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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW 4,4-DIPHENYLBUT-3-ENYL DERIVATIVES OF 4-HYDROXYBUTANAMIDES AS GABA UPTAKE INHIBITORS*

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γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter concerned with the control of neuronal activity in the mammalian central nervous system (CNS). There is considerable direct and indirect evidence that impair activity of GABA-mediated inhibitory synapses may be an important causative factor in experimental and clinical seizure disorders (1, 2).

Enhancement of GABA transmission by inhibition of GABA uptake has gained much attention as a therapeutic strategy, since it functionally increases the effect of GABA in a use-dependent manner, and GABA uptake inhibition has proved effective as anticonvulsant in a variety of experimental models of epilepsy and in epileptic patients. Pharmacological intervention along these lines may be also beneficial in other CNS disorders and malfunctions such as Parkinson's disease, Huntington's chorea, some forms of schizophrenia, chronic pain and sleep disorders (3–6).

To date, four subtypes of membrane-bound proteins transporting GABA have been identified and, if they are cloned from murine brain cells, are termed mGAT1, mGAT2, mGAT3, and mGAT4. They display different physiological activities and distribution in CNS. mGAT1 and mGAT4 are located in CNS, while mGAT2 and mGAT3 are also found in peripheral tissues (7, 8).

The therapeutic potential of GAT inhibition has been confirmed with the successful development of the GAT1 selective drug tiagabine(**1**) which is one of the most potent drugs used in the treatment of epilepsy. Although the extensively investigated GAT1 seems to be the most promising one, the three latter still remain in the area of interest of current medicinal chemistry (1).

The present work is a part of an extended search for new anticonvulsant agents and potential antiepileptics in the group of derivatives of 4-hydroxybutanamide (9–11). In this paper, we describe the synthesis of substituted 4-hydroxybutanamides and biological evaluation of their influence on murine GABA uptake proteins mGAT1-mGAT4. The structure of compounds designed is based on the 4-hydroxybutyric acid (γ-hydroxybutyric acid, GHB), which is a metabolite of GABA, and acts as an inhibitory neurotransmitter in mammalian CNS. In the position 2 of GHB, a 4,4-diphenylbut-3-enylamine was introduced as a part mimicking the biaryl moieties of known for selective mGAT1 inhibitors such as SKF 89976-A and tiagabine (Fig. 1).

EXPERIMENTAL

Chemistry

Melting points were determined in open glass capillaries on the Büchi 353 melting point apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out within 0.4% of the theoretical values and were performed on an Elementar Vario EL III (Elementar Analysensysteme, Hanau, Germany).

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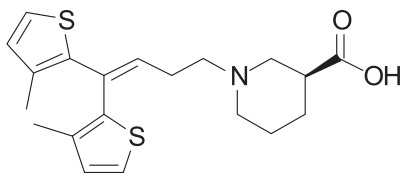
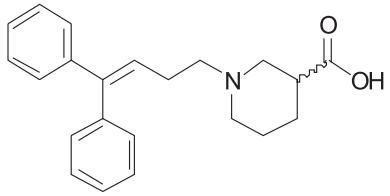


Tiagabine



SKF 89976 A

Figure 1. Structure of tiagabine and SKF 89976 A

^{1}H-NMR and ^{13}C-NMR spectra were recorded on Varian Mercury VX 300 MHz instrument in $CDCl_3$ at ambient temperature using solvent signal as an internal standard. Thin layer chromatography was carried out on Merck silica gel pre-coated F_{254} plates (0.2 mm) using chloroform/acetone (1:1, v/v), as a developing system. The plates were visualized with UV light or ninhidrin solution (0.3 g ninhidrin in 100 mL of *n*-butanol and 3 mL of acetic acid).

3-(4,4-diphenylbut-3-enylamino)dihydrofuran-2(3H)-one (**3**)

A mixture of 2-amino-4-butyrolactone hydrobromide (3.17 g, 17.4 mmol), potassium carbonate (12.04 g, 87.1 mmol), TBAB (0.56 g, 1.7 mmol) and acetonitrile (30 mL) was stirred at room temperature for 1.5 h. Then, the reaction mixture was cooled and 4-bromo-1,1-diphenylbut-1-en (**2**) (5 g, 17.4 mmol) was added dropwise. After the reaction was completed, the precipitate was filtered off, washed with acetone (10 mL) and the filtrate was concentrated in vacuum. The residue was purified by column chromatography on silica gel using a mixture of acetone and CH_2Cl_2 (9:1, v/v) giving (**3**) as a yellow oil.

Yield 2.39 g (44 %). Analysis: calcd. for $C_{20}H_{21}NO_2$: C 78.15, H 6.89, N 4.56%; found: C 78.21, H 6.93, N 4.60%; R_f (acetone:CH_2Cl_2 1:9, v/v) 0.67. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.28–2.46 (m, 2H, CHCH_2CH$_2$), 2.63–2.88 (m, 2H, =CHCH_2), 3.33–3.69 (m, 2H, CH_2NH), 3.72–3.77 (m, 1H, C*H*NH), 4.17–4.38 (m, 2H, CH_2O), 6.06–6.16 (m, 1H, =C*H*CH$_2$), 7.14–7.44 (m, 10H, Ar).

Synthesis of 2-(4,4-diphenylbut-3-enylamino)-4-hydroxybutanamides (general procedure)

3-(4,4-Diphenylbut-3-enylamino)dihydrofuran-2(3H)-one (**3**) (1 eq.) and relevant benzylamine (1.3 eq.) were refluxed in THF (5 mL) for 24 h. The progress of the reaction was monitored by TLC. Then, the solvent was evaporated and the residue was purified by column chromatography on a silica gel using a mixture of acetone and $CHCl_3$ (1 : 1, v/v). The resulting product was crystallized from the mixture of ethyl acetate and *n*-hexane (7:3, v/v).

N-benzyl-2-(4,4-diphenylbut-3-enylamino)-4-hydroxybutanamide (**4a**)

White solid, yield 0.5 g (70%). Analysis: calcd. for $C_{27}H_{30}N_2O_2$: C 78.23, H 7.29, N 6.76%; found: C 78.21, H 7.32, N 6.83%. R_f (acetone:$CHCl_3$ 1:1, v/v) 0.50, m.p. 89.7–90.1°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.72–1.91 (m, 2H, CH_2NH)), 2.22–2.31 (m, 2H, CH_2CH$_2$OH), 2.58–2.79 (m, 2H, =CHCH_2), 3.22 (t, 1H, C*H*CO), 3.65–3.80 (m, 2H, CH_2OH), 4.39–4.50 (m, 2H, NHCH_2), 6.02 (t, 1H, =C*H*CH$_2$), 7.14–7.37 (m, 15H, Ar), 7.44 (br s, 1H N*H*CH$_2$).

N-(2-chlorobenzyl)-2-(4,4-diphenylbut-3-enylamino)-4-hydroxybutanamide (**4b**)

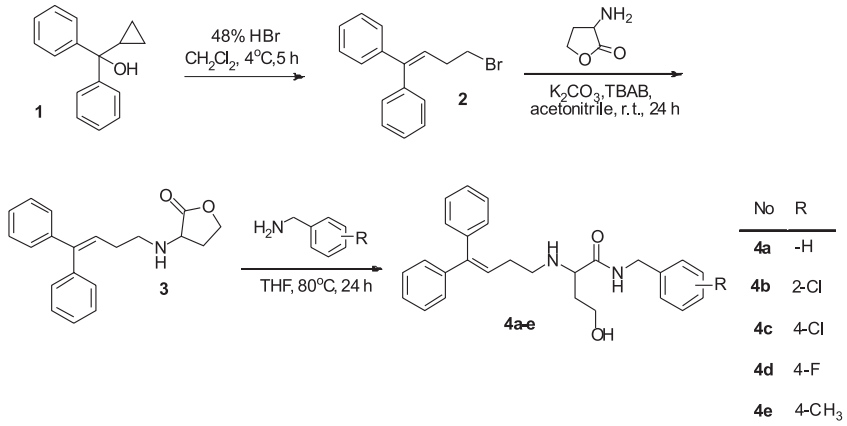
White solid, yield 0.21 g (56 %). Analysis: calc. for $C_{27}H_{29}ClN_2O_2$: C 72.23, H 6.51, N 6.24%; found: C 72.15, H 6.52, N 6.23%. R_f (acetone:$CHCl_3$ 1:1, v/v) 0.58, m.p. 103.0 – 103.5°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.74–1.86 (m, 2H, CH_2NH), 2.27 (q, 2H, CH_2CH$_2$OH), 2.59–2.70 (m, 2H, =CHCH_2), 3.21 (t, 1H, C*H*CO), 3.70–3.77 (m, 2H, CH_2OH), 4.51 (d, 2H, NHCH_2), 6.02 (t, 1H, =C*H*CH$_2$), 7.20–7.36 (m, 14H, Ar), 7.53–7.61 (br s, 1H N*H*CH$_2$).

N-(4-chlorobenzyl)-2-(4,4-diphenylbut-3-enylamino)-4-hydroxybutanamide (**4c**)

White solid, yield 0.32 g (48%). Analysis: calc. for $C_{27}H_{29}ClN_2O_2$: C 72.23, H 6.51, N 6.24%; found: C 72.25, H 6.57, N% 6.29%. R_f (acetone:$CHCl_3$ 1:1, v/v) 0.71, m.p. 142.4–142.8°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.99 (dd, 2H, CH_2NH)), 2.43 (q, 2H, CH_2CH$_2$OH)), 3.66–3.73 (m, 2H, =CHCH_2), 4.08–4.16 (m, 3H, (C*H*CO; CH_2NH)), 4.38 (d, 2H, CH_2OH), 5.77 (d, 2H, NHCH_2), 6.05 (t, 1H, =*CH*CH$_2$) 7.13–7.46 (br s, 15H, Ar, N*H*CH$_2$).

N-(4-fluorobenzyl)-2-(4,4-diphenylbut-3-enylamino)-4-hydroxybutanamide (**4d**)

White solid, yield 0.36 g (51 %). Analysis: calc. for $C_{27}H_{29}FN_2O_2$: C 74.97, H 6.76, N 6.48%; found: C 75.05, H 6.77, N 6.49%. R_f (acetone:$CHCl_3$ 1:1,



Scheme 1. Synthesis of 2-substituted 4-hydroxybutanamides **4a**–**e**.

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

Compound	mGAT1 uptake[a]	mGAT2 uptake[a]	mGAT3 uptake[a]	mGAT4 uptake[a]	GAT1 NO711 binding[b]
4a	4.15 ± 0.12	4.44 ± 0.05	4.60 ± 0.11	4.72 ± 0.10	4.47 ± 0.05
4b	4.54 ± 0.13	4.69 ± 0.03	4.73 ± 0.04	4.96 ± 0.02	47%
4c	4.43 ± 0.10	4.54 ± 0.10	4.80 ± 0.07	67%	92%
4d	4.43 ± 0.05	4.32 ± 0.14	4.49 ± 0.04	4.89 ± 0.13	75%
4e	74%	54%	46%	97%	86%
Tiagabine[14]	6.88 ± 0.12	52%	64%	73%	–

[a] % of remaining GABA uptake at 100 mM concentration of tested compound (the means; n = 3) or pIC_{50} (the mean ± SEM; n = 3). [b] % of NO711 bound to GAT1 at 100 mM concentration of tested compound (the means; n = 3) or pK_i (the mean ± SEM; n = 3).

v/v) 0.70, m.p. 129.1–130.1°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.79 (s, 2H, C*H*$_2$NH), 2.22–2.29 (q, 2H, C*H*$_2$CH$_2$OH), 2.59–2.73 (m, 2H, =CHC*H*$_2$), 3.21 (t, 1H, C*H*CO), 3.70–3.81 (m, 2H, (C*H*$_2$OH), 4.38 (d, 2H, NHC*H*$_2$), 6.02 (t, 1H, =C*H*CH$_2$), 6.94–7.37 (m, 14H, Ar), 7.47 (br s., 1H N*H*CH$_2$).

N-(4-methylbenzyl)-2-(4,4-diphenylbut-3-enylamino)-4-hydroxybutanamide (**4e**)

White solid, yield 0.10 g (14%). Analysis: calc. for $C_{28}H_{32}N_2O_2$: C 78.43, H 7.51, N 6.54%; found: C 78.35, H 7.57, N 6.59%. R_f (acetone:$CHCl_3$ 1:1, v/v) 0.73, m.p. 130.0–131.5°C. ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 1.26 (s, 3H, C*H*$_3$), 1.83 (s, 2H, C*H*$_2$NH), 2.27 (s, 2H, C*H*$_2$CH$_2$OH), 2.68 (s, 2H, =CHC*H*$_2$), 3.20 (t, 1H, C*H*CO), 3.73 (s, 2H, C*H*$_2$OH), 4.40 (d, 2H, NHC*H*$_2$), 6.02 (t, 1H, =C*H*CH$_2$), 7.15 (m, 14H, Ar), 7.37 (br s, 1H, N*H*CH$_2$).

Pharmacology

In vitro activity

[^{3}H]GABA uptake assay

[^{3}H] GABA uptake assays with GAT1-4 were performed as previously described (12). Binding assays for mGAT1 based on NO 711 (1-[2-{[(diphenylmethylene)imino]oxy}ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid) as native marker were performed as described earlier (13). NO 711 was analyzed by LC-MS/MS using an API 3200 triple quadrupole mass spectrometer according to the method described previously (14).

RESULTS AND DISCUSSION

Chemistry

The herein presented compounds (**4a**–**e**) were obtained by a simple three stage reaction pathway. Firstly, 4-bromo-1,1-diphenylbut-1-en (**2**) was syn-

thesized by solvolysis of cyclopropyldiphenylcarbinol (**1**) in concentrated hydrobromic acid (15). Then, the 3-(4,4-diphenylbut-3-enylamino)dihydrofuran-2(3H)-one (**3**) was obtained by *N*-alkylation of 2-amino-4-butyrolactone with compound **2**. The reaction was carried out in acetonitrile in the presence of anhydrous K_2CO_3 at room temperature for 24 h. Finally, the aminolysis of the lactone (**3**) by relevant substituted benzylamines resulted in amides of 2-substituted-4-hydroxybutanoic acid (**4a–e**). The aminolysis was performed by heating substrates in THF for 24 h. The course of the performed reactions is presented in Scheme 1.

Biological evaluation

Inhibitory potency of the compounds **4a–e** was tested at four murine GABA transporter subtypes mGAT1–mGAT4. The study was performed as the [^{3}H]GABA uptake assay based on stably transfected HEK cells, according to the procedure recently described (12). The affinity to mGAT1 was determined by MS-binding assay with NO711 as a non-labeled marker (13, 14). The compounds were considered active, when at a concentration of 100 mM reduced GABA uptake or NO711 binding at least by 50%. For the active compounds, pIC_{50} values were assessed. Based on results obtained, compounds **4a–d** showed inhibitory potency of mGAT1–4 (Tab. 1).

The newly synthesized compounds **4a-d** were found to be GABA uptake inhibitors, while compounds **4e** was not active in those tests. It is also worth to know that compounds **4a**, **4b** and **4d** were also slightly selective towards GAT4. The highest potency towards GAT4 (pIC_{50} 4.96) was observed for compound **4b**, which contains a chlorine atom at the *ortho-* position of benzyl fragment of the molecule. In comparison to unsubstituted compound **4a**, introduction of halide atom in the *ortho-* (**4b**) or *para-* (**4c**, **4d**) position of benzyl fragment resulted in increasing inhibitory activity of the compounds obtained. Among compounds tested only compound **4a** displaced NO 711 from binding with $pK_i = 4.47$.

CONCLUSION

In summary, various substituted *N*-benzyl-2-(4,4-diphenylbut-3-enylamino)-4-hydroxybutanamides (**4a-4e**) have been synthesized. All compounds have been evaluated regarding their inhibitory potency and subtype selectivity at the four murine GABA transporters subtypes mGAT1-mGAT4. The compounds obtained displayed activity at the GABA transport proteins. Although no general dependency from substituent in benzyl fragment of molecules could be determined, these compounds might represent a good starting point for the development of new class of GABA-uptake inhibitors.

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