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

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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 (IC₅₀ value, 6.864 ± 0.020 µM) as compared to standard ascorbic acid (IC₅₀ 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 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.

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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-Butoxy carbonyl)-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,
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 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, 1.06 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
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.
(2) Substitution with aromatic amine at para position increased the activity with increase in electronegativity 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 (Schroedinger Inc. U.S.A. Maestro version 11). Dock score and binding of compound 1, 2, 3 and 4

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**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 (l-ascorbic acid) | 8.110 ± 0.069 |

$^a$ Values are expressed as mean ± SEM, n = 3
Table 2 pMIC values (μM/mL) of synthesized naringenin derivatives against different standard microbial strains

| Compound(s) | 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. 3 IC_{50} value of different synthesized compounds with respect to standard L-ascorbic acid

Fig. 4 Antimicrobial activity (pMIC in μM/mL) of synthesized naringenin derivatives against different microorganisms
| 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 ± 0.16 | 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 $1 \times 10^5$-$1 \times 10^6$
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.
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 NO2 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  | ![Structure of Compound 1](image1)      | -7.42      |
| 2.    | Compound 2  | ![Structure of Compound 2](image2)      | -4.29      |
| 3.    | Compound 3  | ![Structure of Compound 3](image3)      | -3.30      |
| 4.    | Compound 4  | ![Structure of Compound 4](image4)      | -4.02      |
| 5.    | Naringenin  | ![Structure of Naringenin](image5)      | -6.36      |
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 Chemie (Mumbai, India), SRL (Mumbai, India), and Sigma Aldrich (Germany). Nutrient agar, nutrient broth, sabouraud 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](image)
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. \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra were confirmed in DMSO and deuterated CDCl\textsubscript{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.

**Table 5** ADME profile of various newly synthesized naringenin derivatives

| Compound(s) | Mol. Wt. | No. of rotatable bond | DonorHB | AcceptorHB | 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   | 0.251    | 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  |

**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 precipitated compounds 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-nitrophenyl)imino) chroman-5,7-diol

\( R_f \) TLC mobile phase: Chloroform: Acetone (8:5) = 0.66; Yield = 50%; M.P. = 173–175 \( ^\circ \)C; M.Wt. = 345.32; IR (KBr pellets) cm\(^{-1}\): 1074 (-C=O-C=), 1251 (-C=C-), 1513 (-C=C-), 1631 (-C==C-), 1631 (-C=C=C-), 2831 (-C=H-), 2925 (-OH-); \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta = 11.60 \) (s, 1H), 11.10 (s, 1H), 8.04 (s, 1H), 7.28 (d, \( J = 7.2 \) Hz, 2H), 6.80 (d, \( J = 7.2 \) Hz, 2H), 6.28 (s, 1H), 6.27 (s, 1H), 5.28 (t, \( J = 9.0 \) Hz, 1H), 3.15 (d, \( J = 7.6 \) Hz, 1H), 3.00 (d, \( J = 7.0 \) Hz, 1H); \textsuperscript{13}C NMR (400 MHz, CDCl\textsubscript{3}) \( \delta = 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\textsuperscript{+}2]; CHNS: Calc (C\textsubscript{18}H\textsubscript{19}NO\textsubscript{6}): C, 62.60; H, 5.52; N, 4.09; O, 27.82.

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 \( ^\circ \)C; M.Wt. = 345.32; IR (KBr pellets) cm\(^{-1}\): 1074 (-C=O-C=), 1251 (-C=C-), 1513 (-C=C-), 1631 (-C=C=C-), 2831 (-C=H-), 2925 (-OH-); \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta = 11.60 \) (s, 1H), 11.10 (s, 1H), 8.04 (s, 1H), 7.28 (d, \( J = 7.0 \) Hz, 2H), 6.80 (d, \( J = 7.2 \) Hz, 2H), 6.28 (s, 1H), 6.27 (s, 1H), 5.28 (t, \( J = 9.0 \) Hz, 1H), 3.15 (d, \( J = 7.6 \) Hz, 1H), 3.00 (d, \( J = 7.0 \) Hz, 1H); \textsuperscript{13}C NMR (400 MHz, CDCl\textsubscript{3}) \( \delta = 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\textsuperscript{+}2]; CHNS: Calc (C\textsubscript{18}H\textsubscript{19}NO\textsubscript{6}): C, 62.60; 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 \( ^\circ \)C; M.Wt. = 365.35; IR (KBr pellets) cm\(^{-1}\): 753 (-F-), 1082 (-C=O=C-), 1241 (-C=C=), 1612 (-C=C=), 1632 (-C=C=), 2833 (-C=H-), 3350 (-OH-); \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta = 11.78 \) (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); \textsuperscript{13}C NMR (400 MHz, CDCl\textsubscript{3}) \( \delta = 165.92, 165.91, 152.59, 151.39, 146.42, 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\textsuperscript{+}2]; CHNS: Calc (C\textsubscript{18}H\textsubscript{19}NO\textsubscript{6}): C, 62.60; H, 5.52; N, 4.09; O, 27.82.
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 (C12H16ClNO4): C, 69.04; H, 4.41; Cl, 9.17; N, 3.67; O, 16.76; Found C, 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

4.7 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–Cl), 1602 (–C–N–), 1633 (–C–), 2834 (–C–H–), 3284 (–OH–); 1H NMR (400 MHz, DMSO-d6) 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); 13C NMR (400 MHz, CDCl₃) 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 (C12H15ClNO4): C, 69.04; H, 4.41; Cl, 9.17; N, 3.67; O, 16.76; Found C, C, 69.01; H, 4.44; F, 5.23; N, 3.84; O, 17.55.

Antioxidant activity

DPPH radical scavenging assay

Antioxidant activity of the synthesized compounds was determined by DPPH (2, 2-diphenyl-1-pyril diamidazole) 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 IC₅₀ values of given samples were calculated by using formula:

\[ IC_{50} = \left( \frac{A_c - A_s}{A_c} \right) \times 100/A_c \]

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 sulphurated potash were dissolved in 450 mL of water separately and filtered. Then, sulphurated 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 testing in the pharmaceutical preparations.

Preparation of inoculums

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
 inoculated with 0.5–1% volume of microbial inoculum having a concentration of $1 \times 10^5$–$1 \times 10^6$ 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:

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

**Protein preparation**

The X-ray protein structure co-ordinates of pdb id 1moq were downloaded from Protein Data Bank from [www.rcbs.org](http://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 Å) 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.

**Abbreviations**

ADMET: Absorption, distribution, metabolism, excretion & toxicity; G-6-P: Glucose-6-phosphate synthase; CYP: Cytochromes P450; OATP1B1: Solute carrier organic anion transporter family member 1B1; DHEAS: Dehydroepiandrosterone; PPAR: Peroxisome proliferator-activated receptors; DPH: 2,2-Diphenyl-1-picrylhydrazyl; UDP-N-acetyl glucosamine: Uridine diphosphate N-acetylglucosamine; FTIR: Fourier-transform infrared spectroscopy; 1H NMR: Proton nuclear magnetic resonance; 13C NMR: Carbon 13 nuclear magnetic resonance; UV: Ultra violet; TLC: Thin layer chromatography; IC50: Inhibitory concentration; MIC: Minimum inhibitory concentrations; CFU: Colony forming unit; HBA: Hydrogen bond acceptor; HBD: Hydrogen bond donor; MW: Molecular weight; MTCC: Microbial type culture collection; DMSO: Dimethyl sulfoxide; BOD: Biological oxygen demand; USP: United States Pharmacopoeia; PDB ID: Protein Data Bank Identification; OPLS: Optimized potential for liquid simulations; Q.S.: Quantity sufficient.

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**Authors’ contributions**

The authors AL, SS and AK have designed, synthesized and carried out the work in equal contribution. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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