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Synthesis and biological evaluation of 4-amino derivatives of benzimidazoquinoxaline, benzimidazoquinoline, and benzopyrazoloquinazoline as potent IKK inhibitors

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Abstract—We have recently identified BMS-345541 (**1**) as a highly selective and potent inhibitor of IKK-2 (IC_{50} = 0.30 μM), which however was considerably less potent against IKK-1 (IC_{50} = 4.0 μM). In order to further explore the SAR around the imidazoquinoxaline tricyclic structure of **1**, we prepared a series of tetracyclic analogues (**7**, **13**, and **18**). The synthesis and biological activities of these potent IKK inhibitors are described.

The nuclear transcription factor NF-κB plays a key role in regulating the expression of many pro-inflammatory genes. Examples of genes modulated by NF-κB include the cytokines tumor necrosis factor (TNF-α), interleukins IL-1, IL-6, IL-8, intercellular adhesion molecule (ICAM-1), and vascular cellular adhesion molecule (VCAM-1).[1] NF-κB is normally retained in the cytoplasm as an inactive form associated with the IκB inhibitory proteins. However, upon cellular stimulation IκB is phosphorylated by the IκB kinase (IKK) for which IKKα (IKK-1) and IKKβ (IKK-2) are the two most common isoforms,[2] and subsequently phosphorylated IκB is ubiquitinated and degraded. NF-κB is then released from the IκB/NF-κB complex into the cell, where it translocates to the nucleus and activates a number of genes.[3] IKK-2 has been shown to be required for the pro-inflammatory cytokine-induced activation of NF-κB in inflammatory cells through the so-called 'classical' NF-κB activation pathway (i.e., degradation of IκB-alpha), whereas IKK-1 appears to be involved in the 'alternative' NF-κB activation pathway, related to the development and organization of secondary lymphoid organs and B-cell maturation.[4] This suggests that inhibitors of IKK could in principle be used in the treatment of inflammatory and related disorders.[5]

We have recently identified BMS-345541, 4-(2′-aminoethyl)amino-1,8-dimethylimidazo[1,2-*a*]quinoxaline (**1**, Fig. 1) as a highly selective and potent inhibitor of IKK-2 (IC_{50} = 0.3 μM), but which showed considerably less potency against IKK-1 (IC_{50} = 4.0 μM).[6] BMS-345541 has also been reported to show dose-dependent efficacy in terms of reducing disease severity in a murine model of dextran sulfate sodium-induced colitis[7] and in a model of collagen-induced arthritis.[8] Our objective was to pre-

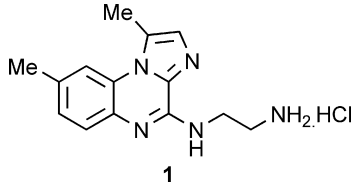

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Figure 1. BMS-345541.

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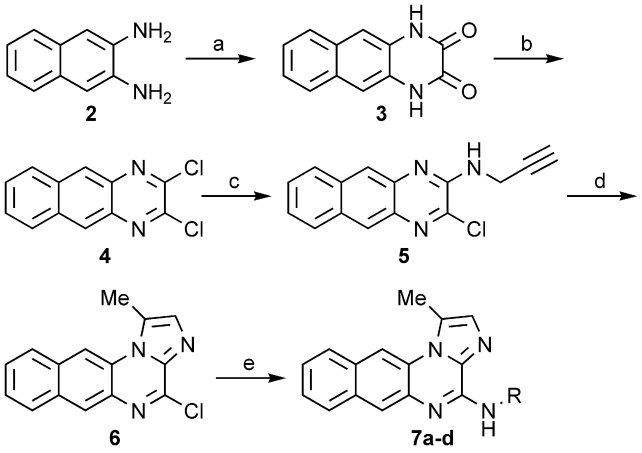
pare tetracyclic analogues related to compound **1** and we report herein the synthesis and biological activities of 4-amino-substituted benzimidazoquinoxaline, benzopyrazoloquinazoline, and benzimidazoquinoline inhibitors of IκB kinase.[9]

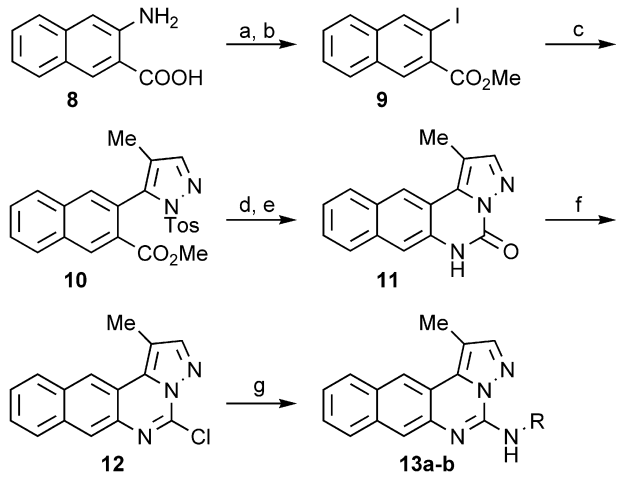
Based on a reported procedure,[10] the synthesis of the benzimidazoquinaxoline series is described in Scheme 1. Commercially available diaminonaphthalene **2** was reacted with diethyl oxalate to give annulated quinoxalinedione **3**, which under treatment with phosphorus oxychloride afforded dichloride **4**. Compound **4** was then reacted with propargylamine to give amino-chloro derivative **5**, which was cyclized under acidic conditions to provide 1-methyl-4-chlorobenzimidazoquinaxoline **6**. Compound **6** then served as a key intermediate to generate 4-amino-substituted analogues **7a**–**d**.[11]

The benzopyrazoloquinazoline series **13a**–**b** was prepared as shown in Scheme 2. 3-Amino-2-naphthoic acid (**8**) was converted to 3-iodo-2-naphthoic acid via diazotization followed by treatment with potassium iodide. Esterification of the resulting acid then gave the ester **9** which was cross-coupled with 4-methyl-1-(4-toluenesulfonyl)-5-trimethylstannylpyrazole[12] under Stille-type conditions to afford the coupled product **10**. Upon hydrolysis, the resulting pyrazole acid intermediate was heated with diphenylphosphoryl azide, producing an intermediate isocyanate which spontaneously cyclized under the reaction conditions to give compound **11**. Exposure of compound **11** to phosphorus oxychloride then gave the chloride intermediate **12**, which was finally reacted with primary amines to provide benzopyrazoloquinazoline analogues **13a**–**b**.

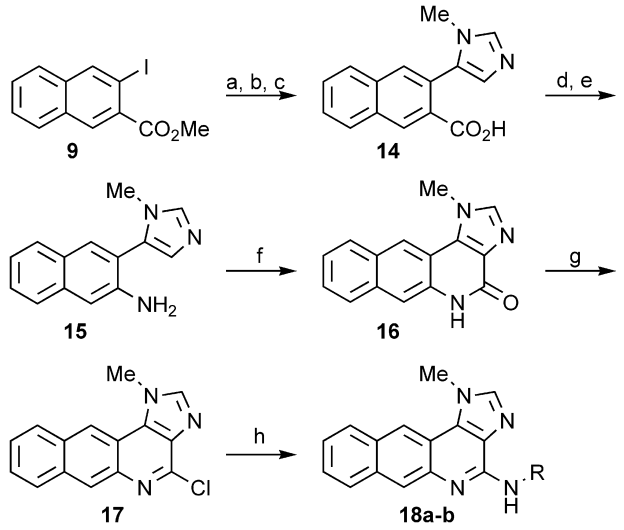
The preparation of the benzimidazoquinolines is outlined in Scheme 3. Previously prepared methyl 3-iodo-2-naphthoate (**9**) was converted to the corresponding boronate derivative using bis(pinacolato)diboron and palladium catalysis. This boronate intermediate was subsequently cross-coupled with 5-bromo-1-methyl-1*H*-imidazole under Suzuki conditions, and the resulting ester was hydrolyzed to give the acid **14**. A Curtius reaction was carried out by reacting the acid **14** with diphenylphosphoryl azide in *tert*-butanol, and this was followed by acid treatment to afford amine **15**. Heating **15** in 1,2-dichlorobenzene with carbonyldiimidazole provided cyclized product **16**, which was subsequently treated with phosphorus oxychloride to give chloride **17**. This intermediate was subsequently reacted with primary amines to give benzimidazoquinolines **18a-b**.



Scheme 1. Synthesis of the benzimidazoquinoxaline series. Reagents and conditions: (a) diethyl oxalate, reflux, 14 h, 82%; (b) $POCl_3$, reflux, 5 h, 85%; (c) propargylamine, Et_3N, 1,4-dioxane, reflux, 4.5 h, 73%; (d) concd H_2SO_4, 80 °C, 1 h, 30%; (e) RNH_2, THF, 80 °C, 18 h, 26–94%.



Scheme 2. Synthesis of the benzopyrazoloquinazoline series. Reagents and conditions: (a) concd HCl, H_2O, $NaNO_2$, 0 °C, KI, rt, 0.1 h then 95 °C, 1 h; (b) concd H_2SO_4, MeOH, reflux, 13 h, 69% over two steps; (c) 4-methyl-1-(4-toluenesulfonyl)-5-trimethylstannylpyrazole, $Pd_2(dba)_3$, Ph_3As, CuI, DMF, 90 °C, 12 h, 55%; (d) 1 N NaOH, THF, MeOH, 80 °C, 4.2 h, 40%; (e) $(PhO)_2PON_3$, Et_3N, benzene, 50 °C, 2 h, then 1,2-dichlorobenzene, 150 °C, 4 h, 67%; (f) $POCl_3$, $PhNEt_2$, reflux, 46 h, 77%; (g) RNH_2, THF, 60–75 °C, 2–5 h, 98%.

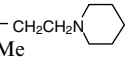


Scheme 3. Synthesis of the benzimidazoquinoline series. Reagents and conditions: (a) bis(pinacolato)diboron, $PdCl_2(dppf)$, KOAc, DMSO, 85 °C, 18 h, 58%; (b) 5-bromo-1-methyl-1*H*-imidazole, $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, H_2O, reflux, 20 h, 72%; (c) 1 N NaOH, THF, MeOH, rt, 20 h, 74%; (d) $(PhO)_2PON_3$, Et_3N, *t*-BuOH, 80 °C, 30 min, 51%; (e) TFA, CH_2Cl_2, rt, 18 h, 70%; (f) CDI, 1,2-dichlorobenzene, 180 °C, 5 h, 42%; (g) $POCl_3$, $PhNEt_2$, reflux, 4 h, 90%; (h) RNH_2, THF, 60–80 °C, 18 h, 5%.

The analogues described above (**7**, **13**, and **18**) were evaluated in a primary screen assay measuring the (IKK-2 and IKK-1) enzyme-catalyzed phosphorylation of GST-IκBα as substrate.[13] The secondary assay measured the inhibition of lipopolysaccharide(LPS)-induced TNF-α secretion in THP-1 cells.[14] The IC_{50} values for all tetracyclic analogues are compared with that of BMS-345541 in Table 1. All tetracyclic analogues tested showed more potent activity than the tricyclic compound **1**, against both IKK-2 and IKK-1.[15] The first series of tetracyclic analogues (**7**), which are related to BMS-345541 by the addition of a fused benzene ring, resulted in an order of magnitude increase in potency in the IKK-2 and THP-1 cell assays. Compound **7a** showed good IKK-2 potency, with a 13-fold selectivity versus IKK-1, comparable to the ratio seen with BMS-345541. Benzimidazoquinoxalines bearing a solubilizing sidechain (i.e., **7b**–**d**) showed no particular advantage in IKK-2 activity over the 4-NHMe analogue **7a**, however, the hydroxyethylamine analogue **7c** gave an improved 48-fold selectivity for IKK-2 versus IKK-1. When the imidazoquinoxaline core was modified to a pyrazoloquinazoline (**13**) or imidazoquinoline (**18**) scaffold, the IKK-2 in vitro activities in general remained comparable to the tetracyclic structures **7**, although a significant loss in cellular potency was observed for compounds **13a** and **18b**.[16]

Since the tetracyclic compounds **7a** and **7b** were more potent IKK-2 inhibitors than the corresponding tricyclic analogue BMS-345541, we examined their in vivo biological activities in mice. As shown in Figures 2 and 3, we measured the effect of compounds **7a** and **7b** on serum TNF-α concentrations induced by intraperitoneal injection of LPS-treated mice.[17] As shown in Figure 2, compound **7a** produced the same effect at 10 mg/kg as did BMS-345541 at 30 mg/kg, which corresponds to approximately a 50% reduction of TNF-α levels versus vehicle control animals. Figure 3 shows that a dose of 100 mg/kg resulted in a nearly complete inhibition of serum TNF-α for BMS-345541 and **7b**, with a good dose-proportional response being observed.

Table 1. IKK-2, IKK-1, and THP-1 cell inhibitory potencies of tetracyclic analogues **7**, **13**, and **18**

Compound	R	IKK-2 IC_{50}, μM[a]	IKK-1 IC_{50}, μM[a]	THP-1 cell IC_{50}, μM[a]
1		0.30	4.0	4.0
7a	–Me	0.018	0.23	0.34
7b	$-CH_2CH_2NHMe\cdot HCl$	0.023	0.39	1.0
7c	$-CH_2CH_2OH$	0.018	0.87	1.4
7d		0.046	1.7	0.60
13a	–Me	0.011	0.67	8.3
13b	$-CH_2CH_2NHMe$	0.035	nd[b]	1.5
18a	–Me	nd[b]	0.99	0.95
18b	$-CH_2CH_2NH_2$	0.058	1.6	5.9

[a] Single experiment.
[b] Not determined.

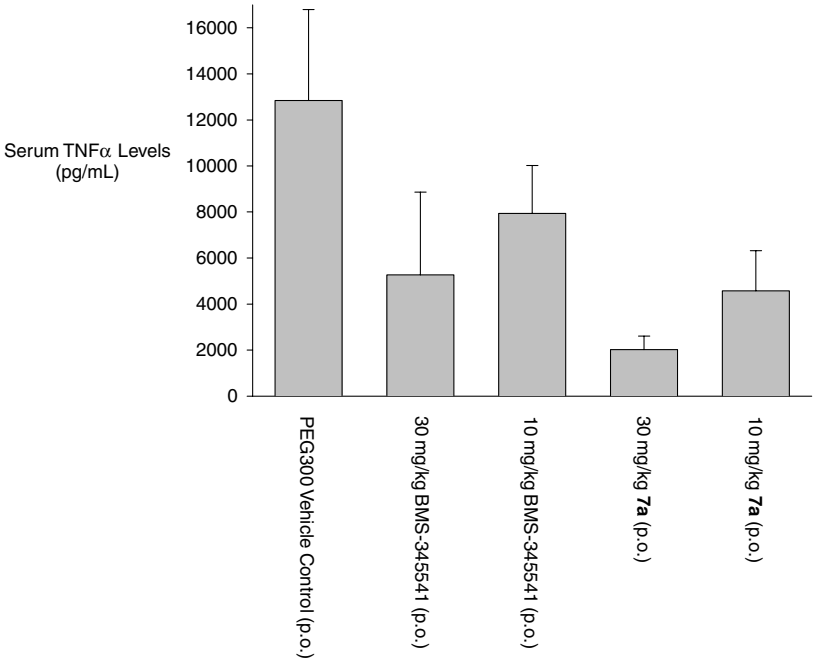


Figure 2. The effect of BMS-345541 and compound **7a** on serum TNF-α concentrations induced by intraperitoneal injection of LPS.

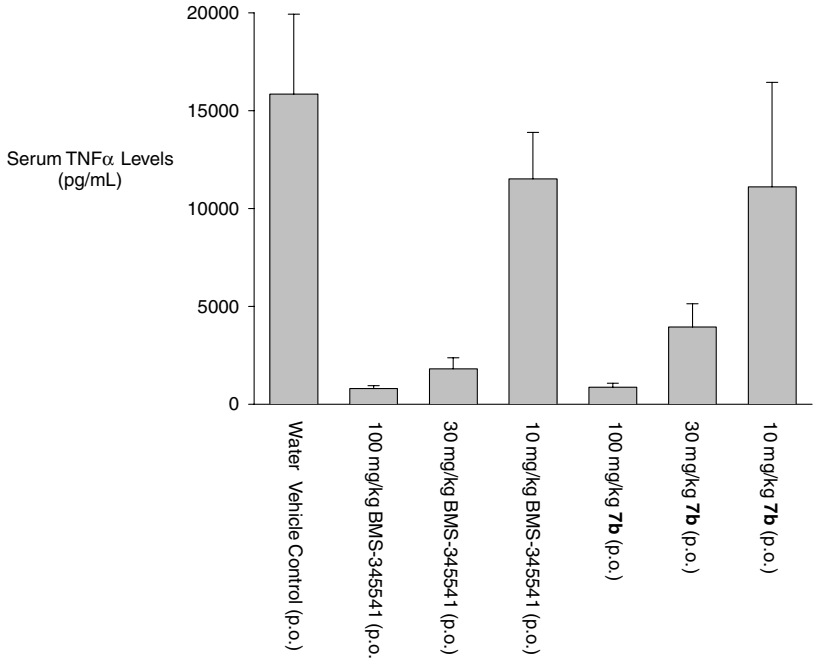


Figure 3. The effect of BMS-345541 and compound **7b** on serum TNF-α concentrations induced by intraperitoneal injection of LPS.

In summary, a series of tetracyclic structures, based on BMS-345541 as a structural lead, were efficiently synthesized and subsequently evaluated as IKK-2 inhibitors in vitro and in vivo. Most of the tetracyclic compounds were more potent than the parent in vitro and two new benzimidazoquinoxalines showed improved overall activity when compared to BMS-345541. Future studies will be directed toward further optimization of these tetracyclic scaffolds.

Acknowledgment

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11. Synthesis of **7a**: To a solution of benzo[*g*]-4-chloro-1-methylimidazo[1,2-*a*]quinoxaline (0.063 g, 0.236 mmol) in 4 mL THF was added $MeNH_2$ (40% in H_2O, 0.16 mL, 1.88 mmol). The reaction tube was sealed, and the mixture was stirred at 80 °C for 18 h. The cooled mixture was then taken up in EtOAc, washed with water and brine, dried (Na_2SO_4), filtered, and evaporated. The resulting residue was recrystallized using *i*-PrOH to give the desired product

as beige needles (0.043 g, 69%); mp 201–202 °C, IR (KBr, cm^{-1}) 3324, 1575, 1557, 1411, 1121; ^1H NMR (400 MHz, DMSO-d_6) δ 8.62 (s, 1H), 8.10 (s, 1H), 8.08 (d, $J = 8.1$ Hz, 1H), 7.94 (d, $J = 8.1$ Hz , 1H), 7.77 (br d, $J = 4.5$ Hz, 1H), 7.49–7.45 (m, 2H), 7.35 (s, 1H), 3.06 (d, $J = 4.6$ Hz, 3H), 2.98 (s, 3H); MS ($^+$ESI, M + H^+) *m/z* 263; HPLC purity: 99.5% (230 nm); HRMS calcd for $C_{16}H_{14}N_4$: 262.1219; found 262.1226.

12. This compound was prepared in 43% yield from 4-methyl pyrazole in two steps (a, *p*-TsCl, pyridine; b, *t*-BuLi, Me_3SnCl).
13. Enzyme Assays. Assays measuring the enzyme-catalyzed phosphorylation of GST-IκBα were performed essentially as described in reference [5], by adding enzyme (IKK-2 or IKK-1, typically to a final concentration of 0.5 μg/mL) at 30 °C to solutions of 50 μg/mL GST-IκBα and 20 μM ATP in 30 mM Tris–HCl, pH 8, containing 7.5 mM $MgCl_2$, 30 mM sodium phosphate, 3 mM NaCl, 0.6 mM potassium phosphate, 1 mM KCl, 1 mM dithiothreitol, 5% (w/v) glycerol, and 475 μg/mL bovine serum albumin.
14. Cytokine production in THP-1 cells. THP-1 cells in 96-well plates (2.5×10^5 cells/well in 180 μL RPMI-1640/10% FBS) were treated with inhibitors for 1 h prior to stimulation with 100 ng/mL LPS. Measurements of the amount of TNF-α in the supernatants were made after 6 h of LPS stimulation. Specific enzyme immunoassay kits from Pharmingen (OptEIA) were used in quantitating each cytokine.
15. Compound **7b** was screened against other kinases (IKK-ε, LCK, EMT) and failed to show inhibition at concentrations as high as 50 μM (data not shown). Enzyme kinetic analysis of inhibition was not performed to investigate the binding mechanism of these new inhibitors.
16. Based on inspection of the structures and physicochemical properties, the reason for the significant loss in cellular potency for **13a** and **18b** remains unclear.
17. LPS-induced serum TNF-α in mice. The general procedure of Ghezzi et al. (*Cytokine* **2000**, 12, 1205–1210) was followed. Briefly, 0.2 mL of an aqueous solution of an inhibitor was administered by peroral gavage to 6- to 8-week-old female BALB/c mice (Harlan) 1 h prior to a challenge with an intraperitoneal dose of 1 μg *Escherichia coli* LPS (from strain O111:B4, Sigma) in 500 μL phosphate-buffered saline. Blood was collected from mice 90 min after LPS challenge and the levels of TNF-α in the serum measured by EIA (R&D systems). Inhibition by the inhibitor was calculated from control animals which received LPS challenge but no inhibitor (vehicle only). Mice receiving no LPS challenge gave no detectable serum TNF-α.