ELSEVIER

Il Farmaco 57 (2002) 973–977

IL FARMACO

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Synthesis antimicrobial and antifungal activity of some new 3-substituted derivatives of 4-(2,4-dichlorophenyl)-5-adamantyl-1*H*-1,2,4-triazole

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Received 17 November 2001; accepted 4 February 2002

Abstract

The synthesis of a series of substituted hydrazones and thiazolidinones is described, starting from *N*-[4-(2,4-dichlorophenyl)-5-adamantyl-1*H*-1,2,4-triazol-3-ylmercaptoacetyl)hydrazine. The new compounds were tested for antimicrobial and antifungal activity and some of them exhibited moderate activity against *Candida albicans*.

Keywords: Substituted 1,2,4-triazoles; Antimicrobial activity; Antifungal activity

1. Introduction

A large number of heterocyclic compounds containing the 1,2,4-triazole ring are associated with diverse pharmacological properties, such as anti-inflammatory, fungicidal, antimicrobial and antiviral activity [1–5]. In previous papers, we have also reported on the preparation of some 5-aryl-3-iminoxyethyl-1,2,4-triazoles with antimicrobial and antifungal activity [6] and some 4-aryl-5-piperidinylmethyl-3-benzylthio-1,2,4-triazoles with antioxidant and moderate antiviral properties [7]. We have also described the synthesis of a number of 4-aryl-5-adamantyl-3-mercapto-1,2,4-triazoles with antifungal and antimicrobial activity [8]. On the other hand, it has been reported that certain cyclohexylidene hydrazides and their cyclization products, the corresponding spirothiazolidinones, with a heterocyclic ring incorporated into their structure, possess interesting antifungal properties [9].

Prompted by the observed biological activities of the above mentioned derivatives and in continuation of our ongoing studies on novel biologically active molecules, we have designed and synthesized some new 5-adamantyl-4-aryl-1,2,4-triazoles, bearing a 3-mercaptoacetic acid substitution, as potential antimicrobial and antifungal agents. The results of this study are discussed in this paper.

2. Chemistry

The preparation of the target compounds is outlined in Scheme 1. The 4,5-disubstituted 3-mercaptotriazole **1** [8] was first reacted with ethyl bromoacetate in alkaline medium, to give the corresponding ethyl ester **2** in very good yield (90%). This ester was then converted almost quantitatively to the hydrazide **3** after treatment with hydrazine hydrate. The reaction of the hydrazide **3** with cyclic ketones (cyclobutanone, cyclopentanone, cyclohexanone, indanone, fluorenone) afforded the corresponding substituted hydrazides **4a**–**e** in good yield (63–75%). The *E*-isomer of the indane analog **4d** was, as expected, the major product of the reaction and it was separated from traces of the corresponding *Z*-isomer by column chromatography. The hydrazides **4a**–**e** were then treated with thioglycolic acid in dry benzene, to provide the corresponding spiro-derivatives **5a**–**e**. The structures of the synthesized compounds were confirmed on the basis of their physical and spectral (IR, ^{1}H

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0014-827X/02/$ - see front matter
PII: S0014-827X(02)01227-2

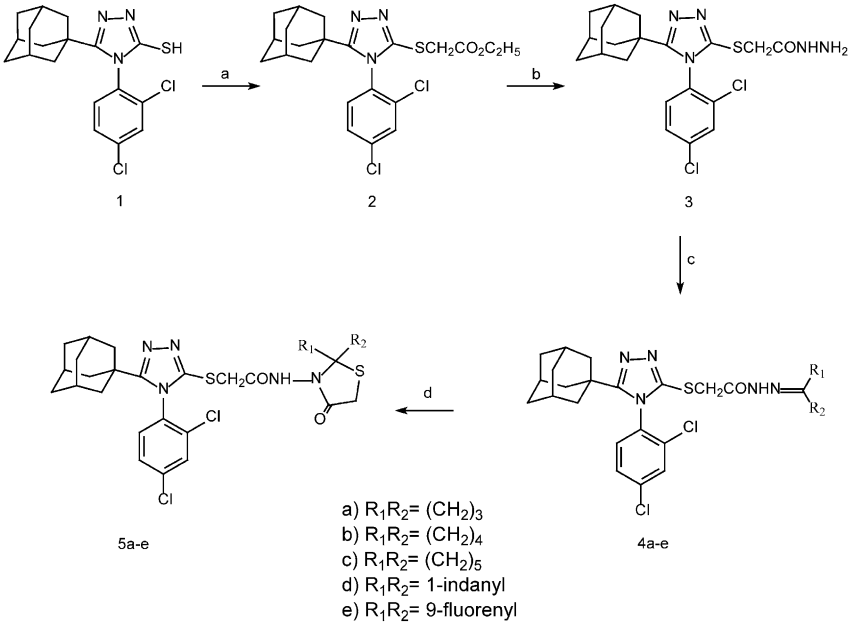
NMR) data, as well as by elemental analyses. The IR spectra of compounds **4a**–**e** and **5a**–**e** showed absorption bands resulting from the amide's NH and CO functionalities at about regions 3250–3215 and 1690–1663 cm^{-1}, respectively. Concerning compounds **5a**–**e**, a new CO band appeared at 1732–1720 cm^{-1}, due to the formation of the spirothiazolidinone moiety. In the ^1H NMR spectra of compounds **2**–**5**, recorded in $CDCl_3$ solution, the CH_2 of the mercaptoacetylamino side-chain clearly resonated as an AX system presenting two separate doublets at 3.65–3.98 ppm, with a coupling constant of 14 Hz, indicative of a geminal coupling, suggesting that the two protons are magnetically inequivalent. On the other hand, in the corresponding spectra of compounds **5a**–**e**, the CH_2 of the spirothiazolidinone structure resonated as two doublets at 3.40–3.57 ppm, with a coupling constant of 16 Hz, presumably because the two protons, which are adjacent to the introduced carbonyl, are not magnetically equivalent. The NH signal of compounds **4a**–**e** and **5a**–**e** appeared as a singlet at 10–11.7 ppm, disappearing after addition of D_2O.

3. Experimental

All reagents used were purchased from Aldrich Chemical Company. Melting points were taken in glass capillary tubes on a Büchi 530 apparatus and are uncorrected. Silica gel TLC was performed on $60_{F\text{-}254}$ precoated sheets and column chromatography was carried out on silica gel (60–120 mesh). IR spectra were recorded on a Perkin–Elmer 833 spectrophotometer. All proton NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer, using tetramethylsilane (TMS) as internal standard, in chloroform ($CDCl_3$) and chemical shifts were reported as δ (ppm) values. The elemental analyses (C, H, N) of all compounds were performed by the Service Centrale de Microanalyses (CNRS, Vernaison, France) and are within the range of experimental error ($\pm 0.4\%$ of the calculated values).

3.1. [4-(2,4-Dichlorophenyl)-5-(tricyclo[3.3.1.1^{3,7}]-decan-1-yl)-4H-1,2,4-triazol-3-yl]mercaptoacetic acid ethyl ester (2)

Ethyl bromoacetate (0.73 g, 41.3 mmol) and potassium carbonate (1.72 g) were added to a solution of 4-(2,4-dichlorophenyl)-3-mercapto-5-(tricyclo[3.3.1.1^{3,7}]decan-1-yl)-4*H*-1,2,4-triazole (**1**) (1.31 g, 3.44 mmol) [8] in dry dimethylformamide (15 ml) and the mixture was refluxed for 16 h. The reaction mixture was then poured into ice-water, allowed to stand for 6 h and the precipitate was filtered, washed with water and air-dried to give compound **2** (1.46 g, 91%). M.p.: 99 °C (EtOH). IR (Nujol, ν cm^{-1}): 1735 (C=O). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 1.16 (t, 3H, $J = 7$ Hz, OCH_2CH_3), 1.55 (m, 8H, adamantane H), 1.75 (m, 3H, adamantane H), 1.82 (m, 4H, adamantane H), 3.87, 3.98 (2d, 2H, $J = 14$ Hz, SCH_2CO), 4.10 (q, 2H, $J = 7$ Hz, OCH_2CH_3), 7.34 (d, 1H, $J = 8.5$ Hz, 6′-aromatic H), 7.47 (dd, 1H, $J = 8.5$ Hz, 2.2 Hz, 5′-aromatic H), 7.63 (d, 1H, $J = 2.2$ Hz, 3′-aromatic H). *Anal.* Calc. for $C_{22}H_{25}Cl_2N_3O_2S$: C, 56.65; H, 5.40; N, 9.01. Found: C, 56.33; H, 5.19; N, 8.87%.



Scheme 1. (a) $BrCH_2CO_2C_2H_5$, K_2CO_3, DMF; (b) $NH_2NH_2 \cdot xH_2O$, EtOH; (c) $R_1R_2C{=}O$, EtOH; (d) $HSCH_2CO_2H$, C_6H_6.

3.2. [4-(2,4-Dichlorophenyl)-5-(tricyclo[3.3.1.1^{3,7}]-decan-1-yl)-4H-1,2,4-triazol-3-yl]mercaptoacetic acid hydrazide (**3**)

Hydrazine hydrate (0.22 g, 4.4 mmol) was added to a solution of **2** (0.98 g, 2.1 mmol) in absolute ethanol (30 ml) and the mixture was refluxed for 8 h. After cooling,the precipitate was filtered, washed with water and air-dried to give the hydrazide **3** (0.8 g, 84%). M.p.: 215–217 °C (EtOH). IR (Nujol, ν cm^{-1}): 3315 (NH), 1655 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.67 (m, 8H, adamantane H), 1.80 (m, 3H, adamantane H), 1.98 (m, 4H, adamantane H), 3.72, 3.84 (2d, 2H, J = 14.3 Hz, SCH_2CO), 3.95 (br.s., 2H, NH_2, D_2O exch.), 7.33 (d, 1H, J = 8.6 Hz, 6′-aromatic H), 7.47 (dd, 1H, J = 8.6 Hz, 2.2 Hz, 5′-aromatic H), 7.64 (d, 1H, J = 2.2 Hz, 3′-aromatic H), 9.06 (br.s., 1H, NH, D_2O exch.). *Anal.* Calc. for $C_{20}H_{23}Cl_2N_5OS$: C, 53.10; H, 5.12; N, 15.48. Found: C, 53.26; H, 4.97; N, 15.20%.

3.3. [4-(2,4-Dichlorophenyl)-5-(tricyclo[3.3.1.1^{3,7}]-decan-1-yl)-4H-1,2,4-triazol-3-yl]mercaptoacetic acid cyclobutylidene hydrazide (**4a**)

Cyclobutanone (84 mg, 1.2 mmol) was added to a solution of **3** (500 mg, 1.15 mmol) in absolute ethanol (20 ml). The mixture was refluxed for 24 h and then, allowed to stand overnight. The solvent was vacuum-evaporated and the residue was purified by column chromatography (silica gel), using a mixture of dichloromethane/cyclohexane: 3/10 (v/v) as the eluent to give compound **4a** (370 mg, 65%) as a white solid. M.p.: 141–143 °C (EtOH). IR (Nujol, ν cm^{-1}): 3250 (NH), 1670 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.60 (m, 8H, adamantane H), 1.77 (m, 3H, adamantane H), 2.04 (m, 6H, cyclobutyl CH_2, 4 × adamantane H), 3.02 (m, 4H, 2 × cyclobutyl CH_2), 3.71, 3.82 (2d, 2H, J = 14.5 Hz, SCH_2CO), 7.31 (d, 1H, J = 8.5 Hz, 6′-aromatic H), 7.46 (dd, 1H, J = 8.5 Hz, 2 Hz, 5′-aromatic H), 7.58 (d, 1H, J = 2.2 Hz, 3′-aromatic H), 11.10 (s, 1H, NH, D_2O exch.). *Anal.* Calc. for $C_{24}H_{27}Cl_2N_5OS$: C, 57.14; H, 5.39; N, 13.88. Found: C, 56.91; H, 5.25; N, 13.60%.

The following derivatives were prepared by a procedure similar to **4a**.

3.4. [4-(2,4-Dichlorophenyl)-5-(tricyclo[3.3.1.1^{3,7}]-decan-1-yl)-4H-1,2,4-triazol-3-yl]mercaptoacetic acid cyclopentylidene hydrazide (**4b**)

Yield: 72%. M.p.: 225–228 °C (EtOH). IR (Nujol, ν cm^{-1}): 3247 (NH), 1663 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.90 (m, 19H, 15 adamantane H, cyclopentyl 3″-CH_2, 4″-CH_2), 2.47 (t, 2H, J = 6.7 Hz, cyclopentyl 2″-CH_2), 2.55 (t, 2H, J = 6.7 Hz, cyclopentyl 5″-CH_2), 3.79, 3.93 (2d, 2H, J = 14 Hz, SCH_2CO), 7.41 (d, 1H, J = 8.3 Hz, 6′-aromatic H), 7.53 (dd, 1H, J = 8.3 Hz, 1.9 Hz, 5′-aromatic H), 7.70 (d, 1H, J = 2.0 Hz, 3′-aromatic H), 11.01 (s, 1H, NH, D_2O exch.). *Anal.* Calc. for $C_{25}H_{29}Cl_2N_5OS$: C, 57.91; H, 5.64; N, 13.51. Found: C, 57.72; H, 5.88; N, 13.25%.

3.5. [4-(2,4-Dichlorophenyl)-5-(tricyclo[3.3.1.1^{3,7}]-decan-1-yl)-4H-1,2,4-triazol-3-yl]mercaptoacetic acid cyclohexylidene hydrazide (**4c**)

Yield: 75%. M.p.: 134–136 °C (EtOH). IR (Nujol, ν cm^{-1}): 3249 (NH), 1667 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.70 (m, 21H, 15 adamantane H, cyclohexyl 3″-CH_2, 4″-CH_2, 5″-CH_2), 2.35 (m, 4H, cyclohexyl 2″-CH_2, 6″-CH_2), 3.67, 3.81 (2d, 2H, J = 13.8 Hz, SCH_2CO), 7.29 (d, 1H, J = 8.1 Hz, 6′-aromatic H), 7.36 (dd, 1H, J = 8.1 Hz, 2 Hz, 5′-aromatic H), 7.55 (d, 1H, J = 2.1 Hz, 3′-aromatic H), 11.11 (s, 1H, NH, D_2O exch.). *Anal.* Calc. for $C_{26}H_{31}Cl_2N_5OS$: C, 58.64; H, 5.87; N, 13.15. Found: C, 58.83; H, 6.21; N, 12.86%.

3.6. [4-(2,4-Dichlorophenyl)-5-(tricyclo[3.3.1.1^{3,7}]-decan-1-yl))-4H-1,2,4-triazol-3-yl]mercaptoacetic acid indanylidene E-hydrazide (**4d**)

Yield: 42%. M.p.: 208–210 °C (EtOH). IR (Nujol, ν cm^{-1}): 3250 (NH), 1668 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.65 (m, 8H, adamantane H), 1.86 (m, 3H, adamantane H), 1.95 (m, 4H, adamantane H), 2.97 (t, 2H, J = 6.2 Hz, 3″-indane-CH_2), 3.20 (t, 2H, J = 6.2 Hz, 2″-indane-CH_2), 3.82, 3.96 (2d, 2H, J = 14.3 Hz, SCH_2CO), 7.35 (m, 4H, 6′-aromatic H, 4″,5″,6″-indane H), 7.46 (dd, 1H, J = 8.4 Hz, 2.2 Hz, 5′-aromatic H), 7.55 (d, 1H, J = 2.2 Hz, 3′-aromatic H), 7.97 (d, 1H, J = 7.7 Hz, 7″-indane H), 11.23 (s, 1H, NH, D_2O exch.). *Anal.* Calc. for $C_{29}H_{29}Cl_2N_5OS$: C, 65.58; H, 5.51; N, 13.19. Found: C, 65.43; H, 5.10; N, 13.02%.

3.7. [4-(2,4-Dichlorophenyl)-5-(tricyclo[3.3.1.1^{3,7}]-decan-1-yl)-4H-1,2,4-triazol-3-yl]mercaptoacetic acid fluorenylidene hydrazide (**4e**)

Yield: 45%. M.p.: 296–298 °C (EtOH). IR (Nujol, ν cm^{-1}): 3238 (NH), 1672 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.60 (m, 15H, adamantane H), 3.70, 3.83 (2d, 2H, J = 14 Hz, SCH_2CO), 7.28 (d, 1H, J = 8.2 Hz, 6′-aromatic H), 7.44 (m, 7H, 5′-aromatic H, 2″,3″,4″,5″,6″,7″-fluorene H), 7.60 (d, 1H, J = 2 Hz, 3′-aromatic H), 7.99 (d, 1H, J = 7.4 Hz, 1″-fluorene H), 8.61 (d, 1H, J = 7.4 Hz, 8″-fluorene H), 11.65 (s, 1H, NH, D_2O exch.). *Anal.* Calc. for $C_{33}H_{29}Cl_2N_5OS$: C, 64.49; H, 4.76; N, 11.39. Found: C, 64.21; H, 4.95; N, 11.11%.

*3.8. 4-[[4-(2,4-Dichlorophenyl)-5-(tricyclo[3.3.1.1^{3,7}]-decan-1-yl)-4H-1,2,4-triazol-3-yl]mercaptoacetyl-amino]-4-aza-1-thiaspiro[4,3]octan-3-one (**5a**)*

To a solution of **4a** (210 mg, 0.42 mmol) in dry benzene (40 ml), was added thioglycolic acid (77.4 mg, 0.84 mmol) and the mixture was refluxed for 40 h in a Dean–Stark apparatus. The solvent was then vacuum-evaporated, the residue was dissolved in dichloromethane and extracted with 10% sodium bicarbonate solution (3 × 40 ml). The organic phase was washed with water, dried (Na_2SO_4) and the solvent evaporated to dryness to give a crude solid which was purified by column chromatography (silica gel), using a mixture of dichloromethane/methanol: 98/2 (v/v) as the eluent to give pure compound **5a** (113 mg, 53%). M.p.: 175–177 °C (EtOH). IR (Nujol, ν cm^{-1}): 3215 (NH), 1720, 1690 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.75 (m, 15H, 15 adamantane H), 1.99 (m, 2H, cyclobutyl-CH_2), 2.30 (m, 2H, cyclobutyl-CH_2), 2.72 (m, 2H, cyclobutyl-CH_2), 3.57, 3.61 (2d, 2H, J = 16 Hz, thiaz. SCH_2), 3.83, 3.97 (2d, 2H, J = 14.3 Hz, SCH_2CONH), 7.39 (d, 1H, J = 8.4 Hz, 6′-aromatic H), 7.47 (dd, 1H, J = 8.4 Hz, 2.2 Hz, 5′-aromatic H), 7.63 (d, 1H, J = 2.2 Hz, 3′-aromatic H), 10.15 (s, 1H, NH, D_2O exch.). *Anal.* Calc. for $C_{26}H_{29}Cl_2N_5O_2S_2$: C, 61.10; H, 5.72; N, 13.70. Found: C, 60.93; H, 5.65; N, 13.47%.

The following derivatives were prepared by a procedure similar to **5a**.

*3.9. 4-[[4-(2,4-Dichlorophenyl)-5-(tricyclo[3.3.1.1^{3,7}]-decan-1-yl)-4H-1,2,4-triazol-3-yl]mercaptoacetyl-amino]-4-aza-1-thiaspiro[4,4]nonan-3-one (**5b**)*

Yield: 49%. M.p.: 180–183 °C (EtOH). IR (Nujol, ν cm^{-1}): 3230 (NH), 1732, 1680 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.70 (m, 23H, 15 adamantane H, 4 × cyclopentyl CH_2), 3.55, 3.63 (2d, 2H, J = 16 Hz, thiaz. SCH_2), 3.74, 3.89 (2d, 2H, J = 14.1 Hz, SCH_2CONH), 7.33 (d, 1H, J = 9 Hz, 6′-aromatic H), 7.45 (dd, 1H, J = 9 Hz, 2 Hz, 5′-aromatic H), 7.58 (d, 1H, J = 1.8 Hz, 3′-aromatic H), 10.06 (s, 1H, NH, D_2O exch.). *Anal.* Calc. for $C_{27}H_{31}Cl_2N_5O_2S_2$: C, 54.72; H, 5.27; N, 11.82. Found: C, 55.39; H, 5.32; N, 11.65%.

*3.10. 4-[[4-(2,4-Dichlorophenyl)-5-(tricyclo-[3.3.1.1^{3,7}]decan-1-yl)-4H-1,2,4-triazol-3-yl]-mercaptoacetylamino]-4-aza-1-thiaspiro[4,5]decan-3-one (**5c**)*

Yield: 55%. M.p.: 178–180 °C (EtOH). IR (Nujol, ν cm^{-1}): 3237 (NH), 1725, 1685 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.75 (m, 25H, 15 adamantane H, 5 × cyclohexyl CH_2), 3.38, 3.46 (2d, 2H, J = 16 Hz, thiaz. SCH_2), 3.71, 3.83 (2d, 2H, J = 14 Hz, SCH_2CONH), 7.30 (d, 1H, J = 8.4 Hz, 6′-aromatic H), 7.39 (dd, 1H, J = 8.4 Hz, 1.9 Hz, 5′-aromatic H), 7.55 (d, 1H, J = 1.8 Hz, 3′-aromatic H), 9.88 (s, 1H, NH, D_2O exch.). *Anal.* Calc. for $C_{28}H_{33}Cl_2N_5O_2S_2$: C, 55.44; H, 5.48; N, 11.55. Found: C, 55.09; H, 5.84; N, 11.37%.

*3.11. 3′-[[4-(2,4-Dichlorophenyl)-5-(tricyclo-[3.3.1.1^{3,7}]decan-1-yl)-4H-1,2,4-triazol-3-yl]-mercaptoacetylamino]spiro[indane-1,2′-thiazolidine-4′-one] (**5d**)*

Yield: 46%. M.p.: 290–292 °C (EtOH). IR (Nujol, ν cm^{-1}): 3227 (NH), 1726, 1688 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.70 (m, 15H, adamantane H), 2.92 (t, 2H, J = 6 Hz, 3″-indane-CH_2), 3.23 (t, 2H, J = 6 Hz, 2″-indane-CH_2), 3.41, 3.52 (2d, 2H, J = 16 Hz, thiaz. SCH_2), 3.80, 3.88 (2d, 2H, J = 14 Hz, SCH_2CONH), 7.37 (m, 5H, 6′-aromatic H, indane H), 7.51 (dd, 1H, J = 8.2 Hz, 2.1 Hz, 5′-aromatic H), 7.59 (d, 1H, J = 2.1 Hz, 3′-aromatic H), 9.90 (s, 1H, NH, D_2O exch.). *Anal.* Calc. for $C_{31}H_{31}Cl_2N_5O_2S_2$: C, 58.12; H, 4.88; N, 10.93. Found: C, 58.61; H, 4.59; N, 11.21%.

*3.12. 3′-[[4-(2,4-Dichlorophenyl)-5-(tricyclo-[3.3.1.1^{3,7}]decan-1-yl)-4H-1,2,4-triazol-3-yl]-mercaptoacetylamino]spiro[9H-fluorene-9,2′-thiazolidine-4′-one] (**5e**)*

Yield: 42%. M.p.: 320–322 °C (EtOH). IR (Nujol, ν cm^{-1}): 3228 (NH), 1725, 1687 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.75 (m, 15H, adamantane H), 3.40, 3.48 (2d, 2H, J = 16 Hz, thiaz. SCH_2), 3.72, 3.84 (2d, 2H, J = 14 Hz, SCH_2CONH), 7.40 (m, 10H, 5′,6′-aromatic H, 2″,3″,4″,5″,6″,7″-fluorene H), 7.66 (d, 1H, J = 1.8 Hz, 3′-aromatic H), 7.79 (d, 1H, J = 7.3 Hz, 1″-fluorene-H), 8.08 (d, 1H, J = 7.3 Hz, 8″-fluorene-H), 10.95 (s, 1H, NH, D_2O exch.). *Anal.* Calc. for $C_{35}H_{31}Cl_2N_5O_2S_2$: C, 61.04; H, 4.54; N, 10.17. Found: C, 60.88; H, 4.51; N, 9.93%.

3.13. In vitro antimicrobial evaluation

The antimicrobial activity of the compounds was assayed using the agar dilution method against the following bacteria and fungi. Ampicillin and miconazole were used as reference compounds respectively.

3.13.1. Test organisms

Bacteria. Staphylococcus aureus, from our laboratory strains collection. *Bacillus subtilis* CCM 2216 (Czechoslovak collection of Microorganisms, Brno, Czechia). *Escherichia coli*, from our laboratory strains collection. *Pseudomonas aeruginosa* CCM 1960 (Czechoslovak collection of Microorganisms, Brno, Czechia).

Fungi. Candida albicans CNCM 1180-79 (American Type Culture Collection, Rockville, Maryland, USA).

All tested microorganisms were first incubated at 37 °C in Müller Hinton broth (bacteria) or at 25 °C in Sabouraud dextrose broth (*C. albicans*) for 18 h. The antibacterial test was performed on Müller Hinton agar and the antifungal on Sabouraud dextrose agar, using inocula of 10^6 bacteria or 10^5 fungi with a multipoint inoculator. Cultures were incubated for 24 h at 37 °C and 25 °C for *C. albicans*. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of compound at which there was no growth expressed in μg/ml. MIC was the mean of three measurements. Blanks were prepared in the test medium without test compound.

4. Results and discussion

The antimicrobial activity of the hydrazides **4a**–**e** and the derivatives **5a**–**e** was assayed using the agar dilution method against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and a strain of *C. albicans*. Ampicillin and miconazole were used as reference compounds, respectively. Compounds **4a**, **4b** and **5e** showed only weak activity against *C. albicans* (MIC = 365 μg/ml), while the rest of the compounds were inactive. Concerning the antibacterial tests, compounds **4a**–**c** showed MIC values ranging from 215 to 280 μg/ml, against all four tested bacteria. On the other hand the spiro-derivatives **5a**–**d** showed a weak activity (MIC values: 280–350 μg/ml) against *B. subtilis*, but not against the other strains tested.

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