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Short Note

(*E*)-*N*′-(4-Fluorobenzylidene)-5-methyl-2-(pyridin-3-yl) thiazole-4-carbohydrazide

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Abstract: 5-methyl-2-(pyridin-3-yl)-1,3-thiazole-4-carbohydrazide (**1**) on treatment with 4-fluorobenzaldehyde in presence of catalytic amount of acetic acid, accessed the target compound (**2**) with the yield of 79%. The target compound was confirmed by ^{1}H-NMR, ^{13}C-NMR, FT-IR and LCMS. In vitro antibacterial activity against *Staphylococcus aureus* (*S. Aureus*), *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) were carried out and compound **2** showed promising activity against *B. subtilis*. In addition, compound **2** was analyzed for DNA binding study. It revealed that compound **2** has a promising affinity towards DNA double helix.

Keywords: thiazole; condensation; antibacterial; DNA binding

1. Introduction

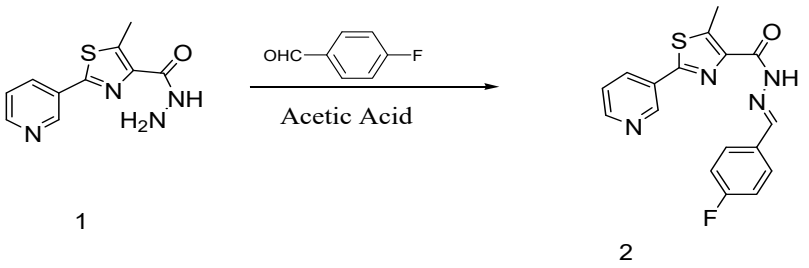
Most of modern medicine deals with heterocyclic compounds, as they play an important role in drug development. Heterocyclic compounds are easily prepared and a few of them can be handled easily such as thiazoles, oxadiazoles, triazoles, and many more. Thiazoles are used in various fields such as dyes [1], fungicides [2,3], agrochemical industries [4], catalyst [5], and in the development of peptidomimetics [6]. As reported earlier, thiazole, incorporated with other scaffolds, shows remarkable potency in biological systems such as anticancer [7,8], anti-inflammatory [9], antioxidant, antibacterial [10,11], antifungal [12], herbicidal [13], hypertensive [14].

In order to discover new lead molecules that have potential antibacterial property, researchers should first inhibit the synthesis of the bacterial cell wall peptidoglycan [15]. Due to increased antibiotic resistance of the bacteria, infections may lead to life-threatening diseases. This severe impact leads researchers to synthesize more effective antibacterial agents.

The important step in the functioning of many drugs is to observe the interaction of target compounds with DNA, as a majority of the drugs specifically target DNA [16]. To understand the extensive information produced, DNA binding mechanism was required. These observations promoted us to design and synthesize target compound **2**.

2. Results

Intermediate 5-methyl-2-(pyridin-3-yl)-1,3-thiazole-4-carbohydrazide (**1**) was prepared as reported earlier [17,18] by the condensation of ethyl 5-methyl-2-(pyridin-3-yl)thiazole-4-carboxylate with hydrazine hydrate as outlined in Scheme 1. Furthermore, both 5-methyl-2-(pyridin-3-yl)-1,3-thiazole-4-carbohydrazide (**1**) and 4-fluorobenzladehyde were taken in equal quantities and a catalytic amount of acetic acid was added and refluxed for 4 h. Following this, the synthesized compound was confirmed by elemental analysis, FT-IR, LCMS, ^{1}H-NMR, and ^{13}C-NMR.



Scheme 1. Synthesis of (*E*)-*N*′-(4-fluorobenzylidene)-5-methyl-2-(pyridin-3-yl)thiazole-4-carbohydrazide (**2**).

3. Discussion

FT-IR spectrum of the title compound exhibited an absorption of 3272 and 3160 cm^{-1}, which corresponds to N–H, whereas peaks at 3040, 2941, 1664, 1609 and 651 cm^{-1} corresponds to Ar–H, CH, NHCO, C=N, and C–S–C stretching respectively. ^{1}H-NMR spectrum confirms the formation of the title compound. A singlet at δ 11.97 and 9.21 ppm, corresponds to NH, and pyridine ring proton respectively, whereas two doublets at δ 8.73 and 8.39 ppm are due to pyridine ring proton with *J* value 4 and 7.2 Hz respectively, at δ 8.14 ppm, a singlet, which corresponds to CH was observed, at δ 7.85 ppm, a triplet for pyridine ring of *J* value 6.4 Hz was observed, at δ 7.57 to 7.60 ppm, a multiplet for Ar–H was observed, at δ 7.33 to 7.38 ppm, a multiplet for Ar–H was observed, at δ 2.81 ppm, a singlet for methyl proton was observed. The ^{13}C-NMR spectrum of the synthesized compound showed a singlet at δ 19.3 ppm, corresponding to $–CH_3$. Aromatic carbons appeared in the range of δ 112.5 to 152.0 ppm, a singlet at δ 167.45 ppm, corresponding to CONH, a doublet at δ 161.7 ppm, corresponding to C=N. These results show that the structure was confirmed. Mass spectrum of the synthesized compound confirms the formation of the title compound by the formation of molecular ion peak, which appeared at *m*/*z* value of 341.05 $[M + H]^+$ (in positive ion mode).

Antibacterial activity: The synthesized compound was screened for antibacterial assay against two positive Gram-positive (*S. aureus* (MTCC No. 9760), and *B. subtilis* (MTCC No.441)) and two Gram-negative (*E. coli* (MTCC No. 443) and *P. aeruginosa* (MTCC No. 424)) strains. The minimum inhibitory value was expressed in terms of μg/mL and the results were compared with standard Tetracycline and Streptomycin and it is tabulated in Table 1.

Table 1. Minimal inhibitory concentration (MIC) value of the synthesized compound **2**.

Tested Organism	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
Tetracycline	1	0.5	1	1
Streptomycin	1	0.5	1	1
Compound **2**	3	6	3	3

The title compound showed promising bacterial activity against tested bacterial strains, but the standard showed more activity than the tested compound.

The binding affinity of compound **2** with CT-DNA was evaluated by using UV-Visible spectrophotometer in the range 250 to 375 nm. As DNA concentration increased, the intensity of the band decreased. The change in the absorbance values with increasing amounts of CT-DNA was used to calculate binding constant of **2**.

The compound **2** showed a decrease in the intensity of the band, but the bands were gradually shifted to higher wavelength region. Hence, bathochromic shift was observed. The change in absorbance values with increasing amounts of CT-DNA was used to calculate binding constant of compound **2**. Typical absorption spectra of compound **2** are given in Figure S5. Due to the strong stacking interaction between the base pairs of DNA and aromatic chromophores, the DNA binding constants of compound **2** (Table 2) concluded that the compound had interacted with CT-DNA through intercalation mode. It was also indicated that the compound formed adducts with DNA

through intercalation and was stabilized by hydrophobic and hydrogen bonds interactions. Hence, the compound revealed a stronger binding affinity for DNA double helix.

Table 2. Wavelength shifts %, hypochromism (H%), and binding constants of compound **2** using calf thymus DNA.

Compound	Free	Bound	Δλmax (nm)	H%	$K_b \times 10^6$ M^{-1}
2	316	320	4	70.58	2.26

Δλmax (nm) = (Bound−Free), 2. H% = $[(A_f - A_b)/A_f)] \times 100$, Where A_f and A_b represent the absorbance of free and bound compounds, 3. K_b = Intrinsic binding constant.

4. Materials and Methods

All the reagents for the present study were purchased from commercial suppliers of Sigma-Aldrich and Spectrochem. Melting point was determined in an open capillary tube and was uncorrected. Thin layer chromatography (Merck silica gel 60 F254 coated aluminum plates) confirmed the purity of the products. Synthesized compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, FT-IR, LCMS, and Elemental analysis. FT-IR spectrum was recorded on Perkinelmer, FT-IR Infrared spectrometer (γ_{max} in cm^{-1}). ^{1}H-NMR (400 MHz), and ^{13}C-NMR spectrum, was recorded on a Bruker Advance II 400 spectrometer, with 5 mm PABBO BB-1H Tubes, using DMSO as a solvent, using trimethylsilane as internal standard (Chemical shift in δ ppm). Elemental analysis was carried out by using VARIOEL-III (Elemental analyze system GmBH).

Synthesis of *N*′-[(*E*)-(4-fluorophenyl)methylidene]-5-methyl-2-(pyridin-3-yl)-1,3-thiazole-4-carbohydrazide (**2**): Equimolar quantities of 5-methyl-2-(pyridin-3-yl)-1,3-thiazole-4-carbohydrazide (**1**) (0.31 g, 0.0013 mol) and 4-fluorobenzaldehyde (0.14 mL, 0.0013 mol) was dissolved in ethanol and glacial acetic acid was added and refluxed for 4 h. Completion of the reaction was monitored by TLC, the reaction mass was allowed to attain room temperature and quenched to crushed ice. The precipitate form was filtered, dried, and recrystallized to get pure compound **2**. (89%) pale yellow, m.p. 212–214 °C; FT-IR (KBr cm^{-1}): 3272, 3160 (N–H), 3040 (Ar–H), 2941 (C–H), 1664 (NHCO), 1609 (C=N), 651(C–S–C); ^{1}H-NMR (DMSO-d_6, 400 MHz): 11.97(s, 1H, N–H), 9.21(s, 1H, pyridine ring), 8.73(d, 1H, pyridine ring, *J* 4), 8.39(d, 1H, pyridine proton *J* 7.2), 8.14 (s, 1H, CH), 7.85 (t, 2H, Ar–H of *J* 6.4), 7.57–7.60 (m, 1H, pyridine proton), 7.33–7.38 (m, 2H, Ar–H), 2.50 (s, 3H); ^{13}C-NMR (DMSO-d_6, 100 MHz):167.45, 161.79, 161.61, 151.95, 147.49, 145.37, 134.32, 129.22, 124.87, 121.60, 120.35, 112.49, 19.28; LCMS (*m*/*z*): 341.05 $[M + H]^+$; Elemental analysis of $C_{17}H_{13}FN_4OS$; Calcd: C, 59.99 H, 3.85 N, 16.46. Found: C, 59.97 H, 3.88 N, 16.49%.

Antimicrobial assay: In order to evaluate the antimicrobial susceptibility of the title compound **2**, a National Committee on Clinical Laboratory Standards (NCCLS) macro dilution broth method was carried out. The Minimal Inhibitory Concentration (MIC) for the title compound **2** was evaluated according to the Clinical and Laboratory Standards Institute (CLSI), formerly the NCCLS Macro dilution broth method. In brief, sterile test tubes containing different concentrations of the title compound **2** in Sterile Mueller Hinton Broth (MHB) medium for bacteria was prepared to obtain a wide range of concentrations, from 0.125 to 256 μg/mL for which 1 mL of the test microorganisms were added. MHB without title compound **2** were used as a control. The MIC was detected as the lowest concentration of the title compound **2** containing tube showing no visible growth of the test microorganism. The experiment was performed in triplicate and the data were analyzed by SPSS 20.0 software. Tetracycline was used as the reference drugs for the bacteria.

5. Conclusions

1,3-thiazole containing carbohydrazide (**2**) was prepared by a convenient method and confirmation of the compound was done by spectral analysis. Antibacterial assay of the title compound (**2**) revealed promising activity towards both Gram-positive and Gram-negative bacterial strains. Binding results reveal compound **2** showed good binding interaction.

Supplementary Materials: The following are available online at http://www.mdpi.com/1422-8599/2019/2/M1058/s1. Figure S1: FT-IR, Figure S2: LC-MS, Figure S3: ^{1}H-NMR, Figure S4: ^{13}C-NMR, Figure S5: Absorption spectra were available online.

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Conflicts of Interest: Authors declares no conflict of interest.

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