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SYNTHESIS OF BIOLOGICALLY ACTIVE HETEROCYCLES AS USEFUL LEADS TOWARDS THE DEVELOPMENT OF POTENT ANTIMICROBIAL AGENTS

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ABSTRACT

The chemical structures of the produced products were determined by the analysis of their IR, ^{1}H NMR, and ^{13}C NMR spectra and the detection of their physicochemical attributes. We used the agar dilution technique to test the antibacterial efficacy of coumacines in vitro against a panel of typical aerobic and anaerobic bacterial strains, with ciprofloxacin and metronidazole serving as positive controls. From what can be seen in the data, compounds 1–12 had only moderate antibacterial efficacy against the strains used in the tests, with compound 12 showing the most promise. The MIC values against S for all of these drugs ranged from 32 to 64 lg/ml. E. bovis E. coli and Klebsiella pneumoniae. Except for compound 12, which had a MIC of 16 µg/ml against all strains of T. tropicalis tested, all other compounds showed no activity. These chemicals were tested for their minimum inhibitory concentrations (MICs) against S. aureus and P. aeruginosa biofilms. mutans were found to be between 64 and 256 µg/ml and 64 and 128 µg/ml, respectively, which appears high, leading one to the conclusion that these compounds have enormous potential to be used as a two in one formulation of antibacterial and antifungal agents.

Keywords: Heterocyclic compounds, Antifungal activity, Antibacterial, Thiazoles, Pyrazoline

I. INTRODUCTION

Bacterial infections are a major cause of death and illness, especially in underdeveloped regions. Salmonella, diarrhea, pandemics, rheumatic fever, and food poisoning are just a few of the ailments that can be caused by a bacterial infection. Because of this widespread usage and misuse of antibacterial medications, a rising number of microbes have emerged with diverse resistances to previously effective treatments.

Conventional antimicrobials work by blocking the creation of proteins, RNA, DNA, cell wall, and folic acid, which is a rather narrow range of biological functions. Inhibitors against such targets continue to be discovered, albeit at a far lower rate than during the "golden era" of antibiotic drug discovery.

Antimicrobial resistance is a serious problem since both established and emerging infectious illnesses continue to pose a significant risk to human health all over the world. It's important to note that during the past few decades, the prevalence of systemic fungal infections has grown.

Researchers in the field of medicinal chemistry often look to heterocyclic compounds as potential targets. Over the past decade, scientists have synthesized a large number of heterocyclic compounds, making it more difficult to discover novel antimicrobials with the potential to treat illnesses caused by bacterial strains that are resistant to existing treatments. Key structural units in many pharmacological preparations include five-membered heterocyclic containing two or three heteroatoms, such as thiazoles, benzothiazoles, thiazolidinones, and triazoles. Recently, azoles have received considerable interest, particularly in the

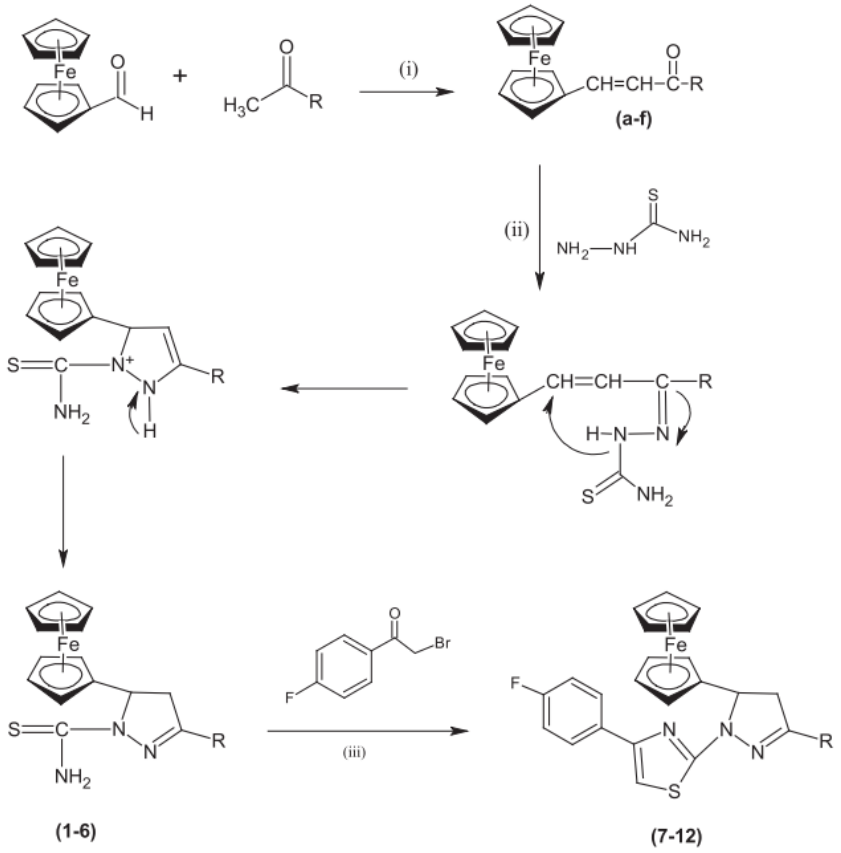
context of the development and synthesis of compounds with noteworthy biological activity.

II. SYNTHESIS

Scheme 1 depicts the synthetic sequences used to create the aforementioned chemicals. A Claisen-Schmidt condensation of substituted acetophenones/1-phenyl butane1-one/2-acetyl ferrocene with ferrocene carboxaldehyde in the presence of KOH and 100% ethanol yielded the ferrocenyl chalcones (a-f). The pyrazoline analogues of ferrocenyl chalcones (a-f) were obtained by cyclizing them with thiosemicarbazide and sodium hydroxide in the presence of absolute ethanol, yielding the corresponding 5-ferrocenyl-3-aryl4, 5-dihydro-1H-pyrazole-1-carbothioamides (1-6), which were then cyclized with 2-bromo-40 fluro acetophen (7–12). The mechanism suggests that thiosemicarbazone production promotes the synthesis of pyrazoline analogues by undergoing cyclization under basic condition to generate the required pyrazoline ring in all the compounds.

All the synthesized compounds were characterized by spectroscopic methods such as IR, ^{1}H NMR ^{13}C NMR and Mass and the purity of compounds was confirmed by elemental analysis and melting points. The melting temperatures of all the compounds were quite distinct, and the elemental analyses agreed to within 0.30 percentage points. The experimental part presents the analytical data.

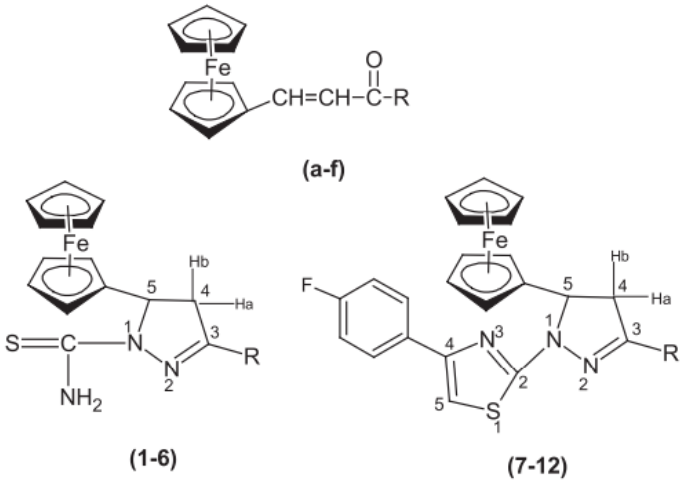
Condensation of substituted ketones/1-phenyl butane-1-one/2-acetyl ferrocene with ferrocene carboxaldehyde is suggested by the presence of distinctive bands at 1642-1655 cm^{-1} and 1568-1580 cm^{-1} in the IR spectra of ferrocenyl chalcones (a-f). The ^{1}H NMR spectra were used to verify the structures of all of these compounds. Hα and Hβ are transisomers because doublets arise in the area d δ 6.75-7.35 ppm and 6.82-7.71 ppm, respectively, with coupling constants (J) in the range of 15.2-16 Hz. The experimental data shows that the rest of the proton levels fell within the predicted range. 13C NMR data provided further confirmation of the structures of the compounds (a-f). In the region of d 189.81-192.00 ppm, a signal indicative of ferrocenyl chalcones (C=O) was detected. All compounds had an α, β unsaturated keto function, as evidenced by the δ 120.23–122.43 and 142.46–146.82 ppm signals (a–f).354



Scheme 1: General synthesis of 5-ferrocenyl-3-substituted aryl-4, 5-dihydro-1H-pyrazol-1-carbothioamides (1–6) and their cyclized derivatives (7–12).

IR spectra of pyrazoline analogues (1-6) of ferrocenyl chalcones (a-f) revealed important information on the structures of the compounds through the presence of certain diagnostic bands. The ν (C=S) stretch of the thiocarboxamide group was clearly visible in the area 1032-1078 cm^{-1} for all the compounds. All of the

compounds had the same ν (C=N) stretch in their IR spectra, visible between 1524 and 1587 cm^{-1}, as a result of the ring closure. Moreover, the 1125-1215 cm^{-1} absorption bands were assigned to the ν (C=N) stretch vibrations, proving the production of the required pyrazoline ring in all the compounds. The ^{1}H NMR spectra gave diagnostic tools for the positional elucidation of the protons, providing further support for the structures of the pyrazoline analogues. Signal identification is performed using chemical changes and intensity patterns. Fig. 1 shows that the geminal protons Hα and Hβ at the C-4 carbon of pyrazolines emerged as doublets of doublets in the 3.35–3.36 ppm and 3.50–3.83 ppm ranges, respectively, in all the compounds. Due to vicinal interaction with two nonequivalent germinal protons of C-4 carbon, the C-H proton (H_x) of the pyrazoline ring also showed as a doublet of doublets in the area 6.30-5.91 ppm. In the experimental part, we see that the integral values and chemical shifts of the protons in the aromatic ring and ferrocenyl group fall within the predicted range. Spectral analysis was also used to verify the pyrazoline derivatives' (7-12) structures. The m (C=N) stretch at 1582–1598 cm^{-1} in the IR spectra of all these compounds is indicative of thiazole ring formation. These chemicals' suggested structures are corroborated by their ^{1}H NMR spectra. To verify the development of the thiazole ring, the H-5 proton was detected as a singlet between 6.68 and 6.82 ppm in all the compounds. Furthermore, five protons of unsubstituted Cp emerged as a singlet in the range of 4.24-4.27 ppm, while four protons in mono-substituted Cp of ferrocene moiety of compounds (7-12) appeared as three singlet peaks in the region of 4.68-4.82, 4.64-4.69, and 4.59-4.61 ppm. Additionally, mass spectrometry validated the structures of all freshly synthesized compounds (1-6) and (7-12) by seeing all the predicted aromatic and aliphatic protons with the expected chemical shift and integral values, as demonstrated in the data supplied in the experimental section. Every substance had a characteristic molecular ion peak that matched its molecular formula.



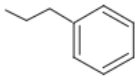
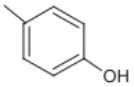
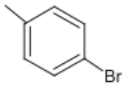
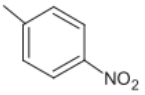
Compounds	R	Compounds	R	Compounds	R
a, 1, 7		b, 2, 8		c, 3, 9	
d, 4, 10		e, 5, 11		f, 6, 12	

Figure 1: Novel nitrogen and sulphur containing organometallic heterocycles (1–12).

III. IN VITRO ANTIMICROBIAL STUDY

By using the broth microdilution method, all of the recently synthesised compounds (1-6) and (7-12) were

tested for antimicrobial activity against 15 ATCC strains (8 bacteria; P. aeruginosa; S. bovis; E. faecalis; K. pneumonia; E. coli; E. cloacae; MRSA; S. mutans; and 7 fungi; C. albicans; C. dubliniensis; C. The effectiveness of the antibiotics was tested on eight different bacteria: four Gram-positive (Streptococcus bovis, Enterococcus, faecalis, Methicillin-resistant Staphylococcus aureus, and Streptococcus mutans) and four Gram-negative (Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, and Enterobacter cloacae). The effectiveness against microorganisms was measured against that of the most common antibiotic, amoxicillin. Both Gram-positive and Gram-negative bacteria can be treated effectively with this antibiotic. The MICs it produced in our trials ranged from 8 to 64 µg/mL against the different strains we used. All of the compounds tested (1-12) with ferrocenyl, phenyl ethyl, phenyl, hydroxy, or bromo group substituted at position 4 in the benzene ring in the pyrazoline moiety showed average antimicrobial activity against the strains used in the study, with the exception of compound 12, whose activity was in close proximity to that of Amoxicillin. The minimum inhibitory concentrations (MICs) of the other drugs against P. aeruginosa and S. aureus biofilm-forming bacteria mutans ranged from 64 to 256 µg/ml and 64 to 128 µg/ml, all of which are considered high. The MIC values against S for all of these drugs ranged from 32 to 64 µg/ml. E. bovis E. coli and Klebsiella pneumoniae. Except for compound 12, which had a MIC of 16 µg/ml against all strains of T. tropicalis tested, all other compounds showed no activity. Table 1 presents a summary of the data on antibacterial efficacy.

Table 1: MIC (lg/ml) value of synthesized compounds (1–12) on the tested ATCC strains using broth microdilution method.

Compounds	Bacterial strains								Fungal strains						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	256	64	32	128	64	128	256	128	128	256	128	128	64	128	128
2	128	32	32	128	32	128	128	64	128	256	64	64	32	64	128
3	128	64	32	64	32	128	128	128	128	256	64	128	64	128	128
4	128	32	32	64	32	64	64	128	128	256	64	64	32	256	128
5	128	64	32	64	64	64	64	128	128	128	64	128	64	128	128
6	128	64	64	128	32	64	128	128	128	128	64	64	32	128	128
7	128	64	32	128	32	128	64	128	128	128	64	128	32	128	128
8	128	32	32	128	32	128	128	256	256	256	64	64	32	128	128
9	256	64	64	128	32	64	128	256	128	256	64	128	32	128	128
10	128	32	32	64	64	64	64	128	256	256	64	64	64	64	128
11	128	32	32	64	32	128	128	128	128	256	64	128	32	128	128
12	64	16	16	64	16	32	32	64	64	64	32	32	16	64	64
Amoxycillin	64	8	8	16	8	16	16	16	–	–	–	–	–	–	–
Fluconazole		–	–	–	–	–	–	–	64	64	64	64	64	64	64

IV. IN VITRO ANTIFUNGAL ACTIVITY

Seven fungal strains were used to test the antifungal activity of each drug (Candida albicans, Candida dubliniensis, Candida glabrata, Candida parapsilosis,

Candida tropicalis, Candida kefyr and Candida krusei). Fluconazole, the gold standard antifungal drug, was utilised as a comparison. Treatment of Candida infections with this method is considered standard first-line therapy. The MIC that we found in our studies was 64 µg/mL across all of the tested strains. Compound 12, which included a nitro group at position 4 on the benzene ring, in the pyrazoline moiety, demonstrated antifungal efficacy against C that was equivalent to or better than that of Fluconazole. C. glabrata (32 µg/mL), and C. s. C. parapsilosis (32 µg/mL), and P. 16 µg/mL of tropicalis. Table 1 summarises the antifungal activity data.

V. CONCLUSION

Using spectroscopic techniques such infrared spectroscopy, proton nuclear magnetic resonance, mass spectrometry, and elemental analysis, we synthesized and studied some new sulphur and nitrogen containing ferrocenyl linked heterocyclic compounds. The in vitro antimicrobial activity of the newly synthesised compounds (1-12) was evaluated using the broth microdilution method against 15 ATCC strains; 8 were bacterial (P. aeruginosa, S. bovis, E. faecalis, K. pneumonia, E. coli, E. cloacae, MRSA, and S. mutans), and 7 were fungal (C. albicans, C. dubliniensis, C. Since these compounds have shown antimicrobial action, they may be used as an antibacterial and antifungal combination therapy. In addition, their novelty makes them a potential answer to the growing resistance that has emerged as a worldwide issue.

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