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Original research paper

Antimicrobial activity of some synthesized glucopyranosyl-pyrimidine carbonitrile and fused pyrimidine systems

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3-Amino-5-(4-chlorophenylamino)-4-cyanofuran-2-carboxamide (**2**) was used as the key molecule for preparation of various furopyrimidines **3**-**9** and formation of spiro-cycloalkane furopyrimidines **10**, **11**. Also, poly fused heterocyclic compounds **13**-**17** were prepared from compound **2**. The synthesized compounds were screened for their antimicrobial activity.

Keywords: pyrimidine, glucopyranosyl-pyrimidine, oxazine, antimicrobial activity

This research is an extension of our studies on pyrimidine and pyrimidinethione derivatives as synthons to prepare fused heterocyclic compounds (1, 2). Some of the pyrimidine and fused heterocyclic pyrimidine derivatives have proved to be active antiviral, antitumor, analgesic and antimicrobial agents (3–6). In addition, pyrimidothiazine, thiazolopyrimidine and oxazolidinone derivatives are of great antimicrobial activity (7). Recently, we have found that certain substituted pyrimidines and their heterocyclic derivatives show antimicrobial and antiinflammatory (8, 9) as well as antitumor activities (10, 11). On the other hand, thioxopyrimidine and thiazolopyrimidine derivatives have anticancer activities (12, 13). In view of these observations and in continuation of our previous work in heterocyclic chemistry, we have synthesized some new pyrimidine and thiazolopyrimidine derivatives and tested their antimicrobial activity.

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EXPERIMENTAL

All melting points are uncorrected and were measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Perkin-Elmer, USA). ^{1}H NMR was recorded on a Jeol-Ex-270 NMR spectrometer (Jeol, Japan) and chemical shifts were expressed as part per million (ppm δ values) against TMS as the internal reference standard. Mass spectra were recorded on a VG 2AM-3F mass spectrometer (Thermo Electron, USA). Microanalyses were operated using a Yanaco CHN Corder Elemental Analyzer (Japan) and the results were within the accepted range (± 0.2 %) of calculated values. Follow-up of the reactions and purity checking of the compounds were done by TLC on silica gel-coated aluminum sheets (Type 60 F254, Merck, Germany).

Syntheses

2-[3-(4-Chlorophenyl)-4-oxooxazolidin-2-ylidene]malononitrile (**1**). – To a stirred mixture of malononitrile (0.66 g, 0.01 mol), *p*-chlorophenylisocyanate (1.53 g, 0.01 mol) in distilled water (20 mL) containing KOH (1 g) at room temperature, ethyl chloroacetate (1.22 g, 0.01 mol) in ethanol (25 mL) was added after 1h. The reaction mixture was heated under reflux for 2 h, the obtained solid was collected by filtration and crystallized from methanol to give compound **1** as white crystals.

3-Amino-5-(4-chlorophenylamino)-4-cyanofuran-2-carboxamide (**2**). – A solution of compound **1** (2.56 g, 0.01 mol) in ammonia solution (25 %, 30 mL) was stirred at room temperature for 6 h. The solid formed was filtered off, dried and crystallized from chloroform to give compound **2** as a brown powder.

*6-(4-Chlorophenylamino)-4-oxo-3,4-dihydrofuro[3,2-*d*]pyrimidin-7-carboxamide* (**3**). – A mixture of compound **2** (2.77 g, 0.01 mol) and formic acid (20 mL) was refluxed for 6 h. The reaction mixture was concentrated under reduced pressure, the obtained precipitate was collected by filtration, dried and crystallized from dioxane to give compound **3** as yellow crystals.

*6-(4-Chlorophenylamino)-4-oxo-3-phenyl-2-thioxo-1,2,3,4-tetrahydro-furo[3,2-*d*]pyrimidine-7-carbonitrile* (**4**). – A mixture of compound **2** (2.77 g, 0.01 mol) and phenylisothiocyanate (1.35 g, 0.01 mol) in glacial acetic acid (25 mL) was refluxed for 6 h. The reaction mixture was allowed to cool; the formed solid was filtered off, dried and crystallized from ethyl acetate to give **4** as a light brown powder.

*6-(4-Chlorophenylamino)-2,4-dioxo-1,2,3,4-tetrahydro-furo[3,2-*d*]pyrimidine-7-carbonitrile* (**5**). – A mixture of compound **2** (2.77 g, 0.01 mol) and ethylchloroformate (1.08 g, 0.01 mol) in acetic acid (15 mL) was refluxed for 10 h. The reaction mixture was allowed to cool, the solid product was filtered off and crystallized from DMSO to give the corresponding compound **5** as a brown powder.

*1-(3-Chloro-2-oxopropyl)-6-(4-chlorophenylamino)-4-oxo-3-phenyl-2-thioxo-1,2,3,4-tetrahydro-furo[3,2-*d*]pyrimidine-7-carbonitrile* (**6**) and *6-(4-chlorophenylamino)-1-(oxiran-2-yl-methyl)-4-oxo-3-phenyl-2-thioxo-1,2,3,4-tetrahydro-furo[3,2-*d*]pyrimidine-7-carbonitrile* (**7**). – A mixture of compound **4** (3.95 g, 0.01 mol) and sodium hydride (0.24 g, 0.01 mol) in dry

dioxane (20 mL) was stirred at 60 °C for 3 h. The reaction mixture was cooled at room temperature, then dichloroacetone or epichlorohydrine (0.01 mol) was added under stirring. Stirring was continued for 5 h at room temperature. The reaction mixture was evaporated under reduced pressure; the residue was washed with distilled water, filtered off and crystallized from methanol to give compound **6** or **7**.

6-(4-Chlorophenylamino)-4-oxo-3-phenyl-2-(2′,3′,4′,6′-tetra-O-*acetyl-1′-thio-β-D-glucopyranosyl)-1,2,3,4-tetrahydrofuro[3,2-*d*]pyrimidine-7-carbonitrile* (**8**). – To a solution of **2** (2.77 g, 0.01 mol) in aqueous potassium hydroxide (0.56 g, 0.01 mol) in distilled water (5 mL), a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (4.11 g, 0.011 mol) in acetone (30 mL) was added. The reaction mixture was stirred at room temperature for 14 h (under TLC control). The solvent was evaporated under reduced pressure at 40 °C and the crude product was filtered off and washed with distilled water to remove KBr formed. The product was dried, and crystallized from diethyl ether as a brown powder.

*6-(4-Chlorophenylamino)-4-oxo-2-phenyl-1,2,3,4-tetrahydrofuro[3,2-*d*]pyrimidine-7-carbonitrile* (**9**). – A mixture of compound **2** (2.77 g, 0.01 mol) and benzaldehyde (1.06 g, 0.01 mol) in glacial acetic acid (20 mL) was refluxed for 4 h. The reaction mixture was evaporated under reduced pressure and the obtained residue was triturated with diethyl ether. The formed solid was collected by filtration, dried and crystallized from dioxane to give compound **9** as yellow crystals.

*6-(4-Chlorophenylamino)-7-cyano-1,3,4-trihydrospirocyclopentane(1′,2)-furo[3,2-*d*]pyrimidin-4-one* (**10**) and *6-(4-chlorophenylamino)-7-cyano-1,3,4-trihydrospirocyclohexane(1,2)furo[3,2--*d*]pyrimidin-4-one* (**11**). – A mixture of compound **2** (2.77 g, 0.01 mol), cyclopentanone or cyclohexanone (0.01 mol) and sodium acetate (2 g) in glacial acetic acid (20 mL) was refluxed for 6 h. The reaction mixture was cooled, then poured into ice-water. The precipitated solid was filtered off, washed with water, dried and crystallized from ethanol to give compound **10** or **11**.

*6-(4-Chlorophenyl)-6-(7-cyano-2-methyl-4-oxo-4*H*-furo[3,2-*d*][1,3]oxazin-6-yl)acetamide* (**12**). – A solution of compound **2** (2.77 g, 0.01 mol) in acetic anhydride (20 mL) was refluxed for 8 h. The reaction mixture was concentrated under reduced pressure. The precipitated solid was collected by filtration, dried and crystallized from ethanol to give compound **12** as orange crystals.

*1-(4-Chlorophenyl)-4-imino-2,6-dithioxo-1,4,6,7-tetrahydropyrimido[5,4-*b*]furo[5,4-*d*]-1,3-thiazin-8-one* (**13**). – A mixture of compound **2** (2.77 g, 0.01 mol), carbon disulfide (1.56 g, 0.02 mol) and dry pyridine (20 mL) was refluxed for 20 h. The reaction mixture was cooled then poured into ice-water. The precipitated solid was filtered off, washed with water, dried and crystallized from DMF.

*1-(4-Chlorophenyl)-2,3-dithioxo-8-oxo-2,2′,10,11-tetrahydrothiazolo[1,3-*b*]pyrimido[1,3-*el*]-furo-[5,4-*e*]pyrimidine* (**14**) and *1-(4-chlorophenyl)-2,3-dithioxo-8-oxo-2,2′,3,3′,11,12-hexahydro-thiazino[1,3-*b*]-pyrimido[1,3-*e*]furo[5,4-*e*]pyrimidine* (**15**). – A mixture of compound **13** (3.95 g, 0.01 mol), chloroacetic acid or chloropropionic acid (0.01 mol) and anhydrous sodium acetate (2 g) was refluxed in glacial acetic acid/acetic anhydride (40 mL, 3:1) for 3 h. The reaction mixture was cooled and poured into water; the obtained precipitate was filtered off and crystallized from methanol to give compound **14** or **15**.

7-[Phenylmethylene]-1-(4-chlorophenyl)-2,3-dithioxo-8-oxo-2,2′,10,11-tetrahydrothiazolo[1,3-b]-pyrimido[1,3-e]furo-[5,4-e]pyrimidine (**16**) and *8-[phenylmethylene]-1-(4-chlorophenyl)-2,3-dithioxo-8-oxo-2,2′,3,3′,11,12-hexahydrothiazino[1,3-b]-pyrimido[1,3-e]furo[5,4-e]pyrimidine* (**17**). – A mixture of compound **14** or **15** (0.01 mol), benzaldehyde (1.06 g, 0.01 mol) and anhydrous sodium acetate (2 g) in glacial acetic acid/acetic anhydride (40 mL, 3:1) was

Table I. Physical and analytical data of new compounds

Compd. No.	Formula (M_r)	M. p. (°C)	Yield (%)	Analysis (%) (calcd./found)			
				C	H	N	Cl
1	$C_{12}H_6ClN_3O_2$ (259.65)	222–224	80	55.51 55.42	2.33 2.30	16.18 16.13	13.65 13.62
2	$C_{12}H_9ClN_4O_2$ (276.68)	253–254	75	52.09 52.00	3.28 3.26	20.25 20.18	12.81 12.77
3	$C_{13}H_9ClN_4O_3$ (304.69)	277–278	70	51.25 51.30	2.98 3.00	18.39 18.45	11.64 11.60
4	$C_{19}H_{11}ClN_4O_2S$ (394.83)	289–290	78	57.80 57.72	2.81 2.79	14.19 14.12	8.98 8.90
5	$C_{13}H_7ClN_4O_3$ (302.67)	282–283	68	51.59 51.55	2.33 2.31	18.51 18.60	11.71 11.55
6	$C_{22}H_{14}Cl_2N_4O_3S$ (485.34)	256–258	60	54.44 54.40	2.91 2.90	11.54 11.60	14.61 14.63
7	$C_{22}H_{15}ClN_4O_3S$ (450.90)	236–238	55	58.60 58.55	3.35 3.33	12.43 12.40	7.86 7.80
8	$C_{33}H_{29}ClN_4O_{11}S$ (725.12)	258–257	70	54.66 54.65	4.03 4.01	7.73 7.80	4.89 4.88
9	$C_{19}H_{13}ClN_4O_2$ (364.79)	297–299	73	62.56 62.60	3.59 3.60	15.36 15.30	9.72 9.65
10	$C_{17}H_{15}ClN_4O_2$ (342.78)	284–285	70	59.57 59.55	4.41 4.40	16.34 16.33	10.34 10.29
11	$C_{18}H_{17}ClN_4O_2$ (356.81)	303–304	80	60.59 60.63	4.80 4.82	15.70 15.58	9.94 10.01
12	$C_{16}H_{10}ClN_3O_4$ (343.72)	267–268	60	55.91 55.70	2.93 2.96	12.23 12.50	10.31 10.28
13	$C_{14}H_7ClN_4O_2S_3$ (394.88)	213–214	65	42.58 42.60	1.79 1.80	14.19 14.15	8.98 8.90
14	$C_{16}H_7ClN_4O_3S_3$ (434.90)	208–210	53	44.19 44.15	1.62 1.61	12.88 12.90	8.15 8.10
15	$C_{17}H_9ClN_4O_3S_3$ (448.93)	226–228	55	45.48 45.52	2.02 2.00	12.48 12.53	7.90 7.87
16	$C_{23}H_{11}ClN_4O_3S_3$ (523.01)	278–280	78	52.82 52.80	2.12 2.14	10.71 10.80	6.78 6.77
17	$C_{24}H_{13}ClN_4O_3S_3$ (537.03)	293–295	80	53.68 53.72	2.44 2.47	10.43 10.50	6.60 6.62

refluxed for 3 h. The reaction mixture was allowed to cool and poured into water. The solid substance was filtered off and crystallized from acetic acid/water to give aryl methylene derivative **16** or **17**.

Physico-chemical and spectral data of the synthesized compounds are given in Tables I and II.

Antimicrobial activity

The antibacterial activity of synthesized compounds was tested against *Escherichia coli* NRRL B-210 (Gram-negative bacterium), *Bacillus subtilis* NRRL B-543 and *Staphylococcus aureus* NRRL B-313 (Gram-positive bacteria) using a nutrient agar medium. The

Table II. Spectral data of the new compounds

Compd. No.	IR (ν_{max}, cm^{-1})	^1H NMR (δ, ppm)[a]	MS (*m/z*, %)
1	2221, 2225 (2CN), 1685 (C=O)	4.9 (s, 2H, oxazolidine), 7.15 (d, 2H, *J* = 5.8 Hz, Ar-H), 7.62 (d, 2H, *J* = 5.9 Hz, Ar-H)	259 [M^+] (100), 261 [M^++2] (31), 224(14), 195 (11), 148 (8), 111 (19)
2	3350, 3340, 3110 ($2NH_2$, NH), 2217 (CN), 1686 (C=O)	6.50 (s, 2H, NH_2, D_2O exchangeable), 7.2 (d, 2H, *J* = 5.8 Hz, Ar-H), 7.6 (d, 2H, *J* = 5.8 Hz, Ar-H), 7.8 (s, 2H, NH_2 amide, D_2O exchangeable), 8.0 (s, 1H, NH, D_2O exchangeable)	276 [M^+] (80), 278 [M^++2] (26), 241 (5), 232 (52), 165 (10), 150 (25), 126 (19), 111 (4)
3	3242, 3160 (NH_2, 2NH), 1682, 1702 (2C=O)	6.5 (d, 2H, *J* = 5.3 Hz, Ar-H), 7.0 (d, 2H, *J* = 5.4 Hz, Ar-H), 7.57 (br, 2H, NH_2, D_2O exhangeable), 7.91 (s, 1H, pyrimidine proton), 8.1, 10.8 (2s, 2H, 2NH, D_2O exchangeable)	304 [M^+] (70), 306 [M^++2] (20) 260 (19), 193 (8), 178 (28), 126 (11), 111 (16)
4	3214, 3186 (2NH), 2222 (CN), 1695 (C=O), 1256 (C=S)	6.40 (d, 2H, *J* = 5.8 Hz, Ar-H), 6.8 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H) 7.7 (d, 2H, *J* = 5.7 Hz, Ar-H), 8.0, 10.6 (2s, 2H, 2NH, D_2O exchangeable)	394 [M^+] (60), 396 [M^++2] (20), 283 (23), 268 (14), 126 (10), 111 (24)
5	3240, 3217, 3153 (3NH), 2219 (CN), 1690, 1705 (2C=O)	6.63 (d, 2H, *J* = 5.5 Hz, Ar-H), 7.2 (d, 2H, *J* = 5.5 Hz, Ar-H), 7.8, 10.3, 11.0 (3s, 3H, 3NH, exchangeable)	302 [M^+] (49), 304 [M^++2] (14), 191 (10), 176 (5), 126 (8), 111 (15)
6	3115 (NH), 2220 (CN), 1698 (C=O), 1237 (C=S)	4.50 (s, 2H, CH_2), 4.63 (s, 2H, CH_2), 6.5 (d, 2H, *J* = 5.8 Hz, Ar-H), 6.75 (m, 2H, Ar-H), 7.21 (m, 3H, Ar-H), 7.6 (d, 2H, *J* = 5.8 Hz, Ar-H), 7.9 (s, 1H, NH, D_2O exchangeable)	484 [M^+] (90), 486 [M^++2] (27), 393 (30), 358 (55), 373 (9), 358 (12), 126 (23), 111 (14)
7	3122 (NH), 2221 (CN), 1691 (C=O), 1244 (C=S)	2.50 (m, 2H, CH_2-oxirnyl ring), 2.80 (m, 1H, CH-oxirnyl ring), 3.58 (d, 2H, *J* = 3.7 Hz, CH_2), 6.4 (d, 2H, *J* = 5.7 Hz, Ar-H), 6.64 (m, 2H, Ar-H), 7.01 (m, 3H, Ar-H), 7.7 (d, 2H, *J* = 5.6 Hz, Ar-H), 7.91 (s, 1H, NH, D_2O exchangeable)	450 [M^+] (27), 439 (45), 424 (10), 393 (9), 226 (25), 211 (24)

8	3192 (NH), 2211 (CN), 1718–1667 (5C=O)	2.04–2.20 (4s, 12H, 4COCH_3), 3.75 (m, 2H, H-6′, H-6″), 3.81 (m, 1H, H-5′), 3.98 (m, 2H, H-4′, H-3′), 5.49 (m, 1H, H-2′), 5.70 (d, 1H, $J_{1'\text{-}2'}$ = 9.8 Hz, H-1′), 6.58 (d, 2H, *J* = *5.8* Hz, Ar-H), 6.71 (m, 2H, Ar-H), 6.98 (m, 3H, Ar-H), 7.65 (d, 2H, *J* = *5.9* Hz, Ar-H), 8.1 (s, 1H, NH, D_2O exchangeable)	725 [M^+] (68), 727 [M^++2] (18) 598 (23), 393 (14), 331 (5), 163 (41), 126 (25), 111 (11)
9	3275, 3213, 3127 (3NH), 2218 (CN), 1689 (C=O)	5.79 (s, 1H, pyrimidine proton), 6.59 (d, 2H, *J* = *5.6* Hz, Ar-H), 6.84 (m, 2H, Ar-H), 7.10 (m, 3H, Ar-H), 7.45(d, 2H, *J* = *5.5* Hz, Ar-H), 7.9, 9.2, 10.3 (3s, 3H, 3NH, D_2O exchangeable)	364 [M^+] (38), 366 [M^++2] (9) 287 (25), 253 (55) 238 (9), 126 (46), 111 (23)
10	3280, 3219, 3121 (3NH), 2219 (CN) 1695 (C=O)	1.50–1.96 (m, 8H, aliphatic), 6.89 (d, 2H, *J* = *5.9* Hz, Ar-H), 7.2 (d, 2H, *J* = *5.9* Hz, Ar-H), 7.93, 9.3, 10.4 (3s, 3H, 3NH, pyrimidine, D_2O exchangeable)	342 [M^+] (20) 344 [M^++2] (7), 286 (10), 231 (14), 216 (8), 126 (10), 111 (25)
11	3277, 3217, 3119 (3NH), 2215 (CN) 1679 (C=O)	1.30–1.74 (m, 10H, aliphatic), 6.66 (d, 2H, *J* = *5.4* Hz, Ar-H), 7.31 (d, 2H, *J* = *5.5* Hz, Ar-H), 8.1, 9.6, 10.1 (3s, 3H, 3NH, pyrimidine, D_2O exchangeable)	356 [M^+] (38), 358 [M^++2] (12), 286 (45), 245 (11), 230 (23), 126 (24), 111 (42)
12	2218 (CN), 1695, 1705 (2C=O)	1.30 (s, 3H, CH_3), 2.4 (s, 3H, OCH_3), 6.6 (d, 2H, *J* = *5.8* Hz, Ar-H), 7.3 (d, 2H, *J* = *5.8* Hz, Ar-H)	343 [M^+] (65), 345 [M^++2] (22), 328 (58), 232 (43), 175 (54), 168 (12), 111 (23)
13	3225, 3150, 3125 (3NH), 1698 (C=O), 1260, 1255 (2C=S)	6.54 (d, 2H, *J* = *5.6* Hz, Ar-H), 7.23 (d, 2H, *J* = *5.7* Hz, Ar-H), 9.3, 10.7, 11.2 (3s, 3H, 3NH, D_2O exchangeable)	394 [M^+] (30), 396 [M^++2] (9), 378 (10), 361 (8), 282 (14), 111 (9)
14	3210 (NH), 1697, 1690 (2C=O), 1256, 1253 (2C=S)	3.30 (s, 2H, CH_2), 6.48 (d, 2H, *J* = *5.9* Hz, Ar-H), 7.14 (d, 2H, *J* = *5.9* Hz, Ar-H), 10.7 (s, 1H, NH, D_2O exchangeable)	434 [M^+] (30), 436 [M^++2] (12), 322 (64), 267 (14), 165 (42), 111 (18)
15	3215 (NH), 1700, 1694 (2C=O), 1253, 1258 (2C=S)	2.9 (t, 2H, *J* = *3.3* Hz, CH_2), 3.12 (t, 2H, *J* = *4.2* Hz, CH_2), 6.78 (d, 2H, *J* = *5.9* Hz, Ar-H), 7.07 (d, 2H, *J* = *5.9* Hz, Ar-H), 10.8 (s, 1H, NH, D_2O exchangeable)	448 [M^+] (21), 450 [M^++2] (8), 336 (12), 267 (8), 180 (10), 111 (5)
16	3207 (NH), 1715, 1698 (2C=O), 1254, 1252 (2C=S)	6.48 (d, 2H, *J* = *5.6* Hz, Ar-H), 6.90 (m, 2H, Ar-H), 7.07 (m, 3H, Ar-H), 7.42 (d, 2H, *J* = *5.7* Hz, Ar-H), 7.98 (s, 1H, methylene proton), 10.5 (s, 1H, NH, D_2O exchangeable).	523 [M^+] (49), 524 [M^++2] (18), 431 (54), 410 (32), 267 (41), 254 (24), 111 (21)
17	3217 (NH), 1713, 1693 (2C=O), 1255, 1249 (2C=S)	3.20 (s, 2H, CH_2), 6.57 (d, 2H, *J* = *5.9* Hz, Ar-H), 6.90 (m, 2H, Ar-H), 7.07 (m, 3H, Ar-H), 7.42 (d, 2H, *J* = *5.9* Hz, Ar-H), 8.3 (s, 1H, methylene proton), 10.45 (s, 1H, NH, D_2O exchangeable)	537 [M^+] (47), 538 [M^++2] (15), 445 (64), 424 (25), 268 (14), 111 (21)

[a] The solvent for compounds **1, 2, 3, 5, 7, 9, 14** is $CDCl_3$ and for compounds **4, 6, 10, 11, 12, 13, 15, 16, 17, 18** is DMSO-d_6.

antifungal activity of the compounds was tested against *Candida albicans* NRRL Y-477 using the Sabouraud dextrose agar medium.

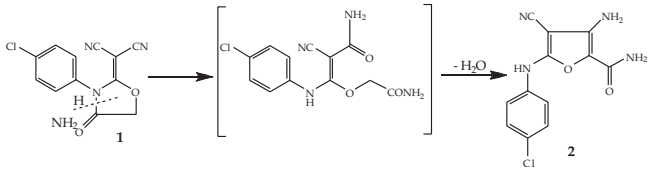
Agar diffusion medium. – Nine compounds were screened *in vitro* for their antimicrobial activity by the agar diffusion method (14). A suspension of organisms was added to a sterile nutrient agar medium at 45 °C and the mixture was transferred to a sterile Petri dish and allowed to solidify. Holes of 10 mm in diameter were made using a cork borer and filled with the solution of synthesized compounds (100 μg mL^{-1}). A hole filled with DMSO was used as control. The plates were left for 1 hour at room temperature as a period of pre-incubation. The plates were then incubated at 37 °C for 24 hours and observed for antibacterial activity. Diameters of the zone of inhibition were measured and compared with that of the standard. Ciprofloxacin (50 μg mL^{-1}) and ketoconazole (50 μg mL^{-1}) were used as standards for antibacterial and antifungal activity, respectively. The observed zones of inhibition are presented in Table III.

Minimum inhibitory concentration. – Minimum inhibitory concentration (*MIC*) of the test compounds was determined by the agar streak dilution method. Stock solutions of synthesized compounds were made using DMSO as a solvent (68 mg mL^{-1}). From this stock solution, a series of concentrations was prepared (0.17, 0.34, 0.68, 0.85 and 1.7 mg mL^{-1}) and mixed with known quantities of molten sterile agar medium aseptically. About 20 mL of the medium containing the tested compound was dispensed into a sterile Petri dish. Then, the medium was allowed to solidify. Microorganisms were then streaked one by one on the agar plates aseptically. After streaking, all the plates were incubated at 37 °C for 24–48 h for antibacterial and antifungal activity, respectively. The lowest concentration of the synthesized compound that inhibits the growth of the given bacterium/fungus was considered as the minimum inhibitory concentration (*MIC*) of the test compounds. The *MIC* values are tabulated in Table IV.

RESULTS AND DISCUSSION

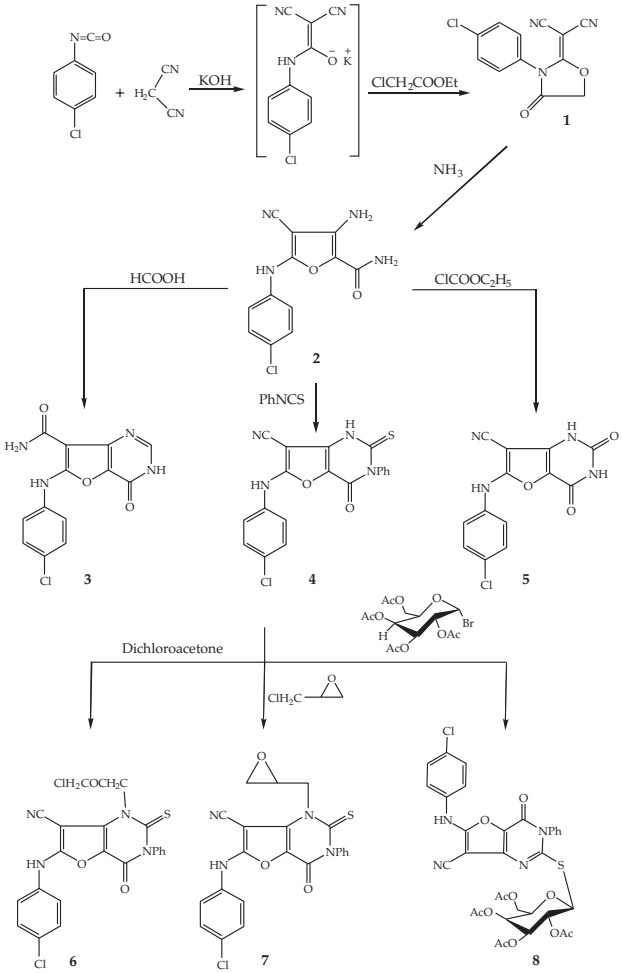
Chemistry

4-Oxo-oxazolidin-2-ylidene malononitrile derivative **1** was prepared *via* condensation of malononitrile with *p*-chlorophenylisocyanate in alkaline medium, followed by addition of ethyl chloroacetate. Ammoniation of compound **1** afforded 3-amino-5-(4-chlorophenylamino)-4-cyanofuran-2-carboxamide (**2**) according to the mechanism given in Scheme 1. Compound **2** was used to synthesize some fused heterocyclic derivatives. Thus, heating of compound **2** with an aliphatic acid, namely formic acid, resulted in the formation of 3,4-dihydrofuro[3,2-*d*]pyrimidine **3**. Also, when reacted with phenylisothiocyanate in refluxing acetic acid it afforded the corresponding 1,2,3,4-tetrahydrofuro[3,2-*d*] pyrimidine-7-carbonitrile **4** (Scheme 2). All the synthesized compounds were characterized by their physical, chemical and spectral data (Tables I and II). IR spectra of compound **3** showed the presence of absorption bands at 1682 and 1702 cm^{-1} (2 C=O) and the absence of a characteristic band (CN) due to hydrolysis of the cyano group; the MS gave the molecular ion peak at *m*/z (%) = 394 (60).



Scheme 1

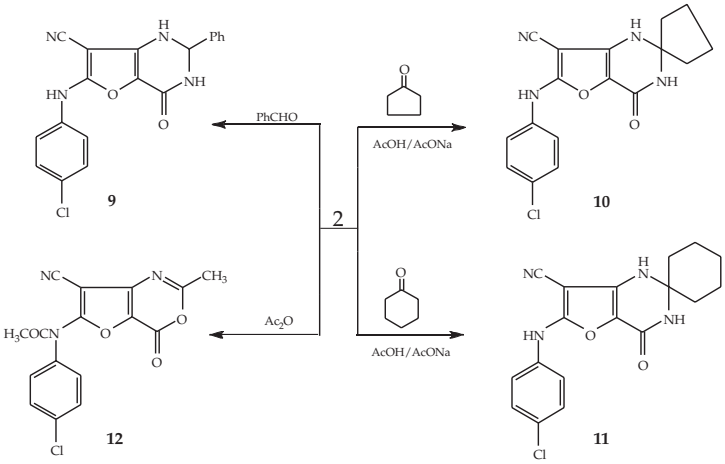
Furthermore, 4-cyanofuran-2-carboxamide **2** reacted with ethylchloroformate in refluxing acetic acid to yield 2,4-dioxofuro[3,2-*d*]pyrimidine-7-carbonitrile **5**. Beside correct values of elemental analyses, the spectral data for **5** are in agreement with the assigned structure (Scheme 2).



Scheme 2

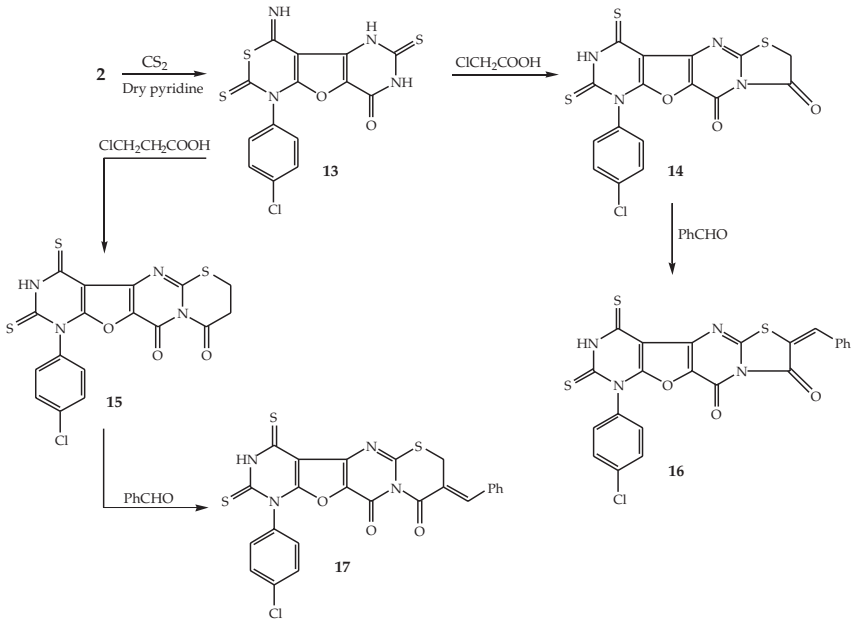
In addition, alkylation of compound **4** with dichloroacetone or epichlorohydrine in the presence of sodium hydride afforded the corresponding *N*-alkyl pyrimidine derivatives **6** and **7**, respectively. On the other hand, alkylation of **4** with 2,3,4,6-tetra-*O*-acetyl--α-D-glucosyl bromide in acetone and in the presence of aqueous potassium hydroxide afforded the corresponding *S*-glucosides **8** in good yield (Scheme 2). Attachment of the glucosyl residues to the sulfur atom rather than to the nitrogen atom was supported by the value of the chemical shift of the anomeric protons which should other-wise appear at a lower field. The anomeric proton of β-*N*-glucosides having an adjacent C=S was reported (15) to appear at a higher chemical shift (δ 6.90–7.20 ppm) due to the anisotropic deshielding effect of the C=S. The structure of **8** was confirmed by elemental analysis and spectral data. The IR spectrum showed absorption bands at 3192, 2211 and 1718–1667 cm^{-1} due to the presence of NH, CN and C=O groups, respectively. The ^1H NMR spectrum showed the anomeric proton of the glucose moiety as a doublet at δ 5.70 ppm with a coupling constant $J_{1'\text{-}2'}$ = 9.8 Hz, indicating that H-1′ is trance-dioxal to H-2′. The other six glucose protons resonated at δ 3.75–5.49 ppm and four acetyl groups appeared as four singlet peaks at δ 2.04–2.20 ppm. In addition, its C, H, N analysis data revealed the molecular formula $C_{33}H_{29}ClN_4O_{11}S$.

When compound **2** was allowed to react with benzaldehyde, it afforded 6-(4-chloro--phenylamino)-4-oxo-2-phenyl-1,2,3,4-tetrahydrofuro[3,2-*d*]pyrimidine-7-carbonitrile (**9**) in good yield (Scheme 3). Also, compound **2** reacted with cyclopentanone or cyclohexanone in refluxing acetic acid and sodium acetate to afford the corresponding pyrimidine derivatives **10** and **11**, respectively. On the other hand, when compound **2** was treated with acetic anhydride, it afforded the corresponding furo[3,2-*d*][1,3]oxazine derivative **12** (Scheme 3). The ^1H NMR spectrum of compound **12**, for example, showed a singlet at δ 1.30 ppm, which supported the methyl protons and signal at δ 2.4 ppm for the methoxy group, supporting the acetylation of NH group. The MS gave the molecular ion peak at *m/z* (%) = 343 (65).



Scheme 3

A very interesting reaction occurred when compound **2** was allowed to react with carbon disulfide in dry pyridine to produce 1-(4-chlorophenyl)-4-imino-2,6-dithioxo-1,4,6,7-tetrahydro-pyrimido[5,4-*b*]furo[5,4-d]-1,3-thiazin-8-one (**13**) (Scheme 4). The structure of compound **13** was confirmed from its spectral data. On the other hand, when treated with aliphatic acids, namely chloroacetic acid or chloropropionic acid compound **13** afforded polycyclic fused compounds **14** and **15**, respectively. Beside the values in elemental analyses and spectral data, the latter compounds were confirmed chemically *via* condensation with benzaldehyde to give the corresponding aryl methylene compounds **16** and **17**, respectively (Scheme 4).



Scheme 4

Antimicrobial activity

The antimicrobial results as the average diameter of inhibition zones, expressed in mm are given in Table III. It is evident that all tested compounds display activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* while only compounds **6**, **9**, **11**, **17** and **18** were active against *Candida albicans* as well. Compounds **5**, **6**, and **8** were the most active ones against all the listed bacteria. Mean inhibition zones and minimal inhibitory concentrations of these compounds ranged from 20 to 30 mm and from 0.17 to 0.68 mg mL^{-1}, respectively. Compounds **6** and **8** also exhibited activity against the fungus *C. albicans*. All tested compounds showed activity lower than that of the standard drugs (ciprofloxacin and ketaconazole).

Table III. Inhibition zones of the newly synthesized compounds

Compd. No.	Inhibition zone			
	Gram positive bacteria		Gram negative bacteria	Fungus
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
DMSO(solvent)	–	–	–	–
2[a]	14	21	21	–
3[a]	13	19	16	–
5[a]	23	25	30	–
6[a]	25	25	20	17
8[a]	20	25	24	14
9[a]	14	18	19	–
10[a]	19	24	20	16
16[a]	16	20	14	16
17[a]	17	22	21	20
Ciprofloxacin[b]	23	23	25	–
Ketaconazole[b]	–	–	–	23

[a] 100 μg mL^{-1}
[b] 50 μg mL^{-1}

Table IV. MIC *of the newly synthesized compounds*

Compd. No.	Minimum inhibitory concentration (mg mL^{-1})			
	Gram positive bacteria		Gram negative bacteria	Fungus
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
5	0.34	0.34	0.17	1.7
6	0.34	0.34	0.68	0.85
8	0.68	0.34	0.34	1.7
10	0.68	0.34	0.68	0.85
17	0.85	0.68	0.68	0.68
Ciprofloxacin (μg mL^{-1})	0.12	0.15	0.01	–
Ketaconazole (μg mL^{-1})	–	–	–	0.03

CONCLUSIONS

Screening data of the prepared compounds show promising antibacterial and antifungal activity. Compounds **8**, **10** and **16** showed significant antibacterial activity due to the presence of the sugar moiety and addition of cycloalkane or thiazole to the furanopyrimidine ring. Efficacy of compound **6** is probably due to addition of chloroacetone to the furopyrimidine ring.

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S A Ž E T A K

Antimikrobno djelovanje nekih glukopiranozil-pirimidin karbonitrila i fuzioniranih pirimidinskih sustava

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3-Amino-5-(4-klorfenilamino)-4-cijanofuran-2-karboksamid (**2**) upotrebljen je kao ključni spoj za pripravu različitih furo-pirimidina **3-9** i spiro-cikloalkan furopirimidina **10** i **11**. Fuzionirani heterociklički spojevi **13-17** pripravljeni su također polazeći iz spoja **2**. Sintetizirani spojevi ispitani su na antimikrobno djelovanje.

Ključne riječi: pirimidin, glukopiranozil-pirimidin, oksazin, antimikrobno djelovanje

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