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2-Substituted 4-hydroxybutanamides as potential inhibitors of γ-aminobutyric acid transporters mGAT1–mGAT4: Synthesis and biological evaluation

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ABSTRACT

A series of 2-substituted 4-hydroxybutanamide derivatives has been synthesized by the aminolysis of appropriate 2-substituted dihydrofuran-2(3*H*)-one derivatives with various substituted benzylamines. The final compounds have been evaluated for their capability of inhibiting the GABA transport proteins GAT1-4 stably expressed in HEK-239 cell lines. The pIC_{50} values determined were in the range 4.21–5.14. Two compounds (**16a** and **16d**), which displayed the most interesting profiles in in vitro tests, have also been subjected to further preliminary behavioral studies, evaluating their antinociceptive activity in hot-plate, writhing, and formalin tests. Their influence on motor coordination has also been assessed.

1. Introduction

γ-Aminobutyric acid (GABA) plays a significant role in the inhibitory function within the mammalian central nervous system (CNS). As 20–50% of all central synapses use GABA as their neurotransmitter, it controls the activity of a large percentage of neurons.[1] A decrease of GABA-ergic neurotransmission appears to be involved in the development and outbreak of several neurological and psychiatric disorders, such as epilepsy, anxiety, neuropathic pain, Parkinson's disease, and Huntington Chorea.[2,3]

Currently, a number of strategies are available for increasing the GABAergic tone, which include agonism for GABAergic receptors, inhibition of GABA catabolism, or its reuptake. Since the identification of GABA transporter proteins (GATs) and the realization of their importance in the regulation of GABA concentration in the brain, these have become interesting targets as new drugs for the treatment of epilepsy or neuropathic pain syndromes.[4,5] To date, five GABA transporters are known, of which one is the vesicular GABA transporter (VGAT) and the other four (GAT1, 2, 3, and 4) are membrane proteins. GAT1-4 belong to the SLC6 superfamily of Na^+-dependent transporters.[6,7] For other species, including humans and rats, a different nomenclature, also adopted by the Human Genome Organization (HUGO), is used. According to this, these transporters are named as GAT1, GAT2, GAT3, and BGT1, respectively.

The GABA transporters display different physiological activities and distributions in the CNS. mGAT1 and mGAT4 are located in the CNS, while mGAT2 and mGAT3 are poorly expressed in the CNS and can also be found in peripheral tissues.[8,9] In view of the fact that the biological characterization of the test compounds was performed using murine GABA transporters, the 'mouse' nomenclature is used in the present paper.

Since the identification of the GABA transport system as a pharmacological target, a number of acyclic and cyclic structures have been tested as potential GAT inhibitors[10–13] (Fig. 1). However, the therapeutic potential of GAT inhibition has been confirmed with the successful development of the GAT1-selective drug tiagabine, which is used in the treatment of partial seizures and neuropathic pain.[14] The therapeutic potential of other GAT1–GAT4 inhibitors is still unclear. However, anticonvulsant activity has been demonstrated for (*S*)-SNAP-5114, which is slightly selective for mGAT4[4,15] (Fig. 1).

In recent years, much attention has been paid to the function of mGAT1 and mGAT3 in nociception. It was shown that the overexpression of mGAT1 is responsible for hyperalgesic effects in mice, whereas in mice lacking GAT1 hypoalgesia was observed.[16]

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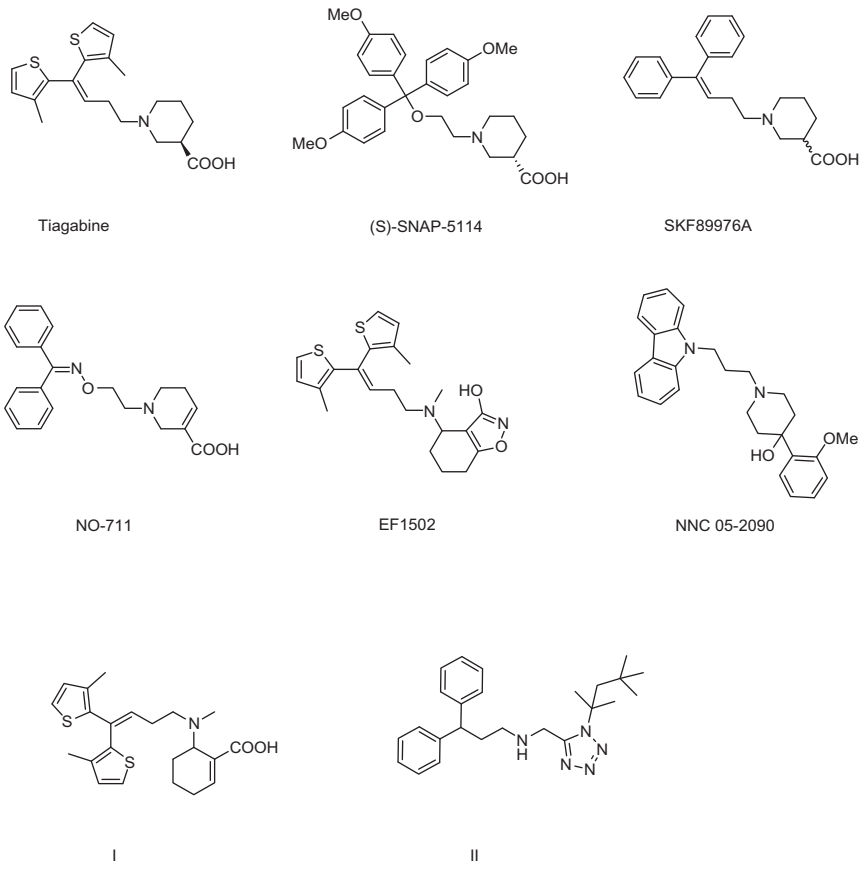


Figure 1. Selected structure of lipophilic GABA uptake inhibitors.[10,11]

Based on this knowledge, numerous mGAT1 inhibitors, including tiagabine, have been tested for their antinociceptive activity in hot-plate, tail flick, formalin, and writhing tests in mice.[16–18] GAT1-selective inhibitors, such as ethyl nipecotate and NO-711, exhibited significant antinociceptive effects in these nociceptive assays in naïve and GAT1-overexpressing transgenic mice. The results indicated that GAT1 is involved in the regulation of pain processes, and pointed to the possibility of developing analgesic drugs that target GAT1.

The nociceptive responses of GAT1-knockout mice (GAT1(−/−)) were compared with those of heterozygous (GAT(+/−)) and wild-type (GAT(+/+)) mice in some of these pain screening models. These data demonstrated that GAT1 deficiency (due to genetic knockout or acute blockade by selective inhibitors) leads to hypoalgesia in mice. These results confirmed the crucial role of GAT1 in the regulation of nociceptive threshold and indicated that two GAT1-selective inhibitors, NO-711 and tiagabine, have potential for clinical use in pain therapy.[17]

In addition, it was demonstrated that a peripherally located mGAT3 transporter might play a regulatory role in peripheral GABAergic mechanisms controlling numerous pathological processes, including pain.[19]

Our earlier studies, which were focused on the search for novel biologically active compounds, demonstrated that several 2-substituted 4-hydroxybutanamides with affinity for GAT display not only anticonvulsant but also antinociceptive properties in some rodent models of seizures,[20] as well as thermally- and chemically-induced acute and tonic pain models.[20–21] Promising results obtained by our research team confirmed that the pharmacological activity of some derivatives from this group extends far beyond the well-known therapeutic targets, which suggests that there are more opportunities for their use.

The present work is part of an extensive search for new GABA uptake inhibitors among derivatives of 4-hydroxybutanamide, and is based on the results of our earlier studies.[21–23]

Previously, we reported the synthesis and biological evaluation of the series of 4-hydroxybutanamide derivatives **III** and **IV** (Fig. 2) as potential GABA uptake inhibitors.[21–23] Preliminary SAR studies on this group of compounds indicated that the benzyl substituent on the amide group and an aromatic substituent at the 2-position of the 4-hydroxybutanamide moiety are crucial for their activity.

In order to verify the above hypothesis, we present herein the synthesis and biological evaluation of new 4-hydroxybutanamide derivatives with further structural modifications. An *N*-bulky and lipophilic biaryl group was introduced at the 2-position of 4-hydroxybutanamide in order to mimic the biaryl moieties of known GAT inhibitors.[24] Other modifications included the introduction of substituents on the benzyl fragment of the molecules. Schematic structures of the designed and synthesized compounds are presented in Fig. 3. All of the designed compounds were synthesized and subsequently tested for their inhibitory potency and selectivity towards cloned murine GABA transporters GAT1–

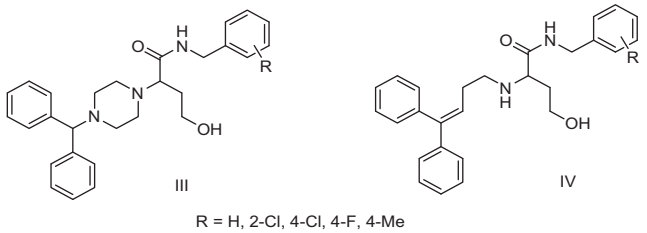


Figure 2. The structure of the 4-hydroxybutanamide derivatives (III and IV).[21–23]

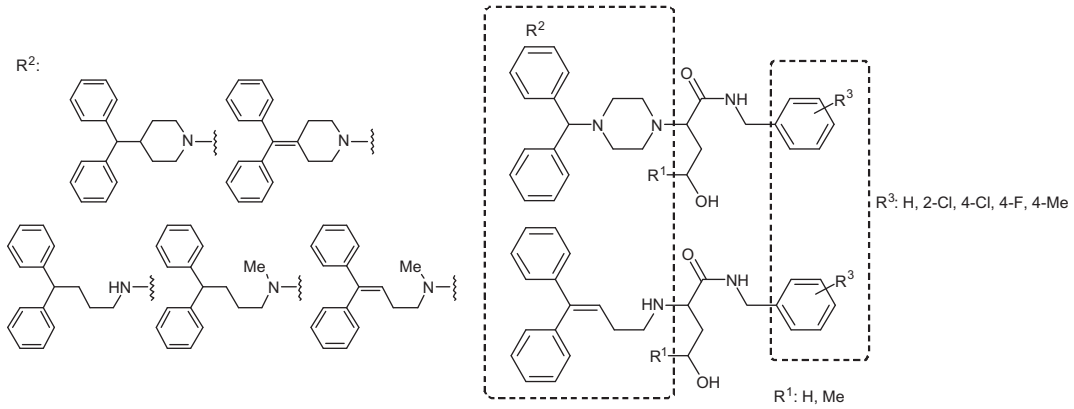
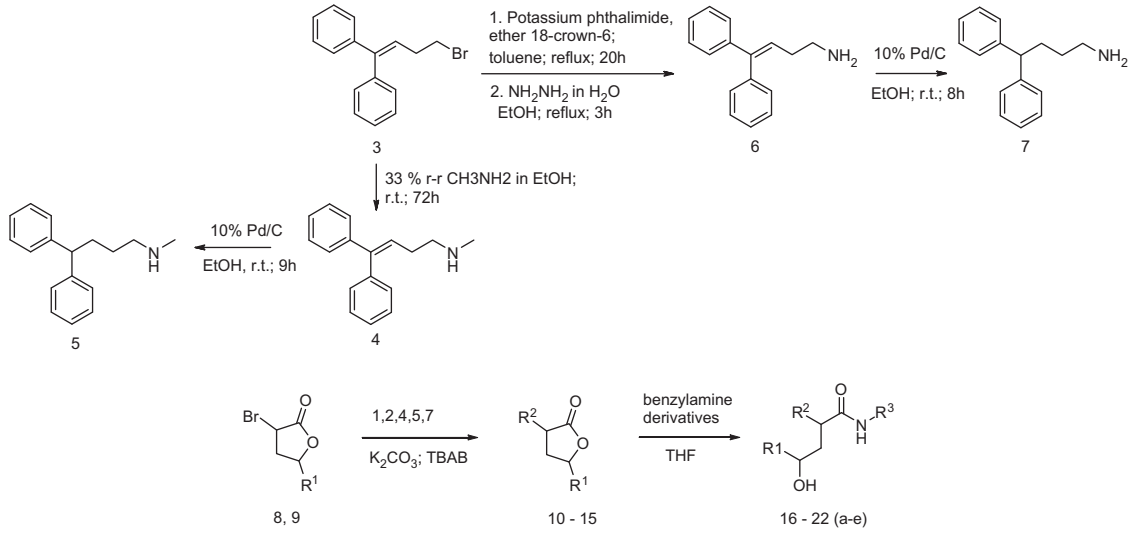


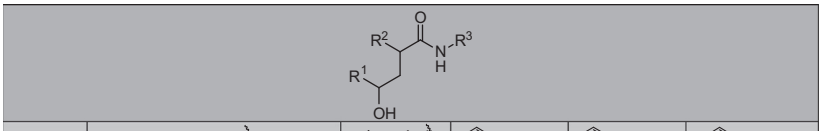
Figure 3. Schematic structure of designed 2-substituted 4-hydroxybutan- or 4-hydroxypentanamides.

GAT4 in uptake assays, and their affinity for GAT1 in an MS binding assay was evaluated. As the next step, the most interesting compounds were further investigated in vivo in some behavioral assays designed to evaluate their antinociceptive properties, namely hot-plate, writhing, and formalin tests. For a preliminary evaluation of the safety profiles of these compounds, the rotarod test was used.

2. Chemistry

All of the derivatives of 4-hydroxybutanamides or 4-hydroxypentanamides presented herein were synthesized by aminolysis of the appropriate substituted butyrolactones (**10**–**15**). The lactones were prepared by *N*-alkylation of the corresponding amines





R²							
R¹	H	CH₃		H	H	H	H
	Lactones						
R³	10	*cis*-11	*trans*-11	12	13	14	15
	Amides						
	16a	18a	19a	17a	20a	21a	22a
	16b	18b	19b	17b	20b	21b	22b
	16c	18c	19c	17c	20c	21c	22c
	16d	18d	19d	17d	20d	21d	22d
	16e	18e	19e	17e	20e	21e	22e

Scheme 1. The synthesis of 2-substituted 4-hydroxybutanamides **16a**–**e** to **22a**–**e**.

Table 1
Results of [^{3}H] GABA uptake and NO711 MS-binding assays

Compound	mGAT1 uptake[a]	mGAT2 uptake[a]	mGAT3 uptake[a]	mGAT4 uptake[a]	mGAT1 NO-711 binding[b]
16a	4.83 ± 0.05	4.33 ± 0.05	4.43 ± 0.05	4.48 ± 0.01	5.04 ± 0.13
16b	100 μM: 58%	4.12 ± 0.05	4.25 ± 0.08	4.17 ± 0.08	100 μM: 68%
16c	4.47 ± 0.07	4.71 ± 0.11	4.49 ± 0.10	4.75 ± 0.05	4.12 ± 0.05
16d	5.01 ± 0.09	4.78 ± 0.12	4.55 ± 0.05	4.58 ± 0.03	4.94 ± 0.13
16e	4.42 ± 0.18	4.51 ± 0.11	4.48 ± 0.06	4.47 ± 0.05	4.36 ± 0.08
17a	4.88 ± 0.11	100 μM: 51%	4.38 ± 0.11	4.23 ± 0.09	100 μM: 55%
17b	4.71 ± 0.07	4.31 ± 0.09	4.44 ± 0.05	4.17 ± 0.05	100 μM: 89%
17c	4.62 ± 0.07	4.22 ± 0.10	4.49 ± 0.13	4.16 ± 0.10	100 μM: 68%
17d	4.74 ± 0.05	4.26 ± 0.06	4.46 ± 0.13	4.09 ± 0.07	100 μM: 49%
17e	4.52 ± 0.11	4.42 ± 0.10	4.37 ± 0.08	4.06 ± 0.10	100 μM: 55%
18a (3*RS*,5*RS*)	4.62 ± 0.10	4.21 ± 0.10	4.51 ± 0.05	4.14 ± 0.11	100 μM: 51%
18b (3*RS*,5*RS*)	100 μM: 47%	100 μM: 73%	4.54 ± 0.13	100 μM: 71%	100 μM: 95%
18c (3*RS*,5*RS*)	4.18 ± 0.08	4.14 ± 0.04	4.37 ± 0.12	4.16 ± 0.10	100 μM: 69%
18d (3*RS*,5*RS*)	4.46 ± 0.09	4.00 ± 0.07	4.26 ± 0.14	100 μM: 65%	100 μM: 52%
18e (3*RS*,5*RS*)	4.27 ± 0.04	4.30 ± 0.02	4.48 ± 0.09	4.46 ± 0.08	100 μM: 57%
19a (3*RS*, 5*SR*)	5.11 ± 0.05	4.31 ± 0.10	4.66 ± 0.07	4.05 ± 0.07	100 μM: 50%
19b (3*RS*, 5*SR*)	100 μM: 95%	100 μM: 69%	100 μM: 79%	100 μM: 99%	100 μM: 99%
19d (3*RS*, 5*SR*)	4.79 ± 0.04	4.19 ± 0.10	4.57 ± 0.11	4.38 ± 0.07	4.37 ± 0.09
19e (3*RS*, 5*SR*)	100 μM: 46%	4.62 ± 0.09	4.24 ± 0.06	100 μM: 616%	100 μM: 58%
20a	4.98 ± 0.10	4.57 ± 0.10	4.87 ± 0.14	4.64 ± 0.04	100 μM: 50%
20b	4.72 ± 0.07	4.90 ± 0.13	5.14 ± 0.10	5.00 ± 0.04	100 μM: 54%
20c	4.63 ± 0.03	4.88 ± 0.11	5.02 ± 0.07	4.95 ± 0.08	100 μM: 61%
20d	4.66 ± 0.10	4.67 ± 0.12	4.95 ± 0.12	4.70 ± 0.10	100 μM: 73%
20e	4.65 ± 0.09	4.84 ± 0.13	4.93 ± 0.09	4.78 ± 0.08	100 μM: 50%
21a	4.23 ± 0.06	4.40 ± 0.02	4.49 ± 0.06	4.44 ± 0.07	100 μM: 79%
21b	4.43 ± 0.10	4.54 ± 0.05	4.79 ± 0.04	4.59 ± 0.07	100 μM: 85%
21c	4.41 ± 0.03	4.64 ± 0.04	4.88 ± 0.08	4.64 ± 0.11	100 μM: 67%
21d	4.01 ± 0.09	4.38 ± 0.11	4.41 ± 0.10	4.23 ± 0.11	100 μM: 74%
21e	4.43 ± 0.07	4.54 ± 0.09	4.70 ± 0.10	4.69 ± 0.02	4.09 ± 0.07
22a	4.21 ± 0.11	4.54 ± 0.07	4.45 ± 0.06	4.41 ± 0.05	100 μM: 62%
22b	4.48 ± 0.04	4.66 ± 0.06	4.74 ± 0.02	4.68 ± 0.10	4.33 ± 0.02
22c	4.63 ± 0.03	4.96 ± 0.01	4.83 ± 0.05	4.78 ± 0.09	4.25 ± 0.10
22d	4.41 ± 0.05	4.57 ± 0.06	4.64 ± 0.08	4.60 ± 0.04	100 μM: 82%
22e	4.26 ± 0.08	4.77 ± 0.09	4.63 ± 0.10	4.63 ± 0.11	4.29 ± 0.04
Tiagabine[c]	6.88 ± 0.12	52%	64%	73%	7.41 ± 0.06
(*S*)-SNAP-5114[d]	4.07 ± 0.09	56%	5.29 ± 0.04	5.71 ± 0.07	4.56 ± 0.02

[a] % of remaining GABA uptake at 100 μM concentration of tested compound (means; $n = 2$) or pIC_{50} (means ± SEM; $n \geqslant 3$ if not specified otherwise).
[b] % of NO-711 binded to GAT1 at 100 μM concentration of tested compound or pK_i (means ± SEM; $n = 3$).
[c] Data from.[18]
[d] Data from.[41]

(**1**, **2**, **4**, **5**, and **7**) with 3-bromodihydrofuran-2(3*H*)-one (**8**) or 3-bromo-5-methyldihydrofuran-2(3*H*)-one (**9**). The reactions were carried out in acetonitrile in the presence of anhydrous K_2CO_3 and tetrabutylammonium bromide (TBAB) at room temperature for 12–48 h. In the case of lactone **11**, two geometric isomers *cis* (**11**-*cis*) and *trans* (**11**-*trans*) were obtained.

The compounds needed for the reactions, namely 4-diphenylmethylene piperidine (**1**) and 4-benzhydryl piperidine (**2**), were prepared according to a literature procedure.[25]

N-Methyl-4,4-diphenylbut-3-en-1-amine (**4**) was prepared according to a literature procedure using 1,1′-(4-bromobut-1-ene-1,1-diyl)dibenzene (**3**) as a starting material.[23,26,27] *N*-Methyl-4,4-diphenylbutan-1-amine (**5**) and 4,4-diphenylbutan-1-amine (**7**) were obtained by the hydrogenation of their derivatives **4** and **6**.

Aminolyses of the obtained lactones **10**–**15** were performed under different conditions depending on the reactivity and solubility of both the lactone and the amine, using conventional methods of heating or microwave irradiation. The course of the performed reactions is presented in Scheme 1.

3. Biological evaluation

4-Hydroxybutanoic acid and 4-hydroxypentanoic acid derivatives **16a**–**e** to **22a**–**e** were evaluated for their inhibitory potency towards the four GABA transporter subtypes mGAT1–mGAT4. The assay system used was based on [^{3}H]GABA uptake in HEK-293 cells stably expressing the individual GABA transporters.[28] The affinity for GAT1 was determined by MS binding assay with NO711 as a non-labeled marker.[29] The compounds were considered as active if GABA uptake or NO711 binding was reduced by at least 50% at a concentration of 100 μm. For the active compounds, pIC_{50} or pK_i values were assessed. The results are listed in Table 1.

The compounds (**16a** and **16d**) that displayed the most interesting profile in in vitro tests were then subjected to further behavioral studies in vivo. The performed investigations included pain (hot plate, writhing, formalin) and rotarod tests.

4. Results and discussion

With the aim of identifying GABA uptake inhibitors with improved pharmacological properties, a wide range of new GABA uptake inhibitors has been prepared. The core structure of the designed compounds is 4-hydroxybutanamide (**16a**–**e**, **17a**–**e**, **20a**–**e** to **22a**–**e**) or 4-hydroxypentanamide (**18a**–**e**, **19a**–**e**).

The biological evaluation revealed that among the obtained amides with the 4,4-diphenylbutan-1-amine moiety (**22a**–**e**), the highest potency towards transporters mGAT1-4 was shown by **22c** with a chloro substituent at the *para*-position of the *N*-benzylamide moiety ($pIC_{50} = 4.63–4.96$) (Table 1). The introduction of a double bond in the amino moiety at the 2-position of the acidic fragment of the amides obtained and a tertiary amine led to an increase in the inhibitory potency of compounds **20a**–**e**.

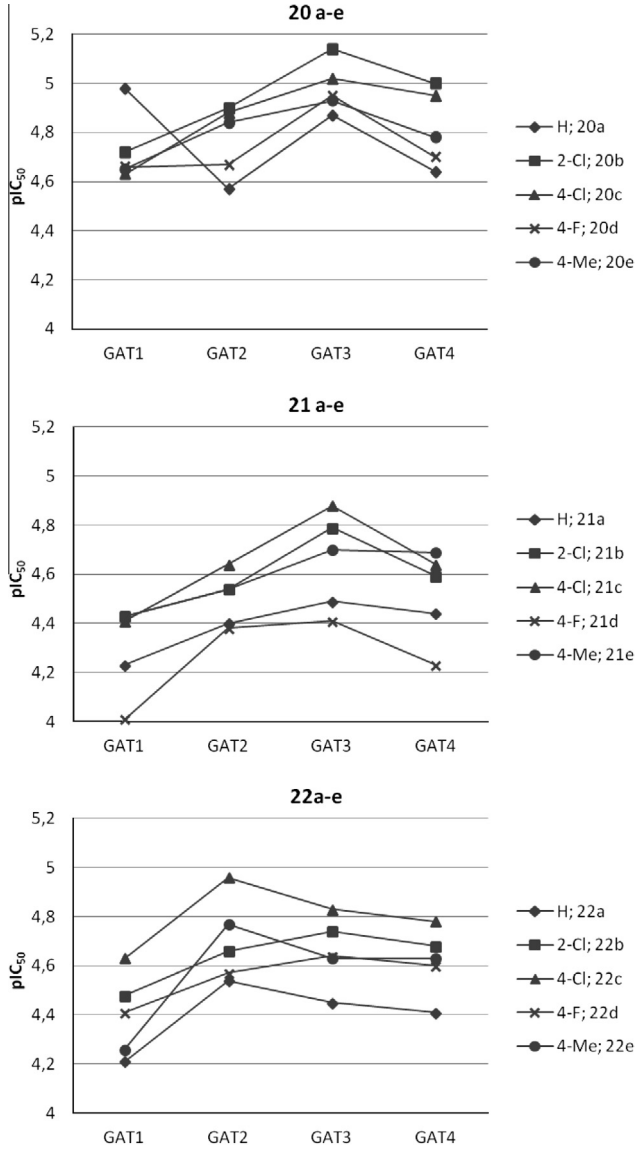


Figure 4. Potencies of compound series **20**, **21** and **22**.

Increased inhibitory activity of GABA uptake was observed towards mGAT3 and mGAT4 transporters. Moreover, the compound bearing a chloro substituent at the *ortho*-position of the benzyl fragment of the molecule showed the highest potency towards mGAT3 (**20b**, $pIC_{50} = 5.14$). Structural modification involving hydrogenation of the double bond in the amino moiety of the series discussed above was found to have a negative effect as the potencies of compounds **21a**–**e** were greatly reduced. The rigid amine group of 4-(diphenylmethylidene)piperidine (**16a**–**e**) in the 2-position of the acidic fragment of the amides obtained provided optimal results only towards mGAT1. Compound **16d**, which contains a fluoro substituent at the *para*-position of the benzyl fragment of the molecule, showed significant potency towards mGAT1 ($pIC_{50} = 5.01$). Structural modification involving hydrogenation of the double bond in the amino moiety of 4-(diphenylmethyl)piperidine **17a**–**e** caused only a slight increase in the inhibitory activity towards mGAT1 compared with **16a**–**e**. Unfortunately, this modification proved to be unfavorable for the other transporters. A marked decrease in activity was observed towards GAT4.

The dependences of the potency towards inhibition of GAT for the compound series **20**, **21**, and **22** on the nature of the substituents on the benzyl amide function are presented in Fig. 4.

The introduction of an additional methyl group at the 4-position of 4-hydroxybutanamide enabled the synthesis of two series of geometric isomers **18a**–**e** (from **11-*cis***) and **19a**, **b**, **d**, **e** (from **11-*trans***). Among the obtained compounds containing a 4-(diphenylmethylidene)piperidine moiety, unsubstituted amide **19a** showed the highest inhibitory activity towards mGAT1 ($pIC_{50} = 5.11$); however, this compound was not active in NO711 binding studies.

Taking into consideration the results of in vitro tests, two compounds **16a** (the most active in NO711 binding and active in uptake studies) and **16d** (active in both uptake and binding studies), which displayed the most interesting uptake profiles, were subjected to further in vivo tests.

Behavioral studies concerning the antinociceptive properties of compounds **16a** and **16d** proved their activity in some tests in mice. In the hot-plate assay, both **16a** and **16d** at 30 mg/kg prolonged the latency time to nocifensive response in mice, but this effect did not reach statistical significance ($F(2,25) = 1.332$; $p > 0.05$). The reference (*S*)-SNAP-5114 showed no antinociceptive activity in this test.

A statistically significant and dose-dependent analgesic activity of the test compound **16d** was observed in the acetic acid-induced writhing test (Table 2). ED_{50} values of 18.1 mg/kg for **16d** and 7.1 mg/kg for (*S*)-SNAP-5114 were determined. The analgesic potency of **16d** in the writhing test ($ED_{50} = 18.1$ mg/kg) (Table 2) indicates that it has the ability to attenuate the symptoms of inflammatory pain. This observation was confirmed using another model of chemogenic pain, namely the formalin test (Table 3, Fig. 5). The latter is a tonic pain model, which in rodents reflects persistent pain dependent on both sensory C-fiber activation as well as sensitization within the spinal cord dorsal horn and the brain; however, peripheral inflammation is also an important contributor 30–32. In the formalin test, both **16a** and **16d** demonstrated significant analgesic properties. **16a** was antinociceptive in both phases of the test, but its activity was particularly pronounced in the second (inflammatory) phase of this assay (Table 3; Fig. 5). This can be ascribed to the formalin-evoked combination of inflammatory reactions in the peripheral tissues and functional changes in the dorsal horn of the spinal cord (central sensitization of pain and neuroplasticity of the CNS).[30,31,33] **16d** did not influence the duration of the licking response in the first phase of the test, but it had significant antinociceptive properties in the inflammatory phase (Fig. 5). (*S*)-SNAP-5114 at 30 mg/kg (ip) displayed weak antinociceptive activity in the second phase of the formalin test (40% vs vehicle-treated mice).

Several lines of evidence indicate that there is a strong concentration dependence of nociceptive responses induced by formalin in both mice and rats. There is also evidence that distinct nociceptive mechanisms might underlie animals' pain reaction in response to low or high concentrations of formalin 32. In our study, we used 5% formalin solution. At this concentration of formalin, in the late phase of the test, a significant dependence of nociceptive responses on peripheral inflammatory changes and to a lesser degree on central sensitization is observed 33,34. In our research, this fact was confirmed by the observed significant plasma extravasation after formalin injection into the hind paws of control animals (data not shown). This observation is consistent with results reported by other authors 32, who demonstrated that 5% but not 1% formalin produced significant inflammation in peripheral tissues and plasma extravasation, which were attenuated by high doses of anti-inflammatory drugs 32,34.

Based on the obtained results, it is worth noting that the antinociceptive effect is of a rather peripheral origin, as indicated by the lack of activity in the hot-plate test (this test reveals compounds with central analgesic properties), yet statistically significant activities in two chemogenic pain models, i.e. the writhing test and in the second phase of the formalin test.

Table 2
Antinociceptive activity of **16a**, **16d** and (*S*)-SNAP-5114 in the acetic acid-induced writhing test

Compound	Dose (mg/kg)	Number of writhes (mean ± SEM)	Analgesic effect (%)	ED_{50} (mg/kg)
Vehicle (0.5% MC)	—	55.3 ± 3.9	—	—
16a	30.0	38.8 ± 8.3	29.8	—
16d	15.0	39.3 ± 6.6	28.9	18.1 (14.1–23.4)
$F(3,44) = 13.51$;	20.0	16.6 ± 8.1***	69.9	
$p < 0.0001$	30.0	11.4 ± 7.6***	79.4	
(*S*)-SNAP-5114	3.75	42.4 ± 8.5	23.3	7.1 (4.2–12.2)
$F(3,37) = 13.12$;	7.5	20.6 ± 3.9***	62.7	
$p < 0.0001$	15.0	16.3 ± 4.9***	70.5	

Each value represents the mean ± SEM. The test compounds and the vehicle were administered ip 30 min before the assay. ED_{50} value: the dose that reduced the number of stretches (i.e., the dose that diminished the pain reaction) for 50% as compared to vehicle-treated animals. MC: methylcellulose (vehicle).
Statistical analysis: Student's *t*-test (**16a**) and one-way ANOVA, followed by post hoc Dunnett's comparison (**16d**) and (*S*)-SNAP-5114). Significant difference compared to MC-treated mice: ***$p < 0.0001$.

The lack of motor coordination impairments in animals pretreated with **16a** or **16d**, together with their antinociceptive activity in two chemogenic pain models, indicate that these molecules are interesting lead structures for the further development of novel analgesic drugs (Table 4).

5. Conclusion

Seven series of new compounds have been produced. An *N*-bulky and lipophilic biaryl group in the 2-position of 4-hydroxybutanamide proved to be significant for inhibitory potency towards GAT1-4. The highest activities against the mGAT1 transporter were observed for 4-(diphenylmethylidene)piperidine- and 4-(diphenylmethyl)piperidine-substituted 4-hydroxybutanamides (**16** and **17**) and the 4-(diphenylmethylidene)piperidine-substituted 4-hydroxypentanamide (**18**). Modification that involved opening of the piperidine ring was well-tolerated against mGAT2 (**22**). Also, the introduction of an additional methyl group on the amino group in the 2-position of 4-hydroxybutanamide improved the activity with respect to mGAT3 and mGAT4 (**20**, **21**).

Moreover, the introduction of substituents on the benzyl fragment of molecules is essential for activity against mGAT1. The introduction of an *ortho*- or *para*-chloro substituent led to a decrease in activity towards mGAT1. However, replacement of the chloro substituent in the *para*-position by a fluoro substituent yielded better results, but a methyl group in the *para*-position did not significantly affect the activity. Among the obtained compounds containing a 4-(diphenylmethylidene)piperidine moiety, unsubstituted amide **19a** showed the highest inhibitory activity towards mGAT1 (**19a**; $pIC_{50} = 5.11$).

The presence of a chloro substituent at the *para*-position of the *N*-benzylamide moiety had a beneficial effect on the activity against mGAT2. Slightly lower values were obtained by switching the chloro substituent to the *ortho* position. Nevertheless, this substitution afforded compound **22c**, the most active agent against mGAT2 (**22c**; $pIC_{50} = 4.96$). However, replacement of the chloro substituent in the *para*-position by a fluoro substituent or reverting to an unsubstituted *N*-benzylamide moiety caused a decrease in activity. As in the case of mGAT1, a *para*-methyl group showed no significant effect on the activity towards mGAT2.

Among the obtained series, substitution on the *N*-benzylamide moiety did not produce a significant effect on the activity towards mGAT3. However, compound **18b**, bearing a chloro substituent at the *ortho*-position, exhibited a slightly improved subtype selectivity with respect to mGAT3 (**18b**; $pIC_{50} = 4.54$). Among the obtained compounds, compound **20b**, which contains a chloro substituent in the *ortho* position, was the most active toward mGAT3 (**20b**; $pIC_{50} = 5.14$). It is worthy of note that the *N*,*N*-(methyl)(4,4-diphenyl)but-3-enylamine moiety is the most preferred lipophilic biaryl group toward mGAT3, regardless of the substituent on the benzyl fragment of the molecule.

The effect on the activity toward mGAT4 was comparable to that toward mGAT3. Increased activity toward mGAT4 was also observed for compound **20b** (**20b**; $pIC_{50} = 5.00$).

The results of in vivo studies indicated that the test compounds displayed antinociceptive properties in these pain models, in which pain is induced by chemical stimuli acting within peripheral

Table 3
Antinociceptive activity of **16a**, **16d** and (*S*)-SNAP-5114 in the formalin test

Compound	Dose (mg/kg)	Duration of licking response (s) ±SEM (phase 1)	Analgesic activity (%)	ED_{50} (mg/kg) (phase 1)	Duration of licking response (s) ±SEM (phase 2)	Analgesic activity (%)	ED_{50} (mg/kg) (phase 2)
Vehicle (0.5% MC)	—	55.4 ± 6.6	—		111.6 ± 17.1	—	
16a	7.5	39.0 ± 6.1	29.6	27.1 (7.8–94.4)	75.1 ± 6.5	32.7	11.1 (7.1–17.3)
	15.0	31.0 ± 4.9*	44.0		39.6 ± 7.2***	64.5	
	30.0	27.7 ± 5.0**	50.0		16.3 ± 8.1***	85.4	
16d	7.5	59.9 ± 7.4	NA	—	30.9 ± 14.4***	72.3	—
	15.0	50.4 ± 8.4	NA		17.8 ± 7.3***	84.1	
	30.0	58.1 ± 5.6	NA		24.0 ± 3.3***	78.5	
Vehicle (0.5% MC)	—	34.8 ± 9.4	—	—	78.8 ± 15.9	—	—
(*S*)-SNAP-5114	30.0	35.6 ± 5.9	NA	—	47.7 ± 11.6	39.5	—

Each value represents the mean ± SEM. The test compounds and the vehicle were administered ip 30 min before the assay. ED_{50} value: the dose that reduced the duration of licking response (i.e., the dose that diminished the pain reaction) for 50% as compared to vehicle-treated animals. MC: methylcellulose (vehicle). NA: not active.
Statistical analysis: Student's *t*-test ((*S*)-SNAP-5114) and one-way ANOVA, followed by post hoc Dunnett's comparison (**16a** and **16d**). **16a**: $F(3,29) = 4.777$; $p < 0.01$ (phase 1) and $F(3,32) = 11.38$; $p < 0.0001$ (phase 2).
16d: $F(3,29) = 0.3149$; $p > 0.05$ (phase 1) and $F(3,31) = 12.39$; $p < 0.0001$ (phase 2). Significant difference compared to MC-treated mice: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

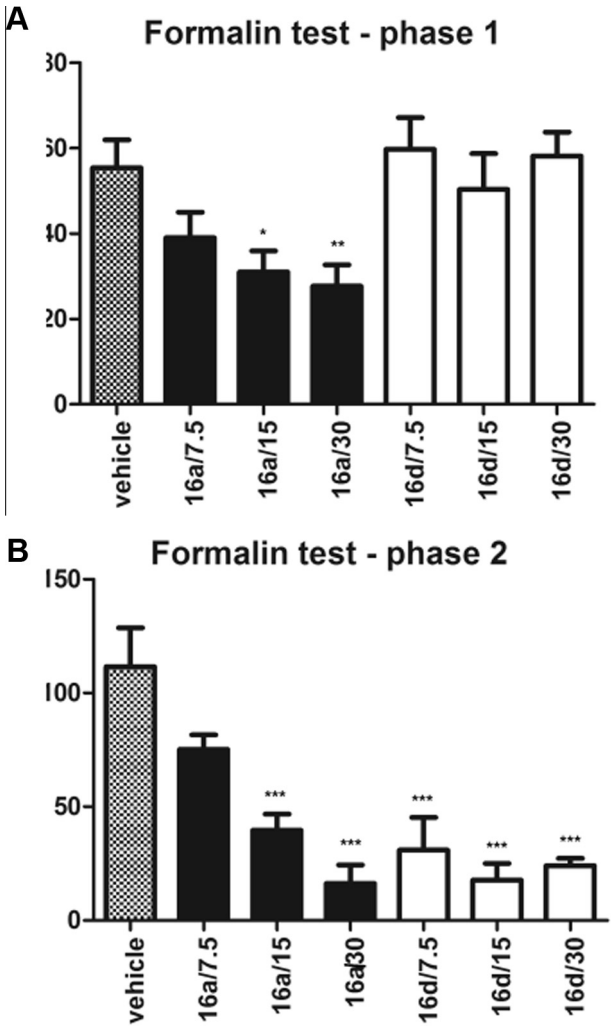


Figure 5. Analgesic activity of the test compounds in the neurogenic (0–5 min) phase (Fig. 5A) and in the second—inflammatory (15–30 min) phase (Fig. 5B) of the formalin test. Significant difference compared to MC-treated mice: $^{*}p$ <0.05; $^{**}p$ <0.01; $^{***}p$ <0.001.

tissues. The effect of GAT inhibition as the mechanism underlying antinociception requires further study. Nevertheless, our research indicates a potential role of GAT subtypes in peripheral pain and inflammation.

6. Experimental

6.1. Chemistry

Reactions were monitored by thin layer chromatography (TLC) using silica gel pre-coated 60 F_{254} plates (0.2 mm; Merck Kieselgel) and following solvent system: S_1 (PE/EtOAc 7:3, v/v); S_2 (*n*-hexan/ethanol/triethylamine 7:2:1, v/v/v), S_3 (chloroform/acetone 1:1, v/v), S_4 (PE/EtOAc 1:1, v/v), S_5 (DCM/acetone 9:1, v/v), S_6 (chloroform/acetone 9:1, v/v), S_7 (DCM/acetone 7:3, v/v). The plates were visualized with the UV light and mixture of ninhydrin (0.3 g ninhydrin in 100 mL of *n*-butanol and 3 mL of acetic acid). NMR spectra were recorded with VX Merkury Varian (300 MHz) instrument in $CDCl_3$ at ambient temperature using solvent signal as an internal standard. Elemental analyses (C, H, N) were carried out within 0.4% of the theoretical values and were performed on an ElementarVario EL III (Elementar Analysensysteme, Hanau, Germany). Mass spectra were recorded on MDX SCILEX API 2000 (Concord, ON, Canada) using the ESI method. Melting points were determined in open glass capillaries on a Melting Point Büchi 535 apparatus and are uncorrected. Reactions under microwave irradiation were carried out in Discover LabMate (CEM Corporation, USA).

The synthesis of the 4-(diphenylmethylidene)piperidine (**1**) and 4-benzhydryl piperidine (**2**) was performed based on method described in literature.[25] The synthesis of the 1,1′-(4-bromobut-1-ene-1,1-diyl)dibenzene (**3**) was performed according to methods described in literature.[26] Synthesis of *N*-methyl-4,4-diphenylbut-3-en-1-amine (**4**) and 4,4-diphenylbut-3-en-1-amine (**6**) was performed as previously described.[23,27]

6.1.1. General procedure for the synthesis of amines (5) and (7)

To the solution of 1 mmol (**4** or **6**) in ethanol (6 ml) 70 mg Pd/C (10%) was added. After hydrogenation for 8 h at room temperature, the catalyst was removed through celite and the filtrate was concentrated in vacuo to afford (**5**) or (**7**) as oils, which were used for the next step without further purification.

6.1.1.1. *N*-Methyl-4,4-diphenylbutan-1-amine (5). Thick yellow oil; yield: 80%; R_f: 0.40 (S_2); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.43–1.53 (m, 2H(CH_2CH_2NH)) 2.05–2.14 (m, 2H($CHCH_2$)) 2.39 (s, 3H(*Me*)) 2.60 (t, J = 7.18 Hz, 2H(CH_2NH)) 3.91 (t, J = 7.82 Hz, 1H($CHCH_2$)) 7.13–7.36 (m, 10H(*Ar*)), ESI-MS (*m*/*z*) 239.1 $[M+H]^+$.

6.1.1.2. 4,4-Diphenylbutan-1-amine (7). Thick yellow oil; yield: 92%; R_f: 0,27 (S_2); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.37–1.44 (m, 2H($CH_2CH_2NH_2$)) 2.03–2.14 (m, 2H($CHCH_2$)) 2.71 (t, J = 7.05 Hz, 2H(CH_2NH_2)) 3.90 (t, J = 7.82 Hz, 1H($CHCH_2$)) 7.13–7.40 (m, 10H(Ar)), ESI-MS (*m*/*z*) 226.1 $[M+H]^+$.

6.1.2. General procedure for the synthesis of 3-subsituted dihydrofuran-2(3*H*)-one derivatives and 3-subsituted 5-methyldihydrofuran-2(3*H*)-one derivatives (10–15)

Anhydrous K_2CO_3 (5 equiv) was added to the solution of relevant amine (1 equiv) and tetrabutylammonium bromide (TBAB) (0.01 equiv) in the acetonitrile and the mixture was stirred at at 0 °C for 1.5 h. Then a solution of 3-bromodihydrofuran-2(3*H*)-one (**8**) or 3-bromo-5-methyldihydrofuran-2(3*H*)-one (**9**) (1 equiv) was added dropwise and stirring was continued for 12–48 h at room temperature. After the reaction was completed, the precipitate was filtered off and the filtrate was concentrated under vacuum. Obtained crude products were purified by column chromatography.

6.1.2.1. 3-[4-(Diphenylmethylidene)piperidin-1-yl]dihydrofuran-2(3*H*)-one (10). Reagents and conditions: 21.25 mmol **1** (5.30 g), 85 mmol K_2CO_3 (11.75 g), 0.65 mmol TBAB (0.21 g), 21.25 mmol **8** (3.50 g), 50 ml MeCN, 24 h; purification by column chromatography (S_7); Yield 98%; yellow solid; mp 164.1–165.3 °C; R_f: 0.89 (S_3); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.30–2.39 (m, 2H($NCHCH_2CH_2$)) 2.41–2.52 (m, 4H(*piperidine*)) 2.60–2.69 (m, 2H(*piperidine*)) 2.89 (dt, J = 10.64, 5.45 Hz, 2H(*piperidine*)) 3.66 (t, J = 9.62 Hz, 1H(N*CH*)) 4.16–4.25 (m, 1H(CH_2CH_2O)) 4.33–4.40 (m, 1H(CH_2CH_2O)) 7.09–7.31 (m, 10H(Ar)), ESI-MS (*m*/*z*) 334.1 $[M+H]^+$.

6.1.2.2. 3-[4-(Diphenylmethylidene)piperidin-1-yl]-5-methyldihydrofuran-2(3*H*)-one (cis-11 or trans-11). Reagents and conditions: 16 mmol **1** (3.98 g), 16 mmol K_2CO_3 (2.21 g), 0.16 mmol TBAB (0.05 g), 16 mmol **9** (2.85 g), 20 ml MeCN, 20 h; purification by column chromatography (S_6) and recrystallization from *n*-hexane/EtOAc 1:1; Yield 71%; white solid; *cis*-**11**: 0.83; *trans*-**11** 0.78 (S_7); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm (*cis*-18): 1.43 (d, J = 6.16 Hz, 3H(*Me*)) 2.20–2.54 (m, 6H(*piperidine*))

Table 4
Influence of **16a**, **16d** and (*S*)-SNAP-5114 on motor coordination in the rotarod test

Compound	Dose	Rotations per minute (rpm)	Mean time spent on the rotarod (±SEM) (s)	Number of animals with motor impairments/number of animals tested (*X*/*Y*)
Vehicle (0.5% MC)	—	6	60.0 ± 0.0	0/8
		18	60.0 ± 0.0	0/8
		24	60.0 ± 0.0	0/8
16a	30.0	6	60.0 ± 0.0	0/8
		18	60.0 ± 0.0	0/8
		24	60.0 ± 0.0	0/8
16d	30.0	6	60.0 ± 0.0	0/8
		18	60.0 ± 0.0	0/8
		24	60.0 ± 0.0	0/8
(*S*)-SNAP-5114	30.0	6	59.6 ± 0.4	1/8
		18	56.3 ± 3.8	1/8
		24	59.0 ± 1.0	1/8

Motor coordination measured on the rotarod apparatus rotating at 6, 18 and 24 rpm 30 min after the ip administration of each compound. Data are expressed as: mean time spent on the rotarod (±SEM) or *X*/*Y*: the number of animals falling off the rod (*X*) per the number of mice tested (*Y*). Animals per group were: 8.
Statistical analysis of data: average time spent on the rotarod: one-way ANOVA followed by Dunnett's test, $F(3,28) = 1.000$; $p > 0.05$ (6, 18, 24 rpm). Qualitative variables (i.e., the number of mice showing motor impairments vs number of mice that performed the test) were statistically evaluated by means of the Fisher's exact probability test (differences not significant).

2.61 (dt, *J* = 10.45, 5.42 Hz, 2H(*piperidine*)) 2.82–2.92 (m, 2H(*piperidine*)) 3.71 (dd, *J* = 12.18, 8.34 Hz, 1H(NC*H*)) 4.38–4.51 (m, 1H(*CH*Me)) 7.08–7.14 (m, 4H(Ar)) 7.18–7.31 (m, 6H(Ar)); (*trans*-**11**): 1.43 (d, *J* = 6.16 Hz, 3H(*Me*)) 2.20–2.54 (m, 6H(*piperidine*)) 2.61 (dt, *J* = 10.45, 5.42 Hz, 2H(*piperidine*)) 2.82–2.92 (m, 2H(*piperidine*)) 4.09–4.16 (q, 1H(NC*H*)) 4.64–4.73 (m, 1H(*CH*Me)) 7.08–7.14 (m, 4H(Ar)) 7.18–7.31 (m, 6H(Ar)), ESI-MS (*m*/*z*) 338.1 $[M+H]^+$.

6.1.2.3. 3-[4-(Diphenylmethyl)piperidin-1-yl]dihydrofuran-2(3*H*)-one (12). Reagents and conditions: 4.85 mmol **2** (1.22 g), 24.25 mmol K_2CO_3 (3.35 g), 0.05 mmol TBAB (0.02 g), 4.85 mmol **8** (0.80 g), 20 ml MeCN, 20 h; purification by column chromatography (S_5); Yield 72%; yellow oil; R_f: 0.76 (S_7); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.27–1.39 (m, 2H(*piperidine*)) 1.54–1.65 (m, 2H(*piperidine*)) 2.15 (dt, *J* = 11.41, 3.53 Hz, 1H(*piperidine*)) 2.22–2.35 (m, 3H (*piperidine*)) 2.60 (td, *J* = 11.41, 2.56 Hz, 1H(CHC*H*)) 2.71–2.80 (m, 1H(NCHCH_2CH$_2$)) 2.95–3.02 (m, 1H(NCHCH_2CH$_2$)) 3.47–3.52 (d, 1H (*CH*CH)) 3.54–3.61 (t, 1H (NC*H*)) 4.17 (td, *J* = 8.91, 7.82 Hz, 1H(CH$_2$CH_2O)) 4.27–4.38 (m, 1H (CH$_2$CH_2O)) 7.07–7.41 (m, 10H(Ar)), ESI-MS (*m*/*z*) 336.2 $[M+H]^+$.

6.1.2.4. 3-[(4,4-Diphenylbut-3-en-1-yl)(methyl)amino]dihydrofuran-2(3*H*)-one (13). Reagents and conditions: 6.03 mmol **4** (1.43 g), 30.19 mmol K_2CO_3 (4.17 g), 0.06 mmol TBAB (0.02 g), 6.03 mmol **8** (1.00 g), 15 ml MeCN, 48 h; purification by column chromatography (S_6); Yield 78%; yellow oil; R_f: 0.70 (S_6); ^{1}H NMR ($CDCl_3$) δ [ppm]: 2.17–2.20 ppm (m, 2H(CH_2CH$_2$N)), 2.23–2.30 (m, 2H(NCHCH_2)) 2.31 (s, 3H (Me)), 2.68–2.74 ppm (t, 2H (CH_2N)), 3.62–3.68 ppm (t, 1H(NC*H*)), 4.13–4.37 ppm (m, 2H(CH$_2$-CH_2O), 6.13 (t, 1H(C=C*H*)), 7.17–7.40 ppm (m, 10H (Ar)), ESI-MS (*m*/*z*) 322.1 $[M+H]^+$.

6.1.2.5. 3-[(4,4-Diphenylbutyl)(methyl)amino]dihydrofuran-2(3*H*)-one (14). Reagents and conditions: 1.78 mmol **5** (0.40 g), 8.90 mmol K_2CO_3 (1.23 g), 0.02 mmol TBAB (0.01 g), 1.78 mmol **8** (0.29 g), 7 ml MeCN, 24 h; purification by column chromatography (S_7); Yield 75%; yellow oil; R_f: 0.76 (S_7); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.41–1.53 (m, 2H(CH_2CH$_2$N)) 2.03–2.13 (m, 2H(CHCH_2CH$_2$)) 2.16–2.25 (m, 2H(NCHCH_2)) 2.29 (s, 3H(Me)) 2.59 (t, *J* = 7.44 Hz, 2H(CH_2N)) 3.59 (t, *J* = 9.62 Hz, 1H(NC*H*)) 3.90 (t, *J* = 7.82 Hz, 1H(ArC*H*CH$_2$)) 4.09–4.19 (m, 1H(CH$_2$-CH_2O)) 4.29–4.37 (m, 1H(CH$_2$CH_2O)) 7.05–7.42 (m, 10H(Ar)), ESI-MS (*m*/*z*) 324.2 $[M+H]^+$.

6.1.2.6. 3-[(4,4-Diphenylbutyl)amino]dihydrofuran-2(3*H*)-one (15). Reagents and conditions: 8.17 mmol **7** (1.84 g), 40.37 mmol K_2CO_3 (5.58 g), 0.08 mmol TBAB (0.03 g), 8.18 mmol **8** (0.80 g), 10 ml MeCN, 48 h; purification by column chromatography (S_5); Yield 60%; yellow oil; R_f: 0.74 (S_3); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.42–1.54 (m, 2H(CH_2CH$_2$NH)) 2.03–2.17 (m, 3H(NHCHCH_2); (CHCH_2CH$_2$)) 2.37–2.50 (m, 1H(CHCH_2CH$_2$)) 2.64 (dt, *J* = 11.22, 7.21 Hz, 1H(CH_2NH)) 2.76 (dt, *J* = 11.16, 6.99 Hz, 1H(CH_2NH)) 3.49 (dd, *J* = 10.39, 8.08 Hz, 1H(NHC*H*)) 3.89 (t, *J* = 7.82 Hz, 1H(ArC*H*CH$_2$)) 4.17 (td, *J* = 9.81, 6.28 Hz, 1H(CH$_2$CH_2-O)) 4.37 (td, *J* = 8.91, 2.18 Hz, 1H(CH$_2$CH_2O)) 7.09–7.40 (m, 10H(Ar)), ESI-MS (*m*/*z*) 310.2 $[M+H]^+$.

6.1.3. 2-Substituted 4-hydroxybutanamides (16a–e to 22a–e)

General procedure 1 (GP1): 2-Substituted dihydrofuran-2(3*H*)-one derivative (1 equiv) was heated with relevant amine (2.5 or 1.2 equiv) in THF at reflux for 48–72 h. Then the solvent was evaporated under vacuum and crude product was purified by column chromatography and recrystallized from *n*-hexane/EtOAc 1:1.

General procedure 2 (GP2): The process vial was charged with 2-substituted dihydrofuran-2(3*H*)-one derivative (1 equiv) and relevant benzylamine derivative (3 equiv). The vial was then closed and its content was magnetically stirred and microwave heated at 160 °C for 1 h. After the reaction was finished, the crude product was purified by column chromatography (S_3) and recrystallized from *n*-hexane/EtOAc 1:1.

6.1.3.1. *N*-Benzyl-4-hydroxy-2-(4-(diphenylmethylidene)piperidin-1-yl)butanamide (16a). Procedure GP2. Yield: 34%; white solid; mp 157–160 °C; R_f: 0.62 (S_3). Anal. Calcd for $C_{29}H_{32}N_2O_2$: C, 79.06; H, 7.32; N, 6.36. Found: C. 79.15; H, 7.46; N, 6.42. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.81–1.99 (m, 2H(CH_2CH$_2$OH)) 2.25–2.42 (m, 4H(*piperidine*)) 2.49–2.67 (m, 4H(*piperidine*)) 3.26 (dd, *J* = 9.49, 3.08 Hz, 1H(NC*H*)) 3.59–3.68 (m, 1H(CH_2OH)) 3.84–3.91 (m, 1H(CH_2OH)) 4.39–4.55 (m, 2H(NHCH_2)) 7.07–7.14 (m, 4H(Ar)) 7.14–7.36 (m, 11H(Ar)) 7.84 (br s, 1H(CO*NH*)); ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 33.5, 56.9 (piperidine) 34.6 (CHCH$_2$CH$_2$) 43.6 (*C*H$_2$Ph) 58.3 (*C*H$_2$OH) 70.1 (CHCH$_2$) 129.3 (*C*=CPh$_2$) 137.8 (*C*Ph$_2$) 126.7, 126.9, 127.9, 128.3, 128.6, 137.9, 142.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 441.2 $[M+H]^+$.

6.1.3.2. *N*-(2-Chlorobenzyl)-4-hydroxy-2-(4-(diphenylmethylidene)piperidin-1-yl)butanamide (16b). Procedure GP2. Yield: 36%; white solid; mp 174–175 °C; R_f: 0.72 (S_3). Anal. Calcd for $C_{29}H_{31}ClN_2O_2$: C, 73.33; H, 6.58; N, 5.90. Found: C, 73.25; H, 6.66; N, 5.82. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.76–1.87 (m, 1H(CH_2CH$_2$OH)) 1.89–1.98 (m, 1H(CH_2CH$_2$OH)) 2.29–2.41 (m,

4H(*piperidine*)) 2.48–2.60 (m, 4H(*piperidine*)) 3.23 (dd, *J* = 9.49, 3.08 Hz, 1H(NC*H*)) 3.62 (ddd, *J* = 11.28, 8.34, 3.46 Hz, 1H(*CH*$_2$OH)) 3.80–3.88 (m, 1H(*CH*$_2$OH)) 4.49–4.62 (m, 2H(NH*CH*$_2$)) 7.05–7.14 (m, 4H(Ar)) 7.15–7.33 (m, 10H(Ar)) 8.00–8.10 (m, 1H(CO*NH*)); ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 33.5, 56.9 (piperidine) 34.6 (CH*C*H$_2$CH$_2$) 38.5 (*C*H$_2$Ph) 58.3 (*C*H$_2$OH) 70.1 (*C*HCH$_2$) 129.3 (*C*=CPh$_2$) 137.8 (*C*Ph$_2$) 126.6, 127.9, 128.3, 128.6, 132.2, 142.0, 142.4 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 375.5 [M+H]$^+$.

6.1.3.3. *N*-(4-Chlorobenzyl)-4-hydroxy-2-(4-(diphenylmethylidene)piperidin-1-yl)butanamide (16c). Procedure GP2. Yield: 34%; white solid; mp 130-132 °C; R_f: 0.64 (S_3). Anal. Calcd for $C_{29}H_{31}ClN_2O_2$: C, 73.33; H, 6.58; N, 5.90. Found: C, 73.47; H, 6.56; N, 5.94. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.79–1.92 (m, 1H(*CH*$_2$CH$_2$OH)) 1.93–2.02 (m, 1H(*CH*$_2$CH$_2$OH)) 2.23–2.45 (m, 4H(*piperidine*)) 2.47–2.66 (m, 4H(*piperidine*)) 3.25 (dd, *J* = 9.49, 3.33 Hz, 1H(NC*H*)) 3.60–3.69 (m, 1H(*CH*$_2$OH)) 3.81–3.91 (m, 1H(*CH*$_2$OH)) 4.31–4.55 (m, 2H(NH*CH*$_2$)) 7.09 (d, *J* = 9.49 Hz, 4H(Ar)) 7.14–7.41 (m, 10H(Ar)) 7.84 (s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 33.5, 56.9 (piperidine) 34.6 (CH*C*H$_2$CH$_2$) 43.6 (*C*H$_2$Ph) 58.3 (*C*H$_2$OH) 70.1 (*C*HCH$_2$) 129.3 (*C*=CPh$_2$) 137.8 (*C*Ph$_2$) 127.9, 128.6, 127.9, 132.2, 134.6, 136.0, 142.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 375.5 [M+H]$^+$.

6.1.3.4. *N*-(4-Fluorobenzyl)-4-hydroxy-2-(4-(diphenylmethylidene)piperidin-1-yl)butanamide (16d). Procedure GP2. Yield: 29%; white solid; mp 68–69 °C; R_f: 0.71 (S_3). Anal. Calcd for $C_{29}H_{31}FN_2O_2$; C, 75.96; H, 6.81; N, 6.11. Found: C, 75.89; H, 6.96; N, 6.28 ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.79–2.01 (m, 2H(*CH*$_2$CH$_2$OH)) 2.23–2.44 (m, 4H(*piperidine*)) 2.49–2.64 (m, 4H(*piperidine*)) 3.25 (dd, *J* = 9.62, 3.21 Hz, 1H(NC*H*)) 3.58–3.69 (m, 1H(*CH*$_2$OH)) 3.81–3.93 (m, 1H(*CH*$_2$OH)) 4.32–4.55 (m, 2H(NH*CH*$_2$)) 6.94–7.14 (m, 5H(Ar)) 7.14–7.34 (m, 9H(Ar)) 7.84 (br s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 33.5, 56.9 (piperidine) 34.6 (CH*C*H$_2$CH$_2$) 43.6 (*C*H$_2$Ph) 58.3 (*C*H$_2$OH) 70.1 (*C*HCH$_2$) 129.3 (*C*=CPh$_2$) 137.8 (*C*Ph$_2$) 115.3, 127.9, 128.3, 128.5, 128.6, 133.5, 142.0, 160.9 (arom) 171.3 (carbonyl);ESI-MS (*m*/*z*) 459.3 [M+H]$^+$.

6.1.3.5. *N*-(4-Methylbenzyl)-4-hydroxy-2-(4-(diphenylmethylidene)piperidin-1-yl)butanamide (16e). Procedure GP2. Yield: 30%; white solid; mp 140–142 °C; R_f: 0.64 (S_3). Anal. Calcd for $C_{30}H_{34}N_2O_2$: C, 79.26; H, 7.54; N, 6.16. Found: C, 79.34; H, 7.69; N, 6.28. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.85 (d, *J* = 18.72 Hz, 1H(*CH*$_2$CH$_2$OH)) 1.94–2.03 (m, 1H(*CH*$_2$CH$_2$OH)) 2.28–2.43 (m, 7H(*piperidine; Me*)) 2.47–2.65 (m, 4H(*piperidine*)) 3.26 (d, *J* = 3.08 Hz, 1H(NC*H*)) 3.62 (d, *J* = 7.95 Hz, 1H(*CH*$_2$OH)) 3.81–3.91 (m, 1H(*CH*$_2$OH)) 4.38–4.52 (m, 2H(NH*CH*$_2$)) 7.04–7.32 (m, 14H(Ar)) 7.77 (br s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 21.3 (CH$_3$) 33.5, 56.9 (piperidine) 34.6 (CH*C*H$_2$CH$_2$) 43.6 (*C*H$_2$Ph) 58.3 (*C*H$_2$OH) 70.1 (*C*HCH$_2$) 129.3 (*C*=CPh$_2$) 137.8 (*C*Ph$_2$) 127.9, 128.1, 128.3, 128.6, 128.8, 134.9, 136.4, 142.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 455.1 [M+H]$^+$.

6.1.3.6. *N*-Benzyl-4-hydroxy-2-(4-(diphenylmethyl)piperidin-1-yl)butanamide (17a). Procedure GP1. Reagents and conditions: **12** (2.33 mmol, 0.78 g), benzylamine (2.80 mmol, 0.30 g), THF (15 mL), 72 h; purification by column chromatography (S_7); recrystallization from *n*-hexane/EtOAc 1:1; yield: 76%; white solid; mp 112.1 °C; R_f: 0.62 (S_3). Anal. calcd for $C_{29}H_{34}N_2O_2$: C, 78.70; H, 7.74; N, 6.33. Found: C, 79.19; H, 7.56; N, 6.42. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.06–1.21 (m, 2H(*piperidine*)) 1.51–1.67 (m, 2H(*piperidine*)) 1.77–2.24 (m, 4H(*piperidine*)) 2.38–2.48 (m, 1H(NC*H*CO)) 2.57–2.81 (m, 2H(NCHC*H*$_2$CH$_2$))) 3.19 (dd, *J* = 9.49, 3.08 Hz, 1H(ArCHC*H*)) 3.46 (d, *J* = 11.03 Hz, 1H(ArC*H*)) 3.56–3.67 (m, 1H(*CH*$_2$OH)) 3.80–3.90 (m, 1H(*CH*$_2$OH)) 4.43–4.50 (m, 2H (NH*CH*$_2$)) 7.05–7.41 (m, 15H(*Ar*)) 7.76 (br s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 26.7, 38.0, 44.2 (piperidine) 34.6 (CH*C*H$_2$CH$_2$) 43.6 (*C*H$_2$Ph) 58.3 (*C*H$_2$OH) 53.7 (*C*Ph$_2$) 70.0 (*C*HCH$_2$) 126.2, 126.7 126.9, 128.2, 128.5, 137.9, 143.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 443.1 [M+H]$^+$

6.1.3.7. *N*-(2-Chlorobenzyl)-4-hydroxy-2-(4-(diphenylmethyl)-piperidin-1-yl)butanamide (17b). Procedure GP1. Reagents and conditions: **12** (2.50 mmol, 0.84 g), 2-chlorobenzylamine (3 mmol, 0.42 g), THF (15 mL), 72 h; purification by column chromatography (S_7); recrystallization from *n*-hexane/EtOAc 1:1; yield: 60%; white solid; mp 173.6 °C; R_f: 0.53 (S_3). Anal. Calcd for $C_{29}H_{33}ClN_2O_2$: C, 73.02; H, 6.97; N, 5.87. Found: C, 73.17: H, 7.01: N, 5.99. ^{1}H NMR (300 MHz, chloroform-*d*) δppm 1.08–1.30 (m, 2H(*piperidine*)) 1.52–1.61 (m, 2H(*piperidine*)) 1.73–1,97 (m, 2H(*piperidine*)) 2.02–2.21 (m, 2H(*piperidine*)) 2.34–2.47 (m, 1H(NC*H*CO)) 2.52–2.62 (m, 1H(NCHC*H*$_2$CH$_2$)) 2.64–2.75 (d, 1H (NCHC*H*$_2$CH$_2$)) 3.12–3.21 (m,1H (ArCHC*H*))3.41–3.52 (d, *J* = 11.03 Hz, 1H(ArC*H*)) 3.54–3.65 (m, 1H(*CH*$_2$OH)) 3.77–3.87 (m, 1H(*CH*$_2$OH)) 4.41–4.62 (m, 2H (NH*CH*$_2$)) 7.09–7.40 (m, 14H(*Ar*)) 7.96 (br s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 26.7, 38.0, 44.2 (piperidine) 34.6 (CH*C*H$_2$CH$_2$) 38.5 (*C*H$_2$Ph) 58.3 (*C*H$_2$OH) 53.7 (*C*Ph$_2$) 70.0 (*C*HCH$_2$) 126.2, 126.6, 128.1, 128.2, 128.3, 129.2, 132.2, 143.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 477,5 [M+H]$^+$

6.1.3.8. *N*-(4-Chlorobenzyl)-4-hydroxy-2-(4-(diphenylmethyl)piperidin-1-yl)butanamide (17c). Procedure GP1. Reagents and conditions: **12** (2.50 mmol, 0.84 g), 4-chlorobenzylamine (3 mmol, 0.42 g), THF (15 mL), 72 h; purification by column chromatography (S_7); recrystallization from n-hexane/EtOAc 1:1; yield: 69%; white solid; mp 141.4 °C; R_f: 0.47 (S_3). Anal. Calcd for $C_{29}H_{33}ClN_2O_2$: C, 73.02; H, 6.97: N, 5.87. Found: C, 73.20; H, 7.16; N, 6.01. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.06–1.24 (m, 2H(*piperidine*)) 1.49–1.66 (m, 2H(*piperidine*)) 1.74–1,98 (m, 2H(*piperidine*)) 2.02–2.21 (m, 2H(*piperidine*)) 2.35–2.48 (t, 1H(NC*H*CO)) 2.51–2.64 (m, 1H(NCHC*H*$_2$CH$_2$)) 2.65–2.76 (d, 1H (NCHC*H*$_2$CH$_2$)) 3.11–3.23 (m,1H (ArCHC*H*)) 3.42–3.50 (d, 1H(ArC*H*)) 3.55–3.68 (m, 1H(*CH*$_2$OH)) 3.77–3.90 (m, 1H(*CH*$_2$OH)) 4.33–4.50 (m, 2H (NH*CH*$_2$)) 7.06–7.40 (m, 14H(*Ar*)) 7.76 (br s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 26.7, 38.0, 44.2 (piperidine) 34.6 (CH*C*H$_2$CH$_2$) 43.6 (*C*H$_2$Ph) 58.3 (*C*H$_2$OH) 53.7 (*C*Ph$_2$) 70.0 (*C*HCH$_2$) 126.2, 128.2, 128.6, 129.2, 132.3, 134.6, 136.0, 143.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 477,5 [M+H]$^+$.

6.1.3.9. *N*-(4-Fluorobenzyl)-4-hydroxy-2-(4-(diphenylmethyl)-piperidin-1-yl)butanamide (17d). Procedure GP1. Reagents and conditions: **12** (2.33 mmol, 0.78 g), 4-fluorobenzylamine (2.80 mmol, 0.35 g), THF (15 mL), 72 h; purification by column chromatography (S_7); recrystallization from *n*-hexane/EtOAc 1:1; yield: 70%; white solid; mp 131.1 °C; R_f: 0.51 (S_3). Anal. Calcd for $C_{29}H_{33}FN_2O_2$: C, 75.62; H, 7.22; N, 6.08. Found: C, 75.91; H, 7.43; N, 6.22. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.96–1.31 (m, 2H(*piperidine*)) 1.50–1.64 (dd, *J* = 13.08, 2.31 Hz, 2H(*piperidine*)) 1.86–1,99 (m, 2H(*piperidine*)) 2.01–2.24 (m, 2H(*piperidine*)) 2.37–2.48 (m, 1H(NC*H*CO)) 2.55–2.64 (m, 1H(NCHC*H*$_2$CH$_2$)) 2.65–2.76 (m, 1H (NCHC*H*$_2$CH$_2$)) 3.15–3.23 (dd, *J* = 9.62, 3.21 Hz, 1H (ArCHC*H*)) 3.43–3.50 (d, *J* = 11.03 Hz, 1H(ArC*H*)) 3.56–3.68 (m, 1H(*CH*$_2$OH)) 3.80–3.89 (m, 1H(*CH*$_2$OH)) 4.33–4.49 (m, 2H (NH*CH*$_2$)) 6.96–7.09 (m, 2H(*Ar*)) 7.09–7.40 (m, 12H(*Ar*)) 7.76 (br s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 26.7, 38.0, 44.2 (piperidine) 34.6 (CH*C*H$_2$CH$_2$) 43.6 (*C*H$_2$Ph) 58.3 (*C*H$_2$OH) 53.7 (*C*Ph$_2$) 70.0 (*C*HCH$_2$) 115.3, 126.2, 128.5, 129.2, 143.0, 160.9 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 461,5 [M+H]$^+$.

6.1.3.10. *N*-(4-Methylbenzyl)-4-hydroxy-2-(4-(diphenylmethyl)-piperidin-1-yl)butanamide (17e). Procedure GP1. Reagents and conditions: **12** (2.60 mmol, 0.87 g), 4-methylbenzylamine

(3.12 mmol, 0.38 g), THF (15 mL), 72 h; purification by column chromatography (S_7); recrystallization from *n*-hexane/EtOAc 1:1; yield: 64%; white solid; mp 147.4 °C; R_f: 0.69 (S_3). Anal. Calcd for $C_{30}H_{36}N_2O_2$: C, 78.91; H, 7.95; N, 6.13. Found: C, 79.04; H, 7.83; N, 6.17;^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.48–1.67 (m, 3H(*piperidine*)) 1.77–1.86 (m, 1H(*piperidine*)) 1.89–1,97 (m, 1H(*piperidine*)) 1.97–2.25 (m, 3H(*piperidine*)) 2.33 -2.36 (s, 3H (*Me*)) 2.38–2.47 (m, 1H(NC*H*CO)) 2.58–2.74 (m, 2H(NCHCH_2CH_2)) 3.14–3.21 (m,1H (ArCH*CH*)) 3.42–3.49 (d, *J* = 10.77, 1H(Ar*CH*)) 3.57–3.65 (m, 1H(CH_2OH)) 3.81–3.88 (m, 1H(CH_2OH)) 4.38–4.44 (dd, *J* = 5.77, 2.44 Hz, 2H (NHCH_2)) 7.13–7.27 (m, 14H(*Ar*)) 7.71 (br s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 21.3 (CH_3) 26.7, 38.0, 44.2 (piperidine) 34.6 (CHCH_2CH_2) 43.6 (CH_2Ph) 58.3 (CH_2OH) 53.7 (CPh_2) 70.0 (*C*HCH$_2$) 126.2, 128.1, 128.2, 128.5, 128.8, 134.9, 136.4, 143.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 456,1 [M+H]$^+$.

6.1.3.11. N-benzyl-4-hydroxy-2-(4-(diphenylmethylidene)piperidin-1-yl)pentanamide (18a; 19a). Procedure GP1. Reagents and conditions: **11** (1.50 mmol, 0.52 g), benzylamine (1.80 mmol, 0.19 g), THF (8 mL), 72 h. Crude product was a mixture of diastereomers which were separated by column chromatography (S_5) and recrystallized from *n*-hexane/EtOAc 1:1; yield: 60% (**18a**); 40% (**19a**)*;* white solid; mp 136.4-139 °C (**18a**);136.8-140.5 °C (**19a**); R_f: 0.74 (**18a**); 0.70 (**19a**) (S_3). Anal. Calcd for $C_{30}H_{34}N_2O_2$: C, 79.26; H, 7.54; N, 6.16. Found: (**18a**) C, 79.28; H, 7.69; N, 6.17. Found: (**19a**) C, 79.31; H, 7.70; N, 6.24. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm (**18a**); 1.26 (s, 3H(Me)) 1.70–1.85 (m, 2H(CH_2CH_2-OH)) 2.22–2.43 (m, 4H(*piperidine*)) 2.43–2.69 (m, 4H(*piperidine*)) 3.32 (dd, *J* = 9.10, 2.44 Hz, 1H(NC*H*)) 3.73–3.83 (m, 1H(*CH*OH)) 4.48–4.59 (m, 2H(NHCH_2)) 7.06–7.15 (m, 5H(Ar)) 7.15–7.39 (m, 10H(Ar)) 7.99 (br s, 1H(CO*NH*)); (**19a**) 1.19 (d, *J* = 6.16 Hz, 3H(Me)) 1.74–1.83 (m, 2H(CH_2CH_2OH)) 2.23–2.41 (m, 4H(*piperidine*)) 2.46–2.74 (m, 4H(*piperidine*)) 3.37 (dd, *J* = 6.80, 1H(NC*H*)) 3.70–3.83 (m, 1H(*CH*OH)) 4.40–4.54 (m, 2H(NHCH_2)) 7.10–7.49 (m, 15H(Ar)) 7.73 (br s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 22.8 ($CHCH_3$) 33.5, 56.9 (piperidine) 43.8 (CHCH_2-CH) 43.6 (CH_2Ph) 62.6 (CH_2OH) 67.6 (*C*HCH$_2$) 129.3 (*C*=CPh$_2$) 137.8 (*C*Ph$_2$) 126.7, 126.9, 127.9, 128.3, 128.5, 128.6, 137.9, 142.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 455,1 [M+H]$^+$.

6.1.3.12. *N*-(2-Chlorobenzyl)-4-hydroxy-2-(4-(diphenylmethylidene)piperidin-1-yl)pentanamide (18b; 19b). Procedure GP1. Reagents and conditions: **11** (1.50 mmol, 0.52 g), 2-chlorobenzylamine (3.67 mmol, 0.52 g), THF (10 mL), 72 h. Crude product which was a mixture of diastereomers were separated by column chromatography (S_5) and recrystallized from *n*-hexane/EtOAc 1:1; yield: 50% (**18b**); 30% (**19b**)*;* white solid; mp 190.1–192.6 °C (**18b**);198.2–207.3 °C (**19b**); R_f: 0.74 (**18b**); 0.72 (**19b**) (S_3). Anal. Calcd for $C_{30}H_{33}ClN_2O_2$: C, 73.68; H, 6.80: N, 5.73. Found: (**18b**) C, 73.74; H, 7.99; N, 5.81. Found: (**19b**) C, 73.61; H, 6.70; N, 5.64. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm(**18b**) 1.25 (d, *J* = 6.16 Hz, 3H(Me)) 1.66–1.85 (m, 2H(CH_2CH_2OH)) 2.20–2.43 (m, 4H(*piperidine*)) 2.43–2.66 (m, 4H(*piperidine*)) 3.29 (dd, *J* = 9.36, 2.18 Hz, 1H(NC*H*)) 3.71–3.82 (m, 1H(*CH*OH)) 4.43–4.64 (m, 2H(NHCH_2)) 6.76–7.16 (m, 4H(Ar)) 7.16–7.32 (m, 8H(Ar)) 7.37 (d, *J* = 5.64 Hz, 2H(Ar)) 8.21 (s, 1H(CO*NH*)); (**19b**) 1.17 (d, *J* = 6.16 Hz, 3H(Me)) 1.71–1.80 (m, 2H(CH_2CH_2OH)) 2.26–2.44 (m, 4H(*piperidine*)) 2.46–2.66 (m, 4H(*piperidine*)) 3.29–3.36 (m, 1H(NC*H*)) 4.03 (dd, *J* = 12.18, 5.00 Hz, 1H(*CH*OH)) 4.46–4.61 (m, 2H(NHCH_2)) 6.99–7.14 (m, 4H(Ar)) 7.14–7.47 (m, 10H(Ar)) 7.61 (s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 22.8 ($CHCH_3$) 33.5, 56.9 (piperidine) 43.8 (CHCH_2CH) 38.5 (CH_2Ph) 62.6 (CH_2OH) 67.6 (*C*HCH$_2$) 129.3 (*C*=CPh$_2$) 137.8 (*C*Ph$_2$) 126.6, 127.9, 128.1, 128.3, 128.6, 132.2, 142.0. 142.4 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 489,5 [M+H]$^+$.

6.1.3.13. *N*-(4-Chlorobenzyl)-4-hydroxy-2-(4-(diphenylmethylidene)piperidin-1-yl)pentanamide (18c). Procedure GP1. Reagents and conditions: **11** (1.50 mmol, 0.52 g), 4-chlorobenzylamine (3.32 mmol, 0.47 g), THF (10 mL), 72 h. Crude product which was a mixture of diastereomers were separated by column chromatography (S_5) and recrystallized from *n*-hexane/EtOAc 1:1; yield: 47%*;* white solid; mp 150.1–156.6 °C; R_f: 0.72 (*cis*); (S_3). Anal. Calcd for $C_{30}H_{33}ClN_2O_2$: C, 73.68; H, 6.80; N, 5.73. Found: C, 73.64; H, 6.83; N, 5.77. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm: 1.26 (d, *J* = 6.16 Hz, 3H(Me)) 1.71–1.82 (m, 2H(CH_2CH_2OH)) 2.28–2.42 (m, 4H(*piperidine*)) 2.46–2.63 (m, 4H(*piperidine*)) 3.31 (d, *J* = 11.54 Hz, 1H(NC*H*)) 3.72–3.85 (m, 1H(*CH*OH)) 4.35–4.46 (m, 1H(NHCH_2)) 4.46–4.54 (m, 1H(NHCH_2)) 7.07 (m, 3H(Ar)) 7.20 (m, 2H(Ar)) 7.14 (m, 3H(Ar)) 7.28 (m, 6H(Ar)) 8.00 (br s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 22.8 ($CHCH_3$) 33.5, 56.9 (piperidine) 43.8 (CHCH_2CH) 43.6 (CH_2Ph) 62.6 (CH_2OH) 67.6 (*C*HCH$_2$) 129.3 (*C*=CPh$_2$) 137.8 (*C*Ph$_2$) 127.9, 128.3, 128.6, 132.3, 134.6, 136.0, 142.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 489,5 [M+H]$^+$.

6.1.3.14. *N*-(4-Fluorobenzyl)-4-hydroxy-2-(4-(diphenylmethylidene)piperidin-1-yl)pentanamide (18d; 19d). Procedure GP1. Reagents and conditions: **11** (1.50 mmol, 0.52 g),4-fluorobenzylamine (1.80 mmol, 0.22 g), THF (10 mL), 72 h. Crude product which was a mixture of diastereomers were separated by column chromatography (S_5) and recrystallized from *n*-hexane/EtOAc 1:1; yield: 60% (**18d**); 44% (**19d**)*;* white solid; mp 152.2–162.4 °C (**18d**);153.4–163.6 °C (**19d**); R_f: 0.77 (**18d**); 0.76 (**19d**) (S_3). Anal. Calcd for $C_{30}H_{33}FN_2O_2$: C, 76.24; H, 7.04; N, 5.93. Found: (**18d**) C, 76.28; H, 7.19; N, 5.97. Found: (**19d**) C, 76.31; H, 7.10; N, 5.84. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm (**18d**) 1.28 (s, 3H(Me)) 1.68–1.89 (m, 2H(CH_2CH_2OH)) 2.06–2.42 (m, 4H(*piperidine*)) 2.42–2.76 (m, 4H(*piperidine*)) 3.31 (dd, *J* = 9.23, 2.31 Hz,1H(NC*H*)) 3.72–3.84 (m, 1H(*CH*OH)), 4.31–4.42 (m, 1H(NHCH_2)) 4.45–4.57 (m, 1H(NHCH_2)) 6.80–7.15 (m, 5H(Ar)) 7.15–7.39 (m, 9H(Ar)) 7.98 (br s, 1H(CO*NH*)); (**19d**)1.19 (d, *J* = 6.16, 3H(Me)) 1.72–1.83 (m, 2H(CH_2CH_2OH)) 2.34 (q, *J* = 5.13 Hz, 4H(*piperidine*)) 2.57 (t, *J* = 5.51 Hz, 4H(*piperidine*)) 3.32–3.39 (m, 1H(NC*H*)) 3.97–4.12 (m, 1H(*CH*OH)), 4.32–4.54 (m, 2H(NHCH_2)) 6.98–7.15 (m, 5H(Ar)) 7.15–7.32 (m, 9H(Ar)) 7.40 (br s, 1H(*CH*OH)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 22.8 ($CHCH_3$) 33.5, 56.9 (piperidine) 43.8 (CHCH_2CH) 43.6 (CH_2Ph) 62.6 (CH_2OH) 67.6 (*C*HCH$_2$) 129.3 (*C*=CPh$_2$) 137.8 (*C*Ph$_2$) 115.3, 127.9, 128.3, 128.5, 128.6, 133.5, 142.0, 160.9 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 473,1 [M+H]$^+$.

6.1.3.15. *N*-(4-Methylbenzyl)-4-hydroxy-2-(4-(diphenylmethylidene)piperidin-1-yl)pentanamide (18e; 19e). Procedure GP1. Reagents and conditions: **11** (1.01 mmol, 0.35 g), 4-methylobenzylamine (2.31 mmol, 0.29 g), THF (10 mL), 48 h. Crude product which was a mixture of diastereomers were separated by column chromatography (S_5) and recrystallized from *n*-hexane/EtOAc 1:1; yield: 64% (**18e**); 31% (**19e**)*;* white solid; mp 165.8–167.7 °C (**18e**);151.3–154.5 °C (**19e**); R_f: 0.73 (**18e**); 0.71 (**19e**) (S_3). Anal. Calcd for $C_{31}H_{36}N_2O_2$: C, 79.45; H, 7.74; N, 5.98. Found: (**18e**) C, 79.58; H, 7.79; N, 6.07. Found: (**19e**) C, 79.51; H, 7.70; N, 5.94. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm (**18e**) 1.26 (d, *J* = 6.16 Hz, 3H(Me)) 1.69–1.86 (m, 2H(CH_2CH_2OH)) 2.21–2.31 (m, 2H(*piperidine*)) 2.33 (s, 3H(Ar*Me*)) 2.34–2.45 (m, 2H(*piperidine*)) 2.45–2.68 (m, 4H(*piperidine*)) 3.30 (dd, *J* = 8.98, 2.56 Hz, 1H(NC*H*)) 3.73–3.83 (m, 1H(*CH*OH)) 4.34–4.43 (m, 1H(NHCH_2)) 4.43–4.55 (m, 1H(NHCH_2)) 6.98–7.36 (m, 14H(Ar)) 7.93 (br s, 1H(*CH*OH)); (**19e**) 1.18 (d, *J* = 6.41 Hz, 3H(Me)) 1.75–1.82 (m, 2H(CH_2CH_2OH)) 2.33 (s, 3H(Ar*Me*)) 2.6 (m, *J* = 7.44 Hz, 4H(*piperidine*)) 2.63 (m, *J* = 5.00, 4H(*piperidine*)) 3.41 (dd, *J* = 9.49 Hz, 1H(NC*H*)) 3.99–4.07 (m, 1H(*CH*OH)) 4.35–4.48 (m, 2H(NHCH_2)) 7.05–7.31 (m, 14H(Ar)) 7.43 (br s, 1H(*CH*OH)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 21.3 (CH_3) 22.8 ($CHCH_3$) 33.5,

56.9 (piperidine) 43.8 (CHCH$_2$CH) 43.6 (*C*H$_2$Ph) 62.6 (*C*H$_2$OH) 67.6 (*C*HCH$_2$) 129.3 (*C*=CPh$_2$) 137.8 (*C*Ph$_2$) 127.9, 128.3, 128.6, 128.8, 134.9, 136.4, 142.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 469,1 [M+H]$^+$.

6.1.3.16. *N*-Benzyl-4-hydroxy-2-(*N*-methyl-4,4-diphenylbut-3-en-1-amino)butanamide (20a). Procedure GP1. Reagents and conditions: **13** (2.12 mmol, 0.68 g), benzylamine (2.54 mmol, 0.27 g), THF (7 mL), 48 h; purification by column chromatography (S_3); yield: 63%; yellow oil; R_f: 0.77 (S_3). Anal. Calcd for $C_{28}H_{32}N_2O_2$: C, 78.47; H, 7.53; N, 6.54. Found: C, 78.54; H, 7.43; N, 6.67. ^{1}H NMR ($CDCl_3$) δ [ppm]: 1.82–1.85 (m, 2H (*CH*$_2$CH$_2$N) 2.21 (s, 3H (Me)) 2.54–2.58 (m, 2H (*CH*$_2$CH$_2$OH)) 3.20–3.29 (m, 1H (*CH*$_2$N)) 3.50–3.56 (m, 1H (*CH*$_2$N)) 3.79–3.81 (m, 1H (N*CH*)) 4.10–4.15 (m, 2H (*CH*$_2$OH)) 4.29–4.32 (m, 2H (NH*CH*$_2$)) 6.03 (t, 1H (C=*CH*)) 7.14–7.37 (m, 15H (Ar)) 7.82 (s, 1H (CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 24.2 (CH*C*H$_2$CH$_2$) 34.3 (CH*C*H$_2$CH) 41.7 (*C*H$_3$) 43.6 (*C*H$_2$Ph) 57.3 (CH$_2$*C*H$_2$N) 58.3 (*C*H$_2$OH) 72.6 (*C*HCH$_2$) 115.3 (*C*=CPh$_2$) 133.8 (*C*Ph$_2$) 126.7, 126.9, 127.9, 128.5, 128.6, 137.9, 140.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 429,3 [M+H]$^+$.

6.1.3.17. *N*-(2-Chlorobenzyl)-4-hydroxy-2-(*N*-methyl-4,4-diphenylbut-3-en-1-amino)butanamide (20b). Procedure GP1. Reagents and conditions: **13** (1.56 mmol, 0.50 g), 2-chlorobenzylamine (1.88 mmol, 0.27 g), THF (5 mL), 48 h; purification by column chromatography (S_3); yield: 60%; yellow oil; R_f: 0.79 (S_3). Anal. Calcd for $C_{28}H_{31}ClN_2O_2$: C, 72.63; H, 6.75; N, 6.05. Found: C, 72.64; H, 6.73; N, 6.07. ^{1}H NMR ($CDCl_3$) δ [ppm]: 1.81 (m, 2H (*CH*$_2$CH$_2$N)) 2.15 (s, 3H (Me)) 2.52 (m, 2H (*CH*$_2$CH$_2$OH)) 3.23 (m, 1H (*CH*$_2$N)) 3.53 (m, 1H (*CH*$_2$N)) 3.81 (m, 1H (N*CH*)) 4.15 (q, 2H (*CH*$_2$OH)) 4.29 (d, 2H (NH*CH*$_2$)) 6.01 (t, 1H (C=*CH*)) 7.04–7.41 (m, 14H (Ar)) 7.71 (s, 1H (CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 24.2 (CH*C*H$_2$CH$_2$) 34.3 (CH*C*H$_2$CH) 41.7 (*C*H$_3$) 38.5 (*C*H$_2$Ph) 57.3 (CH$_2$*C*H$_2$N) 58.3 (*C*H$_2$OH) 72.6 (*C*HCH$_2$) 115.3 (*C*=CPh$_2$) 133.8 (*C*Ph$_2$) 126.6, 127.9, 128.1, 128.3, 128.6, 140.0, 142.4 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 463,5 [M+H]$^+$.

6.1.3.18. *N*-(4-Chlorobenzyl)-4-hydroxy-2-(*N*-methyl-4,4-diphenylbut-3-en-1-amino)butanamide (20c). Procedure GP1. Reagents and conditions: **13** (1.56 mmol, 0.50 g), 4-chlorobenzylamine (1.88 mmol, 0.27 g), THF (5 mL), 48 h; purification by column chromatography (S_3); yield: 58%; yellow oil; R_f: 0.76 (S_3). Anal. Calcd for $C_{28}H_{31}ClN_2O_2$: C, 72.63; H, 6.75; N, 6.05. Found: C, 72.69; H, 6.77; N, 6.11. ^{1}H NMR ($CDCl_3$) δ [ppm]: 1.83 (m, 2H (*CH*$_2$CH$_2$N)) 2.11 (s, 3H (Me)) 2.53 (m, 2H (*CH*$_2$CH$_2$OH)) 3.21 (m, 1H (*CH*$_2$N)) 3.53 (m, 1H (*CH*$_2$N)) 3.82 (m, 1H (N*CH*)) 4.13 (q, 2H (*CH*$_2$OH)) 4.31 (d, 2H (NH*CH*$_2$)) 6.03 (t, 1H (C=*CH*)) 6.97–6.60 (m, 14H (Ar)) 7.84–7.87 (s, 1H (CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 24.2 (CH*C*H$_2$CH$_2$) 34.3 (CH*C*H$_2$CH) 41.7 (*C*H$_3$) 43.6 (*C*H$_2$Ph) 57.3 (CH$_2$*C*H$_2$N) 58.3 (*C*H$_2$OH) 72.6 (*C*HCH$_2$) 115.3 (*C*=CPh$_2$) 133.8 (*C*Ph$_2$) 126.6, 127.9, 128.5, 128.6,132.3, 134.6, 136.0, 140.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 463,5 [M+H]$^+$.

6.1.3.19. *N*-(4-Fluorobenzyl)-4-hydroxy-2-(*N*-methyl-4,4-diphenylbut-3-en-1-amino)butanamide (20d). Procedure GP1. Reagents and conditions: **13** (1.68 mmol, 0.54 g), 4-fluorobenzylamine (2.02 mmol, 0.25 g), THF (5 mL), 48 h; purification by column chromatography (S_3); yield: 66%; yellow oil; R_f: 0.76 (S_3). Anal. Calcd for $C_{28}H_{31}FN_2O_2$: C, 75.31; H, 7.00; N, 6.27. Found: C, 75.34; H, 7.03; N, 6.27. ^{1}H NMR ($CDCl_3$) δ [ppm]: 1.84-1.87 (m, 2H (*CH*$_2$CH$_2$N)) 2.25 (s, 3H (Me)) 2.53-2.56 (m, 2H (*CH*$_2$CH$_2$OH)) 3.22-3.27 (m, 1H (*CH*$_2$N)) 3.53- 3.58 (m, 1H (*CH*$_2$N)) 3.82- 3.89 (m, 1H (N*CH*)) 4.11–4.15 (q, 2H (*CH*$_2$OH)) 4.30-4.34 (d, 2H (NH*CH*$_2$)) 6.03-6.07 (t, 1H (C=*CH*)) 7.04–7.37 (m, 14H (Ar)) 7.83 (s, 1H (CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 24.2 (CH*C*H$_2$CH$_2$) 34.3 (CH*C*H$_2$CH) 41.7 (*C*H$_3$) 43.6 (*C*H$_2$Ph) 57.3 (CH$_2$-*C*H$_2$N) 58.3 (*C*H$_2$OH) 72.6 (*C*HCH$_2$) 115.3 (*C*=CPh$_2$) 133.8 (*C*Ph$_2$) 115.3, 127.9, 128.3, 128.5, 128.6, 133.5, 140.0, 160.9 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 445,3 [M+H]$^+$.

6.1.3.20. N-(4-methylbenzyl)-4-hydroxy-2-(N-methyl-4,4-diphenylbut-3-en-1-amino)butanamide (20e). Procedure GP1. Reagents and conditions: **13** (1.56 mmol, 0.50 g), 4-methylbenzylamine (1.88 mmol, 0.23 g), THF (5 mL), 48 h; purification by column chromatography (S_3); yield: 74%; yellow oil; R_f: 0.78 (S_3). Anal. Calcd for $C_{29}H_{34}N_2O_2$: C, 78.70; H, 7.74; N, 6.33. Found: C, 78.72; H, 7.78; N, 6.47. ^{1}H NMR ($CDCl_3$) δ [ppm]: 1.36 (s, 3H (Me)) 1.81–1.84 (m, 2H (*CH*$_2$CH$_2$N)) 2.11 (s, 3H (Me)) 2.53–2.57 (m, 2H (*CH*$_2$CH$_2$OH)) 3.23–3.28 (m, 1H (*CH*$_2$N)) 3.54–3.58 (m, 1H (*CH*$_2$N)) 3.82–3.87 (m, 1H (N*CH*)) 4.13–4.19 (q, 2H (*CH*$_2$OH)) 4.31–4.35 (d, 2H (NH*CH*$_2$)) 6.03–6.09(t, 1H (C=*CH*)) 6.97–6.60 (m,14H (Ar)), 7.84-7.87 (s, 1H (CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 21.3 (CH$_3$) 24.2 (CH*C*H$_2$CH$_2$) 34.3 (CH*C*H$_2$CH) 41.7 (*C*H$_3$) 43.6 (*C*H$_2$Ph) 57.3 (CH$_2$*C*H$_2$N) 58.3 (*C*H$_2$OH) 72.6 (*C*HCH$_2$) 115.3 (*C*=CPh$_2$) 133.8 (*C*Ph$_2$) 127.9, 128.1, 128.3, 128.6, 128.8, 134.9, 136.4, 140.0 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 443,1 [M+H]$^+$.

6.1.3.21. *N*-Benzyl-4-hydroxy-2-(*N*-methyl-4,4-diphenylbutylamino)butanamide (21a). Procedure GP1. Reagents and conditions: **14** (1.5 mmol, 0.50 g), benzylamine (3.75 mmol, 0.40 g), THF (10 mL), 72 h; purification by column chromatography (S_7); yield: 78%; yellow oil; R_f: 0.59 (S_3). Anal. Calcd for $C_{28}H_{33}N_2O_2$: C, 78.10; H, 7.96; N, 6.51. Found: C, 78.12; H, 7.98; N, 6.57. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.32–1.44 (m, 2H(*CH*$_2$CH$_2$N)) 1.81–1.89 (m, 2H(*CH*$_2$CH$_2$OH)) 1.90–2.01 (m, 2H(CH*CH*$_2$)) 2.13 (s, 3H(Me)) 2.39 (t, *J* = 7.18 Hz, 2H(*CH*$_2$N)) 3.18–3.25 (m, 1H(N*CH*)) 3.52–3.61 (m, 1H(*CH*CH$_2$)) 3.78–3.88 (m, 2H(*CH*$_2$OH)) 4.39–4.49 (m, 2H(NH*CH*$_2$)) 7.13–7.35 (m, 15H(Ar)) 7.76 (br s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 24.6 (CH*C*H$_2$CH$_2$) 34.3 (CH*C*H$_2$CH) 36.3 (*C*H$_2$CHPh$_2$) 41.6 (*C*H$_3$) 43.6 (*C*H$_2$Ph) 50.0 (*CH*Ph$_2$) 56.5 (CH$_2$*C*H$_2$N) 58.3 (*C*H$_2$OH) 72.5 (*C*HCH$_2$) 126.2, 126.7, 126.9, 128.2, 128.5, 128.7, 137.9, 145.1 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 430,1 [M+H]$^+$.

6.1.3.22. *N*-(2-Chlorobenzyl)-4-hydroxy-2-(*N*-methyl-4,4-diphenylbutylamino)butanamide (21b). Procedure GP1. Reagents and conditions: **14** (1 mmol, 0.33 g), 2-chlorobenzylamine (2.50 mmol, 0.35 g), THF (10 mL), 72 h; purification by column chromatography (S_7); yield: 75%; yellow oil; R_f: 0.63 (S_3). Anal. Calcd for $C_{28}H_{33}ClN_2O_2$: C, 72.32; H, 7.15; N, 6.02. Found: C, 72.42; H, 7.18; N, 6.07. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.35–1.44 (m, 2H(*CH*$_2$CH$_2$N)) 1.78–1.86 (m, 2H(*CH*$_2$CH$_2$OH)) 1.95–2.04 (m, 2H(CH*CH*$_2$)) 2.11 (s, 3H(Me)) 2.39 (t, *J* = 7.18 Hz, 2H(*CH*$_2$N) 3.16–3.21 (m, 1H(N*CH*)) 3.50–3.59 (m, 1H(*CH*CH$_2$)) 3.79–3.87 (m, 2H(*CH*$_2$OH)) 4.52 (dd, *J* = 6.16, 2.82 Hz, 2H(NH*CH*$_2$)) 7.08–7.41 (m, 14H(Ar)) 7.91 (br s, 1H(CO*NH*)), ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 24.6 (CH*C*H$_2$CH$_2$) 34.3 (CH*C*H$_2$CH) 36.3 (*C*H$_2$CHPh$_2$) 41.6 (*C*H$_3$) 43.6 (*C*H$_2$Ph) 50.0 (*CH*Ph$_2$) 56.5 (CH$_2$-*C*H$_2$N) 58.3 (*C*H$_2$OH) 72.5 (*C*HCH$_2$) 126.2, 126.6, 128.1, 128.2, 128.6, 129.2, 132.2, 142.4, 145.1 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 465,5 [M+H]$^+$.

6.1.3.23. *N*-(4-Chlorobenzyl)-4-hydroxy-2-(*N*-methyl-4,4-diphenylbutylamino)butanamide (21c). Procedure GP1. Reagents and conditions: **14** (1 mmol, 0.33 g), 4-chlorobenzylamine (2.50 mmol, 0.35 g), THF (10 mL), 72 h; purification by column chromatography (S_7); yield: 80%; yellow oil; R_f: 0.66 (S_3). Anal. Calcd for $C_{28}H_{33}ClN_2O_2$: C, 72.32; H, 7.15; N, 6.02. Found: C, 72.39; H, 7.14; N, 6.11. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.32–1.46 (m, 2H(*CH*$_2$CH$_2$N)) 1.79–1.88 (m, 2H(*CH*$_2$CH$_2$OH))

1.92–2.04 (m, 2H(CHCH_2)) 2.13 (s, 3H(Me)) 2.39 (t, J = 7.31 Hz, 2H(CH_2N)) 3.17–3.23 (m, 1H(NCH)) 3.52–3.61 (m, 1H($CH$$CH_2$)) 3.79–3.87 (m, 2H($CH_2$OH)) 4.31–4.43 (m, 2H(NH$CH_2$)) 7.09–7.35 (m, 14H(Ar)) 7.78 (br s, 1H(CONH)), ^{13}C NMR (300 MHz, chloroform-d) δ ppm 24.6 (CHCH_2CH_2) 34.3 (CHCH_2CH) 36.3 (CH_2CHPh$_2$) 41.6 (CH_3) 43.6 (CH_2Ph) 50.0 (CHPh$_2$) 56.5 (CH_2CH_2N) 58.3 (CH_2OH) 72.5 ($CH$$CH_2$) 126.2, 128.2, 128.6, 129.2, 132.3, 134.6, 136.0, 145.1 (arom) 171.3 (carbonyl); ESI-MS (m/z) 465,5 [M+H]$^+$.

6.1.3.24. *N*-(4-Fluorobenzyl)-4-hydroxy-2-(*N*-methyl-4,4-diphenylbutylamino)butanamide (21d). Procedure GP1. Reagents and conditions: **14** (2,3 mmol, 0.77 g), 4-fluorobenzylamine (5,75 mmol, 0.72 g), THF (10 mL), 72 h; purification by column chromatography (S_7); yield: 71%; yellow oil; R_f: 0.63 (S_3). Anal. Calcd for $C_{28}H_{33}FN_2O_2$: C, 74.97; H, 7.42; N, 6.25. Found: C, 74.92; H, 7.48; N, 6.27; ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.32–1.46 (m, 2H(CH_2CH_2N)) 1.78–1.88 (m, 2H(CH_2CH_2OH)) 1.91–2.01 (m, 2H(CHCH_2)) 2.12 (s, 3H(Me)) 2.39 (t, J = 7.18 Hz, 2H(CH_2N)) 3.16–3.23 (m, 1H(NCH)) 3.51–3.60 (m, 1H($CH$$CH_2$)) 3.78–3.87 (m, 2H($CH_2$OH)) 4.37 (dd, J = 6.16, 3.59 Hz, 2H(NHCH_2)) 6.95–7.02 (m, 2H(Ar)) 7.14–7.29 (m, 12H(Ar)) 7.77 (br s, 1H(CONH)), ^{13}C NMR (300 MHz, chloroform-d) δ ppm 24.6 (CHCH_2CH_2) 34.3 (CHCH_2CH) 36.3 (CH_2CHPh$_2$) 41.6 (CH_3) 43.6 (CH_2Ph) 50.0 (CHPh$_2$) 56.5 (CH_2CH_2N) 58.3 (CH_2OH) 72.5 ($CH$$CH_2$) 115.3, 126.2, 128.2, 128.5, 129.2, 133.5, 145.1 (arom) 171.3 (carbonyl); ESI-MS (m/z) 449,1 [M+H]$^+$.

6.1.3.25. *N*-(4-Methylbenzyl)-4-hydroxy-2-(*N*-methyl-4,4-diphenylbutylamino)butanamide (21e). Procedure GP1. Reagents and conditions: **14** (1 mmol, 0.33 g), 4-methylbenzylamine (2.50 mmol, 0.30 g), THF (10 mL), 72 h; purification by column chromatography (S_7); yield: 86%; yellow oil; R_f: 0.67 (S_3). Anal. Calcd for $C_{29}H_{38}N_2O_2$: C, 77.99; H, 8.58; N, 6.27. Found: C, 77.96; H, 8.58; N, 6.26; ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.31–1.44 (m, 2H(CH_2CH_2N)) 1.81–1.89 (m, 2H(CH_2CH_2OH)) 1.90–2.02 (m, 2H(CHCH_2)) 2.12 (s, 3H(NMe)) 2.33 (s, 3H(ArMe)) 2.38 (t, J = 7.18 Hz, 2H(CH_2N)) 3.16–3.23 (m, 1H(NCH)) 3.52–3.61 (m, 1H($CH$$CH_2$)) 3.77–3.89 (m, 2H($CH_2$OH)) 4.33–4.44 (m, 2H(NH$CH_2$)) 7.06–7.38 (m, 14H(Ar)) 7.72 (br s, 1H (CONH)), ^{13}C NMR (300 MHz, chloroform-d) δ ppm 21.3 (CH_3) 24.6 (CHCH_2CH_2) 34.3 (CHCH_2CH) 36.3 (CH_2CHPh$_2$) 41.6 (CH_3) 43.6 (CH_2Ph) 50.0 (CHPh$_2$) 56.5 (CH_2CH_2N) 58.3 (CH_2OH) 72.5 ($CH$$CH_2$) 126.2, 128.1, 128.2, 128.8, 129.2, 134.9, 136.4, 145.1 (arom) 171.3 (carbonyl); ESI-MS (m/z) 442,2 [M+H]$^+$.

6.1.3.26. *N*-Benzyl-4-hydroxy-2-(4,4-diphenylbutylamino) butanamide (22a). Procedure GP1. Reagents and conditions: **15** (0.97 mmol, 0.30 g), benzylamine (2.42 mmol, 0.26 g), THF (5 mL), 72 h; purification by column chromatography (S_5); recrystallization from *n*-hexane/EtOAc 1:1; yield: 54%; white solid; mp 97.3 °C; R_f: 0.36 (S_3). Anal. Calcd for $C_{27}H_{32}N_2O_2$: C, 77.85; H, 7.74; N, 6.73. Found: C, 77.92; H, 7.79; N, 6.78. ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.37–1.47 (m, 2H(CH_2CH_2NH)) 1.77–1.84 (m, 2H(CH_2CH_2OH)) 2.51–2.63 (m, 2H(CHCH_2)) 3.18–3.30 (m, 2H(CH_2NH)) 3.69–3.75 (m, 1H(NHCH)) 3.77–3.94 (m, 2H(CH_2OH)) 4.12 (q, J = 7.18 Hz, 1H($CH$$CH_2$)) 4.36–4.51 (m, 2H(NH$CH_2$)) 7.19–7.33 (m, 15H($Ar$)) 7.39–7.49 (m, 1H(CO$NH$)), ^{13}C NMR (300 MHz, chloroform-d) δ ppm 27.1 (CHCH_2CH_2) 36.8 (CHCH_2CH) 36.0 (CH_2CHPh$_2$) 43.6 (CH_2Ph) 50.0 (CHPh$_2$) 48.2 (CH_2CH_2N) 58.0 (CH_2OH) 64.2 ($CH$$CH_2$) 126.2, 126.9, 126.7, 128.2, 128.5, 129.2, 137.9, 145.1 (arom) 171.3 (carbonyl); ESI-MS (m/z) 417,1 [M+H]$^+$.

6.1.3.27. *N*-(2-Chlorobenzyl)-4-hydroxy-2-(4,4-diphenylbutylamino)butanamide (22b). Procedure GP1. Reagents and conditions: **15** (0.97 mmol, 0.30 g), 2-chlorobenzylamine (2.42 mmol, 0.34 g), THF (5 mL), 72 h; purification by column chromatography (S_5); recrystallization from *n*-hexane/EtOAc 1:1; yield: 60%; white solid; mp 93.5 °C; R_f: 0.47 (S_3). Anal. Calcd for $C_{27}H_{31}ClN_2O_2$: C, 71.90; H, 6.93; N, 6.21. Found: C, 71.98; H, 6.98; N, 6.27. ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.36–1.47 (m, 2H(CH_2CH_2NH)) 1.75–1.85 (m, 2H(CH_2CH_2OH)) 1.95–2.06 (m, 2H(CHCH_2)) 2.46–2.60 (m, 2H(CH_2NH)) 3.19 (t, J = 6.80 Hz, 1H(NHCH)) 3.68–3.78 (m, 2H(CH_2OH)) 3.83 (t, J = 7.82 Hz, 1H($CH$$CH_2$)) 4.47–4.58 (m, 2H(NH$CH_2$)) 7.13–7.38 (m, 14H($Ar$)) 7.54 (d, J = 11.54 Hz, 1H(CONH)), ^{13}C NMR (300 MHz, chloroform-d) δ ppm 27.1 (CHCH_2CH_2) 36.8 (CHCH_2CH) 36.0 (CH_2CHPh$_2$) 38.6 (CH_2Ph) 50.0 (CHPh$_2$) 48.2 (CH_2CH_2N) 58.0 (CH_2OH) 64.2 ($CH$$CH_2$) 126.2, 126.6, 128.1, 128.2, 128.6, 129.2, 142.4, 145.1 (arom) 171.3 (carbonyl); ESI-MS (m/z) 451,5 [M+H]$^+$.

6.1.3.28. *N*-(4-Chlorobenzyl)-4-hydroxy-2-(4,4-diphenylbutylamino)butanamide (22c). Procedure GP1. Reagents and conditions: **15** (1.5 mmol, 0.46 g), 4-chlorobenzylamine (3.75 mmol, 0.53 g), THF (10 mL), 72 h; purification by column chromatography (S_5); recrystallization from *n*-hexane/EtOAc 1:1; yield: 57%; white solid; mp 105.9 °C; R_f: 0.38 (S_3). Anal. Calcd for $C_{27}H_{31}ClN_2O_2$: C, 71.90; H, 6.93; N, 6.21. Found: C, 71.87; H, 7.02; N, 6.17. ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.35–1.45 (m, 2H(CH_2CH_2NH)) 1.78–1.86 (m, 2H(CH_2CH_2OH)) 1.97–2.08 (m, 2H(CHCH_2)) 2.48–2.62 (m, 2H(CH_2NH)) 3.20 (t, J = 6.80 Hz, 1H(NHCH)) 3.71–3.79 (m, 2H(CH_2OH)) 3.80–3.86 (m, 1H($CH$$CH_2$)) 4.39 (t, J = 5.51 Hz, 2H(NHCH_2)) 7.14–7.31 (m, 14H(Ar)) 7.46 (br s, 1H(CONH)), ^{13}C NMR (300 MHz, chloroform-d) δ ppm 27.1 (CHCH_2CH_2) 36.8 (CHCH_2CH) 36.0 (CH_2CHPh$_2$) 43.6 (CH_2Ph) 50.0 (CHPh$_2$) 48.2 (CH_2CH_2N) 58.0 (CH_2OH) 64.2 ($CH$$CH_2$) 126.2, 128.2, 128.6, 129.2, 132.3, 136.0, 145.1 (arom) 171.3 (carbonyl); ESI-MS (m/z) 451,5 [M+H]$^+$.

6.1.3.29. *N*-(4-Fluorobenzyl)-4-hydroxy-2-(4,4-diphenylbutylamino)butanamide (22d). Procedure GP1. Reagents and conditions: **15** (1.5 mmol, 0.46 g), 4-fluorobenzylamine (3.75 mmol, 0.50 g), THF (10 mL), 72 h; purification by column chromatography (S_5); recrystallization from *n*-hexane/EtOAc 1:1; yield: 65%; white solid; mp 87.0 °C; R_f: 0.37 (S_3). Anal. Calcd for $C_{27}H_{31}FN_2O_2$: C, 74.63; H, 7.19; N, 6.45. Found: C, 74.67; H, 7.23; N, 6.58; ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.34–1.44 (m, 2H(CH_2CH_2NH)) 1.77–1.85 (m, 2H(CH_2CH_2OH)) 1.98–2.08 (m, 2H(CHCH_2)) 2.47–2.61 (m, 2H(CH_2NH)) 3.20 (t, J = 6.80 Hz, 1H(NHCH)) 3.70–3.78 (m, 2H(CH_2OH)) 3.83 (t, J = 7.82 Hz, 1H($CH$$CH_2$)) 4.39 (dd, J = 5.90, 3.33 Hz, 2H(NHCH_2)) 6.92–7.04 (m, 2H(Ar)) 7.09–7.35 (m, 12H(Ar)) 7.43 (br s, 1H(CONH)), ^{13}C NMR (300 MHz, chloroform-d) δ ppm 27.1 (CHCH_2CH_2) 36.8 (CHCH_2CH) 36.0 (CH_2CHPh$_2$) 43.6 (CH_2Ph) 50.0 (CHPh$_2$) 48.2 (CH_2CH_2N) 58.0 (CH_2OH) 64.2 ($CH$$CH_2$) 115.3, 126.2, 128.2, 129.2, 133.5, 145.1 (arom) 171.3 (carbonyl); ESI-MS (m/z) 435,1 [M+H]$^+$.

6.1.3.30. *N*-(4-Methylbenzyl)-4-hydroxy-2-(4,4-diphenylbutylamino)butanamide (22e). Procedure GP1. Reagents and conditions: **15** (0.5 mmol, 0.15 g), 4-methylbenzylamine (1.25 mmol, 0.15 g), THF (5 mL), 72 h; purification by column chromatography (S_5); recrystallization from *n*-hexane/EtOAc 1:1; yield: 65%; white solid; mp 107.3 °C; R_f: 0.53 (S_3). Anal. Calcd for $C_{28}H_{34}N_2O_2$: C, 78.10; H, 7.96; N, 6.51. Found: C, 78.18; H, 6.99; N, 6.57. ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.36–1.43 (m, 2H(CH_2CH_2NH)) 1.82 (td, J = 6.92, 4.87 Hz, 2H(CH_2CH_2OH)) 1.97–2.06 (m, 2H(CHCH_2)) 2.33 (s, 3H(Me)) 2.50–2.59 (m, 2H(CH_2NH)) 3.20 (t, J = 6.80 Hz, 1H(NHCH)) 3.72–3.79 (m, 2H(CH_2OH)) 3.79–3.86 (m, 1H($CH$$CH_2$)) 4.36–4.43 (m, 2H(NH$CH_2$)) 7.12–7.21 (m, 10H($Ar$)) 7.23–7.28 (m, 4H($Ar$)) 7.29 (t, J = 1.41 Hz, 1H(CONH)), ^{13}C NMR (300 MHz, chloroform-d) δ ppm 21.3 (CH_3) 27.1 (CHCH_2CH_2) 36.8 (CHCH_2CH) 36.0 (CH_2CHPh$_2$) 43.6 (CH_2Ph) 50.0 (CHPh$_2$) 48.2 (CH_2CH_2N) 58.0 (CH_2OH) 64.2 ($CH$$CH_2$) 126.2, 126.7, 128.1, 128.5, 128.8,

129.2, 134.9136.4, 145.1 (arom) 171.3 (carbonyl); ESI-MS (*m*/*z*) 431,1 $[M+H]^+$.

6.2. Pharmacology

6.2.1. In vitro activity

6.2.1.1. [^{3}H]GABA uptake assay. Inhibitory potency of compounds (**16a**–**e** to **22a**–**e**) were tested at four murine GABA transporter subtypes mGAT1–mGAT4. The study was performed as a [^{3}H] GABA uptake assay based on stably transfected HEK cells, according to the procedure recently described.[29] Briefly, the affinity for mGAT1 was determined by MS-binding assay with NO 711 as a non-labeled marker.[35] Binding assays for mGAT1 based on NO 711 as native marker were performed as described earlier.[29] NO 711 was analyzed by LC–MS/MS using an API 3200 triple quadrupole mass spectrometer according to the method described previously.[35]

The compounds were considered active if GABA uptake or NO 711 binding was reduced at least by 50% at a concentration of 100 μM. For the active compounds, pIC_{50} values were assessed.

6.2.2. In vivo activity

6.2.2.1. Animals. The behavioral experiments were carried out at the Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University in Kraków. Adult male Albino Swiss (CD-1) mice weighing 18–25 g were used in the experiments. The animals were kept in groups of 15 mice in cages at a room temperature of 22 ± 2 °C, under light/dark (12:12) cycle and had free access to food and water. The ambient temperature of the room and the humidity were kept consistent throughout all the tests. For the experiments the animals were selected in a random way and killed by cervical dislocation immediately after the assay. Experimental groups consisted of 6–12 animals/dose and all the animals were used only once. The number of animals was kept at minimum to obtain definite results with the test utilized. Prior to the test, the mice were allowed to acclimate to the holding cages for a minimum of 2 h. The experiments were performed between 8 a.m. and 3 p.m. All the procedures were approved by the Local Ethics Committee of the Jagiellonian University in Kraków (ZI/595/2011).

6.2.2.2. Chemicals used in pharmacological assays. For the pharmacological studies the test compounds were suspended in 0.5% methylcellulose solution (Loba Chemie, Germany) and administered intraperitoneally (ip) 30 min before the test. Control mice were given an appropriate amount of vehicle (i.e., methylcellulose solution).

Acetic acid was purchased from Polskie Odczynniki Chemiczne (Poland). It was diluted to obtain a 0.9% solution which was used in the subsequent behavioral test. Morphine hydrochloride was provided by Polfa Kutno (Poland). 37% formaldehyde was provided by Polskie Odczynniki Chemiczne (Poland). It was diluted to obtain a 5% solution which was used in the subsequent behavioral test.

6.2.2.3. Behavioral tests. The behavioral measures were scored by trained observers blind to the experimental conditions.

6.2.2.3.1. Hot plate test. In the hot plate test mice were treated ip either with the test compound or the vehicle 30 min before placing the animal on the hot plate apparatus (Hot Plate 2A Type Omega, Poland). This apparatus has an electrically heated surface and is supplied with a temperature-controller that maintains the temperature at 55–56 °C. The latency time until the animal licked its back paws or jumped was recorded by means of the stop-watch. In this assay the cut-off time was established (30 s) to avoid tissue damage and mice not responding within 30 s were removed from the apparatus and assigned a score of 30 s.[36]

6.2.2.3.2. Writhing test. In this test mice were placed individually into glass beakers and 30 min before the experimentation were allowed to habituate. Then, each mouse was weighed, injected with the test compound or vehicle (10 ml/kg) and then placed back into the cylinder. 30 min later, 0.9% acetic acid (in saline) was injected ip (10 ml/kg). Mice were placed in the beakers once again and observed continuously for 30 min. Stereotypical writhes (lengthwise constrictions of the torso with a concomitant concave arching of the back) were counted over this period.[37]

6.2.2.3.3. Formalin test. In mice the injection of diluted formalin produces a biphasic nocifensive behavioral response (i.e., licking or biting the injected paw). The acute nociceptive phase lasts for the first 5 min, whereas the second (inflammatory) phase occurs between 15 and 30 min after formalin injection. The analgesic activity is indicated by the reduction of time spent on licking or biting the injected paw in drug-treated groups compared to control animals.

The formalin test in mice was performed according to.[38] Briefly, 20 μl of a 5% formalin solution was injected intraplantarly (ip) into the right hind paw using a 26-gauge needle. Immediately after formalin injection, the animals were placed individually into glass beakers and were observed for the next 30 min. The time (in seconds) spent on licking the injected paw during periods of 0–5 min, and then 15–30 min was measured and was an indicator of nociceptive behavior.

6.2.2.3.4. Rotarod test. The test was performed according to the method described by Talarek et al.[39] with some minor modifications. The mice were trained daily for 3 days on the rotarod apparatus (Rotarod apparatus, May Commat RR0711, Turkey; rod diameter: 2 cm) rotating at a constant speed of 18 rotations per minute (rpm). During each training session, the animals were placed on a rotating rod for 3 min with an unlimited number of trials. The proper experimentation was conducted at least 24 h after the final training trial. On the test day, 30 min before the rotarod test the mice were intraperitoneally pretreated with the test compound. Then the animals were tested on the rotarod revolving at 6, 18, 24 rpm. Motor impairments, defined as the inability to remain on the rod for 1 min, were measured at each speed and were expressed as the number of animals falling from the roller per the number of mice tested. The mean time to fall off the rotating rod was also counted for each dose.

6.2.2.3.5. Data analysis. The data obtained in behavioral experiments are expressed as mean ± SEM (standard error of the mean). To compare the results between three or more groups of animals (the investigated compound groups vs. the vehicle-treated group) one-way ANOVA, followed by Dunnett's post hoc statistics were used. To compare the results between only two groups (drug-treated group vs control group) Student's *t*-test was used. Qualitative variables from the rotarod test were compared by the use of the Fisher's exact probability test. In each assay the difference of means was statistically significant if $p < 0.05$.

In the writhing test and in the formalin test ED_{50} values were calculated using log-probit method.[40]

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