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THE ANTIFUNGAL ACTIVITY OF 2,2′-DIAMINO-4,4′-DITHIAZOLE DERIVATIVES IS DUE TO THE POSSIBLE INHIBITION OF LANOSTEROL-14-α-DEMETHYLASE

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Aryl/alkyl sulfonylamido-, arylsulfenylamido-, arylcarboxamido- and ureido/thioureido/guanidino derivatives of 2,2′-diamino-4,4′-dithiazole were prepared by reaction of the title compound with sulfonyl/sulfenyl halides, sulfonic acid anhydrides, acyl chlorides, tosyl isocyanate, aryl/allyl isocyanates or isothiocyanates. Mono- as well as bis-derivatized compounds have been obtained. Several of the newly synthesized compounds act as effective antifungal agents against *Aspergillus* and *Candida spp.*, some of them showed activities comparable to ketoconazole (with minimum inhibitory concentrations in the range of 0.2–1.8 μg/mL) but possessed lower activity as compared to itraconazole. Greatest activity was detected against *A. niger*, and least activity against *C. albicans*. The mechanism of action of these compounds probably involves inhibition of ergosterol biosynthesis, and interaction with lanosterol-14-α-demethylase (CYP51A1), since reduced amounts of ergosterol were found by means of HPLC in cultures of the sensitive strain *A. niger* treated with some of these inhibitors. Thus, the compounds reported here and the azole antifungal derivatives might possess a similar mechanism of action at molecular level.

Keywords: 2,2′-Diamino-4,4′-dithiazole; Sulfonamides; (Thio)ureas; Antifungal compounds; Lanosterol-14-α-demethylase; Ergosterol biosynthesis inhibitors

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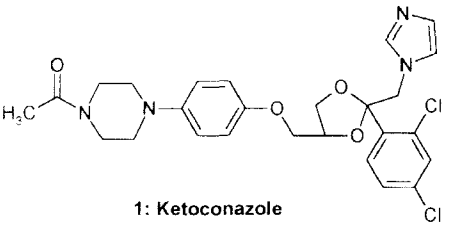
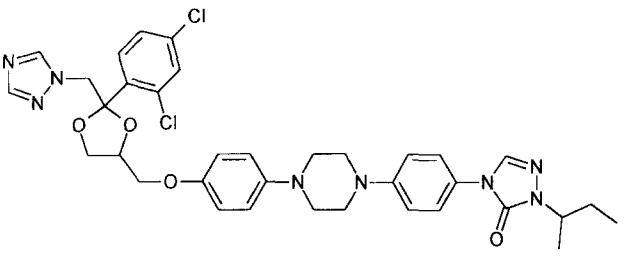
INTRODUCTION

Opportunistic fungal infections are an increasingly important cause of morbidity and mortality, with *Aspergillus* and *Candida* species being the most common such pathogens.[1] Members of the genus *Aspergillus* are associated with an impressive spectrum of diseases in humans, ranging from benign colonization of the lung to severe pathologies such as invasive aspergillosis or allergic bronchopulmonary aspergillosis.[2] Although *A. fumigatus* has been identified as the most common etiological agent in the human diseases, being considered a pathogen and allergen at the same time,[2,3] recent data showed the apparently benign *A. niger* and *flavus* to be involved in life-threatening conditions such as fungal endocarditis[4] as well as endogenous endophthalmitis, leading in many cases to an irreversible loss of visual outcome.[5] Moreover, these and other fungi developed resistance to many of the clinically used drugs, such as ketoconazole **1** or itraconazole **2**, so that novel pharmacological agents of this type are permanently needed.[3–7]

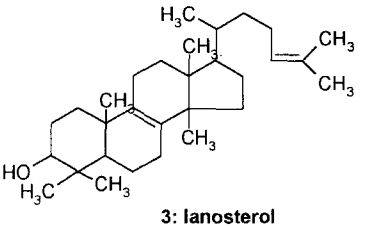
The mechanism of action of many fungistatic drugs, such as the widely clinically used azoles ketoconazole **1**, itraconazole **2**,[6–9] consists in inhibition of sterol 14-α-demethylase (CYP51A1), a microsomal cytochrome P-450 dependent enzyme system belonging to a gene superfamily involved in sterol biosynthesis in fungi, plants and animals.[10–12] CYP51A1 has been shown to catalyze the conversion of lanosterol **3** to the 14-desmethylated derivative, ergosterol **4**, through a complicated oxidative sequence involving 4,4-dimethylcholesta-8,24-dienol and 4,4-dimethyl-cholesta-8,14,24-trienol, as well as the CH_2OH, CHO and COOH derivatives corresponding to the 14-methyl carbon atom of lanosterol, followed by decarboxylation of the latter compound and release of formic acid.[13–15] Inhibition of CYP51A1 by azole antifungals causes thus depletion of ergosterol and accumulation of 14-methylsterols in the membrane of fungal cells, disturbing the membrane function and causing the death of these organisms.[1,6–12]



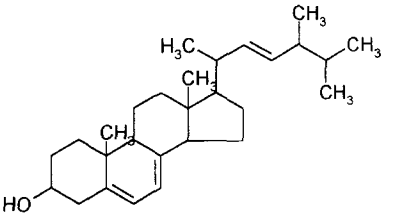
1: Ketoconazole



2: Itraconazole

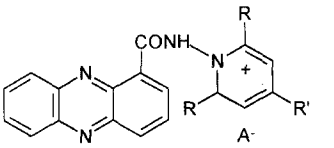


3: lanosterol



4: ergosterol

Several polynuclear heterocyclic derivatives recently reported by this group such as phenazines of type **5**,[16] phenoxathiins 6,[17] phenoxathiin-10,10-dioxides **7**,[18] or aminoglutethimide derivatives **8**[19] showed a large variety of interesting biological activity, possessing among others antifungal activity of the azole type.[16–19] Some of these compounds were shown to interact with the ergosterol synthesis pathway in the sensitive fungi *A. niger*,[17] so possessing a similar mechanism of action with the azole antifungals.



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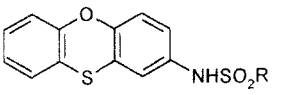

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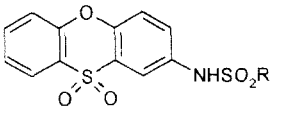

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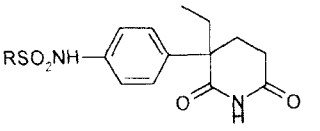

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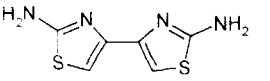

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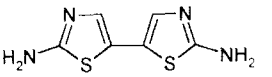

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It appeared thus of interest to extend research in this group of biologically active compounds. In this paper we report the preparation of derivatives of 2,2′-diamino4,4′-dithiazole **9** obtained by reaction of **9** with sulfonyl halides or sulfonic acid anhydrides. Related compounds were prepared from **9** and sulfenyl chlorides, acyl chlorides, aryl/allyl isocyanates and isothiocyanates.

The newly obtained compounds were assayed for inhibition of growth against several widespread fungi, such as *Aspergillus* and *Candida spp.*, showing interesting activity for some of them. For the most active compound against *A. niger*, the amount of ergosterol after treatment with different concentrations of the new and azole type inhibitors have been determined by means of HPLC. In this way it was shown that the antifungal effect of the new class of compounds is probably due to inhibition of ergosterol biosynthesis. Whether CYP51A1 is the inhibited enzyme, or whether other enzymes involved in the ergosterol pathway interact with our compounds, is for the moment an unresolved problem.

MATERIALS AND METHODS

Melting points were obtained with a heating plate microscope and are uncorrected. IR spectra were recorded in CsBr pellets with a Nicolet 2DXFT-IR apparatus. ^{1}H-NMR spectra were recorded with a Bruker CPX 200 instrument operating at 200 MHz. Elemental analysis was done by combustion with a Carlo Erba Instrument. Reverse-phase HPLC has been performed with a Beckman 1057 instrument, on a μ-Bondapak-C18 column, with acetonitrile as eluting solvent.

2,2′-Diamino-4,4′-dithiazole **9** unlike its isomer **10**,[20] is a new compound and was prepared by reaction of 1,4-dibromo-butanedione with thiourea by the general synthesis for this ring system described in the literature.[21] Ergosterol and lanosterol were from Sigma, whereas butanedione, bromine, thiourea, sulfonyl halides, sulfonic acid anhydrides, tosyl isocyanate, triethylamine, allyl isothiocyanate, 3,4-dichorophenyl isocyanate, and acyl halides were commercially available from Acros, E. Merck or Aldrich, and were used without further purification. Ketoconazole and itraconazole were from Janssen. Solvents were kept on molecular sieves to keep them anhydrous. Chromatography grade acetonitrile was from E. Merck.

Synthesis of 1,4-Dibromobutanedione 12

A volume of 50 ml (49.05 g, 0.57 mol) of butanedione **11** in 100 mL chloroform, heated at 70°C, was treated dropwise with a solution obtained from 60 mL of bromine dissolved in 60 mL chloroform, over a 3 h period. The mixture was heated at 60°C for 30 min, then left at room temperature overnight. 41 g of crystals of **12** precipitated, and were filtered off. The remaining solution was evaporated under reduced pressure and 39 g of product was further isolated. Recrystallization from chloroform yielded **12** (overall yield = 57%), as white crystals, m.p. 118–119°C (lit.[22] m.p. 118–119°C).

Synthesis of 2,2′-diamino-4,4′-dithiazole dihydrobromide (9 · 2HBr)

24.88 g (0.326 mol) of thiourea was partially dissolved in 160 mL of isopropanol by gently warming the solution on a water bath with mechanical stirring. 40 g (0.164 mol) of **12** dissolved in 335 mL isopropanol were then added and stirring was continued for 1 h. The reaction mixture was left overnight at room temperature and the precipitate was filtered off to give 59 g of dihydrobromide of **9** (yield = 98%) as tan crystals, m.p. > 300°C. Neutralization of the dihydrobromide with aqueous NaOH or sodium

ethoxide/propoxide quantitatively gave the free base **9** as tan crystals, m.p. 247–248°C (dec.). IR (KBr), cm^{-1}: 1640 (C=N), 3065 and 3320 (NH_2). ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.39 (s, 4H, $2H_2N$); 7.85 (s, 2H, 2CH thiazole). Found, C, 35.99; H, 3.40; N, 28.39. $C_6H_6N_4S_2$ requires C, 36.35; H, 3.05; N, 28.26 percent.

Synthesis of derivatives 13–50

Methods A and B

198 mg (10 mmol) of 2,2′-diamino-4,4′-dithiazole **9** suspended in 10 mL of acetonitrile were treated with 10 mmol of sulfonyl/sulfenyl or acyl chloride/carboxylic acid anhydride (Method A) or sulfonyl fluoride (Method B) dissolved in a small amount of anhydrous acetonitrile. The stoichiometric amount of triethylamine was added, and the mixture was stirred at 40°C for 4 h (A) or at 60°C for 6 h (B). The solvent was then evaporated *in vacuo* and the reaction mixture poured into 40 mL of water and ice. The precipitated derivatives were purified by repeatedly dissolving them in the stoichiometric amount of aqueous 2 N NaOH solution and reprecipitation with 5 N HCl solution. Recrystallization was generally unsuccessful due to the low solubility of these derivatives in solvents other than DMSO and DMF.

Method C

198 mg (10 mmol) of 2,2′-diamino-4,4′dithiazole **9** and 0.84 mL (5 mmol) of triflic anhydride were suspended in 10 mL of acetone and magnetically stirred at 4°C for 15 h. The solvent was then evaporated *in vacuo*, and the tan residue treated with 10 mL of cold water. The triflate salt of 2,2′-diamino-4,4′-dithiazole being water soluble was thus separated from **15** by filtration. The latter compound was recrystallized from ethanol, since it possessed an acceptable solubility in this solvent.

Method D

99 mg (5 mmol) of 2,2′diamino-4,4′-dithiazole **9** and 5 mmol of sulfobenzoic cyclic anhydride or tetrabromo-*O*-sulfobenzoic cyclic anhydride were heated under reflux in 50 mL of anhydrous acetonitrile for 2 h, with a small amount of *p*-toluenesulfonic acid added as catalyst. After evaporation of the solvent, the products **29**, **30** were recrystallized from ethanol.

Method E

99 mg (5 mmol) of 2,2′-diamino-4,4′-dithiazole **9** and 5 mmol of isocyanate or isothiocyanate were heated under reflux in 50 mL of anhydrous acetonitrile for 2–10 h, with a small amount (0.5 mL) of triethylamine added as catalyst. After evaporation of the solvent, the crude products were recrystallized from ethanol or methanol with a small amount (5%) of DMSO added to increment solubility.

Method F

99 mg (5 mmol) of 2,2′-diamino-4,4′-dithiazole dihydrobromide, **9** · 2HBr, suspended in 15 mL of ethanol, and 15 mmol of KCNO or KSCN or cyanamide dissolved in 5 mL of water were heated under reflux for 2 h. The solvent was then evaporated under reduced pressure down to half the initial volume and the reaction mixture left overnight at 4°C. The precipitated derivatives were filtered and recrystallized from ethanol.

Method G

As in Method A, but working at a molar ratio of 2,2′-diamino-4,4′-dithiazole **9**: RSO_2Cl of 1 : 2, instead of 1 : 1.

Method H

As in Method C, but working at a molar ratio of 2,2′-diamino-4,4′-dithiazole **9**: triflic anhydride of 1 : 2 and in the presence of triethylamine.

Method I

As in Method D, but working at a molar ratio of 2,2′-diamino-4,4′-dithiazole **9**: benzenesulfonic cyclic anhydride of 1 : 2, instead of 1 : 1.

Method J

As in Method E, but working at a molar ratio, of 2,2′-diamino-4,4′-dithiazole **9**: tosyl isocyanate of 1 : 2, instead of 1 : 1.

2-(N,N-Dimethylsulfamoylamido)-2′-amino-4,4′-dithiazole **13**

As tan crystals, m.p. 279–281°C (dec.). IR (KBr), cm^{-1}: 1156 (SO_2^{sym}), 1342 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.80 (s, 6H, Me_2N), 6.40 (s, 2H, H_2N), 7.85 (s, 2H, 2CH thiazole), 8.09 (s, 1H, SO_2NH). Found, C, 31.34; H, 3.54; N, 22.79. $C_8H_{11}N_5O_2S_3$ requires C, 31.46; H, 3.63; N, 22.93 percent.

***2-Phenylmethylsulfonylamido-2′-amino-4,4′-dithiazole* 14**

As tan crystals, m.p. 266–267°C. IR (KBr), cm^{-1}: 1179 (SO_2^{sym}), 1364 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.21 (s, 2H, PhCH_2), 6.40 (s, 2H, H_2N), 7.12–7.48 (m, 5H, ArH from Ph), 7.85 (s, 2H, 2CH thiazole), 8.10 (s, 1H, SO_2NH). Found, C, 44.42; H, 3.25; N, 15.80. $C_{13}H_{12}N_4O_2S_3$ requires C: 44.30; H, 3.43; N, 15.90 percent.

***2-Trifluoromethylsulfonylamido-2′-amino-4,4′-dithiazole* 15**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1169 (SO_2^{sym}), 1355 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.85 (s, 2H, 2CH thiazole), 8.38 (s, 1H, SO_2NH). Found, C, 25.30; H, 1.46; N, 16.68. $C_7H_5F_3N_4O_2S_3$ requires: C, 25.45; H, 1.53; N, 16.96 percent.

***2-(4-Flurophenylsulfonylamido)-2′-amino-4,4′-dithiazole* 16**

As tan crystals, m.p. 290–292°C (dec.). IR (KBr), cm^{-1}: 1171 (SO_2^{sym}), 1366 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.11–7.49 (m, AA′BB′, J_{AB} = 7.4 Hz, 4H, ArH, *p*-F-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.05 (s, 1H, SO_2NH). Found, C, 40.21; H, 2.70; N, 15.65. $C_{12}H_9FN_4O_2S_3$ requires: C, 40.44; H, 2.55; N, 15.72 percent.

***2-(4-Chlorophenylsulfonylamido)-2′amino-4,4′-dithiazole* 17**

As tan crystals, m.p. 291–292°C (dec.). IR (KBr), cm^{-1}: 1175 (SO_2^{sym}), 1367 (SO_2^{as}), 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.09–7.44 (m, AA′BB′, J_{AB} = 7.4 Hz, 4H, ArH, *p*-Cl-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.06 (s, 1H, SO_2NH). Found, C, 38.53; H, 2.69; N, 15.00. $C_{12}H_9ClN_4O_2S_3$ requires: C, 38.65; H, 2.43; N, 15.03 percent.

***2-(4-Bromophenylsulfonylamido)-2′-amino-4,4′-dithiazole* 18**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1179 (SO_2^{sym}), 1376 (SO_2^{as}), 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.12–7.59 (m, AA′BB′, J_{AB} = 7.4 Hz, 4H, ArH, *p*-Br-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.10 (s, 1H, SO_2NH). Found, C, 34.19; H, 2.10; N, 13.25. $C_{12}H_9BrN_4O_2S_3$, requires: C, 34.54; H, 2.17; N, 13.43 percent.

***2-(4-Iodophenylsulfonylamido)-2′-amino-4,4′-dithiazole* 19**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1185 (SO_2^{sym}), 1380 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.17–7.48 (m, AA′BB′, J_{AB} = 7.4 Hz, 4H, ArH, *p*-I-phenylene), 7.85 (s, 2H, 2CH thiazole),

8.09 (s, 1H, SO_2NH). Found, C, 31.12; H, 1.83; N, 12.20. $C_{12}H_9IN_4O_2S_3$ requires: C, 31.04; H, 1.95; N, 12.07 percent.

***2-p-Tosylamido-2′-amino-4,4′-dithiazole* 20**

As tan crystals, m.p. >278–279°C. IR (KBr), cm^{-1}: 1165 (SO_2^{sym}), 1350 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me from tosyl) 6.40 (s, 2H, H_2N), 7.17–7.49 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-Me-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.08 (s, 1H, SO_2NH). Found, C, 44.25; H, 3.46; N, 15.82. $C_{13}H_{12}N_4O_2S_3$ requires: C, 44.30; H, 3.43; N, 15.90 percent.

***2-(4-Nitrophenylsulfonylamido)-2′-amino-4,4′-dithiazole* 21**

As yellow crystals, m.p. > 300°C. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1350 (NO_2), 1366 (SO_2^{as}), 1525 (NO_2), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.08–7.49 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-O_2N-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.13 (s, 1H, SO_2NH). Found, C, 37.56; H, 2.45; N, 18.15. $C_{12}H_9N_5O_4S_3$ requires: C, 37.59; H, 2.37; N, 18.27 percent.

***2-(3-Nitrophenylsulfonylamido)-2′-amino-4,4′-dithiazole* 22**

As yellow crystals, m.p. >282–284°C. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1355 (NO_2), 1374 (SO_2^{as}), 1525 (NO_2), 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.08–7.50 (m, 4H, ArH, *m*-O_2N-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.12 (s, 1H, SO_2NH). Found, C, 37.28; H, 2.34; N, 18.20. $C_{12}H_9N_5O_4S_3$ requires: C, 37.59; H, 2.37; N, 18.27 percent.

***2-(2-Nitrophenylsulfonylamido)-2′-amino-4,4′-dithiazole* 23**

As yellow crystals, m.p. >279–281°C. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1350 (NO_2), 1362 (SO_2^{as}), 1520 (NO_2), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.00–7.54 (m, 4H, ArH, *o*-O_2N-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.09 (s, 1H, SO_2NH). Found, C, 37.66; H, 2.08; N, 18.01. $C_{12}H_9N_5O_4S_3$ requires: C, 37.59; H, 2.37; N, 18.27 percent.

***2-(3-Chloro-4-nitrophenylsulfonylamido)-2′-amino-4,4′-dithiazole* 24**

As yellow crystals, m.p. >290–292°C. IR (KBr), cm^{-1}: 1171 (SO_2^{sym}), 1350 (NO_2), 1369 (SO_2^{as}), 1525 (NO_2), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.08–7.69 (m, 3H, ArH, 3-Cl-4-O_2N-phenyl), 7.85 (s, 2H, 2CH thiazole), 8.10 (s, 1H, SO_2NH). Found, C, 34.21; H, 1.60; N, 16.52. $C_{12}H_8ClN_5O_4S_3$ requires: C, 34.49; H, 1.93; N, 16.76 percent.

***2-(4-Acetylaminophenylsulfonylamido)-2′-amino-4,4′-dithiazole* 25**

As tan crystals, m.p. $>300°C$. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1291 (amide III), 1350 (SO_2^{as}), 1533 (amide II), 1680 (amide I); 3066 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.83 (s, 3H, Me from Ac), 6.40 (s, 2H, H_2N), 7.07–7.50 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-AcNH-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.08 (s, 1H, SO_2NH). Found, C, 42.75; H, 3.14; N, 17.65. $C_{14}H_{13}N_5O_3S_3$ requires: C, 42.52; H, 3.31; N, 17.71 percent.

***2-(4-Aminophenylsulfonylamido)-2′-amino-4,4′-dithiazole* 26**

As tan crystals, m.p. $>300°C$. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1347 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.42 (s, 2H, H_2N-phenylene), 6.40 (s, 2H, H_2N-thiazole), 7.05–7.50 (m, AA′BB′, $J_{AB} = 7.3$ Hz, 4H, ArH, *p*-H_2N-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.11 (s, 1H, SO_2NH). Found, C, 40.51; H, 3.14; N, 19.57. $C_{12}H_{11}N_5O_2S_3$ requires: C, 40.78; H, 3.14; N, 19.81 percent.

***2-(3-Aminophenylsulfonylamido)-2′-amino-4,4′-dithiazole* 27**

As tan crystals, m.p. 287–290°C (dec.). IR (KBr), cm^{-1}: 1172 (SO_2^{sym}), 1360 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.11 (s, 2H, H_2N-phenylene), 6.40 (s, 2H, H_2N-thiazole), 7.21–7.45 (m, 4H, ArH, *m*-H_2N-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.09 (s, 1H, SO_2NH). Found, C, 40.82; H, 3.02; N, 19.76. $C_{12}H_{11}N_5O_2S_3$ requires: C, 40.78; H, 3.14; N, 19.81 percent.

***2-Pentaflurophenylsulfonylamido-2′-amino-4,4′-dithiazole* 28**

As tan crystals, m.p. 275–277°C (dec.). IR (KBr), cm^{-1}: 1156 (SO_2^{sym}), 1330 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.85 (s, 2H, 2CH thiazole), 8.29 (s, 1H, SO_2NH). Found, C, 33.27; H, 1.20; N, 12.87. $C_{12}H_5F_5N_4O_2S_3$ requires: C, 33.65; H, 1.18; N, 13.08 percent.

***2-(2-Carboxyphenylsulfonylamido)-2′-amino-4,4′-dithiazole* 29**

As tan crystals, m.p. $>300°C$. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1355 (SO_2^{as}), 1720 (COOH), 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.15–7.43 (m, 4H, ArH, *o*-HOOC-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.08 (s, 1H, SO_2NH), 10.12 (br s, 1H, COOH). Found, C, 40.54; H, 2.41; N, 14.35. $C_{13}H_{10}N_4O_4S_3$ requires: C, 40.83; H, 2.64; N, 14.65 percent.

***2-(2-Carboxytetrabromophenylsulfonylamido)-2′-amino-4,4′-dithiazole* 30**

As tan crystals, m.p. $>300°C$. IR (KBr), cm^{-1}: 1184 (SO_2^{sym}), 1371 (SO_2^{as}), 1720 (COOH); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H,

H_2N), 7.85 (s, 2H, 2CH thiazole), 8.10 (s, 1H, SO_2NH), 10.27 (br s, 1H, COOH). Found, C, 22.16; H, 0.80; N, 8.05. $C_{13}H_6Br_4N_4O_4S_3$ requires: C, 22.37; H, 0.87; N, 8.03 percent.

***2-(4-Methoxyphenylsulfonylamido)-2′-amino-4,4′-dithiazole* 31**

As tan crystals, m.p. 287–289°C (dec.). IR (KBr), cm^{-1}: 1167 (SO_2^{sym}), 1357 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.50 (s, 3H, Me), 6.40 (s, 2H, H_2N), 7.05–7.48 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-MeO-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.12 (s, 1H, SO_2NH). Found, C, 42.58; H, 3.02; N, 15.15. $C_{13}H_{12}N_4O_3S_3$ requires: C, 42.38; H, 3.28; N, 15.21 percent.

***2-(2,4,6-Trimethylphenylsulfonylamido)-2′-amino-4,4′-dithiazole* 32**

As tan crystals, m.p. 270–271°C. IR (KBr), cm^{-1}: 1169 (SO_2^{sym}), 1358 (SO_2^{as}), 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, 4-Me), 2.71 (s, 6H, 2,6-Me_2), 6.40 (s, 2H, H_2N), 7.35 (s, 2H, ArH, 3,5-H from mesityl), 7.85 (s, 2H, 2CH thiazole), 8.08 (s, 1H, SO_2NH). Found, C, 47.65; H, 4.53; N, 14.61. $C_{15}H_{16}N_4O_2S_3$ requires: C, 47.35; H, 4.24; N, 14.72 percent.

***2-(N,N-Diphenylcarbamoylamido)-2′-amino-4,4′-dithiazole* 33**

As tan crystals, m.p. 273–274°C. IR (KBr), cm^{-1}: 1295 (amide III), 1520 (amide II), 1680 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 6.61 (br s, 1H, CONH), 7.18–7.43 (m, 10H, ArH from 2Ph), 7.85 (s, 2H, 2CH thiazole). Found, C, 50.52; H, 3.24; N, 16.11. $C_{19}H_{15}N_5OS_2$ requires: C, 50.33; H, 3.52; N, 16.30 percent.

***2-(Isonicotinoylamido)-2′-amino-4,4′-dithiazole* 34**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1294 (amide III), 1545 (amide II), 1680 (amide I), 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.15–7.72 (m, AA′BB′, $J_{AB} = 7.9$ Hz, 4H, ArH), 7.85 (s, 2H, 2CH thiazole), 7.98 (s, 1H, CONH). Found, C, 47.21; H, 3.06; N, 22.82. $C_{12}H_9N_5OS_2$ requires: C, 47.51; H, 2.99; N, 23.09 percent.

***2-(Nicotinoylamido)-2′-amino-4,4′-dithiazole* 35**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1290 (amide III), 1540 (amide II), 1677 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.03–7.60 (m, 4H, ArH from nicotinoyl), 7.85 (s, 2H, 2CH thiazole), 8.03 (s, 1H, CONH). Found, C, 47.36; H, 3.00; N, 22.76. $C_{12}H_9N_5OS_2$ requires: C, 47.51; H, 2.99; N, 23.09 percent.

***2-(2,4-Dichlorophenylcarboxamido)-2′-amino-4,4′-dithiazole* 36**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1300 (amide III), 1543 (amide II), 1720 (amide I), 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.40 (s, 2H, H_2N), 7.07–7.69 (m, 3H, ArH), 7.85 (s, 2H, 2CH thiazole), 7.95 (s, 1H, CONH). Found, C, 42.34; H, 2.29; N, 15.08. $C_{13}H_8Cl_2N_4OS_2$ requires: C, 42.06; H, 2.17; N, 15.09 percent.

***2-(3,4-Dichlorophenylureido)-2′-amino-4,4′-dithiazole* 37**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1290 (amide III), 1550 (amide II), 1736 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.23 (s, 2H, HN–CO–NH), 6.40 (s, 2H, H_2N), 7.25–7.50 (m, 3H, ArH, dichlorophenyl), 7.85 (s, 2H, 2CH thiazole). Found, C, 40.30; H, 2.45; N, 18.05. $C_{13}H_9Cl_2N_5OS_2$ requires: C, 40.42; H, 2.35; N, 18.13 percent.

***2-[4-(Tosylsulfonylureido)]-2′-amino-4,4′-dithiazole* 38**

As pale yellow crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1290 (amide III), 1364 (SO_2^{as}), 1570 (amide II), 1735 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me from tosyl), 5.83 (s, 2H, HN–CO–NH), 6.40 (s, 2H, H_2N), 7.05–7.48 (m, AA′BB′, $J_{AB}=7.1$ Hz, 4H, ArH, phenylene from tosyl), 7.85 (s, 2H, 2CH thiazole). Found, C, 42.80; H, 3.03; N, 18.04 $C_{14}H_{13}N_5O_3S_3$ requires: C, 42.52; H, 3.31; N, 17.71 percent.

***2-(Allylthioureido)-2′-amino-4,4′-dithiazole* 39**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1040 (thioamide III), 1554 (thioamide I), 3294 (NHCSNH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.45–4.60 (m, 2H, CSNHCH_2), 5.60–5.99 (m, 2H, CH=CH_2), 6.40 (s, 2H, H_2N), 6.60–6.89 (br s, 2H, NHCSNH), 7.85 (s, 2H, 2CH thiazole). Found, C, 40.29; H, 3.48; N, 23.25. $C_{10}H_{11}N_5S_3$ requires: C, 40.38; H, 3.73; N, 23.55 percent.

***2-(4-Nitrobenzenesulfenylamido)-2′-amino-4,4′-dithiazole* 40**

As yellow crystals, m.p. 291–293°C (dec.). IR (KBr), cm^{-1}: 1075 and 1350 (NO_2), 1490 and 1520 (NO_2), 1580 (C=C), 3230 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.19 (br s, 1H, SNH), 6.40 (s, 2H, H_2N), 7.15–7.46 (m, AA′BB′, 4H, ArH from nitro-phenylene), 7.85 (s, 2H, 2CH thiazole). Found, C, 41.12; H, 2.80; N, 19.83. $C_{12}H_9N_5O_2S_3$ requires: C, 41.01; H, 2.58; N, 19.93 percent.

***4-(2-Nitrobenzenesulfenylamido)-2′-amino-4,4′-dithiazole* 41**

As yellow crystals, m.p. 285–286°C (dec.). IR (KBr), cm^{-1}: 1080 and 1350 (NO_2), 1490, 1520 (NO_2), 1585 (C=C), 3260 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.24 (br s, 1H, NH), 6.40 (s, 2H, H_2N), 7.29–7.66 (m, 4H, ArH from *ortho*-substituted phenyl), 7.85 (s, 2H, 2CH thiazole). Found, C, 41.00; H, 2.34; N, 19.61. $C_{12}H_9N_5O_2S_3$ requires: C, 41.01; H, 2.58; N, 19.93 percent.

***2,2′-Bisureido-4,4′-dithiazole* 42**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1290 (amide III), 1560 (amide II), 1730 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.96 (br s, 6H, 2HN–CO–NH_2), 7.85 (s, 2H, 2CH thiazole). Found, C, 33.52; H, 2.79; N, 29.63. $C_8H_8N_6O_2S_2$ requires: C, 33.80; H, 2.84; N, 29.56 percent.

***2,2′-Bisthioureido-4,4′-dithiazole* 43**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1044 (thioamide III), 1553 (thioamide I), 3302 (NHCSNH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.51–6.96 (br s, 6H, 2NHCSNH_2), 7.85 (s, 2H, 2CH thiazole). Found, C, 30.29; H, 2.18; N, 26.45. $C_8H_8N_6S_4$ requires: C, 30.36; H, 2.55; N, 26.56 percent.

***2,2′-Bisguanido-4,4′-dithiazole* 44**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1035, 1545, 1710, 3260; ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.20–6.59 (br s, 8H, $2H_2NC(=NH)NH$–), 7.85 (s, 2H, 2CH thiazole). Found, C, 34.21; H, 3.40; N, 39.58. $C_8H_{10}N_8S_2$ requires: C, 34.03; H, 3.57; N, 39.69 percent.

***2,2′-Bisacetamido-4,4′-dithiazole* 45**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1295 (amide III), 1540 (amide II), 1680 (amide I) 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.95 (s, 6H, 2Me from 2Ac), 7.85 (s, 2H, 2CH thiazole). Found, C, 42.72; H, 3.27; N, 19.70. $C_{10}H_{10}N_4O_2S_2$ requires: C, 42.54; H, 3.57; N, 19.84 percent.

***2,2′-Bistrifluoromethylsulfonylamido-4,4′-dithiazole* 46**

As tan crystals, m.p. >300°C. IR (KBr), cm^{-1}: 1173 (SO_2^{sym}), 1355 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.85 (s, 2H, 2CH thiazole), 8.39 (s, 2H, $2SO_2NH$). Found, C, 20.60; H, 0.69; N, 12.04. $C_8H_4F_6N_4O_4S_4$ requires: C, 20.78; H, 0.87; N, 12.12 percent.

***2,2′-Bispentafluorolphenylsulfonylamido-4,4′-dithiazole* 47**

As tan crystals, m.p. 266–269°C (dec.). IR (KBr), cm^{-1}: 1162 (SO_2^{sym}), 1339 (SO_2^{as}). 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.85 (s, 2H, 2CH thiazole), 8.45 (s, 2H, $2SO_2NH$). Found, C, 32.75; H, 0.56; N, 8.50. $C_{18}H_4F_{10}N_4O_4S_4$ requires: C, 32.83; H, 0.61; N, 8.51 percent.

***2,2′-Bis(2-carboxyphenylsulfonylamido)-4,4′-dithiazole* 48**

As tan crystals, m.p. > 300°C. IR (KBr), cm^{-1}: 1166 (SO_2^{sym}), 1350 (SO_2^{as}), 1720 (COOH), 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.15–7.43 (m, 8H, ArH, 2*o*-HOOC-phenylene), 7.85 (s, 2H, 2CH thiazole), 8.12 (s, 2H, $2SO_2NH$), 10.21 (br s, 1H, COOH). Found, C, 42.27; H, 2.40; N, 9.71. $C_{20}H_{14}N_4O_8S_4$ requires: C, 42.40; H, 2.49; N, 9.89 percent.

***2,2′-Bis(2-carboxytetrabromophenylsulfonylamido)-4,4′-dithiazole* 49**

As tan crystals, m.p. > 300°C (dec.). IR (KBr), cm^{-1}: 1188 (SO_2^{sym}), 1370 (SO_2^{as}), 1720 (COOH), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.85 (s, 2H, 2CH thiazole), 8.30 (s, 2H, $2SO_2NH$), 10.48 (br s, 1H, COOH). Found, C, 20.12; H, 0.60; N, 4.49. $C_{20}H_6Br_8N_4O_8S_4$ requires: C, 20.06; H, 0.50; N, 4.68 percent.

***2,2′-Bis[4-(tosylsulfonylureido)]-4,4′-dithiazole* 50**

As tan crystals, m.p. > 300°C. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}), 1296 (amide III), 1362 (SO_2^{as}), 1575 (amide II), 1740 (amide I), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 6H, 2Me from 2 tosyl), 5.20–5.96 (br s, 4H, 2 HN–CO–NH), 7.05–7.48 (m, AA′BB′, $J_{AB} = 7.1$ Hz, 8H, ArH, 2 phenylene from 2 tosyl), 7.85 (s, 2H, 2CH thiazole). Found, C, 44.49; H, 3.62; N, 13.97. $C_{22}H_{20}N_6O_6S_4$ requires: C, 44.58; H, 3.40; N, 14.18 percent.

Assay of Fungistatic Activity of Compounds 12–50

Fungistatic activity was determined by a modification of the growth method recently reported by us,[16–19,23] utilizing two *Aspergillus* and one *Candida spp.* Minimum inhibitory concentrations (MICs) were determined by the agar dilution method with Iso-Sensitest agar as described by Kinsman *et al.*[24] The fungi/moulds were cultivated in agar plates at 37°C for 5 days, in the nutrient broth (NB, Diagnostic Pasteur), in the absence and in the presence of 100–0.01 μg/mL of compounds **12–50**. Stock solutions of inhibitors were obtained in DMSO (100 mg/mL) and dilutions up to 0.01 μg/mL were done with distilled deionized water. The minimum

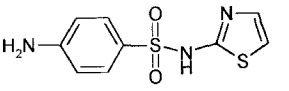
concentration at which no growth was observed was taken as the MIC value (μg/mL), and represents the mean of at least two determinations. Standard deviations were generally around 2–3% (data not shown).

Assay of Sterols Present in the Fungi Cultures

A reverse-phase HPLC method adapted from the literature,[25] was used to determine the amount of sterols (ergosterol **4** and lanosterol **3**) present in the fungi cultures. HPLC was performed with a Beckman 1057 instrument, using a Rheodyne pump and column (reverse phase μ-Bondapak C18). The fungi were cultivated as mentioned above for 5 days, with or without inhibitors added to the nutrient broth. Culture media were suspended in a small volume of MOPS buffer (pH 7.4) and the cells centrifuged at 20 000× *g* for 30 min. Cells were weighed (wet paste) and broken by sonication. Sterols present in the homogenate were then extracted in chloroform, the solvent evaporated to a small volume and the extracts applied on a μ-Bondapak-C18 column, with acetonitrile as eluting solvent. Authentic ergosterol and lanosterol (from Sigma) were used as standards. The flow rate was of 3 mL/min. The retention times were: 8.87 min for ergosterol; and 7.62 min for lanosterol, respectively. Blank assay were done for cultures which were not treated with inhibitors in order to assess the normal levels of sterols present. The amount of ergosterol present in the same amount of wet cells from the culture grown in the absence of inhibitor was taken as 100%.[26,27]

RESULTS AND DISCUSSION

Although sulfathiazole **51** is an antibacterial sulfonamide[28,29] widely used clinically, derivatives containing the dithiazole ring system have been relatively little studied, so that the 2,2′-diamino-4,4′-dithiazole **9** has not been reported previously, whereas the isomeric 2,2′-diamino-5,5′dithiazole **10** and its bis-phthalimido derivative were prepared in 1951 by Beyer *et al.*,[20] although their biological activity has not been evaluated.



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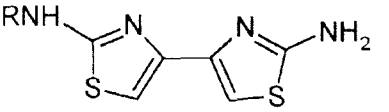
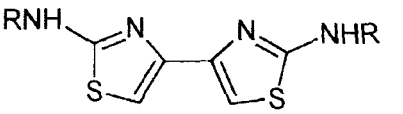
It appeared thus of great interest to synthesize derivatives containing the 4,4′-dithiazole ring system and evaluate them for biological activity. Thus, 2,2′-diamino-4,4′-dithiazole **9** has been synthesized by a classical ring

closure reaction involving condensation of 1,4-dibromo-butanedione with thiourea.[21] The obtained dihydrobromide was subsequently transformed into the free base **9**, which has been further derivatized at one or both amino groups by reaction with sulfonyl/sulfenyl halides, sulfonic acid anhydrides, acyl chlorides, tosyl isocyanate, aryl/allyl isocyanates or isothiocyanates, potassium cyanate/thiocyanate or cyanamide. The obtained derivatives **13–50** as well as their synthetic method are shown in Table I.

TABLE I Compounds **13–50** prepared in the present study and their methods of synthesis



13–41



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Compound	*R*	*Yield*	*Synthetic method*
13	Me_2NSO_2	26	A
14	$PhCH_2SO_2$	31	B
15	CF_3SO_2	15	C
16	*p*-F-C_6H_4-SO_2	62	A
17	*p*-Cl-C_6H_4-SO_2	66	A
18	*p*-Br-C_6H_4-SO_2	61	A
19	*p*-I-C_6H_4-SO_2	80	A
20	*p*-CH_3-C_6H_4-SO_2	54	A
21	*p*-O_2N-C_6H_4-SO_2	60	A
22	*m*-O_2N-C_6H_4-SO_2	49	A
23	*o*-O_2N-C_6H_4-SO_2	48	A
24	3-Cl-4-O_2N-C_6H_3-SO_2	57	A
25	*p*-AcNH-C_6H_4-SO_2	42	A
26	*p*-H_2N-C_6H_4-SO_2	19	B
27	*m*-H_2N-C_6H_4-SO_2	22	B
28	C_6F_5-SO_2	56	A
29	*o*-HOOC-C_6H_4-SO_2	89	D
30	*o*-HOOC-C_6Br_4-SO_2	84	D
31	*p*-CH_3O-C_6H_4-SO_2	60	A
32	2,4,6-$(CH_3)_3$-C_6H_2-SO_2	35	A
33	Ph_2N-CO	70	A
34	Isonicotinoyl	51	A
35	Nicotinoyl	43	A
36	2,4-$Cl_2C_6H_3CO$	37	A
37	3,4-$Cl_2C_6H_3NHCO$	85	E
38	*p*-Me-$C_6H_4SO_2NHCO$	97	E
39	CH_2=$CHCH_2NHCS$	40	E
40	*p*-O_2N-C_6H_4-S	51	A
41	*o*-O_2N-C_6H_4-S	43	A
42	H_2N-CO	76	F
43	H_2N-CS	79	F
44	H_2N-C(=NH)	52	F
45	CH_3CO	84	G

TABLE I (*Continued*)

Compound	*R*	*Yield*	*Synthetic method*
46	CF_3SO_2	25	H
47	C_6F_5-SO_2	40	G
48	*o*-HOOC-C_6H_4-SO_2	79	I
49	*o*-HOOC-C_6Br_4-SO_2	85	I
50	*p*-Me-$C_6H_4SO_2NHCO$	94	J

A – 2,2′-diamino-4,4′-dithiazole + RSO_2Cl (or RCOCl, or $(RCO)_2O$ or RSCl); B – 2,2′-diamino-4,4′-dithiazole + RSO_2F; C – 2,2′-diamino-4,4′-dithiazole + triflic anhydride; D – 2,2′-diamino-4,4′-dithiazole + sulfobenzoic cyclic anhydride; E – 2,2′-diamino-4,4′dithiazole + RNCO (or RNCS); F – 2,2′-diamino-4,4′-dithiazole dihydrobromide + KCNO (or KSCN, or cyanamide); G – 2,2′-diamino-4,4′-dithiazole + 2 RSO_2Cl (or 2 RCOCl); H – 2,2′-diamino-4,4′-dithiazole + 2 moles of triflic anhydride; I – 2,2′-diamino-4,4′-dithiazole + 2 moles of sulfobenzoic cyclic anhydride; J – 2,2′-diamino-4,4′-dithiazole + 2 moles of TsNCO.

Since many compounds containing alkyl/arylsulfonylamido and related moieties in their molecule, recently reported by this group, such as **6**,[17] **7**[18] and **8**,[19] possessed interesting antifungal activity, the new derivatives **13–50** reported here have also been assayed for their capacity to inhibit the growth of three wide-spread fungi species, i.e., *Aspergillus flavus*, *A. niger* and *Candida albicans*.

Biological activity data with the new derivatives **13–50** and the standard azole CYP51A1 inhibitors, ketoconazole **1** and itraconazole **2** are shown in Table II.

From the data of Table II, it should be noted that the new compounds **13–50** reported here represent a new class of antifungals, with good antifungal activity against *Aspergilli* (for the most active derivatives of the series, such as **28–30**, **37–39** and **45–47**) but much less activity than the azoles against *Candida albicans*. In this respect compounds **13–50** show the same activity profile as the recently reported phenoxathiin and phenoxathiin-10,10-dioxide derivatives **6** and **7**.[17,18] Groups substituting the amino moieties of **9** which led to good antifungal activity in the new compounds **13–50** included: tryfluoromethylsulfonyl, pentafluorophenylsulfonyl, dichlorophenylureido or 2-carboxyphenylsulfonylamido among others. Generally the bis-substituted derivatives were less active as compared to the corresponding mono-substituted derivatives (compare for instance the pairs: **29–48**, **30–49** or **38–50**), and this was the reason that prompted us not to show here the bis-substituted derivatives synthesized, which possessed poor biological activity (data not shown). An important exception from the above rule is represented by the polyfluorinated derivatives (the compound pairs **15–46** and **28–47**), where the bis-substituted compounds possessed a greater inhibitory activity as compared to the mono-substituted derivatives

TABLE II Antifungal activity of compounds **12–50** against several organisms

Compound	*MIC* (μg/mL)		
	A. flavus C1150	*A. niger* C418	*Candida albicans* C316
12	25	20	35
13	12	12	9
14	11	9	15
15	9	8	10
16	13	9	12
17	8	7	10
18	9	6	10
19	8	5	9
20	18	8	10
21	6	6	4
22	4	6	5
23	11	9	7
24	2	2	5
25	14	10	14
26	12	9	15
27	15	11	16
28	1	0.5	1.5
29	2	1	3
30	2	1	1.5
31	15	7	15
32	15	5	13
33	9	8	14
34	11	10	12
35	12	7	10
36	6	5	8
37	5	1.5	6
38	1	0.5	3
39	2	1.5	4
40	4	2	3
41	0.5	0.2	1.5
42	7	5	12
43	5	4	9
44	3	2	7
45	3	1.5	4
46	0.5	0.3	0.8
47	0.4	0.2	1.5
48	21	19	24
49	20	17	21
50	23	19	23
Ketoconazole **1**	1.2	1.8	0.06
Itraconazole **2**	0.9	0.2	0.02

against all three investigated organisms. For all the new derivatives reported here, the nature of the substituent in the 2-position greatly influenced biological activity, but effective compounds were found in all the chemical classes investigated here (i.e. the sulfonamides **28–30**, the ureas **37** and **38**, the thiourea **39** or the sulfenamides **40**, **41** showed comparable activities,

TABLE III Levels of ergosterol in *A. niger* cultures after treatment with different concentrations of the azole CYP51A1 inhibitor itraconazole and compounds **38** and **47**

Inhibitor	*Concentration* (μg/mL)	% *Ergosterol**
Itraconazole	0.01	78 ± 5
Itraconazole	0.05	41 ± 7
Itraconazole	0.10	11 ± 4
38	0.01	96 ± 3
38	0.10	66 ± 5
38	0.25	28 ± 6
38	0.75	8 ± 2
47	0.01	79 ± 8
47	0.05	30 ± 4
47	0.15	7 ± 2

*Mean ± standard deviation ($n = 3$); The amount of ergosterol present in the same amount of wet cells from the culture grown in the absence of inhibitor is taken as 100%.

although they belong to very different structural classes, with the carboxamides **33–35** showing moderate activity.

In order to test the hypothesis that the compounds reported here act as ergosterol biosynthesis inhibitors, similarly to the azole antifungals, the amounts of ergosterol present in *A. niger* cultures after treatment with different concentrations of new inhibitors (itraconazole **2**, a potent CYP51A1 inhibitor[11–13] has also been included in the study as standard) have been determined by means of a HPLC method (Table III).[25] These data show that at low concentrations of inhibitor around 80–96% of ergosterol (as compared to the amount of sterol formed in cultures in which inhibitors have not been added, and which was considered 100%) is still synthesized. By increasing the concentrations of inhibitors used in the experiments, the amount of synthesized ergosterol decreased dose-dependently. A similar effect has been observed for the well-known CYP51A1 inhibitor itraconazole **2** as well as for the compounds **38** and **47** synthesized in the present study. These data allow us to propose a similar mechanism of action for the two classes of antifungal compounds, i.e., the inhibition of lanosterol-14-α-demethylase, although it is not improbable that our compounds might interfere with other enzyme(s) involved in the ergosterol biosynthetic pathway.

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