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Synthesis and screening for antioxidant and cytotoxic activities of novel 2-thioxo-benzo[f]chromeno[2,3-*d*]pyrimidin-4-ones derived by cetylpyridinium chloride catalyzed multicomponent reactions in aqueous micellar media

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The cetylpyridinium chloride (CPC) has been used as micellar catalyst in three-component one-pot reaction involving thiobarbituric acids, aromatic aldehydes and β-napthol for synthesizing the novel 2-thioxo-benzo[5,6]chromeno[2,3-*d*]pyrimidin-4-one derivatives in aqueous media. These 2-thioxo-benzo[5,6]chromeno[2,3-*d*]pyrimidin-4-one derivatives have been screened for antioxidant and cytotoxic activities. It has been found that 2-thioxo-5-(4-nitrophenyl)-benzo[f]chromeno[2,3-d]pyrimidin-4-one (**4a**) has shown highest scavenging activity value to reduce DPPH free radicals, which may be due to the presence of nitro group at *para*-position. Three compounds, **4a**, **4b** and **4o** show significant *in vitro* antitumor activity. The study of the antioxidant and cytotoxic activities reveal that all the compounds show moderate to good activity indicating that the presence of thiouriedo linkage in the pyrimidine nucleus and the chromeno group attached to pyrimidene moiety are responsible for these activities.

Keywords: Cetylpyridinium chloride, benzochromenopyrimidinones, multicomponent reactions, antioxidant, cytotoxicity

Development of newer methods for the synthesis of biologically active compounds and catalysis in water medium is an intense area of research[1-4]. One of the drawbacks of water as solvent is the low solubility of most organic substrate in this medium. This can be overcome by using organic solvent as co-solvent or surfactants which can solubilize organic solvents under miceller condition[5-7]. The role of surfactants in organic reactions may be explained that it is possible to make the insoluble and less soluble compounds soluble in aqueous water by penetrating through the hydrophobic core of micelles. It is suggested that the reagent having either polar or non-polar counterparts can be incorporated in micelles making it easier to solubilize. The solubilising capacity can also be increased by using co-solvents and even with variable temperature conditions. In micellar solution, the organic substrates solvated in the interior lipophilicity (hydrophobicity) of micelles created by the surfactants, and thereby facilitates a smooth reaction[8]. Increased reaction rates are usually observed when organic reactions are performed in aqueous solutions in presence of surfactants[9].

Benzochromenopyrimidines are biologically important compounds with anti-microbial[10-15], anti-convulsant activities[16] and are used as antagonists for Neuropeptide S receptor (Figure 1)[17]. Recently, racemic benzochromenopyrimidinones are reported as non-hepatotoxic, acetylcholinesterase inhibitors with anti-oxidative properties (Figure 2)[18].

Although the benzochromenopyrimidinones are biologically important compounds, very few literatures are reported for the synthesis of these compounds. It was reported that the sulfonic acid-functionalized nanoparticles could be used as catalysts for the synthesis of 5-aryl-1*H*-benzo [f]chromeno[2,3-d]pyrimidine-2,4(3*H*,5*H*)-dione derivatives in terms of activity and recyclability[19]. Syntheses of naphthopyranopyrimidine derivatives by three component cyclocondensation of *β*-naphthol, aldehydes, and cyclic 1,3-dicarbonyl compounds using various catalysts such as $ZnAl_2O_4$ nanoparticles[20], $ZrOCl_2$/nano-TiO_2[21], Iodine[22], alum $KAl(SO_4)_2$. $12H_2O$[23] and $InCl_3$ or P_2O_5[24] were recently reported. One-pot, three-component synthesis of naphthopyranopyrimidines was also reported from *β*-naphthol, aldehydes, and 6-amino-1,3-dimethyluracil

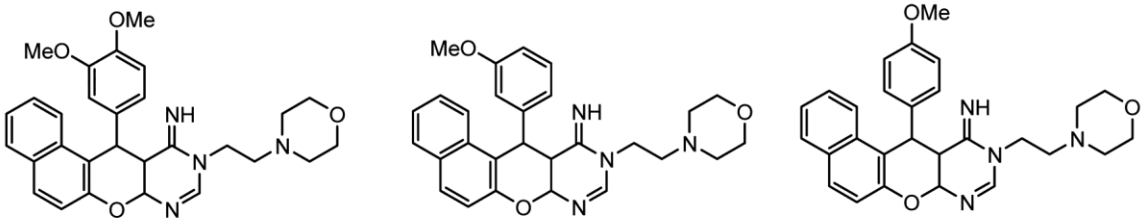


Figure 1 — Antagonists for neuropeptide S-receptor

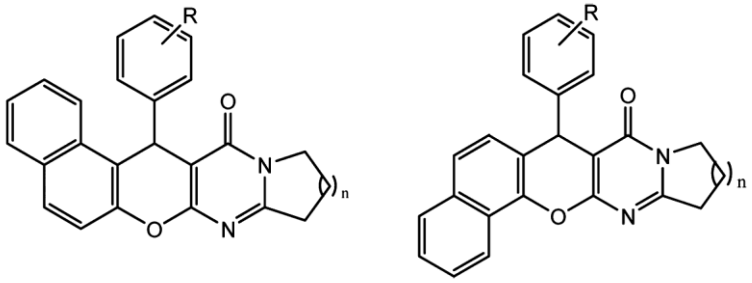


Figure 2 — Cholinesterase inhibitors

using various catalysts such as $InCl_3$[25], silicotungstic acid ($H_4[SiW_{12}O_{40}]$)[26], $Al(H_2PO_4)_3$[27] and proline[28] under solvent-free conditions. However, these methods used for the synthesis of benzochromenopyrimidine derivatives were accomplished with expensive catalysts, complex, strong acids, long reaction time and of moderate yields. Moreover, the synthesis of 2-thioxo-5-aryl-benzo[f]chromeno[2,3-*d*]pyrimidin-4-one derivatives is not known to the best of our knowledge as reported syntheses were only for benzochromenopyrimidines and benzochromenopyrimidinones.

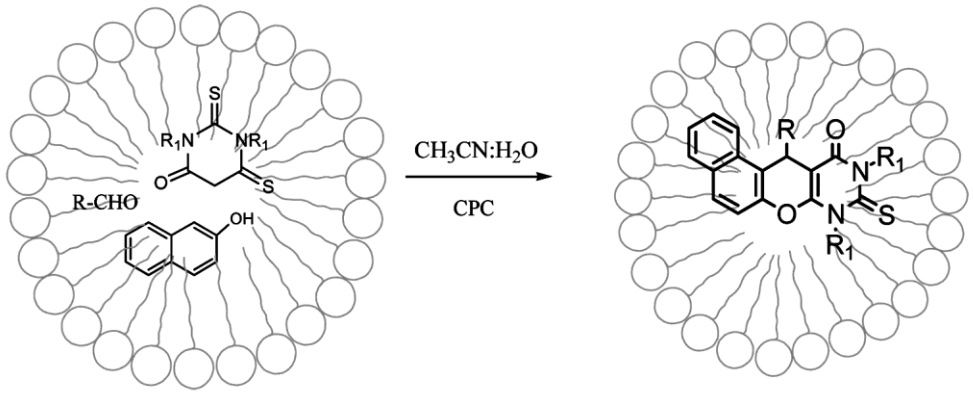
In continuation of our work on the development of newer methods for the synthesis of heterocyclic compounds[29-32] and fused heterocyclic compounds[33-35], herein we are reporting the surfactant miceller catalyzed synthesis of novel 2-thioxo-benzochromenopyrimidine-4-ones by one-pot three-component reaction of thiobarbituric acids, aldehydes and β-napthol in aqueous media. The methodology adopted for the synthesis of 2-thioxo-benzochromenopyrimidine-4-ones offers several advantages such as use of environmentally benign non-toxic catalyst, efficient, short reaction time, simple experimental and purification procedure, and excellent yields. Furthermore, the anti-oxidant properties and the cytotoxicity of the synthesized compounds were determined. It was found that the pyrimidine nucleus containing thiouriedo linkage (-NH-C(S)-NH-) is pharmaceutically important because the development of medicine mainly arose from the heterocyclic compounds containing nitrogen and sulphur atoms. Thus, for the first time the catalytic effect of surfactant in the synthesis of 2-thioxo-benzochromenopyrimidine-4-ones is described.

Results and Discussion

Chemistry

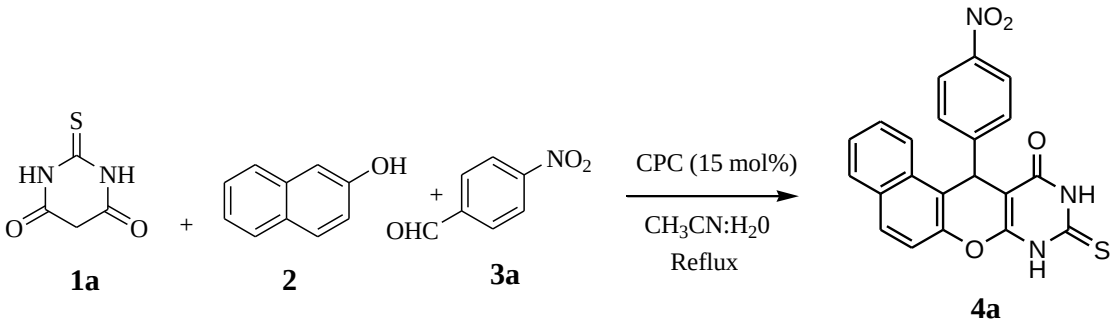
Initial attempt for optimisation towards the synthesis of 2-thioxo-benzochromenopyrimidinone derivatives (**4**) was carried out using one pot multi-component reaction of 2-thiobarbituric acid (**1a**), *p*-nitrobenzaldehyde (**2a**) and β-napthol (**3**) under micellar condition (Scheme I).

First, the reaction was stirred at room temperature and in refluxing condition in water without any catalyst to establish the real efficiency of catalysis, it was found that no desired product was obtained (Table I, entries 1 and 2). The model reaction was again carried out at room temperature using water:acetonitrile (1:1) as solvent, even in this condition, no desired product was formed (Table I, entry 3). The reaction was then optimized with different surfactants as catalyst, so as to help in reducing the reaction time and improved yield of the target product. The less expensive and easily available anionic and cationic surfactants, such as, sodium



Scheme I — Synthesis of 2-thioxo-5-aryl-2,3-dihydro-1*H*-benzo[6,7]chromeno[2,3-*d*]pyrimidin-4(5*H*)-one **4**

Table I — Optimization of reaction condition



1a **2** **3a** **4a**

Entry	Surfactant (mol%)	Solvent	Temperature (°C)	Time (h)	Yield of **4a** (%)
1	No catalyst	H_2O	RT[a]	48	NP[b]
2	No catalyst	H_2O	reflux	48	NP[b]
3	No catalyst	CH_3CN: H_2O	reflux	48	NP[b]
4	SDS (15)	H_2O	RT[a]	48	<10
5	SDS (15)	H_2O	reflux	48	32
6	TTAB (15)	H_2O	reflux	48	40
7	CTAB (15)	H_2O	reflux	48	35
8	CPC (15)	H_2O	reflux	48	53
9	TEAB (15)	H_2O	reflux	48	45
10	SDS (15)	CH_3CN: H_2O	RT[a]	48	46
11	SDS (15)	CH_3CN: H_2O	80°C	48	52
12	SDS (15)	CH_3CN: H_2O	reflux	24	65
13	TTAB (15)	CH_3CN: H_2O	reflux	12	80
14	CTAB (15)	CH_3CN: H_2O	reflux	12	85
15	CPC (15)	CH_3CN: H_2O	reflux	8	94
16	TEAB (15)	CH_3CN: H_2O	reflux	12	85
17	CPC (20)	CH_3CN: H_2O	reflux	12	88
18	CPC (10)	CH_3CN: H_2O	reflux	12	82

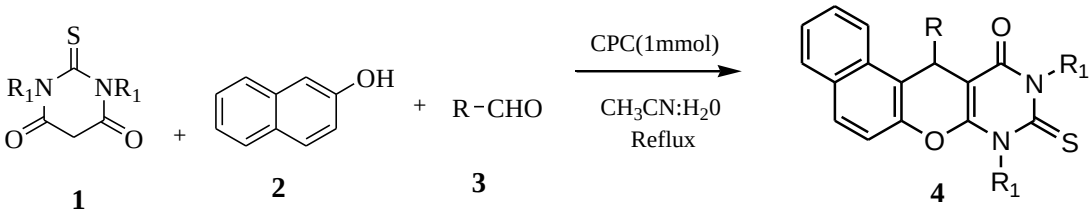
[a] Room temperature (RT) was 40°C; [b] No desired product (NP)

dodecyl sulphate (SDS), cetyl trimethyl ammonium bromide (CTAB), cetyl pyridinium chloride (CPC), tetradecyl trimethyl ammonium bromide (TTAB), tetra ethyl ammonium bromide (TEAB), etc. were employed as catalysts (15 mol%) for this reaction. After screening several catalysts in water, it can be noted that the reaction gave the product in low yields (Table I, entries 4-9). The improvement of the yield can be seen when water with medium polarity solvent acetonitrile is used in 1:1 volume. The reason for this may be attributed to the enhance solubility of substrates by acetonitrile.

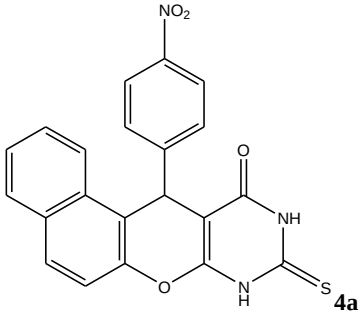
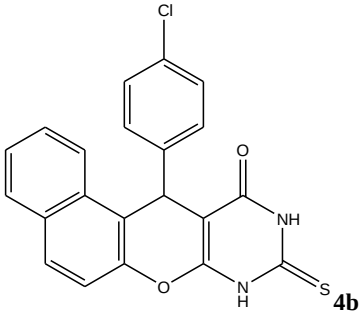
To acquire the best effect of reaction, a number of surfactants in water: acetonitrile solvent was tried by refluxing (Table I). The longer time of reaction with lesser yields were found when surfactants, like SDS, TTAB, CTAB, TEAB (Table I, entries 12, 13, 14, 16) were used. It may be noted that the anionic surfactant SDS either at ambient temperature or under refluxing condition gave the desired product in low yields, 46%, 52% and 65%, respectively (Table I, entries 10, 11 and 12). It was observed that among the cationic surfactants, CPC was found to be the efficient catalyst to yield the desired product in high 94% yield (Table I, entry 15). The effect of amount of the catalyst loading was also evaluated in model reaction by refluxing in water:acetonitrile solvent. It was shown that 15 mol% of the catalyst was the best choice for the completion of reaction. When the amount of the catalyst was increased to 20 mol%, it was found that there was no effect on the yield as well as on the duration of the reaction (Table I, entry 17). Again, on reducing the amount of the catalyst (10 mol%), it gave lesser yield of the desired product (Table I, entry 18).

To expand the scope and versatility of the reaction under the optimized condition, reactions were performed using various aryl aldehydes to give the products in good to excellent yields (Table II). It may be noted that the reaction when carried out with bulky groups has no effect on the steric hinderance of the product (**4c**, 4**d**). In addition, when N,N-dimethylbenzaldehyde and 2,5-dimethoxybenzaldehyde, that is, in presence of strong electron donating groups, the reactions were tolerated and gave the yields in 85% and 80%, respectively (Table II, entries 5 and 8). Interestingly, when aliphatic aldehydes, such as, butyaldehyde was used, the

Table II — Reaction of barbituric acid **1** with aldehydes **3** to give the products, **4**

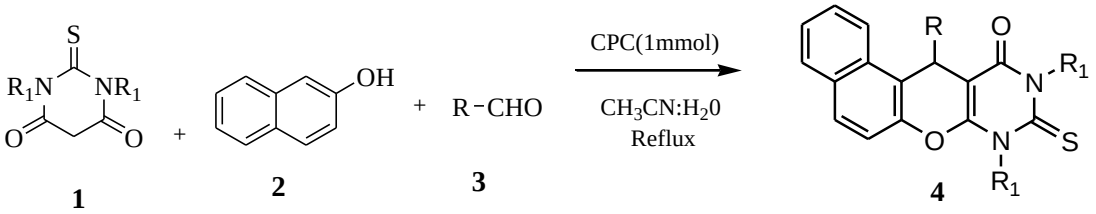


1 **2** **3** **4**

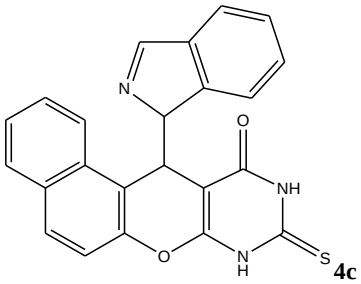
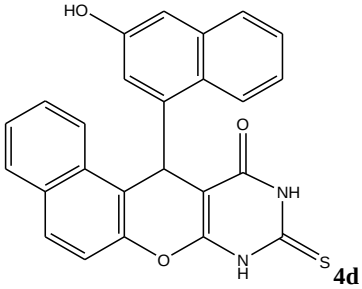
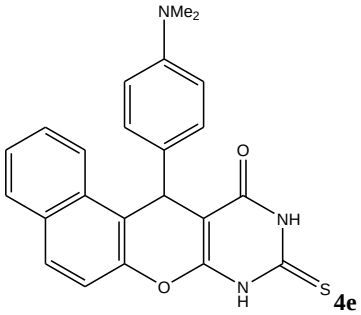
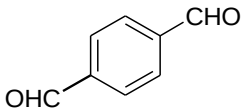
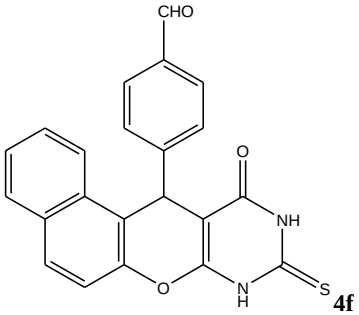
Entry	**1**	**3**	**4**	Time (h)	Yield (%)	m.p. (°C)
1	**1a**, R_1 = H			8	85	207-210
		3a	 **4a**			
2	**1b**, R_1 = H			10	80	198-203
		3b	 **4b**			

(*Contd*.)

Table II — Reaction of barbituric acid **1** with aldehydes **3** to give the products, **4** *(Contd.)*

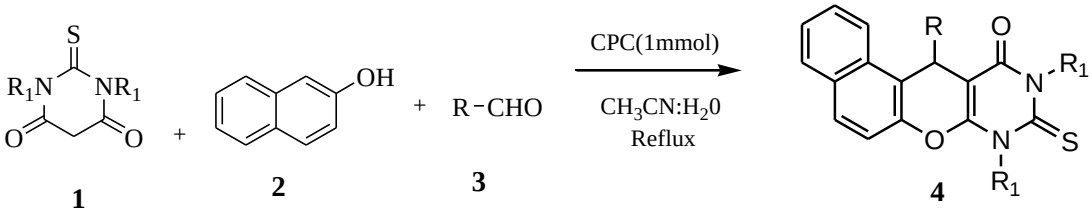


1 **2** **3** **4**

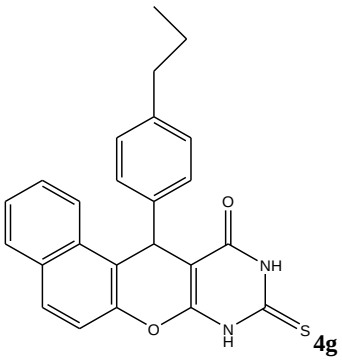
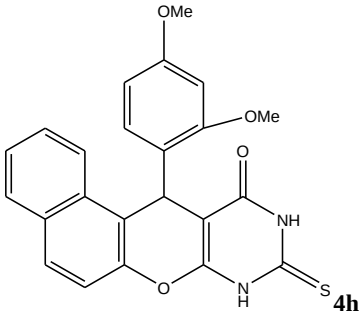
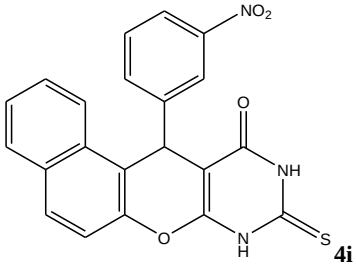
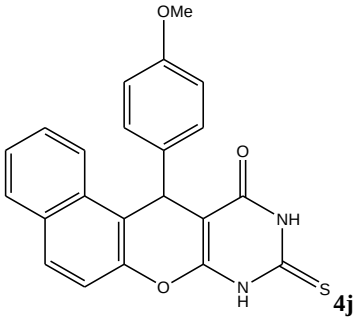
Entry	**1**	**3**	**4**	Time (h)	Yield (%)	m.p. (°C)
3	**1c**, R_1 = H	**3c**	 **4c**	4	93	188-191
4	**1d**, R_1 = H	**3d**	 **4d**	3	85	195-197
5	**1e**, R_1 = H	**3e**	 **4e**	4	85	205-208
6	**1f**, R_1 = H	**3f** 	 **4f**	4	92	201-204

(*Contd.*)

Table II — Reaction of barbituric acid **1** with aldehydes **3** to give the products, **4** *(Contd.)*

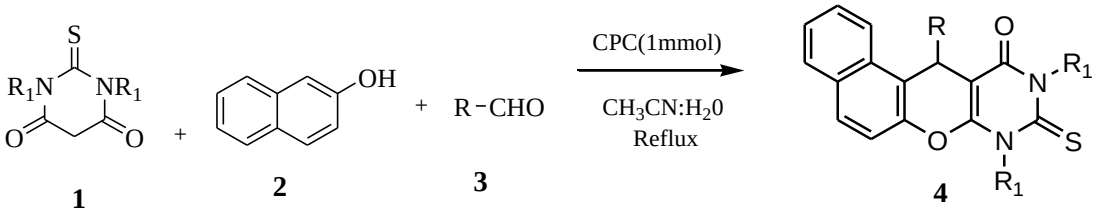


1 **2** **3** **4**

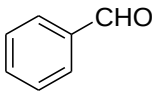
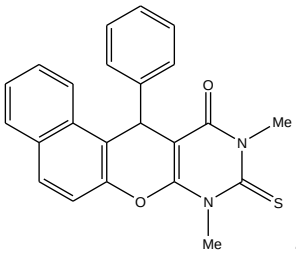
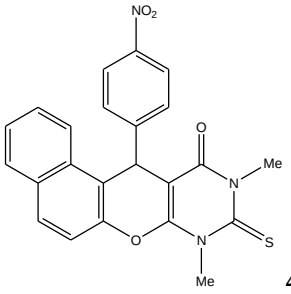
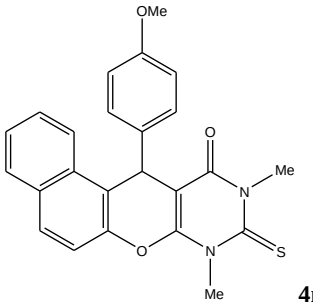
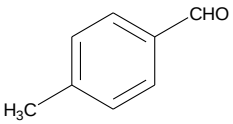
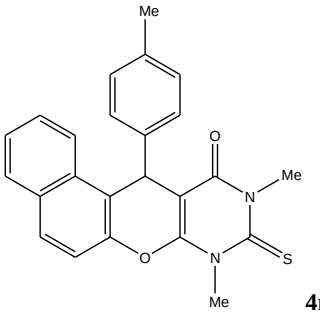
Entry	**1**	**3**	**4**	Time (h)	Yield (%)	m.p. (°C)
7	**1g**, R_1 = H	**3g**	 **4g**	5	75	188-191
8	**1h**, R_1 = H	**3h**	 **4h**	4	80	185-187
9	**1i**, R_1 = H	**3i**	 **4i**	4	95	205-209
10	**1j**, R_1 = H	**3j**	 **4j**	4	90	200-203

(*Contd.*)

Table II — Reaction of barbituric acid **1** with aldehydes **3** to give the products, **4** *(Contd.)*

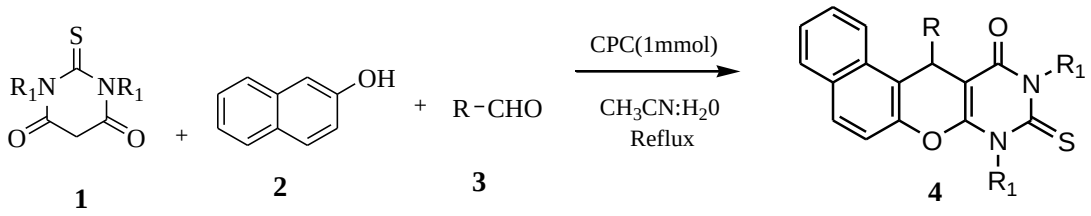


1 **2** **3** **4**

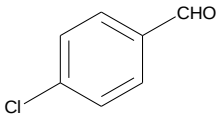
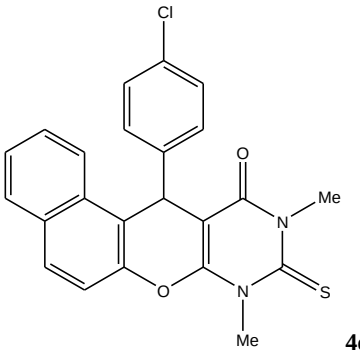
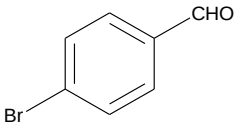
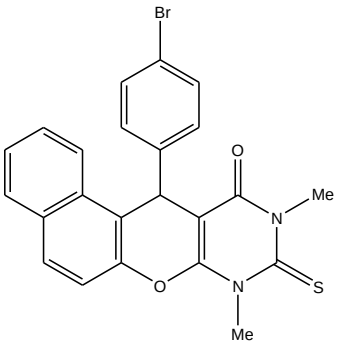
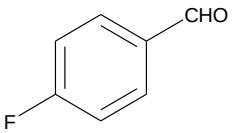
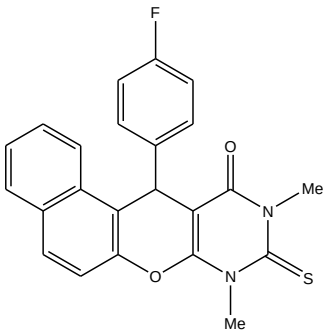
Entry	1	3	4	Time (h)	Yield (%)	m.p. (°C)
11	**1k**, $R_1 = CH_3$	 **3k**	 **4k**	9	90	215-217
12	**1l**, $R_1 = CH_3$	**3a**	 **4l**	8	92	
13	**1m**, $R_1 = CH_3$	**3j**	 **4m**	12	90	257-259
14	**1n**, $R_1 = CH_3$	 **3l**	 **4n**	11	92	

(*Contd*.)

Table II — Reaction of barbituric acid **1** with aldehydes **3** to give the products, **4** *(Contd.)*

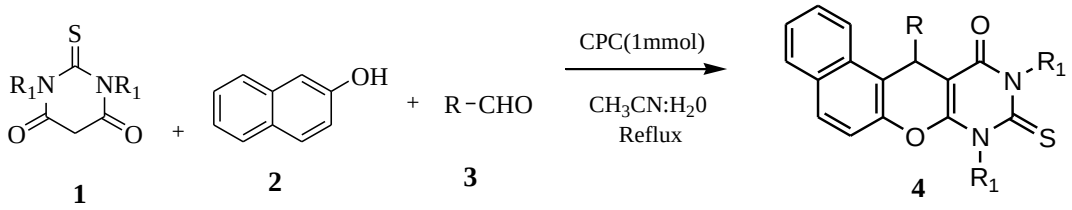


1 **2** **3** **4**

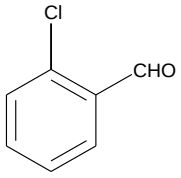
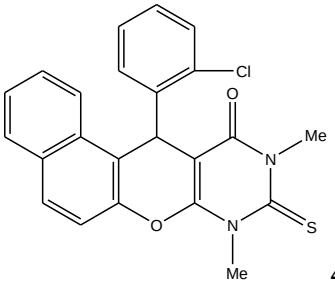
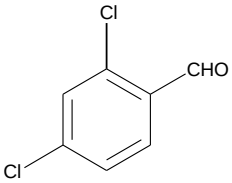
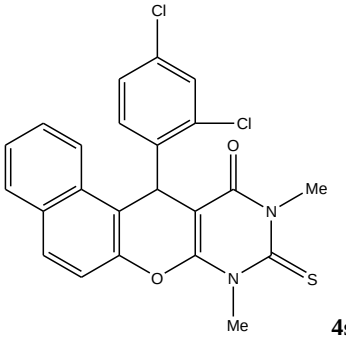
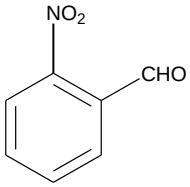
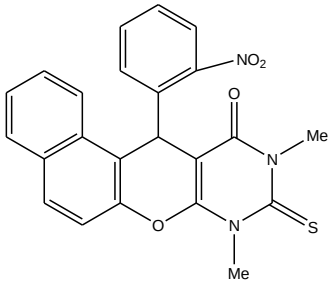
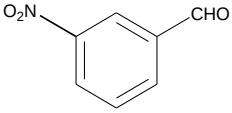
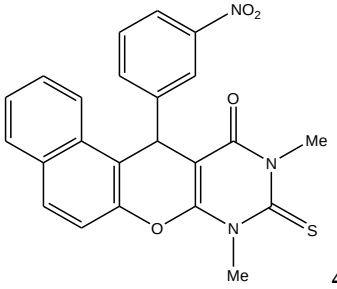
Entry	**1**	**3**	**4**	Time (h)	Yield (%)	m.p. (°C)
15	**1o**, $R_1 = CH_3$	 **3b**	 **4o**	9	90	
16	**1p**, $R_1 = CH_3$	 **3m**	 **4p**	8	89	250-252
17	**1q**, $R_1 = CH_3$	 **3o**	 **4q**	6	90	

(*Contd.*)

Table II — Reaction of barbituric acid **1** with aldehydes **3** to give the products, **4** *(Contd.)*

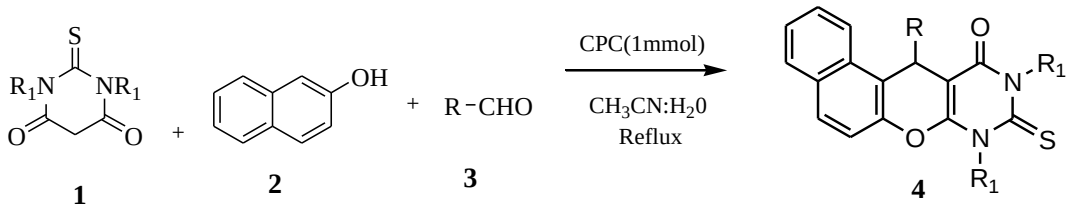


1 **2** **3** **4**

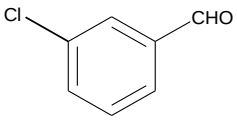
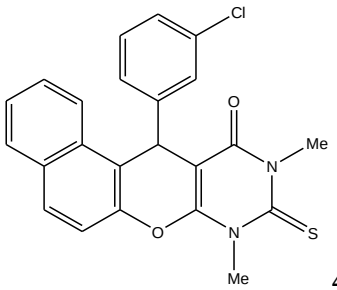
Entry	**1**	**3**	**4**	Time (h)	Yield (%)	m.p. (°C)
18	**1r**, $R_1 = CH_3$	 **3p**	 **4r**	7	91	
19	**1s**, $R_1 = CH_3$	 **3q**	 **4s**	5	86	
20	**1t**, $R_1 = CH_3$	 **3r**	 **4t**	7	88	
21	**1u**, $R_1 = CH_3$	 **3i**	 **4u**	8	92	309-312

(*Contd.*)

Table II — Reaction of barbituric acid **1** with aldehydes **3** to give the products, **4** *(Contd.)*



1 **2** **3** **4**

Entry	**1**	**3**	**4**	Time (h)	Yield (%)	m.p. (°C)
22	**1v**, $R_1 = CH_3$	 **3s**	 **4v**	9	91	250-252

desired product was formed but in lesser yield (Table II, entry 7, **4g**).

A plausible mechanism for the synthesis of 2-thioxo-5-aryl-2,3-dihydro-1*H*-benzo[6,7]chromeno [2,3-*d*]pyrimidin-4(5*H*)-one (**4**) from 2-thiobarbituric acid (**1**), aldehyde (**3**) and β-napthol (**2**) under micellar condition is shown in Scheme II.

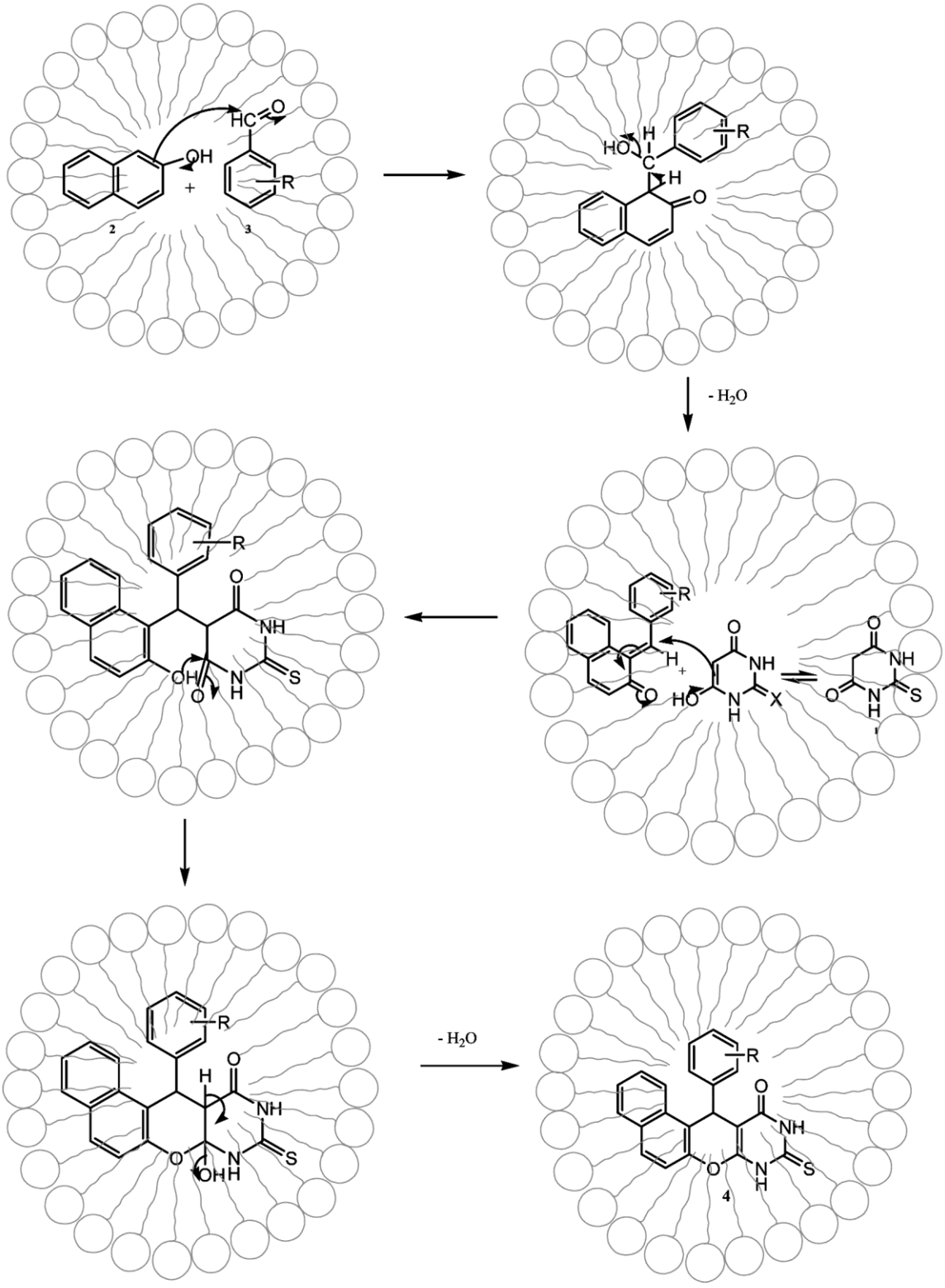
Antioxidant activity

Antioxidants, free radical scavengers, are thought to have a role in the prevention of cellular damage - the common pathway for cancer, aging, and a variety of diseases. Many heterocyclic compounds containing free radical scavengers are gaining importance for the treatment of such diseases. The primary anti-oxidative activity can be evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The free radical scavenging activity of the compound was evaluated for hydrogen ion donating or free radical scavenging ability using the stable free radical DPPH method of Lee *et al*.[36] with some modifications. The methanolic solution of DPPH was rapidly mixed with the methanolic sample and the absorbance at 515 nm was measured after 5 mins. BHT (Butylated hydroxy toluene) solution was used as a reference corresponding to 100% radical scavenging activity.

It was found that 2-thioxo-5-aryl-2,3-dihydro-1*H*-benzo[6,7]chromeno[2,3-*d*]pyrimidin-4(5*H*)-ones (**4a-4o**) reduced DPPH free radicals in a concentration dependent manner. The scavenging activities of these compounds are shown in Table III. The results showed that compound **4a** (78.3%) having highest scavenging activity value followed by **4b** (66.4%), **4l** (64.1%), and **4o** (62.5%) are more active; and **4d** (54.1%), **4f** (43.3%), **4i** (42.2) are as active as the positive control, **4c** (30.8%), **4k** (24.7%), **4e** (16.1%), **4m** (8.4%), **4n** (7.7%), **4g** (4.9%), **4h** (2.1%) and **4j** (1.6%) showed very less activity. The mechanism of reaction between antioxidants and DPPH is dependent upon the structural conformation of the antioxidants which could be attributed to their hydrogen donating ability.

Cytotoxicity

The anti-tumour activities of the target compounds against human hepatoma cell line (HepG2) were determined using cytotoxicity assay and were assessed in comparison to Taxol on the basis of monitoring the inhibition of the growth of human cancer cells by using MTT assay[37,38]. MTT assay is a rapid and high accuracy colorimetric approach that widely used to determine cell growth and cell cytotoxicity, particularly in the development of new drug. It measures cell membrane integrity by determining mitochondrial activity through enzymatic reaction on the reduction of MTT to formazan. Synthesized compounds (**4a**-**4o**) were subjected to a screening system for investigation of their antitumor potency against liver (HepG2) cell line. The concentrations that induce 50% inhibition of cell growth (IC_{50}) in mM are reported in Table IV. The antitumor activity results indicated that most of the



Scheme II — Plausible mechanism for the synthesis of 2-thioxo-5-aryl-2,3-dihydro-1*H*-benzo[6,7]chromeno[2,3-*d*]pyrimidin-4(5*H*)-one **4**

Table III — DPPH free radical scavenging activity of different concentrations of **4**

Sl. No.	Compd	Radical scavenging activity[a] (%)
1	**4a**	78.3
2	**4b**	66.4
3	**4c**	30.8
4	**4d**	54.1
5	**4e**	16.1
6	**4f**	43.3
7	**4g**	4.9
8	**4h**	2.1
9	**4i**	42.2
10	**4j**	1.6
11	**4k**	24.7
12	**4l**	64.1
13	**4m**	8.4
14	**4n**	7.7
15	**4o**	62.5

[a] All analyses were performed in triplicate and the data were recorded as mean

Table IV — *In vitro* antitumor activity of the synthesized compounds

Compd	Viability %						IC_{50} (μg/mL)[a]
	Sample concentration (μg m^{-1})						
	50	25	12.5	6.25	3.12	1.56	
4a	22.40	67.74	86.99	97.83	100.0	100.0	17.4
4b	27.81	84.66	93.29	99.12	100.0	100.0	23.6
4c	28.72	46.99	73.91	89.63	98.12	100.0	34.8
4d	26.60	71.09	91.41	98.18	100.0	100.0	36.5
4e	19.87	69.49	84.74	91.09	98.46	100.0	34.8
4f	27.81	84.66	93.29	99.12	100.0	100.0	40.2
4g	15.83	62.88	79.62	87.44	96.92	100.0	31.8
4h	28.33	72.31	81.54	95.86	100.0	100.0	37.7
4i	26.60	71.09	91.41	98.18	100.0	100.0	36.9
4j	45.77	71.28	86.92	97.24	100.0	100.0	45.9
4k	28.82	77.60	91.30	98.11	100.0	100.0	38.3
4l	31.85	76.16	89.79	96.84	100.0	100.0	29.8
4m	26.43	73.86	87.43	96.07	100.0	100.0	37.6
4n	18.38	68.45	77.82	90.53	98.51	100.0	35.7
4o	27.43	79.04	92.27	98.05	100.00	100.0	24.2
Taxol[b]	10.95	14.29	16.90	21.03	30.32	100.0	1.2

[a]IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.
[b]Positive control. Experiments were performed in triplicate.

compounds showed inhibition activity against the tested cell line but varying intensity extents in comparison to the known anticancer drug (Taxol). Compounds **4a**, **4b** and **4o** showed significant *in vitro* antitumor activity (IC_{50}, 17.4 μg/mL, 23.6 μg/mL, and 24.2 μg/mL), and thus, the presence of thiouredo moiety and fusion of chromeno-ring in pyrano-pyrimido structures enhance the antitumor activity.

Experimental Section

The melting points were determined on a Buchi M-560 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on Shimadzu FT-IR spectrophotometer in the range of 200 cm^{-1} to 4000 cm^{-1}. All the samples were run on a sodium chloride plate as a liquid film. Absorption maxima were recorded in wave numbers (cm^{-1}). Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on BRUCKER–ACF-300 (300 MHz). Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on BRUCKER-ACF-300 (75 MHz). All chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (TMS), reference to the chemical shifts of residual solvent resonances (^{1}H and ^{13}C NMR). Coupling constants are given in Hz. All samples are run in deutero-chloroform ($CDCl_3$) and DMSO. The FAB mass spectra were recorded at 6000 Mass Spectrometed data systems using Argon/Xenon (6KV, 10mA) as the FAB gas. The accelerating voltage was 10 KV and the spectra were recorded at room temperature. All the commercial chemicals were distilled before use.

General procedure for the synthesis of 2-thioxo-bezochromenopyrimidin-4-ones, 4a-j

In a typical experiment, *p*-nitrobenzaldehyde **3a** (1.0 mmol), β-napthol **2** (0.144g, 1.0 mmol), thiobarbituric acid **1a** (1.0 mmol) and CPC (0.015 mmol %) were taken in a round bottom flask using water (2.5 mL) and acetonitrile (2.5 mL) as solvent in 1:1 ratio. The reaction mixture was refluxed and the progress of the reaction was monitored by TLC. After completion of reaction (8 h), the solid obtained was collected by filtration and washed successively with water (3 × 10 mL) and with acetonitrile (3 × 5 mL). The crude product was recrystallized from ethanol to afford the pure compound **4a** which required no further purification.

2-Thioxo-5-(4-nitrophenyl)-benzo[f]chromeno [2,3-d]pyrimidin-4-one, 4a: Yellow solid; m.p. 207-210°C; IR (KBr): ν_{max} 3541, 1651, 1537, 1445, 1350, 1198, 1132, 1015, 849 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): ∂ 5.70 (s, 1H), 6.72 (m, 1H), 6.85 (m, 1H), 7.01 (d, 2H, *J*=7.6 Hz), 7.24 (m, 4H), 7.67 (d, 2H, *J*=7.6 Hz), 10.90 (br s, 1H), 11.20 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 173.6, 152.9, 145.7, 128.3, 123.5, 95.6, 31.6; Anal. Calcd. for $C_{21}H_{13}N_3O_4S$: C 65.12, H 3.38, N 10.85; Found: C 64.83, H 3.64, N 10.54.

2-Thioxo-5-(4-chlorophenyl)-benzo[f]chromeno [2,3-d]pyrimidin-4-one, 4b: White solid; m.p: 198-203°C; IR (KBr): ν_{max} 3524, 1660, 1537, 1443, 1359, 1201, 1134, 1013, 866 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): ∂ 5.80 (s, 1H), 6.97-7.22 (m, 10H), 11.32 (br s, 1H), 11.69 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂173.4, 164.0, 163.0, 142.7, 129.8, 128.9, 128.0, 96.0, 30.6; HRMS (EI) cald. 393.0464; found 393.0478. Anal. Calcd. for $C_{21}H_{13}ClN_2O_2S$: C 65.12, H 3.38, N 10.85; Found: C 64.83, H 3.64, N 10.54.

2-Thioxo-5-(1H-indol-3-yl)-benzo[f]chromeno [2,3-d]pyrimidin-4-one, 4c: White solid; m.p: 188-192°C; IR (KBr): ν_{max} 3538, 1655, 1528, 1377, 1277, 1213, 1157 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): ∂ 5.80 (s, 1H), 6.97-7.22 (m, 10H), 12.28 (br s, 1H), 12.32 (br s, 1H), 13.01 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 178.2, 163.3, 161.5, 145.1, 141.5, 137.1,129.5, 124.5, 123.6, 118.4, 113.9, 112.9, 109.2, 29.5; HRMS (EI) cald for $C_{21}H_{13}ClN_2O_2S$: 304.3127; found 304.3238.

2-Thioxo-5-(3-hydroxynaphthalen-1-yl)-benzo[f] chromeno[2,3-d]pyrimidin-4-one, 4d: White solid; m.p: 195-198^{0}C; IR (KBr): ν_{max} 3452, 3032, 1678, 1564, 1458, 1211, 1138, 814 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): ∂ 5.56 (s, 1H), 7.15 (m, 2H), 7.22 (m, 2H), 7.55 (m, 2H), 7.60 (m, 2H), 7.88 (m, 2H), 8.22 (m, 2H), 11.55 (br s, 1H), 12.20 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 174.9, 173.5, 160.3, 156. 8, 148.3, 131.4, 129.8, 128.0, 126.6, 126.5, 123.1, 119.1, 109.1, 43.2, 29.6; HRMS (EI) cald for $C_{25}H_{18}N_2O_3S$: 426.1038; found 426.4870.

2-Thioxo-5-(4-N,N-dimethylanilino)-benzo[f] chromeno[2,3-d]pyrimidin-4-one, 4e: White solid; m.p: 205-208^{0}C; IR (KBr): ν_{max} 3457, 3119, 1634, 1537, 1495, 1373, 1194, 1142, 1011 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): ∂ 3.00 (s, 3H), 3.02 (s, 3H), 5.90 (s, 1H), 6.75 (m, 2H), 7.22 (m, 2H), 7.40 (m, 2H), 8.22 (m, 2H), 8.48 (m, 2H), 11.98 (br s, 1H), 12.05 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 178.0, 173.5, 163.5, 161.0, 156.7, 155.3, 140.3, 128.6, 120.9, 112.0, 109.8, 95.9, 46.1, 30.9; HRMS (EI) cald for $C_{23}H_{19}N_3O_2S$: 401.1198; found 401.4809.

2-Thioxo-5-(4-carbaldehydophenyl)-benzo[f] chromeno[2,3-d]pyrimidin-4-one, 4f: White solid; m.p: 201-203^{0}C; IR (KBr): ν_{max} 3522, 3148, 1670, 1574, 1518, 1433, 1298, 1207, 1148 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): ∂ 4.50 (s, 1H), 5.90 (s, 1H), 7.12-8.25 (m, 10H), 11.62 (br s, 1H), 12.25 (br s, 1H), 12.42 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 178.4, 173.5, 162.5, 145.2, 132.7, 128.6, 61.3, 29.6; HRMS (EI) cald for $C_{22}H_{14}N_2O_3S$: 386.4232; found 386.0725.

2-Thioxo-5-propyl-benzo[f]chromeno[2,3-d] pyrimidin-4-one, 4g: White solid; m.p: 188-192^{0}C; IR (KBr): ν_{max} 3178, 1686, 1582, 1522, 1316, 1165 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): ∂ 0.82 (m, 3H), 1.18 (m, 2H), 2.28 (m, 2H), 5.78 (m, 1H), 7.12 (m, 2H), 7.32-7.45 (m, 2H), 7.65-7.72 (m, 2H), 11.98 (br s, 1H), 12.05 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 178.2, 157.3, 145.5, 135.3, 129.8, 128.6, 128.0, 126.6, 126.5, 123.1, 119.1, 109.1, 31.8, 29.6, 22.4, 14.5; HRMS (EI) cald for $C_{18}H_{16}N_3O_2S$: 324.0932; found 324.3968.

2-Thioxo-5-(2,4-dimethoxyphenyl)-benzo[f] chromeno[2,3-d]pyrimidin-4-one, 4h: White solid; m.p: 185-187^{0}C; IR (KBr): ν_{max} 3181, 1688, 1576, 1543, 1495, 1379, 1260, 1175, 1034, 949 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): ∂ 3.73 (s, 3H), 3.85 (s, 3H), 4.56 (s, 1H), 7.08-7.23 (m, 5H), 7.40-8.13 (m, 4H), 12.34 (br s, 1H), 12.48 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 178.5, 161.7, 159.4, 155.2, 154.1, 150.1, 145.4, 129.2, 128.0, 126.1, 125.9, 122.6, 121.6, 121.2, 118.4, 117.0, 56.3, 55.5, 28.8; HRMS (EI) cald for $C_{23}H_{20}N_2O_4S$: 420.4809; found 420.1144.

2-Thioxo-5-(3-nitrophenyl)-benzo[f]chromeno [2,3-d]pyrimidin-4-one, 4i: White solid; m.p: 185-187^{0}C; IR (KBr): ν_{max} 3181, 1688, 1576, 1543, 1495, 1379, 1260, 1175, 1034, 949 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): ∂ 5.75 (s, 1H), 7.38-7.43 (m, 4H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.88-8.07 (m, 4H), 8.17 (d, *J* = 9.2 Hz, 1H), 12.14 (br s, 1H), 12.38 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 173.1, 163.8, 153.6, 148.7, 138.4, 133.8, 131.3, 125.6, 123.1, 116.9, 89.4, 33.8; Anal. Calcd. for $C_{21}H_{13}N_3O_4S$: C 65.12, H 3.38, N 10.85; Found: C 65.04, H 3.63, N 10.26.

2-Thioxo-5-(4-methoxyphenyl)-benzo[f]chromeno [2,3-d]pyrimidin-4-one, 4j: White solid; m.p: 185-187^{0}C; IR (KBr): ν_{max} 3181, 1688, 1576, 1543, 1495, 1379, 1260, 1175, 1034, 949 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): ∂ 3.63 (s, 3H), 5.56 (s, 1H), 6.82 (d, *J* = 8.2 Hz, 2H), 7.11-7.26 (m, 4H), 7.32-7.88 (m, 4H), 11.34 (br s, 1H), 12.18 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 177.8, 164.3, 158.2, 153.8, 150.4, 146.7, 132.2, 131.0, 129.8, 129.2, 127.6, 125.8, 124.2, 118.7, 114.5, 89.6, 56.3, 55.6, 33.8; Anal. Calcd. for $C_{22}H_{16}N_2O_3S$: C 65.12, H 3.38, N 10.85; Found: C 65.04, H 3.63, N 10.26.

1,3-Dimethyl-2-thioxo-5-phenyl-benzo[f]chromeno [2,3-d]pyrimidin-4-one, 4k: White solid; m.p: 215-217^{0}C; IR (KBr): ν_{max} 3178, 1686, 1582, 1522, 1316, 1165 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): ∂ 0.82 (m, 3H), 1.18 (m, 2H), 2.28 (m, 2H), 5.78 (m, 1H), 7.12 (m, 2H), 7.32-7.45 (m, 2H), 7.65-7.72 (m, 2H), 11.98 (br s, 1H), 12.05 (br s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): ∂ 178.2, 157.3, 145.5, 135.3, 129.8, 128.6, 128.0, 126.6, 126.5, 123.1, 119.1, 109.1, 31.8, 29.6, 22.4, 14.5; HRMS (EI) cald for $C_{18}H_{16}N_3O_2S$: 324.0932; found 324.3968.

Antioxidant activity

Aliquots (50 mL) of the extract dilution at a concentration range of 0.1– 2 mg/mL were mixed with 450 mL Tris–HCl buffer (pH = 7.4) and 1 mL of the methanolic DPPH solution (0.1 mM). The mixtures were left for 30 min at room temperature in the dark and the absorbance at 517 nm was measured using MeOH as blank. Extract concentration providing 50% inhibition (IC_{50}) was calculated using the graph by plotting inhibition percentage against extract concentration. Synthetic antioxidant reagent butylated hyroxytoluene (BHT) was used as a positive control. The measurements were performed in triplicate and the results were averaged. Radical scavenging activity was expressed as percentage inhibition of DPPH radical and was calculated by the following equation:

$$\text{DPPH Scavenged (\%)} = [(A_{cont} - A_{test})/ A_{cont}] \times 100$$

where, A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the compounds.

Cytotoxic activity

Cell Line Culture

Human ovarian carcinoma cell line, A2780 was obtained from the European Collection of Cell Culture (ECACC). Cells were cultured in RPMI 1640 media supplemented with 10% foetal bovine serum, glutamine (2mM) and 1 % penicillin-streptomycin in static 75 cm^2 TFlask (GIBCO, USA). The cells were incubated in a humidified atmosphere with 5 % CO_2 at 37^{o}C.

Cell Cytotoxicity Assay

Cells were plated in a 96-well-plate with 1 X 105 cells/well of concentration. The cells were left to adhere for 48 hours before exposed to the plant extracts (0-1000 μg/ml) administered in media containing 1% of FBS and returned to the incubator for 48 hrs. Subsequently, 3-(4,5-dimethylthaizol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent (0.5 mg/mL in sterile Phosphate buffer salin, PBS) was added directly to the wells. Cells were returned to the incubator for 4 hrs. The formation of insoluble purple formazan from yellowish MTT by enzymatic reduction was dissolved in DMSO after removal of supernatant. The optical density of solution was measured at 590 nm using a microplate reader (ELx808, BioTek, USA).

Cell Viability Assay

After treatment with the synthesized compounds, the cells were pooled together and the remaining attached cells were detached from the culture plates by exposure to trypsin-EDTA. The resultant cells were then stained with trypan blue at the concentration of 0.2%. Then, the trypan blue-excluded viable cells were counted using a hemacytometer (FORTUNA® GERMANY) under microscope.

Conclusion

In conclusion, an efficient, simple way of synthesizing the substituted 2-thioxo-benzochromeno [2,3-d]pyrimidin-4-one derivatives in good to excellent yields was developed. Here, for the first time, the use of CPC as a catalyst for the synthesis of a series of novel 2-thioxo-bezochromenopyrimidin-4-one derivatives as one pot multi component reaction has been described without using any harmful and toxic organic solvents. It was found that the compounds having electron withdrawing group in the ring (**4a**, **4b**, **4l** and **4o**) showed high antioxidant activities. The cytotoxic activity results indicated that most of the tested compounds (**4a**, **4b** and **4o**) showed anti-tumour activity against the tested cell line. Thus the study of the antioxidant and cytotoxic activities revealed that all the tested compounds showed moderate to good activities indicating that the presence of thiouriedo linkage in the pyrimidine nucleus and the chromeno group attached to pyrimidene moiety are responsible for these activities.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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