การสังเคราะห์บนวัฏภาคของแข็งและฤทธิ์ต้านมะเร็งของอนุพันธ์ไดเอริลเฮปทานอยด์เอไมด์

Solid-phase synthesis and anticancer activity of diarylheptanoid amide derivatives

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บทคัดย่อ

สามารถสังเคราะห์อนุพันธ์ของสารไดเอริลเฮปทานอยด์เอไมด์โดยเทคนิคการสังเคราะห์บนวัฏภาคของแข็งที่นำกลับมาใช้ใหม่ โดยอนุพันธ์ของสารไดเอริลเฮปทานอยด์เอไมด์ได้ทดสอบความเป็นพิษต่อเซลล์ของเซลล์มะเร็งและเซลล์ปกติ อนุพันธ์ 4-คลอโรเบนโซอิล แสดงฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งลำไส้ (HT29) และมะเร็งเต้านม (MCF-7) ที่ค่า IC_{50} 38.61 และ 40.15 ไมโครกรัมต่อมิลลิลิตรตามลำดับ นอกจากนั้นอนุพันธ์ 4-คลอโรเบนโซอิล ไม่แสดงความเป็นพิษต่อเซลล์ปกติ (HEK293) ที่ค่า IC_{50} 747.20 ไมโครกรัมต่อมิลลิลิตร ค่าดัชนีความจำเพาะของสาร 4-คลอโรเบนโซอิล แสดงค่าสูงกว่าสารมาตรฐานด็อกโซรูบิซินและเคอร์คูมิน

ABSTRACT

A library of diarylheptanoid amide derivatives were synthesized by solid-phase synthesis technique using reusable linker. Diarylheptanoid amide derivatives obtained were screened for cytotoxicity activity against cancer and normal cell lines. 4-Chlorobenzoyl analogues exhibited moderate activity against human colon adenocarcinoma cell line (HT29) and human breast adenocarcinoma cell line (MCF-7) with IC_{50} values of 38.61 and 40.15 µg/ml, respectively. Moreover, 4-chlorobenzoyl analogues showed no cytotoxic activity against the normal cell (HEK293) with IC_{50} value of 747.20 µg/ml. The selectivity index value of 4-chlorobenzoyl analogues was greater than that of doxorubicin and curcumin.

คำสำคัญ: การสังเคราะห์บนวัฏภาคของแข็ง อนุพันธ์ไดเอริลเฮปทานอยด์เอไมด์ ฤทธิ์การต้านเซลล์มะเร็ง ค่าดัชนีความจำเพาะ

Keywords: Solid-phase synthesis, Diarylheptanoid amide derivatives, Anticancer activity, Selectivity index Value

INTRODUCTION

Diarylheptanoids are organic compounds containing two aromatic rings connected by a seven-carbons chain (Claeson et al., 1994; Claeson et al., 2002; Keserü and Nógrádi, 1995; Lv and She, 2010). Several diarylheptanoids compounds can be purified from rhizome of the the Zingiberaceae family e.g., *Curcuma longa* L. and *Curcuma comosa* Roxb. (Priyadarsini, 2009; Ravindran et al., 2010). Diarylheptanoids are highly hydrophobic compounds (Figure 1) and exhibit many interesting biological activities such as anti-HIV activity (Jordan and Drew, 1996; Mazumder et al., 1995), anti-inflammatory activity (Chan et al., 1995; Chan et al., 1998) and anticancer activity (Kuttan et al., 1985; Karunagaran et al., 2005).

Solid-phase organic synthesis has emeraged as a powerful tool for organic synthesis of organic molecules (Yingyongnarongkul et al., 2006; Yingyongnarongkul et al., 2008). Reusable resin is the linker structure can be regenerated upon product release. With this method, additional synthesis cycle can be performed on the same resin. Also, reusable resin was reasonably cost-effectiveness for large scale solid-phase organic synthesis in that multigram quantities of product could be realized by repeated synthesis (Scheme 1). Eriksson et al. (2006) reported that ortho-nitrophenol resin was the reusable linker that can be used to synthesized amide product (Scheme 2).

In this work, we reported the synthesis of diarylheptanoid amide derivatives with different diamines and benzoyl analogues and related aromatic carboxylic acids using reusable resin as well as evaluation of their cytotoxicity activity against various cancer and normal cell lines.

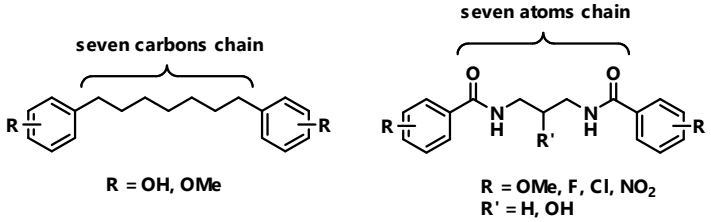
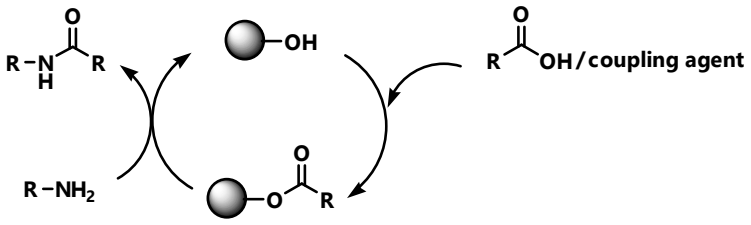
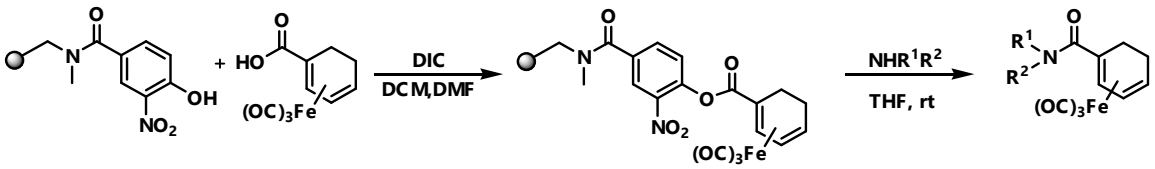


Figure 1 Diarylheptanoids and diarylheptanoids amide derivatives



Scheme 1 Reusable linker for amide compound synthesis



Scheme 2 Synthesis of amide product using ortho-nitrophenol resin

EXPERIMENTAL

All chemicals were purchased from Sigma-Aldrich-Fluka. Solvents were purchased from commercial suppliers and used without further purification. Column chromatography and thin layer chromatography (TLC) were carried out using Merck silica gel 60 (<0.063 mm) and precoated silica gel 60 F254 plates, respectively. Spots on TLC were detected by spraying with anisaldehyde–H_2SO_4 and ninhydrin reagents followed by heating. IR spectra were obtained using a Frontier FT-IR Perkin-Elmer spectrophotometer. NMR spectra were recorded on a Bruker AVANCE400 spectrometer. Electrospray (ES) mass spectra were obtained by using a Finnigan LC-Q mass spectrometer and high resolution mass spectra (HRMS) were obtained by using a Bruker micrOTOF–II mass spectrometer.

Synthesis of reusable linker (**1**)

Aminomethyl polystyrene resin (**2**) (1.10 g, 1.21 mmol, 1.11 mmol/g) was pre-swollen in CH_2Cl_2 (10 ml) for 30 minutes and filtered. The mixture solution of 5-nitrosalicylic acid (**3**) (0.88 g, 4.84 mmol), *N,N*-diisopropylcarbodiimide (DIC) (0.75 ml, 4.84 mmol) and 4-dimethylaminopyridine (DMAP) in DMF (10 ml) was added to the amino resin and shaken overnight. Then, the resin was filtered and washed successively with DMF, CH_2Cl_2, MeOH (3 x 10 ml, each) to give reusable linker. The reusable linker was dried under vacuum for 2 h.

Synthesis of active ester resin 1 (**A-K**)

The mixture solution of various carboxylic acid **A-K** (4 equiv) (Figure 2), DIC (4 equiv) and DMAP in CH_2Cl_2: DMF (4 : 1) were added to dry reusable linker (**1**) (1 equiv), shaken overnight, and then the suspensions were filtered and successively washed with DMF, CH_2Cl_2, MeOH (3 x 10 ml, each), yielding an active ester resin **1** (**A-K**). In the final step, the active ester resin was dried under vacuum for 2 h.

Synthesis of diarylheptanoid amide derivatives (**A-K**) (**a-b**)

Each of active ester resin **1** (**A-K**) derived from previous steps was divided into two equal parts for parallel synthesis. Each portion was individually reacted with diaminopropane (**a-b**) (0.4 equiv) (Figure 2) in CH_2Cl_2 (1.5 ml). The suspensions were shaken overnight. The resins were filtered and solutions were collected. The solvents were removed and the crude products were purified by column chromatography to obtain the desired products (**A-K**)(**a-b**) in high yield. The structures of the synthesized compounds were confirmed by IR, ^{1}H-NMR, MS and HR-MS techniques.

N,N′-Bis(2-naphthoyl)-1,3-diaminopropane (**Aa**)

White amorphous solid; MP: 213-214 °C; IR: ν_{max} 3249, 3081, 3057, 3018, 1618, 1553, 1341, 1316, 1304, 1147, 1119, 953, 912, 871, 839, 781, 737 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.86 (2H, *quint*, *J* = 6.0 Hz, H-3), 3.53 (4H, *t*, *J* = 5.8 Hz, H-2 and H-4), 7.47 (4H, *m*, H-6′ and H-7′), 7.83 (8H, *m*, H-3′, H-4′, H-5′ and H-8′), 8.33 (2H, *s*, H-1′); MS (ESI$^+$) *m/z*: 383.6 ([M + H]$^+$, 100%); HR-MS (ESI$^+$) *m/z*: [M + Na]$^+$ calcd. for $C_{25}H_{23}N_2O_2Na$ 405.1580; Found 405.1573.

N,N′-Bis(2-naphthoyl)-1,3-diaminopropane-2-ol (**Ab**)

White amorphous solid; MP: 210-211 °C; IR: ν_{max} 3295, 3053, 2919, 1618, 1536, 1503, 1427, 1314, 1238, 1085, 954, 905, 868, 834, 783, 758, 737 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.40 (2H, *m*, H-2b and H-4b), 3.66 (2H, *m*, H-2a and H-4a), 3.92 (1H, *m*, H-3), 7.46 (4H, *m*, H-6′ and H-7′), 7.82 (8H, *m*, H-3′, H-4′, H-5′ and H-8′), 8.33 (2H, *s*, H-1′); MS (ESI$^-$) *m/z*: 397.5 ([M - H]$^-$, 100%); HR-MS (ESI$^+$) *m/z*: [M + Na]$^+$ calcd. for $C_{25}H_{23}N_2O_3Na$ 421.1542; Found 421.1522.

N,N′-Bis(3,5-dimethoxybenzoyl)-1,3-diaminopropane (**Ba**)

White amorphous solid; MP: 176-177 °C; IR: ν_{max} 2936, 2843, 1631, 1587, 1544, 1424, 1324, 1304, 1205,

1160, 1125, 1063, 870, 737 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.80 (2H, *quint*, *J* = 6.0 Hz, H-3), 3.51 (4H, *q*, *J* = 6.0 Hz, H-2 and H-4), 3.80 (12H, *s*, 4✕OMe), 6.55 (2H, *s*, H-4′), 6.96 (4H, *d*, *J* = 2.1 Hz, H-2′ and H-6′); MS (ESI^+) *m/z*: 403.4 ($[M + H]^+$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{21}H_{26}N_2O_6Na$ 425.1686; Found 425.1683.

N,N′-Bis(3,5-dimethoxybenzoyl)-1,3-diaminopropane-2-ol (**Bb**)

White amorphous solid; MP: 163-164 °C; IR: ν_{max} 3329, 2969, 2935, 1586, 1541, 1323, 1276, 1261, 1204, 1160, 1125 ,1061, 764, 749 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.58 (4H, *t*, *J* = 5.2 Hz, H-2 and H-4), 3.79 (12H, *s*, 4✕OMe), 3.98 (1H, *m*, H-3), 6.55 (2H, *brs*, H-4′), 6.93 (4H, *d*, *J* = 2.1 Hz, H-2′ and H-6′); MS (ESI^-) *m/z*: 417.3 ($[M - H]^-$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{21}H_{26}N_2O_7Na$ 441.1636; Found 441.1632.

N,N′-Bis(2,3-dimethoxybenzoyl)-1,3-diaminopropane (**Ca**)

Pale yellow amorphous solid; MP: 55-56 °C; IR: ν_{max} 3475, 3373, 1642, 1533, 1456, 1438, 1257, 1220, 1068, 972, 805, 749 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.88 (2H, *quint*, *J* = 6.3 Hz, H-3), 3.54 (4H, *q*, *J* = 6.3 Hz, H-2 and H-4), 3.86 (6H, *s*, 2✕OMe), 3.91 (6H, *s*, 2✕OMe), 7.00 (2H, *d*, *J* = 7.5 Hz, H-4′), 7.10 (2H, *t*, *J* = 7.9 Hz, H-5′), 7.65 (2H, *d*, *J* = 7.8 Hz, H-6′); MS (ESI^+) *m/z*: 403.2 ($[M + H]^+$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{21}H_{26}N_2O_6Na$ 425.1697; Found 425.1683.

N,N′-Bis(2,3-dimethoxybenzoyl)-1,3-diaminopropane-2-ol (**Cb**)

Pale yellow amorphous solid; MP: 118-119 °C; IR: ν_{max} 2951, 2924, 1649, 1575, 1528, 1457, 1258, 1217, 1063, 983, 805, 746 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.54 (2H, *m*, H-2b and H-4b), 3.65 (2H, *m*, H-2a and H-4a), 3.86 (6H, *s*, 2✕OMe), 3.92 (6H, *s*, 2✕OMe), 3.99 (1H, *m*, H-3), 7.01 (2H, *d*, *J* = 7.9 Hz, H-4′), 7.10 (2H, *t*, *J* = 7.9 Hz, H-5′), 7.64 (2H, *d*, *J* = 7.9 Hz, H-6′); MS (ESI^-) *m/z*: 417.7 ($[M - H]^-$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{21}H_{26}N_2O_7Na$ 441.1639; Found 441.1632.

N,N′-Bis(2,4-dimethoxybenzoyl)-1,3-diaminopropane (**Da**)

White amorphous solid; MP: 175-176 °C; IR: ν_{max} 3274, 2923, 2835, 1649, 1612, 1536, 1492, 1427, 1226, 1205, 1178, 1124, 1051, 1023, 974, 856, 799, 728 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.71 (2H, *br s*, H-3), 3.37 (4H, *br s*, H-2 and H-4), 3.72 (6H, *s*, 2✕OMe), 3.77 (6H, *s*, 2✕OMe), 6.54 (2H, *m*, H-5′), 6.78 (2H, *m*, H-6′), 6.98 (2H, *s*, H-3′); MS (ESI^+) *m/z*: 403.7 ($[M + H]^+$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{21}H_{26}N_2O_6Na$ 425.1685; Found 425.1683.

N,N′-Bis(2,4-dimethoxybenzoyl)-1,3-diaminopropane-2-ol (**Db**)

White amorphous solid; MP: 171-172 °C; IR: ν_{max} 3294, 2905, 2834, 1649, 1608, 1545, 1493, 1428, 1284, 1225, 1117, 1050, 1023, 974, 723 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.30 (2H, *m*, H-2b and H-4b), 3.48 (2H, *m*, H-2a and H-4a), 3.74 (6H, *s*, 2✕OMe), 3.78 (7H, *s*, 2✕OMe, H-3), 6.57 (2H, *m*, H-5′), 6.81 (2H, *m*, H-6′), 6.99 (2H, *s*, H-3′); MS (ESI^-) *m/z*: 417.1 ($[M - H]^-$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{21}H_{26}N_2O_7Na$ 441.1641; Found 441.1632.

N,N′-Bis(3,4-dimethoxybenzoyl)-1,3-diaminopropane (**Ea**)

White amorphous solid; MP: 185-186 °C; IR: ν_{max} 3300, 1630, 1536, 1509, 1316, 1276, 1237, 1135, 1018, 859 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.79 (2H, *br s*, H-3), 3.53 (4H, *q*, *J* = 5.8 Hz, H-2 and H-4), 3.90 (6H, *s*, 2✕OMe), 3.93 (6H, *s*, 2✕OMe), 6.87 (2H, *d*, *J* = 8.3 Hz, H-5′), 7.41 (2H, *d*, *J* = 8.3 Hz, H-6′), 7.48 (2H, *s*, H-2′); MS (ESI^+) *m/z*: 403.4 ($[M + H]^+$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{21}H_{26}N_2O_6Na$ 425.1695; Found 425.1683.

N,N′-Bis(3,4-dimethoxybenzoyl)-1,3-diaminopropane-2-ol (**Eb**)

White amorphous solid; MP: 170-171 °C; IR: ν_{max} 3210, 1639, 1582, 1507, 1274, 1229, 1134, 1088, 1017, 874, 811, 751 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.53 (2H, *m*, H-2b and H-4b), 3.64 (2H, *m*, H-2a and H-4a), 3.88 (12H, *s*, 4✕OMe), 3.98 (1H, *s*, H-3), 6.83 (2H, *d*, *J* = 8.3 Hz, H-5′), 7.39 (2H, *d*, *J* = 8.3 Hz, H-6′), 7.42 (2H, *s*, H-2′); MS (ESI^-) *m/z*: 417.1 ([M - H]$^-$, 100%); HR-MS (ESI^+) *m/z*: [M + Na]$^+$ calcd. for $C_{21}H_{26}N_2O_7Na$ 441.1646; Found 441.1632.

N,N′-Bis(2-picolinoyl)-1,3-diaminopropane (**Fa**)

White amorphous solid; MP: 87-88 °C; IR: ν_{max} 3297, 1650, 1523, 1466, 1431, 1350, 1041, 996, 851, 822, 745 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.89 (2H, *quint, J* = 6.3 Hz, H-3), 3.53 (4H, *m*, H-2 and H-4), 7.36 (2H, *t*, *J* = 7.6 Hz, H-4′), 7.78 (2H, *t*, *J* = 7.6 Hz, H-5′), 8.13 (2H, *d*, *J* = 7.7 Hz, H-6′), 8.49 (2H, *brd*, *J* = 4.6 Hz, H-3′); MS (ESI^+) *m/z*: 307.5 ([M + Na]$^+$, 100%); HR-MS (ESI^+) *m/z*: [M + Na]$^+$ calcd. for $C_{15}H_{16}N_4O_2Na$ 307.1170; Found 307.1165.

N,N′-Bis(2-picolinoyl)-1,3-diaminopropane-2-ol (**Fb**)

White amorphous solid; MP: 75-76 °C; IR: ν_{max} 3407, 3338, 1658, 1526, 1424, 1290, 1245, 1127, 1092, 997, 817, 746 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.58 (4H, *m*, H-2 and H-4), 3.99 (1H, *m*, H-3), 7.36 (2H, *t*, *J* = 6.9 Hz, H-4′), 7.78 (2H, *t*, *J* = 6.9 Hz, H-5′), 8.11 (2H, *d*, *J* = 7.7 Hz, H-6′), 8.50 (2H, *brd*, *J* = 4.3 Hz, H-3′); MS (ESI^+) *m/z*: 323.4 ([M + Na]$^+$, 100%); HR-MS (ESI^+) *m/z*: [M + Na]$^+$ calcd. for $C_{15}H_{16}N_4O_3Na$ 323.1130; Found 323.1114.

N,N′-Bis(3-picolinoyl)-1,3-diaminopropane (**Ga**)

White amorphous solid; MP: 163-164 °C; IR: ν_{max} 3288, 1628, 1588, 1548, 1423, 1320, 1122, 1025, 838, 739 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.82 (2H, *br s*, H-3), 3.47 (4H, *t*, *J* = 6.3 Hz, H-2 and H-4), 7.35 (2H, *m*, H-5′), 8.16 (2H, *d*, *J* = 7.9 Hz, H-6′), 8.61 (2H, *brs*, H-4′), 8.99 (2H, *s*, H-2′); MS (ESI^+) *m/z*: 307.8 ([M + Na]$^+$, 100%); HR-MS (ESI^+) *m/z*: [M + Na]$^+$ calcd. for $C_{15}H_{16}N_4O_2Na$ 307.1166; Found 307.1165.

N,N′-Bis(3-picolinoyl)-1,3-diaminopropane-2-ol (**Gb**)

White amorphous solid; MP: 158-159 °C; IR: ν_{max} 3231, 1657, 1626, 1594, 1550, 1411, 1114, 1017, 900, 830 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.37 (2H, *m*, H-2b and H-4b), 3.57 (2H, *m*, H-2a and H-4a), 3.91 (1H, *quint*, *J* = 5.2 Hz, H-3), 7.35 (2H, *t*, *J* = 7.9 Hz, H-5′), 8.15 (2H, *d*, *J* = 7.9 Hz, H-6′), 8.60 (2H, *brd*, *J* = 4.3 Hz, H-4′), 8.98 (2H, *s*, H-2′); MS (ESI^+) *m/z*: 323.1 ([M + Na]$^+$, 100%); HR-MS (ESI^+) *m/z*: [M + Na]$^+$ calcd. for $C_{15}H_{16}N_4O_3$ Na 323.1119; Found 323.1114.

N,N′-Bis-(4-fluorobenzoyl)-1,3-diaminopropane (**Ha**)

White amorphous solid; MP: 155-156 °C; IR: ν_{max} 3302, 2917, 2850, 1633, 1603, 1543, 1500, 1231, 1160, 851 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.81 (2H, *quint*, *J* = 6.2 Hz, H-3), 3.54 (4H, *q*, *J* = 6.0 Hz, H-2 and H-4), 7.11 (4H, *d*, *J* = 8.5 Hz, H-3′ and H-5′), 7.87 (4H, *d*, *J* = 8.5 Hz, H-2′ and H-6′); MS (ESI^+) *m/z*: 319.3 ([M + H]$^+$, 100%); HR-MS (ESI^+) *m/z*: [M + Na]$^+$ calcd. for $C_{17}H_{16}F_2N_2O_2Na$ 341.1071; Found 341.1072.

N,N′-Bis(4-fluorobenzoyl)-1,3-diaminopropane-2-ol (**Hb**)

White amorphous solid; MP: 172-174 °C; IR: ν_{max} 3296, 2916, 2850, 1734, 1636, 1605, 1548, 1501, 1234, 1160, 1113, 1096 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.30 (2H, *m*, H-2b and H-4b), 3.60 (2H, *m*, H-2a and H-4a), 3.86 (1H, *br s*, H-3), 7.08 (4H, *m*, H-3′ and H-5′), 7.85 (4H, *m*, H-2′ and H-6′); MS (ESI^-) *m/z*: 333.3 ([M - H]$^-$, 100%); HR-MS (ESI^+) *m/z*: [M + Na]$^+$ calcd. for $C_{17}H_{16}F_2N_2O_3Na$ 357.1054; Found 357.1021.

N,N′-Bis(4-chlorobenzoyl)-1,3-diaminopropane (**Ia**)

White amorphous solid; MP: 216-217 °C; IR: ν_{max} 3351, 3280, 1636, 1625, 1595, 1540, 1486, 1088, 1014, 845, 754 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.80

(2H, *quint*, *J* = 5.0 Hz, H-3), 3.50 (4H, *q*, *J* = 6.0 Hz, H-2 and H-4), 7.39 (4H, *d*, *J* = 8.4 Hz, H-3′ and H-5′), 7.78 (4H, *d*, *J* = 8.4 Hz, H-2′ and H-6′); MS (ESI^-) *m/z*: 349.5 ($[M - H]^-$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{17}H_{16}Cl_2N_2O_2Na$ 373.0504; Found 373.0481.

N,N′-Bis(4-chlorobenzoyl)-1,3-diaminopropane-2-ol (**Ib**)

White amorphous solid; MP: 220-221 °C; IR: ν_{max} 3382, 3278, 1625, 1596, 1540, 1485, 1295, 1215, 1093, 1012, 983, 841, 753, 727 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.40 (2H, *m*, H-2b and H-4b), 3.64 (2H, *m*, H-2a and H-4a), 3.92 (1H, *m*, H-3), 7.40 (4H, *d*, *J* = 8.4 Hz, H-3′ and H-5′), 7.77 (4H, *d*, *J* = 8.4 Hz, H-2′ and H-6′); MS (ESI^-) *m/z*: 365.1 ($[M - H]^-$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{17}H_{16}Cl_2N_2O_3Na$ 389.0448; Found 389.0430.

N,N′-Bis(piperonyloyl)-1,3-diaminopropane (**Ja**)

White amorphous solid; MP: 171-172 °C; IR: ν_{max} 3347, 2841, 2916, 1637, 1601, 1542, 1466, 1434, 1254, 1237, 1036, 926, 830, 808, 756 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.75 (2H, *br s*, H-3), 3.43 (4H, *br s*, H-2 and H-4), 5.97 (4H, *s*, O-CH_2-O), 6.78 (2H, *d*, *J* = 8.0 Hz, H-5′), 7.30 (2H, *s*, H-2′), 7.36 (2H, *d*, *J* = 8.0 Hz, H-6′); MS (ESI^+) *m/z*: 371.8 ($[M + H]^+$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{19}H_{18}N_2O_6Na$ 393.1086; Found 393.1057.

N,N′-Bis(piperonyloyl)-1,3-diaminopropane-2-ol (**Jb**)

White amorphous solid; MP: 187-188 °C; IR: ν_{max} 3382, 2915, 1638, 1599, 1544, 1479, 1437, 1256, 1240, 1034, 928, 811, 754 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.26 (2H, *m*, H-2b and H-4b), 3.54 (2H, *m*, H-2a and H-4a), 3.80 (1H, *br s*, H-3), 5.95 (4H, *s*, O-CH_2-O), 6.77 (2H, *d*, *J* = 8.1 Hz, H-5′), 7.28 (2H, *s*, H-2′), 7.36 (2H, *d*, *J* = 8.1 Hz, H-6′); MS (ESI^-) *m/z*: 385.4 ($[M - H]^-$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{19}H_{18}N_2O_7Na$ 409.1035; Found 409.1006.

N,N′-Bis(4-nitrobenzoyl)-1,3-diaminopropane (**Ka**)

White amorphous solid; MP: 221-222 °C; IR: ν_{max} 3327, 1635, 1595, 1543, 1512, 1489, 1349, 1300, 865, 856 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.82 (2H, *quint*, *J* = 5.9 Hz, H-3), 3.46 (4H, *br s*, H-2 and H-4), 8.00 (4H, *d*, *J* = 8.6 Hz, H-2′ and H-6′), 8.24 (4H, *d*, *J* = 8.6 Hz, H-3′ and H-5′); MS (ESI^+) *m/z*: 373.8 ($[M + H]^+$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{17}H_{16}N_4O_6Na$ 395.0988; Found 395.0962.

N,N′-Bis(4-nitrobenzoyl)-1,3-diaminopropane-2-ol (**Kb**)

White amorphous solid; MP: 200-201 °C; IR: ν_{max} 3289, 1639, 1598, 1548, 1517, 1348, 1111, 867 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.31 (2H, *m*, H-2b and H-4b), 3.62 (2H, *m*, H-2a and H-4a), 3.90 (1H, *m*, H-3), 8.01 (4H, *d*, *J* = 8.4 Hz, H-2′ and H-6′), 8.25 (4H, *d*, *J* = 8.4 Hz, H-3′ and H-5′); MS (ESI^-) *m/z*: 387.3 ($[M - H]^-$, 100%); HR-MS (ESI^+) *m/z*: $[M + Na]^+$ calcd. for $C_{17}H_{16}N_4O_7Na$ 411.0915; Found 411.0911.

Cell cultures

Cervical epithelial adenocarcinoma (HeLa), human colon adenocarcinoma (HT29) and human embryonic kidney (HEK293) cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), streptomycin (100 µg/mL) and L-glutamine (4 mM) at 37 °C, 5% CO_2. Human breast adenocarcinoma (MCF-7) was grown using the same medium, but supplemented with 1% of insulin.

Cytotoxic activity testing

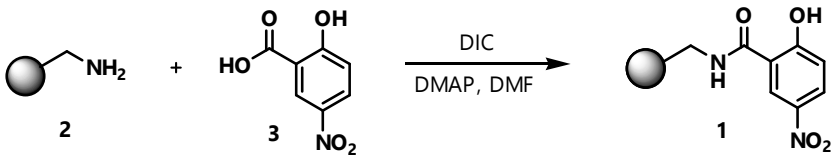
For cytotoxicity testing, cells were seeded onto 96-well culture plates (1×10^5 cell/well) for overnight. Monolayers of cancer and normal cells were exposed to various concentrations of diarylheptanoid amide derivatives (**A**-**K**)(**a**-**b**) in DMSO for 48 h. To determine cell viability, MTT activity assay was performed. Cells were incubated with 0.5% MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide) solution and further incubated at 37 °C under a humidified 5% CO_2 incubator for 4 h. The culture medium was removed, substituted with 100 µL DMSO, and then the absorbance was measured at 550 nm using Tecan U.S., (Durham, NC, USA). Half maximal inhibitory concentration (IC_{50}) values were calculated by regression analysis.

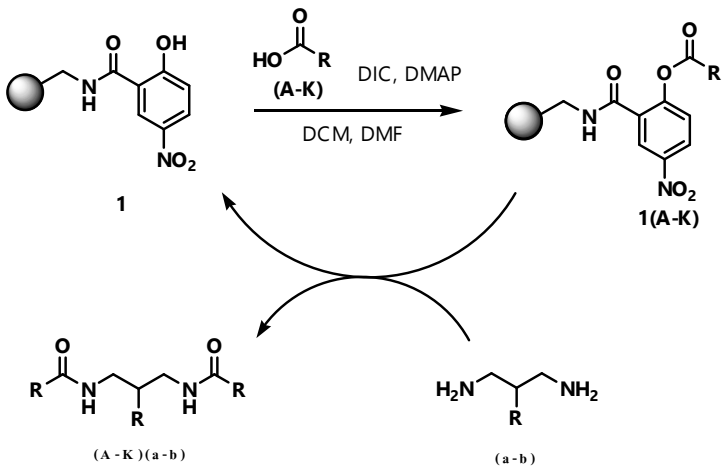
RESULT AND DISCUSSION

Our approach to generate a diarylheptanoid amide derivatives via solid phase synthesis. Reusable linker (**1**) was synthesized by shaking aminomethyl polystyrene resin (**2**) with 5-nitrosalicylic acid (**3**) in the presence of *N*,*N*'-diisopropylcarbodiimide (DIC) as the coupling agent and 4-dimethylaminopyridine (DMAP) as a catalyst at room temperature to generate reusable resin (Scheme 3). The suspension was shaken overnight. Reaction of the reusable linker with various aromatic carboxylic acids (**A**-**K**) (Figure 2) using DIC and DMAP yielded the active ester resin **1**(**A**-**K**) (Scheme 4). The active ester resin was subsequently reacted with diaminopropane (**a**-**b**) (Figure 2) and shaked further for 6 h. After complete of reaction suspension was filtered and collected the liquid phase. Resin was washed with CH_2Cl_2 and the solution was combined with the previous one. The solvent was removed under stream of nitrogen and dried under vacuum. The crude product was purified by column chromatography using CH_2Cl_2 and CH_2Cl_2-MeOH. The diarylheptanoid amide derivatives (**A**-**K**)(**a**-**b**) were obtained in moderate to good yields (60–80%) compared to original resin (loading 1.11 mmol/g). The structures of the synthesized compounds were established on the basis of IR, ^{1}H-NMR, and high resolution mass spectral analysis.

The diarylheptanoid amide derivatives (**A**-**K**)(**a**-**b**) were subjected to cytotoxicity evaluation against cancer cell lines (HeLa, MCF-7, and HT29) and normal cell line (HEK293) (Table 1). The standard agents including doxorubicin and curcumin were also tested with identical conditions for comparison purposes. The IC_{50} values of doxorubicin and curcumin were 3.97 µg/ml and 31.38 µg/ml, respectively, against HT29 cells and 0.11 µg/ml and 8.59 µg/ml, respectively, against HEK293 cells. Most of the synthesized compounds showed low cytotoxicity against tested cell lines. Compound **Db** exhibited weak cytotoxicity with IC_{50} values of 43.36 µg/ml against HT29 cell. The 4-chlorobenzoyl analogues (**Ib**) exhibited moderate cytotoxic activity with IC_{50} values of 38.61 µg/ml and 40.15 µg/ml against HT29 and MCF-7 cell lines, respectively. The results indicated that the hydroxyl group of diaminopropane (**b**) has effect on cytotoxicity. Thus, compounds **Ib** and **Db** were more active than **Ia** and **Da**. The 4-chlorobenzoyl analogues (**Ib**) were also tested against the normal cell lines and the human embryonic kidney (HEK293), the results suggested that compound **Ib** was not toxic to the normal cell with IC_{50} value of 747.20 µg/ml. The selectivity index is a measure of selectivity of the cytotoxicity of agent for normal cells and cancer cells (Lin et al., 2016). It is calculated as a ratio of IC_{50} of normal cell to the IC_{50} the cancer cell (Chaichompoo et al., 2017). The selectivity index value of compound **Ib** was 19.35. In contrast, doxorubicin and curcumin exhibited selectivity index values of 0.02 and 0.27, respectively. The selectivity index of compound **Ib** was almost 950-fold as compared to standard drug, doxorubicin.



Scheme 3 Synthesis of reusable linker



Scheme 4 Synthesis of diarylheptanoid amide derivatives

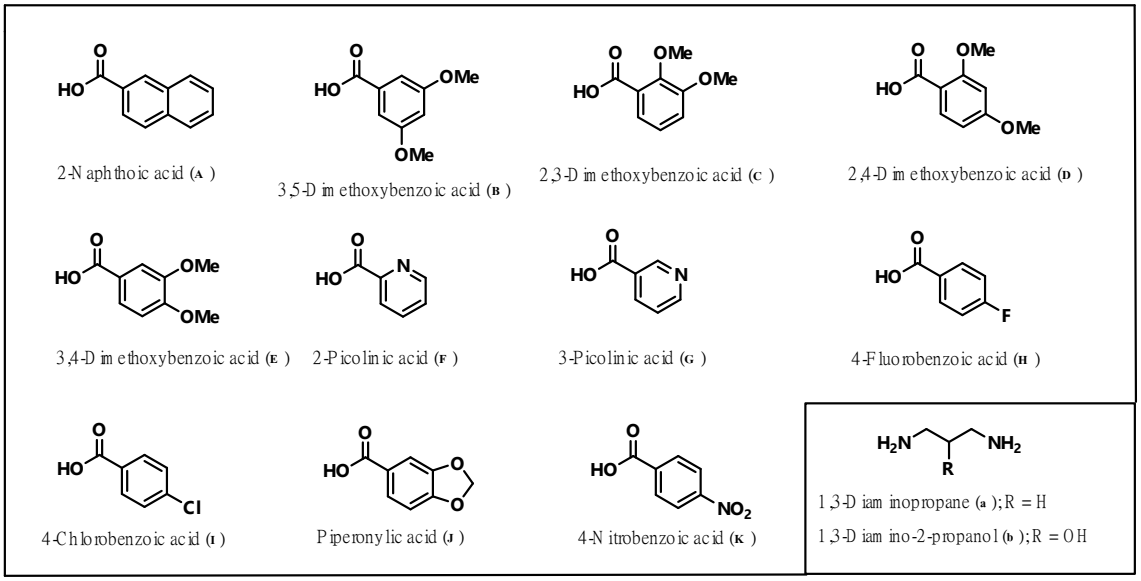


Figure 2 Various aromatic carboxylic acids and diaminopropane used in the synthesis of diarylheptanoid amide derivatives

Table 1 Cytotoxicity activity of diarylheptanoid amide derivatives (**A-K**)(**a-b**) against cell line

Compounds	HT29 cell µg/ml	MCF7 cell µg/ml	HeLa cell µg/ml	HEK293 cell µg/ml
Doxorubicin	3.97	0.68	0.48	0.11
Curcumin	31.38	33.47	31.70	8.59
Aa	IN	IN	IN	IN
Ba	IN	IN	IN	IN
Ca	IN	IN	IN	IN
Da	IN	IN	IN	IN
Ea	IN	IN	IN	IN
Fa	IN	IN	IN	IN
Ga	IN	IN	IN	IN
Ha	IN	IN	IN	IN
Ia	IN	IN	IN	IN
Ja	IN	IN	IN	IN
Ka	IN	IN	IN	IN
Ab	IN	IN	IN	IN
Bb	IN	IN	IN	IN
Cb	IN	IN	IN	IN
Db	43.36	IN	IN	IN
Eb	IN	IN	IN	IN
Fb	IN	IN	IN	IN
Gb	IN	IN	IN	IN
Hb	IN	IN	IN	IN
Ib	38.61	40.15	IN	747.20
Jb	IN	IN	IN	IN
Kb	IN	IN	IN	IN

IN: Inactive at 50 µg/ml

CONCLUSIONS

We have synthesized a library of diarylheptanoid amide derivatives using solid phase synthesis. Compounds **Db** and **Ib** exhibited cytotoxicity activity against cancer cell lines. It should be noted that these compounds exhibited no toxicity to the normal cell with the IC_{50} value of 747.20 µg/ml. Among the synthesized compounds, **Ib** was the most promising drug candidate in terms of cancer selectivity demonstrated by relatively high SI value on HT29 cancer cells lines.

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