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Triazoloquinazolinediones as novel high affinity ligands for the benzodiazepine site of $GABA_A$ receptors

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ABSTRACT

Based on a pharmacophore model of the benzodiazepine-binding site of $GABA_A$ receptors, a series of 2-aryl-2,6-dihydro[1,2,4]triazolo[4,3-*c*]quinazoline-3,5-diones (structure type **I**) were designed, synthesized, and identified as high-affinity ligands of the binding site. For several compounds, K_i values of around 0.20 nM were determined. They show a structural resemblance with the previously described 2-phenyl-2*H*-pyrazolo[4,3-*c*]quinolin-3(5*H*)-ones (**II**) and 2-phenyl-[1,2,4]triazolo[1,5-*a*]quinoxalin-4(5*H*)-one (**III**). The 9-bromo substituted compounds **8a–d** were prepared in an 8-step synthesis in an overall yield of approximately 40%, and a library of 9-substituted analogues was prepared by cross-coupling reactions. Compound **8e**, **21**, **22**, and **24** were tested on recombinant rat $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, and $\alpha_5\beta_3\gamma_2$ subtypes, and displayed selectivity for the $\alpha_1\beta_3\gamma_2$ isoform.

1. Introduction

Most compounds that elicit a pharmacological response by interacting with the benzodiazepine-binding site (BZDR) of the $GABA_A$ receptors are, in spite of the name of the binding-site, not benzodiazepines, and examples are the 2-arylpyrazoloquinolines,[1,2] β-carbolines,[3] pyridodiindoles,[4] triazoloqunioxalines,[5] pyrimidin-5(6*H*)-ones,[6] cyclopyrrolones, and quinolines.[7] They display a continuous range of effects, from being positive allosteric modulators that enhance the GABAergic inhibition exhibiting anxiolytic, anticonvulsant, sedative-hypnotic and myorelaxant activities, to negative allosteric modulators that reduce the GABAergic inhibition and thereby exhibit anxiogenic, somnolytic, and convulsant activities. Neutral allosteric modulators elicit no pharmacological response, but may affect the BZDR by competitively inhibit binding of other ligands.

The $GABA_A$ receptors are pentameric ligand gated chloride ion channels assembled from at least 16 subunits from seven different classes (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, π, and θ).[8–10] The BZDR is believed to be located at an interface between an α and a γ subunit, and ligands of the BZDR are believed to mediate their pharmacological effect predominately through interactions at the $\alpha_1\beta_x\gamma_2$, $\alpha_2\beta_x\gamma_2$, $\alpha_3\beta_x\gamma_2$, and $\alpha_5\beta_x\gamma_2$ subtype assemblies. Studies with transgenic mice and subtype selective compounds clearly suggest that $GABA_A$ receptors with different subtype composition are associated with different physiological effects, α_1-containing receptors mediate sedative and anterograde amnesic effects, α_2-, and/or α_3-containing receptors are involved in anxiolytic activity, while α_5-containing receptors might be associated with cognition and memory.[11,12]

A pharmacophore model of the BZDR based on 10 different compound classes[13] has been further developed and refined based on a SAR study of synthetic flavone derivatives,[14,15] adding additional pharmacophore elements. This model was recently used to identify and optimize novel 4-quinolones and azaflavone derivatives as ligands for the BZDR of the $GABA_A$ receptors with K_i values down to 0.05 nM.[16–18] In the present study, the ability of 2-aryl-2,6-dihydro[1,2,4]triazolo[4,3-*c*]quinazoline-3,5-diones (structure type **I**, Fig. 1) to interact with the BZDR has been investigated. The basic structure of 2-aryl-2,6-dihydro[1,2,4]triazolo[4,3-*c*]quinazolin-3,5-dione (2-aryltriazoloquinazolinedione) resembles those of 2-phenyl-2*H*-pyrazolo[4,3-*c*]quinolin-3(5*H*)-one (2-phenylpyrazoloquinoline, **II**) and 2-phenyl-[1,2,4]triazolo[1,5-*a*]quinoxalin-4(5*H*)-one (2-phenyltriazoloquinoxalone, **III**), and 2-arylpyrazoloquinolines and 2-aryltriazoloquinoxalones have previously been described as BZDR ligands.[1,2,5] The 2-aryltriazoloquinazolinediones retains a [6,6,5]-tricyclic heteroaromatic system and combines the two carbonyl moieties of **II** and **III** into a dicarbonyl compound (Fig. 1). When positioned in the pharmacophore model (Fig. 2), the 2-aryltriazoloquinazolinediones (exemplified by compound **8a**) appear to fulfill the requirements for a strong BZDR affinity, for example, the NH(6), N(1), and the 3- and 5-carbonyl oxygens can be expected to interact with the hydrogen bond donors/acceptors

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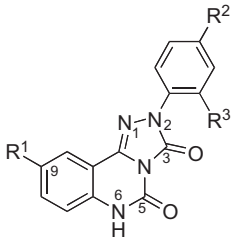
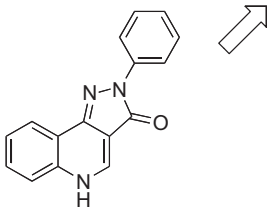
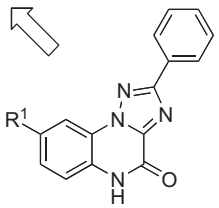


I, 2-aryl-[1,2,4]triazolo[4,3-*c*]quinazoline-3,5(2*H*,6*H*)-dione



II, 2-phenyl-2*H*-pyrazolo[4,3-*c*]quinolin-3(5*H*)-one



III, 2-phenyl-[1,2,4]triazolo[1,5-*a*]quinoxalin-4(5*H*)-one

Figure 1. Design of the 2-aryltriazoloquinazolinediones based on the two previously described BZDR ligands 2-phenylpyrazoloquinolines and 2-phenyl-triazoloquinoxalines.

H1, A2 and H2/A3. The aim of this study was both to develop a synthetic route to the 2-aryltriazoloquinazolinediones and use that for the preparation of new derivatives, as well as to further explore the pharmacophore region called 'interface' (see Fig. 2). A common feature with previously reported potent ligands that reaches into the interface region, is a flexible methylene (e.g., a benzyl group) or ethylene bridge at the position corresponding to the R^1 group in the 2-aryltriazoloquinazolinediones (Fig. 1).[6,16–18] To facilitate the investigation of this region our strategy was to prepare derivatives with R^1 = Br, and to use suitable cross-coupling protocols to attach various groups to the 2-aryltriazoloquinazolinedione scaffold.

2. Results

2.1. Chemical synthesis

All 2-aryltriazoloquinazolinedione derivatives prepared in this study are to our knowledge new compounds. The triazoloquinazolinedione scaffold **8a–e** was synthesized using two similar synthetic routes, as outlined in Scheme 1 and 2. The route leading to the 9-bromo-substituted triazoloquinazolinediones **8a–d** via the nitro-triazole **6** facilitated multi-gram scale synthesis, as the purification of the intermediates by chromatography was not required (most of the compounds have limited solubility).

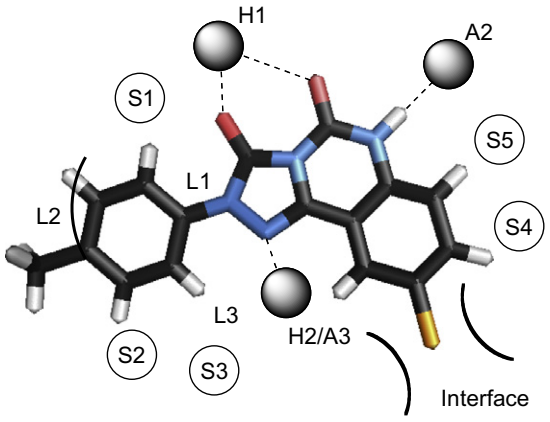
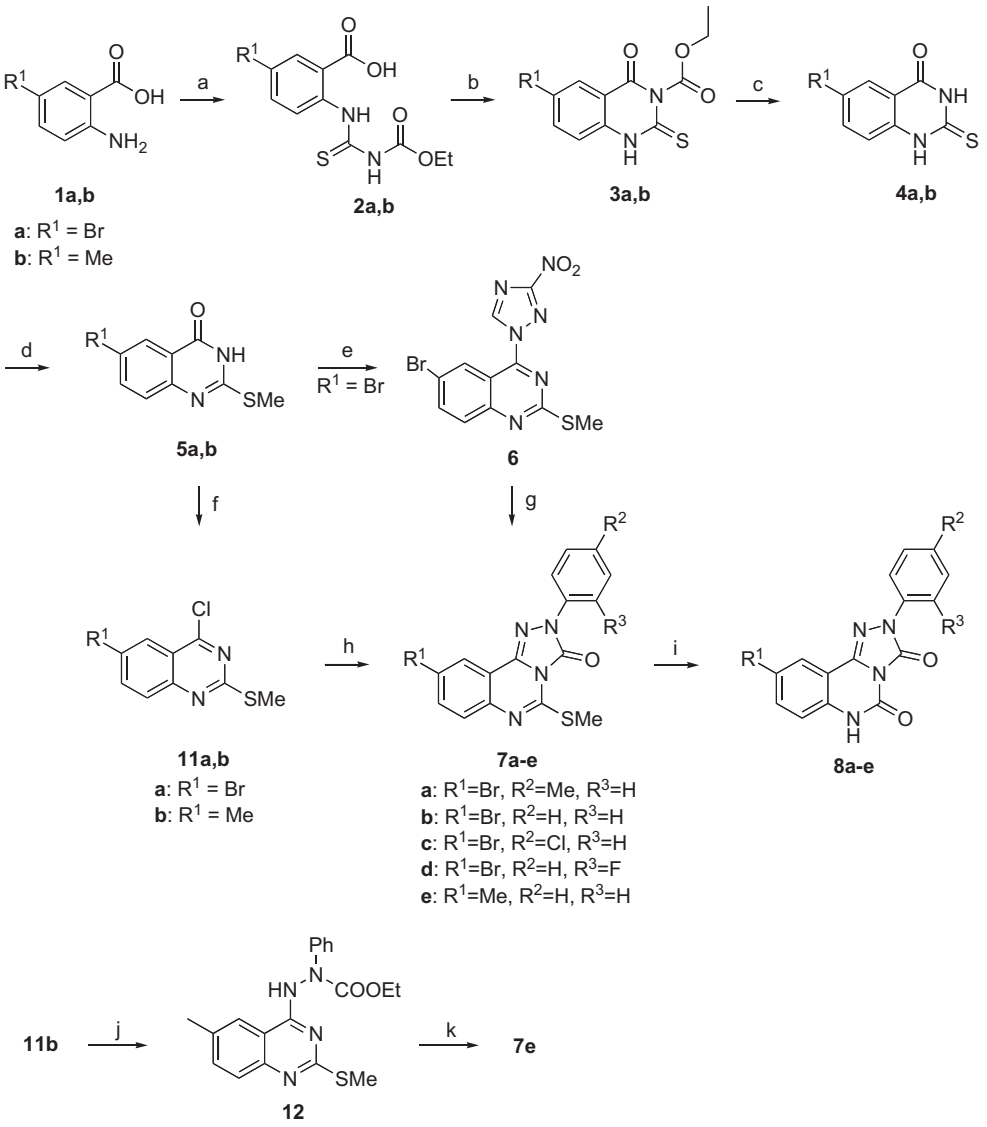


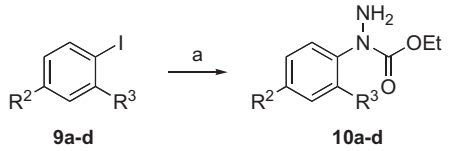
Figure 2. The proposed binding mode of triazoloquinazoline-3,5-dione **8a** in the pharmacophore model representation. H1 and H2 are hydrogen bond donor sites and A2 and A3 are hydrogen bond acceptor sites. L1, L2, and L3 represent lipophilic pockets and S1–S5 denotes regions of steric repulsive ligand–receptor interactions (receptor essential volume). The interface region is a partly lipophilic region in the vicinity of the 9-position of triazoloquinazolinediones. This region has been suggested to represent the interface between the α- and γ-subunit in the $GABA_A$ receptors.

The quinazolinone derivatives **3a** and **3b** were prepared by addition of ethoxycarbonyl isothiocyanate to an anthranilic acid followed by cyclization in acetic anhydride. Deprotection and methylation gave **5a** and **5b**, which were activated for a subsequent S_NAr displacement using two different protocols. Treatment of **5a** with 3-nitro-1*H*-1,2,4-triazole in $I_2/PPh_3/EtN(i\text{-}Pr)_2$/toluene gave the nitro-triazole derivative **6**, and triazole derivatives **7a–d** were subsequently prepared in a one-pot reaction by the treatment of **6** with **10a–d** under solvent-free conditions.[19,20] Alternatively, activation of **5a** and **5b** may be achieved using phosphorous oxychloride to give compound **11a** and **11b**, respectively. The reaction of **11a** with **10b** gave **7b**, while the reaction of **11b** with **10b** gave **7e** in yields that were essentially the same as for the nitro-triazole pathway. It is likely that the initial step of these reactions is a S_NAr displacement followed by a base promoted cyclization, a suggestion that is supported by the isolation of **12** as an intermediate from **11b** to **7e**, using slightly modified conditions. Cyclization of **12** in the presence of lithium hydroxide as base gave **7e** in a yield similar to the one-pot reactions described above.

Treatment of **7a–e** with 1 equiv of *m*CPBA yielded triazoloquinazolinediones **8a–e** in a total yield of 40% over eight steps (for **8a** starting from **1a**). Compounds **10a–d** were prepared utilizing a copper-catalyzed coupling between ethyl carbazate and substituted iodobenzenes in presence of 1,10-phenantroline and cuprous iodide as a catalyst system (Scheme 2).[21] Coupling with 4-iodotoluene and iodobenzene gave **10a** and **b**, respectively, in good yields, whereas coupling with 4-chloro- or 2-fluoro-substituted iodobenzene resulted in significantly lower yields. The triazoloquinazolinedione **8a** was subjected to various cross-coupling reactions including a copper-free Sonogashira coupling,[22] Stille coupling[23] and a Heck reaction[24] as shown in Scheme 3. The copper-free Sonogashira coupling was employed for the synthesis of acetylenes **13–20**. The yield of these reactions was generally somewhat hampered by problems associated with chromatographic purification of the sparingly soluble acetylenes.



Scheme 1. Reagents and conditions: (a) ethoxycarbonyl isothiocyanate, MeCN, 80 °C, 4 h (yield 95% for **2a** and 91% for **2b**); (b) Ac_2O, 60 °C, 1 h (yield 93% for **3a** and 100% for **3b**); (c) 0.5 M NaOMe in MeOH, THF, reflux, 90 min (yield 100% for **4a** and 88% for **4b**); (d) DMF, 0.5 M NaOMe in MeOH, then MeI, rt, 10 h (yield 90% for **5a** and 77% for **5b**); (e) 3-nitro-1*H*-1,2,4-triazole, PPh_3, I_2, toluene, DIPEA, 95 °C, 1 h (yield 89%); (f) $POCl_3$, pyridine, 110 °C, 18 h (yield 75% for **11a** and 77% for **11b**); (g) DIPEA, **10a**–**d**, 110 °C, 60 h (yields 69% for **7a**, 76% for **7b**, 70% for **7c**, and 51% for **7d**); (h) DIPEA, **10b**, 110 °C, 60 h (yields 71% for **7b** and 75% for **7e**); (i) *m*CPBA (77%), CH_2Cl_2, rt, 6 h (quantitative yields); (j) DIPEA, **10a**, 1,4-dioxane, reflux, 48 h (quantitative yield); (k) LiOH, THF, rt, 2 h (yield 78%).

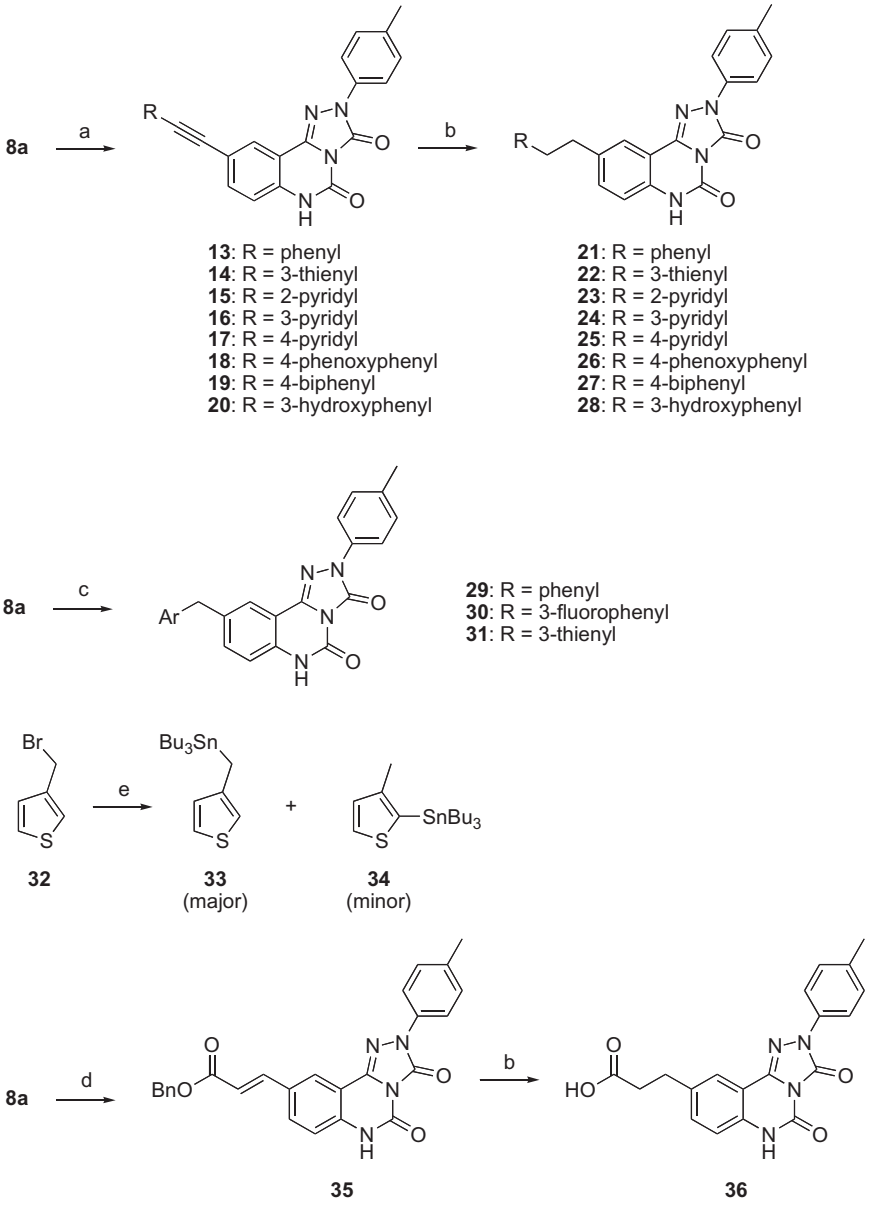


Scheme 2. Reagents and conditions: CuI, 1,10-phenantrolein, ethyl carbazate, Cs_2CO_3, DMF, 80 °C, 16 h (yields 83% for **10a**, 76% for **10b**, 40% for **10c**, and 22% for **10d**).

Hydrogen reduction over palladium on charcoal yielded the desired ethylene-linked aryl derivatives **21**–**28**. Treatment of arylmethyl bromides (i.e., benzyl bromide, 3-fluorobenzyl bromide, and 3-bromomethylthiophene, respectively), with magnesium turnings and addition of tributyltin chloride to the resultant Grignard solution[25–27] gave the corresponding arylmethyl Stille-reagents. Reaction with the 3-thienyl Grignard reagent led to formation of two products, of which the desired **33** was the major product. The high reactivity of the 2-position in 3-thienylmagnesium bromide has previously been recognized in the synthesis of 3-methyl-2-thenoic acid.[27] The methylene-linked aryl derivatives **29**–**31** were then prepared by a Stille coupling with **8a**, and carboxylic acid **36** by a Heck reaction with benzyl acrylate followed by a hydrogen reduction over palladium on charcoal.[23]

2.2. Receptor binding

Affinities for the BZDR were determined in vitro by displacement of ^{3}H-Flumazenil in rat cortical tissue (Table 1). Subtype affinity testing was performed with compounds **8e**, **21**, **22**, and **24** on recombinant $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, and $\alpha_5\beta_3\gamma_2$ receptor subtypes (Table 2).



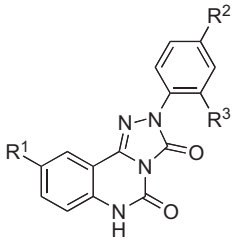
Scheme 3. Reagents and conditions: (a) $Pd(OAc)_2$, PPh_3, RC≡CH, NEt_3, DMF, 100 °C, 18 h (yields 32–75%); (b) H_2 (1 atm) 10% Pd/C, CH_2Cl_2, MeOH, rt, 18 h (yields 79–98%); (c) $Pd(PPh_3)_4$, NEt_3, DMF, $PhCH_2SnBu_3$, tributyl(3-fluorobenzyl)tin, or **33**, 110 °C, 18 h (yields 65% for **29**, 70% for **30**, and 62% for **31**); (d) $Pd(OAc)_2$, PPh_3, benzyl acrylate, NEt_3, DMF, 120 °C, 15 h (yield 47%); (e) Mg, THF, 35 °C, 30 min, then Bu_3SnCl, 1 h (yield 36%).

3. Discussion

The 2-thioxoquinazolin-4(1*H*)-one **3b** was previously identified as the best virtual BZDR ligand[28] in a 3D database search employing the program Catalyst®. A structurally similar quinazoline-2,4(1*H*,3*H*)-dione (with oxygen instead of sulfur) was prepared and found to be inactive, and when **3b** now became available it was also assayed with the same result (K_i value >30,000 nM). Both compound types fit into the pharmacophore model in a similar fashion as the triazoloquinazolinediones, with NH(1) interacting with A2, the 4-carbonyl oxygen interacting with H2, and the 3-carbamate carbonyl oxygen interacting with H1. The low affinity of the quinazoline-2,4(1*H*,3*H*)-dione as well as of **3b** can most likely be attributed to the steric repulsion between the 3-carbamate carbonyl oxygen and the 2/4 oxygen/sulfur atom, which forces the molecule to adopt a non-planar conformation and hence disables the H1 interaction. The 2-aryltriazoloquinazolinediones are structurally related to the 2-arylpyrazoloquinolines, and it has previously been shown that the presence of a hydrogen, a chlorine or a methoxy group in position 4′ as well as a fluorine in position 2′ of the 2-arylpyrazoloquinolines (**II**, Fig. 1) results in subnanomolar affinities.[1,2] Inspired by this, compounds **8a**–**e** were prepared and were all found to display subnanomolar affinities (Table 1). **8a** (K_i = 0.47 nM) was chosen as the lead structure for the exploration of various substituents in position 9. Not surprisingly, the difference between a methyl group (**8e**) and a bromine (**8b**) in position 9 is small.

Among the most potent compounds presented in this study are the pyridyl substituted compounds **23**–**25** which inhibit BZDR binding at concentrations as low as 0.17 nM. The high affinity of these compounds may be best understood in the light of the suggestion that the interface region located in this area is a water filled channel-like cavity.[15] This hypothesis is also supported by the high affinity of other polar compounds, such as the carboxylic acid derivative **36** (1.4 nM). Compared to **21** and **29**, which are substituted with lipophilic side-chains in position 9, the more polar

Table 1
K_i values of triazoloquinazolinediones tested on ^{3}H-Flumazenil binding in vitro to rat cortical membranes



8a-e, 13, 21-31, 36

Compds	R^1	R^2	R^3	K_i value[a] (nM)
8a	Br-	Me-	H-	0.47 ± 0.09
8b	Br-	H-	H-	0.24 ± 0.07
8c	Br-	Cl-	H-	0.6 ± 0.26
8d	Br-	H-	F-	0.20 ± 0.07
8e	CH_3-	H-	H-	0.62 ± 0.13
13	Phenyl-C≡C–	Me-	H-	40 ± 11
21	Phenyl-CH_2CH_2–	Me-	H-	4.0 ± 1.2
22	(3-Thienyl)-CH_2CH_2–	Me-	H-	0.94 ± 0.27
23	(2-Pyridyl)-CH_2CH_2–	Me-	H-	0.53 ± 0.11
24	(3-Pyridyl)-CH_2CH_2–	Me-	H-	0.20 ± 0.04
25	(4-Pyridyl)-CH_2CH_2–	Me-	H-	0.17 ± 0.03
26	(4-PhOPh)-CH_2CH_2–	Me-	H-	123 ± 12
27	(4-Biphenyl)-CH_2CH_2–	Me-	H-	1570 ± 150
28	(3-Hydroxyphenyl)-CH_2CH_2–	Me-	H-	12.4 ± 5.9
29	Benzyl-	Me-	H-	1.0
30	3-Fluorophenyl-CH_2–	Me-	H-	2.1 ± 0.3
31	(3-Thienyl)-CH_2–	Me-	H-	0.23 ± 0.18
36	HOOC–CH_2CH_2–	Me-	H-	1.4 ± 0.15

[a] Each K_i value is the mean ± SD of three determinations.

Table 2
The affinity of selected triazoloquinazolinediones tested on ^{3}H-Flumazenil binding to $\alpha_1\beta_3\gamma_{2s}$, $\alpha_2\beta_3\gamma_{2s}$, $\alpha_3\beta_3\gamma_{2s}$, and $\alpha_5\beta_3\gamma_{2s}$ $GABA_A$ receptor subtypes

Compds	K_i α_1[a] (nM)	K_i α_2[a] (nM)	K_i α_3[a] (nM)	K_i α_5[a] (nM)
8e	0.43 ± 0.18	1.0 ± 0.17	1.3 ± 0.38	n.d.
21	3.6 ± 2.2	65 ± 8.5	9.7 ± 5.0	66 ± 21
22	0.14 ± 0.05	1.7 ± 0.7	0.71 ± 0.25	3.6 ± 1.5
24	0.68 ± 0.02	4.4 ± 1.7	2.3 ± 0.90	9.1 ± 4.1

[a] Each K_i value is the mean ± SD of three determinations. n.d., not determined.

22–**25** and **31** may more readily participate in a continuous network of hydrogen bonds through their heteroatoms. From our previous experiences with other classes of BZDR ligands, the change in affinity observed upon introducing a lipophilic $PhCH_2$- or $PhCH_2CH_2$-group into the interface region, ranges from unaffected to a 10-fold increase. This indicates that the formation of a favorable interaction between the lipophilic side-chain of the ligand and the partly lipophilic interface region is possible.[16–18] For the 2-aryltriazoloquinazolinediones, the same substitutions actually decrease the affinity, ninefold for **21** compared to **8a** and twofold for **29** compared to **8a**. The 2-aryltriazoloquinazolinediones are, compared to the other BZDR ligands,[16–18] larger and more rigid, and a possible explanation for this observed difference in binding strength is that smaller and more flexible BZDR ligands more easily may adapt a conformation in which favorable interactions are formed between the lipophilic side-chain and the partially lipophilic area in the interface region.

The larger phenoxyphenyl- (**26**) and biphenyl- (**27**) groups are evidently discriminated, indicating a sterical repulsive interaction further out in the interface region. Worth noticing is also the large tolerance for rigid planar substituents such as the phenylacetylene **13**, with an affinity of 40 nM. Although not favoured, this still tolerated non-flexible substituent indicates that the extension of the channel-like cavity in the interface region is considerable.

Subtype affinity testing was performed with compounds **8e**, **21**, **22**, and **24** on recombinant $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$ receptor subtypes (Table 2). All compound investigated in this study display selectivity for $\alpha_1\beta_3\gamma_2$ over the other receptor subtypes with α_2/α_1 K_i ratios ranging from 18 for **21** to 2 for **8e**, α_3/α_1 K_i ratios ranging from 5 for **22** to 3 for **24**, and α_5/α_1 K_i ratios ranging from 26 for **22** to 13 for **24**.

4. Conclusion

Triazoloquinazolinediones, a novel class of BZDR ligands, have been designed using a pharmacophore model, and a small library of compounds have been synthesized and assayed. This set of compounds has permitted additional characterization of the BZDR, in particular of the interface region (as defined in the pharmacophore model), which has been showed to be a highly interesting region for optimization of BZDR binding ligands. The decreased affinity displayed by the derivatives with the largest substituents in position 9, **26** and **27**, indicate a repulsive interaction with the receptor essential volumes further out in the interface region. The highest in vitro affinity observed for the set of compound investigated here was 0.17 nM (**24**) and a few compounds were selected and assayed on recombinant $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$ receptor subtypes. All compound tested displayed selectivity for $\alpha_1\beta_3\gamma_2$ over the other receptor subtypes, and it is reasonable to suggest that this specific pharmacophore has a certain selectivity for this subtype.

5. Experimental

Reagents and solvents (except THF) were used from commercial sources without purification. THF was distilled from sodium/benzophenone prior to use. ^{1}H and ^{13}C NMR were recorded at room temperature unless otherwise specified with a Bruker DR400 spectrometer. The spectra were recorded in $CDCl_3$, DMSO–d_6, and C_6D_6, and the solvent signals (7.27 and 77.0, 2.50 and 39.5 or 7.18 and 128.1 ppm, respectively) were used as reference. Analytical thin layer chromatography (TLC) was performed on Kiselgel 60 F_{254} plates (Merck). Column chromatography was performed on SiO_2 (Matrex LC-gel: 60A, 35-70 MY, Grace). Melting points (uncorrected) were determined with a Reichert microscope. EI mass spectra were recorded at 70 eV with a Jeol SX102 spectrometer and ESI spectra were recorded with Micromass Q-TOF Micro.

5.1. 5-Bromo-2-({[(ethoxycarbonyl)amino]carbonothioyl}-amino)-benzoic acid (2a)

To a solution of 2-amino-5-bromobenzoic acid (8.09 g, 37.4 mmol) in 60 mL of dry MeCN was added ethoxycarbonyl isothiocyanate (4.32 mL, 37.4 mmol) and the mixture was stirred at reflux for 5 h. Heating was removed and a white precipitate was filtered off, to give **2a** as a white solid (12.3 g, 95%). Mp: 180 °C (decomp.). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (1H, s), 12.25 (1H, s), 8.07 (1H, d, J = 8.8 Hz), 7.99 (1H, d, J = 2.4 Hz), 7.78 (1H, dd, J = 8.7 and J = 2.4 Hz), 4.20 (2H, q, J = 7.1 Hz), 1.25 (3H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.2, 165.9, 152.9, 137.7, 134.5, 132.5, 129.5, 126.4, 117.9, 62.0, 14.1; HRMS (ESI): for $C_{11}H_{11}BrN_2O_4SNa$ calcd: 368.9521 [M+H]; found: 368.9523.

5.2. 2-({[(Ethoxycarbonyl)amino]carbonothioyl}amino)-5-methylbenzoic acid (2b)

Compound **2b** was prepared and purified according to the procedure described for **2a**, starting from 2-amino-5-methylbenzoic

acid. The reaction yielded **2b** (91%) as a white solid (mp: 197.0 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.43 (1H, s), 8.3 (1H, s), 8.15 (1H, s), 7.91 (1H, d, J = 1.8 Hz), 7.45 (1H, dd, J = 8.38 and 1.8 Hz), 4.32 (2H, q, J = 7.1 Hz), 2.41 (3H, s), 1.36 (3H, t, J = 7.1 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 178.4, 167.0, 152.0, 137.1, 136.3, 134.4, 131.9, 126.9, 121.3, 63.2, 21.1, 14.4; HRMS (ESI): for $C_{12}H_{15}N_2O_4S$ calcd: 283.0753 [M+H]; found 283.0758.

5.3. Ethyl 6-bromo-4-oxo-2-thioxo-1,4-dihydroquinazoline-3(2*H*)-carboxylate (3a)

Compound **2a** (12.3 g, 35.5 mmol) was dissolved in 150 mL of acetic anhydride and stirred at 60 °C for 4 h. The mixture was slowly cooled to 4 °C during crystallization and the white crystals formed were filtered off, washed with cold acetic anhydride and dried under vacuum, to give **3a** (10.8 g, 32.8 mmol, 93%). Mp: 214.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.87 (1H, s), 8.06 (1H, d, J = 2.4 Hz), 7.97 (1H, dd, J = 8.7 and J = 2.4 Hz), 7.47 (1H, d, J = 8.7 Hz), 4.19 (2H, q, J = 7.1 Hz), 1.25 (3H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.4, 154.1, 153.3, 146.9, 138.8, 131.1, 126.3, 119.8, 118.9, 62.0, 14.2; HRMS (ESI): for $C_{11}H_9BrN_2O_3S$ calcd: 327.9517 [M+H]; found: 327.9524.

5.4. Ethyl 6-methyl-4-oxo-2-thioxo-1,4-dihydroquinazoline-3(2*H*)-carboxylate (3b)

Compound **3b** was prepared and purified according to the procedure described for **3a**, starting from **2b**. The reaction yielded **3b** (100%) as a white solid (mp: 164.0 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (1H, br s), 7.95 (1H, s), 7.54 (1H, dd, J = 8.3 and 2.0 Hz), 7.42 (1H, d, J = 8.3 Hz), 4.30 (2H, q, J = 7.1 Hz), 2.45 (3H, s), 1.34 (3H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.3, 152.8, 152.6, 145.7, 137.4, 137.2, 129.0, 124.8, 119.4, 62.9, 21.3, 14.4; HRMS (ESI): for $C_{12}H_{13}N_2O_3S$ calcd: 265.0647 [M+H]; found 265.0647.

5.5. 6-Bromo-2-thioxo-2,3-dihydroquinazolin-4(1*H*)-one (4a)

A solution of sodium methoxide (0.5 M, 36.1 mmol) in methanol (72 mL) was added to a solution of **3a** (10.8 g, 32.8 mmol) in 150 mL of dry THF and the mixture was heated at reflux for 90 min. The mixture was allowed to reach room temperature and quenched by addition of acetic acid (2.1 mL, 36.1 mmol). The mixture was concentrated under reduced pressure and 200 mL of ethyl alcohol and 100 mL of water was added and the mixture was heated at reflux for 30 min. The slurry was cooled to rt and filtrated to give **4** white solid (8.43 g, 100%). Mp: 350 °C (decomp.), ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.67 (2H, br s), 7.97 (1H, d, J = 2.3 Hz), 7.88 (1H, dd, J = 8.7 and 2.3 Hz), 7.29 (1H, d, J = 8.7 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.4, 158.6, 139.8, 137.9, 128.7, 118.4, 118.1, 115.9; HRMS (FAB+): $C_8H_6ON_2BrS$ calcd: 256.9382 [M+H]; found 256.9382.

5.6. 6-Methyl-2-thioxo-2,3-dihydroquinazolin-4(1*H*)-one (4b)

Compound **4b** was prepared and purified according to the procedure described for **4a**, starting from **3b**. The reaction yielded **4b** (88%) as a white solid [mp: 312 °C, (decomp.)]; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.65 (1H, s), 12.40 (1H, s), 7.75 (1H, s), 7.55 (1H, d, J = 8.4 Hz), 7.27 (1H, d, J = 8.4 Hz), 2.35 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.6, 160.5, 139.3, 137.3, 134.8, 127.0, 116.9, 116.7, 21.3; $C_9H_8ON_2S$ calcd: 192.0357 [M+H]; found 192.0354.

5.7. 6-Bromo-2-(methylsulfanyl)quinazolin-4(3*H*)-one (5a)

To a solution of compound **4a** (8.43 g, 32.8 mmol) in 130 mL of DMF was added a solution sodium methoxide (0.50 M, 32.8 mmol) in methanol (65.6 mL) and the mixture was stirred at room temperature for 15 min. Iodomethane (2.04 mL, 32.8 mmol) was added and the reaction mixture was stirred at room temperature for 22 h. The solvents were evaporated under reduced pressure and the remaining solid was dissolved in 500 mL of EtOAc and washed with 400 mL of a saturated solution of aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$, concentrated under reduced pressure and precipitated from 50 mL of ethyl alcohol, to give **5a** as a white solid (8.03 g, 90%). Mp: 230 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (1H, s), 8.09 (1H, J = 2.4 Hz), 7.89 (1H, dd, J = 8.7 and 2.4 Hz), 7.48 (1H, d, J = 8.7 Hz), 2.57 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.0, 157.3, 147.4, 137.3, 128.3, 128.1, 121.5, 117.6, 12.8; HRMS (ESI): $C_9H_8N_2OSBr$ calcd: 270.9541 [M+H]; found 270.9539.

5.8. 6-Methyl-2-(methylsulfanyl)quinazolin-4(3*H*)-one (5b)

Compound **5b** was prepared and purified according to the procedure described for **5a**, starting from **4a**. The reaction yielded **5b** (77%) as a white solid (mp: 206 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.63 (1H, s), 8.05 (1H, s), 7.55 (2H, m), 2.69 (3H, s), 2.47 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 154.4, 147.5, 136.5, 136.1, 126.39, 126.35, 119.7, 21.4, 13.6; HRMS (ESI): for $C_{10}H_{11}N_2OS$ calcd: 207.0592 [M+H]; found 207.0600.

5.9. 6-Bromo-2-(methylsulfanyl)-4-(3-nitro-1*H*-1,2,4-triazol-1-yl)quinazoline (6)

Iodine (16.5 g, 65.1 mmol) was added to a suspension of compound **5a** (8.03 g, 29.6 mmol), 3-nitro-1*H*-1,2,4-triazole (11.8 g, 103.6 mmol), triphenylphosphine (18.1 g, 69.1 mmol) in 630 mL of toluene. The reaction mixture was rapidly heated to 95 °C for 15 min after which 25 mL of *N,N*-diisopropylethylamine was added and the mixture was stirred for another 50 min. The reaction was cooled to room temperature and concentrated under reduced pressure. The crude product was precipitated from ethyl alcohol, to give **6** as a yellow solid (9.67 g, 89%). Mp: 204 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (1H, s), 8.97 (1H, dd, J = 2.2 and 0.3 Hz), 8.18 (1H, dd, J = 9.0 and 2.2 Hz), 7.88 (1H, dd, J = 9.0 and 0.3 Hz), 2.70 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.0, 163.2, 152.3, 151.1, 148.2, 139.1, 129.1, 127.9, 120.5, 114.3, 14.0; HRMS (FAB+): $C_{11}H_8N_6O_2BrS$ calcd: 366.9613 [M+H]; found 366.9615.

5.10. 9-Bromo-2-(4-methylphenyl)-5-(methylsulfanyl)[1,2,4]-triazolo[4,3-*c*]quinazolin-3(2*H*)-one (7a)

N,N-Diisopropylethylamine (0.95 mL, 5.45 mmol) was added to a mixture of compound **6** (2.0 g, 5.447 mmol) and **10a** (1.164 g, 5.99 mmol) and the reaction mixture were heated at 110 °C for 60 h. The mixture was cooled to rt, concentrated under reduced pressure and precipitated from ethyl alcohol to give **7a** as a white solid (1.51 g, 69%). Mp: 213 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (1H, d, J = 2.2 Hz), 7.94 (2H, dd, J = 8.6 and 1.9 Hz), 7.70 (1H, dd, J = 8.6 and J = 2.2 Hz), 7.50 (1H, d, J = 8.6 Hz), 7.28 (2H, d, J = 8.6 Hz), 2.65 (3H, s), 2.40 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.9, 147.1, 141.7, 138.5, 136.5, 135.7, 134.9, 129.9, 129.9, 128.8, 125.3, 120.6, 119.5, 119.5, 116.1, 21.2, 13.6; HRMS (FAB+): $C_{17}H_{14}ON_4BrS$ calcd: 401.0072 [M+H]; found 401.0073.

5.11. 9-Bromo-2-phenyl-5-(methylsulfanyl)[1,2,4]triazolo[4,3-*c*]-quinazolin-3(2*H*)-one (7b)

Compound **7b** was prepared and purified according to the procedure described for **7a**, starting from **10b**. The reaction yielded **7b** (76%) as a white solid (mp: 222 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ

8.26 (1H, d, J = 1.6 Hz), 8.07 (2H, d, J = 7.9 Hz), 7.69 (1H, dd, J = 8.5 and 1.6 Hz), 7.48 (3H, m), 7.30 (1H, t, J = 7.4 Hz), 2.64 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.9, 147.1, 141.7, 138.6, 137.3, 135.8, 129.3, 129.3, 128.8, 126.6, 125.3, 120.7, 119.5, 119.5, 115.9, 13.6; HRMS (ESI): $C_{16}H_{12}ON_4BrS$ calcd: 386.9915 [M+H]; found 386.9912.

5.12. 9-Bromo-2-(4-chlorophenyl)-5-(methylsulfanyl)[1,2,4]-triazolo[4,3-*c*]quinazolin-3(2*H*)-one (7c)

Compound **7c** was prepared and purified according to the procedure described for **7a**, starting from **10c**. The reaction yielded **7c** (70%) as a white solid (mp: 247 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (1H, d, J = 2.2 Hz), 8.06 (2H, dt, J = 9.0 and 2.0 Hz), 7.72 (1H, dd, J = 8.7 and 2.2 Hz), 7.51 (1H, d, J = 8.7 Hz), 7.44 (2H, dt, J = 9.0 and 2.0 Hz), 2.65 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.8, 147.0, 141.8, 138.9, 136.0, 135.9, 132.0, 129.5, 129.5, 128.9, 125.4, 120.8, 120.6, 120.6, 115.8, 13.7; HRMS (ESI): $C_{16}H_{11}ON_4BrClS$ calcd: 420.9525 [M+H]; found 420.9525.

5.13. 9-Bromo-2-(2-fluorophenyl)-5-(methylsulfanyl)[1,2,4]-triazolo[4,3-*c*]quinazolin-3(2*H*)-one (7d)

Compound **7d** was prepared and purified according to the procedure described for **7a**, starting from **10d**. The reaction yielded **7d** (51%) as a white solid (mp: 256 °C). NMR (500 MHz, DMSO-d_6) δ 8.11 (1H, d, J = 2.1 Hz), 7.83 (1H, dd, J = 8.7 and 2.1 Hz), 7.67 (1H, dt, J = 7.7 and 1.2 Hz), 7.56 (2H, m), 7.43 (1H, t, J = 9.6 Hz), 7.39 (1H, t, J = 7.7 Hz), 2.64 (3H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.9 (d, J = 251.4 Hz), 150.5, 146.8, 140.9, 138.8, 135.1, 130.6 (d, J = 7.9 Hz), 128.3, 127.9, 124.6 (d, J = 3.7 Hz), 123.8, 123.3 6 (d, J = 11.8 Hz), 119.1, 116.3 (d, J = 19.3 Hz), 115.6, 12.3; HRMS (ESI): $C_{16}H_{11}ON_4BrFS$ calcd: 404.9821 [M+H]; found 404.9820.

5.14. 9-Methyl-5-(methylthio)-2-phenyl[1,2,4]triazolo[4,3-*c*]-quinazolin-3(2*H*)-one (7e)

A suspension of **12** (0.25 g, 0.68 mmol) and lithium hydroxide (0.057 g, 1.35 mmol) in 15 mL of dry THF was stirred at room temperature for 2 h. The mixture was poured onto 100 mL of a saturated solution of aqueous $NaHCO_3$ and extracted twice with 200 mL of EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to afford **7e** as a white solid (0.17 g, 0.5 mmol, 78%). Mp >400 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (2H, dd, J = 8.8 and 1.14 Hz), 7.49 (3H, m), 7.91 (1H, s), 7.41 (1H, dd, J = 8.3 and 1.6 Hz), 7.29 (1H, t, J = 7.4 Hz), 2.63 (3H, s), 2.46 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.0, 147.3, 140.9, 139.9, 137.6, 137.5, 134.0, 129.2, 129.2, 126.9, 126.3, 122.3, 119.5, 119.5, 114.1, 21.5, 13.5; HRMS (ESI): for $C_{17}H_{15}N_4OS$ calcd: 323.0967 [M+H]; found 323.0962.

5.15. 9-Bromo-2-(4-methylphenyl)-2,6-dihydro[1,2,4]triazolo-[4,3-*c*]quinazoline-3,5-dione (8a)

To a solution of **7a** (0.534 g, 1.33 mmol) in 60 mL of CH_2Cl_2 was added (77%) *m*CPBA (0.597 g, 2.67 mmol) and the mixture was stirred at rt for 6 h. A white precipitate was slowly formed. Saturated solutions of aqueous $Na_2S_2O_3$ (20 mL) and $NaHCO_3$ (20 mL) were added, subsequently, and the mixture was stirred vigorously for 30 min. The precipitate was filtered off, washed several times with water and heated in a mixture of 25 mL ethyl alcohol and 25 mL of water at reflux for 30 min. The mixture was cooled to rt and filtered to give **8a** as a white solid (0.488 g, 99%). Mp: 369 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.52 (1H, s), 7.96 (1H, d, J = 2.2 Hz), 7.86 (2H, d, J = 8.4 Hz), 7.58 (1H, dd, J = 8.8 and 2.2 Hz), 7.30 (2H, d, J = 8.4 Hz), 7.02 (1H, d, J = 8.8 Hz), 2.33 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.6, 143.7, 139.4, 136,6 135.2, 135.1, 134.7, 129.6, 129.6, 124.5, 118.9, 117.9, 117.9, 114.9, 111.3, 20.5; HRMS (ESI): $C_{16}H_{12}N_4O_2Br$ calcd: 371.0144 [M+H]; found 371.0143.

5.16. 9-Bromo-2-phenyl-2,6-dihydro[1,2,4]triazolo[4,3-*c*]quinazoline-3,5-dione (8b)

Compound **8b** was prepared and purified according to the procedure described for **8a**, starting from **7b**. The reaction yielded **8b** (100%) as a white solid (mp: 405 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.55 (1H, s), 8.06 (1H, d, J = 2.2 Hz), 7.97 (2H, d, J = 7.7 Hz), 7.73 (1H, dd, J = 8.7 and 2.2 Hz), 7.52 (1H, t, J = 8.0 Hz), 7.34 (2H, t, J = 7.4 Hz), 7.13 (1H, d, J = 8.7 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.6, 143.6, 139.6, 137.1, 136.6, 135.2, 129.2, 129.2, 125.8, 124.6, 118.8, 118.8, 117.9, 114.9, 111.2; HRMS (ESI): $C_{15}H_{10}N_4O_2Br$ calcd: 356.9987 [M+H]; found 356.9993.

5.17. 9-Bromo-2-(4-chlorophenyl)-2,6-dihydro[1,2,4]triazolo-[4,3-*c*]quinazoline-3,5-dione (8c)

Compound **8c** was prepared and purified according to the procedure described for **8a**, starting from **7c**. The reaction yielded **8c** (100%) as a white solid [mp: 387 °C (decomp.)]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (1H, br s), 8.02 (2H, d, J = 7.4 Hz), 7.74 (1H, br d, J = 7.8 Hz), 7.59 (2H, d, J = 7.4 Hz), 7.13 (1H, d, J = 7.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.6, 143.6, 139.9, 136.8, 136.0, 135.3, 129.7, 129.2, 129.2, 124.7, 120.2, 120.2, 118.1, 114.9, 111.1; HRMS (ESI): $C_{15}H_9N_4O_2BrCl$ calcd: 390.9597 [M+H]; found 390.9599.

5.18. 9-Bromo-2-(2-fluorophenyl)-2,6-dihydro[1,2,4]triazolo-[4,3-*c*]quinazoline-3,5-dione (8d)

Compound **8d** was prepared and purified according to the procedure described for **8a**, starting from **7d**. The reaction yielded **8d** (100%) as a white solid [mp: 347 °C (decomp.)]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (1H, s), 7.63 (2H, m), 7.54 (1H, m), 7.47 (1H, t, J = 8.7 Hz), 7.38 (1H, t, J = 8.7 Hz), 7.07 (2H, d, J = 8.7 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.3 (d, J = 250.6 Hz), 147.8, 145.3, 141.7, 141.1, 134.7, 130.7 (d, J = 7.8 Hz), 128.5, 125.0 (d, J = 3.5 Hz), 124.1, 123.9 (d, J = 11.7 Hz), 120.6, 116.7 (d, J = 19.1 Hz), 113.0, 111.5; HRMS (ESI): $C_{15}H_9N_4O_2BrF$ calcd: 374.9893 [M+H]; found 374.9889.

5.19. 9-Methyl-2-phenyl-2,6-dihydro[1,2,4]triazolo[4,3-*c*]quinazoline-3,5-dione (8e)

Compound **8e** was prepared and purified according to the procedure described for **8a**, starting from **7e**. The reaction yielded **8e** (100%) as a white solid [mp. 330 °C (decomp.)]; ^{1}H NMR (500 MHz, DMSO-d_6, 323 K) δ 7.96 (1H, d, J = 7.7 Hz), 7.77 (1H, s), 7.50 (2H, t, J = 7.7 Hz), 7.35 (3H, m), 7.21 (1H, d, J = 8.36 Hz), 2.33 (3H, s); ^{13}C NMR (125 MHz, DMSO-d_6, 323 K) δ 146.5, 143.3, 140.3, 136.9, 135.0, 133.0, 132.4, 128.6, 128.6, 125.3, 121.7, 118.7, 118.7, 115.5, 108.6, 19.8; HRMS (ESI): for $C_{16}H_{13}N_4O_2$ calcd: 293.1039 [M+H]; found 293.1033.

5.20. Ethyl 1-(4-methylphenyl)hydrazinecarboxylate 10a

N,*N*-Dimethylformamide (20 mL) was added to a mixture of 4-iodotoluene (3.0 g, 13.8 mmol), ethyl carbazate (1.72 g, 16.5 mmol), 1,10-phenanthroline (0.50 g, 2.76 mmol), copper(I) iodide (0.131 g, 0.69 mmol) and cesium carbonate (6.3 g, 19.3 mmol) and the mixture was heated under N_2 at 80 °C for 18 h. The crude mixture was concentrated in vacuo and purified by chromatogra-

phy on a silica gel column. Elution with *n*-heptane/EtOAc (4:1) as eluent afforded **10a** as a white solid (2.22 g, 83%). Mp. 35 °C, ^{1}H NMR (400 MHz, C_6D_6) δ 7.53 (2H, d, J = 8.3 Hz), 6.98 (2H, d, J = 8.3 Hz), 4.00 (2H, q, J = 7.1 Hz), 3.96 (2H, br s), 2.08 (3H, s), 0.94 (3H, t, J = 7.1 Hz), ^{13}C NMR (100 MHz, C_6D_6) δ 155.9, 141.2, 134.0, 129.0, 129.0, 123.4, 123.4, 62.1, 20.8, 14.6; HRMS (ESI): $C_{10}H_{15}N_2O_2$ calcd: 195.1134 [M+H]; found 195.1129.

5.21. Ethyl 1-phenylhydrazinecarboxylate (10b)

Compound **10b** was prepared and purified according to the procedure described for **10a**, starting from iodobenzene. The reaction yielded **10b** (76%) as a white solid (mp: 25 °C). ^{1}H NMR (400 MHz, C_6D_6) δ 7.66 (2H, br d, J = 7.55 Hz), 7.18 (2H, m), 6.94 (1H, tt, J = 7.4 and 1.1 Hz), 3.99 (2H, q, J = 7.1 Hz), 3.93 (2H, br s), 0.95 (3H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, C_6D_6) δ 155.8, 143.6, 128.4, 124.5, 124.5, 123.2, 123.2, 62.2, 14.5; HRMS (ESI): $C_9H_{13}N_2O_2$ calcd: 181.0977 [M+H]; found 181.0980.

5.22. Ethyl 1-(4-chlorophenyl)-hydrazinecarboxylate (10c)

Compound **10c** was prepared and purified according to the procedure described for **10a**, starting from 1-chloro-4-iodobenzene. The reaction yielded **10c** (40%) as a white semisolid. ^{1}H NMR (400 MHz, C_6D_6) δ 7.43 (2H, d, J = 7.0 Hz), 7.11 (2H, dt, J = 7.0 and 2.2 Hz), 3.92 (2H, q, J = 7.1 Hz), 3.79 (2H, br s), 0.91 (3H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, C_6D_6) δ 155.3, 142.1, 129.5, 128.4, 128.4, 124.0, 124.0, 62.3, 14.5; HRMS (ESI): $C_9H_{12}N_2O_2Cl$ calcd: 215.0587 [M+H]; found 215.0586.

5.23. Ethyl 1-(2-fluorophenyl)-hydrazinecarboxylate (10d)

Compound **10d** was prepared and purified according to the procedure described for **10a**, starting from 1-fluoro-2-iodobenzene. The reaction yielded **10d** (22%) as a white semisolid. ^{1}H NMR (400 MHz, C_6D_6) δ 7.22 (1H, m), 6.80–6.91 (3H, m). 4.27 (2H, br s), 4.11 (2H, q, J = 7.1 Hz), 1.12 (3H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, C_6D_6) δ 157.9 (d, J = 248 Hz), 156.8, 131.8 (d, J = 12 Hz), 128.8, 128.0 (d, J = 23.6 Hz), 124.1 (d, J = 3.8 Hz), 116.2 (d, J = 20.3 Hz), 62.5, 14.5; HRMS (ESI): $C_9H_{12}N_2O_2F$ calcd: 199.0885 [M+H]; found 199.0888.

5.24. 6-Bromo-4-chloro-2-(methylsulfanyl)quinazoline (11a)

To a suspension of compound **5a** (0.65 g, 2.4 mmol) in 6.2 mL of $POCl_3$ was added 10 μL of pyridine and the mixture was heated at 110 °C for 18 h. The reaction mixture was cooled to rt and concentrated under reduced pressure. A saturated solution of 50 mL of aqueous $NaHCO_3$ was added to the crude solid and the mixture was extracted with 50 mL of EtOAc. The organic layer was dried over $MgSO_4$, concentrated under reduced pressure and purified by chromatography on a silica gel column. Elution with *n*-heptane/EtOAc (4:1) as eluent afforded **11a** as a white solid (0.52 g, 75%). Mp: 124 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (1H, J = 2.2 Hz), 7.92 (1H, dd, J = 8.9 and 2.2 Hz), 7.73 (1H, J = 8.9 Hz), 2.66 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 160.7, 150.6, 138.9, 129.0, 128.4, 122.2, 120.7, 14.6; HRMS (FAB+): $C_9H_7N_2ClBrS$ calcd: 288.9202 [M+H]; found 288.9200.

5.25. 4-Chloro-6-methyl-2-(methylsulfanyl)quinazoline (11b)

Compound **11b** was prepared and purified according to the procedure described for **11a**, starting from **5b**. The reaction yielded **11b** (77%) as a white solid (mp: 205 °C, decomp). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (1H, s), 7.73 (1H, d, J = 8.6 Hz), 7.66 (1H, d, J = 8.6 Hz), 2.65 (3H, s), 2.55 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 161.2, 150.6, 137.5, 137.5, 127.0, 124.9, 121.0, 21.8, 14.5; HRMS (ESI): for $C_{10}H_{10}N_2SCl$ calcd: 225.0253 [M+H]; found 225.0256.

5.26. Ethyl 2-[6-methyl-2-(methylthio)quinazolin-4(3*H*)-ylidene]-1-phenylhydrazinecarboxylate (12)

N,*N*-Diisopropylethylamine (0.104 mL, 0.6 mmol) was added to a solution of **11b** (0.071 g, 0.3 mmol) and **10a** (0.057 g, 0.3 mmol) in 5 mL of dioxane and the solution was heated at reflux for 48 h, during which a white precipitate fell out. The white slurry was filtered and the precipitate was washed with dioxane to give **12** as a white solid (0.110 g, 100%). Mp. 223 °C; ^{1}H NMR (500 MHz, DMSO-d_6, 323 K) δ 8.48 (1H, s), 7.78 (2H, s), 7.59 (2H, d, J = 7.8 Hz), 7.37 (2H, t, J = 7.7 Hz), 7.22 (1H, t, J = 7.4 Hz), 4.19 (2H, q, J = 7.1 Hz), 2.56 (3H, s), 2.46 (3H, s), 1.13 (3H, t, J = 7.1 Hz); ^{13}C NMR (125 MHz, DMSO-d_6, 323 K) δ 165.2, 158.3, 153.1, 141.6, 140.8, 136.8, 136.2, 128.2, 125.6, 123.3, 122.8, 120.5, 109.7, 62.0, 20.6, 13.9, 13.1; HRMS (ESI): for $C_{19}H_{21}N_4O_2S$ calcd: 369.1385 [M+H]; found 369.1380.

5.27. 2-(4-Methylphenyl)-9-phenylethynyl-2,6-dihydro[1,2,4]-triazolo[4,3-*c*]-quinazoline-3,5-dione (13)

To a stirred solution of phenylacetylene (15.0 μL, 0.137 mmol) and **8a** (25.3 mg, 0.0682 mmol) in a mixture of 2 mL of DMF and 1 mL of NEt_3, was added $Pd(OAc)_2$ (1.53 mg, 6.82 μmol) and triphenylphosphine (3.57 mg, 13.6 μmol) in a seal tube. The tube was sealed under argon and the mixture was stirred at 100 °C for 18 h. The mixture was cooled and concentrated to dryness in vacuo and the crude product was purified by chromatography on a silica gel column. Elution with *n*-heptane/EtOAc (2:1) as eluent yielded **13** as a white solid (14 mg, 70%). Mp: >300 °C (decomp.), ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.61 (1H, s), 8.06 (1H, d, J = 1.9 Hz), 7.85 (2H, d, J = 8.5 Hz), 7.70 (1H, dd, J = 8.6 and 1.9 Hz), 7.58 (2H, m), 7.44 (3H, m), 7.31 (2H, d, J = 8.5 Hz), 7.20 (1H, d, J = 8.6 Hz), 2.34 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.5, 143.7, 139.7, 137.2, 135.1, 134.7, 131.4, 131.4, 129.5, 129.5, 128.9, 128.8, 128.8, 125.2, 122.0, 118.8, 118.8, 117.0, 116.3, 109.8, 89.5, 88.1, 20.5; HRMS (ESI): for $C_{24}H_{17}N_4O_2$ calcd: 393.1352 [M+H]; found: 393.1350.

5.28. 2-(4-Methylphenyl)-9-(3-thienylethynyl)-2,6-dihydro-[1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (14)

Compound **14** was prepared and purified according to the procedure described for **13**, starting from 3-ethynylthiophene and **8a**. The reaction yielded **14** (40%) as a white solid (mp: >320 °C, decomp). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (1H, s), 8.06 (1H, d, J = 1.8 Hz), 7.93 (1H, dd, J = 2.9 and 1.2 Hz), 7.87 (2H, d, J = 8.4 Hz), 7.67 (2H, m), 7.34 (2H, d, J = 8.4 Hz), 7.30 (1H, dd, J = 5.0 and 1.2 Hz), 7.21 (1H, d, J = 8.5 Hz), 2.35 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.6, 143.7, 139.7, 137.2, 135.1, 134.9, 134.8, 130.2, 129.6, 129.6, 129.6, 127.0, 125.0, 120.9, 118.8, 118.8, 117.1, 116.3, 109.8, 87.4, 85.1, 20.5; HRMS (ESI): for $C_{22}H_{15}N_4O_2S$ calcd: 399.0916 [M+H]; found: 399.0920.

5.29. 2-(4-Methylphenyl)-9-(pyridin-2-ylethynyl)-2,6-dihydro-[1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (15)

Compound **15** was prepared and purified according to the procedure described for **13**, starting from 2-ethynylpyridine and **8a**. The reaction yielded **15** (75%) as a white solid (mp: 330 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (1H, s), 8.63 (1H, ddd, J = 4.9, 1.8 and 1.0 Hz), 8.14 (1H, br d, J = 1.9 Hz), 7.86 (3H, m), 7.77 (1H, dd, J = 8.5 and 1.9 Hz), 7.69 (1H, dt, J = 7.8 and 1.0 Hz), 7.43 (1H,

ddd, J = 7.6, 4.9 and 1.2 Hz), 7.33 (2H, d, J = 8.4 Hz), 7.24 (1H, d, J = 8.5 Hz), 2.34 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.2, 146.6, 143.7, 142.1, 139.7, 137.8, 136.8, 135.4, 135.1, 134.8, 129.6, 129.6, 127.4, 125.7, 125.7, 123.6, 118.8, 116.4, 116.1, 109.9, 89.2, 87.2, 20.5; HRMS (ESI): for $C_{23}H_{16}N_5O_2$ calcd: 394.1304 [M+H]; found: 394.1310.

5.30. 2-(4-Methylphenyl)-9-(pyridin-3-ylethynyl)-2,6-dihydro-[1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (16)

Compound **16** was prepared and purified according to the procedure described for **13**, starting from 3-ethynylpyridine and **8a**. The reaction yielded **16** (50%) as a white solid (mp: 339 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.62 (1H, s), 8.76 (1H, br s), 8.59 (1H, dd, J = 4.9 and 1.5 Hz), 8.06 (1H, d, J = 1.7 Hz), 7.97 (1H, dt, J = 7.8 and 1.5 Hz), 7.83 (2H, d, J = 8.4 Hz), 7.69 (1H, dd, J = 8.5 and 1.7 Hz), 7.46 (1H, dd, J = 7.8 and 4.9 Hz), 7.29 (2H, d, J = 8.4 Hz), 7.19 (1H, d, 8.5), 2.32 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 151.7, 149.0, 146.4, 143.6, 139.6, 139.6, 138.5, 137.5, 135.1, 134.7, 129.5, 129.5, 125.4, 123.6, 119.2, 118.7, 118.7, 116.4, 116.3, 109.7, 91.1, 86.4, 20.5; HRMS (ESI): for $C_{23}H_{16}N_5O_2$ calcd: 394.1304 [M+H]; found: 394.1305.

5.31. 2-(4-Methylphenyl)-9-(pyridin-4-ylethynyl)-2,6-dihydro-[1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (17)

Compound **17** was prepared and purified according to the procedure described for **13**, starting from 4-ethynylpyridine hydrochloride and **8a**. The reaction yielded **17** (39%) as a white solid (mp: 330 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.66 (1H, s), 8.63 (2H, d, J = 5.9 Hz), 8.15 (1H, d, J = 1.7 Hz), 7.85 (2H, d, J = 8.4 Hz), 7.76 (1H, dd, J = 8.5 and 1.8 Hz), 7.55 (2H, d, J = 5.9 Hz), 7.32 (2H, d, J = 8.4 Hz), 7.23 (1H, d, J = 8.5 Hz), 2.34 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.9, 149.9, 146.5, 143.6, 139.6, 137.9, 135.3, 135.1, 134.7, 130.0, 129.5, 129.5, 125.8, 125.3, 125.3, 118.7, 118.7, 116.4, 115.9, 109.9, 92.4, 86.9, 20.5; HRMS (ESI): for HRMS (ESI): for $C_{23}H_{16}N_5O_2$ calcd: 394.1304 [M+H]; found: 394.1298.

5.32. 2-(4-Methylphenyl)-9-[(4-phenoxyphenyl)ethynyl]-2,6-dihydro[1,2,4] triazolo[4,3-*c*]-quinazoline-3,5-dione (18)

Compound **18** was prepared and purified according to the procedure described for **13**, starting from 1-ethynyl-4-phenoxybenzene and **8a**. The reaction yielded **18** (60%) as a white solid (mp: 216 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (1H, s), 8.06 (1H, d, J = 1.9 Hz), 7.85 (2H, d, J = 8.5 Hz), 7.69 (1H, dd, J = 8.5 and 1.9 Hz), 7.59 (2H, dt, J = 8.8 and 1.9 Hz), 7.44 (2H, m), 7.32 (2H, d, J = 8.3 Hz), 7.20 (2H, m), 7.09 (2H, dd, J = 8.7 and 1.1 Hz), 7.02 (2H, dt, J = 8.8 and 1.9 Hz), 2.34 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.5, 155.6, 146.6, 143.7, 139.8, 137.1, 135.1, 135.0, 134.8, 133.4, 133.4, 130.3, 130.3, 129.6, 129.6, 125.1, 124.3, 119.5, 119.5, 118.8, 118.8, 118.2, 118.2, 117.2, 116.6, 116.3, 109.8, 89.2, 87.5, 20.5; HRMS (ESI): for $C_{30}H_{21}N_4O_3$ calcd: 485.1614 [M+H]; found: 485.1614.

5.33. 9-(Biphen-4-ylethynyl)-2-(4-methylphenyl)-2,6-dihydro-[1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (19)

Compound **19** was prepared and purified according to the procedure described for **13**, starting from 4-ethynylbiphenyl and **8a**. The reaction yielded **19** (69%) as a white solid (mp: >350 °C, decomp.). ^{1}H NMR (400 MHz, DMSO-d_6, 373 K) δ 11.30 (1H, s), 8.12 (1H, br s), 7.86 (2H, d, J = 8.2 Hz), 7.72 (5H, m), 7.67 (2H, dt, J = 8.2 Hz), 7.49 (2H, t, J = 7.6 Hz), 7.40 (1H, d, J = 7.4 Hz), 7.33 (2H, d, J = 8.1 Hz), 7.28 (2H, d, J = 8.5 Hz), 2.38 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6, 373 K) δ 146.1, 143.0, 140.1, 139.2, 138.8, 136.9, 134.8, 134.5, 134.4, 131.4, 131.4, 128.9, 128.9, 128.4, 128.4, 127.2, 126.3, 126.3, 126.1, 126.1, 124.7, 120.8, 118.8, 118.8, 117.0, 115.9, 109.3, 89.1, 88.4, 19.8; HRMS (ESI): for $C_{30}H_{21}N_4O_2$ calcd: 469.1665 [M+H]; found: 469.1665.

5.34. 9-(3-Hydroxyphenyl)ethynyl-2-(4-methylphenyl)-2,6-dihydro[1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (20)

Compound **20** was prepared and purified according to the procedure described for **13**, starting from 3-*tert*-butyldimethylsilyloxy phenylacetylene and **8a**.[29] The reaction yielded **20** (32%) as a white solid [mp: 300 °C (decomp.)]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (1H, s), 9.72 (1H, s), 8.05 (1H, s), 7.86 (2H, d, J = 8.0 Hz), 7.70 (1H, d, J = 8.3 Hz), 7.32 (2H, d, J = 8.0 Hz), 7.22 (2H, m), 7.00 (1H, d, J = 7.5 Hz), 6.93 (1H, s), 6.84 (1H, d, J = 7.8 Hz), 2.34 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.4, 146.6, 143.7, 139.7, 137.2, 135.1, 135.1, 134.8, 129.9, 129.5, 129.5, 125.2, 122.9, 122.3, 118.8, 118.8, 117.8, 117.1, 116.4, 116.3, 109.8, 20.5; HRMS (ESI): for $C_{24}H_{17}N_4O_3$ calcd: 409.1301 [M+H]; found: 409.1299.

5.35. 2-(4-Methylphenyl)-9-(2-phenylethyl)-2,6-dihydro[1,2,4]-triazolo[4,3-*c*]-quinazoline-3,5-dione (21)

A catalytic amount of 10% Pd/C and **13** (20.0 mg, 0.0510 mmol) in a mixture of 2 mL of dichloromethane and 2 mL of methyl alcohol was stirred during 12 h under H_2 at atmospheric pressure. The mixture was filtered through Celite, washed with additionally methyl alcohol and triturated from ethyl alcohol to afford **21** (16 mg, 0.0404 mmol, 79%) as a white solid. Mp: 303 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.34 (1H, s), 7.85 (2H, d, J = 8.4 Hz), 7.83 (1H, d, J = 1.7 Hz), 7.43 (1H, dd, J = 8.4 and 1.7 Hz), 7.33 (2H, d, J = 8.4 Hz), 7.27 (3H, m), 7.18 (1H, m), 7.10 (1H, d, J = 8.4 Hz), 2.92 (4H, m), 2.35 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.7, 143.9, 141.2, 140.5, 136.9, 135.4, 135.0, 134.8, 133.0, 129.5, 129.5, 128.4, 128.4, 128.2, 128.2, 125.9, 121.9, 118.8, 118.8, 115.6, 109.0, 37.0, 36.2, 20.5; HRMS (ESI): for $C_{24}H_{21}N_4O_2$ calcd: 397.1665 [M+H]; found: 397.1669.

5.36. 2-(4-Methylphenyl)-9-[2-(3-thienyl)ethyl]-2,6-dihydro-[1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (22)

Compound **22** was prepared and purified according to the procedure described for **21**, starting from **14**. The reaction yielded **22** (88%) as a white solid (mp: 281 °C).^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (1H, s), 7.85 (3H, m), 7.44 (2H, m), 7.32 (2H, d, J = 7.9 Hz), 7.18 (1H, s), 7.12 (1H, d, J = 8.3 Hz), 7.05 (1H, d, J = 4.34 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.7, 143.9, 141.6, 140.5, 136.9, 135.4, 135.0, 134.8, 132.9, 129.5, 129.5, 128.4, 125.8, 121.8, 120.8, 118.8, 118.8, 115.6, 109.0, 35.3, 31.4, 20.5; HRMS (ESI): for $C_{22}H_{19}N_4O_2S$ calcd: 403.1229 [M+H]; found: 402.1226.

5.37. 2-(4-Methylphenyl)-9-[2-(2-pyridyl)ethyl]-2,6-dihydro-1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (23)

Compound **23** was prepared and purified according to the procedure described for **21**, starting from **15**. The reaction yielded **23** (85%) as a white solid (mp: >300 °C, decomp). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.39 (1H, s), 8.58 (1H, d, J = 4.42 Hz), 7.84 (4H, m), 7.43 (2H, m), 7.34 (3H, m), 7.11 (1H, d, J = 8.4 Hz), 3.10 (4H, m), 2.34 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.4, 147.4, 146.7, 143.8, 140.4, 138.5, 136.5, 135.5, 135.0, 134.8, 132.9, 129.5, 129.5, 123.8, 122.1, 121.9, 118.8, 118.8, 115.7, 109.1, 39.1, 34.0, 20.5; HRMS (ESI): for $C_{23}H_{20}N_5O_2$ calcd: 398.1617 [M+H]; found: 398.1621.

5.38. 2-(4-Methylphenyl)-9-[2-(3-pyridyl)ethyl]-2,6-dihydro-[1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (24)

Compound **24** was prepared and purified according to the procedure described for **21**, starting from **16**. The reaction yielded **24** (91%) as a white solid (mp: 292 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (1H, s), 8.57 (1H, s), 8.50 (1H, br s), 7.91 (1H, d, J = 7.8 Hz), 7.82 (3H, m), 7.50 (1H, dd, J = 7.8 and 5.0 Hz), 7.41 (1H, dd, J = 8.4 and 1.7 Hz), 7.30 (2H, d, J = 8.4 Hz), 7.11 (1H, d, J = 8.4 Hz), 2.97 (4H, m), 2.32 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.3, 146.7, 145.0, 143.8, 140.4, 138.9, 137.9, 136.2, 135.5, 135.0, 134.8, 132.9, 129.5, 129.5, 124.4, 121.9, 118.8, 118.8, 115.7, 109.0, 35.5, 33.7, 20.5; HRMS (ESI): for $C_{23}H_{20}N_5O_2$ calcd: 398.1617 [M+H]; found: 398.1620.

5.39. 2-(4-Methylphenyl)-9-[2-(4-pyridyl)ethyl]-2,6-dihydro-[1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (25)

Compound **25** was prepared and purified according to the procedure described for **21**, starting from **17**. The reaction yielded **25** (91%) as a white solid (mp: 294 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (1H, s), 8.47 (1H, d, J = 6.0 Hz), 7.85 (3H, m), 7.45 (1H, dd, J = 8.4 and 1.9 Hz), 7.34 (4H, m), 7.11 (1H, d, J = 8.4 Hz), 2.97 (4H, m), 2.35 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 151.0, 148.8, 148.8, 146.7, 143.8, 140.4, 136.2, 135.5, 135.0, 134.8, 132.9, 129.5, 129.5, 124.3, 124.3, 121.8, 118.8, 118.8, 115.7, 109.0, 36.0, 34.8, 20.5; HRMS (ESI): for $C_{23}H_{20}N_5O_2$ calcd: 398.1617 [M+H]; found: 398.1615.

5.40. 2-(4-Methylphenyl)-9-[2-(4-phenoxyphenyl)ethyl]-2,6-dihydro[1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (26)

Compound **26** was prepared and purified according to the procedure described for **21**, starting from **18**. The reaction yielded **26** (80%) as a white solid (mp: 213 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (1H, s), 7.83 (3H, m), 7.44 (1H, d, J = 8.5 Hz), 7.33 (3H, m), 7.27 (2H, d, J = 8.2 Hz), 7.11 (3H, m), 6.94 (4H, m), 2.35 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.1, 154.6, 146.8, 143.9, 140.5, 136.9, 136.5, 135.4, 135.0, 134.8, 133.0, 130.0, 130.0, 130.0, 130.0, 129.5, 129.5, 123.1, 121.9, 118.9, 118.9, 118.8, 118.8, 118.2, 118.2, 115.7, 109.0, 36.4, 36.3, 20.5; HRMS (ESI): for $C_{30}H_{25}N_4O_3$ calcd: 489.1927 [M+H]; found: 489.1929.

5.41. 9-[2-(Biphen-4-yl)ethyl)]-2-(4-methylphenyl)-2,6-dihydro[1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (27)

Compound **27** was prepared and purified according to the procedure described for **21**, starting from **19**. The reaction yielded **27** (98%) as a white solid (mp: 345 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.07 (1H, s), 7.82 (3H, m), 7.62 (2H, d, J = 6.3 Hz), 7.56 (2H, d, J = 6.5 Hz), 7.43 (3H, m), 7.31 (5H, m), 7.18 (1H, d, J = 7.9 Hz), 3.01 (4H, m), 2.36 (3H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 146.2, 143.1, 139.9, 139.8, 139.7, 137.5, 136.4, 135.0, 134.6, 134.5, 132.3, 128.8, 128.8, 128.3, 128.3, 128.1, 128.1, 126.4, 125.9, 125.9, 125.8, 125.8, 121.3, 118.8, 118.8, 115.2, 108.5, 35.7, 35.3, 19.8; HRMS (ESI): for $C_{30}H_{25}N_4O_2$ calcd: 473.1978 [M+H]; found: 473.1981.

5.42. 9-[2-(3-Hydroxyphenyl)ethyl]-2-(4-methylphenyl)-2,6-dihydro[1,2,4]triazolo[4,3-*c*]-quinazoline-3,5-dione (28)

Compound **28** was prepared and purified according to the procedure described for **21**, starting from **20**. The reaction yielded **28** (96%) as a white solid [mp: 300 °C (decomp.)]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.48 (1H, s), 9.41 (1H, s), 7.83 (2H, d, J = 8.3 Hz), 7.79 (1H, s), 7.41 (1H, d, J = 8.4 Hz), 7.31 (2H, d, J = 8.3 Hz), 7.18 (1H, d, J = 8.4 Hz), 7.03 (1H, dd, J = 7.5 and 8.0 Hz), 6.65 (2H, m), 6.59 (1H, br d, J = 7.0 Hz), 2.84 (4H, m), 2.33 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.5, 146.8, 143.9, 142.6, 140.6, 137.0, 135.1, 134.9, 133.0, 129.6, 129.6, 129.2, 126.9, 121.8, 119.1, 119.0, 119.0, 115.8, 115.5, 113.0, 109.1, 37.1, 36.2, 20.6; HRMS (ESI): for $C_{24}H_{21}N_4O_3$ calcd: 413.1614 [M+H]; found: 413.1619.

5.43. 9-Benzyl-2-(4-methylphenyl)-2,6-dihydro[1,2,4]triazolo-[4,3-*c*]quinazoline-3,5-dione (29)

To a stirred solution of benzyltributyltin (69 mg, 0.184 mmol) and **8a** (19.5 mg, 0.0525 mmol) in a mixture of 2 mL of DMF and 1 mL of NEt_3, was added $Pd(PPh_3)_4$ (6.0 mg, 5.3 μmol). The seal tube was sealed under argon and the mixture was stirred at 110 °C for 18 h.[26] The reaction mixture was cooled and concentrated to dryness in vacuo and the crude product was purified by chromatography on a silica gel column. Elution with *n*-heptane/EtOAc (2:1) as eluent yielded **29** as a white solid (13 mg, 65%). Mp: 299 °C, ^{1}H NMR (500 MHz, DMSO-d_6, 340 K) δ 7.82 (3H, m), 7.43 (1H, d, J = 8.0 Hz), 7.29 (5H, m), 7.20 (1H, m), 7.15 (1H, d, J = 8.0 Hz), 4.01 (2H, s), 2.34 (3H, s); ^{13}C NMR (125 MHz, DMSO-d_6, 340 K) δ 146.4, 143.4, 140.5, 140.1, 136.3, 135.3, 134.8, 134.6, 132.8, 129.1, 129.1, 128.3, 128.3, 128.1, 128.1, 125.8, 121.8, 118.9, 118.9, 115.6, 108.9, 39.9, 20.1; HRMS (ESI): for $C_{23}H_{19}N_4O_2$ calcd: 383.1508 [M+H]; found: 383.1512.

5.44. 9-(3-Fluorobenzyl)-2-(4-methylphenyl)-2,6-dihydro-[1,2,4]triazolo[4,3-*c*] quinazoline-3,5-dione (30)

Compound **30** was prepared and purified according to the procedure described for **29**, starting from **8a** and (3-fluorobenzyl)tributyltin.[26] The reaction yielded **30** (70%) as a white solid (mp: 324 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (1H, s), 7.86 (1H, s), 7.83 (2H, d, J = 8.2 Hz), 7.46 (d, J = 8.2 Hz), 7.33 (3H, m), 7.13 (3H, m), 7.02 (1H, t, J = 8.3 Hz), 4.04 (2H, s), 2.34 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.2 (d, J = 243.5 Hz), 146.7, 144.0 (d, J = 7.3 Hz), 143.9, 140.4, 136.1, 135.7, 135.0, 134.8, 133.2, 130.4. (d, J = 8.4 Hz), 129.5, 129.5, 124.8 (d, J = 2.6 Hz), 122.2, 118.9, 118.9, 116.0, 115.4 (d, J = 21.1 Hz), 112.9 (d, J = 20.9 Hz), 109.3, 40.4, 20.5; HRMS (ESI): for $C_{23}H_{18}N_4O_2F$ calcd: 401.1414 [M+H]; found: 401.1417.

5.45. 2-(4-Methylphenyl)-9-(3-thienylmethyl)-2,6-dihydro-[1,2,4]triazolo[4,3*c*] quinazoline-3,5-dione (31)

Compound **31** was prepared and purified according to the procedure described for **29**, starting from **8a** and **33**. The reaction yielded **31** (62%) as a white solid (mp: 287 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37 (1H, s), 7.82 (3H, m), 7.46 (2H, m), 7.32 (2H, d, J = 8.3 Hz), 7.25 (1H, br s), 7.12 (1H, d, J = 8.3 Hz), 6.98 (1H, d, J = 4.5 Hz), 4.01 (2H, s), 2.34 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.7, 143.9, 141.1, 140.5, 136.3, 135.5, 135.0, 134.8, 133.1, 129.5, 129.5, 128.4, 126.4, 122.0, 121.6, 121.6, 118.9, 115.9, 109.2, 34.8, 20.5; HRMS (ESI): for $C_{21}H_{17}N_4O_2S$ calcd: 389.1072 [M+H]; found: 389.1074.

5.46. Tributyl-(methyl-3-thienyl)tin (33)

A solution of **32** (620 mg, 3.5 mmol) in 3 mL of tetrahydrofuran were added to magnesium turnings (170 mg, 7.0 mmol) and the mixture was heated at 35 °C for 1 h.[25,27] The mixture was cooled to room temperature and a solution of tributyltin chloride (0.760 mL, 2.8 mmol) in 3 mL of tetrahydrofuran was added to the Grignard solution. The mixture was stirred at rt for 1 h and was then poured onto a saturated aqueous NH_4Cl solution and the mixture was extracted twice with EtOAc. The organic layers

were washed with brine, dried over $MgSO_4$, concentrated and purified by chromatography on a silica gel column. Elution with *n*-heptane/EtOAc (100:1) as eluent yielded **33** as a clear oil (490 mg, 36%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (1H, dd, J = 4.9 and 2.95 Hz), 6.77 (1H, dd, J = 4.9 and 1.33 Hz), 6.62 (1H, m), 2.33 (2H, s), 1.44 (6H, m), 1.27 (6H, m), 0.88 (9H, m), 0.83 (6H, m), ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.9, 128.7, 125.1, 115.8, 29.2, 27.5, 17.7, 13.9, 9.6.

5.47. Benzyl (2E)-3-[2-(4-methylphenyl)-3,5-dioxo-2,6-dihydro-[1,2,4]triazolo[4,3-*c*]quinazolin-9-yl]acrylate (35)

To a stirred solution of benzyl acrylate (44 μg, 0.274 mmol) and **8a** (50.6 mg, 0.137 mmol) in a mixture of 8 mL of DMF and 4 mL of NEt_3, was added $Pd(OAc)_2$ (3.06 mg, 13.7 μmol) and triphenylphosphine (7.14 mg, 27.4 μmol) in a sealed tube. The tube was sealed under argon and the mixture was stirred at 120 °C for 15 h. The mixture was cooled and concentrated to dryness in vacuo and the crude product was precipitated from acetone to give **35** as a white solid (29 mg, 47%). Mp: 242 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.58 (1H, br s), 8.29 (1H, s), 7.95 (1H, br d, J = 8.5 Hz), 7.87 (2H, d, J = 8.3 Hz), 7.78 (1H, d, J = 16.0 Hz), 7.35–7.46 (5H, m), 7.32 (2H, d, J = 8.3 Hz), 7.19 (1H, d, J = 8.5 Hz), 6.74 (1H, d, J = 16.0 Hz), 5.23 (2H, s,), 2.35 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 146.7, 144.0, 143.5, 140.2, 139.5, 136.2, 135.0, 134.8, 131.7, 131.5, 129.5, 129.5, 129.0, 128.5, 128.5, 128.1, 128.1, 123.2, 118.8, 118.8, 117.5, 116.7, 109.8, 65.6, 20.5; HRMS (ESI): for $C_{26}H_{21}N_4O_4$ calcd: 453.1563 [M+H]; found: 453.1559.

5.48. 3-[2-(4-Methylphenyl)-3,5-dioxo-2,6-dihydro[1,2,4]-triazolo[4,3-*c*]quinazolin-9-yl]propanoic acid (36)

Compound **36** was prepared and purified according to the procedure described for **21**, starting from **35**. The reaction yielded **36** (71%) as a white solid (mp: 275 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.43 (1H, br s), 7.85 (2H, d, J = 8.4 Hz), 7.82 (1H, br s), 7.44 (1H, br d, J = 8.2 Hz), 7.32 (2H, d, J = 8.4 Hz), 7.14 (1H, d, J = 8.2 Hz), 3.58 (1H, s, COO*H*), 2.90 (2H, m), 2.68 (1H, m), 2.56 (1H, m), 2.34 (3H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.5, 146.8, 143.9, 140.5, 135.9, 135.0, 134.9, 134.8, 132.9, 129.5, 129.5, 121.8, 118.9, 118.9, 115.8, 109.1, 34.7, 29.5, 20.5; HRMS (ESI): for $C_{19}H_{17}N_4O_4$ calcd: 365.125 [M+H]; found: 365.130.

5.49. Benzodiazepine receptor binding in vitro

Binding of ^{3}H-Flumazenil (87 Ci/mmol) to rat cortical membranes and to a membrane suspension of HEK 293 cells expressing human $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, or $\alpha_5\beta_3\gamma_2$ $GABA_A$ receptors was done following methods previously described by Kahnberg et al.[28] In brief: Tissue is homogenized in 20 mL Tris–HCl (30 mM, pH 7.4) using an Ultra-Turrax homogenizer. The suspensions are centrifuged at 27,000*g* for 15 min followed by three centrifugations resuspension cycles. The washed pellet is resuspended in 20 mL buffer, incubated at 37 °C for 30 min and then centrifuged for 10 min (27,000*g*). The pellet is washed once and the final pellet is resuspended in 30 mL Tris–HCl buffer (50 mM, pH 7.1) and stored at -20 °C until use. For binding studies frozen membrane suspensions were thawed and centrifuged (27,000*g*, 10 min). The pellet was resuspended into Tris–citrate buffer (50 mM, pH 7.1) at a tissue concentration: cortex preparation ca. 50 μg protein/0.55 mL assay (1 mg original tissue/0.55 mL assay) and HEK cells ca. 25 μg protein per 0.55 mL assay. Aliquots of 0.5 mL membrane preparation are added to 25 μL of ^{3}H-Flumazenil solution (1 nM final concentration) and 25 μL containing test substance and incubated at an ice-bath (0–4 °C) for 40 min. The incubated samples were added 5 mL ice-cold buffer (Tris–citrate, 50 mM pH 7.1), poured directly onto Whatman GF/C glass fiber filters under suction and immediately washed with 5 mL ice-cold buffer. Non-specific binding was determined by adding Clonazepam (1 μM final concentration) to separate samples. Protein was estimated by conventional protein assay method using Bovine serum albumin as standard.

IC_{50} values were determined using 4–6 different concentrations of test substance. K_i values were calculated according to $K_i = IC_{50}/(1 + (L)/K_D)$, (L) is the concentration (nM) of ^{3}H-Flumazenil; K_D is binding affinity constant of ^{3}H-Flumazenil (1.6 nM).

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Supplementary data

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