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Synthesis of Coumarins Linked With 1,2,3-Triazoles Under Microwave Irradiation and Evaluation of Their Antimicrobial and Antioxidant Activity

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Supplementary Information

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General Considerations

The antibacterial activity of the newly synthesized compounds was determined by well plate method and the minimum inhibitory concentration (MIC) of the organic compounds was determined by broth dilution method. The antifungal studies of the newly synthesized compounds **4a-t** were determined by well plate method and the antioxidant activity was carried out by the conventional colorimetric DPPH radical scavenging capacity assay.

Experimental procedure and initial results of Biological activities

Antibacterial activity

The antibacterial activity of the newly synthesized compounds **4a-t** was determined by well plate method in nutrient agar media [1]. The compounds were tested against a panel of pathogenic microorganisms, including *E. coli*, *S. aureus, B. subtilis* and *P. aeruginosa*. Microorganism strains were maintained on nutrient agar medium at 37 °C. The cultures were inoculated in fresh 10 mL nutrient broth to yield an initial suspension of approximately 10-100 cfu/mL. All broths were then incubated statically at the aforementioned temperatures for microorganisms for 18-24 h. so that all cells are in the stationary phase. Susceptibility of the test organism to the compounds was determined by employing the well plate technique. The bacterial suspensions were diluted tenfold in sterilized distilled water and 0.1 mL from the appropriate dilution was spread plated on nutrient agar in order to give a population approximately 10^6 cfu/plate. Six millimeter diameter well was then punched carefully using a sterile cork borer and 30 µL of test solutions of different concentrations were added into each labeled well. The same procedure was repeated for different micro-organisms. Each experiment was carried out in triplicate. After the incubation, the inhibition zone was measured and the values for control were subtracted to get the actual values. Ciprofloxacin was used as the standard drug and the results are summarized in Table 1.

Table 1. Determination of antibacterial activity of the synthesized organic compounds[a]

Compounds	*Escherichia coli*		*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Bacillus subtilis*	
Conc. in mg/mL	1	0.5	1	0.5	1	0.5	1	0.5
Control	00		00		00		00	
Ciprofloxacin	22±0.2	17±0.1	21±0.2	18±0.2	21±0.2	18±0.2	23.8±0.3	14.2±0.2
4a	07±0.3	05±0.2	08±0.2	06±0.4	05±0.3	03±0.3	10±0.5	08±0.3
4b	00	00	00	00	00	00	00	00
4c	00	00	00	00	00	00	00	00
4d	00	00	00	00	00	00	00	00
4e	03±0.4	01±0.2	05±0.6	03±0.6	07±0.3	04±0.4	08±0.2	05±0.3
4f	13±0.1	10±0.2	14±0.3	12±0.2	16±0.2	13±0.3	12±0.4	10±0.1
4g	13±0.2	10±0.1	09±0.2	07±0.1	10±0.2	08±0.1	07±0.4	04±0.2
4h	04±0.5	02±0.3	03±0.2	02±0.1	05±0.4	03±0.2	05±0.3	03±0.3
4i	10±0.2	07±0.2	10±0.1	08±0.	07±0.2	03±0.2	08±0.	06±0.2
4j	00	00	00	00	00	00	00	00

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4k	15±0.2	12±0.2	12±0.1	11±0.2	16±0.2	13±0.1	10±0.3	07±0.4
4l	10±0.2	08±0.2	08±0.1	06±0.2	09±0.2	07±0.1	09±0.5	06±0.5
4m	11±0.2	09±0.2	09±0.2	07±0.1	10±0.2	07±0.2	11±0.5	08±0.3
4n	06±0.1	02±0.2	09±0.2	07±0.2	08±0.2	06±0.1	08±0.3	06±0.4
4o	00	00	00	00	00	00	00	00
4p	09±0.2	07±0.1	08±0.2	07±0.2	10±0.1	07±0.2	07±0.3	05±0.1
4q	06±0.2	04±0.1	08±0.1	05±0.1	08±0.2	04±0.2	10±0.5	07±0.3
4r	00	00	00	00	00	00	00	00
4s	09±0.2	06±0.2	06±0.1	04±0.2	07±0.2	05±0.2	07±0.3	05±0.4
4t	10±0.1	07±0.1	11±0.2	08±0.2	10±0.1	08±0.2	12±0.4	09±0.5

[a] The experiment was performed in triplicate and the values are expressed as Mean ±SD

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of the organic compounds was determined by broth dilution method recommended by EUCAST according to the International Standard ISO 20776-1 using MH-F broth [2]. The MIC value, representing the lowest concentration that completely inhibited the formation of visible growth or turbidity, was evaluated after 18 h. of incubation at 37 °C.

Antifungal activity

Antifungal studies of the newly synthesized compounds **4a-t** were determined by well plate method against *A. flavus*, *C. keratinophilum* and *C. albicans*. Normal saline was used to make a suspension of spore of fungal strains for lawning [3]. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliters of agar media were poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for 1 hour. Using sterile cork, the borer was punched carefully, wells were made on these seeded agar plates and different concentrations of the test compounds in DMSO were added into each labeled well. A control was also prepared for the plates in the same way using DMSO as solvent. The Petri dishes were prepared in triplicate and maintained at 25 °C for 72 hours. Antifungal activity was determined by measuring the diameter of inhibition zone. The activity of each compound was compared with Fluconazole as the standard and the results are depicted in Table 2.

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Table 2. Determination of antifungal activity of the synthesized compounds[a]

Compounds	*Aspergillus flavus*		*Chrysosporium keratinophilum*		*Candida albicans*	
Con in mg/mL	1	0.5	1	0.5	1	0.5
Control	00		00		00	
Fluconazole	13±0.	10±0.1	17±0.2	15±0.2	22±0.2	20±0.2
4a	05±0.2	03±0.4	06±0.3	05±0.1	04±0.1	02±0.4
4b	00	00	00	00	00	00
4c	00	00	00	00	00	00
4d	00	00	00	00	00	00
4e	0	00	00	00	00	00
4f	0±0.2	08±0.2	09±0.1	07±0.1	11±0.1	09±0.1
4	04±0.1	03±0.1	05±0.1	03±0.1	06±0.2	04±0.1
h	00	00	00	00	00	00
4i	03±0.1	00	02±0.1	00	03±0.1	01±0.1
4j	00	00	00	00	00	00
4k	10±0.1	07±0.2	08±0.2	07±0.2	09±0.2	07±0.1
4l	05±0.1	03±0.1	04±0.2	03±0.1	06±0.2	05±0.1
4m	05±0.2	02±0.1	03±0.2	01±0.1	04±0.2	02±0.1
4n	10±0.3	07±0.3	08±0.3	06±0.3	12±0.2	07±0.2
4o	00	00	00	00	00	00
4p	05±0.1	04±0.1	04±0.1	03±0.1	06±0.1	05±0.2
4q	03±0.1	01±0.1	04±0.2	03±0.1	05±0.1	03±0.1
4r	00	00	00	00	00	00
4s	09±0.5	06±0.3	09±0.3	06±0.4	11±0.4	07±0.5
4t	04±0.1	02±0.1	03±0.1	01±0.1	04±0.1	01±0.1

[a] The experiment was performed in triplicate and the values are expressed as Mean ±SD

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was determined by broth dilution method recommended by EUCAST according to the International Standard ISO 20776-1 using MH-F broth [2]. The MIC value was evaluated after 18 h. of incubation at 37 °C.

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Antioxidant activity

The conventional colorimetric DPPH• scavenging capacity assay was performed according to a previously described laboratory protocol [4]. Briefly, a 100 µL (100 µg concentration) sample of organic compounds in methanol was added to 3 mL of 0.004 % w/v DPPH• solution and each test tube were made up to a final volume of 4 mL. BHT was used as a reference standard and dissolved in methanol to make the same concentration as that of the remaining extracts. Each mixture was vortexed for a few seconds and left to stand in the dark for 10 min. at ambient temperature. The absorbance of each reaction mixture at 517 nm was measured against a blank of methanol using a UV-visible spectrometer (Shimadzu UV-1800). The level of DPPH• remaining for each reaction was calculated as:

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance of the control - Absorbance of the test sample}}{\text{Absorbance of the control}} \times 100$$

The inhibition curve was plotted for triplicate experiments and represented as percentage of mean inhibition ± standard deviation.

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