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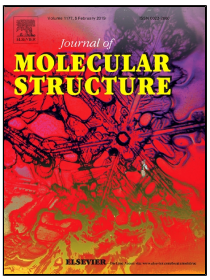

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Ahmad M. Farag, Asmaa M. Fahim

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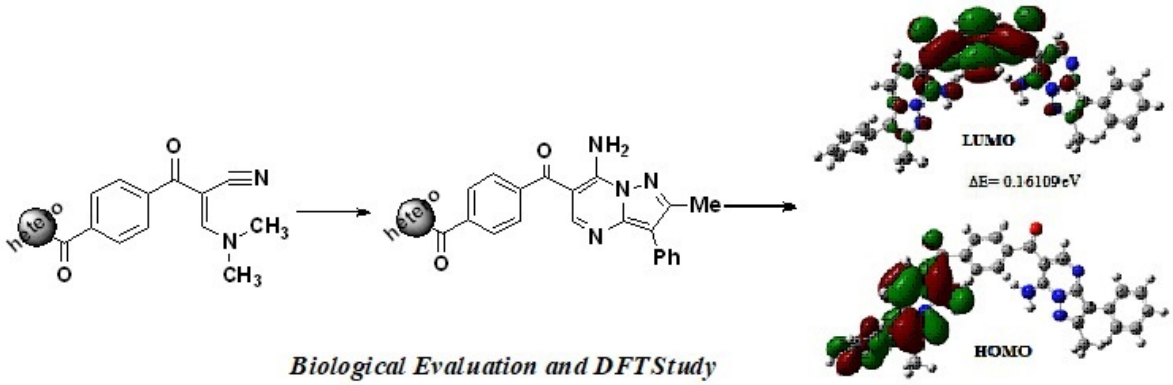
Graphical Abstract:



Synthesis, Biological Evaluation and DFT Calculation of Novel Pyrazole and Pyrimidine Derivatives

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Abstract:

The utility of the enaminonitriles **3a** and **3b** for the synthesis of the pyrazole derivatives **5a,b, 7a,b**, diaminopyrimidine derivatives **9, 11**, pyrazolo[1,5-*a*]pyrimidines **12, 15**, triazolo[4,3-*a*]pyrimidines **13, 16**, imidazo[1,2-*a*]pyrimidine derivatives **14** and **17**, was explored. Most of the synthesized compounds showed excellent *in vitro* antitumor activity against MCF-7 cell line. They also exhibted high antimicrobial and antioxidant activities. Density functional theory (DFT) calculations at the B3LYP/6-31G level of theory have been carried out to investigate the equilibrium geometry of the novel phenylpyrazolo[1,5-*a*]pyrimidin-6-yl)methanone derivative **12** and phenylpyrazolo[1,5-*a*]pyrimidine derivative **15** . The structure-activity relationship (SAR) has been used to correlate the biological activity with the appropriate quantum such as total energy. The energy of the HOMO and LUMO and Mulliken atomic charges were also calculated.

Keywords: Enaminone, Pyrazole, Pyrimidine, MCF-7 cells, Antimicrobial, Antioxidant, DFT Calculations.

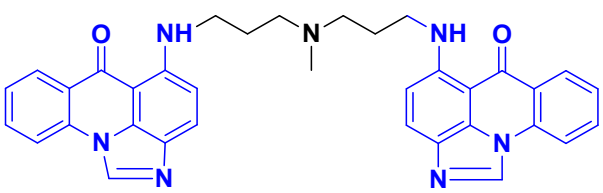
1. Introduction:

Enaminonitriles are versatile reagents for the synthesis of heteroaromatics [1-9]. On the other hand, pyrazole derivatives showed broad spectrum of biological activities, such as oxidase inhibitor [10], anticonvulsant [11], antibacterial [12], hypotensive [13], antipyretic [14] anti-inflammatory [15], antimicrobialand anthelmintic activities [16]. Moreover, the nucleus of pyrazole represent the central unit in a variety of drugs [17] such as celecobix (Celebrex) **I**, and sildenafil (Viagra) **II** (Figure **1**). Also, pyrimidine nucleus exhibited a wide range of pharmacological activities including antifungal, anti-inflammatory [18], antihypertensive antiviral, antidiabetic, antioxidant, anticancer activities [20]. Several

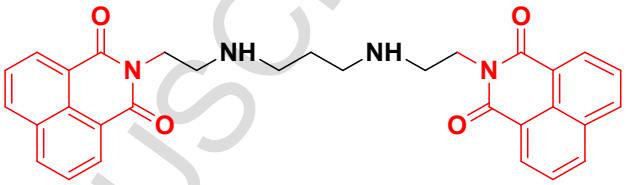
antibacterial drugs containing pyrimidine derivatives **III-IV** [20-22] are shown in Figure **1**.

Figure 1. Some drugs incorporating pyrazole and pyrimidine heterocycles

The physicochemical and pharmacological properties of bis-heterocyclic compounds [23] such as WMC-26 and LU 79553, both showing high effectiveness against *in vivo* antitumor xenografts [24-26] (**Figure 2**).



(WMC-26) V



(LU 79553) VI

Figure 2. Some bis-heterocycles with antitumor activity.

The main goal of the research reported here was to synthesize and characterize mono- and bis-heterocyclic compounds and to estimate of the energies of these molecules which is very important both in theoretical studies and their chemical reactivity [27-29]. In this context, we report the synthesis of pyrazolopyrimidine derivatives starting from dimethyl terephthalate **1a** and 4-(methoxycarbonyl)benzoic acid **1b** and their conversion to the corresponding enaminonitrile derivatives [30-32]. DFT calculations of selected examples the synthesized pyrazolo[1,5-*a*]pyrimidin derivatives have also been carried out [33].

2. Results and Discussion:

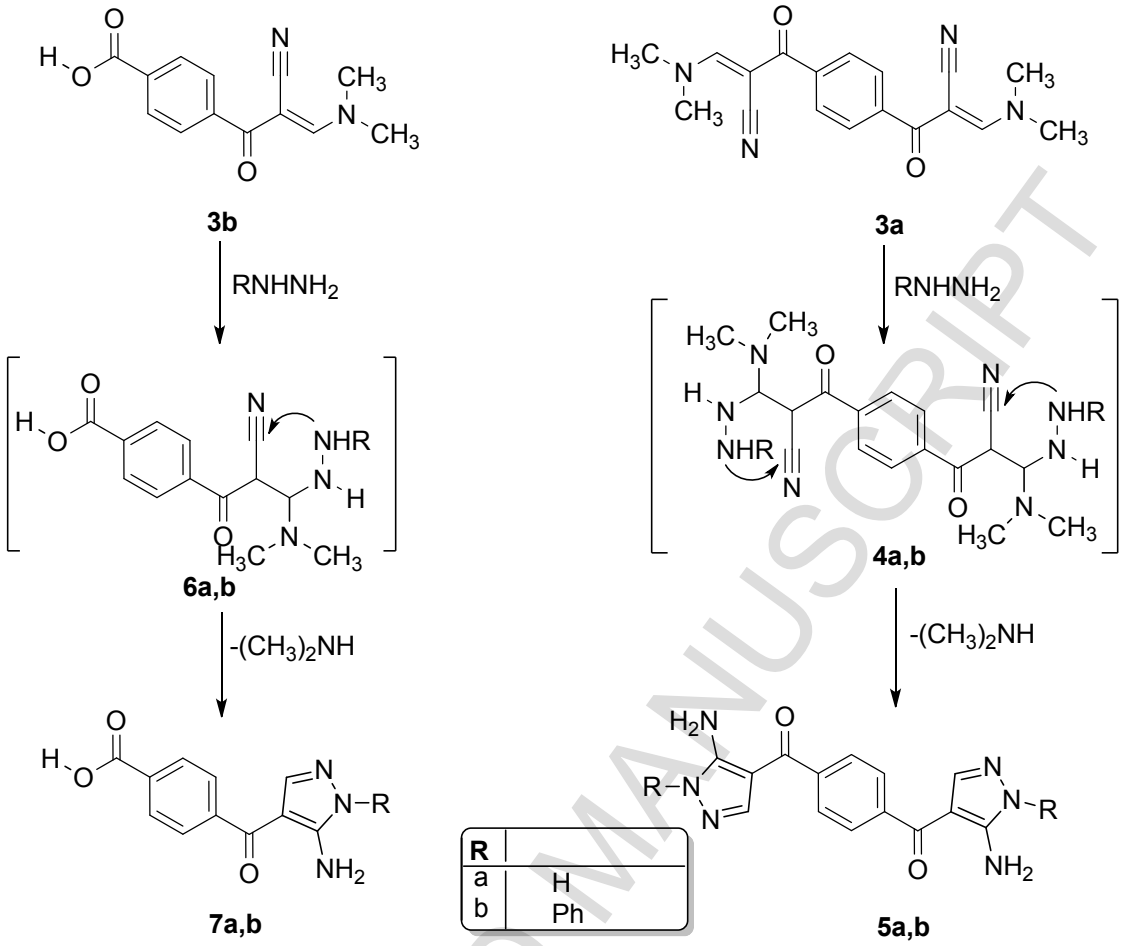
2.1. Chemistry:

The adopted route for the reaction of dimethyl terephthalate **1a** and 4-(methoxycarbonyl) benzoic acid **1b** with acetonitrile, in the presence of Sodium hydride (NaH), furnished the corresponding 3,3'-(1,4-phenylene)bis(3-oxopropanenitrile) (**2a**) and 4-(2-cyanoacetyl) benzoic acid (**2b**),[34] respectively (Scheme **1**). Treatment of each of compound **2a** and **2b** with *N,N*-dimethylformamide dimethyl acetal (*DMF-DMA*) afforded high yields of the corresponding enaminonitriles **3a** and **3b**, respectively.

The IR spectrum of the product **3b** exhibited a strong C=O absorption bands at 1723 cm^{-1} and 1677; respectively, a nitrile absorption band at 2359 cm-1 and OH band at 3406 cm^{-1} Its 1H NMR revealed signals at δ 3.37 and at δ 7.89 due to *N,N*-dimethylamino and CH proton, respectively, in addition to an aromatic protons in the region 7.93-8.12 ppm. Its mass spectrum revealed a peak at *m/z* 244 corresponding to its molecular ion. The reactivity of the enaminonitriles **3a** and **3b** towards some nitrogen nucleophiles was investigated. Thus, when the enaminonitrile 3a was treated with hydrazine and with phenyl hydrazine, it afforded the corresponding pyrazole products **5a** and **5b,** respectively as shown in Scheme **2**. The 1H NMR spectrum of compound **5a** taken as an example, revealed a singlet signal at δ 8.08 (pyrazole-CH-2) and two singlet signals at δ 7.9-8.07 due to aromatic protons, respectively The IR spectrum of the same compound revealed the presence of a strong absorption band at 1720 cm^{-1} due to carbonyl and a broad absorption band in the region 3262-3211cm^{-1} due to amino group and signal at 3365 due to NH group.

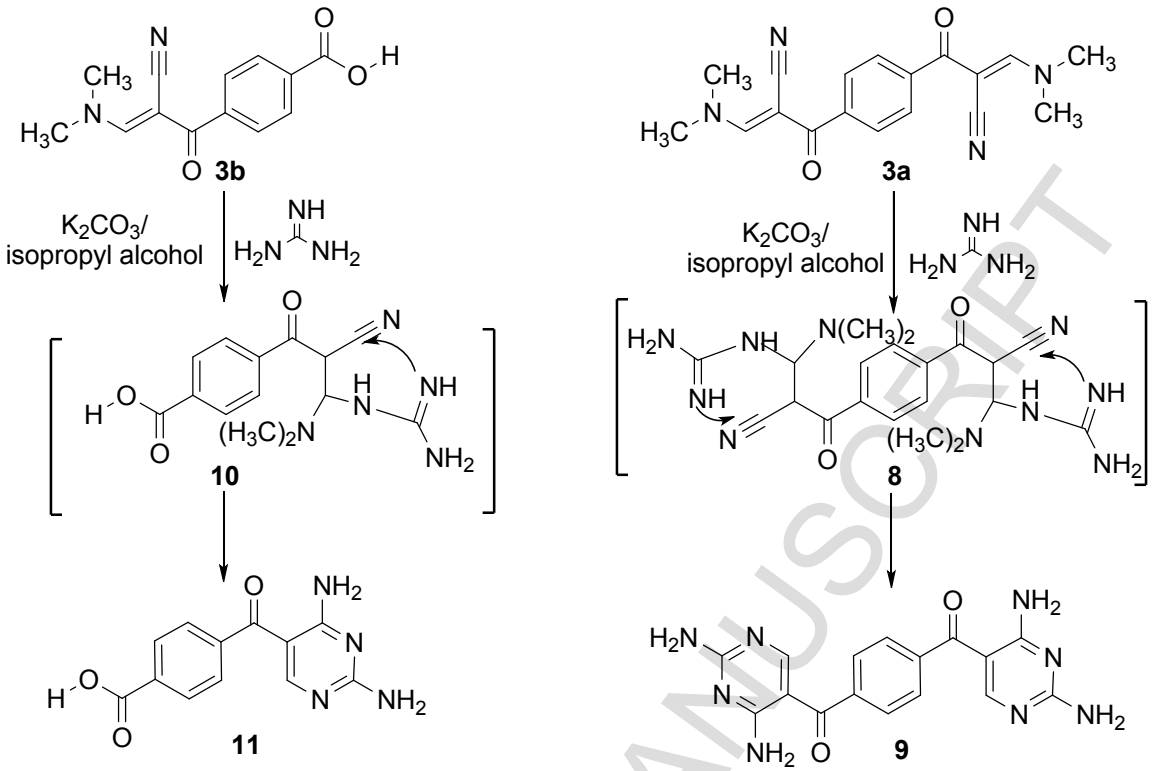
Scheme1. Synthesis of the oxopropanenitriles **2a,b** and the enaminonitrile derivatives **3a,b.**

When an ethanolic solution of the enaminonitrile **3b** was treated with hydrazine derivatives, it afforded the corresponding pyrazole derivatives **7a** and **7b**, respectively. The IR spectrum of compound **7b**, taken as a typical example, showed a characteristic absorption band at 3423 cm^{-1} due to OH function, at 3262-3216 cm^{-1} due to amino group and two strong absorption bands of carbonyl groups at 1722 cm^{-1} and 1698 cm^{-1}. The ^{1}H NMR spectrum of the same compound revealed singlet signal at δ 9.80 due to pyrazole proton and δ 12.30 (D_2O-exchangable) due to OH protons. Its mass spectrum revealed a peak at *m/z* 307 corresponding to its molecular ion (Scheme **2**).



Scheme 2. Synthesis of the pyrazole derivatives **5** and **7.**

Treatment of the enaminonitriles **3a** and **3b** with guanidine, in basic medium, afforded an excellent yield of a products identified as 1,4-phenylenebis((2,4-diaminopyrimidin-5-yl)methanone) (**9**) and 4-(2,4-diaminopyrimidine-5-carbonyl) benzoic acid (**11**), respectively. The formation of the latter products was assumed to take place *via* addition of the *N*-nuclophile to the activated ethylenic double bond of the enaminonitriles **3a** and **3b** followed by cyclization and elimination of dimethylamine molecule as depicted in Scheme **3**.

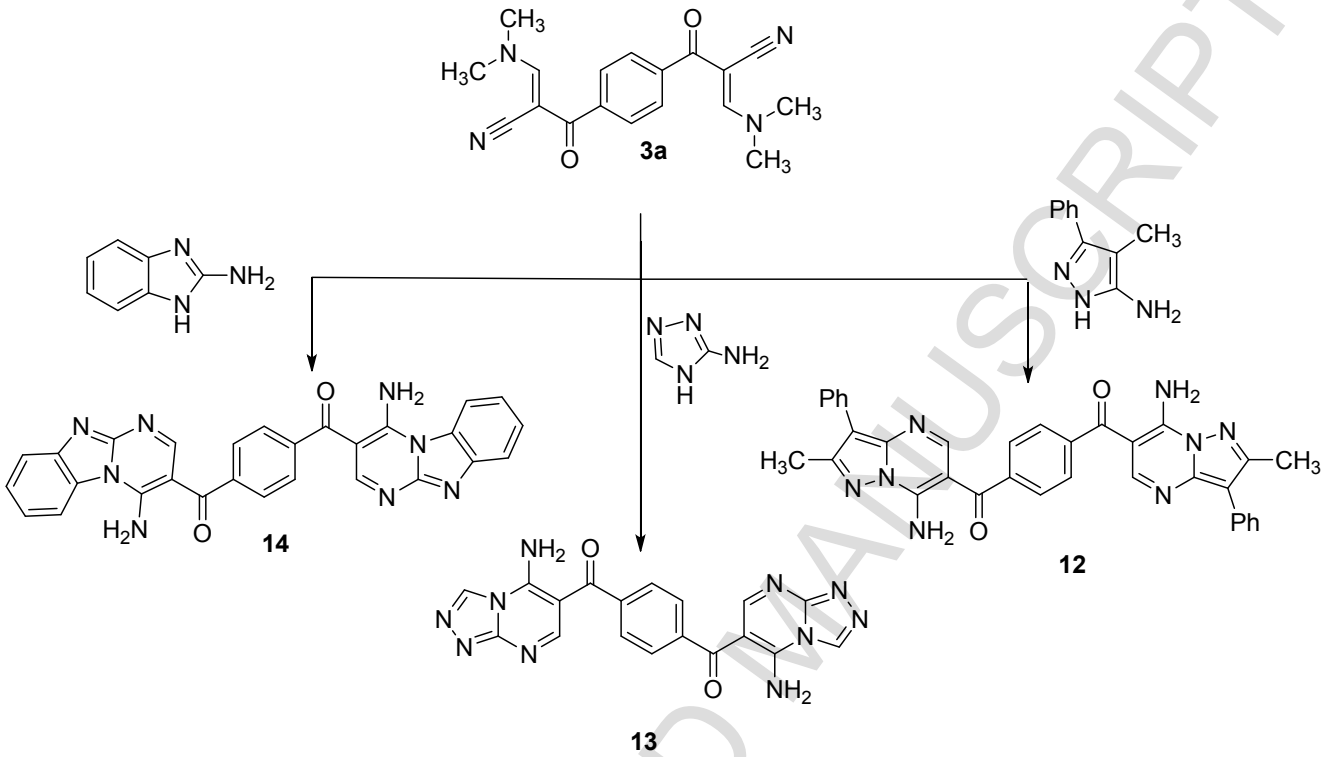


Scheme 3. Synthesis of diaminopyrimidine derivatives **9** and **11**.

The IR spectrum of compound **9** showed strong absorption bands at 3366-3300 cm^{-1} and 3206-3039 cm^{-1} due to two amino group, in addition of carbonyl band at 1710 cm^{-1}. The ^1H NMR spectrum of the same compound revealed singlet signal at δ 7.87 due to pyrimidine proton and Its mass spectrum revealed a peak at *m/z* 350 corresponding to its molecular ion

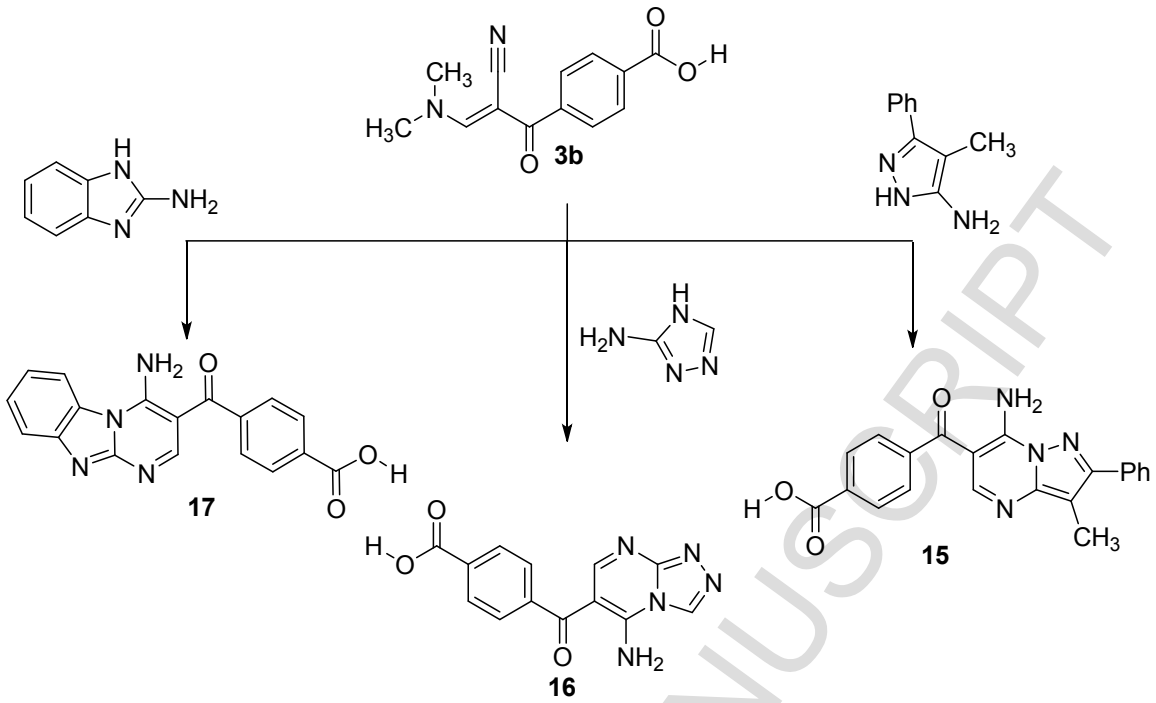
In the same manner, the enaminonitrile **3a** reacts regioselectively with heterocyclic amines such as methyl-aminopyrazole, 1,2,4-aminotraizole and 2-aminobenzimidazole, in refluxing pyridine, to afford the corresponding bis-heterocyclic systems **12**, **13**, and **14** (**Scheme** 4). The spectral data of the isolated compounds are in complete agreement with the assigned structure. The ^1H NMR spectrum of compound **13,** taken as a typical example**,** revealed a singlet signal at δ 8.33 (triazole-CH-2) and two singlet signals at δ 8.83, 8.97 due to pyrimidine protons (CH-6, CH-5) respectively, in addition to aromatic protons as a multiplet in the region 8.02-8.12 ppm. The IR spectrum of the same compound revealed a presence of strong absorption band at 1716 cm^{-1} corresponding to a carbonyl group.

The IR spectrum of compound **14** revealed bands due to amino function at 3250-3100 (NH_2) and carbonyl groups at 1733 and 1699 cm^{-1}, respectively. Its ^1H NMR spectrum revealed an aromatic a multiplet in region δ 7.96, in addition to triplet at δ 7.43 and doublet 8.66 due to imdazole protons [*cf.* Experimental part].



Scheme 4. Synthesis of bis(pyrimidine) derivatives **12**, **13** and **14.**

The enaminonitrile **3b** reacts also with heterocyclic amines to afford the corresponding pyrazolo[1,5-*a*] pyrimidine derivatives **15,** 4-(5-amino-[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonyl)benzoic acid (**16**) and 4-(4-aminobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonyl)benzoic acid (**17**), respectively (Scheme **5**). The elemental analyses and spectral data are in complete agreement with the assigned structures (*cf.* Experimental part).



Scheme 5. Synthesis of the pyrimidine derivatives **15**, **16** and **17**

3. Biological evaluation:

3.1 Anticancer screening studies:

Selected examples of the newly synthesized heterocyclic compounds (**2a, 2b, 3a, 3b, 5a, 5b, 7a, 7b, 9, 11, 12, 13, 14, 15, 16** and **17**) were screened for their anticancer activity on breast cell line by one dose assay at the National Institute of Cancer (Cairo, Egypt) by SRB assay. [35] Most of compounds showed potential cytotoxicity effect at four concentrations. The cell line used in the present investigation was MCF-7 (breast carcinoma). The IC50 was calculated with respect to the saline control group and the potency was calculated with respect to the percentage of change for *Tamoxifen* and tested compounds, as presented in Table **1** and illustrated in Figure. **3.** In comparison to standard drugs, the tested compounds exhibited high to a low antitumor activity toward the one cell line, with IC50 values ranging from 3-50 µg/ml for MCF-7 breast cell line.

Table 1. *In vitro* anti-proliferative activities of the newly prepared derivatives against MCF-7cell line.

Compound	IC_{50}(***μg/ml***)[a]	Compound	IC_{50}(***μg/ml***)[a]
MCF-7		MCF-7	
2a	28±0.13	**7b**	45.6±0.14
2b	36.1±0.19	**9**	6.3±0.01
3a	11.8±0.03	**11**	9.97±0.03
3b	16.9±0.06	**12**	3.0±0.0051
5a	38.6±0.11	**13**	5.4±0.01
5b	29.5±0.12	**14**	18.2±0.03
7a	7.6±0.01	**15**	50±0.13
16	16.2±0.05	**17**	41±0.119
(TAM)[a]	8.31±0.0102	(TAM)[a]	8.31±0.0102

TAM (tamoxifen, standard drug for breast cancer [a] IC_{50} values are the mean ± S.D. of three separate experiments.

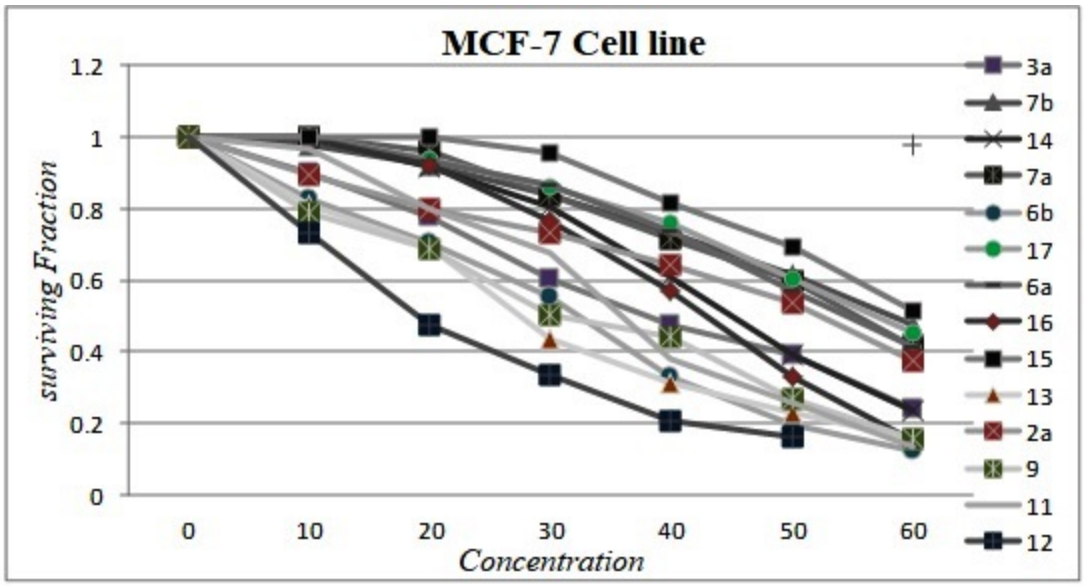


Figure 3. Survival Plot of MCF7 cells grown for 48 h in the presence of increasing concentrations of compounds **3a**,**7b**,**14**,**7a**,**6b**, **12**, **17**,**6a**,**16**,**15**,**13**, **2a**, **9** and **11** in comparison to tamoxifen.

Anti-proliferative activity of the newly heterocyclic derivatives **2a, 2b, 3a, 3b, 5a, 5b, 7a, 7b, 9, 11, 12, 13, 14, 15, 16** and **17** was examined in the MCF-7 breast cancer using sulforhodamine B (SRB) colorimetric assay as described by Skehan *et al.*[36] From the obtained results. Compound **12** exhibited the highest antitumor activity compared to standard drugs for the breast cancer cell lines with (IC50 = 3.0 µg/ml for MCF-7) compared to the mono-substituent of pyrazolo[1,5-*a*]pyrimidine **15** (IC50 = 50.0 µg/ml for MCF-7) which exhibited low activity as shown in **Table 1**. The benzoic acid derivatives **5a, 5b, 7b, 15** and **17** decreased the anticancer activity against MCF-7(IC50 =38.6, 29.5, 45.6, 50 and 41 µg/ml), whereas compounds **7a**, **9**, **11** and **13** possessed excellent anti-proliferative activities against MCF-7 cells (IC50= 7.6, 6.3, 9.97 and 5.4 µg/ml, respectively). Moreover, compounds **3a, 3b, 14** and **16** displayed good activities against MCF-7 cell line with IC50 of 11.8, 16.9, 18.2 and 16.2 µg/ml; respectively. It seems that the benzoic acid derivatives decrease the anticancer activity rather than the novel bis-heterocyclic derivatives which enhanced biodegradation and thus affected drug availability to the target sites. The obtained results are in good agreement with previous studies.[37,38]

3.2. Antimicrobial activity and structure–activity relationship (SAR):

The synthesized compounds **5a**, **5b**, **9**, **12**, **13**, **14**, **7a**, **7b**, **14**, **11**, **15** and **17** were examined for antimicrobial strains. For the optimization purpose, the most active agent is compound

17 to all strains and was selected for further modification, hoping to increase the the antimicrobial activities due to carboxyl group. On the other hand, compounds **5b** and **11** are almost having the same activity. It is worth mentioning that incorporation of carboxyl group in compounds **16, 5b, 11** and **15** showed a high antimicrobial activity than the bis-derivatives. Compounds **14** and **12** show moderate activities against all strains. These results indicate that additional bulky substituents decrease the antibacterial activity. *Geotricum candidum* is largely responsible for increased the incidence of invasive aspergillosis (IA) in immune compromised patients[39]. Moreover, the mortality rate due to invasive fungal diseases is still unacceptable high, because of a limited number of antifungal agents.[40] Data in Tables **2** revealed that compounds **5b, 16** showed remarkable activity.

Table 2. Antimicrobial activities of the synthesized compounds against the pathological organisms expressed as inhibition diameter zonesin millimeters

Compound	Gram Positive Bacteria		Gram Negative Bacteria		Fungi			
	Bs	Sp	Ec	Pa	Af	Ca	Ss	Gc
5a	11.3±0.25	NA*	NA	9.3±0.58	NA	NA	11.3±0.25	12.5±0.19
5b	19.3±0.19	17.8±0.44	NA	16.7±0.58	NA	16.8±0.58	18.7±0.44	20.6±0.19
9	12.0±0.25	10.6±0.37	NA	11.8±0.58	NA	10.3±0.37	11.4±0.34	12.6±0.37
12	18.9±0.25	16.8±0.19	NA	18.3±0.25	NA	14.9±0.58	17.2±0.37	18.7±0.37
13	16.9±0.44	14.2±0.44	NA	16.8±0.58	NA	11.9±0.25	13.4±0.19	14.8±0.58
14	18.7±0.25	16.2±0.58	NA	17.3±0.44	NA	14.8±0.37	16.3±0.25	18.8±0.44
7a	15.2±0.37	13.6±0.58	NA	14.3±0.58	NA	11.2±0.25	12.4±0.44	14.6±0.37
7b	16.3±.37	12.3±0.44	NA	14.6±0.58	NA	12.4±0.25	13.4±.44	15.8±0.44
11	20.3±0.58	17.9±0.63	NA	18.7±0.19	NA	14.8±0.19	17.8±0.63	19.3±0.44
15	14.9±0.25	11.7±0.37	NA	13.4±0.37	NA	11.4±0.25	11.7±0.19	12.9±0.19
16	23.4±0.37	19.1±0.25	NA	20.9±0.58	NA	17.8±0.25	21.9±0.25	29.8±0.19
17	16.2±0.44	13.9±0.19	NA	15.8±0.19	NA	13.3±0.25	14.7±0.58	16.2±0.44
Amphotericin B	25.4±0.1	28.7±0.2	19.7±0.2	23.7±0.1	-	-	-	-
Ampicilline	-	-	-	-	-	-	23.8±0.2	32.4±0.3
Gentamicin	-	-	-	-	17.3±0.1	19.9±0.3	-	-

NA*: No Activity; The screening organisms, Mould: Gram positive bacteria: B. subtilis (RCMB 010069,Bs) and S. pneumonia (RCMB 010010,Sp), two Gram-negative bacteria: P. aeruginosa (RCMB 010043, Pa), and E. coli (RCMB 010052,Ec),four fungi A. fumigatus (RCMB 02568,Af), , Candida albicans (RCMB 05036,Ca), Syncephala strumracemosum(RCMB 05922,Sa) and Geotricum candidum(RCMB 05097,Gc).

The preliminary SAR study has focused on the effect of mono-carboxyl subsituent is essential for the activity against Fungi and Bacteria. Amongst the dozens of phenylpyrazoles found in recent few years, Compound **16** showed one of the highest observed activities against *B. subtilis* and *Geotricum candidum.* It can be considered as a lead compound in this field. Further studies are in progress on the same compound to increaseits efficacy and understand its QSAR. Also, benzoic acid moiety is important to increase antipseudomonal activity as observed in compound **11**. The overall results of the present study can be considered very promising in the perspective of new drugs discovery, with respect to the medical importance of the tested microorganisms. *P. aeruginosa* has emerged as one of the most problematic Gram-negative pathogens, with the alarmingly high antibiotics resistance rates [41]. Even with the most effective antibiotics against this pathogen, namely carbapenems (imipenem and meropenem), the resistance rates were detected as 15-20.4% amongst 152 *P. aeruginosa* strains [42b]. This pathogen was found to be sensitive to compounds **16, 11,14,18** and **5b** (Table **2**). *Candida albicans* and other Candida species causing candidiasis are increasingly important diseases that are distributed worldwide since they are frequent opportunistic pathogens in AIDS patients. This fungus was found to be sensitive to most of the synthesized compounds especially triazolo[4,3-*a*]pyrimidine **16** (Table **2**).

3.3 DPPH radical scavenging:

The aim of this study is to identify the potential heterocyclic compound for antioxidant activity. Amongst the tested compounds 1,4-phenylenebis-((5-amino-[1,2,4]triazolo[4,3-*a*]pyrimidin-6-yl)methanone) (**13**) (27.7 µM ±2.4) was found to be potential antioxidant agents. This may be due to effective conjugation. On the other hand, 4-(5-amino-1*H*-pyrazole-4-carbonyl)benzoic acid (**5b**) (10.3 µM ±6.3) exhibited comparatively higher antioxidant activity than 1,4-phenylene-bis((5-amino-1*H*-pyrazol-4-yl)methanone (**5a**) (20.6 µM ±11.3). The presence of similarity substituent on the aromatic ring enhanced the activity which may be due to +M effect. This evidenced that compound **3a** showed excellent radical scavenging activity when compared with the standard ascorbic acid. It was also perceived that compounds **2a** exhibited good activity. However, compound **14** displayed the least activity, The IC50 value of the standard drug ascorbic acid in DPPH

method was found to be (34µ M ±6.17), respectively. The data presented in Table **3** showed that all new synthesized compounds showed good to moderate anti-oxidative activity [42,43].

Table 3._DPPH radical scavenging activity of new synthesized compounds

Compound	Inhibition%	DPPH IC_{50} (µM)[a]
Ascorbic acid	90.20%	34±6.17[b]
2a	80.9%	47.0±7.8
3a	88.9%	10.4±1.1
5a	84.8%	20.6±11.3
5b	99.5%	10.3±6.3
9	82.25%	37.5±10.08
13	91.3%	27.7±2.4
14	73.16%	66.4±10.4

[b]IC_{50} values represent as mean±SD of three determinations [b]Reported IC_{50} = 15.3µM[b]

The overview of the results presented in Table **1, 2** and **3** revealed that, in general, most of the bis-heterocyclic compounds were more active than those containing aromatic rings. More studies are needed to be carried out to find the correlation between IC50 of the evaluated noval pyrazole and pyrimidine derivatives and their molecular descripts, such as electronic, lipophilic, and steric parameters.

4. Molecular Orbital Calculations:

Goal studies of structure-activity relationship (SAR) to correlate the biological activity of a series of compounds with some appropriate descriptors such as the electronic properties of molecules and the quantum chemical descriptors based on DFT have been found useful in several SAR studies.[44] From the results of DFT calculations, different descriptors such as some of optimized geometries (bond lengths and bond angles) as well as ground state

energies (total energy ET, energy of highest occupied MO EHOMO, energy of lowest unoccupied MO ELUMO, energy gap (Eg), dipole moment (μ), and net charge utilizing Gaussian 09 [45] on fused dihydropyrazolo[1,5-*a*]pyrimidin (**12**) and phenylpyrazolo[1,5-*a*]pyrimidine (**15**) using B3LYP/6-31G were selected for SAR analysis. The computed quantum chemical descriptors based upon DFT calculations are presented in **Tables 4** and **5**. The energies of frontier molecular orbitals are important parameters in several chemical and pharmacological processes. EHOMO measures the electron donating character of a compound, while ELUMO measures its electron accepting character. Also, DFT calculation was employed to study the stability of the (7-amino-2-methyl-3-phenyl-3,7-dihydropyrazolo[1,5-*a*]pyrimidin-6-yl)(4-(7-amino-2-methyl-3-phenylpyrazolo[1,5-*a*]pyrimidine-6-carbonyl)phenyl)methanone (**12)** and 4-(7-amino-2-methyl-3-phenylpyrazolo[1,5-*a*]pyrimidine-6-carbonyl)benzoic acid (**15**) which they are both have pyrazolo[1,5-*a*]pyrimidine moiety, but they differ in the arrangement of the rings.

From the results listed in Tables **4**,**5** and Figures. **4**,**5**; the following conclusions were inferred:

(1) For both optimized structures **DHP** (**12**) of fused dihydropyrazolo[1,5-*a*]pyrimidine derivative is shown in Figure **4**, and the **PCA** (**15**) of phenylpyrazolo[1,5-*a*]pyrimidine derivative (Figure **5**), the two compounds are non-planar, where both the pyrazolo[1,5-*a*]pyrimidine are out of the molecular plane .

(2) The optimized bond length of both dihydropyrazolo[1,5-*a*]pyrimidine derivatives **12** fall in range 1.050 to 1.394 the same agreement with experimental [46] also phenyl-pyrazolo[1,5-*a*]pyrimidine **15** alls in the range of 0.971to 1.531 Å which are in good agreement with the experimental data 0.930-1.47 Å, for C=O bonds the optimized length obtained by B3LYP/6-31G is 1.247-1.240 Å were slightly shorter than the experimental value.[47,48]

(3) For dihydropyrazolo[1,5-*a*]pyrimidine derivatives **12**, the pyrazolo[1,5-*a*]pyrimidine are out of the molecular plane by -0.00227 (N29-N25-C26-N30) and 0.00259 (N12-N8-C9-N13), respectively, which allow the interaction with the appropriate dihydropyrazolo[1,5-*a*]pyrimidine **12** to afford the corresponding target molecule.

(4)- For phenylpyrazolo[1,5-*a*]pyrimidine **PCA** (**15**), the the pyrazolo[1,5-*a*]pyrimidine was out of the molecular plane by -179.968 (N8-N7-N24-C25) and -0.25288 (H41-O40-

C38-O39), respectively which may be due the stability of 4-(7-amino-2-methyl-3-phenyl pyrazolo[1,5-*a*]pyrimidine-6-carbonyl)benzoic acid (**15**) between pyrazolo[1,5-*a*]pyrimidine and benzoic acid ring.

Table4: Optimized bond length Å, and bond angle degrees dihedral angle degrees of Compound **DHP** (**12**) and **PCA**(**15**) using DFT/B3LYP/6-31G(d)

	DHP (12)			**PCA(15)**			
	B3LYP/6-31G(d)			B3LYP/6-31G(d)			
Parameters of bond lengths	Å	Parameters of bond angles	Degrees(°)	Parameters of bond lengths	Å	Parameters of bond angles	Degrees(°)
$N_{12} - N_8$	1.3428	$<C_{21}$-C_{19}-C_{20}	119.998	N_{24}-N_7	1.3665	$<C_2$-C_{26}-C_{27}	118.532
N_8-C_7	1.3795	$<C_{21}$-C_{26}-N_{30}	27.262	N_7-N_8	1.4303	$<N_{24}$-C_{25}-C_2	124.008
N_{13}-C_9	1.2660	$<N_{25}$-C_{26}-N_{30}	122.194	C_5-N_8	1.3826	$<N_8$-N_7-N_{24}	128.326
N_{13}-H_{46}	1.0500	$<C_{28}$-N_{29}-N_{25}	102.803	C_{26}-O_{27}	1.2473	$<C_5$-N_8-N_7	106.282
N_6-C_7	1.3497	$<C_{27}$-C_{24}-N_{25}	105.485	C_{38}-O_{39}	1.2421	<C5-C4-C1	109.012
C_9-C_4	1.3948	$<C_{21}$-C_{19}-O_{20}	119.998	C_{38}-O_{40}	1.4033	$<C_2$-C_{26}-C_{27}	118.532
C_2-O_3	1.2081	$<N_{29}$-N_{25}-C_{24}	33.440	N_7-C_1	1.4264	$<O_{40}$-C_{38}-O_{39}	120.936
C_1-C_2	1.3509	$<N_{12}$-N_8-C_9	123.520	C_4-C_1	1.4167	$<H_{41}$-O_{40}-C_{38}	108.942
N_{12}-C_{11}	1.3406	$<N_{12}$-N_8-C_9	122.453	C_5-C_4	1.4411	$<C_5$-C_4-C_{20}	27.8105
N_{25}-C_{26}	1.3888	$<N_{13}$-C_9-C_4	112.178	C_{26}-C_{28}	1.5317	$<C_{10}$-C_9-C_5	122.307
N_{30}-H_{54}	1.0500	$<N_{29}$-N_{25}-C_{26}-N_{30}	-0.00227	O_{40}-H_{41}	0.9718	$<N_8$-N_7-N_{24}-C_{25}	-179.968
N_{25}-N_{29}	1.3395	$<N_{12}$-N_8-C_9-N_{13}	0.00259	C_{25}-N_{24}	1.3442	$<H_{41}$-O_{40}-C_{38}-O_{39}	-0.25288
C_{19}-O_{20}	1.2081	$<N_6$-C_5-C_4-C_2	179.997	C_{26}-C_2	1.5299	$<N_7$-N_8-C_5-C_4	0.21265

From the above results (Tables **1**-**4**), it is clear that as the dihydropyrazolo[1,5-*a*]pyrimidine (DHP**) 12** and phenylpyrazolo[1,5-*a*]pyrimidine **(**PCA) **15**, come close to the molecular plane of the pyrazolo[1,5-*a*]pyrimidine moiety. On the other hand, compounds **12** and **15** moieties go far from the molecular plane of the pyrazolo[1,5-*a*]pyrimidine ring, by increasing its dihedral angles.

(5) The two *p*-isoelectronic structures **12** and **15** are though dihydropyrazolo[1,5-*a*]pyrimidine **12** seems a more stable than phenylpyrazolo[1,5-*a*]pyrimidine **15** by 3.0717 eV (≈70.835kcal) (Table **5**).[49]

Table 5: Quantum-chemical descriptors based on DFT calculations used for SAR studies at DFT/ B3LYP/6-31G(d).

Compound **12**					Compound **15**	
E_T (au)	-1883.705			E_T (au)	-1254.570	
E_{HOMO} (eV)	-5.4423			E_{HOMO} (eV)	-5.1598	
E_{LOMO} (eV)	-2.3706			E_{LOMO} (eV)	-2.3399	
Eg (eV)	3.0717			Eg (eV)	3.1799	
μ (D)	6.4786			μ (D)	4.8810	
Net charges		N30	-0.884	Net charges	N13	-0.817
		N13	-0.812		N8	-0.443
		N6	-0.502		N6	-0.503
		N25	-0.455		N12	-0.430
		O20	-0.461		O3	-0.461
		O3	-0.452		O20	-0.465
		C19	0.313		O43	-0.562
		C24	0.415		C10	-0.125
		C10	-0.125		C7	0.549

[a] Eg= E_{LOMO} - E_{HOMO}.

(**6**) From the calculations of the energy gap, E_g, which measure the chemical activity, the dihydropyrazolo[1,5-*a*]pyrimidine (DHP**)** **12** was found to be more reactive than phenylpyrazolo[1,5-*a*]pyrimidine (PCA) **15** by -2.4951 kcal as shown in Figures **4** and **5**. (**7**) The polarity or charge separation over the molecule, which is measured by the dipole moment μ, showed that μ of dihydropyrazolo[1,5-*a*]pyrimidine (DHP) **12** > μ of amino phenylpyrazolo[1,5-*a*]pyrimidine (PCA) **15** by 1.5976 D.

From the above results (**5**–**7**), one can conclude that although the total energy (E_T) of dihydropyrazolo[1,5-*a*]pyrimidine (DHP) **12** is less than that of phenylpyrazolo[1,5-*a*]pyrimidine (PCA) **15** by a factor of -394788.257 kcal together with the fact that polarity, as well as the reactivity of compound **12**, are more than that of **15**, experimental results showed that dihydropyrazolo[1,5-*a*]pyrimidine (DHP) **12** give excellent biological activity rather than compound **15** which its energy is more so give less activity

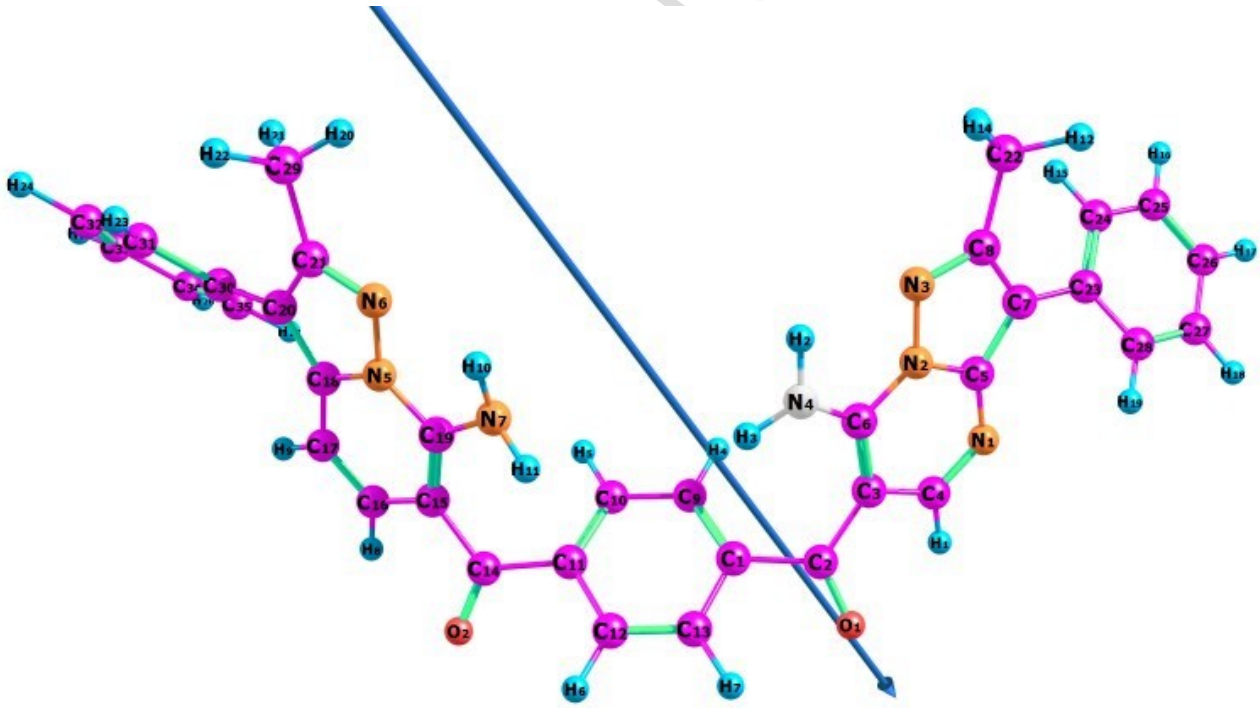


Figure 4. The optimized geometry, numbering system, and and the vector of the dipole moment of DHP (**12)** using B3LYP/6-31G.

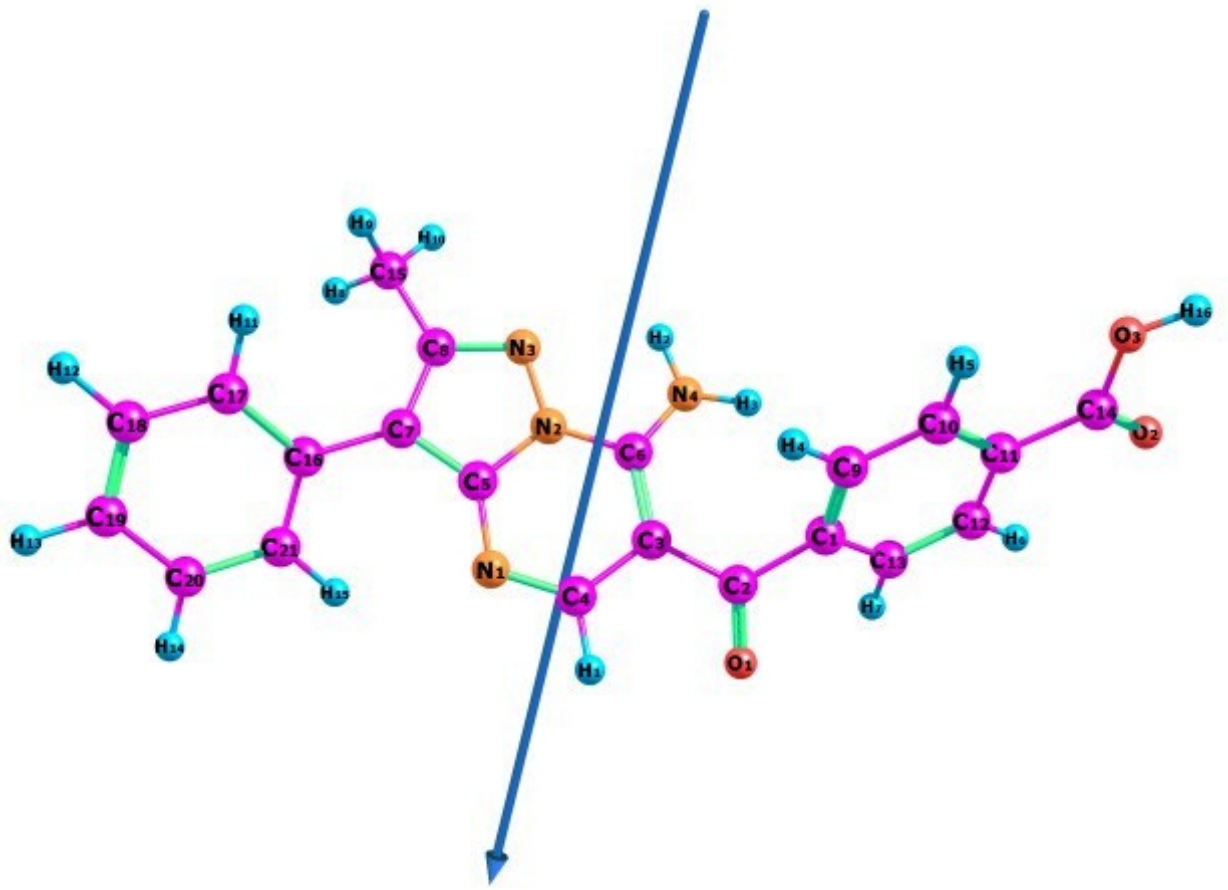


Figure 5. The optimized geometry, numbering system, and and the vector of the dipole moment **PCA** (**15**) using B3LYP/6-31G.

4.1 Frontier Molecular Orbitals *(FMO):*

The importance of the frontier molecular orbital (FMO) contribution is lying in determining the charge-separated states and reflecting the chemical activity of the studied molecules. General, hybrid funcionals were used to improve the description of the ground state energies for small molecules,[50] One of the most commonly hybrid functional used is B3LYP which leads to improved band gaps as good as that obtained from sophisticated correlated calculation. Therefore, in order to rationalized this increased accuracy by comparison with results from time-dependent DFT (TD-DFT) calculations so we found the HOMO-LUMO gap energy for compound **12** was (0.16109 eV) rather than compound **15** which has energy gap (1.0753 eV). The small energy gap in compound **12** expose a numeral of unique properties such as infrequent electronic properties, for instance thermo-excited

intermolecular electron transfer (in solution) and metal conductivity (in the solid state),[51] as demonstrated in Figure **6**.

Figure 6. illustrated the electronic structures of HOMO and LUMO of compounds **12** and **15**. As can be seen from that figure, strong delocalization of the HOMO for compound **12** occurs on the pyrazolopyrimidine donor moiety while strong delocalization of the LUMO occurs on the benzene bridge (acceptor moiety) between the pyrazolopyrimidine moieties demonstrating the flow of electron density along compound **12**. Furthermore, strong delocalization of the HOMO for compound **15** occurs on the pyrazolopyrimidine donor moiety while strong delocalization of the LUMO occurs on the benzoic acid as an acceptor moiety. Consequently, the lowering of the HOMO-LUMO energy gap is essentially a consequence of the large stabilization of the LUMO due to the strong electron-accepting capability of the electron-acceptor group and influences the biological activity of the molecule.

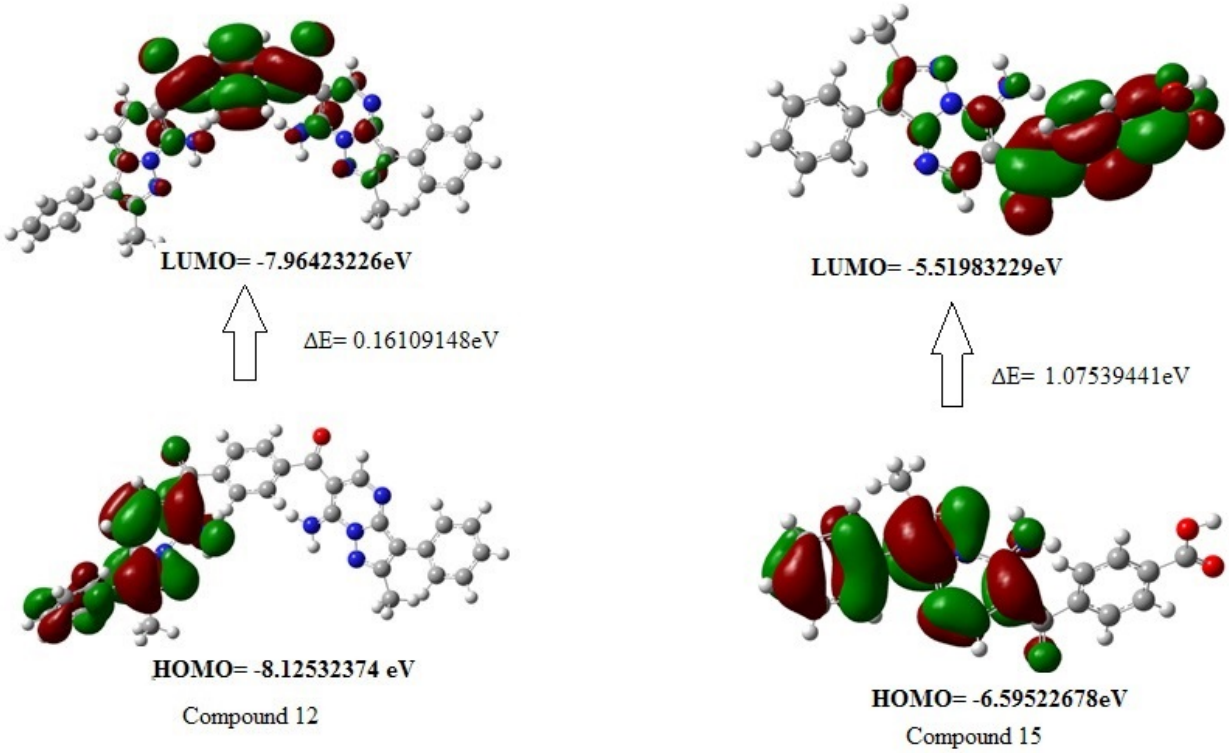


Figure 6. Gap energy (HOMO–LUMO) (eV) are calculated for compounds **12** and **15** using TD-DFT B3LYP/6-31G (d).

5. Conclusion

In this investigation, we have developed an efficient and simple method for the synthesis of a series of novel heterocycles of 5-amino-1*H*-pyrazol-4-yl derivatives **5 a,b** and **7 a,b**, 2,4-diaminopyrimidin **9** and **11**, pyrazole[1,5-*a*] pyrimidine **12** and **15**, triazolo [4,3-*a*]pyrimidine **13** and **16** and dihydropyrimido[1,2-*b*]indazole **15** and **18** from the ester derivatives **1a,b**. (7-Amino-2-methyl-3-phenyl-3,7-dihydropyrazolo[1,5-a] pyrimidin-6-yl)(4-(7-amino-2-methyl-3-phenylpyrazolo[1,5-*a*]pyrimidine-6-carbonyl)phenyl) methanone (**12**) showed a higher antitumor activity than tamoxifen standard drug, while 4-(5-amino-[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonyl)benzoic acid (**16**) showed high antimicrobial activity in all strain media comparable with commercial compounds. Also, 1,4-phenylenebis((5-amino-1-phenyl-1*H*-pyrazol-4-yl)methanone) (**5b**) showed high activity against DPPH as antioxidant activity. DFT calculations at B3LYP/6-31G predicted that the dihedral angles of fused pyrazolo[1,5-*a*]pyrimidine rings of both *p*-isoelectronic structures **12** and **15** are a new building block heterocyclic compounds responsible for that chemical and biological activities and therefore, become lead molecules for further synthetic and biological evaluation. It can be concluded that this class of compounds certainly holds great promise towards pursuit to discover a novel class of antitumor, antimicrobial and antioxidant agents. Further studies are being conducted to acquire more information about quantitative structure-activity relations.

6. Experimental:

6.1. General

All melting points were measured on a Gallenkamp melting point apparatus and were uncorrected. The infrared spectra were recorded in potassium bromide disks on a PyeUnicam SP-3-300 and Shimadzu FT-IR 8101 PC infrared spectrophotometers. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}HNMR spectra were run at 300 MHz and ^{13}CNMR spectra were run at 75.46 MHz in dimethyl sulphoxide (DMSO-d_6). Chemical shifts were related that of the solvents. Mass spectra were recorded on a Shimadzu GCMS-QP-1000EX mass spectrometer at 70 e.V.

6.1.1 Material and reagents:

Dimethyl terephthalate, 4-(methoxycarbonyl)benzoic acid, acetonitrile, sodium hydride, dimethylformamide dimethyl acetal (*DMF-DMA*), hydrazine hydrate, phenylhydrazine, triethylamine, 2-aminobenzimidazole, aminopyrazole and aminotriazole were purchased from Aldrich Chemical CO. Ethanol, pyridine, toluene, THF and piperidine purchased from Aldrich Chemical CO. Methanol, petroleum ether; chloroform where BDH reagents.

6.1.2. Preparation of 3-oxopropanenitrile derivatives **2a, b**:

General procedure:

A three-necked, round bottomed flask (500-ml), equipped with a magnetic stirrer, thermometer, decanter and condenser was charged with the proper ester **1a** or **1b** (10g)**,** acetonitrile (10 ml, 10 mmole), in dry THF and stirred well, then an equivalent weight of NaH and DMF were added then refluxed to 100^{o}C for 2 h then left to cool, and filtered washed with petroleum ether. The formed salt was dissolve in ice-cold water and acidified HCl to afford the corresponding 3-oxo-propanenitrile derivatives **2a** and **2b**; respectively.

3,3'-(1,4-Phenylene)bis(3-oxopropanenitrile) (**2a**) [33] pale brown powder (EtOH/DMF), m.p:175-177^{o}C, yield:75% $C_{12}H_8N_2O_2$, IR (KBr, cm^{-1}): ν= 2549 (C≡N), 1717 (C=O) ; ^{1}HNMR (DMSO-d_6) 3.875 (s, 4H, H_2C), 8.03-8.07 (s, 4H, *H*-Ar), ^{13}C NMR (DMSO-d_6): 29.3 (*C*H$_2$), 129.9 (*C*H), 134.4(*C*≡N), 135.4 (*C*H), 167.10 (*C*=O); MS, (*m/z*): 149 (M^{+}, 100.0%),163(38.46%), 212 (25.23%), 104(60.52%); Anal calcd $C_{12}H_8N_2O_2$ (212.20); C, 67.92; H,3.80; N, 13.20%; Found: C, 67.88; H, 3.77; N, 13.24%.

4-(2-Cyanoacetyl)benzoic acid (**2b**), white crystals (EtOH), m.p:170-172^{o}C, yield: 75%, $C_{10}H_7NO_3$ IR (KBr, cm^{-1}) ν=3374(OH), 2237(C≡N), 1715 (C=O) , 1689 (C=O); ^{1}HNMR (DMSO-d_6): 3.480 (s, 2H, H_2C), 7.93-8.00(d, 2H, *H*C), 8.01-8.15(d, 2H, *H*C), 12.30(s, 1H, *H*OOC D_2O exchangeable); ^{13}C NMR (DMSO-d_6):δ 29.77 (*C*H$_2$), 129-130 (*C*H), 134 (*C*N), 135 (*C*H),139 (*C*H), 165 (*C*=O), 170(*C*=O); MS (*m/z*): 149 (M^{+}, 100.0%), 189 (94.3%),179 (83.8%), 120 (70.4%); Anal calcd $C_{10}H_7NO_3$ (189.17) C, 63.49; H, 3.73; N, 7.40%; Found C, 63.53 %; H, 3.77%; N, 7.45%;

6.3. Synthesis of Enaminonitrile derivatives **3a,b:**

General procedure:

A solution of the 3-oxopropanenitrile derivatives **2a** or **2b** (10 mmol) in dry THF (50 ml) and dimethylformamide-dimethyl acetal (*DMF-DMA*) (10mmol) was refluxed for 2 h and then left to cool. The white precipitated was filtered off, washed with dry petroleum ether (40-60 ^{o}C) and dried.

(*Z*)-2-(4-((*E*)-2-cyano-3-(dimethylamino)acryloyl)benzoyl)-3-(dimethylamino) acrylonitrile (**3a**) brown crystals (EtOH), m.p:197-199^{o}C, yield: 80%: $C_{18}H_{18}N_4O_2$; IR (KBr, cm^{-1}) ν=: 2200(C≡N), 1712(C=O); ^{1}HNMR (DMSO-d_6): 3.9 (s, 12H, H_3C), 7.9 (s, 4H, *H*-Ar), ^{13}C NMR (DMSO-d_6): 41 (*C*H$_3$), 85.2 (*C*H), 115.8 (*C*≡N), 129.3 (*C*H), 137 (*C*H), 141 (*C*H), 168 (*C*=O); MS (*m/z*): 322.36 (M^{+}, 100%), 228(14.3%) Anal calcd $C_{18}H_{18}N_4O_2$ (322.36) C, 67.07; H, 5.63; N, 17.38%; Found: C, 67.03; H, 5.67; N, 17.42%

(*E*)-4-(2-cyano-3-(dimethylamino)acryloyl)benzoic acid (**3b**) pale white crystals (EtOH), m.p: 182-184^{o}C, yield: 88%; $C_{13}H_{12}N_2O_3$; IR (KBr, cm^{-1}), ν=3406 (OH), 2359 (C≡N), 1723(C=O), 1677 (C=O); ^{1}HNMR (DMSO-d_6): 3.37(s, 6H, H_3C), 7.89 (s, 1H, *H*C), 7.93-8.03(d, 2H, *H*C aromatic), 8.06-8.12(d, 2H, *H*C aromatic), 12.30(s, 1H, 1H, *H*OOC D_2O exchangeable); ^{13}CNMR (DMSO-d_6): δ 42.7 (*C*H$_3$), 85.2 (*C*H), 115.8 (*C*N), 129.8 (*C*H), 136 (*C*H), 141 (*C*H), 157.2 (*C*H), 169.3 (*C*=O), 186.4 (*C*=O); (*m/z*): 244 (M^{+}, 100.0%), 55(74%), 57 (6.86%); Analysis for $C_{13}H_{12}N_2O_3$ (244.25); C, 63.93;%; H, 4.95;% N, 11.47%; Found C, 63.98;%; H, 5.00;%; N, 11.43%.

6.4. Reaction of the enaminonitrile derivatives **3** with hydrazine derivatives:

General procedure:

To an ethanolic solution of the appropriate enaminonitrile **3** (0.44 g, 2 mmol), (20 ml), hydrazine hydrate (80 %, 0.2 ml), phenylhydrazine (0.2 ml, 2 mmol) was added and the resulting mixture was refluxed for 6h then left to cool. The solid formed was filtered off, and washed with ethanol and dried, to afford the corresponding pyrazoles derivatives **5a, b** and **7a**, **b**.

1,4-phenylenebis((5-amino-1*H*-pyrazol-4-yl)methanone) (**5a**) pale brown crystals: (EtOH), m.p: 211-213^{o}C yield: 66%, $C_{14}H_{12}N_6O_2$; IR (KBr, cm^{-1}) ν= 3365(NH), 3262-3211(NH_2), 1720 (C=O); ^{1}HNMR (DMSO-d_6): 7.34 (s, 4H, H_2N D_2O-exchangeable), 7.9-8.07(s, 4H, *H*C aromatic), 8.08 (s, 2H, H_2C pyrazole), 13.26(s, 2H, *H*N D_2O-exchangeable); ^{13}C NMR (DMSO-d_6): δ 96.3 (*C*H), 128.3(*C*H), 129.6 (*C*H), 135.1 (*C*H), 138.2 (*C*H), 145.2 (*C*H), 196.3 (*C*=O), MS (*m/z*): 296 (M^+, 100.0%), 187 (25%), 110(12.3%); Anal calcd $C_{14}H_{12}N_6O_2$ (296.28); C, 56.75; H, 4.08; N, 28.36% Found: C, 56.79; H, 4.03; N, 28.40%.

1,4-Phenylenebis((5-amino-1-phenyl-1*H*-pyrazol-4-yl)methanone) (**5b**), dark brown crystals (EtOH), m.p: 231-233^{o}C yield:64%, $C_{26}H_{20}N_6O_2$, IR (KBr, cm^{-1}) ν=3362-3330 (NH_2), 1646 (C=O): ^{1}HNMR (DMSO-d_6): 6.43-6.49 (t, 2H, *H*C, *J*=3.3Hz), 6.7-7.3(m, 8H, *H*C), 7.4-7.8(s, 4H, *H*C), 7.93(s, 4H, H_2N D_2O-exchangeable), 8.41(s, 2H, *H*C-pyrazole); MS (*m/z*): 448 (M^+, 100%), 290(36%), 158(12.3%) Anal calcd $C_{26}H_{20}N_6O_2$ (448.16): C, 69.63; H, 4.49; N, 18.74, Found C, 69.68; H, 4.52; N, 18.79.

4-(5-Amino-1*H*-pyrazole-4-carbonyl)benzoic acid (**7a**) white crystals (EtOH), m.p:198-199^{o}C , yield: 69%,$C_{11}H_9N_3O_3$; IR (KBr, cm^{-1}) ν=: 3423 (OH), 3330 (NH), 3262-3216 (NH_2), 1723(C=O), 1693(C=O); ^{1}HNMR (DMSO-d_6): 7.43 (s, 2H, H_2N D_2O-exchangeable), 7.62-7.96 (d, 2H, *H*C aromatic, *J*=12Hz), 7.9-8.1 (d, 2H, *H*C aromatic, *J*=12Hz), 9.80 (s, 1H, *H*C pyrazole), 12.30 (s, 1H, *H*O D_2O-exchangeable), 13.09(s, 1H, *H*ND_2O- exchangeable); MS (*m/z*): 233(M^+, 100.0%), 205 (47.39%), 64 (35.98%); Anal calcd $C_{11}H_9N_3O_3$ (231.21) C, 57.14; H, 3.92 ; N, 18.17%, Found: C, 57.17; H, 3.96; N, 18.20%.

4-(5-Amino-1-phenyl-1*H*-pyrazole-4-carbonyl)benzoic acid (**7b**) brown solid crystals (EtOH), m.p: 235-237^{o}C, yield:60%,$C_{17}H_{13}N_3O_3$, IR (KBr, cm^{-1}) ν=: 3423 (OH), 3262-3216 (NH_2), 1722 (C=O), 1698 (C=O); ^{1}HNMR (DMSO-d_6): 7.43-7.83(m, 5H, *H*-Ar), 7.93 (s, 2H, H_2N D_2O-exchangeable), 8.07-8.12 (m, 4H, *H*-Ar), 9.80 (s,1H, *H*C pyrazole), 12.30 (s, 1H, *H*O D_2O-exchangeable); MS (*m/z*): 307(M^+, 100%), 311

(7.57%), 68 (33.2%); Anal calcd $C_{17}H_{13}N_3O_3$ (307.30); C, 66.44; H, 4.26;N, 13.67%; Found :C, 66.48; H, 4.31; N, 13.63%.

6.5. Reaction of the enaminonitriles **3** with guanidine:

General procedure:

To a mixture of the appropiate enaminonitrile **3a** or **3b** (0.44 g, 2 mmol) and guanidine nitrate (2.3 mmol), in isopropyl alcohol (30 ml), anhydrous potassium carbonate (0.552 g, 4 mmol) was added. The resulting mixture was refluxed for 6h and then allowed to cool to room temperature and diluted with water (20ml). The solid product was collected by filtration, washed with water and dried. Recrystallization from ethanol afforded the corresponding (2,4-diaminopyrimidin-5-yl) derivatives **9** and **11**; respectively.

1,4-Phenylenebis((2,4-diaminopyrimidin-5-yl)methanone) (**9**) yellow crystals (EtOH), m.p: 221-223^{o}C yield: 65%, $C_{16}H_{14}N_8O_2$; IR (KBr, cm^{-1}) ν = 3366-3300 (NH_2), 3206-3039 (NH_2), 1710 (C=O) ;^{1}HNMR (DMSO-d_6): 6.98 (s, 4H, H_2N D_2Oexchangeable),7.63(s, 4H,H_2N-D_2Oexchangable) 7.87 (s, 2H, *H*C-pyrimidine), 8.07 (s, 4H, *H*-Ar); MS (*m/z*): 350 (M^+, 100.0%),213(29.3%), 137.2(9.6%); Anal calcd $C_{16}H_{14}N_8O_2$ (350.33); C, 54.85; H, 4.03; N, 31.98 % Found: C, 54.88; H, 4.00; N, 32.02%.

4-(2,4-Diaminopyrimidine-5-carbonyl)benzoic acid (**11**) white crystals (EtOH), m.p: >300^{o}C, yield: 66%**,** $C_{12}H_{10}N_4O_3$, IR (KBr, cm^{-1}) ν=: 3699(OH), 3411-3396(NH_2), 3256-3102 (NH_2), 1723 (C=O), 1688 (C=O); ^{1}HNMR (DMSO-d_6): 6.96(s, 2H, H_2N D_2O-exchangeable), 7.085 (s, 1H, *H*C pyrimidine7.83(s, 2H, H_2N D_2O-exchangeable), 7.9-8.02 (d, 2H, *H*C aromatic, *J*=1.2Hz), 8.02-8.06 (d, 2H, *H*C aromatic, *J*=3Hz), 12.36(s, 1H, *H*O D_2O-exchangeable),; MS (*m/z*): 258 (M^+, 100.0%), 149 (50.02%), 104(77.3%); Anal calcd $C_{12}H_{10}N_4O_3$ (258.23); C, 55.81;H, 3.90; N, 21.70% Found: C, 55.85; H, 3.94; N, 21.74%.

6.6. Reaction of the enaminonitrile **3a**, **b** with heterocyclic amines:

General procedure:

A mixture of the enaminonitrile **3** (2 mmol) and the corresponding heterocyclic amine [aminoyrazole or 2-aminobenzimidazole or 1,2,4- aminotriazole] (2 mmol) in pyridine (15

ml) was refluxed for 6h, then left to cool to room temperature. The precipitated product was filtered off, washed with ethanol and recrystallized from the proper solvent to afford the products **12**, **13**, **14**, **15**, **16** and **17.**

1,4-phenylenebis((7-amino-2-methyl-3-phenylpyrazolo[1,5-*a*]pyrimidin-6-yl)methanone) (**12)** brown powder (EtOH/DMF), m.p: 263-265^{o}C yield: 71%; $C_{34}H_{26}N_8O_2$, IR (KBr, cm^{-1}) ν=: 3421-3319 (NH_2), 3309-3260 (NH_2), 1714(C=O); ^{1}HNMR (DMSO-d_6): 2.23 (s, 6H, H_3C), 7.23-7.44 (m, 10H, *H*-Ar), 7.75(s, 4H, H_2ND_2O-exchangeable), 8.11-8.17 (s, 4H, *H*-Ar), 8.34 (s, 2H, *H*C pyrimidine); ^{13}C NMR (DMSO-d_6): δ 11.0 (*C*H_3), 115.3 (*C*H), 126.5 (*C*H), 128.7 (*C*H), 129 (*C*H), 132.6 (*C*H), 137.3 (*C*H), 160 (*C*H), 164 (*C*H), 169 (*C*H), 190 (*C*=O), 196.3 (*C*=O) MS (*m/z*): 578(M^+, 100.0%), 357(14.3%), 255 (25.15%), 166 (13.55%), 149(63.2%), 121(54.92%); Anal calcd. $C_{34}H_{26}N_8O_2$ (578.62); C, 70.58; H, 4.53; N, 19.37% Found: C, 70.62; H, 4.57; N, 19.41%

1,4-phenylenebis((5-amino-[1,2,4]triazolo[4,3-*a*]pyrimidin-6-yl)methanone) (**13**) pale yellow crystals (EtOH/DMF), m.p: 268-270^{o}C, yield:72%, $C_{18}H_{12}N_{10}O_2$, IR (KBr, cm^{-1}) ν=3320-3214(NH_2), 3125-3065 (NH_2), 1716 (C=O); ^{1}HNMR (DMSO-d_6): 7.95 (s, 4H, H_2N, D_2O exchangeable), 8.02-8.12 (m,4H, *H*-Ar), 8.33 (s, 2H, triazole- *H*C-2, *J* = 7.2Hz), 8.83(s,1H, *H*C, pyrimidine), 8.97(s,1H, *H*C, pyrimidine), MS (*m/z*): 400 (M^+, 100.0%), 340 (47.68%), 166 (4512%), 149 (10.2%), 79.20 (99.34%); ^{13}C NMR (DMSO-d_6); 111.7 (*C*H), 121(*C*H), 129 (*C*H), 136 (*C*H), 146 (*C*H), 154 (*C*H), 166 (*C*H), 168 (*C*H), 169 (*C*=O); Anal calcd $C_{18}H_{12}N_{10}O_2$ (400.35); C,54.00; H, 3.02; N, 34.99% Found: C, 54.04; H, 3.06; N 34.95%.

1,4-phenylenebis((4-aminobenzo[4,5]imidazo[1,2-*a*]pyrimidin-3-yl)methanone) (**14**) dark yellow crystals (EtOH/DMF), m.p: 298-299^{o} C; yield: 69%; $C_{28}H_{18}N_8O_2$, IR (KBr, cm^{-1}) ν=3250-3100 (NH_2), 1733 (C=O), 1699 (C=O); ^{1}HNMR (DMSO-d_6): 7.21-7.29 (d, 4H, *H*C), 7.43(t, 2H, *H*C, *J*=6.3Hz), 7.71(s, 4H, H_2N D_2O exchangeable),7.96 (s, 4H, *H*-Ar), 8.12 (s, 2H, *H*C), 8.66 (d, 2H, *H*C, *J* =7.8 Hz), MS (*m/z*): 149 (M^+, 100.0%), 166 (23.08%); Anal calcd $C_{28}H_{18}N_8O_2$ (498.49); C, 67.46; H, 3.64; N, 22.48%; Found: C, 67.50; H,3.60; N, 22.42%.

4-(7-amino-2-methyl-3-phenylpyrazolo[1,5-*a*]pyrimidine-6-carbonyl)benzoic acid (**15**) white crystals (EtOH), m.p: >300° C yield: 61%,($C_{21}H_{16}N_4O_3$), IR (KBr, cm^{-1}) ν =3419 (OH), 3329-3201(NH_2), 1721(C=O), 1689 (C=O); ^{1}HNMR (DMSO-d_6): 1.04 (s, 3H, H_3C), 7.63-7.77 (s, 5H, *H*-Ar), 7.88(s, 2H, H_2N D_2O-exchangeable), 8.29-8.30 (dd, 4H, *H*-Ar, *J* = 3.2 Hz), 8.33(d, 1H, *H*C, *J* =1.2Hz), 12.32(s, 1H, *H*O D_2O- exchangeable), MS(*m/z*): 372(M^+, 100.0%), 75(85.37%); Anal calcd $C_{21}H_{16}N_4O_3$ (372.38); C,67.73; H,4.33; N,15.05% Found: C,67.77; H, 4.38; N, 15.01%

4-(5-Amino-[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonyl)benzoic acid (**16**) white crystals (EtOH), m.p:> 300°C yield: 68%; $C_{13}H_9N_5O_3$; IR (KBr, cm^{-1}): 3423(OH), 3210-3065 (NH_2), 1722 (C=O), 1685 (C=O); ^{1}HNMR (DMSO-d_6): 7.89 (s, 2H, H_2N D_2O-exchangeable) 7.83- 7.86 (m, 4H, *H*-Ar), 7.99 (s, 1H, *H*C pyrimidine), 8.08 (d, 1H, triazole-*H*C-2, *J* = 3.2Hz), 12.28 (s, 1H, *H*O D_2O-exchangeable), ^{13}C NMR (DMSO-d_6), 115 (*C*H), 130 (*C*H), 134 (*C*H), 139 (*C*H), 152 (*C*H), 160 (*C*H), 168 (*C*H), 169 (*C*=O), 196 (*C*=O); MS (*m/z*): 283(M^+, 100.0%), 84(43%), 149 (28.67%); Anal calcd $C_{13}H_9N_5O_3$ (283.24); C, 55.13; H,3.02; N,24.73% Found: C, 55.10; H, 3.06; N,24.77%.

4-(4-aminobenzo[4,5]imidazo[1,2-*a*]pyrimidine-3-carbonyl)benzoic acid (**17**) yellow crystals (EtOH), m.p: 305-308 yield: 67%; $C_{18}H_{12}N_4O_3$; IR (KBr, cm^{-1}): 3237 (OH), 3128-3087(NH_2), 1712(C=O), 1682(C=O); ^{1}HNMR (DMSO-d_6): 7.20-7.31 (d, 2H, *H*C), 7.47 (d, 1H, *H*C), 7.89 (s, 2H, H_2N D_2O-exchangeable), 8.13-8.25(m, 4H, *H*-Ar), 8.31 (s, 1H, *H*C), 8.9 (d, 1H, *H*C-pyrimidine, *J* = 7.8 Hz), 12.32(s, 1H, *H*O D_2O-exchangeable), ^{13}C NMR (DMSO-d_6), 111.9 (*C*H), 119 (*C*H), 123 (*C*H), 129(*C*H), 134(*C*H), 140 (*C*H), 155 (*C*H), 166 (*C*H), 169(*C*=O), 196(*C*=O); MS (*m/z*): 332 (M^+, 100.0%), 166 (23.08%), 149(23.6%); Anal calcd $C_{18}H_{12}N_4O_3$ (332.31); C, 65.06; H, 3.64; N, 16.86% Found: C,65.10; H,3.70; 16.82%.

***6.7. In vitro Cytotoxic Activity**:*

MCF-7 breast cancer cell lines, obtained from the National Cancer Institute (Cairo, Egypt), were grown in RPMI-1640. Media were supplemented with 10% heat-inactivated

FBS, 50 units/ml of penicillin and 50 g/mL of streptomycin and maintained at 37°C in a humidified atmosphere containing 5% CO2. Cytotoxicity was determined using SRB method as previously described *via* Skehan *et al* [35]. Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96-well plates at 1000-2000 cells/well in RPMI-1640 supplemented medium. After 24 h, cells were incubated for 72 h with various concentrations of the tested compounds as well as tamoxifen as reference drug. Following 72 h of treatment, the cells will be fixed with 10% trichloroacetic acid for 1 h at 4 ºC. Wells were stained for 10 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The plates were air dried for 24 h and the dye was solubilized with Tris-HCl for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well was measured spectrophotometrically at 564 nm with an ELISA microplate reader (ChroMate-4300, FL, USA) The IC50 values were calculated according to the equation for Boltzmann sigmoidal concentration response curve using the nonlinear regression fitting models (Graph Pad, Prism Version 5). The results reported are means of at least three separate experiments. Statistical differences were analyzed according to one way ANOVA test wherein the differences were considered to be significant at $p< 0.05$.

6.8 Antimicrobial Screening:

Antibacterial and antifungal activities were performed at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Initially, target compounds **2a-17** and reference drugs were evaluated *in vitro* for their antibacterial and antifungal activity, by inhibition zone technique and minimum inhibitory concentration (MIC), using four fungi: *A. fumigates* (RCMB 02568), *Candida albicans* (RCMB 05036), *Syncephala strumracemosum* (RCMB 05922) *and Geotricum candidum* (RCMB 05097), two Gram-positive bacteria: *S. pneumonia* (RCMB 010010) and *B. subtitles* (RCMB 010069), two Gram-negative bacteria: *P. aeruginosa* (RCMB 010043), and *E. coli* (RCMB 010052) and the results were compared with respect to those of Amphotericin B, Ampicillin and Gentamicin as a standard drugs. Suspension of the above-mentioned micro-organisms was prepared by inoculating fresh stock cultures into separate broth tubes, each containing 7 ml of nutrient broth (pepton, 0.3%) beef extract (0.3%). The inoculated tubes

were incubated at 37 ºC for 24 h. Solutions of the tested compounds and reference drugs were prepared by dissolving 0.5 g of the compound in 10 ml DMF.[39,40]

6.9. Antioxidant Activity:

Organic acids and esters and *O*-*N*-heterocycles, in particular, have been recognized as antioxidant activity . Furthermore, their mechanism of action and the structure-activity relationships (SAR) were extensively studied the antioxidant activity of synthesized heterocyclic was evaluated using DPPH radical scavenging assays. This widely used method determines antioxidant activity by measuring the hydrogen donating ability of the compound being studied. IC50 values are displayed in Table **3** Vitamin C was used as a positive standard for the antioxidant activity in all experiments. Antioxidant assay by ABST method % Inhibition = {[*A* sample – *A*test] / *A* control} × 100. Compounds **2a**, **3a**, **5a**, **5**, **9**, **13 and 14** were evaluated for antioxidant property by 2,2'-diphenyl-1-picrylhydrazyl (DPPH). [42] The observed data on the antioxidant activity of the compounds and control drug are displayed in Table **3** .

6.10. Computational method:

Calculations have been performed using Khon–Sham's DFT method subjected to the gradient-corrected hybrid density functional B3LYP. This function is a combination of the Becke's three parameters non-local exchange potential with the non-local correlation functional of Lee et al. [52] For each structure, a full geometry optimization was performed using this function and the 6-31G bases set as implemented by Gaussian 09 package.[45] All geometries were visualized either using Gauss View5.0.9 [53] and chemcraft1.6 53 [54]. No symmetry constrains were applied during the geometry optimization.

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Highlights:

- A series of pyrazole and pyrimidine derivatives was synthesized.
- The synthesized compounds exhibited potent antitumor activity.
- Most of compounds showed antimicrobial and antioxidant activities.
- DFT calculations of two examples of the new compounds was carried.