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# Discovery of novel 1,2,4-triazole tethered $\beta$ -hydroxy sulfides as bacterial tyrosinase inhibitors: synthesis and biophysical evaluation through *in vitro* and *in silico* approaches†

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In this study, a series of 1,2,4-triazole-tethered  $\beta$ -hydroxy sulfide scaffolds **11a–h** was synthesized in good to remarkable yields (69–90%) through the thiolysis of oxiranes by the thiols in aqueous basic catalytic conditions. The synthesized 1,2,4-triazole-tethered  $\beta$ -hydroxy sulfides were screened against bacterial tyrosinase enzyme, and Gram-positive and Gram-negative bacterial cultures *i.e.*, (*S. aureus*) *Staphylococcus aureus* & (*E. coli*) *Escherichia coli*. Among the synthesized derivatives, the molecules **11a** ( $IC_{50} = 7.67 \pm 1.00 \mu\text{M}$ ), **11c** ( $IC_{50} = 4.52 \pm 0.09 \mu\text{M}$ ), **11d** ( $IC_{50} = 6.60 \pm 1.25 \mu\text{M}$ ), and **11f** ( $IC_{50} = 5.93 \pm 0.50 \mu\text{M}$ ) displayed the better tyrosinase inhibitory activity in comparison to reference drugs ascorbic acid ( $IC_{50} = 11.5 \pm 1.00 \mu\text{M}$ ) and kojic acid ( $IC_{50} = 30.34 \pm 0.75 \mu\text{M}$ ). The molecule benzofuran-triazol-propan-2-ol **11c** proved to be the most potent bacterial tyrosinase inhibitory agent with a minimum  $IC_{50}$  of  $4.52 \pm 0.09 \mu\text{M}$ , as compared to other synthesized counterparts and both standards (kojic acid and ascorbic acid). The compound diphenyl-triazol-propan-2-ol **11a** and benzofuran-triazole-propan-2-ol **11c** showed comparable anti-bacterial chemotherapeutic efficacy with minimum inhibitory concentrations ( $MIC = 2.0 \pm 2.25 \text{ mg mL}^{-1}$  and  $2.5 \pm 0.00 \text{ mg mL}^{-1}$ , respectively) against *S. aureus* bacterial strain in comparison with standard antibiotic penicillin ( $MIC = 2.2 \pm 1.15 \text{ mg mL}^{-1}$ ). Furthermore, among the synthesized derivatives, only compound **11c** demonstrated better anti-bacterial activity ( $MIC = 10 \pm 0.40 \text{ mg mL}^{-1}$ ) against *E. coli*, which was slightly less than the standard antibiotic *i.e.*, penicillin ( $MIC = 2.4 \pm 1.00 \text{ mg mL}^{-1}$ ). The compound **11c** demonstrated a better binding score ( $-7.08 \text{ kcal mol}^{-1}$ ) than ascorbic acid ( $-5.59 \text{ kcal mol}^{-1}$ ) and kojic acid ( $-5.78 \text{ kcal mol}^{-1}$ ). Molecular docking studies also validate the *in vitro* anti-tyrosinase assay results; therefore, the molecule **11c** can be the lead bacterial tyrosinase inhibitor as well as the antibacterial agent against both types of bacterial strains after suitable structural modifications.

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## 1. Introduction

Tyrosinase is a Cu-containing polyphenol oxidase that is ubiquitously distributed in insects, plants, animals, and microorganisms.<sup>1–4</sup> It catalyzes the initial two steps required for melanin biosynthesis, which is responsible for the pigmentation of eyes, skin and hair.<sup>5,6</sup> Unregulated stimulation of tyrosinase can lead to severe dermatological afflictions (*i.e.*, scarring with hyperpigmentation and ephelides) and neurodegenerative diseases (*i.e.*, Parkinson's disease).<sup>7–9</sup> Tyrosinase inhibitors have become vital in the agriculture, cosmetics, food business, and pharmaceutical industries to prevent hyperpigmentation.<sup>10,11</sup> Lately, surplus natural and laboratory-generated tyrosinase inhibitors have been documented, including kojic acid, aromatic acids, ascorbic acid, polyphenols, arbutin, hydroquinone, aromatic aldehydes, tropolone, and aromatic alcohols.<sup>12</sup>



However, several widely recognized brightening substances *i.e.*, kojic acid and hydroquinone, are regarded as dangerous agents attributable to the undesired adverse effects *i.e.*, neurological disorders, dermatological cancer, skin irritation, and cytotoxicity.<sup>13–16</sup> The exploration of novel, manageable and harm-less tyrosinase inhibitors has become a matter of grave concern.

Triazole derivatives are recognized for their remarkable heterocyclic structures, which offer a diverse array of therapeutic and biological effects.<sup>17–19</sup> These compounds have demonstrated significant roles in combating viruses, cancers, bacteria, seizures, pain, inflammation, diabetes, fungal infections, oxidative stress, and hypertension. Their versatility in addressing such wide-ranging health concerns underscores

their importance in pharmaceutical research and development.<sup>20–22</sup>

Previous research has identified various heterocyclic compounds such as benzofuran-oxadiazole as bacterial tyrosinase inhibitors,<sup>23</sup> oxadiazoles as human tyrosinase inhibitors,<sup>24</sup> and piperazines as bacterial tyrosinase inhibitors.<sup>25</sup> Nitrogenous triazole scaffolds have been found to exhibit mushroom tyrosinase inhibition, such as 1,2,4-triazole hydrazones and 1,2,4-triazole clubbed *N*-phenylacetamides (Fig. 1).<sup>26,27</sup> The anti-tyrosinase inhibition of 1,2,4-triazole derivatives lies in the fact that the N-atoms of the triazole functionality can coordinate with the two Cu ions constituting the catalytic hub (active site) of enzyme. Furthermore, van der Waals interactions between

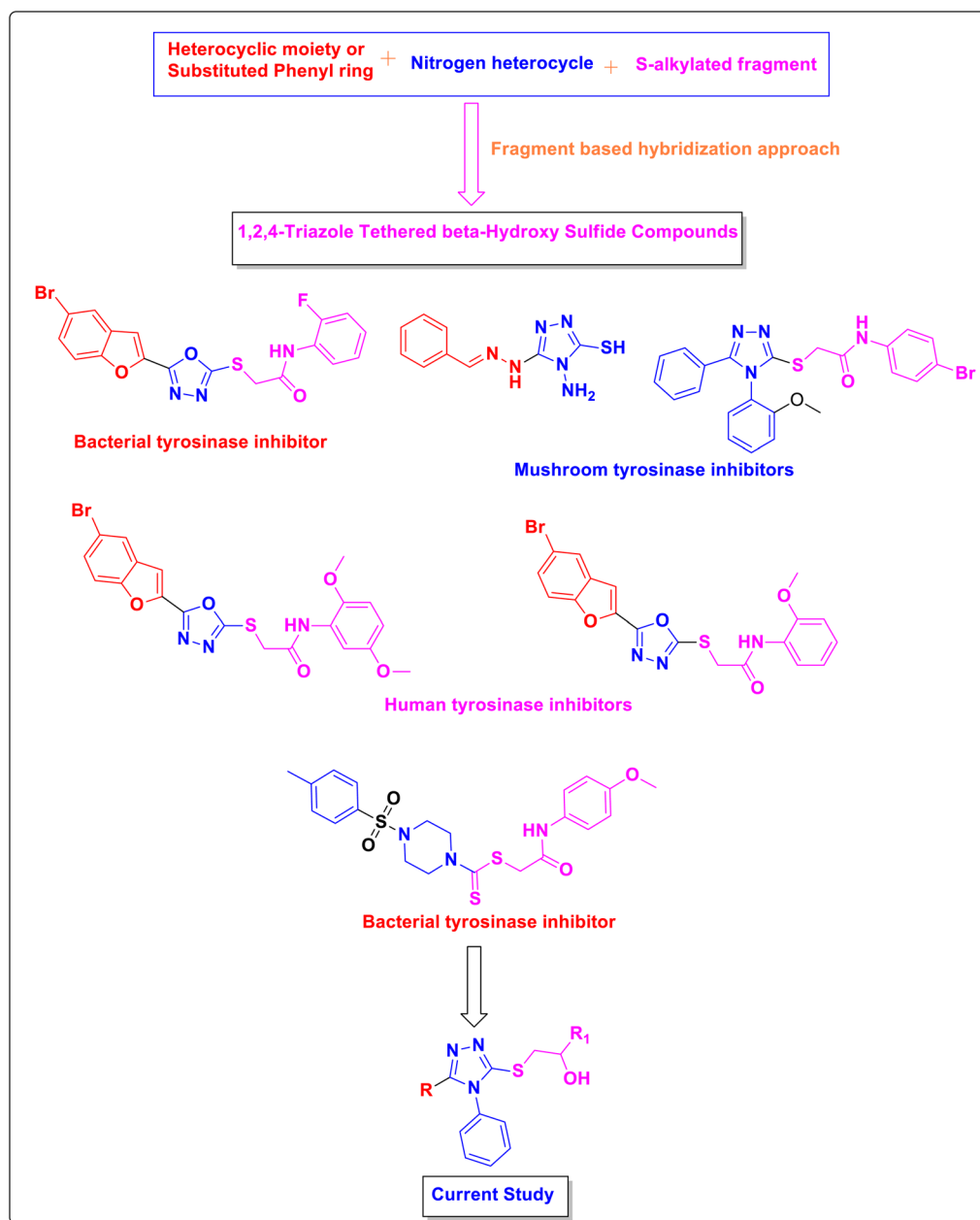


Fig. 1 Rational design of fragment-based hybridization approach.



the residues covering the hydrophobic void and phenyl group, augment the binding capability of enzyme.

In organic synthesis, epoxides facilitate the generation of several organic frameworks by acting as pivotal forerunners. They undergo treatment with a variety of nucleophiles including thiols, alcohols and amines to afford several organic functionalities.<sup>28,29</sup> The ring cleavage of epoxides with -SH (thiols) lead to the generation of  $\beta$ -hydroxy sulfides which act as fascinating precursors in synthetic organic chemistry and can be employed to achieve the natural products, biologically potent molecules and other products including leukotrienes such as LTC<sub>4</sub> and LTD<sub>4</sub>.<sup>30-32</sup>

Here, a fragment-based hybridization approach was applied to develop rationale of the current study by the incorporation of various heterocyclic moieties such as benzofuran, bromobenzofuran, naphthofuran, and acefylline along with substituted phenyl ring around the 1,2,4-triazole core. The thiol functionality of triazole core was modified by the incorporation of  $\beta$ -hydroxy sulfides as depicted in Fig. 1.

Encouraged by the biological importance and applications of the above compounds in various fields, the aspiration of the current study involves the functional layout and preparation of some novel anti-bacterial and anti-tyrosinase agents through the coupling of 1,2,4-triazole derivatives with different epoxides *e.g.* 2-methyloxirane, 2-((naphthalen-2-yloxy)methyl)oxirane, and 2-(phenoxy)methyl)oxirane *via* thiolysis. Molecular docking studies were utilized to validate the tyrosinase inhibition activity of the potent compound and finally, a detailed structure-activity relationship was inferred.

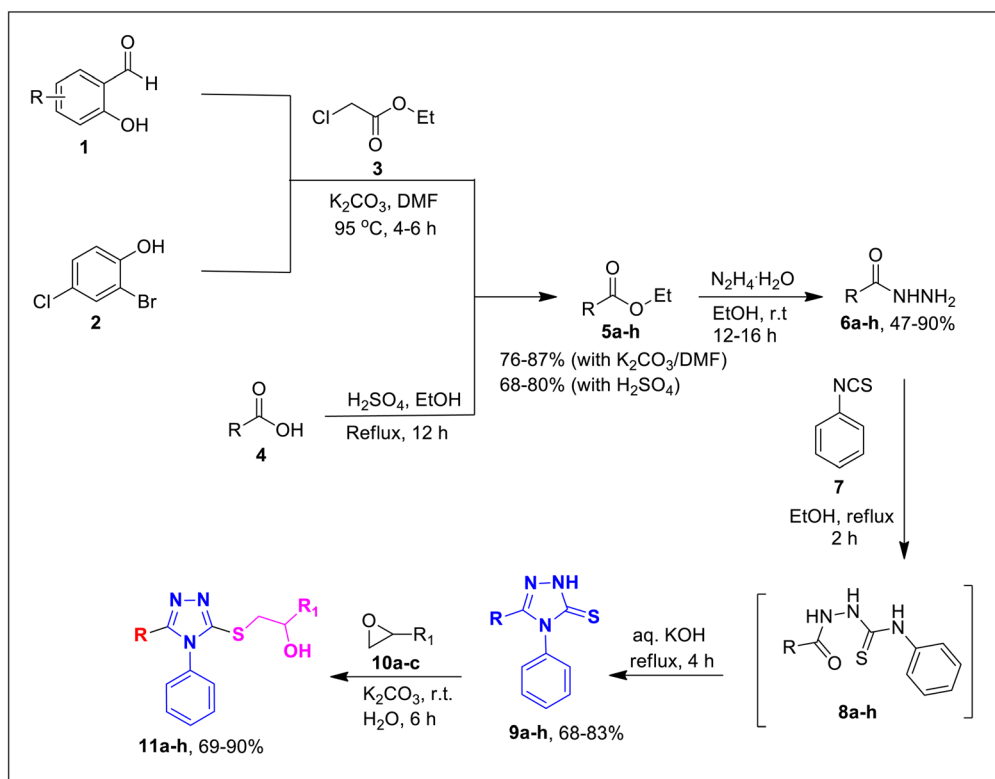
## 2. Results and discussion

### 2.1. Chemistry

The designed framework to access  $\beta$ -hydroxy sulfides **11a-h** has been depicted in Scheme 1. Various substituted aromatic aldehydes **1** and phenol **2** were reacted with ethyl chloroacetate **3** using potassium carbonate in DMF to obtain corresponding esters in 76–87% yield.<sup>33,37</sup> Besides this, various carboxylic acids were transformed into respective esters (68–80% yield) *via* Fischer esterification, by using a catalytic amount of sulfuric acid.<sup>38</sup> Corresponding hydrazides **6a-h** were synthesized in 47–90% yield by treating various esters **5a-h** with hydrazine monohydrate in ethanol.<sup>39</sup> These synthesized compounds were refluxed with aryl isothiocyanate **7** in ethanol to get key intermediates (thiosemicarbazide) **8a-h**. These intermediates, after refluxing with KOH solution, were further transformed into the desired 1,2,4-triazoles **9a-h** (68–83%).<sup>40</sup> Targeted 1,2,4-triazole-based  $\beta$ -hydroxy sulfides **11a-h** (Table 1) were prepared in excellent yield (69–90%) by ring opening of epoxides **10a-c** with triazoles **9a-h**, in the presence of potassium carbonate.<sup>41</sup>

### 2.2. Bacterial tyrosinase inhibition activity of 1,2,4-triazole-tethered $\beta$ -hydroxy sulfides (**11a-h**)

The *in vitro* anti-tyrosinase activity of the  $\beta$ -hydroxy sulfides **11a-h** containing the 1,2,4-triazole moiety was examined and compared with that of ascorbic acid and kojic acid. Table 2 depicts the inhibitory effects of the novel compounds against bacterial tyrosinase and their IC<sub>50</sub> values ranged from 4.52  $\pm$  0.09 to 51.40  $\pm$  0.16  $\mu$ M. Interestingly, the tested compounds



Scheme 1 Schematic layout of the synthesis of 1,2,4-triazoles tethered  $\beta$ -hydroxy sulfides (**11a-h**).

Table 1 Synthesis of 1,2,4-triazoles tethered  $\beta$ -hydroxy sulfides **11a–h**

Compound	R	R <sub>1</sub>	Product	Yield (%)
<b>11a</b>		CH <sub>3</sub> -		79
<b>11b</b>		CH <sub>3</sub> -		79
<b>11c</b>		CH <sub>3</sub> -		85
<b>11d</b>		CH <sub>3</sub> -		86
<b>11e</b>				90
<b>11f</b>				81
<b>11g</b>		CH <sub>3</sub> -		69
<b>11h</b>		CH <sub>3</sub> -		71

**11a**, **11c**, **11d** and **11f** were found to be more potent inhibitors of bacterial tyrosinase than the reference inhibitors, ascorbic acid and kojic acid. Over all, compound **11c** exhibited the most robust anti-tyrosinase activity, demonstrating  $IC_{50} = 4.52 \pm 0.09$   $\mu$ M, whereas, compound **11e** displayed poor anti-tyrosinase effect with  $51.40 \pm 0.16$   $\mu$ M  $IC_{50}$  value. The remaining tested compounds displayed moderate inhibitory activity.

### 2.3. Structure–activity relationship

The anti-tyrosinase efficacy of prepared compounds **11a–h** relied significantly on the key parameters, including the

position of substituents, the nature of substituents as electron withdrawing or donating, and the electronegativity of attached moieties to the triazole ring. These factors play crucial roles in establishing the structure activity relationship (SAR).

Regarding the nature of the substituent, the benzofuran moiety conferred a remarkable tyrosinase inhibition potential over other functionalities. Interestingly, compound **11c** bearing a benzofuran moiety was found to be the most promising and potent bacterial tyrosinase inhibitory agent, (which) exhibited the minimum inhibitory concentration ( $IC_{50} = 4.52 \pm 0.09$   $\mu$ M) as displayed in Fig. 2. The replacement of benzofuran moiety in



Table 2 Retardant action of 1,2,4-triazole based- $\beta$ -hydroxy sulfides (11a–h) on the tyrosinase activity

Compounds	Tyrosinase inhibition $IC_{50}$ ( $\mu M$ )
11a	$7.67 \pm 1.00$
11b	$14.43 \pm 1.50$
11c	$4.52 \pm 0.09$
11d	$6.60 \pm 1.25$
11e	$51.40 \pm 0.16$
11f	$5.93 \pm 0.50$
11g	$13.50 \pm 0.50$
11h	$13.41 \pm 1.15$
Ascorbic acid	$11.5 \pm 1.00$
Kojic acid	$30.34 \pm 0.75$

compound **11c** with aryl (**11a**,  $IC_{50} = 7.67 \pm 1.00 \mu M$ ), naphthofuran (**11d**,  $IC_{50} = 6.60 \pm 1.25 \mu M$ ), and benzofuran and propan-2-ol of compound **11c** with naphthofuran and 3-phenoxypropan-2-ol (**11f**,  $IC_{50} = 5.93 \pm 0.50 \mu M$ ) led to a dramatic decline in (the) tyrosinase inhibitory activity (Fig. 2). Additionally, compound **11e** ( $IC_{50} = 51.40 \pm 0.16$ ) with 5-bromobenzofuran and 3-(naphthalen-2-yloxy)propan-2-ol substituents was found to be less active as compared to other synthesized compounds as displayed in Fig. 2. The placement of electron-donating groups on the aryl ring, *i.e.*, 4- $CH_3$  (**11b**,  $IC_{50} = 14.43 \pm 1.50 \mu M$ ), also resulted in decreased inhibitory activity. Whereas, the introduction of halogens (bromo and chloro) at the 2- and 4-position of the phenyl ring **11g** (attached to the 1,2,4-triazole ring), respectively, diminished the inhibitory effect against the tested enzyme with an  $IC_{50}$  of  $13.50 \pm 0.50 \mu M$  as in compound **11g**. The anti-tyrosinase effect of **11h** ( $IC_{50} = 13.41 \pm 1.15 \mu M$ ) bearing acefylline scaffold was found to be

almost similar in activity to that of the compound **11g**, that is,  $13.50 \pm 0.50 \mu M$ .

#### 2.4. Molecular docking studies of the most bioactive 1,2,4-triazole-tethered $\beta$ -hydroxy sulfide **11c**

The *in vitro* investigations of the prepared compounds reflected that the compound **11c** showed a significantly good inhibition profile against the tyrosinase enzyme. Encouraged by the results, we further explored the binding modes and the molecular interactions of compound **11c** with the target enzyme utilizing the computation molecular docking investigations. The results of molecular docking investigation revealed that the compound **11c** did bind to the catalytic hub (active site) of the tyrosinase enzyme with an anchoring affinity score of  $-7.08 \text{ kcal mol}^{-1}$  in comparison to the reference standard tyrosinase inhibitors (*i.e.*) kojic acid ( $-5.78 \text{ kcal mol}^{-1}$ ) and ascorbic acid ( $-5.59 \text{ kcal mol}^{-1}$ ), respectively. The binding affinities showed that compound **11c** has a significantly better affinity towards the tyrosinase enzyme in comparison with the control compounds.

The conformational analysis of the compound **11c** inside the substrate-binding site of the target enzyme showed that it binds strongly and makes robust molecular interactions of diverse types with the catalytic hub residues of the tyrosinase enzyme. It was noted that during the conformational analysis, the Ser360 active site residue was engaged by the  $-OH$  group of this compound with a hydrogen bond contact. Moreover, the nearby sulfur atom of compound **11c** was able to interact *via* a pi-sulfur contact with the Phe347 residues of the target enzyme. The triazole moiety of compound **11c** exhibited several interactions within the tyrosinase active site and formed notable molecular

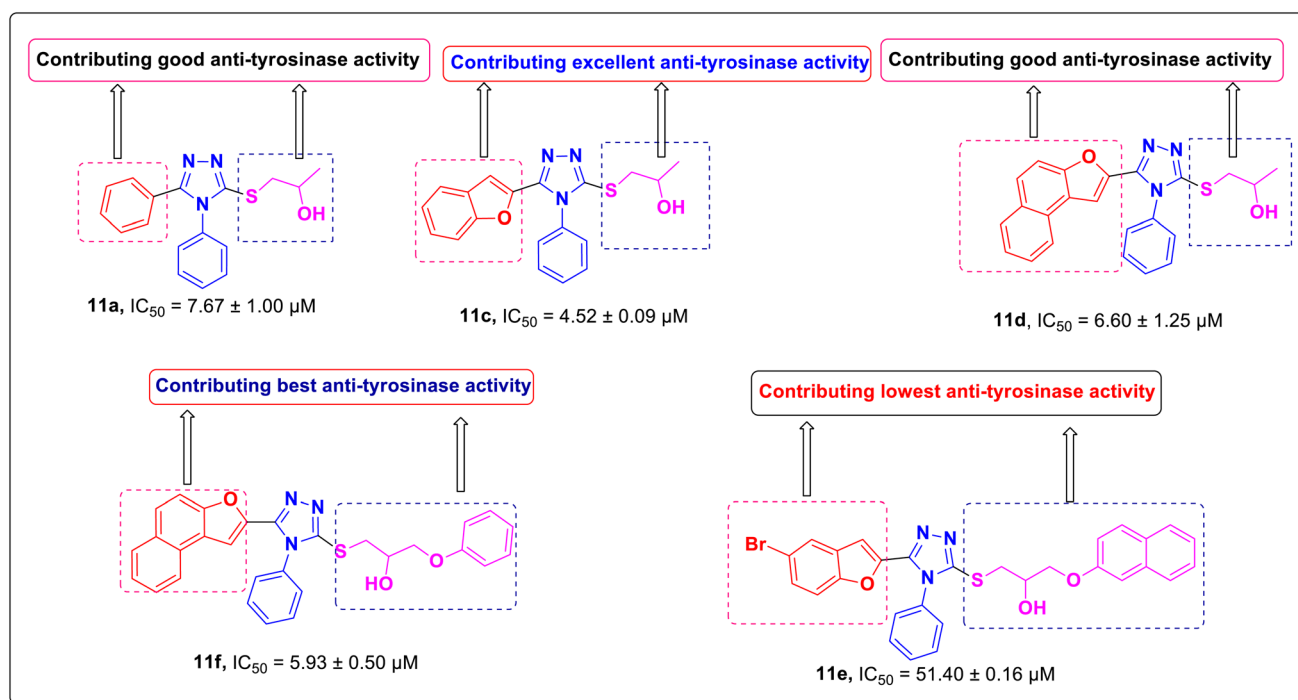


Fig. 2 Structure–activity relationship of the excellent to least potent tyrosinase inhibitors.





contacts of  $\pi$ - $\pi$ , T-shaped coordination with Phe347 and a  $\pi$ -alkyl water-repellant contact (hydrophobic) with Ile368. Additionally, the triazole-linked phenyl ring also participated in a similar type of interaction with Val377. Furthermore, the benzofuran moiety, the main pharmacophore of this compound, significantly contributed to binding through dual  $\pi$ -alkyl water-repellant interactions (hydrophobic) with Val377. The benzofuran moiety of compound **11c** further engaged with His367, a critical active-site histidine in the tyrosinase enzyme. This residue gets coordinated to a copper ion, which is essential for the enzyme's catalytic activity. Moreover, the benzofuran moiety established a dual interaction with His367 *i.e.*,  $\pi$ - $\pi$ , T-shaped and a  $\pi$ - $\pi$  stacked type interactions. Other weak types of van der Waals (interactions) were also noted (observed) with the copper ions that are located in the substrate-binding site of tyrosinase enzyme along with the other amino acid vestiges of the active site. Further, preliminary computational SAR studies showed that the substitution of bulky groups on the benzofuran ring lowers the binding affinities of the derivatives towards the catalytic hub (active site) of the tyrosinase while the introduction of a small polar group like -OH (hydroxyl) on the benzofuran moiety enhances the binding of these compounds with the tyrosinase enzyme. The binding energies of the evaluated compounds are presented in Table 1, while the graphical representation of the compound **11c** inside the tyrosinase active site can be viewed in Fig. 3.

The probable mechanism of action of compound **11c** against the tyrosinase enzyme can be explained by the direct contact of the benzofuran moiety with the important histidine residue and other stronger interactions with the active site residues as seen in the molecular docking studies. This compound may obstruct the association of the catalytic Cu ions inside the catalytic hub of tyrosinase enzyme by interfering with the co-ordination process of these metal ions with the coordinating histidine residues. This may result in altering the oxidation states of these ions, resulting in the loss of activity of the tyrosinase enzyme.

### 2.5. Anti-bacterial activity

The outcomes of the anti-bacterial action of newly synthesized compounds presented as MIC values in comparison to the control (penicillin) are illustrated in Table 3. Among the evaluated compounds, most of the derivatives showed strong to moderate inhibition in defiance of gram +ve and -ve negative bacterial cultures *i.e.*, *S. aureus* and *E. coli* respectively. All-encompassing, compound **11a** demonstrated remarkable effectiveness in inhibiting the growth of the Gram-positive bacterium *S. aureus*, thereby exhibiting minimum inhibitory concentration (MIC) =  $2.0 \pm 2.25$  mg mL<sup>-1</sup>. However, against Gram-negative bacteria, compound **11c** exhibited notable potency, displaying an MIC of  $10 \pm 0.40$  mg mL<sup>-1</sup>. These findings underscore the diverse anti-bacterial properties of these compounds, with **11a** showing specificity towards Gram-positive strains and **11c** demonstrating efficacy against Gram-negative bacteria as depicted in Table 4.

SAR for  $\beta$ -hydroxy sulfides (**11a-h**) regarding antibacterial activity demonstrated that tested compounds exhibited desirable

activity, in which compound **11a** (MIC =  $2.0 \pm 2.25$  mg mL<sup>-1</sup>) having an aryl group at the triazole (ring), showed the most potent activity against *S. aureus* (Gram-positive) comparable to penicillin (MIC =  $2.2 \pm 1.15$  mg mL<sup>-1</sup>). The *p*-tolyl moiety, when introduced in place of aryl(ring), decreased the activity from  $2.2 \pm 1.15$  mg mL<sup>-1</sup> to  $3.5 \pm 0.25$  mg mL<sup>-1</sup>. The association of a benzofuran heterocycle at the 5th locale of the triazole ring in compound **11c** (MIC =  $2.5 \pm 0.00$  mg mL<sup>-1</sup>) revealed promising anti-bacterial activity. The introduction of the naphthofuran moiety in compound **11d** has resulted in a decreased activity (MIC =  $10 \pm 1.00$  mg mL<sup>-1</sup>). Similarly, the incorporation of 2-bromo-4-chlorophenoxymethyl **11g** (MIC =  $12 \pm 1.00$  mg mL<sup>-1</sup>) and acefylline moiety **11h** (MIC =  $9.0 \pm 1.50$  mg mL<sup>-1</sup>) did not significantly enhance the activity. All  $\beta$ -hydroxy sulfides (**11a-h**) were found to be less potent against Gram-negative bacteria than the standard drug. Among the synthesized derivatives, however, compound **11c** was interpreted to be functional with  $10 \pm 0.40$  mg mL<sup>-1</sup> MIC value. Other hybrids with *p*-tolyl **11b**, benzofuran **11c**, naphthofuran **11d**, 2-bromo-4-chlorophenoxymethyl **11g**, and acefylline **11h** showed moderate activity with MIC values ranging between  $11.0 \pm 1.00$  to  $20.5 \pm 1.00$  mg mL<sup>-1</sup> towards similar strain, whereas, compounds 5-bromobenzofuran **11e** and naphthofuran **11f** were found inactive against both strains.

## 3. Experimental

Reagents and starting materials used in this research were obtained from Merck and Sigma-Aldrich. All the reactions were conducted in a flask (round bottom) with magnetic stirring. All experiments were kept under surveillance by thin-layer chromatographic analysis on silica gel (60 F254) precoated plates. TLC spots were detected under ultraviolet (UV) light after elution. The melting points (m.p.) were determined with the help of the Galenkamp equipment. <sup>1</sup>H and <sup>13</sup>C-NMR spectra of prepared derivatives were chronicled on a Bruker spectrophotometer functioning at 400 MHz and 100 MHz for proton and carbon correspondingly. Chemical shifts values were measured in ppm (parts per million), which were obtained with respect to the internal reference *i.e.*, tetramethylsilane (TMS). <sup>1</sup>H-NMR signal multiplicities were expressed in "s", "d", "t", and "m" symbols for singlet, doublet, triplet, and multiplet correspondingly.

### 3.1. Synthesis of compounds

**3.1.1. General synthesis of benzofuran/naphthofuran esters.** For the preparation of substituted benzofuran/naphthofuran esters, previously published synthetic protocol was utilized. A solution of corresponding 2-hydroxybenzaldehyde/2-hydroxy-1-naphthaldehyde (1 mmol), ethyl chloroacetate (1 mmol), dimethylformamide, and K<sub>2</sub>CO<sub>3</sub> (1.5 mmol) was subjected to stirring for 4–6 h at 95 °C. Upon the indication of reaction completion (*via* TLC), the reaction mixture was streamed into chilled water and the resulting precipitates were filtered and their purification was attained by column chromatography (*n*-hexane/ethylacetate).<sup>34,35,42,43</sup>

**3.1.2. Synthesis of esters from carboxylic acids.** Substituted benzoic acid/acefylline (1 mmol) was dissolved in ethanol and



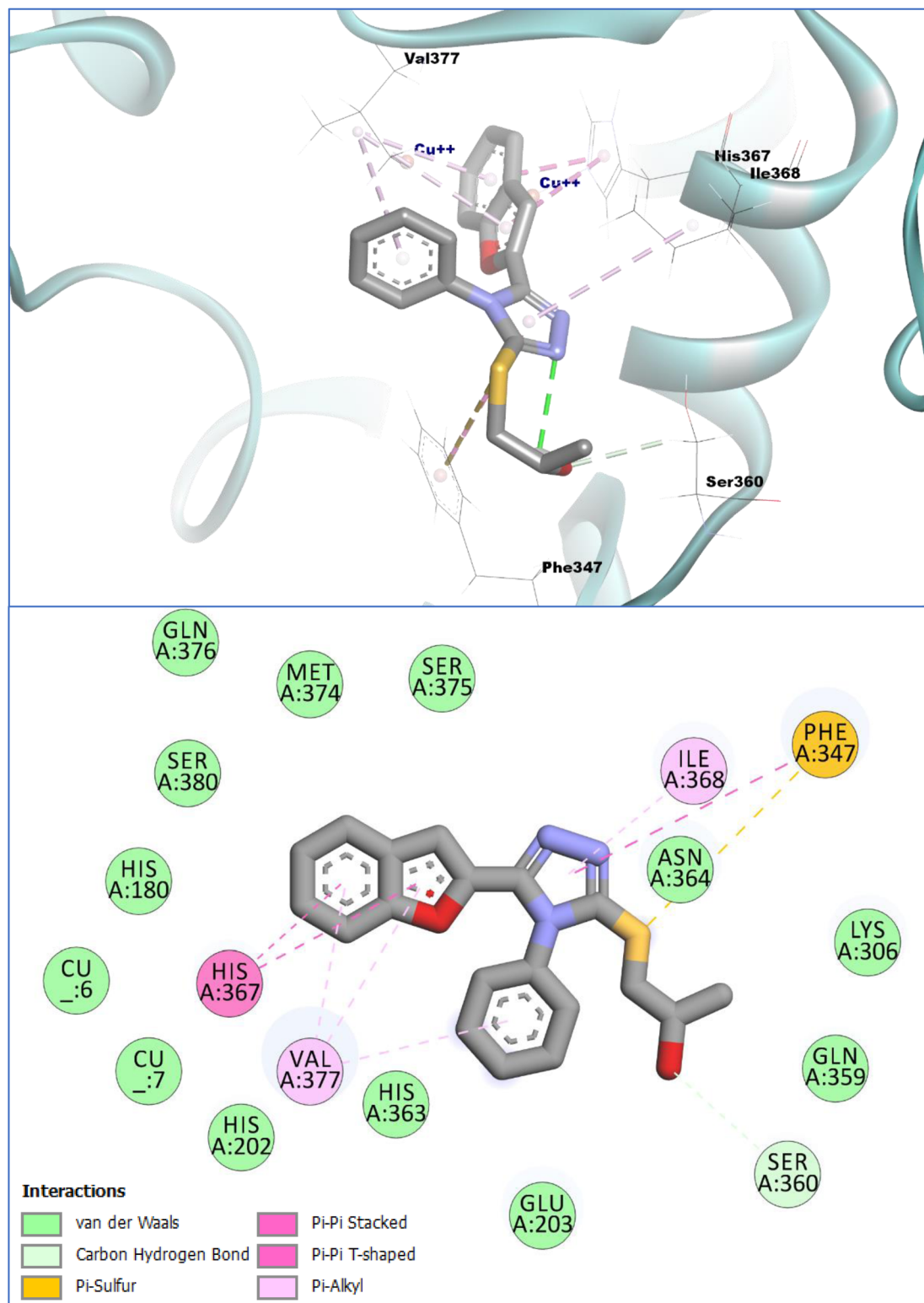


Fig. 3 Binding conformation and interaction profile of compound 11c with the tyrosinase enzyme. Molecular docking was performed using Webina (an online version of Autodock Vina).

refluxed with a catalytic amount (few drops) of concentrated sulfuric acid for 12 h. After monitoring the completion of reaction *via* TLC, sodium carbonate (saturated solution) was

added, until basic ( $\text{pH} \geq 8$ ). The solvent extraction of crude product was carried out with ethyl acetate (3 times), followed by washing of organic layer by using water and brine. Later, the

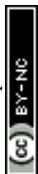


Table 3 Binding affinities of the evaluated compounds with the tyrosinase enzyme

Sr. No.	Compounds	Binding affinities
1	Compound <b>11c</b>	-7.08 kcal mol <sup>-1</sup>
2	Ascorbic acid	-5.59 kcal mol <sup>-1</sup>
3	Kojic acid	-5.78 kcal mol <sup>-1</sup>

organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> (anhydrous) which was purified by column chromatography using *n*-hexane/EtOAc.<sup>38,39,44,45</sup>

**3.1.3. Synthesis of ester from 2-bromo-4-chlorophenol.** A solution of 2-Br-4-Cl-phenol (1 mmol) and potassium carbonate (1.2 mmol), methyl/ethyl chloroacetate (1.5 mmol) in DMF was stirred and subjected to heat at 95 °C for 4–6 h. Upon reaction completion, excess of DMF was evaporated and the solvent extraction of unrefined product was carried out with ethyl acetate (3 times), followed by washing of organic layer with water. The washed organic layer was then dried over Na<sub>2</sub>SO<sub>4</sub> (anhydrous) which was subjected to purification by column chromatography using *n*-hexane/EtOAc.<sup>36,38,39,44,45</sup>

**3.1.4. Synthesis of hydrazides 6a–h.** Hydrazine monohydrate (6 mmol) was added dropwise to the solution of respective ester (1 mmol) in ethanol 0 °C and the reaction solution was stirred for 12–16 h/overnight. After the reaction completion (signaled by TLC), the solvent was evaporated to attain the product in crude form, which was employed as such in subsequent reaction(s).<sup>36,37,39,45–47</sup>

**3.1.5. Synthesis of triazoles 9a–h.** Hydrazide **6a–h** (1 mmol) was mixed to the solution of aryl isothiocyanate (1 mmol) in ethanol and refluxed for 2 hours. The resulting precipitates (thiosemicarbazide) were filtered, washed, re-dissolved in an aqueous solution of KOH (1.5 mmol) and refluxed for 4 h. The reaction mixture was then brought to 0–5 °C and acidified with dil. HCl. The resulting precipitates were drained away and recrystallized to attain the respective triazoles **9a–h**.<sup>34,40,48,49</sup>

**3.1.6. General protocol for the preparation of 1,2,4-triazole tethered β-hydroxy sulfides 11a–h.** Corresponding epoxide **10a–c** (1.2 mmol) was incorporated into a suspension of substituted 1,2,4-triazole-3-thione (1 mmol) and potassium carbonate (1

mmol) in water. Afterwards, the reaction was allowed to stir for 4–6 hours until completion. The solvent extraction of unrefined product was carried out with ethyl acetate (3 times), followed by washing of organic layer with water. Later, the corresponding layer was then dried over Na<sub>2</sub>SO<sub>4</sub> (anhydrous) and evaporated to get the crude product, which was purified by recrystallization (ethanol) or *via* column chromatography using *n*-hexane/EtOAc.<sup>41</sup>

**3.1.6.1. Synthesis of 1-((4,5-diphenyl-4H-1,2,4-triazol-3-yl)thio)propan-2-ol 11a.** According to the general procedure in Section 3.1.6, 3,4-diphenyl-1H-1,2,4-triazole-5(4H)-thione **9a** (0.1 g, 0.39 mmol), 2-methyloxirane **10a** (0.028 g, 0.47 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.05 g, 0.39 mmol) in water (2 mL) were reacted to obtain **11a** in 79% yield.

**3.1.6.2. Synthesis of 1-((4-phenyl-5-(*p*-tolyl)-4H-1,2,4-triazol-3-yl)thio)propan-2-ol 11b.** According to the general procedure in Section 3.1.6, 4-phenyl-3-(*p*-tolyl)-1H-1,2,4-triazole-5(4H)-thione **9b** (0.1 g, 0.37 mmol) was made to react with 2-methyloxirane **10a** (0.026 g, 0.45 mol) & K<sub>2</sub>CO<sub>3</sub> (0.05 g, 0.37 mmol) in water (2 mL) to attain **11b** in 79% yield.

**3.1.6.3. Synthesis of 1-((5-(benzofuran-2-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)propan-2-ol 11c.** In accordance with the general procedure Section 3.1.6, benzofuran-based triazole **9c** (0.1 g, 0.34 mmol), 2-methyloxirane **10a** (0.024 g, 0.41 mol) and potassium carbonate (0.047 g, 0.34 mmol) were reacted in water (2 mL) to obtain **11c** in 85% yield.

**3.1.6.4. Synthesis of 1-((5-(naphtho[2,1-*b*]furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)propan-2-ol 11d.** In accordance with the general procedure Section 3.1.6, naphthofuran-based triazole **9d** (0.1 g, 0.29 mmol) was reacted with 2-methyloxirane **10a** (0.02 g, 0.35 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.04 g, 0.29 mmol) in water (2 mL) to afford **11d** in 86% yield.

**3.1.6.5. Synthesis of 1-((5-(5-bromobenzofuran-2-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-3-(naphthalen-2-yloxy)propan-2-ol 11e.** In accordance with the general procedure Section 3.1.6, the reaction of benzofuran-based triazole **9e** (0.1 g, 0.26 mmol) upon treatment with 2-((naphthalen-2-yloxy)methyl)oxirane **10b** (0.065 g, 0.32 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.037 g, 0.26 mmol) in water (2 mL) yielded **11e** in 90% yield.

**3.1.6.6. Synthesis of 1-((5-(naphtho[2,1-*b*]furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-3-phenoxypropan-2-ol 11f.** In

Table 4 Anti-bacterial evaluation of 1,2,4-triazole-tethered β-hydroxy sulfide compounds 11a–h

Compound	<i>S. aureus</i>		<i>E. coli</i>	
	ZI (mm)	MIC (mg mL <sup>-1</sup> )	ZI (mm)	MIC (mg mL <sup>-1</sup> )
<b>11a</b>	10 ± 0.0	2.0 ± 2.25	12 ± 1.0	11.0 ± 1.00
<b>11b</b>	10 ± 1.0	3.5 ± 0.25	11.33 ± 1.52	12.5 ± 1.25
<b>11c</b>	11.33 ± 1.52	2.5 ± 0.00	10.33 ± 0.57	10 ± 0.40
<b>11d</b>	9.33 ± 0.57	10 ± 1.00	15.66 ± 2.08	16 ± 1.00
<b>11e</b>	ND	ND	ND	ND
<b>11f</b>	ND	ND	ND	ND
<b>11g</b>	19.33 ± 2.08	12 ± 1.00	14.33 ± 2.51	20.5 ± 1.00
<b>11h</b>	12.66 ± 1.52	9.0 ± 1.50	11 ± 1.0	15.0 ± 0.75
Penicillin	26 ± 0.75	2.2 ± 1.15	24 ± 0.50	2.4 ± 1.00





accordance with the general procedure Section 3.1.6, the reaction of naphthofuran-linked triazole **9d** (0.1 g, 0.29 mmol) was carried out with 2-(phenoxy)methyl oxirane **10c** (0.052 g, 0.35 mmol) and potassium carbonate (0.04 g, 0.29 mmol) in water (2 mL). Resultantly, targeted compound **11f** was formed in 81% yield.

**3.1.6.7. Synthesis of 1-((5-((2-bromo-4-chlorophenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)propan-2-ol **11g**.** According to the general procedure in Section 3.1.6, triazole derivative **9g** (0.1 g, 0.25 mmol) was subjected to react with 2-methyloxirane **10a** (0.018 g, 0.30 mmol), and  $K_2CO_3$  (0.035 g, 0.25 mmol) in water (2 mL) to yield desired compound **11g** (69% yield).

**3.1.6.8. Synthesis of 7-((5-((2-hydroxypropyl)thio)-4-phenyl-4H-1,2,4-triazol-3-yl)methyl)-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione **11h**.** According to the general procedure in Section 3.1.6, acefylline bearing triazole **9h** (0.1 g, 0.27 mmol) upon treatment with 2-methyloxirane **10a** (0.019 g, 0.32 mmol) and  $K_2CO_3$  (0.037 g, 0.27 mmol) in water (2 mL) afforded **11h** in 71% yield.

## 3.2. Characterization data

**3.2.1. 1-((4,5-Diphenyl-4H-1,2,4-triazol-3-yl)thio)propan-2-ol (**11a**).** White solid; yield 79%; mp 190 °C;  $^1H$ -NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.25 (d,  $J = 6.2$  Hz, 3H,  $CH_3$ ), 2.10 (s, 1H, OH), 3.17 (dd,  $J = 6.9, 14.4$  Hz, 1H,  $CH_2$ ), 3.37 (dd,  $J = 3.3, 14.3$  Hz, 1H,  $CH_2$ ), 4.16–4.20 (m, 1H, CH), 7.17–7.46 (m, 10H, aryl-H).  $^{13}C$ -NMR (100 MHz,  $CDCl_3$ )  $\delta$  22.34, 40.92, 67.29, 126.16, 127.24, 128.10, 128.55, 129.91, 130.04, 130.09, 133.99, 153.77, 154.90. MS (ESI)  $m/z$ : 312.11  $[M + 1]^+$ ; anal. calcd. For  $C_{17}H_{17}N_3OS$ : C, 65.57; H, 5.50; N, 13.49; found; C, 65.59; H, 5.52; N, 13.47.

**3.2.2. 1-((4-Phenyl-5-(*p*-tolyl)-4H-1,2,4-triazol-3-yl)thio)propan-2-ol (**11b**).** White solid; yield: 79%; mp 191 °C;  $^1H$ -NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.26 (d,  $J = 6$  Hz, 3H,  $CH_3$ ), 2.02 (s, 3H,  $CH_3$ ), 3.23 (dd,  $J = 6.8, 14.1$  Hz, 1H,  $CH_2$ ), 3.42 (dd,  $J = 2.4, 14.1$  Hz, 1H,  $CH_2$ ), 4.18–4.41 (m, 1H, CH), 6.43–7.59 (m, 9H, aryl-H).  $^{13}C$ -NMR (100 MHz,  $CDCl_3$ )  $\delta$  20.82, 22.29, 40.94, 67.16, 121.73, 123.56, 126.18, 127.17, 127.46, 130.21, 130.89, 133.24, 142.26, 147.75, 154.49, 154.80. MS (ESI)  $m/z$ : 326.11  $[M + 1]^+$ ; anal. calcd. For  $C_{18}H_{19}N_3OS$ : C, 66.43; H, 5.88; N, 12.91; found; C, 66.40; H, 5.86; N, 12.95.

**3.2.3. 1-((5-(Benzofuran-2-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)propan-2-ol (**11c**).** White solid; yield: 85%; mp 181 °C;  $^1H$ -NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.33 (d,  $J = 6.4$  Hz, 3H,  $CH_3$ ), 1.85 (s, 1H, OH), 3.25 (dd,  $J = 7.0, 14.2$  Hz, 1H,  $CH_2$ ), 3.47 (dd,  $J = 3.3, 14.2$  Hz, 1H,  $CH_2$ ), 4.24–4.28 (m, 1H, CH), 6.45–7.67 (m, 10H, aryl/benzofuran-H).  $^{13}C$ -NMR (100 MHz,  $CDCl_3$ )  $\delta$  22.39, 40.94, 67.30, 107.35, 111.73, 121.64, 123.49, 126.02, 127.26, 127.46, 130.15, 130.73, 133.47, 142.69, 147.98, 154.26, 154.76. MS (ESI)  $m/z$ : 352.10  $[M + 1]^+$ ; anal. calcd. For  $C_{19}H_{17}N_3O_2S$ : C, 64.94; H, 4.88; N, 11.96; found; C, 64.95; H, 4.86; N, 11.99.

**3.2.4. 1-((5-(Naphtho[2,1-*b*]furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)propan-2-ol (**11d**).** Off white solid; yield: 86%; mp 159 °C;  $^1H$ -NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.26 (d,  $J = 6.1$  Hz, 3H,  $CH_3$ ), 1.92 (s, 1H, OH), 3.22 (dd,  $J = 6.9, 14.1$  Hz, 1H,  $CH_2$ ), 3.41 (dd,  $J = 2.8, 14.1$  Hz, 1H,  $CH_2$ ), 4.19–4.22 (m, 1H, CH), 6.91–7.80 (m, 12H, aryl/naphthofuran-H).  $^{13}C$ -NMR (100 MHz,  $CDCl_3$ )  $\delta$  22.34, 41.01, 67.17, 106.77, 112.22, 122.80, 123.11, 125.10,

126.78, 127.33, 127.35, 127.56, 128.82, 130.18, 130.37, 130.82, 133.41, 141.76, 147.91, 152.79, 154.20. MS (ESI)  $m/z$ : 402.11  $[M + 1]^+$ ; anal. calcd. For  $C_{23}H_{19}N_3O_2S$ : C, 68.81; H, 4.77; N, 10.47; found; C, 68.82; H, 4.75; N, 10.49.

**3.2.5. 1-((5-(5-Bromobenzofuran-2-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-3-(naphthalen-2-yloxy)propan-2-ol (**11e**).** Off white solid; yield: 90%; mp 98 °C;  $^1H$ -NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.98 (s, 1H, OH), 3.56 (dd,  $J = 5.1, 13.7$  Hz, 1H,  $CH_2$ ), 3.70 (dd,  $J = 13.6$  Hz, 1H,  $CH_2$ ), 4.17–4.24 (m, 2H,  $CH_2$ ), 4.52 (s, 1H, CH), 6.37–7.74 (m, 16H, aryl/benzofuran/naphthalene-H).  $^{13}C$ -NMR (100 MHz,  $CDCl_3$ )  $\delta$  34.49, 67.74, 67.83, 104.94, 105.00, 111.26, 116.67, 121.90, 122.39, 124.53, 124.91, 125.44, 125.69, 127.17, 127.21, 127.55, 128.38, 129.10, 131.16, 132.48, 141.66, 145.58, 151.59, 153.08, 154.32. MS (ESI)  $m/z$ : 574.06  $[M + 1]^+$ ; anal. calcd. For  $C_{29}H_{22}BrN_3O_3S$ : C, 60.84; H, 3.87; N, 7.34; found; C, 60.86; H, 3.85; N, 7.30.

**3.2.6. 1-((5-(Naphtho[2,1-*b*]furan-2-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-3-phenoxypropan-2-ol (**11f**).** Off white solid; yield: 81%; mp 139 °C;  $^1H$ -NMR (400 MHz,  $CDCl_3$ )  $\delta$  2.03 (s, 1H, OH), 3.54 (dd,  $J = 6.3, 14.5$  Hz, 1H,  $CH_2$ ), 3.69 (d,  $J = 14.5$  Hz, 1H,  $CH_2$ ), 4.04–4.08 (m, 1H,  $CH_2$ ), 4.14 (dd,  $J = 9.1, 4.9$  Hz, 1H,  $CH_2$ ), 4.31–4.56 (m, 1H, CH), 6.70–7.88 (m, 17H, aryl/naphthofuran-H).  $^{13}C$ -NMR (100 MHz,  $CDCl_3$ )  $\delta$  36.42, 69.59, 69.71, 107.04, 112.24, 114.50, 121.12, 122.80, 123.13, 125.17, 126.85, 127.35, 127.50, 128.86, 129.51, 130.24, 130.41, 130.94, 133.28, 141.57, 147.96, 152.90, 154.58, 158.32. MS (ESI)  $m/z$ : 494.15  $[M + 1]^+$ ; anal. calcd. For  $C_{29}H_{23}N_3O_3S$ : C, 70.57; H, 4.70; N, 8.51; found; C, 70.54; H, 4.72; N, 8.53.

**3.2.7. 1-((5-((2-Bromo-4-chlorophenoxy)methyl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)propan-2-ol (**11g**).** White semisolid; yield: 69%;  $^1H$ -NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.23 (d,  $J = 6.1$  Hz, 3H,  $CH_3$ ), 3.17 (dd,  $J = 6.8, 14.1$  Hz, 1H,  $CH_2$ ), 3.37 (dd,  $J = 3.2, 14$  Hz, 1H,  $CH_2$ ), 4.15–4.19 (m, 1H, CH), 5.02 (s, 2H,  $CH_2$ ), 6.91–7.50 (m, 8H, aryl-H).  $^{13}C$ -NMR (100 MHz,  $CDCl_3$ )  $\delta$  21.93, 39.64, 60.30, 66.85, 112.55, 114.58, 127.11, 127.56, 128.47, 129.46, 130.12, 130.90, 131.68, 133.12, 136.29, 152.41, 156.15, 161.08. MS (ESI)  $m/z$ : 454.98  $[M + 1]^+$ ; anal. calcd. For  $C_{18}H_{17}BrClN_3O_2S$ : C, 47.54; H, 3.77; N, 9.24; found; C, 47.55; H, 3.78; N, 9.26.

**3.2.8. 7-((5-((2-Hydroxypropyl)thio)-4-phenyl-4H-1,2,4-triazol-3-yl)methyl)-1,3-dimethyl-1H-purine-2,6(3H,7H)-dione **11h**.** White solid; yield: 71%; mp 96 °C;  $^1H$ -NMR (400 MHz,  $CDCl_3$ )  $\delta$  1.25 (d,  $J = 6$  Hz, 3H,  $CH_3$ ), 3.22 (s, 3H,  $CH_3$ ), 3.48 (s, 3H,  $CH_3$ ), 3.66–4.73 (m, 3H,  $CH_2$ , CH), 5.56 (s, 2H,  $CH_2$ ), 7.45, 7.63 (m, 6H, aryl-H/N=CH).  $^{13}C$ -NMR (100 MHz,  $CDCl_3$ )  $\delta$  22.14, 27.82, 29.80, 40.54, 40.76, 66.92, 106.50, 126.92, 130.21, 130.79, 131.52, 141.68, 148.29, 150.93, 151.42, 154.70, 154.84. MS (ESI)  $m/z$ : 428.14  $[M + 1]^+$ ; anal. calcd. For  $C_{19}H_{21}N_7O_3S$ : C, 53.38; H, 4.95; N, 22.94; found; C, 53.39; H, 4.96; N, 22.92.

## 3.3. Tyrosinase inhibition assay

A novel series of naphthofuran based triazole scaffolds was synthesized and assessed for their ability to inhibit tyrosinase activity, an enzyme involved in melanin production. The details about the isolation, purification, characterization, and kinetic constants ( $K_m$  and  $V_{max}$ ) *etc.* of bacterial tyrosinase enzyme are provided as ESI† (Section 2: Bacterial tyrosinase enzyme data).



The evaluation of tyrosinase inhibition followed a modified protocol based on methods as outlined by Kim.<sup>50</sup> In this protocol, a reaction mixture comprising of L-tyrosine (700  $\mu\text{L}$ ; 2 mM), phosphate buffer solution (0.05 M), and synthesized molecules (100  $\mu\text{L}$ ) was prepared and subjected to incubation for ten minutes at room temperature. Afterwards, the 200  $\mu\text{L}$  of bacterial tyrosinase (approximately 48 U  $\text{mL}^{-1}$ ) was introduced to the solution, which was then subjected to incubation at 37  $^{\circ}\text{C}$  for 20 minutes. The dopachrome formation was observed after 2 minutes incubation by gauging the escalation in optical density (OD) at  $\lambda_{\text{max}} = 275$  nm. The prepared derivatives (1 mM) were solubilized in DMSO to furnish stock solution, from which further five different dilutions were prepared. Kojic acid (1 mM), a known tyrosinase inhibitor, was taken as standard for comparison. The effectiveness of inhibition was expressed as  $\text{IC}_{50}$  values, representing the concentration at which 50% inhibition occurred. To quantify the inhibitory activity, the inhibition percentage was evaluated by using the below formula:

$$\% \text{ Inhibition} = [(A - B)/A] \times 100$$

where, *A* represented the enzyme activity in the absence of inhibitor (control), and *B* represented the enzyme activity in the presence of the test sample (inhibitor).

### 3.4. Molecular docking study

The protein structure of the target enzyme human Tyrosinase (hTYR) has no resolved X-ray crystallographic or cryo-EM structure available in the protein databank yet. Therefore, the amino acid sequence of this enzyme was taken from the UniProt database with sequence ID-P14679 and a new protein structure of this enzyme was homology modelled by utilizing the SWISS-MODEL<sup>51</sup> server. The template (used) for modelling this structure, was hTYRP1 (human tyrosinase-related protein-1) with PDB ID-5M8L<sup>52</sup> as this enzyme showed the highest homology with the human tyrosinase. As the hTYRP1 contains the  $\text{Zn}^{++}$  as active catalytic atoms and the hTYR contains the  $\text{Cu}^{++}$  ions utilizing the MOE v2022 software, the copper ions were modelled into the prepared homology modelled hTYR enzyme active site. The protein structure was further optimized *via* the protein preparation function in the MOE v2022. The structures of ligands were prepared in ChemDraw professional v16.0 and then it was imported to MOE for further optimization. After that, the ligands were docked using the online version of AutodockVina (v1.2.3) known as Webina<sup>53-55</sup> by creating a box with XYZ dimensions of 22  $\text{\AA}$  and around the catalytic hub of the modelled tyrosinase enzyme with the grid coordinates of the docking site centered at  $X = 32.154$ ,  $Y = 140.176$ ,  $Z = 215.585$ . The molecular interaction and conformational analysis of the ligand-protein complexes were performed in Biovia DS v2017 software.

### 3.5. Antibacterial assay

The antibacterial efficacy of designated synthesized structural motifs was assessed by using disc diffusion technique.<sup>56,57</sup> A suspension of hundred microliter constituting 108 cfu per

milliliter of bacteria was spread onto nutrient agar using a sterilized loop. Filter paper discs with 5.6 mm diameter, impregnated with the compound solution at a specified concentration of 25  $\mu\text{g}/100$   $\mu\text{L}$  in  $\text{CHCl}_3$ , and were carefully positioned on the agar surface. Penicillin was served as a reference drug (25  $\mu\text{g}$  per disc). Following the one hour incubation at 4  $^{\circ}\text{C}$ , it was proceeded at 27  $^{\circ}\text{C}$  for 24 hours. The diameter of the inhibition zone, including the disc's 5.6 mm diameter, was measured in millimeters using a reference control.

## 4. Conclusion

A mild and efficient synthetic protocol has been utilized to achieve 1,2,4-triazole-tethered  $\beta$ -hydroxy sulfide structural motifs **11a-h** in good to remarkable yields (69–90%) by the thiolysis of oxiranes with substituted 1,2,4-triazole-3-thiol using (basic conditions) and characterized by various spectroscopic techniques. These novel 1,2,4-triazole-tethered  $\beta$ -hydroxy sulfides **11a-h** were screened against bacterial tyrosinases. Among these synthesized triazole derivatives, molecules **11a** ( $\text{IC}_{50} = 7.67 \pm 1.00$   $\mu\text{M}$ ), **11c** ( $\text{IC}_{50} = 4.52 \pm 0.09$   $\mu\text{M}$ ), **11d** ( $\text{IC}_{50} = 6.60 \pm 1.25$   $\mu\text{M}$ ), and **11f** ( $\text{IC}_{50} = 5.93 \pm 0.50$   $\mu\text{M}$ ) demonstrated the higher tyrosinase inhibition efficacy in comparison to the reference standards ascorbic acid ( $\text{IC}_{50} = 11.5 \pm 1.00$   $\mu\text{M}$ ) and kojic acid ( $\text{IC}_{50} = 30.34 \pm 0.75$   $\mu\text{M}$ ). Specifically, the benzofuran (ring) containing compound **11c** unveiled the most significant inhibition activity ( $\text{IC}_{50} = 4.52 \pm 0.09$   $\mu\text{M}$ ), surpassing both kojic acid and ascorbic acid. Moreover, triazole compounds **11a** and **11c** demonstrated comparable anti-bacterial efficacy with MIC of  $2.0 \pm 2.25$   $\text{mg mL}^{-1}$  and  $2.5 \pm 0.00$   $\text{mg mL}^{-1}$ , respectively, against the *S. aureus* strain, comparable to the standard antibiotic penicillin (MIC =  $2.2 \pm 1.15$   $\text{mg mL}^{-1}$ ). Notably, compound **11c** exhibited slightly less anti-bacterial activity (MIC =  $10 \pm 0.40$   $\text{mg mL}^{-1}$ ) against *E. coli*, and was evidently less potent as compared to penicillin (MIC =  $2.4 \pm 1.00$   $\text{mg mL}^{-1}$ ). Furthermore, the benzofuran-moiety based compound **11c** demonstrated a better binding score ( $-7.08$   $\text{kcal mol}^{-1}$ ) than both ascorbic acid ( $-5.59$   $\text{kcal mol}^{-1}$ ) and kojic acid ( $-5.78$   $\text{kcal mol}^{-1}$ ), indicating the strong molecular interactions. Molecular docking studies corroborate the *in vitro* findings, suggesting the compound **11c** as promising candidate for bacterial tyrosinase inhibition. Thus, it could be inferred that compound **11c** shows dual functionality, offering a means to combat melanogenesis and bacterial infections simultaneously. Its versatility underscores its significance in pharmaceutical research, suggesting avenues for novel therapeutic interventions against microbial threats.

## Author contributions

S. S.: methodology, investigation, data curation, writing—original draft preparation. M. J. S.: visualization; writing—review and editing, validation, project administration. A. F. Z.: conceptualization, resources, writing—review and editing, supervision, project administration; H. T.: writing—review and editing, formal analysis, data curation; S. K.: biological activity, writing—review and editing; S. F.: molecular docking software,



writing—original draft, writing—review and editing; R. A.; methodology, writing—review and editing. S. G. K.: resources, data curation, writing—review and editing, funding acquisition; U. N.: analysis, writing—original draft preparation, resources, data curation; A. I.: writing—review and editing, visualization, funding acquisition; M. A. B.: project administration, writing—review and editing, and funding acquisition.

## Conflicts of interest

Authors have no conflict of interest for this research work.

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