Synthesis, antimicrobial and cytotoxic activity of novel 4-phenoxy and 4-(substitutedamino) pyrazolo [3, 4-*d*] pyrimidine derivatives

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ABSTRACT

A series of new pyrazolopyrimidines was synthesized. Antimicrobial screening was done for the novel molecules to discover their activity against some test organisms, *Staphylococcus aureus* (ATCC 25923) (as example for Gram-positive bacteria), *Escherichia. coli (*ATCC 25922), *Enterobacter cloacae* (ATCC 23048), *Klebsiella (*ATCC 23495), *Salmonella typhimurium* (as examples for Gram-negative bacteria) and *Candida albicans* (as example for fungi). The antimicrobial screening results showed that some of these compounds exhibited a significant antimicrobial activity, where, compounds **(5c)** and **(5e)** showed the highest antimicrobial activity with MIC of 16 µg/mL,whilst, compounds **(4d)**, **(5g)** and **(5h)** displayed antifungal activity against *Candida albicans*. Besides, eight of the newly synthesized compounds were selected by National cancer Institute NCI (U.S.A) to be screened for their cytotoxic activity. The test compounds showed limited cytotoxic activity. The cytotoxicity results suggested that substitution at position 4 of pyrazolo [3, 4-*d*] pyrimidine with aryloxy or substitutedanilino moieties was preferred to aralkylamino moiety. Besides, introduction of small lipophilic group like methyl group in the *para* position of aryloxy or anilino moiety enhanced the cytotoxic activity. The results of cytotoxic screening were in good agreement with the calculated log P of the test compounds.   © 2013 Trade Science Inc. - INDIA

INTRODUCTION

The discovery of novel antimicrobial agents represent one of the most important advances in therapeutics, antibiotics represent the main strategy for controlling bacterial infections[1] In spite of the large number of antibiotics introduced to the market, resistance developed by many bacterial strains limited the treatment options for patients and representing a threat to public health[2,3].For example, *Staphylococcus aureus* developed resistance to penicillin and methicillin[4-6]. Therefore, the discovery of new synthetic antimicrobials is important, especially to control hospital infections by such multidrug-resistant strains. In this context, the chemistry of fluorine bearing compounds, aminobenzoic acid fragments, pyrimidines and fused pyrazolopyrimidine ring is of particular interest since these compounds present a variety of antimicrobial activities. Some pyrimidinones were reported to possess promising antibacterial and fungicidal activities[7-12]. Especially against

the gram positive (Gr+) pathogens *Staphylococcus aureus* through inhibition of DNA polymerase IC[11]. Fluorine containing compounds possess promising anti-infective activities which originate from their uniquely high lipophilicity[13-17]. *P*-Aminobenzoic acid (PABA) displayed an important role in the biosynthesis of tetrahydrofolic acid, which is a basic growth factor essential for the metabolic process of bacteria., many synthetic molecules are reported to interfere with this process as antagonist for PABA[18,19].

Moreover, cancer is considered as one of the most common devastating diseases that affect millions of people every year. It is considered as the second leading cause of death in humans. Therefore, there is a continuous need for the development of novel anti-cancer agents to combat the disease[20]. Many chemical classes have been reported to exhibit cytotoxic activity. In particular, 4-aminopyrazolo[3,4-*d*]pyrimidines received considerable attention and many research articles had been published in the last decade describing their effect as cytotoxic agents. In fact, 4-aminopyrazolo[3,4-*d*]pyrimidine core is an isosteric to adenine nucleus, this may be the real cause for the ATP-competitive inhibition of many kinase enzymes by 4-amino pyrazolo[3,4-*d*]pyrimidine derivatives[21]. *N*-substitutedpyrazolo[3,4-*d*]pyrimidin-4-amine derivatives were reported as strong cytotoxic agents which inhibited p38á MAP kinase[21]. Src kinase[22-25], EGFR and erbB2[26-30],cyclin-dependent kinase 2 (CDK2)[31], Or antagonize Adenosine receptor[32,33]. Besides, the presence of 3-methylsulphanyl group on pyrazolo [3,4-*d*]pyrimidine ring was reported to enhance the antimicrobial as well as the anti-proliferative activity[34-36]. On the other hand, little data had been published on the influence of substitution with phenoxy moiety on the anticancer activity of pyrazolo [3,4-*d*]pyrimidine ring. Recently, 6-(2,4-diflourophenoxy)-pyrazolo[3,4-*d*]pyrimidine derivatives were reported to show potent anticancer activity due to inhibition of p38á kinase[37]. Nevertheless, up to our knowledge, the effect of substitution of aryloxy group at 4-position in the presence of 3-methylsulphanyl on the antimicrobial or the cytotoxic activity of pyrazolopyrimidine had not been studied before. Prompted by these claims, we initiated a work to synergize the antibacterial or antitumor activity pyrazolo pyrimidines by preparing hybrid molecules having the

features of pyrazolopyrimidine ring, methylsulphanyl group and either aryloxy fragments or aminosubstiuted aryl moieties (in particular, *p-aminobenzoic moiety*) in an effort to discover antibacterial or antitumor agents (Chart 1).
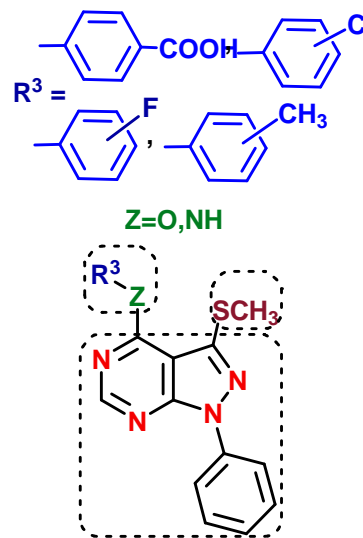
General structural hybrid
for thedesigned molecules

Chart 1: The structural hybrid for the designed molecules

In this work, novel series of 4-aryloxy **(4a-d)**, 4-(substitutedanilino),**(5a-j)** and 4-(aralkyl amino)-3-methylsulphanylpyrazolo[3,4-*d*]pyrimidines **(6a,b)** were synthesized and as searching for new antimicrobial candidates, antimicrobial screening was done to estimate the antimicrobial activity of the synthesized compounds. Representative derivatives of each series were screened by National Cancer Institute (NCI) U.S.A for their possible anticancer activity.

RESULTS AND DISCUSSION

Chemistry

The synthesis of the target compounds is outlined in Scheme 1 and 2. The starting and intermediate compounds 3-amino-3-methylsulphanyl-1-phenyl-1*H*-pyrazole-4-carbonitrile **(1)**[38], 3-methyl sulphanyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **(2)**[38] and 4-chloro-3-methyl sulphanyl-1-phenylpyrazolo[3,4-*d*]pyrimidine **(3)**[36] were prepared according to the reported methods. The target 4-aryloxy derivatives **(4a-d)** were obtained through reacting the 4-chloro derivative 3 with phenol or cresols in ethanol in

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the presence of equimolar amount of NaOH to initiate the phenoxide ions (Scheme 1). The presence of the corresponding molecular ion peaks in the mass spectra

of **(4a-d)** together with the appearance of signals of extra aromatic protons in their ^{1}H NMR spectra confirmed the structure of **(4a-d)**
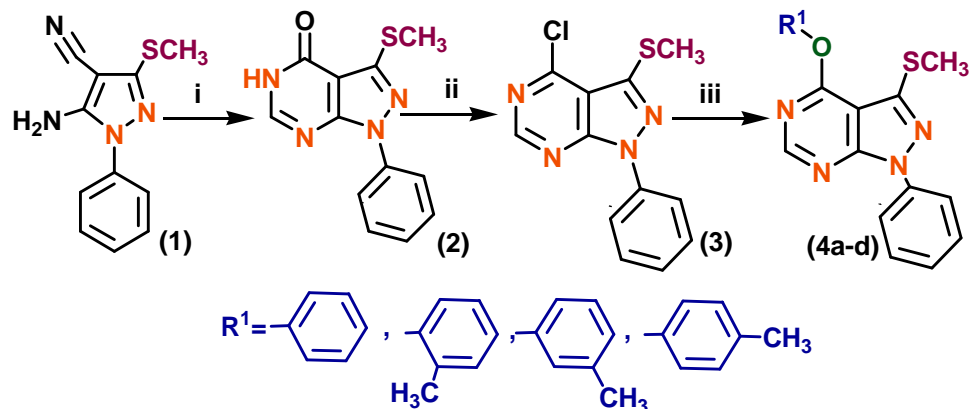
Reagents& conditions : i) HCOOH; ii) POCl$_3$; iii)R^{1}C$_6$H$_4$OH, NaOH, ethanol, stirr at R.T.3h.

**Scheme 1 : Synthesis of intermediates and 4-aryloxy-1-phenylpyrazolopyrimidine derivatives (4a-d)**

On the other hand, the 4-substitutedanilino derivatives **(5a-j)** and the 4-aralkylamino derivatives **(6a,b)** were synthesized via refluxing the 4-chloro derivative 3 with the appropriate amine in ethanol and triethylamine. (Scheme 2). The appearance of NH band in the IR spectra of **(5a-j)** and **(6a,b)** proved the chloro substitution. Besides, the ^{1}H NMR spectra of 5a-j and **(6a,b)** revealed the presence of exchangeable singlet signals at δ 6.99-8.96 ppm corresponding to NH proton. Fur-

thermore, the mass spectra of **(5a-j)** and **(6a,b)** showed the corresponding molecular ion peaks.

Antimicrobial activity

Antimicrobial susceptibility testing

In this work, compounds **(4a-d)**, **(5a-i)** and **(6a,b)** were screened for their possible antimicrobial activity using the disc diffusion technique[39]. The compounds were tested against *Staphylococcus aureus* (ATCC
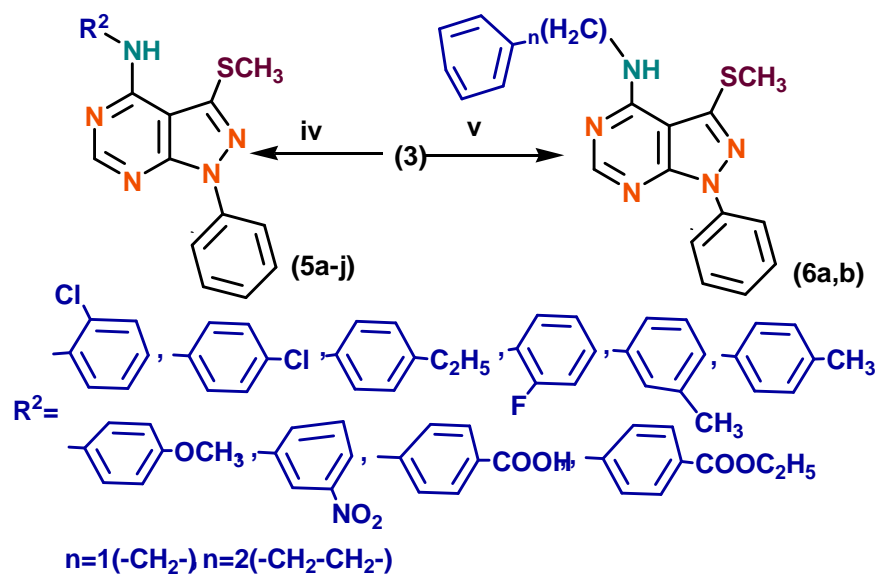
Reagents&conditions : iv) R^{2}C$_6$H$_4$NH$_2$, ethanol,TEA, reflux,4h; v) C$_6$H$_5$(CH$_2$)$_n$NH$_2$, ethanol,TEA,reflux,2 h

Scheme 2 : Synthesis of 4-arylamino-1-phenylpyrazolopyrimidine derivatives (5a-j) and 4-arylalkylamino-1-phenylpyrazolopyrimidine derivatives (6a-b)

25923) (as example for Gram-positive bacteria), *Escherichia. coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 23048), *Klebsiella* (ATCC 23495), *Salmonella typhimurium* (as examples for Gram-negative bacteria) and *Candida albicans* (as example for fungi),Tobramycin and Fluconazole were used as reference standard. The results revealed that most of the synthesized compounds showed variable degrees of inhibition against test organisms where *Staphylococcus aureus* (ATCC 25923) was the most susceptible strain and the zone of inhibition. varied from 7 to 18 (mm). The results against *Staphylococcus aureus* were displayed in (TABLE 1) and represented graphically in (Figure 1).

TABLE 1: The results of organisms susceptibility test for the prepared molecules (conc. 10 mg/disc). Data represent the mean of three replicates.

Cpd. No	Test microorganisms					
	Zone of Inhibition (mm)					
	S.a	S.t	E.c	Ks	En.c	C.a
4a	10 ± 0.6	-	-	-	-	-
4b	7 ± 0.3	-	-	-	-	-
4c	9 ± 1.	-	-	-	-	-
4d	12 ±1	-	-	-	-	7±0.4
5a	7 ± 0.4	-	-	-	-	-
5b	12 ± 1	-	-	-	9±1	-
5c	18 ± 1	7±0.4	-	-	9±0.8	-
5d	12 ± 0.8	-	-	-	7±0.3	-
5e	15 ±1	7±0.3	-	-	-	-
5f	8 ±0.6	-	-	-	13±0.8	-
5g	-	-	-	-	-	7±0.3
5h	-	-	-	-	-	7±0.6
5i	10 ± 0.4	-	-	-	-	-
6a	-	-	-	-	-	-
6b	12 ±1	-	-	-	-	-

No inhibition; *Sa-Staphylococcus aureus* **(ATCC 25923);** *St-Salmonella typhimurium* **;** *Ec-Escherichia coli* **(ATCC25922);** *Ks-Klebsiella* **(ATCC 23495);** *En.c.***-Enterobacter cloacae (ATCC 23048);** *Ca- Candida albicans*

From the results obtained in (TABLE 2), it was found that compounds **(4a-d)**, **(5a-f)**, **(5i)** and **(6b)** exhibited selective antibacterial activity against *Staphylococcus aureus* (ATCC 25923) Compounds **(5c)** and **(5e)** displayed antibacterial activity against *Salmonella typhimurium*, while four compounds, namely **(5b)**, **(5c)**, **(5d)** and **(5f)** showed antibacterial activity against *Enterobacter cloacae* (ATCC 23048). None of the

test compounds showed antibacterial activity against *Escherichia coli* (ATCC 25922) or *Klebsiella* (ATCC 23495). On the other hand, only compounds **(4d)**, **(5g)** and **(5h)** showed potential antifungal activity against *Candida albicans*.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of compounds **(4a-d)**, **(5a-f)**, **(5i)** and **(6b)** against *Staphylococcus aureus* (ATCC 25923) were then determined using broth dilution method[40]. (TABLE 2) displayed the results of MIC determination. The results showed that both 4-ethylanilino **(5c)** and 3-methylanilino **(5e)** derivatives exhibited the low MIC (16 μg/mL). Besides, 4-chloroanilino **(5b)** and 2-flouroanilino derivatives **(5d)**, and 4-aminobenzoic derivative **(5i)** exhibited moderate MIC (32 μg/mL). In addition, compounds **(4a-d)** and **(5a)** high MIC (64 ug/mL).

TABLE 2 : The results of minimum inhibitory concentration (MIC) in (ug/ml) for the synthetic molecules against *staphylococcus aurous* **(ATCC 25923).**

pd. No	Z	R^3	MIC (ug/ml)
4a	-O-	C_6H_{5-}	64
4b	-O-	$2\text{-}CH_3C_6H_4\text{-}$	64
4c	-O-	$3\text{-}CH_3C_6H_4\text{-}$	64
4d	-O-	$4\text{-}CH_3C_6H_4\text{-}$	64
5a	-NH	$2\text{-}ClC_6H_4\text{-}$	64
5b	-NH-	$4\text{-}ClC_6H_4\text{-}$	32
5c	-NH-	$4\text{-}C_2H_5C_6H_4\text{-}$	16
5d	-NH-	$2\text{-}FC_6H_4\text{-}$	32
5e	-NH-	$3\text{-}CH_3C_6H_4\text{-}$	16
5f	-NH-	$4\text{-}CH_3C_6H_4\text{-}$	64
5i	-NH-	$4\text{-}HOOC\ C_6H_4\text{-}$	32
6b	-NH-	$(CH_2)_2\ C_6H_4\text{-}$	64

In vitro cytotoxic activity

Eight compounds **(4a,b,d)**, **(5a,b,f,g)** and **(6a)** were selected by the NCI Developmental Therapeutic Program (www.dtp.nci.nih.gov) to be tested *in vitro* for

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their possible anticancer activity. The anticancer assays were performed according to the protocol of the Drug Evaluation Branch, NCI, Bethesda[41-43]. A 48 h drug exposure protocol was used and sulforhodamine B (SRB) protein assay was applied to estimate the cell viability and growth[44]. The selected compounds were evaluated at one dose (concentration 10^{-5} M) primary anticancer assay towards a panel of approximately 60 cancer lines. The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, central nervous system (CNS), ovarian, renal, prostate and breast cancers. Results for each compound were expressed in terms of the percentage growth of the treated cells compared to the untreated control cells, and presented as mean graph of the growth percent. The screening results are displayed in (TABLE 3). From the obtained results, it was found that all the test compounds showed limited cytotoxic activity. Nevertheless, the test compounds showed moderate to high cytotoxic activity against specific cell lines and the most sensitive panels were the renal cancer, the ovarian cancer and the non-small cell lung cancer. Structurally, the test compounds belong to three series: 4-aryloxy, 4-substitutedanilino and 4-aralkyl amino series. Better activity was displayed by the 4-aryloxy series than by 4-substitutedanilino series. While, the least activity was exhibited by the 4-aralkylamino series. Compound **(6a)** (4-benzylamino) displayed the highest mean growth percentage with limited cytotoxic activity against almost all panels screened. Regarding the influence of the substituents on the phenoxy ring on the cytotoxic activity, it was found that 4-(4-methylphenoxy) derivative showed better cytotoxic activity than 4-phenoxy or 4-(2-methylphenoxy). Similarly, careful examination of the effect of substitution on the anilino moiety showed that the order of potency was $4\text{-CH}_3 > 4\text{-CH}_3\text{O} > 4\text{-Cl} > 2\text{-Cl}$. This order might reflect the importance of the presence of small lipophilic group in the *para* position. Thus, investigation of the substitution effect on 4-phenoxy and the 4-substitutedanilino series on the cytotoxic activity highlighted the following remarks:

- In both series, substitution at *para* position was favored over substitution at *ortho* position.
- In case of *para* substitution, small lipophilic group like methyl group gave better results than methoxy or chloro groups.

In order to explain the effect of lipophilicity on cytotoxic activity, log P of the test compounds was calculated and the values were presented in (TABLE 3). Indeed, the screening results were in good agreement with log P except for compounds **(5a)** and **(5b)** whose log P values were very high (in fact, the highest) and their mean growth percentage were high too. For the rest of compounds tested, increasing the hydrophobicity, reduced the mean growth percentage and thus increased the cytotoxic activity probably due to increase in membrane permeability. Compound **(6a)** had the smallest log P value and the highest mean growth percentage. It is worth noting that the NCI antitumor drug discovery screen has been designed to distinguish between broad-spectrum antitumor and subpanel-selective compounds. In this work, it was found that renal cancer cell lines (UO-31, A498, 786-0 and RXF 393) were the most sensitive cell lines affected by the 4-phenoxy derivatives. Whilst, the ovarian cancer cell line (OVCAR-4) and the non-small cell lung cancer cell line (NCI-H522) were the most sensitive cell lines affected by 4-substitutedanilino derivatives. The most potent cytotoxic activities in this study were exhibited by 4-aryloxy derivative **(4a)** against the renal cancer cell line UO-31 (mean growth %=7.79) and 4-(4-methylphenoxy) derivative 4d against the ovarian cancer cell line OVCAR-4 (mean growth %=9.15).

CONCLUSION

In summary, a series of new 4-aryloxy,4-(substitute danilino) and 4-(aralkylamino) pyrazolo[3,4-*d*]pyrimidines was synthesized. Antimicrobial evaluation was done for the novel molecules against some test organisms to discover their activity. Compounds **(5c)** and **(5e)** showed the highest activity with MIC of 16 μg/mL, whilst, compounds **(5b)**, **(5d)** and **(5i)** exhibited moderate antimicrobial activity with MIC 32 μg/mL. against *Staphylococcus aureus (*ATCC 25923) Moreover, compounds **(4d)**, **(5g)** and **(5h)** displayed potent antifungal activity against *Candida albicans*. The results revealed that the newly synthesized molecules represent potential antimicrobial agents. Eight of the newly synthesized compounds were selected by NCI to be screened for their cytotoxic activity. All the test compounds showed limited cytotoxic activity. The re-

sults suggested that substitution at position 4 of pyrazolo[3,4-*d*]pyrimidine with aryloxy or substitute danilino moieties was preferred to aralkylamino moiety. Besides, introduction of small lipophilic group like methyl group in the *para* position of aryloxy or anilino moiety enhanced the cytotoxic activity. The results of cytotoxic screening were in good agreement with the calculated log P of the test compounds. Renal cancer cell lines (UO-31, A498, 786-0 and RXF 393) were the most sensitive cell lines affected by the 4-aryloxy derivatives. Whilst, the ovarian cancer cell line (OVCAR-4) and the non-small cell lung cancer cell line (NCI-H522) were the most sensitive cell lines affected by 4-substitutedanilino derivatives. The most potent cytotoxic activities in this study were exhibited by 4-aryloxy derivative 4a against the renal cancer cell line UO-31 and 4-(4-methylphenoxy) derivative (**4d**) against the ovarian cancer cell line OVCAR-4.
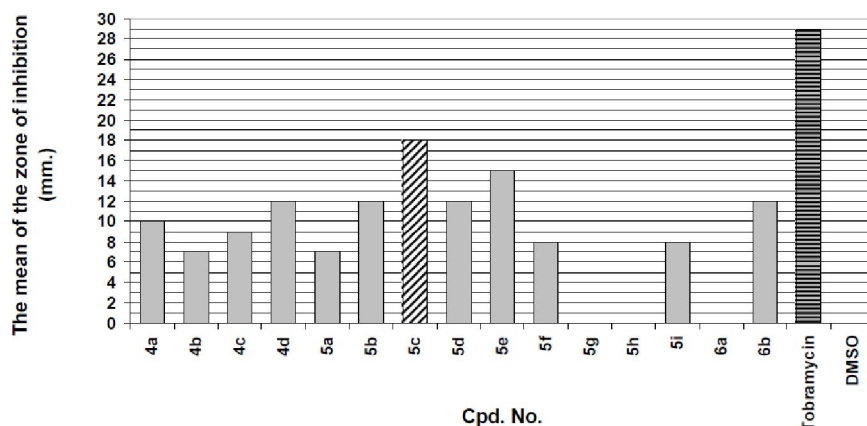
Figure 1 : The results of organism susceptibility test for the synthetic molecules against *staphylococcus aureus* **(ATCC 25923) using tobramycin as reference and DMSO as control**

EXPERIMENTAL

General

Melting points were determined using a Griffin apparatus and were uncorrected. IR spectra were recorded on Shimadzu IR 435 spectrophotometer and values were represented in cm^{-1}. ^{1}H NMR were carried out on Varian Gemini 300 MHz spectrophotometer, Cairo University, Cairo, Egypt, using TMS as an internal standard and chemical shifts were recorded in ppm on δ scale and coupling constants (J) are given in Hz. ^{13}C NMR were carried out on Varian Gemini 300 MHz spectrophotometer, Main Defense Chemical Laboratory, Cairo, Egypt, The electron impact (EI) mass spectra were recorded on Hewlett Packard 5988 spectrometer Microanalytical center, Cairo University, Cairo, Egypt. Elemental microanalyses were performed at Microanalytical center, Cairo University, The results within ±0.4%. Analytical thin layer chromatography (TLC) on silica gel plates containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products. All reagents and solvents were purified and dried by standard techniques.5-Amino-3-methylsulphanyl-1-phenyl-1*H*-pyrazole-4-carbonitrile (**1**)[38], 3-methyl sulphanyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5H)-one (**2**)[38] and 4-chloro-3-methyl sulphanyl-1-phenyl pyrazolo[3,4-*d*]pyrimidine (**3**)[36] were synthesized according to the published methods.

General procedure for the synthesis of 3-(methylsulfanyl)-1-phenyl-4-(substituted aryloxy)-1*H*-pyrazolo [3,4-*d*]pyrimidines (4a-d)

A mixture of 4-chloro-3-methylsulphanyl pyrazolo[3,4-*d*]pyrimidine derivative (**3**) (0.28 g, 0.001 mol), appropriate phenol (0.001 mol) and sodium hydroxide (0.04 g, 0.001 mol) in absolute ethanol (20 mL) was stirred at room temperature for 3 h. The obtained solid was filtered, washed with water (50 mL), dried and crystallized from ethanol.

3-(Methylsulfanyl)-4-phenoxy-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (4a)

Yield: 0.13g (38 %); mp: 114-116 °C; Anal.% calcd. For $C_{18}H_{14}N_4OS$: (334.39) :C, 64.65; H, 4.22; N, 16.75; Found: C, 64.81; H, 4.33; N, 16.80 ; I R

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(cm^{-1}): 2916, 2854 (CH-aliphatic), 1589 (C=N); ^{1}H NMR (DMSO-d_6) δ: 2.75 (s, 3H, SCH_3), 7.32-8.20 (m, 10H, Ar-H), 8.60 (s 1H, CH-6); ^{13}C NMR (DMSO-d_6) δ:14.1(s,CH$_3$),105.3(s), 120.2.(s), 122.2(s),124.0(s)125.6(s),124.4(s), 130.1 (s), 141.2(s), 149.1(s),152.1(s), 155.3(s), 156.3(s), 158.2(s); MS m/z : 334 [M$^+$, 62.0%], 333 [(M-1)$^+$, 10.2%], 77 [C$_6$H$_5$$^+$, 100%].

4-(2-Methylphenoxy)-3-(methylsulfanyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (4b)

Yield: 0.12 g (34%); mp: 120-122 ℃; Anal.% calcd. For C$_{19}$H$_{16}$N$_4$OS (348.42): C, 65.50; H, 4.63; N, 16.08, Found, C,65;40,H, 4.38; N, 16.20;IR (cm^{-1}):2920, 2854 (CH-aliphatic), 1597 (C=N); ^{1}H NMR (DMSO-d_6) δ : 2.35 (s, 3H, CH_3), 2.74 (s, 3H, SCH_3), 7.10-8.19 (m, 9H, Ar-H), 8.60 (s, 1H, CH-6); MS m/z: 348 [M$^+$, 61.5%], 347 [(M-1)$^+$, 19.2%], 77 [C$_6$H$_5$$^+$, 61.5%], 76 [C$_6H_4$$^+$, 34.6%], 58 [100%].

4-(3-Methylphenoxy)-3-(methylsulfanyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (4c)

Yield: 0.13g (37%); mp: 89-90 ℃; Anal.% calcd. ForC$_{19}$H$_{16}$N$_4$OS (348.42): C, 65.50; H, 4.63; N, 16.08 ; Found: C,64.90,H, 4.31; N, 15.82; IR (cm^{-1}): 2920, 2854 (CH-aliphatic), 1593 (C=N); ^{1}H NMR (DMSO-d_6) δ: 2.13 (s, 3H, CH_3), 2.75 (s, 3H, SCH_3), 7.25-8.19 (m, 9H, Ar-H), 8.58 (s, 1H, CH-6); MS m/z: 348 [M$^+$, 100%], 347 [(M-1)$^+$, 12.9%], 91 [CH$_3$C$_6$H$_4$$^+$, 53.0%], 77 [C$_6H_5$$^+$, 87.5%], 76 [C$_6H_4$$^+$, 10.2%].

4-(4-Methylphenoxy)-3-(methylsulfanyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (4d)

Yield: 0.15g (39%); mp: 140-142 ℃; Anal.% calcd. For C$_{19}$H$_{16}$N$_4$OS:(348.42): C, 65.50; H, 4.63; N, 16.08 Found: C,65.81,H, 4.83; N, 16.28;IR (cm^{-1}): 2920, 2854 (CH-aliphatic), 1593 (C=N); ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H, CH_3), 2.74 (s, 3H, SCH_3), 7.18-8.19 (m, 9H, Ar-H), 8.58 (s, 1H, CH-6); ^{13}C NMR (DMSO-d_6) δ: 13.7(q,CH$_3$),22.7 (s, CH$_3$), 105.5(s), 121.1. (s), 123.6 (s), 125.7 (s), 139.2(s), 135.2(s), 130.4(s), 131.2(s), 149.1(s), 152.1(s), 154.3(s), 155.3(s), 171.2(s); MS m/z: 348 [M$^+$, 100%], 347 [(M-1)$^+$,40.9%], 91 [CH$_3$C$_6$H$_4$$^+$,62.1%], 77 [C$_6H_5$$^+$,89.4%], 76 [C$_6H_4$$^+$,24.2%].

General procedure for the synthesis of *N*-substituted-3-(methylsulfanyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amines (5a-j) and (6a,b)

A mixture of 4-chloro-3-methylsulphanyl pyrazolo[3,4-*d*]pyrimidine derivative (3) (0.28 g, 0.001 mol), appropriate amine (0.001 mol) and triethylamine (0.001 mol) in absolute ethanol (20 mL) was heated under reflux for 2 - 4 h. The reaction mixture was cooled and the solid formed was filtered, dried and crystallized from ethanol.

N-(2-Chlorophenyl)-3-(methylsulfanyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (5a)

Yield: 0.11g (30%); mp: 137-139 ℃ Anal.% calcd. For C$_{18}$H$_{14}$ClN$_5$S (367.86): C, 58.77; H, 3.84; N, 19.04; Found: C,58.92;H, 4.04; N, 19.24; IR (cm^{-1}): 3363 (NH), 2947, 2866 (CH-aliphatic); ^{1}H NMR (DMSO-d_6) δ:2.75 (s, 3H, SCH_3), 7.23-8.41 (m, 9H, Ar-H), 8.54 (s, 1H, CH-6), 8.73 (s, 1H, NH, D$_2$O exchangeable); MS m/z: 369 [(M+2)$^+$, 10.0%], 367 [M$^+$, 22.6%], 332 [(M- Cl)$^+$, 100%], 331 [(M- HCl)$^+$, 83.8%], 77 [C$_6$H$_5$$^+$, 61.6%], 76 [C$_6H_4$$^+$, 23.6%].

N-(4-Chlorophenyl)-3-(methylsulfanyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (5b)

Yield: 0.2g (54%); mp: 162-164 ℃; Anal.% calcd. For C$_{18}$H$_{14}$ClN$_5$S (367.86): C, 58.77; H, 3.84; Cl, 9.64; N, 19.04 Found : C,58.97;H,4.00 ; N, 19.20 ; IR (cm^{-1}): 3325 (NH), 2924, 2850 (CH-aliphatic); ^{1}H NMR (DMSO-d_6) δ : 2.73 (s, 3H, SCH_3), 7.32-7.58 (m, 5H, Ar-H), 7.73 (d, 2H, *J*=8.4 Hz, Ar-H), 8.15 (d, 2H, *J*=8.4 Hz, Ar-H), 8.49 (s, 1H, CH-6), 8.76 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ:15.1(s,CH$_3$), 106.2(s), 122.1(s), 122.4(s), 123.2(s), 125.8 (s), 127.6 (s), 130.(s), 140.5(s), 141.2 (s), 149.1(s), 152.1 (s), 155.3 (s), 159.2(s); MS m/z: 369 [(M+2)$^+$, 35.1%], 367 [M$^+$, 98.4%], 77 [C$_6$H$_5$$^+$, 100%], 76 [C$_6H_4$$^+$, 27.9%].

N-(4-ethylphenyl)-3-(methylsulfanyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (5c)

Yield: 0.18g (50%); mp: 188-190 ℃; Anal.% calcd. For C$_{20}$H$_{19}$N$_5$S (361.46): C, 66.46; H, 5.30; N, 19.37; Found: C, 66.74;H,5.60 ; N, 19.67; IR (cm^{-1}): 3344 (NH), 2962, 2870 (CH -aliphatic); ^{1}H NMR (DMSO-d_6) δ :1.13 (t, 3H, *J*= 7.2 Hz, CH$_2$CH_3), 2.51 (q, 2H, *J*= 7.2 Hz, CH_2CH$_3$), 2.74 (s, 3H, SCH_3), 6.99-8.28 (m, 9H, Ar-H), 8.35 (s, 1H, CH-6), 8.47 (s, 1H, NH, D$_2$O exchangeable); MS m/z: 361 [M$^+$, 82.4%], 360

[(M-1)$^+$, 88.2%], 359 [(M-2)$^+$, 41.2%], 347 [(M-CH$_3$)$^+$, 88.2%], 346 [(M-CH$_2$)$^+$, 88.2%], 91 [(CH$_3$C$_6$H$_4$)$^+$, 58.8%], 77 [C$_6$H$_5$$^+$, 100%], 76 [C$_6H_4$, 52.9%].

N-(2-Flourophenyl)-3-(methylsulfanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (5d)

Yield: 0.19g (54%); mp: 136-138 ℃; Anal.% calcd. For C$_{18}$H$_{14}$FN$_5$S (351.40): C, 61.52; H, 4.02; F, 5.41; N, 19.93, Found: C,61.12,H,4.32 ; N, 20.10;IR (cm^{-1}): 3394 (NH), 2924, 2854 (CH-aliphatic); ^{1}H NMR (DMSO-d_6) δ: 2.74 (s, 3H, SCH$_3$), 7.24-8.18 (m, 9H, Ar-H), 8.48 (s, 1H, CH-6), 8.66 (s, 1H, NH, D$_2$O exchangeable);^{13}C NMR (DMSO-d_6) δ :15.1(q, CH$_3$), 106.2 (s), 119.2(s), 121.1(s), 123.5(s), 123.6(s), 126.4 (s), 126.4(s), 129.7(s), 131.2(s), 141.2(s), 149.1(s), 152.1(s), 154.8 (s), 55.3(s), 161.2(s); MS m/z: 351 [M$^+$, 43.9%], 350 [(M-1)$^+$, 50.9%], 332 [(M- F)$^+$, 70.4%], 95 [(FC$_6$H$_4$)$^+$, 23.0%], 77 [C$_6$H$_5$$^+$, 100%].

N-(3-Methylphenyl)-3-(methylsulfanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (5e)

Yield: 0.2g (58%); mp: 141-143 ℃; Anal.% calcd. For C$_{19}$H$_{17}$N$_5$S (347.44): C, 65.68; H, 4.93; N, 20.16; Found: C, 65.40;H, 4.82; N, 19.96; IR (cm^{-1}): 3325 (NH), 2920, 2800 (CH-aliphatic); ^{1}H NMR (DMSO-d_6) δ :2.34 (s, 3H, CH$_3$), 2.74 (s, 3H, SCH$_3$), 7.00-8.46 (m, 10H, Ar-H), 8.56 (s, 1H, NH, D$_2$O exchangeable); MS m/z: 347 [M$^+$, 93.6%], 346[(M-1)$^+$, 100%], 91 [(CH$_3$C$_6$H$_4$)$^+$, 35.1%], 77 [C$_6$H$_5$$^+$, 51.5%].

N-(4-Methylphenyl)-3-(methylsulfanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (5f)

Yield: 0.26g (75%); mp: 167-169 ℃; Anal.% calcd. For C$_{19}$H$_{17}$N$_5$S (347.44): C, 65.68; H, 4.93; N, 20.16 Found: C,65.70;H 5.13; N, 20.24 ; IR (cm^{-1}): 3341 (NH), 2924, 2854 (CH-aliphatic); ^{1}H NMR (DMSO-d_6) δ : 2.31 (s, 3H, CH$_3$), 2.74 (s, 3H, SCH$_3$), 7.02-8.47 (m, 10H, Ar-H), 8.56 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6) δ:14.5(s,CH$_3$), 21.9(s,CH$_3$), 105.2(s), 120.6 (s), 122.1(s), 123.5(s), 126.2 (s), 130.2 (s), 132.1(s), 139.4(s), 141.3(s), 149.1(s), 152.1(s), 155.3 (s), 171.2 (s); MS m/z: 347 [M$^+$, 100%], 346[(M-1)$^+$, 75.7%], 345[(M-2)$^+$, 36.4%], 91 [(CH$_3$C$_6$H$_4$)$^+$, 30.8%], 77 [C$_6$H$_5$$^+$, 64.5%], 76 [C$_6H_4$$^+$, 33.6%].

N-(4-Methoxyphenyl)-3-(methylsulfanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (5g)

Yield: 0.2g (55%); mp: 138-140 ℃; Anal.% calcd. For C$_{19}$H$_{17}$N$_5$OS (363.44): C, 62.79; H, 4.71; N, 19.27; Found: C,62.90;H,4.92 ; N, 19.30,IR (cm^{-1}): 3356 (NH), 2997, 2800 (CH-aliphatic); ^{1}H NMR (DMSO-d_6) δ ppm 2.74 (s, 3H, SCH$_3$), 3.77 (s, 3H, OCH$_3$), 6.69-8.42 (m, 10H, Ar-H), 8.53 (s, 1H, NH, D$_2$O exchangeable);^{13}C NMR (DMSO-d_6) δ :13.5 (s,CH$_3$), 57.6(s,CH$_3$), 104.4(s), 116.2(s), 121.2(s), 122.3(s), 123.7(s), 126.4(s), 134.5(s), 141.3(s), 149.1(s), 152.1(s), 155.3(s), 156.2 (s), 160.2 (s);MS m/z : 363 [M$^+$, 100%], 362 [(M-1)$^+$, 35.0%], 77 [C$_6$H$_5$$^+$, 58.0%], 76 [C$_6H_4$$^+$, 15.0%].

N-(3-Nitrophenyl)-3-(methylsulfanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (5h)

Yield: 0.2g (53%); mp: 187-189 ℃; Anal.% calcd. For C$_{18}$H$_{14}$N$_6$O$_2$S (378.41): C, 57.13; H, 3.73; N, 22.21 Found: C,57.43;H,4.00 ; N, 22.53; IR (cm^{-1}): 3387 (NH), 2924, 2835 (CH-aliphatic), 1531, 1354 (NO$_2$); ^{1}H NMR (DMSO-d_6)δ: 2.76 (s, 3H, SCH$_3$), 7.37-8.74 (m, 9H, Ar-H), 8.75 (s, 1H, CH-6), 9.18 (s, 1H, NH, D$_2$O exchangeable); MS m/z: 378 [M$^+$, 100%], 377 [(M-1)$^+$, 45.5%], 332 [(M- NO$_2$)$^+$, 10.7%], 331 [(M- HNO$_2$)$^+$, 34.6%], 77 [C$_6$H$_5$$^+$, 92.0%], 76 [C$_6H_4$$^+$, 68.6%].

4-{[3-(methylsulfanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl]amino}benzoic acid (5i)

Yield: 0.33g (88%); mp: 290-292 ℃; Anal.% calcd. For C$_{19}$H$_{15}$N$_5$O$_2$S (377.42): C, 60.46; H, 4.01; N, 18.56 Found: C, 60.23;H,4.21 ; N,18.82, IR (cm^{-1}): 3325 (NH/OH), 2989, 2877 (CH-aliphatic), 1689 (C=O); ^{1}H NMR (DMSO-d_6)δ : 2.75 (s, 3H, SCH$_3$), 7.35-8.20 (m, 9H, Ar-H), 8.66 (s, 1H, CH-6), 8.97 (s, 1H, NH, D$_2$O exchangeable), 12.80 (br s, 1H, OH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d_6)ä :13.9(s,CH$_3$), 104.9 (s), 114.3(s), 122.1(s), 122.6(s), 123.2(s), 126.2(s), 133.2(s), 142.1(s), 149.1(s), 149.3(s), 152.1(s),155.3(s), 159.2 (s), 174.2(s); MS m/z: 377 [M$^+$, 100%], 376 [(M- 1)$^+$, 84.3%], 375 [(M-2)$^+$, 52.9%], 77 [C$_6$H$_5$$^+$, 78.4%], 76 [C$_6H_4$$^+$, 35.3%].

Ethyl 4-{[3-(methylsulfanyl)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl] amino} benzoate (5j)

Yield: 0.34g (84%); mp: 196-198 ℃; Anal.%

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calcd. For $C_{21}H_{19}N_5O_2S$ (405.47): C, 62.21; H, 4.72; N, 17.27; Found: C, 62.50;H, 5.02; N, 17.40;IR (cm⁻¹): 3321 (NH), 2931, 2800 (CH-aliphatic), 1701 (C=O); ¹H NMR (DMSO-d_6) δ:1.31 (t, 3H, J= 7.5 Hz, OCH$_2$CH_3), 2.75 (s, 3H, SCH_3), 4.28 (q, 2H, J= 7.2 Hz, OCH_2CH$_3$), 7.35-8.19 (m, 9H, Ar-H), 8.65 (s, 1H, CH-6), 8.96 (s, 1H, NH, D$_2$O exchangeable); ¹³C NMR (DMSO-d_6) δ:14.8(s,CH$_3$),14.3(s,CH$_3$), 61.2(s,CH$_2$), 104.7 (s), 114.7(s), 121.3(s), 121.1(s), 124.2(s), 125.2(s), 132.4(s), 142.1(s), 147.3(s), 149.1(s), 152.1(s), 155.3 (s), 161.2(s), 166.1(s); MS m/z: 405 [M⁺, 31.4%], 404 [(M-1)⁺, 23.3%], 187 100%], 77 [$C_6H_5^+$, 18.4%], 76 [$C_6H_4^+$, 8.5%].

N-Benzyl-3-(methylsulfanyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (6a)

Yield: 0.1g (29%); mp: 234-236 ºC; Anal.% calcd. For ($C_{19}H_{17}N_5S$) (347.44): C, 65.68; H, 4.93; N, 20.16 Found: C, 65.38;H, 5.03; N, 19.92; IR (cm⁻¹): 3380 (NH), 2924, 2835 (CH-aliphatic); ¹H NMR (DMSO-d_6) δ:2.83 (s, 3H, SCH_3), 4.72 (d, 2H, J=5.4 Hz, CH_2C$_6$H$_5$), 7.12-8.44 (m, 11H, Ar-H), 8.17 (s, 1H, NH, D$_2$O exchangeable); ¹³C NMR (DMSO-d_6) δ :13.9(s,CH$_3$), 50.2 (s,CH$_2$), 104.6(s), 122.1(s), 124.2(s), 126.2(s), 126.1(s), 126.8(s), 129.1(s), 141.2(s), 142.1(s), 149.1(s), 152.1 (s), 155.3(s), 171.2(s); MS m/z: 347 [M⁺, 41.2%], 346 [(M-1)⁺, 29.4%],106 [(NHCH$_2$C$_6$H$_5$)⁺, 41.2%], 91 [(CH$_3$C$_6$H$_4$)⁺,23.5%], 77 [$C_6H_5^+$, 70.6%], 65 [100%].

3-(Methylsulfanyl)-1-phenyl-*N*-(2-phenylethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (6b)

Yield: 0.16 g (46%); mp: 234-235 ºC; Anal.% calcd. For $C_{20}H_{19}N_5S$ (361.46) :C, 66.46; H, 5.30; N, 19.37 Found: C, 66.63;H,5.42 ; N, 19.51; IR (cm⁻¹): 3387 (NH), 2924, 2877(CH-aliphatic); ¹H NMR (DMSO-d_6) δ: 2.62 (s, 3H, SCH_3), 2.78 (t, 2H, J=7.0 Hz, CH_2CH$_2$C$_6$H$_5$), 2.93 (t, 2H, J=7.0 Hz, CH$_2$CH_2C$_6$H$_5$), 6.99 (br s, 1H, NH, D$_2$O exchangeable), 7.13-8.26 (t, 11H, Ar-H); ;MS m/z: 361 [M⁺, 28.0%], 91 [(C$_6$H$_5$CH$_2$)⁺,64.0%], 77 [$C_6H_5^+$,84.0%], 57 [100%].

Antimicrobial activity

Antimicrobial susceptibility testing

Antimicrobial activity of the tested compounds was carried out using the disc diffusion susceptibility testing[39]. which involved several steps and was done as follow:

Plates employed for disk susceptibility testing

Muller Hinton agar (MHA, oxoid) is dispensed into glass culture plates to yield a uniform depth of 4 mm. For plates of internal diameters of 9 or 15 cm or 60 ml of media were dispensed, respectively to yield the desired depth. MHA plates were stored at 4 to 8 °C.

Disk employed for susceptibility testing

The newly synthesized compounds were dissolved in DMSO at a concentration 10 mg /mL, (6 mm) filter paper Whatman no. 1 was soaked in 50 µL of each dissolved compound. Both positive and negative control discs were applied using Tobramycin (10 mg/mL), Flucanazole (10mg/mL) and DMSO 10 mg respectively.

Inoculum preparation and standardization

The test microorganisms include *Staphylococcus aureus* (ATCC 25923) (as example for Gram-positive bacteria), *Escherichia. coli (*ATCC 25922), *Enterobacter cloacae* (ATCC 23048), *Klebsiella* (ATCC 23495), *Salmonella typhimurium* (as examples for Gram-negative bacteria) and *Candida albicans*. (as example for fungi) were picked from sub-culture plate. All microorganisms were kindly provided from culture collection of the Microbiology department, Faculty of pharmacy, Cairo University, Cairo, Egypt. The wire loop was used to touch each colony, and was then immersed in about 5 ml of Tryticase Soya broth (TSB). The broth was incubated at 35 until it equals or exceeds the correct turbidity, generally 2-6 hours for rapidly growing pathogens. The broth was then diluted with a fresh TSB until the required turbidity was reached. Inoculate were calibrated to 0.5 McFarland turbidity standard. A photometer was used to achieve this calibration. For instance, at a wave length of 550 nm, a 5 ml glass tube with 2 ml of the inoculum suspension with an optical density of 0.1-0.12 approximates a 0.5 McFarland barium sulfate standard.

Inoculation of plates

Plats are inoculated by streak method, where a sterile cotton swab was dipped into the inoculum (10⁸cfu/ml) and the excess is removed by rotating the swab several times against the inside wall of the tube above the fluid level. The surface of MHA is inoculated by streaking the swab over the surface. Streaking is inoculated by streaking the swab over the surface. Streak-

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ing is repeated three times and each time the plate is rotated 60°

Application of disks

Not more than 15 minutes after inoculation of plates, the discs containing the tested synthesized products were applied using aseptic technique on the surface of agar and plates. The discs were applied with a forceps to ensure complete contact of the disk with the agar surface.

Incubation of plates

Inoculated plates were incubated immediately at 35°C for 14-19 hours (overnight) in an inverted position.

Reading of disk tests

Manual reading of result was done. The diameters of the measured zones showing complete inhibition were record to the nearest millimeter (mm).

Interpretation of results

The test was repeated 3times and the means of the zone of inhibition for the synthesized molecules were tabulated in (TABLE 1).

Determination of the minimum inhibitory concentration (MIC)

MIC values of the synthesized compounds with *Staphylococcus aureus* (ATCC 25923) were determined using broth dilution method[40]. The tested compounds were dissolved in DMSO and further dilutions in Muller Hinton broth were prepared to make 256, 128, 64, 32, 16, 8, 4, 2, 1 µg /mL. Both negative and positive control were prepared to ensure the sterility of the medium and viability of the tested strain respectively, also, a control test was carried out using inoculated broth with DMSO to test the solvent effect which was found to be inactive in culture medium. The results obtained are shown in (TABLE 2).

In vitro cytotoxic activity[45]

Materials and methods

The operation of this screen utilized 60 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. The screening involved the evaluation of the selected compounds against the 60 cell lines at a single dose of 10 µM. The data was reported as a mean graph of the percent growth of treated cells.

Measurement of potential cytotoxicity

The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of test compounds. After 24 h, two plates of each cell line were fixed *in situ* with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of test compound addition (Tz). The test compounds were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of compound addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/mL gentamicin. Additional four, 10-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 µL of the different test compounds dilutions were added to the appropriate microtiter wells already containing 100 µL of medium, resulting in the required final drug concentrations. Following compound addition, the plates were incubated for an additional 48 h at 37°C, 5 % CO_2, 95 % air, and 100 % relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µL of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µL) at 0.4 % (w/v) in 1 % acetic acid was added to each well, and plates were incubated for 10 minutes at room temperature. After staining, unbound dye was removed by washing five times with 1 % acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm.

For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 µL of 80 % TCA (final concentration, 16 % TCA). Using

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the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of the test compounds at the five concentration levels (Ti)], the percentage growth was calculated at each of the test compounds concentrations levels. Percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Tie"Tz
[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

TABLE 3 : The results of cytotoxic activity of the selected synthetic molecules E in concentration (10^{-5} M) against 60 cell cancer lines and their calculated log P values.
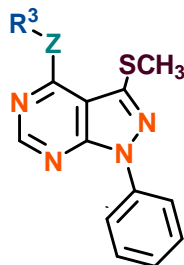
Cpd No.	Z	R^3	Mean growth % (log P)	Range of growth %	The most sensitive cell lines (panel), Growth % of the most sensitive cell lines
4a	-O-	C_6H_5-	95.25 (3.96)	7.79-148.26	NCI-H522 (Non-Small Cell Lung Cancer), 72.57 SNB-75 (CNS Cancer), 70.14 UO-31 (Renal Cancer), 7.79
4b	-O-	$2\text{-}CH_3C_6H_4$-	90.35 (4.36)	44.36-133.41	MDA-MB-435 (Melanoma), 44.36 SNB-75 (CNS Cancer), 55.15 A498 (Renal Cancer), 66.94 UO-31 (Renal Cancer), 69.11
4d	-O-	$4\text{-}CH_3C_6H_4$-	87.05 (4.40)	9.15-113.08	HOP-62 (Non-Small Cell Lung Cancer), 67.52 OVCAR-4 (Ovarian Cancer), 9.15 OVCAR-8 (Ovarian Cancer), 50.74 786-0 (Renal Cancer), 65.44 A498 (Renal Cancer), 67.76 RXF 393 (Renal Cancer), 68.88 MDA-MB-231/ATCC (Breast Cancer), 66.85
5a	NH-	$2\text{-}ClC_6H_4$-	94.98 (4.82)	63.48-112.26	OVCAR-4 (Ovarian Cancer), 63.48
5b	NH-	$4\text{-}ClC_6H_4$-	92.33 (4.87)	70.23-107.32	NCI-H522 (Non-Small Cell Lung Cancer), 72.16 OVCAR-4 (Ovarian Cancer), 70.23 A498 (Renal Cancer), 70.90
5f	NH-	$4\text{-}CH_3C_6H_4$-	85.32 (4.64)	49.26-113.43	K-562 (Leukemia), 66.10 RPMI-8226 (Leukemia), 68.51 SR (Leukemia), 62.18 NCI-H522 (Non-Small Cell Lung Cancer), 62.29 HCT-15 (Colon Cancer), 62.55 SK-MEL-5 (Melanoma), 68.94 A498 (Renal Cancer), 72.45 SN12C (Renal Cancer), 72.65 UO-31 (Renal Cancer), 68.55 PC-3 (Prostate Cancer), 49.26 T-47D (Breast Cancer), 53.44 MDA-MB-468 (Breast Cancer), 61.53
5g	NH-	$4\text{-}H_3OC_6H_4$-	92.11 (4.25)	61.33-136.40	NCI-H522 (Non-Small Cell Lung Cancer), 61.33 SNB-75 (CNS Cancer), 65.79 OVCAR-4 (Ovarian Cancer), 73.31 PC-3 (Prostate Cancer), 70.43 T-47D (Breast Cancer), 70.66 MDA-MB-468 (Breast Cancer), 69.18
6a	NH-	$-CH_2C_6H_5$	99.76 (3.46)	70.25-120.98	SNB-75 (CNS Cancer), 70.25

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Determination of calculated log P values of the test compounds

The log P Figures were obtained from Online Cheminformatics Services provided by Molinspiration Cheminformatics[46]. The results are summarized in (TABLE 3).

ACKNOLEDGMENTS

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