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ANTIMALARIAL ACTIVITY OF 3-HYDROXYALKYL-2-METHYLENE-PROPIONIC ACID DERIVATIVES

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Abstract: Several Baylis-Hillman adducts and their derivatives were synthesized and evaluated as targeted potential anti-malarials. The compounds **4**, **7** and **9** were found to have highest potency against *P. falciparum in vitro*. The *in vivo* test result of compound **4** and **9** against *P. berghei* demonstrated activity at 80 mg/Kg dose level.

Malaria has been a serious disease throughout recorded history and it continues to be one of the most widely spread health hazards. The causative organism was found to be mainly the dreaded parasitic strains of *P. falciparum*. The efficacy of even the most generally useful anti-malarial drugs is continually challenged by the genetic plasticity of this parasite. Therefore, the discovery of the resistance against chloroquine marked the beginning of the modern era of anti-malarial chemotherapy.

Recently, anti-malarial activity of α,β-unsaturated carbonyl systems[1,2] and 4-quinolino-methanols[3] has been reported. The structural similarity between the Baylis-Hillman (BH) adducts and these class of compounds led us to investigate anti-malarial activity of BH adducts. Continuing our studies in the design and synthesis of novel anti-malarials[4] we report herein the synthesis as well as *in vitro* and *in vivo* anti-malarial activity studies of 2-methylene-3-hydroxyalkyl propionic acid derivatives.

The Baylis-Hillman reaction[5] is an carbon-carbon bond formation reaction between an aldehyde and α,β-unsaturated system in the presence of 1,4-diazabicyclo-2, 2, 2 - octane (DABCO) to produce α-hydroxyalkyl vinyl system, commonly known as Baylis-Hillman (BH) adduct.

Chemistry: The compounds **(1-16)** were synthesized through Baylis-Hillman reaction. The compounds were prepared either at room temperature[5] or under microwave irradiation[6]. The compound **4** was also produced from p-nitrobenzaldehyde, acrylonitrile and DABCO (1:1:0.05 mmol) in 4M NaI/water and tetrahydrofuran solution at room temperature.[7] The compound **14**[8] was obtained from piperonal and acrylonitrile in the presence of DABCO at room temperature. Compound **14** showed the absence of -OH peak and the presence of two cyanide peaks at 2260 & 2240 cm^{-1} in IR and the presence of two triplets

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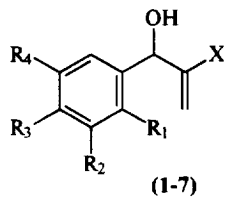
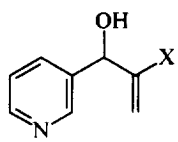
at δ 2.60 & 3.70 in ^{1}H NMR. The compound **15** was obtained through dimerization of methyl vinyl ketone in the presence of DABCO.[9] The compound **16** was prepared from compound **3** by treatment with isopropylamine in methanol at 0-10 °C.[10]

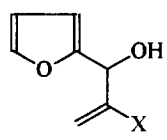


(1-7)

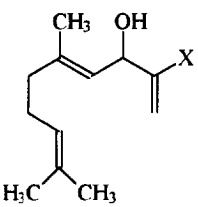
1, $R_1 = R_2 = R_3 = R_4 = H$, $X = CO_2CH_3$
2, $R_1 = R_2 = R_3 = R_4 = H$, $X = CN$
3, $R_1 = R_2 = R_4 = H$, $R_3 = NO_2$, $X = CO_2CH_3$
4, $R_1 = R_2 = R_4 = H$, $R_3 = NO_2$, $X = CN$
5, $R_1 = NO_2$, $R_2 = R_3 = R_4 = H$, $X = CN$
6, $R_1 = R_4 = H$, $R_2 = R_3 = OCH_3$, $X = CN$
7, $R_1 = H$, $R_2 = R_3 = R_4 = OCH_3$, $X = CN$



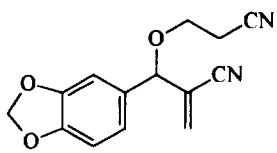
8, $X = CO_2CH_3$
9, $X = CN$



10, $X = CO_2CH_3$
11, $X = CN$



12, $X = CO_2CH_3$
13, $X = CN$



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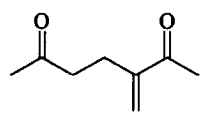

15

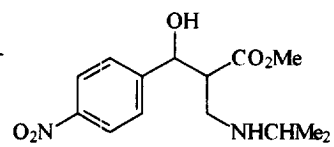

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Fig. 1

Biological Evaluation[11]

Anti-malarial activity: In vitro: Fourteen synthetic compounds (**1-11, 14-16**) were evaluated against *P. falciparum* at different doses starting from 500 μM/well onwards with five fold or two fold serial dilution. Doses were kept constant for all compounds to have a comparative profile. Results obtained from the *in vitro* schizont maturation are listed in **Table 1**. All the compounds displayed anti-malarial activity at 10^{-6} dose level, the compounds **4**, **7** and **9** exhibited more efficacy. The *in vitro* parasite growth inhibition results are summarized in **Table 2**. The most active compounds *in vitro* **4** and

7 were again tested *in vitro* against chloroquine resistant *P. falciparum* strain and the results are summarized in **Table 3and Table 4** respectively.

Table 1: Anti-malarial activity of 3-hydroxyalkyl-2-methylene-propionic acid derivatives (in μmol/well)

% Schizonts Maturation Inhibition (Assay time 24h)											
Compound	500	100	50.0	20.0	10.0	5.00	2.50	1.00	0.50	0.25	0.10
1	----	100	100	100	100	93.0	64.0	43.0	7.00	0.00	0.00
2	----	100	----	85.0	80.0	65.7	62.8	25.7	8.60	0.00	0.00
3	100	81.6	52.6	----	44.7	18.4	----	2.60	----	----	----
4	100	100	100	100	100	100	84.0	75.2	68.0	56.4	41.2
6	100	100	71.0	----	42.0	21.0	----	5.26	0.00	0.00	0.00
7	100	100	100	100	100	82.4	76.5	64.0	42.4	30.0	0.00
8	----	100	100	100	100	100	64.0	43.0	29.0	5.00	0.00
9	100	100	100	100	100	100	100	93.0	50.0	12.0	0.00
14	----	100	100	100	93.0	64.0	57.0	43.0	36.0	6.00	0.00
15	100	100	100	----	100	100	----	52.6	----	----	----
16	----	100	100	80.0	29.0	29.0	25.0	14.0	0.00	0.00	0.00
Chloroquine phosphate (pmol/well)					64	32	16	8	4	2	1
% Schizonts Maturation Inhibition					100	100	100	86.4	39.4	16.0	0.00

Table 2: Anti-malarial activity of 3-hydroxyalkyl-2-methylene-propionic acid derivatives (in μmol/well)

Total Parasite Growth Inhibition (% PGI, Assay Time 72h)										
Compound	100	50	25	20	10	5.0	2.5	1.0	0.5	0.25
1	100	100	----	100	100	82.5	62.5	40.0	7.50	0.00
2	100	----	85.0	----	80.0	65.7	62.8	25.7	8.60	----
4	100	100	100	100	100	100	100	100	92.0	83.0
5	100	----	----	100	100	100	77.2	18.0	----	0.00
7	100	100	100	100	100	100	100	94.0	86.0	73.0
8	100	100	----	100	100	92.5	75.0	50.0	25.0	5.00
9	100	100	----	100	97.5	90.0	82.5	77.5	55.0	20.0
10	100	----	100	----	98.0	85.7	42.8	20.0	----	0.00
11	100	----	84.0	----	79.0	68.6	40.0	31.4	14.0	----
14	100	100	----	95.0	90.0	52.5	40.0	25.0	10.0	0.00
15	100	----	100	----	100	88.6	65.7	8.60	----	----
16	100	100	----	92.5	42.5	25.0	15.0	10.0	0.00	0.00
Chloroquine phosphate (pmol/well)				64	32	16	8.0	4.0	2.0	1.0
Total Parasite Growth Inhibition				100	100	100	100	91.7	58.3	16.7

In vivo activity: The compounds **1**, **4**, **7**, **9**, **12-14** and **16** were tested *in vivo* against *P. berghei* in mice. On comparison of activities (**Table 5**) the compound **4** and **9** were found to be most potent. Both the compounds showed activity at 80 mg/Kg dose level.

Table 3: Anti-malarial activity against chloroquine resistant *P.falciparum* (in μmol/well)

% Schizonts Maturation Inhibition (Assay time 24h)											
Compound	500	100	50.0	20.0	10.0	5.00	2.50	1.00	0.50	0.25	0.10
4	----	100	100	100	100	100	100	86.0	75.0	58.0	47.7
7	----	100	100	100	100	81.0	69.0	65.0	48.6	30.0	4.20
Chloroquine phosphate (pmol/well)					64	32	16	8.0	4.0	2.0	1.0
% Schizonts Maturation Inhibition					100	100	100	86.8	39.4	16.0	0.00

Table 4: Anti-malarial activity against chloroquine resistant *P. falciparum* (in μmol/well)

Total Parasite Growth Inhibition (% PGI, Assay Time 72h)										
Compound	100	50	25	20	10	5.0	2.5	1.0	0.5	0.25
4	100	100	100	100	100	100	100	94.0	82.0	73.0
7	100	100	100	100	100	100	100	90.0	81.0	70.0
Chloroquine phosphate (pmol/well)				64	32	16	8.0	4.0	2.0	1.0
Total Parasite Growth Inhibition				100	100	100	100	91.7	58.3	16.7

Table 5: Anti-malarial activity of selected compounds against P. berghei

Compounds	Untreated control (days)	Mean Survival Time (days)				Remarks
Doses mg/kg		20	40	80	160	
1	6.2	6.0	7.0	10.2	12.0	Inactive
4	6.0	9.4	11.8	12.8	14.4	Active at 80 & 160mg/kg
7	6.2	6.0	7.6	10.4	12.4	Active at 160 mg/kg
9	6.2	6.4	8.8	13.4	15.2	Active at 80 & 160 mg/kg
12	6.2	7.2	8.4	9.6	11.4	Inactive
13	6.2	7.3	8.6	9.8	11.6	Inactive
14	6.2	7.8	9.2	11.4	14.2	Active at 160mg/kg
16	6.2	7.0	8.2	9.8	11.8	Inactive

In conclusion, 3-hydroxyalkyl-2-methylene propionic acid derivatives are found to be novel anti-malarials among which the compound 4 and 9 has the highest potency. Further studies on these adducts and their derivatives are in progress.

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7. *Compound* **4** (1.53 g, 38%), mp: 71-73 °C. IR (neat) cm^{-1}: 3440, 2260, 1610, 1530. ^1H NMR ($CDCl_3$ + D_2O) δ: 8.25 (d, J=~8 Hz, 2H, aromatic H), 7.70 (d, J=~8 Hz, 2H, aromatic H), 6.15 (d, J=6 Hz, 2H, olefinic H), 5.48 (bs, 1H, allylic H), 3.08 (s, 1H, -O<u>H</u>).
8. *Synthesis of [3-cyanoethoxy-3-{(3',4'-methylene-dioxy)-phenyl}-2-methylene]propionitrile (14):* To a stirred mixture of piperonal (600 mg, 4.0 mmol) and acrylonitrile (5 mL, 6.25 g, 117.9 mmol) was added DABCO (240 mg, 2.14 mmol) and the resultant reaction mixture was allowed to stir for 5 days at room temperature. Usual workup followed by removal of excess acrylonitrile in vacuo, extraction with ethyl acetate, washing with brine, drying over sodium sulfate, removal of the organic layer in vacuo and purification by column chromatography over silica gel with petroleum ether/ethyl acetate as eluant gave **14** (560 mg, 56%). IR (neat) cm^{-1}: 2260, 2240, 1620. ^1H NMR ($CDCl_3$ + D_2O) δ: 6.85 (s, 3H, aromatic H), 6.15-5.95 (m, 4H, 2 olefinic H and 2 -O-C<u>H</u>$_2$-O- overlapping signals), 4.85 (s, 1H, allylic H), 3.70 (t, 2H, -OC<u>H</u>$_2$-), 2.60 (t, 2H, -C<u>H</u>$_2$-CN).
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11. ***Biological Methods:*** *In Vitro: P. falciparum culture:* Three *P. falciparum* strains were obtained from well adapted *in vitro* culture lines. Two chloroquine sensitive isolates like FSJ-A6 (Shahjahanpur, U.P.) and FJB-D2 (Jabalpur, M.P.) collected from patients in 1987 and 1988 respectively, were adapted and maintained *in vitro*. The third parasite line was the chloroquine resistant *P. falciparum* clone T-9/106 from Thailand. Parasites were cultured in O(+) erythrocytes in RPMI-1640 media supplemented with 25 mM HEPES buffer and 10% AB(+) serum by candlejar technique.[12] Initial culture was maintained in small vials (2.5 cm dia.) with 10% haematocrit, i.e. 10 µl erythrocytes containing 1.5% ring stage parasite in 100 µl complete media. The culture volume per well for the assay was 100 µl. Parasitamia was determined for each set of culture, number of parasites for the assay were adjusted at 1 to 1.5% by diluting with fresh O(+) RBC. Assay was done in 96 well microtitre flat bottomed tissue culture plates. Parasite culture was synchronized at ring forms using density gradient method[13] and cultured for 24h. in the presence of various doses of compounds **(1-11, 14-16)** and chloroquine for their effect in schizont maturation. Test was done in duplicate wells for each dose of the drugs. Control culture was done with RPMI-164 containing 10% AB(+) serum. Growth of the parasites from duplicate wells of each concentrations was monitored in JSB stained[14] blood smears by counting number of schizont per 200 asexual parasites. Percent schizont maturation inhibition was calculated by the formula: $(1-N_t/N_c)x100$ where, N_t and N_c represents the number of schizont in the test and control well respectively.

In Vivo: Rane's Test: The compounds were evaluated for their activity against virulent strains of *P. berghei yoelli* (NK 65) using Rane's schizontocidal method described by Osdane et al.[15] Four weeks old mice weighing 20-25g each received intraperitoneal innoculum of $1x10^{-6}$ parasitized *P. berghei* red cells. The test solutions of synthesized compounds in distilled water were prepared by homogenization with 2 drops of 1% Tween-80 and injected once subcutaneously 72h post infections. A control group of infected mice was not administered any drug was kept as untreated control. The dose range selected was 20, 40, 80 and 160 mg/Kg and a minimum of five mice per dose were used. Artemisinin (20 and 40 mg/Kg), cycloguanilhydrochloride (25 mg/Kg) and DDS (20 mg/Kg) were kept as standard drugs in trial for comparison. Deaths occurring within 24h of treatment classified as death due to toxicity. All mice receiving synthetic compounds showed survival time of 12-18 days. Testing was evaluated by calculating mean survival time (MST) of the treated and controlled group of mice.

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