European Journal of Medicinal Chemistry 44 (2009) 2178–2183

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Fullerene derivatized *s*-triazine analogues as antimicrobial agents

Anish Kumar, Shobhana Karuveettil Menon*

Department of Chemistry, School of Sciences, Gujarat University, Navrangpura, Ahmedabad 380009, Gujarat, India

ARTICLE INFO

Article history:
Received 29 July 2008
Received in revised form
24 September 2008
Accepted 22 October 2008
Available online 5 November 2008

Keywords:
Fulleropyrrolidine
s-Triazine
Schiff base
Prato reaction
Antibacterial

ABSTRACT

A series of novel fullerene derivatives bearing *s*-triazine moiety have been synthesized by adopting 1,3 dipolar cycloaddition reaction of C_{60} and azomethine ylides generated from the corresponding Schiff bases of 2,4,6 trisubstituted *s*-triazine. All the compounds synthesized were characterized by elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR and FAB-MS. The compounds were then screened for their antibacterial activity against both gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus pumilis*) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacteria by disc diffusion method. All the compounds were found to be active against these strains at very low concentration and were comparable to standard drug ciprofloxacin.

1. Introduction

Ever since its discovery, C_{60} has become a topic of considerable interest in medicinal chemistry as a potential biologically active compound. Its spherical shape together with its amazing physical and chemical properties has prompted scientist world over to exploit it in many areas ranging from material chemistry to biological sciences [1–4]. Interest in using C_{60} for diagnosis and therapeutic use has been accelerated ever since it was discovered that C_{60} derivatives could cross cell membranes [5]. Fullerene derivatives have found potential application as neuroprotective agents [6], anti-HIV agents [7,8], antimycobacterials [9], antibacterials [10–12], bone-disorder drugs [13], X-ray contrast agents [14], transfection vectors [15], photodynamic therapy agents [16], antiproliferative agents [17], drug delivery systems [18,19], inhibitors of DNA enzymes [20], free radical sponge [21], anti-inflammatory agents [22], anti-apoptosis agents [23], immunostimulatory agents [24], vaccine against human papilloma virus [25], anticancer agents [26], radiopharmaceuticals [27] and MRI contrast agents [28]. One of the difficulties faced in using fullerene derivatives for biological application is its low solubility in water or water miscible solvents. This problem can be solved by introduction of (a) hydrophilic group or (b) ionic species in the molecule. There are many reports wherein fullerene derivatives have been used as antibacterials [10–12]. These derivatives were having either hydrophilic functional group or ionic part along with hydrophobic fullerene moiety so that the dual purpose of interaction with cell wall of bacteria as well as their biological activity studies in water or water miscible solvents becomes viable. Derivatized *s*-triazine molecules had been proved efficient in inhibiting the growth of several strains of bacteria [29–31]. Because of the increased bacterial resistance to conventional antibiotics, there is an urgent need to design and develop new antibiotics based on new chemical entities. Amphiphilic peptides have shown great activity as antibacterials at concentrations below μM range [32–34]. Activity of such class of compounds has been attributed to electrostatic and hydrophobic interactions with the bacterial membrane. Hence the presence of both hydrophobic and hydrophilic groups is favorable for developing new chemical scaffolds as antibacterials. Moreover, the presence of Schiff base has resulted in various compounds being applicable to pharmaceutical and medicinal chemistry. Such compounds had shown antibacterial, antifungal and antitumour activities [35–37]. Some of the Schiff base derivatives with appreciable cell membrane permeability were studied in cancer multidrug resistance studies. With these in view, we designed and derivatized fullerene with substituted *s*-triazine having Schiff base group between them so that the purpose of solubility for biological studies as well as proper interaction with cell wall of bacteria could be achieved efficiently.

2. Chemistry

In the synthetic methodology, derivatives of 1,3,5-triazine (**1a–e**) were synthesized from 2,4,6 trichloro 1,3,5 triazine and

* Corresponding author. Tel.: +91 79 26302286; fax: +91 79 26308545.
E-mail address: shobhanamenon07@gmail.com (S.K. Menon).

0223-5234/$ – see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.10.036

different nucleophilic amines ranging from ammonia to *N*,*N* dimethyl amine by reported procedures [38–40].

In general, the first chlorine was displaced with temperature at 0 °C or below and second chlorine was substituted with temperature between 25 °and 50 °C. The third chlorine in all the products was replaced by hydrazine hydrate at reflux temperature of 80 °C.

2,4,6 Trisubstituted 1,3,5-triazine was then treated with terephthalaldehyde in ethanol. Triazine derivative was added portionwise slowly to an ethanolic solution of terephthalaldehyde. Mixtures of products were obtained from which the desired product was isolated by column chromatography using toluene/methanol (50:1) as the eluant. The Schiff base containing free aldehyde group was then reacted with C_{60} by Prato's 1,3 dipolar cycloaddition reaction [41] to get the desired products. The products were purified by column chromatography using toluene/ethyl acetate as the eluant. Reaction sequence is given in Scheme 1. Ionic derivative **3f** was obtained by treatment of **3a** with HCl solution in methanol at 0 °C. The yield and melting point of the series of fulleropyrrolidines synthesized are given in Table 1.

3. Pharmocology

All the novel fullerene derivatives (**3a**–**f**) as well as the Schiff base precursors (**2a**–**e**) were screened for their antibacterial activity against bacterial strains of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus pumilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by disc diffusion method [42,43]. A standard inoculum ($1–2 \times 10^7$ c.f.u/ml 0.5 McFarland standards) was introduced onto the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The disc measuring 6.25 mm in diameter was prepared from Whatmann filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile plates previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were maintained. The plates were inverted and incubated for 24 h at 37 °C. Ciprofloxacin was used as the standard drug. The inhibition zones were measured and compared with the controls. The zone developed by the solution of compound **3f** (100 μg/ml) in the plate against *S. aureus* and *B. subtilis* is as shown in Fig. 1. Bacterial inhibition zone values for all the fulleropyrrolidines are given in Table 2. Minimum inhibitory concentration (MIC) was evaluated by broth dilution technique. The nutrient broth, which contained logarithmic serially twofold diluted amount of test compound and controls, was inoculated with approximately 5×10^5 c.f.u of actively dividing bacterial cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The investigation of antibacterial screening revealed that all the fullerene derivatives showed moderate to good bacterial inhibition. Bacterial inhibition zones developed by fulleropyrrolidines (**3a**–**f**) were found to be much bigger in size as compared to *s*-triazine based Schiff base precursors (Table 3) indicating the contribution of fullerene moiety towards antimicrobial activity of the synthesized fulleropyrrolidines. The minimum inhibitory concentrations of various fulleropyrrolidines are given in given in Table 4. Compound **3f** having with ionic $-NH_3^+$ group was found to be the most active followed by the derivative in which both $-NH_2$ group was free. The activity was found to decrease on increasing the substitution on NH_2 group. This observation can be attributed to the fact that on increasing the substitution on NH_2 group, the hydrophilicity of the molecule as a whole decreases which in turn decreases the disruption caused to the negatively charged bacterial cell membrane (Fig. 2).

4. Results and discussion

2,4,6 Trisubstituted 1,3,5 *s*-triazines (**1a**–**e**) were synthesized by reported procedure. Schiff bases (**2a**–**e**) of the corresponding triazine derivatives (**1a**–**e**) were synthesized by condensation with terephthalaldehyde. Fulleropyrrolidines (**3a**–**e**) were synthesized from these Schiff bases by adopting Prato's 1, 3 dipolar cycloaddition reaction. IR spectra of compounds (**3a**–**e**) showed peaks between 3060 and 3070 cm^{-1} due to aromatic C–H stretching. Other peaks such as 2920–2925 cm^{-1} corresponding to aliphatic C–H stretching were observed in derivatives **3b**–**e**. The peak corresponding to azomethine linkage was observed in the range 1595–1615 cm^{-1} in case of all the fulleropyrrolidines. The peak between 3520 and 3240 cm^{-1} for N–H stretching was observed in all the derivatives.

^{1}H NMR spectra of all the derivatives (**3a**–**e**) showed a singlet in the range δ 8.0–8.1 attributable to (CH═N) proton. Aromatic protons of the only benzene ring molecule appeared as doublets at δ 7.60 and δ 7.20 ($J = 8.4$ Hz) each integrating for two protons, respectively. A singlet at δ 3.85 integrating for one proton corresponding to NH attached with azomethine linkage was observed in all the synthesized fulleropyrrolidine. Other peaks such as δ 5.25 and 4.10 ($J = 9.3$ Hz) appear as doublets for one proton each corresponding to $-CH_2-N$ of the pyrrolidine ring. Peaks at δ 4.35 and 2.20 have been observed in all the derivatives of fullerene, which can be attributed to protons (–CH and $N-CH_3$, respectively) involving the pyrrolidine ring. ^{13}C NMR showed δ between 180.0 and 176.0 corresponding to C of the triazine ring. The values between 160 and 120 are due to sp^2C of the fullerene cage. The peak corresponding to sp^3C of fullerene cage could be found around δ 73.0 and 68.0.The peak corresponding to C of the azomethine linkage was observed at δ 154.7. The mass spectrum of compound **3a** showed molecular ion peak at *m*/*z* 1004, in conformity with the molecular formula $C_{73}N_8H_{16}$. All the synthesized fulleropyrrolidines were screened for antibacterial activity against both gram-positive and gram-negative bacteria. Of all the derivatives tested for activity, it was the ionic fullerene derivative with $-NH_3^+$ groups, which was found to be the most active against both classes of bacteria. The activity was found to decrease on increasing the substitution on $-NH_2$ group (Table 4).

5. Conclusion

The investigation of antibacterial screening data revealed that all the fullerene derivatives bearing *s*-triazine moiety showed moderate to good bacterial inhibition.

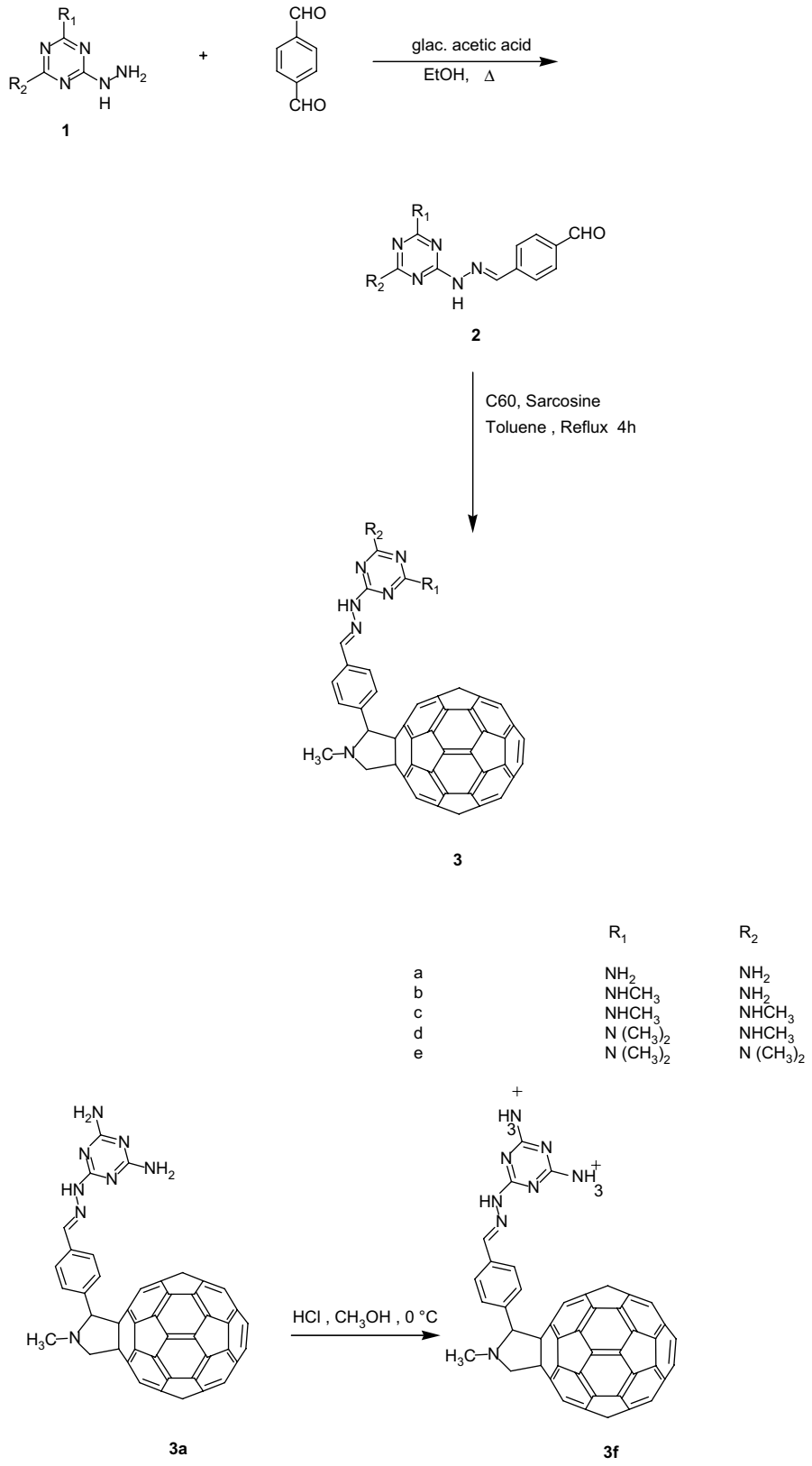
6. Experimental protocols

6.1. Apparatus

All the chemicals used were of analytical grade and purchased from Sigma Aldrich and Merck Co. The cycloaddition reactions were performed under argon. The cycloaddition reactions as well as other preceeding steps were monitored by TLC using Merck silica gel 60-F_{254}. Silica gel (Merck, 0.040–0.063 mm) was used for column chromatography. FAB-MS was recorded on Jeol SX-102/DA 600 mass spectrometer using argon/xenon as the accelerating gas and nitrobenzylalcohol (NBA) as the matrix. EI-MS was recorded on SHIMADZU QP-2020A. ^{1}H NMR spectra were recorded on a DRX 300 spectrophotometer operating at 400 MHz in $CDCl_3$ with TMS as the internal standard. The absorption spectra were recorded on Hitachi U – 3210 UV–vis spectrophotometer using 10-mm quartz cell. Elemental analysis was done on a Carlo Erba 1108 analyzer. FT–IR spectra were recorded on a BRUKER-TENSOR 410 FT–IR spectrophotometer as KBr pellets. Melting point was taken on Veego (VMP-DS) using a Mel-Temp apparatus.

6.2. General procedure for the synthesis of 2,4,6 trisubstituted 1,3,5 s-triazines (*1a*–*e*)

2,4,6 Trisubstituted 1,3,5 *s*-triazine derivatives were synthesized by the reported procedure [27–29].



	R_1	R_2
a	NH_2	NH_2
b	$NHCH_3$	NH_2
c	$NHCH_3$	$NHCH_3$
d	$N(CH_3)_2$	$NHCH_3$
e	$N(CH_3)_2$	$N(CH_3)_2$

Scheme 1. Synthetic protocol of fulleropyrrolidines (**3a**–**f**).

6.3. Synthesis of Schiff bases (*2a*–*e*)

To an ethanolic solution of terephthaldehyde (2.68 g, 0.02 mol) was added 3–4 ml of glacial acetic acid. This mixture was refluxed for a while and in the mean time **1a** (2.82 g, 0.02 mol) was added portionwise slowly for a period of half an hour. The yellow product obtained was isolated and purified by column chromatography (toluene/methanol 9:1). The solvent was then distilled out to get

Table 1
Characterization data of fulleropyrrolidines (**3a–f**)[a].

Compound	R^1	R^2	Molecular formula	M.P. [°C]	Yield [%]
3a	NH_2	NH_2	$C_{73}N_8H_{16}$	215	33
3b	$NHCH_3$	NH_2	$C_{74}N_8H_{18}$	212	39
3c	$NHCH_3$	$NHCH_3$	$C_{75}N_8H_{20}$	208	38
3d	$N(CH_3)_2$	$NHCH_3$	$C_{76}N_8H_{22}$	205	40
3e	$N(CH_3)_2$	$N(CH_3)_2$	$C_{77}N_8H_{24}$	201	39

[a] Characterization data of fulleropyrrolidines (**3a–e**).

2a. Similarly **2b–e** was synthesized by following the above procedure.

6.3.1. ***2a*** *(2-(4,6 Diamino-1,3,5-triazin-2-yl) hydrazinylidene) methyl benzaldehyde*

Calcd for $C_{11}N_7H_{11}O$ (C: 51.4%, H: 4.3%, N: 38.1%). Found (C: 51.2%, H: 4.4%, N: 38.2%); IR (KBr, υ cm^{-1}) 3520 (N–H), 3240 (N–H), 3090 (Ar–H), 1695 (CHO), 1595 (C═N); ^{1}H NMR (δ, DMSO-d_6): 9.87 (s, 1H, CHO), 7.80 (d, $J = 8.32$, 2H, Ar–H), 7.50 (s, 1H, CH═N), 7.40 (d, $J = 8.32$, 2H, Ar–H), 4.0 (s, 4H, $-NH_2$), 3.85 (s, 1H, NH); ^{13}C NMR (δ, 125 MHz, DMSO-d_6) 190.0, 180.8, 176.0, 154.7, 139.0, 137.0, 129.8, 129.5; mass (%) M+ 257 (68).

6.3.2. ***2b*** *(2-(4-Amino-6-(methylamino)-1,3,5-triazin-2-yl) hydrazinylidene)methyl benzaldehyde*

Calcd for $C_{12}N_7H_{13}O$ (C: 53.1%, H: 4.8%, N: 36.2%). Found (C: 53.2%, H: 4.6%, N: 36.4%); IR (KBr, υ cm^{-1}) 3525 (N–H), 3245 (N–H), 3090 (Ar-H), 1695 (CHO), 1595 (C═N); ^{1}H NMR (δ, DMSO-d_6): 9.86 (s, 1H, CHO), 7.80 (d, $J = 8.30$, 2H, Ar-H), 7.50 (s, 1H, CH═N), 7.40 (d, $J = 8.30$, 2H, Ar-H), 4.0 (s, 2H, $-NH_2$), 3.85 (s, 1H, NH), 3.65 (s, 1H, NH), 2.50 (s, 3H, CH_3); ^{13}C NMR (δ, 125 MHz, DMSO-d_6) 190.1, 182.4, 179.2, 176.0, 154.7, 139.0, 137.2, 129.8, 129.5, 35.3; mass (%) M+ 271 (66).

6.3.3. 2 ***c*** *(2-(4,-6-Bis (methylamino)-1,3,5-triazin-2-yl) hydrazinylidene) methyl benzaldehyde*

Calcd for $C_{13}N_7H_{15}O$ (C: 53.6%, H: 5.2%, N: 34.4%). Found (C: 53.5%, H: 5.2%, N: 34.4%); IR (KBr, υ cm^{-1}) 3245 (N–H), 3090 (Ar–H), 1695 (CHO), 1595 (C═N); ^{1}H NMR (δ, DMSO-d_6): 9.86 (s, 1H, CHO), 7.75 (d, $J = 8.30$, 2H, Ar–H), 7.50 (s, 1H, CH═N), 7.40 (d, $J = 8.30$, 2H, Ar–H), 3.80 (s, 1H, NH), 3.65 (s, 2H, NH), 2.48 (s, 6H, CH_3); ^{13}C NMR (δ, 125 MHz, DMSO-d_6) 190.0, 180.8, 176.0, 154.5, 140.0, 138.3, 136.5, 130.0, 129.0, 35.0; mass (%) M+ 285 (70).

6.3.4. ***2d*** *2-((4-Dimethyamino)-6-(methylamino)-1,3,5-triazin-2-yl) hydrazinylidene methyl benzaldehyde*

Calcd for $C_{14}N_7H_{17}O$ (C: 56.1%, H: 5.6%, N: 32.8%). Found (C: 56.0%, H: 5.6%, N: 32.7%); IR (KBr, υ cm^{-1}) 3245 (N–H), 3090 (Ar–H), 1695 (CHO), 1595 (C═N); ^{1}H NMR (δ, DMSO-d_6): 9.85 (s, 1H, CHO), 7.70 (d, $J = 8.34$, 2H, Ar–H), 7.50 (s, 1H, CH═N), 7.40 (d, $J = 8.34$, 2H, Ar–H), 3.80 (s, 1H, NH), 3.65 (s, 1H, NH), 2.45 (s, 3H, CH_3), 2.35 (s, 6H, CH_3); ^{13}C NMR (δ, 125 MHz, DMSO-d_6) 190.1, 180.7, 178.2, 176.0, 154.7, 139.0, 137.0, 129.0, 128.5, 44.0, 35.8; mass (%) M+ 299 (58).

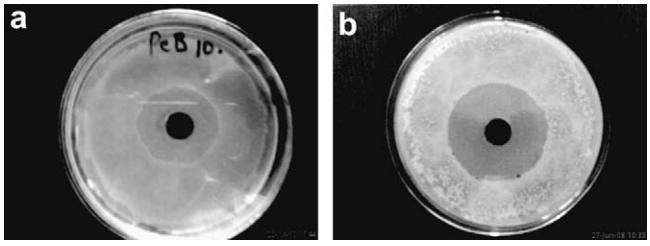


Fig. 1. The zone developed by the solution of compound **3f** (100 μg/ml) in the plate against *S. aureus* (**1a**) and *B. subtilis* (**1b**).

Table 2
Zone of inhibition of fulleropyrrolidines (**3a–f**) (conc. 100 μg/ml)[a]

Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Bacillus pumilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
3a	20	28	28	25	24	16
3b	19	27	24	24	22	15
3c	16	23	20	21	18	14
3d	15	21	18	20	16	12
3e	13	15	13	14	15	–[b]
3f	22	32	31	25	25	16
Standard	22	38	36	27	32	19

[a] Zone of inhibition of fulleropyrrolidines (100 μg/ml).
[b] Bacteria is resistant to compound.

6.3.5. ***2e*** *(2-(4,6-Bis (dimethylamino)-1,3,5-triazin-2-yl) hydrazinylidene) methyl benzaldehyde*

Calcd for $C_{15}N_7H_{19}O$ (C: 57.5%, H: 6.0%, N: 31.3%). Found (C: 57.4%, H: 6.0%, N: 31.4%); IR (KBr, υ cm^{-1}) 3090 (Ar–H), 1695 (CHO), 1595 (C═N); ^{1}H NMR (δ, DMSO-d_6): 9.85 (s, 1H, CHO), 7.70 (d, $J = 8.33$, 2H, Ar–H), 7.50 (s, 1H, CH═N), 7.40 (d, $J = 8.33$, 2H, Ar–H), 3.70 (s, 1H, NH), 2.40 (s, 12H, CH_3); ^{13}C NMR (δ, 125 MHz, DMSO-d_6) 190.0, 181.0, 176.5, 154.0, 139.0, 137.0, 129.0, 128.5, 44.5; mass (%) M+ 313 (58).

*6.4. Synthesis of fulleropyrrolidines (****3a–e****)*

Schiff base **2a** (25.7 mg, 0.1 mmol), *N*-methylglycine(5 mg) and C_{60} (72 mg,0.1 mmol) were refluxed in dry toluene in inert atmosphere for 6 h . The product was first purified by column chromatography (toluene/ethyl acetate 9:1) to get pure product **3a**. In a similar way all other products (**3b–e**) were obtained by the above procedure.

6.4.1. Compound ***3a***. Calcd for $C_{73}N_8H_{16}$ (C: 87.3%, H: 1.6%, N: 11.2%). Found (C: 87.2%, H: 1.6%, N: 11.2%); IR (KBr, υ cm^{-1}) 3520 (N–H), 3240 (N–H), 3090 (Ar–H), 1595 (C═N); ^{1}H NMR (δ, 400 MHz, DMSO-d_6): 8.10 (s, 1H, CH═N), 7.60 (d, $J = 8.32$, 2H, Ar–H), 7.20 (d, $J = 8.32$, 2H, Ar–H), 5.25 (d,1H, $J = 9.3$, *H*HC–N of the pyrrolidine ring), 4.35 (s, 1H, CH of the pyrrolidine ring), 4.10 (d,1H, $J = 9.3$, H*H*C–N of the pyrrolidine ring), 4.0 (s, 4H, $-NH_2$), 3.85 (s, 1H, NH), 2.20 (s, 3H, CH_3 linked to N of pyrrolidine ring); ^{13}C NMR (δ, 125 MHz, DMSO-d_6) 180.8, 176.0, 166.5, 164.8, 164.0, 163.6, 156.2, 155.9, 154.7, 154.0, 152.9, 151.6, 150.5, 147.3, 146.2, 146.0, 144.4, 143.1, 143.0, 142.7, 142.5, 142.4, 142.1, 142.0, 141.8, 141.5, 140.2, 139.9, 139.4, 136.9, 136.7, 136.4, 135.9, 135.6, 132.6, 132.3, 131.1, 130.6, 129.9, 129.5, 129.1, 128.3, 127.7, 127.0, 122.6, 121.2, 120.3,118.9, 114.6, 114.4, 111.0, 82.0, 73.50 (sp^3 C– of C_{60}) 72.7, 72.4, 68.6 (sp^3 C– of C_{60}),33.3; mass (%) M+ 1004 (58).

6.4.2. Compound ***3b***. Calcd for $C_{74}N_8H_{18}$ (C: 87.3%, H: 1.6%, N: 11.2%). Found (C: 87.2%, H: 1.6%, N: 11.2%); IR (KBr, υ cm^{-1}) 3520 (N–H), 3240 (N–H), 3090 (Ar–H), 1595 (C═N); ^{1}H NMR (δ, 400 MHz, DMSO-d_6): 8.00 (s, 1H, CH═N), 7.55 (d, $J = 8.33$, 2H, Ar–H), 7.20 (d,

Table 3
Zone of inhibition of Schiff base precursors (**2a–e**) (conc. 100 μg/ml)[a].

Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Bacillus pumilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
2a	09	16	15	15	11	–[b]
2b	09	18	13	15	13	15
2c	11	12	10	14	11	14
2d	10	10	11	12	10	12
2e	09	10	10	–[b]	–[b]	–[b]
Standard	22	38	36	27	32	19

[a] Zone of inhibition of *s*-triazine Schiff bases(100 μg/ml).
[b] Bacteria is resistant to compound.

Table 4
MIC results of fulleropyrrolidines (**3a–e**)[a].

Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Bacillus pumilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
3a	10	0.1	0.50	10	12.5	12.5
3b	12	0.1	0.50	12.	12.5	12.5
3c	25	0.5	1.0	12.5	25	12.5
3d	25	0.5	1.0	12.5	25	25
3e	50	1.0	5.0	25	25	–[b]
3f	6.25	0.08	0.25	12.5	12.5	12.5
Standard[c]	6.25	0.06	0.25	6.25	6.25	12.5

[a] MIC results of fulleropyrrolidines(**3a–e**).
[b] Bacteria is resistant to compound.
[c] Ciprofloxacin is used as the standard drug.

$J = 8.33$, 2H, Ar–H), 5.20 (d, 1H, $J = 9.3$, *H*HC–N of the pyrrolidine ring), 4.35 (s, 1H, CH of the pyrrolidine ring), 4.10 (d, 1H, $J = 9.3$, H*H*C–N of the pyrrolidine ring), 4.0 (s, 2H, $-NH_2$), 3.80 (s, 1H, NH), 3.65 (s, 1H, NH), 2.50 (s, 3H, CH_3), 2.20 (s, 3H, CH_3 linked to N of pyrrolidine ring); ^{13}C NMR (δ, 125 MHz, DMSO-d_6) 180.8, 176.0, 166.5, 164.8, 164.0, 163.6, 156.2, 155.9, 154.7, 154.0, 152.9, 151.6, 150.5, 147.3, 146.2, 146.0, 144.4, 143.1, 143.0, 142.7, 142.5, 142.4, 142.1, 142.0, 141.8, 141.5, 140.2, 139.9, 139.4, 136.9, 136.7, 136.4, 135.9, 135.6, 132.6, 132.3, 131.1, 130.6, 129.9, 129.5, 129.1, 128.3, 127.7, 127.0, 122.6, 121.2, 120.3,118.9, 114.6, 114.4, 111.0, 82.0, 73.2 (sp^3 C– of C_{60}), 72.7, 72.4, 68.3 (sp^3 C– of C_{60}), 33.3; mass (%) M+ 1018 (42).

6.4.3. Compound ***3c***. Calcd for $C_{75}N_8H_{20}$ (C: 87.3%, H: 1.6%, N: 11.2%). Found (C: 87.2%, H: 1.6%, N: 11.2%); IR (KBr, υ cm^{-1}) 3520 (N–H), 3240 (N–H), 3090 (Ar–H), 1595 (C=N); ^1H NMR (δ, 400 MHz, DMSO-d_6): 8.00 (s, 1H, CH=N), 7.50 (d, $J = 8.34$, 2H, Ar–H), 7.20 (d, $J = 8.34$, 2H, Ar–H), 5.20 (d,1H, $J = 9.3$, *H*HC–N of the pyrrolidine ring) 4.35 (s, 1H, CH of the pyrrolidine ring), 4.00 (d,1H, $J = 9.3$, H*H*C–N of the pyrrolidine ring) 3.75 (s, 1H, NH), 3.65 (s, 2H, NH), 2.50 (s, 6H, CH_3), 2.20 (s, 3H, CH_3 linked to N of pyrrolidine ring); ^{13}C NMR (δ, 125 MHz, DMSO-d_6) 180.8, 176.0, 166.5, 164.8, 164.0, 163.6, 156.2, 155.9, 154.7, 154.0, 152.9, 151.6, 150.5, 147.3, 146.2, 146.0, 144.4, 143.1, 143.0, 142.7, 142.5, 142.4, 142.1, 142.0, 141.8, 141.5, 140.2, 139.9, 139.4, 136.9, 136.7, 136.4, 135.9, 135.6, 132.6, 132.3, 131.1, 130.6, 129.9, 129.5, 129.1, 128.3, 127.7, 127.0, 122.6, 121.2, 120.3,118.9, 114.6, 114.4, 111.0, 82.0, 73.1 (sp^3 C– of C_{60}), 72.7, 72.4, 68.0 (sp^3 C– of C_{60}), 33.3; mass (%) M+ 1032 (45).

6.4.4. Compound ***3d***. Calcd for $C_{76}N_8H_{22}$ (C: 87.3%, H: 1.6%, N: 11.2%). Found (C: 87.2%, H: 1.6%, N: 11.2%); IR (KBr, υ cm^{-1}) 3520 (N–H), 3240 (N–H), 3090 (Ar–H), 1595 (C=N); ^1H NMR (δ, 400 MHz, DMSO-d_6): 8.00 (s, 1H, CH=N), 7.45 (d, $J = 8.33$, 2H, Ar–H), 7.15 (d, $J = 8.33$, 2H, Ar–H), 5.20 (d, 1H, $J = 9.3$, *H*HC–N of the pyrrolidine ring) 4.35 (s, 1H, CH of the pyrrolidine ring), 4.00 (d, 1H, $J = 9.3$, H*H*C–N of the pyrrolidine ring), 3.75 (s, 1H, NH), 3.65 (s, 1H, NH), 2.50 (s, 3H, CH_3), 2.35 (s, 6H, CH_3), 2.20 (s, 3H, CH_3 linked to N of pyrrolidine ring); ^{13}C NMR (δ, 125 MHz, DMSO-d_6) 180.8, 176.0, 166.5, 164.8, 164.0, 163.6, 156.2, 155.9, 154.7, 154.0, 152.9, 151.6, 150.5, 147.3, 146.2, 146.0, 144.4, 143.1, 143.0, 142.7, 142.5, 142.4, 142.1, 142.0, 141.8, 141.5, 140.2, 139.9, 139.4, 136.9, 136.7, 136.4, 135.9, 135.6, 132.6, 132.3, 131.1, 130.6, 129.9, 129.5, 129.1, 128.3, 127.7, 127.0, 122.6, 121.2, 120.3,118.9, 114.6, 114.4, 111.0, 82.0, 73.0 (sp^3 C– of C_{60}), 72.7, 72.4, 68.0 (sp^3 C– of C_{60}), 33.3; mass (%) M+ 1046 (50).

6.4.5. Compound ***3e***. Calcd for $C_{77}N_8H_{24}$ (C: 87.3%, H: 1.6%, N: 11.2%). Found (C: 87.2%, H: 1.6%, N: 11.2%); IR (KBr, υ cm^{-1}) 3520 (N–H), 3240 (N–H), 3090 (Ar–H), 1595 (C=N); ^1H NMR (δ, 400 MHz, DMSO-d_6): 8.00 (s, 1H, CH=N), 7.40 (d, $J = 8.35$, 2H, Ar–H), 7.20 (d, $J = 8.35$, 2H, Ar–H), 5.25 (d, 1H, $J = 9.3$, *H*HC–N of the pyrrolidine ring) 4.35 (s, 1H, CH of the pyrrolidine ring), 3.90 (d,1H, $J = 9.3$, H*H*C–N of the pyrrolidine ring), 3.65 (s, 1H, NH), 2.40 (s, 12H, CH_3), 2.20 (s, 3H, CH_3 linked to N of pyrrolidine ring); ^{13}C NMR (δ, 125 MHz, DMSO-d_6) 180.8, 176.0, 166.5, 164.8 164.0, 163.6, 156.2, 155.9, 154.7, 154.0, 152.9, 151.6, 150.5, 147.3, 146.2, 146.0, 144.4, 143.1, 143.0, 142.7, 142.5, 142.4, 142.1, 142.0, 141.8, 141.5, 140.2, 139.9, 139.4, 136.9, 136.7, 136.4, 135.9, 135.6, 132.6, 132.3, 131.1, 130.6, 129.9, 129.5, 129.1, 128.3, 127.7, 127.0, 122.6, 121.2, 120.3,118.9, 114.6, 114.4, 111.0, 82.0, 72.9 (sp^3 C– of C_{60}), 72.7, 72.4, 67.9(sp^3 C– of C_{60}), 33.3; mass (%) M+ 1060 (48).

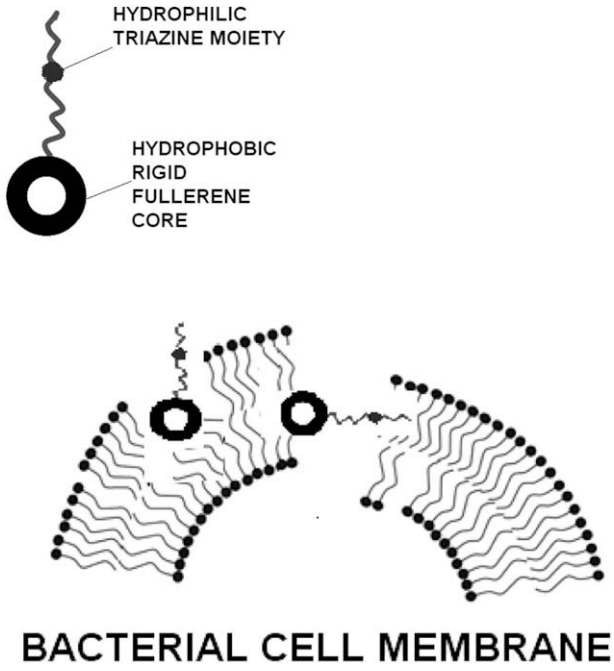


Fig. 2. Interaction of triazine derivatized fulleropyrrolidines with cell wall of bacteria.

Acknowledgement

Authors are thankful to SAIF, CDRI Lucknow for spectral analysis. Authors would also like to thank Department of Microbiology, Gujarat University for antimicrobial studies and Prof. K.H. Chikhaliya, Gujarat University for his support. One of the authors, Anish Kumar is grateful to CSIR, New Delhi for Junior Research Fellowship.

References

[1] N. Tagmatarichis, H. Shinohara, Mini Rev. Med. Chem. 1 (2001) 339–348.
[2] S.R. Wilson, in: K. Kadish, R. Ruoff (Eds.), The Fullerene Handbook, Wiley, New York, 2000, pp. 437–465.
[3] T. Da Ros, M. Prato, J. Chem. Soc. Chem. Commun. (1999) 663–669.
[4] A.W. Jenson, S.R. Wilson, D.I. Schuster, Bioorg. Med. Chem. 4 (1996) 767–779.
[5] S. Folet, C. Crowley, M. Smaihi, C. Bonfils, B. Erlanger, P. Seta, C. Larraque, Biochem. Biophys. Res. Commun. 294 (2002) 116–119.
[6] C. Cusan, T. Da Ros, G. Spalluto, S. Foley, J.M. Janot, P. Seta, C. Larraque, M.C. Tomasini, T. Antonelli, L. Ferraro, M. Prato, Eur. J. Org. Chem. 17 (2002) 2928–2934.
[7] G. Marcorin, T. Da Ros, S. Castellano, G. Stefancich, I. Borin, S. Miertus, M. Prato, Org. Lett. 2 (2000) 3955–3958.
[8] O.A. Troshina, P.A. Troshin, A.S. Peregudov, V.I. Kozlovskiy, J. Balzarini, R.N. Lyubovskaya, Org. Biomol. Chem. 5 (2007) 2783–2791.
[9] S. Bosi, T. Da Ros, S. Castellano, E. Banfi, M. Prato, Bioorg. Med. Chem. Lett. 10 (2000) 1043–1045.
[10] T. Mashino, K. Okuda, T. Hirota, M. Hirobe, T. Nagano, M. Mochizuchi, Bioorg. Med. Chem. Lett. 9 (1999) 2959–2962.
[11] N. Taso, T.Y. Luh, C.K. Chou, J.J. Wu, Y.S. Lin, H.Y. Lei, Antimicrob. Agents Chemother. 45 (2001) 1788–1793.
[12] M.B. Specia, M.E. Milanesio, E.N. Durantini, Eur. J. Org. Chem. 43 (2008) 853–861.
[13] K. Gonzalez, L. Wilson, W. Wu, G. Nancollas, Bioorg. Med. Chem. 10 (2002) 1991–1997.
[14] T. Wharton, L.J. Wilson, Tetrahedron Lett. 43 (2002) 561–564.
[15] E. Nakamura, H. Isobe, N. Tomita, M. Sawamura, S. Jinno, H. Okayama, Angew. Chem., Int. Ed. 39 (2000) 4254–4257.

[16] C.Yu.T. Canteenwala, M.E. El-Khouly, Y. Araki, K. Pritzker, O. Ito, B.C. Wilson, L.Y. Chiang, J. Mater. Chem. 15 (2005) 12508–12509.
[17] H.C. Hsu, Y.Y. Chiang, W.J. Chen, Y.T. Lee, J. Cardiovasc. Pharmacol. 36 (2000) 423–427.
[18] T.Y. Zakharian, A. Seryshev, B. Sitharaman, B.E. Gilbert, V. Knight, L.J. Wilson, J. Am. Chem. Soc. 127 (2005) 12508–12509.
[19] M. Kellermann, W. Bauer, A. Hirsch, B. Shade, K. Ludwig, C. Bottcher, Angew. Chem., Int. Ed. 43 (2004) 2959–2962.
[20] X. Yang, X. Meng, B. Li, Z. Chen, D. Zhao, X. Tan, Q. Yu, Biologicals 36 (2008) 223–226.
[21] Z. Markovic, V. Trajkovic, Biomaterials 29 (2008) 3561–3573.
[22] S.T. Huang, J.S. Liao, H.W. Fang, C.M. Lin, Bioorg. Med. Chem. Lett. 18 (2008) 99–103.
[23] Z. Hu, W. Guan, W. Wang, L. Huang, X. Tang, H. Xu, Z. Zhu, X. Xie, H. Xing, Carbon 46 (2008) 99–109.
[24] A. Babakhin, A. Garmanova, A. Petrukhina, V. Romanova, S. Andreev, L. Dubuske, Clin. Immunol. 127 (2008) S128.
[25] S. Andreev, A. Garmanova, A. Babakhin, A. Petrukhina, S. Korobova, M. Khaitov, L. Dubuske, Clin. Immunol. 127 (2008) S129.
[26] P. Mroz, A. Pawlak, M. Satti, H. Lee, T. Wharton, H. Gali, T. Sarna, M.R. Hamblin, Free Radic. Biol. Med. 43 (2007) 711–719.
[27] D.W. Cagle, T.P. Thrash, M. Alford, L.P.F. Chibante, L.J. Ehrhardt, L.J. Wilson, J. Am. Chem. Soc. 118 (1996) 8043–8047.
[28] M. Mikawa, H. Kato, M. Okumura, M. Narazaki, Y. Kanazawa, N. Miwa, H. Shinohara, Bioconjugate Chem. 12 (2001) 510–514.
[29] K. Srinivas, V. Srinivas, V.J. Rao, K. Bhanuprakash, K.H. Kishore, V.S.N. Murthy, Bioorg. Med. Chem. Lett. 15 (2005) 1121–1123.
[30] S. Jain, D. Bhambi, R. Sharma, G.L. Talesara, Ind. J. Pharm. Sci. 69 (2007) 28–32.
[31] V.K. Pandey, S. Tusi, Z. Tusi, M. Joshi, S. Bajpai, Acta Pharm. 54 (2004) 1–12.
[32] D. Andreu, L. Rivas, Biopolymers 47 (1998) 415–422.
[33] S. Fernandez-Lopez, H.S. Kim, E.C. Choi, M. Delgado, J.R. Granja, A. Khasanov, K. Kraehenbuehl, G. Long, D.A. Wienberger, K.M. Wilcoxen, M.R. Ghadiri, Nature 412 (2001) 452–456.
[34] S.S. Lim, Y.M. Song, M.H. Jang, Y. Kim, K.S. Hahm, S.Y. Shin, Protein Pept. Lett. 11 (2004) 35–39.
[35] K. Singh, M.S. Barwa, P. Tyagi, Eur. J. Med. Chem. 41 (2006) 7482–7489.
[36] S.K. Sridhar, M. Saravan, A. Ramesh, Eur. J. Med. Chem. 36 (2001) 615–625.
[37] O.M. Walsh, M.J. Meegan, R.M. Prendergast, T.A. Nakib, Eur. J. Med. Chem. 31 (1996) 989–1000.
[38] M. Goi, J. Synth. Org. Chem. Jpn 18 (1960) 332–336.
[39] P. de Hoog, P. Gamez, W.L. Driessen, J. Reedijk, Tetrahedron Lett. 43 (2002) 6783–6786.
[40] O. Diels, Ber. Dtsch. Chem. Ges. 32 (1899) 691–702.
[41] M. Maggini, G. Scorrano, J. Am. Chem. Soc. 115 (1993) 9798–9799.
[42] R. Cruickshank, J.P. Duguki, B.P. Marion, R.H.A. Swain, Medicinal Microbiology, 12th ed., vol. 2, Churchill Livingstone, London, 1975, pp. 196–202.
[43] A.H. Collins, Microbiological Methods, second ed. Buttersworth, London, 1976.