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Original article

Synthesis of new pyrrolo[2,3-*d*]pyrimidine derivatives as antibacterial and antifungal agents

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ABSTRACT

A series of new pyrrole derivatives, pyrrolo[2,3-*d*]pyrimidine derivatives, pyrrolotriazolopyrimidines and pyrrolotetrazolopyrimidines were synthesized. The evaluation of their antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* were carried out. Pyrrolo[2,3-*d*]pyrimidines **3a**–**d**, **7a**,**e**, **11d** exhibited excellent activity against *C. albicans* with MIC 0.31–0.62 mg/mL. These compounds displayed better antifungal activity than that of standard drug (fluconazole with MIC 1.5 mg/mL). Furthermore, pyrrolo[2,3-*d*]pyrimidines **3b,c**, **7e** exhibited the best activity against *S. aureus* with MIC 0.31 mg/mL, compared with the standard drug (ampicillin with MIC 0.62 mg/mL). The rest of the compounds were found to be inactive against bacteria and fungi.

1. Introduction

Pyrrolo[2,3-*d*]pyrimidine derivatives attracted organic chemists very much due to their biological and chemotherapeutic importance. Their related fused heterocycles are of interest as potential bioactive molecules. They are known to exhibit biological activities such as enzyme inhibitors [1], cytotoxic [2], antiviral [3–5], anti-inflammatory [6], antialgeric [7], anti-ocular hypertension [8], antitumor [9–12], antibacterial and antifungal agents [13].

As pathogenic bacteria continuously evolve mechanisms of resistance to currently used antibacterials, so the discovery of novel and potent antibacterial drugs is the best way to overcome bacterial resistance and develop effective therapies [14]. On the meanwhile *Candida* infections have increased dramatically over the past three decades. *Candida* species is the fourth common cause of nosocomial bloodstream infections in many hospitals and represents 10% of all bloodstream infections [15,16]. On the basis of these observations, we report the synthesis and antimicrobial activity of a novel series of pyrroles and pyrrolo[2,3-*d*]pyrimidine derivatives.

2. Results and discussion

2.1. Chemistry

Synthesis of compounds **4a**–**e** was achieved through two synthetic pathways, the first of which was the reaction of **1a**–**e** [17] with triethylorthoformate under reflux for 4 h to give N-[5-(4-substituted phenyl)-3-cyano-1-phenyl-1H-pyrrolo-2-yl]-formimidic ethyl ester **2a**–**e**. IR spectrum of **2d** showed absorption band at 2217 cm^{-1} assigned to cyano group (CN). Furthermore, its ^1H NMR spectrum showed a singlet at δ 8.40 ppm indicated the formation of imine (N=CH) and a triplet at δ 1.09–1.20 ppm (CH_3) and a quartet at δ 4.00–4.20 ppm (OCH_2) confirmed the structure. Mass spectrum of **2d** showed molecular ion peak at *m/z* 329 corresponding to its molecular formula $C_{21}H_{19}N_3O$. Reaction of the latter compounds with hydrazine hydrate (99%) in ethanol afforded **3a**–**e**. The IR spectrum of **3d** revealed the absence of the cyano group and the appearance of absorption bands at 3422–3200 cm^{-1} for (NH, NH_2). Its ^1H NMR spectrum also showed two singlets at

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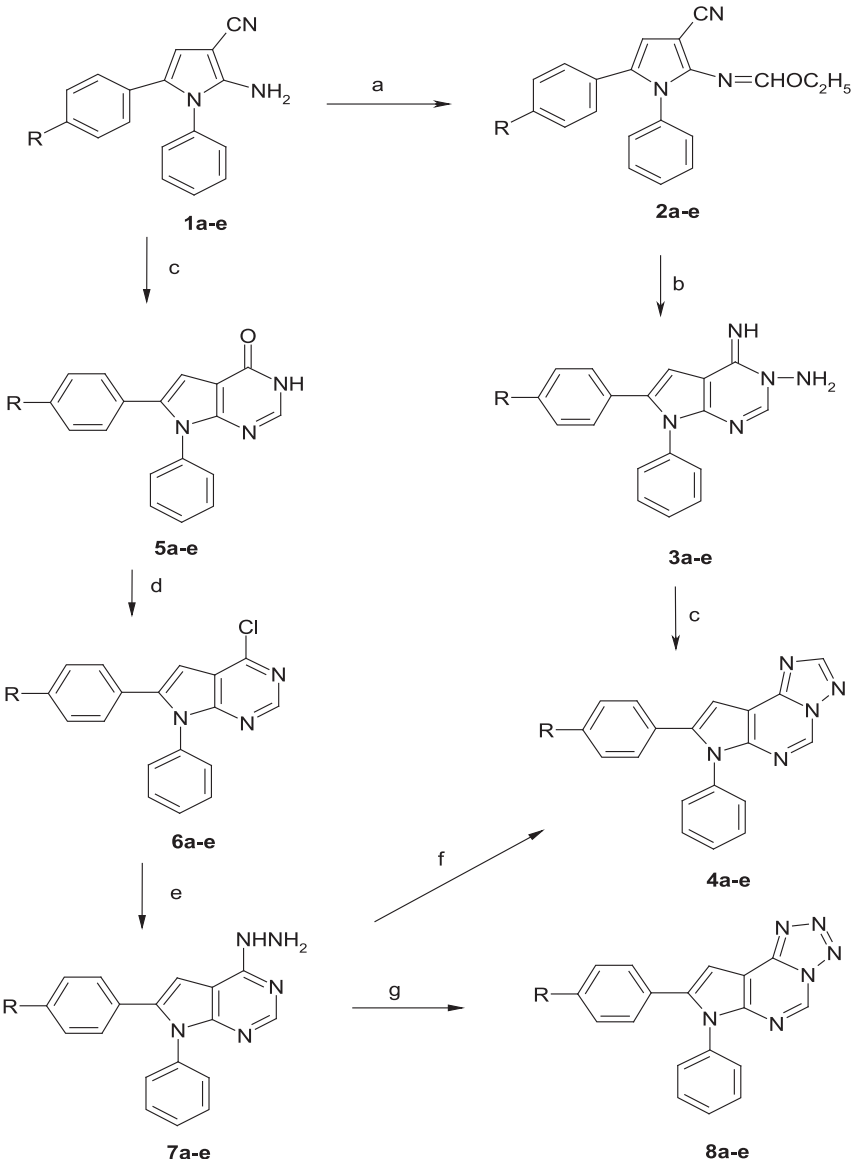
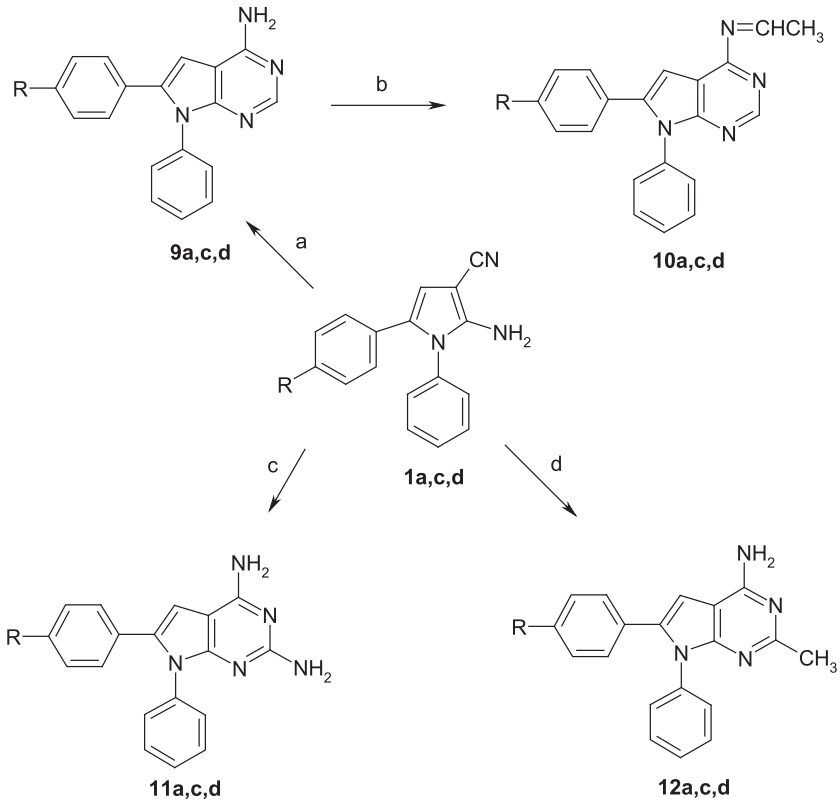
δ 5.68 & 8.40 ppm which indicated the presence of NH_2 & NH groups respectively. Cyclization with formic acid (85%) occurred smoothly by heating under reflux for 8 h to afford our targets **4a**–**e** (Scheme 1). Disappearance of absorption bands at 3400–3200 in the IR spectra as well as in the ^{1}H NMR spectra was the characteristic for the pyrrolotriazolopyrimidines. The second pathway involves the conversion of **1a**–**e** to 6-aryl-7-phenyl-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-4-one derivatives **5a**–**e** through its cyclization with formic acid (85%) for 4 h. Chlorination of **5a**–**e** with phosphorus oxychloride under reflux for 10 h afforded **6a**–**e**. Nucleophilic displacement of the 4-chloro group in **6a**–**e** was achieved by heating under reflux with hydrazine hydrate (99%) to give compounds **7a**–**e**. IR spectrum of the titled compound **7e** showed the appearance of absorption bands at 3424, 3349, 3313, 3196 cm^{-1} for (NH, NH_2) respectively, its ^{1}H NMR spectrum showed the presence of characteristic peaks at δ 4.02, 8.89 ppm of NH_2 and NH groups, the mass spectrum showed molecular ion peak at *m/z* 319 corresponding to its molecular formula $C_{18}H_{14}FN_5$ which confirmed its chemical structure (Scheme 1). 8-Aryl-7-phenyl-7*H*-pyrrolo[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives **4a**–**e** were readily obtained through smooth cyclization of **7a**–**e** under reflux with formic acid (85%) (Scheme 1). Furthermore, reaction of the latter compounds with aqueous solution of sodium nitrite in acetic acid at 0–5 °C afforded 8-aryl-7-phenyl-7*H*-pyrrolo[3,2-*e*] tetrazolo[1,5-*c*]pyrimidine derivatives **8a**–**e**. Absence of absorption bands in the latter series at 3400–3200 cm^{-1} in the IR spectra as well as in the ^{1}H NMR spectra was characteristic for the pyrrolotetrazolpyrimidines. On the other hand, compounds **1a,c,d** were reacted with formamide under reflux to give 4-amino-6-aryl-7-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine derivatives **9a,c,d**, from which **9c** exhibited absorption bands ranging from 3400, 3306, 3200, 3150 cm^{-1} for the amino (NH_2). A singlet at δ 6.85 ppm assigned to NH_2 in the ^{1}H NMR spectrum and molecular ion peak at *m/z* 320 and M^{+2} at 322 confirmed its molecular formula $C_{18}H_{13}ClN_4$. Schiff's bases **10a,c,d** were obtained by the reaction of **9a,c,d** with acetaldehyde in ethanol under reflux for 4 h. Moreover, reaction of **1a,c,d** with urea/acetamide in the presence of acetic acid, hydrochloric acid (3:1) under reflux for 4 h afforded **11a,c,d**, and **12a,c,d** respectively (Scheme 2). Compounds **11a** and **12d** were characterized with absorption bands ranging from 3100 to 3345 cm^{-1} for the



Scheme 1. Compounds: **1a**, R = H; **1b**, R = Br; **1c**, R = Cl; **1d**, R = CH_3; **1e**, R = F. Reagents and conditions: (a) triethylorthoformate, reflux, 4 h; (b) hydrazine hydrate (99%), ethanol, reflux, 2 h; (c) formic acid (85%), reflux, 4 h; (d) $POCl_3$, reflux, 10 h; (e) hydrazine hydrate (99%), ethanol, reflux, 2 h; (f) formic acid (85%), reflux, 7–8 h; (g) $NaNO_2$, glacial acetic acid, 0–5 °C.



Scheme 2. Compounds: **1a**, R = H; **1c**, R = Cl; **1d**, R = CH_3. Reagents and conditions: (a) formamide, reflux, 4 h; (b) acetaldehyde, reflux, 4 h; (c) urea, glacial acetic acid and conc. HCl (3:1), reflux, 4 h; (d) acetamide, glacial acetic acid and conc. HCl (3:1), reflux, 4 h.

amino group (NH_2) respectively. The presence of ^{1}H NMR characteristic singlet at δ 6.70 ppm for 2-NH_2 group in compound **11a**, however, two singlets at 2.30 and 2.50 ppm corresponding to 2 methyl groups, another singlet at δ 6.79 ppm (NH_2) confirmed the structure of **12d**. All structures were assigned by their mass spectrum and elemental analysis.

2.2. Antimicrobial activity

The antimicrobial screenings of the synthesized compounds were undertaken using agar well diffusion assay [18]. Table 1 lists the screening results of the tested compounds against the gram negative bacteria (*Escherichia coli*), gram positive bacteria (*Staphylococcus aureus*) in addition to the pathogenic fungi, *Candida albicans*. The obtained data revealed that most of the compounds showed moderate to excellent activities against the microorganisms used at a dose of 5 mg/mL. Compounds showing inhibition of at least 18 mm were considered active and were further evaluated for their minimal inhibitory concentration (MIC). Ampicillin and Tetracycline were used as a standard antibacterial, while Fluconazole and Nystatin were used as standard antifungal. DMSO was used as a blank exhibited no activity against any of the used organisms. It is well noticed that compounds **3c**, **7e** showed remarkable broad spectrum potency against *E. coli*, *S. aureus* with MIC values of 1.25, 0.31 mg/mL respectively, compared with MIC of Ampicillin 1.25 and 0.62 mg/mL and five times as potent as Fluconazole with MIC value of 0.31 mg/mL relative to 1.5 mg/mL of the latter standard. Compounds **3d, 7a**,**b, 11a** and **12c** with MIC = 0.62 mg/mL were equipotent against *S. aureus* similar to the reference standard. However, **3a,b**,**d** and **11d** were twice as potent as Fluconazole against *C. albicans* with MIC = 0.62 mg/mL Table 2.

3. Conclusion

In this work, we have synthesized a series of new pyrrole derivatives **2a**–**e**, pyrrolo[2,3-*d*]pyrimidine derivatives **3a**–**e**, **5a**–**e, 6a**–**e, 7a**–**e**, **9a,c,d**–**12a,c,d**, pyrrolotriazolopyrimidines **4a**–**e** and pyrrolotetrazolopyrimidines **8a**–**e** which were screened for their antimicrobial activities. The tested compounds **3a**–**d**, **7a,b,c,e**, **9d, 10a, 11a,c,d, 12a** demonstrated potent antifungal activity against *C. albicans* than that of the standard drug. Also, compounds **3b,c,d**, **7a,b,e**, **11a,d,** and **12c** showed antibacterial activity against Gram positive *S. aureus*. Moreover, **3b,c** and **7e**, exhibited antibacterial activity against gram negative *E. coli* similar to the standard drug. It seems obvious that pyrrolo[2,3-*d*]pyrimidine derivatives in which the pyrimidine ring is bearing an amino & imino groups of compounds **3a**–**d**, as well as carrying hydrazino group of compounds **7a,b,c,e** and diamino group as in **11a,c,d** obtained the best results in the biological screening. However, fused systems such as pyrrolotriazolopyrimidines, pyrrolotetrazolopyrimidines as well as pyrrole derivatives didn't have such an influence on activity.

4. Experimental protocols

4.1. Chemistry

Melting points were determined on a Stuart melting point apparatus and are uncorrected. IR spectra (KBr) were measured on Jasco FT/IR 460 plus (Japan), ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Gemini 200 MHz in DMSO-d_6 or $CDCl_3$ as solvent, using tetramethyl-silane (TMS) as internal reference standard. The chemical shifts values are expressed in ppm. Elemental analysis (C, H, N) was performed by a Vario III CHN analyzer (Germany). All compounds were within ±0.4% of the

Table 1
Inhibition zones (IZ) in mm of the synthesized compounds at 5 mg/mL.

Compound (mg/mL)	*Escherichia coli*	*Staphylococcus aureus*	*Candida albicans*
2a	17	16	19
2b	–	–	11
2c	–	–	11
2d	11	–	–
2e	13	13	16
3a	16	21	23
3b	27	25	27
3c	22	21	27
3d	20	21	21
3e	15	17	15
4a	16	13	17
4b	14	19	19
4c	18	17.5	18
4d	–	–	11
4e	16	17	17
6a	14	13	16.5
6b	17	16	17
6c	13	12	12
6d	13	12	16
6e	13	15	15
7a	18	27	31
7b	15	25	23
7c	16	17	19
7d	13	14	17
7e	20	31	32
8a	16	16	17
8b	15	16	17
8c	15	14	17
8e	–	–	11
9a	14	15	20
9c	16	17	16
9d	14	23	25
10a	16	21	21
10c	16	17	17
10d	17	16	15
11a	13	24	24
11c	14	24	23
11d	19	25	23
12a	18	20	21
12c	14	25	24
12d	14	17	16
Ampicillin	9	9	–
Tetracycline	18	20	–
Nystatin	–	–	17
Fluconazole	–		26

–, no inhibition zone.

Table 2
The MIC of the compounds tested against organisms.

Compound (mg/mL)	*Escherichia coli*	*Staphylococcus aureus*	*Candida albicans*
	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)
2a	–	–	2.5
3a	–	1.25	0.62
3b	1.25	0.31	0.62
3c	1.25	0.31	0.31
3d	2.5	0.62	0.62
7a	2.5	0.62	0.31
7b	–	0.62	1.25
7c	–	–	1.25
7e	1.25	0.31	0.31
9a	–	–	2.5
9d	–	2.5	1.25
10a	–	1.25	1.25
11a	–	0.62	1.25
11c	–	1.25	1.25
11d	2.5	0.62	0.62
12a	2.5	1.25	1.25
12c	–	0.62	2.5
Ampicillin	1.25	0.62	–
Fluconazole	–	–	1.5

theoretical values. Mass spectra were run on DI analysis Shimadzu QP-2010 plus mass spectrometer. All spectroscopic analysis were made at the Microanalytical Unit of Cairo University. The progress of the reaction and the purity of the compounds were monitored by TLC analytical silica gel plates 60 F_{254} eluting with CH_2Cl_2/CH_3OH (39:1). The chemical reagents used in synthesis were purchased from Fluka, Sigma, and Aldrich.

*4.1.1. General procedure for the synthesis of compounds (**2a**–**e**)*

A mixture of **1a**–**e** [17] (0.01 mol) in triethylorthoformate (10 mL) was refluxed for 4 h and then excess of ethanol was added. The solution thus obtained was treated with ice cold water. The separated solid was filtered and recrystallized from ethanol.

*4.1.1.1. N-(3-Cyano-1,5-diphenyl-1H-pyrrol-2-yl)-formimidic acid ethyl ester (**2a**).* (2.91 g, 92%); mp: 103–104 °C; IR (KBr) cm^{-1}: 3134 (C–H aromatic), 2980 (C–H aliphatic), 2217 (C≡N), 1622, 1498 (C=C, C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 1.10–1.20 (t, 3H, OCH_2CH_3), 4.00–4.10 (q, 2H, OCH_2CH_3), 6.71 (s, 1H, CH pyrrole), 7.00–7.50 (m, 10H, ArH), 8.40 (s, 1H, N=CHOEt); ^{13}C NMR: 14.12 (CH_3), 63.63 (CH_2), 79.81 (CN), 109.75, 117.44, 127.58, 128.16, 128.74, 128.84, 129.33, 131.47, 131.94, 136.59, 146.33, 160.21 (12 type of C_a), 220.04 (N=CHOEt). MS (*m/z*, %): 315.05 (M^+, 100). Anal. Calcd for $C_{20}H_{17}N_3O$ (315.37): C, 76.17; H, 5.43; N, 13.32. Found: C, 75.94; H, 5.22; N, 12.96.

*4.1.1.2. N-[5-(4-Bromo-phenyl)-3-cyano-1-phenyl-1H-pyrrol-2-yl]-formimidic ethyl ester (**2b**).* (3.78 g, 96%); mp: 142–143 °C, IR (KBr) cm^{-1}: 3067 (C–H aromatic), 2984 (C–H aliphatic), 2211 (C≡N), 1630, 1497 (C=C, C=N), 576 (C–Br); ^{1}H NMR (DMSO-d_6) δ ppm: 1.10–1.20 (t, 3H, OCH_2CH_3), 4.00–4.20 (q, 2H, OCH_2CH_3), 6.80 (s, 1H, CH pyrrole), 7.00–7.50 (m, 9H, ArH), 8.40 (s, 1H, N=CHOEt). MS (*m/z*, %): 392.90 (M+, 100), 394.90 (M^{+2}, 92.06). Anal. Calcd for $C_{20}H_{16}BrN_3O$ (394.26): C, 60.93; H, 4.09; N, 10.66. Found: C, 60.63; H, 4.19; N, 10.35.

*4.1.1.3. N-[5-(4-Chloro-phenyl)-3-cyano-1-phenyl-1H-pyrrol-2-yl]-formimidic ethyl ester (**2c**).* (2.83 g, 84%); mp: 144–145 °C; IR (KBr) cm^{-1}: 3068 (C–H aromatic), 2984 (C–H aliphatic), 2211 (C≡N), 1630,1497 (C=C, C=N), 755 (C–Cl); ^{1}H NMR (DMSO-d_6) δ ppm: 1.10–1.20 (t, 3H, OCH_2CH_3), 4.00–4.20 (q, 2H, OCH_2CH_3), 6.80 (s, 1H, CH pyrrole), 7.00–7.50 (m, 9H, ArH), 8.40 (s, 1H, N=CHOEt). MS (*m/z*, %): 349.00 (M^+, 84.89), 351.00 (M^{+2}, 30.74). Anal. Calcd for $C_{20}H_{16}$ Cl N_3O (349.81): C, 68.67; H, 4.61; N, 12.01. Found: C, 68.99; H, 4.59; N, 11.66.

*4.1.1.4. N-(3-Cyano-1-phenyl-5-p-tolyl-1H-pyrrol-2-yl)-formimidic acid ethyl ester (**2d**).* (3.15 g, 96%); mp: 124–125 °C; IR (KBr) cm^{-1}: 3134 (C–H aromatic), 2980 (C–H aliphatic), 2217 (C≡N), 1622, 1498 (C=C, C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 1.09–1.16 (t, 3H, OCH_2CH_3), 2.20 (s, 3H, CH_3), 4.01–4.20 (q, 2H, OCH_2CH_3), 6.60 (s, 1H, CH pyrrole), 6.91–7.60 (m, 9H, ArH), 8.37 (s, 1H, N=CHOEt). MS (*m/z*, %): 329.00 (M^+, 100). Anal. Calcd for $C_{21}H_{19}N_3O$ (329.15): C, 76.57; H, 5.81; N, 12.76. Found: C, 76.50; H, 5.71; N, 12.85.

*4.1.1.5. N-[3-Cyano-5-(4-fluoro-phenyl)-1-phenyl-1H-pyrrol-2-yl]-formimidic acid ethyl ester (**2e**).* (2.76 g, 83%); mp: 109–110 °C; IR (KBr) cm^{-1}: 3120 (C–H aromatic), 2981 (C–H aliphatic), 2211 (C≡N), 1630, 1509 (C=C, C=N), 1226 (C–F); ^{1}H NMR (DMSO-d_6) δ ppm: 1.10–1.20 (t, 3H, OCH_2CH_3), 4.00–4.20 (q, 2H, OCH_2CH_3), 6.80 (s, 1H, CH pyrrole), 7.00–7.50 (m, 9H, ArH), 8.40 (s, 1H, N=CHOEt). MS (*m/z*, %): 333.13 (M^+, 100). Anal. Calcd for $C_{20}H_{16}FN_3O$ (333.36): C, 72.06; H, 4.84; N, 12.61; Found: C, 72.20; H, 4.51; N, 12.81.

*4.1.2. General procedure for the synthesis of compounds (**3a**–**e**)*

A mixture of **2a**–**e** (0.01 mol) and hydrazine hydrate (99%) (10 mL) was refluxed in ethanol for 2 h. The reaction mixture was allowed to cool, poured onto crushed ice and neutralized with (50%) acetic acid. The solid obtained was filtered, washed with water, dried and recrystallized from dioxane.

*4.1.2.1. 3-Amino-4-imino-6,7-diphenyl-7H-pyrrolo[2,3-d]pyrimidine (**3a**).* (2.67 g, 89%); mp: 122–123 °C; IR (KBr) cm^{-1}: 3400, 3312, 3200 and 3150 (NH, NH_2), 1639 (C=N imine), 1593, 1497 (C=C, C=N); ^1H NMR (DMSO-d_6) δ ppm: 5.60 (s, 2H, NH_2), 6.90 (s, 1H, CH pyrrole), 7.10–7.60 (m, 10H, ArH), 7.90 (s, 1H, H at C_2), 8.10 (s, 1H, NH). MS (*m/z*, %): 301.00 (M^+, 100). Anal. Calcd for $C_{18}H_{15}N_5$ (301.35): C, 71.74; H, 5.02; N, 23.24. Found: C, 71.58; H, 4.87; N, 23.57.

*4.1.2.2. 3-Amino-4-imino-6-(4-bromophenyl)-7-phenyl-7H-pyrrolo [2,3-d]pyrimidine (**3b**).* (3.19 g, 84%); mp: 223–224 °C; IR (KBr) cm^{-1}: 3400, 3312, 3200 and 3150 (NH, NH_2), 1639 (C=N imine), 1593, 1497 (C=C, C=N), 565 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 5.70 (s, 2H, NH_2), 7.00 (s,1H, CH pyrrole), 7.10–7.60 (m, 9H, ArH), 7.89(s, 1H, H at C_2), 8.13 (s, 1H, NH); ^{13}C NMR: 103.68, 106.34, 127.85, 128.20, 128.40, 128.98, 129.44, 130.51, 131.81, 133.16, 135.92, 144.29, 147.03, 153.34 (14 type of C_a). MS (*m/z*, %): 379.04 (M^+, 100), 381.04 (M^{+2}, 97.00). Anal. Calcd for $C_{18}H_{14}$ Br N_5 (380.24): C, 56.86; H, 3.71; N, 18.42. Found: C, 56.48; H, 3.80; N, 18.11.

*4.1.2.3. 3-Amino-4-imino-6-(4-chlorophenyl)-7-phenyl-7H-pyrrolo [2,3-d]pyrimidine (**3c**).* (2.84 g, 85%); mp: 207–208 °C; IR (KBr) cm^{-1}: 3401, 3312 and 3245 (NH, NH_2), 3097 (C–H aromatic), 1644 (C=N imine), 1596, 1500 (C=C, C=N), 749 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm: 5.60 (s, 2H, NH_2), 6.90 (s, 1H, CH pyrrole), 7.10–7.60 (m, 9H, ArH), 7.90 (s, 1H, H at C_2), 8.11 (s, 1H, NH). MS (*m/z*, %): 335.09 (M^+, 100), 337.09 (M^{+2}, 30.30). Anal. Calcd for $C_{18}H_{14}$ Cl N_5 (335.79): C, 64.38; H, 4.20; N, 20.86. Found: C, 64.08; H, 4.15; N, 21.20.

*4.1.2.4. 3-Amino-4-imino-6-(p-tolyl)-7-phenyl-7H-pyrrolo[2,3-d] pyrimidine (**3d**).* (2.77 g, 88%); mp: 204–205 °C; IR (KBr) cm^{-1}: 3422, 3300 and 3200 (NH, NH_2), 1637 (C=N imine), 1595, 1499 (C=C, C=N); ^1H NMR (DMSO-d_6) δ ppm: 2.31 (s, 3H, CH_3), 5.68 (s, 2H, NH_2), 6.81 (s, 1H, CH pyrrole), 6.89–7.70 (m, 9H, ArH), 7.89 (s, 1H, H at C_2), 8.40 (s, 1H, NH). MS (*m/z*, %): 315.15 (M^+, 100). Anal. Calcd for $C_{19}H_{17}N_5$ (315.37): C, 72.36; H, 5.39; N, 22.21. Found: C, 72.66; H, 5.26; N, 21.97.

*4.1.2.5. 3-Amino-4-imino-6-(4-fluorophenyl)-7-phenyl-7H-pyrrolo [2,3-d]pyrimidine (**3e**).* (2.67 g, 84%); mp: 203–204 °C; IR (KBr) cm^{-1}: 3422, 3300 and 3200 (NH, NH_2), 3071 (C–H aromatic), 1637 (C=N imine), 1595, 1499 (C=C, C=N), 1173 (C–F); ^1H NMR (DMSO-d_6) δ ppm: 5.64(s, 2H, NH_2), 6.94 (s, 1H, CH pyrrole), 7.20–7.41 (m, 9H, ArH), 7.90 (s, 1H, H at C_2), 8.00 (s, 1H, NH). MS (*m/z*, %): 319.12 (M^+, 100). Anal. Calcd for $C_{18}H_{14}$ F N_5 (319.34): C, 67.70; H, 4.42; N, 21.93. Found: C, 67.66; H, 4.05; N, 21.64.

*4.1.3. General procedure for the synthesis of (**4a**–**e**)*

Method A: A mixture of **3a**–**e** (0.01 mol) in formic acid (85%) (15 mL) was heated under reflux for 4 h. The cold reaction mixture was poured onto crushed ice and the solid obtained was filtered, washed with water, dried and recrystallized from ethanol.

Method B: A mixture of **7a**–**e** (0.01 mol) in formic acid (85%) (20 mL) was heated under reflux for 8 h. The cold reaction mixture was poured onto crushed ice and the solid obtained was filtered, washed with water, dried and recrystallized from ethanol.

*4.1.3.1. 7,8-Diphenyl-7H-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**4a**).* 2.86 g, 92% (Method A), 2.55 g, 82% (Method B); mp: 177–178 °C; IR (KBr) cm^{-1}: 3073 (C–H aromatic), 1636, 1500 (C=C, C=N); ^1H NMR (DMSO-d_6) δ ppm: 7.13–7.62 (m, 11H, ArH + CH pyrrole), 8.60 (s, 1H, H at C_5), 9.49 (s, 1H, H at C_2). MS (*m/z*, %): 311.12 (M^+, 100). Anal. Calcd for $C_{19}H_{13}$ N_5 (311.34): C, 73.30; H, 4.21; N, 22.49. Found: C, 73.54; H, 4.32; N, 22.89.

*4.1.3.2. 7-Phenyl-8-(4-bromophenyl)-7H-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**4b**).* 3.46 g, 89% (Method A), 3.77 g, 97% (Method B); mp: 217–218 °C; IR (KBr) cm^{-1}: 3061 (C–H aromatic), 1644, 1499 (C=C, C=N), 555 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 7.10–7.70 (m, 10H, ArH + CH pyrrole), 8.60 (s, 1H, H at C_5), 9.50 (s, 1H, H at C_2). MS (*m/z*, %): 389.03 (M^+, 100), 391.03 (M^{+2}, 98.00). Anal. Calcd for $C_{19}H_{12}$ Br N_5 (389.03): C, 58.48; H, 3.10; N, 17.95. Found: C, 58.37; H, 3.05; N, 17.71.

*4.1.3.3. 7-Phenyl-8-(4-chlorophenyl)-7H-pyrrolo[3,2-e][1,2,4]triazolo [1,5-c] pyrimidine (**4c**).* 3.20 g, 93% (Method A), 2.52 g, 73% (Method B); mp: 225–227 °C; IR (KBr) cm^{-1}: 3061 (C–H aromatic), 1644, 1501 (C=C, C=N), 756 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm: 7.20–7.70 (m, 10H, ArH + CH pyrrole), 8.60 (s, 1H, H at C_5), 9.50 (s, 1H, H at C_2). MS (*m/z*, %): 344.90 (M^+, 100), 346.90 (M^{+2}, 32.94). Anal. Calcd for $C_{19}H_{12}$Cl N5 (345.79): C, 66.00; H, 3.50; N, 20.25. Found: C, 66.05; H, 3.65; N, 20.09.

*4.1.3.4. 7-Phenyl-8-(p-tolyl)-7H-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c] pyrimidine (**4d**).* 2.99 g, 92% (Method A), 2.79 g, 86% (Method B); mp: 229–230 °C; IR (KBr) cm^{-1}: 3073 (C–H aromatic), 2919 (C–H aliphatic), 1636, 1500 (C=C, C=N); ^1H NMR (DMSO-d_6) δ ppm: 2.27 (s, 3H, CH_3), 7.09 (s, 1H, CH pyrrole), 7.13–7.60 (m, 9H, ArH), 8.58 (s, 1H, H at C_5), 9.41 (s, 1H, H at C_2); ^{13}C NMR: 21.16 (CH_3), 100.52, 106.05, 128.38, 128.83, 128.95, 129.03, 129.53, 129.62, 136.08, 136.44, 138.18, 139.87, 143.54, 148.50, 155.19 (15 type of C_a). MS (*m/z*, %): 325.20 (M^+, 100). Anal. Calcd for $C_{20}H_{15}N_5$ (325.37): C, 73.83; H, 4.65; N, 21.52. Found: C, 73.52; H, 4.65; N, 21.42.

*4.1.3.5. 7-Phenyl-8-(4-fluorophenyl)-7H-pyrrolo[3,2-e][1,2,4]triazolo [1,5-c]pyrimidine (**4e**).* 2.96 g, 90% (Method A), 2.86 g, 87% (Method B); mp: 206–207 °C; IR (KBr) cm^{-1}: 3060 (C–H aromatic), 1643, 1511 (C=C, C=N), 1229 (C–F); ^1H NMR (DMSO-d_6) δ ppm: 7.11–7.60 (m, 10H, ArH + CH pyrrole), 8.62 (s, 1H, H at C_5), 9.47 (s, 1H, H at C_2). MS (*m/z*, %): 329.95 (M^+, 12.61).Anal.Calcd for $C_{19}H_{12}$ F N_5 (329.33): C, 69.29; H, 3.67; N, 21.27. Found: C, 69.35; H, 3.32; N, 20.95.

*4.1.4. General procedure for the synthesis of compounds (**5a**–**e**)*

A mixture of **1a**–**e** (0.01 mol) in formic acid (85%) (20 mL) was refluxed for 4 h. Then the reaction mixture was cooled and the separated solid was filtered, washed with ethanol, dried and crystallized from ethanol.

*4.1.4.1. 6,7-Diphenyl-3,7-dihydro-pyrrolo[2,3-d]pyrimidin-4-one (**5a**).* (1.37 g, 48%); mp: 292–293 °C; IR (KBr) cm^{-1}: 3108 (NH), 1680 (C=O), 1596, 1484 (C=C, C=N); ^1H NMR (DMSO-d_6) δ ppm: 6.80–7.90 (m, 11H, ArH + CH pyrrole), 8.20 (s, 1H, H at C_2), 10.10 (s, 1H, NH); ^{13}C NMR: 104.31, 106.55, 124.62, 128.01, 130.53, 135.39, 139.12, 140.11, 143.41, 147.73, 149.35, 157.69. MS (*m/z*, %): 287.11 (M^+, 100). Anal. Calcd for $C_{18}H_{13}N_3O$ (287.32): C, 75.25; H, 4.56; N, 14.63. Found: C, 75.50; H, 4.43; N, 14.67.

*4.1.4.2. 6-(4-Bromophenyl)-7-phenyl-3,7-dihydro-pyrrolo[2,3-d]pyrimidin-4-one (**5b**).* (2.23 g, 84%); mp: 340–341 °C; IR (KBr) cm^{-1}: 3427 (NH), 1668 (C=O), 1588, 1484 (C=C, C=N); ^1H NMR ($CDCl_3$) δ ppm: 6.90–7.40 (m, 10H, ArH + CH pyrrole), 7.90 (s, 1H, H at C_2),

11.60 (s, 1H, NH, D_2O exchangeable). MS (*m/z*, %): 365.00 (M^+, 100), 367.00 (M^{+2}, 94.32). Anal. Calcd for $C_{18}H_{12}BrN_3O$ (266.21): C, 59.03; H, 3.30; N, 11.47. Found: C, 59.23; H, 3.50; N, 11.5.

*4.1.4.3. 6-(4-Chlorophenyl)-7-phenyl-3,7-dihydro-pyrrolo[2,3-d]pyrimidin-4-one (**5c**).* (2.79 g, 87%); mp: 320–321 °C; IR (KBr) cm^{-1}: 3427 (NH), 1668 (C=O), 1588, 1484 (C=C, C=N); ^1H NMR ($CDCl_3$) δ ppm: 6.90–8.00 (m, 10H, ArH + CH pyrrole), 8.10 (s, 1H, H at C_2), 11.70 (s, 1H, NH). MS (*m/z*, %): 321.07 (M^+, 100), 323.05 (M^{+2}, 32.00). Anal. Calcd for $C_{18}H_{12}ClN_3O$ (321.76): C, 67.19; H, 3.76; N, 13.06. Found: C, 67.30; H, 3.50; N, 13.20.

*4.1.4.4. 6-(p-Tolyl)-7-phenyl-3,7-dihydro-pyrrolo[2,3-d]pyrimidin-4-one (**5d**).* (2.40 g, 80%); mp: 302–303 °C; IR (KBr) cm^{-1}: 3427 (NH), 1668 (C=O), 1588, 1484 (C=C, C=N); ^1H NMR ($CDCl_3$) δ ppm: 2.30 (s, 3H, CH_3), 6.90–8.00 (m, 10H, ArH + CH pyrrole), 8.10 (s, 1H, H at C_2), 11.70 (s, 1H, NH). MS (*m/z*, %): 301.12 (M^+, 100). Anal. Calcd for $C_{19}H_{15}N_3O$ (301.34): C, 75.73; H, 5.02; N, 13.94. Found: C, 75.50; H, 4.71; N, 13.20.

*4.1.4.5. 6-(4-Fluorophenyl)-7-phenyl-3,7-dihydro-pyrrolo[2,3-d]pyrimidin-4-one (**5e**).* (2.47 g, 83%); mp: 304–305 °C; IR (KBr) cm^{-1}: 3427 (NH), 1668 (C=O), 1588, 1484 (C=C, C=N); ^1H NMR ($CDCl_3$) δ ppm: 6.90–7.50 (m, 10H, ArH + CH pyrrole), 8.00 (s, 1H, H at C_2), 12.30 (s, 1H, NH). MS (*m/z*, %): 305.00 (M^+, 100). Anal. Calcd for $C_{18}H_{12}FN_3O$ (305.31): C, 70.81; H, 3.96; N, 13.76. Found: C, 70.49; H, 3.54; N, 13.52.

*4.1.5. General procedure for the synthesis of compounds (**6a–e**)*

A mixture of **5a–e** (0.01 mol) in phosphorous oxychloride (20 mL) was refluxed for 10 h. Then the cold reaction mixture was poured onto crushed ice, stirred well, filtered, washed with water, dried and recrystallized from ethanol.

*4.1.5.1. 4-Chloro-6,7-diphenyl-7H-pyrrolo[2,3-d]pyrimidine (**6a**).* (2.62 g, 86%); mp: 152–153 °C; IR (KBr) cm^{-1}: 3039 (C–H aromatic), 1561, 1485 (C=C, C=N), 756 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm: 6.90–8.10 (m, 11H, ArH + CH pyrrole), 8.60 (s, 1H, H at C_2). MS (*m/z*, %): 305.05 (M^+, 84.75), 307.05 (M^{+2}, 29.79). Anal. Calcd for $C_{18}H_{12}Cl\ N_3$ (305.76): C, 70.61; H, 3.96; N, 13.74. Found: C, 70.89; H, 3.75; N, 13.64.

*4.1.5.2. 6-(4-Bromophenyl)-4-chloro-7-phenyl-7H-pyrrolo[2,3-d] pyrimidine (**6b**).* (3.15 g, 89%); mp: 179–180 °C; IR (KBr) cm^{-1}: 3048 (C–H aromatic), 1579, 1482 (C=C, C=N), 771 (C–Cl), 594 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 7.10–8.10 (m, 10H, ArH + CH pyrrole), 8.60 (s, 1H, H at C_2). MS (*m/z*, %): 384.00 (M^+, 100), 385.00 (M^{+2}, 80.00), 387.00 (M^{+4}, 27.00). Anal. Calcd for $C_{18}H_{11}Br\ Cl\ N_3$ (384.66): C, 56.20; H, 2.88; N, 10.92. Found: C, 56.46; H, 3.01; N, 10.92.

*4.1.5.3. 4-Chloro-6-(4-chlorophenyl)-7-phenyl-7H-pyrrolo[2,3-d] pyrimidine (**6c**).* (3.06 g, 90%); mp: 160–161 °C; IR [Br] cm^{-1}: 3047 (C–H aromatic), 1581, 1484 (C=C, C=N), 771 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm: 6.89 (s, 1H, CH pyrrole), 7.09–8.00 (m, 9H, ArH), 8.62 (s, 1H, H at C_2). MS (*m/z*, %): 338.00 (M^+, 100), 341.00(M^{+2}, 87.54), 342.00 (M^{+4}, 23.76). Anal. Calcd for $C_{18}H_{11}\ Cl_2N_3$ (340.21): C, 63.55; H, 3.26; N, 12.35. Found: C, 63.52; H, 3.22; N, 12.31.

*4.1.5.4. 4-Chloro-7-phenyl-6-p-Tolyl-7H-pyrrolo[2,3-d]pyrimidine (**6d**).* (2.29 g, 79%); mp: 119–120 °C; IR (KBr) cm^{-1}: 3105 (C–H aromatic), 2919 (C–H aliphatic), 1561, 1485 (C=C, C=N), 756 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm: 2.30 (s, 3H, CH_3), 7.01 (s, 1H, CH pyrrole), 7.20–8.11 (m, 9H, ArH), 8.60 (s, 1H, H at C_2). MS (*m/z*, %): 318.05 (M^+, 100), 320.05 (M^{+2}, 53.54). Anal. Calcd for $C_{19}H_{14}\ Cl\ N_3$ (319.09): C, 71.36; H, 4.41; N, 13.14. Found: C, 71.49; H, 4.13; N, 13.40.

*4.1.5.5. 4-Chloro-6-(4-fluorophenyl)-7-phenyl-7H-pyrrolo[2,3-d] pyrimidine (**6e**).* (3.10 g, 96%); mp: 112–113 °C; IR (KBr) cm^{-1}: 3057 (C–H aromatic), 1581, 1510 (C=C, C=N), 1225 (C–F), 755 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm: 6.90 (s, 1H, CH pyrrole), 7.00–8.10 (m, 9H, ArH), 8.60 (s, 1H, H at C_2). MS (*m/z*, %): 321.95 (M^+, 100), 323.95 (M^{+2}, 50.57). Anal. Calcd for $C_{18}H_{11}N_3ClF$ (323.75): C, 66.78; H, 3.42; N, 12.98. Found: C, 66.75; H, 3.35; N, 12.88.

*4.1.6. General procedure for the synthesis of compounds (**7a–e**)*

A mixture of **6a–e** (0.01 mol) in (99%) hydrazine hydrate (99%) (10 mL) and ethanol (10 mL) was refluxed for 2 h. Then the cold reaction mixture was poured onto crushed ice, stirred well, filtered, washed with water, dried and recrystallized from ethanol.

*4.1.6.1. 6,7-Diphenyl-4-hydrazino-7H-pyrrolo[2,3-d]pyrimidine (**7a**).* (2.64 g, 88%); mp: 188–189 °C; IR (KBr) cm^{-1}: 3404, 3326 and 3200 (NH, NH_2), 3040 (C–H aromatic), 1589, 1496 (C=C, C=N); ^1H NMR (DMSO-d_6) δ ppm: 4.20 (s, 2H, NH_2), 6.90–7.60 (m, 11H, ArH + CH pyrrole), 7.90 (s, 1H, H at C_2), 8.60 (s, 1H, NH). MS (*m/z*, %): 301.00 (M^+, 100). Anal. Calcd for $C_{18}H_{15}N_5$ (301.35): C, 71.74; H, 5.02; N, 23.24. Found: C, 71.62; H, 5.04; N, 22.95.

*4.1.6.2. 6-(4-Bromophenyl)-7-phenyl-4-hydrazino-7H-pyrrolo[2,3-d] pyrimidine (**7b**).* (3.26 g, 86%); mp: 227–228 °C; IR (KBr) cm^{-1}: 3400, 3309 and 3200 (NH, NH_2), 3062 (C–H aromatic), 1594, 1495 (C=C, C=N), 564 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 4.20 (s, 2H, NH_2), 6.90–7.60 (m, 10H, ArH + CH pyrrole), 7.90 (s, 1H, H at C_2), 8.60 (s, 1H, NH). MS (*m/z*, %): 379.00 (M^+, 50.93), 381.00 (M^{+2}, 41.91). Anal. Calcd for $C_{18}H_{14}\ Br\ N_5$ (380.24): C, 56.86; H, 3.71; N, 18.42. Found: C, 56.48; H, 3.34; N, 18.03.

*4.1.6.3. 6-(4-Chlorophenyl)-7-phenyl-4-hydrazino-7H-pyrrolo[2,3-d] pyrimidine (**7c**).* (3.04 g, 91%); mp: 213–214 °C; IR (KBr) cm^{-1}: 3400, 3306, 3200 and 3150 (NH, NH_2), 3040 (C–H aromatic), 1595, 1496 (C=C, C=N), 771 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm: 4.20 (s, 2H, NH_2), 6.85 (s, 1H, CH pyrrole), 7.00–7.60 (m, 9H, ArH), 7.89 (s, 1H, H at C_2), 8.60 (s, 1H, NH). MS (*m/z*, %): 334.90 (M^+, 100), 336.90 (M^{+2}, 37.63). Anal. Calcd for $C_{18}H_{14}\ Cl\ N_5$ (335.79): C, 64.38; H, 4.20; N, 20.86. Found: C, 64.72; H, 4.58; N, 20.66.

*4.1.6.4. 6-(p-Tolyl)-7-phenyl-4-hydrazino-7H-pyrrolo[2,3-d]pyrimidine (**7d**).* (2.83 g, 90%); mp: 172–173 °C; IR (KBr) cm^{-1}: 3404, 3326 and 3200 (NH, NH_2), 3040 (C–H aromatic), 1589, 1496 (C=C, C=N); ^1H NMR (DMSO-d_6) δ ppm: 2.40 (s, 3H, CH_3), 4.70 (s, 2H, NH_2), 6.91 (s, 1H, CH pyrrole), 7.13–8.01 (m, 9H, ArH), 8.23 (s, 1H, H at C_2), 9.23 (s, 1H, NH). MS (*m/z*, %): 315.00 (M^+, 88.36). Anal. Calcd for $C_{19}H_{17}N_5$ (315.37): C, 72.36; H, 5.43; N, 22.21. Found: C, 72.21; H, 5.19; N, 22.12.

*4.1.6.5. 6-(4-Fluorophenyl)-7-phenyl-4-hydrazino-7H-pyrrolo[2,3-d] pyrimidine (**7e**).* (2.55 g, 80%); mp: 225–226 °C; IR (KBr) cm^{-1}: 3424, 3349, 3313 and 3196 (NH, NH_2), 3062 (C–H aromatic), 1589,1511 (C=C, C=N), 1220 (C–F); ^1H NMR (DMSO-d_6) δ ppm: 4.02 (s, 2H, NH_2), 7.03(s, 1H, CH pyrrole), 7.10–7.47(m, 9H, ArH), 8.20 (s, 1H, H at C_2), 8.89 (s, 1H, NH). MS (*m/z*, %): 318.95 (M^+, 100). Anal. Calcd for $C_{18}H_{14}\ F\ N_5$ (319.34): C, 67.70; H, 4.42; N, 21.93. Found: C, 67.32; H, 4.30; N, 21.92.

*4.1.7. General procedure for the synthesis of compounds (**8a–e**)*

An aqueous solution of (20% w/v) sodium nitrite (4.2 mL) was slowly added in portions to a stirred mixture of **7a–e** (0.01 mol) in acetic acid (40 mL) at 0–5 °C. The reaction mixture was then further

stirred for 2 h at the same temperature, then it was diluted with cold water and the solid obtained was filtered, washed with water, dried and crystallized from ethanol.

*4.1.7.1. 7,8-Diphenyl-7H-pyrrolo[3,2-e]tetrazolo[1,5-c]pyrimidine (**8a**).* (2.83 g, 91%); mp: 199–200 °C; IR (KBr) cm^{-1}: 3100 (C–H aromatic), 1629, 1501 (C=C, C=N); ^1H NMR (DMSO-d_6) δ ppm: 6.84 (s, 1H, CH pyrrole), 7.34–7.87 (m, 10H, ArH), 9.85 (s, 1H, H at C_5). MS (*m/z*, %): 312.00 (M^+, 13.44). Anal. Calcd for $C_{18}H_{12}N_6$ (312.23): C, 69.22; H, 3.87; N, 26.91. Found: C, 69.02; H, 3.82; N, 27.11.

*4.1.7.2. 8-(4-Bromo-phenyl)-7-phenyl-7H-pyrrolo[3,2-e]tetrazolo [1,5-c]pyrimidine (**8b**).* (3.44 g, 88%); mp: 202–203 °C; IR (KBr) cm^{-1}: 3061 (C–H aromatic), 1644, 1499 (C=C, C=N), 555 (C–Br); ^1H NMR (DMSO-d_6) δ ppm: 7.20–7.70 (m, 10H, ArH + CH pyrrole), 9.90 (s, 1H, H at C_5). MS (*m/z*, %): 390.00 (M^+, 100), 392.00 (M^{+2}, 94.00). Anal. Calcd for $C_{18}H_{11}$ Br N_6 (391.22): C, 55.10; H, 2.80; N, 21.42. Found: C, 55.10; H, 3.11; N, 21.11.

*4.1.7.3. 8-(4-Chloro-phenyl)-7-phenyl-7H-pyrrolo[3,2-e]tetrazolo [1,5-c]pyrimidine (**8c**).* (3.22 g, 93%); mp: 210–211 °C; IR (KBr) cm^{-1}: 3099 (C–H aromatic), 1631, 1501 (C=C, C=N), 749 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm: 7.33–7.58 (m, 10H, ArH + CH pyrrole), 9.88 (s, 1H, H at C_5). MS (*m/z*, %): 346.00 (M^+, 10.54), 348.00 (M^{+2}, 3.95). Anal. Calcd for $C_{18}H_{11}$ Cl N_6 (346.77): C, 62.34; H, 3.20; N, 24.23. Found: C, 62.71; H, 3.22; N, 23.86.

*4.1.7.4. 7-Phenyl-8-p-tolyl-7H-pyrrolo[3,2-e]tetrazolo[1,5-c]pyrimidine (**8d**).* (2.70 g, 83%); mp: 209–210 °C; IR (KBr) cm^{-1}: 3072 (C–H aromatic), 2925 (C–H aliphatic) 1629, 1502 (C=C, C=N); ^1H NMR (DMSO-d_6) δ ppm: 2.27 (s, 3H, CH_3), 7.00–7.59 (m, 10H, ArH + CH pyrrole), 9.86 (s, 1H, H at C_5). MS (*m/z*, %): 326.00 (M^+, 100). Anal. Calcd for $C_{19}H_{14}N_6$ (326.35): C, 69.92; H, 4.32; N, 25.75. Found: C, 69.78; H, 4.40; N, 25.52.

*4.1.7.5. 8-(4-Fluoro-phenyl)-7-phenyl-7H-pyrrolo[3,2-e]tetrazolo [1,5-c]pyrimidine (**8e**).* (2.97 g, 90%); mp: 162–163 °C; IR (KBr) cm^{-1}: 3077 (C–H aromatic), 1633, 1511 (C=C, C=N), 1229 (C–F); ^1H NMR (DMSO-d6) δ ppm: 7.20–7.60 (m, 10H, ArH + CH pyrrole), 9.86 (s, 1H, H at C_5); ^{13}C NMR: 101.48, 102.85, 115.39, 116.00, 116.46, 128.61, 128.89, 129.35, 130.53, 131.15, 133.60, 140.40, 143.34, 146.03. MS (*m/z*, %): 329.95 (M^+, 12.61). Anal. Calcd for $C_{18}H_{11}$ F N_6 (330.32): C, 65.45; H, 3.36; N, 25.44. Found: C, 65.22; H, 3.40; N, 25.27.

*4.1.8. General procedure for the synthesis of compounds (**9a**,**c**,**d**)*

A mixture of **1a,c,d** (0.01 mol) and formamide (10 mL) was refluxed for 4 h, the reaction mixture was then concentrated and poured into ice cold water. The separated solid was filtered off and recrystallized from ethanol.

*4.1.8.1. 6,7-Diphenyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**9a**).* (2.71 g, 95%); mp: 240–241 °C; IR (KBr) cm^{-1}: 3458, 3299 and 3120 (NH_2), 3050 (C–H aromatic), 1589, 1498 (C=C, C=N); ^1H NMR (DMSO-d_6) δ ppm: 6.85 (s, 2H, NH_2), 6.90 (s, 1H, CH pyrrole), 7.20–8.00 (m, 10H, ArH), 8.52 (s, 1H, CH pyrimidine). MS (*m/z*, %): 286.00 (M^+, 60.20). Anal. Calcd for $C_{18}H_{14}N_4$ (286.33): C, 75.50; H, 4.93; N, 19.57. Found: C, 75.21; H, 4.52; N, 19.38.

*4.1.8.2. 6-(4-Chlorophenyl)-7-phenyl-7H-pyrrolo[2,3-d]pyrimidin4-amine (**9c**).* (2.72 g, 85%); mp: 260–261 °C; IR (KBr) cm^{-1}: 3400, 3306, 3200 and 3150 (NH_2), 3040 (C–H aromatic), 1595, 1496 (C=C, C=N), 771 (C–Cl); ^1H NMR (DMSO-d_6) δ ppm: 6.85 (s, 2H, NH_2), 6.89 (s, 1H, CH pyrrole), 7.20–8.00 (m, 9H, ArH), 8.52 (s, 1H, CH pyrimidine). MS (*m/z*, %): 320.00 (M^+, 100), 322.00 (M^{+2}, 30.00). Anal. Calcd for $C_{18}H_{13}$ Cl N_4 (320.78): C, 67.40; H, 4.08; N, 17.47. Found: C, 67.19; H, 3.85; N, 17.42.

*4.1.8.3. 7-Phenyl-6-p-tolyl-7H-pyrrolo[2,3-d]pyrimidin4-amine (**9d**).* (2.70 g, 90%); mp: 238–239 °C; IR (KBr) cm^{-1}: 3404, 3326 and 3200 (NH_2), 3040 (C–H aromatic), 1589, 1496 (C=C, C=N); ^1H NMR (DMSO-d_6) δ ppm: 2.25 (s, 3H, CH_3), 6.83 (s, 2H, NH_2), 6.84 (s, 1H, CH pyrrole), 7.00–7.90 (m, 9H, ArH), 8.00 (s, 1H, CH pyrimidine). MS (*m/z*, %): 300.00 (M^+, 33.00). Anal. Calcd for $C_{19}H_{16}N_4$ (300.36): C, 75.98; H, 5.37; N, 18.65. Found: C, 75.71; H, 5.22; N, 18.51.

*4.1.9. General procedure for the synthesis of compounds (**10a**,**c**,**d**)*

A mixture of **9a,c,d** (0.01 mol) and acetaldehyde (10 mL) was refluxed in ethanol (20 mL) for 4 h. The cold reaction mixture was concentrated, the separated solid filtered off and recrystallized from ethanol.

*4.1.9.1. N-Ethylidene-6,7-diphenyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**10a**).* (2.96 g, 95%); mp: 121–122 °C; ^1H NMR (DMSO-d_6) δ ppm: 1.12 (d, 3H, -$CHCH_3$), 1.30 (q, 1H, -$CHCH_3$), 6.76 (s, 1H, CH pyrrole), 6.80–8.00 (m, 10H, ArH), 8.45 (s, 1H, CH pyrimidine). MS (*m/z*, %): 312.00 (M^+, 100). Anal. Calcd for $C_{20}H_{16}N_4$ (312.37): C, 76.92; H, 5.13; N, 17.95. Found: C, 76.64; H, 4.89; N, 17.56.

*4.1.9.2. 6-(4-Chlorophenyl)-N-ethylidene-7-phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**10c**).* (3.18 g, 92%); mp: 116–117 °C; ^1H NMR (DMSO-d_6) δ ppm: 1.12 (d, 3H, -$CHCH_3$), 1.30 (q, 1H, -$CHCH_3$), 6.70 (s, 1H, CH pyrrole), 6.80–8.00 (m, 9H, ArH), 8.45 (s, 1H, CH pyrimidine). MS (*m/z*, %): 346.00 (M^+, 100), 348.00 (M^{+2}, 38.00). Anal. Calcd for $C_{20}H_{15}$ Cl N_4 (346.81): C, 69.26; H, 4.36; N, 16.15. Found: C, 69.02; H, 3.98; N, 16.11.

*4.1.9.3. N-Ethylidene-7-phenyl–6-p-tolyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**10d**).* (3.03 g, 93%); mp: 130–131 °C; ^1H NMR (DMSO-d_6) δ ppm: 1.11 (d, 3H, -$CHCH_3$), 1.50 (q, 1H, -$CHCH_3$), 2.25 (s, 3H, CH_3), 6.70 (s, 1H, CH pyrrole), 6.80–7.50 (m, 9H, ArH), 8.40 (s, 1H, CH pyrimidine). MS (*m/z*, %): 326.15 (M^+, 100). Anal. Calcd for $C_{21}H_{18}N_4$ (326.39): C, 77.28; H, 5.56; N, 17.17. Found: C, 76.92; H, 5.28; N, 17.11.

*4.1.10. General procedure for the synthesis of compounds (**11a**,**c**,**d**)*

A mixture of **1a,c,d** (0.01 mol) and urea (0.01 mol) was refluxed in glacial acetic acid and hydrochloric acid (3:1) for 4 h. After that the cold reaction mixture was poured into ice cold water, filtered and recrystallized from chloroform.

*4.1.10.1. 6,7-Diphenyl-7H-pyrrolo[2,3-d]pyrimidine-2,4-diamine (**11a**).* (2.34 g, 78%); mp: 135–136 °C; IR (KBr) cm^{-1}: 3200, 3100 (NH_2), 3060 (C–H aromatic), 1595, 1497 (C=C, C=N); ^1H NMR (DMSO-d_6) δ ppm: 6.70 (s, 4H, $2NH_2$), 7.00 (s, 1H, CH pyrrole), 7.10–7.80 (m, 10H, ArH). MS (*m/z*, %): 301.00 (M^+, 100). Anal. Calcd for $C_{18}H_{15}N_5$ (301.35): C, 71.74; H, 5.02; N, 23.24 Found: C, 71.55; H, 4.82; N, 23.01.

*4.1.10.2. 6-(4-Chlorophenyl)-7-phenyl-7H-pyrrolo[2,3-d]pyrimidine-2,4-diamine (**11c**).* (2.98 g, 89%); mp: 114–115 °C; IR (KBr) cm^{-1}: 3200, 3100 (NH_2), 3065 (C–H aromatic), ^1H NMR (DMSO-d_6) δ ppm: 6.79 (s, 4H, $2NH_2$), 6.81 (s, 1H, CH pyrrole), 6.90–8.40 (m, 9H, ArH). MS (*m/z*, %): 335.00 (M^+, 100), 337.00 (M^{+2}, 14.20). Anal. Calcd for $C_{18}H_{14}$ Cl N_5 (335.79): C, 64.38; H, 4.20; N, 20.86. Found: C, 64.12; H, 3.82; N, 20.68.

*4.1.10.3. 7-Phenyl-6-p-tolyl-7H-pyrrolo[2,3-d]pyrimidine-2,4-diamine (**11d**).* (2.74 g, 87%); mp: 101–102 °C; IR (KBr) cm^{-1}: 3200,

3100 (NH_2), 3041 (C–H aromatic), 2925 (C–H aliphatic), 1602, 1497 (C=C, C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 2.28 (s, 3H, CH_3), 7.00 (s, 4H, $2NH_2$), 7.20 (s, 1H, CH pyrrole), 7.30–7.88 (m, 9H, ArH); ^{13}C NMR: 24.57 (CH_3), 101.41, 104.2, 120.72, 127.83, 128.0, 128.33, 128.45, 128.80, 129.14, 134.10, 143.36, 173.58, 197.84, 207.8. MS (*m/z*, %): 315.00 (M^+, 31.30). Anal. Calcd for $C_{19}H_{17}N_5$ (315.37): C, 72.36; H, 5.43; N, 22.21. Found: C, 72.02; H, 5.58; N, 22.10.

*4.1.11. General procedure for the synthesis of compounds (**12a**,**c**,**d**)*

A mixture of **1a,c,d** (0.01 mol) and acetamide (0.01 mol) was refluxed in glacial acetic acid and hydrochloric acid (3:1) for 4 h. After that the cold reaction mixture was poured into ice cold water, filtered and recrystallized from chloroform.

*4.1.11.1. 2-Methyl-4-amino-6,7-diphenyl-7H-pyrrolo[2,3-d]pyrimidine (**12a**).* (2.85 g, 95%); mp: 135–136 °C; IR (KBr) cm^{-1}: 3345, 3116 (NH_2), 3059 (C–H aromatic), 2923 (C–H aliphatic), 1595, 1497 (C=C, C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 2.50 (s, 3H, CH_3), 6.80 (s, 2H, NH_2), 6.81 (s, 1H, CH pyrrole), 6.84–8.46 (m, 10H, ArH). MS (*m/z*, %): 300.00 (M^+, 16.70). Anal. Calcd for $C_{19}H_{16}N_4$ (300.36): C, 75.98; H, 5.37; N, 18.65. Found: C, 75.92; H, 5.69; N, 18.59.

*4.1.11.2. 2-Methyl-4-amino-6-(4-chlorophenyl)-7-phenyl-7H-pyrrolo[2,3-d]pyrimidine (**12c**).* (2.97 g, 89%); mp: 102–103 °C; IR (KBr) cm^{-1}: 3345, 3116 (NH_2), 3059 (C–H aromatic), 2923 (C–H aliphatic), 1595, 1497 (C=C, C=N), 756 (C–Cl); ^{1}H NMR (DMSO-d_6) δ ppm: 2.49(s, 3H, CH_3), 6.80 (s, 2H, NH_2), 6.81 (s, 1H, CH pyrrole), 6.84–8.47 (m, 9H, ArH). MS (*m/z*, %): 334.00 (M^+, 42.90), 336.00 (M^{+2}, 34.30). Anal. Calcd for $C_{19}H_{15}ClN_4$ (334.80): C, 68.16; H, 4.52; N, 16.73. Found: C, 68.02; H, 4.82; N, 17.1.

*4.1.11.3. 2-Methyl-4-amino-6-(p-tolyl)-7-phenyl-7H-pyrrolo[2,3-d]pyrimidine (**12d**).* (2.73 g, 87%); mp: 279–280 °C; IR (KBr) cm^{-1}: 3345, 3116 (NH_2), 3059 (C–H aromatic), 2850 (C–H aliphatic), 1595,1497 (C=C, C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 2.30 (s, 3H, CH_3), 2.50 (s, 3H, CH_3 at C_2), 6.79 (s, 2H, NH_2), 6.82 (s, 1H, CH pyrrole), 6.85–8.48 (m, 9H, ArH). MS (*m/z*, %): 314.00 (M^+, 53). Anal. Calcd for $C_{20}H_{18}N_4$ (314.38): C, 76.41; H, 5.77; N, 17.82. Found: C, 76.13; H, 5.91; N, 18.15.

4.2. Microbiology

Determination of the minimal inhibitory concentration (MIC) of the compounds was measured by two-fold serial dilution method.

4.2.1. Sample preparation

Each of the test compounds and standards (Ampicillin, Fluconazole) were dissolved in 12.5% DMSO, at concentration of 5 mg/mL. Further dilutions of the compounds and standards in the test medium were prepared at required quantities 2.5, 1.25, 0.62 and 0.31 mg/mL.

4.2.2. Culture of microorganisms

Bacteria and fungal species used were obtained from Microbiology Department of Al-Azahar University, Faculty of Medicine for girls, Nasr City, Cairo, Egypt, namely *S. aureus* (NCTC-7447), *E. coli* (NCTC-10416), *C. albicans* (ATCC-10145). The bacterial strains were maintained on MHA (Mueller-Hinton Agar) medium for 24 h at 37 ± 1 °C and fungi were maintained on SDS (Sabouraud Dextrose Agar) for 48 h at 28 ± 1 °C. The bacteria and fungi inocula were prepared by suspension in 5 mL of sterile saline for colonies from culture on MHA and SDA medium.

4.2.3. Agar well diffusion technique [18]

The *in vitro* antibacterial and antifungal activity of compounds were tested by the diffusion agar technique [18]. The diffusion agar technique was followed to determine the minimum inhibitory concentration (MIC) of all the synthesized compounds. With a sterile loop, pure colonies of the bacteria and fungi culture were picked up. The colonies were suspended in 5 mL of sterile saline. Well containing the material were pre-incubated for 1 h at refrigerator followed by incubation for bacteria 24 h at 37 ± 1 °C and for fungi 48 h at 28 ± 1 °C. After incubation, the diameter of the inhibition zone was measured. The MIC is the lowest concentration (highest dilution) of active compound that makes inhibition for microbial growth i.e. the next dilution not makes inhibition in microbial growth [19–21].

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