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ORIGINAL ARTICLE

Synthesis, spectral studies, *in vitro* and molecular docking studies of novel hydrazinyl carbothioamide derivatives

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Abstract Five novel compounds possessing hydrazinyl carbothioamide moiety were designed and synthesized. All the compounds were tested for *in vitro* biological activities. Most of the tested compounds displayed strong antibacterial and antifungal activities. Molecular docking studies suggested that the hydrazinyl carbothioamide moiety of compounds (**6**–**10**) can in general be accommodated the binding pocket of the breast cancer protein (1JNX) and are responsible for the activity of the whole of the molecule. The docking results provide a new insight into the design of hydrazinyl carbothioamide derivatives as breast cancer drug.

1. Introduction

Bacterial and fungal infections are growing problem in contemporary medicine, as a result of the increasing use of antibacterial agents for all kinds of infectious diseases on mankind, many drug-resistant pathogens have appeared on recent years besides that of various human diseases, cancer has proved to be one of the most intractable diseases to which human beings are subjected, and as yet no practical and generally effective drugs or methods of control are available. Therefore, identification of novel potent, selective and less toxic anticancer agents remains one of the most pressing health problems [1]. Hetero cycles possessing nitrogen and sulfur hetero atoms are found to exhibit a wide spectrum of biological activities including antibacterial [2] and antifungal [3], activities. Many hydrazinyl carbothioamide containing compounds are reported as herbicidal [4], fungicidal [5], anti-tubercular [6], anti allergic [7], anti anaphylactic [8], antiarthritic [9], antibiotic [10], antiviral [11], anti-inflammatory [12], analgesic [13] and psychotropic agents [14]. A series of hydrazine carbothioamide derivatives were synthesized possessing excellent antibacterial and antifungal activities [15–17], they have also been shown to possess antimalarial [18–20], antibiotic [21], anticancer [22], antiinflammatory [23], antihypertensive [24], tyrokinase PDGF–RTK inhibition [25] and anti-HIV [26,27] properties. The molecular docking technique [28,29], plays an important role in the drug design and discovery to predict the conformations of each ligand molecule at the active site. So it was planned to synthesize new hydrazinyl

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carbothioamide derivatives with 1, 4-disubstitution using a green route that is microwave organic reaction enhancement method (MORE) and test them for *in vitro* antifungal and antibacterial activities. The rigid molecular docking studies of newly synthesized hydrazinyl carbothioamide derivatives were carried out to predict the antibacterial activity and molecular docking are reported.

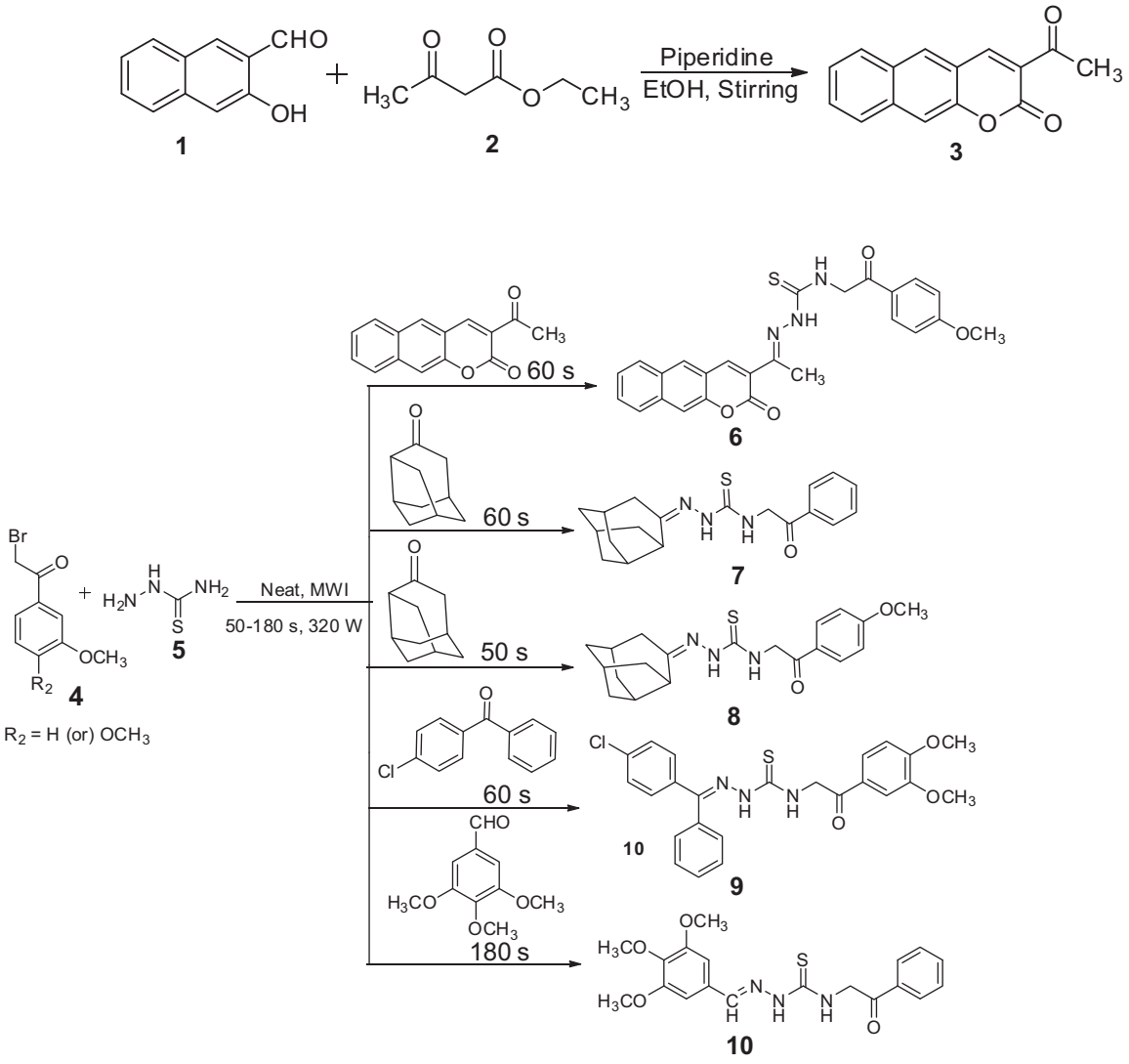
2. Results and discussion

2.1. Chemistry

In view of the large amount of literature that addresses organic-TSCs and their applications as potential antineoplastic and antibacterial agent, it is surprising that aryl ethanone incorporated analogs with these biological targets have not been extensively studied. So we determined to design and synthesize a series of N-14, N-17-disubstituted hydrazine carbothioamide derivatives (**6**–**10**) by the reaction of 2-bromo-1-aryl ethanone either with substituted ketone/aldehyde or with thiosemicarbazide Scheme 1. The synthesized compounds were characterized by (Preparation of 3-acetyl-2H-benzo[g]chromen-2-one), ^{1}H NMR Spectrum of compound **3** (Preparation of 3-acetyl-2H-benzo[g]chromen-2-one) (Fig. S1), IR, ^{1}H NMR, ^{13}C NMR, 2D NMR spectrum of ^{1}H–^{13}C COSY, HR-Mass spectrometry and Elemental analysis. In the IR spectrum of compound **6** the peaks at 1656, 1601, 1315 and 3429 cm^{-1} are due to carbonyl and C═O, C═N C═S and NH stretching frequencies.

2.2. NMR spectral analysis

In the ^{1}H NMR spectrum (Fig. S2) of compound **6** (Fig. 1) there is a singlet observed at 9.30 ppm that should be due to the coumarinyl benzylic proton (H-4). The (H-17a/b) protons of (H-17) appeared as singlet at 4.71 ppm. The two singlets appeared at 2.78 (methyl) and 3.89 (methoxy) each with three protons integral value must be due to the methyl and methoxy protons. The aromatic protons pertaining to the coumarinyl



Scheme 1 Synthesis of hydrazinyl carbothioamide derivatives (**6**–**10**).

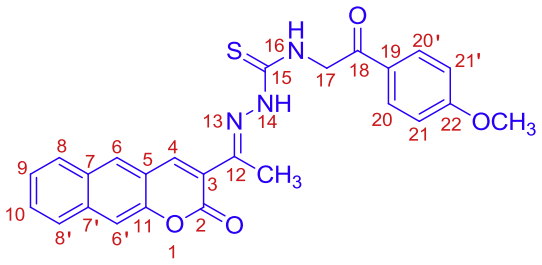


Figure 1 (E)-N-(2-(4-methoxyphenyl)-2-oxoethyl)-2-(1-(2-oxo-2H-benzo[g]chromen-3-yl)ethylidene)hydrazinyl carbothioamide.

and phenyl rings are observed as doublet and triplet at around 6.96–8.36 ppm.

In the ^{13}C NMR spectrum (Fig. S3) of the compound **6** the C-12 carbon is observed around 164.8 ppm, while the C-16 carbon is resonating at around 189.2 ppm. The signals in the region 195.6 ppm in compound **6** are the characteristics of the C-19 carbon. The C-2 carbon is observed at 156.2 ppm. The signal in the upfield region 30.7 ppm is due to the methyl carbon. The methylene carbon (C-17) of hydrazinyl carbothioamide functions on the region 43.0 ppm. The compound also possesses a signal at 55.7 ppm which is a characteristic of the methoxy carbon.

The aromatic carbons appeared at around 112.2–136.3 ppm. The *ipso* carbons of the phenyl ring and the coumarinyl group show a characteristic absorption further down field region. In methoxy compound **6** the most down field signal is assigned to methoxy bearing *ipso* carbon 159.4 ppm. The above assignments are further confirmed by recording ^{1}H–^{13}C COSY spectrum for the representative compound **6**.

Using ^{1}H–^{13}C COSY spectrum (Fig. S4) of the representative compound **6** ^{13}C signals can be assigned without ambiguity. The signal at 143.3 ppm has a cross peak with proton signal pertaining to H-4. Hence the signal at 143.3 ppm should be due to C-4. The signal at 116.5 ppm has a cross peak with the signal at 7.45–7.47 ppm (8.0 Hz) corresponding to H-6 protons and the signal with chemical shift (116.5 ppm) is due to C-6. The signal at 7.59–7.63 ppm (16.0 Hz) exhibits strong cross peak with the triplet 7.59–7.63 ppm pertaining to H-8 and H-8g′. Therefore the signal at 126.7 ppm must be due to C-8 and C-8′ ppm. The signal at 129.8 ppm has cross peak with the signals (triplet) in the region 7.73–7.76 (12.0 Hz) and 7.89–7.93 ppm (16.0 Hz) pertaining to H-9 and H-20 and H-20′ protons. Hence the signal with chemical shift 129.8 ppm must be due to C-9 and C-20 and C-20′ carbons. The more striking observation here is the signal at 136.3 ppm shows correlation with the doublet centered at 8.09–8.11 ppm (8.0 Hz) corresponding to H-10. So the signal at 136.3 ppm should be due to C-10. Likewise the signal at 121.7 ppm has a cross peak with the doublet in the region 8.34–8.36 ppm (8.0 Hz) which is due to the H-6′ proton. Hence the signal at 121.7 ppm is due to C-6′. The signal at 114.3 ppm has a cross peak with the doublet at 6.96–6.98 ppm (8.0 Hz) pertaining to H-21 and H-21′. So the signal at 114.3 ppm is due to C-21 and 21′ carbon. The signal at 30.7 ppm exhibits a correlation with the singlet at 2.78 characteristic of CH_3 protons and hence the signal at 30.7 ppm is due to methyl carbon. It is interesting to note that the signal at 43.0 ppm has a cross peak with the signal at 4.71 ppm due to the protons of the hydrazinyl methyene group. Therefore the signal at 43.0 ppm should be due to the hydrazinyl methyene carbon of the hydrazine carbothioamide. The signal at 55.7 ppm has a cross peak with the singlet at 3.89 ppm characteristic of methoxy protons and hence the signal at 55.7 ppm is assigned to the methoxy carbon. The HR–Mass spectrum (Fig. S5) of compound **6** shows $(M+H)^+$ peak at m/z = 460.1332 exact molecular mass of the compound **6** with its molecular formula ($C_{25}H_{21}N_3O_4S$) is 460.1064. All the other synthesized compounds (**7**–**10**) were characterized similarly and spectral data were given in the experimental sections.

3. Biological evaluation

3.1. Antimicrobial activity

The antibacterial properties of all the newly synthesized compounds (**6**–**10**) were assessed by disk diffusion method [30,31]. The minimum inhibitory concentration (MIC, μg/mL) were determined in comparison with *amikacin* and *amphotericin*. The *in vitro* antibacterial activities of the newly synthesized compounds (**6**–**10**) against *Escherichia coli* and *Pseudomonas aeruginosa* are listed in Table 1 for comparison the MIC of the standard amikacin and amphotericin are also given in (Table 1).

From Table 1 it is clear that all the compounds except **10** exhibit excellent activity compared to the standard *Amphotericin* but compound **10** shows almost similar activities to those of the standard compound. Especially compound **6** having the coumarinyl moiety was found to be more potent among all and

Table 1 Minimal inhibitory concentration (MIC, mg/mL) of hydrazinyl carbothioamide derivatives (**6**–**10**).

	Strains		MIC (μg/mL)				
	Amikacin	Amphotericin	6	7	8	9	10
S. typhi	25	-	1.56	1.56	12.5	25	50
S. aureus	50	-	3.12	6.25	6.25	12.5	50
E. coli	50	-	1.56	12.5	12.5	12.5	100
k.pneumoniae	50	-	3.12	3.12	12.5	25	50
Pseudomonas	25	-	1.56	1.56	3.12	12.5	100
A. flavus	-	50	1.56	3.12	6.25	6.25	50
C. albicans	-	25	1.56	6.25	6.25	25	50
A. fumigatus	-	25	3.12	3.12	6.25	6.25	50
A. niger	-	25	1.56	1.56	12.5	12.5	25

exhibits an enhanced activity than *amikacin* and *amphotericin*. Similarly among the two adamantyl substituted hydrazinyl carbothioamides (**7** and **8**), compound **7** shows very good activity than **8**. The decreased activity of compound **8** may be due to the presence of electron releasing methoxy substituent in the *para* position of the aryl ring.

Compound **10** having the trimethoxy phenyl ring not only shows similar activity but also demonstrated a poor activity against the germs *E. coli* and *P. aeruginosa* when compared to the standard. This also might be due to the presence of three electron releasing methoxy substituent in the phenyl ring attached to the N-1 of compound **10**.

Compounds **6**–**10** were screened for their antifungal activity against *Aspergillus flavus* and *Aspergillus niger*. Among the five compounds (E)-N-(2-(4-methoxyphenyl)-2-oxoethyl)-2-(1-(2-oxo-2H–benzo[g]chromen-3-yl) ethylidene) hydrazine carbothioamide) (**6**) shows excellent activity against the entire tested organism whereas **7** and **8** show only moderate activity. But **10** shows relatively poor activity against the entire tested microorganism. Keeping in view of the above excellent *in vitro* antimicrobial activities shown by the all the synthesized compounds (**6**–**10**), it is thought worthwhile to carry out docking studies to investigate their anticancer activity.

3.2. Molecular docking studies

Unique bioactivities of some compounds can be illuminated by interaction between the compounds and this target protein.

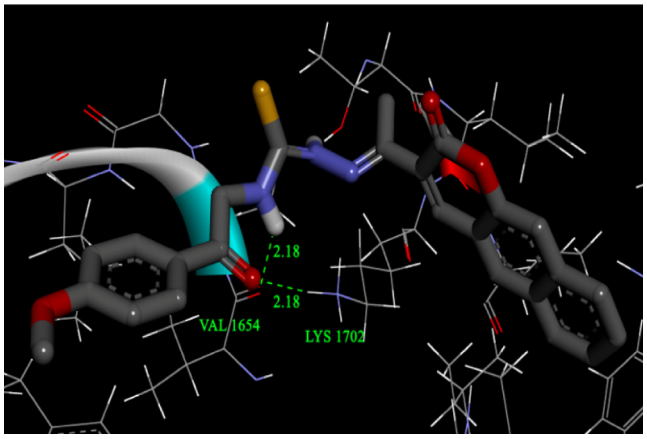


Figure 2 Docking of inhibiting ligand molecules **6**–**10**.

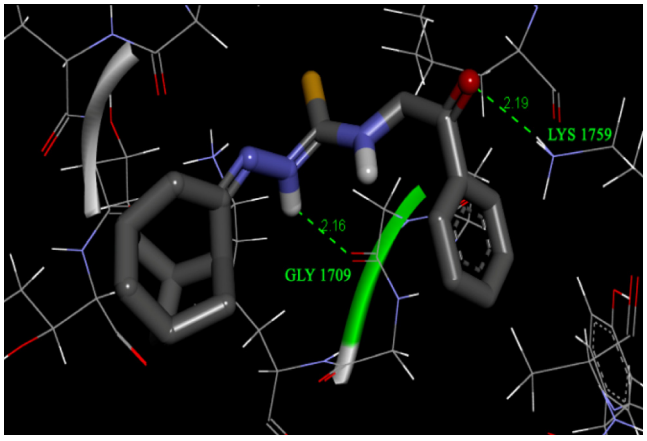


Figure 3 Docking of inhibiting ligand molecules **6**–**10**.

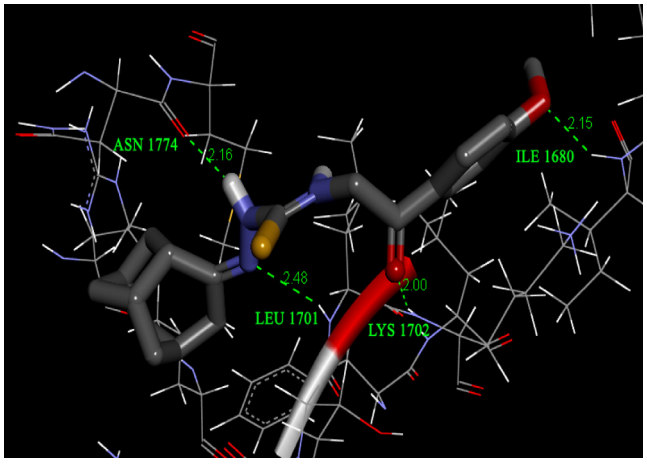


Figure 4 Docking of inhibiting ligand molecules **6**–**10**.

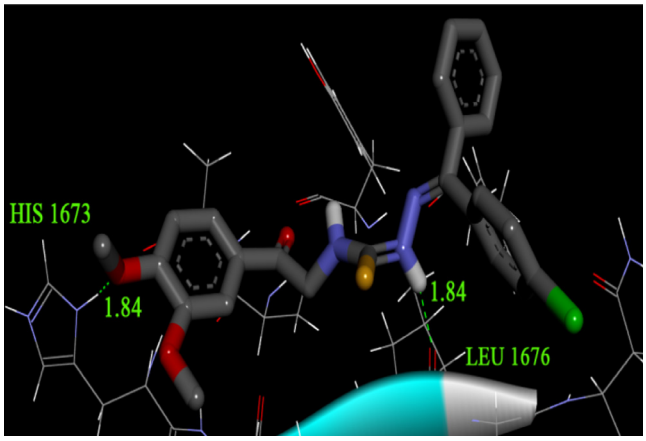


Figure 5 Docking of inhibiting ligand molecules **6**–**10**.

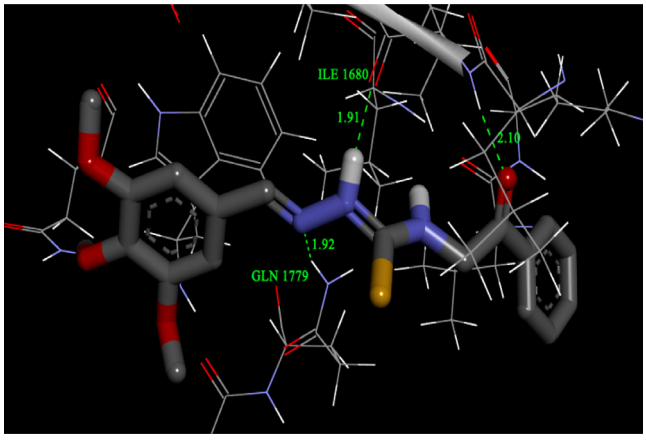


Figure 6 Docking of inhibiting ligand molecules **6**–**10**.

Table 2 Molecular docking binding energy of hydrazinyl carbothioamide derivatives (**6**–**10**).

Drug (ligand)	Breast cancer target protein (Receptor)	Binding energy [kcal/mol]
6	1JNX	−8.40
7		−7.99
8		−6.75
9		−5.79
10		−5.82
	Tamoxifen	

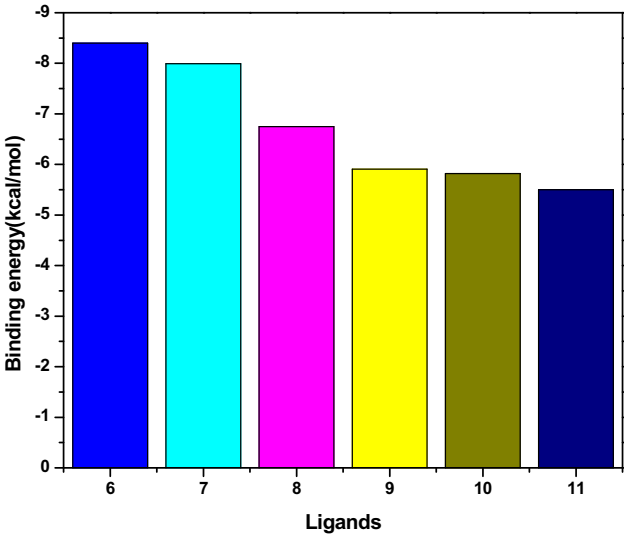


Figure 7 Histogram chart of ligands *vs* binding energy.

Molecular docking is an important strategy for arising potential drug-target interaction which plays a vital role in drug discovery.

Molecular docking studies are performed to find essential active site residues playing role in the activity or selectivity of the newly synthesized compounds against target protein (PDB: 1JNX). It also expresses the orientation of the inhibitors and interaction patterns, which suggest the role of various functional groups present.

All the five compounds were chosen for molecular docking studies using rigid docking method. Auto docking 4.2 [32] was used to determine the orientation of inhibitors bound in the active site of the breast cancer target protein (Receptor) 1JNX. Algorithm genetic method, implemented in the program AutoDock 4.2 was employed. The docking of inhibiting ligand molecules **6**–**10** with the protein 1JNX reveals that all the inhibitors are in bonding with more than one amino acid residues and thereby they occupy the active pockets of the protein 1JNX shown in Figs. 2–6. Theoretically all the five inhibitor molecules (**6**–**10**) exhibit very good binding energy as shown in Table 2.

The inhibitor **6** forms three hydrogen bonds with bond distances 2.179, 2.058 and 2.183 Å, inhibitor **7** and **10** also form three hydrogen bonds with the amino acid residues of protein 1JNX. But the ligands **8** and **9** found form only two hydrogen bonds with the amino acid LYS 1759.

From the histogram (Fig. 7) it is clear that all the inhibitors are found to possess maximum binding energy than the standard Tamoxifen (most popular breast cancer drug). All the newly synthesized compounds are found to be good inhibitors of the target protein 1JNX. In particular the activity gets increased even further when a coumarin moiety is introduced (ligand **6**).

Docking studies revealed that the inhibitors (**6**–**10**) are found to be interacting with active site of residues like LYS 1702: HZ2, ILE1680: HN, LYS1759:HZ3, GLN1779: HE21. The binding energy of the Tamoxifen (**11**) the most popular drug for breast cancer is also given in (Table 2). The Histogram chart of ligand *vs* binding energy is given in Fig. 7.

All the compounds studied were found to form three strong hydrogen bonds with the protein 1JNX except compound **8** which forms only two strong hydrogen bonds with the receptor (breast cancer cell target protein) moiety. Bonded residues, hydrogen bond, bond distance and bond energy are given in (Table 3 **S6**).

Among the five molecules investigated, *(E)-N-(2-(4-methoxyphenyl)-2-oxoethyl)-2-(1-(2-oxo-2H-benzo[g]chromen-3-yl)ethylidene)hydrazine carbothioamide* (**6**) with the best antibacterial activity shows a very good binding energy of −8.40 kcal/mol

Table 3 Bonded residues, hydrogen bond, bond distance and bond energy (**6**).

Compounds	Protein [PDB–ID]	Bonded residues	Hydrogen bond	Bond distance (Å)	Bond energy [kcal/mol]
6	1JNX	Protein:X:LYS1702:HZ2	3	2.179	−0.468
		Ligand:0:H		2.058	−2.075
		Ligand:0:H		2.183	−1.758
7		Protein:X:ILE1680:HN	3	2.154	−2.881
		Protein:X:LYS1702:HN		2.002	−5.558
		Ligand:0:H		2.158	−4.466
8		Protein: X: LYS1759: HZ3	2	2.188	−0.001
		Ligand:0:H		2.163	−0.427
9		Protein:X:HIS1673:HD1	2	1.839	−4.887
		Ligand:0:H		1.84	−1.561
10		Protein: ILE1680: NH	3	2.104	−5.250
		Protein: X: GLN1779:		1.922	−5.119
		HE21		1.912	−6.558
		Ligand:0:H			

and it may be considered as a good inhibitor of the protein 1JNX.

4. Conclusions

We have designed and synthesized new classes of hydrazinyl carbothioamide derivatives by introducing aryl ethanone into the N-4 atom of hydrazinyl carbothioamides and their biological activities are evaluated. Almost all the compounds displayed excellent antimicrobial activities. Molecular docking studies also revealed that all the compounds (**6–10**) possess maximum binding energy than the representative drug Tamoxifen and may all be considered as good inhibitors of 1JNX. One can conclude that these novel ligands are more efficient drugs for breast cancer than Tamoxifen.

5. Experimental section

5.1. Instruments

The IR spectrum was recorded in an AVATAR-330 FT-IR spectrophotometer and only noteworthy absorption levels (reciprocal centimeters) were listed. ^{1}H NMR spectra were recorded at 400 and 500 MHz on a Bruker AMX 400 and 500 MHz spectrophotometer using $CDCl_3$ as solvent and TMS as the internal standard. ^{13}C NMR spectra were recorded at 100 and 125 MHz on a Bruker AMX 400 and 500 MHz spectrophotometer using $CDCl_3$ as a solvent. HR-Mass (ESI) was carried out in a Bruker Maxis instrument in the School of Chemistry, University of Hyderabad. Elemental analyses (CHN) were recorded on a Thermo Finnigan Flash EA 1112 analyzer at the School of Chemistry, University of Hyderabad. Routine monitoring of the reactions was performed by TLC, using silica gel plates (Merck 60 F254) and compounds were visualized with a UV light at 254 nm.

5.2. General procedure for the synthesis of 3-acetyl-2H-benzo[g]chromen-2-one

A mixture of 2-hydroxy naphthaldehyde (**1**) and ethyl acetoacetate (**2**) was prepared. Piperidine was added dropwise to this mixture while stirring (Scheme 1). The reaction mixture was left overnight, resulting in the formation of a yellow colored solid. Purification by recrystallization (in EtOH) gave 3-acetyl-2H-benzo[g]chromen-2-one (**3**) (85%) as yellow crystal.

*5.2.1. 3-acetyl-2H-benzo[g]chromen-2-one (**3**)*

Yellow crystal; yield (85%); m. p 100–102 °C; mf $C_{15}H_{10}O_3$; ^{1}H NMR (400 MHz, $CDCl_3$) 2.78 (3H, CH_3), 7.42–7.45 (d, CH, J = 12.0 Hz), 7.58–7.62 (t, CH, J = 16.0 Hz), 7.71–7.75 (t, CH, J = 12.0 Hz), 7.89–7.91 (d, CH, J = 8.0 Hz), 8.06–8.09 (d, CH, J = 12.0 Hz), 8.30–8.33 (d, CH, J = 12.0 Hz), 9.25 (s, H); IR (KBr); 1643 (C═O), 1720 (C═O, coumarine ring), 3071 (C–H stretching).

5.3. Synthesis of hydrazine carbothioamide derivatives

Equimolar mixture of ketones (or) aldehyde, thiosemicarbazide (**5**) and substituted phenacyl bromide (**4**) are mixed and subjected to microwave irradiation for 50–180 s at a heating of 320 W Scheme 1. After the reaction has completed it is taken out, the solid product was recrystallized from ethanol to get pure compounds.

*5.3.1. (E)-N-(2-(4-methoxyphenyl)-2-oxoethyl)-2-(1-(2-oxo-2H-benzo[g]chromen-3-yl)ethylidene)hydrazine carbothioamide (**6**)*

Pale green solid; yield (78%); m. p 120–122 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 2.80 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 4.72 (s, 2H, CH_3) 6.98–7.00 (d, CH, 2H, J = 8.0 Hz), 7.48–7.50 (d, CH, J = 8.0 Hz), 7.54–7.55 (d, CH, J = 4.0 Hz), 7.62–7.65 (t, CH, J = 12.0 Hz), 8.37–8.39 (d, CH, J = 8.0 Hz), 8.11–8.13 (d, CH, J = 8.0 Hz), 7.91–7.96 (t, CH, 2H, J = 16.0 Hz), 7.72–7.79 (m, CH, 2H), 9.33 (s, coumarine CH); ^{13}C NMR (100 MHz, $CDCl_3$) 30.3, 42.9, 55.6, 112.8, 114.3, 116.5, 121.7, 122.5, 126.6, 126.9, 129.2, 129.8, 130.9, 136.3, 143.3, 156.2, 159.4, 164.0, 189.1, 195.6; IR (KBr). 1601 (C═N), 1656 (C═O), 1315 (C═S), 3429 (NH); HR-MS (ESI-MS) Exact M. W. 459.1253; found: 460.1332 $(M+H)^+$; CHN analysis: $C_{25}H_{21}N_3O_4S$. Anal. Calcd. (%) for: C, 65.34; H, 4.61; N, 9.14; found (%): C, 65.39; H, 4.56; N, 9.29.

*5.3.2. (Z)-2-((7aS)-hexahydro-1H-2,6-methanoinden-4(2H)-ylidene)-N-(2-oxo-2phenylethyl) hydrazine carbothioamide (**7**)*

Pale white solid; yield (71%); m. p 103–105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 0.84–0.89 (m, 3H, CH_2), 1.41–1.42 (d, CH, 2H, J = 4.0 Hz), 1.64 (s, CH) 1.74 (s, CH) 1.85 (s, CH), 1.92 (s, CH), 1.98–2.01 (t, CH, 2H, J = 12.0 Hz), 2.06–2.09 (d, 2H, J = 12.0 Hz), 2.54 (s, 2H), 7.22 (s), 7.43–7.46 (d, CH, 2H, J = 12.0 Hz), 8.06–8.08 (d, CH, 2H, J = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 27.4, 36.3, 39.2, 39.4, 43.0, 47.0, 112.2, 114.0, 114.3, 126.9, 130.9, 164.5, 189.2; IR (KBr). 1601 (C═N), 1656 (C═O), 1315 (C═S), 3429 (NH); LC-MS m/z: 341; CHN analysis: $C_{19}H_{23}N_3OS$. Anal.Calcd. (%) for: C, 66.83; H, 6.79; N, 12.31; found (%): C, 66.72; H, 6.76; N, 12.44.

*5.3.3. (Z)-2-((7aS)-hexahydro-1H-2,6-methanoinden-4(2H)-ylidene)-N-(2-(4-methoxyphenyl)-2-oxoethyl)hydrazinecarbothioamide (**8**)*

White solid; yield (69%); m. p 110–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 0.87–0.91 (m, 3H, CH_2), 1.44–1.47 (d, CH, 2H, J = 12.0 Hz), 1.66 (s, CH) 1.75 (s, CH) 1.86 (s, CH), 1.93 (s, CH), 1.97–2.03 (t, CH, 2H, J = 16.0 Hz), 2.07–2.11 (d, 2H, J = 16.0 Hz), 2.38 (s, 2H), 3.90 (s, 3H, OCH_3), 7.46–7.49 (d, CH, 2H, J = 12.0 Hz), 8.19–8.23 (d, CH, 2H, J = 16.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) 27.4, 36.3, 39.6, 39.7, 43.2, 47.4, 55.3, 112.1, 113.0, 114.1, 127.9, 132.5, 163.3, 188.0; IR (KBr); 1597 (C═N), 1621 (C═O), 1384 (C═S), 3421 and 3452 (NH); LC-MS. m/z = 371; CHN analysis: $C_{20}H_{25}N_3O_2S$. Anal. Calcd. (%) for: C, 64.66; H, 6.78; N, 11.31; found (%): C, 64.62; H, 6.75; N, 11.27.

*5.3.4. (E)-2-((4-chlorophenyl)(phenyl)methylene)-N-(2-(3,4-dimethoxyphenyl)-2-oxoethyl)hydrazinyl carbothioamide (**9**)*

Pale yellow solid; yield (73%); m. p 147–149 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 4.72 (s, 2H, CH_2), 3.94 (s, 6H, OCH_3), 6.81 (s, CH), 6.91–6.96 (m, 3H) 7.35 (s, CH, 2H), 7.48–7.54 (d, CH, 3H, J = 24.0 Hz), 7.59 (s, CH), 7.62–7.63 (d, CH, J = 4.0 Hz), 7.64–7.65 (d, CH, J = 4.0 Hz); ^{13}C NMR

(100 MHz, $CDCl_3$) 42.7, 56.1, 110.1, 110.3, 110.4, 110.5, 111.2, 123.4, 123.62, 123.6, 124.9, 125., 127.1, 128.3, 149.6, 149.8, 154.7, 155.4, 155.8, 186.0, 189.4; IR (KBr) 1585 (C═N), 1649 (C═O), 1399 (C═S), 3391 (NH); LC-MS. m/z = 468; CHN analysis: $C_{24}H_{22}ClN_3O_3S$. Anal. Calcd. (%) for: C, 61.60; H, 4.74; N, 8.98; found (%): C, 61.57; H, 4.77; N, 8.84.

*5.3.5. (E)-N-(2-oxo-2-phenylethyl)-2-(3, 4, 5-trimethoxybenzylidene)hydrazine carbothioamide (**10**)*

Green solid; yield (77%); m. p 160–162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) 3.89 and 3.97 (d, 9H, $(OCH_3)_3$), 4.75 (s, CH, CH_2), 6.98–7.00 (d, CH, 3H, J = 8.0 Hz), 7.17 (s, CH), 7.88–7.91 (d, 2H, J = 12.0 Hz), 7.30 (s, CH) 9.90 (s, N═CH); ^{13}C NMR (100 MHz, $CDCl_3$) 42.9, 55.6, 56.1, 61.0, 106.7, 112.1, 114.3, 126.9, 130.9, 153.6, 164.8, 189.1; IR (KBr). 1595 (C═N), 1665 (C═O), 1327 (C═S), 2975 and 2928 (NH); LC-MS m/z = 387; CHN analysis: $C_{19}H_{21}N_3O_4S$. Anal.Calcd. (%) for: C, 61.77; H, 5.18; N, 11.37 found (%): C, 61.84; H, 5.23; N, 11.26.

5.4. Biological activity

The *in vitro* activities of the compounds were tested in Sabouraud's dextrose broth (SDB) (Hi–media, Mumbai) for fungi and Sabouraud's dextrose agar (SDA) for bacteria by two fold serial dilution method. The test compounds were dissolved in dimethylsulfoxide (DMSO) to obtain 1 mg/mL stock solution. Seeded broth (broth containing microbial spores) was prepared in SDA from 24 objective old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1 °C while fungal spores of 1–7 days old were suspended in SDA. The colony forming units (cfu) of the seeded broth were determined by the plating technique and adjusted in the range of 10^3–10^7 cfu/mL. The final inoculum six was 10^7 cfu/mL for antifungal assay. Testing was performed at pH 8 of the solution of test compound was added to 1.8 ml of seeded broth to form the first dilution. One milliliter of this was diluted with a further 1 mL of the seeded broth to give the second dilution and so on till dilutions of desired volume were obtained. A set of assay tubes containing only incubated broth was kept as control and likewise solvent controls were also used simultaneously. The tubes were incubated in BOD incubators at 37 °C for bacteria and 28 °C for fungi. Amikacin and amphotericin were used as standards.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jscs.2014.10.002.

References

[1] S. Eckhardt, Curr. Med. Chem. Anti Canc. Agents 2 (2002) 419–439.
[2] G. Beck, H. Heitzer, U.S. 4, 748, 243 (1998); Bayer Akleingesellschaft.
[3] H.Ul. Osaka, N.H. Matubara, I.M. Kawabe. U.S. 5180833 (1993), Takela Chemical industries Ltd.
[4] K. Schule, F. Ritchur, R. Seishiet, R. Krause, M. Mahlstadt, J. Prakt, Chemie 332 (1980) 629–637.
[5] R. Murugan, E.F.V. Scriven. WO 9845179/1998, Reilly Industries Inc.; Chem. Abstr. 129 (1998) 3026633v. Downloaded By: [INFLIBNET India Order] At: 15:08 11 October 2009 Possible Antibacterial and Antifungal Agents 1389.
[6] A. Jackson, G. Heys, J.I. Grayson, R. Calrke, U.S. 5, 705, 652/1998, Fine Organics Ltd.
[7] R.M. Leanna, H.E. Morton, WO 9616050/1996, Abott Laboratories, USA, Chem. Abstr., 125 (1996) 114603d.
[8] H. Tripathy, D.G. Pradhan, Agricult. Biol. Chem. 37 (1973) 1375–1383.
[9] J.L. Bayer, J.P. Denonte, G. Mourioux, EP 508901 (1992).
[10] M.Q. Zhang, A. Haemers, D. VadenBerghe, S.R. Pattyn, W. Bolaert, J. Hetero-cycl. Chem. 28 (1991) 673–674.
[11] Y. Katsura, S. Nishino, M. Ohno, K. Sakane, Y. Matsumoto, C. Morinaga, H. Ishikawa, H. Takasugi, J. Med. Chem. 42 (1999) 292–2926.
[12] X.Y. Yu, J.M. Hill, G. Yu, W. Wang, A.F. Kluge, P. Wendler, P. Gallant, Bioorg. Med. Chem. Lett. 9 (1999) 375–380.
[13] B.S. Holla, K.V. Malini, B.S. Rao, B.K. Sarojini, N.S. Kumari, Eur. J. Med. Chem. 38 (2003) 313–318.
[14] N. Adibpour, A. Khalaj, S. Rajabalian, Eur. J. Med. Chem. 45 (2010) 19–24.
[15] B. Narayana, K.K. Vijayaraj, B.V. Ashalatha, N. Suchethakumari, B.K. Sarojini, Eur. J. Med. Chem. 39 (2004) 867–872.
[16] B. Narayana, B.V. Ashalatha, K.K. VijayaRaj, N. SuchethaKumari, Phosphorous Sulfur Silicon 181 (2006) 1381–1389.
[17] B. Narayana, K.K. Vijayaraj, B.V. Ashalatha, N. Suchethakumari, Phosphorus Sulfur Silicon 182 (2007) 7–14.
[18] M.P. LaMontagne, A.M.S. Markovac, M. Sami Khan, J. Med. Chem. 25 (1982) 964–968.
[19] M.P. LaMontagne, P. Blumbergs, R.E. Strube, J. Med. Chem. 25 (1982) 1094–1097.
[20] P. Nasveld, S. Kitchener, Trans. R. Soc. Trop. Med. Hyg. 99 (2005) 2–5.
[21] A. Mahamoud, J. Chevalier, A. Davin-Regli, J. Barbe, Jean-Marie Pages, Curr. Drug Targets 7 (2006) 843–847.
[22] W.A. Denny, W.R. Wilson, D.C. Ware, G.J. Atwell, J.B. Milbank, R.J. Stevenson, U.S. Patent 7064 117, 2006.
[23] P.A. Leatham, H.A. Bird, V. Wright, D. Seymour, A. Gordon, Eur. J. Rheumatol. Inflamm. 6 (1983) 209–211.
[24] N. Muruganantham, R. Sivakumar, N. Anbalagan, V. Gunasekaran, J.T. Leonard, Biol. Pharm. Bull. 27 (2004) 1683–1687.
[25] M.P. Maguire, K.R. Sheets, K. McVety, A.P. Spada, A. Zilberstein, J. Med. Chem. 37 (1994) 2129–2137.
[26] W.D. Wilson, M. Zhao, S.E. Patterson, R.L. Wydra, L. Janda, L. Strekowski, Med. Chem. Res. 2 (1992) 102–110.
[27] L. Strekowski, J.L. Mokrosz, V.A. Honkan, A. Czarny, M.T. Cegla, S.E. Patterson, R.L. Wydra, R.F. Schinazi, J. Med. Chem. 34 (1991) 1739–1746.
[28] B.K. Sarojini, B.G. Krishna, C.G. Darshanraj, B.R. Bharath, H. Manjunatha, Eur. J. Med. Chem. 45 (2010) 3490–3496.
[29] X. Wang, Y. Ling, H. Wang, J. Yu, J. Tang, H. Zheng, X. Zhao, D. Wang, G. Chen, W. Qiu, J. Tao, Bioorg. Med. Chem. Lett. 22 (2012) 6166–6172.
[30] A.H. Collins (Ed.), Microbiological Methods, second ed., Butterworth, London, 1976.
[31] B.A. Arthington, M. Motley, D.W. Warnock, C.J. Morrison, J. Clin. Microbiol. 38 (2000) 2254–2260.
[32] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, J. Comp. Chem. 16 (2009) 2785–2791.