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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Antimicrobial activity of Schiff bases of coumarin-incorporated 1,3,4-oxadiazole derivatives: an in vitro evaluation

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Abstract A series of 3-{5-[(*E*)-(substituted benzylidene) amino]-1,3,4-oxadiazol-2-yl}-2*H*-chromen-2-ones (**4a–r**) were synthesized by the reaction of 3-(5-amino]-1,3,4-oxadiazol-2-yl}-2*H*-chromen-2-one with different substituted benzaldehydes to form Schiff bases of coumarin-incorporated 1,3,4-oxadiazole derivatives. The structures of the compounds were confirmed on the basis of their elemental analysis and spectral data. The compounds were screened against bacterial strains *Staphylococcus aureus* NCTC (10418), *Escherichia coli* NCTC (6571), and fungal strain *Candida albicans* ATCC (10231). Ciprofloxacin and Ketoconazole were used as standards. The test compounds and standards were evaluated at 100 μg/ml concentration. DMF (*N*,*N*-dimethylformamide) was used as solvent and control. Most of the synthesized compounds possess significant antimicrobial activity. Compound (**4m**) without any substitution of the phenyl ring which is attached to 1,3,4-oxadiazole moiety showed highly significant in vitro growth inhibition against *S. aureus* and *E. coli*, while as compound (**4g**) with *para* $N(CH_3)_2$ showed highly significant in vitro growth inhibition against *C. albicans*.

Keywords Coumarin · 1,3,4-Oxadiazoles · Schiff base · Antimicrobial activity

Introduction

The name coumarin originates from caribbian word "*coumaron*" for the tonka tree, which was known botanically at one time as *Caumarouna odorata*. Coumarins are reported to possess important pharmacological activities like antimicrobial (Bhat *et al.*, 2009), anticonvulsant (Bhat *et al.*, 2008a), antitumor (Chimichi *et al.*, 2002), and anti-inflammatory (Cruzzocrea *et al.*, 2000). 1,3,4-Oxadiazole derivatives are reported to show a broad spectrum of biological activities which include COX inhibitor (Boschelli *et al.*, 1993), anticonvulsant (Zarghi *et al.*, 2005), antibacterial (Bhat *et al.*, 2005), and antifungal (Nizamuddin *et al.*, 2001). Biocidal activities of Schiff bases have been well established. These have been attributed to the toxophoric C=N linkage in them. Schiff base acquired broad spectrum of biological activities like antibacterial (Iqbal *et al.*, 2007), antifungal (Mishra *et al.*, 2005), anti-inflammatory (Sharma *et al.*, 2002), antiproliferative (Vicini *et al.*, 2006), antitubercular (Lourenco *et al.*, 2007), and anticonvulsant (Ragavendran *et al.*, 2007). The coumarin-incorporated 1,3,4-oxadiazoles have been reported as potential antimicrobial agents (Cacic *et al.*, 2006; Mashelkar and Audi, 2006).The main idea of this work was to design mutual prodrugs which contain coumarin and 1,3,4-oxadiazole moieties, using Schiff base (C=N) as linkage group to produce synergistic antimicrobial activity.

This prompted us to synthesize and study the antimicrobial activity of compounds incorporating coumarin and 1,3,4-oxadiazoles Schiff bases to get the synergistic effect.

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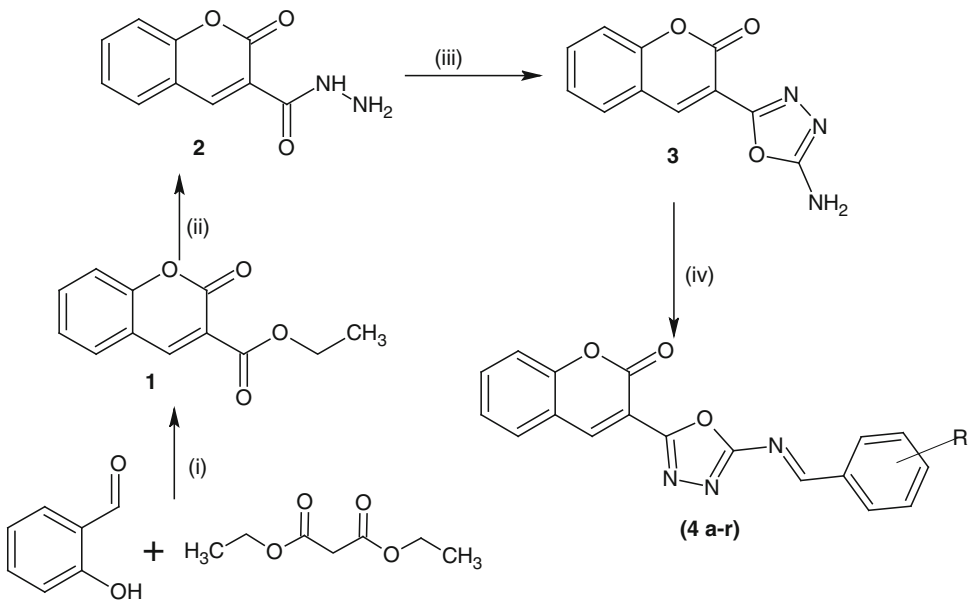

Scheme 1 Synthesis of Schiff bases of coumarin-incorporated 1,3,4-oxadiazole derivatives (**4a–r**) *i* piperidine, C_2H_5OH, reflux 5 h. *ii* $NH_2NH_2{\cdot}H_2O$, C_2H_5OH, reflux 10 h. *iii* CNBr, C_2H_5OH, 55–60 °C for 90 min. *iv* Glacial CH_3COOH, 1,4-dioxan, substituted benzaldehydes, reflux 8 h; **4a**: $R = 3\text{-}NO_2$; **4b**: $R = 3,4\text{-}(OCH_3)_2$; **4c**: $R = 4\text{-}OH$; **4d**: $R = 2\text{-}OH$; **4e**: $R = 2\text{-}NO_2$; **4f**: $R = 3\text{-}OH$; **4g**: $R = 4\text{-}N(CH_3)_2$; **4h**: $R = 4\text{-}F$; **4i**: $R = 4\text{-}OCH_3$; **4j**: $R = 2\text{-}Cl$; **4k**: 3-Cl; **4l**: 4-Cl; **4m**: $R = H$; **4n**: $R = 4\text{-}NO_2$; **4o**: $R = 3\text{-}F$; **4p**: $R = 2\text{-}F$; **4q**: $R = 2\text{-}OCH_3$; **4r**: $R = 3\text{-}OCH_3$

Chemistry

Synthesis of ethyl-2-oxo-2*H*-chromene-3-carbxylate (**1**) (m. p. 120–122 °C), 2-oxo-2*H*-chromene-3-carbohydrazide **2** (m. p. 136–138 °C), and 3-(5-amino-1,3,4-oxadiazole-2-yl)-2*H*-chromen-2-one (**3**) (m. p. 200–202 °C) were performed according to reported methods (Bhat *et al.*, 2008; Khan and Mymmona, 2003). 3-{5-[(*E*)-(substituted benzylidene) amino]-1,3,4-oxadiazol-2-yl}-2*H*-chromen-2-ones (**4a–r**) were synthesized by reacting different substituted benzaldehydes with 3-(5-amino-1,3,4-oxadiazole-2-yl)-2*H*-chromen-2-one (**3**) to form Schiff bases (Scheme 1).

Experimental

All the solvents were of laboratory grade (LR) and were obtained from Merck, Central Drug House Private Ltd. (CDH) and S. D. Fine Chemicals. Melting points were determined in open capillary tubes and thermometer is uncorrected. The elemental analysis (C, H, and N) of all compounds were performed on the CHN Elementar (Analysen systeme GmbH, Germany) and Vario EL III (Elementar Amricas Corporation) and were within limit of ±0.4 % of the theoretical values. Thin layer chromatography was performed on silica gel G (Merck). The FT-IR spectra were recorded in KBr pellets on a (BIO-RAD FTS 135) WIN-IR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker model DPX 300 FT NMR spectrometer in ($CDCl_3$) using tetramethylsilane (Me_4Si, TMS) as an internal standard. Mass spectra were recorded on a Jeol JMS-D instrument fitted with a JMS 2000 data system at 70 eV.

The structures of the synthesized compounds were confirmed on the basis of spectral data and elemental analysis. The additional data of synthesis and analytical data of all the intermediates (**1–3**) and 3-{5-[(*E*)-(substituted benzylidene) amino]-1,3,4-oxadiazol-2-yl}-2*H*-chromen-2-ones (**4a–r**) are given in Online Resource 1.

Antimicrobial activity

The compounds were screened against bacterial strains *Staphylococcus aureus* NCTC (10418), *Escherichia coli* NCTC (6571) and fungal strain C*andida albican* ATCC (10231) by cup plate method [agar diffusion method] (Barry, 1976). Ciprofloxacin and Ketoconazole were used as standards. The test compounds and standards were evaluated at 100 μg/ml concentration. DMF (*N*,*N*-dimethylformamide) was used as solvent and negative control. Data are represented as % inhibition with reference to standards in (Table 1). The microbial strains and standards (Ciprofloxacin and Ketoconazole) were obtained from Biochemistry Department, Majeedia Hospital, Jamia Hamdard, New Delhi.

Results and discussions

It has been observed that most of the compounds showed inhibition against *S. aureus*, *E. coli*, and *C. albicans*. Compound **4m** without any substitution showed 80 % growth inhibition against *S. aureus* which was comparable to Ciprofloxacin while as compounds (**4a**, **4e** and **4n**) showed 64 % growth inhibition against *S. aureus*. Compounds (**4j**, **4k** and **4l**) were devoid of growth inhibition against *S. aureus.*

Table 1 Antibacterial and antifungal activity of synthesized compounds (**4a–r**)

Compounds	Diameter of zone of inhibition (mm)			% Inhibition with reference to standard[a]		
	S. aureus	*E. coli*	*C. albicans*	*S. aureus*	*E. coli*	*C. albicans*
4a	17	12	–	64	48	–
4b	12	10	10	48	40	50
4c	10	10	9	40	40	45
4d	10	12	10	40	48	50
4e	17	14	–	64	56	–
4f	10	15	11	40	60	55
4g	12	12	16	48	48	80
4h	12	15	10	48	60	50
4i	14	10	12	56	40	60
4j	–	17	13	–	64	65
4k	–	17	14	–	64	70
4l	–	17	15	–	64	75
4m	20	20	12	80	80	60
4n	17	12	–	64	48	–
4o	12	10	11	48	40	55
4p	12	10	13	48	40	65
4q	14	15	13	56	60	65
4r	14	15	14	56	60	70
2	10	09	08	40	36	40
Ciprofloxacin[b]	25	25	–	100	100	–
Ketoconazole[b]	–	–	20	–	–	100

Ketoconazole (100 μg/ml), zone of inhibition: 20 mm

[a] The percentage zone of inhibition was calculated against the bacterial strains *Staphylococcus aureus* NCTC (10418), *Escherichia coli* NCTC (6571), and fungal strain C*andida albicans* ATCC (10231). Solvent and negative control: DMF (*N*,*N*-dimethyl formamide)

[b] Standards: Ciprofloxacin (100 μg/ml), zone of inhibition: 25 mm

Compound **4m** without any substitution showed 80 % growth inhibition against *E. coli*, which was comparable to Ciprofloxacin. Compounds (**4j**, **4k**, **4l**) and (**4j**, **4k**, **4l**) showed 64 and 60 % growth inhibition against Gram-negative *E. coli*, respectively. Compound **4g** with para $N(CH_3)_2$ substitution showed 80 % growth inhibition against *C. albicans* which was comparable to Ketoconazole. Compound **4l**, (**4k**, **4r**), (**4j**, **4p**, **4q**), and (**4i**, **4m**) showed 75, 70, 65, and 60 % growth inhibition against *C. albicans*, respectively. Since all the test compounds and standard drugs were prepared in freshly distilled DMF, its zone of inhibition was found to be very negligible and taken as zero mm. All the synthesized compounds were more active than start, 2-oxo-*2H*-chromene-3-carbohydrazide (**2**) against *S. aureus*, *E. coli* and *C. albicans* when compared with standard drugs Ciprofloxacin and Ketoconazole.

Structure activity relationship

(a) Compound without any substitution presented highest growth inhibition against *S. aureus*, *E. coli.* (b) Compounds with nitro substitution presented moderate growth inhibition against *S. aureus.* (c) Compounds with chloro substitution presented moderate growth inhibition against *E. coli.* (d) Compound with para dimethyl amino substitution showed highest growth inhibition against *C. albicans.* (e) Compounds containing Chloro and methoxy substitution also showed moderated activity against *C. albicans.*

Conclusion

Compound **4m** without any substitution of the phenyl ring which is attached to 1,3,4-oxadiazole moiety showed highly significant in vitro growth inhibition against *S. aureus* and *E. coli*, while as compound **4g** with *para* $N(CH_3)_2$ showed highly significant in vitro growth inhibition against *C. albicans*. It can be concluded from this study that unsubstituted compound **4m** and para $N(CH_3)_2$ substituted compound **4g** showed highest in vitro growth inhibition against *S. aureus*, *E. coli*, and *C. albicans*, respectively, compared to Ciprofloxacin and Ketoconazole.

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