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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF α-CYANO SUBSTITUTED CHALCONES

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ABSTRACT

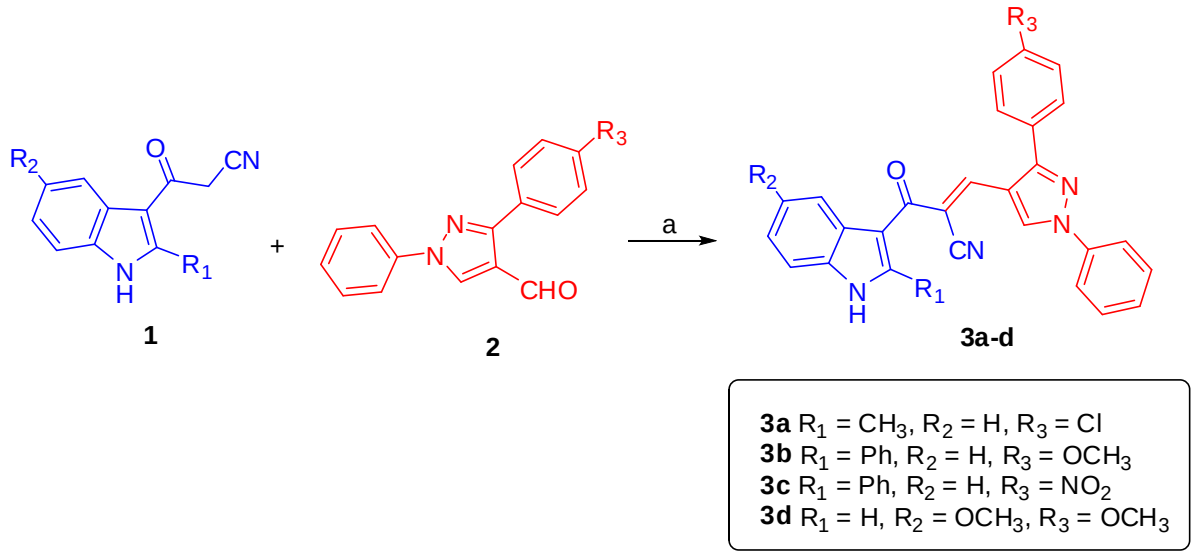
*The series of α-cyano substituted chalcones (**3a-d**) were synthesized by reacting substituted 3-cyanoacetyl indole **1** with substituted pyrazole aldehyde **2** in the presence of piperidine. All the newly synthesized compounds have been characterized by IR, ^{1}H NMR, ^{13}C NMR and HRMS spectroscopy. The synthesized compounds were screened for their antibacterial and antifungal activity.*

KEY WORDS: *Chalcones, antibacterial activity, antifungal activity.*

INTRODUCTION

Chalcones act as precursors in the synthesis of numerous constructive compounds such as flavonoids and isoflavonoids. Flavonoids are the customary constituents of human diet. Chalcones comprise of a three carbon α, β-unsaturated carbonyl system. Chalcones are abundantly present in nature starting from ferns to higher plants and several them are polyhydroxylated in the aryl rings. [1] In plants, chalcones are converted to the corresponding (*2S*)-flavanones in a stereospecific reaction catalyzed by the enzyme *chalconeisomerase*. This close structural and biogenetic relationship between chalcones and flavanones explains why they often co-occur as natural products.

Chalcones act as mediators in the synthesis of beneficial therapeutic compounds. Special attention has been given to chalcones due to their simple structure and diverse pharmacological activities. The compounds with the backbone of chalcones have been reported to possess various biological activities such as anti- inflammatory [2-5], antifungal, antibacterial, antimalarial [6-11], antitumor [12], antimicrobial [13, 14], antiviral [15], antitubercular [16], antioxidant [17], antimitotic [17], anti-leishmanial [18], anti-platelet [19], anticancer [20] and antihypertensive activities [21]. Synthetic and natural chalcones have a significant impact in anticancer drug discovery research. Anticancer activity of chalcones is believed to be a result of binding to the tubulin assembly and thereby preventing it from polymerization to microtubule. Over a period of time, several new chalcones have been reported with structural modifications around the basic enone template. Continuing our on-going research programme on synthesis of bioactive molecules, we have synthesized α-cyano substituted chalcones (**3a-d**) and screened for their antibacterial and antifungal activity (**Scheme 1**).



Scheme 1: Synthesis of α-cyano substituted chalcones

Reagents and condition: a) Piperidine, Ethanol, Reflux 1-4 h

MATERIAL AND METHODS

Experimental

All commercially available chemicals and reagents were purchased from Aldrich and used without further purification. All the solvents were dried and distilled before use. The IR spectra of synthesized compounds were recorded on Shimadzu 8400-S FT-IR Spectrophotometer using potassium bromide. The 1H NMR were recorded in $CDCl_3$ or DMSO-d_6 using NMR Varian-Mercury 300 MHz spectrometer and chemical shifts are reported as parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Reactions were monitored using thin layer chromatography (TLC) carried out on Merck silica gel 60 F254 precoated aluminium plates. The visualization was achieved under UV light or staining with I_2. Chromatographic separations were achieved on silica gel columns (Merck, 100–200 mesh) using gradient of hexane/ethyl acetate as eluent.

General procedure for the synthesis of α-cyano substituted chalcones (3a-d)

To a mixture of substituted 3-cyanoacetyl indole **1** (1 mmol) in ethanol was added piperidine (0.3 mL) and stirred for 10 min. Then, added the pyrazole aldehydes **2** (1 mmol) and this mixture was heated to reflux for 1-4 h. After completion of reaction (monitored by TLC), reaction mixture was poured over crushed ice and acidified with acetic acid. The precipitated solid was filtered, washed with water and oven dried. It was column purified by column chromatography using silica gel mesh size, 100–200 and elution with 10% ethyl acetate in hexane to afford pure product**.**

Spectral data of representative compound:

(E)-3-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2-(2-methyl-1H-indole-3-carbonyl)acrylonitrile

(3a):Yellow solid; 89%; 240–242°C; IR (cm^{-1}): 3319, 2211, 1637, 1588; ^{1}H NMR ($CDCl_3$, 400 MHz): □ = 9.25 (s, 1H), 8.53 (s, 1H), 7.99 (s, 1H), 7.85 (d, J= 8.8 Hz, 2H), 7.78 (d, J= 7.6 Hz, 1H), 7.56-7.52 (m, 2H), 7.44-7.39 (m, 4H), 7.29-7.22 (m, 4H), 2.68 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$,75 MHz): □ = 186.32, 154.01, 150.34, 139.60, 135.22, 134.80, 131.53, 131.07, 129.89, 129.06, 128.90, 128.34, 126.65, 126.18, 121.87, 121.23, 119.92, 119.61, 115.21, 114.12, 111.01, 106.17, 103.36, 15.3; HRMS: Calculated for $C_{28}H_{19}ClN_4O$; Exact mass: 462.1247, found: 463.1310 (ESI M+H).

BIOLOGICAL ACTIVITY

Antibacterial and antifungal activity

Antibacterial activity of the various synthesized compounds was evaluated by pour plate method. Briefly, the test compounds were dissolved in dimethyl sulphoxide (DMSO) to produce 1 mg/ml stock solutions. All bacterial strains were thawed, then bacteria, *Staphylococcus aureus* in soya broth. *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* in nutrient and *Candida albicans* (ATCC 1023) in potato dextrose agar medium were cultured respectively. Broth solution was prepared in bacterial cultures on media at 37 ±2 ^{0}C. The bacterial inoculum was uniformly spread using sterile cotton swab on a sterile Petri dish agar. To this Petri dish, 50 µL of test compounds at various concentrations (0.1 to 1000 µg/ml) were added to each of the 5 wells (7 mm diameter holes cut in the agar gel, having distance of 20-30 mm from another hole). The plates were incubated for 24 hr at 36ºC ± 1ºC for bacteria and 48 hr 36ºC ± 2ºC for fungi , under aerobic conditions. After incubation, confluent bacterial and fungal growths were observed. The zone of inhibition in mm^2 was measured for the test compound and recorded. From these values, the area of inhibition was calculated. Streptomycin and gentamycin were standard for antibacterial agents and Griseofulvin as antifungal agent in comparison.

Minimum Inhibitory concentration (MIC)

The antibacterial activity of test compounds was determined by microdilution method. Test compounds were dissolved in DMSO. The 96 well plates was numbered as per the test compounds and the microorganism used, to which 50µl of various concentrations of test compounds were added to this, 50µl of test organism suspension was added. To this inoculated broth was added. The plate was sealed and incubated for 24 hr at room temperature.

Reading and interpretation

Read the concentration at which there in no visible growth of microorganism in the well of the plate that is known as MIC **(Table 1)**.

Table 1: Antibacterial and antifungal activity of α-cyano substituted chalcones (3a-d)

Compound	*S. aureus*		*B. substalis*		*P. auroginosa*		*E. Coli*		*Candida albicans*	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
3a	438	100	88.0	500	200	1000	1850	1000	78.3	>1000
3b	82.5	500	80.0	500	506	1000	83.33	500	-	-
3c	91.6	50.0	76.66	100	43.0	1000	81.25	1000	80.1	500
3d	326	1000	319	500	-	-	-	-	-	-
Stretomycin	0.65	0.5	0.71	2.0	-	-	-	-	-	-
Gentamycin	-	-	-	-	11.0	1.0	26.0	10.0	-	-
Griseofulvin	-	-	-	-	-	-	-	-	34.93	1.0

RESULTS AND DISCUSSION

Chemistry

In the present study, synthesis of α-cyano substituted chalcones (**3a-d**) accomplished by the Knoevenagel condensation of substituted 3-cyanoacetyl indoles **1** with substituted pyrazole aldehyde **2** in the presence of piperidine in ethanol (**Scheme 1**). The starting compounds for the synthesis of title compounds, namely 3-cyanoacetyl indoles **2** synthesized in good yields from the reaction of substituted indoles **1** with cyanoacetic acid in presence of acetic anhydride. [22] On the other hand, pyrazole aldehydes **2** were synthesized using the method described in the literature with minor modifications. [23] The structures of all the synthesized compounds were confirmed with IR, ^{1}H NMR, ^{13}C NMR, and HRMS techniques.

Antibacterial and Antifungal Activity

All the synthesized compounds (**3a-d**) were screened for their antibacterial and antifungal activity by using pour plate method. Streptomycin and gentamycin were used as reference standard for antibacterial agents and Griseofulvin used as reference standard for antifungal agent. Two parameters namely IC_{50} and MIC were estimated during the screening process and the results are presented in **Table 1**. It is noteworthy that most of the compounds were considerably moderate to low inhibition as compared to standard reference drug. Among the compounds screened, compounds **3a** and **3c** both exhibited inhibition against *S. aureus*, *B. substalis*, *P. auroginosa*, *E. Coli* and *Candida albicans*. Compound **3b** showed inhibition against *S. aureus*, *B. substalis*, *P. auroginosa*, and *E. Coli* and compound **3d** showed only inhibition against *S. aureus*, and *B. substalis*.

CONCLUSION

In conclusion, we synthesized α-cyano substituted chalcones by Knoevenagel condensation and evaluated for their antibacterial and antifungal activity. Investigation of antibacterial and antifungal screening data revealed that the all the synthesized compounds **(3a-d)** showed moderate zone of inhibition against *S. aureus*, *B. substalis*, *P. auroginosa*, *E. Coli* and *Candida albicans*. Further bioassay, optimization and structure-activity relationship of the title compounds are underway.

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