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Syntheses, characterization and evaluation of novel 2,6-diarylpiperidin-4-ones as potential analgesic-antipyretic agents

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ABSTRACT

A novel series of N-(N-methylpiperazinoacetyl)-2,6-diarylpiperidin-4-one derivatives (**1c**–**3c** and **5c**) were synthesized, via base catalyzed nucleophilic substitution of N-chloroacetyl-2,6-diarylpiperidin-4-ones (**1b**–**6b**) with N-methyl piperazine. The newly synthesized compounds were characterized by FTIR, Mass and NMR spectral studies. All the compounds were screened for their possible analgesic and antipyretic activities. The compound **2c** exhibited promising antipyretic activity, comparable to that of paracetamol at 60 mg/kg dose. The compounds **2b** and **2c** showed significant analgesic profile at a dose of 60 mg/kg and were also found to be more potent than the reference drug, diclofenac sodium. Thus, it can be concluded that the synthesized 2,6-diarylpiperidin-4-ones exhibit promising antipyretic and analgesic activities and could be potential drug candidates.

1. Introduction

Inflammation is a normal and essential response to any noxious stimulus that threatens the host and may vary from a localized response to a generalized response [1]. Generally, this response acts to protect the host but at times it may lead to a spectrum of inflammatory diseases, thus necessitating medication to dampen or abolish the inflammatory response [2].

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat the signs and symptoms of inflammation and pain. NSAIDs exert their effect mainly through the inhibition of cyclooxygenase (COX), the key enzyme in prostaglandin (PG) biosynthesis from arachidonic acid (AA). The potentiality of NSAIDs to alleviate pain, inflammation and fever, coupled with a number of pathological conditions, makes them one of the most useful therapeutic agents [3].

NSAIDs are particularly effective when inflammation has caused sensitization of pain receptors to normally painless mechanical or chemical stimuli. Pain, that accompanies inflammation and tissue injury, probably results from local stimulation of pain fibers and enhanced pain sensitivity (hyperalgesia), in part a consequence of increased excitability of central neurons in the spinal cord. Bradykinin, released from plasma kininogen and cytokines, such as TNF-α, IL-1 and IL-8 appear to be particularly important in eliciting the pain related to inflammation. These agents liberate prostaglandins and probably other mediators that promote hyperalgesia. Neuropeptides, such as substance-P, calcitonin gene-related peptide (CGRP) may also be involved in eliciting pain. Non-opoid analgesics act by inhibition of cyclooxygenase (COX) enzyme, and specially COX-2 which plays a key role in the production of inflammatory mediators like prostaglandins and prostacyclins from cyclic endoperoxides. Prostaglandin PGE_2 and prostacyclin PGI_2 sensitize the pain receptors in various organs. Thus, stimuli which are normally painless are able to elicit pain. Some other mediators like bradykinin and cytokines, i.e. tissue necrosis factor (TNF-α), interleukins (IL-1, IL-8) stimulate the COX enzyme and thus produce prostaglandins which act in mediating hyperalgesia [4].

Fever is a complex physiologic response triggered by infectious or aseptic stimuli, due to increased concentrations of prostaglandin E(2) (PGE(2)) within certain areas of the brain. These elevations alter the firing rate of neurons that control thermoregulation in the hypothalamus. Antipyretics, such as aspirin, have been widely used since the late 19th century but the mechanisms by which they relieve fever have only been characterized in the last few decades. It is now clear that most antipyretics work by inhibiting the enzyme cyclooxygenase and reducing the levels of PGE(2) within the hypothalamus. Recently, other mechanisms of action for antipyretic

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drugs have been suggested, including their ability to reduce proinflammatory mediators, enhance anti-inflammatory signals at sites of injury, or boost antipyretic messages within the brain. Analysis of recent data suggests that multiple pathways may be involved in the induction of fever by cytokines, such as local cytokine production leading to signaling through vagal fibers, release of cytokine-induced circulating mediators at the tissue level, the use of membrane-bound cytokines as mediators, or the local release of cytokines in the hypothalamus by circulating activated monocytes. A multi pathway mechanism for the induction of fever is therefore suggested [5].

Traditional NSAIDs such as aspirin, diclofenac, flurbiprofen and ibuprofen are non-selective in their action. Therefore, chronic use of NSAIDs may elicit appreciable gastrointestinal (GI) irritation, bleeding and ulceration. The incidence of clinically significant GI side effects is high (over 30%) and causes some patients to abandon NSAID therapy. Thus, the discovery of COX-2 provided the impetus for the development of drugs devoid of GI disorders while retaining clinical efficacy as anti-inflammatory agents. However, some reports showed that selective COX-2 inhibitors (coxibs) could lead to adverse cardiovascular effects. Therefore, development of novel compounds having anti-inflammatory and analgesic activity with improved safety profile is still a necessity. Synthetic approaches based upon chemical modification of existing NSAIDs have been undertaken with an aim to improve their safety profile [6]. Also, agents with new mechanisms of action are in great demand. Microsomal PGE synthase-1 (mPGES-1), an inducible enzyme for the production of pro-inflammatory PGE_2 from PGH_2, is also upregulated in rheumatoid arthritis and osteoarthritis. The mPGES-1 is a critically important mediator of inflammation, pain, angiogenesis, fever, bone metabolism, tumorigenesis, atherosclerosis, and reproduction. It presents an attractive target to achieve more specific inhibition of PGE_2 production while preserving production of other PGs and, as such, has attracted considerable attention as a "next-generation" anti-inflammatory drug [7]. All this laid the foundation for the search and design of new chemical agents, which are devoid of all the limitations and side effects of the drugs available in the market. Hence, there is an urgent need for mono therapy, with a biologically potent candidate, endowed with analgesic and antipyretic activities together by keeping in view the pharmaco-economic and frequent patient compliance. While exploring for such a compound, it was observed that piperidone nucleus is an integral component of countless alkaloids with marked biological properties [8–15] such as antiviral [16], antitumor [17], anti-inflammatory [18], central nervous system active [19–24], local anesthetic [25], anticancer [26] and antimicrobial activity [27]. Moreover, derivatives of piperidines are also biologically important and act as neurokinin receptor antagonists [28], analgesic and antihypertensive agents [29–31]. Also, substituted piperidin-4-ones are important synthetic intermediates for the preparation of various alkaloids and pharmaceuticals. The utility of substituents at second, third and sixth positions, particularly aromatic substituent at second and/or sixth positions with regard to its biological activity has been well documented [32].

1,3-Dimethyl-2,6-diphenyl-4-piperidone has been found to be a versatile intermediate in different types of reactions since it has two reactive sites, carbonyl and a keto methylene group. This paved the way for the synthesis of some heterocyclic compounds such as tetrahydropyridine, diazepanone, oxazepanone, piperidone, pyridopyrimidone, pyridopyrimidinethione, thiazolopyridine, furanylmethylene and pyridoindole [33].

Generally, all piperidin-4-ones are synthesized by condensing 1 mol of ketone and 2 mol of aldehydes with 1 mol of ammonium acetate, in the presence of ethanol [34]. Noller et al. synthesized a number of 4-piperidones with different substituents on the 1,2,3,5

Table 1
Structure of compounds (1–10).

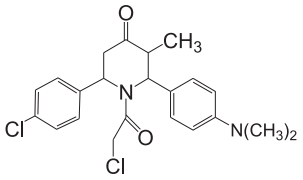
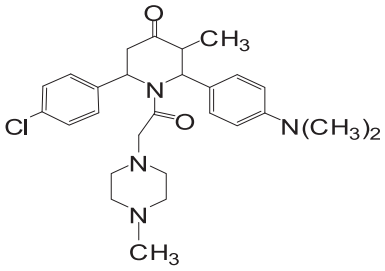
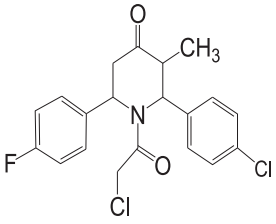
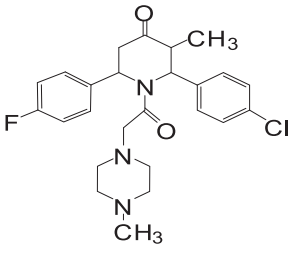
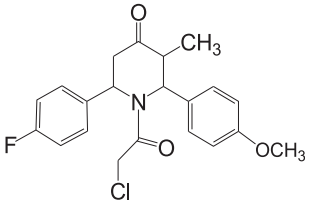
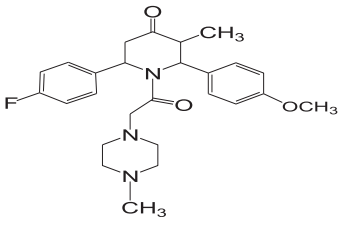
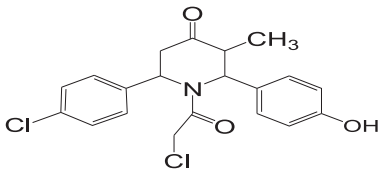
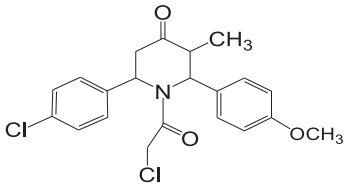
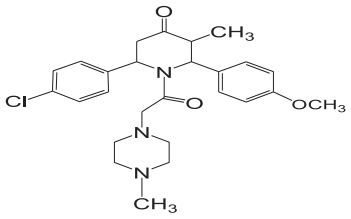
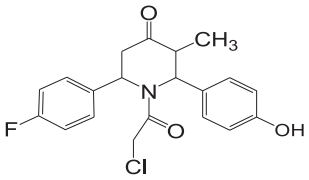
S. No.	Compound code	Structure
1	**1b**	
2	**1c**	
3	**2b**	
4	**2c**	
5	**3b**	
6	**3c**	

Table 1 (*continued*)

S. No.	Compound code	Structure
7	**4b**	
8	**5b**	
9	**5c**	
10	**6b**	

and 6 positions. Glacial acetic acid was used instead of ethanol in their procedure [35].

Piperazine is an interesting heterocyclic moiety as it is a constituent of several biologically active molecules. The polar nitrogen atoms, opposing each other in the piperazine ring, confer bioactivity to the molecules and elicit favorable interactions with macromolecules [36,37]. Slight change in the substitution pattern in piperazine nucleus causes distinguishable change in the pharmacological activities. Compounds with piperazine and its N-substituted derivatives, i.e. N-methyl, N-ethyl piperazines, as an integral part, are the most successfully employed side chains since they exhibit a varied range of activities such as antimicrobial [38,39], anticancer [40], anti-inflammatory [41], antipsychotic [42], CNS active [43], antagonists for CCKB(Cholecystokinin B)/gastrin receptor [44], melanocortin-4-receptor [45] and in the treatment of Alzheimer's disease [46,47].

In the present work, incorporation of both the moieties, i.e. piperidin-4-one and secondary amine i.e. N-methyl piperazine, was carried out in a Mannich base in anticipation of enhanced analgesic and antipyretic activities.

2. Chemistry

By adopting the literature precedent [34,47] 2,6-diarylpiperidin-4-ones (Table 1) were prepared in a one pot multi-component Mannich reaction by condensing suitably substituted aromatic aldehydes, ketone and ammonium acetate in equimolar ratio, using ethanol as the solvent (Scheme 1).

In view of the better basicity and HCl scavenging power of triethylamine (NEt_3), it was used in the nucleophilic reaction of **1a**–**6a** with chloroacetyl chloride. The yields of N-chloroacetyl-2,6-diarylpiperidin-4-ones were significantly better while using NEt_3. Further, nucleophilic substitution of N-methyl piperazine with these chloroacetyl derivatives was performed in different solvents, such as ethanol and toluene, using NEt_3 again as a base. Toluene was chosen as the best solvent for most of the derivatives as it improved the yield of the title compounds (**1b**, **1c**, **2b**, **2c**, **3b**, **3c**, **5a** and **5c**) appreciably when compared to ethanol. For hydroxy derivatives, chloroform was used as the solvent instead of toluene. Increasing the non-polar environment led to easy nucleophilic substitution of these derivatives (**4b** and **6b**). The possible mechanism of the reaction is outlined in Scheme 2.

The FT-IR analysis of the compounds confirmed the absence of NH stretching in piperazine and changes in the magnitude of amide carbonyl stretching at around 1652–1660 cm^{-1} of **1b**–**6b** (for **1c**, **2c**, **3c** and **5c**, it appeared at around 1643 cm^{-1}), besides the appearance of additional aliphatic stretching frequencies in the region 2981–2694 cm^{-1}.

3. Results and discussion

The synthesized compounds were subjected to *in-vitro* studies to predict the log *P* value and hydrolytic profile of the compounds.

3.1. Partition coefficient

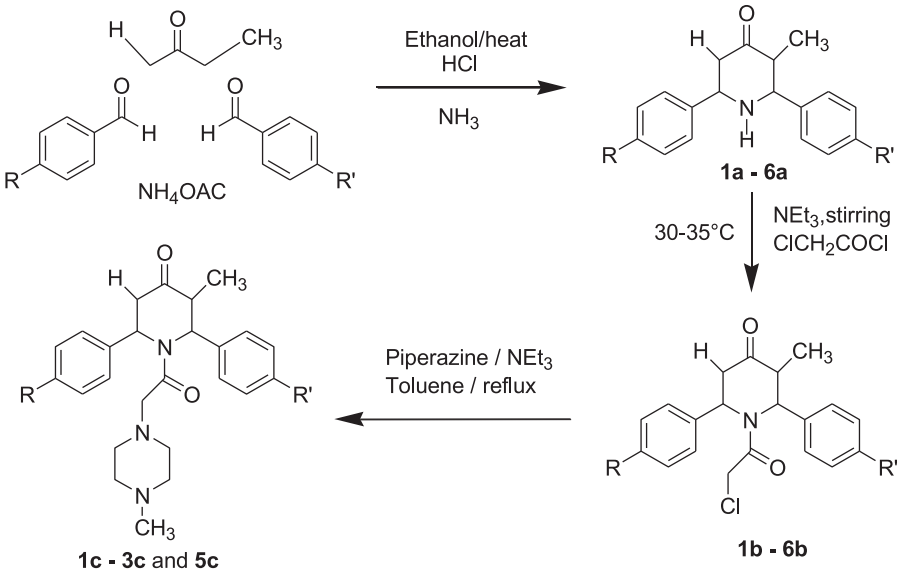
Lipophilicity is represented by the descriptors log *P* (also known as Kow or Pow) and is used to predict *in-vivo* permeability of active compounds in drug discovery.

Partition coefficient of compound **2c**, having 2-(p-chlorophenyl) and 6-(p-fluorophenyl) functionalities with methyl group at C-3 and N-methyl piperazine ring- was found to be 2.09. Thus **2c** was found to be most lipophilic compound of the series, having Log *P* value of 2.09, followed by compound **2b** (having 2-(p-chlorophenyl), 6-(p-fluorophenyl) functionality with methyl at C-3 and N-chloroacetyl moiety), with log *P* value of 1.72. Compound **6b**, having 2-(p-hydroxyphenyl), 6-(p-fluorophenyl) functionalities with a methyl group at C-3 and N-chloroacetyl moiety was the least lipophilic compound with a log *P* value of 1.14. Partition coefficient data are given in Table 2.

3.2. In-vitro hydrolysis

Stability in gastric juice is of prime importance for the drugs intended for oral administration. It is observed that many drugs have low bioavailability as they are degraded in the stomach, due to low pH (1–2). Simulated gastric fluid (SGF) mimics the gastric fluid in terms of acidity and molarity and simulated intestinal fluid (SIF) mimics the intestinal fluid in terms of basicity. These fluids are the perfect media to determine the stability of drug candidates *in vitro*. In the present study, synthesized compounds were tested *in vitro* using SGF and SIF.

Results of *in vitro* hydrolysis (Table 3) showed that compound **1c**, having 6-(p-chlorophenyl), 2-(p-dimethylaminophenyl) functionalities with a methyl group at C-3 and N-methyl piperazine ring was the most hydrolyzed with a hydrolysis of 31.70%. Compound **5c**, having 6-(p-chlorophenyl), 2-(p-methoxyphenyl) functionalities with a methyl group at C-3 and N-methyl piperazine ring was the least hydrolyzed with a hydrolysis of 1.57% in simulated gastric

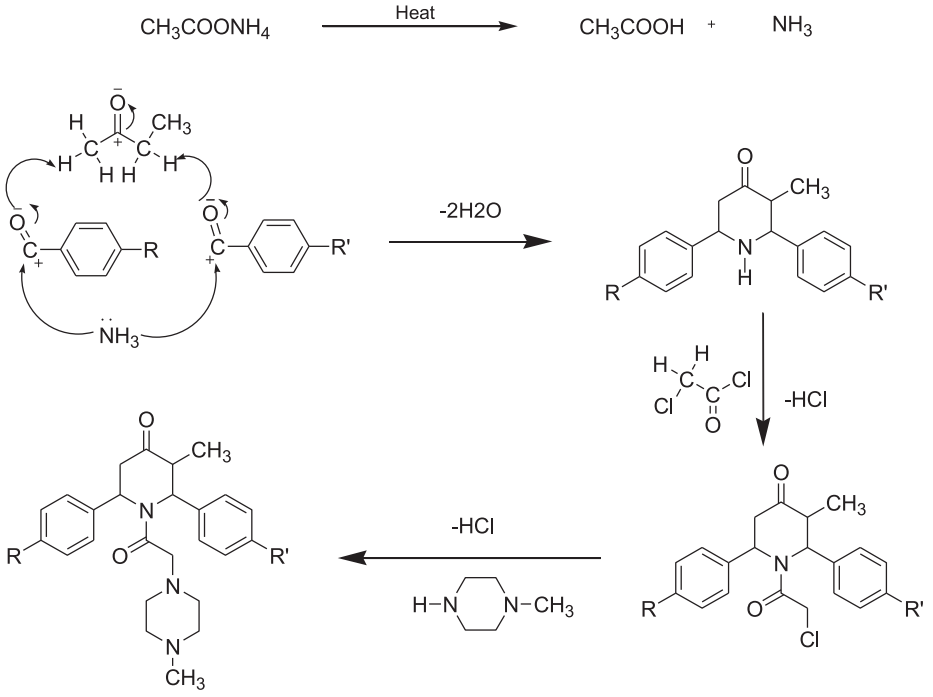


Series	R	R'
1a-1c	Cl	$N(CH_3)_2$
2a-2c	F	Cl
3a-3c	F	OCH_3
4a-4b	Cl	OH
5a-5c	Cl	OCH_3
6a-6b	F	OH

Scheme 1. The synthetic pathway to the title compounds (**1b**-**6b**, **1c**–**3c** and **5c**).

fluid. This indicates their stability in stomach and subsequent bioavailability.

Compound **1c**, having 6-(p-chlorophenyl), 2-(p-dimethylaminophenyl) functionalities with a methyl group at C-3 and N-methyl piperazine ring was the most hydrolyzed with a hydrolysis of 77.60%. Compound **2b**, having 2-(p-chlorophenyl), 6-(p-fluorophenyl) functionalities with a methyl group at C-3 and N-chloroacetyl moiety was the least hydrolyzed with a hydrolysis of 1.17%



Scheme 2. The possible mechanism of the reaction.

Table 2
Partition coefficient of the synthesized compounds.

Comp.	λ_{max} in octanol (nm)	Plotting for standard curve in μg/ml					Abs. of octanol phase after 24 h (y)	Conc. in octanol (x) (μg/ml)	Conc. in water (μg/ml)	Log *P*
		4	8	12	16	20				
1b	338.0	0.102	0.150	0.197	0.248	0.293	0.281	18.92	1.08	1.24
1c	336.5	0.213	0.368	0.532	0.691	0.852	0.816	19.13	0.87	1.35
2b	273.0	0.022	0.051	0.087	0.119	0.148	0.145	19.43	0.57	1.72
2c	282.5	0.145	0.195	0.243	0.294	0.344	0.341	19.84	0.16	2.09
3b	280.5	0.014	0.033	0.053	0.071	0.092	0.086	19.27	0.73	1.42
3c	284.5	0.020	0.041	0.062	0.086	0.108	0.105	19.63	0.37	1.53
4b	303.5	0.023	0.052	0.082	0.109	0.139	0.127	18.37	1.63	1.05
5b	283.0	0.046	0.075	0.101	0.127	0.152	0.147	19.03	0.97	1.29
5c	288.5	0.045	0.087	0.132	0.180	0.219	0.213	19.36	0.64	1.48
6b	340.0	0.062	0.093	0.123	0.150	0.179	0.170	18.66	1.34	1.14

in simulated intestinal fluid. This demonstrates their stability and bioavailability through the intestine.

3.3. Analgesic activity

The analgesic activity of the compounds was determined by acetic acid-induced writhing in mice, using diclofenac sodium as the standard drug. Painful reactions in animals may be produced by chemicals also. Intraperitoneal injection of phenylquinone, bradykinin or acetic acid produces pain reaction which is characterized as a writhing response. The visceral pain induced by acetic acid is due to the release of prostaglandins (PG) via arachidonic acid pathway in the presence of cyclooxygenase enzyme. Constriction of abdomen, turning of trunk (twist) and extension of hind legs are taken as the reactions to chemically induced pain [4]. The test compounds, when administered orally (p.o.) at 60 mg/kg (body weight) 1 h prior to the administration of acetic acid, showed a significant reduction in the writhing movement. Analgesic profiles of the compounds are given in Table 4.

It is evident from Table 4 that analgesic potency estimated by classical acetic acid-induced constriction for certain compounds is comparatively equal or highly related to the reference compound at the test dose. Introduction of chloro/fluoro functionality at the para position of phenyl framework in compound **2b** and **2c** produced statistically significant antinociceptic activity. Compound **2b** displayed almost equipotent analgesic activity, having a percent inhibition of 57.637 ± 1.885, in comparison to the standard drug diclofenac, having a percent inhibition of 56.515 ± 2.029. Further, compound **2b** was substituted with N-methyl piperazine to produce compound **2c.** Significantly, introduction of N-methyl piperazine framework in compound **2b** showed excellent analgesic activity with a percent inhibition of 65.487 ± 1.711. This expressive enhancement of the analgesic activity indicates the pharmacophoric character of para chloro/fluoro phenyl functionality and N-methyl piperazine framework for the analgesic profile in this new series of compounds.

Replacement of one chloro/fluoro phenyl functionality in compounds **3b**, **3c**, **4b**, **5b**, **5c** and **6b** by methoxy/hydroxy phenyl functionality also resulted in diminished analgesic potency, with percent inhibitions of 51.184 ± 1.109, 55.199 ± 1.528, 39.715 ± 2.113, 44.126 ± 1.365, 53.049 ± 1.250 and 51.178 ± 1.156 respectively. However, replacement of one chloro/fluoro phenyl functionality in compounds **1b** and **1c** by para dimethylaminophenyl group resulted in poor analgesic potency, with percent inhibitions of 17.794 ± 2.028 and 28.101 ± 1.636. In all the cases, the para halogen substituted phenyl bearing compounds displayed improved inhibition potency as compared to their analogues, the para dimethylaminophenyl bearing compounds.

Therefore, from this study the importance of the structural units such as fluorine, chlorine, methoxy and hydroxy functions at the para position of the phenyl groups has been established for the antinociceptive profile associated with these compounds.

Furthermore, in the acetic acid-induced writhing test, the index of pain is evaluated through the nociceptive behavior characterized by a motor activity (writhing). The drugs that decrease or impair the motor activity may reduce the acetic acid-induced writhing, without producing antinociception [4]. Therefore, the effect of the synthesized derivatives on motor coordination of the animals was assessed, using the rota rod test. It was evident from the study that none of the synthesized derivatives were involved in altering the motor coordination, as the mean fall-off time of the animals were found to be more than 180 s. The animals treated with Diazepam (positive control), at a dose of 2 mg/kg body weight, failed to maintain the motor coordination in 60 s. Therefore, it may be concluded from the rota rod study that the antinociceptive effect of the synthesized derivatives was not associated with motor impairment [51].

3.4. Antipyretic activity

The antipyretic activity of the compounds was determined by Brewer's yeast-induced hyperthermia in rats, using paracetamol as the standard drug. Hyperthermia was induced in rats by subcutaneous administration of Brewer's yeast in normal saline. This caused an increased production of prostaglandins in the hypothalamus and thus the thermoregulation process was affected [5]. Pretreatment with test compounds at 60 mg/kg dose showed a better antipyretic activity relative to the standard drug at different time intervals. Antipyretic effect of the compounds may be due to the inhibition of prostaglandin synthesis in the central nervous system, thereby reducing the rectal temperature. The observations are recorded in Table 5.

The compounds with para substituted phenyl groups at C-2 and C-6 positions of the piperidone ring system were found to be equipotent to paracetamol in antipyretic activity, in the same pharmacological protocol. Compound **2c**, with para-chloro/fluoro phenyl functionality (with methyl group at C-3), displayed most potent antipyretic activity similar to that of the standard drug (paracetamol) in 1 h, 2 h and 3 h, with rectal temperatures of 37.900 ± 0.072, 37.160 ± 0.075 and 37.180 ± 0.080 respectively, in comparison to the standard drug paracetamol, showing rectal temperatures of 37.880 ± 0.073, 37.140 ± 0.051 and 37.100 ± 0.055 in 1 h, 2 h and 3 h respectively. Further, this compound showed similar onset of hypothermic effect as that of paracetamol immediately after 60 min, which lasted over a period of 150 min. Compound **3c**, with chlorophenyl functionality replaced with paramethoxyphenyl (with methyl group at C-3), also exhibited

Table 3
Data of *In-vitro* hydrolysis.

Compound		λ_{max}	0 min	15 min	30 min	45 min	60 min	75 min	90 min	105 min	120 min	% Hydrolyzed
1b	SGF	349.6	0.289	0.289	0.288	0.287	0.286	0.283	0.282	–	–	2.42%
	SIF	344.8	0.466	0.281	0.208	0.186	0.165	0.124	0.108	0.100	0.100	76.82%
1c	SGF	350.0	0.451	0.353	0.329	0.323	0.316	0.312	0.308	–	–	31.70%
	SIF	342.5	0.375	0.219	0.192	0.151	0.141	0.131	0.115	0.098	0.084	77.60%
2b	SGF	272.0	0.091	0.090	0.090	0.089	0.088	0.086	0.085	–	–	6.59%
	SIF	283.5	0.428	0.426	0.426	0.425	0.424	0.424	0.423	0.423	0.423	1.17%
2c	SGF	288.8	0.189	0.188	0.187	0.186	0.184	0.182	0.181	–	–	4.23%
	SIF	290.5	0.178	0.176	0.176	0.175	0.174	0.172	0.172	0.171	0.170	4.49%
3b	SGF	272.5	0.194	0.193	0.193	0.192	0.192	0.191	0.190	–	–	2.06%
	SIF	274.5	0.398	0.395	0.395	0.394	0.393	0.392	0.392	0.390	0.390	2.01%
3c	SGF	321.2	0.158	0.156	0.153	0.153	0.152	0.151	0.151	–	–	4.43%
	SIF	322.6	0.398	0.397	0.394	0.394	0.393	0.391	0.389	0.389	0.388	2.51%
4b	SGF	273.5	0.087	0.085	0.084	0.083	0.083	0.082	0.080	–	–	8.04%
	SIF	284.0	0.440	0.342	0.281	0.281	0.276	0.262	0.258	0.252	0.252	42.73%
5b	SGF	292.0	0.068	0.068	0.067	0.066	0.064	0.064	0.063	–	–	7.35%
	SIF	286.5	0.298	0.298	0.296	0.294	0.293	0.292	0.291	0.291	0.290	2.68%
5c	SGF	284.0	0.255	0.254	0.254	0.253	0.253	0.252	0.251	–	–	1.57%
	SIF	290.5	0.032	0.032	0.031	0.030	0.029	0.029	0.028	0.026	0.026	18.75%
6b	SGF	271.5	0.092	0.092	0.091	0.090	0.090	0.089	0.088	–	–	4.35%
	SIF	282.0	0.412	0.412	0.282	0.280	0.280	0.242	0.240	0.238	0.234	43.20%

activity comparable to that of the standard in 1 h and 3 h, with rectal temperatures of 38.180 ± 0.059 and 37.100 ± 0.045. Compounds **2b**, **3b**, **4b**, **5b**, **5c** and **6b**, with one chloro/fluoro phenyl functionality replaced with para-methoxyphenyl/para-hydroxyphenyl (with methyl group at C-3), also displayed appreciable antipyretic profile. Compound **1b**, with one chlorophenyl and one dimethylaminophenyl functionality (with methyl group at C-3), displayed the least potent antipyretic activity followed by compound **1c**.

Among the compounds tested for their antipyretic activity, introduction of substituents in the phenyl ring only registered enhanced activity and, in particular, compounds **2c** and **3c** showed superior activity.

4. Conclusion

All the novel target molecules were synthesized by the direct nucleophilic substitution of N-methyl piperazine, with the corresponding N-chloroacetyl amides derived from substituted 2,6-diarylpiperidin-4-ones. In order to probe structural requirements for optimal analgesic and antipyretic activities in this series of compounds, the methyl substituent attached at C-3 position besides introducing electron withdrawing or electron donating functional groups at para position of the phenyl framework was examined.

Partition coefficient of compound **2c** was found to be 2.09. Thus, **2c** was found to be the most lipophilic compound of the series followed by **2b**, having a log *P* value of 1.72. Compound **6b** was the least lipophilic with a log *P* value of 1.14. In the series of synthesized compounds, it was observed that the log *P* values of N-methyl piperazine derivatives were higher than their corresponding N-chloroacetyl derivatives.

Results of *in vitro* hydrolysis showed that compound **1c** was the most hydrolyzed, with a percent hydrolysis of 31.70, and compound **5c** was the least hydrolyzed, with a percent hydrolysis of 1.57, in simulated gastric fluid. This indicated their stability in stomach and subsequent bioavailability. Compound **1c** was the most hydrolyzed with a percent hydrolysis of 77.60, and compound **2b** was the least hydrolyzed, with a percent hydrolysis of 1.17 in simulated intestinal fluid. This demonstrated their stability and bioavailability through the intestine. The percent hydrolysis of the various synthesized compounds in SGF and SIF are depicted in Table 3.

Analgesic potency of the compounds **1b**–**6b** and **1c**–**5c** has been presented in Table 4. The compounds **2b** and **2c** exhibited noticeable analgesic activity at the tested dose of 60 mg/kg. It is probable that these compounds effectively reduced the wave of constriction and elongation passing caudally along the abdominal wall, with twisting of trunk and extension of the hind limb, in mice due to nociceptive property of acetic acid. In the case of antipyretic activity, compounds **2c** and **3c** exhibited significant potency at the tested dose of 60 mg/kg during the period of study. Here also, the antinociceptic and antipyretic activities of these compounds were found to be better than the standard drugs used.

From the preliminary results of the study, it may be concluded that the presence of para substituted phenyl groups at C-2 and C-6 positions, methyl group at C-3 position could result in high log *P* value and less *in-vitro* hydrolysis, which in-turn are the key factors in eliciting valuable biological profiles. The most potent compounds for the analgesic activity (**2b** and **2c**), in addition to the antipyretic activity (**2c** and **3c**), demonstrated modest *in vitro* hydrolysis profiles and enhanced Log *P* values. However, the least active compound of the series (**1c**), exhibited a very high *in vitro* hydrolysis and a low Log *P* value. Therefore, the high potency of these compounds may be attributed to these factors also. Hence, among the ten compounds used in this study, **2b**, **2c** and **3c** were considered to be potent candidates with promising pharmacological properties. Moreover, development of this class of compounds may lead to

Table 4
Analgesic activity of the substituted diaryl piperidin-4-ones.

Group	Treatment	No. of writhes (mean ± SEM)**	% Inhibition (mean ± SEM)**
I	Control CMC (0.5%)	73.800 ± 1.158	_
II	Standard (diclofenac sodium)	32.000 ± 1.000	56.515 ± 2.029
III	Compound 1 (**1b**)	60.600 ± 1.030	17.794 ± 2.028
IV	Compound 2 (**1c**)	53.000 ± 0.0707	28.101 ± 1.636
V	Compound 3 (**2b**)	31.200 ± 1.068	57.637 ± 1.885
VI	Compound 4 (**2c**)	25.400 ± 0.927	65.487 ± 1.711
VII	Compound 5 (**3b**)	36.000 ± 0.707	51.184 ± 1.109
VIII	Compound 6 (**3c**)	33.000 ± 0.707	55.199 ± 1.528
IX	Compound 7 (**4b**)	44.400 ± 0.927	39.715 ± 2.113
X	Compound 8 (**5b**)	41.200 ± 0.860	44.126 ± 1.365
XI	Compound 9 (**5c**)	34.600 ± 0.509	53.049 ± 1.250
XII	Compound 10 (**6a**)	36.000 ± 0.707	51.178 ± 1.156

** Indicates highly significant result with $P < 0.01$.

Table 5
Antipyretic activity of the substituted diaryl piperidin-4-ones.

Gp	Treatment	Initial rectal temp. (°C) (Mean ± SEM)	Rectal temp. after 18 h (°C) (Mean ± SEM)	Rectal temp. 1 h after treatment (°C) (Mean ± SEM)**	Rectal temp. 2 h after treatment (°C) (Mean ± SEM)**	Rectal temp. 3 h after treatment (°C) (Mean ± SEM)**
I	Control CMC (0.5%)	37.260 ± 0.052	39.200 ± 0.089	39.300 ± 0.071	39.320 ± 0.102	39.400 ± 0.071
II	Standard (Paracetamol)	37.240 ± 0.051	39.280 ± 0.087	37.880 ± 0.073	37.140 ± 0.051	37.100 ± 0.055
III	Cpd. 1 (**1b**)	37.280 ± 0.086	39.180 ± 0.058	39.000 ± 0.032	38.560 ± 0.051	37.860 ± 0.051
IV	Cpd. 2 (**1c**)	37.300 ± 0.071	39.140 ± 0.075	38.900 ± 0.070	38.140 ± 0.053	37.520 ± 0.058
V	Cpd. 3 (**2b**)	37.240 ± 0.050	39.220 ± 0.058	38.140 ± 0.051	37.540 ± 0.050	37.160 ± 0.068
VI	Cpd. 4 (**2c**)	37.220 ± 0.086	39.200 ± 0.071	37.900 ± 0.072	37.160 ± 0.075	37.180 ± 0.080
VII	Cpd. 5 (**3b**)	37.200 ± 0.070	39.260 ± 0.051	38.580 ± 0.058	37.900 ± 0.071	37.200 ± 0.070
VIII	Cpd. 6 (**3c**)	37.220 ± 0.058	39.220 ± 0.058	38.180 ± 0.059	37.440 ± 0.074	37.100 ± 0.045
IX	Cpd. 7 (**4b**)	37.180 ± 0.073	39.140 ± 0.052	38.920 ± 0.057	38.500 ± 0.070	37.140 ± 0.081
X	Cpd. 8 (**5b**)	37.200 ± 0.071	39.200 ± 0.045	38.760 ± 0.051	38.360 ± 0.052	37.200 ± 0.072
XI	Cpd. 9 (**5c**)	37.280 ± 0.037	39.180 ± 0.066	38.520 ± 0.058	37.680 ± 0.058	37.280 ± 0.037
XII	Cpd. 10 (**6b**)	37.260 ± 0.068	39.200 ± 0.070	38.720 ± 0.037	38.300 ± 0.045	37.160 ± 0.052

** Indicates highly significant result with $P < 0.01$; Cpd. = Compound; Gp = Group.

some interesting chemical entities with improved biological and pharmacological profiles than the standard drugs. Therefore, this class of compounds may be used as templates to generate better drugs to alleviate pain and fever.

5. Experimental protocols

5.1. General

The chemicals and reagents were procured from S. D. Fine Chemicals, Mumbai and were used as such. Reaction progress was monitored by thin layer chromatography on silica gel G plates, using iodine vapors and UV chamber as the visualizing agents. After physical characterization, the compounds were subjected to spectral analysis. UV spectra were recorded on a Double Beam spectrophotometer (Shimadzu-1700). IR spectra were recorded on an FTIR Spectrophotometer (Shimadzu 8400S) and values are expressed in cm^{-1}, and only noteworthy absorption levels (reciprocal centimeters) are listed. Mass spectra were recorded on JEOL-Accu TOF JMS-T100LC Mass spectrometer and THERMO Finnigan LCQ Advantage max ion trap Mass spectrometer. NMR spectra were recorded on a Bruker DRX-300 spectrometer using $CDCl_3$ as the solvent .Chemical shifts are reported in parts per million (δ values), using TMS as an internal standard (δ-0 ppm for ^1H NMR). The spin multiplicities in ^1H NMR are indicated as follows: s (singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). Elemental analysis was performed on an Elemental Vario EL-III analyzer.

5.2. General procedure for the synthesis of N-chloroacetyl-2,6-diarylpiperidin-4-ones (**1b**, **2b**, **3b** and **5b**)

To a well-stirred solution of (**1a**, **2a**, **3a** and **5a**) substituted 2,6-diphenylpiperidin-4-one (1 equiv., 0.005 mol) and triethylamine (1 equiv., 0.005 mol) in 30 ml toluene, chloroacetyl chloride (1 equiv., 0.005 mol) in 30 ml toluene was added dropwise, for about half an hour, through a dropping funnel. Stirring was continued with mild heating (30–35 °C) till the completion of the reaction. After this, the mixture was poured into 10 ml water and extracted with three 10 ml portions of ether. The collected ether extracts were then washed well with 3% sodium bicarbonate solution and dried over anhydrous sodium sulfate. The same upon evaporation and subsequent recrystallization in distilled ethanol afforded the compounds (**1b**, **2b**, **3b** and **5b**) in pure form with good yields. The completion of the reactions was monitored by TLC, using the solvent system methanol: chloroform (2%).

5.2.1. N-chloroacetyl-2-(4-dimethylaminophenyl)-3-methyl-6-(4-chlorophenyl)-piperidin-4-one (**1b**)

Stirring time 7.0 h; Dark brown colored crystalline solid; Yield 78.47% (Ethanol); Melting range (°C): 115–118; IR (KBr) (ν cm^{-1}): (1654.81) N–C–O Stretching, (1718.46) C=O Stretching, (831.26) Ar–H Bending; ^1H NMR [($CDCl_3$) δ in ppm: 1.052–1.075 (d, J = 6.9 Hz, 3H), 2.854–2.940 (q, J = 25.8 Hz, 1H), 2.970–3.044 (q, J = 22.2 Hz, 1H), 3.087 (s, 6H), 3.131–3.150 (d, J = 11.1 Hz, 1H), 3.948 (s, 2H), 5.359–5.383 (d, J = 7.2 Hz, 1H), 5.863–5.901 (t, J = 11.4 Hz, 1H), 6.818–6.843 (d, J = 7.5 Hz, 2H), 6.690–6.720 (d, J = 9.0 Hz, 2H), 7.304–7.332 (d, J = 8.4 Hz, 2H), 7.164–7.182 (d, J = 5.4 Hz, 2H)]; Anal. Calcd for $C_{22}H_{24}Cl_2N_2O_2$: C = 63.01%, H = 5.77%, N = 6.68%; Found C = 60.50%, H = 5.26%, N = 4.57%; MS: [M^+] at m/z 419.34.

5.2.2. N-chloroacetyl-2-(4-chlorophenyl)-3-methyl-6-(4-fluorophenyl)-piperidin-4-one (**2b**)

Stirring time 20.0 h; Light brown colored crystalline solid; Yield 80.59% (Ethanol); Melting range (°C): 98–101; IR (KBr) (ν cm^{-1}): (1654.81) N–C–O Stretching, (1718.46) C=O Stretching, (835.12) Ar–H Bending; ^1H NMR [($CDCl_3$) δ in ppm: 1.032–1.072 (d, J = 12.0 Hz, 3H), 2.842–2.862 (d, J = 6.0 Hz, 2H), 2.923–2.945 (d, J = 6.6 Hz, 1H), 3.953 (s, 2H), 4.040–4.056 (d, J = 4.8 Hz, 1H), 4.072–4.181 (t, J = 2.7 Hz, 1H), 6.973–6.991 (d, J = 5.4 Hz, 2H), 7.140–7.169 (d, J = 8.7 Hz, 2H), 7.234–7.242 (d, J = 2.4 Hz, 2H), 7.306–7.334 (d, J = 8.4 Hz, 2H)]; MS: [M^+] at m/z 394.16.

5.2.3. N-chloroacetyl-2-(4-methoxyphenyl)-3-methyl-6-(4-fluorophenyl)-piperidin-4-one (**3b**)

Stirring time 21.0 h; Brown colored crystalline solid; Yield 75.84% (Ethanol); Melting range (°C): 121–123; IR (KBr) (ν cm^{-1}): (1654.81) N–C–O Stretching, (1718.46) C=O Stretching, (837.05) Ar–H Bending; ^1H NMR [($CDCl_3$) δ in ppm: 0.822–0.840 (d, J = 5.4 Hz, 3H), 3.159–3.170 (d, J = 3.3 Hz, 2H), 3.782–3.808 (d, J = 7.8 Hz, 1H), 3.895 (s, 3H), 3.956 (s, 2H), 4.511–4.517 (d, J = 1.8 Hz, 1H), 6.781–6.810 (d, J = 8.7 Hz, 2H), 6.872–6.904 (d, J = 9.6 Hz, 2H), 6.952–6.961 (d, J = 2.7 Hz, 2H), 6.996–7.056 (t, J = 18.0 Hz, 1H), 7.827–7.856 (d, J = 8.7 Hz, 2H)]; MS: $[M+1]^+$ at m/z 390.17.

5.2.4. N-chloroacetyl-2-(4-methoxyphenyl)-3-methyl-6-(4-chlorophenyl)-piperidin-4-one (**5b**)

Stirring time 25.0 h; Brown colored crystalline solid; Yield 74.9% (Ethanol); Melting range (°C): 202–205; IR (KBr) (ν cm^{-1}): (1658.67) N–C–O Stretching, (1720.39) C=O Stretching, (833.19) Ar–H Bending; ^1H NMR [($CDCl_3$) δ in ppm: 1.051–1.093 (d, J = 12.6 Hz, 3H), 3.133–3.153 (d, J = 6.0 Hz, 2H), 3.752 (s, 3H), 3.785–3.810 (d, J = 7.5 Hz, 1H), 3.894 (s, 2H), 3.935–3.951 (d,

J = 4.8 Hz, 1H), 4.111–4.176 (t, J = 19.5 Hz, 1H), 6.843–6.871 (d, J = 8.4 Hz, 2H), 6.785–6.809 (d, J = 7.2 Hz, 2H), 7.167–7.182 (d, J = 4.5 Hz, 2H), 7.242–7.269 (d, J = 5.1 Hz, 2H)]; Anal. Calcd for $C_{21}H_{21}Cl_2NO_3$: C = 62.08%, H = 5.21%, N = 3.45%; Found C = 61.64%, H = 5.42%, N = 2.10%; MS: [M^+] at m/z 406.13.

5.3. General procedure for the synthesis of N-chloroacetyl-2,6-diarylpiperidin-4-ones (**4b** and **6b**)

To a well-stirred solution of (**4a** and **6a**) substituted 2,6-diphenylpiperidin-4-one (0.005 mol, 1 equiv.) and triethylamine (0.005 mol, 1 equiv.) in 30 ml chloroform, chloroacetyl chloride (0.005 mol, 1 equiv.) in 30 ml chloroform was added dropwise, for about half an hour, through a dropping funnel. Stirring was continued with mild heating at (30–35 °C) till the completion of the reaction. After this, the mixture was poured into 10 ml water and extracted with three 10 ml portions of ether. The collected ether extracts were then washed well with 3% sodium bicarbonate solution and dried over anhydrous sodium sulfate. The same upon evaporation and subsequent recrystallization in distilled ethanol furnished the compound (**4b** and **6b**) in pure form with good yields. The completion of the reactions was monitored by TLC, using the solvent system methanol: chloroform (4%).

5.3.1. N-chloroacetyl-2-(4-hydroxyphenyl)-3-methyl-6-(4-chlorophenyl)-piperidin-4-one (**4b**)

Stirring time 21.0 h; Orange colored crystalline solid; Yield 45.32% (Ethanol); Melting range (°C): 132–134; IR (KBr) (ν cm^{-1}): (1652.88) N–C–O Stretching, (1716.53) C=O Stretching, (833.19) Ar–H Bending; ^{1}H NMR [($CDCl_3$) δ in ppm: 0.830–0.852 (d, J = 6.6 Hz, 3H), 3.659–3.695 (d, J = 10.8 Hz, 2H), 3.947–3.951 (d, J = 1.2 Hz, 1H), 4.175 (s, 2H), 4.527 (s, 1H), 5.815–5.861 (d, J = 13.8 Hz, 1H), 6.567–6.592 (t, J = 7.5 Hz, 1H), 6.823–6.837 (d, J = 4.2 Hz, 2H), 6.939–6.966 (d, J = 8.1 Hz, 2H), 7.159–7.184 (d, J = 7.5 Hz, 2H), 7.306–7.328 (d, J = 6.6 Hz, 2H)]; Anal. Calcd for $C_{20}H_{19}Cl_2NO_3$: C = 61.24%, H = 4.88%, N = 3.57%; Found C = 60.19%, H = 4.82%, N = 3.82%; MS: [M^+] at m/z 392.12.

5.3.2. N-chloroacetyl-2-(4-hydroxyphenyl)-3-methyl-6-(4-fluorophenyl)-piperidin-4-one (**6b**)

Stirring time 23.5 h; Wine red colored crystalline solid; Yield 73.80% (Ethanol); Melting range (°C): 192–195; IR (KBr) (ν cm^{-1}): (1658.67) N–C–O Stretching, (1720.39) C=O Stretching, (837.05) Ar–H Bending; ^{1}H NMR [($CDCl_3$) δ in ppm: 0.835–0.856 (d, J = 6.3 Hz, 3H), 3.028–3.051 (d, J = 6.9 Hz, 2H), 3.096–3.115 (d, J = 5.7 Hz, 1H), 4.011 (s, 2H), 4.165 (s, 1H), 4.938–4.956 (d, J = 5.4 Hz, 1H), 5.846–5.941 (t, J = 28.5 Hz, 1H), 7.028–7.056 (d, J = 8.4 Hz, 2H), 7.157–7.181 (d, J = 7.2 Hz, 2H), 7.342–7.404 (d, J = 18.6 Hz, 2H), 7.743–7.771 (d, J = 9.6 Hz, 2H)]; MS: $[M+1]^+$ at m/z 376.14.

5.4. General procedure for the synthesis of N-(N-methylpiperazinoacetyl)-2,6-diarylpiperidin-4-ones (**1c**, **2c**, **3c** and **5c**)

A mixture of N-chloroacetyl-2,6-diarylpiperidin-4-one derivative (**1b**, **2b**, **3b** and **5b** – 0.005 mol, 1 equiv.), triethylamine (0.005 mol, 1 equiv.) and N-methyl piperazine (0.005 mol, 1 equiv.) in 30 ml of toluene was refluxed for about 13–20.5 h on water bath. After the completion of the reaction, excess of solvent was removed under reduced pressure. The final mass was poured into water to remove the quaternary ammonium salt formed. This was then extracted with ether three times and dried over anhydrous sodium sulfate. The completion of the reaction was monitored by TLC, using the solvent system n-hexane: ethyl acetate mixture (4:1).

5.4.1. N-(N-methylpiperazinoacetyl)-2-(4-dimethylaminophenyl)-3-methyl-6-(4-chlorophenyl)-piperidin-4-one (**1c**)

Reflux time 13.0 h; Orange colored crystalline solid; Yield 50.62% (Ethanol); Melting range (°C): 105–107; IR (KBr) (ν cm^{-1}): (1643.24) N–CH_2–C=O Stretching, (1714.69) C=O Stretching, (833.19) Ar–H Bending; ^{1}H NMR [($CDCl_3$) δ in ppm: 0.815–0.837 (d, 3H), 2.105 (s, 3H), 2.569–2.626 (q, 8H), 2.947 (s, 6H), 3.026–3.034 (d, 2H), 3.091 (s, 2H), 3.587–3.621 (d, 1H), 6.693–6.722 (d, 1H), 7.142–7.214 (t, 1H), 7.248–7.266 (d, 2H), 7.332–7.354 (d, 2H), 7.378–7.392 (d, 2H), 7.726–7.755 (d, 2H)]; Anal. Calcd for $C_{27}H_{35}ClN_4O_5$: C = 67.13%, H = 7.30%, N = 11.60%; Found C = 79.18%, H = 5.80%, N = 3.09%; MS: [M^+] at m/z 483.35.

5.4.2. N-(N-methylpiperazinoacetyl)-2-(4-chlorophenyl)-3-methyl-6-(4-fluorophenyl)-piperidin-4-one (**2c**)

Reflux time 18.0 h; Brown colored crystalline solid; Yield 63.02% (Ethanol); Melting range (°C): 178–180; IR (KBr) (ν cm^{-1}): (1643.24) N–CH_2–C=O Stretching, (1720.39) C=O Stretching, (833.19) Ar–H Bending; ^{1}H NMR [($CDCl_3$) δ in ppm: 1.078–1.096 (d, J = 5.4 Hz, 3H), 2.291 (s, 3H), 2.793–2.813 (t, J = 6.0 Hz, 4H), 2.860–2.873 (d, J = 3.9 Hz, 2H), 3.09 (s, 2H), 3.294–3.340 (t, J = 13.8 Hz, 4H), 3.587–3.621 (d, J = 10.2 Hz, 1H), 3.683–3.729 (t, J = 13.8 Hz, 1H), 6.880–6.896 (d, J = 4.8 Hz, 2H), 7.026–7.033 (d, J = 2.1 Hz, 2H), 7.169–7.178 (d, J = 2.7 Hz, 2H), 7.233–7.241 (d, J = 2.4 Hz, 2H)]; MS: $[M+1]^+$ at m/z 458.24 .

5.4.3. N-(N-methylpiperazinoacetyl)-2-(4-methoxyphenyl)-3-methyl-6-(4-fluorophenyl)-piperidin-4-one (**3c**)

Reflux time 19.0 h; Dark brown colored crystalline solid; Yield 73.34% (Ethanol); Melting range (°C): 187–189; IR (KBr) (ν cm^{-1}): (1641.31) N–CH_2–C=O Stretching, (1718.46) C=O Stretching, (835.12) Ar–H Bending; ^{1}H NMR [($CDCl_3$) δ in ppm: 0.811–0.833 (d, J = 6.6 Hz, 3H), 2.285 (s, 3H), 2.592–2.615 (t, J = 6.9 Hz, 8H), 3.685–3.698 (d, J = 3.9 Hz, 2H), 3.809 (s, 2H), 3.884–3.894 (d, J = 3.0 Hz, 1H), 4.007 (s, 3H), 4.085–4.097 (d, J = 3.6 Hz, 1H), 6.552–6.588 (t, J = 10.8 Hz, 1H), 6.795–6.824 (d, J = 8.7 Hz, 2H), 6.907–6.914 (d, J = 2.1 Hz, 2H), 7.024–7.032 (d, J = 2.4 Hz, 2H), 7.157–7.182 (d, J = 7.5 Hz, 2H)]; MS: $[M+1]^+$ at m/z 454.30.

5.4.4. N-(N-methylpiperazinoacetyl)-2-(4-methoxyphenyl)-3-methyl-6-(4-chlorophenyl)-piperidin-4-one (**5c**)

Reflux time 20.5 h; Dark brown colored crystalline solid; Yield 71.16% (Ethanol); Melting range (°C): 128–130; IR (KBr) (ν cm^{-1}): (1641.31) N–CH_2–C=O Stretching, (1718.46) C=O Stretching, (831.26) Ar–H Bending; ^{1}H NMR [($CDCl_3$) δ in ppm: 0.857–0.880 (d, J = 6.9 Hz, 3H), 2.285 (s, 3H), 2.353–2.378 (t, J = 7.5 Hz, 8H), 3.722–3.730 (d, J = 2.4 Hz, 2H), 3.772–3.792 (d, J = 6.0 Hz, 1H), 3.799 (s, 2H), 3.894 (s, 3H), 5.869–5.965 (d, J = 1.8 Hz, 1H), 6.532–6.582 (t, J = 15.0 Hz, 1H), 6.734–6.762 (d, J = 8.4 Hz, 2H), 6.793–6.882 (d, J = 26.7 Hz, 2H), 7.016–7.022 (d, J = 1.8 Hz, 2H), 7.376–7.390 (d, J = 4.2 Hz, 2H)]; Anal. Calcd for $C_{26}H_{32}ClN_3O_3$: C = 66.44%, H = 6.86%, N = 8.94%; Found C = 64.98%, H = 6.33%, N = 7.87%; MS: $[M+1]^+$ at m/z 470.30.

5.5. In-vitro studies

The *in-vitro* studies included partition coefficient and hydrolysis profile in SGF (simulated gastric fluid) and SIF (simulated gastric fluid).

5.5.1. Determination of partition coefficient

Partition coefficient of the synthesized compounds was determined by "Shake Flask Method" [48]. Initially, 2 mg of the synthesized compound was dissolved in 100 ml of the solvent (50 ml water + 50 ml octanol). It was then shaken for 30 min and kept

aside for 24 h to attain equilibrium. Two phases were separated and the concentration of the drug was determined in octanol phase by taking absorbance. Finally, found absorbance was extrapolated over a standard curve so as to obtain the concentrations of the organic phase. The log *P* values of the synthesized compounds were then determined from the concentrations of the octanol phase obtained, using the formula given below:

$$\text{Log } P = [\text{conc. of compound in octanol}]/ \times [\text{conc. of compound in distilled water}]$$

Similar procedure was adopted for all the synthesized compounds [49].

5.5.2. Hydrolysis studies

For this, 10 mg of the synthesized compound was dispersed/dissolved in 100 ml of simulated gastric fluid/simulated intestinal fluid. The resulting mixture was shaken on an orbital shaker at a temperature of 37 ± 2 °C. Samples were withdrawn at regular intervals of 0, 15, 30, 45, 60, 75, 90, 105, 120 min. The samples were suitably diluted, filtered and analyzed spectrophotometrically at the designated absorption maxima of each compound [49]. Percent hydrolysis was calculated using the following formulas:

$$\%\ \text{Hydrolysed in SGF} = [(\text{abs. at 0 min} - \text{abs. at 90 min}) \times /\text{abs. at 0 min}]^{*}100.$$

$$\%\ \text{Hydrolysed in SIF} = [(\text{abs. at 0 min} - \text{abs. at 120 min}) \times /\text{abs. at 0 min}]^{*}100.$$

5.6. Pharmacology

Male Albino mice and Wister rats weighing 25–30 g and 200–300 g respectively (unless otherwise specified), were used for the pharmacological studies. The animals were housed in polypropylene cages with steel net, in a temperature controlled room under standard living conditions of temperature 25 ± 5 °C and relative humidity of 55 ± 5% with regular 12 h light and 12 h dark cycles, and allowed free access to standard laboratory food and water. However, the groups of animals chosen for the study were fasted for 20–24 h before the treatment and were divided into parallel groups containing five animals each. All the animals were treated humanely in accordance with the guidelines laid down by the Institutional Animal Ethics Committee (IAEC). Experiments were performed after the protocols were approved by the IAEC and also by strictly adhering to the ethical guidelines.

The reported values are the average of five determinations. The observed results are expressed as mean ± SEM and were analyzed by one-way analysis of variance (ANOVA, Dunnett's test) for the possible significant identification between various groups. The $^{*}P$ value of <0.05 or <0.01 was considered as statistically significant or highly significant, respectively. Statistical analysis was carried out using Graph pad InStat 3.0 (Graph pad software).

5.6.1. Analgesic activity

Antinociceptive activity of the series of compounds was determined by acetic acid-induced writhing test [50] in Albino mice. Groups of 5 animals each were used for control and treated mice. The test compounds were administered at 60 mg/kg body weight, with diclofenac sodium (10 mg/kg) as the reference drug. All the compounds and standard drug were suspended in 0.5% sodium carboxymethyl cellulose (CMC) and administrated orally (p.o.). The writhing syndrome in test animals was elicited by injecting 1% of acetic acid in normal saline (10 ml/kg body weight) intraperitoneally, 1 h after the oral administration of the test compounds. After 5 min of injection of the noxious agent, the number of writhes was counted during the subsequent 30 min by keeping the animals on a flat surface. A significant reduction in the number of writhes by drug treatments, as compared to vehicle control animals, was considered as a positive analgesic response. The mean number of writhes for each experimental group was compared with that of the control group and the analgesic potency was expressed as percent inhibition, determined using the following equation where T is the number of writhes in the test compound treated group and C is the number of writhes in control group.

$$\text{Percentage inhibition of writhing} = [1 - (T/C)]^{*}100.$$

5.6.2. Rota rod test

The test is used to evaluate the activity of drugs interfering with motor coordination. The cardinal feature of the test is to ascertain the impairment of motor performance, ataxia, loss of skeletal muscle strength, and acute neurotoxicity produced by drugs in preclinical studies. The animals were placed on a 1 in. diameter knurled wooden rod, rotating at a speed of 6–10 rpm (Medicraft Rota rod Apparatus). Diazepam at a dose of 2 mg/kg body weight was taken as the positive control. The study was started 60 min after the oral administration of the test compounds. Normal mice remain on a rod rotating at this speed indefinitely. Neurologic toxicity was defined as the failure of the animal to remain on the rod for duration of 1 min or more [51].

5.6.3. Antipyretic activity

Antipyretic activity of the target compounds was determined by measuring the variation in the rectal temperature as per the reported procedure [52]. Fever was induced in the fasted Wistar rats by injecting 15% brewer's yeast in normal saline (10 ml/kg body weight) subcutaneously. Initial rectal temperature was recorded using a lubricated digital thermometer, by inserting it into the rectum of the animal at a depth of about 7 mm. The same thermometer was used for all the animals in each group in order to minimize the possible experimental error. All the values are mean of five independent measurements. After 18 h, the animals that showed rectal temperature of at least 38 °C were selected for this study. Paracetamol (150 mg/kg) was used as the reference drug. The test compounds and standard drug were administered orally as a suspension in 0.5% CMC, at doses of 60 and 150 mg/kg body weight respectively. Control groups received the vehicle (0.5% CMC) only. After the administration of the standard/test compounds, the rectal temperature was measured successively after 30, 60, 120 and 180 min.

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