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Exploration of 2-benzylbenzimidazole scaffold as novel inhibitor of NF-κB

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ABSTRACT

For finding the novel inhibitor of nuclear factor κB activity, a series of benzimidazole derivatives were rationally designed, synthesized and systematically studied for their in vitro activities against LPS induced NF-κB inhibition in RAW 264.7 cells using the SEAP assay based on the flexible chalcone JSH ((*E*)-1-(2-hydroxy-6-(isopentyloxy)phenyl)-3-(4-hydroxy phenyl)prop-2-en-1-one) which was previously reported. Although most of the benzimidazole derivatives showed strong inhibitory activity in low micromolar potency, 2-(4-methoxybenzyl)-1*H*-benzo[*d*]imidazole (**3m**; IC_{50} = 1.7 μM) and 2-(2-methoxybenzyl)-1*H*-benzo[*d*]imidazole (**3n**; IC_{50} = 2.4 μM) showed the best inhibition. The structure activity relationship revealed that 2-benzylbenzimidazole scaffold with hydrogen bonding acceptor on phenyl ring appears as a pharmacophore.

1. Introduction

Nuclear factor κB (NF-κB) transcription factor plays a key role in immune process and cell proliferation and survival.[1,2] NF-κB controls the expression of multiple genes and effector function of lymphocytes.[3–5] More specifically NF-κB regulates interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α).[6] Among transcriptional factors, NF-κB has been known as the first responder to harmful cellular stimuli, such as physiological stress, bacterial or viral infection, and other internal proteins. Furthermore, constitutively active form of NF-κB have been frequently found in cancers[7] and in many inflammatory diseases,[8] such as inflammatory bowel disease, arthritis, sepsis, gastritis, asthma and atherosclerosis. Therefore, the regulation or inhibition of NF-κB transcription should be effective in the treatment of cancers[7] and inflammatory diseases[9] and this approach could be a highly effective way to find a novel immuno-inflammatory agents.[10]

Some of the representative examples for known chemotherapeutic agents acting through the inhibition of NF-κB transcription are shown in Figure 1.[11–15] For a decade, our group have explored the NF-κB inhibitors and discovered a novel small molecule, (*E*)-1-(2-hydroxy-6-(isopentyloxy)phenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one (JSH, Fig. 1)[16] and successfully demonstrated it as an inhibitor of LPS induced NF-κB activation in macrophages with an IC_{50} value of about 10 μM. It is a non-lipid chalcone which not only inhibited the TLR4-mediated NF-κB activating pathway but also suppresses the NF-κB regulated expression of iNOS, COX-2 or pro-inflammatory cytokine genes. Subsequently, we explored structure activity relationship of JSH analogs related to NF-κB inhibition and cytotoxicity against human cancer cell lines.[17]

Since JSH is flexible and eventually has many conformations and hypothetically isomerize to rigid flavanone structure, we assumed a rigid structure with effective conformation might exert better inhibition of NF-κB. Thus, rigid scaffolds like benzimidazole, benzoxazole and benzothiazole were designed retaining HBA and HBD characteristics of chalcone scaffold in JSH (Fig. 2).

2. Chemistry

Preparation of benzimidazole **3a**–**y**, benzoxazole **4a**,**b** and benzothiazole **5** analogs were outlined in Schemes 1–3 and substituents are listed in Table 1. Commercially available substituted benzene-1,2-diamine **1** was reacted with appropriate various substituted 2-phenylacetic acids **2** in xylene solvent in the presence of catalytic amount of boric acid[18] at reflux temperature for 12–16 h

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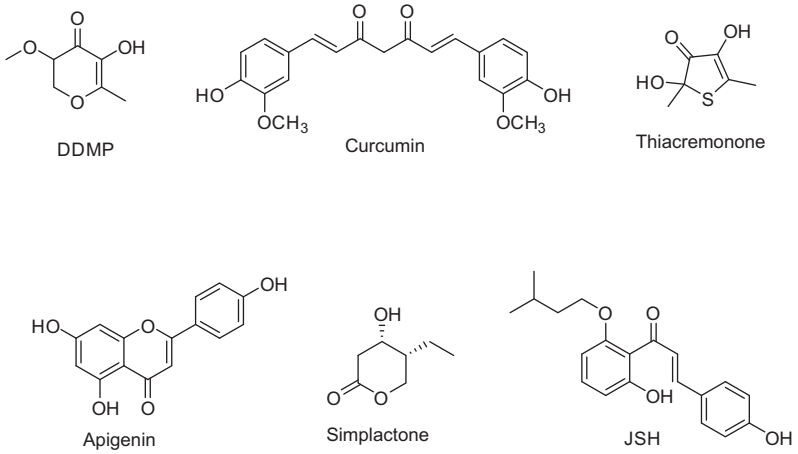


Figure 1. Known chemotherapeutic agents through inactivation of NF-κB.

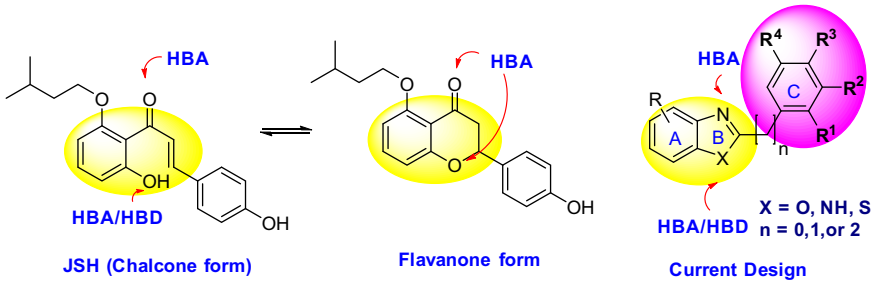
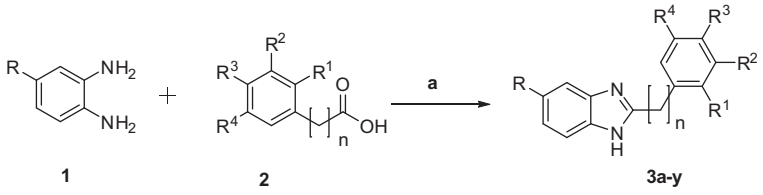
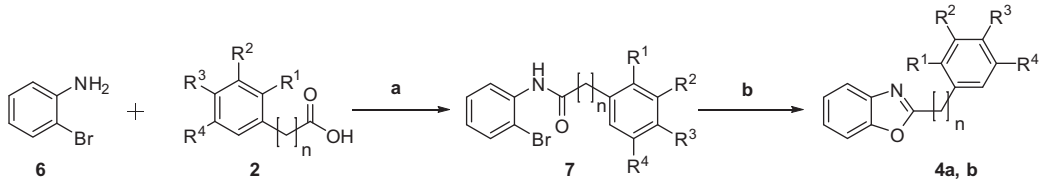


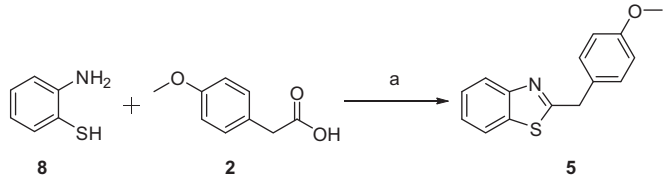
Figure 2. Design of benzimidazoles, benzoxazoles and benzothiazole as NF-κB inhibitor.



Scheme 1. Synthesis of benzimidazole derivatives **3a–y** (substituents are listed in Table 1). Reagents and conditions: (a) boric acid, xylene, reflux, 12–16 h, 45–90%.



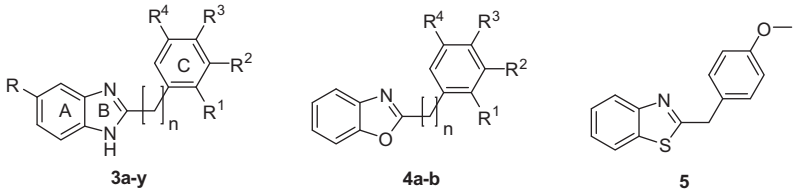
Scheme 2. Synthesis of benzoxazoles derivatives **4a**,**b** (substituents are listed in Table 1). Reagents and conditions: (a) EDC·HCl, DIPEA, DCM, rt, 16 h, 85%; (b) CuI (5 mol %), 1,10-phenanthroline (10 mol %), Cs_2CO_3, toluene, reflux, 16 h, 75–80%.



Scheme 3. Synthesis of benzothiazole **5**. Reagents and conditions: (a) boric acid, xylene, reflux, 16 h, 48%.

to produce desired benzimidazole compounds **3a–y**. In order to obtain benzoxazole **4a** and **4b** compounds, 2-bromoaniline (**6**) was coupled with appropriate substituted phenylacetic acids **2** using EDC·HCl, DIPEA, DCM, condition at room temperature to give key intermediate **7**. This key intermediate was cyclized with CuI, 1,10-phenanthroline, Cs_2CO_3, toluene, at reflux temperature for 16 h to afford benzoxazoles **4a**,**b**.[19] Benzothiazole **5** was obtained by refluxing 2-aminobenzenethiol (**8**) with 4-methoxyphenylacetic acid in xylene.

Table 1
In vitro NF-κB inhibitory activity of benzimidazoles **3a**–**y**, benzoxazoles **4a**,**b** and benzothiazole **5**



Compd No.	R	R^1	R^2	R^3	R^4	*n*	% of inhibition at 30 μM	[a]IC_{50} value μM
3a	H	H	H	H	H	0	22	>30.0
3b	H	H	H	Cl	H	0	12	>30.0
3c	H	H	H	OCH_3	H	0	22	>30.0
3d	H	H	H	H	H	1	43	18.4
3e	H	H	H	Cl	H	1	85	2.4
3f	H	H	Cl	H	H	1	66	10.0
3g	H	Cl	H	H	H	1	33	>30
3h	H	H	Cl	Cl	H	1	63	15.0
3i	H	H	H	CF_3	H	1	55	16.4
3j	H	H	H	NO_2	H	1	64	12.0
3k	H	H	H	SO_2CH_3	H	1	43	28.0
3l	H	H	H	CH_3	H	1	60	19.0
3m	H	H	H	OCH_3	H	1	90	1.7
3n	H	OCH_3	H	H	H	1	85	2.4
3o	H	H	OCH_3	OCH_3	H	1	32	>30.0
3p	H	H	OCH_3	OCH_3	OCH_3	1	32	>30.0
3q	H	H	H	OH	H	1	75	6.0
3r	H	H	H	NH_2	H	1	40	>30.0
3s	H	H	OH	OH	H	1	75	6.0
3t	Cl	H	H	H	H	1	31	>30
3u	OCH_3	H	H	H	H	1	45	18.0
3v	Cl	H	H	Cl	H	1	76	5.0
3w	OCH_3	H	H	OCH_3	H	1	61	>30.0
3x	Cl	H	H	OCH_3	H	1	66	10.0
3y	H	H	H	OCH_3	H	2	50	>30.0
4a	—	H	H	H	H	1	33	>30.0
4b	—	H	H	OCH_3	H	1	42	>30.0
5	—	—	—	—	—	—	67	9

[a] IC_{50} values are taken as a mean from three experiments.

3. Bioassay

Inhibitory effect of compounds **3a**–**y**, **4a**,**b** and **5** on NF-κB transcriptional activity was determined as described previously.[20] In brief, RAW 264.7 monocytic cell line stably transfected with NF-κB-secretory alkaline phosphatase (SEAP) construct, a reporter gene of NF-κB transcriptional activity, was stimulated with LPS (30 ng/mL) for 20 h in the presence of sample. Aliquots of the culture supernatants were heated to 65 °C for 5 min and then reacted with 4-methylumbelliferyl phosphate (500 μM) in the dark. SEAP activity was measured as relative fluorescence units (RFUs) under excitation at 360 nm and emission at 450 nm. The results are summarized in Table 1, representing as the IC_{50} value or inhibition% at the concentration 30 μM from three independent experiments using the average values of triplicate in each experiment.

4. Results and discussion

Initial investigation of 2-phenylbenzimidazole analogs **3a**–**c** without any spacer between the phenyl and benzimidazole rings showed very poor activity (Table 1). Thus, methylene groups were inserted between the benzimidazole and phenyl rings. Surprisingly, 2-benzylbenzimidazole (**3d**, inhibition at 30 μM = 43%, IC_{50} = 18.4 μM) showed considerably increased activity.

With these encouraging results, exploration of the effect of substituents on the C ring was attempted. Accordingly, we prepared 4-chloro substituted compound **3e** (inhibition at 30 μM = 85%, IC_{50} = 2.4 μM) which showed good NF-κB inhibitory activity indicating that mild electron withdrawing or hydrophobic group should be beneficial for the activity.

In order to establish the suitable position of chloro group in the phenyl ring of **3d**, position of chloro group was variated as shown in compounds **3f** and **3g**. Compound **3f** (R_2 = Cl; IC_{50} = 10.0 μM) showed reduced activity compared to **3e** and increased activity compared to **3d** while compound **3g** (R_1 = Cl; IC_{50} = >30.0 μM) did not show activity. These results indicated that chloro substitution at position 4 on phenyl ring of **3d** as shown in **3e** should be optimum for the inhibition of NF-κB and there is a possibility to enhance the activity with dichloro substitution on phenyl ring of **3d**. Thus, additional chloro group was introduced as shown in **3h** (R_2, R_3 = Cl, IC_{50} = 15.0 μM). However, the activity was decreased compared to mono chloro analogs **3e** and **3f**.

Further, to explore the effect of more electron withdrawing groups on the phenyl ring we prepared compounds **3i** (R_3 = CF_3, IC_{50} = 16.4 μM), **3j** (R_3 = NO_2, IC_{50} = 12.0 μM) and **3k** (R_3 = SO_2CH_3, IC_{50} = 28.0 μM). However, all of them showed decrement in activity. These results suggested that hydrophobic character of 4-chloro substituent of benzimidazole **3e** might more contribute to the inhibition of NF-κB rather than electron withdrawing property.

Thus, to increase the hydrophobic character of benzimidazole **3d**, replacement of chloro group with methyl group at R_3 position as shown in **3l** (IC_{50} = 19.0 μM) was performed. However, this analog showed weak inhibitory activity.

Therefore, we examined the role of electron donating group with increasing π constant at R_3 position. Replacement of methyl with methoxy group as shown in **3m** (90% inhibition,

IC_{50} = 1.7 μM) remarkably enhanced the inhibitory activity. Shift of the methoxy group from *para* to *ortho* position as shown in **3n** (R_1 = OCH_3; IC_{50} = 2.4 μM) retained the activity. These results might indicate that the electron donating group with hydrogen bonding acceptor in the phenyl ring of **3d** is important. Since *ortho*- or *para*-substituted methoxy analogs showed good activity, additional methoxy groups on phenyl ring as shown in 3,4-dimethoxy **3o** (IC_{50} = >30 μM) and 3,4,5-trimethoxy analog **3p** (IC_{50} = >30 μM) were introduced. However, these analogs abolished the activity. These results indicated that relatively small binding pocket of the receptor fits well for a mono methoxy group around the benzyl ring of benzimidazole **3d** scaffold.

Considering hydrogen bonding, we further investigated the effect of hydrogen bonding donors and therefore, prepared compound **3q** (R_3 = OH; IC_{50} = 6.0 μM). This analog showed significant activity whereas the amino counterpart **3r** (R_3 = NH_2; IC_{50} = >30 - μM) did not show any activity. In continuation dihydroxy analog **3s** (IC_{50} = 6.0 μM) was prepared and showed the same level of activity as mono hydroxy analog **3q**. Addition of hydrogen bonding donor property did not improve the activity. Therefore, hydrogen bonding acceptor at R_3 position on the phenyl ring of **3d** should be suitable for the activity.

To study the importance of substituents on the A ring of the benzimidazole, compounds **3t** (R = Cl; IC_{50} >30.0 μM) and **3u** (R = OCH_3; IC_{50} = 18.0 μM) were prepared which did not improve the inhibitory activity. Keeping in mind the increment of the activity by substitution with chloro or methoxy group at R_3 position on phenyl ring of **3d** as shown in **3e** and **3m**, benzimidazoles with substitution at 5-position and R_3 position of phenyl ring as shown in **3v** (R, R_3 = Cl, IC_{50} = 5.0 μM), **3w** (R, R_3 = OCH_3, IC_{50} >30.0 μM) and **3x** (R = Cl, R_3 = OCH_3, IC_{50} = 10.0 μM) were prepared. However, none of them improved the activity. These findings strongly emphasized that the substitution on the benzimidazole ring has adverse effect on the inhibitory activity.

To further explore the structure activity relationship, we increased the carbon chain between the two rings as shown in compound **3y** (n = 2, R_3 = OCH_3, IC_{50} >30.0 μM), which lost the activity. This result implied that only one methylene group between the two rings is optimum for the inhibitory activity.

All the above results indicate that the unsubstituted benzimidazole with one carbon spacer to the phenyl ring with methoxy group at R_3 position as shown in compound **3m** demonstrated as a pharmacophore for the potent inhibition of NF-κB activity.

To define the SAR on the B ring of benzimidazole, its isosteres, benzoxazole derivatives (**4a**; IC_{50} >30.0 μM and **4b**; IC_{50} >30.0 μM) and benzothiazole derivative **5** were prepared and these were found to be inactive. These results are attesting that imidazole moiety of benzimidazole is critical for NF-kB inhibitory activity.

5. Conclusion

On the basis of SAR, the 2-benzylbenzimidazole analogs showed moderate to good activity, especially hydrophobic hydrogen bonding acceptor (OCH_3) substituent on C ring showed potent NF-κB inhibition. Substitution on the A ring is not favorable for the inhibitory activity. Altogether, we defined the new rigid 2-benzylbenzimidazole scaffold as a lead from flexible chalcone JSH compound for finding a novel and potent inhibitor of NF-κB.

6. Materials and methods

6.1. General information

Melting points (mp) were determined on an Electro thermal 1A 9100 MK2 apparatus and are uncorrected. All commercial chemicals were used as obtained and all solvents were purified prior to use applying standard procedures. Thin layer chromatography was performed on E Merck silica gel GF-254 pre-coated plates; identification was performed under UV illumination and colorization with 10% phosphomolybdic acid spray followed by heating. Flash column chromatography was performed on E Merck silica gel (230–400 mesh). Infrared spectra were recorded on a Nicolet 380 model FTIR. NMR spectra were measured against the peak of tetramethylsilane using a Varian Unity Inova 400 NMR (400 MHz) spectrometer. We noted that some quaternary carbon signals in ^{13}C NMR spectra of benzimidazoles **3** are not observable possibly due to tautomerism.[21] High resolution mass spectrum (HRMS) was recorded on PE SCIEX API 2000 (triple quadrupole) mass spectrophotometer (Applied Biosystems, Foster City, CA, USA).

6.2. General procedure to synthesis of compounds 3a–y and 5

To a stirred solution of benzene-1,2-diamine **1** (1.85 mmol) in xylenes (10 mL) were added carboxylic acid **2** (2.77 mmol) and boric acid (0.185 mmol). The resulting solution was refluxed for 16 h. After cooling to room temperature, the reaction was concentrated under reduced pressure and diluted with EtOAc (50 mL). The organic phase was washed with saturated $NaHCO_3$ solution (2 × 50 mL), dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (eluting with 10–15% Ethyl acetate in hexanes) to afford the title compounds **3a**–**y** and **5**.

6.2.1. 2-Phenyl-1*H*-benzo[*d*]imidazole (3a)

Yield 60%; Light yellow solid; mp 286–287 °C; IR (KBr) 2950, 2747, 1445, 1421, 1275, 1085, 748 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.92 (br s, 1H), 8.15–8.22 (m, 2H), 7.64–7.71 (m, 1H), 7.46–7.59 (m, 4H), 7.16–7.27 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 151.3, 143.9, 135.1, 130.3, 129.9, 129.0, 126.6, 126.5, 126.5, 122.6, 121.8, 119.0, 111.4; HRMS calcd for $C_{13}H_{10}N_2$ m/z 194.0825, found 194.0823.

6.2.2. 2-(4-Chlorophenyl)-1*H*-benzo[*d*]imidazole (3b)

Yield 65%; Off white solid; mp 289–291 °C; IR (KBr) 2747, 1448, 1428, 1278, 1089, 830, 742 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 13.00 (br s, 1H), 8.16–8.22 (m, 2H), 7.60–7.71 (m, 3H), 7.50–7.58 (m, 1H), 7.17–7.28 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.3, 143.9, 135.1, 134.6, 129.2, 129.2, 128.3, 128.2, 128.2, 122.9, 122.0, 119.1, 111.5; HRMS calcd for $C_{13}H_9ClN_2$ m/z 228.0534, found 228.05131.

6.2.3. 2-(4-Methoxyphenyl)-1*H*-benzo[*d*]imidazole (3c)

Yield 45%; White solid; mp 224–226 °C; IR (KBr) 2940, 2757, 1441, 1426, 1278, 1089, 830, 739 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H), 8.08–8.16 (m, 2H), 7.58–7.66 (m, 1H), 7.46–7.54 (m, 1H), 7.17 (m, 2H), 7.09–7.13 (m, 2H), 3.34 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.7, 151.5, 144.0, 135.1, 128.1, 128.1, 128.1, 122.8, 122.2, 121.5, 118.6, 114.4, 111.1, 55.3; HRMS calcd for $C_{14}H_{12}N_2O$ m/z 224.0932, found 224.0931.

6.2.4. 2-Benzyl-1*H*-benzo[*d*]imidazole (3d)

Yield 85%; White solid; mp 189–190 °C; IR (KBr) 3001, 2857, 1512, 1456, 1415, 1248, 1175, 1029, 738 cm^{-1}; ^1H NMR (400 MHz, CHLOROFORM-d) δ 9.30–9.49 (m, 1H), 7.64–7.80 (m, 1H), 7.26–7.38 (m, 6H), 7.16–7.25 (m, 2H), 4.25–4.29 (m, 2H); ^{13}C NMR (100 MHz, CHLOROFORM-d) δ 153.4, 136.3, 129.1, 127.3, 122.4, 35.8; HRMS calcd for $C_{14}H_{12}N_2$ m/z 208.1025, found 208.1021.

6.2.5. 2-(4-Chlorobenzyl)-1*H*-benzo[*d*]imidazole (3e)

Yield 85%; Off white solid; mp 191–194 °C; IR (KBr) 2850, 2751, 1512, 1451, 1410, 1241, 1181, 1025, 812, 749 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.26 (br s, 1H), 7.52 (br s, 1H), 7.31–7.45 (m, 5H), 7.07–7.16 (m, 2H), 4.17 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.2, 136.7, 134.5, 131.3, 130.8, 128.5, 121.8, 121.1, 118.3, 111.0, 34.1; HRMS calcd for $C_{14}H_{11}ClN_2$ *m*/*z* 242.0625, found 242.0621.

6.2.6. 2-(3-Chlorobenzyl)-1*H*-benzo[*d*]imidazole (3f)

Yield 65%; White solid; mp 158–160 °C; IR (KBr) 3010, 2845, 2751, 1509, 1454, 1410, 1241, 1027, 775 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.32 (br s, 1H), 7.39–7.58 (m, 3H), 7.26–7.39 (m, 3H), 7.09–7.16 (m, 2H), 4.19 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.0, 140.2, 133.1, 130.4, 128.8, 127.7, 126.7, 121.5, 34.4; HRMS calcd for $C_{14}H_{11}ClN_2$ *m*/*z* 242.0637, found 242.0631.

6.2.7. 2-(2-Chlorobenzyl)-1*H*-benzo[*d*]imidazole (3g)

Yield 69%; Off white solid; mp 220–222 °C; IR (KBr) 3009, 2841, 2758, 1511, 1458, 1410, 1241, 1027, 750 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.29 (br s, 1H), 7.36–7.55 (m, 4H), 7.28–7.35 (m, 2H), 7.07–7.17 (m, 2H), 4.30 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.3, 143.5, 135.3, 134.5, 133.4, 131.6, 129.4, 128.8, 127.5, 121.8, 121.1, 118.4, 111.1, 32.7; HRMS calcd for $C_{14}H_{11}ClN_2$ *m*/*z* 242.0627, found 242.0621.

6.2.8. 2-(3,4-Dichlorobenzyl)-1*H*-benzo[*d*]imidazole (3h)

Yield 65%; Off white solid; mp 189–191 °C; IR (KBr) 2851, 2758, 1508, 1451, 1410, 1241, 1183, 1027, 754 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.30 (br s, 1H), 7.64 (d, *J* = 2.20 Hz, 1H), 7.59 (d, *J* = 8.05 Hz, 1H), 7.48–7.56 (m, 1H), 7.39–7.48 (m, 1H), 7.33 (dd, *J* = 2.19, 8.29 Hz, 1H), 7.08–7.18 (m, 2H), 4.21 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.7, 138.8, 131.0, 130.7, 129.5, 129.4, 121.5, 33.7; HRMS calcd for $C_{14}H_{10}Cl_2N_2$ *m*/*z* 276.0225, found 276.0221.

6.2.9. 2-(4-(Trifluoromethyl)benzyl)-1*H*-benzo[*d*]imidazole (3i)

Yield 59%; Pale yellow solid; mp 213–215 °C; IR (KBr) 2858, 2737, 1514, 1448, 1410, 1241, 1183, 1027, 813, 747 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.20–12.50 (br s, 1H), 7.66–7.73 (m, *J* = 8.54 Hz, 2H), 7.54–7.59 (m, *J* = 8.29 Hz, 2H), 7.43–7.52 (m, 2H), 7.06–7.20 (m, 2H), 4.29 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.9, 142.6, 129.8, 127.6, 127.3, 125.8, 125.5, 125.5, 125.5, 125.4, 123.1, 121.5, 34.6; HRMS calcd for $C_{15}H_{11}F_3N_2$ *m*/*z* 276.0925, found 276.0921.

6.2.10. 2-(4-Nitrobenzyl)-1*H*-benzo[*d*]imidazole (3j)

Yield 73%; Brick red solid; mp 217–219 °C; IR (KBr) 2850, 2747, 1505, 1456, 1410, 1241, 1183, 1027, 813, 741 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.37 (br s, 1H), 8.18–8.23 (m, 2H), 7.58–7.65 (m, 2H), 7.51–7.58 (m, 1H), 7.41–7.47 (m, 1H), 7.08–7.19 (m, 2H), 4.35 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.5, 146.5, 145.8, 130.3, 123.8, 121.6, 34.6; HRMS calcd for $C_{14}H_{11}N_3O_2$ *m*/*z* 253.0935, found 253.0931.

6.2.11. 2-(4-(Methylsulfonyl)benzyl)-1*H*-benzo[*d*]imidazole (3k)

Yield 55%; White solid; mp 188–190 °C; IR (KBr) 3010, 2845, 2751, 1509, 1454, 1410, 1241, 1027, 813, 748 cm^{-1};^1H NMR (400 MHz, DMSO-d_6) δ 12.37 (br s, 1H), 7.85–7.92 (m, 2H), 7.57–7.64 (m, *J* = 8.54 Hz, 2H), 7.50–7.57 (m, 1H), 7.39–7.46 (m, 1H), 7.07–7.18 (m, 2H), 4.31 (s, 2H), 3.18 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.7, 143.8, 139.3, 129.9, 127.3, 121.9, 121.2, 118.5, 111.1, 43.6, 34.6; HRMS calcd for $C_{15}H_{14}N_2O_2S$ *m*/*z* 286.0847, found 286.0841.

6.2.12. 2-(4-Methylbenzyl)-1*H*-benzo[*d*]imidazole (3l)

Yield 78%; Off white solid; mp 196–199 °C; IR (KBr) 2854, 2756, 1507, 1451, 1412, 1241, 1183, 1027, 812, 742 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.23 (br s, 1H), 7.49–7.55 (m, 1H), 7.37–7.43 (m, 1H), 7.18–7.23 (m, 2H), 7.07–7.15 (m, 4H), 4.11 (s, 2H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.9, 143.5, 135.7, 134.7, 134.6, 129.1, 128.8, 121.7, 121.0, 118.3, 111.0, 34.6, 20.6; HRMS calcd for $C_{15}H_{14}N_2$ *m*/*z* 222.1225, found 222.1221.

6.2.13. 2-(4-Methoxybenzyl)-1*H*-benzo[*d*]imidazole (3m)

Yield 80%; White solid; mp 163–164 °C; IR (KBr) 2840, 2757, 1509, 1454, 1410, 1241, 1183, 1027, 813, 749 cm^{-1}; ^1H NMR (400 MHz, CHLOROFORM-*d*) δ 9.38 (br s, 1H), 7.52 (br s, 2H), 7.17–7.24 (m, 4H), 6.87 (d, *J* = 8.54 Hz, 2H), 4.22 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (100 MHz, CHLOROFORM-*d*) δ 158.9, 153.8, 130.2, 128.2, 122.4, 114.5, 55.3, 35.0; HRMS calcd for $C_{15}H_{14}N_2O$ *m*/*z* 238.1125, found 238.1123.

6.2.14. 2-(2-Methoxybenzyl)-1*H*-benzo[*d*]imidazole (3n)

Yield 90%; White solid; mp 197–199 °C; IR (KBr) 3010, 2845, 2751, 1509, 1454, 1410, 1241, 1027, 745 cm^{-1}; ^1H NMR (400 MHz, CHLOROFORM-*d*) δ 9.41 (br s, 1H), 7.61 (br s, 1H), 7.23–7.35 (m, 3H), 7.19 (m, 2H), 6.89–6.99 (m, 2H), 4.27 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, CHLOROFORM-*d*) δ 157.0, 153.9, 131.2, 131.2, 131.1, 128.9, 128.8, 128.8, 125.2, 122.1, 121.5, 121.5, 111.1, 55.8, 30.9; HRMS calcd for $C_{15}H_{14}N_2O$ *m*/*z* 238.1145, found 238.1141.

6.2.15. 2-(3,4-Dimethoxybenzyl)-1*H*-benzo[*d*]imidazole (3o)

Yield 75%; Pale yellow solid; mp 160–162 °C; IR (KBr) 3011, 2842, 2751, 1512, 1410, 1241, 1025, 813, 738 cm^{-1}; ^1H NMR (400 MHz, CHLOROFORM-*d*) δ 9.28 (br s, 1H), 7.73 (d, *J* = 8.54 Hz, 1H), 7.29–7.37 (m, 1H), 7.18–7.26 (m, 2H), 6.78–6.88 (m, 3H), 4.23 (s, 2H), 3.86 (s, 3H), 3.79 (s, 3H); ^{13}C NMR (100 MHz, CHLOROFORM-*d*) δ 153.8, 149.3, 148.3, 128.7, 122.4, 121.2, 112.1, 111.4, 55.8, 55.8, 35.5; HRMS calcd for $C_{16}H_{16}N_2O_2$ *m*/*z* 268.1245, found 268.1241.

6.2.16. 2-(3,4,5-Trimethoxybenzyl)-1*H*-benzo[*d*]imidazole (3p)

Yield 76%; Pale yellow solid; mp 151–153 °C; IR (KBr) 3011, 2842, 2758, 1513, 1411, 1241, 1025, 813, 774 cm^{-1}; ^1H NMR (400 MHz, CHLOROFORM-*d*) δ 7.55 (br s, 2H), 7.21–7.27 (m, 2H), 6.49 (s, 2H), 4.21 (s, 2H), 3.81–3.84 (m, 3H), 3.76 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.7, 153.0, 136.3, 133.2, 121.4, 106.3, 60.0, 55.9, 35.4; HRMS calcd for $C_{17}H_{18}N_2O_3$ *m*/*z* 298.1347, found 298.1347.

6.2.17. 4-((1*H*-Benzo[*d*]imidazol-2-yl)methyl)phenol (3q)

Yield 70%; Off white solid; mp 264–266 °C; IR (KBr) 3258, 1511, 1440, 1250, 1027, 786, 736 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.17 (br s, 1H), 9.26 (s, 1H), 7.41–7.49 (m, 2H), 7.08–7.15 (m, 4H), 6.66–6.73 (m, 2H), 4.03 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.1, 154.3, 129.8, 127.8, 121.3, 115.3, 34.1; HRMS calcd for $C_{14}H_{12}N_2O$ *m*/*z* 224.0935, found 224.0931.

6.2.18. 4-((1*H*-Benzo[*d*]imidazol-2-yl)methyl)aniline (3r)

Yield 75%; Off white solid; mp 211–214 °C; IR (KBr) 3446, 3363, 2889, 1621, 1519, 1409, 1271, 1027, 809, 739 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.14 (br s, 1H), 7.37–7.52 (m, 2H), 7.07–7.13 (m, 2H), 6.93–7.00 (m, *J* = 8.29 Hz, 2H), 6.44–6.57 (m, *J* = 8.29 Hz, 2H), 4.93 (br s, 2H), 3.95 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.8, 147.4, 129.4, 124.5, 121.3, 114.1, 34.3 HRMS calcd for $C_{14}H_{13}N_3$ *m*/*z* 223.1135, found 223.1131.

6.2.19. 4-((1*H*-Benzo[*d*]imidazol-2-yl)methyl)benzene-1,2-diol (3s)

Yield 60%; Off white solid; mp 258–260 °C; IR (KBr) 3258, 3248, 1512, 1439, 1248, 1026, 812, 738 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.17 (br s, 1H), 8.87 (s, 1H), 8.75 (s, 1H), 7.45 (br s, 2H), 7.06–7.14 (m, 2H), 6.62–6.71 (m, 2H), 6.52–6.60 (m, 1H), 3.96 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.4, 145.4, 144.1, 128.5, 121.3, 119.6, 116.2, 115.6, 34.4; HRMS calcd for $C_{14}H_{12}N_2O_2$ *m*/*z* 240.0935, found 240.0931.

6.2.20. 2-Benzyl-5-chloro-1*H*-benzo[*d*]imidazole (3t)

Yield 65%; Light orange solid; mp 174–176 °C; IR (KBr) 3004, 2837, 1512, 1410, 1241, 1029, 775, 750 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.41–12.54 (m, 1H), 7.35–7.62 (m, 2H), 7.29–7.34 (m, 4H), 7.20–7.29 (m, 1H), 7.10–7.18 (m, 1H), 4.17 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.3, 137.5, 128.9, 128.7, 126.7, 121.7, 121.6, 34.9; HRMS calcd for $C_{14}H_{11}ClN_2$ *m*/*z* 242.0625, found 242.0622.

6.2.21. 2-Benzyl-5-methoxy-1*H*-benzo[*d*]imidazole (3u)

Yield 67%; Off white solid; mp 109–111 °C; IR (KBr) 3009, 2847, 1510, 1466, 1410, 1248, 1175, 1029, 775 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.04–12.14 (m, 1H), 7.18–7.44 (m, 6H), 6.91 – 7.07 (m, 1H), 6.70–6.79 (m, 1H), 4.12 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.5, 137.9, 128.8, 128.5, 126.6, 118.7, 110.1, 101.3, 94.5, 55.4, 34.9; HRMS calcd for $C_{15}H_{14}N_2O$ *m*/*z* 238.1182, found 238.1180.

6.2.22. 5-Chloro-2-(4-chlorobenzyl)-1*H*-benzo[*d*]imidazole (3v)

Yield 59%; Light grey solid; mp 155–158 °C; IR (KBr) 3004, 2832, 1510, 1466, 1410, 1242, 1029, 813, 769 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.52 (br s, 1H), 7.46–7.58 (m, 2H), 7.33–7.45 (m, 4H), 7.16 (td, *J* = 1.71, 8.54 Hz, 1H), 4.19 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.9, 136.4, 131.5, 130.8, 128.6, 125.9, 121.7, 34.1; HRMS calcd for $C_{14}H_{10}Cl_2N_2$ *m*/*z* 276.0242, found 276.0240.

6.2.23. 5-Methoxy-2-(4-methoxybenzyl)-1*H*-benzo[*d*]imidazole (3w)

Yield 61%; Off white solid; mp 160–161 °C; IR (KBr) 3019, 2857, 1512, 1461, 1410, 1247, 1165, 1028, 813, 776 cm^{-1}; ^1H NMR (400 MHz, METHANOL-d_4) δ 7.36 (d, *J* = 8.78 Hz, 1H), 7.18–7.24 (m, 2H), 6.96–7.01 (m, 1H), 6.84–6.90 (m, 2H), 6.82 (dd, *J* = 2.44, 8.78 Hz, 1H), 4.11 (s, 2H), 3.80 (s, 3H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, METHANOL-d_4) δ 160.3, 157.9, 155.6, 131.0, 130.6, 115.4, 112.9, 56.4, 55.9, 35.4; HRMS calcd for $C_{16}H_{16}N_2O_2$ *m*/*z* 268.1256, found 268.1251.

6.2.24. 5-Chloro-2-(4-methoxybenzyl)-1*H*-benzo[*d*]imidazole (3x)

Yield 68%; Off white solid; mp 166–168 °C; IR (KBr) 3005, 2837, 1512, 1468, 1410, 1242, 1177, 1029, 813 771 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.33–12.47 (m, 1H), 7.35–7.61 (m, 2H), 7.21–7.27 (m, 2H), 7.14 (td, *J* = 1.25, 8.48 Hz, 1H), 6.86–6.91 (m, 2H), 4.10 (s, 2H), 3.70–3.73 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.2, 155.8, 155.8, 130.0, 129.3, 125.8, 125.7, 125.7, 121.6, 121.6, 114.0, 55.1, 34.0; HRMS calcd for $C_{15}H_{13}ClN_2O$ *m*/*z* 272.0728, found 272.0722.

6.2.25. 2-(4-Methoxyphenethyl)-1*H*-benzo[*d*]imidazole (3y)

Yield 55%; White solid; mp 195–196 °C; IR (KBr) 2991, 2838, 2746, 1510, 1452, 1236, 1178, 1035, 818, 748, 738 cm^{-1}; ^1H NMR (400 MHz, CHLOROFORM-*d*) δ 7.53 (br s, 2H), 7.23 (dd, *J* = 3.17, 6.10 Hz, 2H), 7.07–7.12 (m, *J* = 8.78 Hz, 2H), 6.80–6.85 (m, 2H), 3.79 (s, 3H), 3.17–3.23 (m, 2H), 3.09–3.15 (m, 2H); ^{13}C NMR (100 MHz, CHLOROFORM-*d*) δ 158.3, 154.1, 132.6, 129.4, 122.3, 114.1, 55.2, 33.3, 31.4; HRMS calcd for $C_{16}H_{16}N_2O$ *m*/*z* 252.1232, found 252.1231.

6.2.26. 2-(4-Methoxybenzyl)benzo[*d*]thiazole (5)

Yield 48%; Off white solid; mp 60–62 °C; IR (KBr) 3022, 2961, 2929, 2836, 1509, 1450, 1248, 1175, 1126, 1029, 810, 760 cm^{-1}; ^1H NMR (400 MHz, CHLOROFORM-*d*) δ 7.97–8.03 (m, 1H), 7.79 (td, *J* = 0.67, 7.93 Hz, 1H), 7.43–7.49 (m, 1H), 7.28–7.37 (m, 3H), 6.87–6.93 (m, 2H), 4.39 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, CHLOROFORM-*d*) δ 172.1, 159.0, 153.4, 135.7, 130.3, 129.3, 126.0, 124.8, 122.8, 121.6, 114.3, 55.2, 39.7; HRMS calcd for $C_{15}H_{13}NOS$ *m*/*z* 255.0744, found 255.0741.

6.3. General procedure to synthesis of compounds 7

To a stirred solution of 2-bromoaniline **6** (5.81 mmol) in DCM (10 mL) were added DIPEA (8.72 mmol), EDC·HCl (6.976 mmol) and 2-(4-methoxyphenyl)acetic acid **2** (6.39 mmol) at room temperature, and stirred at rt for 16 h. Then the reaction mixture was diluted with DCM (100 mL), washed with 1 N HCl solution (2 × 50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (eluting with 10–15% Ethyl acetate in hexanes) to afford the title compounds **7.**

Yield 85%; off white solid; ^1H NMR (400 MHz, CHLOROFORM-*d*) δ 8.37 (dd, *J* = 1.46, 8.29 Hz, 1H), 7.59–7.71 (m, 1H), 7.27–7.49 (m, 7H), 6.94 (tt, *J* = 1.46, 7.68 Hz, 1H), 3.81 (s, 2H).

6.4. General procedure to synthesis of compounds 4a–b

To a stirred solution of *N*-(2-bromophenyl)-2-(4-methoxyphenyl)acetamide **7** (1.56 mmol) in toluene (10 mL) were added Cs_2CO_3 (3.12 mmol), 1,10-phenanthroline (0.156 mmol) and CuI (0.078 mmol) at room temperature, and refluxed for 16 h. After cooling to rt, the reaction was concentrated under reduced pressure and diluted with EtOAc (50 mL). The organic phase was washed with water (2 × 30 mL), dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (eluting with 10–15% Ethyl acetate in hexanes) to afford the title compounds **4a**–**b**.

6.4.1. 2-Benzylbenzo[*d*]oxazole (4a)

Yield 80%; Pale yellow oil; IR (KBr) 2923, 2831, 1512, 1452, 1242, 1176, 1032, 815, 738 cm^{-1}; ^1H NMR (400 MHz, CHLOROFORM-*d*) δ 7.67–7.73 (m, 1H), 7.45–7.50 (m, 1H), 7.27–7.42 (m, 7H), 4.29 (s, 2H); ^{13}C NMR (100 MHz, CHLOROFORM-*d*) δ 165.3, 151.1, 141.4, 134.8, 129.0, 128.9, 127.4, 124.7, 124.2, 119.9, 110.5, 35.2; HRMS calcd for $C_{14}H_{11}NO$ *m*/*z* 209.0841, found 209.0840.

6.4.2. 2-(4-Methoxybenzyl)benzo[*d*]oxazole (4b)

Yield 75%; Light brown oil; IR (KBr) 2933, 2835, 1510, 1454, 1242, 1176, 1032, 813, 743 cm^{-1}; ^1H NMR (400 MHz, CHLOROFORM-*d*) δ 7.66–7.72 (m, 1H), 7.44–7.50 (m, 1H), 7.27–7.34 (m, 4H), 6.86–6.92 (m, 2H), 4.22 (s, 2H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, CHLOROFORM-*d*) δ 165.7, 158.9, 151.1, 141.4, 130.1, 126.8, 124.7, 124.2, 119.8, 114.3, 110.5, 55.2, 34.4; HRMS calcd for $C_{15}H_{13}NO_2$ *m*/*z* 239.0942, found 224.0941.

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