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New Pyridone, Furopyridine and Pyrazolopyridine Derivatives Bearing 5,6,7,8-Tetrahydronaphthalene Moiety: Synthesis, Antimicrobial and Genotoxicity Evaluation

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REACTION of 2-acetyl-5,6,7,8-tetrahydronaphthalene (1) with different aromatic aldehydes yielded the corresponding cyanopyridone derivatives 2a,b. Upon heating of 2a with chloroacetone, led to the formation of furopyridine derivative 4, while the reaction of compounds 2a,b with ethyl bromoacetate afforded 3–cyanopyridinyl derivatives 5a, b. Condensation of 5a,b with different amines gave the compounds 6a, b, 7 or 8a, b, respectively. Pyrazolopyridine derivatives 9a, b, 10a, b, 11 were obtained upon the reaction of compound 8a with different aromatic aldehydes, ketones and/or substituted malononitrile. The antimicrobial activity evaluation exhibited that 7, 10a,b are promising antibacterial and antifungal agents. Genotoxicity evaluation indicated that both 2a, 8a produced a protection against cytogenetic changes induced by cyclophosphamide in mice.

Keywords: Pyridone, Furopyridine, Pyridine, Pyrazolopyridine and Genotoxicity.

The number of life threatening infections caused by multidrug-resistant Gram-positive bacilli, methicillin-resistant strains of *Staphylococcus aureus* (MRSA) and Gram-negative bacilli (GNB) is a major obstacle in the management of infections in hospitals and the community. Moreover, over the past decade, fungal infections became an important complication and a major cause of morbidity and mortality in immunocompromised individuals such as those suffering from tuberculosis, cancer or AIDS and in organ transplant cases[1]. In recent years, much attention has been focused on addressing the problem of multi-drug resistant (MDR) bacteria and fungi resulting from the widespread use and misuse of classical antimicrobial agents[2]. Such serious global health problem demands a renewed effort seeking the development of new antimicrobial agents that are effective against pathogenic microorganisms resistant to currently available treatments.

Drug discovery programs have reported that different heterocylic derivatives bearing tetralin moiety have been developed and tested as anticancer agents[3-5],

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antiviral[6], antibilharzial[7], antibacterial and antifungal agents[8,9]. Furthermore, novel 2- aminotetralin derivatives were synthesized as antifungal agents and they exhibited much higher antifungal activities against all of the four fluconazole-resistant clinic *Candida albicans* strains than the control drugs including amphotericin B, terbinafine, ketoconazole, and itraconazole[10]. Antibacterial studies showed that tetralin is toxic to bacterial cells and due to its hydrophobicity, it partitions into lipid membranes, thus excessive accumulation of tetralin causes expansion of the membrane and impairment of different membrane functions. Tetralin makes the membrane permeable for ions (protons) and inhibits the respiratory enzymes, which leads to a partial dissipation of the pH gradient and electrical potential, an effect that could lead to the impairment of various metabolic functions and to low growth rates [11].

Different studies of heterocyclic ring systems showed that many 3-cyanopyridone, pyridine and pyrazolo[3,4-*b*]pyridine derivatives exhibited antimicrobial activity[12,13] in addition to other diverse biological and pharmacological activities, such as anti-inflammatory [14], anticancer[15-17], anxiolytic[18] , cardiotonic and Ca2+ channel-blocking properties in vascular smooth muscle[19] glycogen synthesis kinase-3 (GSK-3) and phospholipase A_2 inhibition [20, 21].

In continuation of our search for various biologically active molecules[15, 16, 22, 23], it was considered worthwhile to synthesize new derivatives bearing tetralin moiety incorporated to various heterocycles such as cyanopyridone, pyridine and pyrazolo [3,4-*b*] pyridine to evaluate their antibacterial and antifungal efficiency against a number of pathogenic Gram-positive, Gram-negative and fungi and find the minimum inhibitory concentration of those that exhibited promising antimicrobial activity with the hope to discover new effective antimicrobials that can overcome the resistance problem of the pathogenic microbes.

Chemoprevention of mutation–related diseases and cancer are an important research field and the dietary use of antimutagens and anticarcinogenes has been proposed as the most promising approach for protection of human health[24]. The two derivatives 2a and 8a were selected as representative examples of the role of tetralin nucleus when attached to different types of heterocyclic rings of reported anticancer activity such as: 3-cyano-2-pyridone and pyrazolopyridine, in modulating the genetic damage induced by indirect mutagenic cyclophosphamide. This process was evaluated by applying the mice bone marrow chromosomal aberrations test.
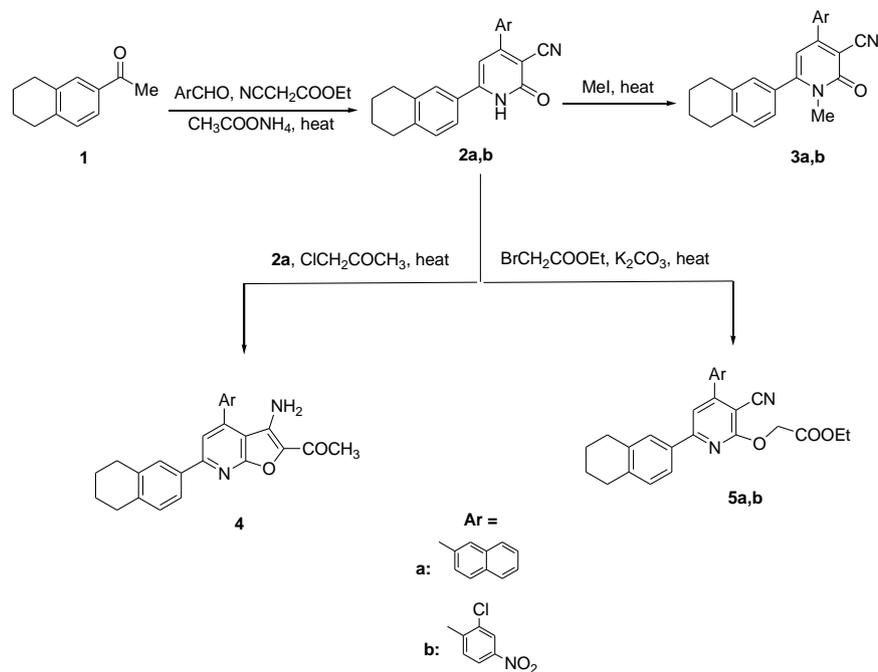
Results and Discussion

Chemistry

The starting material 2-acetyl-5,6,7,8-tetrahydronaphthalene (1) was prepared following the literature method[25], and then underwent cyclocondensation using one pot reaction method with a mixture of the appropriate aromatic aldehydes, namely: naphthalenaldehyde and/or 2-chloro-5-nitrobenzaldehyde, ethylcyano-acetate, excess ammonium acetate in n-butanol to give the corresponding 2-oxo-

pyridine-3-carbonitriles (2a,b).Analytical and spectral data of the aforementioned compounds were in agreement with the proposed structures. IR spectra of 2a,b revealed the presence of the absorption bands corresponding to NH, CN and C=O at $\approx$ 3132, 2220, 1640 cm^{-1}, respectively. Also, ^{1}H NMR spectra of the same compounds showed singlet signals at $\delta \approx$ 8.48 and 12.30 ppm (D$_2$O exchangeable) due to pyridine-H5 and NH protons, respectively. The mass spectra showed the molecular ion peaks of the compounds as base peaks at m/z 376, 405, respectively.

2-Pyridone nucleus has two tautomeric forms (hydroxy and oxo forms), under basic conditions and it normally yields O- or/and N-alkylated products. Thus, N-methylated products (3a,b) were obtained regioselectively in high yields when 2a,b were stirred with methyl iodide in the presence of potassium carbonate at 0^{0}C for 3 hr. The structures of the isolated products were confirmed on the basis of their elemental analyses and spectral data. For example, IR spectra revealed the presence of absorption bands at $\approx$ 1636 cm^{-1} due to C=O groups and the disappearance of NH bands. Also, ^{1}H NMR spectra showed singlet signals at δ 4.17 ppm due to N-Me groups and the disappearance of NH singlet signals. In addition, mass spectra showed the expected molecular ion peaks of 3a,b.

Compound 2a was treated with α-chloroacetone in DMF in the presence of potassium carbonate to yield the furopyridinyl derivative 4 and its structure has been established on the basis of microanalytical and spectral data. IR spectrum revealed two absorption bands at 3426, 3328 cm^{-1} due to NH$_2$ group and at 1668 cm^{-1}due to C=O group. In addition, its ^{1}H NMR spectrum showed singlet signals at δ 2.44 and 5.86 ppm (D$_2$O exchangeable) due to the protons of COCH$_3$ and NH$_2$ groups, respectively. On the other hand, when compounds 2a,b were treated with ethyl bromoacetate in dry acetone, they afforded 3-cyanopyridinyl oxyacetic acid ethyl esters (5a,b). IR spectra of the two compounds showed the appearance of the respective absorption bands at 1752 and 1749 cm^{-1} due to the ester C=O groups and the disappearance of the absorption bands of pyridine C=O groups. Also, ^{1}H NMR spectra showed the characteristic triplet-quartet pattern of the ethyl ester groups at δ 1.22 and 4.22 ppm. A singlet signal referring to OCH$_2$ appeared at $\approx \delta$ 5.12 ppm. Mass spectra showed the molecular ion peak of 5a at m/z 462 and two isotopic peaks for 5b at m/z 491, 493 (3:1)* (Scheme 1).
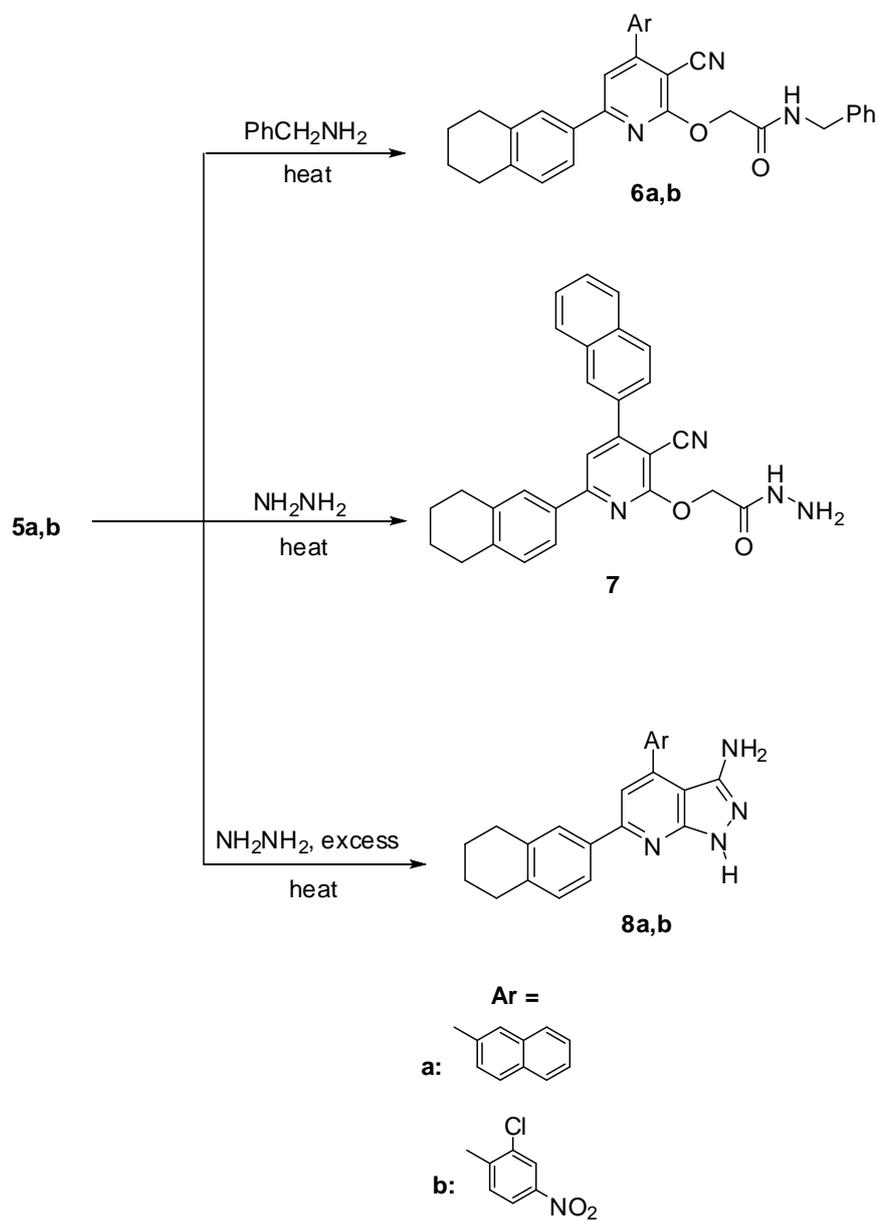
The reaction of compounds 5a,b with benzyl amine under reflux, gave the corresponding pyridin-2-yl oxyacetamido derivatives (6a,b). Their structures were supported by correct elemental analyses and spectral data. Their IR spectra showed the appearance of the absorption bands due to NH, CN, and C=O groups. Also, their ^{1}H NMR spectra showed douplet, singlet signals at δ 4.35 and 5.11 ppm corresponding to -HN-CH$_2$ and OCH$_2$ groups, respectively. Other singlets appeared at $\approx \delta$ 8.70 ppm (exchangeable with D$_2$O) due to NH groups.
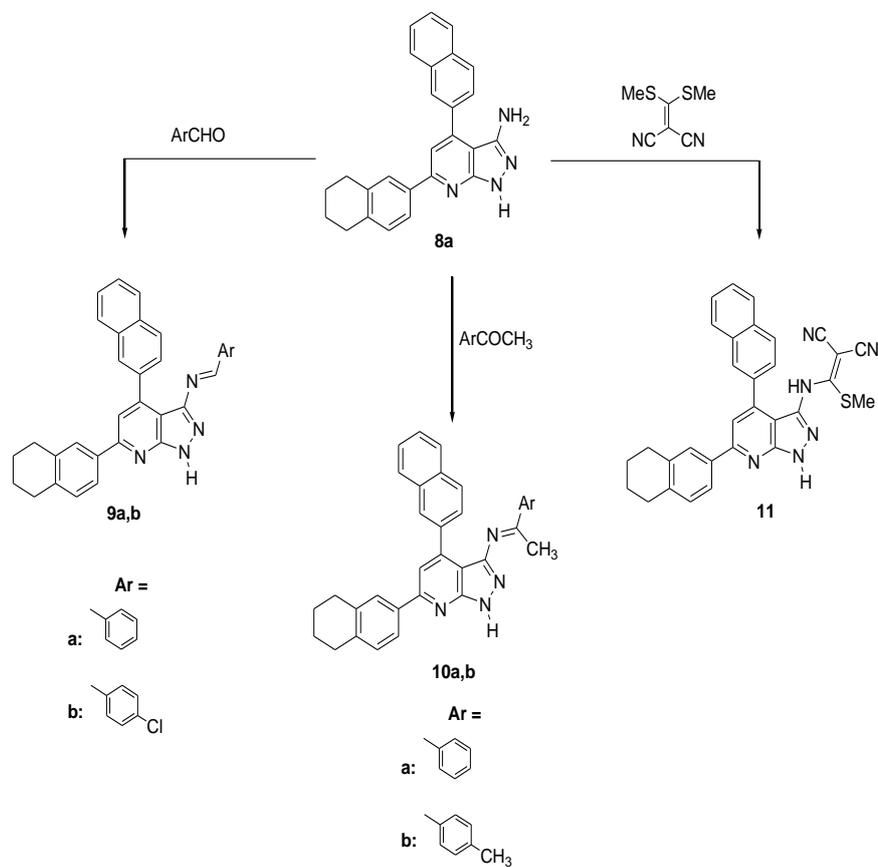
Scheme 1

Hydrazinolysis of compound 5a, with hydrazine hydrate in ethanol, gave the hydrazide derivative 7. Its IR spectrum showed the appearance of three absorption bands at 3438, 3317 and 3266 cm^{-1} due to NH$_2$/NH groups in addition to the CN absorption band at 2222 cm^{-1} and that of C=O appeared at 1653 cm^{-1}. Also, ^{1}H NMR spectrum exhibited singlet signals (exchangeable with D$_2$O) due to NH$_2$ and NH groups at δ 4.30 and 9.42 ppm, respectively. Reflux of 5a,b with excess hydrazine hydrate, afforded the pyrazolopyridine derivatives 8a,b (Scheme 2). The structures of the latter compounds were confirmed on the basis of their elemental analyses and spectral data. IR spectra of 8a,b showed the disappearance of absorption bands due to CN groups and the appearance of absorption bands at $\approx$ 3418, 3333, 3319 cm^{-1} due to NH$_2$, NH, respectively. Also, their mass spectra showed the expected molecular ion peaks.

When compound 8a was refluxed with different aromatic aldehydes; namely: benz- aldehyde and p-chlorobenzaldehyde for 10 hr, it gave the corresponding Schiff's bases 9a,b, respectively. Elemental analyses and spectral data confirmed the chemical structures of the derivatives. IR spectra showed the disappearance of the absorption bands due to NH$_2$, and the appearance of absorption bands due to NH at 3154, 3149 cm^{-1} , respectively. Their ^{1}H NMR spectra revealed singlet signals at $\approx$ δ 8.94 ppm referring to the azomethine proton of -N=CH groups, in addition to the singlet signals at $\approx$ δ 13.62 ppm due to NH groups. Mass spectra

displayed the expected molecular ion peaks at m/z 390, 419, respectively (Scheme 3).

Scheme 2

ArCHO

MeS   SMe

NC   CN

8a

ArCOCH₃

Ar

N

N
H

9a,b

Ar =

a:

b: Cl

Ar

N   CH₃

N
H

10a,b

Ar =

a:

b: CH₃

NC   CN

HN
SMe

N
H

11

Scheme 3

Furthemore, when compound 8a was refluxed with acetophenone or p-methylacetophenone in absolute ethanol in the presence of few drops of glacial acetic acid, afforded the corresponding pyrazolo[3,4-*b*]pyridine derivatives 10a,b. IR spectra of the novel compounds showed the disappearance of the absorption bands due to NH₂ group. ^{1}H NMR spectra showed singlet signals at $\delta \approx 2.44$ and 10.31 ppm (D₂O exchangeable) corresponding to the protons of CH₃ and NH, respectively. Mass spectra revealed the molecular ion peaks of the compounds at m/z 492, , respectively. Moreover, heating of compound 8a with [bis (methylthio) methylene] malononitrile, in ethanol, afforded the corresponding pyrazolopyridine derivative 11 in a good yield (Scheme 3). Its IR spectrum showed absorption bands at 3383, 3272 cm⁻¹ due to the 2NH groups and at 2217 cm⁻¹ for 2C≡N. ^{1}H NMR spectrum revealed singlet signals at δ 2.59 ppm due to SCH₃ protons and at δ 4.55 and 12.32 ppm (D₂O exchangeable) referring to the protons of 2NH groups. These arguments confirmed that the

reaction occurred via loss of a methylmercaptan molecule. Mass spectrum displayed the molecular ion peak at m/z 512.

Biological Evaluation

In vitro antibacterial and antifungal activities

All the newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *Bacillus pumiuls* and *Micrococcus luteus* as examples of Gram-positive bacteria and *Pseudomonas aeruginosa and Sarcina lutea* as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *Penicillium crysogenum* and *Candida albicans* as representatives of fungi. Agar-diffusion method was used for determination of the preliminary antibacterial and antifungal activity. Chloramphenicol (antibiotic) and Nystatin (antifungal) were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the discs in mm (Table 1) (Fig.1 & 2). The minimum inhibitory concentration (MIC) measurement was determined for compounds that showed significant growth inhibition zones (≥19 mm) using the two-fold serial dilution method [26]. The MIC (µg/ml) values of the active compounds against the tested bacterial and fungal strains were recorded in Table 2.
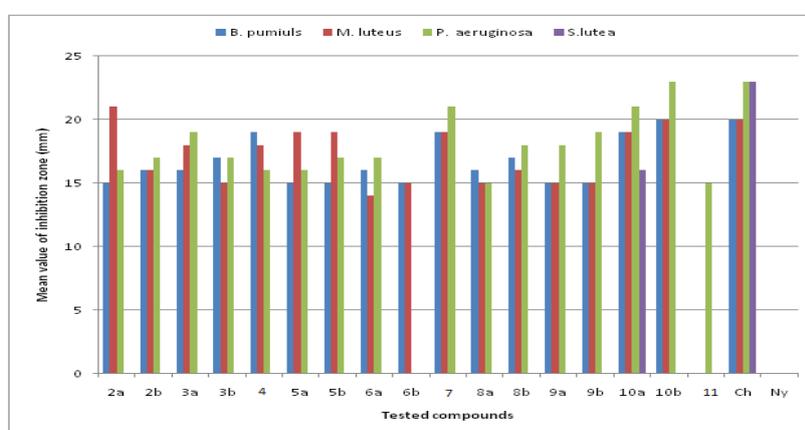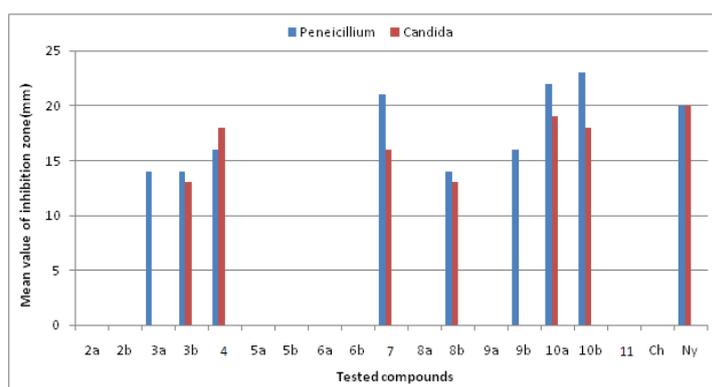
TABLE 1. Inhibition zone in mm as a criterion of antibacterial and antifungal activities of the newly synthesized compounds.

Tested compounds	Gram +ve bacteria		Gram−ve bacteria		Fungi	
	Sarcina lutea	*Pseudomons aeruginosa*	*Micrococcus luteus*	*Bacillus pumiuls*	*Peneicillium*	*Candida*
2a	15.0	21.0	16.0	-ve	-ve	-ve
2b	16.0	16.0	17.0	-ve	-ve	-ve
3a	16.0	18.0	19.0	-ve	14.0	-ve
3b	17.0	15.0	17.0	-ve	14.0	13.0
4	19.0	18.0	16.0	-ve	16.0	18.0
5a	15.0	19.0	16.0	-ve	-ve	-ve
5b	15.0	19.0	17.0	-ve	-ve	-ve
6a	16.0	14.0	17.0	-ve	-ve	-ve
6b	15.0	15.0	-ve	-ve	-ve	-ve
7	19.0	19.0	21.0	-ve	21.0	16.0
8a	16.0	15.0	15.0	-ve	-ve	-ve
8b	17.0	16.0	18.0	-ve	14.0	13.0
9a	15.0	15.0	18.0	-ve	-ve	-ve
9b	15.0	15.0	19.0	-ve	16.0	-ve
10a	19.0	19.0	21.0	16.0	22.0	19.0
10b	20.0	20.0	23.0	-ve	23.0	18.0
11	-ve	-ve	15.0	-ve	-ve	-ve
Ch	20	20	23	23	--	-
Ny	-	-	-	-	20	20

Ch: Chloramphenicol; Ny:Nystatin.

TABLE 2. Minimum inhibitory concentration (MIC) of the most active compounds (µg/ml).

Tested microorganism	Minimum inhibitory concentration (µg/ml)		
	10a	**10b**	**7**
Bacillus pumiuls	75	75	75
Micrococcus luteus	75	75	75
Pseudomonas aeruginosa	75	75	75
Sarcina lutea	1250.0	-ve	-ve
Peneicillium crysogenum	75	75	75
Candida albicans	75	75	75

Fig. 1. Inhibition zone in mm as a criterion of antibacterial activity of the newly synthesized compounds .

Fig. 2. Inhibition zone in mm as a criterion of antifungal activity of the newly synthesized compounds.

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In general, most of the tested compounds revealed better activity against the Gram-positive rather than the Gram-negative bacteria. The tested Gram-positive bacteria and the Gram negative *Pseudomonas aeruginosa* showed relative high sensitivity towards the tested compounds, specially compounds 7, 10a,b which exhibited the highest potency.

All the tested derivatives were devoid of any antibacterial activity against the Gram-negative *Micrococcus luteus* with exception, compound 10a which exhibited a moderate antibacterial activity against this microorganism. According to the structures of the compounds tested, it can be investigated that the presence of tetralin nucleus in combination with pyridine functionality gave derivatives of promising antibacterial activity against the tested bacteria. The attachment of CN at C_3 and oxy acetic acid hydrazide side chain at C_2 of the pyridine ring as compound 7 and the fusion of pyridine ring with furan and/or pyrazole heterocycles to give furo[2,3-b]pyridine-ethanone derivative (4) and pyrazolo[3,4-*b*]pyridin-3-yl]-[1-phenyl (tolyl) eth-(E)-ylidene]-amine derivatives 10a,b, respectively produced an antibacterial activity against the tested Gram positive bacteria that is approximately equipotent to that obtained by the reference chloramphenicol. Meanwhile the activity of the same analogs was slightly less potent than chloramphenicol against the Gram-negative *Pseudomonas aeruginosa* except compound 4 which was moderately active. The replacement of [1-phenyl (tolyl) eth-(E)-ylidene]-amino side chain of 10a,b with amino methylsulfanyl methylene malononitrile substituent as compound 11 led to complete loss of activity against the Gram-positive bacteria and moderate activity against *Pseudomonas aeruginosa*. Furthermore, it can be noticed that the combination of tetralin moiety with 3-cyano-2-oxopyridine as 3a and the attachment of azomethine side chain to the pyrazolo[3,4-*b*]pyridine ring as 9b exhibited high antibacterial profile against *Pseudomonas aeruginosa* but less active than chloramphenicol.

Concerning the antifungal activity of the tested compounds, the analogs 3b, 4, 8b, 9b showed moderate antifungal activity against the two tested fungi in comparison to nystatin; the reference drug used. Meanwhile, the analogs 7 & 10a,b revealed higher potency than that of nystatin against *Penicillium crysogenum* and a slight lower activity against *Candida albicans*.

Since the compound 7 & 10a,b showed the highest antibacterial activity against the tested Gram positive and Gram-negative bacteria, their minimum inhibitory concentration (MIC) measurement was determined for them. These compounds exhibited minimum bacterial and fungal growth inhibition at the same concentration (75µg/ml). The Gram-negative *Sarcina lutea* appeared to be completely insensitive against compounds 7 & 10b and slightly sensitive against 10a.

Cytogentic analysis

The data on chromosomal aberrations are shown in Table 3 and they are presented graphically in Fig. 3. Cytogenetic results showed that the frequencies

of total structural chromosome aberrations were low in the two groups of mice which were treated with single treatments of cyanopyridone 2a and pyrazolopyridine 8a compared to those obtained by the control group of mice.

TABLE 3. **Induction of chromosomal aberration by cyclophosphamide (CP), cyanopyridone 2a and pyrazolopyridine 8a with different treatments in male mice.**

Treatment	No. of animals	No. of examine	Structural aberration			Centric fusion	Total aberration	Numerical aberration Polyploidy
			gap	break	delation			
control ve[-]	10	500	0.20± 0.44[a]	0.20 ± 0.44[a]	0.20 ± 0.44[a]	0.40 ± 0.54[a]	1.00 ± 1.73[a]	0.40 ± 0.54[a]
positive control ve[+] (CP)	10	500	6.0 ± 0.00[d]	5.60 ± 0.54[d]	12.20 ± 0.44[d]	16.60 ± 0.54[d]	40.4 ± 0.54[e]	4.40 ± 0.54[d]
cyanopyridone	10	500	0.40 ± 0.54[a]	0.20 ± 0.44[a]	0.40 ± 0.54[a]	0.60 ± 0.54[a]	1.60 ± 0.54[a]	0.60 ± 0.54[a]
pyrazolopyridine	10	500	0.60 ± 0.54[a]	0.40 ± 0.54[a]	0.60 ± 0.54[a]	0.80 ± 0.44[a]	2.40 ± 0.54[b]	1.20 ± 0.44[b]
cyanopyridone + CP	10	500	0.24 ± 0.54[b]	1.60 ± 0.54[b]	4.60 ± 0.540[b]	14.80 ± 0.83[b]	16.40 ± 0.54[c]	1.40 ± 0.54[b]
pyrazolopyridine + CP	10	500	4.20 ± 0.83[c]	3.00 ± 0.00[c]	8.40 ± 0.54[c]	14.80 ± 0.83[c]	30.40 ± 0.54[d]	3.00 ± 0.00[c]

The different letters (a, b, c, d) in the same column are significantly different at level (P≤0.05) all values were expressed as Mean ± S.E.
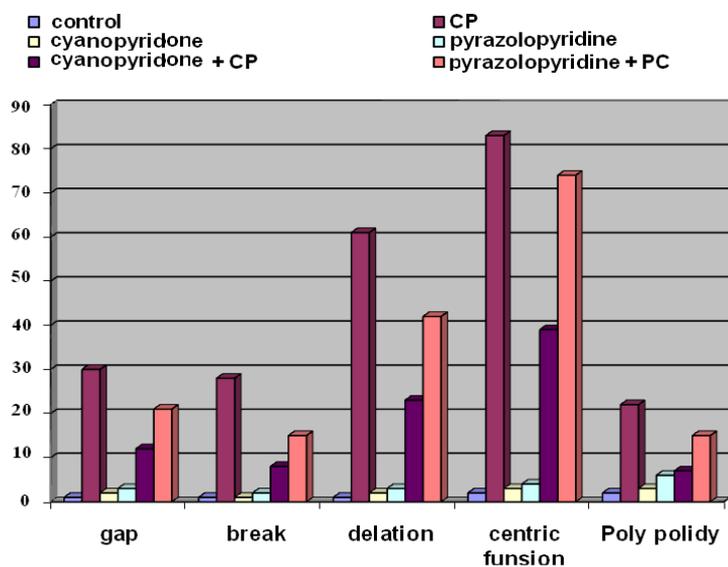

Fig. 3. Induction of chromosomal aberration by cyclophosphamide (CP), canopyridone 2a and pyrazolopyridine 8a with different treatments in male mice .

The established mutagen CP significantly induced chromosomal aberrations in male mice as compared to the control and the two tested derivatives.

The combined treatments of cyanopyridone or pyrazolopyridine 2a & 8a with CP, resulted in a significant decrease in the frequency of the total chromosomal aberrations in bone marrow of male mice induced by CP (25mg/kg b.w.). The decrease of the total chromosomal aberrations observed by cyanopyridone and CP combination is higher than that obtained by pyrazolopyridine and CP combination.

The obtained data of the present study showed that the pyridine-bearing derivatives 2a & 8a have some antimutagenic properties against CP induced chromosomal aberrations, the results that were confirmed by the reported studies[27]. These alterations could be attributed to their chemical structures and biological activity.

Also, no induction of chromosomal aberrations was noted in bone marrow cells of male mice that were given cyanopyridone 2a or pyrazolopyridine 8a. These results are in agreement with the literature [28] that studied the genetic profiling of pyridine derivatives. Moreover, exposure to pyridine compounds in drinking tap water led to reduction of sperm motility at all dose levels in mice and increase of estrous cycle length at the highest dose level in rats[28]. However, the sex linked recessive lethal assay in *Drosophila melanogaster* and aneuploidy in fungal system, all tests covering arrange of end-points for genetic toxicology of pyridine compounds, gave negative results[28]. Also, pyridine compounds do not induce unscheduled DNA synthesis and were found to be non genotoxic in male mice [29]. Anuszewska and Koziorowska[30] reported that pyridine N-oxide showed protective effects against 3-chloropyridine-induced cytotoxicity and clastogenicity *in vitro.*

According to our results, pyridine compounds lack the potential for genetic toxicity tests, tests conducted include Ames assay, rat lymphocyte, chromosomal aberration assay, mouse bone marrow cytogenicity assay and mouse micronucleus assay, all produced negative results[31]. Moreover, the substitution at N-terminal atom in pyrazole ring plays a key role in the antitumor and anti-angiogenic effects[32]. On other hand, the photo-treatment of 2-chloropyridine produced genotoxic products using micronucleus assay [33].

Conclusion

This study deals with synthesis of new derivatives of tetralin that is attached to various heterocyclic ring systems and evaluation of their antibacterial and antifungal activity as a trial to discover new broad spectrum antimicrobial agents. The data obtained revealed that most of the tested compounds showed better activity against the Gram-positive rather than the Gram-negative bacteria. About ten derivatives exhibited moderate activity against the tested Gram-positive bacteria, three compounds 7 & 10a,b showed a remarkable antibacterial effect and only one 11 was completely inactive. With respect to the examined Gram-negative

Pseudomonas aeruginosa: most of the derivatives were moderately active, five derivatives 3a, 7, 9b, 10a,b were highly active and only one 6b was inactive. The whole derivatives, except 10a were completely devoid of antibacterial potency against the Gram negative *Sarcina lutea*. Moderate antifungal activity was obtained by 3a,b, 4, 8b, 9b while the highest antifungal activity appeared by the same compounds 7, 10a,b. The two fungal strains tested showed complete insensitivity towards the other derivatives. The data investigated that the attachment of cyanopyridone, furopyridine and pyrazolopyridine heterocycles to tetralin nucleus produced derivative of a remarkable antimicrobial efficiency.

Another aim of this work is to study the protective effects of some pyridine derivatives against the induction of chromosomal aberration by cyclophosphamide (CP). Cyanopyridone 2a and pyrazolopyridine 8a were selected as representative examples depending on the reported antiacancer activity of these nuclei[15-17]. The present data revealed that both 2a and 8a showed good protective effects against CP-induced cytogenetic disorders, whereas the chromosomal aberrations in animals treated with the two derivatives alone were approximately negligible comparing to the negative control.

Ongoing studies will be to determine the precise mechanism of action of the two compounds to reveal their potential as therapeutical agents.

Experimental

Chemistry

All melting points were uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were performed by Vario El-Mentar apparatus (Shimadzu, Japan), National Research Centre (NRC), Cairo, Egypt. The found values were within ±0.4% of the theoretical values. IR spectra (KBr) were recorded on a Perkin-Elmer 1650 spectrophotometer, NRC. ^{1}H NMR and ^{13}C NMR spectra were determined on a Varian Mercury (300 MHz) spectrometer (Varian, UK) and the chemical shifts were expressed in δ ppm relative to TMS as an internal reference, Faculty of Science, Cairo University, Egypt. Mass spectra were recorded at 70 eV on EI Ms-QP 1000 EX (Shimadzu, Japan), NRC. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-protected glass plates and the spots were detected by exposure to UV-lamp at λ 254.

1-(5,6,7,8-tetrahydronaphthalen-2-yl) ethanone (1)

This compound was prepared according to the previously reported procedure[25].

General procedure for synthesis of compounds (2a,b)

A mixture of 1-(5,6,7,8-tetrahydronaphthalen-2-yl)ethanone (**1**) (1.7g, 10 mmol), aromatic aldehydes namely: 2-naphthaldehyde, 2-chloro-5-nitrobenzaldehyde, ethyl cyanoacetate (1.1g, 10 mmol) and ammonium acetate (6.2g, 80 mmol) in n-butanol (40 ml) was refluxed for 3 hr. The obtained precipitate was filtered off, washed

successively with ethanol and recrystallized from ethanol/DMF to give compounds 2a,b respectively,as yellow crystals.

4-(Naphthalen- 2-yl)-2- oxo-6- (5,6, 7,8- tetrahydronaphthalen- 2-yl)-1,2-dihydropyridine-3-carbonitrile (2a)

Yield (86%); m.p. 304-306°C; IR (KBr, cm^{-1}): 3132 (NH), 2930 (CH alicyclic), 2216 (CN), 1627 (CO); ^{1}H NMR (DMSO-d_6, δ ppm) : 1.75 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 2.77(m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 6.91 (s, 1H, Ar-H), 7.20 (d, J = 7.80 Hz, 1H, Ar-H), 7.60-7.83 (m, 5H, Ar-H), 8.01-8.11 (m, 3H, Ar-H), 8.30 (s, 1H, pyridine-H5), 12.30 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (DMSO- d_6): δ (ppm): 22.34, 22.40, 28.63, 28.68 (4CH$_2$), 127.49 (CN), 105.50, 116.51, 124.55, 125.19, 126.81, 127.58, 128.06, 128.12, 128.53, 129.23, 129.35, 132.37, 133.33, 133.55, 137.37, 140.52 (Ar-C), 161.95 (CO); ^{13}C NMR (DMSO- d_6): δ (ppm): 22.56, 22.64, 28.55, 28.83 (4CH$_2$), 115.9 (CN), 116.1, 116.3, 120.6, 125.7, 127.4, 127.5, 128.5, 128.7, 129.1, 138.4, 154.5 (Ar-H), 160.9 (CO). MS: m/z (%): 377 (M$^+$+1, 28), 376 (M$^+$, 87), 375 (M$^+$-1, 41), 245 (53), 128 (100), 77 (36). Analysis for C$_{26}$H$_{20}$N$_2$O (376.46): Calcd. C, 82.95; H, 5.35; N, 7.44; found: C, 82.86; H, 5.44; N, 7.59.

4-(2-Chloro-5-nitrophenyl)- 2-oxo -6- (5,6,7,8- tetrahydronaphthalen-2-yl)-1,2-dihydropyridine-3-carbonitrile (2b)

Yield (82%); m.p. 218-220°C; IR spectrum (KBr, cm^{-1}): 3130 (NH), 2928 (CH, alicyclic), 2222 (CN), 1640 (CO), 1512, 1341 (NO$_2$); ^{1}H NMR (DMSO-d_6, δ ppm): 1.75 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 2.75 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 6.89 (s, 1H, Ar-H), 7.20 (d, J = 8.10 Hz, 2H, Ar-H), 7.59-8.01 (m, 3H, Ar-H), 8.32(s, 1H, pyridine-H5), 12.92 (s, 1H, NH, D$_2$O exchangeable);); ^{13}C NMR (DMSO- d_6): δ (ppm): 22.5, 22.6, 28.5, 28.8 (4CH$_2$), 115.9 (CN), 115.2, 116.3, 120.4, 124.0, 124.6, 125.8, 128.5, 129.3, 131.1, 135.0, 135.2, 138.6, 149.8, 154.6 (Ar-H), 160.9 (CO); MS: m/z (%): 405 (M$^+$, 100), 407 (M$^+$ +2, 33); Analysis for C$_{22}$H$_{16}$ClN$_3$O$_3$ (405.86): Calcd. C, 65.10; H, 3.97; Cl, 8.73; N, 10.35; found: C, 64.86; H, 4.22; Cl, 8.70; N, 10.21.

General procedure for synthesis of compounds (3a,b)

To a solution of compound 2a or 2b (10 mmol) and potassium carbonate (1 g) in dry DMF (20 ml), methyl iodide (2.8 g, 2 mmol) was added at 0°C. The reaction mixture was stirred at the same temperature for 3 hr. The reaction mixture was kept at room temperature over night, then poured onto ice-cold water and the precipitated solid was collected , recrystallized from ethanol/DMF to give compounds 3a,b, respectively.

1-Methyl-4-(naphthalen-2-yl)- 2- oxo-6-(5,6,7,8- tetrahydronaphthalen-2-yl)-1,2-dihydropyridine-3-carbonitrile (3a)

Yield (86%); m.p. 150-152°C; IR spectrum (KBr, cm^{-1}): 2925 (CH, alicyclic), 2117 (CN), 1636 (C=O); ^{1}H NMR (DMSO-d_6, δ ppm) : 1.77 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 2.78 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 4.17 (s, 3H, NCH$_3$), 7.21 (s, 1H, Ar-H), 7.61-7.67 (m, 2H, Ar-H), 7.78-8.32 (m,

7H, Ar-H); 8.30 (s, 1H, pyridine-H5). MS *m/z* (%): 389 (M$^+$-1, 99); 390 (M$^+$, 100), 391 (M$^+$+1, 23); Analysis for $C_{27}H_{22}N_2O$ (390.49): Calcd.C, 83.04; H, 5.67; N, 7.17; found: C, 83.21; H, 5.56; N, 7.23.

4-(2-Chloro-5- nitrophenyl)-1- methyl-2- oxo- 6-(5,6,7,8-tetrahydronaphthalene-2-yl)-1,2-dihydropyridine-3-carbonitrile (3b)

Yield (81%); m.p. 192-194°C; IR spectrum (KBr, cm^{-1}): 2924 (CH, alicyclic), 2213 (CN), 1622 (C=O), 1508, 1328 (NO$_2$); ^{1}H NMR (DMSO-d_6, δ ppm) : 1.76 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 2.78 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 4.18 (s, 3H, NCH3), 6.90 (s, 1H, Ar-H), 7.22 (d, *J* = 8.20 Hz, 2H, Ar-H), 7.61-7.97 (m, 3H, Ar-H), 8.30 (s, 1H, pyridine-H5). MS *m/z* (%): 419 (M$^+$, 100), 421 (M$^+$+2, 33); Analysis for $C_{23}H_{18}ClN_3O_3$ (419.86): Calcd. C, 65.79; H, 4.32; Cl, 8.44; N, 10.00; found: C, 65.66; H, 4.52; Cl, 8.60; N, 10.11.

1-[3-Amino-4-naphthalen-2-yl-6- (5,6, 7,8-tetrahydronaphthalen- 2-yl)- furo [2,3-b] pyridin-2-yl]-ethanone (4)

To a stirred suspension of compound 2a (1.13 g, 0.003 mol) and chloroacetone (0.278 g, 0.003 mol) in dry DMF (20 ml), potassium carbonate (1gm) was added. The reaction mixture was stirred at reflux temperature for 4 hr. The mixture was cooled, poured onto crushed ice-water and neutralized with dil. HCl to deposit the required solid, which was recrystallized from ethanol/DMF to give compound 4. Yield (80%); m.p. 210-212°C; IR (KBr, cm^{-1}): 3426, 3328 due to (NH$_2$), 2924 (CH alicyclic), 1668 (C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 1.77 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 2.44 (s, 3H, COCH$_3$), 2.79 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 5.86 (s, 2H, NH$_2$, exchangeable with D$_2$O), 7.20 (d, *J* = 7.80 Hz, 1H, Ar-H), 7.63-8.28 (m, 10H, Ar-H); ^{13}C NMR (DMSO- d_6): δ (ppm) 23.3 (CH$_3$), 22.5, 22.6, 28.5, 28.8 (4CH$_2$), 124.3, 125.8, 125.9, 127.4, 128.7, 129.1, 130.2, 138.4, 149.9, 151.1 (Ar-H), 160.2 (CO). MS *m/z* (%): 432 (M$^+$, 5); 417 (M$^+$-CH$_3$, 53), 294 (100); Analysis for $C_{29}H_{24}N_2O_2$ (432.53): Calcd. C, 80.53; H, 5.59; N, 6.48; found: C, 80.68; H, 5.51; N, 6.52.

General procedure for synthesis of compounds 5a,b

A mixture of compound 2a or 2b (10 mmol), ethyl bromoacetate (10 mmol), potassium carbonate (40 mmol) in dry acetone (30 ml) was refluxed for 20 hr. After cooling, water was added to the mixture and the formed solid was filtered off, recrystallized from the ethanol to give compounds 5a, b, respectively.

[3-Cyano-4-naphthalen-2-yl-6-(5,6, 7,8-tetrahydronaphthalen-2-yl) -pyridin-2-yl oxy]-acetic acid ethyl ester (5a)

Yield (74%); m.p. 170-172°C; IR (KBr, cm^{-1}): 2926 (CH, alicyclic), 2219 (CN), 1752 (C=O); ^{1}H NMR (DMSO-d_6, δ ppm): 1.22 (t, *J* = 6.9 Hz, 3H, CH$_3$ of ethyl gp), 1.76 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 2.78 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 4.22 (q, *J* = 6.9 Hz, 2H, CH$_2$, of ethyl gp), 5.12 (s, 2H, OCH$_2$), 7.20 (s, 1H, Ar-H), 7.61-7.68 (m, 2H, Ar-H), 7.83-8.14 (m, 7H, Ar-H), 8.34 (s, 1H, pyridine H5); ^{13}C NMR (DMSO- d_6): δ (ppm): 16.3, 61.2 (CH$_2$CH$_3$), 64.5 (OCH$_2$), 22.56, 22.64, 28.55, 28.83 (4CH$_2$), 115.9 (CN),

116.1, 116.3, 120.6, 125.7, 127.4, 127.5, 128.5, 128.7, 129.1, 138.4, 154.5 (Ar-H), 160.9 (CO). MS: m/z (%): 462 (M$^+$, 100); Analysis for C$_{30}$H$_{26}$N$_2$O$_3$ (462.55): Calcd. C, 77.90; H, 5.66; N, 6.05; found: C, 78.01; H, 5.62; N, 6.12.

[4-(2- Chloro-5- nitrophenyl)- 3-cyano -6-(5,6, 7,8-tetrahydronaphthalen-2-yl)-pyridin-2-yl oxy]-acetic acid ethyl ester (5b)

Yield (68%); m.p. 172-174˚C; IR spectrum (KBr, cm^{-1}): 2932 (CH, alicyclic), 2220 (CN), 1749 (C=O), 1539, 1337 (NO$_2$); ^{1}H NMR (DMSO-d_6, δ ppm) : 1.22 (t, J = 7.2 Hz, 3H, CH$_3$ of ethyl gp), 1.76 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 2.77 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 4.22 (q, J = 7.2 Hz, 2H, CH$_2$ of ethyl gp), 5.18 (s, 2H, OCH$_2$), 7.20 (d, J = 8.7 Hz, 1H, Ar-H), 7.86-8.04 (m, 4H, 3Ar-H + pyridine H5), 8.40-8.54 (m, 2H, Ar-H); ^{13}C NMR (DMSO- d_6): δ (ppm): 16.3, 61.2 (CH$_2$CH$_3$), 64.5 (OCH$_2$), 22.5, 22.6, 28.5, 28.8 (4CH$_2$), 115.9 (CN), 115.2, 116.3, 120.4, 124.0, 124.6, 125.8, 128.5, 129.3, 131.1, 135.0, 135.2, 138.6, 149.8, 154.6 (Ar-H), 160.9 (CO); MS: m/z (%): 491 (M$^+$, 81); 493 (M$^+$+2, 27); Analysis for C$_{26}$H$_{22}$ClN$_3$O$_5$ (491.93): Calcd. C, 63.48; H, 4.50; Cl, 7.20; N, 8.54; found: C, 63.41; H, 4.89; Cl, 7.12; N, 8.74.

General procedure for synthesis of compounds (6a,b)

To a solution of compound 5a or 5b (10 mmol) in dry ethanol (30 ml), benzyl amine (20 mmol) was added. The reaction mixture was heated at the reflux temperature for 6 hr, cooled, poured onto ice-cold water acidified with HCl. The separated solid was filtered off and recrystallized from acetic acid to give compounds 6a, b, respectively.

N-Benzyl-2-[3-cyano-4-naphthalen-2-yl-6-(5,6,7,8-tetrahydronaphthalen-2-yl)- pyridin-2-yl oxy]-acetamide (6a)

Yield (82%); m.p. 228-230˚C; IR (KBr, cm^{-1}): 3265 (NH), 2929 (CH, alicyclic), 2220 (CN), 1655 (C=O); ^{1}H NMR spectrum (DMSO-d_6, δ ppm): 1.76 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 2.77 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 4.35 (d, J = 5.4 Hz, 2H, NCH$_2$), 5.11 (s, 2H, OCH$_2$), 7.09-7.22 (m, 5H, Ar-H), 7.63-7.66 (m, 3H, Ar-H), 7.83-8.15 (m, 7H, Ar-H), 8.33 (s, 1H, pyridine H5), 8.73 (s, 1H, NH, exchangeable with D$_2$O); ^{13}C NMR (DMSO- d_6): δ (ppm): 34.2 (N-CH2), 66.3 (OCH2), 22.56, 22.64, 28.55, 28.83 (4CH$_2$), 115.9 (CN), 116.1, 116.3, 120.6, 125.7, 127.4, 127.5, 128.5, 128.7, 129.1, 138.4, 151.1, 154.5 (Ar-H), 168.9 (CO). MS: m/z (%): 523 (M$^+$, 5), 294 (100); Analysis for C$_{35}$H$_{29}$N$_3$O$_2$ (523.64): Calcd. C, 80.28; H, 5.58; N, 8.02; found: C, 80.11; H, 5.62; N, 8.12.

N-Benzyl-2-[4-(2-Chloro-5-nitrophenyl)-3-cyano-6-(5,6,7,8-tetrahydronaphthalen -2-yl)- pyridin-2-yl oxy]-acetamide (6b)

Yield (74%); m.p. 236-238˚C; IR (KBr, cm^{-1}): 3293 (NH), 2929 (CH, alicyclic), 2227 (CN), 1652 (C=O), 1522, 1344 (NO$_2$); ^{1}H NMR (DMSO-d_6, δ ppm): 1.75 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 2.76 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 4.34 (d, J = 5.4 Hz, 2H, NCH$_2$), 5.10 (s, 2H, OCH$_2$), 7.11-7.19 (m, 6H, Ar-H), 7.89-8.04 (m, 4H, Ar-H), 8.40-8.47 (m,

2H, Ar-H + pyridine H5), 8.76 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO- d_6): δ (ppm): 34.4 (N-CH2), 66.9 (OCH2), 22.5, 22.6, 28.5, 28.8 (4CH2), 115.9 (CN), 115.2, 116.3, 120.4, 124.0, 124.6, 125.8, 128.5, 129.3, 131.1, 135.0, 135.2, 138.6, 149.8, 15105, 154.6 (Ar-H), 169.7 (CO);   MS: *m/z* (%): 552 (M^+, 10), 554 (M+2, 3.2) Analysis for $C_{31}H_{25}ClN_4O_4$ (552.02): Calcd. C, 67.45; H, 4.56; Cl, 6.42; N, 10.14; found: C, 67.21; H, 4.89; Cl, 6.22; N, 9.94.

[3-Cyano-4-naphthalen-2-yl-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-pyridin-2-yl oxy]- acetic acid hydrazide (7)

A solution of compound 5a (10 mmol) and hydrazine hydrate (1 ml, 99%) in absolute ethanol (30 ml) was refluxed for 6 hr. The separated solid after cooling was filtered off and recrystallized from acetic acid to give compound 7. Yield (73%) ; m.p. 228-230˚C; IR (KBr, cm^{-1}): 3438, 3317, 3266 (NH_2, NH), 2222 (CN), 1653 (C=O); ^{1}H NMR spectrum (DMSO-d_6, δ ppm): 1.77 (m, 4H, alicyclic $2CH_2$ of tetrahydronaphthalene), 2.79 (m, 4H, alicyclic $2CH_2$ of tetrahydronaphthalene), 4.30 (s, 2H, NH_2, D_2O exchangeable), 5.02 (s, 2H, OCH_2), 7.19 (d, 1H, *J* = 8.7 Hz, Ar-H), 7.64-7.66 (m, 2H, Ar-H), 7.83-8.16 (m, 7H, Ar-H), 8.33 (s, 1H, pyridine H5), 9.42 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO- d_6): δ (ppm): 66.9 (OCH2), 22.56, 22.64, 28.55, 28.83 (4CH2), 115.9 (CN), 116.1, 116.3, 120.6, 125.7, 127.4, 127.5, 128.5, 128.7, 129.1, 138.4, 154.5 (Ar-H), 170.9 (CO). MS: *m/z* (%): 448 (M^+, 15), 83 (100). Analysis for $C_{28}H_{24}N_4O_2$ (448.53): Calcd. C, 74.98; H, 5.39; N, 12.49; found: C, 75.11; H, 5.22; N, 12.42.

General procedure for synthesis of compounds (8a,b)

To a solution of compound 5a or 5b (10 mmol) in absolute ethanol (30 ml), excess hydrazine hydrate (10 ml, 99%) was added and the reaction mixture was refluxed for 12 hr. After cooling, the separated solid was filtered off and recrystallized from acetic acid to give compounds 8a,b, respectively.

4-Naphthalen-2-yl-6-(5,6,7,8-tetrahydronaphthalen-2-yl)-1H-pyrazolo[3,4-b] pyridin-3-ylamine (8a)

Yield (75%); m.p. 198-200˚C; IR spectrum (KBr, cm^{-1}): 3418, 3333 (NH_2), 3199 (NH), 2924 (CH, alicyclic), 1600, 1566 (C=N); ^{1}H NMR spectrum (DMSO-d_6, δ ppm): 1.77 (m, 4H, alicyclic $2CH_2$ of tetrahydronaphthalene), 2.79 (m, 4H, alicyclic $2CH_2$ of tetrahydronaphthalene), 4.56 (s, 2H, NH_2, D_2O exchangeable), 7.18 (d, *J* = 8.1 Hz, 1H, Ar-H), 7.56-7.63 (m, 3H, Ar-H), 7.81-8.13 (m, 6H, Ar-H), 8.26 (s, 1H, pyridine H5), 12.32 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO- d_6): δ (ppm): 22.5, 28.5 (4CH2), 124.3, 125.2, 128.7, 129.7, 127.4, 138.4, 149, 151.1 (Ar-H), 155 (C-NH2); MS: *m/z* (%): 390 (M^+, 100), 389 (M^+-H, 29). Analysis for $C_{26}H_{22}N_4$ (390.49): Calcd. C, 79.97; H, 5.67; N, 14.34; found: C, 80.10; H, 5.62; N, 14.12.

4-(2-Chloro-5-nitrophenyl)-6-(5,6, 7,8-tetrahydronaphthalen-2- yl)-1H-pyrazolo [3,4-b]pyridin-3-ylamine (8b)

Yield (68%); m.p. 228-230˚C; IR (KBr, cm^{-1}): 3433, 3327(NH_2), 3215 (NH), 2925 (CH, alicyclic), 1522, 1344 (NO_2); ^{1}H NMR (DMSO-d_6, δ ppm) : 1.76 (m, 4H, alicyclic $2CH_2$ of tetrahydronaphthalene), 2.78 (m, 4H, alicyclic $2CH_2$ of

tetrahydronaphthalene), 5.48 (s, 2H, NH_2, D_2O exchangeable), 6.64-6.73 (m, 2H, Ar-H), 7.15-7.34 (m, 3H, Ar-H), 7.83 (s, 1H, Ar-H), 7.85 (s, 1H, pyridine H5), 12.21 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO- d_6): δ (ppm): 22.5, 28.5 ($4CH_2$), 124.0, 124.3, 125.2, 128.7, 129.7, 131.1, 135.0, 135.3, 138.4, 149.3, 149.9, 151.1 (Ar-H), 155 (C-NH2); MS: m/z (%): 419 (M^+,100), 421(M^++2,33); Analysis for $C_{22}H_{18}ClN_5O_2$ (419.86): Calcd. C, 62.93; H, 4.32; Cl, 8.44; N, 16.67; found: C, 62.61; H, 4.89; Cl, 8.22; N, 16.84.

General procedure for synthesis of compounds (9a,b)

   A mixture of compound 8a (0.67 g, 1.5 mmol) and the appropriate aromatic aldehyde namely: benzaldehyde and/or p-chlorobenzaldehyde (1.5 mmol) in absolute ethanol (30 ml) was refluxed for 6 hr. After cooling, the formed solid was filtered off and recrystallized from acetic acid to give derivatives **9a,b**, respectively.

   [4-Naphthalen-2- yl -6-(5,6, 7,8- tetrahydronaphthalen- 2-yl)-1H-pyrazolo[3,4-b]pyridin-3-yl]-[1-phenylmeth-(E)-ylidene]-amine (9a)

   Yield (85%); m.p. 256-258˚C; IR (KBr, cm^{-1}): 3154 (NH), 2923 (CH, alicyclic); ^1H NMR spectrum (DMSO-d_6, δ ppm): 1.78 (m, 4H, alicyclic $2CH_2$ of tetrahydronaphthalene), 2.80 (m, 4H, alicyclic $2CH_2$ of tetrahydronaphthalene), 7.19-8.02 (m, 15H, Ar-H), 8.47 (s, 1H, pyridine H5), 8.94 (s, 1H, N=CH), 13.62 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO- d_6): δ (ppm): 22.5, 28.5 ($4CH_2$), 124.3, 125.2, 128.7, 129.7, 127.4, 138.4, 149, 151.1 (Ar-H), 155 (C-NH2); 163.7 (CH=N); MS: m/z (%): 478 (M^+, 84);   Analysis for $C_{33}H_{26}N_4$ (478.60): Calcd. C, 82.81; H, 5.47; N, 11.70; found: C, 82.75; H, 5.62; N, 11.82.

   [1-(4- Chlorophenyl)- meth- (E)- ylidene]-4-naphthalen-2- yl)-6- (5,6, 7,8-tetra hydronaphthalen-2-yl)-1H-pyrazolo[3,4-b]pyridin-3-yl]-amine (9b)

   Yield (82%); m.p. 280-282˚C; IR spectrum (KBr, cm^{-1}): 3149 (NH), 2918 (CH, alicyclic); ^1H NMR (DMSO-d_6, δ ppm) : 1.79 (m, 4H, alicyclic $2CH_2$ of tetrahydronaphthalene), 2.82 (m, 4H, alicyclic $2CH_2$ of tetrahydronaphthalene), 7.21-8.06 (m, 14H, Ar-H), 8.45 (s, 1H, pyridine H5), 8.93 (s, 1H, N=CH), 13.64 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO- d_6): δ (ppm): 24.3 (CH_3), 22.5, 28.5 ($4CH_2$), 124.3, 125.2, 128.7, 129.7, 127.4, 138.4, 149, 151.1 (Ar-H), 155 (C-NH2); 163.7 (CH=N); MS: m/z (%): 512 (M^+, 81); 514 (M^++2, 27); Analysis for $C_{33}H_{25}ClN_4$ (513.04): Calcd. C, 77.25; H, 4.91; Cl, 6.90; N, 10.92; found: C, 77.41; H, 4.89; Cl, 6.82; N, 10.84.

General procedure for synthesis of compounds (10a,b)

   A mixture of compound 8a (0.67g, 1.5 mmol) and acetophenone or p-methylacetophenone (1.5 mmol) in absolute ethanol in the presence of a few drops of glacial acetic acid was refluxed for 6-8 hr. After cooling, the formed solid was filtered off and recrystallized from acetic acid to give 10a,b, respectively.

[4-Naphthalen-2- yl-6- (5, 6, 7, 8-tetrahydronaphthalen- 2-yl)-1H- pyrazolo[3,4-b]pyridin-3-yl]-[1-phenyleth-(E)-ylidene]-amine (10a)

Yield (78%); m.p. 240-242°C; IR spectrum (KBr, cm^{-1}): 3331 (NH), 2920 (CH, alicyclic); ^{1}H NMR spectrum (DMSO-d_6, δ ppm): 1.78 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 2.44 (s, 3H, CH$_3$), 2.80 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 7.20-8.12 (m, 15H, Ar-H), 8.27 (s, 1H, pyridine H5), 10.31 (s, 1H, NH, exchangeable with D$_2$O); MS: *m/z* (%): 492 (M$^+$, 30); ^{13}C NMR (DMSO- d_6): δ (ppm): 22.0 (CH$_3$), 22.5, 28.5 (4CH$_2$), 124.3, 125.2, 128.7, 129.7, 127.4, 138.4, 149, 151.1 (Ar-H), 155 (C-NH2); 163.7 (CH=N); Analysis for C$_{34}$H$_{28}$N$_4$ (492.62): Calcd. C, 82.89; H, 5.72; N, 11.37; found: C, 82.75; H, 5.62; N, 11.32.

[4-Naphthalen-2-yl-6- (5, 6, 7, 8-tetrahydronaphthalen- 2- yl)-1H-pyrazolo[3,4-b] pyridin-3-yl]-[1-p-tolyl-eth-(E)-ylidene]-amine (10b)

Yield (76%); m.p. 242-244°C; IR spectrum (KBr, cm^{-1}): 3363 (NH), 2920 (CH, alicyclic); ^{1}H NMR spectrum (DMSO-d_6, δ ppm): 1.78 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 2.30 (s, 3H, CH$_3$), 2.42 (s, 3H, CH$_3$), 2.80 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 7.18-8.09 (m, 14H, Ar-H), 8.26 (s, 1H, pyridine H5), 10.24 (s, 1H, NH, exchangeable with D$_2$O); ^{13}C NMR (DMSO- d_6): δ (ppm): 24.3, 22.0 (2CH$_3$), 22.5, 28.5 (4CH$_2$), 124.3, 125.2, 128.7, 129.7, 127.4, 138.4, 149, 151.1 (Ar-H), 155 (C-NH2); 163.7 (CH=N); MS: *m/z* (%): 506 (M$^+$, 10); Analysis for C$_{35}$H$_{30}$N$_4$ (506.66): Calcd. C, 82.97; H, 5.96; N, 11.05; found: C, 82.75; H, 5.85; N, 11.12.

2-{Methylsulfanyl- [4- naphthalen-2-yl- 6-(5,6,7,8-tetrahydronaphthalene- 2-yl)-1H-pyrazolo[3,4-b]pyridin-3-ylamino]-methylene}-malononitrile (11)

To a solution of compound 8a (0.50 g, 10mmol) in absolute ethanol (20 ml), [bis(methylthio)methylene]malononitrile (0.02 g, 1 mmol) was added and the reaction mixture was refluxed for 5 hr. On cooling, the separated product was collected by filteration and recrystallized from ethanol/DMF to give 11. Yield (84%); m.p. 268-270°C; IR (KBr, cm^{-1}): 338, 33272 (2NH), 2930 (CH, alicyclic), 2217 (2CN); ^{1}H NMR (DMSO-d_6, δ ppm): 1.77 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 2.59 (s, 3H, SCH$_3$), 2.79 (m, 4H, alicyclic 2CH$_2$ of tetrahydronaphthalene), 4.55 (s, 1H, NH, exchangeable with D$_2$O), 7.16-7.25 (m, 1H, Ar-H), 7.55-8.19 (m, 9H, Ar-H), 8.29 (s, 1H, pyridine H5), 12.32 (s, 1H, NH, exchangeable with D$_2$O); ^{13}C NMR (DMSO- d_6): δ (ppm): 19.9 (SCH$_3$), 22.5, 28.5 (4CH$_2$), 119.1 (CN), 100.5, 113.2, 124.3, 125.2, 128.7, 129.7, 127.4, 138.4, 149, 151.1 (Ar-H), 155 (C-NH2); 163.7 (CH=N); MS: *m/z* (%): 512 (M$^+$, 100), 513 (M$^+$+1, 44); Analysis for C$_{31}$H$_{24}$N$_6$S (512.64): Calcd. C, 72.63; H, 4.71; N, 16.39; S, 6.25; found: C, 72.75; H, 4.85; N, 16.22, S, 6.02.

Biological Evaluation

In vitro antibacterial and antifungal activities
Inhibition zone (IZ) measurement

Standard sterilized filter paper discs (5 mm diameter) impregnated with a solution of the tested compound in DMSO (5 mg/ml) was placed on an agar

plate, seeded with the appropriate test organism in triplicates. The utilized test organisms were Gram-positive bacteria *Bacillus pumilus*, NCTC8214 & *Micrococcus luteus*, ATCC-25922 and Gram-negative bacteria *Pseudomonas aeruginosa*, ATCC10145 & *Sarcina lutea*, ATCC-9341. The compounds were also evaluated for their *in vitro* antifungal potential against *Penicillium crysogenum* & *Candida albicans IMRU3669* as representatives of fungi. Chloramphenicol (antibiotic) and nystatin (antifungal) were used as reference drugs. The plates were incubated at 37°C for 24 hr for bacteria and for 7 days for fungi. Compounds that showed significant growth inhibition zones (≥ 19 mm) using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

In vitro antibacterial activity (minimal inhibitory concentration (MIC) measurement)

The microdilution susceptibility test in Müller–Hinton Broth (Oxoid) was used for the determination of antibacterial activity[26]. Stock solutions of the tested compounds were prepared in DMSO at concentration of 800 µg/ml followed by two-fold dilution at concentrations of (400, 200, . . . 6.25 lg/ml). The microorganism suspensions at 10^6 CFU/ml (Colony Forming Unit/ml) concentrations were inoculated to the corresponding wells. Plates were incubated at 36°C for 24–48 hr and the minimal inhibitory concentrations (MIC) were determined. All assays were performed in duplicate.

In vitro antifungal susceptibility assay

MIC of the tested compounds against *Penicillium crysogenum* & *Candida albicans IMRU3669* was determined by broth microdilution testing in accordance with the guidelines in NCCLS document M27-A and M38-P[34, 35]. Briefly stock solutions were prepared in DMSO of the test compounds. Serial twofold dilution of each compound was made in RPMI1640 medium buffered to pH 7.0 with 0.165 M 4-morpholinepropanesulfonic acid (MOPS) buffer as outlined in NCCLS M27-A document. Aliquots of 0.1 ml of each compound were dispensed into the wells of plastic microdilution microtiter plates so that the final concentration of solvent did not exceed 1% in any well. An inoculum of the organism at 10^6 CFU/ml (Colony Forming Unit/ml) concentrations was prepared and 100 µl of the individual fungal inoculum was added to each well of the microtiter plate containing the tested compound. The plates were incubated at 25°C for 72 hr. After the completion of incubation, the broth microdilution wells were examined. The MIC of each compound was defined as the lowest concentration that produced 80% inhibition in the growth of the organism. All assays were performed in duplicate.

Genotoxicity assay

   Materials

   Cyclophosphamide (CP: CAS No. 6055-19-12) was obtained from Sigma Chemical Company (St. Louis, Mo, U.S.A).

Experimental animals

Sixty (six to eight weeks old) swiss albino male mice (20-25g), purchased from animal house colony, Giza, Egypt were maintained on standard lab diet (Protein: 160, 4; Fat: 36.3; Fibre: 4/g/kg and metabolizable energy 12.09 MJ), and housed in a room free from any source of chemical contamination, artificially illuminated and thermally controlled, at the Animal House Lab., National Research Centre (NRC), Cairo, Egypt. After an acclimation period of one week, the animals were divided into six groups (10 mice / group) and housed in filter–top poly carbonate cages. All animals received humane care in compliance with the guide lines of the Animal care and use committee of the NRC, Cairo, Egypt.

Experimental design

Animals within different groups were orally treated for 5 weeks as follows:

Group 1: Served as negative control.

Group 2: Treated with intraperitoneal injection with CP (25mg/kg b.w.) after 5 weeks.

Group 3: Male mice were exposed to cyanopyridone 2a in tap drinking water at concentration 50 ppm (equivalent to average daily dose 20mg/kg b.w.).

Group 4: Male mice were exposed to the pyrazolopyridine 8a in tap drinking water at concentration 50ppm (equivalent to average daily dose 20mg/kg b.w.).

Group 5: Treated with cynopyridone 2a (20mg/kg b.w.) for 5 weeks then injected with CP (25mg/kg b.w.).

Group 6: Treated with pyrazolopyridine 8a (20mg/kg b.w.) for 5 weeks then injected with CP (25mg/kg b.w.).

Cytogenetic analysis

For chromosomal analysis both treated and control animals were sacrificed by cervical dislocation. Two hours before sacrifice, mice were injected with 5mg colchicines/kg b.w.

The bone marrow cells were aspirated using saline solution. Metaphase spreads were prepared using the method cited by the reference.[28] Fifty metaphase spreads per animal were analyzed for scoring the different types of chromosomal aberrations.

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مشتقات البيريدون ، الفيوروبيريدون وبيرازولوبيريدون الحاملة لحلقة ٥ ، ٦ ، ٧ ، ٨ـ رباعـى هـيدرونفثالـين الجديـدة: التشيـيـد و التقييم المضاد للميكروبات والسمية الجينية

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معمل الكيمياء العضوية التطبيقية ، *معمل الكيمياء العلاجية و **معمل بيولوجية الخلية ـ المركز القومى للبحوث – القاهرة ـ مصر .

يتناول هذا البحث تفاعل ٢ـ اسيتيل- ٥ ، ٦ ، ٧ ، ٨ـ رباعى هيدرونفثالين مع مجموعة مـن الالد يهيـدات الأروماتيـة ليعطى مشتقات السيانوبيريـدون المقابلـة (2a,b) . بتسخين المركب 2a مع كلوريد الاسيتون أدى إلى تكوين مشتق الفيوروبيريدين 4 وعند تسخين المركبات 2a,b مع ايثيل برومو اسيتات تم الحصول على مشتقات السيانو بيريدون المقابلة 5a,b والتى تم تكثيفها مع مجموعة من الامينات لتكوين المركبات المقابلة 6a,b, 7, 8a,b. تم أيضا الحصول على المركبات 9a,b, 10a,b, 11 وذلك بتفاعل المركب 8a مع مجموعة من الألدهيدات الأروماتية و الكيتونات و مالونونيتريل. التقييم المضاد للميكروبات أثبت أن المركبات 7, 10a,b لها تأثير قوى كمضادات للبكتيريا والفطريات. تم أيضا تقييم السمية الجينية للمركبين 2a, 8a اللذين أظهرا حماية ضد سمية الدواء سيكلوفوسفاميد المعالج للسرطان فى الفئران.