SYNTHESIS AND LEUKEMIA CELL GROWTH INHIBITION OF A SERIES OF 1,3-DITHIAZOLYLBENZENE DERIVATIVES

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By a slightly modified Hantzsch thiazole synthesis either 1,3-bis[(thiazol-2-yl)amino]benzene derivatives **2** or 1,3-bis[2-iminothiazol-3(2*H*)-yl]benzene derivatives **3** were exclusively obtained. The compounds can be distinguished by NMR spectroscopy. Compounds **2a**–**2d** and **3a**–**3d** were evaluated for their potential antitumor activity, DNA interaction, and for their activity against DNA and RNA viruses and against HIV-1 and HIV-2.

Keywords: Heterocycles; Anticancer agents; DNA-interaction; NMR spectroscopy; Thiazoles; Hantzsch synthesis.

The 2-aminothiazole scaffold is present in several pharmacophores. Derivatives of 2-aminothiazole have been studied as antiinflammatory[1], antibacterial[2] and antiviral agents[3]. Likewise, this functional group is present in antagonists of fibrinogen receptor showing antithrombosis activity[4] as well as in new inhibitors of bacterial DNA-gyrase B [5] and cyclin-dependend kinases[6]. Bleomycin, belonging to the family of glycoprotein antibiotics and containing a bisthiazole structure, binds to DNA and causes single and double strand breaks in DNA by a free radical mechanism[7]. Bis-2-aminothiazoles have been less studied, partly because they are less easily available. Therefore, we developed a straightforward synthetic procedure to these compounds and evaluated their activity in human leukemia cell system together with their potential DNA binding and antiviral properties. The compounds were obtained in a one-step reaction from α-halo ketones with potassium thiocyanate and 1,3-phenylenediamine. Depending on reaction conditions either 1,3-bis[(thiazol-2-yl)amino]benzene derivatives **2**

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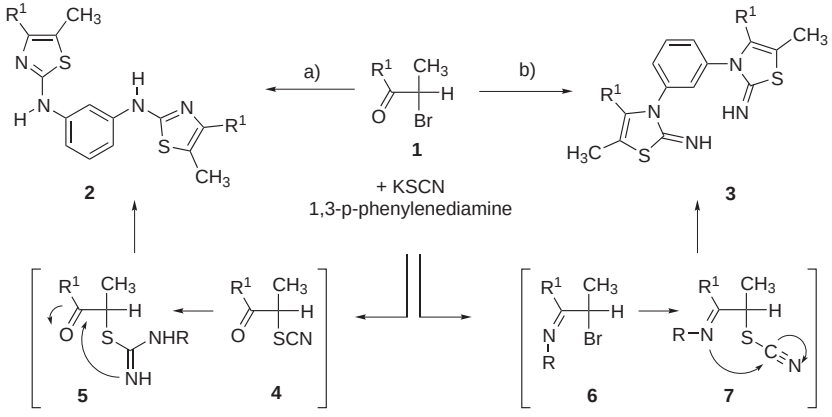
or 1,3-bis[2-iminothiazol-3(2*H*)-yl]benzene derivatives **3** were exclusively obtained.

Chemistry

In a slightly modified version of the Hantzsch thiazole synthesis[8], the condensation of 2 equivalents of α-halo ketones with potassium thiocyanate (2.5 equivalents) and 1,3-phenylenediamine hydrochloride (1 equivalent) in refluxing methanol yielded 1,3-bis[(thiazol-2-yl)amino]benzene derivatives **2** or 1,3-bis[2-iminothiazol-3(2*H*)-yl]benzene derivatives **3** (Scheme 1). Starting from the thiocyanato ketone **4** (pathway a), upon the reaction with the aniline derivative, the imidothiocarbamate intermediate **5** is formed, which, after cyclization, yields the corresponding 1,3-bis[(thiazol-2-yl)-amino]benzene derivative **2**. This reaction pathway has been previously described for the efficient synthesis of compounds of type **2** [8c]. When the Schiff base **6** is formed first (pathway b), nucleophilic exchange of the halo atom for the thiocyanate moiety gives rise to an arylimino thiocyanate **7**, which cyclizes to the corresponding 1,3-bis[2-iminothiazol-3(2*H*)-yl]-benzene derivative **3**.



2, 4, 5	R^1	**3, 6, 7**	R^1
a	2,4-$Cl_2C_6H_3$	**a**	4-FC_6H_4
b	3-BrC_6H_4	**b**	4-ClC_6H_4
c	CH_3	**c**	4-BrC_6H_4
d	CO_2CH_3	**d**	CH_3

Pathway a) 1. α-bromoketone, KSCN, MeOH, r.t. 1 h; 2. 1,3-phenylenediamine·HCl, reflux 18 h. Pathway b) 1. α-bromoketone, 1,3-phenylenediamine·HCl, $NaHCO_3$, MeOH, reflux 3 h; 2. KSCN, reflux 18 h

SCHEME 1

The synthesis can be carried out as a one-pot procedure and, depending on the chosen reaction conditions, either compounds **2** or **3** are obtained in good yields. This is demonstrated by the synthesis of **2c** and **3d**, starting from the same α-halo ketone (3-chlorobutan-2-one). These findings are in contrast to earlier findings[9] where only the formation of iminothiazole derivatives similar to compounds **3**, is claimed. The reaction is suitable for aliphatic as well as aromatic α-halo ketones. The selection of α-bromo ketones **1** that were used in the reaction was based both on availability and on attempts to obtain a considerable diversity of the substitution pattern. All compounds of series **2** and **3** were characterized by NMR spectroscopy; all proton and carbon resonances could be assigned by means of gHMQC and gHMBC spectroscopy[10]. Inspection of chemical shifts and spectral line shapes of compounds **2** and **3** showed several characteristics which distinguish both families of compounds. Aminothiazoles **2** showed sharp, amino-proton line shapes and rather large chemical shifts (δ ≈ 10.1 ppm) whereas the imino protons of iminothiazole compounds **3** produced very broad signals at lower chemical shifts (δ ≈ 9.7 ppm).

Large chemical shift differences between compounds **2** (δ 135.5, 129.4, 131.8 and 132.7 ppm – C-1/3, C-2, C-4/6 and C-5, respectively) and **3** (δ 141.8, 104.7, 110.2 and 129.6 ppm – C-1/3, C-2, C-4/6 and C-5, respectively) could be observed in the central aromatic ring. These differences are due to better electron donor properties of the amino group in series **2** than those of the imino functional group in series **3**. The two isomers can also be easily distinguished by comparing the signal corresponding to carbon 2 of the thiazole moiety. For aminothiazoles **2**, it was found in the δ 160 ppm region, whereas the corresponding signal of the 2-iminothiazoles **3** was found in the δ 169 ppm region. Overlap of the resonance signals of the thiazole moieties and their substituents shows these molecules to be symmetrical. An energy minimized drawing of compounds **2** and **3** is shown in Fig. 1. Together with NMR spectra and high resolution mass spectrometry, the sharp melting points are confirming the high purity of the compounds.

Biological Results and Discussion

Compounds **2a**–**2d** and **3a**–**3c** were evaluated for their potential antitumor activity. The drug concentration required to inhibit the growth of CEM human leukemia cells by 50% (IC_{50}) was determined and the data are listed in Table I.

In the 1,3-bis[(thiazol-2-yl)amino]benzene series, **2a** gave IC_{50} values in the low micromolar range, whereas **2c** and **2d** were of lower activity. In

the 1,3-bis[2-iminothiazol-3(2*H*)-yl]benzene series, marked cytotoxic effects were observed with all congeners; the bromo (**3c**) and chloro (**3b**) derivatives are somewhat more active than the fluoro derivative (**3a**).

DNA interaction was also evaluated by melting temperature (T_m) and DNase I footprinting but little or no interaction was observed. DNA is apparently not a direct target for these molecules. However, cytometry measurements using bromodeoxyuridine-labeled cells indicated that the most cytotoxic compounds **3b** and **3c** inhibit DNA synthesis during the S phase of the cell cycle (Fig. 2a). Moreover, up to 75% of CEM cells treated with the same compounds were positively stained with annexin V (Fig. 2b) and in the cell cycle measurements (propidium iodide staining), appearance of a

TABLE I
Concentration required inhibiting the growth of CEM human leukemia cells by 50% (IC_{50})

Compound	R^1	IC_{50}, μM
2a	2,4-$Cl_2C_6H_3$	2.53
2b	3-BrC_6H_4	>60
2c	CH_3	23.66
2d	CO_2CH_3	12.85
3a	4-FC_6H_4	5.22
3b	4-ClC_6H_4	1.63
3c	4-BrC_6H_4	1.43

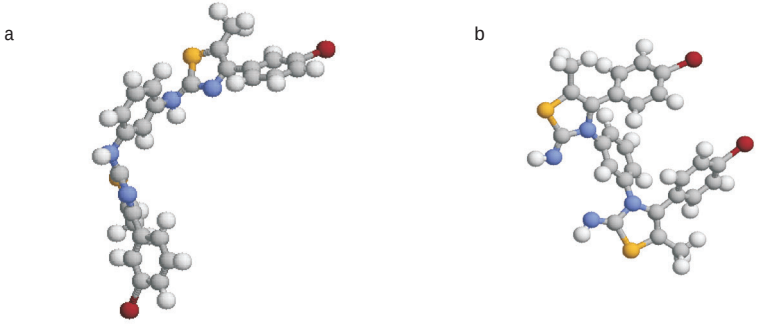


FIG. 1
Energy minimized drawing[11] of a N^1,N^3-bis[4-(3-bromophenyl)-5-methyl-1,3-thiazol-2-yl]-benzene-1,3-diamine (**2b**) and b 1,3-bis[4-(4-bromophenyl)-2-iminothiazol-5-methyl-3(2*H*)-yl]-benzene (**3c**)

sub-G1 cell population was noted, indicating induction of apoptosis by these molecules. Compounds **2a**–**2d** and **3a**–**3c** have also been evaluated for their activity against DNA and RNA viruses as well as against HIV-1 and HIV-2. No specific antiviral activity was observed.

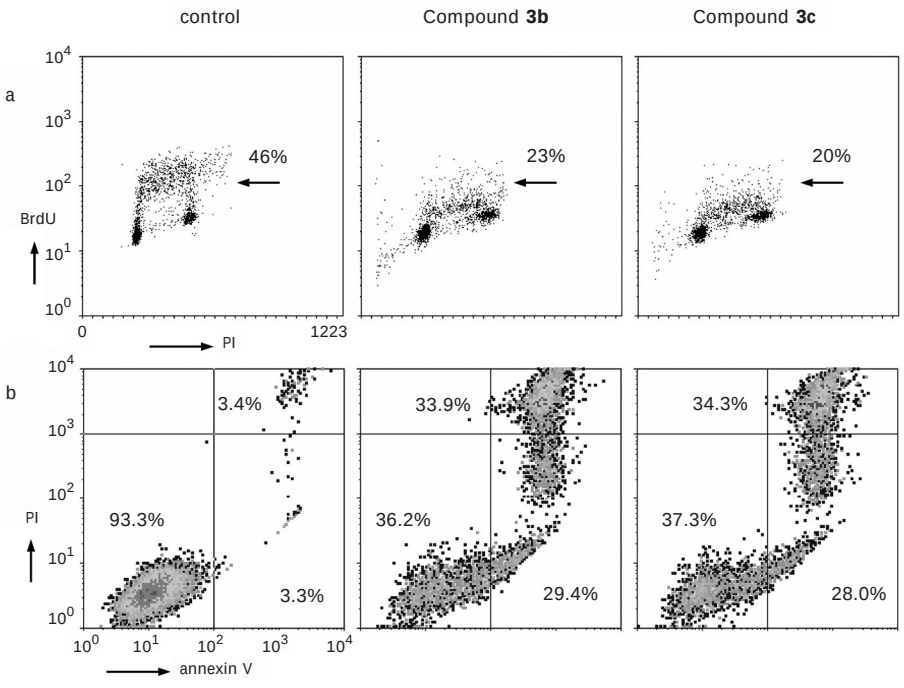


FIG. 2
Cytometry experiments using bromodeoxyuridine as marker for DNA synthesis (a) and annexin V for apotosis (b)

Conclusion

Using a modified Hantzsch synthesis, dithiazolyl benzene derivatives are available in good yields. Control of the reaction conditions allows directing the reaction either to the formation of 1,3-bis[(thiazol-2-yl)amino]benzene derivatives **2** or 1,3-bis[2-iminothiazol-3(2*H*)-yl]benzene derivatives **3**. These dithiazolylbenzene derivatives are a novel series of potential anti-tumor agents; further pharmacological studies are ongoing to elucidate their mechanism of action.

EXPERIMENTAL

NMR spectra (δ, ppm; *J*, Hz) were recorded on a Varian, Gemini 200 spectrometer (^{1}H at 200 and ^{13}C at 50 MHz). All NH protons were assigned by exchange with D_2O. In AA′BB′ systems, determination of *J* is based on the assumption of an AB quartet[12]. gHMQC and gHMBC NMR spectra were recorded on a Varian 500 MHz Unity spectrometer, operating at 499.505 MHz. Quadrature detection was achieved by States–Haberkorn hypercomplex mode[13]. The gHMQC and gHMBC spectra in DMSO consisted of 1024 data points in t_2 and 512 increments in t_1. The data were apodized with a shifted sine-bell square function in both dimensions and processed to a 2K × 2K matrix. Exact mass measurements were performed on a quadrupole–time of flight mass spectrometer (Q-Tof-2, Micromass, Manchester, U.K.) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a propan-2-ol–water (1:1) mixture at 3 μl/min. TLC was performed with TLC aluminum sheets (Silica gel 60 F_{254}, Merck) and silica (200–425 mesh) was used for column chromatography. Melting points were determined with a Kofler block. All starting materials are commercially available (e.g. from ACROS, Aldrich, Fluka) or can be obtained as outlined further[14].

N,N′-Bis(thiazol-2-yl)benzene-1,3-diamines **2a**–**2d**. General Procedure (Path a)

A solution of the α-halo ketone (4.69 mmol) and potassium thiocyanate (684 mg, 7.03 mmol) in methanol (20 ml) was stirred at room temperature for 1 h. Subsequently, 1,3-phenylenediamine hydrochloride (424 mg, 2.34 mmol) was added and the reaction mixture was refluxed overnight. Progress of the reaction was checked by TLC (ether–petroleum ether, 3:1). After cooling to ambient temperature, the precipitate was filtered off and washed with water. The crude products were dried and crystallized from MeOH–water.

N^1,N^3-Bis[4-(2,4-dichlorophenyl)-5-methyl-1,3-thiazol-2-yl]benzene-1,3-diamine (**2a**). Yield 80%. R_F (ether–petroleum ether, 3:1) 0.42. M.p. 240 °C (MeOH, water). HRMS $[M+H]^+$ calculated: 590.9805; found: 590.9814. ^{1}H NMR: 2.11 s, 6 H (CH_3); 7.13 m, 3 H (H-4,5,6 central benzene); 7.81 s, 1 H (H-2 central benzene); 7.49 m, 4 H (H-5,6 R^1); 7.72 m, 2 H (H-3 R^1); 10.10 s, 2 H (ex, NH). ^{13}C NMR: 11.4 (CH_3); 118.9, 142.7, 160.1 (C-5, C-4, C-2 thiazole); 104.7, 110.2, 129.6, 141.8 (C-2, C-4,6, C-5, C-1,3 central benzene ring); 127.4, 129.4, 133.4, 133.5, 133.6, 133.9 (C-5, C-3, C-2, C-6, C-1, C-4 R^1).

N^1,N^3-Bis[4-(3-bromophenyl)-5-methyl-1,3-thiazol-2-yl]benzene-1,3-diamine (**2b**). Yield 76%. R_F (ether–petroleum ether, 3:1) 0.38. M.p. 246 °C (MeOH, water). HRMS $[M+H]^+$ calculated: 610.9574; found: 610.9576. ^{1}H NMR: 2.40 s, 6 H (CH_3); 7.19 m, 3 H (H-4,5,6 central benzene); 7.36 dd, 2 H (H-5 R^1); 7.47 d, 2 H (H-6 R^1); 7.63 d, 2 H (H-4 R^1); 7.82 s, 2 H (H-2 R^1); 8.05 s, 1 H (H-2 central benzene); 10.13 s, 2 H (ex, NH). ^{13}C NMR: 12.0 (CH_3); 117.9, 143.7, 159.6 (C-5, C-4, C-2 thiazole); 105.0, 110.3, 129.9, 141.9 (C-2, C-4,6, C-5, C-1,3 central benzene ring); 121.8, 127.0, 129.5, 130.6, 130.7, 137.6 (C-3, C-6, C-1, C-2, C-5, C-4 R^1).

N^1,N^3-Bis(4,5-dimethyl-1,3-thiazol-2-yl)-1,3-benzenediamine (**2c**)[8c]. Yield 75%. R_F (ether–petroleum ether, 3:1) 0.30. M.p. 178 °C (MeOH, water). HRMS $[M+H]^+$ calculated: 331.1051; found: 331.1046. ^{1}H NMR: 2.12 s, 6 H (CH_3 R^1); 2.18 s, 6 H (CH_3); 6.65–6.89 m, 3 H (H-4,5,6 central benzene); 7.85 s, 1 H (H-2 central benzene); 9.81 s, 2 H (ex, NH). ^{13}C NMR: 10.5 (CH_3); 14.6 (CH_3 R^1); 113.5, 142.6, 159.2 (C-5, C-4, C-2 thiazole); 104.4, 109.4, 129.1, 141.9 (C-2, C-4,6, C-5, C-1,3 central benzene ring).

Methyl 2-(3-{[4-(methoxycarbonyl)-5-methyl-1,3-thiazol-2-yl]amino}anilino)-5-methyl-1,3-thiazole-4-carboxylate (**2d**). Yield 73%. R_F (ether–petroleum ether, 3:1) 0.28. M.p. 210 °C (MeOH, water). HRMS $[M+H]^+$ calculated: 419.0848; found: 419.0845. ^{1}H NMR: 2.57 s, 6 H

(CH_3); 3.78 s, 6 H (CH_3CO_2 R^1); 7.24 m, 3 H (H-4,5,6 central benzene); 7.65 s, 1 H (H-2 central benzene); 10.27 s, 2 H (ex, NH). ^{13}C NMR: 12.2 (CH_3); 51.6, 162.7 (CH_3, CO_2 R^1); 133.1, 138.6, 159.1 (C-5, C-4, C-2 thiazole); 105.3, 110.3, 129.8, 141.5 (C-2, C-4,6, C-5, C-1,3 central benzene ring).

1,3-Bis[2-iminothiazol-3-(2*H*)-yl]benzenes **3a**–**3d**. General Procedure (Path b)

A solution of the α-halo ketone (4.69 mmol), 1,3-phenylenediamine hydrochloride (424 mg, 2.34 mmol) and $NaHCO_3$ (394 mg, 4.69 mmol) in methanol (20 ml) was refluxed for 3 h. After disappearance of the starting material (TLC), potassium thiocyanate (684 mg, 7.03 mmol) was added and heating under reflux continued overnight. Progress of the reaction was checked by TLC (ether–petroleum ether, 3:1). After cooling to ambient temperature, the precipitate was filtered off and washed with water. The crude products were dried and crystallized from MeOH–water.

1,3-Bis[4-(4-fluorophenyl)-2-iminothiazol-5-methyl-3(2H)-yl]benzene (**3a**). Yield 74%. R_F (ether–petroleum ether, 3:1) 0.13. M.p. 246 °C (MeOH, water). HRMS $[M+H]^+$ calculated: 491.1175; found: 491.1176. ^{1}H NMR: 2.10 s, 6 H (CH_3); 7.12 m, 8 H (H-2,6,3,5 R^1); 7.46 m, 3 H (H-4,5,6 central benzene); 7.99 s, 1 H (H-2 central benzene); 9.60 s, 2 H (ex, NH). ^{13}C NMR: 11.8 (CH_3); 116.6, 132.7, 168.0 (C-5, C-4, C-2 thiazole); 129.4, 131.8, 132.7, 135.5 (C-2, C-4,6, C-5, C-1,3 central benzene ring); 116.0 (d), 133.5 (d), 135.4 (d), 165.1 (d) (C-3,5, C-2,6, C-1, C-4 R^1).

1,3-Bis[4-(4-chlorophenyl)-2-iminothiazol-5-methyl-3(2H)-yl]benzene (**3b**). Yield 73%. R_F (ether–petroleum ether, 3:1) 0.12. M.p. 252 °C (MeOH, water). HRMS $[M+H]^+$ calculated: 523.0584; found: 523.0585. ^{1}H NMR: 2.11 s, 6 H (CH_3); 7.12 d, 4 H (H-3,5 R^1); 7.39 d, 4 H (H-2,6 R^1); 7.46 m, 3 H (H-4,5,6 central benzene); 8.07 s, 1 H (H-2 central benzene); 9.85 s, 2 H (ex, NH). ^{13}C NMR: 11.3 (CH_3); 116.6, 134.4, 167.9 (C-5, C-4, C-2 thiazole); 129.4, 131.7, 132.6, 135.5 (C-2, C-4,6, C-5, C-1,3 central benzene ring); 126.5, 128.9, 132.5, 134.8 (C-4, C-3,5, C-2,6, C-1 R^1).

1,3-Bis[4-(4-bromophenyl)-2-iminothiazol-5-methyl-3(2H)-yl]benzene (**3c**). Yield 77%. R_F (ether–petroleum ether, 3:1) 0.09. M.p. 258 °C (MeOH, water). HRMS $[M+H]^+$ calculated: 610.9574; found: 610.9577. ^{1}H NMR: 2.11 s, 6 H (CH_3); 7.05 d, 4 H (H-3,5 R^1); 7.46 d, 4 H (H-2,6 R^1); 7.48 m, 3 H (H-4,5,6 central benzene); 8.10 s, 1 H (H-2 central benzene); 9.78 s, 2 H (ex, NH). ^{13}C NMR: 12.0 (CH_3); 116.6, 132.7, 167.9 (C-5, C-4, C-2 thiazole); 129.4, 131.6, 132.7, 135.5 (C-2, C-4,6, C-5, C-1,3 central benzene ring); 123.7, 126.8, 131.9, 134.4 (C-3,5, C-4, C-2,6, C-1 R^1).

1,3-Bis[4,5-dimethyl-2-iminothiazol-3(2H)-yl]benzene (**3d**). Yield 70%. R_F (ether–petroleum ether, 3:1) 0.09. M.p. 193 °C (MeOH, water). HRMS $[M+H]^+$ calculated: 331.1051; found: 331.1049. ^{1}H NMR: 2.14 s, 6 H (CH_3 R^1); 2.20 s, 6 H (CH_3); 7.15 m, 2 H (H-4, 6 central benzene); 7.40 m, 1 H (H-5 central benzene); 7.90 s, 1 H (H-2 central benzene); 10.0 s, 2 H (ex, NH). ^{13}C NMR: 10.5 (CH_3); 13.5 (CH_3 R^1); 114.0, 131.2, 165.0 (C-5, C-4, C-2 thiazole); 129.3, 131.6, 131.8, 135.4 (C-3,5, C-4, C-2,6, C-1 central benzene ring).

Experimental procedures for the cell cultures[15a] and survival assay[15b], cell cycle analysis[15c], externalization of phosphatidylserines and bromodeoxyuridine incorporation[15d] have been described previously.

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