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Isolation of 4-chloro-3-formyl-2-(2-hydroxyethene-1-yl) quinolines by Vilsmeier Haack reaction on quinaldines: Construction of diazepino quinoline heterocycles and their antimicrobial and cytogenetic studies

RAJU NANDHA KUMAR
THANGARAJ SURESH
PALATHURAI SUBRAMINIAM MOHAN*

Department of Chemistry, Bharathiar University, Coimbatore – 641 046, India

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Application of Vilsmeier conditions to 4-hydroxyquinaldines gives 4-chloro-3-formyl-2-(2-hydroxyethene-1-yl)--quinolines as an intermediate. The latter is utilized to prepare diazepino quinolines on treatment with phenylhydrazine hydrochloride. All the synthesized compounds have been screened for their antibacterial and antifungal as well as cytogenetic activities.

Keywords: quinaldines, Vilsmeier Haack reaction, 4-chloro-3-formyl-2-(2-hydroxyethene-1-yl)quinolines, diazepinoquinolines, antimicrobial activity, cytogenetic activity

Literature search of the last decade reveals sustained interest in the application of the Vilsmeier Haack reagent in organic synthesis. It has been proven to be a mild and efficient reagent for the formylation of reactive aromatic and heteroaromatic substrates (1–3). The versatility of the reagent has been further extended to an activating agent for acylhalo addition (4) and annelation (5–7). Besides aromatic formylation, a wide variety of alkene derivatives (8), carbonyl compounds (9), activated methyl and methylene groups (10) as well as oxygen and nitrogen nucleophiles (11) efficiently react with the Vilsmeier Haack reagent to yield the corresponding iminium salts. The intramolecular cyclization potential of halomethyleniminium salts formed under the Vilsmeier condition and microwave-induced Vilsmeier conditions has been reported (12–18). The classical Vilsmeier Haack reaction involves electrophilic substitution of an activated aromatic ring with a halomethyleniminium salt to yield the corresponding iminium species, which facilitates its inclusion into a large number of novel heterocyclic systems.

In this report we aim to proceed with the iminium species to get the hitherto unknown quinoline intermediate, as this would be a part of a synthetic strategy for the construction of [*c*] annelated nitrogen and oxygen heterocycles *via* 4-chloro-3-formylquinaldines obtainable from 4-hydroxyquinaldines (19) by the Vilsmeier Haack reaction, and for evaluating its pharmacological studies.

* Correspondence, e-mail: ps_mohan_in @yahoo.com

EXPERIMENTAL

Thin-layer chromatography was used to access the reactions and the purity of products. Melting points were determined on a Boetius Microheating Table (Japan) and are uncorrected. IR spectra were recorded with a Shimadzu – 8201FT instrument (Japan) in KBr discs and only noteworthy absorption levels (reciprocal centimeter) are listed. ^{1}H NMR spectra were recorded with a Bruker-AMX-400 MHz spectrometer (USA) in $CDCl_3$ solution; chemical shifts are expressed in ppm (δ) relative to TMS, coupling constants (*J*) in Hz. Signal multiplicities are represented by bs (broad singlet), s (singlet), d (doublet), t (triplet) and m (multiplet). ^{13}C NMR spectra were recorded on the Bruker-AMX-400 MHz spectrometer in $CDCl_3$ with TMS as internal standard. Mass spectra were recorded using a Jeol-D-300 mass spectrometer (70 eV) (Japan). CHN analyses were carried out on a Perkin-Elmer Model 240 analyzer (UK).

Vilsmeier Haack reaction on 4-hydroxyquinaldine

The Vilsmeier reagent was prepared by adding 3.86 mL (0.05 mol) *N*,*N*-dimethylformamide in a round bottomed flask in an ice-cold condition (0–5 °C) under constant stirring. To this, 13.04 mL (0.014 mol) phosphorus oxychloride was added dropwise over a period of half-an-hour and the resultant mixture was stirred for a further hour. Appropriate 4-hydroxyquinaldines (**1a-e)** were added to the Vilsmeier reagent and stirred for further half an hour and the reaction mixture was kept on a water bath at 100 °C for the period of time stated in Table I. After the reaction was completed (TLC monitoring), the reaction mixture was poured into 500 g of crushed ice under constant manual stirring. The reaction mixture was kept aside overnight. After neutralization with 4 mol L^{-1} sodium hydroxide solution, the precipitate obtained was washed well with water and ex-

*Table I. Vilsmeier Haack reaction of 4-hydroxyquinaldines (**1a-e**) and synthesis of diazepino[4,5-b]quinolines (**8a-e**)*

Substrate	R_1	R_2	R_3	R_4	Reaction time (h)	Yield[b] (%)			
						2	**3**	**4**	**8**
1a	H	H	H	H	15	70	15	10	–
1b	CH_3	H	H	H	12.5	73.5	18	5	–
1c	H	CH_3	H	H	16	55	12	17	–
1d	H	H	Cl	H	17.5	78	12	5	–
1e	CH_3	H	H	CH_3	20	65	10	15	–
2a	H	H	H	H	3	–	–	–	90
2b	CH_3	H	H	H	2.5	–	–	–	85
2c	H	CH_3	H	H	2	–	–	–	95
2d	H	H	Cl	H	1.5	–	–	–	60
2e	CH_3	H	H	CH_3	3	–	–	–	70

[a] All reactions were carried out at 100 °C.
[b] Yields after column chromatography on silica gel.

Table II. Analytical data for compounds **2a-e**

Compd. No.	M.p. (°C)	IR (KBr) ν_{max} (cm^{-1})	Analysis (%) Calculated/found			MS (70 eV) M^+ (m/z)	^1H NMR ($CDCl_3$) (δ, ppm)	^{13}C NMR ($CDCl_3$, 400 MHz, δ, ppm)
			C	H	N			
2a	155	3438 1664 1595	61.69 61.62	3.45 3.38	5.99 5.92	233	7.6 (t, 1H, C_7–H, *J* = 8.3 Hz), 7.7 (d, 1H, C_8–H, J = 8.1 Hz), 7.9 (t, 1H, C_6–H, *J* = 7.3 Hz), 8.2 (d, 1H, C_5–H, *J* = 8.3 Hz), 9.2 (s, 1H, C_3–CHO), 9.4 and 9.6 (2s, 2H, vinylic protons), 16.5 (bs, vinylic–OH, D_2O exchangable)	192.42, 189.33, 189.08, 146.24, 137.30, 135.90, 133.32, 126.93, 125.21, 122.61, 119.32, 118.99
2b	132	3450 1660 1595	63.04 62.02	4.07 3.98	5.66 5.63	247	2.8 (s, 3H, CH_3), 7.5 (t, 1H, C_6–H, *J* = 7.92 Hz), 7.7 (d, 1H, C_7–H, *J* = 7.16 Hz), 8.1 (d, 1H, C_5–H, J = 8.24 Hz), 9.2 (s, 1H, C_3–CHO), 9.4 and 9.5 (2s, 2H, vinylic protons), 16.5 (bs, vinylic–OH, D_2O exchangable)	192.36, 189.45, 89.01, 147.22, 136.95, 33.95, 130.23, 126.61, 26.39, 122.99, 121.96, 18.59, 18.46
2c	148	3480 1670 1590	63.04 62.98	4.07 4.01	5.66 5.59	247	2.6 (s, 3H, CH_3), 7.6 (d, 1H, C_6–H, *J* = 7.56 Hz), 7.8 (s, 1H, C_8–H), 8.1 (d, 1H, C_5–H, *J* = 8.16 Hz), 9.2 (s, 1H, –CHO), 9.3 and 9.5 (2s, 2H, vinylic protons), 16.5 (bs, vinylic–OH, D_2O exchangable)	192.28, 189.41, 189.09, 145.26, 135.68, 134.06, 131.22, 126.28, 126.05, 122.75, 22.34, 119.70, 19.52
2d	180	3470 1670 1590	53.76 53.71	2.63 2.65	5.26 5.22	269	7.3 (d, 1H, C_7–H, *J* = 7.68 Hz), 7.5 (d, 1H, C_8–H, *J* = 7.96 Hz), 8.1 (s, 1H, C_5–H), 9.2 (s, 1H, C_3–CHO), 9.3 and 9.5 (2s, 2H, vinylic protons), 16.5 (bs, vinylic–OH, D_2O exchangable)	192.27, 189.26, 189.08, 133.90, 133.21, 129.80, 129.04, 124.41, 121.13, 120.86, 19.87, 118.34
2e	170	3525 1680 1595	64.25 64.21	4.62 4.57	5.35 5.28	261	2.6 (s, 6H, 2xCH_3), 7.6 (d, 1H, C_6–H, *J* = 7.46 Hz), 7.9 (d, 1H, C_7–H, *J* = 7.96 Hz), 9.3 (s, 1H, –CHO), 9.4 and 9.5 (2s, 2H, vinylic protons), 16.5 (bs, vinylic–OH, D_2O exchangable)	192.34, 189.38, 189.07, 144.34, 136.38, 133.86, 130.95, 127.54, 126.01, 123.11, 122.45, 118.60, 19.80, 8.95

*Table III. Analytical data for compounds **3a-e**, **4a-e** and **8a-e***

Compd. No.	M.p. (°C)	IR (KBr) ν_{max} (cm^{-1})	Analysis (%) Calculated/found			MS (70 eV) M^+ (m/z)	^{1}H NMR ($CDCl_3$, δ, ppm)
			C	H	N		
3a	140	3520 1695 1610	70.58 70.54	4.85 4.81	7.48 7.43	187	2.4 (s, 3H, CH_3), 7.3 (d, 1H, C_8-H, *J* = 7.84 Hz), 7.5 (t, 1H, C_7–H, *J* = 7.58 Hz), 7.7 (t, 1H, C_6–H, *J* = 7.6 Hz), 8.2 (d, 1H, C_5–H, *J* = 8.28 Hz), 9.4 (s, 1H, CHO), 14.1 (bs, 1H, OH)
3b	195	3500 1710 1590	71.63 71.58	5.51 5.44	6.96 6.98	201	2.6 (s, 6H, 2xCH_3), 7.4 (t, 1H, C_6–H, *J* = 7.6 Hz), 7.6 (d, 1H, C_7–H, *J* = 7.28 Hz), 8.0 (d, 1H, C_5–H, *J* = 8.24 Hz), 9.3 (s, 1H, CHO), 15.3 (bs, 1H, OH)
3c	215	3350 1700 1600	71.63 71.61	5.51 5.46	6.96 6.94	201	2.5 (s, 6H, 2xCH_3), 7.6 (d, 1H, C_6–H, *J* = 7.14 Hz), 7.8 (s, 1H, C_8–H), 8.0 (d, 1H, C_5–H, *J* = 8.20 Hz), 9.4 (s, 1H, CHO), 15.1 (bs, 1H, OH)
3d	230	3480 1715 1595	59.61 59.52	3.64 3.57	6.32 6.27	222	2.5 (s, 3H, CH_3), 7.4 (d, 1H, C_7–H, *J* = 8.76 Hz), 7.7 (d, 1H, C_8–H, *J* = 7.64 Hz), 8.2 (s, 1H, C_5–H), 9.4 (s, 1H, CHO), 14.2 (bs, 1H, OH)
3e	155	3510 1702 1610	72.54 72.47	6.09 6.01	6.51 6.42	215	2.7 (s, 9H, 3xCH_3), 7.5 (d, 1H, C_6–H, *J* = 7.84 Hz), 7.7 (d, 1H, C_7–H, *J* = 7.68 Hz), 9.2 (s, 1H, CHO), 14.5 (bs, 1H, OH)
4a	65	1590	67.62 67.54	4.54 4.41	7.89 7.81	177	2.5 (s, 3H, CH_3), 7.3 (s, 1H, C_3–H), 8.1 (d, 1H, C_5–H, *J* = 8.2 Hz), 7.7 (t, 1H, C_6–H, *J* = 7.54 Hz), 7.9 (t, 1H, C_7–H, *J* = 7.82 Hz), 7.5 (d, 1H, C_8–H, *J* = 8.14 Hz)
4b	47	1570	68.94 68.85	5.26 5.38	7.31 7.49	191	2.7 (s, 3H, C_2–CH_3), 2.8 (s, 3H, C_8–CH_3), 7.4 (s, 1H, C_3–H), 8.1 (d, 1H, C_5–H, *J* = 7.92 Hz), 7.4 (t, 1H, C_6–H, *J* = 7.2 Hz), 7.6 (d, 1H, C_7–H, *J* = 6.28 Hz)
4c	92	1585	68.94 68.82	5.26 5.12	7.31 7.23	191	2.6 (s, 6H, 2xCH_3), 7.3 (s, 1H, C_3–H), 7.5 (s, 1H, C_8–H), 7.8 (d, 1H, C_6–H, *J* = 7.24 Hz), 8.1 (d, 1H, C_5–H, *J* = 8.06 Hz)

Compd. No.	M.p. (°C)	IR (KBr) ν_{max} (cm^{-1})	Analysis (%) Calculated/found			MS (70 eV) M^+ (m/z)	^1H NMR ($CDCl_3$, δ, ppm)
			C	H	N		
4d	74	1575	56.63 56.64	3.33 3.27	6.61 6.74	211	2.4 (s, 3H, CH_3), 7.4 (s, 1H, C_3–H), 7.7–8.0 (m, 3H, Ar–H)
4e	80	1580	70.07 70.19	5.88 5.81	6.81 6.93	205	2.6 (s, 9H, 3xCH_3), 7.4 (s, 1H, C_3–H), 7.6–7.8 (m, 2H, Ar–H)
8a	180	1570 1595	70.71 70.65	3.96 3.91	13.74 13.68	305	7.3 (s, 1H, C_1–H), 8.0 (d, 1H, C_{10}–H, *J* = 8.16 Hz), 7.2–7.8 (m, 10H, Ar–H)
8b	148	1565 1590	71.36 71.22	4.41 4.33	13.14 13.06	319	2.8 (s,3H, CH_3), 8.0 (d, 1H, C_{10}–H, *J* = 8.24 Hz), 7.2–7.9 (m, 10H, Ar–H)
8c	165	1570 1585	71.36 71.24	4.41 4.29	13.14 13.02	319	2.7 (s, 3H, CH_3), 8.1 (d, 1H, C_{10}–H, *J* = 7.56 Hz), 7.3–7.9 (m, 10H, Ar–H
8d	172	1580 1595	63.55 63.48	3.26 (3.19	12.35 (12.21	339	8.2 (d, 1H, C_1–H, *J* = 7.92 Hz), 7.1–7.9 (m, 10H, Ar–H)
8e	192	1563 1587	71.96 71.84	4.83 4.75	12.59 12.51	333	2.6 (s, 6H, 2x CH_3), 7.3–8.2 (m, 10H, Ar–H)

tracted using ethyl acetate. The combined organic layers were collected and dried over anhydrous sodium sulfate. The silica gel chromatography of the reaction mixture afforded three products, **2**, **3** and **4**, using petroleum ether/ethyl acetate (85:15, *V*/*V*), petroleum ether/ethyl acetate (94:6, *V*/*V*) and petroleum ether respectively. The products were recrystallized from methanol. The products were identified by elemental analyses and spectral data (Tables II and III).

Preparation of diazepino[4,5-b]quinolines

Phenylhydrazine hydrochloride (0.002 mol) and the appropriate 4-chloro-3-formyl--2-(2-hydroxyethene-1-yl)quinolines (0.002 mol) (**2a-e**) were dissolved in 50 mL of absolute ethanol. The reaction mixture was kept at the reflux temperature for the period of time given in Table I. After completion of the reaction, monitored by TLC, the ethanol was removed under reduced pressure and the residue was extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate and purified by silica gel column chromatography using petroleum ether/ethyl acetate (98:2, *V*/*V*) as eluent. The products were further recrystallized with chloroform and their structures were confirmed by analytical data (Table II).

Antibacterial and antifungal studies

Antibacterial and antifungal activities of all synthesized compounds were screened for the *in vitro* growth inhibitory activity against *Aeromonas hydrophilla, Escherichia coli, Salmonella typhi, Aspergillus flavus* and *Penicillium funiculosum* using the disc diffusion method (20, 21). The bacteria were cultured in a nutrient agar medium and used as inoculum for this study. Bacterial cells were swabbed onto the nutrient agar medium [prepared from NaCl (5.0 g), peptone (5.0 g), beef extract powder (3.0 g) and yeast extract powder (3.0 g), agar (20.0 g) in 100 mL distilled water; pH = 7.5 ± 0.2)] in Petri dishes. The compounds to be tested were dissolved in chloroform to a final concentration of 0.125, 0.25 and 0.5% and soaked in filter paper discs of 5 mm diameter and 1 mm thickness. The discs were placed into the already seeded dishes and incubated at 35 ± 2 °C for 24 hours. The diameter (mm) of the inhibition zone around each disc was measured after 24 hours and the results are listed in Table IV. Streptomycin was used as a standard and the solutions of the same concentration as that of the tested compounds were prepared in $CHCl_3$.

The fungi were cultured in a potato dextrose agar medium (potato 150 g, dextrose 5 g and agar 2 g in 200 mL distilled water). It was poured into sterilized Petri dishes and allowed to solidify. The dishes were inoculated with a spore suspension of *Aspergillus flavus* or *Penicillium funiculosum* (10^6 spores *per* mililiter of medium). The compounds to be tested were dissolved in acetone to a final concentration of 0.5, 1 and 2% and soaked in filter paper discs (Whatman No. 4, 5 mm diameter). The discs were placed on the already seeded dishes and incubated at 35 ± 2 °C for 4 days. After 4 days, the inhibition zone that appeared around the discs in each dish was measured. A solvent-only treated dish and untreated control plate were also maintained in order to calculate the percentage inhibition (Table IV). Carbendazim was used as the standard solutions in concentrations 0.5, 1 and 2%.

Cytogenetic analysis

Cytogenetic analysis was performed for compounds **8a**, **8b** and **8d** in human peripheral blood leucocyte culture. Cultures of leucocytes were obtained from peripheral blood set-up following the method of Hungerford (22, 23). Three experiments were carried out using three non-smoking healthy male donors, aged 23, 24, and 28 years. The compound tested was dissolved in 1% acetone. Four different concentrations of 0.02, 0.2, 2 and 20 $\mu g\ mL^{-1}$ were added to the culture medium (0.1 mL solution of compounds **8a**, **8b**, and **8d** *per* 8 mL of the medium) 0, 24, 48 hours after culture initiation, indicated as treatment for 72, 48 and 24 hours, respectively. Triplicate cultures for each dose from three donors were maintained for the study of chromosomal aberrations. The cultures were incubated at 37 °C for a period of 72 hours. Water and 1% acetone were applied to controls I and II, respectively.

The cultures were shaken periodically three times a day. The dividing cells were arrested in the metaphase, 45 minutes before the culture harvest, by adding 0.05 mL of colchicine solution (w = 0.01%). The contents in the vial were centrifuged at 1000 rpm for 5 minutes at the end of the colchicine treatment. The supernatant was discarded and 7 mL of pre-warmed hypotonic solution (0.075 mol L^{-1} KCl) was added to the cell button.

Table IV. Antibacterial and antifungal activity of compounds ***2a-e*** *and* ***8a-e***

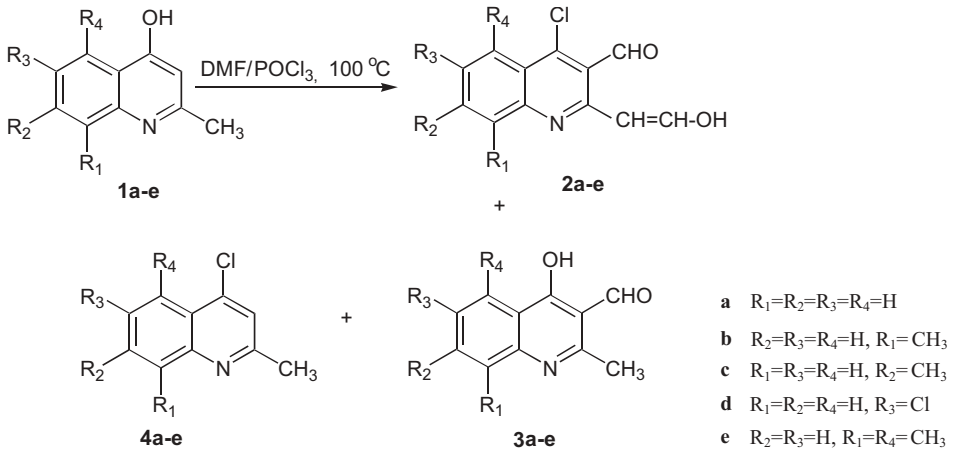
Compd. No.	Diameter of inhibition zone (mm)[a]														
	Aeromonas hydrophilla			*Escherichia coli*			*Salmonella typhi*			*Aspergillus flavus*			*Penicillium funiculosum*		
	0.125%	0.25%	0.5%	0.125%	0.25%	0.5%	0.125%	0.25%	0.5%	0.5%	1%	2%	0.5%	1%	2%
2a	4	6	9	–	–	–	3	5	9	2	3	8	–	1	5
2b	2	5	8	–	–	–	4	7	11	1	2	5	–	2	6
2c	3	7	10	–	–	2	4	8	13	3	5	9	1	3	8
2d	3	5	11	1	5	8	3	5	10	3	6	10	2	5	10
2e	4	6	10	–	–	4	2	6	10	4	7	12	3	7	11
8a	4	10	12	–	–	3	4	7	12	2	4	7	1	3	8
8b	4	9	12	–	–	3	5	8	13	2	5	9	2	4	9
8c	5	10	14	3	5	8	6	9	16	3	6	9	2	5	8
8d	6	8	11	–	–	5	5	9	12	6	10	14	4	7	14
8e	6	9	12	3	7	11	5	8	13	4	8	13	3	5	12
Streptomycin	8	12	19	7	10	18	8	10	18	NT	NT	NT	NT	NT	NT
Carbendozim	NT	NT	NT	NT	NT	NT	NT	NT	NT	6	14	21	9	16	21

[a] Mean of three replicates
– no inhibition;
NT – not tested

The cells were incubated for 7 minutes, sedimented after centrifugation at 1000 rpm for 5 minutes and then fixed in a freshly prepared fixative (methanol/glacial acetic acid, 3:1, *V*/*V*). Two or three changes of the fixative were applied. Slides were prepared by placing a drop of the cell suspension on a clean chilled slide and immediately drying the slide at 40 °C for a few seconds. The slides were routinely stained in a 4% buffered solution of Giemsa (22, 23). Three hundred and fifty well-banded metaphases were analyzed for each treatment under an oil immersion lens.

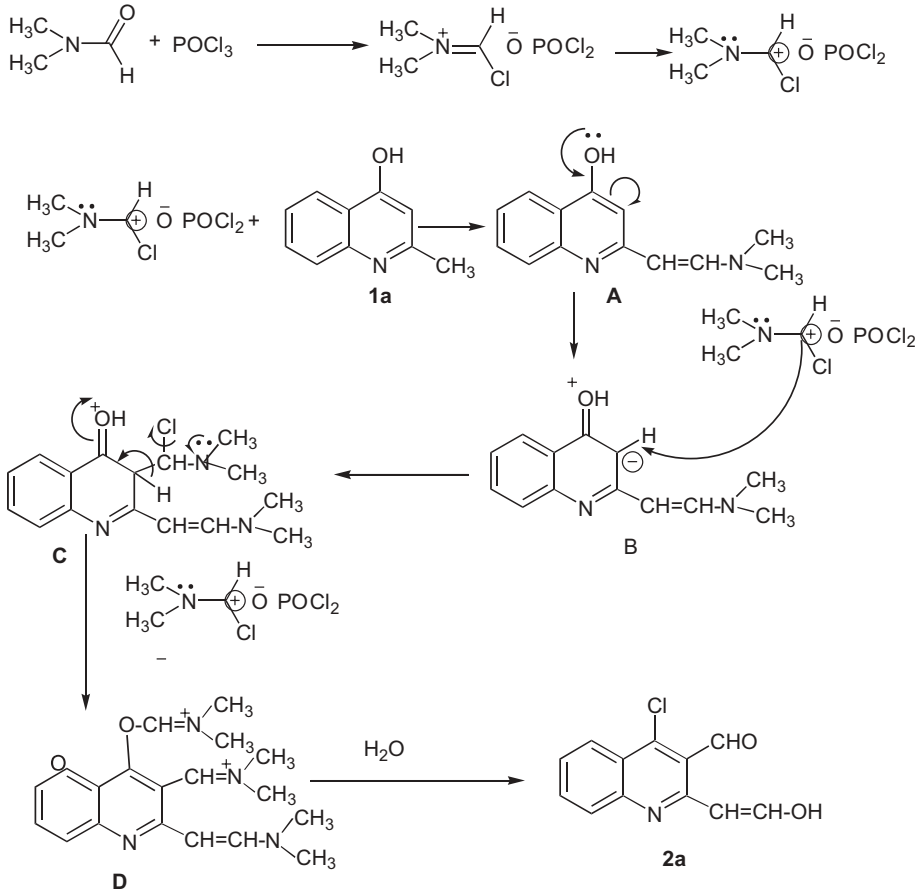
RESULTS AND DISCUSSION

The Vilsmeier Haack reaction of 4-hydroxyquinaldines (**1**) (previously prepared from the corresponding aniline and ethyl acetoacetate) followed by subsequent cyclization of β-anilinocrotonates would provide an efficient intermediate for the preparation of several substituted [*c*] annelated heterocyclic compounds. The reaction was carried out at 100 °C for 15–20 h, using the Vilsmeier Haack reagent derived from phosphorus oxychloride-dimethyl formamide *in situ*. The reaction yielded a mixture of products. These were isolated using silica gel column chromatography. The analytical and spectroscopic data confirmed the products as 4-chloro-3-formyl-2-(2-hydroxyethene-1-yl)quinoline (**2**), 4-hydroxy-3-formylquinaldine (**3**) and 4-chloroquinaldine (**4**) in good yields. (Scheme 1). Thus, the treatment with the Vilsmeier reagent at 100 °C provided an efficient and facile method for the generation of 4-chloro-3-formyl-2-(2-hydroxyethene-1-yl)quinolines (**2a**-**e**).



Scheme 1

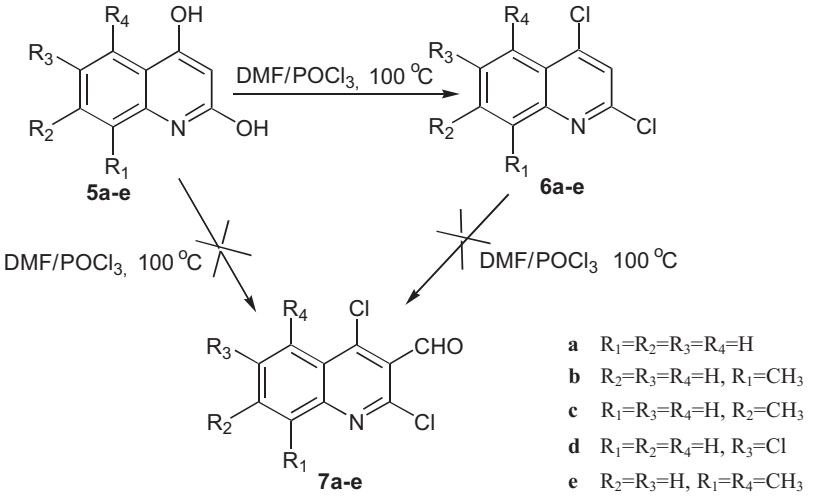
The Vilsmeier Haack reagents are usually applied for the formylation of aromatic and heteroaromatic compounds. These are the chloromethyleniminium species responsible for the formylation (Scheme 2). Like in our reaction, the chloromethyleniminium spe-



Scheme 2

cies obtained *in situ* from phosphorus oxychloride/dimethylformamide reacts with the active methyl group of 4-hydroxyquinaldine (**1a**) to yield **A**. The another formylation occurs at the aromatic C_3 of the quinaldine leading to the iminium compound **D**. Simultaneously, the iminium salts with a special capability to replace the hydroxyl group at aromatic C_4 by the nucleophiles like chlorine, bromine, *etc.*, might have led to the formation of 4-chloroquinaldine (**4a**) in minor yields and also in the conversion of hydroxy moiety to the chloro moiety in the case of 4-chloro-3-formyl-2-(2-hydroxyethene-1-yl)quinoline (**2a**). In the case of 4-hydroxyquinaldine (**1a**) and 8-methyl-4-hydroxyquinaldine (**1b**), the reaction was completed within 15 h and with 5,8-dimethyl-4-hydroxyquinaldine (**1e**), in 20 h, as monitored by the TLC (Table I).

Similarly, we studied the formylation of 2,4-dihydroxy quinolines (**5**). Hence, 2,4-dihydroxy quinolines (24) (**5**) were treated with the classical Vilsmeier Haack reagent under similar conditions as above. Only 2,4-dichloro quinolines (**6**), but not the 2,4-dichlo-



Scheme 3

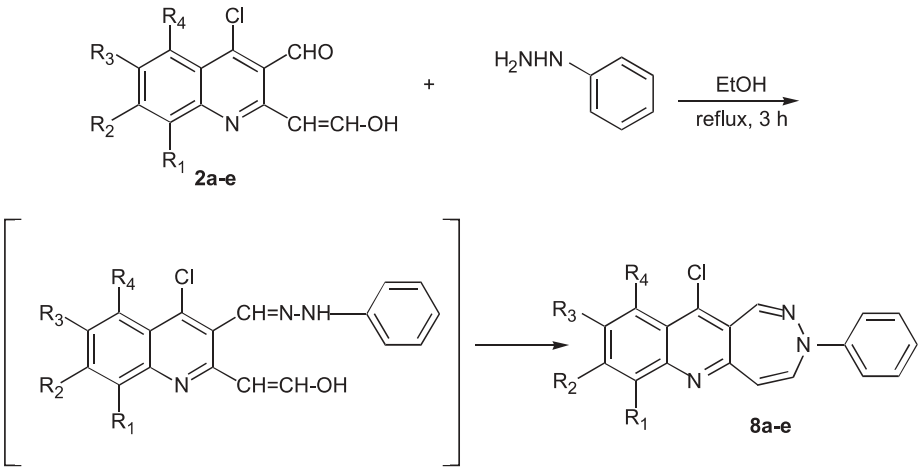
ro-3-formyl quinolines (**7**), were obtained (Scheme 3). In continuation of the reaction sequence, the Vilsmeier Haack reaction was carried out with 2,4-dichloro quinolines, but no expected characteristic change was observed. These observations may be either due to the steric hindrance of bulky groups at C_2 and C_4 positions, or the electron withdrawing nature of chloro groups at the same positions restricts the electrophilic attack of the iminium species at C_3-H.

With the new intermediates, **2a-e**, at our disposal we were able to start the intended synthesis of some annelated quinolines. The corresponding vinyl derivative was treated with phenyl hydrazine hydrochloride in absolute ethanol at reflux temperature for three hours. After the solvent removal, under reduced pressure, the residue was extracted with ethyl acetate. The silica gel column chromatography afforded the desired compounds **8a-e** using the petroleum ether/ethyl acetate (98:2, *V*/*V*) as the eluent (Scheme 4).

The reaction proceeded *via* the corresponding phenyl hydrazone and the subsequent aromatization yielded the diazepine. The yields, reaction time and the temperature at which the reaction was carried out are shown in Table I.

Almost all the tested compounds exhibited moderate activity against all species of bacteria used in this study and only a few showed no activity against *Escherichia coli*. All the compounds exhibited good activity against both the pathogenic fungi used. Although the majority of the compounds were active, they did not reach the effectiveness of the conventional bacteriostatic streptomycin. Table IV indicates that the diazepines show more prominent results than the intermediate quinolines. The higher activities may be due to the presence of azepine ring and the electron donating nitrogen atoms. The effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or on the diffusion of ribosomes in microbial cells.

The clastogenic properties and the mutagenecity of compounds **8a**, **8b** and **8d** were studied on human chromosomes *in vitro* using human peripheral blood in leucocyte cultures. The results presented in Table V show that **8b** and **8d** are mild mutagens at concentrations not lower that 20 μg mL^{-1}.



a – R_1=R_2=R_3=R_4=H;
b – R_2=R_3=R_4=H, R_1=CH_3;
c – R_1=R_3=R_4=H, R_2=CH_3
d – R_1=R_2=R_4=H, R_3=Cl;
e – R_2=R_3=H, R_1=R_4=CH_3

Scheme 4

Table V. Chromosomal aberrations in vitro *following addition of compounds* ***8b*** *and* ***8d*** *(20 μg mL^{-1}) zero and 24 h after human peripheral blood leucocyte culture initiation*

Experiment (μg mL^{-1})	Average No. of metaphases (n = 3)	Mean number of abberations (%)	
		Chromatid gaps	Chromatid breaks
Control I[a]	350	–	–
Control II[a]	350	–	–
	0 h		
8b	350	5 (14)	2 (0.6)
8d	350	7 (2.0)	3 (0.9)
	24 h		
8b	350	2 (0.6)	1 (0.3)
8d	350	3 (0.9)	1 (0.3)

[a] Control I was given an equal volume of distilled water; control II was given an equal volume of 1% acetone.

CONCLUSIONS

Utility of the Vilsmeier Haack reaction on 4-hydroxyquinaldine, resulting in some efficient and potential intermediates towards the synthesis of novel diazepino systems, has been demonstrated. The suggested reaction path could be of use in the construction of other heterocycles. The antibacterial, antifungal and cytogenetic studies of all synthesized compounds indicate their biological importance.

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S A Ž E T A K

Izolacija 4-kloro-3-formil-2-(2-hidroksieten-1-il)kinolina Vilsmeier Haackovom reakcijom na kinaldinima: Stvaranje diazepino kinolinskih heterocikala i njihovo antimikrobno i citogenetsko djelovanje

RAJU NANDHA KUMAR, THANGARAJ SURESH i PALATHURAI SUBRAMINIAM MOHAN

Primjenom Vilsmeierovih reakcijskih uvjeta na 4-hidroksikinaldin pripravljeni su 4-kloro-3-formil-2-(2-hidroksieten-1-il)kinolini koji su u reakciji s fenilhidrazino hidrokloridom dali diazepino kinoline. Ispitano je antibakterijsko, antifungalno i citogenetsko djelovanje sintetiziranih spojeva.

Ključne riječi: kinaldini, Vilsmeier Haackova reakcija, 4-kloro-3-formil-2-(2-hidroksieten-1-il)kinolini, diazepinokinolini, antimikrobno djelovanje, citogenetsko djelovanje

Department of Chemistry, Bharathiar University, Coimbatore – 641 046, India