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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Potential anti-inflammatory activity and ulcerogenicity study of some novel pyrimido[4′,5′:4,5]pyrimido[1,6-*a*]azepine derivatives

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Abstract A series of novel tricyclic pyrimido[4′,5′:4,5]pyrimido[1,6-*a*]azepine derivatives were synthesized using the starting compound 3-amino-1-oxo-2-phenyl-5-(pyrrolidin-1-yl)-1,2,4a,5,6,7,8,9-octahydropyrimido[1,6-*a*]azepine-4-carbonitrile **4**. This series includes the 3-aryl derivatives **6a**, **b**, the 3-cycloaminoalkyl derivatives **8a–f**, the 3-mercaptomethyl derivatives **10** and **11a**, **b**, the 2-cycloaminomethyl derivatives **13a–c**, the 1-cycloamino derivatives **15a–c** and the 1-amino derivative **16**. The structures of the newly synthesized compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy and elemental analyses. The anti-inflammatory activity of all newly synthesized compounds was evaluated using the carrageenan-induced paw oedema test in rats using diclofenac sodium as the reference drug. Ulcer indices for the most active compounds were calculated. The 3-mercaptomethylacetic acid derivative **10** was the most active compound, showing activity comparable to diclofenac sodium with minimal ulcerogenic effect while the rest of the tested compound exhibited moderate anti-inflammatory activity.

Keywords Pyrimidopyrimidoazepine · Anti-inflammatory activity · Ulcerogenicity

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Introduction

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants (Ferrero-Miliani *et al.,* 2007). Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. On the other hand, chronic inflammation leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process (Lacerda *et al.,* 2009).

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain and fever. However, long term usage of NSAIDs is associated with significant side effects of gastrointestinal lesions, bleeding and nephrotoxicity (Alagarsamy *et al.,* 2007).

Therefore, there is an unrelenting effort to discover new and safe NSAIDs. In this field, several studies were reported on the nitrogen bridgehead condensed pyrimidine derivatives which unveil new potent anti-inflammatory agents belonging to the pyrimido[4′,5′:4,5]pyrimido[1,6-*a*]azepines **I–III** (Fig. 1) (Ebeid *et al.,* 1991; El-Sayed *et al.,* 1993, 2003). A later study revealed that the introduction of a morpholino group at position 12 of the tricyclic system afforded derivatives that were found markedly more potent than unsubstituted ones, as exemplified by compound **IV** and at the same time they are of minimal ulcerogenic potential (El-Sayed *et al.,* 2006). In continuation to our previous reports and for further exploration of

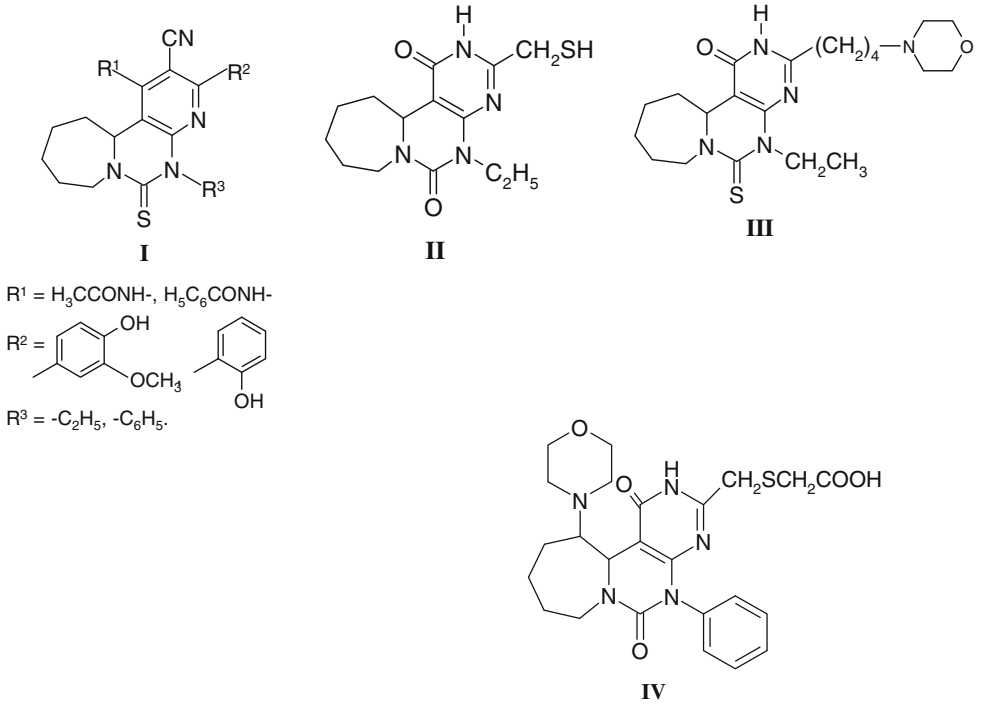
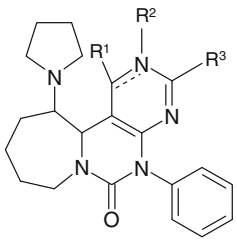


Fig. 1 Examples of pyrimido[4′,5′:4,5]pyrimido[1,6-*a*]azepine compounds with anti-inflammatory activity

this fused tricyclic ring system, it deemed of interest to prepare a set of new tricyclic pyrimido[4′,5′:4,5]pyrimido[1,6-*a*]azepine derivatives substituted with the more lipophilic pyrrolidine moiety at position 12 instead of the morpholino group to study the influence of this replacement on the anti-inflammatory activity. This approach of increasing lipophilicity by introducing the more lipophilic pyrrolidine ring has been recently explored by the authors and proved beneficial in improving the anti-inflammatory activity which urged us to continue in this direction (El-Sayed *et al.,* 2010). In addition, various substituents were introduced at positions 1, 2 or 3 of the pyrimido[4,5-*d*]pyrimido[1,6-*a*]azepine nucleus, which have been modified to evolve our target compounds (Fig. 2).

Fig. 2 General structure of the target compounds



Results and discussion

Chemistry

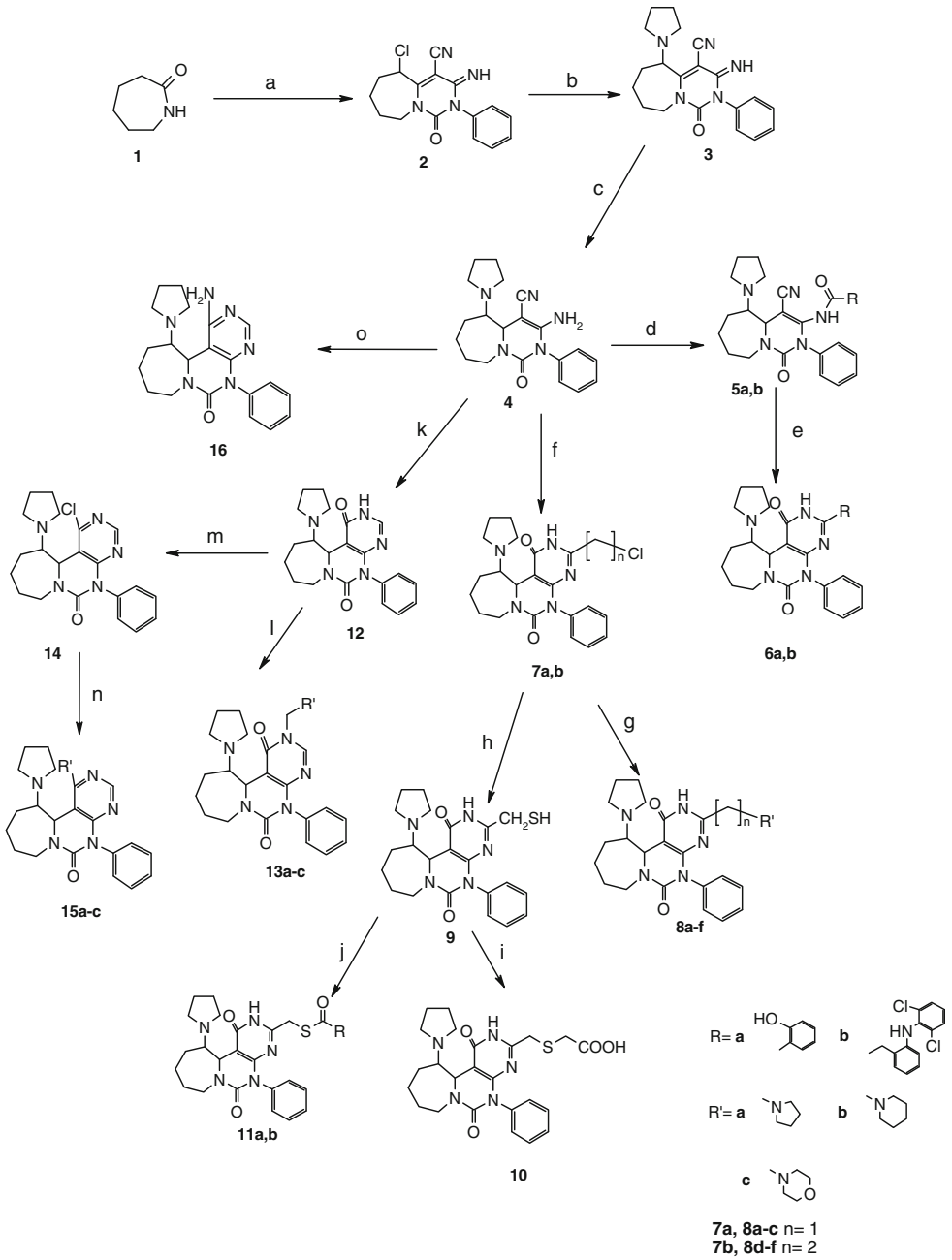
The synthetic pathways adopted for the preparation of the target compounds are illustrated in Scheme 1. The known chloropyrimidoazepine intermediate **2** was prepared according to the previously reported method from caprolactam **1** in several steps (Ebeid and Bitter, 1978; Ebeid *et al.,* 1995; El-Sayed and Hussein, 2003; El-Sayed *et al.,* 2006).

The 5-pyrrolidino derivative **3**, the key intermediate aminonitrile **4** and the amide derivatives **5a**, **b** were prepared as previously reported by the authors (El-Sayed *et al.,* 2010).

Refluxing of compounds **5a**, **b** in ethanolic hydrochloric acid gave the tricyclic compounds **6a**, **b**, respectively. IR spectra of the target compounds supported the postulated structure due to the disappearance of the nitrile absorption band.

Synthesis of compounds **7a**, **b** was achieved by refluxing the enaminonitrile derivative **4** with the appropriate chloroacyl chloride in dry benzene, using triethylamine as a catalyst. The data drawn from IR spectra of **7a** supported the postulated structure. Moreover, ^{1}H-NMR spectrum of **7a** revealed a singlet signal resonating at 4.35 ppm attributed to the two protons of the chloromethyl moiety. Also, ^{1}H-NMR of **7b** showed two triplets at 2.12 and 4.23 ppm assigned to the four protons of the chloroethyl moiety.

Condensation of the chloro derivatives **7a**, **b** with different cyclic secondary amines in absolute ethanol in the presence of anhydrous potassium carbonate resulted in the formation of compounds **8a**–**f**. Physical, elemental and



Scheme 1 Reagents and solvents: **a** *1* $(CH_3)_2SO_4$, *2* $CH_2(CN)_2$, *3* SO_2Cl_2, *4* PhNCO; **b** pyrrolidine in abs. ethanol; **c** $NaBH_4$ in abs. ethanol; **d** RCOCl, dry benzene, triethylamine; **e** ethanolic HCl; **f** $ClCO(CH_2)_nCl$, dry benzene, triethylamine; **g** appropriate amine, methylene chloride, anh. potassium carbonate; **h** thiourea, ethanol, 10% NaOH; **i** $ClCH_2COOH$, ethanol, KOH; **j** RCOCl, dry benzene, triethylamine; **k** 85% formic acid; **l** appropriate amine, HCHO, acetonitrile, acetic acid; **m** $POCl_3$; **n** appropriate amine, abs. ethanol, anh. potassium carbonate; **o** $HCONH_2$

spectral data were in accordance with the structure of the titled compounds.

The reaction of thiourea with the 3-chloromethyl derivative **7a** yielded the corresponding 3-mercaptomethyl derivative **9**. The IR spectral analysis of compound **9** revealed a stretching band of the SH group, weakly absorbing around at 2455 cm^{-1}. In addition, its ^{1}H-NMR spectrum showed a singlet signal resonating at 10.62 ppm, assigned to the proton of the SH group, in addition to another singlet signal at 2.82 ppm related to the methylene protons of the mercaptomethyl group.

The 3-mercaptomethylacetic acid derivative **10** was obtained through the reaction of the mercapto derivative **9** with chloroacetic acid in absolute ethanol in the presence of potassium hydroxide. The ^{1}H-NMR spectrum of **10** revealed a singlet signal at 4.21 ppm correlated to the methylene protons of the acetic acid moiety, beside another singlet resonating at 6.82 ppm corresponding to the proton of the carboxylic function group.

On the other hand, reaction of **9** with the appropriate acid chloride in dry benzene in the presence of triethylamine afforded the thioesters **11a**, **b**.

Reaction of the key enaminonitrile **4** with formic acid yielded the corresponding tricyclic derivative **12**. IR spectrum of compound **12** exhibited the disappearance of the characteristic stretching vibration band of the cyano group, in addition to the presence of vibrations bands absorbing at 3265, 1656 cm^{-1} related to the NH and two carbonyl groups. ^{1}H-NMR spectrum of **12** showed a singlet signal at 8.15 ppm attributed to the proton at C3.

In a successful method to obtain the Mannich bases **13a–c**, compound **12** was reacted with the appropriate amine, formaldehyde and acetic acid, in acetonitrile. The suggested structure of the target compounds was referred from the IR spectra which revealed the disappearance of the characteristic NH stretching band. ^{1}H-NMR spectra of **13a–c** were in consistency with their structure.

The preparation of the chloro derivative **14** was achieved via heating compound **12** with excess phosphorous oxychloride under dry conditions. The synthesized compound was characterized by its physical, analytical and spectral data. IR spectrum demonstrated only one band at 1675 cm^{-1} attributed to one carbonyl group, in addition to the vanishing of the NH band. ^{1}H-NMR spectrum was complying with the postulated structure of our titled compound.

Nucleophilic substitution of the chloro group in **14** with the appropriate secondary amine was performed to afford the target compounds **15a–c**. ^{1}H-NMR spectra of **15a–c** were complying with their expected structure as that of **15c** showed two triplets at 2.75 and 4.15 ppm related to the 8 protons of the morpholino group.

Alternatively, fusion of the key compound **4** with formamide gave the target 1-amino derivative **16**. Reaction was confirmed by the IR spectrum which revealed the disappearance of the characteristic stretching vibration of the cyano group; and the ^{1}H-NMR spectrum which showed a singlet signal at 8.46 ppm related to the proton C3.

Pharmacological screening

Anti-inflammatory activity

Evaluation of anti-inflammatory activity of the synthesized compounds was performed using the carrageenan-induced rat paw oedema model using diclofenac sodium as the reference drug (Winter *et al.,* 1962; Kasahara *et al.,* 1985; Tozkoparan *et al.,* 2007; Abdel-Megeed *et al.*, 2009; El-sayed *et al.,* 2010). Mean changes in paw oedema thickness of animals pretreated with the tested compounds after 1, 2 and 3 h from induction of inflammation was measured and the inhibition percent of oedema by the tested compounds was calculated. The relative potencies % of the tested compounds compared to diclofenac sodium at the third hour was also calculated (Table 1).

The results of the preliminary anti-inflammatory activity revealed that the intermediate bicyclic derivatives **5a**, **b**, which could be considered as hybrid molecules between the pyrimidoazepine nucleus and salicylic acid or diclofenac moieties, respectively, possessed higher activity than their cyclized tricyclic derivatives **6a**, **b**. However, when these moieties were separated from the pyrimidine ring by a carbonyl thiomethyl spacer as in compounds **11a**, **b**, the activity slightly increased. Introduction of a saturated five- or six-membered aza heterocyclic ring separated by a methyl spacer from the pyrimidine ring **8a–c** were slightly more active than those with an ethyl spacer **8d–f**. However, movement of the azaheterocyclic methyl moiety to position 2 as in compounds **13a–c** or to position 1 as in compounds **15a–c** led to a decrease in activity.

No significant difference was perceived in the activity of the primary amino derivative **16** compared to its tertiary amino analogues **15a–c**. Finally the 3-mercaptomethyl acetic acid derivative **10** retains good potency comparable to that of diclofenac sodium (relative potency = 97.71%). The high activity of this compound could suggest that this compound is acting mechanistically as the classical NSAIDs. Compound **IV**, the morpholino analogue of **10**, was previously reported to have 84.5% relative potency which evidenced the positive impact of lipophilicity on the activity of these compounds and which supported our rationale of improving activity by increasing lipophilicity.

The IC 50 of the most active compound **10** was calculated using doses of 12, 18 and 24 μmol/kg after 3 h from induction of inflammation (Table 2).

Acute ulcerogenicity study

The most active compounds, **5a**, **5b**, **8c**, **8f**, **10** and **11a**, **b**, were evaluated for gastric ulcerogenic potential in rats (Table 3) (Robert *et al.*, 1968; Meshali *et al.,* 1983; El-Sayed *et al.,* 2010). In comparison with diclofenac sodium all tested compounds showed better gastric tolerance. The highest ulcer index was exhibited by the acetic acid derivative **10** but it is still much lower than that of diclofenac sodium.

Conclusions

Various 1, 2 or 3 substituted pyrimido[4′,5′:4,5]pyrimido[1,6-*a*]azepine derivatives were prepared and screened for their anti-inflammatory activity and ulcerogenic potential. The 3-mercaptomethylacetic acid derivative **10** showed activity comparable to diclofenac sodium while the rest of the tested compound exhibited moderate anti-inflammatory activity. Taking the results of the ulcerogenicity study into consideration it could be claimed that

Table 1 Oedema thickness, inhibition percent in oedema thickness and relative potency % of control, diclofenac sodium and tested compounds

Comp.	Oedema thickness ($\times 10^{-2}$ mm) ± SEM (% inhibition)			Relative potency % at 3 h
	1 h	2 h	3 h	
Control	137.80 ± 1.067	156.60 ± 1.967	171.40 ± 1.435	
Diclofenac-sodium	43.20 ± 1.562 (68.6)	25.80 ± 1.392 (83.5)	21.09 ± 1.428 (87.6)	
5a	76.21 ± 1.392 (44.7)	71.22 ± 1.71 (53.8)	59.32 ± 1.870 (65.4)	74.65
5b	93.81 ± 2.596 (31.9)	80.65 ± 1.326 (48.5)	56.22 ± 2.267 (67.2)	76.71
6a	112.82 ± 1.319 (21.9)	111.83 ± 2.083 (27.9)	107.64 ± 2.976 (37.19)	42.45
6b	101.43 ± 1.939 (29.7)	96.81 ± 2.457 (35.2)	93.62 ± 2.227 (45.3)	51.71
8a	90.83 ± 1.241 (36.2)	89.01 ± 1.288 (42.2)	87.80 ± 1.933 (48.7)	53.59
8b	99.22 ± 1.414 (28.01)	98.62 ± 1.536 (37.2)	97.96 ± 1.772 (42.4)	48.40
8c	71.61 ± 1.363 (51.9)	66.43 ± 1.536 (57.5)	64.33 ± 1.984 (62.4)	71.23
8d	107.61 ± 2.267 (20.5)	105.62 ± 2.358 (32.5)	95.41 ± 2.315 (44.3)	50.57
8e	105.80 ± 2.059 (26.8)	101.26 ± 1.913 (35.7)	100.21 ± 2.014 (41.4)	47.26
8f	84.23 ± 2.477 (38.8)	74.82 ± 1.854 (52.2)	64.22 ± 1.773 (52.5)	59.93
10	46.41 ± 1.860 (66.4)	41.20 ± 2.064 (73.2)	20.43 ± 1.661 (85.6)	97.71
11a	98.81 ± 1.772 (29.8)	96.72 ± 1.655 (36.9)	86.21 ± 1.714 (49.7)	56.37
11b	92.23 ± 1.827 (33.09)	84.83 ± 2.498 (45.8)	80.43 ± 1.568 (53.07)	61.30
12	122.60 ± 1.949 (11.03)	120.40 ± 1.029 (26.8)	114.60 ± 1.990 (33.13)	37.81
13a	124.40 ± 1.886 (16.2)	121.63 ± 1.720 (22.3)	115.41 ± 1.923 (32.6)	37.21
13b	150.20 ± 1.319 (11.9)	130.82 ± 2.011 (16.4)	121.23 ± 1.861 (29.27)	33.41
13c	127.61 ± 1.772 (13.06)	131.22 ± 1.593 (18.5)	119.81 ± 2.973 (30.09	34.34
15a	145.60 ± 1.631 (9.5)	133.45 ± 2.785 (14.8)	124.61 ± 2.420 (27.29)	31.15
15b	137.81 ± 1.714 (10.3)	129.20 ± 1.462 (17.4)	123.51 ± 2.863 (27.94)	31.89
15c	127.81 ± 2.477 (17.7)	123.41 ± 1,568 (21.2)	113.42 ± 1.861 (33.82)	38.60
16	128.81 ± 2.395 (17.6)	120.61 ± 1.854 (22.8)	113.52 ± 2.024 (33.7)	38.47

Data analyzed by one way ANOVA, ($n = 5$), all compounds were significantly different from control and from diclofenac sodium using Student's t-test, $P < 0.05$

Table 2 IC 50 values of compound **10** and Diclofenac sodium

Comp	% Inhibition in oedema thickness at			IC 50 (μmol/kg)
	12 μmol/kg	18 μmol/kg	24 μmol/kg	
Diclofenac-sodium	59.5	79.3	88.2	7.26
10	51.2	69.3	89.6	11.73

Table 3 Ulcer indices of compounds **5a**, **5b**, **8c**, **8f**, **10**, **11a** and **11b**

Compound	% Incidence/10	Average no. of ulcer	Average severity	Ulcer index
Control	–	–	–	Nil
Diclofenac-sodium	6	1.2	1.8	9
5a	2	0.4	1	3.4
5b	2	0.6	1.5	4.1
8c	2	0.8	1.5	4.3
8f	1	1.2	1.2	3.4
10	2	2.2	1.5	2.7
11a	1	1.2	1.2	3.4
11b	1	0.2	1.4	2.6

compound **10** outweigh diclofenac sodium as a safe anti-inflammatory agent of comparable potency.

Experimental protocols

Chemistry

Melting points were uncorrected and were carried out by open capillary tube method using IA 9100MK-Digital Melting Point Apparatus. Microanalyses were carried out at the Microanalytical Center, Faculty of Science, Cairo University. Infrared spectra were made on BRUKER Vector 22 (Japan), infrared spectrophotometers and were expressed in wave number (cm^{-1}) using potassium bromide disc. ^{1}H-NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 300 MHz in the specified solvent. Vulnerable protons (NH, OH, SH) were deuterium exchanged. Chemical shifts were reported on the δ scale and were related to that of the solvent and J values are given in Hz. ^{13}C-NMR spectra were obtained on a

Varian Mercury VX-300 NMR spectrometer at 100 MHz in the specified solvent. Mass spectra were recorded on Fennigan MAT, SSQ 7000, Mass spectrometer, at 70 eV (EI). IUPAC chemical nomenclature was assigned using CS Chemdraw ultra version 5.0. Reaction time was monitored using thin layer chromatography; performed using Macherey–Nagel Alugram Sil G/UV254 silica gel plates and chloroform-ethanol (9:1) as the eluting system.

*3-(2-Hydroxyphenyl)-5-phenyl-12-(pyrrolidin-1-yl)-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido [4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**6a**) and 3-[2-(2,6-dichlorophenylamino) benzyl]-5-phenyl-12-(pyrrolidin-1-yl)-1,2,5,6,8,9,10,11,12, 12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a] azepine-1,6-dione (**6b**)*

Compound **5a/5b**(10 mmol) was refluxed in ethanolic hydrochloric acid solution (15 ml) for about 4 h and left to cool. The solution was diluted with water and neutralized with sodium carbonate and the separated solid was filtered and crystallized from aqueous ethanol.

3-(2-Hydroxyphenyl)-5-phenyl-12-(pyrrolidin-1-yl)-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido [1,6-a]azepine-1,6-dione (**6a**): m.p. 175–177°C, yield 62.2%. IR (KBr, cm^{-1}): 3445 (OH); 3310 (NH); 3017 (CH aromatic); 2912–2866 (CH aliphatic); 1702, 1645 (2CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.41–1.53 (m, CH_2-10, 2H); 1.73–2.24 (m, CH_2-9, CH_2-11, $–CH_2CH_2–$ pyrrolidine, 8H); 2.64 (t, $J = 3.2$ Hz, $2CH_2N–$ pyrrolidine, 4H); 3.12 (t, $J = 4.2$ Hz, CH_2-8, 2H); 3.48–3.52 (m, CH-12, 1H); 4.34 (d, $J = 4.7$ Hz, CH-12a, 1H); 6.82–7.52 (m, aromatic protons, 9H); 9.21 (s, NH, 1H, disappeared on deuteration); 9.73 (s, OH, 1H, disappeared on deuteration). Anal. Calcd. for $C_{27}H_{29}N_5O_3$ (471.55): C, 68.77; H, 6.20; N, 14.85. Found: C, 68.45; H, 6.31; N, 14.61.

3-[2-(2,6-Dichlorophenylamino)-benzyl]-5-phenyl-12-(pyrrolidin-1-yl)-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**6b**): m.p. 173–175°C, yield 58.5%. IR (KBr, cm^{-1}): 3180, 3218 (2NH); 3043 (CH aromatic); 2933–2857 (CH aliphatic); 1675, 1630 (2C=O). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.38–1.42 (m, CH_2-10, 2H); 1.82–2.31 (m, CH_2-9, CH_2-11, $–CH_2CH_2–$ pyrrolidine, 8H); 2.62 (t, $J = 4.1$ Hz, $2CH_2N–$ pyrrolidine, 4H); 2. 81 (s, $–CH_2–C_6H_4$, 2H); 3.21 (t, $J = 4.5$ Hz, CH_2-8, 2H); 3.58–3.64 (m, CH-12, 1H); 4.61 (d, $J = 4.9$ Hz, CH-12a, 1H); 6.42–7.31(m, aromatic protons, 12H); 9.12 (s, NH, 1H, disappeared on deuteration); 10.34 (s, NH, 1H, disappeared on deuteration). Anal. Calcd. for $C_{34}H_{34}Cl_2N_6O_2$ (629.58): C, 64.86; H, 5.44; N, 13.35. Found: C, 64.41; H, 6.02; N, 12.96.

*3-Chloroalkyl-5-phenyl-12-(pyrrolidin-1-yl)-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′: 4,5]pyrimido[1,6-a]azepine-1,6-dione (**7a, b**)*

A mixture of the aminonitrile **4** (3.5 g, 10 mmol), the appropriate chloroacyl chloride (15 mmol) and triethylamine (2.5 ml) in dry benzene (10 ml) was refluxed at a temperature not exceeding 70°C for 2 h and evaporated under vacuum to dryness. The residue was triturated with petroleum ether; the separated solid was filtered and crystallized from aqueous ethanol.

3-Chloromethyl-5-phenyl-12-(pyrrolidin-1-yl)-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**7a**): m.p. 184–186°C, yield 72.8%. IR (KBr, cm^{-1}): 3265 (NH); 3055 (CH aromatic); 2933–2857 (CH aliphatic); 1675 (2CO). ^{1}H-NMR ($CDCl_3$: δ, ppm): 1.28–1.41(m, CH_2-10, 2H); 1.61–2.28 (m, CH_2-9, CH_2-11, $–CH_2CH_2–$ pyrrolidine, 8H); 2.75 (t, $J = 3.6$ Hz, $2CH_2N–$ pyrrolidine, 4H); 3.33 (t, $J = 4.3$ Hz, CH_2-8, 2H); 3.71-3.78 (m, CH-12, 1H); 4.35 (s, $–CH_2Cl$, 2H); 4.72 (d, $J = 5.4$ Hz, CH-12a, 1H); 7.21–7.75 (m, aromatic protons, 5H); 8.53 (s, NH, 1H, disappeared on deuteration). MS: *m/z* (%) M^+ 427 (23), 191 (100). Anal. Calcd. for $C_{22}H_{26}ClN_5O_2$ (427.93): C, 61.75; H, 6.12; N, 16.37. Found: C, 61.45; H, 6.66; N, 16.85.

3-(2-Chloroethyl)-5-phenyl-12-(pyrrolidin-1-yl)-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**7b**): m.p. 183–185°C, yield 54.5%. IR (KBr, cm^{-1}): 3235 (NH); 3094 (CH aromatic); 2963–2875 (CH aliphatic); 1681, 1641 (2CO). ^{1}H-NMR ($CDCl_3$: δ, ppm): 1.08–1.24 (m, CH_2-10, 2H); 1.54–1.93 (m, CH_2-9, CH_2-11, $–CH_2CH_2–$ pyrrolidine, 8H); 2.12 (t, $J = 2.9$ Hz, $–CH_2CH_2Cl$), 2H); 2.54 (t, $J = 3.5$ Hz, $2CH_2N–$ pyrrolidine, 4H); 3.21 (t, $J = 4.8$ Hz, CH_2-8, 2H); 3.51–3.58 (m, CH-12, 1H); 4.23 (t, $J = 2.9$ Hz, $–CH_2CH_2Cl$, 2H); 4.81 (d, $J = 5.2$ Hz, CH-12a, 1H); 7.15–7.82 (m, aromatic protons, 5H); 8.76 (s, NH, 1H, disappeared on deuteration). Anal. Calcd. for $C_{23}H_{28}ClN_5O_2$ (441.95): C, 62.51; H, 6.39; N, 15.85. Found: C, 62.88; H, 6.12; N, 15.63.

*5-Phenyl-12-(pyrrolidin-1-yl)-3-[(cycloalkylamino)methyl]-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′: 4,5]pyrimido[1,6-a]azepine-1,6-dione (**8a–f**)*

A mixture of the 3-chloroalkyl derivative **7a/7b** (10 mmol), the appropriate amine (10 mmol) and anhydrous potassium carbonate (1.9 g, 20 mmol), in methylene chloride (15 ml) was refluxed for about 7 h and filtered while hot. The filtrate was distilled under vacuum and the remaining residue was crystallized from ethanol.

5-Phenyl-12-(pyrrolidin-1-yl)-3-[(pyrrolidin-1-yl)methyl]-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**8a**): m.p. 205–207°C, yield 73%. IR (KBr, cm^{-1}): 3229 (NH); 3054 (CH aromatic); 2912–2853 (CH aliphatic); 1655 (2CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.23–1.29 (m, CH_2-10, 2H); 1.72–2.14 (m, CH_2-9, CH_2-11, –CH_2CH_2– of 2 pyrrolidine rings, 12H); 2.55 (t, $J = 4.6$ Hz, 2CH_2-N of 2 pyrrolidine rings, 8H); 2.84 (s, N=C(NH)–*CH_2*–N, 2H); 3.41 (t, $J = 4.1$ Hz, CH_2-8, 2H); 3.73–3.79 (m, CH-12, 1H); 4.51 (d, $J = 5.7$ Hz, CH-12a, 1H); 7.17–7.64 (m, aromatic protons, 5H); 9.14 (s, NH, 1H, disappeared on deuteration). MS: *m/z* (%) M^+ 462 (15), 191 (100). Anal. Calcd. for $C_{26}H_{34}N_6O_2$ (462.59): C, 67.51; H, 7.41; N, 18.17. Found: C, 67.22; H, 7.31; N, 17.74.

5-Phenyl-12-(pyrrolidin-1-yl)-3-[(piperidin-1-yl)methyl]-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**8b**): m.p. 163–166°C, yield 56%. IR (KBr, cm^{-1}): 3312(NH); 3098 (CH aromatic); 2921–2866 (CH aliphatic); 1640, 1623 (2CO). ^{1}H-NMR (C*D*Cl_3: δ, ppm): 1.01–1.23 (m, CH_2-10, 2H); 1.53–1.96 (m, CH_2-9, CH_2-11, –CH_2CH_2– pyrrolidine, –$CH_2CH_2CH_2$– of piperidine, 14H); 2.22 (t, $J = 3.9$ Hz, 2CH_2–N of piperidine, 2CH_2–N of pyrrolidine, 8H); 2.71 (s, N=C(NH)–*CH_2*N–, 2H); 3.26 (t, $J = 4.5$ Hz, CH_2-8, 2H); 3.58–3.61 (m, CH-12, 1H); 4.58 (d, $J = 5.4$ Hz, CH-12a, 1H); 6.96–7.32 (m, aromatic protons, 5H); 8.77 (s, NH, 1H, disappeared on deuteration). Anal. Calcd. for $C_{27}H_{36}N_6O_2$ (476.61): C, 68.04; H, 7.61; N, 17.63. Found: C, 68.31; H, 7.32; N, 17.23.

5-Phenyl-12-(pyrrolidin-1-yl)-3-[(morpholin-4-yl)methyl]-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**8c**): m.p. 185–186°C, yield 51%. IR (KBr, cm^{-1}): 3215 (NH); 3094 (CH aromatic); 2944–2862 (CH aliphatic); 1661, 1641 (2CO). ^{1}H-NMR (C*D*Cl_3: δ, ppm): 1.32–1.37 (m, CH_2-10, 2H); 1.53–1.96 (m, CH_2-9, CH_2-11, –CH_2CH_2– pyrrolidine, 8H); 2.22 (t, $J = 4.1$ Hz, 2CH_2–N pyrrolidine, 4H); 2.51 (t, $J = 4.7$ Hz, N=C(NH)–*CH_2*N–, 2CH_2–N morpholine, 6H); 3.18 (t, $J = 5.5$ Hz, CH_2-8, 2H); 3.50–3.56 (m, CH-12, 1H); 4.23 (t, $J = 4.5$ Hz, 2CH_2–O morpholine, 4H); 4.61 (d, $J = 5.2$ Hz, CH-12a, 1H); 7.14–7.73 (m, aromatic protons, 5H); 8.24 (s, NH, 1H, disappeared on deuteration). Anal. Calcd. for $C_{26}H_{34}N_6O_3$ (478.59): C, 65.25; H, 7.16; N, 17.56. Found: C, 65.72; H, 6.94; N, 17.82.

5-Phenyl-12-(pyrrolidin-1-yl)-3-[2-(pyrrolidin-1-yl)ethyl]-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**8d**): m.p. 166–169°C, yield 63%. IR (KBr, cm^{-1}): 3294 (NH); 3084 (CH aromatic); 2915–2867 (CH aliphatic); 1656 (2CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.14–1.21 (m, CH_2-10, 2H); 1.45 (t, $J = 2.8$ Hz,–*CH_2*CH_2–N, 2H); 1.83–2.32 (m, CH_2-9, CH_2-11, –CH_2CH_2– of 2 pyrrolidine rings, 12H); 2.64 (t, $J = 4.6$ Hz, 2CH_2–N of 2 pyrrolidine rings, 8H); 2.96 (t, $J = 2.8$ Hz, CH_2*CH_2*–N, 2H); 3.23 (t, $J = 4.2$ Hz, CH_2-8, 2H); 3.58–3.62 (m, CH-12, 1H); 4.52 (d, $J = 5.6$ Hz, CH-12a, 1H); 7.15–7.91 (m, aromatic protons, 5H); 8.47 (s, NH, 1H, disappeared on deuteration). Anal. Calcd. for $C_{27}H_{36}N_6O_2$ (476.62): C, 68.04; H, 7.61; N, 17.63. Found: C, 68.54; H, 7.45; N, 17, 41.

5-Phenyl-12-(pyrrolidin-1-yl)-3-[2-(piperidin-1-yl)ethyl]-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**8e**): m.p. 172–174°C, yield 33%. IR (KBr, cm^{-1}): 3313 (NH); 3045 (CH aromatic); 2916–2865 (CH aliphatic); 1685, 1641 (2CO).^{1}H-NMR (DMSO-d_6: δ, ppm): 1.01–1.23 (m, CH_2-10, 2H); 1.51 (t, $J = 3.2$ Hz, –*CH_2*CH_2–N, 2H); 1.82–2.36 (m, CH_2-9, CH_2-11, –CH_2CH_2– pyrrolidine, –$CH_2CH_2CH_2$– piperidine, 14H); 2.62 (t, $J = 4.5$ Hz, 2CH_2–N piperidine, 2CH_2–N pyrrolidine, 8H); 2.93 (t, $J = 3.2$ Hz, –CH_2*CH_2*–N, 2H); 3.37 (t, $J = 4.3$ Hz, CH_2-8, 2H); 3.68-3.74 (m, CH-12, 1H); 4.62 (d, $J = 5.9$ Hz, CH-12a, 1H); 7.21–7.76 (m, aromatic protons, 5H); 9.15 (s, NH, 1H, disappeared on deuteration). Anal. Calcd. for $C_{28}H_{38}N_6O_2$ (490.31): C, 68.54; H, 7.81; N, 17.13. Found: C, 68.72; H, 7.35; N, 17.26.

5-Phenyl-12-(pyrrolidin-1-yl)-3-[2-(morpholin-4-yl)ethyl]-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**8f**): m.p. 191–193°C, yield 54%. IR (KBr, cm^{-1}): 3281 (NH); 3062 (CH aromatic); 2945–2862 (CH aliphatic); 1675, 1623 (2CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.11–1.34 (m, CH_2-10, 2H); 1.56 (t, $J = 2.6$ Hz, *CH_2*CH_2–N, 2H); 1.74–2.31 (m, CH_2-9, CH_2-11, –CH_2CH_2– pyrrolidine, 8H); 2.61 (t, $J = 4.3$ Hz, 2CH_2–N pyrrolidine, 4H); 2.93 (m, CH_2*CH_2*–N–, 2CH_2–N morpholine, 6H); 3.34 (t, $J = 4.7$ Hz, CH_2-8, 2H); 3.63–3.72 (m, CH-12, 1H); 4.15 (t, $J = 3.4$ Hz, 2CH_2–O morpholine, 4H); 4.61 (d, $J = 5.5$ Hz, CH-12a, 1H); 7.11–7.61 (m, aromatic protons, 5H); 8.82 (s, NH, 1H, disappeared on deuteration). Anal. Calcd. for $C_{27}H_{36}N_6O_3$ (492.28): C, 65.83; H, 7.37; N, 17.06. Found: C, 65.61; H, 6.85; N, 16.67.

*5-Phenyl-12-(pyrrolidin-1-yl)-3-sulfanylmethyl-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**9**)*

A mixture of compound **7a** (4.27 g, 10 mmol) and thiourea (0.76 g, 10 mmol) in ethanol (10 ml) was heated under reflux for about 3 h then left to cool. The separated crystals were filtered, dissolved in ice cooled solution of 10% sodium hydroxide and filtered; the alkaline filtrate was acidified with dilute hydrochloric acid to pH 4. The formed precipitate was filtered, washed with water and crystallized from aqueous ethanol to give 2.76 g (64.6%) of **9**, m.p. 205–209°C. IR (KBr, cm^{-1}): 3218 (NH); 3044 (CH aromatic); 2913–2843 (CH aliphatic); 2455 (SH), 1670, 1625 (2CO). ^{1}H-NMR (C*D*Cl_3: δ, ppm): 1.23–1.31 (m, CH_2-10,

2H); 1.98-2.29 (m, CH_2-9, CH_2-11, –CH_2CH_2– pyrrolidine, 8H); 2.55 (t, $J = 4.1$ Hz, 2CH_2–N pyrrolidine, 4H); 2.82 (s, –CH_2SH, 2H); 3.43 (t, $J = 4.4$ Hz, CH_2–8, 2H); 4.10–4.13 (m, CH-12, 1H); 4,84 (d, $J = 5.2$ Hz, CH-12a, 1H); 6.94–7.58 (m, aromatic protons, 5H); 8.64 (s, NH, 1H, disappeared on deuteration); 10.62 (s, SH, 1H, disappeared on deuteration). MS: *m/z* (%) M^+ 425.19 (14), 160 (100). Anal. Calcd. for $C_{22}H_{27}N_5O_2S$ (425.19): C, 62.09; H, 6.40; N, 16.46. Found: C, 62.21; H, 6.34; N, 15.97.

*(1,6-Dioxo-5-phenyl-12-pyrrolidino-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5] pyrimido[1,6-a]azepin-3-ylmethylsulfanyl)acetic acid (**10**)*

A mixture of compound **9** (4.2 g, 10 mmol), chloroacetic acid (0.94 g, 10 mmol) and potassium hydroxide (0.72 g, 20 mmol), in absolute ethanol (20 ml) was refluxed for about 8 h then filtered while hot. The filtrate was evaporated to dryness; the residue was diluted with water and neutralized with dilute hydrochloric acid. The separated crystals were filtered, washed with water and recrystallized from aqueous ethanol to give 2.46 g (50.9%) of **10**, m.p. 212–215°C. IR (KBr, cm^{-1}): 3235 (OH), 3145 (NH), 3072 (CH aromatic); 2915, 2863 (CH aliphatic); 1660, 1618 (3CO). ^{1}H-NMR (C*D*Cl_3: δ, ppm): 1.28–1.34 (m, CH_2-10, 2H); 1.58–1.99 (m, CH_2-9, CH_2-11, –CH_2CH_2– pyrrolidine, 8H); 2.45 (t, $J = 4.7$ Hz, 2CH_2–N pyrrolidine, 4H); 2.91(s, –CH_2SCH_2COOH, 2H); 3.23 (t, $J = 5.3$ Hz, CH_2-8, 2H); 3.61–3.65 (m, CH-12, 1H); 4.21 (s, –CH_2 SCH_2COOH, 2H); 4.51 (d, $J = 5.6$ Hz, CH-12a, 1H); 6.82 (s, –COOH, 1H, disappeared on deuteration); 7.25–7.73 (m, aromatic protons, 5H); 8.71 (s, NH, 1H, disappeared on deuteration). ^{13}C-NMR(C*D*Cl_3: δ): 22.4 (C-10), 24.1 (–CH_2CH_2– pyrrolidine), 29.1 (C-9, C-11), 35.2 (–CH_2S–CH_2), 37.8 (CH_2S–CH_2), 45.3 (C-8), 48.3 (C-12a), 50.2 (2CH_2–N pyrrolidine), 52.3 (C-12), 106.2 (C-12b), 119.1 (C-2′, C-6′), 123.8 (C-4′), 129.2 (C-3′, C-5′), 140.2 (C-1′), 152.3 (C-4a), 154.6 (C-1), 165.1 (C-3), 170.4 (C=O), 177.2 (–*C*OOH). MS: *m/z* (%) M^+ 483.19 (20), 162 (100). Anal. Calcd. for $C_{24}H_{29}N_5O_4S$ (483.19): C, 59.61; H, 6.04; N, 14.48. Found: C, 59.15; H, 6.41; N, 14.97.

*3-[(Substituted benzoyl/substituted acetyl)sulfanylmethyl]-5-phenyl-12(pyrrolidin-1-yl)-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**11a, b**)*

A mixture of compound **9** (4.2 g, 10 mmol), the appropriate acid chloride (11 mmol) and triethylamine (0.5 ml) in dry benzene (10 ml) was refluxed for 4 h. The solvent was removed under vacuum and the obtained residue was triturated with ether; the separated solid was filtered and crystallized from aqueous ethanol.

3-[(2-Hydroxybenzoyl)sulfanylmethyl]-5-phenyl-12 (pyrrolidin-1-yl)-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**11a**): m.p. 190–193°C, yield 44%. IR (KBr, cm^{-1}): 3415 (OH); 3315 (NH); 3052 (CH aromatic); 2934-2882 (CH aliphatic); 1710, 1664, 1633 (3CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.28–1.41 (m, CH_2-10, 2H); 1.52–1.91 (m, CH_2-9, CH_2-11, –CH_2CH_2– pyrrolidine, 8H); 2.38 (t, $J = 3.5$ Hz, 2CH_2–N pyrrolidine, 4H); 2.93 (s, –CH_2S–CO, 2H); 3.48 (t, $J = 4.2$ Hz, CH_2-8, 2H); 3.78–3.82 (m, CH-12, 1H); 4.65 (d, $J = 5.1$ Hz, CH-12a, 1H); 6.94–7.63 (m, aromatic protons, 9H); 8.56 (s, NH, 1H, disappeared on deuteration); 10.13 (s, OH, 1H, disappeared on deuteration). Anal. Calcd. for $C_{29}H_{31}N_5O_4S$ (545.21): C, 63.83; H, 5.73; N, 12.83. Found: C, 63.41; H, 5.32; N, 12.52.

3-{[2-(2,6-Dichlorophenylamino)phenyl]acetyl]sulfanylmethyl}-5-phenyl-12-(pyrrolidin-1-yl)-1,2,5,6,8,9,10,11, 12,12a-decahydroprimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**11b**): m.p. 206–209°C, yield 56%. IR (KBr, cm^{-1}): 3371 (NH); 3081 (CH aromatic); 2913–2833 (CH aliphatic); 1680, 1655, 1630 (3CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.22–1.34 (m, CH_2-10, 2H); 1.42–1.84 (m, CH_2-9, CH_2-11, –CH_2CH_2– pyrrolidine, 8H); 2.45 (t, $J = 4.7$ Hz, 2CH_2–N pyrrolidine, 4H); 2.88 (s, CH_2S-CO, 2H); 3.28 (t, $J = 4.3$ Hz, CH_2-8, 2H); 3.61–3.72 (m, CH-12, 1H); 4.12 (s, –S–CO–CH_2–, 2H); 4.55 (d, $J = 4.9$ Hz, CH-12a, 1H); 6.92–7.55 (m, aromatic protons, 12H); 8.66 (s, NH, 1H); 9.22 (s, NHCO, 1H). Anal. Calcd. for $C_{36}H_{36}Cl_2N_6O_3S$ (702.19): C, 61.45; H, 5.16; N, 11.94. Found: C, 61.12; H, 4.78; N, 11.61.

*5-Phenyl-12-(pyrrolidin-1-yl)-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1, 6-dione (**12**)*

A mixture of the aminonitrile **4** (3.48 g, 10 mmol) and formic acid (85%, 30 ml) was refluxed for 10 h, cooled and then poured onto ice-water. The precipitate was filtered, washed several times by water, dried and then crystallized from ethanol to afford 1.58 g (41.6%) of **12**, m.p. 152–155°C. IR (KBr, cm^{-1}): 3265 (NH); 3083 (CH aromatic); 2913-2873 (CH aliphatic); 1656 (2CO). ^{1}H-NMR (C*D*Cl_3: δ, ppm): 1.28–1.41 (m, CH_2-10, 2H); 1.63–2.28 (m, CH_2-9, CH_2-11, –CH_2CH_2– pyrrolidine, 8H); 2.55 (t, $J = 4.5$ Hz, 2CH_2–N pyrrolidine, 4H); 3.25 (t, $J = 4.8$ Hz, CH_2-8, 2H); 3.58–3.64 (m, CH-12, 1H); 4,67 (d, $J = 5.7$ Hz, CH-12a, 1H); 6.94–7.65 (m, aromatic protons, 5H); 8.15 (s, NH-*CH*=N, 1H); 9.26 (s, NH, 1H). MS: *m/z* (%) M^+ 379.2 (16), 196 (100). Anal. Calcd. for $C_{21}H_{25}N_5O_2$ (379.2) : C, 66.47; H, 6.64; N, 18.46 Found: C, 65.82; H, 6.25; N, 18.24.

*5-Phenyl-12-(pyrrolidin-1-yl)-2-[(cycloalkylamino)methyl]-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**13a–c**)*

To a stirred solution of compound **12** (3.8 g, 10 mmol) in acetonitrile (20 ml), a mixture of the appropriate amine (10 mmol), formaldehyde (10 mmol) and acetic acid (5 ml) was added drop wise. The mixture was stirred at room temperature for 10 h then treated with a solution of 20% sodium hydroxide and extracted with ethyl acetate. The organic extracts were collected, evaporated under vacuum and the residue was crystallized from aqueous ethanol.

5-Phenyl-12-(pyrrolidin-1-yl)-2-[(pyrrolidin-1-yl)methyl]-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**13a**): m.p. 175–179°C, yield 56%. IR (KBr, cm^{-1}): 3081 (CH aromatic); 2932–2817 (CH aliphatic); 1680, 1650 (2CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.21-1.29 (m, CH_2-10, 2H); 1.85–2.21 (m, CH_2-9, CH_2-11, –CH_2CH_2– 2 pyrrolidines, 12H); 2.63 (t, J = 3.8 Hz, 2CH_2–N 2 pyrrolidines, 8H); 3.14 (t, J = 4.8 Hz, CH_2-8, 2H); 3.49–3.54 (m, CH-12, 1H); 4.25 (s, N–CH_2–N, 2H); 4,67 (d, J = 5.3 Hz, CH-12a, 1H); 6.98–7.72 (m, aromatic protons, 5H); 8.27 (s, N–CH=N, 1H). MS: *m/z* (%) M^+ 462.27 (32), 195 (100). Anal. Calcd. for $C_{26}H_{34}N_6O_2$ (462.59): C, 67.51; H, 7.41; N, 18.17. Found: C, 67.11; H, 7.25; N, 18.36.

5-Phenyl-12-(pyrrolidin-1-yl)-2-[(piperidin-1-yl)methyl]-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**13b**): m.p. 164–168°C, yield 58%. IR (KBr, cm^{-1}): 3075 (CH aromatic); 2923–2877 (CH aliphatic); 1670, 1635 (2CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.22–1.34 (m, CH_2-10, 2H); 1.63–1.96 (m, CH_2-9, CH_2-11, CH_2CH_2– pyrrolidine, –$CH_2CH_2CH_2$– piperidine, 14H); 2.36 (t, J = 4.2 Hz, 2CH_2–N piperidine, 2CH_2–N pyrrolidine, 8H); 3.18 (t, J = 5.2 Hz, CH_2-8, 2H); 3.49–3.58 (m, CH-12, 1H); 4.22(s, N–CH_2–N, 2H); 4.63 (d, J = 5.8 Hz, CH-12a, 1H); 6.92–7.32 (m, aromatic protons, 5H); 8.77 (s, N=CH–N, 1H). Anal. Calcd. for $C_{27}H_{36}N_6O_2$ (476.61): C, 68.04; H, 7.61; N, 17.63. Found: C, 67.74; H, 7.35; N, 17.33.

5-Phenyl-12-(pyrrolidin-1-yl)-2-[(morphlin-4-yl)methyl]-1,2,5,6,8,9,10,11,12,12a-decahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepine-1,6-dione (**13c**): m.p. 171–174°C, yield 41%. IR (KBr, cm^{-1}): 3072 (CH aromatic); 2935–2861 (CH aliphatic); 1685, 1645 (2CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.21–1.36 (m, CH_2-10, 2H); 1.62–1.99 (m, CH_2-9, CH_2-11, –CH_2CH_2– pyrrolidine, 8H); 2.29 (t, J = 4.3 Hz, 2CH_2–N of pyrrolidine, 4H); 2.64 (t, J = 3.9 Hz, 2CH_2–N morpholine, 4H); 3.25 (t, J = 4.6 Hz, CH_2-8, 2H); 3.68-3.76 (m, CH-12, 1H); 4.23 (t, J = 3.9 Hz, 2CH_2–O morpholine, 4H); 4.42 (s, N–CH_2–N, 2H); 4.81 (d, J = 5.5 Hz, CH-12a, 1H); 6.92–7.54 (m, aromatic protons, 5H); 8.31 (s, N=CH–N, 1H). Anal. Calcd. for $C_{26}H_{34}N_6O_3$, (478.59): C, 65.25; H, 7.16; N, 17.56. Found: C, 65.73; H, 7.52; N, 17.34.

*1-Chloro-5-phenyl-12-pyrrolidino-5,6,8,9,10,11,12,12a-octahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepin-6-one (**14**)*

A mixture of compound **12** (0.38 gm, 1 mmol) and phosphorus oxychloride (10 ml) was heated at 110°C for about 6 h. The mixture was evaporated under vacuum and triturated with sodium carbonate solution (5%). The precipitate was filtered, dried and crystallized from aqueous ethanol to yield 0.18 g (47%) of **14**, m.p. 150–152°C. IR (KBr, cm^{-1}): 3072 (CH aromatic); 2954–2831 (CH aliphatic); 1675 (CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.19–1.32 (m, CH_2-10, 2H); 1.72–2.15 (m, CH_2-9, CH_2-11, CH_2CH_2–pyrrolidine, 8H); 2.62 (t, J = 4.5 Hz, 2CH_2–N pyrrolidine, 4H); 3.31 (t, J = 4.7 Hz, CH_2-8, 2H); 3.60–3.68 (m, CH-12, 1H); 4,51 (d, J = 5.3 Hz, CH-12a, 1H); 7.14–7.68 (m, aromatic protons, 5H); 8.31 (s, N–CH=N, 1H). MS: *m/z* (%) M^+ 397.17 (21), 161 (100). Anal. Calcd. for $C_{21}H_{24}ClN_5O$ (397.17): C, 63.39; H, 6.08; N, 17.60. Found: C, 62.97 H, 6.34; N, 18.01.

*5-Phenyl-1-(cycloalkylamino)-12-(pyrrolidin-1-yl)-5,6,8,9,10,11,12,12a-octahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepin-6-one (**15a–c**)*

A mixture of compound **14** (3.97 g, 10 mmol), the appropriate amine (10 mmol) and anhydrous potassium carbonate (1.9 g, 20 mmol), in absolute ethanol (15 ml) was refluxed for about 8 h and filtered while hot. The filtrate was distilled under vacuum; the residue was triturated with ethanol and the obtained solid was filtered, dried and crystallized from aqueous ethanol.

5-Phenyl-1,12-di(pyrrolidin-1-yl)-5,6,8,9,10,11,12,12a-octahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]azepin-6-one (**15a**): m.p. 165–168°C, yield 75%. IR (KBr, cm^{-1}): 3073 (CH aromatic); 2913–2865 (CH aliphatic); 1645 (CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.29–1.36 (m, CH_2-10, 2H); 1.72–2.13 (m, CH_2-9, CH_2-11, –CH_2CH_2– of 2 pyrrolidine rings, 12H); 2.33 (t, J = 4.6 Hz, 2CH_2–N of 12-pyrrolidinyl, 4H); 2.72 (t, J = 3.8 Hz, 2CH_2–N of 1-pyrrolidinyl, 4H); 3.25 (t, J = 5.7 Hz, CH_2-8, 2H); 3.31–3.48 (m, CH-12, 1H); 4,67 (d, J = 5.3 Hz, CH-12a, 1H); 7.23–7.81 (m, aromatic protons, 5H); 8.42 (s, N–CH=N, 1H). ^{13}C-NMR(DMSO-d_6: δ): 22.6 (C-10), 23.5 (2C-12-pyrrolidinyl), 24.1 (2C-1-pyrrolidinyl), 29.3 (C-9, C-11), 43.6 (C-12a), 47.2 (C-8), 50.3 (2*C*-N-1-pyrrolidinyl), 53.8 (2*C*-N-12-pyrrolidinyl), 60.2 (C-12), 104.3 (C-12b), 118.8 (C-2$^\backslash$, C-6$^\backslash$), 125.1 (C-4$^\backslash$), 129.6 (C-3$^\backslash$, C-5$^\backslash$), 139.1 (C-1$^|$), 155.2

(C-1), 157.2 (C-3), 165.4 (C-4a), 172.2 (C=O). MS: *m/z* (%) M^+ 432.26 (25), 196 (100). Anal. Calcd. for $C_{25}H_{32}N_6O$ (432.26): C, 69.42; H, 7.46; N, 19.43. Found: C, 69.12; H, 7.54; N, 18.96.

5-Phenyl-1-(piperidin-1-yl)-12-(pyrrolidin-1-yl)-5,6,8,9,10,11,12,12a-octahydropyrimido[4′,5′:4,5]pyrimido[1,6-a]-azepin-6-one (**15b**): m.p. 174–176°C, yield 65%. IR (KBr, cm^{-1}): 3083 (CH aromatic); 2933–2857 (CH aliphatic); 1680 (CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.19–1.29 (m, CH_2-10, 2H); 1.62–1.98 (m, CH_2-9, CH_2-11, CH_2CH_2–pyrrolidine, –$CH_2CH_2CH_2$– of piperidine, 14H); 2.36 (t, $J = 4.6$ Hz, 2CH_2–N of pyrrolidine, 4H); 2.82 (t, $J = 3.9$ Hz, 2CH_2–N of piperidine, 4H); 3.29 (t, $J = 4.4$ Hz, CH_2-8, 2H); 3.59–3.64 (m, CH-12, 1H); 4.82 (d, $J = 5.4$ Hz, CH-12a, 1H); 7.26–7.75 (m, aromatic protons, 5H); 8.25 (s, N=CH–N, 1H). Anal. Calcd. for $C_{26}H_{34}N_6O$ (446.28): C, 69.93; H, 7.67; N, 18.82. Found: C, 69.74; H, 8.11; N, 19.21.

5-Phenyl-1-(morpholin-4-yl)-12-(pyrrolidin-1-yl)-5,6,8,9,10,11,12,12a-octahydropyrimido[4′,5′:4,5]pyrimido[1,6-a] azepin-6-one (**15c**): m.p. 181–183°C, yield 53%. IR (KBr, cm^{-1}): 3055 (CH aromatic); 2925–2857 (CH aliphatic); 1665 (CO). ^{1}H-NMR (DMSO-d_6: δ, ppm): 1.13–1.24 (m, CH_2-10, 2H); 1.65–1.99 (m, CH_2-9, CH_2-11, CH_2CH_2–pyrrolidine, 8H); 2.34 (t, $J = 4.2$ Hz, 2CH_2–N of pyrrolidine, 4H); 2.75 (t, $J = 4.5$ Hz, 2CH_2–N morpholine, 4H); 3.16 (t, $J = 5.2$ Hz, CH_2-8, 2H); 3.58–3.64 (m, CH-12, 1H); 4.15 (t, $J = 4.5$ Hz, 2CH_2–O morpholine, 4H); 4.86 (d, $J = 5.6$ Hz, CH-12a, 1H); 6.98–7.67 (m, aromatic protons, 5H); 8.24 (s, N=CH–N, 1H). Anal. Calcd. for $C_{25}H_{32}N_6O_2$ (448.25): C, 66.94; H, 7.19; N, 18.74. Found: C, 66.61; H, 7, 53; N, 18.34.

*1-Amino-5-phenyl-12-(pyrrolidin-1-yl)-5,6,8,9,10,11,12,12a-octahydropyrimido[4′,5′:4,5] pyrimido[1,6-a]azepin-6-one (**16**)*

A mixture of the aminonitrile **4** (1.75 g, 5 mmol) and formamide (20 ml) was refluxed for 2 h; cooled and the reaction mixture was poured into cold water. The solid precipitate was filtered, washed with water, dried and crystallized from aqueous ethanol to yield 1.03 g (54%) of **12**, m.p. 163–165°C. IR (KBr, cm^{-1}): 3215 (NH_2); 3064 (CH aromatic); 2940–2865 (CH aliphatic); 1635 (CO). ^{1}H-NMR (C*D*Cl_3: δ, ppm): 1.29–1.36 (m, CH_2-10, 2H); 1.83–2.35 (m, CH_2-9, CH_2-11, –CH_2CH_2– pyrrolidine, 8H); 2.64 (t, $J = 4.5$ Hz, 2CH_2–N pyrrolidine, 4H); 3.27 (t, $J = 4.6$ Hz, CH_2-8, 2H); 3.69–3.74 (m, CH-12, 1H); 4.25 (d, $J = 5.7$ Hz, CH-12a, 1H); 7.12–7.73 (m, aromatic protons, 5H); 8.46 (s, N–CH=N, 1H); 8.89 (s, NH_2, 2H, disappeared on deuteration). MS: *m/z* (%) M^+ 380.23 (22%), 166 (100%). Anal. Calcd. for $C_{21}H_{26}N_6O$ (380.23): C, 66.29; H, 7.42; N, 22.09. Found: C, 65.83; H, 6.83; N, 21.73.

Pharmacological screening

Anti-inflammatory activity

Adult albino rats of both sexes weighing between 120 and 150 g were used. Rats were uniformly hydrated by giving 3 ml water/rat through gastric inoculation to reduce variability to oedema response. Animals were divided into 23 groups each of five animals. The control group was given saline solution containing few drops of 1% Tween 80. Diclofenac sodium (20 μmol/kg) was taken as standard drug for comparison and compounds under examination (20 μmol/kg) were suspended in distilled water by the aid of few drops of Tween 80 and were given intraperitoneally 1 h before induction of inflammation. Induction of inflammation was performed by S.C. injection of 50 μl of 1% carrageenan-sodium gel (Sigma-Aldrich, USA), into the sub-plantar region of the right hind paw. The dorso-ventral diameter (thickness) of the right and left hind paw of each rat was measured using a pair of dial thickness gauge calipers accurate to 0.001 cm 1 h, 2 h and 3 h after induction of inflammation. The left hind paw diameter served as a control for the degree of inflammation in the right hind paw. The percentage of anti-inflammatory activity (% inhibition of inflammation) was calculated according to the following equation:

$$\%\ \text{Inhibition} = (1 - L_t/L_c) \times 100$$

where L_t is the mean increase in paw thickness in rats treated with the tested compounds and L_c is the mean increase in paw thickness in control group.

Data were analyzed by SPSS statistical package version 10. Results are presented in Table 1.

Acute ulcerogenicity study

Adult albino rats of both sexes weighing between 120 and 150 g were used. Animals were divided into groups each of five animals. Rats were fasted 20 h before drug administration. The tested compounds and diclofenac sodium were given orally in a dose of 20 μmol/kg suspended in 1% Tween while one group received vehicle (1% Tween). Rats were fasted for 2 h; allowed to feed for 2 h then fasted for another 20 h. Rats were given another two doses in the second and third day. In the fourth day, rats were killed, the stomach removed, opened along with the greater curvature and rinsed with 0.9% saline. The number of mucosal damage (red spots) was counted and their severity (ulcerogenic severity) was graded from 0 to 4 according to the following score assignment:

	Score		Score
Normal (no injury)	0	Slight injury	3
Latent small red spot	1	Severe injury	4
Wide red spot	2		

The following figures were calculated:

- *% Incidence/10* = [number of rats showing ulcer of any grade divided by total number of rats in the group × 100]/10.
- *Average number of ulcers*: number of ulcers in the group/total number of rats in the group.
- *Average severity*: ∑[each ulcer multiplied by its score of severity]/number of ulcers in the group.

Ulcer index = the sum of the three figures

Results are tabulated in Table 3.

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