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Benzofuran fused phenyl pyrimidine/pyrazole schiff base derivatives as bioactive agents: anticancer, antimicrobial and molecular docking studies

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ABSTRACT: Benzofuran fused phenyl pyrimidine/pyrazole schiff base derivatives **8(a-e)** and **9(a-e)** have been synthesized by a three step reaction. The structures of the synthesized compounds were established based on the analytical and spectral data. The *in-vitro* anticancer and antimicrobial activity of compounds against MCF-7 and HeLa cancer cell lines and microbes were studied. The compounds **8c** and **9c** showed significant levels of anticancer activity compared to the standard. Molecular docking studies were performed on the active site of transferase (PDB ID: 4ANM) with the synthesized ligands **8(a-e)** and **9(a-e)** for the optimization of the investigated compounds as potential cancer cell inhibitors. The antimicrobial activity was performed by using well diffusion method, compounds **8d** and **9d** showed maximum inhibition effect against the tested microbial pathogens, respectively.

KEYWORDS: Benzofuran fused; pyrimidine; pyrazole; anticancer; antimicrobial; molecular docking.

1. INTRODUCTION

Heterocyclic compounds are widely distributed in nature and consumed by mammals through dietary materials. They are essential for health and life as they provide the basic skeletons for most of the existing available drugs. The presence of nitrogen, sulphur and oxygen makes heterocyclic compounds potential agents to be used in biological field [1]. Among these, benzofuran and its derivatives has emerged as potent source in the field of drug discovery. The benzofuran derivatives have attracted many researchers due to their broad scope for biological activities like anticancer, antimicrobial, antiviral, antioxidant, anti-inflammatory, and other properties [2-7]. A number of drugs containing benzofuran skeleton such as saprisartan (used in the treatment of hypertension and heart failure), benzbromarone (used in the treatment of gout), vilazodone (used for the treatment of depressive disorder in adults), 6-(2-aminopropyl) benzofuran or 6-APB (used as psychoactive drug), amiodarone and dronedarone (respectively, used for the treatment of arrhythmia), psoralen, methoxsalen, and trioxsalen for used for skin diseases like vitiligo [8,9]. Therefore, the vast range of biological effects associated with this scaffold has resulted in the benzofuran ring system being considered as a privileged structure. In addition to this Schiff bases also exhibit a variety of biological activities, including antibacterial, anti-inflammatory, antifungal, antitumor, trypanocidal, antimalarial, anti-HIV and anti-urease activities [10, 11]. Indeed, the imine group present in schiff base ligands is critical for their biological applications [12]. Also, theoretical calculations of structure-based molecular docking approach were followed for analyzing structure activity relationship (SAR) of molecules for finding potentially active compounds [13-15].

Thus, along with continuation of our research interest on heterocyclic compounds, our focus goes on the benzofuran moieties as they have wide biological activities [16-19]. Hence, here we present synthesized benzofuran fused phenyl pyrimidine/pyrazole Schiff base derivatives (Fig 1) along with their anticancer, antimicrobial activity and employ computational methods to investigate the interference of benzofuran derivatives with active site of transferase. We believe that the results obtained from this work could be a help to the researchers in adding knowledge and further which could serve as a platform for medicinal chemists to design better anticancer drugs.

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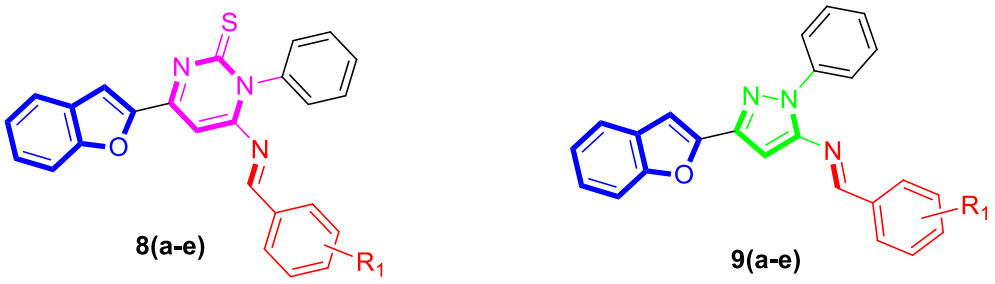
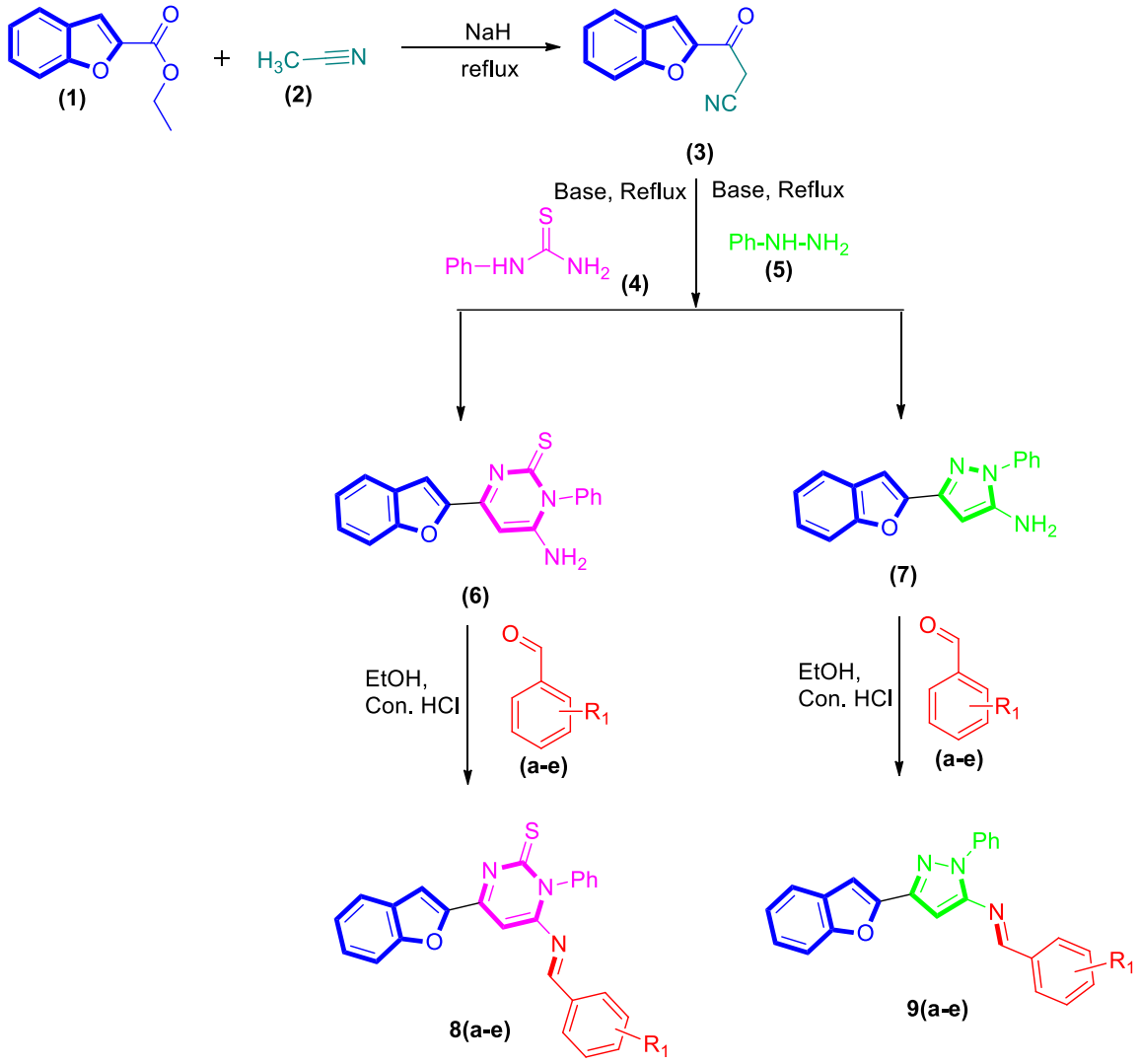


Figure 1. Benzofuran fused phenyl pyrimidine **8(a-e)**/ pyrazole **9(a-e)** Schiff base derivatives

2. RESULTS AND DISCUSSION

2.1. Chemistry

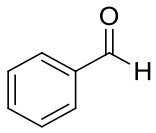
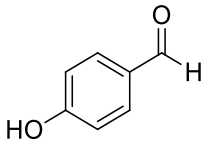
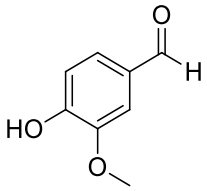
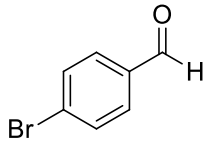
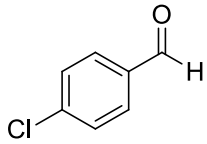
Initially, ethyl benzofuran-2-carboxylate **(1)** was added to acetonitrile **(2)** in the presence of sodium hydride (NaH) resulting in the compound 3-(benzofuran-3-yl)-3-oxopropanenitrile (**3**). Further compound **(3)** was made to react with phenyl thiourea **(4)** and phenyl hydrazine hydride **(5)** along with a base yielding corresponding compound (6) and **(7).** Finally, the target compounds **8(a-e)** and **9(a-e)** were achieved by the condensation of commercially available substituted benzaldehydes **(a-e)** with compounds (**6**) and **(7)** containing linker bond -NH_2 (**Scheme 1**).



Scheme 1. Synthetic pathway of benzofuran fused pyrimidine/ pyrazole Schiff base **8(a-e)** and **9(a-e)**.

The synthesized derivatives were furnished in moderate to high yields and are reported in Table 1. The structures of the compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analysis.

Table 1. Chemical structures of benzofuran fused pyrimidine/ pyrazole Schiff base **8(a-e)** and **9(a-e)**

Compound (8a-e)	Compound (9a-e)	Substituted aldehydes (R-CHO)
8a	**9a**	
8b	**9b**	
8c	**9c**	
8d	**9d**	
8e	**9e**	

2.2. Biological activity

2.2.1. Anticancer activity

The anticancer activity of the synthesized compounds was investigated. It was assessed by (MTT) cleavage assay with MCF-7 and HeLa cell lines. Anticancer activity included measurement of % of the *in vitro* cell inhibition by using MTT colorimetric assay at 10µM concentration. All the benzofuran fused pyrimidine/pyrazole schiff base derivatives **8(a-e)** and **9(a-e)** synthesised, exhibited some degree of anticancer effects as shown in Table 2. Compared to the standard, anticancer potency of the compounds (**8c**) and (**9c**) were found to be the highest. According to the structure–activity Relationship (SAR), it is clear that the presence of hydroxyl groups and methoxy substituents on phenyl ring of benzaldehydes when further fused to pyrimidine **(6)/**pyrazole **(7)** skeletons showed better anticancer activity than the other analogues. Among the tested compounds, (**8c**) and (**9c**) showed pronounced anticancer activity against both the cell lines. Whereas, compounds (**8b**) and (**9b**) had also exhibited good activity due to the presence of hydroxyl group within the same ring system. İn the other analogues (**8d-e**), (**9d-e**) it was noticed that the introduction of bromo and chloro substituted benzaldehydes to compounds (**6 & 7**) decreased their anticancer activity. It can be therefore concluded that the presence of 3-methoxy,4-hydroxy benzaldhyde or 4-hydroxy benzaldehyde substitution to NH_2 bond in compounds (**6 & 7**) ring system caused significant increase in the anticancer activity.

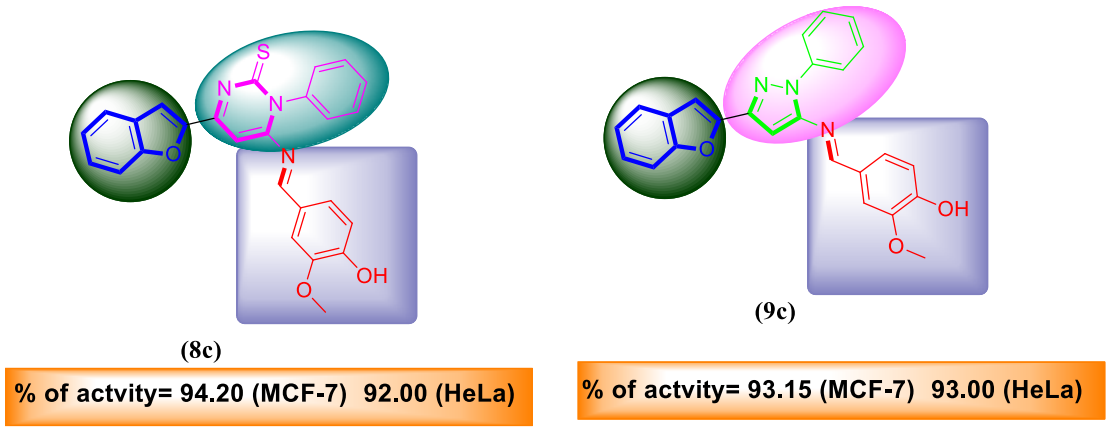


Figure 2. Compounds (**8c**) and (**9c**) was found to be the highest anticancer potency

Table 2. (%) anticancer activity of benzofuran fused piperidone/pyrazole Schiff base **8(a-e)** and **9(a-e)**

Sl. No	Compounds	(%) of Activity	
	10 µM	MCF-7	HeLa
1	6	45.15	53.10
2	7	35.37	33.54
3	8a	54.00	79.15
4	8b	85.54	82.10
5	8c	94.20	92.00
6	8d	62.13	60.45
7	8e	63.45	58.11
8	9a	58.15	53.20
9	9b	80.14	79.55
10	9c	93.15	93.00
11	9d	70.15	72.30
12	9e	62.70	65.50
13	Cisplatin	95.45	96.10

2.2.2. Antimicrobial activity

The antimicrobial activity of benzofuran fused phenyl pyrimidine/pyrazole schiff base derivatives **8(a-e)** and **9(a-e)** were evaluated by agar well diffusion technique, in which the inhibitory activity was calculated as the diameter of inhibition zone (in mm). In the presence of phenyl pyrimidine/pyrazole schiff base derivatives **8(a-e)** and **9(a-e)**, the growth of bacterial and fungal species was pointedly inhibited without any interruption of other external agents. Among the tested compounds, **8d** and **9d** showed the highest microbial inhibition effect against the tested bacterial and fungal strains. This may be due to the presence of 'Br' substituent at *para* position of the phenyl ring. Compounds **8e** and **9e** showed good antimicrobial activity, the reason may be the presence of 'Cl' group on phenyl ring. Table 3 shows the antimicrobial activity of the synthesised derivatives in comparison to Streptomycin/Fluconazole. Our study suggests that when the bromo and chloro group was introduced to the phenyl ring, the inhibitory activity was improved for the benzofuran fused phenyl pyrimidine/pyrazole schiff base derivatives. When the hydroxy group is substituted with phenyl ring, the antimicrobial activity was found to be lower.

Table 3. Antimicrobial activity of benzofuran fused phenyl pyrimidine/pyrazole schiff base derivatives **8(a-e)** and **9(a-e)**

Sl. No.	Compounds	Zone of inhibition (mm)			
		Bacteria		Fungi	
		E. coli	*S. aureus*	*A. niger*	*C. albicans*
1	**6**	4.0	3.2	2.8	3.5
2	**7**	2.5	2.0	3.0	2.7
3	**8a**	3.0	3.1	3.5	4.2
4	**8b**	2.5	2.7	NA	NA
5	**8c**	2.0	1.8	NA	NA
6	**8d**	15.0	16.5	13.6	15.2
7	**8e**	13.5	15.3	11.8	10.2
8	**9a**	5.0	4.7	3.5	2.5
9	**9b**	3.3	3.0	NA	NA
10	**9c**	2.8	2.5	NA	NA
11	**9d**	17.0	18.0	14.8	13.5
12	**9e**	12.0	13.8	13.0	11.0
13	**Streptomycin**	16.8	19.0	--	--
14	**Fluconazole**	--	--	14.5	16.0

Note: NA= Not active

2.2.3. Molecular docking studies

Molecular docking studies was performed on the active site of transferase (PDB ID: 4ANM) with the synthesized ligands **8(a-e)** and **9(a-e)** in order to determine the possible binding interactions of highly potent molecules.

Most of the compounds showed good docking scores and potent interactions with different amino acid residues and the results were tabulated in Table 4. Compound **8c** and **9c** showed carbon- hydrogen bond interactions with Gly 48, Asn 118 and Val 116, p-anion with Asp 175 and pi–pi stacking interactions with Phe 113 (Fig. 3.4). Compound **8b** exhibited hydrogen bond interactions with Asn 118, Arg 47, Ser 51 and Val 116 and p-anion interactions with Asp 175 (Fig. 3.5). These molecules showed the highest docking scores because of the involvement of hydroxyl and methoxy groups on the phenyl ring in the hydrogen bond interactions. Docking score and potent interactions with different amino acid residues for all the compounds were presented in Table 4.

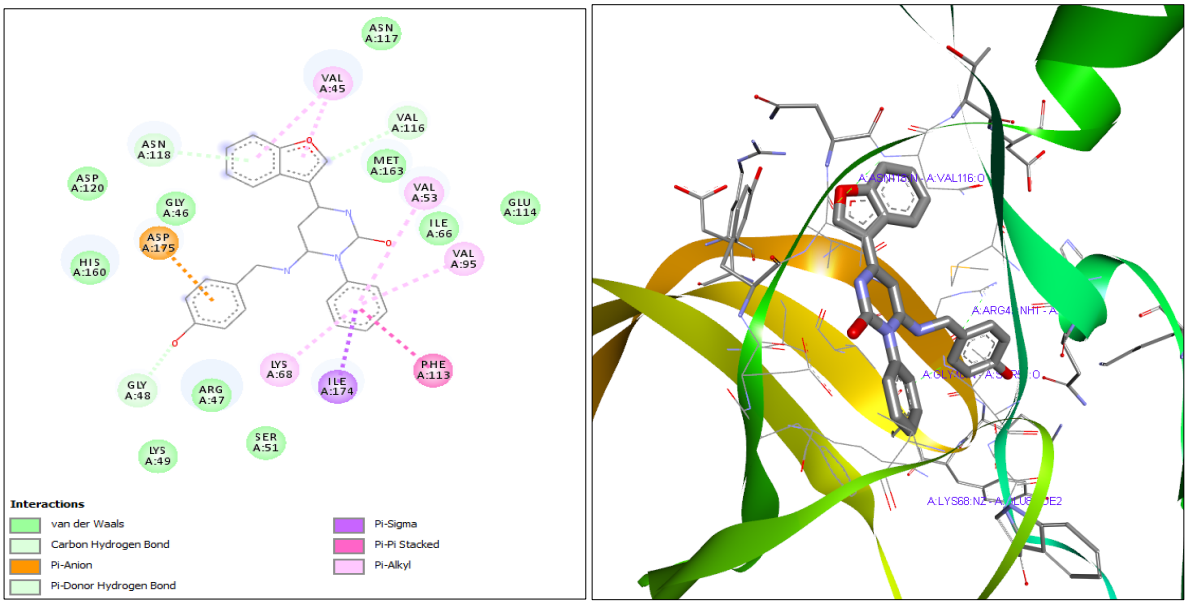


Figure 3. 2D and 3D images of compounds **8b** with 4ANM.

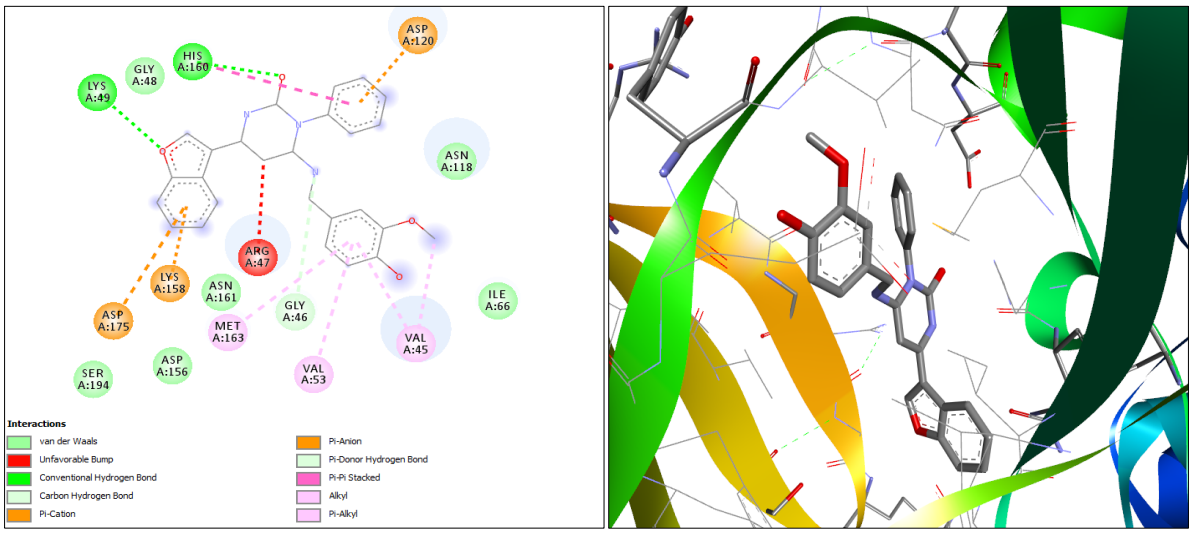


Figure 4. 2D and 3D images of compounds **8c** with 4ANM.

Table 4. Anticancer docking studies of benzofuran fused piperidone/ pyrazole Schiff base **8(a-e)** and **9(a-e)** with 4ANM protein.

Compounds No	Molecular docking with 4ANM		
	Docking Score	Hydrogen bond interaction with amino acid residues	Pi-pi stacked/ pi-anion/cation interaction
6	-5.10	Gly 48, Asn 118, Arg 47, Ser 51, Lys 49	Asp 120/Phe 113
7	-7.81	Asn 118, Arg 47, Ser 51 and Val 116	Asp 120/Phe 113
8a	-6.12	Gly 48, Asn 118, Arg 47, Ser 51 and Val 116	Asp 175/Phe 113
8b	-10.70	Asn 118, Arg 47, Ser 51 and Val 116	Asp 175/Phe 113
8c	-11.54	Gly 48, Arg 47, Ser 51 and Val 116	Asp 175/Phe 113
8d	-6.85	Asn 118, Arg 47, Ser 51, Val 116 and Lys 49	Asp 175/Phe 113
8e	-6.55	Gly 46, Asn 118, Arg 47, Ser 51 and Val 116	Asp 175/Phe 113
9a	-5.32	Gly 48, Asn 118, Arg 47, Ser 51 and His 160	Asp 120, Lys 150/Met 163
9b	-9.50	Gly 48, Asn 118, Arg 47, Ser 51 and Val 116	Asp 175, Asp 120, Lys 150/Met 163
9c	-9.87	Asn 118, Arg 47, Ser 51 and His 160	Asp 175, Asp 120, Lys 150/Met 163
9d	-5.45	Gly 46, Asn 118, Arg 47, Ser 51 and Ile 66	Asp 120, Lys 150/Met 163
9e	-4.68	Gly 48, Asn 118, Arg 47, Ser 51 and Val 116	Asp 175, Lys 150/Met 163
Cisplatin	-12.30	Gly 48, Asn 118, Arg 47, Ser 51, Val 116 and Ile 66	Asp 175, Asp 120, Lys 150/Met 163

3. CONCLUSION

In the present article, we have reported the synthesis of benzofuran fused pyrimidine/pyrazole schiff base derivatives **8(a-e)** and **9(a-e)** by choosing proper experimental conditions and investigated them for anticancer and antimicrobial assays with a hope of discovering new structures which can serve as better anticancer and antimicrobial agents. Compounds (**8c**) and (**9c**) showed pronounced anticancer activity against both the cell lines. Whereas, compounds (**8d**) and (**9d**) have exhibited highest microbial inhibition effect against the tested bacterial and fungal strains. Meanwhile, SAR studies revealed the critical role of hydroxyl groups at para position in the target compounds for the elevated anticancer activity. The presence of bromo and chloro groups at para position on the phenyl ring exhibited better antimicrobial potency in comparison to Streptomycin/Fluconazole. In short, we can say that hydroxyl groups, methoxy groups as well as Br and Cl atoms at para positions respectively showed better anticancer as well as antimicrobial activity in phenyl pyrimidine/pyrazole schiff base derivatives.

4. MATERIALS AND METHODS

4.1. Chemistry

All chemicals, reagents and solvents were purchased from Avra synthesis, AR grade and were used as provided. Thin Layer Chromatography (TLC) analysis was performed on alumina sheets precoated with silica gel 60F-254 with visualization under UV (254 nm) and SiO_2, 200-400 mesh (Merck) was used for column chromatography Hexane: Ethyl acetate (EA), 8:2 used as mobile phase. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) was obtained AC Bruker spectrometer in the appropriate dimethyl sulfoxide (DMSO-d_6) solvent. Melting points were obtained on a Reichert thermopan melting point apparatus, equipped with a microscope and are uncorrected. Elemental analysis was performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. Mass spectra were obtained on an Electron Impact mass spectrometer at 70 ev ionizing beam and using a direct insertion probe.

4.2. Synthesis procedure

4.2.1. Synthesis of 3-(benzofuran-3-yl)-3-oxopropanenitrile ***(3)***

To ethyl-2-benzofurancarboxylate (**1**)10 (20.04 g, 0.1 mol) and acetonitrile (4.1 mL, 0.1 mol) in dry benzene (250 mL), dimethylformamide (10 mL) was added along sodium hydride (4.8 g, 60%). The reaction mixture was refluxed for 4h, and then allowed to cool to room temperature. The solid formed was collected by filtration, washed with ether and dried. This material was dissolved in water and then neutralized with concentrated hydrochloric acid to pH 7. The precipitated product was collected by filtration, washed with water and dried. Recrystallisation from ethanol gave compound (**3)** [20]

(68% yield), m.p. 115–117 °C. IR (KBr): vmax/cm^{-1}: 2260 (C≡N), 1682 (C=O). ^{1}H NMR (DMSO-*d*6): δ 2.54 (s, 3H, CH3), 4.3 (s, 2H, CH2), 7.25–7.71 (m, 4H, ArH). MS: *m/z* (%) 199 (M+, 19.5), 159 (100), 131 (55), 51 (18.5). Calc. for $C_{12}H_9NO_2$: C, 72.35; H, 4.55; N, 7.03. Found: C, 72.26; H, 4.6; N, 6.9 %

4.2.2. Synthesis of piperidone/ pyrazole benzofuran (6&7)

A mixture of 3-(benzofuran-3-yl)-3-oxopropanenitrile (**3**) (10 mmol), phenyl hydrazine (**4**) or phenyl thiourea (**5**) (12.0 mmol), anhydrous K_2CO_3 (2.0 g, 15.0 mmol), and absolute ethanol (20 mL) was refluxed for 7 h. After cooling, the mixture was poured into ice water and the resulting solid was filtered off and recrystallized from EtOH [21].

6-amino-4-(benzofuran-2-yl)-1-phenylpyrimidine-2(1H)-thione (6)

Off white solid. m.p.: 150-152 ^{o}C Spectroscopic analysis: IR (KBr)ν_{max}(cm^{-1}): 3110-2950 (Ar-CH), 1618 (N-H); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 8.50 (s, 2H, NH_2) 6.25-7.90 (m, 10H, Ar-H), 5.10 (s, 1H, CH of pyrimidine); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 66.0, 104.5, 111.0, 120.9, 123.0, 124.5, 128.4, 129.0, 133.0, 133.8, 134.6, 155.0, 159.5, 164.6, 172.0; MS (ESI) m/z: 319 (M^+); Anal.calcd. for $C_{18}H_{13}N_3OS$: C, 67.69; H, 4.10; N, 13.16; found: C, 67.71; H, 4.15; N, 13.12%.

3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-5-amine (7)

Light yellow solid. m.p.: 162-164 ^{o}C Spectroscopic analysis: IR (KBr)ν_{max}(cm^{-1}): 3168-2954 (Ar-CH), 1625 (C=N pyrazole); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 7.74 (s, 2H, NH_2) 7.30-7.90 (m, 10H, Ar-H), 6.70 (s, 1H, CH of pyrazole); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 95.9, 104.5, 111.5, 120.5, 123.5, 124.9, 126.4, 129.5, 135.0, 139.7, 147.0, 150.0, 155.5; MS (ESI) m/z: 319 (M^+); Anal.calcd. for $C_{17}H_{13}N_3O$: C, 74.17; H, 4.76; N, 15.26; found: C, 74.21; H, 4.80; N, 15.21%.

2.2.3. Synthesis of benzofuran fused piperidone/ pyrazole Schiff base **(8a-e & 9a-e)**

Compound **(6 & 7)** (1.6mmol) dissolved in absolute ethanol(10 mL)was added dropwise to the solution of substituted aldehydes **(a-e)** (1.6mmol) in absolute ethanol (10 mL) with constant stirring after 5 mins. Con.HCl was added dropwise along with heating at 60^{0}C for 4-5 hours. A crystalline powder was collected by vaccum filtration and dried overnight in vacuum. The compounds **(8a-e & 9a-e)** were purified by using column chromatography hexane: ethylacetate (8:2.) used as mobile phase and recrystallized from ethanol.

(E)-4-(benzofuran-3-yl)-6-(benzylideneamino)-1-phenylpyrimidine-2(1H)-thione ***(8a)***

Light yellow solid. m.p.: 180-182 ^{o}C Spectroscopic analysis: IR (KBr)ν_{max}(cm^{-1}): 3130-2960 (Ar-CH), 1618 (C=N azomethine); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 8.10 (s, 1H, HC=N) 6.25-7.80 (m, 14H, Ar-H), 5.10 (s, 1H, CH of pyrimidine); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 84.5, 102.2, 111.0, 120.5, 123.3, 125.0, 125.5, 129.0,

129.5, 131.0, 133.7, 151.4, 154.3, 160.0, 163.8, 164.8, 172.0; MS (ESI) m/z: 407 (M^+); Anal.calcd. for $C_{25}H_{17}N_3OS$: C, 73.69; H, 4.21; N, 10.31;; found: C, 73.71; H, 4.17; N, 10.35; %.

(E)-4-(benzofuran-3-yl)-6-((4-hydroxybenzylidene)amino)-1-phenylpyrimidine-2(1H)-thione ***(8b)***

Brown solid. m.p.: 212-214 °C Spectroscopic analysis: IR (KBr)ν_{max}(cm^{-1}): 3125-2950 (Ar-CH), 1626 (C=N azomethine); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 8.15 (s, 1H, HC=N) 6.30-7.85 (m, 13H, Ar-H), 5.35 (s, 1H, OH), 5.10 (s, 1H, CH of pyrimidine), ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 84.5, 102.0, 111.5, 121.0, 123.0, 125.7, 129.0, 129.8, 131.2, 133.5, 151.5, 154.0, 160.0, 161.0, 163.8, 164.8, 172.0; MS (ESI) m/z: 423 (M^+); Anal.calcd. for $C_{25}H_{17}N_3O_2S$: C, 70.90; H, 4.05; N, 9.92; found: C, 70.87; H, 4.09; N, 9.90%.

(E)-4-(benzofuran-3-yl)-6-((4-hydroxy-3-methoxybenzylidene)amino)-1-phenylpyrimidine-2(1H)-thione ***(8c)***

Brown semi solid. Spectroscopic analysis: IR (KBr)ν_{max}(cm^{-1}): 3120-2950 (Ar-CH), 1620 (C=N azomethine); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 8.10 (s, 1H, HC=N) 6.30-7.80 (m, 12H, Ar-H), 5.30 (s, 1H, OH), 5.10 (s, 1H, CH of pyrimidine), 3.81 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 56.0, 84.0, 102.5, 111.5, 117.0, 121.5, 123.0, 125.5, 129.0, 130.0, 131.2, 133.5, 149.3, 151.8, 154.5, 160.0, 161.3, 163.5, 164.8, 172.5; MS (ESI) m/z: 453 (M^+); Anal.calcd. for $C_{26}H_{19}N_3O_3S$: C, 68.86; H, 4.22; N, 9.27; found: C, 68.80; H, 4.20; N, 9.30 %.

(E)-4-(benzofuran-3-yl)-6-((4-bromobenzylidene)amino)-1-phenylpyrimidine-2(1H)-thione ***(8d)***

Brown solid. m.p.: 225-227 °C Spectroscopic analysis: IR (KBr)ν_{max}(cm^{-1}): 3125-2965 (Ar-CH), 1624 (C=N azomethine); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 8.13 (s, 1H, HC=N) 6.25-7.88 (m, 13H, Ar-H), 5.12 (s, 1H, CH of pyrimidine); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 84.5, 102.0, 111.3, 117.0, 121.5, 123.0, 125.0, 125.5, 129.0, 130.3, 131.5, 134.0, 149.3, 151.8, 154.5, 160.0, 161.3, 163.5, 164.5, 172.0; MS (ESI) m/z: 485. (M^+); Anal.calcd.for $C_{25}H_{16}BrN_3OS$: C, 61.73; H, 3.32; N, 8.64; found: C, 61.75; H, 3.30; N, 8.60 %.

(E)-4-(benzofuran-3-yl)-6-((4-chlorobenzylidene)amino)-1-phenylpyrimidine-2(1H)-thione ***(8e)***

Dark brown solid. m.p.: 202-204 °C Spectroscopic analysis: IR (KBr)ν_{max}(cm^{-1}): 3120-2970 (Ar-CH), 1620 (C=N azomethine); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 8.10 (s, 1H, HC=N) 6.32-7.85 (m, 13H, Ar-H), 5.10 (s, 1H, CH of pyrimidine); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 84.0, 102.2, 111.5, 117.5, 121.0, 123.5, 125.7, 126.2, 129.0, 130.5, 131.9, 134.0, 151.5, 154.0, 160.5, 161.5, 163.5, 164.5, 172.2; MS (ESI) m/z: 441. (M^+); Anal.calcd.for $C_{25}H_{16}ClN_3OS$: C, 67.94; H, 3.65; N, 9.51; found: C, 67.91; H, 3.62; N, 9.50;%.

(E)-3-(benzofuran-3-yl)-N-benzylidene-1-phenyl-1H-pyrazol-5-amine ***(9a)***

Dark brown solid. m.p.: 195-197 °C Spectroscopic analysis: IR (KBr)ν_{max}(cm^{-1}): 3130-2980 (Ar-CH), 1618 (C=N azomethine); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 8.60 (s, 1H, HC=N) 7.40-8.15 (m, 14H, Ar-H), 6.70 (s, 1H, CH of pyraozle); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 102.5, 111.5, 120.5, 123.5, 124.7, 126.3, 128.5, 129.2, 131.0, 135.4, 136.4, 139.7, 142.5, 149.2, 156.5, 158.5; MS (ESI) m/z: 363. (M^+); Anal.calcd.for $C_{24}H_{17}N_3O$: C, 79.32; H, 4.72; N, 11.56; found: C, 79.30; H, 4.75; N, 11.59%.

(E)-4-(((3-(benzofuran-3-yl)-1-phenyl-1H-pyrazol-5-yl)imino)methyl)phenol ***(9b)***

Light brown solid. m.p.: 155-157 °C Spectroscopic analysis: IR (KBr)ν_{max}(cm^{-1}): 3125-2975 (Ar-CH), 1620 (C=N azomethine); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 8.60 (s, 1H, HC=N) 7.30-8.10 (m, 13H, Ar-H), 6.75 (s, 1H, CH of pyraozle), 5.25 (s, 1H, OH); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 102.0, 111.3, 120.0, 123.3, 124.5, 126.5, 128.5, 129.0, 131.5, 135.5, 136.4, 139.7, 142.1, 149.5, 156.0, 158.5, 160.8; MS (ESI) m/z: 379. (M^+); Anal.calcd.for $C_{24}H_{17}N_3O_2$: C, 75.97; H, 4.52; N, 11.08; found: C, 75.95; H, 4.50; N, 11.12%.

(E)-4-(((3-(benzofuran-3-yl)-1-phenyl-1H-pyrazol-5-yl)imino)methyl)-2-methoxyphenol ***(9c)***

Light brown solid. m.p.: 215-217 °C Spectroscopic analysis: IR (KBr)ν_{max}(cm^{-1}): 3125-2970 (Ar-CH), 1625 (C=N azomethine); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 8.63 (s, 1H, HC=N) 7.30-8.14 (m, 12H, Ar-H), 6.70 (s, 1H, CH of pyraozle), 5.30 (s, 1H, OH), 3.80 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 56.5, 102.0, 111.5, 120.0, 123.3, 124.5, 126.0, 128.5, 129.0, 131.3, 135.5, 136.2, 139.7, 142.5, 149.8, 156.5, 158.5, 160.5; MS (ESI) m/z: 409. (M^+); Anal.calcd.for $C_{25}H_{19}N_3O_3$: C, 73.34; H, 4.68; N, 10.26; found: C, 73.32; H, 4.71; N, 10.22; %.

(E)-3-(benzofuran-3-yl)-N-(4-bromobenzylidene)-1-phenyl-1H-pyrazol-5-amine ***(9d)***

Brown solid. m.p.: 188-190 °C Spectroscopic analysis: IR (KBr)ν_{max}(cm^{-1}): 3120-2975 (Ar-CH), 1624 (C=N azomethine); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 8.60 (s, 1H, HC=N) 7.30-8.10 (m, 13H, Ar-H), 6.70 (s, 1H, CH of pyraozle); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 102.0, 111.8, 120.5, 123.0, 124.5, 126.0, 128.5, 129.0, 131.0, 135.8, 136.0, 139.7, 143.8, 149.8, 156.5, 158.5, MS (ESI) m/z: 409. (M^+); Anal.calcd.for $C_{24}H_{16}BrN_3O$: C, 65.17; H, 3.65; N, 9.50; found: C, 65.15; H, 3.60; Br, 18.11; N, 9.47%.

(E)-3-(benzofuran-3-yl)-N-(4-chlorobenzylidene)-1-phenyl-1H-pyrazol-5-amine ***(9e)***

Brown solid. m.p.: 188-190 ^{o}C Spectroscopic analysis: IR (KBr)ν_{max}(cm^{-1}): 3135-2970 (Ar-CH), 1615 (C=N azomethine); ^{1}H NMR (DMSO-d_6 400 MHz) δ ppm: 8.62 (s, 1H, HC=N) 7.35-8.14 (m, 13H, Ar-H), 6.75 (s, 1H, CH of pyraozle); ^{13}C NMR (DMSO-d_6 100 MHz) δ ppm: 102.0, 111.0, 120.5, 123.5, 124.5, 126.0, 128.5, 129.0, 131.0, 135.8, 136.0, 139.7, 143.8, 149.5, 156.4, 158.0, MS (ESI) m/z: 397 (M^+); Anal.calcd. for $C_{24}H_{16}ClN_3O$: C, 72.45; H, 4.05; N, 10.56; found: C, 72.41; H, 4.00; N, 10.52%.

4.3. Biological activity

4.3.1. Anticancer assay

The inhibition of cell growth by benzofuran fused pyrimidine/pyrazole Schiff bases **(8a-e & 9a-e)** were evaluated for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl terazolium bromide (MTT) cleavage assay with MCF-7 and HeLa cell lines [22]. The cells were seeded at 1x10^4 cells/well in 96 well plates in RPMI medium supplemented with 10% Fetal Bovine Serum (FBS). After 20 hrs of culture, synthesized compounds **(8a-e & 9a-e)** at 10 µM concentration were added in triplicate and the cells were further cultured for 72 hrs. The cells were then exposed to 5mg/mL MTT in Phosphate buffer saline (PBS) at a final concentration of 1 mg/mL in culture for 5 hrs. Formazan crystals formed during the incubation period were dissolved overnight at 37^{o}C by adding 10% SDS containing 20mM HCl. The absorbance was measured at 570nm.

4.3.2. Antimicrobial activity

Benzofuran fused phenyl pyrimidine/pyrazole schiff base derivatives **8(a-e)** and **9(a-e)**were screened for their antibacterial activity using Muller-Hilton (MH) method.The pathogenic bacterial strains (*Escherichia coli and Staphylococcus aureus)* andtwo fungal strains (*AspergillusNiger and Candida albicans)*were used for screening of antimicrobial activity of synthesized compounds [23, 24].

4.3.3. Antibacterial activity

Briefly, 20 mL of the sterilized agar media was poured into pre-sterilized petri-plates. Excess of the suspension was decanted and the plates were dried in an incubator at 37 ^{o}C for 1 hr. 20 mL of the bacterial suspension (in sterilized agar media) was poured into, swabbed neatly and allowed to solidify. Wells were made on each petri-plate using 6 mm sterile cork-borer. 100 µL of the test compounds (5mg/mL in DMSO) were added to each of them. The plates were incubated at 37 ^{o}C for 24 h and the zone of inhibition (ZI) was measured in millimeter (mm) using digital micrometer and compared it with the reference standard streptomycin. (5mg/mL) [25,26].

4.3.4. Antifungal activity

Antifungal screening of benzofuran fused phenyl pyrimidine/pyrazole schiff base derivatives**8(a-e)** and **9(a-e)** were carried out by well diffusion method against three fungal strains in sterilized Asthana and Hawker's agar media as reported earlier [27]. Briefly, the fungal suspension (in 3 mL of saline solution) mixed with 20 mL sterile agar media was poured into petri-plates and allowed to solidify. Later, the plates were dried at 37 ^{o}C for 1 hr in incubator. Wells were made using 6 mm sterile cork borer to which 100 µL of the test compounds (5mg/mL in DMSO) were added and incubated at 25 ^{o}C for 72 hrs. The Zone of inhibition (in mm) was measured and compared with the reference fluconazole [28, 29].

4.4. Molecular Docking

4.4.1. Docking procedure

The interactions of the synthesized compounds with proteins (PDB ID: 4ANM) were analyzed using the HEX docking software. Molecular docking involves the following steps [30].

- Identify a target protein
- Downloaded the protein PDB file and saving the PDB file
- All the ligands were drawn in ChemDraw Ultra software in SD Molfile and minimum energy configuration was set for the 3D structure.
- Stored the 3D optimized structure as .PDB file
- Opening both the .PDB files in HEX software
- Finally running the Docking software with desired parameters

The parameters used for the docking process via HEX docking software were followed:

- Correlation type – Shape only

- Grid Dimension – 0.6
- Receptor range – 180
- Ligand Range – 180
- Twist range – 360
- Distance Range – 40

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