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Synthesis, Characterization and Biological Evaluation of some Novel Substituted Indole-Coumarin Derivatives as Potential Antibacterial and Antifungal Agents

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: In the present work, we have designed and synthesized indole-fused coumarin derivatives as potential antibacterial and antifungal agents.
Place and Duration of Study: The present work was carried out at PEA's Modern college of Pharmacy, Sector 21, Yamunanagar, Nigdi- 411044 between the duration of January-2021 to May-2021.
Methodology: The *in vitro* antibacterial and antifungal activity was performed by disc diffusion method. The antibacterial activities were tested using agar nutrient medium against *Bacillus subtilis, Bacillus pumilus, Escherichia coli,* and *Pseudomonas aeruginosa* that are representative types of gram-positive and gram-negative organisms respectively. The anti-fungal activity of all the compounds were determined on potato dextrose agar medium against *Aspergillus niger* and *Candida albicans*. Clotrimazole 100 µg/ml was used as a standard and DMF was used as control.
Results: It was observed that all the compounds were sensitive to the gram +ve bacteria. Compound 2c, 2e, and 2f were sensitive to gram –ve bacteria. Compounds 2a, 2b, and 2f were sensitive to both the fungal strains while all the derivatives were sensitive to *Candida albicans*.

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Conclusion: From present investigation it has been observed that compound 2f was sensitive against gram +ve, gram –ve, and both the strains of fungal strains. Structurally, compound 2f possess aldehyde functional group at indole nucleus. Therefore, from present study we have concluded that compound 2f is a lead molecule for the further development of potential antibacterial and antifungal agents.

Keywords: Coumarin; indoles; antibacterial; antifungal; In vitro.

1. INTRODUCTION

The development of multidrug-resistant bacteria and the effects of global warming are two of the most significant problems that contemporary scientists have faced in recent decades. The fact that many pathogenic microorganisms responsible for a variety of human and animal diseases have developed mechanisms of resistance to conventional therapies has prompted extensive research in the fields of natural and synthetic chemistry with the goal of discovering new drug classes with significantly improved therapeutic profiles than those currently available [1]. In this regard, the antimicrobial activities of numerous classes of organic substances, including phenols [2,3], coumarins [4–8], thiophenes [9], quinones [10], and azo compounds [11], are currently the focus of drug discovery efforts around the world.

We are currently seeing the development of an entirely new class of medicines with hybrid molecular architectures, which combine the biological properties of two or more small molecules, or even small molecules and biologics, to produce a single treatment with improved efficacy. Molecular and/or biologic conjugates of this kind, it is anticipated, will become the main form of targeted infectious disease therapy in the long run, according to the researchers. Combining two or more potentially bioactive substructures to form an integrated new molecular framework with increased anticipated therapeutic firepower is a conceptually simple concept that has proven to be extremely successful in the context of infectious diseases that necessitate effective killing of microbes [1].

In the present work, we have designed and synthesized indole-fused coumarin derivatives as potential antibacterial and antifungal agents. The synthesized products have been subjected for in vitro antibacterial and antifungal activities.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The chemicals of synthetic grades such as 2-hydroxybenzaldehyde, chloroacetyl chloride, dichloromethane, trimethylamine, chlorosulphonic acid, NH_4OH, and substituted indoles were purchased and procured from Lab Trading Chemicals, Auranagabad, Maharashtra.

2.2 Reaction Scheme and Synthesis of Indole-fused Coumarin Derivatives

2.1.1 Step-I: Synthesis of 3-chloro-2H-chromen-2-one

Solution of 2-hydroxybenzaldehyde (3.75 mol) in dichloromethane (4 ml) was added in triethylamine (8.6 mol) and a solution of chloroacetyl chloride (5.02 moles) under continuous stirring in cold condition. The above cold solution was added in 2ml methylene chloride. The mixture was stirred at room temperature for 30 min. and then heated to reflux for 8-10 hrs. The solvent was removed under reduced pressure and the dark brown oily residue was chromatographed over silica gel (100-200 mesh) using n-hexane as an eluent to give some amount of starting compound and 3-chlorocoumarin as crystalline products. The yields reported are based on recovered starting compound. All 3-chlorocoumarins were recrystallized from n -hexane [12]. Completion of reaction was monitored by using TLC.

2.1.2 Step-II: Synthesis of 3-chloro-2-oxo-2H-1-benzopyran-6-sulfonyl chloride

Chlorosulfonic acid (4 ml, 0.06 mol) was cooled in ice bath and treated with 4.32-4.40 gm of 3-chlorocoumarin separately (1 mol) at such a rate that the reaction temperature was maintained between 10-15°C for almost 35 minutes. The ice bath was removed and the solution was stirred at room temperature for 16 hrs. The mixture was diluted with dichloromethane and the solution

was slowly added to ice water with stirring. From the two phases which were separated, the dichloromethane portion was collected and cooled to 5°C and then treated with conc. NH_4OH and stirred at 15°C for 15 min. The mixture was then extracted with dichloromethane, filtered and concentrated to give crude products. The crude products were dissolved to 2-butanone and then recrystallized using isopropyl alcohol [13]. Completion of reaction was monitored by using TLC.

2.1.3 Step-III: Synthesis of 3-chloro-2-oxo-2H-1-benzopyran-6-sulfonamide

The prepared 3-chloro-2-oxo-2H-1-benzopyran-6-sulfonyl chloride (0.5 mol) was boiled for ten minutes with concentrated ammonium hydroxide (5 cc). After cooling to room temperature, and adding cold water (10 cc), the resultant solid sulphonamide was filtered with suction and thoroughly washed. It was then recrystallized to constant melting point from dilute ethanol and dried at 105^0C[14]. Completion of reaction was monitored by using TLC.

2.1.4 Step-IV: Synthesis of Substituted Indole-fused Coumarin Derivatives

A solution of substituted indoles (10mol) in dichloromethane (50 ml) was added drop wise to a stirred mixture of 3-chloro-2-oxo-2H-1-benzopyran-6-sulfonyl chloride (10 mol) and sodium carbonate (14 mol) in dichloromethane (20 ml). The reaction mixture was stirred at room temperature overnight, and distilled water (20 ml) was added. The organic phase was separated and the aqueous phase was extracted with dichloromethane (20-30 ml). The organic extracts were combined and washed with water (30 ml). The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated to dryness under reduced pressure. The crude product was further purified by column chromatography on silica gel using 1:9 acetone/hexane to obtain the pure product [15]. Completion of reaction was monitored by using TLC. The proposed reaction scheme is depicted in Fig. 1. The characterization of some representative compounds have been performed by IR, NMR and Mass. The physicochemical data of synthesized product is tabulated in Table 1. The characterization data is given below:

2a: IR (KBr): v(cm -1) 3497 (NH Stretch); 3121 (NH Bend w); 2987 (Ar Stretch); 1751 (C=O Stretch) 765 (C=O), 1H NMR: δ 3.70 (3H, s), 6.79 (1H, ddd, J = 8.5, 1.4, 0.5 Hz), 6.99-7.19 (2H, 7.14 (ddd, J = 8.4, 7.6, 1.4 Hz), 7.04 (ddd, J = 8.5, 7.6, 1.1 Hz)), 7.42 (1H, dd, J = 7.9, 0.5 Hz), 7.84 (1H, dd, J = 7.9, 2.0 Hz), 8.21 (1H, ddd, J = 8.4, 1.1, 0.5 Hz), 8.55 (1H, s), 8.59 (1H, dd, J = 2.0, 0.5 Hz). Mass m/e 408.81 (100.0%), 409.02 (21.1%), 407.01 (1.9%). Elemental analysis (*calc.*): C(49.95%), H(3.21%), Cl(8.67%), N(6.85%), O(23.48%), S(7.84%).

2b: IR (KBr): v(cm -1) 3497 (NH Stretch); 3121 (NH Bend w); 2987 (Ar Stretch); 1751 (C=O Stretch) 765 (C=O), 1H NMR: δ 6.57 (1H, ddd, J = 8.0, 7.7, 1.2 Hz), 6.67 (1H, ddd, J = 7.9, 1.2, 0.5 Hz), 7.00 (1H, ddd, J = 7.9, 7.7, 1.1 Hz), 7.37-7.45 (2H, 7.40 (ddd, J = 8.0, 1.1, 0.5 Hz), 7.42 (dd, J = 7.9, 0.5 Hz)), 7.84 (1H, dd, J = 7.9, 2.0 Hz), 8.55 (1H, s), 8.59 (1H, dd, J = 2.0, 0.5 Hz). Mass m/e 493.80 (100.0%), 494.02 (20.0%), 492.01 (1.7%). Elemental analysis (*calc.*): C(48.80%), H(3.07%), Cl(9.00%), N(10.67%), O(20.31%), S(8.14%).

2c: IR (KBr): v(cm -1) 3497 (NH Stretch); 3121 (NH Bend w); 2987 (Ar Stretch); 1751 (C=O Stretch) 765 (C=O), 1H NMR: δ 2.21 (3H, s), 6.99-7.16 (3H, 7.03 (ddd, J = 7.8, 7.5, 1.1 Hz), 7.08 (ddd, J = 7.8, 1.8, 0.5 Hz), 7.13 (ddd, J = 8.4, 1.1, 0.5 Hz)), 7.26 (1H, ddd, J = 8.4, 7.5, 1.8 Hz), 7.42 (1H, dd, J = 7.9, 0.5 Hz), 7.84 (1H, dd, J = 7.9, 2.0 Hz), 8.55 (1H, s), 8.59 (1H, dd, J = 2.0, 0.5 Hz). Mass m/e 392.81 (100.0%), 393.03 (32.2%), 391.01 (17.63%). Elemental analysis (*calc.*): C(51.98%), H(3.34%), Cl(9.03%), N(7.13%), O(20.37%), S(8.16%).

2.3 Antibacterial Activity

All the cultures were procured from NCL, Pune. The antibacterial activities were tested using agar nutrient medium against *Bacillus subtilis(*NCIM 5458*), Bacillus pumilus*(NCIM 5319*), Escherichia coli*(NCIM 5346*),* and *Pseudomonas aeruginosa*(NCIM 5740) that are representative types of gram-positive and gram-negative organisms respectively. The antibacterial activity of the compounds was assessed by disc diffusion method. The weighed quantities of bacteriological peptone (20gm) and beef extract (5gm) were dissolved in distilled water by gentle warming and then specified amount of agar (20gm) was dissolved by heating on water bath. Then the p^H of the solution was adjusted to 7.2 to 7.4 by adding the sodium chloride (5gm) and the volume of the final solution was made up to 1000 ml with distilled water. Then it was transferred in to a suitable

container, plugged with non-adsorbent cotton and the media was sterilized by in autoclave at 121^{0}C for 20 minutes at 15 lbs pressure. The test solutions were prepared by dissolving specified quantity of compounds in DMF to make concentration of 100µg/ml. Ciprofloxacin of 100µg/ml was used as standard. Discs of 6-7 mm in diameter were punched from NO: 1 Whatman filter paper with sterile cork-borer of same size. These discs were sterilized by keeping in oven at 140^{0}C for 60 minutes. Then the standard and test solutions were added to each disc and discs were air-dried. The sterilized media was cooled to 45^{0}C with gentle shaking for uniform cooling and then inoculated for 18-24 hrs old culture under aseptic conditions, then mixed well by gentle shaking. This was poured into a sterile Petri dishes (properly labeled) and allowed the medium to set. After solidification all the Petri dishes were transferred to laminar flow unit. Then the discs which were previously prepared were carefully kept on the solidified media by using sterilized forceps. These Petri dishes were kept as it is for one-hour diffusion at room temperature and then for incubation at 37^{0}C for 24 hours in an incubator. The extent diameter of inhibition after 24 hours was measured as the zone of inhibition in millimeters[16–19].

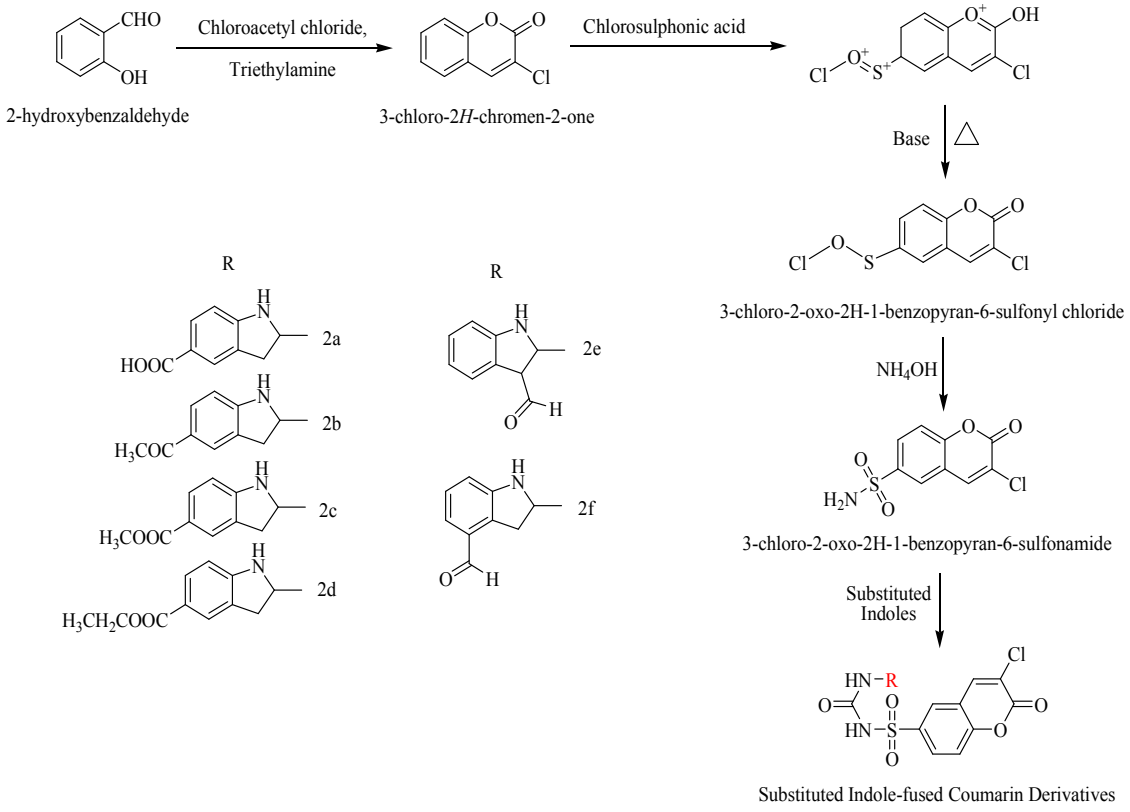

Fig. 1. The reaction scheme for the synthesis of substituted indole-fused coumarin derivatives

Table 1. The physicochemical data of synthesized products

Compound Code	Molecular Formula	Molecular Weight	Melting Point (^{o}C)	R_f Value	% Yield
2a	$C_{18}H_{12}ClNO_6S$	405.81	163-165	0.76	71
2b	$C_{19}H_{14}ClNO_6S$	419.84	146-148	0.59	59
2c	$C_{20}H_{16}ClNO_6S$	433.86	178-180	0.78	84
2d	$C_{21}H_{18}ClNO_6S$	447.89	177-179	0.62	86
2e	$C_{18}H_{12}ClNO_5S$	389.81	131-133	0.72	63
2f	$C_{18}H_{12}ClNO_5S$	389.81	117-119	0.43	56

2.4 Antifungal Activity

The anti-fungal activity of all the compounds were determined on potato dextrose agar medium against *Aspergillus niger*(NCIM 1269) and *Candida albicans*(NCIM 3628). Clotrimazole 100 µg/ml was used as a standard and DMF was used as control. The sterile molten potato dextrose medium was cooled to 45^0C and inoculated with test organism and mixed the contents thoroughly and poured into the sterile Petri dishes under aseptic conditions. All the inoculated Petri dishes were incubated at 28^0C for 4 days and the extent diameter of inhibition was measured as the zone of inhibition in millimeters. Peeled potato (200-300gm) were cut into pieces and boiled for 30 min to get extract. The extract is filtered through muslin cloth. The dextrose (5gm) was added to the filtrate and final volume is adjusted to 1000 ml with distilled water. Then it was sterilized by autoclave at 121^0 C for 20 min (two days before testing the culture is prepared by inoculating the fungus from master culture into potato dextrose medium and incubated for 48 hrs at room temperature). The test solutions were prepared by dissolving specified quantity of compounds in DMF to make concentration of 100µg/ml. Clotrimazole lotion (1%) was used as standard anti-fungal for comparison and solution were prepared by using DMF; 1 ml of clotrimazole lotion was dissolved in 9 ml of DMF so that the concentrations of the solution were 100µg/ml. The method of testing for fungicidal activity is the same as that of antibacterial testing[16–19].

3. RESULTS AND DISCUSSION

LD_{50} value was calculated by plotting a graph of conc. (microgm/ml) Vs. Zone diameter in mm.

3.1 Antibacterial Activity

All the synthesized compounds were subjected for antibacterial activity at a 100µg/ml concentration and DMF as a control against, *Bacillus subtilis, Bacillus pumilus, Escherichia coli* and *Pseudomonas aeruginosa* by disc-diffusion method. The antibacterial activity of compounds is illustrated in Table 2. It was observed that all the compounds were sensitive to the gram +ve bacteria. Compound 2c, 2e, and 2f were sensitive to gram –ve bacteria. Although, the compounds did not demonstrated significant activity compared to Ciprofloxacin but can be treated as lead molecule for the further development of more potent and selective derivatives. As compounds 2c, 2e, and 2f were sensitive to both the bacteria (gram +ve and gram –ve) they can be considered as lead molecules for further development as potential antibacterial agents to overcome the resistance.

Table 2. The antibacterial activity of compounds against gram +ve and gram -ve

Compound Code	Zone of Inhibition (mm)*			
	B. subtilis	***B. pumilus***	***E. coli***	***P. aeruginosa***
	Gram +ve		**Gram -ve**	
2a	8	7	NA	NA
2b	7	11	NA	NA
2c	8	8	7	9
2d	9	9	NA	NA
2e	12	7	9	10
2f	13	11	9	8
Ciprofloxacin	33	24	31	32

*Where, *Average of triplicate ± Standard deviation. NA, No Activity*

Table 3. The antifungal activity of synthesized compounds

Compound Code	***Aspergillus niger***	***Candida albicans***
2a	9	7
2b	7	11
2c	NA	13
2d	NA	11
2e	NA	14
2f	9	11
Clotrimazole	26	27

Where, NA, No Activity

3.2 Antifungal Activity

All the synthesized compounds were screened for antifungal activity at a concentration of 100 µg/ml using DMF as a control against *Aspergillus niger* and *Candida albicans* on potato dextrose agar media. Clotrimazole 100 µg/ml was used as standard. The antifungal activity of all the synthesized compounds is tabulated in Table 3. Compounds 2a, 2b, and 2f were sensitive to both the fungal strains while all the derivatives were sensitive to *Candida albicans*.

4. CONCLUSION

In the present study, we have designed and synthesized indole-fused coumarin derivatives as potential antibacterial and antifungal agents. From present investigation it has been observed that compound 2f was sensitive against gram +ve, gram –ve, and both the strains of fungal strains. Structurally, compound 2f possess aldehyde functional group at indole nucleus. Therefore, from present study we have concluded that compound 2f is a lead molecule for the further development of potential antibacterial and antifungal agents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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