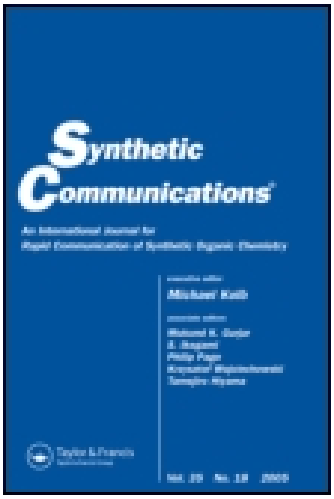

Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

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Accepted author version posted online: 07 Dec 2011.Published online: 26 Mar 2012.

To cite this article: Hyeong Kyu Lee, Eunju Yun, Ji Hye Min, Kab Seog Yoon, Dong-Ho Choung & Sangku Lee (2012) Convenient Synthesis of an Isoxazole Compound, KRIBB3, as an Anticancer Agent, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 42:13, 1890-1894, DOI: 10.1080/00397911.2010.546551

To link to this article: http://dx.doi.org/10.1080/00397911.2010.546551

Synthetic Communications®, 42: 1890–1894, 2012

ISSN: 0039-7911 print/1532-2432 online
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CONVENIENT SYNTHESIS OF AN ISOXAZOLE COMPOUND, KRIBB3, AS AN ANTICANCER AGENT

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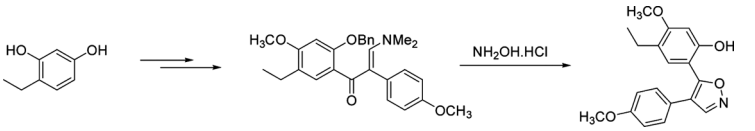
GRAPHICAL ABSTRACT



Abstract *A diaryl isoxazole compound, KRIBB3, which exhibits strong antimigratory and antimitotic activities against cancer cells, was prepared in a practical synthetic way. The synthetic method may provide easy access to KRIBB3 analogs with various substituents at an aryl moiety for structure–activity relationships (SAR), as well as a large quantity of KRIBB3 for in vivo studies.*

Keywords Anticancer; antimigratory; cyclization; isoxazole

INTRODUCTION

In the course of searching for an antimigratory compound using cell-based screening, we synthesized a series of diaryl isoxazole compounds. One of them, 5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)isoxazole (**1**), named KRIBB3, showed strong antimigratory and antimitotic activities against cancer cells (Figure 1).[1] KRIBB3 showed inhibition of proliferation of HCT-116 colorectal cancer cells with GI_{50} value of 0.1 μM and showed six times stronger inhibitory activity than nocodazol as a reference. As we have been interested in further biological studies including in vivo activities, we need a practical synthetic approach to KRIBB3, thereby also enabling access to KRIBB3 analogs for examining structure–activity relationships. Herein we describe a practical synthesis of KRIBB3.

The synthesis of 5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl) isoxazole (**1**) was reported by two research groups.[2] They constructed the core diaryl isoxazole ring system via ring opening and cyclization by reaction of isoflavones with hydroxylamine. Their approaches have a drawback in modifying substituents of

Received October 19, 2010.
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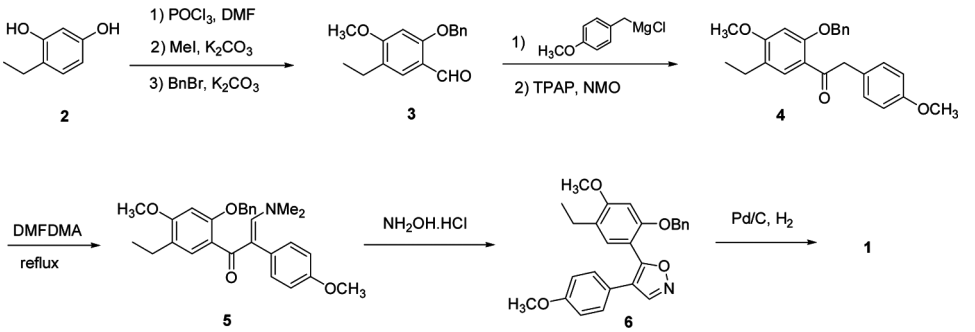

Scheme 1. Synthesis of KRIBB3.

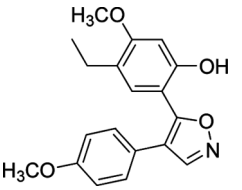

Figure 1. Structure of KRIBB3 (**1**).

an aryl moiety for structure–activity relationships of KRIBB3. The substituent at the 2-position of an aryl moiety of diaryl isoxazole derivatives is fixed with a hydroxyl group because of use of isoflavones in the reaction routes. However, our synthetic route to the isoxazole ring involves the reaction of diaryl enaminone with hydroxylamine, providing a way to prepare diaryl isoxazole derivatives with various substituents at an aryl moiety.

The synthesis started with formylation of commercially available 4-ethylresorcinol (**2**) using $POCl_3$ and dimethylformamide (DMF),[3] followed by methylation using methyl iodide and potassium carbonate to yield 5-ethyl-2-hydroxy-4-methoxybenzaldehyde, which by treatment with benzyl bromide and potassium carbonate afforded compound **3** (Scheme 1). Aldehyde **3** was converted to ketone **4** by addition of 4-methoxybenzylmagnesium chloride and tetrapropylammonium perruthenate (TPAP) oxidation[4] of the resulting alcohol. Enaminoketone **5** was prepared in 70% yield from diaryl ethanone **4** by treatment with dimethylformamide dimethyl acetal (DMFDMA) in refluxing toluene.[5] Finally, heterocyclization of compound **5** with hydroxylamine in refluxing methanolic AcOH in the presence of Na_2CO_3 afforded diary isoxazole **6**,[5b] and subsequent debenzylation provided KRIBB3 (**1**). This synthetic pathway afforded a practical route easily amenable to large scale and produced 10-g quantities of KRIBB3 starting from 25 g of 4-ethylresorcinol (**2**).

In conclusion, KRIBB3 (**1**), an antimigratory agent, was prepared by a convenient synthetic method that would bring a large quantity of **1**.

EXPERIMENTAL

Compound 3

$POCl_3$ (18.5 mL, 0.33 mol) was slowly added to dried dimethylformamide (DMF) (61 mL, 0.75 mol) at 10 °C. The mixture was stirred for 30 min. Then, a solution of 4-ethylresorcinol (**2**) (12.5 g, 0.095 mol) in DMF (40 mL) was added, and the reaction mixture was stirred at room temperature for 1 h. 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. 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–EtOAc 7:1) to give 10 g of formylated compound. Methyl iodide (9.4 g, 0.065 mol) was added to a mixture of the formylated compound (10 g, 0.065 mol) and potassium carbonate (17.5 g, 0.13 mol) in DMF (100 mL), and the reaction mixture was stirred at room temperature for 5 h. The reaction solution was filtered to remove inorganic salts, and the filtrate was diluted with water, 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–EtOAc 20:1) to give 6.6 g of methylated compound. Benzyl bromide (7.2 g, 0.05 mol), was added to a mixture of the methylated compound (6.6 g, 0.04 mol) and potassium carbonate (15 g, 0.11 mol) in DMF (65 mL), and the reaction mixture was stirred at room temperature for 1 h. The reaction solution was filtered to remove inorganic salts, and the filtrate was diluted with water, 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–EtOAc 5:1) to give 9.4 g (35% yield in three steps) of compound **3**: white solid, mp 92-93 °C; IR (film) 2868, 1667, 1602, 1443, 1271, 1198, 1112, 1057 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.39 (s, 1H), 7.66 (s, 1H), 7.46-7.34 (m, 5H), 6.45 (s, 1H), 5.18 (s, 2H), 3.85 (s, 3H), 2.56 (q, $J = 7.8$ Hz, 2H), 1.16 (t, $J = 7.8$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.3, 163.7, 161.7, 136.2, 128.7, 128.3, 128.2, 127.2, 125.8, 118.4, 95.6, 70.8, 55.5, 22.2, 13.8; ESIMS m/z 293.3 ($M^+ + Na$).

Compound 4

A solution of 4-methoxybenzylchloride (16 g, 0.11 mol) in THF (50 mL) was slowly added to a mixture of magnesium turnings (7.5 g, 0.32 mol) in THF (100 mL) at room temperature. 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. The Grignard reagent was added slowly to a solution of the compound **3** (9.25 g, 0.035 mol) in THF (100 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 1 h. Saturated ammonium chloride solution was added to the reaction solution,

and the mixture was extracted with ethyl acetate three times 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–EtOAc 7:1) to give 13 g of alcoholic compound. Tetrapropylammonium perruthenate (0.55 g) was added to a mixture of the alcoholic compound (13 g, 0.042 mol), 4-methylmorpholine N-oxide (5.7 g, 0.05 mol) and anhydrous powdered 4 Å molecular sieves (13 g) in dichloromethane (80 mL). The reaction mixture was stirred for 30 min, passed through a short silica-gel pad by washing with ethyl acetate, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (dichloromethane as an eluent) to give 12 g (88% yield in two steps) of compound **4**: white solid, mp 67–68 °C; IR (film) 2969, 1661, 1598, 1516, 1249, 1125, 1020 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.45–7.36 (m, 5H), 7.11–7.02 (m, 2H), 6.85–6.78 (m, 2H), 6.45 (s, 1H), 5.19 (s, 2H), 4.23 (s, 2H), 3.81 (s, 3H), 3.77 (s, 3H), 2.56 (q, $J = 7.8$ Hz, 2H), 1.16 (t, $J = 7.8$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.3, 161.8, 158.1, 136.2, 131.4, 130.5, 129.2, 128.7, 128.2, 127.8, 127.6, 125.2, 120.1, 113.6, 95.9, 71.0, 55.3, 55.1, 49.0, 22.3, 13.9; HRMS (FAB) m/z 391.1906 [$(M + H)^+$, calcd for $C_{25}H_{27}O_4$ 391.1909].

Compound 5

Dimethylformamide dimethylacetal (DMFDMA) (6.25 mL, 0.065 mol) was added to a solution of compound **4** (12 g, 0.03 mol) in toluene (40 mL). The reaction mixture was refluxed with heating for 16 h at 135 °C. The reaction solution was cooled to 0 °C, concentrated, and purified by silica-gel column chromatography (hexane–EtOAc 2:1) to give 9.4 g (70% yield) of compound **5**: yellow oil; IR (film) 2960, 2233, 1566, 1511, 1384, 1240, 1147 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.44–7.26 (m, 5H), 7.15 (s, 1H), 7.09–7.06 (m, 2H), 7.04 (s, 1H), 6.81–6.78 (m, 2H), 6.40 (s, 1H), 5.04 (s, 2H), 3.78 (s, 3H), 3.76 (s, 3H), 2.63 (s, 6H), 2.56 (q, $J = 7.8$ Hz, 2H), 1.13 (t, $J = 7.8$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.0, 158.1, 158.0, 154.4, 154.0, 137.6, 132.9, 129.4, 129.0, 128.2, 127.5, 127.0, 125.1, 124.8, 113.3, 112.9, 97.9, 71.4, 55.3, 55.1, 43.2, 22.4, 14.1; ESIMS m/z 446.5 ($M^+ + H$).

Compound 6

Sodium carbonate (0.7 g, 12 mmol) and NH_2OH HCl (16 g, 23 mmol) were added to a solution of compound **5** (9.4 g, 21 mmol) in methanol (125 mL). The mixture was adjusted to pH 4-5 using acetic acid and then refluxed with heating for 2 h. The mixture was concentrated in vacuo, diluted with water, adjusted to pH 8 using saturated ammonium hydroxide aqueous solution, extracted with dichloromethane four times, dried over anhydrous sodium sulfate, and concentrated in vacuo. The concentrated residue was purified by silica-gel column chromatography (hexane–EtOAc 8:1) to give 8.0 g (90% yield) of isoxazole compound **6**: white solid, mp 99–100 °C; IR (film) 2960, 1605, 1507, 1444, 1247, 1195 cm^{-1}; ^1H 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 1

To the solution of the isoxazole compound **6** (8.0 g, 19 mmol) prepared in ethyl acetate (75 mL) was added 10% Pd/C (0.4 g). The reaction was performed under 60 psi of hydrogen atmosphere for 14 h. The reaction solution was passed through a short silica-gel pad by washing with ethyl acetate and concentrated in vacuo. The residue was purified by silica-gel column chromatography (hexane–EtOAc 3:1) to give 5.3 g (90% yield) of the final compound **1**: white solid, mp 149–150 °C; IR (film) 3162, 1613, 1598, 1515, 1242, 1207, 1142 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.28 (dd, $J=6.9$, 2.4 Hz, 2H), 7.06 (s, 1H), 6.88 (dd, $J=6.9$, 2.4 Hz, 2H), 6.48 (s, 1H), 5.19 (s, 2H), 3.80 (s, 6H), 2.42 (q, $J=7.8$ Hz, 2H), 0.98 (t, $J=7.8$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.3, 160.3, 159.4, 153.6, 151.5, 129.3, 128.5, 125.2, 121.6, 115.2, 114.4, 105.3, 99.6, 55.3, 55.2, 22.0, 13.7; HRMS (FAB) m/z 326.1389 [$(M+H)^+$, calcd. for $C_{19}H_{20}NO_4$ 326.1392].

ACKNOWLEDGMENT

This research was supported by a grant from the KRIBB Research Initiative Program.

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