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Original article

New pyrimido[5,4-*e*]pyrrolo[1,2-*c*]pyrimidines: Synthesis, 2D-QSAR, anti-inflammatory, analgesic and ulcerogenicity studies

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ABSTRACT

New pyrimido[5,4-*e*]pyrrolo[1,2-*c*]pyrimidines were synthesized. A series of ylidene carbohydrazides **14a**–**i**, and hydrazonate **15**, were obtained from the prepared 3-carbohydrazide derivative **13**. Pyrazole derivatives **12**, **16a**,**b**, **18**, **19**, **20**, were also prepared through different reactions. The anti-inflammatory and analgesic activities of all new compounds were evaluated and most of them exerted comparable activity to indomethacin and celecoxib. Ulcer indexes for the most active compounds were calculated and most of them showed less ulcerogenic effect than the reference drugs. The most potent anti-inflammatory compound **12** showed an IC_{50} of 6.00 μmol/kg and low ulcer index. COX-1/COX-2 activity ratio of compounds **12** and **16b** showed almost equal inhibitory effect on both isoenzymes. 2D-QSAR studies revealed good predictive and statistically significant QSAR models.

1. Introduction

Inflammation is part of the non-specific immune response that occurs as a reaction to any exogenous or endogenous insult which threatens the host. It is a complex phenomenon involving biochemical as well as immunological factors [1]. Non-steroidal anti-inflammatory drugs (NSAIDs) have a wide clinical use for the treatment of inflammatory and painful conditions including rheumatoid arthritis, soft tissue lesions, fever and respiratory tract infections [2]. However, long term administration of NSAIDs gives rise to unacceptable side effects mainly gastric lesions [3]. The effectiveness of NSAIDs in reducing pain and swelling lies in their ability to inhibit cyclooxygenases COX-1 and COX-2 that are the key enzymes involved in the prostaglandin biosynthesis [4]. COX-1 inhibition is thought to be a major mechanism of gastric damage by NSAIDs [5]. Selective COX-2 inhibitors were developed to decrease the incidence of gastrointestinal toxicity. However, recent concerns regarding these drugs and their association with significant cardiovascular side effects led to reconsideration of their appropriate use [6]. Therefore, there is always a constant need for discovery of novel and safer anti-inflammatory drugs.

Pyrimidines and condensed pyrimidines constitute an important skeleton for compounds with diverse biological activities [7–9] including anti-inflammatory [10–12] and analgesic activities [13–16]. In previous studies, pyrimido[5,4-*e*]pyrrolo[1,2-*c*]pyrimidine derivatives prepared in our lab were found to possess significant anti-inflammatory and analgesic activities, whereby, some of them were superior to the reference drugs and with low ulcerogenic potential [17]. Compounds **I** and **II** (Fig. 1) are shown as examples [18]. As a continuation of this work and aiming to further explore this ring system in the field of non-ulcerogenic anti-inflammatory agents, it was of interest to investigate a hybrid pharmacophoric approach by combining the pyrimido[5,4-*e*]pyrrolo[1,2-*c*]pyrimidine a privileged lead molecule with several other important pharmacophoric moieties and to evaluate their analgesic and anti-inflammatory activities. Therefore, active pharmacophoric groups were introduced at position 3 of the pyrimidopyrrolopyrimidine core including carboxylic and acetic acid moieties and their esters, as possible prodrugs with low ulcerogenic activity. Furthermore, different substituted ylidene groups were also incorporated as it was reported that hydrazone moiety present in some compounds was suggested to be anti-inflammatory pharmacophoric element [19]. Additionally, pyrazole derivatives, including antipyrine molecule, that are well known to be important integral moieties in many analgesic and anti-inflammatory drugs [20,21] were also used and hybridized to the tricyclic core.

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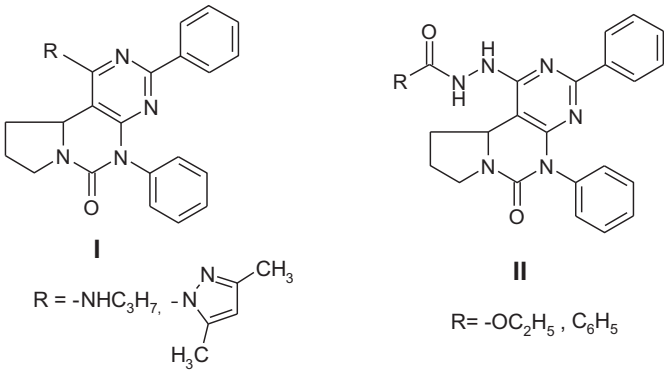


Fig. 1. Examples of tricyclic pyrimidopyrrolopyrimidine derivatives with anti-inflammatory and analgesic activities.

Ulcerogenic studies were carried out for compounds with the best activity profile. 2D-QSAR studies were also performed to correlate between the structures of the synthesized compounds and their pharmacological activities.

2. Results and discussion

2.1. Chemistry

The synthetic pathways for the synthesis of the targeted compounds are illustrated in Schemes 2 and 3. The known starting compound pyrrolo[1,2-*c*]pyrimidine-4-carbonitrile derivative **5**, was synthesized according to previously reported procedures. 2-Pyrrolidin-2-ylidine malononitrile **3** was prepared by methylation of 2-pyrrolidinone **1** into the ether intermediate **2** followed by condensation with malononitrile. The reaction of **3** with phenyl isocyanate followed by reduction of the obtained enaminonitrile **4** with sodium borohydride, via 1,4-addition reaction, afforded the key starting compound **5** (Scheme 1) [22–24].

Cyclization and formation of the 3-chloroacetyl and 3-chlorocarbonyl pyrimido[5,4-*e*]pyrrolo[1,2-*c*]pyrimidine intermediates **6** and **9**, respectively, was achieved by acylation of **5** with either malonyl or oxalyl chloride in dry benzene at room temperature (Scheme 2).

The ester derivatives **7** and **10** were prepared by refluxing the non-isolated intermediates **6** and **9**, with absolute ethanol. The IR spectrum of **7** revealed the presence of an additional C=O stretching band at 1743 cm^{-1}, furthermore, the disappearance of the nitrile band confirmed intramolecular cyclization. ^1H NMR of **7** showed a singlet signal at 3.51 attributed to CH_2 protons of the acetate group and the characteristic triplet and quartet signals of the ethyl protons at 1.08 and 3.93 ppm, respectively. Alkaline hydrolysis of **7** and **10** with 10% NaOH solution afforded the acetic acid and carboxylic acid analogues **8** and **11**, respectively. The IR spectrum of **8** revealed the presence of the OH and C=O absorption bands at 2785–2600 cm^{-1} and 1743 cm^{-1} respectively. ^1H NMR of **8** showed an additional exchangeable singlet at 12.28 ppm assigned to the COOH proton (Scheme 2).

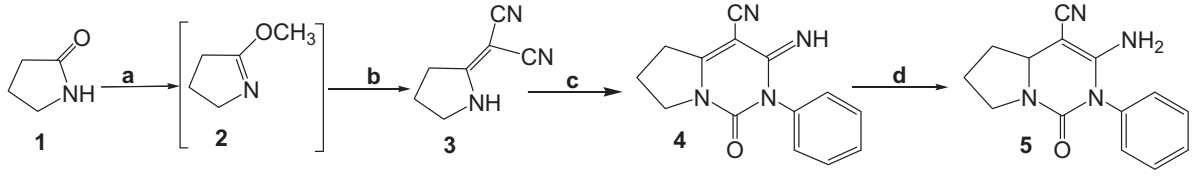
The reaction of the 3-chlorocarbonyl intermediate **9** with 4-aminoantipyrine, in dry dimethylformamide, generated the target compound 3-*N*-pyrazolylcarboxamide derivative **12**. The ^1H NMR spectrum of **12** showed 2 singlet peaks at 0.94 and 3.16 ppm corresponding to the 2 methyl protons (C–CH_3 and N–CH_3), respectively.

The synthesis of the 3-carbohydrazide derivative **13** was carried out by treating the carboxylate ester **10** with hydrazine hydrate in absolute ethanol. The presence of absorption bands at 3398, 3317and 3244 cm^{-1} in the IR spectrum were attributed to the NH and NH_2 groups, furthermore, the 3 C=O bands appeared in the range of 1700–1681 cm^{-1} (Scheme 2).

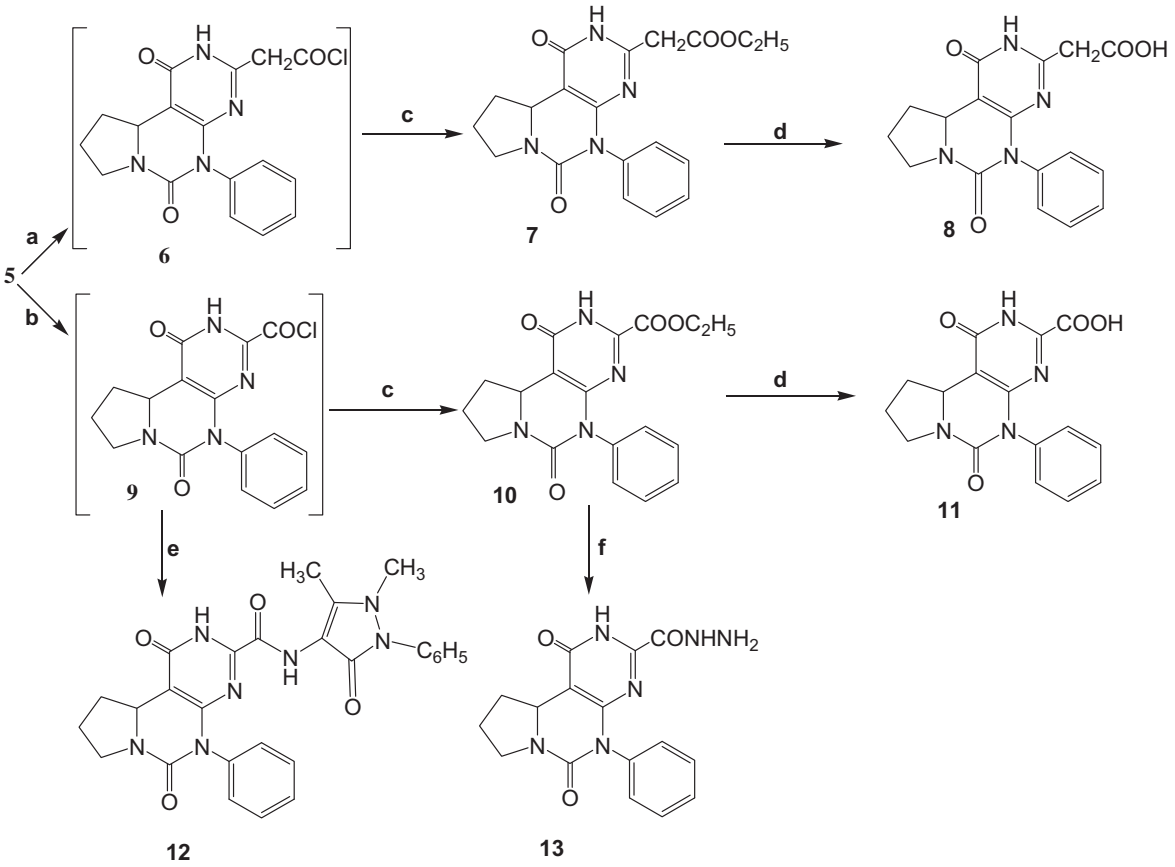
Condensation of the obtained acid hydrazide derivative **13** with different aldehydes, ketones and triethylorthoformate in the presence of absolute ethanol and catalytic amount of glacial acetic acid furnished the ylidene **14a–i** and the formamidic acid ethyl ester **15**. The IR spectra of **14a–i** and **15** revealed the disappearance of NH_2 bands. ^1H NMR of **14c** showed 2 singlets at 3.87 and 8.61 ppm attributed to the 3 protons of the OCH_3 group and the CH=N proton, respectively, in addition to multiplets of 9 aromatic protons in the range of 6.91–7.80 ppm denoting the presence of the 2 phenyl groups. The ^1H NMR of **15** displayed the triplet and quartet signals corresponding to the ethyl group at 1.32 and 4.09 ppm, respectively (Scheme 3).

The 3-carbohydrazide **13** was also reacted with different reagents in order to obtain the desired substituted pyrazolyl derivatives **16a**,**b**, **18**, **19** and **20**. Therefore, 1,3-diketones, namely acetylacetone and benzoylacetone were allowed to react with **13** in dry DMF to give the 3,5-dimethylpyrazole-1-carbonyl derivative **16a** and its 5-methyl-3-phenyl analogue **16b**. The IR spectrum of **16b** revealed the disappearance of the amino stretching bands. ^1H NMR of **16b** showed the presence of 2 singlet peaks at 2.21 and 6.18 ppm corresponding to the methyl group and the pyrazolyl proton respectively. The 3-methyl-5-hydroxypyrazolyl derivative **18** was obtained from the reaction of **13** with ethyl acetoacetate, whereby, the uncyclized ester derivative **17** was first separated. Subsequently, ring closure to the corresponding pyrazole derivative **18** was successfully performed by refluxing **17** with 2 M KOH. The IR spectrum of **17** showed the presence of the ester carbonyl stretching band at 1720 cm^{-1} and the disappearance of the amino band. The ^1H NMR of **17** revealed the presence of triplet and quartet signals at 1.18 and 4.09 ppm attributed to ethyl group confirming that condensation occurred with the acetyl group. ^1H NMR of **18** showed the disappearance of the characteristic pattern of the ethyl group in addition to the presence of 2 singlet peaks of the CH_3 and CH pyrazole protons at 2.49 and 8.00 ppm, respectively. Furthermore, the 3-hydroxypyrazolone derivative **19** was synthesized by treating the carbohydrazide **13** with diethyl malonate. The structure of **19** was confirmed by the presence of the OH absorption band at 3294 cm^{-1} in the IR spectrum and the ^1H NMR that revealed the signals of the CH_2 pyrazole and OH protons at 1.90 and 12.85 ppm respectively (Scheme 3).

Finally, the reaction of ethyl cyanoacetate with **13** yielded the 5-amino-3-hydroxypyrazole-1-carbonyl derivative **20**. The IR spectrum of **20** showed the absorption bands of OH and NH_2 group in the range of 3425–3367 cm^{-1} (Scheme 3).



Scheme 1. Reagents and solvents. a: Dimethyl sulphate, dry benzene; b: malonnitrile; c: phenyl isocyanate, methylene chloride; d: sodium borohydride, absolute ethanol.



Scheme 2. Reagents and solvents. a: Malonyl chloride, dry benzene; b: oxalyl chloride, dry benzene; c: absolute ethanol; d: 10% NaOH; e: 4-aminoantipyrine, DMF, K_2CO_3; f: hydrazine hydrate, absolute ethanol.

2.2. Pharmacological screening

2.2.1. Anti-inflammatory activity

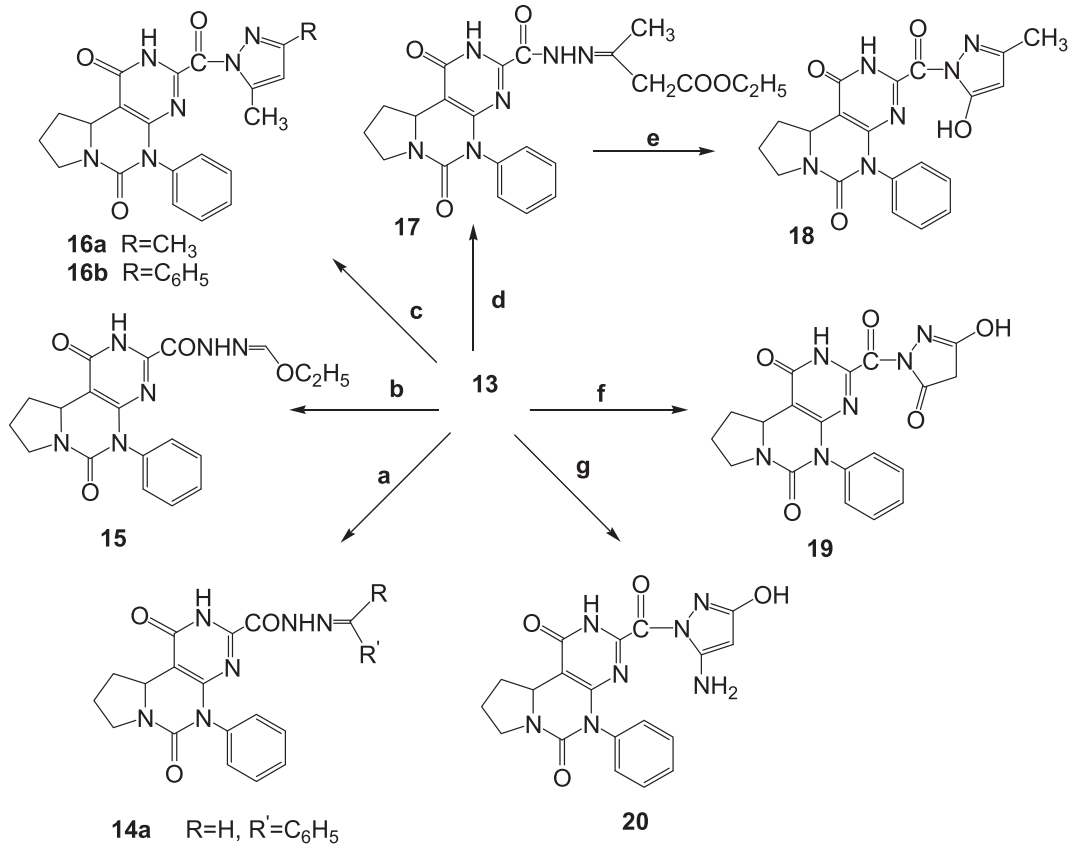
Evaluation of anti-inflammatory activity of the newly synthesized compounds was determined by the carrageenan-induced rat paw oedema model using indomethacin and celecoxib as reference drugs [25–27]. Mean changes in paw oedema thickness after 1, 2, 3, and 4 h, from induction of inflammation and inhibition% of oedema by the tested compounds were recorded in Table 1.

The newly synthesized compounds presented significant anti-inflammatory profile and most of the tested compounds revealed anti-inflammatory activity comparable to or higher than that of the reference drugs at the same dose level. The ethyl acetate ester derivative **7** and its acetic acid analogue **8** exerted an excellent activity that was the highest among the rest of tested compounds and the 2 reference drugs. Reduction of the chain length in **7** and **8** to the ethyl carboxylate derivative **10** and its 3-carboxylic acid analogue **11**, led to some decrease in activity, suggesting the importance of the acetic acid moiety for optimum activity; nevertheless, they were still more potent than indomethacin, celecoxib and most of the tested compounds.

The 3-carbohydrazide derivatives **13** and **14a–i** displayed good to moderate activity when compared with the reference drugs. The disubstituted methylene derivatives **14g–i** were noticed to be more potent than the monosubstituted benzylidene analogues **14a–e** and the reference drugs. Unexpectedly, the dimethylmethylene derivative **14f** showed a marked decrease in activity being the least active in the series, suggesting that the presence of an aromatic phenyl group might be important for optimum activity. Regarding the effect of the electronic nature of the substituent on the activity, it was observed that substitution on the phenyl group of benzylidene derivative **14a** with an electron withdrawing group led to the less active 4-chlorobenzylidene derivative **14b**. On the other hand, a non-significant change in activity was recorded for electron donating substituents as shown in compounds **14c**, **14d**, and **14e** that were equipotent having % inhibition values approaching compound **14a** and indomethacin. The ethyl formohydrazonate derivative **15** revealed activity higher than the standard drugs.

As expected, all pyrazolyl derivatives, exhibited remarkable anti-inflammatory profile and were more active than indomethacin and celecoxib as shown in compounds **12**, **16a**, **16b**, **18** and **19** or equipotent as compound **20**. The pyrazolone derivative **12** proved to exert excellent activity higher than the other pyrazole derivatives and the 2 reference drugs. The 3,5-dimethyl pyrazolyl derivative **16a** showed activity higher than its 3-phenyl analogue **16b**. Replacing one methyl group in **16a** with a hydroxyl group in **18** or replacing both methyl groups by OH and C=O in compound **19**, led to slight decrease in activity; however, it was still more potent than indomethacin. On the other hand, the presence of an additional hydrophilic amino group as shown in compound **20** further decreased the activity approaching that of indomethacin. The uncyclized ethyl hydrazono butanoate derivative **17** showed anti-inflammatory activity equal to that of indomethacin but less than its cyclized pyrazolyl bioisostere **18**.

IC_{50} values of the most active compounds **7**, **8**, **12**, **16b**, were calculated and the results were recorded in Table 2. The pyrazolone derivatives **12** exhibited the highest activity.



14a R=H, R'=C_6H_5
14b R=H, R'=4-ClC_6H_4
14c R=H, R'=4-$OCH_3C_6H_4$
14d R=H, R'=2-OHC_6H_4
14e R=H, R'=3-OH, 4-$OCH_3C_6H_3$
14f R=CH_3, R'=CH_3
14g R=CH_3, R'=C_6H_5
14h R=CH_3, R'=4-ClC_6H_4
14i R=C_6H_5, R'=C_6H_5

Scheme 3. Reagents and solvents. a: Aldehydes or ketones, absolute ethanol, glacial acetic acid; b: triethylorthoformate; c: acetylacetone or benzoylacetone, DMF; d: ethyl acetoacetate; e: 2 M KOH; f: diethyl malonate; g: ethyl cyanoacetate, DMF.

2.2.2. Analgesic activity

The newly synthesized compounds were evaluated for their analgesic activity by applying the acetic acid-induced writhing test in mice using indomethacin as a reference standard (Table 3) [28,29]. The tested compounds exhibited significant analgesic activity, whereby, some of them **7**, **14c**, **14d**, **14g**, **14i**, **20**, showed excellent activity exceeding that of indomethacin at the same dose level. Furthermore, compounds **8**, **11**, **12**, **14a**, **14f**, **14h**, **16a**, **18** also exhibited good analgesic activity with % inhibition values approaching or equal to that of the standard drug. The 3-ethyl acetate derivative **7** revealed the highest activity compared with the carboxylate ester **10** and the acid derivatives **8** and **11**. The unsubstituted benzylidene derivative **14a** exerted good activity, furthermore, substitution on the phenyl ring with electron donating groups, either OCH_3 group **14c** or OH group **14d**, increased the activity to exceed that of indomethacin. On the other hand, the presence of these 2 groups together on the phenyl ring, as shown in compound **14e**, or the presence of an electron withdrawing substituent **14b** led to decrease in activity. The diphenyl methylene derivative **14i** showed the best analgesic profile compared with all tested compounds and indomethacin. Replacement of one phenyl group with methyl group **14g** decreased the activity; however, the compound was still more active than indomethacin. On the other hand, a further decrease in activity was noted when the 2 phenyl were replaced by methyl groups **14f**. Introduction of electron withdrawing group on the phenyl ring **14h** also decreased the activity.

All pyrazole derivatives displayed good activity; compounds **12**, **16a**, 18 and **19** were nearly equipotent. However, substitution of one methyl group in **16a** by a phenyl group **16b**, led to a decrease in activity, on the other hand, substitution with hydrophilic NH_2 and OH groups **20** led to increased in activity that exceeded that of the other pyrazole derivatives and indomethacin.

2.2.3. Ulcerogenicity study

The newly synthesized compounds were tested for their ulcerogenic effect. The ratio of ulceration was recorded in Table 1. It was observed that the ester derivatives **7** and **10** had gastric safety profile better than their acid analogues **8** and **11**. The carbohydrazide derivatives **14b**, **14c**, **14e**, **14f** and **15** were found safe however, the unsubstituted carbohydrazide **13** and compounds **14a**, **14d**, **14g** and **14h** revealed certain ulcerogenic effect. On the other hand, pyrazoles **12**, **16a**, **16b**, **18**, **19**, **20**, showed good gastric tolerance.

Table 1
Anti-inflammatory activity of the synthesized compounds against carrageenan-induced paw oedema in rats and ratio of ulceration ($n = 6$).

Compd No	Ratio of ulceration	Oedema thickness (mm) ± SEM (% inhibition)			
		1 h	2 h	3 h	4 h
Control	0/6	3.573 ± 0.231[b,c]	3.808 ± 0.052[b,c]	4.060 ± 0.152[b,c]	4.162 ± 0.233[b,c]
7	1/6	0.660 ± 0.191[a,b,c] (81)	0.763 ± 0.143[a,b,c] (80)	0.580 ± 0.173[a,b,c] (90)	0.452 ± 0.163[a,b,c] (89)
8	3/6	0.805 ± 0.189[a,b,c] (77)	1.143 ± 0.199[a,b,c] (70)	1.126 ± 0.116[a,b,c] (72)	0.168 ± 0.800[a,b,c] (80)
10	1/6	1.020 ± 0.277[a] (71)	1.293 ± 0.537[a,b,c] (67)	1.292 ± 0.518[a,b,c] (68)	1.398 ± 0.466[a,b,c] (66)
11	2/6	1.377 ± 0.216[a] (68)	1.661 ± 0.912[a,c] (56)	1.375 ± 0.147[a,b,c] (66)	1.133 ± 0.923[a,b,c] (72)
12	0/6	0.780 ± 0.280[a,b,c] (78)	1.190 ± 0.252[a,b,c] (68)	0.863 ± 0.192[a,b,c] (78)	0.803 ± 0.208[a,b,c] (83)
13	2/6	1.580 ± 0.308[a] (56)	2.092 ± 0.282[a] (45)	2.022 ± 0.298[a] (50)	1.831 ± 0.274[a] (56)
14a	2/6	1.140 ± 0.264[a] (68)	2.243 ± 0.241[a] (41)	1.900 ± 0.240[a] (53)	2.072 ± 0.245[a] (50)
14b	0/6	1.640 ± 0.127[a] (54)	2.603 ± 0.274[a] (32)	2.270 ± 0.150[a] (44)	2.572 ± 0.160[a] (38)
14c	0/6	1.748 ± 0.279[a] (51)	3.000 ± 0.203[a,b] (21)	2.271 ± 0.208[a] (44)	2.223 ± 0.208[a] (47)
14d	2/6	1.692 ± 0.206[a] (53)	2.520 ± 0288[a] (34)	2.197 ± 0.168[a] (46)	2.208 ± 0.185[a] (47)
14e	0/6	2.933 ± 0.206[b,c] (18)	3.242 ± 0.099[a,b,c] (15)	2.177 ± 0.154[a] (46)	2.177 ± 0.154[a] (46)
14f	0/6	2.653 ± 0.235[a,b,c] (26)	2.937 ± 0.205[a] (23)	2.623 ± 0.199[a] (35)	2.652 ± 0.170[a,b] (36)
14g	2/6	1.402 ± 0.102[a] (61)	2.115 ± 0.261[a] (44)	1.913 ± 0.141[a] (53)	1.608 ± 0.130[a] (61)
14h	1/6	2.042 ± 0.254[a] (43)	1.898 ± 0.209[a] (50)	1.997 ± 0.258[a] (51)	1.790 ± 0.0.125[a] (57)
14i	0/6	2.013 ± 0.223[a] (44)	1.853 ± 0.233[a] (51)	1.997 ± 0.160[a] (51)	1.960 ± 0.217[a] (53)
15	0/6	1.888 ± 0.425[a] (47)	1.690 ± 0.312[a,c] (55)	1.456 ± 0.208[a,b,c] (64)	1.863 ± 0.247[a] (55)
16a	0/6	1.493 ± 0.182[a] (58)	1.631 ± 0.163[a,b,c] (57)	1.426 ± 0.217[a,b,c] (65)	1.341 ± 0.253[a,b,c] (67)
16b	0/6	0.776 ± 0.034[a] (78)	1.311 ± 0.171[a,b,c] (65)	1.636 ± 0.241[a] (59)	1.555 ± 0.201[a,c] (62)
17	0/6	1.961 ± 0.231[a] (45)	2.223 ± 0.311[a] (41)	2.120 ± 0.169[a] (47)	1.993 ± 0.144[a] (52)
18	0/6	1.545 ± 0.166[a] (57)	2.498 ± 0.226[a] (34)	1.765 ± 0.188[a] (57)	1.582 ± 0.192[a] (62)
19	0/6	1.138 ± 0.246[a] (68)	2.131 ± 0.179[a] (44)	2.090 ± 0.215[a] (49)	1.456 ± 0.190[a,c] (65)
20	0/6	1.780 ± 0.168[a] (50)	3.303 ± 0.090[a,b] (20)	2.228 ± 0.057[a] (45)	2.073 ± 0.146[a] (50)
Indo.	5/6	1.540 ± 0.300[a] (57)	2.300 ± 0.133[a] (39)	2.160 ± 0.161[a] (47)	2.020 ± 0.161[a] (51)
Celec.	5/6	1.650 ± 0.170 (54)	2.51 ± 0.172[a] (34)	2.16 ± 0.186[a] (47)	2.15 ± 0.175[a] (48)

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

Acute ulcerogenicity study was performed for compounds **7**, **8**, **12** and **16b** with the best overall anti-inflammatory profile. The ulcerogenic effect was evaluated for gastric ulcerogenic potential in rats and all compounds were found less ulcerogenic than the standard drugs (Table 4) [30,31]. The ester derivative **7** exhibited gastric ulceration lower than that produced by its acid analogue **8** and the reference drugs indomethacin and celecoxib at the same dose level.

2.2.4. COX-1/COX-2 selectivity assay

Compounds **12** and **16b** were evaluated for their selectivity to inhibit COX-1 and COX-2 isoenzymes using Cayman's COX activity assay kit according to the manufacturer's instructions [32]. The ratio of % inhibition of COX-1/COX-2 would suggest the non-selectivity of the tested compounds. The COX-1/COX-2 ratio showed that compounds **12** and **16b** have almost equal inhibitory activity on both isoenzymes compared with indomethacin (Table 5).

2.3. QSAR studies

In an attempt to correlate the anti-inflammatory and analgesic activities of the new compounds with their physicochemical properties, QSAR study was undertaken. Descriptors of the molecular modelling software, Molecular Operating Environment (MOE version 2008.10.2), 57 were used. The anti-inflammatory and analgesic activity data were expressed as % inhibition. The most relevant descriptors derived for modelling the anti-inflammatory activity are shown in Table 6; and those derived for the analgesic activity are shown in Table 7. The relation between the experimental and predicted values for anti-inflammatory and analgesic activities is illustrated in Figs. 2 and 3, respectively. These models were validated with the leave-one-out (LOO) method.

As per the anti-inflammatory activity, the best derived QSAR model for the 21 pyrimidopyrrolopyrimidine derivatives was presented by the following estimated penta-parametric equation with

Table 2
IC_{50} of the most active compounds **7**, **8**, 1**2**, **16b**.

Compd. No.	Response (% inhibition) at doses of				IC_{50} mg/kg (μmol/kg)
	1 mg/kg	2 mg/kg	4 mg/kg	6 mg/kg	
7	12	32	60	64	4.07 (11.00)
8	20	40	64	74	3.56 (10.00)
12	30	48	78	77	3.16 (6.00)
16b	20	45	65	60	3.90 (8.00)

Table 3
Effects of the compounds on acetic acid induced abdominal writhing in mice.

Compound No	Dose (mg/kg)	Writhing reflex ± SEM	Inhibition %	Potency %
Control	–	70.83 ± 1.45[b]	–	–
Indomethacin	10	24.00 ± 0.93[a]	66.12	100
7	10.68	20.66 ± 0.88[a,b]	70.83	107.12
8	9.86	30.00 ± 1.15[a,b]	57.65	87.19
10	10.26	50.00 ± 2.22[a,b]	29.41	44.48
11	9.46	25.00 ± 0.96[a]	64.70	97.85
12	15.29	26.00 ± 0.96[a]	63.29	95.72
13	9.86	48.00 ± 0.85[a,b]	32.23	48.74
14a	12.42	30.00 ± 0.96[a,b]	57.65	87.19
14b	13.42	46.16 ± 1.16[a,b]	34.83	52.68
14c	13.29	20.00 ± 1.06[a,b]	71.76	108.53
14d	12.88	22.00 ± 0.96[a]	68.94	104.26
14e	13.75	39.83 ± 0.90[a,b]	43.77	66.20
14f	11.03	29.16 ± 1.13[a,b]	58.83	88.97
14g	12.83	23.00 ± 0.96[a]	67.53	102.13
14h	13.83	26.83 ± 0.60[a]	62.12	93.95
14i	14.63	12.83 ± 0.60[a,b]	81.89	123.85
15	11.49	53.16 ± 0.07[a,b]	24.95	37.73
16a	11.72	26.83 ± 0.94[a,b]	62.12	93.95
16b	13.51	42.76 ± 0.83[a,b]	39.63	59.79
17	13.12	73.00 ± 0.96[a,b]	1.17	1.77
18	11.78	27.71 ± 0.60[a]	61.00	92.25
19	11.84	27.00 ± 0.66[a]	61.88	93.58
20	11.81	21.00 ± 0.96[a,b]	70.35	106.40

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

Table 4
Ulcer index of the most active compounds **7**, **8**, **12**, **16b**.

Cpd	% incidence	Average	Severity	Ulcer index
Indo.	10	3.33	1.40	14.73
Celec.	6.66	3.16	1.31	11.13
7	5	1	1	7
8	8.33	1.16	1	10.49
12	5	1	1.16	7.16
16b	6.66	3	1.28	10.94

correlation coefficient (R^2) = 0.7528 and root mean square error (RMSE) = 0.06641.

Anti-inflammatory (% inhibition) = −5.43095−1.54154 a_nO − 10.18241 log *P* + 464.65895 vsurf_CP + 2.64391 mr + 9.17934 dipole.

From the equation positive correlation of vsurf_CP (i3D critical packing parameter), mr (2D molar refractivity) and dipole (i3D dipole moment) with the anti-inflammatory activity was observed. On the other hand, the activity was negatively correlated with a_nO (2D number of oxygen atoms) and log *P* (2D log octanol/water partition coefficient). The high coefficient value of vsurf_CP reflected its high impact on the anti-inflammatory activity i.e. it was the most influencing parameter. Critical packing parameter defines a ratio between the hydrophilic and lipophilic part of a molecule. In contrast to the hydrophilic−lipophilic balance, critical packing refers just to molecular shape. It is defined as: volume (lipophilic part)/[(surface)(hydrophilic part)(length of lipophilic part)]. Lipophilic and hydrophilic calculations are performed at −0.6 and −3.0 kcal/mol, respectively. Critical packing is a good parameter to predict molecular packing such as in micelle formation, and may be relevant in solubility studies in which the melting point plays an important role [33,34]. The influence of CP was in agreement with the experimental data, where the most active compound **7** (inhibition % = 89) had vsurf_CP = 0.1562; whereas, the compounds of low inhibition% (around 50%) possessed vsurf_CP values of 0.0628−0.0981.

Regarding the analgesic activity, the best derived QSAR model was presented by the following estimated penta-parametric equation with correlation coefficient (R^2) = 0.6547 and root mean square error (RMSE) = 0.12196.

Analgesic activity (% inhibition) = 67.43728 + 24.50080 dipole + 14.84819 Slog *P* − 0.9148 vsurf_D1 + 0.22627 TPSA + 0.40038 zagreb.

From the equation positive correlation of dipole (i3D dipole moment), Slog *P* (2D log octanol/water partition coefficient), TPSA (2D topological polar surface area) and Zagreb (2D Zagreb index) with the analgesic activity was observed. The activity was negatively correlated with vsurf_D1 (i3D hydrophobic volume). The most influencing parameter was the dipole moment as revealed from its high coefficient value.

Comparing the QSAR models for both anti-inflammatory and analgesic activities, it could be revealed that the parameters that affected one activity were different from those which affected the other activity. This could explain the experimental biological activities in which the highly active compounds in anti-inflammatory screening (e.g. **8**, **12**) were not as potent in the analgesic activity and vice versa.

Table 5
COX-1/COX-2 ratio of compounds **12**, **16b** and indomethacin.

Compound No	COX-1/COX-2
12	1.30
16b	1.02
Indomethacin	3.98

3. Experimental

3.1. Chemistry

Melting points were uncorrected and were determined on Electrothermal Stuart $5MP_3$ digital melting point apparatus. Elemental microanalyses were performed at the microanalytical center, Faculty of Science, Cairo University and the Regional Center for Mycology and Biotechnology, Al-Azhar University. The infrared spectra were carried out on a Bruker FT-IR spectrophotometer and Shimadzu FT-IR 8400S infrared spectrophotometer and were expressed in wave number (cm^{-1}) using potassium bromide disc. The ^1H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 300 MHz and on NMR JOEL (Eclipse) 400 at 400 MHz in the specified solvent (exchanged with D_2O). ^{13}C NMR spectra were recorded on NMR JOEL (Eclipse) 400 at 100 MHz in DMSO-d_6. Mass spectra were performed on HP MODEL: MS_5988 mass spectrometer and on Shimadzu QP-2010 Plus (EI, 70 eV).

Compounds **2**–**5** were prepared according to the reported procedures [22–24].

3.1.1. General procedure for the preparation of *7*, *10*

To a suspension of **5** (1 mmol) in dry benzene (10 ml) the appropriate acid chloride malonyl or oxalyl chloride (1.1 mmol) was added, and the reaction mixture was stirred for 3 h at room temperature (R.T.). The solvent was removed under vacuum, the residue washed twice with dry benzene (5 ml) and the solvent was removed by distillation at each time. To the obtained 3-chloroacetyl or 3-chlorocarbonyl intermediate **6** or **9**, absolute ethanol (30 ml) was added and the reaction mixture was refluxed for 2 h. The separated precipitate was filtered, washed with water and crystallized from absolute ethanol.

3.1.1.1. Ethyl 2-(1,6-dioxo-5-phenyl-1,2,5,6,8,9,10,10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidin-3-yl)acetate ***7***. Yellow solid, 76% yield, m.p. 239–240 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3515 (NH stretching), 3035 (CH aromatic), 2962–2831 (CH aliphatic), 1743, 1685, 1674 (3C=O). ^1H NMR (DMSO-d_6, 400 MHz): δ 1.08 (t, 3H, $CH_2\underline{CH_3}$, J = 8 Hz), 1.60–1.95 (m, 3H, pyrrolidine), 2.62–2.75 (m, 1H, pyrrolidine), 3.43 (m, 1H, pyrrolidine), 3.51 (s, 2H, $\underline{CH_2}COOC_2H_5$), 3.60–3.67 (m, 1H, pyrrolidine), 3.93 (q, 2H, $\underline{CH_2}CH_3$, J = 8 Hz), 4.42–4.47 (m, 1H, pyrrolidine), 7.12–7.37 (m, 5H, aromatic protons), 12.46 (s, 1H, NH). ^{13}C NMR: 14.3, 32.9, 45.4, 55.5, 61.3, 62.9, 98.8, 128.8, 130.6, 137.7, 142.0, 151.0, 186.5, 195.4. Anal. Calcd. for $C_{19}H_{20}N_4O_4$ (368.39): C, 61.95; H, 5.47; N, 15.21. Found: C, 62.18; H, 5.52; N, 15.43.

3.1.1.2. Ethyl 1,6-dioxo-5-phenyl-1,2,5,6,8,9,10,10a,-octahydropyrimido [5,4-e]pyrrolo[1,2-c]pyrimidine-3-carboxylate ***10***. Light brown solid, 80% yield, m.p. 270–272 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3140 (NH stretching), 3050 (CH aromatic), 2982–2860 (CH aliphatic), 1759, 1688, 1645 (3C=O). ^1H NMR (DMSO-d_6, 300 MHz): δ 1.17 (t, 3H, $CH_2\underline{CH_3}$, J = 10 Hz), 1.60–1.98 (m, 3H, pyrrolidine), 2.62–2.78 (m, 1H, pyrrolidine), 3.23–3.27 (m, 1H, pyrrolidine), 3.60–3.75 (m, 1H, pyrrolidine), 4.14 (q, 2H, $\underline{CH_2}CH_3$, J = 10 Hz), 4.5–4.6 (m, 1H, pyrrolidine), 7.10–7.50 (m, 5H, aromatic protons), 12.90 (s, 1H, NH). MS: m/z (%): 355.30 (M^+ + 1, 1.03), 354.35 (M^+, 2.28), 119.15 (100). Anal. Calcd. for $C_{18}H_{18}N_4O_4$ (354.13): C, 61.01; H, 5.12; N, 15.81. Found: C, 61.27; H, 5.33; N, 16.22.

3.1.2. General procedure for the preparation of *8*, *11*

The ester derivative **7** or **10** (1 mmol) was refluxed with 10% NaOH (20 ml) for 2 h, the solution was cooled, diluted with water (10 ml) and acidified with 1 N HCl. The precipitated solid was filtered, washed with water and crystallized from ethanol.

Table 6
The molecular descriptor values, experimental and predicted activity values of the anti-inflammatory activities of the studied compounds.

Compound No	a_nO	logP	vsurf_CP	Mr	Dipole	Experimental activity (% inhibition)	Predicted activity (% inhibition)	Residual values (RES)
7	4.000	1.5260	0.1562	9.7686	1.0673	89	81.0541	7.9459
8	4.000	0.7490	0.1073	8.7860	1.3034	80	65.8236	14.1764
10	4.000	2.0200	0.1574	9.2891	0.9900	66	74.6393	−8.6393
11	4.000	0.6610	0.1258	8.3080	1.3834	72	74.8131	−2.8131
12	4.000	2.0880	0.1236	14.0352	2.1066	83	80.9965	2.0035
13	3.000	−0.4870	0.0889	8.7324	0.4813	56	63.7136	−7.7136
14a	3.000	3.3520	0.0981	12.0228	1.8238	50	49.9208	0.0792
14b	3.000	3.3630	0.0956	12.1989	1.3925	38	45.1661	−7.1661
14c	4.000	2.7570	0.0893	12.6920	1.7770	47	51.6870	−4.6870
14d	4.000	2.4610	0.0904	11.8350	1.9134	47	54.1963	−7.1963
14e	5.000	2.4150	0.0698	12.4840	2.1237	46	47.2239	−1.2239
14g	3.000	3.2780	0.1215	12.4706	1.5722	61	60.4411	0.5589
14h	3.000	3.2890	0.1362	12.6571	1.4587	57	66.5770	−9.5770
14i	3.000	5.5210	0.1256	14.8254	1.5560	53	45.5488	7.4512
15	4.000	1.4410	0.0992	10.3103	1.2188	55	58.2716	−3.2716
16a	3.000	1.4600	0.1091	10.8234	1.1649	67	65.0872	1.9128
16b	3.000	3.7030	0.1195	13.1991	1.1115	62	52.8851	9.1149
17	5.000	1.2790	0.1023	11.7958	0.6455	52	58.4882	−6.4882
18	4.000	0.7720	0.0809	10.2970	0.8494	62	53.1575	8.8425
19	4.000	0.6930	0.0807	10.4401	1.5504	65	60.6814	4.3187
20	5.000	0.5170	0.0628	10.1106	1.1028	50	47.6276	2.3724

*3.1.2.1. 2-(1,6-Dioxo-5-phenyl-1,2,5,6,8,9,10,10a,-octahydropyrimido [5,4-e]pyrrolo[1,2-c]pyrimidin-3-yl)acetic acid **8**.* Pale yellow solid, 68% yield, m.p. >340 °C (decomposition). IR: $\upsilon_{max.}/cm^{-1}$ 3100 (NH stretching), 3035 (CH aromatic), 2962–2831 (CH aliphatic), 2785–2600 (br, OH), 1743, 1674, 1635 (3C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.62–1.88 (m, 3H, pyrrolidine), 2.05 (s, 2H, $\underline{CH_2}$COOH), 2.68–2.72 (m, 1H, pyrrolidine), 3.21–3.29 (m, 1H, pyrrolidine), 3.58–3.64 (m, 1H, pyrrolidine), 4.39–4.44 (m, 1H, pyrrolidine), 7.13–7.41 (m, 6H, aromatic protons + NH), 12.28 (s, 1H, OH). Anal. Calcd. for $C_{17}H_{16}N_4O_4$ (340.33): C, 59.99; H, 4.74; N, 16.46. Found: C, 60.17; H, 4.92; N, 16.78.

*3.1.2.2. 1,6-Dioxo-5-phenyl-1,2,5,6,8,9,10,10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-3-carboxylic acid **11**.* White solid, 71% yield, m.p. 282–284 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3394 (NH stretching), 3050 (CH aromatic), 2974–2877 (CH aliphatic), 2792–2600 (br, OH), 1759, 1666, 1635 (3C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.70–1.90 (m, 3H, pyrrolidine), 2.70–2.75 (m, 1H, pyrrolidine), 3.24–3.32 (m, 1H, pyrrolidine), 3.60–3.69 (m, 1H, pyrrolidine), 4.45–4.54 (m, 1H, pyrrolidine), 7.16–7.42 (m, 5H, aromatic protons), 7.90 (s, 1H, NH), 12.60 (br, s, 1H, OH). MS: *m/z* (%): 327.20 (M^+ + 1, 0.09), 326.20 (0.05), 281 (100). Anal. Calcd. for $C_{16}H_{14}N_4O_4$ (326.31): C, 58.89; H, 4.32; N, 17.17. Found: C, 59.13; H, 4.41; N, 17.36.

*3.1.3. N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-1,6-dioxo-5-phenyl-1,2,5,6,8,9,10,10a-octahydropyrimido[5,4-e] pyrrolo[1,2-c]pyrimidine-3-carboxamide **12***

To a solution of the acid chloride intermediate **9** (1 mmol) in DMF (10 ml), K_2CO_3 (1 mmol) and 4-aminoantipyrine (1 mmol) were added. The reaction mixture was heated at 100 °C for 8 h and then cooled to R.T. The reaction mixture was poured on cold water; the separated solid was filtered, washed, dried and crystallized from ethanol to give yellowish white crystals, 60% yield, m.p. 228–229 °C. IR: $\upsilon_{max.}/cm^{-1}$ 3205, 3132 (2NH stretching), 3070 (CH aromatic),

Table 7
The molecular descriptor values, experimental and predicted activity values of the analgesic activities of the studied compounds.

Compound No	Dipole	Slog *P*	vsurf_D1	TPSA	Zagreb	Experimental activity (% inhibition)	Predicted activity % inhibition	Residual values (RES)
8	1.3034	1.3056	713.6250	102.3100	138.0000	57.6500	60.5151	−2.8651
10	0.9900	1.7416	847.8750	91.3100	142.0000	29.4100	32.7158	−3.3058
11	1.3834	0.9155	737.8750	102.3100	134.0000	64.7000	50.4388	14.2612
12	2.1066	2.4479	1077.8750	117.6600	206.0000	63.2900	58.1084	5.1816
13	0.4813	−0.1792	653.6250	120.1300	138.0000	32.2300	33.8463	−1.6163
14a	1.8238	2.9033	1013.6250	106.4700	166.0000	57.6500	51.6960	5.9540
14b	1.3925	2.6346	1038.3750	106.4700	180.0000	34.8300	38.0054	−3.1754
14c	1.7770	2.5298	882.6250	115.7000	174.0000	71.7600	75.3796	−3.6196
14d	1.9134	1.6868	957.2500	126.7000	180.0000	68.9400	56.8046	12.1354
14e	2.1237	1.6954	967.0000	135.9300	190.0000	43.7700	66.3104	−22.5404
14f	1.8301	1.3429	834.6250	106.4700	152.0000	58.8300	57.3502	1.4798
14g	1.5722	3.2934	908.0000	106.4700	172.0000	67.5300	73.9510	−6.4210
14h	1.4587	3.0247	1027.2500	106.4700	186.0000	62.1200	49.9520	12.1680
14i	1.5560	5.2439	1080.2500	106.4700	192.0000	81.8900	77.5407	4.3493
15	1.2188	0.9269	747.8750	115.7000	154.0000	24.9500	55.6965	−30.7465
16a	1.1649	1.9846	870.3750	99.9000	170.0000	62.1200	49.4555	12.6645
16b	1.1115	3.7990	1044.8750	99.9000	190.0000	39.6300	49.6817	−10.0517
17	0.6455	1.2762	981.2500	132.7700	174.0000	1.1700	14.0198	−12.8498
18	1.5504	0.9657	808.0000	114.7500	170.0000	61.0000	59.0755	1.9245
19	1.1028	0.4613	738.6250	134.9800	170.0000	61.8800	58.4816	3.3984
20	0.8494	0.6556	796.1250	146.1500	170.0000	70.3500	46.6750	23.6750

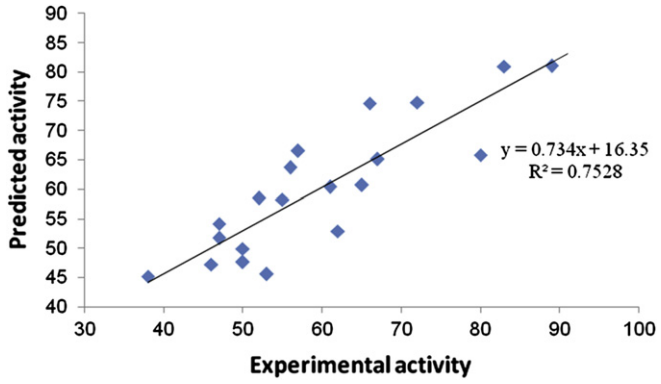


Fig. 2. Experimental vs predicted anti-inflammatory activity represented as inhibition%.

2927–2885 (CH aliphatic), 1766, 1743, 1675, 1651 (4C=O). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.94 (s, 3H, CH_3–C), 1.72–1.90 (m, 3H, pyrrolidine), 2.35–2.42 (m, 1H, pyrrolidine), 3.16 (s, 3H, N–CH_3) 3.32–3.45 (m, 1H, pyrrolidine), 3.96–4.04 (m, 1H, pyrrolidine), 5.32–5.40 (m, 1H, pyrrolidine), 7.05–7.65 (m, 11H, aromatic protons + NH), 11.63 (s, 1H, NH). ^{13}C NMR; 11.3, 35.3, 53.1, 116.2, 126.3, 128.1, 129.6, 134.8, 151.8, 153.3, 156.9, 160.2. MS: *m/z* (%): 526.20 (M^+ − 1, 1.87), 80.00 (100). Anal. Calcd. for $C_{27}H_{25}N_7O_4$ (527.57): C, 61.41; H, 4.73; N, 18.57. Found: C, 61.58; H, 4.85; N, 18.79.

3.1.4. 1,6-Dioxo-5-phenyl-1,2,5,6,8,9,10,10a,-octahydropyrimido [5,4-e]pyrrolo[1,2-c]pyrimidine-3-carbohydrazide ***13***

To a mixture of **10** (0.35 g, 1 mmol) in absolute ethanol (20 ml), hydrazine hydrate (99%) (10 mmol) was added. The mixture was stirred for 5 h at R.T. The separated white precipitate was filtered and crystallized from absolute ethanol to give white crystals, 70% yield, m.p. >340 °C (decomposition). IR: $\upsilon_{max.}$/cm^{-1} 3398, 3317, 3244 (NH_2, 2NH stretching), 3063 (CH aromatic), 2884 (CH aliphatic), 1700–1681 (br, 3C=O). ^{1}H NMR ($CHCl_3$, 300 MHz): δ 1.84–2.04 (m, 3H, pyrrolidine), 2.97–2.99 (m, 1H, pyrrolidine), 3.43–3.50 (m, 1H, pyrrolidine), 3.80–4.10 (m, 3H, pyrrolidine + NH_2), 4.58–4.63 (m, 1H, pyrrolidine), 7.20–7.48 (m, 6H, aromatic protons + NH), 7.90 (s, 1H, NH). Anal. Calcd. for $C_{16}H_{16}N_6O_3$ (340.34): C, 56.47; H, 4.74; N, 24.69. Found: C, 56.72; H, 4.80; N, 25.06.

3.1.5. General procedure for the preparation of ***14a–i***

To a solution of the hydrazide **13** (0.34 g, 1 mmol) in ethanol (20 ml), few drops of acetic acid and the appropriate aldehyde or ketone (1 mmol) were added. The reaction mixture was heated under reflux for 5 h and then cooled. The solvent was removed under vacuum; the separated solid was filtered, dried and crystallized from the appropriate solvent.

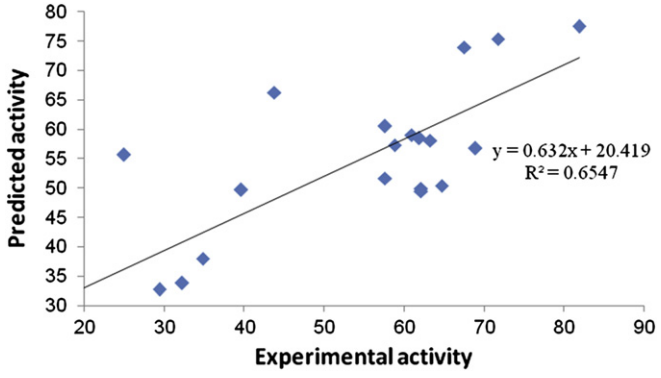


Fig. 3. Experimental vs predicted analgesic activity represented as inhibition%.

3.1.5.1. N′-Benzylidene-1,6-dioxo-5-phenyl-1,2,5,6,8,9,10,10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-3-carbohydrazide ***14a***. Light brown solid, crystallized from ethanol, 69% yield, m.p. 264–265 °C. IR: $\upsilon_{max.}$/cm^{-1} 3425, 3264 (2NH stretching), 3063 (CH aromatic), 2974 (CH aliphatic), 1724–1650 (br, 3C=O). ^{1}H NMR ($CHCl_3$, 300 MHz): δ 1.80–2.10 (m, 3H, pyrrolidine), 2.85–3.20 (m, 1H, pyrrolidine), 3.42–3.57 (m, 1H, pyrrolidine), 3.80–3.98 (m, 1H, pyrrolidine), 4.59–4.69 (m, 1H, pyrrolidine), 7.27–7.74 (m, 10H, aromatic protons), 7.77, (s, 1H, CH=N), 9.61, (s, 1H, NH), 10.25 (s, 1H, NH). Anal. Calcd. for $C_{23}H_{20}N_6O_3$ (428.44): C, 64.48; H, 4.71; N, 19.62. Found: C, 65.08; H, 4.91; N, 19.24.

3.1.5.2. N′-(4-Chlorobenzylidene)-1,6-dioxo-5-phenyl-1,2,5,6,8,9,10, 10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-3-carbohydrazide ***14b***. Light brown solid, crystallized from ethanol, 64% yield, m.p. 261–262 °C. IR: $\upsilon_{max.}$/cm^{-1} 3439–3320 (br, 2NH), 3044 (CH aromatic), 2992–2938 (CH aliphatic), 1691–1620 (3C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.72–1.93 (m, 3H, pyrrolidine), 2.62–2.79 (m, 1H, pyrrolidine), 3.23–3.40 (m, 1H, pyrrolidine), 3.62–3.79 (m, 1H, pyrrolidine), 4.5–4.6 (m, 1H, pyrrolidine), 7.15–7.74 (m, 9H, aromatic protons), 8.08, (s, 1H, CH=N), 11.19, (s, 1H, NH), 13.00 (s, 1H, NH). Anal. Calcd. for $C_{23}H_{19}ClN_6O_3$ (462.89): C, 59.68; H, 4.14; N, 18.16. Found: C, 60.10; H, 4.17; N, 18.38.

3.1.5.3. N′-(4-Methoxybenzylidene)-1,6-dioxo-5-phenyl-1,2,5,6,8,9,10, 10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-3-carbohydrazide ***14c***. Orange solid, crystallized from aqueous ethanol, 72% yield, m.p. 252–254 °C. IR: $\upsilon_{max.}$/cm^{-1} 3474, 3284 (2NH), 3050 (CH aromatic), 2930–2839 (CH aliphatic), 1720, 1646, 1602 (3C=O). ^{1}H NMR ($CHCl_3$, 300 MHz): δ 1.80–2.10 (m, 3H, pyrrolidine), 2.90–3.20 (m, 1H, pyrrolidine), 3.42–3.55 (m, 1H, pyrrolidine), 3.70–3.79 (m, 1H, pyrrolidine), 3.87 (s, 3H, O–CH_3) 4.60–4.69 (m, 1H, pyrrolidine), 6.91–7.80 (m, 9H, aromatic protons), 8.61, (s, 1H, CH=N), 9.53 (s, 1H, NH), 10.1 (s, 1H, NH). ^{13}C NMR: 19.3, 45.7, 56.4, 115.0, 127.2, 128.1, 129.6, 130.8, 137.9, 138.2, 157.0, 162.1, 162.5. Anal. Calcd. for$C_{24}H_{22}N_6O_4$ (458.47): C, 62.87; H, 4.84; N, 18.33. Found: C, 63.02; H, 4.70; N, 18.71.

3.1.5.4. N′-(2-Hydroxybenzylidene)-1,6-dioxo-5-phenyl-1,2,5,6,8,9,10, 10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-3-carbohydrazide ***14d***. Light brown solid, crystallized from acetic acid, 72% yield, m.p. 217–218 °C. IR: $\upsilon_{max.}$/cm^{-1} 3444 (OH), 3290, 3271 (2NH), 3043 (CH aromatic), 2939–2854 (CH aliphatic), 1700–1610 (br, 3C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.70–2.00 (m, 3H, pyrrolidine), 2.65–2.80 (m, 1H, pyrrolidine), 3.29–3.40 (m, 1H, pyrrolidine), 3.70–3.79 (m, 1H, pyrrolidine), 4.50–4.60 (m, 1H, pyrrolidine), 6.89–7.70 (m, 9H, aromatic protons), 9.00 (s, 1H, CH=N), 10.70 (s, 1H, NH), 11.13 (s, 1H, OH), 11.30 (s, 1H, NH). Anal. Calcd. for $C_{23}H_{20}N_6O_4$ (444.44): C, 62.16; H, 4.54; N, 18.91. Found: C, 62.34; H, 4.80; N, 19.37.

3.1.5.5. N′-(3-Hydroxy-4-methoxybenzylidene)-1,6-dioxo-5-phenyl-1,2, 5,6,8,9,10,10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-3-carbohydrazide ***14e***. Light brown solid, crystallized from ethanol, 77% yield, m.p. 278–280 °C. IR: $\upsilon_{max.}$/cm^{-1} 3474 (OH), 3255, 3217 (2NH), 3059 (CH aromatic), 2931–2893 (CH aliphatic), 1681, 1639, 1630 (3C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.71–2.00 (m, 3H, pyrrolidine), 2.63–2.80 (m, 1H, pyrrolidine), 3.29–3.40 (m, 1H, pyrrolidine), 3.65–3.72 (m, 1H, pyrrolidine), 3.80 (s, 3H, O–CH_3), 4.52–4.57 (m, 1H, pyrrolidine), 6.81–7.48 (m, 8H, aromatic protons), 7.92 (s, 1H, CH=N), 9.68 (s, 1H, NH), 10.87 (s, 1H, OH), 12.8 (s, 1H, NH). Anal. Calcd. for $C_{24}H_{22}N_6O_5$ (474.47): C, 60.75; H, 4.67; N, 17.71. Found: C, 60.71; H, 4.80; N, 17.93.

3.1.5.6. 1,6-Dioxo–5-phenyl-N′-(propan-2-ylidene)-1,2,5,6,8,9,10,10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-3-carbohydrazide ***14f***. Light brown solid, crystallized from absolute ethanol, 70% yield, m.p. 268–270 °C. IR: $\upsilon_{max.}$/cm^{-1} 3325 (br, 2NH), 3020 (CH aromatic), 2950–2850 (CH aliphatic), 1690, 1674 (br) (3C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.39, (s, 3H, CH_3), 1.70–1.87 (m, 3H, pyrrolidine), 1.92 (s, 3H, CH_3), 2.65–2.80 (m, 1H, pyrrolidine), 3.29–3.40 (m, 1H, pyrrolidine), 3.63–3.78 (m, 1H, pyrrolidine), 4.48–4.59 (m, 1H, pyrrolidine), 7.26–7.46 (m, 5H, aromatic protons), 9.61 (s, 1H, NH), 12.85 (s, 1H, NH). Anal. Calcd. for $C_{19}H_{20}N_6O_3$ (380.40): C, 59.99; H, 5.30; N, 22.09. Found: C, 60.40; H, 5.90; N, 22.34.

3.1.5.7. 1,6-Dioxo-5-phenyl-N′-(1-phenylethylidene)-1,2,5,6,8,9,10,10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-3-carbohydrazide ***14g***. Brown solid, crystallized from ethanol, 73% yield, m.p. 310–311 °C. IR: $\upsilon_{max.}$/cm^{-1} 3309, 3151 (2NH stretching), 3059 (CH aromatic), 2958–2877 (CH aliphatic), 1720, 1689, 1647 (3C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.79, (s, 3H, CH_3), 1.92–2.00 (m, 3H, pyrrolidine), 2.65–2.80 (m, 1H, pyrrolidine), 3.29–3.40 (m, 1H, pyrrolidine), 3.64–3.71 (m, 1H, pyrrolidine), 4.50–4.59 (m, 1H, pyrrolidine), 7.30–7.78 (m, 10H, aromatic protons), 9.99 (s, 1H, NH), 13.04 (s, 1H, NH). MS: *m*/*z* (%): 442.45 (M^+, 29.69), 77.10 (100). Anal. Calcd. for $C_{24}H_{22}N_6O_3$ (442.47): C, 65.15; H, 5.01; N, 18.99. Found: C, 65.24; H, 4.98; N, 18.99.

3.1.5.8. N′-[1-(4-Chlorophenyl)ethylidene]-1,6-dioxo-5-phenyl-1,2,5,6,8,9,10,10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-3-carbohydrazide ***14h***. Brown solid, crystallized from ethanol, 76% yield, m.p. 157–158 °C. IR: $\upsilon_{max.}$/cm^{-1} 3367, 3305 (2NH), 3039 (CH aromatic), 2958–2881 (CH aliphatic), 1720, 1697, 1643 (3C=O). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.72–2.00 (m, 3H, pyrrolidine), 1.78 (s, 3H, CH_3), 2.65–2.78 (m, 1H, pyrrolidine), 3.35–3.40 (m, 1H, pyrrolidine), 3.65–3.72 (m, 1H, pyrrolidine), 4.53–4.63 (m, 1H, pyrrolidine), 7.31–7.80 (m, 9H, aromatic protons), 10.1 (s, 1H, NH), 12.81 (s, 1H, NH). Anal. Calcd. for $C_{29}H_{21}ClN_6O_3$ (476.91): C, 60.44; H, 4.44; N, 17.62. Found: C, 60.72; H, 4.81; N, 18.09.

3.1.5.9. N′-(Diphenylmethylene)-1,6-dioxo-5-phenyl-1,2,5,6,8,9,10,10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-3-carbohydrazide ***14i***. Brown solid, crystallized from ethanol, 68% yield, m.p. 287–289 °C. IR: $\upsilon_{max.}$/cm^{-1} 3306, 3150 (2NH stretching), 3043 (CH aromatic), 2974–2860 (CH aliphatic), 1710, 1697, 1647 (3C=O). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.65–1.92 (m, 3H, pyrrolidine), 2.65–2.75 (m, 1H, pyrrolidine), 3.24–3.35 (m, 1H, pyrrolidine), 3.55–3.65 (m, 1H, pyrrolidine), 4.45–4.55 (m, 1H, pyrrolidine), 6.95–7.70 (m, 15H, aromatic protons), 9.65 (s, 1H, NH), 12.80 (s, 1H, NH). ^{13}C NMR: 21.3, 45.0, 45.3, 92.9, 127.3, 128.2, 128.5, 128.8, 130.1, 130.2, 136.1, 137.0, 150.0, 150.1, 150.2, 150.3. Anal. Calcd. for $C_{29}H_{24}N_6O_3$ (504.54): C, 69.04; H, 4.79; N, 16.66. Found: C, 69.43; H, 4.88; N, 16.97.

3.1.6. Ethyl N′-(1,6-dioxo-5-phenyl-1,2,5,6,8,9,10,10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-3-carbonyl) formohydrazonate ***15***

A mixture of **13** (0.34 g, 1 mmol) and a catalytic amount of acetic anhydride in triethylorthoformate (5 ml) was heated for 3 h, the mixture was cooled to R.T. and poured on ice water (20 ml). The separated solid was filtered, washed with water and crystallized from ethanol to give orange solid, 72% yield, m.p. 234–235 °C. IR: $\upsilon_{max.}$/cm^{-1} 3450, 3331, 3150 (2NH stretching), 3059 (CH aromatic), 2929–2884 (CH aliphatic), 1720–1639, (3C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.32 (t, 3H, $CH_2\underline{CH_3}$, J = 7.2 Hz), 1.80–2.10 (m, 3H, pyrrolidine), 2.90–3.10 (m, 1H, pyrrolidine), 3.42–3.57 (m, 1H, pyrrolidine), 3.80–3.88 (m, 1H, pyrrolidine), 4.09 (q, 2H, $\underline{CH_2}CH_3$, J = 7.2 Hz), 4.61–4.65 (m, 1H, pyrrolidine), 6.67 (s, 1H, N=CH), 7.25–7.49 (m, 5H, aromatic protons), 9.84 (s, 1H, NH), 10.32 (s, 1H, NH). Anal. Calcd. for $C_{19}H_{20}N_6O_4$ (396.4): C, 57.57; H, 5.09; N, 21.20. Found: C, 57.99; H, 4.79; N, 21.53.

3.1.7. General procedure for the preparation of ***16a,b***

To a solution of **13** (0.34 g, 1 mmol) in dry DMF (5 ml) acetylacetone or benzoylacetone (2 mmol), was added. 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, and crystallized from absolute ethanol.

3.1.7.1. 3-(3,5-Dimethyl-1H-pyrazole-1-carbonyl)-5-phenyl-8,9,10,10a,-tetrahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-1,6(2H,5H)-dione ***16a***. Yellow solid, crystallized from ethanol, 70% yield, m.p. 209–210 °C. IR: $\upsilon_{max.}$/cm-^{1} 3250 (NH), 3050 (CH aromatic), 2939–2827 (CH aliphatic), 1700–1651 (br, 3C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.85–2.24 (m, 3H, pyrrolidine), 2.44 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 2.95–3.00 (m, 1H, pyrrolidine), 3.40–3.52 (m, 1H, pyrrolidine), 3.78–3.92 (m, 1H, pyrrolidine), 4.51–4.71 (m, 1H, pyrrolidine), 6.20 (s, 1H, pyrazole proton), 7.24–7.47 (m, 5H, aromatic protons), 9.61 (s, 1H, NH). MS: *m*/*z* (%): 404.30 (M^+, 2.08), 96.15 (100). Anal. Calcd. for $C_{21}H_{20}N_6O_3$ (404.42): C, 62.37; H, 4.98; N, 20.78. Found: C, 62.49; H, 4.87; N, 21.14.

3.1.7.2. 3-(5-Methyl-3-phenyl-1H-pyrazole-1-carbonyl)-5-phenyl-8,9,10,10a,-tetrahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-1,6(2H,5H)-dione ***16b***. Orange solid, crystallized from ethanol, 80% yield, m.p. 260–262 °C. IR: $\upsilon_{max.}$/cm^{-1} 3329 (NH), 3062 (CH aromatic), 2970–2850 (CH aliphatic), 1720, 1685, 1647 (3C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.86–2.10 (m, 3H, pyrrolidine), 2.21 (s, 3H, CH_3), 2.95–3.00 (m, 1H, pyrrolidine), 3.40–3.52 (m, 1H, pyrrolidine), 3.78–3.93 (m, 1H, pyrrolidine), 4.50–4.71 (m, 1H, pyrrolidine), 6.18 (s, 1H, pyrazole proton), 7.26–7.95 (m, 10H, aromatic protons), 9.68 (s, 1H, NH). Anal. Calcd. for $C_{26}H_{22}N_6O_3$ (466.18): C, 66.94; H, 4.75; N, 18.02. Found: C, 66,69; H, 5.00; N, 18.37.

3.1.8. Ethyl 3-[2-(1,6-dioxo-5-phenyl-1,2,5,6,8,9,10,10a,-octahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-3-carbonyl) hydrazono]butanoate ***17***

A mixture of the hydrazide **13** (0.34 g, 1 mmol) and ethyl acetoacetate (10 ml) were heated under reflux for 5 h. The reaction mixture was cooled to R.T. and the separated solid was filtered washed with water and crystallized from ethanol to give orange solid, 85% yield, m.p. 239–240 °C. IR: $\upsilon_{max.}$/cm^{-1} 3367, 3232 (2NH), 3062 (CH aromatic), 2989–2893 (CH aliphatic), 1720–1685, 1670–1647 (br, 4C=O). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.18 (t, 3H, $CH_2\underline{CH_3}$, J = 8 Hz), 1.45 (s, 3H, CH_3–C), 1.67–1.95 (m, 3H, pyrrolidine), 2.68–2.78 (m, 1H, pyrrolidine), 3.25–3.30 (m, 1H, pyrrolidine), 3.31 (s, 2H, CH_2), 3.64–3.73 (m, 1H, pyrrolidine), 4.09 (q, 2H, $\underline{CH_2}CH_3$, J = 8 Hz), 4.50–4.59 (m, 1H, pyrrolidine), 7.28–7.45 (m, 5H, aromatic protons), 9.75(s, 1H, NH), 10.30 (s, 1H, NH). ^{13}C NMR: 14.6, 15.6, 21.5, 44.3, 45.3, 60.9, 115.1, 129.2, 130.5, 137.4, 150.5, 165.8, 169.9, 195.0. MS: *m*/*z* (%): 454.30 (M^+ + 2, 36.56), 96.15 (100). Anal. Calcd. For $C_{22}H_{24}N_6O_5$ (452.46): C, 58.46; H, 5.35; N, 18.57. Found: C, 58.91; H, 5.49; N, 18.74.

3.1.9. 3-(3-Methyl-5-hydroxy-1H-pyrazole-1-carbonyl)-5-phenyl-8,9,10,10a,-tetrahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-1,6(2H,5H)-dione ***18***

Compound **17** (0.45 g, 1 mmol) and 2 M KOH (20 ml) were refluxed for 5 h, the reaction mixture was then cooled and acidified with HCL. The resulting solid was filtered, washed with water, dried and crystallized from acetic acid to give yellow solid, 65% yield, m.p. 284–285 °C. IR: $\upsilon_{max.}$/cm^{-1} 3441 (OH), 3267 (NH), 3012 (CH

aromatic), 2978–2850 (CH aliphatic), 1675–1600 (3C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.80–1.85 (m, 3H, pyrrolidine), 2.49 (s, 3H, CH_3), 3.13–3.18 (m, 2H, pyrrolidine), 3.86–3.91 (m, 2H, pyrrolidine), 7.07–7.51 (m, 5H, aromatic protons), 8.00 (s, 1H, pyrazole proton), 11.90 (s, 1H, NH), 13.86 (s, 1H, OH). Anal. Calcd. for $C_{20}H_{18}N_6O_4$ (406.39): C, 59.11; H, 4.46; N, 20.68. Found: C, 59.29; H, 4.38; N, 21.04.

3.1.10. 3-(3-Hydroxy-5-oxo-4,5-dihydro-1H-pyrazole-1-carbonyl)-5-phenyl-8,9,10,10a,-tetrahydropyrimido[5,4-e]pyrrolo[1,2-c] pyrimidine-1,6(2H,5H)-dione **19**

A mixture of the hydrazide **13** (0.34 g, 1 mmol) and diethyl malonate (10 ml) was heated under reflux for 5 h. The resulting solid was filtered, washed with water, dried and crystallized from ethyl alcohol to give orange solid, 60% yield, m.p. >350 °C. IR: $\upsilon_{max.}$/cm^{-1} 3294 (OH), 3192 (NH), 3059 (CH aromatic), 2981–2850 (CH aliphatic), 1720–1700, 1680–1665(br, 4C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.68–1.88 (m, 3H, pyrrolidine), 1.90 (s, 2H, pyrazole proton), 2.62–2.78 (m, 1H, pyrolidine), 3.22–3.29 (m, 1H, pyrrolidine), 3.60–3.74 (m, 1H, pyrrolidine), 4.49–4.60 (m, 1H, pyrrolidine), 7.20–7.41 (m, 5H, aromatic protons), 9.89 (s, 1H, NH), 12.85 (s, 1H, OH). Anal. Calcd. for $C_{19}H_{16}N_6O_5$ (408.37): C, 55.88; H, 3.95; N, 20.58. Found: C, 55.96; H, 4.08; N, 21.04.

3.1.11. 3-(5-Amino-3-hydroxy-1H-pyrazole-1-carbonyl)-5-phenyl-8,9,10,10a,-tetrahydropyrimido[5,4-e]pyrrolo[1,2-c]pyrimidine-1,6(2H,5H)-dione **20**

A mixture of the hydrazide **13** (0.34 g, 1 mmol) and ethyl cyanoacetate (1 mmol) in dioxane (20 ml) was heated under reflux for 5 h. The solution was cooled and poured on ice cold water, the resulting solid was filtered, washed with water, dried and crystallized from ethyl alcohol to give brown solid, 60% yield, m.p. >350 °C (decp). IR: $\upsilon_{max.}$/cm^{-1} 3425–3367 (OH, NH_2), 3228 (NH stretching), 3070 (CH aromatic), 2981–2850 (CH aliphatic), 1720–1680 (br, 3C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.68–1.84 (m, 3H, pyrrolidine), 2.62–2.79 (m, 1H, pyrrolidine), 3.20–3.29 (m, 1H, pyrrolidine), 3.58–3.79 (m, 1H, pyrrolidine), 4.05–4.20 (m, 1H, pyrrolidine), 6.30 (s, 2H, NH_2), 7.20–7.52 (m, 6H, aromatic protons + pyrazole proton), 9.89 (s, 1H, NH), 11.25 (s, 1H, OH). Anal. Calcd. for $C_{20}H_{17}N_7O_4$ (407.38): C, 56.02; H, 4.21; N, 24.07. Found: C, 56.15; H, 4.32; N, 24.38.

3.2. Pharmacological screening

3.2.1. Anti-inflammatory activity

Anti-inflammatory activity screening was carried out using carrageenan induced paw oedema in albino rats [25–27]. Adult Wistar albino rats of both sexes weighing 120–150 g were divided into groups of 6 animals each. The animals were acclimatized to the lab conditions for two days with free access to food and water. On the day before the experiment, the food was withdrawn, but the animals were allowed free access to water. The control group was given saline solution containing few drops of Tween 80. The synthesized compounds (0.029 mmol/kg) and the reference drugs indomethacin and celecoxib (0.029 mmol) were suspended in saline solution with few drops of tween 80 and were given orally, using the oral stomach tube, 1 h before induction of inflammation. Induction of inflammation was performed by S.C. injection of 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 1, 2, 3 and 4 h intervals after induction of the inflammation and compared with the initial hind paw thickness of each rat for the determination of oedema thickness. The anti-inflammatory activity was expressed as % inhibition of oedema and was calculated by the following equation:

$$\%\text{Inhibition} = 100 \times [1 - \text{Vt/Vc}]$$

Where Vt is the mean increase of paw thickness of rats after administration of the tested compounds or the reference drug. Vc is the mean increase in paw thickness in rats after administration of carrageenan in the positive control group. Data were analyzed by SPSS statistical package version 10. Results are presented in Table 1.

IC_{50} values was calculated by the same method using the doses of 1, 2, 4, 6 mg/kg (Table 2).

3.2.2. Analgesic activity

Analgesic activity was measured using the acetic acid induced writhing test in mice [28,29]. Six Swiss albino mice of either sex (18–22 g) were used in each group. One hour after oral administration of a suspension of the tested compounds or indomethacin, (0.029 mmol/kg), 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 writhing 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 average number of writhing in control group and n' is average number of writhing in treated groups (Table 3).

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

Potency of the tested compounds was calculated according to the following equation:

$$\text{Potency}\% = \%\,\text{inhibition of the tested compd./}\ \%\,\text{inhibition of Indomethacin} \times 100$$

3.2.3. Gastric-ulcerogenic effect

Five hours after the oral treatment of rats with the tested compounds and standard drugs, they were killed under deep ether anaesthesia and their stomachs were removed. The stomach of each rat was opened through great curvature and examined for lesions or bleedings (Table 1).

3.2.4. Acute ulcerogenicity studies [30,31]

Adult Wistar albino rats of both sexes weighing between 120 and 150 g were used. Animals were divided into groups each of 6 animals. Rats were fasted for 20 h before drug administration. The tested compounds and the reference drugs indomethacin and celecoxib were given orally in a dose of 0.029 mmol/kg suspended in 1% Tween while one group received vehicle (1% Tween). Rats were fasted for 2 h, allowed to feed 2 h then fasted for another 20 h. Rats were given another two doses in the second and third days. In the fourth day, rats were sacrificed, the stomach removed, open 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 3 figures.
Results are presented in Table 4.

3.2.5. In-vitro COX study

The inhibitory COX activity of the tested compounds **12**, **16b** and indomethacin was assayed using Cayman's COX Activity Assay kit (catalogue No. 760151, Cayman chemicals, Ann Arbor, MI, USA) that measures the peroxidase activity of COX by the method of Kulmacz and Lands [32]. COX-1/COX-2 activity ratio of the tested compounds was recorded in Table 5.

3.2.6. 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.

3.3. QSAR studies

3.3.1. Computational method

All the computational works were performed on Molecular Operating Environment software (MOE version 2008.10.2).57 [33,34]. The structures of 21 compounds used as training set were sketched using molecular builder of MOE and each structure was subjected to energy minimization up to 0.01 kcal/mol Å using the MMFF94× force field. Optimization methods were used followed by conformational search of each energy-minimized structure. The most stable conformer of each structure was selected and saved into database to generate the common descriptors. QuaSAR descriptor module of MOE was used to calculate about 180 descriptors for each molecule. The probability density functions used are Gaussian. The RMSD tolerance was set to 0.5 Å. Regression analysis was performed using inhibition% as dependent factor and the calculated physicochemical descriptors as predictable variables. In this study, the pool of descriptors was optimized using principal components analysis (PCA). The optimization started with the reduction in the number of molecular descriptors by the determination of the highly inter-correlated descriptor pairs and only one from each pair was selected; then the descriptors with insignificant variance through the data set were also rejected. QSAR model was then constructed after ensuring reasonable correlation of anti-inflammatory/analgesic activities with the individual descriptors and minimum inter-correlation among the descriptors used in the derived model. The models were validated with the leave-one-out (LOO) method. The quality of the model was assessed using the statistical parameter R^2.

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