Med Chem Res (2014) 23:3065–3081
DOI 10.1007/s00044-013-0892-x

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antimicrobial, and antioxidant activity of benzofuran barbitone and benzofuran thiobarbitone derivatives

R. Kenchappa · Yadav D. Bodke · B. Asha · Sandeep Telkar · M. Aruna Sindhe

Received: 5 October 2013 / Accepted: 12 December 2013 / Published online: 27 December 2013

Abstract The synthesis of novel series of benzofuran derivatives, containing barbitone moiety, 5-[(2/4-substitutedphenyl)(5-substituted-1-benzofuran-2-yl) methylidene] pyrimidin-2,4,6(1*H*,3*H*,5*H*)-trione (**4a–i**) and thiobarbitone moiety, 5-[(2/4-substitutedphenyl)(5-substituted-1-benzofuran-2-yl)methylidene]-2-thioxodihydropyrimidin-4,6(1*H*,5*H*)-dione (**5a–i**) have been reported. The target compounds (**4a–i**) and (**5a–i**) were synthesized by the Knoevenagel condensation of (5-substituted-1-benzofuran-2-yl)(2/4-substitutedphenyl) methanone (**3a–i**) with barbituric acid and thiobarbituric acid, respectively, in acid medium. These compounds were screened for the antimicrobial and antioxidant activities. From antimicrobial activity results it was found that compounds **4a**, **5a**, **4c**, and **5c** displayed good antibacterial and antifungal activity against all tested strains. Further, the synthesized compounds were studied for docking on the enzyme, Glucosamine-6-phosphate synthase and the compounds **4c** and **5c** have emerged has an active antimicrobial agent with least binding energy (-5.27 and -4.85 kJ mol^{-1}). Compounds **4e**, **4f**, **5e**, and **5f** showed promising free radical scavenging activity and compounds **5a** and **5b** showed good chelating ability with Fe^{2+} ions.

Keywords Benzofuran · Barbiturates · Knoevenagel condensation · Antimicrobial · Antioxidant · Docking

R. Kenchappa · Y. D. Bodke (✉) · M. Aruna Sindhe
Department of P.G. Studies and Research in Industrial Chemistry, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shivamogga 577451, Karnataka, India
e-mail: yd_bodke@yahoo.co.in; ydbodke@gmail.in

B. Asha
Department of P.G. Studies and Research in Applied Botany, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shivamogga 577451, Karnataka, India

S. Telkar
Department of P.G. Studies and Research in Biotechnology, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shivamogga 577451, Karnataka, India

Introduction

The resistance of pathogenic bacteria toward available antibiotics is rapidly becoming a major worldwide problem. On the other hand, primary and opportunistic fungal infections continue to grow rapidly because of the increased number of immune compromised patients (Grange and Zumla, 2002; Guzeldemirci and Kucukbasmaci, 2010). In order to combat this new problem, novel, structurally diverse antibiotic compounds are required (Francesca, 2011).

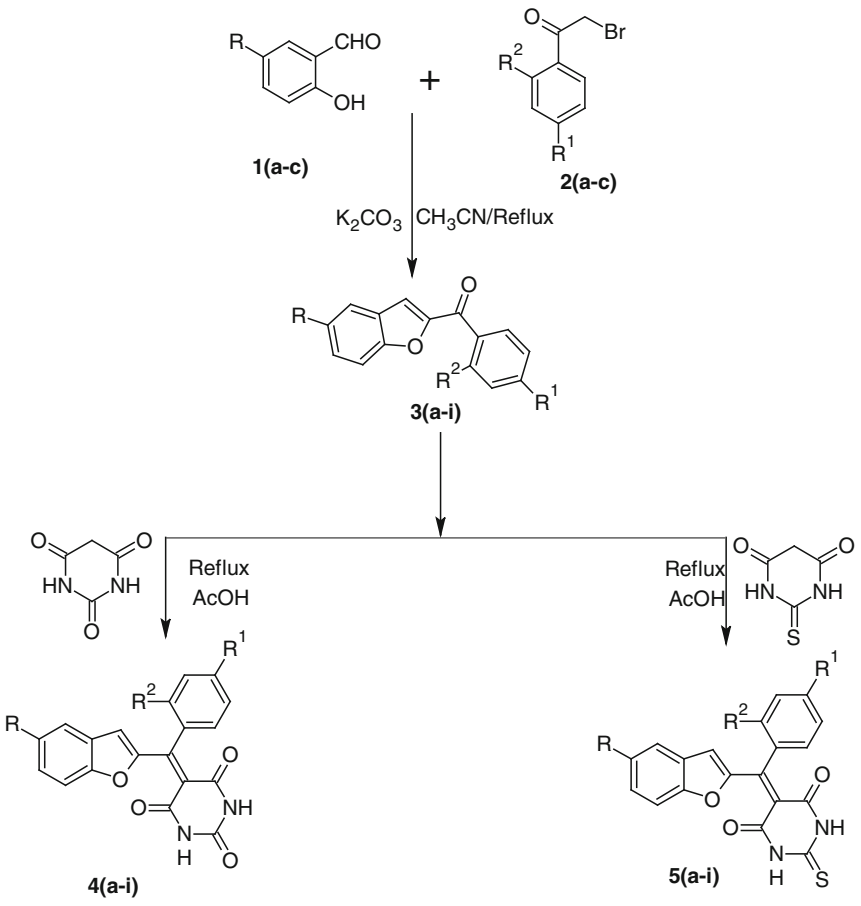
Benzofuran heterocycles exhibit a broad range of biological activities. Recently, compounds containing methanone linkage between the benzofuran at C-2 position and aromatic rings have been investigated and reported as antimicrobials (Koca *et al.*, 2005; Kirilmis *et al.*, 2008; Bondock *et al.*, 2011; Jiang *et al.*, 2011; Liu *et al.*, 2012; Rajanarendar *et al.*, 2013), novel SIRT1 inhibitors (Wu *et al.*, 2013), anticancer (Parekh *et al.*, 2011), and antioxidant agents (Karatas *et al.*, 2006). Also, benzofuran-2-yl(phenyl)methanone analogs displayed anti-tumor (Agatsuma *et al.*, 2004), antitubercular (Manna and Agrawal, 2010, 2011), anti-inflammatory, analgesic (Rajanarendar *et al.*, 2013), CYP19 inhibitory activity, molecular docking (Saberi *et al.*, 2006), and cytotoxicity properties (Bigler *et al.*, 2007; Saberi *et al.*, 2006).Cloridarol, a 2-substituted benzofuran is used for treatment of lipidemia and has

anticoagulant, antifungal and aromatase inhibiting activities (Ghelardoni *et al.*, 1981). Amiodarone, a benzofuran derivative was shown to have antiarrhythmic property (Singh and Vaughan, 1970).

Barbituric acid (BA) derivatives are versatile reagents, capable of condensing with a wide range of carbonyl compounds (Vijaya Laxmi *et al.*, 2012; Mathew *et al.*, 2012; Moskvin *et al.*, 2002) and the reaction of BA with carbonyl compounds was studied nearly 40 years ago (Rezende *et al.*, 2001; Reddy and Nagaraj, 2007). The condensed barbiturates and thiobarbiturates possesses different pharmacological profile such as antimicrobial, selective cell adhesion inhibitors, antioxidant, and DNA cleavage activities (Sangani *et al.*, 2006; Harriman *et al.*, 2008; Biradar *et al.*, 2010; Mathew *et al.*, 2012). They also possess chemical and pharmacological importance due to their anti-neoplastic, anticancer, antitumor, antitubercular (Basavaraja *et al.*, 2010; Vijaya Laxmi *et al.*, 2011), anti-inflammatory (Holy *et al.*, 2002), antiviral activity(Andres and Marhold, 1996) as well as in agrochemical and veterinary products (El-Gaby *et al.*, 2006; Aly and Kamal, 2012; Dhorajiya *et al.*, 2012).

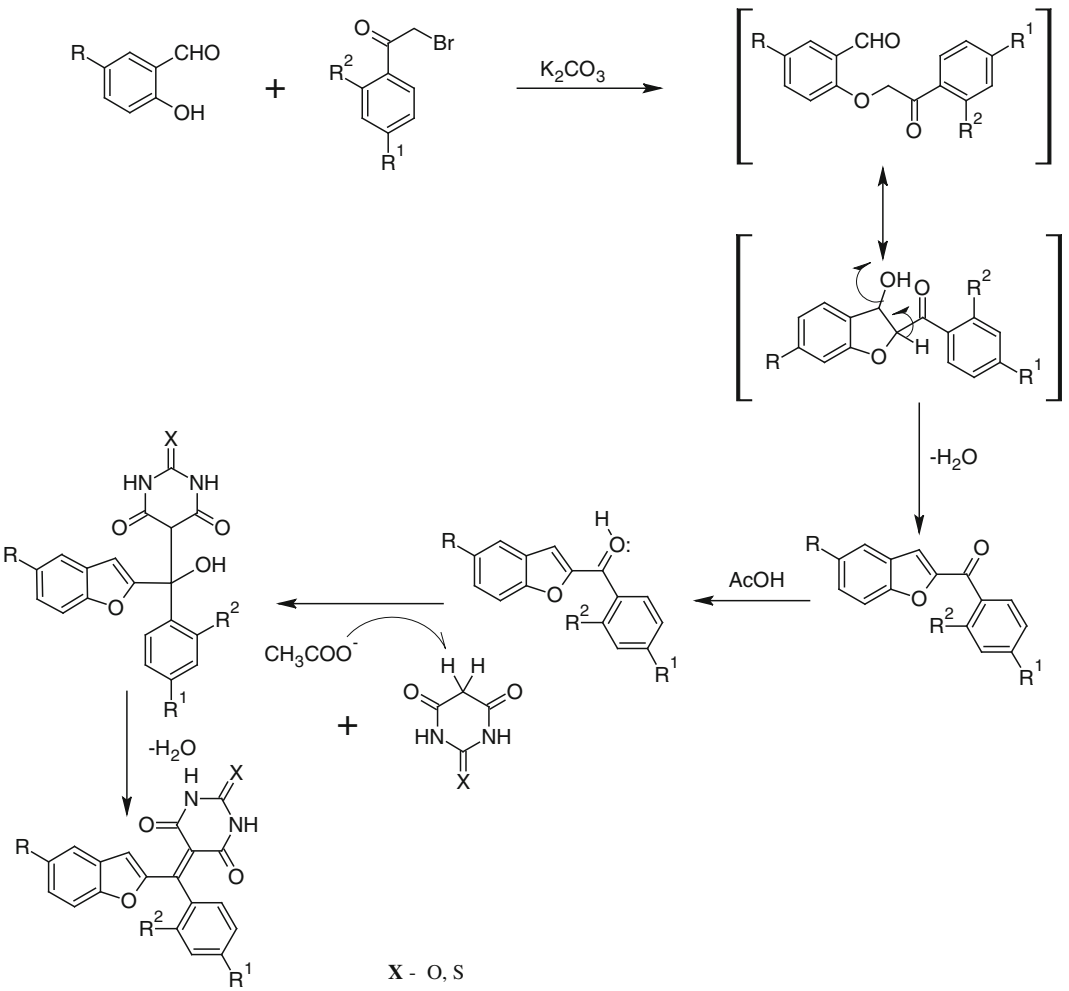
Therefore, it is envisaged that chemical entities with benzofuran and barbituric/thiobarbituric moieties would result in compounds of interesting biological activities. In view of these findings, we have attempted to incorporate these two biologically active components together and to investigate a possible additive effect of these rings on biological activity.

Earlier, our research group has synthesized different derivatives of benzofuran bearing aryl substituents at the C-2 position through a methanone linkage (Kenchappa *et al.*, 2013a, b; Sheelavanth *et al.*, 2013; Chandrashekar *et al.*, 2013; Venkatesh *et al.*, 2010a, b). These results



Scheme 1 Synthesis of benzofuran barbitone (**4a–i**) and benzofuran thiobarbitone (**5a–i**) derivatives

Comp	R	R^1	R^2	Comp	R	R^1	R^2
4a/5a	H	Br	H	**4f/5f**	OH	OCH_3	H
4b/5b	H	OCH_3	H	**4g/5g**	H	H	Br
4c/5c	Br	Br	H	**4h/5h**	Br	H	Br
4d/5d	Br	OCH_3	H	**4i/5i**	OH	H	Br
4e/5e	OH	Br	H				



Comp	R	R^1	R^2	Comp	R	R^1	R^2
4a/5a	H	Br	H	**4f/5f**	OH	OCH_3	H
4b/5b	H	OCH_3	H	**4g/5g**	H	H	Br
4c/5c	Br	Br	H	**4h/5h**	Br	H	Br
4d/5d	Br	OCH_3	H	**4i/5i**	OH	H	Br
4e/5e	OH	Br	H				

Fig. 1 Proposed reaction mechanism of synthesized compounds (**4a–i**) and (**5a–i**)

encouraged us to extend the scope of this methodology to build new systems for improving the spectrum of activity of these scaffolds.

Result and discussion

Chemistry

The reaction pathway used for the synthesis of title compounds (**4a–i**) and (**5a–i**) have been shown in Scheme 1. The key intermediates, 5-substituted-1-benzofuran-2-yl) (2/4-substitutedphenyl) methanone (**3a–i**) were synthesized by a known method (Stille *et al.*, 1996; Mahboobi *et al.*, 2007).

The present study described the Knoevenagel condensation of (5-substituted-1-benzofuran-2-yl) (2/4-substitutedphenyl) methanone (**3a–i**) with barbituric/thiobarbituric acid. Initially, (5-substituted-1-benzofuran-2-yl) (2/4-substitutedphenyl) methanone undergoes protonation by acetic acid. The protonated form of the methanone then facilitates the addition reaction toward a nucleophile. The acetate ion which was formed in the former step can accept a proton from the methylene unit of barbituric/thiobarbituric acid and generate a carbanion. The electron-rich carbanion attacks on the electron deficient carbonyl carbon of (5-substituted-1-benzofuran-2-yl) (2/4-substitutedphenyl) methanone to form an adduct, which upon dehydration furnished the target compounds. The proposed mechanism has been described in Fig. 1.

Table 1 Characterization data of synthesized compounds **4a–i** and **5a–i**

Compd.	R	R^1	R^2	Yield (%)	Mol. wt.
4a	H	Br	H	79	410
4b	H	OCH_3	H	90	362
4c	Br	Br	H	86	488
4d	Br	OCH_3	H	83	440
4e	OH	Br	H	85	426
4f	OH	OCH_3	H	76	378
4g	H	H	Br	91	410
4h	Br	H	Br	75	488
4i	OH	H	Br	83	426
5a	H	Br	H	79	426
5b	H	OCH_3	H	89	378
5c	Br	Br	H	77	504
5d	Br	OCH_3	H	84	456
5e	OH	Br	H	76	442
5f	OH	OCH_3	H	81	394
5g	H	H	Br	75	426
5h	Br	H	Br	87	504
5i	OH	H	Br	85	442

The structures of synthesized compounds were established on the basis of spectral data. In IR spectra of compounds, the characteristic absorption bands between 1670 and 1710 cm^{-1} confirmed the presence of C=O group of BA. In thiobarbiturates (TBA), C=S stretching frequency was observed between 1395 and 1452 cm^{-1}. Bands in the region 3230–3345 cm^{-1} attributed to NH group of the BA and TBA. The broad absorption band observed at 3425–3465 cm^{-1} corresponds to OH group. ^1H NMR spectrum of compound **5d** showed two singlets at δ 10.03 and 10.61 ppm due to two NH protons of TBA, the multiplet between δ 7.13 and 8.07 ppm were assigned to aromatic protons. The singlet observed at δ 3.88 ppm corresponds to OCH_3 protons. In compound **5f**, the –OH proton on the benzofuran ring exhibited as broad singlet at δ 5.8 ppm and a singlet at δ 3.72 ppm corresponding to OCH_3 protons. Further, ^{13}C NMR spectrum of compound **5d** confirmed the proposed structure by appearance of signal at δ 178.2 ppm due to the C=S carbon and another signal at δ 167.1 ppm correspond to C=O carbon of TBA ring. Another signal at δ 55.9 ppm attributed to OCH_3 carbon. In compound **4d**, the signal at δ 165.0 ppm was assignable to C=O carbon and the other signals are in well agreement with the suggested structures. Mass spectrum of compound **5d** displayed a molecular ion peak at *m/z* 457 $[M^+]$ corresponding to the molecular mass of the compound along with an isotopic peak at *m/z* 459 [M+2]. The physical and analytical data of synthesized compounds (**4a–i**) and (**5a–i**) have been given in Table 1.

Pharmacology

In vitro antibacterial and antifungal activity

Though, we have many synthetic drugs in the market, the bacterial mutations are making them resistance. To overcome this problem, there is an urgent need to discover the new and specific enzyme targeting novel synthetic molecules. In view of this, the compounds synthesized in the present investigation (**4a–i**) and (**5a–i**) were evaluated for their antimicrobial activity as primary screening at three different concentrations and the results have been displayed in Tables 2 and 3. The antimicrobial activity was carried out against five bacterial strains *Pseudomonas syringae*, *Salmonella tyhphi*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Escherichia coli*, and four fungal strains *Asperigillus flavous*, *Candida albicans*, *Microspora griseus*, and *Asperigillus terus*.

The investigation of antimicrobial screening revealed that, test compounds showed varying degree of activity against all the tested micro-organisms. The zone of inhibition shown by the test compounds against bacterial and fungal strains has shown in Fig. 2a, b.

Further, the compounds which showed good activity in primary screening were assessed by minimum inhibitory concentration (MIC) at different concentrations to quantify the antimicrobial potency of the compounds. The results of MIC values of antimicrobial activity are given in Table 4.

From literature survey it is clear that, the structural and electronic parameters may have greater impact in varying the efficacy of antimicrobial activity (Tomasz *et al.*, 2011; Stefania *et al.*, 2012; Stefania *et al.*, 2012; Lopez *et al.*, 2001; Sharma *et al.*, 2004; Dhorajiya *et al.*, 2012).

A close investigation of the MIC values indicates that all the compounds exhibited a varied degree of MIC (11.38–199.10 μmol/L) of antibacterial activity against all the tested bacterial strains. The compounds **4c**, **5c** having two bromo substituents on C-5 of benzofuran and C-4 of phenyl ring, respectively were found to exhibit excellent antibacterial activity against all the tested bacterial strains with MIC value 29.76–31.96 μmol/L. Compounds **4a**, **5a** having –Br substituent on C-4 position of the aryl ring showed very good ability to inhibit *S. tyhphi* at MIC 36.61–37.92 μmol/L; the same compounds showed good activity against *P. syringae* with MIC 37.20–38.50 μmol/L. In case of compounds **4e** and **5e**, which are having hydroxyl and bromo-substituent, exhibited moderate to good activity against *S. tyhphi* with MIC value 36.08–36.73 μmol/L. Compounds **4d** and **5d** were inactive against *K. pneumoniae*, *P. syringae*, and *E. coli*.

The MIC of antifungal activity of title compounds indicated that, the compounds **4c** and **5c** exhibited equipotent activity against all the tested fungal strains with MIC value

Table 2 Antibacterial activity data of synthesized compounds **4a–i** and **5a–i**

Comp. no.	Conc. M (Molar)	Zone of inhibition in mm (mean ± SD) $n = 3$				
		P.s ± SD	*S.t* ± SD	*B.s* ± SD	*K.p* ± SD	*E.c* ± SD
4a	3.9	14 ± 0.2	13 ± 0.1	16 ± 0.2	16 ± 0.3	13 ± 0.2
	1.5	11 ± 0.3	12 ± 0.2	14 ± 0.1	14 ± 0.1	12 ± 0.3
	0.6	09 ± 0.1	10 ± 0.3	11 ± 0.3	13 ± 0.1	10 ± 0.1
4b	4.4	09 ± 0.2	10 ± 0.1	10 ± 0.2	11 ± 0.3	–
	1.7	08 ± 0.3	08 ± 0.2	06 ± 0.2	05 ± 0.1	–
	0.7	–	–	–	02 ± 0.1	–
4c	3.2	14 ± 0.2	14 ± 0.1	16 ± 0.2	17 ± 0.3	14 ± 0.2
	1.3	11 ± 0.3	13 ± 0.2	14 ± 0.1	15 ± 0.1	13 ± 0.3
	0.5	10 ± 0.1	11 ± 0.3	12 ± 0.3	14 ± 0.1	10 ± 0.1
4d	3.6	11 ± 0.2	13 ± 0.1	12 ± 0.2	13 ± 0.3	12 ± 0.2
	1.4	10 ± 0.3	11 ± 0.2	10 ± 0.1	12 ± 0.1	10 ± 0.3
	0.5	08 ± 0.1	06 ± 0.3	09 ± 0.3	10 ± 0.1	08 ± 0.1
4e	3.7	13 ± 0.2	13 ± 0.1	15 ± 0.2	15 ± 0.3	13 ± 0.2
	1.5	10 ± 0.3	11 ± 0.2	13 ± 0.1	14 ± 0.1	11 ± 0.3
	0.5	09 ± 0.1	10 ± 0.3	10 ± 0.3	12 ± 0.1	10 ± 0.1
4f	4.2	09 ± 0.2	–	09 ± 0.2	11 ± 0.3	–
	1.7	06 ± 0.3	–	06 ± 0.2	05 ± 0.1	–
	0.6	–	–	–	02 ± 0.1	–
4g	3.9	11 ± 0.2	11 ± 0.1	11 ± 0.2	09 ± 0.3	08 ± 0.2
	1.5	08 ± 0.3	08 ± 0.2	07 ± 0.1	05 ± 0.1	07 ± 0.3
	0.6	05 ± 0.1	06 ± 0.3	03 ± 0.3	02 ± 0.1	02 ± 0.1
4h	3.2	11 ± 0.2	12 ± 0.1	11 ± 0.2	12 ± 0.3	10 ± 0.2
	1.3	09 ± 0.3	09 ± 0.2	10 ± 0.1	08 ± 0.1	10 ± 0.3
	0.5	06 ± 0.1	06 ± 0.3	03 ± 0.3	05 ± 0.1	02 ± 0.1
4i	3.7	10 ± 0.2	09 ± 0.1	10 ± 0.2	08 ± 0.3	07 ± 0.2
	1.5	08 ± 0.3	08 ± 0.2	–	05 ± 0.1	07 ± 0.3
	0.5	–	06 ± 0.3	–	02 ± 0.1	−1
5a	3.7	13 ± 0.2	14 ± 0.1	17 ± 0.2	17 ± 0.3	14 ± 0.2
	1.5	11 ± 0.3	13 ± 0.2	14 ± 0.1	15 ± 0.1	12 ± 0.3
	0.5	09 ± 0.1	11 ± 0.3	12 ± 0.3	13 ± 0.1	09 ± 0.1
5b	4.2	09 ± 0.2	10 ± 0.1	09 ± 0.2	11 ± 0.3	10 ± 0.2
	1.7	07 ± 0.3	04 ± 0.2	08 ± 0.1	09 ± 0.1	04 ± 0.3
	0.6	04 ± 0.3	–	04 ± 0.1	05 ± 0.1	03 ± 0.1
5c	3.1	13 ± 0.2	15 ± 0.1	17 ± 0.2	17 ± 0.3	15 ± 0.2
	1.2	12 ± 0.3	13 ± 0.2	14 ± 0.1	16 ± 0.1	12 ± 0.3
	0.5	10 ± 0.1	12 ± 0.3	11 ± 0.3	13 ± 0.1	11 ± 0.1
5d	3.5	12 ± 0.2	13 ± 0.1	16 ± 0.2	15 ± 0.3	13 ± 0.2
	1.4	11 ± 0.3	12 ± 0.2	13 ± 0.1	13 ± 0.1	11 ± 0.3
	0.5	09 ± 0.1	11 ± 0.3	10 ± 0.3	12 ± 0.1	09 ± 0.1
5e	3.6	13 ± 0.2	14 ± 0.1	16 ± 0.2	16 ± 0.3	14 ± 0.2
	1.4	10 ± 0.3	12 ± 0.2	14 ± 0.1	14 ± 0.1	13 ± 0.3
	0.5	09 ± 0.1	10 ± 0.3	11 ± 0.3	13 ± 0.1	10 ± 0.1
5f	4.0	08 ± 0.2	10 ± 0.1	09 ± 0.2	–	09 ± 0.2
	1.6	07 ± 0.3	–	07 ± 0.1	–	04 ± 0.3
	0.6	05 ± 0.3	–	03 ± 0.1	–	01 ± 0.1

Table 2 continued

Comp. no.	Conc. M (Molar)	Zone of inhibition in mm (mean ± SD) $n = 3$				
		P.s ± SD	*S.t* ± SD	*B.s* ± SD	*K.p* ± SD	*E.c* ± SD
5g	3.7	10 ± 0.2	12 ± 0.1	13 ± 0.2	13 ± 0.3	12 ± 0.2
	1.5	09 ± 0.3	09 ± 0.2	10 ± 0.1	09 ± 0.1	09 ± 0.3
	0.5	–	06 ± 0.3	05 ± 0.3	–	06 ± 0.1
5h	3.1	12 ± 0.2	12 ± 0.1	15 ± 0.2	14 ± 0.3	12 ± 0.2
	1.2	10 ± 0.3	11 ± 0.2	11 ± 0.1	10 ± 0.1	10 ± 0.3
	0.5	–	09 ± 0.3	–	09 ± 0.1	08 ± 0.1
5i	3.6	10 ± 0.2	10 ± 0.1	11 ± 0.2	12 ± 0.3	10 ± 0.2
	1.4	06 ± 0.3	08 ± 0.2	09 ± 0.1	08 ± 0.1	05 ± 0.3
	0.5	03 ± 0.3	07 ± 0.3	–	04 ± 0.1	03 ± 0.1
Streptomycin	2.7	15 ± 0.2	16 ± 0.1	18 ± 0.2	19 ± 0.3	16 ± 0.2
	1.1	13 ± 0.3	15 ± 0.2	16 ± 0.1	17 ± 0.1	14 ± 0.3
	0.4	11 ± 0.1	13 ± 0.3	13 ± 0.3	16 ± 0.1	12 ± 0.1
Control (DMF)	–	00	00	00	00	00

Each value is expressed as mean ± SD of three replicates for zone of inhibition

P.s Psedomonas syringae, *S.t Salmonella typhi*, *B.s Bacillus subtilis*, *K.p Klebsiella pneumoniae*, *E.c Escherichia coli*, *SD* standard deviation

12.50–66.49 μmol/L. Compounds **4a** and **5a** showed excellent activity against the tested organisms with MIC value 14.90–29.92 μmol/L. In case of compounds **4e** and **5e** which are having hydroxyl and bromo substituent exhibited moderate activity against the tested fungal strains with MIC value 15.37–31.22 μmol/L.

From the structure–antimicrobial activity relationship of the synthesized compounds, it revealed that, to evaluate the SAR studies, the effect of structural changes in the target compounds (**4a–i**) and (**5a–i**) play an important role. Regarding the impact of substituents on the benzofuran ring and substituents on *ortho* and *para* position of aryl ring, it was observed that electron withdrawing groups in the *ortho* position of benzofuran ring and in the *para* position of aryl ring have a tendency to increase the potency. Nevertheless, when the –Br group is in *o*-position of aryl ring (**4g**, **4i**, **5g**, and **5i**), we found decrease in activity, suggesting that the presence of a group in the *o*-position of the aryl ring could introduce important steric effects. Interestingly, compounds **4h** and **5h** exhibited moderate activity even though the substituent present on the *o*-position, this could be due to the influence of electron withdrawing group present on the C-5 of benzofuran ring. Compounds containing electron donating groups (**4f**, **4i**, **5f** and **5i**) found to weaken the antimicrobial activity. However, when –Br group is introduced to *o*-position of aryl ring (**4i** and **5i**), the activity did not change significantly.

In correlation to in vitro antimicrobial activity, it thought worthwhile to carryout in silico studies to predict the binding affinity and orientation at the active site of the receptor. Glucosamine-6-phosphate synthase (L-glutamine: D-fructose-6-phosphate amino transferase) catalyze the first step in hexamine biosynthesis, converting D-fructose-6-phosphate (Fru-6-P) into D-glucosamine 6-phosphate (GlcN-6-P) using glutamine as the ammonia source.

Automated docking was used to assess the orientation of inhibitors bound in the active pockets of GlcN-6-P synthase. The molecular docking of ligand molecules **4a**, **4c**, **4e**, **5a**, **5c**, and **5e** with GlcN-6-P synthase revealed that all the tested ligand molecules showed encouraging binding energy and the compounds have exhibited the bonding with one or the other amino acids in the active pockets as shown in Fig. 3.

Among the six molecules, **4a**, **4c**, **4e**, **5a**, **5c**, and **5e**, the docking of GlcN-6-P synthase with compounds **4c** and **5c** was found with least binding energy (−5.27 and −4.85 kJ mol^{-1}). Compound **4c** establishes three hydrogen bonds between barbitone oxygen and with gln 348, ser 349 and thr 352 amino acids in the active site of the target protein with minimum bond length (2.118, 2.207, and 2.195 Å). Compound **5c** establishes two hydrogen bonds between thiobarbitone oxygen with gln 348 and thr 352 amino acids with bond length (2.149, 2.105, and 2.195 Å) with the highest affinity and hence is the best dock conformation. In vitro studies too, compound **4c** and **5c** have emerged has an active antimicrobial agent against all tested organisms. Molecular docking results of synthesized compounds are given in Table 5.

Antioxidant activity

Free radical scavenging activity by DPPH method

All the synthesized compounds were screened for their radical scavenging activity by DPPH method. The freshly

Table 3 Antifungal activity data of synthesized compounds **4a–i** and **5a–i**

Comp. no.	Conc. (mg/mL)	Zone of inhibition in mm (mean ± SD) $n = 3$			
		A.f ± SD	*C.a* ± SD	*M.g* ± SD	*A.t* ± SD
4a	3.9	12 ± 0.2	17 ± 0.1	16 ± 0.2	18 ± 0.3
	1.5	10 ± 0.3	16 ± 0.2	15 ± 0.1	16 ± 0.1
	0.6	09 ± 0.1	14 ± 0.3	14 ± 0.3	15 ± 0.1
4b	4.4	07 ± 0.2	09 ± 0.1	09 ± 0.2	10 ± 0.3
	1.7	06 ± 0.3	06 ± 0.2	06 ± 0.1	–
	0.7	–	02 ± 0.2	–	–
4c	3.2	12 ± 0.2	18 ± 0.1	17 ± 0.2	19 ± 0.3
	1.3	11 ± 0.3	16 ± 0.2	16 ± 0.1	17 ± 0.1
	0.5	08 ± 0.1	15 ± 0.3	14 ± 0.3	16 ± 0.1
4d	3.6	12 ± 0.2	17 ± 0.1	16 ± 0.2	17 ± 0.3
	1.4	10 ± 0.3	15 ± 0.2	15 ± 0.1	16 ± 0.1
	0.5	09 ± 0.1	12 ± 0.3	13 ± 0.3	15 ± 0.1
4e	3.7	12 ± 0.2	17 ± 0.1	17 ± 0.2	18 ± 0.3
	1.5	11 ± 0.3	16 ± 0.2	15 ± 0.1	17 ± 0.1
	0.5	09 ± 0.1	13 ± 0.3	14 ± 0.3	15 ± 0.1
4f	4.2	07 ± 0.2	08 ± 0.1	07 ± 0.2	06 ± 0.3
	1.7	–	–	04 ± 0.1	–
	0.6	–	02 ± 0.2	–	–
4g	3.9	11 ± 0.2	14 ± 0.1	13 ± 0.2	14 ± 0.3
	1.5	09 ± 0.3	12 ± 0.2	10 ± 0.1	12 ± 0.1
	0.6	06 ± 0.1	–	–	–
4h	3.2	11 ± 0.2	15 ± 0.1	13 ± 0.2	15 ± 0.3
	1.3	09 ± 0.3	13 ± 0.2	10 ± 0.1	12 ± 0.1
	0.5	07 ± 0.1	06 ± 0.3	–	10 ± 0.1
4i	3.7	10 ± 0.2	10 ± 0.1	11 ± 0.2	12 ± 0.3
	1.5	07 ± 0.3	09 ± 0.2	09 ± 0.1	10 ± 0.1
	0.5	–	–	–	03 ± 0.1
5a	0.37	13 ± 0.2	18 ± 0.1	17 ± 0.2	19 ± 0.3
	1.5	11 ± 0.3	16 ± 0.2	15 ± 0.1	17 ± 0.1
	0.5	08 ± 0.1	15 ± 0.3	14 ± 0.3	15 ± 0.1
5b	4.2	07 ± 0.2	10 ± 0.1	08 ± 0.2	10 ± 0.3
	1.7	05 ± 0.3	05 ± 0.2	06 ± 0.1	06 ± 0.1
	0.6	01 ± 0.3	02 ± 0.3	01 ± 0.3	02 ± 0.1
5c	3.1	13 ± 0.2	18 ± 0.1	18 ± 0.2	20 ± 0.3
	1.2	10 ± 0.3	17 ± 0.2	16 ± 0.1	17 ± 0.1
	0.5	09 ± 0.1	16 ± 0.3	14 ± 0.3	16 ± 0.1
5d	3.5	12 ± 0.2	16 ± 0.1	15 ± 0.2	16 ± 0.3
	1.4	09 ± 0.3	15 ± 0.2	14 ± 0.1	15 ± 0.1
	0.5	08 ± 0.1	14 ± 0.3	13 ± 0.3	14 ± 0.1
5e	3.6	13 ± 0.2	17 ± 0.1	17 ± 0.2	18 ± 0.3
	1.4	10 ± 0.3	16 ± 0.2	15 ± 0.1	17 ± 0.1
	0.5	08 ± 0.1	14 ± 0.3	13 ± 0.3	16 ± 0.1
5f	4.0	07 ± 0.2	–	09 ± 0.2	11 ± 0.3
	1.6	05 ± 0.3	–	06 ± 0.1	07 ± 0.1
	0.6	–	–	–	03 ± 0.1
5g	3.7	10 ± 0.2	12 ± 0.1	12 ± 0.2	12 ± 0.3
	1.5	08 ± 0.3	09 ± 0.2	09 ± 0.1	10 ± 0.1
	0.5	–	–	06 ± 0.3	09 ± 0.1
5h	3.1	10 ± 0.2	14 ± 0.1	14 ± 0.2	14 ± 0.3
	1.2	08 ± 0.3	12 ± 0.2	13 ± 0.1	13 ± 0.1
	0.5	06 ± 0.1	11 ± 0.3	–	10 ± 0.1
5i	3.6	09 ± 0.2	12 ± 0.1	10 ± 0.2	12 ± 0.3
	1.4	06 ± 0.3	08 ± 0.2	08 ± 0.1	08 ± 0.1
	0.5	–	03 ± 0.3	06 ± 0.3	04 ± 0.1
Fluconazole	5.2	14 ± 0.2	20 ± 0.1	19 ± 0.2	22 ± 0.3
	2.0	12 ± 0.3	19 ± 0.2	17 ± 0.1	19 ± 0.1
	0.8	10 ± 0.1	18 ± 0.3	16 ± 0.3	18 ± 0.1
Control (DMF)	–	00	00	00	00

Each value is expressed as mean ± SD of three replicates for zone of inhibition

A.f Asperigillus flavous, *C.a Candida albicans*, *M.g Microspora griseus*, *A.t Asperigillus terus*, *SD* standard deviation

prepared solution exhibits a deep blue color with the absorption maximum at 517 mm. This purple color generally fades when antioxidant is present in the solution. All compounds have exhibited varied free radial scavenging capacity by comparison with the standard butylated hydroxytoluene (BHT). The variation exhibited in DPPH scavenging activity could be attributed to the effect of different substituents. It has been reported that, the compounds substituted with phenolic hydroxyl group has the high potential for scavenging radicals (Hatano *et al.*, 1989; Sawa *et al.*, 1999; Babita *et al.*, 2011). Phenolic compounds donate hydrogen to reactive radicals and break the chain reaction of lipid oxidation at the initiation step (Gülcin *et al.*, 2004). Thus, the number and configuration of hydrogen donating hydroxyl groups are the main structural features that influence the radical scavenging activity (Cao *et al.*, 1997; Pannala *et al.*, 2001). On the other hand, presence of two amino groups can also donate their free electrons to the reactive ions, making them stable.

The activity results of the newly synthesized compounds are represented in Fig. 4. Among the tested compounds, compounds substituted with hydroxyl group (**4e**, **4f**, **4i**, **5e**, **5f**, and **5i**) at C-5 position of benzofuran ring displayed potent DPPH free radical scavenging activity with the lowest IC50 value (128.87–153.89 μmol/L). The lone pair of electrons present on the ring heteroatom along with electron donating group (–OH) may be responsible for blocking the DPPH free radical by donating electron pair, and hence fading the color. We also observed that, absence of hydroxyl

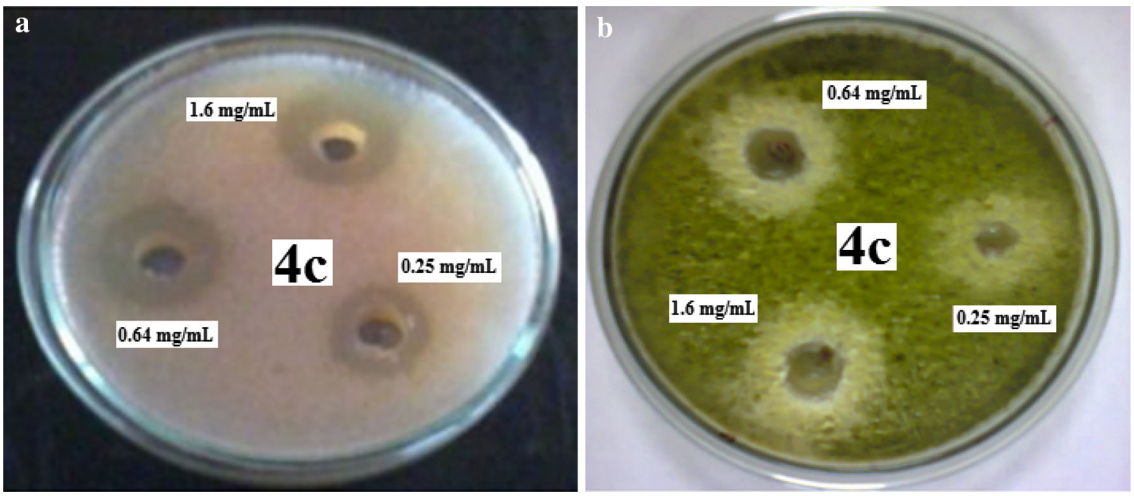


Fig. 2 **a.** Zone of inhibition of compound **4c** against *Salmonella typhi* (bacteria). **b**. Zone of inhibition of compound **4c** against *Microspora griseus* (fungus)

Table 4 Minimum inhibitory concentration (MIC) of synthesized compounds **4a–i** and **5a–i**

Comp. no.	Minimum inhibitory concentration (μmol/L)								
	P.s	*S.t*	*B.s*	*K.p*	E.c	*A.f*	*C.a*	*M.g*	*A.t*
4a	38.50	37.92	39.14	38.90	38.90	30.90	30.85	15.48	15.85
4c	30.22	30.73	31.96	31.76	31.96	25.81	25.81	12.90	13.01
4d	68.97	57.95	41.47	–	46.59	–	55.68	37.50	42.15
4e	38.14	36.73	38.61	38.84	38.73	30.16	30.04	15.84	15.37
4h	12.34	11.38	–	63.21	63.21	–	66.49	52.66	50.61
5a	37.20	36.61	37.79	38.14	37.79	29.92	29.81	14.90	15.37
5c	29.76	29.76	31.64	31.54	31.05	25.00	25.09	12.50	12.59
5d	–	121.16	99.12	101.64	–	51.86	53.72	40.57	45.06
5e	36.99	36.08	36.42	36.31	36.31	31.10	31.22	16.96	16.96
5h	–	199.10	188.98	189.18	169.34	66.07	–	50.99	49.00
Streptomycin	24.95	24.95	26.67	26.24	26.24	–	–	–	–
Flucanazole	–	–	–	–	–	40.84	40.84	20.42	20.42

P.s Psedomonas syringae, *S.t Salmonella typhi*, *B.s Bacillus subtilis*, *K.p Klebsiella pneumoniae*, *E.c Escherichia coli*, *A.f Asperigillus flavous*, *C.a Candida albicans*, *M.g Microspora griseus*, *A.t Asperigillus terus*

group and incorporation of –Br group at C-5 position of benzofuran ring drastically reduced the activity (as observed in compounds **4a**, **5c**, **4h**, and **5h**).On the other hand, compounds **4d** and **5d** displayed moderate to good activity with IC50 value 162.36 and 160.85 μmol/L, respectively.

Iron chelating ability

The iron chelating study measures the ability of antioxidants to compete with ferrozine in chelating ferrous ion (Elmastas *et al.*, 2006). The ferrous ion-chelating activity of the newly synthesized compounds is represented in Fig. 5. The Fe^{2+} chelating capacities varied significantly among different compounds. From the activity results it revealed that, among the tested barbiturate series, compounds **4a**, **4b**, and **4g** showed very good chelating ability with IC50 value 146.63–175.93 μmol/L. In thiobarbiturates series, compounds **5a**, **5b**, and **5g** exhibit excellent activity with IC50 value 126.57–147.67 μmol/L. Furthermore, it was observed that the presence of OH group at C-5 of benzofuran ring and OCH_3/Br group on C-4 of aryl ring (**4e**, **4f**, **5e**, and **5f**) showed good chelating ability. Further, declining trend in chelating ability was found in **4d**, **4i**, **5d**, and **5i**. Compounds with halogen substituents (**4c**, **5c**, **4h**, and **5h**) lowered the activity with higher IC50 value (284.01–458.75 μmol/L).

IC50 values of DPPH radical scavenging and ferrous ion chelating activity of test compounds is given in Table 6.

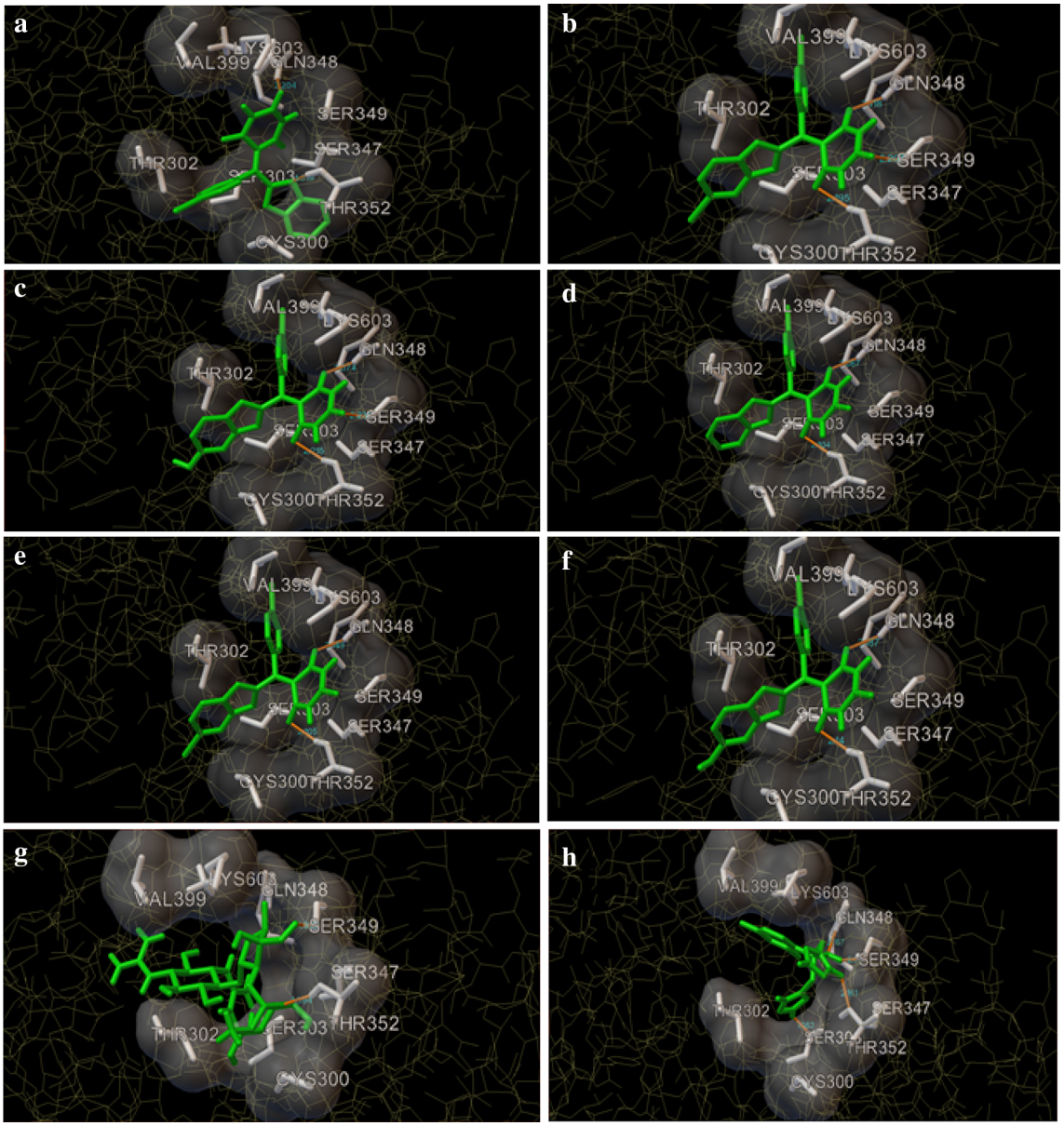


Fig. 3 Interaction of ligand molecules **4a**, **4c**, **4e**, **5a**, **5c**, and **5e** with GlcN-6-P. A: interaction of **4a** with GlcN-6-P, B: interaction of **4c** with GlcN-6-P, C: interaction of **4e** with GlcN-6-P, D: interaction of **5a** with GlcN-6-P, E: interaction of **5c** with GlcN-6-P, F: interaction of **5e** with GlcN-6-P, G: interaction of Streptomycin with GlcN-6-P, H: interaction of Fluconazole with GlcN-6-P

Experimental protocols

The BA, thiobarbituric acid, and phenacyl bromides with 98 % purity were purchased from Sigma Aldrich Company. Melting points were recorded on electro thermal melting point apparatus and are uncorrected. Column chromatography was performed using silica gel (230–400 mesh), silica gel GF254 plates from Merck were used for TLC and spots were identified under ultraviolet radiation. Ethyl acetate:pet ether (1:4) is used as a mobile phase. The FT-IR spectra were taken in KBr pellets (100 mg) using Shimadzu FT-Infrared spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 400 MHz spectrometer and chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as internal standard. LC–MS were obtained using C-18 column on Shimadzu, LCMS 2010A, Japan.

The results of biological studies have been expressed in molar unit. In antimicrobial activity the zone of inhibition and in antioxidant activity the IC50 values are expressed as mean $\pm$ SD of three replicates.

Table 5 Molecular docking results of synthesized compounds with Glucosamine-6-phosphate synthase

Comp. no.	Binding energy (kJ mol^{-1})	Inhibition constant (μM)	RMSd	Ligand efficiency	No. of hydrogen bonds	Bonding residues	Bond length (Å)
4a	−4.71	295.65	0.0	−0.19	2	2VF5: GLN348: HE22: Ligands/4a:: : O	2.204
						2VF5: THR352: HG1: Ligands/4a:: : O	1.919
4c	−5.27	229.07	0.0	−0.18	3	2VF5: GLN348: HE22: Ligands/4c:: : O	2.118
						2VF5:SER349: HG: Ligands/4c:: : O	2.207
						2VF5:THR352: HG1: Ligands/4c:: : O	2.195
4e	−4.53	476.51	0.0	−0.17	3	2VF5:GLN348: HE22: Ligands/4e:: : O	2.074
						2VF5:SER349: HG: Ligands/4e:: : O	2.231
						2VF5:THR352:HG1: Ligands/4c:: : O	2.215
5a	−4.66	353.32	0.0	−0.18	2	2VF5:GLN348:HE22: Ligands/5a:: : O	2.067
						2VF5:THR352:HG1: Ligands/5a:: : O	2.104
5c	−4.85	280.61	0.0	−0.18	2	2VF5:GLN348:HE22: Ligands/5c:: : O	2.149
						2VF5:THR352:HG1: Ligands/5c:: : O	2.105
5e	−4.33	673.95	0.0	−0.16	2	2VF5:GLN348:HE22: Ligands/5c:: : O	2.187
						2VF5:THR352:HG1: Ligands/5c:: : O	2.140
Streptomycin	−6.72	−181.49	0.0	−0.17	2	2VF5:SER349:HG: Ligands/Streptomycin:: : O	1.922
						2VF5:THR352: OG1: Ligands/Streptomycin:: : H	1.894
Fluconazole	−5.65	−197.44	0.0	−0.26	4	2VF5:GLN348:HG: Ligands/Fluconazole:: : O	2.167
						2VF5:THR352:OG1: Ligands/Fluconazole:: : H	2.161
						2VF5:SER349:OG: Ligands/Fluconazole:: : H	2.09
						2VF5: SER303:OG: Ligands/Fluconazole:: : H	1.962

Experimental procedure

Chemistry

*General procedure for the synthesis of benzofuran barbitone derivatives (**4a–i**)*

The mixture of (2/4-substitutedphenyl) methanone (**3a–i**) (0.01 mol) and BA (0.01 mol, 1.44 g) was taken in acetic acid and refluxed in an oil bath for about 8–10 h at 110–115 °C. After the completion of reaction, the reaction mass was cooled to room temperature, poured into crushed ice and neutralized with $NaHCO_3$ solution. The product was filtered, dried and recrystallized using ethyl acetate/ethanol, further purified by silica gel column chromatography eluting with petroleum ether, ethyl acetate mixture (80:20, v/v).

*5-[(Benzofuran-2-yl)(4-bromophenyl)methylidene]pyrimidin-2,4,6(1H,3H,5H)-trione (**4a**)* Yellow solid (EtOH); m.p. 161 °C; IR (KBr, υ cm^{-1}): 3317 (N–H), 3155 (Ar–CH), 1715 (C=O), 1675 (–NHC=O), 684 (C–Br); ^{1}H NMR

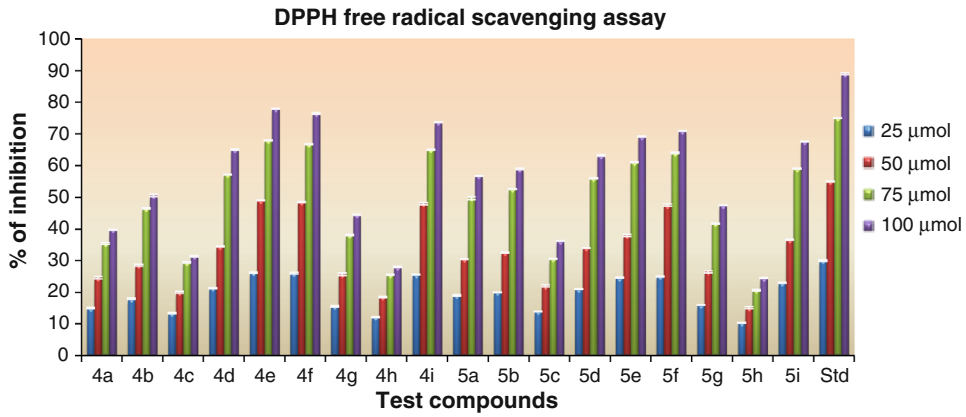


Fig. 4 DPPH free radial scavenging activity of test compounds (**4a–i**) and (**5a–i**)

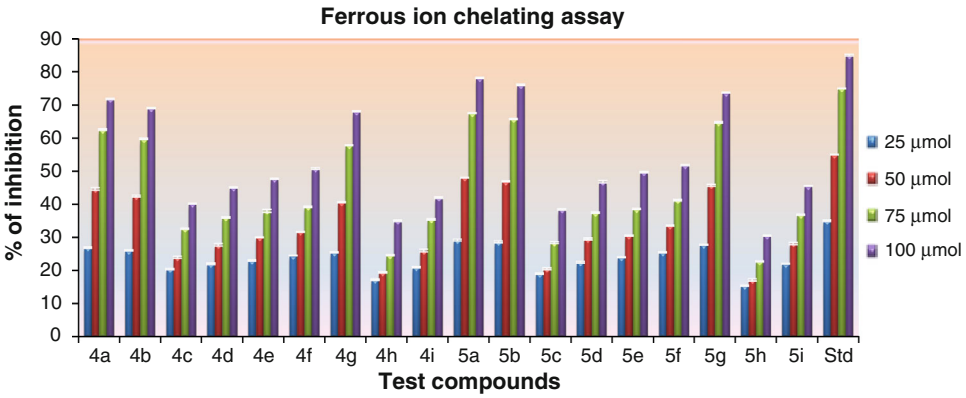


Fig. 5 Ferrous ion chelating assay of test compounds (**4a–i**) and (**5a–i**)

(400 MHz, DMSO-d_6, δ ppm): 10.68 (s, 1H –NH), 10.21 (s, 1H –NH), 7.2-8.3 (m, 9H Ar–H); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 165.0 (C=O, C-11, C-11′), 159.9 (C-9), 158.3 (C-4), 157.3 (C-1), 150.5 (C=O, C-12), 139.0 (C-13), 131.6 (CH, C-15, C-15′), 130.2 (C-3), 128.6 (CH, C-14, C-14′), 124.7 (CH, C-6), 123.3 (CH, C-7), 122.0 (C-10, C-16), 121.0 (CH, C-8), 111.6 (CH, C-5), 106.9 (CH, C-2); MS (LCMS): *m/z* 410 [M], 412 [M+2].

5-[(Benzofuran-2-yl) (4-methoxyphenyl) methylidene] pyrimidin-2,4,6(1H,3H,5H)-trione (***4b***) Off-white solid (EtOH); m.p. 163–165 °C; IR (KBr, υ cm^{-1}): 3310 (N–H), 1710 (C=O), 1648 (–NHC=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.71 (s, 1H –NH), 10.14 (s, 1H –NH), 7.0–8.1 (m, 9H Ar–H), 3.85 (s, 3H –OCH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 167.1 (C=O, C-11, C-11′), 160.0 (C-9, C-16), 158.3 (C-4), 157.3 (C-1), 150.5 (C=O, C-12), 132.3 (C-13), 131.0 (C-3), 127.4 (CH, C-14, C-14′), 124.7 (CH, C-6), 123.3 (CH, C-7), 122.0 (C-10), 121.0 (CH, C-8), 114.5 (CH, C-15, C-15′), 111.6 (CH, C-5), 106.9 (CH, C-2), 55.8 (O–CH_3); MS (LCMS): *m/z* 362 [M].

5-[(5-Bromo-1-benzofuran-2-yl)(4-bromophenyl) methylidene] pyrimidin-2,4,6(1H,3H,5H)-trione (***4c***) Off-white solid (ethyl acetate); m.p. 160–162 °C; IR (KBr, υ cm^{-1}): 3419 (N–H), 1708 (C=O), 1654 (–NHC=O), 669 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.80 (s, 1H –NH), 10.21 (s, 1H –NH), 7.4–8.0 (m, 8H Ar–H); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 165.0 (C=O, C-11, C-11′), 159.9 (C-9), 157.3 (C-1), 150.5 (C=O, C-12), 139.0 (C-13), 133.7 (C-4), 131.6 (CH, C-15, C-15′), 129.0 (CH, C-6), 128.6 (CH, C-14, C-14′), 124.2 (CH, C-8), 122.3 (C-10), 116.5 (C-7), 113.8 (C-5), 106.9 (CH, C-2); MS (LCMS): *m/z* 488 [M], 490 [M+2], 492 [M+4].

5-[(5-Bromo-1-benzofuran-2-yl) (4-methoxyphenyl) methylidene] pyrimidin-2,4,6(1H, 3H, 5H)-trione (***4d***) Colorless solid (ethyl acetate); m.p. 161–163 °C; IR (KBr, υ cm^{-1}): 3308 (N–H), 1705 (C=O), 1669 (–NHC=O), 659 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.65 (s, 1H –NH), 10.02 (s, 1H –NH), 7.2-8.0 (m, 8H Ar–H), 3.86 (s, 3H –OCH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 165.0 (C=O, C-11, C-11′), 159.9 (C-9, C-16), 157.3 (C-4), 150.5 (C=O, C-12), 133.7 (C-3), 132.3 (C-13), 129.0 (CH, C-6), 127.4 (CH, C-14, C-14′), 124.2 (CH, C-8), 122.3 (C-10), 116.5 (C-7), 114.2 (CH, C-15, C-15′), 112.9 (C-5), 106.9 (CH, C-2), 55.6 (O–CH_3); MS (LCMS): *m/z* 440 [M], 442 [M+2].

5-[(4-Bromophenyl) (5-hydroxy-1-benzofuran-2-yl) methylidene] pyrimidin-2,4,6(1H,3H,5H)-trione (***4e***) Off-white solid (EtOH); m.p. 168–169 °C; IR (KBr, υ cm^{-1}):

Table 6 Half-maximum inhibition concentrations (IC50) for of DPPH radical scavenging and ferrous ion chelating activity of test compounds

Compd.	DPPH (IC50 μmol/L)	Fe^{2+} ion Chelating activity (IC50 μmol/L)
4a	304.87 ± 0.19	146.63 ± 0.33
4b	256.74 ± 0.29	175.93 ± 0.18
4c	341.78 ± 0.15	284.01 ± 0.15
4d	162.36 ± 0.14	269.38 ± 0.19
4e	128.87 ± 0.12	256.24 ± 0.14
4f	148.09 ± 0.22	268.28 ± 0.10
4g	269.48 ± 0.40	160.95 ± 0.15
4h	399.95 ± 0.25	355.10 ± 0.25
4i	135.51 ± 0.14	299.90 ± 0.24
5a	193.02 ± 0.11	126.57 ± 0.11
5b	208.54 ± 0.32	147.67 ± 0.31
5c	284.28 ± 0.10	299.78 ± 0.29
5d	160.85 ± 0.15	245.28 ± 0.21
5e	147.78 ± 0.18	104.46 ± 0.15
5f	150.91 ± 0.21	236.33 ± 0.20
5g	238.05 ± 0.13	135.56 ± 0.20
5h	458.75 ± 0.17	402.46 ± 0.17
5i	153.89 ± 0.13	262.08 ± 0.13
Std[a,b]	203.82 ± 0.23	150.93 ± 0.15

Each value is expressed as mean ± SD of three replicates

Std[a] BHT used as standard for DPPH radical scavenging activity; Std[b] EDTA is used as a standard for Fe^{2+} ion chelating activity

3421 (O–H), 3391 (N–H), 1702 (C=O), (–NHC=O), 696 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.55 (s, 1H –NH), 10.12 (s, 1H –NH), 7.2–8.1 (m, 8H Ar–H), 5.2 (s, 1H –OH); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 165.4 (C=O, C-11, C-11′), 159.9 (C-10), 157.6 (C-1), 153.6 (C–OH, C-7), 151.1 (C-4), 150.5 (C=O, C-12), 139.0 (C-13), 132.9 (C-3), 131.2 (CH, C-15, C-15′), 128.6 (CH, C-14, C-14′), 122.3 (C-10, C-16), 114.3 (CH, C-6), 113.0 (CH, C-5), 106.9 (CH, C-2), 106.1 (CH, C-8); MS (LCMS): *m*/*z* 426 [M], 428 [M+2].

*5-[(5-Hydroxy-1-benzofuran-2-yl) (4-methoxyphenyl) methylidene] pyrimidin-2,4,6(1H,3H,5H)-trione (**4f**)* Light yellow solid (EtOH); m.p. 161–163 °C; IR (KBr, υ cm^{-1}): 3380 (N–H), 1703 (C=O), 1670 (–NHC=O), ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.60 (s, 1H –NH), 10.12 (s, 1H –NH), 7.2–8.2 (m, 8H Ar–H), 5.5 (s, 1H -OH); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 165.0 (C=O, C-11, C-11′), 160.1 (C-9, C-16), 157.3 (C-1), 153.6 (C–Br, C-7), 151.8 (C-4), 150.1 (C=O, C-12), 133.0 (C-3), 131.6 (C-13), 127.4 (CH, C– C-14, C-14′), 122.3 (C-10), 114.3 (CH, C-6), 113.0 (CH, C-15, C-15′), 112.0 (CH, C-5), 107.0 (CH, C-2), 106.1 (CH, C-8), 55.9 (O–CH_3); MS (LCMS): *m*/*z* 378 [M].

*5-[1-Benzofuran-2-yl (2-bromophenyl) methylidene] pyrimidin-2,4,6(1H,3H,5H)-trione (**4g**)* Colorless solid (ethyl acetate); m.p. 78–80 °C; IR (KBr, υ cm^{-1}): 3318 (N–H), 1731 (C=O), 1709 (–NHC=O), 688 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.69 (s, 1H –NH), 10.12 (s, 1H –NH), 7.2–8.3 (m, 9H Ar–H); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 165.0 (C=O, C-11, C-11′), 159.9 (C-9), 158.3 (C-4), 157.3 (C-1), 150.1 (C=O, C-12), 138.2 (C-13), 132.0 (CH, C-15), 131.2 (C-3), 130.2 (CH, C-16), 128.6 (CH, C-14′), 127.1 (CH, C-15′), 124.8 (CH, C-6), 123.3 (CH, C-7), 122.0 (C, C-10), 121.0 (CH, C-8), 118.9 (C–Br, C-14), 111.6 (CH, C-5), 106.9 (CH, C-2); MS (LCMS): *m*/*z* 410 [M], 412 [M+2].

*5-[(5-Bromo-1-benzofuran-2-yl)(2-bromophenyl) methylidene] pyrimidin-2,4,6(1H,3H,5H)-trione (**4h**)* Brown solid (ethyl acetate); m.p. 155–157 °C; IR (KBr, υ cm^{-1}): 3412 (N–H), 1718 (C=O), 1675 (–NHC=O), 669 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.80 (s, 1H –NH), 10.22 (s, 1H –NH), 7.5–8.1 (m, 8H Ar–H); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 165.2 (C=O, C-11, C-11′), 159.9 (C-9), 157.3 (C-1), 150.5 (C=O, C-12), 138.2 (C-13), 133.7 (C-3), 131.6 (CH, C-15), 130.1 (CH, C-16), 128.5 (CH, C-6), 127.8 (CH, C-14′), 126.6 (CH, C-15′), 124.2 (CH, C-8), 122.3 (C-10), 118.9 (C–Br, C-14), 116.5 (C–Br, C-7), 113.8 (CH, C-5), 106.5 (CH, C-2); MS (LCMS): *m*/*z* 488 [M], 490 [M+2], 492 [M+4].

*5-[(2-Bromophenyl) (5-hydroxy-1-benzofuran-2-yl) methylidene] pyrimidin-2,4,6(1H,3H,5H)-trione (**4i**)* Green solid (EtOH); m.p. 96–98 °C; IR (KBr, υ cm^{-1}): 3421 (O–H), 3395 (N–H), 1721 (C=O), 1668 (–NHC=O), 694 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.58 (s, 1H –NH), 10.12 (s, 1H –NH), 7.2–8.0 (m, 8H Ar–H), 5.4 (s, 1H -OH); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 166.0 (C=O, C-11, C-11′), 159.9 (C-9), 157.3 (C-1), 153.6 (C–OH, C-7), 151.3 (C-4), 150.5 (C=O, C-12), 138.2 (C-13), 132.9 (C-3), 131.6 (CH, C-15), 130.2 (CH, C-16), 128.6 (CH, C-14′), 127.0 (CH, C-15′), 122.3 (C-10), 118.9 (C–Br, C-14), 114.3 (CH, C-6), 113.0 (CH, C-5), 106.9 (CH, C-2), 106.0 (CH, C-8); MS (LCMS): *m*/*z* 426 [M], 428 [M+2].

*General procedure for the Synthesis of benzofuran thiobarbitone derivatives (**5a–i**)*

The mixture of (2/4-substituted-phenyl) methanone (**3a–i**) (0.01 mol) and thiobarbituric acid (0.01 mol, 1.44 g) was taken in acetic acid and refluxed in an oil bath for about 8–10 h at 110–115 °C. After the completion of reaction, the reaction mass was cooled to room temperature, poured into crushed ice and neutralized with $NaHCO_3$ solution. The product was filtered, dried, and recrystallized using

ethyl acetate/ethanol, further purified by silica gel column chromatography eluting with petroleum ether, ethyl acetate mixture (80:20, v/v).

5-[1-Benzofuran-2-yl (4-bromophenyl) methylidene]-2-thioxodihydropyrimidin-4,6(1H,5H)-dione ***(5a)*** Brown solid (EtOH); m.p. 102–104 °C; IR (KBr, υ cm^{-1}): 3317 (N–H), 1229 (C=S), 1735 (C=O), 686 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.67 (s, 1H –NH), 10.24 (s, 1H –NH), 7.1-8.2 (m, 9H Ar–H); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 178.1 (C=S, C-12), 167.0 (C=O, C-11, C-11′), 159.9 (C-10), 158.3 (C-4), 156.3 (C-1), 139.0 (C-13), 131.6 (CH, C-15, C-15′), 130.1 (C-3), 128.6 (CH, C-14, C-14′), 124.7 (CH, C-6), 123.3 (CH, C-7), 122.0 (C–Br, C-16), 121.0 (CH, C-8), 111.6 (CH, C-5), 106.9 (CH, C-2); MS (LCMS): *m/z* 426 [M], 428 [M+2].

5-[1-Benzofuran-2-yl (4-methoxyphenyl) methylidene]-2-thioxodihydropyrimidin-4,6(1H, 5H)-dione ***(5b)*** Yellow solid (EtOH); m.p. 92–94 °C; IR (KBr, υ cm^{-1}): 3304 (N–H), 1329 (C=S), 1633 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.13 (s, 1H –NH), 10.70 (s, 1H –NH), 7.1–8.0 (m, 9H Ar–H), 3.88 (s, 3H –OCH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 179.1 (C=S, C-12), 167.8 (C=O, C-11, C-11′), 159.9 (C-9, C-16), 158.2 (C-4), 155.7 (C-1), 135.0 (C-13), 130.9 (C-3), 127.4 (CH, C-14, C-14′), 124.4 (CH, C-6), 123.0 (CH, C-7), 122.3 (C-10), 117.9 (CH, C-8), 114. 5 (CH, C-15, C-15′), 111.6 (CH, C-5), 106.9 (CH, C-2), 55. 8 (O–CH_3); MS (LCMS): *m/z* 378 [M].

5-[(5-Bromo-1-benzofuran-2-yl)(4-bromophenyl)methylidene]-2-thioxodihydropyrimidin-4,6(1H,5H)-dione ***(5c)*** Light yellow solid (ethyl acetate); m.p. 100–102 °C; IR (KBr, υ cm^{-1}): 3417 (N–H), 1293 (C=S), 1735 (C=O), 666 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.22 (s, 1H –NH), 10.79 (s, 1H –NH), 7.5-8.0 (m, 8H Ar–H); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 178.1 (C=S, C-12), 167.0 (C=O, C-11, C-11′), 160.0 (C-9), 157.3 (C-1, C-4), 139.2 (C-13), 133.7 (C-3), 131.6 (CH, C-15, C-15′), 129.0 (CH, C-14, C-14′), 128.1 (CH, C-6), 124.2 (CH, C-8), 122. 3 (C-10, C-16), 116.5 (C–Br, C-7), 113.8 (CH, C-5), 106.9 (CH, C-2); MS (LCMS): *m/z* 504 [M] 506 [M+2], 508 [M+4].

5-[(5-Bromo-1-benzofuran-2-yl)(4-methoxyphenyl)methylidene]-2-thioxodihydropyrimidin-4,6(1H,5H)-dione ***(5d)*** Brown solid (ethyl acetate); m.p. 110–112 °C; IR (KBr, υ cm^{-1}): 3302 (N–H), 1286 (C=S), 1641 (C=O), 650 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.03 (s, 1H –NH), 10.61 (s, 1H –NH), 7.1–8.0 (m, 8H Ar–H), 3.88 (s, 3H -OCH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 178. 2 (C=S, C-12), 167.1 (C=O, C-11, C-11′), 159.9 (C, C-9, C-16), 157.3 (C, C-1, C-4), 133.7 (C, C-3), 132.3 (C, C-13), 129.0 (CH, C-6), 127.4 (CH, C-14, C-14′), 124.2 (CH, C-8), 122.3 (C-10), 116.5 (C–Br, C-7), 114.2 (CH, C-15, C-15′), 112.9 (CH, C-5), 106.9 (CH, C-2), 55.9 (O–CH_3); MS (LCMS): *m/z* 456 [M], 458 [M+2].

5-[(4-Bromophenyl) (5-hydroxy-1-benzofuran-2-yl) methylidene]-2-thioxodihydropyrimidin-4,6(1H,5H)-dione ***(5e)*** Colorless solid (EtOH); m.p. 109 °C; IR (KBr, υ cm^{-1}): 3419 (O–H), 3394 (N–H), 1279 (C=S), 1641 (C=O), 694 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.13 (s, 1H –NH), 10.51 (s, 1H –NH), 7.2-8.1 (m, 8H Ar–H), 5.6 (s, 1H –OH); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 178.3 (C=S, C-12), 167.5 (C=O, C-11, C-11′), 159.3 (C-9), 157.6 (C-1), 153.6 (C–OH, C-7), 150.9 (C-4), 139.2 (C-13), 132.8 (C-3), 131.5 (CH, C-15, C-15′), 128.6 (CH, C-14, C-14′), 122.6 (C, C-10, C-16), 114.3 (CH, C-6), 113.0 (CH, C-5), 106.9 (CH, C-2), 106.1 (CH, C-8); MS (LCMS): *m/z* 442 [M], 444 [M+2].

5-[(5-Hydroxy-1-benzofuran-2-yl) (4-methoxyphenyl) methylidene]-2-thioxodihydropyrimidin-4,6(1H,5H)-dione ***(5f)*** Brown solid(EtOH); m.p. 103–106 °C; IR (KBr, υ cm^{-1}): 3384 (N–H), 1310 (C=S), 1653 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.36 (s, 1H –NH), 10.52 (s, 1H –NH), 7.1-8.2 (m, 8H Ar–H), 5.8 (s, 1H –OH) 3.72 (s, 3H –OCH_3); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 178.5 (C=S, C-12), 167.5 (C=O, C-11, C-11′), 160.1 (C-9, C-16), 157.3 (C-1), 153.2 (C–OH, C-7), 150.8 (C-4), 133.2 (C-3), 131.1 (C-13), 128.4 (CH, C-14, C-14′), 122.3 (C-10), 115.3 (CH, C-15, C-15′), 113.1 (CH, C-6), 112.3 (CH, C-5), 107.0 (CH, C-1), 106.1 (CH, C-8), 55.9 (OCH_3); MS (LCMS): *m/z* 394 [M].

5-[1-Benzofuran-2-yl (2-bromophenyl) methylidene]-2-thioxodihydropyrimidin-4,6(1H,5H)-dione ***(5g)*** Pale yellow solid(ethyl acetate); m.p. 105–108 °C; IR (KBr, υ cm^{-1}): 3317 (N–H), 1290 (C=S), 1735 (C=O), 686 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.24 (s, 1H –NH), 10.67 (s, 1H –NH), 7.1–8.2 (m, 9H Ar–H); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 178.1 (C=S, C-12), 167.0 (C=O, C-11, C-11′), 159.9 (C-9), 158.3 (C-4), 157.3 (C-1), 139.2 (C-13), 131.8 (CH, C-14), 131.1 (C-3), 130.2 (CH, C-16), 129.0 (CH, C-14′), 127.6 (CH, C-15′), 124.8 (CH, C-6), 123.5 (CH, C-7), 122.0 (C-10), 121.0 (CH, C-8), 118.9 (C–Br, C-14), 111.6 (CH, C-5), 106.9 (CH, C-2); MS (LCMS): *m/z* 426 [M], 428 [M+2].

5-[(5-Bromo-1-benzofuran-2-yl)(2-bromophenyl)methylidene]-2-thioxodihydropyrimidin-4,6(1H,5H)-dione ***(5h)*** Brown solid (ethyl acetate); m.p. 110–114 °C; IR (KBr, υ cm^{-1}): 3417 (N–H), 1285 (C=S), 1735 (C=O), 666 (C–Br);

^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.22 (s, 1H –NH), 10.79 (s, 1H –NH), 7.5-8.0 (m, 8H Ar–H); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 178.3 (C=S, C-12), 167.2 (C=O, C-11, C-11′), 159.9 (C, C-9), 157.3 (C-1, C-4), 138.2 (C-13), 133.7 (C-3), 131.6 (CH, C-15), 129.0 (CH, C-6) 128.5 (CH, C-14′), 127.2 (CH, C-15′), 124.2 (CH, C-8), 122.3 (C-10), 118.9 (C–Br, C-14), 116.5 (C–Br, C-7), 113.8 (CH, C-5), 106.5 (CH, C-2); MS (LCMS): *m/z* 504 [M] 506 [M+2], 508 [M+4].

5-[(2-Bromophenyl) (5-hydroxy-1-benzofuran-2-yl) methylidene]-2-thioxodihydropyrimidin-4,6(1H,5H)-dione ***(5i)*** Brown solid(EtOH); m.p. 114 °C; IR (KBr, υ cm^{-1}): 3419 (O–H), 3394 (N–H), 1260 (C=S), 1641 (C=O), 694 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.13 (s, 1H –NH), 10.51 (s, 1H –NH), 7.2-8.1 (m, 8H Ar–H), 5.6 (s, 1H –OH); ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 178.1 (C=S, C-12), 167.0 (C=O, C-11, C-11′), 159.9 (C, C-9), 157.3 (C-1), 153.6 (C–OH, C-7), 150.9 (C, C-4), 138.2 (C-13), 132.9 (C-3), 131.6 (CH, C-14), 130.2 (CH, C-16), 128.6 (CH, C-14′), 127.0 (CH, C-15′), 122.3 (C-10), 118.9 (C–Br, C-14), 114.3 (CH, C-6), 113.0 (CH, C-5), 106.9 (CH, C-2), 106.1 (CH, C-8); MS (LCMS): *m/z* 442 [M], 444 [M+2].

Antimicrobial activity

Antimicrobial activity of the synthesized compounds was tested against five bacterial strains using agar well diffusion method (Nasser Khalil, 2010). Dimethylsulfoxide (DMSO) was used as solvent control. The bacterial cultures were inoculated on nutrient agar (Merck) and fungal culture was inoculated on potato dextrose agar media (20 mL). The test compounds were dissolved in DMSO to get a concentration of 12.79 M and 100 µL of this sample was loaded into the wells of agar plates directly. Plates inoculated with the bacteria were incubated at 37 °C for 24 h and the fungal culture was incubated at 25 °C for 72 h. All determinations were done in triplicates. The Streptomycin (1.71 and 0.85 M) and Fluconazole (3.26 and 1.6 M) were used as standard drugs for antibacterial and antifungal activities, respectively.

The MIC was performed by serial broth-dilution method (National Committee for Clinical Laboratory Standards (NCCLS), 1982) at different concentrations like 1, 10, 25, 50, and 100 mol/L. After the incubation period, the minimum inhibition zone at which the micro-organism growth was inhibited was measured in M.

In silico molecular docking studies

The compounds in the present investigation were subjected for molecular docking studies using Auto Dock (version 4.2) with Lamarckian genetic algorithm. The synthesized ligand molecules having 2D structure were converted to energy minimized 3D structures and were further used for in silico protein–ligand docking. The docking of receptor GlcN-6-P with newly synthesized ligands exhibited well established bonds with one or more amino acids in the receptor active pocket. The active pocket was considered to be the site, where glucosamine-6-phosphate complexes with GlcN-6-P of 2VF5. The active pocket consisted of 12 amino acid residues as Ala602, Val399, Ala400, Gly301, Thr302, Ser303, Cys300, Gln348, Ser349, Thr352, Ser347, and Lys603 (Wallace *et al.*, 1995).

The crystal structure of GlcN-6-P synthase (PDB ID 2VF5) from the PDB (http://www.pdb.org/pdb/home/home.do) was selected and edited by removing the heteroatoms and adding C-terminal oxygen (Binkowski *et al.*, 2003). The Graphical User Interface program "AutoDock Tools" was used to prepare, run, and analyze the docking simulations. Kollman united atom charges, solvation parameters, and polar hydrogens were added to the receptor for the preparation of protein in docking simulation. Since ligands are not peptides, Gasteiger charge was assigned and then non-polar hydrogens were merged.

Antioxidant activity

Free radical scavenging activity by DPPH method

Free radical-scavenging capacities of synthesized compounds were determined according to the reported procedure (Braca *et al.*, 2001). The newly synthesized compounds at different concentrations (25–100 µmol/L) were added to each test tube and volume was made up to 4 mL using methanol. To this, 3 mL of 0.004 % DPPH in methanol was added and the mixtures were incubated at room temperature under dark condition for 30 min. The absorbance was recorded at 517 nm using UV–Visible spectrophotometer (Shimadzu UV-1800, Japan). Butylated hydroxytoluene (BHT) dissolved in distilled water was used as a reference. Control sample was prepared using the same volume without any compound and BHT, 95 % methanol served as blank. Test was performed in triplicate and the results were averaged. Radical scavenging activity was calculated using the formula:

$$\% \text{ of radical scavenging activity} = [(A_{\text{control}} - A_{\text{test}})/A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the control sample (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution + test compound).

Iron chelating ability

The chelating effect was determined according to the literature method (Nevcihan *et al.*, 2010). The test solution (2 mL) of different concentrations (25–100 μmol/L) in methanol was added to a solution of 2 mM $FeCl_2$ (0.05 mL), the reaction was initiated by adding 5 mM ferrozine (0.2 mL) and total volume was adjusted to 5 mL with methanol. Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was measured spectrophotometrically at 562 nm. EDTA was used as a standard. The inhibition percentage of ferrozine–Fe^{2+} complex formations was calculated using the formula:

$$\text{Metal chelating effect}\,(\%) = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right] \times 100$$

where A_{control} is the absorbance of control (control contains $FeCl_2$ ferrozine complex) and A_{sample} is the absorbance of test compounds. Ascorbic acid is used as control. Test was performed in triplicate and the results were averaged.

Conclusion

We synthesized novel series of benzofuran derivatives containing barbiturate and thiobarbiturates nucleus. The structure of synthesized compounds was confirmed by FT-IR, NMR, and mass spectroscopic methods. From the antimicrobial study results it revealed that, compounds containing two bromo substituents are responsible for increased antimicrobial activity than those bearing a single halogen/other substituent group. In case of antioxidant screening, compounds containing hydroxyl groups showed very good DPPH radical scavenging activity. In metal chelating assay, compounds having thiobarbiturate nucleus showed comparatively predominant activity than barbiturate series, this could be due to the presence of thione group in thiobarbiturates.

Acknowledgments The authors are thankful to the Chairman, Department of Industrial Chemistry, Kuvempu University, Shankaraghatta for providing the laboratory facilities. One of the authors (Kenchappa. R) is thankful to the UGC for the award of Rajiv Gandhi National Fellowship (RGNF).

References

Agatsuma T, Furukawa H, Hayakawa I, Shioya R, Sugano Y (2004) Thienopyridine and benzofuran derivatives as potent anti-tumor agents possessing different structure–activity relationships. Bioorg Med Chem Lett 14:3411–3414

Aly HM, Kamal MM (2012) Efficient one-pot preparation of novel fused chromeno[2,3-*d*]pyrimidine and pyrano[2,3-*d*] pyrimidine derivatives. Eur J Med Chem 47:18–23

Andres P, Marhold A (1996) A new synthesis of 5-trifluoromethyl-uracil source. J Fluor Chem 77:93–95

Babita P, Hari DB, Hye YK, Sung G, Se Jong H, Hong KL, Hyuncheol O, Hyun WS, Joung HY (2011) Ramalin, a novel nontoxic antioxidant compound from the Antarctic Lichen *Ramalina terebrata*. Phytomedicine 18:1285–1290

Basavaraja HS, Jayadevaiah KV, Mumtaz MH, Vijay Kumar MMJ, Basavaraj P (2010) Synthesis of novel piperazine and morpholine linked substituted pyrimidine derivatives as antimicrobial agents. J Pharm Sci Res 2:5–12

Bigler L, Spirli C, Fiorotto R, Pettenazzo A, Duner E, Baritussio A, Follath F, Riem HH (2007) Synthesis and cytotoxicity properties of Amiodarone analogues. Eur J Med Chem 42:861–867

Binkowski A, Naghibzadeg S, Liang J (2003) CASTp computed atlas of surface topography of proteins. Nucleic Acid Res 31:3352–3355

Biradar JS, Sashidhar BS, Praveen R (2010) Synthesis, antioxidant and DNA cleavage activities of novel indole derivatives. Eur J Med Chem 45:4074–4078

Bondock S, Khalifa W, Fadda AA (2011) Synthesis and antimicrobial activity of some new 4-hetarylpyrazole and furo[2,3-*c*]pyrazole derivatives. Eur J Med Chem 46:2555–2561

Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I (2001) New diterpenes from *Jatropha divaricata*. J Nat Prod 64:892–895

Cao G, Sofic E, Prior RL (1997) Antioxidant and prooxidant behavior of flavonoids: structure–activity relationships. Free Radic Biol Med 22:749–760

Chandrashekar A, Eswarappa B, Bodke YD, Venkatesh KB, Raghu N, Shivakumar MC, Peethambar SK, Telkar S (2013) 5-Phenyl-1-benzofuran-2-yl derivatives: synthesis, antimicrobial, and antioxidant activity. Med Chem Res 22:78–87

Dhorajiya BD, Bhakhar BS, Dholakiya BZ (2012) Synthesis, characterization, solvato chromic properties and antimicrobial evaluation of 5-acetyl-2-thioxo-dihydropyrimidine-4, 6-dione-based chalcones. Med Chem Res 22:4075–4086. doi:10.1007/s00044-012-0395-1

El-Gaby MSA, Abdel-Gawad SM, Ghorab MM, Heiba HI, Aly HM (2006) Synthesis and biological activity of some novel thieno[2,3-*b*]quinoline, quinolino[3,2:4,5]thieno[3,2-*d*]pyrimidine and pyrido[2,3:4,5]thieno[2,3-*b*]quinoline derivatives. Phosphorus, Sulfur Silicon Relat Elem 181(2):279–297

Elmastaş M, Gülçin I, Işildak Ö, Küfrevioğlu ÖI, Ibaoğlu K, Aboul-Enein HY (2006) Radical scavenging activity and antioxidant capacity of bay leaf extracts. J Iran Chem Soc 3:258–266

Francesca SL (2011) Anti-microbial properties of *Scutellaria baicalensis* and *Coptis chinensis*, two traditional Chinese medicines. Biosci Horizons 1:119–127

Ghelardoni M, Del SP, Meli A, Pestellini V (1981) US Patent 4,296,124 Chem Abstr 95:1847720

Grange JM, Zumla A (2002) The global emergency of tuberculosis: what is the cause? J R Soc Promot Health 122:78–81

Gülcin I, Beydemir S, Alici HA, Elmastas M, Büyükokuřolu ME (2004) In vitro antioxidant properties of morphine. Pharmacol Res 49:59–66

Guzeldemirci NU, Kucukbasmacı O (2010) Synthesis and antimicrobial activity evaluation of new 1,2,4-triazoles and 1,3,4-thiadiazoles bearing imidazo[2,1-*b*]thiazole moiety. Eur J Med Chem 45:63–68

Harriman GC, Brewer M, Bennet R, Kuhn C, Luly JR, Gallant D, Cochran N, Skeriker P, Briskin MJ (2008) Selective cell adhesion inhibitors: barbituric acid based α4β7-MAd CAM inhibitors. Bioorg Med Chem 18:2509–2512

Hatano T, Edamatsu R, Mori A, FujitaY Y, Yasuhara E (1989) Effect of interaction of tannins with co-existing substances, VI: effects of tannins and related polyphones on superoxide anion radical and on DPPH radical. Chem Pharm Bull 37:2016–2021

Holy A, Votruba I, Masojidkova M, Andrei G, Snoeck R, Naesens L, De Clercq E, Balzarini J (2002) 6-[2-(Phosphonomethoxy)alkoxy]pyrimidines with antiviral activity. J Med Chem 45:1918–1929

Jiang X, Liu W, Zhang W, Jiang F, Gao Z, Zhuang H, Lei F (2011) Synthesis and antimicrobial evaluation of new benzofuran derivatives. Eur J Med Chem 46:3526–3530

Karatas F, Koca M, Kara H, Servi S (2006) Synthesis and oxidant properties of novel (5-bromobenzofuran-2-yl) (3-methyl-3-mesitylcyclobutyl) ketone thiosemicarbazone. Eur J Med Chem 41:664–669

Kenchappa R, Bodke YD, Peethambar SK, Telkar S, Venkatesh KB (2013a) Synthesis of β-amino carbonyl derivatives of coumarin and benzofuran and evaluation of their biological activity. Med Chem Res 22:4787–4797

Kenchappa R, BodkeYD, Chandrashekar A, Sandeep T, Manjunatha KS, Aruna Sindhe M (2013) Synthesis of some 2,6-bis(1-coumarin-2-yl)-4-(4-substituted-phenyl) pyridine derivatives as potent biological agents. Arab J Chem. doi:10.1016/j.arabjc.2013.03.020

Kirilmis C, Ahmedzade M, Servi S, Koca M, Kizirgil A, Kazaz C (2008) Synthesis and antimicrobial activity of some novel derivatives of benzofuran: part 2. The synthesis and antimicrobial activity of some novel 1-(1-benzofuran-2-yl)-2-mesitylethanone derivatives. Eur J Med Chem 43:300–308

Koca M, Servi S, Kirilmis, Ahmedzade M, Kazaz C, Özbek B, Ötük G (2005) Synthesis and antimicrobial activity of some novel derivatives of benzofuran: part 1. Synthesis and antimicrobial activity of (benzofuran-2-yl) (3-phenyl-3-methylcyclobutyl) ketoxime derivatives. Eur J Med Chem 40:1351–1358

Liu J, Jiang F, Jiang X, Zhang W, Liu J, Liu W, Lei F (2012) Synthesis and antimicrobial evaluation of 3-methanone-6-substituted benzofuran derivatives. Eur J Med Chem 54:879–886

Lopez SN, Castelli MV, ZacchinoS A, Domı′nguez JN, Lobo G, Charris JC, Corte′s JCG, Ribas JC, Devia C, Rodrı′guezd AM, Enrizd RD (2001) In vitro antifungal evaluation and structure–activity relationships of a new series of chalcone derivatives and synthetic analogues with inhibitory properties against polymers of the fungal cell wall. Bioorg Med Chem 9:1999–2013

Mahboobi S, Sellmer A, Höcher H, Garhammer C, Pongratz H, Maier T, Ciossek T, Beckers T (2007) 2-Aroylindoles and 2-aroylbenzofurans with *N*-hydroxyacrylamide substructures as a novel series of rationally designed histone deacetylase inhibitors. J Med Chem 50:4405–4418

Manna K, Agrawal YK (2010) Design, synthesis, and antitubercular evaluation of novel series of 3-benzofuran-5-aryl-1-pyrazolylpyridylmethanone and 3-benzofuran-5-aryl-1-pyrazolylcarbonyl-4-*oxo*-naphthyridin analogs. Eur J Med Chem 45:3831–3839

Manna K, Agrawal YK (2011) Potent in vitro and in vivo antitubercular activity of certain newly synthesized indophenazine 1,3,5-trisubstituted pyrazoline derivatives bearing benzofuran. Med Chem Res 20:300–306

Mathew B, Suresh J, Vinod D (2012) Antitumor activity of 5-[(2*E*)-1-(1*H*-benzimidazol-2-yl)-3-substituted-phenylprop-2-en-1-ylidene] pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones against Dalton's ascetic lymphoma in mice. Med Chem Res 22:3911–3917. doi:10.1007/s00044-012-0407-1

Moskvin AV, Reznikova NR, Ivin BA (2002) Condensation of hydroxypyrimidines with carbonyl compounds: I. Barbituric acids. Russ J Org Chem 38(4):463–3474

Nasser Khalil SAM (2010) Efficient synthesis of novel 1,2,4-triazole fused cyclic and 21–28 membered macrocylic and/or lariat macrocyclic oxaazathia crown compounds with potential antimicrobial activity. Eur J Med Chem 45:5265–5277

National Committee for Clinical Laboratory Standards (NCCLS) (1982) Standard methods for dilution antimicrobial susceptibility tests for bacteria, which grows aerobically. NCCLS Ltd., Villanova

Nevcihan G, Cengiz S, Bektas T, Halil SM (2010) Evaluation of antioxidant activities of three edible mushrooms; *Ramaria flava*, *Rhizopogan roseolus* and *Russula delica*. Food Sci Biotech 19(3):691–696

Pannala AS, Chan TS, O'Brien PJ, Rice-Evans CA (2001) Flavonoid B-ring chemistry and antioxidant activity: fast reaction kinetics. Biochem Biophys Res Commun 282:1161–1168

Parekh S, Bhavsar D, Savant M, Thakrar S, Bavishi A, Parmar M, Vala H, Radadiya A, Pandya N, Serly J, Molnár J, Shah A (2011) Synthesis of some novel benzofuran-2-yl(4,5-dihyro-3,5-substituted diphenylpyrazol-1-yl) methanones and studies on the antiproliferative effects and reversal of multidrug resistance of human MDR1-gene transfected mouse lymphoma cells in vitro 2-acetylbenzofurans: synthesis reactions and applications. Eur J Med Chem 46:1942–1948

Rajanarendar E, Govardhan Reddy K, Krishna SR, Shireesha B, Reddy YN, Rajam MV (2013) Design, synthesis, antimicrobial, anti-inflammatory, and analgesic activity of Novel dihydrobenzofuro[3,2-*e*]isoxazolo [4,5-*b*]azepin-5(5*aH*)-ones. Med Chem Res 22:6143–6153. doi:10.1007/s00044-013-0598-0

Reddy CS, Nagaraj A (2007) Knoevenagel condensation of α,β-unsaturated aromatic aldehydes with barbituric acid under non-catalytic and solvent-free condition. Chin Chem Lett 18:1431–1435

Rezende MC, Campodonico P, Abuin E, Kossanyi J (2001) Merocyanine-type dyes from barbituric acid derivatives. Spectrochem Acta A 57:1183–1190

Saberi MR, Vinh TK, Yee WS, Griffiths BJN, Evans PJ, Simons C (2006) Potent CYP19 (aromatase) 1-[(benzofuran-2-yl) (phenyl methyl) pyridine, -imidazole, and -triazole inhibitors: synthesis and biological evaluation. J Med Chem 49:1016–1022

Sangani HG, Bhimani KB, Khunt RC (2006) Synthesis and characterization of barbitones as antimicrobial agents. J Serb Chem Soc 71:587–591

Sawa T, Nakao M, Akaike T, Ono K, Maeda H (1999) Alkyl peroxyl radical scavenging activity of various flavonoids and other phenolic compounds: implications for the antitumor promoter effect of vegetables. J Agric Food Chem 47:397–402

Sharma P, Rane N, Gurram VK (2004) Synthesis and QSAR studies of pyrimido[4,5-*d*]pyrimidine-2,5-dione derivatives as potential antimicrobial agents. Bioorg Med Chem Lett 14:4185–4190

Sheelavanth S, Bodke YD, Sundar SM (2013) Synthesis, antioxidant, and antibacterial studies of phenolic esters and amides of 2-(1-benzofuran-2-yl) quinoline-4-carboxylic acid. Med Chem Res 22:1163–1171

Singh BN, Vaughan WEM (1970) The effect of amiodarone, a new anti-anginal drug, on cardiac muscle. Br J Pharmacol 39:657–667

Stefania FB, Gabriel S, Gabriela LA, Constantin D, Florica B, Gabriela B (2012) New heterocyclic compounds from 1,2,4-triazole and 1,3,4-thiadiazole class bearing diphenyl sulfone moieties. Synthesis, characterization and antimicrobial activity evaluation. Eur J Med Chem 49:417–423

Stille JR, Ward JA, Leffelman C, Sullivan KA (1996) 5-Formyl salicylaldehyde as a linker for the synthesis of benzofuran containing insulin sensitivity enhancer compounds. Tetrahedron Lett 37(52):9267–9270

Tomasz P, Monika W, Agata S, Urszula K, Anna M (2011) Synthesis and antimicrobial activity of thiosemicarbazides, *s*-triazoles and their Mannich bases bearing 3-chlorophenyl moiety. Eur J Med Chem 46:241–248

Venkatesh KB, Bodke YD, Biradar SA (2010a) Synthesis of 3-[4-(1-Benzofuran-2-yl)-1,3-thiazol-2-yl]-2-(4-aryl)-1,3-thiazolidin-4-one derivatives as biological agents. Phosphorus, Sulfur Silicon Relat Elem 185:1926–1931

Venkatesh KB, Bodke YD, Biradar S, Kumara Swamy BE, Umesh S (2010b) A facile synthesis of bromo-substituted benzofuran containing thiazolidinone nucleus bridged with quinoline derivatives potent analgesic and antimicrobial agents. Phosphorus, Sulfur Silicon Relat Elem 185:110–116

Vijaya Laxmi S, Thirupathi Reddy Y, Suresh Kuarm B, Narsimha Reddy P, Crooks PA, Rajitha B (2011) Synthesis and evaluation of chromenyl barbiturates and thiobarbiturates as potential antitubercular agents. Bioorg Med Chem Lett 21:4329–4331

Vijaya Laxmi S, Suresh Kuarm B, Rajitha B (2012) Synthesis and antimicrobial activity of Coumarin pyrazole pyrimidine 2,4,6(1*H*,3*H*,5*H*)triones and thioxopyrimidine-4,6(1*H*,5*H*)diones. Med Chem Res 22:768–774. doi:10.1007/s00044-012-0078-y

Wallace AC, Laskowski RA, Thornton JM (1995) LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Prot Eng 8:127–134

Wu J, Li Y, Chen K, Jiang H, Ming-Hua X, Liu D (2013) Identification of benzofuran-3-yl(phenyl)methanones as novel SIRT1 inhibitors: binding mode, inhibitory mechanism and biological action. Eur J Med Chem 60:441–450