Full Paper

Novel Cationic Quinazolin-4(3*H*)-one Conjugated Fullerene Nanoparticles as Antimycobacterial and Antimicrobial Agents

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A series of novel cationic fullerene derivatives bearing a substituted-quinazolin-4(3*H*)-one moiety as a side arm were synthesized using the 1,3-dipolar cycloaddition reaction of C_{60} with azomethine ylides generated from the corresponding Schiff bases of substituted quinazolinones. The synthesized compounds **5a–f** were characterized by elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR, and ESI-MS and screened for their antibacterial activity against *Mycobacterium tuberculosis* (H_{37}RV) and antimicrobial activity against selected Gram-positive (*Staphylococcus aureus* and *S. pyogenes*) and Gram-negative (*Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*) bacterial and fungal strains (*Candida albicans*, *Aspergillus clavatus*, and *A. niger*), respectively. All the compounds exhibited significant activity, with the most effective compounds having MIC values and zones of inhibition comparable to those of standard drugs.

Keywords: Antibacterial study / Antifungal study / Antimycobacterial study / Cationic fulleropyrrolidines / Quinazolin-4(3*H*)-one

Received: October 1, 2012; Revised: November 27, 2012; Accepted: November 30, 2012

DOI 10.1002/ardp.201200371

Introduction

Since the discovery of fullerene (C_{60}) in 1985 [1], there have been various reports of chemically modified fullerenes with widespread applications in chemical, biological, and material sciences [2–4]. C_{60} derivatives have been well known for their encouraging applications as antibacterial agents [5], antimycobacterial agents [6], anti-amyloid agents [7], photo-induced DNA cleavage agents [8]. They have also been used for studying various photo-physical properties [9], potentiometric biosensing of glucose [10], and drug delivery systems [11]. There has been a great deal of emphasis in using C_{60} for diagnosis and therapeutic purposes; the main motivation being the ability of the hydrophobic fullerene spheroid in crossing cell membrane [12].

It is known that the lipidic bacterial cell wall, especially of mycobacterium, is robust and impermeable to many antibiotics [13]. But the high hydrophobicity of fullerene allows it to intercalate into the bilayer lipid membrane of the cell wall and inhibits bacterial growth by inducing membrane stress [14]. In addition, fullerenes bearing a cationic derivative have shown enhancement in the interaction with the negatively charged bacterial cell surface and thereby destruct the cell wall [14].

But bacteria are characterized by a robust central metabolism and cell wall, which can resist or adapt to stress sturdily. It can easily cause precipitation of membrane stress inducing molecules and thus adapt to the environment. Hence we need to design a molecule which can rupture the cell wall (cause membrane stress) and also disturb the central metabolism ensuring complete cell death.

Cationic fullerenes, though capable of destroying the integrity of the bacterial cell wall, are less likely to disturb the central metabolism of the bacteria; hence keeping both the above points in view we have synthesized a series of cationic fullerene derivatives having a substituted quinazolin-4(3*H*)-one side arm.

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The quinazolin-4(3*H*)-one side arm was chosen due to the versatility of the quinazolinone scaffold. Quinazolinone derivatives are readily accessible, have diverse chemical reactivity as well as a wide range of biological activities [15–17].

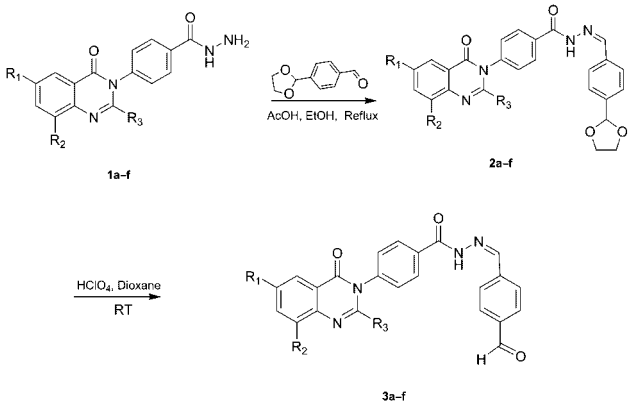
They are known to inhibit enzymes essential for bacterial DNA replication and hence are very potent antibacterial agents having immense therapeutic significance [18]. Furthermore, it is known that substitution at the 2nd, 3rd, and 6th positions of the quinazolinone ring gives improved bacterial activity; hence these sites were considered for derivatization [17, 19].

We have reported earlier a fullerene–isoniazid conjugate with improved activity toward $H_{37}Rv$ mycobacterial strain [6]. Considering the tremendous biological importance of fullerene and quinazolinone for the treatment of tuberculosis and also based on our expertise in functionalization of fullerene [8–10] and calixarene [20], we carried out the synthesis of Schiff base linked cationic fullerene–quinazolinone analogues, which would provide a synergistic enhancement in the antimycobacterial and antibacterial activity of both the moieties. The synthesized compounds were then screened for their antimicrobial activity by using the broth microdilution method and antituberculosis activity against $H_{37}RV$ strain using the L. J. agar dilution method.

Results and discussion

Chemistry

The synthetic route adopted for the synthesis of fullerene quinazolin-4(3*H*)-one conjugates is shown in Schemes 1–3.



Compound	R_1	R_2	R_3
a	H	H	$-CH_3$
b	Br	H	$-CH_3$
c	Br	Br	$-CH_3$
d	H	H	$-C_6H_5$
e	Br	H	$-C_6H_5$
f	Br	Br	$-C_6H_5$

Scheme 1. Synthesis of compounds **3a–f**.

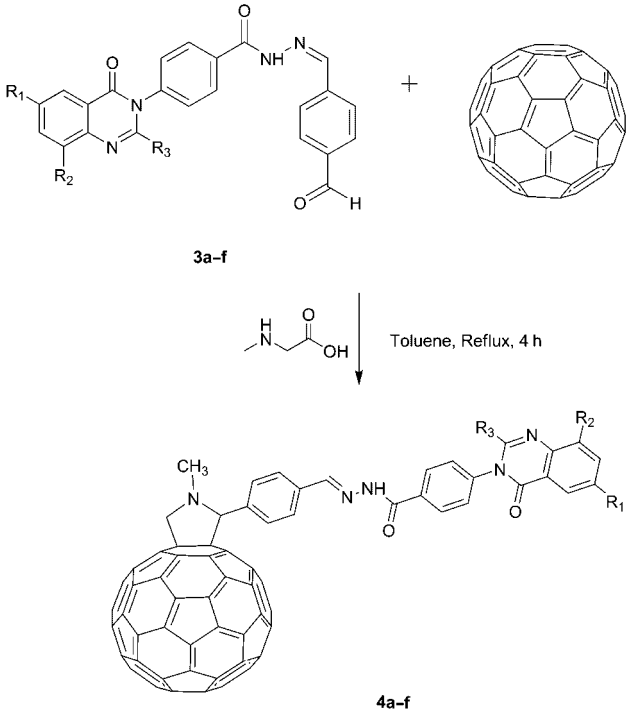
The compounds **1a–f** were synthesized by a reported procedure [17]. Schiff bases **2a–f** were formed by treating **1a–f** with mono-protected terephthaldehyde in ethanol and glacial acetic acid. The deprotection of the aldehyde group was then achieved by treating with perchloric acid using dioxane as solvent to obtain 4-(substituted-4-oxo-(3*H*)-quinazoline-3-yl)-4-formyl-benzylidene hydrazide **3a–f** (Scheme 1).

Compounds **3a–f**, *N*-methyl glycine and C_{60} were then allowed to reflux in toluene wherein they undergo a 1,3-dipolar cycloaddition reaction [21] to give fulleropyrrolidines **4a–f** (Scheme 2).

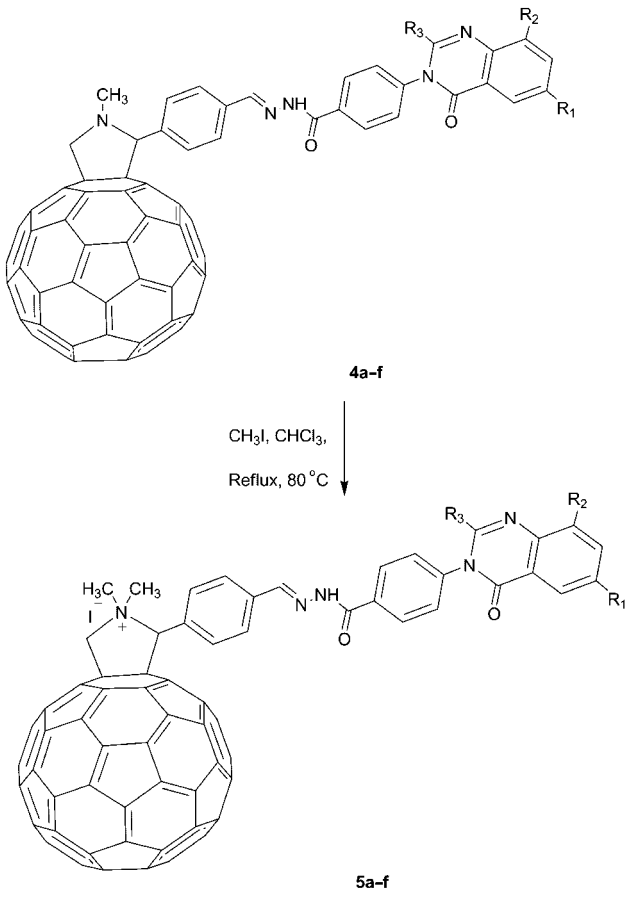
The IR spectra of the novel compounds **4a–f** showed characteristic bands of amide carbonyl between 1695 and 1712 cm^{-1} and organo fullerenes between 524 and 527 cm^{-1}. The ^1H NMR showed the presence of the pyrrolidine protons as two doublets at 3.68 and 4.23 ppm with coupling constants between 9.3 and 10.1 Hz and a singlet at 4.59 ppm.

The cationic derivatives **5a–f** (Scheme 3) was obtained by refluxing **4a–f** with stoichiometric amount of methyl iodide in chloroform for 2 days.

The quaternization of the nitrogen on the pyrrolidine ring was confirmed from the spectral data. The presence of a peak in the ^1H NMR spectra of compound (**5a–f**) at δ 10.0–10.8 attributed to the hydrazide proton and the N–H stretching (3500–3250 cm^{-1}) in the IR spectra showed that the hydra-



Scheme 2. Synthesis of fullerene–quinazolinone conjugates **4a–f**.



Scheme 3. Synthesis of cationic derivatives of fullerene–quinazolinone conjugates **5a–f**.

zide nitrogen has not participated in salt formation. The study of the ^{1}H NMR and ^{13}C NMR for the surrounding atoms of the quinazolinone nitrogen also shows that it has not undergone methylation. Owing to the basic nature of the pyrrolidine nitrogen it is the most susceptible to be attacked by methyl iodide resulting in the cationic center for compounds **5a–f**.

The compounds **4a–f** and **5a–f** have a hydrophilic cationic center but are still highly hydrophobic and were found to be insoluble in water and other polar solvents like DMSO. Hence suspensions in deionized water were prepared for their biological evaluation by a similar procedure as reported earlier [22]. The size of suspended particles of compounds **4a–f** and **5a–f** in water was determined by transmission electron microscopy (TEM) which shows that the particle sizes are 50 nm (Fig. 1). The concentration of suspended aggregates in water was determined spectrophotometrically.

The compounds **4a–f** and **5a–f** gave the characteristic peaks of fullerene moiety at a UV range of 292–329 nm in chloroform from which a standard curve was plotted. 5 mL of chloroform was added to 5 mL of the aqueous suspension to extract the particles and then stirred for 1.5 h. The procedure was repeated using 2.5 mL chloroform. Both the chloroform layers were combined and the concentration of compounds was found by comparing the absorbance from the standard plot.

Pharmacology

Antibacterial activity

The antibacterial activities of compounds **3a–f**, **4a–f**, and **5a–f** are listed in Table 1. The results revealed that there is significant improvement in the activity of the compounds **3a–f**

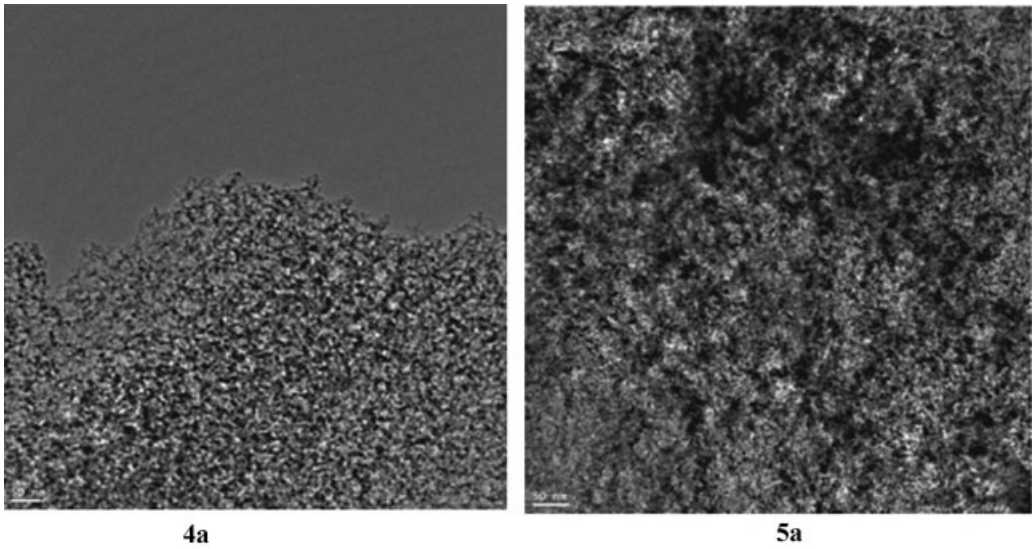


Figure 1. TEM images of compounds **4a** and **5a**.

Table 1. *In vitro* antibacterial activity of compounds **3a–f**, **4a–f**, and **5a–f**.

Compd No	Gram-positive		Gram-negative		
	S. aureus **MTCC 96**	***S. pyogenes*** **MTCC 442**	***P. aeruginosa*** **MTCC 741**	***K. pneumoniae*** **MTCC 109**	***E. coli*** **MTCC 739**
	MIC	**MIC**	**MIC**	**MIC**	**MIC**
3a	125	100	250	500	250
3b	100	125	200	500	250
3c	100	125	200	500	200
3d	125	125	250	500	125
3e	125	100	200	500	125
3f	125	100	200	250	100
4a	100	125	125	125	100
4b	125	125	100	125	125
4c	125	125	100	125	100
4d	125	100	100	125	100
4e	100	100	100	125	100
4f	125	100	100	125	100
5a	100	100	50	100	100
5b	100	100	50	100	100
5c	100	100	50	100	50
5d	100	100	50	100	25
5e	100	100	25	50	25
5f	50	50	25	50	25
Ciprofloxacin[a)]	50	50	25	50	25
DMSO	–	–	–	–	–

MIC (μg/mL), minimum inhibitory concentration.
[a)] Standard drugs.

(200 μg/mL) on conjugation with a fullerene, i.e., **4a–f** (100 μg/mL) for Gram-negative bacteria. In fact the activity increases significantly for the cationic derivative **5a–f** (50–25 μg/mL) compared to **3a–f**. On the other hand, the trend observed for Gram-positive bacteria shows that the addition of the fullerene to the quinazolinone moiety does not cause significant variation in the antibacterial activity.

This can be clearly explained on the basis of the difference in the cell wall structure of Gram-positive and Gram-negative bacteria. The Gram-positive bacterial cell walls are less charged and more permeable while the Gram-negative bacteria have a highly lipidic and negatively charged cell wall. The antimicrobial activity of **3a–f** is majorly due to the ability of the quinazolinone group to inhibit enzymes and disrupt other metabolic activities inside the cell. Since the Gram-positive bacterial cell walls are highly permeable, **3a–f** are potent antibacterials; but for Gram-negative bacteria permeability through the cell wall is less, consequently the concentration of **3a–f** reaching inside the cell decreases and therefore activity decreases relative to Gram-positive bacteria.

However, when a highly lipophilic moiety like the fullerene spheroid is introduced, the molecules **4a–f** can intercalate into the lipid bilayers of the cell wall and channel the accumulation of more concentration of the quinazolinone inside the cell leading to increased potency. Additionally, an introduction of cationic charge on the compound in **5a–f** increases the interaction with the negatively charged cell wall causing an increase in the activity of the molecule (Fig. 2). On the other hand in Gram-positive bacteria permeability is not an issue, the activities remain similar from **3a–f** to **5a–f** (100 μg/mL).

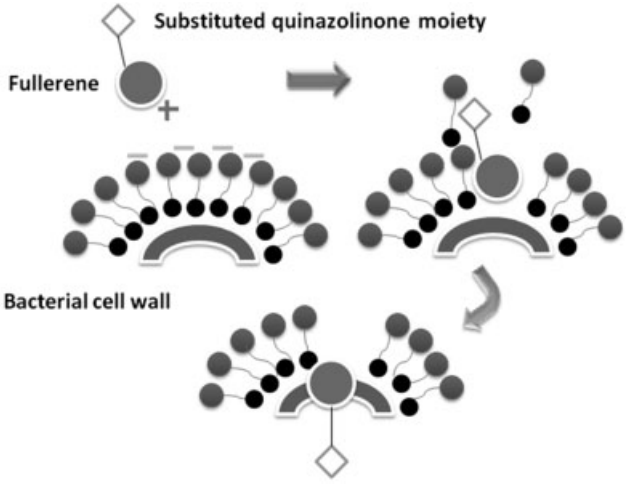


Figure 2. Graphical sketch representing the attack of the compounds **5a–f** on the Gram-negative bacteria.

Table 2. *In vitro* antifungal activity of compounds **3a–f**, **4a–f**, and **5a–f**.

Compound No	*C. albicans* MTCC 183	*A. clavatus* MTC C 1323	*A. niger* MTCC 282
	MIC	MIC	MIC
3a	250	500	500
3b	500	250	200
3c	200	200	250
3d	200	200	200
3e	200	250	250
3f	200	125	200
4a	250	250	200
4b	250	250	200
4c	200	250	250
4d	200	200	200
4e	200	250	250
4f	200	125	200
5a	200	200	200
5b	200	200	200
5c	125	125	125
5d	200	250	250
5e	200	200	200
5f	125	125	125
Nystatine[a)]	100	100	100
DMSO	–	–	–

MIC (μg/mL), minimum inhibitory concentration.
[a)] Standard drugs.

Table 3. *In vitro* antituberculosis activity and % inhibition against *M. tuberculosis* $H_{37}Rv$ at concentration 250 μg/mL and MIC (μg/mL) of compounds **3a–f**, **4a–f**, and **5a–f**.

Compound No	L. J. MIC method	
	MIC (μg/mL)	Inhibition (%) at 250 μg/mL
3a	250	78.12 ± 1.21
3b	250	77.06 ± 0.88
3c	200	79.07 ± 0.91
3d	200	79.42 ± 1.02
3e	200	79.55 ± 1.23
3f	200	79.71 ± 0. 42
4a	25	80.26 ± 0.31
4b	12.5	89.02 ± 1. 15
4c	12.5	91.11 ± 0.99
4d	12.5	90.25 ± 0.28
4e	6.25	92.07 ± 1. 17
4f	6.25	93.08 ± 1. 09
5a	3.125	90.01 ± 1.32
5b	3.125	92.23 ± 0.17
5c	3.125	95.45 ± 1.23
5d	3.125	96.42 ± 0.62
5e	3.125	98.12 ± 1.23
5f	1.562	98.83 ± 0.21
Isoniazid[a)]	0.20	99.00
Rifampicine[a)]	0.25	99.00
DMSO	–	–

MIC (μg/mL), minimum inhibitory concentration.
Mean of five replicates ± standard deviation.
[a)] Standard drugs.

Amongst the activities of fullerene derivatives **5a–f**, it is very obvious that the molecule having more lipophilic substitution, i.e. **5f**, has better activity than the other compounds for Gram-negative bacteria.

Antifungal activity

The compounds **3a–f**, **4a–f**, and **5a–f** were tested for antifungal activity against *Candida albicans*, *Aspergillus niger*, and *A. clavatus.* As shown in Table 2, there is no definite trend observed in the antifungal activities of the compounds **3a–f**, **4a–f**, and **5a–f** suggesting that the attachment of the fullerenes has not resulted in any significant increase in the antifungal activities. This property can be attributed to the cell wall structures of the fungus which are mainly composed of proteins like chitin and contain very less amount of fats. Here again permeability is not a concern hence the activity observed is due to the inherent antifungal property of quinazolinone compounds and the fullerene moiety does not contribute to the potency of the molecule.

Antimycobacterial activity

In vitro evaluation of antimycobacterial activity of the compounds **3a–f**, **4a–f**, and **5a–f** along with the standard drugs is summarized in Table 3. A very pronounced enhancement in the antimycobacterial activity can be seen on attachment of the fullerene spheroid to the quinazolinone group.

The mycobacterium cell wall has a very high concentration of lipids and is associated with the resistance of these bacteria to stains and antibiotics. The waxy cell wall of these bacteria prevents the entry of molecules **3a–f** (250 μg/mL and 78% inhibition); the only activity observed is due to the small amount probably entering through the porin pathway like most of the antitubercular drugs [23]. But on accessorizing the molecule with a fullerene, **4a–f** can easily seep into the waxy cell wall, rupture it, and facilitate the entry of the quinazolinone into the cytoplasm. Inside the cytoplasm quinazolinone can easily cause cell death by inhibiting cell metabolism. Hence the MIC decreases to 6.25 μg/mL and inhibition increases to 93%. The introduction of a cationic charge results in better interaction between the carboxylic groups of mycolic acid present in the cell envelope of mycobacterium with the derivatives **5a–f** (1.5 μg/mL and 98% inhibition), resulting in better activity compared to **3a–f** and **4a–f**.

Conclusion

We have designed and synthesized a novel class of quinazolinone–fullerene conjugates as antimycobacterial/microbial agents. The molecule **5f** inhibits the growth of *M. tuberculosis* effectively at MIC 1.562 μg/mL with inhibition of 98.83%,

which is comparable to that of the existing front-line drug isoniazid and rifampicin which have 99% inhibition.

We conclude that the fullerene moiety here acts as a permeabilizer allowing the transit of the quinazolinone group into the cytoplasm by destroying the cell envelope of mycobacterium as well as for Gram-negative bacteria. Thus these molecules constitute our important ''leads'' for further optimization by structure–activity relationship to develop effective antimycobacterial/microbial agents which can help to shorten the duration of current anti-TB therapy. Further, optimization and pharmacokinetic characterization of this series are in progress in our laboratory.

Experimental

Materials and method

All the chemicals and reagents were of analytical grade of BDH, Aldrich, and Merck unless and otherwise specified. The solvents used for the analysis were purified by standard methods [24]. The melting points (°C, uncorrected) were taken using MPA100 Automated Melting Point Apparatus. The FT-IR spectra were recorded on Bruker Tensor 27 FT-IR spectrometer with KBr pellets. The ESI–MS spectra were recorded on Applied Biosystems, API 2000 LC/MS/MS System. Vario Micro cube elemental analyzer was used as elemental analysis system; ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer in DMSO-d_6 or $CDCl_3$ with tetramethylsilane (TMS) as an internal standard. The nanoparticle size was determined using transmission electron microscope (TEM) JEOL JEM 2100. The progress of the reaction was monitored on readymade silica gel plates (Merck) using toluene/ethyl acetate as a solvent system. Spectral data (IR, ^{1}H NMR, mass spectra, and elemental analysis) confirmed the structure of the synthesized compounds and the purity of these compounds were ascertained by microanalysis. Elemental (C, H, N) analysis indicated that the calculated and observed values were within the acceptable limits (±0.4%).

*Synthesis of **1a–f***

Synthesis of **1a–f** was carried out as reported earlier [17, 25, 26].

*Synthesis of 4-(substituted-4-oxo-(3H)quinazolin-3-yl)-benzoic acid ([1,3]4-dioxolan-2yl-benzylidene)hydrazide (**2a–f**) [6]*

A mixture of 1.79 g (0.01 mol) of 4-(1,3)-dioxolan-2yl-benzaldehyde, 2 mL of glacial acetic acid, 2.941 (0.01 mol) of substituted quinazolinone **1a** dissolved in 50 mL of ethanol was refluxed for about half an hour. Product was isolated and purified by using column chromatography using hexane/ethyl acetate (9:1) as the solvent system. The solvent was then distilled under vacuum to get bright yellow product **2a**. The compounds **2b–f** were synthesized in an analogous manner and were characterized as shown below.

*4-(2-Methyl-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-[1,3]dioxolan-2-yl-benzylidene) hydrazide (**2a**)*

Yield: 84%, mp >240°C; IR (KBr, υ cm^{-1}): 1659 (–C=O cyclic tertiary amide), 1680, 1535 (–C=O acyclic secondary amide), 3300, 3190 (–NH–), 1640 (CH=N); ^{1}H NMR (δ, 400 MHz, DMSO-d_6, Me_4Si) 2.45 (s, 3H, CH_3), 5.82 (s, 1H, O–CH–O), 3.95–4.05 (m, 4H, –OCH_2–CH_2O–); 7.41–8.23 (m, 13H, aromatic H and –N=CH), 9.98 (s, 1H, –NH); ^{13}C NMR (δ, 125 MHz, DMSO-d_6, Me_4Si) 23.33 (–CH_3), 67.47 (OCH_2–CH_2–O), 105.49 (O–CH–O), 121.90, 121.96, 125.06, 126.35, 126.74, 126.81, 127.27, 129.56, 130.23, 130.33, 133.68, 139.44, 142.67, 147.74 (Ar C), 148.27 (–N=CH), 154.04 (–C=N), 161.47 (–C=O–N=), 163.93 (–C=O–NH); MS: *m*/*z* 454.50 (M+). Analysis for $C_{26}H_{22}N_4O_4$, (454.48): Calcd.: % C 68.71; H 4.88; N, 12.33. Found: % C 68.72; H 4.90; N 12.34.

*4-(6-Bromo-2-methyl-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-[1,3]dioxolan-2-yl-benzylidene) hydrazide (**2b**)*

Yield: 80%, mp >240°C; IR (KBr, υ cm^{-1}): 1658 (–C=O cyclic tertiary amide), 1682, 1530 (–C=O acyclic secondary amide), 3429, 3230 (–NH–), 1668 (CH=N); ^{1}H NMR (δ, 400 MHz, DMSO-d_6, Me_4Si) 2.48 (s, 3H, CH_3), 5.89 (s, 1H, O–CH–O), 3.95–4.12 (m, 4H, –OCH_2–CH_2O–); 7.44–8.36 (m, 12H, aromatic H and –N=CH), 10.05 (1H, s, –NH); ^{13}C NMR (δ, 125 MHz, DMSO-d_6, Me_4Si) 23.34 (–CH_3), 67.41 (–OCH_2–CH_2O–), 105.23 (O–CH–O), 121.92, 126.72, 127.27, 127.34, 127.37, 128.26, 130.23, 130.21, 133.36, 133.71, 139.51, 142.62, 146.78 (Ar C), 148.22 (–N=CH), 154.12 (–C=N), 161.32 (–C=O–N=), 163.88; (–C=O–NH); MS: *m*/*z* 533.32, 535.34 (M+), (M+2). Analysis for $C_{26}H_{21}N_4O_4Br$ (533.37): Calcd.: % C 58.55; H 3.97; N 10.50. Found: % C 58.56; H 3.98; N 10.52.

*4-(6,8-Dibromo-2-methyl-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-[1,3]dioxolan-2-yl-benzylidene) hydrazide (**2c**)*

Yield: 79%, mp >240°C; IR (KBr, υ cm^{-1}): 1651 (–C=O cyclic tertiary amide), 1675, 1529 (–C=O acyclic secondary amide), 3323, 3187 (–NH–), 1635 (CH=N); ^{1}H NMR (δ, 400 MHz, DMSO-d_6, Me_4Si) 2.39 (s, 3H, CH_3), 5.79 (s, 1H, O–CH–O), 4.05–4.13 (m, 4H, –OCH_2–CH_2O–); 7.39–8.33 (m, 11H, aromatic H and –N=CH), 9.93 (1H, s, –NH); ^{13}C NMR (δ, 125 MHz, DMSO-d_6, Me_4Si) 22.89 (–CH_3), 67.44 (–OCH_2–CH_2O–), 105.33 (O–CH–O), 126.74, 127.21, 127.29, 130.23, 130.33, 133.68, 136.86, 139.26, 136.44, 142.67 (Ar C), 148.22 (–N=CH), 152.55 (–C=N), 161.42 (–C=O–N=), 163.98 (–C=O–NH); MS: *m*/*z* 612.21, 614.30, 616.10 (M+), (M+2), (M+4). Analysis for $C_{26}H_{20}N_4O_4Br_2$, (612.27): Calcd.: % C 51.00; H 3.29; N 9.15. Found: % C 51.02; H 3.27; N 9.17.

*4-(2-Phenyl-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-[1,3]dioxolan-2yl-benzylidene) hydrazide (**2d**)*

Yield: 85%, mp >240°C; IR (KBr, υ cm^{-1}): 1658 (–C=O cyclic tertiary amide), 1682, 1538 (–C=O acyclic secondary amide), 3312, 3189 (–NH–), 1641 (CH=N); ^{1}H NMR (δ, 400 MHz, DMSO-d_6, Me_4Si) 4.11–4.20 (m, 4H, –OCH_2–CH_2O–), 5.81 (s, 1H, O–CH–O), 7.51–8.29 (m, 18H, aromatic H and –N=CH), 10.91 (1H, s, –NH); ^{13}C NMR (δ, 125 MHz, DMSO-d_6, Me_4Si) 67.44 (–OCH_2–CH_2O–), 105.52 (O–CH–O), 122.07, 122.99, 125.18, 126.29, 126.74, 127.12, 127.27, 128.07, 128.68, 130.13, 130.57, 131.41, 131.73, 133.68, 135.48, 139.99, 142.67, 146.02 (Ar C), 148.17 (–N=CH), 152.19 (–C=N), 163.11 (–C=O–N=), 163.79; (–C=O–NH); MS: *m*/*z* 516.50 (M+). Analysis for $C_{31}H_{24}N_4O_4$, (516.55): Calcd.: % C, 72.08; H, 4.68; N, 10.85. Found: % C, 72.10; H, 4.66; N, 10.84.

*4-(6-Bromo-2-phenyl-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-[1,3]dioxolan-2-yl-benzylidene) hydrazide (**2e**)*

Yield: 83%, mp >240°C; IR (KBr, υ cm^{-1}): 1655 (–C=O cyclic tertiary amide), 1684, 1535 (–C=O acyclic secondary amide),

3412, 3228 (−NH−), 1642 (CH=N); ^{1}H NMR (δ, 400 MHz, DMSO-d_6, Me_4Si) 3.97−4.15 (m, 4H, $-OCH_2-CH_2O-$), 5.81 (s, 1H, O−CH−O), 7.43−8.41 (m, 17H, aromatic H and −N=CH), 9.97 (1H, s, −NH); ^{13}C NMR (δ, 125 MHz, DMSO-d_6, Me_4Si), 67.57 ($-OCH_2-CH_2O-$), 105.47 (O−CH−O), 118.84, 122.07, 126.74, 127.27, 127.62, 127.90, 128.07, 128.68, 130.13, 130.57, 131.41, 131.73, 133.68, 134.20, 135.48, 139.99, 142.67, 145.27 (Ar C), 148.13 (−N=CH), 152.20 (−C=N), 163.03 (−C=O−N=), 163.83; (−C=O−NH); MS: *m*/*z* 595.41, 597.35 (M+), (M+2). Analysis for $C_{31}H_{23}N_4O_4Br$, (595.44): Calcd.: % C, 62.53; H, 3.89; N, 9.41. Found: % C, 62.54; H, 3.88; N, 9.42.

*4-(6,8-Dibromo-2-phenyl-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-[1,3]dioxolan-2yl-benzylidene) hydrazide (**2f**)*

Yield: 80%, mp >240°C; IR (KBr, υ cm^{-1}): 1652 (−C=O cyclic tertiary amide), 1677, 1530 (−C=O acyclic secondary amide), 3399, 3185 (−NH−), 1637 (CH=N); ^{1}H NMR (δ, 400 MHz, DMSO-d_6, Me_4Si) 4.04−4.12 (m, 4H, $-OCH_2-CH_2O-$), 5.77 (s, 1H, O−CH−O), 7.41−8.36 (m, 16H, aromatic H and −N=CH), 9.89 (1H, s, −NH); ^{13}C NMR (δ, 125 MHz, DMSO-d_6, Me_4Si), 67.57 ($-OCH_2-CH_2O-$), 105.48 (O−CH−O), 116.75, 122.07, 122.19, 125.56, 126.62, 126.73, 127.26, 128.06, 128.67, 130.56, 131.41, 131.73, 133.68, 135.47, 136.38, 137.32, 139.98, 142.67, 148.27, 152.34 (Ar C), 148.25 (−N=CH), 152.30 (−C=N), 163.63 (−C=O−N=), 163.87; (−C=O−NH); MS: *m*/*z* 674.28, 676.29, 678.22 (M+), (M+2), (M+4). Analysis for $C_{31}H_{22}N_4O_4Br_2$, (674.34): Calcd.: %C, 55.21; H, 3.29; N, 8.31. Found: %C, 55.22; H, 3.31; N, 8.32.

*General procedure for the synthesis of 4-(substituted-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-formyl-benzylidene) hydrazide (**3a–f**) [6]*

4.51 g (0.01 mol) of compound **2a** was mixed with a solution of 3 mL perchloric acid in 50 mL 1,4-dioxane. The mixture was stirred at room temperature for 12 h. The solution was diluted with chloroform (30 mL) and washed with a saturated solution of sodium bicarbonate. The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed in vacuum and the light yellow solid residue was subjected to column chromatography in toluene/methanol (5:1) to get a light yellow solid product **3a**. In a similar way all other products **3b–f** were obtained by the above procedure.

*4-(2-Methyl-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-formyl-benzylidene) hydrazide (**3a**)*

Yield: 79%, mp >240°C; IR (KBr υ cm^{-1}): 1649 (−C=O cyclic tertiary amide), 1705, 1535 (−C=O acyclic secondary amide), 3351, 3198 (−NH−), 1639 (−C=N); ^{1}H NMR (d, 400 MHz, DMSO-d_6, Me_4Si) 2.40 (s, 3H, CH_3), 7.41−8.23 (m, 13H, Ar−H, CH=N), 9.87 (s, 1 H, −CHO), 10.05 (s, 1H, −NH); ^{13}C NMR (d, 125 MHz, DMSO-d_6, Me_4Si) 23.38 ($-CH_3$), 161.47 (−C=O−N=), 163.93 (−C=O−NH), 148.27 (CH=N), 154.0 (−C=N), 121.90, 121.96, 125.01, 126.35, 126.81, 127.18, 127.07, 129.04, 129.56, 130.23, 130.33, 131.55, 139.44, 139.44, 147.74, 191.17 (CHO); MS: *m*/*z* 410.00 (M+). Analysis for $C_{24}H_{18}N_4O_3$, (410.42): Calcd.: % C, 70.23; H, 4.42; N, 13.65. Found: % C, 70.23; H, 4.44; N, 13.67.

*4-(2-Methyl-6-bromo-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-formyl-benzylidene) hydrazide (**3b**)*

Yield: 82%, mp >240°C; IR (KBr υ cm^{-1}): 1654 (−C=O cyclic tertiary amide), 1699, 1531 (−C=O acyclic secondary amide), 3348, 3190 (−NH−), 1638 (−C=N); ^{1}H NMR (d, 400 MHz, DMSO-d_6, Me_4Si) 2.48 (s, 3H, CH_3), 7.44−8.36 (m, 12H, Ar−H, CH=N), 9.99 (s, 1 H, −CHO), 10.05 (s, 1H, −NH); ^{13}C NMR (d, 125 MHz, DMSO-d_6, Me_4Si) 23.33 ($-CH_3$), 161.02 (−C=O−N=), 163.93 (−C=O−NH), 148.27 (CH=N), 154.0 (−C=N), 116.99, 121.96, 127.19, 127.34, 127.38, 128.26, 129.06, 130.23, 130.33, 131.45, 131.55, 139.44, 139.66, 146.74, 191.14 (CHO); MS: *m*/*z* 489.20, 491.20 (M+), (M+2). Analysis for $C_{24}H_{17}BrN_4O3$, (489.32): Calcd.: % C, 58.91; H, 3.50; N, 11.63. Found: % C, 58.91; H, 3.50; N, 11.63.

*4-(2-Methyl-6,8-dibromo-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-formyl-benzylidene) hydrazide (**3c**)*

Yield: 78%, mp >240°C; IR (KBr υ cm^{-1}): 1647 (−C=O cyclic tertiary amide), 1702, 1529 (−C=O acyclic secondary amide), 3341, 3188 (−NH−), 1641 (−C=N); ^{1}H NMR (d, 400 MHz, DMSO-d_6, Me_4Si) 2.39 (s, 3H, CH_3), 7.39−8.33 (m, 11H, Ar−H, CH=N), 9.93 (s, 1 H, −CHO), 10.01 (s, 1H, −NH); ^{13}C NMR (d, 125 MHz, DMSO-d_6, Me_4Si) 23.33 ($-CH_3$), 161.49 (−C=O−N=), 163.93 (−C=O−NH), 148.27 (CH=N), 152.55 (−C=N), 114.75, 120.07, 121.19, 125.56, 127.73, 127.26, 129.67, 130.56, 131.41, 131.73, 136.68, 139.47, 139.38, 139.32, 139.98 191.16 (CHO); MS: *m*/*z* 568.20, 570.20, 572.20 (M+), (M+2), (M+4). Analysis for $C_{24}H_{16}Br_2N_4O_3$, (568.22): Calcd.: % C, 50.73; H, 2.84; N, 9.86. Found: % C, 50.75; H, 2.86; N, 9.88.

*4-(2-Phenyl-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-formyl-benzylidene) hydrazide (**3d**)*

Yield: 85%, mp >240°C; IR (KBr υ cm^{-1}): 1657 (−C=O cyclic tertiary amide), 16 942, 1535 (−C=O acyclic secondary amide), 3300, 3190 (−NH−), 1640 (−C=N); ^{1}H NMR (d, 400 MHz, DMSO-d_6, Me_4Si) 7.42−8.29 (m, 18H, Ar−H, CH=N), 9.89 (s, 1 H, −CHO), 10.91 (s, 1H, −NH); ^{13}C NMR (d, 125 MHz, DMSO-d_6, Me_4Si), 152.19 (CH=N), 163.74, (−C=O−N=), 163.93 (−C=O−NH), 122.07, 122.99, 125.17, 126.29, 127.19, 128.07, 128.68, 129.07, 130.57, 131.41, 131.55, 131.73, 135.48, 139.66, 139.99, 146.03. 191.18 (CHO); MS: *m*/*z* 472.00 (M+). Analysis for $C_{29}H_{20}N_4O_3$, (472.49): Calcd.: % C, 73.72; H, 4.27; N, 11.86. Found: % C, 73.72; H, 4.27; N, 11.85.

*4-(2-Phenyl-6-bromo-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-formyl-benzylidene) hydrazide (**3e**)*

Yield: 76%, mp >240°C; IR (KBr υ cm^{-1}): 1655 (−C=O cyclic tertiary amide), 1705, 1532 (−C=O acyclic secondary amide), 3350, 3199 (−NH−), 1640 (−C=N); ^{1}H NMR (d, 400 MHz, DMSO-d_6, Me_4Si) 7.43−8.41 (m, 17H, Ar−H, CH=N), 9.97 (s, 1H, −CHO), 9.91 (s, 1H, −NH); ^{13}C NMR (d, 125 MHz, DMSO-d_6, Me_4Si), 152.19 (CH=N), 163.03 (−C=O−N=), 163.93 (−C=O−NH), 118.84, 122.07, 122.99, 125.17, 126.29, 127.19, 128.07, 128.68, 129.07, 130.57, 121.41, 131.55, 131.73, 135.48, 139.66, 139.99, 146.03, 192.11 (CHO); MS: *m*/*z* 551.12, 553.31 (M+), (M+2). Analysis for $C_{29}H_{19}BrN_4O_3$, (551.39): Calcd.: % C, 63.17; H, 3.47; N, 10.16. Found: % C, 65.18; H, 3.49; N, 10.18.

*4-(2-Phenyl-6,8-dibromo-4-oxo-(3H)quinazolin-3-yl)-benzoic acid (4-formyl-benzylidene) hydrazide (**3f**)*

Yield: 83%, mp >240°C; IR (KBr υ cm^{-1}): 1641 (−C=O cyclic tertiary amide), 1705, 1532 (−C=O acyclic secondary amide), 3347, 3189 (−NH−), 1639 (−C=N); ^{1}H NMR (d, 400 MHz, DMSO-d_6, Me_4Si) 7.42−8.86 (m, 16H, J = 8.5 Hz, Ar−H), 9.88 (s, 1 H, −CHO), 9.89 (s, 1H, −NH), ^{13}C NMR (d, 125 MHz, DMSO-d_6, Me_4Si), 152.43 (CH=N), 163.84 (−C=O−N=), 163.93 (−C=O−NH), 116.75, 122.07, 122.19, 125.56, 126.62, 126.73, 127.26, 128.06,

128.67, 130.56, 131.41, 131.41, 131.73, 133.68, 135.47, 136.38, 137.32, 139.98, 142.67, 191.11 (CHO); MS: *m/z* 630.14, 632.15, 634.15 (M+). Analysis for $C_{29}H_{18}Br_2N_2O_4$ (630.29): Calcd.: % C, 55.26; H, 2.88; N, 8.89. Found: % C, 55.26; H, 2.88; N, 8.89.

*General procedure for the synthesis of fulleropyrrolidines (**4a–f**) [21]*

Schiff base **3a** (41.0 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 using toluene/ethyl acetate (9:1) to get pure product **4a**. In a similar way all other products **4b–f** were obtained by the above procedure.

*Compound **4a***

Yield: 38%, mp >240°C; IR (KBr, υ cm^{-1}) 3410, 3300 (N–H), 3148 (Ar–H), 1648 (–C=O cyclic tertiary amide), 1700, 1536 (–C=O acyclic secondary amide), 1640 (C=N), 527 (organo fullerene); ^{1}H NMR (d, 500 MHz, $CDCl_3$): 7.46–8.20 (m, 13H, Ar–H, CH=N), 4.25 (dd, 1H, J = 9.3, HHC–N of the pyrrolidine ring), 3.61 (dd, 1H, J = 9.3, HHC–N of the pyrrolidine ring), 4.51 (s, 1H, CH of the pyrrolidine ring), 9.93 (s, 1H, NH), 2.21 (s, 3H, CH_3 linked to N of pyrrolidine ring), 2.45 (s, 3H, CH_3 of quinazolinone ring); ^{13}C NMR (d, 125 MHz, $CDCl_3$) 163.66, (C=O of quinazolinone ring), 160.64 (CONH), 154.32, 151.43, 150.52, 147.91, 146.81, 145.94, 143.24, 143.13, 142.35, 142.23, 141.18, 140.82, 135.94, 134.22, 133.13, 131.40, 129.23, 128.95, 128.03, 127.62, 126.63, 125.08, 124.06, 123.48, 122.85, 118.93, 114.41, 112.63, 110.40, 28.28 (quinazolinone ring CH_3), 40.25 (CH_3 linked to N of the pyrrolidine ring), 69.21 (NCH_2 of pyrrolidine ring), 83.31 (NCH of the pyrrolidine ring), 77.22, 73.50 (sp^3 C– of C_{60}), 72.73, 72.43, 68.65 (sp^3 C– of C_{60}); ESI *m/z*: 1158.00 (M+). Analysis for $C_{86}H_{23}N_5O_2$ (1158.14).

*Compound **4b***

Yield: 37%, mp >240°C; IR (KBr, υ cm^{-1}) 3418, 3190 (N–H), 1652 (–C=O cyclic tertiary amide), 1698, 1535 (–C=O acyclic secondary amide), 1640 (C=N), 526 (organo fullerene); ^{1}H NMR (d, 500 MHz, $CDCl_3$): 7.43–8.37 (m, 12H, Ar–H, CH=N), 4.25 (dd, 1H, J = 9.6, HHC–N of the pyrrolidine ring), 3.62 (dd, 1H, J = 9.6, HHC–N of the pyrrolidine ring), 4.58 (s, 1H, CH of the pyrrolidine ring), 10.12 (s, 1H, NH), 2.25 (s, 3H, CH_3 linked to N of pyrrolidine ring), 2.52 (s, 3H, CH_3 of quinazolinone ring); ^{13}C NMR (d, 125 MHz, $CDCl_3$) 163.64, (C=O of quinazolinone ring), 161.64 (CONH), 153.82, 151.63, 150.55, 146.98, 146.86, 145.94, 143.29, 143.06, 142.22, 142.03, 141.21, 140.81, 135.93, 134.21, 133.44, 130.65, 129.22, 128.77, 128.20, 127.64, 126.68, 125.45, 124.21, 123.52, 121.81, 118.91, 114.34, 112.63, 111.42, 28.82 (quinazolinone ring CH_3), 40.58 (CH_3 linked to N of the pyrrolidine ring), 67.25 (NCH_2 of pyrrolidine ring), 82.23 (NCH of the pyrrolidine ring), 77.44, 73.50 (sp^3 C– of C_{60}), 72.76, 72.44, 68.62 (sp^3 C– of C_{60}); ESI *m/z*: 1237.10, 1239.20 (M+), (M+2). Analysis for $C_{86}H_{22}BrN_5O_2$ (1237.03).

*Compound **4c***

Yield: 33%, mp >240°C; IR (KBr, υ cm^{-1}) 3450, 3256 (N–H), 1650 (C=N); 1645 (–C=O cyclic tertiary amide) 1707, 1529 (–C=O acyclic secondary amide), 1645 (C=N), 528 (organo fullerene); ^{1}H NMR (d, 500 MHz, $CDCl_3$): 7.38–8.32 (m, 11H, Ar–H, CH=N), 4.22 (dd, 1H, J = 10.1, HHC–N of the pyrrolidine ring), 3.65 (dd, 1H, J = 10.1, HHC–N of the pyrrolidine ring), 4.52 (s, 1H, CH of the pyrrolidine ring), 10.12 (s, 1H, NH), 2.19 (s, 3H, CH_3 linked to N of pyrrolidine ring), 2.41 (s, 3H, CH_3 of quinazolinone ring); ^{13}C NMR (d, 125 MHz, $CDCl_3$) 163.62 (C=O of quinazolinone ring), 160.64 (CONH), 154.34, 151.23, 150.56, 146.95, 146.85, 145.94, 143.54, 143.36, 142.36, 142.23, 141.16, 140.86, 135.93, 134.26, 133.46, 130.46, 129.23, 128.77, 128.24, 127.64, 126.63, 125.41, 124.26, 123.54, 122.84, 118.94, 114.46, 112.62, 111.42, 28.43 (quinazolinone ring CH_3), 41.48 (CH_3 linked to N of the pyrrolidine ring), 67.28 (NCH_2 of pyrrolidine ring), 81.45 (NCH of the pyrrolidine ring), 77.25, 73.50 (sp^3 C– of C_{60}), 71.91, 72.43, 67.48 (sp^3 C– of C_{60}); ESI *m/z*: 1315.40, 1317.40, 1319.20, (M+), (M+2), (M+4). Analysis for $C_{86}H_{21}Br_2N_5O_2$, (1315.93).

*Compound **4d***

Yield: 35%, mp >240°C; IR (KBr, υ cm^{-1}) 3410, 3300 (N–H), 1687 (C=N), 1645 (–C=O cyclic tertiary amide) 1695, 1535 (–C=O acyclic secondary amide), 1640 (C=N), 527 (organo fullerene); ^{1}H NMR (d, 500 MHz, $CDCl_3$): 7.41–8.23 (m, 18H, Ar–H, CH=N), 4.22 (dd, 1H, J = 9.4, HHC–N of the pyrrolidine ring), 3.65 (dd, 1H, J = 9.4 HHC–N of the pyrrolidine ring), 4.52 (s, 1H, CH of the pyrrolidine ring), 9.92 (s, 1H, NH), 2.28 (s, 3H, CH_3 linked to N of pyrrolidine ring); ^{13}C NMR (d, 125 MHz, $CDCl_3$) 163.24, (C=O of quinazolinone ring), 162.15 (CONH), 154.12, 151.23, 150.52, 146.91, 146.81, 145.92, 143.24, 143.00, 142.32, 142.03, 141.11, 140.81, 135.93, 134.21, 133.43, 130.45, 129.23, 128.75, 128.20, 127.62, 126.65, 125.00, 124.06, 123.58, 122.80, 118.91, 114.43, 112.63, 111.40, 28.32 (quinazolinone ring CH_3), 40.23 (CH_3 linked to N of the pyrrolidine ring), 67.63 (NCH_2 of pyrrolidine ring), 82.94 (NCH of the pyrrolidine ring), 77.20, 73.50 (sp^3 C– of C_{60}), 72.78, 72.42, 68.6 (sp^3 C– of C_{60}); ESI *m/z*: 1220.10 (M+). Analysis for $C_{91}H_{25}N_5O_2$, (1220.20).

*Compound **4e***

Yield: 35%, mp >240°C; IR (KBr, υ cm^{-1}) 3420, 3235 (N–H), 2958, (Ar H), 1642 (–C=O cyclic tertiary amide) 1712, 1533 (–C=O acyclic secondary amide), 1677 (C=N), 526 (organo fullerene); ^{1}H NMR (d, 500 MHz, $CDCl_3$): 7.45–8.41 (m, 17H, Ar–H, CH=N), 4.21 (dd, 1H, J = 9.4, HHC–N of the pyrrolidine ring), 3.68 (dd, 1H, J = 9.4 HHC–N of the pyrrolidine ring), 4.51 (s, 1H, CH of the pyrrolidine ring), 9.97 (s, 1H, NH), 2.28 (s, 3H, CH_3 linked to N of pyrrolidine ring); ^{13}C NMR (d, 125 MHz, $CDCl_3$) 163.14, (C=O of quinazolinone ring), 161.24 (CONH), 153.92, 151.33, 150.42, 146.96, 146.80, 145.93, 143.24, 143.32, 142.31, 142.33, 141.13, 140.61, 135.73, 134.25, 133.03, 130.46, 129.53, 128.75, 128.20, 127.67, 126.65, 125.30, 124.76, 123.55, 122.82, 118.94, 114.47, 112.63, 111.49, 28.34 (quinazolinone ring CH_3), 40.23 (CH_3 linked to N of the pyrrolidine ring), 66.34 (NCH_2 of pyrrolidine ring), 81.26 (NCH of the pyrrolidine ring), 77.25, 73.50 (sp^3 C– of C_{60}), 72.7, 72.47, 68.67 (sp^3 C– of C_{60}); ESI *m/z*: 1299.10, 1301.10 (M+), (M+2). Analysis for $C_{91}H_{24}BrN_5O_2$, (1299.10).

*Compound **4f***

Yield: 40%, mp >240°C; IR (KBr, υ cm^{-1}) 3429, 3320 (N–H), 3068, (Ar C–H), 1645 (–C=O cyclic tertiary amide) 1702, 1535 (–C=O acyclic secondary amide), 1675 (C=N), 524 (organo fullerene); ^{1}H NMR (d, 500 MHz, $CDCl_3$): 7.44–8.39 (m, 16H, Ar–H, CH=N), 4.23 (dd, 1H, J = 9.6, HHC–N of the pyrrolidine ring), 3.62 (dd, 1H, J = 9.6 HHC–N of the pyrrolidine ring), 4.52 (s, 1H, CH of the pyrrolidine ring), 9.96 (s, 1H, NH), 2.22 (s, 3H, CH_3 linked to N of pyrrolidine ring); ^{13}C NMR (d, 125 MHz, $CDCl_3$) 163.43 (C=O of

quinazolinone ring), 161.65 (CONH), 154.32, 151.24, 150.54, 146.93, 146.83, 145.96, 143.44, 143.23, 142.42 142.23, 141.13, 140.81, 135.98, 134.25, 133.65 130.46, 129.27, 128.74, 128.22, 127.62, 126.64, 125.03, 124.26, 123.51, 122.85, 118.93, 114.47, 112.66, 111.44, 28.37 (quinazolinone ring CH_3), 40.28 (CH_3 linked to N of the pyrrolidine ring), 69.29 (NCH_2 of pyrrolidine ring), 83.23 (NCH of the pyrrolidine ring), 77.26, 73.50 (sp^3 C– of C_{60}), 72.7, 72.43, 68.65 (sp^3 C– of C_{60}); ESI *m/z*: 1378.00, 1380.10, 1382.30 (M+). Analysis for $C_{91}H_{23}Br_2N_5O_2$, (1378.00).

*General procedure for the synthesis of fullerene-N,N-dimethylpyrrolidines iodide salt (fullerene-quinazolinone conjugates) (**5a–f**)*

A solution of fulleropyrrolidine derivative **4a** (80 mg, 0.0691 mmol) in 30 mL of chloroform and iodomethane (9.8 mg, 0.0691 mmol) was refluxed with stirring for 2 days under argon. Then, the residue was washed with toluene (three times) and hexane (twice). The solvent was removed under vacuum to give fullerene-*N*-dimethylpyrrolidine iodide salt **5a** as brownish solid. In a similar way all other products **5b–f** were obtained by the above procedure.

*Compound **5a***

Yield: 98%, mp 212°C; IR (KBr, υ cm^{-1}) 3410, 3300 (N–H), 3148, (Ar–H), 2945 (N–CH_3), 1701, 1540 (–C=O acyclic secondary amide), 1643 (–C=O cyclic tertiary amide), 1600 (C=N), 525 (organo fullerene); ^{1}H NMR (d, 500 MHz, $CDCl_3$): 7.46–8.20 (m, 13H, Ar–H, CH=N), 4.25 (dd, 1H, J = 9.3, HHC–N of the pyrrolidine ring), 3.61 (dd, 1H, J = 9.3, HHC–N of the pyrrolidine ring), 4.51 (s, 1H, CH of the pyrrolidine ring), 9.93 (s, 1H, NH), 2.21 (s, 3H, CH_3 linked to N of pyrrolidine ring), 2.30 (s, 3H, CH_3 linked to N of pyrrolidine ring), 2.45 (s, 3H, CH_3 of quinazolinone ring); ^{13}C NMR (d, 125 MHz, $CDCl_3$) 163.62 (C=O of quinazolinone ring), 160.66 (CONH), 154.11, 151.20, 150.54, 146.96, 146.83, 145.94, 143.25, 143.00, 142.32, 142.03, 141.11, 140.81, 135.93, 134.21, 133.43, 130.45, 129.23, 128.75, 128.26, 127.67, 126.67, 125.03, 124.06, 123.56, 122.84, 118.93, 114.45, 112.67, 111.42, 28.33 (quinazolinone ring CH_3), 42.54, 41.83 ((CH_3)$_2$ linked to N of the pyrrolidine ring), 69.22 (NCH_2 of pyrrolidine ring), 83.16 (NCH of the pyrrolidine ring), 77.26, 73.50 (sp^3 C– of C_{60}), 72.73, 72.45, 68.66 (sp^3 C– of C_{60}); ESI *m/z*: 1173.20 (M+). Analysis for $C_{87}H_{26}IN_5O_2$, (1300.07).

*Compound **5b***

Yield: 97%, mp >240°C; IR (KBr, υ cm^{-1}) 3410, 3204 (N–H), 3140, (Ar–H), 2945 (N–CH_3) 1701, 1536 (–C=O acyclic secondary amide), 1654 (–C=O cyclic tertiary amide), 1660 (C=N), 526 (organo fullerene); ^{1}H NMR (d, 500 MHz, $CDCl_3$): 7.46–8.20 (m, 12H, Ar–H, CH=N), 4.25 (dd, 1H, J = 9.6, HHC–N of the pyrrolidine ring), 3.62 (dd, 1H, J = 9.6, HHC–N of the pyrrolidine ring), 4.58 (s, 1H, CH of the pyrrolidine ring), 10.12 (s, 1H, NH), 2.25 (s, 3H, CH_3 linked to N of pyrrolidine ring), 2.32 (s, 3H, CH_3 linked to N of pyrrolidine ring), 2.52 (s, 3H, CH_3 of quinazolinone ring); ^{13}C NMR (d, 125 MHz, $CDCl_3$) 163.64 (C=O of quinazolinone ring), 160.59 (CONH), 154.14, 151.22, 150.54, 146.93, 146.83, 145.94, 143.26, 143.02, 142.33, 142.05, 141.13, 140.82, 135.95, 134.25, 133.45, 130.47, 129.26, 128.77, 129.20, 127.64, 126.67, 125.03, 124.05, 123.56, 122.88, 118.95, 114.42, 112.67, 111.46, 28.34 (quinazolinone ring CH_3), 39.54, 41.48 ((CH_3)$_2$ linked to N of the pyrrolidine ring), 67.43 (NCH_2 of pyrrolidine ring), 82.45 (NCH of the pyrrolidine ring), 77.24, 73.50 (sp^3 C– of C_{60}), 72.77, 72.44, 68.62 (sp^3 C– of C_{60}); ESI *m/z*: 1252.40, 1254.50 (M+), (M+2). Analysis for $C_{87}H_{25}BrIN_5O_2$, (1378.97).

*Compound **5c***

Yield: 98%, mp 214°C; IR (KBr, υ cm^{-1}) 3450, 3256 (N–H), 3116, (Ar–H), 2840 (N–CH_3), 1705, 1535 (–C=O acyclic secondary amide), 1657 (–C=O cyclic tertiary amide), 1665 (C=N), 528 (organo fullerene); ^{1}H NMR (d, 500 MHz, $CDCl_3$): 7.38–8.32 (m, 11H, Ar–H, CH=N), 4.22 (dd, 1H, J = 10.1, HHC–N of the pyrrolidine ring), 3.65 (dd, 1H, J = 10.1, HHC–N of the pyrrolidine ring), 4.52 (s, 1H, CH of the pyrrolidine ring), 10.12 (s, 1H, NH), 2.19 (s, 3H, CH_3 linked to N of pyrrolidine ring), 2.23 (s, 3H, CH_3 linked to N of pyrrolidine ring), 2.41 (s, 3H, CH_3 of quinazolinone ring); ^{13}C NMR (d, 125 MHz, $CDCl_3$) 161.22 (C=O of quinazolinone ring), 162.45 (CONH), 154.22, 151.21, 150.54, 146.96, 146.83, 145.95, 143.24, 143.11, 142.35, 142.23, 141.43, 140.36, 135.65, 134.55, 133.83, 130.65, 129.29, 128.74, 128.22, 127.64, 126.66, 125.08, 124.26, 123.59, 122.80, 118.95, 114.43, 112.73, 111.45, 28.39 (quinazolinone ring CH_3), 41.2, 40.5 ((CH_3)$_2$ linked to N of the pyrrolidine ring), 67.51 (NCH_2 of pyrrolidine ring), 81.42 (NCH of the pyrrolidine ring), 77.21, 73.50 (sp^3 C– of C_{60}), 72.75, 72.45, 68.66 (sp^3 C– of C_{60}); ESI *m/z*: 1330.50, 1332.30, 1334.50 (M+), (M+2), (M).

*Compound **5d***

Yield: 94%, mp 218°C; IR (KBr, υ cm^{-1}) 3415, 3300 (N–H), 3128, (Ar–H), 2840, 2740 (N–CH_3), 1701, 1537 (–C=O acyclic secondary amide), 1653 (–C=O cyclic tertiary amide), 1654 (C=N), 524 (organo fullerene); ^{1}H NMR (d, 500 MHz, $CDCl_3$): 7.41–8.23 (m, 18H, Ar–H, CH=N), 4.22 (dd, 1H, J = 9.4, HHC–N of the pyrrolidine ring), 3.65 (dd, 1H, J = 9.4, HHC–N of the pyrrolidine ring), 4.52 (s, 1H, CH of the pyrrolidine ring), 9.92 (s, 1H, NH), 2.28 (s, 3H, CH_3 linked to N of pyrrolidine ring), 2.35 (s, 3H, CH_3 linked to N of pyrrolidine ring); ^{13}C NMR (d, 125 MHz, $CDCl_3$) 161.25 (C=O of quinazolinone ring), 164.65 (CONH), 154.28, 151.28, 150.32, 146.87, 146.89, 145.94, 143.27, 143.12, 142.34, 142.13, 141.17, 140.84, 135.83, 134.25, 133.73, 130.44, 129.28, 128.73, 128.28, 127.52, 126.62, 125.26, 124.45, 123.57, 122.88, 118.71, 114.49, 112.64, 111.46, 38.29, 40.55 ((CH_3)$_2$ linked to N of the pyrrolidine ring), 69.71 (NCH_2 of pyrrolidine ring), 83.00 (NCH of the pyrrolidine ring), 77.23, 73.50 (sp^3 C– of C_{60}), 72.71, 72.44, 68.60 (sp^3 C– of C_{60}); ESI *m/z*: 1235.60 (M+). Analysis for $C_{92}H_{28}IN_5O_2$, (1362.13).

*Compound **5e***

Yield: 97%, mp 212°C; IR (KBr, υ cm^{-1}) 3420, 3235 (N–H), 2958, (Ar–H), 2850 (N–CH_3), 1726, 1536 (–C=O acyclic secondary amide), 1658 (–C=O cyclic tertiary amide), 1647 (C=N), 527 (organo fullerene); ^{1}H NMR (d, 500 MHz, $CDCl_3$): 7.45–8.41 (m, 17H, Ar–H, CH=N), 4.21 (dd, 1H, J = 9.4, HHC–N of the pyrrolidine ring), 3.68 (dd, 1H, J = 9.4 HHC–N of the pyrrolidine ring), 4.51 (s, 1H, CH of the pyrrolidine ring), 9.97 (s, 1H, NH), 2.28 (s, 3H, CH_3 linked to N of pyrrolidine ring), 2.30 (s, 3H, CH_3 linked to N of pyrrolidine ring); ^{13}C NMR (d, 125 MHz, $CDCl_3$) 161.32 (C=O of quinazolinone ring), 164.64 (CONH), 154.12, 151.23, 150.52, 146.91, 146.81, 145.92, 143.24, 143.00, 142.32, 142.03, 141.14, 140.88, 135.83, 134.27, 133.46, 130.55, 129.25, 128.85, 128.24, 127.63, 126.25, 125.30, 124.08, 123.52, 122.81, 118.91, 114.42, 112.66, 111.42, 39.25, 42.52 ((CH_3)$_2$ linked to N of the pyrrolidine ring), 66.22 (NCH_2 of pyrrolidine ring), 81.40 (NCH of the pyrro-

lidine ring), 77.22, 73.50 (sp^3 C– of C_{60}), 72.70, 72.45, 68.66 (sp^3 C– of C_{60}); ESI *m/z*: 1314.40, 1316.20 (M+) (M+2). Analysis for $C_{92}H_{27}BrIN_5O_2$, (1441.04).

Compound ***5f***

Yield: 98%, mp 210°C; IR (KBr, $\upsilon\,cm^{-1}$) 3429, 3320 (N–H), 3068, (Ar C–H), 2921, (N–CH_3), 1715, 1533 (–C=O acyclic secondary amide), 1652 (–C=O cyclic tertiary amide), 1667 (C=N), 524 (organo fullerene); ^{1}H NMR (d, 500 MHz, $CDCl_3$): 7.44–8.39 (m, 16H, Ar–H, CH=N), 4.23 (dd, 1H, J = 9.6, HHC–N of the pyrrolidine ring), 3.62 (dd, 1H, J = 9.6 HHC–N of the pyrrolidine ring), 4.52 (s, 1H, CH of the pyrrolidine ring), 9.96 (s, 1H, NH), 2.22 (s, 3H, CH_3 linked to N of pyrrolidine ring), 2.28 (s, 3H, CH_3 linked to N of pyrrolidine ring); ^{13}C NMR (d, 125 MHz, $CDCl_3$) 162.15 (C=O of quinazolinone ring), 164.26 (CONH), 154.42, 151.22, 150.51, 146.98, 146.81, 145.92, 143.24, 143.11, 142.33, 142.33, 141.41, 140.85, 135.73, 134.27, 133.48, 130.35, 129.63, 128.77, 128.22, 127.52, 126.67, 125.29, 124.06, 123.63, 122.88, 118.99, 114.94, 112.63, 111.60, 28.42 (quinazolinone ring CH_3), 40.4, 40.2 ($(CH_3)_2$ linked to N of the pyrrolidine ring), 69.2 (NCH_2 of pyrrolidine ring), 83.35 (NCH of the pyrrolidine ring), 77.25, 73.50 (sp^3 C– of C_{60}), 72.71, 72.40, 68.63 (sp^3 C– of C_{60}); ESI *m/z*: 1392.00, 1394.40, 1396.30 (M+), (M+2), (M+4) Analysis for $C_{92}H_{26}Br_2IN_5O_2$, (1519.94).

Preparation of 4a–f and 5a–f water suspensions

The aqueous suspensions of compounds **4a–f** and **5a–f** were prepared by a method reported earlier [22]. Fifty milligrams of compound **4a** in 50 mL of Milli-Q water was stirred at 40°C for 2 weeks. The suspension thus obtained was then filtered through a Whatman filter; then through a 0.45 μm Osmonics nylon membrane and a 0.22 μm nylon membrane to remove aggregates larger than 200 nm. The water suspension obtained was then concentrated on a rotavapor to get different concentrations ranging from 1.562 to 2000 μg/mL.

The size of suspended particles in water was determined using TEM. TEM samples were prepared by depositing a drop of dispersed compound **5f** particles in water on the carbon-coated Formvar films on copper grids with electron microscope operated at 200 keV.

In vitro evaluation of antimicrobial activity

The minimum inhibitory concentrations (MIC) of the synthesized quinazolinone and fullerene derivatives (**3a–f**) and (**5a–f**) were determined by broth microdilution method as described by Rattan [27]. Antibacterial activity was screened against two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Streptococcus pyogenes* MTCC 442), and three Gram-negative bacteria (*Pseudomonas aeruginosa* MTCC 741, *Klebsiella pneumoniae* MTCC 109 and *Escherichia coli* MTCC 739). Ciprofloxacin was used as a standard drug.

All MTCC (Microbial Type Culture Collection) cultures were provided by Institute of Microbial Technology, Chandigarh. Mueller–Hinton broth was used as nutrient medium to grow. Inoculum size for test strain was adjusted to 10^8 CFU (colony forming unit) per milliliter by comparing the turbidity. Serial dilutions were prepared for primary and secondary screening and DMSO was used as diluent to get desired concentration of drugs to test upon standard bacterial strains. A stock solution of 2000 μg/mL of each of the synthesized compounds was prepared and diluted as and when required. In primary screening 500, 250, and 125 μg/mL concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5, 6.250, 3.125, and 1.5625 μg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

The control tube, containing no antibiotic, was subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation overnight at 37°C. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. All the tubes not showing visible growth (in the same manner as control tube described above) was subcultured and incubated overnight at 37°C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show similar number of colonies indicating bacteriostatic, a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test included a second set of the same dilutions inoculated with an organism of known sensitivity.

The antifungal activity of the synthesized compounds **3a–f**, **4a–f**, and **5a–f** was screened against three fungal species (*C. albicans* MTCC 183, *A. clavatus* MTCC 1323, and *A. niger* MTCC 282). Nystatine were used as standard drug. The procedure followed was similar to as described for antibacterial studies.

The compounds were screened for the *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* (H_{37}RV), by using L. J. (Lowenstein and Jensen) MIC method [28]. Stock solutions of primary 1000, 500, 250, and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.25, 1.562 μg/mL dilutions of each test compound in DMSO (dimethyl sulfoxide) were added in the liquid L. J. Medium and then media were sterilized by inspissation method. A culture of *M. tuberculosis* H_{37}Rv growing on L. J. medium was harvested in 0.85% saline in bijou bottles. These tubes were then incubated at 37°C for 24 h followed by streaking of *M. tuberculosis* H_{37}Rv (5×10^4 bacilli per tube). These tubes were then incubated at 37 ± 1°C. Growth of bacilli was seen after 12 days, 22 days, and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H_{37}Rv. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The standard strain *M. tuberculosis* H_{37}Rv was tested with known drugs isoniazid and rifampicin.

M.B.P. would like to acknowledge the University Grants Commission, New Delhi, India for a Junior Research Fellowship.

The authors have declared no conflict of interest.

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