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Original article

Synthesis and antimicrobial of new anthraquinone derivatives incorporating pyrazole moiety

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ABSTRACT

Treatment of 2-cyano-*N*-(9,10-dioxo-9,10-dihydro-anthracen-2-yl)-acetamide (**1**) with phenyl isothiocyanate/dimethylsulphate afforded the corresponding ketene *N,S*-acetal **2** which upon treatment with hydrazine hydrate and 4-aminoantipyrine afforded the pyrazolo derivatives **3** and **4**, respectively. 3-aminopyrazole derivative **3** was utilized as key intermediate for the synthesis of pyrazolo[3,4-d]pyrimidine **5**, pentaaza-as-indacene **6**, triaza-cyclopenta[c]phenanthrene **7**, pyrazolo[1,5-a]pyrimidine **8**, **9** and (dimethyl-pyrrol-1-yl)pyrazole **10** derivatives. Furthermore, treatment of **1** with DMF/DMA gave the corresponding acrylamide derivative **11** which upon treatment with hydrazine hydrate afforded the corresponding 3-aminopyrazole derivative **12**. Moreover, coupling of **1** with 4,6-dimethyl-1*H*-pyrazolo[3,4-b]pyridin-3-diazonium chloride gave the hydrazone derivative **13** which upon cyclization with acetic acid afforded the corresponding pentaaza-fluorene derivative **14**. Representative compounds of the synthesized products were evaluated as antimicrobial agents. Some of these compounds exhibited promising activities.

1. Introduction

Aminoanthraquinones [1] have attracted considerable attention from both synthetic and medicinal chemists due to their biological activities covering a wide range of applications anthracenedione moiety [1].

In recent years, the problem of multidrug resistance (MDR) towards numerous antitumor compounds has also become important and much effort has been directed towards incorporation of a five- or six-membered heterocyclic ring in the several aminoanthraquinone derivatives were identified as DNA intercalating agents [2] and the antitumor antibiotics, daunomycin, and adriamycin [3] are examples of derivatives. Anthraquinone derivatives have been utilized for the activation of human telomerase reverse transcriptase expression [4]. Annulated arene heterocycles and carbocycles such as chromanones [5] chromans [6] quinolines [7] and tetrahydroquinolines [8] are present as important core structures in many biologically active natural products and pharmaceuticals. 2*H*-1-Benzopyrans (chromenes) and 3,4-dihydro-2*H*-1-benzopyrans (chromans) are important classes of oxygenated heterocycles that have attracted much synthetic interest because of the biological activity of naturally occurring representatives [9,10]. Numerous 4-aminobenzopyrans and their derivatives have drawn considerable attention in the last decade as the modulators of potassium channels influencing cardiac activity of the heart and blood pressure [11]. Pyrazole nucleus has pronounced pharmacological applications as anti-anxiety and antipyretic [12], analgesic and anti-inflammatory drugs [13–15], and certain alkyl pyrazoles show significant bacteriostatic, bactericidal and fungicidal activities [16].

These biological data prompted us to synthesize some new pyrazole derivatives incorporating 2-amino-9,10-anthraquinone moiety, starting from 2-cyano-*N*-(9,10-dioxo-9,10-dihydro-anthracen-2-yl)acetamide in order to investigate their antimicrobial activity [17].

2. Results and discussion

2.1. Chemistry

The synthetic procedures adopted to obtain the target compounds are depicted in Schemes 1–4. The starting compound, 2-cyano-*N*-(9,10-dioxo-9,10-dihydro-anthracen-2-yl)-acetamide (**1**) [17] was prepared with high yield and purity *via* fusion of 2-aminoanthraquinone with ethylcyanoacetate at 120–130 °C. The structure of compound **1** was established by the spectral data. The IR spectrum showed a characteristic absorption band at 2265 cm^{-1} due to CN group. Its ^1H NMR spectrum exhibited presence of two singlet

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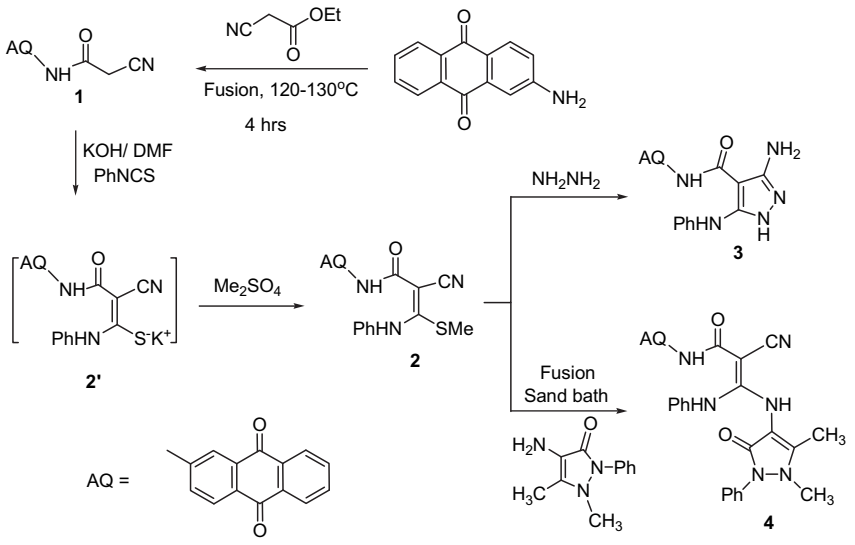


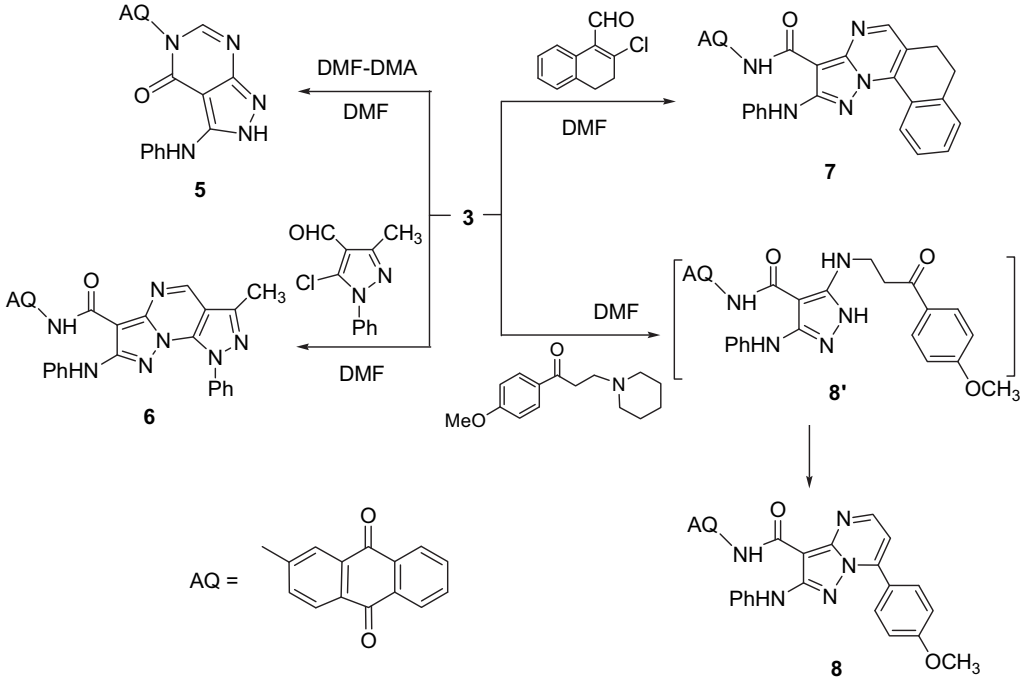
Scheme 1.

signals at δ 4.00 and 10.91 ppm characteristic to CH_2 and NH protons, respectively. Thus, treatment of compound **1** with phenyl isothiocyanate in DMF, and in the presence of potassium hydroxide, at room temperature, followed by treatment with dimethyl sulphate afforded the novel ketene *N*,*S*-acetal **2**. The structure of **2** was established on the basis of its elemental analysis and spectral data. The ^{1}H NMR spectrum displayed singlet signal at δ 2.34 ppm corresponding to the CH_3 protons. Also, its ^{13}C NMR spectrum displayed signals at δ 162.0, 115.2, 79.6 and 18.1 corresponding to acrylonitrile moiety and methyl group, respectively. The Reaction of **2** with hydrazine or 4-aminoantipyrine in refluxing ethanol gave the corresponding pyrazole derivatives **3** and **4**, respectively (Scheme 1).

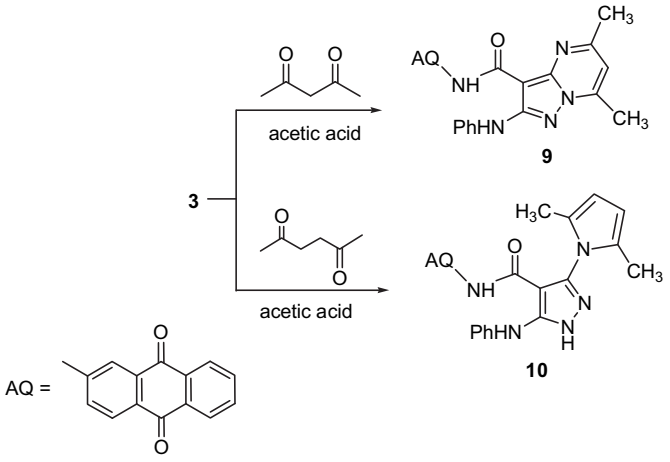
The chemical structure of **3** and **4** were established on the basis of analytical and spectral data. The ^{1}H NMR spectrum of **3** displayed three broad signal at δ 6.17, 9.40 and 11.21 ppm corresponding to the NH_2, NHPh and NH–C=O groups, respectively. The ^{1}H NMR spectrum of **4** displayed two singlet signals at δ 2.53 and 3.32 ppm corresponding to the C=C–CH_3 and (N–CH_3) protons, respectively (Scheme 1).

Pyrazolo[3,4-a]pyrimidine are of considerable chemical and pharmacological importance as purine analogues [18,19]. Various related compounds of these also have antitumor, anti-leukemic activities. Several biological activities have been established for some pyrazolo derivatives [20,21]. Also, the insecticidal activities have been investigated for some pyrazolo derivatives [22,23]. We reported herein the synthesis of some pyrazolo[1,5-a]pyrimidine from 3-amino-5-phenylamino-1*H*-pyrazole-4-carboxylic acid (9,10-dioxo-9,10-dihydro-anthracen-2-yl)-amide (**3**).

Thus, compound **3** was reacted with DMF–DMA in dry DMF afforded the corresponding pyrazolo[3,4-d]pyrimidine derivative **5**.



Scheme 2.



Scheme 3.

The IR and mass spectra were consistent with the proposed structure. Cyclocondensation of **3** with 5-chloro-3-methyl-phenylpyrazolo-4-carboxaldehyde [24], and 2-chloro-3,4-dihydro-naphthalene-1-carbaldehyde [25], in DMF gave the corresponding pentaaza-as-indacene **6** and triaza-cyclopenta[c]phenanthrene **7** derivatives, respectively.

The spectral data were in agreement with the proposed structures. The ^{1}H NMR spectrum of compound **6** revealed singlet signal at δ 3.28 ppm due to methyl protons. The ^{1}H NMR spectrum of **7** displayed multiplite signals at δ 2.73 and 2.89 ppm due to ethene protons of napthalene moiety.

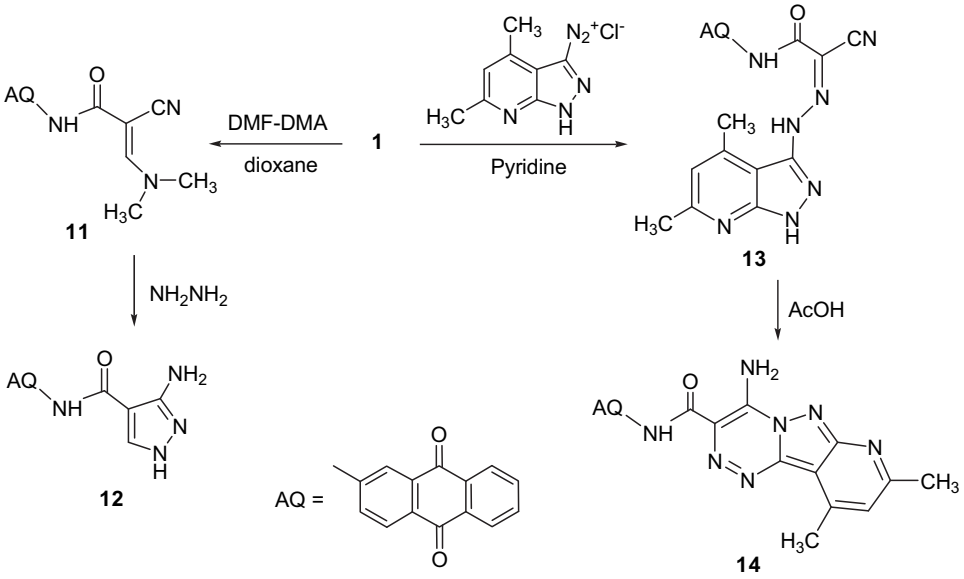
Furthermore, we also investigated the reactivity of aminopyrazole **3** towards Mannich base with the aim of preparing pyrazolo[1,5-a]pyrimidine. Thus, reaction of **3** with 1-(4-methoxyphenyl)-3-piperidin-1-yl-propan-1-one [26], in glacial acetic acid afforded pyrazolo[1,5-a]pyrimidine derivative **8** (Scheme 2). Structure of the latter product was confirmed on the basis of its correct elemental analysis and spectral data. It's ^{1}H NMR spectrum showed singlet signal at δ 3.78 assigned to methoxy protons. This reaction may be explained by the intermediacy of 3-oxo-propylamino]-3-phenylamino-1*H*-pyrazole (**8′**) followed by cyclization and aromatization *via* loss of both water and hydrogen molecules. Although the endocyclic imino group in compound **3** is the most nucleophilic center [27–29], nevertheless, it is the most sterically hindered site [30] (Scheme 2).

Moreover, we also investigated the reactivity of aminopyrazole derivative **3** towards diketo compound with the aim of formation of pyrazolo[1,5-a]pyrimidine Thus, reaction of **3** with acetylacetone or 2,5 hexadione in acetic acid afforded the corresponding pyrazolo[1,5-a]pyrimidine **9** and pyrrolo-1*H*-pyrazole **10** derivatives, respectively (Scheme 3). The structure of compounds **9** and **10** were confirmed on the basis of the analytical and spectral data. The mass spectrum of **9** showed the base peak at *m*/*z* 265 due to 5,7-dimethyl-2-phenylamino-pyrazolo[1,5-a]pyrimidine-3-carbonyl moiety. Also, the mass spectrum of **10** showed the base peak at *m*/*z* 278 due (2,5-dimethyl-pyrrol-1-yl)-5-phenylamino-1*H*-pyrazole-4-carbonyl moiety (Scheme 3).

On the other hand, the reaction of **1** with DMF-DMA in dry dioxane afforded 2-cyano-3-dimethylamino-*N*-(9,10-dioxo-9,10-dihydro-anthracen-2-yl)-acrylamide (**11**), which could be transformed to pyrazole derivative **12** upon heating with hydrazine hydrate. The structure of compound **11** and **12** were elucidated on the basis of spectral data. The ^{1}H NMR spectrum of compound **11** displayed two singlet signals at δ 3.24 and 3.31 ppm due to two methyl groups of dimethylamine group, also, the ^{13}C NMR spectrum showed two signals at δ 43.6 and 43.3 due to dimethylamine moiety. The mass spectrum of compound **11** showed the base peak at *m*/*z* 123 due to 2-cyano-3-dimethylamino-propenone moiety. In addition, the mass spectrum of compound **12** displayed the base peak at *m*/*z* 110 due to 3-amino-1*H*-pyrazole-4-carbonyl moiety.

In continuation of our interest in the synthesis of bridged head nitrogen heterocyclic systems [31], we have found that diazotized heterocyclic amine is an excellent building block for the synthesis of the target compound. Thus, coupling of compound **1** with 4,6-dimethyl-1*H*-pyrazolo[3,4-b]pyridin-3-diazonium chloride [32], in pyridine at 0–5 °C afforded the corresponding hydrazono compound **13**. When compound **13** is refluxed in acetic acid, it can be cyclized to pyrazolo[5,1-c][1,2,4]triazine-derivative **14** (Scheme 4).

The formation of **14** may be interpreted through the nucleophilic attack of ring nitrogen on cyano group. It's ^{1}H NMR spectrum



Scheme 4.

revealed two singlet signals at δ 2.68, and 2.89 ppm assignable for two methyl groups of the pyridine ring.

3. Pharmacology

The new synthesized compounds were screened *in vitro* for their antimicrobial activity. The diameter of inhibition zone was measured as an indicator for the activity of the compounds.

The results for antibacterial activities depicted in Table 1 revealed that compounds **4**, **7**, **8** and **13** exhibited good activities against the reference chemotherapeutics, where **9** and **12** exhibited good activities against *Staphylococcus epidermidis* and moderate against *Pseudomonas aeruginosa*, also compound **5** and **14** exhibited good activities against *P. aeruginosa* and moderate against *S. epidermidis*. On the other hand, most of the prepared compounds exhibited moderate antifungal activities against the reference drugs, whereas, **4**, **7**, **13** and **14** exhibited good antifungal activities against *Alternaria solani*. It is worth mentioning that the incorporation of anthraquinone nucleus to pyrazole moiety caused significant activity against *S. epidermidis*, *P. aeruginosa*, *A. solani* and *Fusarium solani*.

In conclusion, we reported herein a simple and convenient route for the synthesis of some new heterocyclic based on anthraquinone for antimicrobial evaluation.

4. Experimental

4.1. General

All melting points are recorded on Gallenkamp electric melting point apparatus. The IR spectra υ (cm^{-1}) (KBr) were recorded on a Perkin Elmer Infrared Spectrophotometer Model 157. The ^1H NMR spectra were obtained on a Varian Spectrophotometer at 200 MHz, using TMS as an internal reference and DMSO-d_6 as solvent. The ^{13}C NMR spectra were recorded on JEOL-ECA500 (National Research Center, Egypt). The mass spectra (EI) were recorded on 70 eV with Kratos MS equipment and/or a Varian MAT 311 A Spectrometer. Elemental analyses (C, H and N) were carried out at the Microanalytical Center of Cairo Univ., Giza, Egypt.

Table 1
Inhibition zone (mean diameter of inhibition in mm) as a criterion of antibacterial activities of the newly synthesized compounds.

Compound	Inhibition zone in mm			
	Bacteria		Fungi	
	Gram Positive Bacteria; *S. Epidermidis*	Gram negative Bacteria; *P. Auregenosa*	*A. Solani*	*F. Solani*
2	12	13	12	–
3	11	14	14	16
4	24	22	21	16
5	15	20	11	–
6	17	19	–	14
7	25	20	22	14
8	21	20	17	15
9	20	17	14	13
10	19	16	–	–
11	–	14	–	12
12	22	16	17	16
13	19	27	18	13
14	14	21	19	17
Reference drugs				
Ampicillin	19	21	19	17
Chloramphenicol	27	24	18	17
Cloxacillin	22	30	20	22

4.1.1. Synthesis of 2-cyano-N-(9,10-dioxo-9,10-dihydro-anthracen-2-yl)-acetamide (**1**)

A mixture of 2-aminoanthraquinone (0.89 g; 4 mmol) and ethylcyanoacetate (0.566 g; 5 mmol) was refluxed at 120–130 °C for 5 h in air condenser, then dioxane (15 mL) was added. The reaction mixture was refluxed for additional 1 h, then cool; the separated product was filtered, washed with ethanol, dried and crystallized from benzene to give compound (**1**) [17].

Reddish brown powder; Yield 80%; 0.929 g; mp 295 °C [Lit, 290 °C]; IR (KBr): ν/cm^{-1} = 3298 (NH); 2265 (CN), 1695, 1670 (br, 3C=O); ^1H NMR (DMSO): δ_{ppm} = 4.00(s, 2H, CH_2), 7.35–8.36 (m, 7H, Ar–H), 10.91 (s, H, NH). Anal. for $C_{17}H_{10}N_2O_3$ (290.27): calcd.: C 70.34, H 3.47, N 9.65%; found: C 70.42, H 3.53, N 9.76%.

4.1.2. Synthesis of 2-cyano-N-(9,10-dioxo-9,10-dihydro-anthracen-2-yl)-3-methylsulfanyl-3-phenylamino-acrylamide (**2**)

To a cold suspension of finally divided KOH (0.22 g; 4 mmol) in dry DMF (10 mL) were added the cyanoacetamide derivative **1** (1.16 g; 4 mmol) followed by phenyl isothiocyanate (0.54 g; 4 mmol). The mixture was stirred at room temperature over night, then (0.505 g; 4 mmol) dimethyl sulfate was added and stirring over night during which a brown crystalline product separated out. The separated product was filtered, washed with ethanol, dried and crystallized from EtOH–DMF (2:1) to give compound **2**.

Brown crystals; Yield 65%; 1.14 g; mp 232 °C; IR (KBr): ν/cm^{-1} = 3411 (NH), 2195 (CN), 1668, 1639 (3C=O); ^1H NMR (DMSO): δ_{ppm} = 2.34 (s, 3H, S–CH_3), 7.15–8.37 (m, 7H, Ar–H), 10.24 (br, 1H, NH–Ph), 11.40 (br, 1H, NH–C=O); ^{13}C NMR (CF_3COOD, 500 MHz) δ: 182.8, 179.6, 165.3 (3CO), 162.0, 145.7, 134.5, 133.8, 133.6, 130.8, 128.5, 127.1, 124.6, 116.9, 115.2, 108.0, 79.6, 18.1; EIMS (m/z) (%) = 441 (M^+ + 2, 0.5), 440 (M^+ + 1, 2.2), 439 (M^+, 7), 393 (9.1), 392 (30.8), 391 (7.9), 390 (11), 250 (6.3), 249 (26), 248 (2.5), 217 (45.8), 169 (40.2), 77 (100). Anal. for $C_{25}H_{17}N_3O_3S$ (439.49): calcd.: C 68.32, H 3.90, N 9.56%; found: C 68.38, H 3.96, N 9.63%.

4.1.3. Synthesis of 3-amino-5-phenylamino-1H-pyrazole-4-carboxylic acid (9,10-dioxo-9,10-dihydro-anthracen-2-yl)-amide (**3**)

A suspension of product **2** (1.758 g; 4 mmol) in hydrazine hydrate (0.2 g; 4 mmol) was refluxed in water bath for 2 h. Then, (20 mL) ethanol was added, the reaction mixture was refluxed for further 2 h during which a reddish brown crystalline product separated out. The separated product was filtered, washed with ethanol, dried and crystallized from EtOH–DMF (3:1) to give compound **3**.

Reddish brown crystals; Yield 75%; 1.27 g; mp 255 °C; IR (KBr): ν/cm^{-1} = 3425 (NH_2), 3425, 3332, 3175 (3NH), 1663, 1621 (3C=O); ^1H NMR (DMSO): δ_{ppm} = 6.17 (br, 2H, NH_2), 6.77 (br, 1H, pyrazole NH), 7.23–8.51 (m, 12H, Ar–H), 9.40 (br, 1H, NH–Ph), 11.21 (br, 1H, NH–C=O); EIMS (m/z) (%) = 331 (M^+ − NHPh, 27.8), 223 (61.1), 221 (33.3), 164 (88.9), 99 (100). Anal. for $C_{24}H_{17}N_5O_3$ (423.42): calcd.: C 68.08, H 4.05, N 16.54%; found: C 68.14, H 16.60, N 16.62%.

4.1.4. Synthesis of 2-cyano-3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylamino)-N-(9,10-dioxo-9,10-dihydro-anthracen-2-yl)-3-phenylamino-acrylamide (**4**)

A mixture of compound **2** (1.758 g; 4 mmol) and 4-amino-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one (0.813 g; 4 mmol) was fused at 170–180 °C in sand bath for 2 h. Then, (20 mL) ethanol was added, the reaction mixture was refluxed for further 2 h during which a brown crystalline product separated out. The separated product was filtered, washed with ethanol, dried and crystallized from EtOH–DMF (4:1) to give compound **4**.

Brown crystals; Yield 75%; 1.78 g; mp >310 °C; IR (KBr): ν/cm^{-1} = 3244 (br, 3NH), 1700, 1650 (3C=O); ^1H NMR (DMSO): δ_{ppm} = 2.53 (s, 3H, C=C–CH_3), 3.32 (s, 3H, N–CH_3), 7.50–8.20 (m, 19H, 17 Ar–H, 2

NH), 11.61 (br, 1H, NH–CO); EIMS (*m*/*z*) (%) = 596 (M^+ + 2, 20), 595 (M^+ + 1, 16), 411 (24), 410 (20), 266 (44), 265 (76), 264 (64). Anal. for $C_{35}H_{26}N_6O_4$ (594.62): calcd.: C 70.70, H 4.41, N 14.13%; found: C 70.63, H 4.37, N 14.07%.

4.1.5. Synthesis of 2-(4-oxo-3-phenylamino-2,4-dihydro-pyrazolo[3,4-d]pyrimidin-5-yl)-anthraquinone (**5**)

To a solution of **3** (1.694 g; 4 mmol) in DMF (20 mL), dimethoxy-*N*,*N*-dimethylmethanamine (0.477 g; 4 mmol) was added. The reaction mixture was refluxed for 5 h; the reaction mixture was poured into ice. The resulting precipitate was filtered off, dried and crystallized from EtOH–DMF to give compound **5**.

Gray crystals; Yield 70%; 1.21 g; mp 305 °C; IR (KBr): ν/cm^{-1} = 3310–3250 (3NH), 1700, 1671 (3C=O); ^{1}H NMR (DMSO): δ_{ppm} = 7.22–8.34 (m, 15H, 12Ar–H, 2NH, N=CH); EIMS (*m*/*z*) (%) = 435 (M^+ + 2, 22.70), 434 (M^+ + 1, 21.10); 433 (M^+, 61.40), 272 (8.80), 269 (10.50), 224 (10.50), 223 (8.80), 217 (12.30). Anal. for $C_{25}H_{15}N_5O_3$ (433.42): calcd.: C 69.28, H 3.49, N 16.16%; found: C 69.35, H 3.58, N 16.27%.

4.1.6. Synthesis of 3-methyl-1-phenyl-7-phenylamino-1H-1,2,5,8,8a-pentaaza-as-indacene-6-carboxylic acid (9,10-dioxo-9,10-dihydro-anthracen-2-yl)-amide (**6**)

To a suspension of product **3** (1.694 g; 4 mmol) in DMF (20 mL), 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (0.883 g; 4 mmol) were added. The reaction mixture was heated under reflux for 20 h; the mixture was poured into ice. The resulting precipitate was filtered off, dried and crystallized from EtOH–DMF afforded compound **6**.

Brown crystals; Yield 60%; 1.415 g; mp >310 °C; IR (KBr): ν/cm^{-1} = 3358, 3264 (3NH), 1699, 1669 (3C=O); ^{1}H NMR (DMSO): δ_{ppm} = 3.28 (s, 3H, CH_3), 6.88–8.42 (m, 18H, Ar–H), 8.71 (br, 1H, NH–Ph) and 13.14 (s, 1H, NH–C=O); EIMS (*m*/*z*) (%) = 589 (M^+, 7.2), 369 (5.8), 368 (40.6), 367 (42), 366 (33.33), 340 (20.3), 312 (7.2), 253 (7.2), 223 (21.7), 221 (20.3), 139 (27.5), 77 (100). Anal. for $C_{35}H_{23}N_7O_3$ (589.60): calcd.: C 71.30, H 3.93, N 16.63%; found: C 71.38, H 4.02, N 16.69%.

4.1.7. Synthesis of 2-phenylamino-6,7-dihydro-1,4,11c-triaza-cyclopenta[c]phenanthrene-3-carboxylic acid (9,10-dioxo-9,10-dihydro-anthracen-2-yl)-amide (**7**)

To a suspension of product **3** (1.694 g; 4 mmol) in DMF (20 mL), 2-chloro-3,4-dihydro-naphthalene-1-carbaldehyde (0.771 g; 4 mmol) was added. The reaction mixture was heated under reflux for 20 h; the mixture was poured into ice cold-water. The resulting precipitate was filtered off, dried and crystallized from EtOH–DMF afforded compound **7**.

Brown crystals; Yield 63%; 1.415 g; mp 295 °C; IR (KBr): ν/cm^{-1} = 3358, 3226 (3NH), 1700, 1671 (3C=O); ^{1}H NMR (DMSO): δ_{ppm} = 2.73 (m, 2H, C–H_7, triaza-cyclopenta[c]phenanthrene), 2.89 (m, 2H, C–H_6, triaza-cyclopenta[c]phenanthrene), 6.82–8.40 (m, 17 H, Ar–H), 8.70 (br, 1H, NH–Ph), 9.70 (br, 1H, NH–CO); EIMS (*m*/*z*) (%) = 561 (M^+, 35.30), 339 (100), 338 (35.3), 223 (23.50), 221 (35.3), 140 (2.9), 139(26.5), 123 (76.5), 55 (94.1). Anal. for $C_{35}H_{23}N_5O_3$ (561.59): calcd.: C 74.85, H 4.13, N 12.47%; found: C 74.88, H 4.21, N 12.52%.

4.1.8. Synthesis of 7-(4-methoxyphenyl)-2-phenylamino-pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (9,10-dioxo-9,10-dihydro-anthracen-2-yl)-amide (**8**)

A solution of product **3** (1.694 g; 4 mmol) in dioxane (15 mL) with drops of glacial acetic acid which was treated with 1-(4-methoxyphenyl)-3-piperidin-1-yl-propan-1-one (0.989 g; 4 mmol) was heated under reflux for 2 h. The solvent was then evaporated under *vacuo* and triturated with ethanol. The solid deposited was collected by filtration and recrystallized from EtOH–DMF (4:1) afforded compound **8**.

Brown crystals; Yield 90%; 2.036 g; mp 280 °C; IR (KBr): ν/cm^{-1} = 3412, 3318 (3NH), 1700, 1670 (3C=O); ^{1}H NMR (DMSO): δ_{ppm} = 3.78 (s, 3H, CH_3), 6.95–8.90 (m, 18H, Ar–H), 8.91 (br, 1H, NH–Ph), 10.20 (br, 1H, NH–C=O); EIMS (*m*/*z*) (%) = 566 (M^+ + 2, 3.7), 567 (M^+ + 1, 3.7), 565 (M^+, 9.3), 442 (9.3), 441 (11.8), 440 (49.1), 344 (17.4), 343 (30.4), 342 (26.1), 300 (16.8), 258 (22.4), 223 (28), 135 (36), 78 (100). Anal. for $C_{34}H_{23}N_5O_4$ (565.58): calcd.: C 72.20, H 4.10, N 12.38%; found: C 72.26, H 4.19, N 12.45%.

4.1.9. Synthesis of 5,7-dimethyl-2-phenylamino-pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (9,10-dioxo-9,10-dihydro-anthracen-2-yl)-amide (**9**)

A mixture of **3** (1.694 g; 4 mmol) and pentane-2,4-dione (0.4 g; 4 mmol) in DMF (15 mL) with drops of glacial acetic acid was refluxed for 4 h, then the reaction mixture was poured into crushed ice, and the separated solid was filtered off, dried well and recrystallized from DMF-benzene mixture (4:1) afforded compound **9**.

Brown crystals; Yield 82%; 1.599 g; mp 305 °C; IR (KBr): ν/cm^{-1} = 3310, 3226 (3NH), 1700, 1667 (3C=O); ^{1}H NMR (DMSO): δ_{ppm} = 1.87 (br, 6H, 2CH_3), 6.93–8.63 (m, 13H, Ar–H), 9.18 (br, 1H, NH–Ph), 10.45 (br, 1H, NH–C=O); EIMS (*m*/*z*) (%) = 488 (M^+ + 1, 8.0), 487 (M^+, 22.8), 426 (2.5), 395 (3.7), 394 (3.1), 266 (19.1), 265 (100), 264 (92.6). Anal. for $C_{29}H_{21}N_5O_3$ (487.51): calcd.: C 71.45, H 4.34, N 14.37%; found: C 71.37, H 4.32, N 14.35%.

4.1.10. Synthesis of 3-(2,5-dimethyl-pyrrol-1-yl)-5-phenylamino-1H-pyrazole-4-carboxylic acid (9,10-dioxo-9,10-dihydro-anthracen-2-yl)-amide (**10**)

A mixture of compound **3** (1.694 g; 4 mmol) and 2,5-hexanedione (0.457 g; 4 mmol) in DMF (15 mL) with drops of glacial acetic acid was refluxed for 4 h, then the reaction mixture was poured into crushed ice, and the separated solid was filtered off, dried well and recrystallized from DMF–benzene mixture (4:1) to give compound **10**.

Brown crystals; Yield 76%; 1.525 g; mp 295 °C; IR (KBr): ν/cm^{-1} = 3362, 3257 (3NH), 1700, 1664 (3C=O); ^{1}H NMR (DMSO): δ_{ppm} = 2.04 (br, 6H, 2CH_3), 6.11 (m, 2H, pyrole), 7.21–8.12 (m, 13H, 12Ar–H, $NH_{pyrazole}$), 8.9 (br, 1H, NH–Ph), 13.14 (br, 1H, NH–C=O); EIMS (*m*/*z*) (%) = 503 (M^+ + 2, 5.9), 502 (M^+ + 1, 29.8), 501 (M^+, 86.7), 500 (3.7), 499 (0.6), 280 (10.6), 279 (66), 278 (100), 77 (50.4). Anal. for $C_{30}H_{23}N_5O_3$ (501.54): calcd.: C 71.84, H 4.62, N 13.96%; found: C 71.92, H 4.69, N 14.04%.

4.1.11. Synthesis of 2-cyano-3-dimethylamino-N-(9,10-dioxo-9,10-dihydro-anthracen-2-yl)-acrylamide (**11**)

To a solution of 2-cyano-*N*-(9,10-dioxo-9,10-dihydro-anthracen-2-yl)-acetamide (**1**) (1.161 g; 4 mmol) in DMF (20 mL) and dimethoxy-*N*,*N*-dimethylmethanamine (0.477 g; 4 mmol) was added. The reaction mixture was refluxed for 3 h; the mixture was poured into ice. The resulting precipitate was filtered off, dried and crystallized from EtOH-DMF afforded compound **11**.

Gray crystals; Yield 86%; 1.188 g; mp 280 °C; IR (KBr): ν/cm^{-1} = 3431, 3305 (NH), 2188 (CN), 1667 (br C=O); ^{1}H NMR (DMSO): δ_{ppm} = 3.24, 3.31 (2s, 6H, N(CH_3)$_2$), 7.90–8.13 (m, 7H, Ar–H), 8.51 (s, 1H,=CH–N), 9.83 (br, 1H, NH–C=O); ^{13}C NMR (CF_3COOD, 500 MHz) δ: 183.3, 180.1, 164.1 (3CO), 158.2, 138.9, 134.7, 134.4, 133.7, 133.4, 133.0, 129.4, 129.1, 121.3, 116.8, 114.3, 108.7, 43.6, 43.3; EIMS (*m*/*z*) (%) = 347 (M^+ + 2, 0.4), 346 (M^+ + 1, 2.5), 345 (M^+, 12.6), 344 (0.4), 343 (0.2), 124 (7.7), 123 (100), 122 (3.3), 80 (11.6), 77 (1.4). Anal. for $C_{20}H_{15}N_3O_3$ (345.35): calcd.: C 69.56, H 4.38, N 12.17%; found: C 69.50, H 4.33, N 12.14%.

4.1.12. Synthesis of 3-amino-1H-pyrazole-4-carboxylic acid (9,10-dioxo-9,10-dihydro-anthracen-2-yl)-amide (**12**)

A mixture of **11** (1.381 g; 4 mmol) and hydrazine hydrate (0.2 g; 4 mmol) was fused on water bath for 1 h. Then ethanol (10 mL) was

added. The reaction mixture was heated under reflux for further 1 h; the separated product was filtered and crystallized from DMF–EtOH gave compound **12**.

Gray crystals; Yield 78%; 1.037 g; mp >300 °C; IR (KBr): ν/cm^{-1} = 3416 (2NH), 1700, 1610 (3C=O); ^{1}H NMR (DMSO): δ_{ppm} = 7.91–8.47 (m, 8H, Ar–H and =CH–N), 9.83 (br, 1H, NH–C=O); ^{13}C NMR (CF_3COOD, 500 MHz) δ: 183.2, 180.2, 165.3 (3CO), 153.4, 145.3, 140.2, 134.7, 134.1, 133.7, 133.0, 126.5, 126.4, 120.1, 116.8, 115.2, 108.1; EIMS (m/z) (%) = 334 (M^+ + 2, 0.3), 333 (M^+ + 1, 2.5), 332 (M^+, 11.8), 331 (0.4), 290 (1.3), 224 (3.9), 223 (14.4), 222 (2.2), 110 (100), 77 (3.2), 54 (20.3). Anal. for $C_{18}H_{12}N_4O_3$ (332.31): calcd.: C 65.06, H 3.64, N 16.86%; found: C 65.13, H 3.68, N 16.97%.

*4.1.13. Synthesis of 2-cyano-2-[(4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-yl)-hydrazono]-N-(9,10-dioxo-9,10-dihydro-anthracen-2-yl)-acetamide (**13**)*

To a solution compound **1** (1.161 g; 4 mmol) in pyridine (10 mL), an ice-cooled solution of the appropriate diazonium solution [prepared by the addition of a solution of sodium nitrite (0.276 g; 4 mmol) in water (5 mL) to the required arylamine (0.839 g; 4 mmol) in hydrochloric acid (12 mL)] was added drop wise with stirring. Stirring was maintained for 30 min. The precipitated product was filtered, washed with water, dried and crystallized from EtOH-DMF afforded compound **13**.

Brown crystals; Yield 71.5%; 1.325 g; mp >300 °C; IR (KBr): ν/cm^{-1} = 3307 3274, 3259 (NH), 2199 (CN), 1669, 1640 (3C=O), 1527 (N=N); ^{1}H NMR (DMSO): δ_{ppm} = 2.83 (br, 6H, $2CH_3$), 6.87 (br, 1H, $NH_{pyrazole}$) 7.91–8.22 (m, 9H, Ar–H), 8.71 (br, 1H, NH=N), 11.20 (br, 1H, NH–CO); ^{13}C NMR (CF_3COOD, 500 MHz) δ: 183.1, 180.3, 166.1 (3CO), 158.8, 154.2, 146.3, 144, 140.1, 134.4, 133.6, 129.3, 123.9, 121.1, 114.3, 112.5, 108.3, 20.9, 20.1; EIMS (m/z) (%) = 463 (M^+, 40), 163 (66.7), 162 (100), 139 (93.3), 132 (53.3), 131 (86.7), 122 (20), 121 (40). Anal. for $C_{25}H_{17}N_7O_3$ (463.45): calcd.: C 64.79, H 3.70, N 21.16%; found: C 64.83, H 3.76, N 21.28%.

*4.1.14. Synthesis of 8-amino-2,4-dimethyl-1,5,6,8a,9-pentaaza-fluorene-7-carboxylic acid (9,10-dioxo-9,10-dihydro-anthracen-2-yl)-amide (**14**)*

Compound **13** (0.927 g; 2 mmol) was heated under reflux for 6 h in glacial acetic acid (10 mL). The solvent was then evaporated under *vacuo* and triturated with ethanol. The solid deposited was collected by filtration and recrystallized from DMF–EtOH (4:1) afforded compound **14**.

Brown crystals; Yield 84.3%; 0.781 g; mp >300 °C; IR (KBr): ν/cm^{-1} = 3406, 3327, 3291 (NH), 1673, 1633 (3C=O); ^{1}H NMR (DMSO): δ_{ppm} = 2.68 (s, 3H, C_4–CH_3), 2.71 (s, 3H, C_2–CH_3), 6.92 (br, 2H, NH_2), 7.91–8.40 (m, 8H, Ar–H), 10.94 (br, 1H, NH–C=O); EIMS (m/z) (%) = 463 (M^+, 22.7), 229 (22.7), 164 (72.7), 139 (27.3), 133 (31.8), 132 (100), 105 (4.5), 104 (59.1). Anal. for $C_{25}H_{17}N_7O_3$ (463.45): calcd.: C 64.79, H 3.70, N 21.16%; found: C 64.86, H 3.73, N 21.24%.

4.2. In vitro antimicrobial activity

The tested compounds were evaluated by the agar diffusion technique [33] using a 2 mg mL^{-1} solution in DMSO. The test organisms were two bacterial strains: *S. epidermidis* and *P. aeruginosa* and two fungi: *A. solani* and *F. solani*. A control using DMSO without the test compound was included for each organism. *Ampicillin*, *Chloramphenicol* and *Cloxacillin* were purchased form The Egyptian market and used in a concentration 2 mg/mL as reference drugs. The bacteria and fungi were tested on nutrient agar and potato dextrose agar media, respectively. Three plates were used for each compound as replicates. The plates were incubated for 24 h and seven days for bacteria and fungi, respectively. After the incubation period, the diameter of inhibition zone was measured as an indicator for the activity of the compounds.

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