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Synthesis and anti cancer activity of DABCO induced, micellar media intervened Baylis-Hillman adducts of withaferin-A

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ABSTRACT

Baylis-Hillman reaction is one of the important routes to effect structural modifications on biological templates for exploring the possibility of getting effective analogs in drug discovery and designing. It has been carried out on different motifs but is extremely slow on steroidal nuclei. Micellar medium of CTAB in water along with the organic base DABCO was used to effect the Baylis-Hillman reaction on a steroidal nucleus of Withaferin-A with different aromatic aldehydes within 24 hours. A library of BH adducts A_1-A_7 and A_1^*- A_7^* as a mixture of two isomers separated on column was synthesized 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 enhanced activity than parent compound. BH adducts with aromatic systems having methoxy and nitro groups were found to be more active.

***Keywords*: CTAB, Micellar media, steroidal, withaferin A.**

I.INTRODUCTION

The family Solanaceae represents about 84 genera and 3,000 species of annual shrubs distributed Worldwide. The genera *Withania* is known for its use in the Unani system of medicines. The *Withania* species are found in the tropical and subtropical zones, the Mediterranean region and northern Africa to Southwest Asia (1-4).*On account of* medicinal significance *W. Somnifera* is cultivated in several regions(5-7). *W. Somnifera* commonly called as Ashwagandha is in use in indigenous medicine for over 3,000 years (8). Its roots are used in over 200 Unani formulations for various physiological disorders (9-10). *Withania* is widely used as a general energy-enhancing tonic (Medharasayana) and promotes learning and a good memory (11-12). It is helpful in treatment of chronic fatigue, weakness, premature ageing, emaciation, debility, muscle tension and for treatment of tumours and ulcers (13). The decoction of the root boiled with milk is recommended for curing sterility in women, nervous exhaustion, and spermatorrhoea (14). *W somnifera* is cultivated in the drier parts Manasa,

Neemuch and Jawad tehsils of the Mandsaur District of Madhya Pradesh, Punjab, Sind, Rajasthan and Jammu & Kashmir. The leaves are used as a vegetable and as fodder for livestock (15).

The pharmacological activity of *W. Somnifera* extracts has been summarized by Gupta and Rana. It has been shown to possess antioxidant, anti-inflammatory, antibacterial and antitumoral activities . Methanolic extracts of Withania have been found effective in reducing gastric secretions and total acidity. 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 its steroidal structural framework is manifested by the biological functions of adrenal, sex hormones and bile acids. It provides a template for structural modifications on account of its multifunctionality character. Furthermore, biologically significant motifs are nontoxic, multi drug resistant (MDR) and capable of penetrating the biological membranes. There are a number of reports of natural products with α, β unsaturated carbonyl exhibiting chemo-preventive and chemo-protective activities. 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. Sangwan et 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

1.1 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 A_1-A_7 and A_1^*-A_7* 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 A_1, A_2, A_3, A_4, A_5, A_6 and A_7 were found to be more active with A_4 being most active with IC50 values of 0.02-0.5.

1.2 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 (A) 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 IC50 values.

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

4.1 General methods. 1H and 13C 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.

4.2 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 now18.The isolated compound was coded as A.

4.3 Synthesis of Baylis-Hillman adducts of A (A_1-A_7 and A_1^*-A_7*):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 A_1-A_7 and A_1^*-A_7* adducts as a mixture of two isomers which were separated through column chromatography.

Compound A_1 **:** 1HNMR(400 MHz, CDCl3),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) 13CNMR (100 MHz, CDCl3), 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 A_2**:** 1HNMR(400 MHz, CDCl3),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);13CNMR(100MHz,CDCl3)δ=207.19,161.54,152.73,146.24,143.29,142.55,140.97,139.47,139.05,136.89,135.51,135.32,130.06,129.88,129.13,124.18,123.61,122.77,120.69,119.69,116.08,115.53,114.26,112.65,110.01,93.50,55.96,41.42,35.00,34.43,34.02,32.12,31.84,30.53,30.35,29.89,29.82,29.71,29.56,29.36,29.16

Compound A_3:1HNMR(400 MHz, CDCl3),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); 13CNMR (100 MHz, CDCl3), 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 A_4:1HNMR(400 MHz, CDCl3), 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(4H,m)13CNMR(100MHz,CDCl3)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 A_5 : 1HNMR(400 MHz, CDCl3) 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, CDCl3), 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 A_6: 1HNMR(400 MHz, CDCl3),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) 13CNMR (100 MHz, CDCl3), 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.

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Tissue	Lung			Breast		Colon		Leukemia		Normal cell
Cell line	A-549			MCF-7		HCT-116		THP-1		FR-2
Code	Conc µmol									
A	50 10	95 90	IC50	88 80	IC50	97 93	IC50	90 88	IC50	75 58
A_1	50 10 01 0.5 0.1	96 95 74 50 10	2.6	94 92 70 20 05	2.8	95 92 69 37 09	1	90 88 72 58 40	0.06	68 35 - - -
A_2	50 10 01 0.5 0.1	94 92 68 40 30	0.8	96 95 73 20 03	2	94 92 65 40 23	1.9	92 90 70 58 38	0.07	58 30 - - -
A_3	50 10 01 0.5 0.1	98 96 75 48 09	0.7	95 92 65 25 08	1.8	90 86 60 40 10	1.4	95 93 70 50 35	0.13	45 25 - - -
A_4	50 10 01 0.5 0.1	99 98 78 38 36	0.5	96 97 76 50 32	0.38	98 95 74 51 40	0.07	95 92 68 62 48	0.02	45 40 - - -
A_5	50 10 01 0.5	97 93 72 38	0.8	98 96 68 25	2	98 94 69 38	1.8	97 93 70 50	0.09	60 35 - -

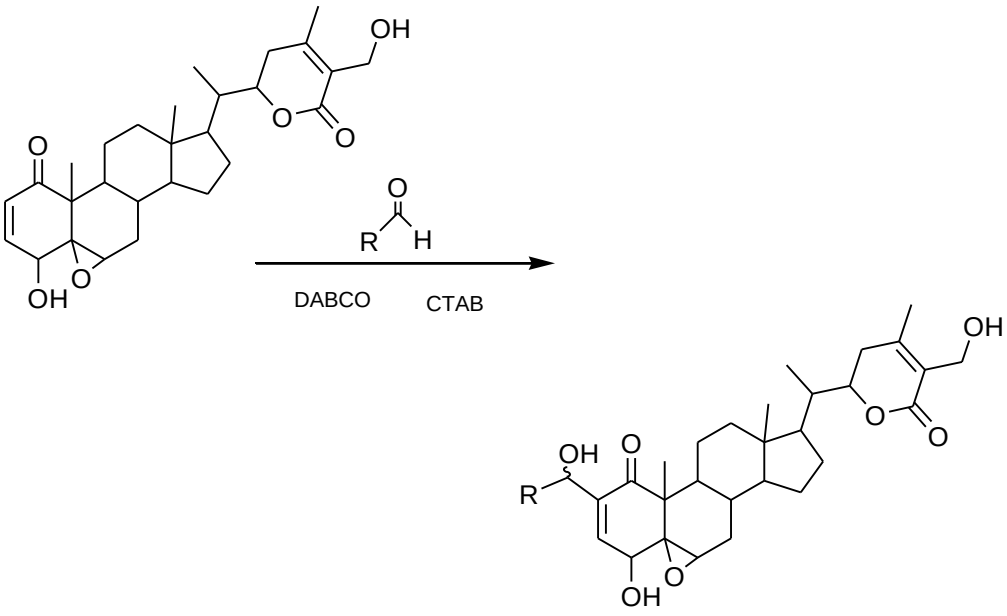
	0.1	29		05		24		42		-
A_6	50 10	99 96		98 94		95 92		96 90		50 40
A_7	50 10	90 87		97 90		98 88		98 95		54 43
5-FU	10	76		68		74		78		58
Mito-C	01	96		74		68		62		45

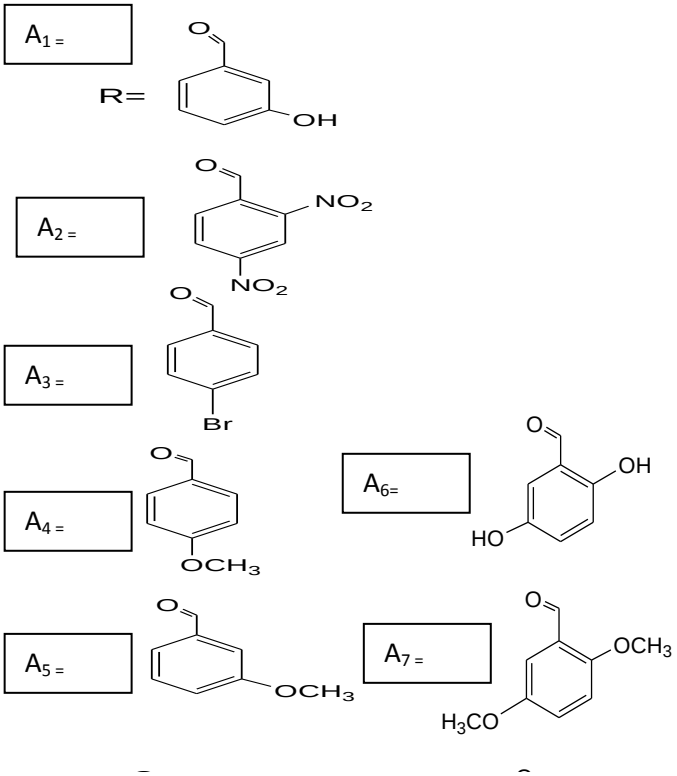
Compound A_7**:** 1HNMR(400 MHz, CDCl3),δ= 7.851 (1H, s), δ= 7.441 (1H, d, j=20.4 Hz), δ= 7.440(1H, d, j=20.3Hz) δ= 6.579 (1H, s),δ= 5.778 (1H, s),δ= 4.892 (1H, m), δ= 4.353 (1H,s) δ= 4.303(6H, m), δ= 3.926 (1H, t), δ= 3.326 (1H, t), δ= 2.924 (1H, t), δ= 2.556 (2H, m), δ=1.999 (7H, m), δ= 1.667 (10H, m), δ= 1.150 (4H, m) 13CNMR (100 MHz, CDCl3), 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.

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

All experiments were carried out in triplicate. 5-FU = 5Fluorouracil , Mitomycin C.

Scheme





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