

Synthesis of organometallic-based biologically active compounds: *In vitro* antibacterial, antifungal and cytotoxic properties of some sulfonamide incorporated ferrocenes

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Abstract

Sulfonamides incorporated ferrocene (SIF) have been synthesized by the condensation reaction of sulfonamides (sulfanilamide, sulfathiazole or sulfamethaxazole) with 1,1'-diacetylferrocene. The synthesized compounds (**SIF**¹–**SIF**⁴) have been characterized by their physical, spectral and analytical properties and have been screened for their *in vitro* antibacterial properties against pathogenic bacterial strains e.g., *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella typhi* and for antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporium canis*, *Fusarium solani* and *Candida glabrata* using Agar-well diffusion method. Most of the compounds showed good antibacterial activity whereas, all the compounds exhibited significant antifungal activity. Brine shrimp bioassay was also carried out for *in vitro* cytotoxic properties against *Artemia salina*.

Keywords: 1,1'-Disubstituted ferrocenes, sulfonamides, antibacterial, antifungal, cytotoxic properties

Introduction

The development and “designing” of new drugs has revolutionized the practice of medicine. In designing new drugs, it is significant to know about the nature of disease or the infectious process. Drug resistance too, has become globally a striking medical and clinical problem. It is therefore, essential specifically and selectively designing such drugs targeting the progression process where the resistance mechanism emerges. The relationship [1,2] between biological activity and metal ions compiles one potential possibility of designing novel therapeutic methodologies in addressing probable mechanism of resistance. In this regard, a number of studies have highlighted the use of ferrocene and its derivatives for the design of biologically active compounds [3–5]. Some reports have signified [6,7] that if certain group in some antibiotics is replaced by ferrocene the antibacterial properties are appreciably improved. Also, many activities such as antibacterial [8–10],

antitumor [11,12], anti-carbonic anhydrase [13,14], hypoglycaemic [15], anti-thyroid [16] and protease inhibitor [17] are related to the potential use and application of sulfonamide drugs. Combining together the chemistry of ferrocene and sulfonamides attracted the attention of the author in designing and introducing new biologically active organometallic compounds that may prove to fight more aggressively against certain bacteria and also resistant ones. Acylferrocene undergoes a condensation reaction with amines to form Schiff bases. Making a successful attempt underlying this transformation, we wish to report some sulfonamide incorporated ferrocenes (**SIF**¹–**SIF**⁴) (Figure 1) along with their structural elucidation. In order to emulate the biological activity of proteins and enzymes, the prepared sulfonamide incorporated ferrocenes have been screened for their antibacterial activity against pathogenic bacterial strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella typhi* and, for antifungal activity against

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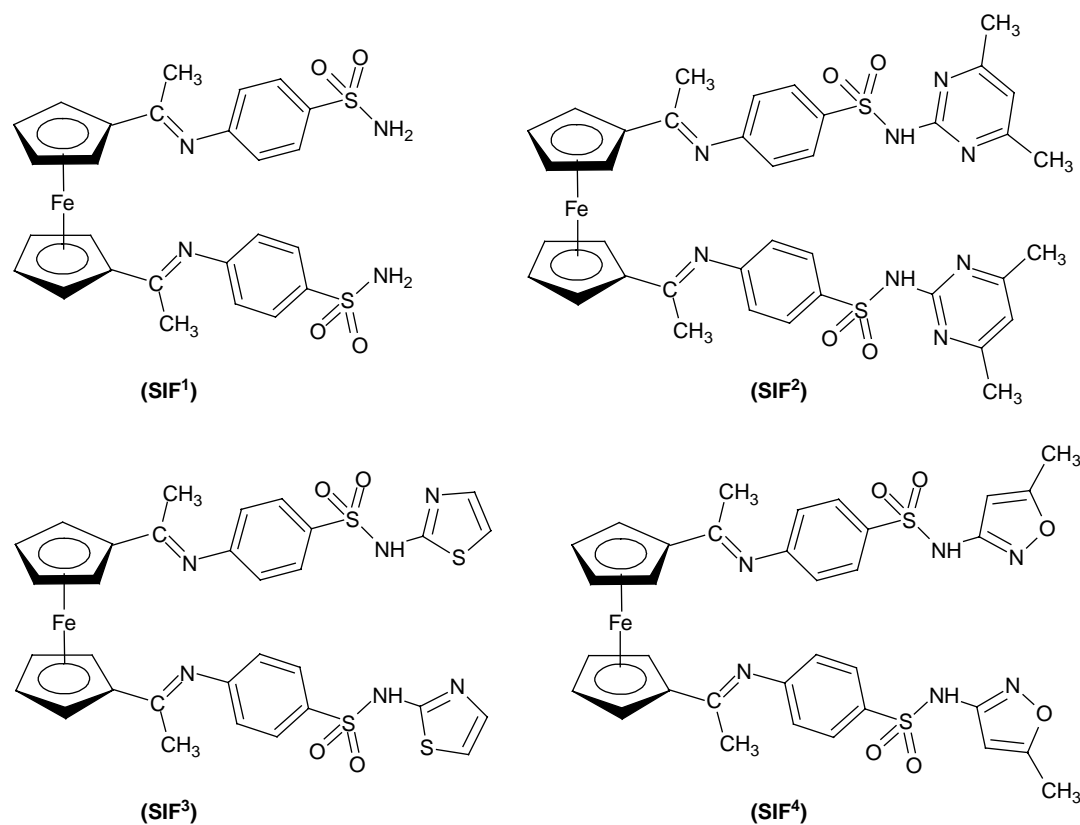


Figure 1. Structures of sulfonamide incorporated ferrocenes (SIF¹–SIF⁴).

Trichophyton longifusus, *Candida albicans*, *Aspergillus flavus*, *Microsporium canis*, *Fusarium solani* and *Candida glabrata* using the agar-well diffusion method [18,19].

Material and methods

All solvents used were of Analytical grade. 1,1'-Diacetylferrocene and sulfonamides were obtained from Aldrich. All metals were used as their chloride salts. IR and NMR spectra were recorded on Perkin Elmer 283B and 300 MHz Varian XL-300 instruments. UV Visible spectra were obtained on a Baush and Lomb spectronic 1001. Conductances of the metal complexes were determined in DMF using a YSI-32 model conductometer. Magnetic measurements were done on solid complexes using the Gouy method. Melting points were recorded on a Gallenkamp apparatus and are uncorrected.

General procedure for the preparation of SIF¹–SIF⁴

A solution of respective sulfonamide (0.01 mol) in ethanol (20 mL) was added drop-wise to a solution of 1,1'-diacetylferrocene (1.35 g, 0.005 mol) in ethanol (15 mL). The reaction mixture was heated under reflux for 8 h, cooled to room temperature, filtered and refrigerated overnight. The precipitated product thus formed during refrigeration was filtered, washed with

ethanol (2 × 3 mL) followed by diethyl ether. After drying, the product was recrystallized from hot ethanol to obtain dark brown solid, which was dried under vacuum. The purity of the product was confirmed by TLC [plastic sheets of a silica gel F₂₅₄ with 0.2 mm layer thickness].

1,1'-Sulfanilamide incorporated ferrocene (SIF¹)

IR (KBr, cm⁻¹): 3390 (NH₂), 1625 (C=N), 1345, 1110 (S=O), 952 (S–N), 833 (C–S); ¹H NMR (DMSO-d₆, δ, ppm): 2.5 (s, 6H, CH₃), 4.4–4.6 (m, 4H, ferrocenyl), 4.9–5.2 (m, 4H, ferrocenyl), 7.4–7.6 (m, 4H, Ph), 7.8–8.0 (m, 4H, Ph), 11.3 (s, 2H, SO₂NH₂); ¹³C NMR (δ, ppm): 15.7 (CH₃), 68.6, 69.6, 83.6 (ferrocenyl), 138.2, 128.6, 122.6, 156.4 (Ph), 161.0 (C=N, azomethine).

1,1'-Sulfamethazine incorporated ferrocene (SIF²)

IR (KBr, cm⁻¹): 3265 (NH), 1625 (C=N), 1345, 1110 (S=O), 952 (S–N), 833 (C–S), 1580 (pyrimidinyl, C=N); ¹H NMR (DMSO-d₆, δ, ppm): 2.5 (s, 6H, CH₃), 2.3 (s, 6H, CH₃-pyrimidinyl), 4.4–4.6 (m, 4H, ferrocenyl), 4.9–5.2 (m, 4H, ferrocenyl), 8.2 (s, 1H, pyrimidinyl), 7.3–7.5 (m, 4H, Ph), 7.6–7.8 (m, 4H, Ph), 11.4 (s, 1H,

SO₂NH); ¹³C NMR (δ, ppm): 25.1 (CH₃-pyrimidinyl), 15.7 (CH₃), 68.6, 69.2, 83.8 (ferrocenyl), 165.2, 103.0, 168.5 (pyrimidinyl), 138.2, 128.6, 122.6, 156.4 (Ph), 161.8 (C=N, azomethine).

1,1'-Sulfathiazole incorporated ferrocene (SIF³)

IR (KBr, cm⁻¹): 3260 (NH), 1620 (C=N), 1345, 1110 (S=O), 952 (S-N), 833 (C-S), 1610 (thiazolyl, C=N); ¹H NMR (DMSO-d₆, δ, ppm): 2.5 (s, 6H, CH₃), 4.4–4.6 (m, 4H, ferrocenyl), 4.9–5.2 (m, 4H, ferrocenyl), 7.4–7.6 (m, 4H, Ph), 7.7–7.9 (m, 4H, Ph), 8.1 (d, 1H, thiazolyl), 8.3 (d, 1H, thiazolyl), 11.3 (s, 1H, SO₂NH); ¹³C NMR (δ, ppm): 15.7 (CH₃), 68.6, 69.2, 83.8 (ferrocenyl), 108.0, 137.8, 171.7 (thiazolyl), 138.2, 128.6, 122.6, 156.4 (Ph), 161.6 (C=N, azomethine).

1,1'-Sulfamethaxazole incorporated ferrocene (SIF⁴)

IR (KBr, cm⁻¹): 3265 (NH), 1620 (C=N), 1345, 1110 (S=O), 952 (S-N), 833 (C-S), 1615 (isoxazolyl, C=N); ¹H NMR (DMSO-d₆, δ, ppm): 2.5 (s, 6H, CH₃), 2.3 (s, 3H, CH₃-isoxazolyl), 4.4–4.6 (m, 4H, ferrocenyl), 4.9–5.2 (m, 4H, ferrocenyl), 7.4–7.6 (m, 4H, Ph), 7.8–8.0 (m, 4H, Ph), 8.2 (s, 1H, isoxazolyl), 11.3 (s, 1H, SO₂NH); ¹³C NMR (δ, ppm): 15.7 (CH₃), 68.6, 69.2, 83.8 (ferrocenyl), 12.8 (CH₃-isoxazolyl), 159.6, 95.0, 150.3 (isoxazolyl), 138.2, 128.6, 122.6, 156.4 (Ph), 162.3 (C=N, azomethine).

Biological properties

Antibacterial bioassay (in vitro)

All the synthesized compounds (SIF¹–SIF⁴) were screened *in vitro* for their antibacterial activity against *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus* and *S. typhi* bacterial strains by the agar-well diffusion method [18,19]. The wells (6 mm in diameter) were dug in the media with the help of a sterile metallic borer with centers at least 24 mm apart. Two to eight hours old bacterial inocula containing approximately 10⁴–10⁶ colony-forming units (CFU/mL) were spread on the surface of the nutrient agar with the help of a sterile cotton swab. The recommended concentration of the test sample (1 mg/mL in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, imipenem, served as negative and positive controls, respectively. The plates were incubated immediately at 37°C for 24 hours. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they

showed no activity against any bacterial strains. Also, the metal salts used for complexation were evaluated for antibacterial activity against the same bacterial strains under the same conditions but none of them showed any significant and/or moderate activity, only insignificant activity was observed.

Antifungal activity (in vitro)

All compounds were studied against six fungal cultures for Antifungal activities. Sabouraud dextrose agar (oxid, Hampshire, England) was seeded with 10⁵ (cfu) mL⁻¹ fungal spore suspensions and transferred to petri plates. Discs soaked in 20 ml (200 µg/mL in DMSO) of all compounds were placed at different positions on the agar surface. The plates were incubated at 32°C for seven days. The results were recorded [20] by measuring the diameter of zones showing complete inhibition (mm). Miconazole and amphotericin B were used as standard drugs for this bioassay.

Minimum inhibitory concentration (MIC)

Compounds containing high antibacterial activity (over 80%) were selected for minimum inhibitory concentration (MIC) studies. The minimum inhibitory concentration was determined using the disc diffusion technique by preparing discs containing 10, 25, 50 and 100 µg/mL of the compounds and applying the protocol [21,22].

Cytotoxicity (in vitro)

Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial seawater, which was prepared with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the matter compartment was opened to ordinary light. After two days nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 mL of DMF. From this stock solutions 500, 50 and 5 µg/mL were transferred to 9 vials (three for each dilutions were used for each test sample and LD₅₀ is the mean of three values) and one vial was kept as control having 2 mL of DMF only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 mL of sea water and 10 shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 mL per vial. After 24 h the number of survivors was counted. Data were analyzed by Finney computer program to determine the LD₅₀ values [23,24].

Results and discussion

Chemistry

The compounds (**SIF**¹–**SIF**⁴) were prepared by refluxing ethanol solution of respective sulfonamide e.g., sulphanilamide, sulfamethazine, sulfathiazole, sulfamethaxazole, with 1,1'-diacetylferrocene (Figure 1) in 2:1 (sulfonamide: diacetylferrocene) molar ratio. All the products were obtained as solids and their purities were checked by TLC (eluent = n-hexane/chloroform/ethylacetate, 1/2/1 v/v). All the synthesized compounds were characterized by spectroscopic techniques (IR, ¹H NMR & ¹³C NMR) given in experimental part and by their elemental analyses. Colour, m.p, yield (%) and CHN analyses for compounds (**SIF**¹–**SIF**⁴), are given in Table I. Microcrystalline powder of these compounds could only be obtained, which were impossible to be used for X-ray structural determinations.

IR spectra

In the IR spectra of compounds (**SIF**¹–**SIF**⁴), a sharp band at 1620–1625 cm⁻¹ is assigned [25] to the stretching of ν(C=N). Presence of this band is an evidence of the formation of condensation product. ν_{asymm}(SO₂) and ν_{symm}(SO₂) vibrations appeared at 1345 and 1110 cm⁻¹, respectively [26,27]. The IR spectra of **SIF**¹ showed peak at 3390 cm⁻¹, due to NH₂ stretchings, whereas the IR spectra of **SIF**²–**SIF**⁴ exhibited peak at 3260–3265 cm⁻¹, due to NH stretching vibrations. In addition, the spectra of **SIF**²–**SIF**⁴ showed bands resulting from the pyrimidinyl (C=N), thiazolyl (C=N), isoxazolyl (C=N) stretchings at 1580, 1610 and 1615 cm⁻¹, respectively. The presence of all these frequencies were supportive [28,29] of the formation of compounds (**SIF**¹–**SIF**⁴).

¹H NMR spectra

The ¹H NMR of all the compounds (**SIF**¹–**SIF**⁴) were taken in DMSO-d₆. The data reported along with the possible assignments in experimental part show that **SIF**¹–**SIF**⁴ displays all expected signals at δ 2.5, 4.4–4.6 and 4.9–5.2 which are assigned to CH₃ and the ferrocenyl moieties, respectively ¹H NMR spectra of the compounds showed multiplet at δ 7.3–7.6 for the phenyl ring system. The SO₂NH₂ and

SO₂NH- protons in all cases appeared as a singlet in the region of δ 11.3–11.4, respectively. The ¹H-NMR spectrum of **SIF**² displayed pyrimidinyl proton as singlet at δ 8.2. In case of **SIF**³, thiazolyl protons appeared as doublet at δ 8.0 and 8.3. In case of compound **SIF**⁴, the isoxazolyl proton appeared as a singlet at δ 8.2. The spectra of all the four compounds displayed methyl protons peaks as singlets at δ 2.5. Furthermore, the number of protons calculated from the integration curves [30] and those obtained from the values of the expected CHN analyses agree well with each other.

¹³C NMR spectra

The ¹³C NMR of all the compounds (**SIF**¹–**SIF**⁴) were also taken in DMSO-d₆. The spectra of all the four compounds displayed azomethine (CH=N) peak at δ 161.0 which further supported the results obtained in IR, ¹H NMR, hence formation of condensation product. ¹³C NMR spectra of the compounds (**SIF**¹–**SIF**⁴) displayed signals of CH₃ and ferrocenyl at δ 15.7, 68.6, 69.2, 83.8 respectively. The phenyl carbons appeared at δ 138.2, δ 128.6, δ 122.6 and δ 156.4, respectively in all the compounds whereas the CH₃-pyrimidinyl and other pyrimidinyl carbons appeared at δ 25.1, 165.2, 103.0, 168.5 respectively in **SIF**². In **SIF**³, the thiazolyl carbons appeared at δ 108.0, 137.8 and 171.7 respectively. In **SIF**⁴, CH₃-isoxazolyl and other isoxazolyl carbons appeared at δ 12.8, 159.6, 95.0 and 150.3 respectively. Furthermore, the number of carbons calculated from the spectra [31] and those obtained from the values of the expected CHN analyses compromise well with each other.

Biological activity

Antibacterial bioassay (in vitro)

All compounds were tested against bacterial strains such as *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus* and *S. typhi* (Table II) according to the literature protocol [18,19]. The results were compared with those of the standard drug imipenem (Figure 2). All compounds (**SIF**¹–**SIF**⁴) showed moderate to significant activity against different bacterial strains except the activity of all compounds against *B. subtilis* where all compounds showed moderate activity. All compounds showed

Table I. Physical measurements and analytical data of sulfonamide incorporated ferrocene (**SIF**¹–**SIF**⁴).

No.	Colour	M.P (°C)	Yield (g) %	Calc. (Found) %			
				C	H	N	
1	[C ₂₆ H ₂₆ FeN ₄ O ₄ S ₂] [578.49]	Brown	248–250	(1.82) 63	53.98 (54.12)	4.53 (4.31)	9.68 (9.22)
2	[C ₃₈ H ₃₈ FeN ₈ O ₄ S ₂] [790.75]	Dark brown	267–269	(2.41) 61	57.72 (58.13)	4.84 (4.47)	14.17 (14.44)
3	[C ₃₂ H ₂₈ FeN ₆ O ₄ S ₄] [744.72]	Dark brown	257–259	(2.31) 62	51.61 (51.76)	3.79 (3.57)	11.28 (11.52)
4	[C ₃₄ H ₃₂ FeN ₆ O ₆ S ₂] [740.64]	Brown	270–272	(2.22) 60	55.14 (55.42)	4.35 (4.26)	11.35 (11.63)

Table II. Antibacterial activity data of (SIF¹–SIF⁴).

Compound	Diameter of zones showing complete inhibition of growth (mm)				
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>S. typhi</i>
SIF ¹	13	14	11	12	12
SIF ²	14	15	13	11	14
SIF ³	16	14	12	14	12
SIF ⁴	15	16	11	13	13
DF	07	06	05	04	06
Imipenem	20	18	19	20	20

14–20 mm = significant activity; 7–13 mm = moderate activity; <7 mm = weak activity. DF = 1,1'-diacetylferrocene.

significant activity against *E. coli* and *P. aeruginosa* however, SIF³ and SIF⁴ were shown to be the most active one (Figure 3).

Antifungal bioassay (in vitro)

The antifungal screening of all compounds was carried out against *T. longifusus*, *C. albicans*, *A. flavus*, *M. canis*, *F. solani* and *C. glaberata* fungal strains according to the literature protocol [20]. All synthesized compounds showed good antifungal activity against different fungal strains. All compounds (SIF¹–SIF⁴) showed significant antifungal activity against all the fungal strains except *C. albicans* where moderate activity was observed. The results of inhibition were compared (Figure 4) with the results of inhibition of standard drugs miconazole and

amphotericin B (Table III). SIF³ was found to be the most antifungal compound (Figure 5).

Minimum inhibitory concentration (MIC) for antibacterial activity

The preliminary antibacterial screening showed that SIF², SIF³, SIF⁴ were the most active ones (above 80%), SIF₂ against *P. aeruginosa*, SIF₃ against *E. coli* and SIF₄ against *P. aeruginosa*. These compounds were therefore, selected for antibacterial minimum inhibitory concentration (MIC) studies (Table IV).

Cytotoxic bioassay (in vitro)

All the synthesized compounds were screened for their cytotoxicity (brine shrimp bioassay) using the protocol

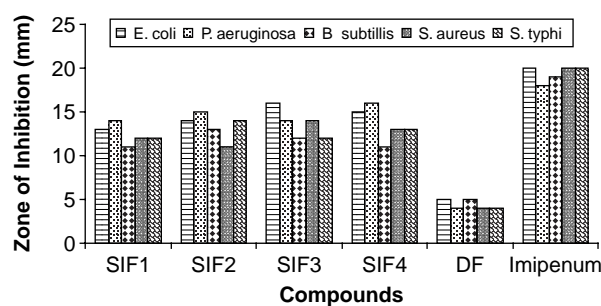


Figure 2. Comparison of antibacterial activity.

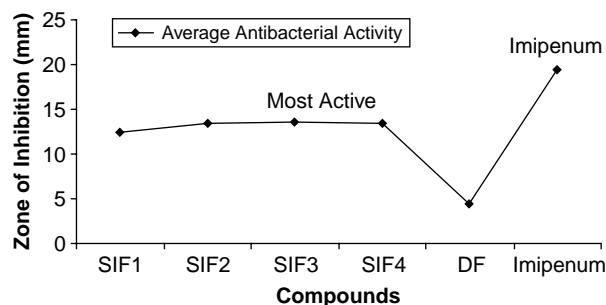


Figure 3. Average antibacterial activity showing the most active compound.

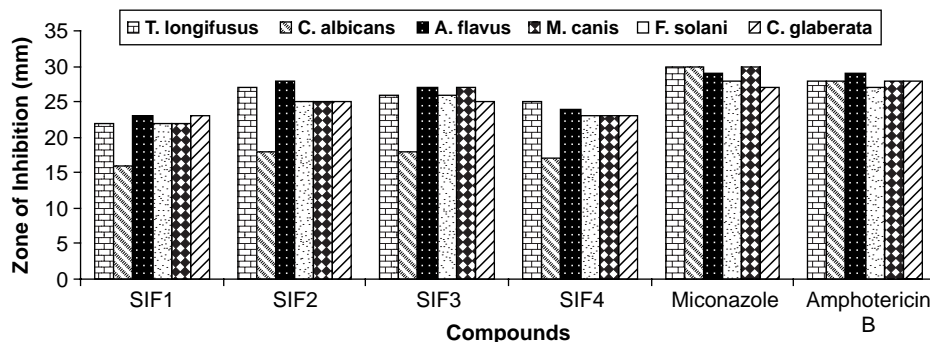


Figure 4. Comparison of antifungal activity.

Table III. Antifungal activity data of (SIF¹–SIF⁴).

Compound	Diameter of zones showing complete inhibition of growth (mm)					
	<i>T. longifusus</i>	<i>C. albicans</i>	<i>A. flavus</i>	<i>M. canis</i>	<i>F. solani</i>	<i>C. glaberata</i>
SIF ¹	22	16	23	22	22	23
SIF ²	27	18	28	25	25	25
SIF ³	26	18	27	26	27	25
SIF ⁴	25	17	24	23	23	23
Miconazole	30	30	29	28	30	27
Amphotericin B	28	28	29	27	28	28

20–30 mm = significant activity; 10–20 mm = moderate activity; <10 mm = weak activity.

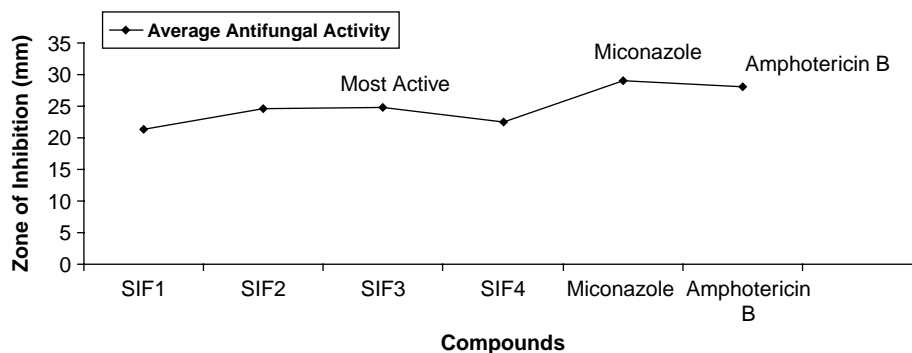


Figure 5. Average antifungal activity showing the most active compound.

Table IV. Minimum inhibitory concentration (M) of the selected compounds against selected bacteria.

Compound	<i>E. coli</i>	<i>P. aeruginosa</i>
SIF ²	–	1.729×10^{-4}
SIF ³	6.714×10^{-5}	–
SIF ⁴	–	6.751×10^{-5}

Table V. Brine shrimp bioassay data of (SIF¹–SIF⁴).

Compound	LD ₅₀ (M)
SIF ¹	1.253
SIF ²	0.794
SIF ³	0.799
SIF ⁴	0.824

of Meyer et al. [23]. From the data recorded in Table V, it is evident that all the compounds displayed potent cytotoxic activity against *Artemia salina*. SIF¹ showed activity (LD₅₀ = 1.253 M), SIF² showed activity (LD₅₀ = 0.794 M), SIF³ showed activity (LD₅₀ = 0.799 M) and SIF⁴ showed activity (LD₅₀ = 0.824 M) in the present series of compounds.

Conclusion

The results of this investigation support the suggested structures of the sulphonamide incorporated ferrocenes. It has been suggested [32,33] that some functional groups such as azomethine or heteroaromatics present in these compounds display extensive

biological activities that may be responsible for the increase of hydrophobic character and liposolubility of the molecules. It ultimately enhances activity of the compounds and biological utilization ratio.

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