Eur. J. Med. Chem. 35 (2000) 333–341

S0223523400001306/FLA

Original article

Substituted 1,2,3-triazolo[1,5-a]quinazolines: synthesis and binding to benzodiazepine and adenosine receptors

Lucia Bertelli[a], Giuliana Biagi[a], Irene Giorgi[a], Oreste Livi[a]*, Clementina Manera[a], Valerio Scartoni[a], Antonio Lucacchini[b], Gino Giannaccini[b], Pier Luigi Barili[c]

[a]Dipartimento di Scienze Farmaceutiche, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy
[b]Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy
[c]Dipartimento di Bioorganica, Università di Pisa, Via Bonanno 33, 56126 Pisa, Italy

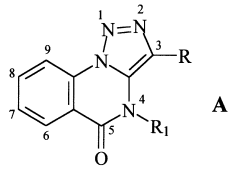
Received 19 May 1999; revised 22 October 1999; accepted 28 October 1999

Abstract – This paper reports the synthesis and evaluation of the biological affinity towards benzodiazepine and A_1 and A_{2A} adenosine receptors of some 3-ethoxycarbonyl or 3-phenyl-substituted 1,2,3-triazolo[1,5-a]quinazolines. Starting from the appropriate chloro-substituted phenylazides, the series of 7 or 8 chloro-substituted triazoloquinazolines were prepared. Nitration reactions of the triazoloquinazoline ring and chlorination reactions of the hydroxyl group in the 5 position of the same ring are also reported. By nucleophilic displacement of halogen, the corresponding 5-amino derivatives and some analogous derivatives bearing cyclohexylamino and *p*-toluidino substituents were obtained. The binding assays showed a generalized decrease in the affinity towards the benzodiazepine receptors and confirmed a moderate affinity towards the A_1 adenosine receptors in comparison with the previously studied triazoloquinazoline derivatives. © 2000 Éditions scientifiques et médicales Elsevier SAS

1,2,3-triazoles / 1,2,3-triazolo[1,5-a]quinazolines / benzodiazepine receptor binding / A_1 and A_{2A} adenosine receptor binding

1. Introduction

In a previous paper of ours [1] concerning a series of 1,2,3-triazolo[1,5-a]quinazoline derivatives (**A**) which had shown an interesting affinity towards benzodiazepine receptors, theoretical calculations based upon the SAR suggested the introduction of an electronegative substituent on the phenyl ring.



A

This tricyclic structure was also tested towards adenosine A_1 and A_{2A} receptors, and the binding results indicated that some triazoloquinazoline derivatives possessed a moderate affinity and selectivity also towards the A_1 receptor subtype. The best R substituent for the A_1 adenosine receptors appeared to be a phenyl group, while for the benzodiazepine receptors, an ethoxycarbonyl group was preferred [1].

The investigation into this nitrogenous tricyclic heterocycle was then continued with the 2-fold purpose of producing compounds more effective towards either benzodiazepine or adenosine receptor binding by the introduction of appropriate substituents on the quinazoline ring in different positions. Electron-withdrawing groups, such as Cl or NO_2, were used to aid the binding to benzodiazepine receptors in accordance with the theoretical suggestions of the previous paper [1] and literature about benzodiazepine [2, 3]. Amino, cycloalkylamino and arylamino groups were used to aid the adenosine receptor binding in accordance with our previous studies

*Correspondence and reprints

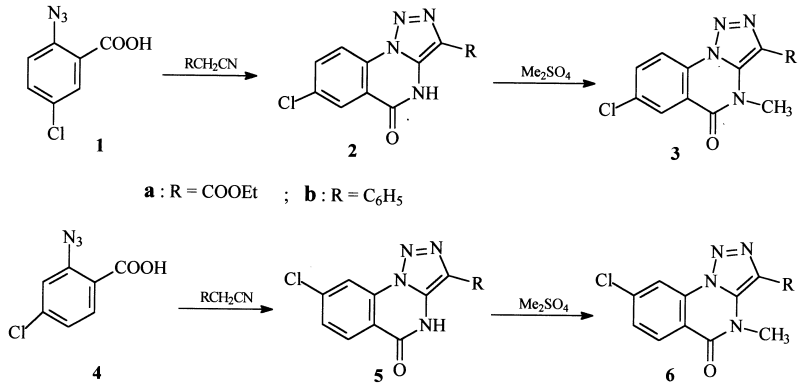


Figure 1. Synthesis of compounds **2**, **3**, **5** and **6**.

[4, 5] and with the substituents present on the common adenosine receptor ligands [6, 7].

2. Chemistry

2-Carboxy-4-chloro-phenylazide **1** and 2-carboxy-5-chloro-phenylazide **4** were prepared starting from the corresponding commercial amines via diazotization and nitrogen displacement by sodium azide [8].

Azides **1** and **4**, by reaction with ethyl cyanacetate in absolute ethanol in the presence of two equivalents of sodium ethoxide at room temperature, afforded the 1-(4- or 5-chloro-2-carboxy-phenyl)-4-ethoxycarbonyl-5-amino-1H-1,2,3-triazole intermediates as sodium salts soluble in the aqueous solution. Acidification of the alkaline solution caused the intramolecular cyclization to give the corresponding tricyclic lactames **2a** and **5a** which precipitated and were isolated in 80% yields (*figure 1*).

Reaction of azide **1** or **4** with phenylacetonitrile required refluxing of the mixture, but it proceeded in the same manner to give the corresponding triazoloquinazoline compounds **2b** and **5b** in high yields (*figure 1*). Methylation of **2a** and **5a** was accomplished with dimethyl sulfate in refluxing butanone in the presence of anhydrous potassium carbonate to give the expected derivatives **3a** and **6a** in good yields; the analogous N-methyl derivatives **3b** and **6b** were prepared in the same manner using DMF at 110 °C (*figure 1*).

The introduction of other substituents on the quinazoline benzofused ring could be achieved via a nitro group and thus the previously prepared compounds 3-ethoxycarbonyl-1,2,3-triazolo[1,5-a]quinazolin-5-one (**7a**) [1] and 3-phenyl-1,2,3-triazolo[1,5-a]quinazolin-5-one (**7b**) [1] underwent nitration (*figure 2*).

Nitration of **7a** with conc. HNO_3 (d = 1.48) in conc. H_2SO_4 at room temperature for 4 h gave a mixture of the mononitro derivatives **8a** and **9a**, substituted in the 7 and 8 positions, respectively, in 60% yields. After crystallization from ethanol, this mixture was found to consist of the isomers **8a** and **9a** in a 70:30 ratio (by ^{1}H-NMR analysis); the subsequent fractionation by flash-chromatography on silica-gel still provided an **8a–9a** mixture in an ≅ 85:15 ratio, which was employed as such. Nitration of **7a** under stronger experimental conditions did not provide a dinitro compound, but gave some demolition products which were not isolated.

On the contrary, nitration of the 3-phenyl-triazoloquinazoline **7b** under the usual mild conditions gave the mononitro derivative **8b** bearing the nitro group in the *para* position of the phenyl substituent. Under stronger experimental conditions (increased equivalents of conc. HNO_3, KNO_3/conc. H_2SO_4, conc. HNO_3 alone, higher temperature and longer reaction time), **8b** lost nitrogen and underwent a rearrangement with formation of essentially the dinitro derivative of the 2-benzoyl-quinazoline **9b**, which was isolated by flash-chromatography; the mononitro derivative **10b** was also characterized. This behaviour had been demonstrated by Tennant [9, 10] for analogous triazoloquinazolines treated with HCl.

The nitrocompound **8b** underwent a chemical reduction with iron powder to obtain the corresponding amine, but under these conditions the hydroxylamino derivative **11b** was isolated (*figure 2*).

Finally, a further functionalization of the triazoloquinazoline system was achieved by the introduction of a chlorine in the 5 position, which was subsequently substituted by an amino group.

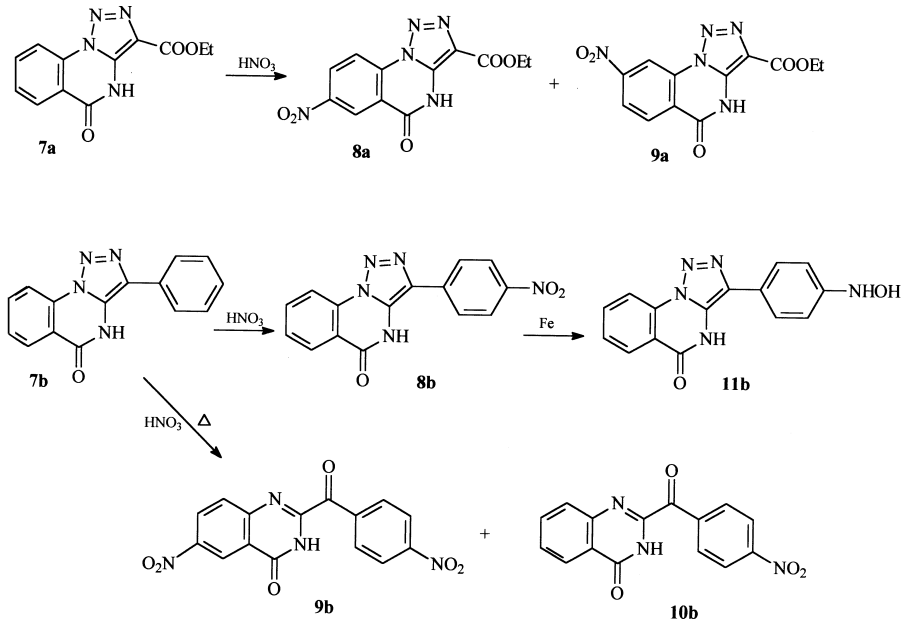


Figure 2. Nitration of **7a** and **7b**.

Thus the 3-ethoxycarbonyl-triazoloquinazoline **7a** [1] and the 3-phenyl-triazoloquinazoline **7b** [1], by reaction with $POCl_3$ or $SOCl_2/CHCl_3$, were converted to the corresponding 5-chloroderivatives **12a** and **12b**, respectively (*figure 3*). Similarly, compound **2b**, bearing a chlorine in the 7 position, provided the dichloro-substituted derivative **14b**, while the analogous 8-chloro-triazoloquinazolines **5a** and **5b** gave the corresponding dichloro derivatives **16a** and **16b** (*figure 3*).

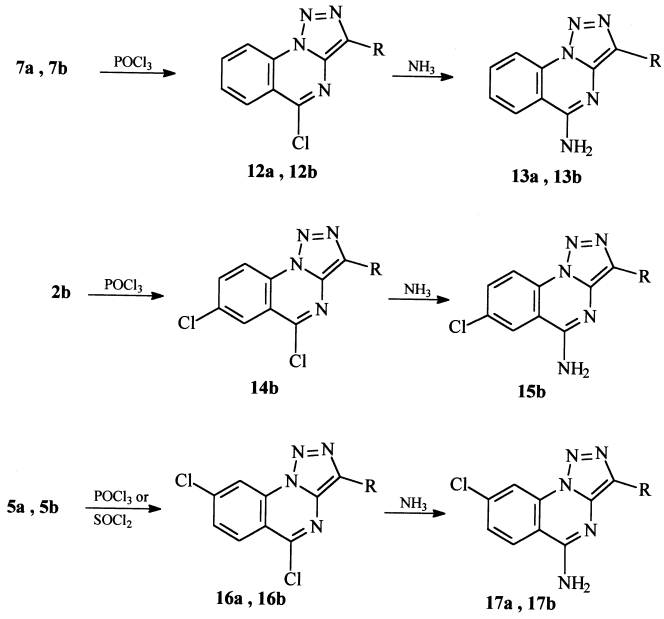


Figure 3. Preparation of the 5-chloro- and 5-amino-triazoloquinazolines **12–17**.

The 5-chloro derivatives, by reaction with NH_3 in a closed tube at 110–120 °C, were converted to the expected 5-amino derivatives **13a**, **13b**, **15b**, **17a** and **17b** in good yields (*figure 3*).

In the same manner, starting from the appropriate chloro derivatives (**12a**, **12b**, **14b** and **16b**), some 5-cyclohexylamino derivatives (**18a**, **18b**, **20b** and **21b**) and 5-(*p*-toluidino) derivatives (**19a** and **19b**) were prepared (*figure 4*).

The structures of all the new compounds were assigned on the basis of the well known reaction mechanisms: 1,3-dipolar cycloaddition of azides to activated methylene compounds, N-methylation, nitration, chlorination and nucleophilic displacement of the halogen by ammonia or primary amines. The structures were also confirmed by analytical and spectroscopic data.

Regarding the position of the nitro groups on the phenyl rings of the nitrocompounds, the AA′BB′ system in the ^{1}H-NMR spectra of compounds **8b**, **9b** and **10b** allowed the assignment of the nitro group in the *para* position of the 3-phenyl substituent. In the spectrum of

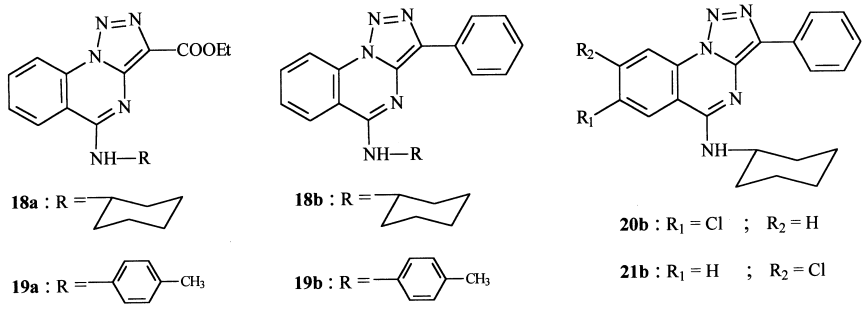


Figure 4. Amino substituted derivatives.

the **8a** and **9a** isomeric mixture, two spin systems, typical of 1,2,4 benzene trisubstitution, clearly indicated the replacement of the 7 and 8 positions. The choice between these positions for the structure assignment to the isomers could appear uncertain if based only on the ^{1}H-NMR data.

On the other hand, in the ^{13}C-NMR spectrum of the mixture, although the complete assignment was difficult, the carbons bearing hydrogen from the major product were easily identifiable by their intensity. Therefore on the basis of the additivity rules [11], structure **8a** was unequivocally assigned to this isomer. ^{1}H-NMR spectral data of some significant compounds are reported in *table I*.

3. Biological results and discussion

All the triazoloquinazoline derivatives underwent binding assays either to benzodiazepine receptors or adenosine A_1 and A_{2A} receptors. Their ability to inhibit benzodiazepine receptor binding was measured by the concentration which was able to displace [^{3}H]-Ro 15–1788 from bovine brain membranes. The inhibition of binding towards adenosine receptors was measured by the ability of compounds to displace [^{3}H]-N^{6}-cyclohexyladenosine (CHA) from A_1 adenosine receptors in bovine cortical membranes and [^{3}H]-2-[p-(2-carboxyethyl)-phenethylamino]-5′-(-N-ethylcarboxamidoadenosine

Table I. ^{1}H-NMR spectral data of some significant compounds.

	3b	**5b**	**7a**	**7b**	**8a**	**8b**	**9a**	**12a**	**12b**	**13a**	**20b**
H-6	8.17	8.21	8.33	8.33	8.84	8.34	8.04	8.71	8.62	8.30	8.65
H-7	–	7.69	8.01	7.97	–	8.00	8.58	8.28	8.19	–	–
H-8	8.10	–	7.71	7.66	8.74	7.69	–	7.98	7.90	7.73	7.93
H-9	8.52	8.31	8.24	8.22	8.52	8.23	8.93	8.43	8.34	8.21	8.38
$J_{6,7}$	–	8.52	8.18	7.17	–	8.10	9.17	8.36	8.34	–	–
$J_{6,8}$	2.34	–	1.12	1.10	2.57	1.00	–	1.14	1.08	1.97	2.16
$J_{7,8}$	–	–	7.43	7.64	–	7.54	–	7.34	7.27	–	–
$J_{7,9}$	–	1.98	1.53	1.48	–	1.38	2.64	1.33	1.30	–	–
$J_{8,9}$	8.80	–	7.96	7.92	8.96	8.22	–	8.25	8.25	8.60	8.86
3-Phenyl											
H-2′	8.28	7.87		7.88		8.16			8.25		8.28
H-3′	7.51	7.48		7.43		8.27			7.54		7.44
H-4′	7.36	7.35		7.35		–			7.41		7.26
3-Ethoxycarbonyl											
CH_2			4.40		4.41		4.45	4.45		4.39	
CH_3			1.36		1.36		1.40	1.39		1.22	

Other signals: **3b**, 4.25 δ N–CH_3; **20b**, 2.15–1.33 δ, cyclohexyl. *Para J* coupling was also detectable in the spectrum of **3b** (0.55 Hz).

(CGS-21680) from A_{2A} adenosine receptors in bovine striatal membranes.

The experimental details of the receptor binding assays are reported in a previous paper [12].

The results of the binding assays towards benzodiazepine receptors have not been tabulated because the introduction of a chlorine atom in the 7 or 8 position of the 1,2,3-triazolo[1,5-a]quinazoline ring caused a marked decrease in receptor affinity, contrary to theoretical suggestions [1]. 3-Ethoxycarbonyl-4-methyl-7-chloro-1,2,3-triazolo[1,5-a]quinazolin-5-one (**3a**) was the most active compound with an inhibition constant Ki = 740 nM and a GABA ratio = 1.9; all the other derivatives showed inhibition percentages so low that the corresponding Ki values were not calculated.

Regarding adenosine receptors, biological evaluation of the previously reported triazoloquinazoline derivatives [1] showed a moderate affinity towards A_1 adenosine receptors of the 3-phenyl derivative **7b** (Ki = 620 nM), followed by the 3-ethoxycarbonyl-4-methyl-1,2,3-triazolo[1,5-a]quinazolin-5-one (Ki = 1 500 nM) [1]. The results of the biological evaluation of the new triazoloquinazoline derivatives are reported in *table II*, expressed as affinity constants towards A_1 and A_{2A} adenosine receptors. Some of these new compounds showed an increased binding affinity towards A_1 adenosine receptors with a marked selectivity, but their Ki values remained higher than 100 nM and therefore their biological activity was moderate. The most active triazoloquinazolin-5-one derivatives were **5b** (Ki = 148 nM) and **6b** (Ki = 264 nM), bearing in the 3 position a phenyl substituent and in the 8 position a chlorine atom, respectively. Compound **5b** can be compared with 9-chloro-2-(2-furyl)-1,2,4-triazolo[1,5-c]quinazolin-5-amine (CGS 15943) [13], which is an effective A_1 adenosine antagonist. The comparison shows that the main differences are the position of the phenyl ring with regard to the furyl substituent which, by steric hindrance, would reduce the receptor binding of the amidic NH, in turn less capable of binding than the free NH_2 group. This structural difference could explain while **5b** fits into the A_1 adenosine receptor site with a difficulty greater than CGS 15943. The most active 5-amino substituted derivatives were **13b** (Ki = 239 nM) and **18a** (Ki = 302 nM), the first bearing a phenyl substituent and an amino group in the 3 and 5 positions, respectively, and the second derivative bearing an ethoxycarbonyl group combined with a cyclohexylamino substituent in the same positions. In conclusion, these triazoloquinazoline derivatives were found to be biologically much less effective than the corresponding 1,2,3-triazolo[1,5-a]quinoxaline derivatives previously experimented [12].

4. Experimental protocols

4.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra in nujol mulls were recorded on a Perkin-Elmer Mod.1310 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker AC 200 spectrometer in δ units from TMS as an internal standard. Mass spectra were performed with a Hewlett Packard MS/System 5988. Elemental analyses (C, H, N) were within ± 0.4% of theoretical values and were performed on a Carlo Erba Elemental Analyzer Mod. 1106 apparatus. Column chromatographies were performed on Silica gel 60 (230–400 mesh). Petroleum ether corresponds to the fraction boiling at 40–60 °C.

4.1.1. 2-Carboxy-4-chloro-phenylazide **1**

To a stirred and cooled (0–5 °C) solution of 2-amino-5-chloro-benzoic acid (0.400 g, 2.34 mmol) in 25 mL of 18% HCl, 10 mL of aqueous solution of $NaNO_2$ (0.230 g, 3.33 mmol) were added dropwise. After 15–20 min 10 mL of a solution of NaN_3 (0.217 g, 3.33 mmol) were added dropwise and **1a** began to precipitate as a white solid. The acidity was lowered (pH 3–4) by adding 32% NH_4OH solution, the ice-bath was removed and the mixture was stirred at room temperature for 1 h. The precipitate was collected by filtration and washed with H_2O (*table III*).

4.1.2. 2-Carboxy-5-chloro-phenylazide **4**

To a stirred and cooled (0–5 °C) solution of 2-amino-4-chloro-benzoic acid (0.500 g, 3.0 mmol) in 30 mL of 18% HCl, 10 mL of aqueous solution of $NaNO_2$ (0.270 g, 3.91 mmol) were added dropwise. After 15–20 min, 12 mL of a solution of NaN_3 (0.250 g, 3.84 mmol) were added and the mixture was worked up as described above (*table III*).

4.1.3. 3-Ethoxycarbonyl-7-chloro-1,2,3-triazolo[1,5-a]-quinazolin-5-one **2a** *and 3-ethoxycarbonyl-8-chloro-1,2,3-triazolo[1,5-a]quinazolin-5-one* **5a**

To a stirred solution of EtONa (0.300 g, 13 mmol of Na in 20 mL of absolute EtOH), 0.75 mL (7.0 mmol) of ethyl cyanacetate were added. After 15 min a solution of **1a** or **1b** (1.15 g, 5.82 mmol) in 35 mL of absolute EtOH was slowly added (≈ 1 h). The suspension was stirred at room temperature for ≈ 20 h, then the precipitate was collected by filtration. The solid was dissolved in 50 mL of H_2O and the solution was acidified (0–5 °C) with 36% HCl (pH = 2) to precipitate the title compounds, which were collected by filtration and washed with H_2O (*table III*).

Table II. Binding to A_1 and A_{2A} adenosine receptors.

	A_1		A_{2A}	
Compound	Inhib. % 10 μM	Ki values (nM)	Inhib. % 10 μM	Ki values (nM)
7a[1]	8	> 10 000	3	> 10 000
7b[1]	87	620 ± 29	28	> 10 000
2a	16	> 10 000	0	–
3a	63	2 269 ± 129	12	> 10 000
5a	23	> 10 000	4	> 10 000
6a	81	808 ± 91	30	> 10 000
2b	2	> 10 000	0	–
3b	6.4	> 10 000	0	–
5b	97	148 ± 16	9	> 10 000
6b	96	264 ± 23	19	> 10 000
8a + **9a**	25	> 10 000	10	> 10 000
8b	30	> 10 000	8	> 10 000
11b	75	1 146 ± 130	14.5	> 10 000
13b	97	239	4	> 10 000
15b	76	900 ± 102	66	1 667 ± 118
17a	78	1 093 ± 148	30	> 10 000
18a	95	302 ± 59	8.5	> 10 000
18b	39	> 10 000	36	> 10 000
19a	74	1 502 ± 110	13.6	> 10 000
19b	69	1 518 ± 147	14	> 10 000
20b	74	1 244 ± 134	6.5	> 10 000
21b	10	> 10 000	9	> 10 000

–: Ki value was not calculated.

4.1.4. 3-Phenyl-7-chloro-1,2,3-triazolo[1,5-a]quinazolin-5-one **2b** *and 3-phenyl-8-chloro-1,2,3-triazolo[1,5-a]-quinazolin-5-one* **5b**

To a stirred solution of EtONa (0.500 g, 21.7 mmol of Na in 15 mL of absolute EtOH), 1.10 mL (9.53 mmol) of phenylacetonitrile was added. After 20 min a solution of **1a** or **1b** (1.20 g, 6.10 mmol) in 50 mL of absolute EtOH was slowly added (≈ 40 min), then the mixture was heated under reflux for 20 h. The reaction mixture was concentrated in vacuo, treated with H_2O and extracted with $CHCl_3$. **2b** precipitated from the aqueous layer by acidification (pH = 2) with 36% HCl, and was collected by filtration and washed with H_2O (*table III*). Compound **5b** precipitated partially, and was consequently recovered from the acid mother liquors by extraction with $CHCl_3$ and evaporation of the solvent (*table III*).

4.1.5. 3-Ethoxycarbonyl-4-methyl-7-chloro-1,2,3-triazolo[1,5-a]quinazolin-5-one **3a** *and 3-ethoxycarbonyl-4- methyl-8-chloro-1,2,3-triazolo[1,5-a]quinazolin-5-one* **6a**

A mixture of **2a** or **5b** (0.300 g, 1.02 mmol), anhydrous K_2CO_3 (0.425 g, 3.0 mmol) and Me_2SO_4 (0.6 mL, 6.30 mmol) in 40 mL of MeCOEt was heated under reflux for 4 h. After 1 night, the reaction mixture was concentrated in vacuo and the residue was treated with 10% NaOH and extracted with $CHCl_3$. The organic layer, dried ($MgSO_4$) and evaporated, gave the crude title compounds (*table III*).

4.1.6. 3-Phenyl-4-methyl-7-chloro-1,2,3-triazolo[1,5-a]-quinazolin-5-one **3b** *and 3-phenyl-4-methyl-8-chloro-1,2,3-triazolo[1,5-a]quinazolin-5-one* **6b**

A mixture of **3a** or **3b** (0.450 g, 1.51 mmol), anhydrous K_2CO_3 (0.625 g, 4.52 mmol) and Me_2SO_4 (0.9 mL, 9.45 mmol) in 20 mL of anhydrous DMF was heated at 110 °C under stirring for 4 h. After 1 night the reaction mixture was concentrated in vacuo and the residue was treated with H_2O to give the crude title compounds as precipitates which were collected by filtration (*table III*).

4.1.7. Nitration of 3-ethoxycarbonyl-1,2,3-triazolo-[1,5-a]quinazolin-5-one **7a***: mixture of* **8a** *and* **9a**

A solution of conc. HNO_3 (d = 1.48, 0.25 mL, 5.3 mmol) and conc. H_2SO_4 (0.5 mL) was added dropwise to an ice-cooled (0–5 °C) and stirred solution of **7a** (0.600 g, 2.32 mmol) in 5 mL of conc. H_2SO_4 (≈ 15 min.). The ice-bath was removed and the mixture was stirred at room temperature for 4 h, and then poured into crushed ice. Addition of conc. NH_4OH (3–4 mL, pH

Table III. Physico-chemical data of azides and triazoloquinazoline derivatives.

Compound	Yield %	Crystall. solvent	M.p. °C	Analysis (C, H, N)	Mass m/z M^+	Base
1	92	EtOH-H_2O	151–153	$C_7H_4N_3O_2Cl$	197	78
4	89	EtOH	139–142	$C_7H_4N_3O_2Cl$	197	78
2a	82	EtOH	239–241	$C_{12}H_8N_4O_3Cl$	292	179
5a	80	EtOH	214–217	$C_{12}H_8N_4O_3Cl$	292	179
2b	82	EtOH-H_2O	255–258	$C_{15}H_9N_4OCl$	296	75
5b	90	EtOH-H_2O	236–240	$C_{15}H_9N_4OCl$	268	75
3a	80	EtOH	178–180	$C_{13}H_{11}N_4O_3Cl$	306	193
6a	88	EtOH	182–185	$C_{13}H_{11}N_4O_3Cl$	306	193
3b	82	DMF-H_2O	240–243	$C_{16}H_{11}N_4OCl$	310	164
6b	80	DMF-H_2O	214–219	$C_{16}H_{11}N_4OCl$	310	164
8b	78	AcOEt	271–274	$C_{15}H_9N_5O_3$	307	76
11b	40	EtOH	259–262	$C_{15}H_{11}N_5O_2$	293	106
12a	53	EtOH	160–164	$C_{12}H_9N_4O_2Cl$	276	163
12b	81	AcOEt	178–180	$C_{15}H_9N_4Cl$	280	114
14b	71	AcOEt	224–226	$C_{15}H_8N_4Cl_2$	314	148
16a	19	*	204–207	$C_{15}H_8N_4O_2Cl_2$	310	29
16b	76	100–140 °C Petr. eth	219–220	$C_{15}H_8N_4Cl_2$	314	148
13a	48	AcOEt	217–222	$C_{12}H_{11}N_5O_2$	258	148
13b	71	AcOEt	226–230	$C_{15}H_{11}N_5$	261	103
15b	96	AcOEt	248–250	$C_{15}H_{10}N_5Cl$	295	137
17a	49	AcOEt	195–198	$C_{12}H_{10}N_5O_2Cl$	292	179
17b	45	*	241–243	$C_{15}H_{10}N_5Cl$	295	137
18a	81	AcOEt	229–233	$C_{18}H_{21}N_5O_2$	339	179
19a	65	AcOEt	225–228	$C_{19}H_{17}N_5O_2$	347	234
18b	87	AcOEt	226–227	$C_{21}H_{21}N_5$	343	233
19b	76	MeOH	213–216	$C_{21}H_{17}N_5$	351	308
20b	78	MeOH	221–224	$C_{21}H_{20}N_5Cl$	377	267
21b	55	MeOH	226–228	$C_{21}H_{20}N_5Cl$	349 (M^+–28)	55

*Isolated by flash-chromatography.

1–2) and vigorous scratching made it possible to separate a yellow solid which was collected by filtration after 1 night. This solid (0.434 g, yield 62%, TLC AcOEt/Petr. eth. 2:1 one large spot) crystallized from 95% EtOH and provided a solid mixture with m.p. = 203–210 °C, Ms *m/e* 303 (M^+). Anal. $C_{12}H_9N_5O_5$ (C, H, N). ^{1}H-NMR see *table I*. ^{13}C-NMR **7a**, δ 134.8 (C-8), 127.7 (C-6), 127.6 (C-7), 114.5 (C-9). **8a** δ 129.2 (C-8), 123.1 (C-6), 114.5 (C-9).

The mixture consisted of the mononitro derivatives **8a** and **9a** in a ratio 70:30. Further fractionation by flash-chromatography under an elution gradient (AcOEt/Petr. eth. 1:2, 1:1, 2:1) again provided a mixture of **8a** and **9a** in a ratio 85:15.

4.1.8. 3-(4- Nitrophenyl)-1,2,3-triazolo[1,5-a]-quinazolin-5-one **8b**

A solution of conc. HNO_3 (d = 1.48, 0.20 mL, 4.2 mmol) and conc. H_2SO_4 (0.4 mL) was added drop by drop to an ice-cooled (0–5 °C) and stirred solution of 3-phenyl-1,2,3-triazolo[1,5-a]quinazolin-5-one **7b** (0.500 g, 1.90 mmol) in 5 mL of conc. H_2SO_4 (≈ 5 min). The ice-bath was removed and the mixture was stirred at room temperature for 4 h. The reaction mixture was poured into crushed ice, the pH was increased (pH 3–4) by the addition of conc. NH_4OH, and by vigorous scratching **8b** precipitated as a yellow solid which was collected by filtration (0.380 g). A further fraction (0.080 g) was obtained from the filtrate by extraction with AcOEt and evaporation of the solvent (*table III*).

When the nitration was carried out with an increased amount of conc. HNO_3 or an increased volume of conc. H_2SO_4, as well as with KNO_3/conc. H_2SO_4 or conc. HNO_3 alone, a mixture of products was obtained. Fractionation of the mixture by flash-chromatography on silica gel eluting with AcOEt/Petr. eth. 1:1 and 2:1 gave principally the dinitro derivative of the 2-benzoyl-quinazoline **9b**. The mononitro derivative **10b** was also characterized. **9b**: m.p. = 235–237 °C (EtOH); Ms *m/e*

340 (M^+), 150 (base). Anal. $C_{15}H_8N_4O_6$ (C, H, N). ^{1}H-NMR (DMSO): δ 8.86 (d, 1H, *J* = 2.7 Hz, H-5); 8.56 (dd, 1H, *J* = 8.9 and 2.7 Hz, H-7); 8.42 and 8.37 (AA′BB′ system, 4H, H-2′ and H-3′); 7.97 (d, 1H, *J* = 8.9 Hz, H-8). **10b:** m.p. = 239–241 °C (EtOH/H_2O); Ms *m/e* 295 (M^+), 90 (base). Anal. $C_{15}H_9N_3O_4$ (C, H, N). ^{1}H-NMR (DMSO): δ 8.43 and 8.36 (AA′BB′ system, 4H, H-2′ and H-3′); 8.23 (dd, 1H, *J* = 6.9 and 1.3 Hz, H-5); 7.90 (ddd, 1H, *J* = 8.0, 7.3 and 1.3 Hz, H-7); 7.79 (dd, 1H, *J* = 7.3 and 1.0 Hz, H-8); 7.68 (ddd, 1H, *J* = 6.9, 8.0, and 1.0 Hz, H-6)

4.1.9. 3-(4-Hydroxylaminophenyl)-1,2,3-triazolo[1,5-a]quinazolin-5-one **11b**

Iron powder (0.200 g) was added to a solution of **8b** (0.300 g, 0.97 mmol) in 60 mL of AcOH and the mixture was heated under reflux. Four more 0.05 g portions of iron powder were added, one every hour, and heating was continued for 18 h. The mixture was filtered, concentrated (1/4 volume) in vacuo, neutralized with 10% NaOH and extracted with $CHCl_3$. The organic layer, after washing (H_2O) and drying, was evaporated to give **11d** (*table III*).

4.1.10. 3-Ethoxycarbonyl-5-chloro-1,2,3-triazolo[1,5-a]-quinazoline **12a**, *3-phenyl -5-chloro-1,2,3-triazolo-[1,5-a]quinazoline* **12b**, *3-phenyl-5,7-dichloro-1,2,3-triazolo[1,5-a]quinazoline* **14b** *and 3-ethoxycarbonyl-5,8-dichloro-1,2,3-triazolo[1,5-a]quinazoline* **16a**

A solution of 1.75 mmol of the appropriate triazoloquinazolin-5-one (**7a**, **7b**, **2b** and **5a**) in 12 mL of TEA was cooled in an ice-bath (0–5 °C), 8 mL of $POCl_3$ were added and the mixture was heated under reflux for 12 h. After cooling, the reaction mixture was poured into crushed ice and quickly extracted with $CHCl_3$. For the isolation of **12a** the chloroform extract was washed with H_2O, dried and evaporated (*table III*). For the isolation of **12b**, the chloroform extract was washed with 6% $NaHCO_3$, H_2O, dried and evaporated (*table III*). The chloroform extract of **14b** was washed with 10% NaOH, H_2O, dried and evaporated (*table III*); by acidification of the alkaline solution, 25% of the starting product **2b** was recovered. The chloroform extract of **16a** was washed with H_2O, dried and evaporated to give a residue which consisted of a mixture. This residue, dissolved in toluene, was fractionated by flash-chromatography through silica gel, eluting with AcOEt/Petr. eth. 3:1. After a small amount of an unidentified product was discarded, **16a** was eluted (*table III*).

4.1.11. 3-Phenyl–5,8-dichloro-1,2,3-triazolo[1,5-a]-quinazoline **16b**

To a suspension of 0.520 g (1.75 mmol) of **5b** in 24 mL of boiling anhydrous $CHCl_3$, 1 mL of anhydrous DMF and 1.5 mL of $SOCl_2$ were added. The reaction mixture was refluxed for 2 h, the solvent was evaporated in vacuo and the semi-solid residue, after cooling at 0 °C, was triturated with acetone to give **16b** as a yellow solid which was collected by filtration (*table III*).

4.1.12. 3-Ethoxycarbonyl-5-amino-1,2,3-triazolo[1,5-a]-quinazoline **13a**, *3-phenyl-5-amino-1,2,3-triazolo-[1,5-a]quinazoline* **13b**, *3-phenyl-5-amino-7-chloro-1,2,3-triazolo[1,5-a]quinazoline* **15b**, *3-ethoxycarbonyl-5-amino-8-chloro-1,2,3-triazolo [1,5-a]quinazoline***17a** *and 3-phenyl-5-amino-8-chloro-1,2,3-triazolo[1,5-a]quinazoline* **17b**

A solution of 1.0 mmol of the suitable chloro derivative (**12a**, **12b**, **14b**, **16a** or **16b**) in 10 mL of anhydrous DMF and 3 mL of TEA was introduced into a 'Sovirel' vial. The vial was cooled (ice-bath 0–5 °C) and the solution was saturated with NH_3 gas for 10–15 min. The vial was quickly closed and heated in an oil bath at 120 °C for 10 h. The reaction mixture was evaporated in vacuo, the residue was treated with H_2O and the insoluble material, consisting of the title compounds (**13a**, **13b**, **15b** or **17a**), was collected by filtration (*table III*). Compound **17b** was obtained as a mixture with an unidentified compound, and as a result it was isolated by flash-chromatography on silica gel. Elution with AcOEt/Petr. eth. 5:1 provided **17a** (*table III*) which was followed by the unidentified product.

4.1.13. 3-Ethoxycarbonyl-5-(p-toluidino)-1,2,3-triazolo[1,5-a]quinazoline **19a** *and 3-phenyl-5-(p-toluidino)-1,2,3-triazolo[1,5-a]quinazoline* **19b**

To a solution of 0.70 mmol of the appropriate chloro-derivative (**12a** or **12b**) in 2 mL of anhydrous DMF and 4 mL of TEA in a closed tube, 0.500 g (4.66 mmol) of *p*-toluidine were added and the mixture was heated at 120 °C for 18 h. The reaction mixture was concentrated in vacuo and the residue was treated with H_2O and extracted with $CHCl_3$. The chloroform layer was washed with 10% HCl and H_2O, dried and evaporated to give the title compounds (*table III*).

4.1.14. 3-Ethoxycarbonyl-5-(cyclohexylamino)-1,2,3-triazolo[1,5-a]quinazoline **18a**, *3-phenyl-5-(cyclohexylamino)-1,2,3-triazolo[1,5-a]quinazoline* **18b** *and 3-phenyl-5-(cyclohexylamino)-7-chloro-1,2,3-triazolo[1,5-a]quinazoline* **20b**

A solution of 0.70 mmol of the appropriate chloro-derivative (**12a**, **12b** or **14b**) in 2 mL of anhydrous DMF,

2 mL of TEA and 0.5 mL (4.40 mmol) of cyclohexylamine in a closed tube was heated at 120 °C for 12 h. The reaction mixture was concentrated in vacuo and worked up as described above to obtain the title compounds (*table III*).

4.1.15. 3-Phenyl-5-(cyclohexylamino)-8-chloro-1,2,3-triazolo[1,5-a]quinazoline **21b**

A solution of 0.300 g (0.96 mmol) of **16b** in 1.5 mL of anhydrous DMF, 3 mL of TEA and 2 mL (17.5 mmol) of cyclohexylamine in a closed tube was heated at 120 °C for 12 h. The reaction mixture was concentrated in vacuo and the residue was extracted with $CHCl_3$. The chloroform solution was washed with 5% HCl and H_2O, dried and evaporated to give a semisolid residue consisting of a mixture which was fractionated by flash-chromatography. Elution with AcOEt/Petr. eth. 2:1 provided the title compound (*table III*) which was followed by an unidentified compound.

References

[1] Biagi G., Giorgi I., Scartoni V., Velo S., De Santis B., Martinelli A., Lucacchini A., Senatore G., Il Farmaco 51 (1996) 131–136.

[2] Haefely W.E., Kyburz E., Gerecke M., Mohler H.P., Adv. Drug Res. 14 (1985) 166–322.

[3] Zhang W., Koehler K.F., Zhang P., Cook J.M., Drug Des. Discovery 12 (1995) 193–248.

[4] Biagi G., Giorgi I., Livi O., Manera C., Scartoni V., Lucacchini A., Senatore G., Il Farmaco 51 (1996) 601–608.

[5] Betti L., Biagi G., Giannaccini G., Giorgi I., Livi O., Lucacchini A., Manera C., Scartoni V., J. Med. Chem. 41 (1998) 668–673.

[6] Jacobson K.J., van Galen P.J.M., Williams M., J. Med. Chem. 35 (1992) 407–422.

[7] Baraldi P.G., Cacciari B., Spalluto G., Brioni A., Viziano M., Dionisotti S., Ongini E., Curr. Med. Chem. 2 (1995) 707–722.

[8] Biffin M.E.C., Miller J., Paul D.B., in: Patai S. (Ed.), The Chemistry of the Azido Group, Interscience Publishers, 1971, pp. 57–190.

[9] Tennant G., J. Chem. Soc. C (1966) 2290–2295.

[10] Sutherland D.R., Tennant G., J. Chem. Soc. Perkin Trans. I (1974) 534–540.

[11] Wehrli F.W., Marchand A.P., Wehrli S., Interpretation of Carbon-13NMR Spectra, 2nd. ed., Wiley, NY, 1988.

[12] Bertelli L., Biagi G., Giorgi I., Manera C., Livi O., Scartoni V. et al., Eur. J. Med. Chem. 33 (1998) 113–122.

[13] Francis J.E., Cash W.D., Psychoyos S., Ghai G., Wenk P., Friedmann R.C. et al., J. Med. Chem. 31 (1988) 1014–1020.