DOI 10.5012/bkcs.2010.31.12.3800

Synthesis and Biological Evaluation of KRIBB3 Analogues on a Proliferation of HCT-116 Colorectal Cancer Cells

Sangku Lee,* Jae Nyoung Kim,† Ji Hye Min, Kab Seog Yoon, Ki Deok Shin,‡ Byoung-Mog Kwon,‡ and Dong Cho Han‡,*

*Immune Modulator Research Center, KRIBB, Daejeon 305-806, Korea. *E-mail: sangku@kribb.re.kr*
†Department of Chemistry and Institute of Basic Science, Chonnam National University, Gwangju 500-757, Korea
*‡Medical Genomics Research Center, KRIBB, Daejeon 305-806, Korea. *E-mail: dchan@kribb.re.kr*
Received September 14, 2010, Accepted October 8, 2010

Key Words: Proliferation, SAR, Anti-mitotic, Isoxazole, Inhibition

Even though target-specific cancer therapies have some progress, only 5% of such therapy show efficacy in the clinic.[1] Therefore, the development of cancer therapy targeted to cytotoxicity is still important. Cell cycle control is the major regulatory mechanism of cell growth. Small chemicals that inhibit cell cycle progression have attracted much attention for cancer therapy because they can block tumor growth. Drug-mediated mitotic-checkpoint-dependent-arrest is often followed by cell death.[2]

In previous studies, we reported a diaryl oxazole compound, KRIBB3 (**1a**) that displayed strong anti-mitotic activity against cancer cells.[3] KRIBB3 showed inhibition of proliferation of HCT-116 colorectal cancer cells with GI_{50} value of 0.1 μM and showed 6 times stronger inhibitory activity than nocodazol. Nocodazole is a well known anti-mitotic chemical and we used it as a control compound. In an effort to further develop this promising compound **1a** toward a potent anti-cancer agent, we have focused on examining structure-activity relationships for KRIBB3. We synthesized a series of diaryl isoxazole derivatives by modifying alkoxy and hydroxyl group in the aryl moiety of KRIBB3 (**1a**), and evaluated their antiproliferative activity on the proliferation of HCT-116 colorectal cancer cells. Herein, we describe the synthesis and biological evaluation of KRIBB3 analogues **1**.

The synthesis of diaryl isoxazole derivative **1b** was achieved through the route in Scheme 1. The formylation[4] of 4-ethylresorcinol (**2**) with DMF and $POCl_3$ gave 5-ethyl-2-hydroxy-4-methoxybenzaldehyde which was dimethylated utilizing methyl iodide and potassium carbonate to furnish compound **3**. Reaction of aldehyde **3** with 4-methoxybenzylmagnesium chloride and the subsequent oxidation of the resulting secondary alcohol with TPAP and NMO afforded ketone **4**.[5] Condensation of compound **4** with dimethylformamide dimethylacetal (DMFDMA) in refluxing toluene gave enaminoketone **5**,[6] which was cyclized in refluxing methanolic AcOH in the presence of Na_2CO_3 to provide isoxazole **1b**.[6b] Compound **1c** and **1f** were prepared from 2-benzyloxy-4-methoxy-5-ethylbenzaldehyde and 3,5-dimethyl-4-hydroxybenzaldehyde, respectively, in same fashion as described for synthesis of **1b**. Compound **1d** and **1e** were prepared by demethylation of **1c** using BBr_3 in 45% and 30% yields, repectively. Compound **1g** was synthesized by debenzylation of compound **1f** using hydrogen (60 psi) and 10% Pd/C.

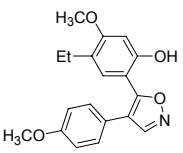
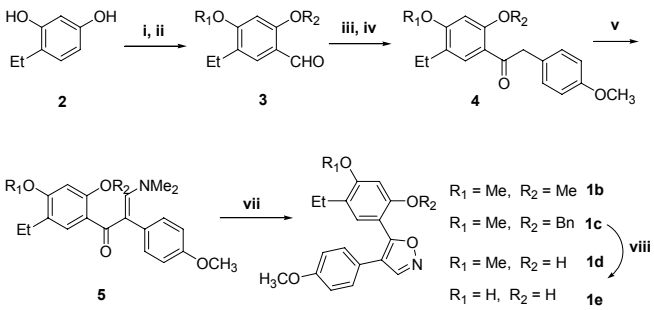


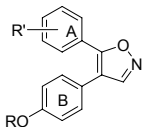
Figure 1. Structure of KRIBB3 (**1a**).

Inhibitory activity of diaryl isoxazole derivatives against proliferaction of HCT-116 colorectal cancer cells was evaluated by measurement of the amount of WST-1 formazan formed by adding cell proliferation reagent WST-1. The GI_{50} values of these compounds were presented in Table 1. The extent of inhibiting proliferaction of HCT-116 colorectal cancer cells was greater in methoxy group (**1a**, **1b** and **1c**) of B-aryl moiety than in hydroxyl group (**1d** and **1e**). The presence of free hydroxyl hydrogen in the B-aryl moiety of KRIBB3 analogues decreased the efficiency of antiproliferative activity. Large benzyloxy substituent (**1c**) at 2-position of A-aryl moiety showed a weak inhibitory activity in comparision with relatively small sized methoxy (**1b**) or hydroxy (**1a**) substituent. Change of substituents in A-aryl moiety of KRIBB3 analogues (**1f** and **1g**) did not enhance antiproliferative activity. Among the evaluated compounds, compound **1b** exhibited the strongest antiproliferative effects and showed 6 times more potent activity than nocodazole as a positive control. The structure-activity analysis indicated



Scheme 1. Reagents and conditions: (i) $POCl_3$, DMF, 10 °C to rt, 1 hr; (ii) CH_3I, K_2CO_3, DMF, rt; (iii) 4-methoxybenzyl magnesium chloride, 0 °C to rt, 1 hr; (iv) tetrapropylammonium perruthenate, 4-methylmorpholine N-oxide, 4Å molecular sieves, CH_2Cl_2, rt, 30 min; (v) DMFDMA, toluene, reflux; (vi) NH_2OH-HCl, MeOH, AcOH, Na_2CO_3, 115 °C, (vii) BBr_3, CH_2Cl_2, −10 °C, 2 h

Table 1. Antiproliferative activity of diaryl isoxazole compounds on the proliferaction of HCT-116 colorectal cancer cells



compound	R	R'	GI_{50} (μM)
1b	CH_3	2,4-$(OCH_3)_2$, 5-Et	0.1
1c	CH_3	2-OBn, 4-OCH_3, 5-Et	3.5
1d	H	2-OH, 4-OCH_3, 5-Et	12
1e	H	2-OH, 4-OH, 5-Et	4.1
1f	CH_3	3,5-$(CH_3)_2$, 4-OBn	75
1g	CH_3	3,5-$(CH_3)_2$, 4-OH	0.7
kribb3 (1a)	CH_3	2-OH, 4-OCH_3, 5-Et	0.1
nocodazole			0.6

that the presence of methoxy group in B-aryl moiety of KRIBB3 analogues and the presence of moderate sized alkoxy group in A-aryl moiety contributed to increase the activity. In conclusion, analogues of KRIBB3 were synthesized and their inhibitory activities on proliferaction of HCT-116 colorectal cancer cells were evaluated. Compound **1b** showed 6 times stronger inhibitory activity than nocodazole and the same potent activity in comparision with KRIBB3 (**1a**).

Experimental Section

Typical procedure for synthesis of compound 1b. To dried DMF (14.5 mL, 0.18 mol) at 10 °C was slowly added of $POCl_3$ (4.43 mL, 0.078 mol) and the mixture was stirred for 30 minutes. Then, a solution of 4-ethylresorcinol (**2**) (3.0 g, 0.022 mol) in DMF (14.5 mL, 0.18 mol) was added and the reaction mixture was stirred at room temperature for 1 hr. The reaction mixture was cooled to 0 °C and 2 M NaOH aqueous solution was added to quench the reaction. The reaction solution was diluted with ethyl acetate and extracted two times with 2 M NaOH aqueous solution. The aqueous solution was neutralized by 3 N HCl, extracted four times with ethyl acetate, and washed with brine. Combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The concentrated residue was purified by silica-gel column chromatography (hexane:ethyl acetate = 7:1), to give 2.5 g of formylated compound. To a mixture of the formylated compound (2.5 g, 0.015 mol) and potassium carbonate (6.24 g, 0.045 mol) in DMF (25 mL) was added methyl iodide (5.55 g, 0.039 mol) and the reaction mixture was stirred at room temperature for 5 hours. The reaction solution was filtered to remove inorganic salts, and the filtrate was diluted with water (200 mL), extracted three times with ethyl acetate and washed with brine. The combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The concentrated residue was purified by silica-gel column chromatography (hexane:ethyl acetate = 20:1) to give 2.63 g (90%) of dimethylated compound **3**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.26 (s, 1H), 7.60 (s, 1H), 6.36 (s, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 2.53 (q, J = 7.2 Hz, 2H), 1.13 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.4, 163.9, 162.5, 128.5, 125.4, 117.9, 93.9, 55.7, 55.5, 22.2, 13.8. To a mixture of magnesium turnings (0.75 g, 0.031 mol) in THF (5 mL) at room temperature was slowly added a solution of 4-methoxybenzylchloride (1.6 g, 10.2 mmol) in THF (10 mL). The reaction solution was refluxed with heating for one hour and then cooled down in a 0 °C water bath. The ashy solution was extracted by using a syringe, which was used as a Grignard reagent. To a solution of the compound **3** (0.67 g, 3.4 mmol) in THF (15 mL) at 0 °C was added the Grignard reagent slowly, and the reaction mixture was stirred at room temperature for 1 hour. Saturated ammonium chloride solution was added to the reaction solution, and the mixture was extracted with ethyl acetate three times and washed with brine. Combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The concentrated residue was purified by silica-gel column chromatography (hexane:ethyl acetate = 7:1), to give 1.05 g of alcoholic compound. To a mixture of the alcoholic compound (1.05 g, 3.3 mmol), 4-methylmorpholine N-oxide (0.57 g, 4.9 mmol) and anhydrous powdered 4 Å molecular sieves (1.64 g) in dichloromethane (10 mL) was added tetrapropylammonium perruthenate (57 mg). The reaction mixture was stirred for 30 minutes, passed through a short silica-gel pad by washing with ethyl acetate, and concentrated to give 0.97 g (88% in two steps) of compound **4**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.14 (d, J = 8.7 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 6.38 (s, 1H), 4.21 (s, 2H), 3.91 (s, 3H), 3.87 (s, 3H), 3.77 (s, 3H), 2.54 (q, J = 7.5 Hz, 2H), 1.14 (t, J = 7.2 Hz, 3H). To a solution of compound **4** (1.1 g, 3.5 mmol) in toluene (10 mL) was added dimethylformamide dimethylacetal (DMFDMA) (1.2 g, 10 mmol). The reaction mixture was refluxed with heating for 16 hours at 135 °C. The reaction solution was cooled to 0 °C, concentrated and purified by silica-gel column chromatography (hexane: ethyl acetate = 1:2) to give 0.65 g (50%) of compound **5**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (s, 1H), 7.08 (d, J = 8.7 Hz, 2H), 6.96 (s, 1H), 6.77 (d, J = 8.7 Hz, 2H), 6.32 (s, 1H), 3.80 (s, 3H), 3.77 (s, 3H), 3.72 (s, 3H), 2.69 (s, 6H), 2.49 (q, J = 7.2 Hz, 2H), 1.10 (t, J = 7.2 Hz, 3H). To a solution of compound **5** (1.0 g, 2.7 mmol) in methanol (35 mL) was added sodium carbonate (190 mg, 1.7 mmol) and NH_2OH HCl (1.9 g, 27.0 mmol). The mixture was adjusted to be pH 4-5 using acetic acid (1.0 mL) and then heated in a heavy-wall screw capped tube at 115 °C for 2 hours. The reaction mixture was cooled to room temperature and methanol was removed under reduced pressure. The residue was extracted with methylene chloride and water, and the organic layer washed with brine, dried over Na_2SO_4, concentrated and purified by silica-gel column chromatography (hexane:ethyl acetate = 5:1) to give 0.9 g (98%) of isoxazole compound **1b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.25 (s, 1H), 7.20 (d, J = 8.7 Hz, 2H), 6.84 (d, J = 8.1 Hz, 2H), 6.43 (s, 1H), 3.87 (s, 3H), 3.79 (s, 3H), 3.51 (s, 3H), 2.56 (q, J = 7.2 Hz, 2H), 1.15 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.3, 159.9, 158.6, 156.3, 150.5, 130.6, 128.2, 124.9, 123.4, 116.3, 113.7, 109.6, 95.2, 55.4, 55.3, 55.1, 22.2, 14.0; HRMS (FAB) *m/z* 340.1558 [(M+H)+, calcd for $C_{20}H_{22}NO_4$ 340.1549].

Compound 1c: Compound **1c** was prepared from 2-benzyloxy-4-methoxy-5-ethylbenzaldehyde according to the typical procedure: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.25-

7.00 (m, 8H), 6.78 (dd, J = 6.9, 2.4 Hz, 2H), 6.48 (s, 1H), 4.84 (s, 2H), 3.80 (s, 3H), 3.78 (s, 3H), 2.56 (q, J = 7.8 Hz, 2H), 1.14 (t, J = 7.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.3, 159.7, 158.7, 155.5, 150.5, 136.5, 130.8, 128.2, 127.6, 127.0, 125.3, 123.3, 116.6, 113.8, 109.3, 97.0, 70.7, 55.3, 55.2, 22.3, 14.0; HRMS (FAB) *m*/*z* 416.1859 [(M+H)+, calcd for $C_{26}H_{26}NO_4$ 416.1862].

Compound 1d and 1e: To a solution of compound **1c** (1.0 g, 2.4 mmol) in dry CH_2Cl_2 (10 mL) at −10 °C was added BBr_3 (1.0 M solution in CH_2Cl_2, 9.6 mL, 9.6 mmol). The reaction mixture was stirred at −10 °C for 2 h, quenched with 0.1 N HCl, extracted with methylene chloride five times, washed with brine, and dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (hexane: ethyl acetate = 2:1) to give 0.33 g (45%) of compound **1d** and 0.2 g (30%) of compound **1e**. compound **1d:** ^{1}H NMR (300 MHz, CD_3OD) δ 8.57 (s, 1H), 7.17 (dd, J = 6.6, 2.4 Hz, 2H), 6.97 (s, 1H), 6.70 (dd, J = 6.6, 2.4 Hz, 2H), 6.48 (s, 1H), 4.86 (s, 2H), 3.81 (s, 3H), 2.48 (q, J = 7.2 Hz, 2H), 1.06 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 161.3, 157.9, 156.2, 151.5, 131.3, 129.5, 125.2, 122.8, 118.1, 116.3, 108.0, 100.0, 55.7, 23.3, 14.7; HRMS (FAB) *m*/*z* 312.1229 [(M+H)+, calcd for $C_{18}H_{18}NO_4$ 312.1236]. compound **1e:** ^{1}H NMR (300 MHz, CD_3OD) δ 8.56 (s, 1H), 7.18 (d, J = 8.4 Hz, 2H), 6.92 (s, 1H), 6.71 (d, J = 8.4 Hz, 2H), 6.39 (s, 1H), 2.48 (q, J = 7.6 Hz, 2H), 1.08 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 164.4, 159.2, 157.8, 155.8, 151.4, 131.7, 129.5, 123.9, 122.9, 117.8, 116.3, 107.6, 103.8, 23.3, 14.7; HRMS (FAB) *m*/*z* 298.1076 [(M+H)+, calcd for $C_{17}H_{16}NO_4$ 298.1079].

Compound 1f: Compound **1f** was prepared from 3,4-dimethyl-4-hydroxybenzaldehyde according to the typical procedure: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.49-7.30 (m, 9H), 6.95 (m, 2H), 4.84 (s, 2H), 3.85 (s, 3H), 2.26 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.6, 159.3, 157.2, 151.8, 137.3, 131.7, 129.7, 128.5, 128.1, 127.8, 127.7, 123.5, 122.5, 115.1, 114.3, 70.0, 63.8, 55.2, 16.4; HRMS (FAB) *m*/*z* 386.1748 [(M+H)+, calcd for $C_{25}H_{24}NO_3$ 386.1756].

Compound 1g: To a solution of the isoxazole compound **1f** (0.7 g, 1.8 mmol) in ethyl acetate (10 mL) was added 10% palladium/c (40 mg). The reaction was performed under 60 psi of hydrogen atmosphere for 14 hours. The reaction solution was passed through a short silica-gel pad by washing with ethyl acetate, concentrated, and purified by silica-gel column chromatography (hexane:ethyl acetate = 1:2) to give 0.47 g (88%) of compound **1g**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.31 (dd, J = 6.6, 1.8 Hz, 2H), 7.28 (brs, 2H), 6.92 (dd, J = 6.6, 2.4 Hz, 2H), 4.91 (s, 1H), 3.85 (s, 3H), 2.21 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.9, 159.2, 153.7, 151.8, 129.7, 127.7, 123.3, 122.7, 119.9, 114.4, 114.3, 53.3, 15.8; HRMS (FAB) *m*/*z* 296.1284 [(M+H)+, calcd for $C_{18}H_{18}NO_3$ 296.1287].

Cell culture and proliferation assay. The cancer cell line was obtained originally from ATCC. HCT-116 (human colon cancer cell line) was maintained in McCoy's 5A (Invitogen) medium supplement with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen) and penicillin (50 units/mL)/streptomycin (50 μg/mL). Cell cultures were maintained at 37 °C under a humidified atmosphere of 5% CO_2 in an incubator. Proliferation assay was done, as described previously (8). Briefly, cells (6,000 cells) were seeded into 96 well plates in McCoy's 5A media containing 10% FBS. After 20 - 24 hrs, cells were replenished with fresh complete medium containing either a test compound or 0.1% DMSO. After incubation for 48 hrs, cell proliferation reagent WST-1 (Roche Applied Science) was added to each well. The amount of WST-1 formazan produced was measured at 450 nm using an ELISA Reader (Bio-Rad, CA).

Acknowledgments. This research was supported by grants from KRIBB Research Initiative Program, the National Chemical Genomics Research Program, and Center for Biological Modulators of the 21st Century Frontier Research Program.

References

1. Reichert, J. M.; Wènger, J. B. *Drug Discovery Today* **2008**, *13*, 30-37.
2. Jordan, M. A.; Wilson, L. *Nat. Rev. Cancer* **2004**, *4*, 253-265.
3. (a) Shin, K. D.; Lee, M.-Y.; Shin, D.-S.; Lee, S.; Son, K.-H.; Koh, S.; Paik, Y.-K.; Kwon, B.-M.; Han, D. C. *J. Biol. Chem*. **2005**, *280*(50), 41439-41448. (b) Shin, K. D.; Yoon, Y. J.; Kang, Y.-R.; Son, K.-H.; Kim, H. M.; Kwon, B.-M.; Han, D. C. *Biochemical Pharmacology* **2008**, *75*(2), 383-394.
4. Nielsen, S. F.; Christensen, S. B.; Cruciani, G.; Kharazmi, A.; Liljefors, T. *J. Med. Chem*. **1998**, *41*, 4819-4832.
5. Sulikowski, M. M.; Ellis Davies, G. E. R.; Smith, A. B., III. *J. Chem. Soc., Perkin Trans. 1* **1992**, 979.
6. (a) SanMartin, R.; Marigorta, E. M.; Dominguez, E. *Tetrahedron* **1994**, *50*, 2255-2264. (b) Olivera, R.; SanMartin, R.; Dominguez, E.; Solans, X.; Urtiaga, M. K.; Arriortua, M. I. *J. Org. Chem.* **2000**, *65*, 6398-6411.