Antimalarial, Anti-trypanosomiasis, Anti-HIV and Cytotoxicity Studies of Some Ferrocenyl Schiff bases

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***Abstract*—**An investigation into the potential biological application of a series of Ferrocenyl Schiff bases (compounds **5-12**) was carried out by evaluating their antimalarial, antitrypanosomiasis, antiHIV activities and toxicity towards HeLa cells. The synthesized ferrocenyl Schiff bases (**5-12**) were found to exhibit higher biological activities investigated in this study than their precursors (Compounds **1-4**). Among the derivatives compound **5** and **7** showed appreciable anti-trypanosomiasis activity. The anti-HIV activity showed that these compounds **5-12** have significant anti-HIV activity up to about 66% enzyme inhibition in the case of compound **7**. All the compounds showed low cytotoxicity towards HeLa cells.

***Keywords*—**Biological activity, Cytotoxicity, Ferrocene, Schiff bases.

I. Introduction

The applications of Schiff bases are reported in coordination [1], [2] and medicinal chemistry [3]-[5]. Aromatic Schiff bases of aromatic aldehydes are more utilised than their aliphatic counterparts [3]. This is because they are more stable which is attributed to the effective conjugation of the aromatic Schiff bases [3]. The presence of the azomethine (C=N) linkage is responsible for the biological activity of the Schiff bases [6]. They showed numerous biological activities such as antibacterial [7], [8], antifungal [9], antitumor [10], [11], anticonvulsants [7], [12], antihistaminic [7], antimalarial [13] [14], [15] anti-HIV [16], and anti-inflammatory activities [3]. Moreover, Schiff bases can be used in enzyme preparation and as vaccines for eradication of viral infections such as smallpox, poliomyelitis [17]. The synthesis of compounds based on the azomethine (C-N) linkage had been studied extensively due to their ease of preparation and stability [18]. Also, the ability to tune the Schiff bases structure both sterically and electronically, had made them ligands of interest in organometallic chemistry [19].

The fascinating chemistry of ferrocene such as its unique electronic properties, powerful donor capacity, ease of functionalisation, stability in aqueous and aerobic media had attracted great interest from synthetic chemists [20]-[24]. Ferrocene derived compounds have found many applications in medicine, sensors, catalysis and aerospace materials [25], [26]. Ornelas recently reviewed on the application of ferrocene and its derivatives in cancer research [11]. Ikhile *et al*. reported on the synthesis of ferrocenylimidazolium salts and their enhanced catalytic activity in transfer hydrogenation of ketones [27]. Also, Abd-Elzaher synthesized ferrocenyl Schiff bases and their use as ligands in the formation of 1:1 complexes with Co(II), Cu(II), Ni(II) and Zn(II) ions [1]. Numerous biological applications of ferrocene derived compounds exist in literature [28]-[30].

Most importantly, applications of these ferrocene based compounds in medicinal chemistry have attracted great interests [31], [32]. Many reports have shown that the substitution of an aromatic group with ferrocenyl moiety in synthetic drugs, improves their biological activity with lesser side effect [33]-[35]. The antitumor activities of a series of ferrocenyl compounds showing lower toxicity in comparison to clinically used drugs has also been reported in literature [11]; [36], [37]. Zaheer *et al*. reported on the low cytotoxicity, antifungal and DNA protection activities of some synthesized ferrocenyl Schiff bases [38]. The novel ferrocene derivatives synthesized by Yavuz and Yilldirim, displayed excellent antimicrobial activity [39]. However the anti-malarial, anti-trypanosomiasis and anti-HIV activities of ferrocenyl Schiff bases have been less widely reported. Therefore in continuation of our interest towards the synthesis of effective drugs with low toxicity, in this study the anti-malarial, anti-trypanosomiasis and anti-HIV activities of some ferrocenyl Schiff bases (Figure 1) previously synthesized in our laboratory were investigated [40]. The cytotoxicity activity of these novel ferrocenyl Schiff bases towards HeLa cells was also determined.

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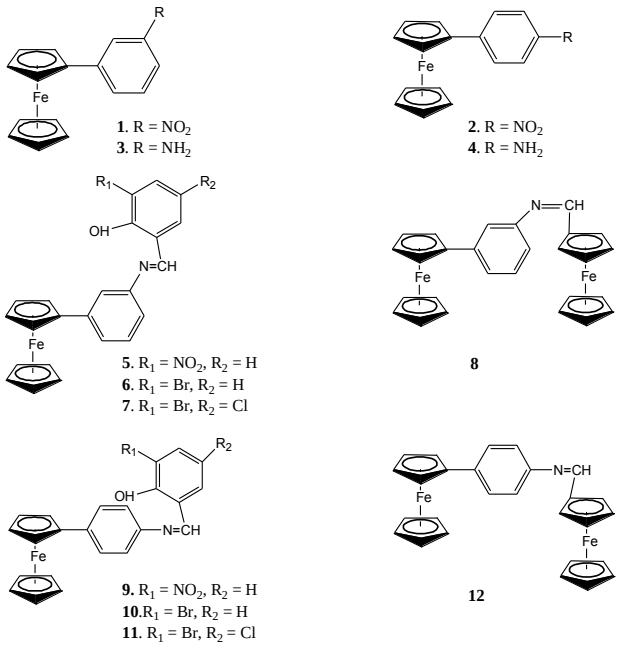

Fig. 1. Ferrocenyl Schiff bases (compounds **1**-**12**)

II. EXPERIMENTAL

A. Synthesis of Compounds *1-12*

The compounds **1-12** (Fig. 1) were synthesized according to a modified procedure [40]. The ferrocenyl Schiff bases **5-12** were synthesized from either 3-ferrocenylaniline or 4-ferrocenylaniline in 15 mL of dried ethanol which was reacted with an equimolar amount of corresponding aromatic aldehydes in 15 mL of dried ethanol. The mixture was heated under reflux for 6 h. Spectroscopic and analytical method were used to confirm the formation of the product [40].

B. Anti-malarial Assay

Malaria parasites (Plasmodium falciparum strain 3D7) were maintained in RPMI 1640 medium containing 2mM L-glutamine and 25 mM Hepes (Lonza). The medium is further supplemented with 5% Albumax II, 20 mM glucose, 0.65 mM hypoxanthine, 60 µg/mL gentamycin and 2-4% hematocrit human red blood cells. The parasites are cultured at 37^{o}C under an atmosphere of 5% CO_2, 5% O_2, 90% N_2 in sealed T25 or T75 culture flasks. For screening compounds **1-12** against malaria parasites, a solution of compounds **1-12** at 20 uM are added to the parasite cultures in 96-well plates and incubated for 48 h in a 37^{o}C CO_2 incubator. After 48 h the plates are removed from the incubator. Twenty µL of culture is removed from each well and mixed with 125 µL of a mixture of Malstat solution and NBT/PES solution in a fresh 96-well plate. These solutions measure the activity of the parasite lactate dehydrogenase (pLDH) enzyme in the cultures. A purple product is formed when pLDH is present, and this product was quantified in a 96-well plate reader by absorbance at 620nm (Abs620). The Abs620 reading in each well is thus an indication of the pLDH activity in that well and also the number of parasites in that well. For each compound concentration, % parasite viability – the PLDH activity in compound-treated wells relative to untreated controls is calculated. Compounds **1-12** were tested in duplicate wells, and a standard deviation (SD) was derived. For comparative purposes, Chloroquine (an anti-malarial drug) is used as a drug standard and yields IC_{50} values in the range 0.01-0.05 µM.

C. Anti-trypanosomiasis Assay

A solution Compounds **1-12** each are added to in vitro cultures of Trypanosoma brucei (T. B.) in 96-well plates at a fixed concentration of 20 µM. After an incubation period of 48 hours, the numbers of parasites surviving drug exposure are determined by adding a resazurin-based reagent. The reagent contains resazurin which is reduced to resorufin by living cells. Resorufin is a fluorophore (Exc560/Em590) and can thus be quantified in a multiwell fluorescence plate reader. Results are expressed as % parasite viability – the resorufin fluorescence in compounds-treated wells relative to untreated controls. Compounds **1-12** are tested in duplicate wells, and a standard deviation (SD) is also included. Generally, compounds/extracts that reduce parasite viability to < 10-20% are considered for further testing (e.g. dose-response and cytotoxicity assays). Pentamidine (an existing drug treatment for trypanosomiasis) at 1µM is used as a positive control drug standard.

D. Anti-HIV Assay

HIV-1 IN assay

The HIV-1 IN strand transfer inhibition assay was adapted from previously described methods [29]. Briefly, 20 nM double-stranded biotinylated donor DNA (5'-5BiotinTEG/ACCCTTTTAGTCAGTGTGGAAAATCTCTAGCA-3' annealed to 5'-ACTGCTAGAGATTTTCCACACTGACTAAAAG-3') was immobilized in wells of streptavidin-coated 96-well microtiter plates (R&D Systems, USA). Following incubation at room temperature for 40 minutes and a stringent wash step, 5 µg/ml purified recombinant HIV-1 subtype C IN in integrase buffer 1 (50 mm NaCl, 25 mM Hepes, 25 mM $MnCl_2$, 5 mM β-mercaptoethanol, 50 µg/ml BSA, pH 7.5) was added to individual wells. Test compounds **1-12** and chicoric acid were added to indi vidual wells to a final concentration of 20 µM. Recombinant HIV-1 subtype C IN was assembled onto the pre-processed donor DNA through incubation for 45 minutes at room temperature. Strand transfer reaction was initiated through the addition of 10 nM (final concentration) double-stranded FITC-labelled target DNA (5'-TGACCAAGGGCTAATTCACT/36-FAM/-3' annealed to 5'-AGTGAATTAGCCCTTGGTCA-/36-FAM/-3') in integrase buffer 2 (same as buffer 1, except 25 mm $MnCl_2$ replaced with 2.5 mm $MgCl_2$). After an incubation period of 60 minutes at 37 °C, the plates were washed using PBS containing 0.05% Tween 20 and 0.01% BSA, followed by the addition of peroxidase-conjugated sheep anti-FITC antibody (Thermo Scientific, USA) diluted 1:1000 in the same PBS buffer. Finally, the plates were washed and peroxidase substrate (SureBlue Reserve™, KPL, USA) was added to allow for detection at 620 nm using a Synergy MX (BioTek®) plate reader. Absorbance values were converted to % enzyme

activity relative to the readings obtained from control wells (enzyme without inhibitor).

HIV protease assay

The HIV protease assay was performed using the flourogenic substrate Arg-Glu(EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(DABCYL)-Arg (Sigma Aldrich) as previously described by Lam *et al.* [30]. The substrate was dissolved in DMSO to make a 500 µM stock. Compounds **1-12** were diluted to desired concentrations in the reaction buffer (0.1 M sodium acetate, 1 M NaCl, 1 mM EDTA, 1 mM DTT and 0.1 % BSA, 5% DMSO, pH 4.7) in a separate plate before being added to a fluorescence assay plate at 50 µl per well. Substrate (25 µl) to the final concentration of 8 µM and enzyme (25 µl) to the final concentration of 50 ng/µl were added. The mixture was incubated at 37 °C for 40 minutes after which fluorescence was read at an excitation wavelength 340 nm and emission wavelength 485 nm using a Synergy MX (BioTek®) plate reader. Ritonavir was used as the standard inhibitor. Flourescence values were converted to % enzyme activity relative to the readings obtained from control wells (enzyme without inhibitor).

E. Cytotoxicity Assay

For the cytotoxicity testing of the compounds **1-12**, they are incubated at a fixed concentration of 20 µM in 96-well plates containing HeLa (human cervix adenocarcinoma) cells for 48 hours. The numbers of cells surviving drug exposure are also determined by using the resazurin based reagent and reading resorufin fluorescence in a multiwell plate reader. The results are also expressed as **% cell viability**, based on fluorescence reading in treated wells vs. untreated control well. Emetine (which induces cell apoptosis) is used as a positive control drug standard.

III. Results and Discussion

All the synthesized ferrocenyl compounds **1-12** were evaluated for their antimalarial, antitrypanosomiasis, and antiHIV activity. The cytotoxicity testing of all the compounds against HeLa cells was also investigated. The result in Table I was obtained for the antimalarial, antitrypanosomiasis and cytotoxicity testing. The result are reported in %parasite viability and the testing were done in duplicate and standard deviation were also included. Malaria disease are usually caused by plasmodium parasite which are transmitted through mosquitoes to vertebrates [41]. However, chloroquine drugs which are usually prescribed for the treatment of malaria have been reported to suffer resistance especially with *P. falciparum* which is the causative agents for most of the life threatening malarial disease to human [42]. Also, other malarial drugs such as amodiaquine was discovered to combat the chloroquine resistant strain but the clinical use of amodiaquine was restricted due to toxicity [41].

Therefore, in continuation of our interest in synthesis of effective drugs with low cytotoxicity, the antimalarial activity of compounds **1-12** was evaluated against *P. falciparum* strain 3D7 by measuring the activity of the parasite lactate dehydrogenase (pLDH) enzyme in the culture. The formation of a purple colour is an indication of the presence of pLDH, which was quantified in a 96-well plate reader by absorbance at 620 nm (Abs620). Compounds **1-12** showed very low antimalarial activity, with %parasite viability ranges from 62.6 – 117.6%. Generally test compounds that reduces parasite viability to < 10-20% implies relatively high antimalarial activity [43]. This might be attributed to the lipophilicity nature of the Schiff bases which restricted permeation into the *P. falciparum* strain cell wall [44].

Also, the anti-trypanosomiasis activity was investigated by addition of compounds **1-12** to *in vitro* cultures of *T.B. brucei* in 96-well plates at a fixed concentration of 20 µM. Penamidine which is an existing treatment for trypanosomiasis was used as positive control drug standard at a concentration of 1 µM. The result (Table I) indicated that some of the compounds **1-12** showed significant activity. However, compounds **5** and **7** showed excellent activity against *T.B. brucei* with %parasite viability of 17.8 and 15.5% respectively. Generally the 3-ferrocenyl Schiff bases reported higher anti-antitrypanosomiasis than their 4-ferrocenyl Schiff bases counterpart. The two electron- withdrawing group (Br and Cl) in compound **7** might have also contributed to the highest anti-trypanosomiasis reported for compound **7**. Arancibia *et al.* had also reported an increase in anti-trypanosomiasis due to the Schiff bases containing more electron-withdrawing group [45].

Furthermore, the antiviral activity of compounds **1-12** were also investigated against human immunodeficiency virus type 1 (HIV-1). This was achieved by examining the inhibitory effect of HIV integrase (HIV-1 IN) and protease (HIV-1 PR) enzymes. The results are shown in Fig. 2 and Table II for HIV-1 IN and HIV-1 PR respectively. Chicoric acid and Ritonavir was used as positive (+ve) control for HIV-1 IN and HIV-1 PR assay respectively for comparison purposes. In general compounds 1-12 were found to showed good inhibitory effects on HIV integrase (Fig. 2) and they showed no significant inhibition effect on HIV protease enzymes (Table II). Compound **7** gave the highest inhibition against HIV-1 IN with %inhibition of 65.6%. Compound **6**, **8**, **10** and **12** were able to showed moderate inhibition against HIV-1 IN with 56.7, 53.0, 50.1 and 50.8% inhibition respectively. The low %inhibition reported for compound **5** and **9** might be attributed to the presence of nitro group in their compounds. Arancibia *et a*l. [45] had also reported a low parasitic activity of some compounds containing $-NO_2$ groups. It seem the presence of bromine/chlorine group (compound **6**, **7** and **10**) and two ferrocenyl moieties (compound **8** and **12**) in the Schiff bases structure is a determining factor for significant inhibition against HIV-1 IN enzyme.

In addition compounds **1-12** were screened for cytotoxicity by testing their effects on human cervix adenocarcinoma cells in order to balance their biological activity and toxicity. The cytotoxicity results are also expressed as %cell viability as showed in Table I. The criteria for cytotoxicity is %cell viability ≤ 50%. All the compounds (**1-12**) showed low toxicity because %cell viability was above 50%. This is not surprising because it had earlier been reported that ferrocenyl derived compounds do possess low toxicity [46]-[48].

TABLE I: %VIABILITY AND STANDARD DEVIATION (SD) RESULT OBTAINED FOR COMPOUNDS 1-12 TESTED FOR ANTIMALARIAL ACTIVITY, ANTI-TRYPANOSOMIASIS ACTIVITY AND CELL TOXICITY.

Compounds	PLDH (Malaria) Viability %	Trypanosomes Viability %	Cell toxicity Viability %
1	117.5745	100.0000	76.2916
2	67.4418	97.1580	102.8157
3	99.6002	98.3452	77.8946
4	65.8183	74.0735	63.9382
5	103.1402	17.7593	84.3532
6	95.6087	100.6046	101.4345
7	65.3178	15.3593	79.51738
8	90.1035	100.4838	95.5278
9	62.6079	56.0207	83.4300
10	74.9733	33.7332	94.7650
11	103.1862	79.0651	86.9199
12	106.2899	107.3187	65.57631

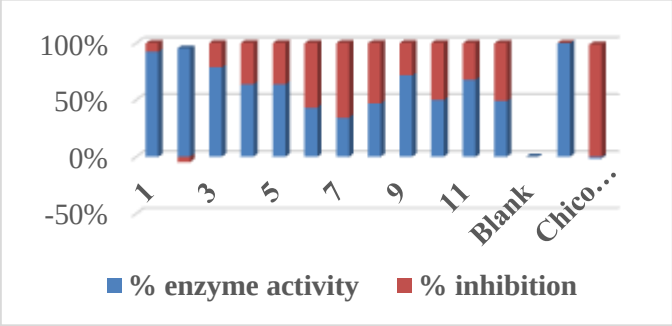

Fig. 2. Comparison of % enzyme activity and % inhibition by compounds **1-12** and controls (blank, -ve and +ve) for HIV-IN assay

IV. Conclusion

The biological activity of ferrocenyl compounds **1-12** were determined by investigating their antimalarial, anti-trypanosomiasis and antiHIV activity. The cytotoxicity testing was also carried out on human cervix adenocarcinoma cells. All the compounds showed low cytotoxicity towards HeLa cells. Compounds **7** was found to show the highest anti-trypanosomiasis and anti-HIV-1 IN activities. Compounds **1-12** showed very low antimalarial activity, with %parasite viability ranges from 62.6 – 117.6%. Compound **6**, **8**, **10** and **12** were able to showed moderate inhibition against HIV-1 IN. The moderate anti-trypanosomiasis, anti-HIV-1 IN activities and low cytotoxicity reported for some of these compounds encourage further assay in determination of IC_{50} for these compounds.

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TABLE II. % ENZYME ACTIVITY, % INHIBITION AND STANDARD DEVIATION (SD) RESULT OBTAINED FOR COMPOUNDS 1-12 TESTED FOR ANTIHIV PRO.

Compounds	% enzyme activity	% inhibition	Standard deviation
1	105,4899646	-5,489964581	369,10974
2	101,3724911	-1,372491145	82,0243866
3	127,1157991	-27,11579914	523,259018
4	120,1261529	-20,12615287	4388,30468
5	125,248242	-25,24824199	4881,15811
6	127,705926	-27,705926	4320,42243
7	118,7197427	-18,71974275	4723,4733
8	104,4930916	-4,493091629	2967,02005
9	92,97324994	7,026750062	3000,96118
10	103,4117813	-3,411781335	3771,70757
11	115,0517686	-15,05176861	2788,82914
12	109,6045797	-9,604579667	3976,06143
Blank	0		529,622979
Normal rxn (-ve ctrl)	100	0	2745,69563
Ritonavir (+ve ctrl)	-4,493091629	104,4930916	287,085353

References

[1] M. M. Abd-Elzaher, "Synthesis and Spectroscopic characterization of some Ferrocenyl Schiff bases containing pyridine moiety and their complexation with coJbalt, nickel, copper and zinc," *J. Chin. Chem. Soc*., vol. 51, pp. 499-504, June 2004. https://doi.org/10.1002/jccs.200400074

[2] F. U. Shah, M. Jamil, J. Aslam, A. Gul, Z. Akhter, and B. Mirza, "Influence of Ferrocene and Transition metals on the biological activities of Schiff bases," *J. Chem. Soc. Pak*., 38, pp. 1112-1120, 2016.

[3] S. Arulmurugan, H. P. Kavitha, and B. R. Venkatraman, "Biological activities of Schiff bases and its complexes: A review," *Rasayan J. Chem*., vol. 3, pp. 385-410, July 2010.

[4] D. M. Aysen, O. Birol, and M. Bedrettin, "Synthesis and antibacterial activity of Schiff base derivatives," *IJDDR*., vol. 2, pp. 102-107, January 2010.

[5] K. Brodowska, and E. Lodyga-Chruscinska, "Schiff bases – Interesting range of applications in various fields of science,"*CHEMIK*., vol. 68, pp. 129-134, 2014.

[6] S. Annapoorani, and C. Krishnan, "Synthesis and spectroscopic studies of trinuclear N4 Schiff base complexes," *international. J. ChemTech Res*., vol. 5, pp. 180-185, January 2013.

[7] D. Sinha, A. K. Tiwari, S. Singh, G. Shukla, P. Mishra, H. Chandra, and A. K. Mishra, "Synthesis, characterization and biological activity of Schiff base analogues of indole-3-carboxaldehyde," *European J. Med. Chem*., vol. 43, pp. 160-165, January 2008. https://doi.org/10.1016/j.ejmech.2007.03.022

[8] S. Kumar, D. N. Dhar, and P.N. Saxena. "Applications of metal complexes of Schiff bases-a review," *J. Sci.Ind. Res*., vol. 68, pp. 181-187, March 2009.

[9] R. K. Kumar, and R. Kant, "Review on synthesis and application of Schiff base and its transition metal complexes," *Res J. Chem. Environ. Sci*. vol. 2(2), pp. 1-4, April 2014. https://doi.org/10.1016/j.ces.2014.05.023

[10] P. Przybylski, A. Huczynski, K. Pyta, B. Brzezinski, and F. Bartl, "Biological properties of Schiff bases and azo derivatives of phenols," *Curr. Org. Chem*., Vol. 13, pp. 124-148, 2009. https://doi.org/10.2174/138527209787193774

[11] C. Ornelas, "Application of ferrocene and its derivatives in cancer research," *New J. Chem*., vol. 35, pp. 1973-1985, May 2011. https://doi.org/10.1039/c1nj20172g

[12] S. K. Sridhar, S. N. Pandeya, J. P. Stables, and A. Ramesh, "Anticonvulsant activity of hydrazones, Schiff and Mannich bases of isatin derivatives," *Eur. J. Pharm. Sci*., vol. 16, pp. 129-132, August 2002. https://doi.org/10.1016/S0928-0987(02)00077-5

[13] W. Qin, S. Long, M. Panunzio, and S. Biondi, "Schiff bases: A short survey on an evergreen chemistry tool," *Molecules*," vol 18, pp. 12264-12289, October 2013.

https://doi.org/10.3390/molecules181012264
[14] P. Rathelot, P., Vanelle, M. Gasquet, F. Delmas, M. P. Crozet, P. Timon-David, and J. Maldonado, "Synthesis of novel functionalised 5-nitroisoquinolines and evaluation of in vitro antimalarial activity," *Eur. J. Med. Chem*., Vol. 30, pp. 503-508, January1995.
https://doi.org/10.1016/0223-5234(96)88261-4
[15] C. Silva da, D. Silva da, L. Modolo, and R. Alves, "Schiff bases: A short review of their antimicrobial activities," *J. Ad. Res*., vol 2, pp. 1-8, July 2002.
[16] S. N. Pandeya, D. Sriram, G. Nath, and E. De Clercq, "Synthesis, antibacterial, antifungal and anti-HIV activity of Schiff and Mannich bases of isatin with N-[6-chlorobenzothiazol-2-yl]thiosemicarbazide.," *Indian J. Pharm. Sci*., vol. 56, pp. 174-177, July1994.
[17] P. Przybylski, M. Maluszynska, and B. Brzezinski, "Spectroscopic and semiempirical studies of new Schiff base of gossypol with allyamine in solution," *J. Mol. Struct.*, vol. 750, pp. 152-157, August 2005.
https://doi.org/10.1016/j.molstruc.2005.04.027
[18] A. S. Thakar, H. B. Friedrich, T. Krishnalal, and G. E. M. Maguire, Isolation, characterization and X-ray structure determination of the Schiff base ligand: 5-methyl-2-phenyl-4-[phenyl-(4-phenyl-thiazol-2-ylamino)-methylene]-2,4-dihydro-pyrazol-3-one. *S. Afri. J. Chem*., vol. 68, pp. 39-44, December 2015.
https://doi.org/10.17159/0379-4350/2015/v68a6
[19] K. K. Upadhyyay, A. Kumar, S. Upadhyay, and P. C. Mishra, "Synthesis, characterization, structural optimization using density functional theory and superoxide ion scavenging activity of some Schiff bases," *J. Mol. Struct*., vol. 873, pp. 5-16, February 2008.
https://doi.org/10.1016/j.molstruc.2007.02.031
[20] E. A. Hillard, A. Vessieres, and G. Jaouen, "The potential of organometallic complexes in medicinal chemistry,"*Top. Organomet. Chem*., vol. 32, pp. 81-117, 2010.
https://doi.org/10.1007/978-3-642-13185-1_4
[21] A. Togni, and T. Hayashi, "Ferrocenes. Homogeneous catalysis," Organic synthesis. Materials science; VCH, Weinheim, 1995.
[22] T.-Y. Dong, P.-H. Ho, and C.-K Chang, "Synthesis of new unsymmetrical 1,1- disubstituted Ferrocenyl ligands," *J. Chin. Chem. Soc*., vol. 47, pp.421-424, June 2000.
https://doi.org/10.1002/jccs.200000056
[23] S. A. Abd-El-Aziz, S. A. Carruthers, P. M. Aguiar, and S. Kroeker, "Hyberbranched polymers containing cyclopenta-dienyliron complexes," *J. Inorg. Organomet. Polym. Mater*, vol. 15, pp. 349-359, September 2005.
https://doi.org/10.1007/s10904-005-7876-3
[24] C. S. Allardyce, A. Dorcier, C. Scolaro, C., and P. J. Dyson, "Development of organometallic (organotransition metal) pharmaceuticals," *Appl. Organomet.Chem*., vol. 19, pp. 1-10, 2005.
https://doi.org/10.1002/aoc.725
[25] S. R. Gupta, P. Mourya, M. M. Singh, and V. P. Singh, "Synthesis, structural, electrochemical and corrosion inhibition properties of two new ferrocene Schiff bases derived from hydrazides," *J. Organomet. Chem*., vol. 767, pp. 136-143, September 2014.
https://doi.org/10.1016/j.jorganchem.2014.05.038
[26] K. Mathiyalagan, S. Gopal, E. Ramasamy, and T. Vennila, "In-vitro antimicrobial screening of ferrocene derived compounds," *Int. J. ChemTech Res*., Vol. 4(4), pp. 1775-1781, October 2012.
[27] M. I. Ikhile, M. D. Bala, V. O. Nyamori, and J. C. Ngila, "Application of ferrocenylimidazolium salts as catalysts for the transfer hydrogenation of ketones," *Appl. Organometal. Chem*., vol. 27, pp. 98-108, February 2013.
https://doi.org/10.1002/aoc.2947
[28] E. M. Njogu, B. Omondi, and V. O. Nyamori, "Synthesis, physical and antimicrobial studies of ferrocenyl-*N*-(pyridinylmethylene) anilines and ferrocenyl-*N*-(pyrinylmethyl) anilines," *S. Afr. J. Chem*., vol. 69, 51-66, January 2016.
https://doi.org/10.17159/0379-4350/2016/v69a7
[29] C. Biot, L. Delhaes, L. A. Maciejewaski, M. Mortuaire, D. Camus, D. Dive, and J. S. Brocard, "Syntheticferrocenic mefloquine and quinine analoguesas potential antimalarial agents," *Eur. J. Med. Chem*., vol. 35, pp. 707-714, August 2000.
https://doi.org/10.1016/S0223-5234(00)00178-1
[30] B. Li, "Preparation, spectroscopy and electrochemistry of some ferrocenyl Schiff base derivatives with NLO responses," *Indian J. Chem. Sec B*., vol. 42B, pp. 2643-2648, August 2003.
[31] Z. H. Chohan, M. E. Jaffery, and C. T. Supuran, "Antibactrial Co(II), Cu(II), Ni(II) ansd Zn(II) complexes of thiadiazoles Schiff bases," *Met.-Based Drugs*., vol. 8, pp. 95, 2001.
https://doi.org/10.1155/MBD.2001.95
[32] Z. H. Chohan, and M. Praveen, "Synthesis, characterization and anti-bacterial properties of symmetric 1,1'-ferrocene derived Schiff-base ligands and their Co(II), Cu(II), Ni(II) and Zn(II) chelates," *Appl. Organomet. Chem*., vol. 14, pp. 376-382, July 2000.
https://doi.org/10.1002/1099-0739(200007)14:7%3C376::AID-AOC995%3E3.0.CO;2-5
[33] H. Yu, L. Shao, and J. Fang, "Synthesis and biological activity research of novel ferrocenyl-containing thiazole imine derivatives," *J. Organomet. Chem*., vol. 692(5), pp. 991-996, February 2007.
https://doi.org/10.1016/j.jorganchem.2006.10.059
[34] Y.-S Xie,, H.-L Zhao, and H.Su et al., "Synthesis, single-crystal characterization and preliminary biological evaluation of novel ferrocenyl pyrazolo[1,5-α]pyrazine-4(5H)-one derivatives," *Eur. J. Med. Chem*., vol. 45(1), pp. 210-218, January 2010.
https://doi.org/10.1016/j.ejmech.2009.09.046
[35] M. Zora, and M. Gormen, "Synthesis of ferrocenyl pyrazoles by the reaction of (2-formyl-1-chlorovinyl) ferrocene with hydrazines," *J. Organomet. Chem*., vol. 692(22), pp. 5026-5032, October 2007.
https://doi.org/10.1016/j.jorganchem.2007.07.029
[36] P. Kopf-Maier, H. Kopf, and E. W. J. Neuse, "Ferricenium complexes: a new type of water-soluble antitumor agent," J. *Cancer Res. Clin. Oncol*., vol. 108, pp. 336-340, 1984.
https://doi.org/10.1007/BF00390468
[37] P. Govender, H. Lemmerhirt, A. T. Hutton, B. Therrien, P. J. Bednarski, and G. S. Smith, "First-and second-generation heterometallic dendrimers containing ferrocenyl-ruthenium(ii)-arene motifs: synthesis, structure, electrochemistry and preliminary cell proliferation studies," *Organometallics*, vol. 33, pp. 5535-5545, October 2014.
https://doi.org/10.1021/om500809g
[38] M. Zaheer, A. Shah, Z. Akhter, R. Qureshi, B. Mirza, M. Tauseef, and M., Bolte, "Synthesis, characterization, electrochemistry and evaluation of biological activities of some ferrocenyl Schiff bases," *Appl. Organometal. Chem*., vol. 25, pp. 61-69, January 2011.
https://doi.org/10.1002/aoc.1690
[39] S. Yavuz, and H. Yildirim, "Ferrocene derivatives carrying Urea, thiourea and sulphonamide moieties: Synthesis and evaluation of antibacterial and antifungal activities," pp. 1-7, 2013. http://dx.doi.org/10.1155/2013/14963
[40] M. I. Ikhile, and J. C. Ngila, "Synthesis and characterization of some ferrocenylphenylimine compounds," *Chin. J. Inorg. Chem*., vol. 31(10), pp. 2079-2088, October 2015.
[41] N. Kaiser, N. Camargo, and S. H. I. Kappe, "Transformation of sporozoites into early exoerythrocytic malaria parasites does not require host cells," *J. Exp. Med*., vol.197, no.8, pp.1045–1050, April 2003.
https://doi.org/10.1084/jem.20022100
[42] H. Marijana S. Kristina, K. P. Sandra, L. Pero, P. Kresimir, and K. Z. Grace, "Synthesis, spectroscopic characterization and antiproliferative evaluation in vitro of novel Schiff bases related to benzimidazoles," *Eur. J. Med. Chem*. Vol. 46, pp. 2274, June 2011.
https://doi.org/10.1016/j.ejmech.2011.03.008
[43] G. Garg, A. Acharya and K. Patel, "New potential antimalarial agents: synthesis and biological evaluation of some Schiff base ligand as antimalarial agents," *IJPSR*, vol. 4(9), pp. 3588-3595, September 2013.
[44] N. Raman, S. J. Raja, and A. Sakthivel, "Transitions metal complexes with Schiff base ligands: 4-aminoantipyrine based derivatives- a review," *J. Coord Chem*., vol. 62, pp. 691-705, February 2009.
https://doi.org/10.1080/00958970802326179
[45] R. Arancibia, A. H. Klahn, G. E. Buono-Core, D. Contreras, G. Barriga, C. Olea-Azar, M. Lapier, J. D. Maya, A. Ibañez, and M. T. Garland, "Organometallic Schiff bases derived from 5-nitrothiophene and 5-nitrofurane: Synthesis, crystallographic, electrochemical, ESR and antiTrypanosoma cruzi studies," *J. of Organomet. Chem*., vol. 743, pp. 49-54, October 2013.
https://doi.org/10.1016/j.jorganchem.2013.06.014
[46] L. V. Popova, V. N. Babin, Y. A. Belousov, Y. S. Nekrasov, A. E. Snegireva, N. P. Borodina, G. M. Shaposhnikova, O. B. Bychenko, P. M. Raevskii, N. B. Morozova, A. I. Liyina, and K. G. Shitkov, "Antitumor effect of binuclear ferrocene derivatives," *Appl. Organomet. Chem.*, vol. 7, pp. 85-94, April 1993.
https://doi.org/10.1002/aoc.590070203

[47] L. V. Snegur, A. A. Simenel, Y. S. Nekrasov, E. A. Morozova, Z. A. Starikova, S. M. Peregudova, Y. V. Kuzmenko, L. A. Babin, L. A. Ostrovskaya, N. V. Blucchterova, and M. M. Fomina, "Synthesis, structure and redox potentials of biologically active ferrocenylalkyl azoles," *J. Organomet. Chem*., vol. 689, pp. 2473-2479, August 2004. https://doi.org/10.1016/j.jorganchem.2004.05.001

[48] A. A. Simenel, S. V. Samarina, L. V. Snegur, Z. A. Starikova, L. A. Ostrovskaya, N. V. Blucchterova, M. M. Fomina, *o*-Carboxybenzoylferrocene, bioactivity and chemical modifications. Appl. Organomet. Chem., vol. 22, pp. 276-280, May 2008. https://doi.org/10.1002/aoc.1397