International Journal of Clinical Medical Research

Pyrrolizines: natural and synthetic derivatives with diverse biological activities

Samir M. El-Moghazy Aly[1], Mohamed A. Azim Mohamed[1], Ahmed M. Shawky[2], Faisal A. Almalki[3], Ashraf N. Abdalla[4,5], Bahaa G.M. Youssif[6], Ahmed H. Abdelazeem[7,8], Nashwa, A. Ibrahim[7], Ahmed M. Gouda[7]

Correspondence:
Ahmed M. Gouda, PhD: Department of Medicinal Chemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt.
Fax: (002)-082-2162133
Email:
ahmed.gouda@pharm.bsu.edu.eg
or amsaid@uqu.edu.sa

1 Pharmaceutical Chemistry Department Faculty of Pharmacy, Cairo University Cairo, Egypt.
2 Science and Technology Unit (STU), Umm Al-Qura University, Makkah 21955, Saudi Arabia
3 Department of Pharmaceutical Sciences, Faculty of Pharmacy, Umm Al-Qura University, Makkah 21955, Saudi Arabia.
4 Department of Pharmacology and Toxicology, Faculty of Pharmacy, Umm Al-Qura University, Makkah 21955, Saudi Arabia.
5 Department of Pharmacology and Toxicology, Medicinal And Aromatic Plants Research Institute, National Center for Research, Khartoum 2404, Sudan.
6 Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt.
7 Medicinal chemistry department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt.
8 Pharmacy Department, College of pharmacy, Nursing and Medical Sciences, Riyadh Elm University, Riyadh 11681, Saudi Arabia.

Volume number 2
Issue number 5
Pages 171-184

10.61466/ijcmr2050003

Received: 09.07.2024
Accepted: 13.09.2024
Published: 17.09.2024
0nline: 01.10.2024

Abstract

The pyrrolizine nucleus is a bicyclic ring system that consists of a pyrrole ring fused to another pyrrolidin ring. It constitutes the basic skeleton in many natural and synthetic compounds with diverse biological activities such as the anti-inflammatory, nootropic, antiemetic, antibacterial, antiviral, anticonvulsant, antiarrhythmic, and anticancer activities. At least two of these derivatives, ketorolac and mitomycin C, have been approved for the treatment of inflammation and cancer, respectively. Licofelone, a dual inhibitor of COX and 5-LOX, was also evaluated in clinical trials for the treatment of osteoarthritis. On the other hand, a large number of the pyrrolizine-based derivatives have displayed anticancer activity against different types of cancer cells. In this review, the pyrrolizine-based derivatives with anticancer activity were classified based on their chemical structure into substituted, fused, and spiro-pyrrolizine. The mechanisms of action of these compounds included alkylation of the DNA, inhibition of COX, or alteration of the permeability of the cytoplasmic membrane in cancer cells. In addition, other pyrrolizines were found to act by inhibiting DNA replication, rRNA, Rac1 kinase, thioredoxin reductase, or oncogenic kinases. The last section of this review also focuses on the reported X-ray crystal structures of these compounds with different proteins. The binding modes and interactions of ketorolac and licofelone were illustrated in this review. To sum up, we anticipate that the data compiled in this review will be useful to researchers in the design of pyrrolizines with potent biological activities.

Keywords: pyrrolizine; mechanism of action; anticancer; anti-inflammatory, binding interactions

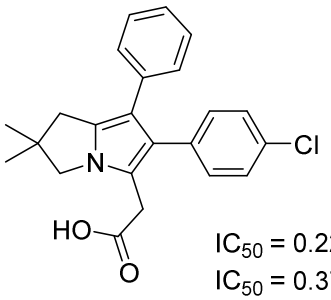
Introduction

Pyrrolizines included a large class of natural and synthetic heterocyclic compounds [1–3]. The pyrrolizine derivatives have exhibited different types of biological activities, such as anti-inflammatory, nootropic, antiemetic, antibacterial, antiviral, anticonvulsant, antiarrhythmic, and anticancer activities [1–8]. The biological activities with representative examples of these compounds will be discussed as follows:

Anti-inflammatory activity

Several pyrrolizines have been reported with anti-inflammatory activity [4,9,10]. Tries *et al*. have reported the anti-inflammatory activity of licofelone (ML3000) **1** in the human whole blood assay [9]. The results revealed the ability of compound **1** (**Figure 1**) to inhibit the synthesis of PGE2 by cyclooxygenase 1 (COX-1) at an IC_{50} value of 3.9 μM compared to indomethacin (IC_{50} = 4.5 μM). Mechanistic studies also revealed the ability of licofelone **1** to inhibit 5-lipoxygenase (5-LOX). The inhibitory activity of licofelone against COX-1 and 5-LOX was evaluated in another study [10]. The results revealed inhibition of COX-1 and 5-LOX at IC_{50} values of 0.22 μM and 0.37 μM, respectively.

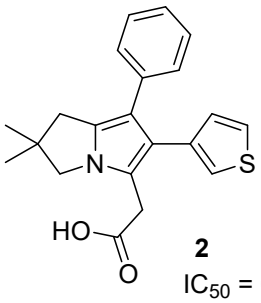
Laufer *et al*. reported the synthesis and biological activity of a series of heterocyclic analogs of **1** (ML3000) with dual inhibitory activity against COX-1 and 5-LOX [10]. The chemical structures of these compounds are depicted in **Figure 2**. The results revealed the ability of the four compounds **2-5** to inhibit 5-LOX at IC_{50} values in the range of 0.03-2.4 μM compared to ML3000 (IC_{50} = 0.37 μM). They also inhibited COX-1 at IC_{50} values in the range of 0.02-0.21 μM compared to ML3000 (IC_{50} = 0.22 μM). Among these compounds, compound **5** with the benzofuran moiety was the most active.



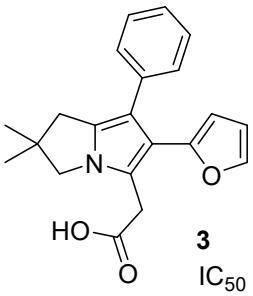
IC_{50} = 0.22 μM (COX-1)
IC_{50} = 0.37 μM (5-LOX)

1 (ML3000)

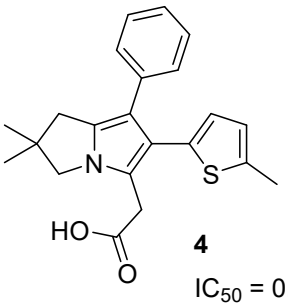
Figure 1. The chemical structure of licofelone (ML3000) **1**.



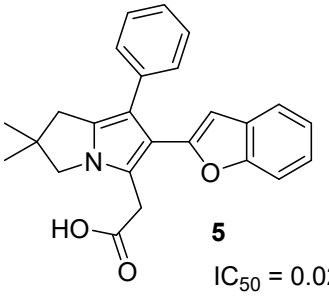
2
IC_{50} = 0.06 μM (COX-1)
IC_{50} = 1.7 μM (5-LOX)



3
IC_{50} = 0.13 μM (COX-1)
IC_{50} = 2.4 μM (5-LOX)



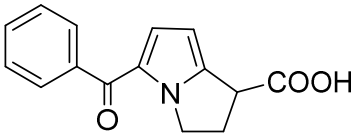
4
IC_{50} = 0.21 μM (COX-1)
IC_{50} = 0.25 μM (5-LOX)



5
IC_{50} = 0.02 μM (COX-1)
IC_{50} = 0.03 μM (5-LOX)

Figure 2. The chemical structures of compounds **2-5**.

Ketorolac **6** (**Figure 3**) is a member of the pyrrolo-pyrrolidine group of nonsteroidal anti-inflammatory drugs (NSAIDs). Ketorolac underwent a number of studies due to its high activity and low side effects. Waterbury *et al*. have investigated the mechanistic of the anti-inflammatory activity of ketorolac [11]. The results revealed inhibition in COX-1 activity at an IC_{50} value of 0.02μM compared to COX-2 (IC_{50} = 0.12μM) [11].

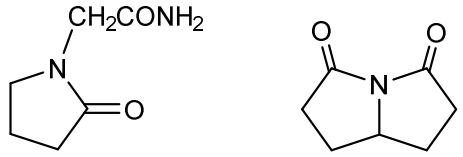


6

Figure 3. The chemical structure of ketorolac **6**.

Nootropic activity

Nootropic drugs are a class of psychostimulants, used to enhance the integrative functions of the brain. Among these drugs, piracetam **7** (**Figure 4**) was found to improves cognitive performance [12]. Compound **8**, a synthetic pyrrolizine-3,5(2*H*)-dione derivative, which is structurally related to piracetam, also showed nootropic activity [13–15].

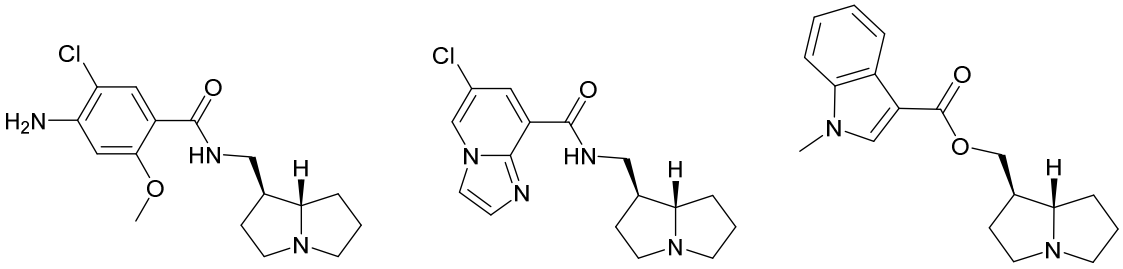


7 (Piracetam) **8** (Rolziracetam)

Figure 4. The chemical structures of piracetam **7** and rolziracetam **8**.

Antiemetic activity

Becker *et al*. reported several pyrrolizine-based derivatives with agonistic and antagonistic activity on the 5-HT4 receptor [16]. Among these derivatives (**Figure 5**), compound **9** exhibited potent and selective partial agonist activity on the 5-HT_4 receptor, which enhanced the gastric emptying in an animal model. On the other hand, compounds **10** and **11** were identified as potent and selective partial agonist and antagonist activity.

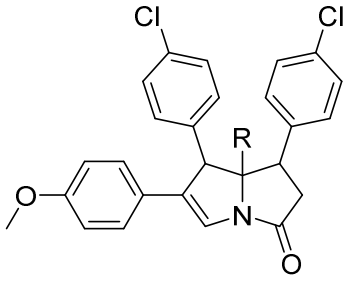


9 **10** **11**

Figure 5. The chemical structures of compounds **9-11**.

Antibacterial activity

Li *et al*. reported the synthesis and antibacterial activity of a pyrrolizidine derivative **12** (PA-1) [17]. The results of the biological evaluation of compound **12** (**Figure 6**) revealed inhibition in the growth of six bacteria (MIC = 0.0039 to 0.025 mg/ml). Among the tested bacteria, the growth of *Escherichia coli* and *Staphylococcus aureus* was inhibited by compound **12** at MIC values of 0.0313 and 0.0039 mg/ml, respectively. Mechanistic studies revealed the ability of compound **12** to damage the cellular membrane of S. aureus.

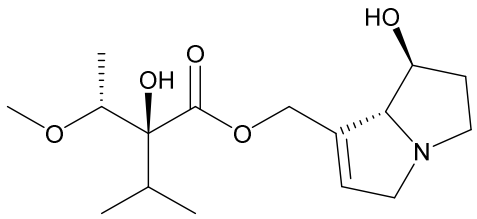


12 (R = $COOC_2H_5$)

Figure 6. The chemical structure of compound **12**.

Antiviral activity

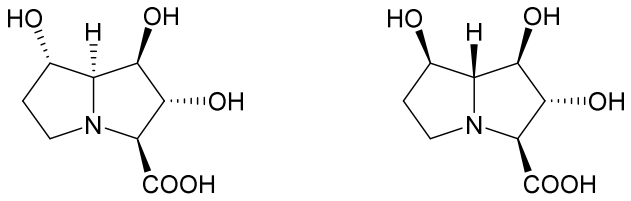
The extracts of several pyrrolizidine-containing herbs and plants have been investigated for antiviral activities [18–20]. In addition, some of the isolated pyrrolizidine derivatives were also investigated for their activity against antiviral activity. Among these, heliotrine **13** (**Figure 7**) exhibited antiviral activity against poliomyelitis [18].



13 (Heliotrine)

Figure 7. The chemical structure of heliotrine **13**.

Taylor *et al*. have also investigated the antiviral activity of alexine **14** and its stereoisomers for their activity against HIV-1 [21]. The results revealed that only the 7,7a-diepialexine **15** inhibits the growth of the virus, **Fig 8**. Compound **15** also inhibited the α-glucosidase 1 enzyme.

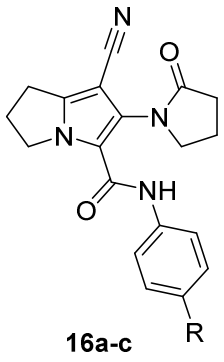


14 (Alexine) **15** (7,7a-diepialexine)

Figure 8. The chemical structure of alexine (**14**) and 7,7a-diepialexine (**15**).

Anticonvulsant activity

Abbas *et al*. have also reported a series of pyrrolizine and fused derivatives [22]. Investigating the ability of these compounds to protect against PTZ-induced seizures revealed weak to moderate protective activity (10.6–67.9%) compared to phenobarbitone (100%). Among these derivatives **16a-c** (**Fig 9**), compound **16b** was the most active.



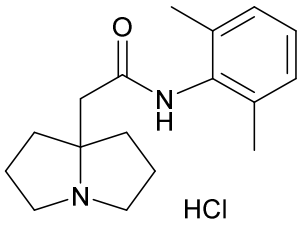
16a-c

16a: R = H;
16b: R = CH_3;
16c: R = Cl

Figure 9. The chemical structure of compounds **16a-c**.

Antiarrhythmic activity

Compound **17** (SUN 1165, **Figure 10**) is a new potent antiarrhythmic agent with sodium channel blocking activity [23]. It reduced the contraction induced by electrical field stimulation and carbachol. Compound **17** also sowed a cardioprotective effect compared to the control group [24].



17

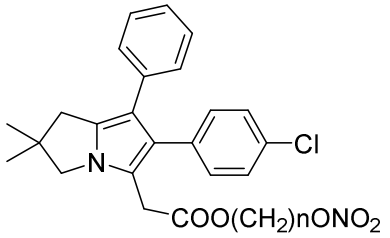
Figure 10. The chemical structure of compound **17** (SUN 1165).

Antitumor activity

Many of the pyrrolizine derivatives have been evaluated for their cytotoxic activity against cancer cell lines. Based on their chemical structures, these compounds could be classified into three groups. In the following, the anticancer potential of each of these three groups will be discussed with representative examples in each one.

Substituted pyrrolizines

Among these, licofelone **1** displayed growth inhibitory activity against prostate cancer cells [25]. This effect was associated with induction of apoptosis and downregulation of the expression of COX-2/5-LOX. Liu *et al*. have reported the synthesis and cytotoxic activity of five nitric oxide donor conjugates with licofelone [26]. The cytotoxicity of esters **18a-e** (**Figure 11**) was evaluated against three cancer cell lines (MDA-MB-231, MCF-7, and HT-29). The results revealed the highest cytotoxicity for **18b** (IC_{50} = 4.6–19.1 μM). Among the three cell lines, MCF-7 cells were the most sensitive to **18b** (IC_{50} = 4.6 μM) compared to licofelone (IC_{50} = 5.5 μM).



18a-e

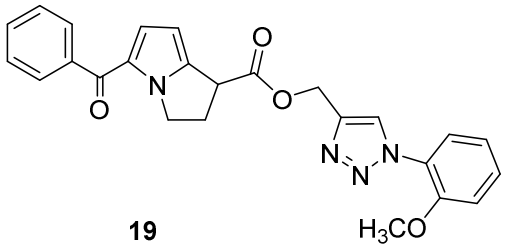
18a: n = 2;
18b: n = 3;
18c: n = 4;
18d: n = 8;
18e: n = 12

Figure 11. The chemical structure of licofelone-nitric oxide donor conjugates **18a-e**.

Guo *et al*. studied the potential chemopreventive effect of ketorolac in ovarian cancer patients [27]. The results revealed that the R-isomer inhibits Rac1 and Cdc42, which were expressed in ovarian cancer patients. The use of ketorolac in these patients was associated with survival benefits.

Ketorolac was also evaluated for its anticancer activity, where the results revealed weak activity [28]. However, the combination of ketorolac and 5-FU showed a synergistic anticancer effect against HT-29 cells [29]. Accordingly, the dose of the anticancer 5-FU may need readjustment when combined with ketorolac.

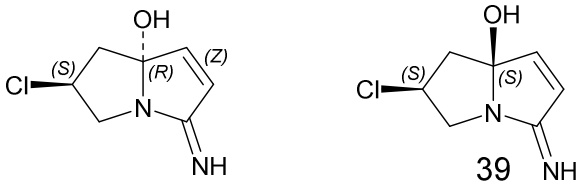
In addition, several derivatives of ketorolac have shown more potent anticancer activity than ketorolac [28]. Among these derivatives, the triazolyl ester of ketorolac (15K) **19** prepared from ketorolac through Click chemistry, **Figure 12**. The triazolyl ester **19** showed a 10-fold increase in permeability compared to ketorolac. Compound **19** prepared also inhibited the growth of A549 cancer cells at an IC_{50} value of 24 nM compared to ketorolac (IC_{50} = 13 μM). Mechanistic studies of compound **19** revealed the association of the anticancer activity with the inhibition of PAK1 activity.



19

Figure 12. The chemical structure of the triazolyl ester of ketorolac **19**.

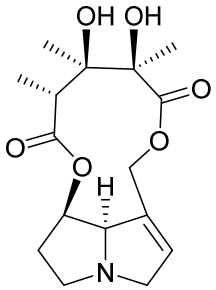
Clazamycin is a natural antitumor antibiotic that was isolated from Streptomyces [30]. In aqueous solutions, clazamycin exists as a mixture of two epimers (clazamycin A **20** and clazamycin B **21**, **Figure 13**). It showed antiviral activity against Herpes simplex and anticancer activity against L1210 cells. Mechanistic studies of clazamycin performed by Hori *et al*. revealed inhibition in DNA replication and cytoplasmic membrane transport [31]. The results also revealed that the effect of clazamycin on L1210-innoculated mice could be due to its effect on the cell membrane.



20 (Clazamycin A) **21** (Clazamycin B)

Figure 13. The chemical structure of clazamycin A **20** and clazamycin B **21**.

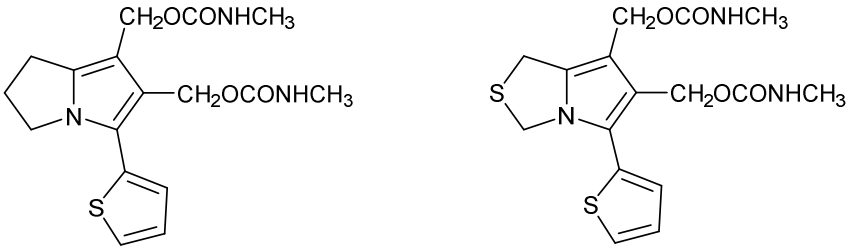
Kusuma *et al*. investigated the antineoplastic activity of monocrotaline **22** (**Figure 14**) using an in silico docking study [32]. The results revealed potential activity against p53, HGF, and TREM1 proteins. The biological evaluation revealed cytotoxicity against HepG2 cells at an IC_{50} value of 24.966 µg/mL.



22 (Monocrotaline)

Figure 14. The chemical structure of monocrotaline **22**.

Ladurée *et al*. reported the synthesis and cytotoxicity of a series of pyrrolizine-6,7-dicarboxylate derivatives [33]. The new compounds (**Figure 15**) were obtained from the reaction of 2,3-dihydro-1*H*-pyrrolizine-6,7-diyl)dimethanol with methyl/isopropyl isocyanate. Among these derivatives, pyrrolizine **23** and pyrrolo[1,2-*c*]thiazole **24** were evaluated for their antileukemic activity *in vivo* using L1210 cancer cells. The results revealed that they have activity similar to that of mitomycin.



23 **24**

Figure 15. The chemical structures of compounds **23** and **24**.

Shawky *et al*. reported two series of 3,4,5-trimethoxyphenyl-bearing pyrrolizines with potent cytotoxicity against MCF-7, A2780, and HCT116 cancer cell lines [5]. Among the reported compounds, **25a**,**b**,**d** (**Figure 16**), the most potent showed inhibitory activity against multiple oncogenic kinases. They also induced cell cycle arrest and apoptosis in MCF-7 cells. Evaluation of the effect of the three compounds on tubulin polymerization revealed weak to moderate inhibitory activity.

Attalah *et al*. reported a series of pyrrolizine-bearing ethyl benzoate moiety with anti-inflammatory and analgesic activities [34]. Among these derivatives, compound **26** (**Figure 17**) was the most active against MCF-7 (IC_{50} = 0.02 µM). In addition, analysis of the MCF-7 cells treated with compound **26** revealed induction of cell cycle arrest and apoptosis.

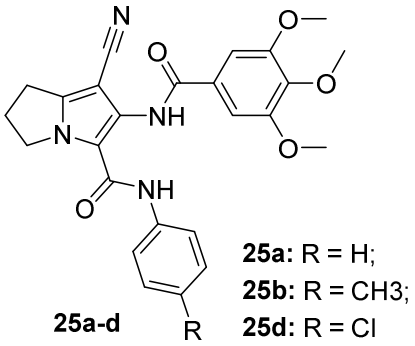


Figure 16. The chemical structure of compounds **25a-d**.

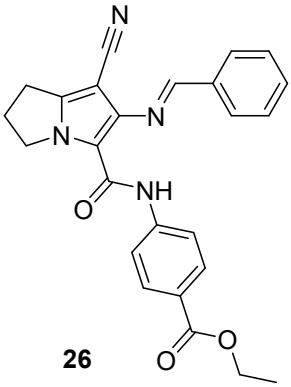


Figure 17. The chemical structure of compound **26**.

Abourehab *et al*. have reported a series of pyrrolizine derivatives hybridized with either ibuprofen or ketoprofen [35]. These hybrids were evaluated for their antiproliferative activity against MCF-7, A549, and HT-29 cancer cell lines. Among these derivatives, compounds **27** and **28** (**Figure 18**) exhibited IC_{50} values of 7.61 and 3.16 µM against MCF-7 cells. They also induced apoptosis and cycle arrest in MCF-7 cells.

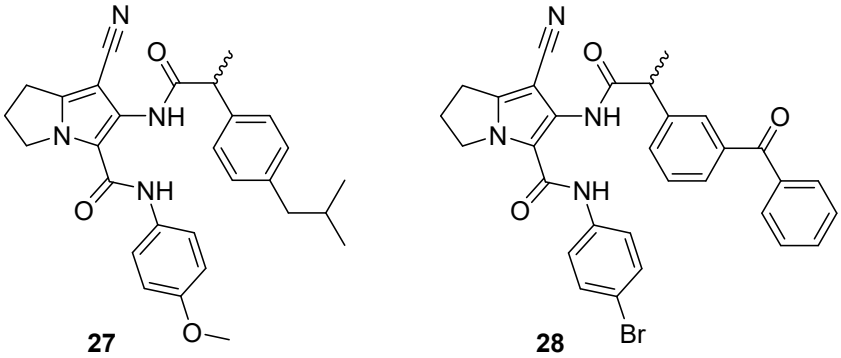


Figure 18. The chemical structure of compounds **27** and **28**.

Shawky *et al*. reported 4-thiazolidinone-bearing pyrrolizines with anti-inflammatory and potent cytotoxicity against MCF-7, A2780, and HT29 cancer cell lines [36]. Compounds **29**, **30**, and **31** (**Figure 19**) displayed inhibitory activity against 20 oncogenic kinases (1-34%). Compound **31** induced G1 cell cycle arrest and apoptosis in MCF-7 cells.

Hanna *et al*. have reported the synthesis and antitumor activity of a series of substituted and fused pyrrolizines [37]. The new compounds were evaluated for their anticancer activity against MCF-7 cells. Among these derivatives, compounds **32** and **33** (**Figure 20**) displayed the highest activity at an IC_{50} value of 0.016 µmol/ml for each of the two compounds.

Compound **34** (**Figure 21**) was synthesized and radiolabeled with ^{131}I, as described by Mahmoud *et al*. [38]. Tumor-bearing mice were used to investigate the biodistribution of [^{131}I]iodo-EZPCA **34**. The results revealed accumulation of the radiolabeled compound in tumor tissue, which indicated its potential use in tumor imaging.

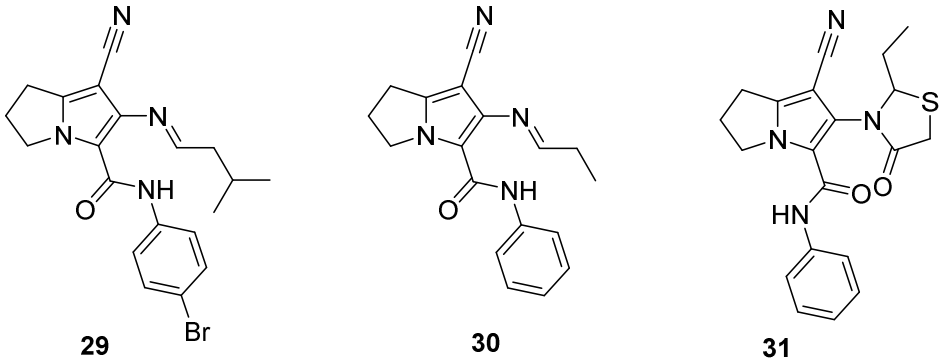


Figure 19. The chemical structure of compounds **29**, **30**, and **31**.

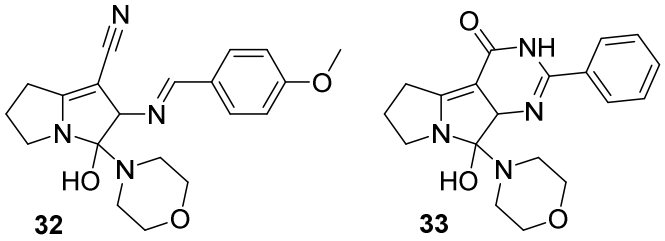


Figure 20. The chemical structures of compounds **32** and **33**.

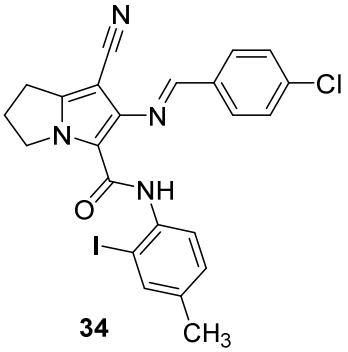


Figure 21. The chemical structure of compound **34**.

Fused pyrrolizines

Shawky *et al*. have also reported several fused pyrrolizines of pyrimidopyrrolizine and diazepinopyrrolizine type with cytotoxic activity against MCF7, A2780, and HT29 cancer cell lines [39]. Among these derivatives, compound **35** (**Figure 22**) induced cell cycle arrest and apoptosis in MCF7 cells. It also inhibited the P-gp in MCF7/ADR cells and increased the accumulation of Rho123.

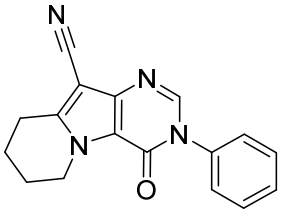
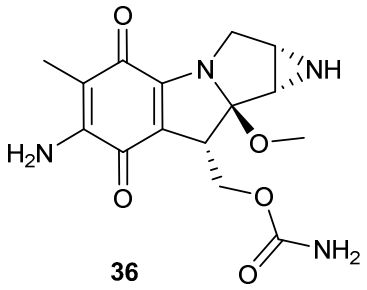


Figure 22. The chemical structure of compound **35**.

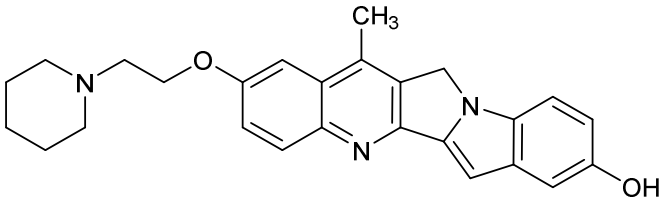
Mitomycin is a class of antibiotics known as isolated from Streptomyces species [40]. Among these compounds, mitomycin C **36** (**Figure 23**), an alkylating agent that was used clinically as an anticancer agent [41,42]. It has broad-spectrum activity against different types of cancers, such as prostate, breast, and non-small cell lung cancers [42]. The mechanisms of action of mitomycin C include its ability to alkylate the DNA and inhibit the rRNA [43]. It also showed inhibitory activity against thioredoxin reductase, which could also play a role in its activity as an anticancer agent [43].



36

Figure 23. The chemical structure of mitomycin C **36**.

Perzyna *et al*. reported a series of benzo[5,6]pyrrolizino[1,2-*b*]quinoline derivatives with cytotoxic activity against L1210, MCF-7, and PC3 cancer cell lines [44]. Among these derivatives, compound **37** (**Figure 24**) was the most active against L1210 and MCF-7 cell lines (IC_{50} = 0.55 µM). Compound **37** also inhibited the growth of PC3 cells at an IC_{50} value of 4.94 µM.

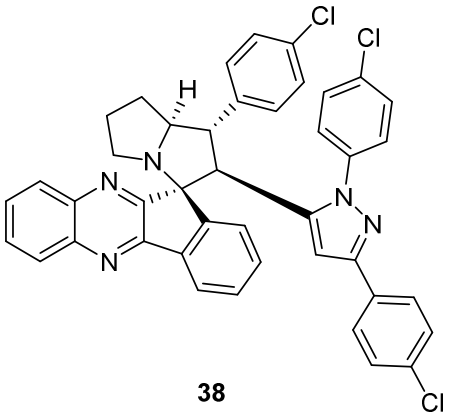


37

Figure 24. The chemical structure of compound **37**.

Spiro derivatives

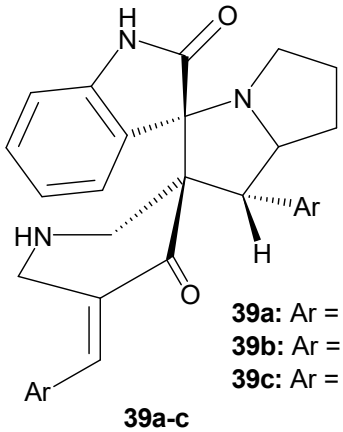
Zimnitskiy *et al*. have reported a series of spiro[indeno[1,2-*b*]quinoxaline-11,3'-pyrrolizine] derivatives [45]. The new compounds were evaluated for their cytotoxicity against the HeLa cancer cell line using the MTT assay. With the exception of four derivatives, the remaining derivatives inhibited the growth of Hela cells at IC_{50} values in the range of 1.93-68.30 µM. Among these derivatives, compound **38** (**Figure 25**) was the most active (IC_{50} = 1.93 µM) compared to camptothecin (IC_{50} = 1.66 µM).



38

Figure 25. The chemical structure of compound **38**.

Almansour *et al*. reported the synthesis and anticancer activity of a series of spiro-pyrrolizine derivatives [46]. These derivatives were evaluated for their antiproliferative activity against three cancer cell lines. The results revealed that these spiro-pyrrolizines have antiproliferative activity similar to those of doxorubicin. Among these derivatives, compound **39a**, **39b** and **39c** were the most potent, **Figure 26**. Among the three compounds, **39b** showed potent activity against leukemia CCRF-CEM cells at an (IC_{50} = 3.6 µM).



39a: Ar = C_6H_5;
39b: Ar = 2-$CH_3C_6H_4$;
39c: Ar = 4-FC_6H_4;

39a-c

Figure 26. The chemical structures of compounds **39a-c**.

Crystal structure of pyrrolizine derivatives

Several crystal structures of pyrrolizine derivatives have been reported in complexes with phospholipase A2 [47], bovine lactoferrin [48,49], human intestinal fatty acid binding protein [50], DNA [51], MMC-binding protein [52], MRD protein [53], methyltransferase [54], and pyrrolizidine alkaloid *N*-oxygenase [55]. The binding modes and interactions of three pyrrolizines into their target proteins were analyzed using Discovery Studio Visualizer (V16.1.0.15350) [56]. Three figures showing the binding modes and interaction of these compounds were generated according to previous reports [57–60].

Licofelone, a dual inhibitor of COX/5-LOX, was reported in complex structure with phospholipase A2 (PDB: 1ZYX) [47]. Phospholipase A2 is responsible for the hydrolysis of fatty acids of phospholipids and play a role in the modulation of inflammatory responses [61]. Analysis of the binding mode and interactions of licofelone into the phospholipase A2 revealed a hydrophobic interaction of the pi-sigma type with Leu2 and several hydrophobic interactions of the pi-alkyl type with Ala18, Ile19, and Trp31, **Figure 27**. In addition, the binding pocket of licofelone into phospholipase A2 includes three molecules of water, which participate in water-mediated hydrogen bonds with the ligand.

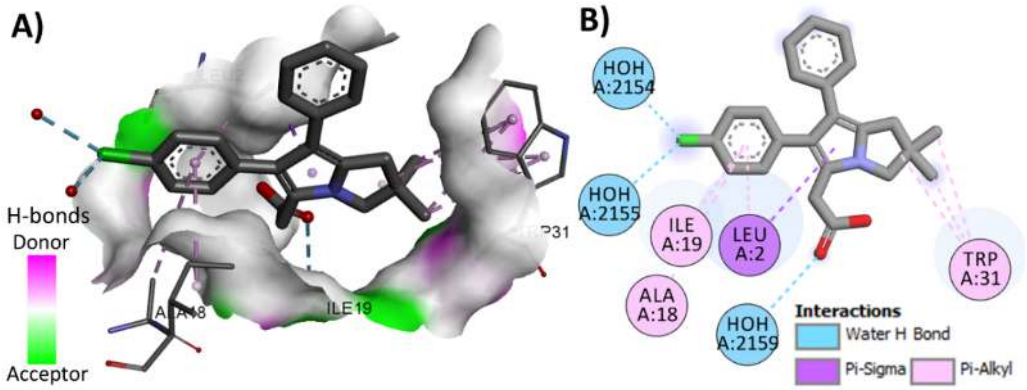


Figure 27. The binding interaction of licofelone in complex with phospholipase A2 (PDB: 1ZYX): **A)** 3D binding mode of licofelone (shown as sticks, colored by element) into its binding pocket in phospholipase A2, water molecules are shown as red spheres; **B)** 2D binding mode of licofelone (shown as sticks), showing the hydrophobic interactions with amino acids in the phospholipase A2; hydrogen atoms were omitted for clarity; this figure was generated using the Discovery Studio Visualizer (V16.1.0.15350).

In addition, ketorolac was reported in a complex structure with human intestinal fatty acid binding protein (hiFABP, PDB: 2MJI) [50]. The binding mode and interaction are illustrated in **Figure 28**. Analysis of the binding interactions revealed one pi-cation interaction with Lys27, one pi-alkyl interaction with Val23, and one pi-pi interaction with Tyr70.

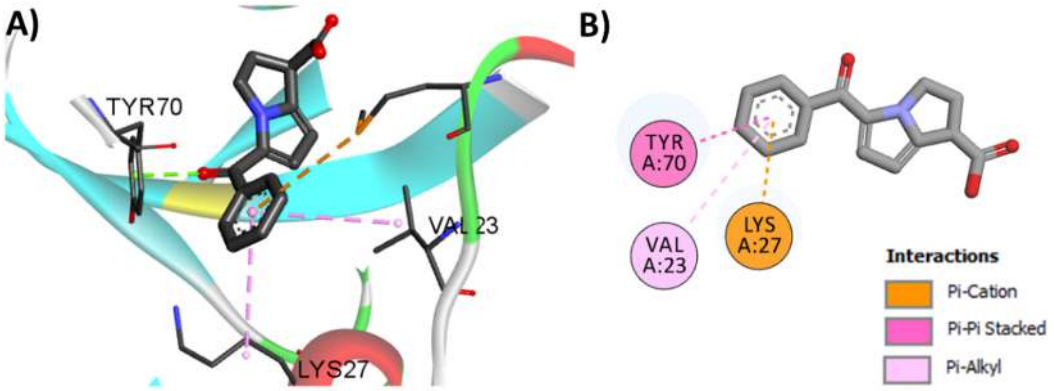


Figure 28. The binding interaction of ketorolac in complex with hiFABP (PDB: 2MJI): **A)** 3D binding mode of ketorolac (shown as sticks, colored by element) into its binding pocket in hiFABP; **B)** 2D binding mode of ketorolac (shown as sticks), showing the hydrophobic interactions with amino acids in hiFABP; hydrogen atoms were omitted for clarity; this figure was generated using the Discovery Studio Visualizer (V16.1.0.15350).

On the other hand, the crystal structure of mitomycin C in complex with DNA (PDB: 199D) was reported by Sastry *et al*. [51]. Analysis of the binding mode and interaction revealed a monoalkylated [MC]dG adduct, **Figure 29**.

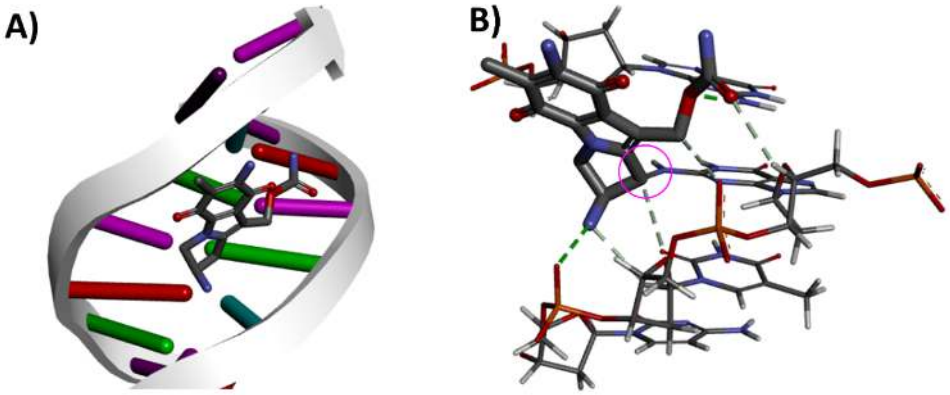


Figure 29. The binding interaction of monoalkylated mitomycin C-DNA complex (PDB: 199D): **A)** 3D binding mode of mitomycin C (shown as sticks, colored by element) into DNA duplex ; **B)** 3D binding mode of mitomycin C (shown as sticks), showing different types of binding interactions; hydrogen atoms were omitted for clarity; this figure was generated using the Discovery Studio Visualizer (V16.1.0.15350).

Conclusions

The pyrrolizine nucleus is a bicyclic ring system that consists of a pyrrole ring fused to another pyrrolidin ring. This nucleus constitutes the basic skeleton in many natural as well as synthetic compounds. In this work, different types of biological activities of these compounds were reviewed with a primary focus on the anticancer activity. Pyrrolizines and the saturated pyrrolizidine alkaloids displayed diverse biological activities such as anti-inflammatory, nootropic, antiemetic, antibacterial, antiviral, anticonvulsant, antiarrhythmic, and anticancer activities. At least two of these derivatives, ketorolac and mitomycin C, have been used clinically as anti-inflammatory and anticancer agents, respectively. The dual COX/5LOX inhibitor licofelone was also evaluated in clinical trials for the treatment of osteoarthritis. Among the diverse activity of these compounds, a large number of the pyrrolizine-based derivatives have displayed cytotoxic activity against different types of cancer cell lines. Based on their chemistry, these compounds could also be classified into substituted, fused, and spiro-pyrrolizine. The cytotoxicity of these pyrrolizines was attributed to different mechanisms of action, such as DNA alkylation, COX inhibition, and alteration of the permeability of the cytoplasmic membrane in cancer cells. In addition, other mechanisms of action such as inhibition of DNA replication, inhibition of rRNA, inhibition of Rac1 kinase, inhibition of thioredoxin reductase, and inhibition of oncogenic kinases have also been reported for members in this class. The last section of this review also focuses on the reported X-ray crystal structure of some pyrrolizines with different target macromolecules. The binding modes and interactions of selected derivatives were illustrated in this review. To sum up, we hope that the data compiled in this review will be useful to researchers in the design of pyrrolizines with potent and selective biological activities.

References

1. A. Robin Mattocks, R. Jukes, Recovery of the pyrrolic nucleus of pyrrolizidine alkaloid metabolites from sulphur conjugates in tissues and body fluids, Chem. Biol. Interact. 75 (1990) 225–239. doi:https://doi.org/10.1016/0009-2797(90)90120-C.
2. E. Vedejs, S. Larsen, F.G. West, Nonstabilized imidate ylides by the desilylation method: a route to the pyrrolizidine alkaloids retronecine and indicine, J. Org. Chem. 50 (1985) 2170–2174. doi:10.1021/jo00212a034.
3. T. Quiroz, D. Corona, A. Covarruvias, J.G. Avila-Zárraga, M. Romero-Ortega, A practical method for the synthesis of pyrrolizidine, indolizidine and pyrroloazepinolizidine nucleus, Tetrahedron Lett. 48 (2007) 1571–1575. doi:https://doi.org/10.1016/j.tetlet.2007.01.015.
4. A.M. Gouda, A.H. Abdelazeem, An integrated overview on pyrrolizines as potential anti-inflammatory, analgesic and antipyretic agents, Eur. J. Med. Chem. 114 (2016) 257–292. doi:10.1016/j.ejmech.2016.01.055.
5. A.M. Shawky, N.A. Ibrahim, A.N. Abdalla, M.A.S. Abourehab, A.M. Gouda, Novel pyrrolizines bearing 3,4,5-trimethoxyphenyl moiety: design, synthesis, molecular docking, and biological evaluation as potential multi-target cytotoxic agents., J. Enzyme Inhib. Med. Chem. 36 (2021) 1313–1333. doi:10.1080/14756366.2021.1937618.
6. F.A. Almalki, A.N. Abdalla, A.M. Shawky, M.A. El Hassab, A.M. Gouda, In Silico Approach Using Free Software to Optimize the Antiproliferative Activity and Predict the Potential Mechanism of Action of Pyrrolizine-Based Schiff Bases, Molecules. 26 (2021). doi:10.3390/molecules26134002.
7. S.M. El-Moghazy, M.A.A. Mohamed, A.E. Farag, A.M. Gouda, Synthesis and antitumor activity of some 5H-pyrrolizine, pyrimido[5,4-a]pyrrolizine, pyrimido[4,5b]pyrrolizine derivatives, Saudi Pharm. J. 17 (2009) 3–18.
8. M.T. Elsaady, A.M. Gouda, F.H. Edrees, N.M.A. Gawad, Design, synthesis and biological evaluation of some novel Schiff base derivatives as potential anticancer agents, J. Chem. Pharm. Res. 8 (2016) 273–282.
9. S. Tries, W. Neupert, S. Laufer, The mechanism of action of the new antiinflammatory compound ML3000: inhibition of 5-LOX and COX-1/2., Inflamm. Res. Off. J. Eur. Histamine Res. Soc. ... [et Al.]. 51 (2002) 135–143. doi:10.1007/pl00000285.
10. S. Laufer, H.G. Striegel, K. Neher, P. Zechmeister, C. Donat, K. Stolingwa, S. Baur, S. Tries, T. Kammermeier, G. Dannhardt, W. Kiefer, Synthesis and evaluation of a novel series of pyrrolizine derivatives as dual cyclooxygenase-1 and 5-lipoxygenase inhibitors., Arch. Pharm. (Weinheim). 330 (1997) 307–312. doi:10.1002/ardp.19973300908.
11. L.D. Waterbury, D. Silliman, T. Jolas, Comparison of cyclooxygenase inhibitory activity and ocular anti-inflammatory effects of ketorolac tromethamine and bromfenac sodium., Curr. Med. Res. Opin. 22 (2006) 1133–1140. doi:10.1185/030079906X112471.
12. K. Scheuer, A. Rostock, R. Bartsch, W.E. Müller, Piracetam improves cognitive performance by restoring neurochemical deficits of the aged rat brain., Pharmacopsychiatry. 32 Suppl 1 (1999) 10–16. doi:10.1055/s-2007-979231.
13. E.W. Thomas, R.H. Rynbrandt, D.C. Zimmermann, L.T. Bell, C.R. Muchmore, E.W. Yankee, Reaction of novel imide reducing reagents with pyrrolizidinediones, J. Org. Chem. 54 (1989) 4535–4543. doi:10.1021/jo00280a018.
14. A.H. Gouliaev, A. Senning, Piracetam and other structurally related nootropics., Brain Res. Brain Res. Rev. 19 (1994) 180–222. doi:10.1016/0165-0173(94)90011-6.
15. R. Verloes, A.-M. Scotto, J. Gobert, E. Wülfert, Effects of nootropic drugs in a scopolamine-induced amnesia model in mice, Psychopharmacology (Berl). 95 (1988) 226–230. doi:10.1007/BF00174514.
16. D.P. Becker, D.L. Flynn, A.E. Moormann, R. Nosal, C.I. Villamil, R. Loeffler, G.W. Gullikson, C. Moummi, D.-C. Yang, Pyrrolizidine esters and amides as 5-HT4 receptor agonists and antagonists., J. Med. Chem. 49 (2006) 1125–1139. doi:10.1021/jm0509501.
17. N. Li, S. Tan, J. Cui, N. Guo, W. Wang, Y. Zu, S. Jin, X. Xu, Q. Liu, Y. Fu, PA-1, a novel synthesized pyrrolizidine alkaloid, inhibits the growth of Escherichia coli and Staphylococcus aureus by damaging the cell membrane, J. Antibiot. (Tokyo). 67 (2014) 689–696. doi:10.1038/ja.2014.49.
18. B. Singh, P.M. Sahu, S.C. Jain, S. Singh, Antineoplastic and Antiviral Screening of Pyrrolizidine Alkaloids from Heliotropium subulatum, Pharm. Biol. 40 (2002) 581–586. doi:10.1076/phbi.40.8.581.14659.
19. S.F. Al-Subaie, A.M. Alowaifeer, M.E. Mohamed, Pyrrolizidine Alkaloid Extraction and Analysis: Recent Updates., Foods (Basel, Switzerland). 11 (2022). doi:10.3390/foods11233873.
20. T. Kopp, M. Abdel-Tawab, B. Mizaikoff, Extracting and Analyzing Pyrrolizidine Alkaloids in Medicinal Plants: A Review., Toxins (Basel). 12 (2020). doi:10.3390/toxins12050320.
21. D.L. Taylor, R. Nash, L.E. Fellows, M.S. Kang, A.S. Tyms, Naturally Occurring Pyrrolizidines: Inhibition of α-Glucosidase 1 and Anti-HIV Activity of One Stereoisomer, Antivir. Chem. Chemother. 3 (1992) 273–277. doi:10.1177/095632029200300504.
22. S.E. Abbas, F.M. Awadallah, N.A. Ibrahim, A.M. Gouda, B.A. Shehata, Design, synthesis and preliminary evaluation of some novel 1,4]diazepino [5,6-b]pyrrolizine and 6-(2-oxopyrrolidino)-1Hpyrrolizine derivatives

as anticonvulsant agents, Med. Chem. Res. 20 (2011) 1015–1023. doi:10.1007/s00044-010-9429-8.
23. Y. Nakashima, S. Sugiyama, J. Shindoh, F. Taki, K. Takagi, T. Satake, T. Ozawa, Effects of sodium channel blockers on electrical field stimulation-induced guinea-pig tracheal smooth muscle contraction., Arch. Int. Pharmacodyn. Ther. 306 (1990) 130–138.
24. S. Sugiyama, Y. Hanaki, T. Ogawa, N. Hieda, K. Taki, T. Ozawa, The effects of SUN 1165, a novel sodium channel blocker, on ischemia-induced mitochondrial dysfunction and leakage of lysosomal enzymes in canine hearts., Biochem. Biophys. Res. Commun. 157 (1988) 433–439. doi:10.1016/s0006-291x(88)80267-5.
25. N.K. Narayanan, D. Nargi, M. Attur, S.B. Abramson, B.A. Narayanan, Anticancer effects of licofelone (ML-3000) in prostate cancer cells., Anticancer Res. 27 (2007) 2393–2402.
26. W. Liu, J. Zhou, Y. Liu, H. Liu, K. Bensdorf, C. Guo, R. Gust, Licofelone-nitric oxide donors as anticancer agents., Arch. Pharm. (Weinheim). 344 (2011) 487–493. doi:10.1002/ardp.201000397.
27. Y. Guo, S.R. Kenney, L. Cook, S.F. Adams, T. Rutledge, E. Romero, T.I. Oprea, L.A. Sklar, E. Bedrick, C.L. Wiggins, H. Kang, L. Lomo, C.Y. Muller, A. Wandinger-Ness, L.G. Hudson, A Novel Pharmacologic Activity of Ketorolac for Therapeutic Benefit in Ovarian Cancer Patients., Clin. Cancer Res. an Off. J. Am. Assoc. Cancer Res. 21 (2015) 5064–5072. doi:10.1158/1078-0432.CCR-15-0461.
28. B.C.Q. Nguyen, H. Takahashi, Y. Uto, M.D. Shahinozzaman, S. Tawata, H. Maruta, 1,2,3-Triazolyl ester of Ketorolac: A “Click Chemistry”-based highly potent PAK1-blocking cancer-killer., Eur. J. Med. Chem. 126 (2017) 270–276. doi:10.1016/j.ejmech.2016.11.038.
29. M. Atefinezhad, A. Moghadamnia, S.P. Eftekhar, A.A. Moghadamnia, S. Kazemi, Effect of Ketorolac on Pharmacokinetics and Pharmacodynamics of 5-Fluorouracil: In Vivo and In Vitro Study., J. Trop. Med. 2022 (2022) 5267861. doi:10.1155/2022/5267861.
30. D.D. Buechter, D.E. Thurston, Studies on the Pyrrolizidine Antitumor Agent, Clazamycin: Interconversion of Clazamycins A and B, J. Nat. Prod. 50 (1987) 360–367. doi:10.1021/np50051a004.
31. M. Hori, K. Naito, N. Sakata, Y. Uehara, H. Umezawa, Inhibition of DNA replication and membrane transport of some nutrients by clazamycin in Escherichia coli., J. Antibiot. (Tokyo). 37 (1984) 260–266. doi:10.7164/antibiotics.37.260.
32. S.S. Kusuma, K. Tanneeru, S. Didla, B.N. Devendra, P. Kiranmayi, Antineoplastic activity of monocrotaline against hepatocellular carcinoma., Anticancer. Agents Med. Chem. 14 (2014) 1237–1248. doi:10.2174/1871520614666140715085907.
33. D. Ladurée, J.C. Lancelot, M. Robba, E. Chenu, G. Mathé, Synthesis and evaluation of antileukemic activity of 5-thienyl- or 5-(2-furyl)-2,3-dihydro-6,7-bis(hydroxymethyl)-1H-pyrrolizine bis(alkylcarbamates) and derivatives., J. Med. Chem. 32 (1989) 456–461. doi:10.1021/jm00122a028.
34. K.M. Attalah, A.N. Abdalla, A. Aslam, M. Ahmed, M.A.S. Abourehab, N.A. ElSawy, A.M. Gouda, Ethyl benzoate bearing pyrrolizine/indolizine moieties: Design, synthesis and biological evaluation of anti-inflammatory and cytotoxic activities., Bioorg. Chem. 94 (2020) 103371. doi:10.1016/j.bioorg.2019.103371.
35. M.A.S. Abourehab, A.M. Alqahtani, F.A. Almalki, D.M. Zaher, A.N. Abdalla, A.M. Gouda, E.A.M. Beshr, Pyrrolizine/Indolizine-NSAID Hybrids: Design, Synthesis, Biological Evaluation, and Molecular Docking Studies, Mol. . 26 (2021). doi:10.3390/molecules26216582.
36. A.M. Shawky, M.A.S. Abourehab, A.N. Abdalla, A.M. Gouda, Optimization of pyrrolizine-based Schiff bases with 4-thiazolidinone motif: Design, synthesis and investigation of cytotoxicity and anti-inflammatory potency, Eur. J. Med. Chem. 185 (2020). doi:10.1016/j.ejmech.2019.111780.
37. M.M. Hanna, N.M. Abdelgawad, N.A. Ibrahim, A.B. Mohammed, Synthesis and antitumor evaluation of some novel pyrrolizine derivatives, Med. Chem. Res. 21 (2012) 2349–2362. doi:10.1007/s00044-011-9761-7.
38. A.F. Mahmoud, M.H. Aboumanei, S. Abdelhalim, Y.A. Hassan, I.T. Ibrahim, Radioiodination and in vivo assessment of the potential of newly synthesized pyrrolizine-5-carboxamides derivative in tumor model, Appl. Radiat. Isot. 166 (2020) 109369. doi:https://doi.org/10.1016/j.apradiso.2020.109369.
39. A.M. Shawky, A.N. Abdalla, N.A. Ibrahim, M.A.S. Abourehab, A.M. Gouda, Discovery of new pyrimidopyrrolizine/indolizine-based derivatives as P-glycoprotein inhibitors: Design, synthesis, cytotoxicity, and MDR reversal activities, Eur. J. Med. Chem. 218 (2021) 113403. doi:https://doi.org/10.1016/j.ejmech.2021.113403.
40. T. HATA, T. HOSHI, K. KANAMORI, A. MATSUMAE, Y. SANO, T. SHIMA, R. SUGAWARA, Mitomycin, a new antibiotic from Streptomyces. I., J. Antibiot. (Tokyo). 9 (1956) 141–146.
41. L. Kabiri, S. Irani, A.R. Nikpoor, J. Tavakkol Afshari, Inhibitory and Apoptotic Effects of Mannan-Mitomycin C Conjugate Against Transitional Cell Carcinoma and Normal Mouse Fibroblasts., Reports Biochem. Mol. Biol. 8 (2019) 63–71.
42. J. Verweij, H.M. Pinedo, Mitomycin C: mechanism of action, usefulness and limitations., Anticancer. Drugs. 1 (1990) 5–13.
43. M.M. Paz, X. Zhang, J. Lu, A. Holmgren, A New Mechanism of Action for the Anticancer Drug Mitomycin C: Mechanism-Based Inhibition of Thioredoxin Reductase, Chem. Res. Toxicol. 25 (2012) 1502–1511. doi:10.1021/tx3002065.

44. A. Perzyna, F. Klupsch, R. Houssin, N. Pommery, A. Lemoine, J.-P. Hénichart, New benzo[5,6]pyrrolizino[1,2-b]quinolines as cytotoxic agents, Bioorg. Med. Chem. Lett. 14 (2004) 2363–2365. doi:https://doi.org/10.1016/j.bmcl.2004.01.097.
45. N.S. Zimnitskiy, A.Y. Barkov, M. V Ulitko, I.B. Kutyashev, V.Y. Korotaev, V.Y. Sosnovskikh, An expedient synthesis of novel spiro[indenoquinoxaline-pyrrolizidine]-pyrazole conjugates with anticancer activity from 1,5-diarylpent-4-ene-1,3-diones through the 1,3-dipolar cycloaddition/cyclocondensation sequence, New J. Chem. 44 (2020) 16185–16199. doi:10.1039/D0NJ02817G.
46. A.I. Almansour, R.S. Kumar, F. Beevi, A.N. Shirazi, H. Osman, R. Ismail, T.S. Choon, B. Sullivan, K. McCaffrey, A. Nahhas, K. Parang, M.A. Ali, Facile, Regio- and Diastereoselective Synthesis of Spiro-Pyrrolidine and Pyrrolizine Derivatives and Evaluation of Their Antiproliferative Activities, Molecules. 19 (2014) 10033–10055. doi:10.3390/molecules190710033.
47. T.P. Singh, N., Jabeen, T., Sharma, S., Bhushan, A., Singh, Crystal structure of the complex of a group IIA phospholipase A2 with a synthetic anti-inflammatory agent licofelone at 1.9A resolution, (2005). doi:https://doi.org/10.2210/pdb1ZYX/pdb.
48. T.P. Gautam, L., Shukla, P.K., Sinha, M., Kaur, P., Sharma, S., Singh, Crystal Structure of C-lobe of Bovine lactoferrin Complexed with ketorolac at 1.68 A Resolution, (n.d.). doi:https://doi.org/10.2210/pdb4GRK/pdb.
49. T.P. Shukla, P.K., Gautam, L., Sinha, M., Kaur, P., Sharma, S., Singh, Crystal structure of C-lobe of bovine lactoferrin complexed with licofelone at 1.88 A resolution, (2012). doi:https://doi.org/10.2210/pdb4G8H/pdb.
50. R. Patil, A. Laguerre, J. Wielens, S.J. Headey, M.L. Williams, M.L.R. Hughes, B. Mohanty, C.J.H. Porter, M.J. Scanlon, Characterization of Two Distinct Modes of Drug Binding to Human Intestinal Fatty Acid Binding Protein, ACS Chem. Biol. 9 (2014) 2526–2534. doi:10.1021/cb5005178.
51. M. Sastry, R. Fiala, R. Lipman, M. Tomasz, D.J. Patel, Solution structure of the monoalkylated mitomycin C-DNA complex., J. Mol. Biol. 247 (1995) 338–359. doi:10.1006/jmbi.1994.0143.
52. N. Danshiitsoodol, C.A. de Pinho, Y. Matoba, T. Kumagai, M. Sugiyama, The mitomycin C (MMC)-binding protein from MMC-producing microorganisms protects from the lethal effect of bleomycin: crystallographic analysis to elucidate the binding mode of the antibiotic to the protein., J. Mol. Biol. 360 (2006) 398–408. doi:10.1016/j.jmb.2006.05.017.
53. T.W. Martin, Z. Dauter, Y. Devedjiev, P. Sheffield, F. Jelen, M. He, D.H. Sherman, J. Otlewski, Z.S. Derewenda, U. Derewenda, Molecular basis of mitomycin C resistance in streptomyces: structure and function of the MRD protein., Structure. 10 (2002) 933–942. doi:10.1016/s0969-2126(02)00778-5.
54. S. Singh, A. Chang, R.D. Goff, C.A. Bingman, S. Grüschow, D.H. Sherman, G.N.J. Phillips, J.S. Thorson, Structural characterization of the mitomycin 7-O-methyltransferase., Proteins. 79 (2011) 2181–2188. doi:10.1002/prot.23040.
55. C. Kubitza, A. Faust, M. Gutt, L. Gäth, D. Ober, A.J. Scheidig, Crystal structure of pyrrolizidine alkaloid N-oxygenase from the grasshopper Zonocerus variegatus., Acta Crystallogr. Sect. D, Struct. Biol. 74 (2018) 422–432. doi:10.1107/S2059798318003510.
56. Dassault systems BIOVIA. Discovery Studio Visualizer, v16.1.0.15350; Dassault Systems: San Diego, CA, USA, (2016).
57. A.M. Shawky, F.A. Almalki, A.N. Abdalla, B.G.M. Youssif, M.M. Abdel-Fattah, F. Hersi, H.A.M. El-Sherief, N. Ibrahim A., A.M. Gouda, Discovery and optimization of 2,3-diaryl-1,3-thiazolidin-4-one-based derivatives as potent and selective cytotoxic agents with anti-inflammatory activity, Eur. J. Med. Chem. 259 (2023) 115712. doi:https://doi.org/10.1016/j.ejmech.2023.115712.
58. M.A.S. Abourehab, A.M. Alqahtani, F.A. Almalki, A.N. Abdalla, A.M. Gouda, Pyrrolizine/indolizine-cinnamaldehyde Schiff bases: design, synthesis, biological evaluation, ADME, and molecular docking study, Eur. J. Med. Chem. Reports. (2022) 100036. doi:https://doi.org/10.1016/j.ejmcr.2022.100036.
59. F.A. Almalki, A.M. Shawky, A.N. Abdalla, A.M. Gouda, Icotinib, Almonertinib, and Olmutinib: A 2D Similarity/Docking-Based Study to Predict the Potential Binding Modes and Interactions into EGFR, Mol. . 26 (2021). doi:10.3390/molecules26216423.
60. A.M. Shawky, F.A. Almalki, H.A. Alzahrani, A.N. Abdalla, B.G.M. Youssif, N.A. Ibrahim, M. Gamal, H.A.M. El-Sherief, M.M. Abdel-Fattah, A.A. Hefny, A.H. Abdelazeem, A.M. Gouda, Covalent small-molecule inhibitors of SARS-CoV-2 Mpro: Insights into their design, classification, biological activity, and binding interactions, Eur. J. Med. Chem. 277 (2024) 116704. doi:https://doi.org/10.1016/j.ejmech.2024.116704.
61. G.Y. Sun, P.B. Shelat, M.B. Jensen, Y. He, A.Y. Sun, A. Simonyi, Phospholipases A2 and inflammatory responses in the central nervous system., Neuromolecular Med. 12 (2010) 133–148. doi:10.1007/s12017-009-8092-z.