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Design, synthesis and pharmacological evaluation of novel pyrrolizine derivatives as potential anticancer agents

Ahmed M. Gouda [a,b], Ahmed H. Abdelazeem [a,c], El-Shaimaa A. Arafa [d], Khaled R.A. Abdellatif [e,*]

[a] Department of Medicinal Chemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt
[b] Department of Pharmaceutical Chemistry, College of Pharmacy, Umm Al-Qura University, Mekkah 21955, Saudi Arabia
[c] Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, Taif 21974, Saudi Arabia
[d] Department of Pharmacology, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt
[e] Department of Pharmaceutical Organic Chemistry, Beni-Suef University, Beni-Suef 62514, Egypt

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ABSTRACT

A new series of novel pyrrolizine derivatives has been synthesized and biologically evaluated as potential anticancer agents. The starting compounds, 6-amino-7-cyano-N-(3,5-disubstitutedphenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamides **11a–b,** were reacted with different acid chlorides, aldehydes and isocyanates to give the target compounds **12–14**. Structural characterizations of the new compounds were performed using spectral and elemental analysis. All compounds were tested for their anticancer activity against human breast cancer and prostate cancer cell lines, MCF-7 and PC-3 respectively. With exception of compounds **11a** and **13a**, results revealed that all the tested compounds showed half maximal inhibitory concentration (IC_{50}) values less than 40 μM. Compound **12b** and the three urea derivatives **14b–d** showed the most potent anticancer activity with IC_{50} values less than 2.73 μM. The anticancer activity of these compounds was mediated, at least in part, via the induction of apoptosis as indicated by its ability to activate caspase-3/7. In light of the high potency of our novel compounds in targeting both breast and prostate cancers, these compounds warrant continued preclinical development as potential anticancer agents.

1. Introduction

Although many anticancer chemotherapeutic agents have been proven to be successful in the clinical trials, the high rate of mortality presents an urgent need for more safe and more efficient agents [1,2]. The clinical use of many chemotherapeutic agents still associated with many severe adverse reactions and rapid development of resistance despite of the great advances in targeted anticancer therapy [3,4]. The design of anticancer agents targeting cancer cell survival pathways improved the clinical promise of these agents as a component of anticancer therapy [5]. The selective induction of apoptosis in cancer cells is one of the main aims of these promising agents [6]. The activation of caspase-3, the main executioner in apoptosis, was useful in the discovery of many potential anticancer agents [7,8].

The pyrrolizine scaffold constitutes the basic skeleton in many compounds with diverse pharmacological activities [9–11]. Many pyrrolizine derivatives exhibit antitumor activities due to their ability to cross link DNA [12,13]. Recently, several pyrrolizine derivatives were developed lacking the alkylating functions and displayed potent antitumor activities. One of the diphenyl substituted pyrrolizine derivatives that showed a good activity against breast cancer is the selective COX-2 inhibitor, Licofelone **1** [14–16]. The high selectively for COX-2 could play the major role in its anticancer effects [14–16]. In addition, licofelone was found to enhance apoptosis in prostate and colon cancer cells through the mitochondrial pathway [17]. The replacement of the acetic acid moiety of licofelone by additional phenyl acyl side chain significantly increased the anticancer activity, compound **2** (Fig. 1) [17].

On the other hand, many nitrile and aminonitrile derivatives showed anticancer activity through induction of apoptosis and activation of caspase-3 compounds [18]. EpiCept Corporation USA has developed the Anticancer Screening Apoptosis Program (ASAP) that helped in the discovery of the 1-benzoyl-3-cyanopyrrolo

Abbreviations: ASAP, Anticancer Screening Apoptosis Program; $CDCl_3$, deuterated chloroform; DMEM/F-12, Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12; DMSO, dimethyl sulfoxide; eV, electron volt; FBS, fetal bovine serum; COX-2, cyclooxygenase-2; IC_{50}, half maximal inhibitory concentration; IR, Infrared spectroscopy; m.p., melting point; *m/z*, mass-to-charge ratio; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; SAR, structural activity relationship.

* Corresponding author. Address: Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt. Fax: +20 (002) 082 2317958.
E-mail address: khaled.ahmed@pharm.bsu.edu.eg (K.R.A. Abdellatif).

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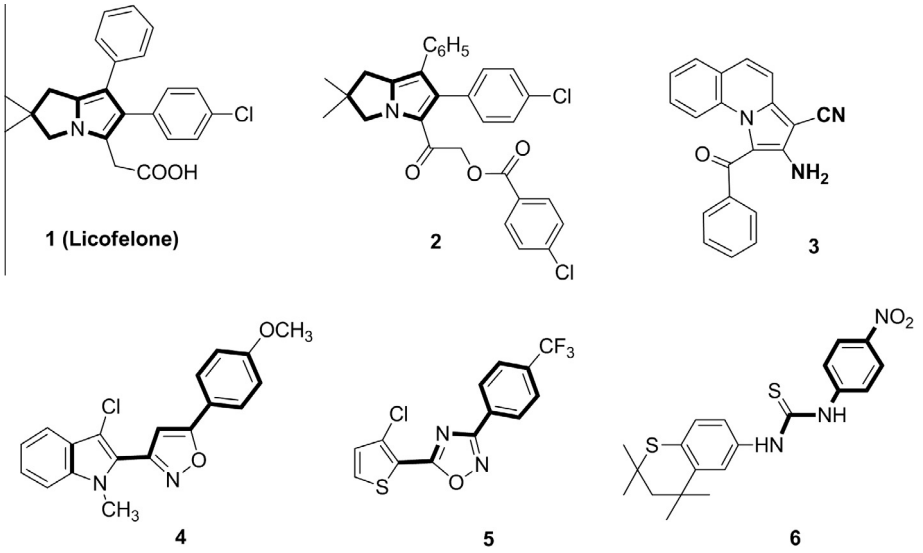


Fig. 1. Structures of some reported apoptosis inducers and caspase activators.

[1,2-a]quinoline **3** as a potent apoptosis inducer [19]. The structural activity relationship (SAR) study of compound **3** revealed that replacing the 3-cyano group by an ester or ketone group led to inactive compounds [18]. Other compounds **4**–**6** sharing certain structural similarity were reported as apoptosis inducers [20–24]. These compounds have a substituted phenyl moiety attached by two or three atoms spacer that contains double bond to another aromatic or heteroaromatic ring. The spacer either consists of sp^2 carbons as in compound **4** or may include one or two nitrogen atoms as in compounds **5** and **6** (Fig. 1). Generally, it was observed that the activities of compounds **1**–**6** vary according to the electronic effect and the position of the monosubstituents where the chloro, methoxy and trifluoromethy substituted derivatives possessed the highest activity [20–24]. In addition, compounds **1**–**2**, **4** and **5** have two phenyl rings attached to the basic nucleus either directly or through three or four atoms spacer.

Promoted by the aforementioned findings and aiming to develop novel and potent anticancer agents, two general scaffolds (**A** and **B,** Fig. 2) incorporating the heterocyclic core, 7-cyano-*N*-(3,5-disubstitutedphenyl)-2,3-dihydro-1H-pyrrolizin-5-carboxamide, were developed. The design concept is based mainly on the structural similarity with compounds **1**–**6** with maximizing the electronic effect of the substituents to investigate their influence on the anticancer activity of the newly designed pyrrolizine derivatives. In order to maximize this electronic effect, we used a disubstituted phenyl ring with two electron withdrawing (dichloro) or electron donating (dimethyl) groups. It was expected that their cumulative effects would be stronger and the impact of these effects on activity would be higher than the monosubstituted pyrrolizine derivatives. Concomitantly, the distance between the additional phenyl ring and pyrrolizine nucleus was varied (two or three atoms), and the substitution pattern of this phenyl ring could affect the target cancer cells and the overall activity.

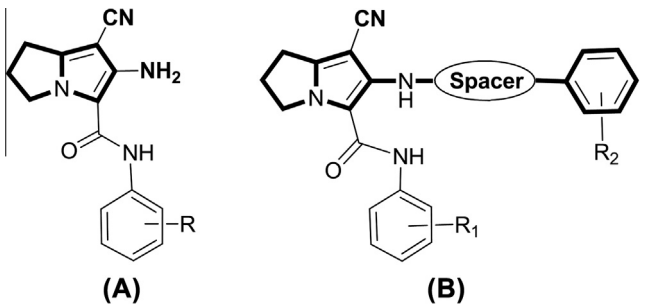


Fig. 2. General scaffolds for the newly designed ligands.

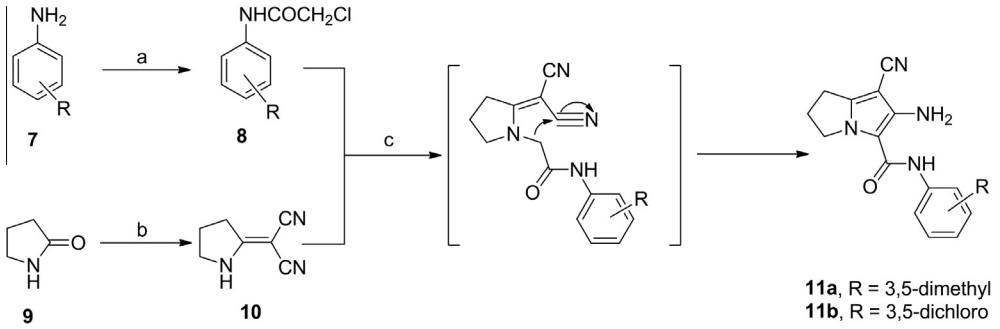
2. Results and discussion

2.1. Chemistry

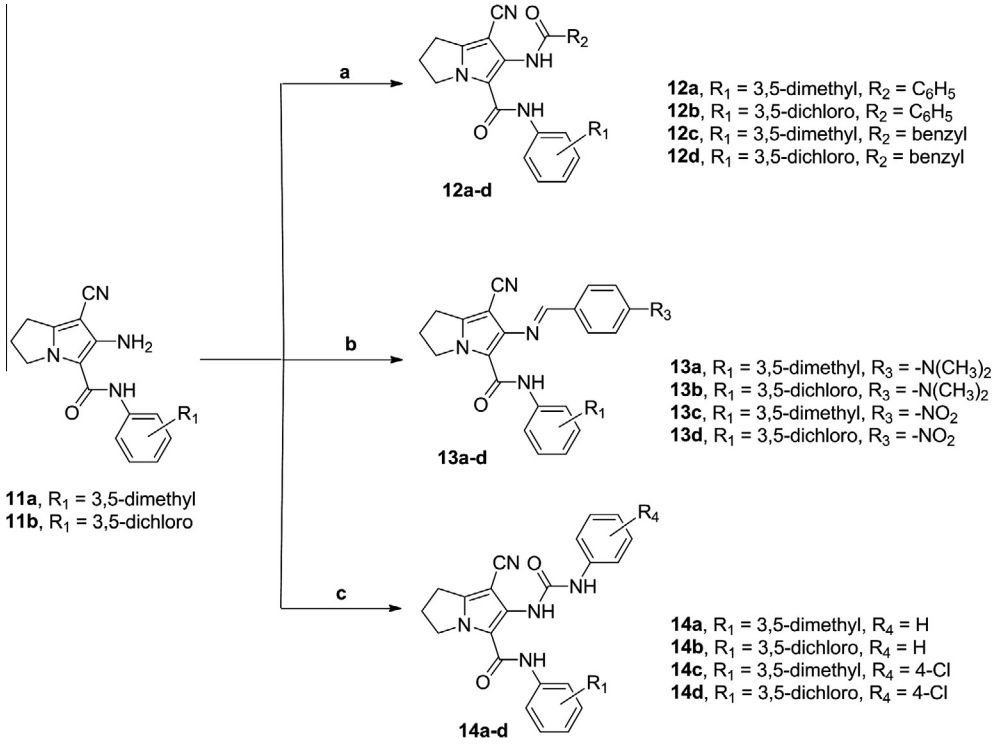
As shown in **Scheme 1**, preparation of the intermediates **8a**, **8b** and **10** was done according to previously reported procedures [25,26]. Compounds **11a** and **11b** were prepared from the reaction of 2-pyrrolidin-2-ylidine malononitrile **10** with the corresponding α-chloroacetanilide **8a** and **8b** in dry acetone according to previously reported procedures [27]. The reaction proceeded directly via the un-isolated intermediate, *N*-substituted pyrrolidin-2-ylidine malononitrile which cyclized spontaneously by addition of the α-methylene group to one of the two nitrile groups to give compounds **11a**–**b**. Characterization of the newly synthesized compounds was carried out using spectral and elemental analysis. The IR spectrum showed the presence of one sharp absorption band at 2217 and 2220 cm^{-1} assigned for the cyano group in the compounds **11a** and **11b** respectively. On the other hand, the mass spectrum revealed the molecular ions of the compounds **11a** and **11b** at 294 and 335 respectively, and the fragmentation patterns were in concordance with the chemical structure. ^{1}H NMR spectra showed the presence of a singlet proton at 9.09 and 9.29 ppm assigned for the amide protons in the compounds **11a** and **11b** respectively. Further characterization of compound **11a** was done using ^{13}C NMR spectrum as well.

Compounds **11a** and **11b** were used as starting materials for preparing the other target compounds **12**–**14**, as illustrated in Scheme 2. The reaction of compounds **11a** and **11b** with the appropriate acid chloride in dry benzene gave compounds **12a**–**d**. The synthesized compounds were characterized by the spectral and elemental analysis. ^{1}H NMR spectra revealed the presence of two protons at the range of 9.29–10.93 ppm assigned for the amide protons in compounds **12a**–**d**.

Moreover, the condensation of the compounds **11a** and **11b** with the appropriate aldehyde in absolute ethanol in the presence



Scheme 1. Reagents and conditions: (a) $ClCH_2COCl$, gl. acetic acid, CH_3COONa; (b) $(CH_3)_2SO_4$, benzene, malononitrile; and (c) K_2CO_3, acetone, reflux, 24 h.



Scheme 2. Reagents and conditions: (a) ArCOCl, dry benzene, 48 h; (b) ArCHO, EtOH, gl. acetic acid, reflux, 4 h; and (c) ArNCO, CH_2Cl_2, $N(Et)_3$, reflux 12 h.

of glacial acetic yielded the compounds **13a–d** which were characterized by spectral and elemental analysis. The ^{1}H NMR spectra showed the appearance of one new proton at the range of 10.37–11.47 ppm assigned for the benzylidene proton C<u>H</u>.

In addition, the target compounds **14a–d** were obtained from the reaction of compounds **11a** and **11b** with the appropriate isocyanate in methylene chloride in the presence of triethylamine. The reaction proceeded without intramolecular rearrangement of the cyano group yielding ureido derivatives. Characterization of compound **14a–d** was done using spectral and elemental analysis. The ^{1}H NMR spectra revealed the presence of two protons in the range of 8.86–10.74 ppm assigned for the two N<u>H</u> protons, while the ^{13}C NMR spectrum of compound **14a** showed two signals at 158.1 and 178.3 ppm assigned for the two carbonyl groups.

2.2. Pharmacological screening

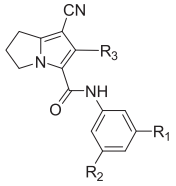
2.2.1. Anticancer activity

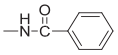
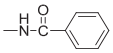
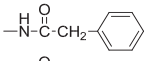
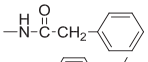
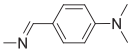
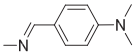
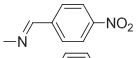
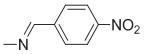
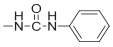
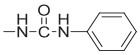
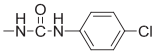
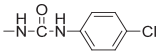
Evaluation of the anticancer activity for the synthesized compounds **11–14** were performed by determination of the growth inhibitory effect on both breast (MCF-7) and prostate cancer (PC-3) cell lines. The effect of the test compounds on cancer cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay in six replicates as previously reported [28]. All tested compound showed an IC_{50} in the micromolar range, going parallel against the two cell lines (Table 1). With exception of compound **13a**, all compounds significantly inhibited the cell viability of cancer cells. The parent compound **11a** showed the lowest anticancer activity while its dichloro analog **11b** showed improved activity on both MCF-7 and PC-3 cells with IC_{50} of 4.17 and 6.12 respectively.

The second substituted phenyl group at position 6 in compounds **12–14** increased the activity compared to the parent compound **11a**. Also, the activity of these compounds varied with the length of the spacer between the second phenyl and the pyrrolizine nucleus, where compounds with three atoms spacer (**14b–d**) showed higher activity than those with two atoms spacer **13a–d**.

The three urea derivatives **14b–d** in addition to 6-benzoylamino-7-cyano-N-[3,5-dichlorophenyl]-1H-pyrrolizine-5-carboxamide **12b**

Table 1
IC_{50} values for the 7-cyano-2,3-dihydro-1H-pyrrolizine-5-carboxamides derivatives on MCF-7 and PC-3 cell lines.



Comp.	R^1	R^2	R^3	IC_{50} (μM)	
				MCF-7	PC-3
11a	CH_3	CH_3	NH_2	59.66 ± 4.2	67.02 ± 3.1
11b	Cl	Cl	NH_2	4.17 ± 0.9	6.12 ± 1.4
12a	CH_3	CH_3		10 ± 1.2	9.61 ± 1.7
12b	Cl	Cl		2.34 ± 0.8	2.73 ± 0.3
12c	CH_3	CH_3		8.81 ± 0.9	7.91 ± 0.7
12d	Cl	Cl		8.58 ± 2.1	8.35 ± 1.8
13a	CH_3	CH_3		>100	>100
13b	Cl	Cl		17.7 ± 1.9	14.3 ± 2.2
13c	CH_3	CH_3		8.76 ± 1.5	6.43 ± 0.9
13d	Cl	Cl		7.9 ± 1.1	8.54 ± 1.4
14a	CH_3	CH_3		38.4 ± 2.3	32.6 ± 2.3
14b	Cl	Cl		2.7 ± 0.3	2.09 ± 0.2
14c	CH_3	CH_3		1.12 ± 0.1	0.98 ± 0.1
14d	Cl	Cl		2.3 ± 0.3	2.0 ± 0.2

Cell were treated with the test compounds or vehicle for 48 h. Data were reported as mean ± S.D. (n = 6).

showed the highest anticancer activity, where their IC_{50} were less than 3 μM. When the additional phenyl were substituted with electron withdrawing group as in compounds **13c–d** and **14c–d**, the observed IC_{50} values were lower than the IC_{50} values of those substituted with electron donating groups (**13a–b**). Taken together, it was found that there are two requirements for the anticancer activity of the pyrrolizine derivatives. First, it is imperative to incorporate two phenyl rings substituted with electron withdrawing groups into the pyrrolizine nucleus. Secondly, the distance between these two phenyl rings and the pyrrolizine nucleus is crucial for activity where the anticancer activity is enhanced with the increase in the length of the spacer. It should be mentioned that the three atoms spacer was the optimal for activity.

2.2.2. Caspase activation assay

Caspase activity was determined for the most active compounds in Cell Viability Analysis. The caspase-3/7 in MCF-7 cells treated with test agents were measured using Caspase-Glo 3/7 luminescence assay kit (Promega, Madison, WI) according to the manufacturer's instructions and as mentioned before [29] (Fig. 3).

The results revealed that compound **12b, 14b–d** stimulate caspase 3/7, where compound **14c** was the most active, and this results were in concordance with the results obtained in the cell viability assay.

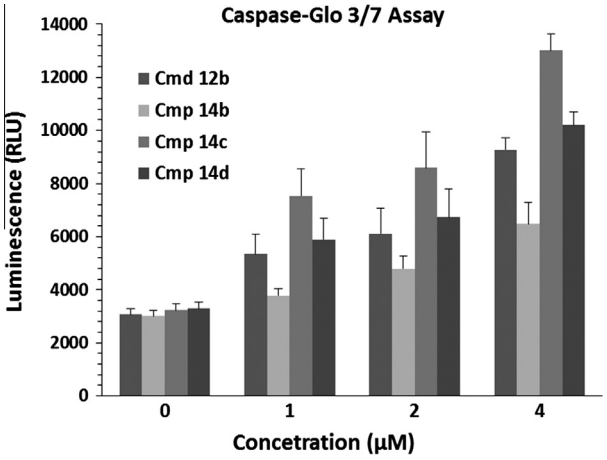


Fig. 3. The caspase activation assay Columns, mean; bars, S.D. (n = 5).

3. Conclusions

Various substituted pyrrolizines were synthesized and screened for anticancer activity. The pyrrolizine scaffold **11b** possesses good anticancer activity in the cell viability assay and activity increase by substitution at position 6 with substituted phenyl. The most active derivatives were those bearing two phenyl rings substituted with electron withdrawing group (**13c**, **13d, 14c** and**14d**). Compounds with three atoms spacer between the pyrrolizine nucleus and the additional phenyl (**12c, 12d** and **12a–d**) showed higher activity than those with two atoms spacer (**12a, 12b** and **13a–d**) and compound **12c** was the most active. Based on the overall results, it was conceptualized that the anticancer activity of the newly synthesized compounds were affected by the length of the spacer, the presence or absence of two phenyl rings attached to the pyrrolizine nucleus and finally the type of the substituents. Combining the results of the cell viability assay and caspase activation assay, we can suggest that, at least in the breast cell line MCF-7, the caspase activation may contribute to the mechanism of anticancer activity of these compounds.

4. Experimental protocol

4.1. Chemistry

Chemical reagents and solvents were obtained from commercial sources. Solvents are dried by standard methods when necessary. Melting points (m.p.) were uncorrected and were carried out by open capillary tube method using IA 9100MK-Digital Melting Point Apparatus. Microanalyses were carried out at the microanalytical Center, Faculty of Science, Cairo University. Infrared spectra were made on BRUKER Vector 22 (Japan), infrared spectrophotometers and were expressed in wavenumber (cm^{-1}) using potassium bromide disc. The proton magnetic resonance ^1H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 400 MHz and BRUKER APX400 spectrometer at 400 MHz in the specified solvent, chemical shifts were reported on the δ scale and were related to that of the solvent and J values are given in Hz. ^{13}C NMR spectra were obtained on a Bruker APX400 at 100 MHz at the faculty of pharmacy, Beni-Suef University. Mass spectra were recorded on Fennigan MAT, SSQ 7000, Mass spectrometer, at 70 eV (EI) at the microanalytical Center, Faculty of Science, Cairo University. All mass spectra were recorded in the EI mode. Thin layer chromatography, was done using Macherey–Nagel Alugram Sil G/UV254 silica gel plates and benzene–ethanol (9.5:0.5) as the eluting system.

*4.1.1. General method for preparation of compounds (**11**)*

A mixture of 2-pyrrolidin-2-ylidenemalononitrile **10** (1 g, 7.5 mmol), the appropriate acetanilide **8a** and **8b** (7.5 mmol) and anhydrous potassium carbonate (1.04 g, 7.5 mmol) in dry acetone (50 ml) was stirred under reflux for 24 h. The mixture was filtered, concentrated and left to cool, whereby a white crystal were formed, collected, dried and recrystallized from ethanol–acetone.

*4.1.1.1. 6-Amino-7-cyano-N-(3,5-dimethylphenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**11a**).* White crystals, m.p. 217–9 °C, yield 71%, IRυ_{max}/cm^{-1} 3338, 3254 (NHs), 2961, 2914 (C—H aliphatic), 2217 (CN), 1664 (CO), 1613 1567, 1542 (C=C, NH), 1436, 1330 (C—N, C—O). ^{1}H NMR (DMSO-d6-400 MHz): δ 2.36 (s, 6H, two Ph$\underline{CH_3}$), 2.44 (m, 2H, CH_2-2), 2.89 (t, 2H, J = 7.5 Hz, CH_2-1), 4.21 (t, 2H, J = 7.2 Hz, CH_2-3), 5.43 (s, 2H, NH_2), 6.71 (s, H, aromatic protons), 7.21 (s, 2H, aromatic protons), and 9.09 (s, H, CON$\underline{H}$)). ^{13}C NMR (DMSO-d_6): δ 21.7 (two Ph$\underline{C}H_3$), 24.8, 25.3, 49.7, 77.9, 108.6, 115.9, 118.1 (two aromatic carbons), 124.9, 138 (two aromatic carbons), 138.6, 144.9, 146.6, 159.7 (CO). MS (EI): *m*/*z* (%): 295 (M$^+$+1, 4), 294 (M$^+$, 23), 174 (100), 146 (12), 121 (14), 92 (11), 65 (12). Anal. Calcd. for $C_{17}H_{18}N_4O$ (294.35): C, 69.37; H, 6.16; N, 19.03. Found: C, 68.93; H, 5.72; N, 18.80.

*4.1.1.2. 6-Amino-7-cyano-N-(3,5-dichlorophenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**11b**).* White crystals, m.p. 244–7 °C, yield 62%. IRυ_{max}/cm^{-1} 3351, 3278 (NHs), 3075 (C—H aromatic), 2957 (C—H aliphatic), 2220 (CN), 1670 (CO), 1587, 1536 (C=C, NH), 1438, 1311 (C—N, C—O), 834 (C—Cl). ^{1}H NMR (DMSO-d6-400 MHz): δ 2.40 (m, 2H, CH_2-2), 2.98 (t, 2H, J = 7.5 Hz, CH_2-1), 4.32 (t, 2H, J = 7.2 Hz, CH_2-3), 5.70 (s, 2H, NH_2), 7.06 (s, H, aromatic protons), 7.65 (s, 2H, aromatic protons), and 9.29 (s, H, NH). ^{13}C NMR (DMSO-d_6): δ 19.7, 36.4, 43.6, 50.7, 59.6, 115.7, 116.3, 118, 121.7, 127.7, 132.2, 138.5, 164.8. MS (EI): *m*/*z* (%): 336 (M$^+$+2, 7), 335 (M$^+$+1, 3), 334 (M$^+$, 10), 161 (10), 174 (100), 145 (11), 146 (21), 147 (10), 92 (16), 77 (3). Anal. Calcd. for $C_{15}H_{12}Cl_2N_4O$ (335.19): C, 53.75; H, 3.61; N, 16.72. Found: C, 54.23; H, 4.07; N, 16.20.

*4.1.2. General method for preparation of compounds (**12a–d**)*

A mixture of 6-Amino-7-cyano-*N*-[3,5-disubstituted-phenyl]-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide **11** (5.3 mmol), appropriate acyl chloride (10.6 mmol) in dry benzene (50 ml) is stirred for one hour and left to stand for 48 h at room temperature. Filter the separated product, wash with water, dry and recrystallize from ethanol/acetone.

*4.1.2.1. 6-Benzoylamino-7-cyano-N-[3,5-dimethylphenyl]-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**12a**).* White crystals, m.p. 263–5 °C, yield 81%. IRυ_{max}/cm^{-1} 3338, 3276, 3254 (NHs), 2997, 2914, 2853 (C—H aliphatic), 2217 (CN), 1664 (broad band due to COs), 1613, 1567, 1542 (C=C, NH), 1436, 1377 (C—N, C—O). ^{1}H NMR (DMSO-d6-400 MHz): δ 2.38 (s, 6H, two Ph$\underline{CH_3}$), 2.42 (m, 2H, CH_2-2), 2.91 (t, 2H, J = 7.5 Hz, CH_2-1), 4.23 (t, 2H, J = 7.2 Hz, CH_2-3), 6.68–7.81 (m, 8H, aromatic protons), and 9.51, 10.23 (two s, 2H, two CON$\underline{H}$). ^{13}C NMR (DMSO-d_6): δ 21.2, 24.8, 25.3, 50.2, 76.7, 109.7, 116.0, 119.2, 123.9, 125.6, 126.8, 129.4, 138.6, 140.1, 148.8, 148.9, 152.1, 157.9 and 160.0. MS (EI): (%) 397 ((M$^+$−1, 10), 396 (M$^+$−2, 29), 304 (70), 209 (8), 130 (58), 102 (61), 91 (26), 65 (100). Anal. Calcd. for $C_{24}H_{22}N_4O_2$ (398.46): C, 72.34; H, 5.57; N, 14.06. Found: C, 72.42; H, 5.26; N, 14.40.

*4.1.2.2. 6-Benzoylamino-7-cyano-N-[3,5-dichlorophenyl]-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**12b**).* White crystals, m.p. 279–81 °C, yield 69%. IRυ_{max}/cm^{-1} 3446, 3274 (NHs), 3070 (C—H aromatic), 2971, 2907 (C—H aliphatic), 2212 (CN), 1668 (CO), 1592, 1537 (C=C, NH), 1434, 1364 (C—N, C—O), 812 (C—Cl). ^{1}H NMR (DMSO-d6-400 MHz): δ 2.40 (m, 2H, CH_2-2), 3.02 (t, 2H, J = 7.5 Hz, CH_2-1), 4.28 (t, 2H, J = 7.2 Hz, CH_2-3), 6.52 (m, H, aromatic proton), 7.24 (s, H, aromatic proton), 7.52 and 760 (two t, 2H, J = 7.2 Hz, aromatic protons), 7.85 (s, 2H, aromatic proton), 8.14 and 8.15 (2 d, 2H, J = 7.2 Hz, aromatic protons), and 9.88, 10.93 (two s, 2H, NH). ^{13}C NMR (DMSO-d_6): δ 24.9, 25.7, 49.6, 85.8, 115.1, 118.3, 118.5, 123.1, 128.4, 128.6, 128.9, 132.6, 133.6, 134.4, 141.7, 146.8, 158.4, 166.3. MS (EI): *m*/*z* (%): 440 (M$^+$+2, 3), 439 (M$^+$+1, 2), 438 (M$^+$, 4), 278 (27), 174 (6), 145 (4), 105 (100), 92 (4), 77 (52). Anal. Calcd. for $C_{22}H_{16}Cl_2N_4O_2$ (439.29): C, 60.15; H, 3.67; N, 12.75. Found: C, 60.31; H, 4.11; N, 12.67.

*4.1.2.3. 6-(2-Benzylacetylamino)-7-cyano-N-[3,5-dimethylphenyl]-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**12c**).* White crystals, m.p. 253–5 °C, yield 72%. IRυ_{max}/cm^{-1} 3408, 3235 (NHs), 3094, 3057 (C—H aromatic), 2961 (C—H aliphatic), 2222 (CN), 1667 (broad band due to C=Os), 1594, 1566, 1525 (C=C, NH), 1490, 1425, 1379 (C—N, C—O). ^{1}H NMR (DMSO-d6-400 MHz): δ 2.24 (s, 6H, two Ph$\underline{CH_3}$), 2.42 (m, 2H, CH_2-2), 2.90 (t, 2H, J = 7.5 Hz, CH_2-1), 3.32 (s, 2H, $COCH_2$), 4.21 (t, 2H, J = 7.2 Hz, CH_2-3), 6.68–7.62 (m, 8 H, aromatic protons), 9.31, 9.67 (two s, 2H, two CON$\underline{H}$). ^{13}C NMR (DMSO-d_6): δ 21.2, 24.8, 25.3, 29.1, 49.7, 79.3, 109.0, 117.1, 118.1, 124.9, 125.3, 138.0, 138.5, 138.7, 139.4, 144.9, 146.6, 147.2, 159.7. MS (EI): *m*/*z* (%): 414 (M$^+$+2, 58), 413 (M$^+$+1, 56), 412 (M$^+$, 65), 365 (50) 340 (58%), 326 (49), 309 (58), 242 (46), 220 (51), 185 (44), 179 (65), 137 (78), 121 (59), 92 (9), 78 (56), 69 (100). Anal. Calcd. for $C_{25}H_{24}N_4O_2$ (412.48): C, 72.79; H, 5.86; N, 13.58. Found: C, 73.22; H, 5.49; N, 13.86.

*4.1.2.4. 6-(2-Benzylacetylamino)-7-cyano-N-[3,5-dichlorophenyl]-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**12d**).* White crystals, m.p. 271–4 °C, yield 66%. IRυ_{max}/cm^{-1} 3294, 3250 (NHs), 3077 (C—H aromatic), 2955 (C—H aliphatic), 2220 (CN), 1680, 1650 (COs), 1588, 1537 (C=C, NH), 1436, 1373 (C—N, C—O), 838 (C—Cl). ^{1}H NMR (DMSO-d6-400 MHz): δ 2.40 (m, 2H, CH_2-2), 2.99 (t, 2H, J = 7.5 Hz, CH_2-1), 3.45 (s, 2H, $COCH_2$), 4.33 (t, 2H, J = 7.2 Hz, CH_2-3), 6.62–7.65 (m, 8H, aromatic protons), and 9.29, 9.54 (two s, 2H, two CON$\underline{H}$). ^{13}C NMR (DMSO-d_6): δ 24.3, 25.2, 28.9, 47.5, 81.4, 116.2, 117.3, 119.0, 122.7, 127.6, 128.3, 128.9, 131.5, 134.1, 136.8, 140.2, 147.5, 156.6, 164.1. MS (EI): *m*/*z* (%): 453 (M$^+$+1, 3), 372 (48), 200 (73), 173 (96), 145 (53), 117 (100), 92 (14), 77 (16). Anal. Calcd. for $C_{23}H_{18}Cl_2N_4O_2$ (453.32): C, 60.94; H, 4.00; N, 12.36. Found: C, 61.43; H, 4.34; N, 11.99.

*4.1.3. General procedure for the preparation of compounds (**13a–d**)*

A mixture of the carboxamide derivatives **11** (3.75 mmol) and the appropriate aldehyde (3.75 mmol) was refluxed in absolute ethanol (20 ml) in the presence of glacial acetic acid (0.5 ml) for 4 h. The reaction mixture was concentrated, set aside to cool, the formed crystals was collected and recrystallized from ethanol.

*4.1.3.1. 7-Cyano-6-[(4-dimethylaminobenzylidene)-amino]-N-[3,5-dimethylphenyl]-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**13a**).* Yellow crystals, m.p. 278–81 °C, yield 83%. IRυ_{max}/cm^{-1} 3244 (NHs), 3095 (C—H aromatic), 2965, 2920, 2852 (C—H aliphatic), 2215 (CN), 1661 (CO), 1617, 1566, 1518 (C=C, NH), 1343 (C—N, C—O). ^{1}H NMR ($CDCl_3$-400 MHz): δ 2.19 (s, 6H, two Ph$\underline{CH_3}$), 2.56 (m, 2H, CH_2-2), 2.93 (t, 2H, J = 7.5 Hz, CH_2-1), 3.29 (s, 6H, N($\underline{CH_3}$)$_2$), 4.53 (t, 2H, J = 7.2 Hz, CH_2-3), 6.77–7.87 (m, 7H, aromatic protons), and 9.02 (s, 1H, CO$\underline{NH}$). 10.92 (s, H, N=C$\underline{H}$). ^{13}C NMR ($CDCl_3$): δ 21.5, 24.6, 25.4, 40.2, 50.0, 77.4, 111.6, 116.8, 117.4, 123.3, 124.3, 130.8, 138.5, 138.6, 140.9, 147.7, 153.2, 158.9, 159.7. MS (EI): *m*/*z* (%): 427 (M$^+$+2, 16), 426 (M$^+$+1, 33), 425 (M$^+$, 60), 395 (13), 362 (100), 334 (33), 305 (24), 272 (42), 255 (22), 174 (16), 148 (76), 105 (127). Anal. Calcd. for $C_{26}H_{27}N_5O$

(425.53): C, 73.39; H, 6.40; N, 16.46. Found: C, 73.71; H, 6.27; N, 16.58.

*4.1.3.2. 7-Cyano-6-[(4-dimethylaminobenzylidene)-amino]-N-[3,5-dichlorophenyl]-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**13b**).* Yellow crystals, m.p. 296–9 °C, yield 72%. IRυ_{max}/cm^{-1} 3408, 3313, 3282, 3226(NHs), 3097, 3032 (C—H aromatic), 2972, 2910 (C—H aliphatic), 2209 (CN), 1678, 1657 (COs), 1592, 1540 (C=C, NH), 1438, 1433, 1316 (C—N, C—O), 835 (C—Cl). ^{1}H NMR (CDCl$_3$-400 MHz): δ 2.58 (m, 2H, CH$_2$-2), 3.07 (t, 2H, *J* = 7.5 Hz, CH$_2$-1), 3.15 (s, 6H, N(CH$_3$)$_2$), 4.51 (t, 2H, *J* = 7.2 Hz, CH$_2$-3), 6.81–7.87 (m, 7H, aromatic protons), and 8.96 (s, 1H, CONH). 11.24 (s, H, N=CH). ^{13}C NMR (CDCl$_3$): δ 25.1, 25.2, 26.0, 41.6, 48.4, 50.2, 114.3, 118.9, 124.1, 127.9, 129.4, 134.9, 136.1, 140.3, 147.5, 150.1, 154.2, 157.6, 162.3. MS (EI): *m*/*z* (%): 467 (M$^+$+2, 25), 466 (M$^+$+1, 17), 465 (M$^+$, 35), 305 (55), 265 (13), 148 (100), 77 (18). Anal. Calcd. for $C_{24}H_{21}Cl_2N_5O$ (466.36): C, 61.81; H, 4.54; N, 15.02. Found: C, 61.70; H, 4.49; N, 14.89.

*4.1.3.3. 7-Cyano-6-[(4-nitrobenzylidene)amino]-N-[3,5-dimethylphenyl]-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**13c**).* Yellow crystals, m.p. 261–4 °C, yield 86%. IRυ_{max}/cm^{-1}3321, 3277 (NHs), 3057 (C—H aromatic), 2929 (C—H aliphatic), 2216 (CN), 1732, 1661 (C=Os), 1591, 1530 (C=C, NH), 1458, 1439, 1303 (C—N, C—O). ^{1}H NMR (CDCl$_3$-400 MHz): δ 2.23 (s, 6H, two PhCH$_3$), 2.61 (m, 2H, CH$_2$-2), 3.01 (t, 2H, *J* = 7.5 Hz, CH$_2$-1), 4.58 (t, 2H, *J* = 7.2 Hz, CH$_2$-3), 6.77–8.38 (m, 7H, aromatic protons), and 9.28 (s, 1H, CONH). 10.37 (s, H, N=CH). ^{13}C NMR (CDCl$_3$): δ 21.5, 24.5, 25.5, 50.3, 116.0, 117.3, 119.3, 124.3, 126.1, 129.2, 130.5, 137.7, 137.8, 139.0, 140.9, 148.7, 149.7, 156.2, 157.9. MS (EI): *m*/*z* (%): 428 (M$^+$+1, 31), 427 (M$^+$, 96), 307 (100), 261 (76), 174 (44), 147 (27), 120 (47), 77 (62). Anal. Calcd. for $C_{24}H_{21}N_5O_3$ (427.46): C, 67.44; H, 4.95; N, 16.38. Found: C, 67.38; H, 4.89; N, 16.80.

*4.1.3.4. 7-Cyano-6-[(4-nitrobenzylidene)amino]-N-[3,5-dichlorophenyl]-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**13d**).* Yellow crystals, m.p. 292–5 °C, yield 73%. IRυ_{max}/cm^{-1} 3351, 3274, 3229 (NHs), 3069 (C—H aromatic), 2217 (CN), 1666 (CO), 1588, 1540 (C=C, NH), 1434, 1345 (C—N, C—O). ^{1}H NMR (CDCl$_3$-400 MHz): δ 2.59 (m, 2H, CH$_2$-2), 3.17 (t, 2H, *J* = 7.5 Hz, CH$_2$-1), 4.58 (t, 2H, *J* = 7.2 Hz, CH$_2$-3), 6.83–7.87 (m, 7H, aromatic protons), and 9.33 (s, 1H, CONH). 11.47 (s, H, N=CH). ^{13}C NMR (CDCl$_3$): δ 24.6, 25.4, 50.3, 117.7, 119.0, 124.1, 124.5, 124.6, 129.2, 129.4, 135.5, 139.8, 140.6, 149.2, 150.0, 157.1, 158.1, 163.4. MS (EI): *m*/*z* (%): 469 (M$^+$+2, 10), 468 (M$^+$+1, 6), 467 (M$^+$, 21), 466 (M$^+$−1, 14), 345 (15), 307 (100), 261 (33), 120 (36), 77 (13). Anal. Calcd. for $C_{22}H_{15}Cl_2N_5O_3$ (468.29): C, 56.43; H, 3.23; N, 14.96. Found: C, 56.52; H, 3.34; N, 15.35.

*4.1.4. General procedure for the preparation of compounds (**14a–d**)*

A mixture of 6-amino-7-cyano-*N*-[3,5-disubstituted-phenyl]-2,3-dihydro-1*H*-pyrrolizine-5-carboxamide **11** (3.8 mmol) in methylene chloride (25 ml), appropriate isocyanate (4 mmol), and three drops of triethylamine is refluxed for 12 h, evaporate the solvent under reduced pressure. The residue is dissolved in acetone, concentrated; set aside where the solid obtained is collected, dried and recrystallized from ethanol–acetone.

*4.1.4.1. 7-Cyano-6-(3-phenylureido)-N-(3,5-dimethylphenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**14a**).* White crystals, m.p. 261–4 °C, yield 79%. IRυ_{max}/cm^{-1}3284 (broad band due to NHs), 3067 (C—H aromatic), 2957 (C—H aliphatic), 2212 (CN), 1671, 1650 (broad band due to COs), 1597, 1560 (C=C, NH), 1498, 1437, 1365 (C—N, C—O). ^{1}H NMR (DMSO-400 MHz): δ 2.39 (s, 6H, two PhCH$_3$), 2.46 (m, 2H, CH$_2$-2), 2.97 (t, 2H, *J* = 7.5 Hz, CH$_2$-1), 4.33 (t, 2H, *J* = 7.2 Hz, CH$_2$-3), 5.81 (bs, 1H, NH), 6.81–7.48 (m, 7H, aromatic protons), and 8.86, 9.23 (two s, 2H, two CONH). ^{13}C NMR (DMSO-d$_6$): δ 21.6, 24.6, 25.9, 50.1, 79.3, 112.4, 115.8, 117.0, 118.6, 122.2, 125.5, 129.3, 138.6, 140.3, 141.4, 147.3, 153.1, 158.1, 178.3. MS (EI): *m*/*z* (%): 413 (M$^+$, 38), 370 (49), 341 (43), 319 (42), 292 (42), 278 (44), 258 (31), 248 (45), 225 (31), 214 (100), 187 (60), 173 (63), 162 (44), 151 (35), 138 (63), 121 (35), 92 (25), 77 (52). Anal. Calcd. for $C_{24}H_{23}N_5O_2$ (413.47): C, 69.72; H, 5.61; N, 16.94. Found: C, 69.63; H, 5.31; N, 17.29.

*4.1.4.2. 7-Cyano-6-(3-phenylureido)-N-(3,5-dichlorophenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**14b**).* White crystals, m.p. 281–4 °C, yield 73%. IRυ_{max}/cm^{-1}3330, 3282 (NHs), 3077 (C—H aromatic), 2221 (CN), 1681, 1650 (COs), 1593, 1538 (C=C, NH), 1498, 1439, 1313 (C—N, C—O). ^{1}H NMR (DMSO-400 MHz): δ 2.45 (m, 2H, CH$_2$-2), 2.97 (t, 2H, *J* = 7.5 Hz, CH$_2$-1), 4.31 (t, 2H, *J* = 7.2 Hz, CH$_2$-3), 5.72 (bs, 1H, NH), 6.94–7.65 (m, 7H, aromatic protons), and 9.12, 10.52 (two s, 2H, two CONH). ^{13}C NMR (DMSO-d$_6$): δ 24.1, 25.2, 44.6, 77.2, 115.7, 116.3, 118.1, 122.7, 124.6, 126.3, 129.5, 132.2, 127.7, 138.5, 140.4, 153.1, 162.1, 174.6. MS (EI): *m*/*z* (%): 456 (M$^+$+3, 20), 455 ((M$^+$+2, 10), 454 ((M$^+$+1, 11), 416 (15), 394 (12), 377 (13), 313 (17), 274 (15), 247 (13), 225 (16), 196 (14), 174 (10), 149 (17), 120 (20), 93 (55), 69 (100). Anal. Calcd. for $C_{22}H_{17}Cl_2N_5O_2$ (454.31): C, 58.16; H, 3.77; N, 15.42. Found: C, 57.83; H, 4.01; N, 15.11.

*4.1.4.3. 7-Cyano-6-[3-(4-chlorophenylureido)]-N-(3,5-dimethylphenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**14c**).* White crystals, m.p. 282–5 °C, yield 85%. IRυ_{max}/cm^{-1} 3295 (broad band, NHs), 3076 (C—H aromatic), 2978, 2914 (C—H aliphatic), 2213 (CN), 1634 (broad band, COs), 1591, 1561(C=C, NH), 1491, 1435, 1397 (C—N, C—O), 823 (C—Cl). ^{1}H NMR (DMSO-400 MHz): δ 2.36 (s, 6H, two PhCH$_3$), 2.50 (m, 2H, CH$_2$-2), 3.33 (t, 2H, *J* = 7.5 Hz, CH$_2$-1), 4.33 (t, 2H, *J* = 7.2 Hz, CH$_2$-3), 5.30 (bs, 1H, NH), 6.71–7.48 (m, 7H, aromatic protons), and 8.96, 9.63 (two s, 2H, two CONH). ^{13}C NMR (DMSO-d$_6$): δ 21.6, 24.7, 25.9, 50.1, 78.3, 112.4, 116.8, 117.3, 119.6, 123.3, 125.9, 130.9, 138.2, 141.3, 143.8, 149.4, 154.8, 165.1, 178.3. MS (EI): *m*/*z* (%): 446 (M$^+$−1, 1), 428 (21), 256 (100), 228 (36), 160 (20). Anal. Calcd. for $C_{24}H_{22}ClN_5O_2$ (447.92): C, 64.36; H, 4.95; N, 15.64. Found: C, 64.10; H, 4.87; N, 15.35.

*4.1.4.4. 7-Cyano-6-[3-(4-chlorophenylureido)]-N-(3,5-dimethylphenyl)-2,3-dihydro-1H-pyrrolizine-5-carboxamide (**14d**).* White crystals, m.p. 287 °C, yield 76%. IRυ_{max}/cm^{-1} 3296 (NHs), 3077 (C—H aromatic), 2955 (C—H aliphatic), 2221 (CN), 1681, 1647 (broad band, COs), 1588, 1536 (C=C, NH), 1491, 1436, 1373 (C—N, C—O), 868. ^{1}H NMR (DMSO-400 MHz): δ 2.46 (m, 2H, CH$_2$-2), 3.32 (t, 2H, *J* = 7.5 Hz, CH$_2$-1), 4.35 (t, 2H, *J* = 7.2 Hz, CH$_2$-3), 5.35 (bs, 1H, NH), 7.26–7.64 (m, 7H, aromatic protons), and 9.29, 10.74 (two s, 2H, two CONH). ^{13}C NMR (DMSO-d$_6$): δ 24.9, 25.6, 44.8, 79.1, 115.8, 117.6, 119.3, 122.7, 124.8, 125.2, 129.9, 133.5, 129.7, 139.1, 141.2, 155.5, 168.3, 177.1. MS (EI): *m*/*z* (%): 489 (M$^+$+2, 15), 488 (M$^+$+1, 19), 464 (12), 442 (15), 418 (17), 392 (15), 376 (12), 357 (20), 290 (16), 272 (17), 236 (19), 223 (18), 178 (14), 149 (15), 137 (13), 121 (21), 93 (19), 69 (100). Anal. Calcd. for $C_{22}H_{16}Cl_3N_5O_2$ (488.75): C, 54.06; H, 3.30; N, 14.33. Found: C, 54.43 H, 3.57; N, 14.40.

4.2. Pharmacological screening

4.2.1. Inhibition of growth

4.2.1.1. Cell culture. MCF-7, human breast cancer cells and PC-3, human prostate cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA) and cultured in Dulbecco's modified Eagle's medium /F12 medium (DMEM/F-12, Gibco, Grand

Island, NY) or DMEM supplemented with 10% fetal bovine serum (FBS; Gibco).All cells were cultured at 37 °C in a humidified incubator containing 5% CO_2.

4.2.1.2. Cell viability analysis. The effect of test compounds on cancer cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay in six replicates as previously reported [28]. Briefly, Cells were seeded in 96-well flat-bottomed plates for 24 h, and treated with test agents in 5% FBS-supplemented DMEM/F-12 or DMEM for the indicated time intervals. Controls received DMSO vehicle at a concentration equal to that in drug-treated cells. After treatment, cells were incubated in the same medium containing 0.5 mg/ml MTT at 37 °C for 2 h. Reduced MTT was solubilized in DMSO (200 μL/well) for determination of absorbance at 570 nm using a microplate reader. Results are presented in Table 1.

4.2.2. Caspase activation assay

Caspase-3/7 and caspase-8 activities in MCF-7 cells treated with test agents were measured using Caspase-Glo 3/7 luminescence assay kit (Promega, Madison, WI) according to the manufacturer's instructions and as mentioned before [29]. Briefly Cells were plated at 1×10^4 (100 μl/well) into clear bottom, opaque wall 96-well tissue culture plates and incubated for 24 h. The medium was removed and the cells were then treated with test compounds for 48 h. Caspase-3/7 activity were then assayed according to the instructions included in the kit. The luminescence of plates was read using a luminometer. Results are represented in Fig. 3.

References

[1] A. Petrelli, S. Giordano, Curr. Med. Chem. 15 (2008) 422–432.
[2] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, M.J. Thun, J. Clin. 59 (2009) 225–249.
[3] H. Wallerand, R.R. Reiter, A. Ravaud, Curr. Opin. Urol. 18 (2008) 524–532.
[4] H.A. Omar, S.A. Arafa el, S.A. Salama, H.H. Arab, C.H. Wu, J.R. Weng, Toxicol. Appl. Pharmacol. 272 (2013) 616–624.
[5] H.A. Omar, S.A. Salama, S.A. Arafa el, J.R. Weng, Biol. Chem. 394 (2013) 865–870.
[6] L.Y. Bai, H.A. Omar, C.F. Chiu, Z.P. Chi, J.L. Hu, J.R. Weng, Cancer Chemother. Pharmacol. 68 (2011) 489–496.
[7] G.S. Salvesen, Essays Biochem. 38 (2002) 9–19.
[8] S.W. Fesik, Nat. Rev. Cancer 5 (2005) 876–885.
[9] H. Ulbrich, B. Fiebich, G. Dannhardt, Eur. J. Med. Chem. 37 (2002) 953–959.
[10] M. Oka, Y. Matsumoto, K. Hirooka, T. Suzuki, Chem. Pharm. Bull. (Tokyo) 48 (2000) 1121–1124.
[11] L. Diafi, C. Rubat, P. Coudert, P. Bastide, N. Margoum, P. Tronchel, Eur. J. Med. Chem. 26 (1991) 231–235.
[12] A. Ghiorghis, A. Talebian, R. Clarke, Cancer Chemother. Pharmacol. 29 (1992) 290–296.
[13] W.K. Anderson, D.C. Dean, T. Endo, J. Med. Chem. 33 (1990) 1667–1675.
[14] S.A. Laufer, J. Augustin, G. Dannhardt, W. Kiefer, J. Med. Chem. 37 (1994) 1894–1897.
[15] G. Rubner, K. Bensdorf, A. Wellner, S. Bergemann, I. Ott, R. Gust, Eur. J. Med. Chem. 45 (2010) 5157–5163.
[16] J.R. Weng, L.Y. Bai, H.A. Omar, A.M. Sargeant, C.T. Yeh, Y.Y. Chen, M.H. Tsai, C.F. Chiu, Oral Oncol. 46 (2010) 748–754.
[17] S. Tavolari, M. Bonafe, M. Marini, C. Ferreri, G. Bartolini, E. Brighenti, S. Manara, V. Tomasi, S. Laufer, T. Guarnieri, Carcinogenesis 29 (2008) 371–380.
[18] W. Kemnitzer, J. Kuemmerle, S. Jiang, N. Sirisoma, S. Kasibhatla, C. Crogan-Grundy, B. Tseng, J. Drewe, S.X. Cai, Bioorg. Med. Chem. Lett. 19 (2009) 3481–3484.
[19] H.Z. Zhang, S. Kasibhatla, J. Kuemmerle, W. Kemnitzer, K. Ollis-Mason, L. Qiu, C. Crogan-Grundy, B. Tseng, J. Drewe, S.X. Cai, J. Med. Chem. 48 (2005) 5215–5223.
[20] N. Dahan-Farkas, C. Langley, A.L. Rousseau, D.B. Yadav, H. Davids, C.B. de Koning, Eur. J. Med. Chem. 46 (2011) 4573–4583.
[21] N.I. Ziedan, H. Kadri, A.D. Westwell, Mini Rev. Med. Chem. 8 (2008) 711–718.
[22] S.F. Md Tohid, N.I. Ziedan, F. Stefanelli, S. Fogli, A.D. Westwell, Eur. J. Med. Chem. 56 (2012) 263–270.
[23] S. Liu, C.W. Brown, K.D. Berlin, A. Dhar, S. Guruswamy, D. Brown, G.J. Gardner, M.J. Birrer, D.M. Benbrook, J. Med. Chem. 47 (2004) 999–1007.
[24] D. Simoni, M. Roberti, F.P. Invidiata, R. Rondanin, R. Baruchello, C. Malagutti, A. Mazzali, M. Rossi, S. Grimaudo, F. Capone, L. Dusonchet, M. Meli, M.V. Raimondi, M. Landino, N. D'Alessandro, M. Tolomeo, D. Arindam, S. Lu, D.M. Benbrook, J. Med. Chem. 44 (2001) 2308–2318.
[25] S.M. El-Moghazy, J. Pharm. Sci. 33 (1992) 527–538.
[26] A. Etienne, Y. Correia, Bull. Soc. Chim. Fr. 10 (1969) 3704–3712.
[27] M.Y. Ebeid, S.M.El. Moghazy, M.M. Hanna, F.A. Romeih, F.F. Barsoum, Bull. Fac. Pharm. Cairo Univ. 35 (1997) 171–183.
[28] H.A. Omar, A.M. Sargeant, J.R. Weng, D. Wang, S.K. Kulp, T. Patel, C.S. Chen, Mol. Pharmacol. 76 (2009) 957–968.
[29] H.A. Omar, C.C. Chou, L.D. Berman-Booty, Y. Ma, J.H. Hung, D. Wang, T. Kogure, T. Patel, L. Terracciano, N. Muthusamy, J.C. Byrd, S.K. Kulp, C.S. Chen, Hepatology 53 (2011) 1943–1958.