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ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 1128–1136
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

Synthesis, antimicrobial activities and cytogenetic studies of newer diazepino quinoline derivatives *via* Vilsmeier–Haack reaction

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Received 11 November 2006; received in revised form 26 December 2006; accepted 2 January 2007
Available online 20 January 2007

Abstract

The study of the Vilsmeier–Haack reagent on 4-hydroxyquinaldines resulted in a new versatile intermediate 4-chloro-3-formyl-2-(2-hydroxy-ethene-1-yl)quinolines, which on further treatment with hydrazine hydrate yielded the desired diazepino quinoline derivatives. All the synthesized diazepino quinoline derivatives are screened for their antibacterial and antifungal activities. Cytogenetic analysis of the samples is also reported.

Keywords: Vilsmeier–Haack reagent; 4-Chloro-3-formyl-2-(2-hydroxy-ethene-1-yl)quinolines; Diazepino[4,5-*b*]quinolines; Antibacterial activities; Antifungal activities; Cytogenetic studies

1. Introduction

Quinolines and their derivatives are important constituents of pharmacologically active synthetic compounds, as these systems have been associated with a wide spectrum of biological activities [1–4] such as DNA binding capability [5], antitumor activities [6,7], DNA-intercalating carrier [8] etc. The quinoline nucleus can also be frequently recognized in the structure of numerous naturally occurring alkaloids and synthetic compounds with interesting pharmacological properties. As a consequence, the development of general methods for the synthesis and biological evaluation of new agents, retaining the 'core' quinoline moiety has been the subject of considerable synthetic effort. An essential component of the search for new leads in the drug designing program is the synthesis of molecules, which are novel yet resemble known biologically active molecules by virtue of the presence of some critical structural features. Certain small heterocyclic molecules act as highly functional scaffolds and are known pharmacophores of a number of biologically active and medicinally useful molecules [9]. In the interest of the above, we planned to synthesize a system which combines these two biolabile components together to give a compact structure like the title compounds *via* Vilsmeier–Haack reaction.

The Vilsmeier–Haack reagent is an efficient, economical and mild reagent for the formylation of reactive aromatic and hetroaromatic substrates [10–12]. Nevertheless, its importance in various synthetic methodologies [13–20] including the microwave chemistry [21–27] is remarkable and inspiring. The classical Vilsmeier–Haack reaction, however, involves electrophilic substitution of an activated aromatic ring with a halomethyleniminium salt to yield the corresponding iminium species, which facilitates easy entry into large number of novel and biologically potential heterocyclic systems. Recently, we have been involved in the synthesis and chemistry of novel quinoline derivatives *via* the Vilsmeier–Haack reaction with a prospect to incorporate diverse bioactive

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doi:10.1016/j.ejmech.2007.01.004

heterocyclic nucleus intact for evaluating their antibacterial and antifungal significance and also as a reagent for effective functional group inter conversion [28–35]. Herein, we wish to report the synthesis, antimicrobial activities and cytogenetic studies of the title compounds.

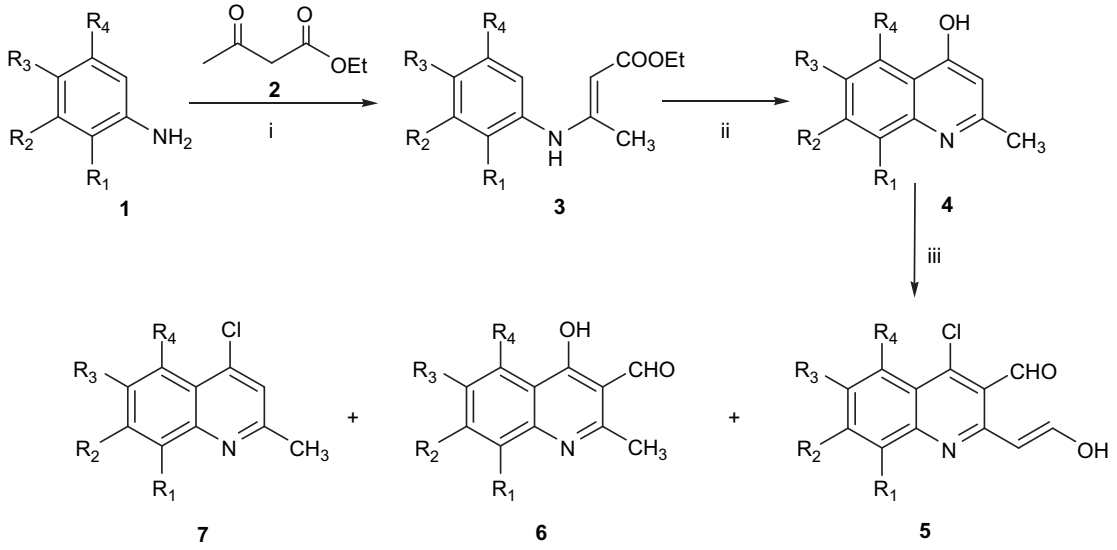
2. Chemistry

The Vilsmeier–Haack reaction on 4-hydroxyquinaldines **4** (prepared from aniline **1** and ethyl acetoacetate **2** and cyclization of the β-anilino crotonates **3**) provided a vital and efficient intermediate for the synthesis of several newer substituted [*b*] and [*c*] annelated heterocyclic compounds. The reaction is performed at 100 °C for 15–20 h, using the typical Vilsmeier–Haack reagent derived from phosphorus oxychloride–dimethylformamide [36]. Although, the reaction proceeded uneventfully, the products formed are isolated using silica gel column chromatography. The analytical and spectroscopic data confirmed the products as 4-chloro-3-formyl-2-(2-hydroxy-ethene-1-yl)quinolines **5**, 4-hydroxy-3-formyl quinaldine **6** and 4-chloroquinaldine **7**. Interestingly, the two singlets around δ 9.5 in the ^{1}H NMR spectra and (M + 2) peak with 3:1 intensity compared to the molecular ion peak in the mass spectra, advocates the presence of the vinyl protons and a chlorine atom for compounds **5**(**a**–**e**) respectively, and are obtained in moderate to high yields whereas compounds **6**(**a**–**e**) and **7**(**a**–**e**) are formed in good to low yields. Therefore, the Vilsmeier–Haack reaction on 4-hydroxyquinaldines **4** at 100 °C provided a facile method for the generation of an efficient and novel quinoline vinyl alcohol. The time taken for each reaction and their yields are shown in Scheme 1 and Table 1.

The Vilsmeier–Haack reagents are normally applied for the formylation of aromatic and heteroaromatic compounds. The *in situ* formation of the chloromethyleniminium species **8** (derived from phosphorus oxychloride–dimethylformamide) is responsible for the formylation, which reacts with the active methyl group of 4-hydroxyquinaldines **4** to yield **9**. The second formylation occurs at the C_3-H moiety of the quinaldine leading to an iminium species **11**. However, these iminium salts having the special capability to replace the hydroxyl group at aromatic C_4 by the nucleophiles [37,38] like chlorine, bromine, etc. that might have led to the formation of 4-chloroquinaldine **7** in minor yields and also in the conversion of hydroxy moiety to the chloro moiety in case of 4-chloro-3-formyl-2-(2-hydroxy-ethene-1-yl)quinoline **5** (Scheme 2).

Despite the success in obtaining a potential quinoline intermediate, our further endeavors towards the formylation of 2,4-dihydroxy quinolines [39] with the classical Vilsmeier–Haack reagent under the similar conditions as the above yielded only 2,4-dichloro quinolines **14** and not the expected 2,4-dichloro-3-formyl quinolines **15** (Scheme 3). Furthermore, as a continuation of the reaction sequence, the Vilsmeier–Haack reaction performed on 2,4-dichloro quinolines **14**, under identical conditions as above had absolutely no reaction. This may be either due to steric hindrance of the bulky groups at C_2 and C_4 positions or the electron withdrawing nature of chloro groups at same positions which restricts the electrophilic attack of the iminium species at C_3-H.

Finally, having realized the above vital intermediate **5** our efforts turned towards the synthesis of novel and biologically potential annulated quinolines. Hence, the intermediate **5** is treated with hydrazine hydrate in absolute ethanol at reflux temperature for 3 h. The removal of the solvent by reduced pressure and recrystallization of the residual mass by chloroform afforded the pure product. The analytical and spectral data verified the compound as 11-chloro-(3*H*)[1,2]diazepino [5,4-*b*]quinoline **17**(**a**–**e**) (Scheme 4). As per our investigation, the reaction may proceed *via* the formation of the Schiff's base between the aldehyde and amino group and subsequent



Scheme 1. (**5**–**7**)**a**: $R_1 = R_2 = R_3 = R_4 = H$, 15 h; **b**: $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$, 12.5 h; **c**: $R_2 = CH_3$; $R_1 = R_3 = R_4 = H$, 16 h; **d**: $R_3 = Cl$; $R_1 = R_2 = R_4 = H$, 17.5 h; **e**: $R_1 = R_4 = CH_3$; $R_2 = R_3 = H$, 20 h. (i) dil. HCl (1:1), rt; (ii) Ph_2O, 240 °C; (iii) Vilsmeier–Haack reagent (DMF–$POCl_3$), 100 °C.

Table 1
Vilsmeier–Haack reaction of 4-hydroxyquinaldines[a]

Entry	Substrates	R_1	R_2	R_3	R_4	Reaction time (h)	Yields[b] (%)		
							5	**6**	**7**
1	**5a**	H	H	H	H	15	70	15	10
2	**5b**	CH_3	H	H	H	12.5	73.5	18	5
3	**5c**	H	CH_3	H	H	16	55	12	17
4	**5d**	H	H	Cl	H	17.5	78	12	5
5	**5e**	CH_3	H	H	CH_3	20	65	10	15

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

aromatization lead to the formation of the diazepino quinoline system. The yields, reaction time taken and the temperature at which the reaction carried are shown in Table 2.

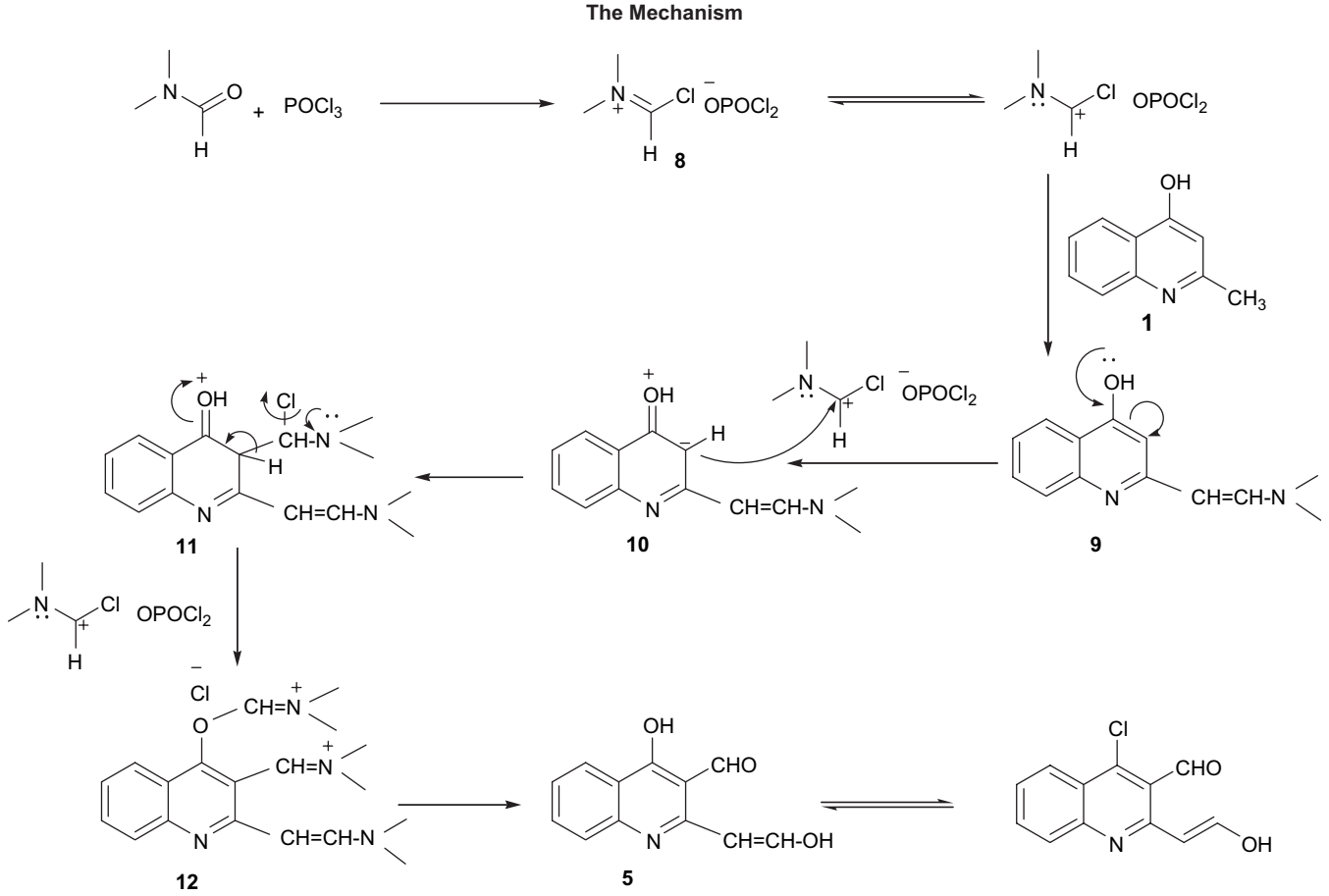
3. Pharmacology

3.1. Antibacterial studies

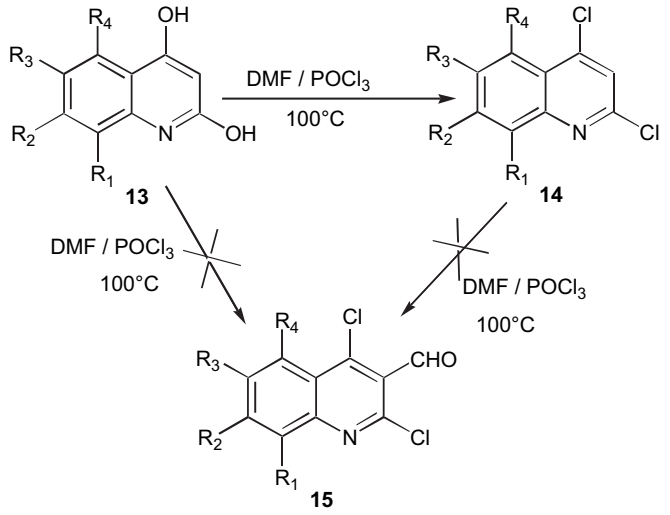
Antibacterial activities of all the synthesized compounds were preliminarily screened for the *in vitro* growth inhibitory activity against *Aeromonas hydrophila*, *Escherichia coli* and *Salmonella typhi* by using the disc diffusion method [40,41]. The bacteria were cultured in nutrient agar medium and used as inoculum for this study. Bacterial cells were swabbed on to nutrient agar medium [prepared from NaCl (5.0 g), peptone (5.0 g), beef extract powder (3.0 g), yeast extract powder (3.0 g), agar (20.0 g) in 100 mL distilled water; pH = 7.5 ± 0.2)] in Petri plates. The compounds to be tested were dissolved in chloroform to a final concentration (weight/volume) of 0.125%, 0.25% and 0.5% and soaked in filter paper discs of 5 mm diameter and 1 mm thickness. These discs were placed on the already seeded plates and incubated at 35 ± 2 °C for 24 h. To avoid the activity of the solvent that is used in the test solutions, a solvent only treated plate was maintained, which showed a 2 mm diameter zone of inhibition. Finally, the diameter (mm) of the inhibition zone around each disc was measured after 24 h and subtracting the diameter of inhibition zone formed by the solvent only treated plate, the results are listed in Table 3. Streptomycin was used as standard.

3.2. Antifungal studies

Antifungal activities of all the synthesized compounds were preliminarily screened for the *in vitro* growth inhibitory activity against *Aspergillus flavus*, *Penicillium funiculosum*, *Alternaria macrospora* and *Fusarium oxysporum* by using the disc diffusion method [40]. The fungi were cultured in potato dextrose agar medium. Potato dextrose agar medium (prepared from



Scheme 2.



Scheme 3. (**13**–**15**)**a**: $R_1 = R_2 = R_3 = R_4 = H$; **b**: $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$; **c**: $R_2 = CH_3$; $R_1 = R_3 = R_4 = H$; **d**: $R_3 = Cl$; $R_1 = R_2 = R_4 = H$; **e**: $R_1 = R_4 = CH_3$; $R_2 = R_3 = H$.

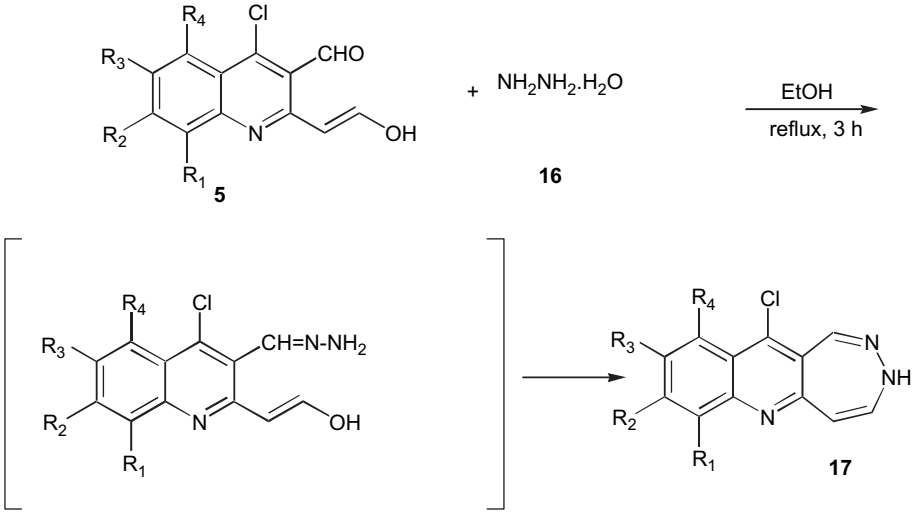
potato 150 g; dextrose 5 g and agar 2 g in 200 mL of distilled water) was poured in the sterilized Petri plates and allowed to solidify. The plates were inoculated with a spore suspension of *A. flavus*, *P. funiculosum*, *A. macrospora* and *F. oxysporum* (10^6 spores'/mL of medium). The compounds to be tested were dissolved in acetone to a final concentration (weight/volume) of 0.5%, 1% and 2% and soaked in filter paper discs (Whatmann no. 4, 5 mm diameter). These discs were placed on the already seeded plates and incubated at 28 ± 2 °C for four days. To avoid the activity of the solvent that is used in the test solutions, a solvent only treated plate was maintained, which showed a 1 mm diameter zone of inhibition. Finally, after four days, the inhibition zone which appeared around the discs in each plate was measured and subtracting the diameter of inhibition zone formed by the solvent only treated plate, the results are tabulated in Table 4. Carbendazim was used as standard.

3.3. Cytogenetic analysis

3.3.1. Cytogenetic analysis of compounds *17(a–e)* in human peripheral blood leukocyte culture (PBLC)

Cultures of leukocytes were obtained from peripheral blood set-up following the method of Hungerford [30,42]. The chromosomal preparations obtained were stained with Giemsa and were processed to obtain G-bands. About 2 mL of venous blood was drawn into a sterile, heparinized syringe. This blood of 0.5 mL (about 30 drops) was inoculated under aseptic conditions into a culture vial containing 5.0 mL of the culture medium (McCoy's 5a, Himedia), 2 mL of AB serum and 0.2 mL of phytohaemagglutinin (PHA, prepared in the laboratory from red kidney bean, *Phaseolus vulgaris*). Three experiments were carried out using three non-smoking healthy male donors' aged 23, 24, and 28 years. The compound was dissolved in 1% acetone. Four different concentrations of 0.02, 0.2, 2 and 20 μg/mL were added to the culture medium (0.1 mL solution of the compounds per 8 mL of the medium), at 0, 24, 48 h after culture initiation, indicated as treatment for 72, 48 and 24 h, respectively. Triplicate cultures for each dose from three donors were maintained for the study of chromosomal aberrations. Control-I was given an equal volume of distilled water. Control-II was given an equal volume of 1% acetone. Acetone solution (0.1 mL) was added to 8 mL of the medium at 0, 24 and 48 h after the culture initiation, indicated as treatment for 72, 48 and 24 h, respectively. The cultures were incubated at 37 °C for a period of 72 h.

The cultures were shaken periodically thrice a day. The dividing cells were arrested at metaphase, 45 min before harvest of culture, by adding 0.05 mL of colchicine solution (0.01%). The contents in the vial were centrifuged at 1000 rpm for 5 min at the end of the colchicine treatment. The supernatant was discarded and 7 mL of prewarmed hypotonic solution (0.075 M KCl) was added to the cell button. The cells were incubated for 7 min, sedimented after centrifugation at 1000 rpm for 5 min and then fixed in freshly prepared fixative



Scheme 4. (**5**,**17**)**a**: $R_1 = R_2 = R_3 = R_4 = H$, 3.5 h, 78%; **b**: $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$, 3 h, 90%; **c**: $R_2 = CH_3$; $R_1 = R_3 = R_4 = H$, 2.5 h, 74%; **d**: $R_3 = Cl$; $R_1 = R_2 = R_4 = H$, 1.5 h, 63%; **e**: $R_1 = R_4 = CH_3$; $R_2 = R_3 = H$, 3.5 h, 82%.

Table 2
Synthesis of diazepino[4,5-*b*]quinolines[a] **17(a–e)**

Entry	Substrates	R_1	R_2	R_3	R_4	Reaction Time (h)	Yields[b] (%)
1	**17a**	H	H	H	H	3.5	78
2	**17b**	CH_3	H	H	H	3	90
3	**17c**	H	CH_3	H	H	2.5	74
4	**17d**	H	H	Cl	H	1.5	63
5	**17e**	CH_3	H	H	CH_3	3.5	82

[a] All reactions were carried out at 80 °C.
[b] Isolated yields after column chromatography on silica gel.

(methanol:glacial acetic acid, 3:1, v/v). Two or three changes of the fixative were given. Slides were prepared by placing a drop of 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 [43]. Three hundred and fifty well-banded metaphases were analyzed for each treatment under oil immersion lens.

4. Results and discussions

4.1. Antibacterial studies

All the synthesized novel diazepino quinolines **17(a–e)** and the intermediate quinolines **5(a–e)** were tested for their antibacterial activity *in vitro* against *A. hydrophila*, *E. coli* and *S. typhi*. Streptomycin was used as a standard drug whose diameter of zone inhibition is furnished in Table 3.

In general, all the synthesized novel diazepino quinolines and the intermediate quinolines exerted a wide range of modest *in vitro* antibacterial activity against all the tested organisms except some compounds against *E. coli*. The compound **5a** without having any substituents at C_5–C_8 did not exhibit any antibacterial activity even at the higher concentration against *E. coli*. There was no further improvement in the activity even after the introduction of the methyl groups (**5b**, **5c**) at positions C_7 or C_8. But all of these compounds showed good activity against *S. typhi* and *A. hydrophila*. Introduction of two methyl groups at C_6 and C_8 positions increased the activity against all the pathogens. Replacement of hydrogen atom at C_6 of the quinoline intermediate by a chlorine atom enhanced the antibacterial activity against all the species. Nevertheless, the compounds **17(a–e)** show similar results but has significant enhancement in their antibacterial activities against all the pathogens when compared to the intermediate quinolines **5(a–e)**. This enhancement may be due the diazepine heterocyclic ring system which has additional electron donating nitrogen atoms. However, according to the observation, the toxicity increases with the increase in concentration of the test solution containing the new compounds. Although, all the compounds are active, they did not reach the effectiveness of the conventional bacteriostatic streptomycin. Table 3 confines that the diazepines show more prominent results than the intermediate quinolines. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or diffusion in ribosomes of microbial cells [44].

4.2. Antifungal studies

The *in vitro* antifungal activity of the novel diazepino quinolines **17(a–e)** and the intermediate quinolines **5(a–e)** were studied against the fungal strains *viz*. *A. flavus*, *P. funiculosum*, *A. macrospora*, *F. oxysporum*. Carbendazim was used as a standard drug whose diameter of zone inhibition is furnished in Table 4.

All the compounds, except compound **5a**, exhibited good activity against all the pathogenic fungi used in this study. The introduction of substituents to diazepino quinolines and the intermediate quinolines such as methyl and chloro groups increased the antifungal activities significantly. The compounds **5(b–d)** exerted modest activity against all the species when compared to compound **5a**. However, the replacement of the hydrogen atom by a chlorine moiety at C_6 position exhibited significant antifungal activity against all the fungal

Table 3
Antibacterial activity of the compounds **5(a–e)** and **17(a–e)**[a]

Compound	Diameter of inhibition zone in mm								
	Aeromonas hydrophila			*Escherichia coli*			*Salmonella typhi*		
	0.125%	0.25%	0.5%	0.125%	0.25%	0.5%	0.125%	0.25%	0.5%
5a	4	6	9	–	–	–	3	5	9
5b	4	8	11	–	–	–	4	7	11
5c	3	7	10	–	–	2	4	8	13
5d	5	9	14	1	5	8	6	11	17
5e	4	6	10	3	5	9	5	8	13
17a	5	8	12	–	2	4	4	6	10
17b	4	7	11	–	2	6	5	7	12
17c	5	9	12	–	1	5	4	9	13
17d	6	11	16	3	7	11	6	12	19
17e	4	8	11	2	5	9	5	9	14
Streptomycin	8	12	19	6	9	15	8	10	18

– No inhibition.
[a] Values of the mean of three replicates.

Table 4
Antifungal activity of the compounds **5**(**a**–**e**) and **17**(**a**–**e**)[a]

Compound	Diameter of inhibition zone in mm											
	Aspergillus flavus			*Penicillium funiculosum*			*Alternaria macrospora*			*Fusarium oxysporum*		
	0.5%	1%	2%	0.5%	1%	2%	0.5%	1%	2%	0.5%	1%	2%
5a	2	3	8	–	1	5	–	1	4	–	–	4
5b	1	2	5	–	2	6	2	5	9	–	3	7
5c	3	5	9	1	3	8	2	4	7	1	4	9
5d	3	6	10	2	5	10	3	7	11	2	5	11
5e	4	7	12	3	7	11	3	8	13	3	7	12
17a	3	5	9	1	4	9	1	3	7	–	3	7
17b	2	4	8	–	3	10	3	6	10	2	6	10
17c	4	7	11	3	6	11	4	10	12	3	7	11
17d	5	8	13	6	9	14	5	9	15	4	8	13
17e	4	9	16	5	10	17	7	11	17	4	11	15
Carbendazim	6	14	21	9	16	21	7	13	18	6	11	18

– No inhibition.
[a] Values of the mean of three replicates.

strains and at all concentrations. As expected, the antifungal activity of the novel diazepino quinolines **17**(**a**–**e**) were enhanced compared to the intermediate quinolines **5**(**a**–**e**). This increased activity in the case of the novel title compounds may be due to the additional diazepine heterocyclic ring system containing two nitrogen atoms when compared to the intermediate quinolines. Nevertheless, the activities of the fungal strains were similar to the activities of the bacterial strains. They also gave a clear picture with regard to the inhibition zone, which increases with the increase in concentration of the test solution containing the new compounds. Although all the compounds show good activity at higher concentrations they did not reach the effectiveness of the standard carbendazim. Table 4 shows that the diazepines show better results than the intermediate quinolines.

4.3. Cytogenetic analysis

4.3.1. Analysis of chromosomal aberrations (CA)

All the prepared slides were stained with Giemsa, 20 well-spread metaphase plates were screened for chromosome aberrations. The aberrations include chromatid gaps, chromatid breaks, isochromatic breaks and chromatid interchanges. Polyploid cells were also screened in all the slides. The clastogenic properties for the compounds **17**(**a**–**e**) were studied by investigating the effects of the compounds on human chromosomes *in vitro* in leukocyte cultures. It was noted that the chromosomal aberrations showed a dose dependant increase. Chromosomal aberrations like chromatid gaps and chromatid breaks were observed. Polyploid cells were absent in all examined slides. There were no chromatid interchanges, isochromatid breaks and gaps found in all the samples and at all concentrations. However, the compounds **17a** and **d** caused no CA at all concentrations. But the compounds **17b**, **c** and **e** are mild mutagens at concentration not lower than 20 μg/mL. Although CA was observed in PBLC treated with the compounds **17b**, **c** and **e**, it was noted that the compound **17e** caused CA even at 48 h of treatment expressing higher genotoxicity. The results are presented in Table 5.

5. Conclusion

We have demonstrated the utility of Vilsmeier–Haack reaction on 4-hydroxyquinaldines resulting in some efficient and potential intermediate towards the synthesis of newer diazepino systems. An examination of the *in vitro* antibacterial and antifungal activity profile of a variety of substituted novel diazepino quinolines and the intermediate quinolines against the bacterial strains *viz*., *A. hydrophila*, *E. coli* and *S. typhi* and the fungal strains *viz*., *A. flavus*, *P. funiculosum*, *A. macrospora*, and *F. oxysporum*, respectively, provide a structure activity correlate, which may be summarized as follows. The compounds with chloro or the dimethyl functional groups in the heterocyclic moiety play an important role in eliciting biological response. Nonetheless, the diazepines show significant antibacterial and antifungal activities when compared to the intermediate quinolines. The cytogenetic studies show its importance, as the compounds do not have any chromosomal

Table 5
Chromosomal aberrations *in vitro* following addition of compounds **17b**, **c** and **e** (20 μg/mL) at 0, 24 and 48 h after human peripheral blood leukocyte culture initiation

Experiment (μg/mL)	Average no. of metaphases ($n = 3$)	Mean number of aberrations (%)	
		Chromatid gaps	Chromatid breaks
Control I[a]	350	–	–
Control II[b]	350	–	–
0 h			
17b	350	6 (1.7)	3 (0.9)
17c	350	8 (2.3)	3 (0.9)
17e	350	11 (3.1)	5 (1.4)
24 h			
17b	350	2 (0.6)	1 (0.3)
17c	350	4 (1.1)	2 (0.6)
17e	350	7 (2.0)	3 (0.9)
48 h			
17e	350	3 (0.9)	1 (0.3)

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

aberrations in the lower doses. Thus, in future this class of quinoline intermediates and novel diazepino quinolines may be used as templates to generate better drugs to combat bacterial and fungal infections.

6. Experimental

Thin layer chromatography was used to access the reactions and the purity of products. Melting points were determined on a Boetius Microheating Table and Mettler-FP5 melting apparatus and are uncorrected. IR spectra were recorded in Shimadzu-8201FT instrument in KBr disc and only noteworthy absorption levels (reciprocal centimeter) are listed. ^{1}H NMR spectra were recorded in a AMX-400 MHz spectrometer in $CDCl_3$ solution; chemical shifts are expressed in parts per million (δ) relative TMS, coupling constants (J) in hertz and signal multiplicities are represented by bs (broad singlet), s (singlet), d (doublet), t (triplet) and m (multiplet). ^{13}C NMR were also recorded on the same AMX-400 MHz spectrometer with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a Jeol-D300 mass spectrometer. CHN analyses were carried out on a Carlo Erba 106 and Perkin–Elmer Model 240 analysers.

6.1. Typical procedures

6.1.1. Vilsmeier–Haack reaction on 4-hydroxyquinaldines

The Vilsmeier reagent was prepared by taking *N*,*N*-dimethylformamide 3.86 mL (0.05 mol) in a round-bottomed flask in an ice cold condition (0–5 °C) with constant stirring. To this, phosphorous oxychloride 13.04 mL (0.014 mol) was added drop by drop for a period of half an hour and the resultant mixture was stirred for a further 1 h. The appropriate 4-hydroxyquinaldines (0.02 mol) **4**(**a**–**e**) were added to the Vilsmeier reagent and stirred for further half an hour and the reaction mixture was kept in a water bath at 100 °C for the stipulated period of time. After the reaction has been completed as inferred through thin layer chromatographic studies (complete disappearance of the starting material), the reaction mixture was poured into 500 g of crushed ice with constant manual stirring. It was kept aside for overnight. After neutralizing the above with 4 N sodium hydroxide solution, the precipitate obtained was washed well with water and extracted using ethyl acetate. The combined organic layers were then collected and dried over anhydrous sodium sulfate. The silica gel chromatography of the reaction mixture afforded three products **7**, **6** and **5** at pet. ether (100), pet. ether–ethyl acetate (94:6) and pet. ether–ethyl acetate (85:5), respectively. The products were recrystallized with methanol.

6.1.2. Synthesis of diazepino[4,5-b]quinolines

Hydrazine hydrate (0.002 mol) **16** and 4-chloro-3-formyl-2-(2-hydroxy-ethene-1-yl)quinoline (0.002 mol) (**5**) were dissolved in 50 mL of absolute ethanol. They were kept at the refluxing temperature for 3 h. After the completion of the reaction inferred through the TLC monitoring, the ethanol was removed under reduced pressure. It was further recrystallized with chloroform.

Compound **5a**: m.p. 155 °C. IR (KBr): 3438, 1664, 1595. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.7 (d, 1H, C_8-H, $J = 8.1$ Hz), 7.6 (t, 1H, C_7-H, $J = 8.3$ 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 exchangeable). ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 192.419 (−CHO), 189.332 (−CH=CH−), 189.08 (−CH=CH−), 146.24, 137.30, 135.90, 133.32, 126.93, 125.21, 122.61, 119.32, 118.99 (aryl carbons). Anal. Calcd. for $C_{12}H_8NO_2Cl$ (%): C, 61.69; H, 3.45; N, 5.99. Found: C, 61.62; H, 3.38; N, 5.92. MS (EI, 70 eV) *m*/*z* 233 (15.2%) (M^+), 235 (4.8%) (M + 2), 206 (66.2%), 164 (35.9%), 190 (100%).

Compound **6a**: m.p. 140 °C. IR (KBr): 3520, 1695, 1610. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 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). Anal. Calcd. for $C_{11}H_9NO_2$ (%): C, 70.58; H, 4.85; N, 7.48. Found: C, 70.54; H, 4.81; N, 7.43. MS (EI, 70 eV) *m*/*z* 187 (M^+).

Compound **7a**: m.p.65 °C. IR (KBr): 1590. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 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).

Compound **17a**: m.p. 182 °C. IR (KBr): 3350, 1580, 1595. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.2 (bs, 1H, −NH), 7.4 (s, 1H, C_1-H), 7.5–8.5 (m, 6H, Ar-H). Anal. Calcd. for $C_{12}H_8N_3Cl$ (%): C, 62.76; H, 3.51; N, 18.30. Found: C, 62.69; H, 3.46; N, 18.41. MS (EI, 70 eV) *m*/*z* 230 (M^+).

Compound **5b**: m.p. 132 °C. IR (KBr): 3450, 1660, 1595. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 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 exchangeable). ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 192.359 (−CHO), 189.451 (−CH=CH−), 189.014 (−CH=CH−), 147.224, 136.948, 133.953, 130.233, 126.611, 126.390, 122.993, 121.964, 118.594 (aryl carbons), 18.459 ($-CH_3$). Anal. Calcd. for $C_{13}H_{10}NO_2Cl$ (%): C, 63.04; H, 4.07; N, 5.66. Found: C, 62.02; H, 3.98; N, 5.63. MS (EI, 70 eV) *m*/*z* 247 (16.5%)(M^+), (M + 2) 249 (5.2%), 220 (66.7%), 204 (100%), 178 (36.5%).

Compound **6b**: m.p.195 °C. IR (KBr): 3500, 1710, 1590. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.6 (s, 6H, $2 \times CH_3$), 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). Anal. Calcd. for $C_{12}H_{11}NO_2$ (%): C, 71.63; H, 5.51; N, 6.96; Found: C, 71.58; H, 5.44; N, 6.98. MS (EI, 70 eV) *m*/*z* 201 (M^+).

Compound **7b**: m.p.47 °C. IR (KBr): 1570. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 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).

Compound **17b**: m.p. 110 °C. IR (KBr): 3390, 1585, 1603. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.3 (s, 3H, CH_3), 5.0 (bs, 1H,

−NH), 7.0 (s, 1H, C_1-H), 7.29 (t, 1H, C_9-H, $J = 7.72$ Hz), 7.45 (d, 1H, C_4-H, $J = 7.56$ Hz), 7.58 (d, 1H, C_5-H, $J = 7.00$ Hz), 7.86 (d, 1H, C_8-H, $J = 8.44$ Hz), 8.02 (d, 1H, C_{10}-H, $J = 8.28$ Hz). Anal. Calcd. for $C_{13}H_{10}N_3Cl$ (%): C, 64.07; H, 4.14; N, 17.24. Found: C, 64.15; H, 4.01; N, 17.11. MS (EI, 70 eV) *m/z* 244 (M^+).

Compound **5c**: m.p. 148 °C. IR (KBr): 3480, 1670, 1590. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 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 exchangeable). ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 192.28 (−CHO), 189.41 (−CH═CH−), 189.09 (−CH═CH−), 145.26, 135.68, 134.06, 131.22, 126.28, 126.05, 122.75, 122.34, 119.70 (aryl carbons), 19.52 ($-CH_3$). Anal. Calcd. for $C_{13}H_{10}NO_2Cl$ (%): C, 63.04; H, 4.07; N, 5.66. Found: C, 62.98; H, 4.01; N, 5.59. MS (EI, 70 eV) *m/z* (M^+) 247, (M + 2) 249.

Compound **6c**: m.p. 215 °C. IR (KBr): 3350, 1700, 1600. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.5 (s, 6H, 2 × CH_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). Anal. Calcd. for $C_{12}H_{11}NO_2$ (%): C, 71.63; H, 5.51; N, 6.96. Found: C, 71.61; H, 5.46; N, 6.94. MS (EI, 70 eV) *m/z* 201 (M^+).

Compound **7c**: m.p. 92 °C. IR (KBr): 1585. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.6 (s, 6H, 2 × CH_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).

Compound **17c**: m.p. 128 °C. IR (KBr): 3400, 1578, 1597. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.5 (s, 3H, CH_3), 5.3 (bs, 1H, −NH), 7.2 (s, 1H, C_1-H), 7.4−8.3 (m, 5H, Ar-H). Anal. Calcd. for $C_{13}H_{10}N_3Cl$ (%): C, 64.07; H, 4.14; N, 17.24. Found: C, 64.01; H, 4.19; N, 17.21. MS (EI, 70 eV) *m/z* 244 (M^+).

Compound **5d**: m.p. 180 °C. IR (KBr): 3470, 1670, 1590. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 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 exchangeable). ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 192.27 (−CHO), 189.26 (−CH═CH−), 189.08 (−CH═CH−), 133.90, 133.21, 129.80, 129.04, 124.41, 121.13, 120.86, 119.87, 118.34 (aryl carbons). Anal. Calcd. for $C_{12}H_7NO_2Cl_2$ (%): C, 53.76; H, 2.63; N, 5.26. Found: C, 53.71; H, 2.65; N, 5.22. MS (EI, 70 eV) *m/z* (M^+) 267, (M + 2) 269, (M + 4) 271.

Compound **6d**: m.p. 230 °C. IR (KBr): 3480, 1715, 1595. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 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). Anal. Calcd. for $C_{11}H_8NO_2Cl$ (%): C, 59.61; H, 3.64; N, 6.32. Found: C, 59.52; H, 3.57; N, 6.27. MS (EI, 70 eV) *m/z* (M^+) 221.

Compound **7d**: m.p. 74 °C. IR (KBr): 1575. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.4 (s, 3H, CH_3), 7.4 (s, 1H, C_3-H), 7.7−8.0 (m, 3H, Ar-H).

Compound **17d**: m.p. 274 °C. IR (KBr): 3478, 1590, 1605. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.1 (bs, 1H, −NH), 7.4 (s, 1H, C_1-H), 7.6−8.5 (m, 5H, Ar-H). Anal. Calcd. for $C_{12}H_7N_3Cl_2$ (%): C, 54.57; H, 2.67; N, 15.91. Found: C, 54.61; H, 2.74; N, 15.81. MS (EI, 70 eV) *m/z* (M^+) 274.

Compound **5e**: m.p. 170 °C. IR (KBr): 3525, 1680, 1595. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.6 (s, 6H, 2 × CH_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 exchangeable). ^{13}C NMR ($CDCl_3$, 400 MHz) δ: 192.34 (−CHO), 189.38 (−CH═CH−), 189.07 (−CH═CH−), 144.34, 136.38, 133.86, 130.95, 127.54, 126.01, 123.11, 122.45, 118.60 (aryl carbons), 19.80 (C_5-CH_3), 18.95 (C_8-CH_3). Anal. Calcd. for $C_{14}H_{12}NO_2Cl$ (%): C, 64.25; H, 4.62; N, 5.35. Found: C, 64.21; H, 4.57; N, 5.28. MS (EI, 70 eV) *m/z* (M^+) 261, (M + 2) 263.

Compound **6e**: m.p. 155 °C. IR (KBr): 3510, 1702, 1610. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.7 (s, 9H, 3 × CH_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); Anal. Calcd. for $C_{13}H_{13}NO_2$ (%): C, 72.54; H, 6.09; N, 6.51. Found: C, 72.47; H, 6.01; N, 6.42. MS (EI, 70 eV) *m/z* (M^+) 215.

Compound **7e**: m.p. 80 °C. IR (KBr): 1580. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.6 (s, 9H, 3 × CH_3), 7.4 (s, 1H, C_3-H), 7.6−7.8 (m, 2H, Ar-H).

Compound **17e**: m.p. 190 °C. IR (KBr): 3375, 1578, 1595. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.6 (s, 6H, 2 × CH_3), 5.0 (bs, 1H, −NH), 7.3 (s, 1H, C_1-H), 7.5−8.4 (m, 4H, Ar-H). Anal. Calcd. for $C_{14}H_{12}N_3Cl$ (%): C, 65.25; H, 4.69; N, 16.30. Found: C, 65.19; H, 4.58; N, 16.19. MS (EI, 70 eV) *m/z* (M^+) 258.

Acknowledgements

TS thank CSIR, New Delhi, India for the award of Senior Research Fellowship. SIF, Indian Institute of Science, Bangalore and Central Drug and Research Institute, Lucknow supported the spectral details.

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