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Synthesis and structure–antibacterial activity relationship studies of 4-substituted phenyl-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(2H)-thiones

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Abstract The synthesis and characterization of a series of 4-substituted phenyl-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(2H)-thiones were presented. Preliminary in vitro antimicrobial activity of the compounds was assessed against a panel of microorganisms including *S. aureus*, *E. faecalis*, *P. aeruginosa*, *E. coli*, and *C. albicans*. Some of the compounds exhibited significantly in vitro antimicrobial activity. The *p*MIC values were correlated with physicochemical descriptors: Hammett substituent constants (σ_m and σ_p) and the lipophilic constant (π). One statistical significant 2D-QSAR model was obtained with *para*-substituted compounds. The *p*MIC values were also correlated with some theoretical descriptors as independent variables and four statistical significant 2D-QSAR models were also obtained with *meta*-substituted compounds.

Keywords Benzoxazepine derivatives · Antimicrobial activity · QSAR

Introduction

The rapid development of drug resistance, the unsatisfactory status of present treatments of bacterial and fungal infections and the drug side effects limit the usage of most antimicrobial agents. Hence, the synthesis of new and effective antimicrobial drugs is a very important objective and many research programs have been directed toward the design of new agents.

Oxazepine derivatives have been attracting much interest due to the wide range of biological activities. Among these activities, it is worth mentioning antithrombotic (Mishra *et al.*, 2010), antiepileptic (Pekcec *et al.*, 2009), anticonvulsant (Sharma *et al.*, 2008), antiinflammatory (Schridhar *et al.*, 1979; Verma *et al.*, 2008), progesterone agonist (Dols *et al.*, 2008), antifungal (Serrano-Wu *et al.*, 2002), antagonist and analgesic (Okada *et al.*, 1994; Hallinan *et al.*, 1994), antipsychotic (Liegeois *et al.*, 1994), anxiolytics (Effland *et al.*, 1982), antihistaminic (Sleevi *et al.*, 1991), antiaggregating (Aono *et al.*, 1991), and epidermal growth factor receptor(EGFR) tyrosine kinase inhibitory (Smith *et al.*, 2006) activities. Considering the structural characteristics of the benzoxazepine-3-ones, the existence of seven-membered heterocyclic ring system, fused aromatic group and the group –N–C(=O)–, similar to protein amide bond, it is reasonable to expect inherent physiological activities. Therefore, the study of substituent effects on antimicrobial activity of these compounds can give better understanding of their structure–activity relationships.

In this research, we synthesized 4-substituted phenyl-4,5-dihydrobenzo[*f*][1,4] oxazepin-3(2H)-thiones (Scheme 1) to determine their antimicrobial activity against some bacteria and fungi and to observe the substituent effects on the activity. We also applied 2D-QSAR analysis to see the relations of the molecular descriptors with the activity.

Synthesis

The synthesis of compounds (**2**–**6**) was carried out as illustrated in Scheme 1. 2-[(E)-(substituted phenylimino)

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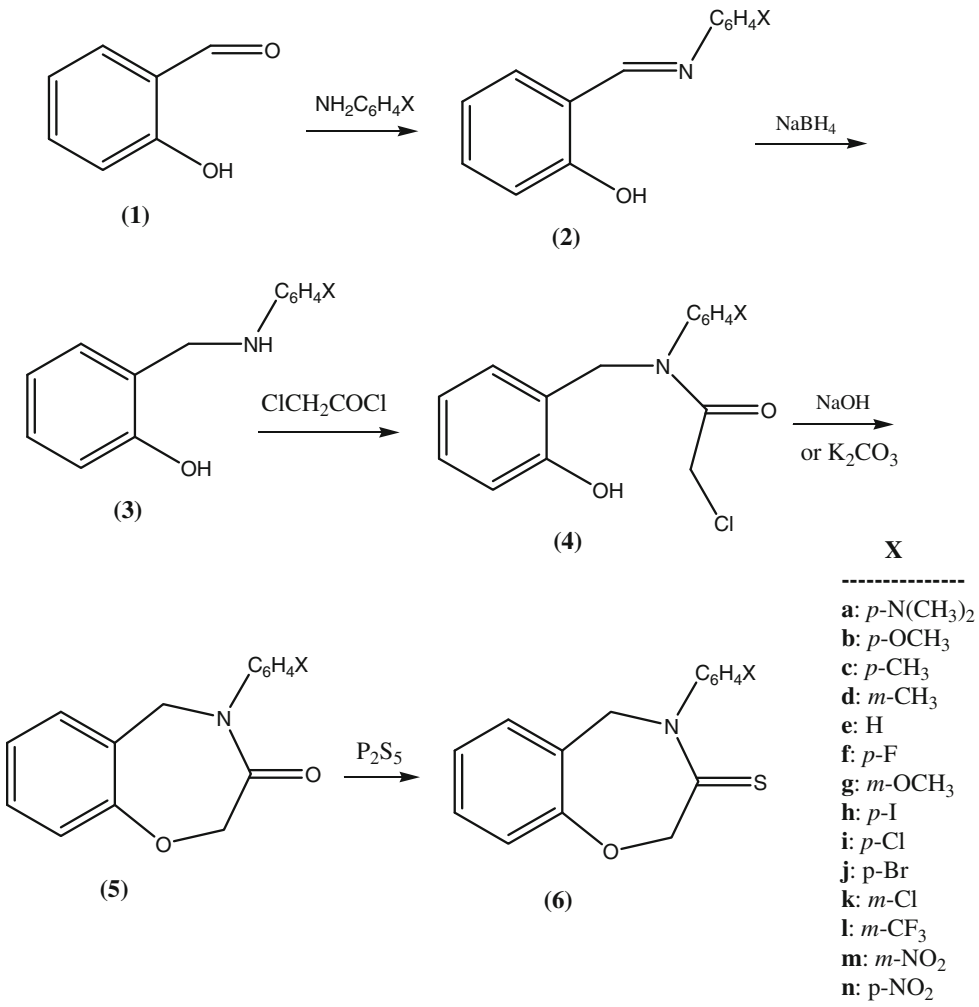
Scheme 1 Synthesis of 4-substituted phenyl-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(2H)-thiones



methyl]phenols (**2a**–**n**) were obtained from the reaction of salicylaldehyde (**1**) with substituted anilines. Then the imines (**2**) were reduced by $NaBH_4$ to give 2-((substituted phenylamino)methyl)phenols (**3a**–**n**) which were reacted with chloroacetyl chloride to have the corresponding amides (**4a**–**n**). 4-Substituted phenyl-4,5-dihydrobenzo[*f*][1,4] oxazepin-3(*2H*)-ones (**5a**–**n**) were obtained in quantitative yields under the basic treatment of the amides. Compounds (**5a**–**n**) were treated with P_2S_5 to give corresponding 4-substituted phenyl-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(*2H*)-thiones (**6a**–**n**). All the compounds obtained in this study were analyzed by their IR (Agirbas *et al.*, 2009) and ^{1}H NMR spectra and representative compounds (**6a**–**n**) by elemental analysis.

Biological Activity

Fourteen of the new synthesized compounds (**6a**–**n**) were evaluated for their in vitro antimicrobial activity against a panel of microorganisms including *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans* by determining their minimal inhibitory concentrations (MIC) by broth microdilution susceptibility tests (CLSI, 2002, 2006). The biological activity results of the compounds are given in Table 1. Compound (**6j**) was found to be the most active derivative against *E. faecalis* at MIC value of 12.5 μg/ml among the tested compounds. All the compounds showed antimicrobial activity with MIC values between 25 and 100 μg/ml against *E. faecalis* and *P. aeruginosa.* Most active compounds are **6f**, **6g**, **6h**, **6j**, **6l**, and **6m** against *E. coli* with the activity of 100 μg/ml. Compounds **6g** and **6j** exhibited highest activity (MIC: 100 μg/ml) against *C. albicans.* All benzoxazepines (**6a**–**n**) displayed low activity against *S. aureus*.

QSAR analysis

We have performed linear regression studies for molecular descriptors with the antimicrobial activity of compounds

Table 1 Antimicrobial activities of compounds(**6a–n**)

Compound (substituent)	Antibacterial and antifungal activities, μg/ml (μmol/ml)				
	S. aureus ATCC25983	*E. faecalis* ATCC 29212	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922	*C. albicans* ATCC 90028
Ampicillin	0.78	0.78	–	6.25	–
Ciprofloxacin	0.25	0.25	0.25	0.04	–
6a (*p*-NMe_2)	400 (1.34)	25 (0.08)	100 (0.34)	200 (0.67)	800 (2.68)
6b (*p*-OMe)	400 (1.40)	50 (0.18)	100 (0.35)	200 (0.70)	800 (2.80)
6c (*p*-Me)	200 (0.74)	25 (0.09)	25 (0.09)	200 (0.74)	200 (0.74)
6d (*m*-Me)	800 (2.97)	50 (0.19)	100 (0.37)	400 (1.49)	800 (2.98)
6e (H)	800 (3.13)	100 (0.39)	100 (0.39)	200 (0.78)	800 (3.13)
6f (*p*-F)	800 (2.93)	50 (0.18)	100 (0.37)	100 (0.37)	400 (1.46)
6g (*m*-OMe)	200 (0.70)	25 (0.09)	50 (0.18)	100 (0.35)	100 (0.35)
6h (*p*-I)	400 (1.05)	50 (0.13)	50 (0.13)	100 (0.26)	800 (2.1)
6i (*p*-Cl)	400 (1.38)	25 (0.09)	100 (0.35)	400 (1.38)	800 (2.76)
6j (*p*-Br)	100 (0.30)	12.5 (0.04)	25 (0.08)	100 (0.30)	100 (0.30)
6k (*m*-Cl)	400 (1.38)	100 (0.35)	200(0.69)	200 (0.69)	800 (2.76)
6l (*m*-CF_3)	400 (1.24)	25 (0.08)	50 (0.15)	100 (0.31)	400 (1.24)
6m (*m*-NO_2)	200 (0.67)	25 (0.08)	25 (0.08)	100 (0.33)	200 (0.67)
6n (*p*-NO_2)	>1600 (5.33)	50 (0.17)	50 (0.17)	200 (0.67)	800 (2.66)
Fluconazole	–	–	–	–	0.25

(**6a–n**). Biological activity data, reported as MIC values (Table 1), are transformed to *p*MIC(−logMIC) on a molar basis used as dependent variables to obtain the linear relationship. The *p*MIC values were first correlated with physicochemical descriptors: Hammett substituent constants (σ_m and σ_p) (Hansch *et al.*, 1991) and the lipophilic constant (π) (Hansch *et al.*, 1973). Non-statistical significant correlations were obtained when the descriptors were studied as independent variables. However, only one statistical significant 2D-QSAR model was obtained (Table 2, Eq. 1) with *para*-substituted compounds.

In order to include theoretical descriptors to the SAR study, a geometry of all the compounds (**6a–n**) has been completely optimized by ab initio (RHF/3-21G) method incorporated in the Hyperchem package (HyperChem, 2002). Surface area (SA), molecular volume(MV), molar refractivity(MR), polarizability (polar), magnitude of dipolar moment (μ), and the calculated log of octanol–water partition coefficient (clogP) of the compounds were also computed by Hyperchem software (Table 3).

Energies of highest occupied molecular orbital (E_{HOMO}) and lowest unoccupied molecular orbital (E_{LUMO}) were calculated using Gaussian 03W program package (Frisch *et al.*, 2004) by means of DFT (B3LYP) with the 6-311G(d,p) basis set (Table 3). Again, non-statistical significant correlations were obtained when these theoretical descriptors were studied as independent variables, but four statistical significant 2D-QSAR models were also obtained (Table 2, Eq. 2–5). The overall quality of the obtained 2D-QSAR models was indicated by the correlation coefficients (r and r^2), the standard deviation (s) of the regression equation, F value (F-statistical analysis; Fischer

Table 2 Significant 2D-QSAR models obtained for antimicrobial activity of 4-substituted phenyl-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(2H)-thiones (**6a–n**)

Equations	Regression equation	Statistic parameter						
		n	*r*	r^2	r^2 adj	*s*	*p*	*F*
1	$pMIC_{S.a.} = 0.6025(\pm 0.18)\pi - 0.24(\pm 0.19)\sigma_p - 0.38(\pm 0.10)$	9	0.817	0.668	0.557	0.224	0.036	6.040
2	$pMIC_{S.a.} = -6.36(\pm 4.54)E_{LUMO} + 0.21(\pm 0.12)$Polar $- 7.26(\pm 3.62)$	6[a]	0.819	0.671	0.452	0.215	0.189	3.061
3	$pMIC_{S.a.} = -4.85(\pm 4.58)E_{LUMO} + 0.01(\pm 0.01)$SA $- 4.24(\pm 1.91)$	6[a]	0.829	0.687	0.479	0.209	0.175	3.299
4	$pMIC_{P.a} = -8.65(\pm 5.10)E_{LUMO} + 0.01(\pm 0.01)$SA $- 3.38(\pm 2.12)$	6[a]	0.846	0.716	0.527	0.233	0.151	3.790
5	$pMIC_{S.a} = -22.94(\pm 15.76)E_{HOMO} - 0.33(\pm 0.16)$clogP $- 4.85(\pm 3.36)$	6[a]	0.822	0.677	0.462	0.212	0.183	3.147

[a] Only *meta*-substituted compounds

Table 3 Theoretical descriptors of 4-substituted phenyl-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(2H)-thiones **6a–n** used for the regression analyses

Compounds	clogP	SA (Å^3)	MV (Å^3)	MR (Å^3)	Polar (Å^3)	E_{HOMO} (au)	E_{LUMO} (au)	μ
6a	−0.15	320.98	276.37	99.32	35.08	−0.1967	−0.0417	6.91
6b	−0.19	292.71	255.32	92.07	32.53	−0.2064	−0.0464	5.94
6c	0.96	280.21	247.45	89.98	31.89	−0.2080	−0.0472	6.23
6d	0.96	280.17	247.54	89.98	31.89	−0.2086	−0.0476	6.05
6e	0.80	256.53	231.01	85.70	30.06	−0.2098	−0.0488	6.19
6f	0.20	261.77	233.36	85.83	29.37	−0.2137	−0.0528	6.31
6g	−0.19	292.62	255.36	92.07	32.53	−0.2071	−0.0459	6.33
6h	1.32	285.31	260.66	98.02	35.09	–	–	6.27
6i	0.58	273.93	245.90	90.42	31.99	−0.2155	−0.0545	6.62
6j	0.85	278.33	252.95	93.23	32.68	−0.2153	−0.0543	6.27
6k	0.58	273.85	245.98	90.42	31.99	−0.2156	−0.0544	6.48
6l	1.37	292.64	254.50	90.91	31.62	−0.2180	−0.0566	6.99
6m	−0.01	288.33	249.71	91.92	31.90	−0.2224	−0.1019	8.12
6n	−0.01	288.34	249.59	91.92	31.90	−0.2243	−0.1027	8.08

test) and the number of data points (n). The predictability of each model was assessed using the cross-validated correlation coefficient (r^2 adj). For the structure-reactivity models, a value of r^2 adj above 0.45 was considered.

In order to evaluate the predictive power of the models, the data was split into the training, and test sets. The regression equations of the training sets gave good coefficient of determination (r^2) and internal cross-validation (r^2 adj) values (Table 4). A fair predicted values of the test sets were also obtained.

Equation 1 (Table 2) may suggest that the resonance effect, which is shown by *para* substituents, reveals to have more influence on the 2D correlation with the electronic and lipophilic descriptors against *S. aureus*. On the other hand, the *meta*-substituted aromatic rings of the compounds could better fit into the pocket in the receptor which should contain polar groups that interact effectively with the *meta* substituents. This may possibly be the reason to get Eqs. 2–5 (Table 2).

The correlation matrix for the descriptors was performed and no cross-relations between the descriptors used in each equation were obtained. Thus, these parameters are orthogonal and allowing its safe use in the multilinear regression relationship (Myers, 1987 and Draper and Smith, 1981). Squared correlation matrix of theoretical descriptors used in the equations is given in Table 5. All the statistical calculations were performed by means of the SigmaPlot program package.

Table 4 The results of the application of training and test sets to 2D-QSAR models (Eqs. 1–5 in Table 2)

Equations	Training set	Regression equation of training set	Test set (MIC)	Predicted value	Statistic parameter		
					r^2	r^2 adj	s
1	**6a**, **6c**, **6e** **6j**, **6n**	$p\text{MIC}_{S.a.} = 1.05\pi - 0.61\sigma_p - 0.42$	**6b** (400)	747	0.986	0.972	0.081
			6f (800)	512			
			6h (400)	1519			
			6i (400)	590			
2	**6d**, **6e**, **6k**, **6m**	$p\text{MIC}_{S.a.} = -9.82E_{LUMO} + 0.09\text{Polar} - 3.80$	**6g** (200)	136	0.891	0.672	0.181
			6l (400)	161			
3	**6d**, **6e**, **6k**, **6m**	$p\text{MIC}_{S.a.} = -9.68E_{LUMO} + 0.004\text{SA} - 1.90$	**6g** (200)	150	0.838	0.515	0.219
			6l (400)	215			
4	**6d**, **6e**, **6k**, **6m**	$p\text{MIC}_{P.a} = -14.09E_{LUMO} + 0.0003\text{SA} - 0.27$	**6g** (200)	561	0.819	0.456	0.296
			6l (400)	899			
5	**6d**, **6e**, **6k**, **6m**	$p\text{MIC}_{S.a} = -75.55E_{HOMO} - 0.33\text{clogP} - 16.64$	**6g** (200)	24	0.993	0.979	0.046
			6l (400)	755			

r^2 coefficient of determination, r^2 adj internal cross-validation, s the standard deviation

Table 5 Squared correlation matrix of the theoretical descriptors used in the QSAR study

E_{LUMO}	1				
Surface area	0.08	1			
clogP	0.14	0.06	1		
Polarizability	0.02	0.67	0.19	1	
E_{HOMO}	0.70	0.08	0.0008	0.005	1
r^2	E_{LUMO}	Surface area	clogP	Polarizability	E_{HOMO}

Conclusion

In conclusion, a series of 14 new 4-substituted phenyl-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(2H)-thiones (**6**) were synthesized from corresponding ones (**5**). The obtained compounds have a great interest because there have been no reports on the antimicrobial studies of these thiones in the literature. In this study, the in vitro antimicrobial activity of compounds (**6**) exhibited good results against *P. aeruginosa*, one of the species that show the most dramatic resistance problems related to nosocomial infections and multiresistant strains (Kiska *et al*., 1999). Moreover, quantitative structure–activity relationship studies allowed to draw the following conclusion about the antimicrobial activity of the synthesized thiones: (i) only *para*-substituted thiones (**6**) showed a fair 2D correlation with the electronic (σ_p) and lipophilic (π) descriptors against *S. aureus*; (ii) *meta*-substituted thiones (**6**) gave fair 2D correlations with some theoretical descriptors against *S. aureus* and *P. aeruginosa*. The QSAR study has provided key information regarding the structure of 4-substituted phenyl-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(2*H*)-thiones which we believe will help to design more potent antimicrobial compounds.

Experimental

Melting points were determined on Electrothermal 9200 apparatus and are uncorrected. The FTIR spectra were recorded using Shimadzu 8201 spectrometer with KBr technique, in the region 4000–400 cm^{-1} that was calibrated by polystyrene. ^1H NMR spectra were recorded on Bruker DPX-400 (400 MHz) High Performance Digital FT-NMR Spectrometer using $CDCl_3$ with Me_4Si as an internal standard. Silica Gel (Fluka or Merck) were used for column chromatography.

Schiff base (**2**) (Scheme 1) were synthesized according to the literature (Vogel, 1972). A band for the azomethine group was observed in IR spectrum at about 1620 cm^{-1}. For the synthesis of compounds (**3**–**5**), literature methods (Derieg and Sternbach, 1966; Davion *et al.*, 2004) were applied with slight modifications.

Synthesis of 2-((phenylamino)methyl)phenol (**3e**) (general procedure)

2-[(E)-(phenylimino)methyl]phenol (**2e**) (25 mmol, 5 g) was dissolved in 100 ml of methanol and dioxan at 1:1 ratio. $NaBH_4$ (25 mmol, 0.8 g) was added to the stirred solution until the yellow color of the Schiff base disappeared (1 h). Cold water was added to the solution to give precipitate. The precipitate was recrystallized from methanol to give 2-((phenylamino)methyl)phenol (**3e**) (4.25 g, 80%). Mp: 112–113°C. IR(KBr), ν (cm^{-1}): 3265 (N–H). ^1H NMR ($CDCl_3$), δ (ppm): 3.89 (s, 1H, NH); 4.36 (s, 2H, CH_2–NH); 6.75–6.81 (m, aromatic, 2H); 6.84–6.97 (m, aromatic, 4H); 7.12–7.15 (m, aromatic, 2H); 7.19–7.24 (m, aromatic, 1H); 8.55 (s, 1H, OH).

*Spectroscopic and analytical data of compounds (**3**)*

*2-((4-Dimethyaminophenylamino)methyl)phenol (**3a**)* Yield: 62%; mp: 74–75°C; IR (KBr), ν (cm^{-1}): 3329 (N–H); ^1H NMR ($CDCl_3$), δ (ppm): 2.86 (s, 6H, $N(CH_3)_2$); 3.47 (s, 1H, NH); 4.35 (s, 2H, CH_2–NH); 6.69–6.82 (m, aromatic, 2H); 6.81–6.90 (m, aromatic, 4H); 7.08–7.11 (m, aromatic, 1H); 7.17–7.29 (m, aromatic, 1H); 8.61 (s, 1H, OH).

*2-((4-Methoxyphenylamino)methyl)phenol (**3b**)* Yield: 60%; mp: 128–129°C; IR (KBr), ν (cm^{-1}): 3254 (N–H); ^1H NMR ($CDCl_3$), δ (ppm): 3.76 (s, 3H, OCH_3); 4.37 (s, 2H, CH_2–NH); 6.81-6.83 (m, aromatic, 4H); 6.85–6.90 (m, aromatic, 2H); 7.10–7.13 (m, aromatic, 1H); 7.18–7.21 (m, aromatic, 1H).

*2-((4-Methylphenylamino)methyl)phenol (**3c**)* Yield: 70%; mp: 122–124°C; IR (KBr), ν (cm^{-1}): 3261.74 (N–H); ^1H NMR ($CDCl_3$), δ (ppm): 2.26 (s, 3H, CH_3); 4.34 (s, 2H, CH_2–NH); 6.71-6.75 (m, aromatic, 2H); 6.82–6.88 (m, aromatic, 2H); 7.02–7.04 (m, aromatic, 2H); 7.09–7.12 (m, aromatic, 1H); 7.16–7.22 (m, aromatic, 1H).

*2-((3-Methylphenylamino)methyl)phenol (**3d**)* Yield: 64%; mp: 113–115°C; IR (KBr), ν (cm^{-1}): 3267.52 (N–H); ^1H NMR ($CDCl_3$), δ (ppm): 2.30 (s, 3H, CH_3); 3.87 (s, 1H, NH); 4.39 (s, 2H, CH_2–NH); 6.63–6.66 (m, aromatic, 2H); 6.72–6.75 (m, aromatic, 1H); 6.84–6.90 (m, aromatic, 2H); 7.10–7.15 (m, aromatic, 1H); 7.19–7.24; 8.48 (s, 1H, OH).

*2-((4-Fluorophenylamino)methyl)phenol (**3f**)* Yield: 60%; mp: 122–123°C; IR (KBr),ν (cm^{-1}): 3259.81 (N–H); ^1H NMR ($CDCl_3$), δ (ppm): 3.89 (s, 1H, NH); 4.36 (s, 2H, CH_2–NH); 6.75-6.81 (m, aromatic, 2H); 6.84–6.97 (m, aromatic, 4H); 7.12–7.24 (m, aromatic, 2H); 8.55 (s, 1H, OH).

*2-((3-Methoxyphenylamino)methyl)phenol (**3g**)* Yield: 68%; mp: 68–69°C; IR (KBr), *v* (cm^{-1}): 3269.45 (N–H); ^1H NMR ($CDCl_3$), δ (ppm): 3.74 (s, 3H, OCH_3); 3.95 (s, 1H, NH); 4.37 (s, 2H, CH_2–NH); 6.37-6.47 (m, aromatic, 3H); 6.84–6.89 (m, aromatic, 2H); 7.11–7.24 (m, aromatic, 3H); 8.24 (s, 1H, OH).

*2-((4-Iodophenylamino)methyl)phenol (**3h**)* Yield: 66%; mp: 130–132°C; IR (KBr), *v* (cm^{-1}): 3255.95 (N–H); ^1H NMR ($CDCl_3$), δ (ppm): 4.00 (s, 1H, NH); 4.36 (s, 2H, CH_2–NH); 6.56–6.61 (m, aromatic, 2H); 6.87–6.92 (m, aromatic, 2H); 7.15–7.25 (m, aromatic, 2H); 7.47–7.52 (m, aromatic, 2H); 7.82 (s, 1H, OH).

*2-((4-Chlorophenylamino)methyl)phenol (**3i**)* Yield: 70%; mp: 122–123°C; IR (KBr), *v* (cm^{-1}): 3257.88 (N–H); ^1H NMR ($CDCl_3$), δ (ppm): 3.97 (s, 1H, NH); 4.36 (s, 2H, CH_2–NH); 6.71–6.76 (m, aromatic, 2H); 6.85–6.91 (m, aromatic, 2H); 7.13–7.24 (m, aromatic, 4H); 8.00 (s, 1H, OH).

*2-((4-Bromophenylamino)methyl)phenol (**3j**)* Yield: 67%; mp: 125–127°C; IR (KBr), *v* (cm^{-1}): 3257.88 (N–H); ^1H NMR ($CDCl_3$), δ (ppm): 3.98 (s, 1H, NH); 4.37 (s, 2H, CH_2–NH); 6.68–6.73 (m, aromatic, 2H); 6.87–6.92 (m, aromatic, 2H); 7.15–7.25 (m, aromatic, 2H); 7.30–7.35 (m, aromatic, 2H); 7.90 (s, 1H, OH).

*2-((3-Chlorophenylamino)methyl)phenol (**3k**)* Yield: 63%; mp: 112–114°C; IR (KBr), *v* (cm^{-1}): 3259.81 (N–H); ^1H NMR ($CDCl_3$), δ (ppm): 4.01 (s, 1H, NH); 4.36 (s, 2H, CH_2–NH); 6.65–6.69 (m, aromatic, 1H); 6.79–6.80 (m, aromatic, 1H); 6.83–6.92 (m, aromatic, 3H); 7.10–7.25 (m, aromatic, 3H); 7.62 (s, 1H, OH).

*2-((3-Trifluorophenylamino)methyl)phenol (**3l**)* Yield: 76%; mp: 80–82°C; IR (KBr), *v* (cm^{-1}): 3269.45 (N–H); ^1H NMR ($CDCl_3$), δ (ppm): 4.15 (s, 1H, NH); 4.41 (s, 2H, CH_2–NH); 6.72–6.85 (m, aromatic, 1H); 6.88–6.97 (m, aromatic, 2H); 7.03–7.08 (m, aromatic, 1H); 7.10–7.13 (m, aromatic, 1H); 7.18–7.26 (m, aromatic, 2H); 7.30–7.35 (m, aromatic, 1H); 7.47 (s, 1H, OH).

*2-((3-Nitrophenylamino)methyl)phenol (**3m**)* Yield: 97%; mp: 117–118°C; IR (KBr), *v* (cm^{-1}): 3263.66 (N–H); ^1H NMR ($CDCl_3$), δ (ppm): 4.37 (s, 1H, NH); 4.44 (s, 2H, CH_2–NH); 6.69–6.82 (m, aromatic, 2H); 6.81–6.90 (m, aromatic, 4H); 7.08–7.11 (m, aromatic, 1H); 7.17–7.29 (m, aromatic, 1H); 8.61 (s, 1H, OH).

*2-((4-Nitrophenylamino)methyl)phenol (**3n**)* Yield: 64%; mp: 135–136°C; IR (KBr), *v* (cm^{-1}): 3365.90 (N–H); ^1H NMR ($CDCl_3$), δ (ppm); 4.46 (s, 2H, CH_2–NH); 4.85 (s, 1H, NH); 6.65–6.68 (m, aromatic, 2H); 6.83–6.86 (m, aromatic, 1H); 6.90–6.96 (m, aromatic, 1H); 7.19–7.25 (m, aromatic, 2H); 8.08–8.11 (m, aromatic, 2H).

Synthesis of 2-Chloro-*N*-(2-hydroxybenzyl)-*N*-phenylacetamide (**4e**) (general procedure)

2-[(E)-(phenylamino)methyl]phenol (**3e**) (20 mmol, 4 g) was dissolved in benzene (150 ml). Triethylamine (20 mmol, 2.05 g) was added to the solution. Chloroacetyl chloride (20 mmol, 2.26 g) in benzene (50 ml) was added dropwise and the reaction mixture was stirred for 2 h at room temperature. Then, precipitated triethylammonium chloride salt was filtered off and the filtrate was evaporated under vacuo. The residual oily matter was crystallized from diethyl ether to give 2-chloro-*N*-(2-hydroxybenzyl)-*N*-phenylacetamide (**4e**) (4.82 g, 87%) Mp: 104–105°C. IR (KBr), *v* (cm^{-1}): 3115(OH), 1635(C=O). ^1H NMR ($CDCl_3$), δ (ppm): 3.83 (s, 2H, CH_2–N–Ar); 4.79 (s, 2H, CH_2–Cl); 6.59–6.61 (m, aromatic, 1H); 6.66–6.72 (m, aromatic, 1H); 6.98–7.01 (m, aromatic, 1H); 7.07–7.10 (m, aromatic, 2H); 7.20–7.25 (m, aromatic, 1H); 7.43–7.47 (m, aromatic, 3H); 9.07 (s, 1H, OH).

*Spectroscopic and analytical data of compounds (**4**)*

*2-Chloro-N-(2-hydroxybenzyl)-N-(4-dimethylaminophenyl) acetamide (**4a**)* Yield: 72%; mp: 106–107°C; IR (KBr), *v* (cm^{-1}): 3144.07 (OH), 1627.97 (C=O); ^1H NMR ($CDCl_3$), δ (ppm): 3.00 (s, 6H, $N(CH_3)_2$); 3.87 (s, 2H, CH_2–N–Ar); 4.74 (s, 2H, CH_2–Cl); 6.65–6.71 (m, aromatic, 4H); 6.86–6.89 (m, aromatic, 2H); 6.97–7.00 (m, aromatic, 1H); 7.19–7.25 (m, aromatic, 1H); 9.22 (s, 1H, OH).

*2-Chloro-N-(2-hydroxybenzyl)-N-(4-methoxyphenyl)acetamide (**4b**)* Yield: 79%; mp: 133–134°C; IR (KBr), *v* (cm^{-1}): 3151.79 (OH), 1629.90 (C=O); ^1H NMR ($CDCl_3$), δ (ppm): 3.84 (s, 2H, CH_2–N–Ar); 3.85 (s, 3H, OCH_3); 4.75 (s, 2H, CH_2–Cl); 6.61–6.73 (m, aromatic, 2H); 6.91–7.00 (m, aromatic, 5H); 7.20–7.25 (m, aromatic, 1H); 9.07 (s, 1H, OH).

*2-Chloro-N-(2-hydroxybenzyl)-N-(4-methylphenyl)acetamide (**4c**)* Yield: 59%; mp: 102–104°C; IR (KBr),*v* (cm^{-1}): 3169.15 (OH), 1629.90 (C=O); ^1H NMR ($CDCl_3$), δ (ppm): 2.41 (s, 1H, CH_3); 3.83 (s, 2H, CH_2–N–Ar); 4.77 (s, 2H, CH_2–Cl); 6.61–6.70 (m, aromatic, 2H); 6.94–7.00 (m, aromatic, 3H); 7.22–7.26 (m, aromatic, 3H); 9.10 (s, 1H, OH).

*2-Chloro-N-(2-hydroxybenzyl)-N-(3-methylphenyl)acetamide (**4d**)* Yield: 47%; mp: 102–104°C; IR (KBr), *v* (cm^{-1}): 3126.71 (OH), 1631.83 (C=O); ^1H NMR ($CDCl_3$),

δ (ppm): 2.37 (s, 1H, CH_3) 3.84 (s, 2H, CH_2–N–Ar); 4.78 (s, 2H, CH_2–Cl); 6.61–6.72 (m, aromatic, 2H); 6.83–6.92 (m, aromatic, 2H); 6.98–7.01 (m, aromatic, 1H); 7.20–7.35 (m, aromatic, 3H); 9.12 (s, 1H, OH).

2-Chloro-N-(2-hydroxybenzyl)-N-(4-fluorophenyl)acetamide ***(4f)*** Yield: 45%; mp: 91–93°C; IR (KBr), v (v (cm^{-1}): 3221.23 (OH), 1633.76 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 3.82 (s, 2H, CH_2–N–Ar); 4.77 (s, 2H, CH_2–Cl); 6.59–6.62 (m, aromatic, 1H); 6.68–6.73 (m, aromatic, 1H); 6.97–7.07 (m, aromatic, 1H); 7.08–7.26 (m, aromatic, 5H); 8.91 (s, 1H, OH).

2-Chloro-N-(2-hydroxybenzyl)-N-(3-methoxyphenyl)acetamide ***(4g)*** Yield: 49%; mp: 69–70°C; IR (KBr), v (cm^{-1}): 3157.58 (OH), 1633.76 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 3.77 (s, 1H, OCH_3) 3.87 (s, 2H, CH_2–N–Ar); 4.78 (s, 2H, CH_2–Cl); 6.58–6.60 (m, aromatic, 1H); 6.64–6.74 (m, aromatic, 3H); 6.97–7.00 (m, aromatic, 2H); 7.20–7.26 (m, aromatic, 1H); 7.32–7.38 (m, aromatic, 1H); 9.06 (s, 1H, OH).

2-Chloro-N-(2-hydroxybenzyl)-N-(4-iodophenyl)acetamide ***(4h)*** Yield: 30%; mp: 122–123°C; IR (KBr), v (cm^{-1}): 3178.79 (OH), 1643.41 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 3.82 (s, 2H, CH_2–N–Ar); 4.77 (s, 2H, CH_2–Cl); 6.60–6.62 (m, aromatic, 1H); 6.63–6.74 (m, aromatic, 1H); 6.82–6.85 (m, aromatic, 2H); 6.97–7.00 (m, aromatic, 1H); 7.21–7.26 (m, aromatic, 1H); 7.78–7.81 (m, aromatic, 2H); 8.87 (s, 1H, OH).

2-Chloro-N-(2-hydroxybenzyl)-N-(4-chlorophenyl)acetamide ***(4i)*** Yield: 41%; mp: 115–117°C; IR (KBr), v (cm^{-1}): 3221.23 (OH), 1637.62 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 3.82 (s, 2H, CH_2–N–Ar); 4.77 (s, 2H, CH_2–Cl); 6.60–6.62 (m, aromatic, 1H); 6.69–6.71 (m, aromatic, 1H); 6.97–7.06 (m, aromatic, 3H); 7.21–7.27 (m, aromatic, 1H); 7.42–7.46 (m, aromatic, 2H); 8.88 (s, 1H, OH).

2-Chloro-N-(2-hydroxybenzyl)-N-(4-bromophenyl)acetamide ***(4j)*** Yield: 51%; mp: 113–115°C; IR (KBr), v (cm^{-1}): 3201.94 (OH), 1643.41 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 3.82 (s, 2H, CH_2–N–Ar); 4.77 (s, 2H, CH_2–Cl); 6.59–6.62 (m, aromatic, 1H); 6.68–6.73 (m, aromatic, 1H); 6.96–7.00 (m, aromatic, 3H); 7.21–7.26 (m, aromatic, 1H); 7.58–7.61 (m, aromatic, 2H); 8.86 (s, 1H, OH).

2-Chloro-N-(2-hydroxybenzyl)-N-(3-chlorophenyl)acetamide ***(4k)*** Yield: 50%; mp: 114–116°C; IR (KBr), v (cm^{-1}): 3128.64 (OH), 1637.62 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 3.84 (s, 2H, CH_2–N–Ar); 4.78 (s, 2H, CH_2–Cl); 6.61–6.64 (m, aromatic, 1H); 6.69–6.75 (m, aromatic, 1H); 6.95–7.00 (m, aromatic, 2H); 7.16–7.17 (m, aromatic, 1H); 7.21–7.27 (m, aromatic, 1H); 7.37–7.48 (m, aromatic, 2H); 8.86 (s, 1H, OH).

2-Chloro-N-(2-hydroxybenzyl)-N-(4-trifluorophenyl)acetamide ***(4l)*** Yield: 47%; mp: 93–94°C; IR (KBr), v (cm^{-1}): 3275.24 (OH), 1639.55 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 3.80 (s, 2H, CH_2–N–Ar); 4.81 (s, 2H, CH_2–Cl); 6.56–6.59 (m, aromatic, 1H); 6.68–6.74 (m, aromatic, 1H); 6.98–7.01 (m, aromatic, 1H); 7.22–7.29 (m, aromatic, 2H); 7.41 (m, aromatic 1H); 7.58–7.64 (m, aromatic, 1H); 7.73–7.76 (m, aromatic, 1H); 8.78 (s, 1H, OH).

2-Chloro-N-(2-hydroxybenzyl)-N-(3-nitrophenyl)acetamide ***(4m)*** Yield: 63%; mp: 105–106°C; IR (KBr), v (cm^{-1}): 3221.23 (OH), 1635.69 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 3.83 (s, 2H, CH_2–N–Ar); 4.85 (s, 2H, CH_2–Cl); 6.56–6.60 (m, aromatic, 1H); 6.68–6.74 (m, aromatic, 1H); 6.97–7.00 (m, aromatic, 1H); 7.22–7.28 (m, aromatic, 1H); 7.41–7.45 (m, aromatic, 1H); 7.65–7.70 (m, aromatic, 1H); 8.06–8.08 (m, aromatic, 1H); 8.33–8.36 (m, aromatic, 1H); 8.59 (s, 1H, OH).

2-Chloro-N-(2-hydroxybenzyl)-N-(4-nitrophenyl)acetamide ***(4n)*** Yield: 40%; mp: 109–111°C; IR (KBr), v (cm^{-1}): 3180.72 (OH), 1643.41 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 3.84 (s, 2H, CH_2–N–Ar); 4.85 (s, 2H, CH_2–Cl); 6.56–6.59 (m, aromatic, 1H); 6.68–6.74 (m, aromatic, 1H); 6.97–7.00 (m, aromatic, 1H); 7.22–7.27 (m, aromatic, 1H); 7.32–7.36 (m, aromatic, 2H); 8.32–8.36 (m, aromatic, 2H); 8.59 (s, 1H, OH).

Synthesis of 4-phenyl-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(2H)-one (**5e**) (general procedure, except **5n**)

2-Chloro-*N*-(2-hydroxybenzyl)-*N*-phenylacetamide (**4e**) (16 mmol, 4.4 g) was dissolved in 100 ml of ethanol and 13 ml of 5% NaOH solution was added. The reaction mixture was stirred for 1 h at room temperature. To separate the inorganic materials, water was added to the solution and the organic part was extracted with chloroform. Then, the organic layer was dried with anhydrous $CaCl_2$. The solvent was evaporated under vacuo. The residual matter was crystallized from ethanol to give 4-phenyl-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(*2H*)-one (**5e**) (2.1 g, 55%). Mp: 148–149°C, Lit. (Derieg and Sternbach, 1966): 147–148°C; IR (KBr), v (cm^{-1}): 1661(C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 4.87 (s, 2H, N–CH_2); 4.89 (s, 2H, O–CH_2); 7.06–7.13 (m, aromatic, 2H); 7.16–7.25 (m, aromatic, 1H); 7.26–7.34 (m, aromatic, 3H); 7.36–7.44 (m, aromatic, 3H).

*4-(4-Nitrophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H) one (**5n**)* 2-Chloro-*N*-(2-hydroxybenzyl)-*N*-(4-nitrophenyl) acetamide (**4n**) (2 mmol, 0.5 g) was dissolved in 100 ml of acetone and K_2CO_3 (6 mmol, 0.83 g) was added. The reaction mixture was refluxed for 2 h. K_2CO_3 was filtered off and acetone was evaporated. The residue was washed with water and then dissolved in chloroform. The solution was dried with anhydrous $CaCl_2$ and the solvent was evaporated under vacuo. The residual matter was crystallized from ethanol to give 4-(4-nitrophenyl)-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(2H)-one (**5n**) (0.25 g, 35 %). Mp: 194–195°C; IR (KBr), *v* (cm^{-1}): 1676.20 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 4.91 (s, 2H, N–CH_2); 4.98 (s, 2H, O–CH_2); 7.09–7.14 (m, aromatic, 2H); 7.20–7.22 (m, aromatic, 1H); 7.34–7.40 (m, aromatic, 1H); 7.46–7.50 (m, aromatic, 2H); 8.26–8.29 (m, aromatic, 2H).

*Spectroscopic and analytical data of compounds (**5**)*

*4-(4-Dimethylaminophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5a**)* Yield: 62%; mp: 213–214°C; IR (KBr), *v* (cm^{-1}): 1660.77 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 2.95 (s, 6H, $N(CH_3)_2$); 4.85 (s, 2H, N–CH_2); 4.86 (s, 2H, O–CH_2); 6.70–6.73 (m, aromatic, 2H); 7.03–7.17 (m, aromatic, 5H); 7.29-7.35 (m, aromatic, 1H).

*4-(4-Methoxyphenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5b**)* Yield: 80%; mp: 175–176°C, Lit. (Davion *et al.*, 2004, 175°C); IR (KBr), *v* (cm^{-1}): 1653.05 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 3.81 (s, 3H, OCH_3); 4.85 (s, 2H, N–CH_2); 4.85 (s, 2H, O–CH_2); 6.90–6.93 (m, aromatic, 2H); 7.07–7.18 (m, aromatic, 5H); 7.30–7.34 (m, aromatic, 1H).

*4-(4-Methylphenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5c**)* Yield: 50%; mp: 142–143°C; IR (KBr), *v* (cm^{-1}): 1654.98 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 2.36 (s, 3H, CH_3); 4.85 (s, 2H, N–CH_2); 4.86 (s, 2H, O–CH_2); 7.05–7.16 (m, aromatic, 5H); 7.17–7.22 (m, aromatic 2H); 7.30–7.36 (m, aromatic 1H).

*4-(3-Methylphenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5d**)* Yield: 80%; mp: 123–125°C; IR (KBr), *v* (cm^{-1}): 1668.48 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 2.36 (s, 3H, CH_3) 4.85 (s, 2H, N–CH_2); 4.86 (s, 2H, O–CH_2); 7.02–7.18 (m, aromatic, 5H); 7.26–7.36 (m, aromatic, 3H).

*4-(4-Fluorophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5f**)* Yield: 65 %; mp: 139–140°C; IR (KBr),*v* (cm^{-1}): 1662.69 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 4.85 (s, 2H, N–CH_2); 4.86 (s, 2H, O–CH_2); 7.06–7.13 (m, aromatic, 4H); 7.16–7.24 (m, aromatic, 3H); 7.32–7.37 (m, aromatic, 1H).

*4-(3-Methoxyphenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5g**)* Yield: 63%; mp: 104–105°C; IR (KBr), *v* (cm^{-1}): 1666.55 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 3.80 (s, 3H, OCH_3) 4.86 (s, 2H, N–CH_2); 4.88 (s, 2H, O–CH_2); 6.79–6.86 (m, aromatic, 3H); 7.08–7.19 (m, aromatic, 3H); 7.29–7.37 (m, aromatic, 2H).

*4-(4-Iodophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5h**)* Yield: 57%; mp: 176–178°C; IR (KBr), *v* (cm^{-1}): 1668.48 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 4.85 (s, 2H, N–CH_2); 4.86 (s, 2H, O–CH_2); 7.06–7.18 (m, aromatic, 5H); 7.32–7.37 (m, aromatic, 1H); 7.51–7.55 (m, aromatic, 2H).

*4-(4-Chlorophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5i**)* Yield: 45%; mp: 154–155°C; IR (KBr), *v* (cm^{-1}): 1668.48 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 4.85 (s, 2H, N–CH_2); 4.86 (s, 2H, O–CH_2); 7.08–7.12 (m, aromatic, 2H); 7.15–7.21 (m, aromatic, 3H); 7.32–7.39 (m, aromatic, 3H).

*4-(4-Bromophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5j**)* Yield: 60%; mp: 161–163°C; IR (KBr),*v* (cm^{-1}): 1668.05 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 4.85 (s, 2H, N–CH_2); 4.86 (s, 2H, O–CH_2); 6.99–7.03 (m, aromatic, 2H); 7.05–7.12 (m, aromatic, 2H); 7.15–7.18 (m, aromatic, 1H); 7.31–7.37 (m, aromatic, 1H); 7.70–7.75 (m, aromatic, 2H).

*4-(3-Chlorophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5k**)* Yield: 71%; mp: 129–130°C; IR (KBr), *v* (cm^{-1}): 1668.48 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 4.85 (s, 2H, N–CH_2); 4.87 (s, 2H, O–CH_2); 7.07–7.20 (m, aromatic, 4H); 7.26–7.38 (m, aromatic, 4H).

*4-(3-Trifluoromethylphenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5l**).* Yield: 49%; mp: 95–96°C; IR (KBr), *v* (cm^{-1}): 1670.41 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 4.87 (s, 2H, N–CH_2); 4.91 (s, 2H, O–CH_2); 7.10–7.25 (m, aromatic, 3H); 7.34–7.39 (m, aromatic, 1H); 7.44–7.47 (m, aromatic, 1H); 7.51–7.55 (m, aromatic, 3H).

*4-(3-Nitrophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5m**)* Yield: 60%; mp: 117–118°C; IR (KBr), *v* (cm^{-1}): 1680.05 (C=O); ^{1}H NMR ($CDCl_3$), δ (ppm): 4.89 (s, 2H, N–CH_2); 4.96 (s, 2H, O–CH_2); 7.09–7.15 (m, aromatic, 2H); 7.20–7.23 (m, aromatic, 1H); 7.34–7.40 (m, aromatic, 1H); 7.55–7.66 (m, aromatic, 2H); 8.13–8.17 (m, aromatic, 2H).

Synthesis of 4-phenyl-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(2H)-thione (**6e**) (general procedure)

4-Phenyl-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-one (**5e**) (6 mmol, 1.45 g) was dissolved in 150 ml of xylene. P_2S_5 (3 mmol, 0.67 g) was added to the solution. The reaction mixture was refluxed for 3 h at 140°C and then filtered instantly. The filtrate was evaporated under vacuo. The residual oily matter was subjected to flash column chromatography (eluent, ethyl acetate:petroleum ether, 1:6) and crystallized from ethyl acetate:petroleum ether (1:9) to give 4-phenyl-4,5-dihydrobenzo[*f*][1,4]oxazepin-3(*2H*)-thione (**6e**) (0.95 g, 62%). Mp: 102–103°C. Selected IR data (cm^{-1}): $v = 1346$ (C=S). ^1H NMR ($CDCl_3$), δ (ppm): 5.12 (s, 2H, N–CH_2); 5.34 (s, 2H, O–CH_2); 7.00–7.08 (m, aromatic, 3H); 7.20–7.23 (m, aromatic, 2H); 7.28–7.40 (m, aromatic, 2H); 7.43–7.48 (m, aromatic, 2H). Anal. Calcd for $C_{15}H_{13}NOS$: C, 70.56; H, 5.13; N, 5.49. Found: C, 70.83; H, 5.04; N, 5.28.

*Spectroscopic and analytical data of compounds (**6**)*

*4-(4-Dimethyaminophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6a**)* Yield: 10%; mp: 195–196°C; IR (KBr), *v* (cm^{-1}): 1342.50 (C=S); ^1H NMR ($CDCl_3$), δ (ppm): 2.97 (s, 6H, N(CH_3)$_2$); 5.11 (s, 2H, N–CH_2); 5.34 (s, 2H, O–CH_2); 6.70–6.73 (m, aromatic, 2H); 6.98–7.08 (m, aromatic, 5H); 7.27–7.30 (m, aromatic, 1H). Anal. Calcd for $C_{17}H_{18}N_2OS$: C, 68.42; H, 6.08; N, 9.39. Found: C, 68.13; H, 5.95; N, 9.29.

*4-(4-Methoxyphenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6b**)* Yield: 18%; mp: 152–154°C; IR(KBr), *v* (cm^{-1}): 1346.36 (C=S); ^1H NMR ($CDCl_3$), δ (ppm): 3.82 (s, 3H, O–CH_3); 5.11 (s, 2H, N–CH_2); 5.34 (s, 2H, O–CH_2); 6.94–7.07 (m, aromatic, 5H); 7.11–7.17 (m, aromatic, 2H); 7.28–7.34 (m, aromatic, 1H). Anal. Calcd for $C_{16}H_{15}NO_2S$: C, 67.34; H, 5.30; N, 4.91; Found: C, 67.17; H, 5.14; N, 4.98.

*4-(4-Methylphenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6c**)* Yield: 26%; mp: 155–156°C; IR (KBr), *v* (cm^{-1}): 1346.36 (C=S); ^1H NMR ($CDCl_3$), δ (ppm): 2.38 (s, 3H, CH_3); 5.10 (s, 2H, N–CH_2); 5.34 (s, 2H, O–CH_2); 6.99–7.24 (m, aromatic, 5H); 7.27–7.34 (m, aromatic, 3H). Anal. Calcd for $C_{16}H_{15}NOS$: C, 71.34; H, 5.61; N, 5.20; Found: C, 71.25; H, 5.83; N, 5.22.

*4-(3-Methylphenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6d**)* Yield: 28%; mp: 129–130°C; IR (KBr),*v* (cm^{-1}): 1344.43 (C=S); ^1H NMR ($CDCl_3$), δ (ppm): 2.37 (CH_3), 5.10 (s, 2H, N–CH_2); 5.33 (s, 2H, O–CH_2); 6.98–7.20 (m, aromatic, 6H); 7.29–7.37 (m, aromatic, 2H). Anal. Calcd for $C_{16}H_{15}NOS$: C, 71.34; H, 5.61; N, 5.20; Found: C, 71.46; H, 5.67; N, 5.02.

*4-(4-Fluorophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6f**)* Yield: 21%; mp: 127–128°C; IR (KBr), *v* (cm^{-1}): 1346.36 (C=S); ^1H NMR ($CDCl_3$), δ (ppm): 5.10 (s, 2H, N–CH_2); 5.34 (s, 2H, O–CH_2); 7.01–7.22 (m, aromatic, 7H); 7.29–7.33 (m, aromatic, 1H) 7.30–7.40 (m, aromatic, 1H). Anal. Calcd for $C_{15}H_{12}FNOS$: C, 65.91; H, 4.43; N, 5.12; Found: C, 65.75; H, 4.67; N, 5.11.

*4-(3-Methoxyphenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6g**)* Yield: 44%; mp: 115–117°C; IR (KBr), *v* (cm^{-1}): 1344.43 (C=S); ^1H NMR ($CDCl_3$), δ (ppm): 3.78 (OCH_3), 5.11 (s, 2H, N–CH_2); 5.33 (s, 2H, O–CH_2); 6.75–6.82 (m, aromatic, 2H); 6.89–6.93 (m, aromatic, 1H); 7.00–7.08 (m, aromatic, 3H); 7.29–7.39 (m, aromatic, 2H). Anal. Calcd for $C_{16}H_{15}NO_2S$: C, 67.34; H, 5.30; N, 4.91; Found: C, 67.38; H, 5.48; N, 4.84.

*4-(4-Iodophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6h**)* Yield: 15%; mp: 163–164°C; IR (KBr), *v* (cm^{-1}): 1340.57 (C=S). ^1H NMR ($CDCl_3$), δ (ppm): 5.09 (s, 2H, N–CH_2); 5.33 (s, 2H, O–CH_2); 6.97–7.08 (m, aromatic, 5H); 7.30–7.34 (m, aromatic, 1H); 7.76–7.80 (m, aromatic, 2H). Anal. Calcd for $C_{15}H_{12}INOS$: C, 47.26; H, 3.17; I, 33.29; N, 3.67; Found: C, 47.17; H, 3.27; N, 3.71.

*4-(4-Chlorophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6i**)* Yield: 33%; mp: 138–139°C; IR (KBr), *v* (cm^{-1}): 1340.57 (C=S); ^1H NMR ($CDCl_3$), δ (ppm): 5.09 (s, 2H, N–CH_2); 5.33 (s, 2H, O–CH_2); 7.00–7.08 (m, aromatic, 3H); 7.15–7.19 (m, aromatic, 2H); 7.30–7.40 (m, aromatic, 1H); 7.41–7.44 (m, aromatic, 2H). Anal. Calcd for $C_{15}H_{12}ClNOS$: C, 62.17; H, 4.17; N, 4.83; Found: C, 62.21; H, 4.35; N, 4.71.

*4-(4-Bromophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6j**)* Yield: 32%; mp: 150–152°C; IR (KBr),*v* (cm^{-1}): 1340.57 (C=S). ^1H NMR ($CDCl_3$), δ (ppm): 5.10 (s, 2H, N–CH_2); 5.33 (s, 2H, O–CH_2); 6.98–7.05 (m, aromatic, 3H); 7.08–7.13 (m, aromatic, 2H); 7.26–7.34 (m, aromatic, 1H); 7.51–7.60 (m, aromatic, 2H). Anal. Calcd for $C_{15}H_{12}BrNOS$: C, 53.90; H, 3.62; N, 4.19; Found: C, 53.83; H, 3.81; N, 4.11.

*4-(3-Chlorophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6k**)* Yield: 10%; mp: 157–159°C; IR (KBr),*v* (cm^{-1}): 1346.36 (C=S); ^1H NMR ($CDCl_3$), δ (ppm): 5.10 (s, 2H, N–CH_2); 5.32 (s, 2H, O–CH_2); 7.00–7.08 (m, aromatic, 3H); 7.11–7.15 (m, aromatic, 1H); 7.23–7.26 (m, aromatic, 1H); 7.30–7.42 (m, aromatic, 3H). Anal. Calcd

for $C_{15}H_{12}ClNOS$: C, 62.17; H, 4.17; N, 4.83; Found: C, 62.13; H, 4.44; N, 4.76.

*4-(3-Trifluoromethylphenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6l**)* Yield: 44%; mp: 112–113°C; IR (KBr), ν (cm^{-1}): 1346.36 (C=S); ^1H NMR ($CDCl_3$), δ (ppm): 5.13 (s, 2H, N–CH_2); 5.34 (s, 2H, O–CH_2); 7.00–7.10 (m, aromatic, 3H); 7.34–7.37 (m, aromatic, 1H); 7.42–7.45 (m, aromatic, 1H); 7.52–7.56 (m, aromatic, 1H); 7.59–7.65 (m, aromatic, 2H). Anal. Calcd for $C_{16}H_{12}F_3NOS$: C, 59.43; H, 3.74; N, 4.33; Found: C, 59.43; H, 3.66; N, 4.27.

*4-(3-Nitrophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6m**)* Yield: 16%; mp: 158–159°C; IR (KBr), ν (cm^{-1}): 1346.36 (C=S); ^1H NMR ($CDCl_3$), δ (ppm): 5.17 (s, 2H, N–CH_2); 5.36 (s, 2H, O–CH_2); 7.04–7.11 (m, aromatic, 3H); 7.33–7.36 (m, aromatic, 1H); 7.59–7.68 (m, aromatic, 2H); 8.14–8.15 (m, aromatic, 1H); 8.22–8.26 (m, aromatic, 1H). Anal. Calcd for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33; Found: C, 60.03; H, 3.52; N, 9.30.

*4-(4-Nitrophenyl)-4,5-dihydrobenzo[f][1,4]oxazepin-3(2H)-thione (**6n**)* Yield: 16%; mp: 182–184°C; IR (KBr), ν (cm^{-1}): 1350.22 (C=S); ^1H NMR ($CDCl_3$), δ (ppm): 5.14 (s, 2H, N–CH_2); 5.35 (s, 2H, O–CH_2); 7.04–7.10 (m, aromatic, 3H); 7.33–7.46 (m, aromatic, 3H); 8.31–8.34 (m, aromatic, 2H). Anal. Calcd for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33; Found: C, 59.83; H, 4.19; N, 9.26.

Biological assays

The antibacterial activities of 14 compounds were determined using broth microdilution susceptibility test outlined by the Clinical and Laboratory Standards Institute M7-A7[13]. Minimal inhibitory concentrations for each compound were investigated against *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25983), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853). For broth microdilution procedures, sterile, disposable, multiwell microdilution plates (96 U-shaped wells) were used. The stock solutions were prepared in pure ethanol (Sigma). Ethanol had no effect on the microorganisms in the concentrations studied.

The antifungal activities of the compounds were determined using broth microdilution susceptibility test outlined by Clinical and Laboratory Standards Institute M27-A2[14]. Minimal inhibitory concentrations for each compound were investigated against *Candida albicans* (ATCC 90028). For broth microdilution procedures, sterile, disposable, multiwell microdilution plates (96 U-shaped wells) were used. The stock solutions were prepared in pure ethanol (Sigma) and again ethanol had no effect on the microorganisms in the concentrations studied.

Dilutions of the compounds

For antibacterial activities, all of the dilutions were done with Mueller–Hinton Broth (Oxoid) in the wells of microdilution plates. The concentrations of the tested compounds were 1600, 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.09, and 0.04 μg/ml. The highest ampicillin and ciprofloxacin were used as a reference compounds which were obtained from the manufacturers.

For antifungal activity, all the dilutions were done with RPMI medium with L-glutamine buffered, pH 7, with MOPS (Sigma) in the wells of microdilution plates. The concentrations of 14 compounds tested are the same as above. The highest fluconazole was used as a reference compound which was also obtained from the manufacturer.

Inoculum preparation

After diluting the compounds, standardized inoculum of each bacterium (0.5 Mc Farland standard unit, 1×10^8 CFU/ml; (colony forming unit/ml) was prepared. Then, the compounds were diluted once more (1/10), and final concentrations became 1×10^7 CFU/ml. Five microliters from each dilution was placed into each well containing 100 μl of dilutions of compounds so that each well contained 5×10^5 CFU/ml of inoculum. All the inoculated plates were incubated at 35°C for 16–20 h. The lowest concentration of compounds that prevents visible growth was considered to be the minimal inhibitory concentration (MIC). Ampicillin and ciprofloxacin are used as reference antimicrobial reagents to compare their parameters with the data that obtained from the method applied in this study and to control the reliability of the results.

For antifungal activity, candida isolate were subcultured in SDA plates, incubated at 35°C for 24–48 h prior to antifungal susceptibility testing and passaged at least twice to ensure purity and viability. An inoculum suspension was prepared from individual five colonies (diameter 1 mm). The suspension was adjusted to 0.5 MacFarland Standard ($1–5 \times 10^6$ CFU/ml) and further diluted 1/20 ($1–5 \times 10^5$ CFU/ml) then 1/50 ($0.5–2.5 \times 10^5$ CFU/ml) in RPMI medium. 100 μl from each dilution was placed into each well containing 100 μl of dilutions of compounds so that each well contained 1×10^3 CFU/ml of inoculum. The MIC plates were incubated at 37°C for 48 h. For the end point was determined as the concentration producing optically clear wells (MIC-0) compared with that of drug-free growth control. Fluconazole was used as reference antifungal reagent to compare its parameters with the data that obtained from the method applied in this study and to control the reliability of the results.

Every experiment for the antibacterial and antifungal assays was replicated twice. MIC values for the activities are given in Tables 1 and 2, respectively.

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