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Original article

Synthesis and *in vitro* antimycobacterial activity of 8-OCH_3 ciprofloxacin methylene and ethylene isatin derivatives

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ABSTRACT

A series of novel 8-OCH_3 ciprofloxacin methylene and ethylene isatin derivatives with remarkable improvement in lipophilicity were synthesized in this study. These derivatives were evaluated for their *in vitro* activity against some mycobacteria. All of the synthesized compounds were less active than the parent 8-OCH_3 ciprofloxacin against *Mycobacterium smegmatis* CMCC 93202, but most of the methylene isatin derivatives were more active than 8-OCH_3 ciprofloxacin, ciprofloxacin, isoniazid and rifampin against MTB H37Rv ATCC 27294. It was noted that compound **3b** (MIC: 0.074 μM) was 2–13 fold more potent than the reference compounds against MTB H37Rv ATCC 27294, and compounds **3f** and **3i–k** (MIC: 6.72–7.05 μM) were around 1.6 fold more potent than the parent 8-OCH_3 ciprofloxacin, 3.5 fold more potent than ciprofloxacin against MDR-MTB 09710.

1. Introduction

Tuberculosis (TB) is a common, communicable and even fatal disease caused by mycobacteria, mainly by *Mycobacterium tuberculosis* (MTB) [1]. The WHO has estimated that approximately one-third of the world population (more than 2 billion) is infected with MTB, and 9.4 million new incident cases occurred in 2008, with around 1.3 million individual deaths in the same year [2,3]. The increasing emergence of drug-resistant TB (DR-TB), especially multidrug-resistant TB (MDR-TB) resistant to at least two major first-line anti-TB drugs (isoniazid/INH and rifampin/RIF) is causing particularly concern [3,4]. MDR-TB has already caused several fatal outbreaks, and posed a significant threat to the treatment and control of the disease in some parts of the world [5]. For example, of the new TB cases in Azerbaijan in 2007, the proportion of MDR-TB reached as high as 22.3% [2]. In addition, TB is also a frequent HIV co-infection, which is having a devastating impact in some regions, such as African where 38% of new TB cases were attributable to HIV co-infection in 2008 [2,6]. Therefore, it's imperative to develop novel, high effective and fast acting anti-TB drugs.

Fluoroquinolones (FQs) have emerged as one of the dominant classes of chemotherapeutic drugs for the treatment of various bacterial infections in both community and hospital settings [7]. These antibacterial agents act by inhibiting bacterial type II DNA topoisomerases, DNA gyrase (the principal target in gram-negative bacteria) and topoisomerase IV (the primary target in gram-positive bacteria) [8]. Surprisingly, there is no evidence of the topoisomerase IV *parC* and *parE* gene homologs in the genome of MTB, so it seems that DNA gyrase is the sole topoisomerase drug target of FQs in MTB [9]. Resistance to FQs remains relatively low in clinical isolates of MTB currently, and there are no reports of cross-resistance or antagonism with other classes of antimycobacterial agents [10,11]. Some early FQs, including ciprofloxacin (CPFX, Fig. 1), ofloxacin and sparfloxacin were recommended as second-line agents for the treatment of TB mainly in cases involving resistance or intolerance to first-line anti-TB therapy by WHO in 1996 [12]. Interestingly, 8-OCH_3 FQ derivatives with N1-cyclopropyl substitution are much more potent against resistant MTB than 8-H analogs [13]. The newer 8-OCH_3 FQs moxifloxacin (MXFX) and gatifloxacin (GTFX) having a particularly strong *in vitro* and *in vivo* activity against MTB, could be promising agents for the treatment of TB [14]. It was noted that 8-OCH_3 ciprofloxacin (8-OCH_3 CPFX, Fig. 1) exhibits excellent *in vitro* activity against gram-positive, gram-negative and anaerobic bacteria [15]. However, to our knowledge, no studies aiming at optimizing 8-OCH_3 CPFX against mycobacteria have been reported in the literature.

Recently, the research concerning FQs has been focused on the basic group at the C-7 position which is the most adaptable site for

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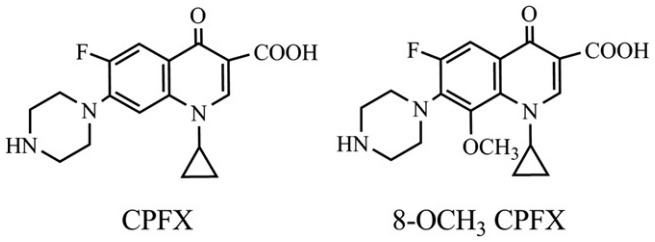


Fig. 1. Chemical structures of CPFX and 8-OCH_3 CPFX.

chemical change and an area that greatly influences their potency, spectrum and safety [16–18]. On the other hand, it was suggested that the lipophilicity of the FQs plays an important role in the penetration of these compounds into bacterial cells, and simply increasing the lipophilic character at C-7 position could also increase the anti-TB activity [11,19]. Therefore, reasonable modification at C-7 position is likely to produce more effective anti-TB agents.

Sriram et al. reported GTFX and CPFX methylene isatin derivatives, and a few compounds were more potent than the parent drugs against MTB [11,19]. In our previous work, a series of balofloxacin (BLFX) ethylene isatin derivatives were synthesized and evaluated for their *in vitro* activity against some mycobacteria. The derivative containing 3-[*N*-methyl-*N*-2-(5-fluorinisatinyl-β-ethoxyimino)ethyl]aminopiperidin-1-yl group at the C-7 position of BLFX was found to be far more potent than the parent BLFX, and comparable to MXFX [20].

Inspired by the above research results, a series of 8-OCH_3 CPFX methylene and ethylene isatin derivatives containing oxime, methyloxime, ethyloxime, semicarbazone and thiosemicarbazone were designed and synthesized in this study. Our primary objective was to optimize the potency of these compounds against mycobacteria.

2. Results and discussion

2.1. Chemistry

Synthetic pathways to 8-OCH_3 CPFX methylene and ethylene isatin derivatives **3a**–**k** and **5a**–**l** are depicted in Schemes 1 and 2, respectively. Condensations of compounds **1a**, **b** with requisite substituted amine hydrochlorides in the presence of $NaHCO_3$ formed isatin derivatives (Schiff's bases) **2a**–**i** (62–88%). Mannich reactions of the compounds **1a**, **b** or **2a**–**i** with paraformaldehyde $[(CH_2O)_n]$ and 8-OCH_3 CPFX were performed in refluxing EtOH under an atmosphere of nitrogen to provide 8-OCH_3 CPFX methylene isatin derivatives **3a**–**k** (40–85%) (Scheme 1) [11,19].

Isatins **1a**, **b** were alkylated with 1,2-dibromoethane to give *N*-(2-bromoethyl)isatins **4a**, **b** (53–68%) according to the reported procedures [20,21]. Nucleophilic substitution reactions of compounds **4a**, **b** with 8-OCH_3 CPFX were performed in DMF at 40 °C to afford 8-OCH_3 CPFX ethylene derivatives **5a**, **b** (34–45%). Subsequent condensations of compounds **5a**, **b** with requisite substituted amine hydrochlorides in the presence of $NaHCO_3$ formed other derivatives (Schiff's bases) **5c**–**l** (48–77%) (Scheme 2).

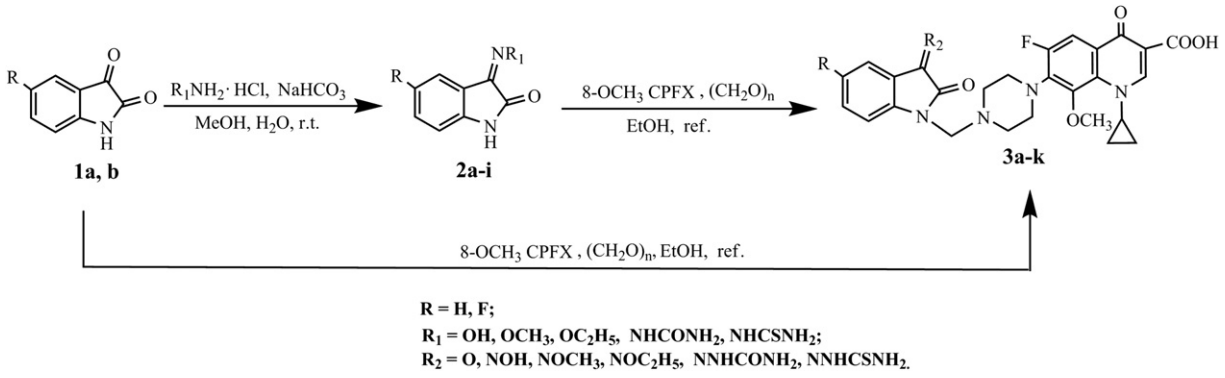
The structures of the synthesized 8-OCH_3 CPFX methylene and ethylene isatin derivatives **3a**–**k** and **5a**–**l** were established by MS, HRMS, ^{1}H NMR and ^{13}C NMR spectra. For example, the ^{1}H NMR chemical shifts of some respective protons in the title compounds were as follows: two sets of singlet at δ 2.66–3.76 ppm for piperazine-4H, two sets of multiplet at δ 0.72–1.22 and 3.88–4.15 ppm for cyclopropyl-5H, a singlet at 3.72–3.82 for 8-OCH_3 group, a singlet at δ 4.48–4.65 ppm corresponding to $-NCH_2N-$ group for methylene derivatives (around 62 ppm in ^{13}C NMR), two sets of triplet at δ 4.07–4.15 and 4.35–4.53 ppm corresponding to $-NCH_2CH_2N-$ group for ethylene derivatives.

2.2. Lipophilicity

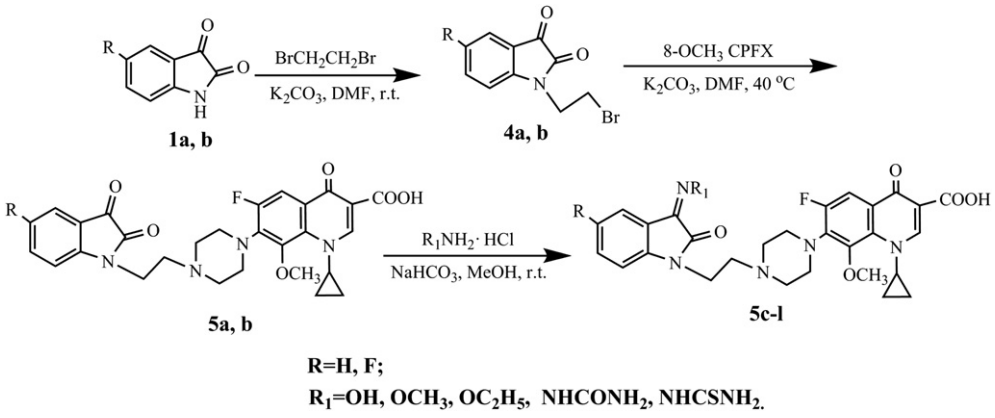
Lipophilicity of the synthesized 8-OCH_3 CPFX derivatives **3a**–**k** and **5a**–**l**, the parent compound 8-OCH_3 CPFX and CPFX is expressed in the term of their log *P* values which were calculated with Chem office 2009 software. As shown in Table 1, a remarkable improvement in the lipophilicity of the derivatives **3a**–**k** and **5a**–**l** as evidenced by log *P* values (1.26–3.33) which were much more than that of the parent 8-OCH_3 CPFX (1.20) (statistically significant at $p < 0.001$ using *t* test).

Compounds **3b**, **3d**, **3e**, **3j** and **3k** were chosen for further evaluation their experimental log *P* values by HPLC, and compared with 8-OCH_3 CPFX (Table 1). These results indicated that the lipophilicity of derivatives **3b**, **3d**, **3e**, **3j** and **3k** (experimental log *P* values arrange from 1.08 to 1.67) was much more than 8-OCH_3 CPFX (0.79), which was consistent with lipophilicity profiles based on log *P* values calculated by softwares. This may be rendering them more capable of penetrating various biomembranes, consequently improving their penetrability towards mycobacterial cell membrane. In other words, the improvement of the lipophilic character of the target compounds **3a**–**k** and **5a**–**l** probably enhances their antimycobacterial activity.

In addition, 8-OCH_3 CPFX methylene isatin derivatives (log *P*: 1.54–3.33) were more lipophilic than that of the corresponding ethylene isatin analogs (log *P*: 1.26–3.05), and compounds containing a 5-fluoroisatin moiety (log *P*: 1.42–3.33) were more than the corresponding isatin analogs (log *P*: 1.26–3.17) (statistically significant at $p < 0.001$ using *t* test).



Scheme. 1. Synthesis of 8-OCH_3 CPFX methylene isatin derivatives **3a**–**k**.

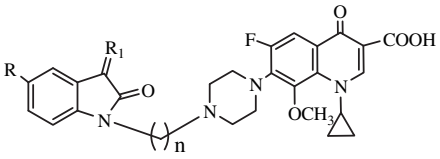


Scheme. 2. Synthetic route of 8-OCH_3 CPFX ethylene isatin derivatives **5a**–**l**.

2.3. Pharmacology

The target compounds **3a**–**k**, **5a**–**l** along with 8-OCH_3 CPFX, CPFX, RIF and INH were initially evaluated for their *in vitro* antimycobacterial activity against *M*ycobacterium *smegmatis* CMCC 93202 using serial double dilution technique in duplicate [20]. Considering the fact that the activity against *M. smegmatis* is often correlated poorly with that against MTB, most of the compounds with relatively good activity, such as **3b**–**k**, **5e**, **5f**, **5k** and **5l**, and compound **3a** with least activity against *M. smegmatis*, were chosen

Table 1
Structures, lipophilicity, antimycobacterial activity and cytotoxicity of compounds **3a**–**k** and **5a**–**l**.



Compd.	n	R	R_1	Log *P*[a]	MIC (μM)			CC_{50}[e] (μM)	SI[f]
					M. s.[b]	MTB[c]	MDR-MTB[d]		
3a	1	H	O	2.18	24.04	0.15	30.77	70.8	472
3b	1	F	O	2.34[g]	2.90	0.074	14.87	70.6	954
3c	1	H	NOH	2.57	2.92	0.15	29.91	74.4	496
3d	1	F	NOH	2.73[g]	1.41	0.14	14.47	73.8	527
3e	1	H	$NOCH_3$	2.83[g]	5.68	0.15	14.57	76.5	510
3f	1	F	$NOCH_3$	2.99	2.75	0.14	7.05	46.8	334
3g	1	H	NOC_2H_5	3.17	2.77	0.14	28.42	91.5	654
3h	1	F	NOC_2H_5	3.33	2.68	0.55		85.8	156
3i	1	H	$NNHCONH_2$	1.54	1.35	0.14	6.93	266.3	1902
3j	1	F	$NNHCONH_2$	1.70[g]	2.62	0.13	6.72	168.4	1295
3k	1	H	$NNHCSNH_2$	2.10[g]	2.63	0.13	6.75	153.6	1182
5a	2	H	O	1.91	5.84				
5b	2	F	O	2.06	2.83				
5c	2	H	NOH	2.29	11.38				
5d	2	F	NOH	2.45	22.05				
5e	2	H	$NOCH_3$	2.56	2.77	1.14		305.6	268
5f	2	F	$NOCH_3$	2.71	2.68	1.10		12.8	12
5g	2	H	NOC_2H_5	2.89	5.41				
5h	2	F	NOC_2H_5	3.05	1.31				
5i	2	H	$NNHCONH_2$	1.26	5.28				
5j	2	F	$NNHCONH_2$	1.42	5.12				
5k	2	H	$NNHCSNH_2$	1.82	2.57	1.05		56.1	53
5l	2	F	$NNHCSNH_2$	1.98	2.50	1.02		57.0	56
8-OCH_3 CPFX				1.20[g]	0.54	0.22	11.08	242.1	1100
CPFX				1.32	2.36	0.97	24.17	600.8	619
RIF					3.79	0.19	>311	180	947
INH					5.69	0.29	467	6282.8	21665

[a] The log *P* is calculated by Chem office 2009 software.
[b] M.s.: M. smegmatis CMCC 93202.
[c] MTB: MTB H37Rv ATCC 27294.
[d] MDR-MTB: MDR-MTB 09710 was resistant to RIF, INH, EMB and SM.
[e] CC_{50}: The 50% cytotoxic concentration.
[f] SI: Selectivity index for MTB H37Rv ATCC 27294, CC_{50}/MIC_{MTB}.
[g] The experimental log *P* values by HPLC of compounds **3b**, **3d**, **3e**, **3j**, **3k** and 8-OCH_3 CPFX are 1.08, 1.57, 1.67, 1.44, 1.34 and 0.79, respectively.

for evaluation of their *in vitro* activity against MTB H37Rv ATCC 27294. Finally, compounds **3a**–**g** and **3i**–**k** with better activity than the parent against MTB H37Rv ATCC 27294, were further evaluated their *in vitro* activity against MDR-MTB 09710 clinical isolate using rapid direct susceptibility test technique [20].

The minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give complete inhibition of mycobacterial growth and MICs of the synthesized compounds along with 8-OCH_3 CPFX, CPFX, RIF and INH for comparison are presented in Table 1. These data indicated that all of the target compounds showed considerable potency in inhibiting the growth of *M. smegmatis* CMCC 93202 (MIC: 1.31–24.04 μM), although less active than the parent 8-OCH_3 CPFX (MIC: 0.54 μM).

As for MTB H37Rv ATCC 27294, all of the methylene isatin derivatives except compound **3h** (MIC: 0.074–0.15 μM) were more active than 8-OCH_3 CPFX, CPFX, RIF and INH (MIC: 0.19–0.97 μM). In particular, the most active compound **3b** (MIC: 0.074 μM) was found to be 2–13 fold more potent than the reference compounds. On the other hand, the activity of four ethylene isatin analogs **5e**, **5f**, **5k** and **5l** was less than the references and the corresponding methylene isatin analogs.

Compounds **3a**–**g** and **3i**–**k** with better activity than the reference compounds against MTB H37Rv ATCC 27294, were also found to have considerable activity (MIC: 6.72–30.77 μM) against MDR-MTB 09710 resistant to INH, RIF, ethambutol/EMB and streptomycin/SM. Among them, compounds **3f** and **3i**–**k** (MIC: 6.72–7.05 μM) were around 1.6 fold more potent than the parent 8-OCH_3 CPFX, and 3.5 fold more potent than CPFX against this strain.

Finally, compounds **3a**–**k**, **5e**, **5f**, **5k** and **5l** were examined for toxicity (CC_{50}) in a mammalian Vero cell line [22]. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a formazan product and the results are reported in Table 1. The fifteen derivatives when tested showed CC_{50} values ranging from 12.8 to 266.3 μM. A comparison demonstrated that the derivatives containing a 5-fluoroisatin moiety were generally more cytotoxic than that of the corresponding isatin analogs. Herein, compounds **3i** and **5e** showed similar toxicity as the parent 8-OCH_3 CPFX, and the selectivity index (SI: 1182–1902) of compounds **3i**–**k** was more than 8-OCH_3 CPFX (1100) for MTB H37Rv ATCC 27294.

3. Conclusion

In summary, a series of novel 8-OCH_3 CPFX methylene and ethylene isatin derivatives were designed, synthesized and characterized by ^{1}H NMR, ^{13}C NMR, MS and HRMS. These derivatives were initially evaluated for their *in vitro* antimycobacterial activity against *M. smegmatis* CMCC 93202, and then some of them against MTB H37Rv ATCC 27294 and MDR-MTB 09710, respectively. The results showed that all of the target compounds with improved lipophilicity were less active than the parent 8-OCH_3 CPFX against *M. smegmatis* CMCC 93202, but most of the methylene isatin derivatives were more active than 8-OCH_3 CPFX, which suggested the activity of these compounds against *M. smegmatis* was correlated poorly with that against MTB. It was noted that compound **3b** (MIC: 0.074 μM) was 2–13 fold more potent than the reference compounds, and the activity of compounds **3f** and **3i**–**k** (MIC: 6.72–7.05 μM) was better than 8-OCH_3 CPFX against MDR-MTB 09710.

The relative contribution of isatin moieties of 8-OCH_3 CPFX methylene isatin derivatives to activity against MDR-MTB 09710 is as follows: thiosemicarbazone ≈ semicarbazone > methyloxime > ketone ≈ oxime ≈ ethyloxime, when R = H; semicarbazone ≈ methyloxime > ketone ≈ oxime, when R = F; 5-fluoroisatin > isatin. In addition, methylene isatin derivatives were more potent than the corresponding ethylene isatin analogs against MTB H37Rv ATCC 27294.

4. Experimental section

4.1. General

Melting points were determined in open capillaries and uncorrected. Log *P* was calculated with Chem office 2009 software. HPLC was preformed using a Shimadzu LC-10Avp with SPD-10Avp UV detector (Shimadzu) and a Class VP 6.x workstation. The column used was a Diamonsil C18 5 μm 250 × 4.6 mm column (Dikma Technologies). ^{1}H NMR and ^{13}C NMR spectra were determined on a Varian Mercury-400 spectrometer in DMSO-d_6 or $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. Electrospray ionization (ESI) mass spectra and high resolution mass spectra (HRMS) were obtained on a MDSSCIEX Q-Tap mass spectrometer and AccuTOF CS JMS-T100CS (JEOL) mass spectrometer, respectively. Fast Atom Bombardment (FAB) mass spectra and high resolution mass spectra (HRMS) were obtained on a MICROMASS AutoSpec Ultima-TOF mass spectrometer. Unless otherwise noted, the reagents were obtained from commercial supplier and used without further purification. TLC was performed on silica gel plates (Merck, ART5554 60F_{254}).

4.2. Synthesis

4.2.1. General procedure for the preparation of compounds *2a*–*i*

To a solution of substituted amine hydrochloride (1.5 mmol) and $NaHCO_3$ (0.12 g, 1.4 mmol) dissolved in water (10 mL) was added dropwise a solution of **1a**, **b** (1 mmol) in MeOH (10 mL) at room temperature over 5 min. The reaction mixture was stirred at the same temperature for 1 h and filtered. The solid obtained was washed with water and EtOH to give the title compounds **2a**–**i** (62–88%).

*4.2.1.1. β-Hydroximinoisatin (**2a**).* Yield: 81%. mp: 215–218 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 6.85–7.93 (4H, m, Ar-H), 10.66 (1H, s, D_2O exchangeable, NH), 13.25 (1H, s, D_2O exchangeable, NOH). FAB-MS: *m*/*z* 163 $(M + H)^+$.

*4.2.1.2. 5-Fluoro-β-hydroximinoisatin (**2b**).* Yield: 88%. mp: 262–264 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 6.84–7.70 (3H, m, Ar-H), 10.70 (1H, s, D_2O exchangeable, NH), 13.53 (1H, s, D_2O exchangeable, NOH). FAB-MS: *m*/*z* 181 $(M + H)^+$.

*4.2.1.3. β-Methoxyiminoisatin (**2c**).* Yield: 80%. mp: 172–174 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 4.17 (3H, s, $NOCH_3$), 6.86–7.83 (4H, m, Ar-H), 10.75 (1H, s, D_2O exchangeable, NH). FAB-MS: *m*/*z* 177 $(M + H)^+$.

*4.2.1.4. 5-Fluoro-β-methoxyiminoisatin (**2d**).* Yield: 82%. mp: 228–231 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 4.19 (3H, s, $NOCH_3$), 6.86–7.63 (3H, m, Ar-H), 10.79 (1H, s, D_2O exchangeable, NH). FAB-MS: *m*/*z* 195 $(M + H)^+$.

*4.2.1.5. β-Ethoxyiminoisatin (**2e**).* Yield: 72%. mp: 136–139 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 1.36 (3H, t, J = 7.2 Hz, $NOCH_2\underline{CH_3}$), 4.42 (2H, q, J = 7.2 Hz, $NO\underline{CH_2}CH_3$), 6.86–7.85 (4H, m, Ar-H), 10.74 (1H, s, D_2O exchangeable, NH). FAB-MS: *m*/*z* 191 $(M + H)^+$.

*4.2.1.6. 5-Fluoro-β-ethoxyiminoisatin (**2f**).* Yield: 62%. mp: 170–174 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 1.36 (3H, t, J = 6.8 Hz, $NOCH_2\underline{CH_3}$), 4.45 (2H, q, J = 6.8 Hz, $NO\underline{CH_2}CH_3$),

6.86–7.62 (3H, m, Ar-H), 10.78 (1H, s, D_2O exchangeable, NH). FAB-MS: *m*/*z* 209 $(M + H)^+$.

*4.2.1.7. β-Semicarbazoisatin (**2g**).* Yield: 85%. mp: 260–265 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 6.87 (2H, brs, D_2O exchangeable, NNHCO$\underline{NH_2}$), 7.00–8.06 (4H, m, Ar-H), 10.66 (1H, s, D_2O exchangeable, NH), 13.25 (1H, brs, D_2O exchangeable, N$\underline{NH}$CONH_2). FAB-MS: *m*/*z* 205 $(M + H)^+$.

*4.2.1.8. 5-Fluoro-β-semicarbazoisatin (**2h**).* Yield: 72%. mp: 273–276 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 6.86 (2H, brs, D_2O exchangeable, NNHCO$\underline{NH_2}$), 6.84–8.08 (3H, m, Ar-H), 10.34 (1H, s, D_2O exchangeable, $\underline{NH}$), 10.69 (1H, brs, D_2O exchangeable, N$\underline{NH}$CONH_2). FAB-MS: *m*/*z* 223 $(M + H)^+$.

*4.2.1.9. β-Thiosemicarbazoisatin (**2i**).* Yield: 77%. mp: 251–253 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 6.90–7.65 (4H, m, Ar-H), 8.66, 9.02 (2H, brs, D_2O exchangeable, NNHCS$\underline{NH_2}$), 11.18 (1H, s, D_2O exchangeable, NH), 12.46 (1H, brs, D_2O exchangeable, N$\underline{NH}$CSNH_2). FAB-MS: *m*/*z* 221 $(M + H)^+$.

*4.2.2. General procedure for the preparation of compounds **3a–k***

A solution of 1-cyclopropyl-6-fluoro-8-methoxy-7-(piperazin-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (8-OCH_3 CPFX, 2.89g, 8 mmol) and $(CH_2O)_n$ (0.36 g, 12 mmol) in EtOH (100 mL) was heated to refluxing for 1.5 h under an atmosphere of nitrogen. To the reaction mixture was added isatin **1a**, **b** or Schiff's base **2a–i** (5 mmol), stirred at refluxing for 12 h, and cooled to room temperature. The precipitate was filtered and recrystallized from DMF and water to give the title compounds **3a–k** (40–85%).

*4.2.2.1. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(isatinylmethyl) piperazin-1-yl]-1,4- dihydro-4-oxoquinoline-3-carboxylic acid (**3a**).* Yield: 85%. mp: 218–222 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ_{ppm}: 1.00 (2H, m, cyclopropyl-H), 1.21 (2H, m, cyclopropyl-H), 2.93 (4H, s, piperazine-4H), 3.53 (4H, s, piperazine-4H), 3.78 (3H, s, OCH_3), 4.01 (1H, m, cyclopropyl-H), 4.64 (2H, s, CH_2 of linker), 7.19–8.82 (6H, m, Ar-H), 14.67 (1H, brs, D_2O exchangeable, COOH). FAB-MS: *m*/*z* 521 $(M + H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{27}H_{26}FN_4O_6$ $(M + H)^+$: 521.1836; Found 521.1801.

*4.2.2.2. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(5-fluoroisatinylmethyl)piperazin-1-yl]-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3b**).* Yield: 78%. mp: 124–127 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ_{ppm}: 1.01 (2H, m, cyclopropyl-H), 1.18–1.22 (2H, m, cyclopropyl-H), 2.88 (4H, s, piperazine-4H), 3.50 (4H, s, piperazine-4H), 3.79 (3H, s, OCH_3), 4.02 (1H, m, cyclopropyl-H), 4.59 (2H, s, CH_2 of linker), 7.26–8.81 (5H, m, Ar-H), 14.68 (1H, brs, D_2O exchangeable, COOH). ^{13}C NMR (DMSO-d_6, 400 MHz) δ_C: 8.93, 40.79, 50.10, 50.50, 61.85, 62.74, 106.48, 111.03, 113.45, 118.53, 120.88, 123.79, 124.03, 134.03, 139.03, 145.93, 147.59, 150.53, 154.34, 157.05, 159.34, 165.62, 176.30, 182.55. FAB-MS: *m*/*z* 539 $(M + H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{27}H_{25}F_2N_4O_6$ $(M + H)^+$: 539.1742; Found 539.1729.

4.2.2.3. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(β-hydroximinoisatinyl) methylpiperazin-1-yl]-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (3c). Yield: 79%. mp: 165–170 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 1.00–1.05 (2H, m, cyclopropyl-H), 1.09–1.12 (2H, m, cyclopropyl-H), 2.73 (4H, s, piperazine-4H), 3.30 (4H, s, piperazine-4H), 3.72 (3H, s, OCH_3), 4.13 (1H, m, cyclopropyl-H), 4.55 (2H, s, CH_2 of linker), 7.09–8.69 (6H, m, Ar-H), 13.46 (1H, s, D_2O exchangeable, NOH), 14.92 (1H, brs, D_2O exchangeable, COOH). FAB-MS: *m*/*z* 536 $(M + H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{27}H_{26}FN_5O_6$ $(M + H)^+$: 536.1945; Found 536.1936.

*4.2.2.4. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(5-fluorinisatinyl-β-hydroximino) methylpiperazin-1-yl]-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3d**).* Yield: 74%. mp: 172–176 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 1.02 (2H, m, cyclopropyl-H), 1.10 (2H, m, cyclopropyl-H), 2.76 (4H, s, piperazine-4H), 3.30 (4H, s, piperazine-4H), 3.74 (3H, s, OCH_3), 4.14 (1H, m, cyclopropyl-H), 4.48 (2H, s, CH_2 of linker), 7.33–8.68 (5H, m, Ar-H), 14.92 (1H, brs, D_2O exchangeable, COOH). FAB-MS: *m*/*z* 554 $(M + H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{27}H_{26}F_2N_4O_6$ $(M + H)^+$: 554.1851; Found 554.1863.

*4.2.2.5. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(β-methoxyiminoisatinyl)methylpiperazin-1-yl]-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3e**).* Yield: 72%. mp: 132–135 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ_{ppm}: 0.99 (2H, m, cyclopropyl-H), 1.19–1.22 (2H, m, cyclopropyl-H), 2.81 (4H, s, piperazine-4H), 3.42 (4H, s, piperazine-4H), 3.75 (3H, s, OCH_3), 4.01 (1H, m, cyclopropyl-H), 4.31 (3H, s, $NOCH_3$), 4.58 (2H, s, CH_2 of linker), 7.06–8.80 (6H, m, Ar-H), 14.75 (1H, brs, D_2O exchangeable, COOH). FAB-MS: *m*/*z* 550 $(M + H)^+$. HRMS-FAB: *m*/*z* Calcd. for for $C_{28}H_{29}FN_5O_6$ $(M + H)^+$: 550.2102; Found 550.2076.

*4.2.2.6. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(5-fluorinisatinyl-β-methoxyimino)methylpiperazin-1-yl]-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3f**).* Yield: 75%. mp: 134–138 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 1.01 (2H, m, cyclopropyl-H), 1.09–1.12 (2H, m, cyclopropyl-H), 2.72 (4H, s, piperazine-4H), 3.31 (4H, s, piperazine-4H), 3.75 (3H, s, OCH_3), 4.14 (1H, m, cyclopropyl-H), 4.23 (3H, s, $NOCH_3$), 4.53 (2H, s, CH_2 of linker), 7.29–8.69 (5H, m, Ar-H), 14.92 (1H, brs, D_2O exchangeable, COOH). ^{13}C NMR (DMSO-d_6, 400 MHz) δ_C: 8.93, 40.77, 50.08, 50.68, 61.44, 62.68, 64.72, 106.47, 111.89, 114.04, 115.47, 119.14, 120.86, 134.00, 138.99, 140.65, 142.89, 145.88, 150.48, 154.29, 156.80, 159.17, 162.99, 165.59, 176.26. FAB-MS: *m*/*z* 568 $(M + H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{28}H_{28}F_2N_5O_6$ $(M + H)^+$: 568.2008; Found 568.2008.

*4.2.2.7. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(β-ethoxyiminoisatinyl)methylpiperazin-1-yl]-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3g**).* Yield: 65%. mp: 117–120 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 1.01 (2H, m, cyclopropyl-H), 1.09 (2H, m, cyclopropyl-H), 1.38 (3H, t, J = 7.2 Hz, $NOCH_2\underline{CH_3}$) 2.73 (4H, s, piperazine-4H), 3.31 (4H, s, piperazine-4H), 3.72 (3H, s, OCH_3), 4.13 (1H, m, cyclopropyl-H), 4.36 (2H, q, J = 7.2 Hz, $NO\underline{CH_2}CH_3$), 4.54 (2H, s, CH_2 of linker), 7.10–8.68 (6H, m, Ar-H), 14.92 (1H, brs, D_2O exchangeable, COOH). FAB-MS: *m*/*z* 564 $(M + H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{29}H_{31}FN_5O_6$ $(M + H)^+$: 564.2258; Found 564.2256.

*4.2.2.8. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(5-fluorinisatinyl-β-ethoxyimino) methylpiperazin-1-yl]-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3h**).* Yield: 57%. mp: 125–128 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 0.98–1.02 (2H, m, cyclopropyl-H), 1.04–1.09 (2H, m, cyclopropyl-H), 1.39 (3H, t, J = 7.6 Hz, $NOCH_2\underline{CH_3}$) 2.66 (4H, s, piperazine-4H), 3.35 (4H, s, piperazine-4H), 3.75 (3H, s, OCH_3), 4.15 (1H, m, cyclopropyl-H), 4.49 (2H, q, J = 7.6 Hz, $NO\underline{CH_2}CH_3$), 4.53 (2H, s, CH_2 of linker), 7.29–8.69 (5H, m, Ar-H), 14.92 (1H, brs, D_2O exchangeable, COOH). FAB-MS: *m*/*z* 582 $(M + H)^+$. HRMS-FAB: *m*/*z* Calcd. for $C_{29}H_{30}F_2N_5O_6$ $(M + H)^+$: 582.2164; Found 582.2159.

*4.2.2.9. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(β-semicarbazoisatinyl)methylpiperazin-1-yl]-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3i**).* Yield: 62%. mp: 174–177 °C. ^1H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 0.98–1.02 (2H, m, cyclopropyl-H), 1.04–1.09 (2H, m, cyclopropyl-H), 2.66 (4H, s, piperazine-4H), 3.35 (4H, s, piperazine-4H), 3.75 (3H, s, OCH_3), 4.14 (1H, m, cyclopropyl-H), 4.61 (2H, s, CH_2 of linker), 7.14 (2H, brs, D_2O exchangeable, NNHCO$\underline{NH_2}$), 7.16–8.69 (6H, m, Ar-H), 11.66 (1H, brs, D_2O exchangeable,

NNHCONH$_2$), 14.92 (1H, brs, D$_2$O exchangeable, COOH). FAB-MS: *m/z* 578 (M + H)$^+$. HRMS-FAB: *m/z* Calcd. for $C_{28}H_{29}FN_7O_6$ (M + H)$^+$: 578.2163; Found 578.2162.

*4.2.2.10. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(5-fluorinisatinyl-β-semicarbazo) methylpiperazin-1-yl]-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3j**).* Yield: 54%. mp: 182–186 °C. ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ_{ppm}: 1.00–1.03 (2H, m, cyclopropyl-H), 1.06–1.10 (2H, m, cyclopropyl-H), 2.74 (4H, s, piperazine-4H), 3.32 (4H, s, piperazine-4H), 3.73 (3H, s, OCH$_3$), 4.14 (1H, m, cyclopropyl-H), 4.59 (2H, s, CH$_2$ of linker), 7.23 (2H, brs, D$_2$O exchangeable, NNHCONH$_2$), 7.25–8.67 (5H, m, Ar-H), 11.58 (1H, brs, D$_2$O exchangeable, NNHCONH$_2$), 14.91 (1H, brs, D$_2$O exchangeable, COOH). FAB-MS: *m/z* 596 (M + H)$^+$. HRMS-FAB: *m/z* Calcd. for $C_{28}H_{38}F_2N_7O_6$ (M + H)$^+$: 596.2069; Found 596.2064.

*4.2.2.11. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(β-thiosemicarbazoisatinyl) methylpiperazin-1-yl]-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**3k**).* Yield: 40%. mp: 180–183 °C. ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ_{ppm}: 0.97–1.00 (2H, m, cyclopropyl-H), 1.09–1.12 (2H, m, cyclopropyl-H), 2.77 (4H, s, piperazine-4H), 3.33 (4H, s, piperazine-4H), 3.73 (3H, s, OCH$_3$), 4.13 (1H, m, cyclopropyl-H), 4.65 (2H, s, CH$_2$ of linker), 7.15–8.67 (6H, m, Ar-H), 8.73, 9.08 (2H, brs, D$_2$O exchangeable, NNHCSNH$_2$), 12.39 (1H, brs, D$_2$O exchangeable, NNHCSNH$_2$), 14.92 (1H, brs, D$_2$O exchangeable, COOH). FAB-MS: *m/z* 594 (M + H)$^+$. HRMS-FAB: *m/z* Calcd. for $C_{28}H_{29}FN_7O_5S$ (M + H)$^+$: 594.1935; Found 594.1934.

*4.2.3. N-(2-Bromoethyl)isatin (**4a**)*

A suspension of isatin (**1a**, 4.41 g, 30 mmol), anhydrous K_2CO_3 (12.51 g, 90 mmol) and 1,2-dibromoethane (16.83 g, 90 mmol) in DMF (100 mL) was stirred at room temperature for 24 h and filtered. The filtrate was concentrated under reduced pressure. The residue was poured into water (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined extracts were washed with saturated brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (*v*: *v* = 5: 1) to give the title compound **4a** (5.19 g, 68%) as anacaratsolid, mp: 126–128 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_{ppm}: 3.62 (2H, t, *J* = 6.8 Hz, CH$_2$CH$_2$Br), 4.15 (2H, t, *J* = 6.8 Hz, CH$_2$CH$_2$Br), 7.00 (1H, d, *J* = 7.6 Hz, Ar-H), 7.15 (1H, t, *J* = 7.6 Hz, Ar-H), 7.60–7.65 (2H, m, Ar-H). ESI-MS: *m/z* 254 (M + H)$^+$, 256 (M+2 + H)$^+$.

*4.2.3. N-(2-Bromoethyl)-5-fluoroisatin (**4b**).* The title compound was obtained in a similar manner as for the preparation of **4a** (53%) as anacaratsolid, mp: 109–110 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_{ppm}: 3.62 (2H, t, *J* = 6.4 Hz, CH$_2$CH$_2$Br), 4.11 (2H, t, *J* = 6.4 Hz, CH$_2$CH$_2$Br), 6.99 (1H, m, Ar-H), 7.26–7.35 (2H, m, Ar-H). ESI-MS: *m/z* 272 (M + H)$^+$, 274 (M+2 + H)$^+$.

*4.2.4. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[4-(2-isatinylethyl) piperazin-1-yl]-1,4- dihydro-4-oxoquinoline-3-carboxylic acid (**5a**)*

A suspension of **4a** (5.08 g, 20 mmol), 8-OCH$_3$ CPFX (7.22 g, 20 mmol), anhydrous K_2CO_3 (8.34 g, 60 mmol) in DMF (200 mL) was stirred at 40 °C for 27.5 h and concentrated under reduced pressure. To the residue was added water (300 mL), stirred at room temperature for 0.5 h and then filtered. The solid obtained was dissolved in 4 N HCl (150 mL), and washed with CH_2Cl_2 (3 × 100 mL). The pH of the aqueous layer was adjusted to 7.0 with 10% NaOH solution and filtered. The solid was washed with water and purified by column chromatography (silica gel) eluted with CH_2Cl_2 and MeOH (*v*: *v* = 20: 1) to give the title compound **5a** (4.81 g, 45%) as anacaratsolid, mp: 165–167 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_{ppm}: 0.91 (2H, m, cyclopropyl-H), 1.15 (2H, m, cyclopropyl-H), 3.40 (4H, s, piperazine-4H), 3.70 (4H, s, piperazine-4H), 3.82 (3H, s, OCH$_3$), 3.90 (1H, m, cyclopropyl-H), 4.15 (2H, t, *J* = 5.2 Hz, CH$_2$ of linker), 4.56 (2H, t, *J* = 5.2 Hz, CH$_2$ of linker), 7.09–8.53 (6H, m, Ar-H). ESI-MS: *m/z* 535 (M + H)$^+$. HRMS-ESI: *m/z* Calcd. for $C_{28}H_{28}FN_4O_6$ (M + H)$^+$: 535.1992; Found 535.2028.

*4.2.4. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(5-fluoroisatinyl) ethyl]piperazin- 1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5b**).* The title compound was obtained in a similar manner as for the preparation of **5a** (34%) as anacaratsolid, mp: 225–227 °C. ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ_{ppm}: 0.87 (2H, m, cyclopropyl-H), 0.98–1.01 (2H, m, cyclopropyl-H), 2.84 (4H, s, piperazine-4H), 3.19 (4H, s, piperazine-4H), 3.76 (3H, s, OCH$_3$), 3.93 (1H, m, cyclopropyl-H), 4.07 (2H, t, *J* = 5.2 Hz, CH$_2$ of linker), 4.36 (2H, t, *J* = 5.2 Hz, CH$_2$ of linker), 7.28–8.32 (5H, m, Ar-H). ESI-MS: *m/z* 553 (M + H)$^+$. HRMS-ESI: *m/z* Calcd. for $C_{28}H_{27}F_2N_4O_6$ (M + H)$^+$: 553.1898; Found 553.1924.

*4.2.5. General procedure for the preparation of compounds **5c–l***

To a solution of substituted amine hydrochloride (3 mmol) and $NaHCO_3$ (0.25 g, 3 mmol) dissolved in water (10 mL) was added dropwise a solution of **5a**, **b** (1 mmol) in MeOH (10 mL) at room temperature over 5 min. The reaction mixture was stirred at the same temperature for 24 h. After removal of the MeOH under reduced pressure, the reaction mixture was diluted with water (20 mL) and stirred for 10 min, and then filtered. The crude product was purified by column chromatography (silica gel) eluted with CH_2Cl_2 and MeOH (*v*: *v* = 20: 1) to give the title compounds **5c–l** (48–77%).

*4.2.5.11-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(β-hydroximinoisatinyl) ethyl]piperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5c**).* Yield: 66%. mp: 220–225 °C. ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ_{ppm}: 0.89 (2H, m, cyclopropyl-H), 1.01–1.06 (2H, m, cyclopropyl-H), 3.23 (4H, s, piperazine-4H), 3.46 (4H, s, piperazine-4H), 3.78 (3H, s, OCH$_3$), 3.94 (1H, m, cyclopropyl-H), 4.07 (2H, t, *J* = 5.2 Hz, CH$_2$ of linker), 4.37 (2H, t, *J* = 5.2 Hz, CH$_2$ of linker), 7.06–8.36 (6H, m, Ar-H), 9.14 (1H, brs, D$_2$O exchangeable, NOH), 13.46 (1H, brs, D$_2$O exchangeable, COOH). ESI-MS: *m/z* 550 (M + H)$^+$. HRMS-ESI: *m/z* Calcd. for $C_{28}H_{29}FN_5O_6$ (M + H)$^+$: 550.2101; Found 550.2133.

*4.2.5.2. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(5-fluorinisatinyl-β-hydroximino) ethyl]piperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5d**).* Yield: 60%. mp: 189–192 °C. ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ_{ppm}: 0.85 (2H, m, cyclopropyl-H), 1.00 (2H, m, cyclopropyl-H), 2.49 (4H, s, piperazine-4H), 3.19 (4H, s, piperazine-4H), 3.75 (3H, s, OCH$_3$), 3.93 (1H, m, cyclopropyl-H), 4.07 (2H, t, *J* = 4.8 Hz, CH$_2$ of linker), 4.36 (2H, t, *J* = 4.8 Hz, CH$_2$ of linker), 7.27–8.32 (5H, m, Ar-H). ESI-MS: *m/z* 568 (M + H)$^+$. HRMS-ESI: *m/z* Calcd. for $C_{28}H_{28}F_2N_5O_6$ (M + H)$^+$: 568.2007; Found 568.2030.

*4.2.5.3. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(β-methoxyiminoisatinyl) ethyl]piperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5e**).* Yield: 71%. mp: 156–159 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_{ppm}: 0.88 (2H, m, cyclopropyl-H), 1.13 (2H, m, cyclopropyl-H), 3.36 (4H, s, piperazine-4H), 3.67 (4H, s, piperazine-4H), 3.81 (3H, s, OCH$_3$), 3.88 (1H, m, cyclopropyl-H), 4.15 (2H, t, *J* = 5.2 Hz, CH$_2$ of linker), 4.29 (3H, s, NOCH$_3$), 4.53 (2H, t, *J* = 5.2 Hz, CH$_2$ of linker), 7.03–8.51 (6H, m, Ar-H). ESI-MS: *m/z* 564 (M + H)$^+$. HRMS-ESI: *m/z* Calcd. for $C_{29}H_{31}FN_5O_6$ (M + H)$^+$: 564.2258; Found 564.2239.

*4.2.5.4. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(5-fluorinisatinyl-β- methoxyimino)ethyl]piperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5f**).* Yield: 65%. mp: 131–132 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_{ppm}: 0.87 (2H, m, cyclopropyl-H), 1.01 (2H, m, cyclopropyl-H), 2.94 (4H, s, piperazine-4H), 3.27 (4H, s,

piperazine-4H), 3.76 (3H, s, OCH_3), 3.94 (1H, m, cyclopropyl-H), 4.06 (2H, t, $J = 4.8$ Hz, CH_2 of linker), 4.21 (3H, s, $NOCH_3$), 4.35 (2H, t, $J = 4.8$ Hz, CH_2 of linker), 7.32–8.32 (5H, m, Ar-H). ESI-MS: *m/z* 582 $(M + H)^+$. HRMS-ESI: *m/z* Calcd. for $C_{29}H_{30}F_2N_5O_6$ $(M + H)^+$: 582.2164; Found 582.2148.

*4.2.5.5. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(β-ethoxyiminoisatinyl) ethyl]piperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5g**).* Yield: 75%. mp: 161–165 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 0.87 (2H, m, cyclopropyl-H), 0.99–1.02 (2H, m, cyclopropyl-H), 1.36 (3H, t, $J = 7.2$ Hz, $NOCH_2\underline{CH_3}$), 3.07 (4H, s, piperazine-4H), 3.36 (4H, s, piperazine-4H), 3.78 (3H, s, OCH_3), 3.94 (1H, m, cyclopropyl-H), 4.07 (2H, t, $J = 4.8$ Hz, CH_2 of linker), 4.36 (2H, t, $J = 4.8$ Hz, CH_2 of linker), 4.43 (2H, q, $J = 7.2$ Hz, $NO\underline{CH_2}CH_3$), 7.06–8.33 (6H, m, Ar-H). ESI-MS: *m/z* 578 $(M + H)^+$. HRMS-ESI: *m/z* Calcd. for $C_{30}H_{33}FN_5O_6$ $(M + H)^+$: 578.2414; Found 578.2391.

*4.2.5.6. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(5-fluorinisatinyl-β-ethoxyimino) ethyl]piperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5h**).* Yield: 61%. mp: 162–164 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_{ppm}: 0.90 (2H, m, cyclopropyl-H), 1.13–1.17 (2H, m, cyclopropyl-H), 1.45 (3H, t, $J = 7.2$ Hz, $NOCH_2\underline{CH_3}$), 3.45 (4H, s, piperazine-4H), 3.73 (4H, s, piperazine-4H), 3.82 (3H, s, OCH_3), 3.89 (1H, m, cyclopropyl-H), 4.15 (2H, t, $J = 4.8$ Hz, CH_2 of linker), 4.53–4.58 (4H, m, CH_2 of linker, $NO\underline{CH_2}CH_3$), 7.07–8.52 (5H, m, Ar-H). ESI-MS: *m/z* 596 $(M + H)^+$. HRMS-ESI: *m/z* Calcd. for $C_{30}H_{32}F_2N_5O_6$ $(M + H)^+$: 596.2320; Found 596.2309.

*4.2.5.7. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(β-semicarbazoisatinyl) ethyl]piperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5i**).* Yield: 59%. mp: 220–225 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 0.86 (2H, m, cyclopropyl-H), 1.01 (2H, m, cyclopropyl-H), 3.20 (4H, s, piperazine-4H), 3.45 (4H, s, piperazine-4H), 3.78 (3H, s, OCH_3), 3.94 (1H, m, cyclopropyl-H), 4.09 (2H, s, CH_2 of linker), 4.37 (2H, s, CH_2 of linker), 6.98 (2H, brs, D_2O exchangeable, $NNHCO\underline{NH_2}$), 7.06–8.33 (6H, m, Ar-H), 9.58 (2H, brs, D_2O exchangeable, $N\underline{NH}CONH_2$, COOH). ESI-MS: *m/z* 592 $(M + H)^+$. HRMS-ESI: *m/z* Calcd. for $C_{29}H_{31}FN_7O_6$ $(M + H)^+$: 592.2319; Found 592.2344.

*4.2.5.8. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(5-fluorinisatinyl-β-semicarbazo) ethyl]piperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5j**).* Yield: 53%. mp: 189–190 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 0.79–0.82 (2H, m, cyclopropyl-H), 1.00 (2H, m, cyclopropyl-H), 2.88 (4H, s, piperazine-4H), 3.33 (4H, s, piperazine-4H), 3.74 (3H, s, OCH_3), 3.93 (1H, m, cyclopropyl-H), 4.10 (2H, t, $J = 4.8$ Hz, CH_2 of linker), 4.36 (2H, t, $J = 4.8$ Hz, CH_2 of linker), 5.78 (2H, brs, D_2O exchangeable, $NNHCO\underline{NH_2}$), 6.88 (1H, brs, D_2O exchangeable, $N\underline{NH}CONH_2$), 7.17–8.31 (5H, m, Ar-H), FAB-MS: *m/z* 610 $(M + H)^+$. HRMS-FAB: *m/z* Calcd. for $C_{29}H_{30}F_2N_7O_6$ $(M + H)^+$: 610.2226; Found 610.2246.

*4.2.5.9. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(β-thiosemicarbazoisatinyl) ethyl]piperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5k**).* Yield: 48%. mp: 160–165 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 0.85–0.89 (2H, m, cyclopropyl-H), 1.03–1.08 (2H, m, cyclopropyl-H), 2.88 (4H, s, piperazine-4H), 3.21 (4H, s, piperazine-4H), 3.75 (3H, s, OCH_3), 3.99 (1H, m, cyclopropyl-H), 4.13 (2H, t, $J = 4.8$ Hz, CH_2 of linker), 4.38 (2H, t, $J = 4.8$ Hz, CH_2 of linker), 7.13–8.39 (6H, m, Ar-H), 8.73, 9.06 (2H, 2s, D_2O exchangeable, $CSNH_2$). ESI-MS: *m/z* 608 $(M + H)^+$. HRMS-ESI: *m/z* Calcd. for $C_{29}H_{31}FN_7O_5S$ $(M + H)^+$: 608.2091; Found 608.2078.

*4.2.5.10. 1-Cyclopropyl-6-fluoro-8-methoxy-7-{4-[2-(5-fluorinisatinyl-β- thiosemicarbazo)ethyl]piperazin-1-yl}-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (**5l**).* Yield: 64%. mp: 218–220 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ_{ppm}: 0.88 (2H, m, cyclopropyl-H), 1.06 (2H, m, cyclopropyl-H), 3.02 (4H, s, piperazine-4H), 3.31 (4H, s, piperazine-4H), 3.78 (3H, s, OCH_3), 3.99 (1H, m, cyclopropyl-H), 4.12 (2H, t, $J = 4.8$ Hz, CH_2 of linker), 4.36 (2H, t, $J = 4.8$ Hz, CH_2 of linker), 7.26–8.38 (5H, m, Ar-H), 8.81, 9.14 (2H, 2s, D_2O exchangeable, $CSNH_2$). FAB-MS: *m/z* 626 $(M + H)^+$. HRMS-FAB: *m/z* Calcd. for $C_{29}H_{30}F_2N_7O_5S$ $(M + H)^+$: 626.1997; Found 626.2057.

4.3. MIC determination

All of the target compounds **3a**–**k**, **5a**–**l** along with 8-OCH_3 CPFX, CPFX, RIF and INH were initially screened for their *in vitro* activity against *M. smegmatis* CMCC 93202 using serial double dilution technique in duplicate [20]. Bacteria were incubated in 20 mL L-broth at 37 °C for 48 h. The culture was diluted to 150 μL, 5×10^5 colony-forming units per well in the 96-well microplates. The tested compounds were dissolved in DMSO and two-fold diluted at the final concentrations of 200, 100, 50, ……, 0.1 μg/mL, and then mixed with the culture. The plates were incubated at 37 °C for 72 h. The MIC was defined as the minimum concentration at which the culture in the well was clear.

Compounds **3a**–**k**, **5e**, **5f**, **5k**, **5l** along with 8-OCH_3 CPFX, CPFX, RIF and INH were chosen for further evaluation of their *in vitro* activity against MTB H37Rv ATCC 27294, and then compounds **3a**–**g** and **3i**–**k** against MDR-MTB 09710 using rapid direct susceptibility test technique [20]. All the tested compounds were two-fold diluted with the two-fold concentration medium (2 × medium, Advanced Middlebrook 7H9 broth) at the final concentrations of 1.28, 0.64, 0.32, ……, 0.005 μg/ml (for MTB H37Rv ATCC 27294) or 256, 128, 64, ……, 1 μg/ml (for MDR-MTB 09710). The wells of a sterile 48-well plate were filled with 100 μL two-fold diluted tested compounds and 100 μL MTB H37Rv ATCC 27294 or MDR-MTB 09710 suspension containing 4×10^{-3} mg cells. Pure medium replaced the diluted compounds in two wells as the positive control of growth, and deionized water instead of the culture in other two wells as the negative control of growth in the plates. The plates were covered and sealed, then incubated at 37 °C in a wet box. The positive and negative control wells should show obvious difference after 3 days. The MIC was determined by observing the quantity and state of the cells in each test well by a continuous visual high magnification system (Nanjing Shengguang Photoelectricity Medical Device Co., Ltd), and redetermined 7 days later. The MIC is defined as the concentration of the compound required to give complete inhibition of bacterial growth. The two strains were obtained from Jiangsu Province Hospital, Nanjing, China.

4.4. Cytotoxicity

Compounds **3a**–**k**, **5e**, **5f**, **5k**, **5l** were further examined for toxicity (CC_{50}) in a mammalian Vero cell line at concentrations from 1000 to 4 μg/mL. The Vero cells were maintained in culture medium (Minimum Essential Medium with Earle's salt, supplemented with 10% fetal bovine serum) at 37 °C under 5% CO_2. Cells were seeded in 96-well plates at the plating density of 1×10^4 cells per well and allowed to recover for 24 h. Culture medium was replaced by assay medium containing the compound to be tested or drug-free. After 72 h of exposure, cells were harvested and cell viability was assessed by MTT assay. The CC_{50} values were calculated by Bliss analyses.

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