Full Paper

Synthesis and Molecular Modeling Studies of Anti-inflammatory Active 1*H*-Pyrrolizine-5-carboxamides

Flora F. Barsoum

Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

A variety of *N*-aryl-7-cyano-2,3-dihydro-1*H*-pyrrolizine-5-carboxamides **5**, **6**, **8**, and **9** were synthesized *via* reaction of the 2-amino derivatives **4** with acid chlorides and aromatic aldehydes. Meanwhile, **4a**,**b** were obtained through the reaction of 2-pyrrolidinylidenepropanedinitrile **1** with chloroacetanilides **2a**,**b**. In addition, the tricyclic pyrimido[5,4-*a*]pyrrolizines were formed through conducting the reaction of **4a**,**b** with 90% formic acid. Anti-inflammatory activity screening of some synthesized compounds utilizing in *vivo* acute carrageenan-induced paw edema standard method in rats exhibited that the prepared heterocycles possess considerable pharmacological properties especially, **4a**, **4b**, **10a**, and **10b** which reveal remarkable activities relative to diclofenac sodium (reference standard). Ulcerogenic liability of the highly promising synthesized anti-inflammatory active agents were evaluated and **4a** and **4b** showed ulcerogenic liability lower than that of the standard used drug. Molecular modeling studies were initiated herein in order to validate the attained pharmacological data and provide understandable evidence for the observed anti-inflammatory behavior.

Keywords: Anti-inflammatory / Chloroacetanilides / Molecular modeling/ 1*H*-Pyrrolizine-5-carboxamides / Ulcerogenic liability

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Introduction

Inflammation is a complex biological response of vascular tissues against harmful stimuli, such as pathogens, damaged cells or irritants, mediated by different physiological and immunological mediators and characterized by the accumulation of fluids and leukocytes leading to edema and pain [1]. Acute inflammation occurs as the initial response to tissue injury, being mediated by the release of autacoids as histamine, bradykinin, prostaglandins and leukotrienes. On the other hand, the chronic inflammatory process involves the release of diverse mediators, as interleukins, interferon and tumor necrosis factor α (TNF-α) [2]. Drugs currently used for reducing inflammation include; steroids, specifically glucocorticoids by binding to cortisol receptors [3–5]; non-steroidal anti-inflammatory drugs (NSAIDs), alleviate pain by counteracting the cyclooxygenase enzyme [6, 7]; immune selective anti-inflammatory derivatives (ImSAIDs) that are class of peptides altering the activation and migration of inflammatory cells which are responsible for amplifying the inflammatory response [8, 9]; in addition to medical drugs, some herbs have anti-inflammatory qualities and can reduce the inflammation [10–12].

Non-steroidal anti-inflammatory drugs are commonly used for the treatment of pain and inflammation, however, long-term therapy may cause gastrointestinal complications ranging from stomach irritation to life-threatening gastrointestinal ulceration and bleeding [13, 14]. Several pharmacological drugs have been synthesized but none of them is free of side effects, so it is important to find new anti-inflammatory drug with a potential for clinical use and not associated with adverse effects. Several bi- and tricyclic compounds have been synthesized using the starting material 2-pyrrolidinylidenepropanedinitrile and they possess anti-inflammatory and analgesic activities [15–20], therefore with the aim of obtaining new anti-inflammatory drugs with greater activity and fewer side effects, in the work described herein, we intended to investigate the synthesis of new

Correspondence: Flora F. Barsoum, Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, 11562, Cairo, Egypt
E-mail: ffbarsoum2@yahoo.com

bi- and tricyclic compounds *via* a facile synthetic approach using the same starting material and evaluating their anti-inflammatory activity. Ulcerogenic properties of the most anti-inflammatory active synthesized compounds will be also considered and a structure-activity relationship analysis was also done in order to understand the structural requirements for optimum activity.

Results and discussion

Chemistry

Reaction of 2-pyrrolidinylidenepropanedinitrile **1** with chloroacetanilides **2a**,**b** in refluxing acetone in the presence of anhydrous potassium carbonate, afforded directly 6-amino-7-cyano-2,3-dihydro-*N*-(2-substituted phenyl)-1*H*-pyrrolizine-5-carboxamides **4a**,**b**. The structure of **4** was established through spectroscopic (IR, ^{1}H-NMR) as well as elemental analyses data. The IR spectra of the products reveal one nitrile stretching vibration band at $\nu = 2221–2220$ cm^{-1} region and the absence of a second band assignable for the second nitrile group which confirmed the cyclized structure, in addition to the appearance of NH_2 bands at 3425 and 3439 cm^{-1}. ^{1}H-NMR spectra exhibit the amino group protons as a singlet signal at $\delta = 3.49–3.86$ region (disappeared upon deuteration). The reaction was assumed to take place *via* dehydrohalogenation under the effect of basic potassium carbonate used in the reaction with subsequent cyclization due to nucleophilic attack of the active acetanilide methylene function at one of the nitrile groups giving eventually **4**. The absence of the methylene acetanilide protons in ^{1}H-NMR spectra, excluded the presence of the open-chain intermediate **3**.

Acylation of **4a**,**b** with acetyl chloride in dry benzene afforded the mono-acetylated derivatives **5a**,**b** which structures were deduced by ^{1}H-NMR spectra exhibiting the acetyl protons as singlet signals at $\delta = 2.10–2.29$ region. Meanwhile, the reaction of **4a**,**b** with aromatic aldehydes (benzaldehyde and *p*-anisaldehyde) in refluxing ethanol in the presence of a catalytic amount of glacial acetic acid gave the corresponding Schiff bases **6a–c** and their structures were confirmed on the basis of their elemental and spectral data. The IR spectra of the products reveal the disappearance of the NH_2 bands at 3425 and 3439 cm^{-1} and ^{1}H-NMR spectra display the azomethine proton as a sharp singlet signal at $\delta = 9.08–9.19$ region.

On the other hand, reaction of **4a**,**b** with chloroacetyl chloride **7a** yielded the chloroacetylamino-1*H*-pyrrolizine derivatives **8a**,**c** and their structures were confirmed on the basis of their elemental and spectral data. The latter upon reaction with secondary amines ''namely morpholine and 1-methylpiperazine'' in refluxing ethanol in the presence of sodium bicarbonate underwent aromatic nucleophilic substitution reaction yielded the corresponding pyrrolizines **9a**,**b**,**d** in good yields. ^{1}H-NMR spectra of **9a**,**b**,**d** exhibit the acetamido methylene protons upfield shifted ($\delta = 3.29, 3.31, 3.55$) compared with the parent chloroacetamido methylene function of **8a**,**c** which appeared at $\delta = 4.26, 4.31$. Similarly, the reaction of **4a**,**b** with 3-chloropropionyl chloride **7b** gave the chloropropionylamino-1*H*-pyrrolizine derivatives **8b**,**d** which underwent nucleophilic substitution reaction with secondary amines, giving the corresponding pyrrolizines **9c**,**e** in good yields.

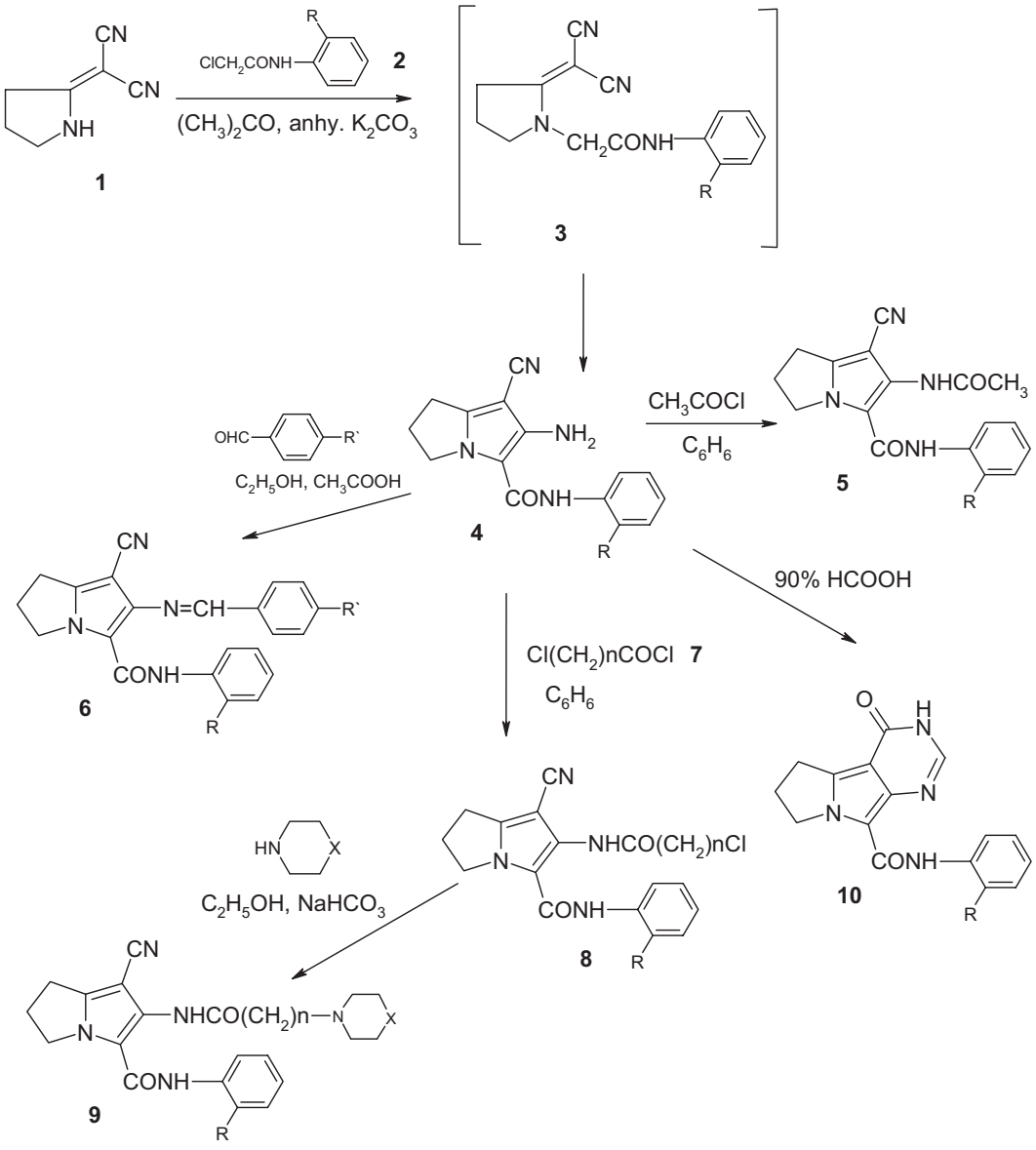
Moreover, refluxing **4a**,**b** with 90% formic acid afforded the tricyclic pyrimido[5,4-*a*]pyrrolizine-9-carboxamides **10a**,**b** through intramolecular cyclization onto the cyano group. The structure of **10** was established through spectroscopic (IR, ^{1}H-NMR) as well as elemental analyses data. The IR spectra reveal two bands attributed to the two carbonyl groups at $\nu = 1699–1658$ cm^{-1} region and the absence of a band assignable for the nitrile group at 2222 cm^{-1} region confirming the cyclized form structure (Scheme 1).

Anti-inflammtory activity screening

The anti-inflammatory activity of the 14 representative compounds **4a–5b**, **8a–9a**, **9c–10b**, was determined *in vivo* by the acute carrageenan-induced paw edema standard method in rats [21–23]. From the obtained results (Table 1), it has been observed that several newly prepared compounds (**4a**, **4b**, **10a**, and **10b**) reveal better anti-inflammatory properties (28.8–36.1% inhibition of edema) comparable to that of diclofenac which was used as a reference standard (28.4% inhibition of edema).

Structure–activity relationships based on the observed results indicated that, the type of aryl group substitution attached to the *N*-position of carboxamide residue plays a controlling role for developing the exhibited pharmacological properties. It has been noticed that, substitution of the phenyl group with an electron-withdrawing group, a chlorine atom, seems more favorable for constructing an anti-inflammatory active agent than the case of substitution with an electron-donating group, a methyl residue, as exhibited in pairs **4a**,**b** (36.1, 31.6% inhibition of edema), **5a**,**b** (23.9, 23.2% inhibition of edema), **8a**,**c** (22.2, 20.3% inhibition of edema), **8b**,**d** (18.0, 17.5% inhibition of edema), **9a**,**d** (7.1, 3.9% inhibition of edema), **9c**,**e** (5.7, 3.2% inhibition of edema) and **10a**,**b** (30.3, 28.8% inhibition of edema, respectively).

However, comparing the activity of **4a** and **4b** with their acetylated products **5a**, **5b**, **8a–d**, it was observed that by blocking the amino group by acetylation, the anti-inflammatory activity decreased and decreased more by nucleophilic substitution of the acetylated compounds **8a–d** with an alicyclic-amino residue (morpholinyl residue) as exhibited in compounds **9a**, **9c–9e**. On the other hand, comparing the activity of the acetylated compounds **5a**, **5b**, **8a–d**, it was



2a, 4a; 5a; R = Cl
2b, 4b; 5b; R = CH_3
6a; R = Cl, R` = H
6b; R = Cl, R` = OCH_3
6c; R = CH_3, R` = H
7a; n = 1
7b; n = 2
8a; R = Cl, n = 1
8b; R = Cl, n = 2
8c; R = CH_3, n = 1
8d; R = CH_3, n = 2
9a; R = Cl, n = 1, X = O
9b; R = Cl, n = 1, X = NCH_3
9c; R = Cl, n = 2, X = O
9d; R = CH_3, n = 1, X = O
9e; R = CH_3, n = 2, X = O
10a; R = Cl
10b; R = CH_3

Scheme 1. Proposed synthesis routes.

observed that replacing the hydrogen atom in compounds **5a** and **5b** by a chlorine atom in compounds **8a** and **8c** reduces the anti-inflammatory activity as exhibited in compounds **5a**, **8a** (23.9, 22.2% inhibition of edema) and **5b**, **8c** (23.2, 20.3% inhibition of edema). In addition, comparing the activity of the acetylated compounds **8a–d**, it was observed that increasing the number of carbon atoms from 1 to 2 of the side chain at position 6, decreased the anti-inflammatory activity as

Table 1. Anti-inflammatory activity of the tested compounds using acute carrageenan-induced paw edema in rats at concentration 20 mg/kg body weight.

Compound	Mean swelling volume (mL)	% Inhibition of edema
Control	2.583 ± 0.095[b]	00.0
Diclofenac sodium	1.850 ± 0.239[a]	28.4
4a	1.650 ± 0.067[a]	36.1
4b	1.767 ± 0.152[a]	31.6
5a	1.967 ± 0.156[a]	23.9
5b	1.983 ± 0.079[a]	23.2
8a	2.010 ± 0.144[a]	22.2
8b	2.117 ± 0.114[a]	18.0
8c	2.060 ± 0.206[a]	20.3
8d	2.130 ± 0.092[a]	17.5
9a	2.401 ± 0.151[b]	7.1
9c	2.435 ± 0.115[b]	5.7
9d	2.483 ± 0.060[b]	3.9
9e	2.500 ± 0.068[b]	3.2
10a	1.800 ± 0.113[a]	30.3
10b	1.840 ± 0.062[a]	28.8

[a] Statistically significant from the control at $p < 0.05$.
[b] Statistically significant from diclofenac sodium at $p < 0.05$.

exhibited in compounds **8a**, **8b** (22.2, 18.0% inhibition of edema) and **8c**, **8d** (20.3, 17.5% inhibition of edema) and the same observation was also recognized by nucleophilic substitution of these compounds with a morpholinyl residue as exhibited in compounds **9a**, **9c** (7.1, 5.7% inhibition of edema) and **9d**, **9e** (3.9, 3.2% inhibition of edema). However, upon cyclization of the most potent compounds **4a** and **4b** using formic acid, the obtained tricyclic compounds **10a** and **10b** showed slight decrease in the anti-inflammatory activity (30.3, 28.8% inhibition of edema) compared to **4a** and **4b** and a slight increase in the activity when compared to diclofenac, reference standard (28.4% inhibition of edema).

As a conclusion, it was found that the bicyclic pyrrolizine-5-carboxamides **4a** and **4b** with a primary amino group at position 6 show the highest anti-inflammatory activity and this activity was also retained by cyclization of these compounds to the tricyclic pyrimido[5,4-*a*]pyrrolizine-9-carboxamides derivatives **10a** and **10b**. However, a decrease in the anti-inflammatory activity was recognized by the substitution of the primary amino group of the bicyclic pyrrolizine-5-carboxamides and this activity decreased more by increasing the number of carbon atoms substituted to the amino group **8b**, **8d**, **9c**, and **9e**.

Ulcerogenic liability

Ulcerogenic liability of the most promising prepared anti-inflammatory active agents (**4a**, **4b**, **10a**, and **10b**) was determined following the previously reported standard method [24–26] using diclofenac sodium as a reference standard. From the obtained data (Table 2) it has been noticed that, compounds **4a** and **4b** reveal the lowest ulcer indexes (9.2, 11.3, respectively) and they are considered more safer than diclofenac sodium (reference standard) itself which reveals ulcer index 13.3.

Molecular modeling studies

Molecular modeling studies were initiated herein in order to validate the attained pharmacological data and provide understandable evidence for the observed anti-inflammatory behavior. Docking studies were performed by Molecular Operating Environment (MOE, Version 2005.06, Chemical Computing Group Inc., Montreal, Quebec, Canada) using IPXX file downloaded from Protein Data Bank, exhibiting COX-2 enzyme co-crystallized with diclofenac (the used reference standard in the anti-inflammatory activity screening study), which used as a template in the present study. 100 Docking interactions for each ligand were performed and the top score docking energy value was recorded (Table 3).

Diclofenac was docked in the active site of COX-2 enzyme using force-field energy MMFX9 with $S = -7.5072$ kcal/mol, exhibiting interaction with two amino acids of the COX-2 active site, which are Ser-530 and Tyr-385. Ser-530 exhibits two hydrogen bonding interactions of its amino function with the carboxylic oxygens of diclofenac ($d = 2.7$, 3.0 Å). However, Tyr-385 exhibits only one hydrogen bond due its amino acid interaction with the carbonyl carboxylate of diclofenac ($d = 2.7$ Å).

Table 2. Ulcerogenic liability of the most promising prepared anti-inflammatory active agents.

Compound	Number of animals with ulcer	% incidence divided by 10	Average of ulcer number	Average severity	Ulcer index
Control	0/6	0.0	0.0	0.0	0.0
Diclofenac sodium	5/6	8.3	3.7	1.3	13.3
4a	4/6	6.7	1.7	0.8	9.2
4b	5/6	8.3	1.8	1.2	11.3
10a	5/6	8.3	4.8	2.0	15.1
10b	6/6	10.0	5.2	2.2	17.4

Table 3. Docking results for the highly effective anti-inflammatory agents.

Compound	Docking energy score (kcal/mol)	Amino acid	Hydrogen bond (Å)
Diclofenac sodium	−7.5072	Ser-530	2.7, 3.0
		Tyr-385	2.7
4a	−10.1032	Tyr-355	3.1
		Arg-120	2.7
4b	−8.856	Ser-530	2.7
10a	−5.4431	Ser-530	2.8
10b	−3.0544	Ser-530	2.7

Docking of the highly observed anti-inflammatory active agents **4a**,**b**; **10a**,**b** were studied using the same mentioned procedure. Docking compound **4a** in the active site of COX-2 revealed docking energy score $S = -10.1032$ kcal/mol, exhibiting two distinguished interactions with two different amino acids. The first observed one is due to interaction of amino function of **4a** with Tyr-355 carboxylic oxygen ($d = 3.1$ Å). The other observed one is due to Arg-120 amino function interaction with carbonitrile nitrogen of **4a** ($d = 2.7$ Å). Meanwhile, docking **4b** in the active site of COX-2, reveals docking energy score $S = -8.856$ kcal/mol providing only one hydrogen bonding due to interaction of amino group of **4b** and carboxylic residue of Ser-530 ($d = 2.7$ Å) (Fig. 1). Meanwhile, docking of either compound **10a** or **10b** (Fig. 2) in the active site of COX-2 reveals only one hydrogen bonding due to interaction of *N*-1 of the pyrimido[5,4-*a*]pyrrolizines with the carboxylic residue of Ser-530 ($d = 2.8$, 2.7 Å, docking score values $S = -5.4431$, −3,0544, respectively).

Alternatively, none of the prepared compounds exhibit any interaction with COX-1 enzyme. Based on the above observations, it could be concluded that the highly observed anti-inflammatory active results (**4a**,**b**, **10a**,**b**) are attributed due to inhibitory properties of these compounds to COX-2 enzyme participated in prostaglandins generations in the living body.

As a conclusion, the molecular modeling studies revealed the importance of the primary amino group in the bicyclic pyrrolizine-5-carboxamides. This primary amino group interacted with the amino acid Tyr-355 by a hydrogen bond 3.1 Å in compound **4a** and with Ser-530 by a hydrogen bond 2.7 Å in compound **4b**. However, upon substitution of this primary

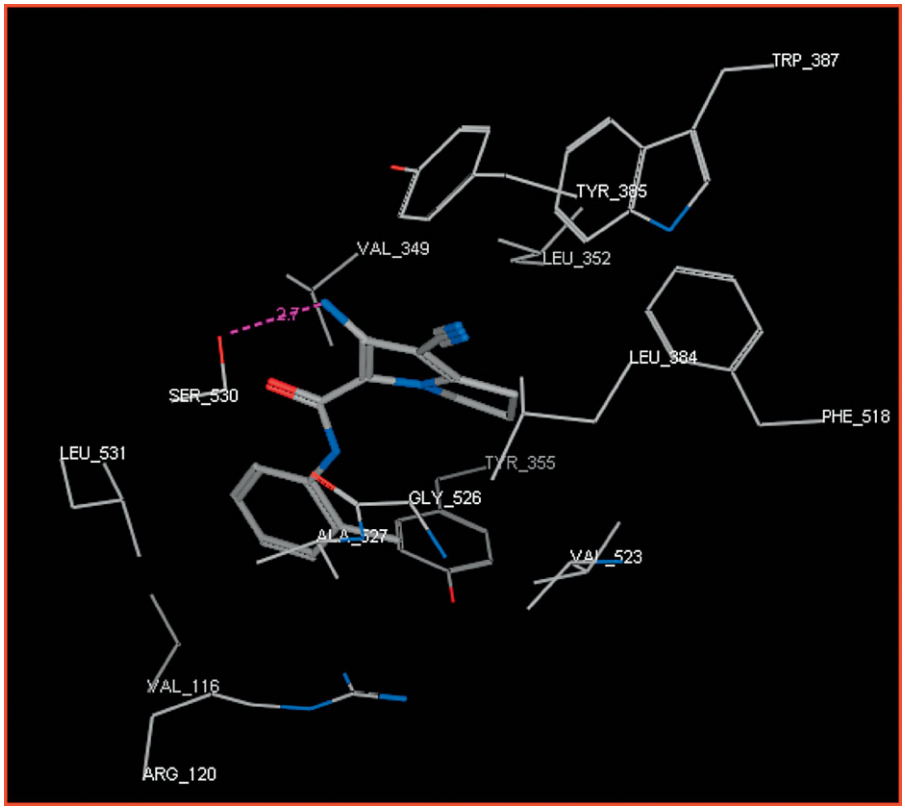


Figure 1. Docking of **4b** in the active site of COX-2 ($S = -8.1856$ kcal/mol) exhibiting interaction with one amino acid, Ser-530 with one hydrogen bond 2.7 Å.

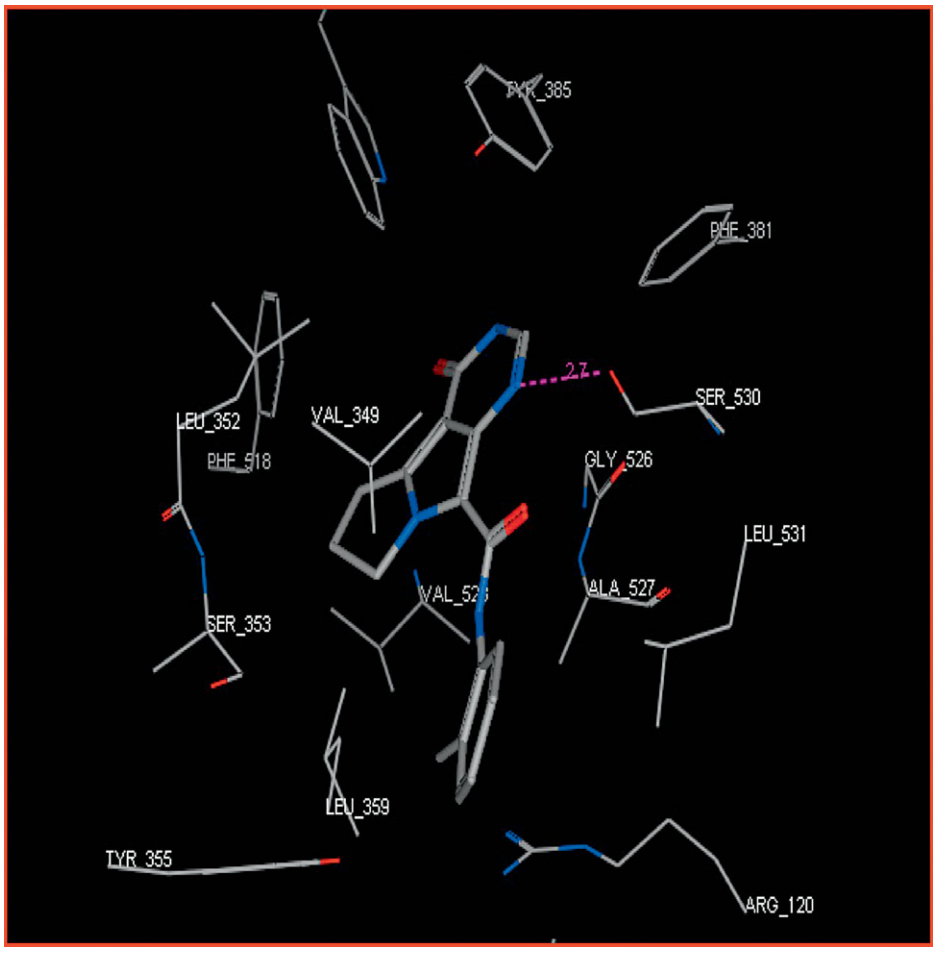


Figure 2. Docking of **10b** in the active site of COX-2 ($S = -3.0544$ kcal/mol) exhibiting interaction with one amino acid, Ser-530 with one hydrogen bond 2.7 Å.

amino group to obtain compounds with secondary amino group, the biological screening showed a marked decrease in the activity to the extent that they were not detected by the molecular modeling. On the other hand, converting this primary amino group to a tertiary one by cyclization of **4a** and **4b**, a slight decrease in the activity was recognized in the biological screening and confirmed in the molecular modeling studies by interacting with the amino acid Ser-530 by a hydrogen bond 2.8 Å for compound **10a** and 2.7 Å for **10b**.

Experimental

Melting points are uncorrected and recorded on an Electrothermal 9100 digital melting point apparatus. IR spectra (KBr) were recorded on a Bruker Vector 22 and Jasco FT/IR plus 460 spectrophotometers. ^{1}H-NMR spectra were recorded on a Varian MERCURY 300 (300 MHz) spectrometer. The starting compounds **1** [27, 28] and **2a**,**b** [29] were prepared according to the previously reported procedures.

*Synthesis of 6-amino-7-cyano-2,3-dihydro-N-(2-substituted phenyl)-1H-pyrrolizine-5-carboxamide **4a,b***

A mixture of equimolar amounts of 2-pyrrolidinylidenepropanedinitrile **1** and the corresponding chloroacetanilides **2a**,**b** (10 mmol) in dry acetone (20 mL) containing anhydrous potassium carbonate (20 mmol), was boiled under reflux for 24 h. The reaction mixture was filtered while hot and the clear solution was evaporated till dryness under reduced pressure. The remaining residue was crystallized from a suitable solvent affording the corresponding **4a**,**b**.

*6-Amino-N-(2-chlorophenyl)-7-cyano-2,3-dihydro-1H-pyrrolizine-5-carboxamide **4a***

Colorless crystals from ethanol, mp 176–178°C, yield 85%. IR: ν_{max} cm^{-1} 3425, 3318 (NH, NH_2), 2221 (C≡N), 1636 (C=O), 1600, 1586 (C=C). ^{1}H-NMR ($CDCl_3$): δ 2.54 (pentat, 2H, pyrrol. *H*-2, $J = 7.5$ Hz), 2.96 (t, 2H, pyrrol. *H*-1, $J = 7.5$ Hz), 3.86 (br.s, 2H, D_2O exchangeable NH_2), 4.37 (t, 2H, pyrrol. *H*-3, $J = 7.2$ Hz), 7.00–8.45 (m, 4H, arom. H), 9.27 (br.s, 1H, D_2O exchangeable

NH). Anal. calcd. for $C_{15}H_{13}ClN_4O$ (300.73): C, 59.90; H, 4.36; N, 18.63. Found: C, 60.19; H, 4.29; N, 18.66.

*6-Amino-7-cyano-2,3-dihydro-N-(2-methylphenyl)-1H-pyrrolizine-5-carboxamide **4b***

Colorless crystals from methanol, mp 165–167°C, yield 87%. IR: ν_{max} cm^{-1} 3439, 3391, 3323 (NH, NH_2), 2220 (C≡N), 1634 (C=O), 1605, 1586 (C=C). ^{1}H-NMR ($CDCl_3$): δ 2.32 (s, 3H, CH_3), 2.51 (pentat, 2H, pyrrol. *H*-2, J = 7.2 Hz), 2.96 (t, 2H, pyrrol. *H*-1, J = 7.2 Hz), 3.49 (br.s, 2H, D_2O exchangeable NH_2), 4.36 (t, 2H, pyrrol. *H*-3, J = 7.2 Hz), 7.01–8.05 (m, 4H, arom. H), 9.28 (br.s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{16}H_{16}N_4O$ (280.32): C, 68.55; H, 5.75; N, 19.99. Found: C, 68.86; H, 5.82; N, 19.83.

*Reaction of **4a,b** with acetyl chloride*

A solution of the appropriate **4** (2.5 mmol) in dry benzene (10 mL) containing acetyl chloride (5 mmol) was stirred at room temperature (25–30°C) for 48 h. The solid separated upon evaporating the reaction mixture till dryness under reduced pressure was collected, washed with water and crystallized from a suitable solvent affording the corresponding **5a**,**b**.

*6-Acetylamino-N-(2-chlorophenyl)-7-cyano-2,3-dihydro-1H-pyrrolizine-5-carboxamide **5a***

Colorless crystals from ethanol/chloroform as 2:1 v/v, mp 222–224°C, yield 81%. IR: ν_{max} cm^{-1} 3378, 3208 (NH), 2226 (C≡N), 1671 (C=O), 1591, 1561 (C=C). ^{1}H-NMR ($CDCl_3$): δ 2.29 (s, 3H, CH_3), 2.56 (pentat, 2H, pyrrol. *H*-2, J = 6.6 Hz), 3.00 (t, 2H, pyrrol. *H*-1, J = 6.9 Hz), 4.42 (t, 2H, pyrrol. *H*-3, J = 6.3 Hz), 7.04–8.35 (m, 5H, arom. H + NH), 8.78 (br.s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{17}H_{15}ClN_4O_2$ (342.77): C, 59.56; H, 4.41; N, 16.35. Found: C, 59.90; H, 4.41; N, 16.51.

*6-Acetylamino-7-cyano-2,3-dihydro-N-(2-methylphenyl)-1H-pyrrolizine-5-carboxamide **5b***

Colorless crystals from methanol, mp 208–210°C, yield 79%. IR: ν_{max} cm^{-1} 3416, 3267 (NH), 2224 (C≡N), 1667 (C=O), 1588, 1540 (C=C). ^{1}H-NMR (DMSO-d_6): δ 2.10 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 2.43–2.52 (m, 2H, pyrrol. *H*-2), 2.99 (t, 2H, pyrrol. *H*-1, J = 7.2 Hz), 4.92 (t, 2H, pyrrol. *H*-3, J = 7.5 Hz), 7.07–7.69 (m, 4H, arom. H), 8.84 (br.s, 1H, D_2O exchangeable NH), 9.96 (br.s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{18}H_{18}N_4O_2$ (322.35): C, 67.06; H, 5.63; N, 17.38. Found: C, 66.98; H, 5.85; N, 17.58.

*Reaction of **4a,b** with aromatic aldehydes*

A mixture of equimolar amounts of the appropriate **4** and the corresponding aromatic aldehyde (2 mmol) in absolute ethanol (10 mL) containing glacial acetic acid (3–4 drops) was boiled under reflux for 4 h. The separated solid was collected and crystallized from a suitable solvent affording the corresponding **6a–c**.

*N-(2-Chlorophenyl)-7-cyano-2,3-dihydro-6-[(phenylmethylene)amino]-1H-pyrrolizine-5-carboxamide **6a***

Yellow crystals from benzene, mp 206–208°C, yield 91%. IR: ν_{max} cm^{-1} 3227 (NH), 2212 (C≡N), 1670 (C=O), 1611, 1590 (C=N, C=C). ^{1}H-NMR ($CDCl_3$): δ 2.58 (pentat, 2H, pyrrol. *H*-2, J = 7.5 Hz), 3.08 (t, 2H, pyrrol. *H*-1, J = 7.5 Hz), 4.55 (t, 2H, pyrrol. *H*-3, J = 7.2 Hz), 7.07–8.30 (m, 9H, arom. H), 9.18 (s, 1H, C*H*=N), 10.38 (br.s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{22}H_{17}ClN_4O$ (388.84): C, 67.95; H, 4.41; N, 14.41. Found: C, 68.29; H, 4.33; N, 14.63.

*N-(2-Chlorophenyl)-7-cyano-2,3-dihydro-6-[[(4-methoxyphenyl)methylene]amino]-1H-pyrrolizine-5-carboxamide **6b***

Yellow crystals from ethanol, mp 208–210°C, yield 95%. IR: ν_{max} cm^{-1} 3226 (NH), 2215 (C≡N), 1665 (C=O), 1594, 1539 (C=N, C=C). ^{1}H-NMR ($CDCl_3$): δ 2.57 (pentat, 2H, pyrrol. *H*-2, J = 7.5 Hz), 3.07 (t, 2H, pyrrol. *H*-1, J = 7.5 Hz), 3.90 (s, 3H, OCH_3), 4.54 (t, 2H, pyrrol. *H*-3, J = 7.2 Hz), 6.98–8.30 (m, 8H, arom. H), 9.08 (s, 1H, C*H*=N), 10.43 (br.s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{23}H_{19}ClN_4O_2$ (418.86): C, 65.95; H, 4.57; N, 13.38. Found: C, 66.28; H, 4.41; N, 13.30.

*7-Cyano-2,3-dihydro- N-(2-methylphenyl)-6-[(phenylmethylene)amino]-1H-pyrrolizine-5-carboxamide **6c***

Yellow crystals from ethanol, mp 169–171°C, yield 89%. IR: ν_{max} cm^{-1} 3290 (NH), 2209 (C≡N), 1661 (C=O), 1592, 1536 (C=N, C=C). ^{1}H-NMR ($CDCl_3$): δ 2.25 (s, 3H, CH_3), 2.57 (pentat, 2H, pyrrol. *H*-2, J = 7.8 Hz), 3.08 (t, 2H, pyrrol. *H*-1, J = 7.5 Hz), 4.56 (t, 2H, pyrrol. *H*-3, J = 7.2 Hz), 7.10–7.89 (m, 9H, arom. H), 9.19 (s, 1H, C*H*=N), 9.99 (br.s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{23}H_{20}N_4O$ (368.42): C, 74.98; H, 5.47; N, 15.21. Found: C, 75.23; H, 5.45; N, 15.13.

*Reaction of **4a,b** with chloroacetyl chloride and 3-chloropropionyl chloride **7a,b***

A mixture of the appropriate **4** (2 mmol) and the corresponding **7a**,**b** (4 mmol) in dry benzene (10 mL) was stirred at room temperature (25–30°C) for 48 h. The separated solid was collected, washed with water and crystallized from a suitable solvent affording the corresponding **8a–d**.

*6-[(Chloroacetyl)amino]-N-(2-chlorophenyl)-7-cyano-2,3-dihydro-1H-pyrrolizine-5-carboxamide **8a***

Colorless crystals from ethanol/chloroform as 3:1 v/v, mp 217–219°C, yield 76%. IR: ν_{max} cm^{-1} 3385, 3212 (NH), 2222 (C≡N), 1667 (C=O), 1592, 1568 (C=C). ^{1}H-NMR ($CDCl_3$): δ 2.59 (pentat, 2H, pyrrol. *H*-2, J = 7.5 Hz), 3.06 (t, 2H, pyrrol. *H*-1, J = 7.5 Hz), 4.31 (s, 2H, CH_2Cl), 4.47 (t, 2H, pyrrol. *H*-3, J = 7.2 Hz), 7.06–8.39 (m, 4H, arom. H), 8.50 (br.s, 1H, D_2O exchangeable NH), 8.66 (br.s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{17}H_{14}Cl_2N_4O_2$ (377.22): C, 54.12; H, 3.74; N, 14.85. Found: C, 54.29; H, 3.67; N, 14.81.

*6-[(Chloropropionyl)amino]-N-(2-chlorophenyl)-7-cyano-2,3-dihydro-1H-pyrrolizine-5-carboxamide **8b***

Colorless crystals from methanol, mp 218–220°C, yield 73%. IR: ν_{max} cm^{-1} 3376, 3225 (NH), 2223 (C≡N), 1669 (C=O), 1592, 1563 (C=C). ^{1}H-NMR ($CDCl_3$): δ 2.57 (pentat, 2H, pyrrol. *H*-2, J = 7.5 Hz), 2.94–3.06 (m, 4H, pyrrol. *H*-1 + CH_2Cl), 3.88 (t, 2H, COCH_2, J = 6.6 Hz), 4.43 (t, 2H, pyrrol. *H*-3, J = 7.2 Hz), 7.05–8.65 (m, 6H, arom. H + 2 NH). Anal. calcd. for $C_{18}H_{16}Cl_2N_4O_2$ (391.25): C, 55.25; H, 4.12; N, 14.32. Found: C, 55.61; H, 3.98; N, 14.30.

6-[(Chloroacetyl)amino]-7-cyano-2,3-dihydro-N-(2-methylphenyl)-1H-pyrrolizine-5-carboxamide ***8c***

Colorless crystals from methanol, mp 184–186°C, yield 74%. IR: ν_{max} cm^{-1} 3429, 3259 (NH), 2224 (C≡N), 1671, 1614 (C=O), 1587, 1527 (C=C). ^{1}H-NMR ($CDCl_3$): δ 2.29 (s, 3H, CH_3), 2.56 (pentat, 2H, pyrrol. *H*-2, J = 7.5 Hz), 3.04 (t, 2H, pyrrol. *H*-1, J = 7.5 Hz), 4.26 (s, 2H, CH_2Cl), 4.40 (t, 2H, pyrrol. *H*-3, J = 7.5 Hz), 7.11–7.27 (m, 4H, arom. H), 8.37 (br.s, 1H, D_2O exchangeable NH), 8.50 (br.s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{18}H_{17}ClN_4O_2$ (356.80): C, 60.59; H, 4.80; N, 15.70. Found: C, 60.79; H, 4.69; N, 15.83.

6-[(Chloropropionyl)amino]-7-cyano-2,3-dihydro-N-(2-methylphenyl)-1H-pyrrolizine-5-carboxamide ***8d***

Colorless crystals from methanol, mp 198–199°C, yield 68%. IR: ν_{max} cm^{-1} 3323, 3289 (NH), 2221 (C≡N), 1704, 1648 (C=O), 1528, 1490 (C=C). ^{1}H-NMR ($CDCl_3$): δ 2.29 (s, 3H, CH_3), 2.54 (pentat, 2H, pyrrol. *H*-2, J = 7.5 Hz), 2.90–3.03 (m, 4H, pyrrol. *H*-1 + CH_2Cl), 3.82 (t, 2H, $COCH_2$, J = 6.3 Hz), 4.37 (t, 2H, pyrrol. *H*-3, J = 7.2 Hz), 7.09–7.62 (m, 4H, arom. H), 8.04 (br.s, 1H, D_2O exchangeable NH), 8.64 (br.s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{19}H_{19}ClN_4O_2$ (370.82): C, 61.54; H, 5.16; N, 15.11. Found: C, 61.39; H, 5.13; N, 14.97.

Reaction of ***8a-d*** *with secondary amines*

A mixture of the appropriate **8a–d** (2 mmol) and the corresponding secondary amine (4 mmol) in absolute ethanol (10 mL) containing sodium bicarbonate (4 mmol), was boiled under reflux for 8 h. The reaction mixture was filtered while hot. The solid separated upon storing the clear reaction mixture at room temperature overnight, was collected and crystallized from a suitable solvent affording the corresponding **9a–e**.

N-(2-Chlorophenyl)-7-cyano-2,3-dihydro-6-[(4-morpholinylacetyl)amino]-1H-pyrrolizine-5-carboxamide ***9a***

Colorless crystals from benzene/ethanol as 1:1 v/v, mp 229–231°C, yield 70%. IR: ν_{max} cm^{-1} 3354, 3228 (NH), 2221 (C≡N), 1712, 1669 (C=O), 1595, 1535 (C=C). ^{1}H-NMR ($CDCl_3$): δ 2.56 (pentat, 2H, pyrrol. *H*-2, J = 7.5 Hz), 2.76 (br. s, 4H, morpholinyl 2 NCH_2), 3.04 (t, 2H, pyrrol. *H*-1, J = 7.5 Hz), 3.31 (s, 2H, $COCH_2$), 3.83 (t, 4H, morpholinyl 2 OCH_2, J = 4.5 Hz), 4.46 (t, 2H, pyrrol. *H*-3, J = 7.2 Hz), 7.05–8.29 (m, 4H, arom. H), 8.89 (br.s, 1H, D_2O exchangeable NH), 9.30 (br.s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{21}H_{22}ClN_5O_3$ (427.88): C, 58.94; H, 5.18; N, 16.37. Found: C, 59.18; H, 5.08; N, 16.24.

N-(2-Chlorophenyl)-7-cyano-2,3-dihydro-6-[(1-methyl-4-piperazinylacetyl)amino]-1H-pyrrolizine-5-carboxamide ***9b***

Colorless crystals from methanol, mp 190–192°C, yield 77%. IR: ν_{max} cm^{-1} 3347, 3251 (NH), 2227 (C≡N), 1710, 1670 (C=O), 1591, 1537 (C=C). ^{1}H-NMR ($CDCl_3$): δ 2.37 (s, 3H, NCH_3), 2.43–2.61 (m, 6H, pyrrol. *H*-2 + piperazinyl 2 NCH_2), 2.80 (br.s, 4H, piperazinyl 2 NCH_2), 3.04 (t, 2H, pyrrol. *H*-1, J = 7.2 Hz), 3.29 (s, 2H, $COCH_2$), 4.45 (t, 2H, pyrrol. *H*-3, J = 7.2 Hz), 7.05–8.28 (m, 4H, arom. H), 8.94 (br.s, 1H, D_2O exchangeable NH), 9.25 (br.s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{22}H_{25}ClN_6O_2$ (440.92): C, 59.92; H, 5.72; N, 19.06. Found: C, 59.89; H, 5.62; N, 19.01.

N-(2-Chlorophenyl)-7-cyano-2,3-dihydro-6-[[3-(4-morpholinyl)propionyl]amino]-1H-pyrrolizine-5-carboxamide ***9c***

Colorless crystals from ethanol, mp 188–190°C, yield 65%. IR: ν_{max} cm^{-1} 3378, 3231 (NH), 2226 (C≡N), 1666 (C=O), 1591, 1562 (C=C). ^{1}H-NMR ($CDCl_3$): δ 2.55 (pentat, 2H, pyrrol. *H*-2, J = 7.5 Hz), 2.67–2.78 (m, 6H, morpholinyl 2 NCH_2 + $COCH_2CH_2$), 2.91 (t, 2H, $COCH_2CH_2$, J = 5.1 Hz), 3.03 (t, 2H, pyrrol. *H*-1, J = 7.5 Hz), 3.80 (t, 4H, morpholinyl 2 OCH_2, J = 3.9 Hz), 4.45 (t, 2H, pyrrol. *H*-3, J = 7.5 Hz), 7.05–8.29 (m, 4H, arom. H), 9.03 (s, 1H, D_2O exchangeable NH), 10.98 (s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{22}H_{24}ClN_5O_3$ (441.90): C, 59.79; H, 5.47; N, 15.85. Found: C, 60.07; H, 5.80; N, 15.99.

7-Cyano-2,3-dihydro-N-(2-methylphenyl)-6-[(4-morpholinylacetyl)amino]-1H-pyrrolizine-5-carboxamide ***9d***

Colorless crystals from methanol, mp 219–221°C, yield 75%. IR: ν_{max} cm^{-1} 3253, 3215 (NH), 2221 (C≡N), 1651 (C=O), 1598, 1566 (C=C). ^{1}H-NMR ($CDCl_3$): δ 2.14 (s, 3H, CH_3), 2.65 (pentat, 2H, pyrrol. *H*-2, J = 7.5 Hz), 2.70 (t, 4H, morpholinyl 2 NCH_2, J = 4.8 Hz), 3.15 (t, 2H, pyrrol. *H*-1, J = 7.5 Hz), 3.55 (s, 2H, $COCH_2$), 3.79 (t, 4H, morpholinyl 2OCH_2, J = 4.5 Hz), 4.42 (t, 2H, pyrrol. *H*-3, J = 7.5 Hz), 7.18–7.38 (m, 4H, arom. H), 9.10 (br.s, 1H, D_2O exchangeable NH), 9.38 (br.s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{22}H_{25}N_5O_3$ (407.46): C, 64.85; H, 6.18; N, 17.19. Found: C, 64.60; H, 5.99; N, 17.16.

7-Cyano-2,3-dihydro-N-(2-methylphenyl)-6-[[3-(4-morpholinyl)propionyl]amino]-1H-pyrrolizine-5-carboxamide ***9e***

Colorless crystals from methanol, mp 197–198°C, yield 71%. IR: ν_{max} cm^{-1} 3237 (NH), 2216 (C≡N), 1649 (C=O), 1575, 1536 (C=C). ^{1}H-NMR ($CDCl_3$): δ 2.31 (s, 3H, CH_3), 2.56 (pentat, 2H, pyrrol. *H*-2, J = 7.5 Hz), 2.62–2.69 (m, 6H, morpholinyl 2 NCH_2 + $COCH_2CH_2$), 2.83 (t, 2H, $COCH_2CH_2$, J = 5.4 Hz), 3.02 (t, 2H, pyrrol. *H*-1, J = 7.5 Hz), 3.79 (t, 4H, morpholinyl 2 OCH_2, J = 4.5 Hz), 4.39 (t, 2H, pyrrol. *H*-3, J = 7.2 Hz), 7.09–7.72 (m, 4H, arom. H), 9.20 (s, 1H, D_2O exchangeable NH), 11.18 (s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{23}H_{27}N_5O_3$ (421.49): C, 65.54; H, 6.46; N, 16.62. Found: C, 65.80; H, 6.37; N, 16.70.

Reaction of ***4a,b*** *with formic acid*

A mixture of **4a**,**b** (2 mmol) and 90% formic acid (4 mmol) was heated in a water bath for 3 h, cooled and neutralized with 10% sodium hydroxide. The separated solid was filtered, washed with water and crystallized from a suitable solvent affording the corresponding **10a**,**b**.

N-(2-Chlorophenyl)-4-oxo-4,5,6,7-tetrahydro-3H-pyrimido[5,4-a]pyrrolizine-9-carboxamide ***10a***

Colorless crystals from benzene, mp 293–295°C, yield 83%. IR: ν_{max} cm^{-1} 3378, 3277 (NH), 1699, 1663 (C=O), 1594, 1536 (C=N, C=C). ^{1}H-NMR (DMSO-d_6): δ 2.52 (pentat, 2H, CH_2-6, J = 7.8 Hz), 3.14 (t, 2H, CH_2-5, J = 7.5 Hz), 4.25 (t, 2H, CH_2-7, J = 7.2 Hz), 7.31–8.20 (m, 7H, arom. H + 2 NH + N = CH). Anal. calcd. for $C_{16}H_{13}ClN_4O_2$ (328.74): C, 58.45; H, 3.98; N, 17.04. Found: C, 58.51; H, 3.93; N, 17.13.

N-(2-Methylphenyl)-4-oxo-4,5,6,7-tetrahydro-3H-pyrimido[5,4-a]pyrrolizine-9-carboxamide ***10b***

Colorless crystals from benzene/methanol as 2:1 v/v, mp 278–280°C, yield 81%. IR: ν_{max} cm^{-1} 3421 (NH), 1689, 1658 (C=O), 1601, 1534 (C=N, C=C). ^{1}H-NMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 2.36–2.54 (m, 2H, CH_2-6), 3.14 (t, 2H, CH_2-5, J = 7.8 Hz), 4.19 (t, 2H, CH_2-7, J = 7.8 Hz), 7.20–7.49 (m, 5H, arom. H + N=CH), 8.33 (s, 1H, D_2O exchangeable NH), 9.75 (s, 1H, D_2O exchangeable NH). Anal. calcd. for $C_{17}H_{16}N_4O_2$ (308.33): C, 66.22; H, 5.23; N, 18.17. Found: C, 66.50; H, 5.02; N, 18.09.

Anti-inflammatory activity screening

Anti-inflammatory activity screening for the prepared compounds **4a–5b**, **8a–9**, **9c–10b** was determined *in vivo* by the acute carrageenan-induced paw edema standard method in rats [21–23]. Wister albino rats of either sex (pregnant female animals were excluded) weighing 160–180 g were divided into 16 groups of 6 animals each. Administration of diclofenac sodium (reference standard) and the tested compounds dissolved in saline solution and 0.2 mL DMSO, at a dose of 20 mg/kg (body weight) was given intraperitoneally 1 h before induction of inflammation. The control group was given saline solution containing 0.2 mL DMSO. Carrageenan paw edema was induced by subcutaneous injection of 1% solution of carrageenan in saline (0.1 mL/rat) into the right hind paw of rats. Paw volumes were measured volumetrically after 4 h of inflammation induction with plethysmometer 7140 (UGO BASILE, Italy) and compared with the initial hind paw volume of each rat for determining the oedema volume. Data were collected, checked, revised and analyzed. Quantitative variables from normal distribution were expressed as means ± SE "standard error". The significant difference between groups was tested by using one-way ANOVA followed by post hoc test and the chosen level of significance was $p < 0.05$.

The anti-inflammatory activity was expressed as percentage inhibition of edema volume in treated animals in comparison with the control group (Table 1).

$$\%\,\text{Inhibition of edema} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_c and V_t are the volumes of edema for the control and drug-treated animal groups, respectively.

Ulcerogenic liability

The ulcerogenic liability of the most promising prepared anti-inflammatory active agents (**4a**, **4b**, **10a**, and **10b**) was determined in albino rats following the previously reported standard method [24–26]. Rats of either sex (pregnant female rats were excluded) weighing 130–150 g were divided into 6 groups of 6 animals each. The animals were fasted 18 h before drug administration. Diclofenac sodium (reference standard) and the tested compounds (at a dose of 20 mg/kg body weight), were suspended in saline solution by the aid of few drops of Tween 80 and were administered orally for three successive days to fasted rats. The control group animals were given saline with few drops of Tween 80. One hour following the last dose, the animals were sacrificed by cervical dislocation and the stomach was removed, opened along the greater curvature and rinsed with saline. The gastric mucosa was examined with a magnifying lens (10×) for the presence of lesions and erosions. The ulcer index was calculated (Table 2) and the degree of ulcerogenic effect was expressed in terms of:

1. Percentage incidence of ulcer divided by 10.
2. Average number of ulcers per stomach.
3. Average severity of ulcers.

The ulcer index is the value that resulted from the sum of the above three values.

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