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SULFONYLAMIDO DERIVATIVES OF 2-AMINOPHENOXATHIIN-10,10-DIOXIDE AND RELATED COMPOUNDS POSSESS ANTIFUNGAL ACTION DUE TO THE POSSIBLE INHIBITION OF LANOSTEROL-14-α-DEMETHYLASE

CLAUDIU T. SUPURAN[a,*], ANDREA SCOZZAFAVA[a], FABRIZIO BRIGANTI[a], GEORGE LOLOIU[b] and OVIDIU MAIOR[b]

[a] *Università degli Studi, Laboratorio di Chimica Inorganica e Bioinorganica, Via Gino Capponi 7, 50121 Firenze, Italia;* [b] *Universitatea Bucuresti, Departamentul de Chimie Organica, Sos. Panduri 92, Bucuresti, Romania*

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Aryl/alkyl-sulfonylamido-, arylsulfenylamido-, arylcarboxamido-, and ureido/thioureido derivatives of 2-aminophenoxathiin-10,10-dioxide were prepared by reaction with sulfonyl/sulfenyl halides, sulfonic acid anhydrides, acyl chlorides, tosyl isocyanate, aryl/allyl isocyanates or isothiocyanates. Some of these derivatives, containing free amino groups, were further derivatized by reaction with 2,4,6-trisubstituted-pyrylium salts, aryl/allyl isocyanate/isothiocyanates or tosyl isocyanate. Several of the newly synthesized compounds act as effective antifungal agents against *Aspergillus* and *Candida* spp., some of them showing activities comparable to ketoconazole, with minimum inhibitory concentrations in the range of 0.3–0.5 μg/mL. Their mechanism of antifungal action is hypothesized to be due to inhibition of lanosterol-14-α-demethylase.

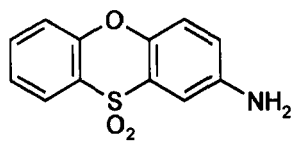
Keywords: 2-Aminophenoxathiin-10,10-dioxide; Sulfonyl halides; Iso(thio)cyanates; (Thio)ureas; Antifungal compounds; Lanosterol-14-α-demethylase

* Corresponding author. Tel.: +39-55-222007. Fax: +39-55-2757555.
E-mail: cts@as.lrm.fi.cnr.it.

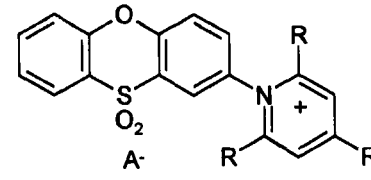
INTRODUCTION

Polynuclear heterocyclic derivatives show a large variety of interesting biological activity, many widely used drugs belonging to this category of compounds. Thus, phenothiazines were found to have antihistaminic and antipsychotic action,[1,2] dibenzazepines and related derivatives are among the most widely used antidepressant and anxiolytic agents,[1,3] but antiarrhythmic,[4] amoebicidic,[5] fungistatic,[6,7] antibiotic/anticancer[8] action for derivatives belonging to the acridine, phenazine, phenothiazine or phenoxazone ring systems have also been reported.[1–8]

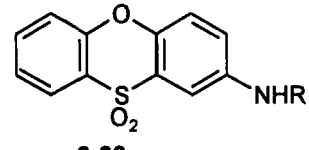
However, phenoxathiin derivatives although structurally similar to the ring systems mentioned above, have been much less investigated for their biological activity. Only recently reported by this group was the preparation of some enzyme inhibitors containing phenoxathiin-2-yl or 10,10-dioxa-phenoxathiin-2-yl moieties in their molecule.[9,10] The interesting biological activity of the above-mentioned compounds prompted us to investigate novel derivatives from this class and 2-aminophenoxathiin-10,10-dioxide **1** constituted the starting material, as it has been recently reported that its pyridinium derivatives of type **2** act as fungistatic agents.[10]



1



2: R, R' = alkyl, aryl



3-30

In this paper we report the preparation of sulfonamido derivatives of 2-aminophenoxathiin-10,10-dioxide, **3–30**, obtained by reaction of **1** with sulfonyl halides or sulfonic acid anhydrides. Related compounds were prepared from **1** and sulfenyl chlorides, acyl chlorides, aryl/allyl isocyanates and isothiocyanates. Some of the above derivatives were further derivatized by means of reactions involving pyrylium salts, allyl/aryl/tosyl isocyanates or isothiocyanates.

Since the mechanism of action of many fungistatic drugs consists in inhibition of sterol 14-α-demethylase, a microsomal cytochrome P-450 dependent enzyme system,[11] we have assayed the obtained derivatives **3–42** for their antifungal activity against several widespread fungi or moulds, such as *Aspergillus* and *Candida* spp., and found interesting activity for some of them. Although the mechanism of action of these compounds is unknown at the present time, it is not improbable that they might inhibit lanosterol-14-α-demethylase (cytochrome P-$450_{14\text{-}\alpha DM}$).[12]

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.

2-Aminophenoxathiin-10,10-dioxide was prepared by literature procedures.[13] Ketoconazole was from Janssen, whereas sulfonyl halides, sulfonic acid anhydrides, tosyl isocyanate, triethylamine, allyl isothiocyanate, 3,4-dichlorophenyl isocyanate, and acyl halides were commercially available from Acros, E. Merck or Aldrich, and were used without further purification. 2,4,6-Trimethyl-, 2,4,6-triphenyl- and 2,6-dimethyl-4-phenyl-pyrylium perchlorates were prepared by literature procedures.[14]

Synthesis of Derivatives 3–30

Methods A and B

124 mg (5 mmol) of 2-aminophenoxathiin-10,10-dioxide suspended in 10 mL of acetonitrile were treated with 5 mmol of sulfonyl/sulfenyl chloride (method A) or 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 sulfonylamido derivatives were recrystallized from ethanol–water (1 : 1, v/v).

Method C

247 mg (10 mmol) of 2-aminophenoxathiine-10,10-dioxide 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-aminophenoxathiine being water soluble was thus separated from **5** by filtration. The latter compound was recrystallized from ethanol.

Method D

124 mg (5 mmol) of 2-aminophenoxathiine-10,10-dioxide and 5 mmol of sulfobenzoic cyclic anhydride or tetrabromo-*O*-sulfobenzoic cyclic anhydride were refluxed 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 **19**, **20** were recrystallized from ethanol.

Method E

124 mg (5 mmol) of 2-aminophenoxathiine-10,10-dioxide and 10 mmol of isocyanate or isothiocyanate were refluxed in 50 mL of anhydrous acetonitrile for 2–10 h, with a small amount (0.2 mL) of triethylamine added as catalyst. After evaporation of the solvent, the crude products were recrystallized from ethanol or methanol.

Synthesis of Derivatives 31–34

2 mmol of amino-derivative **16** or **17** dissolved in 15 mL of anhydrous acetonitrile was heated to reflux and 0.47 g (2.5 mmol) of 3,4-dichlorophenyl isocyanate (or the equivalent amount of allyl isothiocyanate) dissolved in 5 mL of the same solvent was added dropwise. The mixture was refluxed for 3 h, part of the solvent evaporated and the remaining mixture left at 0°C overnight. The precipitated derivatives were filtered and recrystallized from dioxane. Yields were around 80–90%.

Synthesis of Derivatives 35 and 36

2 mmol of amino-derivative **16** or **17** dissolved in 20 mL of anhydrous acetonitrile was treated with 0.30 mL (2 mmol) tosyl isocyanate. The mixture was stirred at room temperature for 1 h, then the precipitated derivatives were filtered off and recrystallized from ethanol. Yields were over 95%.

Synthesis of Derivatives 37–42

2 mmol of amino-derivative **16** or **17** dissolved in 30 mL of anhydrous ethanol was treated with the stoichiometric amount of 2,4,6-trisubstitutedpyrylium

perchlorate dissolved in the minimum amount of the same solvent. The mixture was magnetically stirred at room temperature for 15 min, then 0.27 mL (2 mmol) of triethylamine were added and stirring was continued for another 2 h. After this time, 1.5 mL of acetic acid were added and the reaction mixture was refluxed for 3 h. After cooling, the pyridinium salt was precipitated by addition of 100 mL of diethyl ether. Filtration and recrystallization from *iso*-propanol afforded the title compounds with yields of 55–58%.

***2-(N,N-Dimethylsulfamoylamido)-phenoxathiin-10,10-dioxide* 3**

As tan crystals, mp 142–5°C. IR (KBr), cm^{-1}: 1140 and 1156 (SO_2^{sym}), 1290 and 1342 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.80 (s, 6H, Me_2N); 7.52–7.91 (m, 7H, ArH); 8.06 (s, 1H, SO_2NH). Found, C, 47.58; H, 3.75; N, 7.69. $C_{14}H_{14}N_2O_5S_2$ requires C, 47.44; H, 3.98; N, 7.90%.

***2-Phenylmethylsulfonylamido-phenoxathiin-10,10-dioxide* 4**

As tan crystals, mp 176–7°C. IR (KBr), cm^{-1}: 1156 and 1179 (SO_2^{sym}), 1300 and 1364 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.17 (s, 2H, $PhCH_2$); 7.12–7.49 (m, 5H, ArH from Ph); 7.58–7.93 (m, 7H, ArH, from phenoxathiin); 8.11 (s, 1H, SO_2NH). Found, C, 57.08; H, 3.65; N, 3.45 $C_{19}H_{15}NO_5S_2$ requires C, 56.83; H, 3.76; N, 3.48%.

***2-Trifluoromethylsulfonylamidophenoxathiin-10,10-dioxide* 5**

As colorless crystals, mp 185–6°C. IR (KBr), cm^{-1}: 1160 and 1169 (SO_2^{sym}), 1290 and 1355 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.50–7.93 (m, 7H, ArH); 8.35 (s, 1H, SO_2NH). Found, C, 41.10; H, 2.29; N, 3.48. $C_{13}H_8F_3NO_5S_2$ requires: C, 41.16; H, 2.12; N, 3.69%.

***2-(4-Fluorophenylsulfonylamido)-phenoxathiin-10,10-dioxide* 6**

As colorless crystals, mp 190–2°C. IR (KBr), cm^{-1}: 1158 and 1171 (SO_2^{sym}), 1300 and 1366 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.11–7.43 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-F-phenylene); 7.55–7.94 (m, 7H, ArH, from phenoxathiin); 8.05 (s, 1H, SO_2NH). Found, C, 53.21; H, 2.60, N, 3.45. $C_{18}H_{12}FNO_5S_2$ requires: C, 53.32; H, 2.98; N, 3.45%.

***2-(4-Chlorophenylsulfonylamido)-phenoxathiin-10,10-dioxide* 7**

As colorless crystals, mp 210–2°C. IR (KBr), cm^{-1}: 1160 and 1175 (SO_2^{sym}), 1285 and 1367 (SO_2^{as}), 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.10–7.43

(m, AA′BB′, J_{AB} = 7.4 Hz, 4H, ArH, *p*-Cl-phenylene); 7.50–7.94 (m, 7H, ArH, from phenoxathiin); 8.06 (s, 1H, SO_2NH). Found, C, 51.53; H, 2.69; N, 3.21. $C_{18}H_{12}ClNO_5S_2$ requires: C, 51.24; H, 2.86; N, 3.32%.

***2-(4-Bromophenylsulfonylamido)-phenoxathiin-10,10-dioxide* 8**

As colorless crystals, mp 215–7°C. IR (KBr), cm^{-1}: 1160 and 1179 (SO_2^{sym}), 1295 and 1376 (SO_2^{as}), 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.15–7.52 (m, AA′BB′, J_{AB} = 7.4 Hz, 4H, ArH, *p*-Br-phenylene); 7.55–7.98 (m, 7H, ArH, from phenoxathiin); 8.05 (s, 1H, SO_2NH). Found, C, 46.10; H, 2.44; N, 3.11. $C_{18}H_{12}BrNO_5S_2$ requires: C, 46.36; H, 2.59; N, 3.00%.

***2-(4-Iodophenylsulfonylamido)-phenoxathiin-10,10-dioxide* 9**

As colorless crystals, mp 224–7°C. IR (KBr), cm^{-1}: 1160 and 1185 (SO_2^{sym}), 1300 and 1380 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.17–7.48 (m, AA′BB′ J_{AB} = 7.4 Hz, 4H, ArH, *p*-I-phenylene); 7.54–7.96 (m, 7H, ArH, from phenoxathiin); 8.09 (s, 1H, SO_2NH). Found, C, 41.93; H, 2.13; N, 3.00. $C_{18}H_{12}INO_5S_2$ requires: C, 42.11; H 2.35; N, 2.72%.

***2-p-Tosylamidophenoxathiin-10,10-dioxide* 10**

As tan crystals, mp 233–4°C. IR (KBr), cm^{-1}:1156 and 1165 (SO_2^{sym}), 1300 and 1350 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me from tosyl); 7.05–7.46 (m, AA′BB′, J_{AB} = 7.4 Hz, 4H, ArH, *p*-Me-phenylene); 7.52–7.89 (m, 7H, ArH, from phenoxathiin); 8.05 (s, 1H, SO_2NH). Found, C, 56.51; H, 3.92; N, 3.50. $C_{19}H_{15}NO_5S_2$ requires: C, 56.84; H, 3.76; N, 3.48%.

***2-(4-Nitrophenylsulfonylamido)-phenoxathiin-10,10-dioxide* 11**

As yellow crystals, mp 219–21°C. IR (KBr), cm^{-1}: 1150 and 1160 (SO_2^{sym}), 1300 and 1366 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.08–7.49 (m, AA′BB′, J_{AB} = 7.4 Hz, 4H, ArH, *p*-O_2N-phenylene); 7.53–7.90 (m, 7H, ArH, from phenoxathiin); 8.10 (s, 1H, SO_2NH). Found, C, 51.92; H, 2.76; N, 6.55. $C_{18}H_{12}N_2O_7S_2$ requires: C, 51.91; H, 2.90; N, 6.72%.

***2-(3-Nitrophenylsulfonylamido)-phenoxathiin-10,10-dioxide* 12**

As yellow crystals, mp 214–6°C. IR (KBr), cm^{-1}: 1145 and 1160 (SO_2^{sym}), 1300 and 1374 (SO_2^{as}), 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.08–7.50 (m, 4H, ArH, *m*-O_2N-phenylene); 7.53–7.89 (m, 7H, ArH, from phenoxathiin); 8.10 (s, 1H, SO_2NH). Found, C, 51.58; H, 2.65; N, 6.39. $C_{18}H_{12}N_2O_7S_2$ requires: C, 51.91; H, 2.90; N, 6.72%.

***2-(2-Nitrophenylsulfonylamido)-10,10-dioxide* 13**

As yellow crystals, mp 227–9°C. IR (KBr), cm^{-1}: 1150 and 1160 (SO_2^{sym}), 1300 and 1362 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.02–7.49 (m, 4H, ArH, *o*-O_2N-phenylene); 7.55–7.97 (m, 7H, ArH, from phenoxathiin); 8.07 (s, 1H, SO_2NH). Found, C, 51.60; H, 2.94; N, 6.48. $C_{18}H_{12}N_2O_7S_2$ requires: C, 51.91; H, 2.90; N, 6.72%.

***2-(3-Chloro-4-nitrophenylsulfonylamido)-phenoxathiin-10,10-dioxide* 14**

As yellow crystals, mp 219–20°C. IR (KBr), cm^{-1}: 1160 and 1171 (SO_2^{sym}), 1298 and 1369 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.08–7.67 (m, 3H, ArH, 3-Cl-4-O_2N-phenyl); 7.56–7.98 (m, 7H, ArH, from phenoxathiin); 8.11 (s, 1H, SO_2NH). Found, C, 46.21; H, 2.60; N, 5.63. $C_{18}H_{11}ClN_2O_7S_2$ requires: C, 46.30; H, 2.37; N, 5.99%.

***2-(4-Acetylaminophenylsulfonylamido)-phenoxathiin-10,10-dioxide* 15**

As colorless crystals, mp 237–8°C. IR (KBr), cm^{-1}: 1150 and 1160 (SO_2^{sym}), 1291 (amide III), 1300 and 1350 (SO_2^{as}), 1533 (amide II); 1680 (amide I); 3066 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 1.80 (s, 3H, Me from Ac): 7.07–7.46 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-AcNH-phenylene); 7.53–7.96 (m, 7H, ArH, from phenoxathiin); 8.08 (s, 1H, SO_2NH). Found, C, 54.12; H, 3.44; N, 6.29. $C_{20}H_{16}N_2O_6S_2$ requires: C, 54.04; H, 3.62; N, 6.30%.

***2-(4-Aminophenylsulfonylamido)-phenoxathiin-10,10-dioxide* 16**

As colorless crystals, mp 239–42°C. IR (KBr), cm^{-1}: 1145 and 1160 (SO_2^{sym}), 1300 and 1347 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.42 (s, 2H, H_2N-phenylene); 7.05–7.50 (m, AA′BB′, $J_{AB} = 7.3$ Hz, 4H, ArH, *p*-H_2N-phenylene); 7.54–7.90 (m, 7H, ArH, from phenoxathiin); 8.11 (s, 1H, SO_2NH). Found, C, 53.54; H, 3.40; N, 6.59. $C_{18}H_{14}N_2O_5S_2$ requires: C, 53.72; H, 3.50; N, 6.95%.

***2-(3-Aminophenylsulfonylamido)-phenoxathiin-10,10-dioxide* 17**

As tan crystals, mp 232–3°C. IR (KBr), cm^{-1}: 1160 and 1172 (SO_2^{sym}), 1300 and 1360 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.11 (s, 2H, H_2N-phenylene) 7.21–7.45 (m, 4H, ArH, *m*-H_2N-phenylene); 7.54–7.90 (m, 7H, ArH, from phenoxathiin); 8.05 (s, 1H, SO_2NH); Found, C, 53.48; H, 3.14; N, 6.83. $C_{18}H_{14}N_2O_5S_2$ requires: C, 53.72; H, 3.50; N, 6.95%.

***2-(Pentafluorophenylsulfonylamido-phenoxathiin-10,10-dioxide* 18**

As tan crystals, mp 170–2°C (dec.). IR (KBr), cm^{-1}: 1148 and 1156 (SO_2^{sym}), 1285 and 1330 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.54–7.97 (m, 7H, ArH, from phenoxathiin); 8.38 (s, 1H, SO_2NH). Found, C, 45.26; H, 1.69; N, 2.56. $C_{18}H_8F_5NO_5S_2$ requires: C, 45.28; H, 1.68; N, 2.93%.

***2-(2-Carboxyphenylsulfonylamido)-phenoxathiin-10,10-dioxide* 19**

As tan crystals, mp 215–6°C. IR (KBr), cm^{-1}: 1151 and 1160 (SO_2^{sym}), 1300 and 1355 (SO_2^{as}), 1720 (COOH); 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.15–7.43 (m, 4H, ArH, *o*-HOOC-phenylene); 7.50–7.90 (m, 7H, ArH, from phenoxathiin); 8.05 (s, 1H, SO_2NH); 10.15 (br s, 1H, COOH). Found, C, 52.65; H, 3.40; N, 3.35. $C_{19}H_{13}NO_7S_2$ requires: C, 52.89; H, 3.03; N, 3.24%.

***2-(2-Carboxytetrabromophenylsulfonylamido)-phenoxathiin-10,10-dioxide* 20**

As tan crystals, mp 218–20°C (dec). IR (KBr), cm^{-1}: 1160 and 1184 (SO_2^{sym}), 1300 and 1371 (SO_2^{as}), 1720 (COOH); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.50–7.90 (m, 7H, ArH, from phenoxathiin); 8.09 (s, 1H, SO_2NH); 10.40 (br s, 1H, COOH). Found, C, 30.19; H, 1.30; N, 1.48. $C_{19}H_9Br_4NO_7S_2$ requires: C, 30.54; H, 1.21; N, 1.87%.

***2-(4-Methoxyphenylsulfonylamido)-phenoxathiin-10,10-dioxide* 21**

As white crystals, mp 187–8°C. IR (KBr), cm^{-1}: 1157 and 1167 (SO_2^{sym}), 1300 and 1357 (SO_2^{as}), 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.50 (s, 3H, Me); 7.05–7.48 (m, AA′BB′, $J_{AB} = 7.4$ Hz, 4H, ArH, *p*-MeO-phenylene); 7.52–7.91 (m, 7H, ArH, from phenoxathiin); 8.09 (s, 1H, SO_2NH). Found, C, 54.58; H, 3.69; N, 3.15. $C_{19}H_{15}NO_6S_2$ requires: C, 54.67; H, 3.62; N, 3.36%.

***2-(2,4,6-Trimethylphenylsulfonylamido)-phenoxathiin-10,10-dioxide* 22**

As tan crystals, mp 177–8°C. IR (KBr), cm^{-1}: 1160 and 1169 (SO_2^{sym}), 1300 and 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); 7.35 (s, 2H, ArH, 3,5-H from mesityl); 7.52–7.89 (m, 7H, ArH, from phenoxathiin); 8.05 (s, 1H, SO_2NH). Found, C, 58.65; H, 4.53; N, 3.15. $C_{21}H_{19}NO_5S_2$ requires: C, 58.73; H, 4.46; N, 3.26%.

***2-(N,N-Diphenylcarbamoylamido)-phenoxathiin-10,10-dioxide* 23**

As white crystals, mp 172–4°C. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}); 1295 (amide III); 1300 (SO_2^{as}); 1520 (amide II); 1680 (amide I); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.61 (br s, 1H, CONH); 7.18–7.43 (m, 10H, ArH, from

2 Ph); 7.58–7.96 (m, 7H, ArH, from phenoxathiin). Found, C, 67.93; H, 3.96; N, 6.11. $C_{25}H_{18}N_2O_4S$ requires: C, 67.86; H, 4.10; N, 6.33%.

***2-(Isonicotinoylamido)-phenoxathiin-10,10-dioxide* 24**

As white crystals, mp 181–3°C. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}); 1290 (amide III); 1300 (SO_2^{as}); 1540 (amide II); 1680 (amide I); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.15–7.72 (m, AA′BB′, $J_{AB} = 7.9$ Hz, 4H, ArH); 7.58–7.96 (m, 7H, ArH from phenoxathiin); 7.98 (s, 1H, CONH). Found. C, 61.28; H, 3.46; N, 7.82. $C_{18}H_{12}N_2O_4S$ requires: C, 61.36; H, 3.43; N, 7.95%.

***2-(2,4-Dichlorophenylcarboxamido)-phenoxathiin-10,10-dioxide* 25**

As white crystals, mp 168–70°C. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}); 1300 (amide III and SO_2^{as}); 1540 (amide II); 1700 (amide I); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.05–7.64 (m, 3H, ArH); 7.90 (s, 1H, CONH); 7.58–7.96 (m, 7H, ArH, from phenoxathiin). Found, C, 54.21; H, 2.69; N, 3.40. $C_{19}H_{11}Cl_2NO_4S$ requires: C, 54.30; H, 2.64; N, 3.33%.

***2-(3,4-Dichlorophenylureido)-phenoxathiin-10,10-dioxide* 26**

As white crystals, mp 222–3°C. IR (KBr), cm^{-1}: 1160 (SO_2^{sym}); 1290 (amide III); 1300 (SO_2^{as}); 1550 (amide II); 1730 (amide I); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.23 (s, 2H, HN-CO-NH); 7.25–7.39 (m, 3H, ArH, dichlorophenyl); 7.58–7.96 (m, 7H, ArH, from phenoxathiin). Found, C, 52.39; H, 2.71; N, 6.45. $C_{19}H_{12}Cl_2N_2O_4S$ requires: C, 52.43; H, 2.78; N, 6.44%.

***2-[4-(Tosylsulfonylureido)]phenoxathiin-10,10-dioxide* 27**

As colorless crystals, mp 283–5°C. IR (KBr), cm^{-1}: 1150 and 1160 (SO_2^{sym}), 1290 (amide III); 1300 and 1364 (SO_2^{as}); 1566 (amide II); 1730 (amide I); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me from tosyl); 5.20 (s, 2H, HN–CO–NH); 7.05–7.41 (m, AA′BB′, $J_{AB} = 7.1$ Hz, 4H, ArH, phenylene from tosyl); 7.58–7.96 (m, 7H, ArH from phenoxathiin). Found, C, 53.82; H, 3.60; N, 6.44. $C_{20}H_{16}N_2O_6S_2$ requires: C, 54.04; H, 3.63; N, 6.30%.

***N^1-(10,10-Dioxa-phenoxathiin-2-yl)-N^3-allyl-thiourea* 28**

As white crystals, mp 199–201°C. IR (KBr), cm^{-1}: 1040 (thioamide III), 1160 (SO_2^{sym}); 1300 (SO_2^{as}), 1547 (thioamide I), 3298 (NHCSNH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.45–4.60 (m, 2H, $CSNHCH_2$); 5.60–5.97

(m, 3H, CH=CH_2); 6.70 and 6.82 (br s, 2H, NHCSNH); 7.58–7.96 (m, 7H, ArH, from phenoxathiin). Found, C, 55.29; H, 4.18; N, 8.05. $C_{16}H_{14}N_2O_3S_2$ requires: C, 55.47; H, 4.07; N, 8.09%.

***2-(4-Nitrobenzenesulfenylamido)-phenoxathiin-10,10-dioxide* 29**

As yellow crystals, mp 235–6°C. IR (KBr), cm^{-1}: 1075 and 1250 (NO_2), 1160 (SO_2^{sym}); 1300 (SO_2^{as}); 1490, 1585 (C=C); 3230 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.12 (br 5, 1H, NH); 7.15–7.43 (m, AA′BB′, 4H, ArH from nitro-phenylene); 7.58–7.96 (m, 7H, ArH, from phenoxathiin). Found, C, 54.12; H, 2.90; N, 6.83. $C_{18}H_{12}N_2O_5S_2$ requires: C, 53.99; H, 3.02; N, 7.00%.

***4-(2-Nitrobenzenesulfenylamido)-phenoxathiin-10,10-dioxide* 30**

As pale yellow crystals, mp 195–6°C. IR (KBr), cm^{-1}: 1080 and 1250 (NO_2), 1160 (SO_2^{sym}); 1288 (SO_2^{as}), 1490. 1585 (C=C); 3260 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.19 (br s, 1H, NH); 7.29–7.53 (m, 4H, ArH from *ortho*-substituted phenyl); 7.58–7.96 (m, 7H, ArH from phenoxathiin). Found, C, 54.20; H, 3.07; N, 6.90. $C_{18}H_{12}N_2O_5S_2$ requires: C, 53.99; H, 3.02; N, 7.00%.

***N^1-(10,10-Dioxa-phenoxathiin-2-yl-aminosulfamoyl-phen-3-yl)-N^3-allyl-thiourea* 31**

As white crystals, mp 270–3°C. IR (KBr), cm^{-1}: 1040 (thioamide III); 1160 and 1175 (SO_2^{sym}); 1300 and 1369 (SO_2^{as}); 1540 (thioamide I), 3295 (NHCSNH), 3360 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.40–4.60 (m, 2H, CSNHCH_2); 5.60–5.97 (m, 3H, CH=CH_2); 6.70 and 6.84 (br s, 2H, NHCSNH); 7.28–7.96 (m, 11H, ArH, from phenoxathiin and 1,3-phenylene). Found, C, 52.77; H, 3.75; N, 8.21. $C_{22}H_{19}N_3O_5S_3$ requires: C, 52.68; H, 3.82; N, 8.38%.

***N^1-(10,10-Dioxa-phenoxathiin-2-yl-aminosulfamoyl-phen-4-yl)-N^3-allyl-thiourea* 32**

As white crystals, mp 239–42°C. IR (KBr), cm^{-1}:1040 (thioamide III); 1160 and 1177 (SO_2^{sym}); 1300 and 1368 (SO_2^{as}); 1540 (thioamide I); 3295 (NHCSNH), 3360 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 4.50–4.63 (m, 2H, CSNHCH_2); 5.60–5.93 (m, 3H, CH=CH_2); 6.77 and 6.89 (br s, 2H, NHCSNH); 7.05–7.46 (m, AA′BB′, J_{AB}=7.1 Hz, 4H, ArH, *p*-substituted phenylene): 7.54–7.96 (m, 7H, ArH, from phenoxathiin); Found, C, 52.49; H, 3.67; N, 8.35. $C_{22}H_{19}N_3O_5S_3$ requires: C, 52.68; H, 3.82; N, 8.38%.

N^1-(10,10-Dioxa-phenoxathiin-2-yl-aminosulfamoyl-phen-3-yl)-N^3-(3,4-dichlorophenyl)-urea **33**

As colorless crystals, mp 199–202°C. IR (KBr), cm^{-1}: 1156 and 1165 (SO_2^{sym}), 1290 (amide III), 1300 and 1367 (SO_2^{as}), 1540 (amide II); 1730 (amide I); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.23 (br s, 2H, HN–CO–NH); 7.12–7.45 (m, 7H, ArH, from 1,3-phenylene + 3,4-dichlorophenyl); 7.54–7.97 (m, 7H, ArH from phenoxathiin); 8.08 (s, 1H, SO_2NH). Found, C, 50.77; H, 2.80; N, 7.05. $C_{25}H_{17}Cl_2N_3O_6S_2$ requires: C, 50.85; H, 2.90, N, 7.12%.

N^1-(10,10-Dioxa-phenoxathiin-2-yl-aminosulfamoyl-phen-4-yl)-N^3-(3,4-dichlorophenyl)-urea **34**

As colorless crystals, mp 234–5°C. IR (KBr), cm^{-1}: 1159 and 1171 (SO_2^{sym}); 1290 (amide III); 1300 and 1374 (SO_2^{as}); 1550 (amide II); 1730 (amide I); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 5.20 (br s, 2H, HN–CO–NH); 7.02–7.49 (m, 7H, ArH, from 1,4-phenylene + 3,4-dichlorophenyl); 7.58–7.99 (m, 7H, ArH, from phenoxathiin); 8.12 (s, 1H, SO_2NH). Found, C, 50.96; H, 2.88; N, 7.00. $C_{25}H_{17}Cl_2N_3O_6S_2$ requires: C, 50.85; H, 2.90; N, 7.12%.

N^1-(10,10-Dioxa-phenoxathiin-2-yl-aminosulfamoyl-phen-3-yl)-N^3-[4-(tosylsulfonyl)]-urea **35**

As colorless crystals, mp 251–2°C. IR (KBr), cm^{-1}: 1150 and 1160 (SO_2^{sym}); 1290 (amide III); 1300 and 1360 (SO_2^{as}); 1550 (amide II); 1730 (amide I); 3060 (NH): ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me, from tosyl); 5.20 (br s, 2H, HN–CO–NH); 7.05–7.49 (m, 8H, ArH, 1,4-phenylene from tosyl + 1,3-phenylene); 7.53–7.92 (m, 7H, ArH, from phenoxathiin); 8.06 (s, 1H, SO_2NH). Found, C, 52.21; H, 3.60; N, 6.85. $C_{26}H_{21}N_3O_8S_3$ requires: C, 52.08; H, 3.53; N, 7.01%.

N^1-(10,10-Dioxa-phenoxathiin-2-yl-aminosulfamoyl-phen-4-yl)-N^3-[4-(tosylsulfonyl)]-urea **36**

As colorless crystals, mp 253–5°C. IR (KBr), cm^{-1}: 1152 and 1160 (SO_2^{sym}); 1285 (amide III); 1300 and 1369 (SO_2^{as}); 1546 (amide II); 1730 (amide I); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me, from tosyl), 5.24 (br s, 2H, HN–CO–NH), 7.05–7.40 (m, 8H, ArH, 1,4-phenylene from tosyl + 1,4-phenylene from the arylsulfonamido ring), 7.51–7.95 (m, 7H, ArH, from phenoxathiin), 8.13 (s, 1H, SO_2NH). Found, C, 52.10; H, 3.49; N, 6.94. $C_{26}H_{21}N_3O_8S_3$ requires: C, 52.08; H, 3.53; N, 7.01%.

***1-N-(10,10-Dioxa-phenoxathiin-2-yl-aminosulfamoyl-phen-3-yl)-2,4,6-trimethylpyridinium perchlorate* 37**

As colorless crystals, mp 290–2°C (dec.). IR (KBr), cm^{-1}: 1100 (perchlorate); 1150 and 1159 (SO_2^{sym}); 1300 and 1375 (SO_2^{as}); 1580 (C=C, C=N); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.45 (s, 6H, 2,6-Me_2), 2.70 (s, 3H, 4-Me); 7.09–7.48 (m, 4H, ArH, 1,3-phenylene bound to pyridinium), 7.55 (s, 2H, ArH, 3,5-H from pyridinium), 7.51–7.95 (m, 7H, ArH, from phenoxathiin), 8.08 (s, 1H, SO_2NH). Found, C, 51.20; H, 3.68; N, 4.55. $C_{26}H_{22}N_2O_5S_2^+Cl_4^-$ requires: C, 51.53; H, 3.66; N, 4.62%.

***1-N-(10,10-Dioxa-phenoxathiin-2-yl-aminosulfamoyl-phen-4-yl)-2,4,6-trimethylpyridinium perchlorate* 38**

As colorless crystals, mp 293–5°C (dec.). IR (KBr), cm^{-1}: 1100 (perchlorate); 1148 and 1159 (SO_2^{sym}); 1300 and 1360 (SO_2^{as}); 1595 (C=C, C=N); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.47 (s, 6H, 2,6-Me_2); 2.70 (s, 3H, 4-Me); 7.09–7.48 (m, AA′BB′, J_{AB} = 7.3 Hz, 4H, ArH, 1,4-phenylene bound to pyridinium); 7.60 (s, 2H, ArH, 3,5-H from pyridinium); 7.50–7.95 (m, 7H, ArH, from phenoxathiin); 8.05 (s, 1H, SO_2NH). Found, C, 51.64; H, 3.70; N, 4.39. $C_{26}H_{22}N_2O_5S_2^+CO_4^-$ requires: C, 51.53; H, 3.66; N, 4.62%.

***1-N-(10,10-Dioxa-phenoxathiin-2-yl-aminosulfamoyl-phen-3-yl)-2,6-dimethyl-4-phenyl-pyridinium perchlorate* 39**

As tan crystals, mp 280–2°C. IR (KBr), cm^{-1}: 1100 (perchlorate); 1145 and 1160 (SO_2^{sym}); 1300 and 1375 (SO_2^{as}); 1580 (C=C, C=N); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.47 (s, 6H, 2,6-Me_2); 7.09–7.52 (m, 9H, ArH, 1,3-phenylene bound to pyridinium + 4-Ph); 7.51–7.90 (m, 7H, ArH, from phenoxathiin); 7.95 (s, 2H, ArH, 3,5-H from pyridinium); 8.13 (s, 1H, SO_2NH). Found, C, 59.16; H, 3.78; N, 3.77. $C_{36}H_{26}N_2O_5S_2^+ClO_4^-$ requires: C, 59.22; H, 3.59; N, 3.84%.

***1-N-(10,10-Dioxa-phenoxathiin-2-yl-aminosulfamoyl-phen-4-yl)-2,6-dimethyl-4-phenyl-pyridinium perchlorate* 40**

As colorless crystals, mp 288–90°C (dec.). IR (KBr), cm^{-1}: 1100 (perchlorate); 1151 and 1160 (SO_2^{sym}); 1300 and 1368 (SO_2^{as}); 1590 (C=C, C=N); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.46 (s, 6H, 2,6-Me_2); 7.01–7.49 (m, 9H, ArH, 1,4-phenylene bound to pyridinium + 4-Ph); 7.50–7.91 (m, 7H, ArH, from phenoxathiin); 7.96 (s, 2H, ArH, 3.5-11 from

pyridinium); 8.15 (s, 1H, SO_2NH); Found, C, 59.21; H, 3.60; N, 3.75. $C_{36}H_{26}N_2O_5S_2^+ClO_4^-$ requires: C, 59.22; H, 3.59; N, 3.84%.

***1-N-(10,10-Dioxa-phenoxathiin-2-yl-aminosulfamoyl-phen-3-yl)-2,4,6-triphenylpyridinium perchlorate* 41**

As yellow crystals, mp 255–6°C. IR (KBr), cm^{-1}: 1100 (perchlorate); 1149 and 1158 (SO_2^{sym}); 1300 and 1375 (SO_2^{as}); 1596 (C=C, C=N); 3060 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 6.92–7.54 (m, 19H, ArH, 1,3-phenylene bound to pyridinium + 3 Ph); 7.56–7.95 (m, 7H, ArH, from phenoxathiin); 8.04 (s, 1H, SO_2NH); 8.55 (s, 2H, ArH, 3,5-H from pyridinium). Found, C, 62.10; H, 3.77; N, 3.46. $C_{41}H_{28}N_2O_5S_2^+ClO_4^-$ requires: C, 62.16; H, 3.56; N, 354%.

***1-N-(10,10-Dioxa-phenoxathiin-2-yl-aminosulfamoyl-phen-4-yl)-2,4,6-triphenylpyridinium perchlorate* 42**

As yellow crystals, mp 265–7°C (dec.). IR (KBr), cm^{-1}: 1100 (perchlorate); 1150 and 1159 (SO_2^{sym}); 1300 and 1366 (SO_2^{as}); 1595 (C=C, C=N); 3065 (NH); ^{1}H-NMR (DMSO-d_6), δ, ppm: 7.01–7.58 (m, 19H, ArH, 1,4-phenylene bound to pyridinium + 3 Ph); 7.54–7.96 (m, 7H, ArH, from phenoxathiin); 8.05 (s, 1H, SO_2NH); 8.60 (s, 2H, ArH, 3,5-H from pyridinium). Found, C, 62.20; H, 3.57; N, 3.40. $C_{41}H_{28}N_2O_5S_2^+ClO_4^-$ requires: C, 62.16; H, 3.56; N, 3.54%.

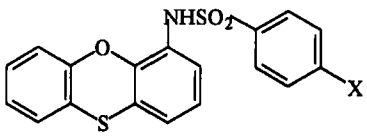
***Assay of Fungistatic Activity of Compounds* 1–42**

Fungistatic activity was determined by a modification of the growth method recently reported by us,[15–17] 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.*[18] 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 **1–42**. The minimum concentration at which no growth was observed was taken as MIC value (μg/mL), and represents the mean of at least two determinations.

RESULTS AND DISCUSSION

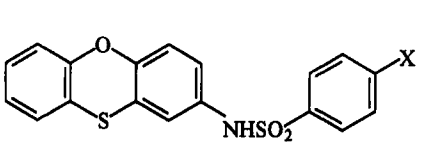
Sulfonylamido derivatives of phenoxathiin or phenoxathiin-10,10-dioxide are little known. Thus, only the 4-(4-aminophenylsulfonylamido)-phenoxathiin **43** (and the corresponding acetamido derivative **44**) have been reported

by Gilman and Stuckwisch[19] in the search of antibacterial sulfonamides,[20] whereas the corresponding 2-amino-phenoxathiin derivatives **45** and **46** have been synthesized by Tomita and Fukunaga, but were poorly characterized.[21]



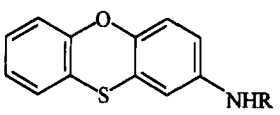
43: X = NH_2

44: X = NHAc



45: X = NH_2

46: X = NHAc



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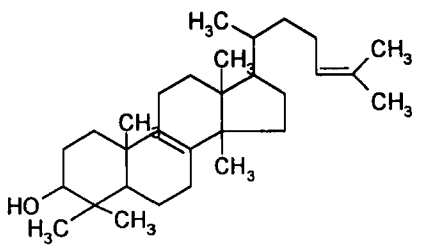

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It appeared thus of interest to prepare a larger series of sulfonylamido derivatives of 2-aminophenoxathiin-10,10-dioxide, since the corresponding derivatives of 2-amino-phenoxathiin **47**, recently reported by this group,[22] showed interesting antifungal activity against several organisms.

The new compounds **3**–**30**, prepared by reaction of 2-aminophenoxathiin-10,10-dioxide **1** with sulfonyl halides, sulfonic acid anhydrides, acyl chlorides as well as isocyanates/isothiocyanates, are shown in Table I. Generally they were synthesized from **1** and the corresponding sulfonyl chloride/fluoride in acetonitrile and in the presence of triethylamine. The only exceptions were the trifluoromethyl derivative **5**, obtained from **1** and triflic anhydride, in acetone as solvent at a molar ratio of 2 : 1, and the 2-carboxyphenyl derivatives **19** and **20**, respectively, prepared from **1** and sulfobenzoic acid cyclic anhydrides in refluxing acetonitrile.

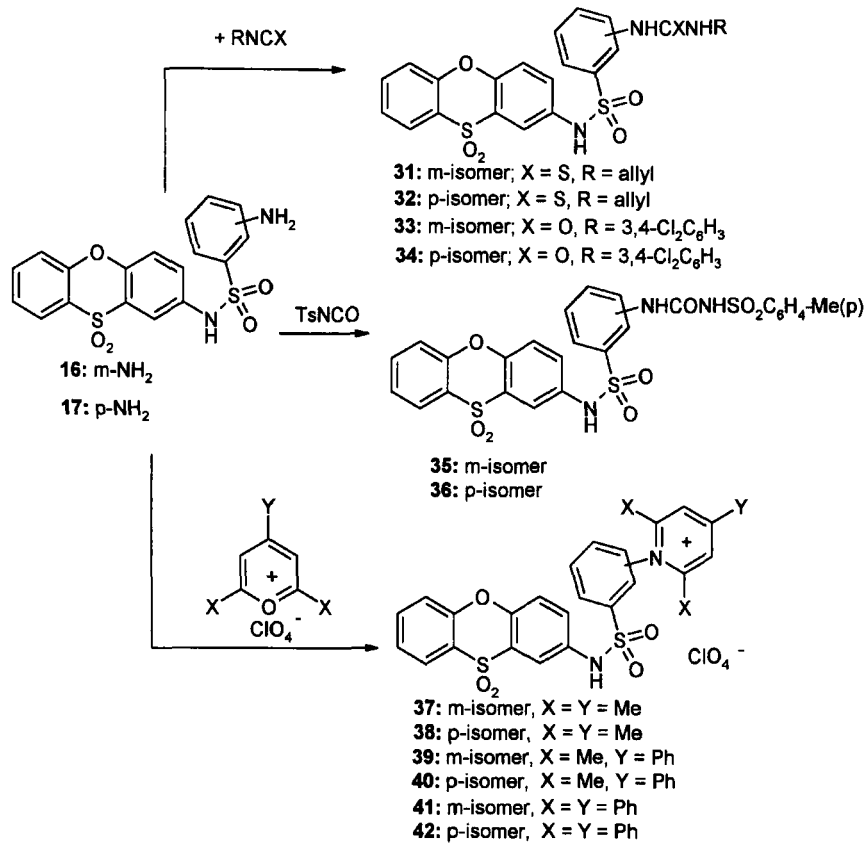
TABLE I 2-Aminophenoxathiin-10,10-dioxide derivatives **3**–**30** prepared in the present study.

Compound	*R*	*Yield*	*Synthetic Method*
3	Me_2NSO_2	48	A
4	$PhCH_2SO_2$	39	B
5	CF_3SO_2	51	C
6	*p*-F–C_6H_4–SO_2	73	A
7	*p*-Cl–C_6H_4–SO_2	70	A
8	*p*-Br–C_6H_4–SO_2	74	A
9	*p*-I–C_6H_4–SO_2	79	A
10	*p*-CH_3–C_6H_4–SO_2	82	A
11	*p*-O_2N–C_6H_4–SO_2	45	A
12	*m*-O_2N–C_6H_4–SO_2	40	A
13	*o*-O_2N–C_6H_4–SO_2	49	A
14	3-Cl-4-O_2N–C_6H_3–SO_2	50	A
15	*p*-AcNH–C_6H_4–SO_2	81	A
16	*p*-H_2N–C_6H_4–SO_2	59	B
17	*m*-H_2N–C_6H_4–SO_2	43	B
18	C_6F_5–SO_2	51	A
19	*o*-HOOC–C_6H_4–SO_2	82	D
20	*o*-HOOC–C_6Br_4–SO_2	80	D
21	*p*-CH_3O–C_6H_4–SO_2	61	A
22	2,4,6-$(CH_3)_3$–C_6H_2–SO_2	42	A
23	Ph_2N–CO	76	A
24	isonicotinoyl	55	A
25	2,4-$Cl_2C_6H_3CO$	38	A
26	3,4-$Cl_2C_6H_3NHCO$	42	E
27	*p*-Me-$C_6H_4SO_2NHCO$	93	E
28	CH_2 = $CHCH_2NHCS$	69	E
29	*p*-O_2N–C_6H_4–S	51	A
30	*o*-O_2N–C_6H_4–S	44	A

A: 2-amino-phenoxathiine-10,10-dioxide + RSO_2Cl (or RCOCl or RSCl); B: 2-amino-phenoxathiine-10,10-dioxide + RSO_2F; C: 2-amino-phenoxathiine-10,10-dioxide triflic anhydride; D: 2-amino-phenoxathiine-10,10-dioxide + sulfobenzoic cyclic anhydride; E: 2-amino-phenoxathiine-10,10-dioxide + RNCO (or RNCS).

Further derivatization of compounds **16** and **17**, containing a free NH_2 group with 2,4,6-trisubstitutedpyrylium perchlorates, tosyl isocyanate, 3,4-dichlorophenyl isocyanate and allyl isothiocyanate afforded the new compounds **31**–**42** (Scheme 1).

Analytical and spectral data confirmed the proposed structure for the newly synthesized derivatives **3**–**42**. In the IR spectra of compounds **3**–**42**, the following bands were detected: (i) the intense sulfonamido vibrations, at 1140–1175 cm^{-1} (SO_2^{sym} from the sulfonamide) and 1156–1160 cm^{-1} (SO_2^{sym} from the 10,10-dioxaphenoxathiin), and 1320–1345 cm^{-1} (SO_2^{sym} from the sulfonamide), and 1285–1300 cm^{-1} (SO_2^{as} from the 10,10-dioxaphenoxathiin) respectively: (ii) the NH vibrations at around 3060 cm^{-1}; (iii) bands of the aromatic rings (C=C) around 1490–1500 cm^{-1}, as well as bands due to the other structural elements present in these molecules (such as NH_2 for



SCHEME 1

derivatives **16, 17** or COOH for **19** and **20**); (iv) the strong amide vibrations at 1680–1700 cm^{-1} (amide I), 1520–1540 cm^{-1} (amide II) and 1290–1300 cm^{-1} (amide III), respectively, for the two carboxamides **24** and **25**, as well as for the ureas **26, 27** and **33–36**. For the latter derivatives (ureas) the amide I bands appeared at 1730 cm^{-1}.

In the 200 MHz ^{1}H-NMR spectra of derivatives **3–30** (in DMSO-d_6) the following signals were detected: (i) the SO_2NH protons as a broad singlet at 8.05–8.35 ppm, in fast exchange with the bulk solvent, which disappears on addition of D_2O into the NMR tube after 5–10 min, (ii) the seven aromatic protons of the phenoxathiin-2-yl moiety as a multiplet in the range 7.50–7.95 ppm, (iii) the aromatic protons of the $ArSO_2NH$ moiety at the 2-position of the phenoxathiine ring appeared as an AA′BB′ multiplet for the *p*-substituted-phenyl derivatives, whereas for other substitution patterns the

expected signals have been evidenced in the NMR spectra. For derivatives **31–42**, the spectral data also confirmed the proposed structures (see Materials and Methods for details).

Antifungal activity of the new compounds reported here was determined by a modification of the growth method, as reported earlier by this group.[15–17] The activity of the new compounds is shown in Table II. Two *Aspergillus* species and one strain of *Candida albicans* were included in the assays, as these are widespread fungi/moulds, which easily develop resistance against many anti-fungal compounds.[18] Ketoconazole, a well known imidazole possessing strong antifungal activity has been included as standard in these assays.

As seen from the data of Table II, 2-amino-phenoxathiin-10,10-dioxide **1** is inactive or possesses very weak antifungal activity, against the three investigated organisms whereas its sulfonamido, carboxamido, sulfenamido and urea/thiourea derivatives are active to different degrees. In the series of synthesized derivatives **3–30**, best activity was correlated with the presence of nitro moieties in the arylsulfonamido/sulfenamido groups linked to the heterocyclic ring (compounds **12–14, 29** and **30**), pentafluorophenylsulfonamido (**18**), or diphenyl-carbamoylamido (**23**) moieties. Monohalogeno atoms or other groups such as amino, alkyls, etc, were less effective in inducing strong antifungal activity in these compounds, but the dichloro-substituted compounds **25** and especially **26** showed good activities. Appreciable inhibitory activity was detected for the compounds obtained by derivatization of the amino compounds **16** and **17**. Thus, thioureas **31** and **32**, as well as ureas **33–36** were among the most active compounds in the whole series, with potencies superior to ketaconazole against the two *Aspergillus* species, but were less effective against *Candida*, as compared to ketaconazole. In the case of the pyridinium salts, the 2,4,6-trisubstituted pyridinium derivatives **37** and **38** were moderately active, but introduction of an increasing number of phenyl moieties in place of the methyl ones led to improved activity, with the 2,4,6-triphenylpyridinium salts **41** and **42** being more effective than ketaconazole against the two *Aspergillus* species. Probably the ineffectiveness of the trimethylpyridinium derivatives **37** and **38** might be due to their very low lipophilicity and inability to penetrate through biological membranes *in vivo*.[23] For compounds **31–42** it should also be noted that generally the *meta*-substituted isomers were more active than the *para*-substituted ones. Mention should be made that the 2-amino-phenoxathiin-10,10-dioxide derivatives prepared in the present study are more active than the corresponding 2-amino-phenoxathiin derivatives **47** previously reported by this group.[22]

TABLE II Antifungal activity of compounds **1**–**42** against several organisms.

Compound	MIC (μg/mL)		
	Aspergillus flavus C1150	*A. niger* C418	*Candida albicans* C316
1	>125	>125	98
3	16	9	10
4	12	9	11
5	26	44	30
6	8	7	6
7	10	7	8
8	15	9	8
9	13	12	7
10	10	9	8
11	6	6	5
12	3	3	4
13	4	2	3
14	2	1	1
15	5	7	8
16	9	10	13
17	10	8	7
18	1	1	0.5
19	44	38	30
20	20	8	12
21	5	6	4
22	5	5	3
23	1.3	1.1	0.4
24	10	4	9
25	2	3	4
26	0.5	0.7	0.4
27	8	9	8
28	7	11	4
29	0.7	1.0	1.2
30	0.5	0.7	0.8
31	0.8	1.2	1.1
32	1.2	1.3	1.0
33	0.3	0.6	0.5
34	0.9	0.8	0.5
35	0.4	0.6	0.5
36	0.5	0.8	0.8
37	21	18	14
38	17	15	11
39	11	9	7
40	13	9	6
41	0.7	0.3	2
42	1.1	0.4	3
Ketoconazole	1.2	1.8	0.06

Earlier it was hypothesized that the compounds reported here act as inhibitors of lanosterol-14-α-demethylase. This hypothesis seems reasonable on consideration of the structural similarities between some of the more potent compounds prepared here and lanosterol **48**.

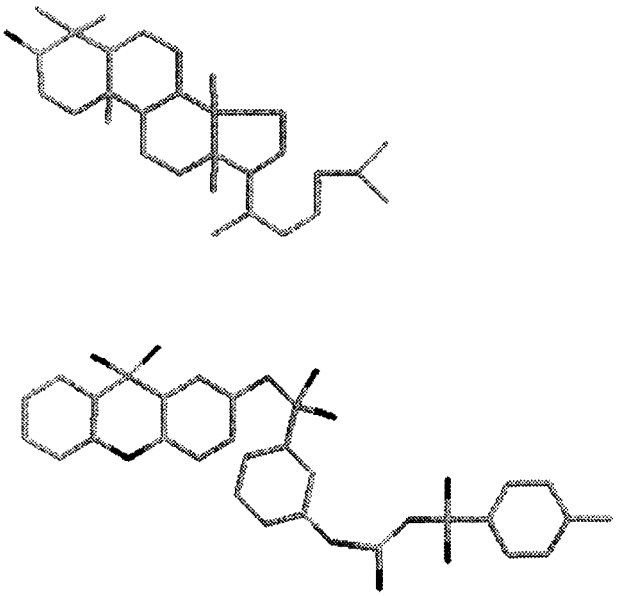


FIGURE 1 Schematic representation of lanosterol **48** (top) and compound **35** (bottom). The program Raswin 2.5 was used to generate the above structures with a Texas Instruments Extensa 390 PC.

As seen from Figure 1, the two structures possess in common a bulky polycyclic system and a relatively large side chain (in 17-position lanosterol) extending from it, which together could be expected to be highly important for the binding of the substrates/inhibitors to the enzyme. The inhibitors of the type described by us here probably meet both these structural requirements and thus are able to strongly bind to the enzyme, and in this manner inhibit the whole steroid synthesis in the organisms investigated here. Unfortunately, at the present time we have been unable to isolate lanosterol-14-α-demethylase from the fungi/moulds investigated by us, mainly due to problems connected with their large scale cultivation. Thus, the hypothesis that it is the inhibition of this enzyme which is responsible for the antifungal effect of our compounds could not, as yet, be verified. Work is in progress in this laboratory to isolate enzymes which might be the target of the inhibitors of the type investigated in this work.

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