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Synthesis, p38 Kinase Inhibitory and Anti-inflammatory Activity of New Substituted Benzimidazole Derivatives

Ravindra G. Kulkarni[a,d*], Stefan A. Laufer[b], Chandrashekhar V. M[c] and Achaiah Garlapati[d*]

[a]*SVERI's College of Pharmacy, Gopalpur Road, Pandharpur- 413304, Maharashtra, India*

[b]*Institute of Pharmacy, Department of Pharmaceutical and Medicinal Chemistry, Eberhard-Karls-University, Tubingen, Auf der Morgenstelle, 8, 72076, Germany*

[c]*BVVS'S Hanagal Shri Kumareshwar College of Pharmacy, BVVS Campus, Bagalkot, Karnataka, 587 101, India*

[d]*University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Andhra Pradesh, India*

Abstract: P38 mitogen activated protein kinases have been found to involve in the production and release of unwarranted levels of pro-inflammatory cytokines including TNFα and IL-1β in numerous inflammatory diseases. A new series of molecules, 5-substituted benzoylamino-2-substituted phenylbezimidazoles has been synthesized from 4-nitro-1, 2-diaminobenzene. The synthesized compounds were characterized by FTIR, ^{1}HNMR and Mass. The final compounds were screened for *in vitro* p38 kinase inhibitory and *in vivo* anti-inflammatory activity. Three compounds from the series demonstrated nearly 50% inhibition of p38 kinase in the *in vitro* screening method at 10 μM concentration and two molecules exhibited greater than 75% inhibition of paw oedema volume during the first hour. The docking study of synthesized molecule revealed a new binding pose in ATP binding pocket.

Keywords: p38 kinase inhibitors, benzimidazoles, anti-inflammatory activity.

1. INTRODUCTION

The elevated levels of pro-inflammatory cytokines like tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β) play a vital role in numerous inflammatory diseases including rheumatoid arthritis. Several peptide based anti-cytokine therapies have been implemented in the clinical treatment of rheumatoid arthritis to suppress such pro-inflammatory cytokines [1-4]. Despite being potent inhibitors of pro-inflammatory cytokines, the protein based anti-cytokines were reported to exhibit severe adverse effects including microbial infections and are quite exorbitant too [5]. However several alternative strategies have been contemplated and quite a few new validated targets were identified for the search of effective anti-inflammatory agents [6]. Studies involving mitogen activated protein kinases (MAPK, proline directed serine-threonine kinases) revealed that MAPKs are involved in the cellular responses to extracellular signals including microbial endotoxins and cytokines [7]. Four isoforms of p38 kinase are known till date viz., p38α, p38β, p38γ and p38δ which are activated by phosphorylation in the activation loop [8-10]. Activated p38 kinase plays a key role in signal transduction pathway leading to production and release of elevated levels of pro-inflammatory cytokines TNFα and IL-1β. Due to this vital role being played by p38 kinase, inhibition of p38 kinase is highly desired in inflammatory diseases. Low molecular weight p38 kinase inhibitors show same therapeutic benefits like biological anti-cytokines but offer advantages in terms of oral dosage and affordable cost [11-13].

The seminal discovery of first generation p38 kinase inhibitors exemplified by **SB203580 [**14**]** has opened a new prospect for the development of next generation agents for inflammatory diseases and pharmaceutical industries pursuing aggressively towards the development of new anti-inflammatory agents by targeting p38 kinase. Further discovery of allosteric binding, noncompetitive N, N′-diarylurea derivatives typified by clinical compound **BIRB796** (Doramapimod) has led to unearthing of several new lead molecules and some of them are in various stages of clinical trials [15-17].

Compounds possessing benzimidazole scaffold reported to possess wide range of pharmacological activities including anti-inflammatory and interleukin-1 inhibitory activity [18-22] and also displayed a variety of kinase inhibitory activities [23-27]. Substituted benzimidazoles have demonstrated potent Raf kinase inhibitory activity which were designed from the modification of most advanced Raf kinase inhibitor **BAY43-9006** [28] and few p38 kinase inhibitors were derived from benzimidazole scaffold [29-33]. In continuation to our previous studies on the development of allosteric binding p38 kinase inhibitors, we designed new benzimidazoles (**7a-j**) from our in house urea derivatives [34] (Fig. **1**). In this communication, we report the synthesis of a series of 2-substituted phenylbenzimidazole derivatives and their evaluation for p38 kinase inhibitory and anti-inflammatory activities.

*Address correspondence to this author at the SVERI's College of Pharmacy, Gopalpur Road, Pandharpur- 413304, Maharashtra, India; Tel: +91-94406 01057, +91-8446765426; Fax: +91 2186 225082;
E-mails: ravigk2000@gmail.com; achaiah_g@yahoo.com

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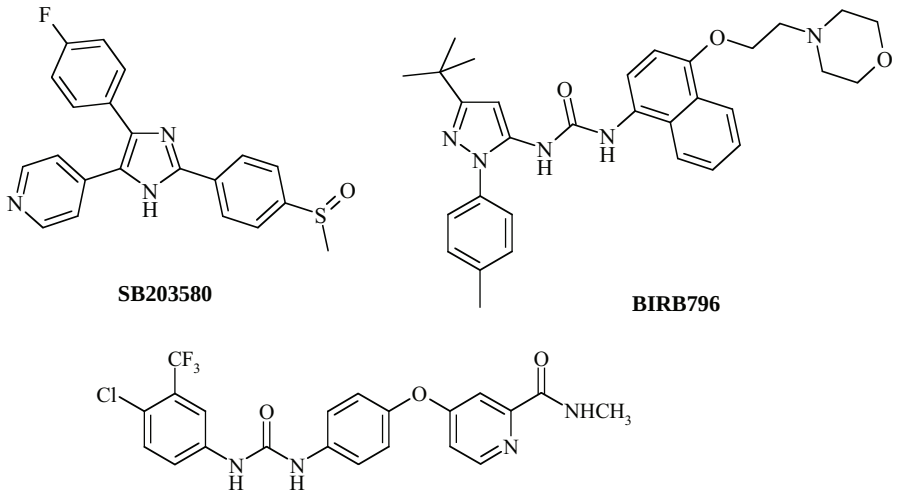


Structures of important p38 kinase inhibitors

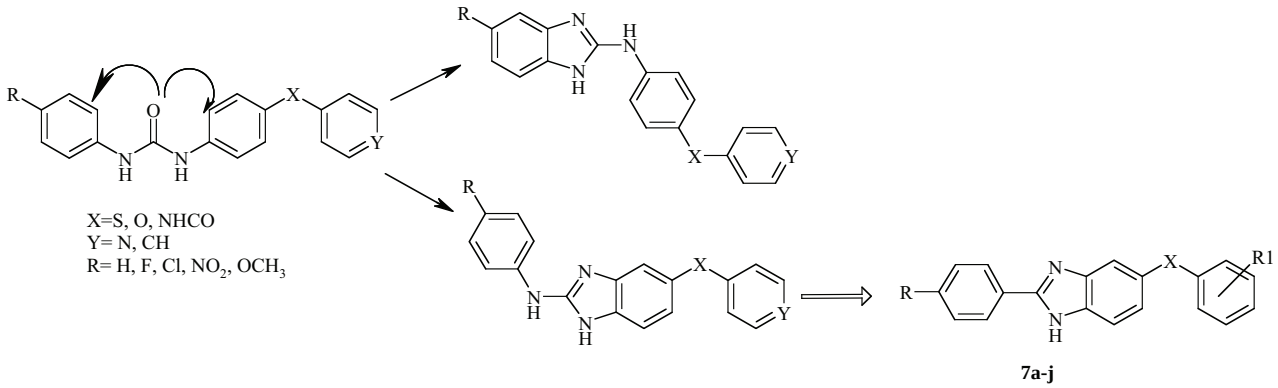


Fig. (1). Design of benzimidazole p38 kinase inhibitors.

MATERIAL AND METHOD

All the reactions were carried out on six stage reaction station of Radleys discovery Technologies, Germany. Melting points were recorded in open glass capillaries on Polmon melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FTIR spectrophotometer using KBr and mass spectra obtained on VG-7070H mass spectromoter. ^{1}HNMR spectra were recorded at 300 MHz on a Bruker Avance NMR spectrometer in $CDCl_3$ (δ 7.26) or DMSO- d_6 (δ 2.49). Thin layer chromatography was performed on pre-coated silica gel F_{254} (Merck) and column chromatography was performed using silica gel 60-120 mesh.

Synthetic Methodology

Synthesis of Substituted Sodium Metabisulfite Adducts of Benzaldehydes (2a-c)

To the solution of benzaldehydes (**1a-c**, 0.015 mol) in 50 ml absolute ethanol, sodium metabisulfite (1.6 g) in 10 mL of water was added drop wise. The reaction mixture was stirred vigorously, more absolute ethanol (20 mL) was added and the reaction mixture was kept in a refrigerator for several hours. The precipitate obtained was separated by filtration, dried to obtain compounds **2a-c** in ~80% yield and were used without purification in the succeeding step [35].

Synthesis of 2-substituted Phenyl-5-nitrobenzimidazoles (4a-c)

A mixture of **2a-c (**0.00317 mol) and 4-nitro-1, 2-benzenediamine (**3**, 0.00314 mol) were heated at 130 °C for 4 h in DMF (20 mL). The reaction mixture was then cooled to room temperature and poured into water. The precipitate thus obtained was filtered, washed with plenty of cold water to remove inorganic salts and dried. The crude compounds were recrystallized from 70% alcohol to get pure **4a-c**.

Synthesis of 5-amino-2-substituted Phenylbenzimidazoles (5a-c)

The Reduction of Nitro Group was Carried by two Different Methods

Method A: Stannous chloride dihydrate (0.0126 mol) was added into appropriate solution of **4a-c** (0.0028 mol) in absolute ethanol and heated at 70 ˚C for 4 h. Cooled reaction mixture was poured over ice, then made alkaline (~pH 8) with 10% aqueous sodium hydroxide and extracted with ethyl acetate [36]. The combined organic phase was washed

with brine and dried over anhydrous sodium sulphate. The crude product thus obtained was purified by passing through silica gel using dichloromethane: acetone as eluent to get respective pure compounds **5a-c**.

Method B: A suspension of an appropriate **4a-c** (0.003 mol) and activated zinc (0.004 mol) in methanol (10 ml) was stirred with ammonium formate (0.5 g) at room temperature [37]. The reaction was monitored by TLC and upon completion of the reaction, the reaction mixture was filtered to remove unreacted zinc. The volatilities were removed by evaporation and the residue was dissolved in dichloro-methane and washed with saturated brine. Evaporation of dichloromethane resulted in crude amines which were purified over silica using dichloromethane: acetone (8:2) as eluent to afford **5a-c**.

Preparation of Benzoylated 5-amino-2-substituted Phenylbenzimidazoles (7a-j)

Appropriate amines (**5a-c**, 0.001 mol) were stirred in tetrahydrofuran (15 mL) for 10 minutes to yield uniform solution and to which triethyl amine (0.002 mol) was added. Solution of substituted benzoyl chlorides (**6a-d**, 0.0011 mol) in 5 mL tetrahydrofuran was added drop wise and the reaction mixture was stirred for 5 h at room temperature. Upon completion of the reaction as indicated by TLC, the volatilities were removed. Then dichloromethane (25 mL) was added, washed with 2-5% sodium bicarbonate and later with brine. The organic component was removed by evaporation and the crude products were subjected to purification by passing through silica gel using dichlromethane: acetone (8: 2) as eluent to afford pure compound (**7a-j**).

In Vitro p38 Kinase Assay

Nonradioactive immunosorbent p38 kinase activity assay method has been followed which is applicable for routine screening of small molecule p38 kinase inhibitors. In the method ATF-2 was used as a substrate for phosphorylation, which showed linearity in 6-24 ng/well range, based on this 12 ng/well and 60 minute incubation period was optimized. Microtiter plates were coated with 50 µL/well of the p38 kinase substrate ATF-2 (10 µg/mL in TBS) for 1.5 h at 37 ˚C. After washing three times with bidistilled water, remaining open binding sites were blocked with blocking buffer (BB; 0.05% Tween 20, 0.25% BSA, 0.02% NaN_3 in TBS) for 30 min at room temperature. Plates were washed again, 50 µL of the respective test solution was filled into the wells and the plates were incubated for 1 h at 37 ˚C. Test solutions containing 12 ng/well p38 MAPK were diluted in kinase buffer (50 mM Tris, pH 7.5, 10 mM $MgCl_2$, 10 mM β-glycerophosphate, 100 µg/mL BSA, 1mM dithiothretol, 0.1 mM Na_3VO_4, 100 µM rATP) with or without test substance (10^{-4} to 10^{-8} M). Test substances were dissolved in dimethyl sulfoxide to form stock solution of 10^{-2} M, all further dilutions were carried out in kinase buffer. After subsequent washing, plates were blocked again with BB for 15 min followed by a fourth washing step. Wells were filled with 50 µL of the specific anti-bis-($Thr^{69/71}$)-phospho-ATF-2 (1. AB, 1:500 in BB) and incubated for 1 h at 37 ˚C followed by washing and consecutive incubation with 50 µL of the secondary antibody (2. AB (alkaline phospatase conjugated), 1:1400 in BB). Then 100 µL of 4-NPP was pipetted in each well after a final washing step and color development was measured 1.5-2 h later with an enzyme linked immunosorbent assay reader equipped with the SOFTmax PRO software at 405 nm. After adapting all solution volumes, this assay was performed automatically with the robotic liquid handling system [38].

Carrageenan Induced Paw Oedema

The anti-inflammatory activity of the test compounds was evaluated as described by Winter *et al*. One hour after the oral administration of test compounds, rats in all groups were challenged with carrageenan (1% prepared in 0.9% NaCl) in the sub-plantar region of right hind paw. The paw volume was measured at different intervals of time (1, 2, 3, and 4 h) using digital plethysmometer (UGO Basil, Italy). The percentage inhibition of paw volume for each test group is calculated using following equation [39-42].

Percentage of inhibition (%) = [1-Volume (test compound) / Volume (control)] x100

MOLECULAR DOCKING

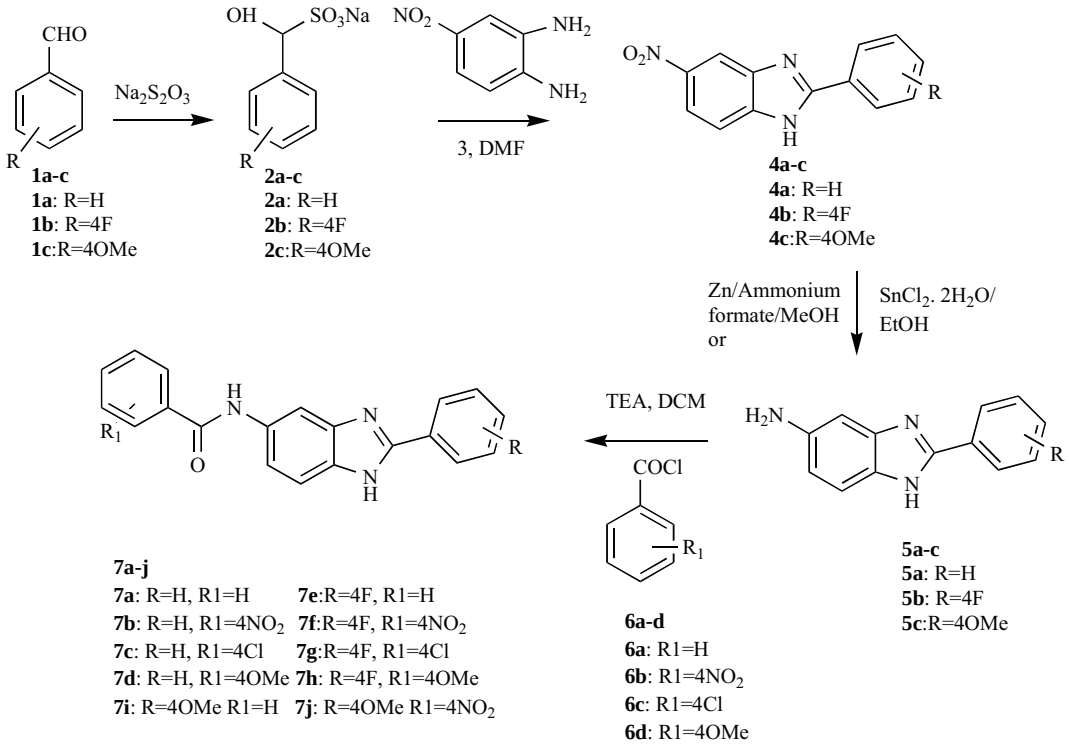
The most effective way to understand the interaction of small molecule with active site is described by docking studies. Previously we integrated both molecular docking and 3D QSAR studies and disclosed the probable strategy to design new p38 kinase inhibitors [43-45]. GOLD 3.5 a genetic algorithm docking program was employed to reveal the binding poses of benzimidazoles in the active site. From the crystal structure 1bl7, the ligand and water molecules were deleted and hydrogen added. To refine the protein structure from undesired contact, local minimization was carried by applying Kollmann partial charges and default parameters were used: 100 population size, 1.1 for selection, 5 number of islands, 100000 number of genetic operations and 2 for the niche size. 10 Å radius from Met109 was assigned to create active site and GOLD score was selected to rank order the docked conformations.

RESULTS AND DISCUSSIONS

Chemistry

The title compounds (**7a-j**) were synthesized as outlined in Scheme **1**. The key intermediates, 5-nitro-2-phenylbenzimidazoles (**4a-c**) were prepared by reaction between 4-nitro-1, 2-benzenediamine (**3**) and benzaldehydes (**1a-c**). Numerous methods are available for the synthesis of 2-phenylbenzimidazole derivatives, however we followed the method in which sodium metabisulfite adducts of substituted benzaldehydes (**2a-c**) were refluxed with **3** to afford **4a-c** in better yields. The nitro derivatives (**4a-c**) were subsequently reduced either by stannous chloride dihydrate or zinc ammonium formate to result **5a-c** in 65-80% yields. Further compounds **5a-c** were benzoylated using appropriate benzoyl chlorides (**6a-d)** in anhydrous tetrahydrofuran to afford the final desired compounds **7a-j** in 60-75% yield.

The ^{1}HNMR spectra of compounds **4a-c** were recorded in $CDCl_3$ and the peak assignment has been mentioned in characterization section**.** All the three molecules exhibited a broad singlet peak around 10 delta value which is attributed



Scheme 1. Synthesis of **7a-j**.

to resonance of NH proton of benzimidazole. C_4, C_6 and C_7 protons of benzimidazole appeared between 8.1 ppm and 8.5 ppm, C_4 was singlet while C_6 and C_7 were in doublets. Protons of aromatic ring C'_3, C'_4 and C'_5 appeared as triplet in **4a,** whereas C'_2, C'_6 and C'_3, C'_5 resonated as doublet in **4b** and **4c**. All the three molecules showed the corresponding M+1 peaks (100%) conforming respective molecular formula. The FTIR spectrum of amines showed two absorption bands at 3400-3200 cm^{-1} which are due to NH_2 stretching and a band at 1580 cm^{-1} indicated NH bending. ^{1}HNMR spectra of compound **5a,** a broad singlet at 3.5 ppm is responsible for protons of primary amine excitation indicating the reduction of nitro group to amine. Protons of C_4, C_6 and C_7 of benzimidazole were located slightly upfield, two doublets were found at 7.9 and 8.0 representing C_6 and C_7 protons of benzimidazole respectively. A triplet peak was noticed at delta 7.1 integrated for three protons due to C'_3, C'_4 and C'_5 protons. A peak at m/z 210 (M+1, 100%) in the mass spectrum of compound **5a** which is agreeing the molecular formula $C_{13}H_{11}N_3$.

FTIR spectrum of **7a** did not show absorption band for primary amine however it showed an absorption band at 3200 cm^{-1} that is responsible for NH stretching and carbonyl group stretching could be assigned for a peak present at 1720 cm^{-1}. ^{1}HNMR of **7a** recorded in DMSO d_6 depicted a broad singlet peak at δ 12.8 indicating amide NH excitation and another broad singlet peak at δ 10.3 was found which could be attributed to benzimidazole NH proton. In the ESI mass spectrum, compound **7a** showed peak at m/z 314.2 (M +1, 100%), which matches with its molecular formula $C_{20}H_{15}N_3O$. FT IR, ^{1}H NMR and Mass spectral results are in concurrence with the projected structures.

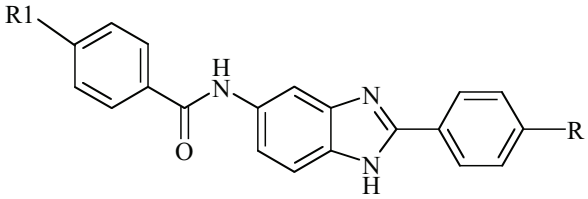
Pharmacology

All the newly synthesized compounds (**7a-j)** were screened for *in vitro* p38 kinase inhibitory and *in vivo* anti-inflammatory activity. The p38 kinase inhibitory activity was determined by robust, nonradioactive and immunosorbent assay method. The antiinflammatory activity was performed using carrageenan in which inflammation response is quantified by increase in paw size (oedema).

In Vitro p38 Kinase Inhibitory Activity

The p38 kinase inhibitory activity of compounds **7a-j** is summarized in Table **1**. Unsubstituted compound **7a** exhibited 30.43% enzyme inhibitory activity at 10 μM. Substitution on either **R** or **R1** led to increase in p38 kinase inhibitory activity except **7h**. Substitution at **R1** (NO_2, Cl and OCH_3) as in **7b**, **7c** and **7d** enhanced the potency drastically and compound **7c** (R=H, R1=Cl) found to be potent among the study compounds with 51.86% activity. Insertion of groups over 4th position of 2-phenyl ring resulted in reduction in the *in vitro* activity however compounds **7e** and **7i** found to be superior when compared with **7a**. Both nitro compounds **7f** and **7j** (R=F and OCH_3 respectively) were less potent than **7b** (R=H) and similarly chloro derivative **7g** (R=F, R1= Cl) exhibited lower potency when compared with **7c**. The above discussion gives the clue that substitution at **R** is not primarily essential which is exceptional to compound **7j** (42.2% kinase inhibitory activity). The initial activity profile suggests that irrespective of nature of groups, bulky substitution at R1 is essential for better activity. New compounds with wide range of bulky substitution at R1 may strengthen our results.

Table 1. ***In vitro*** **p38 Kinase Inhibitory Activity of 5-Substituted Benzoylamino, 2-Substituted Phenylbenzimidazole Derivatives (7a-j)**



Code	R	R1	% Inhibition at 10 μM	GOLD Score
7a	H	H	30.43	48.50
7b	H	NO_2	51.55	45.20
7c	H	Cl	51.86	50.91
7d	H	OCH_3	49.84	47. 05
7e	F	H	39.75	48.26
7f	F	NO_2	35.49	44.40
7g	F	Cl	38.89	45.66
7h	F	OCH_3	22.68	44.32
7i	OCH_3	H	33.33	45.48
7j	OCH_3	NO_2	42.29	41.44
SB 203580			0.041 (IC_{50})	
BIRB 796			0.026 (IC_{50})	

In vivo Anti-inflammatory Activity

In the *in vivo* evaluation, compounds **7a-j** did not display any toxicity till 300 mg/kg dose in acute toxicity studies in mice hence a dose of 50 mg/kg was selected for all the compounds to observe antiinflammatory activity. All the compounds (**7a-j**) exhibited lower anti-inflammatory activity in rats when compared to our library of diarylureas. All the molecules have demonstrated maximum protection during first hour of carrageenan challenge and the activity was found decreasing in the later stage of observation as depicted in Table **2**. Substitution at **R1** position of benzimidazole ring augmented the anti-inflammatory activity as like *in vitro* activity, whereas substitution on 2-phenyl ring showed reduced activity, however compound **7j** (**R=OCH_3** and **R1=NO_2**) exhibited greater protection at the first hour of carrageenan injection (81.26%). Compound **7c** (R=H; R1=Cl, 77.7%) exhibited superior activity over **7b** (R=H; R1=NO_2, 63.3%).

Molecular Docking Studies

During the design, it was speculated that benzimidazole derivatives might bind to ATP binding site of p38 kinase. To ascertain this, all the inhibitors were docked in the ATP site and analyzed for the binding interactions which were found to be different from triarylimidazole derivatives exemplified by **SB203580** [44]. The imidazole derivatives exhibited usual hydrogen bond interaction with NH of Met109 however benzimidazole derivatives did not show such hydrogen bond interaction, some of the compounds demonstrated two different hydrogen bond interactions. N3 of benzimidazole revealed a new hydrogen bond interaction with Asn159 (2.64 Å), N1 of benzimidazole showed hydrogen bond interaction with Glu163 (2.402 Å), both of which seemed to be rare in aryl imidazole derivatives as shown in Fig. **(2a).** The CO and NH group of Met109 were away by ~5.5 Å from amide group of benzimidazoles and the benzimidazole scaffold of all molecules was positioned away from the imidazole group of phenoxypyrimidinyl imidazole derivatives by ~8 Å. The 2-substituted phenyl ring of benzimidazoles was in hydrophobic area surrounded by the backbones of Ala157, Leu164 and Lys165. The benzimidazole nucleus was surrounded by Met109, Asn159 and Glu163 (Fig. **2b**). The methoxy groups of **7d** and **7h** (R1=4-OCH_3) were found exposed into unfavorable hydrophilic environment (Solvent), comparatively **7h** observed to be more exposed to hydrophilic interactions due to displaced orientation than **7d**.

Characterization Data of Synthesized Compounds

5-Nitro-2-Phenylbenzimidazole (4a)

Yield: 84.0 %, Melting Point: 138-142˚C. **^{1}H NMR** ($CDCl_3$) δ (ppm): 10.1 (br. s, 1H, N<u>H</u>), 8.5 (s, 1H, C_4,

Table 2. Anti-inflammatory Activity of 7a-j in Carrageenan Induced Paw Oedema in Rats [a, b].

Groups	Oedema Volume in ml (Mean % Inhibition)			
	1 hr	2 hr	3 hr	4 hr
Control	0.427±0.211	0.495±0.232	0.568±0.277	0.608±0.3
Indomethacin	0.290±0.036*** (35.59±4.6)	0.207±0.047*** (58.18±9.2)	0.212±0.069*** (62.67±9.8)	0.216±0.140*** (64.47±10.0)
7a	0.199±0.126*** (53.4±7.2)	0.288±0.152** (32.6±3.3)	0.434±0.48* (23.6±3.1)	0.505±0.455* (16.9±2.4)
7b	0.160±0.212*** (62.5±5.8)	0.235±0.2*** (52.5±5.3)	0.375± 0.32** (33.9±2.4)	0.432±0.310** (29.0±2.1)
7c	0.112±0.092*** (73.8±6.9)	0.318±0.112** (35.35±5.1)	0.492±0.368* (13.9±2.9)	0.562±0.33 (07.6±1.3)
7d	0.236±0.21*** (44.7±5.7)	0.318±0.112*** (35.75±3.2)	0.445±0.32** (21.6±3.0)	0.507±0.29** (16.63±2.7)
7e	0.220±0.162*** (48.5±3.5)	0.345±0.117** (30.3±3.1)	0.398±0.14* (29.9±3.8)	0.456±0.131** (25.0±2.8)
7f	0.152±0.166*** (64.4±3.7)	0.33±0.2** (33.34±2.6)	0.498±0.428* (12.5±1.8)	0.543±0.36* (10.7±0.9)
7g	0.224±0.221*** (47.54±7.0)	0.332±0.121** (32.9±6.6)	0.415± 0.511* (26.93±5.5)	0.462±0.5** (24.0±5.1)
7h	0.212±0.112*** (50.35±6.7)	0.365±0.11** (26.7±3.5)	0.472±).413** (16.9±3.1)	0.522±0.4** (14.1±2.7)
7i	0.265±0.212** (37.93±7.0)	0.37±0.08* (25.3±5.8)	0.495±0.36* (12.9±4.1)	0.558±0.338 (8.22±3.6)
7j	0.089± 0.092*** (79.15±10.0)	0.156±0.142*** (63.5±7.6)	0.307±0.363*** (45.95±5.8)	0.437±0.27*** (28.1±3.0)

[a] Results expressed in mean ± SEM (n=6), ANOVA followed by Dunnett's multiple comparison test. *<0.05, **p<0.01, *** p<0.001 as compared to control group.
[b] Compounds **7a-j** tested at 50 mg/kg dose and indomethacin at 10 mg/kg dose

benzimidazole), 8.25 (d, 1H, C_6 benzimidazole), 8.22 (d, 1H, C_7 benzimidazole), 7.5-7.6 (t, 3H, aromatic C'_3, C'_4 and C'_5) and 7.3 (d, 2H, aromatic C'_2 and C'_6). **ESI-MS** (m/z): 240.1 $[M+H]^+$.

2-(4-Fluorophenyl)-5-nitrobenzimidazole (4b)

Yield: 79.8 %, Melting Point: >240 ˚C. **^{1}H NMR** ($CDCl_3$) δ (ppm): 10.3 (br. s, 1H, NH), 8.3 (s, 1H, C_4, benzimidazole), 8.20 (d, 1H, C_6 benzimidazole), 8.16 (d, 1H, C_7 benzimidazole), 7.5 (d, 2H, aromatic C'_3, and C'_5) and 7.4 (d, 2H, aromatic C'_2 and C'_6). **ESI-MS** (m/z): 258.0 $[M+H]^+$.

2-(4-methoxyphenyl)-5-nitrobenzimidazole (4c)

Yield: 79.8 %, Melting Point: 228-230 °C. **^{1}H NMR** ($CDCl_3$) δ (ppm): 10.2 (br. s, 1H, NH), 8.4 (s, 1H, C_4, benzimidazole), 8.20 (d, 1H, C_6 benzimidazole), 8.11 (d, 1H, C_7 benzimidazole), 7.3 (d, 2H, aromatic C'_2 and C'_6) and 7.2 (d, 2H, aromatic C'_3, and C'_5), 3.7 (s, 3H, OCH_3). **ESI-MS** (m/z): 270.1 $[M+H]^+$.

5-Amino-2-Phenylbenzimidazole (5a)

Yield: 65.5 %, IR (KBr, ν_{max} cm^{-1}): 3326, 3250 (–NH), 3020 (CH aromatic), 1517 and 1460 (aromatic C=C), and 1598 (C-N bnd). **^{1}H NMR** ($CDCl_3$) δ (ppm): 9.8 (br. s, 1H, NH), 8.0 (d, 1H, C_7, benzimidazole), 7.9 (d, 1H C_6 benzimidazole), 7.7 (s, 1H, C_4, benzimidazole), 7.31-7.4 (t, 3H, aromatic C'_3, C'_4 and C'_5) and 7.1 (d, 2H, aromatic C'_2 and C'_6) and 3.5-3.8 (b, 2H, NH_2). **ESI-MS** (m/z): 210.2 $[M+H]^+$.

5-N-benzoylamino-2-Phenyl-benzimidazole (7a)

Yield: 75.5%, Melting Point: 214-16 ˚C. **IR** (**KBr**): ~3200 cm^{-1} (ν NH str), ~2990 cm^{-1} (ν –CH- aromatic, str), ~1720 cm^{-1} (ν CO str), 1600 cm^{-1} (ν NH bend). **^{1}H NMR** (DMSO d_6) δ (ppm): 12.8 (br. s, 1H, amide NH), 10.3 (br. s, 1H, NH benzimidazole), 8.25 (d, 2H, C''_2, C''_6 aromatic), 8.1-8.2 (t, 3H C''_3, C''_4, C''_5 aromatic), 7.94-8.0 (d, 2H C_6, C_7 benzimidazole), 7.45-7.64 (m, 6H aromatic and C_4 benzimidiazole). **ESI-MS** (m/z): 314.2 $[M+H]^+$.

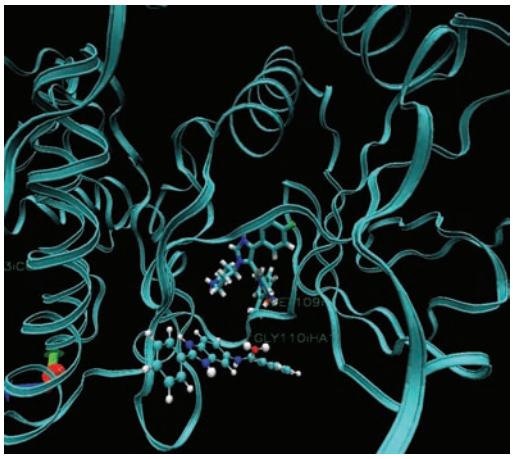


Fig. (2a). The binding pose of **7a** (capped stick) and triaryl imidazole (ball stick).

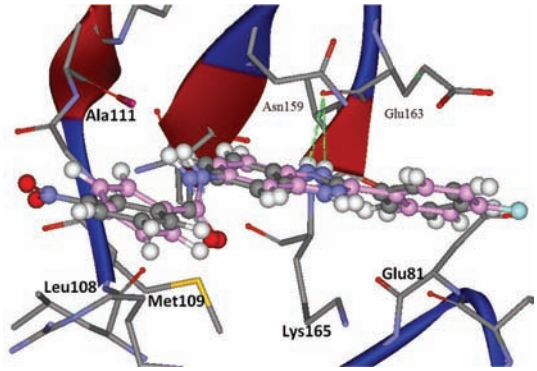


Fig. (2b). The binding pose of **7a** (ash colour) and **7f** (pink colour).

2-Phenyl-5-N-(4″-Nitrobenzoyl)aminobenzimidazole (7b)

Yield: 68.8 %, Melting Point: >300 ˚C, **IR** (**KBr**): ~3250 cm^{-1} (ν NH str), ~2990 cm^{-1} (ν –CH- aromatic, str), ~1700 cm^{-1} (ν CO str), 1600 cm^{-1} (ν NH bend). **^{1}H NMR** (DMSO d_6) δ (ppm): 12.7 (br. s, 1H, amide NH), 10.1 (br. s, 1H, NH benzimidazole), 8.3 (d, 2H C''_3, C''_5 aromatic), 8.20 (d, 2H, C''_2, C''_6 aromatic), 8.0 (d, 2H, C_6, C_7 benzimidazole), 7.8 (s, 1H, C_4 benzimidazole), 7.2-7.5 (m, 5H, aromatic). **ESI-MS** (m/z): 359.4 $[M+H]^+$.

2-Phenyl-5-N-(4″-Chlorobenzoyl)aminobenzimidazole (7c)

Yield: 62.3 %, Melting Point: >300 ˚C, **IR** (**KBr**): ~3180 cm^{-1} (ν NH str), ~3000 cm^{-1} (ν –CH- aromatic, str), ~1750 cm^{-1} (ν CO str). **^{1}H NMR** (DMSO d_6) δ (ppm): 12.4 (br. s, 1H, amide NH), 9.8 (br. s, 1H, NH benzimidazole), 8.2 (d, 2H, C''_2, C''_6 aromatic), 8.1 (d, 2H, C''_3, C''_5 aromatic), 7.9 (s, 1H, C_4 benzimidazole), 7.8 (d, 2H, C_6, C_7 benzimidazole), 7.4 (d, 2H, C'_2, C'_6 aromatic), 7.3 (t, 3H, C'_3, C'_4 and C'_5 aromatic). **ESI-MS** (m/z): 348.4 $[M+H]^+$.

2-Phenyl-5-N-(4″-methoxybenzoyl)aminobenzimidazole (7d)

Yield: 60.5 %, Melting Point: 156-58 ˚C, **IR KBr**): ~3300 cm^{-1} (ν NH str), ~1650 cm^{-1} (ν –CO- str), 1600 cm^{-1} (ν NH bend). **^{1}H NMR** (DMSO d_6) δ (ppm): 12.1 (br. s, 1H, amide NH), 10.0 (br. s, 1H, NH benzimidazole), 8.0 (d, 2H, C''_2, C''_6 aromatic), 7.85 (s, 1H, C_4 benzimidazole), 7.8 (d, 2H, C''_2, C''_6, aromatic), 7.7 (d, 2H, C_6, C_7 benzimidazole), 7.45 (d, 2H, C'_2, C'_6 aromatic), 7.3 (t, 3H, C'_3, C'_4 and C'_5 aromatic), 3.8 (s, 3H, OCH_3). **ESI-MS** (m/z): 344 $[M+H]^+$.

2-(4′-Fluorophenyl)-5-N-benzoylaminobenzimidazole (7e)

Melting Point: 224-26 ˚C. **IR** (**KBr**): ~3200 cm^{-1} (ν NH str), ~3000 cm^{-1} (ν –CH- aromatic, str), ~1680 cm^{-1} (ν CO str), 1600 cm^{-1} (ν NH bend). **^{1}H NMR** (DMSO d_6) δ (ppm): 12.1 (br. s, 1H, amide NH), 9.8 (br. s, 1H,.1 NH benzimidazole), 8.2 (d, 2H, C''_2 and C''_6 aromatic), 8.05- (t, 3H C''_3, C''_4, C''_5 aromatic), 7.9 (d, 2H, C_6, C_7, benzimidazole), 7.8 (s, 1H, C_4 benzimidazole), 7.6 (d, 2H, C'_3, C'_5 aromatic), 7.4 (d, 2H, C'_2, C'_6 aromatic), 7.1 (t, 3H, C''_3, C''_4 and C''_5 aromatic). **ESI-MS** (m/z): 333 $[M+H]^+$.

2-(4′-Fluorophenyl)-5-N-(4″-nitrobenzoyl)aminobenzimidazole (7f)

Yield: 67.7 % Melting Point: 295-97 ˚C, **IR** (**KBr**): ~3250 cm^{-1} (ν NH str), ~3000 cm^{-1} (ν –CH aromatic, str), ~1750 cm^{-1} (ν CO str), 1600 cm^{-1} (ν NH bend). **^{1}H NMR** (DMSO d_6) δ (ppm): 12.8 (br. s, 1H, amide NH), 10.3 (br. s, 1H, NH benzimidazole), 8.35 (d, 2H, C''_3, C''_5 aromatic), 8.15 (d, 2H, C''_2, C''_6 aromatic), 8.0 (s, 1H, C_4 benzimidazole), 7.8 (d, 2H, C_6, C_7 benzimidazole), 7.45 (d, 2H, C'_3, C'_5, aromatic), 7.25 (d, 2H, C'_2, C'_6,aromatic). **ESI-MS** (m/z): 377 $[M+H]^+$.

2-(4'-Fluorophenyl)-5-N-(4"-chlorobenzoyl)*aminobenzimidazole *(7g)

Yield: 66.9%, Melting Point: 298-300 ˚C, **IR** (**KBr**): ~3200 cm^{-1} (ν NH str), ~2990 cm^{-1} (ν –CH- aromatic, str), ~1750 cm^{-1} (ν CO str), 1600 cm^{-1} (ν NH bend). **^{1}H NMR** (DMSO d_6) δ (ppm): 12.28 (br. s, 1H, amide NH), 9.8 (br. s, 1H, NH benzimidazole), 8.15 (d, 2H, C''_2, C''_6 aromatic), 8.04 (d, 2H, C''_3, C''_5 aromatic), 7.87 (s, 1H, C_4 benzimidazole), 7.75 (d, 2H, C_6, C_7 benzimidazole), 7.5 (d, 2H, C'_2, C'_6 aromatic), 7.3 (d, 2H, C'_3, C'_5 aromatic). **ESI-MS** (m/z): 366.5 $[M+H]^+$.

2-(4′-Fluorophenyl)-5-N-(4″-methoxybenzoyl)aminobenzimidazole (7h)

Yield: 59.0%, Melting Point: 232-34 ˚C. **IR** (**KBr**): ~3200 cm^{-1} (ν NH str), ~2990 cm^{-1} (ν –CH- aromatic, str), ~1680 cm^{-1} (ν CO str), 1600 cm^{-1} (ν NH bend). **^{1}H NMR** (DMSO d_6) δ (ppm): 12.2 (br. s, 1H, amide NH), 10.1 (br. s, 1H, NH benzimidazole), 8.04 (d, 2H, C''_2, C''_6 aromatic), 7.9 (s, 1H, C_4 benzimidazole), 7.89 (d, 2H, C''_3, C''_5, aromatic), 7.77 (d, 2H, C_6, C_7 benzimidazole), 7.55 (d, 2H, C'_2, C'_6 aromatic), 7.32 (d, 2H, C'_3, C'_5 aromatic), 3.85 (s, 3H, OCH_3). **ESI-MS** (m/z): 362 $[M+H]^+$.

2-(4′-Methoxyphenyl)-5-N-benzoylaminobenzimidazole (7i)

Yield: 66.0%, Melting Point: 198-200 ˚C. **IR** (**KBr**): ~3000 cm^{-1} (ν –CH- aromatic, str), ~2930 (ν, -CH_3, str), ~1730 cm^{-1} (ν CO str), 1610 cm^{-1} (ν NH bend). **ESI-MS** (m/z): 344.7 $[M+H]^+$

2-(4′-Methoxyphenyl)-5-N-(4″-nitrobenzoyl) aminobenzimidazole (7j)

Yield: 61.0 %, Melting Point: 258-60 ˚C. **IR** (**KBr**): ~3200 cm^{-1} (ν NH str), ~3000 cm^{-1} (ν –CH- aromatic, str),

~2930 (ν, -CH_3, str), ~1760 cm^{-1} (ν CO str), 1600 cm^{-1} (ν NH bend). **^{1}H NMR** (DMSO d_6) δ (ppm): 11.8 (br. s, 1H, amide NH), 10.0 (br. s, 1H, NH benzimidazole), 8.25 (d, 2H, C''_3, C''_5 aromatic), 8.17 (d, 2H, C''_2, C''_6, aromatic), 7.85 (s, 1H, C_4 benzimidazole), 7.7 (d, 2H, C_6, C_7 benzimidazole), 7.33 (d, 2H, C'_2, C'_6, aromatic), 7.1 (d, 2H, C'_3, C'_5,aromatic), 3.75 (s, 3H, OCH_3). **ESI-MS** (m/z): 389 $[M+H]^+$.

CONCLUSION

In summary, we have identified 5-substituted benzoyl-amino-2-substituted phenylbenzimidazoles as new p38 kinase inhibitors. The initial SAR reveals that compounds **7b** and **7c** were found to be moderately active in both models. Substitution at both **R** and **R1** may not be effective however compound **7j** exhibited activity which contradicts our explanation. New molecules with diverse substitution at R1 and methoxy group at R may help to understand incites of SAR.

CONFLICT OF INTEREST

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