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Original article

Synthesis and antimycobacterial activity of a series of ferrocenyl derivatives

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ABSTRACT

In this work we reported the synthesis and evaluation of *Mycobacterium tuberculosis* activities *in vitro* of a series of twenty five ferrocenyl derivatives: ferrocenyl amides derived from nicotinamide and pyrazinamide, ferrocenyl pyridinyl, quinolyl and acridinylhydrazones. In particular ferrocenyl acylhydrazones **7** and **8** and ferrocenylquinoxaline amide **57** showed interesting antimycobacterial activities.

1. Introduction

Tuberculosis (TB) represents a highly contagious, airborne disease that is caused by infection with *Mycobacterium tuberculosis* (Mtb) and is the leading cause of infectious disease mortality in the world. World health organization (WHO) estimates about 8 million new active cases of tuberculosis per year and nearly 2 million deaths each year. Even though improved methods of prevention, detection, diagnosis and treatment have greatly reduced the number of people who contract the disease and die from it, the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis resulted in a major setback in the global fight against TB [1,2]. Currently, TB chemotherapy is made up of a cocktail of first-line drugs, isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (EMB), given for six months. This is a major barrier to full patient compliance and has contributed to the development of drug-resistant strains [3]. The increasing problem of MDR-TB and XDR-TB has focused attention on developing new drugs that are not only active against drug-resistant TB, but also shorten the lengthy therapy [4–6]. There is urgent need and significant interest in developing new TB drugs.

Over the past few years, bioorganometallic chemistry has developed as a rapidly growing and maturing area which links classical organometallic chemistry to biology, medicine, and molecular biotechnology [7]. Among metallocenes, ferrocene has attracted special attention since it is a neutral, chemically stable and nontoxic molecule. Many ferrocenyl compounds display interesting cytotoxic [8,9], antimalarial [10–12], antifungal [13], antitoxoplasmic [14] and DNA-cleaving activity [15].

In spite of toxicity, isoniazid (INH) is still considered to be first-line drug for chemotherapy of tuberculosis. Various isonicotinoylhydrazones have been synthesized because of the development of isoniazid-resistant *M. tuberculosis* strains [16–18]. More recently, the antitubercular activity of quinolylhydrazones has been also reported [19–22]. In previous studies, we reported the synthesis of ferrocenyl diamines and the evaluation of their antimycobacterial activity. These novel derivatives have been based on the modification of ethambutol by incorporating ferrocenyl moiety [23]. To pursue this goal, our effort has been focused on ferrocenyl pyridinyl and quinolylhydrazones. A second series of the ferrocenyl amides derived from nicotinamide and pyrazinamide has also been presented.

2. Chemistry

2.1. Synthesis of ferrocenyl hydrazones

The ferrocenyl acylhydrazones **7**–**11** were synthesized according to the reported procedure (Scheme 1). The hydrazides **1**–**4** were

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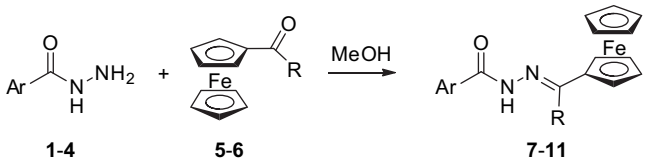
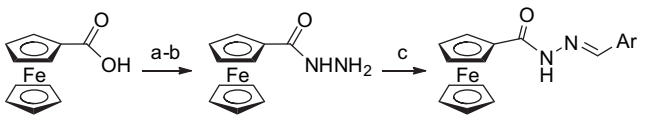


Ar = 4-pyridinyl, R = H **7**
Ar = 4-pyridinyl, R = CH_3 **8**
Ar = 3-pyridinyl, R = H **9**
Ar = 2-aminobenzyl, R = H **10**
Ar = 4-aminobenzyl, R = H **11**

Scheme 1. Synthesis of ferrocenyl hydrazones **7**–**11**.



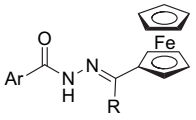
Ar = 4-pyridinyl **14**
Ar = 3-pyridinyl **15**
Ar = 2-aminobenzyl **16**

Scheme 2. Synthesis of ferrocenyl hydrazones **14**–**16**: (a) NH_2NHBoc, EDCI, rt; 24 h (b) trifluoroacetic acid, rt, 1 h; (c) ArCHO, rt, 4 h.

reacted with equimolar amounts of commercially ferrocene carboxaldehyde **5** or acetylferrocene **6** to give **7**–**11** in 52–93% yields (Scheme 1, Table 1) [24–26].

The ferrocenyl hydrazones **14**–**16** were obtained in three steps. First, ferrocene hydrazide **13** was obtained by condensation of ferrocene carboxylic acid with *tert*-butyl hydrazinecarboxylate in the presence of EDCI followed by deprotection of the Boc group by trifluoroacetic acid in 65 % global yield [27]. The ferrocenyl hydrazide was reacted with pyridine-4-carboxaldehyde, pyridine-3-carboxaldehyde and ortho-aminobenzaldehyde to give respectively hydrazones **14**–**16** in 37–80 % yields (Scheme 2).

Table 1
Structure, yield and antimycobacterial *in vitro* activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and three clinical isolates of ferrocenyl acylhydrazones **7**–**11**.



7-11

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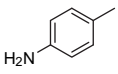
Compound	Ar	R	Yield (%)	MIC $H_{37}Rv$ (μmol/L)	Clinical isolates V	Clinical isolates M	Clinical isolates G
7		H	93	0.75	0.15	12	48
8		CH_3	92	0.72	2.88	11.5	>46
9		H	83	>384	–	–	–
10		H	89	368	–	–	–
11		H	52	184	–	–	–
EMB	–	–	–	9.8	–	–	–
INH	–	–	–	<0.43	0.87	3.6	29

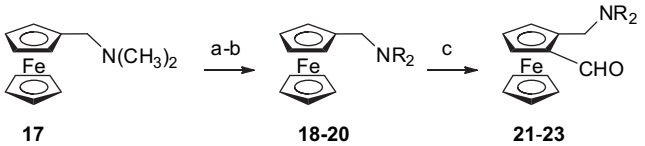
The ferrocenyl amino aldehydes were then obtained in three steps. First, *N*,*N*-dimethylaminomethylferrocene **17** was reacted in acetonitrile with methyl iodide giving the corresponding ammonium. Then, a substitution of the ammonium group with pyrroline, piperidine and morpholine in acetonitrile in the presence of potassium carbonate provided respectively the ferrocenyl amines **18**, **19** and **20** in 57–98% overall yields [28]. The ortholithiation of ferrocenyl amines **18**–**20** by *tert*butyllithium followed by addition of DMF led to aldehydes **21**–**23** in 81–98 % yields (Scheme 3). The hydrazines **24** and **25** were condensed with ferrocene carboxaldehyde, 2-(*N*,*N*-dimethylaminomethylferrocene)carboxaldehyde and ferrocenyl aldehydes **21**–**23** to furnish hydrazones **26**–**35** in 61–98% yields (Scheme 4, Table 2).

2.2. Synthesis of ferrocenyl amides

The synthesis of ferrocenyl amides and esters was achieved by a reaction between aryl carboxylic acid and ferrocenyl amines or alcohols.

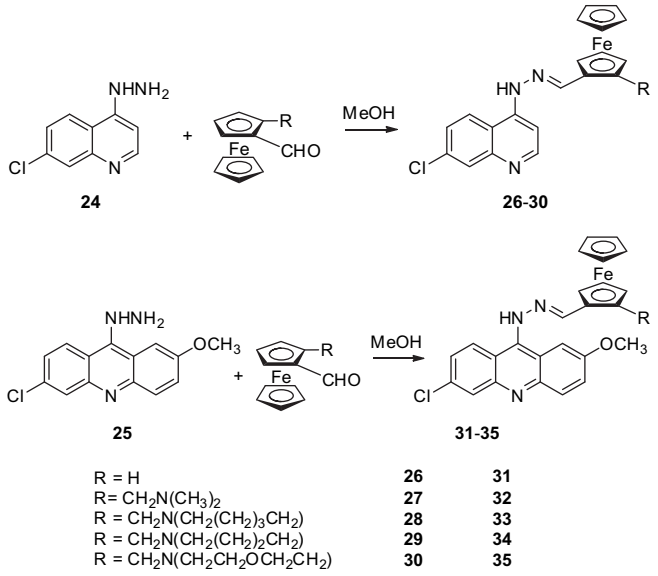
Following the procedure of the literature, ferrocenylmethylamine **37** was first synthesized in 93% global yield by condensation of hydroxylamine on ferrocene carboxaldehyde **36** followed by a reduction with $LiAlH_4$ [14,29]. The ferrocenyl derivative **39** were then prepared with 60% yield by condensation of a large excess of piperazine with the ferrocenyl ammonium **38** in presence of potassium carbonate in CH_3CN (Scheme 5) [10,30].

Esterification of 2-pyrazinecarboxylic acid, 2-quinoxalic acid and 3-nicotinic acid with ferrocenyl methanol in the presence of DCC/DMAP gave esters **43**–**45** in 70–96 % yield (Scheme 6). The ferrocenyl amides **54**–**60** were obtained by condensation of aryl aromatic carboxylic acid with amines **37** and **39** in presence of bromo-tris-pyrrolidinophosphonium hexafluorophosphate (PyBroP) or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI). Compounds **54**–**60** were obtained in 31 to 68 % yields (Scheme 7). Yields and chemical structures have been reported in Table 3.



R = $CH_2(CH_2)_3CH_2$ **18** **21**
R = $CH_2(CH_2)_2CH_2$ **19** **22**
R = $CH_2CH_2OCH_2CH_2$ **20** **23**

Scheme 3. Synthesis of ferrocenyl aldehydes **18**–**20**: (a) lCH_3, MeCN, rt; 1 h (b) secondary amine, K_2CO_3, MeCN; reflux, 12 h (c) *tert*-BuLi, rt, 1 h then DMF.



Scheme 4. Synthesis of ferrocenyl hydrazones **26**–**30** and **31**–**35**.

3. Biological activity

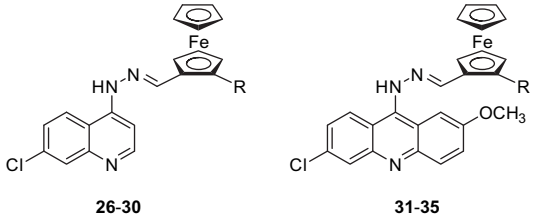
The antimycobacterial *in vitro* activity of ferrocenyl derivatives for tuberculosis inhibition against *M. tuberculosis* $H_{37}Rv$ strain was carried out using the Mycobacteria Growth Indicator Tube system (MGIT) at a concentration of 2 μg/mL. The minimum inhibitory concentration (MIC, μmol/L) was detected by BACTEC 960. The MIC, defined at the lowest concentration of compound inhibiting 90% of the inoculum relative controls, is summarized in Tables 1–3. Isoniazid (INH), ethambutol (EMB) and pyrazinamide (PZA) were included as standard drugs, for comparison.

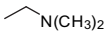
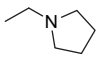
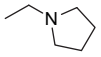
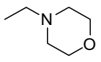
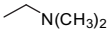
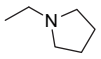
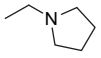
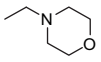
Hydrazone **7**–**11** derived from isoniazid, nicotinohydrazide and anilinohydrazide exhibit modest to promising anti-TB activity (Table 1). For example, analogs **7** and **8** were found to be the most potent compounds (MIC 0.75 μM). Subsequently, these ferrocenyl acylhydrazones were evaluated against clinical isolates with various degrees of resistance. However, when compared with isoniazid, compounds **7** and **8** were less potent. Contrary to the results of the literature with hydrazones of isoniazid derived from benzaldehyde or acetophenone, the replacement of a hydrogen (**7**) by a methyl group (**8**) does not modify the biological activities [31]. Surprisingly, hydrazone derived from nicotinohydrazide exhibits no activity with MIC superior to 384 μM. It is also to be mentioned that the position of the aromatic group (pyridine or anilinophenyl) with regard to the acylhydrazone is essential for the anti-TB activity. Indeed, compounds **14**–**16** do not have any biological activity.

In the second series (Table 2), the most active 4-quinolylhydrazone and 1-acridinylhydrazone derived from ferrocene carboxaldehyde were **26** and **31** with MIC of 20.5 and 17 μM respectively. The presence of a substituent on the ferrocene moiety such as amino groups in **27**–**30** and **32**–**35** could be responsible for a light or significant decrease of the biological activity. In general, a better activity has been obtained from acridinylhydrazones. Moreover, the influence of substituents on the ferrocenyl moiety seems to be different between the quinolyl and acridinyl derivatives.

The Table 3 summarizes biological activities obtained for ferrocenyl esters **43**–**45** and amides **54**–**60**. The MICs were determined at pH 6 with Bactec MGIT 960 PZA. When compared to pyrazinamide (MIC 64 μg/mL or 520 μM), compounds **55**, **56** and **59** were a little more active (MIC 120–172 μM). Contrary to the literature results [32,33], structural modifications of the pyrazine nucleus and the presence of ester functionality have failed to increase the biological activity compared to pyrazinamide. Only compound **57** (MIC 86 μM) with a quinoxaline nucleus presented a higher activity compared to PZA.

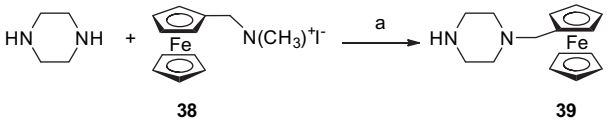
Table 2
Structure, yield and antimycobacterial *in vitro* activity against *Mycobacterium tuberculosis* $H_{37}Rv$ of ferrocenyl hydrazones **26**–**35**.



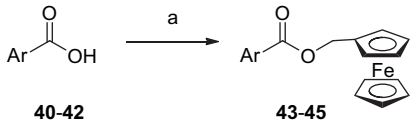
Compound	R	Yield (%)	MIC $H_{37}Rv$ (μmol/L)
26	H	89	20.5
27		61	28.6
28		91	67.7
29		95	263
30		74	131
31	H	90	17
32		80	242.2
33		94	23.1
34		64	56.5
35		94	22.5
EMB	–	–	9.8
INH	–	–	>0.43

4. Conclusion

In summary, a series of novel ferrocenyl derivatives has been synthesized. We explored the antimycobacterial activity for these



Scheme 5. Synthesis of ferrocenyl amines **37** and **39:** (a) K_2CO_3, MeCN, 60 °C, 12 h.



40-42 **43-45**

Ar = 2-pyrazine	**40**	**43**
Ar= 2-quinoxaline	**41**	**44**
Ar = 3-nicotine	**42**	**45**

Scheme 6. Synthesis of ferrocenyl esters **43**–**45:** (a) $FcCH_2OH$, DCC, DMAP, CH_2Cl_2, rt, 12 h.

compounds against *M. tuberculosis* $H_{37}Rv$ and MIC values have been reported. Acylhydrazones **7** and **8** and quinoxaline amide **57** showed interesting antimycobacterial activities. Thus, the quinolinyl and acridinylhydrazones are suited for further modifications to obtain more efficacious and potent antitubercular drugs. Similarly, the synthesis of ferrocenyl amides bearing varied substituents on the ferrocenyl entity is currently underway in our laboratory. The biological activity of selected compounds will be also evaluated against clinical isolates of MDR-TB. Further study of the structure-activity relationship will allow estimating the relative importance of the ferrocene on antitubercular drugs.

5. Experimental

5.1. General methods

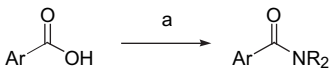
The ^1H and ^{13}C NMR spectra were recorded on a Bruker AC300 spectrometer using tetramethylsilane (TMS) as the internal standard and $CDCl_3$, MeOH-d_4 or DMSO-d_6 as the solvents. MS-MALDI TOF spectra were obtained using a Vision 2000 time-of-flight instrument (Finnigan MAT, Bremen, Germany) equipped with a nitrogen laser operating at wavelength of 337 nm. The matrix used was trihydroxyacetophenone (thap). HRMS were performed on a JEOL JMS-700m Station mass spectrometer. Thin layer chromatography (TLC) was carried out on aluminium-baked Macherey-Nagel silica gel 60. Column chromatography was performed on silica gel (35–70 mesh). Melting points were determined on a Kofler apparatus and are uncorrected. Elemental analyses were performed with a varioMICRO analyser.

5.2. General procedure for the synthesis of hydrazones 7–11

A mixture of the appropriate hydrazine (1 equiv) and ferrocenyl aldehyde (1 equiv) in methanol was left stirring at room temperature in the presence of molecular sieves for 4 h. After filtration, the solvent was evaporated. The residue was purified by column chromatography (eluent:petroleum ether/diethyl ether: 5/5 then diethyl ether/MeOH: 9/1).

5.2.1. N'-(Ferrocenylmethylene)isonicotinohydrazide 7

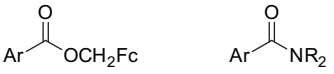
Orange solid. Yield 93%. M.p. 234 °C. ^1H NMR (CD_3OD) δ 8.73 (d, 2H, J = 5.8 Hz), 8.24 (s, 1H), 7.88 (d, 2H, J = 5.9 Hz), 4.79 (m, 2H), 4.49 (m, 2H), 4.2 (s, 5H). ^{13}C NMR (CD_3OD) δ 163.8, 150.5, 150.2, 140.7, 121.5, 78.4, 70.4, 69.0, 67.7. MS (m/z): 333.19 (M^+). Anal. Calcd



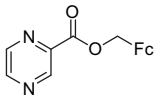
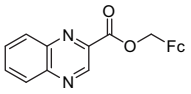
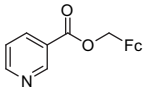
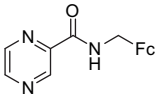
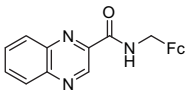
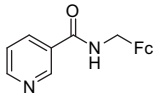
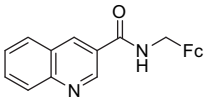
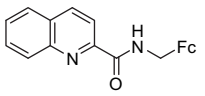
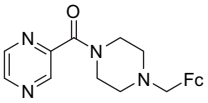
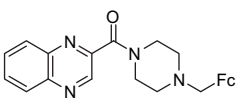
46-53 **54-60**

Scheme 7. Synthesis of ferrocenyl amides **54**–**60:** (a) Ferrocenyl amines **37**or **39**, PyBroP, DIEA, rt for **54**-**55** or EDCI, CH_2Cl_2, rt for **56**–**60**.

Table 3
Structure, yield and antimycobacterial *in vitro* activity against *Mycobacterium tuberculosis* $H_{37}Rv$ of ferrocenyl esters **43**–**45** and amides **54**–**60**.



43-45 **54-61**

Compound	Structure	Yield (%)	MIC $H_{37}Rv$ (μmol/L)
43		79	>397
44		96	344
45		70	399
54		35	398
55		68	172
56		63	120
57		59	86
58		31	>346
59		45	164
60		31	291
PZA	–	–	520

for $C_{17}H_{15}FeN_3O$: C, 61.29; H, 4.54; N, 12.61. Found: C, 61.52; H, 4.65; N, 12.70.

5.2.2. N'-(1-Ferrocenylethylidene)isonicotinohydrazide **8**

Orange solid. Yield 92%. M.p. 183 °C. ^{1}H NMR (DMSO-d_6) δ 10.93 (s, 1H), 8.82 (d, 2H, J = 5.4 Hz), 7.86 (d, 2H, J = 5.4 Hz), 4.78 (m, 2H), 4.49 (m, 2H), 4.29 (s, 5H), 2.30 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 162.1, 161.2, 150.0, 141.4, 121.7, 82.3, 70.1, 69.2, 67.4, 16.0. MS (m/z): 347.20 (M^+). Anal. Calcd for $C_{18}H_{17}FeN_3O$: C, 62.27; H, 4.94; N, 12.10. Found: C, 62.54; H, 4.92; N, 12.20.

5.2.3. N'-(Ferrocenylmethylene)nicotinohydrazide **9**

Red solid. Yield 83%. M.p. 212 °C. ^{1}H NMR (DMSO-d_6) δ 11.74 (s, 1H), 9.06 (s, 1H), 8.75 (d, 1H, J = 3.5 Hz), 8.31 (s, 1H), 8.25 (d, 1H, J = 7.6 Hz), 7.56 (t, 1H, J = 4.7 Hz), 4.68 (m, 2H), 4.47 (m, 2H), 4.25 (s, 5H). ^{13}C NMR (DMSO-d_6) δ 161.4, 152.5, 150.3, 148.9, 135.7, 129.9, 124.1, 79.2, 71.0, 69.4, 67.9. MS (m/z): 333.17 (M^+). Anal. Calcd for $C_{17}H_{15}FeN_3O$: C, 61.29; H, 4.54; N, 12.61. Found: C, 61.11; H, 4.32; N, 12.81.

5.2.4. 2-Amino-N'-(ferrocenylmethylene)benzohydrazide **10**

Red crystals. Yield 89%. M.p. 159–160 °C. ^{1}H NMR ($CDCl_3$) δ 8.98 (s, 1H), 8.05 (s, 1H), 7.44 (d, 1H, J = 7.6 Hz), 7.26 (m, 1H), 6.7 (m, 2H), 5.5 (br, s), 4.70 (m, 2H), 4.41 (m 2H), 4.22 (s, 5H). ^{13}C NMR (DMSO-d_6) δ 165.3, 150.5, 140.4, 132.1, 128.3, 117.1, 115.6, 113.2, 79.4, 70.0, 69.5, 68.5. HRMS, calcd for $C_{18}H_{17}FeN_3O$: 347.0721 [M^+]; found 347.0730.

5.2.5. 4-Amino-N'-(ferrocenylmethylene)benzohydrazide **11**

Brown solid. Yield 52%. M.p. 163–164 ^{1}H NMR ($CDCl_3$) δ 9.88 (br, 1H), 7.70 (s, 1H), 7.64 (d, 2H, J = 3.8 Hz), 7.49 (d, 2H, J = 3.9 Hz), 4.73 (s, 2H), 4.54 (m, 2H), 4.21 (s, 5H), 4.19 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 167.7, 162.1, 150.8, 131.0, 128.9, 120.5, 69.7, 69.4, 69.2. HRMS, calcd for $C_{18}H_{17}FeN_3O$: 347.0721 [M^+]; found 347.0718.

5.3. Ferrocenylcarbohydrazine **13**

To a solution of ferrocene carboxylic acid **12** (346 mg, 1.5 mmol) in dichloromethane (40 mL) were added *tert*-butylcarbazate (204 mg, 1.5 mmol) and EDCI (295 mg, 1.5 mmol). After stirring for 24 h at room temperature, the solution was hydrolyzed by water (20 mL) and extracted with dichloromethane (3 × 15 mL). The organic layers were dried over $MgSO_4$ and evaporated under vacuum. The residue was purified by column chromatography (eluent:CH_2Cl_2/ethyl acetate: 6/4) to give ferrocenyl-(*tert*butyloxy) carbohydrazine (273 mg, 84%) as orange solid. ^{1}H NMR ($CDCl_3$) δ 7.64 (s, 1H), 6.63 (s, 1H), 4.74 (t, 2H, J = 2 Hz), 4.38 (t, 2H, J = 1.9 Hz), 4.29 (s, 5H), 1.52 (s, 9H). To a solution of ferrocenyl-(*tert*butyloxy)carbohydrazine (0.173 g, 0.488 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (1 mL) at 0 °C. After stirring for 1 h at 20 °C, the mixture is neutralized by a saturated Na_2CO_3 solution and extracted with CH_2Cl_2 (3 × 15 mL). The organic layers were dried over $MgSO_4$ and evaporated under vacuum. The residue was purified by column chromatography (eluent: CH_2Cl_2/ethyl acetate/TEA:9/1/1) to give ferrocenylcarbohydrazine **13** (78 mg, 66%) as orange solid. M.p. 174 °C. ^{1}H NMR ($CDCl_3$) δ 6.96 (1H, s); 4.65 (2H, s); 4.35 (2H, s); 4.21 (5H, s); 4.10 (2H, br). ^{13}C NMR ($CDCl_3$) δ 167.7, 71.8, 69.8, 69.3. MS (m/z): 244.08 (M^+).

5.4. N-(Pyridin-4-ylmethylene)ferrocenylhydrazide **14**

To ferrocenylcarbohydrazine **13** (119 mg, 0.488 mmol) in ethanol (30 mL) was added pyridine-4-carboxaldehyde (52 mg, 0.488 mmol). After stirring for 4 h in the presence of molecular sieves (3 Å) at room temperature, the solution was evaporated under reduced pressure to give a brown oil. The product was purified using column chromatography with methanol and ethyl acetate (2/8) as eluent and crystallised with diethyl ether. Compound was obtained as brown crystals (100 mg, 62%). M.p. 220 °C. ^{1}H NMR ($CDCl_3$) δ 9.22 (s, 1H), 8.72 (d, 2H, J = 5.3 Hz), 7.64 (d, 2H, J = 5.9 Hz), 5.04 (br, 1H), 4.53 (m, 2H), 4.29 (m, 2H), 4.31 (s, 5H). ^{13}C NMR ($CDCl_3$) δ 154.8, 152.1, 135.8, 133.8, 123.8, 71.4, 69.9, 69.6. HRMS, calcd for $C_{17}H_{15}FeN_3O$: 333.0565 [M^+]; found 333.0571.

5.5. N-(Pyridin-3-ylmethylene)ferrocenylhydrazide **15**

To ferrocenylcarbohydrazine **13** (145 mg, 0.594 mmol) in ethanol (30 mL) was added pyridine-3-carboxaldehyde (63 mg, 0.589 mmol). After stirring for 4 h in the presence of molecular sieves (3 Å) at room temperature, the solution was evaporated under reduced pressure to give brown oil. The product was purified using column chromatography with methanol and ethyl acetate (2/8) as eluent and crystallised with diethyl ether. Compound was obtained as brown crystals (283 mg, 37%). M.p. 210–212 °C. ^{1}H NMR ($CDCl_3$) δ 9.31 (br, 1H), 8.90 (s, 1H), 8.72 (d, 1H, J = 4.2 Hz), 8.24 (m, 1H), 7.40 (t, 1H, J = 5.2 Hz), 4.91 (br, 1H), 4.53 (m, 2H), 4.30 (m, 7H), 4.28 (s, 5H). NMR (DMSO-d_6) δ 161.4, 154.5, 152.4, 149.8, 135.5, 128.9, 124.4, 79.4, 71.5, 69.1, 68.1. MS (m/z): 333.10 (M^+). Anal. Calcd for $C_{17}H_{15}FeN_3O$: C, 61.29; H, 4.54; N, 12.61. Found: C, 61.45; H, 4.65; N, 12.71.

5.6. N-(2-Aminophenylmethylene)ferrocenylhydrazide **16**

To ferrocenylcarbohydrazine (102 mg, 0.42 mmol) in ethanol (30 mL) was added 2-aminobenzaldehyde (50 mg, 0.42 mmol). After stirring for 4 h in presence the of molecular sieves at room temperature, the solution was filtered and evaporated under reduced pressure to give yellow oil (100 mg, 68%). ^{1}H NMR ($CDCl_3$) δ 9.86 (s, 1H), 7.31 (d, J = 5.4 Hz), 7.11 (s, 1H), 7.09 (m, 1H), 6.91 (m, 1H), 6.71 (d, 1H, J = 6.1 Hz), 4.66 (m, 2H), 4.32 (m, 2H), 4.24 (s, 5H). ^{13}C NMR (DMSO-d_6) δ 163.2, 150.6, 146.3, 130.1, 128.0, 126.7, 117.6, 115.0, 79.6, 70.9, 69.0, 68.2. HRMS, calcd for $C_{18}H_{17}FeN_3O$: 347.0721 [M^+]; found 347.0726.

5.7. General procedure for the synthesis of ferrocenyl hydrazones **26–35**

The hydrazones were prepared by reaction of 7-(chloroquinolein-4-yl)hydrazine (243 mg, 1.22 mmol) or 6-chloro-9-hydrazinyl-2-methoxyacridine (333 mg, 1.22 mmol) with the appropriate ferrocenyl aminoaldehyde [24] (1.22 mmol) in methanol (20 mL) in the presence of molecular sieves 3 Å (4 g). After stirring for 4 h under reflux, the resulting mixture was filtered and concentrated under reduced pressure. Water (10 mL) was added to the residue. The mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried over $MgSO_4$ and evaporated. After purification by column chromatography, the oil was crystallised in petroleum ether and CH_2Cl_2.

5.7.1. 1-(7-Chloroquinolin-4-yl)-2-(ferrocenylmethylene)hydrazine **26**

Red solid. Yield 89%. M.p. 150 °C. ^{1}H NMR ($CDCl_3$) δ 9.86 (s, 1H), 8.68 (d, 1H, J = 4.7 Hz), 8.03 (d, 1H, J = 8.9 Hz), 8.01 (s, 1H), 7.46 (d, 1H, J = 9.1 Hz), 7.38 (d, 1H, J = 4.7 Hz), 4.70 (d, 2H, J = 1.9 Hz), 4.50 (d, 2H, J = 1.8 Hz), 4.17 (s, 5H). ^{13}C NMR ($CDCl_3$) δ 193.4, 150.3, 149.3, 142.6, 136.4, 128.6, 128.5, 125.6, 124.9, 121.4, 79.3, 73.2, 69.6. MS (m/z) 390.01 (M^+ ^{35}Cl). Anal. Calcd for $C_{20}H_{16}ClFeN_3$: C, 61.65; H, 4.14; N, 10.78. Found: C, 61.87; H, 4.21; N, 10.65.

5.7.2. 1-(7-Chloroquinolin-4-yl)-2-(2-N,N-dimethylaminomethylferrocenylmethylene)hydrazine ***27***

Red solid. Yield 61%. M.p. 158–160 °C. ^{1}H NMR ($CDCl_3$) δ 10.02 (s, 1H), 8.07 (d, 1H, J = 4.5 Hz), 8.08 (d, 1H, J = 8.7 Hz), 8.04 (s, 1H), 7.51 (d, 1H, J = 8.7 Hz), 7.40 (d, 1H, J = 4.5 Hz), 4.70 (m, 2H), 4.50 (m, 2H), 4.14 (s, 5H), 3.75 (d, 1H, J = 6.9 Hz), 3.26 (d, 1H, J = 6.9 Hz), 2.09 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 151.0, 149.4, 142.6, 136.5, 128.7, 128.6, 125.6, 124.9, 121.4, 75.9, 71.9, 70.4, 70.2, 44.8, 30.3, 29.7. MS (m/z) 446.90 ($M^{+\,35}$Cl). Anal. Calcd for $C_{23}H_{23}ClFeN_4$: C, 61.83; H, 5.19; N, 12.54. Found: C, 62.01; H, 5.32; N, 12.42.

5.7.3. 1-(7-Chloroquinolin-4-yl)-2-(2-N-pyrrolidinomethylferrocenylmethylene)hydrazine ***28***

Red solid. Yield 95%. M.p. 164–166 °C. ^{1}H NMR (MeOD-d_4) δ 10.04 (s, 1H), 8.43 (d, 1H, J = 6.0 Hz), 8.21 (d, 1H, J = 8.7 Hz), 7.82 (s, 1H), 7.46 (d, 1H, J = 8.8 Hz), 7.37 (d, 1H, J = 6.0 Hz), 4.98 (s, 1H), 4.75 (s, 1H), 4.45 (s, 1H), 4.38 (s, 1H), 4.17 (s, 5H), 4.08 (d, 1H, J = 13.1 Hz), 3.62 (d, 1H, J = 13.1 Hz), 2.60 (m, 4H), 1.78 (m, 4H). ^{13}C NMR (MeOD-d_4) δ 148.5, 145.4, 143.7, 138.5, 135.1, 128.7, 127.3, 124.9, 122.9, 115.4, 84.5, 78.6, 72.2, 69.5, 68.7, 66.1, 53.1, 52.2, 22.8. MS (m/z) 472.94 ($M^{+\,35}$Cl). Anal. Calcd for $C_{25}H_{25}ClFeN_4$: C, 63.51; H, 5.33; N, 11.85. Found: C, 63.25; H, 5.30; N, 11.90.

5.7.4. 1-(7-Chloroquinolin-4-yl)-2-(2-N-piperidinomethylferrocenylmethylene)hydrazine ***29***

Red solid. Yield 91%. M.p. 172–174 °C. ^{1}H NMR (DMSO-d_6) δ 10.97 (s, 1H), 8.88 (d, 1H, J = 4.7 Hz), 8.36 (d, 1H, J = 7.7 Hz), 8.21 (s, 1H), 8.18 (d, 1H, J = 7.7 Hz), 7.81 (d, 1H, J = 4.6 Hz), 4.77 (s, 1H), 4.43 (s, 1H), 4.38 (s, 1H), 4.16 (s, 5H), 3.66 (d, 1H, J = 12.8 Hz), 2.75 (d, 1H, J = 12.8 Hz), 2.36 (m, 4H), 1.43 (m, 4H), 1.32 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 152.4, 149.3, 141.8, 135.8, 129.2, 128.6, 128.0, 126.3, 124.7, 122.6, 80.6, 78.8, 74.7, 73.2, 70.0, 69.2, 57.6, 53.9, 25.8, 24.4. MS (m/z) 486.94 ($M^{+\,35}$Cl). Anal. Calcd for $C_{26}H_{27}ClFeN_4$: C, 64.15; H, 5.59; N, 11.51. Found: C, 64.31; H, 5.71; N, 11.24.

5.7.5. 1-(7-Chloroquinolin-4-yl)-2-(2-N-morphilinomethylferrocenylmethylene)hydrazine ***30***

Red solid. Yield 74%. M.p. 212–214 °C, ^{1}H NMR (MeOD-d_4) δ 10.2 (s, 1H), 8.40 (d, 1H, J = 5.7 Hz), 8.19 (d, 1H, J = 8.0 Hz), 7.80 (s, 1H), 7.44 (d, 1H, J = 7.9 Hz), 7.31 (d, 1H, J = 5.9 Hz), 5.47 (s, 1H), 4.83 (s, 1H), 4.52 (s, 1H), 4.45 (s, 1H), 4.18 (s, 5H), 3.89 (d, 1H, J = 15.7 Hz), 3.64 (m, 4H), 3.49 (d, 1H, J = 14.5 Hz), 3.46 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 152.6, 149.0, 140.6, 134.3, 133.3, 129.8, 126.4, 124.8, 124.7, 122.1, 83.1, 79.6, 73.2, 70.1, 69.4, 66.9, 66.5, 56.5, 53.3. MS (m/z) 488.94 ($M^{+\,35}$Cl). Anal. Calcd for $C_{25}H_{25}ClFeN_4O$: C, 61.43; H, 5.16; N, 11.46. Found: C, 61.32; H, 5.12; N, 11.34.

5.7.6. 1-(6-Chloro-2-methoxyacridin-9-yl)-2-(ferrocenylmethylene)hydrazine ***31***

Red solid. Yield 90%. M.p. 117–119 °C. ^{1}H NMR ($CHCl_3$) δ 9.96 (s, 1H), 8.55 (d, 1H, J = 4.2 Hz), 8.19 (s, 1H), 8.07 (d, 1H, J = 9.2 Hz), 7.85 (d, 1H, J = 8.5 Hz), 7.48 (dd, 1H, J = 8.5 Hz and 4.6 Hz), 7.11 (d, 1H, J = 2.3 Hz), 4.80 (s, 2H), 4.61 (s, 2H), 4.28 (s, 5H), 3.9 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 93.8, 157.3, 147.3, 146.7, 133.7, 131.9, 130.8, 127.9, 127.4, 127.1, 126.2, 125.1, 103.2, 79.4, 73.2, 69.7, 55.0. MS (m/z) 469.90 ($M^{+\,35}$Cl). Anal. Calcd for $C_{25}H_{20}ClFeN_3O$: C, 63.92; H, 4.29; N, 8.95. Found: C, 64.21; H, 4.40; N, 9.25.

5.7.7. 1-(6-Chloro-2-methoxyacridin-9-yl)-2-(2-N,N-dimethylaminomethylferrocenylmethylene)hydrazine ***32***

Red solid. Yield 80%. M.p. 130 °C. ^{1}H NMR (DMSO-d_6) δ 10.98 (s, 1H), 9.15 (d, 1H, J = 11.5 Hz), 8.87 (s, 1H), 8.40 (d, 1H, J = 9.5 Hz), 8.10 (d, 1H, J = 11.4 Hz), 7.81 (s, 1H), 7.17 (d, 1H, J = 10.0 Hz), 5.10 (s, 1H), 4.87 (s, 1H), 4.52 (s, 1H), 4.39 (s, 1H), 4.20 (s, 5H), 3.82 (s, 3H), 3.36 (d, 1H, J = 12.3 Hz), 2.50 (d, 1H, J = 12.1 Hz), 2.14 (s, 6H).^{13}C NMR (DMSO-d_6) δ 195.8, 153.9, 146.2, 145.9, 143.4, 141.0, 134.6, 132.3, 127.7, 120.3, 119.6, 116.4, 114.6, 106.7, 82.4, 78.2, 74.7, 73.1, 69.8, 68.3, 57.3, 56.5, 44.8. MS (m/z) 526.89 ($M^{+\,35}$Cl). Anal. Calcd for $C_{28}H_{27}ClFeN_4O$: C, 63.83; H, 5.17; N, 10.63. Found: C, 63.91; H, 5.22; N, 10.73.

5.7.8. 1-(6-Chloro-2-methoxyacridin-9-yl)-2-(2-N-pyrrolidinomethylferrocenylmethylene)hydrazine ***33***

Red solid. Yield 94%. M.p. 120–122 °C. ^{1}H NMR (MeOD-d_4) δ 10.44 (s, 1H), 8.88 (d, 1H, J = 8.0 Hz), 8.68 (s, 1H), 8.09 (d, 1H, J = 11.0 Hz), 7.59 (s, 1H), 6.98 (d, 1H, J = 8.2 Hz), 6.78 (d, 1H, J = 11.2 Hz), 4.90 (s, 1H), 4.52 (s, 1H), 4.45 (s, 1H), 4.17 (s, 5H), 3.96 (d, 1H, J = 12.8 Hz), 3.87 (s, 3H), 3.57 (d, 1H, J = 12.9 Hz), 3.12 (m, 4H), 2.16 (m, 4H). ^{13}C NMR (MeOD-d_4) δ 195.8, 153.9, 149.2, 145.9, 143.4, 141.0, 134.6, 132.3, 127.7, 120.3, 119.6, 116.5, 114.7, 106.7, 85.0, 78.9, 75.4, 72.8, 69.8, 67.2, 55.7, 54.0, 46.2, 11.5. MS (m/z) 552.97 ($M^{+\,35}$Cl). Anal. Calcd for $C_{30}H_{29}ClFeN_4O$: C, 65.17; H, 5.29; N, 10.13. Found: C, 65.01; H, 5.40; N, 10.24.

5.7.9. 1-(6-Chloro-2-methoxyacridin-9-yl)-2-(2-N-piperidinomethylferrocenylmethylene)hydrazine ***34***

Red solid. Yield 64%. M.p. 130–132 °C. ^{1}H NMR (DMSO-d_6) δ 10.01 (s, 1H), 8.52 (s, 1H), 7.95 (s, 1H), 7.87 (d, 1H, J = 11.4 Hz), 7.81 (d, 1H, J = 9.5 Hz), 7.45 (d, 1H, J = 11.4 Hz), 7.06 (s, 1H), 6.92 (d, 1H, J = 9.0 Hz), 4.91 (s, 1H), 4.57 (s, 1H), 4.52 (s, 1H), 4.21 (s, 5H), 3.92 (s, 3H), 3.56 (d, 1H, J = 13.4 Hz), 3.42 (d, 1H, J = 13.1 Hz), 2.40 (m, 4H), 1.52 (m, 4H), 1.37 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 193.8, 150.9, 145.2, 138.0, 136.3, 130.6, 129.4, 128.2, 127.0, 122.7, 121.8, 120.3, 107.7, 101.0, 80.9, 78.5, 75.5, 74.0, 71.9, 71.1, 57.3, 56.9, 54.7, 26.2, 24.8. MS (m/z) 566.91 ($M^{+\,35}$Cl). Anal. Calcd for $C_{31}H_{31}ClFeN_4O$: C, 65.68; H, 5.51; N, 9.88. Found: C, 65.83; H, 5.44; N, 10.03.

5.7.10. 1-(6-Chloro-2-methoxyacridin-9-yl)-2-(2-N-morphilinomethylferrocenylmethylene)hydrazine ***35***

Red solid. Yield 94%. M.p. 117–119 °C. ^{1}H NMR (DMSO-d_6) δ 10.44 (s, 1H), 8.88 (d, 1H, J = 9.0 Hz), 8.68 (s, 1H), 8.09 (d, 1H, J = 11.0 Hz), 7.45 (s, 1H), 7.03 (d, 1H, J = 8.1 Hz), 6.78 (d, 1H, J = 11.0 Hz), 4.87 (s, 1H), 4.52 (s, 1H), 4.45 (s, 1H), 4.20 (s, 5H), 3.96 (s, 3H), 3.89 (d, 1H, J = 12.3 Hz), 3.84 (m, 4H), 3.74 (d, 1H, J = 11.1 Hz), 3.50 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 195.8, 155.9, 150.2, 145.0, 143.5, 141.4, 1345.6, 132.3, 128.7, 120.3, 119.6, 117.5, 113.7, 109.7, 82.1, 78.4, 74.3, 73.1, 69.8, 68.9, 65.5, 57.5, 55.7, 54.3. MS (m/z) 568.96 ($M^{+\,35}$Cl). Anal. Calcd for $C_{30}H_{29}ClFeN_4O_2$: C, 63.34; H, 5.14; N, 9.85. Found: C, 63.12; H, 5.20; N, 9.75.

5.8. General procedure for synthesis of ferrocenic esters ***43–45***

To a solution of the appropriate carboxylic acid (1.6 mmol) in dichloromethane (20 mL) was added 4-dimethylaminopyridine (0.16 mmol) followed by ferrocenyl methanol (1.5 mmol). The mixture was cooled at 0 °C and dicyclohexylcarbodiimide (1.5 mmol) was added. After stirring at 20 °C for 24 h, the DCU precipitate was removed by filtration. The filtrate was washed by an aqueous K_2CO_3 solution (2 × 20 mL). The organic layer was dried over $MgSO_4$ and evaporated under vacuum to give the crude oil. The product was purified using column chromatography (eluent:-petroleum ether/diethyl ether: 5/5).

5.8.1. Ferrocenylmethyl-2-pyrazinecarboxylate ***43***

Yellow solid. Yield 79%. M.p. 79 °C. ^{1}H NMR ($CDCl_3$) δ 9.26 (d, 1H, J = 1.3 Hz), 8.70 (d, 1H, J = 2.2 Hz), 8.67 (dd, 1H, J = 1.3 and 2.2 Hz), 5.26 (s, 2H), 4.37 (m, 2H), 4.19 (s, 5H), 4.18 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 163.7, 147.5, 146.3, 144.4, 143.6, 80.2, 70.1, 69.1, 68.6, 64.7. HRMS, calcd for $C_{16}H_{14}FeN_2O_2$: 322.0405 [M^+]; found 322.0421.

*5.8.2. Ferrocenylmethyl-2-quinoxalinecarboxylate **44***

Yellow solid. Yield 96%. M.p. 97 °C. ^{1}H NMR ($CDCl_3$) δ 9.49 (s, 1H), 8.26 (dd, 1H, J = 8.0 and 1.7 Hz), 8.16 (dd, 1H, J = 8.8 and 1.9 Hz), 7.80 (m, 2H), 5.34 (s, 2H), 4.42 (m, 2H), 4.21 (s, 5H), 4.12 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 163.9, 145.0, 143.6, 142.7, 141.6, 132.3, 131.0, 130.6, 129.3, 80.4, 70.2, 69.1, 68.7, 64.9. HRMS, calcd for $C_{20}H_{16}FeN_2O_2$: 372.0561 [M^+]; found 372.0545.

*5.8.3. Ferrocenylmethyl-3-nicotinate **45***

Yellow solid. Yield 70%. M.p. 250 °C. ^{1}H NMR ($CDCl_3$) δ 9.24 (s, 1H), 8.90 (m, 2H), 8.01 (d, 1H, J = 7.6 Hz), 5.65 (s, 2H), 4.51 (m, 2H), 4.35 (m, 2H), 4.29 (s, 5H). ^{13}C NMR ($CDCl_3$) δ 166.2, 151.9, 148.2, 134.9, 124.3, 82.3, 70.2, 68.7, 68.9, 68.5. HRMS, calcd for $C_{17}H_{15}FeNO_2$: 321.0452 [M^+]; found 321.0445.

*5.9. (N-Ferrocenylmethyl)-2-pyrazinamide **54***

To a solution of 2-pyrazinecarboxylic acid (152 mg, 1.23 mmol) and ferrocenylmethylamine (290 mg, 1.35 mmol) in dichloromethane (20 mL) was added bromo-tris-pyrrolidinophosphonium hexafluorophosphate (590 mg, 1.26 mmol) at 0 °C. Diisopropyléthylamine (0.66 mL, 2.54 mmol) was slowly added to the mixture for 5 min at 0 °C. After stirring for 1 h at 20 °C, the mixture was hydrolyzed by water and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was washed with 1 N HCl (20 mL), aqueous saturated K_2CO_3 solution (40 mL) and dried over $MgSO_4$ before removing the solvent under reduced pressure. The oil was purified by column chromatography (CH_2Cl_2/MeOH: 1/1) to give **54** as a yellow solid (138 mg, 35%). M.p. 192 °C. ^{1}H NMR ($CDCl_3$) δ 9.44 (d, 1H, J = 1.4 Hz), 8.76 (d, 1H, J = 2.4 Hz), 8.53 (dd, 1H, J = 2.4 and 1.4 Hz), 4.35 (s, 2H), 4.26 (m, 2H), 4.25 (s, 5H), 4.18 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 162.3, 147.3, 144.5, 142.6, 142.5, 84.6, 68.6, 68.2, 68.1, 38.5. HRMS, calcd for $C_{16}H_{15}FeN_3O$: 321.0565 [M^+]; found: 321.0572.

*5.10. (N-Ferrocenylmethyl)-2-quinoxalinenamide **55***

To a solution of 2-quinoxalinecarboxylic acid (100 mg, 0.57 mmol) and ferrocenylmethylamine (136 mg, 0.63 mmol) in dichloromethane (20 mL) was added bromo-tris-pyrrolidinophosphonium hexafluorophosphate (268 mg, 0.57 mmol) at 0 °C. Diisopropylethylamine (0.30 mL, 1.72 mmol) was slowly added to the mixture for 5 min at 0 °C. After stirring for 1 h at 20 °C, the mixture was hydrolyzed by water and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was washed with HCl 1 N (20 mL), aqueous saturated K_2CO_3 (40 mL) and dried over $MgSO_4$ before removing the solvent under reduced pressure. The oil was purified by column chromatography (petroleum ether/diethyl ether/triethylamine: 3/1/2) to give **55** as a yellow solid (143 mg, 68%). M.p. 127 °C. ^{1}H NMR ($CDCl_3$) δ 9.72 (s, 1H), 8.20 (dd, 1H, J = 7.8 and 1.7 Hz), 8.12 (dd, 1H, J = 8.0 and 1.9 Hz), 7.87 (m, 2H), 4.38 (m, 2H), 4.32 (s, 5H), 4.30 (s, 2H), 4.20 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 162.6, 143.9, 143.5, 143.4, 140.3, 131.6, 130.8, 129.6, 139.5, 85.1, 68.6, 68.3, 68.2, 38.5. HRMS, calcd for $C_{20}H_{17}FeN_3O$: 371.0721 [M^+]; found: 371.0742.

*5.11. (N-Ferrocenylmethyl)-3-pyridinamide **56***

To a solution of 3-pyridinecarboxylic acid (412 mg, 3.28 mmol) and ferrocenylmethylamine (705 mg, 3.28 mmol) in dichloromethane (30 mL) was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (643 mg, 3.28 mmol). After stirring overnight at 20 °C, the mixture was hydrolyzed by water and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was washed with HCl 1 N (20 mL), aqueous saturated K_2CO_3 (40 mL) and dried over sodium sulfate before removing the solvent under reduced pressure. The oil was purified by column chromatography (ethyl acetate/diethyl ether/methanol: 5/5/1) to give **56** as a yellow solid (611 mg, 63%). M.p. 155 °C. ^{1}H NMR ($CDCl_3$) δ 8.86 (s, 1H), 8.60 (d, 1H, J = 3.9 Hz), 8.04 (d, 1H, J = 8.0 Hz), 7.29 (dd, 1H, J = 8.0 and 4.1 Hz), 6.65 (s, 1H), 4.28 (s, 2H), 4.18 (m, 2H), 4.11 (s, 5H), 4.09 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 165.1, 152.2, 147.9,135.3, 123.7, 84.3, 68.8, 68.7, 68.5, 39.7. Anal. Calcd for $C_{17}H_{16}FeN_2O$: C, 63.77; H, 5.04; N, 8.75. Found: C, 63.58; H, 5.05; N, 8.73.

*5.12. (N-Ferrocenylmethylquinoline)-3-carboxamide **57***

To a solution of 3-quinolinecarboxylic acid (276 mg, 1.6 mmol) and ferrocenylmethylamine (340 mg, 1.6 mmol) in dichloromethane (30 mL) was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (306 mg, 1.6 mmol). After stirring overnight at 20 °C, the mixture was hydrolyzed by water and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was washed with 1 N HCl (20 mL), aqueous saturated K_2CO_3 (40 mL) and dried over sodium sulfate before removing the solvent under reduced pressure. The oil was purified by column chromatography (ethyl acetate/diethyl ether/triethylamine: 4/4/2) to give **57** as a yellow solid (349 mg, 59%). M.p. 193 °C. ^{1}H NMR ($CDCl_3$) δ 9.29 (d, 1H, J = 2.2 Hz), 8.59 (d, 1H, J = 1.9 Hz), 8.14 (d, 1H, J = 8.5 Hz), 7.89 (d, 1H, J = 8.1 Hz), 7.80 (t, 1H, J = 7.7 Hz), 7.61 (t, 1H, J = 7.7 Hz), 6.56 (br, 1H), 4.43 (m, 2H), 4.21 (m, 2H), 4.20 (s, 5H). ^{13}C NMR ($CDCl_3$) δ 165.0, 155.0, 149.1, 148.0, 135.7, 131.2, 129.3, 128.7, 127.5, 126.9, 71.6, 68.6, 68.4, 39.7. Anal. Calcd for $C_{21}H_{18}FeN_2O$: C, 68.13; H, 4.90; N, 7.57. Found: C, 67.89; H, 4.94; N, 7.51.

*5.13. (N-Ferrocenylmethylquinoline)-2-carboxamide **58***

To a solution of 2-quinolinecarboxylic acid (276 mg, 1.6 mmol) and ferrocenylmethylamine (340 mg, 1.6 mmol) in dichloromethane (30 mL) was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (306 mg, 1.6 mmol). After stirring overnight at 20 °C, the mixture was hydrolyzed by water and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was washed with 1 N HCl (20 mL), aqueous saturated K_2CO_3 (40 mL) and dried over sodium sulfate before removing the solvent under reduced pressure. The oil was purified by column chromatography (ethyl acetate/diethyl ether/triethylamine: 4/4/2) to give **58** as a yellow solid (183 mg, 31%). M.p. 161 °C. ^{1}H NMR ($CDCl_3$) δ 8.56 (br, 1H), 8.26 (m, 2H), 8.05 (d, 1H, J = 8.4 Hz), 7.81 (d, 1H, J = 8.1 Hz), 7.67 (t, 1H, J = 7.5 Hz), 7.54 (t, 1H, J = 7.5 Hz), 4.27 (m, 2H), 4.26 (s, 5H), 4.11 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 163.8, 149.8, 146.5, 137.5, 130.1, 129.7, 129.3, 127.9, 127.7, 118.9, 68.6, 68.1, 68.1, 38.3. Anal. Calcd for $C_{21}H_{18}FeN_2O$: C, 68.13; H, 4.90; N, 7.57. Found: C, 67.73; H, 5.02; N, 7.32.

*5.14. (4-Ferrocenylmethylpiperazin-1-yl)(pyrazin-2-yl)methanone **59***

To a solution of 2-pyrazinecarboxylic acid (49 mg, 0.39 mmol) and ferrocenylmethylpiperazine (102 mg, 0.39 mmol) in dichloromethane (30 mL) was added 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (67 mg, 0.35 mmol). After stirring overnight at 20 °C, the mixture was hydrolyzed by water and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was washed with 1 N HCl (20 mL), aqueous saturated K_2CO_3 (40 mL) and dried over sodium sulfate before removing the solvent under reduced pressure. The oil was purified by column chromatography (diethyl ether/methanol: 8/2) to give **59** as a yellow solid (68 mg, 45%). M.p. 108–110 °C. ^{1}H NMR ($CDCl_3$) δ 8.91 (d, 1H, J = 1.5 Hz), 8.62 (d, 1H, J = 2.6 Hz), 8.52 (dd, 1H, J = 2.6 and 1.5 Hz), 4.20 (m, 2H), 4.12 (m, 2H), 4.13 (s, 5H), 3.70 (m, 4H), 3.41 (s, 2H), 2.47 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 161.2, 149.4, 145.6, 145.2, 142.5, 70.2, 68.6, 68.2, 58.2, 52.6, 52.1, 47.0, 42.3. Anal. Calcd for $C_{20}H_{22}FeN_4O$: C, 61.55; H, 5.68; N, 14.36. Found: C, 59.92; H, 5.70; N, 14.07.

5.15. (4-Ferrocenylmethylpiperazin-1-yl)(quinoxalin-2-yl) methanone **60**

To a solution of 2-quinoxalinecarboxylic acid (61 mg, 0.352 mmol) and ferrocenylmethylpiperazine (100 mg, 0.352 mmol) in dichloromethane (30 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (67 mg, 0.35 mmol). After stirring overnight at 20 °C, the mixture was hydrolyzed by water and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was washed with 1 N HCl (20 mL), aqueous saturated K_2CO_3 (40 mL) and dried over sodium sulfate before removing the solvent under reduced pressure. The oil was purified by column chromatography (ethylacetate/methanol: 8/2) to give **60** as a yellow solid (48 mg, 31%). M.p. 130 °C. ^{1}H NMR ($CDCl_3$) δ 9.16 (s, 1H), 8.12 (m, 2H), 7.85 (m, 1H), 4.20 (m, 2H), 4.18 (m, 2H), 4.14 (m, 2H), 4.13 (s, 5H), 3.81 (m, 4H), 3.45 (s, 2H), 2.53 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 165.2, 148.1, 145.2, 142.2, 140.2, 131.1, 130.7, 129.7, 129.3, 70.2, 68.5, 68.2, 58.2, 52.7, 52.1, 47.1, 42.4. Anal. Calcd for $C_{24}H_{24}FeN_4O$: C, 65.47; H, 5.49; N, 12.72. Found: C, 65.00; H, 5.54; N, 12.34.

5.16. Activity against M. tuberculosis $H_{37}Rv$ strain

Susceptibility testing with the BACTEC MGIT 960 system (Becton Dickinson) was performed according to the manufacturer's recommendations. For pyrazinamide and ferrocenyl derivatives **43**–**45** and **54**–**60**, the pH of the media was 6 using Bactec MGIT 960 PZA (BD). Test compounds were dissolved in dimethyl sulfoxide or in methanol. 390 μl of diluted test compounds was added to MGIT 7 mL tubes supplemented with 0.8 mL of the provided enrichment solution. Susceptibility testing was performed by minimal inhibitory concentration (MIC) determination. Serial twofold dilution of a 1280 mg/l solution of each drugs were added to MGIT tubes to achieve final concentration ranging from 64 to 0.25 mg/L. All the drug-containing tubes were inoculated with 0.5 mL of the positive broth culture. Mycobacterial suspensions were used undiluted from 2 days following the detection of growth, while the suspensions were diluted 1:5 with sterile saline from days 3 to 5. A SIRE drug-free control was inoculated with 0.5 ml of a 10^{-2} dilution of the positive culture broth in sterile saline. The tubes were then placed in a BACTEC 960 set carrier and incubated in the instrument. The tubes were continuously monitored until the results indicating susceptibility or resistance were automatically interpreted and reported using predefined algorithms that compared growth in the drug-containing tube to that in the control tube.

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