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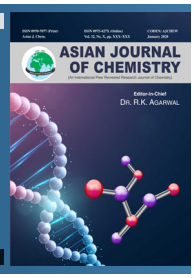

An Efficient and Green Synthesis of Fluorine Containing Benzo[*a*]xanthen-11(12*H*)-ones and Evaluation of their Anticancer Activity

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A facile and an efficient procedure has been developed by one-pot condensation of naphthalene-diols, fluoro substituted benzaldehydes and cyclic 1,3-dicarbonyl compounds for the synthesis of fluorine containing benzo[*a*]xanthen-11(12*H*)-ones under the catalyst and solvent-free conditions. All the synthesized compounds were characterized by IR, NMR (^{1}H & ^{13}C) and mass spectrometry. Several advantages offer the present approach, such as, shorter reaction time, low cost, anticipation of toxic solvents and catalyst, higher yield of products and durability of substrate range. On the other hand, anticancer activity was also performed for the title compounds which demonstrated a significant activity on selected cancer cell lines.

Keywords: Fluorine, Benzo[*a*]xanthen-11(12*H*)-ones, Naphthalene-diols, Anticancer activity.

INTRODUCTION

Nowadays across the world, the most serious health issue is cancer and its leading cause of death with various types of cancer in many high-developed countries [1]. One out of three persons become victims of cancer during their life period. In general, males are more prone to develop cancer than females and also the mortality rate was more in male community. But, increase in female mortality rate owing to cancer has been recorded, whereas in infants cancers are mostly seen during their first year of age. For the treatment of cancer, one of the most widely applied modalities is chemotherapy. However, drug resistance is one of the major obstacles in this standard chemotherapy for successful treatment of cancer patients. Herein, anticancer drug is one of the alterations that is developed during the treatment of patients [2-5]. However, treatment by anticancer drugs didn't adequately increase the success rate [6-8]. Therefore, more effective anticancer drugs are urgently required in order to increase the effectiveness of cancer treatment.

On the other hand, in view of the broad spectrum of pharmaceutical and biological properties of low-cost available xanthene and benzoxanthene compounds from plant sources, they have received significant attention since last two decades [9-13]. Many xanthene derivatives with excellent anticancer activity have been obtained [14-21]. Therefore, a numeral quantity of synthetic modifications have been reported with either by solid or polymer-based catalysts under solvent or solvent-free thermal/ultrasound/microwaves reaction conditions [22-30]. Some of the disadvantages, like, requirement of excess reagents/catalysts, prolonged reaction times, toxic organic solvent usages and lower yields even with harsh conditions influence these methodologies. Therefore, for the better synthesis of benzoxanthene derivatives, there is a requirement of an advanced and environmentally benign simple and efficient technique.

Further, on account of multi-component reactions (MCRs) exceptional practical and synthetic efficiency have attracted considerable attention in synthetic organic chemistry. Moreover they can form the new product in a single flask by involving three or more substrate molecules reacting together. In addition they offer greater potential for molecular assortment by minimum synthetic time per step. Synthesis of heterocyclic compounds is the primary goal of MCRs research, which is crucial in various fields, like, natural products, pharmaceutical drug molecules and agrochemicals [31]. In addition, in the formation

of green chemical carbon-heteroatom bond solvent-free conditions have significantly drawn the attention of chemists [32-35].

The literature survey at this stage revealed that there are no reports on the synthesis of fluorine containing benzo[*a*]-xanthen-11(12*H*)-ones under catalyst and solvent-free conditions at 60 °C. Therefore, herein, a cleaner and relatively faster green one-pot synthetic methodology is reported for the fluorine containing benzo[*a*]xanthen-11(12*H*)-ones *via* three-component coupling of naphthalenediols, fluoro/bromo substituted benzaldehydes and various cyclohexane-1,3-dione under catalyst and solvent-free conditions at 60 °C and tested their cytotoxic activity.

EXPERIMENTAL

Chemicals were obtained from Sd-fine chemicals, Merck, SRL, Aldrich and Alfaaesar chemical companies. All procured chemicals are of analytical grade and the reported melting points were determined in open capillaries using "Stuart Melting Point Apparatus" in °C and are uncorrected. Systronics UV-visible spectrometer was used to record the UV spectra and Perkin-Elmer Spectrum BX-I infrared spectrophotometer was used to record the IR spectra using KBr pellet. The NMR data are collected using the NMR-400 MHz, Jeol, Model: JNM-ECS400. To check the purity of the titled compounds used the glass plates coated by silica gel G and the sample spots were observed by iodine vapour.

General procedure for the preparation of fluorine containing benzo[*a*]xanthen-11(12*H*)-ones (4a-j): The mixture of naphthalenediol (**1a-b**, 1 mmol), fluoro substituted benzaldehyde (**2a-e**, 1 mmol) and 1,3-dicarbonyl compound (**3a-b**, 1 mmol) was stirred in a round bottom flask at 60 °C under catalyst and solvent-free conditions for 20 min. After completion of the reaction (TLC) resulted solid was dissolved in THF, filtered and evaporated the solvent using rotary evaporator. After recrystallization by ethanol we obtained the pure products as white solids (**4a-j**).

12-(3-Flurophenyl)-6-hydroxy-9,10-dihydro-8*H*-benzo-[*a*]xanthen-11(12*H*)-one (4a): Pale yellow solid; Yield: 91 %; m.p.: 230-231 °C; IR (KBr, ν_{max}, cm^{-1}): 3444.8 (OH, *str.*), 2963.2-2875.3 (CH_2, *str.*), 1653.5 (C=O), 1588.7, 1482.2 & 1449.8 (CH_2, bend.), 1371.4, 1237.3 (C-F, *str.*), 1190.5, 1125.7, 954.4, 820.4, 621.4; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 7.21 (q, *J* = 7.3 Hz, 3H), 6.99 (d, *J* = 7.8 Hz, 1H), 6.94-6.87 (m, 5H), 5.56 (s, 1H), 2.67-2.56 (m, 2H), 2.24 (t, *J* = 2.7 Hz, 2H), 1.93-1.79 (m, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.8, 166.1, 153.4, 153.4, 145.1, 139.4, 131.8, 131.2, 129.7, 127.2, 126.3, 125.2, 124.9, 123.4, 123.1, 119.5, 119.4, 115.8, 114.1, 109.2, 36.4, 28.3, 27.0, 20.0 ppm. Anal. calcd. (%) for $C_{23}H_{17}O_3F$: C, 76.65; H, 4.75; F, 5.27; found (%): C, 76.44; H, 4.69; F, 5.05.

12-(2,4-Difluorophenyl)-2-hydroxy-9,9-dimethyl-9,10-dihyro-8*H*-benzo[*a*]xanthen-11(12*H*)-one (4b): Pale yellow solid; Yield: 89 %; m.p.: 231-232 °C; IR (KBr, ν_{max}, cm^{-1}): 3435.5 (OH, *str.*), 2958.88-2871.0 (CH_2, *str.*), 1676.5 (C=O), 1621.1, 1482.3 & 1422.4 (CH_2, bend.), 1357.6, 1227.2 (C-F, *str.*), 1112.8, 963.7, 815.4, 616.7; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.94 (s, 1H), 7.80-7.67 (m, 2H), 7.24-6.84 (m, 5H), 6.54-6.40 (m, 1H), 5.48 (s, 1H), 2.74-2.52 (m, 2H), 2.33 (dd, *J* = 16.0, 3.2 Hz, 1H), 2.10 (d, *J* = 16.5 Hz, 1H), 1.07 (s, 3H), 0.90 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 195.7, 164.4, 156.5, 147.8, 132.9, 132.5, 130.3, 129.8, 129.0, 128.2, 125.3, 117.1, 116.8, 113.8, 113.5, 111.4, 105.6, 104.4, 103.6, 50.0, 40.3, 31.8, 28.8, 28.4, 26.1 ppm. LCMS (*m/z*): 407 (M+.). Anal. calcd. (%) for $C_{25}H_{20}O_3F_2$: C, 73.88; H, 4.96; F, 9.35; found (%): C, 73.45; H, 4.79; F, 9.07.

12-(2-Fluorophenyl)-6-hydroxy-9,10-dihydro-8*H*-benzo[*a*]xanthen-11(12*H*)-one (4c): Pale yellow solid; Yield: 89 %; m.p.: 212-213 °C; IR (KBr, ν_{max}, cm^{-1}): 3435.2 (OH, *str.*), 2958.8-2871.4 (CH_2, *str.*), 1677.2 (C=O), 1622.0, 1481.8& 1422.6 (CH_2, bend.), 1357.9, 1227.5 (C-F, *str.*), 1113.2, 963.9, 815.6, 616.2; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H), 8.11 (dd, *J* = 14.4, 6.2 Hz, 1H), 7.62 (t, *J* = 7.3 Hz, 1H), 7.32-7.12 (m, 3H), 6.99-6.82 (m, 2H), 6.70 (d, *J* = 7.3 Hz, 1H), 6.66-6.55 (m, 1H), 5.73 (s, 1H), 2.83-2.70 (m, 2H), 2.43-2.25 (m, 2H), 2.06-1.79 (m, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.8, 166.1, 153.4, 153.4, 145.1, 139.4, 131.8, 131.2, 129.7, 127.2, 126.3, 125.2, 124.9, 123.4, 123.1, 119.5, 119.4, 115.8, 114.1, 109.2, 36.4, 28.3, 27.0, 20.0 ppm. Anal. calcd. (%) for $C_{23}H_{17}O_3F$: C, 76.65; H, 4.75; F, 5.27; found (%): C, 76.49; H, 4.64; F, 5.04.

12-(4-Bromo-2-fluorophenyl)-6-hydroxy-9,10-dihydro-8*H*-benzo[*a*]xanthen-11(12*H*)-one (4d): Yellow solid; Yield: 88 %; m.p.: 201-202 °C; IR (KBr, ν_{max}, cm^{-1}): 3426.1 (OH, *str.*), 2958.8-2875.3 (CH_2, *str.*), 1598.0 (C=O), 1502.2, 1459.2 & 1422.4 (CH_2, bend.), 1384.9, 1222.9, 1218.6 (C-F, *str.*), 1125.7, 968.0, 852.8, 746.3 (C-Br, *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (s, 1H), 7.71-7.58 (m, 1H), 7.46-7.19 (m, 5H), 7.01-6.78 (m, 2H), 5.69 (s, 1H), 2.77 (d, *J* = 5.0 Hz, 2H), 2.40-2.17 (m, 2H), 2.05-1.80 (m, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.4, 166.1, 154.8, 145.1, 139.4, 131.8, 131.2, 126.4, 125.0, 123.5, 122.9, 122.0, 119.2, 118.1, 109.3, 36.5, 28.4, 27.0, 20.0 ppm. LCMS (*m/z*): 439 (M+.), 441 (M+2). Anal. calcd. (%) for $C_{23}H_{16}O_3BrF$: C, 62.89; H, 3.67; Br, 18.19; F, 4.32; found (%): C, 62.68; H, 3.52; Br, 18.03; F, 4.20.

12-(2,4-Difluorophenyl)-6-hydroxy-9,10-dihydro-8*H*-benzo[*a*]xanthen-11(12*H*)-one (4e): Pale yellow solid; Yield: 86 %; m.p.: 215-217 °C; IR (KBr, ν_{max}, cm^{-1}): 3440.5 (OH, *str.*), 2963.2-2861.6 (CH_2, *str.*), 1657.8 (C=O), 1505.2, 1454.7 & 1422.4 (CH_2, bend.), 1361.4, 1232.3, 1177.6 (C-F, *str.*), 1130.1, 963.7, 838.4, 616.7; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (s, 1H), 8.07 (d, *J* = 8.2 Hz, 1H), 7.63 (d, *J* = 7.3 Hz, 1H), 7.34-7.20 (m, 2H), 7.17 (s, 1H), 6.97 (t, *J* = 7.6 Hz, 1H), 6.50-6.41 (m, 2H), 5.69 (s, 1H), 2.82-2.69 (m, 2H), 2.43-2.25 (m, 2H), 2.06-1.80 (m, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.6, 166.1, 154.9, 145.1, 139.4, 131.2, 131.1, 126.3, 125.1, 124.9, 124.9, 123.5, 123.0, 119.2, 113.9, 109.2, 106.0, 105.8, 102.3, 36.5, 28.0, 27.0, 20.0 ppm. Anal. calcd. (%) for $C_{23}H_{16}O_3F_2$: C, 73.01; H, 4.26; F, 10.04; found (%): C, 72.88; H, 4.04; F, 09.89.

12-(2-Fluorophenyl)-6-hydroxy-9,9-dimethyl-9,10-dihydro-8*H*-benzo[*a*]xanthen-11(12*H*)-one (4f): Pale yellow solid; Yield: 90 %; m.p.: 209-211 °C; IR (KBr, ν_{max}, cm^{-1}): 3431.2 (OH, *str.*), 2954.5-2861.6 (CH_2, *str.*), 1610.8 (C=O), 1510.2, 1449.7 & 1421.6 (CH_2, bend.), 1380.6, 1228.0 (C-F, *str.*), 1176.8, 1121.4, 1065.2, 838.5, 538.2; ^{1}H NMR (400 MHz,

DMSO-d_6) δ 10.13 (s, 1H), 7.69-7.60 (m, 1H), 7.49-6.82 (m, 8H), 5.71 (d, *J* = 9.2 Hz, 1H), 2.78-2.57 (m, 2H), 2.35 (dd, *J* = 16.0, 4.1 Hz, 1H), 2.10 (d, *J* = 15.6 Hz, 1H), 1.07 (d, *J* = 7.8 Hz, 3H), 0.92 (d, *J* = 6.9 Hz, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.2, 164.2, 153.4, 145.2, 139.4, 131.0, 130.7, 129.8, 128.5, 127.2, 126.6, 125.1, 124.8, 123.4, 122.1, 119.4, 117.4, 115.8, 112.8, 109.2, 50.0, 40.2, 31.9, 28.8, 28.5, 26.2 ppm. Anal. calcd. (%) for $C_{25}H_{21}O_3F$: C, 77.30; H, 5.45; F, 4.89; found (%): C, 77.18; H, 5.29; F, 4.73.

12-(3-Fluorophenyl)-6-hydroxy-9,9-dimethyl-9,10-dihyrdo-8*H*-benzo[*a*]xanthen-11(12*H*)-one (4g): Pale yellow solid; Yield: 91 %; m.p.: 210-215 °C; IR (KBr, ν_{max}, cm^{-1}): 3421.8 (OH, *str.*),2963.2-2866.0 (CH_2, *str.*), 1593.0 (C=O), 1523.9, 1482.1 & 1459.0 (CH_2, bend.), 1380.6, 1223.6 (C-F, *str.*), 1186.2, 1112.0, 1074.6, 889.6, 746.3; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.35-7.19 (m, 4H), 7.16-7.02 (m, 2H), 6.90 (t, *J* = 8.5 Hz, 1H), 5.59 (s, 1H), 2.74-2.48 (m, 2H), 2.36 (d, *J* = 15.6 Hz, 1H), 2.15 (d, *J* = 16.5 Hz, 1H), 1.07 (s, 3H), 0.89 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 195.9, 161.5, 145.2, 140.1, 132.6, 131.9, 126.5, 125.2, 123.9, 123.0, 118.5, 115.2, 111.6, 109.9, 50.0, 40.5, 33.9, 31.9, 28.7, 26.1 ppm. Anal. calcd. (%) for $C_{25}H_{21}O_3F$: C, 77.30; H, 5.45; F, 4.89; found (%): C, 77.19; H, 5.32; F, 4.73.

12-(4-Fluorophenyl)-2-hydroxy-9,9-dimethyl-9,10-dihydro-8*H*-benzo[*a*]xanthen-11(12*H*)-one (4h): Pale yellow solid; Yield: 90 %; m.p.: 218-220 °C; IR (KBr, ν_{max}, cm^{-1}): 3435.5 (OH, *str.*), 2926.4-2852.3 (CH_2, *str.*), 1649.5 (C=O), 1588.8, 1482.2 & 1449.7 (CH_2, bend.), 1380.6, 1241.7, 1191.3 (C-F, *str.*), 1107.0, 940.2, 872.6; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 7.79-7.72 (m, 2H), 7.30-7.09 (m, 5H), 7.06-6.94 (m, 2H), 5.35 (s, 1H), 2.72-2.48 (m, 2H), 2.33 (d, *J* = 16.0 Hz, 1H), 2.12 (d, *J* = 16.0 Hz, 1H), 1.05 (s, 3H), 0.86 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 195.9, 163.9, 161.2 (d, *J* = 251.2Hz), 156.4, 147.6, 140.8, 132.3, 130.2, 129.8, 129.7, 128.9, 125.4, 117.1, 115.1, 114.9, 114.7, 113.5, 113.1, 105.0, 50.0, 40.1, 33.4, 31.8, 28.7, 26.1 ppm. Anal. calcd. (%) for $C_{25}H_{21}O_3F$: C, 77.30; H, 5.45; F, 4.89; found (%): 77.16; H, 5.31; F, 4.76.

12-(2-Fluorophenyl)-2-hydroxy-9,10-dihydro-8*H*-benzo[*a*]xanthen-11(12*H*)-one (4i): Yellow solid; Yield: 90 %; m.p.: 210-212 °C; IR (KBr, ν_{max}, cm^{-1}): 3444.8 (OH, *str.*), 2954.5-2866.0 (CH_2, *str.*), 1657.8 (C=O), 1510.2, 1449.7 & 1426.4 (CH_2, bend.), 1366.2, 1213.6, 1177.6 (C-F, *str.*), 1130.0, 959.4, 834.1, 607.8; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (s, 1H), 7.68 (dd, *J* = 14.9, 8.9 Hz, 2H), 7.44 (d, *J* = 14.7 Hz, 1H), 7.19-7.08 (m, 2H), 7.04-6.94 (m, 3H), 6.74-6.59 (m, 1H), 5.55 (s, 1H), 2.72 (s, 2H), 2.42-2.25 (m, 2H), 2.04-1.64 (m, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 197.2, 166.7, 156.2, 153.8, 149.8, 147.8, 132.9, 131.4, 128.2, 127.5, 127.2, 126.9, 125.4, 124.5, 119.3, 116.9, 116.1, 115.1, 114.1, 113.4, 105.7, 36.4, 28.7, 27.0, 19.9 ppm. Anal. calcd. (%) for $C_{23}H_{17}O_3F$: C, 76.65; H, 4.75; F, 5.27; found (%): C, 76.52; H, 4.62; F, 5.14.

12-(4-Fluorophenyl)-2-hydroxy-9,10-dihydro-8*H*-benzo[*a*]xanthen-11(12*H*)-one (4j): Pale yellow solid; Yield: 92 %; m.p.: 217-219 °C; IR (KBr, ν_{max}, cm^{-1}): 3426.2 (OH, *str.*), 2958.8-2852.3 (CH_2, *str.*), 1625.4 (C=O), 1588.7, 1482.1 & 1449.7 (CH_2, bend.), 1384.9, 1233.0 (C-F, *str.*), 1139.5, 1028.5, 829.8, 750.6; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 7.80-7.69 (m, 3H), 7.26-7.17 (m, 3H), 7.05-6.94 (m, 3H), 5.37 (s, 1H), 2.71-2.60 (m, 2H), 2.34-2.23 (m, 2H), 2.02-1.82 (m, 2H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.2, 165.2, 156.6, 147.6, 133.2, 132.4, 131.3, 131.2, 130.3, 129.2, 125.3, 117.7, 115.9, 11.34, 112.2, 104.4, 36.2, 29.2, 26.3, 19.8 ppm. Anal. calcd. (%) for $C_{23}H_{17}O_3F$: C, 76.65; H, 4.75; F, 5.27; found (%): C, 76.53; H, 4.62; F, 5.16.

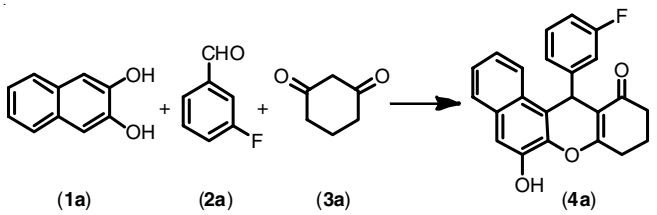
Biological assay

Culture conditions of cell lines: The cancerous cell lines (HepG2/HeLa) and non-cancerous cell lines (HEK 293) are obtained from National Center for Cell Sciences, Pune, India. Then the procured cell lines were grown in Eagle's minimum essential medium, supplemented with 10 % FBS. The cell cultures were maintained with 5 % CO_2 at 37 °C in a humidified atmosphere. The cell lines were subcultured twice in each week, with seeding at a density of about 5×10^3 cells/mL. Before evaluation of activity of synthesized compounds, fresh medium was added for cells which were washed with PBS previously. For the final evaluation, exponentially cells grown were collected and re-suspended in fresh culture with 10 % FBS.

MTT assay: The title compounds (**4a-j**) were evaluated for cytotoxic activity against human hepato cellular carcinoma cell lines (HepG2) and human cervical cancer (HeLa) cells. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazan product. This process requires active mitochondria and even freshly dead cells do not cleave significant amount of MTT. Thus the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods. The compounds were dissolved in DMSO and serially diluted with MEM (minimum essential medium) complete medium to get the concentrations for a range of test concentration. DMSO concentration was kept < 0.1 % in all the compounds. HePG2 hepatocellular carcinoma and human cervical cancer HeLa cells maintained in appropriate conditions were seeded in 96 well plates and treated with different concentrations of the test compounds and incubated in 5 % CO_2 at 37 °C for 96 h. MTT reagent was added to the wells and incubated for 4 h; the dark blue formazan product formed by the cells was dissolved in DMSO under a safety cabinet and read at 550 nm. Percentage inhibitions were calculated and plotted with the concentrations for calculating the IC_{50} values.

RESULTS AND DISCUSSION

To develop the optimal reaction conditions, we carried out a model reaction (**Scheme-I**) for the development of optimal reaction conditions with naphthalene-2,3-diol (**1a**, 1 mmol), 3-fluorobenzaldehyde (**2a**, 1 mmol) and cyclohexane-1,3-dione (**3a**, 1 mmol) under different experimental conditions such as various solvent and catalyst conditions, optimized reaction times and temperatures for an effective reaction completion (Table-1). With these overall results was demonstrated that under catalyst and solvent-free conditions with shorter reaction time at 60 °C are optimal conditions for **4a** synthesis at high yield (Table-1, entry 5). This higher yield could be elucidated



Scheme-I: Synthesis of 12-(3-fluorophenyl)-6-hydroxy-9,10-dihydro-8*H*-benzo[*a*]xanthen-11(12*H*)-one (**4a**)

TABLE-1
OPTIMIZATION OF REACTION CONDITIONS FOR THE SYNTHESIS **4a**[a]

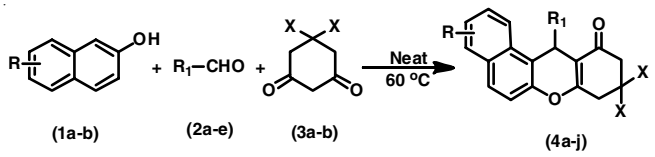
Entry	Catalyst (10 mol %)	Solvent	Temp. (°C)	Time (min)	Yield[b] (%)
1	Neat	Ethanol	80	120	86
2	Neat	THF	60	120	70
3	Neat	DMF	120	80	80
4	Neat	Water	100	130	60
5	Neat	Neat	60	20	91
6	InF_3	Neat	60	20	78
7	*p*-TSA	Neat	60	60	75
8	K-10	Neat	60	40	78
9	$GaCl_3$	Neat	60	35	85
10	MSA	Toluene	60	40	77
11	PSA	CH_2Cl_2	75	20	58
12	SSA	Ethanol	80	20	80

[a]Reaction of naphthalene-2,3-diol (**1a**, 1 mmol), 3-fluorobenzaldehyde (**2a**, 1 mmol) and cyclohexane-1,3-dione (**3a**, 1 mmol) using either neat or various catalysts in 10 mol %; [b]Isolated yields.

by the uniform distribution of reactant's eutectic mixture being in close proximity to react together in solvent-free condition than solvent condition.

In order to extend the above reaction conditions for a library system (Table-2), various kinds of aldehydes (**2b-e**), naphthalene diols (**1a** & **1b**) and cyclohexane-1,3-diones (**3a** & **3b**) were exposed to react (**Scheme-II**) for corresponding fluorine containing benzo[*a*]xanthen-11(12*H*)-ones (**4a-j**). All of the aldehydes gave expected product at high yields regardless of their substitutions under the similar reaction condition. In this procedure, the pure products were isolated by simple filtration using a little amount of THF without chromatography or a cumbersome work-up procedure. All the titled compounds were characterized by IR, NMR (^1H & ^{13}C) and mass spectrometry (for related spectra see supplementary material).

To support the higher product yields of the titled compounds a schematic mechanism (**Scheme-III**) was employed



Scheme-II: Synthesis of fluorinated benzo[*a*]xanthen-11(12*H*)-ones (**4a-j**)

by sequential connection of substrates through the formation of *ortho*-quinone methides intermediate formed by the nucleophilic addition of naphthalene diols (**1a-b**) to aldehydes (**2a-e**). This *ortho*-quinone methides intermediate was processed by a Michael addition with enolic form of cyclic 1,3-dicarbonyls (**3a-b**) and subsequent intramolecular addition of phenolic moiety with carbonyl group of diketone resulted in cyclic hemiketal and later dehydration of it afforded the title compounds (**4a-j**).

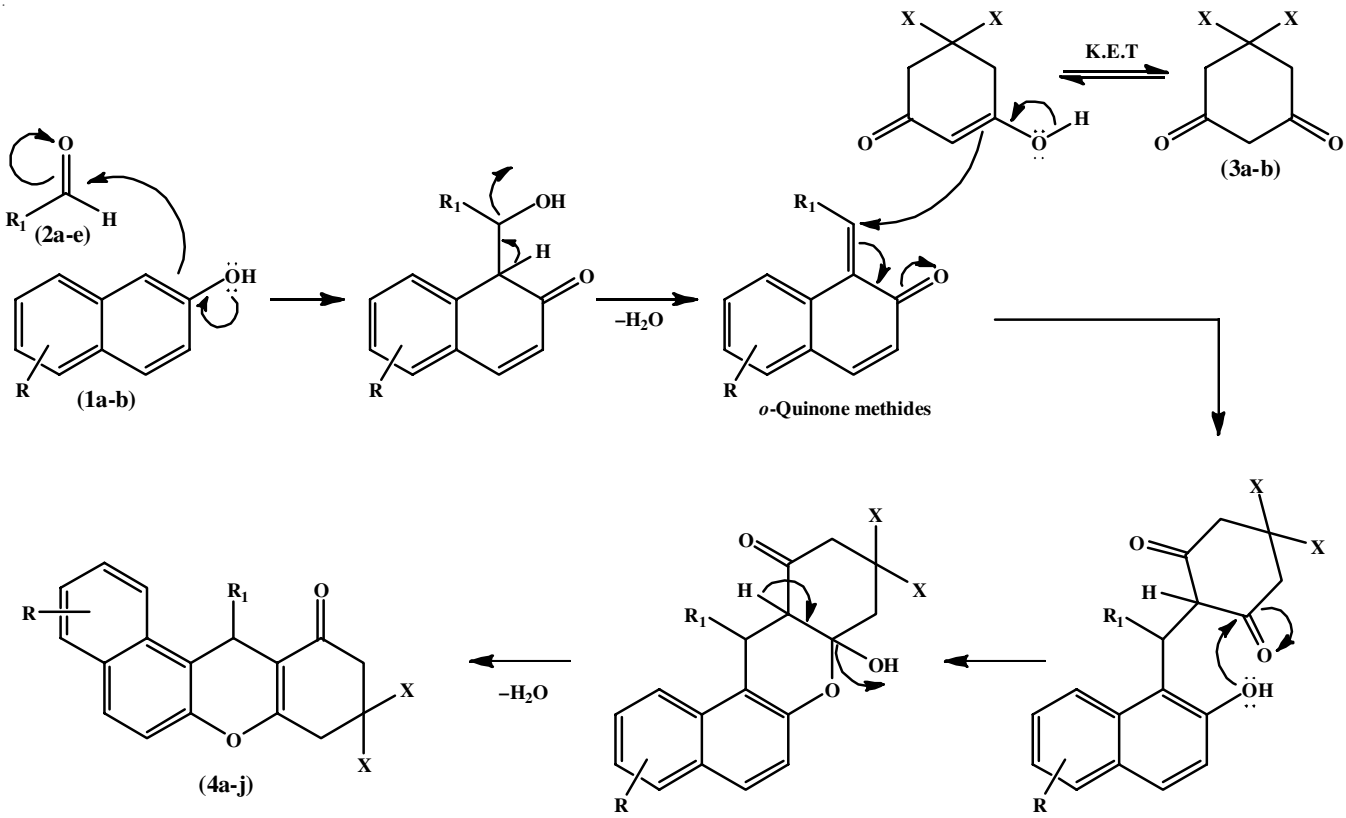
After that, titled compounds (**4a-j**) were screened for *in vitro* cytotoxic activity and which was measured after 96 h by exposure against human hepatocellular carcinoma cell lines (HepG2) and cervical cancer cells of human (HeLa) at 0.001 to 10 μM concentrations. Herein, two independent experiments were performed with duplicates and determined the mean values. The average values of these two determinations are reported by 8-10 % or lesser difference in all cases.

The cell viability graphic method was used to measure the IC_{50} values of titled compounds by plotting the concentrations on X-axis and percentage of inhibition on Y-axis. The obtained linear graph was intersected at 50 % inhibition and then collected the concentration value on X-axis at correlated point of it was recorded and considered as IC_{50} values in μM (Table-3). Table-3 revealed that based on structural differences the compounds showed dissimilar range of substantial IC_{50} values varying from > 10 to 0.6 μM. As evident that the compounds **4g**, **4a**, **4j** and **4f** exhibited maximum activity (Fig. 1) compared to that of HepG2 cell lines (IC_{50} of 0.8, 2.0, 4.0 and 6.0 μM, respectively). Similarly the compounds **4g**, **4j**, **4f** and **4a** exhibited high cytotoxicity against HeLa cell lines with IC_{50} of 0.6, 2.0, 2.0 and 3.0 μM, respectively (Fig. 1). The 4g compound showed high activity (0.6 μM) against HeLa cell lines when compared to HepG2 cell lines. The compound **4g** exhibited highest inhibition of cellular proliferation at 10 μM in both the cell lines.

On the other hand, the normal cells (HEK 293) were unaffected by the treated compounds suggesting that the non-toxic nature of the compounds for healthy cells. Therefore, this study discovered a new family of fluorine containing benzo[*a*]-

TABLE-2
SYNTHESIS OF OXYGEN AND FLUORINE CONTAINING BENZO[*a*]XANTHEN-11(12*H*)-ONE DERIVATIVES (**4a-j**)

Compd.	R	R_1	X	Time (min)	Yield (%)	m.p. (°C)
4a	3-OH	3-F-C_6H_4	H	20	91	230-231
4b	7-OH	2,4-F-C_6H_3	CH_3	23	89	231-232
4c	3-OH	2-F-C_6H_4	H	26	89	212-213
4d	3-OH	4-Br, 2-F-C_6H_3	H	25	88	201-202
4e	3-OH	2,4-F-C_6H_3	H	23	86	215-217
4f	3-OH	2-F-C_6H_4	CH_3	26	90	209-211
4g	3-OH	3-F-C_6H_4	CH_3	19	91	210-215
4h	7-OH	4-F-C_6H_4	CH_3	18	90	218-220
4i	7-OH	2-F-C_6H_4	H	22	90	210-212
4j	7-OH	4-F-C_6H_4	H	20	92	217-219



Scheme-III: Plausible mechanisms of benzo[*a*]xanthen-11(12*H*)-one derivatives (**4a-j**)

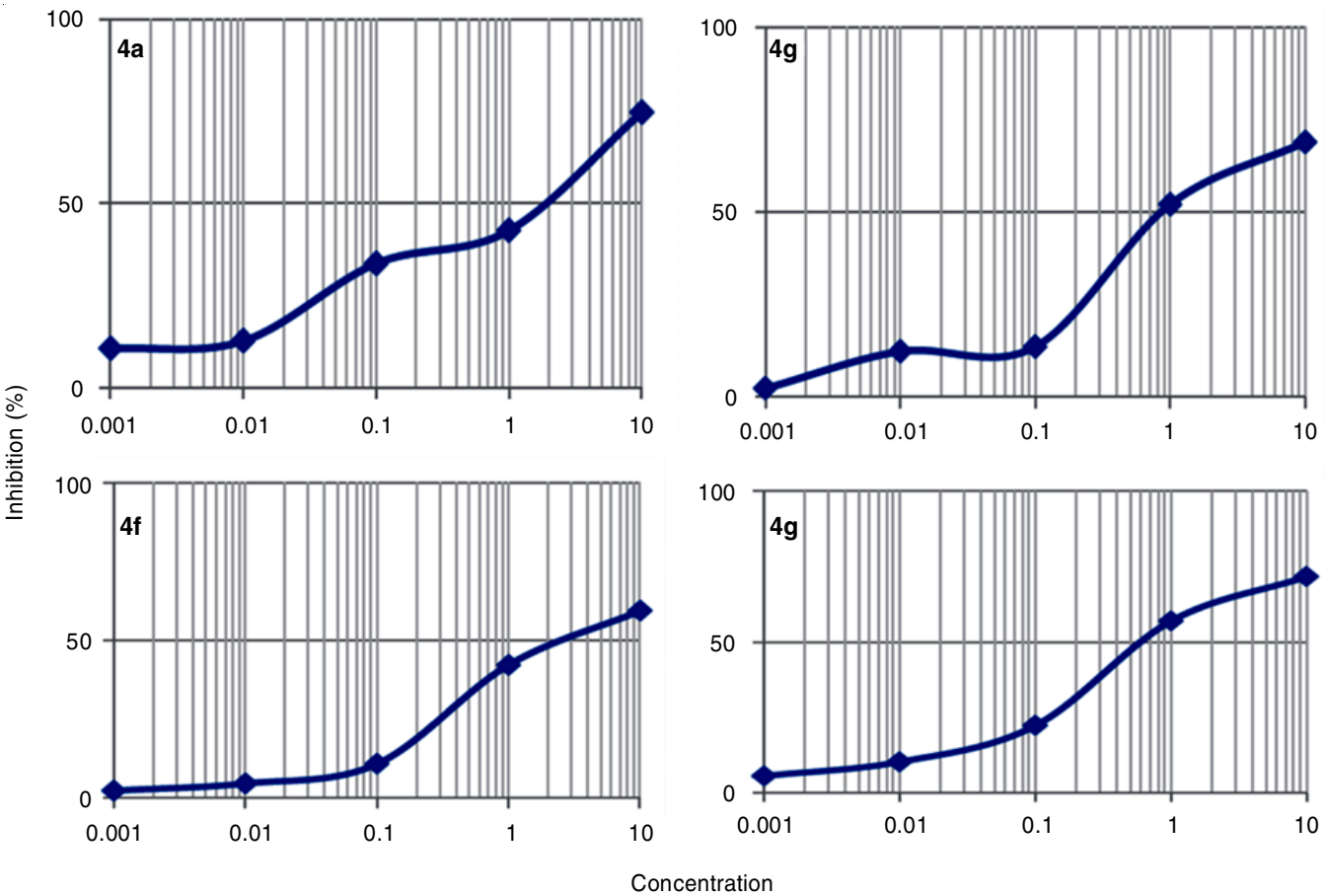


Fig. 1. Cytotoxic activity of compounds **4a** and **4g** against HepG2 (top) and **4f** and **4g** against HeLa (bottom) cell lines using MTT assay on a 96 h culture with an IC_{50} values of 2.0, 0.8 and 2.0 ,0.6 μM, respectively

TABLE-3
in vitro CYTOTOXIC ACTIVITY OF TITLE COMPOUNDS (**4a-j**) AGAINST HepG2 AND HeLa CELL LINES[a]

Compd.	IC_{50} (μM)[b]		Compd.	IC_{50} (μM)[b]	
	HepG2	HeLa		HepG2	HeLa
4a	2.0	3.0	**4f**	6.0	2.0
4b	>10	>10	**4g**	0.8	0.6
4c	>10	>10	**4h**	>10	>10
4d	>10	>10	**4i**	>10	>10
4e	>10	>10	**4j**	4.0	2.0

[a]IC_{50} values were determined from growth inhibition curves (0.001-10 μM); [b]The reported values are the average of two determinations that in all cases differed by 8-10 % or less.

xanthen-11(12*H*)-one derivatives (**4a-j**) with noteworthy target specific cytotoxic activity.

Structure-activity relationship (SAR): The oxygenated tricyclic hydro benzo[*a*]xanthene derivatives are an outstanding class of compounds with diverse captivating pharmacological properties [36-38]. Many natural and synthetic xanthene analogous have been reported in literature and several of them showed antitumor activity [39,40]. In recent years, in the fields of medicinal chemistry and material science, synthesis of this class of compounds gained considerable attention as the number of its applications have increased.

In this work, the antitumor activity of the target molecules with two kinds of human cancer cell lines was tested and the activity probably is attributed to several factors. Therefore, the SAR study of the titled compounds is explained as below. Mostly the SAR studies were carried out at C_2, C_6 and C_{12} positions of the titled compounds (**4a-j**), which revealed that the presence of –OH substituent at position 6 exhibited significant *in vitro* cytotoxic activity on both cancer cell lines. Most of the titled compounds (**4a-j**) structure has stereogenic centers, due to keto-enol tautomerism possibility they show racemization readily (Fig. 2a). Delocalization of non-bonding electrons of the oxygen into various parts of benzo[*a*]xanthen-11(12*H*)-one create nucleophilic and electrophilic centers and facilitate its attack on enzymes and proteins sites with *vice-versa*.

As shown in Fig. 2a the basic core unit of titled compounds have one -OH group on hydrophobic heterocyclic moiety at various positions, which favours the hydrogen bond donation for enzyme or protein of receptor. On the other hand, there are also two oxygen and one fluorine sites ready for hydrogen bond acceptance with enzyme or protein. More electron withdrawing group of fluorine at *meta* position on phenyl ring at C_{12} position, -OH group at C_6 position and an extra hydrophobic methyl groups at C_9 of **4g** showing higher *in vitro* cytotoxic activity on both cancer cell lines. Excitingly, without these methyl groups at C_9 of **4a** have shown little bit lower activity on HepG2 and moderately on HeLa cell lines. It indicates that decrease of an organic moiety on basic core unit results in lowering the hydrophobic interaction with the residues of enzymes/proteins sites (Fig. 2b).

In this connection, the structure, configuration, steric size and nature of attached groups on benzo[*a*]xanthen-11(12*H*)-one ring have critically governed the ease and effectiveness of interaction with enzymes/proteins in living organisms leading to normal metabolic biochemical processes. Therefore, analogue compounds of **4a**, **4j** and **4f** effectively check the abnormal cell metabolic disorders with their necessary steric and elec-

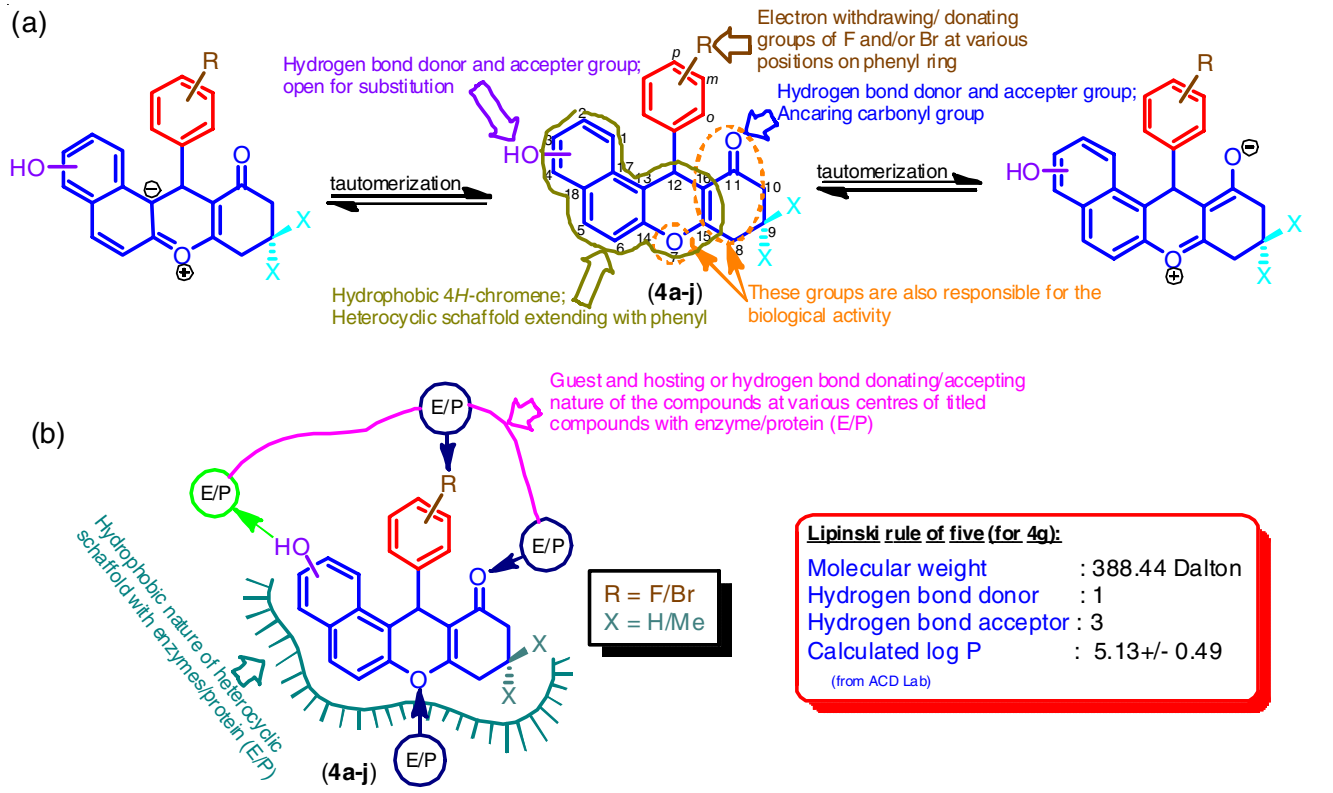


Fig. 2. Illustration of SAR of the titled compounds (**4a-j**), (a) the possible tautomerisation and probable active interactive groups on the basic core unit and (b) the possible interactions of titled compounds with protein/enzyme-ligand in two dimensional view. The calculated Lipinski's properties of **4g** are displayed and log P value is measured by using ACD/Chem Sketch

tronic configuration and also maintain cancer cell components in normal function. In addition, the calculated Lipinski's results also confirm that all the studied compounds possess drug-like properties (Fig. 2b) with the selective two cancer cell lines.

Conclusion

In conclusion, a green and facile synthetic methodology was successfully developed for fluorine containing benzo[*a*]-xanthen-11(12*H*)-ones synthesis under catalyst and solvent-free environments. This methodology has several advantages, such as, operational feasibility, milder reaction conditions, easy work-up and high yields. In addition, a moderate to good anti-cancer activity also showed the titled compounds combating two human cancer cell lines, hepatocellular carcinoma (HepG2) and human cervical cancer (HeLa) cell lines. With active electronic configuration on compounds **4g**, **4a**, **4j** and **4f** arrested effective control of abnormal cell metabolic disorders was noticed.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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