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SYNTHESIS OF 2, 4, 5- TRIPHENYL IMIDAZOLE DERIVATIVES AND BIOLOGICAL EVALUATION FOR THEIR ANTIBACTERIAL AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT: On the basis of various literature survey, imidazole derivatives show various activity such as antimicrobial, anti-inflammatory, analgesic, antitubercular, anticancer etc. The possible improvements in the activity can be further achieved by slight modifications in the substituents on the basic imidazole nucleus. Having structural similarity with histidine imidazole compound can bind with protein molecules with ease compared to the some other heterocyclic moieties. Thus imidazole offers better pharmacodynamic characteristics. Furthermore, some imidazole drugs, at high concentrations, could exert direct inhibitory effects on membranes, without interference with sterols and sterol esters. Various recent new drugs developments in imidazole derivatives show better effect and less toxicity. Prompted by the broad spectrum activities of 2, 4, 5-triphenylimidazole derivatives, it was decided to synthesize various 2, 4, 5-triphenyl-1-substituted imidazoles and to evaluate them for their pharmacological activities.

INTRODUCTION: On the basis of various literature surveys Imidazole derivatives shows various pharmacological activities [1]

- Anti-fungal and anti-bacterial activity
- Anti-inflammatory activity and analgesic activity
- Anti-tubercular activity
- Anti-depressant activity
- Anti-cancer activity
- Anti-viral activity
- Antileishmanial activity

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Biological significance of imidazole: Imidazole is incorporated into many important biological molecules. The most important is the amino acid histidine, which has an imidazole side chain. Histidine is present in many proteins and enzymes play a vital role in the structure and binding functions of hemoglobin. Histidine can be decarboxylated to histamine, which is also a common biological compound. It is a component of the toxin that causes urticaria, i.e. allergic.

Applications of imidazole [2]:

- One of the applications of imidazole is in the purification of His tagged proteins in immobilized metal affinity chromatography (IMAC). Imidazole is used to elute tagged proteins bound to Ni ions attached to the surface of beads in the chromatography column. An excess of imidazole is passed through the column, displaces the His-tagged

from nickel coordination and free the His-tagged proteins.

- Imidazole can be used to prepare buffers in the pH range of 6.2-7.8 at room temperature. It is recommended as a component of a buffer for assay of horseradish peroxides. It is also used as a chelator for the binding of different divalent cations [2].

- The oral administration of imidazole shows beneficial effects on psoriasis and seborrheic dermatitis. In psoriasis the improvement begins after a period of one and a half to three months. In seborrheic dermatitis the patients begin from less redness, itchiness, and scaling within a period of four to six weeks. The benefits of this treatment occur without the need for applications of ointments or other topical applications

- The imidazole nucleus is an important synthetic strategy in drug discovery. Many imidazoles have been prepared as pharmacological agents Azomycine, Clotrimazole, Miconazole, Ergothionine, Clonidine and Moxonidine. One of the most important applications of imidazole derivatives is their used as material for treatment of denture stomatitis [1, 2].

- Imidazole has become an important part of many pharmaceuticals. Synthetic Imidazoles are present in many fungicides and antifungal, antiprotozoal, and antihypertensive medications. Imidazole is part of the theophylline molecule, found in tea leaves and coffee beans, which stimulates the central nervous system. It is present in the anticancer medication mercaptopurine, which used in leukemia by interfering with DNA activities.

MATERIAL AND METHOD: Melting points of the synthesized compound was determined on melting point apparatus and are uncorrected. IR spectra of synthesized compound were determined on FTIR at Bombay College of Pharmacy, Mumbai.

^{1}HNMR were taken on progress and purity of the reaction and the intermediate were analyzed using precoated TLC plates and spots were detected by UV light.

EXPERIMENTAL METHOD:

STEP 1:

Synthesis of 2, 4, 5- triphenyl imidazole: It was synthesized by refluxing benzil, benzaldehyde with ammonium acetate in acetic acid medium at 100°C for 5-24 hrs.

STEP 2:

Synthesis of acid chloride: It was synthesized by refluxing acid with excess of thionyl chloride at 80°C for 2-3 hrs. Excess thionyl chloride was removed by distillation.

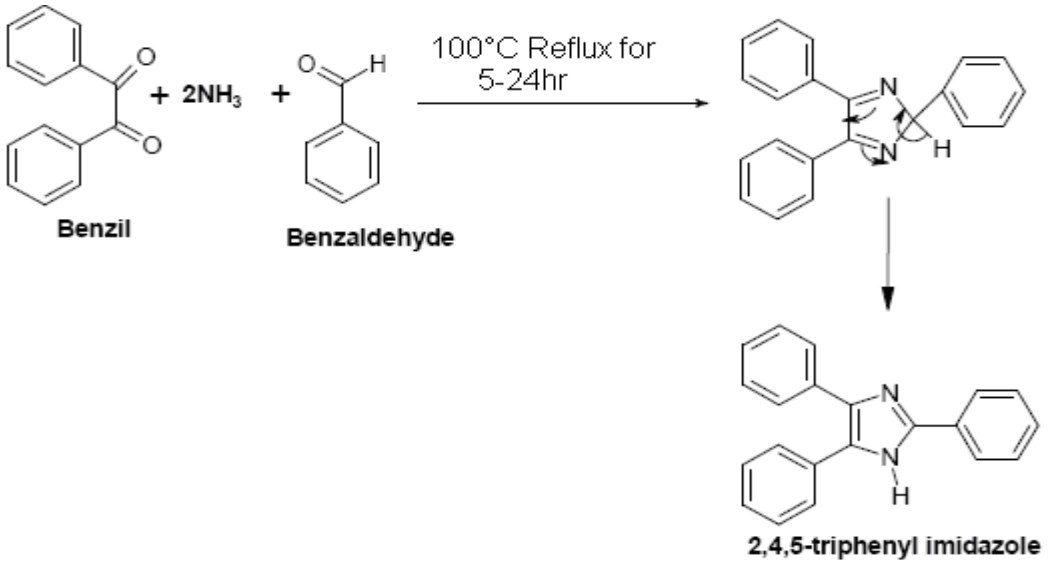
STEP 3:

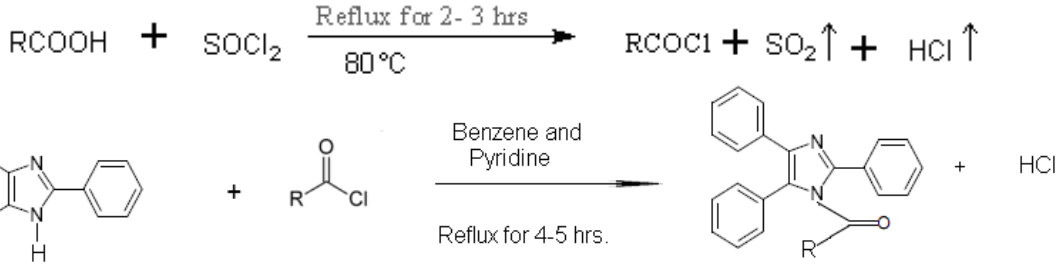
Synthesis of 1-substituted 2, 4, 5 triphenyl imidazoles: It was synthesized by refluxing 2,4,5-triphenyl imidazole and acid chloride by using benzene as solvent and pyridine as a catalyst for 4-5 hrs. The product was isolated, dried and recrystallized from ethanol.

Qualitative analysis of the synthesized compounds was done by using

1. TLC
2. Melting point
3. IR spectroscopy
4. NMR spectroscopy

Biological evaluation of synthesized compounds was done by performing antibacterial, antifungal, analgesic and anti-inflammatory activity.

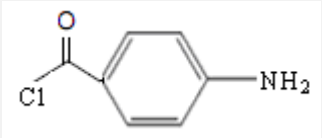
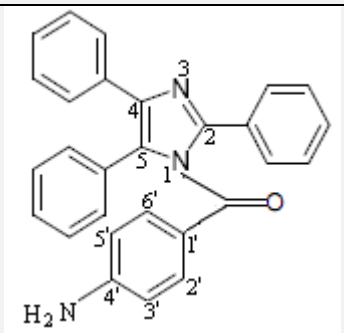
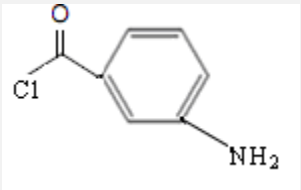
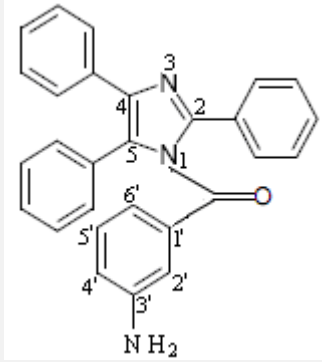


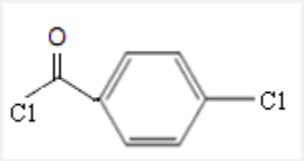
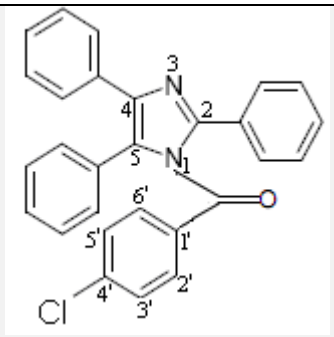
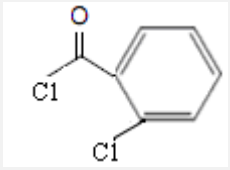
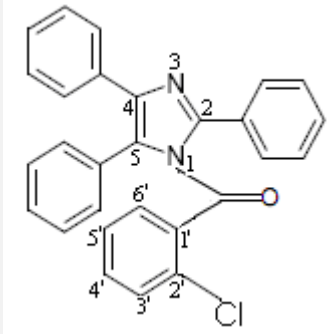
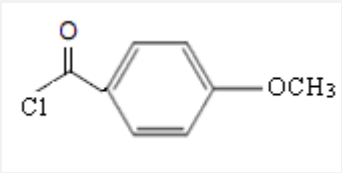
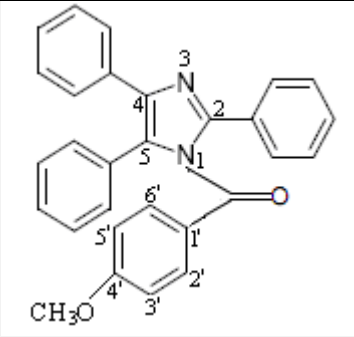


2, 4, 5- triphenylimidazole acid chloride 1-substituted 2, 4, 5- triphenylimidazole

SCHEME:

TABLE 1: REACTANT AND PRODUCT FORM IN THE REACTION.

Sr. no	R	Product
1.	 p- Aminobenzoyl chloride	 1- (4'-Aminobenzoyl)- 2, 4, 5- triphenylimidazole
2.	 m- Aminobenzoyl chloride	 1- (3'-Aminobenzoyl)- 2, 4, 5- triphenylimidazole

3.	 p- Chlorobenzoyl chloride	 1- (4'-Chlorobenzoyl)- 2, 4, 5- triphenylimidazole
4.	 o- Chlorobenzoyl chloride	 1- (2'-Chlorobenzoyl)- 2, 4, 5- triphenylimidazole
5.	 p- Methoxybenzoyl chloride	 1- (4'-Methoxybenzoyl)- 2, 4, 5- triphenylimidazole

SCHEME:

Animal Used: The anti-inflammatory activity was performed on Wistar rats of either sex, weighing between 150- 200 g and the acute oral toxicity studies as well as analgesic activity was performed on Swiss albino mice, of either sex, weighing between 25- 30 g. All the animals were purchased from Haffkine Biopharmaceuticals Ltd., Mumbai, India.

The animals were maintained at 25 ± 2 °C, 50 ± 5 % relative humidity and 12 h light/dark cycle. The animals were fasted for 24 h prior to the experiments and water provided *ad libitum*. The animal study protocols were approved by the Institutional Animal Ethics Committee of C. U. Shah College of Pharmacy, Santacruz (W), Mumbai, India.

An acute Pharmacological toxicity study: An acute toxicity study was performed as per the Organization for Economic Co-operation and Development (OECD) guidelines (OECD Guidelines for the Testing of Chemicals Test No. 423, 2008). Before experimentation, the animals were divided into two groups, each group consisting of six animals. The first group received orally, a single dose of 10 ml/kg body weight of a control (1 % w/v Sodium carboxymethyl cellulose suspension) and was considered as the *negative control group*.

The test compounds, at different dose levels like 500, 1000 and 2000 mg/kg body weighed, were administered orally to the animals present in the *test groups*. After the administration of the test compounds, animals were observed for a period of 14 days for the changes in the skin, eyes and behavioral pattern. Mortality of mice in each group was also observed. A dose leading to these changes or mortality was considered to be a toxic dose [3].

Anti-Inflammatory Activity: The animals were fasted for 24 hrs and divided into a *negative control group* (vehicle), a *positive control group* (indomethacin) and *test groups* (synthesized compounds), each group consisting of six animals. Negative control group received 1 ml of 1 % Sodium carboxymethyl cellulose (Sodium CMC), positive control group received 50 mg/kg body weight of indomethacin and the test groups received 200 mg/kg body weight of the synthesized compounds suspended in 0.5 % sodium CMC. The rats were dosed orally, 30 min prior to 0.1 ml sterile carrageenan (1 % solution) injection in the sub-planter region of the right hind paw of the rat. Carrageenan caused visible redness and pronounced swelling in the rat paw. The paw was marked with ink at the level of the lateral malleolus. The paw volume was measured plethysmographically, immediately after the injection and again at 1, 2, 3, 4 hr after carrageenan challenge.

Antibacterial Activity

Antimicrobial activity of the synthesized compounds was tested against

I. **Gram positive bacteria**

1. *Staphylococcus aureus* (ATCC 25923)
2. *Bacillus subtillus* (ATCC 6633)

II. **Gram negative bacteria**

1. *Escherichia coli* (ATCC 2592)
2. *Klebsiella pneumoniae* (ATCC 13883)

Antimicrobial testing was done by two methods:

1. Microbial sensitivity testing by ditch plate method
2. Microbial inhibition testing by agar cup plate method

Ditch plate method: The sterile nutrient agar (30ml) was poured into a sterile Petri plate and allowed to solidify. A ditch (2x 5 cm) was cut aseptically into the plates by means of a sterile scalpel. A loopful of each inoculum was streaked on the agar surface, outwards from the ditch. A ditch was filled with the solution of a test compound and the Petri plate was then incubated at 37 ˚C for the bacterial cultures and at 25 ˚C for the fungal cultures. The plate was observed after 24 hrs for the inhibition of any bacteria and after 48 hrs for the inhibition of fungi. The bacteria and fungi, which did not grow in presence of the synthesized compounds, were selected for further studies. All the synthesized compounds were tested by the same procedure by using the concentrations of 100, 250, 500 and 1000 µg/ml.

Cup plate method: The sterile molten agar (30ml) was allowed to cool to 40 ˚C. Inoculum (1 ml) was added to the sterile molten nutrient agar, mixed well, poured into the sterile petri plates and allowed to solidify. A sterile cork borer was used to bore wells of 10 mm diameter in the petri plates. The solution of compounds (1 ml) was poured into the wells and the plates were incubated at 37 ˚C for 24 hrs for the bacterial culture and at 25 ˚C for 48 hrs for the fungal cultures. The antibacterial studies were carried out at four levels of concentrations, 100, 250, 500 and 1000µg/ml. The zones of inhibition against each bacterium at four different concentrations of each compound were measured. The zones of inhibition were compared with the standard (100-1000µg/ml) or a positive control (sulfamethoxazole or miconazole).

RESULT AND DISCUSSION: Five derivatives of 1-substituted 2, 4, 5- triphenylimidazole were synthesized using simple reaction conditions and easily available reagent and solvents. These compounds were purified by recrystallization. All the derivatives were obtained in good yields. The purity of the products was checked by recording their melting points, yields and the Rf values from the thin layer chromatography. **Table 2** shows the melting point, yields and Rf values of all the synthesized compounds. The structures of the compounds were characterized by recording their infrared (IR) spectra and nuclear magnetic resonance (^{1}H NMR) spectra.

The anti-inflammatory activity of all the test compounds and indomethacin was evaluated using the carrageenan induced rat paw edema model, reported by Winter *et al*. The results were expressed as the mean ± S.E.M of the difference between the paw volume of the rats in the negative control (vehicle treated) and the test compounds (treated groups). The results were tested statistically by one way ANOVA method and are

shown in **Table 3** and % inhibition of inflammation in rat paw in **table 4**.

The zones of inhibition observed for the test compounds were compared with the zones of inhibition for the standard antibacterial drugs. Compounds showed good activity, which was comparable with the standard drugs.

Table 5 summarizes the antimicrobial activity of all the synthesized compounds at 250µg/ml concentrations respectively.

TABLE 2: PHYSICAL DATA OF SYNTHESIZED COMPOUND

Compound	R_f (chloroform: methanol) (6:4)	Melting point (˚C)	%Yield
1.	0.83	115- 117	85.91%
2.	0.91	80- 90	87.95%
3.	0.88	140- 143	68.24%
4.	0.75	75- 78	45.20%
5.	0.77	150- 152	82.36%

TABLE 3: ANTI INFLAMMATORY ACTIVITY OF SYNTHESIZED COMPOUND

Compound	Mean reduction in paw volume (ml) after treatment with test compounds (mean ± SEM)			
	1hr	2hr	3hr	4hr
1.	1.23 ± 0.072	0.893±0.067	0.290±0.185	0.107±0.081
2.	0.263±0.051	0.597±0.237	0.392±0.075	0.008±0.074
3.	0.497±0.093	0.048±0.060	0.755±0.138	0.292±0.072
4.	1.257±0.123	0.013±0.085	0.088±0.114	0.197±0.050
5.	0.368±0.114	0.275±0.031	0.300±0.059	0.023±0.099
Indomethacin (+ve control)	0.362±0.114	0.762±0.150	0.037±0.102	0.528±0.202
-ve control	1.062±0.142	1.128±0.126	0.967±0.175	0.788±0.168

Data analyzed by one way ANOVA followed by Dunnett's test. Significant at $P \leq 0.05$

TABLE 4: ANTI INFLAMMATORY ACTIVITY OF SYNTHESIZED COMPOUND

Compound no.	Anti- inflammatory activity (% Inhibition)			
	1hr	2hr	3hr	4hr
1.	16.03	19.29	23.49*	41.37*
2.	25.16*	36.98*	60.00*	64.97*
3.	52.83*	64.88*	68.73	74.36*
4.	18.86	43.83*	63.17*	75.12*
5.	55.09*	61.03*	64.76*	72.33*
Indomethacin (+ve control)	15.09*	20.09	45.71*	70.30*
-ve control	-	-	-	-

*P < 0.05 significant from control; ns, not significant.

TABLE 5: ANTIMICROBIAL ACTIVITY OF SYNTHESIZED COMPOUND

Compound No.	Zone of inhibition (in mm) at 250 µg/ml					
	Bacterial species				Fungal species	
	E. coli	*S. aureus*	*B. subtillus*	*K. pneumoniae*	*C. albicans*	*A. niger*
1	07	08	07	03	12	08
2	06	05	05	04	10	08
3	07	07	08	05	8	07
4	05	10	08	04	14	08
5	09	08	05	04	19	09
Sulfamethoxazole (250 µg/ml)	13	12	09	12	-	-
Miconazole (250 µg/ml)	-	-	-	-	22	10

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