Arch. Pharm. Res.
DOI 10.1007/s12272-017-0886-1

RESEARCH ARTICLE

Exploration of SAR for novel 2-benzylbenzimidazole analogs as inhibitor of transcription factor NF-κB

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Received: 25 October 2016 / Accepted: 4 January 2017

Abstract A novel series of 2-benzylbenzimidazole analogs was designed, synthesized and investigated for their in vitro activities against LPS induced NF-κB inhibition in RAW 264.7 cells using the SEAP assay. Among them, 4-((4-(cyclohexylmethoxy)-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenol (**6e**, >100% inhibition at 30 μM, IC_{50} = 3.0 μM), 4-((5-(cyclohexylmethoxy)-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenol (**6j**, 96% inhibition at 30 μM, IC_{50} = 4.0 μM) and 2-((4-(cyclohexylmethoxy)-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenol (**6k**, 95% inhibition at 30 μM, IC_{50} = 5.0 μM) showed strong inhibitory activity. The structure activity relationship confirmed that the substitution on benzimidazole ring A with hydrophobic cyclohexylmethoxy group at position 4 or 5 markedly enhances the activity. In addition, the hydrophilic hydrogen bonding donor group (OH) at position 2 or 4 on phenyl ring B connected with one methylene spacer to the benzimidazole ring is favorable for the inhibitory activity. However, hydrophobic (–OCH_3 and –Cl) groups on phenyl ring B decrease the activity.

Keywords 2-Benzylbenzimidazole · LPS · NF-κB inhibitor

Electronic supplementary material The online version of this article (doi:10.1007/s12272-017-0886-1) contains supplementary material, which is available to authorized users.

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Introduction

Nuclear factor kappa B (NF-κB) is a family of transcription factors and plays a crucial role in inflammation, immune responses, survival and cell proliferation (Hayden and Ghosh 2008; Karin 2006; Li and Verma 2002; Hoesel and Schmid 2013). The NF-κB family is composed of five members, including RelA, RelB, and c-Rel, the precursor and processed products of the NFKB1 (p105/p50) and NFKB2 (p100/p52) genes. Although these proteins form homodimers or heterodimers in various combinations, the main complexes that activate transcription are the p50/RelA and the p52/RelB complexes, which are sequestered in the cytoplasm by the inhibitor of NF-κB (IκB) family or a p100 protein, respectively, in unstimulated cells (Oeckinghaus et al. 2011). Two distinct pathways have been proposed for NF-κB activation. The first one is the canonical pathway that mediates inflammatory responses and the second one is the non-canonical pathway that is involved in immune cell differentiation and maturation and secondary lymphoid organogenesis. The former is dependent on the IκB kinase adaptor molecule NEMO (IKK-γ) and the latter is independent of it (Oeckinghaus et al. 2011; Razani et al. 2011; Sun 2011).

The expression of multiple genes and effector function of lymphocytes (Gerondakis and Siebenlist 2010; Sen 2006) have been controlled by the NF-κB. Especially it modulates the regulation of interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α) (Janssens and Burns 2002; Libermann and Baltimore 1990; Kunsch and Rosen 1993; Schütze et al. 1995). NF-κB has been acknowledged for its response to the harmful cellular stimuli, such as physiological stress, bacterial or viral infection, and other internal proteins, and it is the first ever among all other transcriptional factors

Published online: 20 January 2017

that have recognized to be so. Furthermore, constitutively active form of NF-κB have been frequently found in cancers and in many inflammatory diseases (Hoesel and Schmid 2013; Mantovani et al. 2008) such as inflammatory bowel disease, arthritis, sepsis, gastritis, asthma and atherosclerosis. Therefore, the treatment of cancers and inflammatory diseases, require the regulation or inhibition of NF-κB and this perspective could be the effective approach to find the novel immuno-inflammatory agents. Some of the representative examples for known NF-κB inhibitors are shown in Fig. 1 (Pan et al. 2000; Kunnumakkara et al. 2007; Davis et al. 1999; Li et al. 2005; Rahman et al. 2007; Ban et al. 2007).

Recently, we discovered a novel small molecule, (*E*)-1-(2-hydroxy-6-(isopentyloxy)phenyl)-3-(4-hydroxyphenyl) prop-2-en-1-one (JSH, Fig. 1) (Roh et al. 2011) and successfully proved it as an inhibitor of LPS induced NF-κB activation in macrophages with an IC_{50} value of about 10 μM. Later, we explored the structure activity relationship of JSH analogs related to NF-κB inhibition (Venkateswararao et al. 2012, 2014). Since chalcone JSH and its analogs are flexible molecules, these analogs have many conformations and hypothetically isomerize to a rigid flavanone structure as shown in Fig. 2, which might be an effective form. These aspects therefore dragged our attention to investigate the rigid scaffolds like benzimidazole, benzoxazole and benzothiazole retaining HBA and HBD characteristics of chalcone scaffold in JSH. As a result, 2-benzylbenzimidazoles were identified as novel inhibitors of NF-κB (Boggu et al. 2016). Thus optimization of this scaffold is immediately required to obtain more potent analog for the inhibition of NF-κB. The hydrophobic isopentyloxy group and 4-hydroxyl group on the phenyl ring in the JSH compound were considered to be the important functional groups for the inhibition of LPS induced NF-κB activation (Roh et al. 2011). Based on these characteristics of JSH, we designed and synthesized analogs **6a–s** (Fig. 2; Table 1) by introducing the bulky hydrophobic substituents on the benzimidazole ring A and studied them for their LPS induced NF-κB inhibitory activity.

Materials and methods

Chemistry

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 by distillation prior to use applying the standard procedures (Perrin et al. 1982). Thin layer chromatography was performed on E Merck silica gel GF-254 pre-coated plates; identification was performed under UV illumination (λ = 254 nm), and colorization with iodine. Hydrogenation reaction was performed on Parr shaker type hydrogenator. Flash column chromatography was performed on E Merck silica gel (230–400 mesh). Infrared spectra were recorded on a Nicolet 380 model FTIR. ^{1}H NMR and ^{13}C NMR spectra were measured against the peak of tetramethylsilane using a Bruker Fourier 300 NMR (300 MHz) and JEOL, JNM-AL400 NMR (400 MHz) spectrometer. We noted that some quaternary carbon signals in ^{13}C NMR spectra of benzimidazoles are not observable possibly due to tautomerism. High resolution mass spectrum (HRMS) were measured in ESI ionisation using AB Sciex Triple TOF 5600 LCMS instrument.

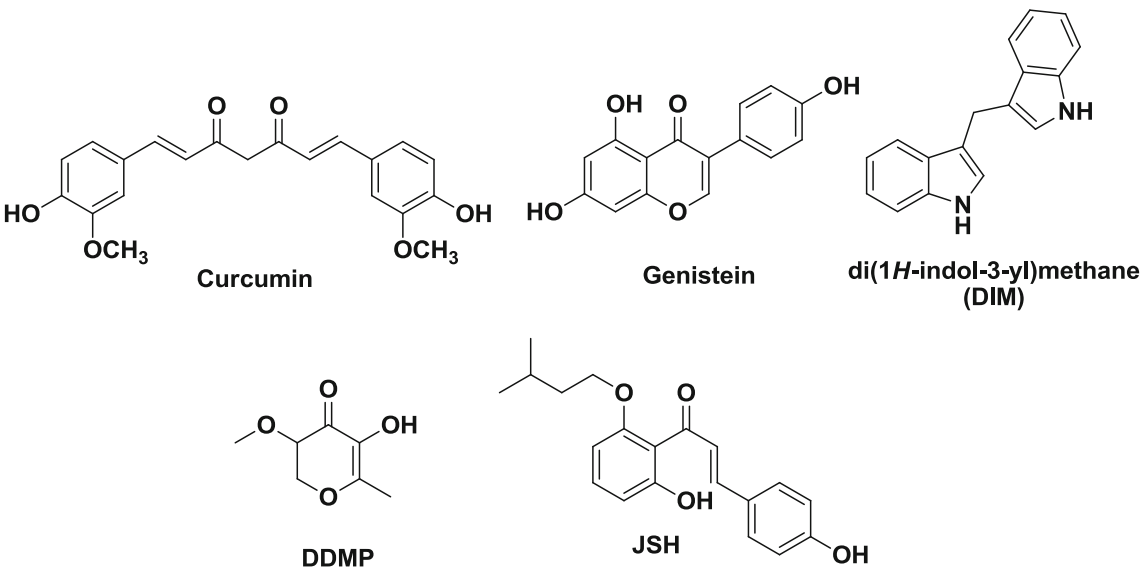


Fig. 1 Representative examples for known NF-κB inhibitors

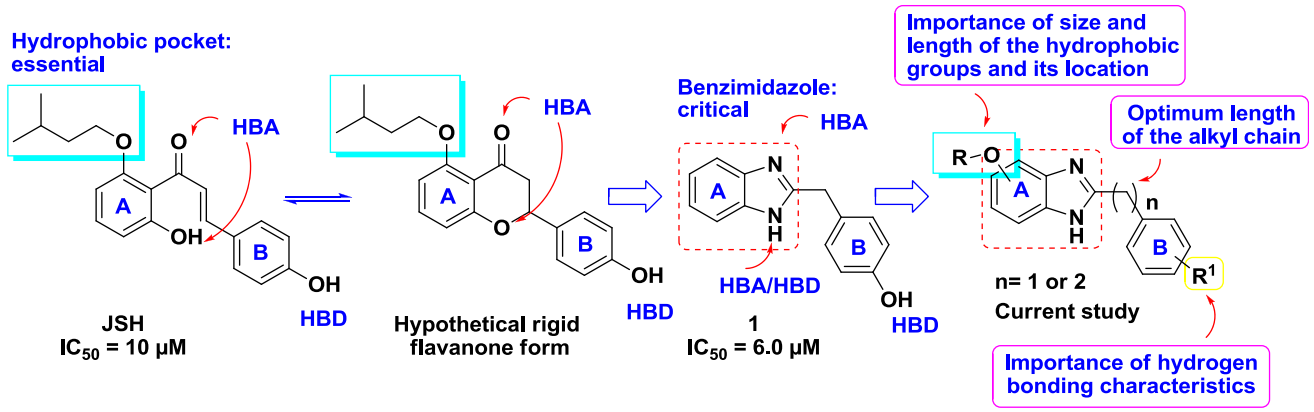


Fig. 2 Structural modification of benzimidazoles as NF-κB inhibitors

Table 1 In vitro NF-κB inhibitory activity of benzimidazoles **6a–s**

Comp. No.	R	R^1	n	% of Inhibition at 30 μM	IC_{50} value[a] (μM)
6a	4-$(CH_2)_2CH(CH_3)_2$	4-OH	1	77	12.0
6b	4-$CH_2CH(CH_3)_2$	4-OH	1	46	>30.0
6c	4-$(CH_2)_2CH_3$	4-OH	1	30	>30.0
6d	4-CH_3	4-OH	1	38	>30.0
6e	4-CH_2Cy	4-OH	1	>100	3.0
6f	4-$(CH_2)_2$Cy	4-OH	1	88	17.0
6g	4-CH_2Ph	4-OH	1	85	12.0
6h	4-$(CH_2)_2$Ph	4-OH	1	87	18.0
6i	5-$(CH_2)_2CH(CH_3)_2$	4-OH	1	84	7.0
6j	5-CH_2Cy	4-OH	1	96	4.0
6k	4-CH_2Cy	2-OH	1	95	5.0
6l	4-CH_2Cy	4-OCH_3	1	71	17.0
6m	5-CH_2Cy	4-OCH_3	1	48	>30.0
6n	4-CH_2Cy	2-OCH_3	1	90	8.0
6o	4-CH_2Cy	4-Cl	1	46	>30.0
6p	–	4-OH	1	12	>30.0
6q	–	2-OH	1	19	>30.0
6r	–	4-OCH_3	1	22	>30.0
6s	4-CH_2Cy	4-OH	2	90	7.0
1	–	–	–	75	6.0
JSH					10.0

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

Cy cyclohexyl, *Ph* phenyl

*General procedure for the synthesis of compounds **3a–j***

To a solution of the corresponding nitro aminophenol **2** (6.49 mmol) in DMF (10 mL), K_2CO_3 (7.79 mmol) and the corresponding alkyl (or benzyl) halide (7.14 mmol) were added. The resulting solution was stirred at 60 °C for 8–12 h. The reaction mixture was cooled, diluted with water, and then extracted with EtOAc. The combined organic extracts were washed with water, brine solution, dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude mixture was subjected to flash silica gel (230–400 mesh) column chromatography to afford the title compounds **3a–j**.

2-(Isopentyloxy)-6-nitroaniline (**3a**) Yield 78%; Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (dd, J = 1.34, 8.90 Hz, 1H), 6.89 (d, J = 7.56 Hz, 1H), 6.60 (dd, J = 7.68, 8.90 Hz, 1H), 6.44 (br. s., 2H), 4.07 (t, J = 6.59 Hz, 2H), 1.81–1.91 (m, 1H), 1.76 (q, J = 6.75 Hz, 2H), 0.99 (d, J = 6.34 Hz, 6H).

2-Isobutoxy-6-nitroaniline (**3b**) Yield 87%; Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, J = 0.98, 9.02 Hz, 1H), 6.87 (d, J = 7.81 Hz, 1H), 6.60 (dd, J = 7.81, 9.02 Hz, 1H), 6.45 (br. s., 2H), 3.81 (d, J = 6.69 Hz, 2H), 2.18 (quind, J = 6.67, 13.34 Hz, 1H), 1.07 (d, J = 6.67 Hz, 6H).

2-Nitro-6-propoxyaniline (**3c**) Yield 85%; Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, J = 1.46, 8.78 Hz, 1H), 6.85–6.92 (dd, J = 1.24, 8.78 Hz, 1H), 6.60 (dd, J = 7.80, 8.78 Hz, 1H), 6.46 (br. s., 2H), 4.01 (t, J = 6.46 Hz, 2H), 1.82–1.96 (m, 2H), 1.08 (t, J = 7.44 Hz, 3H).

2-Methoxy-6-nitroaniline (**3d**) Yield 75%; Light pink solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69–7.78 (dd, J = 0.93, 8.85 Hz, 1H), 6.89 (d, J = 7.71 Hz, 1H), 6.62 (dd, J = 7.71, 8.85 Hz, 1H), 6.44 (br. s., 2H), 3.93 (s, 3H).

2-(Cyclohexylmethoxy)-6-nitroaniline (**3e**) Yield 67%; Yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69–7.75 (dd, J = 0.93, 8.80 Hz, 1H), 6.87 (d, J = 7.64 Hz, 1H), 6.59 (dd, J = 7.78, 8.80 Hz, 1H), 6.44 (br. s., 2H), 3.84 (d, J = 5.96 Hz, 2H), 1.71–1.93 (m, 6H), 1.04–1.39 (m, 5H).

2-(2-Cyclohexylethoxy)-6-nitroaniline (**3f**) Yield 82%; Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.78 Hz, 1H), 6.88 (d, J = 7.56 Hz, 1H), 6.54–6.63 (t, J = 7.80, 8.80 Hz 1H), 6.44 (br. s., 2H), 4.08 (t, J = 6.59 Hz, 2H), 1.66–1.81 (m, 7H), 1.45–1.55 (m, 1H), 1.15–1.33 (m, 3H), 0.93–1.08 (m, 2H).

2-(Benzyloxy)-6-nitroaniline (**3g**) Yield 78%; Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (dd, J = 1.34, 8.90 Hz, 1H), 7.39–7.48 (m, 5H), 6.97 (dd, J = 1.22, 7.80 Hz, 1H), 6.60 (dd, J = 7.80, 8.78 Hz, 1H), 6.45 (br. s., 1H), 5.14 (s, 2H).

2-Nitro-6-phenethoxyaniline (**3h**) Yield 75%; Yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, J = 8.85 Hz, 1H), 7.28–7.42 (m, 5H), 6.89 (d, J = 7.73 Hz, 1H), 6.54–6.63 (t, J = 8.58 Hz, 1H), 6.33 (br. s., 2H), 4.27 (t, J = 6.80 Hz, 2H), 3.18 (t, J = 6.80 Hz, 2H).

4-(Isopentyloxy)-2-nitroaniline (**3i**) Yield 83%; Yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, J = 2.89 Hz, 1H), 7.07 (dd, J = 2.89, 9.03 Hz, 1H), 6.76 (d, J = 9.03 Hz, 1H), 5.89 (br. s., 2H), 3.96 (t, J = 6.66 Hz, 2H), 1.83 (quind, J = 6.61, 13.42 Hz, 1H), 1.67 (q, J = 6.74 Hz, 2H), 0.97 (d, J = 6.52 Hz, 6H).

4-(Cyclohexylmethoxy)-2-nitroaniline (**3j**) Yield 71%; Yellow solid;^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 2.89 Hz, 1H), 7.07 (dd, J = 2.93, 9.08 Hz, 1H), 6.76 (d, J = 9.03 Hz, 1H), 5.88 (br. s., 2H), 3.72 (d, J = 6.33 Hz, 2H), 1.68–1.90 (m, 6H), 1.14–1.37 (m, 3H), 1.00–1.14 (m, 2H).

*General procedure for the synthesis of compounds **4a–f, 4h–j** and **7***

In a 250 mL parr shaker vessel, a mixture of the corresponding alkyl substituted nitro aminophenol **3** (3.99 mmol) in methanol (30 mL) was placed and then added 10% Pd–C (20% w/w). The resulting solution was stirred at ambient temperature for 2 h in the presence of hydrogen gas. After completion of the reaction, the mixture was filtered through celite bed and washed with methanol, and then the filtrate was evaporated under vacuum to afford the title compounds. These compounds were used for the next step without further purification.

3-(Isopentyloxy)benzene-1,2-diamine (**4a**) Yield 85%; Dark brown oil; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.36 (t, J = 7.93 Hz, 1H), 6.17–6.25 (m, 2H), 4.44 (s, 2H), 3.92 (br. s., 2H), 3.90 (t, J = 6.56, 2H), 1.81 (quind, J = 6.69, 13.49 Hz, 1H), 1.61 (q, J = 6.83 Hz, 2H), 0.93 (d, J = 6.59 Hz, 6H).

3-Isobutoxybenzene-1,2-diamine (**4b**) Yield 79%; Black solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.65 (t, J = 8.05 Hz, 1H), 6.38–6.40 (m, 2H), 3.76 (d, J = 6.59 Hz, 2H), 3.45 (br. s., 4H), 2.12 (quind, J = 6.68, 13.26 Hz, 1H), 1.05 (d, J = 6.83 Hz, 6H).

3-Propoxybenzene-1,2-diamine (**4c**) Yield 83%; Black solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.65 (t, J = 8.06 Hz, 1H), 6.36–6.44 (m, 2H), 3.96 (t, J = 6.59 Hz, 2H), 3.45 (br. s., 4H), 1.84 (sxt, J = 7.07 Hz, 2H), 1.06 (t, J = 7.44 Hz, 3H).

3-Methoxybenzene-1,2-diamine (**4d**) Yield 87%; Light brown solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.68 (t, J = 7.8 Hz 1H), 6.41 (td, J = 1.2, 7.5 Hz, 2H), 3.84 (s, 3H), 3.31 (br. s., 4H).

3-(Cyclohexylmethoxy)benzene-1,2-diamine (**4e**) Yield 92%; Dark brown solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.65 (t, J = 7.83 Hz 1H), 6.40–6.37 (m, 2H), 3.79 (d, J = 6.05 Hz, 2H), 3.44 (br. s., 4H), 1.66–1.93 (m, 6H), 1.02–1.39 (m, 5H).

3-(2-Cyclohexylethoxy)benzene-1,2-diamine (**4f**) Yield 82%; Dark brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.66 (t, J = 8.05 Hz, 1H), 6.40 (dt, J = 1.10, 7.62 Hz, 2H), 4.03 (t, J = 6.59 Hz, 2H), 3.44 (br. s., 4H), 1.64–1.81 (m, 7H), 1.51 (m, 1H), 1.13–1.31 (m, 3H), 0.99 (m, 2H).

3-Phenethoxybenzene-1,2-diamine (**4h**) Yield 79%; Dark brown solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22–7.38 (m, 5H), 6.65 (t, J = 8.01 Hz, 1H), 6.38–6.42 (m, 2H), 4.22 (t, J = 6.89 Hz, 2H), 3.40 (br. s., 4H), 3.13 (t, J = 6.85 Hz, 2H).

4-(Isopentyloxy)benzene-1,2-diamine (**4i**) Yield 81%; Dark brown solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.63 (d, J = 8.38 Hz, 1H), 6.33 (d, J = 2.70 Hz, 1H), 6.27 (dd, J = 2.70, 8.38 Hz, 1H), 3.90 (t, J = 6.71 Hz, 2H), 3.53 (br. s., 2H), 3.06 (br. s., 2H), 1.82 (m, 1H), 1.64 (q, J = 6.77 Hz, 2H), 0.95 (d, J = 6.61 Hz, 6H).

4-(Cyclohexylmethoxy)benzene-1,2-diamine (**4j**) Yield 72%; Dark brown solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.63 (d, J = 8.29 Hz, 1H), 6.33 (d, J = 2.61 Hz, 1H), 6.26 (dd, J = 2.70, 8.38 Hz, 1H), 3.67 (d, J = 6.33 Hz, 2H), 3.51 (br. s., 2H), 3.10 (br. s., 2H), 1.69–1.88 (m, 6H), 1.18–1.34 (m, 3H), 0.95–1.08 (m, 2H).

2,3-Diaminophenol (**7**) Yield 95%; Light brown solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (br. s., 1H), 6.23 (t, J = 7.80 Hz, 1H), 6.07 (dd, J = 1.59, 7.93 Hz, 2H), 4.36 (br. s., 2H), 3.81 (br. s., 2H).

3-(Benzyloxy)benzene-1,2-diamine (**4g**) To a solution of the 2-(benzyloxy)-6-nitroaniline **3g** (4.09 mmol) in EtOH (15 mL), $SnCl_2{\cdot}2H_2O$ (20.47 mmol) was added, and stirred at reflux temperature for 5 h. The reaction mixture was cooled, quenched with 1*N* NaOH solution (pH $\sim$ 9), and then extracted with EtOAc. The combined organic extracts were washed with water, brine solution, dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude mixture was subjected to flash silica gel (230–400 mesh) column chromatography to afford the title compound **4g**. Yield 65%; Brown solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47 (d, J = 7.07 Hz, 2H), 7.36–7.41 (m, 2H), 7.28–7.34 (m, 1H), 6.35 (t, J = 7.80 Hz, 1H), 6.29 (dd, J = 1.46, 8.04 Hz, 1H), 6.23 (dd, J = 1.46, 7.80 Hz, 1H), 5.03 (s, 2H), 4.49 (br. s., 2H), 4.03 (br. s., 2H).

*General procedure for the synthesis of compounds **6a–f** and **6h–s***

To a solution of the corresponding substituted alkyloxybenzene-1,2-diamine **4** (2.26 mmol) in 6*N* HCl (10 mL), corresponding acid **5** (6.80 mmol) was added. The resulting solution was refluxed for 16 h. The reaction mixture was cooled to 0 °C, diluted with EtOAc, and then pH was adjusted to $\sim$7 by using solid $NaHCO_3$, extracted with EtOAc. The combined organic extracts were washed with brine solution, dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude mixture was subjected to flash silica gel (230–400 mesh) column chromatography to afford the title compounds **6a–f** and **6h–s**.

4-((4-(Isopentyloxy)-1H-benzo[d]imidazol-2-yl)methyl) phenol (**6a**) Yield 71%; Pale yellow solid; mp 195–197 °C; IR (neat) 3250–2770 (br., peak), 2915, 2843, 1595, 1516, 1441, 1245, 1168, 1098, 1022, 783, 726; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.09–7.15 (m, 2H), 7.03–7.09 (m, 2H), 6.67–6.75 (m, 3H), 4.16 (t, J = 6.66 Hz, 2H), 4.09 (s, 2H), 1.87–2.00 (m, 1H), 1.76 (q, J = 6.80 Hz, 2H), 0.99 (d, J = 6.61 Hz, 6H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 157.7, 155.1, 149.5, 141.7, 131.0, 129.5, 124.1, 116.6, 108.5, 104.9, 67.9, 39.4, 35.2, 26.1, 23.1; HRMS (ESI) Calcd for $C_{19}H_{22}N_2O_2$ $[M+H]^+$ 311.1759, found 311.1784.

4-((4-Isobutoxy-1H-benzo[d]imidazol-2-yl)methyl)phenol (**6b**) Yield 64%; Off white solid; mp 238–241.5 °C; IR (neat) 3255–2780 (br., peak), 2917, 2845, 1595, 1518, 1442, 1245, 1165, 1096, 1023, 783, 724; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.09–7.15 (m, 2H), 7.02–7.09 (m, 2H), 6.64–6.75 (m, 3H), 4.10 (s, 2H), 3.89 (d, J = 6.61 Hz, 2H), 2.17 (quind, J = 6.67, 13.35 Hz, 1H), 1.09 (d, J = 6.80 Hz, 6H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 157.7, 155.1, 149.5, 131.0, 129.5, 124.1, 116.6, 108.6, 105.0, 76.0, 35.2, 29.7, 19.8; HRMS (ESI) Calcd for $C_{18}H_{20}N_2O_2$ $[M+H]^+$ 297.1603, found 297.1627.

4-((4-Propoxy-1H-benzo[d]imidazol-2-yl)methyl)phenol (**6c**) Yield 69%; White solid; mp 228–230 °C; IR (neat) 3250–2780 (br., peak), 2918, 2846, 1596, 1517, 1445, 1244, 1167, 1097, 1021, 782, 725; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.03–7.15 (m, 4H), 6.66–6.75 (m, 3H), 4.06–4.12 (t, J = 6.42 Hz, 4H), 1.89 (sxt, J = 7.08 Hz, 2H), 1.10 (t, J = 7.45 Hz, 3H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 157.7, 155.1, 131.0, 129.5, 124.1, 116.6, 105.0, 71.2, 35.2, 23.8, 11.0; HRMS (ESI) Calcd for $C_{17}H_{18}N_2O_2$ $[M+H]^+$ 283.1446, found 283.1466.

4-((4-Methoxy-1H-benzo[d]imidazol-2-yl)methyl)phenol (**6d**) Yield 61%; Off white solid; mp 226.5–228 °C; IR (neat) 3250–2780 (br., peak), 2918, 2845, 1595, 1515, 1444, 1245, 1168, 1095, 1023, 835, 782, 725; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.06–7.15 (m, 4H), 6.68–6.75 (m, 3H), 4.09 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 157.7, 155.0, 150.2, 141.1, 131.0, 129.3, 124.2, 116.7, 108.3, 104.1, 56.1, 35.2; HRMS (ESI) Calcd for $C_{15}H_{14}N_2O_2$ $[M + H]^+$ 255.1133, found 255.1161.

4-((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl) phenol (**6e**) Yield 68%; White solid; mp 118–121 °C; IR (neat) 3300–2750 (br., peak), 2917, 2848, 1596, 1511, 1441, 1243, 1169, 1097, 1025, 833, 780, 729; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.31 (br. s., 0.5H), 12.11 (br. s., 0.5H), 9.23 (d, 1H), 7.10 (d, J = 7.82 Hz, 2.5H), 6.92–7.03 (m, 1.5H), 6.69 (d, J = 6.15 Hz, 2.5H), 6.59 (d, J = 6.89 Hz, 0.5H), 4.01 (s, 2H), 3.96 (d, J = 5.68 Hz, 1H), 3.90 (d, J = 6.15 Hz, 1H), 1.64–1.94 (m, 6H), 1.18–1.33 (m, 3H), 1.02–1.18 (m, 2H); ^{1}H

NMR (300 MHz, DMSO-d_6-D_2O-exchange) δ 7.12 (d, $J = 8.29$ Hz, 2H), 6.90–7.09 (m, 2H), 6.57–6.70 (m, 3H), 3.99 (s, 2H), 3.86–3.92 (m, 2H), 1.60–1.87 (m, 6H), 1.10–1.25 (m, 5H); ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.12 (d, $J = 8.38$ Hz, 2H), 7.00–7.09 (m, 2H), 6.72 (d, $J = 8.38$ Hz, 2H), 6.62–6.69 (m, 1H), 4.09 (s, 2H), 3.92 (d, $J = 6.24$ Hz, 2H), 1.69–2.01 (m, 6H), 1.13–1.40 (m, 5H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 157.6, 155.1, 149.7, 131.0, 129.5, 124.0, 116.6, 108.5, 104.8, 75.0, 39.2, 35.2, 31.0, 27.7, 27.1; HRMS (ESI) Calcd for $C_{21}H_{24}N_2O_2$ $[M+H]^+$ 337.1916, found 337.1942.

4-((4-(2-Cyclohexylethoxy)-1H-benzo[d]imidazol-2-yl)methyl) phenol (**6f**) Yield 62%; Purple solid; mp 89–93 °C; IR (neat) 3300–2750 (br., peak), 2918, 2847, 1595, 1512, 1441, 1243, 1169, 1097, 1025, 833, 781, 728; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.05–7.14 (m, 4H), 6.71 (d, $J = 8.29$ Hz, 3H), 4.17 (t, $J = 6.57$ Hz, 2H), 4.11 (s, 2H), 1.55–1.82 (m, 8H), 1.17–1.32 (m, 3H), 0.87–1.04 (m, 2H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 157.8, 154.9, 149.6, 140.6, 131.5, 131.1, 128.9, 124.6, 116.7, 108.0, 105.3, 67.4, 37.9, 35.5, 34.9, 34.5, 27.8, 27.4; HRMS (ESI) Calcd for $C_{22}H_{26}N_2O_2$ $[M+H]^+$ 351.2072, found 351.2099.

4-((4-Phenethoxy-1H-benzo[d]imidazol-2-yl)methyl)phenol (**6h**) Yield 58%; Off white solid; mp 220–222 °C; IR (neat) 3500–2600 (br., peak), 2922, 2851, 1598, 1514, 1445, 1247, 1170, 1099, 1021, 838, 782, 731; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.24–7.35 (m, 4H), 7.06–7.22 (m, 5H), 6.72 (d, $J = 7.92$ Hz, 3H), 4.35 (t, $J = 7.03$ Hz, 2H), 4.10 (s, 2H), 3.17 (t, $J = 7.08$ Hz, 2H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 131.6, 131.0, 130.4, 129.7, 129.3, 127.7, 124.3, 116.7, 116.4, 105.5, 70.6, 36.8, 35.1; HRMS (ESI) Calcd for $C_{22}H_{20}N_2O_2$ $[M+H]^+$ 345.1603, found 345.1629.

4-((5-(Isopentyloxy)-1H-benzo[d]imidazol-2-yl)methyl)phenol (**6i**) Yield 74%; Off white solid; mp 237–239 °C; IR (neat) 3350–2770 (br., peak), 2914, 2842, 1594, 1515, 1442, 1243, 1168, 1098, 1022, 783, 726; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.57 (d, $J = 9.69$ Hz, 1H), 7.18–7.25 (d, $J = 8.47$ Hz, 2H), 7.11–7.17 (m, 2H), 6.78–6.87 (d, $J = 8.47$ Hz, 2H), 4.38 (s, 2H), 4.08 (t, $J = 6.47$ Hz, 2H), 1.79–1.94 (m, 1H), 1.71 (q, $J = 6.55$ Hz, 2H), 0.98 (d, $J = 6.61$ Hz, 6H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 159.9, 158.9, 154.2, 133.8, 131.6, 126.8, 125.0, 117.7, 117.4, 115.7, 97.9, 68.5, 39.1, 32.9, 26.3, 23.0; HRMS (ESI) Calcd for $C_{19}H_{22}N_2O_2$ $[M+H]^+$ 311.1759, found 311.1781.

4-((5-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl) phenol (**6j**) Yield 72%; Yellow solid; mp 108–112 °C (shrinking) and 195–198 °C (liquid); IR (neat) 3300–2700 (br., peak), 2918, 2847, 1597, 1512, 1441, 1243, 1169, 1096, 1025, 833, 780, 729; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.34 (d, $J = 8.85$ Hz, 1H), 7.06–7.16 (d, $J = 8.29$ Hz, 2H), 6.92–6.98 (d, $J = 1.82$ Hz, 1H), 6.80 (dd, $J = 1.82$, 8.71 Hz, 1H), 6.69–6.77 (d, $J = 8.29$ Hz, 2H), 4.07 (s, 2H), 3.76 (d, $J = 6.15$ Hz, 2H), 1.68–1.93 (m, 6H), 1.03–1.39 (m, 5H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 157.8, 157.6, 155.6, 131.7, 131.0, 129.3, 123.2, 116.7, 116.5, 113.4, 99.1, 75.4, 39.3, 35.4, 31.1, 27.8, 27.1; HRMS (ESI) Calcd for $C_{21}H_{24}N_2O_2$ $[M+H]^+$ 337.1916, found 337.1944.

2-((4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)methyl) phenol (**6k**) Yield 61%; Off white solid; mp 201–203 °C; IR (neat) 3300–2700 (br., peak), 2918, 2849, 1599, 1512, 1442, 1243, 1169, 1099, 1025, 833, 780, 732; ^{1}H NMR (300 MHz, Methanol-d_4) δ 6.99–7.12 (m, 4H), 6.72–6.84 (m, 2H), 6.63–6.69 (m, 1H), 4.18 (s, 2H), 3.91 (d, $J = 6.24$ Hz, 2H), 1.66–2.02 (m, 6H), 1.07–1.40 (m, 5H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 156.7, 154.8, 131.4, 129.5, 125.2, 124.0, 121.0, 116.4, 105.0, 75.1, 39.3, 31.1, 30.7, 27.8, 27.1; HRMS (ESI) Calcd for $C_{21}H_{24}N_2O_2$ $[M+H]^+$ 337.1916, found 337.1937.

4-(Cyclohexylmethoxy)-2-(4-methoxybenzyl)-1H-benzo[d] imidazole (**6l**) Yield 71%; Light brown solid; 171–173 °C; IR (neat) 2922, 2845, 1599, 1509, 1448, 1242, 1095, 1025, 985, 733; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.25 (br. s., 1H), 7.05–7.27 (m, 4H), 6.86 (d, $J = 8.66$ Hz, 2H), 6.65 (d, $J = 8.57$ Hz, 1H), 4.23 (s, 2H), 3.91 (d, $J = 5.68$ Hz, 2H), 3.80 (s, 3H), 1.65–1.92 (m, 6H), 0.98–1.30 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.7, 152.7, 130.1, 128.6, 122.6, 114.2, 103.6, 73.8, 55.2, 37.4, 34.8, 29.8, 26.3, 25.6; HRMS (ESI) Calcd for $C_{22}H_{26}N_2O_2$ $[M+H]^+$ 351.2072, found 351.2093.

5-(Cyclohexylmethoxy)-2-(4-methoxybenzyl)-1H-benzo[d] imidazole (**6m**) Yield 75%; Pale yellow solid; mp 140–143 °C; IR (neat) 2921, 2843, 1598, 1508, 1448, 1242, 1095, 1025, 985, 733; ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.35 (d, $J = 8.54$ Hz, 1H), 7.18–7.24 (m, 2H), 6.96 (br. s., 1H), 6.84–6.89 (m, 2H), 6.81 (dd, $J = 2.20$, 8.78 Hz, 1H), 4.11 (s, 2H), 3.74–3.78 (m, 5H), 1.83–1.91 (m, 2H), 1.69–1.83 (m, 4H), 1.23–1.36 (m, 3H), 1.05–1.14 (m, 2H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 160.4, 157.6, 155.4, 131.0, 130.6, 115.4, 113.5, 75.4, 55.8, 39.3, 35.4, 31.1, 27.8, 27.1; HRMS (ESI) Calcd for $C_{22}H_{26}N_2O_2$ $[M + H]^+$ 351.2072, found 351.2098.

4-(Cyclohexylmethoxy)-2-(2-methoxybenzyl)-1H-benzo[d] imidazole (**6n**) Yield 63%; Light brown solid; mp 70–74 °C; IR (neat) 2920, 2842, 1597, 1507, 1447, 1241, 1095, 1025, 984, 735; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.19–7.27 (m, 1H), 7.08–7.14 (m, 1H), 7.01–7.08 (m, 2H), 6.95 (d, $J = 8.10$ Hz, 1H), 6.87 (dt, $J = 0.88$, 7.43 Hz, 1H), 6.62–6.70 (m, 1H), 4.18 (s, 2H), 3.92 (d, $J = 6.33$ Hz, 2H), 3.82 (s, 3H), 1.68–2.01 (m, 6H), 1.09–1.39 (m, 5H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 159.0, 154.5, 149.7, 131.4, 129.7, 126.8, 123.9, 121.9,

111.8, 108.3, 104.9, 75.1, 56.0, 39.3, 31.1, 30.5, 27.8, 27.1; HRMS (ESI) Calcd for $C_{22}H_{26}N_2O_2$ $[M+H]^+$ 351.2072, found 351.2095.

2-(4-Chlorobenzyl)-4-(cyclohexylmethoxy)-1H-benzo[d]imidazole (**6o**) Yield 77%; Light yellow solid; mp 82–85 °C; IR (neat) 2920, 2849, 1505, 1490, 1444, 1421, 1241, 1092, 1015, 991, 782, 729; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.28 (s, 4H), 6.99–7.13 (m, 2H), 6.63–6.73 (m, 1H), 4.18 (s, 2H), 3.92 (d, J = 6.24 Hz, 2H), 1.68–2.00 (m, 6H), 1.07–1.39 (m, 5H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 153.8, 149.6, 137.6, 133.9, 131.5, 129.9, 124.2, 108.6, 104.9, 75.0, 39.2, 35.3, 31.0, 27.7, 27.1; HRMS (ESI) Calcd for $C_{21}H_{23}ClN_2O$ $[M + H]^+$ 355.1577, found 355.1598.

2-(4-Hydroxybenzyl)-1H-benzo[d]imidazol-4-ol (**6p**) Yield 75%; Dark green solid; mp 285–287 °C; IR (neat) 3450–2750 (br. peak), 2933, 2851, 1499, 1443, 1421, 1248, 1094, 1014, 783, 732; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.29–7.37 (t, J = 8.15 Hz, 1H), 7.18–7.23 (m, 2H), 7.12 (d, J = 8.20 Hz, 1H), 6.90 (d, J = 7.92 Hz, 1H), 6.80–6.85 (m, 2H), 4.38 (s, 2H); ^{13}C NMR (75 MHz, Methanol-d_4) δ 158.8, 153.7, 146.8, 134.3, 131.3, 128.5, 125.0, 122.6, 117.3, 111.4, 105.1, 32.8; HRMS (ESI) Calcd for $C_{14}H_{12}N_2O_2$ $[M+H]^+$ 241.0977, found 241.0997.

2-(2-Hydroxybenzyl)-1H-benzo[d]imidazol-4-ol (**6q**) Yield 68%; Light brown solid; mp 127–130 °C; IR (neat) 3500–2700 (br. peak), 2923, 2852, 1498, 1442, 1422, 1244, 1093, 1016, 783, 732; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.04–7.14 (m, 2H), 6.93–7.00 (m, 2H), 6.74–6.86 (m, 2H), 6.53–6.60 (m, 1H), 4.17 (s, 2H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 156.8, 154.6, 147.4, 131.6, 129.5, 125.1, 124.1, 121.0, 116.5, 108.2, 106.7, 30.8; HRMS (ESI) Calcd for $C_{14}H_{12}N_2O_2$ $[M+H]^+$ 241.0977, found 241.0999.

2-(4-Methoxybenzyl)-1H-benzo[d]imidazol-4-ol (**6r**) Yield 65%; Light brown solid; mp 58–62 °C; IR (neat) 3200–2700 (br. peak), 2922, 2851, 1491, 1445, 1422, 1244, 1093, 1016, 783, 731; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (d, J = 8.66 Hz, 2H), 7.07–7.14 (m, 1H), 6.79–6.88 (m, 4H), 4.31 (s, 2H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.8, 152.9, 147.6, 136.3, 130.8, 130.2, 127.9, 124.1, 114.4, 108.8, 103.2, 55.2, 34.2; HRMS (ESI) Calcd for $C_{15}H_{14}N_2O_2$ $[M + H]^+$ 255.1133, found 255.1158.

4-(2-(4-(Cyclohexylmethoxy)-1H-benzo[d]imidazol-2-yl)ethyl)phenol (**6s**) Yield 76%; Light yellow solid; mp 118–120 °C; IR (neat) 3350–2700 (br., peak), 2919, 2848, 1598, 1513, 1441, 1242, 1168, 1098, 1024, 833, 780, 731; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.04–7.12 (m, 2H), 7.00 (d, J = 8.38 Hz, 2H), 6.67 (d, J = 8.29 Hz, 3H), 3.92 (d, J = 6.15 Hz, 2H), 3.04 (m, 4H), 1.69–2.02 (m, 6H), 1.06–1.43 (m, 5H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 157.1, 155.5, 149.3, 133.1, 130.5, 123.9, 116.4, 108.7, 104.9, 75.0, 39.3, 35.1, 32.3, 31.1, 27.8, 27.1; HRMS (ESI) Calcd for $C_{22}H_{26}N_2O_2$ $[M+H]^+$ 351.2072, found 351.2096.

4-((4-(Benzyloxy)-1H-benzo[d]imidazol-2-yl)methyl)phenol (**6g**) (Boggu et al. 2016). To a stirred solution of 3-(benzyloxy)benzene-1,2-diamine **4g** (4.67 mmol) in xylenes (10 mL), 2-(4-hydroxyphenyl) acetic acid (9.34 mmol) and boric acid (0.46 mmol) were added. The resulting solution was refluxed for 48 h. After cooling to room temperature, the reaction was concentrated under reduced pressure and diluted with EtOAc. The organic phase was washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The residue was purified by flash silica gel (230–400 mesh) column chromatography to afford the title compounds **6g**. Yield 22%; White solid; mp 105–109 °C; IR (neat) 3400–2600 (br., peak), 2921, 2850, 1594, 1515, 1442, 1245, 1172, 1098, 1022, 837, 784, 732; ^{1}H NMR (300 MHz, Methanol-d_4) δ 7.46–7.57 (m, 2H), 7.26–7.40 (m, 3H), 7.06–7.14 (m, 4H), 6.75–6.80 (m, 1H), 6.68–6.74 (m, 2H), 5.23 (s, 2H), 4.08 (s, 2H); ^{13}C NMR (100 MHz, Methanol-d_4) δ 157.7, 155.3, 149.1, 138.9, 131.0, 129.7, 129.4, 129.2, 129.1, 124.1, 116.8, 116.7, 108.8, 105.7, 71.6, 35.2; HRMS (ESI) Calcd for $C_{21}H_{18}N_2O_2$ $[M + H]^+$ 331.1446, found 331.1471.

Biological study

According to our previous reports, the inhibitory effect of benzimidazole analogs **6a–s** against NF-κB transcriptional activity was evaluated using RAW 264.7 cells containing pNF-κB-SEAP-neomycin phosphotransferase (NTP) (Roh et al. 2011). In brief, RAW 264.7 monocytic cell line stably transfected with NF-κB-dependent secretory alkaline phosphatase (SEAP) construct (Pastukhov and Ropson 2003; Mancek-Keber and Jerala 2006; Doyle and O'Neill 2006), 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 the IC_{50} value and inhibition % at the concentration 30 μM from three independent experiments using the average values of triplicate in each experiment.

Results and discussion

Chemistry

The synthetic routes to obtain the target benzimidazole derivatives (**6a-s,** Table 1) by cyclisation reactions of benzene-1,2-diamine **4** (or **7**) and substituted phenyl acetic acid (**5**) were outlined in Schemes 1 and 2. Commercially available 2-amino-3-nitrophenol (**2a**) or 4-amino-3-nitrophenol (**2b**) was transformed into the corresponding alkoxynitroanilines **3** using alkyl halides with K_2CO_3 in *N,N*-dimethylformamide solvent at 60 °C for 8–16 h. Compounds **3** was then converted into the alkoxybenzene-1,2-diamines **4** using 10% Pd–C in the presence of hydrogen gas in methanol at room temperature for 2 h. The corresponding benzene-1,2-diamine **4** was reacted with appropriate phenyl acetic acid **5** in 6*N* HCl solution at reflux temperature for 16 h to obtain desired benzimidazole derivatives (**6a–f, 6h–o** and **6s**). In order to obtain **6g**, compound **2a** was reacted with benzyl bromide using potassium carbonate in *N,N*-dimethylformamide to give 2-(benzyloxy)-6-nitroaniline **3g**, which was reduced by the treatment with $SnCl_2{\cdot}H_2O$ in ethanol at reflux temperature for 5 h to obtain 3-(benzyloxy)benzene-1,2-diamine **4g**. The reaction of **4g** with 2-(4-hydroxyphenyl) acetic acid under the above same condition (6*N* HCl, reflux) yielded the debenzylated product **6p** instead of **6g**. To overcome this problem, anhydrous condition using boric acid in refluxing xylene was adopted (Boggu et al. 2016) to get the desired product **6g**. The compounds **6p–r** were obtained by reduction of nitro compound **2a** followed by cyclization with appropriate substituted phenyl acetic acid in 6*N* HCl. All these synthesized compounds were characterized by physical and spectral analysis data that confirmed their assigned structures.

Initially the ^{1}H NMR spectrum of compound **6e** was recorded in DMSO-d_6 solvent, which showed approximately 1:1 ratio mixture of two tautomers of benzimidazole motif (Fig. 3, see supplementary material of NMR data of **6e**). In order to avoid these kinds of complications in ^{1}H NMR, we recorded spectra in methanol-d_4 or $CDCl_3$.

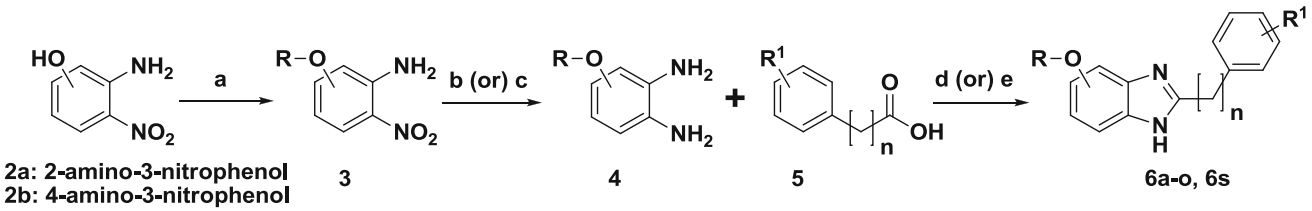
Biological results and SAR

In the first set of experiments, isopentyloxy analog **6a** (77.0% inhibition at 30 μM, IC_{50} = 12.0 μM) was prepared and studied for their LPS induced NF-κB inhibition, which showed almost similar activity as compared to JSH and less activity than the benzimidazole compound **1** (75.0% inhibition at 30 μM, IC_{50} = 6.0 μM). For the confirmation of optimum size of the hydrophobic substituent at position 4 on benzimidazole ring A of **1**, alkoxy groups were variated as shown in isobutoxy **6b** (46.0% inhibition at 30 μM, IC_{50} = > 30.0 μM), propoxy **6c** (30.0% inhibition at 30 μM, IC_{50} = > 30.0 μM) and methoxy **6d** (38.0% inhibition at 30 μM, IC_{50} = >30.0 - μM) analogs. The decrement in the chain length considerably reduced the activity, which is indicating that bulky hydrophobic group appears as an important factor for the inhibition of NF-κB.

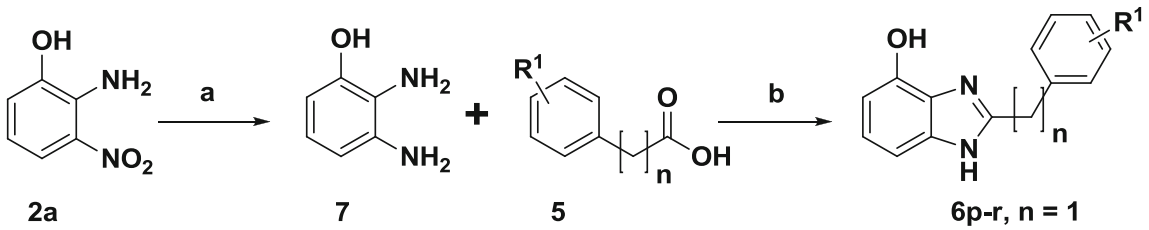
Thereafter, to confirm that the optimum size of the hydrophobic group on benzimidazole ring A of **1**, the bulkier alkoxy groups such as cyclohexylmethoxy **6e** (>100.0% inhibition at 30 μM, IC_{50} = 3.0 μM) and cyclohexylethoxy **6f** (88.0% inhibition at 30 μM, IC_{50} = 17.0 μM) were introduced. Surprisingly, analog **6e** showed more potent inhibition than **1, JSH** or **6a**. However, increasing the chain length as shown in **6f** decreased the activity. These results confirmed our view point that bulky hydrophobic group on benzimidazole ring A of **1** should be important for the activity.

In the next set of experiments, replacement of cyclohexyl group in **6e** and **6f** as shown in benzyl analog **6g** (85.0% inhibition at 30 μM, IC_{50} = 12.0 μM) and phenethyl analog **6h** (87.0% inhibition at 30 μM, IC_{50} = 18.0 μM) decreased the activity as compared to **6e**. These results indicated that the bulkier cyclohexyl group have better activity than the phenyl one.

Next objective of our work was to find the suitable position of bulky hydrophobic substituent on benzimidazole ring A of **1**. Thus, location of hydrophobic alkoxy groups was moved to position 5 of benzimidazole as shown in 5-isopentyloxy analog **6i** and 5-cyclohexylmethoxy analog

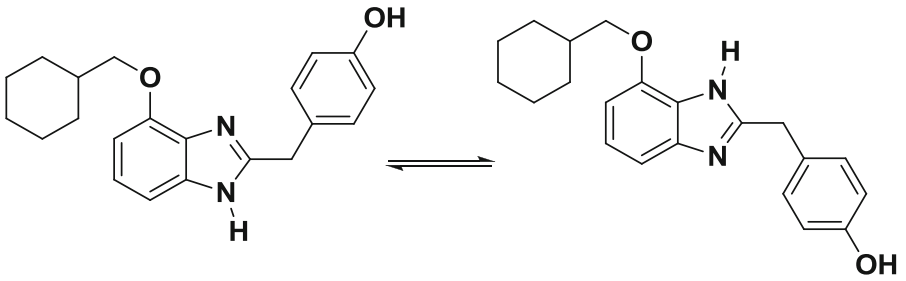


Scheme 1 Synthesis of benzimidazole derivatives **6a–o** and **6s** (substituents are listed in Table 1). Reagents and Conditions: (*a*) R-X, K_2CO_3, DMF, 60 °C, 8–16 h, 67–87%; (*b*) 10% Pd–C, H_2, methanol, rt, 2 h (by using parr shaker), 72–92%; or (*c*) $SnCl_2$, ethanol, reflux, 5 h (for R = benzyl), 65%; (*d*) 6*N* HCl, reflux, 16 h, 58–77%; or (*e*) boric acid, xylene, reflux, 48 h, (for R = benzyl), 22%



Scheme 2 Synthesis of benzimidazole derivatives **6p–r** (substituents are listed in Table 1). Reagents and conditions: (*a*) 10% Pd–C, H_2, methanol, rt, 2 h (by using parr shaker), 95%; (*b*) 6*N* HCl, reflux, 16 h, 65–75%

Fig. 3 Tautomerism of benzimidazole analog **6e**



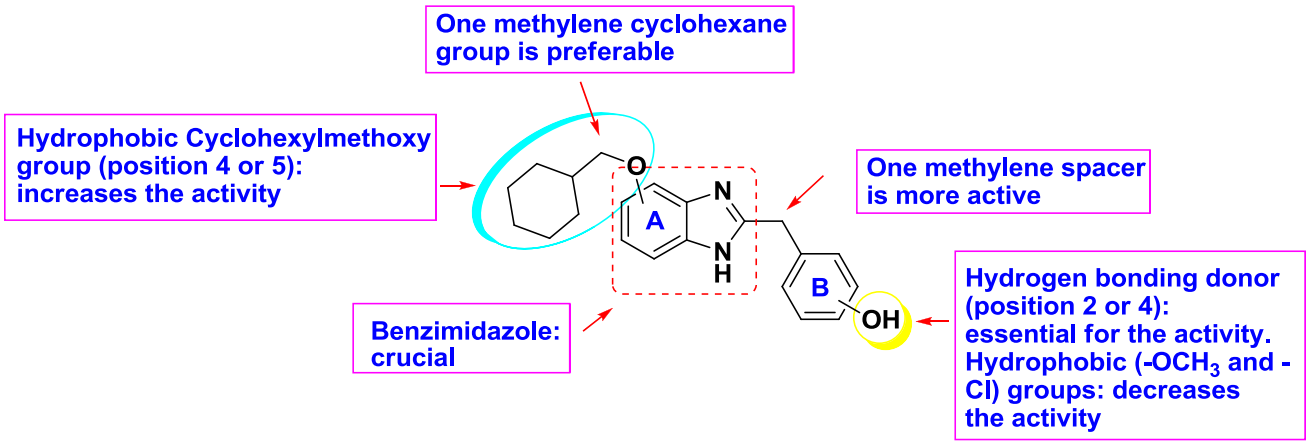


Fig. 4 SAR analysis of the novel benzimidazole **6** analogs

6j. Analog **6i** (84.0% inhibition at 30 μM, IC_{50} = 7.0 μM) showed more potent activity compared to **6a**, whereas less potent activity compared to **6e**. However, compound **6j** (96.0% inhibition at 30 μM, IC_{50} = 4.0 μM) showed almost similar activity as compared to **6e**. These results confirmed that both positions 4 and 5 for bulky hydrophobic cyclohexylmethoxy group on the benzimidazole ring A of **1** are acceptable for the inhibition of LPS induced NF-κB activity.

The promising activity of **6e** led us to shift our focus toward finding the suitable position for the hydroxyl group on phenyl ring B, since the hydroxyl group on phenyl ring in the JSH compound plays an important role for the inhibition of NF-κB activation as we discussed earlier (Roh et al. 2011). Therefore, position of hydroxyl group on benzimidazole **6e** was shifted to position 2 on phenyl ring B as shown in analog **6k** (95.0% inhibition at 30 μM, IC_{50} = 5.0 μM), which resulted in the comparable activity. Thus, this suggests that the position 2 and 4 of phenyl ring B for hydroxyl group should be equally suitable for these benzimidazole analogs in the inhibition of NF-κB.

To prove the importance of hydroxyl functional group on phenyl ring B, the hydrogen bonding ability of hydroxyl function in the highly active compounds **6e**, **6j** and **6k** were masked by the replacement with methoxy group as shown in **6l** (71.0% inhibition at 30 μM, IC_{50} = 17.0 μM), **6m** (48.0% inhibition at 30 μM, IC_{50} = >30.0 μM) and **6n** (90.0% inhibition at 30 μM, IC_{50} = 8.0 μM) or chloro group in **6o** (46.0% inhibition at 30 μM, IC_{50} = >30 μM). Analogs **6l** and **6n** showed moderate activity while the

activity of **6m** and **6o** were nearly abolished. These results indicated that the hydrogen bonding ability of hydroxyl function in ring B should be essential for the NF-κB inhibitory activity of these benzimidazoles.

To further study the importance of hydrophobic group on benzimidazole ring A, analogs containing hydroxyl group at position 4 of benzimidazole motif as shown in **6p** (R^1 = 4-OH, IC_{50} = >30 μM), **6q** (R^1 = 2-OH, IC_{50} = >30 μM) and **6r** (R^1 = 4-OCH_3, IC_{50} = >30 μM) were prepared and did not show any activity. These results again confirmed the importance of bulky hydrophobic group on benzimidazole ring A for the inhibition of NF-κB activity.

Finally, to explore the optimum distance between the benzimidazole ring A and phenyl ring B, the carbon chain between the benzimidazole and phenyl of **6e** was increased with ethylene linkage as shown in **6s** (90.0% inhibition at 30 μM, IC_{50} = 7.0 μM), which did not improve the activity. Thus, it proved that only one methylene group between the two rings is optimum for the inhibitory activity.

Conclusion

In order to discover the optimum functional groups of novel 2-(4-hydroxyphenylmethyl)benzimidazole (**1**) possessing the high inhibitory activity against LPS induced NF-κB activation, the substituents on benzimidazole and phenyl rings and methylene spacer between these two rings were systematically investigated based on structural characteristics of chalcone JSH as previously reported. As a result, 4-((4-(cyclohexylmethoxy)-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenol (**6e**) and 4-((5-(cyclohexylmethoxy)-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenol (**6j**) and 2-((4-(cyclohexylmethoxy)-1*H*-benzo[*d*]imidazol-2-yl)methyl)phenol (**6k**) showed 3–5 micromolar level of IC_{50} values for the inhibition of NF-κB activation and are more potent than **1**. The SAR (Fig. 4) confirmed that the substitution on benzimidazole ring A with bulky hydrophobic cyclohexylmethoxy group at position 4 or 5 showed potent NF-κB inhibition. In addition, the hydrophilic hydrogen bonding donor group (OH) at position 2 or 4 on phenyl ring B with one methylene spacer to the benzimidazole ring is favorable for the inhibitory activity. However, hydrophobic (–OCH_3 and –Cl) groups on phenyl ring B decreases the activity. Taken together, the current study identifies the alkoxy 2-(substituted benzyl)benzimidazoles as novel molecules for inhibiting the transcription factor NF-κB.

Acknowledgement This work was supported by Priority Research Centre Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093815).

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