Synthesis and Computational Analysis of New Antioxidant and Antimicrobial Angular Chromenopyrimidines

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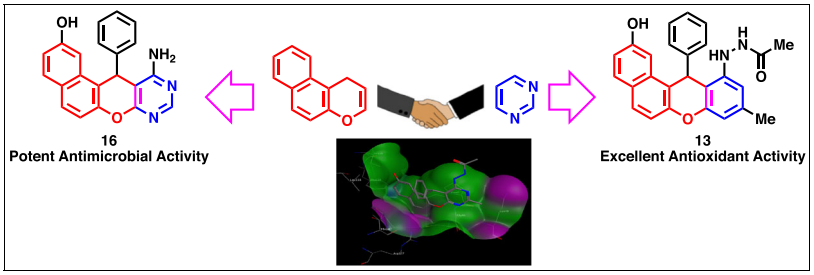

A convenient synthetic protocol for the synthesis of novel chromenopyrimidine derivatives based on angular scaffold has been developed from the readily available enaminonitrile precursor **1**. The skeleton of chromenopyrimidines has been decorated with different moieties in order to approach compounds with improved antioxidant and antimicrobial activities. Compound **1** was reacted with Ac_2O to yield benzochromenopyrimidine **6** that gave chloropyrimidine **7** *via* chlorination reaction. The reaction of chloropyrimidine **7** with different amines afforded a series of benzochromenopyrimidines **9–12**. The hydrazine **11** and hydrazide **13** derivatives showed excellent antioxidant activity in addition to the potent antimicrobial activity revealed by aminopyrimidines **10** and **16**. Computational docking studies indicated the potential recognition of compound **13** towards antioxidant binding site. Dual evaluations of the antimicrobial affinity of compound **16** indicated its promising activity as antimicrobial lead.

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INTRODUCTION

Microbial resistance to the common drugs is recognized as one of the main public health issues in the world. The World Health Organization has reported the microbial resistance as one of the three most serious health threats of the current century. The excessive and uncontrolled use of antimicrobial drugs promoted the appearance of multidrug-resistant microbes, which leaded to continuous need for new drugs. In addition, reactive oxygen species are deleterious oxygen radicals that can mutate DNA as well as altering the cell components.[1] In this regard, the human antioxidant defense system plays the main to neutralize reactive oxygen species and consequently protecting the human body from oxidative stress that is responsible for many diseases such as cancer, hypertension, diabetes, asthma, Alzheimer, and Parkinson.[2] As a result, the development of efficient antioxidants became very important in disease safeguard and medication.

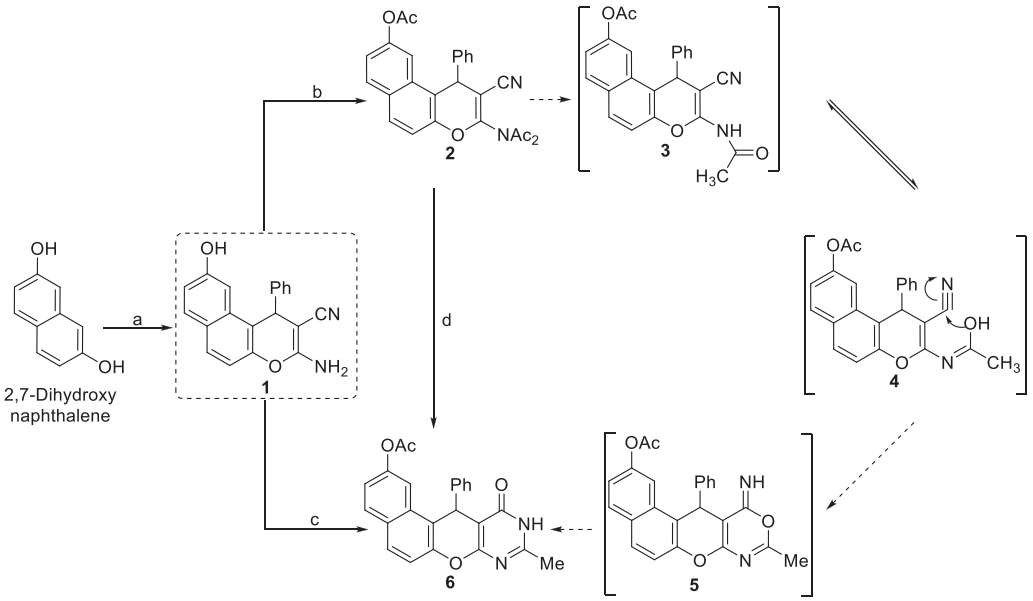
Pyrimidines play a crucial role in the human being life because they are widely distributed in several important organic compounds.[3,4] Many of these compounds exhibit outstanding biological and pharmacological effectiveness including antihypertensive,[5] antiplatelet,[6] antiviral,[7] antidiabetic,[8] antimicrobial, and antioxidant[9] activities. In addition, many vital natural products such as vitamin B1 contain pyrimidine nucleus. Pyrimidine bases such as cytosine, uracil, and thymine are utilized in constructing different nucleotides that are the essential building blocks of the nucleic acid DNA.

In addition, chromene scaffolds became privileged skeletons because of their wide medicinal activities.[10–13] Because enhanced medicinal activity might be produced *via* combining two or more biologically active segments, pyrimidine and chromene units might be appropriate candidates to build an elegant hydrid model.[14] Herein, we disclose our results in the design of new antimicrobial and antioxidant agents based upon an angular chromenopyrimidine hydrid skeleton.

RESULTS AND DISCUSSION

Chemistry. The multicomponent reaction between 2,7-dihydroxynaphthalene, benzaldehyde, and malononitrile in the presence of piperidine afforded the angular

Scheme 1. a. Benzaldehyde, malononitrile, piperidine; b. Ac_2O, 1 h; c. Ac_2O, 7 h; d. AcOH.



enaminonitrile **1** *via* a convenient and high yielding protocol (Scheme 1).[15] The versatile precursor benzo[*f*]chromeno[2,3-*d*]pyrimidine derivative **6** was synthesized from the benzochromene derivative **1** through harsh treatment with acetic anhydride under reflux conditions. Interestingly, the aforementioned reaction was monitored, and it was shown that the triacetylated product **2** was obtained by refluxing for 1 h, whereas the yield of the chromenopyrimidine product **6** increased concurrently with increasing the reaction time that indicated to the formation of the product **6** passing through the triacetylated product **2**.

The plausible reaction mechanism included the gradual hydrolysis of the triacetylated product **2** to give *O*-acetyl-*N*-acetylbenzochromene derivative **3** that paved the way to undergo tautomerization giving the nucleophilic hydroxyl group that attacked the carbonitrile active center giving the benzochromeno[2,3-*d*]oxazine **5** that finally underwent Dimroth rearrangement giving the desired benzochromenopyrimidine derivative **6** as shown in Scheme 1.[16]

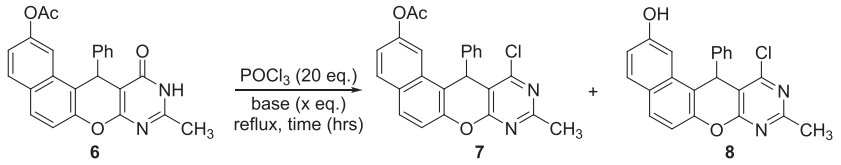
Meanwhile, it is important to finely tune the chlorination reaction conditions of the chromenopyrimidine **6** in order to obtain the corresponding chloropyrimidine **7** in excellent yield as shown in Table 1. Although the reaction did not proceed at all either in the absence of base (entry 1) or in the presence of pyridine (entry 2), triethylamine could promote the reaction upon 19% yield of product **7**. Among the tertiary amines we screened, 1,8-diazabicycloundec-7-ene (DBU) was the most efficient to give the desired product in 60% yield in shorter time (entry 5).[16] However, the yield could be mostly improved by the gradual upgrading of DBU up to 6 equivalents (entries 5–7); the reaction efficacy was downgraded beyond 6 equivalent DBU because of the production of the hydrolyzed phenol **8** (entries 8 and 9). In addition, the nucleophilic bases would be non-efficient in such transformations because of their competing nucleophilic activity.[17]

The replacement of chlorine atom and deacetylation of chloropyrimidine **7** were accomplished by the reaction with piperidine, morpholine, aniline, and hydrazine hydrate yielding *N*-(benzochromenopyrimidinyl) piperidine or morpholine **9a**,**b**, *N*-phenyl-*N*-chromenopyrimidinyl amine **10**, and *N*-benzochromenopyrimidinyl hydrazine **11**, respectively (Scheme 2). Moreover, the deacetylation of chloropyrimidine **7** could be carried out in the presence of triethylamine to yield the hydrolyzed phenol **8** that could afford hydrazine derivative **11** in a good yield by treatment with hydrazine hydrate in ethanol.

The chromenopyrimidinyl hydrazine **11** was exploited to construct different condensed and isolated heterocyclic compounds (Scheme 3). By the reaction with different aldehydes, the corresponding hydrazones **12a**–**d** were produced successfully. Moreover, the pendant hydrazinyl moiety was acetylated in the presence of glacial acetic acid to give the corresponding acetohydrazide **13**. Additionally, the reaction of hydrazine **11** with DMF–DMA or formic acid afforded the corresponding benzochromenotriazolo pyrimidine derivative **14** in a good yield. Moreover, compound **11** was reacted with

Table 1

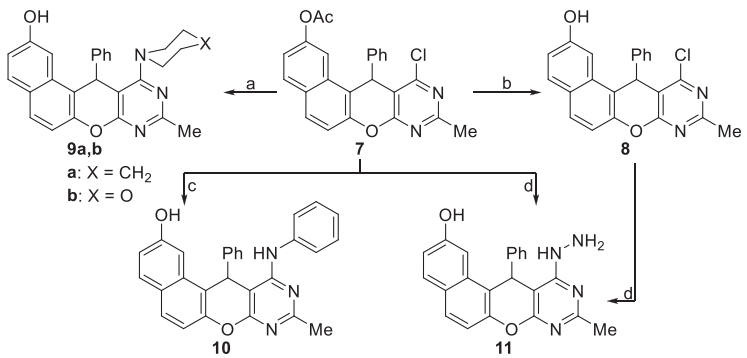
Optimization of chlorination reaction conditions.



Entry	Base	X equivalents	Time (h)	Yield 7 (%)	Yield 8 (%)
1	—	—	48	No reaction	
2	Pyridine	1.5	48	No reaction	
3	Et_3N	1.5	7	19	Traces
4	DMA	1.5	7	28	Traces
5	DBU	1.5	1.5	60	Traces
6	DBU	3	1.5	75	Traces
7	DBU	6	1.5	96	Traces
8	DBU	8	1.5	63	20
9	DBU	10	1.5	40	55

DMA, *N*,*N*-dimethyl aniline; DBU, 1,8-diazabicycloundec-7-ene.

Scheme 2. a. 2 amine; b. 1) Et_3N or DBU, EtOH, 2) HCl; c. aniline, EtOH; d. $H_2NNH_2.xH_2O$, EtOH.



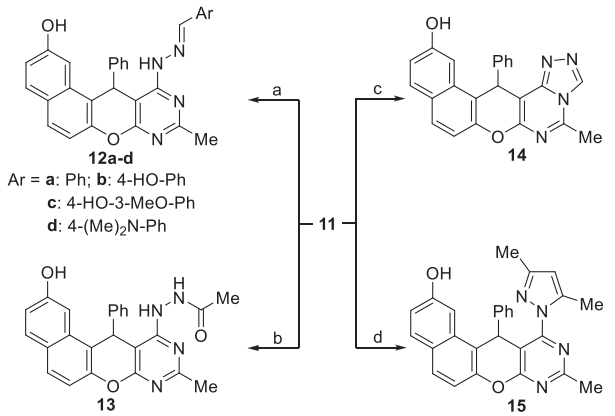
acetylacetone in the presence of acetic acid to give pyrazolylpyrimidine **15**.

Furthermore, chromenopyrimidine derivative **16** containing phenolic and heteroaryl primary amine groups was synthesized *via* the treatment of the enaminonitrile **1** with formamide. Compound **16** was coupled with the diazonium salts of different amines, namely, aniline, 4-methoxyaniline, 4-chloroaniline, and 3-aminopyridine to afford the corresponding azo products **17a–d**. Also, *O*-alkylation of aminopyrimidine **16** with chloroacetone and ethyl chloroacetate afforded the *O*-alkylated products **18** and **19** that could be cyclized into naphthofuran derivatives **20** and **21**, respectively, *via* heating in polyphosphoric acid (Scheme 4).

Biological evaluation and structure–activity relationships. ***Antioxidant activity.*** The antioxidant activities of the synthesized compounds were assessed against L-ascorbic acid as reference drug using two different radical scavenging assays, namely, ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] and DPPH [2,2-diphenyl-1-picrylhydrazyl], as depicted in Table 2. Although hydrazinyl **11** and hydrazide **13** derivatives exhibited excellent antioxidant activities, other products manifested poor to moderate activities.

The advantage of merging chromene and pyrimidine moieties in one scaffold could be observed by the improvement of the antioxidant activities of the hybrid compounds **11** and **13** over chromene-based compounds **1** and **2** (Table 2). Additionally, the presence of free hydroxyl group is very crucial to impart high antioxidant activity, and this could be seen in many cases. For example, conversion of enaminonitrile **1** into compound **2** by full acetylation destroyed its antioxidant activity. Moreover, chromenopyrimidine derivatives **6** and **7** with

Scheme 3. a. ArCHO, EtOH; b. AcOH; c. Method A: DMF–DMA, DMF, Method B: HCOOH; d. acetyl acetone, AcOH, EtOH.



low antioxidant activities showed improved activities (ABTS: 62.1%; DPPH: 57.4) by the removal of acetyl protecting group as seen in compound **8**. The thorough comparison of hydroxychromenopyrimidine derivatives **8**, **11**, and **13** revealed the role of hydrazinyl or hydrazide fragments to improve the antioxidant activity from 62.1% (ABTS) or 57.4% (DPPH) in compound **8** to the range of 80–85% in hydrazinyl **11** and hydrazide **13** derivatives. Also, the transformation of hydrazinyl moiety to other skeletons such as compounds **12a–d**, **14**, and **15** leaded to inferior results. Amino group (compound **16**) failed to enhance the net antioxidant activity as seen by hydrazinyl and hydrazide functions (compounds **11** and **13**). To sum up the structure–activity relationships, the combination

Table 2

Evaluation of antioxidant activity.

	ABTS assay		DPPH assay	
Compound	A[a]	Inhibition (%) [b]	A[a]	Inhibition (%)[b]
1	0.093	81.8	0.155	78.0
2	0.468	8.2	0.613	13.0
6	0.452	11.4	0.583	17.3
7	0.447	12.3	0.567	19.6
8	0.193	62.1	0.300	57.4
9a	0.367	28.0	0.532	24.5
9b	0.203	60.2	0.319	54.8
10	0.184	63.9	0.298	57.7
11	0.090	82.3	0.142	79.8
12a	0.396	22.3	0.576	18.3
12b	0.274	46.3	0.411	41.7
12c	0.224	56.0	0.387	45.1
12d	0.191	62.5	0.254	63.9
13	0.078	84.7	0.121	82.8
14	0.153	70.0	0.290	58.9
15	0.300	41.2	0.489	30.6
16	0.192	62.3	0.301	57.3
17a	0.382	25.1	0.497	29.5
17b	0.361	29.2	0.482	31.6
17c	0.423	17.0	0.551	21.8
17d	0.375	26.5	0.491	30.4
18	0.443	13.1	0.601	14.8
19	0.363	28.8	0.536	23.9
20	0.158	69.0	0.342	51.5
21	0.362	29.0	0.530	28.6
Control	0.510	0.0	0.705	0.0
LAA[c]	0.059	88.4	0.104	85.3

[a]Absorbance of the sample.
[b]Inhibition (%) = $(A_{control} - A_{tested})/A_{control} \times 100$.
[c]L-ascorbic acid.

Scheme 4. a. $HCONH_2$; b. $ArNH_2$, pyridine, $NaNO_2$, HCl; c. Chloroacetone, acetone, K_2CO_3; d. Ethyl chloroacetate, acetone, K_2CO_3; e. PPA.

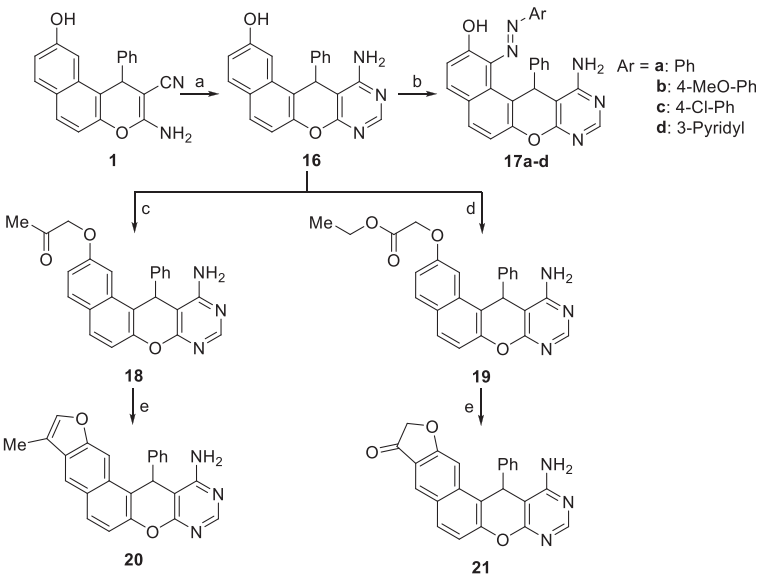


Table 3

Evaluation of antimicrobial activity.

Compound	*E. carotovora*		*B. subtilis*		*C. albicans*	
	D[a] (mm)	AI[b] (%)	D[a] (mm)	AI[b] (%)	D[a] (mm)	AI[b] (%)
1	9	60	10	67	9	56
2	8	53	9	60	9	56
6	NA[c]	NA[c]	9	60	7	44
7	9	60	8	53	9	56
8	9	60	10	67	10	63
9a	7	47	7	47	7	44
9b	8	53	8	53	10	63
10	16	107	15	100	17	106
11	14	93	13	87	10	63
12a	NA[c]	NA[c]	NA[c]	NA[c]	8	50
12b	9	60	9	60	8	50
12c	9	60	9	60	8	50
12d	8	53	NA[c]	NA[c]	NA[c]	NA[c]
13	11	73	10	67	11	69
14	10	67	11	73	10	63
15	7	47	8	53	9	56
16	17	113	15	100	19	119
17a	7	47	8	53	NA[c]	NA[c]
17b	13	87	11	73	12	75
17c	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
17d	10	67	10	67	10	63
18	7	47	8	53	10	63
19	10	67	NA[c]	NA[c]	NA[c]	NA[c]
20	12	80	12	80	15	94
21	7	47	8	53	10	63
Control (DMSO)	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]	NA[c]
Streptomycin	15	100	15	100	16	100

[a]Diameter of inhibition zone.
[b]Activity index (%) = (Diameter of the inhibition zone of the tested compound/Diameter of the inhibition zone of the reference drug) × 100.
[c]No activity.

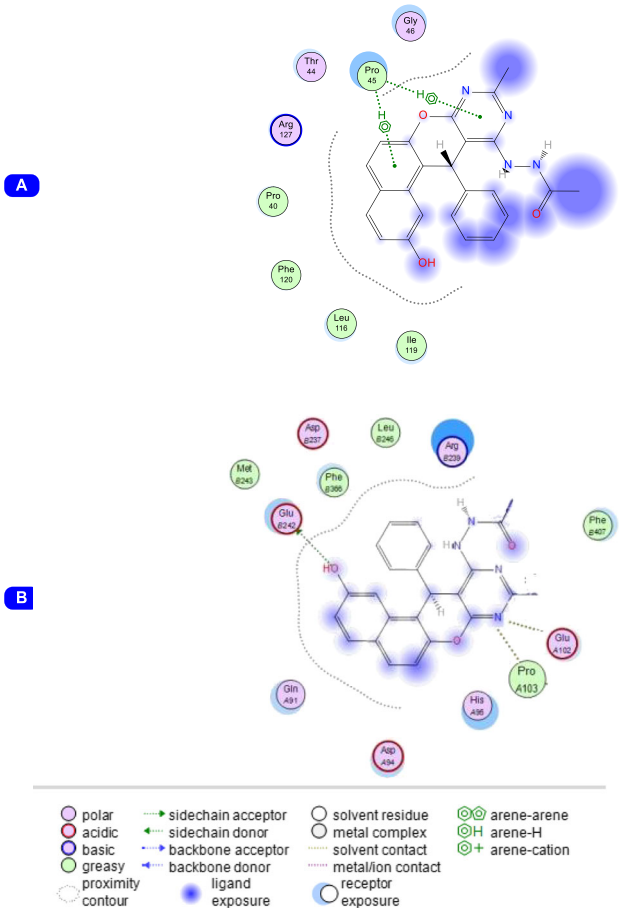


Figure 1. (A) Putative binding complex of compound **13** at the binding site of 1HD2. (B) Putative binding complex of compound **13** at the binding site of 4C1M. [Color figure can be viewed at wileyonlinelibrary.com]

of chromene and pyrimidine pharmacophores in one scaffold bearing free hydroxyl group as well as hydrazinyl or hydrazide functionalities suggested a promising model to build up new leads with enhanced antioxidant efficiency.[18]

Antimicrobial activity. The antibacterial activity of the synthesized bundle was assessed against *Erwinia carotovora* (Gram-negative bacteria) and *Bacillus subtilis* (Gram-positive bacteria) (Table 3). They were also screened for their antifungal activity against *Candida albicans*. The activities of our products were compared with Streptomycin as reference drug.

The results in Table 3 showed that the combination of pyrimidine and chromene scaffolds in products **10** and **16** leaded to super antimicrobial activities. In addition to the excellent antifungal activity of furo derivative **20**, it has very good antibacterial activities against both of *E. carotovora* (Gram-negative bacteria) and *B. subtilis* (Gram-positive bacteria). However, the hydrazinylpyrimidine **11** has very good to excellent activities against both bacterial types; it showed moderate activity against fungal strain *C. albicans*. In addition to the excellent antifungal activity of azo derivative **17b**, it has very good activity against Gram-negative bacteria. The deep speculation indicated that free amino and hydroxyl groups in product **16** were attributed to the best activity against all tested microbial strains.[19] However, capping of both groups as in product **10** did not produce big change in the antimicrobial activity; replacing one of them has an obvious impact on the overall activity. By comparing the structures of the best products **10**, **11**, **16**, and **20** with those of the inactive ones, we could observe that the absence of amino moiety, protection of hydroxyl *via* methyl ketone fragment, or coupling near hydroxyl group has harmful impact on the net antimicrobial activity. Finally, we could conclude that the combination of pyrimidine and chromene moieties where each of them bears polar group is beneficial to create promising antimicrobial compounds.

Molecular docking. *Antioxidant activity.* Docking study of pyrimidinylacetohydyazide **13** was performed using the crystal structure of human peroxiredoxin (code:

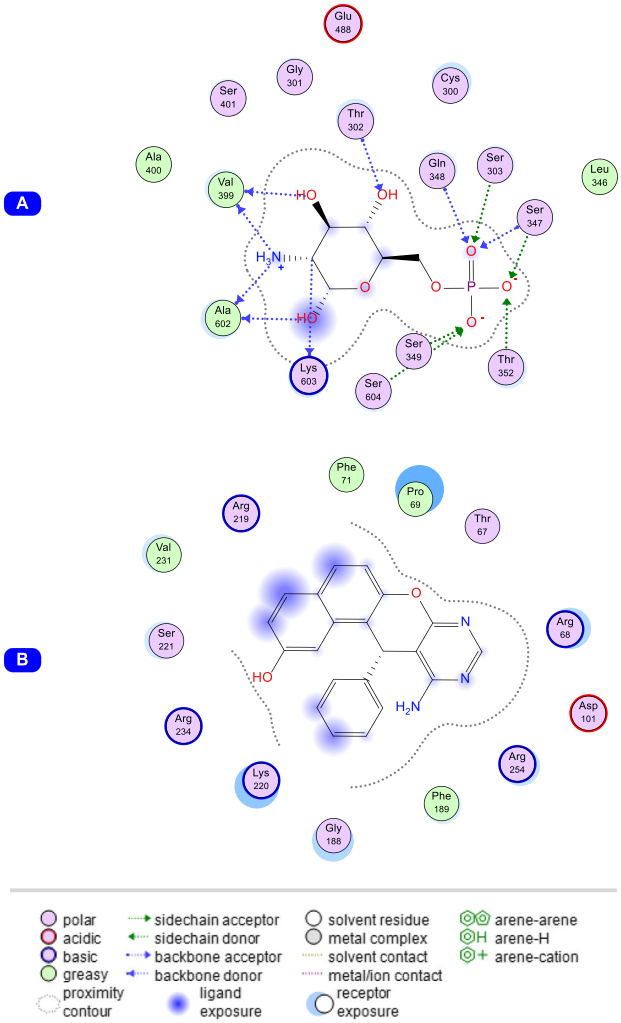


Figure 2. (A) Crystallographic structure of glucose amine-6phosphate at the binding site of 2VF5. (B) Putative binding complex of compound **16** at the binding site of 2VF5. [Color figure can be viewed at wileyonlinelibrary.com]

1HD2), and the results indicated its vast selectivity as antioxidant lead (Fig. 1A). Pro45, one of the essential key binding amino acids, professionally hocked the chromopyrimidine ring by the aromatic–hydrogen interaction. Additionally, the reversible antioxidant activity of compound **13** was examined by docking at oxidoreductase (code: 4C1M). The docking performance is promising as proper antioxidant lead where it professionally was recognized from both chains A and B where the hydroxyl function interacted properly with the key amino acid: GluB242. Atom N_1 of the chromopyrimidine ring showed bifurcated hydrogen bonds with both conserved amino acids GluA102 and ProA103. One of the two nitrogen of the hydrazine function stabilized the Van der Waals interaction with the crucial amino acid: ArgB239 (Fig. 1B).

In conclusion, the 4-hydrazine is occupying strategic location targeting the proper recognition with Arg239. It is clear that bulk substitution on the 4-hydrazino function interferes with its exposure and in turn dramatically lowers the degree of recognition with the conserved amino acid residues and consequently lowers the corresponding antioxidant potency.

Antimicrobial activity. Simulations were performed using the crystal structure of glucosamine-6-phosphate synthase (code: 2VF5) that catalyzes the first step in hexamine biosynthesis converting D-fructose-6-phosphate (Fru-6-P) into D-glucosamine-6-phosphate (GlcN-6-P) using glutamine as the ammonia source. The amino sugars are the significant building blocks of polysaccharides found in the cell wall of most human pathogenic microorganisms. Therefore, not surprising that a number of GlcN-6-P synthase inhibitors of natural or synthetic origin display bactericidal or fungicidal properties.[20] In correlation to *in vitro* antimicrobial activity, it thought worthwhile to carry out *in silico* studies of target molecules to predict the binding affinity and their recognition. Aminopyrimidine **16** performed proper recognition by hydrophilic interaction between free amino function and the facing conserved residues lining the pocket wall Arg254 and Phe189 (Fig. 2).

***In silico* physicochemical evaluation.** Drug-likeness is a term used to define absorption, distribution, metabolism, and excretion properties of a drug molecule.[21,22] The absorption, distribution, metabolism, and excretion properties were calculated by using Osiris Property Explorer server and analyzed by applying Lipinski's rule of five, which is very important standard widely for selecting of lead compounds that are promising for advanced drug design programs (Table 4). As shown in Table 4, chromenopyrimidine analogs exhibited proper molecular properties that are important for a new drug's pharmacokinetics.[23] Most of chromenopyrimidines have molecular weights less than 500 g/mol and logPo/w with the acceptable range. The absorption percentage was calculated based upon the values of polar surface area (PSA) [ABS (%) = 109 − (0.345 × PSA)].[24] However, the number of H-bond acceptors and number of H-bond donors are below to 10 and 5, respectively; some of the synthesized compound showed low levels of violation from Lipinski's rule of five. Water solubility and oral absorption for half of the molecules is 100%.

CONCLUSION

A bundle of novel angular chromenopyrimidine derivatives has been synthesized *via* efficient synthetic plan with the aim to be evaluated as antioxidant and antimicrobial agents. The chromenopyrimidine derivatives

Table 4

In silico drug like (physicochemical) properties.

Comp.	MW	LogP	PSA	ABS (%)	non-HAs	HBA	HBD	Violations	NoRB
1	314.34	3.40	79.28	81.65	24	4	3	0	1
2	440.45	2.77	96.71	75.63	33	7	0	0	4
6	398.42	3.61	81.29	80.95	30	6	1	0	3
7	416.86	4.96	61.32	87.84	30	5	0	0	3
8	374.83	4.93	55.25	89.93	27	4	1	0	1
9a	423.52	5.26	58.48	88.82	32	5	1	1	2
9b	425.49	4.20	67.72	85.64	32	6	1	0	2
10	73.35	6.27	73.35	83.69	36	6	1	1	5
11	370.41	3.49	93.30	76.81	28	6	4	0	2
12a	458.52	5.54	79.64	81.52	35	6	2	1	4
12b	474.52	5.06	99.86	74.55	36	7	3	1	4
12c	504.5	4.88	109.10	71.36	38	8	3	1	5
12d	501.59	5.65	82.88	80.41	38	7	2	2	5
13	412.45	3.23	96.37	75.75	31	7	3	0	3
14	380.41	3.76	72.55	83.97	29	6	1	0	1
15	434.50	4.84	73.07	83.79	33	6	1	0	2
16	341.37	3.65	81.27	80.96	26	5	3	0	1
17a	445.48	6.03	106.00	72.43	34	7	3	1	3
17b	475.51	6.08	115.23	69.25	36	8	3	1	4
17c	479.93	6.71	106.00	72.43	35	7	3	1	3
17d	510.56	5.91	118.36	68.17	39	8	3	2	4
18	397.43	3.73	87.35	78.86	30	6	2	0	4
19	427.46	4.42	96.58	75.68	32	7	2	0	6
20	379.42	4.80	74.18	83.41	29	5	2	0	1
21	381.39	3.50	87.35	78.86	29	6	2	0	1

MW, molecular weight; LogP, logarithm of partition coefficient of compound between *n*-octanol and water; PSA, polar surface area; ABS (%), absorption percentage; non-HAs, number of non-hydrogen atoms; HBA, number of hydrogen bond acceptors; HBD, number of hydrogen bond donors; Violations, number of rule of five violations; NoRB, number of rotatable bonds.

bearing hydrazine or hydrazide moieties (**11** and **13**) revealed excellent antioxidant activities compared with the reference L-ascorbic acid under ABTS and DPPH screening assays and based on the computational recognition at the binding active sites. In addition, the biological evaluation and the docking study indicated that 7-hydroxy-2-methyl-5-phenyl-5*H*-4-phenylamin-*N*-ylbenzo[*f*]chromeno[2,3-*d*]pyrimidine (**10**) and 4-amino-5-phenyl-5*H*-benzo[*f*]chromeno[2,3-*d*]pyrimidin-7-ol (**16**) possess super antimicrobial activities in comparison with the reference antibiotic Streptomycin.

EXPERIMENTAL

General. All melting points are reported in degree Celsius (°C) (uncorrected) and were measured on a Gallenkamp electrical melting point apparatus. Ethanol was dried prior to use based upon the standard techniques. The other chemicals were purchased from commercial suppliers and used without further purification. IR spectra were measured using the KBr disc technique on a Mattson 5000 or Thermo Scientific Nicolet IS10 FTIR Spectrometers at Mansoura University. The ^{1}H NMR spectra were measured on Bruker AC 300 MHz (Cairo University), Bruker Avance III 400 MHz (Beni Suef University), and JEOL ECA II 500 MHz (Mansoura University) using tetramethylsilane as an internal standard in DMSO-d_6 or $CDCl_3$ solvents. The signals' multiplicities are reported as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, and m = multiplet. Exchangeable protons were detected through D_2O test. ^{13}C NMR spectra (δ, ppm) were measured on JEOL ECA II 125 MHz (Mansoura University). Electron impact mass spectra (EIMS) were determined on Thermo Scientific DSQ II S GC/MS with FOCUS GC (70 ev) (Mansoura University) or Kratos MS-80 GC/MS, 70 eV (Al-Azhar University). Elemental analyses (C, H, and N) were executed at Cairo University. Biological screenings were performed at Pharmacognosy Department, Faculty of Pharmacy, Mansoura University. The compounds have been evaluated for the degree of recognition at the binding pockets of the 1HD2, 4C1M, and 2VF5 enzymes using Molecular Operating Environment software 10.2008 (MOE) provided with chemical computing group, Canada.

Synthesis. ***2-Amino-6-hydroxy-4-phenyl-4H-benzo[f] chromene-3-carbonitrile (1).*** A mixture of 2,7-dihydroxynaphthalene (480 mg, 3 mmol), malononitrile (198 mg, 3 mmol), and benzaldehyde (0.32 mL, 3.2 mmol) in EtOH (30 mL) containing few drops of

piperidine was refluxed for 2 h. After cooling, the formed precipitate was filtered off and recrystallized from EtOH affording compound **1**. Colorless crystals; yield (800 mg, 85%); mp = 150–151°C; IR (KBr, ν/cm^{-1}): 3487 (OH), 3381 and 3329 (NH_2), 2189 (CN); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 3.43 (brs, 1H, OH), 4.99 (s, 1H, H-4), 6.91 (s, 2H, NH_2), 6.94–7.76 (m, 10H, ArH); (EIMS) *m/z* (%): 314.1 [M$^+$] (15.9), 313.1 (1.9), 238.2 (16.1), 237.1 (100.0), 221.0 (3.7), 208.1 (3.2), 85.1 (20.4), 84.1 (41.5); Anal.Calcd for $C_{20}H_{14}N_2O_2$ (314.34): C, 76.42; H, 4.49; N, 8.91%. Found: C, 76.31; H, 4.32; N, 8.99%.

3-Cyano-2-(N,N-diacetylamino)-4-phenyl-4H-benzo[f]chromen-6-yl acetate (2). A solution of compound **1** (1.8 g, 5.7 mmol) in acetic anhydride (20 mL) was refluxed for 1 h. The reaction mixture was poured into cold water (150 mL) and stirred vigorously for 1 h. The formed precipitate was filtered off and purified by column chromatography using petroleum ether : ethyl acetate (70:30) to give the triacetylated product. Pale yellow crystals; yield (2.31 g, 92%); mp = 189–190°C; IR (KBr, ν/cm^{-1}): 2221 (CN), 1734 (COO), 1672 (C═O); ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 2.28 (s, 3H, CH_3COO), 2.37 (s, 6H, $(CH_3CO)_2N$), 5.81 (s, 1H, H-4), 7.25–8.06 (m, 10H, ArH); Anal. Calcd for $C_{26}H_{20}N_2O_5$ (440.46): C, 70.90; H, 4.58; N, 6.36%. Found: C, 70.72; H, 4.47; N, 6.51%.

7-Acetoxy-2-methyl-4-oxo-5-phenyl-5H-benzo[f]chromeno[2,3-d]pyrimidine (6). A solution of compound **1** (1.8 g, 5.7 mmol) in acetic anhydride (20 mL) was refluxed for 7 h. The reaction mixture was left to stand overnight. The formed precipitate was filtered off and washed well with hot EtOH several times to afford the desired product. Colorless crystals; yield (2.02 g, 89%); mp = 250–251°C; IR (KBr, ν/cm^{-1}): 3448 (NH), 1758 (COO), 1643 (C═O); ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 2.280 (s, 3H, CH_3), 2.283 (s, 3H, CH_3), 5.66 (s, 1H, H-5), 7.08–7.98 (m, 10H, ArH), 12.49 (s, 1H, NH); (EIMS) *m/z* (%): 398.2 [M$^+$] (44.7), 397.6 (42.3), 396.8 (100.0), 392.9 (11.8), 362.9 (46.2), 362.0 (79.6), 352.2 (28.1), 332.0 (29.6), 283.1 (37.3), 237.8 (19.6), 179.8 (17.0), 138.6 (16.1), 106.0 (27.6), 90.8 (39.9); Anal. Calcd for $C_{24}H_{18}N_2O_4$ (398.42): C, 72.35; H, 4.55; N, 7.03%. Found: C, 72.22; H, 4.31; N, 7.12%.

7-Acetoxy-4-chloro-2-methyl-5-phenyl-5H-benzo[f]chromeno[2,3-d]pyrimidine (7). A mixture of compound **6** (398 mg, 1 mmol), phosphorus oxychloride (3 mL, 20 mmol), and DBU (0.9 mL, 6 mmol) was refluxed for 2 h. After cooling, it was carefully poured into crushed ice (150 g). The formed precipitate was filtered off and washed well with water. The crude product was recrystallized from EtOH to afford the product **7**. White crystals; yield (401 mg, 96%); mp = 252–253°C; IR (KBr, ν/cm^{-1}): 1761 (COO), 1585 (C═N); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.39 (s, 3H, CH_3), 2.69 (s, 3H, CH_3), 5.86 (s, 1H, H-5), 7.15–7.84 (m, 10H, ArH); (EIMS) *m/z* (%): 416.1 [M$^+$] (8.2), 418.1[M$^+$+2] (3.0), 402.0 (7.3), 333.0 (31.6), 332.0 (100.0), 331.0 (22.0), 105.9 (4.6), 90.9 (3.56); Anal.Calcd for $C_{24}H_{17}ClN_2O_3$ (416.86): C, 69.15; H, 4.11; N, 6.72%. Found: C, 69.02; H, 4.05; N, 6.89%.

[*] *Note*: The same compound **7** was prepared also in different yields as mentioned in Table 1 using the same procedure with replacement of the base catalyst.

4-Chloro-2-methyl-5-phenyl-5H-benzo[f]chromeno[2,3-d]pyrimidin-7-ol (8). A mixture of compound **7** (833 mg, 2 mmol) and Et_3N or DBU (6 mmol) in EtOH (15 mL) was refluxed for 30 min. Thereafter, the reaction mixture was poured into cold water (150 mL) and neutralized using conc. HCl. The formed precipitate was filtered off, dried, and recrystallized from EtOH to afford the product **8**. Colorless crystals; yield (726 mg, 97%); mp = 187–188°C; IR (KBr, ν/cm^{-1}): 3407 (OH), 1579 (C═N); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.65 (s, 3H, CH_3), 5.74 (s, 1H, H-5), 7.07–7.74 (m, 11H, ArH, OH); (EIMS) *m/z* (%): 374.0 [M$^+$] (16.8%), 375.0 [M$^+$+1] (4.0), 376.0 [M$^+$+2] (4.5), 307.0 (7.1), 300.0 (4.5), 299.0 (45.0), 297.0 (100.0), 279.0 (4.8), 193.0 (10.7), 164.0 (9.3); Anal. Calcd for $C_{22}H_{15}ClN_2O_2$ (374.82): C, 70.50; H, 4.03; N, 7.47%. Found: C, 70.29; H, 3.89; N, 7.59%.

Synthesis of compounds 9a,b. A suspension of compound **7** (416 mg, 1 mmol) in piperidine or morpholine (6 mmol) was heated at 80°C for 45 min. Thereafter, the mixture was poured into cold water (100 mL) and neutralized by using few drops from conc. HCl. The formed products **9a**,**b** were filtered off, dried, and recrystallized from EtOH to give the products **9a** or **9b**, respectively.

N-(7-Hydroxy-2-methyl-5-phenyl-5H-benzo[f]chromeno[2,3-d]pyrimidin-4-yl)piperidine ***(9a).*** Colorless crystals; yield (400 mg, 95%); mp = 262°C (Decomp); IR (KBr, ν/cm^{-1}): 3447 (OH), 1584 (C═N); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 1.67–1.84 (m, 6H, H-3′, H-4′, H-5′), 2.40 (s, 3H, CH_3), 3.24–3.49 (m, 4H, H-2′, H-6′), 5.67 (s, 1H, H-5), 6.98–7.82 (m, 10H, ArH), 10.00 (s, 1H, OH); (EIMS) *m/z* (%): 423.0 [M$^+$] (16.8), 421.9 (24.9), 409 (25.3), 403.8 (39.9), 402.9 (100.0), 399.0 (42.6), 392.8 (27.5), 384.8 (18.8), 371.0 (49.9), 369.0 (57.1), 366.9 (42.9), 362.8 (25.1), 343.8 (22.1), 300.7 (4.5), 196.7 (15.5), 143.0 (11.6), 42.9 (45.0); Anal.Calcd for $C_{27}H_{25}N_3O_2$ (423.52): C, 76.57; H, 5.95; N, 9.92%. Found: C, 76.66; H, 5.69; N, 10.13%.

N-(7-Hydroxy-2-methyl-5-phenyl-5H-benzo[f]chromeno[2,3-d]pyrimidin-4-yl)morpholine ***(9b).*** Pale yellow crystals; yield (400 mg, 94%); mp = 296°C (Decomp); IR (KBr, ν/cm^{-1}): 3425 (OH), 1580 (C═N);

^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 2.38 (s, 3H, CH_3), 3.17–3.21 (m, 2H, morpholinyl–CH_2), 3.60–3.63 (m, 2H, morpholinyl–CH_2), 3.71–3.74 (m, 2H, morpholinyl–CH_2), 3.84–3.87 (m, 2H, morpholinyl–CH_2), 5.67 (s, 1H, H-5), 7.77–7.72 (m, 10H, ArH), 9.95 (s, 1H, OH); Anal. Calcd for $C_{26}H_{23}N_3O_3$ (425.49): C, 73.39; H, 5.45; N, 9.88%. Found: C, 73.27; H, 5.21; N, 9.97%.

7-Hydroxy-2-methyl-5-phenyl-5H-4-phenylamin-N-ylbenzo[f]chromeno[2,3-d]pyrimidine (10). A mixture of compound **7** (833 mg, 2 mmol) and aniline (0.2 mL, 2.2 mmol) was refluxed in EtOH (10 mL) for 5 h. The mixture was poured into cold water (100 mL) and neutralized using few drops from conc. HCl. The formed precipitate was filtered off and recrystallized from EtOH (80%) to give the product **10**. Dark brown crystals; yield (739 mg, 78%); mp = 236–237°C; IR (KBr, ν/cm^{-1}): 3409 (OH, NH), 1597 (C═N); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 2.39 (s, 3H, CH_3), 6.33 (s, 1H, H-5), 7.06–7.83 (m, 15H, ArH), 9.15 (s, 1H, NH); Anal. Calcd for $C_{28}H_{21}N_3O_2$ (431.50): C, 77.94; H, 4.91; N, 9.74%. Found: C, 77.75; H, 4.78; N, 9.61%.

N-(7-Hydroxy-2-methyl-5-phenyl-5H-benzo[f]chromeno[2,3-d]pyrimidin-4-yl)hydrazine (11). A mixture of compound **7** or **8** (1 mmol) and hydrazine hydrate 80% (2 mL) in EtOH (10 mL) was heated with stirring at 70°C for 1 h. The mixture was poured into crushed ice (100 g). The formed precipitate was filtered off, washed with water, and recrystallized from EtOH to yield compound **11** (293 mg, 79%) or (319 mg, 86%), respectively; pale brown needles; mp = 260–261°C; IR (KBr, ν/cm^{-1}): 3250–3490 (br, OH, NH, NH_2), 1596 (C═N); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 2.33 (s, 3H, CH_3), 4.43 (s, 2H, NH_2), 5.87 (s, 1H, H-5), 6.99–7.73 (m, 10H, ArH); 8.62, 9.86 (2s, 2H, NH, OH); Anal.Calcd for $C_{22}H_{18}O_2N_4$ (370.41): C, 71.34; H, 4.90; N, 15.13%. Found: C, 71.27; H, 4.78; N, 15.22%.

Synthesis of compounds 12a–d. A mixture of compound **11** (740 mg, 2 mmol) and the appropriate aldehydes, namely, benzaldehyde, 4-hydroxybenzaldehyde, vanillin or 4-(dimethylamino)benzaldehyde (2 mmol) in EtOH (20 mL) was refluxed in water bath for 1–4 h (TLC control). After cooling, it was poured into cold water (100 mL). The formed precipitate was filtered off, dried, and recrystallized from EtOH (80%) to form the desired products.

4-(Benzaldehydehydrazono-1-yl)-2-methyl-5-phenyl-5H-benzo[f]chromeno[2,3-d]pyrimidin-7-ol ***(12a).*** Buff crystals; yield (551 mg, 60%); mp = 257°C (Decomp); IR (KBr, ν/cm^{-1}): 3447 (OH), 3202 (NH), 1552 (C═N); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 2.38 (s, 3H, CH_3), 4.35 (s, 1H, H-5), 7.05–7.97 (m, 15H, ArH), 8.31 (s, 1H, –CH═N–), 10.00, 11.22 (2s, 2H, NH, OH); (EIMS) *m/z* (%): 458.4 [M$^+$] (3.7), 451.2 (8.8), 447.7 (5.5), 407.3 (14.7), 383.0 (10.8), 373.0 (19.3), 371.0 (20.1), 369.3 (33.3), 368.1 (100.0), 365.9 (28.4), 349.0 (23.2), 326.0 (37.3), 324.9 (52.6), 305.1 (25.0), 56.8 (25.8), 42.8 (26.6); Anal.Calcd for $C_{29}H_{22}N_4O_2$ (458.52): C, 75.97; H, 4.84; N, 12.22%. Found: C, 75.82; H, 4.61; N, 12.41%.

4-((4-Hydroxybenzaldehyde)hydrazono-1-yl)-2-methyl-5-phenyl-5H-benzo[f]chromeno[2,3-d]pyrimidin-7-ol ***(12b).*** Brown crystals; yield (788 mg, 83%); mp = 295–296°C; IR (KBr, ν/cm^{-1}): 3100–3550 (br, OH, NH), 1580 (C═N); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 2.35 (s, 3H, CH_3), 6.86–7.82 (m, 15H, ArH, H-5), 8.20 (s, 1H, –CH═N–), 9.92, 9.94, 10.99 (3s, 3H, 2OH, NH); Anal. Calcd for $C_{29}H_{22}N_4O_3$ (474.52): C, 73.40; H, 4.67; N, 11.81%. Found: C, 71.29; H, 4.45; N, 11.95%.

4-((4-Hydroxy-3-methoxybenzaldehyde)hydrazono-1-yl)-2-methyl-5-phenyl-5H-benzo[f]chromeno[2,3-d] pyrimidin-7-ol ***(12c).*** Gray crystals; yield (737 mg, 73%); mp = 251°C; IR (KBr, ν/cm^{-1}): 3100–3550 (br, OH, NH), 1582 (C═N); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 2.36 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 6.87–7.81 (m, 14H, ArH, H-5), 8.20 (s, 1H, –CH═N–), 9.52, 9.93, 10.98 (3s, 3H, 2OH, NH); Anal.Calcd for $C_{30}H_{24}N_4O_4$ (504.55): C, 71.42; H, 4.79; N, 11.10%. Found: C, 71.33; H, 4.63; N, 11.29%.

4-((N,N-Dimethyl-4-aminobenzaldehyde)hydrazono-1-yl)-2-methyl-5-phenyl-5H-benzo[f]chromeno[2,3-d] pyrimidin-7-ol ***(12d).*** Pale yellow crystals; yield (601 mg, 60%); mp = 279°C (Decomp); IR (KBr, ν/cm^{-1}): 3446 (OH), 3284 (NH), 1557 (C═N); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 2.34 (s, 3H, CH_3–2), 2.97 (s, 6H, –N $(CH_3)_2$), 4.43 (s, 1H, H-5), 6.79–7.81 (m, 14H, ArH), 8.25 (s, 1H, –CH═N–), 10.13, 11.07 (2s, 2H, OH, NH); Anal.Calcd for $C_{31}H_{27}N_5O_2$ (501.59): C, 74.23; H, 5.43; N, 13.96%. Found: C, 74.11; H, 5.30; N, 14.12%.

N'-(7-Hydroxy-2-methyl-5-phenyl-5H-benzo[f]chromeno[2,3-d]pyrimidin-4-yl)acetohydrazide (13). A solution of compound **11** (740 mg, 2 mmol) in glacial acetic acid (5 mL) was refluxed for 30 min. After finishing the reaction, it was poured into crushed ice (150 g) followed by stirring for additional 30 min. The formed precipitate was filtered off, washed well with water, dried, and recrystallized from EtOH to afford compound **13**. Gray crystals; yield (750 mg, 91%); mp = 240–241°C; IR (KBr, ν/cm^{-1}): 3100–3550 (br, OH, 2NH), 1680 (C═O), 1577 (C═N), 1232 (C–O); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 1.54 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.08 (s, 1H, H-5), 7.05–7.81 (m, 10H, ArH), 9.28, 9.78, 9.86 (3s, 3H, OH, 2NH);

Anal. Calcd for $C_{24}H_{20}N_4O_3$ (412.45): C, 69.89; H, 4.89; N, 13.58%. Found: C, 69.78; H, 4.73; N, 13.77%.

5-Methyl-14-phenyl-14H-benzo[f]chromeno[2,3-e][1,2,4]triazolo[3,4-c]pyrimidin-12-ol (14).

Procedure (A): A mixture of compound **11** (370 mg, 1 mmol) and DMF–DMA (0.22 mL, 1.5 mmol) in dry DMF (10 mL) was refluxed for 4 h. After finishing the reaction, it was poured into crushed ice (100 g) and stirred for 30 min. The formed precipitate was filtered off, dried, and recrystallized from EtOH to afford the product **14** (240 mg, 63%).

Procedure (B): A mixture of compound **11** (370 mg, 1 mmol) and formic acid (7 mL) was refluxed for 3 h, poured into cold water (100 mL), and neutralized using sodium carbonate. The formed precipitate was filtered off, dried, and recrystallized from EtOH to afford compound **14**. Pale brown crystals; yield (340 mg, 89%); mp = 219–220°C; IR (KBr, ν/cm^{-1}): 3425 (OH), 1599 (C═N); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 2.50 (s, 3H, CH_3), 5.95 (s, 1H, H-5), 6.99–7.83 (m, 10H, ArH); 8.76 (s, 1H, H-3), 9.98 (s, 1H, OH); Anal. Calcd for $C_{23}H_{16}N_4O_2$ (380.41): C, 72.62; H, 4.24; N, 14.73%. Found: C, 72.46; H, 4.03; N, 14.91%.

4-(3',5'-Dimethyl-1H-pyrazol-1-yl)-2-methyl-5-phenyl-5H-benzo[f]chromeno[2,3-d]pyrimidin-7-ol (15). A solution of compound **11** (370 mg, 1 mmol) in EtOH (10 mL) was added to a solution of acetyl acetone (0.1 mL, 1 mmol) in glacial acetic acid (15 mL). The reaction mixture was refluxed at 80°C for 1 h; thereafter, it was poured into cold water (100 mL). The formed precipitate was filtered off, dried, and recrystallized from EtOH (70%) to give compound **15**. Colorless crystals; yield (312 mg, 72%); mp = 242–243°C; IR (KBr, ν/cm^{-1}): 3447 (OH), 1596 (C═N), 1631 (C═C); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 1.69 (s, br, 1H, OH), 1.82 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 2.67 (s, 3H, CH_3), 6.11 (s, 1H, H-5), 6.19 (s, 1H, H-4′), 6.53–7.42 (m, 10H, ArH); Anal. Calcd for $C_{27}H_{22}N_4O_2$ (434.50): C, 74.64; H, 5.10; N, 12.89%. Found: C, 74.42; H, 5.01; N, 12.97%.

4-Amino-5-phenyl-5H-benzo[f]chromeno[2,3-d]pyrimidin-7-ol (16). A solution of compound **1** (628 mg, 2 mmol) in excess formamide (15 mL) was refluxed for 4 h. Thereafter, the mixture was poured into cold water (100 mL) and was stirred for 6 h. The formed precipitate was filtered off, dried, and recrystallized from EtOH to give the desired compound. Reddish brown crystals; yield (546 mg, 80%); mp = 288°C (Decomp); IR (KBr, ν/cm^{-1}): 3220–3500 (br, OH, NH_2), 1590 (C═N), 1231 (C–O); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 5.83 (s, 1H, H-5), 7.15 (s, 2H, NH_2), 7.00–7.77 (m, 10H, ArH), 8.06 (s, 1H, H-2), 9.87 (s, 1H, OH); Anal. Calcd for $C_{21}H_{15}N_3O_2$ (341.37): C, 73.89; H, 4.43; N, 12.31%. Found: C, 73.79; H, 4.26; N, 12.53%.

Synthesis of compounds 17a–d. A cold solution (temp. 0–5°C) of aryl diazonium chloride (2 mmol) [prepared by mixing aromatic amine (2 mmol), sodium nitrite (207 mg, 3 mmol), and conc. HCl (0.6 mL, 6 mmol) in 5 mL water] was added to a cold solution of compound **16** (682 mg, 2 mmol) in 10 mL pyridine with continuous stirring for 1–2 h (TLC control). The formed precipitate washed well with water and recrystallized from EtOH to afford the desired products.

*4-Amino-5-phenyl-6-(phenyldiazenyl)-5H-benzo[f]chromeno[2,3-d]pyrimidin-7-ol (**17a**).* Red crystals; yield (739 mg, 83%); mp = 281–282°C; IR (KBr, ν/cm^{-1}): 3461 (OH), 3301, 3126 (NH_2), 1564 (C═N), 1426 (N═N), 1242 (C–O); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 6.65 (s, 1H, H-5), 6.77 (d, 1H, H-8, J = 9 Hz), 6.99 (s, 2H, NH_2), 7.02–7.27 (m, 11H, ArH, OH), 7.42 (d, 1H, H-11, J = 8 Hz), 7.85 (d, 1H, H-10, J = 8 Hz), 7.95 (d, 1H, H-9, J = 9 Hz), 8.11 (s, 1H, H-2); Anal. Calcd for $C_{27}H_{19}N_5O_2$ (445.48): C, 72.80; H, 4.30; N, 15.72%. Found: C, 72.66; H, 4.21; N, 15.89%.

*4-Amino-5-phenyl-6-(4-methoxyphenyldiazenyl)-5H-benzo[f]chromeno[2,3-d]pyrimidin-7-ol (**17b**).* Red crystals; yield (846 mg, 89%); mp = 272°C; IR (KBr, ν/cm^{-1}): 3486 (OH), 3387, 3319 (NH_2), 1564 (C═N), 1429 (N═N), 1248 (C–O); ^{1}H NMR (400 MHz, $CDCl_3$): 3.93 (s, 3H, CH_3), 5.11 (s, 1H, H-5), 6.84–7.70 (m, 16H, ArH, NH_2, OH), 8.34 (s, 1H, H-2); Anal.Calcd for $C_{28}H_{21}N_5O_3$ (475.51): C, 70.73; H, 4.45; N, 14.73%. Found: C, 70.61; H, 4.32; N, 14.81%.

*4-Amino-5-phenyl-6-(4-chlorophenyldiazenyl)-5H-benzo[f]chromeno[2,3-d]pyrimidin-7-ol (**17c**).* Reddish brown crystals; yield (873 mg, 91%); mp = 282–283°C; IR (KBr, ν/cm^{-1}): 3481 (OH), 3312, 3108 (NH_2), 1563 (C═N), 1429 (N═N), 1244 (C–O); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 5.06 (s, 1H, H-5), 6.67–7.61 (m, 16H, ArH, NH_2, OH), 8.34 (s, 1H, H-2); (EIMS) *m/z* (%): 478.9 [M$^+$] (8.3), 481.0 [M$^+$+2] (3.1), 451.0 (3.5), 376.0 (10.9), 373.9 (37.9), 354.0 (18.8), 352.9 (100.0), 338.0 (19.2), 335.0 (16.0), 326.0 (22.0), 311.0 (17.5), 263.9 (46.6), 127.0 (23.8); Anal.Calcd for $C_{27}H_{18}ClN_5O_2$ (479.52): C, 67.57; H, 3.78; N, 14.59%. Found: C, 67.41; H, 3.62; N, 14.70%.

*4-Amino-5-phenyl-6-(pyridin-3-yldiazenyl)-5H-benzo[f]chromeno[2,3-d]pyrimidin-7-ol (**17d**).* Reddish brown crystals; yield (839 mg, 94%); mp = 287–288°C; IR (KBr, ν/cm^{-1}): 3452 (OH), 3296, 3132 (NH_2), 1567 (C═N), 1426 (N═N), 1241 (C–O); ^{1}H NMR (400 MHz, $CDCl_3$): 5.10 (s, 1H, H-5), 6.69–8.91 (m, 16H, ArH, NH_2, OH), 8.34 (s, 1H, H-2); Anal. Calcd for

$C_{26}H_{18}N_6O_2$ (446.47): C, 69.95; H, 4.06; N, 18.82%. Found: C, 69.82; H, 3.88; N, 18.95%.

Synthesis of compounds 18 and 19. A mixture of compound **16** (682 mg, 2 mmol) and chloroacetone (0.3 mL, 3.6 mmol) or ethyl chloroacetate (0.3 mL, 2.8 mmol) was refluxed in dry acetone (20 mL) in the presence of anhydrous K_2CO_3 (1 g) for 7 h. Thereafter, the mixture was poured into cold water (150 mL) and neutralized by using few drops of conc. HCl. The formed precipitate was filtered off, dried, and recrystallized from EtOH to give compounds **18** or **19**, respectively.

1-((4-Amino-5-phenyl-5H-benzo[f]chromeno[2,3-d] pyrimidin-7-yl)oxy)propan-one (18). Gray crystals; yield (683 mg, 86%); mp = 220–221°C; IR (KBr, ν/cm^{-1}): 3530, 3456 (NH_2), 1734 (C═O), 1230 (C–O); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 2.22 (s, 3H, CH_3CO), 4.93 (s, 2H, CH_3COCH_2), 5.85 (s, 1H, H-5), 7.06–7.86 (m, 12H, ArH, NH_2), 8.09 (s, 221H, H-2); Anal.Calcd for $C_{24}H_{19}N_3O_3$ (397.43): C, 72.53; H, 4.82; N, 10.57%. Found: C, 72.40; H, 4.67; N, 10.71%.

Ethyl 2-((4-amino-5-phenyl-5H-benzo[f]chromeno[2,3-d] pyrimidin-7-yl)oxy)acetate (19). Gray crystals; yield (743 mg, 87%); mp = 170°C; IR (KBr, ν/cm^{-1}): 3392, 3188 (NH2), 1738 (C═O), 1587 (C═N), 1220 (C–O); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 1.19 (t, 3H, J = 7 Hz, CH_3CH_2O), 4.15 (q, 2H, J = 7 Hz, CH_3CH_2O), 4.93 (s, 2H, $CH_3CH_2OCOCH_2$), 5.88 (s, 1H, H-5), 7.16–7.87 (m, 12H, ArH, NH_2), 8.09 (s, 1H, H-2); Anal.Calcd for $C_{25}H_{21}N_3O_4$ (427.46): C, 70.25; H, 4.95; N, 9.83%. Found: C, 70.11; H, 4.72; N, 9.98%.

9-Methyl-5-phenyl-5H-1-benzofuro[6,5-f]chromeno[2,3-d] pyrimidin-4-amine (20). A mixture of phosphorus pentoxide (26 g) in phosphoric acid 85% (15 mL) was heated in water bath at 100°C. After 30 min, compound **18** (794 mg, 2 mmol) was added with stirring. The reaction mixture was heated in water bath for 1 h. After finishing the reaction, it was carefully poured into crushed ice (100 g), and the medium was neutralized using sodium acetate. The formed precipitate was filtered off, dried, and purified by preparative chromatography using petroleum ether : ethyl acetate (50:50) to obtain the product **20**. Pale yellow powder; yield (395 mg, 52%); mp = 264°C (Decomp); IR (KBr, ν/cm^{-1}): 3270, 3144 (NH_2), 1561 (C═N), 1245 (C–O); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 1.20 (s, 3H, CH_3), 5.99 (s, 1H, H-5), 7.10–7.27 (br, 2H, NH_2), 7.01–7.81 (m, 9H, ArH), 8.05, 8.07 (2s, 2H, H-8, H-2); Anal. Calcd for $C_{24}H_{17}N_3O_2$ (379.42): C, 75.98; H, 4.52; N, 11.08%. Found: C, 75.73; H, 4.43; N, 11.21%.

4-Amino-5-phenyl-5H-1-benzofuro[6,5-f]chromeno[2,3-d] pyrimidin-9(8H)-one (21). A mixture of phosphorus pentoxide (26 g) in phosphoric acid 85% (15 mL) was heated in water bath at 100°C. After 30 min, compound **19** (854 mg, 2 mmol) was added with stirring. The reaction mixture was heated in water bath for 2 h. After finishing the reaction, it was carefully poured into crushed ice (100 g), and the medium was neutralized using sodium acetate. The formed precipitate was filtered off, dried, and recrystallized from EtOH (85%) to give the product **21**. Black crystals; yield (496 mg, 65%); mp = 293°C (Decomp.); IR (KBr, ν/cm^{-1}): 3458, 3350 (NH_2), 1725 (C═O), 1567 (C═N), 1228 (C–O); ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 4.81 (s, 2H, H-8), 5.84 (s, 1H, H-5), 7.05 (br, 2H, NH_2), 7.05–7.82 (m, 9H, ArH), 8.08 (s, 1H, H-2); Anal. Calcd for $C_{23}H_{15}N_3O_3$ (381.39): C, 72.43; H, 3.96; N, 11.02%. Found: C, 72.33; H, 3.72; N, 11.15%.

Biological evaluation. ***Antioxidant activity.ABTS assay.*** The reaction mixture consisted of 2 mL of ABTS[25] solution (60 μ*M*) was added to MnO_2 (3 mL, 25 mg/mL) solution, all prepared in aqueous phosphate buffer solution (PH 7, 0.1*M*). The mixture was shaken, centrifuged, and filtered. The absorbance ($A_{control}$) of the resulting bluish green solution at 734 nm was adjusted to approximately 0.5. Then, the absorbance was measured upon the addition of 50 μL of (2 m*M*) solution of the tested compounds in spectroscopic grade MeOH/phosphate buffer (1:1) (A_{tested}). The inhibition ratio was calculated using the following equation:

$$\text{Inhibition}\ (\%) = (A_{control} - A_{tested})/A_{control} \times 100$$

L-Ascorbic acid (Vitamin C) was used as standard antioxidant (positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of the tested samples. Negative control sample was run with ABTS and MeOH/phosphate buffer (1:1) only instead of the tested samples.

DPPH assay. Solutions of the synthesized compounds were prepared in methanol at concentration of 60 μg/mL, and then 1 mL of each sample was transferred to 1 mL of 0.012% DPPH[26] in methanol. The mixture was incubated in dark for 30 min at room temperature, and then the absorbance of each mixture was measured at 517 nm. The inhibition percentage of the samples was calculated using the following equation:

$$\text{Inhibition}\ (\%) = (A_{control} - A_{tested})/A_{control} \times 100$$

L-Ascorbic acid was used as a reference to compare the activities of the newly screening compounds. Negative control sample was run with DPPH solution only without the tested compounds.

Antimicrobial activity. The antimicrobial activity of chromenopyrimidines was estimated by filter paper disc method using inoculums containing 10^6 bacterial and fungal cells/mL to spread on nutrient agar.[27] The

sterilized filter paper discs (Whatman no. 1, 6 mm in diameter) were saturated with the solution of the tested compounds in DMSO (0.01 g/mL), and another filter paper disc was saturated with DMSO to serve as control. The discs were placed on the surface of the agar plates seeded with the tested organisms. The plates were incubated at 28°C for fungi and at 37°C for bacteria. Diameters of the inhibition zone (mm) were measured after 18–24 h. The stock cultures of the tested organisms were obtained from the microbiological lab at the Faculty of Medicine in Mansoura University.

Molecular docking. Docking of the chromenopyrimidine analogs into the binding active site of human peroxiredoxin (code: 1HD2),[28] oxidoreductase (code: 4C1M),[29] and glucosamine-6-phosphate synthase (code: 2VF5)[30] has been performed to examine the degree of recognition as antioxidant and antimicrobial leads. The X-ray crystal structure of the 1HD2, 4C1M, and 2VF5 was obtained from the RCSB Protein Data Bank of Brookhaven National Laboratory. The tested compounds were prepared by partial charges and energy minimized using the molecular mechanics force field "AMBER" until the RMS deviation was 0.01 Kcal/mol Å. The docking was carried out on using MOE.

***In silico* physicochemical evaluation.** The physicochemical properties such as molecular weight, partition coefficient value (LogP), which indicates the lipophilicity of the ligand, number of hydrogen bond donor and number of hydrogen bond acceptor, PSA, number of rotatable bonds, and volume for the ligands were obtained as shown in Table 4. For a drug molecule to be orally bioavailable in the systemic circulation, Lipinski's rule of five should apply: "The drug must have molecular weight value of ≤500, hydrogen bond donor ≤5, hydrogen bond acceptor ≤10, and partition coefficient (LogP) value ≤5." From the results in Table 4, the synthesized compounds are in agreement with the Lipinski's rule of five because there was no more than two parameter violations. In addition, the PSA, which reflects the ligand hydrophilicity, is very vital in protein–ligand interaction.

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