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Eco-friendly Solventless Synthesis of 5-Indolylpyrimido[4,5-*d*]pyrimidinones and Their Antimicrobial Activity

Ragini Gupta,* Anshu Jain, Rahul Joshi,† and Meenakshi Jain†

*Department of Chemistry, Malaviya National Institute of Technology, Jaipur-302 017, India. *E-mail: raginigupta@mnit.ac.in*
†Department of Chemistry, University of Rajasthan, Jaipur-302 055, India
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A series of 5-indolylpyrimido[4,5-*d*]pyrimidinones (**4a-h**) were obtained by multi-component reaction of 3-formylindole (**1**), thiobarbituric acid/barbituric acid (**2**) and thiourea/urea (**3**) under microwave irradiation in dry media. Secondly, grinding together neat reactants also gave the titled compounds in good yields. All the synthesized compounds have been characterized on the basis of elemental analyses and spectral data (IR, ^{1}H NMR and Mass). Representative compounds were also evaluated for their antimicrobial activity against *Rhizopus stolonifer*, *Fusarium oxysporum*, *Escherichia coli*, and *Pseudomonas aeruginosa* at different concentrations. Some of the compounds showed promising activity.

Key Words : 5-Indolylpyrimido[4,5-*d*]pyrimidinones, Microwave irradiation on solid support, Grindstone technology, Biginelli reaction

Introduction

Pyrimido[4,5-*d*]pyrimidines are an important class of annulated uracils of biological importance[1] because of their connection with purine pteridine systems.[2] Pyrimido[4,5-*d*]pyrimidinone derivatives are well known as useful bronchodilators,[3] vasodilators,[4] antiallergic,[5] antihypertensive[6] and anticancer[7] agents. Recently pyrimido[4,5-*d*]pyrimidine analogues of folic acid have been screened for antitumour activity.[8]

Perusal of literature revealed that the indole moiety is probably the most common and important feature of a variety of natural products and medicinal agents with significant biological activities including antimicrobial,[9,10] antiviral[11] and antitumour.[12] The presence of two or more different heterocyclic moieties in a single molecule often enhances the biocidal profile remarkably. Besides this, indole derivatives like 8*H*-[4,5-b]indolopyrimidines,[13] and indolo[3,2-*e*][1,2,3]triazolo[1,5-*a*]pyrimidines[14] are also known to show antitumor activity. Recently, Gangjee *et al*.[15] designed, synthesized and evaluated pyrimido[4,5-*b*]indole derivatives that inhibit vascular endothelial growth factor receptor-2 (VEGFR-2) and platelet-derived growth factor receptor beta (PDGFR-beta) for antiangiogenic effects and also inhibit human thymidylate synthase (hTS) for cytotoxic effects and thus afford combination chemotherapeutic potential in single agents. Further, 4*H*-pyrimido[5,4-*b*]indol-4-ones show analgesic and anti-inflammatory activities.[16] Pyrimido[5,4-*b*]-indoles were found to behave as potent alpha (1)-AR antagonists.[17] 2,4-Diamino-9*H*-pyrimido[4,5-*b*]indol-5-ols also show antitumour activity.[18]

Encouraged by all the above facts, we have synthesized novel 5-indolylpyrimido[4,5-*d*]pyrimidinones; drug like scaffolds. Herein, we have incorporated all the above mentioned bioactive moieties together in a single entity by a 'one pot' multi-component reaction (MCR) furnishing 5-indolylpyrimido[4,5-*d*]pyrimidinones **4** expecting to enhance the bioactivity of the newly synthesized compounds.

Biginelli reaction, first reported in 1893, is a one pot three component condensation of benzaldehyde, urea and ethyl acetoacetate in acidic medium which gives 3,4-dihydropyrimidin-2(1*H*)-ones.[19] Several improved procedures over the Biginelli reaction have been reported either by modification of the classical one pot condensation or by new protocols.[20-22]

As an extension to our endeavor towards the synthesis of bioactive molecules[23-25] utilizing green methodologies we now wish to report the synthesis of 5-indolylpyrimido[4,5-*d*]pyrimidinones by grindstone technology in which the carbon atoms carrying the oxo, thio and NH sites can be utilized as centers to obtain various derivatives for the potential use in combinatorial chemistry.[26]

5-Indolylpyrimido[4,5-*d*]pyrimidinones were classically prepared by 'one pot' multi-component reaction in which the three reactants were refluxed in ethanol in acidic medium. Environment protection reasons drive us to use green chemistry protocol like using eco-friendly reagents and catalysts,[27] reaction in aqueous medium,[28] ionic liquids[29] or solvent free reactions[30] because it offers enhanced chemical process economics concomitant with a reduced environmental burden.[31] The emergence of microwave assisted solid supported synthesis[32] is a step forward in this direction. MCR's on solid support directed towards the generation of diverse small organic molecules has generated considerable interest as it relates to efficient lead structure identification.[33,34] In this expeditious and solvent free approach, 3-formylindole **1**, thiobarbituric acid/barbituric acid **2** and thiourea/urea **3** were adsorbed over acidic alumina and then exposed to microwaves to give the desired compounds **4**. The salient features of this high yield protocol are enhanced

reaction rates, greater selectivity and experimental ease of manipulation. Microwave (MW) heating has been used for the rapid synthesis of a variety of compounds, wherein chemical reactions are accelerated because of selective absorption of MW energy by polar molecules, nonpolar molecules being inert to the MW dielectric loss.[35]

But microwave assisted solid support was not absolutely eco-friendly method as we have used requisite amount of solvent for adsorption of reactants at pre-stage of reaction. So, to increase the greenness of the reaction we developed a method by grindstone technology. It is a simplified process for conducting the multi-component Biginelli reaction whereby solvent-free chemical reactions occur by just grinding solid/solid, solid/liquid, or even liquid/liquid reactants together. Required activation energy is provided from friction of the reacting molecules.[36]

Results and Discussion

5-Indolylpyrimido[4,5-*d*]pyrimidinones **4** were prepared by reacting one mole of each 3-formylindole **1** and urea/thiourea **3** with 1.5 mole of barbitiuric/thiobarbituric acid **2** by following the classical Biginelli synthesis using various techniques in acidic medium (5-6 drops of conc. HCl) (Scheme 1). In the traditional approach, reaction proceeds with low yield (55-65%) after refluxing for 5-6 h in ethanol. In an attempt to improve the yield of the reaction and acknowledging the benefits of 'green chemistry', the same reaction was performed by using green chemical techniques (microwave irradiation and grindstone technology).

For solid supported microwave irradiation method, a mixture of 3-formylindole **1** barbituric acid/thiobarbituric acid **2** and urea/thiourea **3** in ethanol was taken in a beaker and adsorbed on acidic alumina (no HCl required). By this method, products were obtained (Scheme 1) in good yield (80-90%) within a few minutes of irradiation, while the same reaction under conventional heating required several hours to give the desired product with comparatively lower yields (55-65%).

Grinding together of one mole of each 3-formylindole **1** and urea/thiourea **3** with 1.5 mole of barbitiuric/thiobarbituric acid **2** with 5-6 drops of conc. HCl without solvent in a mortar and pestle gave 5-indolylpyrimido[4,5-*d*]pyrimidinones **4.** It is not only advantageous from the environmental point of view but also offers rate enhancement and high yields (90-95%). This makes grindstone technology economic and environmentally benign for organic synthesis (Table 1).

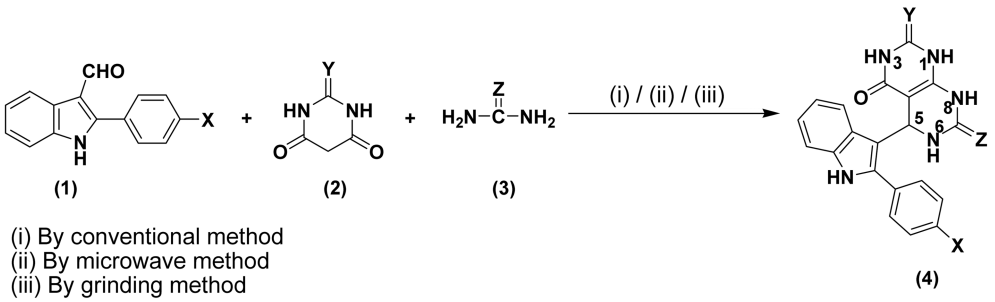
In the IR spectra of 3-formylindole **1** characteristic absorption due to >C=O group appears at 1625 cm^{-1}. This downfield shift from the normal >C=O absorption (1720 cm^{-1}) is attributed to the presence of high degree of conjugation in the formylated indole. An upfield shift in >C=O (1690-1670 cm^{-1}) absorption band of 5-indolylpyrimido-[4,5-*d*]pyrimidinones **4** is observed. Absorption bands due to >C=S and N-H stretching frequencies are observed at 1580-1490 cm^{-1} and 3500-3080 cm^{-1}, respectively.

^{1}H NMR spectra of compounds **4** showed doublets at δ 6.71-6.75 ppm due to methine proton at C-*5* position of 5-indolylpyrimido[4,5-*d*]pyrimidinone moiety. N-H Protons are observed as singlets from δ 2.49-2.64, 11.20-11.23 and 12.14-12.25 ppm, which were D_2O exchangeable. Indolic N-H is observed from δ 8.46-8.49 ppm. Aromatic protons are observed as multiplet from δ 7.30 to 7.92 ppm. The disappearance of peak from δ 10 ppm (indolic formyl) **1** confirmed the formation of title compounds **4**.

Final confirmation was obtained from DART-*MS* spectra

Table 1. Yield (%) and time for the synthesis of 5-indolylpyrimido-[4,5-*d*]pyrimidinones (**4a-h**)

Compound	Conventional		Microwave irradiation		Grindstone technology	
	Yield (%)	Time (min)	Yield (%)	Time (min)	Yield (%)	Time (min)
4a	62	320	86	4.0	92	10
4b	60	340	90	4.0	94	12
4c	65	350	84	4.5	92	12
4d	64	350	88	4.0	90	14
4e	58	360	90	5.0	92	13
4f	65	350	85	4.0	92	14
4g	62	360	88	5.0	94	15
4h	55	360	90	5.5	92	15



4a: X = H, Y = O, Z = O
4b: X = H, Y = O, Z = S
4c: X = H, Y = S, Z = O
4d: X = H, Y = S, Z = S
4e: X = CH_3, Y = O, Z = O
4f : X = CH_3, Y = O, Z = S
4g: X = CH_3, Y = S, Z = O
4h: X = CH_3, Y = S, Z = S

Scheme 1. Synthesis of 5-indolylpyrimido[4,5-*d*]pyrimidinones **4a-h**.

Table 2. Antibacterial activity of 5-indolylpyrimido[4,5-*d*]pyrimidinones (**4a-h**)

Compound	Mean value of zone of inhibition (in cm) against *Escherichia coli* IZ[a] (AI)[b]				Mean value of zone of inhibition (in cm) against *Pseudomonas aeruginosa* IZ[a] (AI)[b]			
	400 ppm	600 ppm	800 ppm	1000 ppm	400 ppm	600 ppm	800 ppm	1000 ppm
Amphicillin	1.0	1.1	1.3	1.5	1.2	1.4	1.5	1.7
4a	–	–	1.0 (0.76)	1.1 (0.73)	–	1.3 (0.92)	1.8 (1.2)	1.8 (1.05)
4b	0.9 (0.9)	1.0 (0.9)	0.9 (0.69)	1.3 (0.86)	1.0 (0.83)	1.1 (0.78)	1.5 (1.0)	1.5 (0.88)
4c	0.8 (0.8)	1.1 (1.0)	1.0 (0.76)	1.4 (0.93)	0.9 (0.75)	–	1.2 (0.8)	1.4 (0.82)
4d	1.0 (1.0)	1.1 (1.0)	1.5 (1.15)	1.6 (1.06)	1.1 (0.91)	1.6 (1.14)	1.9 (1.26)	2.0 (1.17)
4e	–	–	1.1 (0.84)	1.4 (0.93)	–	1.4 (1.0)	1.6 (1.06)	1.7 (1.0)
4f	0.8 (0.8)	1.2 (1.09)	1.3 (1.0)	1.3 (0.86)	0.9 (0.75)	1.3 (0.92)	–	1.8 (1.05)
4g	0.9 (0.9)	1.0 (0.9)	–	1.5 (1.0)	1.0 (0.83)	1.4 (1.0)	1.7 (1.13)	1.8 (1.05)
4h	0.7 (0.7)	0.9 (0.81)	1.0 (0.76)	1.5 (1.0)	1.2 (1.0)	1.5 (1.07)	1.1 (0.73)	1.6 (0.94)

[a]IZ = Inhibition area (zone) excluding diameter of disc. [b]AI (Activity Index) = Inhibition area of sample /Inhibition area of standard

Table 3. Antifungal activity of 5-indolylpyrimido[4,5-*d*]pyrimidinones (**4a-h**)

Compound	Mean value of zone of inhibition (in cm) against *Rhizopus stolonifer* IZ[a] (AI)[b]				Mean value of zone of inhibition (in cm) against *Fusarium oxysporum* IZ[a] (AI)[b]			
	400 ppm	600 ppm	800 ppm	1000 ppm	400 ppm	600 ppm	800 ppm	1000 ppm
Fluconazole	1.2	1.3	1.5	1.6	1.2	1.4	1.5	1.7
4a	–	–	1.4 (0.93)	1.4 (0.87)	–	1.3 (0.92)	1.4 (0.93)	1.6 (0.94)
4b	1.0 (0.83)	1.2 (0.92)	1.2 (0.8)	1.5 (0.93)	1.2 (1.0)	1.4 (1.0)	1.5 (1.0)	1.7 (1.0)
4c	0.9 (0.75)	1.3 (1.0)	1.5 (1.0)	1.6 (1.0)	1.3 (1.08)	1.5 (1.07)	1.6 (1.06)	1.5 (0.88)
4d	1.1 (0.91)	1.5 (1.15)	1.9 (1.26)	2.0 (1.25)	1.2 (1.0)	1.7 (1.21)	1.5 (1.0)	2.1 (1.23)
4e	–	–	1.3 (0.86)	1.6 (1.0)	–	1.6 (1.14)	1.6 (1.06)	1.8 (1.05)
4f	1.0 (0.83)	1.4 (1.07)	1.6 (1.06)	1.8 (1.12)	1.0 (0.83)	–	1.7 (1.13)	1.8 (1.05)
4g	–	1.2 (0.92)	1.8 (1.2)	1.7 (1.06)	1.1 (0.91)	1.6 (1.14)	1.5 (1.0)	2.0 (1.17)
4h	0.8 (0.66)	1.4 (1.07)	–	1.5 (0.93)	0.9 (0.75)	1.4 (1.0)	1.4 (0.93)	1.7 (1.0)

[a]IZ = Inhibition area (zone) excluding diameter of disc. [b]AI (Activity Index) = Inhibition area of sample /Inhibition area of standard

which showed an accurate M+/M+2/M+4 peaks at *m/z* 373 (**4a**), 389/391 (**4b-c**), 405/407/409 (**4d**), 387 (**4e**), 403/405 (**4f-g**), 420/422/424 (**4h**) that agreed well with their corresponding molecular formulae.

On the basis of antimicrobial evaluation, compound **4d** seems to be very active against all microbes at higher concentrations. Compound **4e** showed good activity against *Fusarium oxysporum* at 600, 800 and 1000 ppm concentrations. Compound **4a** and **4g** are active against *Pseudomonas aeruginosa* at 800 ppm and 1000 ppm.

Anti-microbial Activity. The synthesized compounds were screened for their antibacterial and antifungal activity using agar well diffusion method[37] at four different concentrations: (a) 400 ppm (b) 600 ppm (c) 800 ppm (d) 1000 ppm. Amphicillin and Fluconazole were used as standard drugs for antibacterial and antifungal activity, respectively. Zone of inhibition was measured (in cm) against various strains as detailed in Table 2 and 3. The microbial strains used were *Rhizopus stolonifer* MTCC 161, *Fusarium oxysporum* MTCC 284, *Escherichia coli* MTCC 1652, and *Pseudomonas aeruginosa* MTCC 670.

Agar Well Diffusion Method. About 10-15 g of molten agar was spread into each sterilized petri dish by taking the usual precautions to avoid contamination. All the petri dishes were marked in a specific way. Sterile cork borer was used to make well. The agar plates were inoculated with the suspension of particular organism by spread plate technique.

Finally, the agar well plates were filled with 0.5 mL of the test solution. After the addition of the test samples, the plates were kept in freeze for diffusion and incubated at 37 °C for 1 h. The zone of inhibition if any was then measured in cm for the particular compound and specific organism after 24 hr (Table 2 and 3).

All the microbial strains used were of non-invasive species of their genera and thus applicable for analytical work.

Conclusion

In summary, we have developed two simple, novel and eco-friendly synthetic protocols for the synthesis of 5-indolylpyrimido[4,5-*d*]pyrimidinones **4a-h** using solid supported microwave irradiation and grindstone technology which provide higher yields in shorter reaction time with the simplicity of the procedures. On the basis of antimicrobial

evaluation, compound **4d** seems to be very active against all microbes at higher concentrations. Compound **4e** showed good activity against *Fusarium oxysporum* at 600, 800 and 1000 ppm concentrations. Compound **4a** and **4g** are active against *Pseudomonas aeruginosa* at 800 ppm and 1000 ppm.

Experimental

Melting points were determined in open glass capillaries and are uncorrected. The IR spectra (υ_{max} in cm^{-1}) were recorded on FT-IR SHIMADZU-8400S spectrophotometer using KBr pellets. ^{1}H NMR spectra were recorded on JEOL-AL 300 spectrophotometer (300 MHz) using $CDCl_3$/DMSO-d_6 as solvents. TMS was taken as internal standard. DART-*MS* spectra were recorded on JEOL-AccuTOF JMS-T100LC mass spectrometer having a DART (Direct Analyses in Real Time) source. The samples were subjected as such in front of DART source. Dry Helium was used with 4 LPM flow rate for ionization at 350 °C. Elentar Vario EL III automatic CHN analyzer was used for elemental analyses. The DART mass spectra and CHN analyses were recorded at central drug research institute (CDRI), Lucknow, India. Microwave irradiation was carried out in a LG MS-194A house hold microwave oven (2450 MHz, 800 W). The purity of compounds was checked by TLC using silica gel (60-120 mesh) as adsorbent, UV light, or iodine accomplished visualization. All common reagents and solvents were used as obtained from commercial suppliers without further purification. 3-formyl indoles **1** were prepared by the methods in literature.[38-41]

General Procedures for the Preparation of 5-Indolyl-pyrimido[4,5-*d*]pyrimidinones (4a-h).

Method (i): A mixture of 3-formylindole **1** (0.024 mol), barbituric acid/thiobarbituric acid **2** (0.037 mol), and urea/thiourea **3** (0.024 mol) in ethyl alcohol (20 mL) with a few drops of conc. HCl (3-4 drops) was refluxed for 5-6 hours (Table 1). Progress of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was kept in a refrigerator for 24 hours. The solid precipitate was filtered off, dried and recrystallized from ethanol to give pure crystalline desired product.

Method (ii): A mixture of 3-formylindole **1** (0.024 mol), barbituric acid/thiobarbituric acid **2** (0.037 mol), and urea/thiourea **3** (0.024 mol) in ethanol was taken in a beaker and adsorbed on acidic Al_2O_3 (2g). The mixture was stirred well, dried in air and then subjected to MWI for 4-6 mins (intermittently with 1 min cooling interval) at maximum power (800 W) as mentioned in Table 1. Final temperature of the reaction mixture was measured with the help of thermometer at the end of the reaction (110-120 °C). On completion of reaction (TLC monitoring, sampling at an interval of 30 s), the product was extracted with ethanol (3 × 10 mL). Recovery of the solvent under a reduced pressure gave the required product, which was recrystallized from ethanol.

Method (iii): A mixture of 3-formylindole **1** (0.024 mol), barbituric acid/thiobarbituric acid **2** (0.037 mol), and urea/thiourea **3** (0.024 mol) were taken in a mortar and grinded with pestle for 10-15 min with addition of few drops (5-6 drops) of conc. HCl. On completion of the reaction (TLC monitoring), same workup procedure was followed as detailed in the conventional method after completion of the reaction (Fig. 1-2).

Figure 1. Neat Reactants grinded.

Figure 2. After addition of conc. HCl.

5-(2-Phenyl-1*H*-indolyl-3-yl)-5,6-dihydropyrimodo[4,5-*d*]pyrimidine-2,4,7(1*H*,3*H*,8*H*)-trione (4a). Mp 275 °C; IR (cm^{-1}, KBr) 3450 (N-H str.), 3280 (N-H str.), 3045 (aromatic C-H str.), 1670 (C=O), 1650 (aromatic C=C), 1520 (C=S); MS (*m/z*) 373; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.62 (s, NH (6), NH (8), 2H), 6.73 (d, J = 3.3 Hz, H (5), 1H), 7.30-7.81 (m, ArH, 9H), 8.46 (s, 1H, NH (indole)), 11.20 (s, 1H, NH (1)), 12.24 (s, 1H, NH (3)). *Anal*. Calcd. for $C_{20}H_{15}N_5O_3$: C, 64.34; H, 4.05; N, 18.76. Found: C, 64.32; H, 4.01; N, 18.73.

5-(2-Phenyl-1*H*-indol-3-yl)-7-thioxo-5,6,7,8-tetrahydro-pyrimodo[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4b). Mp 288 °C; IR (cm^{-1}, KBr) 3445 (N-H str.), 3285 (N-H str.), 3040 (aromatic C-H str.), 1675 (C=O), 1645 (aromatic C=C), 1510 (C=S); MS (m/z) 389; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.63 (s, NH (6), NH (8), 2H), 6.71 (d, J = 3 Hz, H (5), 1H), 7.31-7.83 (m, ArH, 9H), 8.45 (s, 1H, NH (indole)), 11.23 (s, 1H, NH (1)), 12.22 (s, 1H, NH (3)). *Anal*. Calcd. for $C_{20}H_{15}N_5O_2S$: C, 61.68; H, 3.88; N, 17.98. Found: C, 61.66; H, 3.84; N, 17.93.

4-(2-Phenyl-1*H*-indol-3-yl)-7-thioxo-3,4,7,8-tetrahydro-pyrimido[4,5-*d*]pyrimidine-2,5(1*H*,6*H*)-dione (4c). Mp 310 °C; IR (cm^{-1}, KBr) 3450 (N-H str.), 3250 (N-H str.), 3045 (aromatic C-H str.), 1690 (C=O), 1655 (aromatic C=C), 1515 (C=S). MS (m/z) 389; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.62 (s, NH (6), NH (8), 2H), 6.72 (d, J = 3.1 Hz, H (5), 1H), 7.32-7.82 (m, ArH, 9H), 8.47 (s, 1H, NH (indole)), 11.22 (s, 1H, NH (1)), 12.23 (s, 1H, NH (3)). *Anal*. Calcd. for $C_{20}H_{15}N_5O_2S$: C, 61.68; H, 3.88; N, 17.98. Found:

C, 61.68; H, 3.88; N, 17.98.

5-(2-Phenyl-1*H*-indol-3-yl)-2,7-dithioxo-2,3,5,6,7,8-hexahydropyrimido[4,5-*d*]pyrimidin-4(1*H*)-one (4d). Mp 280 °C; IR (cm^{-1}, KBr) 3440 (N-H str.), 3280 (N-H str.), 3045 (aromatic C-H str.), 1680 (C=O), 1650 (aromatic C=C), 1500 (C=S); MS (m/z) 405; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.64 (s, NH (6), NH (8), 2H), 6.74 (d, J = 3 Hz, H (5), 1H), 7.30-7.81 (m, ArH, 9H), 8.46 (s, 1H, NH (indole)), 11.23 (s, 1H, NH (1)), 12.24 (s, 1H, NH (3)). *Anal*. Calcd. for $C_{20}H_{15}N_5OS_2$: C, 59.24; H, 3.73; N, 17.27. Found: C, 59.20; H, 3.71; N, 17.25.

5-(2-*p*-Tolyl-*1H*-indolyl-3-yl)-5,6-dihydropyrimodo[4,5-*d*]pyrimidine-2,4,7(1*H*,3*H*,8*H*)-trione (4e). Mp 290 °C; IR (cm^{-1}, KBr) 3445 (N-H str.), 3285 (N-H str.), 3045 (aromatic C-H str.), 2950 (C-H str.), 1675 (C=O), 1645 (aromatic C=C), 1495 (C=S); MS (m/z) 387; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (s, CH_3, 3H), 2.52 (s, NH (6), NH (8), 2H), 6.75 (d, J = 3.5 Hz, H (5), 1H), 7.33-7.89 (m, ArH, 9H), 8.49 (s, 1H, NH (indole)), 11.21 (s, 1H, NH (1)), 12.20 (s, 1H, NH (3)). *Anal*. Calcd. for $C_{21}H_{17}N_5O_3$: C, 65.11; H, 4.42; N, 18.08. Found: C, 65.10; H, 4.45; N, 18.05.

7-Thioxo-5-(2-*p*-tolyl-1*H*-indol-3-yl)-5,6,7,8-tetrahydropyrimodo[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (4f). Mp 298 °C; IR (cm^{-1}, KBr) 3440 (N-H str.), 3150 (N-H str.), 3045 (aromatic C-H str.), 2940 (C-H str.), 1670 (C=O), 1650 (aromatic C=C), 1490 (C=S); MS (m/z) 403; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (s, CH_3, 3H), 2.64 (s, NH (6), NH (8), 2H), 6.74 (d, J = 3.2 Hz, H (5), 1H), 7.34-7.83 (m, ArH, 9H), 8.46 (s, 1H, NH (indole)), 11.23 (s, 1H, NH (1)), 12.22 (s, 1H, NH (3)). *Anal*. Calcd. for $C_{21}H_{17}N_5O_2S$: C, 62.52; H, 4.25; N, 17.36. found: C, 62.55; H, 4.24; N, 17.32.

7-Thioxo-4-(2-*p*-tolyl-1*H*-indol-3-yl)-3,4,7,8-tetrahydropyrimodo[4,5-*d*]pyrimidine-2,5(1*H*,6*H*)-dione (4g). Mp 315 °C; IR (cm^{-1}, KBr) 3430 (N-H str.), 3290 (N-H str.), 3045 (aromatic C-H str.), 2980 (C-H str.), 1685 (C=O), 1650 (aromatic C=C), 1510 (C=S); MS (m/z) 403; ^{1}H NMR (300 MHz, CDCl3) δ 1.18 (s, CH_3, 3H), 2.62 (s, NH (6), NH (8), 2H), 6.73 (d, J = 3.2 Hz, H (5), 1H), 7.49-7.92 (m, ArH, 9H), 8.46 (s, 1H, NH (indole)), 11.22 (s, 1H, NH (1)), 12.22 (s, 1H, NH (3)); *Anal*. Calcd. for $C_{21}H_{17}N_5O_2S$: C, 62.52; H, 4.25; N, 17.36. found: C, 62.50; H, 4.23; N, 17.33.

2,7-Dithioxo-5-(2-*p*-tolyl-1*H*-indol-3-yl)-2,3,5,6,7,8-hexahydropyrimodo[4,5-*d*]pyrimidine-4(1*H*)-one (4h). Mp 295 °C; IR (cm^{-1}, KBr) 3400 (N-H str.), 3180 (N-H str.), 3045 (aromatic C-H str.), 2940 (C-H str.), 1690 (C=O), 1645 (aromatic C=C), 1515 (C=S); MS (m/z): 420; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (s, CH_3, 3H), 2.63 (s, NH (6), NH (8), 2H), 6.75 (d, J = 3 Hz, H (5), 1H), 7.30-7.82 (m, ArH, 9H), 8.47 (s, 1H, NH (indole)), 11.23 (s, 1H, NH (1)), 12.25 (s, 1H, NH (3)); *Anal*. Calcd. for $C_{21}H_{17}N_5OS_2$: C, 60.12; H, 4.08; N, 16.69. Found: C, 60.14; H, 4.05; N, 16.64.

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