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Original article

Synthesis, biological evaluation and structure–activity relationship of new GABA uptake inhibitors, derivatives of 4-aminobutanamides

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ABSTRACT

Six series of 2-substituted 4-aminobutanamide derivatives were synthesized and evaluated for their ability to inhibit GABA transport proteins mGAT1–4 stably expressed in HEK-293 cell lines. The pIC_{50} values determined were in the range 4.23–5.23. Two compounds (**15b** and **15c**) were selected for further *in vitro* studies. These compounds were also subjected to preliminary behavioral studies to evaluate their anticonvulsant, antidepressant-like, and antinociceptive activities in mice. Their influence on motor coordination was also assessed. We report that, among a spectrum of *in vivo* activities, both **15b** and **15c** displayed significant activity against pentylenetetrazole (PTZ)-induced seizures.

1. Introduction

4-Aminobutyric acid (GABA) transporters (GATs) have been extensively investigated as prospective tools for studies on their key role in the dysfunction of GABAergic neurotransmission. To date, four different plasma-membrane transport proteins that mediate the uptake of synaptic GABA into neurons and glial cells have been identified and characterized. Following the nomenclature used by the Human Genome Organization (HUGO), these transporters are termed GAT1 (SLC6a1), BGT1 (SLC6a12), GAT2 (SLC6a13) and GAT3 (SLC6a11) which correspond to mouse mGAT1, mGAT2, mGAT3 and mGAT4, respectively [1–6]. Because the characterization of the test compounds was performed using mouse GABA transporters, the mouse nomenclature is used in the present paper.

The four transporter subtypes differ in their localization, affinity for GABA and pharmacological function. mGAT1 and mGAT4 are almost exclusively located in the central nervous system (CNS). Moreover, mGAT1 is primarily localized in presynaptic neurons and to a minor extent in synaptically apposed astrocytes [7,8], whereas mGAT4 is predominantly localized on distal astrocytes which are in direct contact with GABAergic neurons [9]. Thus, mGAT1 and mGAT4 can affect many brain functions including muscle relaxation, cognition, and memory [10,11]. By contrast, mGAT2 and mGAT3 are predominantly expressed in the liver and at lower levels in kidneys, whereas in the brain their significant concentrations are restricted to the leptomeninges (mGAT3 and mGAT2) and to cerebral blood vessels (mGAT3), indicating that these transporters are unlikely to play an important role in the inactivation of GABA [12,13].

Following the identification of GAT inhibitors it was demonstrated that they enhance the GABA tone, and therefore hold promise the treatment of several diseases in which GABA function is reduced, including epilepsy, migraine, neuropathic pain, Huntington's chorea, Parkinson's disease [14–18]. However, to explore both the physiological function of GATs and their individual structure, further compounds that selectively target and modulate GATs are needed.

Tiagabine (Fig. 1), a derivative of nipecotic acid, has been developed and marketed as an add-on treatment for partial epilepsy, and this drug is also in clinical trials for new indications

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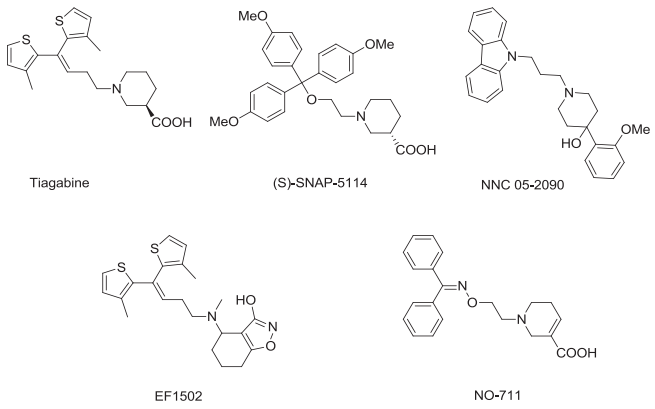


Fig. 1. Structure of lipophilic GABA uptake inhibitors of mGAT1–mGAT4.

including diabetic neuropathy and migraine. Tiagabine is a highly selective blocker of the GAT1 transporter, and this may limit its activity to regions of the CNS in which GAT1 plays a significant role (the cortex, cerebellum, and hippocampus) [19]. This drug strongly inhibits GABA uptake in synaptosomal preparations from rat brain, as well as in cultured neurons and glial cells *in vitro* [20]. Tiagabine generates a dose-dependent increase in extracellular GABA levels in rat brain *in vivo*, and specifically suppresses various chemically-induced seizures evoked by pentylenetetrazole (PTZ) and DMCM (6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate), as well as kindled seizures, whereas it only weakly influences maximal electroshock seizures [19]. Tiagabine demonstrates antinociceptive effects at moderate doses (7.2 and 24.3 μmol/kg) against thermally evoked pain in the mouse hot-plate test [21]. Furthermore, it has an antiallodynic activity and anxiolytic-like properties in rodent models [21–23]. Unfortunately, the utility of tiagabine is limited in part because of adverse effects, such as asthenia, diarrhea, dizziness and tremor [24,25].

Another GAT1 inhibitor which is effective in neuropathic pain is NO711 (Fig. 1). In the chronic constriction injury (CCI) model in mice NO711 induced late-onset and long-lasting analgesic effect. These results indicate that mGAT1 may be involved in the occurrence and development of neuropathic pain [26].

mGAT4 inhibitors are represented by moderately potent (*S*)-SNAP-5114, the most selective mGAT4 inhibitor up to now (Fig. 1) [27,28]. Recently, it was found that (*S*)-SNAP-5114 exerted antinociceptive effects by promoting the activation of $GABA_A$ and $GABA_B$ receptors in the spinal cord. Its analgesic efficacy has been confirmed in the tail flick test (a model of acute thermal nociception), in the late phase of the formalin test (a model of persistent pain) and in CCI neuropathic pain model. Studies conducted by *Kataoka* et al. [29] demonstrated that mGAT4 inhibitors might be useful in treatment of various painful conditions. However, the physiological role of mGAT4 in the CNS is not completely understood [27–29].

Our earlier studies focused on the search for novel biologically active compounds and demonstrated that several 2-substituted 4-hydroxybutanamides with affinity for GATs display not only anticonvulsant effects in some rodent models of seizures [30,31] but also antinociceptive properties in thermally and chemically induced acute and tonic pain models [30,32,33].

We present herein the synthesis and biological evaluation of six series of new 4-aminobutanamide derivatives.

The strategy in the development of these new compounds was motivated by our previous structural characterization and biological evaluation of 2-substituted derivatives of 4-hydroxybutanamides [31,34,35] and of compounds bearing a phthalimide group at the 4-position of the butanamides [36]. Following our previous investigations which showed that 4-(1,3-dioxoisoindolin-2-yl)butanamide derivatives with the 2-(4-benzhydryl)-piperazin-1-yl residue exhibited only weak GAT inhibition [36], we sought to obtain butanamide derivatives with a primary amine group at the 4-position. Thus, drug development commenced from GABA, which shows low selectivity for GATs [37]. Preliminary SAR studies indicated that the benzyl substituent on the amide group and the aromatic and lipophilic substituent at the 2-position of the 4-hydroxybutanamide moiety are crucial for their activity. In addition, the presence of a tertiary amino group, for example *N*-methyl-4,4-diphenylbut-3-en-1-amine (*N*-methyl-*N*-DPB), in analogues of GABA uptake inhibitors is necessary for effective interaction with the GABA uptake system [35,38]. To mimic the biaryl moieties of known GAT inhibitors a different *N*-bulky and lipophilic biaryl group was introduced at the 2-position of 4-aminobutanamide (Fig. 2).

2. Chemistry

To prepare the target compounds **15**–**20**(**a**–**e**), GABA was used as a starting material. The reaction routes to all the target compounds are outlined in Scheme 1.

First, the amine group of GABA was protected by phthalimide. Phthalimide-protected amino acid **1** was easily obtained from the parent amino acid and phthalic anhydride [39]. In the case of substituted α-halo amides **3a**–**e**, 4-(1,3-dioxoisoindolin-2-yl)butanoic acid (**1**) was brominated using *N*-bromosuccinimide. This ionic bromination procedure proved to be tolerant of phthalimido group and was superior to the standard Hell-Vollard-Zielinski procedure [40]. The molecule obtained, 2-bromo-4-(1,3-dioxoisoindolin-2-yl) butanoic acid (**2**), was purified by method described by Kolasa et *al.* [41]. Further treatment with thionyl chloride generated the acid chloride which then was reacted with various substituted derivatives of benzylamine at room temperature in dry THF for 12 h, giving molecules **3a**–**e**. The resulting amides (**3a**–**e**) were a substrate for the *N*-alkylation of earlier obtained amines (**4**–**8**).

The amines necessary for the reactions, namely 4-diphenylmethylene piperidine (**4**) and 4-benzhydryl piperidine (**5**), were prepared according to a published procedure [42]. *N*-Methyl-4,4-diphenylbut-3-en-1-amine (**6**) and 4,4-diphenylbut-3-en-1-amine (**7**) were prepared according to a published procedure using 1,1′-(4-bromobut-1-ene-1,1-diyl)dibenzene as a starting

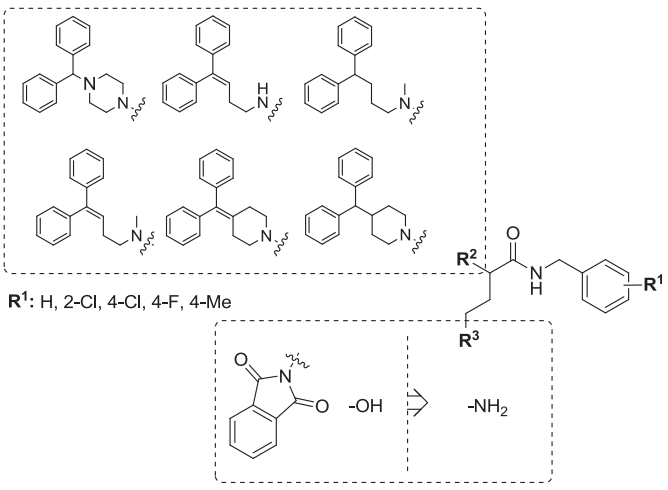
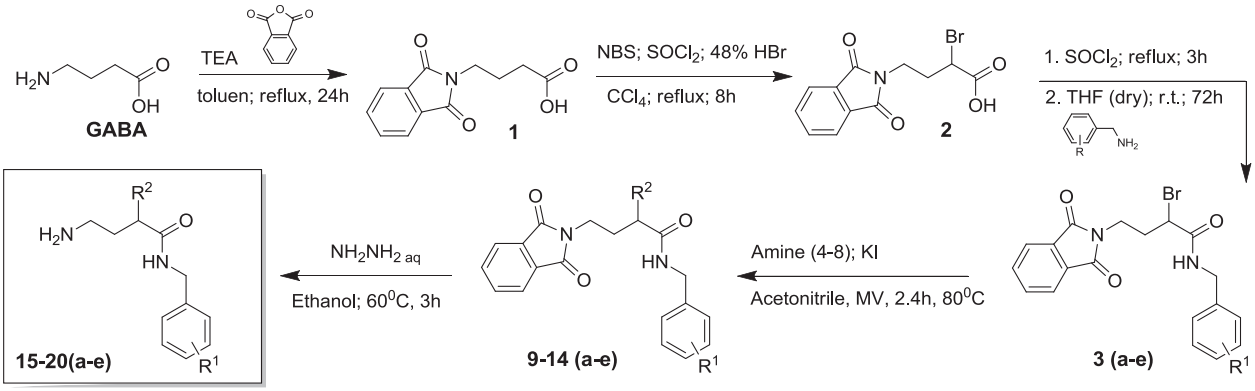
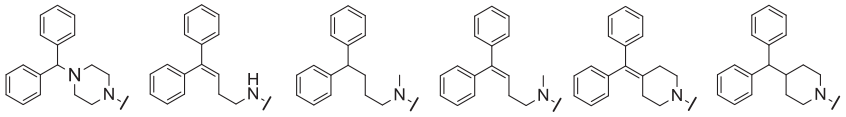


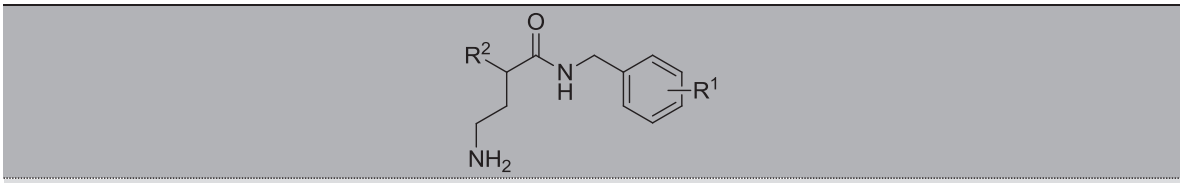
Fig. 2. Schematic structure of designed 2-substituted 4-aminobutanamides.



R¹: H, *o*-Cl; *p*-Cl, *p*-F; *p*-Me

R²:





R² / R¹						
-H	**15a**	**16a**	**17a**	**18a**	**19a**	**20a**
o-Cl	**15b**	**16b**	**17b**	**18b**	**19b**	**20b**
p-Cl	**15c**	**16c**	**17c**	**18c**	**19c**	**20c**
p-F	**15d**	**16d**	**17d**	**18d**	**19d**	**20d**
p-Me	**15e**	**16e**	**17e**	**18e**	**19e**	**20e**

Scheme 1. The synthesis of 2-substituted 4-aminobutanamides **15**–**20(a**–**e)**.

material [31,38,43]. *N*-Methyl-4,4-diphenylbutan-1-amine (**8**) was obtained by the hydrogenation of its derivative **6** [31]. 1-benzhydrylpiperazine (**9**) is commercially available.

The final 2-substituted derivatives of 4-aminobutanamides **15**–**20 (a**–**e)** were obtained by the cleavage of a phthalimide group with hydrazine hydrate.

All the designed compounds were synthesized and subsequently tested for their inhibitory potency and selectivity towards cloned murine GABA transporters mGAT1–mGAT4 in uptake assays, and their affinity for mGAT1 was evaluated in an MS binding assay.

3. *In vitro* inhibitory activity

4-Aminobutanoic acid derivatives **15**–**20 (a**–**e)** were evaluated for their inhibitory potency against 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 [44]. The affinity for GAT1 was determined by an MS binding assay with NO711 as a non-labeled marker [45]. The compounds were considered as active if GABA uptake or NO711 binding was reduced at least by 50% at a concentration of 100 μM. Inhibitory activity was expressed as pIC_{50} (negative log of the 50% inhibitory concentration; IC_{50}); or pK_i values in NO711 MS binding assay. The results are listed in Table 1.

4. Results and discussion

4.1. In vitro inhibitory activity

The purpose of this study was to identify GABA uptake inhibitors with improved pharmacological properties, and a wide range of new GABA uptake inhibitors has been prepared. The core structure of the compounds designed is 4-aminobutanamide (**15**–**20(a**–**e)**).

Compounds were first assessed for the inhibition of mGAT activity in cultured cells stably expressing mGAT1–4.

Among the amides with the 4,4-diphenylbut-3-en-1-ylamine moiety **16(a**–**e)**, compound **16b** with a chloro substituent at the

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 NO711 binding[b]	
15a	4.35	±0.05	4.42	±0.05	4.57	±0.06	4.23	±0.06	100 μM: 83%	
15b	4.68	±0.10	4.66	±0.04	4.98	±0.07	4.82	±0.07	100 μM: 55%	
15c	4.59	±0.10	4.68	±0.07	4.97	±0.07	4.92	±0.04	100 μM: 53%	
15d	4.63	±0.05	4.51	±0.07	4.47	±0.07	4.43	±0.02	4.18	±0.04
15e	4.55	±0.10	4.52	±0.05	4.74	±0.11	4.63	±0.05	4.20	±0.06
16a	4.51	±0.05	5.10	±0.02	4.48	±0.08	4.59	±0.01	4.76	±0.11
16b	4.80	±0.10	5.16	±0.08	4.62	±0.09	4.99	±0.07	100 μM: 53%	
16c	4.78	±0.10	5.02	±0.02	4.75	±0.04	4.93	±0.10	4.79	±0.10
16d	4.56	±0.05	4.92	±0.05	4.61	±0.04	4.50	±0.06	4.64	±0.11
16e	4.71	±0.10	5.05	±0.08	4.53	±0.06	4.82	±0.06	4.96	±0.07
17a	4.65	±0.09	5.02	±0.08	4.53	±0.07	4.59	±0.10	4.51	±0.03
17b	4.90	±0.07	5.11	±0.04	4.72	±0.04	4.94	±0.08	4.67	±0.02
17c	4.76	±0.05	4.76	±0.09	4.67	±0.04	4.84	±0.07	4.75	±0.10
17d	4.78	±0.04	4.94	±0.07	4.57	±0.03	4.87	±0.10	4.67	±0.07
17e	4.91	±0.05	4.85	±0.02	4.64	±0.06	5.08	±0.07	4.81	±0.06
18a	4.73	±0.02	4.92	±0.10	4.62	±0.08	4.80	±0.09	4.90	±0.16
18b	4.89	±0.10	5.19	±0.01	5.00	±0.04	5.04	±0.06	4.96	±0.09
18c	4.89	±0.05	5.01	±0.06	4.95	±0.02	4.90	±0.03	4.95	±0.09
18d	4.86	±0.07	4.89	±0.09	4.96	±0.05	4.90	±0.01	4.80	±0.11
18e	5.07	±0.08	5.02	±0.09	4.92	±0.05	5.01	±0.02	5.04	±0.13
19a	4.96	±0.06	4.81	±0.07	4.86	±0.08	4.92	±0.06	4.78	±0.14
19b	4.87	±0.07	4.99	±0.10	4.81	±0.04	4.96	±0.03	4.98	±0.09
19c	4.96	±0.07	4.98	±0.07	4.89	±0.04	4.92	±0.04	4.62	±0.08
19d	4.96	±0.02	4.99	±0.11	4.91	±0.10	4.87	±0.05	4.60	±0.03
19e	4.95	±0.06	5.23	±0.06	4.79	±0.05	4.94	±0.05	4.53	±0.05
20a	4.71	±0.09	4.92	±0.02	4.69	±0.06	4.70	±0.01	4.49	±0.06
20b	4.86	±0.09	5.23	±0.10	4.96	±0.03	4.99	±0.10	4.59	±0.04
20c	4.95	±0.03	5.01	±0.05	5.02	±0.06	4.99	±0.07	4.52	±0.04
20d	4.80	±0.10	5.03	±0.05	4.67	±0.02	5.07	±0.06	4.47	±0.07
20e	4.88	±0.05	5.10	±0.06	4.84	±0.10	5.13	±0.04	4.78	±0.05
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] pIC_{50} (means ± SEM; $n \geq 3$.
[b] % of NO711 bound to GAT1 at 100 μM concentration of tested compound (means; $n = 2$)or pK_i (means ± SEM; $n = 3$).
[c] Data from Ref. [44].
[d] Data from Refs. [44,45,53].

ortho-position of the *N*-benzylamide moiety showed the highest potency against mGAT2 ($pIC_{50} = 5.16$) (Table 1). *N*-methylation of the analogs **16(a–e)** resulted in the increased inhibitory potency of compounds **18(a–e)**. Increased inhibitory activity against GABA uptake was observed against mGAT1, mGAT3, and mGAT4, whereas activity against mGAT2 was unchanged. Moreover, the compound also bearing a chloro substituent at the *ortho*-position of the benzyl fragment of the molecule showed significant potency against mGAT2 in this series (**18b**, $pIC_{50} = 5.19$) (Fig. 3). Structural modification involving reduction of the double bond in the amino moiety of the series discussed above was found to have a negative effect on the potencies of compounds **17(a–e)**, which were greatly reduced (Fig. 3).

Compound **19e**, which contains a methyl substituent at the *para*-position of the benzyl fragment of the molecule, showed the highest potency towards mGAT2 ($pIC_{50} = 5.23$). Structural modification involving hydrogenation of the double bond in the amino moiety of 4-(diphenylmethyl)piperidine **20(a–e)** caused only a slight decrease in the inhibitory activity against mGAT1, mGAT3, and mGAT4 transporters compared to **19(a–e)**, whereas this modification did not affect potency against mGAT2 transporter. Marked activity against mGAT2 was observed for **20b** with a chloro substituent at the *ortho*-position of the *N*-benzylamide moiety ($pIC_{50} = 5.23$) (Fig. 3).

Taking into consideration the results of *in vitro* tests, two compounds (**15b** and **15c**) were selected for further investigation *in vivo* in behavioral assays.

4.2. Electroconvulsive threshold test

At a dose of 100 mg/kg none of the test compounds elevated the threshold for induction of electroconvulsive seizures. In control mice the CS_{50} value was 6.61 mA. In **15b**-treated group the CS_{50} was 6.22 mA ($p > 0.05$); in addition, no protection against seizures induction was observed with **15c**. For the latter compound, CS_{50} was not calculated because an increased mortality rate compared to control mice was noted in **15c**-treated mice (100 mg/kg) after the animals underwent a single electroconvulsive shock. (*S*)-SNAP5114 elevated the threshold for induction electroconvulsive seizures to 7.95 mA, but this effect was not statistically significant.

4.3. Maximal electroshock seizure test

None of the test compounds at doses 30 mg/kg and 100 mg/kg demonstrated protective properties against electrically induced seizures in the maximal electroshock test. In all **15b**-treated mice tonic hind-limb extension (THLE) was observed after a single electroshock. **15c** at 30 mg/kg was also ineffective in this test. At a dose of 100 mg/kg it reduced the number of mice with THLE by 44% (vs. control group) but an increased mortality rate was observed in this group after the electroshock (65% vs. control, $p < 0.01$).

4.4. Pentylenetetrazole (PTZ)-induced seizures

In contrast to electrical models of seizures, both compounds were effective as anticonvulsants in PTZ-induced seizures. At a dose

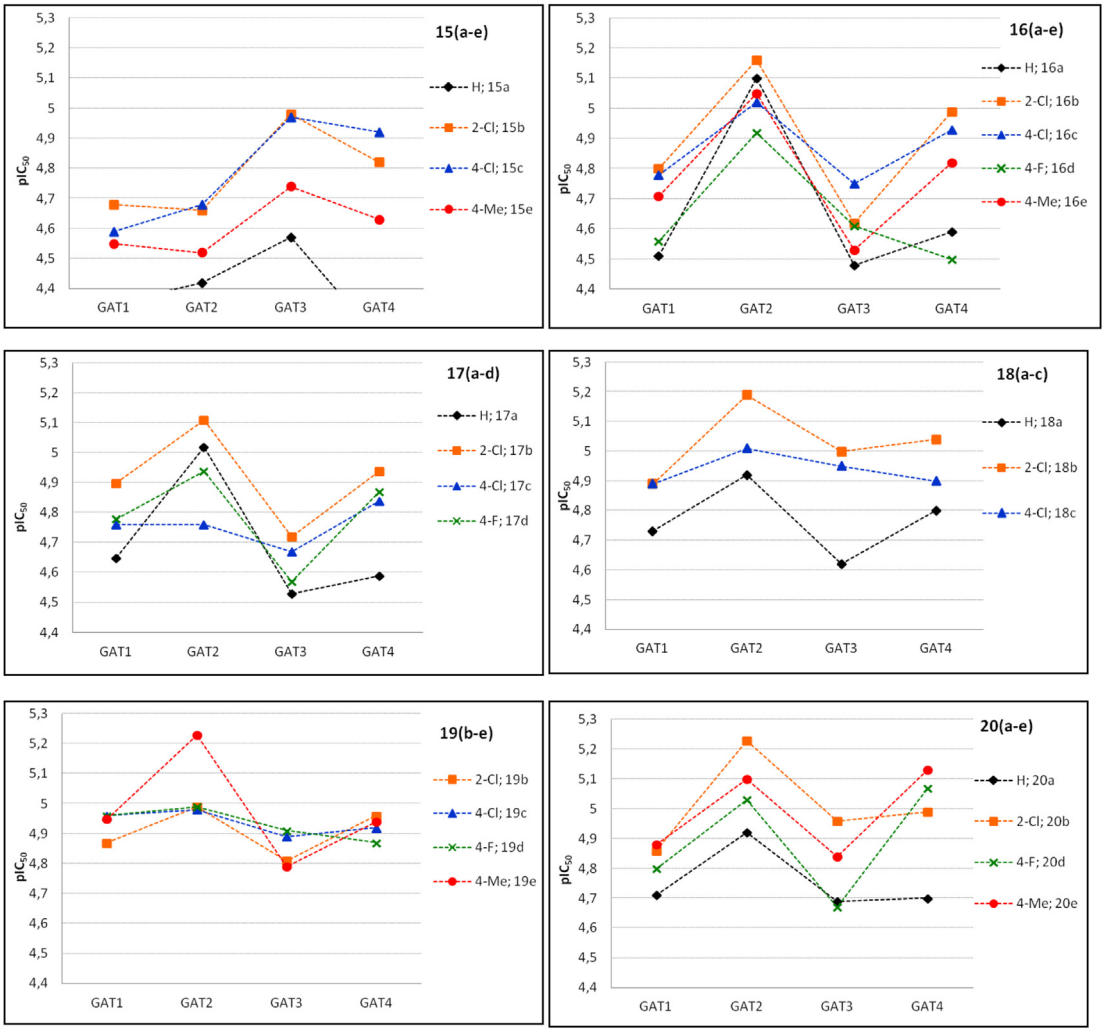


Fig. 3. **(a–f)** Inhibition of GABA uptake; the potency of compounds **15**–**20(a–e)** for GAT inhibition depends on the nature of the substituents at the benzyl fragment of molecule.

of 100 mg/kg **15b** significantly prolonged the latency time to first seizure episode by 100% ($p < 0.05$), whereas at 30 mg/kg it had no effect. Compared to the vehicle-treated group, it also reduced the number of seizure episodes by 53% and 37% at 100 and 30 mg/kg, respectively. **15c** was even more efficacious than **15b** in this test because it was able to delay the onset of seizures by 58% and 217% (vs. control) at doses 15 and 30 mg/kg, respectively. Compared to the vehicle-treated group, it also reduced the number of seizure episodes by 58% and 83%, respectively (Table 2). Both compounds: **15b** at doses 30 and 100 mg/kg and **15c** at 30 mg/kg significantly reduced the mortality rate of experimental animals as compared to control mice ($p < 0.001$; Fig. 4). In this test the anticonvulsant activity of (*S*)-SNAP5114 was not assessed based on literature data indicating that this GAT inhibitor does not display anticonvulsant activity in the PTZ seizure model [46]. Ethosuximide (ETX; 100 mg/kg; i.p.) used as a reference in this test delayed the onset of seizures by 120% ($p < 0.01$) and reduced both the number of seizure episodes by 64% and total mortality rate by 75% (Table 2).

4.5. Pilocarpine-induced seizures

At doses tested the investigated compounds **15c** and (*S*)-SNAP5114 demonstrated a statistically significant protection against seizures, whereas **15b** had no protective effect (Table 3).

Table 2
Anticonvulsant activity of the test compounds in the PTZ-induced seizure model.

Compound	Dose [mg/kg]	Latency to first seizure episode [s][a]	Number of seizure episodes[b]	Mortality rate (X/Y)[c]
Vehicle	–	552 ± 48	2.4 ± 0.4	4/8
15b	30	816 ± 216	1.5 ± 0.5	2/8***
	100	1104 ± 264*	1.1 ± 0.5	1/8***
15c	15	870 ± 114**	1.0 ± 0.0*	0/8***
	30	1752 ± 48***	0.4 ± 0.3***	0/8***
Vehicle	–	614.9 ± 61.9	1.4 ± 0.3	6/8
Ethosuximide	100	1350 ± 187.4**	0.5 ± 0.2*	0/8***

Each value represents the mean ± SEM obtained from 7 to 10 mice. The compounds and the vehicle were administered intraperitoneally 60 min before the assay.

[a, b] Statistical analysis: one-way analysis of variance (ANOVA), followed by Dunnett's *post hoc* comparison (**15b**, **15c**) or Student's *t*-test (ethosuximide). Latency time to first seizure episode: $F[2,29] = 3.339$; $p < 0.05$ (**15b**) and $F[2,28] = 71.38$; $p < 0.0001$ (**15c**). Number of seizure episodes: $F[2,29] = 2.373$; $p > 0.05$ (**15b**) and $F[2,29] = 9.908$; $p < 0.001$ (**15c**). Significant difference compared to the control group: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

[c] Mortality rate is expressed as the number of mice that died during the 30 min observation period (X) as a proportion of the number of mice tested in each group (Y). The results obtained for the drug-treated group were compared to that of vehicle-treated group. These differences were statistically evaluated using Fisher's exact probability test.

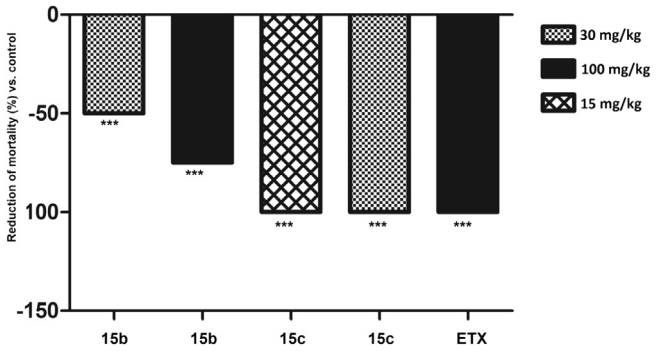


Fig. 4. The influence of the test compounds **15b**, **15c**, and a reference molecule (ethosuximide, ETX) on mortality rate in pentylenetetrazole-induced seizures. Results are shown as the % reduction of mortality rate compared to that observed in vehicle-treated mice. ***$p < 0.001$ (vs. control).

Both **15c** and (*S*)-SNAP5114 prolonged the latency time to seizures by 113% (**15c**; $p < 0.05$), and by 158% and 259%, for 100 mg and 200 mg of (S)-SNAP5114, respectively ($p < 0.05$).

Each value represents the mean ± SEM obtained from 7 to 8 mice. Anticonvulsant activity was assessed by comparison to vehicle-treated mice. Statistical analysis: Student's *t*-test. The compounds and the vehicle were administered i.p. 60 min before pilocarpine. Significant difference *versus* the control group: *$p < 0.05$. NE: not established.

4.6. Forced swim test

Both **15b** and **15c** at the dose of 30 mg/kg showed a statistically significant antidepressant-like effect in the forced swim test ($p < 0.05$; Table 4). In addition, a main overall effect of treatment was observed in the case of (*S*)-SNAP5114 ($F[2,20] = 5.503$; $p < 0.05$). The dose 30 mg/kg of this compound reduced immobility time by 20% *versus* control mice, and this effect was statistically significant at $p < 0.01$.

Each value represents the mean ± SEM obtained from 7 to 8 mice. The compounds and the vehicle were administered intraperitoneally 60 min before the assay. Statistical analysis: one-way analysis of variance (ANOVA), followed by Dunnett's *post hoc* test: $F[2,19] = 4.906$, $p < 0.05$ (**15b**); $F[2,18] = 4.369$, $p < 0.05$ (**15c**); $F[2,20] = 5.503$, $p < 0.05$ ((*S*)-SNAP5114). Significant difference *versus* the vehicle-treated group: *$p < 0.05$, **$p < 0.01$.

4.7. Hot plate test

In this test in the vehicle-treated mice the latency time to pain reaction was 13.9 ± 1.2 s. **15b** tested at 30 mg/kg prolonged the latency time to pain reaction to 18.8 ± 2.3 s ($p > 0.05$) and **15c** at the same dose prolonged the latency time to 27.5 ± 2.9 s ($p < 0.001$). (*S*)-SNAP5114 demonstrated no antinociceptive activity in the hot plate test.

Table 4
Antidepressant-like activity of the investigated compounds in the forced swim test.

Compound	Dose [mg/kg]	Immobility time [s] ± SEM	Reduction of immobility time (%)
Vehicle (1% Tween)	–	159.4 ± 10.1	–
15b	15	117.9 ± 13.5	26.0
	30	98.0 ± 17.10*	38.5
15c	15	153.3 ± 14.8	3.8
	30	113.4 ± 13.3*	28.9
Vehicle	–	198.2 ± 4.5	–
(S)-SNAP-5114	15.0	174.3 ± 11.4	12.1
	30.0	158.7 ± 9.9**	19.9

Each value represents the mean ± SEM obtained from 7 to 8 mice. The compounds and the vehicle were administered intraperitoneally 60 min before the assay. Statistical analysis: one-way analysis of variance (ANOVA), followed by Dunnett's *post hoc* test: $F[2,19] = 4.906$, $p < 0.05$ (**15b**); $F[2,18] = 4.369$, $p < 0.05$ (**15c**); $F[2,20] = 5.503$, $p < 0.05$ ((S)-SNAP5114). Significant difference *versus* the vehicle-treated group: *$p < 0.05$, **$p < 0.01$.

4.8. Rotarod test

In the rotarod test the investigated compounds at doses 30 and 100 mg/kg did not induce detectable motor impairments in experimental animals compared to vehicle-treated mice (Fig. 5a–c).

4.9. Locomotor activity test

At 30 mg/kg **15b** had no influence on locomotor activity (Fig. 6a), whereas a statistically significant ($p < 0.001$) decrease in locomotor activity was observed in **15c**-treated mice at each time-point of the test (Fig. 6b).

5. Conclusion

Six new series of GABA uptake inhibitors have been synthesized based on the structure of 4-aminobutanamides modified by changing the *N*-bulky and lipophilic biaryl side-chain and the position of substituents in the benzyl fragment of the molecules; compounds were tested for activity against stably expressed mGAT1–4 in HEK cells.

The *in vitro* results demonstrated that an *N*-bulky and lipophilic biaryl group in the 2-position of 4-aminobutanamide is significant for the inhibitory potency towards mGAT1–4. The highest activities of 4-(diphenylmethylidene)piperidine, 4-(diphenylmethyl)piperidine, and *N*,*N*-(methyl)(4,4-diphenyl)but-3-enylamine-substituted 4-aminobutanamides were observed against mGAT2 transporter, whereas molecules with a 4-benzhydrylpiperazin-1-yl side chain were the most potent towards mGAT3 transporter.

Table 3
Anticonvulsant activity of the investigated compounds in pilocarpine-induced seizure model.

Compound	Dose [mg/kg]	Latency to seizures [s] ± SEM	Anticonvulsant effect (%)	Latency to death [s] ± SEM	Anticonvulsant effect (%)
Vehicle	–	392.7 ± 41.10	–	519.8 ± 71.58	–
15b	100	342.7 ± 49.88	NE	466.2 ± 26.92	NE
Vehicle	–	368.3 ± 17.96	–	511.1 ± 59.7	–
15c	70	785.9 ± 187.6*	113.4	874.7 ± 142.3*	71.1
(S)-SNAP5114	100	950.3 ± 250.1*	158	1757 ± 544.1*	243.8
	200	1322.0 ± 354.4*	258.9	1547 ± 364.6*	202.7

Each value represents the mean ± SEM obtained from 7 to 8 mice. Anticonvulsant activity compared to vehicle-treated mice. Statistical analysis: Student's *t*-test. The compounds and the vehicle were administered i.p. 60 min before pilocarpine. Significant difference compared to the control group: *$p < 0.05$. NE: not established.

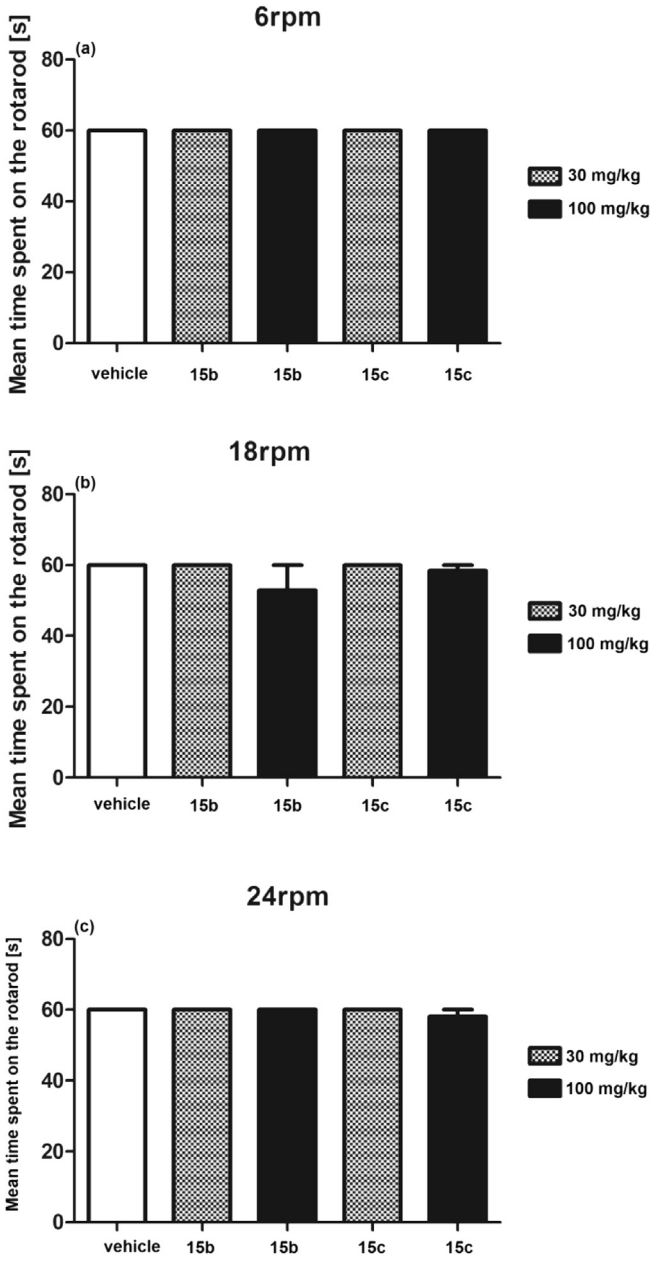


Fig. 5. Influence of the test compounds **15b** and **15c** (at 30 and 100 mg/kg) on motor coordination of experimental animals measured in the rotarod test revolving at 6 rpm (a), 18 rpm (b), or 24 rpm (c). Results are shown as mean time spent on the rotating rod. Statistical analysis: one-way analysis of variance (ANOVA), followed by Dunnett's *post hoc* test: 18 rpm – $F[2,21] = 1.000$, $p > 0.05$ (**15b and 15c**); 24 rpm – $F[2,21] = 1.000$, $p > 0.05$ (**15c**). The results were not significant.

Moreover, the present study indicates that the introduction of an *ortho*-chloro or *para*-methyl substituent on the benzyl fragment of the molecules is essential for activity against mGAT2.

Of note is the finding that, among the molecules obtained, the substitution on the *N*-benzylamide moiety did not produce a difference in activities against mGAT1 and mGAT4, whereas the benzhydrylpiperazin-1-yl substituent in **15(a–e)** was associated with a reduced activity against mGAT1 and mGAT2.

It is also notable that compounds containing the lipophilic biaryl (diphenylmethylidene)piperidine and 4-(diphenylmethyl)piperidine moieties were most active against mGAT2, with comparable activity against mGAT1 and mGAT4. Moreover, our results indicate that the basic character of the amino groups, and the conformational flexibility of the linker connecting the 4-aminobutanamide and the aromatic moiety at the second position of this class of GABA uptake inhibitors, are factors of importance in determining the activity against GABA uptake.

In the pilocarpine-induced seizure test, a rodent model of *status epilepticus*, the molecule **15c**, which displayed the most potent activity against mGAT4, demonstrated a statistically significant anticonvulsant activity, whereas **15b** had no effect.

In the present study we have confirmed that some GAT inhibitors exert an antidepressant-like effect in the forced swim test in mice, in agreement with our recent reports [33] and reports of other authors [47].

A very significant antinociceptive activity in the hot-plate test was demonstrated for **15c**. On the other hand, this compound potently decreased experimental animals' locomotor activity at the dose tested in the hot plate test (30 mg/kg). Taking these sedative properties of **15c** into consideration, the prolongation of the latency time to pain reaction in the hot plate test is likely to be a false positive effect resulting from sedation caused by this compound.

To conclude, given the encouraging results obtained with appropriately 2-substituted GABA derivatives, our study provides a promising starting point for the search for new and highly potent GAT inhibitors.

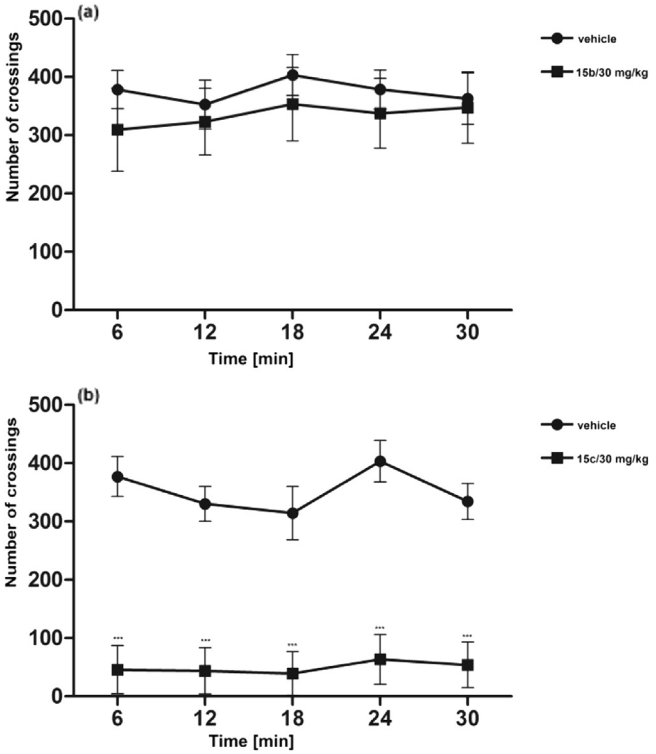


Fig. 6. Influence of the test compounds (both at 30 mg/kg) **15b** (a) and **15c** (b) on locomotor activity in mice. Data are shown as mean number of light-beam interruptions (±SEM) measured in five periods of 6 min. Statistical analysis of the results was conducted using repeated-measures analysis of variance (ANOVA), followed by Bonferroni multiple comparison. **15b**: Drug effect: $F[1,56] = 0.39$; $p > 0.05$; time effect: $F[4,56] = 0.73$; $p > 0.05$; interaction: $F[4,56] = 0.31$; $p > 0.05$. **15c**: Drug effect: $F[1,56] = 35.85$; $p < 0.0001$; time effect: $F[4,56] = 4.71$; $p < 0.01$; interaction: $F[4,56] = 2.23$; $p > 0.05$. Values were compared to vehicle-treated mice at the same time-points: $^{***}p < 0.001$.

6. Experimental section

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 (chloroform:acetone 1:1, v/v), S_7 (DCM:acetone 7:3, v/v); S_8 (DCM:acetone:MeOH 8:2:0.25 v/v/v); S_9 (DCM:acetone 9:1, v/v); N_1:25% NH_3/MeOH/DCM/PE (45:225:600:90); H_7:hexane/ethanol/TEA (7:2:1, v/v/v). The reactions were carried out using Hydrogen Generators PGX-H_2 (Perkin Elmer) and reactions under microwave irradiation were carried out in Discover LabMate (CEM Corporation, USA). 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). ^{1}H NMR and ^{13}C NMR spectra were recorded with VX Mercury 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.

6.2. General procedure for the synthesis of N-benzyl-2-bromo-4-(1,3-dioxoisoindolin-2-yl)butanamide derivatives (**3a**–**e**)

To a solution of 2-bromo-4-(1,3-dioxoisoindolin-2-yl)butanoic acid (**2**) (2.51 g; 8.04 mmol), thionyl chloride (20 mL; 0.27 mol) was added and the resulting solution was refluxed for three hours. The excess unreacted thionyl chloride was removed under reduced pressure and the resulting acid chloride was used in the next step as such. To the ice-cooled and stirred solution of the obtained above acid chloride in dry THF (30 mL), the *N*-benzylamine derivative (24.13 mmol) was added dropwise and stirring continued for three hours and left overnight. The reaction mixture was poured into ice. The organic layer was washed with dil. HCl and evaporated under vacuum. The aqueous layer was extracted with DCM. The combined organic layers were dried ($NaSO_4$) and concentrated in vacuo. The crude product was finally purified by column chromatography (DCM/acetone 9:1 v/v).

6.2.1. N-benzyl-2-bromo-4-(1,3-dioxoisoindolin-2-yl)butanamide (**3a**)

Reagents and conditions: 8.04 mmol **2** (2.51 g), 20 mL $SOCl_2$, 30 mL dry THF, 24.13 mmol *N*-benzylamine (2.58 g); Yield 66%; white solid; mp 152.3–152.5 °C; R_f: 0.87 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.37 (dq, J = 14.33, 7.28 Hz, 1H, CH_2CHBr), 2.59–2.72 (m, 1H, CH_2CHBr), 3.83–3.90 (m, 2H, NCH_2), 4.36 (dd, J = 7.69, 5.90 Hz, 1H, *CH*Br), 4.44 (d, J = 5.39 Hz, 2H, NHCH_2), 6.73 (br. s., 1H, CO*NH*), 7.22–7.42 (m, 5H, arom), 7.72 (m, 2H, *phthalimide*), 7.80–7.88 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 401.04 $[M+H]^+$.

6.2.2. 2-Bromo-N-(2-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl) butanamide (**3b**)

Reagents and conditions: 8.04 mmol **2** (2.51 g), 20 mL $SOCl_2$, 30 mL dry THF, 24.13 N-(2-chlorobenzyl)-amine (3.42 g); Yield 67%; white solid; mp 167.1–167.3 °C; R_f: 0.85 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.30–2.41 (m, 1H, CH_2CHBr), 2.58–2.71 (m, 1H, CH_2CHBr), 3.86 (td, J = 6.54, 1.28 Hz, 2H, NCH_2), 4.36 (dd, J = 7.95, 5.90 Hz, 1H, *CH*Br), 4.53 (d, J = 5.90 Hz, 2H, NHCH_2), 6.86 (br. s., 1H, CO*NH*), 7.22–7.26 (m, 2H, arom), 7.34–7.41 (m, 2H, arom), 7.70–7.74 (m, 2H, *phthalimide*), 7.80–7.87 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 436.70 $[M+H]^+$.

6.2.3. 2-Bromo-N-(4-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl) butanamide (**3c**)

Reagents and conditions: 8.04 mmol **2** (2.51 g), 20 mL $SOCl_2$, 30 mL dry THF, 24.13 N-(4-chlorobenzyl)-amine (3.42 g); Yield 63%; white solid; mp 149.0–149.2 °C; R_f: 0.87 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.36 (dd, J = 14.62, 7.44 Hz, 1H, CH_2CHBr), 2.60–2.70 (m, 1H, CH_2CHBr), 3.86 (td, J = 6.41, 4.10 Hz, 2H, NCH_2), 4.35 (dd, J = 7.44, 6.16 Hz, 1H, *CH*Br), 4.42 (dd, J = 5.90, 1.80 Hz, 2H, NHCH_2), 6.73 (br. s., 1H, CO*NH*), 7.20–7.26 (m, 2H, arom), 7.27–7.36 (m, 2H, arom), 7.71–7.75 (m, 2H, *phthalimide*), 7.81–7.87 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 436.70 $[M+H]^+$.

6.2.4. 2-Bromo-4-(1,3-dioxoisoindolin-2-yl)-N-(4-fluorobenzyl) butanamide (**3d**)

Reagents and conditions: 8.04 mmol **2** (2.51 g), 20 mlLSOCl_2, 30 mL dry THF, 24.13 N-(4-fluorobenzyl)-amine (3.02 g); Yield 61%; white solid; mp 151.5–151.7 °C; R_f: 0.82 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.30–2.43 (m, 1H, CH_2CHBr), 2.59–2.72 (m, 1H, CH_2CHBr), 3.85 (td, J = 6.41, 3.33 Hz, 2H, NCH_2), 4.35 (dd, J = 7.57, 6.28 Hz, 1H, *CH*Br), 4.41 (d, J = 5.64 Hz, 2H, NHCH_2), 6.72 (br. s., 1H, CO*NH*), 6.98–7.08 (m, 2H, arom), 7.24–7.32 (m, 2H, arom), 7.69–7.76 (m, 2H, *phthalimide*), 7.80–7.89 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 420.24 $[M+H]^+$.

6.2.5. 2-Bromo-4-(1,3-dioxoisoindolin-2-yl)-N-(4-methylbenzyl) butanamide (**3e**)

Reagents and conditions: 8.04 mmol **2** (2.51 g), 20 mL $SOCl_2$, 30 mL dry THF, 24.13 N-(4-methylbenzyl)-amine (2.92 g); Yield 60%; white solid; mp 173.7–173.9 °C; R_f: 0.86 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.33 (s, 3H, CH_3), 2.35–2.44 (m, 1H, CH_2CHBr), 2.61–2.72 (m, 1H, CH_2CHBr), 3.87 (td, J = 6.48, 1.15 Hz, 2H, NCH_2), 4.35 (dd, J = 7.82, 5.77 Hz, 1H, *CH*Br), 4.40 (d, J = 5.13 Hz, 2H, NHCH_2), 6.65 (br. s., 1H, CO*NH*), 7.12–7.21 (m, 4H, arom), 7.70–7.74 (m, 2H, *phthalimide*), 7.81–7.87 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 416.28 $[M+H]^+$.

6.3. General procedure for the synthesis of 2-(4-benzhydrylpiperazin-1-yl)-N-benzyl-4-(1,3-dioxoisoindolin-2-yl) butanamide derivatives (**9a**–**e**)

1.24 mmol **3(a–e),** 2.14 mmol 1-benzhydrylpiperazine (**9**; 0.54 g), 29.28 mmol KI (4.86 g) were suspended in acetone (15 mL) and then the reaction mixture was stirred at 55 °C for 2.4 h using MV irradiation. The precipitated salt was filtered and the filtrate was concentrated. The crude product was purified by recrystallization from methanol.

6.3.1. 2-(4-Benzhydrylpiperazin-1-yl)-N-benzyl-4-(1,3-dioxoisoindolin-2-yl)butanamide (**9a**)

1.24 mmol **3a** (0.50 g), 2.14 mmol **9** (0.54 g), 29.28 mmol KI (4.86 g) and acetone (15 mL); Yield 57%; white solid; mp 179.7–179.9 °C; R_f: 0.93 (S_8); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.08–2.21 (m, 2H, NCHCH_2), 2.27–2.48 (m, 4H, *piperazine*), 2.59–2.68 (m, 2H, *piperazine*), 2.71–2.82 (m, 2H, *piperazine*), 3.28 (dd, J = 8.72, 4.10 Hz, 1H, N*CH*), 3.72–3.79 (m, 1H, NCHCH_2), 3.82–3.91 (m, 1H, CH_2N), 4.17 (s, 1H, *C*HPh_2), 4.47 (m, J = 5.39 Hz, 2H, NHCH_2), 7.20–7.40 (m, 15H, arom), 7.52 (br. s., 1H, CO*NH*), 7.66–7.71 (m, 2H, *phthalimide*), 7.77–7.85 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 573.70 $[M+H]^+$.

*6.3.2. 2-(4-Benzhydrylpiperazin-1-yl)-N-(2-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl)butanamide (**9b**)*

1.24 mmol **3b** (0.54 g), 2.14 mmol **9** (0.54vg), 29.28 mmol KI (4.86 g) and acetone (15 mL); Yield 65%; white solid; mp 162.5–162.8 °C; R_f: 0.95 (S_8); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.96–2.13 (m, 2H, NCHC*H*$_2$), 2.25–2.45 (m, 4H, *piperazine*), 2.46–2.63 (m, 4H, *piperazine*), 3.08 (dd, *J* = 7.82, 4.49 Hz, 1H, NC*H*), 3.75–3.84 (m, 1H, NCHC*H*$_2$), 3.86–3.95 (m, 1H, *CH*$_2$N), 4.17 (s, 1H, *CH*Ph$_2$), 4.45–4.57 (m, 2H, NHC*H*$_2$), 7.12–7.30 (m, 9H, arom), 7.34–7.40 (m, 5H, arom), 7.51–7.59 (m, 1H, CO*NH*), 7.66–7.74 (m, 2H, *phthalimide*), 7.79–7.86 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 607.24 [M+H]$^+$.

*6.3.3. 2-(4-Benzhydrylpiperazin-1-yl)-N-(4-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl)butanamide (**9c**)*

1.24 mmol **3c** (0.54 g), 2.14 mmol **9** (0.54 g), 29.28 mmol KI (4.86 g) and acetone (15 mL); Yield 60%; white solid; mp 207.2–207.5 °C; R_f: 0.94 (S_8); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.94–2.15 (m, 2H, NCHC*H*$_2$), 2.21–2.47 (m, 4H, *piperazine*), 2.47–2.76 (m, 4H, *piperazine*), 3.10 (br. s., 1H, NC*H*), 3.69–3.80 (m, 1H, NCHC*H*$_2$), 3.84–3.95 (m, 1H, *CH*$_2$N), 4.17 (s, 1H, *CH*Ph$_2$), 4.37–4.47 (m, 2H, NHC*H*$_2$), 7.08–7.46 (m, 15H, arom, CO*NH*), 7.67–7.75 (m, 2H, *phthalimide*), 7.80–7.87 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 607.24 [M+H]$^+$.

*6.3.4. 2-(4-Benzhydrylpiperazin-1-yl)-4-(1,3-dioxoisoindolin-2-yl)-N-(4-fluorobenzyl)butanamide (**9d**)*

1.24 mmol **3d** (0.52 g), 2.14 mmol **9** (0.54 g), 29.28 mmol KI (4.86 g) and acetone (15 mL); Yield 64%; white solid; mp 202.1–202.3 °C; R_f: 0.93 (S_8); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.99–2.14 (m, 2H, NCHC*H*$_2$), 2.24–2.43 (m, 4H, *piperazine*), 2.47–2.64 (m, 4H, *piperazine*), 3.08 (dd, *J* = 7.82, 4.23 Hz, 1H, NC*H*), 3.75–3.84 (m, 1H, NCHC*H*$_2$), 3.85–3.94 (m, 1H, *CH*$_2$N), 4.16 (s, 1H, *CH*Ph$_2$), 4.34–4.48 (m, 2H, NHC*H*$_2$), 6.95–7.05 (m, 2H, arom), 7.13–7.28 (m, 9H, arom), 7.33–7.39 (m, 4H, arom, CO*NH*), 7.68–7.74 (m, 2H, *phthalimide*), 7.79–7.87 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 591.69 [M+H]$^+$.

*6.3.5. 2-(4-Benzhydrylpiperazin-1-yl)-4-(1,3-dioxoisoindolin-2-yl)-N-(4-methylbenzyl)butanamide (**9e**)*

1.24 mmol **3e** (0.51 g), 2.14 mmol **9** (0.54 g), 29.28 mmol KI (4.86 g) and acetone (15 mL); Yield 66%; white solid; mp 182.8–182.9 °C; R_f: 0.88 (S_8); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.99–2.15 (m, 2H, NCHC*H*$_2$), 2.24–2.44 (m, 7H, C*H*$_3$, *piperazine*), 2.49–2.66 (m, 4H, *piperazine*), 3.08 (dd, *J* = 8.21, 4.36 Hz, 1H, NC*H*), 3.76–3.86 (m, 1H, NCHC*H*$_2$), 3.86–3.96 (m, 1H, *CH*$_2$N), 4.15 (s, 1H, *CH*Ph$_2$), 4.34–4.46 (m, 2H, NHC*H*$_2$), 7.08–7.21 (m, 6H, arom), 7.21–7.28 (m, 5H, arom), 7.33–7.42 (m, 4H, arom; CO*NH*), 7.67–7.74 (m, 2H, *phthalimide*), 7.79–7.87 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 587.62 [M+H]$^+$.

*6.4. General procedure for the synthesis of N-benzyl-4-(1,3-dioxoisoindolin-2-yl)-2-((4,4-diphenylbut-3-en-1-yl)amino) butanamide derivatives (**10a**–**e**)*

0.91 mmol **3(a**–**e**), 0.91 mmol 4,4-diphenylbut-3-en-1-amine (**7,** 0.20 g), 1.82 mmol KI (0.30 g), 2.73 mmol K_2CO_3 (0.38 g) and 15 mL acetonitrile. After the addition was complete, the reaction mixture was heated under reflux for 20 h. The precipitated salt was filtered and the filtrate was concentrated. The crude oily product was purified by column chromatography (SiO_2:DCM/acetone 9:1).

*6.4.1. N-benzyl-4-(1,3-dioxoisoindolin-2-yl)-2-((4,4-diphenylbut-3-en-1-yl)amino)butanamide (**10a**)*

0.91 mmol **3a** (0.37 g), 0.91 mmol **7** (0.20 g), 1.82 mmol KI (0.30 g), 2.73 mmol K_2CO_3 (0.38 g) and 15 mL acetonitrile; Yield 35%; light-yellow oil; R_f: 0.84 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.88–1.96 (m, 1H, NCHC*H*$_2$), 2.09–2.15 (m, 1H, NCHC*H*$_2$), 2.27 (q, *J* = 7.27 Hz, 2H, C=CHC*H*$_2$), 2.58–2.71 (m, 2H, *CH*$_2$NH), 3.10 (dd, *J* = 7.31, 5.77 Hz, 1H, NC*H*), 3.71–3.81 (m, 2H, *CH*$_2$, *phthalimide*), 4.34–4.43 (m, 2H, NHC*H*$_2$), 6.05 (t, *J* = 7.31 Hz, 1H, C=C*H*), 7.06–7.44 (m, 15H, arom), 7.60 (t, *J* = 5.64 Hz, 1H, CO*NH*), 7.68–7.73 (m, 2H, *phthalimide*), 7.77–7.86 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 544.65 [M+H]$^+$.

*6.4.2. N-(2-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl)-2-((4,4-diphenylbut-3-en-1-yl)amino)butanamide (**10b**)*

0.91 mmol **3b** (0.40 g), 0.91 mmol **7** (0.20 g), 1.82 mmol KI (0.30 g), 2.73 mmol K_2CO_3 (0.38 g) and 15 mL acetonitrile; Yield 30%; light-yellow oil; R_f: 0.88 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.89 (dd, *J* = 14.11, 7.44 Hz, 1H, NCHC*H*$_2$), 2.12 (dd, *J* = 14.11, 7.18 Hz, 1H, NCHC*H*$_2$), 2.23–2.33 (m, 2H, C=CHC*H*$_2$), 2.57–2.69 (m, 2H, *CH*$_2$NH), 3.08 (dd, *J* = 7.69, 5.64 Hz, 1H, NC*H*), 3.68–3.82 (m, 2H, *CH*$_2$ phthalimide), 4.47 (d, *J* = 6.16 Hz, 2H, NHC*H*$_2$), 6.06 (t, *J* = 7.44 Hz, 1H, C=C*H*), 7.10–7.43 (m, 15H, *arom*, CO*NH*), 7.67–7.74 (m, 2H, *phthalimide*), 7.77–7.86 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 579.10 [M+H]$^+$.

*6.4.3. N-(4-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl)-2-((4,4-diphenylbut-3-en-1-yl)amino)butanamide (**10c**)*

0.91 mmol **3c** (0.40 g), 0.91 mmol **7** (0.20 g), 1.82 mmol KI (0.30 g), 2.73 mmol K_2CO_3 (0.38 g) and 15 mL acetonitrile; Yield 41%; light-yellow oil; R_f: 0.87 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.85–1.94 (m, 1H, NCHC*H*$_2$), 2.07–2.15 (m, 1H, NCHC*H*$_2$), 2.26 (q, *J* = 7.27 Hz, 2H, C=CHC*H*$_2$), 2.55–2.74 (m, 2H, *CH*$_2$NH), 3.09 (dd, *J* = 7.31, 5.77 Hz, 1H, NC*H*), 3.75 (q, *J* = 6.50 Hz, 2H, *CH*$_2$ phthalimide), 4.33 (d, *J* = 6.16 Hz, 2H, NHC*H*$_2$), 6.05 (t, *J* = 7.31 Hz, 1H, C=C*H*), 7.05–7.39 (m, 14H, arom), 7.63 (t, *J* = 5.77 Hz, 1H, CO*NH*), 7.68–7.75 (m, 2H, *phthalimide*), 7.77–7.87 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 579.10 [M+H]$^+$.

*6.4.4. 4-(1,3-Dioxoisoindolin-2-yl)-2-((4,4-diphenylbut-3-en-1-yl) amino)-N-(4-fluorobenzyl)butanamide (**10d**)*

0.91 mmol **3d** (0.38 g), 0.91 mmol **7** (0.20 g), 1.82 mmol KI (0.30 g), 2.73 mmol K_2CO_3 (0.38 g) and 15 mL acetonitrile; Yield 30%; light-yellow oil; R_f: 0.86 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.83–1.96 (m, 1H, NCHC*H*$_2$), 2.04–2.15 (m, 1H, NCHC*H*$_2$), 2.21–2.30 (m, 2H, C=CHC*H*$_2$), 2.54–2.71 (m, 2H, *CH*$_2$NH), 3.09 (dd, *J* = 7.18, 5.90 Hz, 1H, NC*H*), 3.75 (dq, *J* = 13.79, 7.20 Hz, 2H, *CH*$_2$ phthalimide), 4.34 (d, *J* = 6.16 Hz, 2H, NHC*H*$_2$), 6.05 (t, *J* = 7.31 Hz, 1H, C=C*H*), 6.87–7.04 (m, 2H, arom), 7.08–7.44 (m, 12H, arom), 7.61 (t, *J* = 5.90 Hz, 1H, CO*NH*), 7.66–7.76 (m, 2H, *phthalimide*), 7.77–7.88 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 562.65 [M+H]$^+$.

*6.4.5. 4-(1,3-Dioxoisoindolin-2-yl)-2-((4,4-diphenylbut-3-en-1-yl) amino)-N-(4-methylbenzyl)butanamide (**10e**)*

0.91 mmol **3e** (0.38 g), 0.91 mmol **7** (0.20 g), 1.82 mmol KI (0.30 g), 2.73 mmol K_2CO_3 (0.38 g) and 15 mL acetonitrile; Yield 32%; light-yellow oil; R_f: 0.85 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.85–1.97 (m, 1H, NCHC*H*$_2$), 2.07–2.16 (m, 1H, NCHC*H*$_2$), 2.21–2.28 (m, 2H, C=CHC*H*$_2$), 2.31 (s, 3H, C*H*$_3$), 2.56–2.70 (m, 2H, *CH*$_2$NH), 3.08 (dd, *J* = 7.44, 5.64 Hz, 1H, NC*H*), 3.69–3.82 (m, 2H, *CH*$_2$ phthalimide), 4.34 (d, *J* = 5.90 Hz, 2H, NHC*H*$_2$), 6.05 (t, *J* = 7.44 Hz, 1H, *CH*Ph$_2$), 7.03–7.30 (m, 12H, arom), 7.30–7.42 (m, 2H, arom), 7.53 (t, *J* = 6.03 Hz, 1H, CO*NH*), 7.66–7.76 (m, 2H, *phthalimide*), 7.76–7.92 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 578.65 [M+H]$^+$.

6.5. General procedure for the synthesis of N-benzyl-4-(1,3-dioxoisoindolin-2-yl)-2-((4,4-diphenylbutyl)(methyl)amino) butanamide derivatives (**11a-e**)

1.50 mmol **3(a–e)**, 1.50 mmol *N*-Methyl-4,4-diphenylbutan-1-amine (**8**; 0.36 g), 3.07 mmol KI (0.51 g), 9.11 mmol K_2CO_3 (1.26 g) and 15 mL acetone. After the addition was complete, the reaction mixture was heated under reflux for 20 h. The precipitated salt was filtered and the filtrate was concentrated. The crude oily product was purified by column chromatography (SiO_2:DCM/acetone 9:1).

6.5.1. N-benzyl-4-(1,3-dioxoisoindolin-2-yl)-2-((4,4-diphenylbutyl)(methyl)amino)butanamide (**11a**)

1.50 mmol **3a** (0.60 g), 1.50 mmol **8** (0.36 g), 3.07 mmol KI (0.51 g), 9.11 mmol K_2CO_3 (1.26 g) and 15 mL acetone; Yield 31%; light-yellow oil; R_f: 0.86 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.27–1.39 (m, 2H, CH_2CH_2N), 1.82–1.96 (m, 4H, ArCHCH_2; CH_2CH_2 phthalimide), 2.12 (s, 3H, CH_3), 2.38 (t, J = 7.05 Hz, 2H, CH_2N), 3.15 (dd, J = 8.59, 3.98 Hz, 1H, NC*H*), 3.72–3.85 (m, 2H, CH_2 phthalimide), 3.89–4.00 (m, 1H, C*H*Ph$_2$), 4.35–4.50 (m, 2H, NHCH_2), 7.07–7.32 (m, 15H, arom), 7.33–7.39 (m, 1H, CO*NH*), 7.64–7.73 (m, 2H, *phthalimide*), 7.79–7.87 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 545.67 $[M+H]^+$.

6.5.2. N-(2-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl)-2-((4,4-diphenylbutyl)(methyl)amino)butanamide (**11b**)

1.50 mmol **3b** (0.65 g), 1.50 mmol **8** (0.36 g), 3.07 mmol KI (0.51 g), 9.11 mmol K_2CO_3 (1.26 g) and 15 mL acetone; Yield 35%; light-yellow oil; R_f: 0.88 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.29–1.43 (m, 2H, CH_2CH_2N), 1.88–1.95 (m, 4H, Ph$_2$CHCH_2; CH_2CH_2 phthalimide), 2.10 (s, 3H, CH_3), 2.38 (t, J = 7.18 Hz, 2H, CH_2N), 3.14 (dd, J = 8.59, 3.98 Hz, 1H, NC*H*), 3.78 (quin, J = 7.12 Hz, 2H, CH_2 phthalimide), 3.88–3.98 (m, 1H, C*H*Ph$_2$), 4.44–4.56 (m, 2H, NHCH_2), 7.10–7.39 (m, 14H, arom), 7.49 (t, J = 6.16 Hz, 1H, CO*NH*), 7.64–7.73 (m, 2H, *phthalimide*), 7.77–7.86 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 581.12 $[M+H]^+$.

6.5.3. N-(4-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl)-2-((4,4-diphenylbutyl)(methyl)amino)butanamide (**11c**)

1.50 mmol **3c** (0.65 g), 1.50 mmol **8** (0.36 g), 3.07 mmol KI (0.51 g), 9.11 mmol K_2CO_3 (1.26 g) and 15 mL acetone; Yield 37%; light-yellow oil; R_f: 0.83 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.30–1.39 (m, 2H, CH_2CH_2N), 1.56–1.66 (m, 2H, Ph$_2$CHCH_2), 1.86–1.95 (m, 2H, CH_2CH_2 phthalimide), 2.04 (s, 3H, CH_3), 2.38 (t, J = 7.18 Hz, 2H, CH_2N), 3.14 (dd, J = 8.72, 3.85 Hz, 1H, NC*H*), 3.73–3.84 (m, 2H, CH_2 phthalimide), 3.87–3.98 (m, 1H, C*H*Ph$_2$), 4.30–4.43 (m, 2H, NHCH_2), 7.09–7.20 (m, 6H, arom), 7.20–7.31 (m, 8H, arom), 7.36 (d, J = 4.36 Hz, 1H, CO*NH*), 7.65–7.74 (m, 2H, *phthalimide*), 7.78–7.89 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 581.12 $[M+H]^+$.

6.5.4. 4-(1,3-Dioxoisoindolin-2-yl)-2-((4,4-diphenylbutyl)(methyl) amino)-N-(4-fluorobenzyl)butanamide (**11d**)

1.50 mmol **3d** (0.63 g), 1.50 mmol **8** (0.36 g), 3.07 mmol KI (0.51 g), 9.11 mmol K_2CO_3 (1.26 g) and 15 mL acetone; Yield 52%; light-yellow oil; R_f: 0.87 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.26–1.39 (m, 2H, CH_2CH_2N), 1.85–1.95 (m, 4H, Ph$_2$CHCH_2; CH_2CH_2 phthalimide), 2.12 (s, 3H, CH_3), 2.38 (t, J = 7.18 Hz, 2H, CH_2N), 3.14 (dd, J = 8.72, 3.85 Hz, 1, H, NC*H*), 3.72–3.84 (m, 2H, CH_2 phthalimide), 3.87–3.96 (m, 1H, C*H*Ph$_2$), 4.30–4.42 (m, 2H, NHCH_2), 6.90–7.04 (m, 2H, arom), 7.09–7.29 (m, 12H, arom), 7.33 (t, J = 6.16 Hz, 1H, CO*NH*), 7.66–7.75 (m, 2H, *phthalimide*), 7.76–7.91 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 564.66 $[M+H]^+$.

6.5.5. 4-(1,3-Dioxoisoindolin-2-yl)-2-((4,4-diphenylbutyl)(methyl) amino)-N-(4-methylbenzyl)butanamide (**11e**)

1.50 mmol **3e** (0.62 g), 1.50 mmol **8** (0.36 g), 3.07 mmol KI (0.51 g), 9.11 mmol K_2CO_3 (1.26 g) and 15 mL acetone; Yield 41%; light-yellow oil; R_f: 0.90 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.28–1.37 (m, 2H, CH_2CH_2N), 1.78–1.99 (m, 4H, Ph$_2$CHCH_2; CH_2CH_2 phthalimide), 2.11 (s, 3H, CH_3), 2.31 (s, 3H, CH_3Ph), 2.38 (t, J = 7.31 Hz, 2H, CH_2N), 3.13 (dd, J = 8.59, 3.98 Hz, 1H, NC*H*), 3.73–3.82 (m, 2H, CH_2 phthalimide), 3.89–3.96 (m, 1H, C*H*Ph$_2$), 4.37 (dd, J = 12.31, 5.90 Hz, 2H, NHCH_2), 7.06–7.16 (m, 10H, arom), 7.16–7.18 (m, 1H, CO*NH*), 7.23 (d, J = 7.69 Hz, 4H, arom), 7.67–7.71 (m, 2H, *phthalimide*), 7.80–7.86 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 560.70 $[M+H]^+$.

6.6. General procedure for the synthesis of N-benzyl-4-(1,3-dioxoisoindolin-2-yl)-2-((4,4-diphenylbut-3-en-1-yl)(methyl) amino)butanamide derivatives (**12a–e**)

1.84 mmol **3(a–e)**, 1.88 mmol *N*-Methyl-4,4-diphenylbut-3-en-1-amine (**6**; 0.45 g), 3.88 mmol KI (0.30 g), 4.60 mmol K_2CO_3 (0.64 g) and 15 mL acetone. After the addition was complete, the reaction mixture was heated under reflux for 20 h. The precipitated salt was filtered and the filtrate was concentrated. The crude oily product was purified by column chromatography (SiO_2:DCM/acetone 9:1).

6.6.1. N-benzyl-4-(1,3-dioxoisoindolin-2-yl)-2-((4,4-diphenylbut-3-en-1-yl)(methyl)amino)butanamide (**12a**)

1.84 mmol **3a** (0.74 g), 1.88 mmol **6** (0.45 g), 3.88 mmol KI (0.30 g), 4.60 mmol K_2CO_3 (0.64 g) and 15 mL acetone. Yield 65%; light-yellow oil; R_f: 0.85 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.13–2.31 (m, 4H, CH_2CH_2N; C=CHCH_2) 2.36 (s, 3H, CH_3) 2.55 (t, J = 6.92 Hz, 2H, CH_2N), 3.18 (dd, J = 8.34, 3.98 Hz, 1H, NC*H*), 3.72–3.82 (m, 1H, CH_2 phthalimide), 3.89–3.99 (m, 1H, CH_2 phthalimide), 4.32–4.44 (m, 2H, NHCH_2), 5.98 (t, J = 7.31 Hz, 1H, C*H*Ph$_2$), 7.11–7.34 (m, 15H, arom), 7.42 (t, J = 6.80 Hz, 1H, CO*NH*), 7.66–7.72 (m, 2H, *phthalimide*), 7.80–7.87 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 558.68 $[M+H]^+$.

6.6.2. N-(2-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl)-2-((4,4-diphenylbut-3-en-1-yl)(methyl)amino)butanamide (**12b**)

1.84 mmol **3b** (0.80 g), 1.88 mmol **6** (0.45 g), 3.88 mmol KI (0.30 g), 4.60 mmol K_2CO_3 (0.64 g) and 15 mL acetone. Yield 38%; light-yellow oil; R_f: 0.89 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.77–1.98 (m, 2H, CH_2CH_2N), 2.12 (s, 3H, CH_3), 2.18–2.26 (m, 2H, C=CHCH_2), 2.46–2.58 (m, 2H, CH_2N), 3.17 (dd, J = 8.34, 4.23 Hz, 1H, NC*H*), 3.69–3.82 (m, 1H, CH_2 phthalimide), 3.88–3.98 (m, 1H, CH_2 phthalimide), 4.38–4.54 (m, 2H, NHCH_2), 5.98 (t, J = 7.44 Hz, 1H, C*H*Ph$_2$), 7.04–7.39 (m, 14H, arom), 7.53 (t, J = 6.03 Hz, 1H, CO*NH*), 7.65–7.74 (m, 2H, *phthalimide*), 7.78–7.89 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 593.13 $[M+H]^+$.

6.6.3. N-(4-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl)-2-((4,4-diphenylbut-3-en-1-yl)(methyl)amino)butanamide (**12c**)

1.84 mmol **3c** (0.80 g), 1.88 mmol **6** (0.45 g), 3.88 mmol KI (0.30 g), 4.60 mmol K_2CO_3 (0.64 g) and 15 mL acetone. Yield 45%; light-yellow oil; R_f: 0.80 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.78–1.99 (m, 1H, CH_2CH_2N), 2.11 (s, 3H, CH_3), 2.17–2.27 (m, 3H, CH_2CH_2N, C=CHCH_2), 2.54 (t, J = 6.80 Hz, 2H, CH_2N), 3.17 (dd, J = 8.59, 3.98 Hz, 1H, NC*H*), 3.78 (dd, J = 14.36, 6.92 Hz, 1H, CH_2 phthalimide), 3.88–3.98 (m, 1H, CH_2 phthalimide), 4.22–4.39 (m, 2H, NHCH_2), 5.98 (t, J = 7.44 Hz, 1H, C*H*Ph$_2$), 7.00–7.10 (m, 4H(arom), 7.10–7.36 (m, 10H, *arom*), 7.46 (t, J = 6.28 Hz, 1H, CO*NH*), 7.64–7.73 (m, 2H, *phthalimide*), 7.79–7.90 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 593.13 $[M+H]^+$.

*6.6.4. 4-(1,3-Dioxoisoindolin-2-yl)-2-((4,4-diphenylbut-3-en-1-yl)(methyl)amino)-N-(4-fluorobenzyl)butanamide (**12d**)*

1.84 mmol **3d** (0.77 g), 1.88 mmol **6** (0.45 g), 3.88 mmol KI (0.30 g), 4.60 mmol K_2CO_3 (0.64 g) and 15 mL acetone. Yield 67%; light-yellow oil; R_f: 0.83 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.79–1.99 (m, 2H, CH_2CH_2N), 2.11 (s, 3H, CH_3), 2.17–2.25 (m, 2H, C=CHCH_2), 2.53 (t, J = 6.67 Hz, 2H, CH_2N), 3.16 (dd, J = 8.46, 3.85 Hz, 1H, NC*H*), 3.70–3.82 (m, 1H, CH_2 phthalimide), 3.87–3.99 (m, 1H, CH_2 phthalimide), 4.22–4.39 (m, 2H, NHCH_2), 5.98 (t, J = 7.31 Hz, 1H, $CHPh_2$), 6.87–6.97 (m, 2H, arom), 7.02–7.18 (m, 5H, *arom*), 7.18–7.36 (m, 7H, *arom*), 7.43 (t, J = 5.90 Hz, 1H, CO*NH*), 7.66–7.74 (m, 2H, *phthalimide*), 7.78–7.87 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 596.67 $[M+H]^+$.

*6.6.5. 4-(1,3-Dioxoisoindolin-2-yl)-2-((4,4-diphenylbut-3-en-1-yl)(methyl)amino)-N-(4-methylbenzyl)butanamide (**12e**)*

1.84 mmol **3e** (0.76 g), 1.88 mmol **6** (0.45 g), 3.88 mmol KI (0.30 g), 4.60 mmol K_2CO_3 (0.64 g) and 15 mL acetone. Yield 86%; light-yellow oil; R_f: 0.88 (S_1); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.79–2.00 (m, 2H, CH_2CH_2N), 2.10 (s, 3H, CH_3), 2.30 (s, 3H, CH_3Ph), 2.19–2.21 (m, 2H, C=CHCH_2), 2.48–2.56 (m, 2H, CH_2N), 3.16 (dd, J = 8.46, 4.10 Hz, 1H, NC*H*), 3.78 (dd, J = 14.23, 7.82 Hz, 1H, CH_2 phthalimide), 3.90–3.98 (m, 1H, CH_2 phthalimide), 4.27–4.40 (m, 2H, NHCH_2), 5.97 (t, J = 7.44 Hz, 1H, Ar*CH*), 7.01–7.08 (m, 5H, *arom*), 7.08–7.25 (m, 6H, *arom*), 7.29 (s, 3H, arom), 7.32–7.39 (m, 1H, CO*NH*), 7.67–7.72 (m, 2H (*phthalimide*), 7.80–7.86 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 572.71 $[M+H]^+$.

*6.7. General procedure for the synthesis of N-benzyl-4-(1,3-dioxoisoindolin-2-yl)-2-(4-(diphenylmethylene)piperidin-1-yl) butanamide derivatives (**13a**–**e**)*

3.58 mmol (**3a**–**e**), 3.58 mmol 4-diphenylmethylene piperidine (**4**; 0.89 g), 7.16 mmol KI (1.19 g), 10.74 mmol K_2CO_3 (1.48 g) were suspended in acetone (20 mL). Then the reaction mixture was stirred and heated at reflux for 24 h. The precipitated salt was filtered and the filtrate was concentrated. The crude product was purified by recrystallization from methanol.

*6.7.1. N-benzyl-4-(1,3-dioxoisoindolin-2-yl)-2-(4-(diphenylmethylene)piperidin-1-yl)butanamide (**13a**)*

3.58 mmol **3a** (1.44 g), 3.58 mmol **4** (0.89 g), 7.16 mmol KI (1.19 g), 10.74 mmol K_2CO_3 (1.48 g) and 20 mL acetone. Yield 63%; light yellow solid; mp 154.2 °C; R_f: 0.74 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.93–2.03 (m, 1H, CHCH_2CH_2), 2.09–2.19 (m, 1H, CHCH_2CH_2, 2.21–2.40 (m, 4H, *piperidine*), 2.46–2.67 (m, 4H, *piperidine*), 3.16 (dd, J = 8.85, 3.98 Hz, 1H, *CH*CH_2CH_2), 3.75–3.85 (m, 1H, $CHCH_2CH_2$), 3.95 (dd, J = 13.72, 7.69, 5.77 Hz, 1H, $CHCH_2CH_2$), 4.37 (dd, J = 14.88, 5.64 Hz, 1H, ArCH_2), 4.49 (dd, J = 15.00, 6.28 Hz, 1H,(ArCH_2), 7.01–7.11 (m, 5H, arom), 7.13–7.36 (m, 10H, arom), 7.45 (t, J = 6.03 Hz, 1H, *NH*CO), 7.63–7.76 (m, 2H, *phthalimide*), 7.78–7.89 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 570.69 $[M+H]^+$.

*6.7.2. N-(2-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl)-2-(4-(diphenylmethylene)piperidin-1-yl)butanamide (**13b**)*

3.58 mmol **3b** (1.56 g), 3.58 mmol **4** (0.89 g), 7.16 mmol KI (1.19 g), 10.74 mmol K_2CO_3 (1.48 g) and 20 mL acetone. Yield 73%; light yellow solid; mp 141.9 °C; R_f: 0.69 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.88–2.08 (m, 2H, CHCH_2CH_2), 2.23–2.41 (m, 4H, *piperidine*), 2.44–2.67 (m, 4H, *piperidine*), 3.12–3.21 (m, 1H, *CH*CH_2CH_2), 3.78–3.87 (m, 1H, $CHCH_2CH_2$), 3.93 (dd, J = 8.08, 5.77 Hz, 1H, $CHCH_2CH_2$), 4.47–4.60 (m, 2H, PhCH_2), 7.02–7.12 (m, 3H, arom), 7.15–7.38 (m, 11H, arom), 7.40–7.46 (m, 1H, *NH*CO), 7.67–7.77 (m, 2H, *phthalimide*), 7.84 (td, J = 5.00, 3.08 Hz, 2H, *phthalimide*); ESI-MS (*m*/*z*) 605.14 $[M+H]^+$.

*6.7.3. N-(4-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl)-2-(4-(diphenylmethylene)piperidin-1-yl)butanamide (**13c**)*

3.58 mmol **3c** (1.56 g), 3.58 mmol **4** (0.89 g), 7.16 mmol KI (1.19 g), 10.74 mmol K_2CO_3 (1.48 g) and 20 mL acetone. Yield 54%; light yellow solid; mp 178.3 °C; R_f: 0.71 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.93–2.03 (m, 1H, CHCH_2CH_2), 2.09–2.19 (m, 1H, CHCH_2CH_2), 2.21–2.40 (m, 4H, *piperidine*), 2.46–2.67 (m, 4H, *piperidine*), 3.16 (dd, J = 8.85, 3.98 Hz, 1H, *CH*CH_2CH_2), 3.75–3.85 (m, 1H, $CHCH_2CH_2$), 3.95 (ddd, J = 13.72, 7.69, 5.77 Hz, 1H, $CHCH_2CH_2$), 4.37 (dd, J = 14.88, 5.64 Hz, 1H, PhCH_2), 4.49 (dd, J = 15.00, 6.28 Hz, 1H, PhCH_2), 7.01–7.11 (m, 4H, arom), 7.13–7.36 (m, 10H, arom), 7.45 (t, J = 6.03 Hz, 1H, *NH*CO), 7.63–7.76 (m, 2H, *phthalimide*), 7.78–7.89 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 605.14 $[M+H]^+$.

*6.7.4. 4-(1,3-Dioxoisoindolin-2-yl)-2-(4-(diphenylmethylene) piperidin-1-yl)-N-(4-fluorobenzyl)butanamide (**13d**)*

3.58 mmol **3d** (1.50 g), 3.58 mmol **4** (0.89 g), 7.16 mmol KI (1.19 g), 10.74 mmol K_2CO_3 (1.48 g) and 20 mL acetone. Yield 44%; light yellow solid; mp 168.2 °C; R_f: 0.75 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.92–2.03 (m, 1H, CHCH_2CH_2), 2.09–2.19 (m, 1H, CHCH_2CH_2), 2.21–2.38 (m, 4H, *piperidine*), 2.47–2.64 (m, 4H, *piperidine*), 3.15 (dd, J = 8.72, 3.85 Hz, 1H, *CH*CH_2CH_2), 3.75–3.86 (m, 1H, $CHCH_2CH_2$), 3.90–4.01 (m, 1H, $CHCH_2CH_2$), 4.37 (dd, J = 14.87, 5.64 Hz, 1H, PhCH_2), 4.49 (dd, J = 14.75, 6.28 Hz, 1H, PhCH_2), 6.96–7.11 (m, 4H, arom), 7.15–7.33 (m, 10H, arom), 7.43 (t, J = 6.03 Hz, 1H, *NH*CO), 7.65–7.76 (m, 2H, *phthalimide*), 7.77–7.89 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 588.68 $[M+H]^+$.

*6.7.5. 4-(1,3-Dioxoisoindolin-2-yl)-2-(4-(diphenylmethylene) piperidin-1-yl)-N-(4-methylbenzyl)butanamide (**13e**)*

3.58 mmol **3e** (1.49 g), 3.58 mmol **4** (0.89 g), 7.16 mmol KI (1.19 g), 10.74 mmol K_2CO_3 (1.48 g) and 20 mL acetone. Yield 84%; light yellow solid; mp 149.7 °C; R_f: 0.71 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.98 (td, J = 10.32, 7.05 Hz, 1H, CHCH_2CH_2), 2.09–2.19 (m, 1H, CHCH_2CH_2), 2.19–2.41 (m, 7H, *piperidine*, PhCH_3), 2.45–2.70 (m, 4H, *piperidine*), 3.16 (dd, J = 8.46, 4.10 Hz, 1H, *CH*CH_2CH_2), 3.76–3.87 (m, 1H, $CHCH_2CH_2$), 3.90–4.02 (m, 1H, $CHCH_2CH_2$), 4.37 (dd, J = 14.62, 5.39 Hz, 1H, PhCH_2), 4.49 (dd, J = 14.62, 6.16 Hz, 1H, PhCH_2), 7.01–7.31 (m, 14H, arom), 7.32–7.42 (m, 1H, *NH*CO), 7.66–7.74 (m, 2H, *phthalimide*), 7.78–7.89 (m, 2H, *phthalimide*); ESI-MS (*m*/*z*) 584.72 $[M+H]^+$.

*6.8. General procedure for the synthesis of 2-(4-benzhydrylpiperidin-1-yl)-N-benzyl-4-(1,3-dioxoisoindolin-2-yl) butanamide derivatives (**14a**–**e**)*

2.2 mmol **3a**–**e**, 2.2 mmol **5** (0.55 g), 4.4 mmol KI (0.73 g), 6.6 mmol K_2CO_3 (0.91 g) and 15 mL acetone was stirred and refluxed for 24 h. After the reaction was completed the precipitate was filtered and the filtrate was concentrated under vacuum. Crude product was recrystallized from methanol.

*6.8.1. 2-(4-Benzhydrylpiperidin-1-yl)-N-benzyl-4-(1,3-dioxoisoindolin-2-yl)butanamide (**14a**)*

2.2 mmol **3a** (0.88 g), 2.2 mmol **5** (0.55 g), 4.4 mmol KI (0.73 g), 6.6 mmol K_2CO_3 (0.91 g) and 15 mL acetone. Yield 60%; light yellow solid; mp 184.2 °C; R_f: 0.61 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.92–1.18 (m, 2H, CHCH_2CH_2), 1.47–1.63 (m, 2H, CHCH_2CH_2), 1.91–2.22 (m, 4H, *piperidine*), 2.29–2.41 (m, 1H, *piperidine*),2.61–2.75 (m, 2H, *CH*CH_2CH_2), 3.05–3.12 (m, 1H, *CH*CH_2CH_2), 3.41 (d, J = 10.77 Hz, 1H, Ph_2CH), 3.72–3.83 (m, 1H,

CHCH$_2$*CH$_2$*), 3.83–3.93 (m, 1H, CHCH$_2$*CH$_2$*), 4.40 (dd, J = 11.54, 5.90 Hz, 2H, Ph*CH$_2$*)) 6.96–7.08 (m, 2H, arom), 7.08–7.29 (m, 11H, arom), 7.38 (t, J = 6.16 Hz, 1H, *NH*CO), 7.67–7.75 (m, 2H, *phthalimide*), 7.79–7.87 (m, 2H, *phthalimide*); ESI-MS (m/z) 572.71 [M+H]$^+$.

6.8.2. 2-(4-Benzhydrylpiperidin-1-yl)-N-(2-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl)butanamide (**14b**)

2.2 mmol **3b** (0.96 g), 2.2 mmol **5** (0.55 g), 4.4 mmol KI (0.73 g), 6.6 mmol K_2CO_3 (0.91 g) and 15 mL acetone. Yield 66%; light yellow solid; mp 156.6 °C; R_f: 0.62 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.52 (d, J = 13.34 Hz, 2H, *piperidine*), 1.62 (br. s., 2H, *piperidine*), 1.96 (br. s., 1H, *piperidine*), 2.00–2.21 (m, 3H, *piperidine*), 2.29–2.39 (m, 1H, *piperidine*), 2.58–2.74 (m, 2H, CH*CH$_2$*CH$_2$), 3.01–3.14 (m, 1H, *CH*CH$_2$CH$_2$), 3.44 (d, J = 10.77 Hz, 1H, Ph$_2$*CH*), 3.72–3.83 (m, 1H, CHCH$_2$*CH$_2$*), 3.85–3.97 (m, 1H, CHCH$_2$*CH$_2$*), 4.48–4.57 (m, 2H, Ph*CH$_2$*), 7.13 (d, J = 5.64 Hz, 2H, arom), 7.18–7.29 (m, 9H, arom), 7.29–7.47 (m, 3H, arom), 7.60 (br. s., 1H, *NH*CO), 7.68–7.74 (m, 2H, *phthalimide*), 7.80–7.86 (m, 2H, *phthalimide*); ESI-MS (m/z) 607.15 [M+H]$^+$.

6.8.3. 2-(4-Benzhydrylpiperidin-1-yl)-N-(4-chlorobenzyl)-4-(1,3-dioxoisoindolin-2-yl)butanamide (**14c**)

2.2 mmol **3c** (0.96 g), 2.2 mmol **5** (0.55 g), 4.4 mmol KI (0.73 g), 6.6 mmol K_2CO_3 (0.91 g) and 15 mL acetone. Yield 47%; light yellow solid; mp 208.4 °C; R_f: 0.60 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.42–1.74 (m, 4H, *piperidine*), 1.90–2.07 (m, 2H, CH*CH$_2$*CH$_2$), 2.09–2.17 (m, 3H, *piperidine*), 2.31–2.42 (m, 1H, *piperidine*), 2.60–2.76 (m, 2H, *piperidine*), 3.09 (dd, J = 7.69, 3.33 Hz, 1H, *CH*CH$_2$CH$_2$), 3.42 (d, J = 10.77 Hz, 1H, Ph$_2$*CH*), 3.71–3.83 (m, 1H, CHCH$_2$*CH$_2$*), 3.84–3.96 (m, 1H, CHCH$_2$*CH$_2$*), 4.35–4.46 (m, 2H, Ph*CH$_2$*), 7.11–7.34 (m, 14H, arom), 7.37–7.45 (m, 1H, *NH*CO), 7.67–7.74 (m, 2H, *phthalimide*), 7.79–7.86 (m, 2H, *phthalimide*); ESI-MS (m/z) 607.15 [M+H]$^+$.

6.8.4. 2-(4-Benzhydrylpiperidin-1-yl)-4-(1,3-dioxoisoindolin-2-yl)-N-(4-fluorobenzyl)butanamide (**11d**)

2.2 mmol **3d** (0.92 g), 2.2 mmol **5** (0.55 g), 4.4 mmol KI (0.73 g), 6.6 mmol K_2CO_3 (0.91 g) and 15 mL acetone. Yield 67%; light yellow solid; mp 185.4 °C; R_f: 0.56 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.44–1.76 (m, 4H, *piperidine*), 1.92–2.09 (m, 2H, CH*CH$_2$*CH$_2$), 2.11–2.19 (m, 3H, *piperidine*), 2.33–2.44 (m, 1H, *piperidine*), 2.62–2.78 (m, 2H, *piperidine*), 3.11 (dd, J = 7.67, 3.31 Hz, 1H, *CH*CH$_2$CH$_2$), 3.44 (d, J = 10.78 Hz, 1H, Ph$_2$*CH*), 3.73–3.85 (m, 1H, CHCH$_2$*CH$_2$*), 3.86–3.98 (m, 1H, CHCH$_2$*CH$_2$*), 4.37–4.48 (m, 2H, Ph*CH$_2$*), 7.13–7.36 (m, 14H, arom), 7.39–7.47 (m, 1H, *NH*CO), 7.69–7.76 (m, 2H, *phthalimide*), 7.81–7.88 (m, 2H, *phthalimide*); ESI-MS (m/z) 590.70 [M+H]$^+$.

6.8.5. 2-(4-Benzhydrylpiperidin-1-yl)-4-(1,3-dioxoisoindolin-2-yl)-N-(4-methylbenzyl)butanamide (**14e**)

2.2 mmol **3e** (0.91 g), 2.2 mmol **5** (0.55 g), 4.4 mmol KI (0.73 g), 6.6 mmol K_2CO_3 (0.91 g) and 15 mL acetone. Yield 76%; light yellow solid; mp 179.3 °C; R_f: 0.67 (S_9); ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.95–1.17 (m, 2H, CH*CH$_2$*CH$_2$), 1.45–1.57 (m, 2H, *piperidine*), 1.89–2.23 (m, 4H, *piperidine*), 2.34 (s, 4H, *piperidine*, Ph*CH$_3$*), 2.63–2.75 (m, 2H, *piperidine*), 3.09 (d, J = 4.36 Hz, 1H, *CH*CH$_2$CH$_2$), 3.41 (d, J = 11.03 Hz, 1H, Ph*CH*), 3.77–3.86 (m, 1H, CHCH$_2$*CH$_2$*), 3.86–3.96 (m, 1H, CHCH$_2$*CH$_2$*), 4.32–4.45 (m, 2H, Ph*CH$_2$*), 7.08–7.19 (m, 6H, arom), 7.19–7.29 (m, 9H, arom), 7.31 (t, J = 6.28 Hz, 1H, *NH*CO), 7.66–7.76 (m, 2H, *phthalimide*), 7.77–7.89 (m, 2H, *phthalimide*); ESI-MS (m/z) 443.6 [M+H]$^+$.

6.9. General procedure for the synthesis of 2-substituted 4-aminobutanamide derivatives (**15–20a–e**)

A mixture of hydrazine monohydrate (0.26 g, 0.52 mmol) and **9–14(a–e)** (0.26 mmol) in ethanol (10 mL) was heated under reflux for 5 h. After the reaction mixture was cooled to room temperature, the precipitated was filtered and the filtrate was concentrated. The aqueous solution was extracted with DCM and the combined organic phases were washed with saturated aqueous NaCl, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (SiO_2:EtOAc/MeOH(8:2) → $NH_3 \cdot H_2O$/MeOH/DCM/PE (45:225:600:90).

6.9.1. 4-Amino-2-(4-benzhydrylpiperazin-1-yl)-N-benzylbutanamide (**15a**)

Hydrazine monohydrate (0.26 g, 0.52 mmol) and **9a** (0.15 g; 0.26 mmol), ethanol (10 mL) Yield 58%; white solid; mp 112.5–112.7 °C; R_f: 0.15 (N_1); Anal. Calcd for $C_{28}H_{34}N_4O$: C, 75.98; H, 7.74; N, 12.66. Found: C, 75.87; H, 7.66; N, 12.64. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.82–1.90 (m, 2H, CH*CH$_2$*CH$_2$), 2.36 (br. s., 4H, *Piperazine*), 2.50–2.65 (m, 4H, *piperazine*), 2.81–2.90 (m, 2H, *CH$_2$*NH$_2$), 3.05 (dd, J = 8.21, 5.13 Hz, 1H, N*CH*), 4.19 (s, 1H, Ph$_2$*CH*), 4.42 (dd, J = 5.90, 2.82 Hz, 2H, NH*CH$_2$*), 7.14–7.30 (m, 10H, arom), 7.30–7.43 (m, 6H, arom, CO*NH*); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 29.6 (*C*H$_2$CH), 36.7 (NH$_2$*C*H$_2$), 43.6 (*C*H$_2$NH), 52.5, 54.0 (piperazine), 70.8 (*C*HCO), 84.5 (*C*HPh$_2$), 126.3, 126.7, 127.4, 128.2, 128.5, 129.2, 137.9, 142.7 (arom), 171.3 (*C*=O); ESI-MS (m/z) 443.60 [M+H]$^+$.

6.9.2. 4-Amino-2-(4-benzhydrylpiperazin-1-yl)-N-(2-chlorobenzyl) butanamide (**15b**)

Hydrazine monohydrate (0.26 g, 0.52 mmol) and **9b** (0.16 g; 0.26 mmol), ethanol (10 mL) Yield 61%; white solid; mp 172.1–172.3 °C; R_f: 0.18 (N_1); Anal. Calcd for $C_{28}H_{33}ClN_4O$: C, 70.50; H, 6.97; N, 11.74; Found: C, 70.57; H, 7.00; N, 11.68; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.71–1.87 (m, 2H, CH*CH$_2$*CH$_2$), 2.37 (br. s., 4H, *piperazine*), 2.47–2.62 (m, 4H, *piperazine*), 2.72–2.88 (m, 2H, *CH$_2$*NH$_2$), 3.02 (dd, J = 8.08, 5.26 Hz, 1H, N*CH*), 4.20 (s, 1H, Ph$_2$*CH*), 4.48 (dd, J = 6.28, 1.41 Hz, 2H, NH*CH$_2$*), 7.15–7.41 (m, 14H, arom), 7.61 (t, J = 6.16 Hz, 1H, CO*NH*); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 31.3 (*C*H$_2$CH), 40.1 (NH$_2$*C*H$_2$), 41.3 (*C*H$_2$NH), 52.50 (piperazine), 66.51 (*C*HCO), 76.0 (*C*HPh$_2$), 127.0, 127.1, 127.9, 128.5, 128.9, 130.3, 133.6, 135.8, 142.5, 142.6 (arom), 173.1 (*C*=O); ESI-MS (m/z) 478.04 [M+H]$^+$.

6.9.3. 4-Amino-2-(4-benzhydrylpiperazin-1-yl)-N-(4-chlorobenzyl) butanamide (**15c**)

Hydrazine monohydrate (0.26 g, 0.52 mmol) and **9c** (0.16 g, 0.26 mmol), ethanol (10 mL) Yield 65%; white solid; mp 124.3–124.4 °C; R_f: 0.14 (N_1); Anal. Calcd for $C_{28}H_{33}ClN_4O$: C, 70.50; H, 6.97; N, 11.74 Found: C, 70.53; H, 7.03; N, 11.79 ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.75–1.87 (m, 2H, CH*CH$_2$*CH$_2$), 2.20–2.47 (m, 4H, *piperazine*), 2.47–2.63 (m, 4H, *piperazine*), 2.74–2.87 (m, 2H, *CH$_2$*NH$_2$), 3.05 (dd, J = 7.95, 5.13 Hz, 1H, N*CH*), 4.20 (s, 1H, Ph$_2$*CH*), 4.37 (dd, J = 5.90, 2.82 Hz, 2H, NH*CH$_2$*), 7.13–7.30 (m, 10H, arom), 7.34–7.41 (m, 4H, arom), 7.44 (t, J = 6.16 Hz, 1H, CO*NH*); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 29.6 (*C*H$_2$CH), 37.7 (NH$_2$*C*H$_2$), 43.6 (*C*H$_2$NH), 52.5, 54.0 (piperazine), 70.8 (*C*HCO), 84.5 (*C*HPh$_2$), 126.3, 126.7, 127.4, 128.2, 128.5, 129.2, 132.3, 142.7 (arom), 171.3 (*C*=O); ESI-MS (m/z) 478.04 [M+H]$^+$

6.9.4. 4-Amino-2-(4-benzhydrylpiperazin-1-yl)-N-(4-fluorobenzyl) butanamide (**15d**)

Hydrazine monohydrate (0.26 g, 0.52 mmol) and **9d** (0.15 g, 0.26 mmol), ethanol (10 mL) Yield 69%; white solid; mp

100.3–100.5 °C; R_f: 0.12 (N_1); Anal. Calcd for $C_{28}H_{33}FN_4O$: C, 73.02; H, 7.22; N, 12.16; found C, 73.07; H, 7.26; N,12.20; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.76–1.91 (m, 2H, CH*CH*$_2$CH$_2$), 2.36 (br. s., 4H, *piperazine*), 2.49–2.62 (m, 4H, *piperazine*), 2.77–2.92 (m, 2H, *CH*$_2$NH$_2$), 3.06 (dd, *J* = 8.21, 4.87 Hz, 1H, N*CH*), 4.19 (s, 1H, (Ph$_2$*CH*), 4.36 (dd, *J* = 5.90, 2.56 Hz, 2H, NH*CH*$_2$), 6.94–7.03 (m, 2H, *arom*), 7.16–7.30 (m, 8H, *arom*), 7.34–7.40 (m, 4H, arom), 7.44 (t, *J* = 6.16 Hz, 1H, (CO*NH*)); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 38.4 (*C*H$_2$CH), 39.3 (NH$_2$*C*H$_2$), 42.4 (*C*H$_2$NH), 52.1 (piperazine), 66.3 (*C*HCO), 76.0 (*C*HPh$_2$), 115.6, 127.8, 128.5, 129.3, 134.3, 142.5, 172.7 (arom), 173.3 (*C*=O); ESI-MS (*m*/*z*) 461.59 [M+H]$^+$

6.9.5. 4-Amino-2-(4-benzhydrylpiperazin-1-yl)-N-(4-methylbenzyl)butanamide (**15e**)

Hydrazine monohydrate (0.26 g, 0.52 mmol) and **9e** (0.15 g, 0.26 mmol), ethanol (10 mL) Yield 55%; white solid; mp 129.5–129.6 °C; R_f: 0.18 (N_1); Anal. Calcd for $C_{29}H_{36}N_4O$: C, 76.28; H, 7.95; N, 12.27; found C, 76.32; H, 8.03; N, 12.32; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.74–1.87 (m, 2H, CH*CH*$_2$CH$_2$), 2.32 (s, 3H, C*H*$_3$), 2.33–2.50 (m, 4H, *piperazine*), 2.50–2.67 (m, 4H, *piperazine*), 2.75–2.89 (m, 2H, *CH*$_2$NH$_2$), 3.02 (dd, *J* = 8.21, 4.87 Hz, 1H, N*CH*), 4.18 (s, 1H, Ph$_2$*CH*), 4.37 (dd, *J* = 5.77, 1.92 Hz, 2H, NH*CH*$_2$), 7.09–7.33 (m, 11H, *arom*), 7.33–7.42 (m, 4H, *arom*, CO*NH*); ^{13}C NMR (300 MHz, chloroform-*d*) δ ppm 21.3 (*C*H$_3$), 29.6 (*C*H$_2$CH), 37.6 (NH$_2$*C*H$_2$), 43.4 (*C*H$_2$NH), 52.5, 54.0 (piperazine), 70.8 (*C*HCO), 84.5 (*C*HPh$_2$), 126.3, 126.7, 127.4, 128.1, 128.8, 134.2, 136.9, 142.7 (arom), 171.3 (*C*=O); ESI-MS (*m*/*z*) 457.62 [M+H]$^+$

6.9.6. 4-Amino-N-benzyl-2-((4,4-diphenylbut-3-en-1-yl)amino) butanamide (**16a**)

Hydrazine monohydrate (0.21 g, 0.42 mmol) and **10a** (82 mg, 0.15 mmol), ethanol (5 mL) Yield 55%; yellow oil; R_f: 0.16 (H_7); Anal. Calcd for $C_{27}H_{31}N_3O$: C, 78.42; H, 7.56; N, 10.16; found C, 78.53; H, 7.60, N, 10.23; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.72–1.94 (m, 2H, NCH*CH*$_2$), 2.25 (q, *J* = 6.84 Hz, 2H, C=CH*CH*$_2$), 2.56–2.71 (m, 2H, *CH*$_2$NH), 2.74–2.86 (m, 2H, *CH*$_2$NH$_2$), 3.19 (dd, *J* = 7.57, 5.51 Hz, 1H, N*CH*), 4.42 (d, *J* = 5.90 Hz, 2H, NH*CH*$_2$), 6.02 (t, *J* = 7.44 Hz, 1H, Ph$_2$*CH*), 7.11–7.34 (m, 15H, *arom*), 7.66 (t, *J* = 5.64 Hz, 1H, CO*NH*); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 26.7 (*C*H$_2$CH=C), 31.8 (*C*H$_2$CH) 37.4 (NH$_2$*C*H$_2$), 43.6 (*C*H$_2$NH), 50.9 (NHCH$_2$CH$_2$), 63.6 (*C*HCO), 115.3 (*C*H=CH), 126.9, 128.7, 127.4, 128.5, 129.2, 137.9, 140.7 (arom), 133.8 (CHPh$_2$), 171.3 (*C*=O); ESI-MS (*m*/*z*) 414.55 [M+H]$^+$.

6.9.7. 4-Amino-N-(2-chlorobenzyl)-2-((4,4-diphenylbut-3-en-1-yl) amino)butanamide (**16b**)

Hydrazine monohydrate (0.21 g, 0.42 mmol) and **10b** (87 mg, 0.15 mmol), ethanol (5 mL) Yield 60%; yellow oil; R_f: 0.15 (H_7); Anal. Calcd for $C_{27}H_{30}ClN_3O$: C, 72.39; H, 6.75; N, 9.38; found: C, 72.45; H, 6.87; N, 9.46; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.76–2.01 (m, 2H, NCH*CH*$_2$), 2.18–2.25 (m, 2H, C=CH*CH*$_2$), 2.50–2.70 (m, 2H, *CH*$_2$NH), 2.74–2.97 (m, 2H, *CH*$_2$NH$_2$), 3.24–3.39 (m, 1H, N*CH*), 4.23–4.46 (m, 2H, NH*CH*$_2$), 5.93–6.06 (m, 1H, Ph$_2$*CH*), 6.82–7.05 (m, 3H, arom), 7.05–7.39 (m, 11H, arom), 7.49–7.72 (m, 1H, CO*NH*); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 26.7 (*C*H$_2$CH=C), 31.8 (*C*H$_2$CH) 37.4 (NH$_2$*C*H$_2$), 38.5 (*C*H$_2$NH), 50.9 (NHCH$_2$CH$_2$), 63.6 (*C*HCO), 115.3 (*C*H=CH), 126.9, 128.7, 127.4, 128.5, 129.2, 132.2, 137.9, 140.0, 140.7, 142.4 (arom), 133.8 (CHPh$_2$), 171.3 (*C*=O); ESI-MS (*m*/*z*) 449.00 [M+H]$^+$

6.9.8. 4-Amino-N-(4-chlorobenzyl)-2-((4,4-diphenylbut-3-en-1-yl) amino)butanamide (**16c**)

Hydrazine monohydrate (0.21 g, 0.42 mmol) and **10c** (87 mg, 0.15 mmol), ethanol (5 mL) Yield 51%; yellow oil; R_f: 0.14 (H_7); Anal. Calcd for $C_{27}H_{30}ClN_3O$: C, 72.39; H, 6.75; N, 9.38; found: C, 72.54; H, 6.85; N, 9.43; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.79–2.04 (m, 2H, NCH*CH*$_2$), 2.21–2.28 (m, 2H, C=CH*CH*$_2$), 2.53–2.73 (m, 2H, *CH*$_2$NH), 2.77–3.00 (m, 2H, *CH*$_2$NH$_2$), 3.27–3.41 (m, 1H, N*CH*), 4.26–4.49 (m, 2H, NH*CH*$_2$), 5.96–6.09 (m, 1H, Ph$_2$*CH*), 6.85–7.10 (m, 3H, arom), 7.11–7.42 (m, 11H, arom), 7.52–7.75 (m, 1H, CO*NH*); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 26.7 (*C*H$_2$CH=C), 31.8 (*C*H$_2$CH) 37.4 (NH$_2$*C*H$_2$), 43.6 (*C*H$_2$NH), 50.9 (NHCH$_2$CH$_2$), 63.6 (*C*HCO), 115.3 (*C*H=CH), 127.9, 128.3, 128.6, 129.2, 134.6, 136.0, 140.0 (arom), 133.8 (CHPh$_2$), 171.3 (*C*=O); ESI-MS (*m*/*z*) 414.55 [M+H]$^+$..

6.9.9. 4-Amino-2-((4,4-diphenylbut-3-en-1-yl)amino)-N-(4-fluorobenzyl)butanamide (**16d**)

Hydrazine monohydrate (0.21 g, 0.42 mmol) and **10d** (84 mg, 0.15 mmol), ethanol (5 mL) Yield 46%; yellow oil; R_f: 0.15 (H_7); Anal. Calcd for $C_{27}H_{30}FN_3O$: C, 75.15; H, 7.01; N, 9.74 found: C, 75.02; H, 6.94; N, 9.63; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.78–2.03 (m, 2H, NCH*CH*$_2$), 2.20–2.27 (m, 2H, C=CH*CH*$_2$), 2.52–2.72 (m, 2H, *CH*$_2$NH), 2.76–2.99 (m, 2H, *CH*$_2$NH$_2$), 3.26–3.41 (m, 1H, N*CH*), 4.25–4.48 (m, 2H, NH*CH*$_2$), 5.95–6.08 (m, 1H, Ph$_2$*CH*), 6.84–7.07 (m, 3H, arom) 7.07–7.41 (m, 11H, arom), 7.59–7.74 (m, 1H, CO*NH*); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 26.7 (*C*H$_2$CH=C), 31.8 (*C*H$_2$CH) 37.4 (NH$_2$*C*H$_2$), 43.6 (*C*H$_2$NH), 50.9 (NHCH$_2$CH$_2$), 63.6 (*C*HCO), 115.3 (*C*H=CH), 115.6, 127.9, 128.5, 128.6, 129.2, 137.9, 140.7, 160.9 (arom), 133.8 (CHPh$_2$), 171.3 (*C*=O); ESI-MS (*m*/*z*) 432.55 [M+H]$^+$

6.9.10. 4-Amino-2-((4,4-diphenylbut-3-en-1-yl)amino)-N-(4-methylbenzyl)butanamide (**16e**)

Hydrazine monohydrate (0.21 g, 0.42 mmol) and **10e** (84 mg, 0.15 mmol), ethanol (5 mL) Yield 59%; yellow oil; R_f: 0.16 (H_7); Anal. Calcd for $C_{28}H_{33}N_3O$: C, 78.65; H, 7.78; N, 9.83; found: C, 78.74; H, 7.65; N, 9.79; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.79–1.91 (m, 2H, NCH*CH*$_2$), 2.19–2.29 (m, 2H, C=CH*CH*$_2$), 2.29–2.33 (m, 3H, C*H*$_3$), 2.57–2.68 (m, 2H, *CH*$_2$NH), 2.72–2.93 (m, 2H, *CH*$_2$NH$_2$), 3.18 (dd, *J* = 7.57, 5.51 Hz, 1H, N*CH*), 4.37 (d, *J* = 5.90 Hz, 2H, NH*CH*$_2$), 5.99–6.07 (m, 1H, Ph$_2$*CH*), 7.09–7.36 (m, 14H, arom) 7.57 (d, *J* = 7.18 Hz, 1H, CO*NH*); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 21.3 (*C*H$_3$), 26.7 (*C*H$_2$CH=C), 31.8 (*C*H$_2$CH) 37.4 (NH$_2$*C*H$_2$), 43.6 (*C*H$_2$NH), 50.9 (NHCH$_2$CH$_2$), 63.6 (*C*HCO), 115.3 (*C*H=CH), 128.7, 127.4, 128.5, 129.2, 134.9, 137.9, 140.0 (arom), 133.8 (CHPh$_2$), 171.3 (*C*=O); ESI-MS (*m*/*z*) 428.58 [M+H]$^+$.

6.9.11. 4-Amino-N-benzyl-2-((4,4-diphenylbutyl)(methyl)amino) butanamide (**17a**)

Hydrazine monohydrate (0.26 g, 0.52 mmol) and **11a** (95 mg, 0.17 mmol), ethanol (5 mL) Yield 70%; yellow oil; R_f: 0.12 (H_7); Anal. Calcd for $C_{28}H_{35}N_3O$: C, 78.28; H, 8.21; N, 9.78; found: C, 78.32; H, 8.12; N, 9.63; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.33–1.41 (m, 2H, *CH*$_2$CH$_2$N), 1.71–1.87 (m, 2H, ArCH*CH*$_2$), 1.90–1.99 (m, 2H, *CH*$_2$CH$_2$NH$_2$), 2.13 (s, 3H, C*H*$_3$), 2.35–2.44 (m, 4H, *CH*$_2$N; NH$_2$), 2.79–2.91 (m, 2H, *CH*$_2$NH$_2$), 3.10 (dd, *J* = 8.59, 4.49 Hz, 1H, N*CH*), 3.81 (t, *J* = 7.82 Hz, 1H, Ph$_2$*CH*), 4.39 (dd, *J* = 6.03, 3.21 Hz, 2H, NH*CH*$_2$), 7.12–7.34 (m, 15H, arom), 7.47 (t, *J* = 5.90 Hz, 1H, CO*NH*); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 24.6 (*C*H$_2$CH$_2$CH), 29.3 (*C*H$_2$CH), 36.3 (*C*H$_2$CHPh$_2$), 37.7 (NH$_2$*C*H$_2$), 41.6 (*C*H$_3$N), 43.6 (*C*H$_2$NH), 50.0 (*C*H), 56.5 (NCH$_2$CH$_2$), 73.6 (*C*HCO), 126.9, 128.7, 127.4, 128.5, 129.2, 137.9, 140.7 (arom), 171.3 (*C*=O); ESI-MS (*m*/*z*) 430.60 [M+H]$^+$.

6.9.12. 4-Amino-N-(2-chlorobenzyl)-2-((4,4-diphenylbutyl)(methyl)amino)butanamide (**17b**)

Hydrazine monohydrate (0.26 g, 0.52 mmol) and **11b** (0.10 g, 0.17 mmol), ethanol (5 mL) Yield 62%; yellow oil; R_f: 0.15 (H_7); Anal. Calcd for $C_{28}H_{34}ClN_3O$: C, 72.47; H, 7.39; N, 9.06; found: C, 72.36; H, 7.21; N, 8.95; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.34–1.45 (m, 2H, *CH*$_2$CH$_2$N), 1.67–1.76 (m, 1H, Ph$_2$CH*CH*$_2$), 1.77–1.88 (m, 1H,

Ph_2CHCH_2), 1.92–2.04 (m, 4H, $CH_2CH_2NH_2$, NH_2), 2.11 (s, 3H, CH_3), 2.39 (t, J = 7.31 Hz, 2H, CH_2N), 2.70–2.80 (m, 1H, CH_2NH_2), 2.82–2.91 (m, 1H, CH_2NH_2), 3.07 (dd, J = 8.46, 4.62 Hz, 1H, NCH), 3.83 (t, J = 7.82 Hz, 1H, Ph_2CH), 4.41–4.53 (m, 2H, $NHCH_2$), 7.09–7.40 (m, 14H, arom), 7.61 (t, J = 6.16 Hz, 1H, CONH); ^{13}C NMR (75 MHz, chloroform-d) δ ppm 24.6 (CH_2CH_2CH), 29.3 (CH_2CH), 36.3 (CH_2CHPh_2), 37.7 (NH_2CH_2), 38.5 (CH_2NH), 41.6 (CH_3N), 50.0 (CH), 56.5 (NCH_2CH_2), 73.6 (CHCO), 126.9, 128.7, 127.4, 128.5, 129.2, 137.9, 140.7, 145.1 (arom), 171.3 (C=O); ESI-MS (m/z) 465.04 $[M+H]^+$

*6.9.13. 4-Amino-N-(4-chlorobenzyl)-2-((4,4-diphenylbutyl)(methyl)amino)butanamide (**17c**)*

Hydrazine monohydrate (0.26 g, 0.52 mmol) and **11c** (0.10 g, 0.17 mmol), ethanol (5 mL) Yield 57%; yellow oil; R_f: 0.10 (H_7); Anal. Calcd for $C_{28}H_{34}ClN_3O$: C, 72.47; H, 7.39; N, 9.06; found C, 72.30; H, 7.29; N, 8.99; ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.33–1.41 (m, 2H, CH_2CH_2N), 1.68–1.86 (m, 2H, Ph_2CHCH_2), 1.86–2.05 (m, 4H, $CH_2CH_2NH_2$, NH_2), 2.13 (s, 3H, CH_3), 2.36–2.47 (m, 2H, CH_2N), 2.82–2.91 (m, 2H, CH_2NH_2), 3.11 (dd, J = 8.59, 4.23 Hz, 1H, NCH), 3.82 (t, J = 7.95 Hz, 1H, Ph_2CH) 4.27–4.39 (m, 2H, $NHCH_2$), 7.08–7.32 (m, 14H, arom), 7.49–7.57 (m, 1H, CONH); ^{13}C NMR (75 MHz, chloroform-d) δ ppm 24.6 (CH_2CH_2CH), 29.3 (CH_2CH), 36.3 (CH_2CHPh_2), 37.7 (NH_2CH_2), 41.6 (CH_3N), 43.6 (CH_2NH), 50.0 (CH), 56.5 (NCH_2CH_2), 73.6 (CHCO), 126.2, 128.7, 127.4, 129.2, 137.9, 140.7, 145.1 (arom), 171.3 (C=O); ESI-MS (m/z) 465.04 $[M+H]^+$.

*6.9.14. 4-Amino-2-((4,4-diphenylbutyl)(methyl)amino)-N-(4-fluorobenzyl)butanamide (**17d**)*

Hydrazine monohydrate (0.26 g, 0.52 mmol) and **11d** (0.10 g, 0.17 mmol), ethanol (5 mL) Yield 61%; yellow oil; R_f: 0.22 (H_7); Anal. Calcd for $C_{28}H_{34}FN_3O$: C, 75.14; H, 7.66; N, 9.39; found: C, 75.00; H, 7.59; N, 9.25; ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.32–1.41 (m, 2H, CH_2CH_2N), 1.67–1.87 (m, 2H, Ph_2CHCH_2), 1.89–1.99 (m, 2H, $CH_2CH_2NH_2$) 2.13 (s, 3H, CH_3), 2.36–2.45 (m, 4H, CH_2N, NH_2), 2.78–2.92 (m, 2H, CH_2NH_2), 3.10 (dd, J = 8.46, 4.36 Hz, 1H, NCH), 3.78–3.85 (m, 1H, Ph_2CH), 4.34 (d, J = 6.16 Hz, 2H, $NHCH_2$), 6.92–7.03 (m, 2H, *arom*), 7.13–7.30 (m, 12H, *arom*), 7.49 (t, J = 6.28 Hz, 1H, CONH); ^{13}C NMR (75 MHz, chloroform-d) δ ppm 24.6 (CH_2CH_2CH), 29.3 (CH_2CH), 36.3 (CH_2CHPh_2), 37.7 (NH_2CH_2), 41.6 (CH_3N), 43.6 (CH_2NH), 50.0 (CH), 56.5 (NCH_2CH_2), 73.6 (CHCO), 115.3, 128.7, 127.4, 128.5, 129.2, 145.1, 160.7 (arom), 171.3 (C=O); ESI-MS (m/z) 448.59 $[M+H]^+$.

*6.9.15. 4-Amino-2-((4,4-diphenylbutyl)(methyl)amino)-N-(4-methylbenzyl)butanamide (**17e**)*

Hydrazine monohydrate (0.26 g, 0.52 mmol) and **11e** (98 mg, 0.17 mmol), ethanol (5 mL) Yield 56%; yellow oil; R_f: 0.17 (H_7); Anal. Calcd for $C_{29}H_{37}N_3O$: C, 78.51; H, 8.41; N, 9.47; found: C, 78.41; H, 8.21; N, 9.32; ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.29–1.40 (m, 2H, CH_2CH_2N), 1.71–1.87 (m, 2H, Ph_2CHCH_2), 1.88–1.98 (m, 2H, $CH_2CH_2NH_2$), 2.12 (s, 3H, CH_3), 2.28–2.35 (m, 3H, $PhCH_3$), 2.35–2.44 (m, 2H, CH_2N), 2.56 (br. s., 2H, NH_2), 2.86 (td, J = 6.92, 3.33 Hz, 2H, CH_2NH_2), 3.08 (dd, J = 8.46, 4.36 Hz, 1H, NCH), 3.80 (t, J = 7.69 Hz, 1H, Ph_2CH), 4.35 (dd, J = 5.77, 3.21 Hz, 2H, $NHCH_2$), 7.05–7.30 (m, 14H, arom), 7.43 (t, J = 6.03 Hz, 1H, CONH); ^{13}C NMR (75 MHz, chloroform-d) δ ppm 21.3 (CH_3), 24.6 (CH_2CH_2CH), 29.3 (CH_2CH), 36.3 (CH_2CHPh_2), 37.7 (NH_2CH_2), 41.6 (CH_3N), 43.6 (CH_2NH), 50.0 (CH), 56.5 (NCH_2CH_2), 73.6 (CHCO), 126.9, 128.7, 127.4, 128.5, 136.4, 137.9, 145.1 (arom), 171.3 (C=O); ESI-MS (m/z) 444.62 $[M+H]^+$.

*6.9.16. 4-Amino-N-benzyl-2-((4,4-diphenylbut-3-en-1-yl)(methyl)amino)butanamide (**18a**)*

Hydrazine monohydrate (2.00 g, 4 mmol) and **12a** (0.64 g, 1.17 mmol), ethanol (10 mL) Yield 48%; yellow oil; R_f: 0.74 (N_1); Anal. Calcd for $C_{28}H_{33}N_3O$: C, 78.65; H, 7.78; N, 9.83; found: C, 78.54; H, 7.65; N, 8.68; ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.70–1.81 (m, 1H, $NCHCH_2$), 1.83–1.94 (m, 1H, $NCHCH_2$), 2.11 (s, 3H, CH_3), 2.18–2.30 (m, 2H, $C{=}CHCH_2$), 2.49–2.60 (m, 2H, NH_2), 2.87 (t, J = 6.80 Hz, 2H, CH_2NH), 3.13 (dd, J = 8.59, 4.23 Hz, 1H, NCH), 3.41 (br. s., 2H, CH_2NH_2), 4.23–4.43 (m, 2H, $NHCH_2$), 6.01 (t, J = 7.44 Hz, 1H, Ph_2CH), 7.01–7.41 (m, 15H, arom), 7.60 (t, J = 6.16 Hz, 1H, CONH); ^{13}C NMR (75 MHz, chloroform-d) δ ppm 24.2 ($CH_2CH{=}C$), 29.3 (CH_2CH), 37.7 (NH_2CH_2), 41.7 (CH_3N), 43.6 (CH_2NH), 57.3 (NCH_2CH_2), 73.7 (CHCO), 126.9, 128.7, 127.4, 128.5, 129.2, 137.9, 140.7 (arom), 133.8 (CPh_2), 171.3 (C=O); ESI-MS (m/z) 428.58 $[M+H]^+$.

*6.9.17. 4-Amino-N-(2-chlorobenzyl)-2-((4,4-diphenylbut-3-en-1-yl)(methyl)amino)butanamide (**18b**)*

Hydrazine monohydrate (2.00 g, 4 mmol) and **12b** (0.69 g, 1.17 mmol), ethanol (10 mL) Yield 66%; yellow oil; R_f: 0.76 (N_1); Anal. Calcd for $C_{28}H_{32}ClN_3O$: C, 72.79; H, 6.98; N, 9.09; found: C, 72.65; H, 6.83, N, 8.87; ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.64–1.78 (m, 1H, $NCHCH_2$), 1.78–1.92 (m, 1H, $NCHCH_2$), 2.12 (s, 3H, CH_3), 2.20–2.31 (m, 2H, $C{=}CHCH_2$), 2.54 (t, J = 7.18 Hz, 2H, CH_2NH), 2.63 (br. s, 2H, NH_2), 2.73–2.89 (m, 2H, CH_2NH_2), 3.11 (dd, J = 8.46, 4.36 Hz, 1H, NCH), 4.35–4.52 (m, 2H, $NHCH_2$), 6.02 (t, J = 7.31 Hz, 1H, Ph_2CH), 7.07–7.39 (m, 14H, arom), 7.67 (t, J = 6.16 Hz, 1H, CONH); ^{13}C NMR (75 MHz, chloroform-d) δ ppm 24.2 ($CH_2CH{=}C$), 29.3 (CH_2CH), 37.7 (NH_2CH_2), 41.7 (CH_3N), 43.6 (CH_2NH), 57.3 (NCH_2CH_2), 73.7 (CHCO), 126.9, 128.7, 127.4, 128.5, 129.2, 132.2, 137.9, 140.7, 142.4 (arom), 133.8 (CPh_2), 171.3 (C=O); ESI-MS (m/z) 463.03 $[M+H]^+$

*6.9.18. 4-Amino-N-(4-chlorobenzyl)-2-((4,4-diphenylbut-3-en-1-yl)(methyl)amino)butanamide (**18c**)*

Hydrazine monohydrate (2.00 g, 4 mmol) and **12c** (0.69 g, 1.17 mmol), ethanol (10 mL) Yield 63%; yellow oil; R_f: 0.73 (N_1); Anal. Calcd for $C_{28}H_{32}ClN_3O$: C, 72.79; H, 6.98; N, 9.09; found: C, 72.68; H, 6.81; N, 9.12; ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.63–1.77 (m, 1H, $NCHCH_2$), 1.78–1.91 (m, 1H, $NCHCH_2$), 2.09–2.15 (m, 3H, CH_3), 2.18–2.31 (m, 4H, $C{=}CHCH_2$, NH_2), 2.50–2.60 (m, 2H, CH_2NH), 2.72–2.89 (m, 2H, CH_2NH_2), 3.12 (dd, J = 8.46, 4.36 Hz, 1H, NCH), 4.18–4.36 (m, 2H, $NHCH_2$), 6.02 (t, J = 7.44 Hz, 1H, Ph_2CH), 6.97–7.11 (m, 4H, arom), 7.11–7.43 (m, 12H, arom), 7.59 (t, J = 6.03 Hz, 1H, CONH); ^{13}C NMR (75 MHz, chloroform-d) δ ppm 24.2 ($CH_2CH{=}C$), 29.3 (CH_2CH), 37.7 (NH_2CH_2), 41.7 (CH_3N), 43.6 (CH_2NH), 57.3 (NCH_2CH_2), 73.7 (CHCO), 126.9, 128.7, 127.4, 132.3, 134.6, 136.0, 137.9, 140.7 (arom), 133.8 (CPh_2), 171.3 (C=O); ESI-MS (m/z) 463.03 $[M+H]^+$

*6.9.19. 4-Amino-2-((4,4-diphenylbut-3-en-1-yl)(methyl)amino)-N-(4-fluorobenzyl)butanamide (**18d**)*

Hydrazine monohydrate (2.00 g, 4 mmol) and **12d** (0.67 g; 1.17 mmol), ethanol (10 mL) Yield 54%; yellow oil; R_f: 0.79 (N_1); Anal. Calcd for $C_{28}H_{32}FN_3O$: C, 75.48; H, 7.24; N, 9.43; found: C, 75.21; H, 7.17; N, 9.31; ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.65–1.77 (m, 1H($NCHCH_2$)) 1.80–1.90 (m, 1H($NCHCH_2$)) 2.09–2.13 (m, 3H(Me)) 2.18–2.28 (m, 2H($C{=}CHCH_2$)) 3.36 (s, 2H(NH_2)) 2.50–2.59 (m, 2H(CH_2NH)) 2.73–2.91 (m, 2H(CH_2NH_2)) 3.12 (dd, J = 8.46, 4.36 Hz, 1H(NCH)) 4.20–4.36 (m, 2H($NHCH_2$)) 6.02 (t, J = 7.44 Hz, 1H(ArCH)) 6.85–6.98 (m, 2H(Ar)) 7.03–7.09 (m, 3H(Ar)) 7.11–7.40 (m, 9H(Ar)) 7.57 (t, J = 5.90 Hz, 1H(CONH)); ^{13}C NMR (75 MHz, chloroform-d) δ ppm 24.2 ($CH_2CH{=}C$), 29.3 (CH_2CH), 37.7 (NH_2CH_2), 41.7 (CH_3N), 43.6 (CH_2NH), 57.3 (NCH_2CH_2), 73.7 (CHCO), 115.3, 128.7, 127.4, 128.5, 129.2, 137.9, 160.9 (arom), 133.8 (CPh_2), 171.3 (C=O); ESI-MS (m/z) 446.57 $[M+H]^+$

*6.9.20. 4-Amino-2-((4,4-diphenylbut-3-en-1-yl)(methyl)amino)-N-(4-methylbenzyl)butanamide (**18e**)*

Hydrazine monohydrate (2.00 g, 4 mmol) and **12e** (0.61 g, 1.17 mmol), ethanol (10 mL) Yield 53%; yellow oil; R_f: 0.84 (N_1); Anal. Calcd for $C_{29}H_{35}N_3O$: C, 78.87; H, 7.99; N, 9.52; found: C, 78.78; H, 7.90; N, 9.37; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.72–1.80 (m, 1H, NCH*CH*$_2$), 1.83–1.91 (m, 1H, NCH*CH*$_2$), 2.10 (s, 3H, C*H*$_3$), 2.18–2.26 (m, 2H, C=CH*CH*$_2$), 2.30 (s, 3H, PhC*H*$_3$), 3.68 (s, 2H, N*H*$_2$), 2.50–2.57 (m, 2H, *CH*$_2$NH), 2.88 (dq, J = 12.63, 6.13 Hz, 2H, *CH*$_2$NH$_2$), 3.11 (dd, J = 8.72, 4.36 Hz, 1H, N*CH*), 4.29 (dd, J = 15.39, 5.90 Hz, 2H, NH*CH*$_2$), 6.01 (t, J = 7.44 Hz, 1H, Ph$_2$*CH*), 7.01–7.08 (m, 4H, arom), 7.12–7.37 (m, 10H, arom), 7.56 (t, J = 5.90 Hz, 1H, CO*NH*); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 21.3 (*C*H$_3$Ph), 24.2 (*C*H$_2$CH=C), 29.3 (*C*H$_2$CH), 37.7 (NH$_2$*C*H$_2$), 41.7 (*C*H$_3$N), 43.6 (*C*H$_2$NH), 57.3 (N*C*H$_2$CH$_2$), 73.7 (*C*HCO), 126.9, 128.7, 127.4, 128.5, 129.2, 134.9, 136.4, 140.7 (arom), 133.8 (*C*Ph$_2$), 171.3 (*C*=O); ESI-MS (m/z) 442.61 [M+H]$^+$

*6.9.21. 4-Amino-N-benzyl-2-(4-(diphenylmethylene)piperidin-1-yl)butanamide (**19a**)*

Hydrazine monohydrate (4.21 g, 8.41 mmol) and **13a** (1.41 g, 2.48 mmol), ethanol (30 mL). Yield 58%; white solid; mp 147.8 °C; R_f: 0.74 (N_1); Anal. Calcd for $C_{29}H_{33}N_3O$: C, 79.23; H, 7.57; N, 9.56; found: C, 78.96; H, 7.34; N, 9.46; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.62 (br. s, 2H, *NH*$_2$), 1.77–1.92 (m, 2H, CH*CH*$_2$CH$_2$), 2.24–2.41 (m, 4H, *piperidine*), 2.49–2.69 (m, 4H, *piperidine*), 2.74–2.85 (m, 1H, CHCH$_2$*CH*$_2$), 2.86–2.96 (m, 1H, CHCH$_2$*CH*$_2$), 3.12 (dd, J = 7.69, 5.39 Hz, 1H, *CH*CH$_2$CH$_2$), 4.37–4.52 (m, 2H, Ph$_2$*CH*$_2$), 7.05–7.15 (m, 5H, arom), 7.15–7.40 (m, 10H, arom), 7.54 (t, J = 5.90 Hz, 1H, *NH*CO); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 32.1 (*C*H$_2$CH), 40.4 (piperidine), 43.2 (NH$_2$*C*H$_2$), 51.9 (*C*H$_2$NH), 66.5 (*C*HCO), 126.4, 127.4, 127.6, 128.7, 129.7, 134.5, 136.6, 138.6 (arom), 142.2 (*C*Ph$_2$), 173.0 (*C*=O); ESI-MS (m/z) 440.59 [M+H]$^+$

*6.9.22. 4-Amino-N-(2-chlorobenzyl)-2-(4-(diphenylmethylene) piperidin-1-yl)butanamide (**19b**)*

Hydrazine monohydrate (4.21 g, 8.41 mmol) and **13b** (1.50 g, 2.48 mmol), ethanol (32 mL). Yield 50%; white solid; mp 161.4 °C; R_f: 0.78 (N_1); Anal. Calcd for $C_{29}H_{32}ClN_3O$: C, 73.48; H, 6.80; N, 8.86; found: C, 73.05; H, 6.69; N, 8.65; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.72 (br. s, 2H, *NH*$_2$), 1.76–1.85 (m, 2H, CH*CH*$_2$CH$_2$), 2.27–2.42 (m, 4H, *piperidine*), 2.46–2.63 (m, 4H, *piperidine*), 2.74–2.83 (m, 1H, CHCH$_2$*CH*$_2$), 2.84–2.94 (m, 1H, CHCH$_2$*CH*$_2$), 3.10 (dd, J = 7.57, 5.51 Hz, 1H, *CH*CH$_2$CH$_2$), 4.45–4.57 (m, 2H, Ph$_2$*CH*$_2$), 7.04–7.15 (m, 4H, arom), 7.15–7.42 (m, 10H, arom), 7.77 (t, J = 6.16 Hz, 1H, *NH*CO); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 30.5 (*C*H$_2$CH), 40.4, 32.1 (piperidine); 41.4 (NH$_2$*C*H$_2$), 51.9 (*C*H$_2$NH), 66.4 (*C*HCO), 126.4, 127.1, 128.0, 129.0, 129.5, 130.4, 134.5, 135.9 (arom), 142.2 (*C*Ph$_2$), 173.2 (*C*=O); ESI-MS (m/z) 475.04 [M+H]$^+$.

*6.9.23. 4-Amino-N-(4-chlorobenzyl)-2-(4-(diphenylmethylene) piperidin-1-yl)butanamide (**19c**)*

Hydrazine monohydrate (4.21 g, 8.41 mmol) and **13c** (1.50 g, 2.48 mmol), ethanol (32 mL). Yield 34%; white solid; mp 120.3 °C; R_f: 0.80 (N_1); Anal. Calcd for $C_{29}H_{32}ClN_3O$: C, 73.48; H, 6.80; Cl, 7.48; N, 8.86; found: C, 73.31; H, 6.72; N, 8.59; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.68 (br. s, 2H, *NH*$_2$), 1.76–1.89 (m, 2H, CH*CH*$_2$CH$_2$), 2.24–2.41 (m, 4H, *piperidine*), 2.49–2.66 (m, 4H, *piperidine*), 2.73–2.83 (m, 1H, CHCH$_2$*CH*$_2$), 2.85–2.95 (m, 1H, CHCH$_2$*CH*$_2$), 3.13 (dd, J = 7.69, 5.39 Hz, 1H, *CH*CH$_2$CH$_2$), 4.33–4.49 (m, 2H, PhC*H*$_2$), 7.02–7.14 (m, 4H, arom), 7.14–7.35 (m, 10H, arom), 7.58 (t, J = 6.03 Hz, 1H, *NH*CO); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 30.6 (*C*H$_2$CH), 32.1, 40.1 (piperidine); 42.5 (NH$_2$*C*H$_2$), 51.9 (*C*H$_2$NH), 66.4 (*C*HCO), 126.5, 128.0, 128.8, 128.9, 133.2, 134.3, 136.7, 137.2 (arom), 142.2 (*C*Ph$_2$), 173.2 (*C*=O); ESI-MS (m/z) 475.04 [M+H]$^+$

*6.9.24. 4-Amino-2-(4-(diphenylmethylene)piperidin-1-yl)-N-(4-fluorobenzyl)butanamide (**19d**)*

Hydrazine monohydrate (4.21 g, 8.41 mmol) and **13d** (1.46 g, 2.48 mmol), ethanol (31 mL). Yield 68%; white solid; mp 137.9 °C; R_f: 0.77 (N_1); Anal. Calcd for $C_{29}H_{32}ClN_3O$: C, 76.12; H, 7.05; N, 9.18; found: C, 75.94; H, 7.01; N, 9.01; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.67 (br. s, 2H, *NH*$_2$), 1.76–1.90 (m, 2H, CH*CH*$_2$CH$_2$), 2.23–2.42 (m, 4H, *piperidine*), 2.48–2.67 (m, 4H, *piperidine*), 2.73–2.84 (m, 1H, CHCH$_2$*CH*$_2$), 2.84–2.96 (m, 1H, CHCH$_2$*CH*$_2$), 3.12 (dd, J = 7.69, 5.39 Hz, 1H, *CH*CH$_2$CH$_2$), 4.31–4.49 (m, 2H, PhC*H*$_2$), 6.89–7.14 (m, 6H, arom), 7.14–7.35 (m, 8H, arom), 7.55 (t, J = 5.90 Hz, 1H, *NH*CO); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 30.6 (*C*H$_2$CH), 32.1, 40.4 (piperidine); 42.5 (NH$_2$*C*H$_2$), 51.9 (*C*H$_2$NH), 66.4 (*C*HCO), 115.4, 115.7, 126.5, 128.0, 129.2, 129.3, 129.7, 134.3, 136.7, (arom), 142.2 (*C*Ph$_2$), 173.1 (*C*=O); ESI-MS (m/z) 458.58 [M+H]$^+$.

*6.9.25. 4-Amino-2-(4-(diphenylmethylene)piperidin-1-yl)-N-(4-methylbenzyl)butanamide (**19e**)*

Hydrazine monohydrate (4.21 g, 8.41 mmol) and **13e** (1.45 g, 2.48 mmol), ethanol (31 mL). Yield 49%; white solid; mp 135.8 °C; R_f: 0.73 (N_1); Anal. Calcd for $C_{30}H_{35}N_3O$: C, 79.43; H, 7.78; N, 9.26; fond: C, 79.21; H, 7.63; N, 9.03; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.77 (br. ., 2H, *NH*$_2$), 1.80–1.90 (m, 2H, CH*CH*$_2$CH$_2$), 2.28–2.37 (m, 7H, *piperidine*, PhC*H*$_3$), 2.49–2.67 (m, 4H, *piperidine*), 2.76–2.85 (m, 1H, CHCH$_2$*CH*$_2$), 2.86–2.96 (m, 1H, CHCH$_2$*CH*$_2$), 3.11 (dd, J = 7.57, 5.51 Hz, 1H, *CH*CH$_2$CH$_2$), 4.32–4.46 (m, 2H, Ph$_2$*CH*$_2$), 7.03–7.34 (m, 14H, arom), 7.48 (t, J = 5.64 Hz, 1H, *NH*CO); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 21.1 (*C*H$_3$), 30.7 (*C*H$_2$CH), 32.1, 40.4 (piperidine); 42.9 (NH$_2$*C*H$_2$), 51.9 (*C*H$_2$NH), 66.5 (*C*HCO), 126.4, 127.6, 128.0, 129.4, 129.7, 134.5, 136.5, 137.1 (arom), 142.2 (*C*Ph$_2$), 173.1 (*C*=O); ESI-MS (m/z) 454.62 [M+H]$^+$.

*6.9.26. 4-Amino-2-(4-benzhydrylpiperidin-1-yl)-N-benzylbutanamide (**20a**)*

Hydrazine monohydrate (2.39 g, 4.78 mmol) and **14a** (0.80 g, 1.41 mmol), ethanol (19 mL). Yield 76%; white solid; mp 61.1 °C; R_f: 0.92 (N_1); Anal. Calcd for $C_{29}H_{35}N_3O$: C, 78.87; H, 7.99; N, 9.52; found: C, 78.78; H, 8.07; N, 9.46; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.02–1.19 (m, 2H, CH*CH*$_2$CH$_2$), 1.55 (dt, J = 6.86, 3.11 Hz, 2H, *piperidine*), 1.80 (q, J = 7.18 Hz, 4H, *piperidine*), 2.06–2.19 (m, 2H, *piperidine*), 2.28–2.39 (m, 1H, *piperidine*), 2.68–2.76 (m, 2H, *NH*$_2$), 2.77–2.93 (m, 2H, CHCH$_2$*CH*$_2$), 3.05 (t, J = 6.54 Hz, 1H, *CH*CH$_2$CH$_2$), 3.45 (d, J = 10.77 Hz, 1H, Ph$_2$*CH*), 4.42 (dd, J = 5.90, 3.59 Hz, 2H, PhC*H*$_2$), 7.11–7.37 (m, 15H, arom), 7.47 (t, J = 5.90 Hz, 1H, *NH*CO); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 30.7 (*C*H$_2$CH), 31.7, 31.9, 39.4 (piperidine); 43.1 (NH$_2$*C*H$_2$), 48.3 (*C*H$_2$NH), 58.8 (*C*Ph$_2$), 66.8 (*C*HCO), 126.2, 127.5, 127.9, 128.5, 128.7, 138.6, 143.6 (arom), 173.3 (*C*=O); ESI-MS (m/z) 442.61 [M+H]$^+$.

*6.9.27. 4-Amino-2-(4-benzhydrylpiperidin-1-yl)-N-(2-chlorobenzyl)butanamide (**20b**)*

Hydrazine monohydrate (2.39 g, 4.78 mmol) and **14b** (0.86 g, 1.41 mmol), ethanol (20 mL). Yield 39%; white solid; mp 152.2 °C; R_f: 0.79 (N_1); Anal. Calcd for $C_{28}H_{34}ClN_4O$: C, 73.17; H, 7.20; N, 8.83; found: C, 73.00; H, 7.01; N, 8.72; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.07–1.25 (m, 2H, *piperidine*), 1.54 (d, J = 12.57 Hz, 2H, *piperidine*), 1.78 (q, J = 6.92 Hz, 2H, CH*CH*$_2$CH$_2$), 1.89–2.17 (m, 4H, *piperidine*), 2.32 (t, J = 10.64 Hz, 1H, *piperidine*), 2.68 (t, J = 9.10 Hz, 2H, *NH*$_2$), 2.74–2.89 (m, 2H, CHCH$_2$*CH*$_2$), 3.03 (t, J = 6.54 Hz, 1H, *CH*CH$_2$CH$_2$), 3.48 (d, J = 10.77 Hz, 1H, Ph$_2$*CH*), 4.42–4.56 (m, 2H, PhC*H*$_2$), 7.12–7.30 (m, 10H, arom), 7.30–7.41 (m, 4H, arom), 7.70 (t, J = 6.16 Hz, 1H, *NH*CO); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 30.4

(CH_2CH), 31.7, 31.9, 39.4 (piperidine); 40.3 (NH_2CH_2), 48.3 (CH_2NH), 58.7 (CPh_2), 66.7 (*C*HCO), 126.2, 127.1, 128.0, 128.5, 128.9, 130.1, 133.6, 135.9, 143.6 (arom), 173.4 (*C*=O); ESI-MS (*m*/*z*) 477.05 $[M+H]^+$

*6.9.28. 4-Amino-2-(4-benzhydrylpiperidin-1-yl)-N-(4-chlorobenzyl)butanamide (**20c**)*

Hydrazine monohydrate (2.39 g, 4.78 mmol) and **14c** (0.86 g, 1.41 mmol), ethanol (20 mL). Yield 53%; white solid; mp 130.3 °C; R_f: 0.77 (N_1); Anal. Calcd for $C_{28}H_{34}ClN_4O$: C, 73.17; H, 7.20; N, 8.83; found: C, 73.00; H, 7.01; N, 8.72; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.00–1.21 (m, 2H, CH*CH*$_2$CH$_2$), 1.49–1.61 (m, 2H, *piperidine*), 1.79 (q, *J* = 6.92 Hz, 2H, *piperidine*), 1.99 (br.s, 2H, *NH*$_2$), 2.06–2.19 (m, 2H, CHCH$_2$*CH*$_2$), 2.34 (td, *J* = 11.67, 2.05 Hz, 1H, *piperidine*), 2.64–2.92 (m, 4H, *piperidine*), 3.05 (t, *J* = 6.67 Hz, 1H, *CH*CH$_2$CH$_2$), 3.46 (d, *J* = 11.03 Hz, 1H, Ph$_2$*CH*), 4.29–4.44 (m, 2H, Ph*CH*$_2$), 7.09–7.36 (m, 14H, arom) 7.53 (t, *J* = 5.90 Hz, 1H, *NH*CO); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 30.4 (CH_2CH), 31.7, 31.9, 39.4 (piperidine); 42.4 (NH_2CH_2), 48.3 (CH_2NH), 58.8 (CPh_2), 66.7 (*C*HCO), 126.2, 127.9, 128.6, 128.8, 128.9, 133.1, 137.2, 143.6 (arom), 173.4 (*C*=O); ESI-MS (*m*/*z*) 442.61 $[M+H]^+$

*6.9.29. 4-Amino-2-(4-benzhydrylpiperidin-1-yl)-N-(4-fluorobenzyl)butanamide (**20d**)*

Hydrazine monohydrate (2.39 g, 4.78 mmol) and **14d** (0.83 g, 1.41 mmol), ethanol (19 mL). Yield 42%; white solid; mp 62.5 °C; R_f: 0.90 (N_1); Anal. Calcd for $C_{28}H_{34}FN_3O$: C, 75.79; H, 7.46; N, 9.14; found: C, 75.63; H, 7.31; N, 9.01; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.97–1.21 (m, 2H, CH*CH*$_2$CH$_2$), 1.55 (d, *J* = 13.34 Hz, 2H, *piperidine*), 1.81 (q, *J* = 6.92 Hz, 2H, *piperidine*), 2.06–2.19 (m, 2H, *piperidine*), 2.29–2.44 (m, 3H, *piperidine*), 2.64–2.78 (m, 2H, *NH*$_2$), 2.78–2.93 (m, 2H, CHCH$_2$*CH*$_2$), 3.06 (t, *J* = 6.54 Hz, 1H, *CH*CH$_2$CH$_2$), 3.45 (d, *J* = 11.03 Hz, 1H, Ph$_2$*CH*), 4.32–4.43 (m, 2H, Ph*CH*$_2$), 7.00 (t, *J* = 8.72 Hz, 2H, arom) 7.11–7.30 (m, 12H, arom) 7.51 (t, *J* = 6.16 Hz, 1H, *NH*CO); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 30.0 (CH_2CH), 31.7, 31.9, 39.4 (piperidine); 40.1 (NH_2CH_2), 42.4 (CH_2NH), 58.8 (CPh_2), 66.8 (*C*HCO), 115.4, 115.7, 126.2, 127.9, 128.5, 129.1, 129.3, 134.4, 143.6, 160.4, 163.7 (arom), 173.3 (*C*=O); ESI-MS (*m*/*z*) 460.60 $[M+H]^+$.

*6.9.30. 4-Amino-2-(4-benzhydrylpiperidin-1-yl)-N-(4-methylbenzyl)butanamide (**20e**)*

Hydrazine monohydrate (2.39 g, 4.78 mmol) and **14e** (0.82 g, 1.41 mmol), ethanol (19 mL). Yield 38%; white solid; mp 110.7 °C; R_f: 0.92 (N_1); Anal. Calcd for $C_{28}H_{34}N_4O$: C, 79.08; H, 8.19; N, 9.22; found: C, 78.87; H, 7.92; N, 9.26; ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.00–1.21 (m, 2H, CH*CH*$_2$CH$_2$), 1.46–1.62 (m, 2H, *piperidine*), 1.66–1.76 (m, 2H, *piperidine*), 1.77–1.85 (m, 2H, *piperidine*), 2.03–2.19 (m, 2H, *piperidine*), 2.34 (s, 3H, Ph*CH*$_3$), 2.68–2.80 (m, 4H, *NH*$_2$, CHCH$_2$*CH*$_2$), 2.80–2.92 (m, 1H, *piperidine*), 3.03 (t, *J* = 6.67 Hz, 1H, *CH*CH$_2$CH$_2$), 3.46 (d, *J* = 11.03 Hz, 1H, Ph$_2$*CH*), 4.38 (dd, *J* = 5.77, 2.69 Hz, 2H, Ph*CH*$_2$), 7.09–7.21 (m, 6H, arom), 7.21–7.32 (m, 8H, arom), 7.41 (t, *J* = 5.77 Hz, 1H, *NH*CO); ^{13}C NMR (75 MHz, chloroform-*d*) δ ppm 21.1 (*C*H$_3$), 30.8 (*C*H$_2$CH), 31.7, 31.9, 39.4 (piperidine); 48.4 (NH_2CH_2), 58.8 (CH_2NH), 66.8 (CPh_2), 66.8 (*C*HCO), 126.2, 127.5, 128.0, 128.5, 129.3, 135.5, 137.0, 143.6 (arom), 173.2 (*C*=O); ESI-MS (*m*/*z*) 456.63 $[M+H]^+$.

6.10. [^{3}H] GABA uptake assay

Inhibitory potencies of compounds **15**–**20(a**–**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 [44]. Binding assays for mGAT1 based on NO711 as native marker were performed as described earlier [45]. NO711 was analyzed by LC-MS/MS using an API 3200 triple quadrupole mass spectrometer according to the method described previously [48].

The compounds were considered 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.

6.11. In vivo activity

6.11.1. Animals

Adult male Albino Swiss (CD-1) mice obtained from the Animal Breeding Farm of the Jagiellonian University in Cracow were used for *in vivo* tests. The animals weighed between 18 g and 22 g. They were kept in groups of 10 mice in cages at room temperature of 22 ± 2 °C, under light/dark (12:12) cycle and had free access to food and water before experiments. The ambient temperature of the room and humidity were kept consistent throughout all the tests. For the experiments the animals were selected in a random way. Each group consisted of 6–10 animals/dose and each mouse was used only once. The experiments were performed between 8 a.m. and 3 p.m. Immediately after the assay the animals were killed by cervical dislocation. All the procedures were approved by the Local Ethics Committee of the Jagiellonian University in Cracow (ZI/595/2011).

6.11.2. Anticonvulsant activity

6.11.2.1. Electroconvulsive threshold test. The anticonvulsant efficacy of the test compounds at the dose of 100 mg/kg was evaluated at their previously established time of peak drug effect (60 min after their i.p. injection) according to the procedure recently described [33]. Electroconvulsions were produced by an alternating current (duration of the stimulus: 0.2 s; 50 Hz) delivered *via* standard auricular electrodes by an electroshock generator (Hugo Sachs rodent shocker, Germany). Tonic hind limb extension (i.e., the hind limbs outstretched 180° to the plane of the body axis) was an indicator of seizure episodes. For the evaluation of the electroconvulsive threshold (ECT) at least four groups of animals per dose were used. These mice were challenged with electroshocks of various intensities to yield 10–30%, 30–50%, 50–70% and 70–90% of animals with seizures. Then, a median current strength value (CS_{50} in mA), defined as current intensity required to induce tonic hind limb extension in 50% of the mice challenged, was estimated by means of log-probit method [49].

6.11.2.2. Maximal electroshock seizure test. Maximal electroshock seizure test was performed as previously described [33]. Briefly, the vehicle-treated mice and drug-treated mice received a stimulus of sufficient intensity (25 mA) delivered by an electroshock generator (Hugo Sachs rodent shocker, Germany) to induce maximal seizures (tonic extension) of hind limbs. Electroconvulsions were produced with the use of auricular electrodes and the stimulus duration was 0.2 s. The endpoint was the tonic extension of the hind limbs.

6.11.2.3. Pentylenetetrazole-induced seizures. The test was performed according to Łuszczki et al. [50] with some minor modification. Clonic convulsions were induced in mice by the subcutaneous (*s.c.*) administration of PTZ (Sigma Aldrich, Poland) at a dose of 100 mg/kg. After the administration of PTZ, the mice were placed separately into transparent Plexiglas cages (30 × 20 × 15 cm) and were observed during the next 30 min for the occurrence of clonic seizures. Clonic seizures were defined as clonus of the whole body lasting more than 3 s, with an accompanying loss of the righting reflex. The latency time to first clonus,

the number of seizure episodes and mortality rate were noted and compared between vehicle-treated and drug-treated groups.

6.11.2.4. Pilocarpine-induced seizures. The mice were intraperitoneally treated with the investigated compound or vehicle (1% Tween 80) and 60 min later the animals received pilocarpine (400 mg/kg, i.p.; Sigma Aldrich, Poland). These animals were also pretreated with scopolamine butylbromide (1 mg/kg, i.p.; Sigma Aldrich, Poland) 45 min before pilocarpine to avoid peripheral toxicity and diarrhea, masticatory and stereotyped movements. After the administration of the convulsant, the mice were observed during 1 h for behavioral changes. The latency time to the onset of the tonic–clonic seizure episode was recorded. The latency time to death was also noted in the cut-off of 60 min [51].

6.11.3. Antidepressant-like activity – the forced swim test

The experiment was carried out according to the method described by Porsolt et al. [52] with some minor modification. The mice were dropped individually into glass cylinders (height 25 cm, diameter 10 cm) filled with water to a height of 10 cm (maintained at 23–25 °C) and they were left there for 6 min. In this assay after an initial 2 min period of vigorous activity, each mouse assumes an immobile posture. The total duration of immobility was recorded during the final 4 min of the whole 6 min testing period in control and drug-treated animals. Mice were judged to be immobile when they remained floating passively in the water, making only small movements to keep the heads above the water surface.

6.11.4. Antinociceptive activity – the hot plate test

In the hot plate test the mice were intraperitoneally pretreated either with the test compound or vehicle 60 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 time until the animal licked its hind paws or jumped was recorded by means of the stop-watch [33]. In this assay the cut-off time was established (45 s) to avoid tissue damage and mice not responding within 45 s were removed from the apparatus and assigned a score of 45 s.

6.11.5. Influence on motor coordination – the rotarod test

The test was performed according to the method recently described [33]. 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, 60 min before the rotarod test the mice were intraperitoneally pretreated with the test compound (30 mg/kg and 100 mg/kg) or the vehicle (1% Tween 80). Then the animals were tested on the rotarod revolving at 6, 18 and then 24 rpm. Motor impairments, defined as the inability to remain on the rotating rod for 1 min were measured at each speed and the mean time spent on the rod was counted for each dose.

6.11.6. Influence on locomotor activity

The locomotor activity test was performed using activity cages (40 × 40 × 31 cm, supplied with I.R. beam emitters) (Activity Cage 7441, Ugo Basile, Italy) connected to a counter for the recording of light-beam interruptions. 60 min before the experiment the mice were intraperitoneally pretreated with the test compound (30 mg/kg) or the vehicle (1% Tween 80), then being individually placed in the activity cages in a sound-attenuated room. The animals' horizontal and vertical movements (i.e. the number of light-beam crossings) were counted during the next 30 min in five 6-min intervals [33].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2014.06.024.

References

[1] A.S. Kristensen, J. Andersen, T.N. Jørgensen, L. Sørensen, J. Eriksen, C.J. Loland, K. Strømgaard, U. Gether, SLC6 neurotransmitter transporters: structure, function, and regulation, Pharmacol. Rev. 63 (3) (2011) 585–640, http://dx.doi.org/10.1124/pr.108.000869.
[2] J. Guastella, N. Nelson, H. Nelson, L. Czyzyk, S. Keynan, M.C. Miedel, N. Davidson, H.A. Lester, B.I. Kanner, Cloning and expression of a rat brain GABA transporter, Science 249 (4974) (1990) 1303–1306.
[3] B. Lopez-Corcuera, Q.R. Liu, S. Mandiyan, H. Nelson, N. Nelson, Expression of a mouse brain cDNA encoding novel gamma-aminobutyric acid transporter, J. Biol. Chem. 267 (25) (1992) 17491–17493.
[4] Q.R. Liu, B. López-Corcuera, S. Mandiyan, H. Nelson, N. Nelson, Molecular characterization of four pharmacologically distinct gamma-aminobutyric acid transporters in mouse brain [corrected], J. Biol. Chem. 268 (3) (1993) 2106–2112.
[5] Q.R. Liu, S. Mandiyan, H. Nelson, N. Nelson, A family of genes encoding neurotransmitter transporters, Proc. Natl. Acad. Sci. U. S. A. 89 (14) (1992) 6639–6643.
[6] A. Yamauchi, S. Uchida, H.M. Kwon, A.S. Preston, R.B. Robey, A. Garcia-Perez, M.B. Burg, J.S. Handler, Cloning of a Na(+)- and Cl(−)-dependent betaine transporter that is regulated by hypertonicity, J. Biol. Chem. 267 (1) (1992) 649–652.
[7] R. Radian, O.P. Ottersen, J. Storm-Mathisen, M. Castel, B.I. Kanner, Immunocytochemical localization of the GABA transporter in rat brain, J. Neurosci. 10 (4) (1990) 1319–1330.
[8] F. Conti, M. Melone, S. De Biasi, A. Minelli, N.C. Brecha, A. Ducati, Neuronal and glial localization of GAT-1, a high-affinity gamma-aminobutyric acid plasma membrane transporter, in human cerebral cortex: with a note on its distribution in monkey cortex, J. Comp. Neurol. 396 (1) (1998) 51–63.
[9] A. Minelli, S. DeBiasi, N.C. Brecha, L.V. Zuccarello, F. Conti, GAT-3, a high-affinity GABA plasma membrane transporter, is localized to astrocytic processes, and it is not confined to the vicinity of GABAergic synapses in the cerebral cortex, J. Neurosci. 16 (19) (1996) 6255–6264.
[10] N.O. Dalby, Inhibition of gamma-aminobutyric acid uptake: anatomy, physiology and effects against epileptic seizures, Eur. J. Pharmacol. 479 (1–3) (2003) 127–137.
[11] C.S. Chiu, S. Brickley, K. Jensen, A. Southwell, S. Mckinney, S. Cull-Candy, I. Mody, H.A. Lester, GABA transporter deficiency causes tremor, ataxia, nervousness, and increased GABA-induced tonic conductance in cerebellum, J. Neurosci. 25 (12) (2005) 3234–3245.
[12] Y. Zhou, S. Holmseth, R. Hua, A.C. Lehre, A.M. Olofsson, I. Poblete-Naredo, S.A. Kempson, N.C. Danbolt, The betaine-GABA transporter (BGT1, slc6a12) is predominantly expressed in the liver and at lower levels in the kidneys and at the brain surface, Am. J. Physiol. Ren. Physiol. 302 (3) (2012) 316–328, http://dx.doi.org/10.1152/ajprenal.00464.2011.
[13] Y. Zhou, S. Holmseth, C. Guo, B. Hassel, G. Höfner, H.S. Huitfeldt, K.T. Wanner, N.C. Danbolt, Deletion of the γ-aminobutyric acid transporter 2 (GAT2 and SLC6A13) gene in mice leads to changes in liver and brain taurine contents, J. Biol. Chem. 287 (42) (2012) 35733–35746, http://dx.doi.org/10.1074/jbc.M112.368175.
[14] O.M. Larsson, G.A. Johnston, A. Schousboe, Differences in uptake kinetics of cis-3-aminocyclohexane carboxylic acid into neurons and astrocytes in primary cultures, Brain Res. 260 (2) (1983) 279–285.
[15] O.M. Larsson, P. Krogsgaard-Larsen, A. Schousboe, Characterization of the uptake of GABA, nipecotic acid and cis-4-OH-nipecotic acid in cultured neurons and astrocytes, Neurochem. Int. 7 (5) (1985) 853–860.
[16] A. Schousboe, O.M. Larsson, P. Krogsgaard-Larsen, Lack of a high affinity uptake system for the GABA agonists THIP and isoguvacine in neurons and astrocytes cultured from mouse brain, Neurochem. Int. 7 (3) (1985) 505–508.
[17] A. Schousboe, A. Sarup, O.M. Larsson, H.S. White, GABA transporters as drug targets for modulation of GABAergic activity; White, Biochem. Pharmacol. 68 (8) (2004) 1557–1563.
[18] G.G. Pratt, N.G. Bowery, N.G. Bowery, H.R. Bittiger, P.P. Olpe, $GABA_B$ Receptors in Mammalian Function, Wiley, Chichester, United Kingdom, 1990, p. 319.

[19] B.S. Meldrum, A.G. Chpman, Basic mechanism of gabitril (tiagabine) and future potential developments, Epilepsia 40 (Suppl. 9) (1999) 53–56.
[20] C. Braestrup, E.B. Nielsen, U. Sonnewald, L.J. Knutsen, K.E. Andersen, J.A. Jansen, K. Frederiksen, P.H. Andersen, A. Mortensen, P.D. Suzdak, (R)-N-[4,4-bis(3-methyl-2-tienyl/0but-3-en-1-yl]Nipecotic acid binds with high affinity to the brain γ-aminobutyric acid uptake carrier, J. Neurochem. 54 (1990) 639–647.
[21] W.J. Giardina, M.W. Decker, R.D. Porsolt, S. Roux, S.D. Collins, D.J.B. Kim, A.W. Bannon, An evaluation of the GABA uptake blocker tiagabine in animal models of neuropathic and nociceptive pain, Drug Dev. Res. 44 (1998) 106–113.
[22] A. Ipponi, C. Lamberti, A. Medica, A. Bartolini, P. Malmberg-Aiello, Tiagabine antinociception in rodents depends on GABA(B) receptor activation: parallel antinociception testing and medial thalamus GABA microdialysis, Eur. J. Pharmacol. 368 (2–3) (1999) 205–211.
[23] U. Schmitt, C. Hiemke, Effects on GABA-transporter (GAT) inhibitors on rat behavior in open-field and elevated plus-maze, Behav. Pharmacol. 10 (1999) 131–137.
[24] C. Kellinghaus, T. Loddenkemper, L. Weitemeyer, P. Lüdemann, Experience with tiagabine in the clinical practice; new insights as to the efficacy and safety profile, Nervenarzt 72 (10) (2001) 764–769.
[25] H. Stefan, T.J. Feuerstein, Novel anticonvulsant drugs, Pharmacol. Ther. 113 (1) (2007) 165–183.
[26] Y. Li, Y. Li, P. Gu, B. Fu, F. Liu, E. Li, Analgesic effect of intrathecally γ-aminobutyric acid transporter-1 inhibitor NO-711 administrating on neuropathic pain in rats, Neurosci. Lett. 494 (1) (2011) 6–9.
[27] T.G. Dhar, L.A. Borden, S. Tyagarajan, K.E. Smith, T.A. Branchek, R.L. Weinshank, C. Gluchowski, Design, synthesis and evaluation of substituted triarylnipecotic acid derivatives as GABA uptake inhibitors: identification of a ligand with moderate affinity and selectivity for the cloned human GABA transporter GAT-3, J. Med. Chem. 37 (15) (1994) 2334–2342.
[28] N.O. Dalby, E.B. Nielsen, Comparison of the preclinical anticonvulsant profiles of tiagabine, lamotrigine, gabapentin and vigabatrin, Epilepsy Res. 28 (1) (1997) 63–72.
[29] K. Kataoka, K. Hara, Y. Haranishi, T. Terada, T. Sata, The antinociceptive effect of SNAP5114, a gamma-aminobutyric acid transporter-3 inhibitor, in rat experimental pain models,, Anesth. Analg. 116 (5) (2013) 1162–1169.
[30] K. Sałat, K. Kulig, R. Sałat, B. Filipek, B. Malawska, Analgesic and anticonvulsant activity of new derivatives of 2-substituted 4-hydroxybutanamides in mice, Pharmacol. Rep. 64 (1) (2012) 102–112.
[31] P. Kowalczyk, K. Sałat, G.C. Höfner, N. Guzior, B. Filipek, K.T. Wanner, K. Kulig, 2-Substituted 4-hydroxybutanamides as potential inhibitors of the γ-aminobutyric acid transporters: mGAT1-mGAT4. Synthesis and biological evaluation, Bioorg. Med. Chem. 21 (17) (2013) 5154–5167, http://dx.doi.org/10.1016/j.bmc.2013.06.038.
[32] K. Sałat, A. Więckowska, K. Więckowski, G.C. Hofner, J. Kamiński, K.T. Wanner, B. Malawska, B. Filipek, K. Kulig, Synthesis and pharmacological properties of new GABA uptake inhibitors, Pharmacol. Rep. 64 (4) (2012) 817–833.
[33] K. Sałat, K. Kulig, J. Gajda, K. Więckowski, B. Filipek, B. Malawska, Evaluation of anxiolytic-like, anticonvulsant, antidepressant-like and antinociceptive properties of new 2-substituted 4-hydroxybutanamides with affinity for GABA transporters in mice, Pharmacol. Biochem. Behav. 110 (2013) 145–153.
[34] K. Sałat, K. Kulig, GABA transporters as targets for new drugs, Future Med. Chem. 3 (2) (2011) 211–222, http://dx.doi.org/10.4155/fmc.10.298.
[35] P. Kowalczyk, G.C. Höfner, K.T. Wanner, K. Kulig, Synthesis, pharmacological evaluation of new 4,4-diphenylbut-3-enyl derivatives of 4-hydroxybutanamides as GABA uptake inhibitors, Acta Pol. Pharm. 69 (1) (2012) 157–160.
[36] K. Salat, T. Librowski, A. Moniczewski, K. Stanisz-Wallis, K. Wieckowski, B. Malawska, Analgesic, antioedematous and antioxidant activity of γ-butyrolactone derivatives in rodents, Behav. Pharmacol. 23 (4) (2012) 407–416.
[37] V. N'Goka, G. Schlewer, J.M. Linget, J.P. Chambon, C.G. Wermuth, GABA-uptake inhibitors: construction of a general pharmacophore model and successful prediction of a new representative, J. Med. Chem. 34 (8) (1991) 2547–2557.
[38] E. Falch, P. Krogsgaard-Larsen, GABA uptake inhibitors. Syntheses and structure-activity studies on GABA analogues containing diarylbutenyl and diarylmethoxyalkyl N-substituents, Eur. J. Med. Chem. 26 (1991) 69–78.
[39] E. Guénin, M. Monteil, N. Bouchemal, T. Prangé, M. Lecouvey, Syntheses of phosphonic esters of alendronate, pamidronate and neridronate, Eur. J. Org. Chem. 20 (2007) 3380–3391.
[40] W.J. Kruper Jr., P.R. Rudolf, Ch A. Langhoff, Unexpected selectivity in the alkylation of polyazamacrocycles, J. Org. Chem. 58 (1993) 3869–3876.
[41] T. Kolasa, M.J. Miller, Synthesis of the chromophore of pseudobactin, a fluorescent siderophore from Pseudomonas, J. Org. Chem. 55 (1990) 4246–4255.
[42] J. Lee, S.U. Kang, J.O. Lim, H.K. Choi, M.K. Jin, A. Toth, L.V. Pearce, R. Tran, Y. Wang, T. Szabo, P.M. Blumberg, N-[4-(methylsulfonylamino)benzyl]thiourea analogues as vanilloid receptor antagonists: analysis of structure-activity relationships for the "C-Region", Bioorg. Med. Chem. 12 (2) (2004) 371–385.
[43] G. Chen, H. Xia, Y. Cai, D. Ma, J. Yuan, C. Yuan, Synthesis and SAR study of diphenylbutylpiperidines as cell autophagy inducers, Bioorg. Med. Chem. Lett. 21 (1) (2011) 234–239, http://dx.doi.org/10.1016/j.bmcl.2010.11.029.
[44] A. Kragler, G.C. Höfner, K.T. Wanner, Synthesis and biological evaluation of aminomethylphenol derivatives as inhibitors of the murine GABA transporters mGAT1-mGAT4, Eur. J. Med. Chem. 43 (11) (2008) 2404–2411, http://dx.doi.org/10.1016/j.ejmech.2008.01.005.
[45] C. Zepperitz, G.C. Höfner, K.T. Wanner, MS-binding assays: kinetic, saturation, and competitive experiments based on quantitation of bound marker as exemplified by the GABA transporter mGAT1, ChemMedChem 1 (2) (2006) 208–217.
[46] N.O. Dalby, GABA-level increasing and anticonvulsant effects of three different GABA uptake inhibitors, Neuropharmacology 39 (12) (2000) 2399–2407.
[47] C.K. Thoeringer, A. Erhardt, I. Sillaber, M.B. Mueller, F. Ohl, F. Holsboer, M.E. Keck, Long-term anxiolytic and antidepressant-like behavioural effects of tiagabine, a selective GABA transporter-1 (GAT-1) inhibitor, coincide with a decrease in HPA system activity in C57BL/6 mice, J. Psychopharmacol. 24 (5) (2010) 733–743.
[48] G. Höfner, K.T. Wanner, Using short columns to speed up LC-MS quantification in MS binding assays, J. Chromatogr. 878 (17–18) (2010) 1356–1364, http://dx.doi.org/10.1016/j.jchromb.2009.12.006.
[49] J.T. Litchfield, E. Wilcoxon, A simplified method of evaluating dose-effect experiments, J. Pharmacol. Exp. Ther. 96 (1949) 99–113.
[50] J.J. Łuszczki, R. Czernecki, M. Dudra-Jastrzębska, K.K. Borowicz, S.J. Czuczwar, Influence of agmatine on the protective action of numerous antiepileptic drugs against pentetrazole-induced seizures in mice, Pharmacol. Rep. 61 (2009) 252–260.
[51] E.A. Wilhelm, C.R. Jesse, S.S. Roman, C.F. Bortolatto, C.W. Nogueira, Anticonvulsant effect of (E)-2-benzylidene-4-phenyl-1,3-diselenole in a pilocarpine model in mice, Life Sci. 87 (19–22) (2010) 620–627.
[52] R.D. Porsolt, A. Bertin, M. Jalfre, Behavioral despair in mice: a primary screening test for antidepressants, Arch. Int. Pharmacodyn. Ther. 229 (2) (1977) 327–336.
[53] J. Pabel, M. Faust, C. Prehn, B. Wörlein, L. Allmendinger, G. Höfner, K.T. Wanner, Development of an (S)-1-{2-[tris(4-methoxyphenyl)methoxy] ethyl}piperidine-3-carboxylic acid [(S)-SNAP-5114] carba analogue inhibitor for murine γ-aminobutyric acid transporter type 4, ChemMedChem 7 (7) (2012) 1245–1255, http://dx.doi.org/10.1002/cmdc.201200126.