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Original article

Synthesis and spectral characterization of a new class of *N*-(*N*-methylpiperazinoacetyl)-2,6-diarylpiperidin-4-ones: Antimicrobial, analgesic and antipyretic studies

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Abstract

A series of *N*-(*N*-methylpiperazinoacetyl)-2,6-diarylpiperidin-4-ones (**13c–21c**) were synthesized by the base catalyzed nucleophilic substitution of *N*-chloroacetyl-2,6-diarylpiperidin-4-ones obtained from their corresponding 2,6-diarylpiperidin-4-ones with *N*-methylpiperazine. These newly synthesized compounds were characterized by one- and two-dimensional NMR spectral studies. In all the cases, the piperazine ring adopted normal chair conformation with equatorial orientation of methyl group irrespective of the non-chair conformations of the piperidin-4-one moiety. All the compounds were screened for their possible antibacterial and antifungal activities against a spectrum of microbial agents besides analgesic and antipyretic activities. These biological studies proved that compounds **17c/18c** against bacterial and **18c/20c** against fungal strains exhibited promising antimicrobial activities whereas **17c/19c** and **18c/19c** showed beneficial analgesic and antipyretic profiles, respectively, at a concentration of 60 mg/kg and were also found to be more potent than the reference drug.

Keywords: Piperidin-4-one; *N*-Methylpiperazine; Conformation; Antimicrobial; Analgesic; Antipyretic activities

1. Introduction

Despite the major breakthrough in many areas of modern medicine during the past 10 decades, the successful treatment towards multidrug-resistant pathogens (i.e., microbial isolates such as fungi and bacteria) has become a serious problem and remains a significant challenge over the last 10 years [1]. The potentiality of non-steroidal anti-inflammatory drugs (NSAID) to alleviate pain, inflammation and fever coupled with a number of pathological conditions made them the most useful therapeutic agents in the world [2]. However, the routine use of these agents was reported to be limited because of their associated side effects mainly on gastrointestinal (GI) tracts. Pain is an unpleasant sensory and emotional experience associated with actual or potential organ and tissue damage [3]. Body inflammation is a unique pain inducer which the human kind faces more often as an outcome of tissue damage developed by a series of microbial infections such as anorexia, pain and fever which in turn shoots up the body temperature. In order to combat these diseases caused by pathogens, it is usual that chemotherapeutics, analgesic and antipyretic agents are prescribed separately in clinical practices. However, multidrug treatments for microbial diseases create a significant problem among the patients with impaired organ functions [4]. This laid the foundation for the search and design of new chemical

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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 antimicrobial, analgesic and antipyretic activities together by keeping in view the pharmaco-economic and frequent patient compliance. While exploring for such a compound, we have found that piperidone nucleus is an integral component of countless alkaloids with marked biological properties [5–12]. Interest in 2,6-diarylpiperidin-4-ones **1** (Fig. 1) derives from the varied biological activities of this structural motif containing compounds, that were evaluated for wide range of pharmacological activities [13,14] and in particular their potency towards broad spectrum of microbial strains [15–22]. Generally, compounds possessing an amide bond linkage have a wide range of biological activities such as antimicrobial [23,24], anti-inflammatory [25], antiviral, antimalarial and general anesthetics [26]. Furthermore, the amides derived from chloroacetyl chloride also gain significant importance in medicinal field as evidenced by their varied pharmacological activities [27–32].

Compounds with piperazine and its *N*-substituted derivatives as an integral part are the most successfully employed side chains as they exhibit a varied range of activity such as antimicrobial [23,33], anticancer [34], anti-inflammatory [35], antipsychotic [36], CNS agent [37], antagonists for CCKB/gastrin receptor [38], melanocortin-4-receptor [39] and in the treatment of Alzheimer's disease [40].

Some clinically useful drugs such as Norfloxacin **2**, Ciprofloxacin **3**, Enoxacin **4**, Ofloxacin **5** and Levofloxacine **6** (Fig. 1) having piperazine or its 1-methyl derivative skeleton as a vital part exhibit potential activity for respiratory, urinary, gastrointestinal tracts, skin and soft tissue infections caused by both classes of bacteria [41] while other drugs *viz*. Clothiapine **7**, Loxapine **8** and Clozapine **9** (Fig. 1) with 1-methylpiperazine unit were reported as efficient antipsychotic drugs. Ciprofloxacin **3** possesses modest activity against Gram-positive cocci whereas its *N*-methylpiperazine derivative *viz*., Levofloxacine **6** has enhanced potency against Gram-positive and atypical organisms as it may be due to its better cell permeability and enhanced lipophilicity [41,42]. This indicates that replacement of piperazine moiety by its 1-methyl analogue has well pronounced impact on the microbial potency. Moreover, recent literature [43] reveals that linking of *N*-substituted piperazine to aryl or heteroaryl moiety by two carbon chains having β-keto group **10** (Fig. 1) has additional advantage in eliciting good biological response.

As a part of our ongoing research program to find out potent broad spectrum antimicrobial agents for the past few years, we have made certain modifications on the nitrogen site of **1** by introducing various heterocyclic ring systems through two carbon spacer units (either alkoxy [17,18] or acyl [15,16]), of which, a few compounds were endowed with appreciable and statistically significant antimicrobial activities. However, preliminary in vitro antimicrobial studies of the core structure **1**

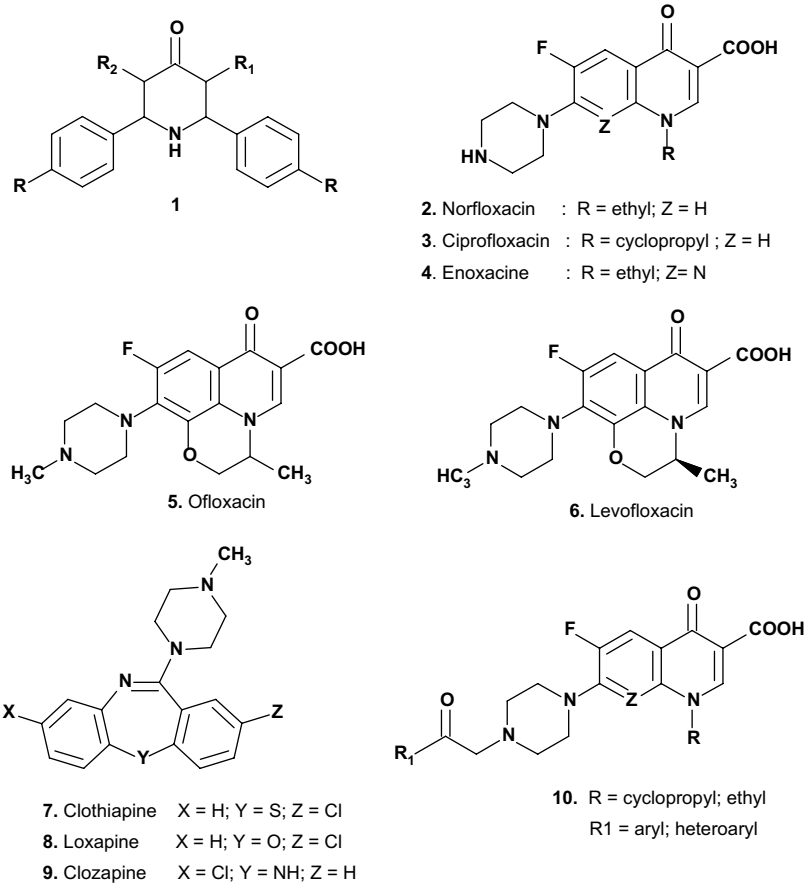


Fig. 1.

with chloroacetyl unit at 'N' (compound **11**) has little effect towards certain selected microbial strains [16] whereas nucleophilic substitution of morpholine [15] in **11** (i.e., compound **12**; Fig. 2) gave encouraging results with improved activity profile. Hence, by considering the potent pharmacological activities of **1**, **12** and piperazine, we have now aimed to widen our knowledge of structure–activity relationships (SAR) in this structural framework by synthesizing a new class of novel target compounds **13c–21c** (Fig. 2) and investigated their possible antimicrobial, analgesic and antipyretic activities.

2. Results and discussion

2.1. Chemistry

The obvious synthetic pathway that leads to the title compounds (**13c–21c**) is represented in Scheme 1. By adopting the literature precedent [44], 2,6-diarylpiperidin-4-ones were prepared in one pot multi-component Mannich reaction by condensing suitably substituted aromatic aldehydes, ketone and ammonium acetate in 1:2:1 ratio using ethanol as solvent. By keeping in view the better basicity and HCl scavenging power of triethylamine, we have used this as a desirable catalyst for the nucleophilic reaction of **13a–21a** with chloroacetyl chloride than the other catalyst. The yields of *N*-chloroacetyl-2,6-diarylpiperidin-4-ones were also significantly good while using this catalyst. Further, nucleophilic substitution of *N*-methylpiperazine with these chloroacetyl derivatives was performed in different solvents such as benzene, ethanol and toluene using NEt_3 again as base. Here, toluene was chosen as the best solvent as it improves the yield of the title compounds (**13c–21c**) appreciably compared to the remaining solvents used.

Preliminary examination of the formation of compounds by FT-IR analysis confirmed the absence of NH stretching in piperazine and changes in the magnitude of amide carbonyl stretching of **13b–21b** (for **13c–21c**, it appeared at around 1660 cm^{-1}) besides the appearance of additional aliphatic stretching frequencies in the region 2981–2694 cm^{-1}. Analytical data of all the synthesized compounds are furnished in Table 1.

2.2. NMR spectral analysis and stereochemistry

It is very clear from our earlier reports [45] that the substitution of chloroacetyl moiety at the heterocyclic 'N' of 2,6-diarylpiperidin-4-ones significantly influences the chemical shift and coupling constant values of **13b–21b** which in turn affect the normal chair conformation of the parent compounds **13a–21a**. By one- and two-dimensional NMR spectral studies of chloroacetyl derivatives **13b–21b**, we have arrived at the following conclusions:

(a) Existence of two rotomers **A** and **B** (Fig. 3) is due to the restricted rotation about N–CO bond.
(b) The obtained average NMR spectra for these two rotomers are mainly due to their faster interconversion on NMR time scale in $CDCl_3$ at room temperature.
(c) Observation of broad singlet for the benzylic protons in the most deshielded region suggested the co-planarity of chloroacetyl moiety with the reference plane of the piperidone ring. Due to this, severe allylic strain may exist between amide carbonyl group and phenyl moieties at C-2 and C-6 positions which consequently alters the chair conformation of piperidone framework into non-chair conformations of its preference.
(d) In these flexible conformations, the ring may become flattened at the nitrogen end. Therefore, the benzylic protons fall in the planar region of the chloroacetyl group thereby deshielded well whereas the methylene protons at C-5 resonated at two different chemical shifts with significant splitting patterns.

By keeping these observations in mind, we have analyzed the chemical shifts and conformations of the final compounds **13c–21c**.

2.2.1. ^1H NMR spectral analysis of *13c–21c*

^1H NMR assignment of compounds **13c–21c** is made based on their one- and two-dimensional NMR spectral studies. In all the cases, the benzylic protons resonated as a broad singlet with very low intensity in the most downfield region 5.44–6.54 ppm. Akin to **13b–21b**, for symmetrically substituted derivatives **16c**, **18c**, **20c**, **21c**, C-2 and C-6 benzylic protons of piperidone ring showed a common resonance whereas for the unsymmetrically substituted compounds **13c–15c**, **17c** and **19c**, they resonated individually. The observed broadening of the benzylic proton signal suggests the existence of restricted rotation about N–CO bond and also the in plane nature of the substituent at heterocyclic nitrogen of piperidone moiety with dynamically averaged plane of the piperidone system.

One-dimensional ^1H NMR spectra of these compounds showed a medium intense unresolved broad singlet and a very sharp singlet in the aliphatic region 2.49–2.55 ppm and 2.31–2.43 ppm with eight and three protons integral, respectively. In **13c–21c**, as the nitrogen of piperazine moiety is having almost similar chemical environment on either side, its ring methylene protons become equivalent and therefore the signals in the former region are assigned to four methylene protons while the signal in the latter region is characteristic for the methyl protons of *N*-methylpiperazine system. Moreover, irrespective of the restricted rotation of the N–CO bond, the rotation about N–C bond of the

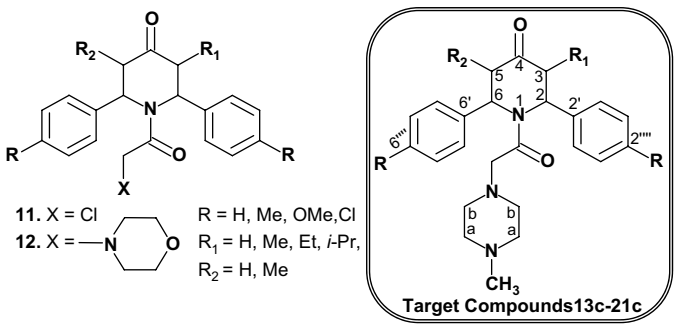
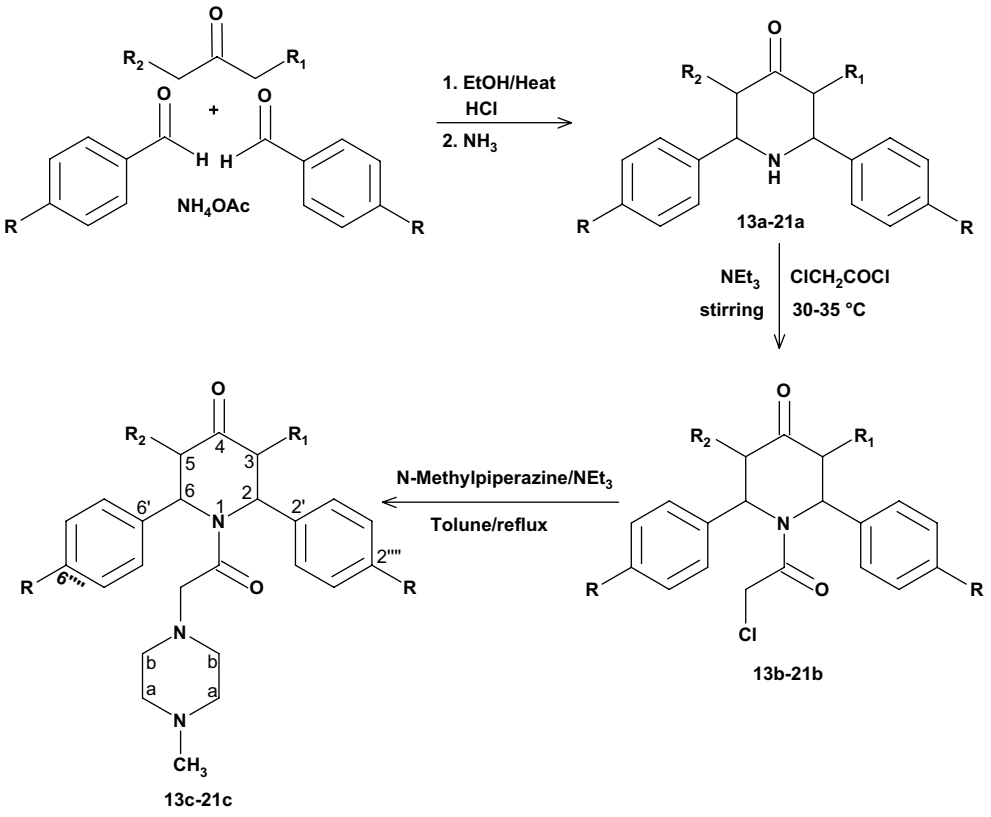


Fig. 2.



Entry	R_1	R_2	R
13 (a-c)	Me	H	H
14 (a-c)	Et	H	H
15 (a-c)	*i*-Pr	H	H
16 (a-c)	Me	Me	H
17 (a-c)	Me	H	Cl
18 (a-c)	Me	Me	Cl
19 (a-c)	Me	H	OMe
20 (a-c)	Me	Me	OMe
21 (a-c)	Me	Me	Me

Scheme 1.

piperazinoacetyl moiety is very fast and prevents the spin–spin coupling of all the methylene protons of piperazine moiety. Owing to this, their resonances were observed as an unresolved broad singlet. All these observations strongly confirm the nucleophilic substitution of piperazine moiety in place of chlorine. Moreover, disappearance of acetyl methylene proton signal of **13b–21b** from its original position (i.e., chemical shift) also strongly confirms this nucleophilic substitution. Our earlier report [45] described clearly that protons of C-3, C-5 and acetyl methylene group of **13b** appeared as well-resolved signals with good splitting pattern at 3.06 (t), 2.83/3.17 (dd) and 3.88/3.93 (d) ppm, respectively. But, for its corresponding piperazinoacetyl derivative (**13c** unsymmetrically substituted), well-resolved signals were not observed except a double doublet centered at 2.81 ppm. By comparing chemical shift and coupling constant values of double doublet at 2.81 ppm ($^2J_{5a,5e} = 17.99$ Hz/$^3J_{5e,6a} = 5.91$ Hz), it is conveniently assigned to H-5e. This is further confirmed by its one proton integral value. For the precise assignment of acetyl methylene, C-3 and H-5a protons of **13c**, HOMOCOSY (Fig. 4) experiment has been performed and the observed correlations are furnished in Table 2. From the ^{1}H–^{1}H

Table 1
Analytical data of bioactive compounds **13c–21c**[a]

Compound	Molecular formula	Molecular weight	Yield (%)	Reaction time (h)	M.p (°C)	Elemental analysis					
						Observed (%)			Calculated (%)		
						C	H	N	C	H	N
13c[b]	$C_{25}H_{31}N_3O_2$	405.53	88	8.0	61	74.04	7.71	10.37	74.04	7.70	10.36
14c	$C_{26}H_{33}N_3O_2$	419.56	82	9.5	128–129	74.45	7.92	10.02	74.43	7.93	10.02
15c	$C_{27}H_{35}N_3O_2$	433.59	81	8.5	105	74.50	8.15	9.70	74.79	8.14	9.69
16c[b]	$C_{26}H_{33}N_3O_2$	419.56	85	9.0	Semisolid	74.41	7.94	10.03	74.43	7.93	10.02
17c	$C_{25}H_{29}N_3O_2Cl_2$	474.42	87	10.0	Semisolid	63.27	6.15	8.86	63.29	6.16	8.86
18c	$C_{26}H_{31}N_3O_2Cl_2$	488.45	80	9.5	82	63.93	6.42	8.58	63.93	6.40	8.60
19c	$C_{27}H_{35}N_3O_4$	465.59	82	8.0	Semisolid	69.67	7.59	9.01	69.65	7.58	9.03
20c	$C_{28}H_{37}N_3O_4$	479.61	79	10.0	Semisolid	70.14	7.79	8.77	70.12	7.78	8.76
21c	$C_{28}H_{37}N_3O_2$	447.61	74	9.5	Semisolid	75.13	8.34	9.39	75.13	8.33	9.39

[a] All the compounds were purified by column chromatography using *n*-hexane:ethyl acetate (4:1) as eluent.
[b] Molecular mass noted for **13c** and **16c** at 406 and 420 $(M+H)^+$ respectively are consistent with the proposed molecular formula.

correlations, it is found that the partially overlapped multiplet in the region 3.02–3.28 ppm has good HOMO correlation with H-2 [5.64 ppm (s)], H-5e [2.81 ppm (dd)] and methyl protons at C-3 [1.12 ppm (d)]. By considering these correlations, the partially overlapped multiplet with four proton integral is assigned beyond doubt to H-3a, H-5a and acetyl methylene protons. As the signals for the remaining unsymmetrically substituted compounds (**14c**, **15c**, **17c** and **19c**) did not vary appreciably, they were assigned in a similar fashion. Likewise, for unambiguous assignment of symmetrically substituted compounds (**16c**, **18c**, **20c**, and **21c**), HOMOCOSY spectrum was recorded for the representative compound **16c** and the noted correlations are illustrated in Table 2. In symmetrically substituted compounds, except shielding of acetyl methylene protons by about 1 ppm, no appreciable change in the chemical shift values of piperidone ring protons was observed.

Therefore, from one- and two-dimensional NMR spectra, the complete and unambiguous assignment of the individual protons in piperidone and piperazine ring system of *N*-(*N*-methylpiperazinoacetyl)-2,6-diarylpiperidin-4-ones was made.

2.2.2. ^{13}C NMR spectral analysis of **13c–21c**

In plane nature of piperazine ring with respect to the amide carbonyl group is evidenced from the two intense signals observed (54.4–55.0/52.9–53.5 ppm) for the methylene carbons of piperazine *viz*. C-a and C-b unlike their corresponding protons. On the basis of the earlier studies [43,46], the upfield signal is assigned to C-b carbons while the downfield signal to C-a carbons because the former carbons are present at γ-position with respect to the amide carbonyl group, thereby experiencing its electronic effect (about 1.5 ppm). The methyl carbon of *N*-methylpiperazine moiety resonated in the region of 45.4–45.9 ppm. All the signals corresponding to the *N*-methylpiperazine moiety are further confirmed from the HSQC (heteronuclear single quantum coherence) spectrum recorded for the representative compounds **13c** (Fig. 5) and **16c** and the observed correlations are reproduced in Table 3. As the proton NMR chemical shift values of the piperidone ring system and the acetyl methylene group in **13c** and **16c** are assigned perfectly, the respective carbon signals are also established on the basis of the observed HSQC correlations given in Table 3. A striking observation from the HSQC correlations is that the resonances of acetyl methylene carbon in **13c–21c** were deshielded to about 30 ppm due to the replacement of chlorine by *N*-methylpiperazine moiety and observed in the region of 61.8–62.4 ppm. The known electronic and substituent effects explain the significant changes in the carbon resonances. Likewise, NMR assignments of the rest of the compounds were also made.

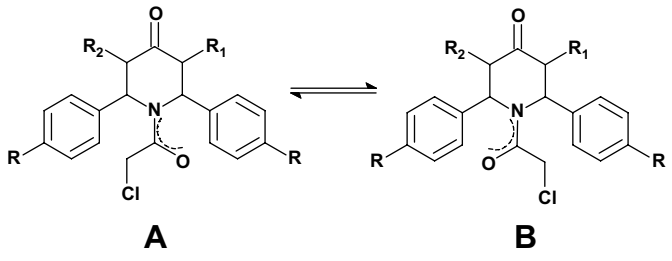


Fig. 3.

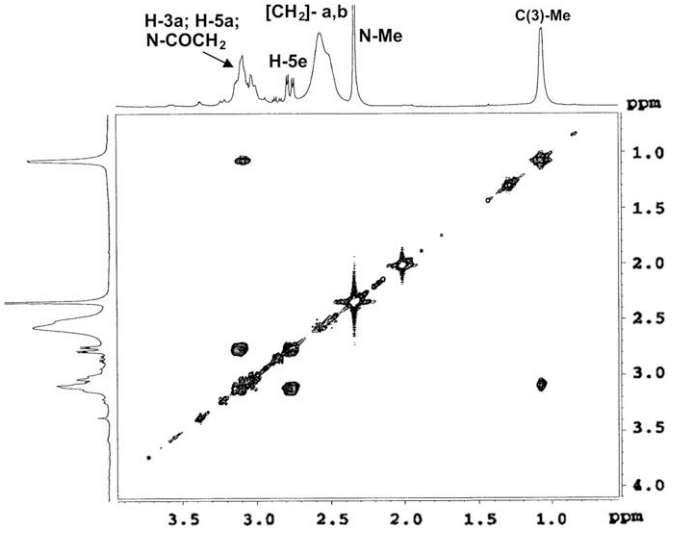


Fig. 4. HOMOCOSY spectrum of **13c**.

Table 2
Correlations in the HOMOCOSY spectrum of compounds **13c** and **16c** [δ (ppm)]

Compound	Signal	Observed correlations
13c	1.12 (d, 3H, CH_3 at C-3)	3.02–3.28
	2.81 (dd, 1H, H-5e)	3.02–3.28
	5.64 (br s, 1H, H-2)	3.02–3.28
	3.02–3.28 (m, 4H, H-3a, H-5a and N–$COCH_2$)	5.64, 2.81, 1.12
16c	1.06 (d, CH_3 at C-3 and C-5)	3.12
	3.12 (t, 2H, H-3a and H-5a)	5.52, 1.06
	5.52 (br s, 2H, H-2 and H-6)	3.12

2.2.3. Conformational analysis of *13c–21c*

Broadening of benzylic protons supports the existence of restricted rotation about N–CO bond and also the in plane nature of the amide group with the dynamically averaged plane of the piperidone ring [45,47]. Depending upon the substituents on the piperidone ring system, the possible non-chair conformations for *N*-chloroacetyl-2,6-diarylpiperidin-4-one system have been established earlier by our group [45]. Replacement of chlorine atom by *N*-methylpiperazine moiety did not change the resonances of carbon and the associated protons appreciably in **13b–21b**. This suggests that the piperidone ring system in *N*-(*N*-methylpiperazinoacetyl)-2,6-diarylpiperidin-4-ones also retains the same flexible non-chair conformations and the substituent at its nitrogen is also in plane with the reference plane of the piperidone.

Though the restricted rotation exists about N–CO bond, it will not affect the chair conformation of the piperazine ring system as evidenced from the unique resonance of the ring methylene protons. However, difference in the chemical shift of C-a and C-b carbons of the same reveals the co-planarity of piperazine moiety with the amide system (here C-b carbons are well within the planar region of the amide carbonyl group)

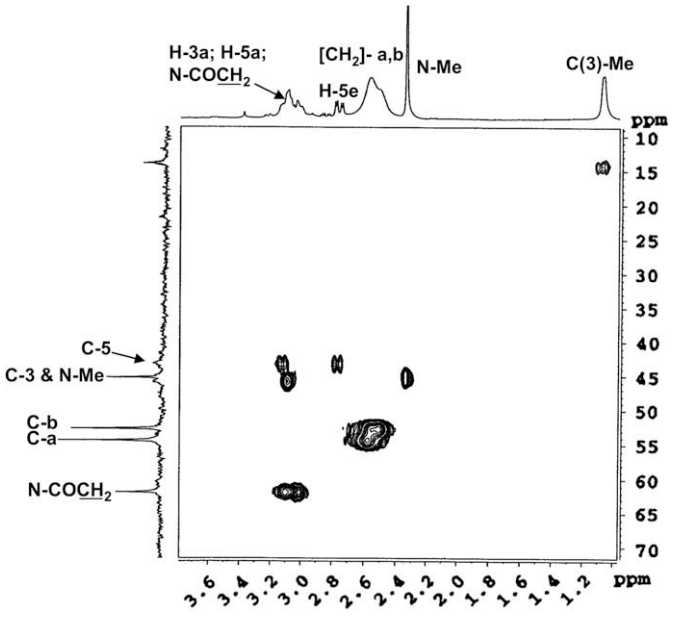


Fig. 5. HSQC spectrum of **13c**.

Table 3
Correlations in the HSQC spectrum of compounds **13c** and **16c** [δ (ppm)]

Compound	^{13}C signal	Correlations in the HSQC spectrum (with ^1H NMR)
13c	126.79–128.88 (aromatic carbons)	7.09–7.41 (aromatic protons)
	62.45 (N–*C*OCH_2)	3.02–3.28 (H-3a, H-5a, N–$COCH_2$)
	60.89 (C-2)	5.64 (H-2a)
	53.45 (C-6)	6.05 (H-6a)
	45.99 (C-3)	3.02–3.28 (H-3a, H-5a, N–$COCH_2$)
	43.28 (C-5)	3.02–3.28 (H-3a, H-5a, N–$COCH_2$), 2.81 (H-5e)
	55.00[(*C*H_2)$_2$-a of piperazine]	2.49 [$(CH_2)_2$-a and b of piperazine]
	53.45 [(*C*H_2)$_2$-b of piperazine]	2.49 [$(CH_2)_2$-a and b of piperazine]
	45.99 (N–*C*H_3)	2.33 (N–CH_3)
	14.09 (*C*H_3 at C-3)	1.12 (CH_3 at C-3)
16c	127.25–128.66 (aromatic carbons)	7.01–7.30 (aromatic protons)
	61.88 (N–*C*OCH_2)	2.99 (N–$COCH_2$)
	60.85 (C-2 and C-6)	5.52 (H-2a and H-6a)
	45.48 (C-3 and C-5)	3.12 (H-3a and H-5a)
	54.54 [(*C*H_2)$_2$-a of piperazine]	2.50 [$(CH_2)_2$-a and b of piperazine]
	52.99 [(*C*H_2)$_2$-b of piperazine]	2.50 [$(CH_2)_2$-a and b of piperazine]
	45.48 (N–*C*H_3)	2.32 (N–CH_3)
	14.14 (*C*H_3 at C-3 and C-5)	1.06 (CH_3 at C-3 and C-5)

which in turn is in plane with the piperidone ring system. Further, ^1H and ^{13}C resonances of *N*-methyl function have almost the same magnitude as that of equatorially oriented *N*-methyl substituted piperazine systems [48]. From this, it is clearly conceived that the methyl group is most preferentially in the equatorial orientation. On the basis of the above made investigations, the compounds **13c–21c** adopt the following three different pairs (which are in equilibrium with each other) of flexible non-chair conformations **A**, **B** and **C** (Fig. 6) in which *N*-methylpiperazine ring adopts normal chair conformation with equatorial orientation of methyl group irrespective of the substituents at C-3 and/or C-5 position of piperidin-4-one ring system.

2.3. Antimicrobial activity (structure–activity relationship)

The new class of compounds described in this paper have been preliminarily evaluated for their antibacterial and antifungal activities against a band of certain selected bacterial (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumoniae*) and fungal (*Candida albicans*, *Rhizopus* sp., *Aspergillus niger* and *Aspergillus flavus*) pathogens in vitro by conventional twofold serial dilution method [49]. The obtained results were compared to Ciprofloxacin and Amphotericin B which were used as the standard drugs for bacterial and fungal strains, respectively. The antibacterial and antifungal potencies

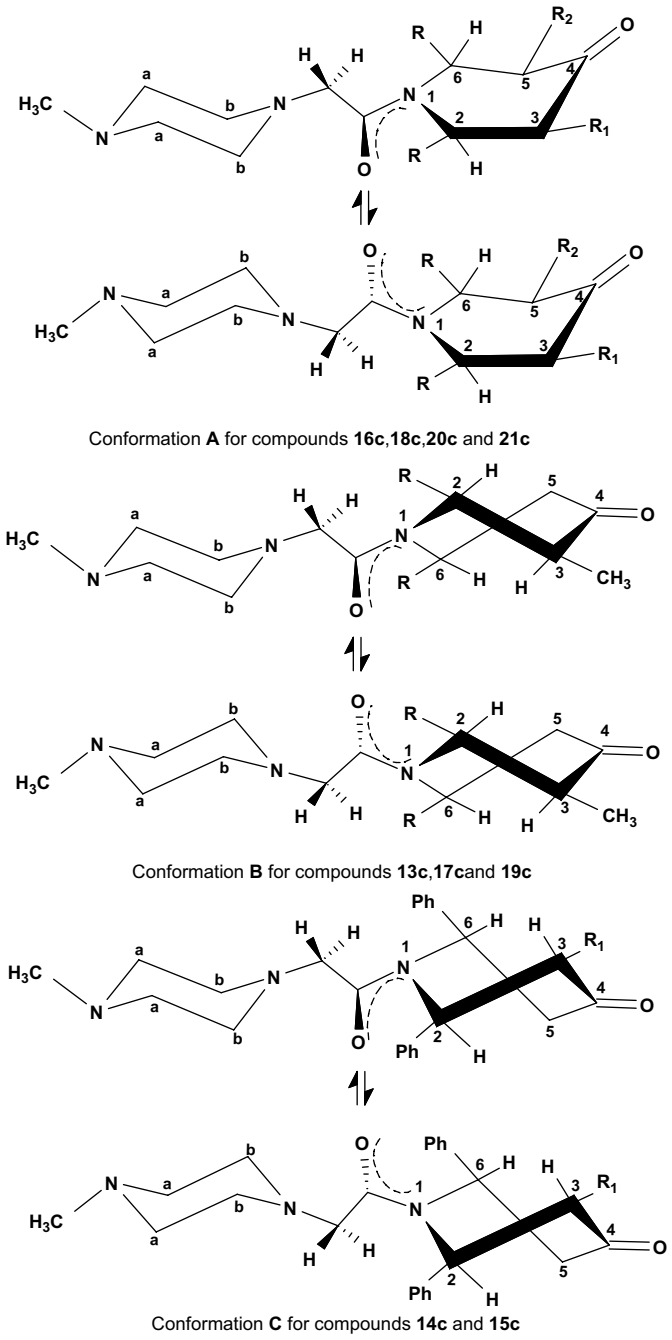


Fig. 6. Different conformations of compounds **13c–21c**.

of the synthesized compounds are reproduced in Tables 4 and 5, respectively.

2.3.1. Antibacterial activity

Among the compounds **13c–16c** with unsubstituted phenyl groups (at C-2 and C-6), compound **16c** only recorded better activity against *S. typhi* and *K. pneumoniae* at a MIC of 25 and 12.5 μg/mL, respectively, whereas the remaining compounds showed very less activity. Removal of one of the methyl groups at C-5 from **16c** (compound **13c**) completely eradicates the antibacterial activity even at a high concentration of 200 μg/mL against *S. aureus* and *P. aeruginosa* (for which compound **16c** recorded MIC at 50 μg/mL) while against rest of the strains, the activity did not eliminate completely. This confirms the importance of methyl functionality at C-5 position besides another methyl group at C-3 for the antibacterial profile of 2,6-diarylpiperidin-4-one derivatives. Furthermore, substitution of bulkier alkyl groups *viz.* ethyl (compound **14c**) or isopropyl (compound **15c**) group in place of methyl in **13c** exerted moderate activity. Compared to **13c**, **14c** and **15c** showed twofold added activity against *K. pneumoniae* and *S. typhi*, respectively, whereas against *E. coli*, the activity was retained as such.

Compounds **17c** and **18c** obtained due to the replacement of phenyl groups in **13c** and **16c**, respectively, by *para*-chlorophenyl group are significant in inhibiting the growth of all the tested bacterial strains, particularly, **17c** against *P. aeruginosa* and **18c** against *S. aureus* and *K. pneumoniae* are most superior (6.25 μg/mL) while towards remaining bacterial strains, their potencies fall in between 12.5 and 50 μg/mL. Moreover, impotency of **13c** and **14c** even at a maximum concentration of 200 μg/mL towards certain selected bacterial strains can remarkably be alleviated by the simple structural modification carried out in compounds **17c** and **18c**.

Substitution of *para*-methoxyphenyl group instead of phenyl ring in **13c** (compound **19c**) showed two- to threefold increased activity against all the tested bacterial strains except against *S. typhi* for which no change in activity was noted. The same kind of phenyl group substitution in **16c** (compound **20c**) registered 200% improved inhibition potency for *S. aureus* and *E. coli* whereas 100–200% decreased activity was noticed in *S. typhi* and *K. pneumoniae*. Introduction of *para*-methylphenyl groups at C-2 and C-6 positions of piperidone ring system in **16c** (compound **21c**) registered onefold increased activity towards *P. aeruginosa* while for rest of the organisms, the same activity was retained.

Among the compounds tested for their inhibition potency towards the chosen bacterial strains, **18c**, **20c** against *S. aureus*, **18c** against *E. coli*, **17c** against *P. aeruginosa*, *S. typhi*, **16c**, **18c** and **21c** against *K. pneumoniae* recorded elevated activity compared to the standard Ciprofloxacin drug whereas the MIC values of **17c**, **20c** against *E. coli*, **18c** against *P. aeruginosa*, **15c**, **18c** against *S. typhi*, **17c** and **20c** against *K. pneumoniae* were at par with the activity of the standard drug.

2.3.2. Antifungal activity

All the compounds were assessed to elicit their antifungal potency against the selected fungal strains and their MIC values fall in the range of 6.25–200 μg/mL. Compound **13c** with methyl group at C-3 position failed to exhibit inhibition potency even at a maximum concentration of 200 μg/mL against *C. albicans* and *Rhizopus* sp. whereas a modest inhibition (100 μg/mL) was noticed against *A. niger* and *A. flavus*. Replacement of methyl by ethyl group (compound **14c**) showed a positive response towards *C. albicans* (100 μg/mL) only but it was inactive against *Rhizopus* sp. Besides, a complete elimination of inhibitory potency against *A. flavus* and onefold decreased activity against *A. niger* was noted in **13c** if methyl group is replaced by ethyl group (i.e., compound

Table 4
In vitro antibacterial activities of compounds **13c–21c** against selected bacterial strains (MIC in μg/mL)

Compound	R_1	R_2	R	Minimum inhibitory concentration (MIC)[a] in μg/mL				
				S. aureus	*E. coli*	*P. aeruginosa*	*S. typhi*	*K. pneumoniae*
13c	Me	H	H	>200[b]	100	>200	200	200
14c	Et	H	H	100	>200	200	100	50
15c	*i*-Pr	H	H	200	100	100	50	200
16c	Me	Me	H	50	100	50	25	12.5
17c	Me	H	Cl	50	25	6.25	12.5	25
18c	Me	Me	Cl	6.25	12.5	12.5	50	6.25
19c	Me	H	OMe	100	50	50	200	100
20c	Me	Me	OMe	12.5	25	50	100	25
21c	Me	Me	Me	50	100	25	25	12.5
Ciprofloxacin				25	25	12.5	50	25

[a] MIC is the lowest concentration of an antimicrobial agent that will significantly inhibit the visible growth of a microorganism after a period of incubation.
[b] No inhibition even at 200 μg/mL.

14c). Due to the introduction of bulkier isopropyl group in place of ethyl function (compound **15c**), a striking decrease in the inhibitory concentration (50 μg/mL) was noted in the control of *Rhizopus* sp. against which compounds **13c** and **14c** were completely inactive. Similarly, it exhibited promising activity towards *A. niger* and *A. flavus* but onefold decreased activity was noted against *C. albicans*. Furthermore, insertion of methyl group at C-5 position in **13c** (compound **16c**) expressed prominent inhibitory activity for all the tested organisms with an MIC ranging from 50 to 100 μg/mL. It is worth mentioning here that among the phenyl (at C-2 and C-6) substituted piperidone systems (compounds **13c–16c**), compound **16c** only recorded moderate to better activity.

It is very surprising from Table 5 that incorporation of chloro functionality at the *para* position of phenyl group in **13c** (compound **17c**) resulted in well-pronounced impact over *C. albicans* and *Rhizopus* sp. by controlling their growth at a very low MIC i.e., at 6.25 and 12.5 μg/mL, respectively. Against *A. niger* and *A. flavus*, MIC value of **17c** was decreased by about 1/4 and 1/2, respectively, compared to **13c**. The same kind of phenyl group modification on **16c** (compound **18c**) also enhanced the biological activity remarkably and MIC was noted in the range of 6.25–25 μg/mL. Compared to **16c**, compound **18c** showed threefold elevated activity against *C. albicans* and *Rhizopus* sp. whereas only onefold improvement was noted against *A. niger* and *A. flavus*. However, compared to **17c**, only 100% improvement was noted in compound **18c** against *Rhizopus* sp. whereas for rest of the strains, an appreciable enhancement was not observed.

Replacement of chlorine by methoxy group in **17c** (compound **19c**) retained the same inhibition potency towards *A. flavus* while for the remaining strains, 200% decreased inhibition potency was noticed. However, the same type of interchange in **18c** (compound **20c**) augments its microbial potency against *C. albicans* and *A. flavus* by reducing its MIC value to half of its magnitude. But twofold decreased activity was noted against *Rhizopus* sp. Compound **21c**, which resulted by means of substituting methyl functionality in place of chlorine in **18c** did not influence the control of the multiplication of *C. albicans* and *Rhizopus* sp. whereas a distinguished activity was noted against *A. niger* and *A. flavus* at an inhibition value of 12.5 μg/mL.

Among the compounds explored for their antifungal potencies, **17c**, **20c** against *C. albicans*, **18c** against *Rhizopus* sp., **21c** against *A. niger* and **20c**, **21c** against *A. flavus* expressed 400% elevated potency compared to the standard drug used whereas rest of the compounds showed moderate to poor activity.

Table 5
In vitro antifungal activities of compounds **13c–21c** against selected fungal strains (MIC in μg/mL)

Compound	R_1	R_2	R	Minimum inhibitory concentration (MIC)[a] in μg/mL			
				C. albicans	*Rhizopus* sp.	*A. niger*	*A. flavus*
13c	Me	H	H	>200[b]	>200	100	100
14c	Et	H	H	100	>200	200	>200
15c	*i*-Pr	H	H	200	50	100	100
16c	Me	Me	H	100	50	50	50
17c	Me	H	Cl	6.25	12.5	25	50
18c	Me	Me	Cl	12.5	6.25	25	25
19c	Me	H	OMe	25	50	100	50
20c	Me	Me	OMe	6.25	25	25	12.5
21c	Me	Me	Me	50	25	12.5	12.5
Amphotericin B				25	25	50	50

[a] MIC is the lowest concentration of an antimicrobial agent that will significantly inhibit the visible growth of a microorganism after a period of incubation.
[b] No inhibition even at 200 μg/mL.

2.4. Analgesic activity

Analgesic activity of the compounds **13c–21c** was determined in a dose-dependent manner by acetic acid induced writhing test in mice. The visceral pain induced by acetic acid is due to the release of arachidonic acid via cyclohexagenase and prostaglandin (PG). The test compounds administered orally (p.o.) at 30, 60 and 90 mg/kg (body weight) 1 h prior to the administration of acetic acid showed a significant reduction in writhing movement. Analgesic profiles of the target compounds are given in Table 6.

It is clearly inferred from Table 6 that analgesic potency estimated by classical acetic acid induced constriction for certain compounds is comparatively equal or highly related to the reference compound at all the three different doses. The compounds with phenyl groups at C-2 and C-6 positions of piperidone ring (**13c–16c**) did not exert appreciable activity in all the three doses. These four compounds showed only 1.2–24.1% analgesic effect.

To our great surprise, introduction of chloro functionality at the *para* position of phenyl framework in **13c** (compound **17c**) produced statistically significant antinociceptic activity among the three doses. Further, this activity is also superior to the standard drug used at the same concentration. However, a strong beneficial effect over analgesic profile was noted at 60 mg/kg (70.4%) than 30 (49.2%) and 90 mg/kg (61.6%) doses. This expressive enhancement of the analgesic activity has indicated us the pharmacophoric character of *para*-chlorophenyl framework for the analgesic profile in this new series of compounds.

But the introduction of another methyl group at C-5 position in **17c** (compound **18c**) showed relatively decreased activity at all the doses. Replacement of chlorine in **17c** by methoxy function (compound **19c**) also resulted in diminished analgesic potency at 30 (48.2%) and 90 mg/kg (48.0%) whereas at 60 mg/kg, the same potency (70.3%) was retained. However, when compared to aspirin, a notable enhancement in inhibition of writhing was observed in **19c**.

Compounds **20c** and **21c** which resulted out of incorporation of methoxy and methyl functions, respectively, instead of chlorine in **18c** registered a drastic improvement in activity at 30 and 60 mg/kg, while at 90 mg/kg a decline in analgesic property was observed. In all the cases, the *para* substituted phenyl bearing compounds display improved inhibition potency compared to their analogues, the unsubstituted phenyl bearing compounds.

Therefore, from this study the importance of the structural units such as chlorine, methoxy or methyl function at the *para* position of the phenyl groups besides methyl group at C-3 is confirmed for the observed antinociceptive profile associated with **17c** and **19c**. Besides, though these compounds exhibited an elevated activity in all the doses, the inhibition of writhing response was proved to be superior at 60 mg/kg in **17c** (70.4%), **19c** (70.3%), **20c** (59.1%) and **21c** (54.6%) compared to aspirin (57.9%) and was considered as an optimum dose to execute antinociceptive activity.

Table 6
Analgesic activity of compounds **13c–21c**

S. no	Compound	Writhing (mean ± SEM)		
		30 mg/kg	60 mg/kg	90 mg/kg
1	**13c**	15.15 ± 0.70** (6.3)	16.92 ± 0.01** (15.6)	17.18 ± 0.05** (17.7)
2	**14c**	14.87 ± 0.55** (8.0)	19.14 ± 0.28** (4.5)	20.62 ± 0.20** (1.2)
3	**15c**	15.24 ± 0.08** (5.6)	16.41 ± 0.80** (18.1)	20.41 ± 0.15** (2.2)
4	**16c**	13.35 ± 0.18** (17.4)	15.21 ± 0.56** (24.1)	17.98 ± 0.25** (13.9)
5	**17c**	8.22 ± 0.90** (49.2)	5.94 ± 0.76** (70.4)	8.02 ± 0.45** (61.6)
6	**18c**	12.54 ± 0.44** (22.5)	12.72 ± 0.52** (36.5)	12.75 ± 0.25** (38.9)
7	**19c**	8.38 ± 0.13** (48.2)	5.96 ± 0.79** (70.3)	10.85 ± 0.10** (48.0)
8	**20c**	8.70 ± 0.84** (46.2)	8.19 ± 0.79** (59.1)	12.80 ± 0.15** (38.7)
9	**21c**	9.29 ± 0.08** (42.6)	9.09 ± 0.56** (54.6)	13.95 ± 0.22** (33.1)
10	Aspirin	8.92 ± 0.12* (44.8)	8.43 ± 0.18* (57.9)	12.81 ± 0.01* (38.6)
11	Control	16.17 ± 0.70	20.04 ± 0.06	20.87 ± 0.25

Significant levels: ** $P < 0.05$, * $P < 0.01$ compared to control. The values in the parentheses are the percentage of inhibition compared to control.

2.5. Antipyretic activity

In the present investigation, hyperthermia was induced by subcutaneous administration of brewer's yeast in normal saline. Pretreatment with test compounds at two different doses (30 and 60 mg/kg) shows a better antipyretic activity in a dose-dependent manner at different time intervals relative to the standard indomethacin drug. The obtained results are furnished in Table 7. A close survey of Table 7 shows that compounds with *para* substituted phenyl groups at C-2 and C-6 positions of piperidone ring system were found to be equi-potent to indomethacin and even possess significant activity compared to the standard drug in the same pharmacological protocol. However, the activity of compounds **18c** and **19c** with *para*-chloro (with methyl at C-3 and C-5) and *para*-methoxy (with methyl at C-3 only) phenyl groups, respectively, exhibited an excellent antipyretic activity at both the concentrations compared to the standard drug. Further, these compounds showed similar pharmacokinetic profile relative to indomethacin as evidenced by the onset of hypothermic effect immediately after 30 min and further it lasted over a period of 150 min. Besides, although introduction of methyl group at C-5 position in **19c** (compound **20c**) and replacement of methoxy by methyl function at the *para* position of phenyl group in **20c** (compound **21c**) showed a reduction in rectal temperature, their activity is less than that of indomethacin. From this study, it is also clearly known that removal of substituents from the phenyl groups significantly reduced the antipyretic potency at both the concentrations throughout the period of study. Among the compounds tested for their antipyretic activity, introduction of substituents in the phenyl group only registered enhanced activity and in particular compounds **18c** and

Table 7
Antipyretic activity of compounds **13c–21c**

S. no.	Compound	Dose (mg/kg)	Body temperature ± SEM[a]				
			30 min	60 min	90 min	120 min	150 min
1	**13c**	30	38.25 ± 0.03	38.20 ± 0.15	38.10 ± 0.20	38.03 ± 0.01	38.01 ± 0.05
		60	38.20 ± 0.30	38.10 ± 0.25	38.02 ± 0.03	38.01 ± 0.06	38.95 ± 0.02
2	**14c**	30	38.27 ± 0.10	38.22 ± 0.15	38.15 ± 0.03	38.01 ± 0.02	38.98 ± 0.10
		60	38.25 ± 0.11	38.20 ± 0.10	38.10 ± 0.12	38.95 ± 0.15	38.90 ± 0.11
3	**15c**	30	38.15 ± 0.15	38.10 ± 0.20	38.01 ± 0.15	38.96 ± 0.20	38.90 ± 0.50
		60	38.05 ± 0.11	38.01 ± 0.10	38.95 ± 0.25	38.90 ± 0.15	38.75 ± 0.10
4	**16c**	30	38.11 ± 0.12	38.00 ± 0.20	38.95 ± 0.10	38.85 ± 0.15	38.70 ± 0.25
		60	38.10 ± 0.25	37.95 ± 0.15	37.90 ± 0.23	37.70 ± 0.25	37.55 ± 0.15
5	**17c**	30	37.05 ± 0.25	37.96 ± 0.20	37.89 ± 0.20	37.70 ± 0.15	37.65 ± 0.15
		60	37.95 ± 0.10	37.92 ± 0.50	37.85 ± 0.25	37.65 ± 0.25	37.50 ± 0.15
6	**18c**	30	37.95 ± 0.25	37.90 ± 0.15	37.80 ± 0.15	37.60 ± 0.15	37.40 ± 0.25
		60	37.92 ± 0.15	37.85 ± 0.25	37.75 ± 0.11	37.55 ± 0.20	37.30 ± 0.50
7	**19c**	30	37.90 ± 0.25	37.80 ± 0.15	37.60 ± 0.25	37.50 ± 0.10	37.20 ± 0.20
		60	37.85 ± 0.15	37.70 ± 0.25	37.50 ± 0.15	37.30 ± 0.20	37.01 ± 0.20
8	**20c**	30	38.10 ± 0.12	38.01 ± 0.15	37.95 ± 0.25	37.80 ± 0.15	37.70 ± 0.15
		60	38.01 ± 0.21	38.95 ± 0.10	37.90 ± 0.22	37.85 ± 0.25	37.60 ± 0.25
9	**21c**	30	38.11 ± 0.22	38.02 ± 0.15	37.95 ± 0.25	37.90 ± 0.15	37.70 ± 0.25
		60	38.01 ± 0.25	38.95 ± 0.50	37.90 ± 0.15	37.80 ± 0.25	37.60 ± 0.15
10	Control	–	38.34 ± 0.12	38.30 ± 0.21	38.90 ± 0.03	38.20 ± 0.01	38.20 ± 0.25
11	Indomethacin	5	38.10 ± 0.15	37.90 ± 0.10	37.80 ± 0.15	37.70 ± 0.20	37.50 ± 0.25

[a] Significant levels: $P < 0.05$, $P < 0.01$ compared to control, respectively, for test compounds and reference drug.

19c showed superior activity as they were expected to inhibit the synthesis of prostaglandin thereby inducing the hypothalamus to regulate the body temperature.

3. Conclusion

All the novel target molecules were synthesized by the direct nucleophilic substitution of *N*-methylpiperazine with the corresponding *N*-chloroacetyl amides derived from 2,6-diarylpiperidin-4-ones. The complete one- and two-dimensional NMR spectral studies reveal that piperazine moiety in all the target molecules adopts chair conformation with equatorial orientation of methyl group irrespective of the various non-chair conformations adopted by the piperidone framework. Moreover, broadening of the benzylic protons (at C-2 and C-6) confirms the existence of restricted rotation in the molecules and also the in plane nature of piperazinoacetyl moiety with the dynamically averaged plane of the piperidone ring.

In order to probe structural requirements for optimal antimicrobial, analgesic and antipyretic activities in this series of compounds, the size of the substituents attached at C-3 and/or C-5 positions besides introducing electron withdrawing or donating functional groups at *para* position of the phenyl framework was examined in detail.

From the close survey of the antibacterial and antifungal results against a panel of microbial strains, the following SAR can be concluded and is also revealed from the bar graphs (Figs. 7 and 8). The antibacterial efficacy of compounds **17c** and **18c** with *para*-chlorophenyl groups (C-2 and C-6) along with methyl at C-3 and/or C-5 towards *P. aeruginosa* and *S. aureus*/*K. pneumoniae*, respectively, is greater when compared to the structurally analogous non-chlorine substituted compounds. Besides, elevated antifungal activities are noted in **18c** and **20c** bearing *para*-chloro and *para*-methoxy functions, respectively, at the phenyl ring besides methyl at C-3 and C-5 positions against all the tested fungal strains. However, the

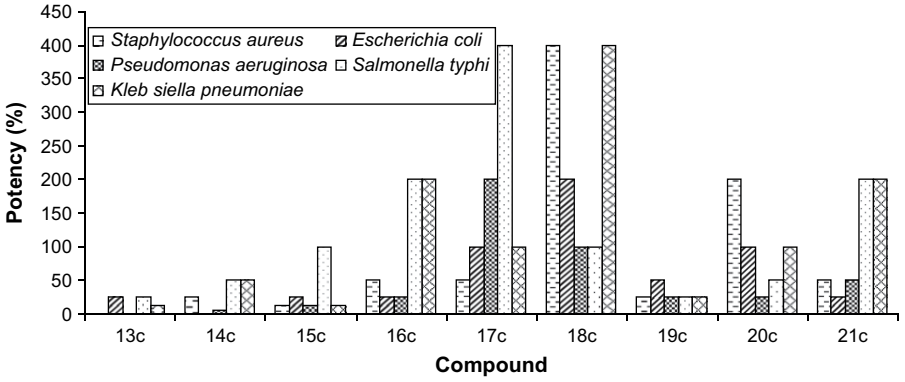


Fig. 7. Comparison of antibacterial potency of compounds **13c–21c** with Ciprofloxacin.

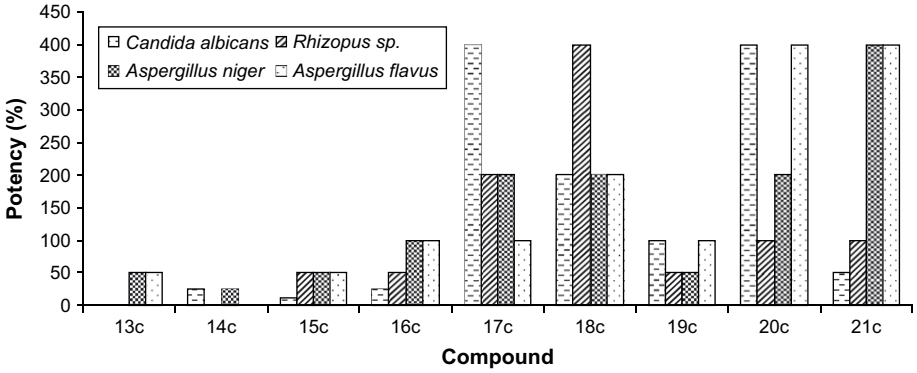


Fig. 8. Comparison of antifungal potency of compounds **13c–21c** with Amphotericin B.

activity of **18c** and **20c** against *Rhizopus* sp. and *C. albicans*, respectively, is remarkable. Further, the above said compounds possess significantly higher activity compared to the standards used in the same experimental protocol. The physicochemical properties like relative hydrophobicity, charge and molecular mass are expected to be the important characteristics for the penetration into the cell wall and execute different effects on the microbial strains. Moreover, conformations of the compounds may also play a crucial role in eliciting good biological responses. Therefore, the poor potency of the compounds with bulkier substituents at the C-3 position may be related to their stereochemical influence which in turn may affect the better penetration of the *N*-(*N*-methylpiperazinoacetyl)-2,6-diarylpiperidin-4-one framework into the cell walls of the respective strains. The elevated antimicrobial profiles of **17c**, **18c** and **20c** may perhaps be due to their appropriate size, lipophilicity, electronic effects of the substituents in the piperidone core structure and the conformations adopted by the respective compounds.

Similarly, analgesic potency of the compounds **13c–21c** is clearly revealed from the pictorial representation in Fig. 9. The compounds **17c** and **19c** exhibited a noticeable analgesic activity at all the three doses. However, the activity at 60 mg/kg (≈70%) was found to be superior among others. It is considered that these compounds effectively reduced the wave of constriction and elongation passing caudally along the abdominal wall with twisting of truck and extension of the hind limb in mice due to nociceptive property of acetic acid. Besides, the analgesic property appears to be peripherally mediated and could result from the combined inhibition effects of prostaglandin and acetylcholine. In the case of antipyretic activity, compounds **18c** and **19c** were endowed with a significant potency at both the doses during the period of study. Here also, the better hypothermic activity of the compounds may be due to the inhibition of prostaglandin synthesis in the central nervous system thereby reducing the rectal temperature. The antinociceptic and antipyretic activities of these compounds are better than the standard drugs used. In these in vivo studies also the compounds without substitution in the phenyl group did not exert an appreciable pharmacological activity.

From the preliminary microbiological and pharmacological studies, it is concluded that presence of *para* substituted phenyl groups at C-2 and C-6 positions besides methyl group at C-3 and/or C-5 positions are the key factors in eliciting beneficial biological profiles. Hence, among the nine compounds used for this study, **18c** and **19c** are considered to be potent candidates with promising biological and pharmacological properties. Moreover, development of this class of compounds may lead to 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 combat microbial infections and to alleviate pain and fever.

4. Experimental

4.1. General

The course of the reactions and purity of the products were assessed by performing TLC. All the reported melting points

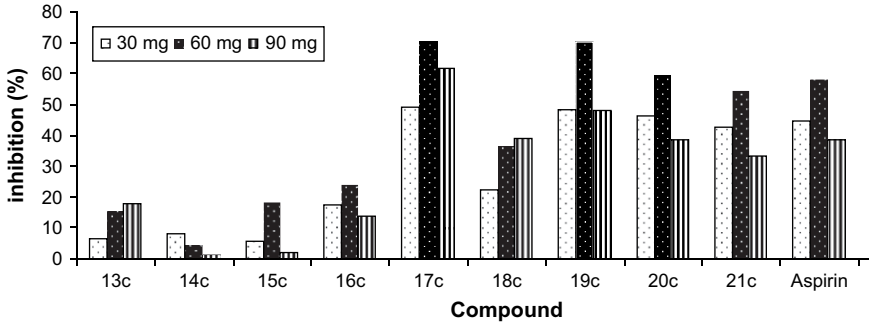


Fig. 9. Comparison of analgesic potency of compounds **13c–21c** with the control at three different doses.

were taken in open capillaries and are uncorrected. IR spectra were recorded in AVATAR-330 FT-IR spectrophotometer (Thermo Nicolet) and only noteworthy absorption levels (reciprocal centimeters) are listed. ^{1}H NMR spectra were recorded at 400 MHz on BRUKER AMX 400 MHz spectrophotometer using $CDCl_3$ as solvent and TMS as internal standard. ^{13}C NMR spectra were recorded at 100 MHz on BRUKER AMX 400 MHz spectrophotometer in $CDCl_3$. ^{1}H–^{1}H COSY and one-bond ^{1}H–^{13}C correlation spectra were recorded on BRUKER DRX 500 MHz NMR spectrometer using standard parameters. The chemical shift values were reported in ppm (parts per million) and the spin multiplicities are indicated as follows: s (singlet), br s (broad singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). 0.05 M solution of the sample in $CDCl_3$ was used for recording two-dimensional NMR spectra. The tubes used for recording NMR spectra were of 5 mm diameter. Mass spectra were recorded on Jeol SX-102 (EI) and microanalyses were performed on Heraeus Carlo Erba 1108 CHN analyzer. Unless otherwise stated, all the reagents and solvents used were of high grade and purchased from Fluka and Merck. They were used as received without any further purification.

By adopting the literature precedent [44], 2,6-diarylpiperidin-4-ones **13a–21a** were prepared by the condensation of appropriate ketones, aldehydes and ammonium acetate in 1:2:1 ratio using ethanol as solvent.

4.2. General procedure for the synthesis of N-chloroacetyl-2,6-diarylpiperidin-4-ones (13b–21b)

To a well-stirred solution of 3-methyl-2,6-diphenylpiperidin-4-one **13a** (1 equiv.) and triethylamine (1 equiv.) in 30 mL of dry benzene, chloroacetyl chloride (1 equiv.) in 20 mL of benzene was added dropwise through the addition funnel for about half an hour. Stirring was continued with mild heating using a magnetic stirrer. After the completion of reaction, it was poured into water and extracted with ether in three 50 mL portions. The combined ether extract was then washed well with 3% sodium bicarbonate solution and dried over anhydrous sodium sulphate. This upon evaporation and subsequent recrystallization in distilled ethanol furnished the compound **13b** in pure form with good yield. The compounds **14b–21b** were also synthesized similarly.

4.3. General procedure for the synthesis of N-(N-methylpiperazinoacetyl)-2,6-diarylpiperidin-4-ones (13c–21c)

A mixture of *N*-chloroacetyl-2,6-diarylpiperidin-4-one (1 equiv.), triethylamine (1 equiv.) and *N*-methylpiperazine [NMP] (1 equiv.) in toluene was refluxed for about 8–10 h. After the completion of 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 sulphate. The residue thus obtained was purified by column chromatography using *n*-hexane:ethyl acetate mixture (4:1) as eluent.

4.3.1. Synthesis of N-(N-methylpiperazinoacetyl)-3-methyl-2,6-diphenylpiperidin-4-one (13c)

By adopting the above general method, compound **13c** was obtained as pale white solid from **13b** (1 g, 2.47 mmol) and NMP (0.247 g, 2.47 mmol). IR (KBr) (cm^{-1}): 3058, 3028, 2935, 2845, 2818, 2791, 2694 (C–H stretching), 1713 (C=O stretching), 1642 (N–C=O stretching), 1498, 1453, 1415, 1347, 1289, 1232, 1163, 1135, 1079, 1015, 945, 908, 829, 747, 697, 650, 531, 463, 417. Mass (*m/z*): 406 $(M+H)^+$, 332, 303, 280, 233, 202, 171, 131, 100 (100%), 77. ^{1}H NMR (δ ppm): 2.49 (br s, 8H, H-a and H-b protons of NMP), 2.33 (s, 3H, CH_3 of NMP), 3.02–3.28 (m, 4H, H-3a, H-5a and N–$COCH_2$), 2.81 (dd, $^2J_{5a,5e} = 17.99$ Hz, $^3J_{5e,6a} = 5.91$ Hz, 1H, H-5e), 5.64 (br s, 1H, H-2), 6.05 (br s, 1H, H-6), 7.09–7.41 (m, 10H, phenyl protons), 1.12 (d, $J = 5.01$ Hz, 3H, CH_3 at C-3). ^{13}C NMR (δ ppm): 55.00 (C-a carbons of NMP), 53.45 (C-b carbons of NMP), 62.45 (N–CO*C*H$_2$), 60.89 (C-2), 53.45 (C-6), 45.99 (C-3), 43.28 (C-5), 209.77 (*C*=O at C-4), 171.44 (N-*C*=O), 141.11 (C-2′ *ipso*), 141.48 (C-6′ *ipso*), 126.79, 127.59, 127.69, 127.82, 128.49, 128.88 (other phenyl carbons), 45.99 (CH_3 of NMP), 14.09 (CH_3 at C-3).

4.3.2. Synthesis of N-(N-methylpiperazinoacetyl)-3-ethyl-2,6-diphenylpiperidin-4-one (14c)

By adopting the above general method, compound **14c** was obtained as pale white solid from **14b** (1 g, 2.38 mmol) and NMP (0.238 g, 2.38 mmol). IR (KBr) (cm^{-1}): 3062, 3028, 2945, 2845, 2791, 2695 (C–H stretching), 1706 (C=O stretching), 1643 (N–C=O stretching), 1560, 1498, 1452, 1414, 1348, 1286, 1229, 1162, 1133, 1077, 1012, 914, 830, 759, 696, 651, 532, 485. ^{1}H NMR (δ ppm): 1.05 (t, 3H, $J = 7.32$ Hz, CH_2CH_3 at C-3), 1.61–1.74 (m, 2H, CH_2CH_3 at C-3), 2.35 (s, 3H, CH_3 of NMP), 2.55 (br s, 8H, H-a and H-b protons of NMP), 2.67 ($^2J_{5a,5e} = 17.35$ Hz, $^3J_{5e,6a} = 5.73$ Hz, 1H, H-5e), 2.88 (dd, $^2J_{5a,5e} = 16.96$ Hz, $^3J_{5a,6a} = 9.15$ Hz, 1H, H-5a), 2.99–3.04 (m, 2H, N–COCH*H*, H-3a), 3.18 (d, $J = 14.06$ Hz, 1H, N–COC*H*H), 5.70 (br s, 1H, H-6), 6.23 (br s, 1H, H-2), 7.03–7.26 (m, 10H, phenyl protons). ^{13}C NMR (δ ppm): 11.83 (CH_2CH_3 at C-3), 23.32 (CH_2CH_3 at C-3), 44.27 (C-5), 45.52 (CH_3 of NMP), 52.57 (C-3), 53.08 (C-b carbons of NMP), 54.66 (C-a carbons of NMP), 55.92 (C-2 and C-6), 62.05 (N–CO*C*H$_2$), 126.41, 127.46, 127.74, 128.45, 128.75, 129.13 (other phenyl carbons), 141.24 (C-2′ *ipso*), 141.69 (C-6′ *ipso*), 171.51 (N–*C*=O), 209.51 (*C*=O at C-4).

4.3.3. Synthesis of N-(N-methylpiperazinoacetyl)-3-isopropyl-2,6-diphenylpiperidin-4-one (15c)

By adopting the above general method, compound **15c** was obtained as pale red solid from **15b** (1 g, 2.31 mmol) and NMP (0.231 g, 2.31 mmol). IR (KBr) (cm^{-1}): 3060, 3032, 2973, 2943, 2815, 2789, 2693 (C–H stretching), 1702 (C=O stretching), 1642 (N–C=O stretching), 1498, 1453, 1412,

1346, 1285, 1228, 1163, 1131, 1081, 1014, 914, 829, 754, 697, 651, 531, 498. ^{1}H NMR (δ ppm): 1.06 {d, $J = 6.72$ Hz, 3H, [CH(CH$_3$′)(*CH$_3$″*)] at C-3}, 1.11 {d, $J = 6.44$ Hz, 3H, [CH(*CH$_3$′*)(CH$_3$″)] at C-3}, 2.02–2.06 [m, 1H, *CH*(CH$_3$)$_2$ at C-3], 2.43 (s, 3H, CH$_3$ of NMP), 2.67 (br s, 8H, H-a and H-b protons of NMP), 2.71 (dd, $^2J_{5a,5e} = 16.89$ Hz, $^3J_{5e,6a} = 6.14$ Hz, 1H, H-5e), 2.83 (dd, $^2J_{5a,5e} = 16.40$ Hz, $^3J_{5a,6a} = 8.56$ Hz, 2H, H-3a and H-5a), 2.98 (br s, 1H, N–COCH*H*), 3.27 (d, $J = 14.56$ Hz, 1H, N–COC*H*H), 5.54 (br s, 1H, H-6), 6.54 (br s, 1H, H-2), 6.95–7.19 (m, 10H, phenyl protons). ^{13}C NMR (δ ppm): 20.31 [CH(CH$_3$′)(*CH$_3$″*) at C-3], 21.73 [CH(*CH$_3$′*)(CH$_3$″) at C-3], 28.84 (*C*H(CH$_3$)$_2$ at C-3), 44.23 (C-5), 45.88 (CH$_3$ of NMP), 53.50 (C-b carbons of NMP), 54.96 (C-a carbons of NMP), 54.96 (C-3), 56.42 (C-6), 58.39 (C-2), 62.25 (N–CO*CH$_2$*), 126.41, 127.22, 127.81, 128.26, 128.54 (other phenyl carbons), 141.29 (C-2′ and C-6′ *ipso*), 171.32 (N–*C*=O), 209.56 (*C*=O at C-4).

4.3.4. Synthesis of N-(N–methylpiperazinoacetyl)-3,5-dimethyl-2,6-diphenylpiperidin-4-one (**16c**)

By adopting the above general method, compound **16c** was obtained as grayish white semisolid from **16b** (1 g, 2.38 mmol) and NMP (0.238 g, 2.38 mmol). IR (KBr) (cm^{-1}): 3059, 3028, 2968, 2938, 2875, 2809 (C–H stretching), 1716 (C=O stretching), 1647 (N–C=O stretching), 1494, 1455, 1387, 1351, 1289, 1214, 1131, 1088, 1012, 918, 826, 762, 736, 704, 663, 624, 534, 422. Mass (*m/z*): 420 $(M + H)^+$, 378, 353, 301, 280 (100%), 224, 202, 148, 133, 106, 87, 77, 57. ^{1}H NMR (δ ppm): 2.50 (br s, 8H, H-a and H-b protons of NMP), 2.32 (s, 3H, CH$_3$ of NMP), 3.12 (t, 2H, H-3a and H-5a), 2.99 (s, 2H, N–COCH$_2$), 5.52 (br s, 2H, H-2 and H-6), 7.01–7.30 (m, 10H, phenyl protons), 1.06 (d, $J = 6.99$ Hz, 6H, CH$_3$ at C-3 and C-5). ^{13}C NMR (δ ppm): 54.54 (C-a carbons of NMP), 52.99 (C-b carbons of NMP), 61.88 (N–CO*CH$_2$*), 60.85 (C-2 and C-6), 45.48 (C-3 and C-5), 211.35 (*C*=O at C-4), 171.77 (N–*C*=O), 141.39 (C-2′ and C-6′), 127.25, 127.74, 128.66 (other phenyl carbons), 45.48 (CH$_3$ of NMP), 14.14 (CH$_3$ at C-3 and C-5).

4.3.5. Synthesis of N-(N-methylpiperazinoacetyl)-3-methyl-2,6-bis(p-chlorophenyl) piperidin-4-one (**17c**)

By adopting the above general method, compound **17c** was obtained as reddish semisolid from **17b** (1 g, 2.11 mmol) and NMP (0.211 g, 2.11 mmol). IR (KBr) (cm^{-1}): 2984, 2941, 2847, 2798 (C–H stretching), 1718 (C=O stretching), 1648 (N–C=O stretching), 1569, 1492, 1457, 1411, 1345, 1289, 1227, 1136, 1092, 1014, 940, 831, 753, 664, 545, 497, 472. ^{1}H NMR (δ ppm): 1.08 (d, $J = 6.82$ Hz, CH$_3$ at C-3), 2.37 (s, CH$_3$ of NMP), 2.58 (br s, 8H, H-a and H-b protons of NMP), 2.82 (dd, $^2J_{5a,5e} = 17.94$ Hz, $^3J_{5e,6a} = 6.19$ Hz, 1H, H-5e), 2.92–3.16 (m, 4H, H-3a, H-5a and N–COCH$_2$), 5.49 (br s, 1H, H-2), 5.96 (br s, 1H, H-6), 7.02, 7.11 (d, 4H, aryl protons *meta* to chlorine), 7.20, 7.27 (2d, 4H, aryl protons *ortho* to chlorine). ^{13}C NMR (δ ppm): 14.23 (CH$_3$ at C-3), 42.49 (C-5), 45.71 (CH$_3$ of NMP), 45.95 (C-3), 53.23 (C-b carbons of NMP), 54.05 (C-6), 54.79 and 54.97 (C-a carbons of NMP), 60.39 (C-2), 62.34 (N–CO*CH$_2$*), 128.21, 128.57, 128.94, 129.17 (other aryl carbons), 133.61 (C-2‴″ *ipso*), 133.78 (C-6‴″ *ipso*), 139.29 (C-2′ *ipso*), 139.51 (C-6′ *ipso*), 171.11 (N–*C*=O), 208.56 (*C*=O at C-4).

4.3.6. Synthesis of N-(N-methylpiperazinoacetyl)-3,5-dimethyl-2,6-bis(p-chlorophenyl) piperidin-4-one (**18c**)

By adopting the above general method, compound **18c** was obtained as pale reddish solid from **18b** (1 g, 2.05 mmol) and NMP (0.205 g, 2.05 mmol). IR (KBr) (cm^{-1}): 2979, 2937, 2833, 2798, 2738, 2694 (C–H stretching), 1716 (C=O stretching), 1646 (N–C=O stretching), 1491, 1456, 1386, 1290, 1211, 1134, 1090, 1011, 980, 930, 828, 798, 727, 727, 650, 542, 516, 464. ^{1}H NMR (δ ppm): 1.06 (d, $J = 6.91$ Hz, 6H, CH$_3$ at C-3 and C-5), 2.36 (s, CH$_3$ of NMP), 2.55 (br s, 8H, H-a and H-b protons of NMP), 3.02–3.13 (m, 4H, H-3a, H-5a and N–COCH$_2$), 5.44 (br s, 2H, H-2 and H-6), 7.11 (d, 4H, aryl protons *meta* to chlorine), 7.30 (d, 4H, aryl protons *ortho* to chlorine). ^{13}C NMR (δ ppm): 14.11 (CH$_3$ at C-3 and C-5), 45.47 (C-3 and C-5), 45.47 (CH$_3$ of NMP), 52.95 (C-b carbons of NMP), 54.48 (C-a carbons of NMP), 60.41 (C-2 and C-6), 61.92 (N–CO*CH$_2$*), 128.93, 129.09 (other aryl carbons), 133.89 (C-2‴″ and C-6‴″ *ipso*), 139.68 (C-2′ and C-6′ *ipso*), 171.59 (N–*C*=O), 210.25 (*C*=O at C-4).

4.3.7. Synthesis of N-(N-methylpiperazinoacetyl)-3-methyl-2,6-bis(p-methoxyphenyl)-piperidin-4-one (**19c**)

By adopting the above general method, compound **19c** was obtained as dark reddish semisolid from **19b** (1 g, 2.15 mmol) and NMP (0.215 g, 2.15 mmol). IR (KBr) (cm^{-1}): 3066, 2940, 2838, 2798 (C-H stretching), 1717 (C=O stretching), 1641 (N–C=O stretching), 1613, 1514, 1459, 1415, 1299, 1253, 1181, 1137, 1032, 1082, 934, 835, 761, 557, 460. ^{1}H NMR (δ ppm): 1.05 (d, $J = 5.18$ Hz, CH$_3$ at C-3), 2.34 (s, 3H, CH$_3$ of NMP), 2.55 (br s, 8H, H-a and H-b protons of NMP), 2.74 (dd, $^2J_{5a,5e} = 18.05$ Hz, $^3J_{5e,6a} = 6.03$ Hz, 1H, H-5e), 3.04–3.26 (m, 4H, H-3a, H-5a and N–COCH$_2$), 3.77, 3.79 (s, 6H, OCH$_3$ at C-2‴″ and C-6‴″), 5.46 (br s, 1H, H-2), 5.93 (br s, 1H, H-6), 6.75, 6.81 (2d, 4H, aryl protons *ortho* to methoxy), 7.02, 7.10 (br s, 4H, aryl protons *meta* to methoxy). ^{13}C NMR (δ ppm): 13.99 (CH$_3$ at C-3), 43.25 (C-5), 45.59 (CH$_3$ of NMP), 46.18 (C-3), 53.07 (C-b carbons of NMP), 53.78 (C-6), 54.76 (C-a carbons of NMP), 55.24 (OCH$_3$ at C-2‴″ and C-6‴″), 60.48 (C-2), 62.19 (N–CO*CH$_2$*), 113.86, 114.23, 128.06, 128.99 (other aryl carbons), 133.26 (C-2′ *ipso*), 133.52 (C-6′ *ipso*), 158.94, 159.06 (C-2‴″ and C-6‴″ *ipso*), 171.19 (N–*C*=O), 209.79 (*C*=O at C-4).

4.3.8. Synthesis of N-(N-methylpiperazinoacetyl)-3,5-dimethyl-2,6-bis(p-methoxyphenyl) piperidin-4-one (**20c**)

By adopting the above general method, compound **20c** was obtained as reddish semisolid from **20b** (1 g, 2.09 mmol) and NMP (0.209 g, 2.09 mmol). IR (KBr) (cm^{-1}): 2938, 2837, 2798 (C–H stretching), 1713 (C=O stretching), 1635 (N–C=O stretching), 1607, 1513, 1458, 1397, 1295, 1250, 1179, 1135, 1031, 929, 834, 879, 654, 591, 539. ^{1}H NMR (δ ppm): 1.05 (d, 6H, $J = 5.24$ Hz, CH$_3$ at C-3 and C-5), 2.31

(s, 3H, CH_3 of NMP), 2.51 (br s, 8H, H-a and H-b protons of NMP), 2.99 (s, 2H, N–$COCH_2$), 3.09 (t, 2H, H-3a and H-5a), 3.79 (s, 6H, OCH_3 at C-2′′′′ and C-6′′′′), 5.45 (br s, 2H, H-2 and H-6), 6.82 (d, 4H, aryl protons *ortho* to methoxy), 7.08 (s, 4H, aryl protons *meta* to methoxy). ^{13}C NMR (δ ppm): 14.13 (CH_3 at C-3 and C-5), 45.47 (C-3 and C-5), 45.71 (CH_3 of NMP), 53.02 (C-b carbons of NMP), 54.58 (C-a carbons of NMP), 55.28 (OCH_3 at C-2′′′′ and C-6′′′′), 60.35 (C-2 and C-6), 61.96 (N–COCH_2), 114.03, 128.94 (other aryl carbons), 133.54 (C-2′ and C-6′ *ipso*), 159.09 (C-2′′′′ and C-6′′′′ *ipso*), 171.68 (N–*C*=O), 211.63 (*C*=O at C-4).

4.3.9. Synthesis of N-(N-methylpiperazinoacetyl)-3,5 -dimethyl-2,6-bis(p-methylphenyl) piperidin-4-one (**21c**)

By adopting the above general method, compound **21c** was obtained as pale reddish semisolid from **21b** (1 g, 2.09 mmol) and NMP (0.209 g, 2.09 mmol). IR (KBr) (cm^{-1}): 3033, 2973, 2935, 2875, 2800 (C–H stretching), 1716 (C=O stretching), 1644 (N–C=O stretching), 1514, 1455, 1383, 1290, 1166, 1138, 1086, 1014, 981, 927, 824, 726, 656, 521, 575, 444. ^{1}H NMR (δ ppm): 1.04 (d, $J = 6.36$ Hz, 6H, CH_3 at C-3 and C-5), 2.33 (s, 6H, CH_3 at C-2′′′′ and C-6′′′′), 2.41 (s, 3H, CH_3 of NMP), 2.59 (br s, 8H, H-a and H-b protons of NMP), 3.00 (s, 2H, N–$COCH_2$), 3.06–3.11 (m, 2H, H-3a and H-5a), 5.45 (br s, 2H, H-2 and H-6), 7.06–7.36 (m, 8H, aryl protons). ^{13}C NMR (δ ppm): 14.12 (CH_3 at C-3 and C-5), 20.89 (CH_3 at C-2′′′′ and C-6′′′′), 45.61 (C-3 and C-5), 45.79 (CH_3 of NMP), 53.35 (C-b carbons of NMP), 54.87 (C-a carbons of NMP), 60.61 (C-2 and C-6), 62.16 (N–COCH_2), 127.71, 129.24 (other aryl carbons), 137.36 (C-2′′′′ and C-6′′′′ *ipso*), 138.57 (C-2′ and C-6′ *ipso*), 171.76 (N–*C*=O), 211.65 (*C*=O at C-4).

4.4. Microbiology: in vitro antimicrobial activity

In vitro activities of the compounds were tested in Sabourauds dextrose broth (SDB, Hi-media, Mumbai) for fungi and in nutrient broth (NB, Hi-media, Mumbai) for bacteria by two-fold serial dilution method [49]. The test compounds were dissolved in dimethylsulphoxide (DMSO) to obtain 1 mg/mL stock solutions. Seeded broth (broth containing microorganisms) was prepared in NB from 24 h old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1 °C while fungal spores from 1–7 days old Sabourauds agar slant cultures were suspended in SDB. The colony forming units (cfu) of the seeded broth were determined by plating technique and adjusted in the range of 10^4–10^5 cfu/mL. The final inoculum size was 10^5 cfu/mL for antibacterial assay and 1.1–1.5×10^2 cfu/mL for antifungal assay. Testing was performed at pH 7.4 ± 0.2 for bacteria (NB) and at pH 5.6 for fungi (SDB). Exactly, 0.4 mL of the solution of the test compound was added to 1.6 mL of seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 mL of seeded broth to give the second dilution and so on till six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control and likewise solvent controls were also run simultaneously. The tubes were incubated in BOD incubators at 37 ± 1 °C for bacteria and 28 ± 1 °C for fungi. The minimum inhibitory concentrations (MICs) were recorded by visual observations after 24 h (for bacteria) and 72–96 h (for fungi) of incubation. Ciprofloxacin and Amphotericin B were used as standards for bacterial and fungal studies, respectively. The percentage of antimicrobial potency of the test compounds compared to reference standard was calculated by adopting the following equation

$$\text{Antimicrobial potency}(\%) = \frac{\text{MIC}(\mu\text{g/mL})\text{of reference compound}}{\text{MIC}(\mu\text{g/mL})\text{of test compound}} \times 100$$

4.5. Pharmacology

Albino mice and Wister rats of both sexes (pregnant females excluded) weighing 20–25 g and 200–250 g, respectively (unless otherwise specified), were used to test the following pharmacological studies. All these animals were provided by Raja Muthia Medical College Hospital (RMMCH) and experiments were performed after getting clearance from IAEC (Institutional Animal Ethical Committee) and also by strictly adhering to the ethical guidelines. The provided animals were allowed to adopt the lab condition for two days before being used for the study and were fed with standard pellet diet 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 six animals each.

4.5.1. Analgesic activity

Antinociceptive activity of the series of compounds was determined by acetic acid induced writhing test [50] in Albino mice. The test compounds were administered at three different doses i.e., at 30, 60 and 90 mg/kg (body weight) and aspirin was chosen as reference drug at the same concentration of the test compounds. All the compounds and standard drug were suspended in 1% sodium carboxymethyl cellulose (CMC) and administrated orally (p.o.). The writhing syndrome in test animals was elicited by injecting 1% acetic acid in normal saline (10 mL/kg body weight) intraperitoneally 1 h after the oral administration of the test compounds. Complete extension of either hind limb was regarded as a writhing response. After 5 min of injection of noxious agent, the number of writhes was counted during the subsequent 30 min by keeping the animals in 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 test compound treated group and C is the number of writhes in control group.

$$\text{Percentage inhibition of writhing} = [1 - T/C] \times 100$$

The reported values were the average of six determinations $\pm$ SEM of n (6) animals per group. The observed results

were statistically analyzed by Student's *t*-test and ANOVA (one way). The $*P$ value of <0.05 or <0.01 was considered as statistically significant or highly significant, respectively.

4.5.2. Antipyretic activity

Antipyretic activity of the target compounds was determined by measuring the variation in the body temperature as per the reported procedure [51]. Fever was induced to fasted Wister rats by administering 2% brewer's yeast in normal saline (10 mL/kg body weight) subcutaneously. Initial rectal temperature was recorded using a lubricated digital thermometer with a precision of 0.1 °C by inserting into the rectum of the animal. The same thermometer was used for all the animals in each group in order to minimize the possible experimental error. After 18 h, the animals that showed an increase of 0.3–0.5 °C in rectal temperature were selected for this study. The test compounds were administered orally (at a dose of 30 and 60 mg/kg body weight) as a suspension in 1% CMC and indomethacin (5 mg/kg) was used as a reference drug. Control groups received the vehicle (1% CMC) only. After the administration of the test compounds, rectal temperature was measured successively after 30, 60, 90, 120 and 150 min. The reported values were the average of six determinations ± SEM of n (6) animals per group. The observed results were statistically analyzed by Student's *t*-test and ANOVA (one way) for a significance level of $*P < 0.05$ or $*P < 0.01$.

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