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Microwave Assisted Parallel Synthesis of 1,4,5-Tri Substituted Imidazoles and their Pharmacological Evaluation

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ABSTRACT

Among the advantages gained by employing microwave organic synthesis, most important one is the dramatic reduction in reaction times. In the present study various tri substituted imidazoles are synthesized using synthetic microwave oven, which showed significant reduction in reaction time, increased yield and synthesis of library of compounds in a very short time. Following microwave assisted parallel synthesis, six compounds were synthesized using six different amines and the yield was found to be 60-70%. The reactions were carried out in millimolar scale for optimization of reaction conditions and in molar scale for the bulk syntheses of the compounds. The synthesized compounds were characterized by Mass, NMR, IR and TLC. The activity check was performed in the area of Pharmacological evaluation, wherein synthesized compounds were screened for anti-inflammatory activity by rat paw edema method and they showed good activity when given orally.

Key words: Microwave, Trisubstituted, Imidazole, Antiinflammatory

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INTRODUCTION

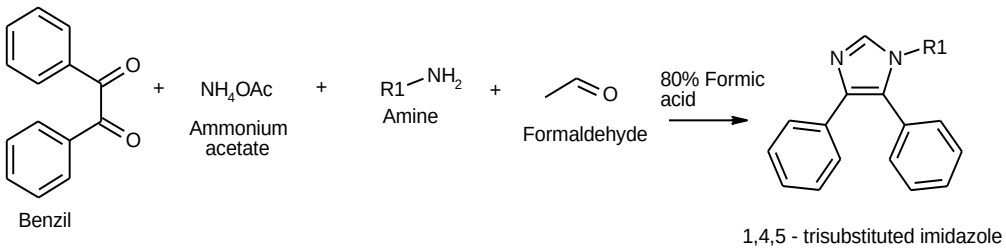
Rational drug designing along with combinatorial chemistry has made possible rapid lead identification and lead optimization. Microwave chemistry with all its advantages when combined with combinatorial chemistry eases the bottleneck in the drug discovery process. For a drug discovery process one among the many requirements from the medicinal chemist's point of view, is high throughput synthetic methods that enable production of large compound libraries in a short time. Substituted imidazoles are one such library of compounds that is being extensively worked on.

Literature findings, especially on its analgesic, anti-inflammatory and antifungal activities and the continued interest in the imidazole molecule after its discovery more than century ago have prompted the synthesis of similar compounds and screen them for their biological activity [1–7]. The microwave reactions can be carried out in high boiling solvents (Dimethylformamide). Heating is fast, but maximum temperatures are chosen below the boiling point of the solvent in order to avoid solvent evaporation. Thus, one could work in open reaction vessels, could choose small amount of solvent when targeting for solubility at the reaction temperature. This method is cost effective (only simple glass ware needed) and environmentally friendly (less solvent needed). Owing to the advantages of microwave assisted organic synthesis, our aim is to synthesize libraries of substituted imidazoles using parallel synthetic procedure in a short time with high purity and high yield [8 – 10].

EXPERIMENTAL

This reaction is a single step four component reaction wherein the imidazole ring is formed.

Scheme -1



The reactants, 0.210g (1 millimole) of benzil, 0.076g(1 millimole) of ammonium acetate, 0.050g(1 millimole) of paraformaldehyde, 2-3 drops of 80% formic acid and 2 millimoles of amine were taken in 1 mL dry DMF in a long necked glass vial. The reaction mixture was irradiated in synthetic microwave oven (Emry's optimizer). The reaction conditions were optimized in Emry's optimizer as Irradiation time: 8 minutes, Temperature: 120 °C, Pre-stirring time: 60 seconds, Absorption: Normal, Pressure: 19 bars.

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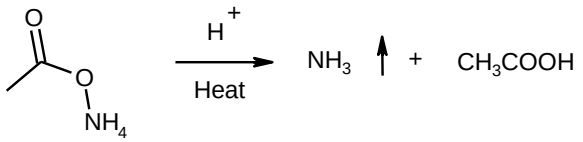
The progress of the reaction was monitored by TLC, using 10% methanol in chloroform as the mobile phase. The reaction mixture was allowed to come back to room temperature and 2g of crushed ice was added. This mixture was then stirred continuously as the sticky solid precipitates out. After allowing standing for one hour, this mixture was extracted with ethyl acetate and organic layer was evaporated using high vacuum rotary evaporator. The residue was recrystallized with aqueous ethanol.

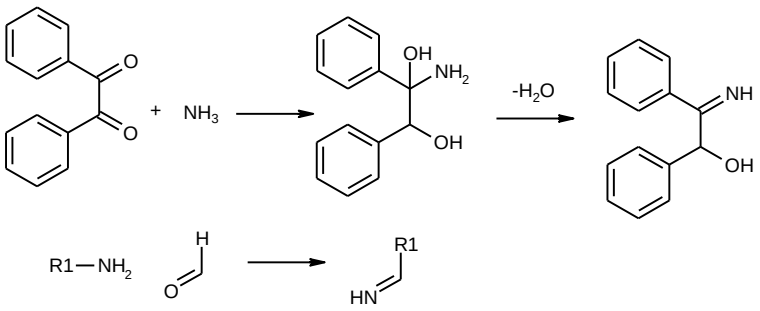
Six different amines were used for the synthesis. The detailed description of amines (name, amount taken) is tabulated in **Table 1**.

PROPOSED MECHANISM

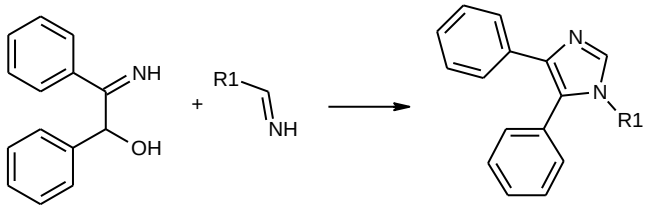
Step 1: Release of ammonia from ammonium acetate



Step 2: Formation of imine



Step 3: Cyclization



Ammonia is released from ammonium acetate when heated in acidic conditions (acidic silica was used). One molecule of ammonia reacts with aldehyde to form imine; other molecule of ammonia reacts with carboxyl group of benzil forming imine. The aldehydic nitrogen (C=NH) has greater reactivity towards C=O group of benzil, whereas imine of benzil reacts with reactive

Methylene group (CH=NH) of aldehyde, loosing one molecule of water which results in cyclization to form imidazole ring.

SCREENING FOR ANTI-INFLAMMATORY ACTIVITY

The synthesized compounds were randomly screened for Anti-inflammatory activity by Formalin induced rat paw edema model [11 – 12].

The drugs were prepared as a suspension using 0.3% sodium carboxy methylcellulose (Na CMC). Standard drug, Rofecoxib 25mg/kg was used for comparison with the synthesized compounds.

Drugs and vehicle were administered orally one hour before the induction of inflammation. Albino Rats were given the drug suspension orally and after one hour, the rats were challenged by a subcutaneous injection of 0.1 mL of 1% v/v formalin solution into the plantar region of left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediately after injection and every hour for four hours. The change in the mercury level was measured by traveling microscope as change in height (in cm). This value obtained was converted into change in volume (in mL) by interpolating them on plethysmograph calibration curve.

Statistical evaluation

The increase of paw volume (in mL) after every hour was determined and compared with the paw volume measured immediately after injection of the irritant. Treated groups showed less edema when compared to vehicle control group. The difference in mean values between treated and control groups were compared by Two-Way ANOVA and unpaired student t-test. The p value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Here we have standardized methods for fast synthesis of Imidazole derivatives under Microwave reaction conditions. Following microwave assisted parallel synthesis, six compounds were synthesized using six different amines. The yield was found to be 60-70% **(Table 1).** More than 20 amines were tested for the synthesis of 20 different 1,4,5-tri substituted imidazoles, but it was found that simple amines like substituted anilines and amines having alkyl chain alone were successful. The reason may be the steric hindrance due to bulky groups or highly electronegative groups on the carbon adjacent to the carbon bearing amine.

TABLE 1: 1-Substituted-4,5-Diphenyl Imidazole Derivatives

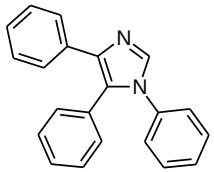
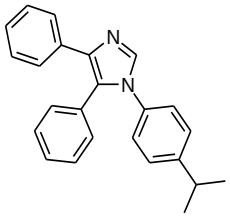
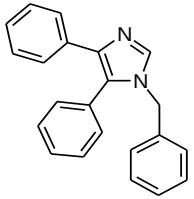
S.No.	Amines	Amount Taken (g or mL)	Compound /Lab Code	Mass	NMR	HPLC Purity %	M.Pt. (°C)	Solubility
1	Aniline	0.18	 Tri-imid-1	296	*HNMR (CDCl3)* = 7.2, 7.3, 7.4, 7.5, 7.6 (m,15H)	92	174	Insoluble in water, ethanol; soluble in methanol
2	4-Isopropyl aniline	0.27	 Tri-imid-4	338	*HNMR (CDCl3)* = 1.2 (d, 6H); 2.9(septet,1H); 7.05(d,2H); 7.1, 7.2, 7.3, 7.5, 7.8 (m,14H)	100	178	Insoluble in water, ethanol; soluble in methanol, chloroform
3	Benzyl amine	0.21	 Tri-imid-8	310	*HNMR (CD3OD)* 5.2 (s, 2H); 7.18(d,2H); 7.2, 7.3, 7.4, 7.5 (m, 15H)	65	160	Insoluble in water, ethanol; sparingly soluble in acetone; soluble in methanol

Table 1 Contd…

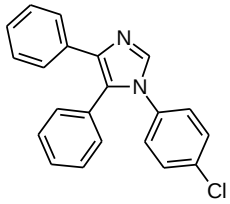
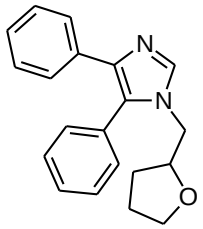
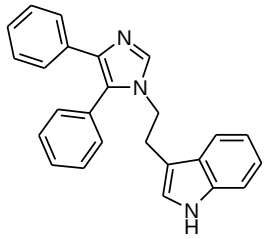
4	4-Chloro aniline	0.25	 Tri-imid-9	331	*HNMR (CDCl3)* = 7.06, 7.1, 7.2, 7.3, 7.5, 7.7(m,15H)	78	191-196	Insoluble in water, ethanol; soluble in acetone, chloroform
5	1(H)-Furfuryl amine	0.2	 Tri-imid-18	304	*HNMR (CD3OD)* = 1.6(m,1H); 1.8(m,3H); 3.8(m,2H); 3.9(m,3H); 7.1, 7.2, 7.3, 7.4, 7.5, 7.8(multiplets,11H)	70	158-160	Insoluble in water,ethanol; soluble in methanol, chloroform
6	Tryptamine	0.32	 Tri-imid-19	363	*HNMR (CD3OD*) = 2.9(t,2H); 4.1(t,2H); 6.9, 7.1, 7.2, 7.3, 7.4, 7.6(m,14H)	89	188-190	Insoluble in water, ethanol; soluble in methanol.

Table 2: Anti-inflammatory Activity of synthesized compounds by Rat paw edema method								
Treatment Group	**Increase in Paw volume (mL) (Mean ± SD)**				**% Inhibition of Edema (%)**			
	1st Hour	**2nd Hour**	**3rd Hour**	**4th Hour**	**1st Hour**	**2nd Hour**	**3rd Hour**	**4th Hour**
Vehicle Control	0.354 ± 0.025	0.439 ± 0.027	0.462 ± 0.037	0.568 ± 0.033	-	-	-	-
Rofecoxib (25mg/kg)	0.098 ± 0.025	0.043 ± 0.023	0.026 ± 0.014	0.005 ± 0.003	72.32	90.21	94.37	99.12
Tri-imid-1	0.086 ± 0.013	0.032 ± 0.013	0.035 ± 0.016	0.064 ± 0.023	75.71	92.71	92.42	88.73
Tri-imid-4	0.058 ± 0.026	0.095 ± 0.040	0.049 ± 0.023	0.009 ± 0.004	83.62	78.36	89.39	98.42
Tri-imid-8	0.102 ± 0.019	0.114 ± 0.032	0.028 ± 0.013	0.025 ± 0.024	71.19	74.03	93.94	95.60
Tri-imid-9	0.041 ± 0.018	0.045 ± 0.026	0.028 ± 0.017	0.072 ± 0.032	88.42	89.75	93.94	87.32
Tri-imid-18	0.136 ± 0.015	0.204 ± 0.016	0.199 ± 0.023	0.204 ± 0.027	61.58	53.53	56.93	64.08
Tri-imid-19	0.159 ± 0.029	0.181 ± 0.029	0.193 ± 0.045	0.172 ± 0.048	55.08	58.77	58.26	69.72

All the six synthesized compounds screened for anti-inflammatory activity showed significant activity by reducing the increase in paw volume using unpaired student t-test, $p < 0.001$.
(Table 2).

CONCLUSION

The synthesis of imidazole by the conventional route takes longer time and tedious work-up. Highly promising series of 1,4,5-trisubstituted imidazoles are synthesized using Microwave induced reactions in synthetic microwave oven. These compounds can be synthesized in high purity and high yield and with diverse substitutions.

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