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Preliminary evaluation of a 3*H* imidazoquinoline library as dual TLR7/TLR8 antagonists

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ABSTRACT

Toll-like receptors (TLR) -7 and -8 are thought to play an important role in immune activation processes underlying the pathophysiology of HIV and several clinically important autoimmune diseases. Based on our earlier findings of TLR7-antagonistic activity in a 3*H* imidazoquinoline, we sought to examine a pilot library of 3*H* imidazoquinolines for dual TLR7/8 antagonists, since they remain a poorly explored chemotype. 2D-NOE experiments were employed to unequivocally characterize the compounds. A quinolinium compound **12**, bearing *p*-methoxybenzyl substituents on *N*3 and *N*5 positions was identified as a lead. Compound **12** was found to inhibit both TLR7 and TLR8 at low micromolar concentrations. Our preliminary results suggest that alkylation with electron-rich substituents on the quinoline *N*5, or conversely, elimination of the fixed charge of the resultant quaternary amine on the quinolinium may yield more active compounds.

1. Introduction

Chronic immune activation is a hallmark of several infectious and autoimmune diseases. Dysregulated cellular and humoral immune responses in progressive HIV infection,[1,2] for instance, leads to accelerated turnover of $CD4^+$ lymphocytes, thereby providing a milieu for HIV replication.[3] The engagement of toll-like receptor-7 (TLR7) by single-stranded viral RNA (ssRNA) has recently been reported to play a central role in immune activation-driven HIV replication.[4] Progressive $CD4^+$ T lymphocyte depletion in non-human primate models is highly correlated with TLR7-mediated interferon-α (IFN-α) production by plasmacytoid dendritic cells, and antagonists of TLR7 have been shown to inhibit immune activation.[4] In contrast to the predominantly $CD4^+$ T lymphocyte-driven activation by TLR7- and -8 mediated recognition of HIV ssRNA, autoreactive B lymphocytes are thought to play an important role in the sustained generation of autoantibodies directed against both cytosolic and nuclear components contributing to the pathophysiology of disease states such as Systemic Lupus Erythematosis and Sjögren's syndrome.[5–10] TLR7 and TLR8 are thus logical targets for pharmacological intervention, and inhibitors for these endosomal receptors are being actively studied for potential use in the therapy of such autoimmune diseases.[6,11,12]

Whereas small molecule agonists of TLR7 are well known,[13–15] the only known class of TLR7 antagonists, until recently, were single-stranded phosphorothioate oligonucleotides.[16–19] En route to the synthesis of a TLR7-agonistic imidazoquinoline, gardiquimod, we synthesized its 3*H* regioisomer, which was found to be a weak TLR7 antagonist.[20] A *des*-amino precursor of the 3*H* regioisomer (**4a**, Scheme 1) was more potent as a TLR7 antagonist, with an IC_{50} value of 7.5 μM;[20] negligible TLR8-antagonstic activity, however, was observed with this compound. Given the potential value of a detailed examination of this poorly explored chemotype toward obtaining leads for novel, and more potent TLR7/8 dual antagonists, we undertook the syntheses and evaluation of a preliminary library of 3*H* imidazoquinolines with the aim of identifying potential chemotypes capable of inhibiting both TLR7 and TLR8.

2. Results and discussion

Our point of departure was **4a** (Scheme 1), a 4-desamino, 3*H* imidazoquinoline with a 2-methyl-propan-2-ol substituent at *N*3,

Abbreviations: CD, cluster of differentiation; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; ESI-TOF, electrospray ionization-time of flight; HATU, 2-(1*H*-7-azabenzo-triazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate; HEK, human embryonic kidney; HIV, human immunodeficiency virus; IC_{50}, half-maximal inhibitory concentration; IFN-α, interferon-α; NF-κB, nuclear factor-kappa B; NOE, nuclear overhauser effect; 2D-NOESY, two-dimensional nuclear overhauser effect spectroscopy; sAP, secreted alkaline phosphatase; ssRNA, single stranded RNA; THF, tetrahydrofuran; TLR, toll-like receptor.

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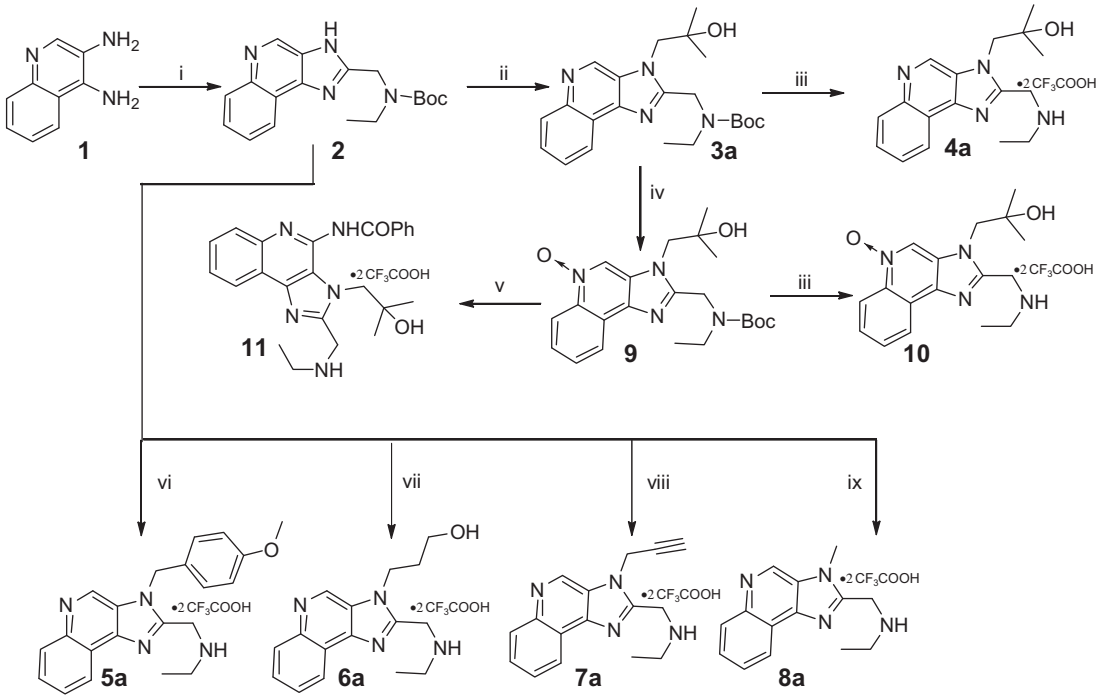


Scheme 1. Syntheses of derivatives of **4a** and *N*3-substituted 3*H* imidazoquinolines. Reagents and conditions: (i) (a) 2-(*ter t* Butoxycarbonyl(ethyl)amino)acetic acid, HATU, DMF (b) $NaOH/H_2O$, EtOH**;** (ii) DBU, 2,2-dimethyloxirane; (iii) CF_3COOH; (iv) 3-chloroperoxybenzoic acid, CH_2Cl_2, $CHCl_3$, MeOH; (v) (a) benzoyl isocyanate, CH_2Cl_2 (b) CF_3COOH. (vi) (a) 1-(chloromethyl)-4-methoxybenzene, THF, 80 °C (b) CF_3COOH; (vii) (a) 3-bromo-1-propanol, DMF, 80 °C (b) CF_3COOH; (viii) (a) propargyl bromide, THF, 90 °C (b) CF_3COOH; (ix) (a) methyl iodide, DBU, THF (b) CF_3COOH.

and a 2-(ethylamino)methylene substituent at C2.[20] We first synthesized the quinoline N-oxide and C4-*N*-benzoyl derivatives (**10**, and **11**, respectively; Scheme 1). Upon finding that neither compound showed any appreciable activity (Table 1), we elected to first examine a small subset of compounds with varying substituents at *N*3 (**5a**–**8a**; Scheme 1). These compounds also were found to be either inactive (**6a**) or of low potency (**5a**, **7a**, **8a**; Table 1).

We next attempted generating a small subset (15 compounds) in which the substituents at *N*3 and C2 were combinatorially varied. Since this was an exploratory library, we chose the C2 substituents from among planar, aromatic (phenyl), a cycloaliphatic (cyclohexyl), and a long-chain aliphatic (nonyl) groups (Scheme 2), while preserving the *N*3 substituents that we had used in Scheme 1. Alkylation of the three series of compounds afforded, as expected, three sets of regioisomers. In order to unambiguously characterize the position of the alkyl groups in these isomers, we correlated the crystal structure of **4b** with its 2D-NOESY spectrum (Fig. 1). The NOESY spectrum showed diagnostic NOEs between the methylene protons on *N*3 (atom 20) with the quinoline proton (atom 9), as well as the phenyl protons (atoms 15, 19). Also seen, as would be expected, are NOEs between the methylene protons on *N*3 (atom 20) with the terminal dimethyl protons on the *N*3 substituent (Fig. 1). NOESY spectra for compounds **6b**, **7d**, and **8c** (Supplementary data) with differing C2 and *N*3 substituents are consistent with the regioselectivity observed with the alkylation reactions.

Most of these compounds displayed modest activity, with exceptions being the C2-nonyl-substituted **7d** and **8d** compounds exhibiting low micromolar TLR7-inhibitory activity. **7d** was also found to be TLR8-antagonistic (IC_{50}: 10 μM, Table 1).

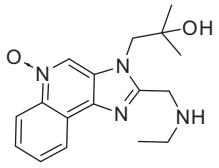
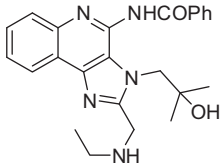
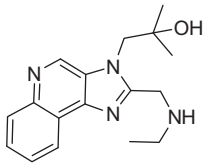
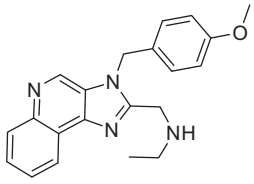
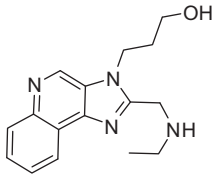
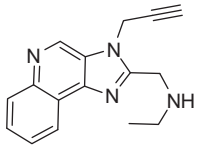
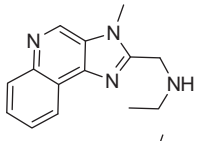
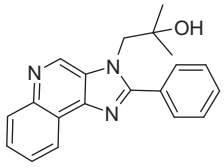
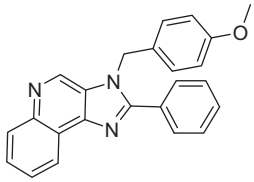
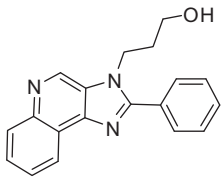
During the synthesis of **5b**, one of the side-products, **12**, corresponded in mass- and NMR-spectral characteristics to a *bis*-alkylated compound. This was isolated and was found to be the best-in-class in both TLR7- and TLR8-antagonism assays, with IC_{50} values of 2.79 and 4.55 μM, respectively (Table 1). We verified that the inhibition was competitive by classic Schild analyses; the IC_{50} values for **12** were determined to be 2.79 and 0.82 μM, at agonist (gardiquimod) concentrations of 1 μg/mL and 250 ng/mL, respectively. In order to determine whether **12** was a quinolinium or imidazolinium species, we synthesized the mono-alkylated quinolinium **13** and the 1*H* regioisomer **17** (Scheme 3). The dialkyl species **12** was obtained using an excess of 1-(chloromethyl)-4-methoxybenzene and DBU as a base in THF at 150 °C, whereas **13** was obtained in the absence of DBU and in DMF at 120 °C. The 1*H* regioisomer **17** was synthesized by pre-installing the *p*-methoxybenzyl substituent on *N*1 (Scheme 3). We characterized the monoalkylated regioisomers **5b**, **13**, and **17** (Scheme 3) via 2D-NOESY experiments (Fig. 2).

Compounds **5b**, **13**, and **17** are all monoalkylated species, with the *p*-methoxybenzyl substituent at the *N*3 (imidazole), *N*5 (quinolinium), *N*1 (imidazole), respectively. Each of these compounds showed characteristic and diagnostic NOE crosspeaks. In **5b** (as in **4b**, discussed earlier), the NOESY spectrum showed NOEs between the methylene protons on *N*3 (atom 20) with the quinoline proton (atom 9), as well as the phenyl protons (atoms 15, 19); compound **13** showed NOEs between the methylene protons on *N*5 (atom 20) with the quinoline protons (atoms 9 and 6); compound **17** was distinguished by crosspeaks between phenyl protons (atoms 15, 19) and the quinoline proton (atom 3) (Fig. 2). The clear NOE patterns helped establish unequivocally that the additional *p*-methoxybezyl substituent in compound **12** was on the quinoline nitrogen (*N*5) because of NOEs similar to both **13** and **5b** (Fig. 2).

Compound **12**, to our considerable surprise, showed the highest potency in simultaneously inhibiting both TLR7 and TLR8 (Table 1, Fig. 3). This was unexpected since the quinolinium compound with its fixed charge is generally thought to be relatively membrane-impermeant and, as discussed earlier, both TLR7 and TLR8 are compartmentalized in the endosome.

In conclusion, a pilot library of 3*H* imidazoquinolines have been synthesized, characterized, and evaluated for biological activity. Although possessing modest activity, a dual TLR7/TLR8 antagonist,

Table 1
TLR7- and TLR8-antagonistic activities of the title compounds. ND denotes no significant activity detected at the highest concentration tested (250 μM). All samples were tested in duplicate, except for **7b**, **7d**, and **12** which were assayed in quadruplicate

Structure	Compound number	TLR7 antagonistic activity (μM)	TLR8 antagonistic activity (μM)
	**10**	ND	46.5
	**11**	ND	21.17
	**4a**	7.5	56.31
	**5a**	28.02	59.64
	**6a**	ND	ND
	**7a**	26.1	28.13
	**8a**	30.44	35.43
	**4b**	ND	38.42
	**5b**	ND	ND
	**6b**	ND	37.24

(*continued on next page*)

Table 1 (*continued*)

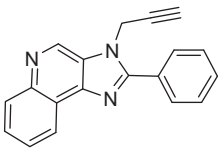
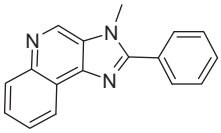
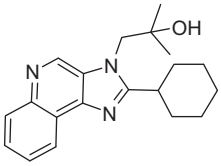
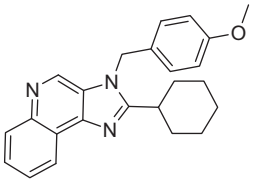
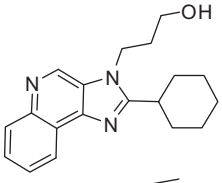
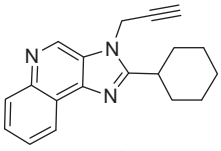
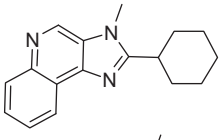
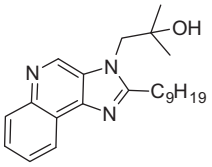
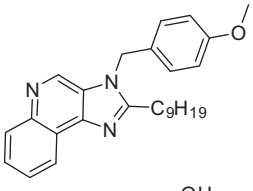
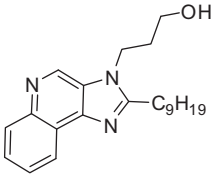
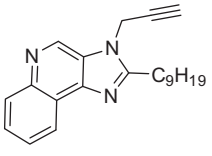
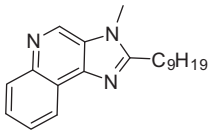
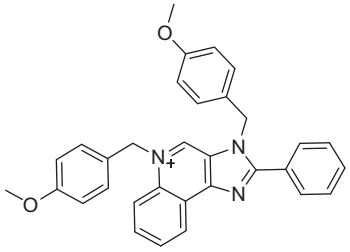
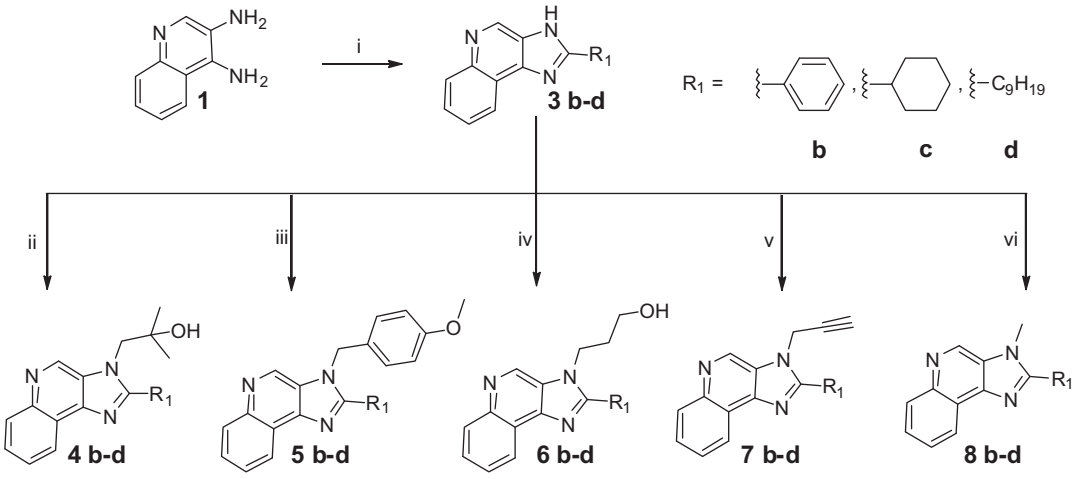
Structure	Compound number	TLR7 antagonistic activity (μM)	TLR8 antagonistic activity (μM)
	**7b**	14.85 ± 0.74	13.71 ± 0.75
	**8b**	21.63	25.81
	**4c**	ND	38.73
	**5c**	19.9	40.01
	**6c**	ND	20.32
	**7c**	21.87	21.12
	**8c**	22.04	24.3
	**4d**	14.4	52.94
	**5d**	19.44	46.4
	**6d**	ND	4.81
	**7d**	8.58 ± 0.43	10.07 ± 0.50

Table 1 (*continued*)

Structure	Compound number	TLR7 antagonistic activity (μM)	TLR8 antagonistic activity (μM)
	**8d**	10.14	68.8
	**12**	2.79 ± 0.12	4.55 ± 0.13

SD values are given for these latter compounds.



Scheme 2. Syntheses of *N*3 and C2 modified 3*H* imidazoquinoline compounds. Reagents and conditions: (i) polyphosphoric acid, R_1-COOH, 180 °C; (ii) DBU, 2,2-dimethyloxirane; (iii) 1-(chloromethyl)-4-methoxybenzene, DBU, THF, 80 °C; (iv) 3-bromo-1-propanol, DBU, DMF, 80 °C; (v) propargyl bromide, DBU, THF, 90 °C; (vi) methyl iodide, DBU, THF.

12, has been identified with micromolar potencies. These preliminary results have been instructive in that they already point to strategies for improvement in potency. For instance, the monoalkylated compounds **7b** and **7d**, bearing propargyl groups on *N*3, ranked next in potency to **12**, are attractive leads for additional alkylation with electron-rich substitutents on the quinoline *N*5. As mentioned earlier, the quaternary amine on the quinolinium of **12** may deter optimal trans-membrane transport and concentration in the endosomal compartment, and carbocyclic analogues may be evaluated to carefully examine the effect of the fixed charge.

3. Experimental section

All of the solvents and reagents used were obtained commercially and used as such unless noted otherwise. Moisture- or air-sensitive reactions were conducted under nitrogen atmosphere in oven-dried (120 °C) glass apparatus. The solvents were removed under reduced pressure using standard rotary evaporators. Flash column chromatography was carried out using RediSep Rf 'Gold' high performance silica columns on CombiFlash Rf instrument unless otherwise mentioned, while thin-layer chromatography was carried out on silica gel CCM pre-coated aluminum sheets. Microwave reactions were done in Synthos 3000 instrument (Anton Paar). Purity for all final compounds was confirmed to be greater than 97% by LC-MS using a Zorbax Eclipse Plus 4.6 × 150 mm, 5 μm analytical reverse phase C_{18} column with H_2O–isopropanol or H_2O–CH_3CN gradients and an Agilent ESI-TOF mass spectrometer (mass accuracy of 3 ppm) operating in the positive ion acquisition mode. Compounds **2**, **3a**, **4a** and **14** were synthesized as published by us earlier.[20] All reported yields are unoptimized.

3.1. Synthesis of compound 10: 2-((ethylamino)methyl)-3-(2-hydroxy-2-methylpropyl)-3*H*-imidazo[4,5-*c*]quinoline 5-oxide

Compound **3a** (145 mg, 0.36 mmol) was dissolved in 10 mL of anhydrous dichloromethane/chloroform (1:1) and 1 mL of anhydrous MeOH. 3-Chloro-peroxybenzoic acid (188 mg, 1.09 mmol) was added and the reaction mixture was refluxed for 30 min. The solvent was then removed under vacuum and the residue was purified using column chromatography (4% MeOH/dichloromethane) to obtain the compound **9** (100 mg, 66%). Compound **9** (28 mg, 0.07 mmol) was then dissolved in 5 mL of trifluoroacetic acid and stirred for 30 min. The solvent was then removed and the residue was washed with diethyl ether to obtain the compound **10** (40 mg, 95%). ^{1}H NMR (400 MHz, MeOD) δ 9.46 (s, 1H),

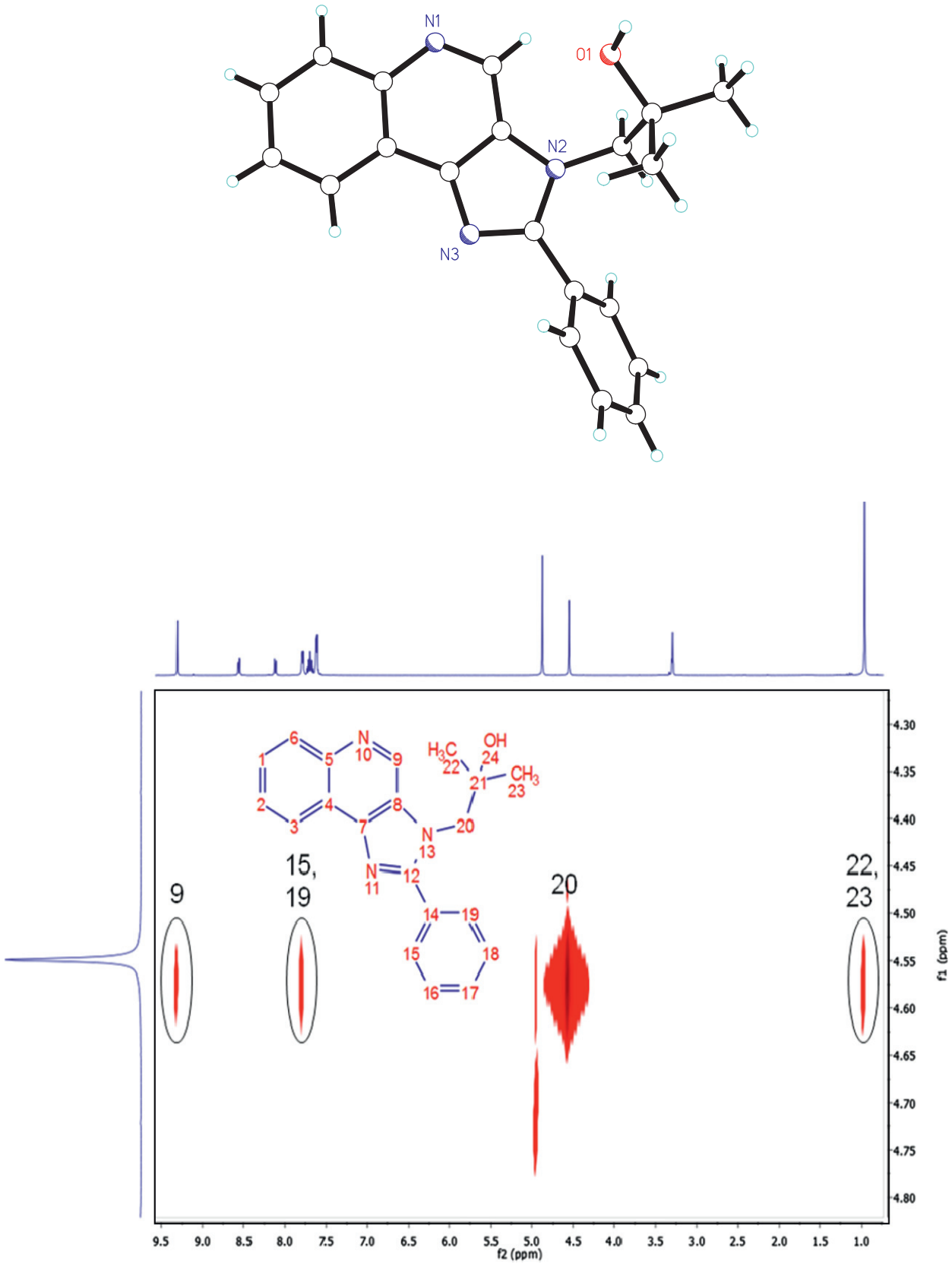
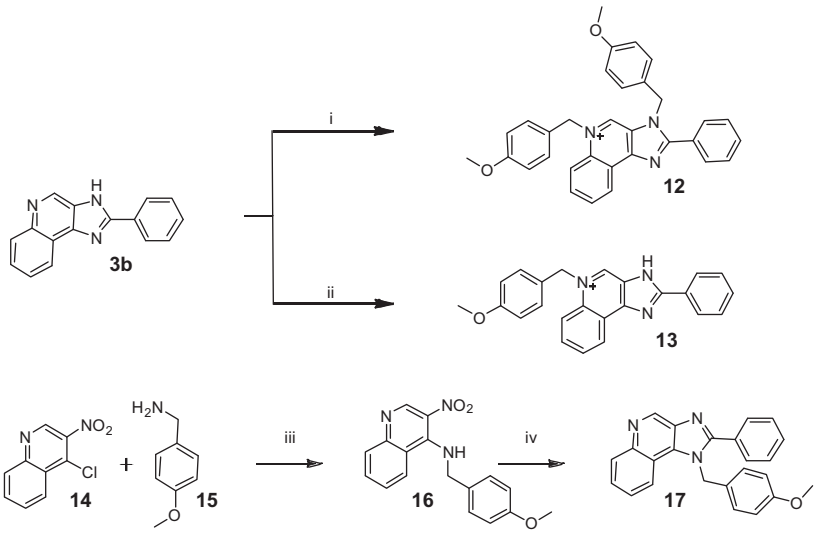


Figure 1. Crystal structure (top) and 2D-NOESY spectrum (bottom) of Compound **4b**.

8.77–8.67 (m, 1H), 8.66–8.55 (m, 1H), 8.00–7.86 (m, 2H), 4.81 (s, 2H), 4.41 (s, 2H), 3.39 (q, J = 7.3, 2H), 1.48 (t, J = 7.3, 3H), 1.31 (s, 6H). ^{13}C NMR (101 MHz, MeOD) δ 151.64, 139.07, 137.10, 129.59, 129.53, 128.75, 127.64, 122.22, 122.06, 119.15, 70.58, 54.56, 43.12, 43.07, 26.06, 10.09. MS (ESI) calcd for $C_{17}H_{22}N_4O_2$, $(M+H)^+$: 315.1816; observed: 315.1764.



Scheme 3. Syntheses of analogues of **5b**. Reagents and conditions: (i) 1-(chloromethyl)-4-methoxybenzene, DBU, THF, 150 °C; (ii) 1-(chloromethyl)-4-methoxybenzene, DMF, 120 °C; (iii) *N*,*N*-diethylpropan-2-amine, toluene/2-propanol (4:1), 70 °C; (iv) (a) H_2, Pd/C, 60 psi, MeOH; (b) Benzoic acid, HBTU, Et_3N, DMAP, DMF; (c) NaOH/H_2O, EtOH.

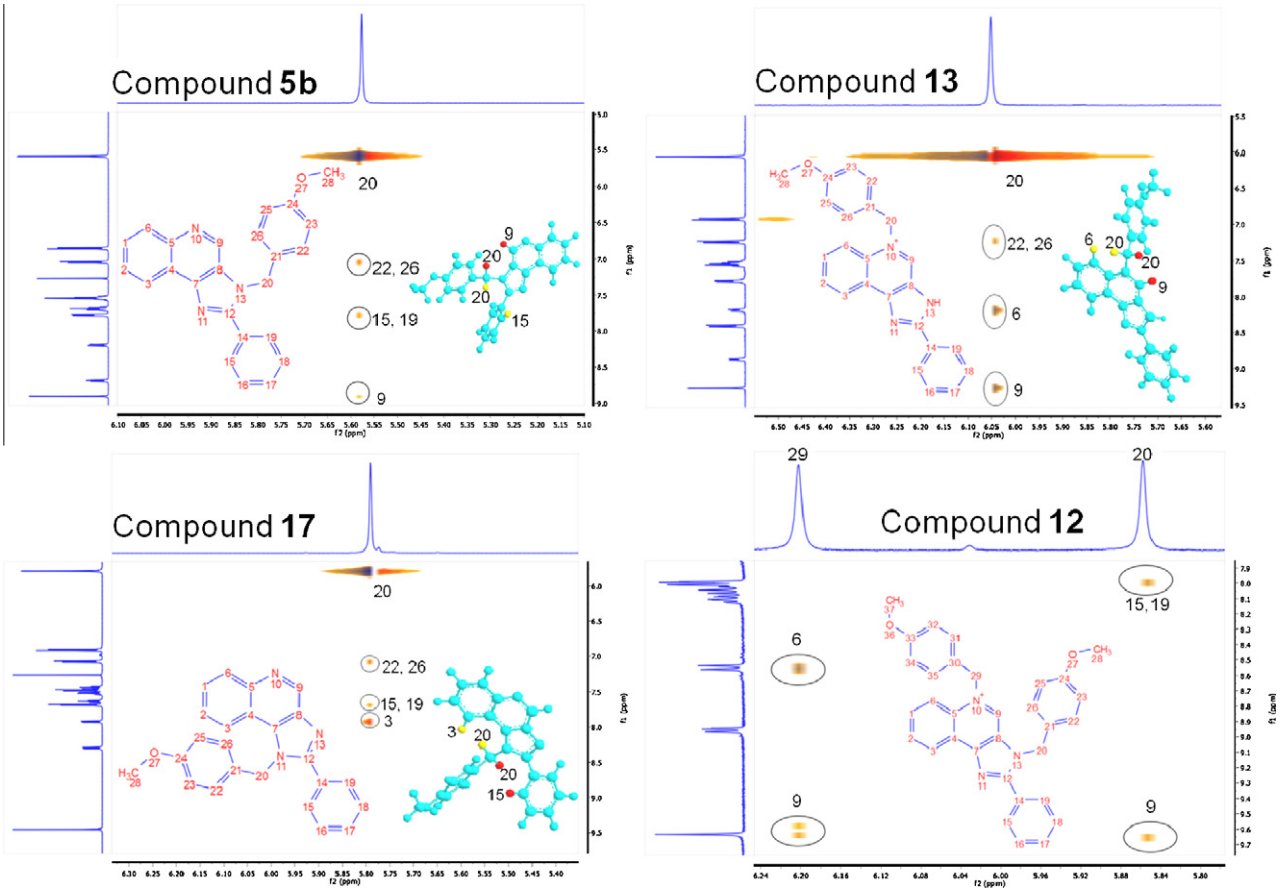


Figure 2. 2D-NOESY spectra of Compounds **5b**, **13**, **17**, and **12**.

3.2. Synthesis of compound 11: *N*-(2-((ethylamino)methyl)-3-(2-hydroxy-2-methylpropyl)-3*H*-imidazo[4,5-*c*]quinolin-4-yl)benzamide

To a solution of **9** (68 mg, 0.16 mmol) in 5 mL of anhydrous dichloromethane, was added benzoylisocyanate (36 mg, 0.25 mmol) and the reaction mixture was refluxed for 30 min. The solvent was then removed under vacuum and the residue was purified using column chromatography (35% EtOAc/dichloromethane) to obtain *tert*-butyl (4-benzamido-3-(2-hydroxy-2-methylpropyl)-3*H*-imidazo[4,5-*c*]quinolin-2-yl)methyl(ethyl)carbamate (30 mg, 35%), which was *N*-Boc deprotected by stirring in 5 mL of trifluoroacetic acid for 30 min The solvent was then removed and the residue was washed with diethyl ether to obtain

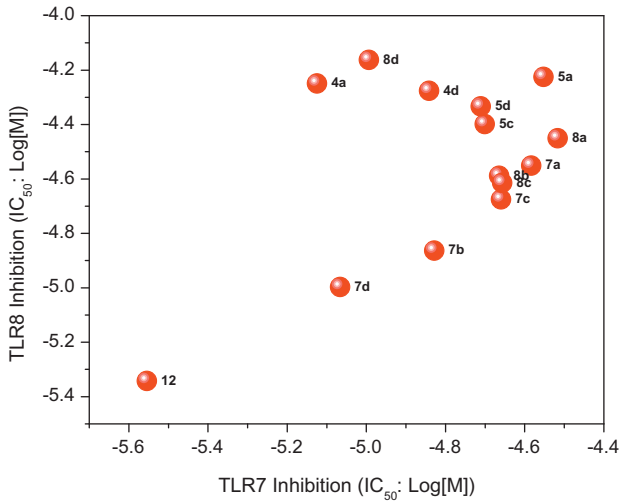


Figure 3. 2D-Scatter plot of TLR7 (abscissa) and TLR8 (ordinate) -antagonistic activities of the title compounds. Compounds that do not show significant inhibitory activity have been omitted.

the compound **11** (35 mg, 95%). ^{1}H NMR at 50 °C (400 MHz, MeOD) δ 8.42 (d, J = 8.1 Hz, 1H), 8.25 (d, J = 7.3 Hz, 2H), 7.81–7.73 (m, 1H), 7.72–7.65 (m, 1H), 7.57 (t, J = 6.7 Hz, 2H), 7.50 (t, J = 7.4 Hz, 2H), 5.19 (s, 2H), 4.80 (s, 2H), 3.35 (q, J = 7.3 Hz, 2H), 1.45 (t, J = 7.3 Hz, 3H), 1.31 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.99, 155.88, 151.18, 145.60, 137.98, 133.28, 131.79, 129.13, 128.93, 128.15, 125.10, 122.17, 118.43, 117.99, 70.60, 56.37, 45.68, 44.04, 27.42, 14.81. MS (ESI) calcd for $C_{24}H_{27}N_5O_2$, $(M+H)^+$: 418.2238; observed: 418.2137.

3.3. Synthesis of compound 5a: *N*-((3-(4-methoxybenzyl)-3*H*-imidazo[4,5-*c*]quinolin-2-yl)methyl)ethanamine

To a solution of **2** (50 mg, 0.15 mmol) in 1 mL of anhydrous THF, were added DBU (47 mg, 0.31 mmol) and 1-(chloromethyl)-4-methoxybenzene (96 mg, 0.61 mmol). The solution was then heated in a sealed vessel at 80 °C for 1 h. After cooling to room temperature, and removing solvent under vacuum, the residue was dissolved in EtOAc and washed with water, dried over sodium sulfate, concentrated and purified using column chromatography (1% MeOH/dichloromethane) to obtain the compound *tert*-butyl ethyl ((3-(4- methoxybenzyl)-3*H*-imidazo [4,5-*c*]quinolin-2-yl)methyl)carbamate, which was dissolved in 5 mL of trifluoroacetic acid and stirred for 30 min. The solvent was then removed and the residue was washed with diethyl ether to obtain the compound **5a** (18 mg, 21%). ^{1}H NMR (400 MHz, MeOD) δ 9.46 (s, 1H), 8.85–8.71 (m, 1H), 8.28 (d, J = 8.0, 1H), 8.07–7.93 (m, 2H), 7.31 (d, J = 8.8 Hz, 2H), 7.04–6.95 (m, 2H), 5.77 (s, 2H), 4.81 (s, 2H), 3.80 (s, 3H), 3.40 (q, J = 7.3 Hz, 2H), 1.48 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 160.28, 153.97, 147.68, 133.33, 130.79, 129.07, 128.77, 128.59, 125.90, 122.61, 122.29, 121.52, 114.47, 54.42, 47.95, 43.33, 42.91, 10.00. MS (ESI) calcd for $C_{21}H_{22}N_4O$, $(M+H)^+$: 347.1866; observed: 347.1890.

3.4. Synthesis of compound 6a: 3-(2-((ethylamino)methyl)-3*H*-imidazo[4,5-*c*]quinolin-3-yl)propan-1-ol

To a solution of **2** (50 mg, 0.15 mmol) in 1 mL of anhydrous THF, were added DBU (47 mg, 0.31 mmol) and 3-bromopropan-1-ol (32 mg, 0.23 mmol). The solution was then heated in a sealed vessel at 80 °C for an hour. After cooling to room temperature, and removing solvent under vacuum, the residue was dissolved in EtOAc and washed with water, dried over sodium sulfate, concentrated and purified using column chromatography to obtain *tert*-butyl ethyl((3-(3-hydroxypropyl)-3*H*-imidazo[4,5-*c*]quinolin-2-yl)methyl)carbamate (9 mg, 15%), which was dissolved in 8 mL of trifluoroacetic acid and stirred for 30 min. The solvent was then removed and the residue was washed with diethyl ether to obtain compound **6a** (11 mg, 95%). ^{1}H NMR (400 MHz, MeOD) δ 9.24 (s, 1H), 8.60–8.52 (m, 1H), 8.21–8.09 (m, 1H), 7.81–7.67 (m, 2H), 4.68 (t, J = 6.8 Hz, 2H), 4.31 (s, 2H), 3.58–3.50 (m, 2H), 2.88 (q, J = 7.2 Hz, 2H), 2.28–2.14 (m, 2H), 1.25 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 154.25, 143.61, 136.25, 128.49, 128.17, 127.45, 126.88, 121.74, 121.20, 57.03, 44.36, 43.42, 40.91, 32.25, 13.04. MS (ESI) calcd for $C_{16}H_{20}N_4O$, $(M+H)^+$: 285.1710; observed: 285.1752.

3.5. Synthesis of compound 7a: *N*-((3-(prop-2-ynyl)-3*H*-imidazo[4,5-*c*]quinolin-2-yl) methyl)ethanamine

To a solution **2** (65 mg, 0.2 mmol) in 1 mL of anhydrous THF, were added DBU (61 mg, 0.4 mmol) and 80% propargyl bromide in toluene (119 mg, 1.0 mmol). The solution was then heated in a sealed vessel at 90 °C for 30 min. After cooling to room temperature, and removing the solvent under vacuum, the residue was dissolved in EtOAc and washed with water, dried over sodium sulfate, concentrated and purified using column chromatography to obtain *tert*-butyl ethyl((3-(prop-2-ynyl)-3*H*-imidazo[4,5-*c*]quinolin-2-yl)methyl)carbamate, which was dissolved in 5 mL of trifluoroacetic acid and stirred for 30 min. The solvent was then removed and the residue was washed with diethyl ether to obtain the compound **7a** (23 mg, 43%). ^{1}H NMR (400 MHz, MeOD) δ 9.74 (s, 1H), 8.77 (dd, J = 8.1, 1.1 Hz, 1H), 8.30 (d, J = 8.1 Hz, 1H), 8.08–7.92 (m, 2H), 5.54 (d, J = 2.6 Hz, 2H), 4.95 (s, 2H), 3.45 (q, J = 7.2 Hz, 2H), 3.27 (t, J = 2.5 Hz, 1H), 1.52 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 153.17, 147.34, 137.95, 133.55, 130.69, 129.01, 128.14, 123.11, 122.21, 121.49, 76.40, 75.07, 43.38, 42.62, 34.26, 10.02. MS (ESI) calcd for $C_{16}H_{16}N_4$, $(M+H)^+$: 265.1448; observed: 265.1553.

3.6. Synthesis of compound 8a: *N*-((3-methyl-3*H*-imidazo[4,5-*c*]quinolin-2-yl)methyl) ethanamine

To a solution of **2** (50 mg, 0.15 mmol) in 1 mL of anhydrous THF, were added DBU (47 mg, 0.31 mmol) and iodomethane (33 mg, 0.23 mmol). The solution was stirred for an hour and the solvent was then removed under vacuum. The residue was dissolved in EtOAc, washed with water, dried over sodium sulfate, concentrated and purified using column chromatography (30% EtOAc/dichloromethane) to obtain *tert*-butyl ethyl((3-methyl-3*H*- imidazo[4,5-*c*]quinolin-2-yl) methyl)carbamate (7 mg, 20%), which was dissolved in 5 mL of trifluoroacetic acid and stirred for 30 min. The solvent was then removed and the residue was washed with diethyl ether to obtain the compound **8a** (13 mg, 95%). ^{1}H NMR (400 MHz, MeOD) δ 9.72 (s, 1H), 8.79 (d, J = 7.9 Hz, 1H), 8.30 (d, J = 8.2 Hz, 1H), 8.14–7.91 (m, 2H), 4.91 (s, 2H), 4.18 (s, 3H), 3.45 (q, J = 7.3 Hz, 2H), 1.52 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 154.89, 147.95, 136.50, 132.91, 130.97, 129.23, 129.14, 122.38, 121.87, 121.36, 43.38, 42.48, 30.57, 10.01. MS (ESI) calcd for $C_{14}H_{16}N_4$, $(M+H)^+$: 241.1448; observed: 241.1476.

3.7. Synthesis of compound 3b: 2-phenyl-3*H*-imidazo[4,5-*c*] quinoline

To a mixture of **1** (450 mg, 2.83 mmol) and benzoic acid (691 mg, 5.66 mmol), was added polyphosphoric acid (approx. 25 mL) and heated to 180 °C for 1 h. The reaction mixture was then slowly cooled to room temperature and the polyphosphoric acid was slowly neutralized with ammonium hydroxide until the pH

was around 8. The compound was then extracted using EtOAc and the EtOAc fraction was then washed with water, dried over sodium sulfate, concentrated and purified using column chromatography (4% MeOH/ dichloromethane) to afford the compound **3b** (315 mg, 45%). ^{1}H NMR (400 MHz, MeOD) δ 9.07 (s, 1H), 8.44 (d, *J* = 6.9 Hz, 1H), 8.18–8.16 (m, 1H), 8.16–8.15 (m, 1H), 8.11–8.06 (m, 1H), 7.72–7.62 (m, 2H), 7.60–7.52 (m, 3H). ^{13}C NMR (101 MHz, MeOD) δ 143.22, 130.45, 129.06, 128.84, 128.14, 127.48, 126.74, 126.63, 121.39. MS (ESI) calcd for $C_{16}H_{11}N_3$, $(M+H)^+$: 246.1026; observed: 246.1025.

3.8. Compounds 3c and 3d were synthesized similarly as described for compound 3b

3c (409 mg, 58%): ^{1}H NMR (500 MHz, MeOD) δ 8.93 (s, 1H), 8.25 (s, 1H), 7.98 (t, *J* = 8.2 Hz, 1H), 7.63–7.52 (m, 2H), 3.02–2.87 (m, 1H), 2.09–2.01 (m, 2H), 1.83 (dd, *J* = 10.4, 7.3 Hz, 2H), 1.69 (dd, *J* = 14.8, 8.0 Hz, 1H), 1.67–1.59 (m, 2H), 1.46–1.35 (m, 2H), 1.34–1.22 (m, 1H). ^{13}C NMR (126 MHz, MeOD) δ 144.54, 129.56, 128.68, 127.95, 122.57, 40.03, 32.89, 27.16, 26.91. MS (ESI) calcd for $C_{16}H_{17}N_3$, $(M+H)^+$: 252.1495; observed: 252.1565.

3d (355 mg, 38%): ^{1}H NMR (500 MHz, MeOD) δ 8.93 (s, 1H), 8.22 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 7.62–7.53 (m, 2H), 2.91 (t, *J* = 7.7 Hz, 2H), 1.83–1.75 (m, 2H), 1.36–1.22 (m, 4H), 1.14–1.17 (m, 8H), 0.75 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 158.61, 144.52, 129.56, 128.72, 128.00, 122.49, 33.02, 30.55, 30.41, 30.37, 30.32, 29.92, 29.41, 23.74, 14.45. MS (ESI) calcd for $C_{19}H_{25}N_3$, $(M+H)^+$: 296.2121; observed: 296.2178.

3.9. Synthesis of compound 4b: 2-methyl-1-(2-phenyl-3*H*-imidazo[4,5-*c*]quinolin-3-yl)propan-2-ol

To a solution of **3b** (50 mg, 0.2 mmol) in 1 mL of 2,2-dimethyloxirane, was added DBU (62 mg, 0.41 mmol) and the solution was heated under microwave conditions (600 W, 80 °C, 1 h). After cooling to room temperature, and removing the solvent under vacuum, the residue was dissolved in EtOAc and washed with water, dried over sodium sulfate, concentrated and purified using column chromatography (4% MeOH/dichloromethane) to obtain the compound **4b** (24 mg, 38%). ^{1}H NMR (400 MHz, MeOD) δ 9.34 (s, 1H), 8.65–8.51 (m, 1H), 8.17–8.13 (m, 1H), 7.87–7.79 (m, 2H), 7.78–7.69 (m, 2H), 7.67–7.63 (m, 3H), 4.59 (s, 2H), 1.00 (s, 6H). ^{13}C NMR (101 MHz, MeOD) δ 155.90, 143.40, 143.02, 139.02, 130.15, 129.95, 129.85, 129.76, 128.72, 128.04, 127.46, 126.80, 121.65, 121.37, 70.71, 55.09, 26.19. MS (ESI) calcd for $C_{20}H_{19}N_3O$, $(M+H)^+$: 318.1601; observed: 318.1631.

3.10. Compounds 4c and 4d were synthesized similarly as described for compound 4b

4c (42 mg, 65%): ^{1}H NMR (400 MHz, MeOD) δ 9.19 (s, 1H), 8.70–8.56 (m, 1H), 8.14–8.05 (m, 1H), 7.74–7.61 (m, 2H), 4.43 (s, 2H), 3.33 (dt, *J* = 3.3, 1.6 Hz, 1H), 2.07–1.80 (m, 7H), 1.62–1.42 (m, 3H), 1.32 (s, 6H). ^{13}C NMR (101 MHz, MeOD) δ 162.55, 143.37, 143.26, 137.65, 128.97, 127.93, 127.12, 126.50, 121.56, 121.47, 70.32, 53.88, 36.16, 31.55, 26.26, 25.84, 25.53. MS (ESI) calcd for $C_{20}H_{25}N_3O$, $(M+H)^+$: 324.2070; observed: 324.2084.

4d (11 mg, 18%): ^{1}H NMR (400 MHz, MeOD) δ 9.17 (s, 1H), 8.58 (d, *J* = 6.4 Hz, 1H), 8.14–8.09 (m, 1H), 7.69 (dd, *J* = 6.8, 3.2 Hz, 2H), 4.40 (s, 2H), 3.13 (t, *J* = 6.4 Hz, 2H), 2.02–1.88 (m, 2H), 1.49 (d, *J* = 6.3 Hz, 2H), 1.39 (d, *J* = 15.1 Hz, 2H), 1.38–1.20 (m, 14H), 0.92–0.86 (m, 3H). ^{13}C NMR (101 MHz, MeOD) δ 158.89, 143.39, 143.19, 137.44, 129.30, 127.99, 127.27, 126.65, 121.52, 121.46, 70.72, 54.40, 31.66, 29.27, 29.22, 29.15, 29.05, 27.97, 27.32, 26.51, 22.37, 13.24. MS (ESI) calcd for $C_{23}H_{33}N_3O$, $(M+H)^+$: 368.2696; observed: 368.2733.

3.11. Synthesis of compound 5b: 3-(4-methoxybenzyl)-2-phenyl-3*H*-imidazo[4,5-*c*]quinoline

To a solution of **3b** (50 mg, 0.2 mmol) in 1 mL of anhydrous THF, were added DBU (62 mg, 0.41 mmol) and 1-(chloromethyl)-4-methoxybenzene (128 mg, 0.82 mmol). The solution was then heated in a sealed vessel at 80 °C for an hour. After cooling to room temperature, and removing the solvent under vacuum, the residue was dissolved in EtOAc and washed with water, dried over sodium sulfate, concentrated and purified using column chromatography (8% EtOAc/dichloromethane) to obtain the compound **5b** (36 mg, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H), 8.73–8.69 (m, 1H), 8.24–8.20 (m, 1H), 7.82–7.77 (m, 2H), 7.74–7.69 (m, 2H), 7.59–7.53 (m, 3H), 7.06 (d, *J* = 8.8 Hz, 2H), 6.90–6.84 (m, 2H), 5.60 (s, 2H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.52, 154.86, 144.69, 144.54, 136.63, 130.42, 129.67, 129.62, 129.55, 129.11, 129.01, 128.30, 127.66, 127.53, 127.45, 126.86, 122.42, 121.87, 114.64, 55.33, 48.60. MS (ESI) calcd for $C_{24}H_{19}N_3O$, $(M+H)^+$: 366.1601; observed: 366.1491.

3.12. Compounds 5c and 5d were synthesized similarly as described for compound 5b

5c (9 mg, 12%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.70–8.60 (m, 1H), 8.18 (dd, *J* = 6.6, 2.9 Hz, 1H), 7.72–7.60 (m, 2H), 7.04 (d, *J* = 8.8 Hz, 2H), 6.92–6.80 (m, 2H), 5.50 (s, 2H), 3.79 (s, 3H), 2.96 (s, 1H), 2.00–1.67 (m, 7H), 1.41 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.52, 135.86, 129.47, 127.60, 127.07, 126.42, 122.31, 121.95, 114.57, 55.33, 47.06, 36.74, 31.84, 26.25, 25.61. MS (ESI) calcd for $C_{24}H_{25}N_3O$, $(M+H)^+$: 372.2070; observed: 372.1990.

5d (21 mg, 30%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 1H), 8.66–8.58 (m, 1H), 8.22–8.15 (m, 1H), 7.73–7.62 (m, 2H), 7.05 (d, *J* = 8.7 Hz, 2H), 6.91–6.82 (m, 2H), 5.47 (s, 2H), 3.79 (s, 3H), 3.05–2.95 (m, 2H), 1.87 (dt, *J* = 15.6, 7.7 Hz, 2H), 1.49–1.38 (m, 2H), 1.33–1.26 (m, 10H), 0.89 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.57, 156.76, 144.46, 144.11, 135.74, 129.54, 128.59, 127.69, 127.33, 127.14, 126.59, 122.20, 121.75, 114.57, 55.32, 47.36, 31.85, 29.54, 29.42, 29.29, 29.25, 28.28, 27.86, 22.66, 14.11. MS (ESI) calcd for $C_{27}H_{33}N_3O$, $(M+H)^+$: 416.2696; observed: 416.2560.

3.13. Synthesis of compound 6b: 3-(2-phenyl-3*H*-imidazo[4,5-*c*]quinolin-3-yl)propan-1-ol

To a solution of **3b** (60 mg, 0.25 mmol) in 2 mL of anhydrous DMF, were added DBU (62 mg, 0.41 mmol) and 3-bromopropan-1-ol (139 mg, 1.0 mmol). The solution was then heated in a sealed vessel at 80 °C for 2 h. After cooling to room temperature, and removing the solvent under vacuum, the residue was dissolved in EtOAc and washed with water, dried over sodium sulfate, concentrated and purified using column chromatography (20%acetone/dichloromethane) to obtain the compound **6b** (13 mg, 18%). ^{1}H NMR (400 MHz, MeOD) δ 9.30 (s, 1H), 8.60 (dt, *J* = 5.0, 2.2 Hz, 1H), 8.21–8.17 (m, 1H), 7.89–7.85 (m, 2H), 7.79–7.71 (m, 2H), 7.67 (dd, *J* = 4.2, 2.4 Hz, 3H), 4.74–4.60 (m, 2H), 3.55 (t, *J* = 4.6 Hz, 2H), 2.12–2.03 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 155.37, 143.79, 143.53, 136.59, 130.46, 129.65, 129.38, 129.15, 128.78, 128.22, 127.59, 126.93, 121.70, 121.40, 58.02, 42.26, 32.70. MS (ESI) calcd for $C_{19}H_{17}N_3O$, $(M+H)^+$: 304.1444; observed: 304.1440.

3.14. Compounds 6c and 6d were synthesized similarly as described for compound 6b

6c (12 mg, 16%): ^{1}H NMR (400 MHz, MeOD) δ 9.18 (s, 1H), 8.66–8.59 (m, 1H), 8.12 (dt, *J* = 4.8, 2.8 Hz, 1H), 7.72–7.67 (m, 2H), 4.61 (t, *J* = 7.2 Hz, 2H), 3.64 (dd, *J* = 12.7, 7.0 Hz, 2H), 3.27–3.16 (m,

1H), 2.15–2.09 (m, 2H), 2.05–1.85 (m, 8H), 1.66–1.39 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 161.46, 143.61, 135.86, 128.04, 127.90, 127.22, 126.61, 121.63, 121.44, 57.72, 40.52, 35.95, 32.95, 31.62, 25.82, 25.49. MS (ESI) calcd for $C_{19}H_{23}N_3O$, $(M+H)^+$: 310.1914; observed: 310.1927.

6d (11 mg, 22%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 8.58 (dt, *J* = 17.0, 8.1 Hz, 1H), 8.17 (dt, *J* = 18.1, 9.6 Hz, 1H), 7.78–7.55 (m, 2H), 4.53 (t, *J* = 6.8 Hz, 2H), 3.72 (t, *J* = 5.6 Hz, 2H), 3.11–2.96 (m, 2H), 2.22–2.03 (m, 2H), 1.94 (dt, *J* = 15.6, 7.7 Hz, 2H), 1.54–1.43 (m, 2H), 1.35–1.28 (m, 10H), 0.89 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.89, 144.17, 144.08, 135.51, 129.19, 128.53, 127.16, 126.59, 122.15, 121.79, 58.16, 40.70, 32.85, 31.86, 29.62, 29.46, 29.36, 29.26, 28.43, 27.50, 22.66, 14.10. MS (ESI) calcd for $C_{22}H_{31}N_3O$, $(M+H)^+$: 354.2540; observed: 354.2401.

3.15. Synthesis of compound 7b: 2-phenyl-3-(prop-2-ynyl)-3*H*-imidazo[4,5-*c*]quinoline

To a solution of **3b** (50 mg, 0.21 mmol) in 1 mL of anhydrous THF, were added DBU (62 mg, 0.41 mmol) and 80% propargyl bromide in toluene (98 mg, 0.82 mmol). The solution was then heated in a sealed vessel at 90 °C for an hour. After cooling to room temperature, and removing the solvent under vacuum, the residue was dissolved in EtOAc and washed with water, dried over sodium sulfate, concentrated and purified using column chromatography (35% EtOAc/dichloromethane) to obtain the compound **7b** (6 mg, 10%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.72–8.67 (m, 1H), 8.30–8.25 (m, 1H), 7.97–7.90 (m, 2H), 7.69–7.77 (m, 2H), 7.67–7.60 (m, 3H), 5.15 (d, *J* = 2.5, 2H), 2.59 (t, *J* = 2.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.90, 136.03, 130.66, 129.72, 129.69, 129.17, 128.63, 127.60, 126.95, 121.90, 75.11, 35.35. MS (ESI) calcd for $C_{19}H_{13}N_3$, $(M+H)^+$: 284.1182; observed: 284.1046.

3.16. Compounds 7c and 7d were synthesized similarly as described for compound 7b

7c (8 mg, 12%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1H), 8.65–8.56 (m, 1H), 8.28–8.11 (m, 1H), 7.75–7.60 (m, 2H), 5.09 (d, *J* = 2.5 Hz, 2H), 3.00 (m, 1H), 2.48 (t, *J* = 2.5 Hz, 1H), 2.11 (m, 2H), 2.04–1.89 (m, 6H), 1.47 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.48, 144.62, 135.35, 129.54, 127.76, 127.20, 126.51, 121.95, 76.47, 74.50, 36.58, 33.42, 31.62, 26.20, 25.65. MS (ESI) calcd for $C_{19}H_{19}N_3$, $(M+H)^+$: 290.1652; observed: 290.1740.

7d (10 mg, 15%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1H), 8.65–8.53 (m, 1H), 8.29–8.16 (m, 1H), 7.76–7.61 (m, 2H), 5.07 (d, *J* = 2.5 Hz, 2H), 3.13–2.97 (m, 2H), 2.48 (t, *J* = 2.5 Hz, 1H), 1.96 (dt, *J* = 15.5, 7.7 Hz, 3H), 1.57–1.46 (m, 2H), 1.46–1.38 (m, 2H), 1.30 (dd, *J* = 5.8, 4.2 Hz, 7H), 0.90 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.03, 144.63, 144.11, 135.27, 129.59, 127.89, 127.30, 126.70, 122.10, 121.76, 76.17, 74.63, 33.73, 31.86, 29.51, 29.44, 29.31, 29.25, 28.17, 27.67, 22.66, 14.11. MS (ESI) calcd for $C_{22}H_{27}N_3$, $(M+H)^+$: 334.2278; observed: 334.2291.

3.17. Synthesis of compound 8b: 3-methyl-2-phenyl-3*H*-imidazo[4,5-*c*]quinoline

To a solution of **3b** (40 mg, 0.16 mmol) in 1 mL of anhydrous THF, were added DBU (73 mg, 0.48 mmol) and iodomethane (114 mg, 0.8 mmol). The solution was stirred for 30 min and the solvent was then removed under vacuum to obtain the residue, which was dissolved in EtOAc, washed with water, dried over sodium sulfate, concentrated and purified using column chromatography (20% EtOAc/dichloromethane) to obtain the compound **8b** (15 mg, 36%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92–8.85 (m, 1H), 8.75 (s, 1H), 8.53 (dd, *J* = 8.3, 1.4 Hz, 2H), 7.83–7.67 (m, 3H), 7.55–7.44 (m, 3H), 4.29 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.40, 154.97, 139.30, 135.84, 134.27, 134.02, 129.75, 129.35, 128.61, 128.17, 126.36, 124.91, 121.36, 116.61, 43.88. MS (ESI) calcd for $C_{17}H_{13}N_3$, $(M+H)^+$: 260.1182; observed: 260.1188.

3.18. Compounds 8c and 8d were synthesized similarly as described for compound 8b

8c (8 mg, 12%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.70–8.57 (m, 1H), 8.25–8.13 (m, 1H), 7.74–7.57 (m, 2H), 3.97 (s, 3H), 3.00–2.94 (m, 1H), 2.17–1.87 (m, 7H), 1.57–1.37 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.11, 144.46, 143.99, 135.12, 129.50, 128.88, 126.96, 126.37, 122.25, 121.97, 36.55, 31.38, 30.24, 26.24, 25.68. MS (ESI) calcd for $C_{17}H_{19}N_3$, $(M+H)^+$: 266.1652; observed: 266.1666.

8d (21 mg, 40%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.60–8.56 (m, 1H), 8.19 (dt, *J* = 8.0, 4.3, 1H), 7.73–7.61 (m, 2H), 3.96 (s, 3H), 3.08–2.93 (m, 2H), 1.90 (dt, *J* = 15.6, 7.8, 2H), 1.48 (dt, *J* = 15.0, 7.0, 2H), 1.44–1.35 (m, 2H), 1.35–1.20 (m, 8H), 0.88 (t, *J* = 7.0, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.38, 143.89, 143.52, 134.55, 128.98, 128.49, 126.67, 126.14, 121.63, 121.31, 31.39, 30.06, 29.08, 28.99, 28.87, 28.80, 27.74, 27.22, 22.20, 13.65. MS (ESI) calcd for $C_{20}H_{27}N_3$, $(M+H)^+$: 310.2278; observed: 310.2306.

3.19. Synthesis of compound 12: 3,5-*bis*(4-methoxybenzyl)-2-phenyl-3H-imidazo[4,5-*c*]quinolin-5-ium

To a solution of **3b** (50 mg, 0.2 mmol) in 1 mL of anhydrous THF, were added DBU (91 mg, 0.61 mmol) and 1-(chloromethyl)-4-methoxybenzene (159 mg, 1.02 mmol), and the solution was heated under microwave conditions (600 W, 150 °C, 0.5 h). After cooling to room temperature, and removing the solvent under vacuum, the residue was purified using column chromatography (10% MeOH/dichloromethane) to obtain the compound **5b** (21 mg, 22%). ^{1}H NMR (400 MHz, MeOD) δ 9.63 (s, 1H), 8.96 (d, *J* = 7.8 Hz, 1H), 8.55 (d, *J* = 9.1 Hz, 1H), 8.13–8.08 (m, 1H), 8.07–8.02 (m, 1H), 8.02–7.99 (m, 2H), 7.77–7.68 (m, 3H), 7.24 (d, *J* = 8.8 Hz, 2H), 7.10 (d, *J* = 8.7 Hz, 2H), 6.94 (d, *J* = 8.7 Hz, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 6.20 (s, 2H), 5.86 (s, 2H), 3.82 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 163.08, 160.44, 159.97, 150.30, 135.98, 135.52, 132.27, 131.96, 129.73, 129.40, 129.10, 129.03, 128.45, 127.98, 127.57, 126.49, 124.96, 123.80, 122.18, 119.33, 114.43, 114.39, 59.82, 54.46, 54.43, 49.46. MS (ESI) calcd for $C_{32}H_{28}N_3O_2^+$, (M^+): 486.2176; observed: 486.2140.

3.20. Synthesis of compound 13: 5-(4-methoxybenzyl)-2-phenyl-3H-imidazo[4,5-*c*]quinolin-5-ium

To a solution of **3b** (20 mg, 0.08 mmol) in 1 mL of anhydrous THF, was added 1-(chloromethyl)-4-methoxybenzene (19 mg, 0.12 mmol) and the solution was heated in a sealed vessel at 120 °C for an 12–14 h. After cooling to room temperature and removing the solvent under vacuum, the residue was subjected to column chromatography (8% MeOH/dichloromethane) to obtain the compound **13** (12 mg, 40%). ^{1}H NMR (400 MHz, MeOD) δ 9.28 (s, 1H), 8.92–8.84 (m, 1H), 8.41 (d, *J* = 7.0 Hz, 2H), 8.24–8.14 (m, 1H), 7.84–7.74 (m, 2H), 7.54 (dq, *J* = 14.2, 7.0 Hz, 3H), 7.23 (d, *J* = 8.6 Hz, 2H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.05 (s, 2H), 3.76 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 159.99, 138.62, 137.14, 134.14, 133.69, 129.64, 129.32, 128.37, 128.00, 127.75, 126.66, 123.91, 121.38, 118.59, 114.25, 58.48, 54.35. MS (ESI) calcd for $C_{24}H_{20}N_3O^+$, (M^+): 366.1601; observed: 366.1760.

3.21. Synthesis of compound 16: *N*-(4-methoxybenzyl)-3-nitroquinolin-4-amine

To a solution of **14** (147 mg, 0.71 mmol) in 4:1 mixture of toluene and 2-propanol were added *N*,*N*-diethylpropan-2-amine

(0.14 mL, 6 mmol) and **15** (121 mg, 0.88 mmol). The reaction mixture was heated to 70 °C for half an hour until a solid started to precipitate. The reaction mixture was then cooled, filtered and washed with toluene/2-propanol (7:3), ether and cold water. The residue was dried at 80 °C to obtain the compound **16** (200 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (s, 1H), 9.41 (s, 1H), 8.34 (d, J = 8.4, 1H), 8.03 (d, J = 8.3, 1H), 7.79 (dd, J = 11.2, 4.1 Hz, 1H), 7.48 (dd, J = 11.3 Hz, 4.2, 1H), 7.34 (d, J = 8.6 Hz, 2H), 6.97 (d, J = 8.6 Hz, 2H), 5.07 (d, J = 5.4 Hz, 2H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.76, 150.82, 150.64, 147.43, 132.72, 130.46, 128.83, 128.68, 126.99, 126.20, 125.52, 119.22, 114.72, 55.37, 52.74. MS (ESI) calcd for $C_{17}H_{15}N_3O_3$, $(M+H)^+$: 310.1186; observed: 310.1268.

3.22. Synthesis of compound 17: 1-(4-methoxybenzyl)-2-phenyl-1*H*-imidazo[4,5-*c*]quinoline

Compound **16** (120 mg, 0.39 mmol) was dissolved in MeOH and hydrogenated over Pd/C as a catalyst at 60 psi hydrogen pressure for 4 h. The solution was then filtered using Celite, followed by evaporation of the solvent under reduced pressure to afford N^4-(4-methoxybenzyl) quinoline-3,4-diamine (85 mg, 94%). N^4-(4-methoxybenzyl)quinoline-3,4-diamine (85 mg, 0.31 mmol), benzoic acid (41 mg, 0.34 mmol), HBTU (129 mg, 0.34 mmol), triethylamine (34 mg, 0.34 mmol) and a catalytic amount of DMAP were dissolved in 5 mL of DMF and stirred for 10–12 h. The solvent was then removed under vacuum. The residue was dissolved in EtOAc and washed with water, dried over sodium sulfate and concentrated under reduced pressure to obtain the residue, which was dissolved in 10 mL of ethanol, and a solution of excess of sodium hydroxide in 1 mL of water was added. The reaction mixture was refluxed for 5–6 h and then the solvent was removed to obtain the residue, which was purified using column chromatography (4% MeOH/dichloromethane) to obtain the compound **17** (82 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.47 (s, 1H), 8.31 (d, J = 7.7 Hz, 1H), 7.94 (d, J = 7.7 Hz, 1H), 7.71 (dd, J = 8.1 Hz, 1.4, 2H), 7.68–7.62 (m, 1H), 7.58–7.42 (m, 4H), 7.10 (d, J = 8.7 Hz, 2H), 6.95–6.91 (m, 2H), 5.81 (s, 2H), 3.82 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.35, 154.84, 145.54, 137.06, 130.86, 130.33, 129.46, 128.91, 127.76, 127.07, 126.71, 126.52, 120.53, 117.76, 114.91, 55.30, 50.12. MS (ESI) calcd for $C_{24}H_{19}N_3O$, $(M+H)^+$: 366.1601; observed: 366.1785.

2D-NOESY experiments: The 2D-NOESY experiments were performed on the Bruker Avance 400 or Avance AV-III 500 NMR instruments. Compounds were dissolved in appropriate deuterated solvents and experiments were performed with mixing time (d8) of 0.5 sec (400 MHz) or 0.7 sec (500 MHz). The data generated was processed using MestReNova 6.2.1 (Mestrelab Research S.L.).

TLR-7/8 antagonism assay: A reporter gene assay using TLR7[15,20,21] (or TLR8)-dependent NF-κB induction was used. The inhibition of induction of NF-κB, a key transcriptional activator of the innate immune system, was quantified using human embryonic kidney 293 cells stably transfected with plasmids encoding TLR7 as well as an NF-κB reporter gene coupled to secreted alkaline phosphatase (sAP) (InvivoGen, San Diego, CA), and were maintained in HEK-Blue™ Selection medium containing zeocin and normocin. Stable expression of secreted alkaline phosphatase (sAP) under control of NF-κB/AP-1 promoters is inducible by the gardiquimod (TLR7 agonist) or CL075 (TLR8 agonist), and extracellular sAP in the supernatant is proportional to NF-κB induction. HEK-Blue-7 (or HEK-Blue-8) cells were incubated at a density of $\sim 10^5$ cells/mL in a volume of 80 μL/well, in 384-well, flat-bottomed, cell culture-treated microtiter plates until confluency was achieved, and subsequently stimulated with 1 μg/mL of gardiquimod or CL075. Concurrent to stimulation, serially diluted concentrations of test compounds were added to the cell medium using a rapid-throughput, automated protocol employing a Bio-Tek P2000 liquid handler and left to incubate overnight. sAP was assayed spectrophotometrically using an alkaline phosphatase-specific chromogen (present in HEK-detection medium as supplied by the vendor) at 620 nm.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.04.052.

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