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Parallel Synthesis of Tetra Substituted Imidazoles by Microwave Irradiation and Evaluation of their Anti-inflammatory Activity

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ABSTRACT

Green chemistry is the design of chemical products and processes that reduce or eliminate the use and/or generation of hazardous substances. Imidazoles are one such library of compounds that is being extensively worked on. In the present study various tetra substituted imidazoles are synthesized using domestic microwave oven which showed significant reduction in reaction time, increased yield and very short time for the synthesis of library of compounds. All the compounds are synthesized by microwave assisted parallel synthetic method in solid phase. These multi component, one pot 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 and were screened for anti-inflammatory activity by rat paw edema method. Some of these compounds showed anti-inflammatory activity comparable to that of the market standard.
Keywords: Imidazole, Microwave, anti-inflammatory, tetrasubstituted

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INTRODUCTION

Microwave irradiation is becoming an increasingly popular method of heating samples in the laboratory. It offers a clean, cheap, and convenient method of heating, which often results in higher yields and shorter reaction times.

Avoiding organic solvent during the reactions in organic synthesis leads to a clean, efficient and economical technology (green chemistry); safety is largely increased, work up is considerably simplified, cost is reduced, increased amounts of reactants can be used in the same equipment, the reactivities and sometimes selectivities are enhanced without dilution. In reactions absorbed on solid supports, the microwave-absorbing solid support e.g. alumina, silica, etc., is added to a solution of the reactants in a volatile solvent such as methanol or acetone. After thorough mixing, the absorbed material is allowed to dry by evaporation of the solvent and is then irradiated with microwaves. On completion of the reaction, the product is extracted with an appropriate solvent.

The recent interest in the old molecule, imidazole is because of newer application and putting them through newer assays for the activities. It is evident from the recent Patent applications [1 - 4] and publications related to imidazoles about its vitality [5-9].

Libraries of substituted imidazoles are synthesized using parallel synthetic procedure in a short time with high purity and high yield using the method of microwave induced organic reaction.

EXPERIMENTAL

All the compounds were synthesized by microwave assisted parallel synthetic method in solid phase.

One pot synthesis of tetra-substituted imidazoles is a single step four-component condensation reaction wherein the cyclization occurs to form imidazole ring [10 - 11]. 0.210g (1 millimole) of benzil, 0.076g (1 millimole) of ammonium acetate, aldehydes (1 millimole) and amine (1 millimole) were triturated with 1g of silica and this reaction mixture was taken in a long necked glass vial irradiated in domestic microwave oven for 6 minutes following the cycle of 30 seconds heating and 10 seconds cooling. The progress of the reaction was monitored by TLC using 15% ethyl acetate in hexane as the mobile phase. The reaction mixture was extracted with chloroform 3-4 times and the organic layer was evaporated to dryness using rotary evaporator. The residue obtained was recrystallized using aqueous ethanol.

In microwave assisted parallel synthesis, different aldehydes and different amines can be used to synthesize a library of substituted tetra aryl imidazoles [12,13]. Various synthetic methods followed for the synthesis of polyaryl imidazoles or 1, 2,4,5-Tetra-substituted imidazoles are enumerated below.

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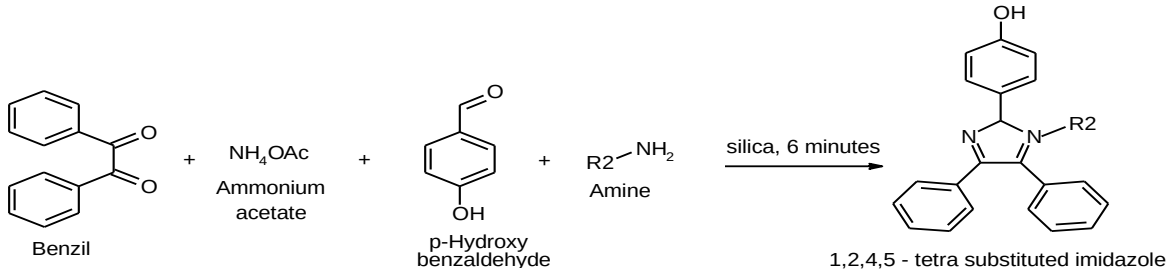
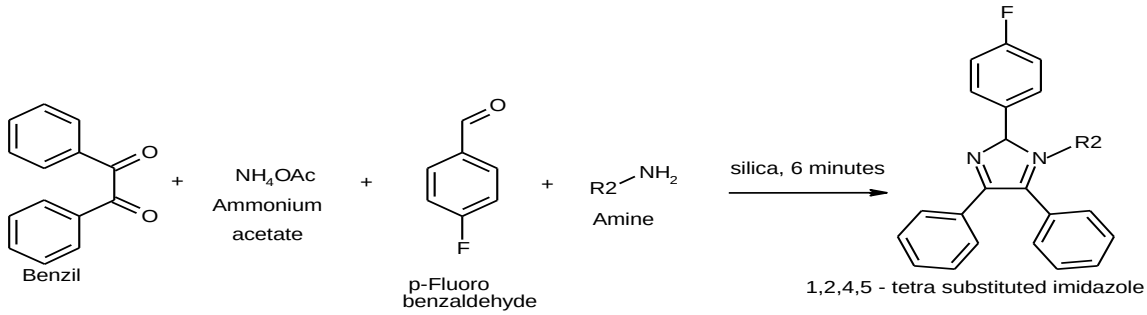
Scheme A: Synthesis of N-substituted 4,5 diphenyl-2-(4-hydroxyphenyl)imidazole derivatives **(Illustration 1).** Five different amines were used with p-Hydroxy benzaldehyde (0.122g, 1Mm) to produce a library of tetra substituted imidazoles. The detailed description of amines used (name, amount taken) is tabulated in **Table 1.**

Illustration 1



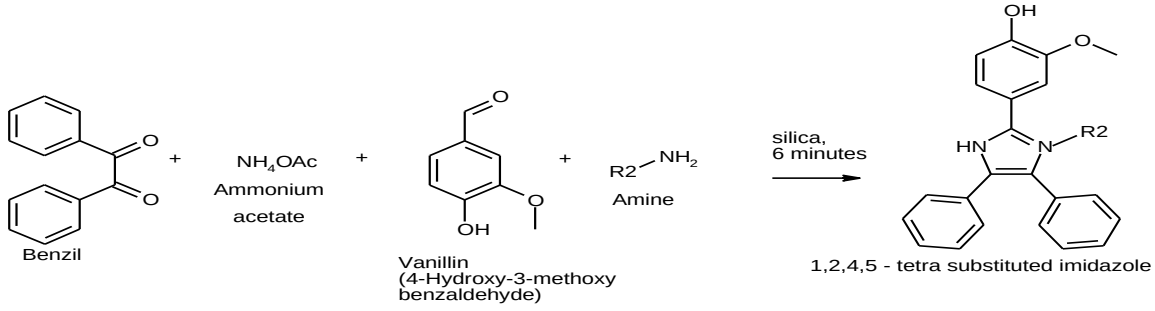
Scheme B: Synthesis of N-substituted 4,5 diphenyl-2-(4-fluorophenyl) imidazole derivatives **(Illustration 2).** Two different amines were used with p-Fluoro benzaldehyde (0.124g, 1 millimole) to synthesize a library of tetra substituted imidazoles. The detailed description of amines used (name, amount taken) is tabulated in **Table 2.**

Illustration 2



Scheme C: Synthesis of N-substituted 4,5 diphenyl-2-(4-hydroxy-3- Methoxy phenyl) imidazole derivatives **(Illustration 3).** Seventeen different amines were used with vanillin (0.152g, 1 millimole), to synthesize a library of tetra substituted imidazoles. The detailed description of the amines used (name, amount taken) is tabulated in **Table 3.**

Illustration 3



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SCREENING FOR ANTIINFLAMMATORY ACTIVITY

The synthesized compounds were screened by Formalin induced rat paw edema model [14-16]Experiments were carried out on female Wistar rats of albino strain. The rats were kept 3 to 5 per cage. They were fed with standard pellet diet and water *ad libitum.* They were kept in a well-aerated room and a 12-hour light and dark cycle was maintained. The room temperature was maintained at 22±2°C.

The drugs were prepared as a suspension using 0.3% sodium carboxy methylcellulose (Na CMC). Standard drug, Rofecoxib 2mg/kg (human single dose 25mg) was used as positive control. Since this dose did not give good results, a higher dose of Rofecoxib 25mg/kg was used for comparison with the synthesized compounds.

Drugs and vehicle were administered orally one hour before the induction of inflammation. 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. 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 calculated and compared with the paw volume measured immediately after injection of the irritant. Treated groups show 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. p value < 0.05 was considered significant.

RESULTS AND DISCUSSION

The synthesis of imidazole by the conventional route takes longer time and tedious work-up. Here we have standardized methods for fast synthesis of Imidazole derivatives under Microwave reaction conditions. Conventional method for the synthesis of 1,2,4,5-tetra-substituted imidazoles requires refluxing for 2 hours. These compounds can be synthesized in domestic microwave oven in 6 minutes. We have employed the same reaction conditions and have synthesized a library of 24 compounds with diverse substitutions. Several complex amines were used for the substitution at position 1 of imidazole ring. It was observed that the simple substituted anilines and alkylamines give higher yields than the complex heterocyclic amines. Among them 4-substituted anilines gave maximum yield and required minimum purification.
Following microwave assisted parallel synthesis five compounds were synthesized using five different amines with p-Hydroxy benzaldehyde **(Table 1).** The yield was 80-85%. Two compounds were synthesized using two different amines with p-Fluoro benzaldehyde **(Table 2).**

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Table-1: N-substituted 4,5 diphenyl-2-(4-hydroxyphenyl) imidazole derivatives synthesized

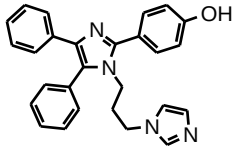
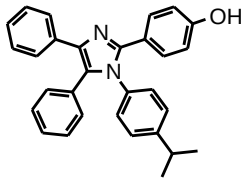
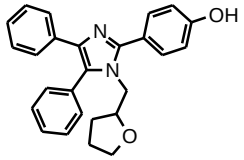
S.No.	Amines	Amount Taken (g or mL)	Compound/Lab Code	Mass	*NMR*	HPLC Purity	M.Pt.	Solubility
1	3(1-imidazolyl) propylamine	0.12	 B1	420	*HNMR (CD3OD)* = 1.8(m,2H); 3.7(t,2H); 3.7(t,2H); 6.9, 7.1, 7.2, 7.4,7.5 (m,16H)	100	239	Insoluble in water,ethanol; soluble in methanol.
2	4-Isopropyl amine	0.13	 B2	430	*HNMR (CDCl3)* = 1.2(d,6H); 2.9(septet,1H); 6.9,7.1,7.2,7.6 (m,19H)	100	261	Insoluble in water, ethanol;soluble in chloroform, methanol
3	2-Furfuryl amine	0.1	 B5	396	*HNMR (CD3OD)* = 1.2(m,1H); 1.7(m,3H); 3.4(m,1H); 3.8(m,1H); 4.0(t,2H); 6.9, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6(m,14H)	100	236-238	Insoluble in water, ethanol,methanol; soluble in acetone.

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Table 1 Contd…

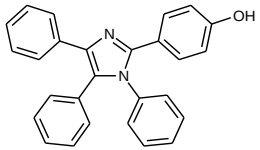
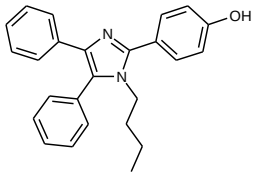
4	Aniline	0.09	 B6	388	*HNMR (CD3OD)* = 6.9, 7.2, 7.3, 7.4, 7.9 (m, 19H)	100	284	Insoluble in water, ethanol,methanol; soluble in chloroform, acetone
5	n-Butyl amine	0.07	 B7	368	*HNMR (CD3OD)* = 0.6(t,3H); 0.9(m,2H); 1.3(m,2H); 3.9(m,2H); 6.9, 7.0, 7.1, 7.2, 7.4, 7.5 (m,14H)	88.5	196	Insoluble in water, ethanol;soluble in chloroform, acetone, methanol

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Table-2: N-substituted 4,5 diphenyl-2-(4-fluorophenyl) imidazole derivatives synthesized

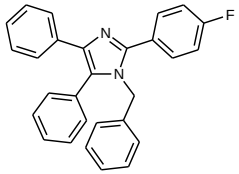
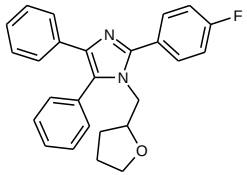
S.No.	Amines used	Amount Taken (g or mL)	Compound/ Lab Code	Mass	NMR	HPLCPurity	M.Pt.	Solubility
1	Benzy amine	0.11	 F1	404	*HNMR (CDCl3)* = 5.08 (s,2H); 7.05(d,2H); 6.8(d,2H); 7.1, 7.2, 7.3, 7.5, 7.6 (m,19H)	54	189	Insoluble in water, soluble in chloroform, acetone, methanol, ethanol
2	2-Furfuryl amine	0.1	 F3	397	*HNMR (CD3OD)* = 1.2 (m,1H); 1.7(m,3H); 3.4(m,2H); 3.8(m,1H); 3.9(m,2H); 6.9, 7.1, 7.2, 7.4, 7.5, 7.7(m,14H)	97.2	143.5-144	Insoluble in water, ethanol; soluble in methanol

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Table-3: N-substituted 4,5 diphenyl-2-(4-hydroxy-3-methoxy phenyl) imidazole derivatives synthesized

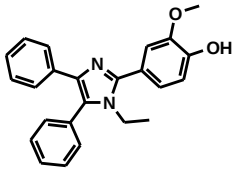
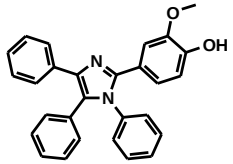
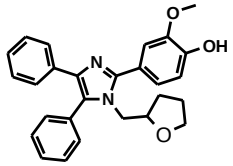
S. No	Amines used	Amount Taken (g or mL)	Compound/ Lab Code	Mass	NMR	HPLC Purity (%)	M.Pt. (°C)	Solubility
1	Ethyl amine	0.045	 T-IMID-9	370	*HNMR ($CDCl_3$)* = 1.02(t,3H); 3.95(q,2H); 3.98(s ,3H); 7.1, 7.2, 7.4, 7.5(m,13H)	97.7	191-193.5	Insoluble in water, ethanol; soluble in methanol, acetone.
2	Aniline	0.09	 T-IMID-1	418	*HNMR (CD_3COCD_3)* = 3.6(s,3H); 6.9, 7.2, 7.3, 7.4, 7.9(m,18H)	100	208-209.5	Insoluble in water, ethanol; sparingly soluble in methanol; soluble in acetone, chloroform
3	2-Furfuryl amine	0.1	 T-IMID-2	424	*HNMR ($CDCl_3$)* = 1.3(s,1H); 1.6(m,4H); 3.3(m,1H); 3.8(m,1H); 3.9(m,2H); 4.0(s,3H); 7.1, 7.2, 7.3, 7.4, 7.5(m,13H)	90	189-190	Insoluble in water, methanol, acetone, soluble in chloroform

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Table 3 Contd..

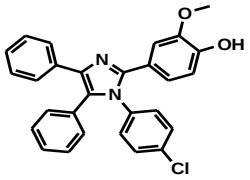
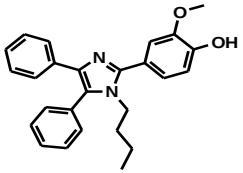
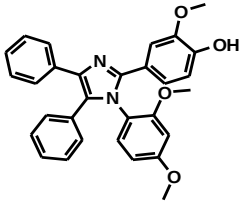
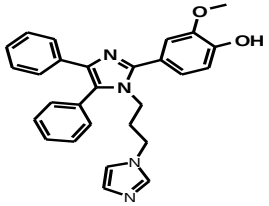
4	4-Chloro aniline	0.13	 T-IMID-13	453	*HNMR ($CDCl_3$)* = 3.78(s,3H); 4.1(d,2H); 6.7,7.2(m,17H)	97.7	191.4-194	Insoluble in water; sparingly soluble in ethanol; soluble in methanol, acetone, chloroform
5	n-Butyl amine	0.07	 T-IMID-19	398	*HNMR ($CDCl_3$)* = 0.6(t,3H);0.9(m,2H); 1.3(m,2H); 3.8(m,2H); 3.98(s,3H); 7.0, 7.1, 7.2, 7.4, 7.5(m,13H)	100	187.7-189	Insoluble in water, ethanol; sparingly soluble in methanol, acetone.
6	2,4-Dimethoxy niline	0.15	 T-IMID-16	479	*HNMR ($CDCl_3$)* = 3.5(s,3H); 3.78(s,6H); 6.3, 7.1, 7.2(m,16H)	98.8	254-255.5	Insoluble in water, ethanol, methanol, acetone, acetonitrile; soluble in DMSO, chloroform
7	3-(1-imidazolyl) propyl amine	0.12	 T-IMID-10	450	*HNMR ($CDCl_3$)* = 1.9(m,2H); 3.6(t, 2H); 3.8(t,2H); 3.9(s,4H); 7.2, 7.5(m,16H)	79	220-221	Insoluble in water; soluble in methanol, ethanol, chloroform; sparingly soluble in acetone

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Table 3 Contd..

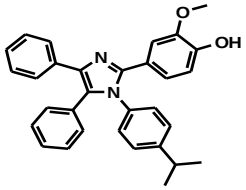
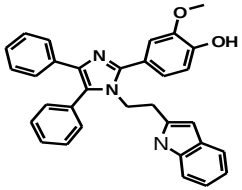
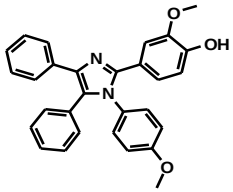
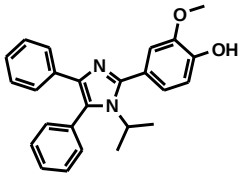
8	4-Isopropyl aniline	0.13	 T-IMID-17	460	*HNMR ($CDCl_3$)* = 1.2(d,6H); 2.9(septet,1H); 3.65(s,3H); 3.78(s,6H); 6.9, 7.1, 7.2(m,17H)	90.8	198-198.4	Insoluble in water; soluble in methanol, acetone, chloroform, ethanol.
9	Tryptamine	0.16	 T-IMID-12	485	*HNMR ($CDCl_3$)* = 2.4(t,2H); 3.89(s,3H); 4.1(t,2H); 7.1,7.2,7.5(m,18H)	85	223-225	Insoluble in water, ethanol, methanol; soluble in acetone, chloroform
10	4-Methoxy aniline	0.12	 T-IMID-14	449	*HNMR ($CDCl_3$)* = 3.78(d,6H); 6.7, 7.2(m,17H)	79	227-228.5	Insoluble in water, ethanol, acetone; soluble in methanol, chloroform
11	Isopropyl amine	0.06	 T-IMID-23	384	*HNMR ($CDCl_3$)* = 1.2(d,6H); 3.8(septet,1H); 4.1 (d,3H); 7.1, 7.2, 7.3, 7.4(m,13H)	71.6	254.7-257	Insoluble in water, ethanol; sparingly soluble in methanol, acetone

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Table 3 Contd..

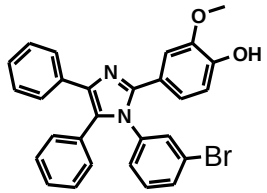
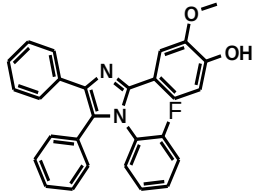
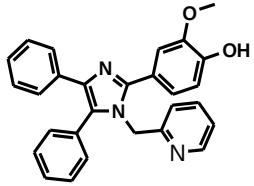
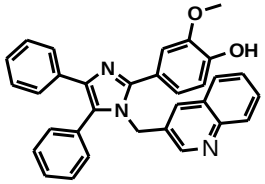
12	3-Bromo aniline	0.17	 T-IMID-21	497	*HNMR ($CDCl_3$)* = 3.78(s,3H); 6.7, 7.2(m,17H)	76	201-203.5	Insoluble in water; soluble in methanol, acetone, ethanol, chloroform
13	2-Fluoro aniline	0.11	 T-IMID-22	436	*HNMR ($CDCl_3$)* = 4.0 (s,3H); 7.2,7.3 (m,17H)	65	254.7-257	Insoluble in water, ethanol; soluble in chloroform, methanol
14	2-Aminomethyl pyridine	0.11	 T-IMID-25	433	*HNMR ($CDCl_3$)* = 3.7 (s,3H); 3.9 (s,2H); 6.2 (s,2H); 7.1, 7.2, 7.3, 7.5 (m,17H)	85	231-232	Insoluble in water, ethanol; soluble in chloroform, methanol
15	3-Aminoquinoline	0.14	 T-IMID-30	469	*HNMR ($CDCl_3$)* = 3.69 (s,3H); 6.6, 7.1, 7.2, 7.6, 8.5 (m,19H)	93	235-240	Insoluble in water, ethanol; soluble in chloroform, methanol

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Table 3 Contd..

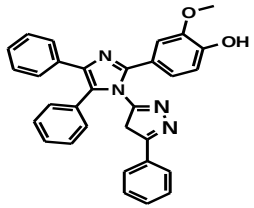
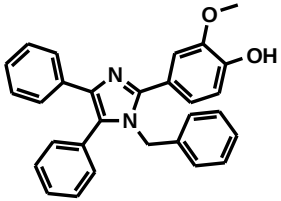
16	3-Amino-5-phenylpyrazole	0.16	 T-IMID-31	484	*HNMR ($CDCl_3$)* = 3.84 (s,3H); 5.2 (s,2H); 6.9, 7.1, 7.2, 7.3, 7.5, 7.6 (m,17H)	100	296-298	Insoluble in water, ethanol; soluble in chloroform, methanol
17	Benzyl amine	0.11	 T-IMID-24	432	*HNMR ($CDCl_3$)* = 3.74 (s,3H); 5.1 (s,2H); 6.9,7.1,7.2,7.3,7.5 (multiplets,18H)	88.8	201.5-202.7	Insoluble in water, ethanol, acetone; soluble in methanol, chloroform

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Table 4. 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
T-imid-9	0.136 ± 0.015	0.204 ± 0.016	0.199 ± 0.023	0.204 ± 0.027	61.58	53.53	56.93	64.08
T-imid-12	0.036 ± 0.019	0.108 ± 0.035	0.029 ± 0.026	0.024 ± 0.019	89.83	75.4	93.72	95.77
T-imid-19	0.159 ± 0.029	0.181 ± 0.029	0.193 ± 0.045	0.172 ± 0.048	55.08	58.77	58.23	69.72
B-1	0.070 ± 0.025	0.104 ± 0.028	0.133 ± 0.024	0.079 ± 0.023	80.23	76.31	71.21	86.09
B-5	0.141 ± 0.047	0.116 ± 0.025	0.109 ± 0.044	0.116 ± 0.031	60.17	73.58	76.41	79.58
F-1	0.218 ± 0.033	0.179 ± 0.046	0.082 ± 0.038	0.064 ± 0.033	38.42	59.23	82.25	88.73

The yield was found to be 75-80%. Seventeen compounds were synthesized using seventeen different amines with vanillin **(Table 3).** The yield was found to be 70-75%.

Six of the synthesized compounds screened for anti-inflammatory activity were found to be active when given orally and significantly reduced the increase in paw volume (unpaired student t-test, $p < 0.001$) **(Table 4)**

CONCLUSION

Microwave accelerated solvent-free reactions are an environment friendly methods which allow minimal solvent waste and energy consumption. One pot method of synthesizing tetra substituted imidazoles is presented with excellent yields, purity and short reaction times. These compounds showed good antiinflammatory activity when given orally and show a great promise for further studies.

REFERENCES

[1] Fitzi K. United States Patent, 1976,3,940,486.
[2] Adams LJ, Boehm CJ, Gallaghar FT. United States Patent, 2000, 6,046,208.
[3] Fitzi K. United States Patent, 1976, 3,940,486.
[4] Lambardino GJ, United States Patent, 1972,3,707,475.
[5] Atwell Gj, Fan JY, Tan K. Denny J Med Chem 1998; 41: 4744 – 54
[6] Baldwin JJ, Christy ME, Denny GH. J Med Chem 1986; 29:1065-80.
[7] Akhavan MM, Mojarrad JS, Rouzrokh A. Farmaco 2003;58: 1193-9
[8] Belgodere E, Bossio R, Parrini V. Arzneimittelforschung 1980, 30: 1051-6
[9] Bender PE, Hill DT, Offen PH. J Med Chem 1985; 28:1169-77.
[10] Balalaie S, Hashemi MM, Akhbari MM. Tetrahydron Letters 2003;44:1709-1711
[11] Balalaie S, Arabanian A. Green Chemistry 2000; 2: 274-276.
[12] Ucucu U, Karaburun GN, Isikdag I. Farmaco 2001;56:285-290.
[13] Isikdag I, Mereic A. Boll Chem Farm 1999;138:24-9
[14] Khanna IK, Yu Y, Huff RM. J Med Chem 2000;43:3168-85.
[15] Roberts LJ, Morrow JD, Goodman &Gillman's The Pharmacological Basis of Therapeutics, 2001, pp. 687-731
[16] Vogel HG, Drug Discovery and Evaluation; Pharmacological Assays, 2002, pp. 759-762.