Synthesis and Characterization of some *N*-Mannich Bases of [1,2,3]Triazoloquinolines

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Some novel *N*-Mannich bases of [1,2,3]-triazolo[4,5-*f*] and [4,5-*h*]quinolines were synthesized following the classical experimental procedure for Mannich base preparation: triazoloquinoline with formaldehyde and secondary amine. Tricyclic nuclei were obtained starting from two protected isomeric amino-quinolines, which were nitrated, reduced and diazotated. Two *N*-anilinomethyltriazoloquinolines were also synthesized *via* the *N*-hydroxymethyl intermediate.

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In the field of synthesis of substituted pyrroloquinolines as biologically active compounds, our recent good results [1,2] prompted us to continue research, moving towards relatively new, similar polyheterocyclic [1,2,3]triazolo[4,5-*f*]quinolines, which have been proposed as potential antimicrobial and anticancer drugs [3], due to their isosteric nature in comparison with other known bioactive azoloquinolines [4].

Our interest in the preparation of some *N*-Mannich bases of [1,2,3]triazoloquinolines arose from continued studies on the chemical and pharmacological properties of both benzazoles [4] and heterocyclic Mannich bases [5].

The aims of the present work were the synthesis, characterization and study of the probable biological activity of some novel *N*-Mannich bases, prepared by the Mannich reaction between triazoloquinolines having a reactive

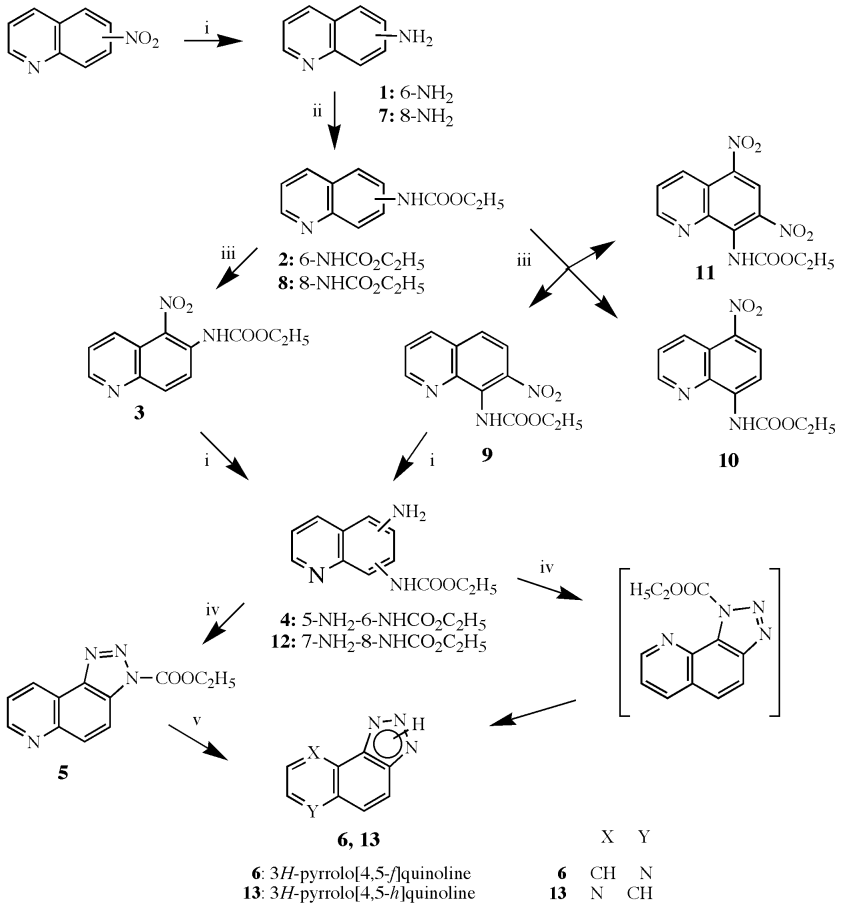


Figure 1. Reagents and conditions: i: H2, 5% Pd/C, ethanol, rt, 1-2h; ii: $ClCOOC_2H_5$, THF, rt, 4-5h; iii: HNO_3/ H_2SO_4, 0 °C, 30'; iv: $NaNO_2/CH_3COOH$, 0 °C, 4-7h; v: 2 *N* NaOH, 1h.

hydrogen atom, formaldehyde, and either a secondary dialkyl or a primary aryl amine.

We examined [1,2,3]triazolo[4,5-*f*]quinoline **6** and [1,2,3]triazolo[4,5-*h*]quinoline **13**, prepared following a well-established method, starting from *o*-diaminoquinolines **14** and **12** *via* a diazotization reaction with simultaneous intramolecular cyclization. As primary aryl amine for the synthesis of **19** and **25**, we chose an aniline derivative, known on the analogy of our previous compounds showing antiproliferative activity [1,2]. The bioactivity of compounds showing suitable physico-chemical characteristics is being assessed. Preliminary results of their antiproliferative activity against various cell lines are reported.

The key intermediates for the synthesis of [1,2,3]triazolo[4,5-*f*]quinoline **6** and [1,2,3]triazolo[4,5-*h*]quinoline **13** (Figure 1) were (5-aminoquinolin-6-yl)-carbamic acid ethyl ester **4** and (7-aminoquinolin-8-yl)-carbamic acid ethyl ester **12**. The known syntheses of **6** and **13**, starting from the appropiate nitroquinolines **3** and **9**, followed multistep synthetic pathways consisting of the same classical reactions. Hence, commercial materials were catalytically reduced by hydrogen at atmospheric pressure and room temperature to aminoderivatives **1** and **7**, which were then protected before being nitrated by a nitric and sulfuric acid mixture. A carbamate was chosen as protecting group, due to the mild conditions required for its formation by ethyl chloroformate and its removal by either refluxing in a dilute sodium hydroxide water solution or by adding water only (see experimental section). Compounds **2** and **8** were obtained in this way and then submitted to nitration. Only one nitroderivative (**3**) was formed from nitration of compound **2**, whereas the mono nitroproducts **9**, **10** and the dinitro product **11** were isolated from the nitration mixture of **8**. Compounds **9** and **10** were isolated by flash chromatography of the precipitate formed after cooling of the reaction mixture, and bis-nitrated **11** by alkalinization of the filtrate. Compounds **9** and **10** were identified by TLC analysis and ^{1}H and ^{13}C nmr spectroscopy. Rf values and eperimental chemical shifts (Table 1) agree with the expected and calculated values for similar isomeric compounds respectively. The ^{1}H nmr spectrum of **11** showed a singlet at 8.96 δ due to the HC-6, while the ^{1}H-nmr spectra of **9** and **10** showed two doublets with the same *ortho*-J coupling due to an A-B system:7.89 and 8.03 δ J=9Hz for HC-5 and HC-6 of **9**, and 8.51 and 8.6 δ, J=8.8Hz for HC-7 and HC-8 of **10**. Uncoupled ^{13}C-nmr showed signals at 123 (C-5) and 124.3 δ (C-6) for **9** and 115 (C-7) and 129 δ (C-6) for **10**. The desired mono-nitrated compound **9** was obtained in a 30% yield. Successively, **3** and **9,** after their reduction to *o*-aminoderivatives **4** and **12**, underwent diazotization by sodium nitrite in dilute acetic acid and simultaneous intramolecular cyclization to triazoloquinolines. [1,2,3]Triazolo[4,5-*f*]quinoline-3-carboxylic acid ethyl ester **5** was separated and had to be treated with alkaline water in order to obtain **6**. In the case of the isomeric triazolo[4,5-*h*]quinoline, unsubstituted compound **13** was directly separated by diluting

Table 1

Physical and Spectral Data of Compounds **2** and **8-11**

Compound	Molecular formula	mw	Yield %	mp °C	Solvent [a]	Rf [b]	^{1}H nmr [c]	^{13}C nmr [d]
2	$C_{12}H_{12}N_2O_2$	216.24	95	169-70	A	0.46	1.26 (t, 3H, J=7.2Hz, CH_3), 4.2 (q, 2 H, J=7.1Hz, CH_2), 7.98 (dd, 1H, J=5.3 and 8.4Hz, HC-3), 8.09 (dd, 1H, J=2.2 and 9.2Hz, HC-7), 8.38 (d, 1H, J=9.2Hz, HC-8), 8.44 (d, 1H, J=2.2Hz, HC-5), 9.07 (m, 2H, HC-2 and HC-4), 10.46 (s, 1H, NH)	
8	$C_{12}H_{12}N_2O_2$	216.24	83	68-70	A	0.70	1.34 (t, 3H, J=7.1 Hz, CH_3), 4.28 (q, 2H, J=7.1Hz, CH_2), 7.64 and 7.53 (m, 3H, HC-3, HC-5 and HC-7), 8.46 and 8.34 (m, 2H, HC-4 and HC-6), 8.88 (dd, 1H, J=1.5 and 4.2Hz, HC-2), 9.17 (bs, 1H, NH)	
9	$C_{12}H_{11}N_3O_4$	261.34	40	116	A	0.73	1.31 (t, 3H, J=7.1Hz, CH_3), 4.23 (q, 2H, J=7.1Hz, CH_2), 7.81 (dd, 1H, J=4.2 and 8.3Hz, HC-3), 7.89 (d, 1H, J=9.1Hz, HC-5), 8.03 (d, 1H, J=9.1Hz, HC-6), 8.54 (dd, 1H, J=1.7 and 8.4Hz, HC-4), 9.07 (dd, 1H, J=1.6 and 4.2Hz, HC-2), 9.26 (bs, 1H, NH)	14 (CH_3) 63 (CH_2), 123 (C-5), 124.3 (C-6), 126 (C-3), 139 (C-4), 152 (C-2), 206 (C=O)
10	$C_{12}H_{11}N_3O_4$	261.34	50	108-10	A	0.60	1.37 (t, 3H, J=7.1Hz, CH_3), 4.15 (q, 2H, J=7.1Hz, CH_2), 7.95 (dd, 1H, J=4.3 and 8.7Hz, HC-3), 8.51 (d, 1H, J=8.8Hz, HC-7), 8.61 (d, 1H, J=8.8Hz, HC-6), 9.02 (dd, 1H, J=1.6 and 4.2Hz, HC-2), 9.21 (dd, 1H, J=1.5 and 8.8Hz, HC-4), 9.56 (bs, 1H, NH)	14 (CH_3) 63 (CH_2), 115 (C-7), 126 (C-3), 129 (C-6), 134 (C-4), 151 (C-2), 206 (C=O)
11	$C_{12}H_{10}N_4O_6$	306.34	20	158-60	B	0.40	1.34 (t, 3H, J=71Hz, CH_3), 4.29 (q, 2H, J=7.1Hz, CH_2), 8.1 (dd, 1H, J=4.3 and 8.8Hz, HC-3), 8.96 (s, 1H, HC-6), 9.21 (dd, 1H, J=1.5 and 4.3Hz, HC-2), 9.25 (dd, 4.3 and 8.8Hz, HC-4), 9.85 (bs, 1H, NH)	

[a] A=ethyl alcohol, B=methyl alcohol; [b] Eluant: ethyl acetate/*n*-hesane 70:30 (TLC); [c] Solvent: hexadeutero-dimethylsulfoxide (**2**) and hexadeutero-acetone (**8-11**); [d] Solvent: hexadeutero-acetone.

with water. Compounds **6** and **13** were obtained in moderate to good overall yields of 60% and 20%, respectively. This difference was mainly attributed to the formation of three nitrated products in the nitration step of **8**. This drawback was partly compensated, because **13** was obtained directly during diazotization of **12**. Unlike the ^{1}H nmr spectrum of compound **6,** which seemed to be due to only one of three isomers 1*H*-, 2*H*- and 3*H*-[1,2,3]triazolo[4,5-*f*]quinoline (see experimental), the ^{13}C nmr spectrum showed two isomers, as there were double signals for C-4 (123.54 and 123.19 δ), C-9 (131.31 and 131.4 δ), and C-7 (151.03 and 150.63 δ). So far, these isomers have not been identified. Instead, the ^{13}C nmr spectrum of **13** indicated only one isomeric form (see experimental).

In previous works, by a different method than ours, triazolo[4,5-*f*]quinoline **6** was prepared starting from benzotriazole amino-derivatives *via* a Gould-Jacobs reaction [11-13], and triazole[4,5-*h*]quinoline **13** by a laborious procedure starting from 5,7-diamino-6-*p*-toluensulfonamidoquinoline with nitrous acid [10]. These syntheses resulted in poor yields and, particularly for **13**, were characterized with very little chemical-physical data.

The aminoalkylation reaction of **6** and **13** leads to Mannich bases of isomeric triazoloquinolines **14-17** and **20-23** with formaldehyde and acyclic or cyclic dialkylamines RH in a 1:1:1 ratio in methanol at room temperature. *N*-Arylaminomethyl-derivatives **19** and **25** were prepared *via N*-hydroxymethyl compounds **18** and **24** (Figure 2).

The synthesis, isolation and purification procedures for the two sets of *N*-Mannich bases **14-17** and **20-23** of triazolo[4,5-*f*] and triazolo[4,5-*h*]quinoline (Scheme 2) generally required particular care, due to their susceptibility to heating, pH, solvents, and various kinds of chromatographic separation, like the corresponding partly formed *N*-hydroxymethyl derivatives **18** and **24** (shown by ^{1}H-nmr data). As each base behaved differently, appropriate procedures (extraction or recrystallization) were used each time to obtain pure samples. Dimethylaminomethyl-triazoloquinolines **14** and **20-23** almost always furnished hydroxy methylene derivatives and were not obtained in pure form. DMSO-d_6 ^{1}H nmr of pure **14** was obtained once, from a very small sample after fractionated recrystallization with methanol. Table 2 lists significant chemical shifts for CH_2N and CH_2O protons of **20-23**.

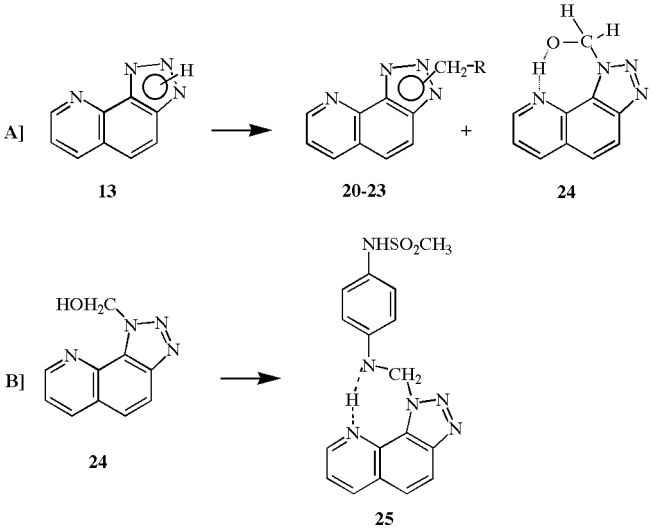
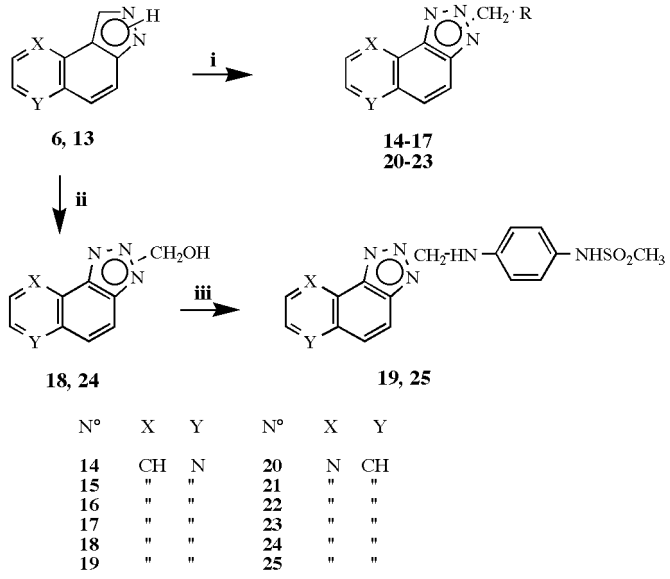


Figure 3. Probable hydrogen bonds between pyridine N and hydroxile in 24 A] and in 25 B].

In these derivatives, it was noted that the stability of compounds increased on binding increasingly bulky side-chains, from dimethylamino- to *N*(4)-phenylpiperazino groups.

These *N*-substituted triazoloquinolines showed another particular kind of behaviour, also verified for benzotriazole analogs [7,8], *i.e.*, in solid state, they exist as one compound (shown by ir and mp). However, in solution they exist as two isomeric forms of the theoretically possible (1-, 2- and 3-aminoalkyl-triazoles) that still have not been definitively identified by ^{1}H nmr with respect to the binding positions of the side-chain to the triazolo moiety. This is observed in compounds **14-17** and **20-23**. In the ^{1}H nmr spectra of these compounds, the presence of two close singlets for N-CH_2-N methylene in the 6-5.5 δ range are observed which together integrate for two protons. In the aromatic, but not in the aliphatic zone, there are double signals, some of which partially overlap, show the expected splitting and integrate for only one compound (see Table 2). In order to explain the mechanism by which these isomers occurred in solution, we hypothesized a dissociation equilibrium, as also proposed by Smith [7]. Further, on the basis

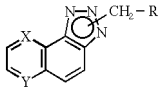


N°	X	Y	N°	X	Y
14	CH	N	**20**	N	CH
15	"	"	**21**	"	"
16	"	"	**22**	"	"
17	"	"	**23**	"	"
18	"	"	**24**	"	"
19	"	"	**25**	"	"

Figure 2. Reagents and conditions: i: CH_2O, dialkylamine, rt, 2-48h; ii: CH_2O; iii: *p*-methansulfonamidoaniline, rt, 48h. R-key: see Table 2

Table 2

Physical and Spectral Data of *N*-Substituted Triazoloquinolines **14-25**



Compound	R	Molecular formula	mw	Yield %	mp °C	^{1}H nmr [e]	ms, m/z ^{13}C nmr [f]
14	dimethyl-amino-	$C_{12}H_{13}N_5$	227.16	56	-	2.31 and 2.38 (2s, 6H, $2CH_3$), 5.61 and 5.65 (2s, 2H, CH_2), 7.77 (dd, 2H, J=4.3 and 8 Hz, HC-8), 7.95 (d, 2H, J=9.1 Hz, HC-4), 8.17 (d, 2 H, J=9 Hz, HC-5), 8.90 (dd, 2H, HC-9), 9 (m, 2H, J=1.8 and 8.4 Hz, HC-7)	m/z: 227.6 [M^+, 25%], 184 [M^+-(R), 63%], 170 [M^+- (CH_2-R), 70%]
15	morpholino-	$C_{14}H_{15}N_5O$	269.31	48	104	2.73 (m, 4H, CH_2-N-CH_2), 3.63 (t, 4H, CH_2-O-CH_2), 5.65 and 5.69 (2s, 2H, CH_2), 7.67 and 7.73 (2dd, 1H, HC-8), 7.90 and 8.26 (3d, 2H, J=9.3 Hz, HC-4 e HC-5), 8.84 and 9.02 (1dd, 1m, 2H, HC-9 and HC-7)	m/z: 269.4 [M^+, 1%], 183 [M^+-R, 1%], 170 [M^+- (CH_2-R), 3%], 101 [CH_2-R, 100%]
16	*N*-methyl-piperazino-	$C_{15}H_{18}N_6$	282.35	64	122	2.13 (s, 3H, CH_3), 2.36 and 2.72 (2m, 4H, CH_2-N-CH_2), 5.64 and 5.68 (2s, 2H, CH_2), 7.66 and 7.79 (2dd, 1H, HC-8), 7.89 and 8.24 (3d, 2H, J=9.2 Hz, HC-4 and HC-5), 8.83 and 9.01 (1dd, 1m, 2H, HC-9 and HC-7)	m/z: 282.3 [M^+, 5%], 170 [M^+-(CH_2-R), 3%], 112 [CH_2-R, 100%]
17	*N*-phenyl-piperazino-	$C_{20}H_{20}N_6$	344.42	65	141.5	2.74 (m, 4H, CH_2-N-CH_2), 3.05 (m, 4H, CH_2-N-CH_2), 5.59 and 5.63 (2s, 2H, CH_2), 6.61 (t, 1H, CH-4' phe), 6.75 (m, 2H, HC-3' and HC-5' phe), 7.03 (m, 2H, HC-2' and HC-6' phe), 7.52 e 7.65 (2dd, 1H, HC-8), 7.76 and 8.16 (3d, 2H, J=9.3 Hz, HC-4 and HC-5), 8.70 and 8.88 (1dd, m, 2H, HC-9 and HC-7)	m/z: 345 [M^+, 5%], 170 [M^+]-(CH_2-R), 72%], 162 [R, 31%], 119 [C_8H_9N, 100%]
18 [a]	hydroxy-	$C_{10}H_8N_4O$	200.20	97	259-260	6.12 (d, 2H, J=7.7 Hz, CH_2), 7.42 (t, 1H, J=7.7 Hz, OH), 7.85 (dd, 1H, J= 4.7 and 8.2 Hz, CH-8), 8.14 (d, 1H, J=9.2 Hz, CH-4), 8.28 (d, 1H, J=9.2 Hz,CH-5), 9.01 (m, 2H, HC-7 e HC-9)	^{13}C: 71.73 and 58.82 (-$\underline{C}H_2$), 115.95 (C-8), 121.09 (C-3a), 123.69 and 124.15 (C-4), 127.39 (C-9b), 129.25 (C-5), 132.20 and 131.33 (C-9), 133.39 (C-9a), 145.62 (C-5a), 150.08 and 149.84 (C-7) m/z: 201.12 (MH^+, 4%), 171.07 ($C_9H_7N_4$, 22%), 143.06 (C_9H_7N, 55%), 142.06 (C_9H_6N, 100%)
19 [b]	*p*-methan-sulfonamido-anilino-	$C_{17}H_{16}N_6O_2S$	368.42	35	211-212	2.77 and 2.79 (2s, 3H, -CH3), 6.12 and 6.22 (2d, 2H, -CH2-), 6.81 and 7.01 (m, 4H, phenyl), 7.36 and 7.51 (2t, 1H, HN amin.), 7.77 (m, 1H, HC-8), 7.86 and 8.38 (4d, 2H, HC-4 and HC-5), 8.75 and 9.08 (m, 3H, HC-9 and HC-7 and HN aminic.)	m/z: 198 [-CH_2-NH-(C_6H_4)-NH-SO_2-CH_3, 12%], 170 [$C_9H_6N_4$, 67%], 119 [-CH_2-NH-(C_6H_4)-NH, 100%]
20	dimethyl-amino-	$C_{12}H_{13}N_5$	227.16	-	-	2.33 and 2.40 (2s, 6H, $2CH_3$), 5.60 and 5.70 (2s, 2H, $2CH_2$)	
21	morpholino-	$C_{14}H_{15}N_5O$	269.31	-	-	5.6 and 5.63 (2s, 2H, CH_2-N), 6.3 (s, 2H, CH_2-O)	
22	N-methyl-piperazino-	$C_{15}H_{18}N_6$	282.35	-	-	5.57 and 5.59 (2s, 2H, CH_2-N), 6.3 (s, 2H, CH_2-O)	
23	*N*-phenyl-piperazino-	$C_{20}H_{20}N_6$	344.42			5.71 and 5.75 (2s, 2H, CH_2-N),6.3 (s, 2H, CH_2-O)	
24 [c]	hydroxy-	$C_{10}H_8N_4O$	200.20	97	265-266	6.02 (d, 2H, J=7.9 Hz, CH_2), 6.13 (d, 2H, J=7,6 Hz, CH_2), 6.54 (d, 2H, J=7.6 Hz, CH_2)	^{13}C: 71.53, 73.00 and 78.83 (CH_2), 112.46 (C-7), 118.48 and 119.31 (C-3a), 122.63, 123.47 and 123.52 (C-4), 126,21, 126,28 and 129.44 (C-5), 128.86, 128.46 and 127.98 (C-9b), 134.17 (C-5a), 137.74, 137.82 and 138.02 (C-6), 141.59, 142.33 and 142.70 (C-9a), 150.77, 150.93 and 151.75 (C-8); m/z: 171.07 ($C_9H_7N_4$, 11%), 143.06 (C_9H_7N, 43%), 142.06 (C_9H_6N, 100%)
25 [d]	*p*-methansul-fonamido-anilino-	$C_{17}H_{16}N_6O_2S$	368.42	30	196-197	2.75 and 2.78 and 2.90 (3s, 3H CH_3), 6.12 and 6.20 and 6.55 (3d, 2H, N-CH_2-N), 6.80 and 6.99 (m, 4H, phe), 7.22 and 7.45 and 7.49 (3t, 1H, NH aminic.)	m/z: 198 [-CH_2-NH-C_6H_4-NH-SO_2 CH_3, 10%], 170 [$C_9H_6N_4$, 48%], 119 [CH_2-NH-(C_6H_4)-NH]

Table 2 (continued)

[a] IR (KBr): 3097 (OH), 2842 (CH_2),. 1586 and 1533 (Ar) cm^{-1}. [b] IR (KBr): 3410 (NH amin.), 3270 (NH amid.), 1616 and 1526 (Ar), 1320 and 1152 (SO_2) cm^{-1}. [c] IR (KBr): 3120 (OH), 2845 (CH_2), 1585 and 1533 (Ar) cm^{-1}. [d] IR (KBr): 3362 (NH amin.), 3230 (NH amid.), 1616 and 1526 (Ar), 1321 and 1152 (SO_2) cm^{-1}. [e] Solvent: hexadeutero-dimethylsulfoxide (**14,18,19,24** and **25**); hexadeutero-acetone (**15-17** and **20-23**). [f] Solvent: hexadeutero-dimethylsulfoxide.

of simple electronic considerations, the more greatly deshielded signal between two singlets in the series **14-17** may be assigned to the methylene protons of the 2-substituted isomer, because of the strong withdrawing effect of N(1) and N(3) atoms adjacent to N(2) binding methylene. However, further investigations are needed in order to confirm this and to identify the other isomer.

It should be emphasized that, in analogous derivatives of benzotriazoles, a few symmetrical elements, the polar character and steric hindrance considerations with spectral data often allow definite identification of isomeric forms in solution [8,9]. In the case of various angular *N*-substituted triazoloquinolines, the presence of a fused pyridine ring makes identification of isomers more complicated.

Although more detailed investigations are required, on the basis of previous studies on benzotriazoles, a preliminary explanation for the stability of the new compounds may be proposed: due to the withdrawing effect of the pyridine ring, the acidity of the tricyclic system increases in comparison with that of benzotriazole [9] (Table 3), reducing the stability of all the *N*-Mannich bases as they decompose into hydroxymethyl derivatives.

Table 3

pKa Values for Compounds **6** and **13**

Compound	pka [a]
6	4.96
13	4.68
Benzotriazole [7]	8.2

[a] Calculated by pH measurements of 5% alcoholic solution.

Compounds **20-23** were also obtained as mixtures with the corresponding 1-hydroxy methylene compound **24** in a constant ratio, even in the crude materials (shown by ir and nmr). It is difficult to state whether the hydroxymethylene derivative forms directly by reaction between triazolo[4,5-*h*]-quinoline and formaldehyde, or by *in situ* decomposition of various *N*(1)-substituted Mannich bases **20-23**. However, in both cases, the formation of a hydrogen bond between the atoms (pyridine N and hydroxyle O) of the 1-hydroxymethylene derivative **24** makes it more stable (Figure 3A).

Compounds **18** and **24** were obtained quantitatively from triazoloquinolines and an excess of formaldehyde in neutral conditions at room temperature. Their ^1H nmr spectral data again show two isomers for **18** and the three possible forms for **24.**

In the case of **18**, ^1H nmr spectra in DMSO-d_6 show two doublets: for the same reason described above for **14-17,** the doublet at 6.12 δ may be assigned to methylene protons of 2-hydroxymethyl-triazoloquinoline (**18a**, Figure 4) (present in a yield of 90% from integrals), more greatly deshielded than the doublet at 6.01 δ (10 %). Until now, we are unable to assign the second doublet at 6.01 δ definitely to the 1- or 3-substituted isomer on electronic considerations alone, so that further investigations will have to be carried out. The ^{13}C nmr spectrum of **18** also confirmed the presence of two isomeric forms (see Table 2) from the presence of two signals for methylene carbon at 71.73 and 58.82 δ and double signals for the aromatic ones.

The ^1H nmr in DMSO-d_6 of **24** obtained with various solvents shows three doublets at 6.02 (18%), 6.13 (70%) and 6.54 δ (12%), with one isomer constantly present to a greater extent than the others.

This evidence, with the probable explanation of the hydrogen bond, allows us to assign the doublet at 6.13 δ to 1-hydroxymethyl-triazolo[4,5-*h*]quinoline (**24a)** and to propose it as the main reaction product. For the same electronic considerations made above**,** the doublet at 6.54 δ may be assigned to 2-hydroxymethyl isomer **24b** and that at 6.02 δ to 3-hydroxymethyl isomer **24c** (Figure 4).

The ^{13}C nmr spectrum of **24** shows three signals for methylenic carbon at 71.53, 73.00 and 78.83 δ, and triple signals in the aromatic zone (Table 2).

As previously reported [8], *N*-hydroxymethylene derivatives of the fused triazole nucleus are good precursors for the synthesis of interesting active biological compounds. We therefore followed this path in order to obtain first the two *N*-(*p*-methansulfonamido)anilinomethyl derivatives **19** and **25**, analog isosters of previously studied bioactive pyrroloquinolines [1,2]. It is interesting to note that both compounds showed the same in-solution behaviour seen for the respective hydroxymethylene derivatives - that is, the presence of two *N*-substituted isomers for **19** (doublets at 6.12 and 6.22 δ for methylene protons) and the contemporaneous presence of all three isomers for **25** (doublets at 6.12, 6.20 and 6.55 δ for methylene protons and three distinct triplets at 7.22, 7.45 and 7.49 δ for the amine proton). In the latter, the formation of a hydrogen bond between the pyridine N and amine N of the secondary amine, stabilizing the 1N- isomeric form in solution, is again hypothesized, and indicates that the 1-substituted derivative is the main reaction product (Figure 3B). In analogy with *N*-Mannich bases **14-17, 20-23** and hydroxymethyl derivatives **18** and **24,** the same assignments in ^1H nmr spectra (DMSO-d_6) for

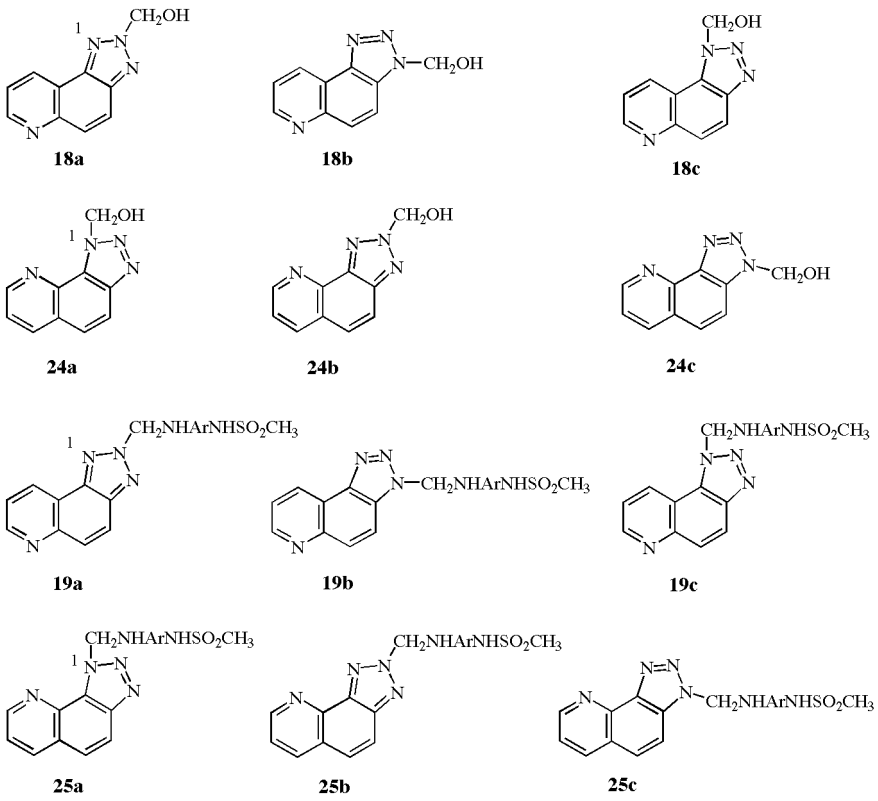


Figure 4. In solution isomeric forms proposed for **18**, **24**, **19** and **25**.

Table 4

Cell sensitivity to 100 μM drugs [a]

Compound	HeLa	Hep-G2	Aou-373
6	78	15	0
13	8	22	3
14	94	98	72
15	88	83	8
16	30	5	0
17	75	65	0
18	99	88	1
19	81	90	48
20	73	10	12
21	88	72	41
22	15	0	0
23	21	33	6
24	59	65	5
25	93	87	55

[a] Results are expressed as % reduction cell survival over controls.

in-solution isomers of **19** and **25** were made: the doublet at 6.22 δ refers to **19a**, that at 6.20 δ with the triplet at 7.49 δ to **25a**, that at 6.55 δ with the triplet at 7.45 δ to **25b,** and that at 6.12 δ with the triplet at 7.22 δ to **25c** (Figure 4).

As regards of biological activity (Table 4), unsubstituted triazoloquinolines **6** and **13** are only poorly active against the three cell lines used for assays, at a concentration of 100 μM/ml.

All other compounds show variable capability in reducing cell survival and generally poorly active in the Aou-373 line. **14**, **19**, **21** and **25** are quite active in all three lines, whereas **15**, **18** and **24** are active in HeLa and Hep-G2 but not in Aou-373. Considering these early data, we can see similar behavior for some analog compounds, that is, for those having the same-side chain: **15** and **21**, **18** and **24**, **19** and **25**.

EXPERIMENTAL

Chemistry.

Melting points were determined on a Gallenkamp MFB 595 010M/B capillary melting point apparatus, and are not corrected. Infrared spectra were recorded on a Perkin-Elmer 1760 FTIR spectrometer as potassium bromide pressed disks; values are expressed in cm^{-1}. ^1H nmr spectra were recorded on Varian Gemini (200 MHz) and Bruker (300 MHz) spectrometers, using the indicated solvents; chemical shifts are reported in δ (ppm) downfield from tetramethylsilane as internal reference. **J** values are given in Hertz. In the case of multiplets, the chemical shift quoted was measured from the approximate centre. Integrals corresponded satisfactorily to those expected on the basis of compound structure. Elemental analyses were performed in the Microanalytical Laboratory of the Department of Pharmaceutical Sciences of the University of Padova, using a Perkin-Elmer Elemental Analyzer Model 240B; results fell in the range ± 0.4% with respect to calculated values.

Mass spectra were obtained with a Mat 112 Varian Mat Bremen (70Ev) mass spectrometer and Applied Biosystems Mariner System 5220 LC/MS (nozzle potential 250.00). Column flash chromatography was carried out on Merck silica gel (250-400 mesh ASTM); reactions were monitored by analytical thin-layer chromatography (TLC) using Merck silica gel 60 F-254 glass plates. Solutions were concentrated in a rotary evaporator under reduced pressure. Starting nitro-quinolines and the four secondary amines were purchased from Aldrich Chimica and Janssen Chimica (now Acros) respectively. P-methanesulfonamidoaniline was prepared by us [1].

Biological Activity.

Human cell lines (epitheloid carcinoma cervix: HeLa, hepatocellular carcinoma: Hep-G2, glioblastoma Aou-373, obtained from the American Type Culture Collection) were plated at a density of 5 x 10^3 cells/well with 100 µM of drugs in flat-bottomed 96-well culture plates. After incubation for 6 days, cell sensitivity to the drugs was evaluated using the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit (Roche). Cell viability was determined by comparing the number of viable cells with and without drugs. Data are the means of three separate experiments (performed in triplicate) and results are expressed as % reduction cell survival over controls [10].

General Procedure for Synthesis of Quinolinyl-6-carbamic Acid Ethyl Esters **2** and **8**.

An ethanol solution of starting nitro-compounds (3.00 g, 17.2 mmoles) was slowly added to 5% palladium on a charcoal ethanol suspension saturated with hydrogen, and the mixture was hydrogenated at atmospheric pressure and 40°C until the starting material disappeared at TLC analysis. After 1-2 h, the mixture was filtered and the filtrate evaporated to dryness to give almost pure crude solids **1** and **7** (2.6 g, 98 %) which were directly transformed into urethane derivatives with ethyl chloroformate in anhydrous THF. After some hours of stirring at room temperature, the solvent was removed in vacuum to yield 2.49 g of raw material. Physical and spectroscopic data : see table 1.

Anal. Calcd. for **2** and **8**, $C_{12}H_{12}N_2O_2$: C, 66.55; H, 5.59; N, 12.96. Found: C, 66.38; H, 5.73; N, 12.68; and C, 66.75; H, 5.56; N, 12.88 respectively.

Synthesis of (5-nitro-quinolin-6-yl)-carbamic Acid Ethyl Ester (**3**).

Quinolinyl-6-carbamic acid ethyl ester **2** (2.00 g, 9.24 mmoles) was dissolved in 5 ml of cold conc. sulfuric acid, and then slowly added to 3 ml of a 1:1 cold mixture of concentrated sulfuric acid/fuming nitric acid. After about 30 minutes of stirring in an ice bath, the mixture was poured into 50 ml ice/water and made alkaline using dilute ammonium hydroxide. A yellow precipitate formed, which was collected, washed and dried (1.4 g). Yield 69%; mp 97-98°C (methyl alcohol); rf 0.67 (TLC, ethyl acetate/n-exane 70:30); ^{1}H nmr ($CDCl_3$), d: 1.36 (q, 3H, J=7.1Hz, CH_3), 4.3 (q, 2H, J=7,1Hz, CH_2), 7.56 (dd, 1H, J=4.2 and 8.8Hz, HC-3), 8.29 (d, 1H, J=8.5Hz, HC-7), 8.49 (dd, 1H, J=1.6 and 8.7Hz, HC-4), 8.72 (d, 1H, J=8.5Hz, HC-8), 8.84 (bs, 1H, NH), 8.92 (dd, 1H, J=1.5 and 4.2Hz, HC-2).

Anal. Calcd. for $C_{12}H_{11}N_3O_4$: C, 55.17; H, 4.24; N, 16.09. Found: C, 55.35; H, 4.21; N, 16.39.

Synthesis of (5-Amino-quinolin-6-yl)-carbamic Ethyl Ester (**4**).

By the same procedure adopted to obtain **1** and **7**, compound **3** (2.2 g, 8.4 mmoles) was reduced to its amino-derivative using ethyl acetate as solvent. After a time varying from 10 to 20 hours, the catalyst was removed by filtration and the filtrate was evaporated until dryness to give 2.07 g of product. Yield 96%; mp 73-74°C (ethyl alcohol); rf 0.30 (TLC; ethyl acetate/*n*-hexane 70:30); ^{1}H nmr (DMSO-d_6) d: 1.25 (t, 3H, J=7.0Hz, CH_3), 4.12 (q, 2H, J=7.0Hz, CH_2), 5.68 (bs, 2H, NH_2), 7.22 (d, 1H, J=8.8Hz, CH-8), 7.38 (dd, 1H, J=8.7 and 4.1Hz, HC-3), 7.55 (d, 1H, J=8.9Hz, HC-7), 8.58 (d, 1H, J=8.8 and 1.6Hz, HC-4), 8.75 (dd, 2H, J=4.1 and 1.6Hz, HC-2 and NH amid.).

Anal. Calcd. for $C_{12}H_{13}N_3O_2$: C, 62.33; H, 5.67; N, 18.17. Found: C, 62.48; H, 5.37; N, 17.95.

Synthesis of Ethyl-3*H*-[1,2,3]-triazolo[4,5-*f*]quinoline-3-carboxylate (**5**).

In a 100-ml round-bottomed flask, 1.9 g (8.2 mmoles) of **4** were dissolved in a 1:1 mixture of glacial acetic acid/water and the red solution was cooled in an ice bath. A 2ml cold water solution of stoichiometric sodium nitrite (0.579 g. 8.4 mmoles) was slowly added to the acetic acid solution, and the reaction mixture was stirred at 0°C for 4-7 hours. In the meantime, a light precipitate separated which was collected by filtration, washed and dried. Yield 85%; mp 101°C (ethyl alcohol); rf 0.23 (TLC; ethyl acetate/n-hexane 70:30); ^{1}H nmr (DMSO-d_6) d: 1.61 (t, 3H, J=7.1Hz, CH_3), 4.75 (q, 2H, J=7.1Hz, CH_2), 7.7 (dd, 1H, J=4.6 and 8.3Hz, HC-8), 8.28 (d, 1H, J=9.2Hz, HC-5), 8.39 (d, 1H, J=9.3Hz, HC-4), 9.06 (dd, 1H, J=1,7 and 4.4Hz, HC-7), 9.13 (dd, 1H, J=1.8 and 8.2Hz, HC-9).

Anal. Calcd. for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.33; H, 4.19, N, 23.36.

Synthesis of [1,2,3]Triazolo[4,5-*f*]quinoline (**6**).

In a 25-ml round-bottomed flask, 2.6 g (10.7 mmoles) of **5** were dissolved in 5-10 ml of 2N sodium hydroxide and the solution was refluxed for 1hour. After cooling, the reaction mixture was acidified with dilute acetic acid and then extracted by ethyl acetate. The organic phase was washed, dried with anhydrous sodium sulfate and evaporated to dryness, yielding a white crystalline residue purified by flash chromatography (L 13 cm, i.d. 3 cm, acetone/dichloromethane 70:30 as eluent). Yield 96%; mp 268-269°C (methyl alcohol) (lit.[14] 260°C); rf 0.26 (TLC; ethyl acetate/n-exane 70:30); ^{1}H nmr (DMSO-d_6) d: 7.7 (dd, 1H, J=4.6 and 8.3Hz, HC-8), 8.28 (d, 1H, J=9.2Hz, HC-5), 8.39 (d, 1H, J=9.3Hz, HC-4), 9.06 (dd, 1H, J=1.7 and 4.4Hz, HC-7), 9.13 (dd, 1H, J=1.8 and 8.2Hz, HC-9); ^{13}C nmr (DMSO-d_6) d: 118.54 (2bs, 3C, C-8, C-9b and C3a)), 123.55 and 123.19 8(C-4), 129.19 (2bs, C-5), 131.31 and 131.04 (C-9), 137.03 (bs, C-9a), 147.84 (C-5a), 151.03 and 150,63 (C-7); uv (ethyl alcohol): lmax nm 213, 249; lmin nm 229; ir (KBr): 2511 (NH), 1548,1401 (Ar) cm^{-1}; ms, m/z (relative abundance): 171.07 ($C_9H_7N4^+$, 28%), 143.06 ($C_9H_7N_2$, 42%), 142.06 ($C_9H_6N_2$, 100%).

Anal. Calcd. for $C_9H_6N_4$: C, 63.52; H, 3.55; N, 32.92. Found: C, 63.61; H, 3.55; N, 32.97.

Procedure for nitration of Compound **8**.

In a 100-ml round-bottomed flask placed in an ice-bath, 1.2 g (5.5 mmoles) of **8** were dissolved in 4 ml of previously cooled (0°C) concentrated sulfuric acid and a 1:3 cold mixture of concentrated sulfuric acid/fuming nitric acid was added dropwise. The mixture was stirred for 30 minutes at 0°C and was then poured into 20 ml ice/water. After a short time, a yellow precipitate separated out that was collected, washed and dried. This raw

material (1.15 g, 80% yield) was composed of two compounds, as shown by TLC, and was later identified by ^1H and ^{13}C nmr data as **9** and **10**, following their separation by flash chromatography (silica gel, L 13 cm, i.d. 3 cm, ethyl acetate/n-hexane 7:3). The filtrate was made alkaline with dilute ammonium hydroxide and another precipitate formed (0.3 g, 20%), identified as di-nitro derivative **11**. Spectral data: see Table 1.

Anal. Calcd. for **9** and **10** $C_{12}H_{11}N_3O_4$: C, 55.17; H, 4.24; N, 16.08. Found: C, 55.13; H, 4.28; N, 16.12; and C, 54.93; H, 4.38; N, 15.88 respectively.

Anal. Calcd. for **11** $C_{12}H_{10}N_4O_6$: C, 47.06; H, 3.30; N, 18.30. Found: C, 47.38; H, 3.30; N, 18.42.

Synthesis of (7-Aminoquinolin-8-yl)-carbamic Acid Ethyl Ester (**12**).

Following the same reducing procedure previously described for **3**, 0.95 g (3.6 mmoles) yielded 3.2 g of titled compound **12**. Yield 98%; mp 163°C (ethyl alcohol); rf 0.50 (TLC; ethyl acetate/*n*-hexane 70:30); ^1H nmr (DMSO-d_6) d: 1.3 (t, 3H, J=7.1Hz, CH_3), 4.2 (q, 2H, J=7.1Hz, CH_2), 5.39 (2H, NH_2), 7.23 (d, 1H, J=9Hz, HC-6), 7.24 (dd, 1H, J=4.3 and 8.1Hz, HC-3), 7.58 (d, 1H, J=8.9Hz, HC-5), 8.12 (dd, 1H, J=1.7 and 8Hz, HC-4), 8.21 (bs, 1H, NH), 8.72 (dd, 1H, J=1.7 and 4.3Hz, HC-2).

Anal. Calcd. for $C_{12}H_{13}N_3O_2$: C, 62.33; H, 5.67; N, 18.17. Found: C, 62.41; H, 5.50; N, 17.87.

Synthesis of [1,2,3]Triazolo[4,5-*h*]quinoline (**13**).

Amino-derivative **12** (0.30 g, 1.3 mmoles) was submitted to the same diazotization procedure as **4**. From the reaction mixture, kept under stirring at 0°C, a light crystalline precipitate separated out, which was collected, washed and dried, yielding 0.15 g of pure compound **13**. Yield 64%; mp 277-280°C (ethyl alcohol) (lit. [11] 250°C, lit. [12] 242-244°C); rf 0.42 (TLC; ethyl acetate/n-exane 70:30); ^1H nmr (DMSO-d_6) d: 7.71 (dd, 1H, J=4.4 and 8.3Hz, HC-7), 7.88 (d, 1H, J=8.9Hz, HC-5), 8.08 (d, 1H, J=8.9Hz, HC-4), 8.54 (dd, 1H, J=1,7 and 8.2Hz, HC-6), 9.02 (dd, 1H, J=4.4 and 8.3Hz, HC-8); uv (ethyl alcohol): lmax nm 213, 249; lmin nm: 226; ir (KBr): 2804 (NH), 1528-1373 (Ar) cm^{-1}; ms, m/z (relative abundance): 171.07 ($C_9H_7N4^+$, 10%), 143.06 ($C_9H_7N_2$, 42%), 142.06 ($C_9H_6N_2$, 100%).

Anal. Calcd. for $C_9H_6N_4$: C, 63.52; H, 3.55; N, 32.92. Found: C, 63.13; H, 3.50; N, 32.78.

General Procedure for Synthesis of 1/(2)/3-Ylmethyl-amino-derivatives of [1,2,3]Triazolo[4,5-*f*]quinoline **14**-**17** and Triazolo[4,5-*h*]quinolines **20**-**23**.

A mixture of stoichiometric quantities of triazoloquinoline (**6** or **13**) (about 0.3 g, 1.76 mmoles) and one of the four secondary amines (see Table 2) was dissolved in 15-20 ml methanol and cooled in an icebath. Under stirring, a water solution of slight excess 37% formaldehyde was added and the reaction mixture was stirred at 0°C for 2 hours and then for 48 hours at room temperature. The reaction mixture reaction was then worked up each time by extraction or recrystallization. In all cases, *N*-hydroxymethyl-substituted triazoloquinolines formed to variable extents. Yields of raw material are given in Table 2.

Compounds **14** and **20-23** were not obtained as pure samples, due to their ease of cleavage to hydroxymethyl derivatives; **15** and **16** were isolated by several extractions using ethyl acetate, and **17** was purified by precipitation from methanol by slow addition of water at room temperature.

Procedure for Synthesis of [1,2,3]Triazolo[4,5-*f*]quinolin-1-yl-methanol **18** and [1,2,3]Triazolo[4,5-*h*]quinolin-1-ylmethanol **24**.

To about 0.308 g (1.81 mmoles) of triazoloquinolines **6** or **13** dissolved in 10 ml methanol was added a slight excess of 37% formaldehyde and the mixture was stirred for 24 hours at room temperature. After removing the solvent in vacuum, the solid residue was recrystallized with ethanol.

Anal. Calcd. for **18** and **24**, $C_{10}H_8N_4O$: C, 60.00; H, 4.03; N, 27.99. Found: C, 60.28; H, 4.33; N, 27.72 and C, 60.18; H, 4.25; N, 27.81, respectively.

Procedure for Ssynthesis of *N*-{4-[([1,2,3]Triazolo[4,5-*f*]quinolin-3-ylmethyl)-amino]-phenyl}-methanesulfonamide **19** and *N*-{4-[([1,2,3]triazolo[4,5-*h*]quinolin-3-ylmethyl)-amino]-phenyl}-methanesulfonamide **25**.

About 0.3 g (1.49 mmoles) of **18** or **24** was directly added to a ethanol solution of a stoichiometric quantity of p-methansulfonamido-aniline. After 48 hours stirring at room temperature, the solvent was evaporated in vacuum at 30-40°C and the residues were purified by means of precipitation from absolute ethanol.

Anal. Calcd. for **19** and **25** $C_{17}H_{16}N_6O_2S$: C, 55.45; H, 4.38; N, 22.81; S, 8.70. Found: C, 55.64; H, 4.31; N, 22.57; S, 8.68 and C, 55.59; H, 4.17; N, 22.65; S, 8.66, respectively.

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