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Anti-malarial Activity of New Emodin Derivatives against *Plasmodium falciparum* Chloroquine Resistant Strain

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Abstract

Emodin (1) is the major bioactive compound of several herb species, which belongs to anthraquinone class of compound. As a part of our drug discovery program, large quantities of emodin (1) was isolated from the roots of Rheum emodi and a library of novel emodin derivatives 2–24 were prepared to evaluate their in-vitro antimalarial activity, among them, compound 17, 18 and 20 showed potent antimalarial activity against chloroquine resistant strain PfK1with the IC50 of 2.28, 2.49 and 2.48 μM respectively with a high safety index.

Keywords: Emodin derivatives; Antimalarial agents; Anthraquinones; Cytotoxicity; *Rheum emodi*

Introduction

Anthraquinones are widely distributed secondary metabolites of plants (aloe, cascara sagrada, senna and rhubarb), microbes, lichens and insects, which possess various biological activities [1-3]. Emodin (**1**) an anthraquinone class of compound is the major bioactive compounds of numerous herb species such as Rheum, Polygonum (polygonaceae), Rhamnus (Rhamnaceae) and Cassieae (senna) [4-6] and possesses immunosuppressive, anticancer [7], anti-inflammatory, anti-atherosclerotic, and vasorelaxant effects [8-11].

As a part of our drug discovery program on antimalarial agents from Indian medicinal plants, we isolated large quantities of anthraquinone i.e. emodin (**1**) from the roots of *Rheum emodi* and planned to carry out chemical transformation to improve its therapeutic application. Chemical transformation of bioactive compounds of medicinal herbs is one of the most common approaches in drug discovery to improve the therapeutic properties. Towards this goal, we have synthesized a library of novel emodin derivatives **2-24** and their antimalarial activity was evaluated.

Materials and Methods

General chemistry

IR spectra were recorded on perkin-Elmer RX-1 spectrometer. Using either KBr pellets (or) in neat. ^{1}H-NMR,^{13}C-NMR, DEPT-90 and DEPT-135 spectra were run on Bruker Advance DPX 300 MHz and 200 MHz in $CDCl_3$.Chemical shifts are reported as values in ppm relative to $CHCl_3$ (7.26) in $CDCl_3$ and TMS was used as internal standard.ESI mass spectra were recorded on JEOL SX 102/DA-6000. Chromatography was executed with silica gel (60-120 mesh) using mixtures of chloroform, ethyl acetate and hexane as eluants.

Background of plant

Rheum emodi wall (Family: Polygonaceae, commonly known as revand-chini and English name rhubarb) is a stout herb, distributed in the alpine and sub-alpine zones of the Himalayas. The roots of this species are used widely in ayurvedic medicine. Roots of the Indian rhubarb is darker, inferior in aroma, and is a well-known stomachic, bitter, cathartic and used all over the world.

Collection of medicinal plant

Rheum emodi wall (Bark) was purchased from the local market of Lucknow, U.P, India and the authentification was done by Botany Division of Central Drug Research Institute, Lucknow.

Extraction

Powdered *Rheum emodi* wall (Bark) (3 kg) were placed in glass percolator with 95% ethanol (10 lit) and allowed to stand for 24hr at room temperature. The percolate was collected and these processes were repeated for four times. The combined percolate was evaporated under reduced pressure at 50°C to afford ethanol extract. The weight of extract was found to be 200 g.

Isolation and purification of Emodin

The alcoholic extract was (200 g) chromatographed on a column of silica gel (60-120 mesh), eluted with hexane and chloroform (70:30); recrystallization from methanol afford emodin (3g). The compound visualization was obtained under UV light, also shown orange spot by spraying with 10% sulphuric acid in methanol.

1, 3, 8-trihydroxy-6-methylanthracene-9-10-dione (1)

IR (KBr) 3613, 2925, 1625, 1461, 1373, 1277, 1029, 767, 672 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.03 (s, OH), 11.95 (s, OH), 7.41 (s, 1H), 7.10 (s, 1H), 7.06 (s, 1H), 6.55 (s, 1H), 2.38 (s, 3H); ^{13}C NMR(DMSO-d_6, 75 MHz) δ 189.40, 180.92, 165.51, 164.35, 161.30, 148.04, 134.77, 132.47, 123.91, 120.25, 113.04, 108.09, 107.75, 21.45; MS (ESI) m/z 270.

Preparation of emodin derivatives

General method for O-alkylation (Method A): A stirred solution of compound **1** (100 mg, 0.00037 moles) in DMF (5 mL) at room temperature was treated with respective alkyl halide (RX) (0.00033 moles) and K_2CO_3 (0.00092 moles). The reaction mixture was stirred at 60-70°C for 4 hr. It was then extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhydrous Na_2SO_4

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and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound.

Ethyl 2 - (4, 5-dihydroxy-7-methyl-9, 10-dioxo-9, 10 dihydroanthracen-2-yloxy)-2-methylpropanoate (2): IR (KBr) 3688, 3400, 2374, 1722, 1625, 1468, 1382, 1283, 1217, 1133, 766, 671 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz) δ 12.22 (s, OH), 12.10 (s, OH), 8.02 (s, 1H), 7.62 (s, 1H), 7.09 (s, 1H), 6.56 (s, 1H), 4.31-4.24 (q, 2H), 2.45 (s, 3H), 1.71 (s, 6H), 1.42 (t, 3H); ESI - MS: Cacld for $C_{21}H_{20}O_7$ $[M+H]^+$: 384, Found 384; Yield: 65% .

3-(2-(diethyl amino) ethoxy)-1, 8-dihydroxy-6-methylanthracene-9, 10-dione (3): IR (KBr) 3869, 3413, 1638,1333, 1216, 1028, 925, 764, 672cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz,) δ 12.27 (s, OH), 12.09 (s, OH), 7.61 (s, 1H), 7.35 (s, 1H), 7.07 (s, 1H), 6.68 (s, 1H), 4.25 (t, 2H), 2.86 (t, 2H), 2.62 (s, 4H), 2.45 (s, 3H), 1.40 (t, 6H); ESI - MS: Cacld for $C_{21}H_{23}NO_5$ $[M+H]^+$: 370, Found 370; Yield: 70%.

1,8-dihydroxy-3-methyl-6-(2-(pyrrolidin-1-yl)ethoxy) anthracene-9, 10-dione (4): IR (KBr) 3438, 2341, 1720, 1630, 1463, 1385, 1285, 1218, 1130, 1074, 765, 668 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz,) δ 7.59 (s, 1H), 7.32 (s, 1H), 7.05 (s, 1H), 6.66 (s, 1H), 4.29 (t, 2H), 2.95 (t, 2H), 2.66 (t, 4H), 2.43 (s, 3H), 2.04 (t, 4H); ESI - MS: Cacld for $C_{21}H_{21}NO_5$ $[M+H]^+$: 368, Found 368; Yield: 68%.

1, 8-dihydroxy-3-methyl-6-(2-(piperidin-1-yl) ethoxy) anthracene-9, 10-dione (5): IR (KBr) 3285, 2923, 1725, 1623, 1461, 1376, 1218, 909, 764, 669 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz,) δ 7.61 (s, 1H), 7.35 (s, 1H), 7.08 (s, 1H), 6.68 (s, 1H), 4.31 (t, 2H), 2.96 (t, 2H), 2.68 (t, 4H), 2.45 (s, 3H), 1.43 (t, 6H); ESI - MS: Cacld for $C_{22}H_{23}NO_5$ $[M+H]^+$: 383, Found 383; Yield: 68%.

1, 8-dihydroxy-3-methyl-6-(2-morpholinoethoxy) anthracene-9, 10-dione (6): IR (KBr) 3677, 3646, 3018, 1626, 1462, 1217, 920, 767, 670 cm^{-1}; ^1H NMR ($CDCl_3$, 300MHz,) δ 7.60 (s, 1H), 7.34 (s, 1H), 7.06 (s, 1H), 6.67 (s, 1H), 4.24 (t, 2H), 3.74 (t, 4H), 2.85 (t, 3H), 2.62 (t, 4H), 2.44 (s, 3H); ESI - MS: Cacld for $C_{21}H_{21}NO6$ $[M+H]^+$: 384, Found 384; Yield: 70%.

1, 8-dihydroxy-3-methyl-6-(nonyloxy) anthracene-9, 10-dione (7): IR (KBr) 3365, 2359, 1628, 1461, 1217, 1020, 769, 671 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 12.27 (s, OH), 12.10 (s, OH), 7.59 (s, 1H), 7.32 (s, 1H), 7.05 (s, 1H), 6.64 (s, 1H), 4.07 (t, 2H), 2.43 (s, 3H), 1.82 - 1.78 (m, 2H), 1.58 (s, 2H), 1.45 (s, 2H), 1.25 (s, 6H), 0.85 (t, 3H); MS (ESI) m/z 382; Yield:66%.

1, 8-dihydroxy-3-methyl-6-(undecyoxy) anthracene-9, 10-dione (8): IR (KBr) 3761, 3414, 2952, 1624, 1318, 1216, 766, 671 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz,) δ 12.49 (s, OH), 12.32 (s, OH), 7.81 (s, 1H), 7.54 (s, 1H), 7.46 (s, 1H), 6.84 (s, 1H), 4.27 (t, 2H), 2.64 (s, 3H), 2.02 - 1.99 (m, 2H), 1.76 (s, 2H), 1.66 (s, 2H), 1.45 (s, 16H), 1.07 (t, 3H)); ^{13}C NMR($CDCl_3$, 75 MHz) δ 191.06, 183.04, 167.18, 166.19, 163.45, 149.31, 140.27, 136.16, 134.23, 125.44, 122.21, 115.06, 114.70, 111.00, 109.72, 108.10, 70.04, 34.02, 32.93, 32.63, 30.70, 30.50, 30.53, 30.36, 30.29, 30.16, 29.96, 29.89, 26.89, 23.69, 23.13, 15.11; MS (ESI) m/z 438; Yield: 65%.

3-(dodecyloxy)-1, 8-dihydroxy-6-methyl anthracene-9, 10-dione (9): IR (KBr) 3429, 1627, 1473, 1386, 1303, 1264, 1218, 1032, 769, 670 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz,) δ 12.30 (s, OH), 12.13 (s, OH), 7.62 (s, 1H), 7.35 (s, 1H), 7.07 (s, 1H), 6.85 (s, 1H), 4.08 (t, 2H), 2.44 (s, 3H), 2.05 (s, 2H), 1.85 - 1.80(m, 2H), 1.57 (s, 2H), 1.45 - 1.41 (m, 4H), 1.29 (s, 2OH), 0.86 (t, 3H); ^{13}C NMR($CDCl_3$, 75 MHz) δ 190.65, 182.01, 166.16, 165.17, 162.44, 148.31, 136.12, 133.19, 124.43, 121.21, 106.71, 107.08, 69.03, 31.92, 31.62, 29.66, 29.36, 28.88, 25.88, 22.69, 22.13, 14.12; MS (ESI) m/z 452; Yield: 65%.

1,8-dihydroxy-3-methyl-6-(pentadecyloxy)anthracene-9, 10-dione (10): IR (KBr) 3490, 2924, 1627, 1452, 1217, 769, 670 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz,) δ 7.61 (s, 1H), 7.34 (s, 1H), 7.06 (s, 1H), 6.65 (s, 1H), 4.07 (t, 2H), 2.44 (s, 3H), 2.02 (t, 2H), 1.84 - 1.79 (m, 2H), 1.56 (s, 2H), 1.25 (s, 24H), 0.87 (t, 3H); ^{13}C NMR($CDCl_3$, 75 MHz) δ 190.69, 182.05, 166.19, 166.20, 162.47, 148.32, 139.27, 135.17, 133.24, 124.45, 121.22, 114.06, 113.70, 110.03, 108.72, 107.10, 69.04, 33.83, 31.93, 31.63, 29.70, 29.54, 29.37, 29.30, 29.16, 28.96, 28.89, 25.89, 22.70, 22.13, 14.12; MS (ESI) m/z 494; Yield: 62%.

1,8-dihydroxy-3-methyl-6-(octadecyloxy)anthracene-9, 10-dione (11): IR (KBr) 3407, 2952, 1631, 1363, 1217, 1012, 769, 670 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz,) δ 12.29 (s, OH), 12.12 (s, OH), 7.61 (s, 1H), 7.34 (s, 1H), 7.07 (s, 1H), 6.66 (s, 1H), 4.09 (t, 2H), 2.45 (s, 3H), 1.86 - 1.82 (m, 2H), 1.60 (s, 2H), 1.47 - 1.43 (m, 2H), 1.27 (s, 28H), 0.87 (t, 3H) ; MS (ESI) m/z 522; Yield: 65%.

1, 8-dihydroxy-3-methyl-6-(icosyloxy) anthracene-9, 10-dione (12): IR (KBr) 3289, 2368, 1658, 1584, 1458, 1218, 769, 670 cm^{-1};^1H NMR ($CDCl_3$, 300 MHz,) δ 12.31 (s, OH), 12.15 (s, OH), 7.64 (s, 1H), 7.37 (s, 1H), 7.09 (s, 1H), 6.68 (s, 1H), 4.10 (t, 2H), 2.46 (s, 3H), 2.05 (t, 2H), 1.87 - 1.82 (m, 2H), 1.59 (s, 2H), 1.34 (s, 31H), 0.88 (t, 3H)); ^{13}C NMR($CDCl_3$, 75 MHz) δ 190.11, 181.47, 165.61, 164.62, 161.89, 147.74, 138.69, 134.59, 132.66, 123.87, 120.54, 113.48, 113.12, 109.45, 108.14, 106.52, 68.45, 32.25, 31.35, 31.05, 29.12, 29.0, 28.79, 28.72, 28.59, 28.38, 28.31, 25.31, 22.12, 21.55, 13.54; MS (ESI) m/z 550; Yield: 63%.

3-(docosyloxy)-1, 8-dihydroxy-6-methyl anthracene-9, 10-dione (13): IR(KBr) 3388, 2362, 1624, 1365, 1217, 767, 672 cm^{-1};^1H NMR ($CDCl_3$, 300 MHz,) δ 12.29 (s, OH), 12.12 (s, OH), 7.61 (s, 1H), 7.34 (s, 1H), 7.06 (s, 1H), 6.65 (s, 1H), 4.08 (t, 2H), 2.44 (s, 3H), 2.05 (t, 2H), 1.82 (s, 2H), 1.25 (s, 34H), 0.88 (t, 3H); MS (ESI) m/z 578; Yield: 64%.

(E)-3-(3, 7-dimethylocta-2, 6-dienyloxy) 1, 8-dihydroxy-6-methyl anthracene-9, 10-Dione (14): IR (KBr) 3349, 2367, 1718, 1624, 1364, 1218, 770, 650 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz,) δ ^1H 12.32 (s, OH), 12.16 (s, OH), 7.64 (s, 1H), 7.28 (s, 1H), 7.10 (s, 1H), 6.70 (s, 1H), 5.51 (t, 1H), 5.11 (s, 1H), 4.71 (t, 2H), 2.47 (s, 3H), 2.14 (s, 4H), 1.80 (s, 6H), 1.73 (s, 3H); MS (ESI) m/z 406; Yield: 62%.

1, 8-dihydroxy-3-methyl-6-(prop-2-ynyloxy) anthracene-9, 10-dione (15): IR (KBr) 3282, 2922, 2125, 2357, 1717, 1631, 1390, 1218, 1078, 766, 680 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 12.12 (s, OH), 12.01 (s, OH), 7.80 (s, 1H), 7.66 (s, 1H), 7.64 (s, 1H), 7.096 (s, 1H), 4.03 (s, 2H), 2.85 (s, 1H), 2.46 (s, 3H); MS (ESI) m/z 308; Yield: 60%.

General method for C-alkylation (Method B)

A stirred solution of amine (0.0022 moles) cooled at 0°C was slowly treated with formalin (0.00148 moles), glacial acetic acid (0.0192 moles) and compound **1** (100 mg, 0.00037 moles). The whole reaction mixture was brought to room temperature and stirred for 4 hr, after pH was adjusted to 8 by addition of 20% aq. NaOH. It was then extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound.

1,3,8-trihydroxy-6-methyl-2-(pyrrolidin-1-ylmethyl) anthracene-9, 10-dione (16): IR (KBr) 3556, 3023, 2925, 2357, 1625, 1379, 1278, 1104, 767, 671 cm^{-1};^1H NMR (DMSO-d^6,300MHz) δ 7.40

(s, 1H), 6.85 (s, 1H), 6.78 (s, 1H), 4.14 (s, 2H), 3.21 (t, 4H), 2.36 (s, 3H), 1.96 (t, 4H); ESI - MS: Cacld for $C_{20}H_{19}NO_5$ $[M+H]^+$: 354, Found 354; Yield: 80%.

1,3,8-trihydroxy-6-methyl-2-(piperidin-1-ylmethyl) anthracene-9, 10-dione (17): IR(KBr) 3401, 2927, 2372, 1628, 1535, 1459, 1369, 1222, 1112, 768 cm^{-1};^{1}H NMR (DMSO-d^6,300MHz) δ 7.39 (s, 1H), 6.90 (s, 2H), 3.90 (s, 2H), 2.74 (t, 4H), 2.33 (s, 3H), 1.68 (t, 4H), 1.52 (t, 2H); ESI - MS: Cacld for $C_{21}H_{21}NO_5$ $[M+H]^+$: 369, Found 369; Yield: 78%.

1,3,8-trihydroxy-6-methyl-2-(morpholinomethyl) anthracene-9, 10-dione (18): IR (KBr) 3565, 3021, 2929, 2357, 1720, 1618, 1461, 1377, 1283, 1217, 1122, 765, 671 cm^{-1}; ^{1}H NMR ($CDCl_3$ & DMSO-d^6, 300MHz) δ 7.43 (s, 1H), 6.86 (s, 1H), 6.70 (s, 1H), 3.65 (t, 2H), 3.49 (t, 4H), 2.54 (t, 2H), 2.27 (t, 2H), 2.17 (s, 3H); ESI - MS: Cacld for $C_{21}H_{23}NO_5$ $[M+H]^+$: 370, Found 370; ESI - MS: Cacld for $C_{20}H_{19}NO_6$ $[M+H]^+$: 370, Found 370; Yield: 80%.

1,3,8-trihydroxy-6-methyl-2-(piperazin-1-ylmethyl) anthracene-9, 10-dione (19): IR (KBr) 3463, 3020, 2929, 2356, 1720, 1649, 1458, 1380, 1284, 1217, 1123, 765, 670 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz) δ 12.78 (s, OH), 12.05 (s, OH), 7.66 (s, 1H), 7.02 (s, 1H), 6.86 (s, 1H), 3.97 (s, 2H), 3.03 (t, 4H), 2.52 (t, 2H), 2.48 (t, 2H), 2.32 (s, 3H), 2.10(s, 1H); ESI - MS: Cacld for $C_{20}H_{20}N_2O_5$ $[M+H]^+$: 369, Found 369; Yield: 75%.

1, 3, 8-trihydroxy-6-methyl-2-((4-methylpiperazin-1-yl) methyl) anthracene-9, 10-dione (20): IR (KBr) 3484, 3021, 2403, 1678, 1526, 1427, 1216, 929,762, 672 cm^{-1}; ^{1}H NMR ($CDCl_3$ & DMSO-d^6, 300MHz) δ 7.84 (s, 1H), 7.69 (s, 1H), 7.20 (s, 1H), 4.11 (s, 2H), 2.92 (t, 4H), 2.76 (t, 4H), 2.61 (s, 3H), 2.49 (s, 3H)); ^{13}C NMR(CDCl3 & DMSO, 75 MHz) 190.62, 189.40, 168.45, 163.03, 162.67, 148.47, 134.58, 133.71, 124.73, 121.31, 113.25, 110.66, 108.59, 54.88, 53.96, 52.92, 46.22, 22.58; ESI - MS: Cacld for $C_{21}H_{22}N_2O_5$ $[M+H]^+$: 383, Found 383; Yield: 78%.

2-((4-(4-fluorophenyl) piperazin-1-yl) methyl)-1, 3, 8-trihydroxy-6-methylanthracene-9, 10-dione (21): IR (KBr) 3557, 2931, 2837, 2355, 1650, 1510, 1455, 1367, 1222, 1018, 926, 766, 669 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300MHz,) δ 12.66 (s, OH),12.05 (s, OH), 7.52 (s, 1H), 7.15 (s, 1H), 6.98 (s, 1H), 6.89 (m,4H), 3.93 (s, 2H), 3.13 (t, 4H), 2.77 (t, 4H), 2.36 (s, 3H); ESI - MS: Cacld for $C_{26}H_{23}FN_2O_5$ $[M+H]^+$: 463, Found 463; Yield: 80%.

General method for esterification and acid formation (Method C): A stirred solution of compound **1** (100mg, 0.00037 moles) in pyridine (2 mL) and acetic anhydride (0.00185 moles) at 60-70°C for 4 hr. The reaction mixture was put into cold water for crystallization, then filtered and dried. To the resultant crude acetate was gradually added 10 ml of acetic anhydride and glacial acetic acid mixture (1:1) and CrO_3 at 45°C and stirred for 10 hr at 70°C. Acetic anhydride and glacial acetic acid mixture was removed by vacuum. It was then extracted with ethyl acetate (3 × 25 mL), the organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound.

(E)-4,5-dihydroxy-7-methyl-9,10-dioxo-9, 10-dihydroanthracen-2-yl 2-methylbut-2-enoate (22): IR (KBr) 3408, 3023, 2929, 2370, 1731, 1639, 1464, 1373, 1217, 1119, 926, 764, 672 cm^{-1};^{1}H NMR ($CDCl_3$, 300MHz) 12.57 (s, OH), 12.23 (s, OH), 8.02 (s, 1H), 8.01 (s, 1H), 7.63 (s, 1H), 7.33-7.19 (q, 1H), 7.11 (s, 1H), 2.46 (s, 3H), 2.05 (s, 3H), 1.98 (d, 3H); ESI - MS: Cacld for $C_{20}H_{16}O_6$ $[M+H]^+$: 352, Found 352; Yield: 65%.

6-methyl-9, 10-dioxo-9,10-dihydroanthracene-1,3,8-triyl triacetate (23): IR (KBr) 3407, 2927, 1761, 1668, 1605, 1457, 1324, 1206, 1029, 908, 766, 674 cm^{-1};^{1}H NMR ($CDCl_3$, 300MHz) 8.01 (s, 1H), 7.95 (s, 1H), 7.23 (s, 1H), 7.22 (s, 1H), 2.50 (s, 3H), 2.43 (s, 6H), 2.35 (s, 3H); ESI - MS: Cacld for $C_{21}H_{16}O_8$ $[M+H]^+$: 397, Found 397; Yield: 98%.

4,5,7-triacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (24): IR (KBr) 3416, 3024, 2927, 2367, 1770, 1648, 1460, 1372, 1215, 1029, 923, 761, 672 cm^{-1};^{1}H NMR (DMSO-d^6,300MHz) 8.12 (s, 1H), 7.95 (s, 1H), 7.93 (s, 1H), 7.63 (s, 1H), 2.39 (s, 9H); ^{13}C NMR (DMSO-d^6,75 MHz) 180.14, 179.37, 168.40, 166.29, 165.02, 154.69, 150.99, 149.81, 136.56, 135.26, 134.43, 132.51, 124.98, 123.96, 123.08, 121.93, 120.39, 20.88; ESI - MS: Cacld for $C_{21}H_{14}O_{10}$ $[M+H]^+$: 425, Found 425; Yield: 80%.

Biological Study

Materials

RPMI-1640 medium, sodium bicarbonate, glucose, hypoxanthine, resazurin, chloroquine diphosphate and MEM medium were purchased from Sigma (St. Louis, MO, USA). Albumax II was procured from Gibco BRL (Grand Island, NY, USA). Giemsa stain was purchased from Merck (USA). SYBR Green l nucleic acid gel stain was purchased from Invitrogen molecular probe (Carlsbad, USA).

In-vitro cultivation of *P. falciparum*

The chloroquine sensitive (*Pf*3D7) and chloroquine resistant (*Pf*K1) strains of *P. falciparum* were grown in continuous culture according to Trager and Jensen [12]. Parasites were cultured in RPMI-1640 (HEPES modified) medium (Sigma) supplemented with 0.5% AlbuMaxII, 0.2% glucose, 0.2% $NaHCO_3$ and 15 µM hypoxanthine. Parasite growth rate and stage was determined by the examination of Giemsa's stained thin smears of the RBCs.

Antimalarial activity of compounds

In-vitro assessment of antimalarial activity of compounds towards *P. falciparum* was performed by the determination of fifty percent inhibitory concentration (IC_{50}) according to the method of Johnson [13] with some modifications. Compounds were tested in range between 20 µM to 0.31 µM. To determine IC_{50} initially 20 µM concentration were used and two fold serially diluted till 0.31 µM and chloroquine were prepared in 96 well plates and then 50 µl asynchronous culture of infected erythrocytes with 1-1.5% parasitaemia and 2-3% haematocrit was added to each well (100 µl-final volume). Eight wells were treated as positive control (without drug) and 4 wells as negative controls (without parasite and drug). These plates were incubated in CO_2 incubator maintained at 37°C for 72 h. After 72 h, 100µl lytic buffer containing SYBR Green 1X final concentration was added to each well and incubated for 1-2h at room temperature in dark. Plates were read under fluorescence reader (Synergy HT BioTek) at Ex. 485nm, Em. 535nm. IC_{50} was determined on the basis of DNA content of the parasite by using MS-Excel template.

Cytotoxicity assay

Cytotoxic level of active compounds would be assessed according to protocol defined by O'Brien [14], with some modifications. The monkey kidney cell line will be maintained *in vitro* in MEM medium supplied with 15% Fetal Bovine Serum (FBS) and 5% CO_2 at 37°C. An appropriate serial drug dilution was prepared in culture plates and the cells were exposed to these concentrations of particular compounds for two days, 10% of cell viability marker resazurin was added and read under fluorescent reader at excitation wavelength 530 ± 25nm and emission at 590 ± 25 nm for

calculation of the median cytotoxic concentration (CC_{50}). The selective index (SI) would be calculated by using the formula-

$$\text{Selective index} = \frac{\text{Median cytotoxic concentration}\left(CC_{50}\right)}{\text{Median inhibitory concentration}\left(IC_{50}\right)}$$

Statistical analysis

Fifty percent inhibitory concentration (IC_{50}) of tested compounds were obtained by transferring the data into a graphic program (e.g. Excel) and expressed as percentage of the untreated controls and then evaluated by Logit regression analysis using pre-programmed Excel spreadsheet obtained from MMV group at Swiss Tropical Institute, Basel, Switzerland [15].

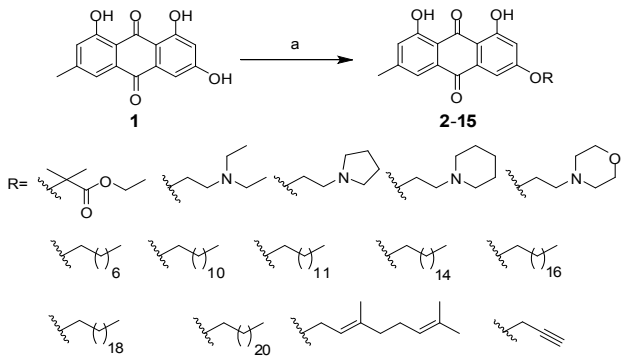
Results and Discussion

Chemistry

In order to prepare O-alkylated derivatives (**2-6**), (**7-15**) hydroxyl group at meta position of emodin (**1**) was alkylated with various amine side chains (Figure 1) and long chain alkyl halides (Figure 1) under basic conditions using K_2CO_3 and DMF as a solvent.

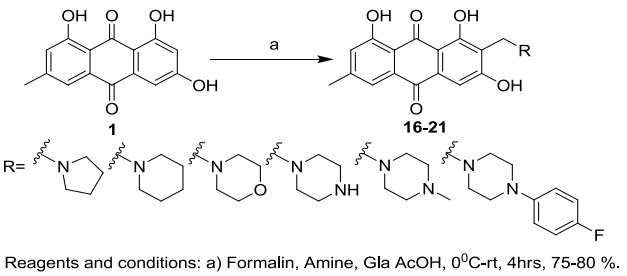
To study the substituent effect on anthraquinone nucleus C-alkylated derivates **16-21** were prepared using Mannich reaction protocol [16-20] (Figure 2).

"Since the emodin (**1**) contain free hydroxyl group we planned to" prepare few esters to study their antimalarial activity. To prepare ester derivative **22** and **23**, emodin (**1**) was reacted with tiglic acid in presence of triethylamine at 0°C and acetic anhydride in presence of pyridine respectively. To transform the methyl group in to acid functionality, emodin triacetate (**23**) was oxidized with chromium trioxide in presence of glacial acetic acid and acetic anhydride to provide acid (**24**).



Reagents and conditions: a) RX, K_2CO_3, DMF,60^{0}C, 4hrs.

Figure 1: Preparation of O- alkylated derivatives 2-15 of emodin (1).

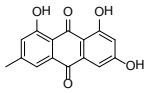
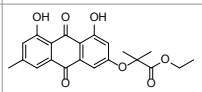
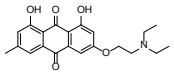
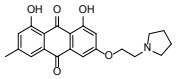
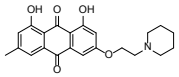
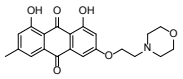
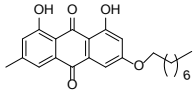
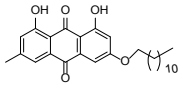
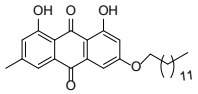
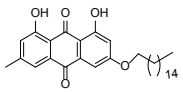
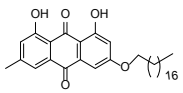
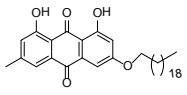
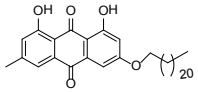
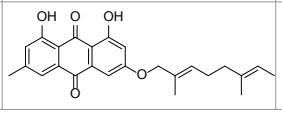


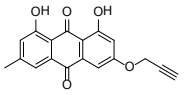
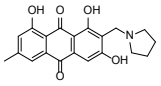
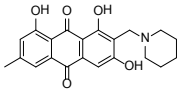
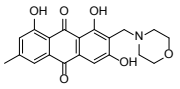
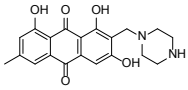
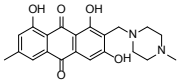
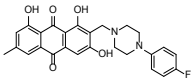
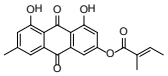
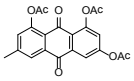
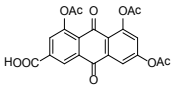
Reagents and conditions: a) Formalin, Amine, Gla AcOH, 0^{0}C-rt, 4hrs, 75-80 %.

Figure 2: Preparation of C-alkylated derivatives 16-21 of emodin (1).

Biological evaluation

***In vitro* antimalarial activity:** All the compounds were screened

Comp. No	Chemical Structure	IC_{50}		CC_{50}
		*Pf*3D7 (CQS)	*Pf*K1 (CQR)	VERO cell line
		µM	µM	µM
1		16.2	37	nd
2		16.2	>52	nd
3		22.4	48.8	nd
4		12.6	30.6	nd
5		5.7	12.1	69.8
6		10.6	18.41	nd
7		36.5	>52.35	nd
8		>45.6	>45.6	nd
9		>44.24	>44.24	nd
10		>40.4	>40.4	nd
11		>38.31	>38.31	nd
12		2.1	>36.36	909
13		>34.6	>34.6	nd
14		>49.26	>49.26	nd

15		11.6	>64.93	1623
16		10.1	6.26	nd
17		2.74	2.28	>1358
18		5.36	2.49	>1355
19		10.05	9.32	nd
20		3.58	2.48	782.7
21		18.74	8.29	nd
22		13.55	52.89	nd
23		25.93	19.09	nd
24		30.04	34.50	nd
Chloroquine (Standard antimalarial)		0.014	1.12	226.7

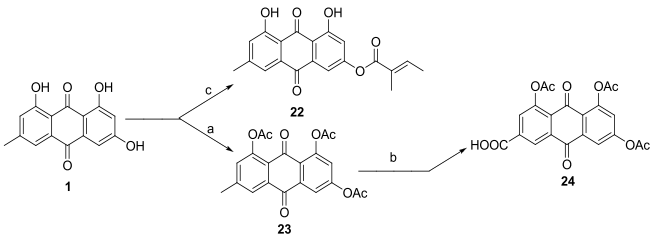
nd= not determined

Table 1: Chemical structures and *in vitro* antimalarial activity (IC_{50} in μM) of emodin (1) and its derivatives (2–24).

against chloroquine sensitive (*Pf*3D7) and chloroquine resistant (*Pf*K1) strain of *P. falciparum.* The parent compound, emodin (**1**) has an IC_{50} of 16.2 and 37 μM against *Pf*3D7 and *Pf*K1 strain respectively (Table 1). Except **2, 3, 7-11, 13**, **14 & 24** all other derivatives exhibited improved *in-vitro* antimalarial activity against *Pf*3D7 and *Pf*K1 strains with better therapeutic index (Table 1). Derivatives **4** (IC_{50}=12.6 μM), **6** (IC_{50}=10.6 μM), **15** (IC_{50}=11.6 μM), **16** (IC_{50}=10.1 μM), **19** (IC_{50}=10.05 μM), **22** (IC_{50}=13.55 μM) shows moderate activity against *Pf*3D7. Derivatives **5** (IC_{50}=12.1 μM), **23** (IC_{50}=19.09 μM) shows moderate activity against *Pf*K1. Derivatives **5** (IC_{50}=5.7 μM), **12** (IC_{50}=2.1 μM) have IC_{50} below 10 μM against *Pf*3D7. Derivatives **16** (IC_{50}=6.26 μM), **19** (IC_{50}=9.32 μM), **21** (IC_{50}=8.29 μM) have IC_{50} below 10 μM against *Pf*K1.Where as derivatives **17** (IC_{50}=2.74 μM, 2.28 μM), **18** (IC_{50}=5.36 μM, 2.49 μM), **20** (IC_{50}=3.58 μM, 2.48 μM) also have IC_{50} below 10 μM against *Pf*3D7 and *Pf*K1 strain. Our structure activity relationships indicated that C-alkylation of emodin (**1**) improves the activity in both strains, where as O-alkylation only improves the activity in *Pf*3D7 strain. It is noteworthy to mention here that Mannich base derivatives **17, 18** and **20** exhibited comparable *in-vitro* antimalarial activity with the marketed drug chloroquine (IC_{50}= 1.12) with good therapeutic index against CQ resistant strain (Table 2, Figure 3 and 4).

Compound No.	IC_{50} (*Pf*3D)(μM)	IC_{50} (*Pf*k1) (μM)	SI (*Pf*3D7) (μM)	SI (*Pf*k1) (μM)
17	2.74	2.28	495.6	595.6
18	5.36	2.49	>252	>544
20	3.58	2.48	218.6	315
Chloroquine	0.014	1.12	16192.8	201.7

Table 2: Antimalarial profile and safety index of active compounds against CQS (Pf3D7) and CQR (P*f*K1) strains of *P.falciparum.*



Reagents and conditions: a) Ac_2O, Pyridine, 60-70^{0}C, 4hrs, 98 % b) Gla AcOH: Ac_2O (1:1), CrO_3, 70^{0}C, 10hrs, 80 % c) RCOCl, Et_3N, DCM, 0^{0}C, 2hrs, 65%.

Figure 3: Preparation of ester derivatives 22, 23 and acid (24) from emodin (1).

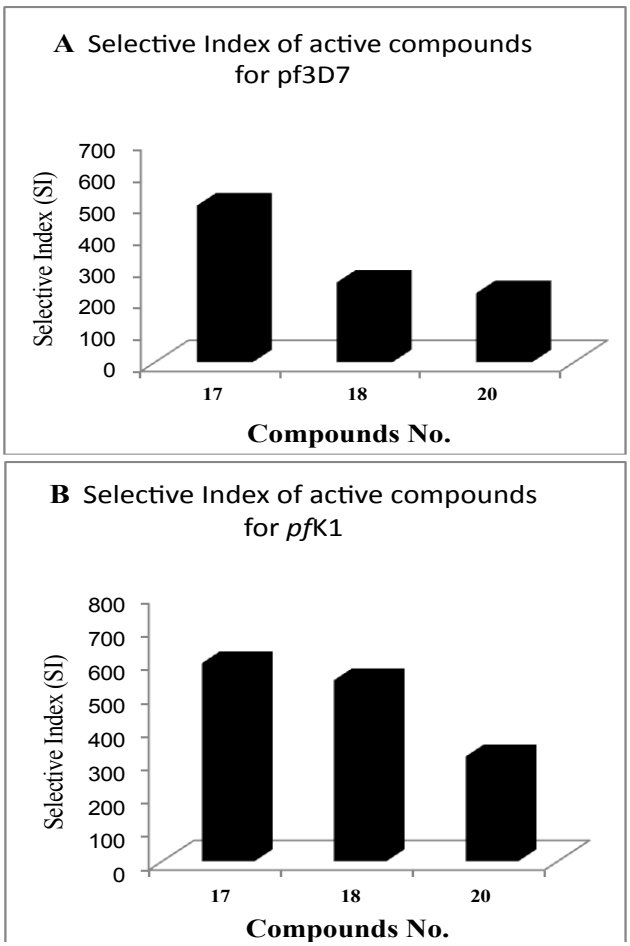


Figure 4: (A) Selective Index of active compounds for Pf3D7 (B). Selective Index of active compounds for PfK1.

Cytotoxicity assay and selective index (SI)

In order to characterize the *Pf*3D7 and *Pf*K1 strain basis of antimalarial effects of selected derivatives **5, 12, 15, 17, 18** and **20** we investigated the cytotoxic level of these active compounds. Derivatives **5** (CC_{50}=69.8 μM), **12** (CC_{50}=909 μM), **15** (CC_{50}=1623 μM), **17** (CC_{50}= >1358 μM), **18** (CC_{50}= >1355 μM), **20** (CC_{50}=782.7 μM) against VERO cell line. These compounds did not show any significant cytotoxicity against vero cell line (Table 4).

Conclusions

In conclusion, we have isolated larger quantities of emodin (**1**) from the roots of *Rheum emodi* and a library of novel emodin O-alkylated derivatives **2**-**6**, **7**-**15**, C-alkylated Mannich derivatives **16**-**21**, acyl derivatives **22**-**23**, and acid derivative **24** (Figure 3) from **1** were synthesized and evaluated their *in-vitro* antimalarial activity against chloroquine sensitive strain and chloroquine resistant strain. Among these 24 derivatives, C-alkyl Mannich bases **17**, **18** and **20** showed potent antimalarial activity against chloroquine resistant strain *Pf*K1with an IC_{50} of 2.28, 2.49, 2.48 μM respectively, which is comparable to marketed drug chloroquine (IC_{50} of 1.12). Further work is in progress in our laboratory to prepare more Mannich bases of emodin to develop a potent lead for inhibition of chloroquine resistant malaria.

Acknowledgment

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