Naringenin derivatives as glucosamine-6-phosphate synthase inhibitors: synthesis, antioxidants, antimicrobial, preservative efficacy, molecular docking and in silico ADMET analysis

Amit Lather[1], Sunil Sharma[2] and Anurag Khatkar[3]*

Abstract

Background: Preservatives have to be added in food, pharmaceuticals and cosmetics products to maintain their shelf life. However, the existing chemical based preservatives have been associated with severe side effects that compel the researchers to find better safe preservatives based on natural products. G-6-P synthase is an important enzyme for bacterial and fungal cell wall synthesis and offers as a potential target to find better G-6-P synthase inhibitors based antimicrobial compounds. Naringenin, a flavanone, has been reported for a wide range of pharmacological activities including antimicrobial activity, which makes it a potential candidate to be explored as novel G-6-P synthase inhibitor.

Results: The synthesis of naringenin derivatives with potent G-6-P synthase inhibitor having remarkable antioxidant, antimicrobial and preservative efficacy was performed. Among the synthesized compounds, the compound **1** possessed good antioxidant activity (IC_{50} value, 6.864 ± 0.020 μM) as compared to standard ascorbic acid (IC_{50} value, 8.110 ± 0.069 μM). The antimicrobial activity of synthesized compounds revealed compound **1** as the most potent compound (pMIC 1.79, 1.79, 1.49, 1.49, 1.49 and 1.49 μM/mL for *P. mirabilis, P. aeruginosa, S. aureus, E. coli, C. albicans* and *A. niger* respectively) as compared to standard drugs taken. The compound **2** showed comparable activity against *P. mirabilis* (pMIC 1.14 μM/mL), *C. albicans* (pMIC 1.14 μM/mL) while the compound **3** also showed comparable activity against *C. albicans* (pMIC 1.16 μM/mL) as well *A. niger* (pMIC 1.46 μM/mL), likewise the compound **4** showed comparable activity against *P. mirabilis* (pMIC 1.18 μM/mL) as compared to the standard drugs streptomycin (pMIC 1.06, 1.36, 1.06 and 1.96 μM/mL for *P. mirabilis, P. aeruginosa, S. aureus* and *E. coli* respectively), ciprofloxacin (pMIC 1.12, 1.42, 1.12 and 1.42 μM/mL for *P. mirabilis, P. aeruginosa, S. aureus* and *E. coli* respectively), ampicillin (pMIC 1.14, 0.84, 0.84 and 1.74 μM/mL for *P. mirabilis, P. aeruginosa, S. aureus* and *E. coli* respectively) and fluconazole (pMIC 1.08 and 1.38 μM/mL for *C. albicans* and *A. niger* respectively). The molecular docking with the target G-6-P synthase pdb id 1moq resulted with an better dock score for compound **1** (-7.42) as compared to standard antimicrobial drugs, ciprofloxacin

*Correspondence: dranuragkhatkarmdurtk@gmail.com;
anuragpharmacy@gmail.com
[3] Laboratory for Preservation Technology and Enzyme Inhibition Studies, Department of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, Haryana, India
Full list of author information is available at the end of the article

(— 5.185), ampicillin (— 5.065) and fluconazole (— 5.129) that supported the wet lab results. The preservative efficacy test for compound **1** in White Lotion USP showed the log CFU/mL value within the prescribed limit and results were comparable to standard sodium benzoate, ethyl paraben and propyl paraben as per USP standard protocol.

Conclusions: The synthesized naringenin derivatives exhibited significant G-6-P synthase inhibitory potential with good selectivity towards the selected target G-6-P synthase. Compound **1**, bearing nitro group showed good antioxidant, antimicrobial and preservative efficacy compared with the standard drugs taken. The mechanistic insight about the compounds within the active site was completed by molecular docking that supported the results for novel synthesized G-6-P synthase inhibitors.

Keywords: G-6-P synthase, Naringenin derivatives, DPPH, Preservative efficacy

Introduction

The use of packaged foods containing various additive's viz. artificial sweeteners, colorants, stabilizers, preservatives etc. has greatly increased in recent years. As per recent data available it is estimated that 75% of the contemporary diet is packaged food and on an average every person consumes 3.6 to 4.5 kg of food additives per year [1].

Among other additives the preservative such as sodium benzoate, ethyl paraben, propyl paraben, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), etc. plays a vital role to maintain the shelf life of various food, pharmaceuticals and cosmetic products [2–4]. However, the existing chemical preservatives have been associated with serious side effects viz. estrogenic effect, breast cancer, malignant melanoma, contact eczema, endocrine disruption, etc. [5–12]. Hence, there is an urgent need for the discovery of novel and safer preservatives for use in food, pharmaceuticals and cosmetic products.

G-6-P synthase is a complex enzyme involved in the formation of UDP-N-acetyl glucosamine and catalyzes the initial step in hexosamine biosynthesis. One of these catalyzed products, N-acetyl glucosamine, is an important part of the peptidoglycan layer of bacterial and fungal cell wall. Hence, G-6-P synthase may act as potential target for discovery of novel antimicrobial compounds which could be evaluated for their preservative efficacy to find better and safe preservatives [13, 14].

The complex 3-D crystal structure of G-6-P synthase can be utilized for molecular docking to explore the structural requirements for the pharmacophore complex. Flavonoids such as luteolin, catechin,   (4S)-2-Methyl-2-phenylpentane-1,4-diol, 7-Methoxy-2,3-dihydro-2-phenyl-4 quinolone, 3-(tert-Butoxycarbonyl)-6-(3 benzoylprop-2-yl)phenol and (3R,4S)-4-(methylamino)-1-phenylpent-1-en-3-ol also have been explored for G-6-P synthase inhibition [15–18]. Some flavonoids along with their G-6-P synthase inhibitory dock score have been shown in Fig. 1.

Naringenin is a naturally occurring bioflavonoid present in various fruits, vegetables and honey which is used as a dietary supplement due to its low toxicity [19–21]. Naringenin has been reported for its diverse pharmacological profile including its antibacterial property as shown in Fig. 2 [22–41].

Further, naringenin could be utilized as a potential candidate for evaluation of its G-6-P synthase inhibitory response. Hence, it was planned to synthesize and investigate the naringenin derivatives for their antioxidant, antimicrobial, preservative efficacy and in silico evaluation for G-6-P synthase inhibition.
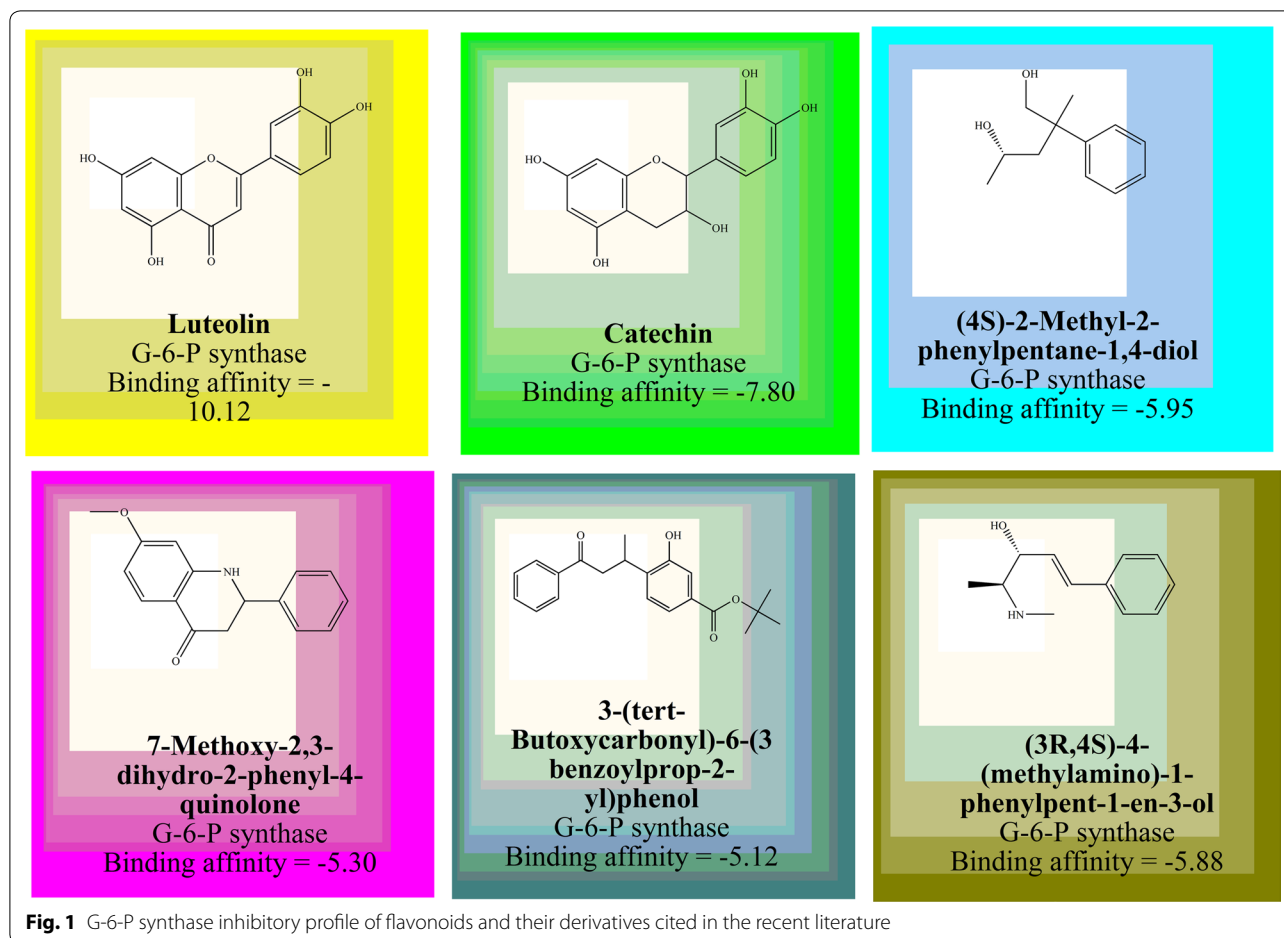
Results and discussion

Chemistry

Naringenin derivatives were synthesized according to Kriza et al. 2011 with slight modifications as shown in Scheme 1 [42]. The chemical structures of all the synthesized compounds were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy and elemental analysis which were in agreement with the structures.

For the synthesis of naringenin derivatives substituted aniline (0.01 mol) was taken in a round bottom flask and concentrated hydrochloric acid was added drop wise with continuous stirring. Equimolar concentration of naringenin (0.01 mol) was dissolved in ethanol (50 mL) and was refluxed for 80–100 h at 80 °C on heating mantle. All the compounds in series were synthesized according to the standard procedure outlined in Scheme 1. Completion of reaction was confirmed by TLC under UV lamp and FTIR spectra.

Formation of compound **1, 2, 3** and **4** was confirmed by peaks of IR, NMR, mass spectroscopy. In positive chemical ionization most of the naringenin derivatives showed (M++1), M+ (molecular ion peak), (M++2) and in negative chemical ionization mode showed (M+1), (M+2), M+. The elemental analysis established the synthesis of naringenin derivatives where the percentage of C, H and N in the synthesized compounds was observed within defined limits. The reaction mixture was concentrated,

Lather *et al. BMC Chemistry*   (2020) 14:41

Page 3 of 15

Fig. 1 G-6-P synthase inhibitory profile of flavonoids and their derivatives cited in the recent literature

after that precipitates formed were filtered off and dried. Crude products were recrystallized by alcohol which yielded the final compounds **1–4**.

Antioxidant activity
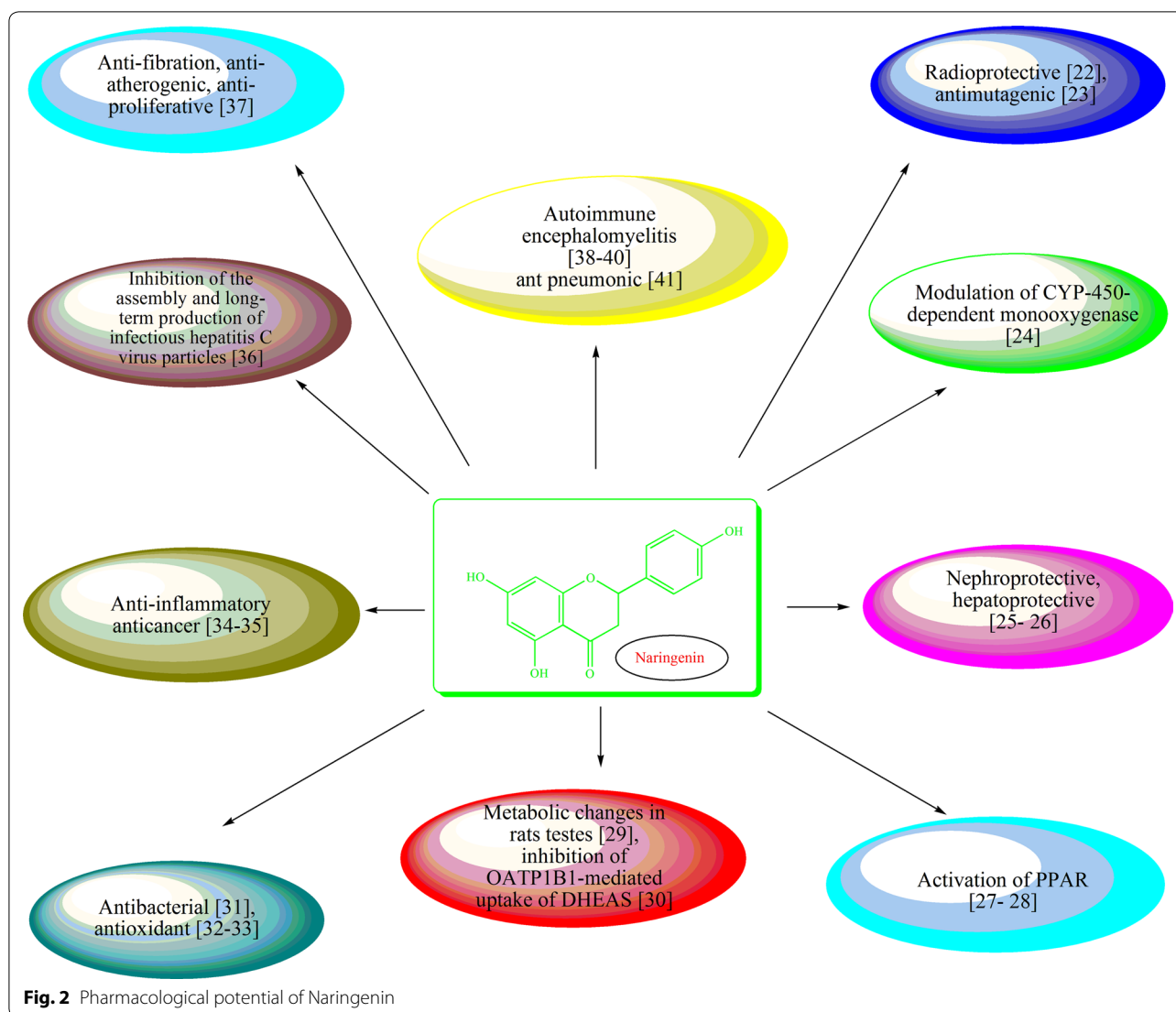### DPPH radical scavenging activity
All the synthesized compounds were evaluated for antioxidant profile by using DPPH radical scavenging assay method (Table 1). The compound **1** was observed as the most potent antioxidant compound (IC_{50} 6.864 ± 0.020 μM) as compared to standard L-ascorbic acid (IC_{50} 8.110 ± 0.069 μM). However, compounds **3** and **4** showed moderate antioxidant activity (IC_{50} 7.170 ± 0.028 μM and 7.801 ± 0.077 μM, respectively) as compared to standard. The electron withdrawing strongly deactivating nitro group in compound **1** may be responsible for better antioxidant activity. The presence of weakly deactivating electron withdrawing chloro and fluoro groups present in compound 3 and 4 have moderate antioxidant activity. IC_{50} value of synthesized naringenin derivatives has been shown in Fig. 3.

Antimicrobial activity
Minimum inhibitory concentration
The antimicrobial activity of synthesized compounds revealed compound **1** as the most potent compound (pMIC 1.79, 1.79, 1.49, 1.49, 1.49 and 1.49 μM/mL for *P. mirabilis, P. aeruginosa, S. aureus, E. coli, C. albicans* and *A. niger* respectively) as compared to standard drugs taken. The compound **2** showed comparable activity against *P. mirabilis* (pMIC 1.14 μM/mL), *C. albicans* (pMIC 1.14 μM/mL) while the compound **3** also showed comparable activity against *C. albicans* (pMIC 1.16 μM/mL) as well *A. niger* (pMIC 1.46 μM/mL), likewise the compound **4** showed comparable activity against *P. mirabilis* (pMIC 1.18 μM/mL) as compared to the standard drugs streptomycin (pMIC 1.06, 1.36, 1.06 and 1.96 μM/mL for *P. mirabilis, P. aeruginosa, S. aureus* and *E. coli,* respectively), ciprofloxacin (pMIC 1.12, 1.42, 1.12 and 1.42 μM/mL for *P. mirabilis, P. aeruginosa, S. aureus* and *E. coli,* respectively), ampicillin (pMIC 1.14, 0.84, 0.84 and 1.74 μM/mL for *P. mirabilis, P. aeruginosa, S. aureus* and *E. coli,* respectively) and fluconazole (pMIC 1.08 and 1.38 μM/mL for *C. albicans* and *A. niger,* respectively). In

Lather *et al. BMC Chemistry*   (2020) 14:41

Page 4 of 15

Fig. 2 Pharmacological potential of Naringenin

general, the results of MIC studies (Table 2) revealed that the synthesized compounds have better anti bacterial and anti fungal potential as compared to standard drugs streptomycin, ciprofloxacin, ampicillin and fluconazole. The graphically representation of the pMIC values of test and standard compounds have been shown in Fig. 4.

Preservative efficacy study

The most active antimicrobial compound **1** was selected for the evaluation of its preservative efficacy. The results of preservative efficacy testing performed in triplicate and were reported as mean values in Table 3.
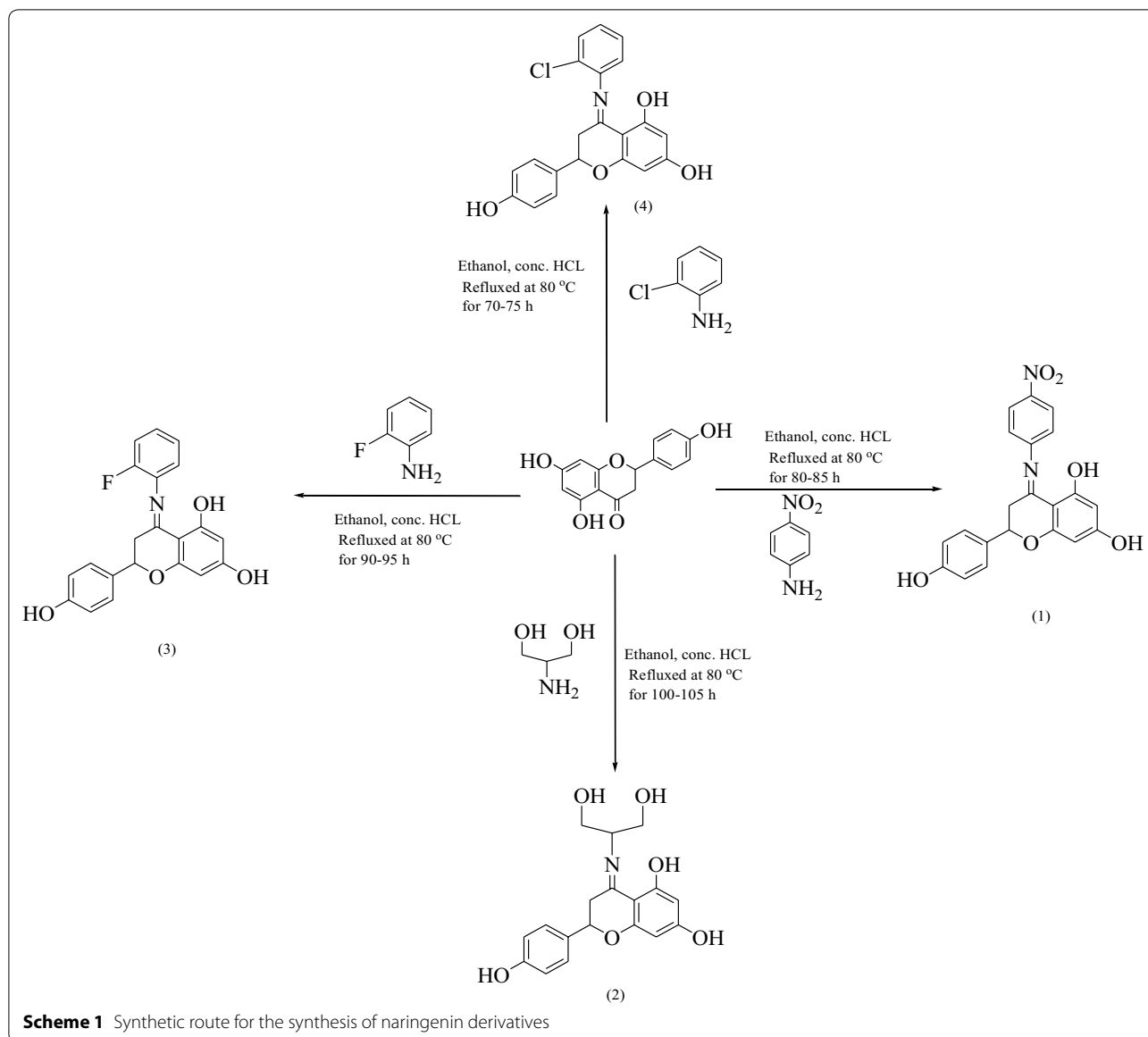
Compound **1** showed the values of log CFU/mL reduction within the prescribed limit and the results were comparable to that of the standard preservatives sodium benzoate, propyl paraben and methyl paraben. The preservative efficacy of compound **1** in White lotion USP

and degree of microbial log reduction has been represented in Fig. 5.

Structure activity relationship (SAR) studies

Design strategy of naringenin derivative for G-6-P inhibition and antioxidant activity has been represented in Fig. 6. The structure activity relationship of the synthesized naringenin derivatives with their antioxidant activity results were summarized as:

(1) Substitution of naringenin with aliphatic amines produced biological activity but aromatic substitution showed greater activity than aliphatic i.e. compound **2** showed the lowest activity as compared to other.

Lather *et al. BMC Chemistry*   (2020) 14:41

Page 5 of 15

Scheme 1 Synthetic route for the synthesis of naringenin derivatives

Table 1 Antioxidant IC$_{50}$ values of synthesized compounds

S. no.	Compound(s)	IC$_{50}$ (µM)[a]
1.	Compound **1**	*6.864 ± 0.020*
2.	Compound **2**	26.210 ± 0.151
3.	Compound **3**	7.170 ± 0.028
4.	Compound **4**	7.801 ± 0.077
5.	Naringenin	*13.765 ± 0.408*
6.	Standard (ʟ-ascorbic acid)	*8.110 ± 0.069*

[a] Values are expressed as mean ± SEM, n = 3

(2) Substitution with aromatic amine at para position increased the activity with increase in electronega-tivity i.e. compound **1** was more active than compound **3** and **4**.

(3) Replacement of para position with nitro group produced the highest activity i.e. compound **1** was most active in the series.

(4) Exchange at para position produced more activity as compared to ortho position substitution.

Molecular docking study

Molecular docking studies were carried out to identify the binding affinities and interaction between the inhibitors and pdb id 1moq of G-6-P synthase protein by using Glide software (Schrodinger Inc. U.S.A. Maestro version 11). Dock score and binding of compound 1, 2, 3 and 4

Lather *et al. BMC Chemistry*   (2020) 14:41

Page 6 of 15
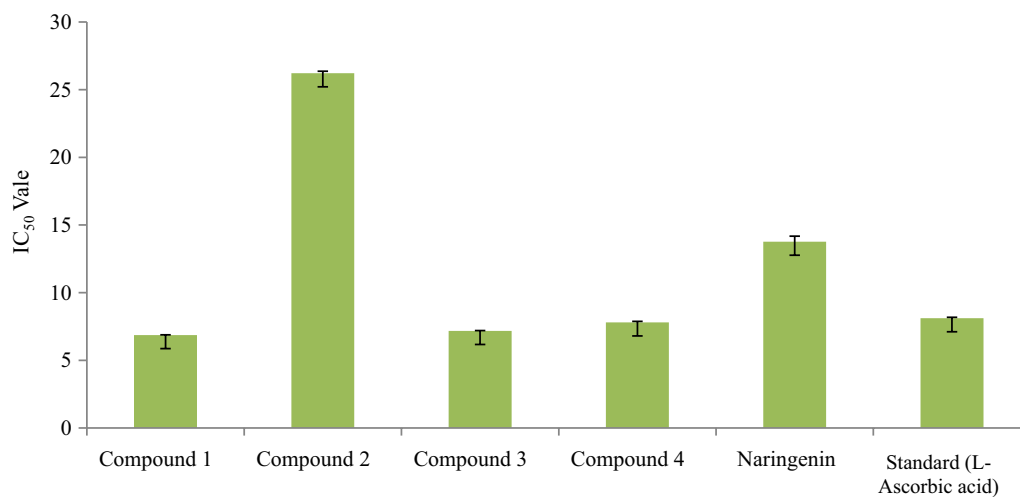
Fig. 3 IC_{50} value of different synthesized compunds with respect to standard L-ascarbic acid

Table 2 pMIC values (µM/mL) of synthesized naringenin derivatives against different standard microbial strains

| Compound(s)	PMIC values in µM/mL					
	P. mirabilis	*P. aeruginosa*	*S. aureus*	*E. coli*	*C. albicans*	*A. niger*
Compound **1**	*1.79*	*1.79*	*1.49*	*1.49*	*1.49*	*1.49*
Compound **2**	1.14	1.14	0.83	1.14	1.14	0.83
Compound **3**	0.86	1.16	0.86	0.86	1.16	1.46
Compound **4**	1.18	0.88	0.88	1.18	0.88	1.18
Naringenin	*< 0.73*	*< 0.73*	*< 0.73*	*< 0.73*	*< 0.73*	*< 0.73*
Streptomycin	*1.06*	*1.36*	*1.06*	*1.96*	–	–
Ciprofloxacin	*1.12*	*1.42*	*1.12*	*1.42*	–	–
Ampicillin	*1.14*	*0.84*	*0.84*	*1.74*	–	–
Fluconazole	–	–	–	–	*1.08*	*1.38*
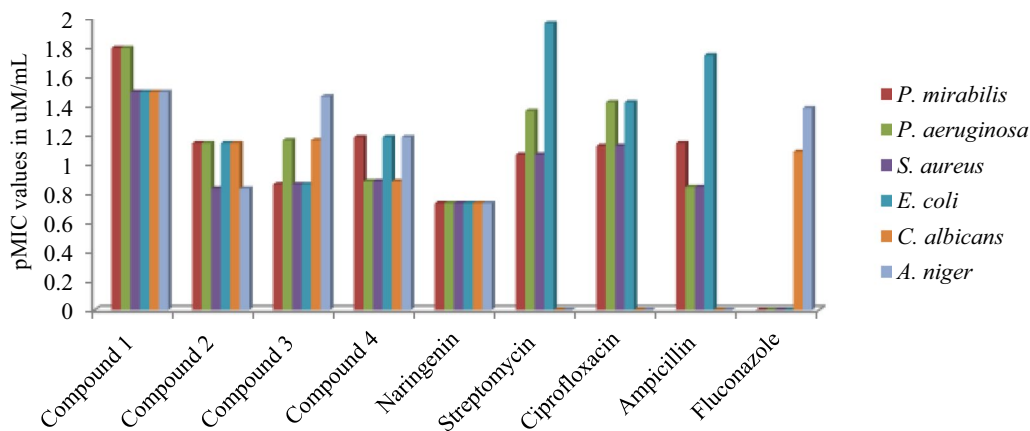
Fig. 4 Antimicrobial activity (pMIC in µM/mL) of synthesized naringenin derivatives against different microorganisms

Lather *et al. BMC Chemistry*   *(2020) 14:41*

Page 7 of 15

Table 3 Log CFU/mL values of the selected compound 1

Compound(s)	E. coli		P. aeruginosa		S. aureus		C. albicans		A. niger	
CFU/mL after days	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days
Compound **1**	3.190 ± 0.008	3.496 ± 0.12	3.306 ± 0.16	3.406 ± 0.016	3.486 ± 0.012	3.486 ± 0.012	3.200 ± 0.081	3.313 ± 0.016	3.306 ± 0.016	3.463 ± 0.020
Sodium Benzoate	3.213 ± 0.012	3.323 ± 0.24	3.282 ± 016	3.210 ± 0.037	3.863 ± 0.044	3.166 ± 0.047	3.076 ± 0.088	2.800 ± 0.081	3.166 ± 0.012	3.320 ± 0.014
Propyl Paraben	3.280 ± 0.57	3.246 ± 0.36	3.310 ± 0.016	3.306 ± 0.016	3.883 ± 0.023	3.516 ± 0.012	3.940 ± 0.028	3.530 ± 0.016	3.113 ± 0.065	3.403 ± 0.012
Ethyl Paraben	3.336 ± 0.020	3.090 ± 0.148	3.246 ± 0.36	3.340 ± 0.014	3.166 ± 0.047	3.210 ± 0.008	3.520 ± 0.014	3.200 ± 0.018	3.043 ± 0.041	3.300 ± 0.081

Initial microbial count in inoculums $1 \times 10^5 - 1 \times 10^6$

Lather *et al. BMC Chemistry*   (2020) 14:41

Page 8 of 15
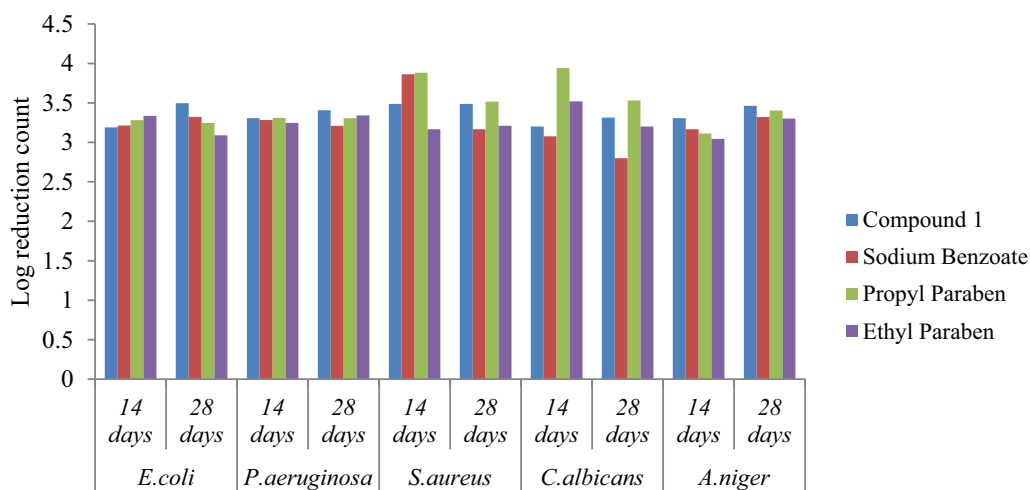
Fig. 5 Preservative efficacy of compound **1** in White lotion USP and degree of microbial log reduction
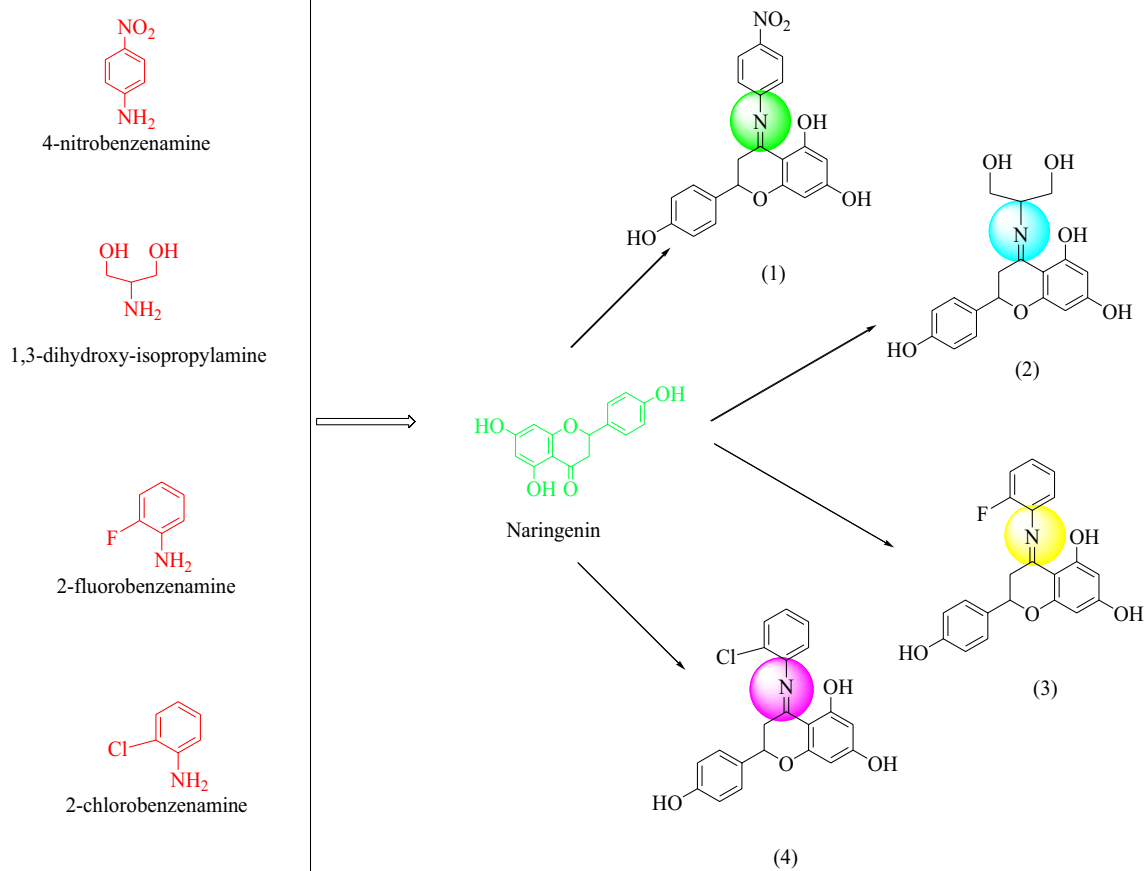
Fig. 6 Design strategy of Naringenin derivatives for G-6-P synthase inhibition and antioxidant activity

Lather *et al. BMC Chemistry* (2020) 14:41

Page 9 of 15

with G-6-P synthase have been shown in Table 4 and Fig. 7. After, docking results of compound **1** with G-6-P synthase protein suggested the formation of the hydrogen bond between NO_2 and Thr 402. Additionally, the molecule has been stabilized by residues such as Ser 347, Thr 352, Ser 303, Gln 348, Ala 602, Asn 600 and Asp 354. The binding orientation of compound **2** within the catalytic site of G-6-P synthase exhibited backbone hydrogen bonding with Glu 488. The molecule is stabilized by residues such as Asp 354, Lys 603, Glu 488, Lys 487 and Ala 400. The compound **3** showed interaction with Arg 599. The molecule was enclosed by residues such as Val 399, Thr 302, Lys 487 and Leu 484. In compound **4** hydrogen bonding was shown by Thr 606 and ligand was entrapped by the residue sequence of Val 399, Lys 487, Cys 300 and Ser 328. Docking results of G-6-P synthase showed that the synthetic compounds have comparable docking score as compared to the standard drugs taken. All the ligands showed variable degrees of hydrogen bond interaction, hydrophobic interactions, electrostatic interactions, ionic interactions and π–π stacking with the various amino acid residues in the binding pockets of G-6-P synthase.
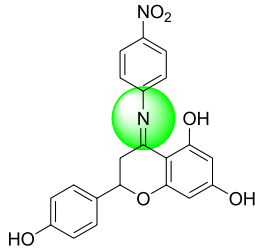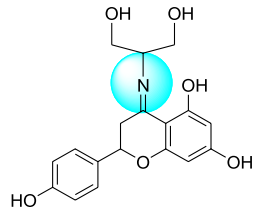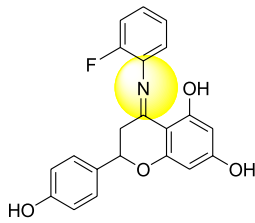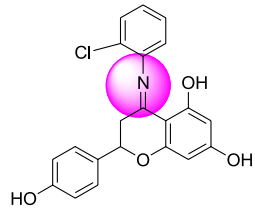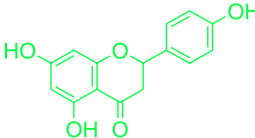
ADME study

The evaluation of different ADME parameters has been represented in Table 5. It was observed that all the synthesized compounds fulfilled the standard Rule of Five [43]. All the synthesized compounds qualified the conditions for various descriptors like LogP, HBA, HBD and MW. All these parameters were in suitable range for drug-like characteristics. In addition, according to Veber et al., 2002 for better bioavailability rotatable bonds should be ≤ 10 as the rotatable bonds in ligand impart elasticity [44]. The values of QPlogBB should be > 1.0 CNS active compounds and value < 1.0 CNS inactive compounds. QPPCaco cell permeability should be in a range from 4–70 [45–47]. In the present study, all the synthesized compounds exhibited a suitable drug-like profile.

Conclusion

In conclusion, the above mentioned wet and dry laboratory studies highlight the underlying mechanism of G-6-P synthase inhibition. The rational development of inhibitors and specificity of naringenin derivatives to be discovered as the novel preservatives. Moreover, the synthesized compounds were also found as wonderful antioxidants towards DPPH with remarkable potential as compared to the reference compounds.

Table 4 G-6-P synthase inhibition showed by synthesized naringenin derivatives

| S. no.	Compound(s)	Structure of G-6-P synthase inhibitors	Dock score
1.	Compound **1**		− 7.42
2.	Compound **2**		− 4.29
3.	Compound **3**		− 3.30
4.	Compound **4**		− 4.02
5.	Naringenin		− 6.36

Lather *et al. BMC Chemistry*   (2020) 14:41

Page 10 of 15

Table 4 (continued)

S. no.	Compound(s)	Structure of G-6-P synthase inhibitors	Dock score
6.	Standard	Streptomycin	− 5.795
		Ciprofloxacin	− 5.185
		Ampicillin	− 5.065
		Fluconazole	− 5.129

Experimental

Materials and methods

All the chemicals required for experiments were of analytical grade and were purchased from Loba Che-mie (Mumbai, India), SRL (Mumbai, India), and Sigma Aldrich (Germany). Nutrient agar, nutrient broth, sab-ouraud dextrose agar and sabouraud dextrose broth required for antimicrobial and preservative efficacy were obtained from Hi-media Laboratories. Streptomycin,
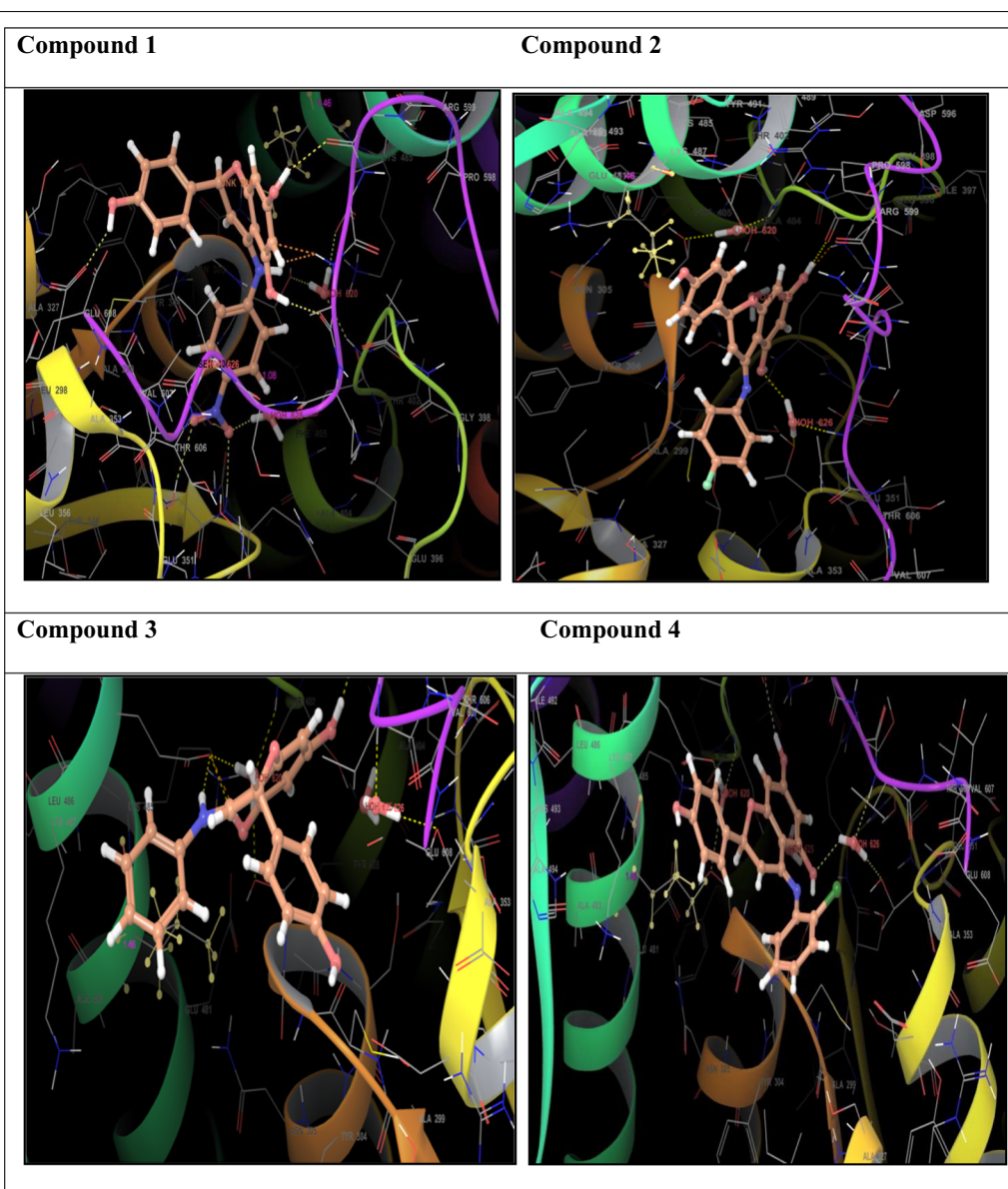
Fig. 7 Binding of compounds **1, 2, 3** and **4** with G-6-P synthase

Table 5 ADMET profile of various newly synthesized naringenin derivatives

Compound(s)	Mol. Wt.	No. of rotatable bond	DonorHB	AcceptHB	QPlogPo/w	QPlogBB	QPPMDCK	QPPCaco
Compound **1**	392.10	5	5	4	2.084	0.081	0.053	1.877
Compound **2**	345.12	3	3	3	2.490	0.138	11.251	2.773
Compound **3**	365.11	4	2	4	4.29	2.445	0.282	10.982
Compound **4**	381.08	3	4	2	1.278	3.355	0.162	20.169

ciprofloxacin, ampicillin and fluconazole were obtained as gift sample from Belco Pharma, Bahadurgarh, India. Microbial strains *S. aureus MTCC* 3160, *P. aeruginosa MTCC* 1934, *E. coli MTCC* 45, *C. albicans MTCC* 183 and *A. niger MTCC* 282 strains were purchased from MTCC, Chandigarh, India. Chemical reactions were monitored by TLC on silica gel plates in iodine and UV chambers. Sonar melting point apparatus in open capillary tube was used for the recording of melting points. ^{1}H NMR and ^{13}C NMR spectra were confirmed in DMSO and deuterated $CDCl_3$ on Bruker Avance II 400 NMR spectrometer at a frequency of 400 MHz downfield to tetramethyl silane standard. FTIR spectra were recorded on Perkin Elmer FTIR spectrophotometer with the help of KBr pellets technique. Waters Micromass Q-ToF Micro instrument was used for Mass spectrum recording.

General procedure for the synthesis of naringenin derivatives

Substituted aniline (0.01 mol) was taken in a round bottom flask, concentrated hydrochloric acid was added drop wise with continuous stirring. Equimolar concentration of naringenin (0.01 mol) was dissolved in ethanol (50 mL) and was re fluxed for 80-100 h on heating mantle. All the compounds in the series were synthesized according to the standard procedures as outlined in Scheme 1. Completion of reaction was monitored by TLC. Reaction mixture was concentrated and the precipitates formed were filtered off and dried. The crude product was recrystallized using alcohol which yielded the final compounds **1-4**.

Spectral data

2-(4-hydroxyphenyl)-4-(4-nitrophenylimino) chroman-5, 7-diol

R_f TLC mobile phase: Chloroform: Acetone (8:5) = 0.63; Yield = 55%; M.P. = 190–192 °C; M.Wt. = 317.29; IR (KBr pellets) cm^{-1}: 1081 (–C–O–C), 1156 (–C–C–), 1305 (–NO_2), 1599 (–C=C–), 1632 (–C=N–), 2921 (–C–H–), 3479 (–OH–); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.94 (s, 1H), 11.10 (s, 1H), 8.17 (d, *J* = 8.5 Hz, 2H),

8.04 (s, 1H), 7.29 (d, *J* = 9.3 Hz, 2H), 7.28 (d, *J* = 7.5 Hz, 2H), 6.80 (d, *J* = 7.3 Hz, 2H), 6.28 (s, 1H), 6.27 (s, 1H), 5.28 (t, *J* = 9.0 Hz, 1H), 3.15 (d, *J* = 7.3 Hz, 1H), 3.00 (d, *J* = 7.3 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 166.11, 165.34, 163.98, 161.90, 153.71, 152.59, 146.42, 133.96, 131.14, 126.43, 125.72, 124.08, 123.64, 117.42, 103.05, 97.89, 95.36, 77.13, 38.79, 27.19, 22.70; MS ES + (ToF): m/z 392.10 [M$^+$+2]; CHNS: Calc ($C_{12}H_{16}N_2O_2$): C, 64.28; H, 4.11; N, 7.14; O, 24.47; Found C, 64.25; H, 4.14; N, 7.17; O, 24.44.

4-(1,3-dihydroxypropan-2-ylimino)-2-(4-hydroxyphenyl) chroman-5,7-diol

R_f TLC mobile phase: Chloroform: Acetone (8:5) = 0.66; Yield = 50%; M.P. = 173–175 °C; M.Wt. = 345.32; IR (KBr pellets) cm^{-1}: 1074 (–C–O–C–), 1251 (–C–C–), 1513 (–C=C–), 1631 (–C=N–), 2831 (–C–H–), 3295 (–OH–); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.60 (s, 1H), 11.10 (s, 1H), 8.04 (s, 1H), 7.28 (d, *J* = 7.2 Hz, 2H), 6.80 (d, *J* = 7.3 Hz, 2H), 6.28 (s, 1H), 6.24 (s, 1H), 5.25–5.24 (m, 1H), 3.95 (d, *J* = 8.1 Hz, 2H), 3.64 (q, *J* = 9.0 Hz, 2H), 3.50 (q, *J* = 9.6 Hz, 2H), 3.47–3.45 (m, 1H), 3.13 (d, *J* = 8.6 Hz, 1H), 2.86 (d, *J* = 8.6 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 164.81, 162.47, 161.87, 161.58, 158.28, 130.84, 128.42, 115.98, 107.15, 103.33, 96.95, 78.20, 72.30, 63.75, 37.92, 27.60, 22.32, 14.16; MS ES + (ToF): m/z 345.12 [M$^+$+2]; CHNS: Calc ($C_{18}H_{19}NO_6$): C, 62.60; H, 5.55; N, 4.06; O, 27.80; Found C, 62.63; H, 5.52; N, 4.09; O, 27.82.

4-(2-fluorophenylimino)-2-(4-hydroxyphenyl) chroman-5,7-diol

R_f TLC mobile phase: Chloroform: Acetone (8:5) = 0.64; Yield = 23%; M.P. = 165-167 °C; M.Wt. = 365.35; IR (KBr pellets) cm^{-1}: 753 (–F–), 1082 (–C–O–C), 1241 (–C–C–), 1612 (–C=C–), 1632 (–C=N–), 2833 (–C–H–), 3350 (–OH–); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.78 (s, 1H), 11.10 (s, 1H), 8.04 (s, 1H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.31 (dt, *J* = 15.7, 8.4 Hz, 2H), 7.28–7.26 (m, 3H), 6.80 (d, *J* = 7.4 Hz, 2H), 6.31 (s, 1H), 6.28 (s, 1H), 5.33 (t, *J* = 8.5 Hz, 1H), 3.04 (d, *J* = 7.7 Hz, 1H), 2.92 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 165.92, 165.91,

165.24, 163.73, 161.86, 132.64, 132.61, 126.96, 126.94, 126.50, 126.48, 125.25, 114.89, 114.86, 102.91, 97.83, 95.53, 72.64, 39.18, 20.46; MS ES+ (ToF): m/z 365.11 [M^++2]; CHNS: Calc ($C_{21}H_{16}FNO_4$): C, 69.04; H, 4.41; F, 5.20; N, 3.83; O, 17.52; Found C, 69.01; H, 4.44; F, 5.23; N, 3.84; O, 17.55.

4-(2-chlorophenylimino)-2-(4-hydroxyphenyl) chroman-5,7-diol

R_f TLC mobile phase: Chloroform: Acetone (8:5) = 0.66; Yield = 60%; M.P. = 155-157 °C; M.Wt. = 381.81; IR (KBr pellets) cm^{-1}: 754 (–Cl–Str), 1062 (–C–O–), 1155 (–C–C–), 1602 (–C=C–) 1633 (–C=N–), 2834 (–C–H–), 3284 (–OH–); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.78 (s, 1H), 11.10 (s, 1H), 8.04 (s, 1H), 7.55 (d, J = 6.9 Hz, 1H), 7.39 (t, J = 8.0 Hz, 1H), 7.28 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 8.3 Hz, 1H), 7.17 (d, J = 7.6 Hz, 1H), 6.80 (d, J = 7.5 Hz, 2H), 6.19 (s, 1H), 6.17 (s, 1H), 5.34 (t, J = 8.9 Hz, 1H), 3.04 (d, J = 8.7 Hz, 1H), 2.94 (d, J = 9.1 Hz, 1H); ^{13}C NMR (400 MHz, $CDCL_3$) δ = 165.10, 163.08, 161.26, 159.81, 143.28, 139.86, 129.24, 128.98, 128.45, 128.28, 127.73, 127.42, 126.85, 124.29, 107.38, 102.08, 95.02, 76.72, 38.77, 17.39, 14.71; MS ES+ (ToF): m/z 381.08 [M^++2]; CHNS: Calc ($C_{21}H_{16}ClNO_4$): C, 66.06; H, 4.22; Cl, 9.29; N, 3.67; O, 16.76; Found C, C, 66.09; H, 4.20; Cl, 9.26; N, 3.69; O, 16.72.

Antioxidant activity
DPPH radical scavenging assay

Antioxidant activity of the synthesized compounds was determined by DPPH (2, 2-diphenyl-1-pycrilhydrazil hydrate) radical scavenging method. Briefly, 0.1 mM solution of DPPH in methyl alcohol was prepared and 1 mL of this solution was added to 3 mL of sample or standard with a concentration of 12.5, 25, 50, 75 and 100 µg/mL. Discolorations were measured at 517 nm after incubation for 30 min at 30 °C in the dark. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The IC_{50} values of given samples were calculated by using formula:

$$IC_{50} = (A_c - A_s) \times 100/A_c$$

Here, A_c was the absorbance of the control and A_s was the absorbance of the sample [48, 49].

Antimicrobial activity
Minimum inhibitory concentration (MIC)

The antimicrobial activity of the synthesized compounds were performed against *S. aureus MTCC* 3160, *P. aeruginosa MTCC* 1934, *E. coli MTCC* 45, *P. mirabilis MTCC* 3310, *C. albicans MTCC* 183 and *A. niger MTCC* 282 by using the tube dilution method [50]. Dilutions of test and standard compounds were prepared in double

strength nutrient broth I.P. (bacteria) or sabouraud dextrose broth I.P. (fungi) [51, 52]. The slants of *E. coli, P. aeruginosa, P. mirabilis* and *S. aureus* were incubated at the 30-35 °C for 24 h. The slants of *C. albicans* were incubated at 20–25 °C for 48 h whereas; the slants of *A. niger* were incubated at 20–25 °C for 5 days. After the incubation period sterilized 0.9% NaCl solution was used to harvest the bacterial and fungal cultures from agar slant through proper shaking and then the suspensions of microorganisms were diluted with the sterile 0.9% NaCl solution to CFU count was adjusted by adjusting the density of microorganism suspension to that of 0.5 McFarland standards by adding distilled water. The number of CFU was determined by dilution pour-plate method [53]. A serial dilution of 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.12 µg/mL and 1.62 µg/mL was used for determination of MIC. The samples tubes were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 days (*A. niger*), and at 37 °C for 48 h (*C. albicans*) and the results were recorded in pMIC.

Preservative effectiveness

White lotion USP was utilized as the medium for the testing of preservative effectiveness.

Ingredients: Zinc sulfate 40 gm, sulfurated potash 40 gm and purified water q.s. to 1000 mL.

Firstly, zinc sulphate and sulfurated potash were dissolved in 450 mL of water separately and filtered. Then, sulfurated potash solution was added to zinc sulfate with stirring. At last, the required amount of water was added and mixed thoroughly and sterilized. For preservative efficacy testing, the White lotion USP was prepared using the equimolar amount of compounds **1**-**4** as novel preservatives by replacing sodium benzoate, methyl paraben and propyl paraben from the formula [54].

Challenge microorganism

Staphylococcus aureus MTCC 3160, *P. aeruginosa MTCC* 1934, *E. coli MTCC* 45, *C. albicans MTCC* 183 and *A. niger MTCC* 282 were used as common contaminants in the study as prescribed in USP for preservative efficacy testing in the pharmaceutical preparations.

Preparation of ioculums

The slants of *E. coli, P. aeruginosa* and *S. aureus* were incubated at the 30–35 °C for 24 h. The slants of *C. albicans* were incubated at 20–25 °C for 48 h whereas; the slants of *A. niger* were incubated at 20–25 °C for 5 days [55].

Test procedure

White lotions USP was added in final containers and were used in challenge test. The preparation was

Lather *et al. BMC Chemistry*   (2020) 14:41

Page 13 of 15

inoculated with 0.5–1% volume of microbial inoculum having a concentration of 1×10^5–1×10^6 CFU/mL [56]. Inoculated samples were mixed thoroughly to ensure homogeneous microorganism distribution and incubated. The CFU/mL of the product was determined at an interval of 0 days, 7 days, 14 days, 21 days, and 28 days in agar plates. Log CFU/mL of white lotion USP was calculated as not less than 2.0 log reductions from initial count at 14 days of incubation and no increase in CFU from 14 days count at 28 days in case of bacteria and no increase from the initial calculated count at 14 and 28 days [57].

In silico molecular docking studies
The Schrodinger, Inc. (New York, USA) software Maestro 11 was used for the computational calculations and docking studies. Laboratory for Enzyme Inhibition Studies, Department of Pharmaceutical Sciences, M.D. University, Rohtak, INDIA was used for the computational work. The receptor-grid files were generated by grid-receptor generation program Glide [58]. Grid-based ligand docking utilized the hierarchical sequence of filters to produce possible conformations of the ligand in the active-site region of the protein receptor. At this stage, crude score values and geometric filters were prepared out unlikely binding modes. The next filter phase involves a grid-based force field evaluation and refinement of docking experiments including torsional and rigid-body movements of the ligand [59]. The remained docking evaluations were subjected to a Monte Carlo procedure to minimize the energy score. A conjugate gradient minimization protocol was used in all calculations [60].

The energy differences were calculated using the equation:

$$\Delta E = E_{complex} - E_{ligand} - E_{protein}$$

Protein preparation
The X-ray protein structure co-ordinates of pdb id 1moq were downloaded from Protein Data Bank from www.rcbs.org [61] and were prepared with the help of the Schrödinger protein preparation wizard 'Prepwiz' [62, 63]. PDB id 1moq (resolution 1.57 A°) was selected on the basis of the lowest resolution and availability. All the waters molecules except metals co-ordinated and present between the ligand and protein were removed. The energy-restrained structure of the protein G-6-P synthase was constructed with the help of OPLS-2005 force field.

Ligand Preparation
The three-dimensional structural library was prepared using the Chemdraw software and proceeded for energy minimization using the LigPrep tool for the correction of coordinates, ionization, stereochemistry and tautomeric structure to gain the appropriate conformation through the addition or removal of hydrogen bonds. The partial charges were computed according to the OPLS-2005 force field (32 stereo isomers, tautomers and ionization) at biological pH and used for molecular docking studies.

Author details
[1] Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, Haryana, India. [2] Department of Pharmaceutical Sciences, G.J.U.S.&T., Hisar, India. [3] Laboratory for Preservation Technology and Enzyme Inhibition Studies, Department of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, Haryana, India.

References
1. Zengin N, Yuzbasıoglu D, Unal F, Yilmaz S, Aksoy H (2011) The evaluation of the genotoxicity of two food preservatives: sodium benzoate and potassium benzoate. Food Chem Toxicol 49(4):763–769

Lather *et al. BMC Chemistry* (2020) 14:41

Page 14 of 15

2. Reddy MV, Aruna G, Parameswari SA, Banu BH, Jayachandra PR (2015) Estimated daily intake and exposure of sodium benzoate and potassium sorbate through food products in school children of Tirupati, India. Int J Pharm and Pharmaceut Sci 7(7):129–133

3. Denyer SP, King RO (1988) Microbial quality assurance in pharmaceuticals, cosmetics and toiletries. Ed. Bloomfield SF, Baird R, Leak RE and Leech R. Chichester: Ellis Horwood 156–170

4. Pawar HA, Shenoy AV, Narawade PD, Soni PY, Shanbhag PP, Rajal VA (2011) Preservatives from nature: a review. Int J Pharm Phytopharmacol Res 1(2):78–88

5. The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (2002) The determination of certain formaldehyde releasers in Cosmetic products, 1–9

6. Gue L (2010) What's linside? That counts a survey of toxic ingredients in our cosmetics. David Suzuki Foundation, Vancouver, pp 1–26

7. Sedlewicz LB (2011) Current trends in cosmetic preservation. Schulke inc

8. David Suzuki Foundation (2010) What's inside? The "dirty dozen" ingredients investigated in the david Suzuki foundation survey of chemicals in cosmetics 1-19

9. Rastogi SC, Jensen GH, Petersen MR, Worsoe IM, Christoffersen C (1999) Preservatives in skin creams. Analytical Chemical Control of Chemical Substances and Chemical Preparations. National Environmental Research Institute. Denmark. NERI Technical Report No. 297:1–67

10. Darbre PD, Harvey PW (2008) Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks. J Appl Toxi 28(5):561–578

11. Tavares RS, Martins FC, Oliveira PJ, Ramalho-Santos J, Peixoto FP (2009) Parabens in male infertility-is there a mitochondrial connection. Reprod Toxicol 27(1):1–7

12. Lundov MD, Moesby L, Zachariae C, Johansen JD (2009) Contamination versus preservation of cosmetics: a review on legislation, usage, infections, and contact allergy. Cont Derm 60(2):70–80

13. Satyendra RV, Vishnumurthy KA, Vagdevi HM, Rajesh KP, Manjunatha H, Shruthi A (2012) In vitro antimicrobial and molecular docking of dichloro substituted benzoxazole derivatives. Med Chem Res 21(12):4193–4199

14. Bearne SL, Blouin C (2002) Inhibition of *Escherichia coli* glucosamine-6-phosphate synthase by reactive intermediate analogues, the role of the 2-amino function in catalysis. J Biol Chem 75(1):135–140

15. Krishna PKV, Harish BGV, Kumar SSR, Kumar GK (2012) Antibacterial activity of leaf extract of *Delonix elata* and molecular docking studies of luteolin. J Biochem Tech 3(5):S193–S197

16. Fikrika H, Ambarsari L, Sumaryada T (2016) Molecular docking studies of catechin and its derivatives as anti-bacterial inhibitor for glucosamine-6-phosphate synthase, IOP Conf. Series. Earth and Environmental Science 31:012009

17. Deepa M, Devi PR, Alam Md A (2016) *In silico* antimicrobial activity of active phyto compounds from the leaf extract of *Vitex negundo* linn. against glucosamine-6-phasphate synthase. World J Pharm Pharmaceut Sci 5(1):1144–1156

18. Cushnie TPT, Lamb AJ (2005) Antimicrobial activity of flavonoids. Int J Antimicrob Agents 26:343–356

19. Bugianesi R, Catasta G, Spigno P, D'Uva A, Maiani G (2002) Naringenin from cooked tomato paste is bioavailable in men. J Nutr 132:3349–3352

20. Xu XH, Ma CM, Han YZ, Li Y, Liu C, Duan ZH, Wang HL, Liu DQ, Liu RH (2015) Protective effect of naringenin on glutamate-induced neurotoxicity in cultured hippocampal cells. Arch Biol Sci Belgrade 67(2):639–646

21. Tomas-Barberan FA, Clifford MN (2000) Flavanones, chalcones and dihydrochalcones- nature, occurence and dietary burden. J Sci Food and Agric 80:1073–1080

22. Kumar S, Tiku BA (2016) Biochemical and molecular mechanisms of radio protective effects of naringenin, a phytochemical from citrus fruits. J Agric Food Chem 64(8):1676–1685

23. Bear WL, Teel RW (2000) Effects of citrus flavonoids on the mutagenicity of heterocyclic amines and on cytochrome P450 1A2 activity. Anticancer Res 20:3609–3614

24. Ueng YF, Chang YL, Oda Y, Park SS, Liao JF, Lin MF (1999) *In vitro* and in vivo effects of naringin on cytochrome P450-dependent monooxygenase in mouse liver. Life Sci 65:2591–2602

25. Pandey KB, Rizvi SI (2009) Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2(5):270–278

26. Al-Harbi MS (2016) Hepatoprotective effect and antioxidant capacity of naringenin on arsenic induced liver injury in rats. Int J Pharm Pharmaceut Sci 8(4):103–108

27. Goldwasser J, Cohen PY, Yang E, Balaguer P, Yarmush ML, Nahmias Y (2010) Transcriptional regulation of human and rat hepatic lipid metabolism by the grapefruit flavonoid naringenin: role of PPAR alpha, PPAR gamma and LXR alpha. PLoS ONE 5(8):e12399

28. Casas M, Prat G, Robledo P, Barbanoj M, Kulisevsky J, Jane F (1999) Scopolamine prevents tolerance to the effects of caffeine on rotational behavior in 6-hydroxydopamine-denervated rats. Eur J Pharmacol 366(1):1–11

29. Papiez MA (2004) Influence of naringenin on the activity of enzymes participating in steroidogenesis in male rats. Annales Academiae Medicae Bialostocensis 49:120–122

30. Wang X, Wolkoff AW, Morris ME (2005) Flavonoids as a novel class of human organic anion-transporting polypeptide OATP1B1 (OATP-C) modulators. Drug Metab Dispos 33:1666–1672

31. Celiz G, Daz M, Audisio MC (2011) Antibacterial activity of naringin derivatives against pathogenic strains. J Appl Microbiol 1(3):731–738

32. Pietta PG (2000) Flavonoids as antioxidants. J Nat Prod 63:1035–1042

33. Burda S, Oleszek W (2001) Antioxidant and antiradical activity of flavonoids. J Agric Food Chem 49:2774–2779

34. Kanno S, Tomizawa A, Hiura T, Osanai Y, Shouji A, Ujibe M, Ohtake T, Kimura K, Ishikawa M (2005) Inhibitory effects of naringenin on tumor growth in human cancer cell lines and sarcoma S-180-implanted mice. Biol Pharm Bull 28:527–530

35. Zhang S, Jiang ZF, Pan Q, Song CY, Zhang WH (2016) Anti-cancer effect of naringenin chalcone is mediated via the induction of autophagy, apoptosis and activation of PI3K/Akt signalling pathway. Bangladesh J Pharmacol 11:684–690

36. Goldwasser J, Cohen PY, Lin W, Kitsberg D, Balaguer P, Polyak SJ (2011) Naringenin inhibits the assembly and long-term production of infectious hepatitis C virus particles through a PPAR-mediated mechanism. J Hepatol 55:963–971

37. Du G, Jin L, Han X, Song Z, Zhang H, Liang W (2009) Naringenin: a potential immune modulator for inhibiting lung fibrosis and metastasis. Cancer Res 69:3205–3321

38. Cui W, Zhang J, Wang Q, Gao K, Zhang W, Yang J (2014) A novel synthesis of naringenin and related flavanones. J Chem Res 38:686–689

39. Wang HK, Yeh CH, Iwamoto T, Satsu H, Shimizu M, Totsuka M (2012) Dietary flavonoid naringenin induces regulatory T cells via an aryl hydrocarbon receptor mediated pathway. J Agric Food Chem 60(9):2171–2178

40. Yilma AN, Singh SR, Morici L, Dennis VA (2013) Flavonoid naringenin: a potential immunomodulator for Chlamydia trachomatis inflammation. Mediat Inflamm 2013:102457

41. Zhang Y, Wang JF, Jing D, Wei JY, Wang YN, Dai XH, Wang X, Luo MJ, Tan W, Deng XM, Niu XD (2013) Inhibition of α-toxin production by sub inhibitory concentrations of naringenin controls *Staphylococcus aureus* pneumonia. Fitoterapia 86:92–99

42. Kriza A, Ignat I, Stanica N, Draghici C (2011) Synthesis and characterization of Cu(II), Co(II) and Ni(II) complexes with Schiff bases derived from isatin. Rev Chim 62:696–701

43. Hopkins AL, Groom CR (2002) The drug gable genome. Nat Rev Drug Discov 1:727–733

44. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD (2002) Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem 45:2615–2623

45. Irvine JD, Takahashi L, Lockhart K, Cheong J, Tolan JW, Selick HE, Groove R (1999) MDCK (Madin Darby Canine Kidney) cells: a tool for membrane permeability screening. J Pharm Sci 88(1):28–33

46. Kulkarni A, Han Y, Hopfinger AJ (2002) Predicting Caco-2 cell permeation coefficients of organic molecules using membrane-interaction QSAR analysis. J Chem Inf Comput Sci 42(2):331–342

47. Teague SJ, Davis AM, Leeson PD, Opera TA (1999) The design of lead like combinatorial libraries. Chem Int Ed Eng 38:3743–3748

48. Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature 181(4617):1199–1200

49. Mohamed SK, Ahmed AAA, Yagi SM, Alla AEWHA (2009) Antioxidant and antibacterial activities of total polyphenols isolated from pigmented sorghum (*Sorghum bicolor*) Lines. J Genet Eng Biotechn 7(1):51–58

50. Cappucino JG, Sherman N (1999) Microbiology - A laboratory manual. Addison Wesley, Boston, p 263

Lather *et al. BMC Chemistry*   (2020) 14:41

Page 15 of 15

51. Indian Pharmacopoeia Vol-I (2007) The controller of publications, New Delhi 37
52. Kowser MM, Fatema N (2009) Determination of MIC and MBC of selected azithromycin capsule commercially available in Bangladesh. The ORION Med J 32(1):619–620
53. Andrews JM (2001) Determination of minimum inhibitory concentration. J Antimicrob Chem 48(S1):5–16
54. Narang R, Narasimhan B, Judge V, Ohlan S, Ohlan R (2009) Evaluaton of preservatve effectiveness in an official antacid preparaton. Acta Pharmaceutica Sciencia 51:225–229
55. Indian Pharmacopoeia (2010) Indian Pharmacopoeia Commission, Ghaziabad, India 27-28
56. Dafale NA, Semwal UP, Agarwal PK, Sharma P, Singh GN (2014) Valuation of preservative effectiveness in antacid, cough syrup and ophthalmic solution by microbial challenge test. Int J Pharm 1(3):193–199
57. The United States Pharmacopoeia (2004) Antmicrobial effectiveness testing. United States Pharmacopoeial Conventon Inc., Rockville, pp 2148–2150
58. Glide, version 6.6 (2015) Schrodinger, LLC, New York, America
59. Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz DT (2006) Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. J Med Chem 49:6177–6196
60. Godschalk F, Genheden S, Soderhjelm P, Ryde U (2013) Comparison of MM/GBSA calculations based on explicit and implicit solvent simulations. Phy Chem 15:7731–7739
61. Teplyakov A, Obmolova G, Badet-Denisot MA, Badet B, Polikarpov I (1998) Involvement of the C terminus in intramolecular nitrogen channeling in glucosamine 6-phosphate synthase: evidence from a 1.6 Å crystal structure of the isomerase domain. Structure 6:1047–1055
62. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE (2004) Glide: a new approach for rapid, accurate docking and scoring. Method and assessment of docking accuracy. J Med Chem 47:1739–1749
63. Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, Banks JL (2004) Glide: a new approach for rapid, accurate docking and scoring. Enrichment factors in database screening. J Med Chem 47:1750–1759

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