Original Articles

SYNTHESIS AND BIOLOGICAL EVALUATION OF CERTAIN NEW SUBSTITUTED PYRIDO[2,3-*d*]PYRIMIDIN-4(1*H*)-ONE AND PYRIDO [2,3-*d*]TRIAZOLO[3,4-*b*]PYRIMIDINE ANALOGS

Fatma E. Goda[1*] and Farid A Badria[2]

تحتوي هذه المقالة على وصف لعملية التشييد الكيميائي لمجموعة جديدة من مشتقات بايريدو [3,2 - د] بايريميدين 8-13 وكذلك الأشكال المطابقة لها والمحتوية على مجموعة ميثايلية مرتبطة بذرة كبريت 14-19 كما تم كذلك تحضير مشتقات بايريدو- [3,2 - د] ترايازولو [4,3 - ب] بايريميدين 26 ,27. تم التأكد من التركيب البنائي لهذه المركبات الجديدة بواسطة التحليل الدقيق للعناصر وطيف الأشعة تحت الحمراء وطيف الطنين النووي المغناطيسي لذرة الهيدروجين- 1 ، كما تم إنجاز دراسة عن كيفية ارتباط هذه المركبات المشيدة مع الحامض النووي المنقوص ذرة الأكسجين.

The synthesis of a new series of Pyrido[2,3-*d*]-pyrimidine derivatives **8-13** and their corresponding S-methyl analogs **14-19** is described. The pyrido[2,3-*d*] triazolo[3,4-*b*]pyrimidines **26, 27** are also prepared. The structures of the newly synthesized compounds have been confirmed by elemental analysis, IR, and ^{1}H-NMR spectra. DNA-binding activity of the synthesized compounds was performed.

Key words: Pyrido[2,3-*d*]pyrimidine, pyrido[2,3-*d*]triazolo[3,4-*b*]pyrimidines, synthesis, anti-microbial activity, DNA-binding assay.

Introduction

In the course of identify of various chemical structures which may serve as leads for designing novel antitumor agents. Fused pyrimidines are important class of compounds that attracted the attention of medicinal chemists as chemo-therapeutic agents. The antifungal, antibacterial and antitumor activities are well documented (1-11). Moreover some of its substituted derivatives act as DNA intercalating antitumor agents (12-14).

Benzofuran analogs are recently known to contribute to the DNA binding activity (15) and hence to the antitumor potency, they proved to possess the ability in inhibiting estrogen-dependent tumor growth (16). Natural benzofuran lignans isolated form aglaia species inhibit cell proliferation and alter cell cycle distribution in human monocytic leukemia cell lines (17). Dihydrobenzofuran lignans and related compounds also proved to inhibit tubulin polymerization (18).

With the view that thioxopyridopyrimidine may modify the anticancer activity, it was thought worthwhile to undertake the synthesis of some 4-oxo-2-thioxo-substituted pyrido[2,3-*d*]pyrimidines, in addition to the synthesis of substituted pyrido[2,3-*d*]triazolo[3,4-*b*]pyrimidines all carrying a benzo-furan moiety, with the hope to obtain new candidate(s) as antimicrobial and antitumor agents.

Experimental

Melting points were recorded on a Fisher-Johns apparatus (°C, uncorrected). IR spectra (KBr) were recorded on Mattson 5000 FT-IR spectrophotometer (υ in cm^1). ^{1}H-NMR spectra were recorded on a Varian EM-390 (90MHz) spectrometer using TMS as internal standard (chemical shifts in δ ppm). Microanalytical data (C, H, N) agreed with the proposed structures within ± 0.4% of the theoretical values. The following materials were used in the

[1]Department of Pharmaceutical Organic Chemistry . [2]Pharmacognosy, Faculty of Pharmacy, University of Mansoura, Mansoura 35515, Egypt.

*To whom correspondence should be addressed.

biological screening: Giemse stain (Fischer Scientific Co., Fair Lawn, NY, USA).TLC Plates ($RP\text{-}18_{F254}$, 0.25 mm, Merck), DNA (Calf thymus type I, sigma, 100 µg/ml), DNA/methyl green (Sigma, St. Louis, MO, USA).

Synthesis:

*2-Amino-4-aryl-6-(benzofuran-2-yl)-3-cyano-pyridines **(2-4):***

A mixture of 2-acetylbenzofuran **1** (1.6 g, 0.01 mole) and the appropriate aromatic aldehydes (0.01 mole) in EtOH (40 ml) was treated with malononitrile (0.66 g, 0.01 mole) and ammonium acetate (2g). The reaction mixture was refluxed for 10 h, where a crystalline precipitate separated. The formed precipitate was filtered, washed with ethanol, dried and recrystallized from AcOH-EtOH (Table 1). ^{1}H-NMR, **2** (DMSO-d_6): δ 3.70 (s, 6H, 2 OCH_3), 5.52 (s, 2H, NH_2, D_2O-exchangeable), 7.20-7.90 (m, 9H, ArH). **3** (DMSO-d_6): δ 6.11 (s, 2H, NH_2, D_2O-exchangeable), 7.0-8.0 (m, 9H, ArH), **4** (DMSO-d_6): δ 6.30 (s, 2H, NH_2, D_2O-exchangeable), 7.20-7.90 (m, 9H, ArH).

*2-Amino-4-aryl-6-(benzofuran-2-yl)pyridine-2-pyridine-**2**-carboxamide**s*** **(5-7):**

2-Amino-4-aryl-6-(benzofuran-2-yl)-3-cyanopy-ridines **2-4** (0.04 mole), KOH (39.2g, 0.7 mole) and EtOH (150 ml) were refluxed for 7 h. After cooling to room temperature, the reaction mixture was poured into excess water, and the obtained solid was filtered and recrystallized from EtOH (Table 1). ^{1}H-NMR, **5** (DMSO-d_6): δ 3.90 (s, 6H, OCH_3), 6.60 (s, 2H, NH_2, D_2O-exchangeable), 7.20-7.90 (m, 9H, ArH), 10.8 (s, 2H, NH_2, D_2O-exchangeable). **6** (DMSO-d_6): δ 4.11 (s, 2H, NH_2, D_2O-exchangeable), 7.50-8.12 (m, 9H, ArH), 11.10 (s, 2H, NH_2, D_2O-exchangeable). **7** (DMSO-d_6): δ 4.30 (s, 2H, N H_2, D_2O-exchangeable), 7.90-8.70 (m, 9H, ArH), 11.50 (s, 2H, NH_2, D_2O-exchangeable).

5-Aryl-7-(benzofuran-2-yl)-3(-n-butyl or phenyll)-4-oxo-2-thioxo-1,2,3.4-tetrahydro-pyrido[2,3-d] pyrimidines **(8-13):**

A mixture of compounds **5-7** (0.01 mole) and the appropriate *n*-butyl or phenyl isothiocyanate (0.01 mole) in pyridine (15 ml) was refluxed for 8-10 h. The reaction mixture was poured into ice-cold water and the separated solid was filtered, washed with water, dried and recrystallized from DMF-H_2O (Table 1). ^{1}H-NMR, **8** (DMSO-d_6): δ 4.0 (s, 6H, OCH_3), 6.80-7.72 (m, 14H, ArH), 9.0 (s, 1H, NH, D_2O-exchangeable), **9** (DMSO-d_6): δ 7.70-8.20 (m, 14H, ArH), 9.20 (s, 1H, NH, D_2O-exchangeable).**10** (DMSO-d_6): δ 7.80-8.40 (m, 14H, ArH), 9.40 (s, 1H, NH, D_2O-exchangeable). **11 (**DMSO-d_6**):** δ 1.30 (t3H, CH_3), 1.80 (t, 2H, CH_2), 2.4 (m, 2H, CH_2), δ 4.0 (s, 6H, OCH_3), 7.07-7.81 (m, 9H, ArH),9.0 (s, 1H, NH, D_2O-exchangeable). **12 (**DMSO-d_6**):** δ 1.31 (t 3H, CH_3), 1.82 (t, 2H, CH_2), 2.40 (m, 2H, CH_2), 7.0-7.90 (m, 9H, ArH), 8.80 (s, 1H, NH, D_2O-exchangeable).

5-Aryl-7-(benzofuran-2-yl)-3-(n-butyl or phenyll) -2-mehylthio-4-oxo-3,4-dihydropyrido [2,3-d] pyrimidines **(14-19).**

To a solution of compounds **8-13** (0.01 mole) in DMF (10 ml), methyl iodide (1.43 g, 0.01 mole) and K_2CO_3 (1.38 g, 0.01 mole) were added, and the reaction mixture was heated under reflux for 8-10 h. After cooling, the solution was poured into ice-cold water. The precipitated products were filtered washed with water, dried and recrystallized from DMF-EtOH (Table 1). ^{1}H-NMR, **14** (DMSO-d_6): δ 2.60 (s, 3H, SCH_3), 4.10 (s, 6H, OCH_3), 7.0-8.20 (m, 14H, ArH), **15** (DMSO-d_6): δ 2.60 (s, 3H, CH_3), 7.20-8.20 (m, 14H, ArH). **16** (DMSO-d_6): δ 2.60 (s, 3H, CH_3), 7.0-8.20 (m, 14H, ArH). **17** (DMSO-d_6): δ 1.30 (t, 3H, CH_3), 1.80 (t, 2H, CH_2), 2.30 (m, 2H, CH_2), 2.60 (s, 3H, SCH_3), 4.10 (s, 6H, OCH_3), 7.30-7.90 (m, 9H, ArH) ,2.30 (m, 2H, CH_2), 2.60 (s, 3H, SCH_3), 4.0 (s, 6H, OCH_3), 7.30-7.91 (m, 9H, ArH). **18** (DMSO-d_6): δ 1.30 (t, 3H, CH_3), 1.80 (t, 2H, CH_2), 2.0-2.40 (m, 2H, CH_2), 2.61 (s, 3H, SCH_3), 7.0-8.0 (m, 9H, ArH). **19** (DMSO-d_6): δ 1.30 (t, 3H, CH_3), 1.80 (t, 2H, CH_2), 2.01-2.40 (m, 2H, CH_2), 2.60 (s, 3H, SCH_3), 7.20-8.0 (m, 9H, ArH).

7-(Benzofuran-2-yl)-3-(n-Butyl or phenyl)-5-(2,4-dimethoxyphenyl)-4-imino-2-oxo-3,4-dihydro-pyrido dihydropyrido[2,3-d]pyrimidines **(20,21**) *and 7-(benzofuran-2-yl)-3-(n-butyl or phenyl)-5-(2,4-dimethoxyphenyl)- 4-imino-2-thioxo-3,4-dihydro-pyrido[2,3-d]-pyrimidines* **(22, 23)**:

A mixture of 2-amino-4-(2,4-dimethoxyphenyl)-3-cyanopyridine **2** (3.7g, 0.01 mole) and the appropriate isocyanate or isothiocyanate (0.01 mole) in pyridine (10 ml), was heated under reflux for 12 h. After cooling, the mixture was poured into water. The precipitated solid was filtered, washed with water and recrystallized from methanol. ^{1}H-NMR, **20** ($CDCl_3$): δ 4.0 (s, 6H, 2 OCH_3), 7.0-8.20 (m,

15H, ArH, NH), 9.90 (s, 1H, NH, D_2O-exchangeable). **21** ($CDCl_3$ δ 1.60 (t, 3H, CH_3), 1.80 (t, 2H, CH_2), 2.0 (m, 2H, CH_2), 4.0 (s, 6H, OCH_3), 7.2-8.0 (m, 10H, ArH, NH), 10.0 (s, 1H, NH, D_2O-exchangeable). **22** ($CDCl_3$): δ 3.20 (s, 6H, OCH_3), 7.0-8.10 (m, 15H, ArH, NH), 10.50 (s, 1H, NH, D_2O-exchangeable). **23** ($CDCl_3$**):** δ 1.60 (t, 3H, CH_3), 1.80 (t, 2H, CH_2), 2.0 (m, 2H, CH_2), 3.91 (s, 6H, OCH_3), 7.0-7.80 (m, 10H, ArH, NH), 9.81 (s, 1H, NH, D_2O-exchangeable).

7-(Benzofuran-2-yl)-3-(n-butyl or pheny)-3,4-dihydro-4-imino-2-methylthio-5-(2,4-dimethoxypheny)pyrido[2,3-d]pyrimidines **(24, 25):**

To a solution of compound **22** or **23** (0.01 mole) in DMF (10 ml), methyl iodide (1.43 g, 0.01 mole) was added together with K_2CO_3 (1.38 g, 0.01 mole). After 12 h, at reflux, the reaction mixture was poured into ice- cold water. The precipitated product was filtered, washed with water and recrystallized from DMF (Table 1). ^{1}H-NMR, **24** (DMSO-d_6): δ 2.80 (s, 3H, SCH_3), 3.91 (s, 6H, 2 OCH_3), 6.50 (s, 1H, NH, D_2O-exchangeable), 7.20-8.0 (m, 14H, ArH). **25** (DMSO-d_6): δ 1.62 (t, 3H, CH_3), 1.80 (t, 2H, CH_2), 2.11 (m, 2H, CH_2), 2.7 (s, 3H, SCH_3), 4.0 (s, 6H, OCH_3), 6.5 (s, 1H, NH, D_2O-exchangeable), 7.0-7.90 (m, 9H, ArH).

8-(Benzofuran-2-yl)-4-(n-butyl or phenyl)-5-imino-6-(2,4-dimethoxyphenyl)- 1-phenylpyrido[2,3-d]-1,2,4-triazolo[3,4-b]pyrimidines **(26, 27**):

Benzoic acid hydrazide (4.08 g, 0.03 mole) was added to a solution of **24** or **25** (0.01 mole) in n-butanol (5 ml) and the resulting mixture was heated under reflux for 15 h. Excess solvent was removed in *vacuo* and the residue was purified by crystallization from DMF-H_2O, (Table 1). ^{1}H-NMR, **26** ($CDCl_3$): δ 3.91 (s, 6H, OCH_3), 6.52 (s, 1H, NH, D_2O-exchangeable), 7.08-8.20 (m, 19H, ArH). **27** ($CDCl_3$): δ 1.40 (t, 3H, CH_3), 1.60 (t, 2H, CH_2), 2.0-2.24 (m, 2H, CH_2), 2.60 (s, 3H, SCH_3), 4.0 (s, 6H, OCH_3), 6.22 (s, 1H, NH, D_2O-exchangeable), 7.20-8.20 (m, 14H, ArH).

Antimicrobial testing:

Nutrient agar plates were seeded using 0.1 ml of the diluted organism. Cylindrical plugs were removed from the agar using a sterile cork bore, and 100 µl of the tested compounds (1 mg/ml DMSO) and blank solvent were added to each well in triplicate. Plates of *P. aeruginosa*, *E. Coli*, *S. aureus* and *B. subtilis* were incubated at 37°C. Those of *C. albicans* was incubated at 30°C. After 24 h, the average diameter of the inhibiton zone was measured in millimeters (Table 3).

Minimum inhibitory concentration (MIC) measurements:

The substances dissolved in DMF at 1 mg/ml were diluted in broth in the range 200-10 µg/ml. Inocula were prepared from well-growing overnight cultures of each test organism such that the final inoculum size was ca 10^6 cells/ml. The tubes were then inoculated with 0.1 of inoculum and the results are presented as µg/ml and the lowest concentration of compounds that completely inhibits the visible growth of microorganisms was considered to be the minimum inhibitory concentration (MIC) expressed in ug/ml (Table 3). MIC was the mean of three measurements. Ampicillin, streptomycin and nystatin were used as reference antibiotics.

DNA Binding Assay:

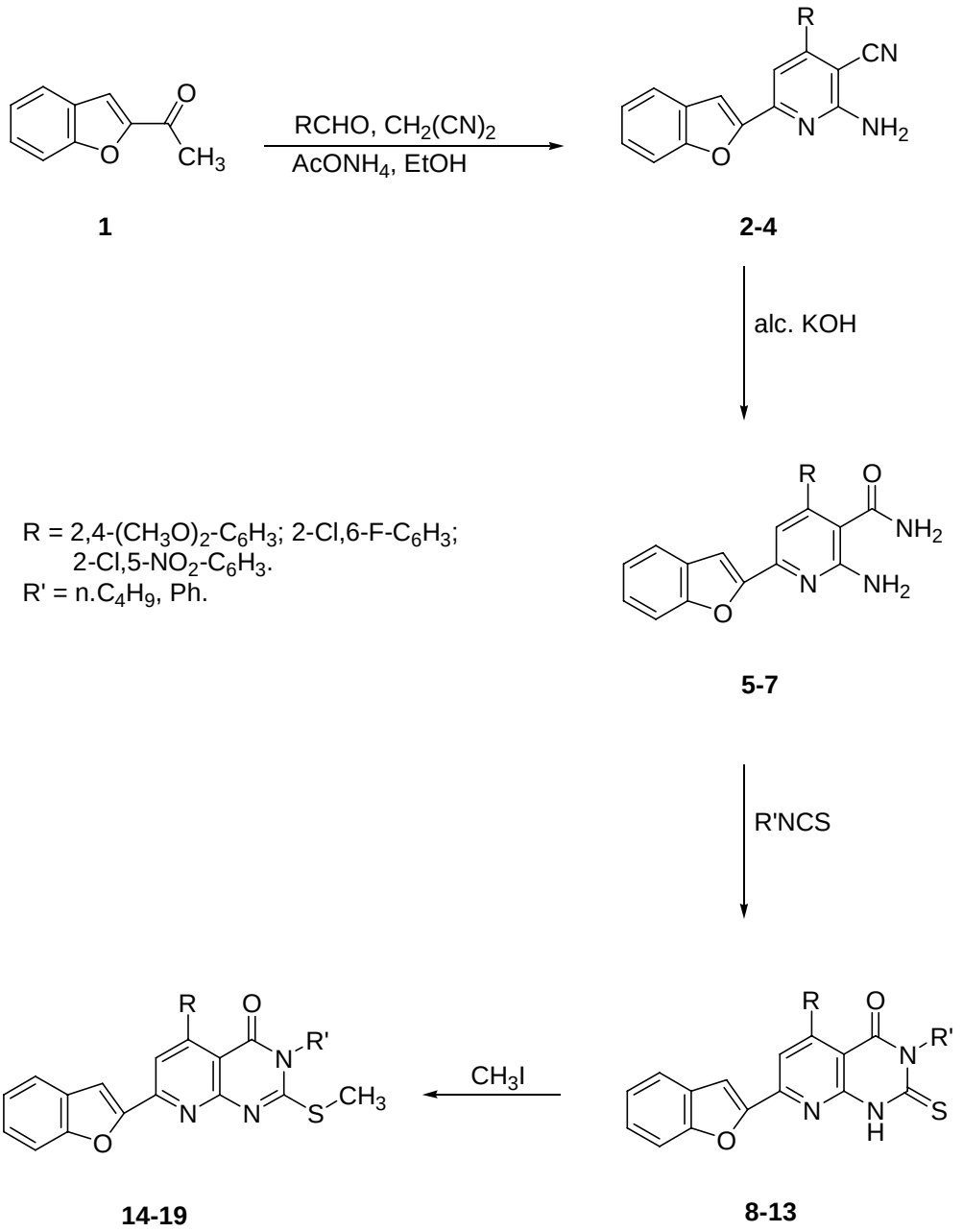
Analysis of the DNA binding affinity of the tested compounds was performed using RP-TLC; TLC plates were predeveloped with MeOH-H_2O (8:2). Test compounds were then applied (5 mg/ml in MeOH) at the origin, followed by the addition of DNA (1mg/ml in H_2O-MeOH mixture) at the same position at the origin. The plates were then developed with the same solvent system and the position of the DNA was determined by spraying with anisaldehyde reagent. The reagent yields a blue color spot with DNA, and the intensity of the color was proportional to the quantity of DNA added to the plate. Ethidium bromide was used as a positive control .

Methyl green-DNA displacement assay:

DNA methyl green (20 mg) was suspended in 100 ml of 0.05 M Tris-HCl buffer, (PH 7.5), containing 7.5 mM $MgSO_4$ and stirred at 37 °C for 24 h. Unless otherwise indicated, samples to be tested were dissolved in EtOH in Ippendorf tubes. Solvent was removed in *vacuo*, and 200 µl of the DNA/ methyl green solution was added to each tube. The absorption maximum for the DNA/methyl green complex is 642.5-645 nm. Samples were incubated in the dark at ambient temperature. After 24 h. the final absorbance of sample was determined. Readings were corrected for initial absorbance and normalized as a % of the untreated DNA/methyl

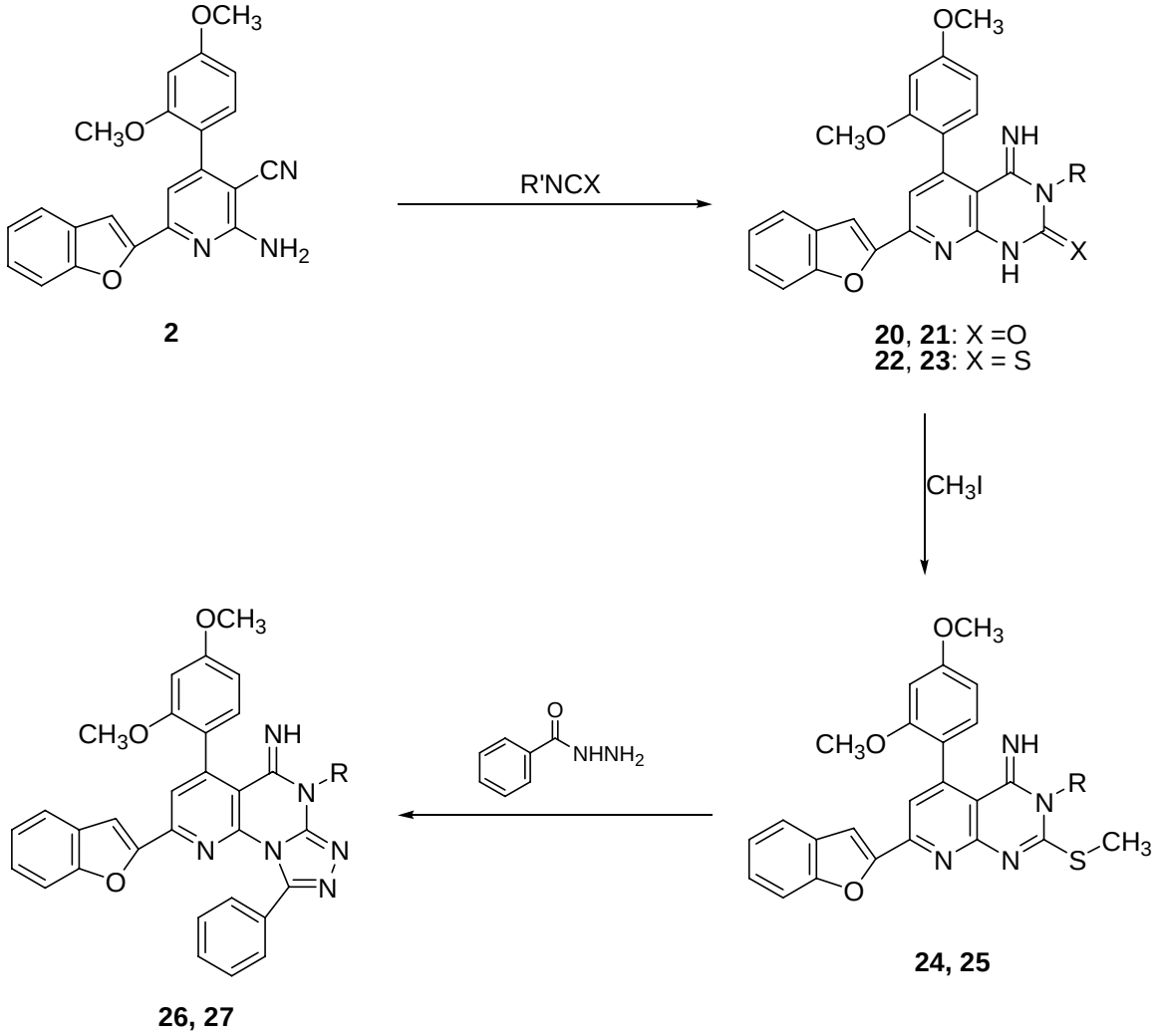
green absorbent value. IC_{50} were determined for each compound as shown in (Table 4) Daunomycin was used as a positive control.

Scheme I



1 **2-4** **5-7** **8-13** **14-19**

Scheme II



Result and Discussion

Chemistry:

2-Amino-4-aryl-3-cyano-6-benzofuran-2-yl) pyridines (**2-4) w**ere obtained *via* the reaction of 2-acetylbenzofuran **(1**) and the appropriate aromatic aldehyde with malononitrile and excess ammonium acetate in ethanol (19,20). IR spectra of compounds **2-4** showed sharp bands at 2220-2110 cm^{-1} (CN), 3440-3300 cm^{-1} (NH_2). Compounds **2-4** were refluxed in alcoholic KOH solution to obtain 2-amino-4-aryl-6-(benzofuran-2-yl)pyridine-2-carbox-amides (**5-7)**, which showed a strong absorption peak at 1685-1670 cm^{-1} (C=O) indicating the carbonyl group and showed the disappearance of CN absorption band. Upon heating compounds **5-7** with *n*-butyl or phenyl isothiocyanate in pyridine, 5-aryl-7-(benzofuran-2-yl)-3-(*n*-butyl or phenyl)-4-oxo-2-thioxo-1,2,3,4-tetra-hydropyrido[2,3-*d*]pyrimidines (**8-13)** were obtained. The IR spectra of compounds **8-13** showed peaks at 1720-1675 cm^{-1} (C=O), 1400-1200 cm^{-1} (C=S), 3400-3320 cm^{-1} (NH). The reaction of compound **(8-13)** with methyl iodide afforded the corresponding S-methyl derivatives (**14-19)** (Scheme I, Table 1). Treatment of compound **2** with n-butyl or phenyl isocyantes or isothiocyantes

in pyridine afforded compounds (**20-23).** The IR spectra of compounds **(20-23)** gave band at 3400-3330 cm^{-1} (NH) and showed disappearance of (CN) absorption band while compounds **20, 21** showed band at 1640 cm^{-1} corresponding to (C=O). Interaction of compounds **22, 23** with methyl iodide in DMF in the presence of K_2CO_3 yielded the S-methyl analogs (**24**, **25)** in good yields. Treatment of compounds **24**, **25** with benzoic acid hydrazide gave the required 8-(benzofuran-2-yl)-4-(n-butyl or phenyl)-5-imino-6-(2,4-dimethoxyphenyl)-1-phenyl-pyrido[2,3-*d*]-1,2,4-triazolo[3,4-*b*]pyrimidines **(26, 27)** (Scheme II, Table 1).

Biological Investigations
Antimicrobial activity:

The antimicrobial screening of all the synthesized compounds was done using the agar diffusion assay (21). This screening was performed against the Gram-negative bacteria *Pesudomonas aeruginosa* ATCC 15442 and *Escherechia coli* ATCC 10536 and the Gram-positive bacteria *Staphylococcus aureus* ATCC 06538, *Bacillus Subtilis* ATCC 6633, in addition to the pathogenic fungi *Candida albicans* ATCC 1023. The maximum activity was observed with compounds **2-7**, which proved to possess marked activity against *E .coli* and *C. Albicans* (Table 2). The minimum inhibitory concentration (MIC) (22) was determined for each of the active compounds along with ampicillin, streptomycin and nystatin as positive controls; results are shown in (Table 3).

DNA-binding assay:

The mechanism of several antitumor compounds such as alkylating agents and antitumor antibiotics involves their interaction with DNA. Based on the interaction of small molecular weight ligands with DNA, some short-term procedures have been applied for the discovery and evaluation of naturally occuring synthetic compounds that function by this method. In this work, the antitumor activity of the newly synthesized compounds was determined using DNA binding assay and methyl green DNA displacement assay (23). In this method, a fixed amount of the ligand is spotted on the RP-18 TLC plates followed by addition of known amount of DNA on the same spot. The plate was then developed and the position of DNA was determined by spraying the plates with anisaldehyde reagent. (The response of the test system is dependent on the dose of the test substance). In the presence of strong DNA intercalator, a greater portion of DNA is bound to form a complex, and consequently, the free DNA was detected as a blue spot (R_f MeOH-H_2O, 8:2) on RP-18 TLC after spraying with anisaldehyde reagent. Compounds with high binding affinity to DNA retained on the base line. It was demonstrated that, when DNA was mixed with compounds which known to interact e.g ethidium bromide, the complex was retained at the origin when MeOH-H_2O (8:2) was used for elution, but inactive compounds did not cause the DNA to be retained at the origin. Results obtained from DNA-binding assay revealed that all compounds in this study showed variable affinity to DNA which was demonstrated by retaining the complex at the origin or by its migration for a very short distance. Compounds **17-19** are the most active members of this series.

Methyl green reversibly binds to polymerized DNA forming a stable complex at neutral pH. The maximum absorption for the DNA/methyl green complex is 642.5-645 nm. This assay was used to measure the displacement of methyl green from DNA by compounds having ability to bind with DNA. The degree of displacement was determined spectrophotometrically by measuring the change in initial absorbance of the DNA/methyl green solution in the presence of reference compound.. The activity of compounds **17-19** which showed high affinity for DNA have been determined and expressed as IC_{50} (concentration required for 50% decrease in the initial absorbance of the DNA/methyl green solution) (Table 4).

These results indicated that compounds **17-19** proved to have binding affinity for DNA and their structures are characterized by the presence of n-butyl nucleus at position 3 of the pyridopyrimidine ring .

In conclusion, compounds with the basic structures 5- (aryl) -7- (benzofuran-2-yl) -3- (n-butyl) -2-methyl-thio-4-oxo-3,4-dihydropyrido[2,3-*d*] pyrimi dine **(17-19)**, showed significant activity and will be considered as a guide for further future synthetic design.

Table 1. Physicochemical properties of the new compounds.

Compd. No.	R̀	R	Yield (%)	MP (°C)	Mol.Formulae (Mol.Wt.)
2	-	2,4-$(CH_3O)_2$-C_6H_3	80	280–2	$C_{22}H_{17}N_3O_3$ (371.38)
3	-	2-Cl, 6-F-C_6H_3	84	>300	$C_{20}H_{11}ClFN_3O$ (363.76)
4	-	2-Cl, 5-NO_2-C_6H_3	82	295–7	$C_{20}H_{11}ClN_4O_3$ (390.77)
5	-	2,4-$(CH_3O)_2$-C_6H_3	65	250–2	$C_{22}H_{19}ClN_3O_4$ (389.39)
6	-	2-Cl, 6-F-C_6H_3	60	285–7	$C_{20}H_{13}ClFN_3O_2$ (381.77)
7	-	2-Cl, 5-NO_2-C_6H_3	63	240–2	$C_{20}H_{13}ClN_4O_4$ (408.78)
8	C_6H_5	2,4-$(CH_3O)_2$-C_6H_3	75	>300	$C_{29}H_{21}N_3O_4S$ (507.54)
9	C_6H_5	2-Cl, 6-F-C_6H_3	76	>300	$C_{27}H_{15}ClFN_3O_2S$ (499.92)
10	C_6H_5	2-Cl, 5-NO_2-C_6H_3	73	>300	$C_{27}H_{15}ClN_4O_4S$ (526.93)
11	n-C_4H_9	2,4-$(CH_3O)_2$-C_6H_3	62	253–5	$C_{27}H_{25}N_3O_4S$ (487.49)
12	n-C_4H_9	2-Cl, 6-F-C_6H_3	65	227–9	$C_{25}H_{19}ClFN_3O_2S$ (479.93)
13	n-C_4H_9	2-Cl, 5-NO_2-C_6H_3	67	210–2	$C_{25}H_{19}ClN_4O_4S$ (506.94)
14	C_6H_5	2,4-$(CH_3O)_2$-C_6H_3	70	190–3	$C_{30}H_{23}N_3O_4S$ (521.56)
15	C_6H_5	2-Cl, 6-F-C_6H_3	75	161–3	$C_{28}H_{17}ClFN_3O_2S$ (513.95)
16	C_6H_5	2-Cl, 5-NO_2-C_6H_3	73	141–3	$C_{28}H_{17}ClN_4O_4S$ (540.96)
17	n-C_4H_9	2,4-$(CH_3O)_2$-C_6H_3	69	152–3	$C_{28}H_{27}N_3O_4S$ (501.58)
18	n-C_4H_9	2-Cl, 6-F-C_6H_3	70	130–2	$C_{26}H_{21}ClFN_3O_2S$ (493.96)
19	n-C_4H_9	2-Cl, 5-NO_2-C_6H_3	72	127–9	$C_{26}H_{21}ClN_4O_4S$ (520.97)
20	-	C_6H_5	68	>300	$C_{29}H_{22}N_4O_4$ (490.50)
21	-	n-C_4H_9	61	>300	$C_{27}H_{26}N_4O_4$ (470.51)
22	-	C_6H_5	45	>300	$C_{29}H_{22}N_4O_3S$ (506.56)
23	-	n-C_4H_9	48	>300	$C_{27}H_{26}N_4O_3S$ (486.57)
24	-	C_6H_5	40	95–7	$C_{30}H_{24}N_4O_3S$ (520.58)
25	-	n-C_4H_9	45	67–70	$C_{25}H_{28}N_4O_3S$ (500.59)
26	-	C_6H_5	55	290–3	$C_{36}H_{26}N_6O_3$ (590.62)
27	-	n-C_4H_9	60	282–5	$C_{34}H_{30}N_6O_3$ (570.63)

Table 2. Antimicrobial screening results of the tested compounds at 1000 μg/ml.*

Comp. No.	*P .aeruginosa*	*E .coli*	*S. aureus*	*B. subtilis*	*C. albicans*
2	-	I2	-	-	20
3	-	16	-	-	18
4	9	20	10	-	19
5	-	18	-	-	14
6	-	19	-	-	19
7	-	18	-	-	19
8	-	-	-	-	-
9	-	8	-	-	10
10	-	-	-	-	-
11	-	-	-	-	-
12	-	-	-	-	-
13	8	9	-	-	-
14	-	9	-	-	-
15	-	-	-	-	10
16	-	9	-	-	-
17	-	8	-	-	-
18	-	8	-	-	-
19	-	8	-	-	9
20	-	9	-	-	-
21	-	-	-	-	-
22	-	-	-	-	-
23	-	8	-	-	10
24	-	9	-	-	10
25	-	-	-	-	-
26	-	-	-	-	8
27	-	9	-	-	-
Ampicillin	-	-	22	-	-
Streptomycin	-	20	21	-	-
Nystatin	-	-	-	-	22

-, no activity, (inhibition zone < 7mm), weak activity (7-10 mm), moderate activity (11-15mm), strong activity (>15mm), solvent: DMSO (6mm).

Table 3. Antimicrobial activity for compounds 2-7 in terms of MIC (μg/ml) after 48h.

Comp. No.	*E. Coli*	*C. albicans*
2	2.0	1
3	2.1	1
4	1.5	1.6
5	2	1.9
6	2.1	2.2
7	2.4	1.9
Ampicillin	2.0	-
Streptomycin	1	-
Nystatin	-	2

Table 4. DNA binding activity of compounds **17-19** using methyl green-DNA displacement assay.

Compd. No.	C_{50} (μg/ml)[a]
17	53 ± 2
18	40 ± 4
19	45 ± 1
Daunomycin	30 ± 7

[a]Values represent the concentration (mean ± SD, n = 3) required for 50% decrease in the initial absorbance of DNA-methyl green solution.

References

1. Fry DW., Jackson R., Biological and biochemical properties of new anticancer folate antagonists, Cancer Metastasis Rev.1987; 5 (3): 251-70.
2. Golankiewicz B., Ostrowski T., Goslinski T., Januszczyk P., Zeidle J., Baranowski D., De Clerq E., Fluorescent tricyclic analogues of acyclovir and anciclovirg Astructure-antiviral activity study. J. Med. Chem. 2001; 44:4284-7.
3. Badawy E., Rida S. M., Hazza A. A., Fahmy H.T., Gohar YM., Potential antmicrobial agents I. Synthesis and structure-activity studies of some new Thiazolo[4,5-d]pyrimidine derivatives. Eur. J. Med.Chem.1993; 28:9, 1-6.
4. Ismail A. K., Aboulwafa M O., Synthesis and antimicrobial activity of some tetramethylenethieno[2,3-d]pyrimidine derivatives. Farmaco 1995; 50(9): 611-16.
5. Papalardo MS., Vittorio F., Pasquinucci L., Bousquet E., Synthesis of substituted pyrimidines pyrazole[3,4-d]pyrimidines and evaluation of their antifungal activity. Farmaco 1989; 44(4):483-90.
6. Andrson G. L., Shim J. L., Broom A. D., Pyrido[2,3-d]pyrimidine IV. Synthesis studies leading to various oxo-pyrido[2,3-d]pyrimidine. J. Org. Chem.1976; 23: 327.
7. Grivsky E. M., Lees Sigel C. W., Duch D. S., Nichol C. A., Synthesis and antitumor activity of 2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido-[2,3-d]pyrimidine, J. Med. Chem.1980; 23(3):227-9.
8. El-Sherbeny M. A., El-Ashmawy M. B., El-Subbagh H. I., El-Emam A. A., Badria F. A., Synthesis, antmicrobial and antiviral evaluation of certain thienopyrimidine derivatives. Eur. J. Med. Chem.1995; 30:445-9.
9. Rival Y., Michael G., Synthesis and antibacterial activity of some imidazo[1,2-a]pyrimidine derivatives. Chem. Pharm. Bull.1992; 40:1170-76.
10. Gupta A., Shaihla A., Mital R. L., Parkash L., Condensation products of 2-amino-3-cyano-4,6-disbustituted pyridine with carbon disulphide thiourea, urea and formamide and their antibacterial activity. Farmaco 1992;47:979-83.
11. Aso K., Hitaka T., Yukishige K., Ostu k., Akimoto H., Synthesis and antitumor activity of pyrrolo[2,3-d]pyrimidine antifolate with bridage chain contaning a nitrogen atom.Chem. Pharm. Bull. 1995; 43:256-61.
12. Da Settimo A., Da Settimo F., Marini A. M., Primofiore G., Salerno S., Viola G., Via LD., Magno SM., Synthesis, DNA binding and in vitro antiproliferative activity of purinoquinazoline, pyridopurine and pyrido-pyimido-benzimidazole derivatives as ponential antitumor agents. Eur. J. Med. Chem. 1998; 33:685-96.
13. El-Bendary E. R., El-Sherbeny M. A., Badria F. A, Synthesis and biological evaluation of certain new Thiazolo-pyrimidines. Boll. Chim Farmaceutico.1998; 137:115-9
14. El-Bendary E. R., Badria F. A., Synthesis, DNA-binding and antiviral activity of certain pyrazolo[3,4-d]pyrimidine derivatives. Arch. Pharm. Med. Chem. 2000; 333:99-103.
15. Denny W. A., DNA minor groove alkylating agents. Curr. Med. Chem. 2001; 8:516- 33.
16. Palma C., Cri scuoli M., Lippid A., Muratori M., Mauro S., Maggi C. A. Effect of the aromatase inhibitor,MEN 11066, on growth of two different MCF-7 sublines. Eur.J.Pharmacol. 2000; 409: 93- 101
17. Bohnenstengel F. I., Steube K. G., Meyer C., Quentmeier H.,Nugroho B. W., Proksch P. 1H-Cyclopenta[b]benzofuran lignans from Agalia species inhibit cell broliferation and alter cell cycle distribution in human mono-cyticleukemia cell lines. Z Naturforsch. [c]. 1999; 54:1075-83.
18. Pieters L., Van Dyck S., Gao M., Bai R., Hamel E., Vlietinck A., Lemi G. Synthesis and biological evaluation of dihydrobenzofuran lignans and related compounds as potential antitumor agents that inhibit tubulin polymerization. J. Med. Chem. 1999; 42: 5475-81.
19. Omar M. T., Kassem E. M., Kamel M. M., Khalifa N. M., Synthesis of some new substituted anilinoquinolines of expected biological activity. Egypt. J. Pharm. Sci. 1997; 48:251-9.
20. Goda F. E., New benzofurylpyridines as possible anti-microbial agent. Alex. J. Pharm. Sci. 1999; 13 (2): 145-8.
21. Clark A. M., El-Feraley F. S., .Iu W. S., Antimicrobial activity of phenolic constituents of magnolia grandiflora L. J. Pharm. Sci. 1981;70,951.
22. Blair J. E, Lennette E. H., Traunt, J. P., Manual of Clinical Microbiology Am. Soc. for microbiology, Bethesda MD, 1970; 300.
23. Burres N. S., Frigo A., Rasmussen R. R., Mcalpine J. B., A colorimetric microassay for the detection of agents that Interact with DNA. J. Nat. Prod. 1992; 55: 1583 -720.