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Synthesis, docking and evaluation of antioxidant and antimicrobial activities of novel 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl) selenopheno[2,3-*d*]pyrimidines☆

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ABSTRACT

A series of 1,2,4-(triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl)selenopheno[2,3-*d*]pyrimidines (**10a**–**j**) were synthesized with various substituted anilines and benzoic acids. Structures of newly synthesized compounds were established by IR, ^{1}H & ^{13}C NMR and LC–MS spectral data. The antioxidant activity of the synthesized compounds was evaluated by DPPH, NO and H_2O_2 radical scavenging methods. The newly synthesized compounds were evaluated for their antimicrobial activity against Gram +ve and Gram −ve bacteria and antifungal activity by well diffusion method. Compounds **10d**, **10h** and **10i** showed promising antioxidant, antibacterial as well as antifungal activity and these were found to be the most potent activity molecules when compared with that of standard drugs. Molecules docking studies have been performed on *Staphylococcus aureus* (SA) of Gram +ve bacteria.

1. Introduction

In the past few decades, the incidence of microbial infection has increased on frightening levels over the world as a result of antimicrobial resistance. Microbial infections are a growing problem in contemporary medicine and the use of antibiotics is common across the world. Consequently, there is an urgent need to widen new antimicrobial agents, which have a broad spectrum of activity against the resistant micro-organisms. The current literature is enriched with progressive findings about the synthesis and pharmacological action of fused heterocycles. It is well known that the N-bridged heterocycles consequent from 1,2,4-triazoles find applications in the field of medicine, agriculture and industry. There is existing therapeutically important medicines Terconazole, Itraconazole, Fluconazole, Cefazoline, Ribavarin, Triazolam, Alprazolam, Etizolam and Furacylin are some of the examples which contain anyone of these heterocyclic nucleuses. Moreover, triazolo-thiadiazoles are a class of fused heterocyclic compounds, which have attracted great interest in medicinal chemistry owing to their wide range of pharmacological activities including antitumor/antiproliferative/anticancer activities [1–9].

In contrast, selenium has attracted great interest as an essential element and certain diseases have been eradicated by dietary supplementation of this element. Selenium is essential for cell metabolism as a component of glutathione peroxidase and other enzyme systems. Current interest lies in the prevention of certain cancers by supplementation with selenium [10–12]. Furthermore, Selenium is noted to regulate the expression and activity of selenoenzymes and thus provides protection from oxidative stress induced cell damage, which otherwise would lead to neuropsychiatric diseases and disorders like cerebrovascular disease, Alzheimer's disease, Parkinson's disease, obsessive compulsive disorders, stroke and epilepsy [13,14]. It is noted that selenium modulates endogenous antioxidant enzyme systems in quenching reactive oxygen species in the neuronal cells [15–17]. Similarly, organo selenium heterocycles have attracted much attention and have been widely used in medicinal chemistry research. Synthesis of selenophenes has been of considerable interest during the past few decades as they exhibit better biological activity compared to other heterocycles.

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Prompted by these observations and continuation of our research [18,19] program on the synthesis of novel heterocyclic compounds exhibiting biological activity, it was thought worthwhile to prepare a new type of hybrid that clubbed both of selenopheno[2,3-*d*]pyrimidin as well as 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazole ring systems with a view to produce promising biologically active agents.

In order to know the binding interactions of these molecules docking studies were performed on *Staphylococcus aureus* (SA) of Gram +ve bacteria. Pathogenicity for majority of diseases caused by SA is multifunctional. Hence, it is difficult to define precisely the role of any specific enzyme in the inhibition. In this regard docking was performed on four different proteins namely dehydrosqualene synthase (DHSS), Thymidine kinase (TK), hydroxymethylpterin pyrophosphokinase (HPPK) and dihydropteroate synthetase (DHPS). Among the four different proteins considered for the study DHSS of SA considered to be important and aimed for targeting the virulence of SA. Due to high structural similarity with the human DHSS which is involved in cholesterol synthesis pathway, cholesterol lowering drugs found to have inhibitory effect on DHSS of SA [20]. Drugs reported and marketed are of phosphosulfonates, they considered as most potent and selective inhibitors of DHSS of SA. According to the study of Kahlon et al. [21] amino acids involved in the functionality of this enzyme are hydrophilic, polar and charged residues. In order the molecules to be active towards DHSS it must and should have the hydrophilic functional groups in its structure. Due to less hydrophilic character of these molecules, active sites of other proteins mentioned earlier have been considered.

2. Results and discussion

2.1. Chemistry

In order to generate the desired compounds, eight steps synthetic strategies are adopted are depicted in Scheme 1. The basic compound ethyl 5-amino-4-cyano-3-methylselenophene-2-carboxylate **1** was synthesized *via* expedient procedure of ethyl acetoacetate, malononitrile, Selenium metal powder in DMF as solvent and catalytic amount of imidazole by the standard reaction of Gewald method [22]. In the next step, compound **1** was refluxed with triethyl orthoformate [23,24] afforded 4-cyano-5-ethoxymethyleneimino-3-methylselenophene-2-carboxylic acid ethyl ester **2** with good yield. Further, compound **2** was converted in to corresponding selenopheno[2,3-*d*]pyrimidines **3a** and **3b** by treating with appropriate substituted anilines in the presence of acetic acid gave the satisfactory yields. The ester hydrolysis of compounds **3a** and **3b** with aqueous sodium hydroxide in methanol yielded the corresponding substituted carboxylic acids **4a** and **4b** with an excellent yield. Meanwhile, compounds **9a**–**e** was prepared from the appropriate substituted benzoic acids by employing well known methods available in the literature [25,26]. Finally, the triazolo thiadiazolyl selenopheno[2,3-*d*]pyrimidines were prepared from the reaction of compounds **3a** & **3b** with compounds **9a**–**e** in the presence of $POCl_3$ afforded the compounds (**10a**–**j**) in good yields. The detailed synthesis and spectral data analysis were described in the experimental section.

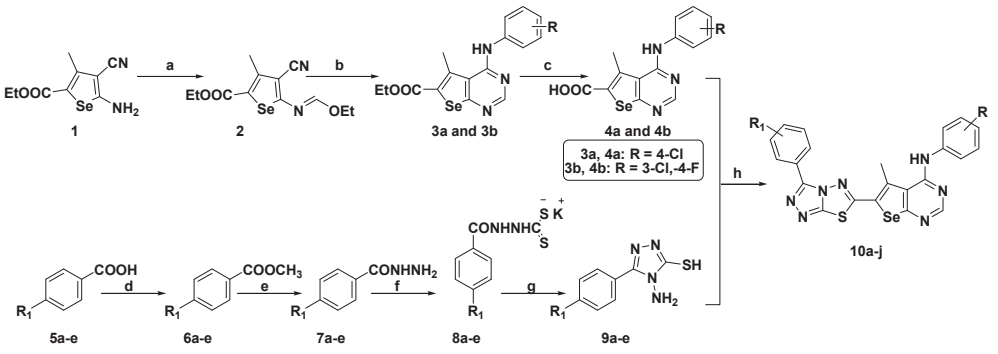
Table 1
Antimicrobial activity (MIC profiles) of the synthesized compounds (**10a**–**j**).

Minimum inhibitory concentration (MIC) (μg ml^{-1} sample)						
Compound	Antibacterial activity				Antifungal activity	
	Gram +ve bacteria		Gram −ve bacteria			
	S. aureus	*B. subtilis*	*K. pneumoniae*	*E. coli*	*A. flavus*	*C. albicans*
10a	50	>100	>100	>100	50	>100
10b	25	50	25	50	50	>100
10c	12.5	25	25	12.5	6.25	6.25
10d	6.25	3.12	6.25	6.25	3.12	1.56
10e	>100	>100	>100	>100	>100	>100
10f	50	50	100	100	50	25
10g	6.25	12.5	12.5	12.5	12.5	25
10h	6.25	3.12	6.25	6.25	3.12	3.12
10i	3.12	3.12	3.12	3.12	1.56	1.56
10j	100	100	50	50	>100	>100
Ciprofloxacin	6.25	6.25	6.25	6.25	Nt	Nt
Fluconazole	Nt	Nt	Nt	Nt	3.12	3.12
Control	–	–	–	–	–	–

Nt – denotes not tested.
Lower MIC values indicate higher the antimicrobial activity.

2.2. Antimicrobial activity

All the synthesized compounds (**10a**–**j**) were screened for their antimicrobial activity determined by well plate method [27,28]. The



Reagents and conditions: (a) $HC(OEt)_3$, (10 eq.), reflux, 6 h, (b) AcOH, halo substituted anilines, 4 h, reflux; (c) NaOH, MeOH, RT, 16 h; (d) $SOCl_2$, MeOH, reflux 2 h; (e) $NH_2NH_2\ H_2O$, reflux, 1 h; (f) CS_2, KOH, reflux, 12 h; (g) $NH_2NH_2\ H_2O$, reflux, 12 h; (h) $POCl_3$, reflux, 5-6 h.

Compound	10a	10b	10c	10d	10e	10f	10g	10h	10i	10j
R	4-Cl	4-Cl	4-Cl	4-Cl	4-Cl	3-Cl,4-F	3-Cl,4-F	3-Cl,4-F	3-Cl,4-F	3-Cl,4-F
R_1	H	4-CH_3	4-OCH_3	4-OH	4-NO_2	H	4-CH_3	4-OCH_3	4-OH	4-NO_2

Scheme 1. Synthesis of novel 1,2,4-(triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl)selenopheno[2,3-*d*]pyrimidines.

potentiality of the synthesized compounds as antimicrobials was appraised for their antimicrobial studies against various Gram +ve such as *S. aureus* and *Bacillus subtilis* and Gram −ve such as *Escherichia coli* and *Klebsiella pneumoniae* strains of human pathogens. The result obtained as MIC is presented in Table 1. It is more attractive to speculate the observation that the result of the antimicrobial activity of the various derivatives appeared to be related the nature of substituents on the phenyl ring. It is evident from Table 1 that four compounds viz. **10d**, **10g**, **10h** and **10i** were found more potent with either less or equal MIC as compared to control drug ciprofloxacin. DMSO was also taken in a control experiment which showed no effect in the experiment. The compound **10i** containing 3-chloro-4-fluoro substituted aniline on selenopheno [2,3-*d*]pyrimidine and hydroxyl substituted benzene ring on triazolothiadiazol was found potent against all the tested organisms with MIC 3.12 μg ml^{-1} while the standard Ciprofloxacin showed 6.25 μg ml^{-1}. Further, the compounds **10h** and **10d** having $-OCH_3$ and −OH respectively found equal and considerable antibacterial activity against all the tested microorganisms at MIC 6.25 μg ml^{-1}. The compound **10g** found equal and moderate activity against tested microorganisms at MIC in the range 6.25–12.5 μg ml^{-1} while the standard showed 6.25 μg ml^{-1}. Further, the compounds **10b**, and **10c** were found moderate to good activity against all the tested organisms at a MIC range 50 to 12.5 μg ml^{-1}. The compounds **10a**, **10e**, **10f** and **10j** were found inactive with MIC 50 μg ml^{-1}, 100 μg ml^{-1} and >100 μg ml^{-1} against all strains. In general, control drug ciprofloxacin showed MIC 6.25 μg ml^{-1} against all the tested microorganisms. It is obvious from the analysis of activity results that electron withdrawing groups such as chloro and chloro-fluoro on selenopheno[2,3-*d*]pyrimidine these have strong effects in determining the antibacterial activity. This observation is supported by the highest activity shown by the compound **10i** against both the Gram +ve and Gram −ve bacterial strains. Thus, we hypothesize that the presence of electron withdrawing groups such as chloro and chloro-fluoro and triazolothiadiazol with electron releasing groups such as OH, OCH_3 and CH_3 has significant effect on antibacterial activity as compared to withdrawing NO_2 group.

Further, these compounds **10a**–**j** were also screened for their antifungal activity against two fungal strains such as *Aspergillus flavus* and *Candida albicans*. Fluconazole was taken as a standard drug throughout the experiment. Compound **10d** was found to be the equal and potent antifungal activity with MIC 3.12 μg ml^{-1} and 1.56 μg ml^{-1} against *A. flavus* and *C. albicans* respectively, while standard drug Fluconazole with MIC 3.12 μg ml^{-1}. The reason might be the presence of electron releasing hydroxyl group present on the triazolothiadiazol ring in the moiety. The compounds **10h** and **10i** containing 3-chloro-4-fluoro substitution on selenopheno[2,3-*d*] pyrimidine but only compound **10i** showed potent activity with MIC 1.56 μg ml^{-1} whereas compound **10d** showed similar activity with MIC 3.12 μg ml^{-1} against both the fungal strains. The compound **10c** showed moderate activity with MIC 6.25 μg ml^{-1} against both the fungal strains.

2.3. Antioxidant screening (in vitro)

In the present study, the antioxidant activity of the synthesized compounds was assessed *in vitro* by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, nitric oxide (NO) and hydrogen peroxide (H_2O_2) [29–33]. These methods were based on measuring the continual absorbance decrease of the methanolic solution of the DPPH at 517 nm, in the presence of antioxidant compound. The DPPH has an odd electron so it can accept an electron or hydrogen free radical. In the presence of antioxidant, this odd electron becomes paired due to H transfer from antioxidant and hence DPPH absorbance decreases. The ability of newly synthesized compounds (**10a**–**j**) to act as hydrogen donors or free radical scavengers was tested by *in vitro* antioxidant assays involving DPPH radical, NO radical, H_2O_2, and the results were compared with that of standard antioxidants including natural antioxidant Ascorbic acid and synthetic antioxidant BHT (Butylated Hydroxy Toluene). All the synthesized compounds showed interesting antioxidant activity compared to the standard (Table 2). Among the synthesized compounds, **10d**, **10h** and **10i** were found to be the most potent antioxidant activity compared with that of the standard (AA and BHT 12.27 ± 0.86 and 16.53 ± 1.74 respectively) with the least values of IC_{50} 11.02 ± 0.27, 10.41 ± 0.23 and 9.46 ± 0.91 μg ml^{-1}, inhibition concentration respectively. Table 2 gives an account for this antioxidant evolution and indicates that compounds were capable with significant scavenging properties towards DPPH. The reason would be the presence of electron releasing hydroxy and methoxy groups on phenyl ring which is linked with triazolothiadiazole along with halo (4-Cl and 3-Cl,4-*F*-phenyl) substituted seleneopheno[2,3-*d*]pyrimidines. Further, insertion of substituted phenyl on both sides to that of the moiety endowed notable improvement in radical scavenging activity. Almost, all the tested compounds (**10a**–**j**) exhibited optimistic efficacy for scavenging DPPH free radical. The next promising antioxidant activity was showed by compounds **10a**, **10b**, **10c**, **10g** and **10f** were found to be moderate to good. This may be due to the presence of unsubstituted and electron donating groups such as $-CH_3$ and $-OCH_3$ on phenyl ring which linked to the triazolothiadiazole. Compounds **10d** and **10e** which are having NO_2 group on phenyl ring showed least activity compared to other compounds. This may due to the presence of electron withdrawing nature of nitro group would decrease the scavenging ability.

The IC_{50} value is the concentration of sample required to inhibit 50% of the NO radicals. Except **10d** and **10e** all the tested compounds exhibited strong NO radical scavenging with low IC_{50} values. The greater NO radical scavenging activity of the tested compounds was showed by compounds **10d**, **10h** and **10i** showed 13.72 ± 1.26, 12.74 ± 0.18 and 8.20 ± 1.60 respectively. This may due to the presence of the electron donating groups such as $-CH_3$ and $-OCH_3$ on phenyl ring. Almost all the synthesized compounds have capable to exhibit the NO radical scavenging activity with satisfactory results. The synthesized compounds (**10a**–**j**) having various concentrations (5, 10, 25, 50 and 100 μg ml^{-1}) were subjected to H_2O_2 radical scavenging activity. The findings of present study (Table 2) indicated that the most of the synthesized compounds exhibited moderate to good radical scavenging ability. Biological

Table 2
Antioxidant activities of the compounds in IC_{50} values.

Compound	Scavenging activity (IC_{50} μg ml^{-1})		
	DPPH	NO	H_2O_2
10a	14.82 ± 0.26	18.41 ± 0.78	17.03 ± 0.40
10b	13.31 ± 0.17	21.64 ± 0.42	19.14 ± 0.81
10c	12.75 ± 0.36	19.82 ± 0.61	21.09 ± 0.44
10d	11.02 ± 0.27	13.72 ± 1.26	15.38 ± 0.96
10e	33.28 ± 0.85	46.92 ± 1.02	48.31 ± 0.93
10f	13.24 ± 0.48	20.27 ± 0.81	22.92 ± 0.74
10g	12.86 ± 0.17	16.52 ± 0.49	18.36 ± 0.22
10h	10.41 ± 0.23	12.74 ± 0.18	17.08 ± 0.12
10i	9.46 ± 0.91	8.20 ± 1.60	12.54 ± 1.17
10j	26.94 ± 0.67	30.17 ± 0.44	36.42 ± 0.92
AA[a]	12.27 ± 0.86	14.62 ± 0.97	15.24 ± 0.44
BHT[b]	16.53 ± 1.74	19.06 ± 1.04	17.82 ± 0.28
Blank	–	–	–

(−) Showed no scavenging activity. Lower IC_{50} values indicate higher radical scavenging activity. Values were the means of three replicates ± SD.
[a] Ascorbic acid.
[b] Butylated Hydroxy Toluene.

systems can produce hydrogen peroxide. The generation of H_2O_2 by activated phagocytes is known to play an important part in the killing of several bacterial and fungal strains. Additionally, H_2O_2 is formed in vivo by a variety of enzymes, including superoxide dismutase. H_2O_2 is in general measured as a powerful oxidizing agent. It is known that H_2O_2 is toxic and induces cell death *in vitro*. It can attack many cellular energy-producing systems. Thus, removing hydrogen peroxide, as well as superoxide anion, is very important for the protection of pharmaceuticals and food systems. The highest H_2O_2 scavenging effect was detected in compounds **10i** and **10d** (12.54 ± 1.17, and 15.38 ± 0.96) than the standard Ascorbic acid and Butylated Hydroxy Toluene 15.24 ± 0.44 and 17.82 ± 0.28 respectively. The reason would be the presence of electron releasing hydroxyl group present on the phenyl ring. Almost all the tested compounds exhibited moderate to weak antioxidant activity in the H_2O_2 radical scavenging activity.

2.4. Docking results

The analysis of docking score and hydrogen bond interactions were performed for all the molecules (**10a**–**10j**) including the standard ciproflaxin. Re-dock pose of the crystal structure ligands showed RMSD values in range and further confirmed the docking validation. Docking results were analyzed with different parameters like dock score and hydrogen bond interactions. Combined analysis of these two parameters for TK, HPPK and DHSS gave comparable results in accordance with one another. This is not seen in the case of DHSS where a high dock score and less hydrogen bond interactions were observed compare to existing cholesterol lowering drugs. This was analyzed and confirmed to be the structural disparity between the molecule and the active site of the DHSS protein. As the molecules taken for the study were hydrophobic in nature could not participate in hydrogen bond interactions with the active site residues which are hydrophilic in character. Docking analysis of all the molecules in the set emphasized that molecule **10i** in the series is showing good results compared to other candidates of the set. This is due to the hydrophilic (OH) substituent in the molecule, which makes the hydrogen bond contribution during the calculation of dock score that is missing in other molecules of the series. This OH group is participating in hydrogen bond interaction with His 18 and Arg 265 of DHSS in molecule **10i** (Fig. 1), but are not seen with the other molecules due to lack of polar groups this further giving an evidence for hydrophilic requirement in the molecule towards protein active site. The docking results for all the four proteins were given in Table 3 (Supplementary material). As per the dock score is concerned these molecules showed good results towards DHSS ranging −8.99 to −5.68 whereas the other proteins showing dock score as −5.03 to −3.62, −5.90 to −3.59 and −4.52 to −2.84 for TK, HPPK and DHPS respectively.

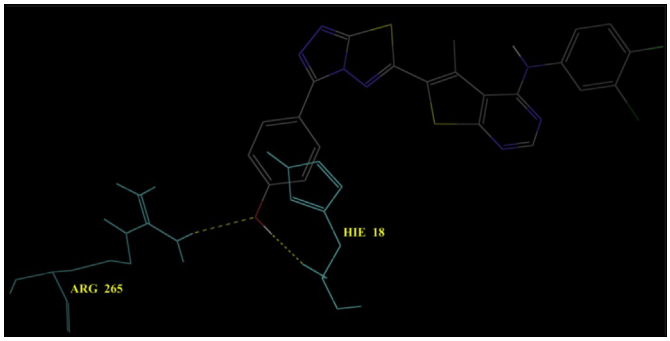


Fig. 1. Docking results.

3. Conclusions

In summary, we have synthesized a series of ten 1,2,4-(triazolo[3,4-*b*][1,3,4]thiadiazol-6-yl)selenopheno[2,3-*d*]pyrimidine derivatives and evaluated for their *in vitro* antimicrobial and antioxidant activities were successfully achieved. Three compounds **10d**, **10h** and **10i** showed excellent antibacterial activities against all the tested strains with MIC value comparable to those of Ciprofloxacin and Fluconazole. Specially, compound **10i** displayed the strain-specific to both bacterial and fungal strains (MIC 3.12 μg ml^{-1} and 1.56 μg ml^{-1} respectively). The radical scavenging activities of the synthesized compounds also showed that the tree compounds such as **10d**, **10h** and **10i** with potent IC_{50} values than that of the standard drugs in all the three methods. The structure activity relationship (SAR) studies showed that the electron releasing groups such as hydroxyl, methoxy and methyl were essential to activities, and the functional groups presented on phenyl ring plays important role in the antibacterial selectivity of these compounds. Further, complete structure activity relationship (SAR) and mechanistic approach should be taken into account while considering designing and screening of much more compounds. Due to multifunctional factorization of SA virulence docking studies were performed on DHSS, TK, HPPK and DHPS to know the binding interactions of these molecules. More attention has been paid for DHSS among other proteins here we discussed the docking interactions of these molecules with DHSS. Due to high hydrophobic character of these molecules showing limited interactions with the active site of DHSS. Compare to other molecules in the series **10i** showed good interactions with the active site amino acids of the four proteins. This confirms that the hydrophilic character should be possessed by the molecules for the inhibition of DHSS from SA. Hence, further modification of these molecules with the incorporation of hydrophilic substituents will further result in increasing the activity towards DHSS. The development of the second generation analogs is in progress. More research in this direction is under progress and results will be published in due course.

4. Experimental section

4.1. Chemistry

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 (mesh); spots were visualized with UV light. Merck silica gel (60–120 mesh) was used for column chromatography. The IR spectra were recorded on a Perkin–Elmer BX1 FTIR Spectrophotometer as KBr pellets and the wave numbers were given in cm^{-1}. ^1H NMR (400 MHz), and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker AMX 400 MHz NMR spectrometer in $CDCl_3$/

Table 3
Dock score of ten analogues (**10a**–**10j**) taken for the study.

Mol. name	DHSS	TK	DPHS	HPPK	MIC of SA
10a	−7.0514	−3.9187	−2.8396	−4.3213	50
10b	−5.6861	−3.4578	−3.6544	−3.5958	25
10c	−6.5804	−3.7924	−2.9203	−3.8199	12.5
10d	−7.8957	−4.108	−3.6576	−4.5312	6.25
10e	−6.618	−4.5428	−3.6719	−4.0565	>100
10f	−6.5028	−3.2665	−3.0626	−3.9658	50
10g	−6.8441	−4.3323	−3.0722	−3.8094	6.25
10h	−6.0305	−3.479	−3.902	−3.8645	6.25
10i	−8.9898	−4.2737	−4.0857	−4.6417	3.12
10j	−8.4159	−3.5492	−4.3743	−4.2034	100
Ciproflaxin	−8.4888	−5.0387	−4.521	−5.9075	6.25

DMSO-d_6 solution using TMS as an internal standard. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Agilent 1100 LC/MSD instrument with method API-ES at 70 eV. The microanalyses were performed on a Perkin–Elmer 240C elemental analyzer. The antioxidant property was carried out by using Shimadzu UV-2450 spectrophotometer.

4.1.1. Synthesis of ethyl 5-amino-4-cyano-3-methylselenophene-2-carboxylate (**1**)

The starting ethyl 5-amino-4-cyano-3-methylselenophene-2-carboxylate **1** was prepared according to reported Gewald synthetic procedure (Huang et al., 2011). A mixture of ethyl acetoacetate (3.0 mmol), dicyanomethane (3.3 mmol), selenium powder (4.5 mmol), and imidazole (0.3 mmol) in DMF (3.0 ml) was stirred at 60 °C under nitrogen atmosphere for 16 h. After completion of starting materials, the reaction mixture was cool to room temperature, and then the unreacted selenium powder was filtered. The filtrate was then poured onto ice cold water, and stirred for 15 min. The solid obtained was collected by filtration and recrystallized from ethanol. Yellowish solid Yield 53%; m.p. = 212–214 °C; IR (Chloroform) ν (cm^{-1}): 3435 (NH_2), 2206 (C≡N), 1641 (C=O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 (t, 3H, $-CH_2-\underline{CH_3}$), 2.48 (s, 3H, selenophene$-CH_3$), 4.27 (q, 2H, $-\underline{CH_2}-CH_3$), 5.50 (br s, 2H, NH_2); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 14.14, 14.59, 60.02, 88.65, 106.88, 114.90, 146.25, 161.24 and 166.57; LC–MS (negative ion mode): m/z 257 $(M-H)^-$ for $C_9H_{10}N_2O_2Se$.

4.1.2. Synthesis of 4-cyano-5-ethoxymethyleneimino-3-methylselenophene-2-carboxylic acid ethyl ester (**2**)

A mixture of 5-amino-4-cyano-3-methylselenophene-2-carboxylicacid ethyl ester **1** (2.0 g, 7.72 mmol) and triethyl orthoformate (7.15 ml, 38.61 mmol) was refluxed for 16 h. After completion of starting compound the reaction mixture was cooled, the excess amount of triethyl orthoformate was concentrated and the solid obtained was recrystallized from ethanol. Yield: 86%; m.p.: 106–108 °C; IR (KBr) ν (cm^{-1}): 2958, 2894, 2210, 1690, 1558; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (t, 3H, $-OCH_2\underline{CH_3}$), 1.43 (t, 3H, CH_3–ester), 2.58 (s, 3H, CH_3–selenophene), 4.31(q, 2H, $-O\underline{CH_2}CH_3$), 4.47 (q, 2H, CH_2–ester), 7.90 (s, 1H, N=$\underline{CH}$); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 14.28, 15.84, 16.25, 61.31, 62.12, 109.86, 114.23, 115.67, 145.12, 153.51, 156.49 and 167.21; LC–MS (positive ion mode): m/z 315 $(M+H)^+$ for $C_{12}H_{14}N_2O_3Se$.

4.2. General procedure for the synthesis of ethyl 4-(substituted phenylamino)-5-methylselenopheno[2,3-d]pyrimidine-6-carboxylate (**3a** and **3b**)

A mixture of 4-cyano-5-ethoxymethyleneimino-3-methylselenophene-2-carboxylic acid ethyl ester **2** (1.6 g 5.09 mmol) was dissolved in AcOH to this added (6.11 mmol) of appropriate halo substituted anilines and refluxed for 4 h. After completion of the starting compounds, then the total reaction mixture was cooled to room temperature for 2 h, poured into ice cold water and stirred for 15 min. The product was separated by filtration and washed with water, dried well and recrystallized from chloroform and n-hexane to give compounds (**3a** and **3b**) in good yields.

4.2.1. Ethyl 4-(4-chlorophenylamino)-5-methylselenoenopheno [2,3-d]pyrimidine-6-carboxylate (**3a**)

Yield 86%; White crystalline solid m.p. = 166–168 °C; IR (KBr) ν (cm^{-1}): 3421, 2926, 1710, 1565; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (t, 3H, $-CH_2-\underline{CH_3}$), 3.08 (s, 3H, selenophene$-CH_3$), 4.37 (q, 2H, $-\underline{CH_2}-CH_3$), 7.37 (d, 2H, Ar–H, J = 12.0 Hz), 7.50 (s, 1H, N–H), 7.59 (d, 2H, Ar–H, J = 8.0 Hz), 8.51 (s, 1H, C–H); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 14.05, 15.28, 61.10, 114.73, 117.79, 120.61, 124.18, 133.73, 140.12, 154.35, 158.62, 161.03, 162.35 and 166.36; LC–MS (negative ion mode): m/z 394 $(M-H)^-$ for $C_{16}H_{14}ClN_3O_2Se$.

4.2.2. Ethyl 4-(3-chloro-4-fluorophenylamino)-5-methlyselenopheno[2,3-d]pyrimidine-6-carboxylate (**3b**)

Yield 68%; Pale-yellow solid m.p. = 158–160 °C; IR (KBr) ν (cm^{-1}): 3412, 2926, 1706, 1555; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (t, 3H, $-CH_2-\underline{CH_3}$), 3.07 (s, 3H, selenophene$-CH_3$), 4.37 (q, 2H, $-\underline{CH_2}-CH_3$), 7.16 (t, 1H, Ar–H, J = 8.0 Hz), 7.42 (m, 1H, Ar–H), 7.47 (s, 1H, N–H), 7.81 (q, 1H, Ar–H, J = 4.0 Hz), 8.52 (s, 1H, C–H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.25, 15.96, 60.09, 112.36, 115.82, 117.08, 118.49, 122.84, 141.06, 144.62, 146.54, 150.76, 154.38, 156.61, 160.44 and 170.18; LC–MS (negative ion mode): m/z 412 $(M-H)^-$ for $C_{16}H_{13}ClFN_3O_2Se$.

4.3. General procedure for the synthesis of 4-(substituted phenylamino)-5-methylselenopheno[2,3-d]pyrimidine-6-carboxylic acid (**4a** and **4b**)

The compound (**3a** and **3b**) was dissolved in MeOH/H_2O (12 ml: 6 ml), and 15% v/v NaOH aq (2 ml) was added. Stirring was continued for 16 h at rt, then $CHCl_3$ was added. The aqueous layer was acidified with 1 N HCl, stirred for 15 min, the product was separated by vacuum filtration and washed with water, dried well and recrystallized from chloroform and methanol to give compounds (**4a** and **4b**) in good yields.

4.3.1. 4-(4-Chlorophenylamino)-5-methylselenopheno[2,3-d] pyrimidine-6-carboxylic acid (**4a**)

Yield 92%; Off-white solid, m.p. = 324–326 °C; IR (KBr) ν (cm^{-1}): 3446, 2926, 1705, 1539; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.12 (s, 3H, selenophene$-CH_3$), 7.45 (d, 2H, Ar–H, J = 8.0 Hz), 7.71 (d, 2H, Ar–H, J = 12.0 Hz), 8.52 (s, 1H, N–H), 8.69 (s, 1H, C–H), 13.60 (br s, 1H, $-COO\underline{H}$); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 16.62, 120.93, 124.00, 127.50, 127.72, 127.99, 137.41, 140.61, 153.39, 157.24, 164.77and 171.55; LC–MS (positive ion mode): m/z 368 $(M+H)^+$ for $C_{14}H_{10}ClN_3O_2Se$.

4.3.2. 4-(3-Chloro-4-fluorophenylamino)-5-methylselenenopheno [2,3-d]pyrimidine-6-carboxylic acid (**4b**)

Yield 87%; White solid m.p. = 315–317 °C; IR (KBr) ν (cm^{-1}): 3423, 2924, 1693, 1568; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.04 (s, 3H, selenophene$-CH_3$), 7.43 (t, 1H, Ar–H, J = 8.0 Hz), 7.64 (t, 1H, Ar–H, J = 4.0 Hz), 7.92 (d, 1H, Ar–H, J = 4.0 Hz), 8.52 (s, 1H, N–H), 8.70 (s, 1H, C–H), 13.56 (br s, 1H, $-CO\underline{OH}$); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.07, 113.61, 117.84, 123.57, 127.78, 138.52, 142.51, 146.34, 151.24, 154.70, 156.66, 163.71, 166.81 and 169.23; LC–MS (positive ion mode): m/z 386 $(M+H)^+$ for $C_{14}H_9ClFN_3O_2Se$.

4.4. General procedure for the synthesis of compounds **10a–j**

To a mixture of compound (**4a** and **4b**) (0.01 mol) and appropriate substituted phenyl triazoles (0.01 mol), phosphorus oxychloride (10 ml) was added and the reaction contents were refluxed for 5–6 h. After completion of the starting compounds, then the total reaction mixture was cooled to room temperature for 2 h, poured into ice cold water, basified with 10% NaOH and stirred for 15 min. The product was separated by filtration and washed with ice cold water, dried well and recrystallized from chloroform and methanol to give compounds (**10a–j**) in good yields.

*4.4.1. N-(4-Chlorophenyl)-5-methyl-6-(3-phenyl-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazol-6-yl)selenopheno[2,3-d]pyrimidin-4-amine (**10a**)*

Yield 71%; Pale yellow solid, m.p. = 246–248 °C; IR (KBr) ν (cm^{-1}): 3438, 2937, 1605, 1538; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.96 (s, 3H, selenophene–CH_3), 7.46 (d, 2H, Ar–H, J = 8.0 Hz), 7.57–7.68 (m, 5H, Phenyl), 8.26 (d, 2H, Ar–H, J = 8.0 Hz), 8.49 (s, 1H, N–H), 8.91 (s, 1H, C–H), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.05, 120.84, 124.30, 125.46, 125.81, 128.39, 129.18, 129.79, 130.38, 137.76, 137.89, 140.58, 147.16, 151.95, 154.09, 157.15, 161.42, and 166.20; LC–MS (positive ion mode): m/z 524 $(M + H)^+$ for $C_{22}H_{14}ClN_7SSe$.

*4.4.2. N-(4-Chlorophenyl)-5-methyl-6-(3-p-tolyl-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazol-6-yl)selenopheno[2,3-d]pyrimidin-4-amine (**10b**)*

Yield 68%; Off-white solid, m.p. = 240–242 °C; IR (KBr) ν (cm^{-1}): 3437, 2922, 1603, 1543; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.41 (s, 3H, Phenyl–CH_3), 2.98 (s, 3H, selenophene–CH_3), 7.39 (d, 2H, Ar–H, J = 8.0 Hz), 7.46 (d, 2H, Ar–H, J = 8.0 Hz), 7.68 (d, 2H, Ar–H, J = 8.0 Hz), 8.16 (d, 2H, Ar–H, J = 8.0 Hz), 8.41 (s, 1H, N–H), 8.50 (s, 1H, C–H), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.23, 20.96, 117.81, 123.04, 125.77, 126.60, 127.38, 129.52, 129.92, 131.43, 137.07, 137.46, 141.26, 148.50, 152.03, 153.14, 156.84, 162.10, and 167.48; LC–MS (positive ion mode): m/z 538 $(M + H)^+$ for $C_{23}H_{16}ClN_7SSe$.

*4.4.3. N-(4-Chlorophenyl)-6-(3-(4-methoxyphenyl)-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazol-6-yl)-5-methylselenopheno[2,3-d] pyrimidin-4-amine (**10c**)*

Yield 74%; Off-white solid, m.p. = 262–264 °C; IR (KBr) ν (cm^{-1}): 3448, 2928, 1657, 1543; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.90 (s, 3H, selenophene–CH_3), 3.79 (s, 3H, Phenyl–OCH_3), 7.13 (d, 2H, Ar–H, J = 8.0 Hz), 7.36 (d, 2H, Ar–H, J = 8.0 Hz), 7.59 (d, 2H, Ar–H, J = 8.0 Hz), 8.11 (d, 2H, Ar–H, J = 8.0 Hz), 8.41 (s, 1H, N–H), 8.91 (s, 1H, C–H), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 17.68, 56.80, 116.53, 121.39, 125.12, 125.98, 128.31, 129.14, 130.44, 131.68, 136.24, 138.72, 140.80, 149.02, 151.37, 154.40, 156.81, 162.94, and 168.22; LC–MS (positive ion mode): m/z 554 $(M + H)^+$ for $C_{23}H_{16}ClN_7OSSe$.

*4.4.4. 4-(6-(4-(4-Chlorophenylamino)-5-methylselenopheno[2,3-d] pyrimidin-6-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phenol (**10d**)*

Yield 62%; White solid, m.p. = 242–244 °C; IR (KBr) ν (cm^{-1}): 3512, 3425, 2922, 1604, 1566; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.99 (s, 3H, selenophene–CH_3), 6.24 (s, 1H, Phenyl–OH), 7.44 (d, 2H, Ar–H, J = 8.0 Hz), 7.70 (d, 2H, Ar–H, J = 8.0 Hz), 8.22 (d, 2H, Ar–H, J = 8.0 Hz), 8.48 (d, 2H, Ar–H, J = 8.0 Hz), 8.51 (s, 1H, N–H), 9.17 (s, 1H, C–H), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.13, 120.76, 124.45, 127.39, 127.87, 128.36, 129.67, 131.43, 132.05, 137.76, 138.06, 140.26, 148.84, 153.14, 153.57, 157.63, 161.08, and 167.40; LC–MS (positive ion mode): m/z 539 $(M + H)^+$ for $C_{22}H_{14}ClN_7OSSe$.

*4.4.5. N-(4-Chlorophenyl)-5-methyl-6-(3-(4-nitrophenyl)-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazol-6-yl)selenopheno[2,3-d]pyrimidin-4-amine (**10e**)*

Yield 68%; Yellow solid, m.p. = 226–228 °C; IR (KBr) ν (cm^{-1}): 3436, 2912, 1607, 1547; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.97 (s, 3H, selenophene–CH_3), 6.74 (d, 2H, Ar–H, J = 8.0 Hz) 7.45 (d, 2H, Ar–H, J = 8.0 Hz), 7.68 (d, 2H, Ar–H, J = 8.0 Hz), 7.93 (d, 2H, Ar–H, J = 8.0 Hz), 8.50 (s, 1H, N–H), 9.08 (s, 1H, C–H), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.04, 112.19, 113.58, 120.89, 124.21, 124.45, 127.08, 127.62, 128.32, 137.58, 137.80, 139.27, 141.92, 147.17, 150.93, 153.97, 156.99 and 172.09; LC–MS (positive ion mode): m/z 569 $(M + H)^+$ for $C_{22}H_{13}ClN_8O_2SSe$.

*4.4.6. N-(3-Chloro-4-fluorophenyl)-5-methyl-6-(3-phenyl-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazol-6-yl)selenopheno[2,3-d]pyrimidin-4-amine (**10f**)*

Yield 74%; Off-White solid, m.p. = 228–230 °C; IR (KBr) ν (cm^{-1}): 3442, 2923, 1607, 1547; ^1H NMR (DMSO-d_6, 400 MHz) δ 3.00 (s, 3H, selenophene–CH_3), 6.58–6.90 (m, 5H, Phenyl), 7.45 (t, 1H, Ar–H, J = 8.0 Hz), 7.63 (dd, 1H, Ar–H, J = 4.0 Hz), 7.89 (dd, 1H, Ar–H, J = 8.0 Hz), 8.28 (s, 1H, N–H), 8.51 (s, 1H, C–H), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.13, 115.83, 116.05, 118.17, 120.91, 121.28, 123.87, 124.08, 127.11, 129.70, 132.22, 136.39, 137.13, 140.08, 143.84, 147.48, 153.60, 155.52, 157.71 and 162.04; LC–MS (positive ion mode): m/z 542 $(M + H)^+$ for $C_{22}H_{13}ClFN_7SSe$.

*4.4.7. N-(3-Chloro-4-fluorophenyl)-5-methyl-6-(3-p-tolyl-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazol-6-yl)selenopheno[2,3-d]pyrimidin-4-amine (**10g**)*

Yield 82%; Brown solid, m.p. = 272–274 °C; IR (KBr) ν (cm^{-1}): 3412, 2936, 1608, 1546; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.41 (s, 3H, Phenyl–CH_3), 3.00 (s, 3H, selenophene–CH_3), 7.39 (t, 1H, Ar–H, J = 8.0 Hz), 7.42 (dd, 1H, Ar–H, J = 4.0 Hz), 7.53 (dd, 1H, Ar–H, J = 8.0 Hz), 7.86 (d, 2H, Ar–H, J = 8.0 Hz), 8.15 (d, 2H, Ar–H, J = 8.0 Hz), 8.40 (s, 1H, N–H), 9.18 (s, 1H, C–H), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.14, 21.01, 116.36, 116.57, 119.30, 120.17, 121.40, 123.34, 124.46, 125.70, 129.68, 129.97, 131.14, 137.10, 138.87, 141.22, 145.66, 147.87, 152.28, 154.13, 158.76 and 161.48; LC–MS (positive ion mode): m/z 556 $(M + H)^+$ for $C_{23}H_{15}ClFN_7SSe$.

*4.4.8. N-(3-Chloro-4-fluorophenyl)-6-(3-(4-methoxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)-5-methylselenopheno [2,3-d]pyrimidin-4-amine (**10h**)*

Yield 76%; Pale yellow solid, m.p. = 262–264 °C; IR (KBr) ν (cm^{-1}): 3446, 3150, 2836, 1610, 1547; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.98 (s, 3H, selenophene–CH_3), 3.86 (s, 3H, Phenyl–OCH_3), 7.21 (d, 2H, Ar–H, J = 8.0 Hz), 7.46 (t, 1H, Ar–H, J = 8.0 Hz), 7.61 (dd, 1H, Ar–H, J = 4.0 Hz), 7.90 (dd, 1H, Ar–H, J = 8.0 Hz), 8.21 (d, 2H, Ar–H, J = 8.0 Hz), 8.52 (s, 1H, N–H), 8.91 (s, 1H, C–H), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.06, 52.98, 113.84, 117.40, 121.72, 123.61, 128.17, 119.20, 120.83, 121.62, 122.80, 124.07, 125.22, 129.41, 130.18, 131.77, 137.21, 138.29, 140.85, 144.38, 147.20, 153.12, 156.24, 157.68 and 162.07; LC–MS (positive ion mode): m/z 572 $(M + H)^+$ for $C_{23}H_{15}ClFN_7OSSe$.

*4.4.9. 4-(6-(4-(3-Chloro-4-fluorophenylamino)-5-methylselenopheno[2,3-d]pyrimidin-6-yl)-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazol-3-yl)phenol (**10i**)*

Yield 69%; White solid, m.p. = 225–227 °C; IR (KBr) ν (cm^{-1}): 3394, 3158, 2948, 1609, 1570; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.99 (s, 3H, selenophene–CH_3), 5.99 (br s, 1H, Phenyl–OH), 7.22 (t, 1H, Ar–H, J = 8.0 Hz), 7.44 (d, 2H, Ar–H, J = 8.0 Hz), 7.62 (dd, 1H, Ar–H, J = 4.0 Hz), 7.92 (dd, 1H, Ar–H, J = 8.0 Hz), 8.24 (d, 2H, Ar–H, J = 8.0 Hz), 8.52 (s, 1H, N–H), 9.12 (s, 1H, C–H), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.13, 116.40, 116.62, 118.76, 120.73, 121.08, 123.48, 124.64, 127.31, 135.93, 137.90, 142.68, 146.94, 149.27, 153.06, 153.70, 155.00,157.04 161.22 and 166.52; LC–MS (positive ion mode): m/z 558 $(M + H)^+$ for $C_{22}H_{13}ClFN_7OSSe$.

*4.4.10. N-(3-Chloro-4-fluorophenyl)-5-methyl-6-(3-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl) selenopheno[2,3-d]pyrimidin-4-amine (**10j**)*

Yield 61%; Yellow solid, m.p. = 236–238 °C; IR (KBr) ν (cm^{-1}): 3206, 2957, 1602, 1576; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.93 (s, 3H, selenophene–CH_3), 7.26 (t, 1H, Ar–H, J = 8.0 Hz), 7.38 (d, 2H, Ar–H, J = 8.0 Hz), 7.58 (dd, 1H, Ar–H, J = 4.0 Hz), 8.03 (dd, 1H, Ar–H, J = 8.0 Hz), 8.16 (d, 2H, Ar–H, J = 8.0 Hz), 8.79 (s, 1H, N–H), 9.01 (s, 1H, C–H), ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.24, 115.84, 116.40,

119.29, 121.35, 122.82, 123.04, 126.27, 129.60, 134.88, 138.92, 141.84, 146.29, 148.90, 152.74, 153.81, 156.22, 157.41 163.07 and 167.08; LC–MS (positive ion mode): m/z 587 $(M + H)^+$ for $C_{22}H_{12}ClFN_8O_2SSe$.

4.5. Biological essay

4.5.1. Antimicrobial activity

Microdilution broth susceptibility assay was used for the antibacterial evaluation of the compounds [27], whereas antifungal susceptibility of the fungus yeasts was examined according to NCCLS reference method for broth dilution antifungal susceptibility testing of yeasts [28]. Ciprofloxacin was used as standard antibacterial agent whereas flucanazol was used as antifungal agent and both are prepared as described in the related references.

4.5.2. DPPH radical scavenging activity

The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of 1,1-diphenyl-1-picrylhydrazyl (DPPH). The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 ml of various concentrations of the test compounds (5, 10, 25, 50 and 100 μg ml^{-1}) in methanol was added to 4 ml of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. The percent of inhibition (I%) of free radical production from DPPH was calculated by the following equation.

$$\%\ \text{of scavenging} = [(\text{A control} - \text{A sample})/\text{A blank}] \times 100 \quad (1)$$

where A control is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. Tests were carried at in triplicate.

4.5.3. Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity was measured by slightly modified methods of Green et al. and Marcocci et al. Nitric oxide radicals (NO) were generated from sodium nitroprusside. 1 ml of sodium nitroprusside (10 mM) and 1.5 ml of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (5, 10, 25, 50 and 100 μg ml^{-1}) of the test compounds and incubated for 150 min at 25 °C and 1 ml of the reaction mixture was treated with 1 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromatophore was measured at 546 nm. Nitric oxide scavenging activity was calculated using Eq. (1).

4.5.4. Hydrogen peroxide (H_2O_2) scavenging activity

The H_2O_2 scavenging ability of the test compound was determined according to the method of Ruch et al. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 5, 10, 25, 50 and 100 μg ml^{-1} concentrations of the test compounds in 3.4 ml phosphate buffer were added to H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. The percent of scavenging of H_2O_2 was calculated using Eq. (1).

4.5.5. Molecular docking studies

The crystal structures of *S. aureus* DHSS, TK, HPPK and DHPS (PDB ID: 3ACX, 2CCJ, 4DH6 & 1AD4) were retrieved from protein data bank [34–37] and these target proteins were used for molecular docking study using Glide5.6 [38]. The ligands were built using Maestro build panel and prepared by Lig Pep 2.0 application which uses MMFF 94s [39] force field and gave the corresponding low energy 3D conformers of the ligands. These ligands were then neutralized and checked for their ADME [40] properties using Qikprop. Qikprop helps in analyzing the pharmacokinetics and pharmacodynamics of the ligand by accessing the drug like properties. Before docking the screened ligands into the protein active site, the protein was prepared by deleting the substrate cofactor as well as the crystallographically observed water molecules and then the active site of the protein was defined for generating the grid. Receptor van der Waals scaling for the non polar atoms was set to 0.9 [41] which makes the protein site "roomier" by moving back the surface of non polar regions of the protein and ligand. This kind of adjustments emulate to some extent the effect of breathing motion to the protein site, it is a kind of giving breathing to the receptor, this approach softens the active site region of the receptor making it flexible [42]. The screened ligands were docked into the prepared grid, for which "standard precision mode" of docking was selected. The dock pose of each ligand docked into protein was analyzed for their hydrogen bond interactions with the receptor. The analysis of dock poses of all the molecules showed hydrogen bond interaction with the active site residues mentioned in the literature.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.01.006.

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