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Synthesis and Biological Evaluation of Some Novel Substituted Pyrrolizines and Pyrimidopyrrolizines as Chemotherapeutic Agents

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ABSTRACT

A novel series of 3-ethyl ester substituted pyrrolizines (4, 5, 8-11, 14a,b, 15-19c), 3-oxadiazinyl substituted pyrolizines (21-23b, 25-27b), 3-carboxilic acid substituted pyrrolizine (7) and pyrimidopyrrolizines (6, 12, 14a,b, 24a,b, 28a,b) were synthesized. Their chemical structures were confirmed by spectral and elemental analysis. Ten compounds of these new derivatives showed activity against MCF7 cancer cell line with IC50 value less than 60 µM. Moreover, compound ethyl 2-{[aminomethylene]amino}-1-cyano -6,7-dihydro-5H-pyrrolizine-3-carboxylate (11) that was prepared by facile chemical reaction of the ethyl ester start 3 with formamide, showed activity on candida albicans equipotent with that of clotrimazole.

Keywords: Pyrrolizine, Pyrimidopyrrolizine, Oxadiazine, Ethyl ester, Antitumor, Antimicrobial, Antibacterial, Antifungal

INTRODUCTION

The pyrrolizine derivatives constitute an important group of heterocyclic compounds. Pyrrolizidine skeleton is a naturally occurring system; it was separated from a number of plants [1] in the form of alkaloids [2-4]. Pyrrolizine derivatives which are not naturally occurring have attracted considerable attention in medicinal chemistry as well. They have several pharmacological activities as antineoplastic activity [5-7], anti-inflammatory [8-10], antiviral [11,12], CNS activity [13-17] and immunomodulator activity [18].

Mitomycin-C, **Figure 1(I)**, is naturally occurring and has a pyrrolizine scaffold in its structure. It is an antibiotic and anticancer drug at the same time, it is used for treatment of esophageal carcinoma, anal cancers, breast cancers and superficial bladder tumors. Mitomycin-C 0.02% can be applied topically in eye surgery in order to prevent scarring during glaucoma [19,20]. It also has strong bactericidal action against both gram-negative and gram-positive organisms [21]. 5-thienyl 2,3-dihydro-1H-pyrrolizine derivatives **Scheme 1 (II)** showed antitumor activity comparable with that of mitomycin [22]. Also compound N-(3-Benzoyl-1-cyano-6,7-dihydro-5H-pyrrolizin-2-yl)-2-thiomorpholin-4-yl-acetamide **Figure 1 (III)** was reported to have a broad spectrum anticancer activity against breast (MCF7), colon (HCT116) and liver (HEPG2) cancer cell lines [23]. In addition, clazamycins A and B are pyrrolizidines with antitumor and antibiotic activity [24]. Moreover, Pyrimido (4,5-b) pyrrolizine derivative **Figure 1 (IV)** showed neoplasm inhibiting activity [25] and compound **Figure 1 (V)** showed significant antitumor activity in vivo [26].

Based on these findings that proved the importance of this heterocyclic scaffold, we designed and synthesized new series based on pyrrolizine as the main skeleton and with different substituents at position 1, 2 and 3, the synthesized new derivatives general formula A, B and C are represented in **Figure 1**. Exploration of the biological activity on MCF7 cancer cell line and also the antimicrobial activity were discussed in this study.

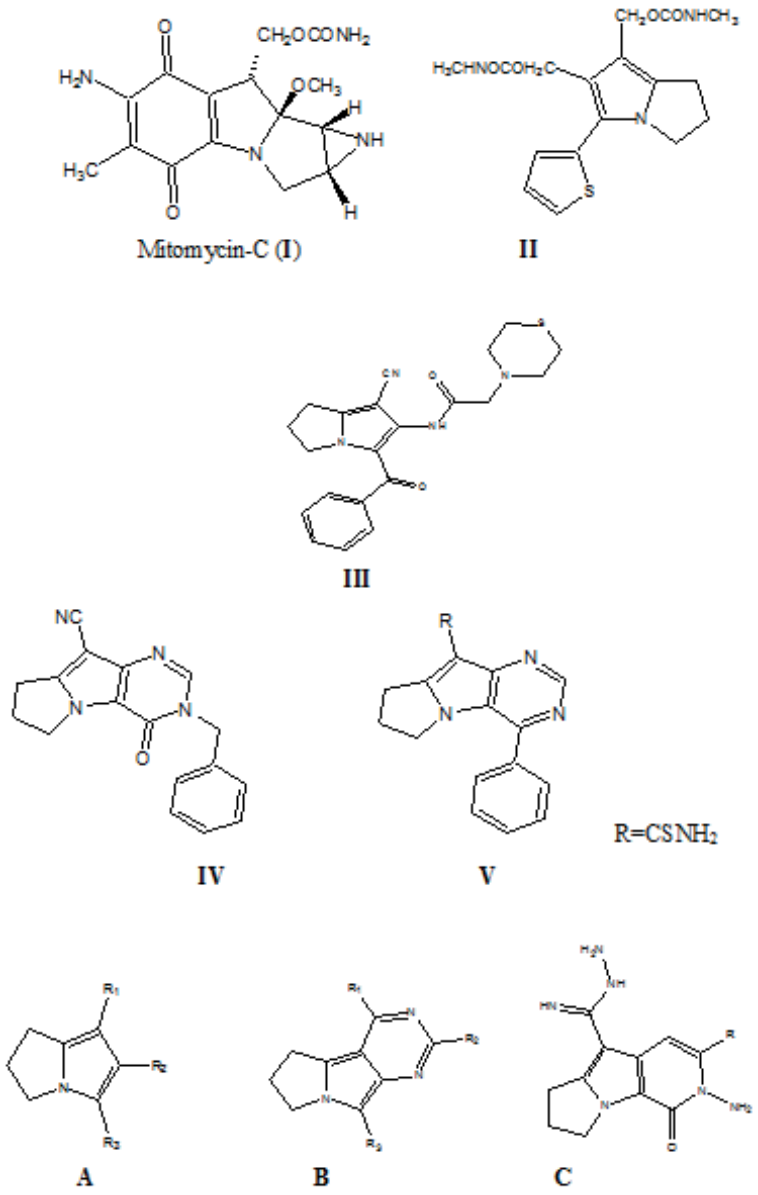


Figure 1: Reported (I-V) and designed pyrrolizines (A-C).

MATERIALS AND METHODS

Chemistry

Melting points are uncorrected and were determined by open capillary tube method using Electrothermal IA9100MK-digital melting point apparatus. Elemental microanalyses were performed at the micro analytical center, Faculty of Science, Cairo University. The infrared (IR) spectra were recorded on Schimadzu-435 IR and Bruker FT-IR spectrophotometers and expressed in wave number (cm^{-1}) using potassium bromide disc. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a varian mercury spectrophotometer at 300 MHZ using tetramethylsilane (TMS) as internal reference. Chemical shift values were given in parts per million (ppm). Mass spectra were performed on Fennigan MAT, SSQ 7000 mass spectrophotometer at 70 eV. IUPAC chemical nomenclature were assigned using ACD/1-Labs program, version 8 (1995). Pyrrolidin-2-ylidenemalononitrile **(1)** was prepared according to reported procedure from 2-pyrrolidinone, its m.p. is 159-61° (reported 158-9°) [27,28]. Compound 3 also have been previously reported [25].

Ethyl [2-(dicyano methylene) pyrrolidin-1-yl] acetate (2)

A mixture of compound 1 (1 g, 7.5 mmol), powdered anhydrous potassium carbonate (2.1 g, 15 mmol) and ethylchloroacetate (0.92 g.,7.5 mmol.) in dry acetone (30 ml) was stirred under reflux for 8 hours and filtered. The filtrate was concentrated and set aside to cool; the formed white crystals were collected, dried, and recrystallized from ethanol. Yield (72%), m.p. 87-89°C. IR(cm^{-1}): 2986 (CH_2, CH_3), 2211, 2190 (2CN), 1750 (CO).

Ethyl 2-amino-1-cyano-6,7-dihydro-5H-pyrrolizine-3-carboxylate (3)

Compound **2** (1g, 4.5mmol) was treated with 1% sodium ethoxide solution (0.3 g sodium metal in 30 ml absolute ethanol) at room temperature. The obtained crystals were collected, dried and washed with ethanol, yield (90%), m.p. 237-239°C (reported 239-41°C) I R (Cm^{-1}): 3431,3338 (NH_2), 2982 (CH_2,CH_3), 2210 (CN), 1668 (CO).

Ethyl 2- (acetylamino)-1-cyano-6,7-dihydro-5H-pyrrolizine-3-carboxylate (4)

A mixture of compound **3** (1 g, 4.5 mmol) and acetyl chloride (0.7 g, 9 mmol) in dioxan (15 ml) was stirred for 24 hours at room temperature. The formed white crystals were filtered, dried and recrystallized from acetone. Yield (83%), m.p. 137-139. I R (Cm^{-1}): 3268 (NH), 2228 (CN), 1697 ($COOC_2H_5$), 1668 (CONH). ^{1}H-NMR (DMSO-d_6): δ = 1.24 (q, 3H, CH_2CH_3), 2.01 (s, 3H, $COCH_3$), 2.49 (m, 2H, C6), 2.94 (t, 2H C7), 4.19 (m, 4H,C5, CH_2CH_3), 9.56 (s,1H, NH). Microanalysis (%): Calculated for $C_{13}H_{15}N_3O_3$ (261.28) Cal. C 59.75, H 5.78 N 16.08 Found, C 59.76 H 5.94 N 15.93.

Ethyl 2-(benzoylamino)-1-cyano-6,7-dihydro-5H-pyrrolizine-3-carboxylate (5)

A mixture of compound **3** (1 g, 4.5 mmol) and benzoyl chloride (1.26g, 9 mmol) in dioxin, was refluxed for 3 hours, then cooled to room temperature. The formed crystals were filtered, dried and recrystallized from ethanol. Yield (78%), m.p. 193-195. I R (Cm^{-1}): 3295 (NH), 2221 (CN), 1662 (2CO). MS: m/z (%), M^+ 323 (43); 105 (100). Microanalysis (%): Calculated for $C_{18}H_{17}N_3O_3$ (323.35), Calc, C 66.86 H 5.29 N 12.99, Found, C 67.01H 5.11 N 12.89.

Ethyl 3- methyl-1-oxo-2,7,8,9- tetrahydro-1H- pyrimido[5,4-a]pyrrolizine-5-carboxylate (6)

A solution of compound **4** (1 g) in 0.5% sodium ethoxide was refluxed for 2 hours, the formed crystals were filtered, dried and recrystallized from methanol. Yield (70%), m.p. 316-318°C. IR (Cm^{-1}): 3146 (NH), 2969, 2927 (CH aliphatic), 1672 ($COOC_2H_5$), 1615 (CONH). MS: m/z (%), M^+ 261 (70) ; 189 (100). ^{1}H-NMR($DMSOd_6$), 1.25 (t, 3H, CH_2CH_3), 2.25 (s, 3H, CH_3), 2.49 (m, 2H, C8), 3.05 (t, 2H, C9), 4.23 (q, 2H, CH_2CH_3), 4.37 (t, 2H, C7),11.45(s,1H,NH). Microanalysis (%): Calculated for $C_{13}H_{15}N_3O_3$; (261.28), C 59.76 H 5.78 N 16.08, Found C 59.58 H 5.37 N 16.20.

2- (Benzoylamino)-1-cyano-6,7-dihydro-5H-pyrrolizine-3-carboxylic acid (7)

Compound **5** (0.5 g, 3.38 mmol) in 0.5% sodium ethoxide solution was refluxed for 2 hours, the formed product was collected and dissolved in water (30 ml), the solution was neutralized with dilute HCl. The produced precipitate was filtered, dried and recrystallized from acetone. Yield (46%), m.p. 199-201°C. IR (Cm^{-1}): 3340 (NH), 2800-3500 (OH stretching), 2238 (CN), 1662(COOH,CONH). MS: m/z (%), M^+ 295 (3); 106 (100). Microanalysis (%): Calculated for $C_{16}H_{13}N_3O_3$. 1.5 H_2O (322), C 59.62 H 5.00 N 13.03, Found C 59.43 H 5.37 N 12.55.

Ethyl 2-[chloroacetamido]-1-cyano-6,7-dihydro-5H-pyrrolizine-3-carboxylate (8)

A mixture of compound **3** (1 g, 4.5 mmol.) and chloroacetyl chloride (1 g, 9 mmol.) in dioxan was stirred at room temperature for 24 hours, the formed white precipitate was filtered, dried and recrystallized from acetone. Yield (89%), m.p. 176-178°C IR (Cm^{-1}) : 3240 (NH), 2982,2961 (CH aliphatic), 2225 (CN), 1701 ($COOC_2H_5$), 1683 (CONH), 769 (Cl). ^{1}H-NMR (DMSO-d_6); δ=1.25 (t,3H,CH_2CH_3), 2.47 (m,2H,C6), 2.98 (t,2H,C7), 4.21(m,4H,C5,CH_2CH_3), 4.32(s,2H,CH_2Cl), 9.99(s,1H,NH). Microanalysis (%): Calc. for $C_{13}H_{14}CLN_3O_3$ (295.73), C 52.79 H 4.77 N 14.20, Found C 52.52 H 4.84 N 14.12.

General method for the preparation of compounds 9a-c

A mixture of compound **8** (1g, 3.4mmol) and the secondary amine (8 mmol) in absolute ethanol (20 ml) was refluxed for 8 hours. The reaction mixture was filtered while hot and left to cool; the separated crystals were collected, dried and crystallized from ethanol.

Ethyl 1-cyano-2-[morpholin-yl acetamido]-6,7- dihydro-5H- pyrrolizine-3-carboxylate (9a)

White crystals, yield (68%), m.p. 167-168. IR (cm^{-1}): 3299(NH), 2984, 2918 (CH aliphatic), 2223(CN), 1683(2CO). MS: m/z (%), M^{+1} 347 (2.7); 100 (100). ^{1}H-NMR(DMSO-d_6); δ=1.27(t,3H,CH_2CH_3), 2.49(m,2H,C6), 2.99(t,2H,C7),

3.14(s,2H,$COCH_2N$), 3.67(2t, 8H,$O(CH_2)_4N$), 4.28(m,C5,CH_2CH_3), 10.14(s,1H,NH). Microanalysis(%) cal. For, $C_{17}H_{22}N_4O_4$(346.39), C 58.94, H 6.40 N 16.17 found, C 58.75 H 6.62 N 16.20.

Ethyl 1-cyano-2-[piperidinyl acetamido]-6,7-dihydro-5H-pyrrolizine-3-carboxylate (9b)

White crystals, yield (74%), m.p. 167-169, IR (cm^{-1}): 3227 (NH), 2976, 2940 (CH aliphatic), 2225 (CN), 1703(COO), 1673 (NHCO). MS: = m/z (%) = M^{+1} 347 (2.7); 100 (100). Microanalysis (%) cal. For $C_{18}H_{24}N_4O_3$ (344.41), calc. C 62.77 H 7.02 N 16.26, Found, C 62.30 H 7.36 N 16.15.

Ethyl 1- cyano-2-[piperazin-yl acetamido] -6,7- dihydro-5H-pyrrolizine-3-carboxylate (9c)

White crystals, yield (73%), m.p. 199-200, MS: m/z (%), M^{+1} 347 (2.7) ; 100 (100). Microanalysis(%) cal. For $C_{17}H_{23}N_5O_3$ (345.40), calc. C 59.11 H 6.71 N 20.27, found C 59.12 H 6.41 N 20.18.

Ethyl 1- cyano-2- [(ethoxycarbonyl)amino]-6,7-dihydro-5H-pyrrolizine-3-carboxylate (10)

A mixture of compound **3** (1 g, 4.5 mmol) and excess ethylchloroformate (10 ml) was refluxed for 5 hours, the formed crystals were filtered, dried and recrystallized from acetone. Yield 76 (%), m.p. 143-144°C. IR(cm^{-1}): 3318(NH), 2978, 2936 (CH aliphatic), 2223(CN), 1742 ($COOC_2H_5$), 1668 ($NHCOOC_2H_5$). Microanalysis(%): calculated for $C_{14}H_{17}N_3O_4$(291.31), C 57.72 H 5.88 N 14.42, Found C 57.75 H 5.74 N 14.13.

Ethyl 2-{[aminomethylene]amino}-1-cyano-6,7-dihydro-5H-pyrrolizine-3-carboxylate (11)

A mixture of compound **3** (1 g, 4.5 mmol) and formamide (80%) (30 ml) was refluxed for 20 hours, the reaction mixture was poured over water (50 ml) then cooled. The produced precipitate was filtered, dried, and recrystallized from ethanol. Yield (64%), m.p.245-247°C. I R (cm^{-1}):3502, 3420(NH_2), 2982, 2935 (CH aliphatic), 2220 (CN), 1677(CO). MS: = m/z (%) = M^+ 246 (30) ; 174 (100). ^{1}H-NMR (DMSO-d_6); δ=1.26(t,3H,CH_2CH_3), 2.37(m,2H,C6), 2.85(t,2H,C7), 4.07(t,2H,C5), 4.19(q,2H,CH_2CH_3), 5.75(s,2H,NH_2), 8.09(s,1H,CH-NH_2) Microanalysis(%): Calculated. for $C_{12}H_{14}N_4O_2$ (246.27), Calc. C 58.52 H 5.72 N 22.75, Found C 58.82 H 5.44 N 22.46.

1-Imino-2,7,8,9-tetrahydro-1H-pyrimido[5,4-a]pyrrolizine-5-carboxylic acid (12)

The solution of compound **11** (1g, 4.1mmol) in 0.5% sodium ethoxide (0.15 g in 30 ml absolute ethanol) was refluxed for 3 hours, the formed crystals were collected, dissolved in water then neutralized with dil HCl, the liberated solid was filtered, dried and recrystallized from ethanol/acetone. Yield (56%), m.p. 266-268 °C I R (cm^{-1}) : 3314 (NH_2), 2800-3400 (OH stretching), 1656 (CO). Microanalysis(%): calculated for $C_{10}H_{10}N_4O_2$. (218.22), Calc. C 55.04 H 4.61 N 25.67, Found C 55.01 H 4.14 N 25.38.

Ethyl 1-imino-2-phenyl-3-thioxo-2,3,4,7,8,9-hexahydro-1H-pyrimido[5,4-a]pyrrolizine-5-carboxylate (13)

Compound **3** was refluxed in excess phenylisothiocyanate (10 ml) for 3 hours, the formed crystalline product was filtered, dried and recrystallized from acetone. Yield (69%), m.p. 243-245°C IR(cm^{-1}): 3380, 3258(2NH), 3091(CH,aromatic), 2985, 2927(CH aliphatic), 1678(CO). MS: m/z (%), M^+ 354 (100) ; 354 (100). ^{1}H-NMR(DMSO-d_6); δ=1.29 (t, 3H, CH_2CH_3), 2.48(m, 4H, C6, C7), 4.29 (m,4H,CH_2CH_3,C5), 7.19-7.39 (m,5H,aromatic), 7.42(s,2H,2NH). Microanalysis (%): Calculated for $C_{18}H_{18}N_4O_2S.H_2O$ (372.45), Calc. C 58.04 H 5.41 N 15.04, Found C 58.49 H 5.10 N 15.54

General method for the preparation of 14a,b

A mixture of compound **4** (0.5 g, 1.91 mmol) or 5 (0.5 g, 3.38 mmol) and hydrazine hydrate 98%, (20 ml) was refluxed for 5 hours, then filtered. The formed white crystals were dried and crystallized from methanol.

2-Amino-3-methyl-1-oxo-4,6,7,8-tetrahydro-1H-pyrimido [4,5-b]pyrrolizine-5-carbox imidohydrazide (14a)

Yield (74%), m.p. 286-288°C IR(cm^{-1}): 3365, 3255 (2NH_2), 3205 (2NH) 2966(CH aliphatic), 1686 (C=O). ^{1}H-NMR (DMSO-d_6); δ=2.43(m,2H,C6), 2.56(s,3H,CH_3), 3.05(t,2H,C7), 4.21(t,2H,C5), 5.70(s,2H,HN=C-NH), 7.17(s,2H,HN-NH_2), 7.69(s,2H,N-NH_2). Microanalysis (%): Calculated for $C_{11}H_{15}N_7O$ (261.28) cal, C 50.56 H 5.78 N 37.52, found, C, 50.77 H, 5.94 N, 37.48.

phenyl-1-oxo-4,6,7,8-tetrahydro-1H-pyrimido[4,5-b]pyrrolizine-5-carboximido hydrazide (14b)

Yield (73%), m.p. 253-255°C IR(cm^{-1}): 3451, 3414, 3369, 3339 (2NH_2), 3315, 3186 (2NH), 1653 (C=O). MS: = m/z (%) = M^{+1} 324 (20) ; 175 (100). Microanalysis (%): Calculated for $C_{17}H_{17}N_7O$ (323.35) C 59.43 H 5.29 N 30.32, found, C 59.56 H 5.30 N 30.10.

Ethyl-2-amino-1-(aminocarbonyl)-5,6-dihydro-5H-pyrrolizine-3-carboxylate (15)

A mixture of **3** (10 mmol) and 90% sulfuric acid (10 ml) was stirred for few minutes and left to stand for 48 hours at room temperature. The reaction mixture was cooled, poured over cold and stirred ammonia solution, left overnight in a refrigerator. The product was filtered, washed with water and crystallized from ethanol. Yield (77%), m.p 224-226°C. I R (cm^{-1}): 3473, 3363, 3318, 3260 (NH_2, $CONH_2$), 2982, 2929 (CH aliphatic), 1658 (2CO). ^{1}H-NMR(DMSO-d_6); δ=1.25 (t,3H,CH_2CH_3), 2.34 (m,2H,C6), 2.96 (t,2H,C7), 4.03 (t,2H,C5) 4.19 (q,2H,CH_2CH_3), 5.87 (s,2H,NH_2), 6.51 (s,2H,$CONH_2$). Microanalysis (%) : Calculated. for $C_{11}H_{15}N_3O_3$ (237.26), Calc. C 55.68 H 6.37 N 17.71 Found C 55.51 H 6.62 N 17.42.

Ethyl-2-(acetylamino)-1-(aminocarbonyl)-5,6-dihydro-5H-pyrrolizine-3-carboxylate (16)

A mixture of **15** (10 mmol) and acetyl chloride (0.8 ml, 11 mmol) in dioxan (10 ml),was left to stand overnight, at room temperature. The obtained crystalline product was filtered, washed with water and crystallized from ethanol. Yield (72%), m.p 199- 201°C. I R (cm^{-1}): 3418,3185,(NH_2, NH), 1712 (CO of ester),1653 (2CO of amide). MS: m/z (%), M^+ 279 (7.7); 148 (100). ^{1}H-NMR(DMSO-d_6); δ=1.29 (t, 3H, CH_2CH_3), 2.01 (s, 3H, $COCH_3$), 2.46 (m, 2H,C6), 2.96(t, 2H, C7), 4.17(m, 4H,CH_2CH_3, C5), 9.56 (s,3H,NH,NH_2). Microanalysis (%): Calculated. for $C_{13}H_{17}N_3O_4$ (279.30), C 55.90 H 6.13 N 15.04, Found, C 56.24 H 5.84 N 15.07.

Ethyl-1-(aminocarbonyl)-2-(benzoylamino)-5,6-dihydro-5H-pyrrolizine-3-carboxylate (17)

Compound **15** (2.37 g, 10 mmol) and benzoyl chloride (1.3 ml, 11 mmol) in dioxan (20 ml) were refluxed for 2 hours, the reaction mixture was concentrated by distillation and cooled. The separated crystals were collected, dried and recrystallized from acetone. Yield (78%), m.p 237-239°C. I R (cm^{-1}): 3144, 3044 (NH_2, NH), 1738 (COO),1653 (2CO of amide). Microanalysis (%) : Calculated. for $C_{18}H_{19}N_3O_4$ (341.37), C 63.33 H 5.60 N 12.30, Found, C 63.40 H 5.50 N 12.39.

Ethyl 1-(aminocarbonyl)-2-[(chloroacetyl)amino]-6,7-dihydro-5H-pyrrolizine-3-carboxylate (18)

This compound was prepared by the same method used for the preparation of **8** starting from **3** (0.9g, 3.8 mmol) and chloroacetylchloride (0.9 g, 7.6 mmol). It was crystallized from acetone. Yield (76%), m.p.211-213 ºC. IR (cm^{-1}): 3408,3302,3195(NH_2,NH), 2982(CH aliphatic), 1692(COO), 1652(2CO amide). ^{1}H-NMR(DMSO-d_6); δ=1.27(t,3H,CH_2CH_3), 2.36(m,2H,C6), 3.03(t,2H,C7), 4.12-4.26(m,4H,CH_2CH_3,C5), 4.53(s,2H,CH_2Cl), 9.71(s,3H,NH_2,NH). Microanalysis (%): Calculated for $C_{13}H_{16}N_3O_4Cl$(313.74); Calc. C 49.76 H 5.14 N 13.39 Found C 49.75 H 5.42 N 13.39.

General method for the preparation of comp. 19a-c

A mixture of **18** (2.9 mmol.) and the appropriate amine (3.5 mmol.) in absolute ethanol (10 ml) was refluxed for 8 hours. The reaction mixture was filtered while hot, concentrated and cooled. The separated crystals were collected, dried, and recrystallized from ethanol.

Ethyl-1-(aminocarbonyl)-2-[(morpholin-4-ylacetyl)amino]-6,7-dihydro-5H-pyrrolizine-3-carboxylate (19a)

White crystals, Yield (83%), m.p 219-221°C. I R (cm^{-1}): 3567, 3488, 3158 (NH_2,NH), 1669, 1610 (3CO). MS: = m/z (%) = M^{+1} 365 (20); 77 (100). ^{1}H-NMR (DMSO-d_6); δ=1.28 (t,3H,CH_2CH_3), 2.49 (m,2H,C6), 3.05 (t,2H,C7), 3.37 (s,2H,$COCH_2N$), 3.57 (2t,8H,N$(CH_2)_4$O),4.20 (q,2H,CH_2CH_3), 4.39(t,2H,C5), 11.05(s,3H,NH_2,NH). Microanalysis (%): Calculated for $C_{17}H_{24}N_4O_5$ (364.40), C 56.03 H 6.63 N 15.37, found, C 55.67 H 7.08 N 15.30.

Ethyl-1-(aminocarbonyl)-2-[(piperidin-4-ylacetyl)amino]-6,7-dihydro-5H-pyrrolizine-3-carboxylate (19b)

White crystals, Yield (72%), m.p. 229-231°C. IR (cm^{-1}): 3349, 3272, 3158 (NH_2, NH), 1671, 1611(3CO). Microanalysis (%): Calculated for $C_{18}H_{26}N_4O_4$(362.43) C 59.65 H 7.23 N 15.45, found C 59.82 H 6.94 N 15.98.

Ethyl-1-(aminocarbonyl)-2-[(piperazin-4-ylacetyl)amino]-6,7-dihydro-5H-pyrrolizine-3-carboxylate (19c)

White crystals, Yield (79%), m.p 253-255° C. I R (cm^{-1}): 3416, 3263, 3154 (NH_2,NH),1714,1686,1650 (3CO). Microanalysis (%): Calculated for $C_{17}H_{25}N_5O_4$ (363.42), C 56.18 H 6.93 N 19.26. Found, C 56.21 H 7.01 N 19.25

2-Chloro-N'-(chloroacetyl)-N-phenylacetohydrazide (20)

To a mixture of phenylhydrazine (1 g, 9.3 mmol) in dry benzene (20 ml), chloroacetylchloride (2.19, 19 mmol) was added drop wise, while stirring at room temperature. Shortly after the addition, a heavy precipitate was formed and stirring was continued for an additional one hour. The separated product was filtered, washed with water, dried

and recrystallized from ethanol/water. Yield (92%), m.p. 116-118°C. IR(cm^{-1}): 3212(NH), 3005 (CHaromatic),1710, 1677(2CO), 767(Cl). MS: = m/z (%) M^+ 261 (64); 215 (100). ^{1}H-NMR ($DMSOd_6$); δ =4.16 (s,2H,CH_2Cl), 4.26 (s,2H,$NHCOCH_2Cl$), 6.93-7.46 (m, 5H, aromatic), 8.61(s,1H,NH). Microanalysis (%): Calculated for $C_{10}H_{10}Cl_2N_2O_2$ (261.11), Calc. C 45.99 H 3.86 N 10.72, Found C 46.01 H 4.05 N 10.55.

2-Amino-3-(5-oxo-4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)-6,7-dihydro-5H-pyrrolizine-1-carbonitrile (21)

A mixture of **1** (1 g, 7.5 mmol), powdered anhydrous potassium carbonate (2.1g, 15mmol), and compound **20** (1.95g., 7.5mmol) in dry acetone (50 ml) was stirred under reflux for 24 hours and filtered, The filtrate was concentrated, set aside to cool, the formed reddish brown crystals were collected, dried and recrystallized from acetone. Yield (78%), m.p. 219-221°C. I R (cm^{-1}): 3429, 3335(NH_2), 3075(CH aromatic), 2208(CN), 1688 (CO). MS: = m/z (%) = M^+ 321 (100) ; 321 (100). ^{1}H-NMR (DMSO-d_6); δ = 2.36(m,2H,C6),2.88(t,2H,C7),4.14(t, 2H, C5),4.87(s,2H, OCH_2CO), 5.59 (s,2H,NH_2) disappears with D_2o, 7.28-7.66(m,5H, aromatic). Microanalysis (%): Calculated for $C_{17}H_{15}N_5O_2$ (321.34), Calc. C 63.54 H 4.70 N 21.79, Found C 63.62 H 4.38 N 21.77

General method for the preparation of compo. 22a-c

A mixture of compound **21** (1 g, 3.12 mmol), glacial acetic acid (0.5 ml) and p-substitutedbenzaldhyde (3.12 mmol) in absolute ethanol (20 ml) was refluxed for 10 hours, the reaction mixture was then concentrated and set aside to cool, yellow crystals were formed, collected, dried and crystallized from ethanol.

2-{[(4-Chloro phenyl)methylene]amino}-3-(5-oxo-4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)-6,7-dihydro-5H-pyrrolizine-1-carbonitrile (22a)

Yellow crystals, Yield (92%), m.p. 202-204°C. I R (cm^{-1}): 3061(CH aromatic), 2215 (CN), 1690 (CO). MS: = m/z (%) = M^+ 443 (39) ; 77 (100). ^{1}H-NMR(DMSO-d_6): δ = 2.44 (m, 2H, C6), 3.04 (t, 2H, C7), 4.29(t, 2H, C5), 4.88(s,2H,OCH_2CO),7.24-7.94 (m,9H,aromatic), 8.70(s,1H,N=CH). Microanalysis (%) : Calculated for $C_{24}H_{18}ClN_5O_2$ (443.89) C 64.93 H 4.08 N 15.77, found, C 64.68 H 4.33 N 15.68.

2-{[(4- Bromo phenyl) methylene] amino } -3-(5-oxo-4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)-6,7-dihydro-5H-pyrrolizine-1-carbonitrile (22b)

Yellowish green crystals, Yield (85%), m.p. 199-201°C. I R (cm^{-1}): 3069 (CH aromatic), 2209(CN),1684(CO). Microanalysis (%): Calculated for $C_{24}H_{18}BrN_5O_2$ (488.34), C 59.02 H 3.71 N 14.34, Found, C 58.82 H 3.68 N 14.19.

2-{[(4- Nitro phenyl) methylene] amino } -3-(5-oxo-4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)-6,7-dihydro-5H-pyrrolizine-1-carbonitrile (22c)

Orange crystals, Yield (88%), m.p. 226-228°C. I R (cm^{-1}): 3069(CH aromatic), 2209(CN),1682(CO). Microanalysis (%): Calculated for $C_{24}H_{18}N_6O_4$ (454.44), C 63.43 H 3.99 N 18.49, Found, C 63.22 H 4.03 N 18.34.

General method for the preparation of compounds 23a,b

To 1 g of compound 21 (3.12 mmol) in dioxan (15 ml), acyl chloride (3.12 mmol) was added, the reaction mixture was left to stand overnight at room temperature. The separated product was filtered, washed with water, dried and crystallized from ethanol.

N-[1-Cyano-3-(5-oxo-4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)-6,7-dihydro-5H-pyrrolizin-2-yl]acetamide (23a)

Yellowish crystals, Yield (75%), m.p. 264-266°C. I R (cm^{-1}): 3310 (NH), 3070(CH aromatic), 2222(CN), 1684, 1632 (2CO). MS: = m/z (%) = M^+ 363 (49) ; 321 (100). ^{1}H-NMR (DMSO-d_6);=1.97 (s, 3H, CH_3), 2.38 (m,2H,C6), 3.00(t,2H,C7), 4.11(t,2H,C5), 4.75(s,2H,OCH_2CO), 7.23-7.65 (m,5H,aromatic),9.45(s,1H,NH). Microanalysis (%): Calculated for $C_{19}H_{17}N_5O_3$(363.38), C 62.80 H 4.71 N 19.27, Found, C 63.51 H 4.62 N 19.17.

N-[1-Cyano-3-(5-oxo-4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)-6,7-dihydro-5H-pyrrolizin-2-yl] benzamide (23b)

Yellowish white crystals, Yield (78%), m.p. 270-272°C. I R (cm^{-1}): 3227 (NH), 3061(CHaromatic), 2220 (CN), 1691,1674 (2CO). Microanalysis (%): Calculated for $C_{24}H_{19}N_5O_3$ (425.45), C 67.75 H 4.50 N 16.46, Found, C 67.30 H 4.90 N 15.98.

General method for the preparation of compounds 24a,b

A solution of compound **23 a,b** (0.36 mmol.), in sodium ethoxide 0.5% (20 ml) was refluxed for 2 hours. The formed

precipitate was filtered and dissolved in water (20 ml) and dilute HCl was added. The obtained solid product was filtered, dried and crystallized from ethanol.

3-Methyl-1-oxo-2,7,8,9-tetrahydro-1H-pyrimido[5,4-a]pyrrolizine-5-carboxylic acid (24a)

White crystals, Yield (46%), m.p. 251-253°C. I R (cm^{-1}): 3400 (NH), 2800-3500(OH), 1601(2CO). Microanalysis (%): Calculated for $C_{11}H_{11}N_3O_3$ (233.23), C 56.64 H 4.75 N 18.01, Found, C 56.84 H 4.94 N 17.92.

3-phenyl-1-oxo-2,7,8,9-tetrahydro-1H-pyrimido[5,4-a]pyrrolizine-5-carboxylic acid (24b)

White crystals, Yield (52%), m.p. 265-267°C. IR (cm^{-1}): 3422(NH),3200-3500(OH), 1678,1623(2CO). Microanalysis (%): Calculated for $C_{16}H_{13}N_3O_3$ (295.30), C 65.07 H 4.43 N 14.22, Found, C 65.46 H 4.08 N 14.19.

2-Chloro-N-[1-cyano-3-(5-oxo-4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)-6,7-dihydro-5H-pyrrolizin-2-yl] acetamide (25)

To compound **21** (1.2 g, 3.8 mmol) in dioxan (20 ml), chloroacetyl chloride (0.9 g, 7.6 mmol) was added, The reaction mixture was left to stand for 24 hours at room temperature. The separated product was filtered, washed with water and crystallized from ethanol. Yield (68%), m.p 228-231°C. IR (cm^{-1}):3271 (NH), 3062 (CHaromatic), 2216(CN), 1688 ($CONC_6H_5$), 1630 (CONH), 766(Cl). ^{1}H-NMR(DMSO-d_6); δ=2.42(m,2H,C6), 2.97(t,2H,C7), 3.55(s,2H,CH_2Cl), 4.23(t,2H,C5), 4.84(s,2H,OCH_2), 7.28-7.65(m,aromatic), 10.21(s,1H,NH). Microanalysis(%); Calculated for $C_{19}H_{16}ClN_5O_3$ (397.82), Calc. C 57.36 H 4.05 N 17.60, Found C 57.60 H 4.40 N 17.33

General method for the preparation of compounds 26a-c

A mixture of compound **25** (1.2 g, 2.9 mmol) and the secondary amine (3.2 mmol) in absolute ethanol (10 ml) was refluxed for 8 hours. The reaction mixture was filtered while hot, concentrated and cooled. The separated crystals were collected, dried and crystallized from ethanol.

2-(Morpholin-4-yl)-N-[1-cyano-3-(5-oxo-4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)-6,7-dihydro-5H-pyrrolizin-2-yl]acetamide (26a)

Yellowish crystals, Yield (72%), m.p 195-197°C. IR(cm^{-1}): 3345(NH),3073(CH aromatic), 2220 (CN), 1698,1642 (2CO). MS:=m/z (%)=M^+ 448 (2) ;93 (100). ^{1}H-NMR (DMSO-d_6); 2.45 (m, 2H, C6), 2.96 (t,2H,C7), 3.09(s,2H,$COCH_2N$), 3.50-3.53 (2t,8H,$N(CH_2)_4O$), 4.21 (t,2H,C5),4.87 (s,2H, OCH_2CO),7.29-7.64(m,aromatic), 9.61(s,1H,NH). Microanalysis(%); Calculated for $C_{23}H_{24}N_6O_4$ (448.48), C 61.59, H 5.39, N 18.73, found, C 61.30 H 5.11 N 18.48

2-(piperidin-1-yl)-N-[1-cyano-3-(5-oxo-4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)-6,7-dihydro-5H-pyrrolizin-2-yl]acetamide (26b)

Yellowish white crystals, Yield (64%), m.p 204-206°C. Microanalysis(%); Calculated for $C_{24}H_{26}N_6O_3$ (446.51), C 64.55 H 5.86 N 18.82, found C 64.70, H 5.70 N 18.80.

2-(Piperazin-1-yl)-N-[1-cyano-3-(5-oxo-4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)-6,7-dihydro-5H-pyrrolizin-2-yl]acetamide (26c)

Yellowish white crystals, Yield (62%), m.p 222-224°C. Microanalysis(%); Calculated for $C_{23}H_{25}N_7O_3$ (447.50), C 61.73 H 5.63 N 21.91, found, C 61.50 H 5.60 N 21.85.

General method for the preparation of compounds 27a,b

A mixture of compound 21 (1 g, 0.32 mmol), in dioxan (30 ml), (0.35 mmol.), phenyl or p-chlorophenylisocyanate and 3-4 drops triethylamine, was refluxed for 15 hours, concentrate, leave to set aside collect the formed crystals, dry and crystallize from acetone.

1-[1-Cyano-3-(5-oxo-4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl)-3a,4,5,6- tetrahydropentalen -2-yl] -3-phenyl urea (27a).

Yellowish crystals, Yield (62%), m.p 249-251°C. 1688, 1648(2CO). MS:=m/z (%) IR (cm^{-1}): 3301 (NHs), 3060 (CH aromatic), 2224 (CN)=M^+ 440 (54); 77 (100). ^{1}H-NMR(DMSO-d_6) 88a; δ=2.45 (m,2H,C6), 3.02(t,2H,C7), 4.24 (t,2H,C5), 4.88 (s,2H,OCH_2CO), 6.95,7.66(m,aromatic protons),8.24(s,1H,NH),8.98(s,1H,NH). Microanalysis(%); Calculated for $C_{24}H_{20}N_6O_3.H_2O$ (458.48), C 62.87 H 4.83 N18.33, found, C 63.35 H 4.56 N 17.91.

1-[1-Cyano-3-(5-oxo-4-phenyl-5,6-dihydro-4H-1,3,4-oxadiazin-2-yl) -3a,4,5,6- tetrahydropentalen -2-yl] -3-p-chlorophenyl urea (27b)

Yellowish white crystals, Yield (65%), m.p 164-166°C. IR(cm^{-1}): 3453,3341(2NH), 2223(CN),1703,1677, (2CO). Microanalysis(%); Calculated for $C_{24}H_{19}ClN_6O_3$. H_2O (492.92), C 58.48 H 4.29 N 17.04, found, C 58.6 H 74.61 N 17.02.

General methods for the preparation of compounds 28a,b

A solution of compound **27a,b** (1 g) in 0.05% sodium ethoxide (20 ml), stirred for 2 hours at room temperature, filter, dissolve in water (15 ml), neutralize with dilute HCl. The collected precipitate, was washed with water, dry and crystallize from ethanol.

1-Imino-3-oxo-2- phenyl-1,2,4,7,8,9-hexahydro-1H-pyrimido[5,4-a]pyrrolizine-5-carboxylic acid (28a)

White crystals, Yield (46%), m.p 265-267°C. IR(cm^{-1}): 3422(NH),2900-3500(COOH), 1650(2CO). Microanalysis(%); Calculated for $C_{16}H_{14}N_4O_3$ (310.31), C 61.93 H 4.54 N 18.05, found, C 62.20 H 4.77 N 18.06

1-Imino-3-oxo-2-p.chlorophenyl-1,2,4,7,8,9-hexahydro-1H-pyrimido[5,4-a]pyrrolizine-5-carboxylic acid (28b)

White crystals, Yield (48%), m.p 274-276°C. IR(cm^{-1}): 3426(2NH),3100-3500(OH),1624(2CO). Microanalysis(%); Calculated for $C_{16}H_{13}ClN_4O_3$ (344.76), C 55.74 H 3.79 N 16.25, found, C 55.77 H 3.91 N 16.12.

Biological screening

Potential cytotoxicity of the selected compounds on MCF7 cancer cell line was performed using the method of Skehan et al, (1990) [29]. Cells were plated in 96-multiwell plate (10^4cells/well) for 24 hours before treatment with the compounds to allow attachment of cell to the wall of plate. Different concentration of the compounds under test (0, 1, 2.5, 5 and 10 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hours at 37 ˚C and in atmosphere of 5% CO_2. After 48 hours, cells were fixed, washed and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with tri EDTA buffer. Color intensity was measured by an ELISA reader. The relation between the surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line after the specified compound. Data were reviewed, compared and the biological activity was determined.

Antimicrobial screening

The antimicrobial activity for the selected newly synthesized compounds was evaluated in vitro against *Staphylococcus aureus* and *Bacillus subtilis* (as representatives of gram positive bacteria). *Escherichia coli* and *Pseudomonas aeruginosa* (as representatives of gram negative bacteria) and the fungus *Candida albicans* by the MIC method. The MIC is the minimal concentration at which the microbial growth was completely inhibited. For each tested compound, a stock solution of 20000 µg/ml in dimethyl formamide (DMF) was prepared and two folds serial dilution were made in the same solvent. Each dilution was added to 10 ml of nutrient agar medium to yield final test concentrations in agar medium in range of 12, 5-400 µg/ml. Controls were prepared with the same quantities of dimethylformamide but without the tested compounds. The mixtures were mixed, poured into sterile Petri dishes and allowed to harden at room temperature. The agar surface was inoculated with 10 ml of standardized suspension of test organisms (10^6cell/ml) and incubated at 37°C for 24 to 48 hours. Control plates containing tetracycline and clotrimazole were seen side by side.

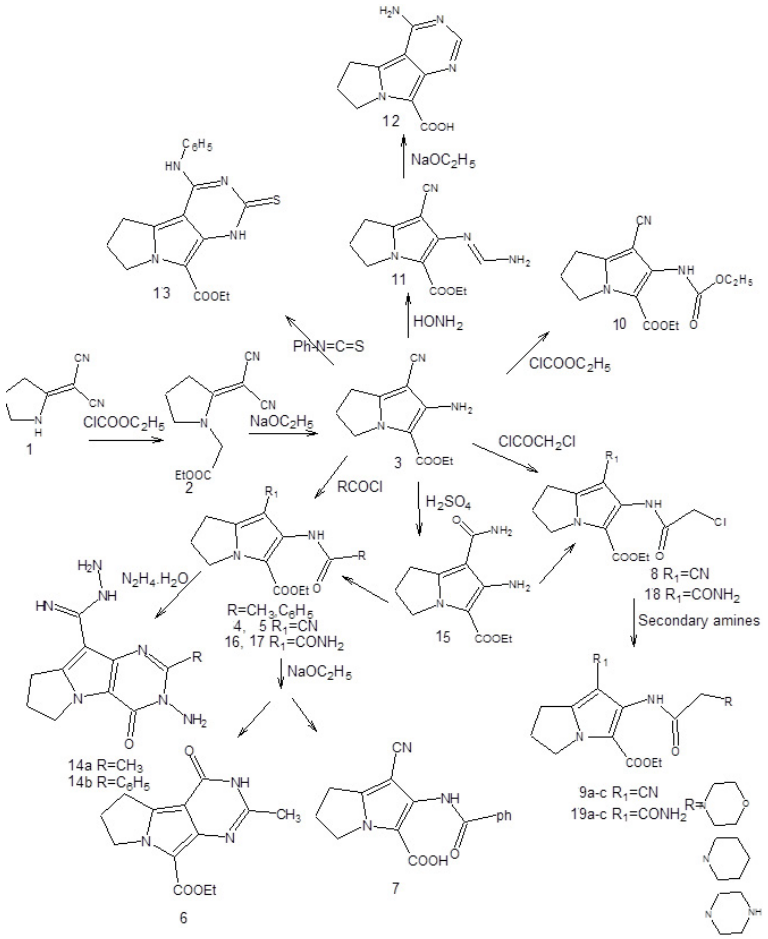
RESULTS AND DISCUSSION

Chemistry

Compound (1) was prepared according to previously reported methods [27,28]. In the present work the *o*-aminonitrile derivative of pyrrolizine (3) (25) was synthesized via the condensation of pyrrolidin-2-ylidenemalononitrile (1) with ethyl chloroacetate, the reaction mixture was refluxed for 24 hours in dry acetone and potassium carbonate as an acid binding agent, however, the product was obtained in a low yield. Therefore, jn a trial to improve the yield, the reaction duration was decreased to reach only 8 hours, where the uncyclized ethyl [2-(dicyanomethylene)pyrrolidin-1-yl] acetate intermediate (2) was separated. Cyclization was achieved by stirring this intermediate (2) with sodium ethoxide at r.t. to afford the bicyclic pyrrolizine derivative (3) in a good yield (**Scheme 1**). The IR spectrum of the pyrrolidine derivative (2) revealed a carbonyl absorption band of the acetate group at 1750 cm^{-1} in addition to characteristic absorption bands of the geminal cyano groups at 2190 and 2211 cm^{-1}, this confirmed the uncyclized structure. On the other hand, the IR spectrum of the o-aminonitrile pyrrolizine derivative (3) showed the disappearance of the

characteristic absorption bands of the geminal cyano group and showed only one sharp band at 2210 cm^{-1}, indicating that cyclization happened successfully. In addition, the IR spectrum revealed the sharp stretching absorption bands of the 2-amino group at 3431 and 3338 cm^{-1}.

In this work, the *O*-aminonitrile (3) (**Scheme 1**), was acetylated by reacting it with acetyl chloride at room temperature to give the open bicyclic 2-acetylamino pyrrolizine derivative (4). Cyclization was achieved by refluxing the obtained 2-acetylamino derivative (4) with sodium ethoxide to give the tricyclic ethyl 3-methyl-1-oxo-2,7,8,9-tetrahydro-1*H*-pyrimido[5,4-*a*]pyrrolizine-5- carboxylate (6). The IR spectrum of (4) showed a strong and sharp band for the cyano group at 2228 cm^{-1}, indicating that intramolecular cyclization did not occur and that the separated compound was the uncyclized 2-acetylamino derivative (4). In addition, the IR spectrum revealed stretching bands at 1697 and 1668 cm^{-1}, this indicated the presence of the 2 CO groups and another absorption band at 3268 cm^{-1} indicating the NH group. On the other hand, The IR spectrum of compound (6) showed the disappearance of the characteristic absorption band of the cyano group. The ^{1}H-NMR of compound (4) revealed a singlet at 2.0 ppm representing the three protons of $COCH_3$ group. In addition, the ^{1}H-NMR spectrum of compound (6) revealed a singlet at 2.2 ppm, corresponding to the 3 protons of N=C-CH_3 group, the triplet and quartet pattern of the ethyl ester group appeared at 1.2 and 4.2 ppm.



Scheme 1: Synthesis of compounds 3-19c.

Benzoylation was performed by reacting the *o*-aminonitrile derivative (3) with benzoyl chloride at elevated temperature to give 2- benzoylamino pyrrolizine derivative (5), which upon treatment with sodium ethoxide afforded the free 3-carboxylic acid derivative (7). The IR spectrum of (5) revealed the characteristic absorption band of the cyano group at 2221 cm^{-1}. However, the IR spectrum of (7) showed the appearance of a broad band at 2800-3500 cm^{-1} indicating the presence of OH group in addition to the cyano band at 2238 cm^{-1}, therefore confirming hydrolysis of the ethyl carboxylate ester group to its carboxylic acid analog. The mass spectrum of (5) and (7) showed the molecular ion peak at m/z=323 and m/z=295 respectively.

Compound (3) reacted readily with chloroacetyl chloride in dioxan at room temperature to afford the 2-chloroacetylamino pyrrolizine derivative (8), the structure of (8) was confirmed by microanalytical and spectral data. The IR spectrum showed the characteristic absorption band of the cyano group at 2225 cm^{-1}, also it revealed absorption bands at 3240 cm^{-1} corresponding to the NH group and 1683, 1701 cm^{-1} corresponding to the two CO groups. Furthermore, the ^{1}H-NMR spectrum showed a singlet at 4.3 ppm corresponding to the two protons of the CH_2Cl group, in addition to the characteristic signals of the ethyl group at 1.25 and 4.21 ppm.

Condensation of compound (8) with excess secondary amine in absolute ethanol was performed, the IR spectrum revealed the presence of the absorption band of the cyano group at 2223 cm^{-1}. ^{1}H-NMR spectrum of (9a) revealed two triplets at 3.64-3.67 ppm indicates morpholino protons, a singlet at 3.25 ppm indicating $COCH_2N$ protons and the ethyl group protons at 1.27 and 4.21 ppm.

Compound (3) was refluxed in excess ethylchloroformate, the 2-ethoxycarbonylamino derivative (10) was obtained. The IR spectrum of the obtained product (10) showed an absorption band of the cyano group at 2223 cm^{-1}, in addition it revealed another absorption band at 3318 cm^{-1} corresponding to the NH group and two absorption bands at 1742 and 1668 cm^{-1} indicating the presence of the 2 carbonyl groups.

Formamide was reacted with the aminonitrile derivative (3) in the absence of solvent to give the uncyclized aminomethyleneaminopyrrolizine derivative (11), the obtained compound was cyclized to the pyrimidopyrrolizine derivative (12) by refluxing it in a solution of sodium ethoxide. The IR spectrum of compound (11) showed the characteristic absorption band of cyano group at 2220 cm^{-1} and the forked absorption bands corresponding to the NH_2 group appeared at 3502 and 3420 cm^{-1}. The ^{1}H-NMR spectrum revealed the characteristic signals of the ethyl ester group appeared as a triplet and quartet at 1.2 and 4.2 ppm, in addition to a singlet signal corresponding to one proton of the N=CH-NH_2 appeared at 8.0 ppm, another singlet with an integration of two protons corresponding to the amino group appeared at 5.7 ppm. On the other hand, the IR spectrum of the tricyclic structure (12) revealed the disappearance of the cyano group and the appearance of a broad band at 2700-3500 cm^{-1}, corresponding to an OH stretching band, indicating that hydrolysis of the ester group occurred and the free carboxylic acid derivative was obtained.

Refluxing the aminonitirile derivative (3) in excess phenyl isothiocyanate gave the tricyclic pyrimido (5,4-a) pyrrolizine derivative (13). The IR spectrum of (13) showed the disappearance of the cyano band indicating that addition of phenyl isothiocyanate occured followed by intramolecular cyclization. ^{1}H-NMR revealed the presence of ethyl ester protons as a triplet at 1.3 ppm (CH_3) and a quartet at 4.2 ppm (CH_2), indicating that no hydrolysis occurred to the ester group. The mass spectrum also showed a molecular ion peak at 354 confirming the tricyclic pyrimidopyrrolizine structure.

2-acylamino pyrrolizine carboxylate derivatives (4) and (5) were refluxed in excess hydrazine hydrate. Cyclization occurred as expected but an additional molecule of hydrazine hydrate was added to the nitrile group giving the 5-carboximido hydrazide pyrimido(4,5b) pyrrolizine derivatives (14a,b). The IR spectrum of (14a) showed absorption bands for the 2 NH_2 and 2 NH groups at 3365-3205 cm^{-1}, in addition to the CO absorption band at 1686 cm^{-1}. The ^{1}H-NMR spectrum of methyl derivative (14a) showed no signals for the ethyl carboxylate protons but it revealed the presence of a singlet signal at 2.5 ppm with an integration of three protons corresponding to the CH_3 group, another singlet at 5.7 ppm for the two protons of the 2 NH groups and two singlets at 7.1 and 7.6 corresponding to the 4 protons of the two NH_2 groups. The mass spectrum of (14b) showed a molecular ion peak at 324 indicating m^{+1}.

In the present work, ethyl 2-amino-1-(aminocarbonyl)-6,7-dihydro-5*H*-pyrrolizine-3-carboxylate (15) was prepared by treating the aminonitrile (3) with sulfuric acid, at room temperature, then treated with ammonia solution. The IR spectrum of this compound revealed the presence of stretching absorption bands at 3473, 3363, 3318, and 3260 cm^{-1} indicating the presence of two amino groups, on the other hand the nitrile absorption band was not observed. Moreover, a strong absorption band of the two carbonyl groups appeared at 1658 cm^{-1}. The ^{1}H-NMR revealed two singlets at 5.8 and 6.5 ppm corresponding to the 4 protons of the 2 amino groups, in addition signals of the ethyl carboxylate protons appeared as triplet and quartet at 1.29 and 4.19 ppm respectively indicating that no hydrolysis occurred to the ester group.

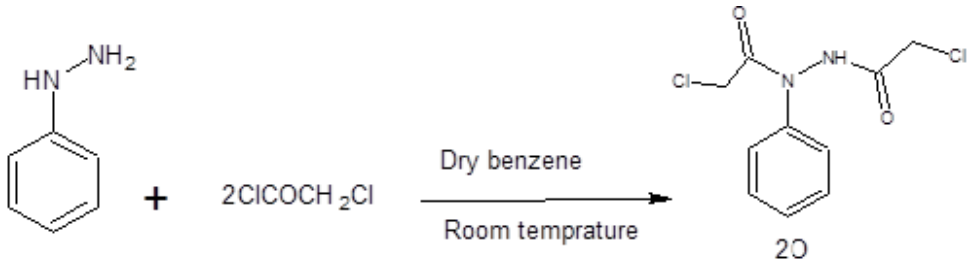
Acetylation to the 2-amino pyrrolizine derivative (15) was achieved by reacting it with acetyl chloride in dioxan at room temperature to give the 2-acetyl aminopyrrolizine derivative (16). Benzoylation using benzoyl chloride occurred in the same way but at elevated temperature yielding 2-benzamidopyrrolizine derivative (17). The IR spectrum of (16) showed the presence of two absorption bands at 3418, 3185 cm^{-1} corresponding to NH_2 and NH groups, the absorption bands of the CO groups were also observed at 1712 and 1653 cm^{-1}.The ^{1}H-NMR spectrum of (16) revealed the presence of C_2H_5 group as a quartet and a triplet at 1.29 and 4.26 ppm respectively, it also showed a singlet at 2 ppm

corresponding to $COCH_3$. The mass spectrum of (16) showed a molecular ion peak at 279 that confirmed the structure.

Chloroacetylation of the 2-amino-1-carboxamidepyrrolizine derivative (15) was carried out by reacting it with chloroacetyl chloride at room temperature to yield the chloroacylamino derivative (18). The IR spectrum of (18) revealed an absorption band at 3195 cm^{-1} corresponding to the NH group. In addition to the two absorption bands of the carboxamide amino group at 3408 and 3302 cm^{-1}. In addition the stretching absorption bands of the 3 carbonyl groups appeared at 1698, 1652 and 1602 cm^{-1}.

The ^{1}H-NMR spectrum of (18) showed a singlet at 4.5 ppm corresponding to the 2 protons of $COCH_2Cl$ group, in addition to signals characterizing the ethyl group at 1.2 and 4.1ppm. The chloroacylamino derivative (18) was refluxed with excess secondary amines in absolute ethanol to obtain (19a-c), the IR spectrum of (19a) revealed the appearance of the absorption bands of the carboxamide amino group at 3567 and 3488 cm^{-1}. Also the mass spectrum showed molecular ion peak m/z=365. The ^{1}H-NMR spectrum of (19a) revealed the presence of two triplets at 3.37-3.59 ppm corresponding to the morpholino protons, in addition to the signals of the ethyl group at 1.28 and 4.2 ppm respectively and $COCH_2$ signal at 3.3 ppm.

Preparation of 2-chloro-*N*'-(chloroacetyl)-*N*-phenylacetohydrazide, (20) (**Scheme 2**). In the present work acylation of phenylhydrazine was performed by stirring it with chloroacetyl chloride in dry benzene to yield 2-chloro-*N*'-(chloroacetyl)-*N*-phenylacetohydrazide (20). The IR spectrum of (20) showed a stretching absorption band at 3212 cm^{-1} corresponding to the carboxamide NH group. In addition, it revealed the absorption bands of the 2 CO groups at 1710 and 1677cm^{-1}. The ^{1}H-NMR spectrum revealed two singlets at 4.1 and 4.2 ppm corresponding to the 4 protons of the two CH_2Cl group. Mass spectrum showed a molecular ion peak at m/z=261 confirming that chloroacetylation of phenylhydrazine occurred with 2 moles of chloroacetyl chloride. The obtained hydrazide derivative (20) was reacted with the dinitrile (1) in dry acetone and potassium carbonate as an acid binding agent. It was expected to give the 2-chloro-*N*'-{[2-(dicyanomethylene)pyrrolidin-1-yl]acetyl}-*N*-phenylacetohydrazide, however spontaneous cyclization of this intermediate occurred yielding the 3-oxadiazine pyrrolizine derivative (21). The proposed mechanism is presented in **Scheme 4**. The IR spectrum revealed a strong and sharp absorption band at 2208 cm^{-1} characteristic for the cyano group and another two absorption bands appeared at 3429 and 3335 cm^{-1} indicating the presence of the amino group. The ^{1}H-NMR spectrum revealed a singlet at 4.8 ppm, that didn't disappear upon deuteration and its integration showed two protons corresponding to the 2 protons of the OCH_2CO group. On the other hand the two protons of NH_2 group appeared as a singlet at 5.5 ppm that disappeared upon deuteration. The structure was also confirmed by the mass spectrum that showed the molecular ion peak at 321 m/z revealing the loss of a mole of HCl and formation of the cyclized oxadiazine derivative.

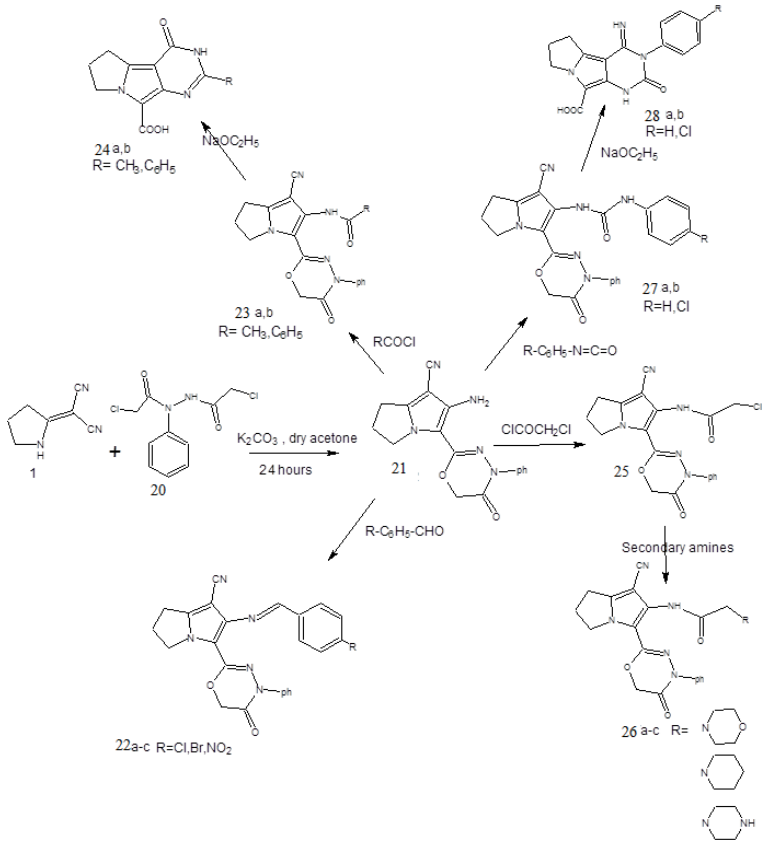


Scheme 2: Synthesis of compound 20

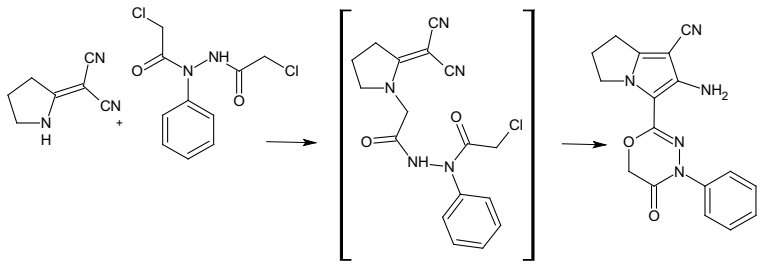
In the present work, compound (21) (**Scheme 3**) was condensed with para substituted benzaldehydes in absolute ethanol and few drops of glacial acetic acid, to yield the 2-(4-substituted phenyl methylene amino) derivatives (22a-c). The IR spectrum of (22a) showed that the absorption band characteristic to the primary amino group at 3335 cm^{-1} was disappeared. The ^{1}H-NMR spectrum showed the appearance of a singlet at 8.7 ppm, with an integration of one proton corresponding to the proton of the $N{=}CHC_6H_5$ group, in addition it revealed a multiplet between 7-8 ppm. Corresponding to the aromatic protons of the phenyl group.

Acetylation of the primary amino group was achieved using acetyl chloride at room temperature and the produced compound was 2-acetyl amino pyrrolizine derivative (23a). The IR spectrum of (23a) revealed the characteristic absorption band of the cyano group at 2222 cm^{-1}, another two absorption bands were observed at 1684 and 1632 cm^{-1} corresponding to the 2CO groups and another peak at 3310 cm^{-1} indicating NH groups. The ^{1}H-NMR spectrum of (23a) showed a singlet at 1.9 ppm, with integration of three protons indicating the protons of the methyl ($COCH_3$) group and another singlet appeared at 9.4 ppm with integration of one proton indicating the proton of NH group, in addition

to the aromatic protons appeared at 7.3-7.6 ppm and a signal for OCH_2CO appeared at 4.7 ppm. Benzoylation of (21) also occurred at room temperature using benzoyl chloride in dioxan. The IR spectrum of (23b) showed absorption peak at 3227 cm^{-1} corresponding to NH group and the cyano group absorption band at 2220 cm^{-1} and also showed two absorption bands at 1691 and 1674 cm^{-1} corresponding to the 2CO groups. Intramolecular cyclization of the acylated compounds (23a,b), was achieved by refluxing them in sodium ethoxide solution, however the reaction was accompanied by hydrolysis of the oxadiazine ring to carboxylic acid derivatives (24a,b). The IR spectrum revealed the disappearance of the characteristic absorption band of the cyano group and the appearance of broad band at 3000-3500cm^{-1} indicating the presence of OH stretching absorption band of the carboxylic group.



Scheme 3: Synthesis of compounds 21-28b.



Scheme 4: Proposed mechanism for synthesis of compound 21.

Acylation with chloro acetyl chloride to compound (21) occurred at room temperature in dioxan, the IR spectrum of compound (25) revealed absorption bands at 2216, 3271, 1688 cm^{-1} indicating CN, NH, 2CO respectively. The ^{1}H-NMR spectrum of (25) showed a singlet at 3.58 ppm indicating two protons of CH_2Cl group, in addition to the other signals characterizing the structure. The chloroacetyl chloride derivative (25) obtained was reacted with different secondary amines, the products obtained (26a-c), were also the bicyclic derivatives. The IR spectrum showed the presence of cyano group absorption band at 2220 cm^{-1}. The ^{1}H-NMR spectrum of morpholino derivative (26a) showed the appearance of two triplets at 3.5-3.53 ppm indicating morpholino protons. Mass spectrum of morpholino derivative showed molecular ion peak m/z=448.

In this work, addition to isocyanates in dioxan using triethyl amine produced the uncyclized urea derivatives, the IR spectrum of (27a) showed absorption bands at 2224, 3301 cm^{-1} indicating CN and NH group respectively and another two absorption bands at 1688 and 1648 cm^{-1} corresponding to 2CO groups. The ^{1}H-NMR spectrum revealed additional aromatic protons 7-8 ppm and two singlets at 8.2 and 8.9 ppm with integration of one proton indicates NH, NH groups. Mass spectrum of phenyl urea derivative showed molecular ion peak m/z=440. Intramolecular cyclization and oxadiazine ring cleavage for compounds (27a, b) to give the free carboxylic acid group was achieved by action of sodium ethoxide to give products (28a, b), the IR spectrum of both revealed the broad stretching absorption band of the OH group and disappearance of the cyano group. Supporting data are presented in supplementary data file in the online version of the manuscript **(Supplimentary Data).**

Biological activity

Anticancer activity

The selected new compounds were screened on MCF7 cancer cell line using sulpharudamine-B method, the survival curve was plotted then the obtained data were expressed using death curve instead of the survival curve to provide good illustration. A plot of the concentration of the drug expressed in µg/ml against the measured test values expressed as percentage of the dead fraction was drawn after treatment of the data using a specified computerized program analysis (probit analysis) [30,31] (see Supp. Data). The obtained IC_{50} and IC_{90} values (**Table 1**) for the tested compounds are presented in **Table 1**, From the previous data It was concluded that: All the tested compounds showed good activity on MCF7 cancer cell line with IC_{50} values less than 60 µm. The best active compounds are 1-aminocarbonyl-3-Ethyl ester pyrrolizine substituted at position 2 with acetyl morpholine 19a and 1-cyano-3-oxadiazin pyrrolizine substituted also at position 2 with acetyl morpholine 26a with IC_{50} values equal 32 and 39 respectively. Both the ortho amino nitrile pyrrolizin-3-ethyl ester 3 and the benzoylated pyrrolizin-3- carboxylic acid derivative showed to be equipotent on MCF7 cancer cell line with IC_{50} value equal 43 µM. The other tested compounds 6, 21, 10, 9a, 14a and 15 showed a closely similar activity, their IC_{50} range was from 45 to 58 µM.

Table 1: IC_{50} and IC_{90} of the tested compounds on MCF7 cell line

Tested comp.	IC_{50} (µM)	IC_{90} (µM)
3	43.195	93.806
4	55.305	123.316
6	45.047	94.726
7	43.831	104.068
9a	51.589	91.458
10	49.123	95.843
14a	57.142	101.806
15	58.164	111.102
19a	39.215	72.558
21	46.119	93.608
26a	32.042	62.834

Table 2: Antibicrobial activity results for the tested compounds (MIC method, concentrations by µg/ml)

Candida albicans	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*	Tested Compounds
400<	200	400<	400<	400<	4
400<	400	400<	400<	400<	5
400	400<	400<	400	400<	7
200	400	400<	400	400	8
400	400<	400<	400<	400<	9b
100	400	400<	400<	400<	10
12.5	400<	400	400<	400<	11
100	400<	400	400<	200	14a
400<	400<	400<	400<	400<	14b
400<	400	400<	400<	400<	15
400<	400<	400<	400<	400<	16
400<	50	400<	400	400<	18
400	400<	400	400<	400<	19a
100	400	200	400	400	19b
400	400<	400<	400<	400<	21
200	400<	400<	400<	400	22a
200	400<	400	400<	400<	22b

400	400	400	400<	400<	23a
400	400<	400<	400<	400<	23b
100	400<	400<	400<	400<	26a
400<	400	400<	400	400	27a
400	400<	400<	400<	400<	27b
400<	12.5	12.5	12.5	12.5	Tetracycline
12.5	400<	400<	400<	400<	Clotrimazole

Analysis of data

Data were collected, checked, revised and analyzed by SPSS statistical package version 11. Excel computer program was used to tabulate the results and represent them graphically. Probit regression analysis procedure was introduced to select the best model that can describe the relationship between the drug concentration and the probit (percentage of protection) as a dependent variable in order to be used for prediction of the (IC_{50}) or (IC_{10}) of cancer cells.The in vitro growth inhibition properties of each drug were described by IC_{50} or IC_{10} the relation between drug concentration and the degree of inhibition of cancer cell line was described by the equation:

The probit (p)= intercept+regression coefficient(conc.)

Antimicrobial activity

The antimicrobial activity for the newly synthesized compounds was evaluated in vitro against *Staphylococcus aureus* and *Bacillus subtilis* (as representatives of gram positive bacteria). *Escherichia coli* and *Pseudomonas aeruginosa* (as representatives of gram negative bacteria) and the fungus *Candida albicans* by the MIC method. The MIC is the minimal concentration at which the microbial growth was completely inhibited. From these results (**Table 2**), some observations are noticed, Compounds 5, 8,10, 23a, 27a, 15, 19b inhibited the growth of *Escherichia coli* at 400 µg/ml, Compound 4 showed a greater activity and compound 18 was the best active one against *Escherichia coli*. Compounds 8, 22a, 27a,19b are active against *Bacillus subtilis* at 400 µg/ml and compound 14a showed better activity as it inhibited *Bacillus subtilis* growth at 200 µg/ml. Compounds 7, 8, 27a,18,19b are the active compounds against *Staphylococcus aureus*. Compounds 14a, 11, 22b, 23a, 19a are active against *Pseudomonas aeruginosa* and compound 19b showed to be the best active one. Compounds 7, 9b, 21, 23a, 23b, 27b, 19a are active against *Candida albicans*, compounds 8, 22a, 22b showed good activity, compounds 10,14a, 26a, 19b showed better activity, however, compound 11 is highly active and is found to be equipotent to the known antifungal clotrimazole. It is noticed that the antifungal activity of the tested compounds is much better than their antibacterial activity.

CONCLUSION

In this research work several new pyrrolizines were synthesized using accessible chemicals and facile chemical reactions, four different classes of pyrrolizines were designed and synthesized, 3-ethyl ester bicyclic substituted pyrrolizine, 3- carboxylic acid substituted pyrrolizine, 3-oxadiazinyl substituted bicyclic pyrrolizines and pyrimidopyrrolizines. Combining morpholine with 3-ethyl ester pyrrolizine 19a or 3-oxadiazinyl pyrrolizine 26a in one molecule lead to obtaining new active compounds on MCF7 cancer cell line with IC_{50} values equal 39 and 32 µM respectively. Moreover, compound 11 showed equipotent activity with clotrimazole against *Candida albicans* and this represent a good achievement for this work to synthesize a new pyrrolizine derivative with equipotent activity as clotrimazole on *Candida albicans*. Hoping for further investigations for this new compound 11 to be approved as an effective antifungal agent in the future.

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