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Microwave-Assisted, Rapid Synthesis of Benzimidazole based Potential Anticancer Agent Methyl 1-benzyl-2-(4-fluoro-3-nitrophenyl)-1*H*-benzo[*d*]imidazole-5-carboxylate (TJ08) *via* T3P Mediated Cyclization

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A novel microwave assisted protocol for the rapid synthesis of methyl 1-benzyl-2-(4-fluoro-3-nitrophenyl)-1*H*-benzo[*d*]imidazole-5-carboxylate (**TJ08**) with potent antileukemic activity has been developed with excellent yields in 31 min of reaction time over 5 steps, whereas the conventional heating method required around 17 h. In this method, *n*-propanephosphonic acid anhydride (T3P) was used as a coupling reagent for amidation, during this reaction the *in situ* generated byproduct *n*-propylphosphonic acid subsequently catalyzes the cyclization reaction to form benzimidazole ring and hence this novel protocol affords to synthesize the novel benzimidazole derivatives expeditiously to develop new druggable compounds.

Keywords: Benzimidazole derivative, T3P, Anticancer agent, Antileukemic activity.

INTRODUCTION

In recent years, the microwave assisted organic synthesis is one of the methods of choice in the field of drug discovery due to its high efficiency, drastically reduced reactions time, higher products yield, energy efficient and environmentally benign green chemistry method to develop the new drugs required by the society in a short periods of time [1]. This technology opens up new opportunities to the synthetic chemists in the form of new reactions that are not possible using conventional heating [2]. It has enormous applications in other branches of chemistry such as microwave assisted organic synthesis [3-7], spectroscopy [8-10], electrochemistry [11-13], peptide synthesis [14,15], nanotechnology [16], flow chemistry [17], polymer chemistry [18], carbohydrate chemistry [19], combinatorial chemistry [20], process development and scaling up [21,22]. Some of the researchers developed the microwave assisted method for the commercial drugs such as well known anticancer drug imatinib [23].

These microwave reactor applications has inspired us to synthesize bioactive heterocyclic compounds under microwave conditions. Recently, we have synthesized the libraries of designed 1,2,5-trisubstituted benzimidazole derivatives and evaluated their anticancer effect against panel of cancer cell lines such as Jurkat, K-562, MOLT-4, HeLa, HCT116 and MIA PaCa-2 cancer cells and identified the potential antileukemic agent methyl 1-benzyl-2-(4-fluoro-3-nitrophenyl)-1*H*-benzo[*d*]imidazole-5-carboxylate (**TJ08**) with IC_{50} 1.88 μM concentration. The accomplished results revealed that compound **TJ08** decreases foci formation, cell migration and invasion abilities and it induces cell cycle arrest at G1/S phase and helps apoptosis in diverse cancer cell lines [24].

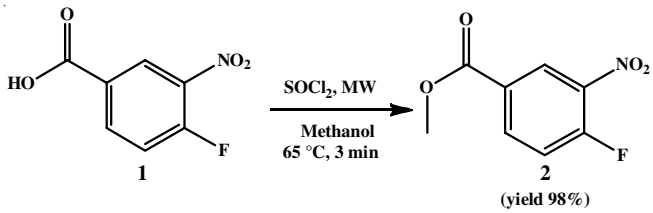
The above results inspired us to develop **TJ08** molecule further to improve its potency, however the synthesis of library of *in silico* designed novel substituted benzimidazoles takes lot of time and energy, which slows downs the process of structure activity and relationship (SAR) study and the identification

of potent molecule. In order to expedite the structure activity relationship study (SAR) process, herein an energy efficient, rapid, cost effective, feasible protocol is developed to synthesize methyl 1-benzyl-2-(4-fluoro-3-nitrophenyl)-1*H*-benzo[*d*]imidazole-5-carboxylate (**TJ08**). This environmentally sustainable method will help to synthesize the substituted benzimidazoles in a short duration of time and to identify druggable compounds through structure activity relationship study.

EXPERIMENTAL

Starting chemicals and reagents were purchased from Sigma-Aldrich,while the dry solvents were procured from Sonia Industries, India. The organic reactions were carried out in the Microwave instrument (make: Anton Paar Monowave 200). The reaction progress was monitored by TLC on Merck brand TLC plates (silica gel 60 F_{254}) and visualized under UV lamp, but in some cases TLC were visualized by dipping in aqueous $KMnO_4$ solution followed by heating. The solvents were removed under *vacuo* using rotary evaporator. The final product was purified by flash column chromatography (silica gel 230-400 mesh). The ^{1}H NMR spectra were recorded on Bruker 400 MHz, 300 MHz whereas ^{13}C NMR spectra were recorded on Bruker 100 MHz spectrometer (DMSO-d_6). The LC-MS and HPLC analysis were performed on Agilent Technologies Infinity 1290 and Agilent Technologies, respectively. The FTIR spectra were recorded on Bruker Alpha 2 spectrometer and HRMS analysis were done on Waters SYNAPTG2.

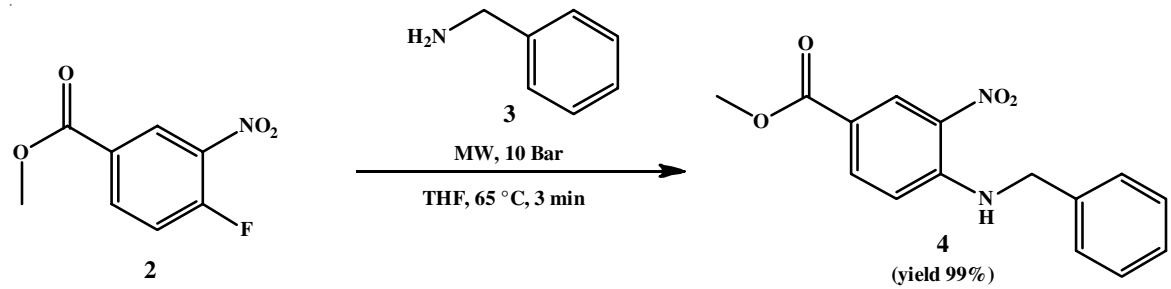
Synthesis of methyl 4-fluoro-3-nitrobenzoate (2): A solution of compound **1** (500 mg, 2.70 mmol) in methanol (5 mL) was taken in a 20 mL microwave reaction vial followed by a catalytic amount of DMF and thionyl chloride (0.4 mL, 5.50 mmol) were added dropwise to the reaction mixture under nitrogen atmosphere. The resulting reaction mixture was sealed, pre-stirred for a minute and then heated to 65 ºC for 3 min under microwave irradiation. After that, the reaction mixture was cooled at 0 ºC, quenched with ice-cold water and the organic product was extracted with ethyl acetate (15 mL × 3). The combined organic phase was washed with saturated aqueous sodium bicarbonate and brine solution. The organic phase was desiccated on anhydrous Na_2SO_4, solvent was concentrated under *vacuo* to obtain compound **2** as an off-white solid, with 98% yield (526 mg); (TLC-R_f = 0.46 in petroleum ether:ethyl acetate, 5:5 v/v) (**Scheme-I**). FTIR (KBr, ν_{max}, cm^{-1}): 1614.05 (-C=C-), 1712.91 (-C=O), 2884.34 (-C-H), 3409.84 (C=C-H); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 8.59-8.56 (m, 1H, Ar-H), 8.36-8.31 (m, 1H, Ar-H), 7.78-7.72 (m, 1H, Ar-H), 3.91 (s, 3H, -OCH_3); Found ESI/MS (*m/z*): 198.0 $[M]^-$; calculated for $[C_8H_6FNO_4]$ $[M]^-$: 198.02; LC-MS purity = 99.9%.



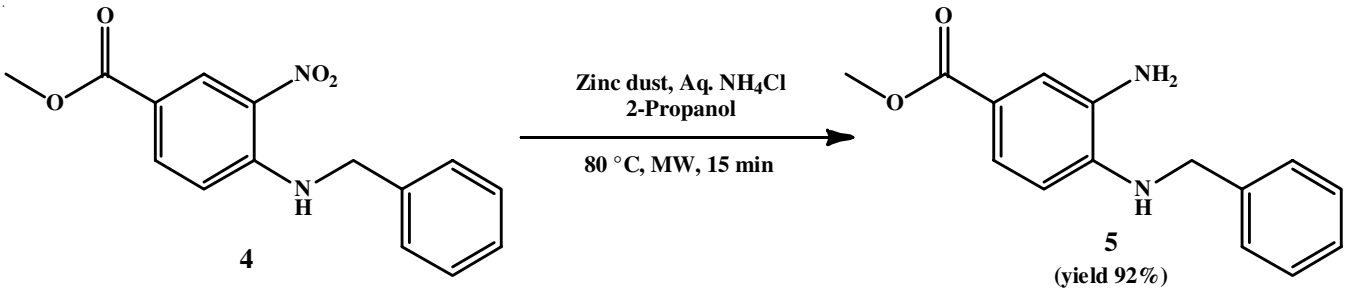
Scheme-I: Synthesis of methyl 4-fluoro-3-nitrobenzoate (**2**)

Synthesis of methyl 4-(benzylamino)-3-nitrobenzoate (4): A solution of compound **2** (505 mg, 2.53 mmol) in THF (8 mL) was taken in a 20 mL microwave reaction vial followed by compound **3** (283 mg, 2.64 mmol) and Cs_2CO_3 (1.1 g, 3.37 mmol) were added successively. The resulting reaction mixture was sealed, pre-stirred for a minute and then heated to 65 ºC for 3 min under microwave irradiation. After that, the reaction mixture was cooled to room temperature, quenched with water and the organic phase was extracted with ethyl acetate (10 mL × 3 times) the combined organic phase was washed with water and brine solution (**Scheme-II**). The organic phase was desiccated over anhydrous Na_2SO_4, solvent was concentrated under *vacuo* to obtain compound **4** as yellow solid, with 99% yield (716 mg); (TLC-R_f = 0.51 in petroleum ether:ethyl acetate, 5:5 v/v). FTIR (KBr, ν_{max}, cm^{-1}): 1622.89 (-C=C-), 1768.96 (-C=O), 3029.26 (-C-H), 3367.02 (-N-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 9.08-9.11 (t, *J* = 6.0 Hz, 1H, Ar-H), 8.643-8.648 (d, *J* = 2.0 Hz, 1H, Ar-H) 7.88-7.91 (m, 1H, Ar-H), 7.25-7.39 (m, 5H, Ar-H), 7.00-7.02 (d, *J* = 8.8 Hz, 1H, Ar-H, -CH_2), 4.70-4.71 (d, *J* = 6.4 Hz, 2H, -CH_2), 3.81 (s, 3H, -OCH_3); Found ESI/MS (*m/z*): 286.9 $[M+H]^+$; calculated for $[C_{15}H_{14}N_2O_4]$ $[M+H]^+$: 286.09; LC-MS purity = 99.8%.

Synthesis of methyl 3-amino-4-(benzylamino)benzoate (5): A solution of compound **4** (650 mg, 2.27 mmol) in 2-propanol (9 mL) was taken in a 30 mL microwave reaction vial followed by saturated aq. NH_4Cl solution (7 mL) and zinc dust (65 mg, 10 mol%) were added successively. The resulting reaction mixture was sealed, pre-stirred for a minute and then it was heated at 80 ºC for 15 min under microwave irradiation. The reaction mixture was then cooled to room temperature, diluted with water:ethyl acetate (1:1, 20 vol.) and filtered through celite pad, the organic phase was separated and the aqueous phase was extracted with ethyl acetate (10 mL × 2). The combined organic phase was suspended in activated charcoal (65 mg, 10% by weight) and stirred for 5 min, then filtered on celite bed and washed with ethyl acetate (**Scheme-III**). The filtrate



Scheme-II: Synthesis of methyl 4-(benzylamino)-3-nitrobenzoate (**4**)

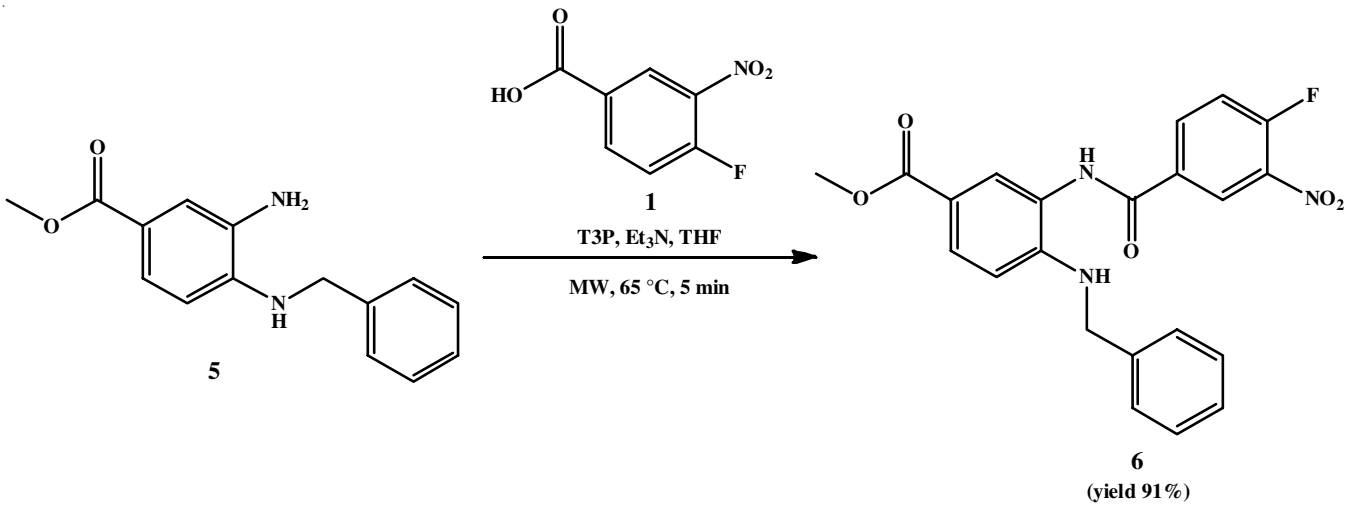


Scheme-III: Synthesis of methyl 3-amino-4-(benzylamino)benzoate (**5**)

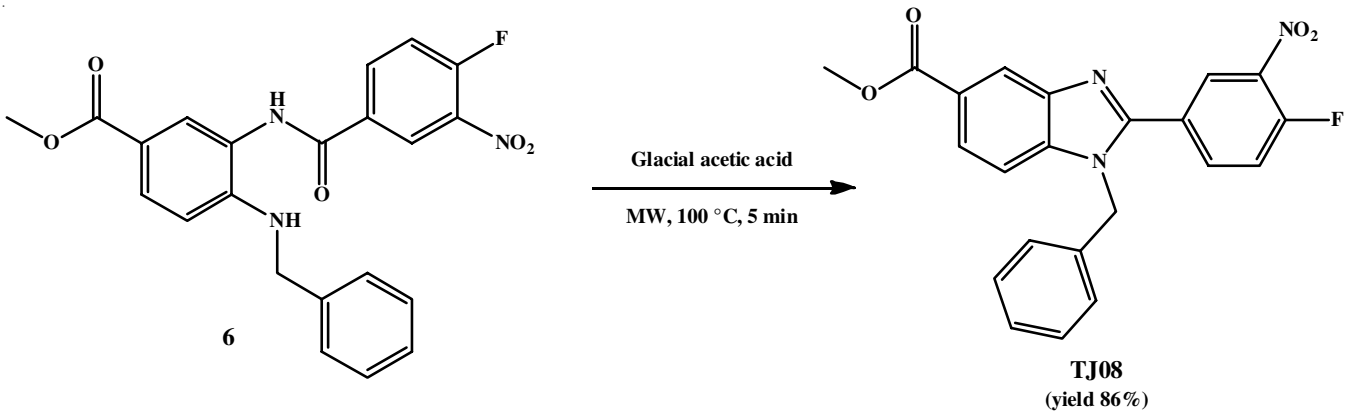
was concentrated under *vacuo* to obtain compound **5** as pale-brown solid with 92% yield (533 mg); (TLC-R_f = 0.29 in petroleum ether: ethyl acetate 5:5, v/v). FTIR (KBr, ν_{max}, cm^{-1}): 1693.57 (-C=O), 2944.16 (-C-H), 3346.98 (-N-H), 3399.28 (-N-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.31-7.36 (m, 4H, Ar-H), 7.19-7.27 (m, 2H, Ar-H), 7.10-7.14 (m, 1H, Ar-H), 6.35-6.37 (d, *J* = 8.0 Hz, 1H, Ar-H), 5.91-5.94 (t, *J* = 5.8 Hz, 1H, -NH), 4.83 (s, 2 H, -NH_2), 4.38-4.39 (d, *J* = 5.6 Hz, 2H, -CH_2), 3.88 (s, 3H, -OCH_3). Found ESI/MS (*m/z*): 257.0 $[M+H]^+$; calculated for $[C_{15}H_{16}N_2O_2]$ $[M+H]^+$: 257.12; LC-MS purity = 96.1%.

Synthesis of methyl 4-(benzylamino)-3-(4-fluoro-3-nitrobenzamido)benzoate (6): A solution of compound **5** (300 mg, 1.17 mmol) in THF (3 mL) was taken in a 5 mL microwave reaction vial followed by compound **1** (218 mg, 1.17 mmol) T3P (50% solution in ethyl acetate, 0.42 mL 1.32 mmol) and triethylamine (0.22 mL, 1.57 mmol) were added successively. The resulting reaction mixture was sealed, pre-stirred for a minute and then heated to 65 ºC for 5 min under microwave irradiation. After that the reaction mixture was cooled to room temperature, quenched with water and the organic phase was extracted with ethyl acetate (10 mL × 3) the combined organic phase was washed with water and brine solution (**Scheme-IV**). The organic phase was desiccated over anhydrous Na_2SO_4, solvent was concentrated under *vacuo* to obtain compound **6** as pale-brown solid with 91% yield (449 mg); (TLC-R_f = 0.36 in petroleum ether: ethyl acetate 5:5 v/v). FTIR (KBr, ν_{max}, cm^{-1}): 1609.47 (-C=C-), 1642.12 (-C=O), 2944.88 (-C-H), 3347.47 (-N-H), 3399.56 (-N-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.12 (s, 1H, -NH), 8.81-8.83 (m, 1H, Ar-H), 8.44-8.48 (m, 1H, Ar-H), 7.80-7.82 (m, 1H, Ar-H), 7.702-7.707 (d, *J* = 2.0 Hz 1H, Ar-H), 7.60-7.63 (m, 1H, Ar-H), 7.30-7.38 (m, 4H, Ar-H), 7.20-7.24 (t, *J* = 7.0 Hz 1H, Ar-H), 6.85-6.88 (t, *J* = 6.2 Hz 1H, -NH), 6.53-6.55 (d, *J* = 8.8 Hz, 1H, Ar-H) 4.44-4.46 (d, *J* = 5.8 Hz, 2H, -CH_2), 3.74 (s, 3H, -OCH_3). Found ESI/MS (*m/z*): 424.1 $[M+H]^+$; calculated for $[C_{22}H_{18}FN_3O_5]$ $[M+H]^+$: 424.1; LC-MS purity = 98.3%.

Synthesis of methyl 1-benzyl-2-(4-fluoro-3-nitrophenyl)-1*H*-benzo[*d*]imidazole-5-carboxylate (TJ08): A solution of compound **6** (400 mg, 0.94 mmol) in glacial acetic acid (4 mL) was taken in a 10 mL microwave reaction vial, the resulting reaction mixture was sealed, pre-stirred for a minute and then heated at 100 ºC for 5 min under microwave irradiation. After that the reaction mixture was cooled to room temperature, quenched slowly with saturated aqueous $NaHCO_3$ solution, it was diluted with ethyl acetate organic phase was separated and aqueous phase was extracted with ethyl acetate (10 mL × 3) the combined organic phase was washed with water and brine solution. The organic phase was desiccated over anhydrous Na_2SO_4, solvent was filtered and concentrated under *vacuo* to obtain crude product (**Scheme-V**). The crude product obtained was further purified by flash column chromatography (silica gel 230-400 mesh, 25-30% ethyl acetate in petroleum ether) to obtain comp-ound **TJ08** as off-white solid with 86% yield (328 mg); TLC (R_f = 0.32, 6:4 petroleum ether:ethyl acetate, 5:5 v/v). FTIR (KBr, ν_{max}, cm^{-1}): 1619.41 (-C=C-), 1716.68 (-C=O), 2952.8 (-C-H), 3029.5 (=C-H), 3399.56 (-N-H); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.45-8.48 (m, 1H, Ar-H); 8.362-



Scheme-IV: Synthesis of methyl 4-(benzylamino)-3-(4-fluoro-3-nitrobenzamido)benzoate (**6**)



6

TJ08
(yield 86%)

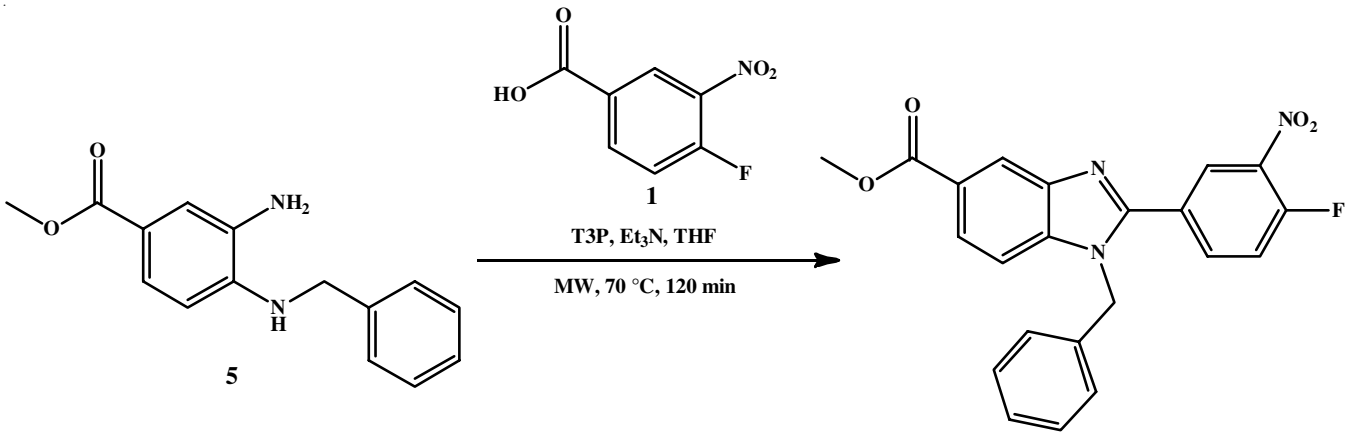
Scheme-V: Synthesis of methyl 1-benzyl-2-(4-fluoro-3-nitrophenyl)-1*H*-benzo[*d*]imidazole-5-carboxylate (**TJ08**) from compound **5**

8.366 (d, *J*= 1.6 Hz, 1H, Ar-H); 8.16-8.20 (m, 1H, Ar-H); 7.92-7.95 (m, 1H, Ar-H); 7.75-7.80 (m, 1H, Ar-H); 7.691-7.713 (d, *J*= 8.8 Hz, 1H, Ar-H); 7.25-7.32 (m, 3H, Ar-H); 7.034-7.038 (d, *J*= 1.2 Hz, 1H, -Ar-H); 7.01 (s, 1H, Ar-H); 5.70 (s, 2H, $-CH_2$); 3.89 (s, 3H, $-OCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 167.01, 157.23-154.59 (J_{C-F}= 264 Hz), 152.78, 142.47, 139.87, 137.51-137.43 (J_{C-F}= 8 Hz), 137.35-137.25 (J_{C-F}= 10 Hz), 136.72, 129.37, 128.21, 126.71, 124.75, 124.71, 121.66, 119.97-119.76 (J_{C-F}= 21 Hz), 112.02, 52.58, 48.28. Found $[M+H]^+$. ESI/MS (*m/z*): 406.0 $[M+H]^+$, calculated for $[C_{22}H_{16}FN_3O_4]$; 406.1 $[M+H]^+$. ESI/HRMS (*m/z*): 406.12 $[M+H]^+$, calculated for $[C_{22}H_{16}FN_3O_4]$; 406.11 $[M+H]^+$. HPLC (0.1% TFA in water: acetonitrile): RT = 4.750 min, 99.724%.

Synthesis of methyl 1-benzyl-2-(4-fluoro-3-nitrophenyl)-1*H*-benzo[*d*]imidazole-5-carboxylate (TJ08): A solution of compound **5** (200 mg, 0.78 mmol) in THF (10 vol.) was taken in a 5 mL microwave reaction vial followed by compound **1** (146 mg, 0.78 mmol), T3P (50% solution in ethyl acetate, 0.5 mL, 1.57 mmol) and triethylamine (0.14 mL, 1.0 mmol) were added successively. The resulting reaction mixture was sealed, pre-stirred for 1 min and then heated at 70 ºC for 120 min. The reaction mixture was cooled to room temperature, quenched with slow addition of saturated aqueous $NaHCO_3$ solution, diluted with ethyl acetate and then organic phase was separated, while aqueous phase was extracted with ethyl acetate (10 mL × 3). The combined organic phase was washed with water and brine solution. The organic phase was desiccated over Na_2SO_4, solvent was concentrated under *vacuo* to obtain crude product. (**Scheme-VI**). The crude product obtained was purified by flash column chromatography (silica gel 230-400 mesh, 25-28% ethyl acetate in petroleum ether) to obtain compound **TJ08** as off-white solid with 56% yield (176 mg). TLC (R_f = 0.32, 6:4 petroleum ether: ethyl acetate, 5:5 v/v).

RESULTS AND DISCUSSION

Initially, the esterification of 4-fluoro-3-nitrobenzoic acid (**1**, 1.0 mmol) by treating with thionyl chloride (1.0 mmol) in methanol as solvent at 45 ºC under microwave irradiation, the obtained product **2** was very less yield (Table-1, entry 1). However, upon increasing thionyl chloride contents at 45 ºC for 3 min (Table-1, entry 2 & 3), the results were again not satisfactory, even though changing the solvent medium did not increase the yield (Table-1, entry 4). Further upon increasing the temperature to 55 and 65 ºC, a gradual improvement of yield was observed (Table-1, entries 5 & 6). When the reaction was carried out at 65 ºC with thionyl chloride (2.0 mmol) in methanol under microwave irradiation 5 M bar pressure for 3 min, we observed the complete conversion of starting materials with maximum yield (Table-1, entry 6). Further the temperature was increased to 75 ºC, no improvement in the yield was obser-ved (Table-1,



5

TJ08
(yield 53%)

Scheme-VI: Synthesis of methyl 1-benzyl-2-(4-fluoro-3-nitrophenyl)-1*H*-benzo[*d*]imidazole-5-carboxylate (**TJ08**) from compound **6**

TABLE-1
OPTIMIZATION OF THE REACTION CONDITIONS FOR THE SYNTHESIS OF METHYL 4-FLUORO-3-NITROBENZOATE (**2**) FROM 4-FLUORO-3-NITROBENZOIC ACID (**1**)

Entry	Reagent	Reagent (equiv.)	Solvent (10 vol.)	Temp. (°C)	Time (min)	Yield (%)
1	$SOCl_2$	1.0	Methanol	45	3	79
2	$SOCl_2$	1.5	Methanol	45	3	85
3	$SOCl_2$	2.0	Methanol	45	3	88
4	$SOCl_2$	2.0	Methanol: DCM	45	3	80
5	$SOCl_2$	2.0	Methanol	55	3	94
6[a]	$SOCl_2$	2.0	Methanol	65	3	98
7	$SOCl_2$	2.0	Methanol	75	3	96
8	$(COCl)_2$	2.0	Methanol	65	3	90

[a]Reaction condition: Reaction was performed with **1** (1.0 mmol) and thionyl chloride (2.0 mmol) in Methanol (10 vol.) at 65 °C for 3 min under microwave irradiation.

entry 7). In spite of changing acylation agent thionyl chloride to oxalyl chloride did not increase the product yield (Table-1, entry 8).

In continuation, in order to find the best reaction condition to synthesize methyl 4-(benzylamino)-3-nitrobenzoate (**4**) from methyl 4-fluoro-3-nitrobenzoate (**2**) *via* aromatic nucleophilic substitution. Started with solvent screening, compound **4** (1.0 mmol) with benzylamine (**3**) (1.0 mmol, 1.0 equiv.) with 3 different solvents in absence of base (Table-2, entry 1-3). It was found that THF is the best solvent for this convention, however isolated yields are low in absences of base. Now, the trial reactions with 3 different bases (Table-2, entry 4-8) were performed and found that caesium carbonate had given the best result. When the reaction was carried out at 65 °C with methyl methyl 4-fluoro-3-nitrobenzoate (**2**) (1.0 mmol), benzylamine (1.0 mmol), caesium carbonate (1.3 mmol) in THF (15 vol.) under microwave irradiation 10 M bar pressure for 3 min, a complete conversion of starting materials with good yield was observed (Table-2, entry 8). Further, when the reaction time was reduced to 2 min and a decreased in product yield was observed (Table-2, entry 9).

The best optimized reaction conditions to synthesize methyl 3-amino-4-(benzylamino)benzoate (**5**) from methyl 4-(benzylamino)-3-nitrobenzoate (**4**) was done by reducing nitro to amine by catalytic hydrogenation. Initially, the catalytic hydrogenation reaction of compound **4** (1.0 mmol) was performed using Zn dust (10 mol%), saturated aqueous NH_4Cl solution (10 vol.) in four different solvents (Table-3, entry 1-4) under microwave irradiation 10 M bar pressure for 15 min. It was found that 2-propanol is the best solvent out of conducted trials for this

TABLE-2
OPTIMIZATION OF THE REACTION CONDITIONS FOR THE SYNTHESIS OF METHYL 4-(BENZYLAMINO)-3-NITROBENZOATE (**4**) FROM METHYL 4-FLUORO-3-NITROBENZOATE (**2**)

Entry	Base	Solvent (10 vol.)	Temp. (°C)	Time (min)	Yield (%)
1	–	MeCN	80	3	28
2	–	DMF	100	3	30
3	–	THF	65	3	36
4	K_2CO_3	MeCN	80	3	86
5	K_2CO_3	DMF	100	3	93
6	K_2CO_3	THF	65	3	97
7	Et_3N	THF	65	3	95
8[b]	Cs_2CO_3	THF	65	3	99
9	Cs_2CO_3	THF	65	2	95

[b]Reaction condition: Reaction was performed with **2** (1.0 mmol), **3** (1.0 mmol) and Cs_2CO_3 (1.3 mmol) in THF (15 vol.) at 65 °C for 3 min under microwave irradiation.

TABLE-3
OPTIMIZATION OF THE REACTION CONDITIONS FOR THE SYNTHESIS OF METHYL 3-AMINO-4-(BENZYLAMINO)BENZOATE (**5**) FROM METHYL 4-(BENZYLAMINO)-3-NITROBENZOATE (**4**)

Entry	Solvent	Solvent (vol.)	Temp. (°C)	Time (min)	Yield (%)
1	Methanol	20	65	15	70
2	Ethanol	15	75	15	87
3	1-Propanol	20	80	15	73
4[c]	2-Propanol	13	80	15	92
5	Ethanol:THF (1:1)	30	80	15	80
6	2-Propanol:THF (1:1)	20	80	15	84

[c]Reaction condition: Reaction was performed with **4** (1.0 mmol), zinc dust (10 mol%), saturated aqueous ammonium chloride solution (10 vol.), 2-Propanol (13 vol.) at 80 °C for 15 min under microwave irradiation.

Note: Saturated aqueous ammonium chloride solution (10 vol.) was used for all the trials, catalyst zinc dust was used (10 mol %) by weight. Solvent volume used depends on the solubility of compound **4** in the respective solvents.

conversion with maximum isolated yield (Table-3, entry 4). When this conversion was tried with the mixture of solvents (Table-3, entry 5 & 6), the obtained results were not satisfactory.

To establish the best conditions to synthesize methyl 4-(benzylamino)-3-(4-fluoro-3-nitrobenzamido)benzoate (**6**) from methyl 3-amino-4-(benzylamino)benzoate (**5**) and 4-fluoro-3-nitrobenzoic acid (**1**) *via* amidation was performed in three different solvents in presence of coupling reagent *n*-propanephosphonic acid anhydride (T3P) and triethylamine as base under microwave irradiation (Table-4, entry 1-3). Out of 3 trials, the reaction between compound **5** (1.0 mmol) and compound **1** (1.0 mmol) in presence of coupling agent T3P (50% solution in ethyl acetate, 1.1 mmol) and triethylamine (1.3 mmol) as base in THF under microwave irradiation for 5 min (Table-4, entry 1) gives the best result.

In this work, two synthetic routes were established to synthesize methyl 1-benzyl-2-(4-fluoro-3-nitrophenyl)-1*H*-benzo[*d*]imidazole-5-carboxylate **(TJ08)** from methyl 4-(benzyl-amino)-3-(4-fluoro-3-nitrobenzamido)benzoate (**6**) *via* cyclization to form benzimidazole derivative. A reaction between compound **6** and glacial acetic acid (10 vol.) under microwave irradiation for 5 min had given the best result (Table-5, entry 1). When the reaction between compound **6** with glacial acetic acid (5 vol.) in THF (5 vol.) (Table-5, entry 2) was conducted, the isolated yield was relatively less.

In second synthetic route of **TJ08**, using methyl 3-amino-4-(benzyl-amino)benzoate **(5)** *via* amidation using T3P as coupling reagent and triethylamine as base followed by cyclization to form benzimidazole ring. In the amidation reaction, the byproduct propylphosphonic acid catalyzes the cyclization reaction to form benzimidazole. Initially, the reaction between compound **5** (1 mmol) and compound **1** (1 mmol), triethyl-amine (1.3 mmol) with different equivalence of coupling agent T3P (Table-6, entry 1-4) was performed. Solvents screening trials (Table-6, entry 4-7) showed that THF is the best solvent for this conversion. After optimizing the suitable solvent and equivalence of coupling agent, the duration of the reaction was further analyzed (Table-6, entry 8-11) at 70 ºC under microwave irradiation 10 M bar pressure for 120 min (Table-6, entry 10) had given the best results. Further increasing the reaction duration (Table-6, entry 11) did not increase the product yield.

TABLE-4
OPTIMIZATION OF THE REACTION CONDITIONS FOR THE SYNTHESIS OF METHYL 4-(BENZYLAMINO)-3-(4-FLUORO-3-NITROBENZAMIDO)BENZOATE (**6**) FROM METHYL 3-AMINO-4-(BENZYLAMINO)BENZOATE (**5**)

Entry	Solvent	Solvent (vol.)	Temp. (°C)	Time (min)	Yield (%)
1[d]	THF	10	65	5	91
2	2-Methyl THF	10	65	5	88
3	Ethyl acetate	10	65	5	82

[d]Reaction condition: Reaction was performed with compound **5** (1.0 mmol), compound **1** (1.0 mmol), T3P (50% solution in ethyl acetate, 1.1 mmol), triethyl amine (1.3 mmol), THF (10 vol.) at 65 °C for 5 min under microwave irradiation.

TABLE-5
OPTIMIZATION OF THE REACTION CONDITIONS FOR THE SYNTHESIS OF METHYL 1-BENZYL-2-(4-FLUORO-3-NITROPHENYL)-1*H*-BENZO[*d*]IMIDAZOLE-5-CARBOXYLATE (**TJ08**) FROM METHYL 4-(BENZYLAMINO)-3-(4-FLUORO-3-NITROBENZAMIDO)BENZOATE (**6**)

Entry	Solvent	Solvent (vol.)	Temp. (°C)	Time (min)	Yield (%)
1[e]	Glacial acetic acid	10	100	5	86
2	Glacial acetic acid: THF 1:1	10	80	5	63

[e]Reaction condition: Reaction was performed with compound **6** (1.0 mmol), glacial acetic acid (10 vol.) at 100 °C for 5 min under microwave irradiation.

TABLE-6
OPTIMIZATION OF THE REACTION CONDITIONS FOR THE SYNTHESIS OF METHYL 1-BENZYL-2-(4-FLUORO-3-NITROPHENYL)-1*H*-BENZO[*d*]IMIDAZOLE-5-CARBOXYLATE (**TJ08**) FROM METHYL 3-AMINO-4-(BENZYLAMINO)BENZOATE (**5**)

Entry	Solvent	T3P (Equivalance)	Temp. (°C)	Time (min)	Yield (%)
1	THF	1.1	70	30	5
2	THF	1.5	70	30	16
3	THF	2.0	70	30	28
4	THF	2.5	70	30	26
5	2-Methyl THF	2.0	80	30	23
6	1,4-Dioxane	2.0	100	30	26
7	DMF	2.0	100	30	18
8	THF	2.0	70	60	35
9	THF	2.0	70	90	44
10[f]	THF	2.0	70	120	53
11	THF	2.0	70	150	51

[f]Reaction condition: Reaction was performed with compound **5** (1.0 mmol), compound **1** (1.0 mmol), T3P (50% solution in ethyl acetate, 2.0 mmol), triethylamine (1.3 mmol), 1,4-dioxane (10 vol.) at 65 °C for 120 min under microwave irradiation.

Conclusion

An efficient, environmentally benign microwave-assisted protocol for the synthesis of 1, 2 and 5-trisubstituted benzimidazole derivative (**TJ08**) was carried out successfully characterized. *n*-Propylphosphonic acid, a byproduct of T3P was used effectively in the amidation reaction for subsequent cyclization reaction to form benzimidazole derivative under microwave irradiation, which was further used to synthesize methyl 1-benzyl-2-(4-fluoro-3-nitrophenyl)-1*H*-benzo[*d*]imidazole-5-carboxylate (**TJ08**) molecule in 31 min of reaction time over 5 steps under microwave assisted synthesis, whereas the conventional heating method took around 17 h. This novel protocol will help to synthesize the novel benzimidazole derivatives expeditiously in order to develop new druggable compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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