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TIME EFFICIENT BAYLIS-HILLMAN REACTION ON STEROIDAL NUCLEUS OF WITHAFERIN-A TO SYNTHESIZE ANTICANCER AGENTS.

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Abstract: Baylis-Hillman reaction has been achieved on different organic motifs but with completion times of three to six days. Micellar medium of CTAB in water along with the organic base DABCO has been used to effect the Baylis-Hillman reaction on a steroidal nucleus of Withaferin-A for the first time with different aromatic aldehydes within a day to synthesize a library of BH adducts (W1a –W14a) and (W1b-W14b) as a mixture of two isomers and W15 as a single compound. The isomers were separated on column and the major components were chosen for bio-evaluation. Cytotoxic activity of the synthesized compounds was screened against a panel of four cancer cell lines Lung A-549, Breast MCF-7, Colon HCT-116 and Leukemia THP-1 along with 5-florouracil and Mitomycin-C as references. All the compounds exhibited promising activity against screened cell lines and were found to possess enhaunced activity than parent compound. BH adducts with aromatic systems having methoxy and nitro groups were found to be more active.

Keywords: Baylis-Hillman, steroidal, withanolides, withaferin A, W. Somnifera.

I. INTRODUCTION

The Solanaceae is a family of about 84 genera and 3,000 species of annual shrubs distributed Worldwide. The genera *Withania* and *Physalis* are known for their use in the Unani and Ayurvedic systems. The *Withania* species are widely distributed in the tropical and subtropical zones, the Mediterranean region and northern Africa to Southwest Asia[1-4]. *W. Somnifera* and *W. coagulans*, are cultivated in several regions[5-7] for their medicinal significance. *W. somnifera*, commonly known as Ashwagandha has been in use in Ayurvedic and indigenous medicine for over 3,000 years[8]. Ashwagandha roots are present in over 200 formulations in Ayruvedha, Siddha and Unani medicine, which are used for various physiological disorders[9-10]. *Withania* appears in WHO monographs on Selected Medicinal Plants and an American Herbal Pharmacopoeia monograph is also forthcoming[11]. In Ayurveda, *Withania* is widely used as a general energy-enhancing tonic (Medharasayana), believed to promote learning and a good memory[12-13]. The plant resembles in its restorative properties of increasing the production of vital fluids, muscle fat, blood, lymph, semen and cells to ginseng roots and has led to Ashwagandha roots being called Indian ginseng. It aids in treatment of chronic fatigue, weakness, premature ageing, emaciation, debility, and muscle tension. The bruised leaves have also been reported for treatment of tumours, carbuncles and ulcers[14]. The decoction of the root boiled with milk is recommended for curing sterility in women, constipation, senile debility, rheumatism, nervous exhaustion, and spermatorrhoea[15]. *W somnifera* is cultivated in the drier partsManasa, Neemuch and Jawad tehsils of the Mandsaur District of Madhya Pradesh, Punjab, Sind, Rajastan and Jammu & Kashmir. The leaves are used as a vegetable and as fodder for livestock[16]. The crude preparation of the plant has been found to be active against a number of pathogenic bacteria.

W. Somnifera has been known for its antioxidant, anti-inflammatory, antibacterial and antitumoral activities. The pharmacological activity of *W. Somnifera* extracts has been summarized by Gupta and Rana. The antioxidant activity of withanolides has been tested with increased levels of enzymes like gluthione, superoxide dismutase in rats. Methanolic extracts of Withania have been found effective in reducing gastric secretions and total acidity. Ashwagandhahasbeen traditionally used as neurotonic and is associated with improvements inscopolamine-induced memory deficits in mice. On account of its antiper oxidative, radical scavenging properties, *W. Somnifera* has also been shown to possess antiparkinonian effect. Withaferin A has been reported to be associated with activation of caspace-3 and translocation of cytochrome-c from mitochondria to cytosol in its apoptosis inducing mechanism by oh-et al

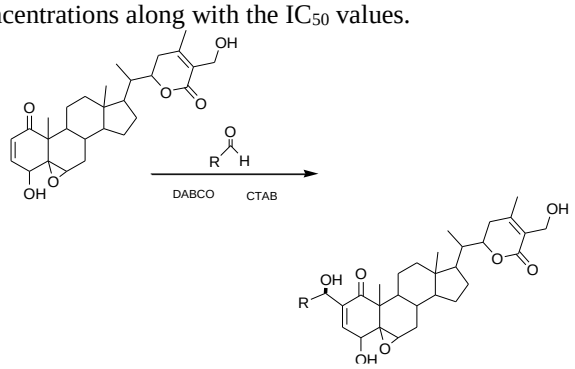
The importance of steroidal structural framework is manifested by the biological functions of adrenal, sexhormones and bile acids. It being a functional group rich entity provide template for structural modifications. Furthermore, biologically significant motifs are nontoxic, multidrugresistant (MDR) and capable of penetrating the biological membranes[g]. There are a number of reports of natural products with α, β unsaturated carbonyl exhibiting chemo-preventive and chemo-protective activities[h]. There are few reports of structural modification of Withaferin-A with much emphasis on C-27 Hydroxyl group and β epoxide ring. Yasuno Yokota et al used C-27 OH to develop probes for studying angiogenesis. Sangwanet al have studied Michael addition to α, β unsaturated carbonyl of ring A to show the importance of double bond in anticancer activity. We in present study report Baylis-Hilman adducts of Withaferin-A for screening as anticancer agents.

II. RESULTS AND DISCUSSION

CHEMISTRY: Baylis-Hillman adducts of the parent compound withaferrin A were synthesized at a reasonable pace using micellar medium of CTAB in water. To 0.074 mmoles of parent compound withaferin-A in 4ml of micellar media of CTAB in water, 1.5 equivalents of different aromatic aldehydes were added in presence of 10 mmol% of organic base DABCO and the mixture was stirred constantly for 24 hrs at room temperature. This resulted in the formation of Baylis-Hillman adducts $W1_a$ to $W14_a$ and $W1_b$

to $W14_b$ as an isomeric mixture.The isomeric mixture was separated on column resulting in isolation of R and S forms of all the adducts. The major isomers of adducts were screened for cytotoxicactivity against a panel of four human cancer cell lines along with the normal cell line and were found to be promisingly more active than parent compound. BH adducts W1, W2, W3, W4, and W5 were found to be more active with W4 being most active with IC_{50} values of 0.02-0.5.

Biology: The sulphorhodamine B assay was used to screen the library of ring-A modified withaferin-A adducts for cytotoxicity. The cytotoxic activity of compounds was studied using cultured A-549(lung), MCF-7(breast), HCT-116(colon) and THP-1(leukaemia) cancer cell lines along with normal cell lines(FR-2) by using sulforhodamine –B assay. 5-fluorouracil and mitomycin-C along with compound (W) were taken as reference standards in this study Table 1. The cell lines were exposed to 50 and 10 µmol concentrations of compounds for 48 h and percentage of dead cells was evaluated. On account of very promising activities against the four human cancer cell lines, the first five compounds were screened for their activities against the same cell lines but at lower concentrations of 1 µmol. Since these five compounds exhibited very good cytotoxic activity against all cell lines at 1(µmol) concentration, the activity of these compounds was determined at even lesser concentrations along with the IC_{50} values.



Scheme. Baylis Hillman reaction of withaferin-A

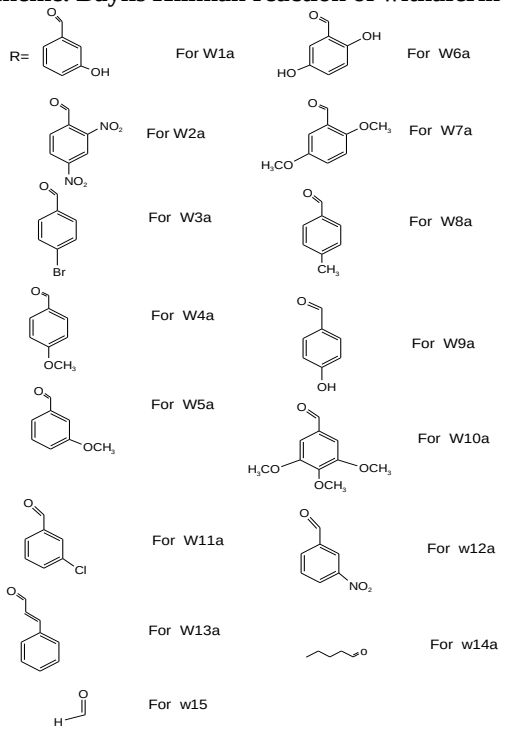


Figure 1.Nature of R in the aldehyde.

III. CONCLUSIONS

To sum up Baylis-Hilman reaction has been achieved on the steroidal system at a reasonable pace using micellar media to get a library of adducts. Cytotoxic activity of all the synthesized compounds was screened against a panel of four cancer cell lines Lung A-549, BreastMCF-7, Colon HCT-116 and Leukaemia THP-1 along with the 5-Fluorouracil, Mitomycin-C as references. All the compounds exhibited a promising activity against all screened cell lines. BH adducts showed more activity than the parent compound. BH adducts with aromatic systems having methoxy, nitro groups were found to be most active.

IV. EXPERIMENTAL SECTION

General methods. ^1H and ^{13}C NMR spectra were recorded on 400 MHz spectrometers with TMS as the internal standard. Chemical shifts are expressed in parts per million (□ppm). MS were recorded on LC Mass spectrometer. IR spectra (KBr) discs were recorded on Bruker Vector 22 instrument.Silica gel coated aluminium plates were used for TLC. Elemental analyses were performed on Elementar. Reagents and solvents used were mostly of LR grade.The chromatograms were visualised under UV-254-366nm and ceric ammonium sulphate spray.

Plant material, extraction and isolation of Withaferin-A:*Withania somnifera* leaves were collected from Jammu, India and its identification were done by the taxonomists at IIIM Jammu. The accession ( ) is being maintained in the institute farm. The shade-dried leaves (1kg) were ground and defatted three times with n-hexane (3 x 1.5 l) by soaking overnight at room temperature. The spent material was further extracted with MeOH (3 x 1 l) overnight at room temperature. The desired compound withaferin-A was isolated by using the patented procedure by our parent institute in collaboration with CIMAP Luck now[18].The isolated compound was coded as W.

Synthesis of Baylis-Hillman adducts of W ($W1_a$-$W15_a$ and $W1_b$-$W14_b$):20mg of compound W(0.074 m moles) was added to micellar medium of CTAB in water along with the 1.5 equivalents' of aromatic aldehyde and 10mmol% of organic base DABCO and the reaction mixture was stirred constantly for 24 hrs at room temperature to get the adducts$W1_a$-$W14_a$ and $W1_b$-$W14_b$as a mixture of two isomers which were separated through column chromatography and W15 as a single compound.

Compound W1**:** ^{1}HNMR(400 MHz, $CDCl_3$),δ= 7.751 (1H, d, j=15.6 Hz),δ= 7.519 (1H, s),δ= 7.441 (1H, d, j=20.4 Hz),δ= 7.090 (1H, t),δ= 6.579 (1H, s),δ= 5.778 (1H, s),δ= 4.892 (1H, m), δ= 4.353 (1H,s) δ= 4.303(3H, m), δ= 3.926 (1H, t), δ= 3.326 (1H, t), δ= 2.924 (1H, t), δ= 2.556 (2H, m), δ=1.999 (7H, m), δ= 1.667 (10H, m), δ= 1.150 (4H, m) ^{13}CNMR (100 MHz, $CDCl_3$), δ= 207.19, 166.90, 147.97, 147.17, 139.52, 130.95, 129.20, 128.55, 124.86, 124.21, 123.93, 119.33, 117.00, 116.13, 114.29, 76.92, 61.06, 57.70, 56.33, 52.12, 50.82, 43.79, 42.94, 41.61, 39.15, 38.97, 35.09, 34.74, 32.15, 31.84, 31.65, 30.41, 29.92, 29.46, 29.18.

Compound W2**:** ^{1}HNMR(400 MHz, $CDCl_3$),δ=8.245 (1H, s), δ= 7.770 (1H, s),δ= 7.581(1H, d, j= 6.4 Hz) δ= 7.334 (1H, d, j=30.8 Hz) δ= 7.091 (1H, t) δ= 6.580 (1H, s) δ= 5.721 (1H, s) δ= 4.892 (1H, m)δ= 4.353(1H,s)δ=4.303(3H,m) δ=3.926(1H,t) δ=3.326(1H,t)

δ=2.924(1H,t)δ=2.556(2H,m)δ=1.999(7H,m)δ=1.667(10H, m)δ=1.150(4H,m);^{13}CNMR(100MHz,$CDCl_3$)δ=207.19,161. 54,152.73,146.24,143.29,142.55,140.97,139.47,139.05,136. 89,135.51,135.32,130.06,129.88,129.13,124.18,123.61,122. 77,120.69,119.69,116.08,115.53,114.26,112.65,110.01,93.5 0,55.96,41.42,35.00,34.43,34.02,32.12,31.84,30.53,30.35,29 .89,29.82,29.71,29.56,29.36,29.16

Compound W3:^{1}HNMR(400 MHz, $CDCl_3$),δ=7.479 (2H, d), δ=7.338 (1H, d), δ=7.024 (1H,d), δ=6.579 (1H, s),δ=5.566 (1H, s), δ=4.692 (1H, s),δ= 4.318 (3H, m),δ= 3.850 (1H, t),δ=3.234 (1H,t),δ=2.897 (1H, t),δ=2.520 (2H, m),δ=2.290 (2H, m),δ=1.999(7H, m),δ=1.667 (10H, m),δ=1.150 (4H, m); ^{13}CNMR (100 MHz, $CDCl_3$), δ=207.69, 167.25, 153.21, 134.44,131.85,129.25, 125.89, 124.82, 124.67, 124.34, 123.61. 114.24, 104.24,82.69, 78.90, 76.91, 72.87, 61.15, 57.50, 56.30, 52.08, 50.76, 50.18, 49.75, 43.72, 42.90, 41.63, 39.14, 38.94, 35.13, 34.71, 32.11, 31.61, 30.37, 29.14,

Compound W4:^{1}HNMR(400 MHz, $CDCl_3$), δ=7.489 (2H, d), δ=7.330 (1H, d), δ=7.034 (1H,d), δ=6.579 (1H, s),δ=5.566 (1H, s), δ=4.692 (1H, s),δ= 4.318 (3H, m),δ= 3.850 (1H, t),δ=3.234 (1H,t),δ=2.897 (1H,t),δ=2.520(2H,m), δ=2.290(2H,m),δ=1.999(7H,m),δ=1.667(10H,m)δ=1.150(4 H,m)^{13}CNMR(100MHz,$CDCl_3$)δ=207.19,152.02,147.83,147 .31,139.51,132.23,129.09,124.85,124.20,123.73,119.46,116. 13,114.55,,78.92,66.20,57.70,56.39,55.81,52.15,43.43,38.98 ,35.17,35.09,34.95,34.75,34.05,33.80,32.15,31.84,31.65,30. 67,30.52,30.42,30.08,29.92,29.47,29.29

Compound W5 : ^{1}HNMR(400 MHz, $CDCl_3$) δ=7.509 (1H, s), δ=7.288 (1H, d,), δ=7.075 (1H, d, j=14.4Hz), δ=6.924 (1H, t) δ=6.077 (1H, s), δ=4.754 (1H, s), δ=4.612 (1H, s), δ=4.355 (2H, d, j=8 Hz), δ=3.77 (3H, s), δ=3.140 (1H, t), δ=2.913 (1H, t), δ=2.496 (1H, t), δ=2.263 (2H, t), δ= 2.248 (5H, m) δ=1.958 (6H, m), δ=1.150 (10H, m).^{13} CNMR(100 MHz, $CDCl_3$), δ=208.58, 167.15, 159.91, 152.86, 140.29, 129.74, 125.97, 123.71, 118.35, 116.11, 114.95, 111.41, 104.12, 80.87, 78.90, 73.88, 61.40, 57.59, 56.80, 55.52, 52.11, 50.48, 44.12, 42.93, 41.73, 39.17, 38.97,35.16, 34.74, 32.14, 31.83, 31.65, 30.66, 30.09, 29.91, 29.87,

Compound W6: ^{1}HNMR(400 MHz, $CDCl_3$),δ= 7.851 (1H, s), δ= 7.441 (1H, d, j=20.4 Hz), δ= 7.440(1H, d, j=20.3Hz) δ= 6.579 (1H, s),δ= 5.778 (1H, s),δ= 4.892 (1H, m), δ= 4.353 (1H,s) δ= 4.303(3H, m), δ= 3.926 (1H, t), δ= 3.326 (1H, t), δ= 2.924 (1H, t), δ= 2.556 (2H, m), δ=1.999 (7H, m), δ= 1.667 (10H, m), δ= 1.150 (4H, m) ^{13}CNMR (100 MHz, $CDCl_3$), δ= 207.19, 166.90, 147.97, 147.17, 139.52, 130.95, 129.20, 128.55, 124.86, 124.21, 123.93, 119.33, 117.00, 116.13, 114.29, 76.92, 61.06, 57.70, 56.33, 52.12, 50.82, 43.79, 42.94, 41.61, 39.15, 38.97, 35.09, 34.74, 32.15, 31.84, 31.65, 30.41, 29.92, 29.46, 29.18.

Compound W7**:** ^{1}HNMR(400 MHz, $CDCl_3$),δ= 7.851 (1H, s), δ= 7.441 (1H, d, j=20.4 Hz), δ= 7.440(1H, d, j=20.3Hz) δ= 6.579 (1H, s),δ= 5.778 (1H, s),δ= 4.892 (1H, m), δ= 4.353 (1H,s) δ= 4.303(6H, m), δ= 3.926 (1H, t), δ= 3.326 (1H, t), δ= 2.924 (1H, t), δ= 2.556 (2H, m), δ=1.999 (7H, m), δ= 1.667 (10H, m), δ= 1.150 (4H, m) ^{13}CNMR (100 MHz, $CDCl_3$), δ= 207.19, 166.90, 147.97, 147.17, 139.52, 130.95, 129.20, 128.55, 124.86, 124.21, 123.93, 119.33, 117.00, 116.13, 114.29, 76.92, 61.06, 57.70, 56.33, 52.12, 50.82, 43.79, 42.94, 41.61, 39.15, 38.97, 35.09, 34.74, 32.15, 31.84, 31.65, 30.41, 29.92, 29.46, 29.18.

Compound W8:^{1}HNMR(400 MHz, $CDCl_3$), δ=7.479 (2H, d), δ=7.338 (1H, d), δ=7.024 (1H,d), δ=6.579 (1H, s),δ=5.566 (1H, s), δ=4.692 (1H, s),δ= 4.318 (3H, m),δ= 3.850 (1H, t),δ=3.234 (1H,t),δ=2.897 (1H,t),δ=2.520(2H,m),δ=2.290(2H,m),δ=1.999(7H,m),δ=1.6 67(10H,m)δ=1.150(4H,m)^{13}CNMR(100MHz,$CDCl_3$)δ=207. 19,152.02,147.83,147.31,139.51,132.23,129.09,124.85,124. 20,123.73,119.46,116.13,114.55,,78.92,66.20,57.70,56.39,5 5.81,52.15,43.43,38.98,35.17,35.09,34.95,34.75,34.05,33.80 ,32.15,31.84,31.65,30.67,30.52,30.42,30.08,29.92,29.47,29. 29

Compound W9:^{1}HNMR(400 MHz, $CDCl_3$),δ= 7.753 (1H, d, j=15.6 Hz),δ= 7.529 (1H, d),δ= 7.443 (1H, d, j=20.4 Hz),δ= 7.090 (1H, d),δ= 6.578 (1H, s),δ= 5.768 (1H, s),δ= 4.882 (1H, m), δ= 4.353 (1H,s) δ= 4.303(3H, m), δ= 3.926 (1H, t), δ= 3.326 (1H, t), δ= 2.924 (1H, t), δ= 2.556 (2H, m), δ=1.999 (7H, m), δ= 1.667 (10H, m), δ= 1.150 (4H, m) ^{13}CNMR (100 MHz, $CDCl_3$), δ= 207.19, 166.90, 147.97, 147.17, 139.52, 130.95, 129.20, 128.55, 124.86, 124.21, 123.93, 119.33, 117.00, 116.13, 114.29, 76.92, 61.06, 57.70, 56.33, 52.12, 50.82, 43.79, 42.94, 41.61, 39.15, 38.97, 35.09, 34.74, 32.15, 31.84, 31.65, 30.41, 29.92, 29.46, 29.18.

Compound W10:^{1}HNMR(400 MHz, $CDCl_3$), δ=7.589 (1H, s), δ=7.330 (1H, s), δ=6.579 (1H, s),δ=5.566 (1H, s), δ=4.692 (1H, s),δ= 4.318 (9H, m),δ= 3.850 (1H, t),δ=3.234 (1H,t),δ=2.897 (1H, t),δ=2.520 (2H, m),δ=2.290 (2H, m),δ=1.999(7H,m),δ=1.667(10H,m),δ=1.150(4H,m);^{13}CNM R(100MHz,$CDCl_3$δ=207.19,152.02,147.83,147.31,139.51,1 32.23,129.09,124.85,124.20,123.73,119.46,116.13,114.55,,7 8.92,66.20,57.70,56.39,55.81,52.15,43.43,38.98,35.17,35.09 ,34.95,34.75,34.05,33.80,32.15,31.84,31.65,30.67,30.52,30. 42,30.08,29.92,29.47,29.29

Compound W11:^{1}HNMR(400 MHz, $CDCl_3$),δ= 7.751 (1H, d, j=15.6 Hz),δ= 7.519 (1H, s),δ= 7.441 (1H, d, j=20.4 Hz),δ= 7.090 (1H, t),δ= 6.579 (1H, s),δ= 5.778 (1H, s),δ= 4.892 (1H, m), δ= 4.353 (1H,s) δ= 4.303(3H, m), δ= 3.926 (1H, t), δ= 3.326 (1H, t), δ= 2.924 (1H, t), δ= 2.556 (2H, m), δ=1.999 (7H, m), δ= 1.667 (10H, m), δ= 1.150 (4H, m) ^{13}CNMR (100 MHz, $CDCl_3$), δ= 207.19, 166.90, 147.97, 147.17, 139.52, 130.95, 129.20, 128.55, 124.86, 124.21, 123.93, 119.33, 117.00, 116.13, 114.29, 76.92, 61.06, 57.70, 56.33, 52.12, 50.82, 43.79, 42.94, 41.61, 39.15, 38.97, 35.09, 34.74, 32.15, 31.84, 31.65, 30.41, 29.92, 29.46, 29.18.

Compound W12: ^{1}HNMR(400 MHz, $CDCl_3$),δ=8.245 (1H, s), δ= 7.770 (1H, s),δ= 7.581(1H, d, j= 6.4 Hz),δ= 7.334 (1H, d, j=30.8 Hz),δ= 7.091 (1H, t),δ= 6.580 (1H, s),δ= 5.721 (1H, s),δ= 4.892 (1H, m), δ= 4.353 (1H,s) δ= 4.303(3H, m), δ= 3.926 (1H, t), δ= 3.326 (1H, t), δ= 2.924 (1H, t), δ= 2.556 (2H, m),), δ=1.999 (7H, m), δ= 1.667 (10H, m), δ= 1.150 (4H, m); ^{13}CNMR (100 MHz, $CDCl_3$), δ= 207.33, 167.14, 152.83, 147.30, 137.71, 133.36, 129.93, 125.99, 124.68, 123.73, 119.33, 116.13, 114.28, 103.39, 82.98, 78.89, 76.91, 61.06, 57.70, 56.33, 52.12, 50.82, 43.79, 42.94, 41.61, 39.15, 38.97, 35.09, 34.74, 32.15, 31.84, 31.65, 30.41, 29.92, 29.46

Compound W13: ^{1}HNMR(400 MHz, $CDCl_3$), δ=7.475 (2H, d), δ=7.336 (2H, t), δ=7.024 (1H,d), δ=6.579 (1H, s),δ=5.566 (1H, s), δ=4.692 (1H, s),δ= 4.318 (3H, m),δ= 3.850 (1H, t),δ=3.234 (1H,t),δ=2.897 (1H,t), δ=2.520(2H,m), δ=2.290(2H,m), δ=1.999(7H,m), δ=1.667(10H,m), δ=1.150(4H,m)^{13} CNMR(100MHz,$CDCl_3$)δ=207.19,152.02,147.83,147.31,139.51,132.23,129.09,124.85,124.20,123.73,119.46,116.13,114.55,,78.92,66.20,57.70,56.39,55.81,52.15,43.43,38.98,35.17,35.09,34.95,34.75,34.05,33.80,32.15,31.84,31.65,30.67,30.52,30.42,30.08,29.92,29.47,29.29

Compound W14: ^{1}HNMR(400 MHz, $CDCl_3$),δ= 9.702 (1H, s),δ= 6.587 (1H, s), δ= 5.078 (1H, s),δ= 4.888 (1H, m),δ= 4.571 (1H, s),δ= 4.329 (3H, m),δ= 3.802 (1H, t),δ= 3.189 (1H, t), δ= 2.825 (1H, t),δ= 2.484 (2H, m), δ= 2.403 (5H, m),δ= 2.278 (5H, m),δ=1.999 (7H, m), δ= 1.667 (10H, m), δ= 1.150 (4H, m) ^{13}CNMR (100 MHz, $CDCl_3$), δ= 207.61, 167.10, 152.93, 125.93, 124.15, 114.24, 95.63, 81.78, 78.85,76.91, 72.57, 61.12, 57.59, 57.26, 57.17, 56.29, 52.07, 50.51, 44.16, 42.88, 39.12, 38.94, 35.04, 34.70, 33.88, 31.61, 30.06, 29.67,29.47, 29.27, 28.69, 27.50, 25.88, 24.40, 22.76.

Compound W15: ^{1}HNMR(400 MHz, $CDCl_3$), δ=7.655 (1H, d), δ=7.535 (1H, d), δ=7.465 (1H, d), δ=7.365 (1H, t), δ=7.321 (1H, t), δ=6.107 (1H,s), δ=4.768 (1H, s) δ=4.304 (4H,m), δ=3.759 (1H, t) δ=2.913 (1H, t), δ=2.496 (1H, t), δ=2.263 (2H, t), δ= 2.248 (5H, m) δ=1.958 (6H, m), δ=1.150(10H,m), ^{13}CNMR (100 MHz, $CDCl_3$), δ=207.60, 166.93, 152.59, 138.47, 132.20, 132.10, 129.01, 128.68, 128.39, 126.19, 125.90, 125.78, 104.12, 80.69, 78.69, 76.69, 73.71, 61.22, 57.50, 56.56, 56.16, 51.90, 50.27, 43.93, 42.72, 41.55, 38.96, 38.76, 33.31, 31.93, 31.44, 30.20, 29.88, 29.39, 29.24, 29.07.

Table -1: In vitro determination of cytotoxicity of structurally modified derivatives of withaferin-A against panel of human cancer cell lines.

Tissue		Lung	Breast	Colon	Leukemia	Normal cell
Cell-Line		A-549	MCF-7	HCT-116	THP-1	FR-2
Code	Conc(µmol)					
W	50	95	88	97	90	75
	10	90	80	93	88	58
W1	50	96	94	95	90	68
	10	95	92	92	88	35
W2	50	94	96	94	92	58
	10	92	95	92	90	30
W3	50	98	95	90	95	45
	10	96	92	86	93	25
W4	50	99	96	98	95	45
	10	98	97	95	92	40
W5	50	97	98	98	97	60
	10	93	96	94	93	35
W6	50	99	98	95	96	50
	10	96	94	92	90	40
W7	50	90	97	98	98	54
	10	87	90	88	95	43
W8	50	97	99	97	97	43
	10	93	96	90	92	40
W9	50	90	92	96	90	50
	10	75	87	80	78	40
W10	50	80	90	97	90	45
	10	67	58	54	60	35
W11	50	85	78	80	85	57
	10	67	54	45	59	34
W12	50	90	98	98	90	45
	10	80	86	87	80	30
W13	50	75	74	67	87	40
	10	68	58	45	53	38
W14	50	70	68	69	70	45
	10	67	59	56	60	38
W15	50	60	56	67	60	40
	10	56	45	60	50	35
5-FU	10	76	68	74	78	58
Mito-C	01	96	74	68	62	45

All experiments were carried out in triplicate.
5-FU = 5Fluorouracil,Mitomycin C.
On account of very promising activities against the four human cancer cell lines, the first Five compounds were screened for their activities against the same cell lines but at lower Concentrations of 1 µmol as shown in table-2.

Table-2

Tissue		Lung	Breast	Colon	Leukemia
Cell-Line		A-549	MCF-7	HCT-116	THP-1
Code	Conc(µmol)				
W1	1	74	70	69	72
W2	1	68	73	65	70
W3	1	75	65	60	72
W4	1	78	76	74	68
W5	1	72	68	69	70
MitoC	1	96	74	68	62

Since these five compounds exhibited very good cytotoxic activity against all cell lines at 1(µmol) concentration, the activity of these compounds was determined at even lesser concentrations and is shown in table-3 along with the IC_{50} values.

Table-3

Tissue		Lung		Breast		Colon		Leukemia	
Cell-Line		A-549		MCF-7		HCT-116		THP-1	
Code	Conc µmol			percentage		growth		inhibition	
			IC_{50}		IC_{50}		IC_{50}		IC_{50}
W1	0.5	50		20		37		58	
	0.1	10	2.6	05	2.8	09	1	40	0.06
W2	0.5	40		20		40		58	
	0.1	30	0.8	03	2	23	1.9	38	0.07
W3	0.5	48		25		40		50	
	0.1	09	0.7	08	1.8	10	1.4	35	0.13
W4	0.5	38		50		51		62	
	0.1	36	0.5	32	0.38	40	0.07	48	0.02
W5	0.5	38		25		38		50	
	0.1	29	0.8	05	2	24	1.8	42	0.09

It can be concluded from above studies that all the compounds showed cytotoxic activity against the cancer cell much more than the parent compound with W4 being most active with IC_{50} values of 0.02-0.5.

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