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Synthesis and Antimicrobial Activities of Some Thiopyrimidine and Thiazolopyrimidine Derivatives From 1-(2-chloro-6-ethoxypyridin-4-yl)-3-(4-fluorophenyl) Prop-2-en-1-one

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Abstract: A series of substituted pyridine derivatives were prepared from 1-(2-chloro-6-ethoxypyridin-4-yl)-3-(4-fluorophenyl) prop-2-en-1-one 2, which was prepared from the reaction of acetylpyridine 1 with 4-flourobenzaldehyde. Acryloylpyridine 2 was treated with urea or guanidine hydrochloride in refluxing ethanolic potassium hydroxide to give the corresponding pyrimidinone and aminopyrimidine derivatives 3 and 4, respectively. Compound 2 was reacted with malononitrile or phenylhydrazine to afford the cyanoaminopyrane and *N*-phenylpyrazoline derivatives 5 and 6, respectively. Finally, cycloaddition reaction of acryloylpyridine 2 with thiourea yielded thioxopyrimidine 7, which was treated with 2-bromopropionic acid, 3-bromopropionic acid, or bromoacetic acid to yield methylthiazolo-, thiazino- and thiazolopyrimidines 8-10, respectively. Aryl methylene 11 was prepared by reacting of thiazolopyrimidine 10 with benzaldehyde or by reacting of thioxopyrimidine 7 with benzaldehyde and bromoacetic acid in one step. The antimicrobial screening showed that many of these obtained compounds have good activity against bactiria, fungi and yeast comparable to and as reference drugs.

Key words: 1-(2-Chloro-6-ethoxypyridin-4-yl)-3-(4-fluorophenyl) prop-2-en-1-one · Thiazolo-pyrimidine · Antimicrobial activity

INTRODUCTION

In previous work we have reported that certain substituted pyridines and their chiral macrocyclic derivatives have antimicrobial [1-5], anticancer [6, 7], analgesic and anticonvulsant [8, 9] activities. In addition, the biological and antiandrogenic activities of many heterocyclic compounds have been reviewed [10, 11]. On the other hand, thioxopyrimidine and thiazolopyrimidine derivatives have promising biological activities, e.g. anticancer properties [12-16] and androgenic anabolic activities [17]. Recently, some new thienopyrimidinone derivatives have been synthesized and tested for their analgesic, anticonvulsant, antiparkinsonian [18, 19] and antiinflammatory [20-23] agents. In view of these observations and in continuation of our previous work in heterocyclic chemistry, we synthesized some new thiopyrimidine, pyrane, pyrazoline and thiazolopyrimidine derivatives using 1-(2-chloro-6-ethoxypyridin-4-yl)-3-(4-fluorophenyl) prop-2-en-1-one starting material and tested their antimicrobial activities.

Experimental: All melting points were taken on Electrothermal IA 9000 series digital melting point apparatus. Elemental analytical data (in accord with the calculated values) were obtained from the microanalytical unit, Cairo University, Cairo, Egypt. The IR spectra (KBr,ν, cm^{-1}) were recorded on a Pye Unicam SP-1000 spectrophotometer. The ^1H NMR spectra (DMSO-d_6,δ, ppm) were recorded at 270 MHz on Varian EM-360 Spectrometer using *TMS* as an internal standard. The Central Services Laboratory, Cairo University, Egypt. The mass spectra were performed using VG 2AB-3F spectrometer. All reactions were followed by *TLC* (silica gel, aluminum sheets 60 F_{254}, Merck). Starting material 2 was prepared from acetylpyridine 1 according to published procedures [4, 24].

Synthesis of substituted pyrimidines 3 and 4: Diamino compounds, namely, urea and guanidine hydrochloride (1 mmole) were added to 2 (0.31 g, 1 mmol) in 100 ml ethanolic sodium hydroxide (1%). The reaction mixture was refluxed for 4-6 h. and then poured gradually with stirring onto cold water. The solid formed was filtered

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off, washed with H_2O and crystallized to give 3 and 4, respectively.

6-[(2-Chloro-6-ethoxypyridin-4-yl)]-1,2,3,4-tetrahydro-2-oxo-4-(4-flourophenyl)pyrimidine(3): Yield 68%, mp 246-8 °C (*Et*OH/H_2O); IR: 3345-3260 (NH), 1678 (C=O); ^{1}H NMR: 1.32 (t, 3H, CH_3), 3.86 (q, 2H, CH_2), 5.38 (d, 1H, H-a, pyrimidine), 6.95-7.24 (m, 5H, Ph-H + H-b pyrimidine), 8.10-8.22 (m, 2H, pyr-H), 8.32 and 8.44 (2s, 2H, 2NH-exchangeable with D_2O); MS (EI, 70 eV): *m/z* = 347 [M$^+$, 22], 252 (10), 224 (18), 196 (54), 156 (32), 110 (34) and at 77 [100, base peak]. Elemental analysis for $C_{17}H_{15}ClFN_3O_2$ (347.77): Calcd. C, 58.71; H, 4.35; Cl, 10.19; N, 12.08. Found: C, 58.65; H, 4.28; Cl, 10.14; N, 11.98.

2-Amino-6-[(2-chloro-6-ethoxypyridin-4-yl)-3,4-dihydro-4-(4-flourophenyl)pyrimidine: (4). Yield 70%, mp 198-200°C (*Ac*OH/H_2O); IR: 3465-3325 (NH, NH_2); ^{1}H NMR: 1.31 (t, 3H, CH_3), 3.80 (q, 2H, CH_2), 4.56 (s, NH_2 exchangeable with D_2O), 5.28 (d, H-a, pyrimidine), 6.98-7.26 (m, 5H, Ph-H + H-b pyrimidine), 8.14-8.28 (m, 2H, pyr-H), 8.52 (s, NH- exchangeable with D_2O); MS (EI, 70 eV): *m/z* = 346 [M$^+$, 8], 251 (10), 235 (24), 156 (45), 121 (32) and at 79 [100, base peak]. Elemental analysis for $C_{17}H_{16}ClFN_4O$ (346.79): Calcd. C, 58.88; H, 4.65; Cl, 10.22; N, 16.16. Found: C, 58.82; H, 4.58; Cl, 10.15; N, 16.16.

Synthesis of 2-amino-4-(4-flourophenyl)-6-[4-(2`-chloro-6`-ethoxypyridinyl)]-3-carbonitrile (5): A solution of 2 (0.31 g, 1 mmol) and malononitrile (0.06 g, 1 mmol) in 30 ml absolute ethanol in the presence of 2 ml pipredine was stirred at room temperature for 3 h. The solvent was concentrated under reduced pressure; the formed product was collected by filtration, washed with water, dried and crystallized to give 5. Yield 75%, mp 124-6 °C (*Et*OH); IR: 3385-3340 (NH_2), 2218 (C≡N); ^{1}H NMR: 1.30 (t, 3H, CH_3), 3.74 (q, 2H, CH_2), 4.48 (d, 1H, H-a pyrane), 4.76 (s, 2H, NH_2 exchangeable with D_2O), 6.98-7.28 (m, 5H, Ph-H + H-b pyrane), 8.14-8.28 (m, 2H, pyr-H); MS (EI, 70 eV): *m/z* = 372 [Mþ$^+$+1, 22], 355 (6), 329 (8), 284 (26), 189 (34), 165 (45), 130 (65) and at 76 [100, base peak]. Elemental analysis for $C_{19}H_{15}ClFN_3O_2$ (371.79): Calcd. C, 61.38; H, 4.07; Cl, 9.54; N, 11.30. Found: C, 61.30; H, 3.98; Cl, 9.48; N, 11.25.

Synthesis of 2-phenyl-3-(4-flourophenyl)-3, 4-dihydro-5-[(2-chloro-6-ethoxypyridin-4-yl) pyrazoline (6): A mixture of 2 (0.31 g, 1 mmol) and phenyl hydrazine (1.6 g, 1.5 mmol) in 15 ml glacial acetic acid was heated under reflux for 5 h. The reaction mixture was poured into ice; the obtained solid was filtered off, washed with water, dried under pressure and crystallized to give compound 6. Yield 58%, mp 234-6°C (*Me*OH/H_2O); IR: 1675 (C=N), 1618 (C=C); ^{1}H NMR: 1.34 (t, 3H, CH_3), 1.86-2.10 (m, 2H, CH_2-pyrazoline), 3.75 (q, 2H, CH_2), 3.86-3.88 (m, 1H, CH-pyrazoline), 6.98-7.36 (m, 9H, 2 Ph-H), 8.12-8.24 (m, 2H, pyr-H); MS (EI, 70 eV): *m/z* = 397 [M$^+$+2, 8], 318 (22), 273 (15), 254 (16), 219 (12) and at 143 [100, base peak]. Elemental analysis for $C_{22}H_{19}ClFN_3O$ (395.86): Calcd. C, 66.75; H, 4.84; Cl, 8.96; N, 10.61. Found: C, 66.68; H, 4.80; Cl, 8.88; N, 10.54.

Synthesis of 6-[(2-chloro-6-ethoxypyridin-4-yl)-1,2,3,4-tetrahydro-2-thioxo-4-(4-flourophenyl)-pyrimidine (7): Dry hydrogen chloride gas was passed through a mixture of 2 (0.31 g, 1 mmol) and thiourea (0.076 g, 1 mmol) in 25 ml absolute ethanol at room temperature for 6 h. The reaction mixture was poured gradually with stirring onto cold water. The solid formed was filtered off, washed with water, dried under pressure and crystallized to give thioxopyrimidine 7. Yield 85%, mp. 265-7°C (*Ac*OH/H_2O); IR: 3386-3298 (NH), 1222 (C=S); ^{1}H NMR: 1.31 (t, 3H, CH_3), 3.84 (q, 2H, CH_2), 5.32 (d, 1H, H-a, pyrimidine), 6.97-7.18 (m, 5H, Ph-H + H-b pyrimidine), 8.06-8.24 (m, 2H, pyr-H), 8.28 and 8.54 (2s, 2H, 2NH exchangeable with D_2O); MS (EI, 70 eV): *m/z* = 363 [M$^+$, 16], 318 (22), 283 (8), 209 (64) and at 101 [100, base peak]. Elemental analysis for $C_{17}H_{15}ClFN_3OS$ (363.84): Calcd. C, 56.12; H, 4.16; Cl, 9.74; N, 11.55; S, 8.81. Found: C, 56.05; H, 4.06; Cl, 9.68; N, 11.48; S, 8.76.

Synthesis of methylthiazolo-, thiazino- and thiazolopyrimidines 8-11: A mixture of 7 (0.36 g, 1 mmol) and halo-compounds, namely, 2-bromopropionic acid, 3-bromopropionic acid and bromoacetic acid (1 mmol) was dissolved in 40 ml of a mixture of *Ac*OH/Ac_2O (1:3) in the presence 3 g anhydrous sodium acetate was refluxed for 6-7 hr. The reaction mixture was cooled and poured onto cold water with stirring; the solid formed was filtered off and crystallized to give the corresponding title compounds 8-11, respectively.

7-[(2-Chloro-6-ethoxypyridin-4-yl)-5-(4-flourophenyl)-2,3-dihydro-5H-3-methylthiazolo[3,2-a]-pyrimidine (8): Yield 74%, mp. 82-4°C (*Ac*OH/H_2O); IR: 1718 (C=O); ^{1}H NMR: 1.34 (t, 3H, CH_3), 1.36 (d, 3H, CH_3), 3.55 (m, 1H, CH-thiazole), 3.79 (q, 2H, CH_2), 5.55 (d, 1H, H-a pyrimidine),

6.92-7.28 (m, 5H, Ph-H + H-b pyrimidine), 8.05-8.23 (m, 2H, pyr-H); MS (EI, 70 eV): *m/z* = 417 [M$^+$, 4], 402 (24), 357 (14), 329 (10), 284 (55), 173 (48) and at 78 [100, base peak]. Elemental analysis for $C_{20}H_{17}ClFN_3O_2S$ (417.88): Calcd. C, 57.48; H, 4.10; Cl, 8.48; N, 10.06; S, 7.67. Found: C, 57.40; H, 3.98; Cl, 8.42; N, 9.96; S, 7.60.

2-Chloro-6-ethoxy-4-[6-(4-flourophenyl)-2,3-dihydro-6H-thiazino[3,2-a]pyrimidin-4-one-8-yl)]pyridine (9): Yield 82%, mp. 165-7°C (*Ac*OH/H_2O); IR: 1718 (C=O); ^{1}H NMR: 1.29 (t, 3H, CH_3), 3.38-3.56 (m, 4H, 2CH_2-thiazine ring), 3.78 (q, 2H, CH_2), 5.34 (d, 1H, H-a pyrimidine), 6.88-7.26 (m, 5H, Ph-H + H-b pyrimidine), 8.12-8.26 (m, 2H, pyr-H); MS (EI, 70 eV): *m/z* = 416 [M$^+$-1, 8], 372 (12), 344 (24), 284 (55), 173 (25), 154 (13) and at 114 [100, base peak]. Elemental analysis for $C_{20}H_{17}ClFN_3O_2S$ (417.88): Calcd. C, 57.48; H, 4.10; Cl, 8.48; N, 10.06; S, 7.67. Found: C, 57.39; H, 4.01; Cl, 8.40; N, 9.97; S, 7.60.

7-[(2-Chloro-6-ethoxypyridin-4-yl)-5-(4-flourophenyl)-2,3-dihydro-5H-thiazolo[3,2-a]pyrimidine (10): Yield 65%, mp. 212-4°C (*Ac*OH/H_2O); IR: 1712 (C=O); ^{1}H NMR: 1.30 (t, 3H, CH_3), 3.72 (s, 2H, CH_2-thiazole), 3.78 (q, 2H, CH_2), 5.36 (d, 1H, H-a pyrimidine), 6.92-7.28 (m, 5H, Ph-H + H-b pyrimidine), 8.08-8.24 (m, 2H, pyr-H); MS (EI, 70 eV): *m/z* = 405 [M$^+$+2, 15], 358 (18), 330 (23), 284 (54), 189 (100, base peak), 111 (63), 95 (87). Elemental analysis for $C_{19}H_{15}ClFN_3O_2S$ (403.86): Calcd. C, 56.51; H, 3.74; Cl, 8.78; N, 10.40; S, 7.94. Found: C, 56.42; H, 3.68; Cl, 8.70; N, 10.32; S, 7.87.

7-[(2-Chloro-6-ethoxypyridin-4-yl)-2-(phenylmethylene)-5-(4-flourophenyl)-2,3-dihydro-5-thiazolo[3,2-a]pyrimidine (11): *Method A:* A mixture of 7 (0.36 g, 1 mmol), bromoacetic acid (0.138 g, 1 mmol), anhydrous sodium acetate (1.5 g) in a mixture of *Ac*OH/Ac_2O (40 ml, 1:3) and benzaldehye (0.106 g, 1 mmol) was refluxed for 6 h. The reaction mixture was cooled and poured onto ice-water; the obtained solid was collected by filtration and crystallized to give the corresponding arylmethylene 11. Yield 82%, mp. 232-4°C (*Ac*OH/H_2O); IR: 3375-3324 (NH), 1715 (C=O); ^{1}H NMR: 1.28 (t, 3H, CH_3), 3.82 (q, 2H, CH_2), 5.40 (d, 1H, H-a pyrimidine), 6.65-7.54 (m, 11H, 2 Ph-H + H-b pyrimidine + benzylic-H), 8.08-8.22 (m, 2H, pyr-H); MS (EI, 70 eV): *m/z* = 491 [M$^+$, 100, base peak], 414 (65), 361 (45), 316 (23), 244 (16), 133 (48), 111 (35). Elemental analysis for $C_{26}H_{19}ClFN_3O_2S$ (491.96): Calcd. C, 63.48; H, 3.89; Cl, 7.21; N, 8.54; S, 6.52. Found: C, 63.40; H, 3.82; Cl, 7.16; N, 8.45; S, 6.46.

Method B: A mixture of 10 (g, 1 mmol) and benzaldehyde (0.106 g, 1 mmol) in a mixture of *Ac*OH/Ac_2O (40 ml, 1:3) was refluxed for 5 h, allowed to cool, then poured onto water the solid formed was collected by filtration and crystallized from AcOH/H_2O to yield compound 11 in 74% yield as identified by its m.p.; mixed m.p. and R_f value on TLC by comparison with authentic sample from method A.

Antimicrobial Screening

***Microor Ganisms Species*:**

- Bacteria
  - Gram a negative bacteria, *Eschericha coli. Salmonella typhi*
  - Gram-positive bacteria, *Bacillus subtitles, Staphylococcus aureus* and *Streptococcus*
- Fungi: *Aspergillus Niger*
- Yeast: *Candida albicans,* Sacchromyces

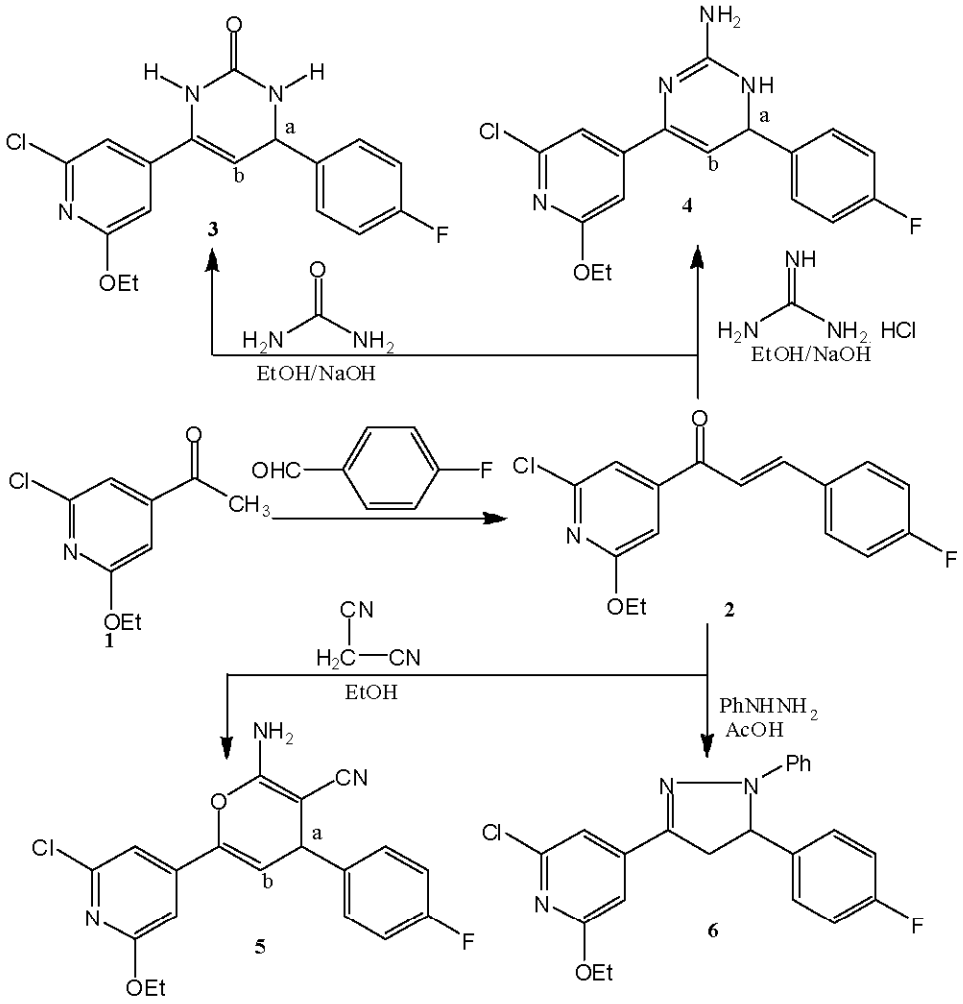
Medium: The cap-assay method containing (g/l): peptone 6.0, yeast extract 3.0, meat extract 1.5, glucose 1.0 and agar 20.0 were used. The medium was sterilized and divided while hot (50-60°C) in 15 ml. Portions among sterile petri-diches of 9 cm diameter.

One ml of the spore suspension of each microorganism was spread all over the surface of the cold solid medium placed in the petri-dish.

RESULTS AND DISCUSSION

In our previous work we reported the synthesis and a preliminary biological activity screening of several pyridine derivatives based 4-β-(4-flourophenyl) acryloylpyridine (2), which was prepared as starting material from the corresponding 2-chloro-6-ethoxy-4-acetylpyridine (1) according to literature methods [24]. Treatment of 2 with diamines, namely, urea, or guanidine hydrochloride in refluxing ethanolic potassium hydroxide to afford the 2-carbonyl- 3 and 2-aminopyrimidines 4, respectively. Compound 2 was reacted with malononitrile in refluxing ethanol with two drops of pipredine gave the cyanoaminopyrane 5 and also treated with phenyl hydrazine in refluxing glacial acetic acid to give *N*-phenylpyrazoline 6 (Scheme 1).

Also, compound 2 was condensed with thiourea in ethanol and dry HCl gas to give thioxopyrimidine 7, which was condensed with 2-propionic acid, 3-propionic acid, or chloroacetic acid in a mixture of acetic acid/acetic anhydride in the presence of anhydrous sodium acetate

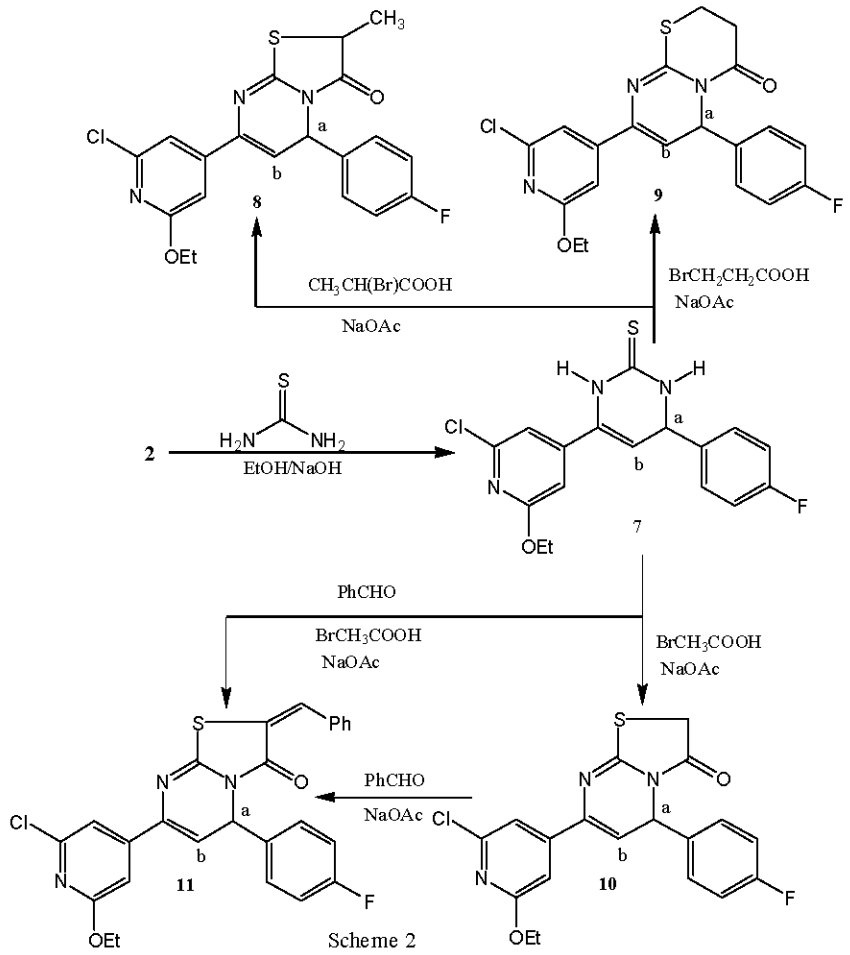


Scheme 1

Scheme 1:

to yield the corresponding methylthiazolo-, thiazino- and thiazolopyrimidines 8-10, respectively. Compound 10 contain an active methylene group. As such it condensed with benzaldehyde in the presence of anhydrous sodium acetate and glacial acetic acid/acetic anhydride mixture to yield the arylmethylene 11. However, the latter was also prepared directly from 7 by the action of chloroacetic acid, benzaldehyde and anhydrous sodium acetate in the presence of acetic acid/acetic anhydride mixture (Scheme 2).

Antimicrobial Screening: Some of the synthesized compounds 4, 5, 7, 9 and 11 were evaluated for its antimicrobial activity against five bacterial strains *E. Coli, S. typhi, Bacillus subtilis, S. aureus and Staphylococcus aureus* and one fungal strain *Aspergillus niger* and also two strains of yeast *Candida albicans* and Sacchro-myces at 50-6 μg cm^{-1} concentration, according to modified Kirby-Bauer's disk diffusion method [25]. MIC values of tested compounds were determined by tube dilution technique. The solvents DMSO/DMF were used as negative controls and Streptomycin®, Erythromycin® and Fusidic acid were used as standards. Calculated average diameters (for triplicate sets) of the zones of inhibition (in mm) for test samples were compared with that produced by the standard drugs. Almost, all the tested compounds were found to exhibit antimicrobial activities. Analysis of antimicrobial data suggested that compounds 5 and 7 possessed higher significant antibacterial and antifungal activities than some known standard drugs. The results of antimicrobial screening were recorded as average diameter of inhibition zone in mm and summarized in Table 1.



Scheme 2

Scheme 2:

Table 1: Antimicrobial activities of some new synthesized compounds

	Inhibition Zone (mm)							
	Microorganism							
	Bacteria							
	Gram-negative		Gram-positive			Fungi	Yeast	
Tested Compounds and positive drugs	*E.coli*	*S.typhi*	*B.subtitls*	*S. aureus*	*Streptococcus*	*A.niger*	*C.albicans*	*Sacchro-myces*
Control (DMSO)	-	-	-	-	-	-	-	-
Streptomycin	35	38	37	39	38	-	-	-
Erythromycin	18	-	36	22	24	-	-	-
Fusidic Acid	-	-	-	-	-	37	38	36
4	-	25	14	-	-	-	23	-
5	34	37	38	25	22	16	36	28
7	36	25	35	38	26	18	39	37
9	-	-	15	-	26	14	15	17
11	18	25	-	16	-	13	24	27

Highly sensitive (inhibition zone = 25-40 mm). Fairly sensitive (inhibition zone = 16-24 mm).

Slightly sensitive (inhibition zone = 10-15 mm). No sensitive (inhibition zone ≤ 10 mm).

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REFERENCES

1. Amr, A.E., A.M. Mohamed and A.A. Ibrahim, 2003. Z. Naturforsch. 58b: 861.
2. Amr, A.E., M.H. Abo-Ghalia and M.M. Abdalla, 2006. Z. Naturforsch., 61b: 1335.
3. Amr, A.E., O.I. Abdel-Salam, A. Attia and I. Stibor, 1999. Collect. Czech. Chem. Commun. 64: 288.
4. Amr, A.E., 2000. Ind. J. Heterocyc. Chem., 10: 49.
5. Attia, A., O.I. Abdel-Salam and A.E. Amr, 1997. Egypt. J. Chem., 40: 317.
6. Abo-Ghalia, M.H. and A.E. Amr, 2004. Amino Acids, 26: 283.
7. Amr, A.E., M.M. Ashraf, F.M. Salwa, A.A. Naglaa and A.G. Hammam, 2006. Bioorg. Med. Chem., 14: 5481.
8. Amr, A.E., 2005. Z. Naturforsch. 60b: 990.
9. Nehad, A.A., A.E. Amr and A.I. Alhusien, 2007. Monatsh. Chem., 138: 559.
10. Amr, A.E., N.A. Abdel-Latif and M.M. Abdalla, 2006. Acta Pharm., 56: 203.
11. Amr, A.E., N.A. Abdel-Latif and M.M. Abdalla, 2006. Bioorg. Med. Chem., 14: 273.
12. Hammam, A.G., A.F. Fahmy, A.E. Amr and A.M. Mohamed, 2003. Ind. J. Chem. Sec. B. 42B: 1985.
13. Hammam, A.G., A.A. Naglaa, M.H. Wanda and M. Marian, 2000. Z. Naturforsch., 55b: 417.
14. Ali, M.I. and A.G. Hammam, 1981. J. Chem. Eng. Data, 26: 352.
15. Hammam, A.G., A.S. Mohie and A.A. Naglaa, 2001. Ind. J. Chem. Sec., B. 40B: 213.
16. Hammam, A.G., S.M. Hussain and I.R. Kotob, 1990. Phosphorus Sulfur and Silicon, 47: 47.
17. Amr, A.E. and M.M. Abdulla, 2002. Ind. J. Heterocycl. Chem., 12: 129.
18. Amr, A.E., M.I. Hegab, A.A. Ibrahim, M.M. Abdulla, 2003. Monatsh. Chem., 134: 1395.
19. Amr, A.E., H.H. Sayed and M.M. Abdulla, 2005. Arch. Pharm. Chem. Life Sci., 338: 433.
20. Abo-Ghalia, M.H., A.E. Amr and M.M. Abdulla, 2003. Z. Naturforsch., 58b: 903.
21. Amr, A.E. and M.M. Abdalla, 2006. Bioorg. Med. Chem., 14: 4341.
22. Amr, A.E. and M.M. Abdalla, 2006. Arch. Pharm. Chem. Life Sci., 339: 88.
23. Amr, A.E., M.S. Nermien and M.M. Abdulla, 2007. Monatsh. Chem., 138: 699.
24. Amr, A.E., S.S. Maigali and M.M. Abdulla, 2008. Monatsh. Chem., 139: 1409.
25. Bauer, A.W., M.M. Kirby, J.C. Sherris and M. Turck, 1996. Am. J. Clin. Pathol., 45: 493.