ELSEVIER

Il Farmaco 53 (1998) 346–349

IL FARMACO

New cyclohexylidenehydrazide and 4-aza-1-thiaspiro[4.5]decan-3-one derivatives of 3-phenyl-4(3*H*)-quinazolinones

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Received 17 December 1997; accepted 14 January 1998

Abstract

In this study, the synthesis of (3-phenyl-4(3*H*)-quinazolinon-2-yl)mercaptoacetic acid cyclohexylidenehydrazides and 4-[(3-phenyl-4(3*H*)-quinazolinon-2-yl)mercaptoacetylamino]-4-aza-1-thiaspiro[4.5]decan-3-ones and the results of the study on their antifungal activity are reported. Most of the tested compounds were found to be active against *Microsporum gypseum* NCPF-580, *Microsporum canis*, *Tricophyton mentagrophytes* NCPF-375 *var. erinacei* and *Tricophyton rubrum* at 25 μg/ml.

Keywords: 3-Phenyl-4(3*H*)-quinazolinones; Cyclohexylidenehydrazides; Spirothiazolidinones; Antifungal activity

1. Introduction

Since the discovery of 4(3*H*)-quinazolinones and their important clinical properties, there has been intense effort to find new compounds of this type which are more potent, more selective and, therefore, without the concomitant effects associated with these drugs. Among the different structural variations carried out on these molecules, the main and more fruitful trends were those based on the change in the 4(3*H*)-quinazolinone nucleus. Some of the compounds synthesized following these criteria (methaqualone, phenquizone, etc.) have exceeded the clinical trials and are used at present as hypnotic–sedative and diuretic agents. Furthermore, it has been shown that 4(3*H*)-quinazolinonylhydrazides and related heterocyclic systems could be considered as antimicrobials [1,2]. On the other hand, the presence of the thiazole ring in compounds with a wide range of biological activities has also contributed to the enlargement of interest in the closely related 4-thiazolidones which are reported to display useful antimicrobial properties [3,4]. These observation prompted us to synthesize cyclohexylidenehydrazides containing the 4(3*H*)-quinazolinone nucleus and their cyclization products, spirothiazolidinones, which may possess antifungal activity.

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0014-827X/98/$19.00
PII S0014-827X(98)00032-9

2. Experimental

2.1. Chemistry

Melting points were determined with a Büchi 530 apparatus in open capillaries and are uncorrected. IR spectra were recorded on KBr discs, using a Perkin-Elmer 1600 spectrometer. ^{1}H NMR spectra were run on a Bruker AC 200 (200 MHz) or Bruker DPX 400 (400 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Bruker AC 200 (50.3 MHz) spectrophotometer. EI/MS were determined on a VG Zab Spec (70 eV) or a VG-Platform (70 eV). Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer. Analyses indicated by the symbols were within ±0.4% of the theoretical values.

2.1.1. Synthesis of (3-phenyl-4(3H)-quinazolinon-2-yl)-mercaptoacetic acid cyclohexylidenehydrazides 2a–c

Cyclohexanone (0.01 mol) was added to a solution of (3-phenyl-4(3*H*)-quinazolinon-2-yl)mercaptoacetic acid hydrazides **1** (0.01 mol) in absolute ethanol. The reaction mixture was refluxed over a water bath for 4 hours and allowed to stand overnight. The white needles formed were filtered and recrystallized from absolute ethanol.

The following abbreviations are used: cyclohex. = cyclo hexane, quin. = quinazolinone, thiaz. = thiazolidone, arom. = aromatic.

Spectral data of **2a**. IR (ν cm^{-1}, KBr): 3236 (NH), 1687, 1667 (C=O). ^{1}H NMR (200 MHz, δ ppm, DMSO-d$_6$): 1.58

(s, 6H, cyclohex.), 2.21, 2.36 (2s, 4H, cyclohex. 2-CH_2, 6-CH_2), 3.98, 4.35 (2s, 2H, SCH_2), 7.44–8.01 (m, 8H, arom.), 10.41, 10.48 (2s, 1H, NH). ^{13}C NMR (APT) (50.3 MHz, δ ppm, DMSO-d_6): 24.91 (s, cyclohex. C_4), 25.49, 26.73 (2s, cyclohex. C_3, C_5), 27.22, 34.75 (2s, cyclohex. C_2, C_6), 35.05, 35.22 (2s, SCH_2), 120.72 (s, quin. C_{4a}), 125.38 (s, quin. C_8), 127.95 (s, phenyl C_4), 129.16 (s, phenyl C_2, C_6), 129.41 (s, phenyl C_3, C_5), 129.93 (s, quin. C_5), 134.77 (s, quin. C_7), 135.43 (s, phenyl C_1, quin. C_6), 145.73 (s, quin. C_{8a}), 156.60 (s, quin. C_2), 157.95 (s, cyclohex. C=N), 159.57 (quin. CO), 162.95, 168.59 (2s, amide CO). EI/MS [*m/z* (% relative intensity)]: 440 [M^+, 6 (442, 2)], 329 [100 (331, 47)], 287 [82 (289, 44)].

Spectral data of **2c**. IR (ν cm^{-1}, KBr): 3215 (NH), 1690, 1667 (C=O). ^{1}H NMR (400 MHz, δ ppm, DMSO-d_6): 1.00, 1.02 (2d, J=6.4, 6.5 Hz, 3H, CH_3), 1.02–2.42 (m, 8H, cyclohex.), 2.95 (q, 1H, cyclohex. 4-CH), 3.40, 4.08 (2s, 2H, SCH_2), 7.56–7.71 (m, 6H, quin. 8-CH, C_6H_5), 7.94, 7.97 (2dd, J=2.4, 8.7 Hz; J=2.4, 8.7 Hz, 1H, quin. 7-CH), 8.11 (t, J=3.1 Hz, 1H, quin. 5-CH), 10.56, 10.63 (2s, 1H, NH). EI/MS [*m/z* (% relative intensity)]: 454 [M^+, 0.3 (456, 0.1)], 329 [2 (331, 0.7)], 287 [3 (289, 1)], 77 (100).

*2.1.2. Synthesis of 4-[(3-phenyl-4(3H)-quinazolinon-2-yl)-mercaptoacetylamino]-4-aza-1-thiaspiro[4.5]decan-3-ones **3a–c** and **4a,b***

A mixture of **2** (0.005 mol), thioglycolic acid or thiolactic acid (0.01 mol) in dry benzene (30 ml) was refluxed for 48 hours by a water separator which removes the liberated water. Benzene was removed by distillation under reduced pressure. The oil thus obtained was washed with 10% sodium bicarbonate solution in order to remove the excess acid. The precipitate was filtered, washed with water and recrystallized from absolute ethanol.

Spectral data of **3a**. IR (ν cm^{-1}, KBr): 3224 (NH), 1738, 1694 (C=O). ^{1}H NMR (200 MHz, δ ppm, DMSO-d_6): 1.22 (t, J=7.1 Hz, 1.5H, 0.5CH_3CH_2OH), 1.38–1.74 (m, 10H, cyclohex.), 3.54 (s, 2H, SCH_2), 3.99 (s, 2H, thiaz. SCH_2), 4.14 (q, J=7.1 Hz, 1H, 0.5CH$_3$CH_2OH), 7.45–8.03 (m, 9H, arom.), 10.29 (s, 1H, NH).

Spectral data of **3b**. IR (ν cm^{-1}, KBr): 3237 (NH), 1727, 1684 (C=O). ^{1}H NMR (200 MHz, δ ppm, DMSO-d_6): 1.45–1.70 (m, 10H, cyclohex.), 3.53, 3.99 (2s, 2H, SCH_2), 3.66 (s, 2H, thiaz. SCH_2), 7.36–8.02 (m, 8H, arom.), 10.36 (br s, 1H, NH). ^{13}C NMR (APT) (50.3 MHz, δ ppm, DMSO-d_6): 22.69 (s, cyclohex. C_4), 23.84 (s, cyclohex. C_3, C_5), 27.64 (s, cyclohex. C_2, C_6), 33.98, 34.25 (2s, SCH_2), 36.63 (s, thiaz. SCH_2), 72.01 (s, spiro C), 120.76 (s, quin. C_{4a}), 125.23 (s, quin. C_8), 127.86 (s, phenyl C_4), 128.87 (s, quin. C_5), 129.11 (s, phenyl C_2, C_6), 129.37 (s, phenyl C_3, C_5), 134.63 (s, quin. C_7), 135.67 (s, phenyl C_1, quin. C_6), 145.53 (s, quin. C_{8a}), 156.75 (s, quin. C_2), 158.13 (s, quin. CO), 167.29 (s, amide CO), 182.17 (s, thiaz. CO). EI/MS [*m/z* (% relative intensity)]: 514 [M^+, 10 (516, 4)], 329 [100 (331, 40)], 287 [67 (289, 37)].

Spectral data of **3c**. IR (ν cm^{-1}, KBr): 3228 (NH), 1725, 1677 (C=O). ^{1}H NMR (200 MHz, δ ppm, DMSO-d_6): 0.86 (d, J=5.6 Hz, 3H, CH_3), 1.09–2.50 (m, 9H, cyclohex.), 3.56 (s, 2H, thiaz. SCH_2), 3.64, 3.73 (2s, 2H, SCH_2), 7.24–8.01 (m, 8H, arom.), 10.18 (br s, 1H, NH). EI/MS [*m/z* (% relative intensity)]: 528 [M^+, 10 (530, 4)], 329 [97 (331, 46)], 287 [100 (289, 52)].

Spectral data of **4a**. IR (ν cm^{-1}, KBr): 3241 (NH), 1732, 1685 (C=O). ^{1}H NMR (200 MHz, δ ppm, DMSO-d_6): 1.19–1.72 (m, 13H, CH_3, cyclohex.), 3.68, 4.11 (2s, 2H, SCH_2), 3.84 (q, J=5.1 Hz, 1H, thiaz. SCH), 7.16–8.24 (m, 9H, arom.), 10.18, 10.33 (2s, 1H, NH).

Spectral data of **4b**. IR (ν cm^{-1}, KBr): 3235 (NH), 1729, 1682 (C=O). ^{1}H NMR (200 MHz, δ ppm, DMSO-d_6): 1.24–1.72 (m, 13H, CH_3, cyclohex.), 3.69, 4.12 (2s, 2H, SCH_2), 3.86 (q, J=4.8 Hz, 1H, thiaz. SCH), 7.24–8.02 (m, 8H, arom.), 10.18, 10.38 (2s, 1H, NH). ^{13}C NMR (APT) (50.3 MHz, δ ppm, DMSO-d_6): 19.53 (s, CH_3), 22.58 (s, cyclohex. C_4), 23.01, 23.85 (2s, cyclohex. C_3, C_5), 25.18, 34.31 (2s, cyclohex. C_2, C_6), 36.52 (s, SCH), 36.76, 37.70 (2s, SCH_2), 70.69 (s, spiro C), 120.59 (s, quin. C_{4a}), 125.37 (s, quin. C_8), 128.07 (s, phenyl C_4), 128.78 (s, quin. C_5), 129.15 (s, phenyl C_2, C_6), 129.45 (s, phenyl C_3, C_5), 134.71 (s, quin. C_7), 135.54 (s, phenyl C_1, quin. C_6), 145.89 (s, quin. C_{8a}), 156.83 (s, quin. C_2), 159.87 (s, quin. CO), 169.72 (s, amide CO), 180.34 (s, thiaz. CO). EI/MS [*m/z* (% relative intensity)]: 528 [M^+, 10 (530, 4)], 329 [100 (331, 43)], 287 [50 (289, 25)].

2.2. Microbiology

2.2.1. Antifungal activity

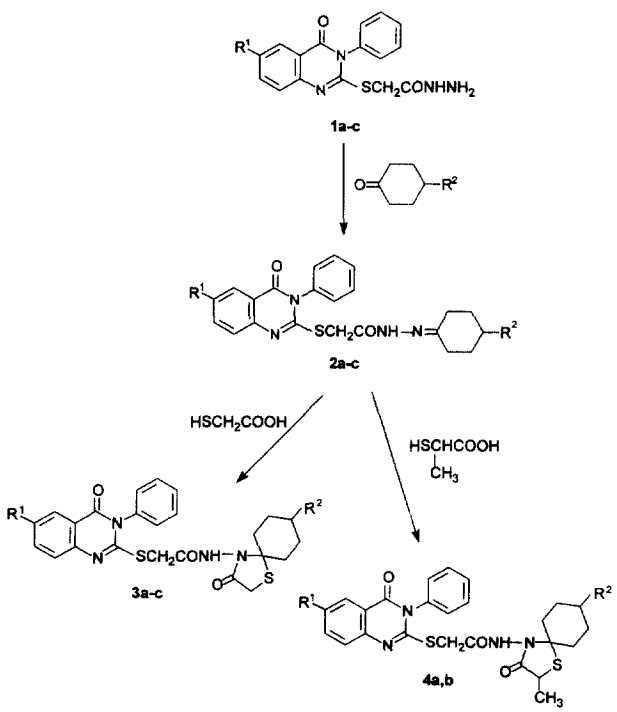
All the compounds to be tested were dissolved in DMSO at a concentration of 4000 μg/ml and the final concentration was reduced to 200 μg/ml with sterile distilled water. No effect of DMSO (5%) was observed upon growth of dermatophytes. The dermatophyte strains which were grown on slant medium of Sabouraud (Difco) were transferred to 3.5 ml nutrient broth (NB, Diagnostic Pasteur) and incubated for three to five days at 25°C. At the end of the incubation period these strains were transferred into screwcapped bottles containing sterilized beads and shaken for 4–5 min in a vortex (IKA-VF, Germany). The suspensions of the cultures were adjusted to have an absorbance degree of 0.6 at 450 nm in the spectrophotometer. Eight different dilutions between 25 and 0.2 μg/ml were prepared in microplates by serial dilutions from top to bottom. Then all the wells except the 12th wells (positive control) were filled with 10 μl of the standardized strains. These plates were incubated at 25°C for 5 or 6 days.

The minimum concentration at which no growth was observed was taken as the MIC value. It should be noted, however, that these techniques leave a variable number of broken hyphae and therefore even an identical optical density of such hyphal suspensions could lead to a considerable var-

iation in the number of viable cells; this would obviously prevent proper standardization of the inoculum.

3. Results and discussion

The reaction of (3-phenyl-4(3*H*)-quinazolinon-2-yl)-mercaptoacetic acid hydrazides **1** [5] with cyclohexanone afforded the corresponding (3-phenyl-4(3*H*)-quinazolinon-2-yl)mercaptoacetic acid cyclohexylidenehydrazides **2a–c** [6]. 4-[(3-Phenyl-4(3*H*)-quinazolinon-2-yl)mercaptoacetylamino]-4-aza-1-thiaspiro[4.5]decan-3-ones **3a–c** and 4-[(3-phenyl-4(3*H*)-quinazolinon-2-yl)mercaptoacetylamino]-4-aza-2-methyl-1-thiaspiro[4.5]decan-3-ones **4a,b** were synthesized by treatment of **2** with thioglycolic acid and thiolactic acid in dry benzene, respectively (Scheme 1). The structures of the synthesized compounds were confirmed by physical (Table 1) and spectral (IR, ^{1}H NMR, ^{13}C NMR, EI/MS) data.



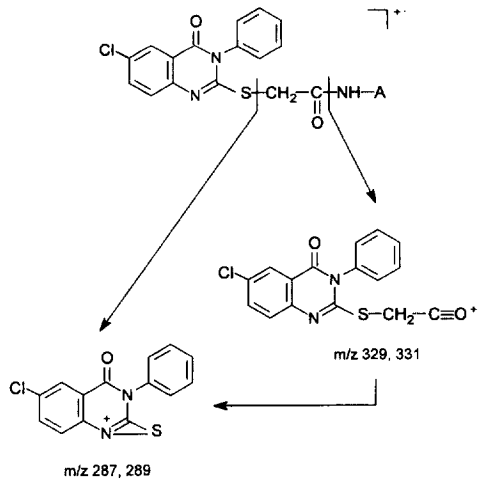
Scheme 1. Synthesis of **2a–c**, **3a–c** and **4a,b**.

The IR spectra of **2**, **3** and **4** showed two separate bands resulting from the NH and CO bands of the amide function at about regions 3241–3194 and 1694–1655 cm^{-1}, respectively [1]. The lactam CO band was observed at about 1694–1677 cm^{-1} [7]. A new CO band at 1738–1725 cm^{-1} in the spectra of **3** and **4** provided evidence for the spirothiazolidone structure [8,9]. In the ^{1}H NMR spectra of **2**, **3** and **4** the SCH_2 (δ 3.54 or 3.53–3.98 and 3.73–4.35 ppm) and NH protons (δ 10.18–10.36 or 10.18–10.41 and 10.33–10.48 ppm) of the mercaptoacetylamino moiety were observed as a singlet or a double singlet presumably due to the partial double bond character of the C–N bond and the bulk of the attached cyclohexyl or 1-thia-4-azaspiro[4.5]decan-3-one structure which can disrupt free rotation about the cited bond [10]. The spectra displayed the SCH_2 (δ 3.56–3.99 ppm) and SCH (δ 3.84–3.86 ppm) resonances as a singlet and quartet, confirming cyclization to **3** and **4**, respectively [9,11]. In the APT ^{13}C NMR spectra of **2a**, **3b** and **4b** chosen as prototypes, all the carbons resonated in the expected regions [11–14]. The spectra of **3b** and **4b** did not display the cyclohexylidene C=N peak, whereas they displayed the SCH_2 (δ 36.63 ppm), spiro C (δ 72.01 ppm) and C=O (δ 182.17 ppm) peaks in **3b** and the CH_3 (δ 19.53 ppm), SCH (δ 36.52 ppm), spiro C (δ 70.69 ppm) and C=O (δ 180.34 ppm) peaks in **4b**, which verified the proposed spirothiazolidone structures [11,14]. The EI/MS spectra of **2a**, **2c**, **3b**, **3c** and **4b** showed molecular ions (M^+) with low intensity and two common fragmentation routes, which was

Table 1
Physical constants of compounds **2**, **3** and **4**

Comp.	R^1	R^2	Yield (%)	M.p. (°C)	Formula (mol. wt.)
2a	Cl	H	92	226–230	$C_{22}H_{21}ClN_4O_2S$ (440.95)
2b	CH_3	CH_3	84	>300	$C_{24}H_{26}N_4O_2S \cdot 1.5H_2O$ (461.58)
2c	Cl	CH_3	79	173	$C_{23}H_{23}ClN_4O_2S$ (454.97)
3a [a]	H	H	82	125	$C_{24}H_{24}N_4O_3S_2 0.5C_2H_5OH$ (503.63)
3b	Cl	H	75	195	$C_{24}H_{23}ClN_4O_3S_2 \cdot 2H_2O$ (551.07)
3c	Cl	CH_3	86	146	$C_{25}H_{25}ClN_4O_3S_2 \cdot 3H_2O$ (583.13)
4a	H	H	81	106	$C_{25}H_{26}N_4O_3S_2 \cdot 2H_2O$ (530.67)
4b	Cl	H	77	130	$C_{25}H_{25}ClN_4O_3S_2 \cdot 2.5H_2O$ (574.11)

[a] The ethanol peak was observed in the ^{1}H NMR spectrum.



Scheme 2. Mass fragmentation routes of compounds **2**, **3** and **4**.

Table 2
MIC values (μg/ml) of compounds **2**, **3** and **4**

Comp.	Fungi [a]			
	A	B	C	D
2a	>25	25	25	25
2b	25	25	>25	25
2c	>25	25	25	25
3a	>25	25	>25	>25
3b	25	25	25	25
3c	25	25	–	25
4a	>25	>25	>25	>25
4b	25	25	25	25
Ketoconazole	0.2	0.4	0.8	1.6

[a] A = *Microsporum gypseum* NCPF-580, B = *Microsporum canis*, C = *Tricophyton mentagrophytes* NCPF-375 *var. erinacei*, D = *Tricophyton rubrum*.

consistent with the literature [5]. In the first route the CO–NH linkage was broken, yielding the peak at *m/z* 329 (331) which was the base peak in most cases. In the second route, fragment ion at *m/z* 287 (289) was formed by the cleavage of the S–CH_2 bond (Scheme 2).

Compounds **2**, **3** and **4** were evaluated for in vitro antifungal activity against *Microsporum gypseum* NCPF 580, *Microsporum canis*, *Trichophyton mentagrophytes var. erinacei* NCPF 375 and *Trichophyton rubrum* using the microdilution method [15]. Ketoconazole was used as the standard in the tests. As can be seen in Table 2, most of the tested compounds showed inhibition against the tested fungi at 25 μg/ml. The preliminary results indicated that R^1- and/or R^2-substituted compounds were more active than entries which were unsubstituted. **3b** (R^1 = Cl, R^2 = H) was the most potent compound and was effective against all fungi, whereas **4a** (R^1 = H, R^2 = H) was devoid of antifungal activity.

Acknowledgements

This work was supported by Istanbul University Research Fund Project Number Ö-244/221196.

References

[1] A.M. Mahmoud, H.A.H. El-Sherief, G.M. El-Naggar, A.E. Abdel-Rahman, Synthesis of 5-mercapto-1,2,4-triazole derivatives containing 4(3*H*)-quinazolone nucleus, Indian J. Chem. B 22 (1983) 491–493.
[2] N.S. Habib, M.A. Khalil, Synthesis and antimicrobial activity of novel quinazolinone derivatives, J. Pharm. Sci. 73 (1984) 982–985.
[3] R.M. Abdel-Rahman, Z. El-Gendy, M.B. Mahmoud, Biologically active thiazolidone. Part I. Synthesis and fungi toxicities of thiazolidinones and their derivatives derived from *o*-aminothiophenol, J. Indian Chem. Soc. 67 (1990) 61–64.
[4] M.M. Dutta, B.N. Goswami, J.C.S. Kataky, Studies on biologically active heterocycles. Part III. Synthesis and antibacterial activity of some 2-aryl/aralkyl-3-substituted-4-thiazolidinones, J. Indian Chem. Soc. 67 (1990) 332–334.
[5] A. Gürsoy, N. Karalı, Synthesis and anticonvulsant activity of new acylthiosemicarbazides and thiazolidones, Farmaco 50 (1995) 857–866.
[6] S.A. El-Feky, M.I. Al-Ashmawi, B. Abd El-Fattah, Synthesis of certain derivatives of 3-aryl-2-mercapto-4(3*H*)-quinazolinone of expected pharmacological activity, Egypt. J. Pharm. Sci. 24 (1983) 57–65.
[7] R.M. Shafik, A.A.B. Hazzaa, N.S. Habib, 2-(Mono- and dinitrophenylthio)-3-substituted-4(3*H*)-quinazolinones: potential sedative–hypnotic agents, Pharmazie 34 (1979) 148–150.
[8] A. Ruab, M.N. Bharati, Synthesis and fungicidal activity of some 1′-(substituted aryloxyaceto)spiro-[3*H*-indole-3,2′-thiazolidine]-2,2′-(1*H*)-diones and 2-aryl[1,3,4]oxadiazino[5,6-b]indoles, Indian J. Chem. B 28 (1989) 526–528.
[9] M.F. El-Zohry, I.M.A. Awad, A.A. Abdel-Hafez, Spiroheterocycles related to 1-oxa-4-thiaspiro[4,4]nonan-2-one and 1-oxa-4-thiaspiro-[4,5]decan-2-one, Arch. Pharm. (Weinheim) 326 (1993) 115–118.
[10] L. Somogyi, Notes on the reactions of ketone acylhydrazones under acylation conditions, Tetrahedron 41 (1985) 5187–5190.
[11] İ. Doğan, S. İçli, Conformational and spectral investigations of thiazolidinone derivatives by ^1H and ^{13}C NMR spectroscopy, Spectrosc. Lett. 16 (1983) 499–511.
[12] J. Petridou-Fischer, E.P. Papadopoulos, Carbon-13 nuclear magnetic resonance spectra of N-substituted 2-amino-4*H*-3,1-benzoxazin-4-ones and 3-substituted 2,4-(1*H*,3*H*)quinazolinediones, J. Heterocycl. Chem. 19 (1982) 123–126.
[13] S.L. Spassov, I.A. Atanossova, M.A. Haimosa, Carbon-13 and proton NMR spectra of 1(2*H*)-isoquinolinone, 1(2*H*)-phthalazinone, 4(3*H*)-quinazolinone and their substituted derivatives, Magn. Reson. Chem. 23 (1985) 795–799.
[14] A.M. Le Maréchal, A. Robert, I. Leban, Gem dicyano époxydes équivalents synthétiques des dications cétènes: Synthèse de thiazolidinone-4 par reaction avec des thiourées substituées et évolution particulière en (aryl-cyanoformyl) méthylène-2 benzoxazole par reaction avec le thioxo-2 benzoxazole, Tetrahedron 46 (1990) 453–464.
[15] T.C. Granade, W.M. Artis, Antimycotic susceptibility testing of dermatophytes in microcultures with a standardized fragmented mycelial inoculum, Antimicrob. Agents Chemother. 17 (1980) 725–729.