International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 2, 2011

Research Article

EXPLORING THE EFFECTS OF NEWER THREE COMPONENT AMINOBENZYLATED REACTIONS OF TRIPHENYL IMIDAZOLE MOTIF AS POTENT ANTIMICROBIAL AND ANTI-INFLAMMATORY AGENTS

N.UMARANI*, K.ILANGO, GYANESH GARG, BOMPADA K. SRINIVAS AND V.HEMALATHA

***Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRM University, Kattankulathur-603203, Tamilnadu, India**
Email:umaarun79@gmail.com

Received: 20 Nov 2010, Revised and Accepted: 24 Dec 2010

ABSTRACT

An elegant synthesis of newer desired aminobenzylated triphenyl imidazole hybrids is described. Cyclization of benzil with appropriate aromatic aldehydes in the presence of ammonium acetate yielded 2-substituted - 4, 5-diphenyl imidazole entities, which further undergoes mannich condensation reaction with benzaldehyde and various aromatic secondary amines afforded the title compounds. The constituents of the newly synthesised compounds have been established on the basis of their physical and spectral data. All the newly synthesised heterocycles have been screened for *invitro* antimicrobial and anti-inflammatory activities. Among all the synthesized compounds, the compound TPI-IV exhibited good anti-inflammatory activity and better anti-microbial activity against bacterial strains *Staphylococcus aureus, Pseudomonas aeruginosa* and fungal strain *Candida albicans*.

Keywords: Triphenyl imidazole hybrids, Synthesised heterocycles, Mannich condensation, *In vitro* antimicrobial activity, Anti-inflammatory activity.

INTRODUCTION

Triphenyl imidazole is a privileged structural motif, which has played a pivotal role in the drug discovery process. Nitrogen containing heterocycles paved way for the active research in Pharmaceutical Chemistry. The study of triphenyl imidazole derivatives has been a developing field within the realm of heterocyclic chemistry for the past several decades because of their ready accessibility through synthesis, wide range of chemical reactivity and manifold biological activities. This structural template shows remarkable pharmacological activities such as antibacterial[1,2], anti-inflammatory[3,4], anticonvulsive[5], anthelmintic[6], antiulcer[7], antiviral[8], antitumour[9], antispasmodic[10], antioxidant[11] and antitubercular[12]. Intrigued by these investigations and as a part of our initial efforts to discover potentially active new agents, we decided to synthesise with this functionality coupled with mannich base could furnish better therapeutic results. To our knowledge mannich reaction using benzaldehyde have not been reported as yet. This initiated us to explore the aminobenzylated reaction as well as anti-inflammatory and antimicrobial properties of target compounds.

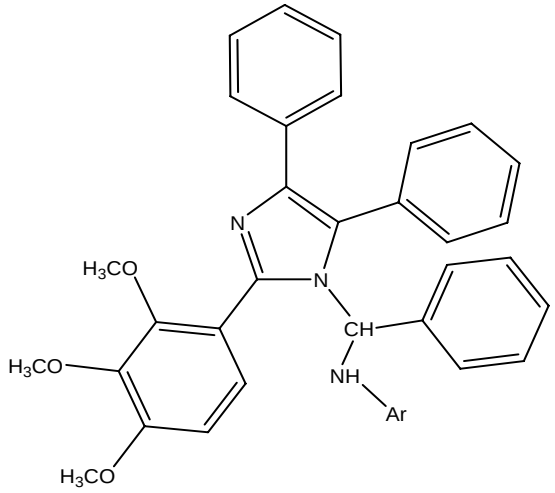


Fig. 1: Parent nucleus of triphenyl imidazole

MATERIALS AND METHODS

All the reagents used were of analytical grade. Melting points of the title compounds were determined using Veego-Digital VMP-D melting point apparatus and are uncorrected. Infra red spectra (cm^{-1}) were recorded on Perkin-Elmer spectrophotometer as pellets on KBr discs. The ^{1}HNMR (400MHz) spectra were recorded on Bruker-Avance II spectrometer in DMSO-d_6 using TMS as an internal standard (chemical shifts in δ ppm). The splitting patterns are designated as follows: s, (singlet), d, (doublet), t, (triplet), m, (multiplet). Mass spectra were recorded on Shimadzu LCMS-SL2010A (70ev) mass spectrometer. The reactions were monitored by thin layer chromatography (TLC) using precoated silica gel G plates of E-Merck. The spots were developed in iodine chamber.

Synthesis of 4,5-diphenyl-2-(2,3,4-trimethoxyphenyl)-4H-imidazole DPI

Benzil (25mmol, 5.25g) and 2,3,4-trimethoxy benzaldehyde (25mmol) were refluxed with ammonium acetate (10g) and glacial acetic acid (5 ml) for 4 hr afforded 2-substituted 4, 5-diphenyl imidazole. After refluxing, the reaction mixture was left overnight and filtered. The filtrate was neutralized with ammonium hydroxide and the second crop of the precipitate were combined and recrystallised from ethanol. Yield 67%, m.p 136^{o}c, R_f value 0.69.

Synthesis of N-((4,5-diphenyl-2-(2,3,4-trimethoxyphenyl)-1H-imidazol-1-yl)phenyl)methyl)substituted amine (TPI-I-TPI-V)

4,5-diphenyl-2-(2,3,4-trimethoxyphenyl)-4H-imidazole derivatives were dissolved in methanol and undergoes mannich condensation reaction with benzaldehyde and appropriate aromatic secondary amines yielded the corresponding N,N-disubstituted - 2,4,5-triphenyl -1H-imidazol-1-yl methanamine analogues by refluxing for 2 hr. Volatiles are removed under reduced pressure and the resulting dense oily product was recrystallised from ethanol to afford a white solid substance. The physicochemical parameters of the target compounds were tabulated in Table 1.

N-((4, 5- diphenyl-2-(2, 3, 4-trimethoxyphenyl)-1H-imidazol-1-yl) phenyl) methyl)-N-phenyl benzenamine (TPI-I).

IR (KBr) cm^{-1}: 3083.60 (Aromatic -CH stretching), 2933.57 (Aliphatic -CH stretching), 1519.64 (aromatic C=C stretching), 1432.44 (C=N stretching), 1041.84 (C-N stretching), 1243.44 (C-O-C stretching).^{1}HNMR(400 MHz,DMSO-d_6) δ ppm: 6.14 (s,1H,CH), 3.84 (s,9H,$(OCH_3)_3$), 6.54-8.26 (m,27H,Ar-H). Mass: m/z 643.

N-((4, 5- diphenyl-2-(2, 3, 4-trimethoxyphenyl)-1H-imidazol-1-yl) phenyl) methyl) piperazine (TPI-II).

IR (KBr) cm^{-1}: 3030.48 (Aromatic -CH stretching), 2969.39 (Aliphatic -CH stretching), 1596.06 (aromatic C=C stretching), 1490.06 (C=N stretching), 1071.10 (C-N stretching), 1238.18 (C-O-C stretching).^{1}H-NMR(400 MHz,DMSO-d_6) δ ppm: 6.12 (s,1H,CH), 3.86 (s,9H,$(OCH_3)_3$), 2.16(s,1H,NH), 6.34-8.26 (m,25H,Ar-H). Mass: m/z 561.

N-((4, 5-diphenyl -2- (2, 3, 4-trimethoxy phenyl)-1H-imidazol-1-yl) phenyl) methyl) –N-napthalen-1-amine (TPI-III).

IR (KBr) cm^{-1}: 3445.62 (Aromatic -CH stretching), 2926.82 (Aliphatic -CH stretching), 1596.32 (aromatic C=C stretching), 1444.66 (C=N stretching), 1242.24 (C-N stretching), 1238.64 (C-O-C stretching).^{1}H-NMR(400 MHz,DMSO-d_6) δ ppm: 6.42 (s,1H,CH), 3.82 (s,9H,$(OCH_3)_3$, 7.12-8.26 (m,29H,Ar-H). Mass: m/z 693.

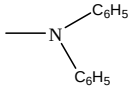
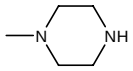
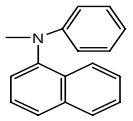
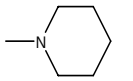
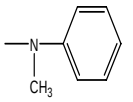
N-((4, 5-diphenyl-2-(2, 3, 4-trimethoxyphenyl)-1H-imidazol-1-yl) phenyl) methyl) piperidine (TPI-IV).

IR (KBr) cm^{-1}: 3010.71 (Aromatic -CH stretching), 2992.28 (Aliphatic -CH stretching), 1656.32 (aromatic C=C stretching), 1478.24 (C=N stretching), 1238.14 (C-N stretching), 1177.96 (C-O-C stretching).^{1}H-NMR(400 MHz,DMSO-d_6) δ ppm: 6.16 (s,1H,CH), 3.82 (s,9H,$(OCH_3)_3$), 6.82-8.26 (m,29H,Ar-H). Mass: m/z 560.

N-((4, 5-diphenyl -2- (2, 3, 4-trimethoxy phenyl)-1H-imidazol-1-yl) phenyl) methyl) –N-methyl benzenamine (TPI-V).

IR (KBr) cm^{-1}: 3020.8 2 (Aromatic -CH stretching), 2960.42 (Aliphatic -CH stretching), 1588.86 (aromatic C=C stretching), 1465.24 (C=N stretching), 1210.15 (C-N stretching), 1180.60 (C-O-C stretching).^{1}H-NMR(400 MHz,DMSO-d_6) δ ppm: 6.14 (s,1H,CH), 3.78 (s,9H,$(OCH_3)_3$), 6.92-8.26 (m,22H,Ar-H).Mass: m/z 581.

Table 1: Physicochemical parameters of Triphenyl imidazole derivatives

Compound Code	Ar	m.p (°c)	Yield (%)	Mol. formula	Mol. Wt.	R_f value
TPI-I		128-132	59.24	$C_{43}H_{37}N_3O_3$	643.77	0.82
TPI-II		134-138	68.70	$C_{35}H_{36}N_4O_3$	560.69	0.73
TPI-III		220-223	63.24	$C_{47}H_{39}N_3O_3$	693.83	0.88
TPI-IV		210-213	46.21	$C_{36}H_{35}N_3O_3$	559.71	0.79
TPI-V		205-208	54.18	$C_{38}H_{35}N_3O_3$	581.70	0.71

Antibacterial activity

The *invitro* antibacterial activity of the compounds was carried out by the agar cup plate method[13]. The concentration of the compound (250µg/ml) was prepared in dimethyl sulfoxide solvent (DMSO) and ofloxacin was used as standard. The antibacterial activity was evaluated using 24 hr cultures of *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and *Escherichia coli* using Muller Hinton agar medium. The medium was sterilised by autoclaving at 120°c for 30 minutes. About 30 ml of molten nutrient agar medium inoculated with the respective strains of bacteria (6ml of inoculums to 300ml of nutrient agar medium) was transferred aseptically into each sterilised petridish (10 cm diameter). The plates were left at room temperature to allow solidification of the media. In each plate 3 wells of 6mm diameter were made using a sterile cork borer. Accurately 0.1ml of test and standard solutions were transferred to the wells aseptically by micropipette and labelled accordingly. The plates were then maintained at room temperature for 2 hr to allow the diffusion of the solution in the medium. The petridish used for antibacterial screening were incubated at 37±1°c for 24 hrs. The diameter of zone of inhibition surrounding each well was recorded.

Antifungal activity

Aspergillus niger and *Candida albicans* were employed for testing fungicidal activity using cup plate method[14]. The cultures were maintained on Sabouraud's agar slants. Sterilised sabouraud's agar medium was inoculated with 72 hr old suspension of fungal spores in a separate flask. About 25 ml of the inoculated medium was evenly spread in a sterilised petridish and allowed to settle down for 2 hr. The cups (10mm in diameter) were punched in petridish and loaded with sample solution in DMSO. The plates were incubated at room temperature (30°c) for 48 hr. After the completion of the incubation period, the zone of inhibition of growth of the synthesized compounds (TPI I-V) in the form of diameter in mm was measured. Along the test solution in each petridish, one cup was filled with solvent which acted as control. The antifungal activity of compounds was compared with a standard drug Griseofulvin. The results of antimicrobial profile were depicted in Table 2.

***In vitro* anti-inflammatory activity**

The *invitro* anti-inflammatory activity was evaluated by human red blood cell membrane (HRBC) stabilisation method[15]. This method involves the stabilisation of the human red blood cell membrane by hypotonicity induced membrane lysis. The lysosomal enzymes released during inflammatory condition produces a variety of disorders. The extra cellular activity of these enzymes were said to be related to acute or chronic inflammation. The anti-inflammatory agents act by either inhibiting the lysosomal enzymes or by stabilising the lysosomal membrane, since the human red blood cell membranes are similar to lysosomal membrane components. The prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of anti-inflammatory activity of the drug.

The synthesised target compounds were made into dose of 250µg/ml with 5% DMSO as solvent. Diclofenac sodium was taken as a standard drug. The percentage membrane stabilization activity was calculated by the following formula and the results were tabulated in Table: 2

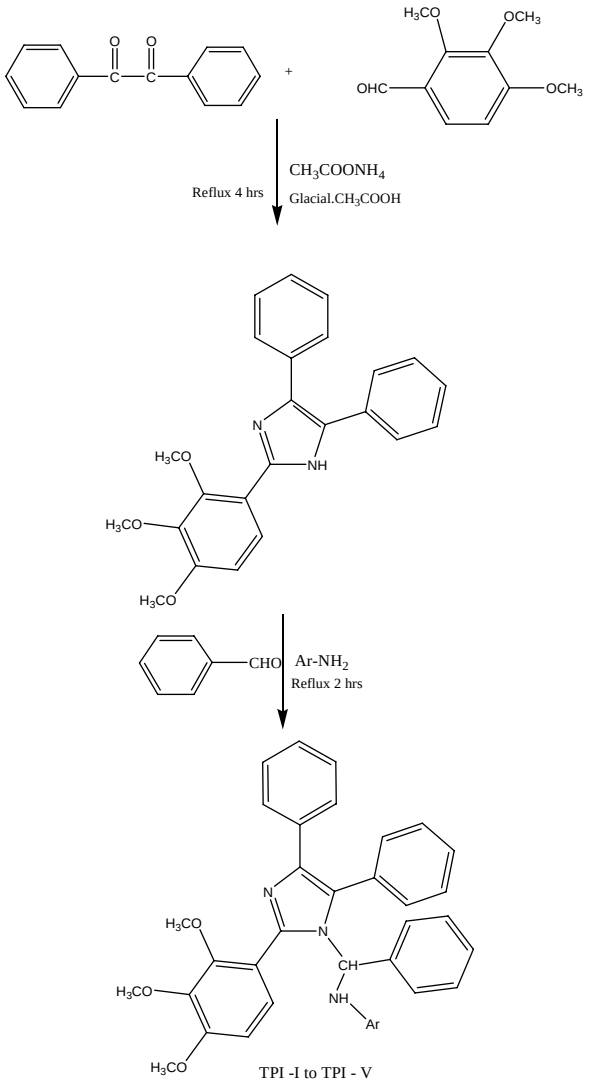
Percentage stabilisation = 100 - (OD of sample-OD of the product control) x 100/OD of test control

Table 2: *In vitro* antimicrobial and anti-inflammatory activity of the titled compounds

Compound Code	Anti bacterial activity Zone of inhibition mm				Antifungal activity Zone of inhibition mm		Anti-inflammatory activity Percentage Stabilisation
	S. aureus	*K. Pneumoniae*	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albicans*	
TPI-I	15	14	15	14	27	28	40.50
TPI-II	18	20	19	19	27	28	59.33
TPI-III	16	18	15	15	24	20	27.56
TPI-IV	20	19	18	23	26	29	61.24
TPI-V	17	15	17	16	16	17	37.00
Ofloxacin	44	39	38	37	-	-	-
Griseofulvin	-	-	-	-	36	39	-
Diclofenac sodium	-	-	-	-	-	-	76.80

CHEMISTRY

2-substituted-4,5-diphenyl imidazole root nucleus were synthesised by refluxing benzil with 2,3,4-trimethoxy benzaldehyde in presence of cyclising agents ammonium acetate and glacial acetic acid. In the next step, the prepared diphenyl imidazole analogues undergoes mannich condensation reaction with benzaldehyde and various substituted aromatic secondary amines resulted in the formation of target compounds (Scheme 1).



Scheme 1: Synthetic scheme of Triphenyl imidazole derivatives

RESULTS AND DISCUSSION

In the current study, totally a series of five different N, N –di substituted 2,4,5-triphenyl-1H-imidazole-1yl-methanamine Hybrids were achieved with a versatile and efficient synthetic route (Mannich condensation reaction). The yields of all the synthesized heterocycles were found to be in the range of 46-68%. The title compounds were characterized by physicochemical parameters like mp and R_f value. The spectral data also supported the assigned structures by showing the characteristic absorption peaks. The synthesized compounds were subjected to *invitro* anti-inflammatory, antibacterial and antifungal activities.

By visualizing the antimicrobial activity index it was noticed that the synthesized scaffolds elicits mild to good activity against gram positive, gram-negative bacterial strains and fungal strains at a concentration of 250 µg/ml.

Anti-inflammatory activity revealed that all the synthesized entities showed significant activity when compared with that of the standard drug Diclofenac Sodium.

It can be concluded that triphenyl imidazole as a useful template for further development through modification or derivatisation to design more potent biologically active compounds.

ACKNOWLEDGEMENT

The authors are grateful to Dr. R. Shivakumar, Pro-Vice Chancellor, SRM University and Dr. K. S.Lakshmi, Dean, College of Pharmacy, SRM University Kattankulathur for providing the facilities to carry out this study.

REFERENCES

1. Ghoniem K M, Essawi M V H, Mohamed M S, Kamal A M. Synthesis of 2-[(4-amino or 2, 4-diaminophenyl) sulfonyl derivatives of benzimidazole, benzothiazole and 6-methyl uracil as potential antimicrobial agents. Indian J. Chem. 1998; 37B: 904-911.
2. Aruna Devi M, Narayana Reddy A, Sarangapani M, Reddy V M. Synthesis and antimicrobial evaluation of some new imidazole-2-sulfones. Indian J. Hetero. Chem. 1998; 7: 181-184.
3. Narayana Reddy A, Sujata Patnaik, Narmath Kalyani, Reddy V M. Synthesis of some substituted imidazolino-(3,4)-2,3-dihydroimidazoles/Benzimidazoles as possible non-steroidal non acidic anti-inflammatory agents. Indian J. Hetero. Chem. 2003; 12: 347-350.
4. Puratchikody A, Yasodha A, Rukmani K. Synthesis and bioactivity of 2, 4, 5-triphenyl imidazoles. Indian J. Hetero. Chem. 2003; 13: 79-80.
5. Puratchikodi A, Nallu M, Gopalakrishnan S. Synthesis of some new triphenyl imidazoles of biological interest. Indian J. Hetero. Chem. 2004; 14: 149-150
6. Himaja M, Rajiv C, Ramana M V. Synthesis of 6-nitrobenzimidazol-1-acetyl amino acids and peptides as potent anthelmintic agents. Indian J. Hetero. Chem. 2002; 12: 121-124.
7. Murugan V, Ramaprasad K, Rama Sharma G V S, Ramanathan M, Suresh B. Synthesis of Triazole, Thiadiazole and Oxadiazole

bearing 2-Thio methyl benzimidazole and their biological evaluation. Indian J. Hetero. Chem. 2001; 11: 169-170.

8. Pandey V K, Zehea Tusi, Sarah Tusi. Synthesis of 8-(Alkyl, aryl amido/imido)-7-hydroxy-4-methyl quinolinyl(1,5-c)benimidazoles as potential antiviral compounds. Indian J. Hetero. Chem. 2002; 12: 194-196.
9. Suthakaran R, Nagarajan G, Balasubramaniam V, Suganthi K, Marikannan, Velrajan G. Synthesis and antitumor activity of 4-aryl/alkyl 2"-D aldo sugar disubstituted bis benzimidazoles. Indian J. Hetero. Chem. 2005; 15: 173-176.
10. Sharma R L, Meena Kumari, Naresh Kumar, Sharma B K. Synthesis of some condensed benzimidazoles containing bridge head nitrogen atom Indian J. Hetero. Chem. 1998; 8: 155-156.
11. Baviskar B A, Bhagyesh Baviskar, Shiradkar M R, Deokate U A, Khandabadi S S. Synthesis and antimicrobial activity of some novel Benzimidazolyl chalcones. E- J.Chem. 2009; 6(1): 196-200.
12. Bhat A R, Gautham Shenoy G, Mohan Kotian. Synthesis and biological activities of mannich bases of 7-nitro-2-methyl-4(3H) quinazolinone. Indian J. Hetero. Chem. 2000; 9: 319-320.
13. Pelczar M J, Chan E C S, Krig N R. Text book of Microbiology, Mc Graw Hill Publication, 5th edition, New York, 1993.P.504-508.
14. Satish Gupte. Text book of Medical Microbiology, Jaypee brother Medical Publisher, 6thedtion, New Delhi, 1995.P.82-87.
15. Umarani, Ilango K. Bridge head nitrogen heterocyclic systems; Facile synthesis, bioactivity of some newer derivatives of 1-substituted benzylidine hydrazino tetrazolo[1,5-a]quinoxalines. International Journal of Pharmaceutical Sciences Review and Research. 2010; 2(2): 24-28.