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Synthesis and Anti-Inflammatory Activity of 1-Benzhydryl-Piperazine Urea Derivatives

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ABSTRACT

*A series of novel 1-benzhydryl-piperazine urea derivatives **1(a-h)** were synthesised and evaluated for in vivo anti-inflammatory activity in carrageenan induced rat paw edema. The results of the present study is interesting and clearly indicated that compound **1f** shows maximum inhibition of 60.8% and compound **1h** with dichloro substitution shows 57.5% inhibition. Remaining compounds shows comparable activity.*

Keywords: 1-benzhydryl-piperazine, Urea derivatives, anti-inflammatory activity, carrageenan, Nimesulide.

INTRODUCTION

Inflammation contributes to play an important pathogenetic role in many inflammatory disorders including asthama, gout, rheumatoid arthritis, multiple sclerosis, ischemia-reperfusion injury etc [1]. During inflammation, production of free radicals [e.g., hydrogen peroxide (H_2O_2), superoxide anion (O_2^-)] and Nitric Oxide (NO) plays a pivotal role for microorganism killing and also signals the activation of leukocytes and macrophage. Conversely, over production of these toxic reactive oxygen and nitrogen metabolites/species (ROS/RNS) may cause more damage of the surrounding tissue than the microbe [1,2]. Besides, activated leukocytes are preferentially to secrete cytokines, interferon-γ and tissue necrosis factor-α for recruitment of inflammatory cells to vascular endothelium and subsequently transmigrate/ infiltrate to injured tissue where they release hydrolytic enzymes and enormous quantities of free radicals (ROS/RNS) that result in remarkable tissue damage and immunopathogenesis [3]. Thus anti-inflammatory therapy is also comprehensive pharmacological approaches in the treatment of these inflammation-related disorders [3,4]. One of these important strategies is to look for the synthetic heterocyclic derivatives that are capable

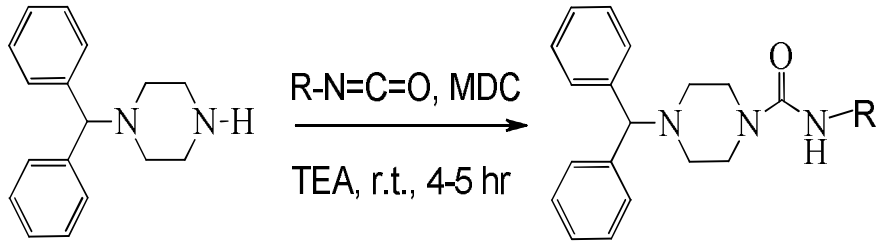
of inhibiting oxidative stress for the prevention of free radicals- and cytokine-induced cellular damage by inflammatory cell infiltrating to the injured tissue.

Non steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, fever and inflammation, particularly arthritis [5,6]. The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme cyclooxygenases (COXs) [7,8]. Due to serious adverse effects like gastric irritation, cardiovascular complications, nephropathy, hypertension hepatotoxicity and edema many NSAIDs are failed to stand in the market place for long time and since from last ten years many researchers are putting more efforts to bring safest NSAIDs in the market place [9-13]. In continuation of our research [14-23], herein we have evaluated in vivo anti-inflammatory activity for 1-benzhydryl-piperazine urea derivatives.

MATERIALS AND METHODS

Chemical and instrumentation: Melting points were determined by SELACO-650 hot stage melting point apparatus and were uncorrected. Infra red (IR) spectra were recorded using a Jasco FTIR-4100 series. Nuclear magnetic resonance (^{1}HNMR) spectra were recorded on Shimadzu AMX 400-Bruker, 400MHz spectrometer using DMSO as a solvent and TMS as a internal standard (chemical shift in ppm). Spin multiplets are given as a (singlet), d (doublet), t (triplet) and m (multiplet). Mass and purity were recorded on a LC-MSD-Trap-XCT. Silica gel column chromatography was performed by using Merck 7734 silica gel (60-120 mesh) and Merck made TLC plates. The crystallographic measurements were made on a DIPLabo using imaging plate system with graphite monochromatic radiation (MoKα). Streptozotocin HCl (STZ) were purchased from sigma chemical Co (St. Louis, MO, USA). Metformin was procured as a gift sample from Dr. Reddy`s labs Hyderabad. Plasma glucose was measured by using commercially available diagnostic kits (Drasania biomedicals Limited, Daman).

Synthesis of 1-benzhydryl-piperazine urea derivatives 1(a-h): The reaction of 1-benzhydryl-piperazine **(1)** with substituted aromatic isocyanates **(R-N=C=O)** were carried out in the presence of triethylamine and dichloromethane as solvent in a good yield with good purity **(Scheme 1)**. Synthesized molecules **1(a-h)** were characterized by ^{1}H NMR, LC/MS and IR. In compounds **1(a-h),** carbonyl group shows the stretching frequency at 1630-1650 cm^{-1} and –NH group shows at 3250-3300 cm^{-1} in IR spectra. The chemical structures, physical data and purity of all the synthesized compounds are given in **Table 1**. Compounds **1(a-h)** were evaluated for anti-inflammatory activity **(Table 2 and 3)**.



1 **1(a-q)**

Scheme 1

Table 1. Chemical structure, physical data and purity of compounds 10(a-q)

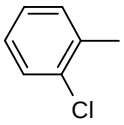
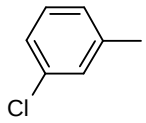
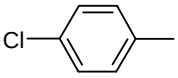
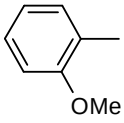
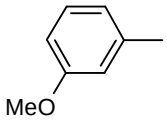
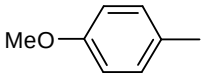
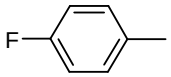
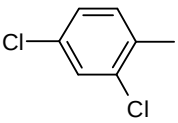
Compound	R	Yield (%)	M.P (^{0}C)	Purity
1a		88	219-221	99.0
1b		84	252-254	98.1
1c		87	232-234	96.8
1d		89	196-198	98.3
1e		90	210-212	97.8
1f		93	189-191	96.8
1g		79	229-231	99.3
1h		76	245-247	98.2

Table 2. Anti-inflammatory activity in carrageenan induced rat paw edema

Sl. No.	Groups	Dose mg kg^{-1}	Difference in Paw Edema Volume (Mean±SEM)			
			0.5 h	**1 h**	**3 h**	**5 h**
1	**Control**	0.5% CMC	1.100 ±0.05	1.167 ±0.03	0.966 ±0.03	0.500 ±0.05
S2	**Standard (Nimesulide)**	50	0.400** ±0.05	0.600** ±0.05	0.400** ±0.03	0.233** ±0.03
3	**1a**	50	0.819 ±0.10	0.666** ±0.06	0.5333** ±0.05	0.300* ±0.05
4	**1b**	50	0.833 ±0.10	0.800 ±0.05	0.766 ±0.03	0.300* ±0.05
5	**1c**	50	0.866 ±0.03	0.866 ±0.03	0.833 ±0.03	0.466 ±0.03
6	**1d**	50	0.800 ±0.03	0.733 ±0.03	0.733 ±0.03	0.486 ±0.03

7	**1e**	50	0.966 ±0.04	0.966 ±0.06	0.833 ±0.03	0.466 ±0.03
8	**1f**	50	0.433** ±0.08	0.666** ±0.03	0.466** ±0.03	0.266** ±0.03
9	**1g**	50	0.566** ±0.03	0.766** ±0.03	0.500** ±0.05	0.266** ±0.03
10	**1h**	50	0.466** ±0.03	0.800** ±0.05	0.466** ±0.03	0.266** ±0.03

Values are expressed as Mean±SEM, **P<0.01 when compared with control.*P<0.05 when compared with control.

Table 3. Anti-inflammatory activity in carrageenan induced rat paw edema

Sl. No.	**Groups**	**Dose mg kg^{-1}**	**Percentage inhibition of paw edema volume at different time intervals (%)**			
			0.5 h	**1 h**	**3 h**	**5h**
1	**Control**	0.5% CMC	-	-	-	-
2	**Standard (Nimesulide)**	50	63.6	48.5	58.6	53.4
3	**1a**	50	25.54	42.8	44.8	40.0
4	**1b**	50	24.24	31.44	20.68	40.0
5	**1c**	50	21.20	25.73	13.83	6.66
6	**1d**	50	27.27	37.18	24.17	2.80
7	**1e**	50	12.11	17.16	13.79	6.80
8	**1f**	50	60.6	42.8	51.7	46.6
9	**1g**	50	48.4	34.3	48.2	46.6
10	**1h**	50	57.5	31.4	51.7	46.6

General procedure for the synthesis of 1-benzhydryl-piperazine urea derivatives 1(a-h): A solution of 1-benzhydryl-piperazine **(1)** (1.0 eq) in dry dichloromethane was taken and cooled to 0-5 ^{o}C in an ice bath. Triethylamine (3 eq) was added to the cold reaction mixture and stirred for 10 min, then different isocyanates (1.0 eq) were added, the reaction mixture was allowed to stir at room temperature for 4-5 h. The progress of the reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. The organic layer was washed with water and dried over anhydrous sodium sulphate. The solvent was evaporated to get crude product which was purified by column chromatography over silica gel using hexane: ethyl acetate (8:2) as an eluent.

Synthesis of 4-benzhydryl-*N*-(2-chlorophenyl) piperazine-1-carboxamide (1a): The general experimental procedure described herein afforded **1a** from 1-benzhydryl-piperazine **(1)** (0.5 g, 1.98 mmol), 2-chlorophenylisocyanate (0.304 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was white pluffy solid (0.706 g). ^{1}H NMR (DMSO-d_6, 400MHz) δ: 8.32 (s, 1H, -NH), 7.45 (d, 5H, Ar-H), 7.36 (t, 5H, Ar-H), 7.27 (s, 2H, Ar-H), 7.23 (m, 2H, Ar-H), 4.36 (s, 1H, -CH-), 3.32 (br s, 4H, -CH_2-), 2.50 (br s, 4H, -CH_2-). MS (ESI + ion): m/z = 406.1. IR (KBr, cm^{-1}): 3290, 1650.

Synthesis of 4-benzhydryl-*N*-(3-chlorophenyl)piperazine-1-carboxamide (1b): The general experimental procedure described herein afforded **1b** from 1-benzhydryl-piperazine **(1)** (0.5 g, 1.98 mmol), 3-chlorophenylisocyanate (0.304 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product

obtained was off-white puffy solid (0.674 g). ^{1}H NMR (DMSO-d_6, 400MHz) δ: 8.18 (s, 1H, -NH), 7.43 (d, 5H, Ar-H), 7.35 (t, 8H, Ar-H), 7.22 (m, 1H, Ar-H), 4.33 (s, 1H, -CH-), 3.40 (br s, 4H, -CH_2-), 2.50 (br s, 4H, -CH_2-). MS (ESI + ion): m/z = 406.0. IR (KBr, cm^{-1}): 3295, 1643.

Synthesis of 4-benzhydryl-*N*-(4-chlorophenyl)piperazine-1-carboxamide (1c): The general experimental procedure described herein afforded **1c** from 1-benzhydryl-piperazine **(1)** (0.5 g, 1.98 mmol), 4-chlorophenylisocyanate (0.304 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was white pluffy solid (0.698 g). ^{1}H NMR (DMSO-d_6, 400MHz) δ: 8.45 (s, 1H, -NH), 7.48 (d, 4H, Ar-H), 7.40-7.35 (m, 6H, Ar-H), 7.27 (t, 2H, Ar-H), 7.15 (m, 2H, Ar-H), 4.38 (s, 1H, -CH-), 3.46 (br s, 4H, -CH_2-), 2.52 (br s, 4H, -CH_2-). MS (ESI + ion): m/z = 406.2. IR (KBr, cm^{-1}): 3290, 1637.

Synthesis of 4-benzhydryl-*N*-(2-methoxyphenyl)piperazine-1-carbox- amide (1d): The general experimental procedure described herein afforded **1d** from 1-benzhydryl-piperazine **(1)** (0.5 g, 1.98 mmol), 2-methoxyphenylisocyanate (0.295 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was light brown crystalline solid (0.706 g). ^{1}H NMR (DMSO-d_6, 400MHz) δ: 8.18 (s, 1H, -NH), 7.43 (d, 5H, Ar-H), 7.32 (t, 5H, Ar-H), 7.25 (s, 2H, Ar-H), 7.20 (m, 2H, Ar-H), 4.36 (s, 1H, -CH-), 3.32 (br s, 4H, -CH_2-), 2.50 (br s, 4H, -CH_2-). MS (ESI + ion): m/z = 402. IR (KBr, cm^{-1}): 3302, 1644.

Synthesis of 4-benzhydryl-*N*-(3-methoxyphenyl)piperazine-1-carbox- amide (1e): The general experimental procedure described herein afforded **1e** from 1-benzhydryl-piperazine **(1)** (0.5 g, 1.98 mmol), 3-methoxyphenylisocyanate (0.295 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was light brown pluffy solid (0.714 g). ^{1}H NMR (DMSO-d_6, 400MHz) δ: 8.20 (s, 1H, -NH), 7.43 (d, 4H, Ar-H), 7.33 (t, 4H, Ar-H), 7.22-7.15 (m, 3H, Ar-H), 6.90 (t, 2H, Ar-H), 6.84 (m, 1H, Ar-H), 4.34 (s, 1H, -CH-), 3.74 (s, 3H, -OCH_3), 3.33 (br s, 4H, -CH_2-), 2.50 (br s, 4H, -CH_2-). MS (ESI + ion): m/z = 402.1. IR (KBr, cm^{-1}): 3290, 1631

Synthesis of 4-benzhydryl-*N*-(4-methoxyphenyl)piperazine-1-carbox- amide (1f): The general experimental procedure described herein afforded **1f** from 1-benzhydryl-piperazine **(1)** (0.5 g, 1.98 mmol), 4-methoxyphenylisocyanate (0.295 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was light brown pluffy solid (0.738 g). ^{1}H NMR (DMSO-d_6, 400MHz) δ: 8.22 (s, 1H, -NH), 7.45 (d, 4H, Ar-H), 7.35-7.29 (m, 6H, Ar-H), 7.22 (t, 2H, Ar-H), 6.87 (m, 2H, Ar-H), 4.36 (s, 1H, -CH-), 3.72 (s, 3H, -OCH_3), 3.44 (br s, 4H, -CH_2-), 2.32 (br s, 4H, -CH_2-). MS (ESI + ion): m/z = 402. IR (KBr, cm^{-1}): 3288, 1636.

Synthesis of 4-benzhydryl-*N*-(4-fluorophenyl)piperazine-1-carboxamide (1g): The general experimental procedure described herein afforded **1g** from 1-benzhydryl-piperazine **(1)** (0.5 g, 1.98 mmol), 4-fluorophenylisocyanate (0.271 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was off-white crystalline solid (0.609 g). ^{1}H NMR (DMSO-d_6, 400MHz) δ: 8.48 (s, 1H, -NH), 7.45-7.40 (m, 6H, Ar-H), 7.33-7.29 (t, 4H, Ar-H), 7.22 (t, 2H, Ar-H), 7.11 (m, 2H, Ar-H), 4.35 (s, 1H, -CH-), 3.45 (br s, 4H, -CH_2-), 2.50 (br s, 4H, -CH_2-). MS (ESI + ion): m/z = 390.1. IR (KBr, cm^{-1}): 3310, 1648.

Synthesis of 4-benzhydryl-*N*-(2,4-dichlorophenyl)piperazine-1-carbox- amide (1h): The general experimental procedure described herein afforded **1h** from 1-benzhydryl-piperazine **(1)** (0.5 g, 1.98 mmol), 2,4-dichlorophenylisocyanate (0.372 g, 1.98 mmol) and triethylamine (0.601 g, 5.94 mmol). The product obtained was white pluffy solid (0.661 g). ^{1}H NMR (DMSO-d_6, 400MHz) δ: 8.20 (s, 1H, -NH), 7.59 (s, 1H, Ar-H), 7.51-7.44 (m, 5H, Ar-H), 7.36-7.29 (m, 5H, Ar-H), 7.22 (t, 2H, Ar-H), 4.35 (s, 1H, -CH-), 3.46 (br s, 4H, -CH_2-), 2.51 (br s, 4H, -CH_2-). MS (ESI + ion): m/z = 440.1. IR (KBr, cm^{-1}): 3300, 1644.

RESULTS AND DISCUSSION

The anti-inflammatory activity of the synthesised compounds **1(a-h)** was evaluated by carrageenan induced Paw Edema method [25]. The compounds were tested at 50 mg kg^{-1} oral dose and were compared with the reference drug Nimesulide. The tested compounds showed anti-inflammatory activity ranging from 2.2 to 60.8% inhibition **(Table 3)** whereas the reference drug Nimesulide showed 53.4% inhibition after 5 h. Among the tested compounds, **1f** showed 42.8 - 60.6%, **1g** showed 34.3 – 48.4% and **1h** showed 31.4 – 57.5% inhibition. Compound **1f** was found to possess highest anti-inflammatory activity by 60.6% inhibition against the carrageenan induced paw edema.

As most of the tested compounds of the present study showed higher anti-inflammatory activity and some of them showed comparable activity, oral bioavailability was considered to play an important role for the development of bioactive molecules as therapeutic agents.

The inhibition is mainly due to the presence of methoxy group in **1f** at para position on the phenyl ring, electronegative fluoro at para position on the phenyl ring in **1g**, electron withdrawing chloro groups at ortho and para position on the aromatic ring in **1h**. The compounds **1(a-e)** displays lesser potency than the **1f**. From the SAR findings seemingly suggest that the 4-OCH_3 compounds could interact with the inflammatory mediators, where as the 2-OCH_3 in **1d** and 3-OCH_3 in **1e** doesn't shows the interaction with the inflammatory mediators. The one more important aspect is that fluoro substituted derivative **1g** showed enhanced activity compared to the chloro substituted derivatives **1(a-c),** this might be presence of more electronegative atom. Thus, in order to verify whether influence was exerted by the nature of substituents on benzene rings, we have explored other mono substituted and disubstituted compounds, among which disubstituted **1h** displays good activity compare to those of monosubstituted **1(a-c)** derivatives. This suggests that disubstituted compounds are more potent. Finally the para substituted derivatives play key role in the potent anti-inflammatory activity.

APPLICATIONS

Antidiabetic activity: All the experimental procedures were carried out in accordance with committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee. K.L.E.S's College of Pharmacy, Belagavi, India.

Animals: Male wistar rats procured from National institute of Nutrition, Hyderabad (210-250 gms) were used in the present study. The animals were kept in individual cages for one week to acclimatize for the laboratory conditions. They were allowed to free access of water and food.

Carrageenan induced Paw Edema: The rats were divided into 10 groups of 6 animals each. First group received 1ml of 0.5% CMC, second group received Nimusulide 50 mg/kg and remaining groups received test compounds 50 mg/kg in 1ml of 0.5% CMC, by oral gavage. Thirty minutes after drug administration, 0.1ml of 1% carrageenan in normal saline solution was injected into the sub plantar region of left hind paw. The paw edema volume was recorded using a plethysmometer (UGO Basile, Italy) at different time intervals. The difference between the initial and subsequent reading gave the actual edema volume. Then at each time interval the anti-inflammatory activity was expressed as percentage inhibition (%) of inflammation induced by carrageenan [25], using the formula, % inhibition = (1 – Vt / Vc) x 100, Where 'Vc' represents edema volume in control and 'Vt' represents edema volume in group treated with standard or test compounds.

Drugs and Reagents used: Carrageenan was purchased from High Media, Mumbai and nimusulide was procured as a gift sample from Dr Reddy's labs Hyderabad.

Statistical Analysis: Results are expressed as mean ± SEM. Statistical Analysis was carried out using One-Way ANOVA followed by dunnets't' tests. Results were considered highly significant if P< 0.01 and less significant if P<0.05.

CONCLUSIONS

From the results obtained, it could be noted that compounds **1f, 1g** and **1h** as potent anti-inflammatory agents. Amongst the test compounds, substitution at para position of the phenyl ring favours the higher anti-inflammatory activity. Further studies are required to establish their exact mechanism of action.

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