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An investigation of the biological effect of structural modifications of isothiosemicarbazones and their cyclic analogues

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Abstract

Several arylideneisothiosemicarbazones and arylidenehydrazothiazoles have been synthesised to obtain new antimicrobial agents. Their activity against both bacteria and fungi has been tested and some interesting informations about their biological activity have been obtained.

Keywords: Arylideneisothiosemicarbazones; Arylidenehydrazothiazoles; Anti-fungal and anti-microbial activity

1. Introduction

In previous communications we reported the synthesis and antimicrobial activity of new arylideneisothiosemicarbazones. In particular we investigated the influence of structural modification, such as the introduction of substituents on the sulfur atom, the aromatic ring, or the terminal nitrogen atom, on their biological activity (Compounds **A** in Scheme 1) [1].

Most of the synthesised compounds show interesting MIC values against some strains of Staphylococcus, Bacillus, and Streptococcus, while none was active against fungi.

More recently we synthesised and studied some isothiosemicarbazones [2], where the arylidene group was replaced with a cycloalkyl and the sulfur atom either differently substituted or enclosed in a thiazole ring (Compounds **B** and **C** in Scheme 1).

In most cases the cycloalkylisothiosemicarbazones (**B**) were inactive against the tested bacteria and fungi, except *S. agalactiae.* Interestingly we observed that the higher the lipophilicity, the higher the activity.

On the contrary the corresponding cyclic analogues (cycloalkyl hydrazo-thiazoles (**C**)) are active against several species of Candida (*C. albicans* and *C. krusei*) with MIC values comparable to those exhibited by miconazole and clortrimazole.

To obtain further information on the influence of structural modification on the biological activity, we synthesised and tested a new series of 2-arylidencycloalkylisothiosemicarbazones (**3**–**27**), 2-hydrazothiazoles (**33**–**44**), and arylidenehydrazothiazoles (**45**–**62**).

The group on the sulfur atom is either a mono- or disubstituted benzyl, these being the most effective substitutions in the previously tested compounds [1,2].

2. Results and discussion

2.1. Chemistry

2-Benzalcyclopentyliden-isothiosemicarbazones (**3**–**27**) (Scheme 2) were synthesised by reacting isothiosemicarbazides with the appropriate 2-arylidencyclopentanone in refluxing isopropyl alcohol and a catalytic amount of acetic acid.

The starting cyclopentanones were synthesised either by reacting aldehydes with ketones in basic media or via enamines as described in the literature.

In the case of compounds **28**–**62**, ketones or aldehydes are directly reacted with thiosemicarbazide, and

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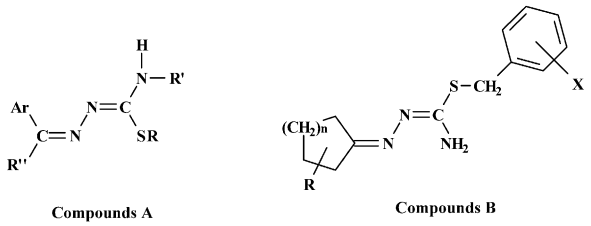
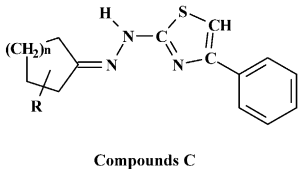




Scheme 1.

the obtained thiosemicarbazones reacted with α-halogenoketones to yield the 4-substituted thiazole ring.

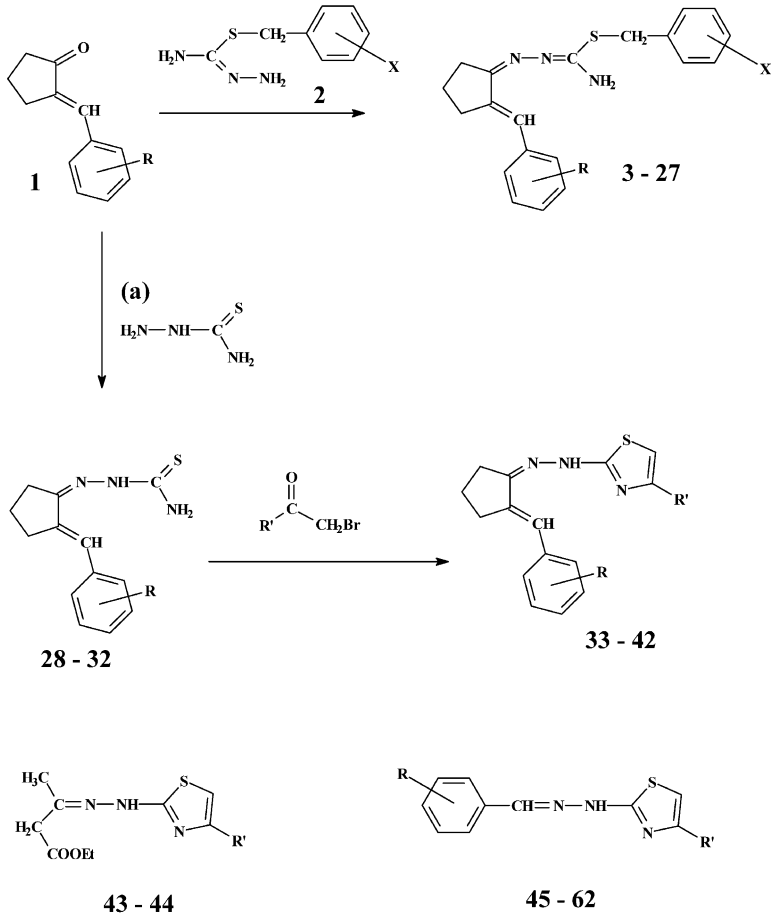
Also in this case, isopropyl alcohol proved to be the best solvent for our purpose. In fact, the reaction products precipitate on cooling down, and can be filtered and purified by crystallisation from ethanol or ethanol/isopropanol.

All the synthesised products have been characterised by analytical methods, Infrared (IR), ^{1}H NMR, and Mass Spectrometry.

2.2. Microbiology

All the synthesised compounds were tested against several microbial species to investigate the influence of structural modifications on biological activity.

In particular the antibacterial activity of compounds **3**–**62** was tested against five Gram-positive species (*Staphylococcus aureus*, *S. epidermidis*, *Streptococcus agalactiae*, *S. faecalis*, and *B. subtilis*), and five Gram-negative species (*Escherichia coli*, *Pseudomonas aerugi-*



R, X, R' = see tables 1, 2, 3

Scheme 2.

nosa, *Salmonella typhi*, *Proteus mirabilis*, and *Klebsiella pneumoniae*), while their antifungal activity was evaluated against *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *Saccharomyces cerevisiae*, and *C. tropicalis*. All the microorganisms were isolated from clinical specimens.

In the case of compounds **3**–**27** a good antibacterial activity was expected. As a matter of fact, in a previous study on cycloalkylisothiosemicarbazones, we observed that the higher the lipophilicity, the lower the MICs [2]. Compounds **3**–**27** have a higher lipophilic character than the previously synthesised derivatives, due to the introduction of the arylidene group on the cycloalkane ring. For this reason an increase in antibacterial activity was predictable.

Unfortunately a dramatic decrease in activity was observed, the MICs being higher than 200 μg/ml for all the tested microorganisms. This suggests that though important lipophilicity is not the only parameter that plays a key role in the structure–activity relationships of these derivatives.

Other factors deriving from the introduction of the arylidene group, such as an increase in both molecular weight and conformational constriction, can seriously affect the biological activity of these molecules.

Compounds **28**–**32** are intermediates and have not been tested for antimicrobial activity.

As well as the previously synthesised thiazole derivatives [2], compounds **33**–**62** were not active against the tested bacteria, but in contrast with previous observations, almost no activity against fungi was measured.

Only compounds **34** and **44** exhibited antifungal activity, but only at relatively high concentrations.

As a matter of fact, their MIC against *C. albicans* was 25 μg/ml, while Miconazole and Clotrimazole, used as reference compounds, each exhibit a MIC of 3.12 μg/ml.

In compounds **45**–**62** the cycloalkane ring was eliminated and the arylidene group was directly condensed with the hydrazo-thiazole moiety to obtain more active compounds.

Also in this case unfortunately the activity was lower than expected, not only against bacteria but also against fungi.

Once more the presence of the planar aromatic ring, with or without interposition of a cycloalkane ring, lead to a complete loss both of antibacterial and antifungal activity.

3. Conclusions

Several arylideneisothiosemicarbazones and arylidenehydrazothiazoles were synthesised.

Unfortunately, none of the synthesised compounds exhibited interesting biological properties, in particular compared to analogous derivatives.

Nevertheless, interesting data can be obtained. In particular, in the case of arylideneisothiosemicarbazones, the reduction in molecular flexibility and the introduction of the arylidene moiety led to an almost complete loss of antibacterial activity, while, in the case of thiazole derivatives the antifungal activity is still present in some of the tested compounds, meaning that most probably the 2-hydrazo-4-substituted thiazole is the active part of the molecule. However, the introduction of substituents with high steric hindrance reduces biological activity.

4. Experimental

4.1. Materials and methods

Melting points are uncorrected and were determined on a Reichert Kofler thermopan apparatus. IR spectra were recorded on a Perkin–Elmer 1640 FT spectrometer (KBr discs, in 1/cm). ^{1}H NMR spectra were recorded on a Bruker AMX (300 MHz) using tetramethylsilane (TMS) as internal standard (chemical shifts in δ values). Electron ionisation (EI) mass spectra were obtained by a Fisons QMD 1000 mass spectrometer (70 eV, 200 μA, ion source temperature 200 °C). The samples were introduced directly into the ion source. Elemental analyses were obtained on a Perkin–Elmer 240 B microanalyser.

4.2. Chemistry

The structures of all compounds were assigned on the basis of IR, NMR, Mass spectra, and elemental analysis.

Analytical data of the synthesised compounds are reported in Tables 1–3.

4.3. Synthesis of starting enamines, arylidencycloketones and isothiosemicarbazides

1-*N*-morpholinocyclopent-1-ene [3], 2-benzylidencyclopentanone [4–6], and isothiosemicarbazides were synthesised according to previously reported methods [2]: 1 equiv. of thiosemicarbazide is refluxed in isopropyl alcohol with 1 equiv. of the appropriate benzyl chloride. The mixture is refluxed until complete dissolution (30–90 min) and then allowed to cool down to r.t. The required isothiosemicarbazide precipitates. After filtration, the product can be used without further purification, or crystallised for characterisation with analytical and spectroscopic methods.

General method for the synthesis of compounds **3**–**27**.

Table 1
2-Benzalcyclopentyliden-*S*-benzylisothiosemicarbazones

Comp.	Formula	R	X	*m/z*	Yield %	M.p., °C	C, %	H, %	N, %
3	$C_{20}H_{20}N_3ClS$	H	2-Cl	369	89	218–219	64.94 (65.05)	5.45 (5.48)	11.36 (11.43)
4	$C_{20}H_{20}N_3ClS$	H	4-Cl	369	88	221	64.94 (64.71)	5.45 (5.41)	11.36 (11.31)
5	$C_{20}H_{19}N_3Cl_2S$	H	3,4-Cl	404	95	206–210	59.41 (59.37)	4.74 (4.71)	10.39 (10.35)
6	$C_{20}H_{20}N_3Cl_2S$	H	2,4-Cl	404	83	218–219	59.41 (59.35)	4.74 (4.77)	10.39 (10.42)
7	$C_{22}H_{25}N_3S$	H	3,4-CH_3	363	81	206–208	72.69 (72.55)	6.93 (6.90)	11.55 (11.60)
8	$C_{20}H_{18}N_4Cl_2O_2S$	4-NO_2	3,4-Cl	449	90	218–220	53.46 (53.29)	4.04 (4.01)	12.47 (12.45)
9	$C_{20}H_{18}N_4Cl_2O_2S$	3-NO_2	3,4-Cl	449	88	225	53.46 (53.32)	4.04 (4.07)	12.47 (12.50)
10	$C_{20}H_{18}N_3Cl_3S$	4-Cl	3,4-Cl	438	92	214	54.74 (54.92)	4.13 (4.16)	9.58 (9.61)
11	$C_{20}H_{17}N_3Cl_4S$	2,4-Cl	3,4-Cl	479	95	206–210	50.76 (50.61)	3.58 (3.61)	8.77 (8.80)
12	$C_{20}H_{18}N_3Cl_3S$	2,4-Cl	4-Cl	438	95	204–206	54.74 (54.89)	4.13 (4.11)	9.58 (9.62)
13	$C_{20}H_{18}N_4Cl_2O_2S$	4-NO_2	2,4-Cl	449	88	221–222	53.46 (53.61)	4.04 (4.01)	12.47 (12.50)
14	$C_{20}H_{18}N_4Cl_2O_2S$	3-NO_2	2,4-Cl	449	92	228–229	53.46 (53.64)	4.04 (4.07)	12.47 (12.43)
15	$C_{20}H_{18}N_3Cl_3S$	4-Cl	2,4-Cl	438	95	211–213	54.74 (54.60)	4.13 (4.15)	9.58 (9.54)
16	$C_{20}H_{17}N_3Cl_4S$	2,4-Cl	2,4-Cl	479	87	218–219	50.76 (50.86)	3.58 (3.61)	8.77 (8.83)
17	$C_{20}H_{19}N_4ClO_2S$	4-NO_2	4-Cl	414	93	220–221	57.90 (57.73)	4.62 (4.64)	13.50 (13.47)
18	$C_{20}H_{19}N_4ClO_2S$	4-NO_2	2-Cl	414	87	229–231	57.90 (58.00)	4.62 (4.59)	13.50 (13.45)
19	$C_{20}H_{19}N_4ClO_2S$	3-NO_2	2-Cl	414	85	220–221	57.90 (57.72)	4.62 (4.59)	13.50 (13.48)
20	$C_{20}H_{19}N_3Cl_2S$	4-Cl	2-Cl	404	90	232	59.41 (59.55)	4.74 (4.76)	10.39 (10.36)
21	$C_{20}H_{18}N_3Cl_3S$	2,4-Cl	2-Cl	438	95	223–226	54.74 (54.63)	4.13 (4.10)	9.58 (9.62)
22	$C_{22}H_{24}N_4O_2S$	4-NO_2	3,4-CH_3	408	85	222–224	64.68 (64.75)	5.92 (5.96)	13.72 (13.69)
23	$C_{22}H_{24}N_4O_2S$	3-NO_2	3,4-CH_3	408	81	233–235	64.68 (64.80)	5.92 (5.90)	13.72 (13.78)
24	$C_{20}H_{19}N_4ClO_2S$	3-NO_2	4-Cl	414	86	218–221	57.90 (58.07)	4.62 (4.66)	13.50 (13.56)
25	$C_{20}H_{19}N_3Cl_2S$	4-Cl	4-Cl	404	92	217	59.41 (59.18)	4.74 (4.72)	10.39 (10.44)
26	$C_{22}H_{24}N_3ClS$	4-Cl	3,4-CH_3	397	87	216–218	66.40 (66.29)	6.08 (6.10)	10.56 (10.52)
27	$C_{22}H_{23}N_3Cl_2S$	2,4-Cl	3,4-CH_3	432	89	210	61.11 (61.27)	5.36 (5.38)	9.72 (9.69)

*4.4. Synthesis of 2-benzalcyclopentyliden-S-(2-chlorobenzyl)-isothiosemicarbazone (**3**)*

In a flask equipped with a refluxing condenser and a magnetic stirrer, 2-benzalcyclopentanone (5 mmol) and isothiosemicarbazide are reacted in 60 ml of isopropyl alcohol and a catalytic amount of acetic aid. The mixture is stirred at room temperature for 5–10 min, and then refluxed to complete dissolution. The solution is further refluxed for 20 min. On cooling, a solid is formed, which can be filtered, washed with water, and crystallised from ethanol.

^{1}H NMR (DMSO): δ 1.91–2.00 (m, 2H, CH_2); 2.47–2.90 (m, 4H, CH_2); 4.86 (s, 2H, CH_2–S); 7.39–8.42 (m, 10H, phenyl-H and phenyl-CH=); 10.05 (s, 1H, NH_2, D-exch.); 12.63 (s, 1H, NH_2, D-exch.).

According to the same procedure, the following listed compounds have been synthesised.

*4.4.1. 2-Benzalcyclopentyliden-S-(4-chlorobenzyl)-isothiosemicarbazone (**4**)*

^{1}H NMR (DMSO): δ 1.95–2.00 (m, 2H, CH_2); 2.46–2.90 (m, 4H, CH_2); 4.88 (s, 2H, CH_2–S); 7.36–8.40 (m,

Table 2
Thiazoles-2,4-substituted **33**–**44**

Comp.	Formula	R	R′	*m/z*	M.p., °C	C, %	H, %	N, %
33	$C_{21}H_{19}N_3S$	H	C_6H_5	345	228–230	73.02 (72.75)	5.54 (5.57)	12.16 (12.20)
34	$C_{16}H_{17}N_3S$	H	CH_3	283	219–220	67.81 (68.03)	6.05 (6.02)	14.83 (14.77)
35	$C_{21}H_{18}N_4O_2S$	4-NO_2	C_6H_5	390	260–262	64.60 (64.32)	4.65 (4.68)	14.35 (14.40)
36	$C_{16}H_{16}N_4O_2S$	4-NO_2	CH_3	328	217–218	58.52 (58.70)	4.91 (4.89)	17.06 (16.99)
37	$C_{21}H_{18}N_4O_2S$	3-NO_2	C_6H_5	390	249–250	64.60 (64.91)	4.65 (4.62)	14.35 (14.29)
38	$C_{16}H_{16}N_4O_2S$	3-NO_2	CH_3	328	218–220	58.52 (58.72)	4.91 (4.94)	17.06 (17.10)
39	$C_{21}H_{17}N_3Cl_2S$	2,4-Cl	C_6H_5	414	190–191	60.87 (61.03)	4.14 (4.11)	10.14 (10.10)
40	$C_{16}H_{15}N_3Cl_2S$	2,4-Cl	CH_3	352	206–207	54.55 (54.36)	4.29 (4.32)	11.93 (11.98)
41	$C_{21}H_{18}N_3ClS$	4-Cl	C_6H_5	379	230–231	66.39 (66.15)	4.77 (4.79)	11.06 (11.08)
42	$C_{16}H_{16}N_3ClS$	4-Cl	CH_3	317	206–208	60.46 (60.72)	5.07 (5.04)	13.22 (13.16)
43	$C_{10}H_{15}N_3O_2S$	–	CH_3	241	247–248	49.77 (50.01)	6.27 (6.31)	17.41 (17.16)
44	$C_{15}H_{17}N_3O_2S$	–	C_6H_5	303	220–223	59.39 (59.59)	5.65 (5.68)	13.85 (13.91)

Table 3
Thiazoles 3,4-disobstituted **45**–**62**

Comp.	Formula	R	R_1	*m/z*	M.p., °C	C, %	H, %	N, %
45	$C_{11}H_{11}N_3S$	H	CH_3	217	192–194	60.80 (61.02)	5.10 (5.14)	19.34 (19.28)
46	$C_{16}H_{13}N_3S$	H	C_6H_5	279	252–255	68.79 (68.58)	4.69 (4.73)	15.04 (14.99)
47	$C_{11}H_{10}N_3ClS$	4-Cl	CH_3	251	207–209	52.49 (52.55)	4.00 (4.03)	16.70 (16.65)
48	$C_{16}H_{12}N_3ClS$	4-Cl	C_6H_5	313	246–248	61.24 (61.07)	3.86 (3.84)	13.39 (13.44)
49	$C_{11}H_9N_3Cl_2S$	2,4-Cl	CH_3	286	224–226	46.17 (45.98)	3.17 (3.19)	14.68 (14.64)
50	$C_{16}H_{11}N_3Cl_2S$	2,4-Cl	C_6H_5	348	244–246	55.18 (54.98)	3.18 (3.20)	12.07 (12.04)
51	$C_{11}H_{10}N_4O_2S$	2-NO_2	CH_3	262	215–217	50.37 (50.26)	3.84 (3.87)	21.36 (21.31)
52	$C_{16}H_{12}N_4O_2S$	2-NO_2	C_6H_5	324	263–265	59.25 (59.13)	3.73 (3.70)	17.27 (17.23)
53	$C_{11}H_{10}N_4O_2S$	3-NO_2	CH_3	262	205–206	50.37 (50.29)	3.84 (3.82)	21.36 (21.30)
54	$C_{16}H_{12}N_4O_2S$	3-NO_2	C_6H_5	324	241–244	59.25 (59.34)	3.73 (3.71)	17.27 (17.29)
55	$C_{11}H_{10}N_4O_2S$	4-NO_2	CH_3	262	231–232	50.37 (50.30)	3.84 (3.81)	21.36 (21.41)
56	$C_{16}H_{12}N_4O_2S$	4-NO_2	C_6H_5	324	245	59.25 (59.29)	3.73 (3.75)	17.27 (17.30)
57	$C_{12}H_{13}N_3OS$	4-OCH_3	CH_3	247	199–203	58.28 (58.19)	5.30 (5.27)	16.99 (17.04)
58	$C_{17}H_{15}N_3OS$	4-OCH_3	C_6H_5	309	213–217	66.00 (65.95)	4.89 (4.93)	13.58 (13.62)
59	$C_{13}H_{15}N_3O_2S$	3,4-OCH_3	CH_3	277	223–226	56.30 (56.22)	5.45 (5.43)	15.15 (15.09)
60	$C_{18}H_{17}N_3O_2S$	3,4-OCH_3	C_6H_5	339	236–238	63.70 (63.63)	5.05 (5.08)	12.38 (12.34)
61	$C_{12}H_{13}N_3S$	4-CH_3	CH_3	231	181–184	62.31 (62.19)	5.66 (5.63)	18.17 (18.12)
62	$C_{17}H_{15}N_3S$	4-CH_3	C_6H_5	293	202–205	69.60 (69.48)	5.15 (5.12)	14.32 (14.37)

10H, phenyl-H and phenyl-CH=); 10.00 (s, 1H, NH_2, D-exch.); 12.60 (s, 1H, NH_2, D-exch.).

*4.4.2. 2-Benzalcyclopentyliden-S-(3,4-dichlorobenzyl)-isothiosemicarbazone (**5**)*

^{1}H NMR (DMSO): δ 1.99–2.07 (m, 2H, CH_2); 2.48–2.95 (m, 4H, CH_2); 4.87 (s, 2H, CH_2–S); 7.32–8.39 (m, 9H, phenyl-H and phenyl-CH=); 10.03 (s, 1H, NH_2, D-exch.); 12.69 (s, 1H, NH_2, D-exch.).

*4.4.3. 2-Benzalcyclopentyliden-S-(2,4-dichlorobenzyl)-isothiosemicarbazone (**6**)*

^{1}H NMR (DMSO): δ 1.94–1.99 (m, 2H, CH_2); 2.60–2.95 (m, 4H, CH_2); 4.75 (s, 2H, CH_2–S); 7.36–8.33 (m, 9H, phenyl-H and phenyl-CH=); 10.25 (s, 1H, NH_2); 12.58 (s, 1H, NH_2).

4.4.4. 2-Benzalcyclopentyliden-S-(3,4-dimethylbenzyl)-isothiosemicarbazone (7)

^{1}H NMR ($CDCl_3$): δ 1.88–1.99 (m, 2H, CH_2); 2.22–2.85 (m, 10H, CH_2 and phenyl-CH_3); 4.53 (s, 2H, CH_2–S); 7.12–7.45 (m, 9H, phenyl-H and phenyl-CH=); 9.95 (s, 1H, NH_2, D-exch.); 12.42 (s, 1H, NH_2, D-exch.).

*4.4.5. 2-(4-Nitrobenzalcyclopentyliden)-S-(3,4-dichlorobenzyl)-isothiosemicarbazone (**8**)*

^{1}H NMR (DMSO): δ 1.96–2.10 (m, 2H, CH_2); 2.40–3.05 (m, 4H, CH_2); 4.86 (s, 2H, CH_2–S); 7.35–8.40 (m, 8H, phenyl-H and phenyl-CH=); 10.10 (s, 1H, NH_2, D-exch.); 12.76 (s, 1H, NH_2, D-exch.).

*4.4.6. 2-(3-Nitrobenzalcyclopentyliden)-S-(3,4-dichlorobenzyl)-isothiosemicarbazone (**9**)*

^{1}H NMR (DMSO): δ 1.87–1.95 (m, 2H, CH_2); 2.67–2.86 (m, 4H, CH_2); 4.68 (s, 2H, CH_2–S); 7.50–8.27 (m, 8H, phenyl-H and phenyl-CH=); 9.95 (s, 1H, NH_2, D-exch.); 12.65 (s, 1H, NH_2, D-exch.).

*4.4.7. 2-(4-Chlorobenzalcyclopentyliden)-S-(3,4-dichlorobenzyl)-isothiosemicarbazone (**10**)*

^{1}H NMR (DMSO): δ 1.84–2.20 (m, 2H, CH_2); 2.51–2.99 (m, 4H, CH_2); 4.78 (s, 2H, CH_2–S); 7.35–7.99 (m, 8H, phenyl-H and phenyl-CH=); 9.96 (s, 1H, NH_2, D-exch.); 12.58 (s, 1H, NH_2, D-exch.).

*4.4.8. 2-(2,4-Dichlorobenzalcyclopentyliden)-S-(3,4-dichlorobenzyl)-isothiosemicarbazone (**11**)*

^{1}H NMR (DMSO): δ 1.90–1.95 (m, 2H, CH_2); 2.60–2.85 (m, 4H, CH_2); 4.74 (s, 2H, CH_2–S); 7.45–7.92 (m, 7H, phenyl-H and phenyl-CH=); 9.79 (s, 1H, NH_2, D-exch.); 12.52 (s, 1H, NH_2, D-exch.).

*4.4.9. 2-(2,4-Dichlorobenzalcyclopentyliden)-S-(4-chlorobenzyl)-isothiosemicarbazone (**12**)*

^{1}H NMR (DMSO): δ 1.89–2.02 (m, 2H, CH_2); 2.56–2.98 (m, 4H, CH_2); 4.77 (s, 2H, CH_2–S); 7.44–8.00 (m, 8H, phenyl-H and phenyl-CH=); 9.97 (s, 1H, NH_2, D-exch.); 12.57 (s, 1H, NH_2, D-exch.).

*4.4.10. 2-(4-Nitrobenzalcyclopentyliden)-S-(2,4-dichlorobenzyl)-isothiosemicarbazone (**13**)*

^{1}H NMR (DMSO): δ 1.98–2.17 (m, 2H, CH_2); 2.22–2.97 (m, 4H, CH_2); 4.89 (s, 2H, CH_2–S); 7.42–8.40 (m, 8H, phenyl-H and phenyl-CH=); 10.04 (s, 1H, NH_2, D-exch.); 12.69 (s, 1H, NH_2, D-exch.).

*4.4.11. 2-(3-Nitrobenzalcyclopentyliden)-S-(2,4-dichlorobenzyl)-isothiosemicarbazone (**14**)*

^{1}H NMR (DMSO): δ 1.99–2.04 (m, 2H, CH_2); 2.38–2.99 (m, 4H, CH_2); 4.74 (s, 2H, CH_2–S); 7.50–8.40 (m, 8H, phenyl-H and phenyl-CH=); 9.69 (s, 1H, NH_2, D-exch.); 12.61 (s, 1H, NH_2, D-exch.).

*4.4.12. 2-(4-Chlorobenzalcyclopentyliden)-S-(2,4-dichlorobenzyl)-isothiosemicarbazone (**15**)*

^{1}H NMR (DMSO): δ 1.93–2.05 (m, 2H, CH_2); 2.50–3.20 (m, 4H, CH_2); 4.78 (s, 2H, CH_2–S); 7.50–7.60 (m, 8H, phenyl-H and phenyl-CH=); 9.83 (s, 1H, NH_2, D-exch.); 12.48 (s, 1H, NH_2, D-exch.).

*4.4.13. 2-(2,4-Dichlorobenzalcyclopentyliden)-S-(2,4-dichlorobenzyl)-isothiosemicarbazone (**16**)*

^{1}H NMR (DMSO): δ 1.60–2.20 (m, 2H, CH_2); 2.45–3.20 (m, 4H, CH_2); 4.79 (s, 2H, CH_2–S); 7.30–8.35 (m, 7H, phenyl-H and phenyl-CH=); 10.30 (s, 1H, NH_2, D-exch.); 12.60 (s, 1H, NH_2, D-exch.).

*4.4.14. 2-(4-Nitrobenzalcyclopentyliden)-S-(4-chlorobenzyl)-isothiosemicarbazone (**17**)*

^{1}H NMR (DMSO): δ 1.88–1.93 (m, 2H, CH_2); 2.88–3.27 (m, 4H, CH_2); 4.88 (s, 2H, CH_2–S); 7.33–8.37 (m, 9H, phenyl-H and phenyl-CH=); 9.65 (s, 1H, NH_2, D-exch.); 12.85 (s, 1H, NH_2, D-exch.).

*4.4.15. 2-(4-Nitrobenzalcyclopentyliden)-S-(2-chlorobenzyl)-isothiosemicarbazone (**18**)*

^{1}H NMR (DMSO): δ 1.92–2.03 (m, 2H, CH_2); 2.40–3.09 (m, 4H, CH_2); 4.79 (s, 2H, CH_2–S); 7.35–8.46 (m, 9H, phenyl-H and phenyl-CH=); 10.01 (s, 1H, NH_2, D-exch.); 12.68 (s, 1H, NH_2, D-exch.).

*4.4.16. 2-(3-Nitrobenzalcyclopentyliden)-S-(2-chlorobenzyl)-isothiosemicarbazone (**19**)*

^{1}H NMR (DMSO): δ 1.87–1.92 (m, 2H, CH_2); 2.40–2.85 (m, 4H, CH_2); 4.64 (s, 2H, CH_2–S); 7.43–8.30 (m, 9H, phenyl-H and phenyl-CH=); 10.00 (s, 1H, NH_2, D-exch.); 12.77 (s, 1H, NH_2, D-exch.).

*4.4.17. 2-(4-Chlorobenzalcyclopentyliden)-S-(2-chlorobenzyl)-isothiosemicarbazone (**20**)*

^{1}H NMR (DMSO): δ 1.91–2.01 (m, 2H, CH_2); 2.39–2.72 (m, 4H, CH_2); 4.73 (s, 2H, CH_2–S); 7.38–8.22 (m, 9H, phenyl-H and phenyl-CH=); 10.09 (s, 1H, NH_2, D-exch.); 12.53 (s, 1H, NH_2, D-exch.).

*4.4.18. 2-(2,4-Dichlorobenzalcyclopentyliden)-S-(2-chlorobenzyl)-isothiosemicarbazone (**21**)*

^{1}H NMR (DMSO): δ 1.96–2.03 (m, 2H, CH_2); 2.43–2.79 (m, 4H, CH_2); 4.83 (s, 2H, CH_2–S); 7.41–8.34 (m, 8H, phenyl-H and phenyl-CH=); 10.13 (s, 1H, NH_2, D-exch.); 12.64 (s, 1H, NH_2, D-exch.).

*4.4.19. 2-(4-Nitrobenzalcyclopentyliden)-S-(3,4-dimethylbenzyl)-isothiosemicarbazone (**22**)*

^{1}H NMR (DMSO): δ 1.90–2.85 (m, 12H, CH_2 and phenyl-CH_3); 4.70 (s, 2H, CH_2–S); 7.21–8.30 (m, 8H, phenyl-H and phenyl-CH=); 10.10 (s, 1H, NH_2, D-exch.); 12.77 (s, 1H, NH_2, D-exch.).

*4.4.20. 2-(3-Nitrobenzalcyclopentyliden)-S-(3,4-dimethylbenzyl)-isothiosemicarbazone (**23**)*

^{1}H NMR (DMSO): δ 2.01–2.04 (m, 2H, CH_2); 2.27–2.96 (m, 10H, CH_2 and phenyl-CH_3); 4.63 (s, 2H, CH_2–S); 7.20–8.38 (m, 8H, phenyl-H and phenyl-CH=); 9.79 (s, 1H, NH_2, D-exch.); 12.71 (s, 1H, NH_2, D-exch.).

*4.4.21. 2-(3-Nitrobenzalcyclopentyliden)-S-(4-chlorobenzyl)-isothiosemicarbazone (**24**)*

^{1}H NMR (DMSO): δ 1.90–1.93 (m, 2H, CH_2); 2.66–2.85 (m, 4H, CH_2); 4.62 (s, 2H, CH_2–S); 7.40–8.27 (m, 9H, phenyl-H and phenyl-CH=); 9.68 (s, 1H, NH_2, D-exch.); 12.48 (s, 1H, NH_2, D-exch.).

*4.4.22. 2-(4-Chlorobenzalcyclopentyliden)-S-(4-chlorobenzyl)-isothiosemicarbazone (**25**)*

^{1}H NMR (DMSO): δ 1.89–2.03 (m, 2H, CH_2); 2.37–2.84 (m, 4H, CH_2); 4.70 (s, 2H, CH_2–S); 7.52–8.33 (m, 9H, phenyl-H and phenyl-CH=); 9.77 (s, 1H, NH_2, D-exch.); 12.42 (s, 1H, NH_2, D-exch.).

*4.4.23. 2-(4-Chlorobenzalcyclopentyliden)-S-(3,4-dimethylbenzyl)-isothiosemicarbazone (**26**)*

^{1}H NMR (DMSO): δ 1.96–2.00 (m, 2H, CH_2); 2.20–2.88 (m, 10H, CH_2 and phenyl-CH_3); 4.79 (s, 2H, CH_2–S); 7.23–7.95 (m, 8H, phenyl-H and phenyl-CH=); 9.80 (s, 1H, NH_2, D-exch.); 12.44 (s, 1H, NH_2, D-exch.).

*4.4.24. 2-(2,4-Dichlorobenzalcyclopentyliden)-S-(3,4-dimethylbenzyl)-isothiosemicarbazone (**27**)*

^{1}H NMR (DMSO): δ 1.97–2.05 (m, 2H, CH_2); 2.13–2.91 (m, 10H, CH_2 and phenyl-CH_3); 4.70 (s, 2H, CH_2–S); 7.13–8.35 (m, 7H, phenyl-H and phenyl-CH=); 9.82 (s, 1H, NH_2, D-exch.); 12.73 (s, 1H, NH_2, D-exch.).

General method for the synthesis of compounds **33**–**62**.

*4.5. Synthesis of 2-[2-(benzalcyclopentyliden)hydrazo]-4-phenylthiazole (**33**)*

2-Benzalcyclopentylidenthiosemicarbazide (0.02 mol) and ω-bromo-acetophenone (0.02 mol) are stirred in refluxing isopropyl alcohol (70–100 ml) to complete dissolution, and then until a white foaming product is formed. The mixture is then allowed to cool down and the solid is filtered, washed with saturated $NaHCO_3$ water solution and then with cool water, dried, and crystallised from ethanol.

^{1}H NMR ($CDCl_3$): δ 1.98–2.09 (m, 2H, CH_2); 2.83–2.95 (m, 4H, CH_2); 6.74 (s, 1H, H^5-thiaz.); 7.25–7.75 (m, 11H, phenyl-H and phenyl-CH=); 12.18 (s, 1H, NH, D-exch.).

According to this method, the following listed compounds have been synthesised.

*4.5.1. 2-[2-(Benzalcyclopentyliden)hydrazo]-4-methylthiazole (**34**)*

^{1}H NMR ($CDCl_3$): δ 2.02–2.22 (m, 2H, CH_2); 2.41 (s, 3H, CH_3); 2.81–2.91 (m, 4H, CH_2); 6.25 (s, 1H, H^5-thiaz.); 7.32–7.52 (m, 6H, phenyl-H and phenyl-CH=); 12.56 (s, 1H, NH, D-exch.).

*4.5.2. 2-[2-(4-Nitrobenzalcyclopentyliden)hydrazo]-4-phenylthiazole (**35**)*

^{1}H NMR ($CDCl_3$): δ 1.98–2.00 (m, 2H, CH_2); 2.75–2.80 (m, 4H, CH_2); 6.64 (s, 1H, H^5-thiaz.); 7.07–8.14 (m, 10H, phenyl-H and phenyl-CH=); 12.47 (s, 1H, NH, D-exch.).

*4.5.3. 2-[2-(4-Nitrobenzalcyclopentyliden)hydrazo]-4-methylthiazole (**36**)*

^{1}H NMR ($CDCl_3$): δ 1.99–2.16 (m, 2H, CH_2); 2.37 (s, 3H, CH_3); 2.82–2.93 (m, 4H, CH_2); 6.24 (s, 1H, H^5-thiaz.); 7.25–8.25 (m, 5H, phenyl-H and phenyl-CH=); 13.02 (s, 1H, NH, D-exch.).

*4.5.4. 2-[2-(3-Nitrobenzalcyclopentyliden)hydrazo]-4-phenylthiazole (**37**)*

^{1}H NMR ($CDCl_3$): δ 2.03–2.13 (m, 2H, CH_2); 2.88–2.95 (m, 4H, CH_2); 6.77 (s, 1H, H^5-thiaz.); 7.25–8.31 (m, 10H, phenyl-H and phenyl-CH=); 12.64 (s, 1H, NH, D-exch.).

*4.5.5. 2-[2-(3-Nitrobenzalcyclopentyliden)hydrazo]-4-methylthiazole (**38**)*

^{1}H NMR ($CDCl_3$): δ 2.05–2.15 (m, 2H, CH_2); 2.43 (s, 3H, CH_3); 2.87–2.99 (m, 4H, CH_2); 6.30 (s, 1H, H^5-thiaz.); 7.31–8.35 (m, 5H, phenyl-H and phenyl-CH=); 12.99 (s, 1H, NH, D-exch.).

*4.5.6. 2-[2-(2,4-Dichlorobenzalcyclopentyliden)-hydrazo]-4-phenylthiazole (**39**)*

^{1}H NMR ($CDCl_3$): δ 2.02–2.10 (m, 2H, CH_2); 2.85–2.93 (m, 4H, CH_2); 6.72 (s, 1H, H^5-thiaz.); 7.18–7.55 (m, 9H, phenyl-H and phenyl-CH=); 12.70 (s, 1H, NH, D-exch.).

*4.5.7. 2-[2-(2,4-Dichlorobenzalcyclopentyliden)-hydrazo]-4-methylthiazole (**40**)*

^{1}H NMR ($CDCl_3$): δ 1.90–2.15 (m, 2H, CH_2); 2.44 (s, 3H, CH_3); 2.60–2.71 (m, 4H, CH_2); 6.08 (s, 1H, H^5-thiaz.); 7.10–7.42 (m, 4H, phenyl-H and phenyl-CH=); 12.85 (s, 1H, NH, D-exch.).

*4.5.8. 2-[2-(4-Chlorobenzalcyclopentyliden)hydrazo]-4-phenylthiazole (**41**)*

^{1}H NMR ($CDCl_3$): δ 1.98–2.08 (m, 2H, CH_2); 2.63–2.88 (m, 4H, CH_2); 6.74 (s, 1H, H^5-thiaz.); 7.25–8.00 (m, 10H, phenyl-H and phenyl-CH=); 12.47 (s, 1H, NH, D-exch.).

*4.5.9. 2-[2-(4-Chlorobenzalcyclopentyliden)hydrazo]-4-methylthiazole (**42**)*

^{1}H NMR ($CDCl_3$): δ 1.94–2.15 (m, 2H, CH_2); 2.37 (s, 3H, CH_3); 2.62–2.84 (m, 4H, CH_2); 6.72 (s, 1H, H^5-thiaz.); 7.54–7.85 (m, 5H, phenyl-H and phenyl-CH=); 13.28 (s, 1H, NH, D-exch.).

*4.5.10. 2-[1-(Carbetoxymethylethyliden)hydrazo]-4-methylthiazole (**43**)*

^{1}H NMR ($CDCl_3$): δ 1.16 (t, 3H, OCH_2CH_3); 2.29 (s, 3H, CH_3); 2.35 (s, 3H, CH_3); 3.54 (s, 2H, CH_2CO); 3.96–4.02 (q, 2H, OCH_2); 6.70 (s, 1H, H^5-thiaz.); 12.58 (s, 1H, NH, D-exch.).

*4.5.11. 2-[1-(Carbetoxymethylethyliden)hydrazo]-4-phenylthiazole (**44**)*

^{1}H NMR ($CDCl_3$): δ 1.29 (t, 3H, OCH_2CH_3); 2.29 (s, 3H, CH_3); 3.41 (s, 2H, CH_2CO); 4.17–4.24 (q, 2H, OCH_2); 6.73 (s, 1H, H^5-thiaz.); 7.25–7.73 (m, 5H, phenyl-H); 12.63 (s, 1H, NH, D-exch.).

*4.5.12. 2-Benzylidenhydrazo-4-methylthiazole (**45**)*

^{1}H NMR (DMSO): δ 2.27 (s, 3H, CH_3); 6.74 (s, 1H, H^5-thiaz); 7.45–7.79 (m, 5H, phenyl-H); 8.47 (s, 1H, phenyl-CH=); 10.46 (s, 1H, NH, D-exch.).

*4.5.13. 2-Benzylidenhydrazo-4-phenylthiazole (**46**)*

^{1}H NMR ($CDCl_3$): δ 6.77 (s, 1H, H^5-thiaz.); 7.43–7.74 (m, 10H, phenyl-H); 8.20 (s, 1H, phenyl-CH=); 10.39 (s, 1H, NH, D-exch.).

*4.5.14. 2-(4-Chlorobenzyliden)hydrazo-4-methylthiazole (**47**)*

^{1}H NMR (DMSO): δ 2.27 (s, 3H, CH_3); 6.72 (s, 1H, H^5-thiaz.); 7.51–7.83 (m, 4H, phenyl-H); 8.40 (s, 1H, phenyl-CH=); 11.02 (s, 1H, NH, D-exch.).

*4.5.15. 2-(4-Chlorobenzyliden)hydrazo-4-phenylthiazole (**48**)*

^{1}H NMR (DMSO): δ 7.40–7.99 (m, 9H, phenyl-H); 7.46 (s, 1H, H^5-thiaz.); 8.15 (s, 1H, phenyl-CH=); 12.36 (s, 1H, NH, D-exch.).

*4.5.16. 2-(2,4-Dichlorobenzyliden)hydrazo-4-methylthiazole (**49**)*

^{1}H NMR (DMSO): δ 2.27 (s, 3H, CH_3); 6.72 (s, 1H, H^5-thiaz.); 7.52–8.07 (m, 3H, phenyl-H); 8.66 (s, 1H, phenyl-CH=); 11.85 (s, 1H, NH, D-exch.).

*4.5.17. 2-(2,4-Dichlorobenzyliden)hydrazo-4-phenylthiazole (**50**)*

^{1}H NMR (DMSO): δ 6.80 (s, 1H, H^5-thiaz.); 7.42–8.05 (m, 8H, phenyl-H); 8.46 (s, 1H, phenyl-CH=); 12.30 (s, 1H, NH, D-exch.).

*4.5.18. 2-(2-Nitrobenzyliden)hydrazo-4-methylthiazole (**51**)*

^{1}H NMR (DMSO): δ 2.26 (s, 3H, CH_3); 6.72 (s, 1H, H^5-thiaz); 7.50–8.47 (m, 4H, phenyl-H); 8.74 (s, 1H, phenyl-CH=); 11.75 (s, 1H, NH, D-exch.).

*4.5.19. 2-(2-Nitrobenzyliden)hydrazo-4-phenylthiazole (**52**)*

^{1}H NMR (DMSO): δ 7.42–8.14 (m, 9H, phenyl-H); 7.51 (s, 1H, H^5-thiaz.); 8.55 (s, 1H, phenyl-CH=); 12.50 (s, 1H, NH, D-exch.).

*4.5.20. 2-(3-Nitrobenzyliden)hydrazo-4-methylthiazole (**53**)*

^{1}H NMR (DMSO): δ 2.27 (s, 3H, CH_3); 6.70 (s, 1H, H^5-thiaz.); 7.65–8.56 (m, 4H, phenyl-H); 8.64 (s, 1H, phenyl-CH=); 11.63 (s, 1H, NH, D-exch.).

*4.5.21. 2-(3-Nitrobenzyliden)hydrazo-4-phenylthiazole (**54**)*

^{1}H NMR ($CDCl_3$); 7.50 (s, 1H, H^5-thiaz.); 7.56–8.34 (m, 9H, phenyl-H); 8.59 (s, 1H, phenyl-CH=); 10.36 (s, 1H, NH, D-exch.).

*4.5.22. 2-(4-Nitrobenzyliden)hydrazo-4-methylthiazole (**55**)*

^{1}H NMR (DMSO): δ 2.25 (s, 3H, CH_3); 6.70 (s, 1H, H^5-thiaz); 8.13–8.36 (m, 5H, phenyl-H and phenyl-CH=); 11.74 (s, 1H, NH, D-exch.).

*4.5.23. 2-(4-Nitrobenzyliden)hydrazo-4-phenylthiazole (**56**)*

^{1}H NMR ($CDCl_3$): δ 6.84 (s, 1H, H^5-thiaz.); 7.25–8.30 (m, 9H, phenyl-H); 8.59 (s, 1H, phenyl-CH=); 12.48 (s, 1H, NH, D-exch.).

*4.5.24. 2-(4-Methoxybenzyliden)hydrazo-4-methylthiazole (**57**)*

^{1}H NMR (DMSO): δ 2.26 (s, 3H, CH_3); 3.80 (s, 3H, OCH_3); 6.71 (s, 1H, H^5-thiaz.); 7.02–7.75 (m, 4H, phenyl-H); 8.43 (s, 1H, phenyl-CH=); 11.80 (s, 1H, NH, D-exch.).

*4.5.25. 2-(4-Methoxybenzyliden)hydrazo-4-phenylthiazole (**58**)*

^{1}H NMR (DMSO): δ 3.91 (s, 3H, OCH_3); 7.42 (s, 1H, H^5-thiaz.) 7.10–7.97 (m, 9H, phenyl-H); 8.13 (s, 1H, phenyl-CH=); 11.42 (s, 1H, NH, D-exch.).

*4.5.26. 2-(3,4-Dimethoxybenzyliden)hydrazo-4-methylthiazole (**59**)*

^{1}H NMR (DMSO): δ 2.32 (s, 3H, CH_3); 3.85 (s, 6H, OCH_3); 6.74 (s, 1H, H^5-thiaz.); 7.05–7.50 (m, 3H, phenyl-H); 8.41 (s, 1H, phenyl-CH=); 11.75 (s, 1H, NH, D-exch.).

*4.5.27. 2-(3,4-Dimethoxybenzyliden)hydrazo-4-phenylthiazole (**60**)*

^{1}H NMR (DMSO): δ 3.90 (s, 6H, OCH_3); 7.11–7.97 (m, 8H, phenyl-H); 7.41 (s, 1H, H^5-thiaz.); 8.08 (s, 1H, phenyl-CH=); 11.70 (s, 1H, NH, D-exch.).

*4.5.28. 2-(4-Methylbenzyliden)hydrazo-4-methylthiazole (**61**)*

^{1}H NMR (DMSO): δ 2.31 (s, 3H, CH_3); 2.51 (s, 3H, phenyl-CH_3); 6.70 (s, 1H, H^5-thiaz); 7.27–7.69 (m, 4H, phenyl-H); 8.40 (s, 1H, phenyl-CH=); 10.83 (s, 1H, NH, D-exch.).

*4.5.29. 2-(4-Methylbenzyliden)hydrazo-4-phenylthiazole (**62**)*

^{1}H NMR ($CDCl_3$): δ 2.41 (s, 3H, CH_3); 6.75 (s, 1H, H^5-thiaz.); 7.26 (s, 1H, phenyl-CH=); 7.43–7.74 (m, 9H, phenyl-H); 10.96 (s, 1H, NH, D-exch.).

4.6. Microbiology

4.6.1. Compounds

Compounds for antimicrobial studies were dissolved in dimethylsulfoxide at 10 mg/ml and stored at −20 °C.

The working solutions were prepared in the same medium used for the tests. To avoid interference with the solvent [7], the highest DMSO concentration was 1%.

4.6.2. Microorganisms

The antimicrobial activity of compounds **3**–**27** and **33**–**62** was evaluated against five Gram-positive species (*Staphylococcus aureus*, *S. epidermidis*, *Streptococcus agalactiae*, *S. faecalis*, and *B. subtilis*), and five Gram-negative species (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus mirabilis*, and *Klebsiella pneumoniae*) isolated from clinical specimens.

C. albicans, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *Saccharomyces cerevisiae*, and *C. tropicalis*, were all isolated from clinical specimens, and were used in the evaluation of antifungal activity.

4.6.3. Determination of MICs

The MICs of the compounds against Gram-positive bacteria, Gram-negative bacteria, and fungi, were determined by a standard broth macrodilution method [8,9]. Tests with Gram-positive and Gram-negative bacteria were carried out in Mueller Hunton broth (Difco Laboratories, Detroit, MI). Antifungal activity was evaluated in Sabouraud Dextrose broth (Difco

Laboratories) [10]. In the case of fungi MFC (minimum fungicidal concentration) values, or more commonly MLC (minimum lethal concentration) values were also measured.

The compounds were diluted in the test medium to obtain a final concentration ranging between 100 and 0.19 μg/ml. Tubes containing 1 ml of the diluted compounds were inoculated with 1×10^5 bacteria and incubated at 37 °C for 18 or 24 h.

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