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Synthesis and antibacterial property of quinolines with potent DNA gyrase activity

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ABSTRACT

Synthesis of a series of novel tetrahydroquinoline annulated heterocycles has been accomplished by intramolecular imino and bisimino Diels–Alder reaction. These compounds were evaluated for their antibacterial activity. All the synthetic compounds, exhibited good antibacterial activity against microorganisms of which one of them **7** was found to be as active as the antibiotic ciplofloxacin and is found to have MIC value of 2.5 mg/mL against *Escherichia coli.*

1. Introduction

In recent years the emergence of multi-drug resistant organisms poses a challenge in the treatment of infectious diseases.[1] Hence there arises a need to synthesise new and effective antimicrobial drugs to overcome this problem. Over the past decade bacterial DNA gyrase has drawn much attention as a selected target of potent antibacterial agents.[2] A number of synthetic quinolone antibacterial agents have been developed and are now widely used in the treatment of infectious diseases.[3–7] Quinolones are known to inhibit DNA gyrase and topoisomerase IV and cause bacterial cell death. Novobiocin, a naturally occurring antibacterial compound, has been identified as a potential inhibitor[8] of DNA gyrase. In our study we have synthesized five different types of quinolines **(4, 7, 10, 11, 12)** and examined their antibacterial activity against various bacterial strains considering gyrase as a target. All these compounds were synthesised by inter and intramolecular imino Diels–Alder reaction starting from aromatic amines and various carbonyl compounds.

Synthesis of hexahydropyranotetrahydroquinoline derivative **4** was accomplished by a one pot reaction of indane 1,2,3-trione *p*-bromo aniline, dihydropyran in the presence of indium trichloride as a catalyst, using acetonitrile as solvent (Scheme 1). Similarly uracil annulated quinoline derivative **7** was synthesized by imino Diels–Alder reaction of *N*-prenyl pyrrolopyrimidine-6-carbaldehyde with *p*-chloroaniline in the presence of indium trichloride as catalyst (Scheme 2). In the same way the quinoline derivatives **10, 11** and **12** were synthesized by imino Diels–Alder reaction (Schemes 3 and 4). All the compounds were characterised by spectroscopic and elemental analysis.

2. Results and discussion

Antibacterial activity for the above synthesized compounds (**4, 7, 10, 11, 12**) were evaluated against six different bacterial strains 1. *Staphylcoccus aureus,* 2. *Escherichia coli*, 3. *Pseudomonas aeruginosa, 4. Klebsiella pneumonia, 5. Salmonella typhimurium, 6. Bacillus subtilis.* Disc diffusion assay was carried out and the zones of inhibition for different concentrations of the synthetic compounds were measured and the results are given in Table 1. Ciprofloxicin was used as a reference drug.

From Table 1 it can be seen that all the compounds exhibited antibacterial activity against various strains. Good inhibitory characteristics are observed for the compounds even for small concentration of 5 μg. Compounds **4**, **10**, **11**, **12** inhibited the growth of the pathogens particularly *P. aeruginosa*, while compounds **7, 10, 11** showed good growth inhibition against *K. pneumonia*. The inhibitory effect was more pronounced for gram −ve strains than gram +ve strains.

2.1. Minimum inhibitory concentration (MIC) of the compounds using broth micro dilution assay

We further determined the MIC values of the quinoline derivatives against different bacterial strains and these are given in Table 2. The effect of various compounds on the growth of the organisms

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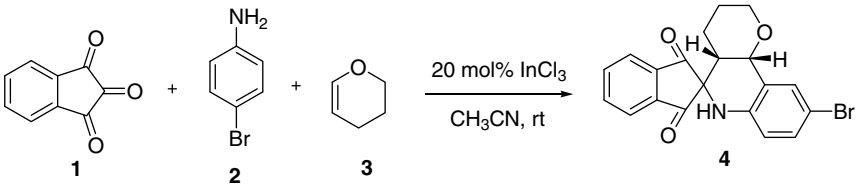
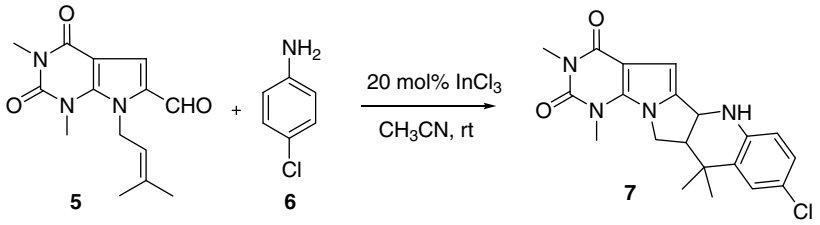
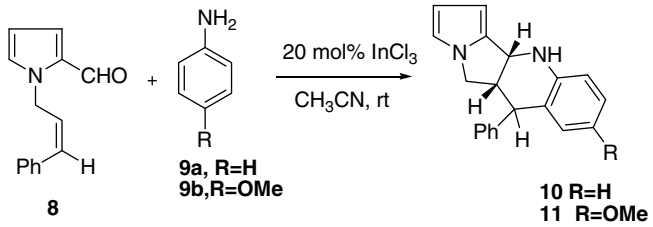


Scheme 1.



Scheme 2.



Scheme 3.

was analysed. Uniform amount of inoculum was added to each tube and after adding the compounds in the mid-log phase, the turbidity of the medium was measured spectrophotometrically at A_{600}. The concentration at which the growth of the organisms was inhibited by 50% and 90% and the range of the concentrations within which there was no visible growth were determined and provided in Table 2. From the values it can be seen that significant inhibition was observed at lower concentrations for the tested strains, particularly, against *E. coli*. The average percentage of growth was found to be 44.1%, 50%, 42.3%, 45.7% and 49% for the compounds **4**, **7**, **10**, **11**, 12, respectively (Fig. 1.1).

2.2. Gyrase supercoiling assay

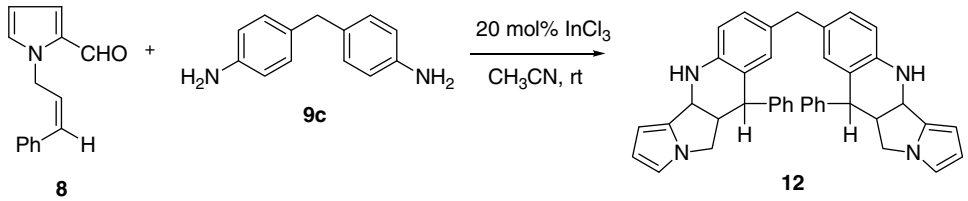
DNA gyrase supercoiling activity was assessed by measuring the conversion of relaxed plasmid Pbr322 DNA to the supercoiled form. The supercoiling assay was performed as described in Section 3 and inhibition of gyrase supercoiling due to the compounds **4, 7, 10 11, 12** was analysed. The results (Figs. 1.2–1.7) showed that compound **10** inhibited DNA gyrase supercoiling while the compounds **7, 12** were found to inhibit gyrase supercoiling with potencies similar to that of known drugs such as ciprofloxacin, and nalidixic acid. The other compounds **4, 10** also inhibited DNA gyrase supercoiling, but to a lesser extent when compared to ciprofloxacin. It is interesting to note that compound **7** consists of an uracil moiety that might be responsible for the inhibition of gyrase.

2.3. Gyrase relaxation assay

To ascertain the effect of the synthetic compounds on the other activity of gyrase, namely DNA relaxation, different concentrations of the drugs were incubated with the enzyme and the relaxation of the supercoiled DNA was evaluated in the absence of ATP. Ciprofloxacin, novabiocin and nalidixic acid were used for comparison. Inhibition in DNA relaxation was observed for compounds **7** and **12** at a concentration of 1 μM. In the case of compounds **4** and **11**, inhibition was not observed even at a higher concentration of 5 μM while for the compound **10** inhibition was found at a concentration greater than 2.5 μM. Novabiocin did not inhibit even at a concentration of 5 μM. Nalidixic acid showed inhibition at concentration greater than 2.5 μM and ciprofloxacin showed inhibition at a concentration of 1 μM.

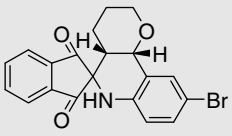
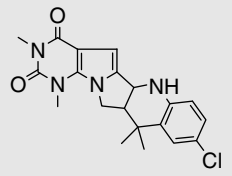
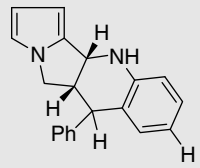
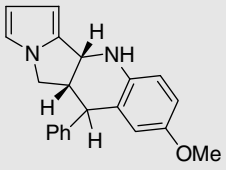
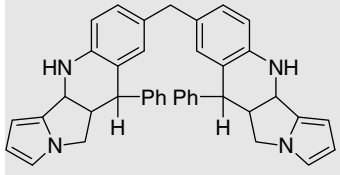
2.4. Reverse transcriptase-polymerase chain reaction analysis

Total RNA isolated from the bacterial cells was treated with the synthetic compounds and RT-PCR was carried out to assess gyrase gene expression, as outlined in Section 3. The products of RT-PCR were electrophoresed on a 1% agarose gel keeping GAPDH as the housekeeping gene. The results showed a very significant decrease in the intensity of the band of 661 that corresponds to gyrase DNA. The reduction was higher for compounds **7**, **11**, compared to that observed for **4**, **10**, **12**. These results (Fig. 2.8) correlate well with those obtained from gyrase supercoiling and relaxation assay. Total RNA without drug treatment was used as control.



Scheme 4.

Table 1
The zone of inhibition for different strains

Compound	Strain	Zone of inhibition (in cm)				
		5 μg	10 μg	15 μg	20 μg	25 μg
 **4**	*Staphylcoccus aureus*	0.8	0.9	1	1.2	1.4
	E. coli	0.8	0.9	1	1.2	1.3
	Pseudomonas aeruginosa	1	1.4	1.6	2	2.3
	Klebsiella pneumonia	0.8	1.0	1.3	1.4	1.6
	Salmonella typhimurium	0.8	1.0	1.3	1.4	1.6
	Bacillus subtilis	–	–	0.8	0.9	1.0
	*Staphylcoccus aureus*	2.0	2.4	2.6	3.0	3.6
	E. coli	1	1.6	1.8	2	2.3
	Pseudomonas aeruginosa	1.0	1.4	1.6	2.0	2.3
	Klebsiella pneumonia	2.0	2.6	3.0	3.4	3.6
	Salmonella typhimurium	1.0	1.6	1.8	2.2	2.4
	Bacillus subtilis	–	–	0.6	0.7	0.9
	*Staphylcoccus aureus*	0.9	1.2	1.3	1.4	1.7
	E. coli	1.0	1.6	1.8	2.0	2.3
	Pseudomonas aeruginosa	1	1.6	1.7	2.2	2.4
	Klebsiella pneumonia	2.0	2.6	3.0	3.4	3.6
	Salmonella typhimurium	1.0	1.6	1.8	2.2	2.4
	Bacillus subtilis	–	0.7	0.6	0.7	0.9
	*Staphylcoccus aureus*	2.0	2.4	2.6	3.0	3.6
	E. coli	1.2	1.4	1.6	2.2	2.3
	Pseudomonas aeruginosa	2.4	2.6	2.8	3.4	3.6
	Klebsiella pneumonia	1.0	1.4	1.6	2.4	2.6
	Salmonella typhimurium	1.0	1.6	1.8	2.2	2.4
	Bacillus subtilis	–	0.8	0.9	1.0	1.1
	*Staphylcoccus aureus*	0.9	1.3	1.5	1.7	2.1
	E. coli	1.1	1.4	1.6	2.1	2.2
	Pseudomonas aeruginosa	2.0	2.4	2.6	2.8	3.0
	Klebsiella pneumonia	1.2	1.6	1.9	2.4	2.6
	Salmonella typhimurium	1.0	1.6	1.8	2.2	2.4
	Bacillus subtilis	0.7	0.9	1.0	1.2	1.3
Ciplofloxicin	*Staphylcoccus aureus*	2.2	2.4	2.6	3.0	3.6
	E. coli	1.4	1.6	1.8	2.0	2.3
	Pseudomonas aeruginosa	1.2	1.4	1.6	2.0	2.3
	Klebsiella pneumonia	2.4	2.6	3.0	3.4	3.6
	Salmonella typhimurium	1.4	1.6	1.8	2.2	2.4
	Bacillus subtilis	0.6	0.7	0.9	1.0	1.2

All the five quinoline derivatives showed antimicrobial activity as indicated by disc diffusion assay. The compounds **7** and **11** showed significant activity against *S. aureus*, *E. coli*, *K. Pneumonia*, *P. aeruginosa, S. typhi* and reduced activity against *B. subtilis*.

The MIC values, were determined for the compounds **4, 7, 10, 11, 12** for the above mentioned organisms and the values of MIC required to inhibit 50% and 90% ($MIC_{50,}$ MIC_{90}) of the growth are shown in Table 2. Analysis has suggested that gram-negative bacteria especially *Pseudomonas* was inhibited strongly by the five quinoline derivatives. All the quinoline derivatives showed good activity against members of *Enterobacteriaceae*. The inhibitory action increases with increase in the concentration of the compounds. Considerable antimicrobial activity was observed against *E. coli*. The percentage of growth was found to be 44.1%, 50%, 42.3%, 45.7% and 49% for the compounds **4, 7, 10**, 11 and **12**, respectively. The MIC values and the observed growth inhibition against *E. coli* has demonstrated that the compounds **7, 12** might act as potent antibacterial agents.

The gyrase supercoiling is inhibited by all the quinoline derivatives of which compounds **7** and **12** inhibited gyrase supercoiling with potencies similar to that of known drugs ciprofloxacin and nalidxic acid. Gyrase relaxation is noticed for all synthesized quinoline derivatives with considerable activity for **11** and **12** at μM concentration, For compounds **4, 7** inhibition was not observed even at a concentration of 5 μM and for the compound **10** the inhibition was found at a concentration greater than 2.5 μM.

Table 2
The MIC of the compound tested against organisms

Organisms	Compound	Range	MIC (mg/mL)	
			50%	90%
S. aureus	**4**	8–128	64	64
	7	0.125–8	0.250	—
	10	16–128	64	128
	11	0.125–1	0.5	0.5
	13	32–128	128	128
	Ciprofloxacin	0.125–1	0.5	1
E. coli	**4**	>128	2	4
	7	0.03–4	0.06	0.25
	10	>128	2	4
	11	0.06–16	0.125	0.25
	13	1–>128	2	8
	Ciprofloxacin	0.0075–1	0.015	0.03
K. pneumonia	**4**	>125–16	0.25	4
	7	0.015–8	0.125	2
	10	0.5–>128	2	32
	11	0.015–16	0.25	2
	13	0.5–128	0.125	2
	Ciprofloxacin	0.0075–2	0.03	0.5
P. aeruginosa	**4**	0.5–16	1	8
	7	0.06–2	0.125	1
	10	0.06–64	0.5	32
	11	0.06–2	0.125	1
	13	0.25–32	1	8
	Ciprofloxacin	0.015–8	0.125	1
S. typhi	**4**	0.3–5	1.64	2.5
	7	0.01–0.3	0.09	0.15
	10	0.3–5	1.64	2.5
	11	<0.0025–0.15	0.06	0.15
	13	0.8–40	2.51	5
	Ciprofloxacin	<0.0025–0.3	0.04	0.15
B. subtilis	**4**	2.5–5	5	5
	7	0.6–1.2	0.25	1.2
	10	1.2–5	2.5	5
	11	0.15–5	0.30	2
	13	0.6–1.2	0.25	5
	Ciprofloxacin	<0.0025–0.3	0.04	0.15

The results are suggestive of down regulation of gyrase gene by compounds **7** and **12** which is significantly higher than that obtained for **4** and **10**. Thus compounds 7 and **12** can be considered as potent gyrase inhibitors whose activity is similar to the quinolones ciprofloxacin in inhibiting gyrase supercoiling and relaxation. Thus the quinoline derivatives synthesized presently might rapidly inhibit bacterial deoxyribonucleic acid replication, thereby leading to cell death.

With the increasing incidence of drug resistance, the development of new drugs is essential to successfully combat bacterial diseases and the current analysis has demonstrated the ability of the synthesised compounds to inhibit DNA gyrase. Thus gyrase inhibitors especially modified quinolines, might gain importance as a new class of highly potent gyrase inhibitors that can serve as lead compounds for drug development.

3. Materials and experimental methods

All melting points are uncorrected. IR spectra were recorded on a SHIMADZU FT-IR 8300 instrument. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as an internal standard on Bruker 300 at 300 MHz and at 75 MHz, respectively. Mass spectra were recorded on a JEOL DX 303 HF-mass spectrometer. Most of the chemicals used were purchased commercially. Column chromatography was performed on silica gel (ACME, 100–200 mesh). Flash column chromatography was performed on silica gel (Sisco, 230–40 mesh). Routine monitoring of the reactions was made using thin layer chromatography developed on glass plates coated with silica gel-G (ACME) of 0.25 mm thickness and visualized with iodine. For the dry experiments, glasswares used were thoroughly dried in a hot air oven, cooled and assembled under a stream of nitrogen. The organic extracts of crude products were dried over anhydrous $MgSO_4$. Solvents were reagent grade and were purified according to standard procedures. All the chemicals used were commercially available.

3.1. Procedure for the synthesis of compound 4

A mixture of indane 1, 2, 3 trione (1 mmol), 4-bromo aniline (1 mmol) and 3,4-dihydro-2*H*-pyran (1 mmol) in the presence of 20 mol % $InCl_3$ in acetonitrile (6 mL) was stirred at room temperature until the completion of the reaction as evidenced by TLC. The reaction mixture was quenched with water and extracted with ethyl acetate (3 × 10 mL), washed with brine and dried over Na_2SO_4. After the removal of solvent under reduced pressure, the crude residue obtained was eluted through column chromatography with petroleum ether ethyl acetate mixture (7:3) to afford **4**. Yield 75%.

3.2. (4*aS*,10*bS*)-9-Bromo-3,4,4*a*,5,6,10*b*-hexahydro-5-indane 1,3 dione-2*H*-pyrano[3,2-*c*]quinoline 4

Red orange powder; mp 224 °C; yield: 69% IR (KBr): 1732 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.13–1.42 (m, 4H), 1.73–2.12 (m, 2H), 3.12 (dt, J = 12.2, 3.5 Hz, 1Hb), 4.33 (s, 1H, NH), 5.12 (d, J = 3.5 Hz, 1Ha), 7.44–7.38 (m, 7H),; ^{13}C NMR (75 MHz, $CDCl_3$): 21.3, 23.1, 33.3, 60.7, 62.4, 65.5, 10.6, 111.5, 114.7, 120.3, 124.0, 124.8, 126.8, 130.7, 133.4, 137.4, 142.8, 153.5, 158.9, 177.8, 181.61. Mass m/z: 397.05 (M^+). Calculated for $C_{20}H_{16}BrNO_3$: C, 60.32; H, 4.05; N, 3.52; Br, 20.06. Found: C, 60.45; H, 4.13; N, 3.43.

3.3. General procedure for synthesis of compound 7

$InCl_3$ (20 mol %, 44 mg) was added to a mixture of *p*-chloro aniline (1 mmol, 270 mg) and *N*-alkenyl pyrrolo[2,3-*d*]pyrimidine-6-carbaldehyde (500 mg) in acetonitrile (5 mL). The reaction mixture was stirred at room temperature until the completion of the reaction as indicated by TLC. The mixture was then quenched with water and extracted with ethyl acetate. The organic layer was washed with water and dried over $MgSO_4$. The solvent was evaporated in vacuo and the crude product was chromatographed on silica gel using petroleum ether ethyl acetate mixture (7:3) to afford compound **7**.

3.4. 7*a*,13*b*-8,8-Dimethyl pyrrolo[2,3-*d*]pyrimidine-[2,1-*a*]pyrrolo[4,3:2,3]-7*a*,8,13,13*b*-tetrahydroquinoline 7

Mp 191 °C, Yield: 92% ^{1}H NMR (MHz, $CDCl_3$): δ 1.52 (s, 3H), 1.55 (s, 3H), 3.23 (td, J = 9.7, 10.2 Hz, $1H_b$), 3.41 (s, 3H), 3.46 (s, 3H), 3.61 (t, J = 10.2 Hz, $1H_d$), 4.10 (dd, J = 7.7, 9.7 Hz, $1H_c$), 4.69 (d, J = 11.2 Hz, $1H_a$), 5.18 (s, 1H), 6.47 (d J = 8.2 Hz, 1H), 6.84 (s, 1H), 7.12 (d, J = 8.2 Hz, 1H). ^{13}C NMR (MHz, $CDCl_3$): δ 25.68, 27.17, 29.22, 30.51, 35.18, 36.64, 55.14, 60.14, 104.14, 107.46, 120.46, 121.27, 123.37, 126.41, 130.79, 132.34, 137.46, 139.49, 154.79, 159.20 ppm. Mass *m/z*: 384.14 (M^+). Calculated for $C_{20}H_{21}ClN_4O_2$: C, 62.42; H, 5.50; N, 14.51. Found: C, 62.56; H, 5.62; N, 14.38.

3.5. Procedure for synthesis of compound 10 and 11

$InCl_3$ (20 mol %, 44 mg) was added to a mixture of aniline (1 mmol, 270 mg) or *p*-methoxy aniline and *N*-cinnamyl pyrrole-2-carbaldehyde (1 mmol, 500 mg) in acetonitrile (5 mL). The reaction mixture was stirred at room temperature until the completion of the reaction as indicated by TLC. The work up procedure is same as mentioned above for **7** afford the product **10** and **11**.

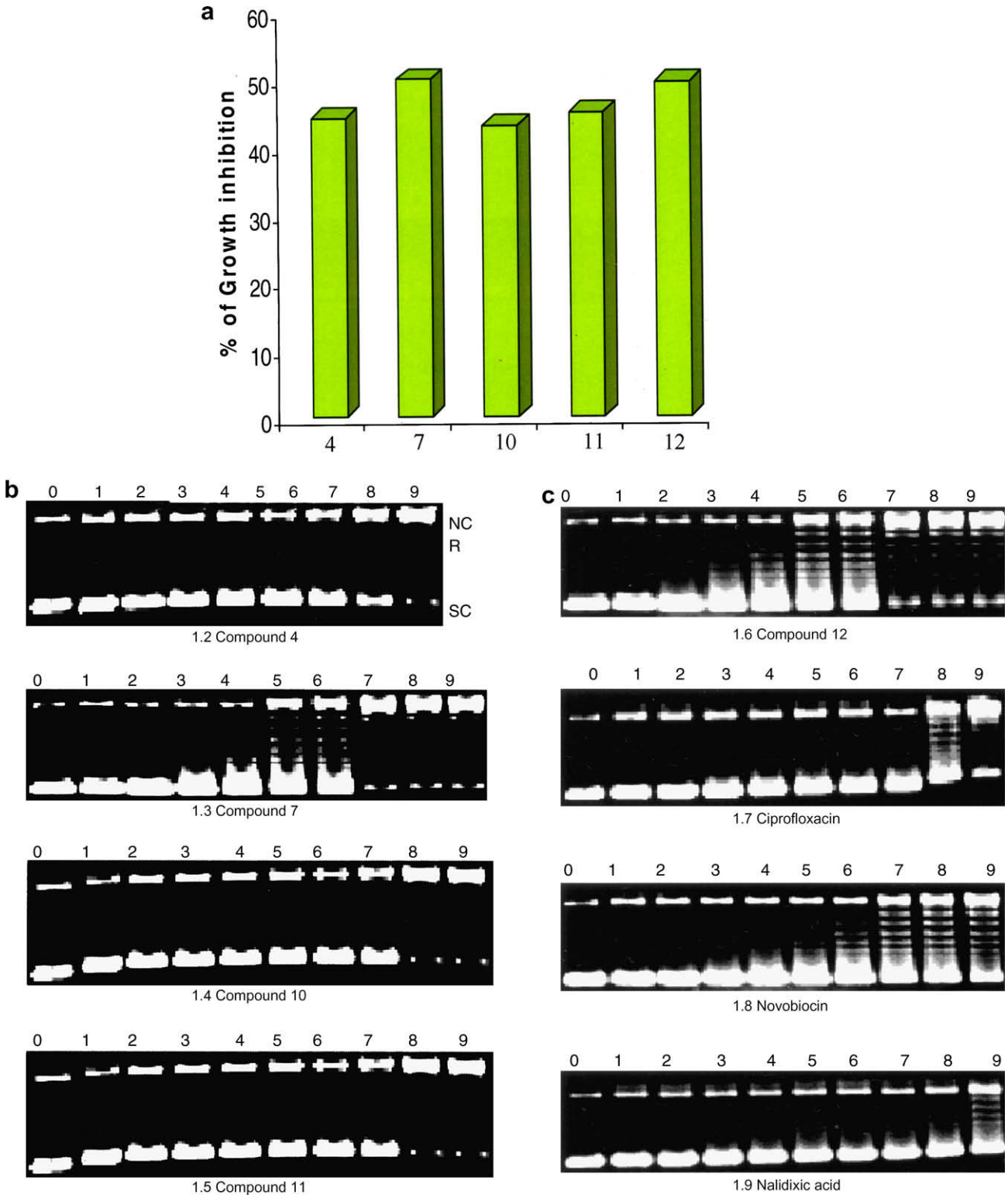


Figure 1. (a) Growth inhibitory effects of synthetic compounds **4**, **7**, **10**, **11**, **12** on *E. coli*. (b–c) Inhibition of DNA gyrase supercoiling assay by synthetic compounds. Pbr322 topoisomers were analysed by agarose gel electrophoresis. Lane 1–9 increasing concentration of synthetic compounds at 0, 1, 2.5, 5, 10, 20, 40, 60, 80, 100 mM, respectively.

3.6. (3*bR*,9*aR*)-4,9,9*a*,10-Tetrahydro-9-phenyl-3*bH*-pyrrolizino[1,2-*b*]quinoline 10

For compound **10**: Liquid, yield: 80%, ^{1}H NMR ($CDCl_3$): δ 4.23 (d, J = 6.1 Hz, 1H$_a$), 5.63 (dd, J = 14.1, 7.5, 1H$_c$), 5.63 (t, J = 6.3, 1H$_d$), 5.07 (d, J = 9.2 Hz, 1H), 5.12–5.28 (m, 1H$_b$), 6.44–7.25 (m, 7H). ^{13}C NMR ($CDCl_3$): δ 42.30, 45.81, 46.60, 63.21, 106.16, 107.67, 113.68, 115.23, 121.34, 125.10, 127.23, 126.36, 125.42, 126.41, 124.91, 131.24, 146.06. Mass m/z: 286.15 (M^+). Calculated for $C_{20}H_{18}N_2$: C, 83.88; H, 6.34; N, 9.78. Found: C, 83.99; H, 6.43; N, 9.65.

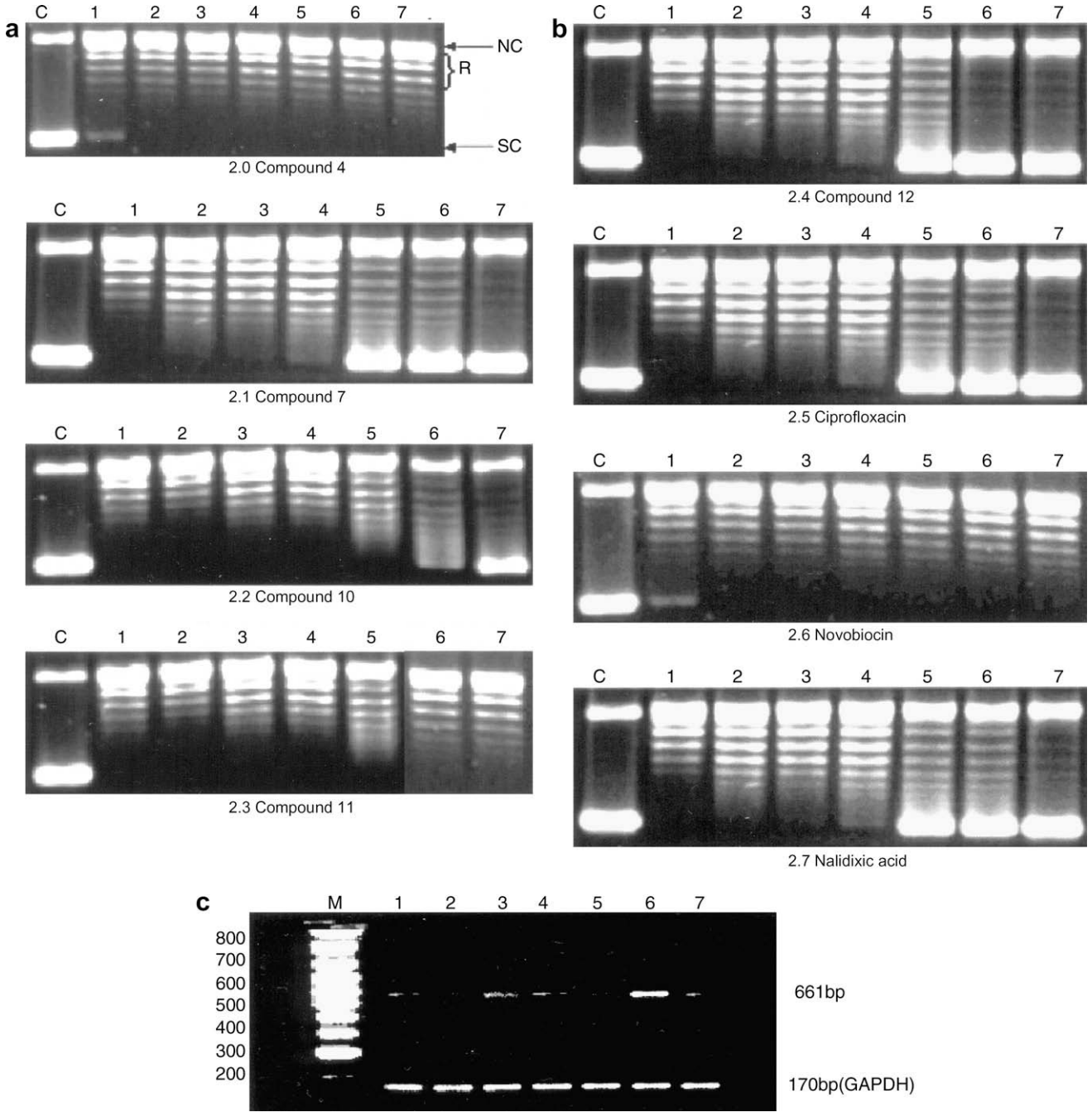


Figure 2. (a–b) Effect of synthetic compounds on gyrase relaxation. Gyrase (60 nM) and supercoiled pBR322 DNA (6 nM) were incubated with increasing concentrations of drugs and ciprofloxacin, novabiocin and nalixidic acid. Concenration—0, 0.1, 0.25, 0.5, 2.5 mM for 1 h at 37 °C. (c) RT-PCR amplification of the total RNA from the drug (0.5 mM) treated *E. coli*. Lane M: marker, Lane 1: cells treated with ER1, Lane 2: cells treated with ER2, Lane 3: cells treated with RD1, Lane 4: cells treated with RD2, Lane 5: cells treated with RD3, Lane 6: negative control (cells without any drug treatment), Lane 7: cells treated with ciprofloxacin. *Reverse transcriptase-polymerase chain reaction analysis*: RT-PCR was carried out on the total RNA from the cells treated with the synthesis drugs and known compounds to assess gyrase expression and the products of the RT-PCR were electrophoresed on a 1% agarose gel. GAPDH was used as the housekeeping gene. The results showed a very significant decrease in the band intensity in the cells treated with **2**, **4**. These results (2.8) correlated well the gyrase inhibition assays namely supercoiling and the relaxation assay. There was also decrease in band intensity in the cells treated with **4**, **10**, **11**. These results were compared with ciprofloxacin, novabiocin and nalidixic acid. Total RNA without any drug treatment was used as control.

3.7. (3*bR*,9*aR*)-4,9,9*a*,10-Tetrahydro-7-methoxy-9-phenyl-3*bH*-pyrrolizino[1,2-*b*]quinoline 11

For compound **11**: Liquid, yield: 87%, ^{1}H NMR ($CDCl_3$): δ 3.12 (s, 3H), 4.36 (d, J = 5.8 Hz, 1H$_a$), 5.45 (dd, J = 13.9, 7.2, 1H$_c$), 5.59 (t, J = 5.9, 1H$_d$), 5.23 (d, J = 9.0 Hz, 1H), 5.01–5.23 (m, 1H$_b$), 6.15–7.21 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 30.14, 41.45, 44.80, 45.55, 62.10, 112.14, 117.63, 112.64, 114.16, 121.13, 125.78, 127.16, 126.72, 127.42, 128.47, 126.90, 134.15, 145.16. Mass *m/z*: 316.16 (M^+). Calculated for $C_{20}H_{18}N_2O$: C, 79.72; H, 6.37; N, 8.85. Found: C, 79.83; H, 6.46; N, 8.74.

3.8. Procedure for synthesis of compound 12

$InCl_3$ (20 mol %, 44 mg) was added to a mixture of 4,4′-methylenedianiline (1 mmol, 230 mg) and *N*-cinnamyl pyrrole-2-carb-

aldehyde (2 mmol, 500 mg) in acetonitrile (5 mL). The reaction mixture was stirred at room temperature until the completion of the reaction as indicated by TLC. Similarly the work up mentioned in the above procedure to afford the product compound **12**.

3.9. Bis(4,9,9*a*,10-Tetrahydro-9-phenyl-3*bH*-pyrrolizino[1,2-*b*]quinolin-7-)methane 12

Yield: 75%; mp 182 °C, ^{1}H NMR ($CDCl_3$): δ 3.33 (s, 2H, $-CH_2-$), 4.84 (d, J = 9.9 Hz, 2H), 4.73 (dd, J = 14.8, 7.6 Hz, 2H), 4.84 (t, J = 6.5 Hz, 2H), 5.12–5.18 (m, 2H), 5.12 (d, J = 8.9 Hz, 2H), 6.33–7.96 (m, 22H). ^{13}C NMR: δ 30.23, 33.13, 41.23, 42.84, 46.23, 98.32, 113.45, 113.23, 120.76, 122.55, 122.78, 123.21, 123.54, 126.18, 127.45, 128.12, 132.84, 131.80, 135.98, 141.60 ppm. Mass m/z: 584.29 (M^+). Calculated for $C_{41}H_{36}N_4$: C, 84.21; H, 6.21; N, 9.58. Found: C, 84.33; H, 6.32; N, 9.42.

4. Methods

4.1. Diffusion method

Disc diffusion assay was performed to determine the rate of inhibition in the growth of bacteria. Inoculum was prepared in 0.85% saline using McFarland standard and spread uniformly on nutrient agar plates with cotton swabs. Sterile Whatman no. 4 filter paper discs were placed at equidistance on the agar surface and drugs of equal volume and varying concentration was placed on each disc. The zone of inhibition for each concentration was measured after 24 h incubation at 37 °C.

4.2. MIC of the compounds by broth micro dilution assay

Broth micro dilution was carried out to determine the MIC of the synthetic compounds as per the protocol of National Committee of Clinical Laboratory Standards (NCCLS), 1997. A 96 well sterile microtiter plate with appropriate drug dilution was labeled. Antibiotic solution was added to the well. A final inoculum of 105 cfu/mL of the organism was prepared. The test organism suspension was added to one row and the control organism was added to the other row. It was incubated at 35.7 °C in air for 18–20 h. The MIC end point was read as the lowest concentration of antibiotic at which there was no visible growth.

4.3. Gyrase supercoiling assay

The gyrase supercoiling was assessed by measuring the conversion of relaxed plasmid pBR322 DNA to the supercoiled form, as described previously (bazile et al., Revel-Viravau et al., 1996). Supercoiling assays were carried out in 15 μL reaction mixtures containing the DNA gyrase assay buffer, 150 ng relaxed pBR322, and 2–3 μL purified DNA gyrase. The mixture was incubated for 3 h at 37 °C for *E. coli*, the reaction was stopped by the addition of 50% glycerol containing 0.25% bromophenol blue, and the total reaction mixture was subjected to electrophoresis on 1% agarose gel in 1×TBE buffer (Tris/borate/EDTA, pH 8.3). After a run of 3 h at 90 V, the gel was stained with ethidium bromide (0.7 μg mL^{-1} supercoiling activity was assessed by tracing the brightness of the bands corresponding to the supercoiling pBR322 DNA, using a Densylab densitometer (Bio-Rad). One unit (U) of enzyme activity was defined as the amount of DNA gyrase that converted 150 ng relaxed pBR322 to the supercoiling form in 30 min at 37 °C for *E. coli*.

4.4. RT-PCR analysis of gyrase gene

Total RNA was isolated from the transformed strains that were collected at regular intervals upto 24 h, Two microgram of total RNA was used as template for the synthesis of first strand cDNA by reverse transcriptase using MMLV. Primers were designed using primer Express 1.5 software (Applied Biosystems, CA, USA). PCR was performed using 1 μL of Taq DNA polymerase in 20 μL reaction containing 2 μL 10× Taq buffer, 1 μL of each primer, 1 μL of dNTPs, 0.5 μL of Taq polymerase, 12.5 μL of sterile water and 2 μL cDNA template. PCR amplification of gyrase gene was conducted under the following conditions: an initial cycle of denaturation step at 94 °C for 4 min and final extension step at 72 °C for 7 min. The RT-PCR products were run on 1.5% agarose gel. Bands were visualized and quantitative data normalized against GAPDH. Densitometric analysis of the band was performed using National Institute of Health (NIH)—Densitometric Image software, available in the public domain (http://rsb.info.nih.gov/nihimage). The values are expressed in arbitrary units. This analysis was carried out for all strains mentioned above.

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