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Structural modifications and antimicrobial activity of *N*-cycloalkenyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles

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Abstract

A series of *N*-cycloalkenyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles **5a–j**, *N*-cycloalkyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles **8a–e**, and *N*-alkyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles **8f–h**, were synthesized and tested for in vitro antibacterial and antifungal activities against four gram-positive and five gram-negative bacteria (*Bacillus subtilis* 6633, *Enterococcus faecalis* 29212, *Staphylococcus aureus* 6538, *Staphylococcus aureus* 25923, *Escherichia coli* 25922, *Acinetobacter calcoaceticus* a1, *A. calcoaceticus* a2, *Pseudomonas aeruginosa* 27835, *Klebsiella oxytoca* 49131), four yeast-like fungi and one fungus (*Candida tropicalis* 750, *C. albicans* 14053, *C. albicans* 10231, *Criptococcus laurentii* 18803, and *Saccharomyces cerevisiae*). Microdilution broth and agar dilution methods were used for antimicrobial tests. The findings obtained showed that some of the tested compounds **5** and **8** were effective against some of the bacterial strains used, whereas, only compounds **8b–g** exhibited a moderate antifungal activity against the yeast strains evaluated.

Keywords: *N*-Cycloalkenyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles; *N*-Cycloalkyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles; Antibacterial activity; Antifungal activity

1. Introduction

The small and simple benzothiazole nucleus is present in compounds involved in research aimed at evaluating new products that possess interesting biological activities: antitumoral [1,2], antimicrobial [3], antitubercular [4], and antimalarial [5]. As part of our program aimed at the synthesis and biological evaluation of azasulfurated heterocyclic compounds [6–9], we have recently reported that also *N*-cycloalkenyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles **A** (Fig. 1), obtained by reaction of *N*-acyl-2,3-dihydro-1,3-benzothiazoles with carboxylic anhydrides [10], showed interesting activity against some gram-positive and gram-negative bacteria. Some of the evaluated compounds [11] exhibited a minimal inhibitory concentration (MIC) lower than the reference compounds chloramphenicol and gentamicine, whereas all the tested compounds proved devoid of antifungal activity. The preliminary structure–antibacterial activity relationship [11] suggested the lipophilicity as an important property modulating the activity of the reported compounds, together with the observation that the antibacterial activity disappeared on replacing the hydrogen atom of the structure **A** (R′ = H) with an alkyl or acyl group.

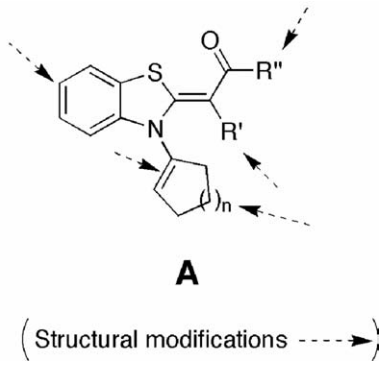

Fig. 1. Structural modifications (- - - →) of the N-cycloalkenyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazole.

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Consistent with the previous studies, and in view of the continuous interest for new antimicrobial agents, we deemed it worthwhile to investigate other *N*-substituted-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles to better evaluate the main structural requirements needful to improve and extend antimicrobial activity. We have therefore enlarged our investigations to determine if the increasing of the lipophilic character of the previously evaluated compounds [11], together with some structural modifications of **A** might influence the antimicrobial activity of the *N*-cycloalkenyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles. In this work, we have designed and synthesized a new series of 1,3-benzothiazole compounds to evaluate the effects of their structural modifications on activity against some gram-positive and gram-negative bacteria, as well as against some fungi. The structural changes of compound **A** comprised: (i) the ring enlargement of the *N*-cycloalkenyl moiety (**5a–d**); (ii) the introduction of different substituents on the benzene ring (**5f–j**); (iii) the variation in the length of acyl chain (**5e**); (iv) reduction of the *N*-cycloalkenyl moiety **8a–e,** including a cyclic acyl chain **8d**, or a *N*-alkyl moiety **8h**, containing also an heterocyclic acyl chain **8g**. Antimicrobial activity of the new compounds and of the known compounds **8c,e,f** [9] was evaluated using both the microdilution broth and agar dilution methods.

2. Experimental

2.1. Chemistry

Melting points were determined on a Büchi apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 257 Spectrophotometer as KBr disks or liquid films. ^{1}H NMR spectra were obtained on a Varian EM 390 spectrometer using TMS as internal standard (chemical shifts are expressed in δ units). Elemental analyses (C, H, N) were performed on Carlo Erba 1106 analyser, and the results agreed with the theoretical values ± 0.4%. GC–MS analysis was carried out on a Hewlet–Packard 5995C-GC–MS instrument. Column chromatography was performed using silica gel (Merck 70–235 mesh) as stationary phase and light petroleum ether/ethyl acetate 8:2 v/v and 9:1 v/v as eluent for compounds **5** and **8**, respectively. The progress of the reactions was monitored by thin-layer chromatography (TLC) with F_{254} silica gel precoated sheets (Merck) using light petroleum ether–ethyl acetate (85:15 v/v) as the mobile phase.

The starting 5-substituted-2-aminobenzenethiols**1f–j** were obtained from the corresponding 6-substituted-2-aminobenzothiazoles (Scheme 1), according to the reported procedure [12]. The *N*-cycloalkenyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles **5** were prepared according to the literature procedure [10] by treatment of the *N*-acetyl-2,3-dihydro-1,3-benzothiazole compounds **4** with appropriate carboxylic anhydride as in Scheme 1. Compound **8h** was prepared, according to a known procedure [13], by reaction of the appropriate acyl chloride with *N*-benzyl-2-methyl-benzothiazole bromide (Scheme 2) obtained by a modified reported method [14]. This last modified method [14] failed in the synthesis of the other compounds **8**, which alternatively were prepared according to the previously reported procedures, by ring cleavage of the 2,3-dihydro-1,3-benzothiazole compounds **3** (Scheme 1) with sodium borohydride to yield the corresponding *N*,*N*′-dialkyldithiodianiline **6** [15]. The reaction of compounds **6** with the appropriate β-ketoester **7** led to compounds **8** (and by products of reaction **9**) [9] in roughly equimolar amount, as in Scheme 1.

2.1.1. General procedure for the synthesis of N-cycloalkenyl-compounds *5a–j*

A mixture of 3-acetyl-2,3-dihydro-1,3-benzothiazole **4** (10 mmol) and the appropriate carboxylic anhydride (10 ml) was refluxed until the disappearance of the starting material monitored by TLC. Then, the excess of the anhydride was evaporated under reduced pressure, and the residue was purified by column chromatography. Physical and spectral data of the new compounds **5** are reported below.

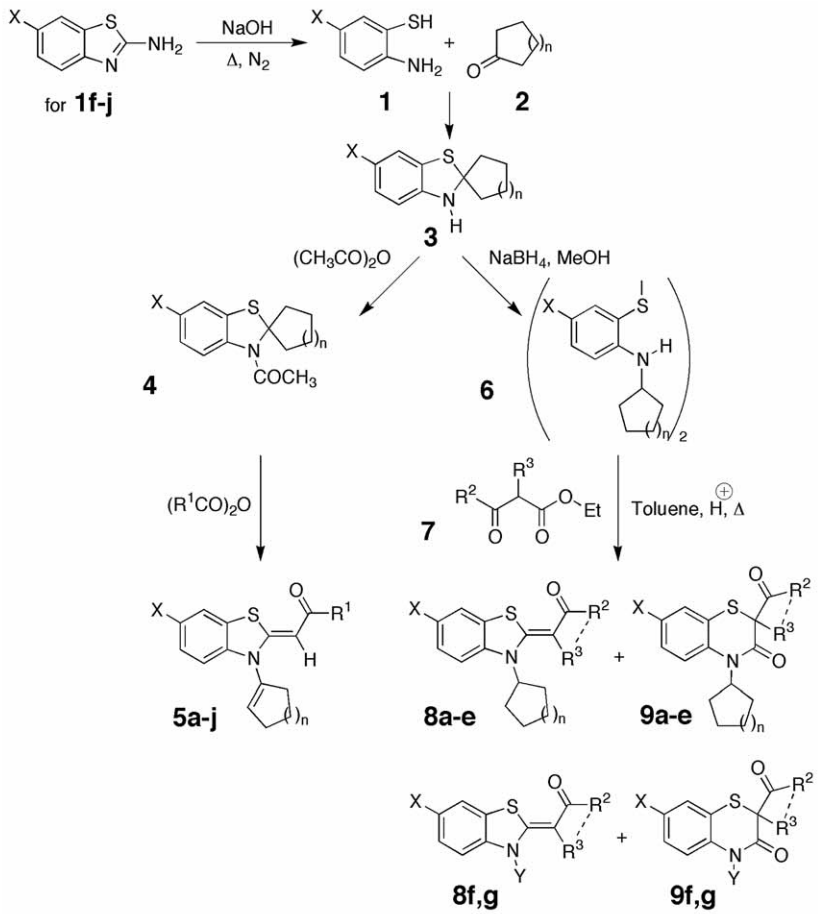
*2.1.1.1. 2-(Acetylmethylene)-N-(cyclododec-1-enyl)-2,3-dihydro-1,3-benzothiazole (**5a**).* Yield: 75%; m.p. 177–178 °C; Anal (C, H, N) $C_{22}H_{29}NOS$. IR (cm^{-1}) 1610 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.20–1.95 (m, 18H, CH_2), 2.20 (s, 3H, CH_3), 2.32–2.70 (m, 4H, CH_2), 5.70–5.90 (m, 1H, =CH–CH_2), 5.92 (s, 1H, =CH), 7.0–7.7 (m, 4H, CHAr); *m/z* 355 (M^+ 31), 312 (M^+ –(CH_3CO), base).

*2.1.1.2. 2-(Propionylmethylene)-N-(cyclododec-1-enyl)-2,3-dihydro-1,3-benzothiazole (**5b**).* Yield: 35%, m.p. 171–172 °C; Anal (C, H, N) $C_{23}H_{31}NOS$. IR (cm^{-1}) 1610 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.10 (t, 3H, CH_3), 1.20–1.95 (m, 18H, CH_2), 2.25–2.70 (m, 4H, CH_2), 5.7–5.9 (m, 1H, =CH–CH_2), 5.93 (s, 1H, =CH), 7.0–7.7 (m, 4H, CHAr); *m/z* 369 (M^+, 29), 312 (M^+ –(CH_3CH_2CO), base).

*2.1.1.3. 2-(Butyrylmethylene)-N-(cyclododec-1-enyl)-2,3-dihydro-1,3-benzothiazole (**5c**).* Yield: 65%, m.p. 167–168 °C; Anal (C, H, N) $C_{24}H_{33}NOS$. IR (cm^{-1}) 1610 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 0.95 (t, 3H, CH_3), 1.2–1.9 (m, 18H, CH_2), 2.42–2.70 (m, 6H, CH_2), 5.70–5.90 (m, 1H, =CH–CH_2), 5.92 (s, 1H, =CH), 7.0–7.70 (m, 4H, CHAr); *m/z* 383 (M^+, 30), 312 (M^+ –($CH_3CH_2CH_2CO$), base).

*2.1.1.4. 2-(Isobutyrylmethylene)-N-(cyclododec-1-enyl)-2,3-dihydro-1,3-benzothiazole (**5d**).* Yield: 42%, m.p. 162–163 °C; Anal (C, H, N) $C_{24}H_{33}NOS$. IR (cm^{-1}) 1610 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.20 (d, 6H, CH_3), 1.22–1.90 (m, 18H, CH_2), 2.24–2.80 (m, 5H, CH + CH_2), 5.70–5.90 (m, 1H, =CH–CH_2), 6.0 (s, 1H, =CH), 7.0–7.7 (m, 4H, CHAr); *m/z* 383 (M^+, 30), 312 (M^+ –((CH_3)$_2$CHCO), base).

*2.1.1.5. 2-(Heptanoylmethylene)-N-(cyclohept-1-enyl)-2,3-dihydro-1,3-benzothiazole (**5e**).* Yield: 55%, oil; Anal (C, H, N) $C_{22}H_{29}NOS$. IR (cm^{-1}) 1610 (C=O); ^{1}H NMR ($CDCl_3$) δ



5			
	X	n	R^1
a	H	8	CH_3
b	H	8	C_2H_5
c	H	8	C_3H_7
d	H	8	*i*-C_3H_7
e	H	3	C_6H_{13}
f	F	3	C_3H_7
g	OCH_3	3	C_3H_7
h	OCH_3	3	*i*-C_3H_7
i	CH_3	3	*i*-C_3H_7
j	CH_3	3	C_4H_9

8				9			
X	n	R^2	R^3	X	n	R^2	R^3
H	4	C_2H_5	H	H	4	C_2H_5	H
H	3	C_2H_5	H	H	3	C_2H_5	H
H	3	CH_3	H	H	3	CH_3	H
H	3	-$(CH_2)_3$-		H	3	-$(CH_2)_3$-	
H	3	C_6H_5	H	H	3	C_6H_5	H
X	Y	R^2	R^3	X	Y	R^2	R^3
H	C_4H_9	C_6H_5	H	H	C_4H_9	C_6H_5	H
H	C_4H_9	-CH_2-N($CH_2C_6H_5$)-$(CH_2)_2$-		H	C_4H_9	-CH_2-N($CH_2C_6H_5$)-$(CH_2)_2$-	

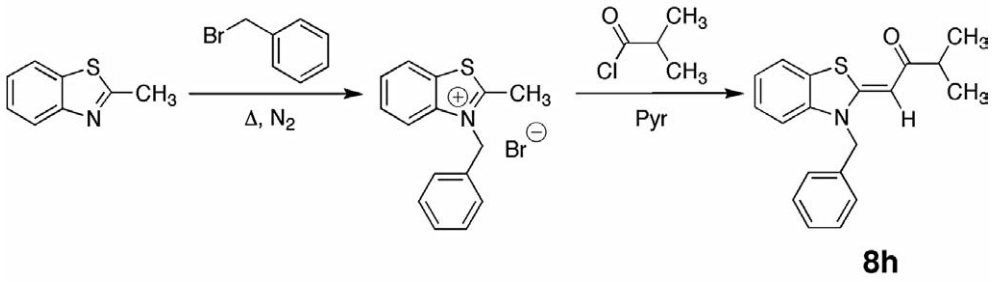
Scheme 1. Synthesis of compounds **5**,**8** and **9**.

(ppm): 0.85 (t, 3H, CH_3), 1.05–1.45 (m, 6H, CH_2), 1.50–1.90 (m, 8H, CH_2), 2.23–2.60 (m, 6H, CH_2), 5.85 (s, 1H, =CH), 6.0–6.20 (m, 1H, =CH–CH_2), 7.0–7.70 (m, 4H, CHAr); *m/z* 355 (M^+, 20), 242 (M^+ –(CH3$(CH2)_5$CO), base).

*2.1.1.6. 2-(Butyrylmethylene)-N-(cyclohept-1-enyl)-6 fluoro-2,3-dihydro-1,3-benzothiazole (**5f**).* Yield: 30%, m.p. 88–89 °C; Anal (C, H, N) $C_{19}H_{22}NOSF$. IR (cm^{-1}) 1590 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 0.95 (t, 3H, CH_3), 1.50–2.02 (m, 8H, CH_2), 2.30–2.63 (m, 6H, CH_2), 5.83 (s, 1H, =CH), 6.10 (t, 1H, =CH–CH_2), 6.90–7.33 (m, 3H, CHAr); *m/z* 331 (M^+, 18), 260 (M^+ –($CH_3CH_2CH_2CO$), base).

*2.1.1.7. 2-(Butyrylmethylene)-N-(cyclohept-1-enyl)-6 methoxy-2,3-dihydro-1,3-benzothiazole (**5g**).* Yield: 35%, oil; Anal (C, H, N) $C_{20}H_{25}NO_2S$. IR (cm^{-1}) 1600 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 0.95 (t, 3H, CH_3), 1.50–2.0 (m, 8H, CH_2), 2.30–2.63 (m, 6H, CH_2), 3.90 (s, 3H, CH_3), 5.80 (s, 1H, =CH), 6.10 (t, 1H, =CH–CH_2), 6.80–7.20 (m, 3H, CHAr); *m/z* 343 (M^+, 19), 272 (M^+ –($CH_3CH_2CH_2CO$), base).

*2.1.1.8. 2-(Isobutyrylmethylene)-N-(cyclohept-1-enyl)-6 methoxy-2,3-dihydro-1,3-benzothiazole (**5h**).* Yield: 40%, m.p. 95–96 °C; Anal (C, H, N) $C_{20}H_{25}NO_2S$. IR (cm^{-1}) 1600 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.20 (d, 6H, CH_3), 1.50–



Scheme 2. Synthesis of compound **8h**.

2.0 (m, 6H, CH_2), 2.20–2.50 (m, 4H, CH_2), 2.50–2.80 (m, 1H, CH), 3.82 (s, 3H, CH_3), 5.80 (s, 1H, =CH), 6.10 (t, 1H, =CH–CH_2), 6.80–7.20 (m, 3H, CHAr); *m/z* 343 (M^+, 22), 272 (M^+ –((CH_3)$_2$CHCO), base).

*2.1.1.9. 2-(Isobutyrylmethylene)-N-(cyclohept-1-enyl)-6 methyl-2,3-dihydro-1,3-benzothiazole (**5i**).* Yield: 70%, m.p. 108–110 °C; Anal (C, H, N) $C_{20}H_{25}NOS$. IR (cm^{-1}) 1610 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.20 (d, 6H, CH_3), 1.60–2.03 (m, 6H, CH_2), 2.20–2.80 (m, 8H, CH_2 + CH_3 + CH), 5.80 (s, 1H, =CH), 6.10 (t, 1H, =CH–CH_2), 6.80–7.40 (m, 3H, CHAr); *m/z* 327 (M^+, 21), 256 (M^+ –((CH_3)$_2$CHCO), base).

*2.1.1.10. 2-(Valerylmethylene)-N-(cyclohept-1-enyl)-6 methyl-2,3-dihydro-1,3-benzothiazole (**5j**).* Yield: 80%, m.p. 72–73 °C; Anal (C, H, N) $C_{21}H_{27}NOS$. IR (cm^{-1}) 1610 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 0.95 (t, 3H, CH_3), 1.20–2.03 (m, 12H, CH_2), 2.30–2.60 (m, 7H, CH_2 + CH_3), 5.80 (s, 1H, =CH), 6.10 (t, 1H, =CH–CH_2), 6.80–7.40 (m, 3H, CHAr); *m/z* 341 (M^+, 21), 256 (M^+ –(CH_3(CH_2)$_3$CO), base).

*2.1.2. General one-flask procedure for the synthesis of N,N′-dialkyldithiodianilines **6***

A solution of 2-aminobenzenethiol **1** (10 mmol), appropriate carbonyl compound **2** (10.1 mmol) and catalytic amount of *p*-toluenesulfonic acid in toluene (60 ml), was refluxed for 3 h under nitrogen, while the water formed was continuously separated. The solvent was then evaporated and methanol (80 ml) was added to the residue. Then, the methanolic solution was treated with sodium borohydride (50 mmol) portionwise under nitrogen, monitoring the progress of the reaction by TLC. Removal of the solvent was followed by dilution with ice water (100 ml), acidification with acetic acid, and extraction with ether (3 × 50 ml). The combined extracts were washed with water (3 × 30 ml), dried with sodium sulfate, stirred with exposure to air overnight and concentrated. The residual yellow oil was purified by column chromatography on silica gel 60, using light petroleum ether/ethyl acetate 95:5 v/v as eluent to give pure compound **6**. Physical and spectral data of the new compound **6a** are reported below.

*2.1.2.1. N,N′-dicyclo-octyl-2,2′-dithiodianiline (**6a**).* Yield: 85%, oil; Anal (C, H, N) $C_{28}H_{40}N_2S_2$. IR (cm^{-1}) 3390 (NH); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.40–1.90 (m, 14H, CH_2), 3.40–3.60 (m, 1H, CH), 4.90 (bs, 1H, NH), 6.40–6.60 (m, 2H, CHAr), 7.10–7.30 (m, 2H, CHAr); *m/z* 436 (M^+, 88), 202 (base).

*2.1.3. General procedure for the synthesis of N-alkyl-1,3-benzothiazole compounds **8***

A solution of the *N,N′*-dialkyl-dithiodianiline **6** (10 mmol) and the appropriate β-ketoester **7** (22 mmol) in toluene containing catalytic amounts of *p*-toluenesulfonic acid, was refluxed by stirring, whilst monitoring the reaction progress by TLC. The solvent was then evaporated under reduced pressure, and the resulting crude was purified by column chromatography on silica gel to obtain the by products of the reaction **9** and compounds **8** in the order given. Physical and spectral data of the new compounds **8a,b,d,g** and **9a,b,d,g** are reported below, while compounds **8c,e,f** and **9c**,**e**,**f** have been reported previously [9].

*2.1.3.1. 1-(3-Cyclo-octyl-1,3-benzothiazole-2(3H)-ylidene)butan-2-one (**8a**).* Yield: 20%, m.p. 110–111 °C; Anal (C, H, N) $C_{19}H_{25}NOS$. IR (cm^{-1}) 1615 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.20 (t, 3H, CH_3), 1.50–2.13 (m, 12H, CH_2), 2.30–2.70 (m, 4H, CH_2), 4.4–4.9 (m, 1H, CH), 5.90 (s, 1H, =CH), 7.0–7.7 (m, 4H, CHAr); *m/z* 315 (M^+, 20), 149 (base).

*2.1.3.2. 1-(3-Cycloheptyl-1,3-benzothiazole-2(3H)-ylidene)-butan-2-one (**8b**).* Yield: 25%, m.p. 84–86 °C; Anal (C, H, N) $C_{18}H_{23}NOS$. IR (cm^{-1}) 1615 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.20 (t, 3H, CH_3), 1.50–2.13 (m, 10H, CH_2), 2.30–2.70 (m, 4H, CH_2), 4.3–4.7 (m, 1H, CH), 5.90 (s. 1H, =CH), 7.0–7.70 (m, 4H, CHAr); *m/z* 301 (M^+, 17), 149 (base).

*2.1.3.3. 2-(N-Cycloheptyl-1,3-benzothiazol-2(3H)-ylidene)-cyclopentanone (**8d**).* Yield: 15%, m.p. 155–156 °C; Anal (C, H, N) $C_{19}H_{23}NOS$. IR (cm^{-1}) 1630 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.50–2.10 (m, 12H, CH_2), 2.2–2.6 (m, 4H, CH_2), 3.15 (t, 2H, CH_2), 4.80–5.22 (m, 1H, CH), 7.0–7.7 (m, 4H, CHAr); *m/z* 313 (M^+, 2), 162 (base).

*2.1.3.4. 1-Benzyl-4-(3-butyl-1,3-benzothiazol-2(3H)-ylidene)-piperidin-3-one (**8g**).* Yield: 35%, oil; Anal (C, H, N) $C_{23}H_{26}N_2OS$. IR (cm^{-1}) 1660 (C=O); ^{1}H NMR ($CDCl_3$) δ (ppm): 0.90 (t, 3H, CH_3), 1.10–1.90 (m, 4H, CH_2), 2.70 (t,

2H, CH_2), 3.0 (t, 2H, CH_2), 3.30 (s, 2H, CH_2), 3.60 (s, 2H, CH_2), 4.22 (m, 2H, CH_2), 7.0–7.40 (m, 7H, CHAr), 7.50–7.60 (m, 2H, CHAr); *m/z* 378 (M^+, 26), 287 ($M^+ - CH_2C_6H_5$, base).

*2.1.3.5. 4-(Cyclo-octyl)-2-propionyl-2H-1,4-benzothiazin-3(4H)-one (**9a**).* Yield: 25%, oil; Anal (C, H, N) $C_{19}H_{25}NO_2S$. IR (cm^{-1}) 1670, 1730 (C=O); ^1H NMR ($CDCl_3$) δ (ppm): 1.0 (t, 3H, CH_3), 1.40–2.80 (m, 16H, CH_2), 4.22 (s, 1H, CH), 4.40–4.80 (m, 1H, CH), 6.90–7.40 (m, 4H, CHAr); *m/z* 331 (M^+, 12), 165 (base).

*2.1.3.6. 4-(Cycloheptyl)-2-propionyl-2H-1,4-benzothiazin-3(4H)-one (**9b**).* Yield: 20%, oil; Anal (C, H, N) $C_{18}H_{23}NO_2S$. IR (cm^{-1}) 1670, 1720 (C=O); ^1H NMR ($CDCl_3$) δ (ppm): 1.0 (t, 3H, CH_3), 1.40–2.80 (m, 14H, CH_2), 4.22 (s, 1H, CH), 4,30–4.60 (m, 1H, CH) 6.90–7.40 (m, 4H, CHAr); *m/z* 317 (M^+, 22), 165 (base).

*2.1.3.7. 4-(Cycloheptyl)-2′H-spiro-[1,4-benzothiazin-2,1′-cyclopentane]-2′,3(4H)-dione (**9d**).* Yield: 35%, oil; Anal (C, H, N) $C_{19}H_{23}NO_2S$. IR (cm^{-1}) 1650, 1730 (C=O); ^1H NMR ($CDCl_3$) δ (ppm): 1.3–3.0 (m, 18H, CH_2), 4.20–4.70 (m, 1H, CH), 6.80–7.50 (m, 4H, CHAr); *m/z* 329 (M^+, 42), 177 (base).

*2.1.3.8. 1′-Benzyl-4-butyl-3′H-spiro[1,4-benzothiazine-2,4′-piperidin]-3,3′(4H)-dione (**9g**).* Yield: 30%, m.p. 58–60 °C; Anal (C, H, N) $C_{23}H_{26}N_2O_2S$. IR (cm^{-1}) 1660, 1720 (C=O); ^1H NMR ($CDCl_3$) δ (ppm): 0.90 (t, 3H, CH_3), 1.20–2.10 (m, 6H, CH_2), 2.70–3.10 (m, 4H, CH_2), 3.50-3.70 (m, 2H, CH_2), 3.90–4.10 (m, 2H, CH_2), 6.90–7.40 (m, 9H, CHAr); *m/z* 394 (M^+, 71), 91 (base).

*2.1.4. Procedure for the synthesis of compound **8h***

Benzyl bromide (12 mmol) was added under nitrogen to 2-methyl benzothiazole (10 mmol) with stirring at 60–70 °C for 3 h. The mixture was then cooled, the precipitate filtered off, and washed with ether to give 3-benzyl-2-methyl-benzothiazolium bromide. To the suspension of 3-benzyl-2-methylbenzothiazolium bromide (5 mmol) in pyridine (5 ml), isobutyryl bromide (10 mmol) was added under nitrogen with stirring for 30 min. Then, ice water and dilute HCl were added to the reaction mixture and extracted with chloroform (3 × 20 ml). The organic layer was washed with 10% aqueous sodium bicarbonate (3 × 30 ml) and with water (3 × 30 ml), dried over sodium sulfate, filtered, and the solvent evaporated to give a solid, which crystallized from ligroine afforded compound **8h** whose physical and spectral data are reported below.

*2.1.4.1. 1-(3-Benzyl-1,3-benzothiazol-2(3H)-ylidene)-3-methylbutan-2-one (**8h**).* Yield: 55%, m.p. 133–135 °C; Anal (C, H, N) $C_{19}H_{19}NOS$. IR (cm^{-1}) 1610 (C=O); ^1H NMR ($CDCl_3$) δ (ppm): 1.1 (d, 6H, CH_3), 2.60 (hept, 1H, CH), 5.20 (s, 2H, CH_2), 5.92 (s, 1H, =CH), 7.0–7.40 (m, 8H, CHAr), 7.48–7.60 (m, 1H, CHAr); *m/z* 309 (M^+, 44), 266 (M^+; $-(CH_3)_2CH$, 83), 91 ($CH_2C_6H_5$, base).

2.2. Antimicrobial assays

Compounds **5** and **8** were tested to evaluate their antimicrobial activity against a variety of gram-positive (*B. subtilis* 6633, *E. faecalis* 29212, *S. aureus* 6538p, *S. aureus* 25923) and gram-negative bacteria (*E. coli* 25922, *A. calcoaceticus* a1, *A. calcoaceticus* a2, *P. aeruginosa* 27853, *K. oxytoca* 49131), as well as against the yeasts (*C. tropicalis* 750, *C. albicans* 14053, *C. albicans* 10231, *S. cerevisiae* 2366, *C. laurentii* 18803).

The MICs (μg/ml) of compounds **5** and **8** were determined by both microdilution broth method (MBM) and agar dilution method (ADM), the latter being a useful standardized reliable susceptibility testing that may be used as a reference for the evaluation of insoluble compounds of a series. Mueller Hinton Broth (MHB) and Mueller Hinton Agar (MHA) were used for the testing methods for bacteria, and Yeast Malt Broth (YMB) and Yeast Malt Agar (YMA) for yeast strains, respectively.

For bacteria and yeast strains, the inocula were prepared as follows: colonies derived from fresh mature strain on MHA for bacteria or potato dextrose agar (PDA) for yeast plates were suspended in saline solutions (0.85%) and suspensions were adjusted approximately to 10^8 cfu/ml (0.5 Mac Farland) [16] for bacteria and 10^6 cfu/ml for yeast strain, as determined by viable count.

In the ADM, a series of twofold dilution in dimethyl sulfoxide (DMSO) for the bacteria, or ethanol for yeasts, of each tested product were prepared in MHA plates for bacteria, or YMA plates for yeasts. Final product concentrations in the Petri dish ranged 100–1.56 μg/ml. After drying for 30 min, plates were spot inoculated by pipetting the desired test microorganism (40 μl for bacteria and 10 μl for yeasts) from the prepared inoculum onto the plates [17]. The MICs were recorded as the lowest concentration of antimicrobial agent that inhibited visible growth for the two types of microorganisms after incubation for 24 h at 37 °C for bacteria and 48 h at 30 °C for yeasts.

In the MBM, for bacteria, a series of twofold dilution of each product were prepared in DMSO and put in 96-well microdilution trays containing broth media. Each well was inoculated with 20 μl of a final inoculum concentration that approximately contained 2.5×10^6 cfu/ml. The trays were incubated at 37 °C for 24 h for bacteria. For yeasts, 100 μl of inoculum containing 10^3 cfu/ml was used, then incubated for 48 h at 30 °C. The MICs were determined as the lowest concentration of the agent resulting in no growth. To determine the MIC, we compared the growth in every well visually with that of the growth control well for bacteria, and for yeasts, the visual comparison of growth (50% inhibition) with that of growth control [17].

Appropriate controls involved cefepime for bacteria and fluconazole as international reference standards [18] for yeasts. Also DMSO and ethanol alone were tested for their activities against bacteria and yeasts, and consequently quantities of these two solvents under 5% for DMSO and 2% for

ethanol were used during the assays. Each species was tested at least three times in duplicate. The MBM and ADM assays for bacteria were based on the recommended procedures by NCCLS [17], whereas the modified reference [18] of the ADM was used for yeasts.

3. Results and discussion

The two different series of compounds **5a–j** and **8a–h** were tested by both the microdilution broth and agar dilution methods to evaluate their antimicrobial activity against some microbial species.

The findings obtained, reported in Table 1, showed that the *N*-cycloalkenyl compounds (**5a–j**) exhibited no activity against fungi analogously to the previously reported compounds [11]. Moreover, an enhancement in antimicrobial activity was observed by increasing the length of the acyl chain R^1, particularly against three gram-positive (*E. faecalis* 29212, *S. aureus* 6538p, *S. aureus* 25923) and one gram-negative bacteria (*E. coli* 25922) (compare **5a,b** with **5c,d**). Surprisingly, the most lipophilic cyclododecenyl moiety on the nitrogen atom (**5a–d**), as well as the heptanoil chain (**5e**) did not lead to more effective compounds than the *N*-cycloheptyl and *N*-cyclo-octyl analogues previously reported [11]. Besides, the presence of fluorine (**5f**) on the benzene ring dramatically reduced the activity in comparison with the analogue not fluorinated, whilst the presence of the methoxy group (**5g,h**) led to effective compounds against all the bacteria tested. Finally, the presence of methyl group on the benzene ring increased the antibacterial activity only for compound **5j** more lipophilic than the **5i** analogue. The *N*-cycloalkyl compounds **8b–e** as well as the *N*-alkyl compounds **8f–g**, differently from the *N*-cycloalkenyl analogue compounds, showed a certain activity against the tested fungi (*C. tropicalis* 750, *C. albicans* 14053, *C. albicans* 10231, *S. cerevisiae* 2366, *C. laurentii* 18803), independent from the length of the acyl chain (see **8b** and **8c**), as well as the presence of the cyclic acyl chain **8d**. The absence of the antifungal activity for compound **8a** (if other reasons are not involved) seemed attributable probably to the most lipophilic *N*-cyclo-octyl moiety.

Furthermore, a moderate antimicrobial activity was observed for compound **8a,b** particularly against the gram-negative bacteria tested. This activity appeared related to the length of the acyl chain. In fact, compound **8c** in which the acyl chain is limited to a methyl group is devoid of any antibacterial activity, whereas the presence of the cyclic acyl chain (compound **8d**) resulted in weak activity against the gram-positive and gram-negative bacteria, with surprisingly high activity only against *E. faecalis* 29212.

4. Conclusions

The structural modifications of the *N*-cycloalkenyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazole **A**, indicate that on increasing the ring size of the *N*-cycloalkenyl moiety, as well as on increasing the length of the acyl chain, or on inserting a substituent on the benzene ring, an increase in antibacterial potency in comparison with the previously reported data [11] is not observed. In particular, the antibacterial activity of the new *N*-cyclododecenyl compounds **5a–d** is comparable with the early reported activity for the *N*-cycloheptenyl and *N*-cyclo-octenyl analogues [11]. Moreover, the length of the acyl chain seems conclusive for the antibacterial activity of the *N*-cycloalkenyl compounds **5**, whereas it does not appear influent on the antifungal activity of the *N*-cycloalkyl compounds **8**. The substitution of the *N*-cycloalkenyl moiety with the *N*-cycloalkyl group causes a new weak antifungal activ-

Table 1
Antimicrobial activity of compounds **5** and **8**

	B. subtilis 6633	*E. faecalis* 29212	*S. aureus* 6538p	*S. aureus* 29213	*E. coli* 25922	*A. calcoaceticus* a1	*A. calcoaceticus* a2	*P. aeruginosa* 27853	*K. oxytoca* 49131	*C. tropicalis* 750	*C. albicans* 14053	*C. albicans* 10231	*S. cerevisiae* 2366	*C. laurentii* 18803
5c	100	12.5	12.5	12.5	12.5	100	100	100	100	> 100	> 100	> 100	>100	>100
5d	100	12.5	12.5	12.5	12.5	100	100	100	100	> 100	> 100	> 100	>100	>100
5e	100	12.5	12.5	12.5	12.5	100	100	100	100	> 100	> 100	> 100	> 100	> 100
5g	6.2	12.5	12.5	12.5	6.2	6.2	12.5	12.5	12.5	> 100	> 100	> 100	> 100	> 100
5h	6.2	12.5	12.5	6.2	6.2	12.5	12.5	12.5	12.5	> 100	> 100	> 100	> 100	> 100
5j	12.5	25	25	50	12.5	50	25	25	50	> 100	> 100	> 100	> 100	> 100
8a	100	100	100	100	25	25	25	25	25	> 100	> 100	> 100	> 100	> 100
8b	100	100	100	100	50	50	25	100	50	50	25	50	25	25
8c	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	50	25	50	25	25
8d	100	12.5	100	100	50	50	50	1500	50	100	50	100	50	25
8e	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	25	50	50	50	25
8f	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	100	50	50	50	25
8g	100	100	100	> 100	100	> 100	> 100	> 100	100	25	50	100	100	50
Cef	1.4	2.3	3.7	3.7	0.1	2.8	2.8	1	0.2					
Flu										2	0.25	0.25	8	4

The omitted compounds 5**a**,**b**,**f**,**i**, and 8**h**, showed a MIC against the reported bacteria and yeasts ≥ 100. The reported MIC values refer to the agar dilution method.
Cef: cefepime; Flu: fluconazole.

ity for some of these compounds. Finally, both microdilution broth and agar dilution methods used are suitable for testing the antimicrobial activity of a series of hydrophilic–lipophilic compounds.

Compound	Formula	Molecular weight	Elemental analyses					
			% Calculated value			% Actual value		
			C	H	N	C	H	N
5a	$C_{22}H_{29}NOS$	355.46	74.33	8.22	3.94	74.88	8.50	3.97
5b	$C_{23}H_{31}NOS$	369.49	74.76	8.46	3.79	75.01	8.55	3.72
5c	$C_{24}H_{33}NOS$	383.51	75.16	8.67	3.65	75.30	8.84	3.61
5d	$C_{24}H_{33}NOS$	383.51	75.16	8.67	3.65	74.85	8.68	3.57
5e	$C_{22}H_{29}NOS$	355.46	74.33	8.22	3.94	74.65	8.41	3.86
5f	$C_{19}H_{22}NOSF$	331.38	68.88	5.74	4.20	68.55	6.01	4.14
5g	$C_{20}H_{25}NO_2S$	343.41	69.95	7.33	4.08	70.12	7.42	4.02
5h	$C_{20}H_{25}NO_2S$	343.41	69.95	7.33	4.08	69.75	7.39	3.90
5i	$C_{20}H_{25}NOS$	327.41	73.36	7.70	4.28	73.05	7.59	4.15
5j	$C_{21}H_{27}NOS$	341.43	73.87	7.97	4.10	73.73	7.95	4.08
6a	$C_{28}H_{40}N_2S_2$	468.62	71.76	8.60	5.98	71.45	8.38	6.03
8a	$C_{19}H_{25}NOS$	315.40	72.35	7.99	4.44	72.45	8.16	4.25
8b	$C_{18}H_{23}NOS$	301.37	71.73	7.69	4.65	71.52	7.83	4.51
8d	$C_{19}H_{23}NOS$	313.38	72.82	7.40	4.47	72.71	7.55	4.33
8g	$C_{23}H_{26}N_2OS$	378.45	72.99	6.93	7.40	72.80	6.81	7.55
8h	$C_{19}H_{19}NOS$	309.34	73.76	6.15	4.52	73.68	6.24	4.72
9a	$C_{19}H_{25}NO_2S$	331.40	68.86	7.60	4.23	68.63	7.73	4.20
9b	$C_{18}H_{23}NO_2S$	317.38	68.12	7.30	4.41	68.04	7.43	4.53
9d	$C_{19}H_{23}NO_2S$	329.38	69.28	7.04	4.25	69.05	7.15	4.11
9g	$C_{23}H_{26}N_2O_2S$	394.45	70,03	6.64	7.10	70.20	6.85	7.21

References

[1] M.S. Chua, D.F. Shi, S. Wrigley, T.D. Bradshaw, I. Hutchinson, P. Nicholas, D.A. Barret, L.A. Stanley, M.F.G. Stevens, Antitumor benzothiazoles. 7. Synthesis of 2-(4-acylaminophenyl)benzothiazoles and investigations into the role of acetylation in the antitumor activities of the parent amines, J. Med. Chem. 42 (1999) 381–392.

[2] I. Hutchinson, S.A. Jennings, B.R. Vishnuvajjala, A.D. Wetsell, M.F.G. Stevens, Antitumor benzothiazoles. 16. Synthesis and pharmaceutical properties of antitumor 2-(4-aminophenyl) benzothiazole amino acids prodrugs, J. Med. Chem. 45 (2002) 744–747.

[3] G. Grandolini, V. Ambrogi, C. Rossi, M.C. Tiralti, L. Tuttobello, Synthesis and antibacterial and antifungal activities of some new derivatives of benzothiazole, 1,4-benzothiazine and 1,5-benzothiazepine, Eur. J. Med. Chem. 21 (1986) 455–460.

[4] F.J. Palmer, R.B. Trigg, J.V. Warrington, Benzothiazolines as antitubercular agents, J. Med. Chem. 14 (1971) 248–251.

[5] A. Burger, S.N. Sawhey, Antimalarials. 3. Benzothiazole amino alcohols, J. Med. Chem. 11 (1968) 270–273.

[6] G. Trapani, A. Reho, F. Morlacchi, A. Latrofa, P. Marchini, F. Venturi, et al., Synthesis and antiinflammatory activities of some 1,4-benzothiazine derivatives, Il Farmaco, 40 (1985) 369–376, Chem. Abstr. 103 (1985) 87826r.

[7] (a) G. Trapani, A. Latrofa, M. Franco, G. Liso, G. Genchi, Synthesis and benzodiazepine receptor binding of 1H-pyrrolo[2,1-c] [1,4] benzothiazines, Farmaco 45 (1990) 577–588; (b) G. Trapani, A. Latrofa, M. Franco, G. Liso, G. Genchi, Synthesis and benzodiazepine receptor binding of 5H-pyrido[2,1-c] [1,4] benzothiazines, Farmaco 45 (1990) 589–602.

[8] G. Trapani, A. Latrofa, M. Franco, G. Liso, Synthesis of 2-substituted-*N*-carboxymethyl-1,5-benzothiazepin-4-ones and 1,4-benzothiazin-3-ones and their evaluation as angiotensin converting enzyme inhibitors, Farmaco 50 (1995) 107–112.

[9] G. Trapani, A. Latrofa, A. Reho, M. Franco, G. Liso, Reaction of N,N′-dialkyldithiodianilines with β-ketoesters. A new synthesis of N-alkyl-2-acyl-3-oxo-3,4-dihydro-1,4-benzothiazines, J. Heterocyclic Chem. 29 (1992) 1155–1159.

[10] G. Trapani, A. Latrofa, A. Reho, M. Franco, G. Liso, F. Stasi, *N*-alkenyl acylketene *S*,*N*-acetals from 2,3-dihydro-1,3-benzothiazoles and carboxylic anhydrides. X-ray molecular structure of 2-(butyrylmethylene)-*N*-(cyclohex-1-enyl)-2,3-dihydro-1,3-benzothiazoles, J. Chem. Soc. Perkin Trans. 1 (1991) 1505–1510.

[11] G. Trapani, A. Latrofa, M. Franco, D. Armenise, F. Morlacchi, G. Liso, Synthesis and antimicrobial activity of some *N*-alkenyl-2-acylalkylidene-2,3-dihydro-1,3-benzothiazoles, Arzneimittelforschung 44 (1994) 969–971.

[12] R.R. Gupta, R. Kumar, Synthesis of 2-amino-3-ethoxybenzenethiol and its conversion into 4H-1,4-benzothiazines, Heterocycles 22 (1984) 87–91.

[13] L.G.S. Brooker, F.L. White, U.S. Patent 2, 112, 139, (1938), Chem. Abstr. 32 (1938) 3713.

[14] O. Tsuge, M. Tanaxa, H. Shimorakada, S. Kanemasa, Reactions of 2-methylenebenzothiazolines with methylenecyclopropenes, Bull. Chem. Soc. Jpn. 56 (6) (1983) 1688–1693.

[15] G. Liso, G. Trapani, A. Reho, A. Latrofa, 2-Alkylaminobenzenethiols by ring cleavage of 2,3-dihydro-1,3-benzothiazoles with sodium borohydride, Synthesis (Mass.) (1985) 288–290.

[16] J. McFarland, Nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines, JAMA 14 (1907) 1176–1178.

[17] National Committee for Clinical Laboratory Standard, Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically, fourth ed., Approved standard M7-A4, Wayne, PA, 1997.

[18] National Committee for Clinical Laboratory Standard, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved standard M27A, NCCLS, Villanova, PA, 1997.