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Synthesis of Some Alkoxylated Pyrazoles

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ABSTRACT

The synthesis of pyrazoles: 1-(3, 4, 5-trimethoxyphenyl)- 3-(2, 4-dimethoxy phenyl) dihydropyrazole was executed by reaction of hydrazine hydrate with chalcones followed by concomitant dehydration and cyclization. The structure of the synthesized compounds were elucidated using infrared, nuclear magnetic resonance (^{1}H and ^{13}C), and mass spectrometry. The chalcones and pyrazoles were screened for antibacterial activities using the agar-well diffusion method at a serial dilution of 106cfu/ml. The pyrazoles showed no significant bacteriocidal or bacteriostatic activity against the clinical isolates and typed culture of bacteria tested against.

INTRODUCTION

Pyrazoles are an important class of heteroaromatic systems that find extensive use in the pharmaceutical industry. They posses antibiotic, antiviral, anti-inflammatory and antiamoebic properties [1]. Pyrazofurin; 3-(amino-hydroxy-methylidene)-5-(3, 4-dihydroxy-5-hydroxymethyl) oxolan-2-yl]-2H-pyrazol-4-one, a naturally occurring pyrazole is used clinically as an anticancer agent while Celecoxib; 4-[5-(4-methyphenyl)-3-(trifluoromethyl) pyrazol-I-yl] benzenesulfonamide, a synthetic pyrazole is used as a non steroidal anti-inflammatory agent [2]. Chalcones, the intermediate product in the synthesis of pyrazoles are also known to posses anticancer, antileishmanial, antimalarial and anti-inflammatory activities.

The isolation and characterization of the naturally occurring pyrazole: pyrazofurin, a naturally occurring antibiotic with potent antimicrobial and broad spectrum antiviral activities [3], renewed the interest to study the anti-microbial activity of substituted pyrazoles. The rise in microbial resistance is partly because there have been no new classes of antibiotics since 1960 [4]. There is a need to maintain a stride ahead of microbial pathogens by constantly synthesizing new antimicrobial agents [5].

MATERIALS AND METHODS

Melting points were determined with a kofler electro thermal melting point apparatus and were uncorrected. The reactions and purity of the product were monitored by TLC using pre-coated silica gel plates (Merck 60 F254). The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 (TMS). IR spectra were measured on a Perkin- Elmer type 457 and the mass spectra were determined using Varian MAT 44S, EI: 70eV

Chemistry

The general synthetic strategy employed to prepare the chalcones was based on Claisen Schmidt's condensation, which has been previously reported [6]. The chalcones and hydrazine hydrate were refluxed in absolute ethanol and monitored with TLC until complete disappearance of starting materials (Fig 1).

The mixture were then left at room temperature overnight and the crystalline precipitate were collected and recrystallized from ethanol to give the products as colourless or brownish plates.

1-(3, 4, 5-Trimethoxyphenyl)-3-(2, 4-dimethoxyphenyl)-2-propen-1-one (2a)

To 3, 4, 5-trimethoxy acetophenone (1) (0.012 moles, 2.53g) dissolved in methanol (30.00ml) was added (0.012 moles, 2.00g) of 2, 4-dimethoxy-benzaldehyde and followed with (10%) potassium hydroxide (4ml). After 2 hours, yellow crystals of 2a were obtained.

IR (KBr) 1458, 1710, 3095 cm–1, 1H-NMR (250.13MHz, CDCl3) 3.82-3.93 (m, 15H) 6.46-6.47 (d, 1H, J = 2.35), 6.51 – 6.55 (dd, IH, J = 2.35), 7.26 (s, 2H), 7.43-7.49 (d, 1H, J = 15.75), 7.55-7.58 (d, 1H, J = 8.56), 8.01-8.07 (d, 1H, J = 15.78) 13C-nmr (62.90 MHz, CDCl3) 55.52, 56.30, 60.92, 98.43, 105.47, 106.04, 117.11, 120.27, 130.78, 134.15, 140.42.01, 153.02, 160.33, 163.05 190.05, MS. m/z: (%) 359 (M+1)(3), 358 (M+) (16) 343 (8), 328 (21), 327 (100), 311 (3), 297 (4), 266 (4), 220 (6), 205 (2), 191 (10), 176 (5), 165 (6), 148 (7), 133 (6), 121 (5), 105 (2), 91 (2), 77 (6), 66 (3) Anal. Calc for C20H22O6 (358.391): C.66.96 H. 6.14 Found C.66.76 H. 6.04.

1-(3, 4, 5-Trimethoxyphenyl) -3-(4-nitrophenyl)-2-propen-1-one (2b)

To 3, 4, 5-trimethoxy-acetophenone (0.0066 moles, 1.39g) dissolved in methanol (30.00ml) in a round bottom flask was added 4-nitrobenzaldehyde (0.0066 moles, 1.00g) and stirred. 10% Potassium hydroxide (4.00ml) was added and after 1.5 hours the product 2b was formed.

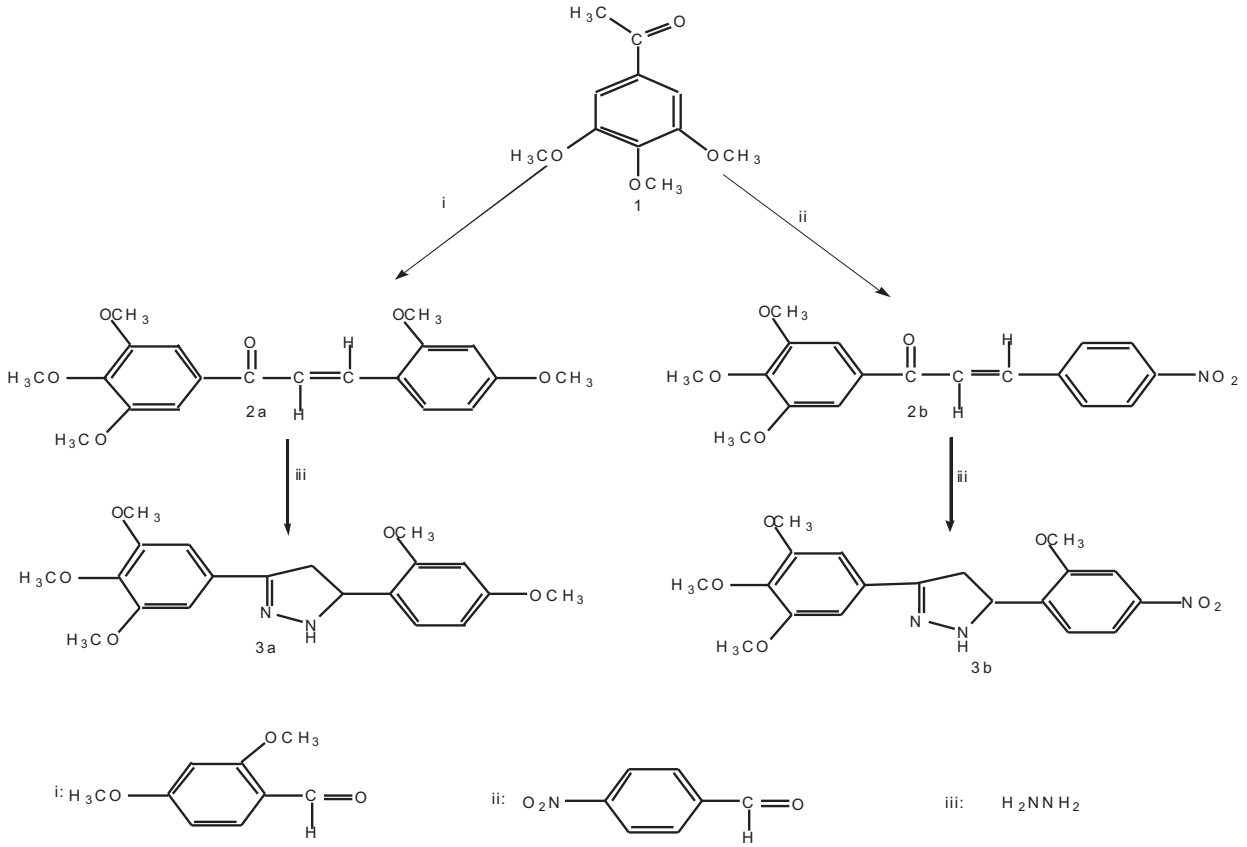

Fig. 1: Scheme of synthesis of pyrazoles: 1-(3, 4, 5-trimethoxyphenyl)- 3-(2, 4-dimethoxy phenyl) dihydropyrazole

IR (KBr) 1200, 1715, 1458, 28401499, 1570, 3095cm 11H-NMR (250.13MHZ, CDCl3) 3.95 – 3.94 (m, 9H), 7.28 (s, 2H), 7.55 – 7.62 (d, 1H, J = 15), 7.76 – 7.79 (d, 2H, J = 8), 7.77 – 7.84 (d, 1H, J = 15), 8.24 – 8.28 (d, 2H, J = 8), 13C-NMR (62.90 MHz, CDCl3) 56.33, 60.99), 105.91, 123.79, 125.42, 128.63, 132.75, 141.06, 141.37, 143.12, 148.52, 153.27, 188.25 (C=O), MS. m/z: (%) 344 (M+1) (12), 343 (M+) (100), 328 (14), 312 (9), 300 (22), 272 (4), 240 (4), 211 (4), 195 (17), 176 (8), 152 (5), 139 (3), 109 (4%), 102 (13%), 76 (4), 30 (4). Anal. Calc for: C18H17NO6 (343.336) Calc: C. 62.91 H. 4.95 N 4.08. Found: C. 62.97 H. 4.96 N 4.08.

1-(3, 4, 5-Trimethoxyphenyl)-3-(2, 4-dimethoxyphenyl)-dihydropyrazole (3a)

1-(3, 4, 5-Trimethoxyphenyl)-3-(2, 4-dimethoxyphenyl)-2-propen-1-one (0.0028 moles, 1.00g) was refluxed in absolute ethanol (10.00ml). 80% Hydrazine hydrate (5.00ml) was gradually added. After 1 hour, 3a was obtained as colourless crystals.

IR (KBr) 1458, 1499, 1570, 3400H cm–1 NMR (250.13 MHz, CDCl3). 2.87 – 2.97 (dd, 1H, J = 10), 3.34 – 3.44 (dd, 2H, J = 10), 3.79 – 3.88 (m, 15H), 5.21 (t, 1H, J = 9), 6.43 (s, 2H), 6.91 (s, 2H), 7.26 – 7.43 (d, 1H, J = 8), 13C-NMR (62.90 MHz, CDCI3) 39.09, 55.38, 56.16, 58.25, 60.89, 98.57, 103.21, 104.07, 122.67, 126.81, 128.82, 138.75, 151.51, 153.21, 157.79, 160.29 MS. m/z: (%) 373 (M + 1) (25%), 372(M+) (100), 357 (19), 341 (20), 193(7), 170(7) 121(7) Anal. Calc for: C20H24N2O5 (372.413) Calc: C.64.44H.6.44N7.52. Found: C. 64.51 H. 6.40 N 7.50.

1-(3, 4, 5-trimethoxyphenyl) -3-(3, 4, 5-trimethoxyphenyl) dihydropyrazole (3b)

1-(3, 4, 5-Trimethoxyphenyl)-3-(3, 4, 5-trimethoxyphenyl)-2-propen-1-one (0.0027 moles, 1.00g) was dissolved in absolute ethanol (10.00ml) and brought to reflux. 80% Hydrazine hydrate (5.00ml) was gradually added and treated as in the general procedure for 0.75 hours.

IR (KBR) 1200, 1458, 2840, 1499, 1570, 3400 cm–1 1H-NMR (250.13 MHz, CDCl3) 2.92 – 3.02 (dd, 1H, J = 9), 3.54 – 3.60 (dd, 1H, J = 9), 3.87 – 3.87 (m, 9H), 5.04 – 5.08 (t, 1H), 6.09 (s, 1H), 6.88 (s, 2H) 7.54 – 7.549 (d, 2H, J = 13), 8.16 – 8.21 (d, 2H, J = 13) 13C-NMR (62.90 MHz, CDCl3) 41.83, 56.19, 60.91, 63.74, 103.35, 124.05, 127.37, 127.84, 139.21, 149.94, 147.47, 150.96, 153.32, MS: m/z (%) 358 (M + 1) (16), 357 (M+) (100), 342 (52) 314 (10), 296 (6), 267 (6), 235 (6), 193 (6), 165 (6), 134 (6) 89 (6) 77 (10) Anal. Calc for: C18H19N3O5 (357.358) C. 60.44 H. 5.32 N 11.75. Found: C. 60.50. H. 5.12 N 11.60.

Anti-bacterial evaluation

Clinical isolates of Staphylococcus aureus, Bacillus subtilis, Streptococcus pneumoniae, Klebsiella pneumonia and Candida albicans were supplied by the Department of Pharmaceutical Microbiology Faculty of Pharmacy, University of Benin. The agar-well diffusion method was used to determine the antibacterial activity [7-9].

Inocula of test organisms obtained from source were prepared by growing each pure isolates in nutrient-broth for 18 hours at 37C. The overnight broth culture was matched with Macfarland turbidity standard to give an approximate 108cfu/ml. (0.2ml) was

then used to seed a molten medium which has cooled to 45°C to obtain approximately 106cfu/ml. This was poured into sterile Petri dishes and used for the analysis.

A stock solution of the chalcones and pyrazoles were made by dissolving 100mg in 5ml dimethylsulphoxide (DMSO) to give a concentration of 20mg ml–1. DMSO (0.1ml) was delivered into wells (6mm in diameter) bored unto the surface of the already seeded agar plates. Equal volumes of dimethylsulphoxide (DMSO) were assayed along as controls. Gentamycin 10 µg and Ciprofloxacin 5 µg were used in parallel in the agar-well diffusion method. Staphylococcus aureus (NCTC 10788) was set up along with test organisms as a check on media and inherent sensitivity of isolates produced by the substances.

Statistical analysis

The results were expressed as mean ± SEM. The statistical analysis was by one way analysis of variance (ANOVA) followed by student t - test using the statistical package for social science (SPSS). The statistical level of significance was $P < 0.5$.

RESULT

The physicochemical properties of the synthesized compounds are shown in Table No.1. The compounds were recrystallized from ethanol. The time of synthesis varied from 45minutes to 2 hours. The melting points also range from 110°C to 136°C, while the yields of the compounds were between 64% and 86%. Table No.2 shows the results of the antimicrobial evaluation of the synthesized compounds. None of the compounds showed significant antibacterial activity.

Table No.1: Physicochemical data of synthesised compounds

Synthesized compounds	Reaction medium	Reaction time (hours)	Yied (%)	Melting point (°C)	Solvent for crystallization
2a	Methanol	2	76	114 –115	Ethanol
2b	Methanol	1.5	86	135-136	Ethanol
3a	Absolute Ethanol	1	64	110 –111	Ethanol
3b	Absolute Ethanol	0.75	78	116 – 117	Ethanol

Table No. 2: Result of Anti-bacterial Assessment

Compounds	Zones of Inhibition (mm)						
	Staphylococus aureus	*Bacillus subtilis*	*Streptococcus pneumonia*	*Escherichia coli*	*Pseudomonas aeroginosa*	*Klebsiella pneumonia*	*Staphylococcus aureus* NCTC 10788
2a (2mg)	NIL	NIL	NIL	NIL	NIL	NIL	NIL
2b (2mg)	NIL	NIL	NIL	NIL	NIL	NIL	NIL
3a (2mg)	NIL	NIL	NIL	NIL	NIL	NIL	NIL
3b (2mg)	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Gentamycin (Oxoid 10µg)	30	26	32	22	24	26	25
Ciprofloxacin (Oxoid5µg)	26	31	30	30	28	27	27

Mean value of three independent experiments was used.

DISCUSSION

In the synthesis of the chalcone, the reaction was carried out at room temperature (25°C) because the yield of the chalcone is higher if the reaction is carried out below 30°C. If the temperature is higher than 30°C, secondary reactions occur and this reduces the yield [10]. At elevated temperatures the acetophonones and benzaldehydes will undergo self condensation as the carbonyl group in each compound reacts with the carbanion that has been generated from the same compound.

1-(3, 4, 5-Trimethoxypheny)-3-(2, 4-dimethoxyphenyl)-2-propen-1-one) (2a) had melting point of 114 - 115°C with a percentage of yield of 76. The mass spectra of the compound revealed the molecular ion at m/z 358 in agreement with the proposed molecular formula C20H22O6. The presence of the carbonyl absorption in the IR (1710 cm–1) and ^{13}C NMR (190.05 ppm) were diagnostic of the carbonyl group of the chalcones. The five methoxy protons were centred in the ^{1}H NMR at about 3.82ppm.

When 1-(3, 4, 5-trimethoxypheny)-3-(2, 4-dimethoxy phenyl)-2-propen-1-one) (2a) was cyclised to the corresponding pyrazole 3-(3, 4, 5-trimethoxypheny)-5-(2, 4-dimethoxy phenyl) pyrazoline (3a), the compound had a melting point of 110 - 111°C and m/z 372 which agrees with the molecular formula of C20H24N2O5. A strong absorption at 3400cm–1 in the IR strongly indicates the presence of an NH group in the pyrazole. The fifteen protons of the methoxy group were identified at about 3.79 ppm in the proton NMR. Spectroscopic data confirmed the absence of a carbonyl group from both the IR and ^{13}C NMR.

Similarly 1-(3, 4, 5-trimethoxypheny)-3-4-nitrophenyl)-2-propen-1-one) (2b) gave a carbonyl absorption at 1715cm–1 in the IR and 188.25 ppm in the 13C NMR which were absent in the corresponding pyrazole 3-(3, 4, 5-trimethoxypheny) -5- (4-nitrophenyl) pyrazoline (3b). The molecular ion (M+) of 343 for the chalcone and 357 for the pyrazole were indicative of the proposed molecular formula of C18H17O6 and C18H19N3O5 respectively.

All the compounds showed no bactericidal or bacteristatic activity against all the bacteria (clinical isolates and typed) tested.

CONCLUSION

The synthesis of novel compounds with different mechanism of action helps in drug discovery and development as well as in the understanding of structure activity relationship.

Electron withdrawing substituents increase both the reaction rate as well as the yield of the products. The cyclization of the chalcones to the corresponding pyrazoles were successfully carried out by reacting with hydrazine hydrate. The structures of the synthesized compounds were elucidated unequivocally by infrared, nuclear magnetic resonance (^{1}H and ^{13}C), mass spectrometry and elemental analysis.

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