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One-Pot Three Component Synthesis of 2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-Arylbenzamides in Glycerol Medium

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In this work, a series of 2-(1*H*-benzo[d]thiazole-2-yl)-*N*-arylbenzamides is synthesized by using diethyl phthalate, anilines and 2-aminobenzenethiol by one-pot three component synthesis in glycerol medium. Phosphoric acid is used as an effective reagent for this one-pot three component reaction. This reaction got completed in a short time, easy workup and gave an excellent yield in glycerol medium. The *N*-arylbenzamides was found to have significant cytotoxic potentials against various cancer cells *viz*., A549 (lung cancer), HeLa (cervical cancer) and MCF-7 (breast cancer) using MTT assay. The molecular docking study is also employed to understand the binding mechanism of *N*-arylbenzamides against the antibacterial target. The docking result shows the binding energy of compound **4a** is -8.6 kcal/mol. The binding affinity is a major concern and it shows that Asn and Thr residues have an interaction with compound **4a**.

Keywords: Glycerol, Diethyl phthalate, Anilines, 2-Aminobenzenethiol, Green chemistry.

INTRODUCTION

Green chemistry [1] state that chemical reactions are experiencing a deep change to meet environmentally benign criteria. This indicates that the "Green Chemistry" process involves the choice of a safe, non-toxic and low-cost solvents [2-4]. However, these green solvents have to be low-cost and easy to handle. In the past decade, water [5-7], glycerol [8], ionic liquids [9], polyethylene glycol [10] have appeared as the most promising options for current solvents. Glycerol was an environmental and biodegradable solvent is produced as a byproduct in the biodiesel industries [11]. Moreover, glycerol is non-volatile under normal atmospheric pressure and has a high boiling point (290 ºC), thus making the distillation of the reaction products a feasible separation technique. Reactions in glycerol is carried out at high temperature, thus allowing acceleration of the reaction or making possible reactions that do not proceed in low boiling point solvents [11]. In particular, low toxicity of glycerol has allowed to utilize as a solvent in the synthesis of pharmaceutically active ingredients, in which the toxicity and residue of solvent have to be carefully controlled [11]. Because of these properties, there are a large number of reports available in literature on the applications of glycerol as an efficient and convenient solvents in organic transformations [11,12]. On the other hand, benzothiazoles have attracted much attention as important structural motifs in medicinal chemistry. Many compounds bearing this heterocyclic unit have revealed diverse and interesting biological activities, such as anti-inflammatory, anti-microbial, anti-tumour, neuroprotective and anti-convulsant activities [13-17]. Due to their importance, various synthetic strategies have been developed. Commonly used methods include: (a) condensation of *o*-amino benzenethiol with carboxylic acids by dehydration catalyzed by acids [18,19]; (b) copper- or palladium-catalyzed intramolecular cyclization of 2-halobenzothioureas [20,21]; (c) coupling of amylamines with 2-halobenzothiazoles [22] or *o*-amino benzothiazoles with aryl halides [23-25]; (d) oxidative cyclization of intermediates generated by *o*-amino

thiophenols with isothiocyanates [26]; and (e) Cu(I)- or Fe(III)-catalyzed cross-coupling reactions of *o*-halo anilines with isothiocyanates [27,28]. We now report our synthetic studies on reactions of diethyl phthalate with anilines and 2-amino-thiophenol. This is probably the first report to synthesize 2-(1*H*-benzo[*d*]thiazole-2-yl)-*N*-aryl benzamides in glycerol. Furthermore, the antibacterial and antifungal activities of reported compounds were also evaluated in present work.

EXPERIMENTAL

Melting points were uncorrected and found in H_2SO_4 bath in open capillary tubes. TLC was run on silica gel-G and visualizations were found using UV light. Infrared spectra were done by instrument Elmer-Perkin 1000 in KBr pellets. ^{1}H NMR spectra were recorded using TMS as standard in DMSO-d_6 with 400 MHz. Mass spectra were recorded by Agilent instrument. Starting materials were received from commercial sources and treated as such for reactions.

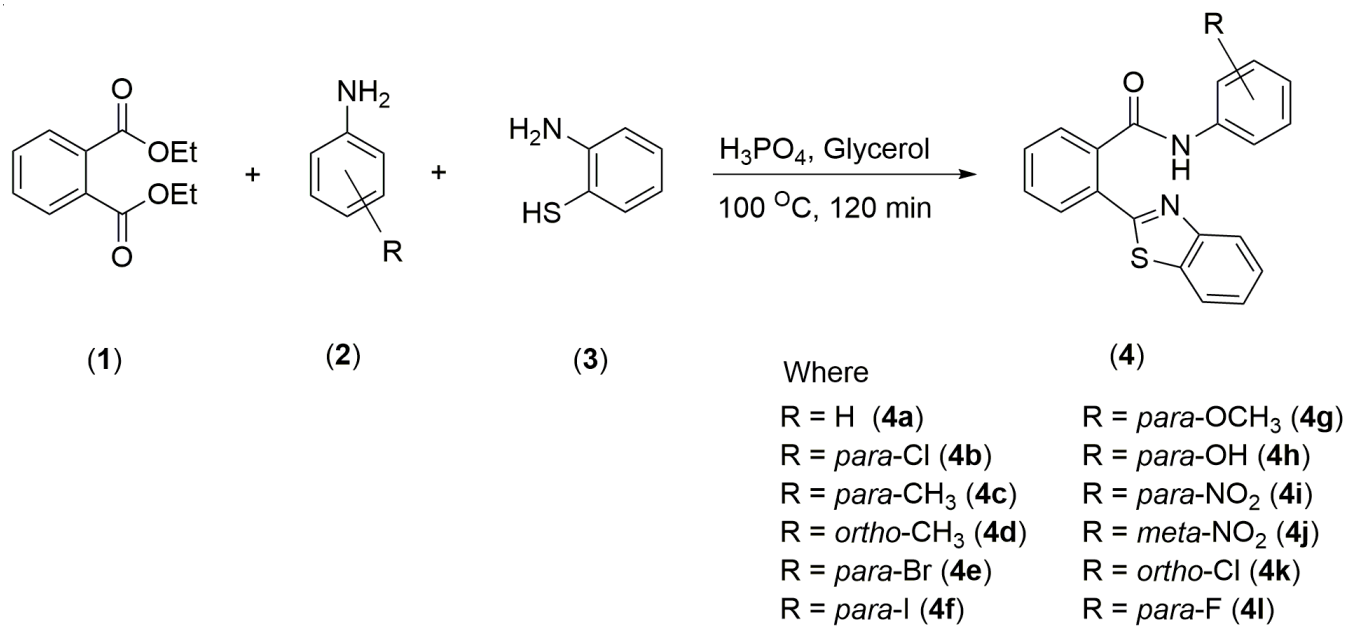
Synthesis of compound 4 from compounds 1, 2 and 3 by one-pot reaction: Charged compounds **1** (5 mmol), **2** (5 mmol) and **3** (5 mmol) in glycerol (20 mL) and heated at 100 ºC for 120-150 min in the presence of H_3PO_4 (5 mmol). Reaction progress was monitored by TLC. After completion of the reaction water (50 mL) was added and stirred for 10-15 min to separate out colourless solid from the reaction mixture, which was collected by filtration. The isolated solid was washed with water (10 mL) twice and dried at 50 ºC under above 650 mmHg vacuum for 10-12 h. The crude compound was purified by recrystallization method using methanol as a solvent to get the desired compound **4** (**Scheme-I**).

Synthesis of compound 5a: Charged compounds **1** (5 mmol) and **2a** (5 mmol) in glycerol (20 mL) and heated at 100 ºC for 20 min in the presence of H_3PO_4 (5 mmol). Reaction progress was monitored by TLC. After completion of the reaction water (50 mL) was added and stirred for 10-15 min to separate out colourless solid from the reaction mixture which was collected by filtration. The isolated solid was washed with water (10 mL) twice and dried at 50 ºC under above 650 mmHg vacuum for 10-12 h. The crude compound was purified by recrystallization method using methanol as a solvent to get the desired compound **5a**.

Synthesis of compound 4a by step-wise synthesis: Charged compounds **5** (5 mmol) and **3** (5 mmol) in glycerol (20 mL) and heated at 100 ºC for 120 min in the presence of H_3PO_4 (5 mmol). Reaction progress was monitored by TLC. After completion of the reaction water (50 mL) was added and stirred for 10-15 min to separate out colourless solid from the reaction mixture, which was collected by filtration. The isolated solid was washed with water (10 mL) twice and dried at 50 ºC under above 650 mmHg vacuum for 10-12 h. The crude compound was purified by recrystallization method using methanol as a solvent to get the desired compound **4a**.

Synthesis of compound 6a: Charged compounds **1** (5 mmol) and **3** (5 mmol) in glycerol (20 mL) and heated at 100 ºC for 20 min in the presence of H_3PO_4 (5 mmol). Reaction progress was monitored by TLC. After completion of the reaction added water (50 mL) and stirred for 10-15 min to separate out colourless solid from the reaction mixture which was collected through filtration. The isolated solid was washed with 10 mL water twice and dried at 50 ºC under above 650 mmHg vacuum for 10-12 h. The crude compound was purified by recrystallization method using methanol as a solvent to get the desired compound **6a**.

Synthesis of compound 4a by step-wise synthesis: Charged compounds **6** (5 mmol) and **2a** (5 mmol) in glycerol (20 mL) and heated at 100 ºC for 120 min in the presence of H_3PO_4 (5 mmol). Reaction progress was monitored by TLC. After completion of the reaction added water (50 mL) and stirred for 10-15 min to separate out colourless solid from the reaction mixture which was collected by filtration. The isolated solid was washed with water (10 mL) twice and dried at 50º C under above 650 mmHg vacuum for 10-12 h. The crude compound was purified by recrystallization method using methanol as a solvent to get the desired compound **4a**.



Scheme-I: Synthesis of various 2-(1*H*-benzo[*d*]thiazole-2-yl)-*N*-phenylbenzamides (**4a–l**) in glycerol medium *via* one-pot synthesis

2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-phenylbenzamide (4a): Yield 90%, m.p.: 217-219 ºC; IR (KBr, ν_{max}, cm^{-1}): 3429-3050 (br, medium, -NH-), 1643 (s, strong, -CO-NH-); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 6.7-7.9 (m, 13H, aromatic-H), 9.7 (s, 1H, -NH-); ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.4, 118.8, 119.1, 123.3, 127.3, 128.1, 1128.5, 130.3, 131.5, 131.6, 134.4, 134.5, 151.9, 165.1; HRMS calcd. (found) for $C_{20}H_{14}N_2O_2$ $[M+H]^+$: 331.0460 (331.0423).

2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-(4-chlorophenyl)-benzamide (4b): Yield 89%, m.p.: 196-198 ºC; IR (KBr, ν_{max}, cm^{-1}): 3455-3045 (br, medium, -NH-), 1648(s, strong, -CO-NH-); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 6.7-7.9 (m, 12H, aromatic-H), 9.8 (s, 1H, -NH-); ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.3, 118.6, 119.3, 123.4, 127.5, 128.3, 128.7, 130.2, 131.6, 131.9, 134.1, 134.2, 151.3, 165.3; HRMS calcd. (found) for $C_{20}H_{13}N_2O_2Cl$ $[M+H]^+$: 365.3513 (365.3818).

2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-(4-methylphenyl)-benzamide (4c): Yield 90%, m.p.: 204-205 ºC; IR (KBr, ν_{max}, cm^{-1}): 3458-3046 (br, medium, -NH-), 1642 (s, strong, -CO-NH-); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 2.3 (s, 3H, $-CH_3$), 6.7-7.9 (m, 12H, aromatic-H), 9.8 (s, 1H, -NH-); ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.0, 116.1, 118.1, 119.1, 123.0, 127.1, 128.0, 128.3, 130.0, 131.8, 131.9, 134.5, 134.9, 151.1, 165.2; HRMS calcd. (found) for $C_{21}H_{16}N_2O_2$ $[M+H]^+$: 345.2887 (345.2843).

2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-(2-methylphenyl)-benzamide (4d): Yield 88%, m.p.: 208-210 ºC; IR (KBr, ν_{max}, cm^{-1}): 3451-3041 (br, medium, -NH-), 1649 (s, strong, -CO-NH-); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 2.2(s, 3H, $-CH_3$), 6.7-7.9 (m, 12H, aromatic-H), 9.7 (s,1H,-NH-); ^{13}C NMR(100 MHz, DMSO-d_6): δ 22.3, 116.4, 118.6, 119.1, 123.0, 127.0, 128.6, 128.9, 130.3, 131.5, 131.9, 134.2, 134.4, 151.2, 165.4; HRMS calcd. (fond) for $C_{21}H_{16}N_2O_2$ $[M+H]^+$: 345.2897 (345.2844).

2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-(4-bromophenyl)-benzamide (4e): Yield 86%, m.p.: 205-207 ºC; IR (KBr, ν_{max}, cm^{-1}): 3459-3040 (br, medium, -NH-), 1650 (s, strong, -CO-NH-); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 6.7-7.9 (m, 12H, aromatic-H), 9.8 (s, 1H, -NH-); ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.2, 118.1, 119.9, 123.8, 127.8, 128.3, 128.7, 130.2, 131.4, 131.9, 134.1, 134.0, 151.3, 165.8; HRMS calcd. (found) for $C_{20}H_{13}N_2O_2Br$ $[M+H]^+$: 408.3774 (408.3727).

2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-(4-iodophenyl)-benzamide (4f): Yield 85%, m.p.: 210-212 ºC; IR (KBr, ν_{max}, cm^{-1}): 3420-3030 (br, medium, -NH-), 1652 (s, strong, -CO-NH-); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 6.7-7.9 (m, 12H, aromatic-H), 9.8 (s,1H, -NH-); ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.8, 118.8, 119.2, 123.1, 127.3, 128.4, 128.7, 130.1, 131.4, 131.9, 134.8, 134.9, 151.4, 165.5; HRMS calcd. (found) for $C_{20}H_{13}N_2O_2I$ $[M+H]^+$: 456.2781 (456.2735).

2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-(4-methoxyphenyl)-benzamide (4g): Yield 90%, m.p.: 162-164 ºC; IR (KBr, ν_{max}, cm^{-1}): 3040-3463 (br, medium, -NH-), 1655 (s, strong, -CO-NH-); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 3.7 (s, 3H, $-OCH_3$) 6.7-7.9 (m, 12H, aromatic-H), 9.8 (s, 1H, -NH-); ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.5, 116.1, 118.3, 119.4, 123.5, 127.1, 128.2, 128.6, 130.7, 131.1, 131.2, 134.4, 134.6, 151.9, 165.1; HRMS calcd. (found) for $C_{21}H_{16}N_2O_3$ $[M+H]^+$: 361.2852 (361.2826).

2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-(2-hydroxyphenyl)-benzamide (4h): Yield 90%, m.p.: 218-220 ºC; IR (KBr, ν_{max}, cm^{-1}): 3455-3045 (br, medium, -NH-), 1645 (s, strong, -CO-NH-); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 6.7-7.9 (m, 12H, aromatic-H), 8.30 (s, 1H, -OH), 9.8(s,1H,-NH-); ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.6, 118.8, 119.6, 123.8, 127.0, 128.4, 128.8, 130.4, 131.4, 131.8, 134.5, 134.8, 151.1, 165.8; HRMS calcd. (found) for $C_{20}H_{14}N_2O_3$ $[M+H]^+$: 347.4685 (347.4621).

2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-(4-nitrophenyl)-benzamide (4i): Yield 88%, m.p.: 156-158 ºC; IR (KBr, ν_{max}, cm^{-1}): 3458-3042 (br, medium, -NH-), 1654 (s, strong, -CO-NH-); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 6.7-7.9 (m, 12H, aromatic-H), 9.8 (s,1H,-NH-); ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.4, 118.1, 119.4, 123.5, 127.6, 128.0, 128.2, 130.2, 131.6, 131.5, 134.2, 134.8, 151.4, 165.9; HRMS calcd. (found) for $C_{20}H_{13}N_3O_4$ $[M+H]^+$: 376.3526 (376.3562).

2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-(3-nitrophenyl)-benzamide (4j): Yield 89%, m.p.: 160-162 ºC; IR (KBr, ν_{max}, cm^{-1}): 3475-3042 (br, medium, -NH-), 1655 (s, strong, -CO-NH-); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 6.7-7.9 (m, 12H, aromatic-H), 9.8 (s, 1H, -NH-); ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.6, 118.6, 119.1, 123.8, 127.3, 128.1, 128.2, 130.3, 131.8, 131.9, 134.1, 134.3, 151.2, 165.4; HRMS calcd. (found) for $C_{20}H_{13}N_3O_4$ $[M+H]^+$: 376.3516 (376.3563).

2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-(2-chlorophenyl)-benzamide (4k): Yield 88%, m.p.: 205-207 ºC; IR (KBr, ν_{max}, cm^{-1}): 3452-3043 (br, medium, -NH-), 1649 (s, strong, -CO-NH-); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 6.7-7.9 (m, 12H, aromatic-H), 9.8 (s, 1H, -NH-); ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.0, 118.9, 119.1, 123.2, 127.3, 128.3, 128.6, 130.0, 131.3, 131.5, 134.3, 134.6, 151.0, 165.1; HRMS calcd. (found) for $C_{20}H_{13}N_2O_2Cl$ $[M+H]^+$: 349.2610 (349.2644).

2-(1*H*-Benzo[*d*]thiazole-2-yl)-*N*-(4-fluorophenyl)-benzamide (4l): Yield 86%, m.p.: 156-158 ºC; IR (KBr, ν_{max}, cm^{-1}): 3456-3048 (br, medium, -NH-), 1643 (s, strong, -CO-NH-); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 6.7-7.9 (m, 12H, aromatic-H), 9.8 (s, 1H, -NH-); ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.3, 118.0, 119.4, 123.1, 127.5, 128.3, 128.5, 130.1, 131.4, 131.6, 134.2, 134.9, 151.1, 165.3; HRMS calcd. (found) for $C_{20}H_{13}N_2O_2F$ $[M+H]^+$: 333.3625 (333.3649).

Computational studies: The Schrodinger molecular modelling suite was employed to carry out the molecular docking protocols.

Ligand background: The binding affinity plays a crucial role to determine the biological activity between the receptor and inhibitor. It was influenced by the geometrical positions, steric and the physical properties. The small molecule was synthesized in a laboratory. It was redrawn using ACD/Chemsketch ver 12.0 and was geometrically optimized and converted into 3D format.

Preparation methodologies: A small molecule was similar to ciproflaxcin. Hence, the PDBID:3G7B was extracted from Protein Data Bank (www.rcsb.org). Later, the protein molecule was prepared using Protein preparation wizard, Schrodinger. The molecule was optimized, and it was minimized with the OPLS-2005 force field.

Docking methodologies: The molecular docking was performed using GLIDE, Schrödinger with the vdW scaling of

0.8 and partial cut-off of 0.15 to soften the potential for non-polar sites and implemented without the constraints. The docking was performed using GLIDE-SP. An attention paid to obtain the better GLIDE energy and Interactions to favour the outcome.

RESULTS AND DISCUSSION

The feasibility of three component reaction between diethyl phthalate (**1**), aniline (**2a**) and 2-aminobenzene thiol (**3**) was examined with H_3PO_4 in glycerol. Initially with H_3PO_4 (0.5 equiv.) at 100 ºC afforded 2-(1*H*-benzo[*d*]thiazole-2-yl)-*N*-phenylbenzamide (**4a**) with 75% yield. The structure and chemical composition of 2-(1*H*-benzo[*d*]thiazole-2-yl)-*N*-phenylbenzamide (**4a**) was confirmed by spectroscopic and analytical techniques.

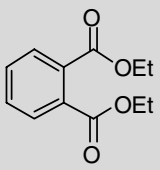
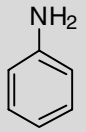
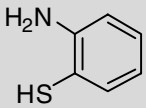
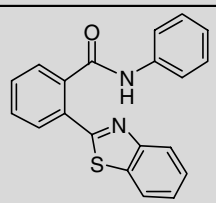
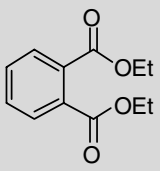
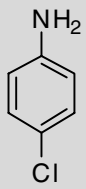
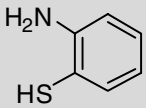
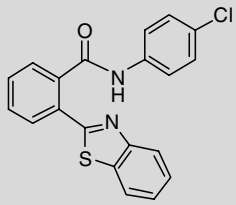
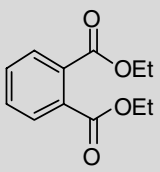
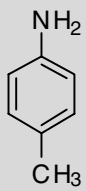
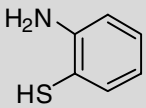
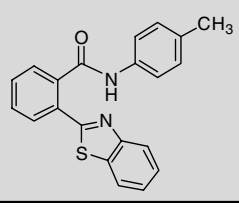
The reaction was further optimized with different solvents such as glycerol, PEG-600, ethylene glycol, DMF and DMSO at different temperatures in the presence of H_3PO_4 (1 eq.). Table-1 shows that at room temperature no product is obtained (entry 1, 8, 10 & 14). However at higher temperature, required compound **4a** was obtained in moderate to good yield (65-90%) under different solvent medium. Higher yield was obtained while performing the reaction at 100 ºC (entry 3). While there was an increase in the reaction temperature, decrement in yield was observed (entry 4). On contrary, there was no product formation when the reaction temperature was performed at room temperature (entry 1, 8, 10 & 14). A number of solvents such as polar protic and polar aprotic solvents were screened and found that the maximum yield was obtained with glycerol (entry 2). Exploration of various acids such as acetic acid, H_2SO_4 and H_3PO_4 were studied and found that the phosphoric acid is the optimum choice (entry 3, 6 and 7) for this reaction.

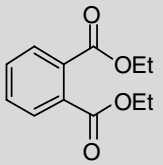
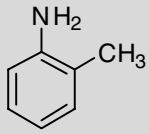
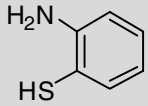
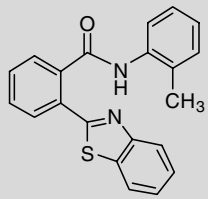
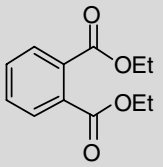
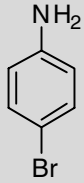
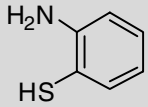
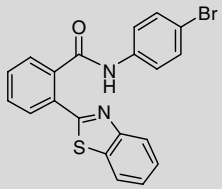
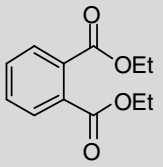
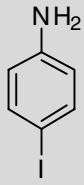
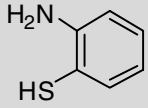
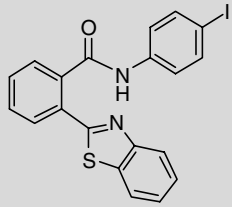
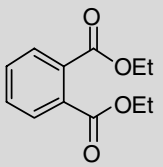
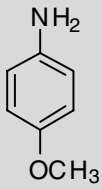
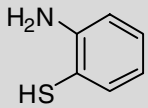
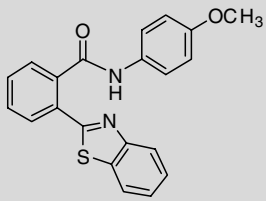
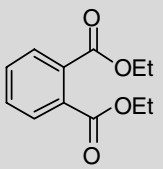
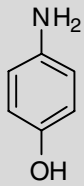
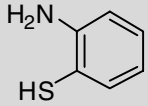
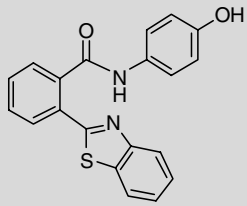
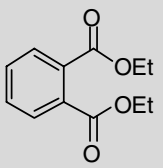
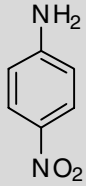
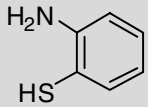
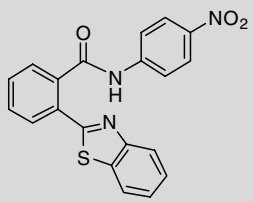
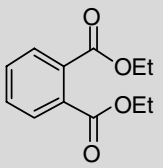
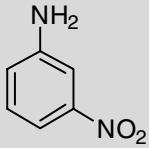
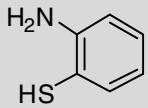
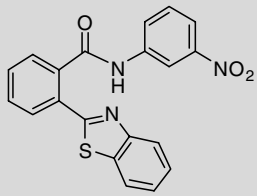
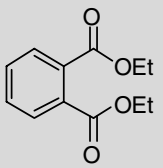
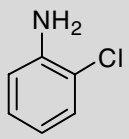
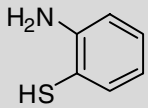
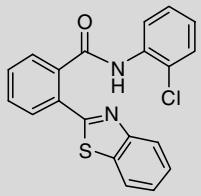
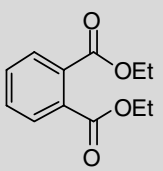
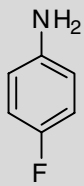
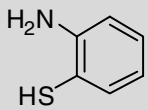
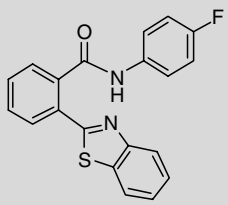
Having optimized three component reaction condition in hand, the scope and limitations with a series of substituted

TABLE-1
OPTIMIZATION OF REACTION CONDITIONS

Entry	Solvent	Temperature (°C)	Catalyst	Reaction time (h)	Yield (%)
1	Glycerol	RT	1 eq. of H_3PO_4	5	–
2	Glycerol	100	0.5 eq of H_3PO_4	3	75
3	Glycerol	100	1 eq. of H_3PO_4	2	90
4	Glycerol	150	1 eq. of H_3PO_4	2	80
5	Glycerol	100	2 eq of H_3PO_4	1.5	80
6	Glycerol	100	1 eq of H_2SO_4	2	75
7	Glycerol	100	1 eq of AcOH	3	84
8	PEG-600	RT	1 eq. of H_3PO_4	5	–
9	PEG-600	100	1 eq. of H_3PO_4	2.5	80
10	Ethylene glycol	RT	1 eq. of H_3PO_4	5	–
11	Ethylene glycol	100	1 eq. of H_3PO_4	2.5	75
12	DMF	RT	1 eq. of H_3PO_4	5	20
13	DMF	100	1 eq. of H_3PO_4	1.5	65
14	DMSO	RT	1 eq. of H_3PO_4	5	–
15	DMSO	100	1 eq. of H_3PO_4	2.5	63

TABLE-2
LIST OF VARIOUS BENZOTHIAZOLE DERIVATIVES OBTAINED BY THE ONE-POT THREE COMPONENT REACTION AND THEIR YIELDS

S. No.	Reactant 1	Reactant 2	Reactant 3	Product 4	Yield (%)
1					90
2					89
3					90

4					88
5					86
6					85
7					90
8					90
9					88
10					89
11					88
12					86

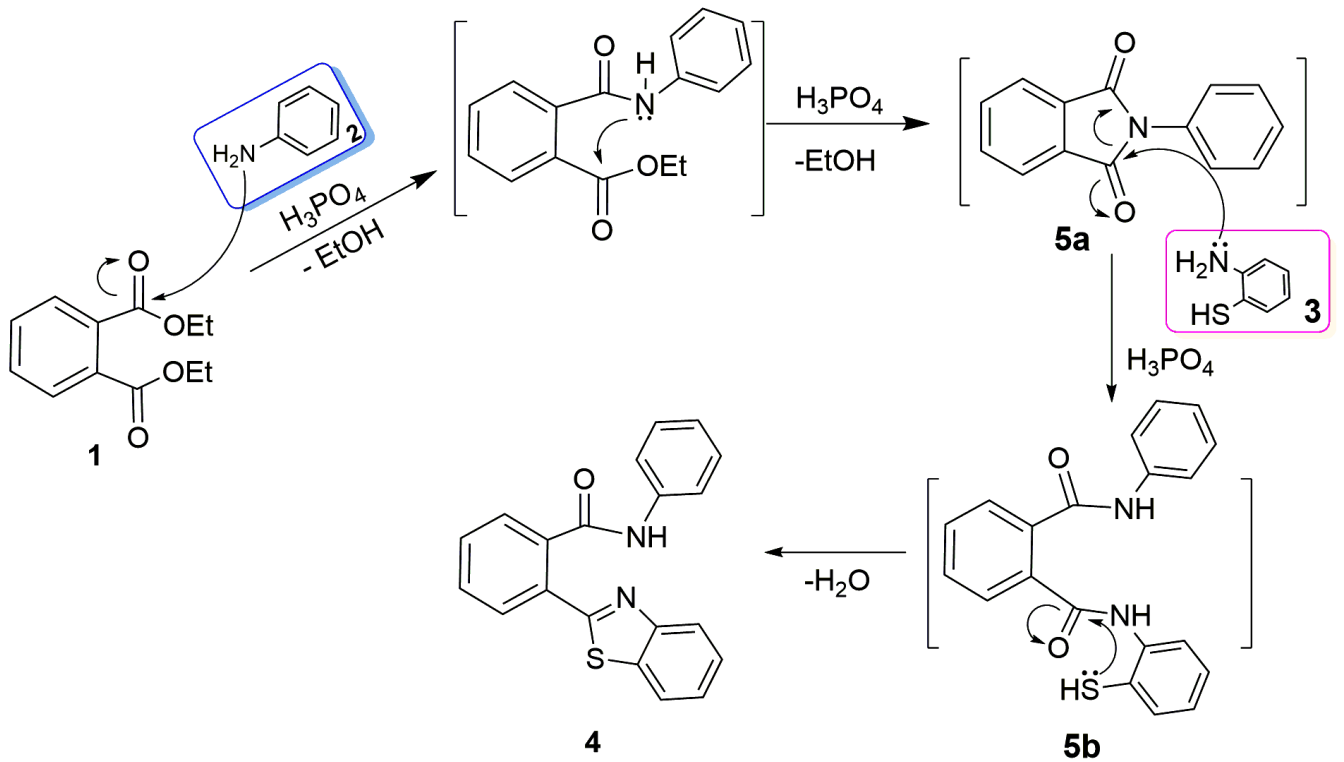
anilines **2a-l** was explored. Therefore, synthesis of compounds **4a-l** was carried out by using diethyl phthalate (**1**), different substituted anilines (**2a-l**) and 2-aminobenzenethiol (**3**) in glycerol at 100 ºC in the presence of 1 eq. H_3PO_4 for 120 min (Table-2). It was found that both electron-deficient and electron-rich anilines were applicable for this optimized condition and afforded the corresponding benzothiazole derivatives in good yield (85-90%).

The plausible mechanism for the formation of benzothiazole derivatives (**4a-l**) from the reactants **1**, **2a-l** and **3** by one-pot three component reaction is presented in **Scheme-II**. As shown, diethyl phthalate (**1**) first reacted with aniline (**2**) in the presence of H_3PO_4 in glycerol medium to afford the intermediate compound 2-phenylisoindoline-1,3-dione (**5a**) and ethanol was eliminated as byproduct. The further reaction of 2-phenylisoindoline-1,3-dione (**5a**) with 2-aminobenezenthiol (**3**) in the presence of H_3PO_4 and glycerol by simple condensation and cyclization mechanism produces the desired compound **4** as major product *via* the formation (**5b**) as an intermediate compound. To confirm the above stated mechanism in **Scheme-II**, an intermediate compound (**5a**) was isolated in good yield separately by the reaction between diethyl phthalate (**1**) and aniline (**2**) in the presence of H_3PO_4 in glycerol medium. The obtained compound (**5a**) was then further treated with 2-aminobenzenethiol (**3**) under the similar reaction conditions to afford the desired compound **4** in a step-wise synthetic method.

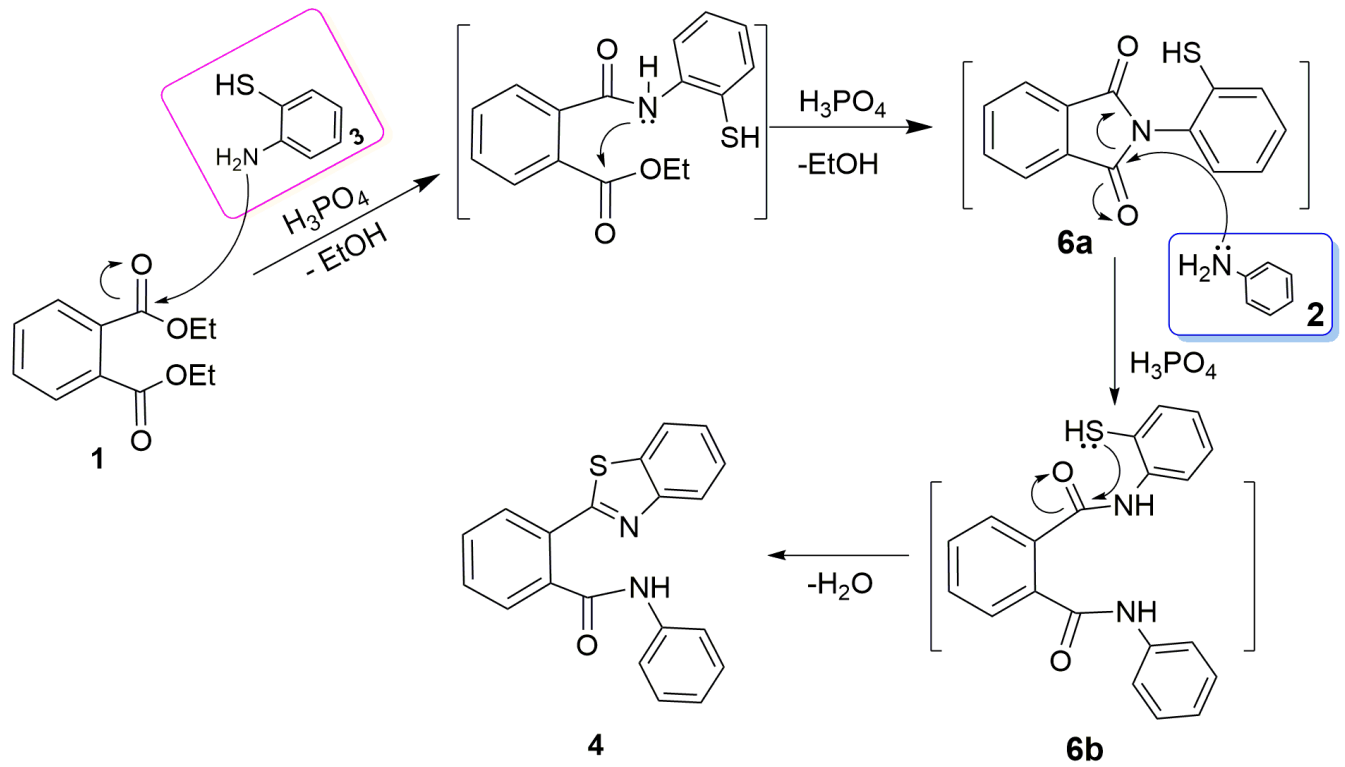
However, the selective formation of desired benzothiazole derivatives (**4a-l**) from the reactants **1**, **2a-l** and **3** could also be possible through one-pot three component reaction *via* an alternative mechanism as shown in **Scheme-III**. According to alternative approach, diethyl phthalate (**1**) initially reacts with 2-aminobenezenthiol (**3**) to form the compound such as 2-(2-mercaptophenyl)-isoindoline-1,3-dione (**6a**) as an intermediate compound. The 2-(2-mercaptophenyl)-isoindoline-1,3-dione (**6a**) was further reacted with aniline (**2**) to form a desired benzothiazole derivative (**4**) as a major product. To confirm the above stated mechanism in **Scheme-III**, an intermediate compound **6a** was isolated in good yield separately by the reaction between diethyl phthalate (**1**) and 2-aminobenzenethiol (**3**) in the presence of H_3PO_4 in glycerol medium. The obtained compound **6a** was then further treated with aniline (**2**) under the similar reaction conditions to afford the desired compound 4 in a stepwise method.

Biological activity: The benzothiazole compound (**4a**) was screened for their *in vitro* antibacterial and antifungal activity against pathogenic bacterial species (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*) and pathogenic fungal species (*Candida albicans*, *Trichophyton rubrum*, *Aspergillus niger*, *Aspergillus fumigatus*, *Candida tropicalis*) at three different concentrations using standard disc diffusion method described elsewhere [29]. The antibacterial and antifungal activity of compound **4a** was determined using sterile 2 µL 96-well plates [30]. The screening solution of compound **4a** was prepared in 10% aqueous DMSO and the results of the antibacterial and antifungal activities were expressed in terms of zone of inhibition and minimum inhibitory concentration (MIC). From these results (Tables 3 and 4), it is concluded that the newly synthesized benzothiazole compound **4a** has a significant antibacterial and antifungal action but they did not match the effectiveness of conventional bactericide gentamicin and fungicide ketoconazole.

***in vitro* Cytotoxicity activity of benzothiazole derivative (4a):** All cancer cell lines used in this study were procured



Scheme-II: Plausible mechanism of one-pot three reaction of **1**, **2** and **3** to obtain benzothiazole compound (**4**)



Scheme-III: Plausible mechanism of one-pot three reaction of **1**, **3** and **2** to obtain benzothiazole compound (**4**)

TABLE-3
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF BENZOTHIAZOLE COMPOUND (**4a**)[a]

Microbial pathogen	Zone of inhibition (mm)			
	S, 10 µg	25 µg	50 µg	100 µg
Staphylococcus aureus	21.02 ± 0.11	12.12 ± 0.15	16.11 ± 0.43	21.54 ± 0.55
Streptococcus pneumoniae	20.29 ± 0.55	08.14 ± 0.36	12.16 ± 0.25	18.11 ± 0.21
Pseudomonas aeruginosa	20.22 ± 0.54	06.01 ± 0.41	14.41 ± 0.32	20.51 ± 0.23
Acinetobacter baumannii	20.87 ± 0.59	11.41 ± 0.23	15.78 ± 0.34	21.17 ± 0.24
Candida albicans	21.43 ± 0.52	11.22 ± 0.98	15.55 ± 0.23	21.08 ± 0.03
Trichophyton rubrum	20.39 ± 0.93	08.44 ± 0.36	15.13 ± 0.35	19.11 ± 0.42
Aspergillus niger	20.21 ± 0.33	07.52 ± 0.85	13.44 ± 0.65	18.61 ± 0.61
Aspergillus fumigatus	20.24 ± 0.14	07.53 ± 0.96	14.34 ± 0.35	18.67 ± 0.56
Candida tropicalis	20.39 ± 0.43	06.97 ± 0.81	14.24 ± 0.32	19.33 ± 0.12

[a]Gentamicin for bacterial strains and Ketoconazole for fungal strains was used as positive controls.

TABLE-4
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF BENZOTHIAZOLE COMPOUND (**4a**)

Microbial pathogen	MIC	Microbial pathogen	MIC	Microbial pathogen	MIC
Staphylococcus aureus	06.34	*Acinetobacter baumannii*	08.25	*Aspergillus niger*	13.26
Streptococcus pneumoniae	13.56	*Candida albicans*	09.14	*Aspergillus fumigatus*	13.89
Pseudomonas aeruginosa	10.32	*Trichophyton rubrum*	14.05	*Candida tropicalis*	10.65

from NCCS, Pune, India and maintained with their selective medium (HiMedia, India). To ensure growth and viability of the cells, the mediums were supplemented with 10% FBS (HiMedia, India) and incubated in a humidified atmosphere with 5% CO_2 at 37 ºC.

MTT assay: All the cells were seeded in 96-well plates and allowed to adhere overnight at 37 ºC. Briefly, following treatment of cells with cisplatin and all the other compounds for 24 h, MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] was added to each well and incubated for 4 h at 37 ºC. Dimethyl sulphoxide was used as control. Absorbance was recorded at 595 nm and sensitivity to cisplatin and the compounds were calculated based on cell proliferation measurements at 24 h. Compound **4a** was evaluated for their cytotoxicity with three human derived cell lines namely human cervical carcinoma (HeLa), human lung carcinoma (A549) and human breast cancer cells (MCF-7) by using standard MTT assay. For comparison purpose, the commercially available drug, cisplatin was used as a positive control. The dose dependent response of compound **4a** against the various cancer cells tested is represented in Fig. 1. Furthermore, the IC_{50} values for the complex for MCF-7, HeLa and A549 showed that the

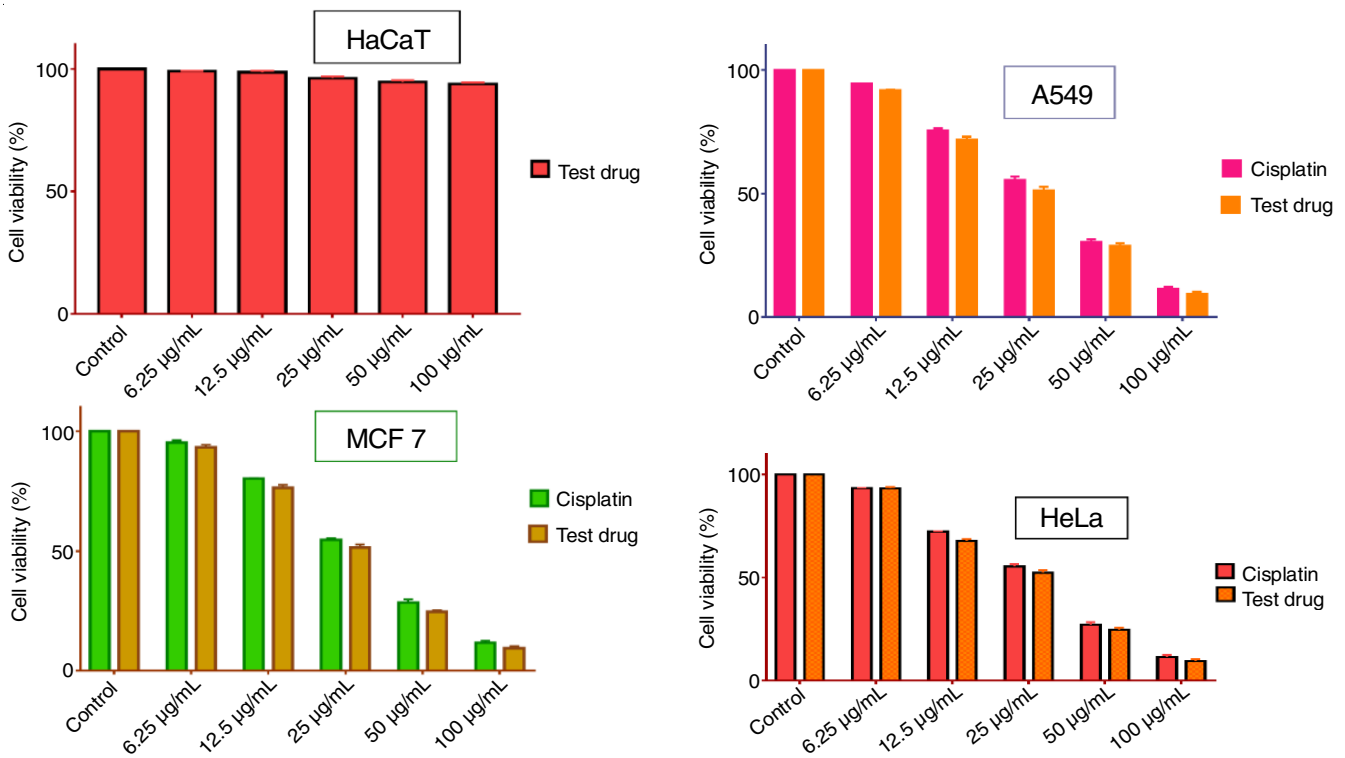


Fig. 1. *in vitro* Cytotoxicity activity of compound **4a** and standard drug (*i.e.*, cisplatin)

4a compound was cytotoxic to these cells (Fig. 2). Compound **4a** was also screened for their cytotoxic potential on the human normal keratinocyte cells (HaCaT), in order to investigate the selectivity of the compound. In the non-cancerous cell line, compound **4a** showed its non-toxic nature.

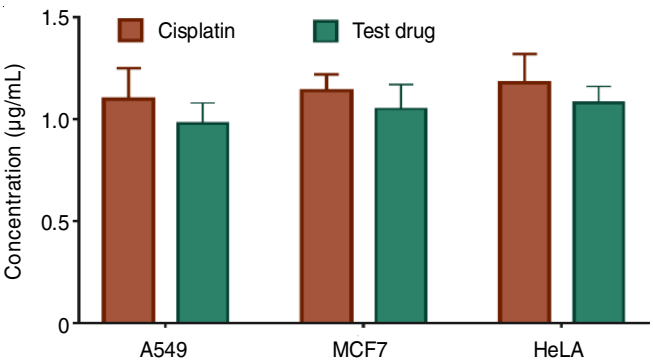


Fig. 2. IC_{50} values of compound **4a** and cisplatin on A549, MCF7 and HeLA cell lines

Molecular docking analysis: The molecular docking was carried out to identify the mechanism of action of small molecule with protein. The study aimed to identify the significance of the synthesized small molecule against anti-bacterial target. The molecular docking has initiated with reproducing the crystal structure of 3G7B. The hydrogen bonding interactions have been observed from the in-sights of the conformation. The complex results of docking score shows -6.6 kcal/mol and hydrogen bonding with Asn54 and Asp81 with the distance of 1.9 Å, respectively (Table-5). Therefore, the residues are considered as an active-sites to proceed further. The docking was performed with ciprofloaxcin and observed the interaction with Asp81 and Ser129 with the distance ~2.0 Å. The test molecule was docked with the reference molecules. The test molecule shows the interaction with Thr173 and Asn54 with the distance of 1.9 and 2.4 Å, respectively, and the docking score has observed to be -8.6 kcal/mol. The test molecule clearly shows as more stable among the earlier molecules with respect to the docking score and strong hydrogen bonding of O–H···O (Fig. 3).

Conclusion

A facile and diverse one-pot three component method is developed for the synthesis of benzothiazole derivatives by using low-cost and conveniently available starting materials in glycerol medium. The one-pot reaction takes short time and easy work up. Compound **4a** was obtained with good yield while performing the reaction at 100 ºC in the presence of H_3PO_4 in glycerol medium. The dose dependent response of compound **4a** against the various cancer cells have shown the

TABLE-5
MOLECULAR DOCKING RESULTS

Complex	Interactions	Docking score (Kcal/mol)
3G7B_X-ray_structure	N54 (N–H···O) 1.87 Å; D81 (O···H–N) 1.9	–
3G7B_reproduced crystal structure	N54 (N–H···O) 1.9 Å; D81 (O···H–N) 1.9	-6.6
3G7B_test_molecule	T173 (O–H···O) 1.9; N54 (O···H–N) 2.4	-8.6
3G7B_ciproflaxcin	D81 (N–H···O) 2.3; D81 (N–H···O) 2.1; S129 (O–H···O) 1.8	-6.9

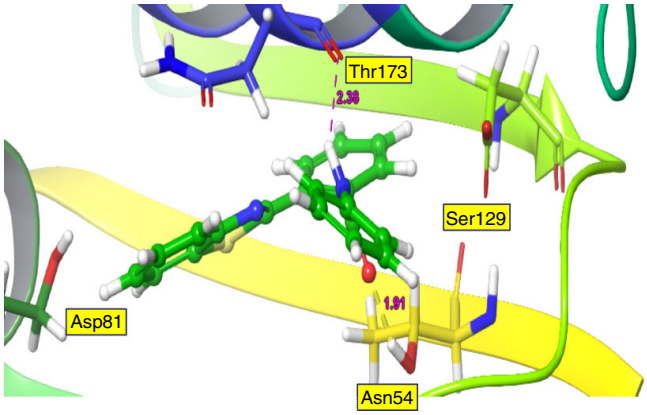


Fig. 3. Binding interactions of benzothiazole compound **4a** with protein molecule having non-covalent interactions with Asp81, Asn54, Ser129 and Thr173

good results compared with standard drug. The results of molecular docking for compound **4a** have observed with a docking score of -8.6 kcal/mol and the strong hydrogen bonding with the Asn54 and Thr173 in the binding site.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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