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Anti-malarial activity of Baylis–Hillman adducts from substituted 2-chloronicotinaldehydes☆

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Abstract—New Baylis–Hillman adducts are synthesized based on substituted 2-chloronicotinaldehydes and screened for their in vitro anti-malarial activity against chloroquine sensitive and chloroquine resistant *Plasmodium falciparum*. Out of the six new compounds synthesized and screened, **2b**, **2c** and **2d** compounds showed substantial anti-malarial activity.

Malaria remains a major problem exacting an unacceptable toll on the health and economic welfare of the world's poorest communities. Over 200–500 million cases and 0.7–2.7 million deaths occur each year due to malaria.[1–3] Every 30 s one child is killed by this disease. Malaria in pregnancy kills up to 2,00,000 newborn babies each year. *Plasmodium falciparum* and *Plasmodium vivax* are the two major human malarial parasites of which *P. falciparum* is responsible for most of the malarial deaths. Over the years chloroquine has been used as an anti-malarial drug due to its availability, effectiveness and low toxicity. Presently it is found by many users that chloroquine is no longer effective in most of the world because of the resistance to it that has developed.[4] The mechanism of chloroquine resistance in *Plasmodium* was first reported by Donald Krogstad.[5] The development of safe and effective anti-malarial agents has been realized as a challenge in recent years because of the rapid spread of drug resistant *P. falciparum* strains.[6] Recently, several groups have contributed to the development of elegant anti-malarial agents.[7–9] Mrinal et al.[10] reported the anti-malarial activity of Baylis–Hillman adducts. Our interest is in the design and synthesis of a diverse range of biologically active heterocyclic compounds,[11] and to this end we have developed a new method for the synthesis of substituted 2-chloronicotinaldehyde[12] from enamide; these substituted 2-chloronicotinaldehydes served as synthons for the present Baylis–Hillman (BH) adducts prepared. In this paper, we report the synthesis and anti-malarial activity of new Baylis–Hillman (BH) adducts.

The new compounds **2a**–**2f** were synthesized by using the Baylis–Hillman reaction[13] between substituted 2-chloronicotinaldehydes **1a**–**1d** and acrylonitrile or methyl acrylate (Scheme 1 and Table 1). Substituted 2-chloronicotinaldehydes are found to be highly active towards Baylis–Hillman reaction and the reaction went to completion within 10–15 min with excellent yields (>96%; Table 1). It is known that the protic solvents or water accelerate the Baylis–Hillman reaction, either through stabilization of the enolate by hydrogen bonding or by activation of aldehyde, again through hydrogen bonding or indeed both.[14] More interestingly, the reaction is completed within 10 min under neat conditions (without any added solvent) with excellent yields.[15,16] Until now this was the fastest Baylis–Hillman reaction reported with respect to substrate aldehyde. The Baylis–Hillman reaction using substituted 2-chloronicotinaldehydes was also examined using various organic bases like DABCO, DBU, DMAP, 3-hydroxy quinuclidine (HQD), etc. Among these, except for DMAP, all the remaining bases were found to work very well at room temperature. Crystal structure solved for one of the compounds **2c** is given in Figure 1.[17]

The compounds **2a**–**2f** were evaluated for in vitro anti-malarial activity[18] against both chloroquine sensitive (FDL-B) and chloroquine resistant (FDL-NG) strains

Keywords: Substituted 2-chloronicotinaldehydes; Baylis–Hillman adducts; Anti-malarial activity.
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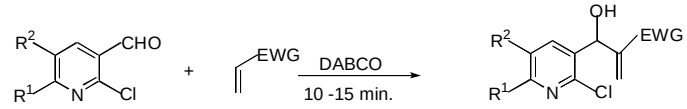
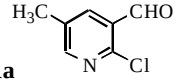
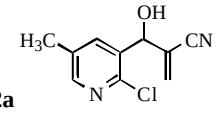
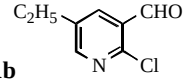
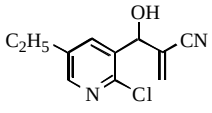
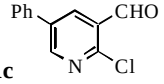
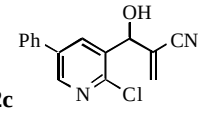
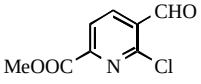
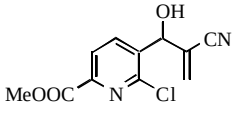
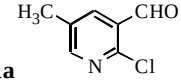
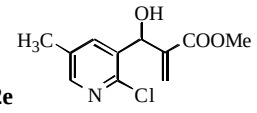
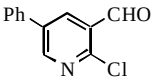
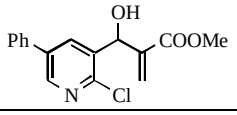


1a : R^1 = H, R^2 = CH_3,
1b : R^1 = H, R^2 = C_2H_5,
1c : R^1 = H, R^2 = Ph,
1d : R^1 = COOMe, R^2 = H

2a: R^1 = H, R^2 = CH_3, EWG = CN
2b: R^1 = H, R^2 = C_2H_5, EWG = CN
2c: R^1 = H, R^2 =Ph, EWG = CN
2d: R^1 = COOMe, R^2 =H, EWG = CN
2e: R^1 = H, R^2 = CH_3, EWG = COOMe
2f: R^1 = H, R^2 = Ph, EWG = COOMe

Scheme 1.

Table 1. Synthesis of Baylis-Hillman (BH) adducts

No.	Substituted 2-chloronicotinaldehyde (**1a**–**d**)	Activated alkene	BH adduct (**2a**–**2f**)	Mp (°C)	Yield[a] (%)
1	 **1a**		 **2a**	143	98
2	 **1b**		 **2b**	96	98
3	 **1c**		 **2c**	136	98
4	 **1d**		 **2d**	88	98
5	 **1a**		 **2e**	92	97
6	 **1c**		 **2f**	86	97

[a] Isolated yields.

of *P. falciparum* at different doses starting from 100 μg/well (500 μg/ml) onwards with 5-fold serial dilutions up to 0.0064 μg/well (0.032 μg/ml). Doses were kept constant for all compounds to have a comparative profile of their activities. Results obtained from the in vitro schizont maturation inhibition (SMI) and total parasite growth inhibition (PGI) of both FDL-B and FDL-NG strains are summarized in Tables 2 and 3. Among all the six compounds screened, **2b**, **2c** and **2d** exhibited significant anti-malarial activity with low IC_{50} and IC_{90} values in both chloroquine sensitive and chloroquine resistant strains.

The compounds studied for anti-malarial activity showed a good structure–activity relationship. The Baylis–Hillman adduct **2c** with electron-withdrawing cyano and R^2 as phenyl groups exhibited substantial anti-malarial activity against both strains. The IC_{50} values of **2c** for resistant strain are 0.9 and 2.2 μg/ml for schizont maturation inhibition (SMI) and total parasite growth inhibition (PGI), respectively. Changing the substitution of cyano to methyl ester in compound **2f** (R^2 remains phenyl only) showed a relatively lower anti-malarial activity in the resistant strain and its IC_{50} values are 5 and 10.75 μg/ml for SMI and PGI. The compounds **2b** and **2d** having a cyano group along with different R^1 and R^2 groups exhibited good anti-malarial activity. The remaining compounds also displayed comparable activity. These results show that the 2-chloronicotinaldehyde-based Baylis–Hillman adducts having a cyano group exhibit anti-malarial activity and further their activity is improved with the phenyl group as R^2 substituent rather than R^2 as alkyl group.

In conclusion, six new Baylis–Hillman adducts based on substituted 2-chloronicotinaldehydes were synthesized. All six compounds were found to exhibit anti-malarial

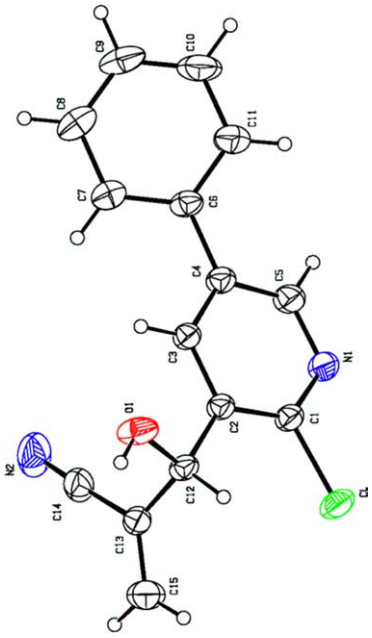


Figure 1. Crystal structure of **2c**.

Table 2. Anti-malarial activities, IC_{50} and IC_{90} of BH adducts **2a**–**2f**, against chloroquine sensitive (CQS) *Plasmodium falciparum* strain (FDL-B)

Compounds	Inhibitory activity in μg/ml			
	IC_{50}		IC_{90}	
	SMI	PGI	SMI	PGI
2a	1.25	1.3	25.5	85
2b	1.8	4	15.5	31
2c	3.6	8	25.5	23
2d	3.4	7	22	29
2e	18	28.5	32	115
2f	45	115	125	350
Chloroquine	0.004	0.005	0.018	0.02

SMI, schizont maturation inhibition determined after 24 h; PGI, total parasite growth inhibition determined after 48 h.

Table 3. Anti-malarial activities, IC_{50} and IC_{90} of BH adducts **2a**–**2f**, against chloroquine resistant (CQR) *Plasmodium falciparum* strain (FDL-NG)

Compounds	Inhibitory activity in μg/ml			
	IC_{50}		IC_{90}	
	SMI	PGI	SMI	PGI
2a	22.5	10.5	125	300
2b	2.5	5.5	6.5	26
2c	0.9	2.2	1.5	6.75
2d	5.75	6.75	25.5	29
2e	3.05	5.75	7	27.5
2f	5	10.75	30	77.5

SMI, schizont maturation inhibition determined after 24 h; PGI, total parasite growth inhibition determined after 48 h.

activity. Interestingly, the compounds **2b**, **2c** and **2d** displayed a relatively significant activity. Evaluation of activity results informs us that cyano- and phenyl groups on the Baylis–Hillman adducts play a role in determining the activity. These molecules are very useful for further optimization work in malarial chemotherapy.

Acknowledgments

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15. General procedure for the synthesis of **2a**: to a mixture of 2-chloro-5- methylnicotinaldehyde **1a** (10 mmol; 1.55 g) and DABCO (10 mmol; 1.12 g) was added to acrylonitrile (60 mmol) at room temperature and the progress of the reaction was monitored by TLC. Upon completion of the reaction (~10 min) the reaction mixture was diluted with diethyl ether (300 ml) and washed with water 3× 50 ml. The organic layer was dried over Na_2SO_4, and concentrated and the residue was subjected to column chromatography over silica gel, eluting with ethyl acetate and hexane (2:8, v/v) to give the desired product in 98%

(1.98 g). The same procedure was adopted to synthesize the remaining compounds **2b**–**2f**.

16. Physical and spectral data for all the six new compounds **2a**–**2f**:
*2-[(2-Chloro-5-methylpyridine-3-yl) (hydroxy) methyl] acrylonitrile **2a***: mp: 143 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.2 (s, 1H), 7.78 (s, 1H), 6.08 (s, 1H), 6.09 (s, 1H), 5.64 (s, 1H), 2.40 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 148.4, 145.3, 137.0, 133.0, 132.2, 130.3, 124.1, 115.8, 68.6, 16.8; MS (EI), *m/z*: 208 [M+], 156, 120, 93, 65; IR (KBr): 3185, 2968, 2243, 1572, 1425, 1059, 971, 788 cm^{-1}; Anal. Calcd for $C_{10}H_9ClN_2O$: C, 57.57; H, 4.34; N, 13.43. Found: C, 57.85; H, 4.68; N, 13.52.
*2-[(2-Chloro-5-ethylpyridine-3-yl) (hydroxy) methyl] acrylonitrile **2b**:* mp: 96 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.18 (s, 1H), 7.82 (s, 1H), 6.1 (s, 1H), 6.08 (s, 1H), 5.66 (s, 1H), 2.72 (q, 2H), 1.3 (t, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 148.7, 146.4, 139.5, 136.9, 133.3, 131.6, 124.5, 116.2, 70.1, 25.3, 14.8; MS (EI), *m/z*: 222 [M+], 170, 134, 106, 77; IR (KBr): 3159, 2979, 2222, 1572, 1432, 1402, 1070, 963, 790 cm^{-1}; Anal. Calcd for $C_{11}H_{11}ClN_2O$: C, 59.31; H, 5.02; N, 12.58. Found: C, 59.70; H, 5.33; N, 12.75.
*2-[(2-Chloro-5-phenylpyridine-3-yl)(hydroxy)methyl] acrylonitrile **2c**:* mp: 136 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.58 (s, 1H), 8.2 (s, 1H), 7.4–7.6 (m, 5H), 6.14 (s, 1H), 6.1 (s, 1H), 5.66 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 147.7, 136.8, 135.9, 135.6, 133.3, 132.1, 129.3, 128.8, 127.2, 124.1, 116.2, 70.4; MS (EI), *m/z*: 270 [M+], 245, 218, 182, 154, 141, 115, 77; IR (KBr): 3331, 2930, 2363, 1680, 1530, 1460, 1255, 1057, 749 cm^{-1}; Anal. Calcd for $C_{15}H_{11}ClN_2O$: C, 66.53; H, 4.13; N, 10.34. Found: C, 66.67; H, 4.38; N, 10.70.
*Methyl 6-chloro-5-(2-cyano-1-hydroxyprop-2-en-1-yl)pyridine-2-carboxylate **2d**:* mp: 88 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.15 (d, 1H), 8.22 (d, 1H), 6.1 (d, 2H), 5.73 (d, 1H), 3.98 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 164.0, 149.1, 147.3, 138.4, 138.0, 132.6, 124.4, 123.5, 116.0, 70.0, 53.2; MS (EI), *m/z*: 252 [M+], 222, 200, 194, 164, 112, 76; IR (KBr): 3435, 3016, 2229, 1731, 1563, 1368, 1311, 1140, 1051, 759 cm^{-1}; Anal. Calcd for $C_{11}H_9ClN_2O_3$: C, 52.29; H, 3.59; N, 11.09. Found: C, 52.56; H, 3.68; N, 11.34.
*Methyl 2-[(2-chloro-5-methylpyridine-3-yl)(hydroxy) methyl] acrylate **2e**:* mp: 92 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.71 (s, 1H), 6.32 (s, 1H), 5.8 (s, 1H), 5.56 (s, 1H), 3.8 (s, 3H), 2.35 (s,3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.1, 148.2, 146.5, 140.2, 137.8, 134.8, 132.4, 126.7, 67.9, 51.7, 17.3; MS (EI), *m/z*: 241 [M+], 226, 206, 156, 146, 120, 92; IR (KBr): 3350, 2953, 1727, 1433, 1053, 972, 753 cm^{-1}; Anal. Calcd for $C_{11}H_{12}ClNO_3$: C, 54.68; H, 4.99; N, 5.80. Found: C, 54.86; H, 5.10; N, 5.98.
*Methyl 2-[(2-chloro-5-phenylpyridine-3-yl)(hydroxy) methyl] acrylate **2f**:* mp: 86 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.48 (s, 1H), 8.12 (s, 1H), 7.34–7.58 (m, 5H), 6.34 (s, 1H), 5.88 (s, 1H), 5.62 (s, 1H), 4.18 (b, 1H), 3.77 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.6, 148.4, 146.7, 139.9, 136.2, 136.1, 135.7, 135.1, 129.1, 128.4, 127.5, 127.0, 69.0, 52.1; MS (EI), *m/z*: 303 [M+], 268, 236, 218, 182, 153, 127, 115; IR (KBr): 3337, 3075, 2951, 1722, 1430, 1040, 770 cm^{-1}; Anal. Calcd for $C_{16}H_{14}ClNO_3$: C, 63.24; H, 4.68; N, 4.61. Found: C, 63.42; H, 4.88; N, 4.86.
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18. Anti-malarial activity was checked in two well-adopted chloroquine sensitive (CQS) and chloroquine resistant (CQR) in vitro culture lines of *P. falciparum* following the published method.[19] The CQS isolate, FDL-B and CQR isolate, FDL-NG, were collected from patients reported with symptomatic malaria in a local clinic of Delhi in 1994 and 1995, respectively. They were adapted and maintained in vitro by the candle-jar technique.[20] Parasites were cultured in human O+ RBCs in RPMI 1640 media enriched with 10% (v/v) AB+ serum and supplemented with 25 mM Hepes buffer and 25 mM sodium bicarbonate. Assay was done in synchronous culture with ring form at 5% haematocrit containing 1% parasitaemia in a 96-well flat-bottomed tissue culture plate. Compounds were dosed in wells in duplicate at concentrations of 100, 20, 4, 0.8, 0.16, 0.032 and 0.0064 μg/well. The volume of culture per well was kept at 200 μl including media, drug and parasite inoculum. Parasite culture only in enriched media was taken as control. Chloroquine was used as reference anti-malarial for comparison. To determine the activity of various compounds, assay was done in two sets. The first set was to determine the effect of compounds on schizont maturation after 24 h and the second set of assay was to determine the effect on total parasite growth after 48 h. Growth of the parasite from each well was monitored microscopically in Giemsa-stained smears by counting the number of schizonts per 200 asexual parasites and the total number of parasites per 5000 RBCs. Percent schizont maturation inhibition (SMI) and parasite growth inhibition (PGI) were calculated by the formula: $(1-N_t/N_c)\times 100$, where N_t and N_c represent the number of schizont and number of parasites in the test and control wells, respectively. Inhibitory concentrations at 50% (IC_{50}) and 90% (IC_{90}) were calculated.
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