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Potential bioisosteres of β-uracilalanines derived from 1*H*-1,2,3-triazole-*C*-carboxylic acids

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ABSTRACT

The 1*H*-1,2,3-triazole-originated derivatives of willardiine were obtained by: (i) construction of the 1*H*-1,2,3-triazole ring in 1,3-dipolar cycloaddition of the uracil-derived azides and the carboxylate-bearing alkynes or α-acylphosphorus ylide, or (ii) *N*-alkylation of the uracil derivative with the 1*H*-1,2,3-triazole-4-carboxylate-derived mesylate. The latter method offered: (i) reproducible results, (ii) a significant reduction of amounts of auxiliary materials, (iii) reduction in wastes and (iv) reduction in a number of manual operations required for obtaining the reaction product. Compound **6a** exhibited significant binding affinity to hHS1S2I ligand-binding domain of GluR2 receptor (EC_{50} = 2.90 μM) and decreased viability of human astrocytoma MOG-G-CCM cells in higher extent than known AMPA antagonist GYKI 52466.

1. Introduction

Willardiine (Fig. 1) belongs to the class of nucleoamino acids [1]. It was isolated from Acacia willardiana or Mimosa asperata in 1961 [2]. Since its agonistic activity at AMPA glutamate receptors was discovered [3], willardiine and its derivatives became useful tools for elucidating the molecular mode of action of glutamate ionotropic receptors. While 5-halowillardiines **1** preserved the agonistic activity of willardiine [4], the N^3-modified derivatives **2** and **3** (Fig. 1) acted as selective antagonists of the ionotropic glutamate AMPA receptors (compounds **2a** [5]) or kainate receptors (compounds **2b** or **3** [6]). Antagonists of glutamate ionotropic receptors were postulated to be useful in the therapy of neurodegenerative diseases, such as Alzheimer's disease [7], owing to the role of these receptors in activity of mammalian brains, i.e. in learning processes and in memory formation.

The concept of bioisosterism has been extensively exploited in the drug design and development process. According to the definition of Friedman, "[bioisosteres are structural moieties] which fit the broadest definition of isosteres and have the same type of biological activity" [8]. The bioisostere concept has been employed to increase potency or selectivity of the native drug (or drug-candidate), to improve ADME properties (adsorption, distribution, metabolism or excretion), or annihilate toxicity of the parent compound. The 1*H*-1,2,3-triazole scaffold has met the criteria of the Friedman definition with a broad range of the structural motifs. It acted as bioisostere of an amide bond, ester bond, double bond, pyrophosphate unit [9], carboxylic group, or a heteroaromatic ring [10]. Further, ability of the 1,4- or 1,5-disubstituted 1*H*-1,2,3-triazole ring to bind proteins *via* hydrogen bonds was reported [11]. In general, the sp^2-nitrogen atom(s) of the 1*H*-1,2,3-triazole ring served as the *H*-bond acceptor(s), while the tertiary carbon atom acted as the *H*-bond donor (Fig. 2a). When *C*-substituted with the carboxamide function as the *H*-bond donor (Fig. 2b), the 1*H*-1,2,3-triazole ring strongly interacted with guanine [12]. The 1*H*-1,2,3-triazole-derived carboxamides were approved as drugs (carboxyamidotriazole [13], rufinamide [14]) or reported as drug candidates (1,2,3-triazole-ribavirin [15], 1,2,3-triazole-bredinin [15], or 1,2,3-triazole-TSAO [16]).

Considering both the *H*-bond-mediated interactions of willardiine(s) with the receptor proteins [17] and the *H*-bonding potential of the 1*H*-1,2,3-triazole ring, we envisaged compounds **4**–**7** (Fig. 3) as potential bioisosteres of willardiine or the wiilardiine-derived glutamate receptor modulators. The *C*-carboxylate- or *C*-carboxamide-substituted 1*H*-1,2,3-triazole moiety was designed as a surrogate of the willardiine amino acid function [18] (compounds **4** and **5**) or the heteroaromatic function of the willardiine derivative **2c** or **3** (compounds **6** and **7**).

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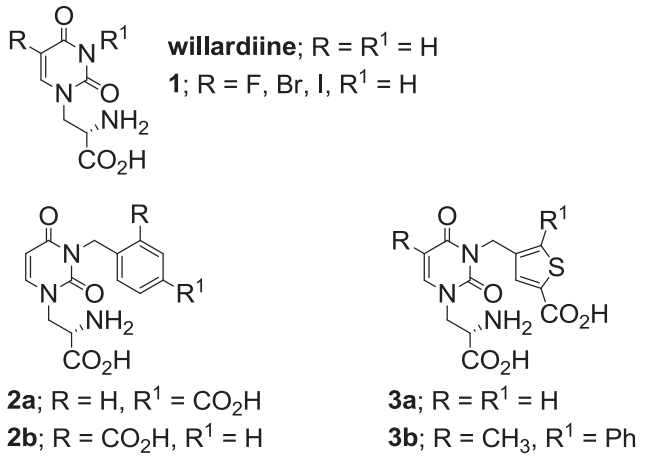


Fig. 1. Willardiine and its uracil C^5- and/or N^3-modified derivatives of medicinal interest.

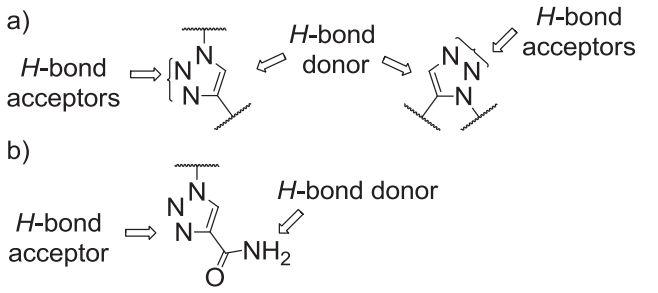
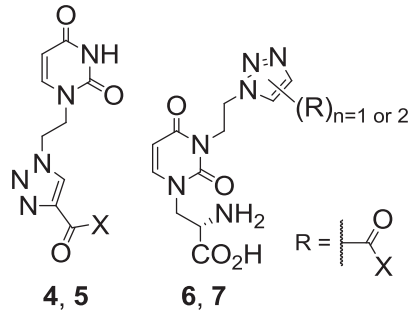


Fig. 2. The reported *H*-bonding properties of: a) the 1,4- or 1,5-disubstituted 1*H*-1,2,3-triazole ring, b) the 1-substituted 1*H*-1,2,3-triazole-4-carboxamide moiety.



Compound	X
4	OH
5	NH_2
6	OH
7	NH_2

Fig. 3. Target compounds in the current project.

2. Results and discussion

2.1. Synthesis

The preparation of the 1*H*-1,2,3-triazole-bearing uracils as precursors of the target compounds **4**–**7** was crucial in our project. Two approaches were considered (Scheme 1): (i) reaction of the N^1- or N^3-protected uracil with the 1*H*-1,2,3-triazole-alkylating agent; or (ii) 1,3-dipolar cycloaddition of the azido-uracil to the carboxylate dipolarophile, such as ethyl propiolate or ethyl acetylenedicarboxylate or phosphorous α-acyl ylide. We decided to examine the former approach because literature data on employing of (2-haloethyl)- [19] or (2-tosyloxyethyl)-1,2,3-triazoles [20] as alkylating agents have been extremely limited. Further, the reported alkylations gave satisfactory yields solely when conducted under a microwave irradiation conditions [19a], or in the absence of a base [20a]. The latter requirement resulted from considerable proclivity of (2-haloethyl)- or (2-tosyloxyethyl)-2*H*-1,2,3-triazoles to elimination reaction leading to the corresponding vinyl-1,2,3-triazoles.

The alkylation approach was developed for the synthesis of compounds **4** and **5** (Scheme 2) because the literature data revealed low yield of 1-(2-azidoethyl)uracil [21], a potential substrate in the cycloaddition approach. Thus, the K_2CO_3-mediated alkylation of 3-(pivaloyloxymethyl)uracil **8** [22] with 1*H*-1,2,3-triazole-bearing mesylate **9** [23] afforded 1,3-disubstituted uracil **10** in 77% yield. Next, treatment of **10** with aqueous LiOH, followed by acidification of the reaction mixture with hydrochloric acid gave the target acid **4** in 69% yield. On the other hand, two-step one-pot treatment of compound **10** with ammonium hydroxide at elevated temperature followed by hydrolysis of the crude reaction products with LiOH gave amide **5** in 69% yield.

The synthesis of compound **15a** *via* the cycloaddition approach is shown in Scheme 3. The starting azide **12** was obtained by azidoethylation of compound **11a** [24]. N^1-deprotection of azide **12** followed by cycloaddition of the resulting product **13** with ethyl propiolate was the most effective synthetic variant (74% two-step yield from azide **12**). The reversed reaction sequence via compound **14a** gave product **15a** in 51% two-step yield owing to non selective formation of 1,2,3-triazoles **14a** and **14b** from azide **12**.

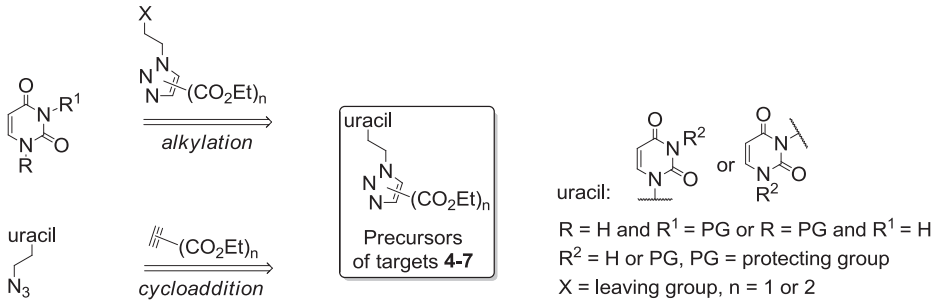
The alkylation approach to compounds **14a** is shown in Scheme 4. The K_2CO_3-mediated reaction of compound **11a** with mesylate **9** produced compound **14a** in 75% yield. From the preparative point of view, the most satisfactory protocol for preparation of compound **15a** (60% total yield) was developed from 1-Boc-uracil **11b** [25] and mesylate **9**. The two-step one-pot process involved the N^3-alkylation of compound **11b** with mesylate **9** under the previously used conditions followed by treatment of the crude post-alkylation mixture with trifluoroacetic acid.

1,5-Disubstituted-1*H*-1,2,3-triazole **15b** (Scheme 5) was synthesized in a two-step process employing the 1,3-dipolar cycloaddition of azide **12** to ethyl 2-oxo-3-(triphenylphosphoranylidene) propanoate [26,27], followed by trifluoroacetic acid-mediated N^1-deprotection of compound **14b**. The overall yield of compound **15b** was 53%. The preparation of the intermediate **14b** (64% yield) was the yield-limiting step of this process.

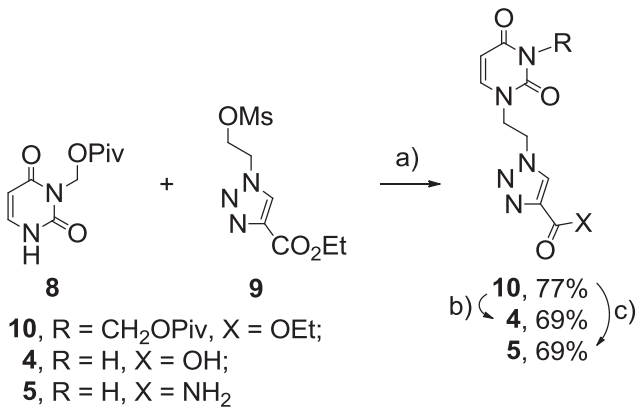
Diester **15c** was obtained from ethyl acetylenedicarboxylate and azide **12** or **13** (Scheme 6). Both synthetic routes were similarly efficient and gave compound **15c** in the overall yields exciding 70% [28].

Next, having the synthesis of compounds **15a-c** optimized, we converted them to the corresponding acids **16a-c** or amides **17a-b** (Scheme 7, Table 1), precursors of the target compounds **6** or **7**, respectively. Thus, carboxylic acids **16a-c** were synthesized in yields exceeding 90% by the LiOH-mediated hydrolysis of esters **15a-c** (Table 1, entries 1–3), followed by treatment of the reaction mixture with acidic ion exchange resin. On the other hand, amides **17a** and **17b** were obtained from ammonolysis of esters **15a** or **15c** at elevated temperature, respectively.

The thus obtained acids **16a-c** and amides **17a**,**b** were subjected to the NaH-promoted reaction with (*S*)-*tert*-butyl-(2-oxooxetan-3-yl)carbamate [29], followed by treatment of the crude reaction mixture with diluted hydrochloric acid (Scheme 8). The type of the acid used in the latter step was crucial in this process. Hydrochloric acid was used less frequently than trifluoroacetic acid in the literature syntheses of willardiine derivatives [30]. However in our project, hydrochloric acid was more efficient than trifluoroacetic acid and gave pure compound **6a** in the 20% yield (Table 2, entry 2 *vs* entry 1, respectively). According to the procedure employing hydrochloric acid for the acidification of the post-coupling mixture, products **7a**,**b** were obtained in 20% yields (Table 2, entries 4 and 5). Yields of compounds **6a** and **7a**,**b** are not surprising. Based on the published syntheses of the willardiine derivatives, we believed that efficiency of the reaction between the pyrimidine derivative **16** (or **17**) and aminolactone was the yield-limiting step. Low stability of the aminolactone under the basic conditions was postulated in the literature as the main reason for the limited reactivity of this compound [31]. Probably for this reason, the reaction between



Scheme 1. Synthetic approach to the 1*H*-1,2,3-triazole-precursors of the target compounds **4–7**.

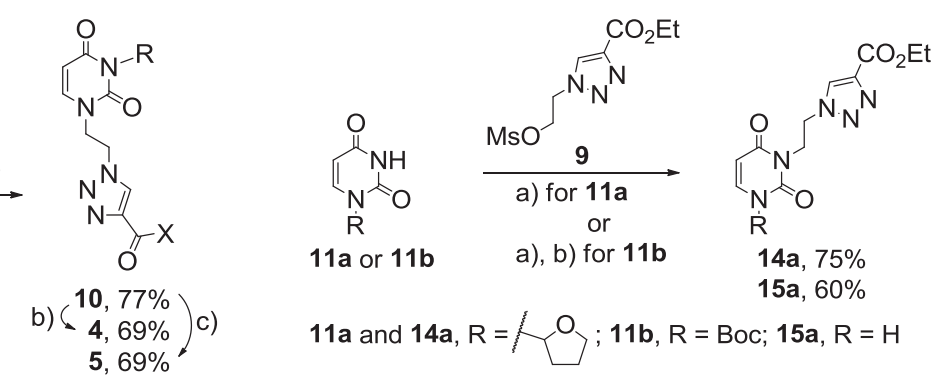


Scheme 2. Synthesis of compounds **4** and **5**. Reagents and conditions. (a) K_2CO_3, DMF, 50 °C, 2 days. (b) (1) LiOH, H_2O, THF, room temperature, 1 day; (2) 2% aqueous HCl. (c) (1) NH_4OH, THF, 50 °C, 1 day; (2) LiOH, H_2O, THF, room temperature, 1 day; (3) 2% aqueous HCl.

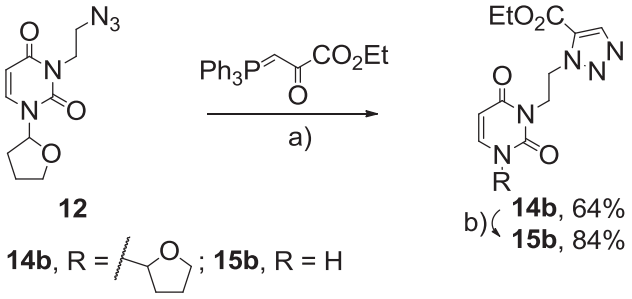
the tri-substituted 1*H*-1,2,3-triazole derivative **16b** and aminolactone did not afford the expected product. Substrate **16b** was recovered from the reaction mixture in 78% yield (Table 2, entry 3).

2.2. Structure elucidation

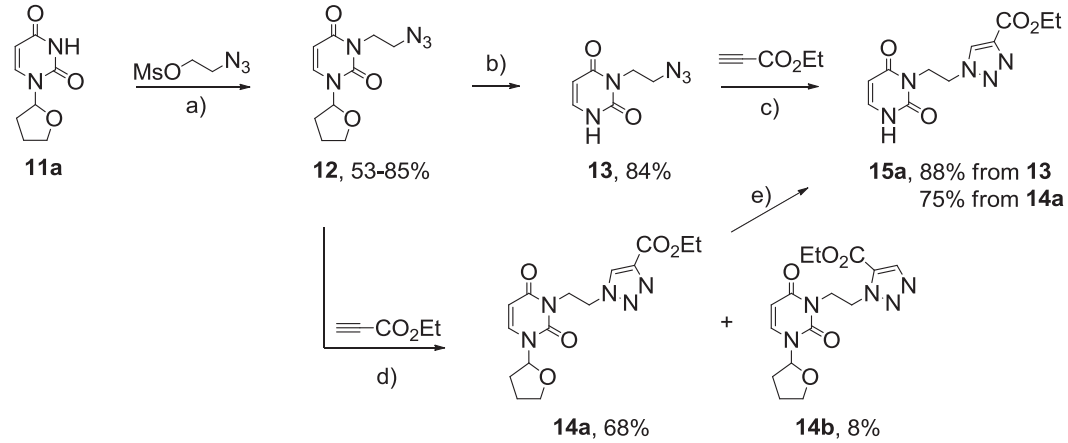
NMR spectroscopy was employed to distinguish compounds **15a** and **15b**. These compounds were identified and fully characterized by [^{1}H]-coupled ^{13}C NMR spectroscopy, ^{1}H–^{13}C HMBC spectroscopy and ^{1}H–^{13}C HMQC spectroscopy. In the [^{1}H]-coupled ^{13}C NMR spectrum of compound **15a**, the signal assigned to the 1,2,3-triazole *H*-bonded-C(5) carbon nucleus appeared as a doublet of triplets at 129.66 ppm, owing to couplings of H(5)↔C(5) ($^1J_{H(5)-C(5)}$ = 198.8 Hz) and H(1a)↔C(5)



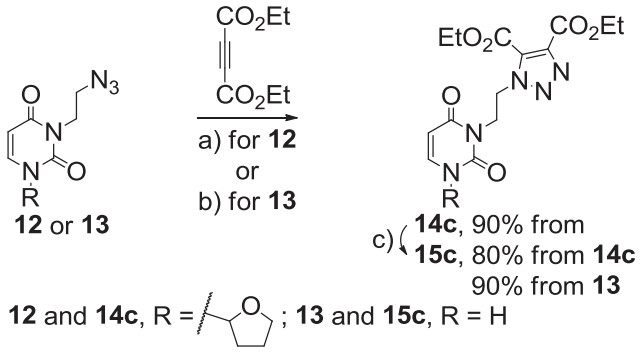
Scheme 4. Alkylation approach to compounds **14a** and **15a**. Reagents and conditions. (a) NaH, DMF, room temperature, 1 day. (b) CF_3CO_2H, CH_2Cl_2, room temperature, 2 h.



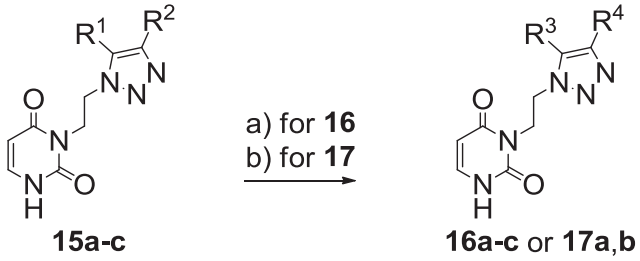
Scheme 5. Cycloaddition approach to compound **15b**. Reagents and conditions. (a) Toluene, 110 °C, 2 days. (b) CF_3CO_2H, room temperature, 3 days.



Scheme 3. Cycloaddition approach to compound **15a**. Reagents and conditions. (a) NaH, NaI, DMF, 60–65 °C, 2 days. (b) conc. HCl, MeOH, 24 h. (c) MeOH, room temperature, 3 days. (d) Hexane-AcOEt (1:1, v/v), room temperature, 3 days. (e) CF_3CO_2H, room temperature, 3 days.



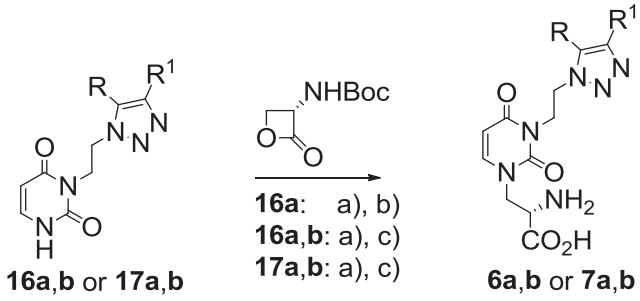
Scheme 6. Cycloaddition approach to compound **15c**. Reagents and conditions. (a) Hexane-AcOEt (1:1, v/v), room temperature, 3 days. (b) MeOH, room temperature, 3 days. (c) CF_3CO_2H, room temperature, 3 days.



Scheme 7. Synthesis of compounds **16** and **17**. Reagents and conditions. (a) (1) LiOH, H_2O, THF, room temperature, 40 min (**15a**) or 1 day (**15b**) or 2 h (**15c**); (2) Dowex 50WX2, room temperature, 1 h. (b) NH_4OH, MeOH, 65 °C, 1 day.

Table 1
Yields of compounds **16** and **17**.

Entry	**15**, R^1, R^2	**16** or **17**, R^3, R^4, yield
1	**a**, H, CO_2Et	**16a**, H, CO_2H, 98%
2	**b**, CO_2Et, H	**16b**, CO_2H, H, 91%
3	**c**, CO_2Et, CO_2Et	**16c**, CO_2H, CO_2H, 95%
4	**a**, H, CO_2Et	**17a**, H, $C(O)NH_2$, 96%
5	**b**, CO_2Et, H	**17b**, $C(O)NH_2$, H, 57%



Scheme 8. Synthesis of compounds **6** and **7**. Reagents and conditions. (a) NaH, DMF, room temperature, 1 day. (b) CF_3CO_2H, H_2O, room temperature, 1 day. (c) 2% HClaq, room temperature, 1 day.

($^3J_{H(1a)-C(5)}$ = 2.5 Hz) (Fig. 4a, green arrows). The H(5)↔C(5) interaction was confirmed from the ^1H–^{13}C HMQC spectrum [32]. The H(1a)↔C(5) interaction was also observed in the ^1H–^{13}C HMBC spectrum (Fig. 4a, blue arrow). In the ^{13}C NMR [1H]-coupled spectrum of isomer **15b**, the resonance due to the 1,2,3-triazole *H*-bonded-C(4) carbon nucleus appeared as a doublet at 137.45 ppm owing to the H(4)↔C(4) coupling ($^1JH_{(4)-C(4)}$ = 196.25 Hz) (Fig. 4b, green arrow). The H(4)↔C(4) interaction was also present in the ^1H–^{13}C HMBC spectrum (Fig. 4b, blue arrow). In contrast to the literature data [33,34],

Table 2
Yields of compounds **6** and **7**.

Entry	R, R^1	**16** or **17**	**6** or **7**, yield
1	H, CO_2H	**16a**	**6a**, 16%[a]
2	H, CO_2H	**16a**	**6a**, 20%[b]
3	CO_2H, CO_2H	**16b**	**6b**, —[c]
4	H, $C(O)NH_2$	**17a**	**7a**, 20%
5	$C(O)NH_2$,H	**17b**	**7b**, 20%

[a] TFA-mediated removal of the Boc protection.
[b] HCl-mediated removal of the Boc protection.
[c] **16b** recovered in 78% yield.

interactions between the carbonyl group-bonded 1,2,3-triazole carbon nucleus and the adjacent protons could also be observed in the [^{1}H]-coupled ^{13}C NMR spectra of isomers **15a** and **15b** (Fig. 4a and 4b, respectively), owing to the well-separated signals. The C(4) carbon nuclei in isomer **15a** was represented by a doublet at 138.68 ppm owing to the H(5)↔C(4) coupling ($^3J_{H(5)-C(4)}$ = 10.0 Hz) in the ^{13}C NMR [^{1}H]-coupled spectrum (Fig. 4a, green arrow). The H(5)↔C(4) interaction was strongly manifested in the ^1H–^{13}C HMBC spectrum (Fig. 4a, blue arrow). The C(5) carbon nuclei in isomer **15b** was represented by a broad doublet at 128.49 ppm in the ^{13}C NMR [1H]-coupled spectrum. The H(4)↔C(5) coupling ($^3J_{H(4)-C(5)}$ = 13.8 Hz) (Fig. 4b, green arrow) in isomer **15b** was accompanied by the H(1a)↔C(5) coupling (Fig. 4b, green arrow). The H(1a)↔C(5) coupling was relatively weak ($^3J_{H(1a)-C(5)}$ < 2 Hz) and manifested by a broadening of the doublet due to the C(5) carbon nuclei. The H(1a)↔C(5) interaction was ultimately confirmed from the ^1H–^{13}C HMBC spectrum (Fig. 4b, blue arrow).

2.3. Receptor-binding assays

The affinity of compounds **4–7** and **15–17** to hHS1S2I ligand-binding domain of GluR2 receptor was determined using reported *in vitro* assay (Table 3) [35,36]. Binding experiments were performed with DL-α-[5-methyl-^{3}H]-AMPA (final concentration 10 nM, 10.6 Ci/mmol). L-Glutamic acid (EC_{50} = 1.66 μM) and willardiine (EC_{50} = 3.22 μM) were tested as binding positive controls. Among the examined compounds, derivative **6a** showed significant binding activity with EC_{50} = 2.90 μM (Supporting Information, Table 1). The obtained results revealed importance of both the amino acid moiety and the carboxyl group in the 1*H*-1,2,3-triazole ring in effective binding to the examined receptor. Amides **7a** and **7b** at concentration of 100 μM showed 99.8% and 94.1%, respectively, of remaining DL-α-[5-methyl-^{3}H]-AMPA bound to hHS1S2I compared to 3.3% for **6a**. It should be noted that this test indicated only binding affinity. Additional experiments are required in order to determine agonistic or antagonistic mode of action of compound **6a**.

2.4. Docking studies

In order to rationalize our findings on the binding assays, molecular modeling study was performed by docking compound **6a** (Fig. 5), willardiine and AMPA in the binding domain of hGluR2 receptor (hGluR2-S1S2/L-glutamate crystal structure, PBD entry 3r7x [38]) [39]. The ranking of these compounds obtained from the *in vitro* radioligand-displacement assays (Section 2.3) was reflected by the predicted free-energies of binding (ΔG_{bind}): **6a** (−9.2 kcal/mol) < willardiine (−8.5 kcal/mol) < AMPA (−6.5 kcal/mol) [40]. The proposed mode of the **6a**-hGluR2-S1S2 binding involved anchoring of the **6a** amino acid residue by Pro89 (Table 4, entry 2), Thr91 (Table 4, entries 3 and 4) and Arg96 (Table 4, entries 5 and 6), i.e. the well established protein residues anchoring L-glutamate [38], AMPA [41] as well as willardiine [42] and its derivatives [43] in the GluR2 binding domain. Unlike the willardiine-derived GluR2 antagonists UBP277 [43] and UBP282 [43], the side chain carboxyl group of compound **6a** was expected to interact

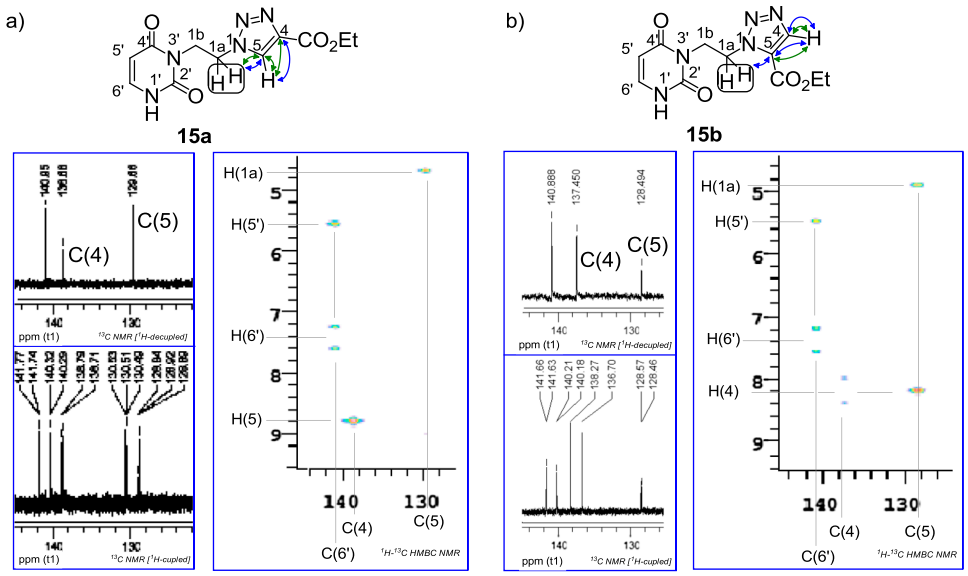


Fig. 4. (a) ^{13}C NMR, [^{1}H]-coupled ^{13}C NMR and ^{1}H–^{13}C HMBC spectra of compound **15a** (in DMSO-d_6). (b). ^{13}C NMR, [^{1}H]-coupled ^{13}C NMR and ^{1}H–^{13}C HMBC spectra of compound **15b** (in DMSO-d_6). The ^{1}H–^{13}C couplings denoted as green. The ^{1}H–^{13}C HMBC interaction denoted in blue.

Table 3
Binding of the newly synthesized compounds to hHS1S2I binding domain of GluR2 receptor. Results are shown as percent of remaining DL-α-[5-methyl-^{3}H]-AMPA bound to hHS1S2I [37].

Compound	Concentration		EC_{50} [μM]
	100 μM	10 μM	
L-glutamic acid	0.9	9.3	1.66
Willardiine	6.9	40.5	3.22
4	109	nd	nd
5	108	nd	nd
6a	3.3	26.9	2.90
7a	100	nd	nd
7b	94	nd	nd
15a	108	nd	nd
15b	102	nd	nd
16a	135	nd	nd
16b	100	nd	nd
16c	94	nd	nd
17a	99	nd	nd
17b	118	nd	nd

nd = not determined.

Table 4
Proposed mode of the **6a**-GluR2-hS1S2 binding and distances of potential ligand-protein *H*-bonds (≤3.3 Å).

Entry	Protein residue	Atomic group of 6a	Distance (Å)[a]
1	Glu193)$O^{\varepsilon 2}$	(N2)H	2.2
2	(Pro89)C═O	(N2)H	2.7
3	(Thr91)$O^{\gamma 1}$	(N2)H	2.2
4	(Thr91)NH	(C1)O11	2.8
5	(Arg96)$N^{\eta 1}$H	(C1)O11	2.3
6	(Arg96)$N^{\eta 2}$H	(C1)O12	1.9
7	(Ser142)NH	(C1)O12	2.0
8	(Ser142)O^{γ}H	(C1)O12	2.1
9	(Leu138)NH	(C7)O	2.1
10	(Thr174)$O^{\gamma 1}$H	(N13)	2.3
11	(Tyr61)O^{η}H	(N14)	2.1
12	(Met196)NH	(C17)O171	2.6

[a] From the docking experiment.

with Met196-*NH via* the *H*-bond of the 2.6 Å distance (Table 4, entry 12). The *H*-bonds between the 1,2,3-triazole sp^2-nitrogen atoms and the side chain hydroxyls form Thr174 and Tyr61 were expected to play a

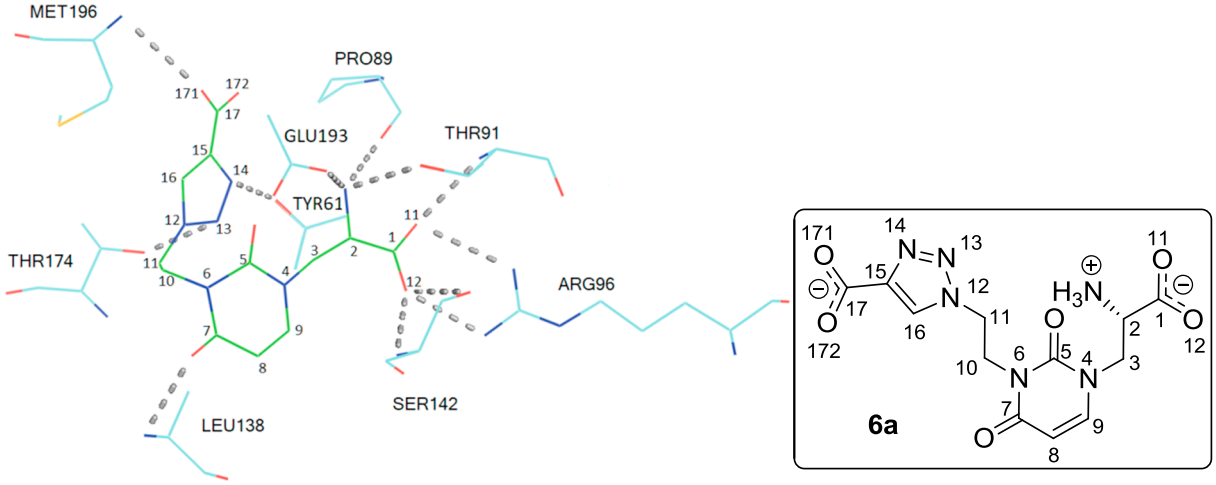


Fig. 5. Proposed mode of the **6a**-GluR2-hS1S2 binding. Colours: C (ligand) = green, C (protein residue) = light blue, N = dark blue, O = red, S = yellow, *H*-bond = gray.

role at the location of compound **6a** in the protein binding domain (Table 4, entries 10 and 11).

2.5. Anticancer activity assays

Antagonists of glutamate ionotropic receptors have recently been suggested as potent anticancer therapy targets owing to their ability to decrease proliferation and migration of tumors derived from the central nervous system or some peripheral tissues [44]. To the best of our knowledge, an effect of willardiine-derived antagonists on such tumors have not been reported. On the other hand, 5-fluorowillardiine – the AMPA agonist – was reported to increase proliferation, migration and invasion of pancreatic cancer cells *in vitro* [45]. In the light of these sparse data, the evaluation of anticancer properties of compounds **4–7** and **15–17** were performed with the use of the selected CNS cancer cell lines expressing GluR1 and GluR2 AMPA subunits at different protein levels [46]: (i) SK-N-AS – human neuroblastoma (lack of AMPA GluR1 and GluR2 subunits), (ii) U251MG – human glioblastoma (AMPA GluR2 subunit), (iii) MOG-G-CCM – human brain astrocytoma (AMPA GluR1 subunit). Additionally, the all newly obtained compounds were tested against (iv) human normal lung fibroblast cells MRC-5 pd30 [47]. Willardiine (AMPA agonist) and GYKI 52,466 [48] (AMPA antagonist) were used as the reference compounds in assays with MOG-G-CCM cells, SK-N-AS cells and MRC-5 pd30 cells. Treatment of SK-N-AS cells with compound **6a** at 100 μM concentration resulted in decrease in cell viability (84% live cells was determined, Table 4 in Supporting information). The same effect (86% viable cells determined) required 200 μM concentration of GYKI 52466. Compound **16c** showed low cytotoxicity toward U251MG cells that was concentration-dependent (in the range of 10–500 μM) but time of exposure-independent (Table 5 in Supporting information). Compounds **6a** and **7b** demonstrated slightly better cytotoxic effect against MOG-G-CCM cells than GYKI 52,466 (Table 4 in Supporting information). After exposure to compound **6a** or **7b** at 100 μM cncentration, 79% or 76% viable cells was determined, respectively. To gain reduction of cell viability to 83% viable cells, 200 μM concentration of GYKI 52,466 was required. While GYKI 52,466 was cytotoxic to MRC-5 pd30 cells at 200 μM concentration (67% of viable cells was determined), compounds **15b** and **16c** were non-cytotoxic for these cells at 200 μM concentration (Table 4 in Supporting information). The remaining compounds were comparatively or less cytotoxic for MRC-5 pd30 cells.

3. Conclusions

In summary, utility of 1*H*-1,2,3-triazole-4-carboxylate-derived mesylate (compound **9**) as the building block in the synthesis of 1,2,3-trazole derivatives has been revealed. The presented approach was base-tolerant, efficient and offered: (a) reproducible results, (b) a significant reduction of amounts of the used auxiliary materials, (c) reduction in wastes and (d) reduction in a number of manual operations required for obtaining the reaction product. The biological evaluation of newly synthesized compounds revealed that compound **6a**: (i) exhibited significant binding affinity to hHS1S2I binding domain of GluR2 receptor, comparable to affinity of L-Glutamic acid or willardiine, and (ii) reduced the viability of human brain astrocytoma cells MOG-G-CCM more effectively than AMPA antagonist GYKI 52466. To the best of our knowledge, willardiines were not considered as anticancer agents to date.

4. Materials and methods

Pre-coated Merck silica gel 60 F254 plates were used for thin-layer chromatography (TLC, 0.2 mm); spots were detected under UV light (254 nm) or by the use of ninhydrin [49] as the visualizing agent. Silica gel (200–400 mesh, Merck) was used for column chromatography. Optical rotations were measured on Anton Paar MCP-150 polarimeter; α values are given in deg·cm^3·g^{-1}·dm^{-1}, concentration *c* in g/(100 mL). High Resolution Mass Spectra (Electrospray Ionisation, ESI) were performed on a Mariner® spectrometer. The NMR spectra were measured on a Varian VNMRS spectrometer (^{1}H NMR at 500 MHz and ^{13}C NMR at 125 MHz). ^{1}H and ^{13}C chemical shifts (δ) were reported in parts per million (ppm) relative to the solvent signals: CDCl3, δH (residual $CHCl_3$) 7.26 ppm, δC 77.16 ppm; DMSO-d_6, δH (residual DMSO) 2.50 ppm, δC 39.52 ppm. The 3-trimethylsilyl-2,2,3,3-tetradeuteropropionic acid (TSP; sodium salt) was used as an internal standard in water/deuterochloric acid soluble samples. The NMR signals were quoted as 's' (singlet), 'd' (doublet), 't' (triplet), 'm' (multiplet), 'br s' (broad singlet), 'dd' (doublet of doublets) and 'ddd' (doublet of doublets of doublets). Coupling constants J were reported in Hertz. The ^{1}H–^{13}C HMBC (Heteronuclear Multiple Bond Correlation) spectra were measured on a Varian VNMRS spectrometer. Anhydrous $MgSO_4$ was employed as a drying agent. Volatiles were distilled off under reduced pressure on a rotating evaporator. Solvents and ammonium hydroxide (25 wt%) were purchased from Avantor Performance Materials Poland S.A., Gliwice. N,N-Dimethylformamide (DMF) and tetrahydrofuran (THF) were dried in accordance with the literature procedures [50]. Dimethyl sulphoxide (DMSO), Molecular Biology grade, used as a solvent for all stocks of the chemical agents, was obtained from Roth. GYKI 52,466 was obtained from Tocris. DL-α-[5-methyl-^{3}H]-AMPA (55,4 Ci/mmol) was purchased from Perkin Elmer. Human glioblastoma U251MG cell line was from Cell Lines Service, Germany. Cells were cultivated at 37 °C with 5% CO2 in Dulbecco's Modified Eagle Medium (DMEM; Gibco, New York, USA) supplemented with GlutaMAX-1, 10% heat inactivated fetal bovine serum (FBS; Gibco, New York, USA) and antibiotics, 1% penicilin and 1% streptomycin (Gibco, New York, USA). The cell viability of U251MG cells after exposure on the newly synthesized compounds were tested by MTS assay. MOG-G-CCM – human brain astrocytoma, SK-N-AS – human neuroblastoma, MRC-5 pd30 – human lung fibroblast (normal) were purchased from ECACC General Cell Collection. MOG-G-CCM, SK-N-AS and MRC-5 cells were cultured in Ham's F10: DMEM (1:1), DMEM with 1% Non Essential Amino Acids (NEAA) and in EMEM (EBSS) medium (Lonza), respectively. All media were supplemented with 10% fetal bovine serum (EuroClone), 2 mM L-glutamine and antibiotics (100 U/ml penicillin, 100 μg/mL streptomycin) and grown in 75 cm^2 cell culture flasks (Sarstedt), in a humidified atmosphere of CO_2/air (5/95%) at 37 °C. The cell viability of MOG-G-CCM, SK-N-AS and MRC-5 cells after exposure on the newly synthesized compounds were tested by MTT assay.

4.1. 1-(2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)ethyl)-1H-1,2,3-triazole-4-carboxylic acid 4

Lithium hydroxide (16 mg, 0.40 mmol) was added to a mixture of compound **10** (120 mg, 0.29 mmol), THF (3 mL) and water (3 mL). The mixture was stirred at room temperature for 1 day and 2% aqueous HCl (0.7 mL) was added. Volatiles were distilled off under reduced pressure. Crystallization of the residue from a mixture of ethanol and water (10:1, v/v) gave compound **4** as a white solid (52 mg, 69%, mp 160–175 °C dec). ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 11.26 (br s, 1H), 8.69 (s, 1H), 7.30 (d, ^3J = 7.0 Hz, 1H), 5.46 (d, ^3J = 7.0 Hz, 1H), 4.69–4.66 (m, 2H), 4.17–4.14 (m, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 163.64, 161.73, 150.85, 145.20, 140.11, 129.43, 101.14, 48.17, 47.50. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_9H_{10}N_5O_4$: 252.0727; found 252.0729.

4.2. 1-(2-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)ethyl)-1H-1,2,3-triazole-4-carboxamide 5

Ammonium hydroxide (2 mL) was added to a mixture of compound **10** (232 mg, 0.59 mmol) and ethanol (3 mL). The mixture was stirred at 50 °C for 1 day in a sealed tube and volatiles were distilled off under

reduced pressure. THF (3 mL), water (3 mL) and lithium hydroxide (17 mg, 0.40 mmol) were added to the residue. The mixture was stirred at room temperature for 1 day and 2% aqueous HCl (0.9 mL) was added. Volatiles were distilled off under reduced pressure. Crystallization of the residue from a mixture of ethanol and water (5:1, v/v) gave compound **5** as a white solid (102 mg, 69%, mp 219–225 °C dec). ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 11.25 (br s, 1H), 8.53 (s, 1H), 7.82 (br s, 1H), 7.43 (br s, 1H), 7.27 (d, ^3J = 8.0 Hz, 1H), 5.45 (d, ^3J = 8.0 Hz, 1H), 4.69–4.67 (m, 2H), 4.13–4.11 (m, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 163.60, 161.45, 150.83, 145.18, 143.02, 127.15, 101.11, 48.09, 47.54. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_9H_{11}N_6O_3$: 251.0887; found 252.0885.

4.3. (S)-1-(2-(3-(2-Amino-2-carboxyethyl)-2,6-dioxo-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-4-carboxylic acid 6a

4.3.1. Method A

A mixture of compound **16a** (200 mg, 0.80 mmol), anhydrous DMF (5 mL) and sodium hydride (60% suspension in mineral oil, 63 mg, 1.58 mmol) was stirred at room temperature for 1 day under argon atmosphere. (*S*)-*tert*-butyl-(2-oxooxetan-3-yl)carbamate (179 mg, 0.96 mmol) was added and the mixture was stirred at room temperature for 1 day under argon atmosphere. Volatiles were distilled off under reduced pressure and a mixture of water (2 mL), ethanol (2 mL) and hydrochloric acid (2%, 2 mL) was added to the residue. The mixture was stirred at room temperature for 1 day and volatiles were distilled off under reduced pressure. Water (5 mL) and ion exchange resin (Dowex 50WX-8-200, 10 g) were added to the residue. The mixture was shaken for 40 min and applied on a top of a chromatographic column containing ion exchange resin (Dowex 50WX-8-200, 10 g). The column was eluted with water, a water–ethanol mixture (1:1 v/v), a water-tetrahydrofuran mixture (1:1 v/v) and 2 M aqueous solution of pyridine. Ninhydrin-positive fractions were collected and volatiles were distilled off under reduced pressure. The residue was dissolved in a water-ethanol mixture (1:10, v/v, 7 mL) and diethyl ether (3 mL) was added to the solution. The white solid thus precipitated was filtered off and dried on air to give compound **6a** (50 mg, 20%). [α] = −3.7 (*c* 0.22, 6 M HCl aq). ^{1}H NMR (DCl/H_2O, 500 MHz): δ = 8.95 (s, 1H), 7.69 (d, ^3J = 8.0 Hz, 1H), 5.88 (d, ^3J 8.0 Hz, 1H), 4.94–4.91 (m, 2H), 4.64–4.58 (m, 1H), 4.52 (dd, ^2J = 15.0 Hz, ^3J = 5.0 Hz, 1H), 4.50–4.40 (m, 2H), 4.40 (dd, ^2J = 15.0, ^3J = 10.0 Hz, 1H). ^{13}C NMR (DCl/H_2O, 125 MHz): δ = 170.23, 166.91, 162.00, 154.62, 147.72, 139.38, 133.14, 103.79, 54.20, 52.08, 51.27, 43.21. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{12}H_{15}N_6O_6$: 339.1048; found 339.1046.

4.3.2. Method B

A mixture of compound **16a** (183 mg, 0.73 mmol), anhydrous DMF (5 mL) and sodium hydride (60% suspension in mineral oil, 61 mg, 1.53 mmol) was stirred at room temperature for 1 day under argon atmosphere. (*S*)-*tert*-butyl-(2-oxooxetan-3-yl)carbamate (150 mg, 0.80 mmol) was added and the mixture was stirred at room temperature for 1 day under argon atmosphere. Volatiles were distilled off under reduced pressure and trifluoroacetic acid (5 mL), water (1 mL) were added to the residue. The mixture was stirred at room temperature for 1 day and volatiles were distilled off under reduced pressure. Water (5 mL) and ion exchange resin (Dowex 50WX-8-200, 10 g) were added to the residue. The mixture was shaken for 40 min and applied on a top of a chromatographic column containing ion exchange resin (Dowex 50WX-8-200, 10 g). The column was eluted with water, a water-ethanol mixture (1:1 v/v), a water-tetrahydrofuran mixture (1:1 v/v) and 2 M aqueous solution of pyridine. Ninhydrin-positive fractions were collected and volatiles were distilled off under reduced pressure. The residue was dissolved in a water–ethanol mixture (1:10, v/v, 7 mL) and diethyl ether (3 mL) was added to the solution. The off-white solid thus precipitated was filtered off and dried on air to give compound **6a** (38 mg, 16%).

4.4. (S)-2-amino-3-(3-(2-(4-carbamoyl-1H-1,2,3-triazol-1-yl)ethyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)propanoic acid 7a

A mixture of compound **17a** (166 mg, 0.72 mmol), anhydrous DMF (5 mL) and sodium hydride (60% suspension in mineral oil, 30 mg, 0.75 mmol) was stirred at room temperature for 1 day under argon atmosphere. (*S*)-*tert*-butyl-(2-oxooxetan-3-yl)carbamate (143 mg, 0.76 mmol) was added and the mixture was stirred at room temperature for 1 day under argon atmosphere. Volatiles were distilled off under reduced pressure and a mixture of water (2 mL), ethanol (2 mL) and hydrochloric acid (2%, 2 mL) was added to the residue. The mixture was stirred at room temperature for 1 day and volatiles were distilled off under reduced pressure. Water (5 mL) and ion exchange resin (Dowex 50WX-8-200, 10 g) were added to the residue. The mixture was shaken for 1 h and applied on a top of a chromatographic column containing a ion exchange resin (Dowex 50WX-8-200, 10 g). The column was eluted with water, a water-ethanol mixture (1:1 v/v), a water-tetrahydrofuran mixture (1:1 v/v) and 2 M aqueous solution of pyridine. Ninhydrin-positive fractions were collected and volatiles were distilled off under reduced pressure. The residue was dissolved in a water–ethanol mixture (1:10, v/v, 10 mL) and diethyl ether (5 mL) was added to the solution. The white solid thus precipitated was filtered off and dried on air to give compound **7a** (49 mg, 20%). [α] = −5.0 (*c* 0.46, 6 M HCl aq). ^{1}H NMR (DCl/H_2O, 500 MHz): δ = 8.32 (s, 1H), 7.41 (d, ^3J = 8.0 Hz, 1H), 5.64 (d, ^3J = 8.0 Hz, 1H), 5.00–4.45 (5H), 4.27–4.23 (m, 1H), 4.21–4.08 (m, 1H). ^{13}C NMR (DCl/H_2O, 125 MHz): δ = 169.68, 164.65, 164.01, 152.57, 145.29, 141.65, 127.90, 101.28, 52.53, 49.25, 48.08, 41.07. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{12}H_{16}N_7O_5$: 338.1207; found 338.1203.

4.5. (S)-2-amino-3-(3-(2-(5-carbamoyl-1H-1,2,3-triazol-1-yl)ethyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)propanoic acid 7b

A mixture of compound **17b** (98 mg, 0.39 mmol), anhydrous DMF (9 mL) and sodium hydride (60% suspension in mineral oil, 17 mg, 0.39 mmol) was stirred at room temperature for 1 day under argon atmosphere. (*S*)-*tert*-butyl-(2-oxooxetan-3-yl)carbamate (88 mg, 0.47 mmol) was added and the mixture was stirred at room temperature for 1 day under argon atmosphere. Volatiles were distilled off under reduced pressure and a mixture of water (2 mL), ethanol (2 mL) and hydrochloric acid (2%, 2 mL) was added to the residue. The mixture was stirred at room temperature for 1 day and volatiles were distilled off under reduced pressure. Water (5 mL) and ion exchange resin (Dowex 50WX-8-200, 10 g) were added to the residue. The mixture was shaken for 1 h and applied on a top of chromatographic column containing a ion exchange resin (Dowex 50WX-8-200, 10 g). The column was eluted with water, a water-ethanol mixture (1:1 v/v), a water-tetrahydrofuran mixture (1:1 v/v) and 2 M aqueous solution of pyridine. Ninhydrin-positive fractions were collected and volatiles were distilled off under reduced pressure. The residue was dissolved in the water–-ethanol mixture (1:10, v/v, 10 mL) and diethyl ether (5 mL) was added to the solution. The white solid thus precipitated was filtered off and dried on air to give compound **7b** (49 mg, 20%). [α] = −8.3 (*c* 0.22, 2% HCl aq). ^{1}H NMR (DCl/H_2O, 500 MHz): δ = 8.01 (s, 1H), 7.42 (d, ^3J = 8.0 Hz, 1H), 5.59 (d, ^3J = 8.0 Hz, 1H), 4.99–4.94 (m, 1H), 4.88 (ddd, ^2J = 14.0 Hz, ^3J = 6.5 Hz, ^3J = 3.5 Hz, 1H), 4.70–4.55 (m, 1H), 4.34 (dd, ^2J = 15.0 Hz, ^3J = 4.5 Hz, 1H), 4.30–4.21 (m, 2H), 4.16 (dd, ^2J = 15.0 Hz, ^3J = 5.5 Hz, 1H). ^{13}C NMR (DCl/H_2O, 125 MHz): δ = 169.31, 164.60, 160.76, 152.63, 145.08, 135.05, 130.99, 101.31, 52.56, 48.78, 47.30, 41.14. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{12}H_{16}N_7O_5$: 338.1207; found 338.1204.

4.6. Ethyl 1-(2-(2,4-dioxo-3-((pivaloyloxy)methyl)-3,4-dihydropyrimidin-1(2H)-yl)ethyl)-1H-1,2,3-triazole-4-carboxylate 10

Anhydrous K_2CO_3 (420 mg, 3.04 mmol) and compound **9** (170 mg,

0.74 mmol) were added to a solution of compound **8** (140 mg, 0.62 mmol) in anhydrous DMF (5 mL). The mixture was stirred at 50 °C for 2 days and filtered through a Celite pad. The pad was washed with DMF (5 mL) and volatiles were distilled off from the filtrate under reduced pressure. Column chromatography of the residue (chloroform-methanol, 100:1 followed by 95:5, v/v) gave compound **10** as a white solid (230 mg, 77%, mp 153–155 °C). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 8.04 (s, 1H), 6.82 (d, ^3J = 8.0 Hz, 1H), 5.93 (s, 2H), 5.60 (d, ^3J = 8.0 Hz, 1H), 4.77–4.74 (m, 2H), 4.41 (q, ^3J = 7.5 Hz, 2H), 4.37–4.33 (m, 2H), 1.40 (t, ^3J = 7.5 Hz, 3H), 1.20 (s, 9H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 177.62, 161.46, 160.33, 150.01, 143.12, 140.86, 128.68, 102.43, 64.72, 61.73, 49.87, 48.45, 39.02, 27.14, 14.41. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{17}H_{24}N_5O_6$: 394.1721; found 394.1721.

4.7. 3-(2-Azidoethyl)-1-(tetrahydrofuran-2-yl)pyrimidine-2,4(1H,3H)-dione 12

A mixture of **11a** (1.2 g, 6.07 mmol), anhydrous DMF (25 mL) and sodium hydride (60% suspension in mineral oil, 288 mg, 7.28 mmol) was stirred at room temperature for 30 min under argon atmosphere. Next, 2-azidoethyl methanesulfonate [51] (2.7 g, 16.27 mmol) and sodium iodide (2.7 g, 18.01 mmol) were added. The mixture was stirred at 60–65 °C for 2 days and filtered through a Celite pad. Volatiles were distilled off from the filtrate under reduced pressure. Column chromatography of the residue (hexane–ethyl acetate, 1:5, v/v) gave compound **12** as a yellowish oil. Yields of compound **12** varied from 55 to 85%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.33 (d, ^3J = 8.0 Hz, 1H), 5.99 (dd, ^3J = 6.5 Hz, ^3J = 3.0 Hz, 1H), 5.75 (d, ^3J = 8.0 Hz, 1H), 4.20–4.15 (m, 3H), 3.99 (ddd, ^2J = 14.5 Hz, ^3J = 8.0 Hz, ^3J = 1.5 Hz, 1H), 3.53 (ddd, ^3J = 8.0 Hz, ^3J = 6.0 Hz, ^3J = 1.5 Hz, 2H), 2.45–2.34 (m, 1H), 2.10–1.95 (m, 2H), 1.95–1.80 (m, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 162.81, 151.00, 137.60, 101.17, 88.22, 70.36, 48.49, 39.60, 33.19, 23.97. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{10}H_{14}N_5O_3$: 252.1091; found 252.1089.

4.8. 3-(2-Azidoethyl)-pyrimidine-2,4(1H,3H)-dione 13

Compound **12** (0.250 g, 0.996 mmol) was dissolved in MeOH (4.0 mL) and conc. HCl was added (1.1 mL). The mixture was stirred at 70 °C for 24 h in a glass pressure tube. Volatiles were distilled off under reduced pressure. The resulting yellow oil was treated with EtOH at −20 °C. The precipitate was filtered off under reduced pressure, washed with cold EtOH and dried on air to give compound **13** as a light yellow solid (0.151 g, 84%, mp 85–87 °C). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 9.51 (bs, 1H), 7.19 (dd, ^3J = 7.6 Hz, ^3J = 5.7 Hz, 1H), 5.79 (dd, ^3J = 7.6 Hz, ^4J = 1.6 Hz, 1H), 4.18 (t, ^3J = 6.1 Hz, 2H), 3.56 (t, ^3J = 6.1 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 163.13, 153.18, 138.91, 102.25, 48.52, 39.36. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_6H_8N_5O_2$: 182.0673; found 182.0672.

4.9. Ethyl 1-(2-(2,6-dioxo-3-(tetrahydrofuran-2-yl)-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-4-carboxylate 14a and ethyl 1-(2-(2,6-dioxo-3-(tetrahydrofuran-2-yl)-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-5-carboxylate 14b

A mixture of compound **12** (487 mg, 1.94 mmol), hexane (3 mL), ethyl acetate (3 mL) and ethyl propiolate (1.76 g, 17.90 mmol, 1.7 mL) was stirred at room temperature for 3 days and volatiles were distilled off under reduced pressure. Column chromatography of the residue (hexane–ethyl acetate, 1:3 followed by 1:5, v/v) gave compound **14a** as a white solid (485 mg, 68%, mp 124–126 °C) and compound **14b** as a colorless oil which solidified on standing (121 mg, 8%, mp 45–47 °C). Compound **14a**. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 8.15 (s, 1H), 7.30 (d, ^3J = 8.5 Hz, 1H), 5.85 (dd, ^3J = 6.5, ^3J = 3.0 Hz, 1H), 5.69 (d, ^3J = 8.5 Hz, 1H), 4.70 (dd, ^3J = 6.0 Hz, ^3J = 6.0 Hz, 2H), 4.41–4.39 (m, 2H), 4.38 (q, ^3J = 7.0 Hz, 2H), 4.18–4.14 (m, 1H), 3.98–3.93 (m, 1H), 2.36–2.28 (m, 1H), 2.06–1.80 (m, 3H), 1.38 (t, ^3J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 162.52, 160.74, 150.59, 140.49, 137.82, 128.10, 100.84, 88.54, 70.44, 61.31, 47.90, 40.04, 33.14, 23.85, 14.41. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{15}H_{20}N_5O_5$: 350.1459; found 350.1461. Compound **14b**. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 8.06 (s, 1H), 7.25 (d, ^3J = 8.5 Hz, 1H), 5.80 (dd, ^3J = 6.0 Hz, ^3J = 3.0 Hz, 1H), 5.63 (d, ^3J = 8.5 Hz, 1H), 5.07–4.94 (m, 2H), 4.44–4.36 (m, 4H), 4.17–4.13 (m, 1H), 3.97–3.92 (m, 1H), 2.31–2.24 (m, 1H), 2.05–1.83 (m, 3H), 1.38 (t, ^3J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 162.66, 158.81, 150.71, 138.04, 137.82, 137.22, 128.50, 100.96, 88.57, 70.42, 61.97, 48.23, 39.81, 33.61, 23.65, 14.35. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{15}H_{20}N_5O_5$: 350.1459; found 350.1456.

4.10. Ethyl 1-(2-(2,6-dioxo-3-(tetrahydrofuran-2-yl)-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-4-carboxylate 14a

A mixture of compound **11a** (104 mg, 0.57 mmol), anhydrous DMF (2 mL) and sodium hydride (60% suspension in mineral oil, 23 mg, 0.57 mmol) was stirred at room temperature for 30 min under argon atmosphere and compound **9** (173 mg, 0.66 mmol) was added. The mixture was stirred at room temperature for 1 day and volatiles were distilled off under reduced pressure. Column chromatography of the residue (hexane–ethyl acetate, 1:5, v/v) gave compound **14a** (150 mg, 75%, mp 124–126 °C). The ^{1}H NMR spectra were identical with those measured for compound **14a** obtained in Section 4.9.

4.11. Ethyl 1-(2-(2,6-dioxo-3-(tetrahydrofuran-2-yl)-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-5-carboxylate 14b

A mixture of compound **12** (1.17 g, 4.66 mmol), toluene (33 mL) and 2-(ethoxycarbonyl)-2-oxoethylidenetriphenylphosphorane (2.17 g, 5.59 mmol) was stirred at 120 °C for 2 days and volatiles were distilled off under reduced pressure. Column chromatography of the residue (hexane-ethyl acetate, 1:1 followed by 1:3, v/v) gave compound **16** as a colorless oil which solidified on standing (1.04 g, 64%, mp 45–47 °C). The ^{1}H NMR spectra were identical with those measured for compound **16** obtained in Section 4.9.

4.12. Diethyl 1-(2-(2,6-dioxo-3-(tetrahydrofuran-2-yl)-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-4,5-dicarboxylate 14c

A mixture of compound **12** (666 mg, 2.65 mmol), hexane (4 mL), ethyl acetate (4 mL) and diethyl acetylenedicarboxylate (1.13 g, 6.62 mmol, 1.06 mL) was stirred at room temperature for 3 days. The precipitated white solid was filtered off, washed with hexane (30 mL) and dried on air. Crystallization (hexane–ethyl acetate, 3:1, v/v) gave compound **14c** as a white solid (1.0 g, 90%, mp 93–96 °C). ^{1}H NMR (CDC_{l3}, 500 MHz): δ = 7.27 (d, ^3J = 8.5 Hz, 1H), 5.79 (dd, ^3J = 6.5 Hz, ^3J = 3.0 Hz, 1H), 5.65 (d, ^3J = 8.5 Hz, 1H), 4.95–2.83 (m, 2H), 4.45–4.37 (m, 6H), 4.17–4.11 (m, 1H), 3.93 (dd, ^2J = 15.0 Hz, ^3J = 8.0 Hz, 1H), 2.30–2.22 (m, 1H), 2.02–1.84 (m, 3H), 1.39 (t, ^3J = 6.5 Hz, 3H), 1.37 (t, ^3J = 6.5 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 162.61, 160.31, 158.56, 150.65, 140.57, 137.65, 130.18, 100.75, 88.39, 70.71, 62.98, 61.87, 48.42, 39.80, 33.11, 23.80, 14.28, 14.01. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{18}H_{24}N_5O_7$: 422.1670; found 422.1668.

4.13. Ethyl 1-(2-(2,6-dioxo-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-4-carboxylate 15a

4.13.1. From compound 11b

A mixture of compound **11b** (777 mg, 3.66 mmol), anhydrous DMF (7 mL) and sodium hydride (60% suspension in mineral oil, 154 mg, 3.85 mmol) was stirred at room temperature for 30 min under argon

atmosphere and compound **9** (1.06 g, 4.06 mmol) was added. The mixture was kept at room temperature for 1 day and volatiles were distilled off under reduced pressure. The residue was dissolved in chloroform (10 mL) and trifluoroacetic acid (0.5 mL) was added. The mixture was stirred at room temperature for 2 h and volatiles were distilled off under reduced pressure. Column chromatography of the residue (chloroform–methanol, 95:5, v/v) gave compound **15a** as a white solid (770 mg, 60%, mp 182–186 °C).

4.13.2. From compound 13

Compound **13** (59 mg, 0.32 mmol) was dissolved in MeOH (2.0 mL) and ethyl propiolate (0.29 g, 2.96 mmol, 0.30 mL) was added. The mixture was stirred at room temperature for 3 days and volatiles were distilled off under reduced pressure. The solid residue was washed with hexanes and crystallized from ethyl acetate to give compound **15a** (80 mg, 88%, mp 182–186 °C).

4.13.3. From compound 14a

A mixture of **14a** (463 mg, 1.32 mmol) and trifluoroacetic acid (7 mL) was stirred at room temperature for 3 days and volatiles were distilled off under reduced pressure. Column chromatography of the residue (chloroform–methanol, 95:5, v/v) gave compound **15a** as a white solid (276 mg, 75%, mp 182–186 °C).^{1}H NMR (DMSO-d_6, 500 MHz, HMBC): δ = 11.10 (br s, 1H; H-1′), 8.79 (s, 1H; H-5), 7.41 (d, ^{3}J = 7.5 Hz, 1H; H-5′), 5.53 (d, ^{3}J = 7.5 Hz, 1H; H-6′), 4.66–4.62 (m, 2H; H-1a), 4.28 (q, ^{3}J = 7.5 Hz, 2H; O-CH_2CH$_3$), 4.22–4.18 (m, 2H; H-1b), 1.30 (t, ^{3}J = 7.5 Hz, 3H; O-CH$_2$CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 162.89 (C-4′), 160.34 (*C*OCH$_2$CH$_3$), 151.28 (C-2′), 140.95 (C-6′), 138.68 (C-4), 129.66 (C-5), 99.57 (C-5′), 60.47 (CO*C*H$_2$CH$_3$), 47.52 (C-1a), 39.42 (C-1b), 14.17 (COCH$_2$*C*H$_3$). HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{11}H_{14}N_5O_4$: 280.1040; found 280.1039.

4.14. Ethyl 1-(2-(2,6-dioxo-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-5-carboxylate 15b

A mixture of compound **14b** (1.00 g, 2.86 mmol) and trifluoroacetic acid (15 mL) was stirred at room temperature for 3 days and volatiles were distilled off under reduced pressure. Column chromatography of the residue (chloroform-methanol, 95:5, v/v) gave compound **15b** as a white solid (670 mg, 84%, mp 154–158 °C). ^{1}H NMR (DMSO-d_6, 500 MHz, HMBC): δ = 11.07–11.06 (m, 1H; H-1′), 8.19 (s, 1H; H-4), 7.37 (dd, ^{3}J = 8.0 Hz, ^{3}J = 6.0 Hz, 1H; H-6′), 5.47 (dd, ^{3}J = 8.0 Hz, ^{4}J = 1.5 Hz, 1H; H-5′), 4.92–4.88 (m, 2H; H-1a), 4.32 (q, ^{3}J = 7.0 Hz, 2H; O-CH_2CH$_3$), 4.25–4.23 (m, 2H; H-1b), 1.31 (t, ^{3}J = 7.0 Hz, 3H; O-CH$_2$CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 162.89 (C-4′), 158.08 (*C*OCH$_2$CH$_3$), 151.28 (C-2′), 140.89 (C-6′), 137.45 (C-4), 128.49 (C-5), 99.50 (C-5′), 61.61 (CO*C*H$_2$CH$_3$), 47.71 (C-1a), 38.88 (C-1b), 13.96 (COCH$_2$*C*H$_3$). HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{11}H_{14}N_5O_4$: 280.1040; found 280.1038.

4.15. Diethyl 1-(2-(2,6-dioxo-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-4,5-dicarboxylate 15c

4.15.1. From compound 13

Compound **13** (60 mg, 0.33 mmol) was dissolved in MeOH (2.0 mL) and diethyl acetylenedicarboxylate (0.14 g, 0.82 mmol, 0.13 mL) was added. The mixture was stirred at room temperature for 3 days and volatiles were distilled off under reduced pressure. The solid residue was washed with hexanes and crystallized from ethyl acetate-hexanes (1:1, v/v) to give compound **15c** as a white solid (104 mg, 90%, mp 120–122 °C).

4.15.2. From compound 14c

A mixture of compound **14c** (616 mg, 1.46 mmol) and trifluoroacetic acid (6 mL) was stirred at room temperature for 3 days. Volatiles was distilled off under reduced pressure. Column chromatography of the residue (chloroform–methanol, 100:1, v/v) gave compound **15c** as a white solid (412 mg, 80%, mp 120–122 °C).^{1}H NMR (DMSO-d_6, 500 MHz): δ = 11.13 (d, ^{3}J = 5.5 Hz, 1H), 7.42 (dd, ^{3}J = 7.5 Hz, ^{3}J = 5.5 Hz, 1H), 5.53 (dd, ^{3}J = 7.5 Hz, ^{4}J = 1.5 Hz, 1H), 4.82–4.80 (m, 2H), 4.34 (q, ^{3}J = 7.0 Hz, 2H), 4.31 (q, ^{3}J = 7.0 Hz, 2H), 4.24–4.21 (m, 2H), 1.30 (t, ^{3}J = 7.0 Hz, 3H), 1.29 (t, ^{3}J = 7.0 Hz, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 162.89, 159.86, 157.85, 151.32, 141.01, 139.19, 130.27, 99.55, 62.73, 61.45, 48.04, 39.95, 13.92, 13.62. HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{14}H_{18}N_5O_6$: 352.1252; found 352.1254.

4.16. 1-(2-(2,6-Dioxo-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-4-carboxylic acid 16a

A mixture of compound **15a** (590 mg, 2.10 mmol), tetrahydrofuran (22 mL), water (22 mL) and lithium hydroxide (130 mg, 5.25 mmol) was stirred at room temperature for 40 min and ion exchange resin (Dowex 50WX2-200 mesh, 3.3 g) was added. The mixture was shaken for 1 h. The resin was filtered off and washed with water (2 × 10 mL). Volatiles were distilled off from the collected filtrates under reduced pressure. Lyophilization of the residue gave compound **16a** as a white solid (520 mg, 98%, mp 185–190 °C dec). ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 13.01 (br s, 1H), 11.10 (d, ^{3}J = 4.5 Hz, 1H), 8.67 (s, 1H), 7.41 (dd, ^{3}J = 7.5 Hz, ^{3}J = 4.5 Hz, 1H), 5.53 (d, ^{3}J = 7.5 Hz, 1H), 4.65–4.63 (m, 2H), 4.21–4.19 (m, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 162.93, 161.77, 151.30, 140.98, 139.58, 129.49, 99.61, 47.44, 39.47. HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_9H_{10}N_5O_4$: 252.0727; found 252.0728.

4.17. 1-(2-(2,6-Dioxo-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-4,5-dicarboxylic acid 16b

A mixture of compound **15b** (568 mg, 1.62 mmol), tetrahydrofuran (19 mL), water (19 mL) and lithium hydroxide (115 g, 4.83 mmol) was stirred at room temperature for 1 day and an ion exchange resin was added (Dowex 50WX2-200 mesh, 3.0 g). The mixture was shaken at room temperature for 1 h. The resin was filtered and washed with water (2 × 10 mL). Volatiles were distilled off from the collected filtrates under reduced pressure. Lyophilization of the residue gave compound **16b** as a white solid (434 mg, 91%, mp 175–185 °C). ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 11.02 (d, ^{3}J = 5.5 Hz, 1H), 7.37 (dd, ^{3}J = 7.5 Hz, ^{3}J = 5.5 Hz, 1H), 5.48 (dd, ^{3}J = 8.0 Hz, ^{4}J = 1.5 Hz, 1H,), 4.92–2.90 (m, 2H), 4.24–4.21 (m, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 162.92, 161.44, 159.23, 151.31, 140.76, 140.28, 132.55, 99.54, 47.73, 39.94. HRMS (ESI): *m/z* [M − H]$^-$ calcd for $C_{10}H_8N_5O_6$: 294.0469; found 294.0481.

4.18. 1-(2-(2,6-Dioxo-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-5-carboxylic acid 16c

A mixture of compound (**15c** 418 mg, 1.50 mmol), tetrahydrofuran (17 mL), water (17 mL) and lithium hydroxide (71 mg, 2.96 mmol) was stirred at room temperature for 2 h and ion exchange resin (Dowex 50WX2-200 mesh, 1.84 g) was added. The mixture was shaken for 1 h. The resin was filtered and washed with water (2 × 10 mL). Volatiles were distilled off from the collected filtrates under reduced pressure. Lyophilization of the residue gave compound **16c** as a white solid (530 mg, 93%, mp 195–205 °C, dec). ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 11.03 (d, ^{3}J = 5.0 Hz, 1H), 8.10 (s, 1H), 7.37 (dd, ^{3}J = 7.5 Hz, ^{3}J = 5.0 Hz, 1H), 5.47 (dd, ^{3}J = 7.5 Hz, ^{4}J = 1.5 Hz, 1H), 4.89–4.87 (m, 2H), 4.26–4.24 (m, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 162.88, 159.46, 151. 28, 140.83, 137.44, 129.31, 99.50, 47.44, 38.90. HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_9H_{10}N_5O_4$: 252.0727; found 252.0728.

4.19. 1-(2-(2,6-Dioxo-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-4-carboxamide 17a

A mixture of compound **15a** (222 mg, 0.795 mmol), methanol (10 mL) and ammonium hydroxide (5 mL) was stirred in a sealed tube at 65 °C for 1 day. The white solid was precipitated. The solid was filtered off and washed with methanol. Crystallization of the solid from a mixture of methanol and water (5:1, v/v) gave compound 17a as white solid (190 mg, 96%, mp 264–275 °C dec). 1H NMR (DMSO-d_6, 500 MHz): δ = 11.01 (br s, 1H), 8.50 (s, 1H), 7.78 (br s, 1H), 7.41–7.42 (m, 2H), 5.53 (d, 3 J = 7.5 Hz, 1H), 4.64–4.62 (m, 2H), 4.21–4.19 (m, 2H). 13C NMR (DMSO-d_6, 125 MHz): δ = 162.93, 161.60, 151.32, 142.78, 140.97, 127.09, 99.63, 47.37, 39.49. HRMS (ESI): m/z [M + H] + calcd for C9H11N6O3: 251.0887; found 251.0885.

4.20. 1-(2-(2,6-Dioxo-2,3-dihydropyrimidin-1(6H)-yl)ethyl)-1H-1,2,3-triazole-5-carboxamide 17b

A mixture of compound **15c** (219 mg, 0.75 mmol), ethanol (4 mL) and ammonium hydroxide (5 mL) was stirred in a sealed tube at 65 °C for 1 day. Volatiles were distilled off from the filtrate under reduced pressure. Column chromatography of the residue (chloroform–methanol, 9:1 followed by 4:1, v/v) gave compound **17b** as a white solid (107 mg, 57%, mp 245–267 °C, dec). ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 11.01 (br s, 1H), 8.15 (s, 1H), 8.12 (br s, 1H), 7.74 (br s, 1H), 7.36 (d, ^3J = 7.5 Hz, 1H), 5.45 (d, ^3J = 7.5 Hz, 1H), 4.90–4.88 (m, 2H), 4.23–4.21 (m, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 162.89 159.05, 151.27, 140.75, 134.41, 130.71, 99.50, 47.15, 39.14. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_9H_{11}N_6O_3$: 251.0887; found 251.0885.

4.21. *DL-α-[5-methyl-^{3}H]-AMPA binding assays* [35,34]

Purified hGluR2 binding domain (hHS1S2I obtained according to Chen, G. Q.; Sun, Y.; Jin, R.; Gouaux, E. *Protein Science* **1998**, *7*, 2623) (1 mg/mL) was dialyzed against binding buffer (30 mM Tris-HCl pH 7.2, 10% glycerol, 100 mM KSCN, 2.5 mM $CaCl_2$) at 4 °C for 48 h. For DL-α-[5-methyl-^{3}H]-AMPA saturation experiments, hHS1S2I (0.5 μg/mL) was incubated in a total volume of 500 μL of the binding buffer with DL-α-[5-methyl-^{3}H]-AMPA (10.6 Ci/mmol, 10 nM) on ice for 1 h. The protein solution was quickly filtrated under reduced pressure through wet GSWP 02,500 membranes (Merck Millipore). The membranes were washed with cold binding buffer (3x2 mL), transferred into scintillation vials, scintillation liquid was added (6 mL) and the radioactivity was counted. The experimental data were analyzed by nonlinear curve fitting (GraphPad Prism).

4.22. Docking of compound 6a, willardiine and AMPA in the binding S1S2 domain of hGluR2 receptor

The experimental crystal structure of hGluR2 LBD (PDB entry 3r7x [38]) was used in docking computation. Docking calculations were performed using AutoDock Vina software with PyMol autodock plugin as graphic user interface. The crystal structure was prepared according to the protein preparation procedure recommended by software creators. The ligands were prepared according to the ligand preparation procedure recommended by software creators. Default input parameters were used in all computations. The best-docked structure was chosen using binding energy score given by AutoDock Vina. All computations were performed on an Intel® Core™ i7-4702MQ 3.2 GHz processor running Fedora 28 Workstation Linux distribution [52].

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Conflict of interest

The authors have declared no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.10.061.

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