Monatsh Chem
DOI 10.1007/s00706-015-1474-z

ORIGINAL PAPER

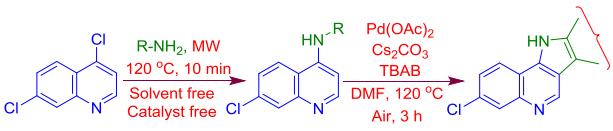
Palladium-catalyzed regioselective aerobic oxidative cyclization via C–H activation in chloroquine analogues: synthesis and cytotoxic study

Gopal Senthil Kumar[1] · Mohamed Ashraf Ali[2,3,4] · Tan Soo Choon[2] · Karnam Jayarampillai Rajendra Prasad[1]

Received: 10 December 2014 / Accepted: 6 April 2015

Abstract The intramolecular regioselective aerobic oxidative cyclization via two types of discrete C–H activation in 7-chloroquinoline analogues is described. All the synthesized compounds are screened against three human cancer cell lines (CCRF-CEM, HT29, and MCF7) for their anticancer activities. Among them, chloroquine analogues with naphthalene ring either substituted/fused form enhance remarkable cytotoxic activity against human breast adenocarcinoma cancer cell line (MCF-7) which outranged positive control doxorubicin. Similarly, 3-chloro-7*H*,12*H*-pyrazolo[5,4-*b*]quinolino[3,2-*c*]indole has possessed significant activity against all the tested cell lines and outranged activity against CCRF-CEM cell line.

Graphical abstract



Keywords Chloroquine analogues · Intramolecular oxidative cyclization · S_NAr reaction · Regioselective · Cytotoxicity

Introduction

Transition metal-catalyzed carbon–carbon (C–C) bond formation is one of the raising areas with great interest in synthetic organic chemistry owing to its region-controlled bond formation in the oxidative coupling reactions [1–8]. Generally, bond formation between two unactivated (hetero)arenes C(sp^2)–C(sp^2) with Pd-catalyzed C–H activation provides a highly attractive strategy for an ideal chemical synthesis [9]. The intramolecular oxidative coupling of two C(sp^2)–H bonds has emerged as an efficient and atom economic approach to construct C–C bond, thereby rendering superior sustainability and environmental compatibility. The development of such C–H activation/arylation reactions, particularly with broad scope, high functional group tolerance, and mild reaction conditions represents an area of significant current interest [10–14]. Quinoline-containing compounds have long been studied for treatment of malaria beginning with quinine [15]. In continuation, the best compound that found for potent antimalarial activity is chloroquine which was discovered in the 1940s [16]. In the last two decades, a number of new

Electronic supplementary material The online version of this article (doi:10.1007/s00706-015-1474-z) contains supplementary material, which is available to authorized users.

✉ Karnam Jayarampillai Rajendra Prasad
prasad_125@yahoo.com

[1] Department of Chemistry, Bharathiar University, Coimbatore 641046, India

[2] Institute for Research in Molecular Medicine, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

[3] New Drug Discovery Research, Department of Medicinal Chemistry, Alwar Pharmacy College, Alwar, Rajasthan 301030, India

[4] New Drug Discovery Research, Department of Medicinal Chemistry, Sunrise University, Alwar, Rajasthan 301030, India

Published online: 17 June 2015

potent quinolines that overcome chloroquine resistance have been reported [17–19]. Most of the compounds contain the 7-chloroquinoline nucleus of chloroquine and vary in the length of basic amine side chain [20–22]. Recently several clinical trials have been made repurposing use of chloroquine as anticancer agent due to their therapeutic efficacy and lower cost [23]. A few literatures have reported that the repositioning 4-amino basic side chain of chloroquine significantly increased the anticancer activity [24, 25].

In recent years, natural and synthetic compounds with the indoloquinoline ring system have attracted growing interest due to their large spectrum of biological activities [26]. Recent studies on the biological effects and DNA binding properties of other naturals and synthetic indoloquinolines have confirmed their remarkable biological potency with promising antiproliferative activities towards various cancer cells in some cases [27–29].

Based on the above biological importance of 7-chloroquinoline scaffolds and indoloquinoline compounds, herein we have described the reaction of various aromatic/heteroaromatic amines with 4,7-dichloroquinoline to afford 7-chloroquinoline compounds (analogues of chloroquine) which subsequently undergo $Pd(OAc)_2$-catalyzed oxidative coupling reaction to give the stable angular products in regioselective manner. The biological significance of this synthesized compound was evaluated on the basis of in vitro cytotoxicity against three human cancer cell lines.

Results and discussion

Nucleophilic displacement (S_NAr reaction) of halogen atom at the C-4 position of quinolines by various amine compounds has traditionally been fashioned to acquire 4-aminoquinoline family [30–32]. Synthetic procedures involved S_NAr reaction in most previous reports have demonstrated at elevated temperature or in the presence of phenol, with or without iodide, anhydrous K_2CO_3 and microwave irradiation [33–38]. Focus on microwave reactions reported for the amination of chloroquinolines have several disadvantages such as requiring organic solvent, need of extraction and chromatographic purification process that involve generation of waste from organic solvents. For the present work, initially, the S_NAr amination reaction of 4,7-dichloroquinoline with aromatic and heteroaromatic amines afforded the corresponding 7-chloro-4-aminoquinoline compounds under catalyst-free, solvent-free microwave irradiation at 120 °C (Scheme 1).

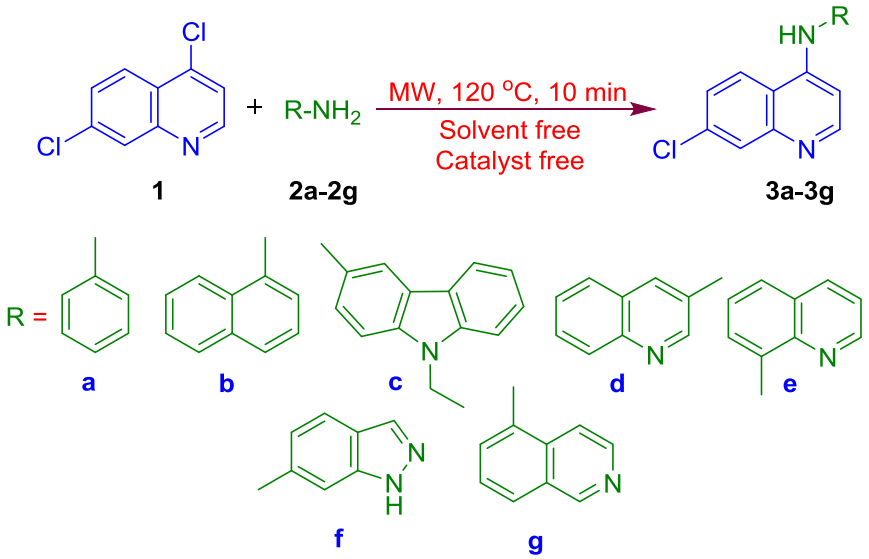
In a typical experiment, 1 equiv. of 4,7-dichloroquinoline was treated with 1.2 equiv. of amine compounds in a sealed microwave vessel and kept at 90 °C for a minute in a Biotage microwave oven to get homogeneous reaction mixture. Then the reaction was prolonged for 10 min with the raise of temperature to 120 °C to furnish the target aminoquinoline compound as a single product with no need of additional purification other than recrystallization from methanol.

After obtaining the aminoquinolines as potential precursors, we subsequently extended the reaction scope to the intramolecular C–H activation field disclosing the direct aryl–heteroaryl C–C bond formation through C–H activation sequences. Previous report suggested the Buchwald–Hartwig amination followed by an intramolecular arylation reaction but have never distinguished the regioselectivity of arylation [39]. To test our hypothesis, we first examined the reaction of 7-chloro-4-[*N*-(9-ethyl-9*H*-carbazol-3-yl)amino]quinoline (**3c**) using 5 mol % of $Pd(OAc)_2$ with Cs_2CO_3 as a base and tetrabutylammonium bromide as an additive in presence of air to yield a single detectable regioisomeric product with the new C–C bond installed in 7-chloro-4-aminoquinoline which was angular product **4c** instead of linear product **4c′** (Scheme 2). The formation of the angular product **4c** was unambiguously confirmed on the basis of spectral data.

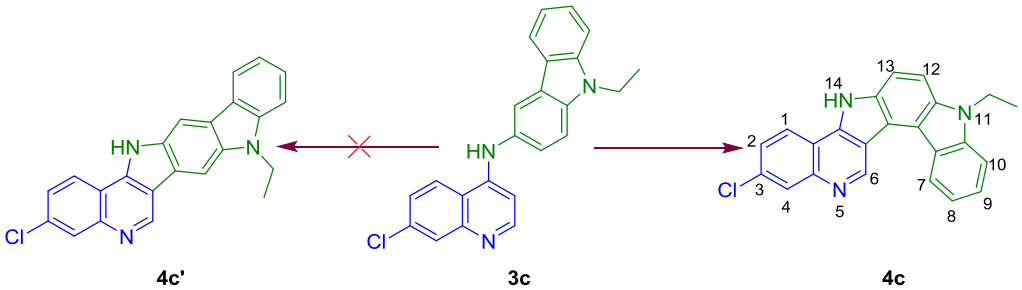
The IR spectrum of the compound showed absorption band at 3140 cm^{-1} for NH group. ^1H NMR of the compound exhibited a doublet at $\delta = 8.27$ ppm ($J = 1.0$ Hz) for C_8–H proton of the quinoline ring. A triplet at 1.38 ppm ($J = 7.5$ Hz) and a quartet at 4.50 ppm ($J = 7.0$ Hz) was corresponding to the N_9-attached methyl and methylene protons, respectively. The disappearance of a doublet of C_3–H in quinoline ring and a singlet of C_4'–H in carbazole ring has confirmed the C–C bond formation occurs at C4-carbon of the carbazole and C3-carbon of quinoline rings to give angular product **4c**. The other possible isomeric linear product **4c′** is ruled out. The observed regioselectivity makes this reaction a potentially valuable curiosity for palladium-mediated oxidative coupling reaction. Next, all the aminoquinolines were examined for this aerobic oxidative coupling reaction. As our expectation, compounds **3d** and **3f** gave the respective angular product as the only product instead of other possibility of linear products in regioselective manner (Scheme 3).

Based on the results observed from our present investigation and literature data ([40], and references therein), the following mechanism has been proposed for the formation of compound **4a** (Scheme 4). Initially, 7-chloro-(*N*-phenylamino)quinoline containing aryl ring undergoes C–H activation at *ortho* position with $Pd(OAc)_2$ in presence of base followed by deprotonation to give *o*-palladation intermediate **A**. Then the second C–H activation occurs at heteroaryl ring to afford intermediate **B**. Subsequently the intermediate **B** undergoes reductive elimination to afford the final product 3-chloro-11*H*-indolo[3,2-*c*]quinoline (**4a**) with generating Pd(0) in presence of TBAB which was used to avoid palladium black generation [41]. Open air as terminal oxidant to oxidize Pd(0) with acetic acid regenerates

Scheme 1



Scheme 2



catalytically active palladium(II)acetate with liberation of hydrogen peroxide. The similar mechanism was also applicable to the formation of other derivatives **4b–4f**.

In vitro cytotoxicity

All the synthesized and characterized compounds were then subjected to cytotoxic evaluation against CCRF-CEM, HT29, and MCF7 cancer cell lines. The results were summarized in Table 1. Doxorubicin, a clinically used antitumor agent was also evaluated for positive control. The anticancer potency of these compounds was indicated by its IC_{50} value. In general, all the cyclization precursors (i.e., intermediate compounds **3a–3g**) showed moderate to weak cytotoxicity in all the three cancer cell lines. Among them, the compound **3b** (in which quinoline 4-position holds naphthylamine moiety) selectively showed excellent activity against MCF7 cancer cell line with IC_{50} value of 25.40 ± 0.82 μM which is outranged to the standard drug doxorubicin (28.09 ± 3.72 μM) and moderate activity against CCRF-CEM and weak activity against HT29 cancer cell lines with the IC_{50} values of 69.66 ± 1.19 and 104.46 ± 4.23 μM, respectively. Both the compounds **3a** and **3b** are simple aromatic amine-substituted compounds in which compound **3b** showed remarkable activity as compared to compound **3a** which may be due to higher electron density of 1-naphthylamine than simple aniline.

It was observed that the presence of heteroaromatic amine skeleton did not influence the activity profile in the panel of intermediate compounds as compared with the simple amine-substituted compounds. The next set of compounds is $Pd(OAc)_2$-catalyzed cyclized products, i.e., various quinoline-fused indoles **4a–4f**. Among them compound **4f** showed strong activity against CCRF-CEM and

Table 1 In vitro cytotoxicity/μM

Compound	CCRF-CEM	HT29	MCF7
3a	76.25 ± 4.87	80.36 ± 3.81	83.88 ± 1.48
3b	69.66 ± 1.19	104.64 ± 4.23	25.40 ± 0.82
3d	67.52 ± 1.99	87.97 ± 3.69	88.89 ± 4.40
3e	87.00 ± 3.59	102.27 ± 6.66	78.07 ± 7.99
3c	60.00 ± 3.99	104.99 ± 8.36	92.30 ± 1.21
3f	61.24 ± 5.44	48.39 ± 12.79	89.08 ± 0.75
3g	82.73 ± 6.30	37.39 ± 3.97	91.43 ± 6.46
4a	90.72 ± 1.83	88.23 ± 5.72	89.50 ± 3.11
4b	67.49 ± 1.23	93.76 ± 1.00	27.82 ± 1.14
4d	84.41 ± 3.19	93.12 ± 2.96	80.61 ± 0.54
4e	62.27 ± 2.76	85.03 ± 7.89	71.48 ± 0.31
4c	42.86 ± 0.79	62.58 ± 1.85	37.56 ± 1.96
4f	36.61 ± 1.61	26.20 ± 1.10	36.82 ± 2.80
DOX	39.12 ± 0.32	13.65 ± 2.11	28.09 ± 3.72
DMSO	99.99 ± 0.52	100.00 ± 8.36	100.00 ± 2.78

MCF7 cancer cell lines with the IC_{50} value of 36.61 ± 1.61 and 36.82 ± 2.80 μM, respectively, which is better than the standard drug (39.12 ± 0.32 and 28.09 ± 3.72 μM) and showed moderate activity against HT29 cell line. Compound **4c** is the next active compound in this series, which displayed good cytotoxicity against CCRF-CEM and MCF7 cancer cell lines with the IC_{50} value of 42.86 ± 0.79 and 37.56 ± 1.96 μM, respectively, which is quite comparable with the standard drug, also showed moderate activity against HT29 cell line, same as compound **4f**. Very interestingly, compound **4b** which is benzoindoloquinoline derived from the intermediate compound **3b**, displayed selectively strong cytotoxicity in MCF7 cancer cell line which outranged the standard drug with the IC_{50} value of 27.82 ± 1.14 μM and showed moderate activity against CCRF-CEM and weak activity against HT29 cancer cell line. From these results, we observed that most of the compounds are selectively active against MCF7 cancer cell lines. Furthermore compound **4f** which is indoloquinoline fused with pyrazole ring enhances the activity profile towards all the three cell lines. In general, mostly all the compounds showed weak activity against HT29 cancer cell line. By comparing the intermediate and the final cyclized compounds, the cyclized compounds showed superior activity than the intermediates.

Conclusion

This paper describes a new palladium-catalyzed reaction for the highly regioselective intramolecular oxidative coupling using Cs_2CO_3 as a base under aerobic condition, which promoted two types of discrete C–H activation in 7-chloro-4-aminoquinoline compounds. The aminoquinolines were developed from a simple catalyst-free and solvent-free microwave irradiation method. The synthesized compounds have showed potent activity against MCF7 cell line among the tested three human cancer cell lines.

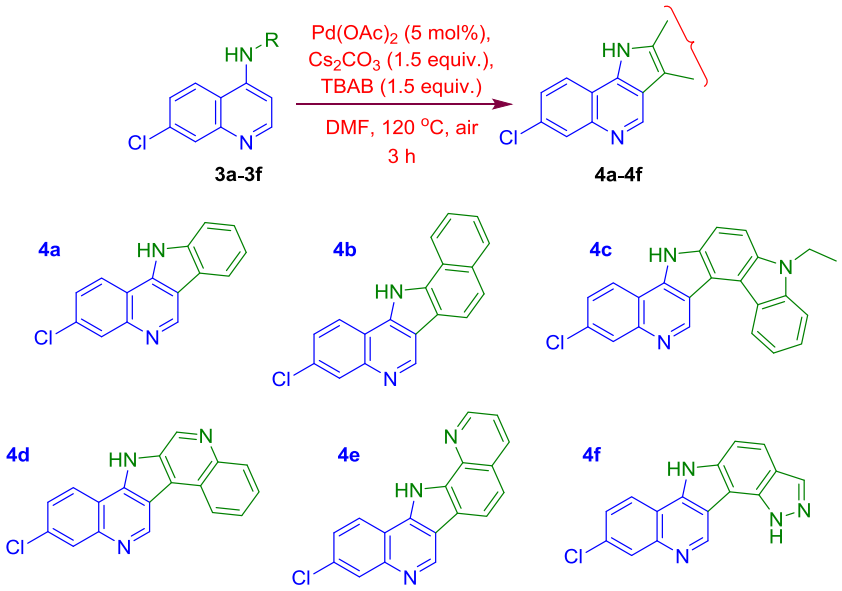
Experimental

Melting points were determined on a Mettler FP 51 apparatus (Mettler Instruments, Switzerland). A Nicolet Avatar Model FT-IR spectrophotometer was used to record the IR spectra (4000–400 cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AV 500 [500 MHz (^1H) and 125 MHz (^{13}C)] and Bruker AV 400 [400 MHz (^1H) and 100 MHz (^{13}C)] spectrometers using tetramethylsilane (TMS) as an internal reference. The chemical shifts are expressed in parts per million (ppm). Coupling constants (J) are reported in hertz (Hz). The terms s, d, t, dd refer to singlet, doublet, triplet, and doublet of doublet, respectively, bs refers to a broad singlet. Mass spectra (MS) were recorded on an Auto Spec EI+Shimadzu QP 2010 PLUS GC–MS and Perkin Elmer Clarus 600 (EI) mass spectrometer. Microanalyses were performed on a Vario EL III model CHNS analyzer (Vario, Germany) at the Department of Chemistry, Bharathiar University. Palladium(II) acetate was purchased from Sigma Aldrich. Unless otherwise specified, other reagents were obtained from commercial suppliers. When known compounds had to be prepared according to the literature procedures, pertinent references are given. The purity of the products was tested by TLC with plates coated with silica gel-G using petroleum ether and ethyl acetate in the ratio of 50:50 as developing solvents. Columns packed with activated silica gel (60–120 mesh) were used to purify the product.

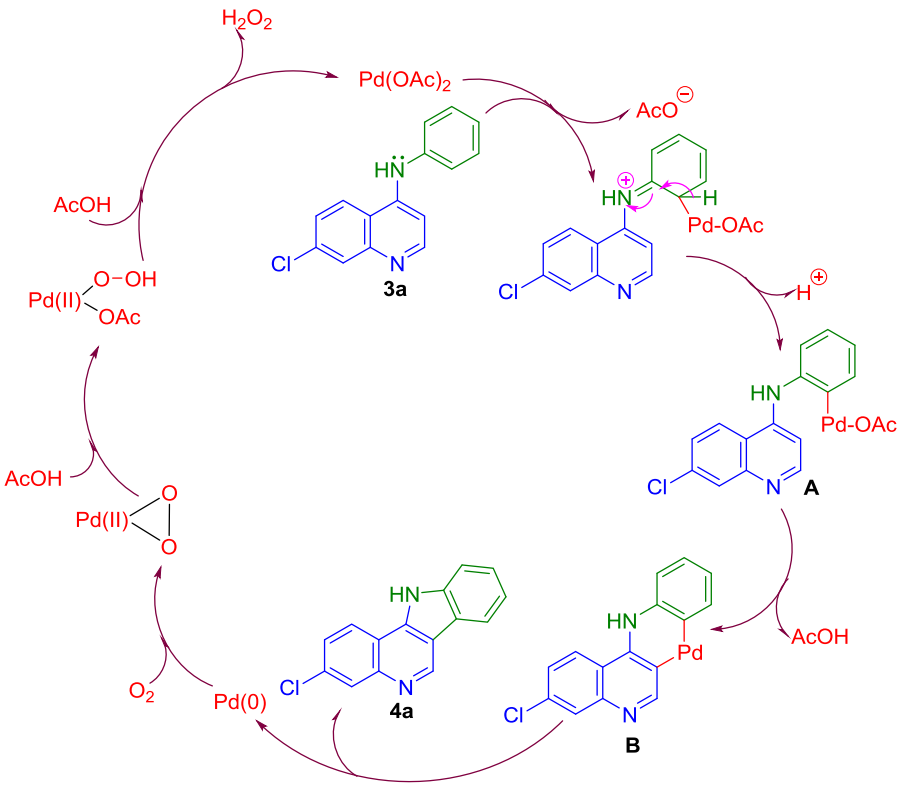
General procedure for the synthesis of 7-chloro-4-aryl amino-substituted quinoline compounds

A mixture of 4,7-dichloroquinoline (**1**, 1.2 mmol) and the respective aryl/heteroaryl amino compounds (**2a**–**2g**, 1.0 mol) was mixed thoroughly with the use of glass rod in a microwave vessel and subjected to microwave irradiation (Biotage microwave oven, 120 °C, 2 bar pressure) for 10 min. The progress of the reaction was monitored by thin-layer chromatography. After completion of the reaction, the reaction mixture was cooled and washed with aqueous methanol. The crude product obtained was dried and recrystallized from methanol to afford pure 4-arylamino substituted quinoline compounds **3a**–**3g**.

Scheme 3



Scheme 4



7-Chloro-N-phenylquinolin-4-amine (**3a**, $C_{15}H_{11}ClN_2$) Yield: 0.244 g (96 %); whitish yellow solid; m.p.: >300 °C; IR (KBr): $\bar{\nu} = 3105$, 1599 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): $\delta = 6.78$ (d, $J = 7.0$ Hz, 1H, C_3–H), 7.45 (t, $J = 7.5$ Hz, 1H, C_4'–H), 7.50 (d, $J = 7.5$ Hz, 2H, C_2'–, C_6'–H), 7.59 (t, $J = 7.0$ Hz, 2H, C_3'–, C_5'–H),

7.88 (dd, $J = 9.0$ Hz, 2.0 Hz, 1H, C_6–H), 8.19 (d, $J = 2.0$ Hz, 1H, C_8–H), 8.52 (d, $J = 7.0$ Hz, 1H, C_2–H), 8.90 (d, $J = 9.5$ Hz, 1H, C_5–H), 11.22 (s, 1H, NH), 14.79 (bs, 1H, iminol) ppm; ^{13}C NMR (DMSO-d_6, 125 MHz): $\delta = 100.72$, 116.42, 119.73, 125.93, 126.63, 127.82, 128.10, 130.46, 137.44, 138.85, 139.60, 143.88, 155.36 ppm; GC–MS (EI): m/z (%) = 254.20 ([M^+], 100).

7-Chloro-N-(1-naphthalenyl)quinolin-4-amine
(**3b**, $C_{19}H_{13}ClN_2$)
Yield: 0.296 g (97 %); brown solid; m.p.: >300 °C; IR (KBr): $\bar{\nu} = 3229$, 1584 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): $\delta = 6.19$ (d, $J = 7.0$ Hz, 1H, C_3–H), 7.58 (t, $J = 7.5$ Hz, 1H, C_6'–H), 7.65 (t, $J = 7.5$ Hz, 1H, C_7'–H), 7.68–7.74 (m, $J = 7.0$ Hz, 2H, C_3'–, C_2'–H), 7.88 (d, $J = 8.0$ Hz, 1H, C_6–H), 7.94 (dd, $J = 9.0$ Hz, 2.0 Hz, 1H, C_4'–H), 8.12 (d, $J = 9.5$ Hz, 2H, C_5'–, C_8'–H), 8.24 (d, $J = 2.0$ Hz, 1H, C_8–H), 8.40 (d, $J = 7.0$ Hz, 1H, C_2–H), 9.06 (d, $J = 9.5$ Hz, 1H, C_5–H), 11.54 (s, 1H, NH), 14.89 (bs, 1H, iminol) ppm; ^{13}C NMR (DMSO-d_6, 125 MHz): $\delta = 101.12$, 116.21, 119.62, 123.03, 125.72, 126.73, 126.92, 127.48, 127.78, 127.87, 129.14, 129.38, 129.47, 133.33, 134.80, 138.92, 139.48, 143.60, 156.95 ppm; GC–MS (EI): m/z (%) = 304.15 ([M^+], 100).

N-(7-Chloro-4-quinolinyl)-9-ethyl-9H-carbazol-3-amine
(**3c**, $C_{23}H_{18}ClN_3$)
Yield: 0.357 g (96 %); yellow solid; m.p.: >300 °C; IR (KBr): $\bar{\nu} = 3325$, 1570 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): $\delta = 1.33$ (t, $J = 7.5$ Hz, 3H, –N–CH_2–CH_3), 4.56 (q, $J = 6.5$ Hz, 2H, –N–CH_2–CH_3), 6.71 (d, $J = 7.0$ Hz, 1H, C_3–H), 7.24 (t, $J = 7.5$ Hz, 1H, C_7'–H), 7.50–7.53 (m, 2H, C_1'–, C_6'–H), 7.68 (d, $J = 8.5$ Hz, 1H, C_5'–H), 7.81 (d, $J = 8.5$ Hz, 1H, C_8'–H), 7.88 (dd, $J = 8.0$ Hz, 1.0 Hz, 1H, C_6–H), 8.12 (s, 1H, C_4–H), 8.19 (d, $J = 8.0$ Hz, 1H, C_2'–H), 8.27 (d, $J = 1.0$ Hz, 1H, C_8–H), 8.45 (d, $J = 7.0$ Hz, 1H, C_2–H), 8.87 (d, $J = 9.0$ Hz, 1H, C_5–H), 11.24 (s, 1H, NH), 14.63 (bs, 1H, iminol) ppm; ^{13}C NMR (DMSO-d_6, 125 MHz): $\delta = 14.24$, 37.68, 100.65, 110.00, 110.86, 116.14, 118.47, 119.64, 121.25, 122.25, 123.44, 124.13, 126.36, 126.96, 127.97, 128.44, 134.34, 138.82, 139.06, 139.54, 140.66, 143.72, 156.17 ppm; GC–MS (EI): m/z (%) = 371.27 ([M^+], 100).

7-Chloro-N-(3-quinolinyl)quinolin-4-amine
(**3d**, $C_{18}H_{12}ClN_3$)
Yield: 0.297 g (97 %); brown solid; m.p.: >300 °C; IR (KBr): $\bar{\nu} = 3285$, 1573 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): $\delta = 7.04$ (d, $J = 7.0$ Hz, 1H, C_3–H), 7.71 (t, $J = 7.5$ Hz, 1H, C_6'–H), 7.84 (t, $J = 7.5$ Hz, 1H, C_7'–H), 7.94 (dd, $J = 9.0$ Hz, 2.0 Hz, 1H, C_6–H), 8.08 (d, $J = 8.0$ Hz, 1H, C_5'–H), 8.12 (d, $J = 8.5$ Hz, 1H, C_8'–H), 8.19 (s, 1H, C_8–H), 8.53 (d, $J = 2.5$ Hz, 1H, C_4'–H), 8.60 (d, $J = 7.0$ Hz, 1H, C_2–H), 8.91 (d, $J = 8.0$ Hz, 1H, C_5–H), 9.04 (d, $J = 2.5$ Hz, 1H, C_2'–H), 11.36 (s, 1H, NH), 14.81 (bs, 1H, iminol) ppm; ^{13}C NMR (DMSO-d_6, 125 MHz): $\delta = 101.39$, 109.68, 116.75, 120.02, 126.62, 128.08, 128.15, 128.65, 129.30, 130.45, 131.37, 131.51, 139.00, 139.74, 144.48, 146.57, 148.75, 155.43 ppm; GC–MS (EI): m/z (%) = 305.23 ([M^+], 100).

7-Chloro-N-(8-quinolinyl)quinolin-4-amine
(**3e**, $C_{18}H_{12}ClN_3$)
Yield: 0.290 g (95 %); brown solid; m.p.: >300 °C; IR (KBr): $\bar{\nu} = 3342$, 1590 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): $\delta = 6.31$ (d, $J = 7.0$ Hz, 1H, C_3–H), 7.70 (dd, $J = 4.5$ Hz, 8.5 Hz, 1H, C_3'–H), 7.83 (t, $J = 7.5$ Hz, 1H, C_6'–H), 7.92 (d, $J = 9.5$ Hz, 1H, C_5'–H), 7.99 (d, $J = 7.5$ Hz, 1H, C_6–H), 8.18 (dd, $J = 8.0$ Hz, 1.0 Hz, 1H, C_4'–H), 8.25 (s, 1H, C_8–H), 8.40 (d, $J = 7.0$ Hz, 1H, C_2–H), 8.61 (d, $J = 8.5$ Hz, 1H, C_5–H), 8.92 (dd, $J = 9.0$ Hz, 1.0 Hz, 1H, C_7'–H), 9.04 (d, $J = 9.0$ Hz, 1H, C_2'–H), 11.52 (s, 1H, NH), 14.92 (bs, 1H, iminol) ppm; ^{13}C NMR (DMSO-d_6, 125 MHz): $\delta = 101.93$, 108.99, 116.12, 120.62, 125.88, 127.64, 128.33, 128.69, 129.72, 130.65, 131.84, 132.59, 139.39, 139.95, 143.88, 146.25, 148.81, 154.48 ppm; GC–MS (EI): m/z (%) = 305.21 ([M^+], 100).

7-Chloro-N-(1H-indazol-6-yl)quinolin-4-amine
(**3f**, $C_{16}H_{11}ClN_4$)
Yield: 0.277 g (94 %); yellow solid; m.p.: >300 °C; IR (KBr): $\bar{\nu} = 3387$, 3182, 1597 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): $\delta = 6.84$ (d, $J = 7.0$ Hz, 1H, C_3–H), 7.21 (dd, $J = 8.5$ Hz, 2.0 Hz, 1H, C_5'–H), 7.67 (s, 1H, C_3'–H), 7.88 (dd, $J = 9.0$ Hz, 2.0 Hz, 1H, C_6–H), 7.94 (d, $J = 8.5$ Hz, 1H, C_4'–H), 8.17 (s, 1H, C_8–H), 8.19 (d, $J = 2.5$ Hz, 1H, C_7'–H), 8.50 (d, $J = 7.0$ Hz, 1H, C_2–H), 8.94 (d, $J = 9.5$ Hz, 1H, C_5–H), 11.37 (s, 1H, N–H), 13.37 (s, 1H, N'–H), 14.86 (bs, 1H, iminol) ppm; ^{13}C NMR (DMSO-d_6, 125 MHz): $\delta = 100.98$, 116.46, 118.76, 119.68, 121.54, 122.58, 126.74, 127.77, 130.58, 133.74, 135.39, 138.82, 139.58, 143.79, 145.62, 155.64 ppm; GC–MS (EI): m/z (%) = 294.24 ([M^+], 100).

7-Chloro-N-(5-isoquinolinyl)quinolin-4-amine
(**3g,** $C_{18}H_{12}ClN_3$)
Yield: 0.287 g (94 %); yellow solid; m.p.: >300 °C; IR (KBr): $\bar{\nu} = 3329$, 1591 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): $\delta = 6.83$ (d, $J = 7.0$ Hz, 1H, C_3–H), 7.44 (d, $J = 8.0$ Hz, 1H, C_6'–H), 7.74–7.82 (m, 2H, C_8'–, C_6–H), 7.87 (t, $J = 7.5$ Hz, 1H, C_7'–H), 8.07 (s, 1H, C_8–H), 8.18 (d, $J = 5.0$ Hz, 1H, C_4'–H), 8.26 (d, $J = 7.0$ Hz, 1H, C_2–H), 8.43 (d, $J = 8.0$ Hz, 1H, C_5–H), 8.91 (s, 1H, C_1'–H), 9.33 (d, $J = 4.5$ Hz, 1H, C_3'–H), 10.23 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 125 MHz): $\delta = 102.80$, 115.23, 118.26, 119.99, 121.23, 125.01, 125.82, 127.12, 128.65, 128.98, 130.15, 133.39, 136.51, 139.61, 149.51, 152.06,

152.96, 158.66 ppm; GC–MS (EI): *m/z* (%) = 305.21 ([M^+], 100).

General procedure for the synthesis of quinolino-fused indole compounds

A mixture of an appropriate 7-chloro-4-amino-substituted compound (**3a**–**3f**, 1.0 mmol) with TBAB (1.5 mmol) and Cs_2CO_3 (1.5 mmol) in 10 cm^3 anhydrous DMF was taken in round-bottom flask and subsequently 5 mol% of $Pd(OAc)_2$ was added. The whole contents were placed in a heating mantle at 120 °C under open air condition for 3 h. After completion of the reaction, the reaction mixture was cooled, diluted with water, and extracted with ethyl acetate. The combined organic extracts were washed with aqueous 1 N HCl and dried over anhydrous sodium sulfate. The solvent was distilled off under vacuum and the crude product subjected to column chromatography over silica gel using petroleum ether:ethyl acetate (85:15) as an eluent to afford the corresponding indolo-fused quinoline **4a**–**4f**.

*3-Chloro-11H-indolo[3,2-c]quinoline (***4a**, $C_{15}H_9ClN_2$)
Yield: 0.205 g (81 %); yellow solid; m.p.: 216–218 °C; IR (KBr): $\bar{\nu}$ = 3167, 1567 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.19 (t, J = 7.6 Hz, 1H, C_8–H), 7.26 (s, 1H, NH), 7.30 (t, J = 7.6 Hz, 1H, C_9–H), 7.40–7.45 (m, 3H, C_6–, C_7–, C_{10}–H), 7.89 (d, J = 8.4 Hz, 1H, C_2–H), 8.01 (d, J = 1.0 Hz, 1H, C_4–H), 8.53 (d, J = 7.6 Hz, 1H, C_1–H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 114.56, 118.22, 121.42, 123.00, 125.16, 126.26, 129.05, 129.92, 135.50, 139.56, 147.89, 149.74, 151.99 ppm; GC–MS (EI): *m/z* (%) = 252.22 ([M^+], 100).

*3-Chloro-13H-benzo[g]indolo[3,2-c]quinoline (***4b**, $C_{19}H_{11}ClN_2$)
Yield: 0.260 g (86 %); brown solid; m.p.: 228–230 °C; IR (KBr): $\bar{\nu}$ = 3210, 1586 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.28 (t, J = 7.5 Hz, 1H, C_{10}–H), 7.34 (s, 1H, NH), 7.45 (t, J = 7.5 Hz, 1H, C_{11}–H), 7.58–7.65 (m, 3H, C_6–, C_9–, C_{12}–H), 7.78 (d, J = 8.0 Hz, 1H, C_8–H), 7.84 (d, J = 8.0 Hz, 1H, C_7–H), 8.24 (d, J = 2.0 Hz, 1H, C_4–H), 8.40 (d, J = 7.0 Hz, 1H, C_2–H), 9.06 (d, J = 9.5 Hz, 1H, C_1–H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 117.63, 119.37, 122.94, 124.61, 125.53, 126.44, 126.98, 127.35, 127.60, 127.82, 129.64, 129.92, 131.17, 132.85, 134.55, 139.71, 140.43, 144.03, 155.38 ppm; GC–MS (EI): *m/z* (%) = 302.30 ([M^+], 100).

*3-Chloro-11-ethyl-14H-indolo[5,4-b]quinolino[3,2-c]indole (***4c**, $C_{23}H_{16}ClN_3$)
Yield: 0.303 g (82 %); yellow solid; m.p.: 246–248 °C; IR (KBr): $\bar{\nu}$ = 3140, 1569 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 1.38 (t, J = 7.5 Hz, 3H, –N–CH_2CH_3), 4.50 (q, J = 7.0 Hz, 2H, –N–CH_2CH_3), 7.23 (t, J = 7.5 Hz, 1H, C_8–H), 7.50–7.55 (m, 2H, C_9–H, NH), 7.67 (d, J = 8.5 Hz, 1H, C_{12}–H), 7.81 (d, J = 8.5 Hz, 1H, C_{13}–H), 7.88–7.91 (m, 2H, C_7–, C_{10}–H), 8.14–8.22 (m, 2H, C_2–, C_6–H), 8.27 (d, J = 1.0 Hz, 1H, C_4–H), 8.69 (d, J = 9.0 Hz, 1H, C_1–H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 19.28, 30.92, 121.88, 122.09, 124.66, 125.19, 126.05, 126.49, 126.87, 127.31, 127.42, 127.60, 127.66, 129.43, 131.01, 131.74, 133.90, 134.41, 141.31, 143.94, 147.07, 147.56 ppm; GC–MS (EI): *m/z* (%) = 369.29 ([M^+], 100).

*3-Chloro-13H-pyrrolo[3,2-c]bisquinoline (***4d**, $C_{18}H_{10}ClN_3$)
Yield: 0.243 g (80 %); brown solid; m.p.: 233–235 °C; IR (KBr): $\bar{\nu}$ = 3198, 1588 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 7.58–7.96 (m, 5H, C_2–, C_7–, C_8–, C_9–, C_{10}–H), 8.01 (d, J = 8.5 Hz, 1H, C_1–H), 8.27 (s, 1H, C_4–H), 8.56 (s, 1H, C_6–H), 8.97 (s, 1H, C_{12}–H), 9.45 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 125 MHz): δ = 115.45, 118.92, 121.58, 123.57, 125.99, 126.87, 127.60, 128.32, 128.74, 129.18, 129.84, 131.32, 133.29, 135.98, 139.77, 141.36, 147.96, 153.43 ppm; GC–MS (EI): *m/z* (%) = 303.25 ([M^+], 100).

*3-Chloro-13H-pyrido[7,6-b]quinolino[3,2-c]indole (***4e**, $C_{18}H_{10}ClN_3$)
Yield: 0.252 g (83 %); brown solid; m.p.: 227–229 °C; IR (KBr): $\bar{\nu}$ = 3233, 1591 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.23–7.53 (m, 5H, C_2–, C_7–, C_8–, C_9–H, NH), 7.95 (dd, J = 6.8 Hz, 4.4 Hz, 1H, C_{10}–H), 8.03 (d, J = 1.6 Hz, 1H, C_4–H), 8.06 (s, 1H, C_6–H), 8.45 (d, J = 7.6 Hz, 1H, C_1–H), 8.87 (dd, J = 4.0 Hz, 1.6 Hz, 1H, C_{11}–H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 119.55, 120.42, 122.61, 124.71, 125.19, 125.84, 127.35, 128.06, 128.69, 132.45, 135.11, 135.47, 135.94, 136.24, 137.79, 142.52, 146.69, 153.90 ppm; GC–MS (EI): *m/z* (%) = 303.24 ([M^+], 100).

*3-Chloro-7H,12H-pyrazolo[5,4-b]quinolino[3,2-c]indole (***4f**, $C_{16}H_9ClN_4$)
Yield: 0.246 g (84 %); yellow solid; m.p.: 207–209 °C; IR (KBr): $\bar{\nu}$ = 3267, 1594 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.23–7.53 (m, 5H, C_6–, C_9–, C_{10}–, C_{11}–H, N_{12}–H), 7.95 (d, J = 8.8 Hz, 1H, C_2–H), 8.03 (d, J = 1.6 Hz, 1H, C_4–H), 8.45 (d, J = 7.6 Hz, 1H, C_1–H), 11.76 (s, 1H, N_7–H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 108.96, 109.60, 117.61, 119.31, 120.74, 121.37, 122.55, 123.74, 123.99, 126.05, 126.47, 138.44, 140.70, 141.98, 143.67, 148.15 ppm; GC–MS (EI): *m/z* (%) = 292.11 ([M^+], 100).

In vitro cytotoxicity

All the tested cell lines were obtained from American Type Culture Collection. The cells were grown on 75 cm^2 cell culture flasks with RPMI-16 medium for CCRF-CEM cells and EMEM (Eagle's minimum essential medium) for HT29 and MCF7 cells, and supplemented with 10 % fetal bovine serum, and 1 % penicillin/streptomycin solution (10,000 units of penicillin and 10 mg of streptomycin in 0.9 % NaCl) in a humidified atmosphere of 5 % CO_2, 95 % air at 37 °C. The cell proliferation assay was carried out using CellTiter 96 aqueous one solution cell proliferation assay kit (Promega, USA). Briefly, upon reaching about 75–80 % confluency, HT29 (5000 cells/well), MCF7 (5000 cells/well), or CCRF-CEM (40,000 cells/well) were plated in 96-well microplate in 100 mm^3 media. After seeding for 72 h, the cells were treated with 50 μM compound in triplicate. Doxorubicin (10 μM) was used as the positive control. At the end of the sample exposure period (72 h), 20 mm^3 CellTiter 96 aqueous solution was added. The plate was returned to the incubator for 1 h in a humidified atmosphere at 37 °C. The absorbance of the formazan product was measured at 490 nm using a microplate reader. The blank control was recorded by measuring the absorbance at 490 nm with wells containing medium mixed with CellTiter 96 aqueous solution but no cells. Results were expressed as the percentage of the control (without compound set at 100 %). The percentage of cell survival was calculated as OD value of cells treated with the test compound − OD value of culture medium/(OD value of control cells − OD value of culture medium) × 100 %. The percentage inhibition was used to determine the IC_{50} values. The results are given as mean ± standard deviation.

Acknowledgments Our sincere thanks go to VIT-SIF Lab, SAS, Chemistry division, VIT University, Vellore, and the SAIF, IIT Madras, Chennai for providing access to their NMR spectral facilities. Dr. K. J. Rajendra Prasad is grateful to UGC-BSR for the one-time research grant.

References

1. Ramirez TA, Zhao BG, Shi Y (2012) Chem Soc Rev 41:931
2. Zhang C, Tang CH, Jiao N (2012) Chem Soc Rev 41:3464
3. Partyka DV (2011) Chem Rev 111:1529
4. Cho SH, Kim JY, Kwak J, Chang S (2011) Chem Soc Rev 40:5068
5. Collet F, Lescot C, Dauban P (2011) Chem Soc Rev 40:1926
6. Zhang XP, Lu H (2011) Chem Soc Rev 40:1899
7. Monnier F, Taillefer M (2009) Angew Chem Int Ed 48:6954
8. Tsang WCP, Zheng N, Buchwald SL (2005) J Am Chem Soc 127:14560
9. de Meijere A, Diederich F (2004) Metal-catalyzed cross-coupling reactions. Wiley-VCH, Weinheim
10. Hung TQ, Thang NN, Hoang DH, Dang TT, Villinger A, Langer P (2014) Org Biomol Chem 12:2596
11. Watanabe T, Ueda S, Inuki S, Oishi S, Fujii N, Ohno H (2007) Chem Commun 43:4516
12. Dwight TA, Rue NR, Charyk D, Josselyn R, Deboef B (2007) Org Lett 9:3137
13. Campeau L-C, Parisien M, Leblanc M, Fagnou K (2004) J Am Chem Soc 126:9186
14. Huang Q, Fazio A, Dai G, Campo MA, Larock RC (2004) J Am Chem Soc 126:7460
15. Coatney GR, Cooper WC, Eddy NB, Greenberg J (1953) Public Health Monogr 15:1
16. Loeb RF, Clarke WM, Coateney GR, Coggeshall LT, Dieuaide FR, Dochez AR, Hankansson EG, Marshall EK, Marvel SC, McCoy OR, Sapero JJ, Serbell WH, Shannon JA, Carden GA (1946) J Am Med Assoc 130:1069
17. O'Neill PM, Ward SA, Berry NG, Jeyadevan JP, Biagini GA, Asadollaly E, Park BK, Bray PG (2006) Curr Top Med Chem 6:479
18. Wipf P, Mo T, Geib SJ, Caridha D, Dow GS, Gerena L, Roncal N, Milner EE (2009) Org Biomol Chem 7:4163
19. Bellot F, Cosledan F, Vendier L, Brocard J, Meunier B, Robert A (2010) J Med Chem 53:4103
20. Kaur K, Jain M, Reddy RP, Jain R (2010) Eur J Med Chem 45:3245
21. Yearick K, Ekoue-Kovi K, Waniuk DP, Natarajan JK, Alumasa J, de Dios AC, Roepe PD, Wolf C (2008) J Med Chem 51:1995
22. Cheruku SR, Maiti S, Dorn A, Scorneaux B, Bhattacharjee AK, Ellis WY, Vennerstrom JL (2003) J Med Chem 46:3166
23. Ridley RG (2002) Nature 415:686
24. Feng T-S, Guantai EM, Nell MJ, van Rensburg CEJ, Hoppe HC, Chibale K (2011) Bioorg Med Chem Lett 21:2882
25. Kimura T, Takabatake Y, Takahashi Y (2013) Cancer Res 73:3
26. Lavrado J, Moreira R, Paulo A (2010) Curr Med Chem 17:2348
27. Lu C-M, Chen YL, Chen H-L, Chen C-A, Lu P-J, Yang C-N, Tzeng C-C (2010) Bioorg Med Chem 18:1948
28. Hu X-W, Chien C-M, Yang S-H, Lin Y-H, Lu C-M, Chen Y-L, Lin S-R (2006) Cell Biol Toxicol 22:417
29. Arzel E, Rocca P, Grellier P, Labaeïd M, Frappier F, Guéritte F, Gaspard C, Marsais F, Godard A, Quéguiner G (2001) J Med Chem 44:949
30. Kumar A, Srivastava K, Kumar SR, Puri SK, Chauhan PMS (2010) Bioorg Med Chem Lett 20:7059
31. Margolis BJ, Long KA, Laird DLT, Ruble CJ, Pulley SR (2007) J Org Chem 72:2232
32. Hashim F, Motiwala A, Raj Kumar A, Chakraborty AK (2007) Aust J Chem 60:369
33. Aillerie A, Pellegrini S, Bousquet T, Pélinski L (2014) New J Chem 38:1389
34. Staderini M, Cabezas N, Bolognesi ML, Meneńdez JC (2013) Tetrahedron 69:1024
35. Musonda CC, Yardley V, de Souza RCC, Ncokazi K, Egan TJ, Chibale K (2008) Org Biomol Chem 6:4446
36. Melato S, Coghi P, Basilico N, Prosperi P, Monti D (2007) Eur J Org Chem 6118
37. Singh T, Stein RG, Hoops JF, Biel JH, Hoya WK, Cruz DR (1970) J Med Chem 4:283
38. Surrey AR, Hammer HF (1946) J Am Chem Soc 68:113
39. Jonckers THM, Maes BUW, Lemière GLF, Robouts G, Pieters L, Haemers A, Dommisse RA (2003) Synlett 5:615
40. Watanabe T, Oishi S, Fujii N, Ohno H (2009) J Org Chem 74:4720 and references therein
41. Feng X, Sun A, Zhang S, Yu X, Bao M (2013) Org Lett 15:108