Research Article

Synthesis and *In Vitro* Biological Activity of (*E*)-1-((4-Methyl-3-(4-(pyridin-3-yl)amino)phenyl)diazenyl)naphthalein-2-ol

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Abstract

(*E*)-1-((4-Methyl-3-(4-(pyridin-3-yl)amino)phenyl)diazenyl)naphthalein-2-ol was synthesized by the reaction of *N*-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidinamine with naphthalene-2-ol in order to determine their *in vitro* antimicrobial activity against clinically isolated strains. The antioxidant activity was also determined by DPPH radical scavenging assay method. The chemical structure was confirmed by different spectral studies. Antimicrobial studies revealed that compound was showed significant activity against tested strains. The compound showed good antioxidant activity in diphenylpicrylhydrazyl (DPPH) radical-scavenging assay method.

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Introduction

Synthesis of most azo dyes involves diazotization, followed by coupling with nucleophiles. Hydroxy and amino groups are commonly used coupling components [1]. Azo compounds are important structures in the medicinal and pharmaceutical field [2] and it has been suggested that the azoimine linkage might be responsible for the biological activities displayed by some reported Schiff bases [3, 4]. In addition, Evans blue and Congo Red are azo dyes being studied as HIV inhibitors of viral replications. This effect is believed to be resulted by binding of azo dyes to both protease and reverse transcriptase of this virus [5]. The existence of an azo moiety in different types of compounds has caused them to show antibacterial and pesticidal activity. In the present time, exploration of azo dye as antibacterial and antifungal agents has received considerable attention [6-8]. In the variety of applications of azo dyes, it is capable to develop synthesis of such naphtholic and phenolic azo dyes and their derivatives in order to unfold many more potentials of such compounds.

N-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidinamine is an intermediate for the preparation of imatinib. It has also been found to be effective in the treatment of gastrointestinal stromal tumors (GISTs) [9]. This selective inhibition of Bcr-Abl kinase by imatinib has been a successful therapeutic strategy for chronic myeloid leukemia because of the high efficacy and mild side effects of this compound [10]. In connection with such studies, the present paper reporting on the synthesis of (*E*)-1-((4-methyl-3-(4-(pyridin-3-yl)amino)phenyl)diazenyl)naphthalein-2-ol (**4**), which is formed during the reaction of diazonium compound **(3)** with naphthalene-2-ol. The synthesized compound was characterized by UV-visible, FT-IR, Mass and ^{1}H NMR studies. An antimicrobial and antioxidant activity of compound was reported.

Experimental

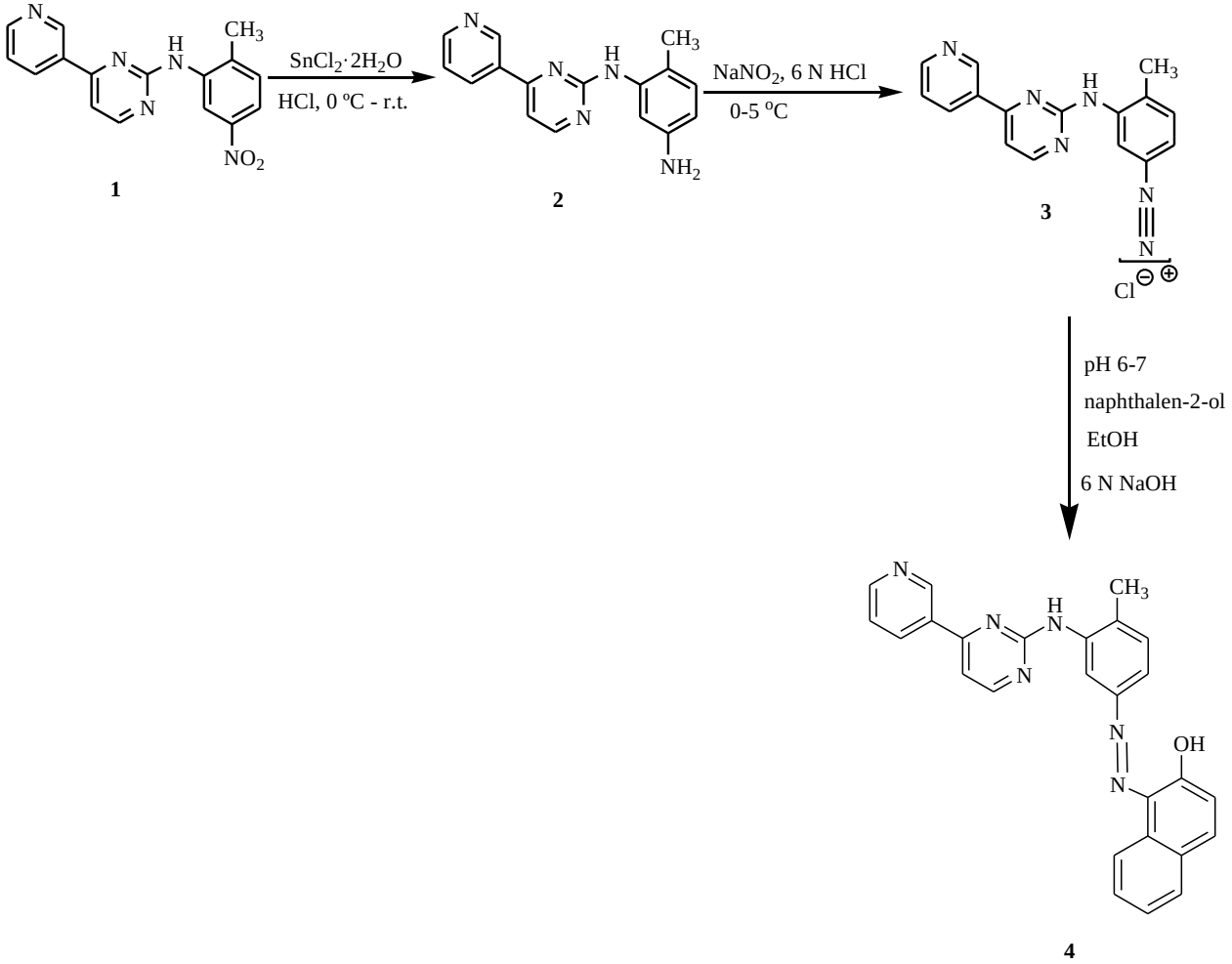
Materials and Reagents

All solvents and reagents were purchased from Merck Chemicals. Melting point was determined by Veego Melting

Point VMP III apparatus. The UV-visible spectrum was recorded on Analytikjena Specord 50 UV–vis spectrophotometer in DMSO solvent. The FT-IR spectra were recorded using KBr discs on FT-IR Jasco 4100 infrared spectrophotometer and were quoted in cm^{-1}. ^1H NMR spectra was recorded on Bruker DMX 300 spectrometer using DMSO-d_6 as solvent. Mass spectral data was recorded by LC/MSD Trap X.

N-(5-Amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidinamine (2)

The target key intermediate, *N*-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidinamine (**2**) was synthesized according to the reported procedure [11] by reacting stannous chloride dihydrate (11.29 g, 50 mmol) in hydrochloric acid (30 mL) and cooled at 0 ºC. *N*-(2-methyl-5-nitrophenyl)-4-pyridin-3-yl-pyrimidin-2-ylamine (**1**, 3.69 g, 12 mmol) was added in portions while the suspension was vigorously stirred for 6 h. The mixture was extracted three times with ethyl acetate (100 mL). The combined organic phase was dried over anhydrous sodium sulphate and the filtrate was evaporated to dryness. The product was recrystallized from methanol solvent (**Scheme 1**).



Scheme 1

Diazotization of N-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidinamine (3)

The amine (**2**, 0.721 mmol) was dissolved in 6 N HCl (25-30 mmol). The mixture was cooled by means of an ice-water bath and an aqueous solution of $NaNO_2$ (0.901mmol, 10 ml) was added drop wise within 15 min. The resulting

yellow to orange red solution was stirred at that temperature for 30 min. Finally the excess of HNO_2 was destroyed by adding solid urea (0.5 g).

Synthesis of (E)-1-((4-methyl-3-(4-(pyridin-3-yl)amino)phenyl)diazenyl)naphthalein-2-ol (4)

The diazonium compound (**3**) was added to the coupling component solution prepared by mixing a naphthalene-2-ol (0.721mmol) in 10 ml ethanol. During the procedure the pH value was maintained within 6-7 by 6N NaOH and the temperature at 0-5 °C. The mixture was stirred for 2 h. The precipitated crude compound was collected by filtration at vacuum and washed with water. The obtained compound was recrystallized from the ethanol**.** FT-IR (KBr, cm^{-1}): 3450 (O-H), 1631 (C=C), 1590 (N=N), 750 (Ar-H bend). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.30 (s, 3H, CH_3), 4.16 (s, 1H, OH), 6.99 (d, 1H, Ar-H), 7.01-7.07 (m, 3H, Ar-H), 7.12-7.36 (m, 6H, Ar-H), 7.52-8.46 (m, 3H, Ar-H), 8.61 (d, 1H, Ar-H), 9.14 (s, 1H, Ar-H). MS (ESI) *m/z*: 433.2.

Antibacterial activity

Antibacterial activity of the synthesized compound was determined against Gram-positive bacteria (*S. aureus* and *S. epidermidis*) and Gram-negative bacteria (*P. vulgaris* and *E. coli*) in Methanol by disc diffusion method on nutrient agar medium [12]. The sterile medium (15 ml) in each petri-plate was uniformly smeared with cultures of Gram positive and Gram negative bacteria. Sterile discs of 10 mm diameter (Hi-Media) was placed in the petriplates, to which 50 µl (1 mg/ml i.e., 50 µg/disc) of the different synthesized compounds were added. The treatments also included 50 µl of DMF as negative control, bacteriomycin and gentamycin as positive control for comparison. The plates were incubated at 37 ± 2 ºC for 24 h and the zone of inhibition was determined.

Antifungal activity

The synthesized compounds were screened for their antifungal activity against *A. niger* and *C. albicans* in DMF by poisoned food technique [13]. Potato Dextrose Agar (PDA) media was prepared and about 15 ml of PDA was poured into each petriplate and allowed to solidify. 5 mm disc of 7 days old culture of the test fungi was placed at the center of the petriplates and incubated at 26 °C for 7 days. The percentage inhibition was measured and three replicates were maintained for each treatment. Nystatin was used as standard drug. The new compound was tested (at the dosage of 500 µl of the novel compounds/petriplate, where concentration was 0.1 mg/ml).

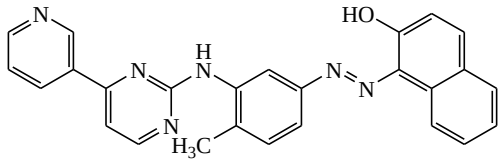
Antioxidant activity

The free radical scavenging activity of the synthesized compounds was studied *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay method [14]. Stock solution of the drug was diluted to different concentrations in the range of 100-200 µg/ml in methanol solvent. Methanolic solution of the synthesized compounds (2 ml) was added to 0.003 % (w/v) methanol solution of DPPH (1 ml). The mixture was shaken and allowed to stand for 30 minutes. Absorbance at 517 nm was determined and the % of scavenging activity was calculated. Ascorbic acid was used as the standard drug. The inhibition ratio (I %) of the tested compounds was calculated according to the following equation: $I\ \% = (Ac\text{-}As) / Ac \times 100$, where *Ac* is the absorbance of the control and *As* is the sample absorbance.

Results and Discussion

(*E*)-1-((4-methyl-3-(4-(pyridin-3-yl)amino)phenyl)diazenyl)naphthalein-2-ol was synthesized by the method summarized in **Scheme 1**. The reaction of compound **3** with naphthalene-2-ol was carried out in the presence of absolute ethanol as a solvent. Synthesized compound was characterized by UV-visible, FT-IR, ^{1}H NMR and mass spectral studies. Compounds were purified by recrystalization method using ethanol. Chemical structure and physical data of the synthesized compound was depicted in **Table 1**.

Table 1 Chemical structure and physical data of the synthesized compound

Compound	Structure	Yield (%)	M. R (°C)	UV-visible (λ_{max})
4		76.0	138-140	495

The first wavelength (λ_{max}) for the compound was found in 200 nm as a result of π-π* transition of the compounds indicating the presence of C=C peculiar to benzene nucleus. This is agreement with earlier report by Mielgo *et al* as per benzenoid UV-visible absorption [15]. The UV-visible absorption spectrum of **4**, as a representative of naphtholic compound, showed a peak at λ_{max} = 230 nm and bathochromic shifts at λ_{max} = 494 nm. The wavelength (λ_{max}) above benzenoid region was as a result of π-n transition and extended conjugation contributed by the C=C and the conjugative linkage performed by the N=N group. Furthermore, the IR spectrum of the compound was run in KBr using single beam FT-IR. The broad band observed at 3450 cm^{-1} was as a result of OH functionality of phenol. The absorption bands at 1631 cm^{-1} and 750 cm^{-1} depicted the present of C=C and Ar-H (bending), respectively. In the present study, the compound give an absorption band in the region of 1590 cm^{-1}. It shows the presence of an azo group (-N=N-) in compound.

The characteristic resonance peaks in ^{1}H NMR for the compound was reported using DMSO-d_6. The expected resonances were assigned by their peak multiplicity and integration. The integration of spectra shows good agreement with the synthesized compound. The proton NMR spectral data of NH_2 in **2** show single resonance at δ 5.30 ppm which is absent in the spectra of **4**, indicating the replacement of the azo group. In addition, the resonance appeared in δ 4.16 ppm as singlet is attributed to the OH proton. The CH_3 protons were resonated as singlet at δ 2.30 ppm. The aromatic ring protons were resonated in the region δ 6.99 - 9.14 ppm. The proton spectral data agree with respect to the number of protons and their chemical shifts with the proposed structure. The synthesized compound was further confirmed by the appearance of molecular ion peak in mass spectra. The appearance of final peak in **4** at m/z = 433.20 ($C_{26}H_{20}N_6O$) (calculated molecular mass at 432.48) and other peak may be due to different fragment.

The antibacterial activity of compound **4** was evaluated and compared with Gentamicin as standard drug. The compound **4** has shown good antibacterial activity against *S. aureus* (Gram +ve) and *E. coli* (Gram +ve) pathogenic bacterial strains when compared with gentamicin. It showed moderate activity against *S. epidermidis* and no zone of inhibition activity against *P. Vulgaris*. The antifungal activity of compound **4** was evaluated and compared with nystatin as standard. Compound **4** showed moderate activity against *C. albicans*. Compared with nystatin the compound **4** showed weak inhibitory activity against *A. niger*. Antimicrobial screening results of the tested compounds are shown in **Table 2**

Antioxidant activity results of the tested compounds are shown in **Table 3**. As antioxidants donate protons to DPPH radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of scavenging. Free radical scavenging effect of the compound was measured by DPPH assay is shown in **Figure 1**. Compound **4** showed good antioxidant activity (82.7 %) compared to standard ascorbic acid (99.3 %) at 200 µg/mL.

Table 2 Antibacterial and antifungal activities

Compound	Zone of inhibition in diameter (mm)					
	S. aureus	*S. epidermidis*	*E. coli*	*P. vulgaris*	*A. niger*	*C. Albicans*
4	12	9	13	-	7	10
Gentamicin	13	14	13	12	-	-
Nystatin	-	-	-	-	14	14

Table 3 Results of DPPH radical scavenging assay

Compound	% Scavenging effect		
	100 µg/mL	150 µg/mL	200 µg/mL
4	64.2	71.1	82.7
Ascorbic acid	74.1	86.4	99.3

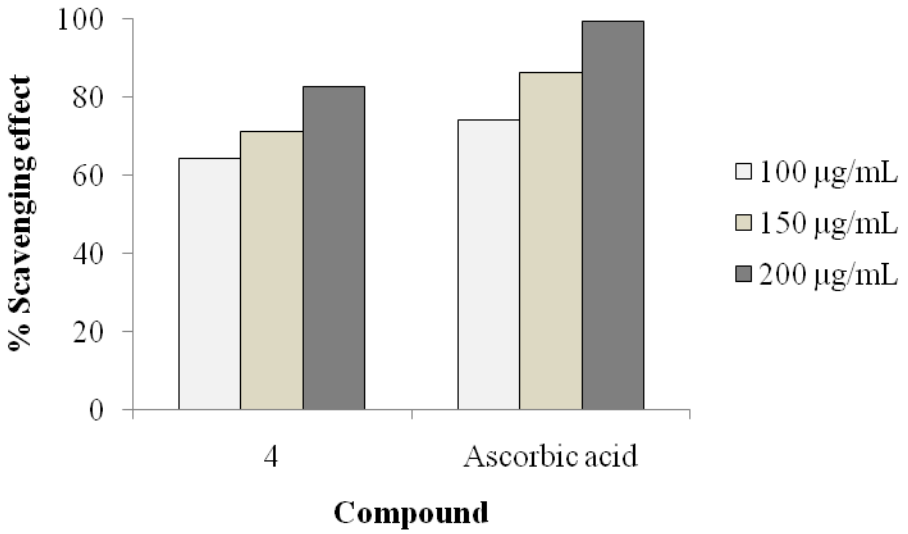


Figure 1 Antioxidant activity of **4**

Conclusion

In conclusion, (*E*)-1-((4-methyl-3-(4-(pyridin-3-yl)amino)phenyl)diazenyl)naphthalein-2-ol (**4**) was synthesized in good yield and characterized by different spectral study. Antimicrobial and antioxidant activities have been evaluated. Compound **4** was demonstrated moderate inhibitions against bacterial and fungal strains tested. The antioxidant activity revealed that compound **4** was weak antioxidant activity.

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