Original Articles

SYNTHESIS AND ANTITUMOR ACTIVITY OF SOME 5*H*-PYRROLIZINE, PYRIMIDO [5,4-*a*] PYRROLIZINE PYRIMIDO[4,5-*b*]PYRROLIZINE DERIVATIVES

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تم في هذا البحث تشييد سبع مجموعات جديدة من مشتقات بيرروليزين مكثفة يتوقع أن يكون لها نشاط مضاد للأورام. وتشتمل هذه المجموعات على مايلي:مشتقات إيثايل-١-سيانو-٣-فينايل كاربامويل-٦ر٧-ثنائي هيدرو-٥ يد-بيروليزين-٢-أيل كاربامات. ومشتقات ٢ر٤-ثنائي أوكسو-ايد-بيريميدو[٤ر٥-ب] بيرروليزين-٩-كاربونايترايل. ومشتقات ١-سيانو-٢-(٣-إحلال يوريدو)-٣ن-فينايل-٦ر٧-ثنائي هيدروبيرروليزين-٣-كاربوكسامايد. ومشتقات ٣-(الكايل/فينايل-إيمينو-٢-اوكسو-٩ن-فينايل-٢ر٣ر٤ر٥ر٦ر٧ سداسي هيدرو-١ يد-بيريميدو [٥ر٤-أ] بيرروليزين-٩-كاربوكسامايد. ومشتقات ٤-أمينو-٣-(ألكايل/فينايل)-٢-أوكسو-٢ر٣ر٤ر٥ر٦ر٧-سداسي هيدو-١ يد-بيريميدو [٥ر٤-أ] بيرروليزين-٩-كابوكساميد.لمشتقات ٢-أمينو-٣ن-فينايل-٦ر٧-ثنائي هيدو-٥ يد-بيرروليزين-١ر٣-ثنائي كاربوكسامايد. و ٤-أوكسو-٩ن-فينايل-٤ر٥ر٦ر٧-رباعي هيدرو-٣يد-بيريميدو [٥ر٤-أ] بيرروليزين-٩-كاربوكساميد. وقد تم إختبار أحد عشر مركباً لمعرفة تأثيرها المضاد للأورام ووجد أن عشرة منها لها فاعلية مضادة للأورام تتراوح بين المتوسطة والضعيفة ضد سرطان الثدي.

Seven new series of condensed pyrrolizine derivatives of anticipated antitumor activity have been synthesized. Comprises ethyl-1-cyano-3-phenylcarbamoyl-6,7-dihydro-5*H*-pyrrolizin-2-yl-carbamate, 2,4-dioxo-1*H*-pyrimido[4,5-*b*]pyrrolizine-9-carbonitrile, 1-cyano-2-(3-substituted ureido)-3N-phenyl-6,7-dihydro-pyrrolizine-3-carboxamide, 3-(alkyl / phenyl)-4-imino-2-oxo-9N-phenyl-2,3,4,5,6,7-hexahydro-1*H*-pyrimido [5,4-*a*]pyrrolizine-9-carboxamide , 4-amino-3-(alkyl / phenyl)-2-oxo-2,3,4,5,6,7-hexahydro-1*H*-pyrimido[5,4-*a*]pyrrolizine-9-carboxamide, of 2-amino-3N-phenyl-6,7-dihydro-5*H*-pyrrolizine-1,3-dicarboxamide and 4-oxo-9N-phenyl-4, 5,6,7-tetrahydro-3*H*-pyrimido[5,4-*a*]pyrrolizin-9-carboxamide derivatives. Eleven were screened for their *in vitro* antitumor activity and ten compounds proved to possess moderate to weak activities.

Keywords: Pyrrolizine, pyrimido[4,5-*b*]pyrrolizine, pyrimido[5,4-*a*]-pyrrolizine, anti-cancer, probit analysis.

Introduction

The search for new antitumor drugs with high activity and selectivity and low side effects is continuous. Over the last three decades pyrrolizines represents a key nucleus for preparation of new drugs. The literature survey revealed that Pyrrolizines have diverse biological activities such as antiinflammatory (1, 2), antiarrhythmic (3-5), nootropic (6), antispasmodic (7), antibacterial (8), and antitumor activity (9-11). The antitumor activity observed in large number of pyrrolizine derivatives had drawn our attention. Mitomycin **1** (9) is a well known pyrrolizine derivative with antileukemic activity.

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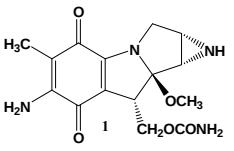
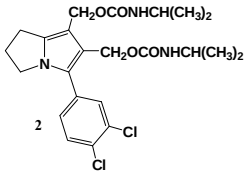
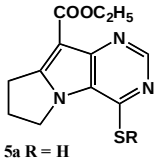
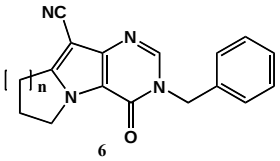
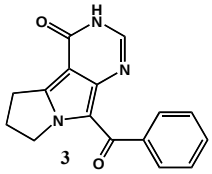
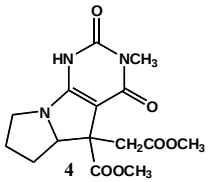








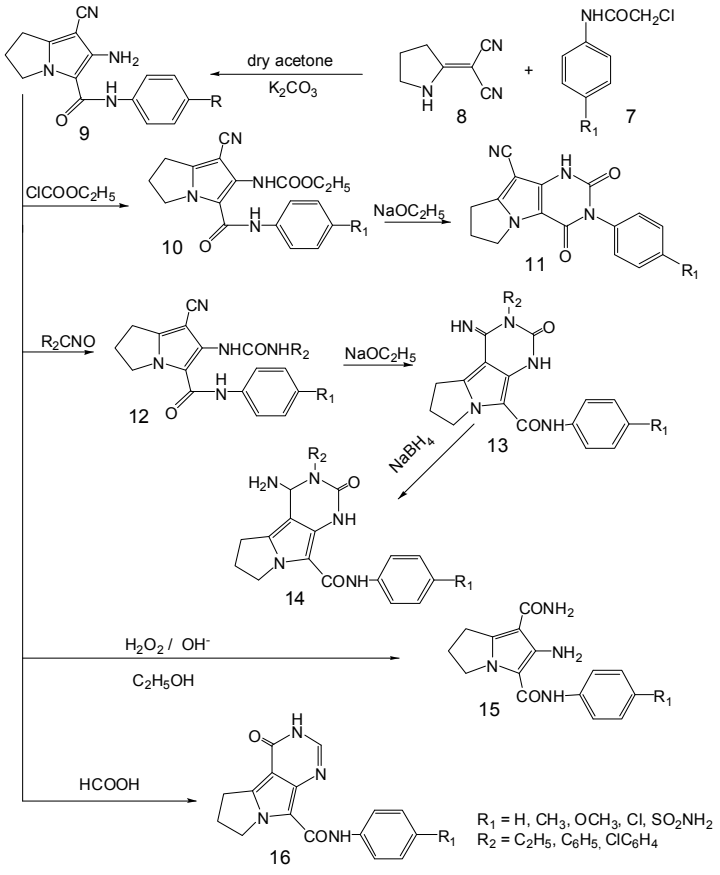




Another group of these compounds represented by the bis-(alkyl carbamoyl) derivative **2** which were found also to have antileukemic activity in addition to its activity against several types of human solid tumors such as Human adenocarcinoma of the colon, oat carcinoma of the lung, and duct cell carcinoma of the breast (12, 13).

Several tricyclic pyrimidopyrrolizines were found to possess antitumor activity. Variable degrees of antitumor activities have been reported for pyrimidopyrrolizine **3**; difference in activity depends on the site of fusion of pyrimidine ring with pyrrolizine nucleus, where certain derivatives showed significant in vivo antitumor activity (14). Pyrimido[b] pyrrolizine **4** represent a new strategy for the synthesis of uracil analogs (15). Compound **5a** showed cumulative toxicity and antitumor activity in mice with B-16 melanoma while compound **5b** (R = $COOC_2H_5$) showed activity against carcinoma 775 (16, 17). The tricyclic pyrolo[3,2-*d*]pyrimidine (n = 2, 3) derivative **6** showed virucidal and antineoplastic activity (18).

Scheme 1



Saudi Pharmaceutical Journal, Vol. 17, No. 1 January 2009

Materials and Methods

Chemistry:

Melting points are uncorrected and were carried out by open capillary tube method using IA 9100MK-Digital Melting Point App. Micro-analyses were carried out at the microanalytical Center, Faculty of Science, Cairo University. Infrared spectra were made on BRUKER Vector 22 (Japan) or Shimadzu 435 or Perkin Elmer 1650 FT, infrared spectrophotometers and expressed in wave number (cm^{-1}) using potassium bromide disc. The proton magnetic resonance ^{1}H-NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}H-NMR spectra were run at 300 MHz in deuterated chloroform ($CDCl_3$) or dimethylsulfoxide (DMSO-d_6). Chemical shifts are quoted in δ units and were related to that of the solvent. Mass spectra were recorded on Fennigan MAT, SSQ 7000, Mass spectrometer, at 70 eV. IUPAC chemical nomenclature were assigned using ACD / Labs program, ACD / Name software, version 1.0 (1995) and CS ChemInfo Pro(tm) version 5.

Synthesis of 2-chloroacetanilide **7a** and its derivatives (19-22), 2-pyrrolidin-2-ylidene-malononitrile **8** (23) were prepared according to the reported procedures. Synthesis of the key compound 2 - amino -1- cyano -3 – phenyl - 6,7 – dihydro - 5*H*-pyrrolizine- 3-carboxamide **9a** and its derivatives were prepared according previously reported procedures (24).

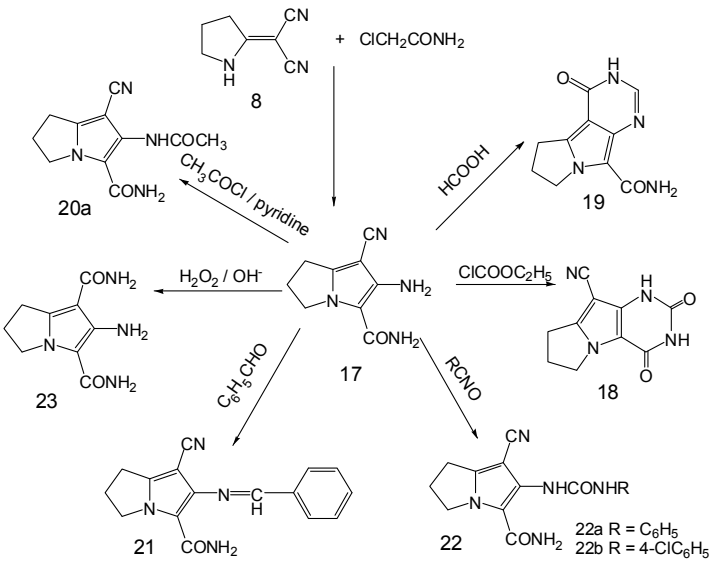
Melting point and spectral analysis were used to confirm the structure of the key compound **9a** excluding the formation of 1,4-diphenyl-piperazine-2,5-dione by-product (24, 25), with the same molecular weight due to condensation of two molecule of the acetanilide **7a** yielded the by-product.

Ethyl (1 - cyano-3 – phenylcarbamoyl -6,7 - dihydro -5H - pyrrolizin-2-yl-carbamate) ***10 a*** *:*

To (1g, 3.8 mmol) of 2-amino-1-cyano-3N-phenyl-6,7-dihydro-5H-pyrrolizine-3-carboxamide **9a** in dry benzene (15 ml), ethyl chloroformate (0.8g, 7.5 mmol.), was added. The reaction mixture was stirred for 3 hour on cold then left to stand over night with stirring at room temperature. The separated product was filtered, washed with water, dried to give 1g (79%), and recrystallized from ethanol-acetone, m.p., 158-60 ° C. IR: 3241, 3178 (NHs), 3072 (Ar-H), 3021 (CH_3), 2855, 2924 (CH_2), 2214 (CN), 1798, 1677 (COs), 1645, 1595, 1548 (NH, C=C), 1315 (C-N), 1254 (C-O). ^{1}H-NMR (DMSO): δ = 1.15 (t, 3H, CH_3), 2.49 (m, 2H, CH_2-6), 3.16 (t, 2H, CH_2-7), 4.08 (q, 2H, CH_2), 4.35 (t, 2H, CH_2-5), 7-7.6 (m, 5H, 5 aromatic protons), 8.13 (s, H, NHCOO), 10.87 (s, H, CONH). MS: m/z (%) = 338 (M, 15), 292 (43), 214 (11), 200 (100)173 (28), 145 (15), 117 (32), 93 (80), 65 (22). Calcd. for $C_{18}H_{18}N_4O_3$ (338.36) C 63.89 H 5.36 N 16.56; found C 64.29 H 5.52 N 17.06%.

Compounds **10b-10e** were similarly prepared (Table 1).

Scheme 2



Ethyl [1-cyano-3-(4-tolylcarbamoyl-6,7- dihydro-5H-pyrrolizin-2-yl-carbamate] ***10 b:***

^{1}H-NMR (DMSO): δ = 2.03 (t, 3H, $\underline{CH_3}$-CH_2), 2.26 (s, 3H, C_6H_4-$\underline{CH_3}$), 2.49 (m, 2H, CH_2-6), 2.96 (t, 2H, CH_2-7) 3.75 (q, 2H, CH_3-$\underline{CH_2}$), 4.25 (q, 2H, CH_2-5), 7.12-7.49 (two d, 4H, 4 aromatic protons), 8.30 (s, H, NH), 10.12 (s, H, CONH-phenyl). MS: m/z (%) = 352 (M, 8.8), 306 (52) 214 (100), 190 (17), 173 (67), 145(34), 105(22), 91(11).

2,4-Dioxo-3-phenyl-2,3,4,6,7,8-hexaydro-1H-pyrimido[4,5-b] pyrrolizine-9-carboonitrile ***11a***:

A solution of ethyl (1-cyano-3- phenyl-carbamoyl- 6,7-dihydro-5H -pyrrolizin-2-yl-carbamate) **10a** (1g, 2.9 mmol.) in sodium ethoxide 1% (15 ml), was refluxed for 3 hours, cooled, neutralized with concentrated hydrochloric acid, then left to stand overnight at room temperature. The solid obtained was filtered, washed with water, and dried to give 0.7g. (81%), recrystallized from ethanol-acetone, m.p., 183-85ºC. IR: 3217 (NHs), 2946 (Ar-H), 2879, 2762 (CH_2), 2227 (CN), 1723, 1673 (COs), 1573, 1493 (NH, C=C), 1350 (C-N). ^{1}H-NMR (DMSO): δ = 2.50 (m, 2H, CH2-7), 3.03 (t, 2H, CH_2-8), 4.18 (t, 2H, CH_2-6), 7.22-7.48 (m, 5H aromatic), 12.03 (s, H, NH) which disappeared on deuteration. MS: m/z (%) = 292 (M, 21), 291 (100), 263 (44), 173 (40), 146 (8), 134 (17), 119 (10), 91 (45). Calcd. for $C_{16}H_{12}N_4O_2$ (292.29) C 65.75 H 4.14 N 19.17; found C 65.90 H 4.50 N 18.97%. Compounds **11b-11e** were similarly prepared (Table 2).

2,4-Dioxo-3-tolyl-2,3,4,6,7,8-hexaydro-1H-pyrimido[4,5-b] pyrrolizine-9-carbonitrile ***11b****:*

^{1}H-NMR (DMSO): δ = 2.09 (s, 3H, CH_3), 2.47 (m, 2H, CH_2-7), 2.95 (t, 2H, CH_2-7), 4.33 (t, 2H, CH_2-7), 7.14-7.46 (two d, 4H, 4-aromatic protons), 9.03 (s, H, NH). MS: m/z (%) = 306 (M, 19), 305 (100), 174 (31), 145 (33), 107 (13), 91 (9).

2,4-Dioxo-3-(4-methoxyphenyl)-2,3,4,6,7,8-hexahy-dro-1H-pyrimido[4,5-b]pyrrolizine-9-carbonitrile ***11c:***

Ms: m/z (%) = 322 (M, 10), 214 (100), 186 (9), 145 (30), 107 (13.8).

2,4-Dioxo-3-(4-methoxyphenyl)-2,3,4,6,7,8-hexahy-dro-1H-pyrimido[4,5-b] pyrrolizine-9-carbo-nitrile ***11 e:***

MS: m/z (%) = 327 (M, 83), 300 (2.4), 214 (100), 127 (51.6), 91 (45).

1-Cyano-2-(3-ethylureido)-3N-phenyl-6,7-dihydro-5H-pyrrolizine-3-carbox-amide ***12a***:

A mixture of 2-amino-1-cyano-3N-phenyl-6,7-dihydro-5H-pyrrolizine-3-carbox-amide **9a** (1g., 3.8 mmol.) in methylene chloride (15ml), ethyl isocyanate (0.27g., 3.8 mmol.), and three drops of triethylamine was refluxed for 12 hours. The solvent was evaporated under reduced pressure. The residue dissolved in acetone, concentrated, set aside where a white crystalline product was formed collected, dried to give 0.81g. (64%), recrystallized from ethanol-acetone m.p., 213-16ºC. IR: 3391, 3340, 3264 (NHs), 2979 (Ar-H), 2944 (CH_3), 2223 (CN), 1674, 1640 (COs), 1602, 1567, 1543 (NH, C=C), 1321 (C-N). ^{1}H-NMR ($CDCl_3$): δ = 1.17 (t, 3H, CH_3), 1.96 (m, 2H, CH_2-6), 3.24 (t, 2H, CH_2-7), 3.65 (t, 2H, CH_2-5), 4.06 (q, 2H, $\underline{CH_2}CH_3$), 4.45 (s, H, NH), 4.66 (s, H, NH), 7.09-7.45 (m, 5H, 5 aromatic protons), 10.04 (s, H, NH). MS: m/z (%) = 337(M, 6), 292 (100), 200 (64), 173 (96), 145 (29), 117 (80), 90 (10). Calcd. for $C_{18}H_{19}N_5O_2$ (337.38) C 64.08 H 5.68 N 20.76; found C 64.39 H 5.60 N 20.74%**.** Compounds **12b-12o** were similarly prepared (Table 3).

1-Cyano-2-(3-phenylureido)-3N-phenyl-6,7-dihydro-5H-pyrrolizine-3-carboxamide ***12f:***

MS: m/z (%) = 385 (M, 100), 292 (37), 264 (29), 174 (11), 146 (9), 92 (10).

1-Cyano-2-(3-(4-chlorophenyl)ureido)-3N-phenyl-6,7-dihydro-5H-pyrrolizine-3-carboxamide ***12k:***

^{1}H-NMR (DMSO): δ = 2.49 (m, 2H, CH2-6), 3.03 (t, 2H, CH2-7), 4.19 (t, 2H, CH2-5), 6.53-7.56 (m, 9H, 9-aromatic protons of the two phenyl rings), 8.57 (s, H, NH), 9.38 (s, H, NH), 12.01 (s, H, NH). On deuteration all the signal of NH protons disappeared. δ = 2.49 (m, 2H, CH_2-6), 2.97 (t, 2H, CH_2-7), 4.17 (CH_2-5), 6.54-7.52 (m, 9H, 9-aromatic protons of the two phenyl rings), MS: m/z (%) = 419 (M, 9), 292 (100), 251 (52), 160 (77).

1-Cyano-2-(3-(4-chlorophenyl)ureido)-3N-tolyl-6,7-dihydro-5H-pyrrolizine-3-carboxamide ***12l:***

1H-NMR (DMSO): δ = 2.32 (s, 3H, CH_3), 2.45 (m, 2H, CH_2-6), 2.90 (t, 2H, CH_2-7), 4.24 (t, 2H, CH_2-5), 4.43 (H, NH), 5.47 (s, H, NH), 7.03-7.75 (m, 8H, 8 aromatic protons), 8.90 (s, H, CONH).

3-Ethyl - 4 –imino -2- oxo- 9N-phenyl -2,3,45,6,7-hexahydro - 1H- pyrimido [5,4-a] pyrrolizine-9-carboxamide ***13 a:***

(1g, 2.9 mmol) of compound **12a** was dissolved

in sodium ethoxide (20 ml, 1%). The reaction mixture was refluxed for 1 hour, then cooled to room temperature and neutralized with dilute hydrochloric acid; the formed precipitate was separated, washed with hot water, dried to give 0.8g, (80%) and recrystallized from ethanol, m.p., 256-9ºC. IR: 3367, 3108 (NHs), 2966 (Ar-H), 1628 (COs), 1593 (C=N), 1556, 1522, 1495 (NH, C=C). ^{1}H-NMR (DMSO): δ = 1.15 (t, 3H, CH_3), 2.56 (m, 2H, CH_2-6), 3.24 (t, 2H, CH_2-5), 3.63 (q, 2H, CH_2), 4.21 (t, 2H, CH_2-7), 5.43 (s, H, C=NH), 7.07-7.61 (m, 5H, C_6H_5), 9.38 (s, H, CONH), 10.33 (s, H, CONH of the anilide). MS: m/z (%) = 337 (M, 34), 266 (40), 245 (34.2), 217 (16.8), 174 (100), 146 (22.6). Calcd. for $C_{18}H_{19}N_5O_2$ (337.38) C 64.08 H 5.68 N 20.76; found C 64.32 H 5.22 N 20.89 %.

Compounds **13b-13o** were similarly prepared (Table 4).

*3-Phenyl-4-imino-2-oxo-9N-phenyl-2,3,4,5,6,7-hexahydro-1H-pyrimido[5,4-a]pyrrolizine-9-carboxamide **13 f** :*

^{1}H-NMR (DMSO): δ = 2.41 (m, 2H, CH_2-6), 2.89 (t, 2H, CH_2-5), 4.41 (t, 2H, CH_2-7), 5.47 (s, H, NH), 6.99-7.66 (m, 10H, 10 aromatic protons), 9.17 (s, H, NH), 9.58 (s, H, CONH of the anilide). MS: m/z (%) = 385 (M, 100), 293 (81.8), 265 (48), 222 (19.5), 119 (17.4), 77 (41.5).

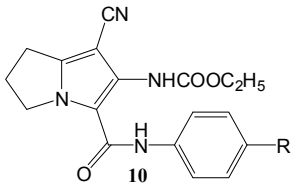
*3- (4-Chlorophenyl) -4- imino -2- oxo-9N-phenyl-2,3,4,5,6,7- hexahydro- 1H- pyrimido [5,4-a] pyrrolizine-9-carboxamide **13 k:***

^{1}H-NMR (DMSO): δ = 2.45 (m, 2H, CH_2-6), 3.01 (t, 2H, CH_2-5), 4.33 (t, 2H, CH_2-7), 7.02-7.66 (m, 10H, 9 aromatic and C=NH), 9-10 (broad band, 2H, NH-1 and CONH of the anilide), disappeared on deuteration. MS: m/z (%) = 419 (M, 65), 385 (92), 327 (60), 300 (34) 293 (78), 265 (60), 173 (39), 145 (31), 119 (59).

*3- (4- Chlorophenyl) -4- imino - 2- oxo - 9N - (4-chlorophenyl)- 2,3,4,5,6,7- hexahydro-1H-pyrimido [5,4-a]pyrrolizine-9-carboxamide **13 n:***

MS: m/z (%) = 454 (M, 14), 327(25), 300 (100), 257(19), 153 (73), 127 (26).

Table 1:



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No.	R	M.P.ºC (Yield %)	Molecular formula (M. wt.)	Microanalysis (%) Calcd.	Found	IR (cm –1)
10b	CH_3	160-2 (85)	$C_{19}H_{20}N_4O_3$ (352.39)	C 64.76 H 5.72 N 15.90	64.44 5.23 16.20	3272, 3127 (NHs), 3076 (Ar-H), 2981 (CH3), 2908 (CH_3), 2199 (CN), 1697, 1657 (COs), 1613, 1570, 1512 (NH, C=C).
c	OCH_3	164-6 (87)	$C_{19}H_{20}N_4O_4$ (368.39)	C 61.95 H 5.47 N 15.21	61.59 5.63 15.32	3283, 3204, 3135 (NHs), 3001 (Ar-H), 2965 (CH_3), 2838 (CH_2), 2208 (CN), 1697 (COs), 1588, 1552, 1506 (NH, C=C), 1303, 1231(C-N, C-O).
d	SO_2NH_2	192-5 (80)	$C_{18}H_{19}N_5O_5S$ (417.44)	C 51.79 H 4.59 N 16.78	51.92 5.12 17.15	3359, 3239, 3383 (NHs), 2999, (CH_3), 2872, (CH_2), 2209 (CN), 1698 (COs), 1305 (SO_2).
e	Cl	187-91 (82)	$C_{18}H_{17}ClN_4O_3$ (372.80)	C 57.99 H 4.60 N 15.03	57.60 4.46 14.56	3316, 3220, (NHs), 3057 (Ar-H), 2971 (CH_3), 2875, 2839 (CH_3), 2220 (CN), 1642 (COs), 1601, 1573, 1516 (NH, C=C), 827, 1029 (C-Cl).

4-Amino-3-ethyl-2-oxo-9N-phenyl-2,3,4,5,6,7-hexahydro-1H-pyrimido[5,4-a]pyrrolizine-9-carboxamide ***14a:***

A mixture of 3-ethyl-4-imino-2-oxo-9N-phenyl-2, 3, 4, 5, 6, 7 – hexahydro - 1*H*-pyrimido [5,4-*a*] pyrrolizine -9-carboxamide **13a** (1g, 2.9 mmol.), and sodium borohydride (0.05g, 1.5 mmol.) in absolute ethanol (20 ml) was stirred occasionally for one hour and left to stand over night at room temperature. The separated crystals were filtered, washed with water, dried to give 0.78g (77%) and recrystallized from ethanol-acetone, m.p., 266-8ºC. IR: 3467, 3437 (NH_2), 3342, 3184 (NHs), 3054 (Ar-H), 1691 (COs), 1552, 1495, 1455 (NH, C=C), 1308 (C-N), 1253 (C-O). 1H – NMR (DMSO): δ = 1.08 (t, 3H, CH_2), 2.46 (m, 2H, CH_2-6), 2.76 (t, H, CH-4), 2.95 (t, 2H, CH_2-5), 3.20 (q, 2H, CH_2), 4.12 (t, H, CH_2-7), 4.26 (t, H, CH), 5-6 (broad band, 2H, NH_2), 7.06-7.57 (m, 7H, 5-aromatic, and 2H of the two CONH groups. MS: m/z (%) = 339 (M, 12), 292 (43), 200(100), 173(14), 117 (32). Calcd. for $C_{18}H_{21}N_5O_2$ (339.39) C 63.70 H 6.24 N 20.64; found C 64.15 H 6.15 N 20.32 %. Compounds **14b-14o** were similarly prepared (Table 5).

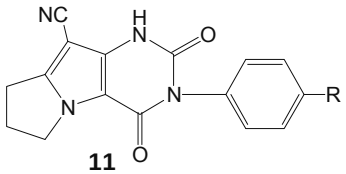
4-Amino-3 – ethyl -2- oxo -9N-(4-methoxy-phenyl)-2, 3, 4, 5, 6, 7- hexahydro- 1H- pyrimido [5,4-a] pyrrolizine-9-carbox-amide ***14c:***

MS: m/z (%) = 369 (M, 9), 368 (36), 339 (23), 313 (45), 285 (27), 212 (59), 118 (24), 92 (100).

4- Amino -3- (4-chlorophenyl) -2- oxo-9N-phenyl-2,3,4,5,6,7-hexahydro-1H-pyrimido[5,4-a]pyrrolizine-9-carboxamide ***14k:***

^{1}H-NMR (DMSO): δ = 2.45 (m, 2H, CH_2-6), 3.20 (t, 2H, CH_2-5), 4.42 (t, 2H, CH_2-7), 6.97-7.65 (m, 10 H, 9- aromatic protons + CH-4), 9.10 (s, H, NH), 9.70 (s, 2H, NH_2), 10.88 (s, H, NH). MS: m/z (%) = 422 (M, 26), 421 (40), 420 (100), 327 (71), 300 (89), 292 (17), 257 (36), 229 (19), 174 (28), 119 (85), 65 (87).

Table 2:



11

No.	R	M.P.ºC (Yield %)	Molecular Formula (M. wt.)	Microanalysis (%) Calcd.	Found	IR(cm-1)
11b	CH_3	190-92 (84)	$C_{17}H_{14}N_4O_2$ (306.32)	C 66.66 H 4.61 N 18.29	66.92 4.93 17.92	3237 (NH), 3063 (Ar-H), 2966 (CH_3), 2923 (CH_2), 2217 (CN), 1646 (COs), 1600, 1514, 1430 (NH, C=C), 1314, 1250 (C-N).
c	OCH_3	197-99 (87)	$C_{17}H_{14}N_4O_3$ (322.32)	C 63.35 H 4.38 N 17.38	63.49 3.98 17.09	3256 (NHs), 3072 (Ar-H), 2997 (CH_3), 2925 ((CH_2), 2218(CN), 1681, 1650 (COs), 1600, 1500 (NH, C=C), 1327 (C-N), 1259 (C-O).
d	SO_2NH_2	228-31 (76)	$C_{16}H_{13}N_5O_4$ S	C 51.75 H 3.53 N 18.86	52.09 4.01 18.80	3367, 3303 (NHs), 3055 (Ar-H), 2962 (CH_2), 2213 (CN), 1662 (COs), 1629, 1597, 1547 (NH, C=C), 1318 (SO_2), 1257 (C-N).
e	Cl	220-22 (71)	$C_{16}H_{11}ClN_4O_2$ (326.74)	C 58.82 H 3.39 N 17.15	58.34 3.88 17.07	3280, NH), 3065 (Ar-H), 2854 (CH_2), 2215 (CN), 1700,1644 (COs), 1597, 1540, 1479 (NH, C=C), 1312 (C-N), 841 (C-Cl).

4- Amino - 3- (4-chlorophenyl) -2- oxo - 9N-(4-methylphenyl) - 2,3,4,5,6,7 - hexahydro -1H-pyrimi-do[5,4-a]pyrrolizine-9-carboxamde ***14l***

^{1}H–NMR (DMSO): δ = 2.25 (s, 3H, CH_3), 2.43 (m, 2H, CH_2-6), 2.89 (t, 2H, CH_2-5), 4.19 (m, 2H, CH_2-7), 4.41 (t, H, CH), 5.43 (s, 2H, NH_2), 7.09-7.74 (m, 8H, 8 aromatic), 9.11 (s, H, CONH), 10.27 (s, H, CONH).

4- Amino – 3 - (4-chlorophenyl) – 2 – oxo - 9N - (4-chlorophenyl -2, 3, 4, 5, 6, 7 – hexahydro - 1H - pyrimido[5,4-a]pyrrolizine-9-carboxamide ***14n:***

^{1}H-NMR (DMSO): δ = 2.41 (m, 2H, CH_2-6), 3.21 (t, 2H, CH_2-5), 4.30 (2H, CH_2-7), 5.45 (s, 2H, NH_2), 7.09-7.73 (m, 10H, 8 aromatic protons, CH-4, NH), 9.6 (s, H, NH). MS: m/z (%) = 457 (M, 15), 456 (23), 327 (24), 300 (100), 257 (19), 174 (8), 153 (9) 146 (9), 112 (7).

2 – Amino - 3N - phenyl-6,7-dihydro-5H-pyrrolizine-1,3-dicarboxamide ***15 a:***

(1g, 3.8 mmol) of 2-amino-1-cyano-3N-phenyl-6,7-dihydro-5H-pyrrolizine-3-carboxamide **9a** was dissolved in ethanol (20 ml) and sodium hydroxide 25% (3 ml) and hydrogen peroxide 30% (10 ml) were added. The reaction mixture was stirred for 3 hours, H_2SO_4 (5%) added until exactly neutralized, the solution was concentrated, cooled, the formed crystals were filtered, washed with water, dried to give 0.85g (80%) and recrystallized from ethanol-acetone m.p., 186-8ºC. IR: 3585, 3473, 3316 (NHs), 2930 (CH_2), 1664 (COs), 1594, 1528, 1482 (NH, C=C), 1313 (C-N). ^{1}H-NMR ($CDCl_3$): δ = 2.55 (m, 2H, CH_2-6), 3.04 (t, 2H, CH_2-7), 3.72 (s, 2H, NH_2), 4.48 (t, 2H, CH_2-5), 7.06-7.58 (m, 5H, 5 aromatic protons), 9.19 (s, 2H, $CONH_2$), 9.50 (s, H, NH). MS: m/z (%) = 284 (M, 50), 192 (90), 175 (100), 147 (26), 119 (19.5), 91 (17.3), 77 (17). Calcd. For $C_{15}H_{16}N_4O_2$ (284.31) C 63.37 H 5.67 N 19.71; found C 63.09 H 5.74 N 19.43%. Compounds **15b-15d** were similarly prepared (Table 6).

2- Amino- 3N - (4-methylphenyl) - 6,7 – dihydro-5H-pyrrolizine-1,3-dicarboxamide ***15 b:***

^{1}H-NMR (DMSO): δ = 2.26 (s, 3H, CH_3), 2.39 (m, 2H, CH_2-6), 3.05 (t, 2H, CH_2-7), 4.23 (t, 2H, CH_2-5), 6.11 (s, 2H, NH_2), 6.54 (s, 2H, $CONH_2$), 7.09, 7.50 (two d, 4H, 4aromatic protons), 9.65 (s, H, CONH of the anilide MS: m/z (%) = 298 (M, 43), 192 (100), 165 (63), 149 (78).

4- Oxo- 9N-phenyl -4, 5, 6, 7- tetrahydro-3H-pyrimido[5,4-a]pyrrolizine-9-carboxamide ***16 a:***

To (1g, 3.8 mmol) of compound 2-amino-1-cyano -3N-phenyl-6,7-dihydro-5H-pyrrolizine-3-carboxamide **9a**, (10.9g, 10 ml) of 90 % formic acid was added. The reaction mixture was heated on a water bath at 100 ºC for three hours and cooled, 10 % sodium hydroxide solution was added slowly, until the mixture was just alkaline to litmus. The formed precipitate was filtered, washed with water dried to give 0.94 g., (85%) and recrystallized from ethanol-acetone m.p., 282-5ºC. IR: 3373, 3143 (NHs), 3059 (Ar-H), 2778 (CH2), 1691, 1667 (COs), 1619 (C=N), 1570, 1537, 1436 (NH, C=C), 1299 (C-N). ^{1}H-NMR (DMSO): δ = 2.57 (m, 2H, CH_2-6), 3.11 (t, 2H, CH_2-5), 4.29 (t, 2H, CH_2-7), 7.28 (s, H, CONH), 7.47-7.58 (m, 6H, C_6H_5 + N=CH), 8.23 (s, H, NH). MS: m/z (%) = 294 (M, 25), 277 (100), 249 (31), 147 (4), 118 (10), 104 (9), 91 (9), 77 (53). Calcd. for C16H14N4O2 (294.31) C 65.30 H 4.79 N 19.04; found C 65.13 H 4.95 N 19.43%. Compounds **16b** and **16c** were similarly prepared (Table 7).

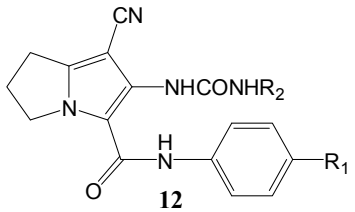
4-Oxo-9N-(4-methylphenyl)-4,5,6,7-tetra-hydro-3H-pyrimido[5,4-a]pyrrolizine-9-carboxamide ***16 b:***

^{1}H-NMR (DMSO): δ = 2.37 (m, 5H, CH_3, CH_2-6), 3.31 (t, 2H, CH_2-5), 4.23 (t, 2H, CH_2-7), 7.08-7.45 (m, 5H, 4 aromatic protons and CH), 7.55 (s, H, NH), 8.16 (s, H, NH). MS: m/z (%) = 308 (M, 18), 291 (100), 214 (81), 186 (14), 145 (22), 119 (9), 92 (6).

2- Amino -1- cyano -6,7- dihydro-5H-pyrrolizine- 3-carboxamide ***17:***

A mixture of 2- pyrrolidin -2- ylidene-malononitrile **8** (1g, 7.5 mmol.), powdered anhydrous potassium carbonate (2.1g, 15 mmol.) and 2- chloroacetamide (0.7g, 7.5 mmol.) in dry acetone (50 ml) was stirred under reflux for 24 hour, filtered. The filtrate was concentrated and set aside to cool, where white crystals were formed, collected, dried, to give 1.1 g. (76%) and recrystallized from ethanol, m.p., 219-21ºC. IR: 3365, 3190 (NHs), 2976, 2876 (CH_2), 2211 (CN), 1697 (CO), 1583, 1447 (NH, C=C), 1304 (C-N). ^{1}H-NMR (DMSO): δ = 2.34 (m, 2H, CH_2-6), 2.81 (t, 2H, CH_2-7), 4.16 (t, 2H, CH_2-5), 5.50 (s, 2H, NH_2), 6.67 (s, 2H, $CONH_2$). MS: m/z (%) = 190 (M, 74), 173 (52), 145 (61), 117. (100), 92 (20). Calcd. for $C_9H_{10}N_4O$ (190.20) C 56.83 H 5.30 N 29.46; found C 57.26 H 5.54 N 29.65%.

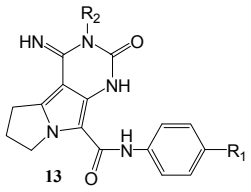
Table 3:



12

No.	R1	R2	M.P.°C (Yield %)	Molecular formula (M. wt.)	Microanalysis (%)		IR (cm-1)
					Calcd.	Found	
12b	CH_3	C_2H_5	221-23 (63)	$C_{19}H_{21}N_5O_2$ (351.40)	C64.94 H 6.02 N19.93	64.81 5.50 20.08	3395, 3343, 3264 (NHs), 2981, 2871 (CH_3, CH_2), 2222 (CN), 1671, 1641 (COs), 1600 (C=C).
c	OCH_3	C_2H_5	229-30 (65)	$C_{19}H_{21}N_5O_3$ (367.40)	C62.11 H 5.76 N19.06	62.51 5.45 19.43	3388, 3336, 3259 (NHs), 2976 (Ar-H), 2942 (CH_3), 2872 (CH_2), 2221 (CN), 1671 (COs).
d	Cl	C_2H_5	249-51 (61)	$C_{18}H_{18}ClN_5O_2$ (371.82)	C58.14 H 4.88 N18.84	58.49 5.00 18.80	3362, 3299 (NHs), 3068 (Ar-H), 2962 (CH_3), 2223 (CN), 1671, 1646 (Cos), 837 (C-Cl).
e	SO_2NH_2	C_2H_5	254-6 (62)	$C_{18}H_{20}N_6O_2S$ (416.45)	C51.91 H 4.84 N20.18	51.92 5.10 20.32	3505, 3414, 3332, 3185 (NHs), 2956 (CH_3), 2891 (CH_2), 2210 (CN), 1640 (COs), 1302 (SO_2).
f	H	C_6H_5	228-30 (79)	$C_{22}H_{19}N_5O_2$ (385.42)	C68.56 H 4.97 N18.17	68.94 5.14 17.82	3405, 3310, 3270 (NHs), 3061 (Ar-H), 2977 (CH_2), 2224 (CN), 1665, 1641 (COs),
g	CH_3	C_6H_5	233-5 (79)	$C_{23}H_{21}N_5O_2$ (399.45)	C69.16 H 5.30 N17.53	68.69 4.94 17.09	3398, 3335, 3294 (NHs), 3030, (Ar-H), 2957 (CH_3), 2863 (CH_2), 2223 (CN), 1656 (COs),1317(C-N).
h	OCH_3	C_6H_5	241-3 (81)	$C_{23}H_{21}N_5O_3$ (415.44)	C66.49 H 5.09 N16.86	66.51 5.19 17.11	3411, 3295 (NHs), 3032 (Ar-H), 2996 (CH_3), 2931 (CH_2), 2223 (CN), 1775, 1639 (COs).
i	Cl	C_6H_5	261-3 (72)	$C_{22}H_{18}ClN_5O_2$ (419.86)	C63.32 H4.33 N16.38	62.93 4.32 16.68	3362, 3330, 3068 (NHs), 2962 (Ar-H), 2222 (CN), 1671, 1646 (COs), 838 (C-Cl).
j	SO_2NH_2	C_6H_5	270-3 (69)	$C_{22}H_{20}N_6O_4S$ (464.50)	C57.18 H4.71 N17.62	56.89 4.34 18.09	3385, 3338, 3259 (NHs), 2979, 2942 (Ar-H), 2873 (CH_2), 2223 (CN), 1670, 1641 (COs), 1315 (SO_2).
k	H	C_6H_4Cl	234-6 (70)	$C_{22}H_{18}ClN_5O_2$ (419.86)	C 62.93 H 4.32 N16.68	62.67 4.69 16.38	3357, 3283, 3205 (NHs), 3034 (Ar-H), 2983, 2918 (CH_2), 2216 (CN), 1704, 1667(COs), 822 (C-Cl).
l	CH_3	C_6H_4Cl	243-5 (81)	$C_{23}H_{20}ClN_5O_2$ (433.89)	C 63.67 H 4.65 N16.14	63.19 4.94 15.75	3408, 3326, 3293 (NHs), 2999 (CH_2), 2867 (CH_2), 2221 (CN), 1890, 1657 (COs), 812 (C-Cl).
m	OCH_3	C_6H_4Cl	248-50 (84)	$C_{23}H_{20}ClN_5O_3$ (449.89)	C 61.40 H 4.48 N 15.57	61.49 4.46 15.77	3330, 3293 (NHs), 3076 (Ar-H), 2955 (CH_3), 2839 (CH_2), 2220 (CN), 1721, 1661 (COs), 822 (C-Cl).
n	Cl	C_6H_4Cl	267-9 (80)	$C_{22}H_{17}Cl_2N_6O_2$ (454.31)	C 58.16 H 3.77 N 15.42	58.06 3.98 15.32	3385, 3338, 3260 (NHs), 2979 (Ar-H), 2941 (CH3), 2874 (CH_2), 2223 (CN), 1670, 1641 (COs), 1315 (C-N), 829, 775 (C-Cl).
o	SO_2NH_2	C_6H_4Cl	265-7 (75)	$C_{22}H_{19}ClN_6O_4S$ (498.94)	C52.96 H 3.84 N16.84	53.04 3.54 16.69	3445, 3240, 3116 (NHs), 3039 (Ar-H), 2917 (CH_2), 2216 (CN), 1722, 1666 (COs), 1320 (SO_2), 819 (C-Cl).

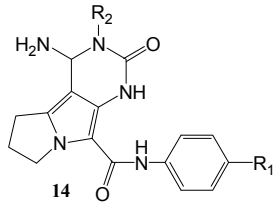
Table 4:



13

No.	R1	R2	M.P.°C (Yield %)	Molecular Formula (M. wt.)	Microanalysis (%)		IR (cm-1)
					Calcd.	Found	
13b	CH_3	C_2H_5	260-3 (63)	$C_{19}H_{21}N_5O_2$ (351.40)	C 64.94 H 6.02 N 19.93	65.00 6.15 20.30	3610, 3473, 3163 (NHs), 2973 (CH3), 1651 (COs), 1300 (C-N).
c	OCH_3	C_2H_5	271-2 (65)	$C_{19}H_{21}N_5O_3$ (367.40)	C 62.11 H 5.76 N 19.06	62.11 5.84 19.67	3376, 3265 (NHs), 3038 (Ar-H), 2880 (CH3), 2787 (CH_2), 1723, 1638 (COs), 1510, 1451 (NH), 1319 (C-N), 1245 (C-O).
d	Cl	C_2H_5	281-3 (61)	$C_{18}H_{18}ClN_5O_2$ (371.82)	C 58.14 H 4.88 N 18.84	58.00 4.48 18.97	3468, 3423, 3235, 3134 (NHs), 3049 (Ar-H), 1653 (COs), 1614, 1552 (C=C).
e	SO_2NH_2	C_2H_5	288-90 (62)	$C_{18}H_{20}N_6O_4S$ (416.46)	C 51.91 H 4.84 N 20.18	51.92 5.12 20.32	3610, 3335, 3162 (NHs), 2973 (Ar-H), 1652 (COs), 1598, 1551, 1514 (NH, C=C), 1301 (C-N).
f	H	C_6H_5	265-7 (79)	$C_{22}H_{19}N_5O_2$ (385.42)	C 68.56 H 4.97 N 18.17	68.29 4.96 17.93	3463, 3356, 3299 (NHs), 3056 (Ar-H), 1650 (COs), 1619, 1494.
g	CH_3	C_6H_5	271-3 (79)	$C_{23}H_{21}N_5O_2$ (399.44)	C 69.16 H 5.30 N N17.53	69.14 5.64 17.58	3470, 3119 (NHs), 3040 (Ar-H), 2914 (CH_3), 1649 (COs), 1616 (C=N), 1596, 1545 (NH, C=C).
h	OCH_3	C_6H_5	279-80 (81)	$C_{23}H_{21}N_5O_3$ (415.44)	C 66.49 H 5.09 N 16.86	66.84 5.10 16.64	3446, 3327, 3201 (NHs), 3048 (Ar-H), 2977 (CH3), 1653, 1626 (COs).
i	Cl	C_6H_5	290-2 (72)	$C_{22}H_{18}ClN_5O_2$ (419.86)	C 62.93 H 4.32 N 16.68	62.88 4.43 16.41	3435, 3135 (NHs), 1671, 1646 (COs), 1618, 1555, 1455 (NH, C=C, C=N), 1317, 1255 (C-N).
j	SO_2NH_2	C_6H_5	311-3 (69)	$C_{22}H_{20}N_6O_4S$ (464.50)	C 56.89 H 4.34 N 18.09	56.43 4.14 17.62	3474, 3431, 3130 (NHs), 3065, 3025 (Ar-H), 1682, 1646 (COs), 1616, 1546, 1490, 1453 (NH, C=C, C=N), 1253 (SO_2).
k	H	C_6H_4Cl	284-6 (70)	$C_{22}H_{18}ClN_5O_2$ (419.86)	C62.93 H 4.32 N 16.68	63.25 4.39 16.75	3468, 3423 3237 (NHs), 3049 (Ar-H), 1652 (COs), 1614 (C=N), 1551, 1460 (NH, C=C), 1310, 1253 (C-N), 769 (C-Cl).
l	CH_3	C_6H_4Cl	286-7 (81)	$C_{23}H_{20}ClN_5O_2$ (433.89)	C63.67 H 4.65 N16.14	63.96 4.18 16.06	3241, 3140 (NHs), 2992 (Ar-H), 2960 (CH_3), 2885 (CH2), 1727, 1643 (COs), 1615, 1514, (NH, C=C), 1308 (C-N), 753 (C-Cl).
m	OCH_3	C_6H_4Cl	297-9 (84)	$C_{23}H_{20}ClN_5O_3$ (449.89)	C61.40 H 4.48 N15.57	61.70 4.32 15.83	3471, 3425, 3326 (NHs), 3035 (Ar-H), 1652 (COs), 1618, 1543, (NH, C=C), 1250, (C-N), 767 (C-Cl).
n	Cl	C_6H_4Cl	321-3 (80)	$C_{22}H_{18}Cl_2N_5O_2$ (454.31)	C58.16 H 3.77 N15.42	58.31 4.12 15.77	3476, 3365, 3267 (NHs), 3053 (Ar-H), 1624 (COs), 1618, 1543, 1490, 1457 (NH, C=C, N-C=O),1767, 1088 (C-Cl).
o	SO_2NH_2	C_6H_4Cl	312-4 (75)	$C_{22}H_{19}ClN_6O_4S$ (498.94)	C52.96 H 3.84 N16.84	52.95 4.17 16.75	3384, 3295, 3186 (NHs), 3013 (Ar-H), 1649 (COs), 1263 (SO_2), 768 (C-Cl).

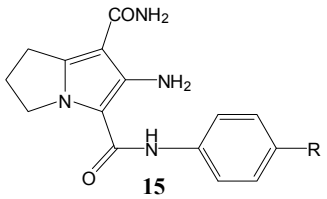
Table 5:



14

No.	R1	R2	M.P.°C (Yield %)	Molecular formula (M. wt.)	Microanalysis (%)		IR (cm-1)
					Calcd.	Found	
14b	CH_3	C_2H_5	272-4 (79)	$C_{19}H_{23}N_5O_2$ (353.42)	C 64.57 H 6.56 N 19.82	64.25 6.84 19.54	3384, 3295, 3186 (NHs), 3013 (Ar-H), 2959 (CH_2), 1649 (CO), 1428, 1402 (NH, C=C).
c	OCH_3	C_2H_5	276-9 (83)	$C_{19}H_{23}N_5O_3$ (369.42)	C 61.77 H 6.28 N 18.96	61.58 6.20 19.43	3320, 3174 (NHs), 1645 (COs), 1547, 1492, 1457 (NH, C=C).
d	Cl	C_2H_5	288-91 (77)	$C_{18}H_{20}ClN_5O_2$ (373.84)	C 57.83 H 5.39 N 18.73	58.07 5.59 18.97	3402, 3368 (NHs), 3097(Ar-H),2982 (CH_3),1630(COs), 1583, 1557, 1521 (NH, C=C), 1304 (C-N), 1230 (C-O).
e	SO_2NH_2	C_2H_5	307-10 (68)	$C_{18}H_{22}N_6O_4S$ (418.47)	C 51.66 H 5.30 N 20.08	51.92 5.12 20.32	3476, 3373, 3267 (NHs), 3059 (Ar-H), 1630(COs),1312 (SO_2).
f	H	C_6H_5	276-7 (72)	$C_{22}H_{21}N_5O_2$ (387.43)	C 68.20 H 5.46 N 18.08	68.18 5.84 17.86	3444 broad band (NHs), 1622 (COs), 1546, 1492 (NH, C=C), 1302 (C-N).
g	CH_3	C_6H_5	283-5 (71)	$C_{23}H_{23}N_5O_2$ (401.46)	C 68.81 H 5.77 N 17.44	69.09 5.79 17.58	3445 (broad band NHs), 2921 (CH_3), 1623 (COs), 1544, 1491, 1458 (NH)
h	OCH_3	C_6H_5	297-8 (79)	$C_{23}H_{23}N_5O_3$ (417.46)	C 66.17 H 5.55 N 16.78	66.20 6.00 16.98	3243, 3180 3129 (NHs), 3070, 3031 (Ar-H), 1671, 1640 (COs), 1315 (C-N).
i	Cl	C_6H_5	301-3 (70)	$C_{22}H_{20}ClN_5O_2$ (421.88)	C 62.97 H 4.85 N 16.21	62.63 4.78 16.60	3491 (NH_2), 3119 (NHs), 3041 (Ar-H), 2914(CH2),1649(COs, 1596, 1545, 1455 (NH, C=C), 1305 (C-N), 1251 (C-O), 774 (C-Cl).`
j	SO_2NH_2	C_6H_5	315-17 (71)	$C_{22}H_{22}N_6O_4S$ (466.51)	C 56.64 H 4.75 N 18.01	57.20 5.21 17.58	3476, 3372, 3266 (NHs), 1628 (COs), 1594, 1548, 1499 (NH, C=C), 1313 (SO2).
k	H	C_6H_4Cl	295-7 (69)	$C_{22}H_{20}ClN_5O_2$ (421.88)	C 62.63 H 4.78 N 16.60	62.91 4.89 16.62	3435, 3135 (NHs), 1646 (COs), 1618, 1555, 1455 (NH, C=C), 770 (C-Cl).
L	CH_3	C_6H_4Cl	317-19 (76)	$C_{23}H_{22}ClN_5O_2$ (435.91)	C 63.37 H 5.09 N 16.07	63.70 5.05 15.75	3473, 3431, 3130 (NHs), 3065, 3025 (Ar-H), 1682, 1647 (COs), 1616, 1546, 1453 (NH, C=C), 1315 (C-N), 770 (C-Cl).
M	OCH_3	C_6H_4Cl	332-4 (82)	$C_{23}H_{22}ClN_5O_3$ (451.90)	C 61.13 H 4.91 N 15.50	61.59 4.46 15.62	3476, 3428, 3241 (NHs), 3065 (Ar-H), 1652 (COs), 1619, 1554, 1491 (NH, C=C), 766 (C-Cl).
N	Cl	C_6H_4Cl	345-8 (65)	$C_{22}H_{19}Cl_2N_5O_2$ (456.32)	C 57.91 H 4.20 N 15.35	57.69 4.39 14.96	3494, 3121(NH_2, NHs), 3043 (Ar-H), 1649 (COs), 1616, 1598, 1546 (NH, C=C), 1307 (C-N), 1251 (C-O).
o	SO_2NH_2	C_6H_4Cl	351-3 (67)	$C_{22}H_{21}ClN_6O_4S$ (500.96)	C 52.75 H 4.23 N 16.78	53.05 4.00 17.07	3477, 3373, 3267 (NHs), 3092, 3067 (Ar-H), 2924 (CH2), 1627 (COs), 1594, 1555, 1498 (C=C, NH), 1312 (SO_2), 834 (C-Cl).

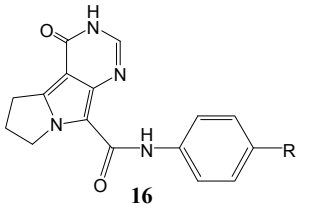
Table 6:



15

No.	R	M.P.°C (Yield %)	Molecular formula (M. wt.)	Microanalysis (%)		IR(cm-1)
				Calcd.	Found	
15b	CH_3	191-2 (78)	$C_{16}H_{18}N_4O_2$ (298.34)	C 64.41 H 6.08 N 18.78	64.57 5.75 18.80	3394, 3317, 3156 (NHs), 3043 (Ar-H), 2974 (CH_3), 2920 (CH2), 1690, 1665 (COs), 1612, 1568, 1533 (NH, C=C).
c	OCH_3	196-8 (82)	$C_{16}H_{18}N_4O_3$ (314.34)	C 61.13 H 5.77 N 17.82	61.22 6.00 17.62	3479, 3430, 3243, 3129 (NHs), 3066 (Ar-H), 2976 (CH3), 2853 (CH2), 1652 (COs), 1308 (C-N).
d	Cl	206-8 (76)	$C_{15}H_{15}ClN_4O_2$ (318.76)	C 56.52 H 4.74 N 17.58	56.21 4.48 17.58	3509, 3430, 3397 (NHs), 3100 (Ar-H), 1652 (COs), 1609, 1543, 1492 (NH, C=C), 1301 (C-N), 831 (C-Cl).

Table 7:



16

No.	R	M.P.°C (Yield %)	Molecular formula (M. wt.)	Microanalysis (%)		IR(cm-1)
				Calcd.	Found	
16b	CH_3	291-3 (87)	$C_{17}H_{16}N_4O_2$ (308.33)	C 66.22 H 5.23 N 18.17	66.32 5.04 17.93	3394, 3156 (NHs), 3042 (Ar-H), 2974 (CH3), 2920 (CH2), 1690, 1665 (COs), 1612, 1568, 1533 (NH, C=C), 1392 (C-N).
c	OCH_3	296-2 (87)	$C_{17}H_{16}N_4O_3$ (324.33)	C 62.95 H 4.97 N 17.27	63.24 4.57 17.07	3278, 3142 (NHs), 2973 (CH2), 1680, 1667) (COs), 1618, 1570, 1530, 1490 (NH, C=C), 1299 (C-N).

9-Cyano-2,3,4,5,6,8-hexahydro-1H-pyrimido[4,5-b]pyrrolizine-2,4-dione ***18:***

A mixture of 2-amino-1-cyano-6,7-dihydro - 5H- pyrrolizine -3-carboxamide **17** (1g, 5.3 mmol.), anhydrous potassium carbonate (1.4g, 10 mmol.) and ethyl chloroformate (1.15g, 10.6 mmol.) in dry acetone (20 ml) was refluxed for 24 hours, filtered while hot, concentrated and set aside to cool, where a white crystals were formed, collected, dried to give 0.72g (63%) and recrystallized from ethanol-acetone, m.p., 224-6ºC. IR: 3357, 3237 (NHs), 2998, 2870 (CH2), 2206 (CN), 1696 (CO), 1628, 1623, 1578, 1444 (NH, C=C), 1303 (C-N), 1159 (C-O). ^{1}H-NMR (DMSO): $\delta = 1.92$ (m, 2H, CH_2-7), 2.95 (t, 2H, CH_2-8), 3.71 (t, 2H, CH_2-6), 7.29 (s, NH-1), 7.54 (s, NH-3). MS: m/z (%) = 216 (M, 76), 174 (74),

119 (22), 93 (100), 85 (16), 71 (24), 65 (25), 55 (29). Calcd. for $C_{10}H_8N_4O_2$ (216.20) C 55.55 H 3.73 N 25.91; found C 55.15 H 4.20 N 25.81%.

4- Oxo -4, 5, 6, 7- tetrahydro- 3H-pyrimido[5,4-a]pyrrolizine-9-carboxamide ***19:***

A mixture of (1g, 5.3 mmol.) of 2-amino-1-cyano-6,7-dihydro-5H-pyrrolizine-3-carbox-amide **17** and (10.9g, 10 ml) of 90 % formic acid was treated using the same procedure adopted for the synthesis of compound **16a**. Compound **19** was obtained in a yield of 0.86g (75%) and recrystallized from ethanol, m.p., 272-4ºC, IR: 3384, 3295, 3186 (NHs), 3013 (CH), 2959 (CH2), 1649 (CONH), 1428, 1402 (NH, C=N, C=C), 1263 (C-N). ^{1}H-NMR: δ = 1.92 (m, 2H, CH_2-6), 2.92 (t, 2H, CH_2-5), 3.67 (t, 2H, CH_2-7), 4.31 (s, 2H, NH_2), 7.39 (s, H, NH), 7.54 (s, H, CH). MS: m/z (%) = 218 (M, 18), 175 (19), 161 (22), 145 (29), 117 (49), 91 (59), 65 (100). Calcd. for $C_{10}H_{10}N_4O_2$ (218.21) C 55.04 H 4.62 N 25.68; found C 55.35 H 4.31 N 25.60%.

2-Acetamido-1-cyano-6,7-dihydro-5H-pyrrolizine-3-carboxamide ***20 a*****:**

A mixture of 2-amino-1-cyano-6,7-dihydro-5H-pyrrolizine -3- carboxamide **17** (1g, 5.3 mmol.), acetyl chloride (0.82g, 10.6 mmol.) in dry pyridine (20 ml) was stirred for one hour and left to stand over night at room temperature. The separated product was filtered, washed with water, dried to give 1g. (81%) and recrystallized from ethanol, m.p., 278-80ºC. IR: 3413, 3332, 3290 (NHs), 2954 (CH_3), 2889 (CH_2), 2209 (CN), 1642 (CO), 1612, 1583, 1549 (NH, C=C), 1300 (C-N). ^{1}H-NMR (DMSO): δ = 2.56 (s, 3H, CH_3), 2.42 (m, 2H, CH_2-6), 3.14 (t, 2H, CH_2-7), 4.28 (t, 2H, CH_2-5), 7.57 (s, 2H, $CONH_2$), 9.58 (s, H, NHCOCH3). MS: m/z (%) = 232 (M, 5.5), 215 (100), 190 (9), 174 (37), 146 (30), 117 (15), 92 (16). Calcd. for $C_{11}H_{12}N_4O_2$ (232.24) C 56.89 H 5.21 N 24.12; found C 56.70 H 5.54 N 24.18%.

Compound **20b** was similarly prepared using benzoyl chloride.

2-Benzoylamido-1-cyano-6,7-dihydro-5H-pyrrolizine-3-carboxamide ***20 b:***

This compound was obtained as a solid in 87% yield, m.p., 293-95ºC IR: 3327 (NH_2), 3193, 3134 (NHs), 3034 (Ar-H), 2211 (CN), 1648 (COs), 1595, 1554, 1494 (NH, C=C), 1310 (C-N). MS: m/z (%) = 294 (M, 27), 188 (15), 175 (100), 147 (81). Calcd. for $C_{16}H_{14}N_4O_2$ (294.31) C 65.30 H 4.79 N 19.04; found C 64.82 H 4.58 N 19.43%.

2-(Benzylideneamino)-1-cyano-6,7-dihydro-5H-pyrrolizine-3-carboxamide ***21:***

A mixture of (1g 5.3 mmol.) of 2-amino-1-cyano-6,7-pyrrolizine-3-carboxamide **17** (5.3 mmol), benzaldehyde (0.56g, 10.5 mmol.) in absolute ethanol (10 ml) and in presence of glacial acetic acid (0.5 ml) was refluxed for 4 hours. The reaction mixture was then concentrated, set aside to cool, where white crystals were formed, collected, dried to give 1.1g (86%) and recrystallized from ethanol, m.p., 253-5ºC. IR: 3365, 3190 (NHs), 2976 (Ar-H), 2876 (CH2), 2211 (CN), 1697 (CO), 1583, 1447 (NH, C=C), 1304 (C-N). ^{1}H-NMR ($CDCl_3$): δ = 2.47 (m, 2H, CH_2-6), 2.96 (t, 2H, CH_2-7), 3.64 (s, 2H, NH_2), 4.35 (t, 2H, $\underline{CH_2}$-5), 7.05-7.55 (m, 5H, aromatic), 9.38 (CH). MS: m/z (%) = 277 (M-1, 90), 276 (100), 190 (52), 173 (60), 145 (69), 104 (65). Calcd. for $C_{16}H_{14}N_4O$ (278.31) C 69.05 H 5.07 N 20.13; found C 68.61 H 4.79 N 20.30%.

1-Cyano-2(3-phenylureido)-6,7-dihydro-5H-pyrrolizine-3-carboxamide ***22 a:***

A mixture of 2-amino-1-cyano-6,7-dihydro-5H-pyrrolizine-3-carboxamide **17** (1g, 5.3 mmol.), and phenyl isocyanate (0.63g, 5.3 mmol.), in methylene chloride (20 ml) and 3 drops of triethylamine (0.2ml) was stirred under reflux for 12 hours. The solvent was evaporated; the residue was dried to give 1.2g (73%) and recrystallized from ethanol to give a white crystalline product, m.p., 257-60ºC. IR: 3382 (NH2), 3193, 3135 (NHs), 3062 (Ar-H), 2212 (CN), 1697, 1648 (COs), 1595, 1555, 1494 (NH, C=C), 1310 (C-N). ^{1}H-NMR (DMSO): δ = 2.38 (m, 2H, CH2-6), 2.87 (t, 2H, CH_2-7), 4.25 (t, 2H, CH_2-5), 5.46 (s, 2H, NH_2), 7.03-7.58 (m, 6H, 5 aromatic + CO$\underline{NH}$), 9.17 (s, H, CONH). MS: m/z (%) = 309 (M, 3), 308 (13), 216 (36), 174 (64), 146 (24), 119 (10), 93(100). Calcd. for $C_{16}H_{15}N_5O_2$ (309.32) C 62.13 H 4.89 N 22.64; found C 61.76 H 4.46 N 22.82%.

Compound **22a** was similarly prepared using 4-chlorophenyl isocyanate.

1-Cyano-2 (3- (4-chlorophenyl)ureido)-6,7-dihydro -5 H- pyrrolizine -3- carboxamide ***22 b:***

Compound **22b** was obtained as a solid in 79 % yield, m.p., 271-3 ºC, IR: 3503, 3412, 3183 (NHs), 3330 (NH2), 2955 (Ar-H), 2890 (CH_2), 2210 (CN), 1640 (COs), 1611, 1582 (NH, C=C), 762, 1087 (C-Cl). MS: m/z = 343(M, 12), 218 (9), 190 (41), 147 (100), 122 (13). Calcd. for $C_{16}H_{14}ClN_5O_2$ (343.77) C 55.90 H 4.10 N 20.37; found C 56.21 H 4.48 N 20.32%.

*2-Amino-6,7-dihydro-5H-pyrrolizine-1,3-dicarboxamide **23**:*

The procedure adopted for the synthesis of compound **15a** was applied using a mixture of 2-amino-1-cyano-6,7-dihydro-5H-pyrrolizine-3-carboxamide **17** (1g, 5.3 mmol.), sodium hydroxide (3 ml, 25 %) and hydrogen peroxide (10 ml, 30 %) in ethanol (20 ml). Compound **23** was obtained in a yield of (0.8, 73 %) and recrystallized from ethanol-acetone m.p., 256-9ºC. IR: 3475, 3318, 3170 (NHs), 2972 (CH_2), 1644 (2$\underline{CO}NH_2$), 1601, 1567, 1533 (NH, C=C), 1379 (C-N) and disappearance of cyano group absorption band. ^{1}H-NMR (DMSO): δ = 2.02 (m, 2H, CH_2-6), 3.33 (t, 2H, CH_2-7), 3.79 (t, 2H, CH_2-5), 4.28 (s, 2H, NH_2), 7.29 (s, 2H, H_2NCOC-1), 7.50 (s, 2H, H_2NCOC-3). MS: m/z (%) = 208 (M, 2.6), 190 (72), 173 (57), 145 (69), 117 (100), 92 (30). Calcd. for $C_9H_{12}N_4O_2$ (208.22) C 51.92 H 5.81 N 26.9; found C 51.92 H 6.12 N 26.91 %.

Results and Discussion

1. Chemistry:

Mitomycin C is a potent DNA crosslinker. A single cross link per genome has shown to be effective in killing bacteria. This is accomplished by intracellular reductive activation to bifunctional or trifunctional alkylating agent (26), followed by two N-alkylation. Both alkylation are sequence specific for a guanine nucleoside in the sequence 5`CpG 3` (27). Carbamoyl group in mitomycin C beside aziridine ring and quinone structure are responsible for its alkylating activity of mitomycin C. In the present work trials were done to design new pyrrolizine derivatives bearing a reactive carbamoyl moiety aiming for obtaining antineoplastic drugs. Compound **10a** and its derivatives were synthesized having ethoxy carbamoyl group and different substituents at the 4-position in the phenyl ring.

The tricyclic 1*H*-pyrimido[4,5-*b*]pyrrolizine **11a** was obtained by intramolecular cyclization of compound **10a** that structurally related to the active analogue **5a, 6**. This cyclization restricts the free rotation of the two side chain in compound **10** provide a mean to study QSAR. The antitumor activity of compound **2** encouraged the authors to synthesize compound **12a** and **22** having a substituted ureidyl moiety at position 2 as an isostere of the carbamate side chain. Two sites of fusion between the pyrimidine ring and pyrrolizine nucleus were found in the reported active compounds (**3**, **4**, **5$_a$**, **5$_b$** and **6)**; pyrimido[a]pyrrolizine and pyrimido[b] pyrrolizine. Synthesis of compounds **10**, **13**, **14**, **16**, **18** and **19** was done aiming to study the effect of this difference of activities. Several derivatives of pyrrolizine have been synthesized from the reaction of compound **9a** and / or **17** with acid chlorides (28), formic acid (14) and aldehydes (29-32). These modifications will result in changes in the physicochemical properties of the new products.

2. Antitumor screening:

The antitumor screening was done in the National Cancer Institute using breast cell line. Eleven compounds were selected and screened using a new, rapid, sensitive and inexpensive colorimetric cytotoxic assay, where sulforhodamine B (SRB) was used to stain cultures that have been fixed with trichloroacetic acid. Protein-bound day was extracted with unbuffered tris(hydroxyl-methyl)-aminomethane and measured colorimetrically. The SRB assay (33) results (optical density) were linear with the number of cells.

Materials and methods for antitumor screening :

1- Potential cytotoxicity of the selected eleven compounds **10a**, **11a**, **12a**, **12f**, **13a**, **13f**, **14a**, **16a**, **15a**, **17** and **20a** were tested using the method of Skehan *et al.* (1990).
2- Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 hours before treatment with the compounds to allow attachment of cell to the wall of the plate.
3- Different concentrations 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.
4- Monolayer cells were incubated with the compounds for 48 hours at 37ºC and in atmosphere of 5% $C0_2$.
5- After 48 hours, cells were fixed, washed and stained with sulforhodamine B stain.

6- Excess stain was washed with acetic acid and attached stain was recovered with Tri EDTA buffer.
7- Color intensity was measured in an ELISA reader.
8- 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 in comparison with other tests done at the same time and determination about activity was made.

It was found that describing the data obtained from the above assay using death curve instead of the survival curve provide a good illustration of the results, 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) (34, 35).

Analysis of data:

Data were collected, checked, revised and entered to a computerized program. Analysis of data toke place by SPSS (statistical package version 11). Excel computer program was used to tabulate the results, and represent them graphically. Probit regression analysis procedure will be introduced to select the best model that describes the relationship among the drug concentration and the probit (percentage of protection or *decrease in no of cancer cells*) as a dependent variable in order to obtain the concentration of the drug that cause inhibition of 50% (IC50) or 90% (IC10) of cancer cells. The in vitro growth inhibition properties of each drug were described by IC50 and IC10. 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.)**

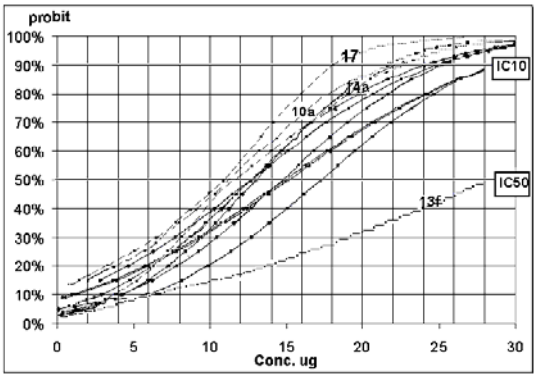


Fig. 1. Relationship between drug conc. (µg) and probit (degree of inhibition in growth of cancer cell line)

The three most active compounds (**17**, **10a** and **14a**) were represented by discontinuous line (---- ----). Compound **10a** has the lowest IC_{50} but compound **17** has the lowest IC10 (90% inhibition)

Compounds **11a**, **12a**, **12f**, **13a**, **15a**, **16a** and **20** exhibiting IC_{50} between 12.68 and 17,27 µg were represented by continuous line (——)

Compound 13f with the highest IC_{50} being the most inactive one was represented by dotted line and dot (—● —)

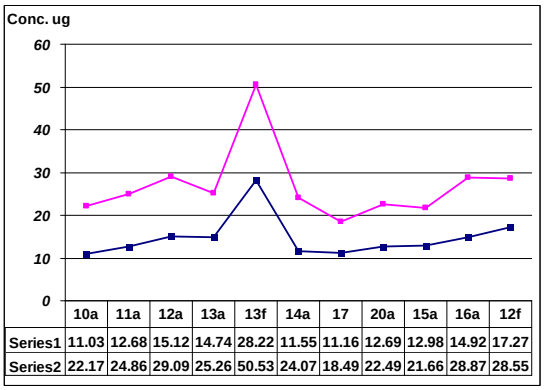
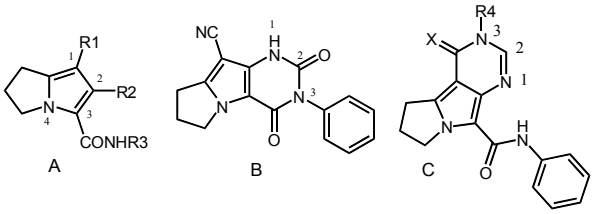


Fig. 2. Comparison of the IC_{50} and IC_{10} of the tested compounds.

Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening considered drug with IC50 ≤ 10 µg to be active as antitumor. Fig. 2 provide

a comparative illustration of the IC_{50} (series 1) and IC_{10} (series 2) for the tested compounds. The tested pyrrolizine and pyrimidopyrrolizine derivatives exhibit weak to moderate level of antitumor activity (all having $IC_{50} > 10$ µg). Substitutions with certain groups on these nuclei were found to modify the activity.



- R_1 = Carboxamides produces moderately active compounds.
- R_2 = ethylcarbamoyl gives the highest antitumor activity complying with the reported model (compound **2**).
- R_2 = Ethylureidyl was less active as antitumor agent and replacement of ethylureidyl by phenylureidyl results in sharp decrease of activity.
- The pyrimido[4,5-b]pyrrolizine exhibit more activity than pyrimido-[5,4-a]pyrrolizines.
- When R_4 = ethyl, a moderate activity was observed but a sharp decrease in activity was observed when R_3 = phenyl ring perhaps due to changes in physicochemical properties.
- Reduction of the imino group in **13a** results in slight increase in antitumor activity.
- When R_3 = H, active compounds were obtained and acylation of the amino group with acetyl group (R_2 = CH_3CONH) resulted in slight decrease in activity.
- When X = O or N only slight difference in activity observed.

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