Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 449–456

BIOORGANIC &
MEDICINAL
CHEMISTRY

Antimicrobial and Antitumor Activity of *N*-Heteroimmine-1,2,3-dithiazoles and Their Transformation in Triazolo-, Imidazo-, and Pyrazolopirimidines

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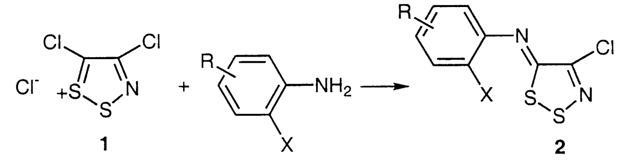
Received 9 July 2001; accepted 15 August 2001

Abstract—The reaction of Appel's salt with *o*-amino nitrile heterocycles **10**–**19** gave the corresponding 4-chloro-5-heteroimmine-1,2,3-dithiazoles **20**–**29** which were evaluated for their antibacterial, antifungal and antitumor activity. Although all these *N*-heteroimines were devoid of significant antibacterial activity, they showed significant antifungal activity. Moreover, the same derivatives represent highly versatile intermediates in heterocyclic synthesis, in fact the pyrazoleimino dithiazoles **20**–**26** can be converted in one step into 2-cyano derivatives of the corresponding 4-methoxy-pyrazolo[3,4-*d*]pyrimidines **30**–**35** by sodium methoxide in refluxing methanol. This provides a general and attractive route to 4-methoxy-6-cyano pyrazolo[3,4-*d*]pyrimidines from 1-substituted 5-amino pyrazoles **10**–**19** in two simple steps. Finally, the isosteric replacement of the pyrazole ring atoms to give the imidazole[3,4-*d*]pyrimidine and triazole [4,5-*d*] pyrimidine ring systems was examined. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

4,5-Dichloro-1,2,3-dithiazolium chloride (Appel's salt, **1**), easily prepared from chloroacetonitrile and disulfur dichloride,[1] is the most studied derivative of the 1,2,3-dithiazolium system[2] and reacts with proton-active compounds by the nucleophilic substitution of the chlorine at carbon atom C-5. Substrates like anilines with electron-withdrawing as well as electron-releasing substituents in the phenyl ring react rapidily with Appel's salt in the presence of pyridine to give very stable 5-(arylimino)-4-chloro-5H-1,2,3-dithiazoles with general formula **2**[1] in very high yield. These heterocyclic compounds are interesting because of their usefulnesss as acaricides, herbicides and for their antimicrobial and antifungal activity,[3] and also because they are useful synthetic intermediates.[2]



R=electron-withdrawing
or electron-releasing group
X=H, OH, CO$_2$H, NHR' and CN

In previous works, Appel[1] found that anilino compounds **2** with X = H, cyclised from an *ortho*-carbon onto sulfur when heated to give the 2-cyanobenzothiazole **3**[2,4] with liberation of the other sulfur atom and hydrogen chloride. For the anilines bearing nucleophilic neighbouring group in the *ortho* position of the aromatic ring, when X = OH the imine **2** was transformed both into the 2-cyanobenzoxazole **4** and dithiazolobenzoxazine **5**,[1,2] while when X = COOH the derivative **2** was converted into the cyanobenzoxazinone **6** or in the cyanobenzothiazinone **7**.[5] When X = NHR′(R′ = alkyl or aryl), the compound **2** rearranged giving the cyanobenzimidazole with general formula **8**[6] by heating or in the presence of a catalytic amount of hydrogen chloride.

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PII: S0968-0896(01)00294-2

The process of cyclisation which led to the synthesis of **4**, **7** and **8** involved the attack of the nucleophilic *ortho*-substituent (X = OH, COOH and NHR′) at the imino-carbon to form a new heterocyclic ring with loss of both sulfur atoms from the dithiazole ring. The treatment with alkoxides of the imine with general formula **2** bearing a cyano group in the *ortho* position (X = CN) of the *N*-aryl group allowed the formation of 4-alkoxy-quinazolines **9**[7] (Scheme 1).

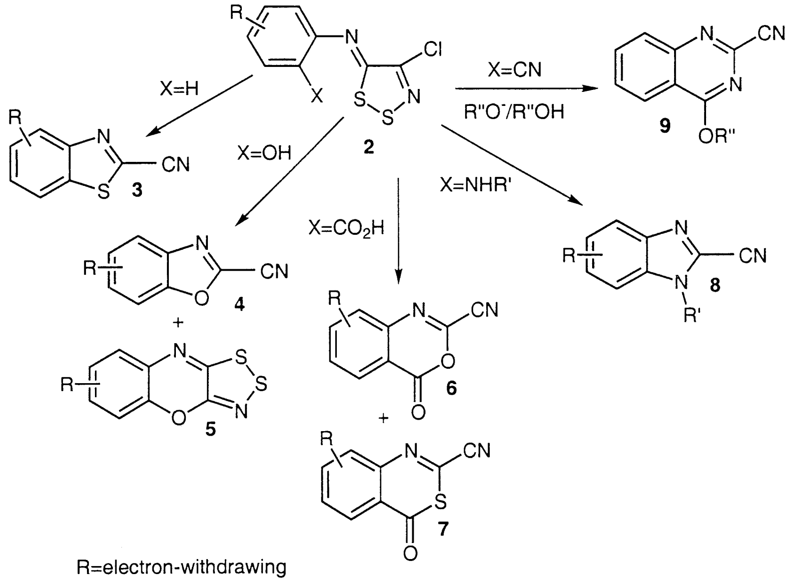
In this article, we have studied the reaction of Appel's salt **1** with amino heterocycles **10**–**19** having a nucleophilic cyano group in the *ortho* position, to give the expected 4-chloro-5-heteroimmine-1,2,3-dithiazoles **20**–**29**, which were then evaluated for their antibacterial, antifungal and antitumor activity. Although the synthesis of 4-chloro-5-heteroimine-dithiazoles has been already reported,[8] only a limited series of examples have been described, and neither the biological activity nor the possible chemical conversion was investigated. For these reasons, we have further explored this method increasing the range of heterocyclic imines that react with Appel's salt, introducing a nucleophilic neighbouring cyano group in the ortho position of the aromatic heterocyclic ring in the hope of enhancing the biological activity of the products **20**–**29** obtained. Since the compounds **20**–**29** are so readily available, we have also explored their use in heterocyclic synthesis heating these derivatives (with the exception of **27**) in a mixture of sodium methoxide in boiling methanol, to give the cyclised compounds **30**–**37**.

Chemistry

The 4-chloro-5-heteroimine-5H-1,2,3-dithiazoles **20**–**29** were prepared clean and in acceptable yield (38–73%) by the reaction of 4,5-dichloro-1,2,3-dithiazolium chloride **1** with the *o*-aminonitrile heterocycles **10**–**19** in methylene chloride (DCM) at room temperature, after which pyridine (2 equiv) was added to the reaction mixture before work-up. The reaction of pyrazolimines **20**–**25** with sodium methoxide (2 equiv) in methanol at reflux to close the pyrimidine ring afforded the 1-alkyl (aryl)-2-cyano-pyrazole[3,4-*c*]pyrimidines **30**–**35** in acceptable yield (52–72%) as outlined in Scheme 2. The general method of synthesis of pyrazolo[3,4-*d*]pyrimidines from a pyrazole intermediate has been further extended. In the same way, the imidazoleimine **26** and triazoleimine **28** were treated with sodium methoxide in methanol to give the corresponding imidazole [3,4-*c*]pyrimidine **36** and triazole [4,5-*c*]pyrimidine **37** in 52 and 69% yields, respectively, confirmed by analysis and spectroscopy. The 2-cyano group can be removed by hydrolysis and decarboxylation with hot concentrated hydrochloric acid, converted into corresponding amides, esters, amidines and ketones and can be displaced by nucleophiles such as methoxide and ethoxide ions.

Results and Discussion

The in vitro antimicrobial activity of the synthesized compounds **20**–**29** was investigated against several pathogenic representative Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 11105 and *Proteus mirabilis* MB81) and Gram-positive bacteria (*Enterococcus faecalis* ATCC 8043 and *Staphylococcus aureus* ATCC 29213). No antibacterial activity was showed by any of the assayed compounds: all MIC values for the Gram-negative bacteria tested were >100 μg/mL, as well as high MIC values, ranging from 75 to >100 μg/mL, were found for the



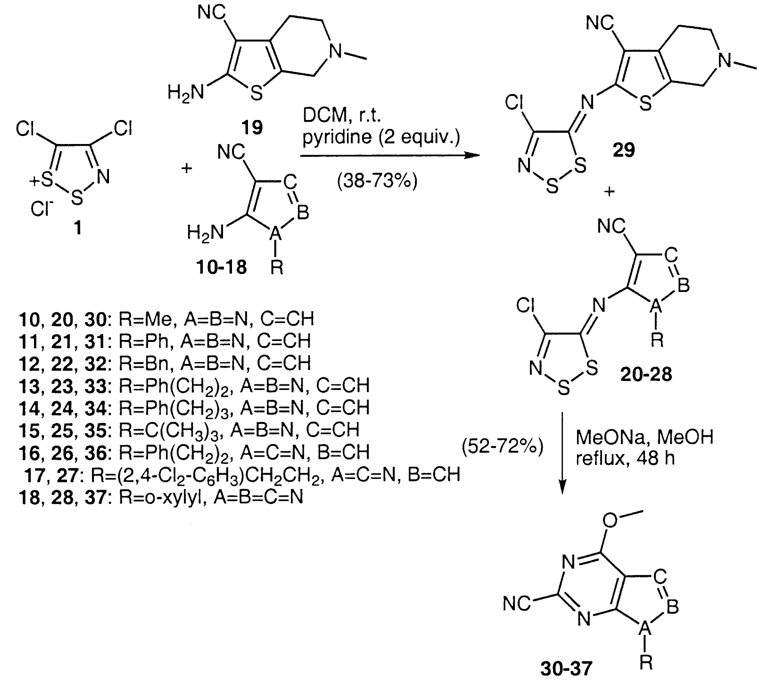
R=electron-withdrawing
or electron-releasing group
R'= Me, C_6H_{11}, Ph or pheny with electron-withdrawing
or electron-releasing groups
R"= Me, Et, Pr^i and *n*-But

Scheme 1.

Gram-positive bacteria (Table 1). None of the derivatives **20–29** showed a better spectrum of activity than the reference drug ampicillin.

The in vitro antifungal activity of compounds **20–29** was performed against yeasts (*Candida albicans* ATCC 10231, *Candida utilis* ATCC 9950, *Candida lipolytica* CBS 6124, *Saccharomyces cerevisiae* ATCC 26785, *Pichia stipitis* CBS 5776) and moulds (*Aspergillus niger* L32 and *Penicillium* sp.) and the results are summarized in Table 2. All the derivatives showed significant antifungal activity against the yeasts tested. The compounds **24–28** exhibited the highest antifungal activity, showing good MIC values included in the range of 10–50 μg/mL. Particularly, the efficacy of these products against the moulds can be compared with that showed by amphotericin B, used as reference compound for inhibitory activity against fungi. **20**, **21** and **23** products appeared to be scarcely active only against some microorganisms such as *Penicillium* and *C. utilis*, whereas **22** and **29** products presented the lowest antifungal activities against all the fungi tested. It is interesting to note that for the compounds **20–25** obtained from *o*-amino nitriles **13–18** which possess the same heterocyclic ring, the biological activity is affected by substituents on the N1-position of the pyrazole ring.

Based on their fungicidal activity, the heteroimine **20–29** may represent interesting leads for further study and, as previously reported,[3e] it seems likely that the 1,2,3-dithiazole ring, having nucleophilic centers at the S1, S2, C4 and C5 positions, could be implicated in inhibitory activity against fungi acting as a potent inhibitor of some enzymes like serine proteases.[3c,d]



10, **20**, **30**: R=Me, A=B=N, C=CH
11, **21**, **31**: R=Ph, A=B=N, C=CH
12, **22**, **32**: R=Bn, A=B=N, C=CH
13, **23**, **33**: R=Ph(CH_2)$_2$, A=B=N, C=CH
14, **24**, **34**: R=Ph(CH_2)$_3$, A=B=N, C=CH
15, **25**, **35**: R=C(CH_3)$_3$, A=B=N, C=CH
16, **26**, **36**: R=Ph(CH_2)$_2$, A=C=N, B=CH
17, **27**: R=(2,4-Cl_2-C_6H_3)CH_2CH_2, A=C=N, B=CH
18, **28**, **37**: R=o-xylyl, A=B=C=N

Scheme 2.

Table 1. Minimum Inhibitory Concentration (MIC) against some strains of Gram–positive and negative bacteria

Compound	MIC (μg/mL)				
	Bacteria tested				
	Pseudomonas aeruginosa ATCC 9027	*Escherichia coli* ATCC 11105	*Proteus mirabilis* MB	*Enterococcus faecalis* ATCC 8043	*Staphylococcus aureus* ATCC 29213
20	>100	>100	>100	>100	100
21	>100	>100	>100	>100	100
22	>100	>100	>100	>100	100
23	>100	>100	>100	>100	75
24	>100	>100	>100	>100	100
25	>100	>100	>100	>100	75
26	>100	>100	>100	>100	100
27	>100	>100	>100	>100	100
28	>100	>100	>100	>100	75
29	>100	>100	>100	>100	>100
Ampicillin	>100	5	10	2.5	0.5

MIC, minimum inhibitory concentration represents the mean from dose–response curves of at least three experiments.

Table 2. Minimum Inhibitory Concentration (MIC) against some fungi

Compound	MIC (μg/mL)						
	Fungi tested						
	Candida albicans ATCC 10231	*Candida utilis* ATCC 9950	*Candida lipolytica* CBS 6124	*Saccharomyces cerevisiae* ATCC 26785	*Pichia stipitis* CBS 5776	*Aspergillus niger*	*Penicilliumsp*
20	50	50	25	25	25	25	100
21	50	> 100	25	25	50	25	100
22	> 100	> 100	100	50	> 100	25	100
23	25	100	25	25	25	25	25
24	25	50	25	25	25	10	25
25	25	25	25	25	25	25	50
26	25	25	25	25	25	10	25
27	25	50	25	25	25	50	50
28	50	25	25	25	50	25	50
29	100	100	100	100	100	100	100
Amphotericin B	2.5	2.5	20	10	2.5	20	20

MIC, minimum inhibitory concentration represents the mean from dose–response curves of at least three experiments.

Table 3. In vitro biological effects on L1210 and K562 cell lines

Compound	IC$_{50}$ (μM)	
	L1210	K562
20	5	5
21	7.5	5
22	8	5
23	5	3
24	3	5
25	5	5
26	3	4
27	5	8
28	7	8
29	8	7
Distamycin A	10	20

IC$_{50}$, 50% inhibitory concentration represents the mean from dose–response curves of at least three experiments.

The antiproliferative activity of compounds **20**–**29** has been tested in vitro on human myeloid leukemia K562 and L1210 murine leukemia cell lines and compared to the antiproliferative effects of the natural product distamycin A. All the imines **20**–**29** are active at a concentration ranging from 3 to 10 μM and they retain antiproliferative activities comparable to those exhibited by distamycin A. The results obtained are summarized in Table 3.

In conclusion, we have described the antimicrobial and antitumor activity of a series of 4-chloro-5-hetero-immine-dithiazoles **20**–**29** and subsequently we have also reported a general and attractive route to 2-cyano-4-methoxy-pyrazole[3,4-*d*]pyrimidines **30**–**35** from 1-substituted-4-cyano-5-amino pyrazoles in two simple steps. Besides alkosides, this method could be extended to the addition of other nucleophiles to the cyano group of the pyrazoleimines **20**–**25**. The conversation of **26** and **28** into **36** and **37**, respectively, is worthy of further investigation. As regards the biological interest of compounds **30**–**37**, biological testing is now in progress and a complete report will appear elsewhere. Moreover, the binding affinity of triazolo[3,4-*d*]- and imidazo[4,5-*d*]pyrimidines for the corticotropin-releasing factor receptor[9] and the anticonvulsant activity[10] of the same compounds has already appeared in several publications.

Experimental

Chemistry

All reactions were carried out under Argon atmosphere, unless otherwise described. Standard syringe techniques were applied for transferring anhydrous solvents. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography on silica gel (precoated F$_{254}$ Merk plates), the spots were examined with UV light and visualized with aqueous KMnO$_4$. ^{1}H NMR spectra were recorded in the given solvent with a Bruker AC 200 spectrometer. Chemical shifts are reported as (δ) values in parts per million. The splitting pattern abbreviations are as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), b (broad), and m (multiplet). Melting points (mp) were determined using a Buchi–Tottoli apparatus and are uncorrected. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. Elemental analyses, conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara, were within 0.4% of the theorical values calculated for C, H, S, Cl and N. Column chromatography was carried out Merck silica gel (230–240 mesh). All compounds obtained commercially were used without further purification. Organic solutions were dried over anhydrous MgSO$_4$. Dioxane was distilled from calcium hydride, methylene chloride was distilled from calcium chloride.

Synthesis of 5-amino-1-phenethylimidazole-4-carbonitrile (15). Following an already reported procedure;[11] dry ammonia was passed through a suspension of aminomalononitrile toluene-*p*-sulphonate (3 g, 15.8 mmol) in dry acetonitrile (150 mL) with stirring and cooling for 20 min. Precipitated amonium toluene-*p*-sulphonate was removed and the filtrate evaporated to ca. 60 mL, then triethyl orthoformate (2.65 mL, 15.8 mmol) was added and the solution refluxed for 10 min. The cooled solution with 2-phenethylamine (2 mL, 15.8 mmol) was set aside at room temperature overnight. The solution was then evaporated to a solide residue which was submitted to purification by flash chromatography (eluent CH_2Cl_2/EtOAc 8:2) to give the compound **15** (1.33 g, 39.5%) recrystallized from aqueous ethanol as brown needles. Mp 197–198 °C (from petroleum ether); IR (KBr) cm^{-1}: 3352, 3160, 2925, 2210, 1657, 1452, 700; ^1H NMR (DMSO-d_6) δ 2.91 (t, 2H, $J=7.3$ Hz), 4.02 (t, 2H, $J=7.3$ Hz), 6.25 (bs, 2H), 7.00 (s, 1H), 7.23 (m, 5H).

Synthesis of 5-amino-1-(2,4-dichlorophenethyl)imidazole-4-carbonitrile (16). Following the procedure reported above, starting from aminomalononitrile toluene-*p*-sulphonate (3 g, 15.8 mmol) in dry acetonitrile (150 mL), triethyl orthoformate (2.65 mL, 15.8 mmol) and 2-(2,4-dichlorophenyl)-ethyl-1-amine (2.38 mL, 15.8 mL), after work-up, the compound **16** (1.23 g, 27.6%) purified by flash chromatography (eluent CH_2Cl_2/EtOAc 8:2) was recrystallized from aqueous ethanol as green needles. Mp 156–158 °C (from petroleum ether); IR (KBr) cm^{-1}: 3343, 3146, 2910, 2200, 1675, 1435, 710; ^1H NMR (DMSO-d_6) δ 2.75 (t, 2H, $J=7.2$ Hz), 4.11 (t, 2H, $J=7.2$ Hz), 6.22 (bs, 2H), 7.00 (s, 1H), 7.04 (d, $J=7.2$ Hz, 1H), 7.22 (d, $J=7.2$ Hz, 1H), 7.34 (s, 1H).

Synthesis of 1-*o*-xylyl-4-cyano-5-amino-1H-1,2,3-triazole (18). Following a procedure previously reported,[12] this compound has been obtained as a white powder. Mp 180–182 °C. IR (KBr) cm^{-1}: 3341, 3183, 2238, 1635, 1553, 752; ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H), 3.36 (bs, 2H), 5.36 (bs, 2H), 6.68 (d, 1H, $J=7.15$ Hz), 7.08 (s, 1H), 7.18 (m,2H)

General procedure for the reaction of 4,5-dichloro-1,2,3-dithiazolium chloride (Appel's salt, 1) with heterocyclic amines 10–19

To a solution of the heterocyclic amine derivative **10**–**19** (5 mmol) in dichloromethane (20 mL) was added 4,5-dichloro-1,2,3-dithiazolium chloride **1**. The mixture was stirred at room temperature for 5 h, then pyridine (5 equiv, 10 mmol, 0.8 mL) was added and the mixture was stirred for a further 2 h. After filtration, the product was purified by column chromatography with a mixture of ethyl acetate and petroleum ether as eluent.

(4-Chloro-[1,2,3]dithiazol-5-ylidene)-(1-methyl-4-cyano-pyrazol-5-yl)-amine (20). Treatment of 1-methyl-4-cyano-5-aminopyrazole[13] **10** (610 mg, 5 mmol) with **1** (1 g, 5 mmol) and pyridine (0.8 mL) in dichloromethane (20 mL), followed by column chromatography (EtOAc/petroleum ether 2:8, 3:7, 1:1 and then only EtOAc) furnished **20** as brown needles (630 mg, 50% of yield). Mp 209–212 °C (from petroleum ether); IR (KBr) cm^{-1}: 3436, 2227, 1584, 1518, 874, 766; ^1H NMR ($CDCl_3$) δ 3.83 (s, 3H), 7.81 (s, 1H). Anal. calcd for $C_7H_4ClN_5S_2$: C, 32.62; H, 1.56; Cl, 13.76; N, 27.17; S, 24.88; found: C, 32.55; H, 1.47; Cl, 13.68; N, 27.03; S, 24.78.

(4-Chloro-[1,2,3]dithiazol-5-ylidene)-(1-phenyl-4-cyano-pyrazol-5-yl)-amine (21). Treatment of 1-phenyl-4-cyano-5-aminopyrazole[13] **11** (920 mg, 5 mmol) with **1** (1 g, 5 mmol) and pyridine (0.8 mL) in dichloromethane (20 mL), followed by column chromatography (EtOAc/petroleum ether 1:9, 2:8, and then 1:1) furnished **21** as green needles (1.06 g, 66% of yield). Mp 195–198 °C (from petroleum ether); IR (KBr) cm^{-1}: 3447, 2218, 1578, 1516, 1497, 1403, 966, 869, 778, 683; ^1H NMR ($CDCl_3$) δ 7.44 (m, 3H), 7.69 d, $J=7.6$ Hz, 2H), 7.98 (s, 1H). Anal. calcd for $C_{12}H_6ClN_5S_2$: C, 45.07; H, 1.89; Cl, 11.09; N, 21.90; S, 20.05; found: C, 44.98; H, 1.81; Cl, 10.96; N, 21.79; S, 19.96.

(4-Chloro-[1,2,3]dithiazol-5-ylidene)-(1-benzyl-4-cyano-pyrazol-5-yl)-amine (22). Treatment of 1-benzyl-4-cyano-5-aminopyrazole[14] **12** (990 mg, 5 mmol) with **1** (1 g, 5 mmol) and pyridine (0.8 mL) in dichloromethane (20 mL), followed by column chromatography (EtOAc/petroleum ether 2:8) furnished **22** as brown needles (1.22 g, 73% of yield). Mp 168–171 °C (from petroleum ether); IR (KBr) cm^{-1}: 3447, 2221, 1583, 1522, 1466, 768, 737, 694; ^1H NMR ($CDCl_3$) δ 5.31 (s, 2H), 7.28 (m, 5H), 7.83 (s, 1H). Anal. calcd for $C_{14}H_{10}ClN_5S_2$: C, 46.77; H, 2.42; Cl, 10.62; N, 20.98; S, 19.21; found: C, 46.68; H, 2.36; Cl, 10.54; N, 20.91; S, 19.12.

(4-Chloro-[1,2,3]dithiazol-5-ylidene)-(1-phenethyl-4-cyano-pyrazol-5-yl)-amine (23). Treatment of 1-phenethyl-4-cyano-5-aminopyrazole[15] **13** (1.06 g, 5 mmol) with **1** (1 g, 5 mmol) and pyridine (0.8 mL) in dichloromethane (20 mL), followed by column chromatography (EtOAc/petroleum ether 2:8) furnished **23** as orange needles (1.06 g, 61% of yield). Mp 120–122 °C (from petroleum ether); IR (KBr) cm^{-1}: 3435, 2216, 1583, 1512, 1430, 1188, 1154, 866, 698; ^1H NMR ($CDCl_3$) δ 3.06 (t, $J=6$ Hz, 2H), 4.46 (t, $J=6.4$ Hz, 2H), 6.93 (m, 2H), 7.06 (m, 3H), 7.04 (s, 1H). Anal. calcd for $C_{14}H_{10}ClN_5S_2$: C, 48.34; H, 2.90; Cl, 10.19; N, 20.13; S, 18.44; found: C, 48.26; H, 2.81; Cl, 10.08; N, 20.06; S, 18.35.

(4-Chloro-[1,2,3]dithiazol-5-ylidene)-[1-(3-phenyl-propyl)-4-cyano-pyrazol-5-yl)-amine (24). Treatment of 1-(3-phenylpropyl)-4-cyano-5-aminopyrazole[15] **14** (1.13 g, 5 mmol) with **1** (1 g, 5 mmol) and pyridine (0.8 mL) in dichloromethane (20 mL), followed by column chromatography (EtOAc/petroleum ether 3:7) furnished **24** as green needles (1.25 g, 69% of yield). Mp 155–160 °C (from petroleum ether); IR (KBr) cm^{-1}: 3431, 2238, 1739, 1554, 1508, 1484, 1260, 884, 769; ^1H NMR ($CDCl_3$) δ 0.78 (m, 2H), 2.12 (t, $J=7.4$ Hz, 2H), 4.07 (t, $J=7$ Hz, 2H), 7.16 (m, 5H), 7.76 (s, 1H). Anal. calcd for $C_{15}H_{12}ClN_5S_2$: C, 49.79; H, 3.34; Cl, 9.80; N, 19.35; S, 17.72; found: C, 49.68; H, 3.28; Cl, 9.71; N, 19.28; S, 17.66.

(4-Chloro-[1,2,3]dithiazol-5-ylidene)-[1-*t*-butyl-4-cyano-pyrazol-5-yl)-amine (25). Treatment of 1-*t*-butyl-4-cyano-5-aminopyrazole[15] **15** (820 mg, 5 mmol) with **1** (1 g, 5 mmol) and pyridine (0.8 mL) in dichloromethane (20 mL), followed by column chromatography (EtOAc/petroleum ether 1:1) furnished **25** as brown needles (1.02 g, 68% of yield). Mp 142–145 °C (from petroleum ether); IR (KBr) cm^{-1}: 3448, 2225, 1572, 1449, 1354, 1235, 1210, 992, 880; ^{1}H NMR (CDCl$_3$) δ 1.63 (s, 9H), 7.72 (s, 1H). Anal. calcd for $C_{10}H_{10}ClN_5S_2$: C, 40.06; H, 3.36; Cl, 11.83; N, 23.36; S, 21.39; found: C, 40.00; H, 3.29; Cl, 11.77; N, 23.31; S, 21.31.

(4-Chloro-[1,2,3]dithiazol-5-ylidene)-(1-phenethyl-4-cyano-imidazol-5-yl)-amine (26). Treatment of 1-phenethyl-4-cyano-5-aminoimidazole **16** (1.06 g, 5 mmol) with **1** (1 g, 5 mmol) and pyridine (0.8 mL) in dichloromethane (20 mL), followed by column chromatography (EtOAc/petroleum ether 7:3) furnished **26** as yellow needles (938 mg, 55% of yield). Mp 85–87 °C (from petroleum ether); IR (KBr) cm^{-1}: 3437, 2219, 1576, 1508, 1174, 764, 700; ^{1}H NMR (CDCl$_3$) δ 3.00 (t, *J* = 6.4 Hz, 2H), 4.28 (t, *J* = 6.4 Hz, 2H), 6.97 (m, 3H), 7.15 (m, 2H), 7.44 (s, 1H). Anal. calcd for $C_{14}H_{10}ClN_5S_2$: C, 48.34; H, 2.90; Cl, 10.19; N, 20.13; S, 18.44; found: C, 48.28; H, 2.83; Cl, 10.09; N, 20.05; S, 18.38.

(4-Chloro-[1,2,3]dithiazol-5-ylidene)-[1-[2-(2,4-dichlorophenyl)-ethyl]-4-cyano-imidazol-5-yl)-amine (27). Treatment of 1-[2-(2,4-dichloro-phenyl)-ethyl]-4-cyano-5-aminoimidazole **17** (1.44 g, 5 mmol) with **1** (1 g, 5 mmol) and pyridine (0.8 mL) in dichloromethane (20 mL), followed by column chromatography (EtOAc/petroleum ether 3:7) furnished **27** as brown needles (1.3 g, 63% of yield). Mp 77–79 °C (from petroleum ether); IR (KBr) cm^{-1}: 3435, 2219, 1577, 1474, 1171, 823; ^{1}H NMR (CDCl$_3$) δ 3.13 (t, *J* = 6.4 Hz, 2H), 4.32 (t, *J* = 6.2 Hz, 2H), 6.81 (d, *J* = 8.2 Hz, 1H), 7.04 (d, *J* = 9.8 Hz, 1H), 7.28 (m, 1H), 7.47 (s, 1H). Anal. calcd for $C_{14}H_{16}Cl_3N_5S_2$: C, 40.35; H, 1.93; Cl, 25.52; N, 16.81; S, 15.39; found: C, 40.28; H, 1.85; Cl, 25.47; N, 16.74; S, 15.31.

(4-Chloro-[1,2,3]dithiazol-5-ylidene)-[1--(2-methyl-benzyl)-4-cyano-1,2,3-triazol-5-yl)-amine (28). Treatment of 1-*o*-xylyl-4-cyano-5-amino-1,2,3-triazole **18** (1.06 g, 5 mmol) with **1** (1 g, 5 mmol) and pyridine (0.8 mL) in dichloromethane (20 mL), followed by column chromatography (EtOAc/petroleum ether 2:8) furnished **28** as yellow needles (1 g, 58% of yield). Mp 146–149 °C (from petroleum ether); IR (KBr) cm^{-1}: 3448, 2240, 1739, 1554, 1485, 1260, 884, 769. ^{1}H NMR (CDCl$_3$) δ 2.42 (s, 3H), 5.53 (s, 2H), 7.17 (m, 4H). Anal. calcd for $C_{13}H_9ClN_6S_2$: C, 44.76; H, 2.60; Cl, 10.16; N, 24.09; S, 18.39; found: C, 44.71; H, 2.52; Cl, 10.08; N, 24.00; S, 18.31.

(4-Chloro-[1,2,3]dithiazol-5-ylidene)-(6-methyl-3-cyano-4,5,6,7-tetrahydro-thieno[2,3-*c*]pyridin-2-yl)-amine (29). Treatment of 2-amino-3-cyano-6-methyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridine[16] **19** (965 mg, 5 mmol) with **1** (1 g, 5 mmol) and pyridine (0.8 mL) in dichloromethane (20 mL), followed by column chromatography (EtOAc/petroleum ether 8:2) furnished **29** as green needles (625 mg, 38% of yield). Mp 151–153 °C (from petroleum ether); IR (KBr) cm^{-1}: 3334, 2199, 1734, 1694, 1559, 1526, 1457, 1374, 1251, 1150, 1047, 792; ^{1}H NMR (CDCl$_3$) δ 2.46 (m, 2H), 2.58 (m, 2H), 2.80 (s, 3H), 3.67 (s, 2H). Anal. calcd for $C_{11}H_9ClN_4S_3$: C, 40.17; H, 2.76; Cl, 10.78; N, 17.04; S, 29.25; found: C, 40.08; H, 2.71; Cl, 10.72; N, 16.96; S, 29.18.

General procedure for the reaction of *N*-heterocyclicimino-1,2,3-dithiazoles (20–26 and 28) with sodium methoxide

A stirred mixture of iminodithiazole (**20**–**26** and **28**) (1 mmol) and sodium methoxide (2 mmol) in methanol (6 mL) was heated at reflux for 36–60 h. After this time, the solution was filtered, the solvent was evaporated from the filtrate and the residue purified by column chromatography.

4-Methoxy-1-methyl-1H-pyrazolo[3,4-*d*]pyrimidine-6-carbonitrile (30). Following the general procedure reported above, treatment of **20** (258 mg, 1 mmol) with MeONa (108 mg, 2 mmol) in MeOH (6 mL) for 48 h followed by column chromatography (EtOAc/petroleum ether 1:1) gave the compound **30** (230 mg, 61% of yield) as a white solid. Mp 192–195 °C, IR (KBr) cm^{-1}: 3436, 1595, 1559, 1369, 1080, 790; ^{1}H NMR (CDCl$_3$) δ 4.14 (s, 3H), 4.22 (s, 3H), 8.12 (s, 1H). Anal. calcd for $C_8H_7N_5O$: C, 50.79; H, 3.73; N, 37.02; found: C, 50.71; H, 3.66; N, 36.93.

4-Methoxy-1-phenyl-1H-pyrazolo[3,4-*d*]pyrimidine-6-carbonitrile (31). Following the general procedure reported above, treatment of **21** (320 mg, 1 mmol) with MeONa (108 mg, 2 mmol) in MeOH (6 mL) for 36 h followed by column chromatography (EtOAc/petroleum ether 2:8) gave the compound **31** (168 mg, 67% of yield) as a white solid. Mp 120–123 °C, IR (KBr) cm^{-1}: 2924, 1592, 1550, 1483, 1372, 1193, 762. ^{1}H NMR (CDCl$_3$) δ 4.25 (s, 3H), 7.44 (t, *J* = 7.2 Hz, 1H), 7.57 (t, *J* = 7.56 Hz, 2H), 8.16 (d, *J* = 8 Hz, 2H), 8.30 (s, 1H). Anal. calcd for $C_{13}H_9N_5O$: C, 62.15; H, 3.61; N, 27.87; found: C, 62.08; H, 3.53; N, 27.79.

4-Methoxy-1-benzyl-1H-pyrazolo[3,4-*d*]pyrimidine-6-carbonitrile (32). Following the general procedure reported above, treatment of **22** (334 mg, 1 mmol) with MeONa (108 mg, 2 mmol) in MeOH (6 mL) for 48 h followed by column chromatography (EtOAc/petroleum ether 3:7) gave the compound **32** (188 mg, 71% of yield) as a white solid. Mp 116–118 °C, IR (KBr) cm^{-1}: 1595, 1561, 1473, 1395, 1375, 1248, 1059, 971, 791, 719.^{1}H NMR (CDCl$_3$) δ 4.19 (s, 3H), 5.63 (s, 2H), 7.31 (m, 5H), 8.11 (s, 1H). Anal. calcd for $C_{14}H_{11}N_5O$: C, 63.39; H, 4.18; N, 26.40; found: C, 63.31; H, 4.11; N, 27.69.

4-Methoxy-1-phenethyl-1H-pyrazolo[3,4-d]pyrimidine-6-carbonitrile (33). Following the general procedure reported above, treatment of **23** (348 mg, 1 mmol) with MeONa (108 mg, 2 mmol) in MeOH (6 mL) for 48 h followed by column chromatography (EtOAc/petroleum ether 1:1) gave the compound **33** (188 mg, 71% of yield) as a white solid. Mp 98–100 °C, IR (KBr) cm^{-1}: 1600, 1560, 1476, 1397, 1378, 1305, 968, 788, 696; ^{1}H NMR (CDCl$_3$) δ 3.25 (t, *J* = 7.4 Hz, 2H), 4.19 (s, 3H), 4.72 (t, *J* = 7.8 Hz, 2H), 7.22 (m, 5H), 8.11 (s, 1H). Anal. calcd for $C_{15}H_{13}N_5O$: C, 64.51; H, 4.69; N, 25.08; found: C, 64.42; H, 4.62; N, 25.00.

4-Methoxy-1-(3-phenyl-propyl)-1H-pyrazolo[3,4-*d*]pyrimidine-6-carbonitrile (34). Following the general procedure reported above, treatment of **24** (362 mg, 1 mmol) with MeONa (108 mg, 2 mmol) in MeOH (6 mL) for 42 h followed by column chromatography (EtOAc/petroleum ether 3:7) gave the compound **34** (164 mg, 54% of yield) as a white solid. Mp 93–95 °C, IR (KBr) cm^{-1}: 2925, 1593, 1557, 133, 1244, 962, 790. ^1H NMR ($CDCl_3$) δ 2.94 (t, $J=7.2$ Hz, 2H), 2.64 (t, $J=7.8$ Hz, 2H), 4.20 (s, 3H), 4.51 (t, $J=7$ Hz, 2H), 7.23 (m, 5H), 8.10 (s, 1H). Anal. calcd for $C_{16}H_{15}N_5O$: C, 65.52; H, 5.15; N, 23.88; found: C, 65.46; H, 4.53; N, 23.81.

4-Methoxy-1-*t*-butyl-1H-pyrazolo[3,4-*d*]pyrimidine-6-carbonitrile (35). Following the general procedure reported above, treatment of **25** (300 mg, 1 mmol) with MeONa (108 mg, 2 mmol) in MeOH (6 mL) for 48 h followed by column chromatography (EtOAc/petroleum ether 6:4) gave the compound **35** (120 mg, 52% of yield) as a white solid. Mp 100–103 °C, IR (KBr) cm^{-1}: 2995, 1598, 1546, 1373, 1240, 1043, 963, 788; ^1H NMR ($CDCl_3$) δ 1.81 (s, 9H), 4.19 (s, 3H), 8.05 (s, 1H). Anal. calcd for $C_{11}H_{13}N_5O$: C, 57.13; H, 5.67; N, 30.28; found: C, 57.04; H, 5.59; N, 30.19.

6-Methoxy-9-phenethyl-9H-purine-2-carbonitrile (36). Following the general procedure reported above, treatment of **26** (348 mg, 1 mmol) with MeONa (108 mg, 2 mmol) in MeOH (6 mL) for 48 h followed by column chromatography (EtOAc/petroleum ether 6:4) gave the compound **36** (140 mg, 52% of yield) as a white solid. Mp 85–87 °C, IR (KBr) cm^{-1}: 1612, 1556, 1388, 1356, 1290, 945, 777, 692. ^1H NMR ($CDCl_3$) δ 3.18 (t, $J=7.2$ Hz, 2H), 4.21 (s, 3H), 4.52 (t, $J=7.2$ Hz, 2H), 7.04 (m, 2H), 7.28 (m, 3H), 7.68 (s, 1H). Anal. calcd for $C_{15}H_{13}N_5O$: C, 64.51; H, 4.69; N, 25.08; found: C, 64.43; H, 4.59; N, 24.98.

7-Methoxy-3-(2-methyl-benzyl)-3H-[1,2,3]triazolo[4,5-*d*]pyrimidine-5-carbonitrile (37). Following the general procedure reported above, treatment of **28** (348 mg, 1 mmol) with MeONa (108 mg, 2 mmol) in MeOH (6 mL) for 60 h followed by column chromatography (EtOAc/petroleum ether 6:4) gave the compound **36** (194 mg, 69% of yield) as a white solid. Mp 105–110 °C, IR (KBr) cm^{-1}: 2924, 1590, 1507, 1376, 1250, 1093, 1053, 739; ^1H NMR ($CDCl_3$) δ 2.53 (s, 3H), 4.21 (s, 3H), 5.71 (s, 2H), 7.20 (m, 4H). Anal. calcd for $C_{14}H_{12}N_6O$: C, 59.99; H, 4.32; N, 29.98; found: C, 59.88; H, 4.25; N, 29.92.

Biological Evaluation

Antimicrobial activity

The in vitro antimicrobial activity of the synthesized compounds was investigated against several pathogenic representative Gram-negative bacteria (*P. aeruginosa* ATCC 9027, *E. coli* ATCC 11105, *P. mirabilis* MB 81), Gram-positive bacteria (*E. faecalis* ATCC 8043, *S. aureus* ATCC 29213), yeasts (*C. albicans* ATCC 10231, *C. utilis* ATCC 9950, *C. lipolytica* CBS 6124, *S. cerevisiae* ATCC 26785, Pichia stipitis CBS 5776) and moulds (*A. niger* L32, *Penicillium* sp. L41). MB and L strains were obtained from our collection. The minimal inhibitory concentrations (MICs) were determined by agar plate dilution method. Mueller–Hinton Agar (BBL) and Sabouraud Dextrose Agar (Difco) were employed for bacterial and fungal growth, respectively. Stock solutions of tested compounds were prepared in *N*,*N*-dimethylformamide (DMF). Inocula containing 10^5–10^6 cfu/mL of bacteria and 10^4–10^5 cfu/mL of fungi were prepared from broth cultures in log phase growth. Bacterial and fungal plates were made in triplicate and incubated at 37 and 28 °C for 48 and 72 h, respectively.

Inhibition of in vitro tumour cell growth

The human chronic myeloid leukaemia K562 and the murine lymphocytic L1210 leukemia cell lines were grown in vitro as a stationary suspension culture in RPMI 1640 medium (GIBCO BRL) supplemented with 10% FCS (GIBCO BRL), 2 mM L-glutamine (GIBCO BRL), 100 U/mL penicillin and 100 mg/mL streptomycin. To determine survival after drug exposure, exponentially growing K562 and Jurkat cells were cultured in the presence of various concentrations of the drugs and the antiproliferative activity of the drugs was evaluated after 4 days by counting surviving cells in a model ZBI Coulter Counter (Coulter Electronics, Hialeah, FL, USA). Results were expressed as IC_{50} (dose causing 50% inhibition of cell growth in treated cultures relative to untreated controls). All experiments were repeated at least twice. For each drug concentration, duplicate cell cultures were used. Vehicle or solvent controls were run with each experiment. All the compounds tested were dissolved in DMSO at 1 mg/mL immediately before the use and diluted just before addition to the cell culture.

Acknowledgements

We wish to thank Pharmacia & Upjohn for providing distamycin A and Ministero Università e Ricerca Scientifica (MURST) (60%) for generous financial support of this work. Maria del Carmen Nuñez is the recipient of a Fundaciòn Ramòn Areces fellowship. R.G. received grants by CNR PF Biotechnology, CNR Agenzia 2000 and Finalized Research funds (2001) from the Italian Ministry of Health.

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