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Original research paper

Synthesis of certain pyrrole derivatives as antimicrobial agents

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In an effort to establish new pyrroles and pyrrolo[2,3-*d*] pyrimidines with improved antimicrobial activity we report here the synthesis and *in vitro* microbiological evaluation of a series of pyrrole derivatives. A series of new 2-aminopyrrole-3-carbonitriles (**1a-d**) were synthesized from the reaction of benzoin, primary aromatic amines and malononitrile, from which a number of pyrrole derivatives (**2a-d** to **5a-d**) and pyrrolo[2,3-*d*]pyrimidines (**6a-d** to **10a,d**) were synthesized. The *in vitro* antimicrobial testing of the synthesized compounds was carried out against Gram-positive, Gram-negative bacteria and fungi. Some of the prepared compounds, [2-amino-1-(2-methylphenyl)--4,5-diphenyl-1*H*-pyrrole-3-carbonitriles (**1b**), 2-amino-3--carbamoyl-1-(3-methylphenyl)-4,5-diphenyl-1*H*-pyrroles (**2b**), *N*-(3-cyano-1-(2-methylphenyl)-4,5-diphenyl-1*H*--pyrrol-2-yl)-acetamides (**3b**), *N*-(3-cyano-1-(3-methylphenyl)-4,5-diphenyl-1*H*-pyrrol-2-yl)-acetamides (**3c**), 2-amino-1-(4-methoxyphenyl)-4,5-diphenyl-3-tetrazolo-1*H*-pyrroles (**5d**), 7-(4-methoxyphenyl)-5,6-diphenyl-7*H*-pyrrolo [2,3-*d*]pyrimidin-4(3*H*)-ones (**7d**), 7-(3-methylphenyl)-5,6--diphenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-thione (**9b**) and *N*-(7-(2-methylphenyl)-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*] pyrimidine)-*N*-aryl amines (**10a**)] showed potent antimicrobial activity.

Keywords: pyrrole, pyrrolopyrimidine, antimicrobial activity

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For several decades, interest in pyrrole derivatives as antimicrobial agents has led to the preparation and antimicrobial evaluation of hundreds of such molecules. For example, monodeoxypyoluteorin and 2-(2'-hydroxybenzoyl) pyrrole bromine derivatives are pyrrole derivatives having antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* and an interesting antifungal activity against *Candida albicans*, as it was shown by Petruso *et al*. (1) and Raimondi *et al*. (2). 3,4,5,3,5-Pentabromo-

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-2-(2-hydroxybenzoyl)pyrrole, a synthetic antibacterial compound related to pyrrolomycins, has significant activity against *Staphylococcus epidermidis* and *Staphylococcus aureus*, as proved by D. Schillaci *et al*. (3).

2-Methyl-1,3,5-trisubstituted pyrroles have significant activity against *Mycobacterium tuberculosis*, as proved by Biava *et al*. (4, 5). Diguanidino-1-methyl-2,5-diaryl-1*H*-pyrrole derivatives have antifungal activity against *Candida* species, as reported by Jana *et al*. (6).

Tubercidin, toyocamycin and sangivamycin are naturally occurring pyrrolo[2,3-*d*]pyrimidine antibiotics having significant activity against *Mycobacterium tuberculosis*, *Candida albicans* and *Streptococcus neoformans*, which was shown in many reports (7–9).

2,4-Diamino-5-methyl-6-substituted-pyrrolo[2,3-*d*]pyrimidines are potent and selective dihydrofolate reductase (DHFR) inhibitors against *Pneumocystis carinii*, *Toxoplasma gondii* and *Mycobacterium avium*, as reported by Gangjee *et al*. (10).

The aim of this study was to synthesize new pyrrole and pyrrolo[2,3-*d*] pyrimidine derivatives, hoping that they could be of promising chemical and biological interest.

EXPERIMENTAL

All melting points are uncorrected and were measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (USA), ^{1}H NMR spectra were determined on a Varian Mercury (300 MHz) spectrometer (Varian, UK) and chemical shifts were expressed as ppm against TMS as internal reference. Mass spectra were recorded on a 70 eV EI Ms-QP 1000 EX (Shimadzu). Microanalyses were operated using Vario, Elementar apparatus (Shimadzu) and the results were within accepted range (± 0.40 %) of the calculated values.

Physicochemical and spectral data for the synthesized compounds are given in Tables I and II. The target compounds were synthesized as outlined in Schemes 1 and 2. Synthesis of compound **1** was performed according to literature (11, 12).

*Synthesis of 2-amino-4,5-diphenyl-1-substituted-1*H*-pyrrole-3-carbonitriles (**1a-d**)*

A mixture of benzoin (2 g, 0.01 mol), the appropriate amine [aniline (0.93 g, 0.01 mol), *o*-toluidine or *m*-toulidine (1.17 g, 0.01 mol), or *p*-anisidine (1.23 g, 0.01mol)] and conc. HCl (6–8 drops) in toluene (50 mL) was heated under reflux for 6 h and cooled. Malononitrile (0.66 mg, 0.01 mol) was added, followed by a catalytic amount (1.5 mL) of pyridine portionwise and left to reflux until a solid was formed. The solvent was evaporated under reduced pressure and the residue was recrystallized from methanol to give compounds **1a-d**, respectively.

*Synthesis of 2-amino-1-aryl-3-carbamoyl-4,5-diphenyl-1*H*-pyrroles (**2a-d**)*

An appropriate cyanopyrrole, **1a** (3.35 g, 0.01 mol), **1b**, **1c** (3.49 g, 0.01 mol) or **1d** (3.65 g, 0.01 mol) in conc. sulphuric acid (40 mL), was refluxed for 4 h, cooled, poured

onto ice-water, neutralized with ammonia to give compounds **2a-d,** respectively in the form of precipitates which were filtered, dried, and recrystallized from methanol.

Synthesis of N-*(1-aryl-3-cyano-4,5-diphenyl-1*H*-pyrrol-2-yl)-acetamides (**3a-d**)*

The appropriate aminopyrrole, **1a** (3.35 g, 0.01 mol), **1b**, **1c** (3.49 g, 0.01 mol) or **1d** (3.65 g, 0.01 mol), in acetic anhydride (40 mL) was refluxed for 4 h, cooled, poured onto ice-water, neutralized with ammonia to give compounds **3a-d**, respectively in the form of precipitates which were filtered off, dried, and recrystallized from methanol.

*Synthesis of 3-(2-amino-1-aryl-4,5-diphenyl-1*H*-pyrrole)amidoximes (**4c,d**)*

The appropriate cyanopyrrole **1c** (3.49 g, 0.01 mol) or **1d** (3.65 g, 0.01 mol), hydroxyl amine hydrochloride (0.33 g, 0.01 mol) and anhydrous sodium carbonate (5.3 g, 0.05 mol) in absolute ethanol (40 mL) was refluxed for 4 h, filtered while hot and the residue was washed with hot ethanol. The collected filtrate was cooled, poured onto ice-water to yield precipitates, which were filtered, dried, and recrystallized from methanol, to give compounds **4c**,**d**, respectively.

*Synthesis of 2-amino-1-aryl-4,5-diphenyl-3-tetrazolo-1*H*-pyrroles (**5a,b,d**)*

A mixture of the appropriate cyanopyrrole **1a** (3.35 g, 0.01 mol), **1b** (3.49 g, 0.01 mol) or **1d** (3.65 g, 0.01 mol), sodium azide (0.65 g, 0.01 mol) and ammonium chloride (1.06 g, 0.02 mol) was refluxed in DMF (30 mL) for 4 h, filtered while hot and the residue was washed with hot DMF. The collected filtrate was concentrated, cooled, poured onto ice--water to yield precipitates, which were filtered, dried and recrystallized from methanol, to give compounds **5a**,**b**,**d**, respectively.

*Synthesis of 5,6-diphenyl-7-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl-amines (**6a-d**)*

A mixture of the appropriate aminopyrrole **1a** (3.35 g, 0.01 mol), **1b**, **1c** (3.49 g, 0.01 mol) or **1d** (3.65 g, 0.01 mol) and formamide (30 mL, 0.066 mol) was heated under reflux for 6 h, cooled and poured onto ice-water to give precipitates, which were filtered off, dried, and recrystallized from ethanol to yield compounds **6a-d**, respectively.

*Synthesis of 5,6-diphenyl-7-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-ones (**7a-d**)*

An appropriate aminopyrrole, **1a** (3.35 g, 0.01 mol), **1b**, **1c** (3.49 g, 0.01 mol) or **1d** (3.65 g, 0.01 mol), in formic acid (20 mL, 85%) was heated under reflux for 3 h, cooled, poured onto ice-water to give compounds **7a-d** in the form of precipitates which were filtered off, dried, and recrystallized from ethanol.

*Synthesis of 4-chloro-5,6-diphenyl-7-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidine (**8a-d**)*

An appropriate pyrrolopyrimidinone, **7a** (3.63 g, 0.01 mol), **7b**, **7c** (3.77 g, 0.01 mol) or **7d** (3.93 g, 0.01 mol), was heated under reflux in phosphorus oxychloride (30 mL) for

Table I. Elemental analysis of the newly prepared compounds ***1-10***

Compd. No.	R	R′	Yield (%)	M.p. (°C)	Mol. formula (M_r)	Microanalysis calcd./found (%)				
						C	H	N	Cl	S
1a	H	–	86	248–250 (13)	$C_{23}H_{17}N_3$ (335.41)	–	–	–	–	–
1b	2-CH_3	–	39	210–212	$C_{24}H_{19}N_3$ (349.44)	82.49 82.83	5.48 5.28	12.03 12.22	– –	– –
1c	3-CH_3	–	58	197–199	$C_{24}H_{19}N_3$ (349.44)	82.49 82.74	5.48 5.62	12.03 11.98	– –	– –
1d	4-OCH_3	–	91	204–206	$C_{24}H_{19}N_3O$ (365.44)	78.89 78.58	5.24 5.39	11.50 11.22	– –	– –
2a	H	–	72	207–209	$C_{23}H_{19}N_3O$ (353.43)	78.16 78.50	5.42 5.12	11.89 12.23	– –	– –
2b	2-CH_3	–	44	231–233	$C_{24}H_{21}N_3O$ (367.45)	78.46 78.21	5.76 5.63	11.44 11.82	– –	– –
2c	3-CH_3	–	59	196–198	$C_{24}H_{21}N_3O$ (367.45)	78.45 78.15	5.76 5.43	11.44 11.61	– –	– –
2d	4-OCH_3	–	79	229–231	$C_{24}H_{21}N_3O_2$ (383.45)	75.18 75.39	5.52 5.22	10.96 10.56	– –	– –
3a	H	–	81	234–236	$C_{25}H_{19}N_3O$ (377.45)	86.20 86.54	5.43 5.08	8.38 8.64	– –	– –
3b	2-CH_3	–	55	193–194	$C_{26}H_{21}N_3O$ (391.48)	86.18 85.89	5.79 5.34	8.04 8.29	– –	– –
3c	3-CH_3	–	67	203–205	$C_{26}H_{21}N_3O$ (391.48)	86.18 86.31	5.79 5.46	8.04 8.36	– –	– –
3d	4-OCH_3	–	87	184–186	$C_{26}H_{21}N_3O_2$ (407.48)	82.39 82.59	5.53 5.45	7.69 7.44	– –	– –
4c	3-CH_3	–	46	207–209	$C_{24}H_{22}N_4O$ (382.47)	85.17 85.35	6.55 6.83	8.28 8.45	– –	– –
4d	4-OCH_3	–	81	191–193	$C_{24}H_{22}N_4O_2$ (398.47)	81.33 81.22	6.26 6.52	7.90 7.71	– –	– –
5a	H	–	64	234–236	$C_{23}H_{18}N_6$ (378.44)	73.00 73.26	4.79 4.87	22.21 22.73	– –	– –
5b	2-CH_3	–	29	219–221	$C_{24}H_{20}N_6$ (392.47)	73.45 73.54	5.14 4.88	21.41 21.78	– –	– –
5d	4-OCH_3	–	72	228–230	$C_{24}H_{20}N_6O$ (408.47)	70.57 70.41	4.94 4.67	20.57 20.31	– –	– –
6a	H	–	86	262–264 (14)	$C_{24}H_{18}N_4$ (362.44)	–	–	–	–	–
6b	2-CH_3	–	39	218–220	$C_{25}H_{20}N_4$ (376.46)	79.76 79.42	5.35 5.17	14.88 15.13	– –	– –

6c	3-CH_3	–	58	235–237	$C_{25}H_{20}N_4$	79.76	5.35	14.88	–	–
					(376.46)	79.53	5.71	14.52	–	–
6d	4-OCH_3	–	91	226–228	$C_{25}H_{20}N_4O$	76.51	5.14	14.28	–	–
					(392.46)	76.91	5.47	13.93	–	–
7a	H	–	72	277–279	$C_{24}H_{17}N_3O$	79.32	4.72	11.56	–	–
					(363.42)	79.12	4.94	11.92	–	–
7b	2-CH_3	–	44	253–255	$C_{25}H_{19}N_3O$	79.55	5.07	11.13	–	–
					(377.45)	79.66	5.39	10.75	–	–
7c	3-CH_3	–	59	269–270	$C_{25}H_{19}N_3O$	79.55	5.07	11.13	–	–
					(377.45)	79.71	4.85	11.51	–	–
7d	4-OCH_3	–	79	261–263	$C_{25}H_{19}N_3O_2$	76.32	4.87	10.68	–	–
					(393.45)	76.65	5.21	10.45	–	–
8a	H	–	81	230–232	$C_{24}H_{18}N_3Cl$	75.49	4.22	11.00	9.28	–
					(381.87)	75.43	4.59	11.23	8.96	–
8b	2-CH_3	–	55	217–219	$C_{25}H_{18}N_3Cl$	75.85	4.58	10.61	8.96	–
					(395.90)	75.88	4.67	10.66	8.71	–
8c	3-CH_3	–	67	221–223	$C_{25}H_{18}N_3Cl$	75.85	4.58	10.61	8.96	–
					(395.90)	75.71	4.92	10.79	9.12	–
8d	4-OCH_3	–	87	209–211	$C_{25}H_{18}N_3ClO$	72.90	4.40	10.20	8.61	–
					(411.89)	73.24	4.21	9.85	8.48	–
9b	2-CH_3	–	76	227–229	$C_{25}H_{19}N_3S$	76.31	4.87	10.68	–	8.15
					(393.51)	76.55	4.63	10.55	–	8.36
9c	3-CH_3	–	32	236–238	$C_{25}H_{19}N_3S$	76.31	4.87	10.68	–	8.15
					(393.51)	76.10	5.12	10.47	–	7.88
9d	4-OCH_3	–	46	205–207	$C_{25}H_{19}N_3SO$	73.33	4.68	10.26	–	7.83
					(409.51)	73.41	4.59	10.39	–	7.59
10a	H	4-OCH_3Ph	87	214–216	$C_{31}H_{24}N_4O$	79.47	5.16	11.96	–	–
					(468.56)	79.17	5.51	12.24	–	–
10d	4-OCH_3	2-CH_3Ph	64	225–227	$C_{32}H_{26}N_4O$	79.64	5.43	11.61	–	–
					(482.59)	79.24	5.62	11.40	–	–

4 h, cooled, poured on ice-water to yield compounds **8a-d** as precipitates, which were recrystallized from ethanol.

*Synthesis of 7-aryl-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(*3H*)-thione (**9b-d**)*

A mixture of 4-chloropyrrolopyrimidines **8b**, **8c** (3.95 g, 0.01 mol) or **8d** (4.11 g, 0.01 mol) and thiourea (1.5 g, 0.02 mol) was refluxed in dry ethanol (30 mL) for 4 h. The solvent was removed under reduced pressure and the residue was recrystallized from methanol to give compounds **9b-d**.

Table II. Spectral data of the newly prepared compounds ***1-10***

Compd. No.	Mass *m*/z (% abundance)	IR (cm^{-1})	^1H NMR (ppm)
1a	335 [M^+] (11.9 %)	3566, 3641 (NH_2) 2202 (CN)	5.04 (br.s, 2H, NH_2, D_2O exchangeable), 7.04–7.67 (m, 15H, Ar-H)
1b	349 [M^+] (100 %), 350 [M^++1] (34.8 %)	3438, 3315 (NH_2) 2209 (CN)	2.3 (s, 3H, CH_3), 6.07 (br.s, 2H, NH_2, D_2O exchangeable), 7.04–8.03 (m, 14H, Ar-H)
1c	349 [M^+] (100 %), 350 [M^++1] (29.7 %)	3441, 3315 (NH_2) 2210 (CN)	2.26 (s, 3H, CH_3), 5.09 (br.s, 2H, NH_2, D_2O exchangeable), 6.5–7.9 (m, 14H, Ar-H)
1d	365 [M^+] (5.6 %)	3526, 3659 (NH_2) 2205 (CN) 1509 (C–O)	3.79 (s, 3H, OCH_3), 5.1 (br.s, 2H, NH_2, D_2O exchangeable), 6.6–7.8 (m, 14H, Ar-H)
2a	353 [M^+] (41 %)	3350 (N-H) 1720 (C=O)	5.0 (s, 2H, NH_2, D_2O exchangeable), 7.0–7.8 (m, 15H, Ar-H), 8.55 (s, 2H, CO-NH_2, D_2O exchangeable)
2b	367 [M^+] (27 %)	3347 (N-H) 1712 (C=O)	2.26 (s, 3H, Ph-CH_3), 5.09 (s, 2H, NH_2, D_2O exchangeable), 6.5–7.9 (m, 14H, Ar-H), 8.5 (s, 2H, CO-NH_2, D_2O exchangeable)
2c	367 [M^+] (36 %)	3410 (N-H) 1725 (C=O)	2.3 (s, 3H, Ph-CH_3), 6.0 (s, 2H, NH_2, D_2O exchangeable), 7.0–7.5 (m, 14H, Ar-H), 8.03 (s, 2H, CO-NH_2, D_2O exchangeable)
2d	383 [M^+] (17 %)	3392 (N-H) 1703 (C=O) 1212 (C-O)	2.25 (s, 3H, Ph-OCH_3), 7.0–8.1 (m, 14H, Ar-H), 4.8 (s, 2H, NH_2, D_2O exchangeable), 8.7 (s, 2H, CO-NH_2, D_2O exchangeable)
3a	377 [M^+] (6.5 %)	3410 (NH) 2225 (CN) 1712 (C=O)	2.22 (s, 3H, CH_3- C=O), 6.9–8.0 (m, 15H, Ar-H), 8.9 (s, 1H, NH, D_2O exchangeable)
3b	391 [M^+](5.6 %)	2223 (CN) 3423 (NH) 1717 (C=O)	2.06 (s, 3H, CH_3-C=O), 2.27 (s, 3H, Ph-CH_3), 6.9–7.9 (m, 14H, Ar-H), 8.1 (s, 1H, NH, D_2O exchangeable)
3c	391 [M^+] (6.8 %) 349 [M^+–Ac] (100 %)	3423 (NH) 2223 (CN) 1717 (C=O)	2.12 (s, 3H, CH_3-C=O), 2.55 (s, 3H, Ph-CH_3), 6.9–7.9 (m, 14H, Ar-H), 8.3 (s, 1H, NH, D_2O exchangeable)
3d	407 [M^+] (33.9 %), 365 [M^+–Ac] (100 %)	3373 (NH) 2137 (CN) 1510 (C–O) 1709 (C=O) 1709 (C=O)	2.1 (s, 3H, CH_3-C=O), 3.75 (s, 3H, CH_3-O), 6.8–7.9 (m, 14H, Ar-H), 8.1 (s, 1H, NH, D_2O exchangeable)
4c	382 [M^+] (43 %)	3323 broad (O-H)	2.34 (s, 3H, CH_3-Ph), 4.2 (s, 1H, OH, D_2O exchangeable), 5.0 (br.s, 2H, NH_2, D_2O exchangeable), 6.5–7.4 (m, 14H, Ar-H), 7.9 (s, 1H, NH, D_2O exchangeable), 8.1 (s, 1H, NH, D_2O exchangeable)
4d	398 [M^+] (36.4 %)	3423 broad (O-H) 1446 (C-O)	3.8 (s, 3H, OCH_3), 5.2 (s, 1H, OH, D_2O exchangeable), 5.9 (br.s, 2H, NH_2, D_2O exchangeable), 6.7–8.1 (m, 14H, Ar-H), 8.8 (s, 1H, NH, D_2O exchangeable), 8.9 (s, 1H, NH, D_2O exchangeable)

5a	378 [M^+] (76 %)	3371 (N-H) 3435, 3449 (NH_2)	5.0 (s, 2H, NH_2, D_2O exchangeable), 7.0–7.6 (m, 15H, Ar-H), 8.5 (s, 1H, NH)
5b	392 [M^+] (28.9 %)	3365 (N-H) 3451, 3459 (NH_2)	2.12 (s, 3H, CH_3), 5.2 (s, 2H, NH_2,D_2O exchangeable), 7.0–7.65 (m, 14H, Ar-H), 8.32 (s, 1H, NH, D_2O exchangeable)
5d	408 [M+] (60 %)	3382 (N-H) 3526, 3445 (NH_2) 1510(C-O)	3.76 (s, 3H, OCH_3), 5.2 (s, 2H, NH_2, D_2O exchangeable), 6.7–7.8 (m, 14H, Ar-H), 8.36 (s, 1H, NH, D_2O exchangeable)
6a	361 [M^+] (45.7 %)	3430, 3330 (NH_2)	5.1 (br.s, 2H, NH_2, D_2O exchangeable), 7.1–7.6 (m, 15H, Ar-H), 8.34 (s, 1H, C-2 H)
6b	376 [M^+] (30 %)	3447, 3331 (NH_2)	2.54 (s, 3H, CH_3), 5.12 (br.s, 2H, NH_2, D_2O exchangeable), 7.0–7.9 (m, 14H, Ar-H), 8.3 (s, 1H, C-2 H)
6c	376 [M^+] (11.5 %)	3415, 3327 (NH_2)	2.31 (s, 3H, CH_3), 5.0 (br.s, 2H, NH_2, D_2O exchangeable), 7.0–7.5 (m, 14H, Ar-H), 8.34 (s, 1H, C_2-H)
6d	392 [M^+] (74.9 %)	3442, 3346 (NH_2)	3.82 (s, 3H, OCH_3), 5.29 (br.s, 2H, NH_2, D_2O exchangeable), 6.7–8.1 (m, 14H, Ar-H), 8.8 (s, 1H, C-2 H)
7a	363 [M^+] (46.3 %)	3227 (NH) 1698 (C=O) 1558 (C=N)	6.9–7.9 (m, 15H, Ar-H), 8.02 (s, 1H, C-2 H), 8.2 (d, 1H, NH, D_2O exchangeable)
7b	377 [M^+] (100 %) 378 [M^++1] (22.7 %)	3328 (NH) 1686 (C=O) 1572(C=N)	2.1 (s, 3H, CH_3), 7.2–8.0 (m, 14H, Ar-H), 8.6 (d, 1H, NH, D_2O exchangeable), 8.7 (s, 1H, C-2 H)
7c	377 [M^+] (21 %)	3362 (NH) 1679 (C=O) 1598 (C=N)	2.32 (s, 3H, CH_3), 6.9–7.67 (m, 14H, Ar-H), 8.02 (d, 1H, NH, D_2O exchangeable), 8.3 (s, 1H, C-2 H)
7d	393 [M^+] (12.9 %)	3130 (NH) 1682 (C=O) 1587 (C=N) 1510 (C-O)	3.76 (s, 3H, OCH_3), 6.6–7.9 (m, 14H, Ar-H), 8.1 (s, 1H, C-2 H), 8.22 (s, 1H, NH, D_2O exchangeable)
8a	381 [M^+] (20 %) 383 [M+2] (8 %)	3076, 2833 (CH) 1615 (C=C) 1585 (C=N)	6.8–7.9 (m, 15H, Ar-H), 8.7 (s, 1H, C-2 H)
8b	395 [M^+] (91 %) 397 [M+2] (28)	3064, 2846 (CH) 1619 (C=C) 1571 (C=N)	2.1 (s, 3H,CH_3), 7.2–8.0 (m, 14H, Ar-H), 8.6 (s, 1H, C-2 H)
8c	395 [M^+] (20 %) 397 [M+2] (5.4 %)	3056, 2857 (CH) 1623 (C=C) 1569 (C=N)	2.3 (s, 3H,CH_3), 7.1–7.55 (m, 14H, Ar-H), 7.9 (s, 1H, C-2 H)
8d	411 [M^+] (40 %) 413 [M+2] (14.5 %)	3045, 2926 (CH) 1629 (C=C) 1605 (C=N) 1226 (C-O)	3.65 (s, 3H, OCH_3), 7.1–7.9 (m, 14H, Ar-H), 8.6 (s, 1H, C-2 H)
9b	393 [M^+] (11.4 %)	3362 (N-H) 1598 (C=N) 3012, 2980 (C-H)	2.26 (s, 3H, CH_3), 6.9–7.7 (m, 14H, Ar-H), 8.0 (s, 1H, C-2 H), 8.1 (d, 1H, NH, D_2O exchangeable)

9c	393 [M^+] (18.7 %)	1560 (C=N) 3357 (N-H) 3023, 2977 (C-H)	2.05 (s, 3H, CH_3), 6.8–7.7 (m, 14H, Ar-H), 8.4 (s, 1H, C-2 H), 8.8 (d, 1H, NH, D_2O exchangeable)
9d	409 [M^+] (6.5 %)	3368 (N-H) 1586 (C=N) 3025, 2983 (C-H)	3.7 (s, 3H, OCH_3), 6.8–7.8 (m, 14H, Ar-H), 8.0 (s, 1H, C-2 H), 9.5 (d, 1H, NH, D_2O exchangeable)
10a	468 [M^+] (46.7 %)	1554 (C=N) 3347 (N-H) 1221 (C-O)	3.77 (s, 3H, OCH_3), 6.5–7.5 (m, 19H, Ar-H), 8.4 (s, 1H, C-2 H), 8.6 (d, 1H, NH, D_2O exchangeable)
10d	482 [M^+] (21 %)	1567 (C=N) 3352 (N-H) 1217 (C-O)	2.5 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 6.9–7.6 (m, 18H, Ar-H), 8.0 (s, 1H, C-2 H), 8.9 (d, 1H, NH, D_2O exchangeable)

Synthesis of N-*(7-aryl-5,6-diphenyl-7*H-*pyrrolo[2,3-*d*]pyrimidine)*-N-*aryl amines (**10a,d**)*

A mixture of the appropriate 4-chloropyrrolopyrimidine, **8a** (3.81 g, 0.01 mol) or **8d** (4.11 g, 0.01 mol), aryl amine [*p*-anisidine (1.23 g, 0.01 mol) or *m*-toluidine (1.07 g, 0.01) and a few drops of triethylamine was refluxed in absolute ethanol for 8 h. The solvent was removed under reduced pressure and the residues were recrystallized from methanol to give **10a,d**.

Antimicrobial evaluation

Antimicrobial activity of the synthesized compounds in comparison with that of some control drugs [amoxicilin (Bioanalyse, Turkey) and fluconazol (Sigma-Aldrich, USA)] was evaluated against Gram positive (*Staphylococcus aureus*, ATCC 29213, *Bacillus subtilis*, ATCC 6633), Gram-negative (*Escherichia coli*, ATCC 25922, *Pseudomonas aeruginosa*, ATCC 278533) bacteria and fungus *(Candida albicans*, ATCC 10231). All microorganisms used were obtained from the culture collection of the Department of Microbiology and Immunology, Faculty of Pharmacy, Helwan University, Cairo, Egypt. The microorganisms were passaged at least twice to ensure purity and viability. The lowest concentration of the compound that completely inhibited macroscopic growth was determined and minimum inhibitory concentrations (*MIC*) was reported. *MIC* for each tested compound was determined on Mueller-Hinton agar (MHA) by the microdilution technique according to NCCLS guidelines 1997 (15).

All bacterial isolates were subcultured in MHA plates and incubated overnight at 37 °C and *Candida* isolates were subcultured in Sabouraud-dextrose agar (SDA) plates at 35 °C for 24–48 h.

Solution of the newly synthesized compounds and standard drugs were prepared at concentrations of 1024, 512, 256, 128, 64, 32 and 16 μg mL^{-1} using serial two-folds dilutions in DMSO. Each concentration was mixed with sterile nutrient agar (Sigma-Aldrich, USA) in a sterile plate and bacteria inoculum was added to each well of the microdilution tray. The trays were incubated at 37 °C in a humid chamber and *MIC* endpoints were read after 24 h of incubation.

DMSO (80 %, Sigma-Aldrich), pure microorganisms, and pure media were used as controls.

Testing the synergistic activity. – To test the synergistic activity of active tested compounds, a mixture of two compounds (1:1 ratio) was prepared in the concentrations of 1024, 512, 256, 128, 64, 32 and 16 μg mL^{-1}, and was tested for by the same method as mentioned before for their antimicrobial activity against the same bacterial and fungal strains (Table III).

*Table III. Antimicrobial activity (*MIC *μg mL^{-1}) of newly synthesized compounds and some mixtures of the synthesized compounds*

Compd. No.	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*
1b	–	–	32	–	32
1c	64	256	128	–	128
1d	256	–	–	–	256
2b	–	–	64	–	32
3a	–	–	–	–	–
3b	–	–	128	–	32
3c	64	–	128	–	64
3d	–	–	128	–	64
4c	128	–	–	–	–
5d	–	–	64	–	–
6a	–	–	–	64	–
6b	32	256	–	–	128
6c	128	128	–	–	128
7a	128	–	256	–	128
7c	256	–	–	–	256
7d	64	–	–	–	32
9b	–	–	–	–	32
10a	–	–	–	–	32
1c+3c	32	–	64	–	64
6c+7a	64	–	512	–	64
3b+3d	–	–	128	–	64
1b+7d	128	–	64	–	32
1c+6b	64	–	256	–	128
9b+10a	–	–	–	–	32
Amoxicillin	64	16	256	64	–
Fluconazol	–	–	–	–	512

RESULTS AND DISCUSSION

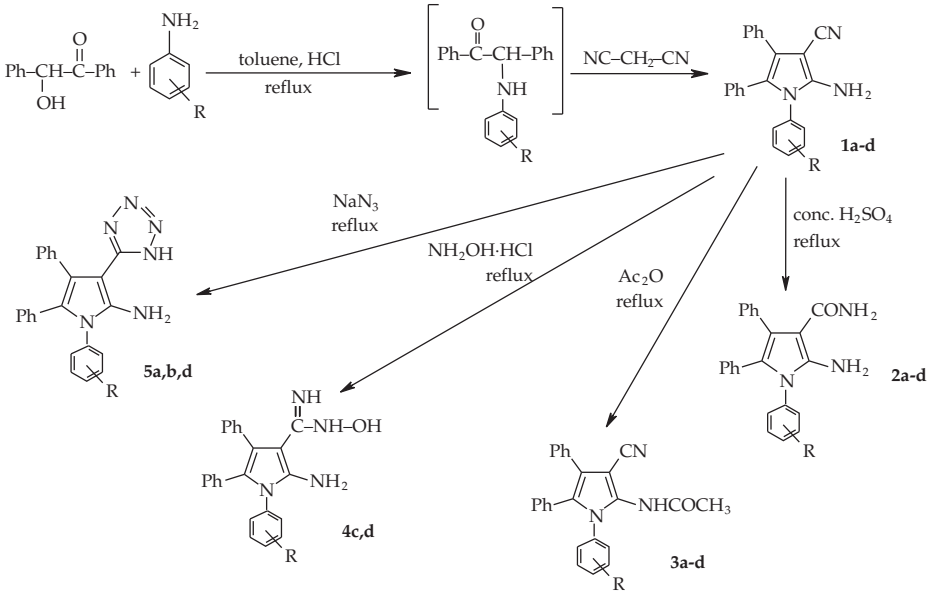
Chemistry

Condensation of benzoin with primary aromatic amines in refluxing toluene resulted in the formation of α-aminoketone intermediates, which were condensed, without isolation, with malononitrile to yield the various 2-amino-4,5-diphenylpyrrole-3-carbonitriles (**1a-d**). Pyrroles **1a-d** reacted with concentrated sulphuric acid to yield 2-amino--3-carbamoyl-4,5-diphenylpyrroles (**2a-d**). Acetylated pyrrole-3-carbonitriles (**3a-d**) were obtained by the reaction of **1a-d** with acetic anhydride.

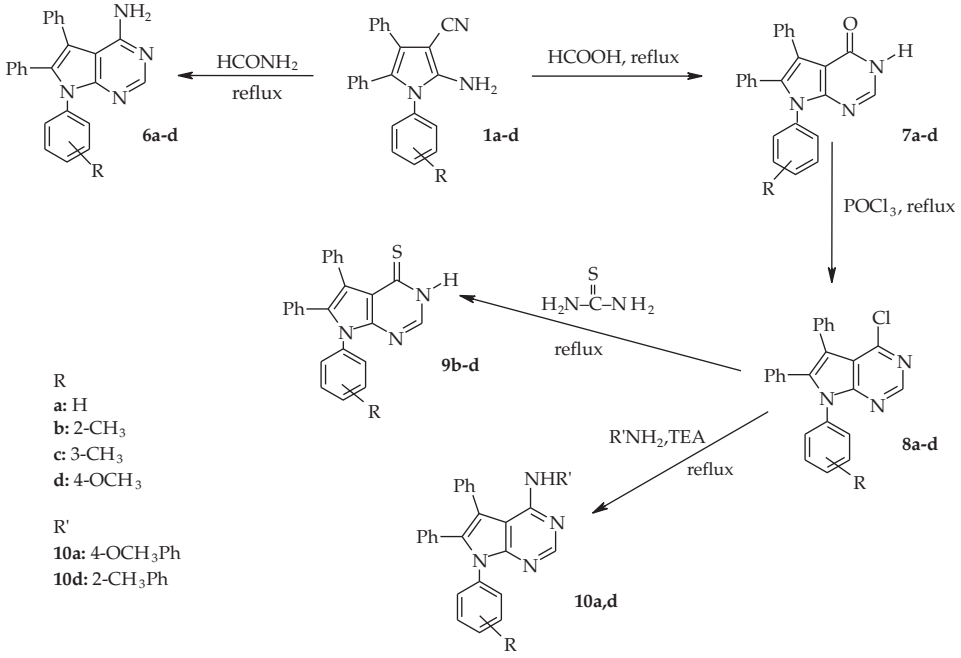
Preparation of 3-(2-amino-4,5-diphenylpyrrole)amidoximes (**4c,d**) was accomplished by the reaction of **1c,d** with hydroxylaminehydrochloride in absolute ethanol using sodium carbonate. On the other hand, synthesis of 2-amino-4,5-diphenyl-3-tetrazolo-pyrroles (**5a,b,d**) was achieved by the reaction of **1a,b,d** with sodium azide in dimethyl formamide using ammonium chloride (Scheme 1).

On the other hand, pyrrolopyrimidines **6a-d** and **7a-d** were obtained by the reaction of **1a-d** with formamide and formic acid, respectively; 4-chloro-pyrrolo-pyrimidines **8a-d** were prepared by the reaction of **7a-d** with phosphorus oxychloride as previously described for the preparation of analogous compounds (14, 16–19).

The pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-thiones **9b-d** were obtained by the reaction of **8b-d** with thiourea in absolute ethanol. Finally, *N*-(pyrrolo[2,3-*d*]pyrimidines)-*N*-aryl amines **10a,d** were obtained from compounds **8a,d** by the previously described method for the preparation of analogous compounds (16) (Scheme 2).



Scheme 1



Scheme 2

Structures of the synthesized compounds were confirmed by microanalytical and spectral data (Tables I and II).

Antimicrobial activity

The antimicrobial activity of some newly synthesized compounds revealed that compounds **1c, 3c**, and 7**d** were similar in their activity to amoxicillin against *Bacillus subtilis* (*MIC* 64 μg mL^{-1}), while compound **6b** was two times more active than amoxicillin against the same organism (Table III). On the other hand, compound **6c** showed lower activity than amoxicillin against *Staphylococcus aureus* (*MIC* 128 *vs*. 16 μg mL^{-1}).

Furthermore, determination of antibacterial activity against *Escherichia coli* indicated that compounds **2b** and **5d** were four times as active as amoxicillin (*MIC* 64 μg mL^{-1}), while compound **1b** was even more active against *Escherichia coli* (*MIC* 32 μg mL^{-1}). Compound **6a** exhibited significant activity against *Pseudomonas aeruginosa*, aquivalent to that of amoxicillin (20).

The results indicated that compounds **1b, 2b, 3b, 7d, 9b** and **10a** were more active against *Candida albicans* (*MIC* 32 μg mL^{-1}) when compared with fluconazol (*MIC* 512 μg mL^{-1}). Compounds **3c** and **3d** were also highly active (*MIC* 64 μg mL^{-1}) and compounds **1c**, **6b**, **6c**, and **7a** were four times as active as fluconazol (*MIC* 128 μg mL^{-1}) whereas

compounds **1d** and **7c** were two times more active than fluconazol (*MIC* 256 μg mL^{-1}). The other compounds did not show significant antibacterial/antimycotic activity.

From the above results, we can see that 2-amino-1,4,5-triphenyl-pyrrole-3-carbonitrile (**1a**) is totally inactive against Gram-positive, Gram-negative bacteria and fungi. On replacing phenyl group at position 1 with 2-methylphenyl in compound **1b**, it acquired high antimicrobial activity against *Escherichia coli* and *Candida albicans*, and on replacing the phenyl group with 3-methylphenyl (in **1c**), it acquired mild antimicrobial activity against *Escherichia coli, Bacillus subtilis* and *Candida albicans*. Replacing this phenyl group by 4-methoxyphenyl and cyano group in position 3 with tetrazole ring (in **5d**) led to high antibacterial activity against *Escherichia coli.*

On the other hand, on converting pyrrole **1a** to 4-amino-pyrrolo[2,3-*d*]pyrimidine (**6a**), it acquired significant activity against *Pseudomonas aeruginosa* pyrrolo[2,3-*d*]pyrimidine-4-one (**7a**) showed mild activity against *Bacillus subtilis* and *Candida albicans.* It was found that pyrrolo[2,3-*d*]pyrimidine-4-thione (**9b**) acquired high antifungal activity against *Candida albicans*. When the slightly active pyrrole derivative **1d** was converted to pyrrolopyrimidine-4-one (**7d**), it acquired significant antimicrobial activity against *Bacillus subtilis* and *Candida albicans.* On replacing the ketonic oxygen atom of **7a** by arylamine in **10a**, it lost its activity against *Bacillus subtilis* but was still active against *Candida albicans.*

The synergistic test revealed that the mixture of compounds **1c** and **3c** acquired higher activity against *Bacillus subtilis* (*MIC* 32 μg mL^{-1}), *Escherichia coli* (*MIC* 64 μg mL^{-1}) and *Candida albicans* (*MIC* 64 μg mL^{-1}) than that of individual compounds (Table III).

The mixture of compounds **6c** and **7a** acquired higher activity against *Bacillus subtilis* (*MIC* 64 μg mL^{-1}) and *Candida albicans* (*MIC* 64 μg mL^{-1}) than that of individual compounds.

The mixtures of compounds **3b** and **3d**, **1b** and **7d**, **1c** and **6b**, and **9b** and **10a** showed similar activities as those of individual compounds (Table III).

CONCLUSIONS

Evaluation of the novel compounds established that some of the synthesized compounds, namely, [2-amino-1-(2-methylphenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitriles (**1b**), 2-amino-3-carbamoyl-1-(3-methylphenyl)-4,5-diphenyl-1*H*-pyrroles (**2b**), *N*-(3-cyano-1-(2-methylphenyl)-4,5-diphenyl-1*H*-pyrrol-2-yl)-acetamide (**3b**), *N*-(3-cyano-1-(3-methylphenyl)-4,5-diphenyl-1*H*-pyrrol-2-yl)-acetamide (**3c**), 2-amino-1-(4-methoxyphenyl)--4,5-diphenyl-3-tetrazolo-1*H*-pyrrole (**5d**), 7-(4-methoxyphenyl)-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4(3*H*)-one (**7d**)] showed improved antimicrobial activity compared to amoxicillin, while the other compounds, 7-(3-methylphenyl)-5,6-diphenyl-7*H*-pyrrolo [2,3-*d*]pyrimidin-4(3*H*)-thione (**9b**) and *N*-(7-(2-methylphenyl)-5,6-diphenyl-7*H*-pyrrolo [2,3-*d*]pyrimidine)-*N*-aryl amine (**10a**), were highly active antimycotic agents compared to fluconazole.

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S A Ž E T A K

Sinteza derivata pirola kao antimikrobnih tvari

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U radu je opisana sinteza novih derivata pirola i pirolo[2,3-d]pirimidina s poboljšanim antimikrobnim djelovanjem. Reakcijom benzoina, primarnih aromatskih amina i malononitrila sintetizirana je serija novih 2-aminopirol-3-karbonitrila (**1a-d**), iz kojih su potom pripravljeni derivati pirola (**2a-d** do **5a-d**) i pirolo[2,3-d]pirimidina (**6a-d** do **10a,d**). Sintetiziranim spojevima ispitano je *in vitro* antimikrobno djelovanje na različite Gram-pozitivne, Gram-negativne bakterije i gljivice. Neki od sintetiziranih spojeva [2-amino-1-(2-metilfenil)-4,5-difenil-1*H*-pirol-3-karbonitril (**1b**), 2-amino-3-karbamoil-1-(3-metilfenil)-4,5-difenil-1*H*-pirol (**2b**), *N*-(3-cijano-1-(2-metilfenil)-4,5-difenil-1*H*-pirol-2-il)-acetamid (**3b**), *N*-(3-cijano-1-(3-metilfenil)-4,5-difenil-1*H*-pirol-2-il)-acetamid (**3c**), 2-amino-1-(4-metoksifenil)-4,5-difenil-3-tetrazolo-1*H*-pirol (**5d**), 7-(4-metoksifenil)-5,6-difenil-7*H*-pirolo[2,3-d]pirimidin-4(3*H*)-on (**7d**), 7(3-metilfenil)-5,6-difenil-7*H*-pirolo[2,3-d]pirimidin-4(3*H*)-tion (**9b**) i *N*-(7-(2-metilfenil)-5,6-difenil-7*H*-pirolo[2,3-d]pirimidin)-*N*-aril amin (**10a**)] imaju snažno antimikrobno djelovanje.

Ključne riječi: pirol, pirolopirimidin, antimikrobno djelovanje

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