Synthesis and Antimicrobial Activity of Novel Derivatives of 7-aryl-10-thioxo-7, 10, 11, 12 – tertahydro-9*H*-benzo[*H*] pyrimido [4,5-*b*] quinoline-8-one

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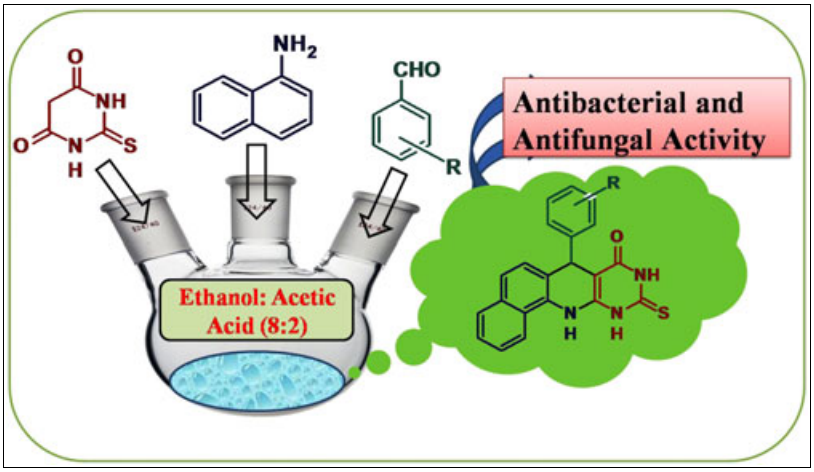


A highly competent synthesis of novel 7-aryl-10-thioxo-7, 10, 11, 12-tertahydro-9*H*-benzo [*H*] pyrimido [4, 5-*b*] quinoline-8-one derivatives has been reported through a Knoevenagel condensation followed by Michael addition and subsequent cyclization using ethanol:acetic acid (8:2 *v*/v). The mentioned protocol has advantages like high yields, cleaner reactions, operational simplicity, and environment friendliness. Moreover, these compounds were further screened against the plant pathogenic fungi like *Colletotrichum truncatum*, *Ustilago maydis*, *Trichosporon*, *Trichothecium sp*., *Aspergillus oryze*, *Aspergillus terreus*, and *Aspergillus niger* by agar well method bioassay. The results were elaborated for minimum inhibitory concentration determination using agar dilution method against fungal strains *C. truncatum* and *U. maydis* as well as broth dilution method for bacteria species Gram-positive *Bacillus megaterium* and Gram-negative *Proteus vulgaris*. Most of the tested compounds showed promising results towards the antimicrobial activity.

INTRODUCTION

Construction of new C─C bonds and C-heteroatom bonds is a key factor for the synthesis of various heterocyclic motifs [1]. It addresses easier and quicker structural framework by a simple and an innovative multicomponent reactions (MCRs) technique. Nowaday's scientist community [2,3] has been utilizing these applicative MCR techniques for the synthesis of novel, bioactive nitrogen containing structural motifs. Current research reveals that the various biological active fused scaffold heterocyclic compounds are essential because the disease causing organisms resists the effect of most powerful drugs due to their ability to cause a range of superficial and systemic infections to the host. This emerges an immense need for synthesizing a definite antifungal, antibacterial drug like molecules which are highly potent and less toxic. However, human cell is much more biochemically similar to fungal and bacterial forms [4]. Hence, many studies are focused on the development of antimicrobial compounds. The literature survey revealed that the some fungi and bacteria are capable to resist the treatment and responsible for diseases [5,6]. Thus, inspired by these aspects and in continuation of our efforts, we put forth a synthesis of antimicrobial active heterocyclic motifs for exhibiting better results to build up medicinally important molecules.

Considering the N heterocycles and great utility of MCR, we have exploited our chemical knowledge to synthesize new fused nitrogen containing molecules with easily accessible starting materials. The nitrogen-containing heterocycles like fused quinolines and pyrimidines are endowed with several bioactivities such as anticancer [7–12], anti-HIV [13], antihypertensive

active [14], antimicrobial [15], antimalarial [16], and others. Fused nitrogen-containing derivatives have been known to depict a broad range of pharmaceutical and agrochemical application [14,15].

The literature study reveals that the synthesis of 7-Methoxy-5-(3-nitrophenyl) pyrimido [4,5-b] quinoline-2,4-(1*H*, 3*H*, 5*H*, 10*H*)-dione [17], 7-aryl-11,12-dihydro-benzo[*h*]pyrimido-[4,5-b]quinoline-8,10(7*H*,9*H*)-dione [18] derivatives, and 12-aryl-7,8,9,10,11,12-hexahydrobenzo[*h*]-pyrimido[4,5-b]quinoline-9,11-diones molecules [19] has been reported using various critical methodologies. Each methodology is abided with its own merits and demerits. Therefore, considering the actual inevitability of synthesis of bioactive nitrogen heterocycles [20–30], herein, we report a simple and efficient synthesis of novel 7-aryl-10-thioxo-7, 10, 11, 12-tertahydro-9H benzo [*H*] pyrimido [4,5-*b*]quinoline-8-one and its antimicrobial activity (Scheme 1).

RESULTS AND DISCUSSION

In a pilot reaction, the mixture of thiobarbituric acid (1 mmol), 4-chloro benzaldehyde (1 mmol), and 1-naphthylamine (1 mmol) in a 25 mL round bottom flask containing 5 mL ethanol with catalytic amount of acetic acid was heated at 80°C on an oil bath for 8 h, and a gray-colored solid is separated and was filtered and washed with water with little diethyl ether to get 7-(4-chlorophenyl)-10-thioxo-7, 10, 11, 12 – tertahydro - 9H – benzo [*H*] pyrimido [4,5-*b*] quinoline-8-one (**4a**), which was recrystallized from EtOH: Benzene (1:1) to get pure desired product.

The structures of the products were confirmed on the basis of spectroscopic data **(**Entry **4a**). The infrared (IR) spectra of the **4a** compound showed the characteristic band at 3462 cm^{-1} which confirmed the presence of ─NH group, whereas another stretching band observed at 1693 cm^{-1} confirmed the presence of amide carbonyl group. The ^1H NMR showed a singlet at δ, 5.205 because of benzylic (CH) proton, which confirms the formation of cyclic C─C bond between two different motifs with aromatic aldehydes. The multiplet appeared between δ, 7.05 and 7.84 represented the aromatic protons, while the singlet at δ, 8.99 was due to ─NH proton. Two singlet's encountered at δ, 11.52 and 11.82 indicated the presence of the two ─NH protons of thiobarbituric acid moiety. In ^{13}C NMR spectrum, the signal appeared at δ, 90.77 confirmed cyno group of carbon, whereas another signal appeared at 118.76, 119.89, 122.24, 123.63, 126.29, 126.59, 127.43, 128.32, 128.69, 129.46, 129.50, 131.76, 132.80, and 145.55 indicated aromatic carbons, while those indicated carbonyl carbon and thio carbonyl carbons appeared at δ, 160.93 and 173.86 ppm (see Supporting Information).

With the preliminary achievement of this reaction, we turn our attention to optimize the reaction conditions in order to improve the yield with a substantial abridgement in the reaction time. In the optimization of reaction condition, initially we study effect of solvent on reaction and then we turn in search of favorable, efficient catalyst/ solvent condition for this reaction. At initial stage, we investigate the reaction of thiobarbituric acid (1 mmol), 4-chloro benzaldehyde (1 mmol), and 1-naphthylamine (1 mmol) under catalyst free condition at room temperature/ reflux condition in water. Unfortunately, the reaction did not proceed according to expectations and no desired product was obtained (entries 1–2, Table 1). When ethanol is used as a solvent, the yield of the desired product increased up to 50% at reflux condition (entries 3–4, Table 1). We examine high yield with minimum reaction time obtained in solvent ethanol than the solvent used for this reaction such as dimethylformamide, acetonitrile and methanol [entries 5–7 Table 1]. Then, the varieties of acid catalysts were investigated for the present transformations. We observed that, in the presence of $AlCl_3$, $FeCl_3$, CuO, HCl, P_2O_5, and PSSA, inferior results were obtained. Then, we tried same reaction in a combination of solvents ethanol: acetic acid. We found that ethanol: acetic acid (8:2) was sufficient to move this reaction forward (entries 15, Table 1). The ethanol: acetic acid (8:2) proved to be an excellent catalyst/solvent system as it furnished better yield in less time (entry 15, Table 1).

To check the generality and functional feasibility of same protocol, the reaction was carried out by employing varying amount of aldehydes. This success of optimized novel reaction encouraged us to work out on atom economy. The results have been summarized in Table 2. The formation of product involves the Knoevenagel

Scheme 1. Synthesis of 7-Aryl-10-thioxo-7, 10, 11, 12-tertahydro-9H- benzo [H] pyrimido [4, 5-b] quinoline-8-one (**4a–h**).

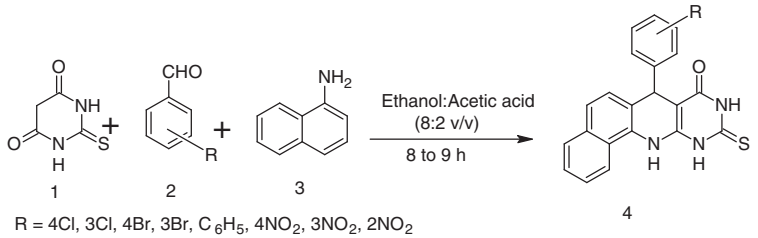


Table 1

Optimization of reaction conditions[a].

Sr. No.	Catalyst	Catalyst Load (mol %)	Solvent Reaction Condition	Reaction time (h)[b]	Yield (%)
1	-	-	Water/RT	15	-
2.	-	-	Water/Refluxs	14	-
3.	-	-	Ethanol/RT	12	-
4.	-	-	Ethanol/ Reflux	11	50
5.	-	-	Methanol/Reflux	11.5	40
6.	-	-	Acetonitrile/Reflux	11.5	24
7.	-	-	DMF/Reflux	12	28
8.	$AlCl_3$	20	Ethanol/ Reflux	10.5	64
9.	$FeCl_3$	20	Ethanol/ Reflux	10.75	70
10.	CuO	20	Ethanol/ Reflux	09	61
11.	HCl	20	Ethanol/ Reflux	08.75	55
12.	P_2O_5	20	Ethanol/ Reflux	09.50	70
13.	PSSA	20	Ethanol/ Reflux	08.25	78
14.	-	-	Ethanol: acetic acid (9:1)	11	82
15.	**-**	**-**	Ethanol: acetic acid **(8:2)**	**08**	**89**
16.	-	-	Ethanol: acetic acid (7:3)	08	89

[a]Reaction of 1-naphthylamine, 4-chloro benzaldehyde and thiobarbituric acid.
[b]Reaction monitor by thin layer chromatography.
Bold font indicates the optimized reaction condition at which maximum yield is obtained in minimum time.

Table 2

Physical data of title compound (**4a–h**) (7-aryl-10-thioxo-7, 10, 11, 12-tertahydro-9H-benzo[*H*] pyrimido [4,5-*b*] quinoline-8-one).

Sr. No.	Compd. code	R	Reaction Time (h)	Yield (%)	Atom economy[a] (%)	M.P.
1.	**4a**	4 -Cl	8	89	91.56	>300°C
2	**4b**	3 -Cl	8.10	90	91.56	>300°C
3.	**4c**	4 -Br	8.30	88	92.35	>300°C
4.	**4d**	3 -Br	8	86	92.35	>300°C
5.	**4e**	4 -NO_2	8.25	91	91.78	>300°C
6.	**4f**	3 -NO_2	8	89	91.78	>300°C
7.	**4g**	2-NO_2	8	87	91.78	294–296°C
8.	**4h**	-H	8	85	90.83	>300°C

[a](molecular mass of desired product molecular mass of all reactants) × 100

condensation of aryl aldehyde with thiobarbituric acid. Knoevenagel intermediate upon Michael addition with 1-naphthylamine followed by cyclisation gives the final product. The plausible mechanism is depicted in Scheme 2.

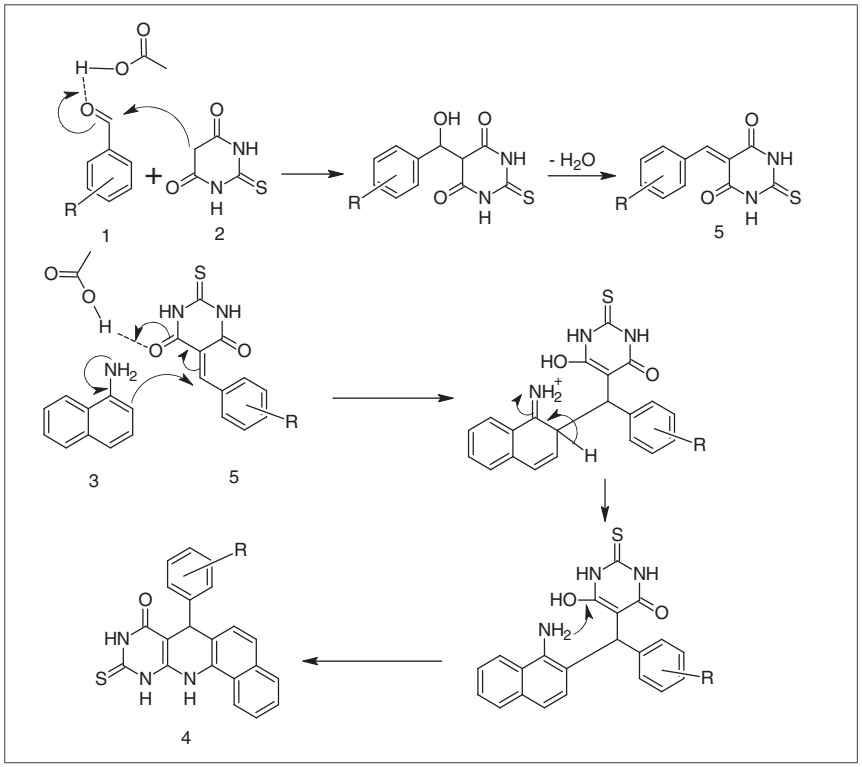
Synthesized compounds having fused quinoline, pyrimidine, phenanthroline, carbamide, and thiocarbamides motifs may exhibit bioactivity because of their structural resemblance with some proficient natural products, antifungal and antibacterial drugs available in market. Thus, we were inspired to undertake biological activity evaluation of the synthesized compounds against agricultural pathogenic fungal strains *Colletotrichum truncatum*, *Ustilago maydis Trichosporon*, *Trichothecium sp*., *Aspergillus oryze*, *Aspergillus terreus*, *Aspergillus niger*, and bacterial strains Gram-positive *Bacillus megaterium* and Gram-negative *Proteus vulgaris*.

Biological assay. The biological assays of synthesized compounds were carried out by agar well method, agar diffusion method, and broth dilution method, as described by Dr M. K. Lalitha [31] with slight modification.

Selection of pathogenic species. For antifungal screening, plant pathogenic fungi *C. truncatum*, *U. maydis*, *Trichosporon sp. Trichothecium sp*., *A. oryze*, *A. terreus*, and *A. niger* were selected, whereas for antibacterial screening, Gram-positive *B. megaterium* and Gram-negative *P. vulgaris* were chosen. The strains were incubated in a test tube having polydopamine (PDA) at 25°C for the antifungal assay. The synthesized compounds were tested for their antimicrobial activity against Gram +ve and Gram −ve pathogenic bacterial strains. Most of pathogenic strains showed good to moderate activity against synthesized compounds.

Antifungal activity. ***Agar well method bioassay [17,19,31–42].*** The synthesized compounds were tested for their antifungal activity by using agar well diffusion method for three different concentrations. The solutions of 100, 200, and 300 ppm concentrations were prepared in dimethyl sulfoxide (DMSO). Initially sterilized molten media PDA was poured in sterilized Petri plates and allowed to cool for 2 h when molten PDA was converted

Scheme 2. Plausible mechanism of 7-aryl-10-thioxo-7, 10, 11, 12-tertahydro-9H- benzo [H] pyrimido [4, 5-b] quinoline-8-one (**4a–h**).



in solid state. Pathogenic fungal strain suspensions were prepared in saline water (0.84%) spread uniformly under sterilized condition and bored with cork borer. The solutions were poured in its respective well. Benomyl (100 µg/ml) was used as a standard. The control of the solvent was performed under parallel conditions to know activity of blank. The diameter (mm) of the zones of inhibition was measured after 48 h for three replications. Most of the listed compounds showed good active against selected strains *C. truncatum*, *U. maydis*, *Trichosporon*, *Trichothecium sp*., *A. oryze*, *A. terreus*, and *A. niger*.

Agar diffusion method bioassay [17,19,31–42]. Then, for detail, the study of MIC by mycelium inhibition for antifungal activity was measured against agricultural pathogenic fungi *C. truncatum* and *U. maydis*. A stock solution of each compound was serially diluted in DMSO and 0.2 mL of each dilution was built in 20 mL of the appropriate potato dextrose agar medium. A final concentration of 0·5% (*v*/v) Tween-20 was established into the culture medium after autoclaving to enhance the compound solubility. The final concentrations of compounds in the medium were 50 to 250 µg/mL and the DMSO concentration was 1% (v/v). Petri dishes containing culture medium with 1% (v/v) DMSO were used as control. Results obtained have been summarized (Table 3).

Antibacterial activity bioassay [17,19,31–42]. Gram-positive bacteria *B. megaterium* and Gram-negative *P. vulgaris* were used for antibacterial study using streptomycin as standard compound. The antibacterial activities were carried out in nutrient broth with standard compositions and procedures. The nutrient agar was sterilized in autoclave at 121°C and 15 psi for 30 min. Inoculums were prepared in nutrient broth medium, and optical density of all pathogens was adjusted to 0.5 McFarland standards on Chemito Spectrascan UV 2600 spectrophotometer (Chemito Instruments Pvt. Ltd. Mumbai, India), which is equivalent to 0.10 at 625 nm. The sensitivity of the compounds was tested by broth dilution method. All the tested compounds were dissolved in DMSO at different concentrations (10 to 100 µg/mL). Ten milligrams of nutrient broth containing test tube was stirred, and 0.1 mL stock solution and 1 mL 0.5 McFarland turbid inoculums was added to test tube after stirring. A final concentration of 0.5% (*v*/v) Tween-20 was established for enhancing the compound solubility and incubated at 37°C for 24 h. The control was run under parallel condition to know activity in blank. The turbidity was recorded on ultraviolet spectrophotometer. The results were compared with streptomycin as a standard (Table 3).

From Table 3, it is clear that, compounds **4b**, **4d**, and **4e** are found to be more active at 250 ppm for both fungal species. The compounds **4b** and **4d** exhibited

Table 3

MIC of Synthesized compounds **4 (a - h)**.

		Antibacterial activity μg/ml		Antifungal activity μg/ml	
Sr. No.	Comp.	*Bacillus megaterium* (gram + ve)	*Proteus vulgaris* (gram - ve)	*Colletotrichum truncatum*	*Ustilago maydis*
1.	**4a**	100	100	>250	>250
2.	**4b**	100	75	250	250
3.	**4c**	100	100	>250	>250
4.	**4d**	75	50	250	250
5.	**4e**	>100	>100	250	250
6.	**4f**	100	100	>250	>250
7.	**4g**	100	100	>250	>250
8.	**4h**	>100	>100	>250	>250
9.	Streptomycin	10	10	-	-
10.	Benomyl	-	-	100	100

In vitro antimicrobial activity and minimum inhibitory concentration (MIC) result against *B. megaterium* (gram +ve), *P. vulgaris* (gram −ve) bacterial strains by using nutrient broth dilution method and fungal strains *C. truncatum* and *U. maydis* by using agar dilution method.

antibacterial activity at 100 and 75 ppm, respectively, against Gram-positive *B. megaterium* and at 75 and 50 ppm, respectively, against Gram-negative *P. vulgari*.

CONCLUSION

The present protocol provides a numerous advantages including high yields, cleaner reactions, operational simplicity, highly pure, minimal environmental impact, and provides convenient protocol for the synthesis of 7-aryl-10-thioxo-7, 10, 11, 12-tetrahydro-9H- benzo (H) pyrimido [4,5-b]quinoline-8-one derivatives. Biological evaluation of the synthesized compounds was done for their antifungal activity against *C. truncatum*, *U. maydis*, *A. niger*, *A. terreus*, *Sclerocium rolfsii*, *Trichosporon*, and *Trichothecium sp*. and antibacterial activity against Gram-positive bacteria *B. megaterium* and Gram-negative *P. vulgaris*. The 7-(3-chlorophenyl)-10-thioxo-7, 10, 11, 12 – tertahydro- 9H – benzo [H] pyrimido [4,5-b] quinoline −8- one (**4b**), 7-(3-Bromo-Phenyl)-10-thioxo-7, 10, 11, 12-tertahydro -9H- benzo [H] pyrimido [4,5-b] quinoline −8- one (**4d**), and 7-(4-Nitro-Phenyl)-10-thioxo-7, 10, 11, 12-tertahydro-9H-benzo[H]pyrimido[4,5-b] quinoline −8-one (**4e**) showed good antifungal activity and promising antibacterial activity exhibited by **4b** and **4d**.

EXPERIMENTAL

All chemicals used were commercially available and purchased from Sigma Aldrich. Melting points were taken on a melting point apparatus and are uncorrected. The reactions were monitored by thin layer chromatography. Proton nuclear magnetic resonance (^{1}H NMR) and ^{13}C NMR spectra were recorded on a Bruker DPX 300 MHz spectrophotometer (Bruker, Karlsruhe, Germany) in hexadeuterated dimethyl sulfoxide (DMSO-d_6) using tetramethylsilane as an internal standard. IR spectra were recorded on a FTIR spectrophotometer. Elemental analysis was done on a Flash elemental analyzer EURO EA-3000 (EuroVector S.p.A., Milan, Italy). Biological assay was carried out using Agar well method with four different concentrations. The solutions were prepared by using DMSO as a solvent. MIC values of the compounds were recorded by broth dilution method for antibacterial activity and Agar diffusion method for antifungal activity using Naanolab Autoclave, Laminar flow and Incubator.

General procedure for the syntheses of compounds (4a–h). In a 25 mL round-bottom flask, thiobarbituric acid **1** (1 mmol), aryl aldehydes **2** (1 mmol), and 1-naphthylamine **3** (1 mmol) in 5 mL ethanol: acetic acid (8:2 *v*/v) were stirred at 80°C for appropriate time. After the completion of reaction as indicated by using thin layer chromatography, the reaction mixture was cooled, and separated solid product was filtered. It was washed with water and further purified by recrystallization from EtOH: Benzene (1:1) mixture to obtain pure product.

Spectroscopic data of the synthesized compounds. ***7-(4-Chlorophenyl)-10-thioxo-7, 10, 11, 12 – Tertahydro - 9H – Benzo [H] pyrimido [4,5-b] quinoline −8- one (4a).*** This compound was obtained as gray color powder; yield 89%; mp >300°C, IR (ν max): 3462, 3267, 3224, 1693, 1574, 1536, 1374, 1143 cm^{-1}**;** ^{1}H NMR (300 MHz, DMSO - d_6): δ 5.20 (s, 1H), 7.05–7.10 (m, 2H), 7.16–7.19 (d, 2H, J = 8.1 Hz), 7.36–7.54 (m, 3H), 7.71–7.74 (d, 2H, J = 8.1 Hz), 7.81–7.84 (d, 1H, J = 8.4 Hz), 8.99 (s, 1H, NH), 11.52 (s, 1H, NH), 11.82 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO): δ, 90.77, 118.76, 119.89, 122.24, 123.63, 126.29, 126.59, 127.43, 128.32, 128.69, 129.46, 129.50, 131.76, 132.80, 145.55, 160.93, 173.86 ppm; *Anal.* calcd. For $C_{21}H_{14}ClN_3OS$: C, 64.36; H, 3.60; N,10.72 Found: C, 64.31; H, 3.52; N,10.58.

7-(3-Chlorophenyl)-10-thioxo-7, 10, 11, 12 – Tertahydro- 9H – Benzo [H] pyrimido [4,5-b] quinoline −8- one (4b). This compound was obtained as gray color powder; yield 90%; mp >300°C, IR (ν max): 3352, 3243, 3156, 3055, 1608, 1582, 1553, 1397 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ, 5.21 (s, 1H), 7.03–7.05 (m, 1H), 7.10–7.12 (m, 3H), 7.20 (s, 1H), 7.39–7.49 (m, 2H), 7.52–7.57 (t, 1H, J = 7.5 Hz), 7.74–7.77 (d, 1H, J = 7.8 Hz), 7.83–7.86 (d, 1H, J = 8.4 Hz), 9.04 (s, 1H, NH), 11.53 (s, 1H, NH), 11.96 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO): δ, 90.56, 120.78, 122.10, 122.72, 123.38, 126.76, 127.76, 127.55, 128.04, 128.65, 128.94, 131.17, 132.68, 133.38, 133.89, 142.71, 167.16, 169.61, 181.51 ppm; *Anal*. calcd. For $C_{21}H_{14}ClN_3OS$: C, 64.36; H, 3.60; N, 10.72 Found: C, 64.16; H, 3.48; N, 10.61.

7-(4-Bromophenyl)-10-thioxo-7, 10, 11, 12 –tertahydro -9H-benzo [H] pyrimido [4,5-b] quinoline −8- one (4c). This compound was obtained as yellow color powder; yield 88%; mp >300°C; IR (ν max): 3384, 3238, 3157, 1663, 1584, 1544, 1395, 1127 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ, 5.19 (s, 1H), 7.07–7.15 (m, 2H), 7.22–7.28 (d, 2H, J = 8.1 Hz), 7.38–7.48 (m, 2H), 7.51–7.56 (t, 1H, J = 8.1 Hz), 7.73–7.76 (d, 1H, J = 8.1 Hz), 7.83–7.88 (m, 2H), 9.05 (s, 1H), 11.52 (s, 1H,NH), 11.90 (s, 1H,NH) ppm; ^{13}C NMR (75 MHz, DMSO): δ, 90.62, 118.69, 119.99, 122.28, 123.58, 126.33, 126.59, 127.48, 128.70, 129.62, 129.90, 131.27, 132.83, 145.52, 146.12, 160.83, 173.94 ppm; *Anal*. calcd. For $C_{21}H_{14}BrN_3OS$: C, 57.81; H, 3.23; N, 9.63 Found: C, 57.69; H, 3.12; N, 9.78.

7-(3-Bromophenyl)-10-thioxo-7, 10, 11, 12-tertahydro -9H-benzo [H] pyrimido [4,5-b] quinoline −8- one (4d). This compound was obtained as yellow color powder; yield 86%; mp >300°C; IR (ν max): 3352, 3241, 3155, 3656, 1607, 1581, 1554, 1396, 1129 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ, 5.20 (s, 1H), 7.04–7.21 (m, 4H), 7.37–7.49 (m, 3H), 7.53–7.58 (t, 1H, J = 8.4 Hz), 7.75–7.78 (d, 1H, J = 8.1 Hz), 7.84–7.87 (d, 1H, J = 8.4 Hz), 9.06 (s, 1H, NH), 11.54 (s, 1H, NH), 11.99 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO): δ, 90.56, 118.52, 119.92, 122.20, 122.25, 123.69, 126.38, 126.64, 126.87, 127.42, 128.71, 129.51, 129.53, 130.23, 130.62, 132.83, 145.61, 149.23, 160.88, 173.92 ppm; *Anal*. calcd. For $C_{21}H_{14}BrN_3OS$: C, 57.81; H, 3.23; N, 9.63 Found: C, 57.94; H, 3.15; N, 9.54.

7-(4-Nitrophenyl)-10-thioxo-7, 10, 11, 12-tertahydro-9H-benzo[H]pyrimido[4,5-b] quinoline −8- one (4e). This compound was obtained as dark green color powder; yield 91%; mp >300°C; IR (ν max) 3316, 3097, 1614, 1559, 1529, 1446, 1398, 1345 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ, 5.35 (s, 1H), 7.03–7.06 (d, 1H, J = 8.4 Hz), 7.37–7.47 (m, 6H), 7.72–7.99 (m, 6H), 9.11 (s, 1H, NH), 11.55 (s, 1H, NH), 11.87 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO): δ, 90.03, 117.53, 117.73, 119.93, 122.28, 122.80, 123.46, 123.62, 123.84, 126.53, 126.73, 27.29,1 28.74, 129.06, 129.72, 132.96, 145.69, 146.36, 153.84, 160.90, 174.02 ppm; *Anal*. calcd. For $C_{21}H_{14}N_4O_3S$: C, 62.68; H, 3.51; N, 13.92 Found: C, 62.51; H, 3.35; N, 13.88.

7-(3-Nitrophenyl)-10-thioxo-7, 10, 11, 12 –tertahydro -9H-benzo [H] pyrimido[4,5-b] quinoline −8- one (4f). This compound was obtained as green color powder; yield 89%; mp >300°C; IR (ν max): 3320, 3080, 1614, 1560, 1528, 1447, 1398, 1345, 1261, 1191 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ, 5.47 (s, 1H), 7.28–7.30 (d, 1H, J = 8.4 Hz), 7.50–7.60 (m, 4H), 7.66–7.74 (m, 2H), 7.87–7.92 (t, 2H, J = 14.4 Hz), 8.15 (s, 1H), 9.24 (s, 1H, NH), 11.64 (s, 1H, NH), 12.21 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO): δ, 90.13, 118.46, 120.34, 121.97, 122.25, 123.41, 123.94, 127.00, 127.22, 127.84, 129.05, 129.75, 130.41, 132.99, 134.95, 145.91, 148.19, 149.40, 160.70, 174.02 ppm; *Anal*. calcd. For $C_{21}H_{14}N_4O_3S$: C, 62.68; H, 3.51; N, 13.92; Found: C, 62.42; H, 3.38; N, 13.79.

7-(2-Nitrophenyl) -10- thioxo-7, 10, 11, 12 –tertahydro -9H-benzo [H] pyrimido[4,5-b] quinoline −8- one (4g). This compound was obtained as green color powder; yield 87%; mp 294°C; IR (ν max): 3344, 3229, 2872, 1701, 1631, 1579, 1523, 1494, 1431 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ, 5.38 (s, 1H), 7.07–7.10 (d, 1H, J = 8.7 Hz), 7.39–7.58 (m, 4H),7.53–7.58 (t, 1H, J = 8.4 Hz), 7.74–7.77 (d, 1H, J = 8.1 Hz), 7.84–7.90 (m, 2H), 7.94–8.01 (d, 1H, J = 8.7 Hz), 9.11 (s, 1H, NH), 11.55 (s, 1H, NH), 12.00 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO): δ, 89.96, 120.10, 122.29, 123.74, 126.87, 127.46, 128.82, 129.15, 132.97, 146.40, 174.04 ppm; *Anal*. calcd. For $C_{21}H_{14}N_4O_3S$: C, 62.68; H, 3.51; N, 13.92 Found: C, 62.56; H, 3.42; N, 13.72.

7-Phenyl-10-thioxo-7, 10, 11, 12-tertahydro-9H-benzo[H] pyrimido[4,5-b]quinoline-8-one (4h). This compound was obtained as brown color powder; yield 85%; mp, >300°C, IR (ν max): 3416, 3089, 2840, 1654, 1591, 1523, 1376, 1166 cm-1; 1H NMR (300 MHz, DMSO-d_6): δ, 5.23 (s, 1H), 7.21–7.23 (d, 3H),7.35–7.38 (d, 2H), 7.47–7.55 (m, 2H), 7.64 (s, 1H), 7.83–7.89 (t, 2H, 8.7 Hz), 8.23 (s, 1H), 9.12 (s, 1H), 11.55 (s, 1H), 12.10 (s, 1H) ppm; ^{13}C NMR (75 MHz, DMSO): δ, 90.01, 113.04, 114.77, 118.56, 118.95, 120.29, 122.07, 127.14, 127.85, 129.02, 130.51, 13110, 145.84, 150.05, 156.42, 157.06, 160.68, 173.94 ppm; *Anal*. calcd. For $C_{21}H_{15}N_3OS$: C, 70.57; H, 4.23; N, 11.76 Found: C, 70.51; H, 4.14; N, 12.01.

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