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Synthesis of N-((2-Substituted Phenyl)-4, 5-Diphenyl-1*H*-Imidazol-1yl)(Phenyl)Methyl) substituted amine derivatives, spectral characterization and their pharmacological evaluation

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ABSTRACT

A series of N-((2-substituted phenyl) -4,5-diphenyl-1H-imidazol-1yl)(phenyl)methyl) substituted amine derivatives TPI (I-V) were synthesized by 2-substituted 4,5-diphenyl imidazole derivatives starting from benzyl and aromatic aldehyde. All these compounds were characterized by IR, ^{1}HNMR, mass and elemental analysis. The newly synthesized compounds were screened for analgesic and anti-inflammatory activities by hot plate and carrageenan induced rat paw oedema methods. Compounds TPI-IV and TPI-V have showed potent anti-inflammatory activity and compounds TPI-III, TPI-IV and TPI-V showed good analgesic activity.

Keywords: *Analgesic activity; Anti-inflammatory activity; Benzyl; Imidazole; Mannich condensation*

1. Introduction

Imidazoles are probably the most well known heterocyclic nuclei, which is a common and important feature of a variety of natural products and medicinal agents. The chemistry of nitrogen heterocyclic compounds, especially imidazoles, has attracted more attention during recent years due to their reactivity and novel biological activities. Compounds having imidazole nucleus present unique antiedema and anti-inflammatory activities [1, 2]. Various substituted imidazoles derivatives have also been found to posses important activities such as antihelmintic [3, 4], analgesic [5], antibacterial [6], antifungal [7], antiviral [8], tuberculostatic [9], and citostatic [10]. There are various methods for synthesis of imidazole derivatives. The Mannich reaction is a three-component condensation in which an active hydrogen atom containing compound reacts with formaldehyde and an NH derivative with the elimination of a water molecule [11]. The Mannich reaction product has the *N*-atom of the nitrogen functionality linked to the substrate through a methylene group. This transformation was first discovered by Carl Mannich in 1912 when he treated a salicylantipyrine pharmaceutical preparation and urotropine with acid [12]. Mannich based products have significant importance in pharmaceuticals and pesticides and in the preparation of natural and synthetic macromolecules. This reaction is an example of nucleophilic addition of an amine to a carbonyl group followed by dehydration to the Schiff base. The Schiff base is an electrophile which reacts in the second step of nucleophilic addition with a compound containing an acidic proton. The Mannich reaction is also considered a condensation reaction. The reason why tertiary amines are not used in Mannich condensation is that they lack an N-H proton to form the intermediate imine. In this paper,

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we will describe the synthesis, screening results of *in vivo* anti-inflammatory and analgesic activities of the above mentioned compound.

Inflammation is a local reaction to injury of the vascular tissue, resulting in the formation of protein-rich exudates; it is a protective response of the nonspecific immune system that serves to localize, neutralize, or to destroy an injurious agent in preparation for the process of healing. The cardinal signs of inflammation are rubor (redness), calor (heat), dolor (pain), tumor (swelling), and functio laesa (loss of function). There are various causes of inflammation such as physical agents, chemical agents, immunological reactions, and infection by pathogenic organisms [13]. Inflammation is divided in two categories such as acute and chronic. The acute inflammation is the exudation of fluid, plasma proteins and the emigration of leukocytes, predominantly neutrophils. Chronic inflammation is considered to be inflammation of prolonged duration in which active inflammation, tissue destruction, and attempts to repair are proceeding simultaneously. Chronic inflammation includes some of the most common and disabling human diseases, such as rheumatoid arthritis, atherosclerosis, tuberculosis, and chronic lung diseases [14]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment in various inflammatory diseases such as arthritis, rheumatisms as well as to relieve the aches and pain [15]. This is essentially brought about by inhibiting the cyclooxygenase (COX) enzyme involved in the inflammatory cascade. Among different types of NSAIDs, imidazole and fused imidazole with six-membered rings [16] occupy central positions among those compounds that are used as analgesic and anti-inflammatory agents. The aforesaid numerous pharmacological activities of imidazoles prompted us to study the *in-vivo* analgesic and anti-inflammatory activities of some important imidazole derivatives.

2. Experimental section

2.1 Methods and Materials. All the chemicals and reagents were obtained from Sigma (Germany) and CDH (India) and were recrystallized/ redistilled as necessary. Melting points were determined by open capillary tube method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates precoated with silica gel G using solvent system ethyl acetate: petroleum ether (1:1 v/v) and n-hexane: petroleum ether (3:7, v/v). The spots were located under iodine vapours and UV light. IR spectra were recorded using KBr on a FTIR Shimadzu 8400S IR spectrophotometer (Japan). A JEOL AL300 FTNMR 300 MHz spectrometer was used to acquire ^{1}H-NMR spectra with acetone as solvent and TMS as internal standard. Chemical shift values are expressed in δ ppm.

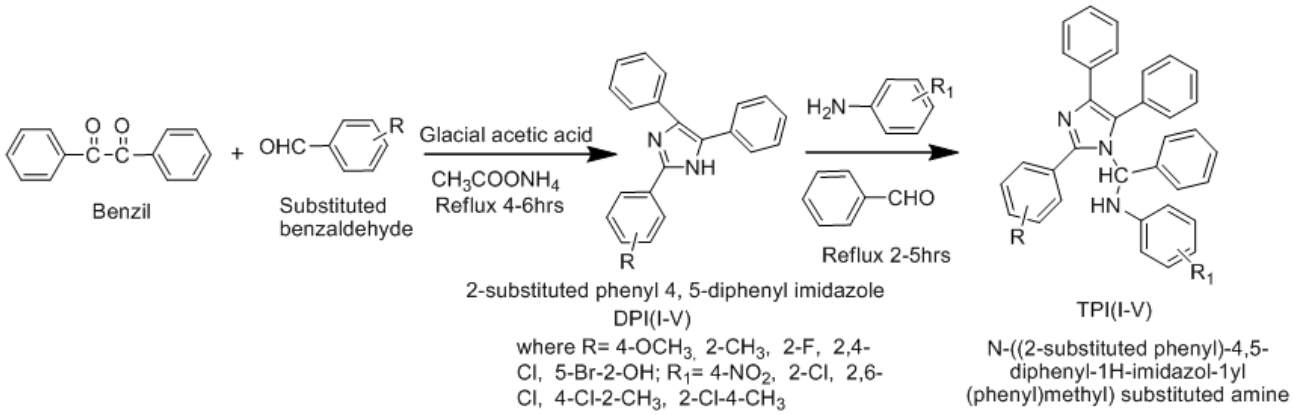
2.2 Synthetic Scheme.

2.2.1. Synthesis of 2-substituted phenyl 4, 5-diphenyl imidazole derivatives (DPI I-V). Benzyl (1 mmol) and substituted benzaldehyde (1 mmol) were refluxed with ammonium acetate (2 mmol) and glacial acetic acid (1mmol) for 4-6 hrs to afford 2-substituted 4, 5-diphenyl imidazole. After reflux, the reaction mixture was allowed cool down to room temperature. The solid that appeared after the addition of water was filtered. The filtrate was neutralized with ammonium hydroxide and was filtered and recrystallised from ethanol **(Scheme 1).**

2.2.2. General procedure for synthesis of N-((2-substituted phenyl)-4,5-diphenyl-1H-imidazol-1yl)(phenyl)methyl)substituted amine (TPI I-V). 2-substituted 4, 5-diphenyl imidazole derivatives were dissolved in methanol and underwent Mannich condensation reaction with

benzaldehyde and appropriate aromatic secondary amines to yield the corresponding triphenyl imidazole derivatives analogues by refluxing for 2-5 hr.

2.3 Anti-inflammatory activity. The results of tested compounds as well as reference standard were measured after the administration of carrageenan injection in rats; the effect was measured in the intervals of 0, 0.5, 1 and 2hr. The percent oedema inhibition was calculated as a regard to control group, and as depicted in Table 2, Figures 1 and 2. Most of the tested compounds have shown good results in comparison with Diclofenac sodium standard drug. Amongst all the compounds, compound TPI-IV and TPI-V have shown potent anti-inflammatory activity. From a view of structure-activity relationship (SAR) studies, Chloro substituted derivatives showed potent activity when compared with other substitution (methoxy, nitro).



Scheme 1: Synthesis of N-((2-substituted phenyl)-4,5-diphenyl-1H-imidazol-1yl)(phenyl)methyl) substituted amine.

2.4. Analgesic activity. The analgesic activity of the synthesized compounds was assessed by the Hot plate method. According to the structure-activity relationship (SAR) studies, almost compounds have shown very potent analgesic activity when compared with standard nimesulide drug. Among the tested compounds, TPI-III, TPI-IV and TPI-V showed most significant activity because of chloro and bromo substituted imidazole derivatives. The results are summarized in Table 1. Graphical representation of reaction time and tested compounds is presented in Figure 3.

Table 1: Analgesic data of synthesized compounds

compounds	Reaction Time (mean±SEM)				
	0min	30min	60min	90min	120min
Control	3.3±0.21	2.6±0.21	3.3±0.33	3.6±0.21	3.5±0.22
Standard	5.1±0.20	6.6±0.29	7.3±0.11	9.9±0.22	12.1±0.27
TPI-I	2.5±0.22	3.5±0.22	3.1±0.30	4.3±0.21	4.8±0.30
TPI-II	3.3±0.33	3.1±0.30	2.6±0.33	3.1±0.30	3.5±0.22
TPI-III	4.3±0.33	4.6±0.33*	5.6±0.55**	6.3±0.33***	7.1±0.65***
TPI-IV	4.5±0.22	4.6±0.21*	6.5±0.34***	8.3±0.33***	11±0.36***
TPI-V	3.6±0.21	4.3±0.33	7±0.63***	9.8±0.30***	10.3±0.33***

Method: Hot plate method; test animal: mice; number of animals per group: 6; route of administration: ip; standard: Nimesulide (50 mg/ kg); test compounds: 50 mg/ kg ***P < 0.001, *P < 0.05. Statistical analysis: the statistical analysis was performed by one-way ANOVA followed by Dunnet's test.

3. Results section

3.1. Spectral Characterization.

3.1.1. (DPI I-V)

2-(4-methoxyphenyl)-4, 5-diphenyl-1H-imidazole (DPI-I): M.W: 326.39 IR (KBr cm^{-1}) 1542 (C=C Ar str.), 829.33 (imidazole ring), 1247.86(C-O-C stretch), ^{1}H NMR (acetone, 300 MHz):

δ11.54(s, 1H, NH (imidazole)), 3.75-3.91(d, 3H, OCH_3), 7.01-8.07 (m, 14H, Ar-H); EIMS m/z: 326.14(M^+)

4, 5-diphenyl-2-*o*-tolyl-*1H*-imidazole (DPI-II): M.W: 310.39; IR (KBr cm^{-1}) 3082.75(C-H aromatic str.), 1517.87(C=C Ar str.), 780.76 (imidazole ring), 1550.30(C-N stretch); ^{1}H NMR (acetone, 300 MHz): δ ppm: 2.36 (s, 3H, CH_3), 7.10-7.48(m, 10H, Ar), 13.1 (s, 1H, imidazole); EIMS m/z: 310.15(M^+)

2-(2-Fluorophenyl)-4, 5-diphenyl-*1H*-imidazole (DPI-III): M.W: 314.36; IR (KBr cm^{-1}) 3072.75(C-H aromatic str.), 1523.87(C=C Ar str.), 760.76 (imidazole ring), 1560.30(C-N stretch) 1068.49(Ar-F str.); ^{1}H NMR (acetone, 300 MHz): δ ppm: 13.31 (s, 1H, NH), 7.03-7.48 (m, 14H, Ar); EIMS m/z: 314.12 (M^+).

2-(2, 4-dichlorophenyl)-4,5-diphenyl-*1H*-imidazole (DPI-IV): M.W: 365.26; IR (KBr cm^{-1}) 3066.75(C-H aromatic str.), 1510.87(C=C Ar str.), 799.76 (imidazole ring), 1560.30(C-N stretch) 1065.49(Ar-Cl str.);^{1}H NMR (acetone, 300 MHz): δ ppm: 13.32 (s, 1H, NH), 7.20-7.48 (m, 14H, Ar); EIMS m/z: 364.05(M^+).

4-bromo-2-(4,5-diphenyl-*1H*-imidazole-2-yl)phenol (DPI-V): M.W: 391.26; IR (KBr cm^{-1}) 3062.15(C-H aromatic str.), 1511.67(C=C Ar str.), 810.76 (imidazole ring), 1560.30(C-N stretch) 1068.49(Ar-Br str.); ^{1}H NMR (acetone, 300 MHz): δ ppm: 13.48 (s, 1H, NH (imidazole)), 4.8(d, 1H, aromatic C-OH), 6.68-7.45 (m, 13H, Ar); EIMS m/z: 390.04(M^+)

3.1.2. (TPI I-V)

***N*-((2-(4-methoxyphenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)(phenyl)methyl)-4-nitro-benzenamine (TPI-I)**

M.W:552.62; m.p-164-166^{0}C; % Yield 59.17; IR (KBr cm^{-1}) 3363.62 (Ar CH str), 1600.81 (Ar C=C str.), 1469.66 (NO_2 str.) 840.91(imidazole ring), 1114.78(C-N str.), 1247.86 (C-O-C str.); ^{1}H NMR (acetone, 300 MHz): δ ppm: 2.84 (s, 3H, OCH_3), 3.85(s, 1H, NH), 6.72-6.75 (d, 1H, CH), 7.01-8.08 (m, 23H, Ar-H)EIMS m/z: 552.22 (M^+)

2-chloro-*N*-(phenyl)(4,5-diphenyl-2-*o*-tolyl-1*H*-imidazol-1-yl)methyl)benzenamine (TPI-II)

M.W: 526.07; m.p 210-212^{0}C; % Yield 71.00; IR (KBr cm^{-1}) 3026.10 (Ar CH str), 1602.74(Ar C=C str.), 730.97 (imidazole ring), 1141.78(C-N str.), 1072.35(Ar-Cl str.); ^{1}H NMR (acetone, 300 MHz): δ ppm: 2.03-2.08 (s, 3H, CH_3), 4.2(d, 1H, NH), 7.19-7.78 (m, 20H, Ar-H); EIMS m/z: 525.20 (M^+)

2,6-dichloro-*N*-((2-(2-fluorophenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)(phenyl)methyl) benzenamine (TPI-III)

M.W: 564.48; m.p 186-188^{0}C; %Yield 39.10; IR (KBr cm^{-1}) 3080.11.10 (Ar CH str), 1602.74(Ar C=C str.), 763.76 (imidazole ring), 1253.64(C-N str.), 1101.28(Ar-Cl str.) ^{1}H NMR (acetone, 300 MHz): δ ppm: 3.77(d, 1H, NH), 6.16 (d, 1H, CH), 7.21-7.65 (m, 22H, Ar-H); EIMS m/z: 563.13

4-chloro-*N*-((2-(2,4-dichlorophenyl)-4,5-diphenyl-1*H*-imidazol-1-yl)(phenyl)methyl)-2-methylbenzenamine (TPI-IV)

M.W: 594.96; m.p 198-200^{0}C; %Yield 61.34; IR (KBr cm^{-1}) 3066.61(CH Ar str.), 1593.09(C=C Ar str.), 767.62(imidazole ring), 1101.28(Ar-Cl str.), 1321.15(CN str.); ^{1}HNMR(acetone, 300 MHz): δ ppm: 3.30-3.36 (d, 1H, NH), 2.83-2.94 (d, 3H, CH_3), 7.21-8.10 (m, 22H, Ar-H); EIMS m/z: 593.12

2-(1-((2-chloro-4-methylphenylamino)(phenyl)methyl)-4,5-diphenyl-1*H*-imidazol-2-yl) -4-bromophenol (TPI-V)

M.W: 620.97; m.p 138-140^{0}C; %Yield 50.63; IR (KBr cm^{-1}) 2921.96(CH Ar str.), 1577.66(C=C Ar str.), 763.76(imidazole ring), 1072.35(Ar-Cl str.), 1247.86 (CN str.), 696.25(C-Br str.); ^{1}HNMR

(acetone, 300 MHz): δ ppm: 4.67 (s, 1H, OH), 2.34 (s, 3H, CH_3), 2.87 (d, 1H, NH), 6.57-6.63(s 1H, CH_3), 6.73-7.46 (m, 21H, Ar-H); EIMS m/z: 621.10.

3.2. Chemistry. A series of N-((2-substituted phenyl)-4,5-diphenyl-1H-imidazol-1yl)(phenyl) methyl) substituted amine derivatives were designed and synthesized from benzyl (Scheme 1). 4-chloro-2-iodo aniline and 2-chloro aniline give better yield, whereas 4-ethyl aniline moderate for the formation of triphenyl imidazole derivatives. The structures of the imidazoles were confirmed by ^{1}H NMR, IR, mass spectral data and melting point. In the IR spectra of all the synthesized compounds showed the absorption band in the region of such as 760-850cm^{-1} and 1731-1652 cm^{-1} assigned for imidazole ring and C=N group respectively providing strong evidence for the formation triphenyl imidazole derivatives. ^{1}H-NMR spectrum showed the most important signal in the range of δ 5.88-6.11 for CH and 3.8-4.3 for NH moiety; different other signals were observed at the expected chemical shift.

3.3. Anti-inflammatory activity. Anti-inflammatory activity of all the synthesized compounds was evaluated by carrageenan induced rat paw oedema method [17]. The oedema hind paw was induced by injection of freshly prepared carrageenan (0.1 ml of 2% w/v) solution in the sub-plantar region of the left hind paw of each rat. One group was kept as control and the animals of the other group were pretreated with the test drugs (50 mg/kg body weight) suspended in 1% carboxymethylcellulose (CMC) given orally, 30min before the carrageenan treatment. The volume was measured after 0, 0.5, 1, and 2hr of carrageenan treatment using a plethysmometer. Diclofenac sodium was used as standard drug. Percent inhibition of the oedema between control group and the compound treated group was calculated and compared with the group receiving standard drug at 50mg/kg b.w.

Table 2: Anti-Inflammatory data of synthesized compounds:

Comp	0hr (X±SEM)	% oedema inhibition	0.5hr (X±SEM)	%oedema inhibition	1hr (X±SEM)	%oedema inhibition	2hr (X±SEM)	%oedema inhibition
Control	1.72±0.16	-	1.87±0.17	-	1.92±0.05	-	2.05±0.16	-
Standard	1.15±0.05	33.13	1.07±0.06	42.78	0.98±0.08	48.95	0.70±0.09	65.83
TPI-I	1.41±0.19	18.02	1.44±0.18	22.99	1.29±0.28	32.81	1.11±0.14**	45.85
TPI-II	1.35±0.13	21.51	1.06±0.16*	43.31	1.21±0.16*	36.97	1.24±0.19	39.51
TPI-III	1.13±0.20	34.30	1.34±0.15	28.34	1.22±0.15*	36.45	0.97±0.11**	52.68
TPI-IV	1.12±0.26	34.88	1.06±0.17*	43.31	0.90±0.16***	53.12	0.93±0.19***	54.63
TPI-V	1.28±0.17	25.58	1.32±0.11*	29.58	1.07±0.14**	44.27	0.94±0.08***	54.14

Data represent mean values(X) ±SEM of six rats per group and the percent changes versus 0, 0.5, 1 and 2hr post-carrageenan injection. Data were analyzed using one-way ANOVA followed by Turkey test ***p< 0.001.

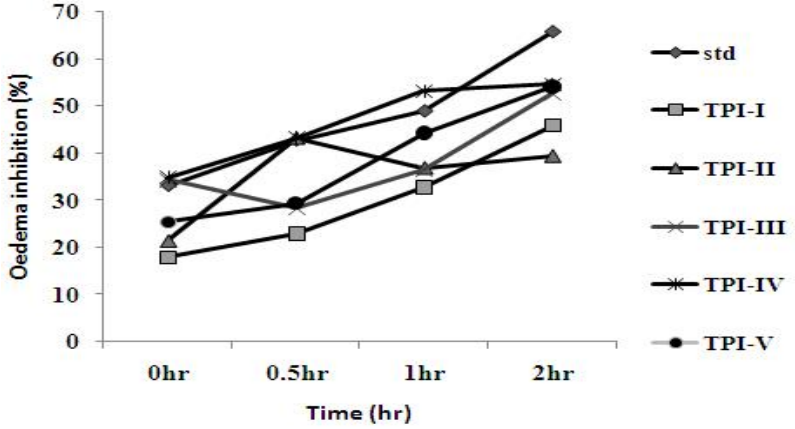


Figure 1: The inhibition of anti-inflammatory activity of the tested compounds and standard drug Diclofenac sodium

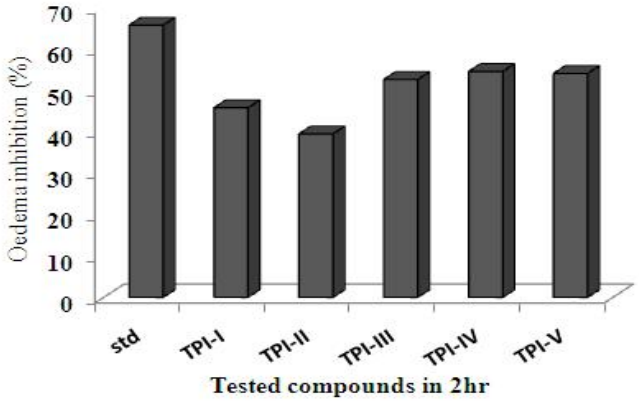


Figure 2: The percentage of inhibition of anti-inflammatory activity of the tested compounds and standard drug Diclofenac sodium in 2hr

3.4. Analgesic activity. Mice were treated and placed on Eddy's hot plate kept at a temperature of 55 ± 0.5 ºC. A cut off period of 15 sec was observed to avoid damage to the paw. The reaction time and the type of response were noted using a stopwatch. The response is in the form of jumping or licking of the paws. The latency or reaction time of licking or jumping response was recorded after 0, 30, 60, 90 and 120 min following the treatments [18]. All tested compounds were compared with control.

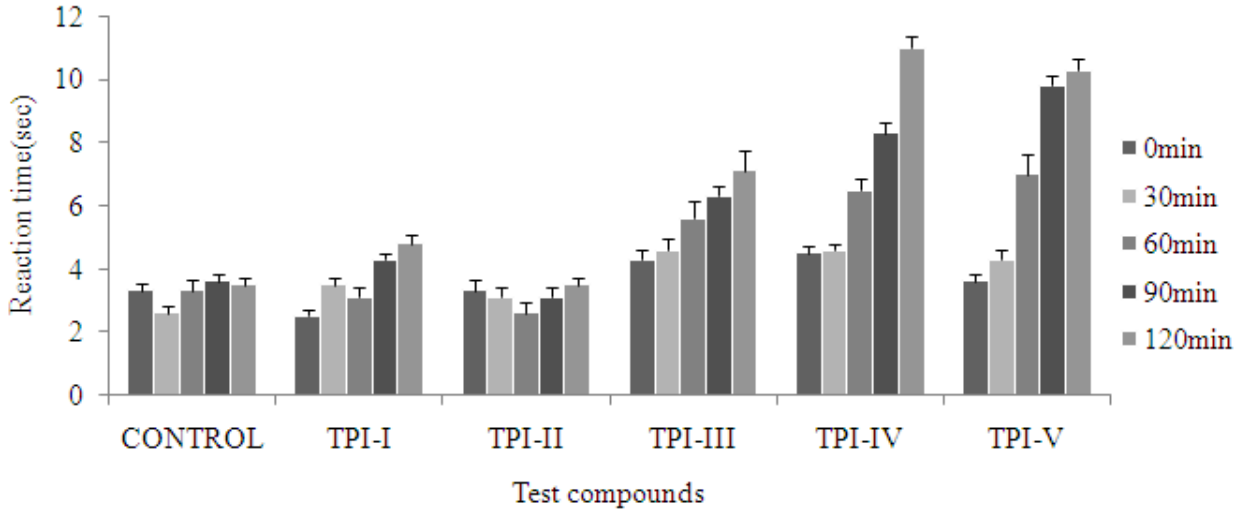


Figure 3: Analgesic activity of tested compounds

4. Conclusions

New N-((2-substituted phenyl)-4,5-diphenyl-1H-imidazol-1yl)(phenyl)methyl)substituted amine derivatives were synthesized by 2-substituted 4,5-diphenyl imidazole derivatives starting from benzyl and aromatic aldehyde. All synthesized compounds have been screened for their *in-vivo* anti-inflammatory and analgesic activities. Amongst all the compounds, compound (TPI-IV) and (TPI-V) have shown potent anti-inflammatory activity. The preliminary *in-vivo* studies of these compounds evidenced that the chloro group enhances the anti-inflammatory activity and might serve as new templates in the synthesis and development of potent therapeutics. Therefore, it can be concluded that such compounds exert their pharmacological effects. These results provide a good impact in the field of medicinal chemistry for further investigations of anti-inflammatory agents containing halo and methyl functional groups. Apart from this compound, Compounds TPI-III, TPI-IV and TPI-V showed good analgesic activity.

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