CSIRO PUBLISHING

Aust. J. Chem.
http://dx.doi.org/10.1071/CH12326

Full Paper

Synthesis, Antimicrobial Activity, and Molecular Docking Study of Some Novel Cyclophanes with Imino Intra-Annular Functionality

Perumal Rajakumar,[A,E] *Ramar Padmanabhan,*[B] *Chandrasekaran Ramprasath,*[C] *Narayanasamy Mathivanan,*[C] *Vaidhyanathan Silambarasan,*[D] *and Devadasan Velmurugan*[D]

[A]Department of Organic Chemistry, University of Madras, Guindy Campus, Chennai, 600 025, India.
[B]Research and Development Centre, Orchid Chemicals and Pharmaceuticals Ltd, Sozhanganallur, Chennai, 600 119, India.
[C]Center for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, 600 025, India.
[D]Center for Advanced Studies in Crystallography and Biophysics, University of Madras, Guindy Campus, Chennai, 600 025, India.
[E]Corresponding author. Email: perumalrajakumar@gmail.com

The synthesis and structural characterisation of novel imino cyclophanes incorporating various spacer units is described. All the imino cyclophanes exhibit comparable antibacterial activity against Gram positive (*Bacillus subtillus*, *Staphylococcus aureus*) and Gram negative (*Escherchia coli*, *Klebsiella pneumonia)* bacterial strains. The imino cyclophanes also exhibit good antifungal activity against human pathogenic fungus, *Candida albicans*.

Manuscript received: 11 July 2012.
Manuscript accepted: 14 August 2012.
Published online: 12 September 2012.

Introduction

Most antibacterials are semisynthetic modifications of various natural compounds.[1] Some common β-lactam antibacterials are penicillins, cephalosporins, penems, and carbapenems.[2] Aminoglycosides are the antibacterials isolated from living organisms whereas other antibacterials, such as sulfonamides, quinolones, and oxazolidinones are obtained by chemical synthesis. The development of bacterial resistance to existing potent antimicrobial drugs is a major concern for managing infections.[3] One of the concerns is severe infections caused by multi drug-resistant (superbug) Gram-positive pathogens, which result in high mortality rates especially in hospital settings. The individual organisms responsible include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus faecalis* (VRE), penicillin-resistant *Streptococcus pneumonia*, and fluconazole resistant *Candida albicans*.[4] This necessitates the continuing research for new classes of antibacterial drugs.[5] There are several antibiotics effective against these organisms but these organisms easily acquire resistance against them. The development of resistance is inherent in the mode of action of all antibiotics and hence poses challenges in the development of new antimicrobial agents to overcome it.[6] Some bacterial strains have been found to be resistant to all currently available antibacterial agents.[7] Several antimicrobials are well documented in the literature.[8] Macrocycles with additional donor atoms appended to the ring have attracted considerable interest because of their capacity to bind and transport metal ions.[9] Imino macrocycles have been greatly attractive for their host–guest complexes and molecular recognition.[10–12] Macrocycles containing hetero atoms are valuable compounds with a broad spectrum of pharmacological activities.[13] Macrocyclic Schiff bases exhibit biological activity as antibiotics, antiviral, antitumour, antifungal, antibacterial, and anticancer agents because of their specific structure.[14] Schiff bases have been reported as potential antimicrobial agents.[15] Recently, the dicationic acridinophanes,[16] quinolinophanes,[17] carbazolophanes,[18] and imidazolophanes[19] using various spacers like pyridine, *m*-terphenyl, and chiral binaphthol have been reported from our laboratory. In a continuation of our on-going investigation into the synthesis of various bioactive cyclophanes, attempts were made to synthesise novel imino cyclophanes by coupling between suitable bis-aldehydes and bis (aminomethyl)*m*-terphenyl and then to study the bioactivity of the synthesised cyclophanes. However, to the best of our knowledge, such imino cyclophanes with bis(aminomethyl)*m*-terphenyl as a spacer have not been reported in the literature. Hence, we report herein the synthesis, antibacterial, and antifungal activity of imino cyclophanes **1**–**7** (Fig. 1) from the corresponding dialdehyde precursors **8**, **9a**, **9b**, **9c**, **10a**, **10b**, **11**, **12**, and **13**.

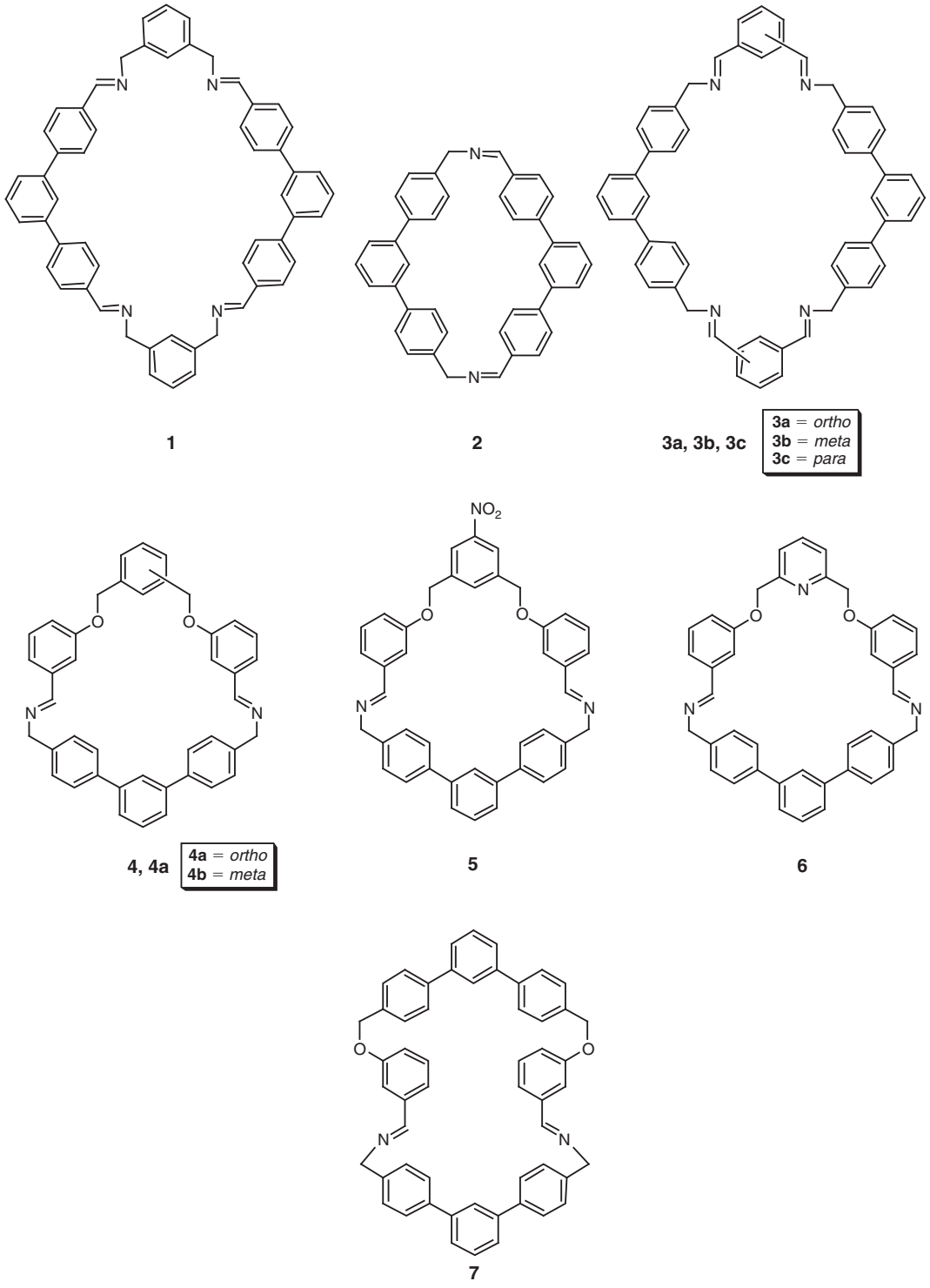


Fig. 1. Structure of imino cyclophanes **1**–**7**.

Results and Discussion

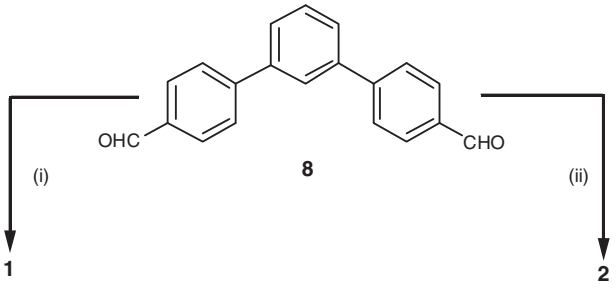
Reaction of 1.0 equiv. of *m*-xylylene diamine and 1.0 equiv. of *m*-terphenyldialdehyde **8** in ethanol under high dilution conditions at room temperature resulted in the formation of cyclophane tetraimine **1** in 85 % yield (Scheme 1). The product slowly precipitated out from the reaction mixture during the course of the reaction. The ^{1}H NMR spectrum of tetraimino cyclophane **1** displayed the *N*-methylene protons as a singlet at δ 4.85 and the N=CH protons as a singlet at δ 8.40. The rest of the aromatic protons appeared in the region δ 7.28–7.81. The mass spectrum of **1** showed the molecular ion peak $[M + H]^+$ at *m*/*z* 773.4. The FT-IR spectrum showed the C=N stretching frequency at 1639 cm^{-1} for the imino cyclophane **1**. The structure of the cyclophane imine **1** was further confirmed from spectroscopic and analytical data. In order to test the synthetic utility of diamine **14** for the synthesis of imino cyclophanes, 1.0 equiv. of *m*-terphenyldiamine **14**[20] was condensed with 1.0 equiv. of *m*-terphenyldialdehyde **8** in ethanol under high dilution

conditions at room temperature. The reaction afforded the formation of imino cyclophane **2** in 70 % yield (Scheme 1). The structure of the cyclophane imine **2** has been confirmed from spectroscopic and analytical data.

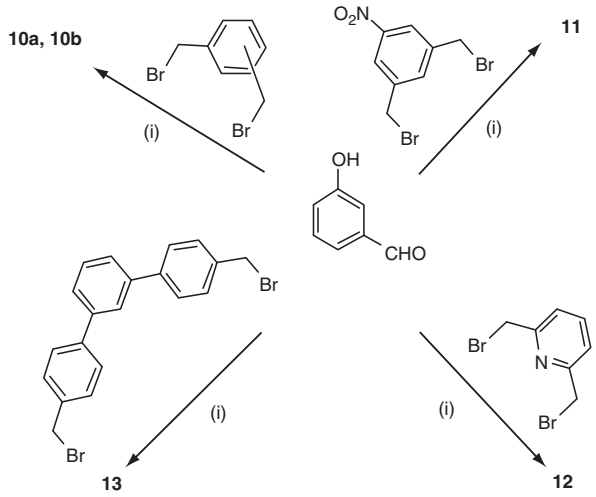
Bis-oxyaldehydes **10a**, **10b**, **11**, **12**, and **13** (Fig. 2) required for the synthesis of imino cyclophanes **4a**, **4b**, **5**, **6**, and **7** were obtained in 86, 81, 93, 90, and 85 % yields after purification by column chromatography by the reaction of 1.0 equiv. of *o*-xylylene dibromide, *m*-xylylene dibromide, 1-nitro-3,5-bis (bromomethyl)benzene, 2,6-(bisbromomethyl)pyridine, and *m*-terphenyl dibromide with 2.1 equiv. of *m*-hydroxybenzaldehyde in the presence of 10.1 equiv. of potassium carbonate in dimethylformamide (DMF) at 60°C for 48 h (Scheme 2).[21] In the ^{1}H NMR spectrum of precyclophane **10b** the *O*-methylene protons appeared as a singlet at δ 5.15 and the aldehyde protons as a singlet at δ 9.97. The rest of the aromatic protons appeared in the region δ 7.24–7.54. The mass spectrum of **10b** showed the molecular ion peak $[M + H]^+$ at *m*/*z* 347.1. The FT-IR spectrum showed the carbonyl stretching frequency at 1695 cm^{-1} for the precyclophane **10b**. The structure of dialdehyde **10b** was further confirmed from spectroscopic and analytical data. Similarly the structures of the precyclophanes **10a**, **11**, **12**, and **13** were also confirmed from spectroscopic and analytical data.

The synthetic pathway leading to imino cyclophane **3a**, **3b**, **4a**, **4b**, **5**, **6**, and **7** (Fig. 1) is outlined in Scheme 3. Cyclophane tetraimines **3a**, **3b**, and **3c** were prepared in ~81, 80, and 82 % yields by the reaction of 1.0 equiv. of *m*-terphenyldiamine **14** and 1.0 equiv. of dialdehyde **9a**, **9b**, and **9c** in ethanol under high dilution conditions at room temperature (Scheme 3). During the course of the reaction, the product slowly crystallized out from the reaction mixture. The ^{1}H NMR spectrum of the tetraimine cyclophane **3b** displayed the *N*-methylene protons as a singlet at δ 4.87 and the N=CH protons appeared as a singlet at δ 8.45. The rest of the aromatic protons appeared in the region δ 7.38–7.86. The mass spectrum of **3b** showed the molecular ion peak $[M + H]^+$ at *m*/*z* 773.4. The FT-IR spectrum showed the C=N stretching frequency at 1642 cm^{-1} for the imino cyclophane **3b**. The structure of imino cyclophane **3b** was further confirmed from spectroscopic and analytical data. Similarly the structures of the imino cyclophanes **3a** and **3c** were also confirmed from spectroscopic and analytical data.

In order to test the synthetic utility of the bis-oxyaldehydes **10a**, **10b**, **11**, **12**, and **13** for the synthesis of bis-oxyimino cyclophanes **4a**, **4b**, **5**, **6**, and **7**, 1.0 equiv. of *m*-terphenyldiamine **14** was condensed with 1.0 equiv. of the bis-oxyaldehydes **10a**, **10b**, **11**, **12**, and **13** in ethanol at room temperature under high dilution conditions. The reaction afforded the bis-oxy cyclophane diimines **4a**, **4b**, **5**, **6**, and **7** in 83, 87, 82, 86, and 71 % yields (Scheme 3). The ^{1}H NMR spectrum of tetraimine



Scheme 1. Reagents and conditions: (i) *m*-xylylene diamine, ethanol, rt, 48 h, **1** (85 %) and (ii) *m*-terphenyl diamine (**14**), ethanol, rt, 48 h, **2** (70 %).



Scheme 2. Reagents and conditions: (i) K_2CO_3, DMF, 60°C, 48 h, **10a** (86 %), **10b** (81 %), **11** (93 %), **12** (90 %), and **13** (85 %).

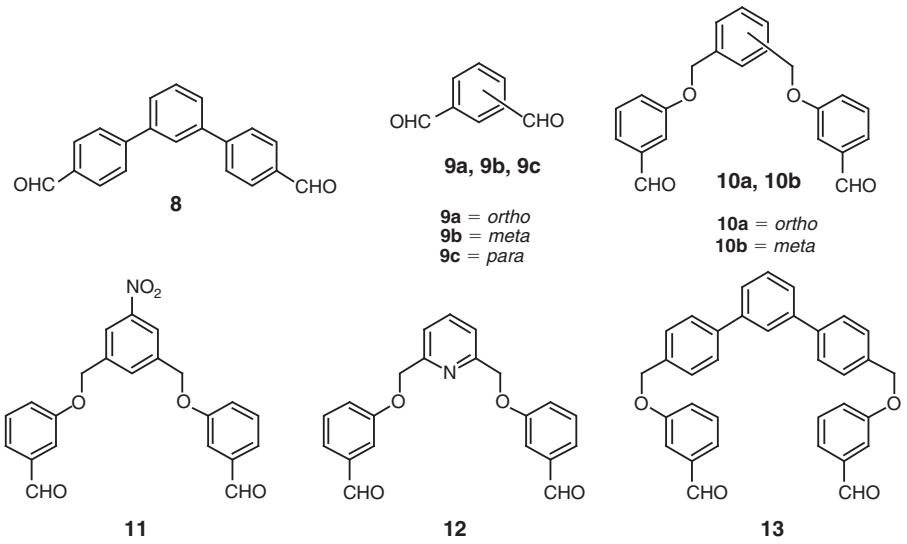
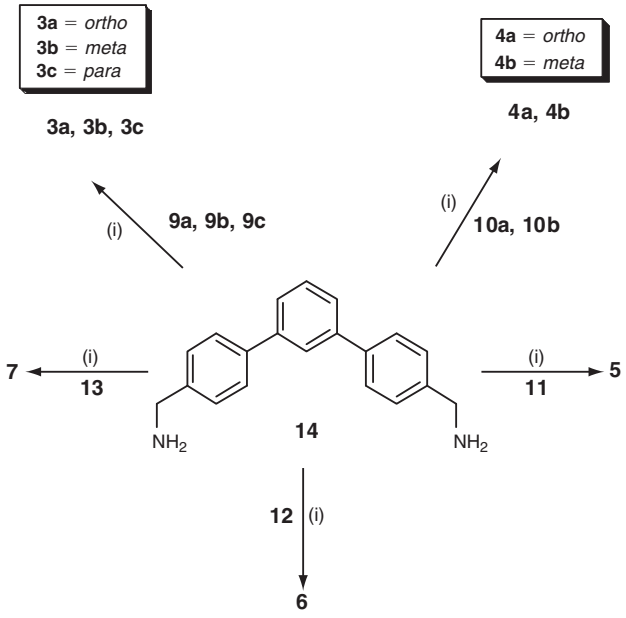


Fig. 2. Structure of dialdehydes **8**–**13**.

cyclophane **4b** displayed the *N*-methylene protons as a singlet at δ 4.85, *O*-methylene protons as a singlet at δ 5.10, and the N=CH protons appeared as a singlet at δ 8.37. The rest of the aromatic protons appeared in the region δ 7.05–7.77. The mass spectrum of **4b** showed the molecular ion peak $[M + H]^+$ at *m*/*z* 599.2. The FT-IR spectrum showed the C=N stretching frequency at 1642 cm^{-1} for the imino cyclophane **4b**. The structure of imino cyclophane **4b** was further confirmed from spectroscopic and analytical data. Similarly the structures of the imino cyclophanes **4a**, **5**, **6**, and **7** were also confirmed from spectroscopic and analytical data.

The in vitro antimicrobial activity of the imino cyclophanes was determined by an agar well diffusion assay against the Gram positive human pathogenic bacteria *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 12600), the Gram negative bacteria *Escherichia coli* (ATCC 11775) and *Klebsiella pnuemonia* (ATCC 13883), and the human fungal pathogen *Candida albicans* (obtained from the culture collections of Biocontrol and Microbial Metabolites Laboratory, Centre for Advanced Studies in Botany, University of Madras, Chennai, India). The primary screening of the antimicrobial activity of the imino cyclophanes was carried out by an agar well diffusion method[22] using Mueller Hinton agar medium. The minimum inhibitory concentration (MIC) for the all the imino cyclophanes **1**–**7** against the same microorganisms was determined using a microdilution susceptibility method.[22] Vancomycin and fluconazole were used as standards for antibacterial and antifungal activity, respectively. The imino cyclophanes **1**–**7** were tested for their in vitro antimicrobial activity at three different concentrations of 50, 75, and 100 $\mu g\ mL^{-1}$. The results revealed that the majority of the imino cyclophanes showed varying degrees of inhibition against the tested human pathogenic microorganisms. The results also revealed that, in general, the inhibitory activity against the Gram-negative bacteria was higher than the Gram-positive bacteria. All the imino cyclophanes **2**, **3a**, **3b**, **3c**, **4a**, **4b**, **6**, and **7** showed significant antimicrobial activity against the test pathogens. Furthermore, all the imino cyclophanes showed a good inhibitory effect against the fluconazole resistant *C. albicans* (Fig. 3).



Scheme 3. Reagents and conditions: (i) ethanol, rt, 48 h, **3** (81 %), **3a** (80 %) and **3b** (82 %), **4** (83 %), **4a** (87 %), **5** (82 %), **6** (86 %), and **7** (71 %).

The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganisms (Table 1). The relationship between the structure of the imino cyclophanes and antimicrobial activity revealed that the imino cyclophane **6** exhibited better antibacterial and antifungal activity against the test pathogens *Bacillus subtillus*, *Staphylococcus aureus*, *Escherchia coli*, and *Klebsiella pneumonia* and the MIC values are comparable to that of the standard drug vancomycin. In fact, fluconazole did not show any inhibitory activity against the tested pathogen *Candida albicans.* However all the imino cyclophanes showed remarkable activity against *Candida albicans*.

All the imino cyclophanes were evaluated for their antifungal activity against *C. albicans* and their MIC was determined. All the tested cyclophanes showed good inhibitory effect against the fluconazole resistant *C. albicans* (Table 1 and Fig. 3). In order to ensure that the solvent had no effect on bacterial and fungal growth a control test was performed with DMSO (dimethyl sulfoxide) which revealed no inhibiting activity.

To study the mechanism of antimicrobial activity of the imino cyclophanes **1**–**7**, docking studies were carried out using a GLIDE application. Imino cyclophanes were tested against the enzyme DNA gyrase B (PDB ID: 2Y3P). These studies revealed that the imino cyclophanes **1**–**7** showed good inhibition of DNA gyrase B enzyme and also showed strong hydrogen bonding with the hydrophobic residues of side chain A of the enzyme dimer DNA gyrase B. In general, the binding of ligand (compounds or drugs) towards the enzyme DNA gyrase B affects its catalytic activity. Prior to unwinding, the DNA binds to the side chain A of DNA gyrase B. As the DNA gyrase B is necessary for the DNA unwinding, the binding of the imino cyclophanes

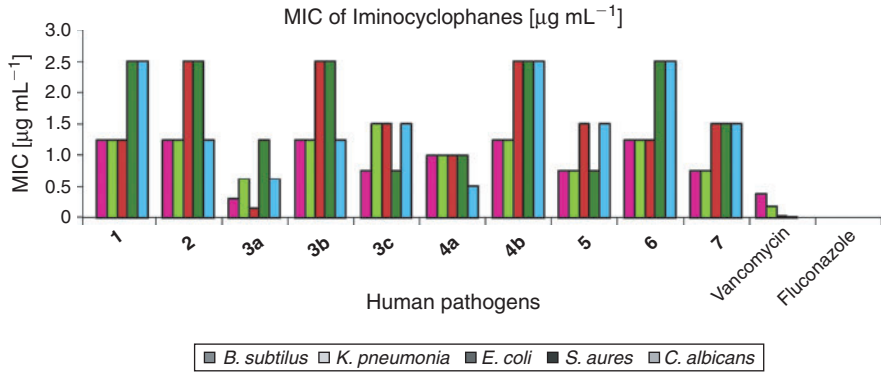


Fig. 3. Minimum inhibitory concentration (MIC) of imino cyclophanes **1**–**7**.

Table 1. Antimicrobial activity (minimal inhibitory concentration (MIC) in μg mL^{-1}) of imino cyclophanes 1–7 against human pathogens

NA: not applicable; –: no inhibition

Imino cyclophanes	*B. subtilus*	*K. pneumonia*	*E. coli*	*S. aureus*	*C. albicans*
1	1.25	1.25	1.25	2.50	2.50
2	1.25	1.25	2.50	2.50	1.25
3a	1.25	1.25	1.25	2.50	2.50
3b	1.25	1.25	2.50	2.50	1.25
3c	0.75	1.50	1.50	0.75	1.50
4a	1.00	1.00	1.00	1.00	0.50
4b	1.25	2.50	2.50	10.00	2.50
5	0.75	0.75	1.50	0.75	1.50
6	0.31	0.63	0.16	1.25	0.63
7	0.75	0.75	1.50	1.50	1.50
Vancomycin	0.39	0.19	0.02	0.002	NA
Fluconazole	NA	NA	NA	NA	–
Control	–	–	–	–	–

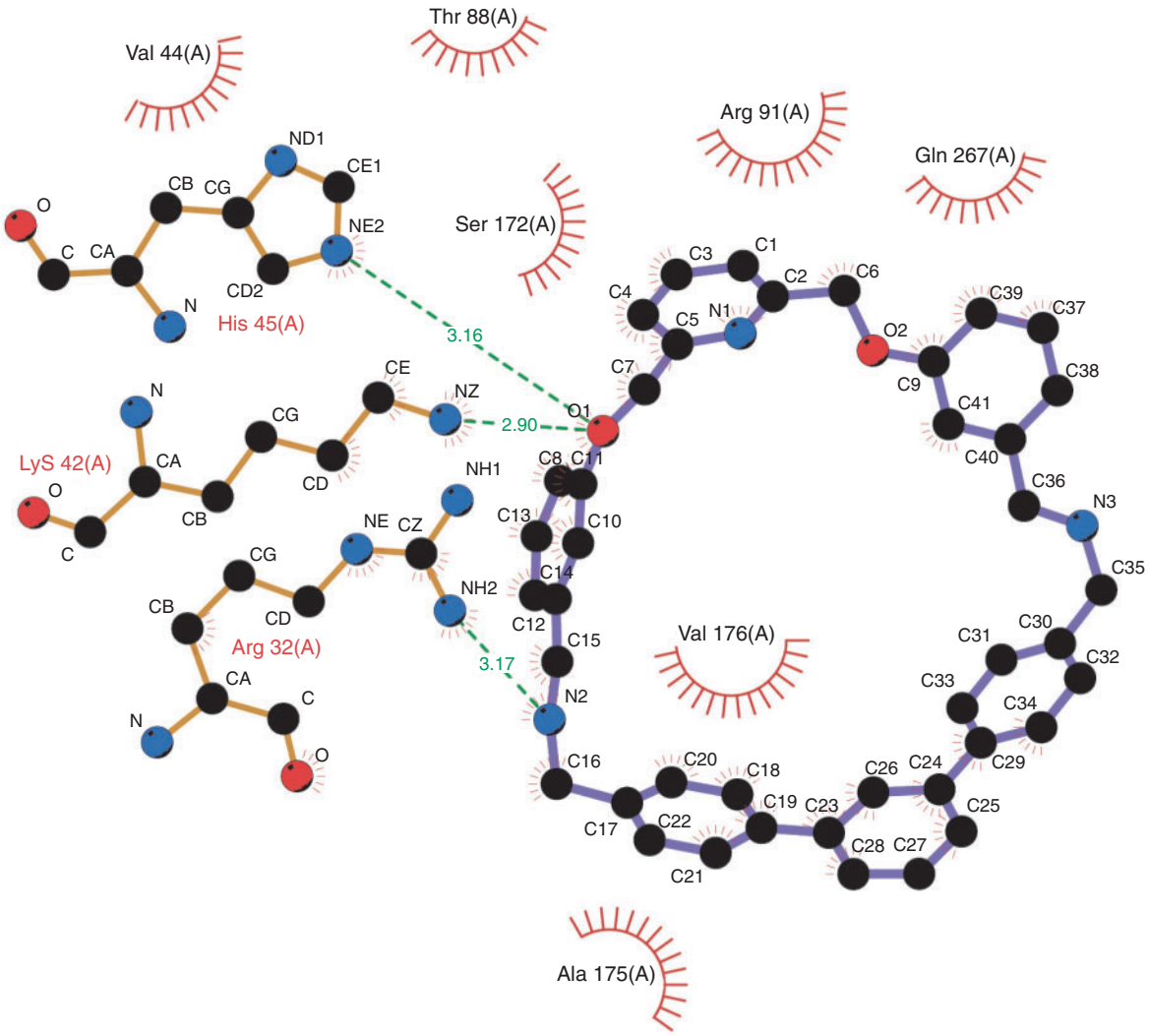


Fig. 4. Ligplot results: Binding of ligand–imino cyclophane **6** on A-chain amino acids of DNA gyrase B (PDB ID: 2Y3P) with three hydrogen bonds and seven hydrophobic interactions.

inhibits the interaction of the DNA enzyme complex.[23] Imino cyclophanes were docked at the active site of the receptor. The imino cyclophane **6** showed strong hydrogen bond interactions with Lys42 (A), Arg32 (A), and His45 (A) and hydrophophic interactions with Val44 (A), Thr88 (A), Ser172 (A), Arg91 (A), and Gln116 (A) (Fig. 4). The Glide energy (binding energy) is −46.64 kcal mol^{-1} and the glide score (docking score) is −3.26 (Table 2 and Fig. 4). The imino cyclophane **4b** also showed

Table 2. Interaction of imino cyclophanes with the target enzyme DNA gyrase B
D: donor; A: acceptor

Imino cyclophane	Glide score	Glide energy [kcal mol^{-1}]	Hydrogen bond	
			D···A	Distance [Å]
3a	−5.02	−57.42	–	–
4b	−4.04	−49.74	His45[N–H···N]	3.14
			Arg91[O–H···N]	2.94
6	−3.26	−46.64	Lys42[N–H···O]	2.90
			Arg32[N–H···N]	3.17
			His45[N–H···O]	3.16
7	−6.27	−70.22	His45[N–H···N]	3.23

strong hydrogen bonding with the residues His45 (A) and Arg91 (A) and hydrophophic interactions with Arg91 (A), Gln267 (A), Thr88 (A), and Asp87 (A). The Glide energy (binding energy) is −49.74 kcal mol^{-1} and the glide score (docking score) is −4.04.

Conclusion

A series of novel imino cyclophanes were synthesised and assayed for their antibacterial activity against the Gram positive human pathogenic bacteria *B. subtilis* and *S. aureus*, the Gram negative bacteria *E. coli* and *K. pneumonia*, and the human fungal pathogen *C. albicans*. Vancomycin and fluconazole were used as standards for antibacterial and antifungal activity, respectively. The imino cyclophane **6** showed good antibacterial activity with a MIC of 0.16–1.25 μg mL^{-1} against the tested bacterial strains and exhibited the most potent inhibitory activity against *E. coli* with 0.16 μg mL^{-1} and *B. subtilus* with 0.31 μg mL^{-1}. The antibacterial assay results also showed that the imino cyclophane **6** could be developed as a potential antmicobial agent against *B. subtilus, E. coli, K. pneumonia*, and *C. albicans.*

Experimental

General

All the reagents and solvents employed were of the best grade available and were used without further purification. The melting points were determined by using a Metler Toledo melting point apparatus by open capillary tube method and were uncorrected. Spectroscopic data were recorded by the following instruments: UV/Vis: Shimadzu 2550 spectrophotometer. IR: Perkin-Elmer series 2000 FTIR spectrophotometer. NMR: Bruker Avance 400 MHz. Mass spectrometry: electrosray ionisation (ESI) PerkinElmer Sciex, API 3000 mass spectrometer and fast atom bombardment (FAB) mass spectra Jeol SX 102/DA-6000 mass spectrometer. The elemental analysis for the compounds was carried out using an Elementar Vario EL III elemental analyzer. Pre-coated silica gel plates from Merck were used for TLC. Column chromatography was carried out using silica gel (100–200 mesh) purchased from ACME.

General Procedure for the Synthesis of Bis-Oxy Aldehydes (**10**–**13**)

A mixture of dibromide (10 mmol), *m*-hydroxybenzaldehyde (21 mmol), and potassium carbonate (101 mmol) in anhydrous DMF (100 mL) was stirred under nitrogen for 48 h at 60°C. The reaction mixture was poured into water (600 mL) and stirred. The resulting precipitate was filtered off, washed with water, and dissolved in diethyl ether (300 mL). The organic layer was washed with water (2 × 250 mL) and then dried over anhydrous sodium sulfate. Removal of the ether gave the corresponding dialdehyde as a crude material, which was purified by column chromatography (SiO_2).

*3,3′-(1,2-Phenylenebis(methylene))bis-oxy) dibenzaldehyde (**10a**)*

Yield 86 %; mp 79°C; ν_{max} (KBr)/cm^{-1} 1696, 1680; δ_H (400 MHz, $CDCl_3$) 9.98 (s, 2H), 7.51–7.55 (m, 2H), 7.46–7.48 (m, 5H), 7.39–7.44(m, 3 H), 7.21–7.24 (m, 2H), 5.24 (s, 4H); δ_C (100 MHz, $CDCl_3$) 192.0, 159.1, 137.9, 134.6, 130.2, 129.3, 128.8, 123.9, 122.1, 113.2, 68.3; MS (ESI) *m/z*: 364.1 ([M + NH_4]$^+$); Anal. Calc. for $C_{22}H_{18}O_4$: C 76.29, H 5.24. Found: C 76.36, H 5.28 %.

*3,3′-(1,3-Phenylenebis(methylene))bis-oxy) dibenzaldehyde (**10b**)*

Yield 91 %; mp 75°C; ν_{max} (KBr)/cm^{-1} 1695, 1681; δ_H (400 MHz, $CDCl_3$) 9.97 (s, 2H), 7.54 (br s, 1H), 7.48 (d, 6H, *J* 1.8), 7.42–7.46 (m, 3H), 7.24–7.27 (m, 2H), 5.15 (s, 4H); δ_C (100 MHz, $CDCl_3$) 192.0, 159.2, 137.9, 136.9, 130.2, 129.1, 127.3, 126.5, 123.8, 122.2, 113.2, 70.0; MS (ESI) *m/z*: 347.1 ([M + H]$^+$); Anal. Calc. for $C_{22}H_{18}O_4$: C 76.29, H 5.24. Found: C 76.35, H 5.28 %.

*3,3′-(5-Nitro-1,3-phenylenebis(methylene))bis-oxy) dibenzaldehyde (**11**)*

Yield 93 %; mp 140°C; ν_{max} (KBr)/cm^{-1} 1698, 1684; δ_H (400 MHz, $CDCl_3$) 9.99 (s, 2H), 8.32 (d, 2H, *J* 0.6), 7.87 (s, 1H), 7.48–7.55 (m, 6H), 7.27–7.30 (m, 2H), 5.25 (s, 4H); δ_C (100 MHz, $CDCl_3$) 191.8, 158.7, 148.8, 139.1, 137.9, 131.5, 130.4, 124.5, 122.1, 121.7, 112.8, 68.7; MS (ESI) *m/z*: 409.0 ([M + NH_4]$^+$); Anal. Calc. for $C_{22}H_{17}NO_6$: C 67.51, H 4.38, N 3.58. Found: C 67.58, H 4.42, N 3.61 %.

*3,3′-(Pyridine-2,6-diylbis(methylene))bis-oxy) dibenzaldehyde (**12**)*

Yield 90 %; mp 146°C; ν_{max} (KBr)/cm^{-1} 1690, 1682; δ_H (400 MHz, $CDCl_3$) 9.98 (s, 2H), 7.76–7.80 (m, 1H), 7.46–7.51 (m, 8H), 7.25–7.30 (m, 2H), 5.28 (s, 4H); δ_C (100 MHz, $CDCl_3$) 191.9, 159.0, 156.3, 137.9, 137.8, 130.3, 123.7, 121.8, 120.5, 113.9, 70.8; MS (ESI) *m/z*: 348.1 ([M + H]$^+$); Anal. Calc. for $C_{21}H_{17}NO_4$: C 72.61, H 4.93, N 4.03. Found: C 72.68; H 4.97, N 4.07 %.

*3,3′-(1,1′:3′,1′-Terphenyl-4,4′-dylbis(methylene))bis (oxy)dibenzaldehyde (**13**)*

Yield 85 %; mp 106°C; ν_{max} (KBr)/cm^{-1} 1697, 1682; δ_H (400 MHz, $CDCl_3$) 9.98 (s, 2H), 7.81 (br s, 1H), 7.68 (d, 4H, *J* 8.2), 7.56–7.60 (m, 2H), 7.53–7.55 (m, 4H), 7.45–7.51 (m, 7H), 7.25–7.30 (m, 2H), 5.18 (s, 4H); δ_C (100 MHz, $CDCl_3$) 192.1, 159.3, 141.3, 141.1, 137.9, 135.6, 130.2, 129.4, 128.1, 127.6, 126.3, 126.1, 123.8, 122.22, 113.3, 70.0; MS (ESI) *m/z*: 516.3 ([M + NH_4]$^+$); Anal. Calc. for $C_{34}H_{26}O_4$: C 81.91, H 5.26. Found: C 81.98, H 5.31 %.

General Procedure for the Synthesis of Imino Cyclophanes (**1**–**7**)

A solution of diamine (15 mmol) in ethanol (400 mL) and a solution of dialdehyde (15 mmol) in ethanol (400 mL) were simultaneously added dropwise to a well stirred volume of

ethanol (800 mL) for 6–8 h. After the addition was complete the reaction mixture was stirred for another 48 h. The precipitated solid was filtered off, washed with ethanol, and dried.

*3,9,13,19-Tetraimine-1,6,11,16(1,3),5,7,15,17(1,4)-octabenzenacycloeicosanaphane (**1**)*

Yield 85 %; mp 175°C; ν_{max} (KBr)/cm^{-1} 1641, 1605; δ_H (400 MHz, $CDCl_3$) 8.40 (s, 4H), 7.80 (8H, d, *J* 8.2), 7.74 (br s, 2H), 7.61 (d, 8H, *J* 8.2), 7.56 (br s, 2H), 7.54 (d, 2H, *J* 1.6), 7.46–7.48(m, 2H), 7.34–7.36 (m, 8H), 4.85 (s, 8H); δ_C (100 MHz, $CDCl_3$) 161.6, 143.0, 138.6, 137.2, 135.5, 129.6, 128.7, 128.2, 126.6, 126.0, 125.6, 124.5, 65.3; MS (ESI) *m/z*: 773.4 ([M + H]$^+$), Anal. Calc. for $C_{56}H_{44}N_4$: C 87.01, H 5.74, N 7.25. Found: C 87.25, H 5.77, N 7.31 %.

*4,9-Dimine-1,7(1,3),2,6,8,12(1,4)-hexabenzenacyclododecanaphane (**2**)*

Yield 75 %; mp 182°C; ν_{max} (KBr)/cm^{-1} 1638, 1601; δ_H (400 MHz, $CDCl_3$) 8.43 (s, 2H), 7.79 (t, 2H, *J* 1.7), 7.62 (d, 8H, d, *J* 8.2), 7.58 (t, 2H, *J* 1.7), 7.56 (d, 2H, *J* 1.5), 7.50–7.52 (m, 2H), 7.41 (d, 8H, *J* 8.2), 4.86 (s, 4H); δ_C (100 MHz, $CDCl_3$) 165.6, 143.3, 137.9, 137.3, 135.4, 129.9, 129.6, 128.5, 128.2, 127.2, 126.4, 65.0; MS (ESI) *m/z*: 539.3 ([M + H]$^+$), Anal. Calc. for $C_{40}H_{30}N_2$: C 89.19, H 5.61, N 5.20. Found: C 89.45, H 5.67, N 5.27 %.

*3,9,13,19-Tetraimine-1,11(1,2),6,16(1,3),5,7,15,17(1,4)-octabenzenacycloeicosanaphane (**3a**)*

Yield 81 %; mp 179°C; ν_{max} (KBr)/cm^{-1} 1641, 1601; δ_H (400 MHz, $CDCl_3$) 8.45 (s, 4H), 8.17–8.29 (m, 2H), 7.75–7.89 (m, 6H), 7.49–7.61 (m, 14H), 7.38–7.48 (m, 12H), 4.86 (s, 8H); δ_C (100 MHz, $CDCl_3$) 161.3, 140.4, 137.7, 137.2, 130.2, 129.7, 129.4, 129.2, 127.6, 126.6, 126.0, 64.7; MS (ESI) *m/z*: 773.4 ([M + H]$^+$), Anal. Calc. for $C_{56}H_{44}N_4$: C 87.01, H 5.74, N 7.25. Found: C 87.27, H 5.80, N 7.30 %.

*3,9,13,19-Tetraimine-1,6,11,16(1,3),5,7,15,17(1,4)-octabenzenacycloeicosanaphane (**3b**)*

Yield 80 %; mp 176°C; ν_{max} (KBr)/cm^{-1} 1596, 1516; δ_H (400 MHz, $CDCl_3$) 8.45 (s, 4H), 7.85 (d, 2H, *J* 1.8), 7.84 (d, 2H, *J* 1.6), 7.75–7.79 (m, 2H), 7.60 (d, 10H, *J* 8.2), 7.57–7.56 (m, 4H), 7.43–7.51 (m, 4H), 7.39 (d, 8H, *J* 8.2), 4.86 (s, 8H); δ_C (100 MHz, $CDCl_3$) 161.6, 141.7, 140.1, 138.5, 136.7, 130.5, 130.0, 129.4, 129.1, 128.7, 127.6, 126.2, 64.9; MS (ESI) *m/z*: 773.4 ([M + H]$^+$), Anal. Calc. for $C_{56}H_{44}N_4$: C 70.36, H 5.33, N 6.00. Found: C 70.50, H 5.41, N 6.08 %.

*3,9,13,19-Tetraimine-6,16(1,3),1,5,7,11,15,17(1,4)-octabenzenacycloeicosanaphane (**3c**)*

Yield 92 %; mp 207°C; ν_{max} (KBr)/cm^{-1} 1642, 1600; δ_H (400 MHz, $CDCl_3$) 8.45 (s, 4H), 7.85 (2H, *J* 1.8), 7.84 (2H, *J* 1.6), 7.75 (t, 2H, *J* 1.4), 7.60 (d, 10H, *J* 8.2), 7.57 (t, 2H, *J* 1.4), 7.56 (d, 2H, *J* 1.6), 7.43–7.51 (m, 4H), 7.39 (d, 8H, *J* 8.2), 4.86 (s, 8H); δ_C (100 MHz, $CDCl_3$) 162.6, 138.4, 137.6, 136.7, 129.6, 129.4, 129.1, 127.5, 126.6, 126.0, 65.1; MS (ESI) *m/z*: 773.4 ([M + H]$^+$), Anal. Calc. for $C_{56}H_{44}N_4$: C 87.01, H 5.74, N 7.25. Found: C 87.29, H 5.81, N 7.31 %.

*3,9-Diimine-12,16-dioxa-1,6,11(1,3),5,7(1,4),14(1,2)-hexabenzenacyclohexadecanaphane (**4a**)*

Yield 83 %; mp 154°C; ν_{max} (KBr)/cm^{-1} 1641, 1579; δ_H (400 MHz, $CDCl_3$) 8.33 (s, 2H), 7.55–7.57 (m, 4H), 7.45–7.51 (m, 8H), 7.32–7.44 (m, 7H), 7.28–7.30 (m, 3H), 7.02–7.06 (m, 2H), 5.20 (s, 4H), 4.85 (s, 4H); δ_C (100 MHz, $CDCl_3$) 162.1, 159.1, 141.7, 138.6, 137.8, 135.2, 130.4, 129.9, 129.3, 128.7, 127.6, 126.2, 126.1, 126.0, 122.2, 118.3, 113.3, 70.0, 64.8; MS (ESI) *m/z*: 599.2 ([M + H]$^+$), Anal. Calc. for $C_{42}H_{34}N_2O_2$: C 84.25, H 5.72, N 4.68. Found: C 84.57, H 5.78, N 4.76 %.

*3,9-Diimine-12,16-dioxa-1,6,11,14(1,3),5,7(1,4)-hexabenzenacyclohexadecanaphane (**4b**)*

Yield 87 %; mp 159°C; ν_{max} (KBr)/cm^{-1} 1642, 1579; δ_H (400 MHz, $CDCl_3$) 8.37 (s, 2H), 7.77 (s, 1H), 7.60 (d, 4H, *J* 6.4), 7.46–7.52 (m, 5H), 7.39–7.41 (m, 8H), 7.32 (br s, 4H), 7.05 (br s, 2H), 5.10 (s, 4H), 4.85 (s, 4H); δ_C (100 MHz, $CDCl_3$) 162.1, 159.2, 141.7, 140.1, 138.6, 137.8, 137.4, 129.8, 128.6, 127.5, 127.3, 126.7, 126.1, 126.0, 122.2, 118.3, 115.0, 113.0, 70.0, 64.8; MS (ESI) *m/z*: 599.2 ([M + H]$^+$), Anal. Calc. for $C_{42}H_{34}N_2O_2$: C 84.25, H 5.72, N 4.68. Found: C 84.59, H 5.79, N 4.75 %.

*3,9-Diimine-12,16-dioxa-1,6,11(1,3),5,7(1,4)-pentaabenzena-14(5-nitro-1,3)benzenacyclohexadecanaphane (**5**)*

Yield 82 %; mp 157°C; ν_{max} (KBr)/cm^{-1} 1643, 1581; δ_H (400 MHz, $CDCl_3$) 8.39 (s, 2H), 8.28 (s, 2H), 7.75 (s, 1H), 7.58–7.66 (m, 4H), 7.49–7.52 (m, 4H), 7.45 (m, 2H), 7.37–7.41 (m, 4H), 7.30–7.35 (m, 4H), 7.03–7.15 (m, 2H), 5.20 (s, 4H), 4.85 (s, 4H); δ_C (100 MHz, $CDCl_3$) 162.3, 161.1, 158.8, 142.1, 140.7, 138.4, 138.0, 129.4, 129.1, 128.7, 128.0, 127.6, 126.2, 125.5, 122.9, 121.7, 118.5, 111.8, 69.1, 64.8; MS (ESI) *m/z*: 644.2 ([M + H]$^+$), Anal. Calc. for $C_{42}H_{33}N_3O_4$: C 78.36, H 5.17, N 6.53. Found: C 78.62, H 5.26, N 6.03 %.

*3,9-Diimine-12,16-dioxa-1,6,11(1,3),5,7(1,4)-pentaabenzena-14(1,3)pyridinacyclo-hexadecanaphane (**6**)*

Yield 86 %; mp 155°C; ν_{max} (KBr)/cm^{-1} 1643, 1581; δ_H (400 MHz, $CDCl_3$) 8.36 (s, 2H), 7.64–7.73 (s, 3H), 7.59–7.64 (m, 4H), 7.51–7.55 (m, 4H), 7.42–7.47 (m, 3H), 7.47–7.40 (m, 3H), 7.24–7.43 (m, 4H), 7.05 (d, 2H, *J* 7.8), 5.23 (s, 4H), 4.84 (s, 4H); δ_C (100 MHz, $CDCl_3$) 162.9, 161.7, 156.4, 141.8, 138.8, 138.2, 137.5, 130.9, 130.8, 130.3, 128.7, 128.1, 127.9, 127.3, 122.6, 122.2, 111.7, 68.9, 64.1; MS (ESI) *m/z*: 600.4 ([M + H]$^+$), Anal. Calc. for $C_{41}H_{33}N_3O_2$: C 82.11, H 5.55, N 7.01. Found: C 82.42, H 5.63, N 7.12 %.

*3,9-Diimine-12,18-dioxa-1,6,11,15(1,3),5,7,14,16(1,4)-octabenzenacyclooctadecanaphane (**7**)*

Yield 71 %; mp 154°C; ν_{max} (KBr)/cm^{-1} 1643, 1581; δ_H (400 MHz, $CDCl_3$) 8.39 (s, 2H), 7.74–7.77 (m, 2H), 7.59–7.64 (m, 8H), 7.46–7.55 (m, 12H), 7.41–7.42 (m, 4H), 7.28–7.35 (m, 4H), 7.02–7.07 (m, 2H), 5.12 (s, 4H), 4.86 (s, 4H); δ_C (100 MHz, $CDCl_3$) 163.7, 160.7, 140.8, 139.7, 137.8, 137.0, 135.6, 129.8, 129.7, 129.5, 128.1, 127.8, 127.6, 126.8, 126.2, 121.5, 116.6, 112.2, 68.6, 64.3; MS (ESI) *m/z*: 751.3 ([M + H]$^+$), Anal. Calc. for $C_{54}H_{42}N_2O_2$: C 86.37, H 5.64, N 3.73. Found: C 86.72, H 5.73, N 3.82 %.

Antimicrobial Activity

Agar Well Diffusion Assay

The antimicrobial activity of imino cyclophanes **1**–**7** against human pathogenic bacteria was done by an agar well diffusion

assay. The human pathogenic Gram-positive bacteria *B. subtilis* and *S. aureus* and Gram-negative bacteria *E. coli* and *K. pneumonia* were obtained from American Type Culture Collection. The human pathogens were inoculated into 5 mL of sterile Muller–Hinton broth, and incubated at 37°C for 8 h. The cultures were swabbed on the surface of sterile nutrient agar plates using a sterile cotton swab. Agar wells were prepared with the help of a sterilized cork borer (9 mm diameter). All the imino cyclophanes **1**–**7** were dissolved in 10 % DMSO which did not affect the microorganism's growth, according to our control experiments. Using a micropipette, 50 mL of different concentrations of imino cyclophanes (50, 75, and 100 μL) were added to each well in the plate. Commercial antibiotic, vancomycin was used as a positive reference standard to compare the efficiency of the test cyclophanes. The plates were incubated at 37°C for 24 h. The antibacterial activities was determined by the zone of inhibition and are shown in Table S1 in the Supplementary Material.

Antifungal Activity

The test human fungal pathogen *C. albican*s was maintained in Sabouraud's dextrose agar (SDA). The *C. albicans* was inoculated in Sabouraud's dextrose broth and incubated at 37°C for 8 h. The pathogen was swabbed on the sterile SDA plates using a sterile swab and wells were made using a sterile cork borer (9 mm diameter). The imino cyclophanes **1**–**7** were tested for their antifungal activity and their MIC was determined. The imino cyclophanes at the concentrations of 50, 75, and 100 μL of 10 % DMSO was used for this study with fluconazole as a positive control. The plates were incubated at 37°C for 24 h. The antifungal activity was determined by the zone of inhibition and are shown in Table S1 in the Supplementary Material.

Minimum Inhibition Assay

The MIC was determined by the methods used by Gabrielson et al.[22b] A sterile 96-well plate was used for the experiment. A volume of 100 μL of test material in 10 % (v/v) DMSO or sterile water was pipetted into the first row of the plate. To all other wells 50 μL of Muller–Hinton broth or normal saline was added. Serial dilutions were performed using a multichannel pipette and the concentration of compound for serial dilution was 10 mg in the first row and tips were discarded after use such that each well had 50 μL of the test material in serially descending concentrations. Aliquots of 10 μL of bacterial suspension (5×10^6 cfu mL^{-1}) were then added to each well to achieve a concentration of 5×10^5 cfu mL^{-1}. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a positive control (vancomycin in serial dilution) in the first column at the last two columns C_1 and C_2. C_1 contained nutrient broth, bacterial suspension and indicator, and C_2 contained nutrient broth and indicator). The plates were prepared in triplicate, and placed in an incubator at 37°C for 18–24 h. Finally, to each well 10 μL of 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide inner salt (MTT) indicator solution was added. After incubation, the colour change was assessed visually. The MTT tetrazolium salt assay measures the cells ability to convert the tetrazolium salt into the fromazan product. Any colour changes from yellow to purple were recorded as positive. The lowest concentration was taken as the MIC value. The bar diagram of MIC value obtained for various imino cyclophanes is shown in Fig. 3.

Molecular Docking Studies

The synthesised imino cyclophanes were subjected to a molecular docking study using the GLIDE application of the Schrödinger package, LLC (New York) version 9.0 and Induced Fit Docking (IFD): one of the modules in the package was employed as a primary docking engine.[24,25] A hierarchical search protocol was utilised by the docking algorithm of the IFD program. The scoring function is called a GLIDE score, for computing the binding affinity is an extension of an empirically based Chem-Score function of Eldridge et al.[26] Optimized potential for liquid simulation (OPLS) – a molecular mechanics potential energy function, was used throughout the calculations.[27] The extra precision mode of GLIDE, which has higher penalties for unfavourable and unphysical interactions, was used for docking. Computations were carried out on a Linux system with a Cent OS 5 computer platform. Interaction pictures were generated using Ligplot.[28] Imino cyclophanes **3a**, **4b**, **6**, and **7** were analysed for their mechanism of antimicrobial action using the above method. The estimated binding energy for each molecule was compared along with the crucial hydrogen and hydrophobic bonds between the protein and ligand to substantiate the experimental results. The interactions were graphically represented in 2D using the Ligplot software (Figs S1–S3 in the Supplementary Material).

Supplementary Material

Zone of inhibition data for antimicrobial activity (Table S1), Ligplot results (Figs S1–S3), and copies of ^{1}H and ^{13}C NMR spectra are available on the Journal's website.

Acknowledgements

The authors thank DST, New Delhi, for financial assistance, RSIC, CDRI, Lucknow for MS, DST-FIST for providing the NMR facility for the Department of Organic Chemistry, University of Madras. R.P. thanks Orchid Chemicals and Pharmaceuticals Ltd, for providing the laboratory and analytical facilities.

References

[1] F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand, D. Häbich, *Angew. Chem. Int. Ed.* **2006**, *45*, 5072. doi:10.1002/ANIE.200600350

[2] (a) P. G. Sammes, *Chem. Rev.* **1976**, *76*, 113. doi:10.1021/CR60299A003
(b) A. Bryskier, *Expert Opin. Investig. Drugs* **1995**, *4*, 705. doi:10.1517/13543784.4.8.705
(c) N. Mealy, J. Castaner, *Drugs Future* **1996**, *21*, 493.

[3] (a) R. M. Klevens, J. R. Edwards, F. C. Tenover, L. C. McDonald, T. Horan, R. Gaynes, *Clin. Infect. Dis.* **2006**, *42*, 389. doi:10.1086/499367
(b) R. A. Weinstein, R. Gaynes, J. R. Edwards, *Clin. Infect. Dis.* **2005**, *41*, 848. doi:10.1086/432803
(c) E. D. Brown, G. D. Wright, *Chem. Rev.* **2005**, *105*, 759. doi:10.1021/CR030116O

[4] (a) S. K. Fridkin, J. R. Edwards, J. M. Courval, H. Hill, F. C. Tenover, R. Lawton, R. P. Gaynes, J. E. McGowan Jr, *Ann. Intern. Med.* **2001**, *135*, 175.
(b) R. N. Jones, *Chest* **2001**, *119*, 397S. doi:10.1378/CHEST.119.2_SUPPL.397S
(c) S. Redding, J. Smith, G. Farinacci, M. Pfaller, M. Rinaldi, A. Fothergill, *Abstr. Gen. Meet. Am. Soc. Microbiol.* **1993**, *93*, 527.
(d) R. Pelletier, J. Peter, C. Antin, C. Gonzalez, L. Wood, T. J. Walsh, *J. Clin. Microbiol.* **2000**, *38*, 1563.

[5] (a) H. W. Boucher, G. H. Talbot, J. S. Bradley, J. E. Edwards, D. Gilbert, L. B. Rice, M. Scheld, B. Spellberg, J. Bartlett, *Clin. Infect. Dis.* **2009**, *48*, 1. doi:10.1086/595011

(b) J. J. Barker, *Drug Discovery Today* **2006**, *11*, 391. doi:10.1016/J.DRUDIS.2006.03.001
(c) I. Masip, E. Pérez-Payá, A. Messeguer, *Comb. Chem. High T. Scr.* **2005**, *8*, 235.

[6] (a) M. Leeb, *Nature* **2004**, *431*, 892. doi:10.1038/431892A
(b) E. D. Brown, G. D. Wright, *Chem. Rev.* **2005**, *105*, 759. doi:10.1021/CR030116O

[7] R. C. Moellering, *Clin. Infect. Dis.* **1998**, *26*, 1196. doi:10.1086/520283

[8] (a) V. Padmavathi, K. Mahesh, G. Dinneswara Reddy, A. Padmaja, *Eur. J. Med. Chem.* **2010**, *45*, 3178. doi:10.1016/J.EJMECH.2010.04.010
(b) V. Padmavathi, P. Thriveni, G. Sudhakar Reddy, D. Deepti, *Eur. J. Med. Chem.* **2008**, *43*, 917. doi:10.1016/J.EJMECH.2007.06.011
(c) N. S. A. M. Khalil, *Eur. J. Med. Chem.* **2010**, *45*, 5265. doi:10.1016/J.EJMECH.2010.08.046
(d) R. Rohini, P. Muralidhar Reddy, K. Shanker, V. Anren Huc, V. Ravinder, *Eur. J. Med. Chem.* **2010**, *45*, 1200. doi:10.1016/J.EJMECH.2009.11.038

[9] (a) K. P. Krishnapriya, M. Kandaswamy, *Polyhedron* **2005**, *24*, 113. doi:10.1016/J.POLY.2004.10.010
(b) P. Rajakumar, A. M. Abdul Rasheed, *Tetrahedron* **2005**, *61*, 5351. doi:10.1016/J.TET.2005.03.064

[10] (a) F. Arico, T. Chang, S. Cantrill, S. I. Khan, J. F. Stoddart, *Chem.–Eur. J.* **2005**, *11*, 4655.
(b) M. Fujita, M. Tominaga, A. Hori, B. Therrien, *Acc. Chem. Res.* **2005**, *38*, 369. doi:10.1021/AR040153H

[11] (a) O. Francesconi, A. Ienco, G. Moneti, C. Nativi, S. Roelens, *Angew. Chem. Int. Ed.* **2006**, *45*, 6693. doi:10.1002/ANIE.200602412
(b) F. P. Schmidtchen, M. Berger, *Chem. Rev.* **1997**, *97*, 1609. doi:10.1021/CR9603845

[12] (a) O. Baudoin, F. Gonnet, M. P. Teulade-Fichou, J. P. Vigneron, J. C. Tabet, J. M. Lehn, *Chem.–Eur. J.* **1999**, *5*, 2762. doi:10.1002/(SICI)1521-3765(19990903)5:9<2762::AID-CHEM2762>3.0.CO;2-F
(b) K. Jantos, R. Rodriguez, S. Ladame, P. S. Shirude, S. Balasubramanian, *J. Am. Chem. Soc.* **2006**, *128*, 13662. doi:10.1021/JA064713E

[13] (a) K. P. Wainwright, *Coord. Chem. Rev.* **1997**, *166*, 35. doi:10.1016/S0010-8545(97)00003-9
(b) N. E. Borisova, M. D. Reshetova, Y. A. Ustynyuk, *Chem. Rev.* **2007**, *107*, 46. doi:10.1021/CR0683616
(c) P. A. Vigato, S. Tamburini, L. Bertolo, *Coord. Chem. Rev.* **2007**, *251*, 1311. doi:10.1016/J.CCR.2006.11.016
(d) N. E. Borisova, M. D. Reshetova, M. D. Ustynyuk, *Russ. Chem. Rev.* **2007**, *76*, 785. doi:10.1070/RC2007V076N09ABEH003705

[14] (a) D. R. Williams, *Chem. Rev.* **1972**, *72*, 203. doi:10.1021/CR60277A001
(b) B. S. Garg, L. Kapur, *Inorg. Chim. Acta* **1990**, *173*, 223. doi:10.1016/S0020-1693(00)80217-0
(c) W. Zishen, L. Zhiping, Y. Zhenhuan, *Trans. Met. Chem. (Weinh.)* **1993**, *18*, 291. doi:10.1007/BF00207949
(d) A. K. Usha, S. Chandra, *Syn. React. Inorg. Met.* **1992**, *22*, 1565. doi:10.1080/15533179208020277

[15] (a) H.-J. Zhang, X. Qin, K. Liu, D.-D. Zhu, H.-L. Zhu, *Bioorg. Med. Chem.* **2011**, *19*, 5708. doi:10.1016/J.BMC.2011.06.077
(b) P. Muralidhar Reddy, Y.-P. Ho, K. Shanker, R. Rohini, V. Ravinder, *Eur. J. Med. Chem.* **2009**, *44*, 2621. doi:10.1016/J.EJMECH.2008.09.035
(c) R. Rohini, K. Shanker, P. Muralidhar Reddy, Y.-P. Ho, V. Ravinder, *Eur. J. Med. Chem.* **2009**, *44*, 3330. doi:10.1016/J.EJMECH.2009.03.022

[16] P. Rajakumar, S. Raja, *Synthesis* **2010**, *2010*, 465. doi:10.1055/S-0029-1218582

[17] P. Rajakumar, R. Raja, S. Selvam, R. Rengasamy, S. Nagaraj, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3466. doi:10.1016/J.BMCL.2009.05.019

[18] (a) P. Rajakumar, K. Sekar, V. Shanmugaiah, N. Mathivanan, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4416. doi:10.1016/J.BMCL.2008.06.047
(b) P. Rajakumar, K. Sekar, V. Shanmugaiah, N. Mathivanan, *Eur. J. Med. Chem.* **2009**, *44*, 3040. doi:10.1016/J.EJMECH.2008.07.031

[19] (a) P. Rajakumar, S. Selvam, M. Dhanasekaran, *Tetrahedron Lett.* **2005**, *46*, 6127. doi:10.1016/J.TETLET.2005.06.159
(b) P. Rajakumar, C. Satheeshkumar, G. Mohanraj, N. Mathivanan, *Eur. J. Med. Chem.* **2011**, *46*, 3093. doi:10.1016/J.EJMECH.2011.03.063

[20] P. Rajakumar, R. Padmanabhan, *Tetrahedron* **2011**, *67*, 9669. doi:10.1016/J.TET.2011.10.046

[21] P. Rajakumar, V. Murali, *Tetrahedron* **2004**, *60*, 2351. doi:10.1016/J.TET.2004.01.008

[22] (a) M. R. Fazeli, G. Amin, M. M. A. Attar, H. Ashtiani, H. Jamalifar, N. Samadi, *Food Contr.* **2007**, *18*, 646. doi:10.1016/J.FOODCONT.2006.03.002
(b) J. Gabrielson, M. Hart, A. Jarelov, I. Kuhn, D. McKenzie, R. Mollby, *J. Microbiol. Methods* **2002**, *50*, 63. doi:10.1016/S0167-7012(02)00011-8

[23] M. J. Edwards, R. H. Flatman, L. A. Mitchenall, C. E. M. Stevenson, T. B. K. L. Thomas, C. A. R. McKay, H.-P. Fiedler, M. J. Buttner, D. M. Lawson, A. Maxwell, *Science* **2009**, *326*, 1415. doi:10.1126/SCIENCE.1179123

[24] *Schrödinger Glide version 2009* **2009**, Vol. 18 (LLC: New York, NY).

[25] *Schrödinger Suite 2009, Induced Fit Docking protocol; prime version 1.5* **2009**, Vol. 19 (LLC: New York, NY).

[26] M. D. Eldridge, C. W. Murray, T. R. Auton, G. V. Paolini, R. P. Mee, *J. Comput. Aided Mol. Des.* **1997**, *20*, 25.

[27] W. L. Jorgensen, D. S. Maxwell, J. Tirado Rives, *J. Am. Chem. Soc.* **1996**, *118*, 11225. doi:10.1021/JA9621760

[28] A. C. Wallace, R. A. Laskowski, J. M. Thornton, *Protein Eng.* **1995**, *8*, 127. doi:10.1093/PROTEIN/8.2.127