Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 41 (2006) 1017–1024
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://france.elsevier.com/direct/ejmech

Preliminary communication

Synthesis of a new series of heterocyclic scaffolds for medicinal purposes

H.S. Hilal [a,*], M.S. Ali-Shtayeh [a], R. Arafat [a], T. Al-Tel [a], W. Voelter [b], A. Barakat [a]

[a] College of Sciences, An-Najah N. University, PO Box 7, Nablus, Palestine
[b] Abteilung für Physikalische Biochemie des Physiologisch-Chemischen Instituts der Universität Tübingen, Hoppe-Seyler Straße 4, 72076 Tübingen, Germany

Received 6 June 2005; received in revised form 11 March 2006; accepted 16 March 2006
Available online 22 June 2006

Abstract

A new series of substituted 8-fluro-4*H*-pyrimido[2,1-b] [1,3]benzothiazole-4-ones () substituted 7-methyl-4*H*-isoxazolo[2,3-*a*]pyrimidin-4-ones, and substituted 2-methyl-5,6,7,8-tetrahydro-9*H*-isoxazolo[2,3-*a*]pyridopyrimidin-9-ones, compounds **I–VII**, have been prepared via condensation of β-keto esters with 2-aminopyridine derivatives, in the presence of polyphosphoric acid. The same technique has also been used to prepare diazepine compounds, **VIII**–**X**, by condensation of a γ-keto ester with 2-aminopyridine derivatives. Details of synthetic procedures are shown. The new compounds have been characterized by elemental analysis, GC–MS, FT-IR and NMR spectrometry. Antibacterial, antifungal and anticancer (cytotoxic) activities, for three of these compounds, have been investigated and are presented.

Keywords: β-Keto esters; 2-Aminopyridine; Diazepine; Heterocyclic; Antifungal; Antibacterial; Cytotoxic

1. Introduction

Naturally occurring hetero-polycyclic compounds are often medically valuable [1]. Cytotoxic, anti-allergic, and anti-malarial activities are documented for hetero-polycyclic compounds such as *colchicin* [2,3], *chalcones* [3], 2-aryl-1,8-naphthyridin-4-one [2] and others [4–7]. Anti-inflammatory [8,9], cardiotonic [10], antiallergic [11], antimalarial [12] and other activities [13–16] are known for heterocyclic compounds. Therefore, it is important to find new efficient methods to synthesize new hetero-polycyclic frameworks. Different synthetic methods are known for such purpose. Examples are: cyclization via reaction of amino-heterocycles with acetylenic compounds, [17,18], base catalyzed isoxazolinyl heterocycle rearrangement [19–21] and intramolecular nucleophilic acyl substitution [22–30]. The main objective of this work is to synthesize and characterize a number of fused heterocyclic compounds that may potentially have medical value, based on structural similarities with earlier compounds.

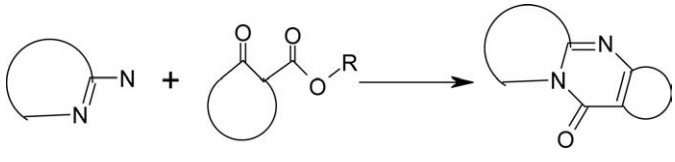
2. Chemistry

Two different groups of compounds have been prepared and characterized [27,31]. Compounds **I–VII** have been prepared by condensation of different β-keto esters with different 2-aminopyridine derivatives, in the presence of polyphosphoric acid, as exemplified in Scheme 1.

On the other hand, compounds **VIII–X** have been prepared by condensing a γ-keto ester with different 2-aminopyridine derivatives, in polyphosphoric acid, as exemplified in Scheme 2. Elemental analysis, GC–MS, FT-IR and NMR indicated the preparation of compounds **I–X** in appreciably pure forms. The structure, expected for each compound, has been confirmed. Details of preparation and characterizations of these compounds are presented in the experimental section.

3. Biological activity results

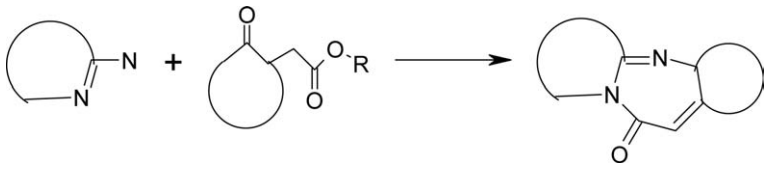
Three compounds, **I**, **IV** and **V,** have been studied for possible future biological functions.



Scheme 1. Structural formulas for I–VII here.

* Corresponding author. Fax: +970 9 238 7982.
E-mail address: hikmathilal@yahoo.com (H.S. Hilal).

0223-5234/$ - see front matter © 2006 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2006.03.025



Scheme 2. Structural formulas for compounds VIII–X here.

3.1. Antibacterial and antifungal activities

Three compounds **I**, **IV** and **V** were all investigated for antibacterial activity against *Staphylococcus aureus*, *Proteus vulgaris* and *Candida albicans.* None of these compounds showed any significant antibacterial activity. The same compounds showed significant antifungal activity against *Microsporum canis*, *Fusarium tricincutum*, *Pythium ultimum*, *P. aphanidermatum* and *P. middletonii.* Results are shown in Tables 1 and 2.

The inhibition effects of compounds **I**, **IV** and **V**, together with the reference values, on *M. canis* and *F. tricincutumare* are shown in Table 1. The data showed significant differences between inhibition effects on *M. canis* and *F. tricincutum*, with a *P*-value < 0.001. On the other hand, all compounds, including the reference, showed same inhibition effects, with no significant differences. Thus, all compounds used including the references, had similar activity, while *M. canis* is less sensitive than the other fungus counterpart.

Inhibition effects of compounds **I**, **IV** and **V**, and references, on *P. ultimum*, *P. aphanider-matum* and *P. middletonii*, are shown in Table 2. The three fungi did not have the same sensitivity to the group of compounds, with a *P*-value < 0.001. Therefore, multiple comparisons, using Tukey's test, had been conducted and indicated that *P. ultimum* and *P. aphanidermatum* had same sensitivity, which is different from that of *P. middletonii* (*P*-value < 0.001).

Table 1
Antifungal activity results (mm day^{-1}) for compounds **I**, **IV** and **V** against *M. canis* and *F. tricincutum,* compared to reference values

Compound	% Inhibition (mean of three replicate plates ± S.E.)*	
	M. canis [a]	*F. tricincutum* [b]
I	100.00 ± 0.0	68.5 ± 1.7
IV	84.5 ± 0.0	42.6 ± 0.6
V	100.00 ± 0.0	48.9 ± 5.8
Ref.	75.5 ± 3.9	67.8 ± 1.9

Compound concentrations used are shown in section 5.4.
[a] Ref. griseofulvin;
[b] Ref. nystatin.

Table 2
Antifungal activity results (mm day^{-1}) for compounds **I**, **IV** and **V** against *P. ultimum*, *P. aphanidermatum* and *P. middletonii* compared to reference values

Compound	% Inhibition		
	P. ultimum	*P. aphanidermatum*	*P. middletonii*
I	80.4 ± 2.3	95.9 ± 0.6	44.4 ± 3.5
IV	100.00 ± 0.0	100.00 ± 0.0	80.0 ± 1.7
V	91.9 ± 0.6	100.00 ± 0.0	78.9 ± 1.0
Ref. (hymexazol)	81.1 ± 5.1	70.0 ± 3.3	24.3 ± 10.1

* Mean of three replicate plates ± S.E. Compound concentrations used are shown in Section 5.4.

Contrary to statistical results obtained from Table 1, the data in Table 2 show statistically significant differences among the group of compounds, including reference, with a *P*-value = 0.001.

Each compound activity was compared to the reference, using the Dunnett's intervals. The activity of **I** showed same activity as reference activity, whereas compound **IV** and **V** showed different activities from reference (with *P*-values < 0.01). Thus, compounds **IV** and **V** are statistically more active than compound **I** and the reference. Furthermore, *P. middletonii* is more resistant than other fungus counterparts.

This shows the future potential of using the prepared compounds in antifungal medical formulations. The hymexazole [32] (3-hydroxy-5-methylisoxazole) reference, resembles the structure of the starting material for compounds **IV** and **V**. No correlation between structures, for **I**, **II** and **V**, and their activities were constructed.

3.2. Cytotoxic activity

Three compounds **IV**, **IX** and **X** have been evaluated for cytotoxic activity. The results are summarized in Table 3. Values of percent mortality of cells against compound concentration for each compound are shown in Fig. 1. Among the three compounds used, **IV** and **X** showed significant cytotoxic activities, whereas **IX** failed to do so. In **IV** and **X**, percent cell mortality increased with compound concentration. The data indicate that **X** is potentially more favorable than **IV**. At 1.5 × 10^{-3} M concentration, **X** showed about 95% mortality, before changing into a plateau. Activity of **IV** was lower than that of **X**, within the concentration range used. To achieve mortality percent of 41%, high concentrations (~3.0 × 10^{-3} M) were needed. This limits the potential value of **IV** in medical applications. Contrary to **IV** and **X**, compound **IX** showed inconsistent correlation between percent mortality and concentration, with only a small peak (40%) for 6.25 × 10^{-4} M concentration.

4. Conclusions

Compounds **I–X** have been prepared. Two compounds, (**III** and **VIII**), occurred in relatively low yields (36% and 35%, respectively), whereas the remaining compounds occurred in high yields. Spectral analyses confirmed the proposed structures for the synthesized compounds. Compounds **I**, **IV** and **X** showed neither antibacterial activity against *Staphylococcus aureus*, *Proteus vulgaris* nor anticandidal activity against *Candida albicans.* When tested against *Microsporum canis*, *Fusarium tricincutum*, *Pythium ultimum*, *Pythium aphanidermatum* and *Pythium middletonii,* compounds **I**, **IV** and **X** showed sig-

Table 3
Cytotoxic activity (MTT assay results) of compounds **IV**, **IX** and **X**

Compound	Stock solution concentration (M)	Final concentration (M)	Absorbance at λ_{max}	% Mortality of cells
IV	6.10×10^{-2}	2.90×10^{-3}	0.030	41.20
		1.50×10^{-3}	0.039	23.50
		7.30×10^{-4}	0.043	15.70
		3.60×10^{-4}	0.050	1.96
IX	5.25×10^{-2}	1.25×10^{-3}	0.040	12.60
		6.25×10^{-4}	0.031	39.20
		3.13×10^{-4}	0.031	39.20
X	6.30×10^{-2}	3.00×10^{-3}	0.002	96.00
		1.50×10^{-3}	0.003	94.10
		7.50×10^{-4}	0.009	82.40
		3.70×10^{-4}	0.028	45.10
		1.90×10^{-4}	0.039	22.90

Absorbance for the reference was 0.051 at 550 nm.

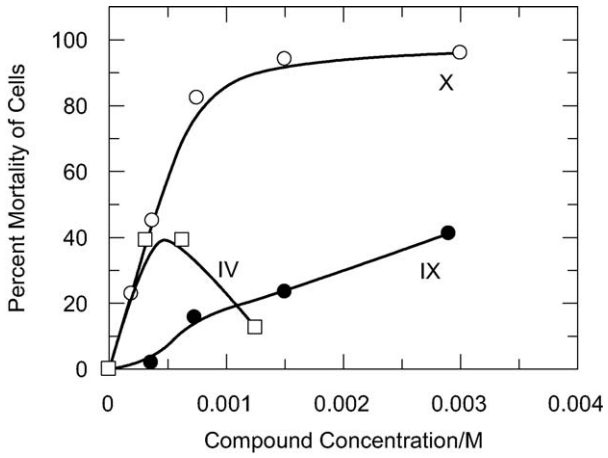


Fig. 1. Values of percent mortality of cells for different compound concentrations.

nificant antifungal activities, as compared to reference compounds. Compounds **IV**, **IX** and **X** were investigated for cytotoxic activity. Compounds **IV** and **X** showed significant cytotoxic activity, whereas **IX** showed low activity. Among the tested compounds, **X** is the most promising candidate showing high cytotoxic activity at relatively low concentrations.

5. Experimental

5.1. Chemicals

Starting materials, 2-amino-6-fluorobenzothiazole, 1-aminoisoquinoline, 3-amino-5-methylisoxazole, ethylacetoacetate, ethyl-2-oxocyclopentane carboxylate, ethyl-2-oxocyclohexanecarboxylate, ethyl-2-cyclohexanone acetate, methyl-1-benzyl-4-oxo-3-piperidinecarboxylate hydrochloride, ethyl-1-benzyl-3-oxo-4-piperidine carboxylate hydrochloride, polyphosphoric acid (PPA) and other solvents (chloroform, petroleum ether, diethylether, ethylacetate and ethanol) were all purchased from Aldrich Ltd., and were used as received. Reference compounds, griseofulvin and nystatin, used in biological activity experiments, were purchased from Aldrich Ltd, with catalogue numbers G4753 and N3503, respectively.

5.2. Equipment

Elemental analysis data were measured on a PE 2400 Perkin Elmer series II CHNS/O analyzer. FT-IR spectra were measured on a Shimadzu 820 PC FT-IR Spectrometer. GC-MS results were recorded on a Shimadzu GC-MS-QP5000, using chloroform and methanol as solvents. The GC runs were conducted using a J&W Scientific DB-5MS 30 m long 0.25 mm in diameter capillary column, equipped with 5% phenyl methyl polysilane as stationery. Helium carrier gas was used at a flow rate of 28.2 ml min^{-1}. The injection unit was kept at 250 °C, and the interface temperature was 230 °C. The oven temperature was initially set at 120 °C, with a ram rate 5 °C min^{-1} reaching a final temperature of 300 °C. The waiting time at each stage was 0.5 min.

^{13}C NMR spectra were recorded on a Bruker 75.48 MHz, using $CDCl_3$ as a solvent, courtesy of the laboratories of Jordan University. The solvent characteristic signal was observed as a triplet at 77.1 ppm. ^{1}H NMR spectra were recorded on a Bruker 300 MHz, using $CDCl_3$ as a solvent.

5.3. Compound preparations and analyses

Compounds **I–X** were prepared by the reaction of the amino-heterocycle with β- or γ-keto esters in the presence of PPA, while continuously stirring for 2 hours at 120 °C [27,31]. Unless otherwise stated the general preparation procedure was as follows: PPA, 3–6 g, was weighed in a 50-ml conical flask. The amino heterocycle (0.5 g) was added. The β-, or γ-keto ester was then added as excess (the molar ratio of the keto ester to the amino heterocycle was 1.1/1, respectively). The mixture was heated, while manually stirring, in an oil bath for 20 min until a 120 °C final temperature was reached. The mixture was kept at this temperature for 2 more hours with continuous stirring [31]. The reaction was monitored periodically by thin layer chromatography (TLC) using 5% ethylacetate/chloroform developing solvent. After completion, the reaction was cooled in an ice bath. Ice water was added to the mixture. The solution was then neutralized by NaOH (4 N) solution. The precipitate product was filtered on a sintered funnel and washed with distilled water. Non-precipitate products were alternatively extracted using the proper solvent. Details of product preparation, description and analysis are shown below.

I: Prepared from 2-amino-6-fluorobenzothiazole (0.5 g, 2.97 mmol) and ethylacetoacetate (0.4 g, 3.08 mmol) in PPA (3.0 g). Product, recrystallized from EtOH, 0.6 g, 86% yield, yellowish ppt., m.p. range 193–195 °C - ^{1}H NMR ($CDCl_3$, 300 MHz), H_a: 3H at 2.38 ppm (s); H_3: 1H at 6.26 ppm (s); H_6: 1H at 7.22 ppm (dd, $J_{H6\text{-}H7} = 8.3$ Hz, $J_{H6\text{-}F} = 7.9$ Hz)); H_7: 1H at 9.05 ppm (dd, $J_{H7\text{-}H6} = 8.8$ Hz, $J_{H7\text{-}F} = 4.7$ Hz); H_9: 1H at 7.39 ppm (d, $J_{H9\text{-}F} = 5.8$ Hz), [33] – ^{13}C NMR (GASPE, $CDCl_3$, 100 MHz, C–C decoupling), C_a: 23.7 ppm; C_2: 159.2 ppm; C_3: 107.3 ppm; C_4: 162.9 ppm; C_b: 125.7 ppm; C_6: 121.4 ppm (d, $J_{C6\text{-}F} = 8.6$ Hz) ; C_7: 108.9 ppm (d, $J_{C7\text{-}F} = 27.3$ Hz); C_8: 161.7 ppm (d, $J_{C8\text{-}F} = 106.2$ Hz); C_9: 114.5 ppm (d, $J_{C9\text{-}F} = 23.3$ Hz) ; C_c: 132.4 ppm; C_d: 160.8 ppm. Further ^{13}C NMR analysis [34] was done using

DEPT 135 technique, and the results were consistent with those listed. – GC-MS (FD), parent ion $m/z = 234$, base peak 28 (CO), fragment at 206 due to CO elimination. – IR, 1681.8 cm^{-1} for C=O str., 1365.5 cm^{-1} for CH_3, and 1600.8 , 1577.7 and 1506.3 cm^{-1} for C=C str. Bands 1725 and 1800 cm^{-1}, characteristic for reactant keto group [35], disappeared. – Anal. $C_{11}H_7ON_2SF$ (MW 234}, Calcd. C 56.40%, H 3.02%, N 11.96%. Found C 56.56%, H 3.54%, N 11.66%.

II: Prepared from 2-amino-6-fluorobenzothiazole (0.5 g, 2.97 mmol) and ethyl-2-oxocyclopentanecarboxylate (0.51 g, 3.27 mmol) in PPA (3.0 g). Product, recrystallized from EtOH, 0.4 g, 52% yield, brown ppt., m.p. range 150–152 °C - ^{1}H NMR ($CDCl_3$, 300 MHz), H_a: 2H at 2.96 ppm (t, $J_{a\text{-}b} = 7$ Hz); H_b: 2H at 2.17 ppm (tt, $J_{a\text{-}b} = J_{b\text{-}c} = 7.4$ Hz); H_c: 2H at 2.93 ppm (t, $J_{b\text{-}c} = 7.2$ Hz); H_6: 1H at 7.00 (dd, $J_{6\text{-}7} = 8.5$ Hz, $J_{6\text{-}F} = 7.8$); H_7: 1H at 9.12 ppm (dd, $J_{7\text{-}6} = 9$ Hz, $J_{7\text{-}F} = 4.7$); H_9: 1H at 7.36 ppm (d, $J_{9\text{-}F} = 5.6$ Hz). – ^{13}C NMR, (GASPE, $CDCl_3$, 100 MHz, C–C decoupling) C_2: 159 ppm; C_a: 34.8 ppm; C_b: 21.7 ppm; C_c: 27.3 ppm; C_3: 132.2 ppm; C_4: 168.1 ppm; C_d: 132.6 ppm; C_6: 121.4 ppm; C_7: 108.9 ppm; C_8: 161.7 ppm; C_9: 114.4 ppm; C_e: 126.1 ppm; C_f: 159.3 ppm.- GC-MS (FD), parent ion $m/z = 260$, base peak 28 (CO), fragment at 232 due to CO elimination.- IR, 1678 cm^{-1} for CO str., 1463.4 cm^{-1} for CH_2 (C-H) bending and 1579.1 and 1501.5 cm^{-1} for aromatic C=C str.- Anal. $C_{13}H_9ON_2SF$ (MW 260), Calcd. C 59.98%, H 3.49%, N 10.77%. Found C 60.42%, H 4.63%, N 11.22%.

III: Prepared from 3-amino-5-methylisoxazole (0.5 g, 5.10 mmol) and ethylacetoacetate (0.73 g, 5.6 mmol) in PPA (3.0 g). Product extracted with $CHCl_3$, and recrystallized from EtOH, 0.3 g, 36% yield, brown ppt., m.p. range 176–178 °C - ^{1}H NMR ($CDCl_3$, 300 MHz), H_a: 3H at 2.38 ppm (s); H_b: 3H at 2.57 ppm (s); H_3: 1H at 6.29 ppm (s); H_8: 1H at 6.15 ppm (s). - ^{13}C NMR, (GASPE, $CDCl_3$, 100 MHz, C–C decoupling), C_a: 24.2 ppm; C_2: 153.4 ppm; C_3: 105.3 ppm; C_4: 165.5 ppm; C_7: 153.6 ppm; C_8: 99.5 ppm; C_9: 166.5 ppm; C_b: 12.8 ppm. - GC-MS (FD), parent ion $m/z \times 164$, base peak 28 (CO), fragment at 121 due to elimination of CO and CH_3 groups. – IR, strong band 1689.5 cm^{-1} for CO str., 1371.3 cm^{-1} for methyl group, 1625.9 cm^{-1} for C=C str.- Anal. $C_8H_8O_2N_2$ (MW 164), Calcd. C 58.52%, H 4.88%, N 17.07%. Found C 57.54%, H 5.12%, N 17.35%.

IV: Prepared from 3-amino-5-methylisoxazole (0.5 g, 5.10 mmol) and ethyl-2-oxocyclopentanecarboxylate (0.87 g, 5.6 mmol) in PPA (3.0 g). Product, recrystallized from EtOH, 0.84 g, 87% yield, brown ppt., m.p.. range 147–150 °C - ^{1}H NMR ($CDCl_3$, 300 MHz), H_a and H_c: 4H at 2.92 ppm (t, $J_{a,c\text{-}b} = 7.5$ Hz); H_b: 2H at 2.16 ppm (q, ($J_{b\text{-}a,c} = 7.5$ Hz); H_d: 3H at 2.57 ppm (s); H_8: 1H at 6.31 (s).- ^{13}C NMR, (GASPE, $CDCl_3$, 100 MHz, C-C decoupling), C2: 152.1 ppm; Ca: 35 ppm; Cb: 22.4 ppm; Cc: 27.4 ppm; C3: 117.6; C4: 154 ppm; C7: 166.1 ppm; C8: 99.4 ppm; C9: 170.4 ppm; Cd: 12.9 ppm. - GC-MS (FD), parent ion $m/z = 190$, base peak 28 (CO), fragment at 147 due to elimination of CO and CH_3 groups.- IR, strong band 1674.1 cm^{-1} for CO str., 1384.8 cm^{-1} for methyl (bend.), 1421.4 and 1438.8 cm^{-1} for CH_2 (bend.), 1622 cm^{-1} for C=C str. – Anal. $C_{10}H_{10}O_2N_2$ (MW 190) Calcd. C 63.14%, H 5.31%, N 14.73%. Found 51.01%, H 7.08%, N 12.51%.

V: Prepared from 3-amino-5-methylisoxazole (0.5 g, 5.10 mmol) and ethyl-2-oxocyclohexane carboxylate (0.95 g, 5.6 mmol) in PPA (3.0 g). Product, recrystallized from EtOH, 0.86 g, yield 83%, pale yellow ppt., m.p. range 98–100 °C.- ^{1}H NMR ($CDCl_3$, 300 MHz), H_a: 2H at 2.71 ppm (distorted t, $J_{a\text{-}b} = 5.9$ Hz); H_b and H_c: 4H at 1.81 ppm (mult.); H_d: 2H at 2.65 ppm (distorted t, $J_{d\text{-}c} = 5.8$ Hz); H_e: 3H at 2.54 ppm (s); H_8: 6.23 ppm (s). - ^{13}C NMR, (GASPE, $CDCl_3$, 100 MHz, C–C decoupling), C2: 150.8 ppm; C_a: 23.2 ppm; C_b: 22.5 ppm; C_c: 21.8 ppm; C_d: 22.3 ppm; C_3: 115.1 ppm; C_4: 161.3; C_7: 153.8 ppm; C_8: 99.2 ppm; C_9: 165.3 ppm; C_e: 12.7 ppm. - GC-MS (FD), parent ion $m/z = 204$, base peak 28 (CO), fragment at 189 due to CH_3 elimination, 176 due to CO elimination and at 161 due to CH_3 and CO elimination. – IR, strong band 1670.2 cm^{-1} for CO Str., 1384.8 cm^{-1} for CH_3, and at 1637.5 cm^{-1} for C=C. – Anal $C_{11}H_{12}O_2N_2$ (MW 204) Calcd. C 64.68%, H 5.93%, N 13.72%. Found C 61.71%, H 6.39%, N 15.17%.

VI: Prepared from3-amino-5-methylisoxazole (0.5 g, 5.10 mmol) and ethyl-1-benzyl-3-oxo-4-piperidine carboxylate hydrochloride (1.67 g, 5.6 mmol) in PPA (6.0 g). Product, extracted with ethylacetate, recrystallized from EtOH and washed with diethylether and petroleum ether, 0.8 g, yield 53%, brown ppt., m.p. range 142–144 °C. - ^{1}H NMR ($CDCl_3$, 300 MHz), H_a: 3H at 2.5 ppm (s); H_3: 1H at 6.19 ppm (broad s); H_7 and H_8: 4H at 2.75 ppm (broad s); H_5: 2H at 3.5 (s); H_e: 2H at 3.7 ppm (s); H_f: 5H at 7.23 ppm (mult.). ^{13}C NMR, (GASPE, $CDCl_3$, 100 MHz, C–C decoupling), C_a: 12.7 ppm; C_2: 153.4 ppm; C_3: 99.2 ppm; C_b: 165.6 ppm; C_c: 151.5 ppm; C_5 : 57.2 ppm; C_7: 49.3 ppm; C_8: 22.7 ppm; C_d: 112.9 ppm; C_9: 159 ppm; C_e: 62.2 ppm; C_{f1}: 137.5 ppm; C_{f2}: 129.1 ppm; C_{f3}: 128.3 ppm; C_{f4}: 127.3 ppm. - GC-MS (FD), parent ion $m/z = 295$, base peak 28 (CO), fragment 176 due to benzyl and CO eliminations, 91 due to benzyl ion. – IR, strong 1679.9 cm^{-1} for CO str., 1382.9 cm^{-1} for CH_3, 1642 cm^{-1} for C=C, 1587.3 and 1541 cm^{-1} for C=C (phenyl). – Anal. $C_{17}H_{17}O_2N_3$ (MW 295) Calcd. C 69.12%, H 5.81%, N 14.23%. Found C 68.01%, H 6.44%, N 16.62%.

VII: Prepared from 3-amino-5-methylisoxazole (0.5 g, 5.10 mmol) and methyl-1-benzyl-4-oxo-3-piperidine carboxylate hydrochloride 1.67 g, 5.6 mmol) in PPA (6.0 g). Product, extracted with ethylacetate, recrystallized from EtOH and washed with dietheylether and petroleum ether, 0.53 g, yield 35%, yellow ppt., m.p. range 176–177 °C. - ^{1}H NMR ($CDCl_3$, 300 MHz), H_a: 3H at 2.52 ppm (s); H_3: 1H at 6.22 ppm (s); H_5: 2H at 2.75 ppm (t, $J_{H5\text{-}H6} = 5.2$ Hz); H_6: 2H at 2.82 ppm (t, $J_{H6\text{-}H5} = 5.0$ Hz); H_8: 2H at 3.61 ppm (s); H_e: 2H at 3.74 ppm (s); H_f: 5H at 7.35 ppm (mult.). - ^{13}C NMR, (GASPE, $CDCl_3$, 100 MHz, C-C decoupling), C_a: 12.7 ppm, C_2: 152.3 ppm; C_3: 99.2 ppm; C_b: 165.7 ppm, C_c: 151.5 ppm; C_5: 32.3 ppm; C_6: 49.2 ppm; C_8: 49.9 ppm; C_d: 113.2 ppm; C_9 : 159.7 ppm; C_e: 62.3 ppm; C_{f1}: 137.9 ppm; C_{f2}: 129.1 ppm; C_{f3}: 128.4 ppm; C_{f4}: 127.2 ppm. - GC-MS (FD), parent ion $m/z = 295$, base peak 28 (CO), fragment 176 due to benzyl and benzyl and CO eliminations, fragment 91 benzyl, fragment 204 due to benzyl elimination. – IR, strong 1666.4 cm^{-1} for CO str., 1359.7 cm^{-1} for CH3, 1581.5 and 1529.4 cm^{-1} for phenyl.

– Anal. $C_{17}H_{17}O_2N_3$ (MW 295). Calcd. C 69.12%, H 5.81%, N 14.23%. Found C 68.55%, H 6.46%, N 15.87%.

VIII: Prepared from 2-amino-6-fluorobenzothiazole (0.5 g, 2.97 mmol) and ethyl-2-cyclohexanone acetate (0.6 g, 3.27 mmol) in PPA (3.0 g). Product, recrystallized from EtOH, 0.68 g, yield 79%, pale yellow ppt., m.p. range 158–160 °C. - ^{1}H NMR ($CDCl_3$, 300 MHz), H_2: 1H at 4.7 ppm (dd, $J_{H2\text{-}Ha}$ = 5.7 Hz, $J_{H2\text{-}Ha'}$ = 11.0 Hz); H_a–H_d: 8H in the range 1.15–3.35 ppm (mult.); H_4: 1H at 5.92 ppm (broad s); H_7: 1H at 7.13 ppm (dd, $J_{H7\text{-}H8}$ = 8.2 Hz, $J_{H7\text{-}F}$ = 8.9 Hz); H_8: 1H at 7.72 ppm (dd, $J_{H8\text{-}H7}$ = 8.6 Hz, $J_{H8\text{-}F}$ = 4.6 Hz); H_{10}: 1H at 7.49 ppm (d, $J_{H10\ \text{–}F}$ = 5.8 Hz). - ^{13}C NMR, (GASPE, $CDCl_3$, 100 MHz, C–C decoupling), C_2: 63.9 ppm; C_a: 34.1 ppm; C_b: 23.1 ppm; C_c: 28 ppm; C_d: 29.1 ppm, C_3: 145.6 ppm; C_4: 116.9 ppm; C_5: 169.7 ppm; C_e: 133 ppm ($J_{Ce\text{-}F}$ = 10.9 Hz); C_7: 121.7 ppm ($J_{C7\text{-}F}$ = 8.3 Hz); C_8: 107.5 ppm ($J_{C8\text{-}F}$ = 26.6 Hz); C_9: 156.9 ppm ($J_{C9\text{-}F}$ = 135.7 Hz); C_{10}: 114.1 ppm ($J_{C10\text{-}F}$ = 24.5 Hz); C_f: 119.7 ppm ($J_{Cf\text{-}F}$ = 33.2 Hz); C_g: 166.9 ppm. - GC-MS (FD), parent ion m/z = 288, base peak 28 (CO), fragment 260 due to CO elimination. - IR, strong 1687 cm^{-1} for CO str., 1458.1 cm^{-1} for CH_2 bend., 1635.5 cm^{-1} for C=C (7-membered ring), 1606.6 and 1541 cm^{-1} for C=C str. (benzene). – Anal. $C_{15}H_{13}ON_2SF$ (MW 288). Calcd. C 62.47%, H 4.55%, N 9.72%. Found C 53.89%, 3.81%, N 14.52%.

IX: Prepared from 3-amino-5-methylisoxazole (0.5 g, 5.10 mmol) and ethyl-2-cyclohexanone acetate (1.03 g, 5.6 mmol) in PPA (3.0 g). Product, recrystallized from EtOH, 0.6 g, yield 54%, brown ppt., m.p. range 87–90 °C. - ^{1}H NMR ($CDCl_3$, 300 MHz), H_a-H_d: 8H in the range 1.12–3.25 ppm (mult.); H_2: 1H at 4.39 ppm (dd, $J_{H2\text{-}Ha}$ = 3.4 Hz, $J_{H2\text{-}Ha'}$ = 10.7 Hz); H_4: 1H at 6.84 ppm (s); H_9: 1H at 5.83 (s); H_e: 3H at 2.4 ppm (s). ^{13}C NMR, (GASPE, $CDCl_3$, 100 MHz, C-C decoupling), C_2: 62.2 ppm; C_a: 33.8 ppm; C_b: 28 ppm; C_c: 23.1 ppm; C_d: 28.8 ppm; C_3: 157.4 ppm; C_4: 117.6 ppm; C_5: 165.5 ppm; C_8: 169.5 ppm; C_9: 95.2 ppm; C_{10}: 169.8 ppm; C_e: 12.6 ppm. - GC-MS (FD), parent ion m/z = 218, base peak 28 (CO), fragment 190 due to CO elimination, fragment m/z 175 due to CO and CH_3 elimination. – IR, strong 1697.2 cm^{-1} for CO str., 1456.2 cm^{-1} for CH_2 bend., 1654.8 and 1635.5 cm^{-1} for C=C str. – Anal. $C_{12}H_{14}O_2N_2$ (MW 218). Calcd. C 66.03%, H 6.48%, N 12.84%. Found C 65.42%, H 6.83%, N 14.54%.

X: Prepared from 1-aminoisoquinoline (0.5 g, 3.47 mmol) and ethyl-2-cyclohexanone acetate (0.7 g, 5.6 mmol) in PPA (3.0 g). Product, recrystallized from EtOH, 0.9 g, yield 97.8%, brown ppt., m.p. range 190–192 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) H_a–H_d: 8H in the range 0.78–2.31 ppm (multi.); H_2: 4.98 ppm (dd, $J_{H2\text{-}Ha}$ = 5 Hz, $J_{H2\text{-}Ha'}$ = 10 Hz)); H_4: 1H at 5.82 ppm (s); H_7–H_{12}: 6H in the range 7.19–8.38 ppm (multi.). – ^{13}C NMR (GASPE, $CDCl_3$, 100 MHz) C_2: 63.5 ppm; C_a: 33.4 ppm; C_b: 23 ppm; C_c: 27.2 ppm; C_d: 28.5 ppm; C_4: 120 ppm; C_7: 126.5 ppm; C_8: 117.5 ppm; C_9: 126.8 ppm; C_{10}: 141 ppm; C_{11}: 127.1 ppm; C_{12}: 130.5 ppm. (MW 266).

It should be noted that the structures for compounds XIII, IX and X are not unequivocally elucidated, and further analytical study is underway to elucidate structures for these and other compounds of similar nature.

5.4. Biological activity experiments

5.4.1. Antibacterial and antifungal activity

5.4.1.1. Test microorganisms. Two bacteria (*S. aureus* and *P. vulgaris*), one isolate of yeast (*Candida albicans*), two isolates of human pathogenic dermatophytes (*Microsporum canis*

Table 4
Test microorganisms and source of collection

Microorganism	Number	Source/researcher	Reference antibiotic (concentration)
Bacteria			
Staphylococcus aureus	ATCC 25923	ATCC [a]	Ampicillin (10 mg per disc)
Proteus vulgaris	ATCC 13315	ATCC	Gentamicin (10 mg per disc)
Yeast			
Candida albicans (Robin) Berkhout	FCCAU [b] R10	Foot swab, patient/Mr. S. Abu-Ghdieb	Nystatin (10 mg per disc)
Dermatophytes			
Microsporum canis Bodin	FCCAU S14	Tinea capitis clinical specimens in Nablus [c] area, patient/Mr. Abu-Ghdieb	Griseofluvin (0.6 μg ml^{-1})
Microsporum gypseum (Bodin) Guiart and Grigorakis	FCCAU S15	Tinea capitis clinical specimens in Nablus area, patient/Mr. Abu-Ghdieb	Nystatin (0.6 μg ml^{-1})
Phytopathogenic fungi			
Fusarium tricinctum (Corda) Sacc.	FCCAU M10	Water pool in Nablus/Mr. T. Khalid	Nystatin (5 mg ml^{-1})
Pythium ultimum var *ultimum* Trow	FCCAU H5R3	Water and soil samples in Nablus area/ Professor Ali-Shtayeh	Hymexazol (25 μg ml^{-1})
P. aphanidermatum (Edson) Fitzp.	FCCAU H739	Water and soil samples in Nablus area/ Professor Ali-Shtayeh	Hymexazol (25 μg ml^{-1})
P. middletonii Sparrow	FCCAU PH 122	Water and soil samples in Nablus area/ Professor Ali-Shtayeh	Hymexazol (25 g ml^{-1})
Phytophthora citrophthora (R. E. Smith and E. H. Smith)	FCCAU AIE 2005	Water and soil samples in Nablus area/ Dr. A-H Hamdan	Metalaxyl (10 μg ml^{-1})

[a] American Type Culture Collection.
[b] FCCAU: Fungal Culture Collection of An-Najah University.
[c] Nablus is largest city in the West Bank, 60 km north of Jerusalem.

and *Microsporum gypseum*), and five isolates of plant pathogenic fungi (*Fusarium tricinctum* , *Pythium ultimum* var *ultimum*, *P. aphanidermatum*, *P. middletonii* and *Phytophthora citrophthora*), were employed in this study (Table 4).

5.4.2. Screening for antimicrobial activities

The test compounds were dissolved in 10% aqueous dimethyl sulfoxide (DMSO) to a final concentration of 200 mg ml^{-1} and sterilized by filtration through a 0.45 um membrane filter. Antimicrobial tests were then carried out by the disc diffusion method [36] using an inoculum containing 10^6 bacterial cells or 10^8 yeast cells ml^{-1} to spread on Muller-Hinton agar plates (1 ml inoculum per plate). Six mm diameter discs were each impregnated with 50 µl of extract (10 mg per disc) at a concentration of 200 mg ml^{-1} and placed on the inoculated agar and incubated at 37 °C for 24 h for bacteria and for 48 h for *Candida albicans*. On each plate, an appropriate reference antibiotic disc was applied depending on the test microorganisms. Each test was carried out in triplicates.

5.4.3. Screening for antifungal activity

Antifungal activity tests were carried out by the poisoned-food technique method [37]. Test isolates were inoculated onto SDA or CMA plates and incubated at 25 °C for 1–4 days for *Pythium* and *Phytophthora*, and for 7–14 days for *Microsporum* and *Fusarium,* to obtain young, actively growing cultures consisting of mycelia and conidia. The required amount of the test compound or reference antimycotic drug was dissolved in 2 ml sterile distilled water or 10% aqueous DMSO, sterilized by filtration through a 0.45 µm membrane filter, and then mixed in requisite amount of pre-sterilized SDA or CMA medium to give a final concentration of 15 µg ml^{-1}. A mycelial disc of 6-mm diameter, cut out from the periphery of 1–4 day old cultures, was aseptically inoculated onto the medium. In controls, sterile DMSO or distilled water was used in place of the compound as negative control and reference antibiotics as a positive control. The inoculated plates were then incubated at 25 °C and colony diameter measured and recorded after 7 days for dermatophytes and keratinophilic fungi, and one day for *Pythium* and *Phytophthora* species. Percentage of mycelial inhibition was calculated as follows: % mycelial inhibition = ((dc–dt)/dc) × 100; where: dc, colony diameter in control (–ve); dt, colony diameter in treatment. Three replicate plates were used for each treatment [37–39].

5.4.4. Statistical analysis

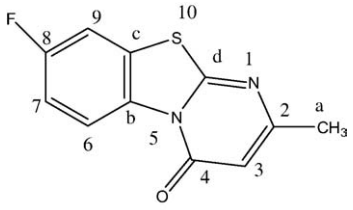
Data were analyzed and treatments compared using analysis of variance with Duncan multiple range test ($P < 0.05$).

5.4.5. Cytotoxic activity

The MTT assay method [40,41] has been used to study the cytotoxic activity of compounds **IV**, **IX** and **X**, against human Caucasian bone marrow neuroblastoma (SK-N-SH) cells (Table 3). Experiments were conducted in the Hospital of the University of Tuebingen. Compound concentrations used are shown in Table 3.

(I)

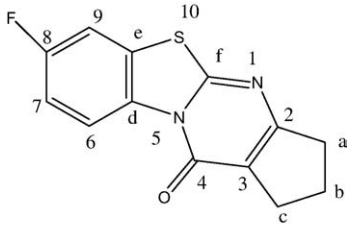
8-Fluoro-2-methyl-4*H*-pyrimido [2,1b][1,3]benzothiazole-4-one.



(I)

(II)

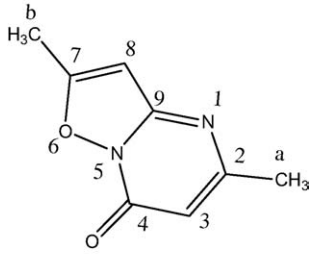
8-Fluoro-2,3-trimethylene-4*H*-pyrimido [2,1b][1,3]benzothiazole-4-one.



(II)

(III)

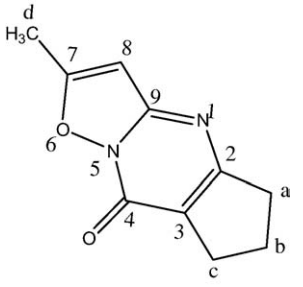
2,7-Dimethyl-4*H*-isoxazolo[2,3-a]pyrimidine-4-one.



(III)

(IV)

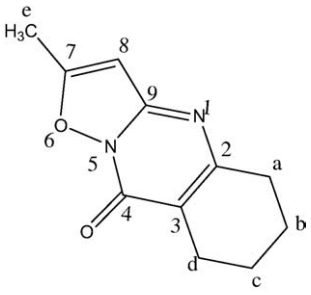
7-Methyl-2,3-trimethylene -4*H*-isoxazolo[2, 3-a]pyrimidine-4-one.



(IV)

(V)

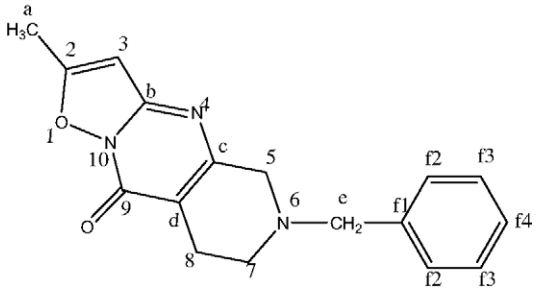
7-Methyl-2,3-tetramethylene-4*H*-isoxazolo[2, 3-a]pyrimidine-4-one.



(V)

(VI)

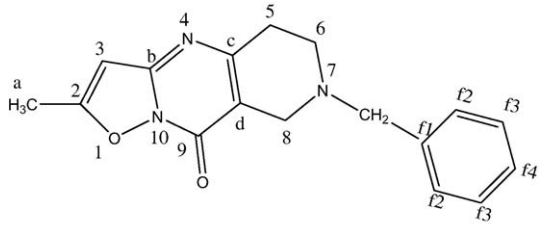
6-Benzyl-2-methyl-5,6,7,8-tetrahydro-9*H*-isoxazolo[2,3-a] pyrido[3,4d]pyrimidine-9-one.



(VI)

(VII)

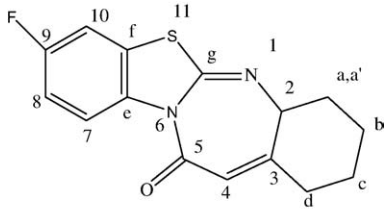
7-Benzyl-2-methyl-5,6,7,8-tetrahydro-9*H*-isoxazolo[2,3-a] pyrido[4,3d]pyrimidine-9-one.



(VII)

(VIII)

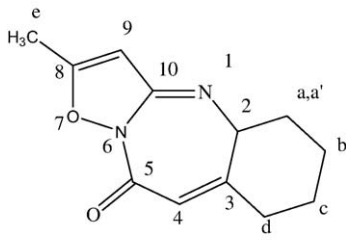
5*H*-9-fluoro-2,3-tetramethylenebenzothiazolo[3,2-a][1,3] diazepin-5-one.



(VIII)

(IX)

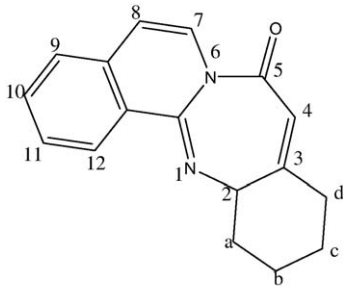
5*H*-8-methyl-2,3-tetramethyleneisoxazolo[2,3-a][1,3]diazepin-5-one.



(IX)

(X)

5*H*-2,3-tetramethyleneisoquinolo[2,1-a][1,3]diazepin-5-one.



(X)

Acknowledgements

Technical help from N. Zatar and R. Zaid (ANU), Dr. M. Abu Zarga and K. Bargathi (Jordan University) and A.R. Matinez (University of Tuebingen) is acknowledged.

References

[1] S.-X. Zhang, K.F. Bastow, Y. Tachibana, S.-C. Kuo, E. Hamel, A. Mauger, V. Narayanan, V. Lee, K.-H. Lee, J. Med. Chem. 42 (1999) 4081–4087.
[2] K. Chem, S.-C. Kuo, M.-C. Hsieh, A. Mauger, C.M. Lin, E. Hamel, K.-H. Lee, J. Med. Chem. 40 (1997) 2266–2275.
[3] M.L. Edwards, D.M. Stemenick, P.S. Sunkara, J. Med. Chem. 33 (1990) 1948–1954.
[4] H.H. Lee, B.D. Palmer, M. Boyd, B.C. Baguley, W.A. Denny, J. Med. Chem. 35 (1992) 258–266.
[5] J.J. Chen, L.W. Deady, M.F. Mackay, Synthesis of some acridone alkaloids and related compounds, Tetrahedron 53 (1997) 12717–12728.
[6] J.J. Chen, L.W. Deady, A Versatile Synthesis of Acridine-1,9-Diones, Synthetic Communications 27 (1997) 95–106.
[7] L.W. Deady, D.M. Werden, J. Org. Chem. 52 (1987) 3930–3932.
[8] F. Suzuki, T. Kuroda, T. Tamura, S. Sato, K. Ohmori, S. Ichikawa, J. Med. Chem. 35 (1992) 2863–2870.
[9] T. Katagi, M. Aoki, M. Kashiwagi, K. Ohata, S. Kohno, T. Murata, T. Inoi, Chem. Pharm. Bull. (Tokyo) 33 (1985) 4878–4888.
[10] V.T. Bandurco, C.F. Schwender, S.C. Bell, D.W. Combs, R.M. Kanojia, S.D. Levine, D.M. Mulvey, M.A. Appollina, M.S. Reed, J. Med. Chem. 30 (1987) 1421–1426.
[11] A. Nohara, T. Ishiguro, K. Ukawa, H. Sugihara, Y. Maki, Y. Sanno, J. Med. Chem. 28 (1985) 559–568.
[12] S.J. Kesten, M.J. Degnan, J. Hung, D.J. McNamara, D.F. Ortwine, S.E. Uhlendorf, L.M. Werbel, J. Med. Chem. 35 (1992) 3429–3447.

[13] Q. Li, D.T.W. Chu, A. Claiborne, C.S. Cooper, C.M. Lee, K. Raye, K.B. Berst, P. Donner, W. Wang, L. Hasvold, A. Fung, Z. Ma, M. Tufano, R. Flamm, L.L. Shen, J. Baranowski, A. Nilius, J. Alder, J. Meulbroek, K. Marsh, D. Crowell, Y. Hui, L. Seif, L.M. Melcher, R. Henry, S. Spanton, R. Faghih, L.L. Klein, S.K. Tanaka, J.J. Plattner, J. Med. Chem. 39 (1996) 3070–3088.
[14] G. Leoncini, M.G. Signorello, G. Roma, M. Di Braccio, Biochem. Pharmacol. 53 (1997) 1667–1672.
[15] G. Roma, N. Cinone, M. Di Braccio, G. Grossi, G. Leoncini, M.G. Signorello, A. Carotti, Bioorg. Med. Chem. 8 (2000) 751–768.
[16] D.W. Fry, J.M. Nelson, V. Slintak, P.R. Keller, G.W. Rewcastle, W.A. Denny, H. Zhous, A.J. Bridges, Biochem. Pharmacol. 54 (1997) 877–887.
[17] H. Ogura, M. Kawaro, T. Itoh, Chem. Pharm. Bull. (Tokyo) 21 (1973) 2019–2025.
[18] D. Dunwell, D. Evans, J. Chem. Soc. C (1971) 2094–2097.
[19] R.H. Prager, T.K. Rosenzweig, Y. Singh, Aust. J. Chem. 45 (1992) 1825–1832.
[20] Y. Singh, R.H. Prager, Aust. J. Chem. 45 (1992) 1811–1823.
[21] C. Donati, W.K. Janowski, R.H. Prager, M.R. Taylor, L.M. Vilkins, Aust. J. Chem. 42 (1989) 2161–2169.
[22] Y.-S. Liu, W.-Y. Huang, J. Chem. Soc. Perkin Trans. 1 (6) (1997) 981–988.
[23] R. Niwa, N. Katagiri, T. Kato, Chem. Pharm. Bull. (Tokyo) 32 (1984) 4153–4194.
[24] K.B. Szwed, M. Krasodomska, Monatsh. Chem. 127 (1996) 1273–1281.
[25] N. Katagiri, T. Kato, R. Niwa, J. Heterocyclic Chem. 21 (1984) 407–412.
[26] T. Morimoto, M. Sekiya, Chem. Pharm. Bull. (Tokyo) 25 (1977) 1607–1609.
[27] M. Shur, S.S. Israelstam, J. Org. Chem. 33 (1968) 3015–3020.
[28] G. Bernath, F. Fulop, I. Hermecz, Z. Meszaros, G. Toth, J. Heterocyclic. Chem. 16 (1979) 137–144.
[29] R.J. Brown, F.W.S. Carver, B.L. Hollingsworth, J. of the Chem. Soc. (Abstracts) (1962) 2624–2626.
[30] R. Bossio, S. Marcaccini, R. Pepino, P. Paoli, J. Heterocyclic Chem. 30 (1993) 33–36.
[31] R. Yasin, M. Sc, Thesis, An-Najah N. University, (1999), pp. 22–23.
[32] S. Kamimura, M. Nishikawa, H. Saeki, Y. Takahi, Phytopathology 64 (1974) 1273–1281.
[33] B.K. Harris, B.E. Mann, in: NMR and the Periodic Table, Academic Press, London, 1978, p. 100.
[34] R.M. Silverstein, G.C. Bassler, T.C. Merrill, Spectrometric Identification of Organic Compounds', 5th ed, J. Wiley, NY, 1991.
[35] C.J. Pouchert (Ed.), The Aldrich Library of Infrared Spectra, 3rd ed, Aldrich Chemical Co. Inc, Milwaukee, 1981, p. 402.
[36] P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, R.H. Yolke, Manual of Clinical Microbiology, 6th ed, ASM press, USA, 1995.
[37] A. Dikshat, A. Husain, Fitoterapia 55 (1984) 171–176.
[38] F.P. Altman, R.G. Butcher, Histochemie 37 (4) (1973) 333–350.
[39] H. Mueller, M.U. Kassack, M. Wiese, J. Biomol. Screen. 9 (6) (2004) 506–515.
[40] M. Čemažar, D. Marolt, M. Lavrič, G. Serša, Radiol. Oncol. 33 (4) (1999) 303–308.
[41] MTT assay protocol, Internet search, (2005), http://www.sghms.ac.uk/depts/immunology/~dash/group/mtt.html.