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Research Article

SYNTHESIS AND ANTIMICROBIAL SCREENING OF SOME NOVEL FERROCENYL DERIVATIVES OF PYRAZOLE ANALOGUES

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ABSTRACT

Pyrazoles have been studied for over a century as an important class of heterocyclic compounds and continue to attract considerable interest due to the broad range of biological activities they possess. The incorporation of the essential structural features of pyrazoles with a ferrocene moiety could provide new derivatives with unexpected and/or enhanced biological activities since several ferrocene derivatives have already been shown to be active against a number of tumors. For this reason, we investigated the synthesis of ferrocenyl-substituted pyrazoles, such as sI-alkyl/aryl-5-ferrocenylpyrazoles, by employing the reaction between (2-formyl-1-chlorovinyl) ferrocene and hydrazine derivatives. Although this reaction is known, it was not studied in much detail and the low yields of ferrocenyl pyrazoles were obtained. In the determination of antimicrobial activity almost all type microbes were used in the present study were sensitive in the newly synthesised compounds and it showed a potential activity against growth of both Gram positive and Gram negative bacteria and fungus Candida albicans and Aspergillus niger. The activity was concentration dependent. Order of activity against the various microorganism used were as follows. M.aureus> C. dipththeriae> E. coil> S. typhi> B. subtilis> P.vulgaris at the diffrent concentration of 10,20 and 30 µg/ well of newly synthesised compounds. It is known for the therapeutic medicinal activities against throat infections especially diphtheria. Now in our screening it was obvious that it potentially inhibited the growth of Gram positive rod C.diptheriiae and gives a scientific basis for use. It also supports its use as antiseptic in wounds. It is also note worthy that the newly synthesised compounds effectively inhibited the fungal growth of cadida albicans and Aspergilus niger at the concentration of 20µg/well and showed doubled activity than that of standard in 30µg/well concentration.

Keywords: Ferrocene, pyrazole, hydrazines, Antimicrobial Screening.

INTRODUCTION

The aim of this work is to synthesize some new ferrocenyl-substituted pyrazole derivatives since the incorporation of the essential structural features of pyrazoles with a ferrocene moiety could provide new derivatives with enhanced antitumor and biological activities. The goal of this work is to synthesize the ferrocenyl-substituted pyrazole derivatives since the incorporation of the essential structural features of pyrazoles with a ferrocene moiety could provide new derivatives with enhanced antitumor and biological activities.[1-5] Although pyrazoles are among the most thoroughly studied compounds, we were surprised that there has been very limited study of the ferrocenyl-substituted pyrazoles. As part of our general involvement in ferrocene containing potential pharmaceuticals, I was investigated the synthesis of ferrocenyl pyrazoles. In particular, although there are numerous methods for the synthesis of pyrazoles, the reaction of (2-formyl-1-chlorovinyl)ferrocene with hydrazines can provide a rapid entry to ferrocenyl pyrazoles.[6] In fact, the reaction of (2-formyl-1-

chlorovinyl)ferrocene with hydrazine and phenyl hydrazine was carried out by Terent'ev and his co-workers for the first time but the low yield of products were obtained since these reactions were not investigated in much detail. We have restudied this reaction under a variety of condition and improved the yields of pyrazoles by optimizing reaction conditions. Moreover, we have examined this reaction with 7 hydrazine derivatives.

EXPERIMENTAL

Synthesis of (2-formyl-1-chlorovinyl) ferrocene.

In the first phase of this study, acetyl ferrocene was synthesized from ferrocene. Ferrocene behaves as an aromatic compound and easily undergoes Friedel-Crafts Acylation reaction to form acetyl ferrocene in 80% yield according to a known literature. The reaction was performed by using $AlCl_3$ under argon condition.

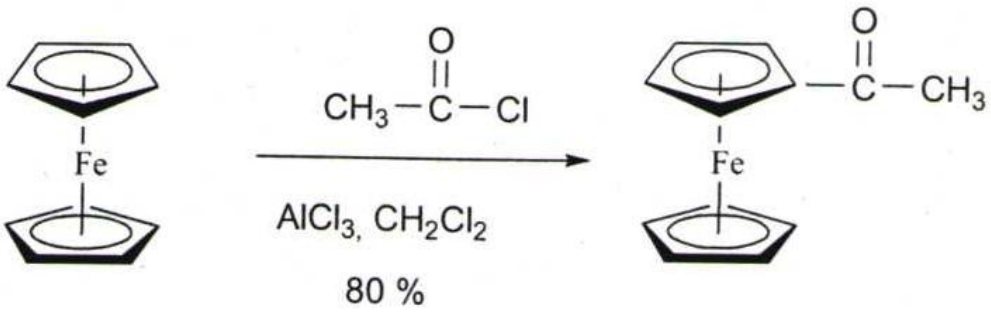

Fig. 1: Synthesis of acetyl ferrocene

Subsequently, (2-formyl-1-chlorovinyl) ferrocene has been prepared from acetyl ferrocene in 93% yield according to known literature. Treatment of acetyl ferrocene with phosphorus oxychloride in dimethyl formamide (DMF) leads to a mixture of (2-formyl-1-chlorovinyl)ferrocene and (1-chlorovinyl)ferrocene with the different product ratio depending on the stoichiometry. However, the formation of (1-chlorovinyl)ferrocene can be effectively suppressed by employing an excess of phosphorus oxychloride. Using DMF as solvent leads to satisfactory results only for small-scale preperations.[7] However, modification of the stoichiometry and experimental conditions led to the above described procedure which is useful for large-scale preperations. Use of conditions employing a comparatively small excess of DMF and phosphorus oxychloride resulting in a heterogeneous reaction mixture, as well as use of solid sodium acetate trihydrate surmount the problems of scale up and enable the removal of organic impurities. The purity and yield of (2-formyl-1-chlorovinyl)ferrocene are substantially improved using the present procedure, and this intermediate is obtained in pure form without need of chromatography.

Synthesis of Acetylferrocene

In a dry flask, ferrocene (2 g, 0, 0108 mol) was added and it was dissolved with stirring in dry dichloromethane (15 ml) under argon. To the resultant dark orange/red solution acetyl chloride (1,03 ml, 0, 0118 mol) was added and then flask was immersed in an ice water bath at 0-5 ^{o}C. Anhydrous aluminium chloride (1, 44g, 0, 0108 mol) was added in 10 portions (2min. between each addition). the reaction mixture darkened. It was stirred for 2 h allowing the ice-water warm to room temperature. Solution was recooled and hydrolized with water by slow addition of 4 x 0, 5 ml of cold water. Then, 3 ml of cold water was added more rapidly. The mixture was transferred to a separating funnel and extracted with dichloromethane then organic extracts were combined and washed with 5% sodium hydroxide solution. Red/orange solution was dried over magnesium sulfate for 10 min, then filtered off. Solvent was removed on a rotary evaporator to give a red/orange solid. This solid was purified by flash chromatography on silica gel using hexane as the eluent.[8] The red/orange fraction (R_f = 0.1in 9:1 hexane/ethyl acetate) was collected to give acetyl ferrocene (1, 96 g, 80%).

^{1}H-NMR ($CDCl_3$): δ 4.60 (s, 2H), 4.32 (s,5H), 2.17 (s, 3H); ^{13}C-NMR ($CDCl_3$): δ 79.2 (C), 72.3 (CH), 69.8 (CH), 69.5 (CH), 27.3 (CH_3). The spectral data is in agreement with those reported previously for this compound.

Synthesis of (2-FORMYL-1-CHLOROVINYL) FERROCENE

To a two necked flask, acetylferrocene (2 g, 8.8 mmol) was placed and addition funnel was

connected. N,N-dimethylformamide (DMF) (2.17 ml, 28.2 mmol) was added on it. The system was flushed with argon, cooled to 0^{o}C by means of an ice bath, and the brown reaction mixture was stirred for several minutes. Separately, in a flask joined with argon, DMF (2.17 ml, 28.2 mmol) was added and cooled to 0^{o}C with good stirring phosphorus oxychloride (2.21 ml, 24 mmol) was added. The resulting viscous, red complex was transferred to the dropping funnel and added to the magnetically stirred mixture of acetylferrocene and DMF dropwise over 30 min. Complete addition was assured by washing the addition funnel and walls of the flask with small amount of DMF. The mixture was stirred at 0^{o}C for 2 hr during which time the colour of the reaction mixture changed from dark brown to olive and ultimately to deep blue. Prior to neutralization, 20 ml portion of diethyl ether was added and viscous mixture was stirred vigorously for several minutes. At 0^{o}C, (10.18 g, 74.6mmol) sodium acetate trihydrate was cautiously added to the reaction mixture in one portion followed by addition of 2 ml water with vigorous stirring. The ice bath was removed whereupon the organic layer undergoes a striking colour change from blue to ruby red indicating the formation of the formyl derivative. After 1 hr, an additional 2 ml of diethyl ether was added and stirring was continued for 3 hr at room temperature to ensure complete quenching. The reaction mixture was transferred to a separator funnel with ether and water and mixed thoroughly, and the organic phase was separated. The aqueous phase was extracted several times with ether. The combined organic phases were carefully washed with 20 ml of saturated aqueous sodium bicarbonate solution. The organic phase was dried over magnesium sulfate, filtered and concentrated using a rotary evaporator. The resulting (2-formyl-1-chlorovinyl)ferrocene was obtained as an only product (2.25 g, 93%).
^{1}H-NMR ($CDCl_3$): δ 10.06 (d, 1H, *J*=7.1 Hz), 6.38 (d, 1H, *J*=7.1 Hz), 4.73 (t, 2H, *J*=1.68 Hz), 4.22 (s, 5H). The spectral data is in agreement with those reported previously for this compound.

Reaction of (2-formyl-1-chlorovinyl)ferrocene with phenyl hydrazine hydrochloride salt.

General Procedure 1 was followed by using (2-formyl-1-chlorovinyl)ferrocene (300 mg, 1.089 mmol), phenyl hydrazine hydrochloride salt (472.4 mg, 327 mmol). After chromatographic purification, a purple fraction (R_f =0.43 in 9:1 hexane/ethyl acetate) was collected to give 1-phenyl-3-ferrocenylpyrazole (18 mg, 15%) and an orange fraction (R_f =0.21 in 9:1 hexane/ethyl acetate) was collected to give 1-phenyl-5-ferrocenylpyrazole (49 mg, 41%).
^{1}H-NMR ($CDCl_3$): δ 7.84 (d, 1H, *J* =2.4 Hz), 7.71 (d, 2H, *J*=7.8 Hz), 7.44 (t, 2H, *J*=7.8 Hz), 7.25 (t, 1H, *J*=7.8 Hz), 6.48 (d, 1H, *J*=2.4 Hz), 4.76 (s, 2H), 4.29 (s, 2H), 4.07 (s, 5H); ^{13}C-NMR ($CDCl_3$): δ 152.5 (C), 140.3 (C), 129.4 (CH), 127.4 (CH), 126.0 (CH), 119.0 (CH), 105.6 (CH), 78.4 (C), 69.6 (CH), 68.7 (CH), 66.9 (CH); IR (neat): 3742 (s), 3669 (w), 3030 (vw), 2959 (vs), 2865 (s), 1719 (vs), 1681 (b), 1506 (s), 1257 (vs), 1129 (w), 1043 (m), 868 (w), 820 (m); MS (EI): 328 (M^{+}), 326, 263, 246, 206, 178, 149, 121, 91, 77, 56; HRMS (EI): Calc. For $C_{19}H_{16}$ $^{56}FeN_2$: 328.0663. Found: 328.0665.
^{1}H-NMR ($CDCl_3$): δ 7.62 (s, 1H), 7.40 (m, 5H), 6.50 (s, 1H), 4.17 (s, 2H), 4.14 (s, 2H), 4.05 (s, 5H); ^{13}C-NMR ($CDCl_3$): δ 141.5 (c), 140.4 (C), 140.0 (CH), 128.8 (CH), 128.0 (CH), 126.1 (CH), 106.8 (CH), 75.1 (C), 69.9 (CH), 68.8 (CH),68.6 (CH); IR (neat): 3744 (w), 3098 (m), 3048 (s), 1737 (vw), 1665 (s), 1597 (s), 1498 (vs), 1402 (s), 1312 (vw), 1259 (vs), 1145 (s), 923 (s), 822 (vs); MS (EI): 328 (M^{+}), 326, 263, 235, 207, 170, 153, 121, 77, 56; HRMS (EI): Calc. For $C_{19}H_{16}$ $^{56}FeN_2$: 328.0663. Found: 328.0661.

Reaction of (2-formyl-1-chlorovinyl)ferrocene with benzyl hydrazine dihydrochloride salt.

General Procedure was followed by using (2-formyl-1-chlorovinyl)ferrocene (100 mg, 0.363 mmol), benzyl hydrazine dihydrochloride salt (212.44 mg, 1.089 mmol). After chromatographic purification, the orange colored fraction (R_f =0.17 in 9:1 hexane/ethyl acetate) was collected to give 1-benzyl-5-ferrocenylpyrazole (68 mg, 55%).
^{1}H-NMR ($CDCl_3$): δ 7.44 (s, 1H), 7.23 (t, 2H, *J*=7.28 Hz), 7.15 (t, 1H, *J*=7.28 Hz), 6.96 (d, 2H, *J*=7.28 Hz), 6.35 (s, 1H, 5.42 (s, 2H), 4.29 (s, 2H), 4.17 (s, 2H), 4.00 (s, 5H); ^{13}C-NMR ($CDCl_3$): δ 141.7 (C), 139.1 (C), 137.7 (CH), 128.6 (CH), 127.3 (CH), 126.3 (CH), 106.0 (CH), 74.9 (C), 70.0 (CH), 68.8 (CH), 68.4 (CH), 53.3 (CH_2); IR(neat): 3096 (w), 2954 (s), 2930 (s), 2858 (w), 1721 (vs), 1673 (b), 1405 (s), 1281 (vs), 1130 (s), 1076 (s), 928 (s), 822 (s); MS (EI): 342 (M^{+}), 277, 252, 223, 185, 157, 121, 91, 65, 56; HRMS (EI): Calc. For $C_{20}H_{18}$ $^{56}FeN_2$: 342.0819. Found: 342.0817.

MATERIALS AND METHODS

Diffusion Method

In the diffusion technique, a reservoir containing the newly synthesized compound to be tested is brought into content with an inoculated (e.g. agar). After incubation, the diameter of the zone around the reservoir (inhibition diameter) is measured. In order to lower the detection limit, the inoculated system is kept at a low temperature during several hours before incubation, which favors diffusion over microbial growth and thus increases the inhibition diameter. Advantages of the diffusion methods are the small size of the sample used in the screening and the possibility of testing five or six compounds per plate against a single microorganism.

In most studies, inhibition zones are compared with those obtained for antibiotics. This is useful in establishing the sensitivity of the test organism, but a comparison of the antimicrobial potency of the samples and antibiotics cannot be drawn from this, since a large inhibition zone may be caused by a highly active substance present in quite small amount or by a highly active substance present in high concentration in the newly synthesised compounds. The zone diameter of inhibition is indeed inversely related to the "minimum inhibitory concentration" (MIC), whereas an appropriate MIC can be extrapolated from the zone diameter.

Organisms

All the organisms were obtained from Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh.

Media

For the study, Nutrient Broth (NB), Nutrient Agar (NA), Muller-Hinton Agar (MHA), Sabouraud's Broth and Sabouraud's Agar media were used. All these media were procured from Himedia, Mumbai.

Nutrient Agar

Suspend 28.0 gm NA in 1000 ml of distilled water and boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure and 121^{o}C temperature for 15 minutes.

Nutrient Blood Agar

Suspend 28.0 gm NA in 1000 ml of distilled water and enrichment medium by 10% blood to this Nutrient Agar, boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure and 121^{o}C temperature for 15 minutes.

Table 1: Composition of media

Media	Constituents	G/L of distilled water
Nutrient Broth (NB)	Peptone	5.00
	Sodium chloride	5.00
	Beef extract	1.50
	Yeast extract	1.50
	pH (at 25^{o}C)	7.4 ± 0.2
Nutrient Agar (NA)	Peptone	5.00
	Sodium chloride	5.00
	Beef extract	1.50
	Yeast extract	1.50
	Agar	15.00
	Final pH (at 25^{o}C)	7.4 ± 0.2
Nutrient Blood Agar (NBA)	Enrichment medium by 10% blood in nutrient Agar	7.4 ± 0.2
Sabouraud Dextrose Broth (SDB)	Peptone	10.00
	Dextrose	20.00
	Final pH	5.6 ± 0.2
Sabouraud Dextrose Broth (SDA)	Mycological peptone	10.00
	Dextrose	40.00
	Agar	15.00
	Final pH (at 25^{o}C)	5.6 ± 0.2

Table2: Evaluation of antibacterial activity of newly synthesized compounds

S. NO.	Microbial culture	Zone of inhibition (mm)			
		Standard Amikacin	Concentration (µg/well)		
		30µg/well	10	20	30
1.	*B. subtilis*	38±1.4	17±1.4	19±0.9	21±0.4
2.	*C. diphtheriae*	24±0.9	16±0.7	30±1.2	39±1.8
3.	*E. coli*	37±1.9	25±0.9	33±1.4	38±1.7
4.	*M. aureus*	22±1.1	25±1.2	32±1.1	40±2.1
5.	*P. vulgaris*	18±0.8	09±0.4	13±0.3	18±1.1
6.	*S. typhi*	34±1.5	11±0.5	25±0.8	36±1.6

n = 3, values are expressed in mean ± SD.

Table 3: Evaluation of antifungal activity of newly synthesised compounds

S.NO.	Microbial culture	Zone of inhibition (mm)			
		Standard Amikacin	Concentration (µg/well)		
		30µg/well	10	20	30
1.	*C. albicans*	22±0.9	18±1.2	25±1.5	38±1.9
2.	*A. niger*	18±0.66	15±0.85	23±0.9	35±0.33

n = 3, values are expressed in mean ± SD.

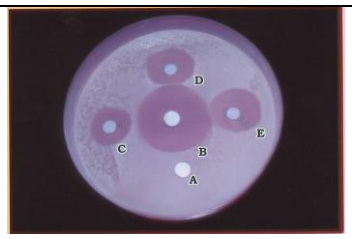

Fig.2 : Antibacterial activity of newly synthesised compounds against B. subtilis

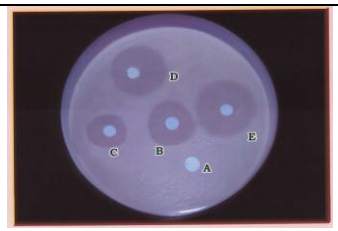

Fig.3 : Antibacterial activity of newly synthesised compounds against C. diphtheriae

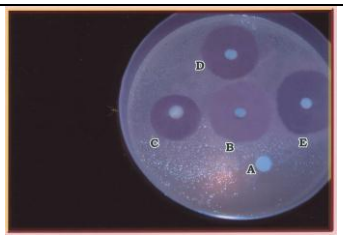

Fig.4 : Antibacterial activity of newly synthesised compounds against E. Coli

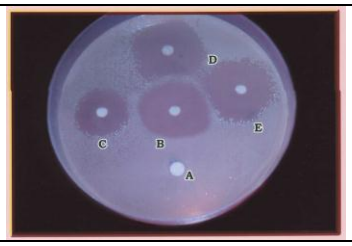

Fig.5 : Antibacterial activity of newly synthesised compounds against M. aureus

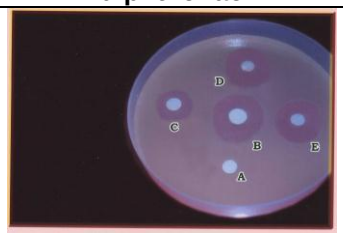

Fig.6 : Antibacterial activity of newly synthesised compounds against P. vulgaris

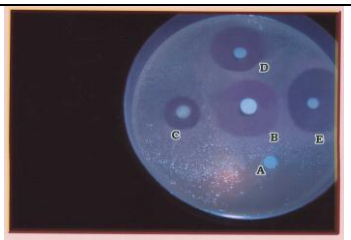

Fig.7 : Antibacterial activity of newly synthesised compounds against S. typhi

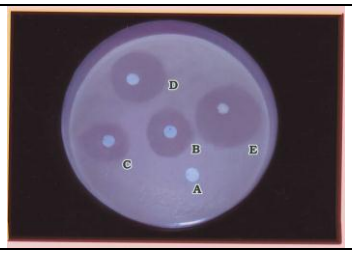

Fig.8 : Antifungal activity of newly synthesised compounds against C. albicans

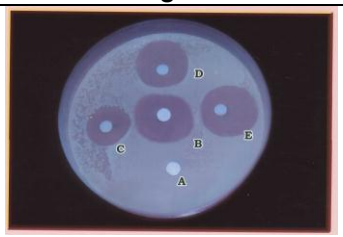

Fig.9 : Antifungal activity of newly synthesised compounds against A. niger

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