioned above were synthesized. The modifications included the exchange of benzhydrylpiperazine for 4-benzylpiperidine (**38**), unsubstituted piperazine (**34**–**36**), or its oxo-analog – morpholine (**37**). In the amide-functionality, apart from differently substituted benzyl- and phenethyl- substituents, primary amide was also obtained (**21**, **30**, **36**).

Biological evaluation revealed that among the tested compounds only the benzhydryl derivatives series A (**13**–**21**) and B (**22**–**30**) were active (Table 1). The derivatives were characterized by $pIC_{50}$ values in range of 3.92–5.06 and slight subtype-selectivity. Among them, (*R*, *S*) 2-(4-benzhydrylpiperazin-1-yl)-*N*-(4-chlorobenzyl)-4-hydroxybutanamide (**15**) was found to be the most potent with an $IC_{50}$ value at GAT3 of about 9 μM. Two amides of

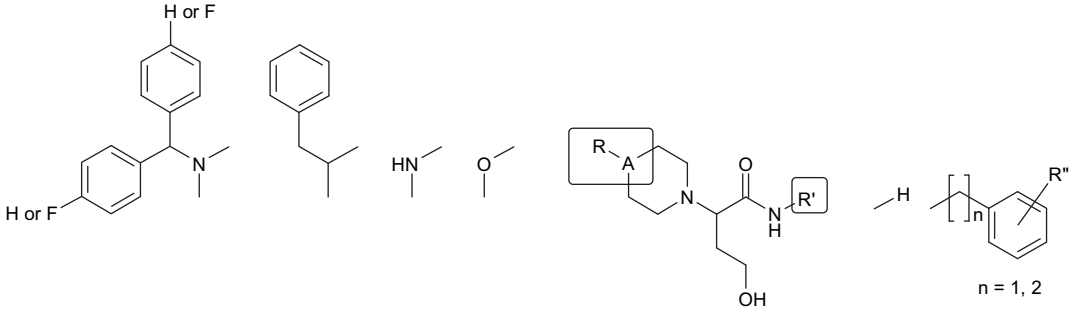
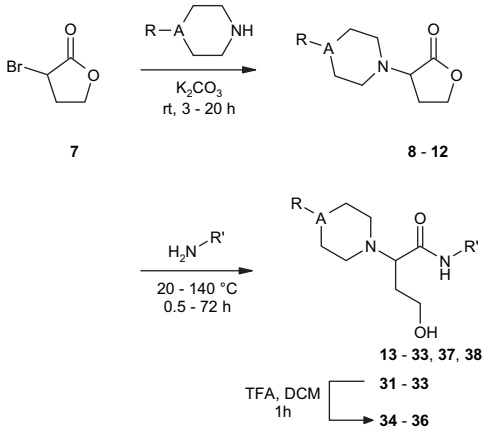
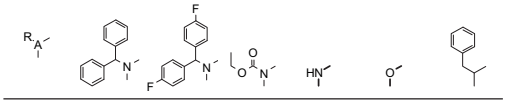



**Fig. 2.** Schematic structure of designed derivatives of 2-substituted-4-hydroxybutanamides as GAT inhibitors.



| | Lactones | | | | | |
|---|---|---|---|---|---|---|
| | **8** | **9** | **10** | **-** | **11** | **12** |
| R' | Amides | | | | | |
| | **13** | **22** | **31** | **34** | **37** | **38** |
| | **14** | **23** | | | | |
| | **15** | **24** | **32** | **35** | | |
| | **16** | **25** | | | | |
| | **17** | **26** | | | | |
| | **18** | **27** | | | | |
| | **19** | **28** | | | | |
| | **20** | **29** | | | | |
| | **21** | **30** | **33** | **36** | | |

**Fig. 3.** Synthesis of 3-substituted dihydrofuran-2(3*H*)-one derivatives (**8**–**12**) and 2-substituted 4-hydroxybutanamides (**13**–**38**).

series A, *N*-benzylamide **13** and *N*-(4-fluorobenzylamide) **17** were found to display a moderate potency at GAT1 with $pIC_{50}$ values of 4.66 and 4.84, i.e. $IC_{50}$ values of 22 and 14 μM, respectively. Comparison of the corresponding amides from series A (**13**–**21**) and B (**22**–**30**) shows that the introduction of the fluorine atoms led to decrease in activity and loss of the binding affinity to GAT1 (**22**–**30**). Among substituents in the amide part, 4-methoxy arylalkylamides were not tolerated (**19**, **28**). The influence of the halogen atom in ortho position on the activity can vary. All further modifications, like degradation, exchange of the piperazine part, or removal of substituent of the amide nitrogen, led to loss of the activity. Since none of the applied modifications gave positive results, we conclude that this kind of bulky and lipophilic biaryl group and substituted amides are crucial for the inhibition of GAT activity in this group of compounds.

## 5. Conclusions

Due to a great number of drug-resistant cases of epilepsy and severe side effects of the available treatment, there is an urgent need for new, more efficient, and safer antiepileptics. GABA transport proteins are among the most promising biological targets in the search for new antiepileptic drugs. In this study, a new group of 2-substituted 4-hydroxybutanamides was synthesized and evaluated for their affinity to GABA transporters GAT1–GAT4. The active compounds were found in the group of *N*-substituted 4-hydroxybutanamides with 4-(diphenylmethyl)piperazin-1-yl and 4-bis(4-fluorophenylmethyl)piperazin-1-yl substituents. Among them, compound **15** showed the highest inhibitory potency against GATs, however was not selective towards the individual subtypes. Preliminary SAR studies for this group of compounds point out that the benzyl substituent in the amide group and benzhydrylpiperazinyl are crucial for the activity. Structural optimization of the active compounds which increases the selectivity and the potency is planned.

## 6. Experimental

### 6.1. Chemistry

Melting points were determined in open glass capillaries on the Electrothermal 9300 apparatus and are uncorrected. Reactions

**Table 1**
Results of [$^3$H] GABA uptake and NO711 MS-binding assays.

| Compound | GAT1 uptake[a] | GAT2 uptake[a] | GAT3 uptake[a] | GAT4 uptake[a] | GAT1 NO711 binding[b] |
|---|---|---|---|---|---|
| **13** | 4.66 ± 0.08 | 4.47$^{(n=1)}$ | 46% | 54% | 4.96 ± 0.07 |
| **14** | 83% | 72% | 73% | 76% | – |
| **15** | 4.88 ± 0.10$^{(n=9)}$ | 4.56 ± 0.13$^{(n=7)}$ | 5.06 ± 0.11$^{(n=5)}$ | 4.91 ± 0.12 | 4.33 ± 0.03 |
| **16** | 4.48 ± 0.11$^{(n=7)}$ | 4.33 ± 0.12$^{(n=7)}$ | 4.26 ± 0.04$^{(n=4)}$ | 82% | 55% |
| **17** | 4.84 ± 0.08 | 52% | 4.34 ± 0.09 | 51% | 4.82 ± 0.12 |
| **18** | 52% | 4.54 | 4.36 ± 0.10 | 52% | 61% |
| **19** | 55% | 78% | 61% | 59% | – |
| **20** | 68% | 93% | 80% | 80% | – |
| **21** | 103% | 49% | 83% | 66% | 98% |
| **22** | 4.19 ± 0.11 | 4.33 ± 0.02 | 4.15 ± 0.06 | 4.10 ± 0.06 | 63% |
| **23** | 4.20 ± 0.13 | 4.17 ± 0.17 | 4.72 ± 0.04 | 4.01 ± 0.08 | 93% |
| **24** | 4.14 ± 0.09 | 4.35 ± 0.08 | 4.54 ± 0.05 | 3.92 ± 0.06 | 102% |
| **25** | 97% | 65% | 75% | 84% | 92% |
| **26** | 4.03 ± 0.07 | 4.13 ± 0.05 | 4.22 ± 0.14 | 4.12 ± 0.14 | 75% |
| **27** | 4.23 ± 0.10 | 4.44 ± 0.11 | 4.52 ± 0.10 | 4.20 ± 0.08 | 92% |
| **28** | 93% | 65% | 75% | 84% | 101% |
| **29** | 69% | 4.43 ± 0.08 | 4.00 ± 0.02 | 4.15 ± 0.90 | 98% |
| **30** | 66% | 84% | 86% | 91% | 106% |
| **34** | 63% | 98% | 62% | 75% | 76% |
| **35** | 79% | 98% | 80% | 80% | – |
| **36** | 84% | 110% | 110% | 98% | – |
| **37** | 113% | 98% | 100% | 100% | 95% |
| **38** | 75% | 49% | 65% | 88% | 86% |
| Tiagabine (**2**)[c] | 6.88 ± 0.12 | 52% | 64% | 73% | – |
| Guvacine (**4**)[c] | 4.87 ± 0.07 | 3.31 ± 0.03 | 4.59 ± 0.05 | 4.59 ± 0.05 | – |

[a] % of remaining GABA uptake at 100 μM concentration of tested compound (means; $n = 2$) or $pIC_{50}$ (means ± SEM; $n = 3$ if not specified otherwise).
[b] % of NO711 binded to GAT1 at 100 μM concentration of tested compound or $pK_i$ (means ± SEM; $n = 3$).
[c] Data from Ref. [17].

were monitored by thin layer chromatography (TLC) using silica gel plates silica gel 60 F254 (Merck) and following solvent systems: $S_1$ ($Me_2CO/CHCl_3$, 1/1) and $S_2$ ($CHCl_3$/MeOH/$CH_3COOH$, 12/2/1). The spots were visualized under UV lamp and by iodine solution (0.05 M in 10% HCl). $^1$H NMR and $^{13}$C NMR spectra were recorded with Varian Mercury 300 spectrometer (300 MHz) in $CDCl_3$ and DMSO-$d^6$, with signal of solvent as an internal standard. Elemental analyses were carried out on a Vario EL III Model Elemental Analyzer. Reactions under microwave irradiation were carried out in Discover LabMate (CEM Corporation).

*6.1.1. Synthesis of 3-substituted dihydrofuran-2(3H)-one derivatives **8**–**12** (general procedure)*

Anhydrous $K_2CO_3$ (1 equiv.) was added to the solution of relevant amine (1 equiv.) in 20 mL of solvent (acetonitrile, DCM or $Me_2CO$) and the mixture was stirred at room temperature for 0.5 h. Then a solution of 3-bromodihydrofuran-2(3*H*)-one (1 equiv.) in 5 mL of appropriate solvent was added dropwise and stirring was continued for 3–20 h. In the synthesis of compounds **11** and **12** tetrabutylammonium bromide (TBAB) (0.1 equiv.) was added. After the reaction was completed, the precipitate was filtered off and the filtrate was concentrated under vacuum. Obtained crude products were recrystallized from suitable solvent (solid) or purified by column chromatography (oil). Lactone **11** was isolated as a hydrochloride salt and recrystallized from DCM. Synthesis of compound **13** was described elsewhere [24].

*6.1.1.1. (R, S) 3-(4-Benzhydrylpiperazin-1-yl)dihydrofuran-2(3H)-one (**8**).* Reagents and conditions: 3-bromodihydrofuran-2(3*H*)-one (1.65 g, 10 mmol), 1-benzhydrylpiperazine (2.52 g, 10 mmol), $K_2CO_3$ (1.38 g, 10 mmol), MeCN, 3 h; yield 65%; mp 161–162 °C (EtOAc); anal. calcd for $C_{21}H_{24}N_2O_2$: C% 74.97, H% 7.19, N% 8.33 found: C% 74.36, H% 7.30, N% 8.22; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 2.30–3.46 (m, 2H, $CHCH_2$), 2.46–2.60 (m, 4H, piperazine), 2.78–2.94 (m, 4H, piperazine), 3.54 (t, $J$ = 9.4 Hz, 1H, C*H*), 4.16–4.20 (m, 1H, C*H*$Ph_2$), 4.32–4.47 (m, 2H, C$H_2$O), 7.15–7.40 (m, 10H, Ar-H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 28.6 ($CHCH_2$), 52.9, 54.4 (piperazine), 66.3 ($OCH_2CH_2$), 78.1 (*C*$HCH_2$), 84.5 (*C*H$Ph_2$), 126.2, 128.2, 129.2, 142.7 (arom), 175.0 (carbonyl); TLC: $R_f$ ($S_1$) = 0.77, $R_f$ ($S_2$) = 0.91.

*6.1.1.2. (R, S) 3-[4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl]dihydrofuran-2(3H)-one (**9**).* Reagents and conditions: 3-bromodihydrofuran-2(3*H*)-one (1.65 g, 10 mmol), 1-[bis(4-fluorophenyl) methyl]piperazine (2.88 g, 10 mmol), $K_2CO_3$ (1.38 g, 10 mmol), MeCN, 20 h; yield 60%; purification by column chromatography ($S_1$); anal. calcd for $C_{21}H_{22}N_2O_2F_2$: C% 67.73, H% 5.95, N% 7.52 found: C% 67.83, H% 5.89, N% 7.49; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 2.28–3.42 (m, 6H, $CHCH_2$, piperazine), 2.55–2.61 (m, 2H, piperazine), 2.83–2.93 (m, 2H, piperazine), 3.53 (t, $J$ = 9.4 Hz, 1H, C*H*), 4.31 (s, 1H, C*H*$Ph_2$), 4.31–4.40 (m, 2H, C$H_2$O), 7.15–7.40 (m, 8H, Ar-H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 28.8 ($CHCH_2$), 53.1, 54.7 (piperazine), 66.2 ($OCH_2CH_2$), 78.6 (*C*$HCH_2$), 84.5 (*C*H$Ph_2$), 116.2, 129.8, 138.2, 160.7 (arom), 175.0 (carbonyl); TLC: $R_f$ ($S_1$) = 0.57, $R_f$ ($S_2$) = 0.83.

*6.1.1.3. (R, S) Ethyl 4-(2-oxotetrahydrofuran-3-yl)piperazine-1-carboxylate (**10**).* Reagents and conditions: 3-bromodihydrofuran-2(3*H*)-one (1.65 g, 10 mmol), ethyl 1-piperazinecarboxylate (1.58 g, 10 mmol), $K_2CO_3$ (1.38 g, 10 mmol), $Me_2CO$, 20 h; yield 66%; purification by column chromatography ($S_1$); anal. calcd for $C_{11}H_{18}N_2O_4$: C% 54.53, H% 7.49, N% 11.56 found: C% 54.40, H% 7.52, N % 11.60; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.22 (t, $J$ = 1.6 Hz, 3H, $CH_2CH_3$), 2.21–2.31 (m, 2H, C$H_2$CH), 2.42–2.50 (m, 4H, piperazine), 2.72–2.84 (m, 4H, piperazine), 3.48 (t, 1H, $J$ = 5.2 Hz, $CH_2CHCO$), 4.05–4.35 (m, 4H, C$H_2$O, C$H_2CH_3$); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 13.8 ($CH_3$), 28.6 ($CHCH_2$), 46.2, 52.0 (piperazine), 62.0 ($OCH_2CH_3$), 66.2 ($OCH_2CH_2$), 78.1 ($CHCH_2$), 156.3, 175.0 (carbonyl); TLC: $R_f$ ($S_1$) = 0.65

*6.1.1.4. (R, S) 3-Morpholinodihydrofuran-2(3H)-one hydrochloride (**11**).* Reagents and conditions: 3-bromodihydrofuran-2(3*H*)-one (1.65 g, 10 mmol), morpholine (0.87 g, 10 mmol), $K_2CO_3$ (1.38 g, 10 mmol), DCM, 3 h; yield 50%; mp 202–204 °C (MeOH); anal. calcd for $C_8H_{13}NO_3 \cdot HCl$: C% 46.27, H% 6.80, N% 6.75 found: C% 45.65, H% 7.16, N% 6.67; $^1$H NMR (DMSO-$d^6$) $\delta$ (ppm): 2.52 (m, 2H, $CHCH_2$), 3.05–3.26 (m, 4H, morpholine), 3.26–3.87 (m, 4H, morpholine),

4.23 (m, 1H, C*H*), 4.47 (m, 2H, C$H_2$O); $^{13}$C NMR (DMSO-$d^6$) $\delta$ (ppm): 28.4 (CH$CH_2$), 53.3, 66.4 (piperazine), 66.8 (O$CH_2CH_2$), 78.4 (*C*H$CH_2$), 175.0 (carbonyl); TLC: $R_f$ ($S_1$) = 0.39, $R_f$ ($S_2$) = 0.61.

*6.1.1.5. (R, S) 3-(4-Benzylpiperidin-1-yl)dihydrofuran-2(3H)-one (**12**).* Reagents and conditions: 3-bromodihydrofuran-2(3*H*)-one (1.65 g, 10 mmol), 4-benzylpiperidine (1.75 g, 10 mmol), $K_2CO_3$ (1.38 g, 10 mmol), $Me_2CO$, 5 h; yield 60%; mp 65–66 °C (iPrOH); anal. calcd for $C_{16}H_{21}NO_2$: C% 74.1, H% 8.16, N% 5.40 found: C% 73.85, H% 8.19, N% 5.37; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.24–1.45 (m, 2H, piperidine), 1.47–1.73 (m, 3H, C$H_2$CH, piperidine), 2.18–2.34 (m, 3H, C$H_2$CH, piperidine), 2.45–2.60 (m, 3H, C$H_2$Ph, piperidine), 2.83 (d, $J$ = 10.3, 1H, piperidine), 2.96 (d, $J$ = 10.5, 1H, piperidine), 3.63 (t, $J$ = 9.5, 1H, C*H*), 4.24 (dd, $J$ = 8.8, 16.8, 1H, C$H_2$O), 4.42 (dt, $J$ = 5.9, 9.1, 1H, C$H_2$O), 7.08–7.36 (m, 5H, Ar-H); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 28.8 (CH$CH_2$), 41.7 (*C*H$_2$Ph), 32.6, 37.2, 44.3 (piperidine), 66.2 (O$CH_2CH_2$), 78.6 (*C*H$CH_2$), 125.9, 128.1, 128.5, 139.7 (arom), 175.0 (carbonyl); TLC: $R_f$ ($S_1$) = 0.66, $R_f$ ($S_2$) = 0.64.

### *6.1.2. Synthesis of 2-substituted 4-hydroxybutanamides **13**–**33**, **37**–**38***

*6.1.2.1. General procedure 1 (GP1).* 2-Substituted dihydrofuran-2(3*H*)-one derivative (1 equiv.) was heated with relevant amine (1.15–2.5 equiv.) at 100–120 °C. The oily residue was then dissolved in 5 mL of EtOAc, 2–5 mL of *n*-hexane was added, and the mixture was refrigerated for about 24 h. The obtained solid was filtered off and purified by recrystallization.

*6.1.2.2. General procedure 2 (GP2).* The process vial was charged with 2-substituted dihydrofuran-2(3*H*)-one derivative (1.0 equiv.), amine (1.4–2 equiv.), and relevant solvent. The vial was then closed and its content was magnetically stirred and microwave heated at 120–140 °C. After the reaction was finished, the mixture was refrigerated, then, the obtained solid was filtered and washed with EtOH and $Et_2O$.

*6.1.2.3. General procedure 3 (GP3).* The mixture of relevant 2-substituted dihydrofuran-2(3*H*)-one derivative and 25% $NH_3$ aq. was stirred in closed vessel at room temperature for 48–72 h. The obtained crude products were purified according to procedures described in the detailed part.

*6.1.2.4. General procedure 4 (GP4).* 2-Substituted dihydrofuran-2(3*H*)-one derivative (1 equiv.) was refluxed in toluene (10 mL) with relevant amine (1.4–2 equiv.) for 5–20 h. Then the solvent was evaporated under vacuum and crude product was refrigerated. The obtained solid was recrystallized from EtOAc.

*6.1.2.5. (R, S) 2-(4-Benzhydrylpiperazin-1-yl)-N-benzyl-4-hydroxybutanamide (**13**).* Procedure GP2; reagents and conditions: compound **8** (0.84 g, 2.5 mmol), benzylamine (0.54 g, 5 mmol), toluene (3 mL), 140 °C; reaction time 25 min; yield 60%; mp 144–145 °C; anal. calcd for $C_{28}H_{33}N_3O_2$: C% 75.82, H% 7.50, N% 9.47 found: C% 75.80, H% 7.70, N% 9.66; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.76–2.03 (m, 2H, CHC$H_2$), 2.21–2.70 (m, 8H, piperazine), 3.19 (dd, $J$ = 3.3, 9.7 Hz, 1H, C*H*), 3.64–3.86 (m, 2H, C$H_2$OH), 4.19 (s, 1H, C*H*Ph$_2$), 4.43 (s, 1H, O*H*), 4.52 (t, $J$ = 6.2 Hz, 2H, C$H_2$Ph), 7.12–7.41 (m, 15H, Ar-H), 7.71 (t, $J$ = 5.5 Hz, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CH$CH_2$), 43.6 (NHC$H_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HOC$H_2CH_2$), 69.6 (*C*H$CH_2$), 84.5 (*C*HPh$_2$), 126.2, 126.7, 126.9, 128.2, 128.5, 129.2, 137.9, 142.7 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.57, $R_f$ ($S_2$) = 0.73.

*6.1.2.6. (R, S) 2-(4-Benzhydrylpiperazin-1-yl)-N-(2-chlorobenzyl)-4-hydroxybutanamide (**14**).* Procedure GP1; reagents and conditions: compound **8** (1.68 g, 5 mmol), 2-chlorobenzylamine (0.99 g, 7 mmol), 120 °C; reaction time 3 h; yield 88%; mp 195–197 °C (toluene); anal. calcd for $C_{28}H_{32}N_3O_2Cl$: C% 70.35, H% 6.75, N% 8.79 found: C% 70.25, H% 6.90, N% 8.82; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.80 (m, 2H, CHC$H_2$), 2.45–2.54 (m, 8H, piperazine), 3.17 (dd, $J$ = 3.2, 9.7 Hz, 1H, C*H*), 3.62–3.83 (m, 2H, C$H_2$OH), 4.21 (s, 1H, C*H*Ph$_2$), 4.43–4.59 (m, 3H, C$H_2$Ph, O*H*), 7.06–7.49 (m, 14H, Ar-H), 7.94 (t, $J$ = 6.1 Hz, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CH$CH_2$), 43.6 (NHC$H_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HOC$H_2CH_2$), 69.6 (*C*H$CH_2$), 84.5 (*C*HPh$_2$), 126.2, 128.2, 129.2, 142.7 (arom), 171.3 (carbonyl); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.8 (CH$CH_2$), 38.5 (NHC$H_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HOC$H_2CH_2$), 69.7 (*C*H$CH_2$), 84.5 (*C*HPh$_2$), 126.2, 126.6, 128.1, 128.2, 128.6, 129.2, 132.2, 142.4, 142.7 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.43, $R_f$ ($S_2$) = 0.76.

*6.1.2.7. (R, S) 2-(4-Benzhydrylpiperazin-1-yl)-N-(4-chlorobenzyl)-4-hydroxybutanamide (**15**).* Procedure GP4; reagents: compound **8** (1.68 g, 5 mmol), 4-chlorobenzylamine (0.99 g, 7 mmol); reaction time 12 h; yield 20%; mp 146–147 °C (EtOAc); anal. calcd for $C_{28}H_{32}N_3O_2Cl$:. C% 70.35, H% 6.75, N% 8.79 found: C% 70.25, H% 6.75, N% 8.34; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.84–2.00 (m, 2H, CHC$H_2$), 2.39–2.56 (m, 8H, piperazine), 3.17–3.20 (m, 1H, C*H*), 3.64–3.85 (m, 2H, C$H_2$OH), 4.21 (s, 1H, C*H*Ph$_2$), 4.35–4.53 (m, 3H, C$H_2$Ph, O*H*), 7.00–7.39 (m, 14H, Ar-H), 7.72 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.9 (CH$CH_2$), 43.4 (NHC$H_2$Ph), 52.6, 54.3 (piperazine), 58.0 (HOC$H_2CH_2$), 69.76 (*C*H$CH_2$), 84.2 (*C*HPh$_2$), 126.2, 128.2, 128.6, 129.2, 132.3, 134.6, 136.0, 142.7 (arom), 171.8 (carbonyl); TLC: $R_f$ ($S_1$) = 0.39, $R_f$ ($S_2$) = 0.75.

*6.1.2.8. (R, S) 2-(4-Benzhydrylpiperazin-1-yl)-N-(2-fluorobenzyl)-4-hydroxybutanamide (**16**).* Procedure GP1; reagents and conditions: compound **8** (1.68 g, 5 mmol), 2-fluorobenzylamine (1.25 g, 10 mmol), 120 °C; reaction time 4 h; yield 36%; mp 148–149 °C (EtOAc); anal. calcd for $C_{28}H_{32}N_3O_2F$: C% 72.86, H% 6.99, N% 9.10 found: C% 72.59, H% 6.74, N% 9.14; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.81–1.99 (m, 2H, CHC$H_2$), 2.40–2.51 (m, 8H, piperazine), 3.16–3.18 (m, 1H, C*H*), 3.62–3.83 (m, 2H, C$H_2$OH), 4.21 (s, 1H, C*H*Ph$_2$), 4.41–4.63 (m, 2H, C*H*Ph, O*H*), 4.49 (m, 1H, C*H*Ph), 7.00–7.40 (m, 14H, Ar-H), 7.81 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.5 (CH$CH_2$), 36.6 (NHC$H_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HOC$H_2CH_2$), 69.9 (*C*H$CH_2$), 84.5 (*C*HPh$_2$), 115.3, 126.2, 128.2, 128.3, 129.2, 129.6, 131.5, 142.7, 159.4 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.54, $R_f$ ($S_2$) = 0.76.

*6.1.2.9. (R, S) 2-(4-Benzhydrylpiperazin-1-yl)-N-(4-fluorobenzyl)-4-hydroxybutanamide (**17**).* Procedure GP1; reagents and conditions: compound **8** (1.68 g, 5 mmol), 4-fluorobenzylamine (1.25 g, 10 mmol), 120 °C; reaction time 4 h; yield 42%; mp 145–147 °C (*n*-hexane/EtOAc, 1/4); anal. calcd for $C_{28}H_{32}N_3O_2F$: C% 72.86, H% 6.99, N% 9.10 found: C% 72.75, H% 6.84, N% 8.93; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.85–2.01 (m, 2H, CHC$H_2$), 2.39–2.57 (m, 8H, piperazine), 3.18–3.20 (m, 1H, C*H*), 3.64–3.86 (m, 2H, C$H_2$OH), 4.19 (s, 1H, C*H*Ph$_2$), 4.40–4.57 (m, 3H, C$H_2$Ph, O*H*), 7.15–7.40 (m, 14H, Ar-H), 7.71 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CH$CH_2$), 43.6 (NHC$H_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HOC$H_2CH_2$), 69.7 (*C*H$CH_2$), 84.5 (*C*HPh$_2$), 115.3, 126.2, 128.2, 128.5, 133.5, 142.7, 160.9 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.38, $R_f$ ($S_2$) = 0.70.

*6.1.2.10. (R, S) 2-(4-Benzhydrylpiperazin-1-yl)-4-hydroxy-N-(4-methylbenzyl)butanamide (**18**).* Procedure GP4; reagents: compound **8** (1.68 g, 5 mmol), 4-methylbenzylamine (0.85 g, 7 mmol); reaction time 5 h; yield 22%; mp 158–160 °C (EtOAc); anal. calcd for $C_{29}H_{35}N_3O_2$: C% 76.12, H% 7.71, N% 9.18 found: C% 76.59, H% 7.74, N% 9.08; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.84–2.00 (m, 2H, CHC$H_2$), 2.33 (s, 3H, C$H_3$), 2.40–2.54 (m, 8H, piperazine), 3.17–3.19 (m, 1H, C*H*), 3.64–3.86 (m, 2H, C$H_2$OH), 4.19 (s, 1H, C*H*Ph$_2$), 4.35–4.53 (m, 3H,

C*H*$_2$Ph, O*H*), 7.13–7.40 (m, 14H, Ar-H), 7.65 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 21.3 (*C*H$_3$), 34.6 (CH*C*H$_2$), 43.6 (NH*C*H$_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HO*C*H$_2$CH$_2$), 69.5 (*C*HCH$_2$), 84.05 (*C*HPh$_2$), 126.2, 128.1, 128.2, 128.8, 129.2, 134.9, 136.4, 142.9 (arom), 171.9 (carbonyl); TLC: $R_f$ ($S_1$) = 0.39, $R_f$ ($S_2$) = 0.78.

*6.1.2.11. (R, S) 2-(4-Benzhydrylpiperazin-1-yl)-4-hydroxy-N-(4-methoxybenzyl)butanamide (**19**).* Procedure GP1; reagents and conditions: compound **8** (1.68 g, 5 mmol), 4-methoxybenzylamine (0.96 g, 7 mmol), 100 °C; reaction time 3 h; yield 37%; mp 138–139 °C (EtOAc); anal. calcd for $C_{29}H_{35}N_3O_3$: C% 73.54, H% 7.45, N% 8.87 found: C% 73.44, H% 7.73, N% 8.87; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.82–2.01 (m, 2H, CHC*H*$_2$), 2.14–2.67 (m, 8H, piperazine), 3.17 (dd, $J$ = 3.3, 9.7 Hz, 1H, C*H*), 3.58–3.69 (m, 1H, C*H*$_2$OH), 3.80 (s, 3H, C*H*$_3$O), 3.81–3.91 (m, 1H, CH$_2$O*H*), 4.19 (s, 1H, C*H*Ph$_2$), 4.37 (t, $J$ = 6.0 Hz, 2H, C*H*$_2$Ph), 4.57 (s, 1H, O*H*), 6.75–6.92 (m, 2H, Ar-H), 7.12–7.41 (m, 12H, Ar-H), 7.63 (t, $J$ = 6.4 Hz, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CH*C*H$_2$), 43.6 (NH*C*H$_2$Ph), 52.5, 54.0 (piperazine), 55.8 (O*C*H$_3$), 58.3 (HO*C*H$_2$CH$_2$), 69.7 (*C*HCH$_2$), 84.5 (*C*HPh$_2$), 114.3, 126.2, 128.2, 130.2, 130.5, 142.7, 159.9 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.49, $R_f$ ($S_2$) = 0.70.

*6.1.2.12. (R, S) 2-(4-Benzhydrylpiperazin-1-yl)-4-hydroxy-N-phenethylbutanamide (**20**).* Procedure GP2; reagents: compound **8** (1.68 g, 5 mmol), 2-phenethylamine (0.85 g, 7 mmol), toluene (3 mL), 120 °C; reaction time 40 min; yield 33%; mp 133–134 °C; anal. calcd for $C_{29}H_{35}N_3O_2$: C% 76.12, H% 7.71, N% 9.18 found: C% 76.32, H% 8.01, N% 9.19; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.66–1.99 (m, 2H, CHC*H*$_2$), 2.00–2.55 (m, 8H, piperazine), 2.85 (t, $J$ = 6.8 Hz, 2H, C*H*$_2$CH$_2$Ph), 3.06 (m, 1H, C*H*), 3.41–3.81 (m, 4H, C*H*$_2$OH, CH$_2$Ph), 4.15 (s, 1H, C*H*Ph$_2$), 4.64 (s, 1H, OH), 7.07–7.55 (m, 16H, Ar-H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CH*C*H$_2$), 35.1 (*C*H$_2$Ph), 40.6 (NH*C*H$_2$), 52.5, 54.0 (piperazine), 58.3 (HO*C*H$_2$CH$_2$), 69.7 (*C*HCH$_2$), 84.5 (*C*HPh$_2$), 125.9, 126.2, 127.7, 128.2, 128.6, 129.2, 139.5, 142.7, (arom), 171.9 (carbonyl); TLC: $R_f$ ($S_1$) = 0.53, $R_f$ ($S_2$) = 0.73.

*6.1.2.13. (R, S) 2-(4-Benzhydrylpiperazin-1-yl)-4-hydroxybutanamide (**21**).* Procedure GP3; reagents: compound **8** (1.34 g, 4 mmol), 25% $NH_3$ aq. (5 g); reaction time 72 h; purification: washed with water, EtOH, Et$_2$O; yield 37%; mp 167–168 °C; anal. calcd for $C_{21}H_{27}N_3O_2$: C% 71.36, H% 7.70, N% 11.89 found: C% 71.69, H% 7.41, N% 11.67; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.99–2.34 (m, 2H, CHC*H*$_2$), 2.43–2.73 (m, 8H, piperazine), 3.20–3.36 (m, 1H, C*H*), 3.64–3.82 (m, 2H, C*H*$_2$OH), 4.22 (s, 1H, C*H*Ph$_2$), 4.67 (s, 1H, O*H*), 6.99–7.48 (m, 12H, Ar-H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.3 (CH*C*H$_2$), 52.5, 54.0 (piperazine), 58.3 (HO*C*H$_2$CH$_2$), 71.7 (*C*HCH$_2$), 84.5 (*C*HPh$_2$), 126.2, 128.2, 129.5, 142.7 (arom), 176.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.68.

*6.1.2.14. (R, S) N-Benzyl-2-[4-(bis(4-fluorophenyl)methyl)piperazin-1-yl]-4-hydroxybutanamide (**22**).* Procedure GP1; reagents: compound **9** (1.86 g, 5 mmol), benzylamine (1.07 g, 10 mmol), 120 °C; reaction time 3 h; yield 20%; mp 108–109 °C (EtOAc); anal. calcd for $C_{28}H_{31}N_3O_2F_2$: C% 70.13, H% 6.52, N% 8.76 found: C% 70.27, H% 6.31, N % 8.82; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.76–1.98 (m, 2H, CHC*H*$_2$), 2.14–2.65 (m, 8H, piperazine), 3.17–3.19 (m, 1H, C*H*), 3.51–3.79 (m, 2H, C*H*$_2$OH), 4.15 (s, 1H, C*H*Ph$_2$), 4.33–4.47 (m, 3H, C*H*$_2$Ph, O*H*), 7.27–7.55 (m, 13H, Ar-H), 7.84 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CH*C*H$_2$), 43.6 (NH*C*H$_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HO*C*H$_2$CH$_2$), 43.6 (*C*HCH$_2$), 84.5 (*C*HPh$_2$), 116.3, 126.7, 128.5, 129.8, 137.5, 138.7, 160.4 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.54.

*6.1.2.15. (R, S) 2-[4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl]-N-(2-chlorobenzyl)-4-hydroxybutanamide (**23**).* Procedure GP1; reagents: compound **9** (1.86 g, 5 mmol), 2-chlorobenzylamine (1.42 g, 10 mmol), 120 °C; reaction time 3 h; yield 22%; mp 138–139 °C (EtOAc); anal. calcd for $C_{28}H_{30}N_3O_2F_2Cl$: C% 65.43, H% 5.88, N% 8.18 found: C% 65.40, H% 5.91, N% 8.12; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.82–2.03 (m, 2H, CHC*H*$_2$), 2.32–2.62 (m, 8H, piperazine), 3.18–3.22 (m, 1H, C*H*), 3.67–3.87 (m, 2H, C*H*$_2$OH), 4.17 (s, 1H, C*H*Ph$_2$), 4.56–4.61 (m, 3H, C*H*$_2$Ph, O*H*), 6.96–7.35 (m, 12H, Ar-H), 7.83 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CH*C*H$_2$), 38.6 (NH*C*H$_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HO*C*H$_2$CH$_2$), 69.7 (*C*HCH$_2$), 84.5 (*C*HPh$_2$), 116.3, 126.6, 128.1, 128.6, 129.8, 132.2, 138.3, 142.4 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.51.

*6.1.2.16. (R, S) 2-[4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl]-N-(4-chlorobenzyl)-4-hydroxybutanamide (**24**).* Procedure GP1; reagents: compound **9** (1.86 g, 5 mmol), 4-chlorobenzylamine (1.42 g, 10 mmol), 120 °C; reaction time 3 h; yield 24%; mp 137–138 °C (EtOAc); anal. calcd for $C_{28}H_{30}N_3O_2F_2Cl$: C% 65.43, H% 5.88, N% 8.18 found: C% 65.39, H% 5.62, N% 8.24; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.82–2.03 (m, 2H, CHC*H*$_2$), 2.36–2.71 (m, 8H, piperazine), 3.18–3.22 (m, 1H, C*H*), 3.67–3.87 (m, 2H, C*H*$_2$OH), 4.20 (s, 1H, C*H*Ph$_2$), 4.40–4.47 (m, 2H, C*H*$_2$Ph), 4.56 (s, 1H, O*H*), 6.96–7.35 (m, 12H, Ar-H), 7.54 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CH*C*H$_2$), 43.6 (NH*C*H$_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HO*C*H$_2$CH$_2$), 69.7 (*C*HCH$_2$), 84.5 (*C*HPh$_2$), 116.0, 128.6, 128.8, 132.3, 134.6, 136.0, 138.3, 160.4 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.48.

*6.1.2.17. (R, S) 2-[4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl]-N-(2-fluorobenzyl)-4-hydroxybutanamide (**25**).* Procedure GP1; reagents and conditions: compound **9** (1.86 g, 5 mmol), 2-fluorobenzylamine (1.25 g, 10 mmol), 120 °C; reaction time 3 h; yield 35%; mp 154–155 °C (EtOAc); anal. calcd for $C_{28}H_{30}N_3O_2F_3$: C% 67.59, H% 6.08, N% 8.45 found: C% 67.69, H% 6.04, N% 8.44; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.83–2.05 (m, 2H, CHC*H*$_2$), 2.37–2.46 (m, 8H, piperazine), 3.16–3.21 (m, 1H, C*H*), 3.65–3.88 (m, 2H, C*H*$_2$OH), 4.21 (s, 1H, C*H*Ph$_2$), 4.42–4.53 (m, 2H, C*H*$_2$Ph), 4.64 (s, 1H, O*H*), 7.00–7.40 (m, 12H, Ar-H), 7.81 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CH*C*H$_2$), 36.8 (NH*C*H$_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HO*C*H$_2$CH$_2$), 69.7 (*C*HCH$_2$), 84.5 (*C*HPh$_2$), 115.3, 116.0, 124.1, 128.3, 129.5, 131.5, 159.7, 160.4 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.57, $R_f$ ($S_2$) = 0.78.

*6.1.2.18. (R, S) 2-[4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl]-N-(4-fluorobenzyl)-4-hydroxybutanamide (**26**).* Procedure GP1; reagents: compound **9** (1.86 g, 5 mmol), 4-fluorobenzylamine (1.25 g, 10 mmol), 120 °C; reaction time 3 h; yield 19%; mp 87–88 °C (EtOAc); anal. calcd for $C_{28}H_{30}N_3O_2F_3$: C% 67.59, H% 6.08, N% 8.45 found: C% 67.42, H% 6.17, N% 8.39; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.85–2.07 (m, 2H, CHC*H*$_2$), 2.42–2.78 (m, 8H, piperazine), 3.18–3.22 (m, 1H, C*H*), 3.67–3.87 (m, 2H, C*H*$_2$OH), 4.18 (s, 1H, C*H*Ph$_2$), 4.40–4.47 (m, 2H, C*H*$_2$Ph), 4.56 (s, 1H, O*H*), 6.96–7.35 (m, 12H, Ar-H), 7.58 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CH*C*H$_2$), 43.6 (NH*C*H$_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HO*C*H$_2$CH$_2$), 69.7 (*C*HCH$_2$), 84.5 (*C*HPh$_2$), 115.3, 116.0, 128.5, 129.8, 133.5, 138.5, 160.4, 160.9 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.52.

*6.1.2.19. (R, S) 2-[4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl]-4-hydroxy-N-(4-methylbenzyl)butanamide (**27**).* Procedure GP1; reagents: compound **9** (1.86 g, 5 mmol), 4-methylbenzylamine (1.21 g, 10 mmol), 120 °C; reaction time 3 h; yield 21%; mp 129–130 °C (EtOAc); anal. calcd for $C_{29}H_{33}N_3O_2F_2$: C% 70.57, H% 6.74, N% 8.51 found: C% 70.27, H% 6.62, N% 8.45; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.80–2.14 (m, 2H, CHC*H*$_2$), 2.30 (s, 3H, C*H*$_3$), 2.36–2.67 (m, 8H, piperazine), 3.21–3.26 (m, 1H, C*H*), 3.63–3.82 (m, 2H, C*H*$_2$OH), 4.20 (s, 1H, C*H*Ph$_2$), 4.40–4.47 (m, 2H, C*H*$_2$Ph), 4.59 (s, 1H, O*H*), 6.96–7.35 (m, 12H, Ar-H), 7.84 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 21.3 (*C*H$_3$), 34.6 (CH*C*H$_2$), 43.6 (NH*C*H$_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HO*C*H$_2$CH$_2$), 69.7 (*C*HCH$_2$), 84.5 (*C*HPh$_2$), 116.0,

125.9, 127.7, 128.6, 129.8, 138.3, 139.4, 160.4 (arom), 173.9 (carbonyl); TLC: $R_f$ ($S_1$) = 0.55.

*6.1.2.20. (R, S) 2-[4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl]-4-hydroxy-N-(4-methoxylbenzyl)butanamide (**28**).* Procedure GP1; reagents and conditions: compound **9** (1.86 g, 5 mmol), 4-methoxybenzylamine (1.37 g, 10 mmol), 100 °C; reaction time 3 h; yield 43%; mp 135–136 °C (EtOAc); anal. calcd for $C_{29}H_{33}N_3O_3F_2$: C% 68.35, H% 6.53, N% 8.25 found: C% 68.44, H% 6.73, N% 8.37; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.85–2.07 (m, 2H, CHC$H_2$), 2.23–2.67 (m, 8H, piperazine), 3.19 (dd, $J$ = 3.3, 9.7 Hz, 1H, C$H$), 3.60–3.74 (m, 1H, C$H_2$OH), 3.80 (s, 3H, C$H_3$O), 3.84–3.98 (m, 1H, $CH_2$OH), 4.19 (s, 1H, C$H$Ph$_2$), 4.37 (t, $J$ = 6.0 Hz, 2H, C$H_2$Ph), 4.59 (s, 1H, O$H$), 6.75–6.92 (m, 2H, Ar-H), 7.12–7.41 (m, 10H, Ar-H), 7.63 (t, $J$ = 6.4 Hz, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CHC$H_2$), 43.6 (NHC$H_2$Ph), 52.5, 54.0 (piperazine), 58.3 (HOC$H_2$C$H_2$), 55.8 (C$H_3$), 69.7 (C$H$C$H_2$), 84.5 (C$H$Ph$_2$), 114.1, 116.3, 129.8, 130.2, 130.5, 158.6, 160.4 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.54, $R_f$ ($S_2$) = 0.73.

*6.1.2.21. (R, S) 2-[4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl]- 4-hydroxy-N-phenethylbutanamide (**29**).* Procedure GP1; reagents: compound **9** (1.86 g, 5 mmol), 2-phenethylamine (1.21 g, 10 mmol), 120 °C; reaction time 4 h; yield 33%; mp 142–143 °C; anal. calcd for $C_{29}H_{33}N_3O_2F_2$: C% 70.57, H% 6.74, N% 8.51 found: C% 70.32, H% 6.83, N% 8.59; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.66–1.99 (m, 2H, CHC$H_2$), 2.00–2.55 (m, 8H, piperazine), 2.85 (t, $J$ = 6.8 Hz, 2H, C$H_2$C$H_2$Ph), 3.06 (m, 1H, C$H$), 3.41–3.81 (m, 4H, C$H_2$OH, $CH_2$Ph), 4.15 (s, 1H, C$H$Ph$_2$), 4.64 (s, 1H, OH), 7.07–7.55 (m, 14H, Ar-H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CHC$H_2$), 35.1 (C$H_2$Ph), 40.6 (NHC$H_2$), 52.5, 54.0 (piperazine), 58.3 (HOC$H_2$C$H_2$), 69.7 (C$H$C$H_2$), 84.5 (C$H$Ph$_2$), 115.3, 126.2, 128.2, 128.5, 133.5, 142.7, 160.9 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.55, $R_f$ ($S_2$) = 0.79.

*6.1.2.22. (R, S) 2-[4-(Bis(4-fluorophenyl)methyl)piperazin-1-yl]-4-hydroxybutanamide (**30**).* Procedure GP3; reagents: compound **9** (1.86 g, 5 mmol), 25% $NH_3$ aq. (5 g); reaction time 72 h; purification: extraction of reaction mixture with EtOAc; yield 12%; mp 148–149 °C (EtOAc); anal. calcd for $C_{21}H_{25}N_3O_2F_2$: C% 64.77, H% 6.47, N% 10.79 found: C% 64.69, H% 6.41, N% 10.67; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.92–2.17 (m, 2H, CHC$H_2$), 2.38–2.73 (m, 8H, piperazine), 3.18–3.22 (m, 1H, C$H$), 3.64–3.82 (m, 2H, C$H_2$OH), 4.22 (s, 1H, C$H$Ph$_2$), 4.63 (s, 1H, O$H$), 6.99–7.48 (m, 10H, Ar-H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CHC$H_2$), 52.5, 54.0 (piperazine), 58.3 (HOC$H_2$C$H_2$), 71.9 (C$H$C$H_2$), 84.5 (C$H$Ph$_2$), 116.0, 129.8, 138.3, 160.4 (arom), 176.7 (carbonyl); TLC: $R_f$ ($S_1$) = 0.71.

*6.1.2.23. (R, S) Ethyl 4-(1-benzylamino-4-hydroxy-1-oxobutan-2-yl) piperazine-1-carboxylate (**31**).* Procedure GP4; reagents: compound **10** (0.97 g, 4 mmol), benzylamine (1.13 g, 8 mmol); reaction time 6 h; yield 53%; mp 132–133 °C (EtOAc); anal. calcd for $C_{18}H_{27}N_3O_4$: C% 61.87, H% 7.79, N% 12.03 found: C% 61.68, H% 7.71 N% 12.16; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.28 (d, $J$ = 6.3 Hz, 3H, $CH_3$), 1.76–1.96 (m, 2H, CHC$H_2$), 2.60–2.78 (m, 4H, piperazine), 3.05–3.24 (m, 5H, piperazine, C$H$), 3.67–3.87 (m, 2H, C$H_2$OH), 4.20 (q, $J$ = 6.3 Hz, 2H, C$H_2$CH$_3$), 4.61 (s, 1H, O$H$), 4.69–4.86 (m, 2H, C$H_2$Ph); 6.96–7.35 (m, 5H, Ar-H), 7.83 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 13.8 (C$H_3$CH$_2$), 34.6 (CHC$H_2$), 43.6 (NHC$H_2$Ph), 45.5, 51.8 (piperazine), 58.3 (HOC$H_2$C$H_2$), 62.0 ($CH_3$C$H_2$), 69.7 (C$H$C$H_2$), 126.7, 126.9, 128.5, 137.9 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.56, $R_f$ ($S_2$) = 0.65.

*6.1.2.24. (R, S) Ethyl 4-[1-(4-chlorobenzylamino)-4-hydroxy-1-oxobutan-2-yl]piperazine-1-carboxylate (**32**).* Procedure GP4; reagents: compound **10** (0.97 g, 4 mmol), 4-chlorobenzylamine (1.13 g, 8 mmol); reaction time 6 h; yield 57%; mp 129–130 °C (EtOAc); anal. calcd for $C_{18}H_{26}N_3O_4Cl$: C% 56.32, H% 6.83, N% 10.95 found: C% 56.20, H% 6.71 N% 10.86; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.24 (d, $J$ = 6.2 Hz, 3H, $CH_3$), 1.70–1.96 (m, 2H, CHC$H_2$), 2.60–2.78 (m, 4H, piperazine), 3.05–3.24 (m, 5H, piperazine, C$H$), 3.67–3.87 (m, 2H, C$H_2$OH), 4.13 (q, $J$ = 6.2 Hz, 2H, C$H_2$CH$_3$), 4.53 (s, 1H, O$H$), 4.67–4.85 (m, 2H, C$H_2$Ph); 6.96–7.35 (m, 4H, Ar-H), 7.83 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 13.8 (C$H_3$CH$_2$), 34.6 (CHC$H_2$), 43.6 (NHC$H_2$Ph), 45.5, 51.8 (piperazine), 58.3 (HOC$H_2$C$H_2$), 62.0 ($CH_3$C$H_2$), 69.7 (C$H$C$H_2$), 128.6, 132.3, 134.6, 136.0 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.50, $R_f$ ($S_2$) = 0.64.

*6.1.2.25. (R, S) Ethyl 4-(1-amino-4-hydroxy-1-oxobutan-2-yl)piperazine-1-carboxylate (**33**).* Procedure GP3; reagents: compound **10** (0.97 g, 4 mmol), 25% $NH_3$ aq. (4 g); reaction time 72 h; purification: extraction of reaction mixture with EtOAc; yield 42%; mp 120–122 °C (EtOAc); anal. calcd for $C_{11}H_{21}N_3O_4$: C% 50.95, H% 8.16, N% 16.21 found: C% 50.86, H% 8.12 N% 16.26; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.24 (d, $J$ = 6.2 Hz, 3H, $CH_3$), 1.80–2.11 (m, 2H, CHC$H_2$), 2.60–2.78 (m, 4H, piperazine), 3.05–3.24 (m, 5H, piperazine, C$H$), 3.76–3.91 (m, 2H, C$H_2$OH), 4.17 (q, 2H, C$H_2$CH$_3$),5.24–5.48 (s wide, 1H, O$H$), 7.58 (s, 2H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 13.8 (C$H_3$CH$_2$), 34.6 (CHC$H_2$), 45.8, 51.6 (piperazine), 58.3 (HOC$H_2$C$H_2$), 62.0 ($CH_3$C$H_2$), 71.9 (C$H$C$H_2$), 156.3 (carbonyl); TLC: $R_f$ ($S_2$) = 0.74.

*6.1.2.26. (R, S) N-Benzyl-4-hydroxy-2-morpholinobutanamide (**37**).* Procedure GP1; reagents: compound **11** (1.28 g, 7.5 mmol), benzylamine (1.07 g, 10 mmol), 115 °C; reaction time 2 h; yield 50%; mp 93–94 °C (EtOAc); anal. calcd for $C_{15}H_{22}N_2O_3$: C% 64.73, H% 7.97, N% 10.06 found: C% 64.60, H% 8.36, N% 10.13; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.83–2.05 (m, 2H, CHC$H_2$), 2.45–2.63 (m, 4H, morpholine), 3.22 (dd, $J$ = 3.5, 9.7 Hz, 1H, C$H$), 3.58–3.87 (m, 6H, C$H_2$OH, morpholine), 4.18 (s, 1H, O$H$), 4.40–4.55 (m, 2H, C$H_2$Ph), 7.19–7.43 (m, 5H, Ar-H), 7.62 (s, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CHC$H_2$), 43.6 (NHC$H_2$Ph), 52.5, 66.8 (morpholine), 58.3 (HOC$H_2$C$H_2$), 70.0 (C$H$C$H_2$), 126.7, 126.9, 128.5, 137.9 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.23, $R_f$ ($S_2$) = 0.60.

*6.1.2.27. (R, S) N-Benzyl-2-(4-benzylpiperidin-1-yl)-4-hydroxybutanamide (**38**).* Procedure GP1; reagents: compound **12** (1.56 g, 6 mmol), benzylamine (1.61 g, 15 mmol), 100 °C; reaction time 3 h; yield 86%; mp 96–97 °C (iPrOH); anal. calcd for $C_{23}H_{30}N_2O_2$: C% 75.38, H% 8.25, N% 7.64 found: C% 75.08, H% 8.34 N% 7.58; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.01–1.36 (m, 2H, piperidine), 1.42–1.57 (m, 1H, C$H$, piperidine), 1.58–1.72 (m, 2H, C$H_2$, piperidine), 1.73–2.00 (m, 2H, CHC$H$2), 2.13–2.38 (m, 2H, piperidine), 2.50 (d, $J$ = 7.1 Hz, 2H, C$H_2$Ph), 2.63–2.73 (m, 2H, C$H_2$, piperidine), 3.19 (dd, $J$ = 3.2, 9.5 Hz, 1H, C$H$), 3.56–3.68 (m, 1H, C$H_2$OH), 3.79–3.91 (m, 1H, C$H_2$OH), 4.39–4.56 (m, 2H, C$H_2$Ph), 4.75 (s, 1H, O$H$), 7.05–7.41 (m, 10H, Ar-H), 7.72–7.86 (s, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CHC$H_2$), 41.6 (CHC$H_2$Ph), 32.2, 37.2, 43.9 (piperidine), 58.3 (HOC$H_2$C$H_2$), 70.7 (C$H$C$H_2$), 126.7, 126.9, 128.1, 128.5, 125.9, 137.9, 139.7 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.36, $R_f$ ($S_2$) = 0.35.

### *6.1.3. Synthesis of 4-Hydroxy-2-(piperazin-1-yl)butanamides **34**–**36** (general procedure)*

To solution of **31**, **32** or **33** (2 mmol) in 5 mL of anhydrous DCM, 5 mL TFA was added and stirred for 1 h at room temperature. Then excess of reagent and solvent was removed under vacuum. The resulting oil was neutralized by 2 M KOH, extracted with DCM (4 × 10 mL), dried over $Na_2SO_4$ and evaporated. The crude product was purified by column chromatography ($Me_2CO$/$CHCl_3$, 1/1).

*6.1.3.1. (R, S) N-Benzyl-4-hydroxy-2-(piperazin-1-yl)butanamide (**34**).* Reagents: compound **31** (0.97 g); yield 34%; mp 121–122 °C; anal. calcd for $C_{15}H_{22}N_3O_2$: C% 64.95, H% 8.36, N% 15.15 found: C% 64.89, H% 8.24 N% 15.21; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.68–1.84 (m,

3H, CHC$H_2$, N$H$ piperazine), 2.64–2.80 (m, 4H, piperazine), 3.09–3.26 (m, 5H, piperazine, C$H$), 3.69–3.93 (m, 2H, C$H_2$OH), 4.24 (s, 1H, O$H$), 4.48–4.59 (m, 2H, C$H_2$Ph), 6.96–7.49 (m, 5H, Ar-H), 7.83 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CHC$H_2$), 43.6 (NHC$H_2$Ph), 45.8, 54.8 (piperazine), 58.3 (HOC$H_2$C$H_2$), 69.7 (C$H$C$H_2$), 126.7, 126.9, 128.5, 137.9 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.49, $R_f$ ($S_2$) = 0.68.

*6.1.3.2. (R, S) N-(4-Chlorobenzyl)-4-hydroxy-2-(piperazin-1-yl)butanamide (**35**).* Reagents: compound **32** (0.77 g); yield 27%; mp 119–120 °C; anal. calcd for $C_{15}H_{22}N_3O_2Cl$: C% 57.78, H% 7.11, N% 13.48 found: C% 57.89, H% 7.04 N% 13.31; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.70–1.96 (m, 3H, CHC$H_2$, N$H$ piperazine), 2.60–2.78 (m, 4H, piperazine), 3.05–3.24 (m, 5H, piperazine, C$H$), 3.67–3.87 (m, 2H, C$H_2$OH), 4.17 (s, 1H, O$H$), 4.42–4.53 (m, 2H, C$H_2$Ph), 6.96–7.35 (m, 4H, Ar-H), 7.83 (t, 1H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.6 (CHC$H_2$), 43.6 (NHC$H_2$Ph), 45.8, 54.8 (piperazine), 58.3 (HOC$H_2$C$H_2$), 69.7 (C$H$C$H_2$), 128.6, 132.3, 134.6, 136.0 (arom), 171.3 (carbonyl); TLC: $R_f$ ($S_1$) = 0.43, $R_f$ ($S_2$) = 0.62.

*6.1.3.3. (R, S) 4-Hydroxy-2-(piperazin-1-yl)butanamide (**36**).* Reagents: compound **33** (0.72 g); yield 26%; mp 94–95 °C; anal. calcd for $C_8H_{17}N_3O_2$: C% 51.32, H% 9.15, N% 22.44 found: C% 51.23, H% 9.10 N% 22.26; $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.80–2.11 (m, 3H, CHC$H_2$, N$H$ piperazine), 2.60–2.78 (m, 4H, piperazine), 3.05–3.24 (m, 5H, piperazine, C$H$), 3.76–3.91 (m, 2H, C$H_2$OH), 5.24–5.48 (s, wide, 1H, O$H$), 8.00 (s, 2H, amide); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 34.3 (CHC$H_2$), 43.6 (NHC$H_2$Ph), 45.8, 54.4 (piperazine), 58.3 (HOC$H_2$C$H_2$), 71.9 (C$H$C$H_2$), 176.7 (carbonyl); TLC: $R_f$ ($S_2$) = 0.74.

### 6.2. Biological evaluations

#### 6.2.1. [$^3$H]GABA uptake

[$^3$H] GABA uptake assays with GAT1-4 were performed as previously described [17].

#### 6.2.2. MS-binding assays

Binding assays for mGAT1 based on NO711 as native marker were performed as described [23]. NO711 was analyzed by LC-MS/MS using an API 3200 triple quadrupole mass spectrometer according to the method described previously [25].

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## References

[1] D.B. Tower, in: E. Roberts, T.N. Chase, D.B. Tower (Eds.), GABA in Nervous System Function, Raven Press, New York, 1976.
[2] A.C. Foster, J.A. Kemp, Curr. Opin. Pharmacol. 6 (2006) 7–17.
[3] F. Conti, A. Minelli, M. Melone, Brain Res. Rev. 45 (2004) 196–212.
[4] L. Iversen, Biochem. Pharmacol. 68 (2004) 1537–1540.
[5] D.W. Volk, D.A. Lewis, Curr. Neuropharmacol. 3 (2005) 45–62.
[6] A. Schouboe, A. Sarup, O.M. Larsson, H.S. White, Biochem. Pharmacol. 68 (2004) 1557–1563.
[7] K.K. Madsen, H.S. White, A. Schouboe, Pharmacol. Ther. 123 (2010) 394–401.
[8] O. Sarup, A. Miller, A. Schousboe, Curr. Drug Targets CNS Neurol. Disord. 2 (2003) 269–277.
[9] K.E. Andersen, C. Braestrup, F.C. Groenwald, A.S. Joergensen, E.B. Nielsen, U. Sonnewald, P.O. Soerensen, P.D. Suzdak, L.J.S. Knutsen, J. Med. Chem. 36 (1993) 1716–1725.
[10] M. Bialer, S.I. Johannessen, H.J. Kupferberg, R.H. Levy, E. Perucca, T. Tomson, Epilepsy Res. 73 (2007) 1–52.
[11] P. Kwan, G.J. Sills, M.J. Brodie, Pharmacol. Ther. 90 (2001) 21–34.
[12] N.O. Dalby, Eur. J. Pharmacol. 479 (2003) 127–137.
[13] K. Kulig, M. Szwaczkiewicz, Mini Rev. Med. Chem. 8 (2008) 1214–1223.
[14] S. Høg, J.R. Greenwood, K.B. Madsen, O.M. Larsson, B. Frølund, A. Schousboe, P. Krogsgaard-Larsen, R.P. Clausen, Curr. Top. Med. Chem. 6 (2006) 1861–1882.
[15] R.P. Clausen, B. Frølund, O.M. Larsson, A. Schousboe, P. Krogsgaard-Larsen, H.S. White, Neurochem. Int. 48 (2006) 637–642.
[16] X. Zhao, C.E. Hösl, G.C. Höfner, K.T. Wanner, Eur. J. Med. Chem. 40 (2005) 231–247.
[17] A. Kragler, G. Höfner, K.T. Wanner, Eur. J. Med. Chem. 43 (2008) 2404–2411.
[18] B. Malawska, K. Kulig, A. Śpiewak, J.P. Stables, Bioorg. Med. Chem. 12 (2004) 625–632.
[19] B. Malawska, K. Kulig, J. Gajda, D. Szczeblewski, A. Musiał, K. Więckowski, J.P. Stables, Acta Polon. Pharm.-Drug Res. 64 (2007) 127–137.
[20] M. Maitre, Prog. Neurobiol. 51 (1997) 337–361.
[21] V. Crunelli, Z. Emri, N. Leresche, Curr. Opin. Pharmacol. 6 (2006) 44–52.
[22] B. Malawska, Front. Med. Chem. 4 (2009) 805–841.
[23] C. Zepperitz, G. Höfner, K.T. Wanner, ChemMedChem. 1 (2006) 208–217.
[24] B. Malawska, S. Gobaille, Pharmazie 50 (1995) 390–393.
[25] G. Höfner, K.T. Wanner, J. Chromatogr. B. 878 (2010) 1356–1364.