TiO_2–SiO_2 Catalyzed Eco-friendly Synthesis and Antioxidant Activity of Benzopyrano[2,3-*d*]pyrimidine Derivatives

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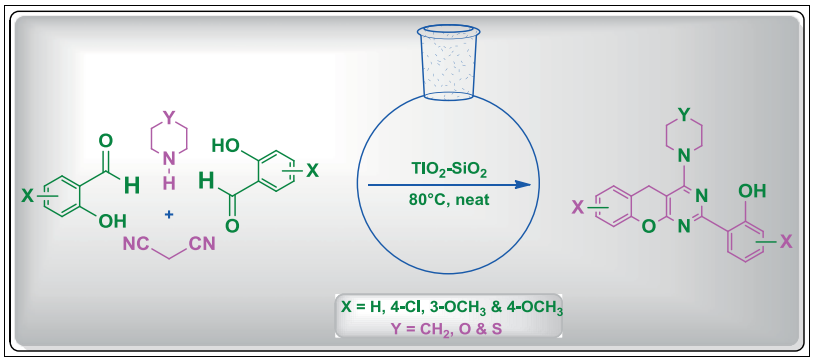

A cost-effective and eco-friendly synthesis of benzopyrano[2,3-*d*]pyrimidine derivatives has been developed *via* three component one-pot tandem approach by condensing different salicylaldehydes and secondary amines with malononitrile in the presence of TiO_2–SiO_2 catalyst at 80°C under solvent-free conditions. Mild experimental conditions, reusability of the catalyst, and cost effectiveness are the merits of this procedure. Compounds **4g**, **4h**, and **4i** bearing 2-OMe group on the hydroxyphenyl group linked to the central carbon present in between the two nitrogen atoms of the pyrimidine ring were found to exhibit good antioxidant activity while other compounds have moderate antioxidant activity.

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INTRODUCTION

The multicomponent reactions (MCRs) are highly important and popular for organic chemical synthetic research due to their wide range of advantages such as increased selectivity, low energy consumption, and minimum wastage [1]. These reactions are also important in pharmaceutical chemistry for rapid generation of several components with structural diversity which are useful for drug discovery [2]. The new MCRs with green synthetic methods in organic chemistry are evoking increased interest due to their eco-friendly nature [3]. Totally, the current organic chemistry research field is well renowned with the application of MCRs, and they serve as a potential method for the synthesis of new molecules with atom economy and time economy [4]. The access of MCRs to design and synthesize the bio-active compounds with diversity orientation has strongly attracted the synthetic community in both industry and academia [5]. Its application for the synthesis of new heterocycles [6] specifically for the pyrimidine derivatives [7] has been turned as an innovative method during the last decade.

Benzopyrano[2,3-*d*]pyrimidines are attracting much attention due to their wide range of pharmacological and biological activities such as antifungal, antiinflammatory, analgesic, and antibacterial activities [8]. Several benzopyrano[2,3-*d*]pyrimidines were tested for cytotoxic activity against a panel of cancer cell lines, and a number of them showed to cause significant perturbation in cell cycle kinetics [9]. In view of their pharmacological importance, few synthetic approaches have been developed for them using different catalysts such as $LiClO_4$ [10], $Na_2MoO_4{\cdot}2H_2O$ [9], [Bmim]BF_4 [11], Fe_3O_4 and SBA-15 [12], Fe(II)–benzoylthiourea complex bound silica nanoparticles [Fe(II)–BTU–SNPs] [13], Brønsted acidic ionic liquids [14], and dibutylamine (DBA) [15]. However, these synthetic methods suffer from one or more drawbacks, such as long reaction times, use of expensive organic solvents, and homogeneous catalyst. Therefore, there arises a need to develop more convenient, environmental friendly, and practically feasible method for the preparation of benzopyrano[4,3-*d*]pyrimidines.

In recent years, heterogeneous catalysts have gained much importance because of their ease of handling, high catalytic activities, reusability, and environmental benefits [16]. Among them, titania–silica (TiO_2–SiO_2) is found to be an efficient heterogeneous catalyst in synthetic chemistry. It received considerable attention because of its nontoxicity, cost effectiveness, recyclability, ease of handling, experimental simplicity, and commercial availability of required chemicals at low cost and remarkable ability to suppress side reactions with acid sensitive substrates [17].

These factors encouraged us to study the synthesis of benzopyrano[4,3-*d*]pyrimidines using TiO_2–SiO_2.

Reactive oxygen species (ROS) such as superoxide, hydroxyl, and nitric oxide (NO) are generated in the human body and cause damage to lipids, proteins, and DNA leading to carcinogenesis, drug-associated toxicity, and inflammation. Furthermore, ROS play a significant role in the inhibition of life-limiting chronic metabolic disorders in the living organisms [18]. Antioxidants are molecules, natural or synthetic, capable of interacting with free radicals and inhibiting their chain reactions ultimately before the damaging of essential vital molecules *in vivo* [19]. Thus, we have studied the antioxidant properties of the title compounds having phenolic groups on them, which easily generates the free radicals and arrests the damage of the vital molecules.

As part of our ongoing research program, we developed some environment benign methodologies for the synthesis of new organic molecules by using silica supported TiO_2 as a catalyst and successfully extended its application again for the synthesis of benzopyranopyrimidine derivatives [20–24]. Finally, to the best of our knowledge, this is the first report on the synthesis of these compounds under solvent-free conditions.

RESULTS AND DISCUSSION

Synthesis. We report a novel synthesis of benzopyrano [4,3-*d*]pyrimidine derivatives **(4a–l)** *via* pseudofour component condensation of salicylaldehydes, malononitrile, and secondary amines catalyzed by 5% TiO_2–SiO_2 at 80°C, under solvent-free conditions (Scheme 1).

As a model reaction, we attempted the condensation of salicyladehyde, malononitrile, and piperdine in the presence of 5 mol% of TiO_2–SiO_2 catalyst under solvent-free conditions. The reaction was slow at room temperature and required a long reaction time (Entry 16, Table 1) for its sustenance, but it was completed in 15 min at 80°C temperature (Entry 14, Table 1). We investigated the same reaction using different catalysts at 80°C, under solvent-free conditions, and the results were listed in Table 1. We found that TiO_2–SiO_2 afforded maximum product yields. In the catalyst optimization studies with 2, 5, and 10 mol% of TiO_2–SiO_2, we obtained the yields 73, 92, and 94, respectively (Entries 13–15, Table 1). Therefore, 5 mol% of TiO_2–SiO_2 was sufficient for completion of the reaction, and excess amount of catalyst did not increase the yields significantly (Entry 14, Table 1).

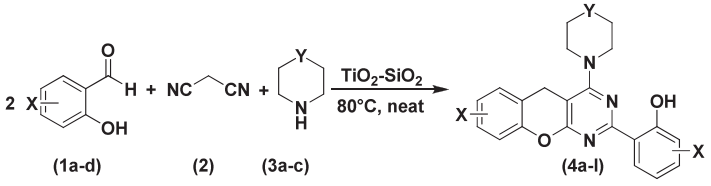
Table 1

Influence of various catalysts on the synthesis of compound **4a** at 80°C.

Entry	Catalyst	Catalyst (mol%)	Time	Yield (%)[a]
1	None	—	15	35
2	*p*-TSA	5	15	48
3	CSA	5	15	60
4	$ZnCl_2$	5	15	58
5	$NiCl_2{\cdot}6H_2O$	5	15	49
6	$AlCl_3$	5	15	29
7	$FeCl_3$	5	15	72
8	$CuBr_2$	5	15	55
9	$Cu(OTf)_2$	5	15	60
10	$InCl_3$	5	15	72
11	BF_3–SiO_2	5	15	68
12	$FeCl_3$–SiO_2	5	15	55
13	TiO_2–SiO_2	2	15	73
[b]**14**	**TiO_2–SiO_2**	**5**	**15**	**92, 90, 85**
15	TiO_2–SiO_2	10	15	94
[c]16	TiO_2–SiO_2	5	12 h	69

[a]Isolated yields.
[b]Yields when catalyst was reused three times.
[c]Reaction was carried out at room temperature.
The bold emphasis is used for easy identification of the optimized catalyst concentration (5mol%).

Scheme 1. Synthesis of benzopyrano[4,3-d]pyrimidine derivatives.



Entry	X	Y	r.t. (min)	Entry	X	Y	r.t. (min)
4a	H	CH_2	12	4g	3(OCH_3)	CH_2	15
4b	H	O	10	4h	3(OCH_3)	O	13
4c	H	S	14	4i	3(OCH_3)	S	20
4d	4-Cl	CH_2	12	4j	4(OCH_3)	CH_2	15
4e	4-Cl	O	15	4k	4(OCH_3)	O	18
4f	4-Cl	S	13	4l	4(OCH_3)	S	20

Examination of the effect of various solvents such as nitromethane, acetone, acetonitrile, 1,4-dioxane, ethanol, toluene, DMF, and THF, in the presence of 5 mol% of TiO_2–SiO_2 at 80°C revealed that presence of solvent retards the reaction (8 h) and results in poor product yields. But the solvent-free reaction was completed within 15 min with 92% yield (Entry 9, Table 2**)**.

After optimizing the reaction conditions, we reacted different salicylaldehydes **(1a–d)** with malononitrile (**2**) and different secondary amines (**3a–d**), and obtained a variety of benzopyrano[2,3-*d*]pyrimidines (**4a–l)**. In all the cases, with either electron-donating or electron-withdrawing groups on salicylaldehydes, reaction proceeded smoothly with malononitrile, and different secondary amines in the presence of 5% TiO_2–SiO_2 at 80°C and formed the corresponding benzopyrano[2,3-*d*]pyrimidines (**4a–l)** in good to excellent yields.

The TiO_2–SiO_2 catalyst was reused for three subsequent runs as it is insoluble in dichloromethane and was easily recovered by simple filtration from the reaction mixture. In our experiment, the successive yields were obtained in 92, 90, and 85% without loss of the effective catalyst activity (Entry 14, Table 1).

Table 2

Effect of various solvents on the synthesis of compound **4a**.

Entry	Solvent (2 mL)	Time (h)	Yield (%)[a]
1	CH_3NO_2	8	25
2	CH_3COCH_3	8	30
3	CH_3CN	8	73
4	1,4-Dioxane	8	67
5	CH_3CH_2OH	8	80
6	Toluene	8	59
7	DMF	8	62
8	THF	8	73
9	Solvent free	15 (min)	92

[a]Isolated yields.

The compounds reported in the literature were characterized by comparing their melting points and NMR, and presently synthesized new compounds were characterized by comparing their melting points, ^1H, ^{13}C NMR, and HRMS spectral studies.

A plausible mechanism is presented in the formation of benzopyrano[4,3-*d*] pyrimidines catalyzed by TiO_2–SiO_2 (Scheme 2). The Knoevenagel condensation of salicylaldehyde (**1**) and malononitrile (**2**) forms (**a**) and then on dehydration forms (**b**), which on Pinner reaction ultimately affords (**c**). The secondary amine (**3**) attacks the cyano group of (**c**) to produce intermediate (**d**), which reacts with another molecule of salicylaldehyde (**1**) to form (**e**). A simple proton transfer in (**e**) affords the benzopyrano[4,3-*d*]pyrimidine (**4)**.

Antioxidant activity. The compounds **4a–l** were tested for *in vitro* antioxidant activity by NO, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing-antioxidant power (FRAP), and H_2O_2 methods. The observed data on the antioxidant activity are given in Table 3. Majority of the compounds showed good activities for NO scavenging, DPPH reduction, and H_2O_2 inhibition as shown in Table 3. Among these the compounds, **4g**, **4h**, and **4i** exhibited these properties to much extent.

Nitric oxide free radical scavenging activity. The NO free radical scavenging activity of **4a–l** reveals that the compounds **4h**, **4g**, **4i**, and **4c** showed the highest NO scavenging activity which is comparable to the reference standard. The remaining compounds exhibited moderate

Scheme 2. Plausible reaction mechanism for the formation of benzopyranopyrimidines.

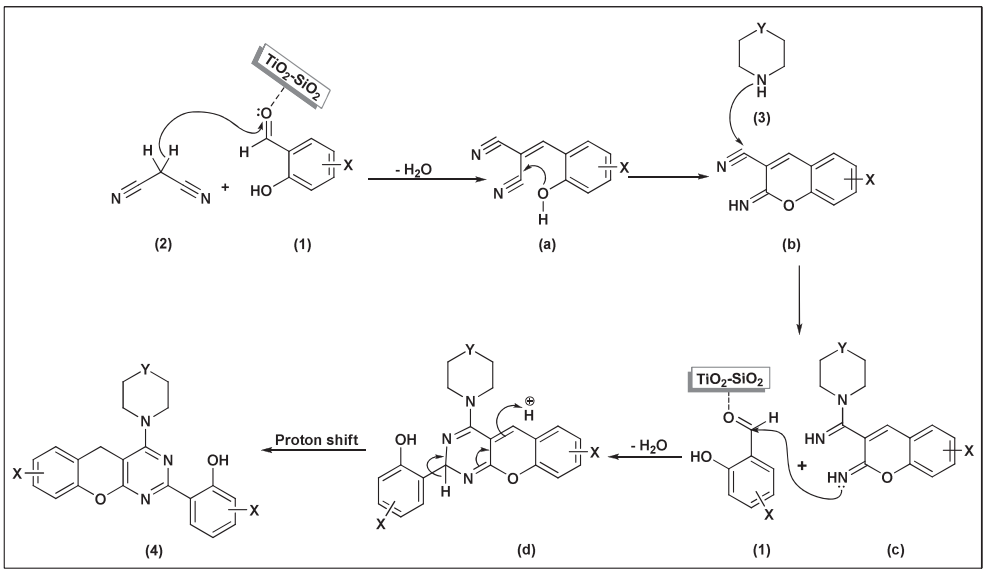


Table 3

Antioxidant activity studies of **4a–l** with various methods.

S. no.	Percentage of inhibition (IC_{50} values)[a,b]			
	Nitric oxide	DPPH	H_2O_2	FRAP
4a	69.53±0.48	89.67±1.14	71.58±0.36	1.145±0.016
4b	68.24±0.35	90.84±1.38	70.46±0.49	1.086±0.036
4c	83.45±0.50	84.55±1.52	73.68±0.37	1.546±0.024
4d	75.63±0.37	87.38±1.14	76.34±0.23	1.323±0.049
4e	66.38±0.29	90.45±1.21	67.85±0.42	1.183±0.026
4f	70.56±0.56	73.47±1.44	71.56±0.61	1.165±0.028
4g	89.44±0.25	80.33±0.72	84.37±0.69	2.124±0.045
4h	91.46±0.57	78.56±1.65	84.58±0.33	2.193±0.036
4i	89.33±0.87	79.56±0.98	86.74±0.67	2.094±0.031
4j	64.27±0.48	94.56±1.39	67.56±0.24	0.923±0.022
4k	67.43±0.59	84.52±1.11	64.77±0.56	1.812±0.023
4l	66.38±0.32	82.55±1.37	61.76±0.74	1.158±0.031
Ascorbic acid	95.57±0.29	97.46±1.25	88.25±0.43	2.362±0.042

[a]IC_{50} values are expressed as mean ± SD of three experiments.
[b]Concentration in 100 μg/mL.

scavenging activity. The results were expressed as a percent of scavenged NO in Table 3.

2,2-Diphenyl-1-picrylhydrazyl free radical scavenging assay. Among the title compounds, **4a–l** tested for antioxidant activity by DPPH method, the compounds **4j**, **4b**, **4e**, and **4a** showed remarkably high antioxidant activity, while the other compounds showed moderate activity when compared with the standard ascorbic acid, and the results were presented in Table 3.

Hydrogen peroxide scavenging assay. The scavenging ability of hydrogen peroxide by compounds **4i**, **4h**, **4g**, and **4d** was found to be comparable with the reference compound ascorbic acid (Table 3). This may be attributed to the phenolics, which can donate electrons to hydrogen peroxide thereby neutralizing it to water. This scavenging activity of hydrogen peroxide is concentration dependent.

Ferric reducing-antioxidant power (FRAP) assay. Ferric reducing-antioxidant power assay was done on the target compounds **4a–l**. Compounds **4h**, **4g**, **4i**, and **4k** showed the highest antioxidant activity as determined by FRAP assay (Table 3). Their capacity for reducing ferric ion was comparable to that of ascorbic acid.

EXPERIMENTAL

General. All reagents were obtained from Sigma-Aldrich and Alfa Aesar and were used directly without further purification. Melting points were recorded on Guna Digital Melting Point apparatus. All the IR spectra of the title compounds were recorded on Bruker Alpha-Eco ATR-FTIR interferometer with a single reflection sampling module equipped with ZnSe crystal. NMR spectra were recorded on Bruker AMX 500-MHz NMR spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR in DMSO, and chemical shifts were referenced to TMS and reported in δ scale. Mass spectra were recorded on a Jeol SX 102 DA/600 mass spectrometer, and elemental analysis was performed on a Thermo Finnigan Instrument.

Chemistry. ***General procedure for the preparation of TiO_2–SiO_2 catalyst.*** TiO_2–SiO_2 catalyst was prepared by adding titanium isopropoxide [$Ti(O\text{-}_i\text{-}C_3H_7)_4$]/isopropanol solution (10 mmol) in dropwise manner to the solution of tetraethylorthosilicate [$Si(O—C_2H_5)_4$]/isopropanol (20 mmol). The obtained mixture was vigorously stirred for 1 h at 90°C under basic p^H conditions, aged for a day, and then the formed precipitate was dispersed by means of ultrasonication over 30 min and then filtered and then dried over muffle furnace at 500°C to get fine TiO_2–SiO_2 catalyst [25,26].

General procedure for the synthesis of benzopyrano[4,3-d] pyrimidines (4a–l). To a mixture of salicylaldehyde **1** (2 mmol), malononitrile **2** (1 mmol), and secondary amine **3** (1 mmol) was added TiO_2–SiO_2 (5 mol%). The mixture was kept stirred at 80°C for the required time (Table 3). The progress of the reactions was monitored by thin layer chromatography (TLC) on 250-μm silica plates using 8:2 n-hexane and ethyl acetate mixture as an eluent. After completion of the reaction as indicated by TLC, methylene dichloride (5 mL) was added. The reaction mixture was filtered to collect the catalyst. It was washed with methylene dichloride (2 × 3 mL) and dried before use. The filtrate was washed with water (8 mL), dried over anhydrous Na_2SO_4, and filtered. The collected filtrate was evaporated under reduced pressure, and the obtained residue was recrystallized from ethanol to get pure benzopyrano[4,3-*d*]pyrimidines **(4a–l)**.

2-(4-(Piperidin-1-yl)-5H-chromeno[2,3-d]pyrimidin-2-yl)phenol (4a). White powder, yield 92%, mp 168–170°C. IR (ZnSe): *v* 3252 (—OH) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 1.70–1.82 (m, 6H), 3.43 (t, *J* = 4.9 Hz, 4H), 3.91 (s, 2H), 5.86 (s, 1H), 6.91 (t, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 7.09 (t, *J* = 7.3 Hz, 1H), 7.16–7.27 (m, 3H), 7.34 (t, *J* = 7.3 Hz, 1H), 8.41 (d, *J* = 8.0 Hz, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.2, 25.5, 25.8, 49.4, 97.3, 116.9, 117.4, 118.5, 118.7, 119.4, 124.2, 128.0, 128.4, 129.1, 132.6, 150.5, 160.3, 161.8, 164.2, 165.0 ppm. MS (EI) *m/z*: 360 $(M + H)^+$. *Anal*. Calcd for $C_{22}H_{21}N_3O_2$ (%): C, 73.52; H, 5.89; N, 11.69. Found: C, 73.44; H, 5.89; N, 11.63.

2-(4-Morpholino-5H-chromeno[2,3-d]pyrimidin-2-yl)phenol (4b). White powder, yield 90%, mp 199–201°C. IR (ZnSe): *v* 3267 (—OH) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 3.49 (t, 4H, *J* = 4.5 Hz), 3.91 (t, 4H, *J* = 4.5 Hz), 3.92 (s, 2H), 5.89 (s, 1H), 6.92 (t, 1H, *J* = 8.0 Hz), 6.98 (d, 1H, *J* = 8.0 Hz), 7.11 (t, 1H, *J* = 7.3 Hz), 7.17–7.29 (m, 3H), 7.36 (t, *J* = 7.3 Hz, 1H), 8.40 (d, *J* = 8.0 Hz, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 25.4, 48.5, 66.6, 97.6, 116.9, 117.5, 118.3, 118.8, 118.9, 124.4, 128.2, 128.5, 129.1, 132.8, 150.2, 160.2, 161.9, 164.0, 164.6 ppm. MS (EI) *m/z*: 361 (M+). *Anal*. Calcd for $C_{21}H_{19}N_3O_3$ (%): C, 69.79; H, 5.30; N, 11.63. Found: C, 69.74; H, 5.22; N, 11.60.

2-(4-Thiomorpholino-5H-chromeno[2,3-d]pyrimidin-2-yl)phenol (4c). Yellow powder, yield 93%, mp 239–241°C. IR (ZnSe): *v* 3276 (—OH) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 2.86 (t, *J* = 4.9 Hz, 4H), 3.76 (t, *J* = 4.9 Hz, 4H), 3.91 (s, 2H), 6.92 (t, *J* = 8.1 Hz, 1H), 6.98 (d, *J* = 8.1 Hz, 1H), 7.12 (t, *J* = 7.3 Hz, 1H), 7.17–7.28 (m, 3H), 7.36 (t, *J* = 7.3 Hz, 1H), 8.39 (d, *J* = 8.0 Hz, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 25.5, 27.3, 50.7, 97.9, 117.1, 117.6, 118.4, 118.8, 119.1, 124.5, 128.3, 128.5, 129.1, 133.0, 150.4, 160.3, 162.1, 165.4 ppm. MS (EI) *m/z*: 377 (M+). *Anal*. Calcd for $C_{21}H_{19}N_3O_2S$ (%): C, 66.82; H, 5.07; N, 11.13. Found: C, 66.72; H, 5.03; N, 11.05.

5-Chloro-2-(8-chloro-4-(piperidin-1-yl)-5H-chromeno[2,3-d]pyrimidin-2-yl)phenol (4d). White powder, yield 89%, mp 255–257°C. IR (ZnSe): *v* 3246 (—OH) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 1.73–1.81 (m, 6H), 3.43 (t, *J* = 4.7 Hz, 4H), 3.87 (s, 2H), 5.91 (s, 1H), 6.89 (d, *J* = 8.8 Hz, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 7.19–7.28 (m, 3H), 8.35 (d, *J* = 3.0, Hz, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.2, 25.5, 25.9, 49.5, 97.0, 118.3, 119.0, 119.4, 121.0, 123.7, 128.2, 128.32, 128.35, 129.4, 132.6, 149.0, 159.0, 161.0, 165.1 ppm. MS (EI) *m/z*: 427 (M+). *Anal*. Calcd for $C_{22}H_{19}Cl_2N_3O_2$ (%): C, 61.69; H, 4.47; N, 9.81. Found: C, 61.55; H, 4.41; N, 9.75.

5-Chloro-2-(8-chloro-4-morpholino-5H-chromeno[2,3-d]pyrimidin-2-yl)phenol (4e). White powder, yield 92%, mp 230–232°C. IR (ZnSe): *v* 3305 (—OH) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 3.48 (t, *J* = 4.5 Hz, 4H), 3.85 (s, 2H), 3.90 (t, *J* = 4.5 Hz, 4H), 5.90 (s, 1H), 6.85 (d, *J* = 8.8 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 7.17–7.25 (m, 3H), 8.24 (d, *J* = 3.0 Hz, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 25.4, 48.5, 66.6, 97.2, 118.3, 119.1, 119.2, 120.4, 123.7, 128.2, 128.3, 128.4, 129.5, 132.7, 148.7, 158.9, 161.0, 163.7, 164.7 ppm. MS (EI) *m/z*: 429 (M+). *Anal*. Calcd for $C_{21}H_{17}C_{l2}N_3O_3$ (%): C, 58.62; H, 3.98; N, 9.77. Found: C, 58.54; H, 3.94; N, 9.70.

5-Chloro-2-(8-chloro-4-thiomorpholino-5H-chromeno[2,3-d]pyrimidin-2-yl)phenol (4f). Yellow powder, yield 90%, mp 235–237°C. IR (ZnSe): *v* 3296 (—OH) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 2.85 (t, *J* = 4.9 Hz, 4H), 3.76 (t, *J* = 4.9 Hz, 4H), 3.87 (s, 2H), 5.88 (s, 1H), 6.91 (d, *J* = 8.8 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.20–7.31 (m, 3H), 8.32 (d, *J* = 3.0 Hz, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 25.4, 27.3, 50.8, 97.5, 118.4, 119.2, 119.3, 120.6, 123.8, 128.3, 128.4, 129.6, 132.8, 148.8, 158.9, 161.0, 165.4 ppm. MS (EI) *m/z*: 445 (M+). *Anal*. Calcd for $C_{21}H_{17}C_{l2}N_3O_2S$ (%): C, 56.51; H, 3.84; N, 9.41. Found: C, 56.42; H, 3.80; N, 9.36.

2-Methoxy-6-(9-methoxy-4-(piperidin-1-yl)-5H-chromeno[2,3-d]pyrimidin-2-yl)phenol (4g). White powder, yield 90%, mp 178–180°C. IR (ZnSe): *v* 3284 (—OH) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 1.66–1.80 (m, 6H), 3.48 (t, *J* = 4.9 Hz, 4H), 3.82 (s, 3H), 3.89 (s, 3H), 3.95 (s, 2H), 5.91 (s, 1H), 6.75 (d, *J* = 7.7 Hz, 1H), 6.78–6.86 (m, 2H), 6.95 (d, *J* = 7.7 Hz, 1H), 7.01 (t, *J* = 8.0 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.1, 25.4, 25.7, 49.3, 55.8, 55.9, 97.3, 110.2, 113.6, 117.5, 118.5, 119.6, 120.2, 120.9, 123.9, 139.9, 147.9, 148.5, 150.6, 161.9, 164.3, 164.7 ppm; MS (EI) *m/z*: 419(M+). *Anal*. Calcd for $C_{24}H_{25}N_3O_4$ (%): C, 68.72; H, 6.01; N, 10.02. Found: C, 68.70; H, 5.99; N, 15.21.

2-Methoxy-6-(9-methoxy-4-morpholino-5H-chromeno[2,3-d]pyrimidin-2-yl)phenol (4h). White powder, yield 89%, mp 210–220°C. IR (ZnSe): *v* 3279 (—OH) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 3.46 (t, *J* = 4.4 Hz, 4H), 3.88 (t, *J* = 4.4 Hz, 4H), 3.85 (s, 3H), 3.87 (s, 3H), 3.91 (s, 2H), 5.93 (s, 1H), 6.70 (d, *J* = 7.7 Hz, 1H), 6.77–6.87 (m, 2H), 6.94 (d, *J* = 7.7 Hz, 1H), 6.99 (t, *J* =8.0 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ: 25.4, 48.5, 55.90, 55.96, 66.5, 97.6, 110.5, 113.9, 117.7, 118.4, 119.6, 119.7, 120.9, 124.1, 139.7, 147.9, 148.5, 150.5, 162.1, 164.2 ppm. MS (EI) *m/z*: 421(M+). *Anal*. Calcd for $C_{23}H_{23}N_3O_5$ (%): C, 65.55; H, 5.50; N, 9.97. Found: C, 65.43; H, 5.44; N, 9.95.

2-Methoxy-6-(9-methoxy-4-thiomorpholino-5H-chromeno[2,3-d]pyrimidin-2-yl)phenol (4i). Yellow powder, yield 92%, mp 220–222°C. IR (ZnSe): *v* 3294 (—OH) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 2.85 (t, *J* = 4.7 Hz, 4H), 3.75 (t, *J* = 4.7 Hz, 4H), 3.89 (s, 2H), 3.93 (d, *J* = 2.2 Hz, 6H), 5.85 (s, 1H), 6.77 (d, *J* = 7.7 Hz, 1H), 6.82–6.88 (m, 2H), 6.97 (d, *J* = 7.7 Hz, 1H), 6.05 (t, *J* =8.0 Hz, 1H), 8.08 (d, *J* = 8.0 Hz, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 25.6, 27.4, 50.8, 56.06, 56.09, 97.9, 110.7, 114.1, 117.9, 118.5,

119.7, 120.0, 121.0, 124.3, 148.2, 148.7, 150.6, 162.3, 164.6, 165.0 ppm. MS (EI) *m/z*: 437 (M+). *Anal*. Calcd for $C_{23}H_{23}N_3O_4S$ (%):C, 63.14; H, 5.30; N, 9.60. Found: C, 63.02; H, 5.22; N, 9.55.

5-Methoxy-2-(8-methoxy-4-(piperidin-1-yl)-5H-chromeno[2,3-d]pyrimidin-2-yl)phenol (4j). White powder, yield 94%, mp 170–172°C. IR (ZnSe): *v* 3298 (—OH) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 1.67–1.81 (m, 6H), 3.40 (t, J = 4.9 Hz, 4H), 3.79 (s, 3H), 3.81 (s, 2H), 3.82 (s, 3H), 4.69 (brs, 1H), 6.45–6.50 (m, 2H), 6.65 (dd, J = 2.5, 8.4 Hz, 1H), 6.72 (d, J = 2.5 Hz, 1H), 7.07 (d, J = 8.4 Hz, 1H), 8.30 (d, J = 8.4 Hz, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.2, 24.7, 25.8, 49.3, 55.2, 55.3, 96.8, 101.1, 101.8, 106.3, 110.8, 111.3, 111.8, 128.9, 130.3, 151.0, 159.3, 161.6, 162.0, 163.3, 164.9 ppm. MS (EI) *m/z*: 419 (M+); *Anal*. Calcd for $C_{24}H_{25}N_3O_4$ (%): C, 68.72; H, 6.01; N, 10.02. Found: C, 68.60; H, 5.97; N, 9.94.

5-Methoxy-2-(8-methoxy-4-morpholino-5H-chromeno[2,3-d]pyrimidin-2-yl)phenol (4k). White powder, yield 90%, mp 227–229°C. IR (ZnSe): *v* 3277 (—OH) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 3.46 (t, J = 4.4 Hz, 4H), 3.80 (s, 3H), 3.83 (s, 5H), 3.89 (t, J = 4.4 Hz, 4H), 4.76 (brs, 1H), 6.46–6.51 (m, 2H), 6.67 (dd, J = 2.5, 8.4 Hz, 1H), 6.72 (d, J = 2.5 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 8.28 (d, J = 8.4 Hz, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.8, 48.5, 55.2, 55.4, 66.6, 97.1, 101.1, 101.9, 106.6, 110.8, 111.1, 111.6, 128.9, 130.4, 151.0, 159.5, 161.8, 162.0, 163.5, 164.6 ppm. MS (EI) *m/z*: 421 (M+). *Anal*. Calcd for $C_{23}H_{23}N_3O_5$ (%): C, 65.55; H, 5.50; N, 9.97. Found: C, 65.42; H, 5.46; N, 9.93.

5-Methoxy-2-(8-methoxy-4-thiomorpholino-5H-chromeno[2,3-d]pyrimidin-2-yl)phenol (4l). Yellow powder, yield 89%, mp 214–216°C. IR (ZnSe): *v* 3258 (—OH) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ 2.84 (t, J = 4.9 Hz, 4H), 3.73 (t, J = 4.9 Hz, 4H), 3.79 (s, 2H), 3.80 (s, 3H), 3.83 (s, 3H), 5.86 (s, 1H), 6.45–6.51 (m, 2H), 6.67 (dd, J = 2.5, 8.4 Hz, 1H), 6.72 (d, J = 2.5 Hz, 1H), 7.07 (d, J = 8.4 Hz, 1H), 8.27 (d, J = 8.4 Hz, 1H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.7, 27.3, 50.6, 55.2, 55.4, 97.2, 101.1, 101.9, 106.5, 110.8, 111.0, 111.6, 128.9, 130.3, 150.9, 159.4, 161.5, 161.7, 162.0, 163.5 ppm. MS (EI) *m/z*: 437 (M+). *Anal*. Calcd for $C_{23}H_{23}N_3O_4S$ (%): C, 63.14; H, 5.30; N, 9.60. Found: C, 63.02; H, 5.25; N, 9.55.

Antioxidant activity. All the *in vitro* antioxidant experiments were performed in triplicate, and the data was expressed with standard deviation, and ascorbic acid was used as the standard in all the four methods. Each method has been performed at four different concentrations and *viz.*, 25, 50, 75, and 100 μg/mL, and the title compounds were observed to enough potent at 100 μg/mL concentration.

Nitric oxide free radical scavenging assay. We have chosen the sodium nitroprusside buffer solution method for the spontaneous generation of NO from it. In the evaluation, initially, 3 mL of the analytical mixture was prepared by mixing 2 mL of 10 mM sodium nitroprusside, 0.5-mL saline phosphate buffer, and 0.5 mL of title compounds and made up to 100 μg/mL concentration and incubated at 25°C for 3 h. After 3 h of incubation, 0.5 mL of the mixture was mixed with 1.5-mL Griess reagent [1.0% sulphanilamide, 2.5% H_3PO_4 and 0.1% N-(1-naphtyl)ethylenediaminedihydrochloride] and subjected for the measurement of absorbance at 546 nm by using UV–visible spectrophotometer [27]. Similarly, the control experiments were also conducted under same conditions and with the same procedure. Then, the scavenging activity was evaluated by using the following equation by taking the difference between the absorbance of the test and the control experiments read at λ 546 nm and expressed the NO percent scavenging activity. The increased absorbance of the compounds with concentration indicates its increased reducing power by this method.

$$\text{NO Radical scavenging } (\%) = \frac{[(\text{Absorbance of control-Absorbance of test sample})]}{(\text{Absorbance of control})} \times 100$$

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), a nitrogen-centered stable free radical has been used to characterize the antioxidant activity of the title compounds; 1.0 mL of 0.1 mM methanolic DPPH solution was added to 3.0 mL of methanolic test solutions of 100 μg/mL concentrations. Then, the absorbance was measured at 517 nm for all the test compounds by spectrophotometric studies. In the analysis, the reduced DPPH free radical gives a strong absorption maximum at λ 517 nm. A radical scavenging antioxidant reacts with DPPH stable free radical and converts into 1,1-diphenyl-2-picrylhydrazine. A blank was prepared without adding compound and tested for the absorbance similarly, and change in the absorbance has been used to measure antioxidant properties. Lower absorbance value of the analytical sample indicates its higher free radical scavenging activity. The antioxidant activity of title compounds was expressed as IC_{50} and compared with standard [28]. The DPPH radical scavenging capacity was calculated using the following equation:

$$\% \text{ DPPH radical scavenging} = \frac{[(\text{Absorbance of control-Absorbance of test sample})]}{(\text{Absorbance of control})} \times 100$$

Hydrogen peroxide (H_2O_2) scavenging activity. The effect of hydrogen peroxide scavenge ability of the title compounds was determined based on the standard procedure [29]. A solution of 100 mM H_2O_2 was prepared, and to this 2 mL of 100 μg/mL title compound

solution was added in phosphate buffer saline of pH 7.4. Absorbance of H_2O_2 at 230 nm was determined spectrophotometrically after 10 min against a blank solution containing phosphate buffer without H_2O_2. The increased absorbance of the compounds with concentration indicates increased reducing power.

$$\text{H2O2 radical scavenging } (\%) = \frac{[(\text{Absorbance of control-Absorbance of test sample})]}{(\text{Absorbance of control})} \times 100$$

Ferric reducing-antioxidant power (FRAP) assay. The capacity of compounds to reduce Fe^{+3} complex [2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ)] to the Fe^{+2} form was considered as an equivalent for the corresponding FRAP value. The reducing power of Fe^{+3} was determined by measuring the change in absorption at 593 nm spectrophotometrically [30]. The reagent was prepared by mixing a 10.0 mL of TPTZ solution, with 10 mL of $FeCl_3 \cdot 6H_2O$ solution and 100 mL of acetate buffer at pH 3.6. Then, 3 mL of 100-μL FRAP reagent is mixed and incubated for 30 min at 37°C, and change in absorbance at 593 nm was recorded and compared to the absorbance of pure reagent. The results were expressed as ascorbic equivalent (mmol/100g compound). In this method, the colorless [Fe(III)–TPTZ] complex was reduced to deep blue colored [Fe(II)–TPTZ] complex by the compounds. The increased absorbance of the compounds with concentration indicates increased reducing power. Ascorbic acid was used as reference, and FRAP value was measured by using the equation:

$$\text{FRAP Value} = \frac{\text{OD at 593nm of test sample}}{\text{OD at 593nm of standard}} \times \frac{\text{standard (mm)}}{\text{sample (mg)}} \times 100.$$

CONCLUSION

We have developed an efficient and environmentally benign new method for the synthesis of benzopyrano [4,3-*d*]pyrimidines by using 5% TiO_2–SiO_2 as a recyclable heterogeneous catalyst, under solvent-free conditions. The major advantages of this methodology are experimental simplicity, simple work-up, reusable catalyst, short reaction times, and high product yields. Interestingly, some compounds **4g**, **4h**, and **4i** are proved to be better antioxidants as evidenced from all the four methods and are comparable to standard ascorbic acid. Analysis of the structure–activity relationship for the structure of the title compounds **4a–l** with the obtained *in vitro* antioxidant activity results reveals that the —OCH_3 substituted compounds have exhibited remarkably high activity when compared to other substituted analogues. It is due to the fact that —OCH_3 group stimulates the formation of reactive free radicals and facilitates to pair up with the other free radicals generated *in vivo*.

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