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Anti-Microbial Activity and Synthesis of 3-(Benzylideneamino)- 2,7-Dimethylbenzo-[4,5] Thieno [2,3-D] Pyrimidine-4-Ones

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Abstract:- **A series of few 3-(benzylideneamino) -2,7-dimethyl-benzo[4,5]thieno[2,3-d]pyrimidine-4-one were prepared in laboratory. They were purified by manual column chromatography as well as re-crystallized from various solvents. Their structures were characterized by spectroscopy techniques such as Proton-NMR and Carbon-NMR. Their masses were detected by using Mass-spectrometry. They were screened for their therapeutic and pharmacological activities as a biological function. Some of the novel target compounds were found to have strong biological activities with respect to Itraconozole and Furacin standard drugs.**

Keywords:- *3-(benzylideneamino)- 2,7-dimethylbenzo[4,5] thieno-[2,3-d]pyrimidine-4-one, Antifungal activity, Antimicrobial activity, Minimal Inhibition Concentration.*

I. INTRODUCTION

Human-being and animals are strongly influenced by the activities of microorganisms. Control on population of microbes is quite necessary to avoid and cure the transmission of disease, infection of disease, their decomposition; their contamination and spoilage caused by them. The comfort and convenience depend upon the quantity and concentration of compound on the control of population of microbes. Some studies on 3-amino-2,7-dimethylbenzo[4,5]thieno[2,3-d]pyrimidin-4-one derivatives came out with interesting pharmacological properties particularly anti-bacterial activity and anti-fungal activity. On the basis of survey of literature and various references and considering the results and the structures of imines of 3-amino-2,7-dimethylbenzo[4,5] thieno[2,3-d]pyrimidine-4-one, The novel final compounds have been synthesized, purified and characterized as described below. The anti-microbial and antifungal activity of these compounds on gram positive bacterias and gram negative bacterias along with one fungal stain with respect to Furacin and Itraconozole standard drugs were evaluated as minimum inhibitory concentration.

II. RESULTS AND DISCUSSION

Int-1 was synthesized by Gewald multi-component reaction among 4-methylcyclohexanone, ethylcyanoacetate and sulphur powder using morpholine as a weak organic base. Int-2 was produced by the acetylation of Int-1 using acetic-anhydride as acetylating reagent. Int-3 was prepared by the reaction between Int-2 and hydrazine-hydrate. Target compounds (4a-j) were synthesized by the final reaction between Intermediate-3 and different aromatic aldehydes to form Schiff's base (imines).

Following reaction scheme was used for the synthesis of target compounds (4a-j) after 4 steps. The reagents and conditions are also mentioned below the scheme.

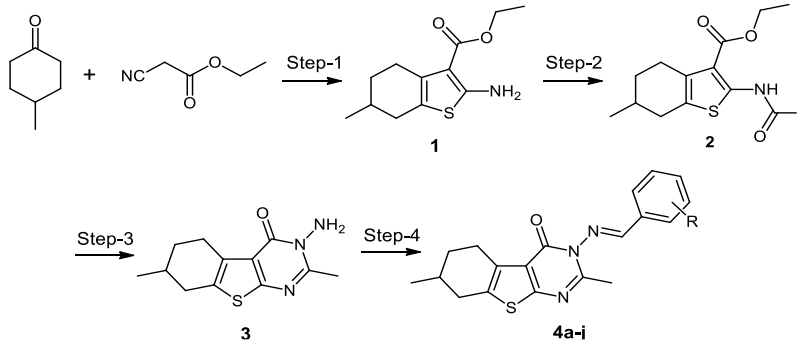

Fig. 1: Scheme Reagents and Conditions (1) Sulphur, Morpholine, Ultrasound irradiation, rt, 1h; (2) $(CH_3CO)_2O$, Reflux, 3h; (3) NH_2NH_2, Ethanol, Reflux, 16h; (4) Aromatic Aldehyde, AcOH, MeOH (1:1), rt, 2-5h.

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We used Pseudomonas Aeruginosa from gram negative group of bacterias. We used KL. Pneumoniae and Staphylococcus Aureus from gram positive group of bacterias along with Escherichia Coli and for the evaluation of anti-bacterial activity and anti-fungal activity Broth dilution method was used to carry out the antibacterial activity. It is actually non-automated in-vitro bacterial susceptibility examination. This conventional method gives a quantitative result for the quantity of anti-microbial reagents which are necessary to stop the growth of specific micro-organisms.

Minimal inhibition concentration was measured by well known and highly used micro broth dilution method. To grow bacterias, Mueller Hinton Broth was used as nutrient media. The same method was also used for testing the strain by comparing the turbidity. S,S-Dimethylsulphoxide was used as a diluent to get the required concentration of various drugs for testing the standard bacterial strains. For the screening of antibacterial and antifungal activities, following common standard strains were used which are given in following Table-1.

Table 1: Standard strains used for the specific bacterial species

E.-Coli	P.-Aeruginosa	KL.-Pneumoniae	S.-Aureus
MTCC443	MTCC1688	MTCC109	MTCC96

Methods used for primary and secondary screening are given below. As a stock solution, each prepared drug was diluted to get concentration of 2000 microgram per ml.

Primary screening process: Two fifty micro-gram per one ml, Five hundred micro-gram per one ml and thousand micro-gram per one ml solutions of the prepared drugs were taken in the primary screening. The prepared drugs which showed activity in this above process were tested further for the Secondary screening process.

Secondary screening process: The active drugs of primary screening process were diluted to get the concentrations of 6.25 micro-gram per ml, 12.5 micro-gram per ml, 25 micro-gram per ml, 50 micro-gram per ml, 100 micro-gram per ml and 200 micro-gram per ml.

Finding Results: The concentration which showed 99% inhibition was considered as MIC. The result was strongly affected by inoculums size. The test mixture must contain at least 108 organisms per ml.

Minimal Inhibition Concentration of each sample in µg per ml unit on two gram negative bacterias Escherichia Coli and Pseudomonas Aeruginosa and two gram positive bacterias Staphylococcus Aureus and KL.Pneumoniae in comparison to standard drug Furacin along with one fungal strain P.Marneffei in comparison to standard drug Itraconozole which are given in following Table-2.

Table 2: Minimal Inhibition Concentration value of each sample in µg/ml

Sr. No.	**Compound Code**	**E. Coli**	**P. Aeruginosa**	**KL. Pneumoniae**	**S. Aureus**	**P. Marneffei**
1	4a	50	25	25	50	100
2	4b	50	50	50	25	200
3	4c	50	50	50	25	200
4	4d	25	50	25	12.5	250
5	4e	50	12.5	50	50	500
6	4f	50	50	12.5	50	50
7	4g	25	25	50	50	100
8	4h	50	12.5	50	50	250
9	4i	25	50	25	25	150
10	4j	50	50	12.5	50	200
11	Furacin	25	25	50	50	-
12	Itraconozole	-	-	-	-	100

III. CONCLUSIONS

3-(benzylideneamino)-2,7-dimethylbenzo-[4,5]thieno[2,3-d] pyrimidine-4-one derivatives were successfully prepared. They were purified by recrystallizaion as well as column chromatography. Their structures were confirmed by various spectroscopy methods such as PMR, CMR, Mass spectrometry and analysis of C,H,N and S elements. Moreover, the therapeutic and pharmacological evaluation of these compounds were carried out as mentioned in above method as compare to standard drugs Furacin and Itraconozole.

Table 2 shows that the minimal inhibition concentration (MIC) of Furacin against E.-Coli and P.-Aeruginosa species is 25.0 µg/ml; against KL.-Pneumoniae and S.-Aureus is 50.0 µg/ml. The minimal inhibition concentration (MIC) of Itraconozole against P.-Marneffei species is 100 µg/ml. Tartet compounds 4d, 4g and 4i showed excellent activity against E.-Coli, P.-Aeruginosa, KL.-Pneumoniae and S.-Aureus. as compare to standard drugs.

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IV. EXPERIMENTAL SECTION

A. Methods and Materials

Physical constants (MP) were measured using open capillaries. Proton NMR spectra and Proton decoupled Carbon NMR spectra of all the intermediates as well as target molecules were recorded on Bruker 400MHz avance III instrument in $DMSOd_6$ or $CDCl_3$ solvents at ambient temperature. In this analysis of the proton and carbon skeleton, Tetramethyl-silane was used as an internal reference standard. Electron-spray ionization mass spectra of all the intermediates as well as target molecules were recorded on mass spectrometer GCMSQP2010. The reagents used were chemically pure having analytical grade. All the chemicals were used without purification. They were purchased from commercial source. The progress of all the chemical reactions were controlled by Thin-Layer Chromatography using Kieselgel. They were visualized with UV light cabinet at 254 nm wavelength. For purification of the compounds, silica gel containing glass column was applied.

B. Synthesis

- *Ethyl 2-amino-6-methyl-4,5,6,7-tetrahydrobenzo [b] thiophene-3-carboxylate (Intermediate-1)*

Sulfur (6.2 g, 0.18 mol) and 1,4-oxazinane (16.9 g, 0.18 mol) were stirred at 28°C. To it, ethyl-cyanoacetate (21 g, 0.18 mol) and 4-methyl-cyclohexanone (21 g, 0.18 mol) were added to it. The reaction mixture was irradiated to ultrasound radiations in sonicator for 45-60 min. The chemical reaction was maintained and observed by TLC technique. It was recrystallised from hot ethanol solvent to afford Int-1 as a white solid (31 g). Yield: 66%; PMR (400 MHz, $DMSOd_6$): δ 0.96-0.98 (3H, d, *J* = 6.4 Hz), δ 1.24-1.28 (3H, t, *J* = 7.2 Hz), δ 1.27-1.30 (1H, m), δ 1.72-1.76 (2H, m), δ 2.02-2.08 (1H, m), δ 2.46-2.51 (2H, m), δ 2.72-2.78 (1H, m), δ 4.12-4.16 (2H, q, *J* = 5.4 & 12.6 Hz), 7.22 (2H, s). Elemental analysis for Int-I having MF $C_{12}H_{17}NO_2S$ Calculated: % C, 60.23; % H, 7.15; % N, 5.84; Found: % C, 60.19; % H, 7.12; % N, 5.88

- *Ethyl 2-acetamido-6-methyl-4,5,6,7-tetrahydrobenzo [b] thiophene-3-carboxylate (Int-2)*

Int-1 (11 g, 0.05 mol) was added to 55 mL of ethanoic anhydride at 25°C. The reaction mass was refluxed for 4.0 h. The chemical reaction was maintained and observed by TLC technique. The reaction mass was poured in to water containing crushed ice. The separated solid material was filtered followed by washing with cold water and drying in high vacuum to yield Int-2 as a light yellow solid (9 g). Yield: 70%; PMR (400 MHz, $DMSOd_6$): δ 1.02-1.04 (3H, d, *J* = 6.4 Hz), δ 1.3-1.33 (3H, t, *J* = 7.0 Hz), 1.31-1.34 (1H, m), δ 1.8-1.82 (2H, m), δ 2.12-2.18 (1H, m), δ 2.21 (3H, s), δ 2.55-2.62 (2H, m), δ 2.64-2.87 (1H, m), δ 4.25-4.31 (2H, q, *J* = 6.8 & 14.0 Hz), δ 10.96 (1H, s). Elemental analysis for Int-2 having MF $C_{14}H_{19}NO_3S$ Calculated: % C, 59.77; % H, 6.8; % N, 4.99; Found: % C, 59.7; % H, 6.77; % N, 5.01.

- *3-amino-2,7-dimethyl-5,6,7,8-tetrahydrobenzo [4,5] thieno [2,3-d]pyrimidin-4(3H)-one (Int-3)*

Int-2 (7.9 g, 0.03 mol) and Hydrazine-hydrate (45 mL) were taken in 50 mL of ethyl alcohol at 27°C. The reaction mass was refluxed overnight. The chemical reaction was maintained and observed by TLC technique. The separated solid material was filtered followed by washing with ethyl alcohol and drying under high vacuum. It was recrystallized using hot ethyl alcohol to give Int-3 as off white solid (4.9 g). Yield: 68%; PMR (400 MHz, $DMSOd_6$): δ 1.02-1.05 (3H, d, *J* = 6.8 Hz), δ 1.35-1.41 (1H, m), δ 1.85-1.88 (m, 2H), δ 2.28-2.34 (1H, m), δ 2.52 (3H, s), δ 2.68-2.83 (2H, m), δ 3.02-3.08 (1H, m), δ 5.81 (2H, s). Mass: 249 m/z. Elemental analysis for Int-3 having MF $C_{12}H_{15}N_3OS$ Calculated: % C, 57.82; % H, 6.05; % N, 16.86; Found: % C, 57.8; % H, 6.1; % N, 16.82.

- *3-((2-bromobenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one*

2-bromobenzaldehyde (0.3 g, 1.62 mmol) and Int-3 (0.41 g, 1.62 mmol) were taken in 6 mL of methyl at 27°C followed by the addition of 1 mL of ethanoic acid. The reaction mass was allowed to stir for 3-5 h. The chemical reaction was maintained and observed by TLC technique. The separated solid material was filtered and washed with methyl alcohol. It was recrystallized from ethyl ethanoate to give 4a as white solid (0.36 g). Yield: 54%; M.p 160-164°C. PMR (400 MHz, $CDCl_3$): δ 1.09-1.12 (3H, d, *J* = 6.8 Hz), δ 1.42-1.48 (1H, m), δ 1.94-1.98 (2H, m), δ 2.36-2.41 (1H, m), δ 2.62 (3H, s), δ 2.82-2.88 (2H, m), δ 3.16-3.2 (1H, m), δ 7.32-7.37 (1H, t, *J* = 8.0 Hz), δ 7.65-7.68 (1H, m), δ 7.72-7.78 (1H, d, *J* = 8.0 Hz), δ 8.06-8.08 (1H, t, *J* = 2.0 Hz), δ 8.98 (1H, s). ^{13}C-NMR (400 MHz, $CDCl_3$): 21.47, 22.66, 25.31, 29.25, 30.51, 33.26, 121.09, 123.13, 127.82, 130.43, 131.09, 131.54, 132.90, 134.73, 135.25, 153.14, 155.55, 160.81, 164.94. Mass: 415 & 417 m/z. Elemental analysis for compound 4a having MF $C_{19}H_{18}BrN_3OS$. Calculated: % C, 54.82; % H, 4.37; % N, 10.07; Found: % C, 54.84; % H, 4.31; % N, 10.01.

- 2,7-dimethyl-3-((2-methylbenzylidene)amino)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one

Yield: 57 %; M.p 188-200°C. Elemental analysis for the compound-4b having MF $C_{20}H_{21}N_3OS$ Calculated: % C, 68.36; % H, 6.03; % N, 11.94; Found: % C, 68.32; % H, 6.06; % N, 11.86.

- 3-((2-methoxybenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one

Yield: 57%; M.p 182-186°C. PMR (400 MHz, $CDCl_3$): δ 1.1-1.12 (3H, d, *J* = 6.4 Hz), δ 1.43-1.45 (1H, m), δ 1.94-1.98 (2H, m), δ 2.37-2.42 (1H, m), δ 2.6 (3H, s), δ 2.82-2.87 (2H, m), δ 3.18-3.22 (1H, m), δ 3.9 (3H, s), δ 7.12-7.14 (1H, m), δ 7.36-7.42 (2H, m), δ 7.45 (brs, 1H), δ 8.89 (1H, s). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 21.5, 22.58, 25.32, 29.28, 30.55, 33.27, 55.44, 122.29, 119.16, 121.14, 122.35, 129.94, 131.52, 132.72, 133.90, 153.08, 155.54, 159.92, 160.86, 167.24 Elemental analysis for compound 4c having MF $C_{20}H_{21}N_3O_2S$ Calculated: % C, 65.38; % H, 5.77; % N, 11.42; Found: % C, 65.31; % H, 5.76; % N, 11.41.

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- 3-((2,4-dimethoxybenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one

Yield: 55%; M.p 162-164°C. PMR (400 MHz, $CDCl_3$): δ 1.09-1.11 (3H, d, *J* = 6.8 Hz), δ 1.36-1.46 (1H, m), δ 1.92-1.99 (2H, m), δ 2.35-2.43 (1H, m), δ 2.58 (3H, s), δ 2.8-2.9 (2H, m); 3.21-3.24 (1H, m), δ 3.84 (3H, s), δ 3.85 (3H, s), δ 6.88-6.91 (1H, t, *J* = 8.8 Hz), δ 7.05-7.10 (1H, dd, *J* = 3.2 & 9.2 Hz), δ 7.67-7.7 (1H, d, *J* = 3.2 Hz), δ 9.16 (1H, s). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 21.50, 22.50, 25.33, 29.29, 30.58, 33.3, 55.88, 56.13, 76.73, 77.04, 77.38, 110.34, 112.68, 121.12, 121.2, 131.52, 132.42, 152.9, 153.56, 154.32, 155.38, 160.85, 164.51. Mass: 397 m/z. Elemental analysis for compound-4d having MF $C_{21}H_{23}N_3O_3S$. Calculated: % C, 63.46; % H, 5.84; % N, 10.55; Found: % C, 63.4; % H, 5.79; % N, 10.57

- 3-((2,3-dimethoxybenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one

Yield: 48%; M.p 156-158°C. PMR (400 MHz, $CDCl_3$): δ 1.08-1.11 (3H, d, *J* = 6.4 Hz), δ 1.42-1.46 (1H, m), δ 1.93-1.98 (2H, m), δ 2.36-2.42 (1H, m), δ 2.6 (3H, s), δ 2.83-2.89 (2H, m), δ 3.2-3.23 (1H, m), δ 3.97 (6H, s), δ 6.91-6.96 (1H, d, *J* = 8.0 Hz), δ 7.32-7.34 (1H, dd, *J* = 1.8 & 8.2 Hz), 7.55 (1H, d, *J* = 2.0 Hz), δ 8.66 (1H, s). Mass: 397 m/z. Elemental analysis for compound-4e having MF $C_{21}H_{23}N_3O_3S$ Calculated: % C, 63.46; % H, 5.84; % N, 10.55; Found: C, 63.41; % H, 5.81; % N, 10.56.

- 3-((2,5-dichlorobenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one

Yield: 50 %; M.p 194-196°C. PMR (400 MHz, $CDCl_3$): δ 1.08-1.12 (3H, d, *J* = 6.8 Hz) δ 1.41-1.46 (1H, m), δ 1.91-1.98 (2H, m) δ 2.36-2.41 (1H, m), δ 2.62 (3H, s), δ 2.82-2.91 (2H, m), δ 3.21-3.23 (1H, m), δ 7.35-7.38 (1H, δ , J = 2.0 & 8.6 Hz), δ 7.46-7.51 (1H, d, *J* = 11.2 Hz), δ 8.15-8.17 (1H, d, *J* = 8.4 Hz), δ 9.4 (1H, s). Mass: 405 m/z. Elemental analysis for compound-4f having MF $C_{19}H_{17}Cl_2N_3OS$ Calculated: % C, 56.15; % H, 4.21; % N, 10.36; Found: % C, 56.13; % H, 4.20; % N, 10.32.

- 3-((3,4-dichlorobenzylidene)amino)-2,7-dimethyl 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one

Yield: 53%; M.p 196-201°C. PMR (400 MHz, $CDCl_3$): δ 1.07-1.12 (3H, d, *J* = 6.8 Hz), δ 1.43-1.45 (1H, m), 1.92-1.98 (2H, m), δ 2.34-2.41 (1H, m), δ 2.58 (3H, s), δ 2.83-2.89 (2H, m), δ 3.2-3.25 (1H, m), δ 7.33-7.39 (1H, δ , J = 2.0 & 8.6 Hz), δ 7.45-7.52 (1H, d, *J* = 11.2 Hz), δ 8.12-8.17 (1H, d, *J* = 8.4 Hz), δ 9.37 (1H, s). Mass: 405 m/z. Elemental analysis for compound-4g having MF $C_{19}H_{17}Cl_2N_3OS$ Calculated: % C, 56.15; % H, 4.21; % N, 10.36; Found: % C, 56.13; % H, 4.2; % N, 10.28.

- 3-((4-ethoxy-3-hydroxybenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidin-4(3H)-one

Yield: 54.5%; M.p 193-196°C. PMR (400 MHz, $CDCl_3$): δ 1.08-1.10 (3H, d, *J* = 6.8 Hz), δ 1.43-1.45 (1H, m), δ 1.45-1.52 (3H, t, *J* = 7.0 Hz), δ 1.91-1.99 (2H, m), δ 2.04-2.42 (1H, m), δ 2.5 (3H, s), δ 2.82-2.9 (2H, m), δ 3.18-3.22 (1H, m), δ 4.18-4.22 (2H, q, *J* = 7.0 & 13.8 Hz), δ 6.32 (brs, 1H), δ 6.96-7.00 (1H, d, *J* = 8.4 Hz), δ 7.25-7.29 (1H, m), δ 7.5-7.54 (1H, d, *J* = 1.6 Hz); δ 8.64 (1H, s). ^{13}C-NMR (400 MHz, $CDCl_3$): 14.77, 21.51, 22.48, 25.31, 29.28, 30.55, 33.28, 64.72, 109.62, 114.49, 121.1, 124.74, 125.42, 131.44, 132.57, 146.34, 150.24, 152.87, 155.57, 160.86, 168.05. Mass: 397 m/z. Elemental analysis for compound-4h having MF $C_{21}H_{23}N_3O_3S$. Calculated: % C, 63.46; % H, 5.84; % N, 10.59; Found: % C, 63.38; % H, 5.79; % N, 10.53.

- 3-((4-chlorobenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one

Yield: 48 %; M.p 204-206°C. PMR (400 MHz, $CDCl_3$): δ 1.08-1.1 (3H, d, *J* = 6.4 Hz), δ 1.44-1.49 (1H, m), δ 1.93-1.95 (2H, m), δ 2.35-2.42 (1H, m), δ 2.54 (3H, s), δ 2.81-2.88 (2H, m), δ 3.22-3.24 (1H, m), δ 6.96-6.99 (1H, m), δ 7.28-7.33 (1H, m), δ 7.3 (brs, 1H), δ 7.46 (brs, 1H), δ 8.63 (1H, s). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 21.48, 22.42, 25.30, 29.23, 30.49, 33.29, 114.73, 120.56, 121.05, 121.82, 129.96, 131.37, 133.22, 152.68, 155.68, 156.81, 161.32, 169.03. Mass: 353 m/z. Elemental analysis for compound-4i having MF $C_{19}H_{19}N_3O_2S$ Calculated: % C, 64.56; % H, 5.41; % N, 11.91; Found: % C, 64.55; % H, 5.41; % N, 11.86

- 3-((4-hydroxybenzylidene)amino)-2,7-dimethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4(3H)-one

Yield: 45 %; M.p 154-156°C. PMR (400 MHz, $CDCl_3$): δ 1.08-1.12 (3H, d, *J* = 6.8 Hz), δ 1.41-1.5 (1H, m), δ 1.92-1.97 (2H, m), δ 2.35-2.43 (1H, m), δ 2.6 (3H, s), δ 2.82-2.87 (2H, m), δ 3.16-3.21 (1H, m), δ 6.98-7.03 (1H, t, *J* = 7.6 Hz), δ 7.05-7.08 (1H, d, *J* = 8.4 Hz), δ 7.36-7.39 (1H, d, *J* = 8.0 Hz), δ 7.45-7.48 (1H, t, *J* = 7.61 Hz), δ 8.91 (1H, s), δ 10.1 (1H, s). ^{13}C-NMR (400 MHz, $CDCl_3$): δ 21.4, 22.7, 25.25, 29.24, 30.47, 33.26, 116.22, 117.52, 119.92, 120.97, 131.52, 133.29, 133.55, 134.65, 151.76, 155.15, 160.17, 160.87, 171.38. Mass: 353 m/z. Elemental analysis for compound-4j having MF $C_{19}H_{19}N_3O_2S$ Calculated: % C, 64.58; % H, 5.43; % N, 11.87; Found: % C, 64.55; % H, 5.47; % N, 11.8.

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