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Research Article

Piperazine containing Murrayanine-Chalcones as Emerging Anti-microbial Agents

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Abstract

Background: Murrayanine is an alkaloid of carbazole constitution obtained from curry tree *Murraya koenigii* L. It is the most active compound among the other 20 alkaloidal compounds in the plant and is highly explored till date. The semi-synthetic derivatives of murrayanine have been developed over the years which demonstrated drastically high activities than that of the parent compound.

Methodology: Piperazine containing chalcone molecule was developed rationally by Claisen-Schmidt reaction where the fusion of the two individual components; murrayanine, a natural product in the A-ring and piperazine containing acetophenone in the B-ring were performed, and screened carefully against virulent bacterial species; *Staphylococcus aureus* and *Escherichia coli*, and virulent fungal species; *Aspergillus niger* and *Candida albicans*.

Results: The molecule exhibited tremendous anti-bacterial activity against *E. coli* with ZOI of 24.39 mm and notable anti-fungal activity against *C. albicans* with ZOI of 22.93 mm. The activity against *S. aureus* was found to be 21.99 mm while fungicidal effect against *A. niger* was observed to be 20.07 mm.

Conclusion: The developed chalcone displayed remarkable anti-bacterial and anti-fungal activity. However, the activity was observed to be quite less than that of the standard anti-bacterial compound (ciprofloxacin) and standard anti-fungal compound (fluconazole).

Keywords: *Murraya koenigii*, Murrayanine, Chalcone, Piperazine, Antifungal, Antibacterial

Introduction

Murrayanine is an alkaloid of carbazole constitution obtained from curry tree *Murraya koenigii* L. (Family: Rutaceae). It is the most active compound (anti-infective, anti-oxidant, anti-inflammatory, anti-diabetic, etc.) among the other 20 alkaloidal compounds in the plant and is highly explored till date. A number of semi-synthetic derivatives of murrayanine have been developed over the years which demonstrated drastically high activities better than that of the parent compound.[1]

Chalcone or prop-2-en-1-one is a famous scaffold owing to their diverse pharmacological potentials such as anti-retroviral, anti-leishmanial, anti-trypanosomal, anti-bacterial, anti-fungal, anti-parasite, anti-malarial, anti-filarial, anti-tubercular, anti-angiogenic, anti-cancer, anti-hyperlipidemic, anti-oxidant, anti-hypertensive, anti-arrhythmic, anti-platelet, anti-obesity, anti-diabetic, anti-inflammatory, anti-gout, analgesic, etc. The popularity of this scaffold can be judged from the volume of patents filed by the enthusiastic investigators. Heterocyclic containing chalcones have been reported to exhibit pronounce biological activities and is currently a hot-topic among the (medicinal)-chemists and pharmacologists. Piperazine is one of the privileged heterocyclic moieties which have received global attention.[2-7]

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In the current research, piperazine containing chalcone molecule was developed rationally by Claisen-Schmidt reaction where the fusion of the two individual components; murrayanine, a natural product in the A-ring and piperazine containing acetophenone in the B-ring were performed, and screened carefully against virulent bacterial species; *Staphylococcus aureus* (*S. aureus,* MTCC 3160) and *Escherichia coli* (*E. coli*, MTCC 2961), and virulent fungal species; *Aspergillus niger* (*A. niger*, MTCC 277) and *Candida albicans* (*C. albicans*, MTCC 227).

Materials and Methods

Chemicals and Instrumentation

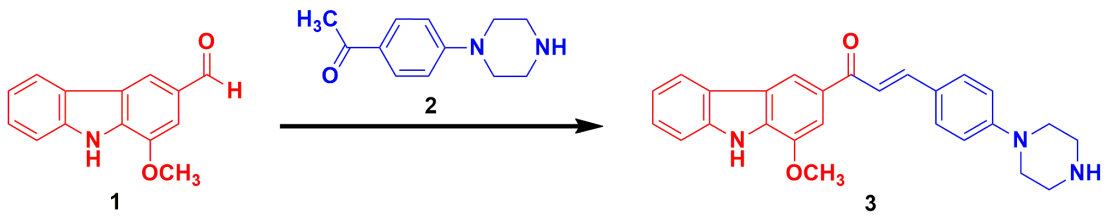
Analytical grade reagents, chemicals, and solvents were procured from HiMedia Ltd., India. 1-(4-(piperazin-1-yl) phenyl)ethanone, the reactant was purchased from Sigma Aldrich, Germany. The progress of the chemical reaction was ensured through pre-coated Merck® Silica gel-G TLC plates. The structure of the fabricated chalcone compound was ascertained by Fourier transformed Infrared Spectroscopy (Shimadzu® IR-Affinity-1), ^{1}H-NMR Spectroscopy (Bruker Avance-II), Mass Spectroscopy (MICROMASS Q-TOF), and Elemental Analyzer (PerkinElmer 2400).

Extraction of murrayanine

By utilizing the silica gel-based column and n-hexane mobile phase, murrayanine was extracted from the powdered stem bark of *M. koenigii*. The previously developed extraction method yielded hexane fractions (B_{21}-B_{37}) which were detected through the thin layer chromatography and afterward concentrated by the vacuum rotary evaporator.[1]

Synthesis of target compounds

The fabrication of prop-2-en-1-one scaffold (3) involved aldol condensation approach where the starting material, murrayanine (1), containing the aldehyde portion reacts with the reactant, piperazine containing acetophenone (2), containing the acetyl part to form a β-hydroxy ketone function, in the presence of ethanolic NaOH solution (Scheme 1).



Scheme 1.Fabrication of piperazine-containing murrayanine-chalcone

Synthetic protocol for (*E*)-1-(1-methoxy-9*H*-carbazol-3-yl)-3-(4-(piperazin-1-yl)phenyl)prop-2-en-1-one

An equal amount (0.01 M) of murrayanine (1) and 1-(4-(piperazin-1-yl)phenyl) ethanone (2) were refluxed in the presence of sodium hydroxide aqueous solution (20 mL) containing 90% ethanol solution (25 mL). Overnight, the mixture was allowed to stand and further added to the beaker containing dilute HCl in crushed ice with vigorous stirring. The product (3) was separated by filtration through Buchner's funnel, and washed thoroughly with cold water, and recrystallized suitably.

65% yield; FTIR (KBr) υ (cm^{-1}): 3350 (-NH, stretching), 3141 (C-H, aromatic), 1704 (C=O), 1633 (C=C, aromatic), 1612 (C=C, alkene), 1588 (-NH, bending), 1351 (C-N), 1201 (C-O); ^{1}H NMR (δ, ppm, $CDCl_3$): 10.15 (9, 1H), 6.7-8.5 (Aromatic, 10H), 3.94 (1, 3H), 3.49 (18, 2H), 2.54 (19, 2H), 1.73 (20, 1H). MS: M^+ 411. Anal. Calcd. for $C_{26}H_{25}N_3O_2$: C, 75.89; H, 6.12; N, 10.21. Found: C, 75.05; H, 5.76; N, 9.83.

Anti-microbial screening

The fabricated compound was screened for anti-bacterial and anti-fungal activity by utilizing the disc diffusion method. For anti-bacterial study, Muller Hinton Agar medium was employed and for the anti-fungal activity, Potato Dextrose Agar medium was used. The nutrient broth media was made use for the culture of microbes and suitably transferred under laminar air flow into the agar plates. The experimental compound was dissolved in dimethyl sulfoxide (DMSO), soaked over a Whatman filter paper, and placed over the cultured microbial plates. The incubation was done performed; 37±1ºC for 24 hrs for bacterial species and 37±1ºC for 72 hrs for fungal species. The minimum inhibitory concentration was estimated using agar streak dilution method. A 10^5 CFU/mL microbial suspension was initially formed by serial dilution with DMSO and the produced suspension was transferred to the petri dish at 40-50ºC temperature, up to 5 mm depth. The average of MIC values was computed properly. The positive control used for anti-bacterial screening was ciprofloxacin and for anti-fungal screening was fluconazole, while the DMSO served as the negative control.[8-9]

Results

Chemistry

The chemical structure of the piperazine containing chalcone was confirmed by the spectroscopic evaluation.

The FT-IR spectra revealed the formation of the proposed compound from the appearance of the carbonyl group of ketone origin at 1704 cm^{-1} and vanishing of the carbonyl group of aldehyde origin which was previously located at 1753 cm^{-1}. The carbazole moiety was substantiated from the NMR spectra where the 3 methoxy protons were confirmed at 3.94 ppm and the -NH component

was discerned from peaks at 10.15 ppm. The 10 aromatic protons of the carbazole and ring-B of chalcone scaffold were corroborated from the peaks in the range of 6.7-8.5 ppm. The presence of heterocycle piperazine was found out from the peaks at 3.49 ppm (reflecting the position 18), 2.54 ppm (revealing the position 19), and 1.73 ppm (depicting the position 20). From the obtained spectral information, it was proved that both the individual parts fused together into a single scaffold. The mass spectra additionally supported the formation of chalcone molecule as evidenced from the base peak which lies corresponding with the molecular mass along with the appearance of some fragmented products (m/z <100). In addition to it, the CHN analysis authenticated the creation of the proposed compound as indicated by the practically obtained ratios of the elements with that of the theoretical values.

Anti-microbial study

The anti-microbial effect of the novel piperazine-containing chalcone compound was observed to be noteworthy. The molecule exhibited tremendous anti-bacterial activity against *E. coli* with ZOI of 24.39 mm and notable anti-fungal activity against *C. albicans* with ZOI of 22.93 mm. The activity against *S. aureus* was found to be 21.99 mm while fungicidal effect against *A. niger* was observed to be 20.07 mm (Table 1). However, the activities of the prop-2-en-1-one derivative were quite low than that of the standard drugs (ciprofloxacin and fluconazole).

Table 1.Anti-microbial activity of piperazine containing chalcone derivative

Compounds	*E. coli*	*S. aureus*	*A. niger*	*C. albicans*
3	24.39± 1.42 (25)	21.99± 1.37 (25)	20.07± 1.66 (25)	22.93± 1.47 (25)
Ciprofloxacin	32.63± 1.71 (6.25)	30.66± 1.58 (6.25)	-	-
Fluconazole	-	-	33.17± 1.82 (6.25)	31.44± 1.26 (6.25)

Zone of inhibition in millimeter, SD = standard deviation.

Discussion

Heterocycles produced by chemical reactions have been known to possess tremendous anti-microbial activity.[10] A number of marketed products have several imperative heterocycle components such as pyrazole, oxazole, pyrimidine, pyridine, thiophene, thiazole, etc which are known to express a wide range of pharmacological activities, including the anti-bacterial, anti-fungal, anti-viral, etc.[11] Numerous well-known antibiotics like macrolide, penicillin, cephalosporin, quinolone, etc. have heteroatoms which amplify the anti-bacterial potential to several extents.[12] Recently, heterocycle containing chalcone molecules have been rationally designed by the researchers which often displayed better activity than the non-heteroatom moiety along with higher potency.[13] The substitution of chalcone with piperazine has been shown to produce better anti-microbial results than that of substitution with piperidine at similar position. Although, the enhancement in the anti-microbial activity was perceived to be quite marginal.[14] The study glorified the anti-infective perspective of the benzylideneacetophenone scaffold against four highly virulent microbial species.

Conclusion

The piperazine containing murrayanine-chalcone [(*E*)-1-(1-methoxy-9*H*-carbazol-3-yl)-3-(4-(piperazin-1-yl) phenyl)prop-2-en-1-one] comprising of a natural product murrayanine in the ring-A and a heterocycle (piperazine) portion in the ring-B displayed remarkable anti-bacterial (*E. coli* and *S. aureus*) and anti-fungal (*A. niger* and *C. albicans*) activity. However, the activity was observed to be quite less than that of the standard anti-bacterial compound (ciprofloxacin) and standard anti-fungal compound (fluconazole). The heterocycle-containing chalcone will positively motivate the researchers in the rational development of chalcones with more substitution of heterocycle rings to obtain pronounce biological activity.

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Conflict of Interest: None

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