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Short communication

Synthesis and in vitro antitumour activity evaluation of 1-aryl-1*H*,3*H*-thiazolo[4,3-*b*]quinazolines

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Abstract – A series of 1*H*,3*H*-thiazolo[4,3-*b*]quinazolines (**2a–i**) were synthesized and evaluated for their in vitro antitumour activity against ca. 60 human tumour cell lines. They exhibited moderate (**2c**, **2d**, **2f** and **2g**) to strong (**2a**, **2b**, **2e**, **2h** and **2i**) cell-growth inhibition at a concentration of 10^{-4} M, but weak activity at lower concentrations. Only 1-(2,6-dichlorophenyl)-1*H*,3*H*-thiazolo[4,3-*b*]quinazoline (**2h**) possesses a significant growth inhibitory activity on 22 cell lines at a concentration of 10^{-5} M. © 2000 Éditions scientifiques et médicales Elsevier SAS

thiazolo[4,3-*b*]quinazolines / antitumour activity / cell

1. Introduction

During our ongoing studies aimed at the discovery of new structures endowed with antitumour activity [1–4], we found that several 1-aryl-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles **1** (*figure 1*) showed an interesting antitumour activity often associated with high or moderate specificity for certain human tumour cell lines [5]. In particular, we found that the introduction of halogens or trifluoromethyl groups on the phenyl ring at C-1 gave a good activity level and that the presence of two identical halogen atoms generally produced favourable effects on selectivity.

On these grounds, in order to evaluate the impact of ring size on activity and selectivity, we synthesized a series of 1-aryl-1*H*,3*H*-thiazolo[4,3-*b*]quinazolines **2** (*figure 1*), where the benzimidazole moiety is replaced by its homologue quinazoline. To the best of our knowledge, few results have been reported [6–9] on the synthesis of 1*H*,3*H*-thiazolo[4,3-*b*]quinazolin-5-ones and only one paper, dealing with the preparation of thiazole unsubstituted 3,3*a*-dihydro-4*H*-thiazolo[4,3-*b*]quinazoline derivatives, appeared in the literature [10].

The synthesis and results of a preliminary screening for anticancer activity of compounds **2a–2i**, selected from a larger series by the National Cancer Institute (NCI), Bethesda, are herein reported.

2. Chemistry

The synthesis of 1-aryl-1*H*,3*H*-thiazolo[4,3-*b*]-quinazolines **2a-2i** (*figure 2*) was achieved with a versatile and efficient synthetic route, by refluxing in anhydrous benzene a mixture of 2-aminobenzylamine, a variety of aromatic aldehydes and an excess of mercaptoacetic acid or 2-mercaptopropionic acid. The structure of all the products was consistent with the analytical and spectroscopic data reported in Section 4.

Due to the different nature of the amino groups of the substrate, two different ring systems, i.e. 1*H*,3*H*-thiazolo[4,3-*b*]quinazoline (**2**) and 1*H*,3*H*-thia-

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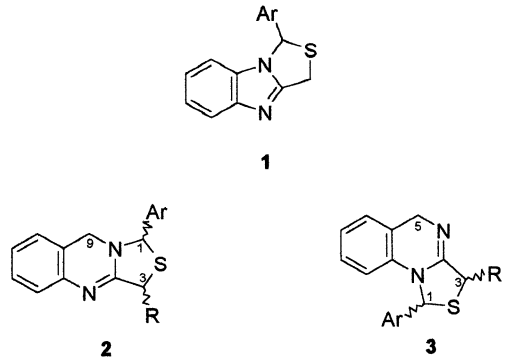

Figure 1. Structure of compounds **1–3**.

zolo[3,4-*a*]quinazoline (**3**) (*figure 1*), could be obtained. However, from the reaction mixture only one of the two possible regioisomers has been isolated and identified as 1*H*,3*H*-thiazolo[4,3-*b*]quinazoline **2** on the basis of COSY and NOE experiments. The key entry point in the structural assignment was the presence in the COSY spectra of correlation peaks between H-1 and the methylene protons of the hexatomic ring (H-9). In addition, the application of the NOE difference spectroscopy unambiguously confirmed the proposed structure. In fact, irradiation of H-1 gave a positive NOE peak at the two protons H-9, as well as at one of the two methylene protons of C-3. These results demonstrated the spatial proximity of H-1 and H-9, which supports the proposed structure **2** and excludes its regioisomer, 1*H*,3*H*-thiazolo[3,4-*a*]quinazoline **3**.

The reaction paths can be rationalized as depicted in *figure 3*. In the first step, owing to the higher nucleophilicity of the aliphatic amino group, the imine derivative **4** is formed. Then, the attack of mercaptoacetic acid at the C=N bond of **4** affords thiazolidinone **5**, which readily cyclizes to compounds **2** upon loss of water. The proposed reaction pathway is supported by the isolation of intermediate **5** from the reaction mixture.

When 2-mercaptopropionic acid was used as the substrate, the reaction furnished two diastereoisomers characterized by a different disposition of the substituents at C-1 and C-3 (*figure 2*). The *trans* and *cis* isomers were separated by column chromatography and their structures were assigned on the basis of ^{1}H-NMR data. In the spectrum of the *trans* isomer (**2g**) it is possible to observe a weak long-range coupling between H-1 and H-3, since they are located pseudo-equatorially, analogous to what is observed in thiazolo[3,4-*a*]benzimidazole derivatives and confirmed by X-ray crystallographic analysis [11]. On the contrary, in the *cis* isomers (**2h** and **2i**) the unfavourable steric interaction between the phenyl ring at C-1 and the methyl group at C-3 forces these two protons to assume a quasi-axial disposition, thus preventing the above-mentioned coupling.

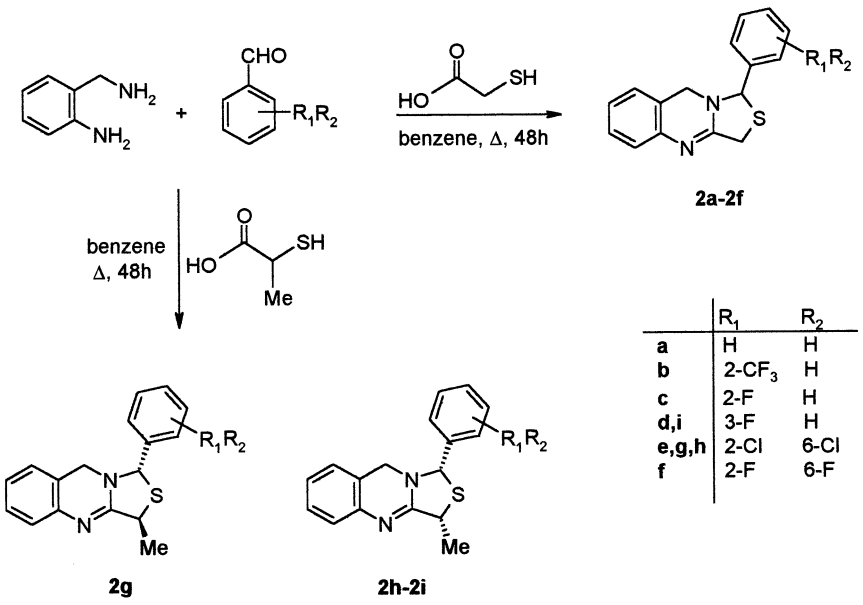

Figure 2. Synthesis of 1*H*,3*H*-thiazolo[4,3-*b*]quinazolines **2a–2i**.

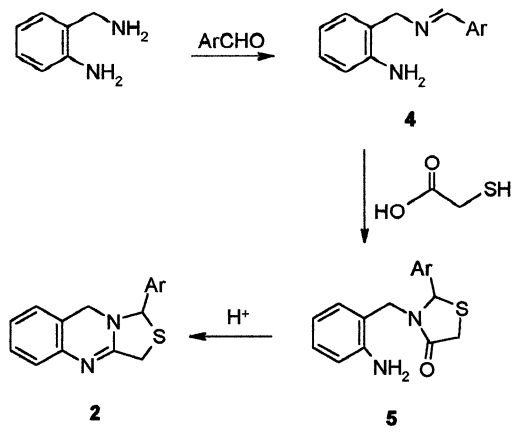

Figure 3. Mechanism for the synthesis of compounds **2**.

Table I. Mean response parameters[a] of in vitro antitumour activity test for compounds **2a–2i**.

Comp.	GI_{50}	TGI	LC_{50}
2a	4.47	4.06	4.00
2b	4.80	4.42	4.17
2c	4.03	4.00	4.00
2d	4.35	4.03	4.00
2e	4.59	4.18	4.03
2f	4.25	4.02	4.00
2g	4.03	4.00	4.00
2h	4.85	4.35	4.09
2i	4.60	4.11	4.01

[a] The response parameters GI_{50}, TGI and LC_{50} are averaged values referred to all cell lines and represent the molar concentration (expressed as $-\log_{10}$) at which the percentage growth is +50, 0 and −50, respectively.

3. Pharmacological results and discussion

The cytotoxic and/or growth inhibitory effects of the compounds **2a–2i** were evaluated in vitro against ca. 60 human tumour cell lines derived from nine neoplastic diseases, namely leukemia, non-small cell lung, colon, central nervous system, melanoma, ovarian, renal, prostate and breast cancers.

The data reported in *table I* are average values referred to all cell lines, obtained by interpolation on dose–response curves and represent the concentrations that produce 50% cell growth inhibition (GI_{50}), total cell growth inhibition (TGI, 0% growth) and 50% cell death ($LC_{50,}$ − 50% growth). The inhibitory activity of the tested compounds falls in the range 10^{-5}–10^{-4} molar concentration.

Most of the tested compounds exhibit a wide range of inhibitory activity recorded at 10^{-4} M. Nevertheless, at the lowest concentrations, sporadic significant values of percent growth inhibition are recorded and only compound **2h** possesses significant activity on 22 cell lines at 10^{-5} M (data not shown).

When compared to the unsubstituted derivative **2a**, the introduction of a trifluoromethyl group (**2b**) or chlorine atoms (**2e**) on the phenyl ring at C-1 produces an increase of growth inhibition activity, while the presence of fluorine atoms (**2c**, **2d** and **2f**) leads to a decrease in activity. When a methyl group is present at C-3, it is noteworthy that in 2,6-dichlorophenyl derivatives **2g** and **2h**, the *cis* isomer **2h** exhibits a wide range of inhibitory activity also at 10^{-5} M; on the contrary the corresponding *trans* isomer **2g** fails also at 10^{-4} M. Interestingly, the introduction of a *cis* methyl group at position 3 in the inactive compound **2c** affords derivative **2i** endowed with significant growth inhibition properties.

4. Experimental protocols

4.1. Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses were carried out on a Carlo Erba 1106 elemental analyser for C, H and N, and the results are within ± 0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (70–230 mesh). ^{1}H-NMR spectra were recorded in $CDCl_3$ by means of a Varian Gemini-300 spectrometer. Chemical shift are expressed in δ (ppm) relative to TMS as internal standard and coupling constants (J) in Hz. COSY and NOE spectra were carried out by using the standard software package.

*4.1.1. General procedure for the synthesis of 1-aryl-1H,3H-thiazolo[4,3-b]quinazolines **2a–2i***

To a stirred solution of 2-aminobenzylamine (1 g, 8 mmol) in dry benzene (40 mL), 2-mercaptoacetic acid (1.1 mL, 16 mmol) and the appropriate aromatic aldehyde (8 mmol) were added. The reaction mixture was refluxed for 48 h using a Dean–Stark apparatus. The solution obtained was washed with 2% sodium hydrogen carbonate, then with water and dried on sodium sulfate. After removal of the solvent under reduced

pressure, the oily residue was triturated by treatment with diethyl ether to afford a solid which was recrystallized from EtOH to give compounds **2a–2f**. Compounds **2g–2i** were obtained by column chromatography on silica gel column eluting with cyclohexane/EtOAc (80:20).

4.1.1.1. 1-Phenyl-1H,3H-thiazolo[4,3-b]quinazoline ***2a***

Mp 127–129 °C (0.48 g, 22%); ^{1}H-NMR: 4.02 and 4.08 (dd, 2H, $J = -15.6$, H-3), 4.28 and 4.42 (dd, 2H, $J = -13.5$, H-9), 5.54 (s, 1H, H-1), 6.78–7.43 (m, 9H, ArH). Anal. calc. for $C_{16}H_{14}N_2S$: C, 72.15; H, 5.30; N, 10.52. Found: C, 71.87; H, 5.43; N 10.39.

4.1.1.2. 1-(2-Trifluoromethylphenyl)-1H,3H-thiazolo[4,3-b]quinazoline ***2b***

Mp 150–152 °C (1.50 g, 56%); ^{1}H-NMR: 4.02 and 4.09 (dd, 2H, $J = -15.2$, H-3), 4.27 and 4.50 (dd, 2H, $J = -13.2$, H-9), 5.96 (s, 1H, H-1), 6.80–7.73 (m, 8H, ArH). Anal. calc. for $C_{17}H_{13}F_3N_2S$: C, 61.07; H, 3.92; N, 8.38. Found: C, 61.32; H, 4.16; N 8.57.

4.1.1.3. 1-(2-Fluorophenyl)-1H,3H-thiazolo[4,3-b]quinazoline ***2c***

Mp 66–68 °C (1.19 g, 54%); ^{1}H-NMR: 3.99 and 4.09 (dd, 2H, $J = -15.2$, H-3), 4.36 and 4.54 (dd, 2H, $J = -13.3$, H-9), 5.90 (s, 1H, H-1), 6.82–7.48 (m, 8H, ArH). Anal. calc. for $C_{16}H_{13}FN_2S$: C, 67.58; H, 4.61; N, 9.85. Found: C, 67.36; H, 4.29; N 10.12.

4.1.1.4. 1-(3-Fluorophenyl)-1H,3H-thiazolo[4,3-b]quinazoline ***2d***

Mp 128–130 °C (0.96 g, 42%); ^{1}H-NMR: 4.00 and 4.06 (dd, 2H, $J = -14.8$, H-3), 4.30 and 4.44 (dd, 2H, $J = -13.3$, H-9), 5.51 (s, 1H, H-1), 6.81–7.42 (m, 8H, ArH). Anal. calc. for $C_{16}H_{13}FN_2S$: C, 67.58; H, 4.61; N, 9.85. Found: C, 67.75; H, 4.38; N 9.72.

4.1.1.5. 1-(2,6-Dichlorophenyl)-1H,3H-thiazolo[4,3-b]quinazoline ***2e***

Mp 184–186 °C (1.11 g, 41%);^{1}H-NMR: 4.09 (d, 1H, $J = -14.8$, H-3$_A$), 4.24 (dd, 1H, J = 1.9 and -14.8, H-3$_B$), 4.32 and 4.58 (dd, 2H, $J = -13.3$, H-9), 6.65 (d, 1H, $J = 1.9$, H-1), 6.85–7.45 (m, 7H, ArH). Anal. calc. for $C_{16}H_{12}Cl_2N_2S$:C, 57.32; H, 3.61; N, 8.36. Found: C, 57.67; H, 3.38; N 8.42.

4.1.1.6. 1-(2,6-Difluorophenyl)-1H,3H-thiazolo[4,3-b]quinazoline ***2f***

Mp 76–78 °C (0.83 g, 34%); ^{1}H-NMR: 3.94 and 4.25 (dd, 2H, $J = -14.9$, H-3), 4.47 and 4.59 (dd, 2H, $J = -13.2$, H-9), 6.03 (s, 1H, H-1), 6.81–7.38 (m, 7H, ArH). Anal. calc. for $C_{16}H_{12}F_2N_2S$: C, 63.56; H, 4.00; N, 9.27. Found: C, 63.38; H, 4.22; N 9.58.

4.1.1.7. Trans-1-(2,6-dichlorophenyl)-3-methyl-1H,3H-thiazolo[4,3-b]quinazoline ***2g***

Mp 176–178 °C (1.19 g, 42%); ^{1}H-NMR: 1.74 (d, 3H, $J = 6.9$, CH_3), 4.30 and 4.55 (dd, 2H, $J = -13.2$, H-9), 4.45 (dq, 1H, $J = 1.6$ and 6.9, H-3), 6.55 (d, 1H, $J = 1.6$, H-1), 6.79–7.40 (m, 7H, ArH). Anal. calc. for $C_{17}H_{14}Cl_2N_2S$: C, 58.46; H, 4.04; N, 8.02. Found: C, 58.65; H, 3.92; N 8.23.

4.1.1.8. Cis-1-(2,6-dichlorophenyl)-3-methyl-1H,3H-thiazolo[4,3-b]quinazoline ***2h***

Mp 163–165 °C (0.54 g, 19%); ^{1}H-NMR: 1.76 (d, 3H, $J = 6.9$, CH_3), 4.30 and 4.50 (dd, 2H, $J = -13.2$, H-9), 4.37 (q, 1H, $J = 6.9$, H-3), 6.50 (s, 1H, H-1), 6.81–7.40 (m, 7H, ArH). Anal. calc. for $C_{17}H_{14}Cl_2N_2S$: C, 58.46; H, 4.04; N, 8.02. Found: C, 58.23; H, 4.21; N 8.14.

4.1.1.9. Cis-1-(2-fluorophenyl)-3-methyl-1H,3H-thiazolo[4,3-b]quinazoline ***2i***

Mp 118–121 °C (1.4 g, 58%); ^{1}H-NMR: 1.73 (d, 3H, $J = 6.9$, CH_3), 4.34 and 4.48 (dd, 2H, $J = -13.5$, H-9), 4.30 (q, 1H, $J = 6.9$, H-3), 5.88 (s, 1H, H-1), 6.82–7.53 (m, 8H, ArH). Anal. calc. for $C_{17}H_{15}FN_2S$: C, 68.43; H, 5.07; N, 9.39. Found: C, 68.21; H, 5.27; N 9.19.

4.2. Pharmacology

Evaluation of anticancer activity of compounds **2a–i** was performed at the National Cancer Institute (NCI) of Bethesda, MD, following the well-known in vitro disease-oriented antitumour screening program [12], which is based upon the use of multiple panels of 60 human tumour cell lines against which our compounds were tested at 10-fold dilutions of five concentrations, ranging from 10^{-4} to 10^{-8} M. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. A 48 h continuous drug exposure protocol was followed and a sulfurhodamine B (SRB) protein assay was used to estimate cell viability or growth.

Acknowledgements

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