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Antifungal study of two synthesized phenolic azo derivatives

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ABSTRACT

Two phenolic azo derivatives consisting of anthracene moiety were synthesized by coupling 9-anthryldiazonium slat with candidate phenols. The synthesized compounds were screened for their antifungal activities against *Aspergillus niger* (*A. niger*). The results were compared with two commercial antibiotics and showed moderate to high levels in inhibitory.

KEYWORDS

Phenolic;
Azo;
Synthesized;
Screened;
Antifungal activities;
Aspergillus niger.

INTRODUCTION

Phenolic derivatives have been commonly considered as good candidates for inhibiting the growth of some types of bacteria and fungi. The antifungal activity of phenolic compounds extracted from olive pomace, a by-product of olive oil production, against *Alternaria solani, Botrytis cinerea* and *Fusarium culmorum* was investigated. These naturally occurring phenolic compound were used in two concentrations 0.1 and 0.2 % (w/v) and tested for their antifungal activity against the three fungi. They showed good levels of inhibition against the growth of the mentioned above three fungi[1]. Various industries rely on azo derivatives due to which large production of such compounds is demanded[2]. The use of azo dyes has increased in foods, pharmaceutical and in textile industries due to their biological activity which gives azo dyes the potential to inhibit the growth of microorganisms that cause a degradation of azo dyes and then forming harmful aromatic amines to the human health[3-5]. The antimicrobial properties of the azo dyes have been intensively studied by many researchers. These studies involved the ability of such dyes to inhibit the growth of both bacteria and fungi[6,7]. Synthesized phenolic azo dyes derived from the salicylic acid showed resistivity against the fungi growth on jowar seeds[8]. Similar type of azo dyes have been synthesized and biologically tested against the fungi affecting some types of seeds. It was found that the synthesized phenolic azo dyes have showed good levels of inhibition against the fungi when the seeds were coated by these azo compounds[9]. Herein, two phenolic azo derivatives consisting of anthracene moiety have been synthesized and their antifungal properties were tested against *A. niger*.

EXPERIMENTAL

Materials

Hydrochloric acid, anthracene, sodium hydroxide and methanol were purchased from Carlo erba. Glacial acetic acid, sodium nitrite and sulfuric acid were purchased from Avonchem. Tin (II) chloride was purchased from PSPARK. *o*-Nitrophenol was

purchased from Riedel De Haen, whereas *m*-nitrophenol and DMSO were purchased from BDH Chemicals. All chemicals were used without further purification.

Instrumentation

Melting point was measured on a Barnstead electrothermal IA 9100. Uv-vis absorptions were recorded on Uv-vis spectrophotometer-uv mini 1240-Shimadzu. pH was measured using Jenway pH meter 3505. ^{1}HNMR spectrum was recorded on a Bruker Avance 300 spectrometer. Residual proton signal from the deuteriated solvents were used as references [DMSO (^{1}H, 2.50ppm, ^{13}C, 39.51 ppm) and $CDCl_3$ (^{1}H, 7.24 ppm, ^{13}C, 77.23ppm)]. Coupling constants were measured in Hz. Infrared spectrum was recorded on Jasco FT/IR-4100 Fourier transform infrared spectrometer. Mass spectrum was recorded on a Micromass Autospec M spectrometer.

Preparation of 4(9-anthrylazo)-2-nitrophenol 5

An adapted literature procedure was followed[10] for synthesizing compound 5. 9-aminoanthracene 1 (0.77 g, 4.00 mmol) was dissolved in concentrated sulfuric acid (10 cm^3). The resulting solution was cooled to 0–5 ºC, to which a precooled (0–5 ºC) aqueous solution of sodium nitrite (0.55 g, 8.00 mmol; in 30 cm^3 water) was added while stirring for 30 min maintaining the temperature between 0–5 ºC. An alkaline solution of the *o*-nitrophenol [0.56 g, 4.00 mmol; in 40 cm^3 of (2N NaOH and 2.5 g sodium carbonate)] was cooled to 0–5 ºC and then added to the reaction vessel. The reaction mixture was stirred for an extra 1½ h at 0–5 ºC. A green precipitate was formed, filtered, washed with cold water (3×10 cm^3) and air dried. The crude material was recrystallized from glacial acetic acid, filtered, washed with water (3×10 cm^3) and air dried to give the azo dye 5 (0.93 g, 2.72 mmol, 68% yield) as a green fine powder. m.p 277 ºC; »$_{max}$ 325 nm ($CHCl_3$); FT-IR (KBr disc): 3447 cm^{-1} (Ar-OH), 1583 cm^{-1} (N=N), 1277 cm^{-1} (C-N); δ_H (400 MHz; DMSO) 7.78-7.75 (3H, m, Ar-C*H*), 7.55-7.44 (4H, m, Ar-C*H*), 7.30-7-22 (2H, m, Ar-C*H*), 7.15-7.13 (1H, m, Ar-C*H*), 7.09-7.07 (1H, m, Ar-C*H*), 7.01-6.97 (1H, m, Ar-C*H*), 5.03 (H, s, Ar-O*H*); δ_C (100 MHz; DMSO) 183.70 (Ar-*C*-OH), 153.13 (Ar-*C*-NO_2), 136.52 (2×Ar-*C*-N=N) 135.86 (4×Ar-*C*), 134.32 (2×Ar-*C*), 129.10 (Ar-*C*), 128.51 (4×Ar-*C*), 127.15 (2×Ar-*C*), 126.50 (2×Ar-*C*), 120.16 (Ar-*C*), m/z ($C_{20}H_{13}N_3O_3$, Mwt. 343.10) 342.89 (65%), 312.33 (17%), 298 (67%), 284.80 (27%).

Preparation of 4-(9-anthrylazo)-3-nitrophenol 6

A modified literature procedure was followed[10] for synthesizing compound 6. 9-aminoanthracene 1 (0.77 g, 4.00 mmol) was dissolved in concentrated hydrochloric acid (20 cm^3). The resulting solution was cooled to 0–5 ºC, to which a pre-cooled (0–5 ºC) aqueous solution of sodium nitrite (0.55 g, 8.00 mmol; in 30 cm^3 water) was added while stirring for 30 min maintaining the temperature between 0–5 ºC. An alkaline solution of the *m*-nitrophenol [0.56 g, 12.00 mmol; in 30 cm^3 of (2N NaOH and 2.5 g sodium carbonate)] was cooled to 0 – 5 ºC and subsequently added to the reaction mixture. The reaction mixture was stirred for further 1½ h at 0–5 ºC. A bright brown precipitate was formed, filtered, washed with cold water (3×10 cm^3) and air dried. The crude material was recrystallized from ethanol 96%, filtered, washed with water (3×10 cm^3) and air dried affording the desired azo dye 6 (0.48 g, 1.40 mmol, 35 % yield) as a fine bright brown powder, m.p 262ºC; λ_{max} 421 nm ($CHCl_3$); FT-IR (KBr disc): 3473 cm^{-1} (Ar-OH), 1530 cm^{-1} (N=N), 1277 cm^{-1} (C-N); δ_H (400 MHz; DMSO) 8.25-8.20 (2H, m, Ar-C*H*), 7.95-7.92 (2H, m, Ar-C*H*), 7.75-7-73 (2H, m, Ar-C*H*), 7.50-7.41 (4H, m, Ar-C*H*), 6.98-7.01 (2H, m, Ar-C*H*), 5.02 (H, s, Ar-O*H*); δ_C (100 MHz; DMSO); 183.94 (Ar-*C*-OH), 141.08 (Ar-*C*-NO_2), 135.85 (4×Ar-*C*), 134.32 (2×Ar-*C*-N=N), 133.44 (2×Ar-*C*), 130.41 (2×Ar-*C*), 129.09 (2×Ar-*C*), 128.04 (4×Ar-*C*), 126.85 (2×Ar-*C*); m/z ($C_{20}H_{13}N_3O_3$, Mwt. 343.10) 342.99 (30%), 344.98 (10%), 312.03 (20%), 298.09 (55%).

General procedure for antimicrobial screening

An adapted literature procedure was followed[11,12].

(a) The use of azo derivatives inhibiting agent as suspensions in water

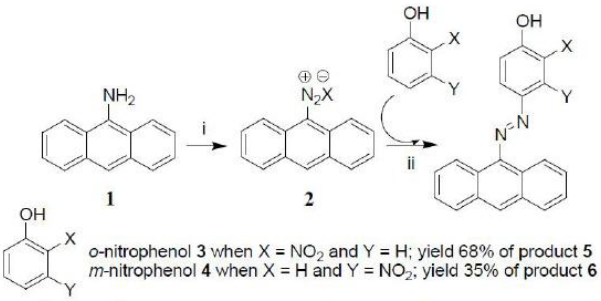
The Czapek Dox Agar with formula (g/l) [sodium nitratr 2.00, potassium chloride 0.50, magnesium glycerophosphate 0.50, ferrous sulphate 0.01, potassium sulphate 0.35, sucrose 30.00, agar 12.00] was dissolved in distilled water (45.4 g/l) and sterilized by autoclaving. The Czapek Dox Agar was poured into petri dishes and mixed with 1 ml containing suspensions of the synthesized azo dye in water (0.025 g/ml). The mixture was allowed to stand at R.T for 1 h. After that a disk of fungi (Aspergillus. niger) with diameter of 8 mm was put in the center of every petri dish. Then, the petri dishes were incubated at 25 ˚C for 24 hrs before measuring the diameter of the fungi growth to determine the percentage of inhibition.

(b) The use of azo derivatives inhibiting agents as solutions in DMSO

The sterilized medium (Czapek Dox Agar) was poured into petri dishes with depth 3-4 mm and mixed with the synthesized compounds [0.025g/ml in DMSO]. The mixture was incubated for 1h at R.T and the disk of fungi A. niger with diameter of 8 mm was placed on the solidified medium. The petri dishes were incubated at 25 ˚C for 24 hrs before measuring the antifungal activity.

RESULTS AND DISCUSSION

Both phenolic azo dyes, the 4-(9-anthrylazo)-2-nitrophenol 5 and 4-(9-anthrylazo)-3-nitrophenol 6 were synthesized upon the treatment of the 9-aminoanthreacene 1with an aqueous solution sodium nitrite under acidic conditions leading to the formation of the corresponding diazonium salt 2 to which an alkaline solution of either *o*-nitrophenol 3 or *m*-nitrophenol 4 was added at about 0 °C (Scheme 1).



Scheme 1 : Synthesis of the desired azo derivatives 5 and 6

Inhibiting the growth of *A.niger* fungi is one of the main objectives of this study. The resulting two azo dyes were screened against the *Aspergillus niger*. The antifungal activity of the obtained azo derivatives 5 and 6 was compared with that for the commonly used antibiotics Nystatin and Griseovin. The azo derivatives 5 and 6 were placed on petri dishes containing Czapek Dox Agar media using a concentration of 0.025 g/ml as a suspension in distilled water, in form three replicates for each azo derivative and the both two references (Nystatin and Griseovin). In addition, three replicates of the control sample (neat Czapek Dox Agar media) were also considered. Having placed a disk of the *A.niger*, with a diameter of 8 mm, in the middle of every petri dish. The samples were monitored every 24 hrs and the diameter of the fungus growth was measured in order to determine the inhibition percentage by applying the following equation[11].

$$\text{Inhibition } \% = \frac{(\text{average of growth diameter for control} - \text{average of growth diameter for sample}) \times 100}{\text{average of growth diameter for control}}$$

The percentage of inhibition for the tested azo derivatives 5 and 6 along with the two references, Nestatin and Griseovin showed low to moderate levels of inhibitory effects for the synthesized azo derivatives compared with Nystatin. Although the azo derivative 5 displayed as about equal inhibitory effect as

TABLE 1 : Inhibition percentage of fungi's growth

Compounds	Inhibition percentage %
5	55
6	18
Nystatin	95
Griseovin	52

that for the Griseovin.

The obtained results (TABLE 1) showed that the azo derivative 5 had a higher level of inhibition than what the other azo dye 6 gave. The presence of the phenolic hydroxyl group in these two azo compounds 5 and 6 could be responsible for inhibiting the growth of the *A. niger*. This finding is in accordance with those in literature were the phenolic compounds have been found to have inhibitory effects against wide variety of fungi[13,14]. Moreover, plants have also been reported to produce phenolic compounds as a defensive system against fungi[15]. However, when the water was replaced with the DMSO as a carrying agent for the azo derivatives. The antifungal activities of the synthesized compounds 5 and 6 were determined by dissolving these two azo compounds in the DMSO with the same concentration 0.025 g/ml in DMSO loaded on Czapek Dox Agar plate. Upon monitoring the growth of the *A. niger*, it was found that there was a remarkable increase in the levels of inhibition for the two azo compounds 5 and 6 in comparison with suspending them in water at the same concentration. Azo derivatives 5 and 6 had much higher inhibition levels 92% and 69%, respectively, than what these compounds showed when they were suspended in water, which gave 55% and 18% inhibitory levels correspondingly. This remarkable increase of the antifungal effect of the synthesized azo dyes could be rationalized to the great capability of the DMSO in penetrating throughout the cell walls of the fungus leading to excellent delivery of the antifungal agent, in this case, the azo derivatives.

CONCLUSION

Two azo dyes consisting of nitrophenol moieties have been synthesized in moderate yields. Both resulting compounds have been tested for their antifungal properties against the *A. niger*. The water suspensions of these azo dyes gave low to moderate inhibition percentages, 18% for azo 6 and 55% for azo 5. However, when the azo dyes were dissolved in the DMSO, the inhibitory effects of both azo dyes increased notably to reach 69% for azo 6 and 92% for azo 5.

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