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SYNTHESIS, CHARACTERIZATION AND ANTIFUNGAL SCREENING OF SOME NOVEL PYRIDINE ASSOCIATED THIAZOLE LINKED 1,2,4-TRIAZOLES

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Abstract: A synthetic and novel protocol consisting a series of six 4-(benzylideneamino)-5-(4-methyl-2-(6-methylpyridin-2-yl)thiazol-5-yl)4*H*-1,2,4-triazole-3-thiols **(7a-f)**in good isolated yields has been reported. The title compounds were synthesized by using 6-methylpyridine-2-carbothioamide **(1)** as initial compound and through the formation of ethyl-4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-carboxylate **(3)**, 4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-carbohydrazide **(4)**, potassium 2-[4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-carbonyl] hydrazine carbodithioate **(5)** and 4-amino-5-(4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-yl)-4*H*-1,2,4-triazole-3-thiol **(6)**as intermediates on extension of the reaction. Structural formulas of all the synthesized compounds were characterized by IR, ^{1}H-NMR, mass spectral data and elemental analysis. Further, the target compounds were used to evaluate their antifungal activity.

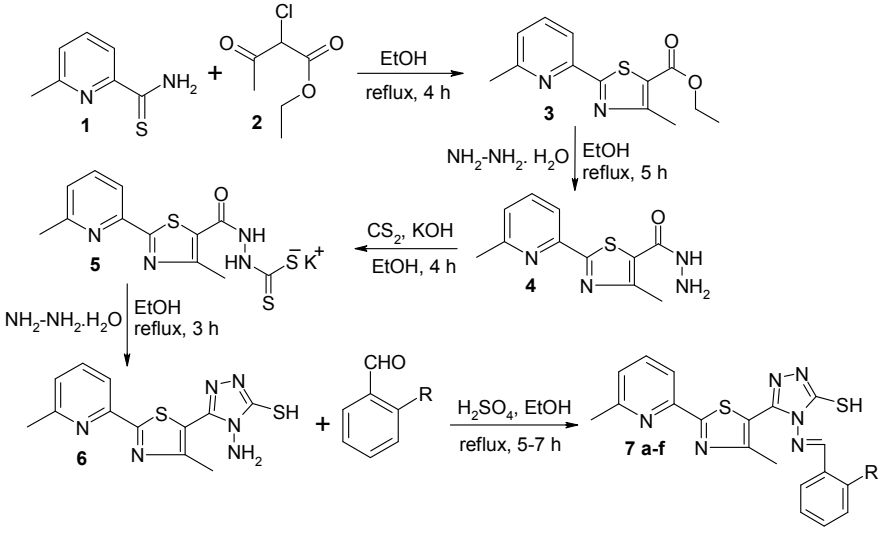
Keywords: Pyridine, 1,2,4-Triazoles, Antifungal activity

Introduction: Small-ring heterocycles including nitrogen and sulphur have been under investigation for a long time on account of their synthetic diversity and therapeutic relevance. Among the wide range of heterocycles explored to privileged candidates in drug discovery, thiazoles have been identified to play a necessary role in medical chemistry [I]. Thiazole ring is a structural fragment of natural compounds such as thiamine (vitamin B1), thiamine pyrophosphate, epothilones, carboxylase and the large family of macrocyclicthiopeptide antibiotics, thiostrepton and micrococcin P1 [II, III]. Thiazole derivatives are associated with a broad spectrum of biological properties, including anticonvulsant, antimicrobial, antituberculous, bacteriostatic activities, antiviral, antimalarial, anticancer, hypertension, inflammation, schizophrenia, HIV infections, hypnotics and more recently for the treatment of pain, as fibrinojen receptor antagonists with antithrombotic activity, as new inhibitors of bacterial DNA gyrase B [IV-VIII]. Thiazole derivatives are also found in application in the drug development for the treatment of allergies [IX]. Biological studies on synthetic analogs of naturally occurring heterocyclic compounds showed that most of them exhibit a broad

spectrum of biological activity. Among compounds of this series, of particular interest are 1,2,4-triazoles which are mostly of synthetic origin. Some 1,2,4-triazole derivatives exhibit hypertensive effect [X] and possess antitumor [XI], fungicidal [XII], antibacterial [XIII], and other kinds of biological activity.

Results and discussion: In continuation of our active research in an area ofnovel heterocyclic compounds, herein we reported the design and synthesis of title compounds, 4-(benzylideneamino)-5-(4-methyl-2-(6-methylpyridin-2-yl)thiazol-5-yl)4*H*-1,2,4-triazole-3-thiol and its derivatives **(7a-f)** according to the synthetic routes as shown in Schemes 1with the purpose of investigating in their possible antifungal activity.

Thus, the initial intermediate, ethyl-4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-carboxylate **(3)**was synthesizedin 79 % yieldfrom cyclization of starting compound, 6-methylpyridine-2-carbothioamide **(1)**and ethyl-2-chloroacetoacetate **(2)**in ethanol at reflux temperature for 4 h on water bath with uniform stirring. 4-Methyl-2-(6-methylpyridine-2-yl)thiazole-5-carbohydrazide **(4)** was obtained in 75 % yield from the reaction of compound **3** with hydrazine hydrate in ethanol under reflux for 5 h on water bath with constant stirring in 75 % of yield. In further attempt, the substitution of compound **4** with carbon disulphide and potassium hydroxide in ethanol with steady stirring at room temperature for 4 h leads to formation of next intermediate, potassium 2-[4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-carbonyl] hydrazine carbodithioate**(5)** in 81 % of yield.According to the next synthetic utility, the cyclization of intermediate **5** with hydrazine hydrate by refluxing in ethanol for 3 h gave the corresponding and expected final intermediate, 4-amino-5-(4-methyl-2-(6-methylpyridine-2-yl)thiazole-5- yl)-4*H*-1,2,4-triazole-3-thiol **(6)** in 85 % of yield.In the final investigation, the treatment of compound **6** with benzaldehyde and its derivatives in boiling ethanol containing sulphuric acid on water bath with stable stirring for 5-7 h offered the anticipated and suitable target compounds, 4-(benzylideneamino)-5-(4-methyl-2-(6-methylpyridin-2-yl)thiazol-5-yl)4*H*-1,2,4-triazole-3-thiol and its derivatives **(7a-f)** through condensation in good yields. The structures of all the newly synthesized intermediates and final compounds were established by elemental analysis and on the basis of their mass, infrared and nuclear magnetic resonance spectra. Finally, the title compounds have been used to test their efficiency against five fungal organisms.



7 R a) = H, b) = CH_3, c) = OH, d) = Cl, e) = Br, f) = NO_2

Scheme 1

Antifungal activity: The antifungal activity of the synthesized target compounds, 4-(benzylideneamino)-5-(4-methyl-2-(6-methylpyridin-2-yl)thiazol-5-yl)4*H*-1,2,4-triazole-3-thiols**(7a-f)**has been evaluated by using agar cup bioassay method [XIV] against five representative fungal strains such as *Aspergillusniger*, *Chysosporiumtropicum*, *Rhizopusoryzae*, *Fusariummoniliforme* and *Curoularialunata*by comparing standard drug *Clotrimazole.* The readymade potato dextrose agar (PDA) medium (Himedia, 39g) was suspended in distilled water (1000 mL) and heated to boiling until it dissolved completely. The medium and petri dishes were autoclaved at pressure of 15 lb/ inc^2 for 20 min. The medium was poured into sterile petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 mL of (week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving plant extract in acetone and different concentrations were made (30, 100 µg/ mL). Agar inoculated, cups were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup different concentrations of test solutions (30, 100 µg/ mL) were added. Controls were maintained with acetone and *Clotrimazole* (100 µg/ mL). The treated and the controls were kept at room temperature for 72-96 h. Inhibition zones were measured and diameter was calculated in millimeter. Three to four replicates were maintained for each treatment.

According to the screening results, it is observed that all the screening compounds **7a-f** exhibited moderate to good activity with a degree of variation. Compound **7a** carrying unsubstituted aromatic ring exhibited highest activity with 15.0 mm zone of inhibition against*R. Oryzae*, least activity towards *C. Lunata*and moderate potential against rest of organisms. Ortho methyl substituted aromating ring containing compound **(7b)** performed good antifungal activity against all fungal organisms within the range of 9.00-15.0 mm zone of inhibition against all fungal strains compare with standard. The title compound **7c** with ortho hydroxyl aromatic ring disclosed high zone of inhibition (18.0 mm) against *C. tropicum* while with notable activity towards other organisms.The target compound **7d** with 2-chloro phenyl group showed least zone of inhibition with 9.00 mm against *C. tropicum* and highest activity (18.0 mm) towards *R. oryzae*. On the other hand orthobromo substituted aromatic ring compound **7e** expressed the antifungal activity with zone of inhibition from 12.0 mm to 16.5 mm against all employed fungal organisms. Finally, among the all compounds, product 7f with 2-NO_2 group exhibited highest activity with 19.5 mm zone of inhibition against *R. Oryzae*. It is interesting to note that none of the compound is inactive towards any fungal organism.

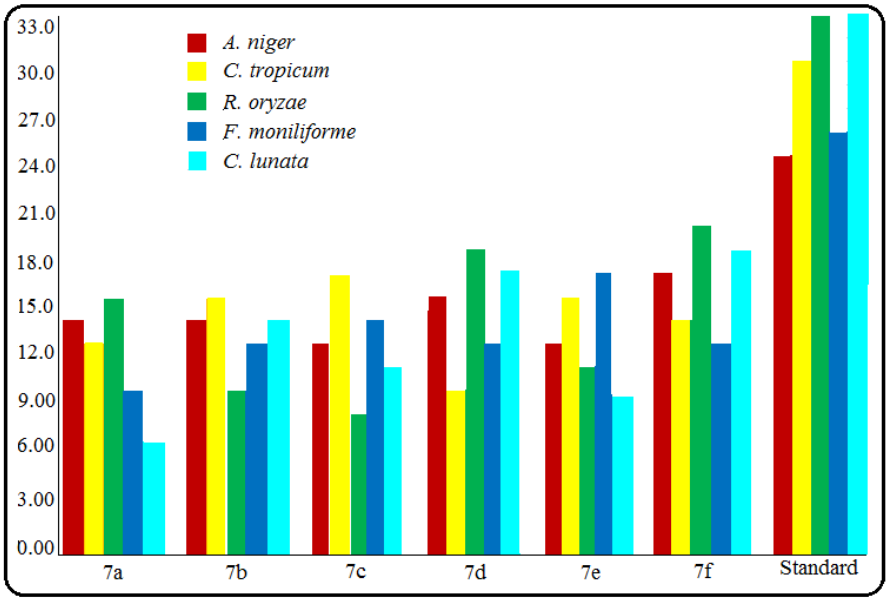


Figure 1:Antifungal activity of compounds 7a-f

Experimental:

General : All starting materials were commercially available research grade chemicals and used without further purification. Silical gel 60 F_{254} (Merck) was used for TLC and the spots were detected with UV light. Column chromatography was carried out on silica gel 60 (Merck). Melting points were determined on a Fischer-John melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer with TMS as internal reference. Multiplicity was denoted by s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were recorded on a VG-Micromass 7070H spectrometer operating at 70 eV.

Ethyl-4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-carboxylate (3): A solution of 6-methylpyridine-2-carbothioamide **(1)** (0.01 mol), ethyl 2-chloroacetoacetate **(2)**(0.01 mol) in ethanol (10 ml)was refluxed with constant stirring on water bath for4 h. After completion of the reaction (monitored by the TLC), cooled the reaction mixture to room temperature, dropped in ice-coldwater to obtain crude precipitate and it is filtered, washed with water, dried and recrystallized form ethanol to offer pure ethyl-4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-carboxylate **(3)**.

4-Methyl-2-(6-methylpyridine-2-yl)thiazole-5-carbohydrazide (4): A mixture of compound **3** (0.01 mol) and hydrazine hydrate (15 ml) in ethanol (20 ml) is maintained at reflux temperature for 5 h on water bath with steady stirring. After fulfilment of the reaction (examined by the TLC), the resulted solution was poured in ice-cold water to achieve crude solid. It is filtered, washed with distilled water, dried and recrystallized from absolute ethanol to get pure 4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-carbohydrazide **(4)**.

Potassium 2-[4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-carbonyl] hydrazine carbodithioate (5): To the solution ofcompound **4** (0.01 mol) in ethanol (10), added alcoholic solution of KOH (5 %, 10 ml) andcarbon disulphide (0.01 mol). The obtained mixture was kept at room temperature for 4 h with uniform stirring. After realization of the reaction (scrutinized by the TLC), resulted solid was poured in water to get precipitation

which is filtered, washed with water, dried and recrystallized form ethyl acetate to yield potassium 2-[4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-carbonyl] hydrazine carbodithioate **(5)** in pure form.

4-Amino-5-(4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-yl)-4*H*-1,2,4-triazole-3-thiol (6): The solution of compound **5** (0.01 mol) in ethanol (10 ml) was added hydrazine hydrate (12 ml) and theresulted mixture was refluxed for 3 h on water bath with continuous stirring. After achievement of the reaction (supervised by the TLC), the mixture was poured in water, acidified with HCl solution to offer cruse precipitation which was filtered, washed with water, dried and recrystallized from ethanol to obtain
pure 4-amino-5-(4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-yl)-4*H*-1,2,4-triazole-3-thiol **(6)**.

4-(Benzylideneamino)-5-(4-methyl-2-(6-methylpyridin-2-yl)thiazol-5-yl)4*H*-1,2,4-triazole-3-thiol (7a): A solution of compound **6** (0.01 mol) and benzaldehyde (0.01 mol) in ethanol (15 ml) was added 2-3 drops of concentrated sulphuric acid. Then the reaction mixture was refluxed for 6 h on water bath with uniform stirring. After completion of the reaction (monitored by the TLC), the solution is dropped into ice-cold water to obtain crude precipitate and it is filtered, washed withwater, dried and recrystallized from ethanol to offer pure 4-(benzylideneamino)-5-(4-methyl-2-(6-methylpyridin-2-yl)thiazol-5-yl)4*H*-1,2,4-triazole-3-thiol **(7a)**. The remaining compounds **(7b-f)** of this series have been prepared by following similar procedure.

Physical and spectral data

Ethyl-4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-carboxylate (3): Yield: 79 %, mp: 110-112 ^{0}C, IR (KBr): 3043 (C-H, Py ring), 2936 (C-H, CH_3), 1742 (C=O), 1580 (C=C), 1428 (C=N), 1275 (C-S), 1139 (C-O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.72-7.26 (m, 3H, Py ring), 4.06 (q, 2H, J = 5.3 Hz, CH_2), 2.89 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 1.29 (t, 3H, J = 5.3 Hz, CH_3); MS: 262m/z (M^+); Elemental analysis: Calculated for $C_{13}H_{14}N_2O_2S$: C-59.52, H-5.38, N-10.68, O-12.20, S-12.22. Found: C-58.76, H-5.37, N-10.65, O-12.18, S-12.20.

4-Methyl-2-(6-methylpyridine-2-yl)thiazole-5-carbohydrazide (4): Yield: 75 %, mp: 131-133^{0}C, IR (KBr): 3258 (N-H), 3048 (C-H, Py ring), 2935 (C-H, CH_3), 1655 (C=O), 1574 (C=C), 1432 (C=N), 1281 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.56 (s, 1H, NH), 7.70-7.29 (m, 3H, Py ring), 4.38 (s, 2H, NH_2), 2.82(s, 3H, CH_3), 2.41 (s, 3H, CH_3); MS: 248m/z (M^+); Elemental analysis: Calculated for $C_{11}H_{12}N_4OS$: C-53.21, H-4.87, N-22.56, O-6.44, S-12.91. Found: C-52.68, H-4.86, N-22.38, O-6.43, S-12.89.

Potassium 2-[4-methyl-2-(6-methylpyridine-2-yl)thiazole-5-carbonyl] hydrazine carbodithioate (5): Yield: 81 %, mp: 122-124^{0}C, IR (KBr): 3263 (N-H), 3045 (C-H, Py ring), 2942 (C-H, CH_3), 1662 (C=O), 1580 (C=C), 1442 (C=N), 1285 (C-S), 1265 (C=S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.72 (s, 1H, NH), 7.75-7.37 (m, 3H, Py ring), 7.35 (s, 1H, NH), 2.85 (s, 3H, CH_3), 2.52 (s, 3H, CH_3); MS: 366m/z (M^+); Elemental analysis: Calculated for $C_{11}H_{11}KN_4O_2S$: C-36.05, H-3.02, K-10.67, N-15.29, O-8.73, S-26.25. Found: C-35.87, H-3.01, K-10.65, N-15.25, O-8.72, S-26.21

4-Amino-5-(4-methyl-2-(6-methylpyridine-2-yl)thiazole-5- yl)-4*H*-1,2,4-triazole-3-thiol (6): Yield: 85 %, mp: 108-110 ^{0}C, IR (KBr): 3252 (N-H), 3038 (C-H, Py ring), 2950 (C-H, CH_3), 2568 (S-H), 1576 (C=C), 1436 (C=N), 1276 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-

d_6): δ 10.72 (s, 1H, SH), 7.79-7.45 (m, 3H, Py ring), 3.68 (s, 2H, NH_2), 2.82(s, 3H, CH_3), 2.57 (s, 3H, CH_3); MS: 304m/z (M^+); Elemental analysis: Calculated for $C_{12}H_{12}N_6S_2$: C-47.35, H-3.97, N-27.61, S-21.07. Found: C-46.58, H-3.96, N-27.38, S-20.85.

4-(Benzylideneamino)-5-(4-methyl-2-(6-methylpyridin-2-yl)thiazol-5-yl)4*H*-1,2,4-triazole-3-thiol (7a): Yield: 85 %, mp: 108-110 ^{0}C, IR (KBr): 3045 (C-H, Ar), 2944 (C-H, CH_3), 2562 (S-H), 1560 (C=C), 1442 (C=N), 1268 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.65 (s, 1H, SH), 7.75-7.47 (m, 3H, Py ring), 7.68-7.38 (m, 5H, Ar-H), 5.26 (s, 1H, CH), 2.79(s, 3H, CH_3), 2.61 (s, 3H, CH_3); MS: 392m/z (M^+); Elemental analysis: Calculated for $C_{19}H_{16}N_6S_2$: C-58.14, H-4.11, N-21.41, S-16.34. Found: C-57.68, H-4.10, N-21.26, S-16.30.

4-(2-Methoxybenzylideneamino)-5-(4-methyl-2-(6-methylpyridin-2-yl)thiazol-5-yl)4*H*-1,2,4-triazole-3-thiol (7b): Yield: 80 %, mp: 130-132^{0}C, IR (KBr): 3038 (C-H, Ar), 2936 (C-H, CH_3), 2558 (S-H), 1565 (C=C), 1448 (C=N), 1272 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.69 (s, 1H, SH), 7.72-7.31 (m, 3H, Py ring), 7.65-7.29 (m, 4H, Ar-H), 5.21 (s, 1H, CH), 2.81 (s, 3H, CH_3), 2.73(s, 3H, CH_3), 2.67 (s, 3H, CH_3); MS: 406m/z (M^+); Elemental analysis: Calculated for $C_{20}H_{18}N_6S_2$: C-59.09, H-4.46, N-20.67, S-15.78. Found: C-58.69, H-4.45, N-20.62, S-15.75.

4-(2-Chlorobenzylideneamino)-5-(4-methyl-2-(6-methylpyridin-2-yl)thiazol-5-yl)4*H*-1,2,4-triazole-3-thiol (7c): Yield: 77 %, mp: 125-127^{0}C, IR (KBr): 3035 (C-H, Ar), 2941 (C-H, CH_3), 2563 (S-H), 1571 (C=C), 1452 (C=N), 1278 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.58 (s, 1H, SH), 7.76-7.37 (m, 3H, Py ring), 7.69-7.32 (m, 4H, Ar-H), 5.25 (s, 1H, CH), 2.77(s, 3H, CH_3), 2.71 (s, 3H, CH_3); MS: 426m/z (M^+); Elemental analysis: Calculated for $C_{19}H_{15}ClN_6S_2$: C-53.45, H-3.54, Cl-8.30, N-19.68, S-15.02. Found: C-52.75, H-3.53, Cl-8.29, N-19.57, S-14.89.

4-(2-Bromobenzylideneamino)-5-(4-methyl-2-(6-methylpyridin-2-yl)thiazol-5-yl)4*H*-1,2,4-triazole-3-thiol (7d): Yield: 81 %, mp: 108-110^{0}C, IR (KBr): 3041 (C-H, Ar), 2945 (C-H, CH_3), 2571 (S-H), 1582 (C=C), 1458 (C=N), 1272 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.55 (s, 1H, SH), 7.72-7.33 (m, 3H, Py ring), 7.67-7.36 (m, 4H, Ar-H), 5.32 (s, 1H, CH), 2.71(s, 3H, CH_3), 2.75 (s, 3H, CH_3); MS: 426m/z (M^+); Elemental analysis: Calculated for $C_{19}H_{15}BrN_6S_2$: C-48.41, H-3.21, Br-16.95, N-17.83, S-13.60. Found: C-47.96, H-3.20, Br-16.88, N-17.76, S-13.57.

4-(2-Nitrobenzylideneamino)-5-(4-methyl-2-(6-methylpyridin-2-yl)thiazol-5-yl)4*H*-1,2,4-triazole-3-thiol (7e): Yield: 75 %, mp: 97-99^{0}C, IR (KBr): 3048 (C-H, Ar), 2952 (C-H, CH_3), 2577 (S-H), 1590 (C=C), 1465 (C=N), 1277 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.47 (s, 1H, SH), 7.75-7.37 (m, 3H, Py ring), 7.72-7.28 (m, 4H, Ar-H), 5.37 (s, 1H, CH), 2.78(s, 3H, CH_3), 2.67 (s, 3H, CH_3); MS: 437m/z (M^+); Elemental analysis: Calculated for $C_{19}H_{15}N_7O_2S_2$: C-52.16, H-3.46, N-22.41, O-7.31, S-14.66. Found: C-51.85, H-3.45, N-22.31, O-7.30, S-14.53.

4-(2-Hydroxybenzylideneamino)-5-(4-methyl-2-(6-methylpyridin-2-yl)thiazol-5-yl)4*H*-1,2,4-triazole-3-thiol (7f): Yield: 83 %, mp: 126-128^{0}C, IR (KBr): 3058 (C-H, Ar), 2947 (C-H, CH_3), 2565 (S-H), 1582 (C=C), 1471 (C=N), 1282 (C-S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.50 (s, 1H, SH), 7.72-7.32 (m, 3H, Py ring), 7.75-7.30 (m, 4H, Ar-H), 5.33 (s, 1H, CH), 2.71(s, 3H, CH_3), 2.62 (s, 3H, CH_3); MS: 408m/z (M^+); Elemental analysis:

Calculated for $C_{19}H_{16}N_6OS_2$: C-55.86, H-3.95, N-20.57, O-3.92, S-15.70. Found: C-54.75, H-3.94, N-20.32, O-3.91, S-15.62.

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