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Original article

Synthesis, analgesic and anti-inflammatory activities evaluation of some bi-, tri- and tetracyclic condensed pyrimidines

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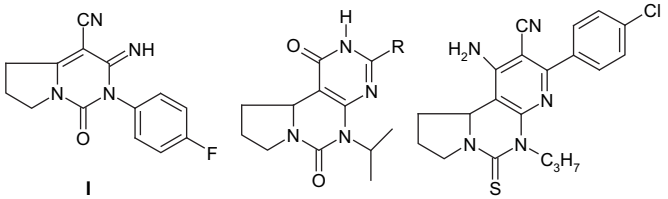
ABSTRACT

Novel series of bicyclic pyrrolo[1,2-c]pyrimidines **3a–g**, **5**, **6a**, **b**, and **7a**, **b**, tricyclic pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidines **8a–c**, **9a–g**, **13a–c**, **17**, **18a**, **b**, **19**, **20a**,**b** and **21** and tetracyclic condensed pyrimidines **14**, **22** and **23** were synthesized through different chemical reactions. Structures of all synthesized pyrimidine derivatives were supported by spectral and elemental analyses. Analgesic activity evaluation was carried out using acetic acid-induced writhing assay, and all compounds exerted comparable activity to indomethacin. The anti-inflammatory activity evaluation was performed using carrageenan-induced paw edema in rats, the potency of the bicyclic derivatives **3a–f** and **7b** revealed comparable activity to indomethacin without gastric ulceration. The tricyclic derivatives **13a** and **20a** exerted good activity, however, they induced gastric ulcers while **13b** and **13c** showed moderate activity without ulceration. In case of tetracyclic derivatives, compound **14** exhibited the highest potency and safety profile.

1. Introduction

Inflammation is a complex defensive mechanism of the body to any noxious stimulus; this process may vary from a localized to a generalized response characterized by the accumulation of fluids and leukocytes leading to edema and pain [1]. This inflammatory response seems to be mediated by different physiological and immunological mediators that play a role in acute and chronic inflammation. The acute inflammation occurs as the initial response to tissue injury, being mediated by the release of autacoids, for example, histamine, bradykinin, prostaglandins and leukotrienes [1,2]. On the other hand, the chronic inflammatory process involves the release of diverse mediators, as interleukins, interferon and tumor necrosis factor α (TNF-α), a cytokine that plays a major role in this kind of inflammatory process and whose production is associated with some inflammatory diseases such as rheumatoid arthritis [1,2]. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of pain and inflammation associated with different diseases particularly rheumatoid arthritis [3], however, their chronic use may cause GIT ulceration, bleeding and renal injury [4,5]. Therefore, although there are a number of anti-inflammatory analgesic drugs available in the market, there is a need to develop novel drugs with better safety profile. Pyrimidine and condensed pyrimidines are important classes of heterocyclic compounds that exhibit a broad spectrum of biological activities such as anticancer [6–10], antiviral [11], antibacterial [12,13], antioxidant [14,15], anxiolytic [16] and antidepressant activities [17]. Furthermore, they possess anti-inflammatory [18–24] and analgesic activities that are well documented in the literature [25–27]. The bicyclic pyrrolo[1,2-c]pyrimidine derivative **I** [28] and the tricyclic pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidines **IIa**, **b** [29] and **III** [30], prepared in our laboratory, were found to have significant analgesic and anti-inflammatory activities, therefore, in continuation of these efforts, we have been interested in the synthesis of further bi-, tri-, and tetracyclic condensed pyrimidine derivatives retaining the pyrrolo[1,2-c]pyrimidine as a basic nucleus and evaluating their analgesic and anti-inflammatory activities.



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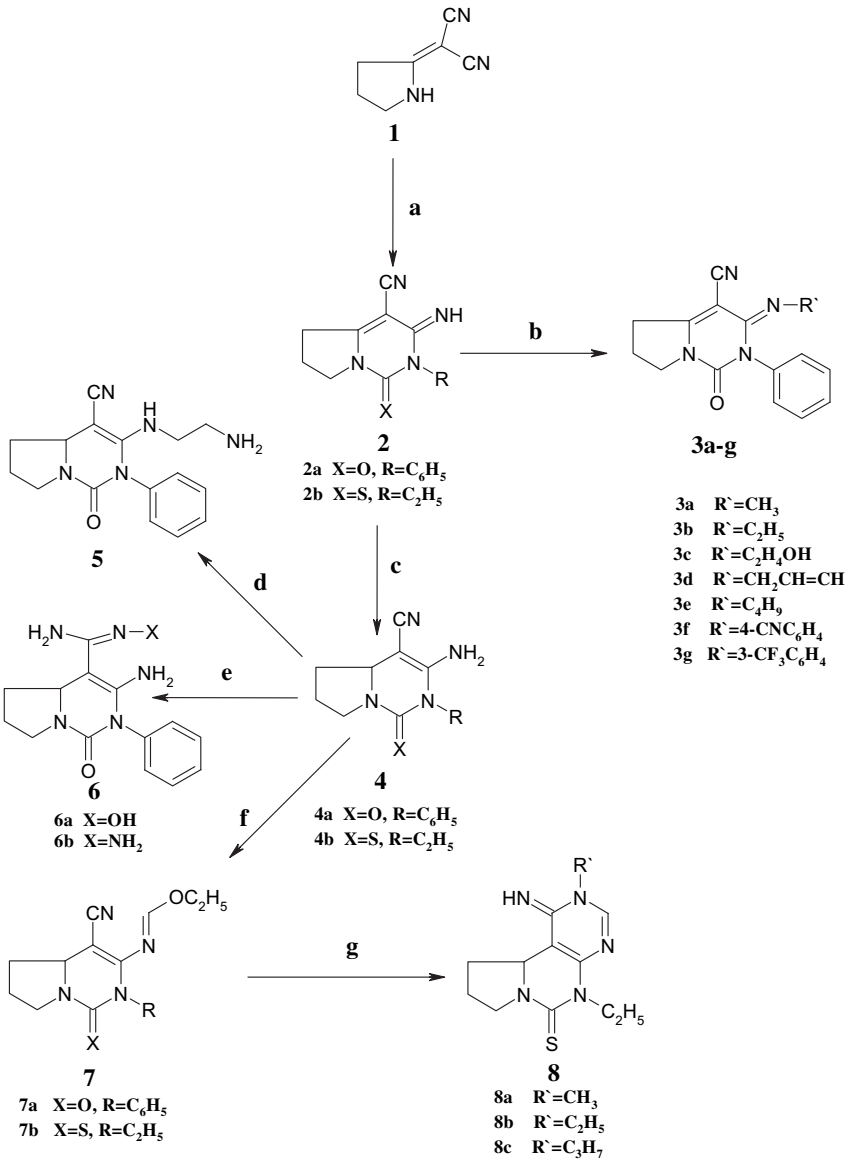


Scheme 1. Reagents and solvents: a: RCNX, CH_2Cl_2; b: DMS, dry benzene for **3a**, R′X, dry DMF for **3b–g**; c: $NaBH_4$, absolute ethanol; d: $ClCH_2CH_2NH_2.HCl$, NaH, dry DMF; e: $NH_2OH.HCl$, $NaHCO_3$,methanol for **6a**, $NH_2NH_2.H_2O$, absolute ethanol for **6b**; f: $CH(OC_2H_5)_3$, acetic anhydride; g: $R'NH_2$, absolute ethanol.

2. Results and discussion

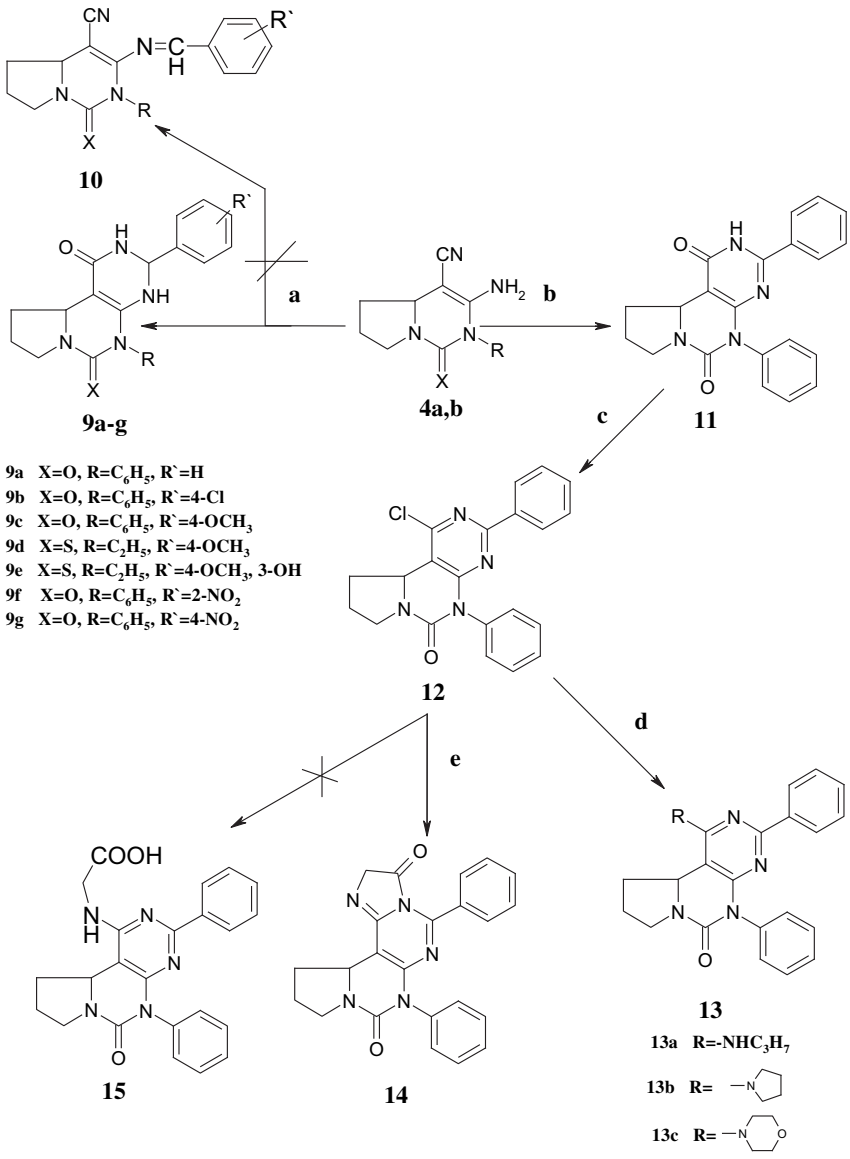
2.1. Chemistry

The synthetic pathways adopted for the preparation of the desired new compounds are illustrated in Schemes 1–3. The known starting materials **2a**, **b**, [30,31] and **4a**, **b** [30,32] proved to be versatile compounds by virtue of their vicinal imino or amino and nitrile functions. Thus, compound **2a** was subjected to react with dimethyl sulfate (DMS) and with alkyl or aryl halides to give **3a** and a number of N-substituted imino derivatives **3b–g,** respectively (Scheme 1). The structures of **3a–g** were confirmed on the basis of their elemental and spectral data. The IR spectrum of **3c** revealed the disappearance of the NH band at 3316 cm^{-1} and the presence of the OH stretching band at 3296 cm^{-1} and an additional nitrile stretching at 2187 cm^{-1} has been observed in case of **3f**. The N-substituted analog **5** was **s**imilarly prepared through reaction of the intermediate **4a** with 2-chloroethylamine hydrochloride in dimethyl formamide (DMF) in the presence of sodium hydride. ^1H NMR spectrum of **5** showed exchangeable bands at 6.21 and 8.42 ppm corresponding to NH, NH_2, respectively.

Furthermore, the target carboxamidines **6a**, **b** were obtained by reaction of the intermediate **4a** with hydroxylamine hydrochloride and hydrazine hydrate, respectively. The ^1H NMR spectra displayed three signals at 4.94, 5.97 and 7.78 ppm corresponding to the two NH_2 and OH groups, respectively, in case of **6a** and at 5.80, 6.10 and 8.38 ppm assigned to the three NH_2 groups of compound **6b**.

On the other hand, condensation of the amino derivatives **4a**, **b** with Triethyl orthoformate in the presence of catalytic amount of acetic anhydride resulted in the formamidic acid ethyl esters **7a**, **b,** respectively. The ^1H NMR spectra showed the presence of signals at 0.98, 3.85 ppm and at 1.32, 4.30 ppm due to OC_2H_5 group of **7a**, **b**, respectively.

Ring closure of **7b** with a proper aliphatic primary amine at room temperature afforded the fused pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidines **8a–c**. The IR spectra of the condensed pyrimidines



Scheme 2. Reagents and solvents: a: the appropriate aldehyde, gl.acetic acid; b: PhCOCl, dry benzene; c: $POCl_3$; d: the appropriate amine, absolute ethanol for **13a**, K_2CO_3, dry DMF for **13b**, **c**; e: glycine, K_2CO_3, dry DMF.

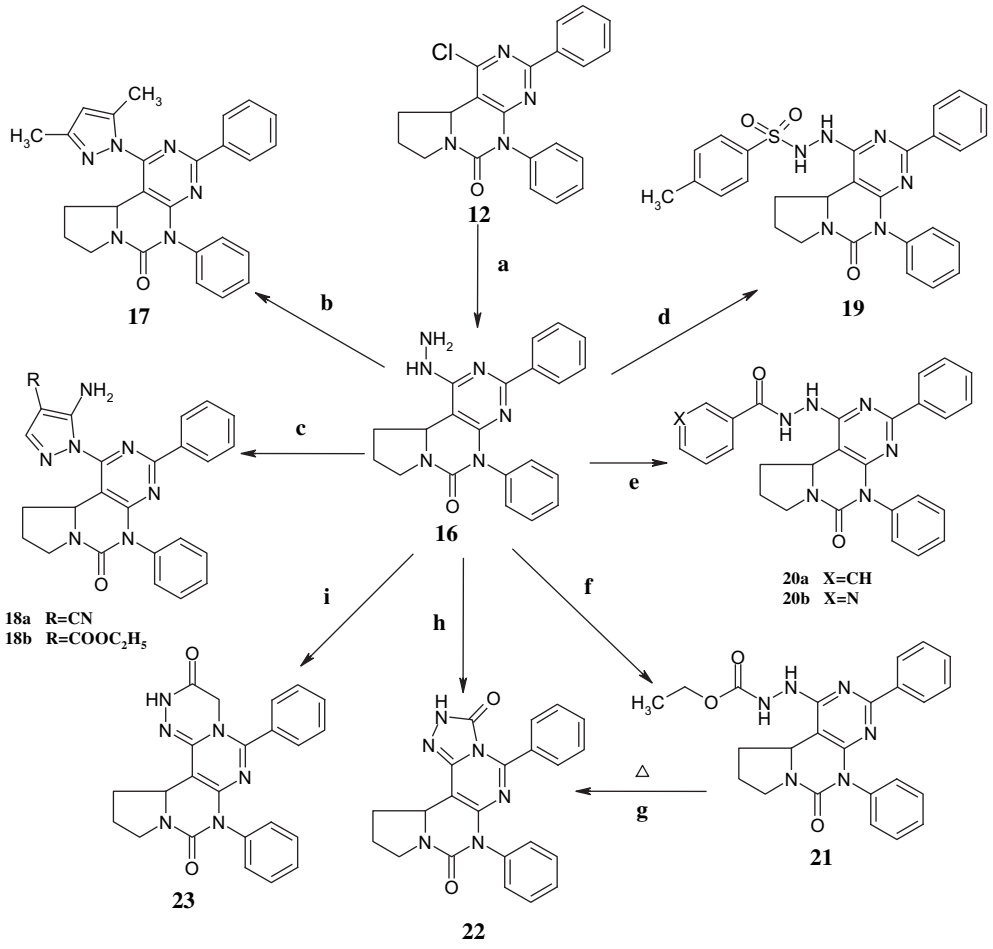
8a–c showed the NH band at 3370 cm^{-1} in addition they lacked the absorption band assigned to the nitrile stretching vibration at 2196 cm^{-1}.

Other target polycyclic azines **9a–g, 11–13** were obtained as depicted in Scheme 2. Thus, condensation of the intermediates **4a**, **b** with different aromatic aldehydes in glacial acetic acid yielded only the tricyclic derivatives **9a–g** instead of the benzylidene derivatives **10** (Scheme 2). The mechanism of this reaction was outlined in Fig. 1 [33]. The proposed structures **9a–g** were confirmed by IR spectra which showed the absence of the nitrile stretching band in the region of 2178 cm^{-1} and the presence of NH and an additional carbonyl stretching bands at 3392 and 1725 cm^{-1}, respectively. Moreover, ^{1}H NMR data showed two exchangeable singlet signals corresponding to the two NH groups.

Furthermore, the reported 1-chloropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine derivative **12** [32], yielded **13a–c** upon alkylation with propylamine, pyrrolidine or morpholine. ^{1}H NMR spectra of the target compounds **13a–c** revealed extra aliphatic bands corresponding to the propyl, pyrrolidino and the morpholino moieties, respectively.

However, treatment of the chloro derivative **12** with glycine in dry DMF in the presence of anhydrous potassium carbonate resulted in a tetracyclic derivative **14** instead of the expected glycine derivative **15**. The proposed structure of **14** was supported by the ^{1}H NMR that showed the characteristic signal of the imidazolidinone CH_2 at 3.03 ppm, and the mass spectrum presented the molecular ion peak at 397.

On the other hand, the known intermediate 1-hydrazino-3,5-diphenyl-8,9,10,10a-tetrahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidin-6-one **16,** [32] was in turn, proved to be a useful intermediate for the synthesis of the designed condensed pyrimidines **17–23** (Scheme 3). Treatment of **16** with acetylacetone in dry DMF resulted in the formation of 1-(3,5-dimethylpyrazol-1-yl) derivative **17** whose structure was confirmed by the presence of singlet peaks at 2.30, 2.53, and 6.07 ppm in the ^{1}H NMR corresponding to the two methyl groups and the pyrazolo proton, respectively. Furthermore,



Scheme 3. Reagents and solvents: a: $NH_2NH_2.H_2O$, absolute ethanol; b: acetylacetone, dry DMF; c: ethoxymethylene malononitrile for **18a**, ethyl ethoxymethylenecyanoacetate, dry DMF for **18b**; d: *p*-toluenesulphonyl chloride, Et3N, dry benzene; e: the acid chloride, pyridine; f: ethyl chloroformate, Et3N, dry benzene; g: dry DMF; h: ethylchloroformate, $NaOCH_3$,CH_3OH; i: chloroacetyl chloride, Et3N, dry benzene.

the 5-aminopyrazolyl derivatives **18a**, **b** were obtained directly by condensation reaction of the hydrazino derivative **16** with ethoxymethylene malononitrile or ethyl ethoxymethylene cyanoacetate, respectively. The mechanism of this reaction is outlined in Fig. 2 [34,35], it is noteworthy that the NH of the hydrazine moiety and not the pyrimidino nitrogen was involved in the cyclization, therefore, resulting in the formation of 1-substituted-5-aminopyrazole-4-carbonitrile or carboxylic acid ethyl ester **18a**, **b** rather than the 1-substituted-3-aminopyrazole-4-carbonitrile or carboxylic acid ethyl ester **18c** nor the triazepine derivative **18d**. The ^{1}H NMR showed the presence of the amino group signal at 7.14 ppm in case of **18a** and signals at 1.31, 4.26, 6.75 ppm corresponding to ethoxycarbonyl and amino groups, respectively, of **18b.** Additionally, mass spectrum of **18b** revealed molecular ion peak at 495; moreover, the structure of the product **18a** was identified on the basis of X-ray crystal structure analysis (Fig. 3) which confirmed that the substituted nitrogen of the pyrazole ring is adjacent to the carbon atom carrying the amino group.

N-acylation of the hydrazino derivative **16** with different acid chlorides as *p*-toluenesulphonyl chloride, benzoyl chloride or nicotinoyl chloride under different conditions resulted in the expected derivatives **19**, **20a**, **b**, respectively (Scheme 3). The ^{1}H NMR spectra of the derivatives **19**, **20a**, **b** confirmed the obtained structures. The hydrazino derivative **16** was also reacted with ethyl chloroformate in dry benzene and in the presence of triethylamine to yield the ethoxyhydrazide derivative **21**, however, when the reaction was performed in methanol in the presence of sodium methoxide the tetracyclic derivative **22** was obtained. It was also possible to obtain compound **22** by refluxing **21** in dry DMF. The IR spectrum of **21** showed stretching bands at 3358, 3328, 1748 cm^{-1} corresponding to the two NHs and carbonyl group of the ester moiety, respectively, and these bands disappeared in the IR spectrum of **22**. In addition, the ^{1}H NMR of **21** revealed the presence of the triplet and quartet peaks of the ethyl group at 1.31 and 4.25 ppm respectively which disappeared in the ^{1}H NMR spectrum of **22**.

Finally, the reaction of the hydrazino derivative **16** with chloroacetyl chloride in dry benzene in the presence of equimolar amount of triethylamine afforded the tetracyclic triazine derivative **23** rather than the open acylated tricyclic derivative. The ^{1}H NMR confirmed the obtained structure by the presence of an exchangeable signal of NH moiety at 9.60 ppm and a singlet peak of CH_2 group at 4.04 ppm.

2.2. Pharmacological screening

2.2.1. Analgesic activity

The analgesic activity of the synthesized compounds **3a**–**g**, **5, 6a**, **b**, **7a**, **b**, **9a**–**g**, **13a**–**c**, **17**, **18a**, **b**, **19**, **20a**, **b**, **21**, **14**, **22**, and **23** was studied by the acetic acid-induced writhing test in mice [36–39] using indomethacin as a reference standard. The tested compounds presented a significant analgesic profile as it was noticed that all compounds revealed analgesic activity comparable to or higher

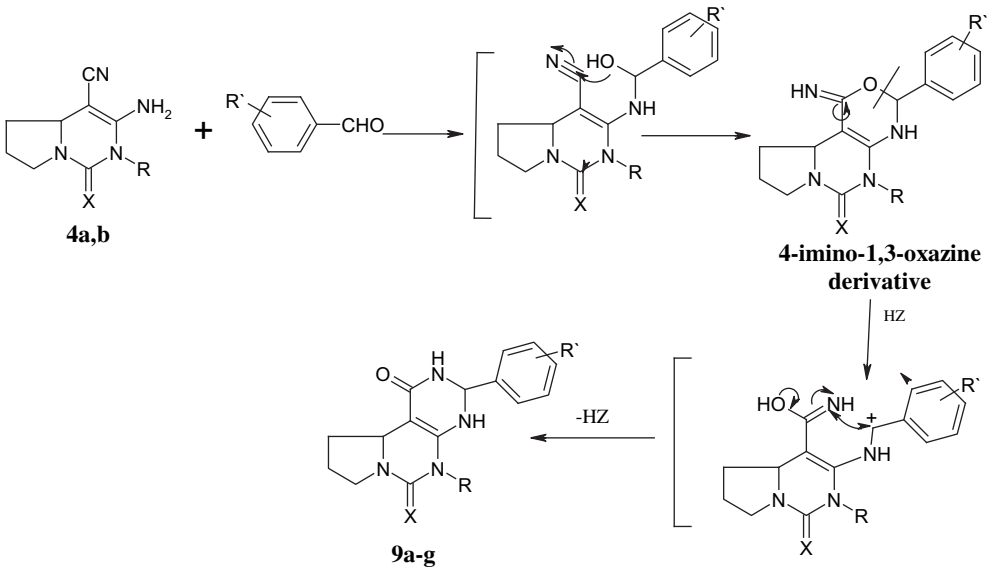


Fig. 1. Mechanism of formation of **9a**–**g**.

than that of indomethacin at the same dose level and test conditions (Table 1). Regarding the bicyclic nucleus, the 3-substituted derivatives **3a**–**f**, **5** and **7b** exhibited higher analgesic activity compared to indomethacin. It is noted that elongation of the aliphatic side chain of the N-alkylated derivatives **3a**–**e** increased the analgesic activity and the N-ethylamino derivative **5** exhibited the highest % inhibition compared to the other bicyclic analogs. However, a decrease in potency was only observed in *m*-aryl substituted derivative **3g** compared with its *p*-substituted analog **3f.** Moreover, replacement of the 1-oxo in the 3-formamidic acid derivative **7a** by 1-thioxo group resulted in **7b** with a higher activity, this may be due to the increase in lipophilicity. It was also noted that the N-hydroxy carboxamidine derivative **6a** had a better analgesic effect than the N-amino analog **6b**. Concerning the tricyclic nucleus, N-methyl derivative **8a** presented higher activity than both the N-ethyl **8b** and N-propyl derivative **8c**, and substitution on 3-aryl group by an electron withdrawing moiety as in **9b**, **9f** and **9g** or electron donating group as in **9c**–**e** resulted in higher potency than the unsubstituted compound **9a**. However, substitution at position 1 with aliphatic amine **13a**, cycloalkylamine **13b**, **c** or pyrazole ring **17**, **18a**, **b** did not affect the activity except for the pyrrolidin-1-yl substituted compound **13b** that exhibited higher significant activity than indomethacin. In addition, the hydrazino derivatives **19**, **20a**, **b**, **21** showed comparable activity to indomethacin while the ethoxyhydrazide derivative **21** had higher potency. All the tetracyclic derivatives exerted comparable activity

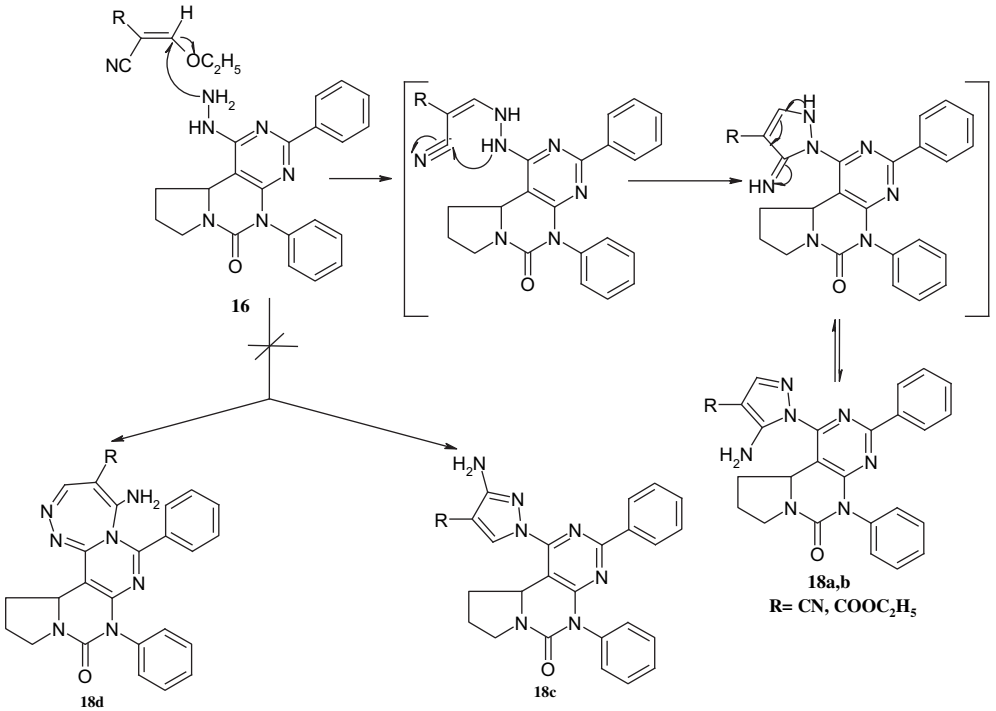


Fig. 2. Mechanism of formation of **18a** and **18b.**

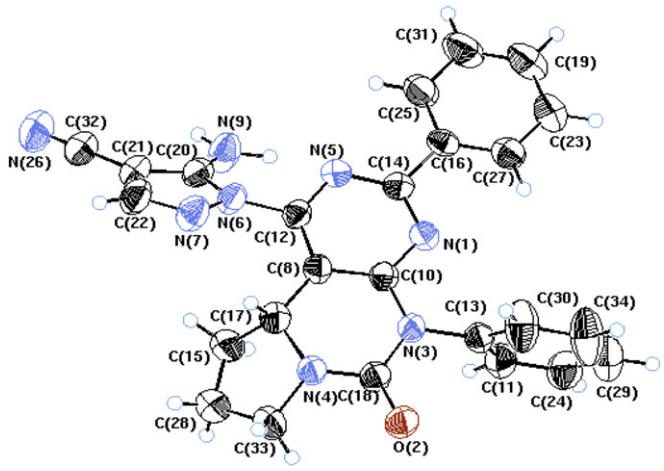


Fig. 3. ORTEP projection of single crystal X-ray diffraction of **18a**.

to indomethacin, however, the triazino derivative **23** displayed the highest activity compared to its 5-membered imidazo and triazolo analogs **14** and **22,** respectively.

Compounds **5**, **9e** and **23** were further tested for their analgesic activity at 0.4, 0.8, 1, 2, 4 and 8 mg/kg in order to determine their IC 50 values. The data revealed that the tricyclic derivative **9e** is more potent than both the bicyclic and the tetracyclic derivatives **5** and **23,** respectively (Table 2).

Table 1
Effects of the compounds on acetic acid-induced abdominal writhing in mice.

Compound	Dose (mg/kg)	Writhing reflex ± SEM	Inhibition %
Control	–	76.83 ± 1.08	–
Indomethacin	10	21.83 ± 0.91[a]	71.58
3a	7.69	20.00 ± 0.58[a]	73.97
3b	8.12	19.67 ± 0.49[a]	74.40
3c	8.59	9.33 ± 0.88[a,b]	87.85
3d	8.47	14.50 ± 0.76[a,b]	81.13
3e	8.94	8.67 ± 0.88[a,b]	88.72
3f	10.24	13.17 ± 0.79[a,b]	82.86
3g	11.49	27.83 ± 0.95[a,b]	63.77
5	8.68	2.83 ± 0.83[a,b]	96.31
6a	8.33	10.83 ± 1.05[a,b]	85.90
6b	8.36	16.17 ± 0.79[a,b]	78.96
7a	9.00	23.17 ± 1.05[a]	69.85
7b	8.12	9.17 ± 0.70[a,b]	88.07
8a	7.63	1.83 ± 0.48[a,b]	97.61
8b	8.04	18.50 ± 0.76[a,b]	75.92
8c	8.45	17.50 ± 0.85[a,b]	77.22
9a	10.45	17.17 ± 0.87[a,b]	77.66
9b	11.45	3.00 ± 0.77[a,b]	96.10
9c	11.32	12.00 ± 0.86[a,b]	84.38
9d	10.39	11.50 ± 0.76[a,b]	85.03
9e	10.86	1.00 ± 0.26[a,b]	98.70
9f	11.75	10.17 ± 0.48[a,b]	86.77
9g	11.75	4.17 ± 0.87[a,b]	94.58
13a	11.66	23.33 ± 0.88 [a]	69.63
13b	12.04	3.83 ± 0.87 [a,b]	95.01
13c	12.40	25.33 ± 0.84 [a,b]	67.02
14	11.52	16.50 ± 0.76[a,b]	78.52
17	12.65	18.00 ± 0.58[a,b]	76.57
18a	13.00	17.83 ± 0.70[a,b]	76.78
18b	14.37	19.33 ± 0.76 [a,b]	74.84
19	15.26	18.00 ± 0.97[a,b]	76.57
20a	13.81	21.67 ± 0.88 [a]	71.80
20b	13.84	17.00 ± 0.73[a,b]	77.87
21	12.87	6.33 ± 0.84[a,b]	91.76
22	11.55	18.50 ± 1.03[a,b]	75.92
23	11.96	6.33 ± 0.42[a,b]	91.76

[a] Statistically significant from control at $p < 0.05$.
[b] Statistically significant from indomethacin at $p < 0.05$.

Table 2
IC 50 values of the analgesic activity for compounds **5**, **9e** and **23**.

Compound	Response (% inhibition) at						IC 50 (mg/kg)
	0.4 mg/kg	0.8 mg/kg	1 mg/kg	2 mg/kg	4 mg/kg	8 mg/kg	
5	49.36	81.33	90.56	94.43	95.97	96.36	0.402
9e	53.65	75.97	86.91	92.00	95.27	97.00	0.359
23	43.13	63.31	76.61	82.40	86.06	88.83	0.446

2.2.2. Anti-inflammatory activity

The tested compounds for analgesic activity were further evaluated for their anti-inflammatory activity using the carrageenan-induced paw edema method in rats [40–43]. The percentage inhibition of edema was calculated and the data were expressed as the mean ± SEM. The anti-inflammatory activity of the test compounds relative to that of indomethacin, as a reference drug, was also calculated. The results are recorded in Tables 3 and 4. It has been noticed that the bicyclic derivatives **3a–c** showed comparable activity to indomethacin, the N-butyl derivative **3e** had higher potency than the N-allyl derivative **3d**, and the N-ethylamino derivative **5** showed moderate activity. The *p*-substituted aromatic derivative **3f** was more potent than the *m*-substituted one **3g**. In case of the formamidic acid ethyl ester derivative **7a,** the activity decreased lower than that of the thioxo analog **7b** and this may be due to the lipophilicity of **7b** (Table 3). Furthermore, the N-hydroxy carboxamidine **6a** was equipotent to indomethacin at the forth hour while the N-amino analog **6b** had the maximum effect at the second hour. On the other hand, the tested tricyclic derivatives displayed moderate to weak anti-inflammatory activity except **13a** and **20a** that exhibited higher activity compared to the reference drug (Table 4). The tetracyclic imidazolo derivative **14** revealed higher potency than the triazolo derivative **22** and its homolog **23** (Table 4). It should be noted that the ethoxyhydrazide derivative **21** had the same activity profile as its cyclized triazole derivative **22**, they may be interconvertable in the body.

Moreover, the IC 50 values for the most active compounds **3a**, **13a** and **14** were calculated using the doses of 10, 15, 20, 25 mg/kg (Table 5). It was found that the bicyclic compound **3a** is nearly equipotent to the tetracyclic one **14** whereas **13a**, the tricyclic derivative, is more potent (IC 50 = 16.50 mg/kg).

In conclusion, all the tested compounds showed remarkable analgesic activity comparable to that of indomethacin in systemic in vivo bioassay at the same dose level. Regarding, the anti-inflammatory activity, generally the bicyclic derivatives exhibited higher activity than the tri- and tetracyclic compounds. Finally, it was concluded that the tested compounds exerted their analgesic and anti-inflammatory activities via different mechanisms.

2.2.3. Ulcerogenic activity

The prepared compounds were tested for their ulcerogenic effect after testing for their anti-inflammatory activity. It was found that the bicyclic N-substituted imino derivatives **3a–f** and the formamidic ethyl esters **7a**, **b** had gastric safety profile. Furthermore, all the tricyclic derivatives were ulcerogenic except **8a**, **8c**, **13b**, **13c** and **18b**, on the other hand, all the tetracyclic derivatives displayed super gastric safety pattern when compared to the ulcerogenic effect of indomethacin.

3. Experimental

3.1. Chemistry

Melting points were determined on Electrothermal Stuart $5MP_3$ digital melting point apparatus and were uncorrected. Elemental microanalyses were performed at the microanalytical center,

Table 3
Anti-inflammatory effect of the bicyclic derivatives against carrageenan-induced paw edema in rats and ratio of ulceration ($n = 6$).

Compound	Ratio of ulceration	Edema thickness (mm) ± SEM (% inhibition)				Potency[c]
		1 h	2 h	3 h	4 h	
Control	0/6	1.483 ± 0.070[b]	2.017 ± 0.070[b]	2.317 ± 0.095[b]	2.683 ± 0.095[b]	–
3a	0/6	1.200 ± 0.052[a,b] (19.08)	1.483 ± 0.054[a] (26.47)	1.617 ± 0.048[a] (30.21)	1.750 ± 0.067[a] (34.77)	1.27
3b	0/6	1.367 ± 0.099[b] (7.82)	1.550 ± 0.120[a] (23.15)	1.750 ± 0.152[a] (24.47)	1.867 ± 0.152[a] (30.41)	1.11
3c	0/6	1.400 ± 0.052[b] (5.60)	1.567 ± 0.067[a] (22.31)	1.717 ± 0.065[a] (25.90)	1.950 ± 0.103[a] (27.32)	1.00
3d	0/6	1.45 ± 0.085[b] (2.23)	1.633 ± 0.080[a,b] (19.04)	1.783 ± 0.048[a] (23.05)	2.300 ± 0.089[a,b] (14.28)	0.52
3e	0/6	1.317 ± 0.105[b] (11.19)	1.533 ± 0.096[a] (24.00)	1.867 ± 0.080[a] (19.42)	2.083 ± 0.079[a] (22.36)	0.81
3f	0/6	1.233 ± 0.092[a,b] (16.86)	1.450 ± 0.067[a] (28.11)	1.667 ± 0.148[a] (28.05)	2.050 ± 0.206[a] (23.59)	0.86
3g	2/6	1.35 ± 0.072[b] (8.97)	1.867 ± 0.080[b] (7.44)	2.117 ± 0.070[b] (8.63)	2.433 ± 0.102[b] (9.31)	0.34
5	3/6	1.250 ± 0.096[b] (15.71)	1.467 ± 0.092[a] (27.27)	1.717 ± 0.087[a] (25.90)	2.230 ± 0.092[a] (16.88)	0.61
6a	4/6	1.417 ± 0.040[b] (4.45)	1.717 ± 0.091[a,b] (14.87)	1.817 ± 0.087[a] (21.58)	1.950 ± 0.062[a] (27.32)	1.00
6b	2/6	1.35 ± 0.081[b] (8.97)	1.467 ± 0.062[a] (27.27)	1.717 ± 0.079[a] (25.90)	2.067 ± 0.156[a] (22.96)	0.84
7a	0/6	1.15 ± 0.112[a,b,c] (22.45)	1.833 ± 0.088[b,c] (9.12)	2.183 ± 0.091[b,c] (5.78)	2.550 ± 0.099[b,c] (4.96)	0.18
7b	0/6	1.300 ± 0.082[b,c] (12.34)	1.633 ± 0.056[a,b,c] (19.04)	1.833 ± 0.092[a] (20.89)	2.133 ± 0.120[a,c] (20.50)	0.75
Indo.	5/6	0.783 ± 0.117[a] (47.20)	1.300 ± 0.179[a] (35.55)	1.600 ± 0.210[a] (30.95)	1.950 ± 0.239[a] (27.32)	1.00

[a] Statistically significant from control at $p < 0.05$.
[b] Statistically significant from indomethacin at $p < 0.05$.
[c] Potency was expressed as % inhibition of the tested compounds relative to % inhibition of indomethacin at 4 h effect.

Faculty of Science, Cairo University. UV spectra were recorded on a Shimadzu UV–Visible spectrophotometer, UV-1650 PC in chloroform. The IR spectra were recorded on a Bruker FT-IR spectrophotometer as potassium bromide discs in case of solids and as thin films in case of oils. The ^{1}H NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 on a Varian Mercury spectrometer (200 and 300 MHz) and ^{13}C NMR spectra were recorded at 75.45 MHz. Mass spectra were performed on HP MODEL: MS_5988 mass spectrometer and on Shimadzu QP-2010 Plus (EI, 70 eV). Silica gel used for column chromatography was obtained from Fluka, 70–230 mesh. Thin layer chromatography was carried out on silica gel TLC plates with fluorescence indicator (F_{254}). Compounds **1** [44], **2a** [31], **2b** [30], **4a** [32], **4b** [30], **11** [32,45], **12**, **16** [32] and the reagents ethyl ethoxymethylene cyanoacetate [46] and nicotinyl chloride hydrochloride [47] were prepared according to the reported procedures.

3.1.1. (E/Z) 3-Methylimino-1-oxo-2-phenyl-1,2,3,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carbonitrile **3a**

To a suspension of **2a** (0.5 g, 2 mmol) in dry benzene (5 ml) dimethyl sulfate (2.2 mmol) was added, and the reaction mixture was refluxed for 3 h. The solvent was removed under vacuum, the residue was dissolved in water, and the pH of the solution was adjusted at 10 using 5 N sodium hydroxide. The obtained precipitate was filtered, washed with water and crystallized from absolute ethanol to give a white solid, 0.47 g, 90% yield, m.p. 186–188 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3053 (CH aromatic), 2937–2866 (CH aliphatic), 2204 (CN),

Table 4
Anti-inflammatory effect of the tri- and tetracyclic derivatives against carrageenan-induced paw edema in rats and ratio of ulceration ($n = 6$).

Compound	Ratio of ulceration	Edema thickness (mm) ± SEM (% inhibition)				Potency[c]
		1 h	2 h	3 h	4 h	
Control	0/6	1.483 ± 0.070[b]	2.017 ± 0.070[b]	2.317 ± 0.095 [b]	2.683 ± 0.095 [b]	–
8a	0/6	1.317 ± 0.048[b] (11.19)	1.733 ± 0.096[a,b] (14.06)	2.050 ± 0.062[b] (11.52)	2.417 ± 0.054[b] (9.93)	0.36
8b	1/6	1.367 ± 0.049[b] (11.63)	1.783 ± 0.065[b] (11.59)	2.067 ± 0.033[b] (10.80)	2.417 ± 0.095[b] (9.91)	0.36
8c	0/6	1.283 ± 0.105[b] (13.46)	1.700 ± 0.097[a,b] (15.72)	2.100 ± 0.107[b] (9.37)	2.500 ± 0.151[b] (6.82)	0.24
9a	2/6	1.417 ± 0.048[b] (4.47)	1.767 ± 0.084[b] (12.41)	2.050 ± 0.089[b] (11.52)	2.400 ± 0.139[b] (10.55)	0.38
9b	3/6	0.850 ± 0.118[a] (42.68)	1.567 ± 0.152[a] (22.31)	2.117 ± 0.172[b] (8.63)	2.417 ± 0.185[b] (9.91)	0.36
9c	2/6	1.383 ± 0.098[b] (6.72)	1.833 ± 0.122[b] (6.64)	2.000 ± 0.134[a,b] (13.68)	2.183 ± 0.087[a] (18.64)	0.68
9d	2/6	1.333 ± 0.115[b] (10.09)	1.833 ± 0.088[b] (9.12)	2.067 ± 0.076[b] (10.79)	2.283 ± 0.087[a,b] (14.90)	0.54
9e	4/6	1.150 ± 0.177[a,b] (22.45)	1.833 ± 0.096[b] (9.12)	2.250 ± 0.106[b] (2.89)	2.600 ± 0.113[b] (3.09)	0.11
9f	3/6	1.300 ± 0.097[b] (12.34)	1.733 ± 0.096[a,b] (14.06)	2.117 ± 0.040[b] (8.65)	2.583 ± 0.060[b] (3.73)	0.13
9g	1/6	1.467 ± 0.084[b] (1.10)	1.733 ± 0.096[a,b] (14.06)	1.983 ± 0.098[a,b] (14.40)	2.167 ± 0.076[a] (19.23)	0.70
13a	3/6	1.283 ± 0.105[b] (13.49)	1.500 ± 0.097[a] (25.63)	1.600 ± 0.103[a] (30.94)	1.700 ± 0.103[a] (36.64)	1.34
13b	0/6	1.367 ± 0.012[b] (7.82)	1.483 ± 0.125[a] (26.47)	1.733 ± 0.115[a] (25.21)	2.267 ± 0.076[a] (15.51)	0.56
13c	0/6	1.317 ± 0.098[b] (11.19)	1.633 ± 0.126[a,b] (19.04)	1.883 ± 0.145[a] (18.73)	2.383 ± 0.164[b] (11.18)	0.40
14	0/6	1.267 ± 0.067 [b] (14.57)	1.567 ± 0.056[a] (22.31)	1.683 ± 0.048[a] (27.36)	1.900 ± 0.113[a] (29.18)	1.06
17	2/6	1.217 ± 0.070[a,b] (17.94)	1.600 ± 0.058[a,b] (20.67)	1.833 ± 0.056[a] (20.89)	2.217 ± 0.114[a] (17.37)	0.63
18a	1/6	1.300 ± 0.052[b] (12.34)	1.817 ± 0.087[b] (9.92)	2.100 ± 0.037[b] (9.37)	2.500 ± 0.068[b] (6.82)	0.24
18b	0/6	1.383 ± 0.133[b] (6.74)	1.65 ± 0.106[a,b] (18.20)	1.767 ± 0.109[a] (23.74)	2.383 ± 0.075[b] (11.18)	0.40
19	2/6	1.150 ± 0.085[a,b] (22.45)	1.683 ± 0.019[a,b] (16.56)	1.783 ± 0.196[a] (23.05)	2.100 ± 0.195[a] (21.73)	0.79
20a	3/6	0.95 ± 0.085[a] (35.94)	1.433 ± 0.152[a] (28.95)	1.683 ± 0.142 [a] (27.36)	2.000 ± 0.144[a] (25.46)	0.93
20b	2/6	1.000 ± 0.058[a] (32.57)	1.783 ± 0.095[b] (11.60)	2.117 ± 0.031[b] (8.63)	2.533 ± 0.115[b] (5.59)	0.20
21	1/6	1.200 ± 0.093[a,b] (19.08)	1.667 ± 0.072[a,b] (17.37)	1.900 ± 0.037[a,b] (17.99)	2.250 ± 0.067[a] (16.14)	0.59
22	0/6	1.330 ± 0.088[b] (10.11)	1.767 ± 0.080[b] (12.39)	1.917 ± 0.048[a,b] (17.26)	2.267 ± 0.080[a] (15.51)	0.56
23	0/6	1.450 ± 0.056 [b] (2.23)	1.867 ± 0.062[b] (7.44)	1.950 ± 0.067[a,b] (15.84)	2.117 ± 0.054[a] (21.10)	0.77
Indo.	5/6	0.783 ± 0.117[a] (47.20)	1.300 ± 0.179[a] (35.55)	1.600 ± 0.210[a] (30.95)	1.950 ± 0.239[a] (27.32)	1.00

[a] Statistically significant from control at $p < 0.05$.
[b] Statistically significant from indomethacin at $p < 0.05$.
[c] Potency was expressed as % inhibition of the tested compounds relative to % inhibition of indomethacin at 4 h effect.

Table 5
IC 50 values of the anti-inflammatory activity for compounds **3a**, **13a** and **14**.

Compound	Response (% inhibition) at				IC 50 (mg/kg)
	10 mg/kg	15 mg/kg	20 mg/kg	25 mg/kg	
3a	36.00	43.04	50.77	62.73	17.77
13a	27.56	41.63	63.29	69.05	16.50
14	17.00	40.00	61.31	68.40	17.62

1706 (C=O), 1636 (C=N), 1590 (C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.22–2.32 (m, 2H, pyrrolidine), 3.24 (t, $J = 7.8$ Hz, 2H, pyrrolidine), 3.38 (s, 3H, CH_3), 4.04 (t, $J = 7.2$ Hz, 2H, pyrrolidine), 7.13–7.51 (m, 5H, aromatic protons). ^{13}C NMR ($CDCl_3$, 75.45 MHz): 20.38, 32.13, 49.75 (pyrrolidine carbons), 36.43 (N–CH_3), 116.50 (CN), 126.02, 128.14, 128.70, 128.97, 129.26 (aromatic protons), 136.90 (C=*C*–CN), 145.26 (*C*=C–CN), 148.71 (C=O), 163.63 (C=N). MS: *m*/*z* (%): 266 (M^+, 18.82), 265 (100), 251 (17.34). Anal. Calcd. for $C_{15}H_{14}N_4O$ (266.31): C, 67.65; H, 5.30; N, 21.04. Found: C, 67.81; H, 4.98; N, 21.03.

3.1.2. General procedure for the preparation of **3b–g**

To a solution of **2a** (0.5 g, 2 mmol) in dry DMF (5 ml), sodium hydride (0.1 g, 60% in mineral oil, washed with hexane, 2.5 mmol) was added, and the reaction mixture was heated at 80 °C for 1 h. Then the appropriate alkyl or aryl halide was added (2.5 mmol), and the mixture was further heated for a specified time, cooled to R.T. and poured on ice water (20 ml). The obtained solid was filtered and recrystallized from ethanol/water in case of **3b–d**, or purified by column chromatography using silica gel (230 mesh size) as stationary phase and chloroform:ethanol (9.5:0.5) as elution system in case of **3e–g**.

3.1.2.1. (E/Z) 3-Ethylimino-1-oxo-2-phenyl-1,2,3,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carbonitrile **3b**.

Compound **3b** was prepared from **2a** and ethyl iodide for 3 h. Brown crystals, 0.20 g, 36% yield, m.p. 264–265 °C (decomposition). UV ($CHCl_3$), Conc. 7.13×10^{-5} M, nm: 227.5 (log ε: 3.65), 241 (log ε: 4.01), 287 (log ε: 3.91). IR: $\upsilon_{max.}$/cm^{-1} 3061 (CH aromatic), 2923–2852 (CH aliphatic), 2203 (CN), 1705 (C=O), 1633 (C=N), 1593–1492 (C=C). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.10 (t, $J = 6.8$ Hz, 3H, CH_2CH_3), 1.94–2.09 (m, 2H, pyrrolidine), 2.90–2.95 (m, 2H, pyrrolidine), 3.63–3.73 (m, 2H, CH_2CH_3), 3.90–4.00 (m, 2H, pyrrolidine), 7.00–7.55 (m, 5H, aromatic protons). MS: *m*/*z* (%): 280 (M^+, 11.87), 57 (100). Anal. Calcd. for $C_{16}H_{16}N_4O$ (280.33): C, 68.55; H, 5.75; N, 19.99. Found: C, 68.20; H, 5.75; N, 19.91.

3.1.2.2. (E/Z) 3-(2-Hydroxyethyl)imino-1-oxo-2-phenyl-1,2,3,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carbonitrile **3c**.

Compound **3c** was prepared from **2a** and 2-chloroethanol for 10 h. Brown crystals, 0.22 g, 38% yield, m.p. >330 °C (decomposition). IR: $\upsilon_{max.}$/cm^{-1} 3296 (OH), 3058 (CH aromatic), 2923–2853 (CH aliphatic), 2206 (CN), 1705 (C=O), 1634 (C=N), 1596–1492 (C=C). ^{1}H NMR (DMSO-d_6, D_2O, 200 MHz): δ 1.95–2.00 (m, 2H, pyrrolidine), 2.91–2.96 (m, 2H, pyrrolidine), 3.80–3.83 (m, 2H, NCH_2CH_2OH), 3.96–4.09 (m, 2H, pyrrolidine), 4.32–4.43 (m, 2H, NCH_2CH_2OH), 6.99–7.51 (m, 5H, aromatic protons), 9.19 (s, 1H, OH exchanged by D_2O). Anal. Calcd. for $C_{16}H_{16}N_4O_2$ (296.33): C, 64.85; H, 5.44; N, 18.91. Found: C, 64.63; H, 5.45; N, 18.55.

3.1.2.3. (E/Z) 3-Allylimino-1-oxo-2-phenyl-1,2,3,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carbonitrile **3d**.

Compound **3d** was prepared from **2a** and allyl bromide for 6 h. Black crystals, 0.15 g, 26% yield, m.p. 177–178 °C (decomposition). IR: $\upsilon_{max.}$/cm^{-1} 3074 (CH aromatic), 2923–2853 (CH aliphatic), 2212 (CN), 1702 (C=O), 1627 (C=N), 1590–1491 (C=C). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 2.00–2.10 (m, 2H, pyrrolidine), 2.40–2.59 (m, 2H, pyrrolidine), 3.67–3.79 (m, 2H, pyrrolidine), 4.56 (br.s, 2H, CH_2CH=CH_2), 5.15 (d, 2H, CH_2CH=CH_2), 5.82–5.86 (m, 1H, CH_2CH=CH_2), 6.99–7.51 (m, 5H, aromatic protons). Anal. Calcd. for $C_{17}H_{16}N_4O$ (292.34): C, 69.85; H, 5.52; N, 19.16. Found: C, 69.71; H, 5.34; N, 18.99.

3.1.2.4. (E/Z) 3-Butylimino-1-oxo-2-phenyl-1,2,3,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carbonitrile **3e**.

Compound **3e** was prepared from **2a** and butyl bromide for 8 h. Brown oil, 0.17 g, 28% yield. IR: $\upsilon_{max.}$/cm^{-1} 3058 (CH aromatic), 2956–2858 (CH aliphatic), 2195 (CN), 1704 (C=O), 1624 (C=N), 1593–1492 (C=C).^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.92 (t, $J = 6.9$ Hz, 3H, $NCH_2CH_2CH_2CH_3$), 1.26–1.45 (m, 4H, $NCH_2CH_2CH_2CH_3$), 1.45–1.75 (m, 2H, pyrrolidine), 3.10–3.15 (m, 2H, pyrrolidine), 4.17–4.20 (m, 4H, $NCH_2CH_2CH_2CH_3$ and 2H pyrrolidine), 7.20–7.58 (m, 5H, aromatic protons). Anal. Calcd. for $C_{18}H_{20}N_4O$ (308.39): C, 70.11; H, 6.54; N, 18.17. Found: C, 70.20; H, 6.31; N, 18.44.

3.1.2.5. (E/Z) 3-(4-Cyanophenyl)imino-1-oxo-2-phenyl-1,2,3,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carbonitrile **3f**.

Compound **3f** was prepared from **2a** and 4-fluorobenzonitrile for 6 h. Reddish brown crystals, 0.36 g, 51% yield, m.p. 185–187 °C. IR: $\upsilon_{max.}$/cm^{-1} 3063 (CH aromatic), 2960–2887 (CH aliphatic), 2212 (CN), 2187(CN), 1683 (C=O), 1602 (C=N), 1542–1494 (C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.14–2.24 (m, 2H, pyrrolidine), 2.93 (t, $J = 7.8$ Hz, 2H, pyrrolidine), 3.69 (t, $J = 7.2$ Hz, 2H, pyrrolidine), 7.09 (d, $J = 8.1$ Hz, 2H, aromatic protons), 7.22–7.44 (m, 5H, aromatic protons), 7.54 (d, $J = 8.1$ Hz, 2H, aromatic protons). MS: *m*/*z* (%): 353 (M^+, 10.85), 215 (100). Anal. Calcd. for $C_{21}H_{15}N_5O.2H_2O$ (389.42): C, 64.77; H, 4.92; N, 17.98. Found: C, 64.47; H, 4.63; N, 17.86.

3.1.2.6. (E/Z) 1-Oxo-2-phenyl-3-(3-trifluoromethylphenyl)imino-1,2,3,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carbonitrile **3g**.

Compound **3g** was prepared from **2a** and 3-fluorobenzotrifluoride for 15 h. Brown crystals, 0.25 g, 32% yield, m.p. 209–211 °C. IR: $\upsilon_{max.}$/cm^{-1} 3062 (CH aromatic), 2955–2853 (CH aliphatic), 2209 (CN), 1704 (C=O), 1629(C=N), 1596–1494 (C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.06–2.13 (m, 2H, pyrrolidine), 2.91 (t, $J = 7.8$ Hz, 2H, pyrrolidine), 3.69 (t, $J = 7.05$ Hz, 2H, pyrrolidine), 7.14–7.59 (m, 9H, aromatic protons). Anal. Calcd. for $C_{21}H_{15}F_3N_4O$ (396.38): C, 63.64; H, 3.81; N, 14.13. Found: C, 64.18; H, 3.63; N, 14.35.

3.1.3. 3-(2-Aminoethylamino)-1-oxo-2-phenyl-1,2,4a,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carbonitrile **5**

Compound **5** was prepared according to the general procedure of preparation of **3b–g** by heating **4a** (0.5 g, 2 mmol), sodium hydride (0.20 g, 60% in mineral oil washed with hexane, 5 mmol) and 2-chloroethylamine hydrochloride (0.28 g, 2.5 mmol) at 80 °C for 10 h. Buff solid, 0.29 g, 50% yield, m.p. 190–192 °C dec. IR: $\upsilon_{max.}$/cm^{-1} 3348 (NH, NH_2 stretching), 3050 (CH aromatic), 2925–2855 (CH aliphatic), 2189 (CN), 1655 (C=O), 1598–1495 (C=C). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.40–1.80 (m, 4H, pyrrolidine), 2.19 (br.s, 1H, pyrrolidine), 2.51–2.72 (m, 2H, $NHCH_2CH_2NH_2$), 2.91–2.93 (m, 2H, $NHCH_2CH_2NH_2$), 3.12 (br.s, 1H, pyrrolidine), 4.00 (br.s, 1H, pyrrolidine), 6.21 (br.s, 1H, NH exchanged by D_2O), 6.88–7.35 (m, 5H, aromatic protons), 8.42 (br.s, 2H, NH_2 exchanged by D_2O). Anal. Calcd. for $C_{16}H_{19}N_5O$ (297.36): C, 64.63; H, 6.44; N, 23.55. Found: C, 64.58; H, 6.62; N, 23.89.

3.1.4. 3-Amino-N-hydroxy-1-oxo-2-phenyl-1,2,4a,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine-4-carboxamidine **6a**

To a suspension of **4a** (0.5 g, 2 mmol) in methanol (5 ml), sodium bicarbonate (0.17 g, 2.1 mmol) and hydroxylamine hydrochloride (0.14 g, 2.1 mmol) were added, and the mixture was refluxed for 24 h. The inorganic precipitate was filtered and the filtrate was concentrated under vacuum, the obtained white crystals were

filtered and recrystallized from ethanol. White solid, 0.28 g, 50% yield, m.p. 166–169 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3403 (OH stretching), 3334–3144 (NH_2 stretching), 2969–2882 (CH aliphatic), 1648 (C=O), 1595–1534 (C = C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.77–2.07 (m, 3H, pyrrolidine), 3.10–3.15 (m, 1H, pyrrolidine), 3.51–3.65 (m, 2H, pyrrolidine), 4.70 (t, J = 6.6 Hz, 1H, pyrrolidine), 4.94 (s, 2H, NH_2 exchanged with D_2O), 5.97 (s, 2H, NH_2 of amidine exchanged with D_2O), 6.88–7.43 (m, 5H, aromatic protons), 7.78 (s, 1H, OH exchanged with D_2O). ^{13}C NMR (DMSO-d_6, 75.45 MHz): 24.50, 31.27, 46.99, 50.47 (pyrrolidine carbons), 82.66 (*C*=C), 117.58, 118.85, 121.56, 128.32, 128.54 (aromatic carbons), 140.20 (C=O), 154.27 (C=*C*), 162.90, 164.81 (C=N). MS: *m*/*z* (%): 287.15 (M^+, 2.87), 93.05 (100). Anal. Calcd. for $C_{14}H_{17}N_5O_2$ (287.32): C, 58.52; H, 5.96; N, 24.37. Found: C, 58.70; H, 5.80; N, 24.87.

3.1.5. 3-Amino-N-amino-1-oxo-2-phenyl-1,2,4a,5,6,7-hexahydropyrrolo[1,2-c]pyrimidine -4-carboxamidine **6b**

A mixture of **4a** (0.5 g, 2 mmol) and hydrazine hydrate (98%, 5 mmol) in absolute ethanol (5 ml) was refluxed for 10 h, the solvent was removed under vacuum and the residue was crystallized from ethanol/water. Buff solid, 0.30 g, 56% yield, m.p. 163–165 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3304–3133 (NH_2 stretching), 3057 (CH aromatic), 2934–2864 (CH aliphatic), 1636 (C=O), 1599 (NH_2 bending), 1562–1496 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.52–1.60 (m, 3H, pyrrolidine), 2.04–2.09 (m, 2H, pyrrolidine), 3.04–3.11(m, 2H, pyrrolidine), 5.80–5.90 (m, 2H, NH_2–C=N exchanged with D_2O), 6.10–6.18 (m, 2H=N–NH_2 exchanged with D_2O), 6.85–7.35 (m, 5H, aromatic protons), 8.38 (s, 2H, NH_2 exchanged with D_2O). MS: *m*/*z* (%): 285 ($M-1^+$, 15.38), 55 (100). Anal. Calcd. for $C_{14}H_{18}N_6O$. 1.5 H_2O (313.36): C, 53.67; H, 6.75; N, 26.82. Found: C, 53.75; H, 6.09; N, 26.83.

3.1.6. General procedure for the preparation of **7a**, **b**

A mixture of **4a** or **4b** (2 mmol) and catalytic amount of acetic anhydride in Triethyl orthoformate (3 ml) is heated for 3 h, the mixture was cooled to R.T and poured on ice water (30 ml). The separated solid was filtered, washed with water and crystallized from ethanol.

3.1.6.1. N-(4-Cyano-1-oxo-2-phenyl-1,2,4a,5,6,7-hexahydropyrrolo-[1,2-c]pyrimidin-3-yl) formimidic acid ethyl ester **7a**.

White solid, 0.54 g, 88% yield, m.p. 103–104 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3053 (CH aromatic), 2983–2894 (CH aliphatic), 2202 (CN), 1697 (C=O), 1628 (C=N), 1613 (C=C). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.98 (t, J = 7 Hz, 3H, OCH_2CH_3), 1.87–2.01(m, 3H, pyrrolidine), 2.35–2.43 (m, 1H, pyrrolidine), 3.47–3.57 (m, 1H, pyrrolidine), 3.64–3.74 (m, 1H, pyrrolidine), 3.85 (q, J = 7 Hz, 2H, OCH_2CH_3), 4.35–4.42 (m, 1H, pyrrolidine), 7.08–7.34 (m, 5H, aromatic protons), 7.72 (s, 1H, N=CH). MS: *m*/*z* (%): 310 (M^+, 18.72), 56 (100%). Anal. Calcd. for $C_{17}H_{18}N_4O_2$ (310.36): C, 65.79; H, 5.85; N, 18.05. Found: C, 65.90; H, 5.92; N, 18.41.

3.1.6.2. N-(4-Cyano-2-ethyl-1-thioxo-1,2,4a,5,6,7-hexahydropyrrolo [1,2-c]pyrimidin -3-yl) formimidic acid ethyl ester **7b**.

Grey solid, 0.48 g, 88% yield, m.p. 87–88 °C. IR: $\upsilon_{max.}/cm^{-1}$ 2977–2829 (CH aliphatic), 2196 (CN), 1634 (C=N), 1594 (C=C), 1248–1219 (C=S). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.13 (t, J = 6.9 Hz, 3H, NCH_2CH_3), 1.32 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 1.80–2.00 (m, 3H, pyrrolidine), 2.35–2.40 (m, 1H, pyrrolidine), 3.78–3.83 (m, 2H, pyrrolidine), 3.98–4.03 (m, 1H, pyrrolidine), 4.11–4.18 (m, 2H, NCH_2CH_3), 4.30 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 7.89 (s, 1H, N=CH). ^{13}C NMR ($CDCl_3$, 75.45 MHz): 13.69 (NCH_2CH_3), 14.33 (OCH_2CH_3), 21.72, 32.57 (pyrrolidine carbons), 42.95 (NCH_2CH_3), 52.53, 54.68 (pyrrolidine carbons), 64.28 (OCH_2CH_3), 69.96 (*C*=C), 117.41 (CN), 155 (N=CH), 159.26 (C=*C*), 175.24 (C=S). Anal. Calcd. for $C_{13}H_{18}N_4OS$ (278.38): C, 56.09; H, 6.52; N, 20.13. Found: C, 56.36; H, 6.32; N, 20.16.

3.1.7. General procedure for the preparation of **8a–c**

To a solution of **7b** (0.25 g, 0.89 mmol) in absolute ethanol (5 ml), the appropriate alkyl amine (0.98 mmol) was added, and the mixture was stirred at R.T. for 3 h. The solvent was evaporated under vacuum and the residue was purified by column chromatography using silica gel as stationary phase and chloroform:ethanol (5.5:4.5) as elution system.

3.1.7.1. 5-Ethyl-1-imino-2-methyl-6-thioxo-1,2,8,9,10,10a-hexahydro-5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine **8a**.

Reddish brown solid, 0.08 g, 32% yield, m.p. 114–115 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3370 (NH stretching), 2923–2853 (CH aliphatic), 1638 (C=N), 1565–1461 (C=C), 1250 (C=S). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.28 (t, J = 6.9 Hz, 3H, NCH_2CH_3), 1.91–2.03 (m, 3H, pyrrolidine), 2.90–2.96 (m, 1H, pyrrolidine), 3.21 (br.s, 1H, NH exchanged with D_2O), 3.52 (s, 3H, NCH_3), 3.73–3.80 (m, 1H, pyrrolidine), 4.20–4.28 (m, 1H, pyrrolidine), 4.45–4.56 (m, 3H, 1H pyrrolidine + NCH_2CH_3), 7.81 (s, 1H, N=CH). MS: *m*/*z* (%): 263 (M^+, 90.07), 234 (100). Anal. Calcd. for $C_{12}H_{17}N_5S$ (263.36): C, 54.73; H, 6.51; N, 26.59. Found: C, 55.18; H, 7.03; N, 26.39.

3.1.7.2. 2,5-Diethyl-1-imino-6-thioxo-1,2,8,9,10,10a-hexahydro-5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine **8b**.

Reddish brown solid, 0.09 g, 36% yield, m.p. 125–127 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3370 (NH stretching), 2923–2853 (CH aliphatic), 1638 (C=N), 1575–1463 (C=C), 1251 (C=S). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.29 (t, J = 6.5 Hz, 3H, NCH_2CH_3), 1.45 (t, J = 7.2 Hz, 3H, NCH_2CH_3), 1.86–2.03 (m, 3H, pyrrolidine), 2.93–3.00 (m, 1H, pyrrolidine), 3.70–3.80 (m, 1H, pyrrolidine), 4.05–4.26 (m, 4H, 1H pyrrolidine + NCH_2CH_3 + NH, exchanged with D_2O), 4.47–4.57(m, 3H, 1H pyrrolidine + NCH_2CH_3), 7.84 (s, 1H, N=CH). Anal. Calcd. for $C_{13}H_{19}N_5S$ (277.39): C, 56.29; H, 6.90; N, 25.25. Found: C, 56.18; H, 6.89; N, 25.13.

3.1.7.3. 5-Ethyl-1-imino-2-propyl-6-thioxo-1,2,8,9,10,10a-hexahydro-5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine **8c**.

Brown solid, 0.10 g, 40% yield, m.p. 137–139 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3361 (NH stretching), 2963–2873 (CH aliphatic), 1637 (C=N), 1569–1472 (C=C), 1254 (C=S). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.98 (t, J = 7.5 Hz, 3H, $NCH_2CH_2CH_3$), 1.25 (t, J = 6.6 Hz, 3H, NCH_2CH_3), 1.76–1.97 (m, 5H, 3H pyrrolidine + $NCH_2CH_2CH_3$), 2.90–2.92 (m, 1H, pyrrolidine), 3.65–3.95 (m, 3H, 1H pyrrolidine + $NCH_2CH_2CH_3$), 4.16–4.20 (m, 1H, pyrrolidine), 4.42–4.50 (m, 3H, 1H pyrrolidine + NCH_2CH_3), 5.40 (br.s,1H, NH, exchanged with D_2O), 7.74 (s, 1H, N=CH). Anal. Calcd. for $C_{14}H_{21}N_5S$ (291.42): C, 57.70; H, 7.26; N, 24.03. Found: C, 58.16; H, 6.70; N, 23.65.

3.1.8. General procedure for the preparation of **9a–g**

A mixture of **4a** or **4b** (2 mmol) and an appropriate aldehyde (2.5 mmol) in glacial acetic acid (5 ml) was heated for 5 h, the solvent was removed under vacuum and the residue was crystallized from ethanol to produce the pure compounds **9 a–g**.

3.1.8.1. 3,5-Diphenyl-3,4,8,9,10,10a-hexahydro-2H,5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-1,6-dione **9a**.

White solid, 0.38 g, 54% yield, m.p. 236–237 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3392 (NH stretching), 3061 (CH aromatic), 2977–2882 (CH aliphatic), 1725 (C=O), 1680 (C=O), 1613–1492 (C=C). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 1.88–2.64 (m, 4H, pyrrolidine), 3.37–3.78 (m, 2H, pyrrolidine), 4.27–4.36 (m, 1H, pyrrolidine), 4.62–4.69 (m, 1H, NH*CH*NH), 4.79 (s, 1H, NH exchanged with D_2O), 4.85 (s, 1H, NH exchanged with D_2O), 7.19–7.74 (m, 10H, aromatic protons). Anal. Calcd. for $C_{21}H_{20}N_4O_2$ (360.42): C, 69.98; H, 5.59; N, 15.54. Found: C, 69.69; H, 5.70; N, 15.44.

3.1.8.2. 3-(4-Chlorophenyl)-5-phenyl-3,4,8,9,10,10a-hexahydro-2H,-5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-1,6-dione ***9b***. White solid, 0.30 g, 37% yield, m.p. 255–256 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3389 (NH stretching), 3061 (CH aromatic), 2978–2880 (CH aliphatic), 1725 (C=O), 1680 (C=O), 1593–1493 (C=C). ^{1}H NMR (DMSO-$\mathbf{d}_6$, 200 MHz): δ 1.88–2.37 (m, 4H, pyrrolidine), 3.47–3.51 (m, 2H, pyrrolidine), 4.32–4.34 (m, 2H, 1H pyrrolidine + NH*CH*NH), 4.81 (s, 1H, NH exchanged with D_2O), 4.88 (s, 1H, NH exchanged with D_2O), 7.20–7.65 (m, 9H, aromatic protons). Anal. Calcd. for $C_{21}H_{19}ClN_4O_2.H_2O$ (412.88): C, 61.09; H, 5.13; N, 13.57. Found: C, 60.86; H, 5.05; N, 13.66.

3.1.8.3. 3-(4-Methoxyphenyl)-5-phenyl-3,4,8,9,10,10a-hexahydro-2H,5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-1,6-dione ***9c***. White solid, 0.27 g, 35% yield, m.p. 255–256 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3389 (NH stretching), 3061(CH aromatic), 2978–2880 (CH aliphatic), 1725 (C=O), 1680 (C=O), 1593–1493 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.88–2.38 (m, 4H, pyrrolidine), 3.40–3.60 (m, 5H, 2H pyrrolidine + OCH_3), 4.23–4.30 (m, 2H, 1H pyrrolidine + NH*CH*NH), 4.78 (s, 1H, NH exchanged with D_2O), 4.82 (s, 1H, NH exchanged with D_2O), 7.18–7.46 (m, 9H, aromatic protons). Anal. Calcd. for $C_{22}H_{22}N_4O_3$ (390.45): C, 67.68; H, 5.68; N, 14.35. Found: C, 67.79; H, 5.33; N, 14.32.

3.1.8.4. 5-Ethyl-3-(4-methoxyphenyl)-6-thioxo-3,4,8,9,10,10a-hexahydro-2H,5H-pyrimido[5,4-e]pyrrolo[1,2-c] pyrimidin-1-one ***9d***. Yellow solid, 0.25 g, 35% yield, m.p. 123–125 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3402 (NH stretching), 3069 (CH aromatic), 2972–2843 (CH aliphatic), 1712 (C=O), 1500–1447 (C=C), 1255 (C=S). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.21 (t, $J = 6.8$ Hz, 3H, $NCH_2\underline{CH_3}$), 1.93–2.21 (m, 3H, pyrrolidine), 2.55–2.63 (m, 1H, pyrrolidine), 3.63 (s, 1H, NH exchanged with D_2O), 3.70 (s, 1H, NH exchanged with D_2O), 3.82–4.36 (m, 9H, 3H pyrrolidine + NH*CH*NH + OCH_3 + NCH_2CH_3), 6.97–7.46 (m, 2H, aromatic protons), 8.04–8.20 (m, 2H, aromatic protons). MS: *m*/*z* (%): 357 ($M-1^+$, 9.92), 355 (100). Anal. Calcd. for $C_{18}H_{22}N_4O_2S$ (358.47): C, 60.31; H, 6.19; N, 15.63. Found: C, 60.36; H, 6.21; N, 15.24.

3.1.8.5. 5-Ethyl-3-(3-hydroxy-4-methoxyphenyl)- 6-thioxo-3,4,8,9,-10,10a-hexahydro-2H, 5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-1-one ***9e***. Yellow solid, 0.21 g, 28% yield, m.p. 126–128 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3504 (OH stretching), 3374 (NH stretching), 3050 (CH aromatic), 2972–2882 (CH aliphatic), 1699 (C=O), 1563–1492 (C=C), 1254 (C=S). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.24 (t, $J = 6.9$ Hz, 3H, NCH_2CH_3), 1.91–2.64 (m, 4H, pyrrolidine), 3.62 (s, 1H, NH exchanged with D_2O), 3.67 (s, 1H, NH exchanged with D_2O), 3.80–4.11 (m, 5H, 2H pyrrolidine + OCH_3), 4.21–4.41 (m, 4H, 1H pyrrolidine + NH*CH*NH + NCH_2CH_3), 6.05 (s, 1H, OH exchanged with D_2O), 7.00–7.20 (m, 2H, aromatic protons), 8.20 (s, 1H, aromatic proton). Anal. Calcd. for $C_{18}H_{22}N_4O_3S$ (374.47): C, 57.74; H, 5.92; N, 14.96. Found: C, 57.82; H, 5.78; N, 14.68.

3.1.8.6. 3-(2-Nitrophenyl)-5-phenyl-3,4,8,9,10,10a-hexahydro-2H,5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-1,6-dione ***9f***. Buff solid, 0.35 g, 45% yield, m.p. 242–244 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3388 (NH stretching), 3062 (CH aromatic), 2978–2881(CH aliphatic), 1724 (C=O), 1681 (C=O), 1596–1493 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.86–2.34 (m, 4H, pyrrolidine), 3.50–3.52 (m, 2H, pyrrolidine), 4.30–4.38 (m, 2H, 1H pyrrolidine + NH*CH*NH), 4.79 (s, 1H, NH exchanged with D_2O), 4.84 (s, 1H, NH exchanged with D_2O), 7.19–7.47 (m, 9H, aromatic protons). ^{13}C NMR (DMSO-d_6, 75.45 MHz): 22.27, 31.15, 40.71 (pyrrolidine carbons), 46.36 (NH*CH*NH), 53.20 (pyrrolidine), 114.86 (O=C–*C*=C), 128.05, 128.10, 128.61, 128.78, 128.95 (aromatic carbons), 135.61 (O=C–C=*C*–NH), 149.68 (N–(*C*O)–N), 163.30 (C–*C*O–NH). Anal. Calcd. for $C_{21}H_{19}N_5O_4$ (405.42): C, 62.22; H, 4.72; N, 17.27. Found: C, 62.00; H, 4.78; N, 17.29.

3.1.8.7. 3-(4-Nitrophenyl)-5-phenyl-3,4,8,9,10,10a-hexahydro-2H,5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-1,6-dione ***9g***. Orange red solid, 0.62 g, 78% yield, m.p. 245–246 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3392 (NH stretching), 3063 (CH aromatic), 2978–2881 (CH aliphatic), 1725 (C=O), 1681 (C=O) 1596–1493 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.86–2.49 (m, 4H, pyrrolidine), 3.43–3.52 (m, 2H, pyrrolidine), 4.31–4.42 (m, 2H, 1H pyrrolidine + NH*CH*NH), 4.79 (s, 1H, NH exchanged with D_2O), 4.83 (s, 1H, NH exchanged with D_2O), 7.15–7.48 (m, 9H, aromatic protons). MS: *m*/*z* (%): 403 ($M-2^+$, 4.41), 401 (100), 283 (12.36). Anal. Calcd. for $C_{21}H_{19}N_5O_4$ (405.42): C, 62.22; H, 4.72; N, 17.27. Found: C, 62.19; H, 4.74; N, 17.28.

3.1.9. 3,5-Diphenyl-1-propyl-8,9,10,10a-tetrahydro-5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidin-6-one ***13a***

A mixture of **12** (0.5 g, 1.32 mmol) and an ethanolic solution of propylamine (5 ml) was refluxed for 5 h, ethanol was removed under vacuum and the residue was crystallized from ethanol/water to give dark brown solid, 0.33 g, 63% yield, mp 102 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3421 (NH stretching), 3061 (CH aromatic), 2957–2852 (CH aliphatic), 1676 (CO stretching), 1657 (NH bending), 1591–1545 (C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.05 (t, J = 7.4 Hz, 3H, $NCH_2CH_2CH_3$), 1.65–2.15 (m, 6H, 3H pyrrolidine + $NCH_2CH_2CH_3$ + NH exchanged with D_2O), 2.55–2.60 (m, 1H, pyrrolidine), 3.40–3.50 (m, 1H, pyrrolidine), 3.55–3.75 (m, 2H, $NCH_2CH_2CH_3$), 4.00–4.10 (m, 1H, pyrrolidine), 4.54–4.60 (m, 1H, pyrrolidine), 7.27–8.08 (m, 10H, aromatic protons). Anal. Calcd. for $C_{24}H_{25}N_5O$ (399.50): C, 72.16; H, 6.31; N, 17.53. Found: C, 71.61; H, 6.34; N, 17.36.

3.1.10. General procedure for the preparation of ***13b, c and 14***

To the mixture of **12** (0.5 g, 1.32 mmol) and anhydrous potassium carbonate (0.23 g, 1.66 mmol) in dry DMF (5 ml) equimolar amount (1.32 mmol) of the corresponding amine was added. The reaction mixture was heated at 100 °C for 7 h, cooled to RT, and poured on ice/water (30 ml). The separated solid was filtered, washed with water and recrystallized from ethanol.

3.1.10.1. 3,5-Diphenyl-1-pyrrolidin-1-yl-8,9,10,10a-tetrahydro-5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidin-6-one ***13b***. Buff solid, 0.38 g, 70% yield, m.p. 205–206 °C (decomposition). IR: $\upsilon_{max.}/cm^{-1}$ 3054 (CH aromatic), 2948–2847 (CH aliphatic), 1678 (CO stretching), 1587–1539 (C=C). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.60–2.15 (m, 7H, 3H pyrrolidine + 4H pyrrolidinyl), 2.40–2.50 (m, 1H, pyrrolidine), 3.40–3.50 (m, 3H, 1H pyrrolidine + 2H pyrrolidinyl), 3.75–3.85 (m, 2H pyrrolidinyl), 4.05–4.20 (m, 1H, pyrrolidine), 4.65–4.80 (m, 1H, pyrrolidine), 7.26–8.08 (m, 10H, aromatic protons). MS: *m*/*z*, (%): 411 (M^+, 100), 383 (53.06), 368 (74.46), 355 (27.07). Anal. Calcd. for $C_{25}H_{25}N_5O$ (411.51): C, 72.97; H, 6.12; N, 17.02. Found: C, 72.55; H, 6.02; N, 17.17.

3.1.10.2. 1-Morpholin-4-yl-3,5-diphenyl-8,9,10,10a-tetrahydro-5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidin-6-one ***13c***. Greyish brown solid, 0.35 g, 63% yield, m.p. 179–180 °C (decomposition). UV ($CHCl_3$), Conc. 3.50×10^{-5} M, nm: 225 (log ε: 3.71), 258 (log ε: 4.43). IR: $\upsilon_{max.}/cm^{-1}$ 3061 (CH aromatic), 2954–2851 (CH aliphatic), 1686 (CO stretching), 1563–1545 (C=C). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.80–2.00 (m, 3H, pyrrolidine), 2.60–2.70 (m, 2H, pyrrolidine), 3.16–3.20 (m, 2H, morpholino NCH_2), 3.42–3.50 (m, 3H, 1H pyrrolidine + morpholino NCH_2), 3.75–3.90 (m, 4H, morpholino OCH_2), 4.60–4.70 (m, 1H, pyrrolidine), 7.18–7.97 (m, 10H, aromatic protons). Anal. Calcd. for $C_{25}H_{25}N_5O_2$ (427.51): C, 70.24; H, 5.89; N, 16.38. Found: C, 69.71; H, 5.62; N, 16.52.

3.1.10.3. 5,7-Diphenyl-2,7,10,11,12,12a-hexahydropyrrolo[1,2-c]imidazo[1′,2′:1,6]pyrimido[5,4-e]pyrimidine-3,8-dione ***14***. Light brown solid, 0.29 g, 53% yield, m.p. 230 °C (decomposition). IR: $\upsilon_{max.}/cm^{-1}$ 3063 (CH aromatic), 2921–2851 (CH aliphatic), 1655 (2 CO), 1595–1507 (C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.70–2.15 (m, 3H, pyrrolidine), 2.65–2.75 (m, 1H, pyrrolidine), 3.03 (s, 2H, CH_2) 3.35–3.45 (m, 1H, pyrrolidine), 3.90–3.95 (m, 1H, pyrrolidine), 4.75–4.80 (m, 1H, pyrrolidine), 7.27–8.09 (m, 10H, aromatic protons). MS: *m/z* (%) = 397.24 (M^+, 1.95), 357.10 (100%). Anal. Calcd. for $C_{23}H_{19}N_5O_2$ (397.43): C, 69.51; H, 4.82; N, 17.62. Found: C, 69.32; H, 5.31; N, 17.92.

3.1.11. General procedure for the preparation of ***17*** *and* ***18a, b***

To a solution of **16** (0.37 g, 1 mmol) in dry DMF (5 ml) was added acetylacetone (10 mmol), ethoxymethylene malononitrile (0.12 g, 1 mmol) or ethyl ethoxymethylene cyanoacetate (0.16 g, 1 mmol). The solution was heated at 100 °C for 8 h, cooled to R.T., and poured on ice water (20 ml). The separated solid was filtered, washed with ethanol to produce **17**, **18a** or **18b**, respectively, in a pure form.

3.1.11.1. 1-(3,5-Dimethyl-pyrazol-1-yl)–3,5–diphenyl-8,9,10,10a-tetrahydro-5H-pyrimido[5,4-e]pyrrolo[1,2-c]pyrimidin-6-one ***17***. Dark brown solid, 0.34 g, 79% yield, m.p. 201–203 °C. UV ($CHCl_3$), Conc. 2.29×10^{-5} M, nm: 228.5 (log ε: 3.89), 260 (log ε: 4.61), 303 (log ε: 4.14). IR: $\upsilon_{max.}/cm^{-1}$ 3062 (CH aromatic), 2923–2853 (CH aliphatic), 1686 (CO stretching), 1583–1554 (C=C). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.46–1.87 (m, 4H, pyrrolidine), 2.30 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 3.42–3.55 (m, 1H, pyrrolidine), 3.80–3.90 (m, 1H, pyrrolidine), 5.15–5.22 (m, 1H, pyrrolidine), 6.07 (s, 1H, pyrazole), 7.26–8.05 (m, 10H, aromatic protons). MS: *m/z*, (%): 436 (M^+, 26.70), 421 (9.42), 394 (100), 329 (21.80). Anal. Calcd. for $C_{26}H_{24}N_6O.H_2O$ (454.53): C, 68.71; H, 5.77; N, 18.49. Found: C, 68.81; H, 5.79; N, 18.72.

3.1.11.2. 5-Amino-1-(6-oxo-3,5-diphenyl-5,6,8,9,10,10a-hexahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidin-1-yl)-1H-pyrazole-4-carbonitrile ***18a***. Brown solid, 0.31 g, 70% yield, m.p. 328–329 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3430, 3259 (NH_2 stretching), 3064 (CH aromatic), 2922–2852 (CH aliphatic), 2215 (CN), 1664 (CO stretching), 1607 (NH bending), 1550–1512 (C = C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.60–1.95 (m, 4H, pyrrolidine), 3.30–3.40 (m, 1H, pyrrolidine), 3.65–3.70 (m, 1H, pyrrolidine), 4.95–5.05 (m, 1H, pyrrolidine), 7.14 (s, 2H, NH_2 exchanged with D_2O), 7.34–7.93 (m, 11H, 10 aromatic protons + 1H pyrazole). Anal. Calcd. for $C_{25}H_{20}N_8O$ (448.49): C, 66.95; H, 4.49; N, 24.98. Found: C, 66.77; H, 4.95; N, 24.90.

3.1.11.3. 5-Amino-1-(6-oxo-3,5-diphenyl-5,6,8,9,10,10a-hexahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidin-1-yl)-1H-pyrazole-4-carboxylic acid ethyl ester ***18b***. Buff solid, 0.35 g, 70% yield, m.p. 238–239 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3451, 3343 (NH_2 stretching), 3061 (CH aromatic), 2920–2850 (CH aliphatic), 1690 (CO stretching), 1608 (NH bending), 1581–1542(C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.31 (t, J = 7.2 Hz, 3H, $COOCH_2CH_3$), 1.60–2.25 (m, 4H, pyrrolidine), 3.35–3.45 (m, 1H, pyrrolidine), 3.85–4.00 (m, 1H, pyrrolidine), 4.26 (q, J = 7.2 Hz, 2H, $COOCH_2CH_3$), 5.20–5.30 (m, 1H, pyrrolidine), 6.75 (s, 2H, NH_2 exchanged with D_2O), 7.18–7.85 (m, 11H, 10 aromatic protons + 1H pyrazole). ^{13}C NMR ($CDCl_3$, 75.45 MHz): 14.29 (CH_2CH_3), 20.83, 29.39, 45.15, 55.30 (pyrrolidine carbons), 59.58 (CH_2CH_3), 95.58 (C=C–NH_2), 104.06 (C=C), 125.76, 127.66, 128.30, 128.63, 129.36, 131.05, 135.75, 136.53 (aromatic carbons), 141.38 (pyrazole carbon), 150.33 (N–(CO)–N), 152.25 (C–NH_2), 154.65 (C-phenyl), 159.48 (C=O ester), 161.47 (C=C), 163.79 (C-pyrazole). MS: *m/z*, (%): 495 (M^+, 100), 467 (96.82), 421 (45.96), 354 (75.71), 329 (58.48). Anal. Calcd. for $C_{27}H_{25}N_7O_3$ (495.55): C, 65.44; H, 5.09; N, 19.79. Found: C, 65.45; H, 5.56; N, 19.11.

3.1.12. General method for the preparation of ***19***, ***21***, *and* ***23***

A mixture of **16** (0.37 g, 1 mmol), *p*-toluenesulphonyl chloride, ethyl chloroformate or chloroacetyl chloride (1.25 mmol) and triethylamine (1 mmol) in dry benzene (5 ml) was refluxed for 6 h; the solvent was removed under vacuum. The residue was triturated with water (15 ml) and filtered, washed with water and crystallized from ethanol.

3.1.12.1. 3,5-Diphenyl-1-p-tolylsulphonylhydrazino-8,9,10,10a-tetrahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidin-6-one ***19***. Pink solid, 0.37 g, 72% yield, m.p. 145–147 °C (decomposition). IR: $\upsilon_{max.}/cm^{-1}$ 3333 (NH stretching), 3063 (CH aromatic), 2923–2852 (CH aliphatic), 1664 (C=O), 1589–1567 (C=C), 1321, 1152 (SO_2 stretching). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.70–2.05 (m, 3H, pyrrolidine), 2.16 (s, 3H, CH_3), 2.70–2.80 (m, 1H, pyrrolidine), 3.45–3.50 (m, 1H, pyrrolidine), 3.97–4.05 (m, 1H, pyrrolidine), 4.52–4.57 (m, 1H, pyrrolidine), 6.80 (s, NH exchanged with D_2O), 7.01 (d, J = 8.1 Hz, 2H, *o*-CH3), 7.20–7.48 (m, 8H, aromatic protons), 7.73–7.80 (m, 4H, aromatic protons), 8.05 (s, NH exchanged with D_2O). MS: *m/z* (%): 526 (M^+, 1.64), 341 (100). Anal. Calcd. for $C_{28}H_{26}N_6O_3S$ (526.55): C, 63.87; H, 4.98; N, 15.96. Found: C, 64.12; H, 4.67; N, 16.14.

3.1.12.2. N′-(3,5-Diphenyl-8,9,10,10a-tetrahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidin-6-one-1-yl)-ethoxyhydrazide ***21***. Yellow solid, 0.39 g, 89% yield, m.p. 226–228 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3358, 3328 (NH stretching), 3059 (CH aromatic), 2922–2852 (CH aliphatic), 1748 (C=O ester), 1645 (C=O), 1591–1562 (C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.31 (t, J = 7.2 Hz, 3H, OCH_2CH_3), 2.00–2.20 (m, 3H, pyrrolidine), 2.60–2.70 (m, 1H, pyrrolidine), 3.40–3.50 (m, 1H, pyrrolidine), 3.95–4.05 (m, 1H, pyrrolidine), 4.25 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 4.65–4.75 (m, 1H, pyrrolidine), 6.50 (s, 1H, NH exchanged with D_2O), 6.85 (s, 1H, NH exchanged with D_2O), 7.27–8.02 (m, 10H, aromatic protons). MS: *m/z* (%): 444 (M^+, 28.67), 342 (100). Anal. Calcd. for $C_{24}H_{24}N_6O_3$ (444.49): C, 64.85; H, 5.44; N, 18.91. Found: C, 64.81; H, 5.54; N, 18.68.

3.1.12.3. 6,8-Diphenyl-11,12,13,13a-tetrahydro-2H-pyrrolo[1,2-c][1,2,4]triazino[4′,3′:1,6]pyrimido[5,4-e]pyrimidine-3,8-dione ***23***. Grey solid, 0.33 g, 80% yield, m.p. 230 °C (decomposition). IR: $\upsilon_{max.}/cm^{-1}$ 3214 (NH stretching), 3063 (CH aromatic), 2924–2853 (CH aliphatic), 1674 (2 C=O), 1577–1548 (C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.95–2.30 (m, 3H, pyrrolidine), 2.50–2.60 (m, 1H, pyrrolidine), 3.50–3.60 (m, 1H, pyrrolidine), 3.75–3.85 (m, 1H, pyrrolidine), 4.04 (s, 2H, CH_2), 4.79–4.83 (m, 1H, pyrrolidine), 7.27–7.98 (m, 10H, aromatic protons), 9.60 (s, 1H, NH exchanged with D_2O). ^{13}C NMR ($CDCl_3$, 75.45 MHz): 21.93, 29.65, 41.12 (pyrrolidine carbons), 45.22 (CH_2), 55.26 (pyrrolidine carbon), 120.94 (*C*=C–N), 125.89, 126.18, 127.92, 128.08, 128.40, 128.89, 129.57, 131.26 (aromatic carbons), 136.59 (C=*C*–N and N–C=N), 150.84 (N–*C*O–N), 159.78 ($NHCOCH_2$), 172.06 (NH–N=*C*–N). Anal. Calcd. for $C_{23}H_{20}N_6O_2$ (412.44): C, 66.98; H, 4.89; N, 20.38. Found: C, 66.52; H, 5.22; N, 20.51.

3.1.13. General procedure for the preparation of ***20a, b***

A mixture of **16** (0.50 g, 1.34 mmol) and the corresponding acid chloride (1.34 mmol) in dry pyridine (5 ml) was stirred at RT for 6 h. The reaction mixture was poured on ice/water (40 ml). The separated solid was filtered, washed with water and crystallized from ethanol.

3.1.13.1. N′-(3,5-Diphenyl-8,9,10,10a-tetrahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidin-6-one-1-yl)benzoic acid hydrazide ***20a***. Buff

solid, 0.50 g, 78% yield, m.p. 230–232 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3276 (NH stretching), 3063 (CH aromatic), 2923–2852 (CH aliphatic), 1669 (2C=O), 1591–1567 (C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.59 (s, 1H, NH exchanged with D_2O), 1.97–2.17 (m, 3H, pyrrolidine), 2.80–2.84 (m, 1H, pyrrolidine), 3.48–3.54 (m, 1H, pyrrolidine), 3.97–4.06 (m, 1H, pyrrolidine), 4.71–4.74 (m, 1H, pyrrolidine), 7.23–7.99 (m, 15H, aromatic protons), 9.26 (s, 1H, NH exchanged with D_2O). ^{13}CNMR ($CDCl_3$, 75.45 MHz): 21.72, 29.67, 45.05, 54.06 (pyrrolidine carbons), 93.11 (*C*=C), 125.99, 127.67, 128.15, 128.65, 128.93, 129.76, 130.61, 132.32, 136.90 (aromatic carbons), 151.40 (N–(*C*O)–N), 156.50 (*C*-phenyl), 157.59 (C=O benzoyl), 161.99 (C=*C*), 164.89 (*C*–NH). MS: *m*/*z* (%): 476.25 (M^+, 3.75), 356.20 (100). Anal. Calcd. for $C_{28}H_{24}N_6O_2$ (476.54): C, 70.57; H, 5.08; N, 17.64. Found: C, 70.22; H, 5.58; N, 17.42.

3.1.13.2. N′-(3,5-diphenyl-8,9,10,10a-tetrahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidin-6-one-1-yl) nicotinic acid hydrazide ***20b***. Reddish brown solid, 0.48 g, 75% yield, m.p. 245–246 °C. UV ($CHCl_3$), Conc. 2.09×10^{-5} M, nm: 227.5 (log ε: 4.02), 257 (log ε: 4.40), 308 (log ε: 3.93). IR: $\upsilon_{max.}/cm^{-1}$ 3415–3271 (NH stretching), 3060 (CH aromatic), 2923–2853(CH aliphatic), 1660 (2C=O), 1590–1565 (C=C). ^{1}H NMR ($CDCl_3$–300 MHz): δ 1.90–2.20 (m, 4H, 3H pyrrolidine + NH exchanged with D_2O), 2.55 (br.s., 1H, pyrrolidine), 3.48–3.52 (m, 1H, pyrrolidine), 3.75–3.85 (m, 1H, pyrrolidine), 4.80 (br.s., 1H, pyrrolidine), 7.20–7.50 (m, 10H, aromatic protons), 7.85 (br.s., 1H, C5′), 8.12 (br.s., 1H, C4′), 8.74 (br.s., 1H, C6′), 9.04 (br.s., 1H, C2′), 10.50 (br.s., 1H, NH amide exchanged with D_2O). Anal. Calcd. for $C_{27}H_{23}N_7O_2$ (477.53): C, 67.91; H, 4.85; N, 20.53. Found: C, 68.14; H, 4.96; N, 20.77.

*3.1.14. 5,7-Diphenyl-10,11,12,12a-tetrahydro-2*H*-pyrrolo[1,2-c][1,2,4]triazolo[4′,3′:1,6]pyrimido[5,4-e]pyrimidine-3,8-dione* ***22***

To a mixture of **16** (0.37 g, 1 mmol) and sodium methoxide (0.05 g, 1 mmol) in methanol (5 ml) was added ethyl chloroformate (1.25 mmol), the reaction was refluxed for 12 h, and the solvent was removed under vacuum. The residue was triturated with water (15 ml) and filtered, washed with water and purified by column chromatography using silica gel as stationary phase and chloroform as mobile phase to give brown solid, 0.32 g, 80% yield, m.p. 182–183 °C. This compound was also obtained by heating a solution of **21** in dry DMF for 12 h then poured on ice water. The obtained solid was filtered, washed with water and crystallized from ethanol to give reddish brown solid, m.p. 180–182 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3420 (NH stretching), 3053–3018 (CH aromatic), 2926–2852 (CH aliphatic), 1692 (2C=O), 1586–1556 (C=C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.90 (br.s., 1H, NH exchanged with D_2O), 2.07–2.12 (m, 3H, pyrrolidine), 2.65–2.75 (m, 1H, pyrrolidine), 3.60–3.67 (m, 1H, pyrrolidine), 3.70–3.77 (m, 1H, pyrrolidine), 4.70–4.80 (m, 1H, pyrrolidine), 7.26–8.11 (m, 10H, aromatic protons). MS: *m*/*z* (%): 398 (M^+, 11.1), 341 (17.8), 77 (100). Anal. Calcd. for $C_{22}H_{18}N_6O_2$ (398.42): C, 66.32; H, 4.55; N, 21.09. Found: C, 66.52; H, 4.52; N, 21.26.

3.2. Single crystal X-ray crystallographic data of *18a*

Compound **18a** was recrystallized as brown cubic crystals from ethanol. The crystallographic data were collected at $T = 298$ K on a Kappa CCD Enraf Nonius FR 590 diffractometer using a graphite monochromator with Mo-K_α radiation ($\lambda = 0.71073$ Å). The crystal structure was determined by SIR92 [48] and refined by maXus [49] (Bruker Nonius, Delft and MacScience, Japan). Chemical formula $C_{25}H_{20}N_8O$, $M_r = 448.490$, monoclinic, crystallizes in space group $P2_1/C$; cell lengths "$a = 10.2720$ (4), $b = 20.8375$ (12), $c = 12.3140$ (9) Å"; cell angles "$\alpha = 90.00$, $\beta = 13.(18) \times 10^{10}$, $\gamma = 90.00°$, $V = 2169.8$ (2) Å^3, $Z = 4$, $D_c = 1.373$ mg/m^3, θ values 2.910–21.726°, absorption coefficient μ (Mo-K_α) = 0.09 mm^{-1}, $F(000) = 720$. The unique reflections measured 4428 of which 1747 reflections with threshold expression $I > 3\sigma(I)$ were used in the structural analysis. Convergence for 307 variable parameters by least-squares refinement on F^2 with $w = 1/[\sigma^2(F_o^2) + 0.10000\,F_o^2]$. The final agreement factors were $R = 0.045$ and $wR = 0.087$ with a goodness-of-fit of 1.422.

3.3. Pharmacological studies

3.3.1. Animals

The albino rats and mice were obtained from the animal house of National Research Center, Dokki, Cairo. The animals were acclimatized to the lab conditions for two days with free access to the food and water. On the day before the experiment, the food was withdrawn, but the animals were allowed free access to water.

3.3.2. Preparation of test samples

All compounds in 0.029 mmol/kg concentration were suspended in saline solution with the aid of few drops of tween 80, and these test samples were given orally to the animals by the oral stomach tube. The control group animals received the same experimental handling as those of the test groups except for the drug treatment. Indomethacin (10 mg/kg, 0.029 mmol) was used as a reference drug.

3.3.3. Analgesic activity

Analgesic activity was measured by the acetic acid-induced writhing test in mice [36–39]. Six albino mice of either sex (18–22 g) were used in each group. One hour after oral administration of a suspension of 0.029 mmol/kg of the test compounds or indomethacin, each mouse was injected with 0.3 ml of 1% acetic acid solution intraperitoneally. Starting 5 min after the acetic acid injection, the number of muscular contractions in each mouse was counted for a period of 30 min. A significant reduction in the number of writhings by any test compound as compared to control animals was considered as a positive analgesic response. Percentage protection was calculated using the following formula, where n is the average number of writhings in control group and n' is average number of writhings in treated group.

$$\%\ \text{Protection} = [(n - n')] \times 100$$

3.3.4. Anti-inflammatory activity

Anti-inflammatory activity screening was carried out using carrageenan-induced paw edema in albino rats [40–43]. Adult albino rats of either sex weighing 120–150 g were divided into 38 groups of 6 animals each. One hour after the oral administration of the test compounds or the reference drug (indomethacin), each rat was injected with freshly prepared suspension of carrageenan (0.1 ml of 1%, Fluka) in saline into subplantar tissue of the right hind paw. The thickness of the paw was measured at successive time intervals (1, 2, 3 and 4 h) after induction of the inflammation and compared with the initial hind paw thickness of each rat for determining the edema thickness. The anti-inflammatory activity was expressed as percentage inhibition of edema and was calculated by the following equation:

$$\%\ \text{Inhibition} = 100 \times [\text{Vc} - \text{Vt}/\text{Vc}]$$

where Vc is the mean of paw thickness of rats after administration of carrageenan in the positive control group, Vt is the mean of paw thickness of rats after administration of the tested compounds or the reference drug. Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods.

Potency of the tested compounds was calculated relative to indomethacin (reference standard) treated group according to the following equation:

$$\text{Potency} = \frac{\%\,\text{inhibition of tested compound treated group}}{\%\,\text{inhibition of indamethacin treated group}}$$

3.3.5. Gastric–ulcerogenic effect

Five hours after the oral treatment of rats with the tested compounds for anti-inflammatory activity tests, they were killed under deep ether anaesthesia and their stomachs were removed. Then the stomach of each rat was opened through great curvature and examined for lesions or bleedings.

3.3.6. Statistical analysis of data

Data obtained from animal experiments were expressed as mean ± standard error (±SEM). Statistical differences between the treatments and the control were tested by one-way analysis of variance (ANOVA) followed by post hoc test using SPSS 11.0 software. A value of $p < 0.05$ was considered to be significant. IC 50 values were calculated by GraphPad Prism 3 software using non-linear regression, sigmoidal dose response (variable slope).

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