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Synthesis and antiproliferative evaluation of new (4-substituted-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-2-yl)methane substituted sulfonamide derivatives

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ABSTRACT

A series of new molecules having 4-substituted-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-2-yl)methane substituted sulfonamide derivatives were synthesized. The structures of the synthesized compounds were elucidated and confirmed by ^{1}H NMR, ^{13}C NMR, Mass spectra, and the purity was checked through HPLC analysis. The compounds were also evaluated for their *in vitro* antiproliferative activity against MCF-7, HeLa, A-549 and DU-145 cancer cell lines by MTT assay. Compounds 4d, 7c and 7d were tested for their activity against a panel of eight human kinase at 10 µM concentrations. Among them the compounds 4d and 7d showed very promising activity against CDK5/P25 kinase with 66 and 70% inhibitions, respectively. Compound 7c also showed promising activity of 59% inhibition. The preliminary bioassay showed that most of the compounds were antiproliferative with different degrees, and some compounds showed better activity than 5-fluorouracil which was used as positive control.

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1. Introduction

Cancer is the major threat globally in 20th century. Its treatment is difficult due to concerns about the toxicity and side effects [1]. Therefore, a good deal of interest has developped among the medicinal chemists for the design, discovery and development of more selective anticancer agents having fewer side effects [2,3]. Various types of anticancer drugs have diverse mechanism of action on the cancerous cells. Hence, finding out such mechanisms will be helpful in the designing of suitable curative remedy for attacking the cancerous cells [4].

Here we have chosen benzo[b][1,4]oxazinyl class for the synthesis as these derivatives have a wide range of biological activities like, treatment of cardiovascular [5-7], antibacterial [8,9], antifungal [10-12], anticancer activity [13-15] and protein methyl transferases inhibitors [16]. Sulphonamide derivatives are considered as an important moiety in drug synthesis and have excellent antimicrobial activity [17].

Literature survey reveals that sulphonamide bearing drug molecules like dihydropyrazole sulphonamide derivatives [18] and pyrazoline substituted benzenesulfonylureas [19] act as potential COX-1/COX-2 inhibitors and antiproloferative agents, respectively. Our research group previously reported antimicrobial and anticancer evaluation for new derivatives of thiazole, piperidone and thiazolidinone [20,21].

Recent literature reveals the synthesis of 1,4-oxazine-2-yl derivatives in water as green solvent [22]. In continuation of our research and owing to the advantages of benzo[b][1,4] oxazinyl as well as sulphonamide moieties, we have synthesized new derivatives containing sulfonamide and benzo[b] [1,4]oxazinyl in one frame work. The synthetic methods adopted for the preparation of the 4-substituted-3,4-dihydro-2*H*-benzo[b][1,4] oxazin-2-yl)methane substituted sulfonamide derivatives (**4a**-**e** and **7a**-**e**) are depicted in Scheme 1 and 2 presented below. These derivatives are screened against a series of five anticancer cell lines and some were tested for its activity against a panel of eight human kinase at 10 µM concentrations. By considering the biological importance of sulfonamides, we have synthesized series of compounds having 4-substituted-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-2-yl)methane substituted sulfonamide derivatives, **4a**-**e** and **7a**-**e**.

2. Experimental

2.1. Chemicals and instrumentation

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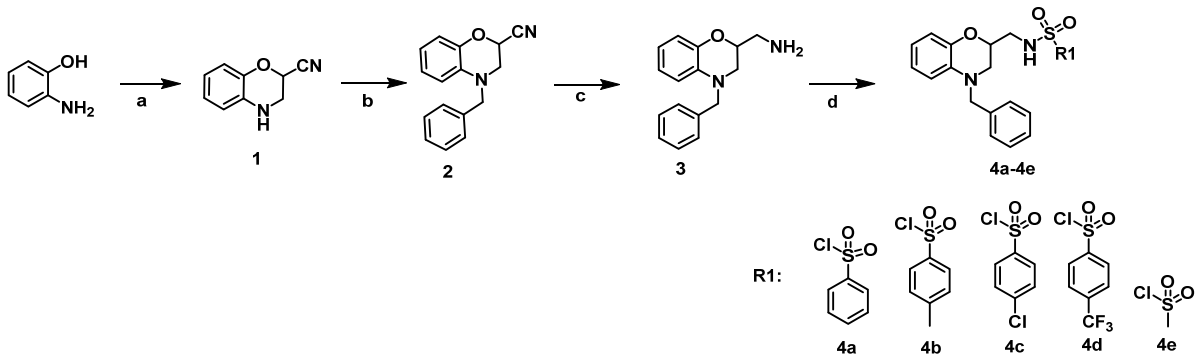
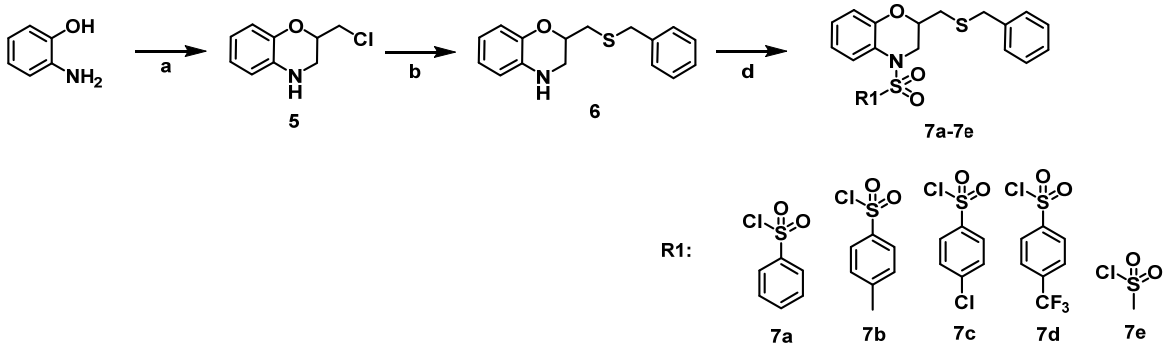


Reagents and conditions: (a) 2-chloroacrylonitrile, CS_2CO_3, toluene, 60 °C, 0.5 h to 110 °C, 4 h; (b) benzyl bromide, 2,6-lutidine, DMAP, acetone, room temperature to 80 °C, 1 h; (c) H_2, Raney Ni, methanolic ammonia, room temperature; (d) substituted sulfonyl chlorides, pyridine, DCM, room temperature. R1 = (a) benzene sulfonyl chloride (b) 4-methyl benzene sulfonyl chloride (c) 4-chloro benzene sulfonyl chloride (d) 4-triflouromethyl benzene sulfonyl chloride (e) methyl sulfonyl chloride.

Scheme 1



Reagents and conditions: (a) 2-chloromethylacrylonitrile, CS_2CO_3, toluene, 60 °C, 0.5 h to 110 °C, 4 h; (b) phenylmethanethiol, NaOH, THF room temperature to 80 °C, 1 h; (d) Substituted sulfonyl chlorides, pyridine, DCM, room temperature. R1 = (a) benzene sulfonyl chloride (b) 4-methyl benzene sulfonyl chloride (c) 4-chloro benzene sulfonyl chloride (d) 4-triflouromethyl benzene sulfonyl chloride (e) methyl sulfonyl chloride.

Scheme 2

All chemicals, unless otherwise specified, were purchased from commercial sources and were used without further purification. The major chemicals were purchased from Sigma Aldrich and Avra Labs. The development of reactions was monitored by thin layer chromatography (TLC) analysis on Merck pre-coated silica gel 60 F_{254} aluminum sheets, visualized by UV light. All reactions were carried out under argon inert atmosphere. Melting points were recorded on SRS OptiMelt, melting point apparatus and are uncorrected. The purity of intermediates was assessed by TLC, NMR, and LC-MS. The purities of final compounds were assessed by NMR, LC-MS and HPLC and all structures are consistent with proposed structures characterization. The ^{1}H NMR spectra were recorded on Varian NMR (400 MHz) spectrometer. The ^{13}C NMR spectra were recorded on Varian NMR (100 MHz) spectrometer. The chemical shifts are reported as NMR spectra δ_{ppm} units. The following abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Mass spectra were taken using Varian VG 7070 spectrometer at nominal 5000 resolution. Purity of final compounds were determined to be >95% by HPLC on an Alltech Alltima C18 column (3.2 × 150 mm, 5 µM) eluting with 5-80% acetonitrile/45 nM sodium bicarbonate.

2.2. Synthesis

2.2.1. Synthesis of 3,4-dihydro-2H-benzo[b][1,4]oxazine-2-carbonitrile (1)

To a stirred solution of 2-aminophenol (5 g, 45.8 mmol) was dissolved in toluene (50 mL) then added cesium carbonate (17.9 g, 55.0 mmol). Stirred reaction mixture at 60 °C for 0.5 h then added 2-chloroacrylonitrile (4.79 g, 55.0 mmol) and heat reaction mixture at 110 °C for 4 h. Progress of reaction was monitored by TLC. After completion the reaction, we evaporated reaction mixture under reduced pressure to obtain crude yellow gummy mass. Added H_2O (50 mL) and extracted it with ethyl acetate (EtOAc) (2 × 50 mL). The organic layer was separated, washed with H_2O (25 mL) and brine (25 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuum. Purification of crude done by silica gel (100-200 mesh) column chromatography by using EtOAc:hexane (10:90, *v*:*v*) to obtain 3,4-dihydro-2*H*-benzo[b][1,4]oxazine-2-carbonitrile (**1**) (Scheme 1). Color: Yellow solid. Yield: 6.66 g, 90 %. MS (ESI, *m/z*): 161 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.42 (dd, 1H, *J* = 12.8, 3.8 Hz, NH-CH_2), 3.58 (dd, 1H, *J* = 12.4, 3.6 Hz, NH-CH_2), 5.57 (s, 1H, CN-CH), 6.19 (s, 1H, NH), 6.58 (q, 1H, Ar-H), 6.64 (d, 1H, *J* = 8.82 Hz, Ar-H), 6.78 (q, 2H, Ar-H). ^{1}H NMR of undesired **1a**: 6.8-6.78 (m, 2H), 6.61-6.58 (m, 2H), 5.61 (m, 1H), 4.38-4.29 (q, 2H), 4.01 (br, 1H, NH).

2.2.2. Synthesis of *4-benzyl-3,4-dihydro-2H-benzo[b][1,4]oxazine-2-carbonitrile* (2)

To a stirred solution of compound **1** (1.6 g, 10.0 mmol) in acetone (32 mL) was added 2,6-lutidine (2.14 g, 20.0 mmol)

and dimethyl aminopyridine (DMAP) (0.24 g, 2.00 mmol) the mixture was stirred at room temperature for 15 min, reaction mixture was cooled to 0 °C and 1-(bromomethyl)benzene (2.05 g, 12.0 mmol) was added drop wise followed by stirring at 80 °C for 1 h. The reaction was monitored by TLC and LC-MS. After completion the reaction, the reaction mass was evaporated under reduced pressure to obtain crude gummy material. The reaction mixture was diluted with H_2O (10 mL) and extracted with dichloromethane (DCM) (2 × 10 mL). The organic layer was washed with aq. saturated $NaHCO_3$ (15 mL), and brine (15 mL) solution. Separated the organic layer and dried it over anhydrous Na_2SO_4 and evaporated under reduced pressure to get 4-benzyl-3,4-dihydro-2*H*-benzo[b][1,4]oxazine-2-carbonitrile (**2**). The product was confirmed by NMR and LC-MS and used as such for next step (Scheme 1). Color: Yellow. Yield: 2.25 g, 90 %. MS (ESI, *m/z*): 251 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.42-7.18 (m, 5H, Ar-H), 6.83-6.79 (m, 2H, Ar-H), 6.63-6.58 (m, 2H, Ar-H), 5.68 (m, 1H, CN-CH), 4.42-4.39 (q, 2H, Ph-CH_2), 3.63-3.57 (q, 2H, N-CH_2).

2.2.3. Synthesis of *(4-benzyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-yl)methanamine* (3)

To a stirred solution of compound **2** (0.25 g, 1.00 mmol) in Parr shaker vessel added methanolic ammonia (2.5 mL) and then added Raney Ni (0.05 g) and kept reaction mixture hydrogen gas pressure of 60 psi for 3 h. Progress of reaction was monitored by TLC. Filtered reaction mass through a pad of celite and obtain filtrate. Evaporated it under reduced pressure to obtain crude yellow gummy mass which was washed by EtOAc:hexane mixture (10:90, *v*:*v*, 5 mL), the gummy mass later solidified on standing and obtain (4-benzyl-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-2-yl)methanamine (**3**) as a yellow solid (Scheme 1). Color: Yellow. Yield: 0.22 g, 85 %. MS (ESI, *m/z*): 255 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.38-7.28 (m, 5H, Ar-H), 6.8-6.73 (m, 2H, Ar-H), 6.64-6.51 (m, 2H, Ar-H), 5.48 (m, 1H, CH_2-CH), 4.12-4.10 (m, 2H, Ph-CH_2), 3.63-3.57 (m, 2H, N-CH_2), 3.15-3.07 (m, 2H, NH_2-CH_2), 2.10 (br, 2H, CH_2-NH_2).

2.2.4. General procedure for the synthesis of *N-((4-benzyl-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-yl)methyl)-4-substituted-sulfonamide* derivatives (4a-4e)

To a stirred solution compound **3** (1 equiv.) in DCM (10 times) was added pyridine (10 times) the mixture was stirred at room temperature for 15 min. Reaction mixture was cooled to 0 °C and substituted sulfonyl chloride (1.2 equiv.) was added drop wise followed by stirring at room temperature for 6 h. The reaction was monitored by TLC and LC-MS, after completion of reaction, the reaction mass was diluted with cold 2 N HCl and stirred it for 30 min. The precipitation formed in reaction mixture. Filtered the obtained solid and washed it with excess of water and cold diethyl ether and cold pentane to obtain all compounds as white solids. For filtrate extracted with DCM twice. The organic layer was washed with brine solution; organic layer was evaporated under reduced pressure to get desired 4-substituted-3,4-dihydro-2*H*-benzo[b][1,4]oxazin-2-yl)methane substituted sulfonamide (**4a**-**e**) derivatives as white solids (Scheme 1).

N-((4-Benzyl-3, 4-dihydro-2H-benzo[b][1, 4]oxazin-2-yl) methyl)benzenesulfonamide (**4a**): Color: White. Yield: 92%. M.p.: 175-176 °C. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.98 (t, 1H, *J* = 6 Hz, NH), 7.82-7.80 (d, 2H, *J* = 6.8 Hz, Ar-H), 7.66-7.57 (m, 3H, Ar-H), 7.34-7.24 (dd, 5H, *J* = 14.4, 6.8 Hz, Ar-H), 6.68-6.62 (m, 3H, *J* = 14.4, 7.8 Hz, Ar-H), 6.51 (q, 1H, *J* = 12.8, 6.8 Hz, Ar-H), 4.48-4.37 (q, 2H, *J* = 16.4, 11.6 Hz, Ph-CH_2), 4.12 (m, 1H, CH_2-CH), 3.37 (m, 1H, N-CH_2), 3.15-3.12 (m, 1H, N-CH_2), 3.02 (t, 2H, *J* = 6 Hz, NH-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 145.12, 143.11, 140.38, 135.36, 129.2, 128.78, 128.12, 127.66, 127.11, 127.10, 127.01, 126.12, 125.11, 125.10, 115.12, 115.10, 110.22, 110.14, 80.30, 60.34, 55.12, 43.15. MS (EI, *m/z* (%)): 395 (M+H). HPLC: Purity = 98.0 %, r.t. = 7.98 min.

N-((4-Benzyl-3, 4-dihydro-2H-benzo[b][1, 4]oxazin-2-yl) methyl)-4-methylbenzenesulfonamide (**4b**): Color: White. Yield: 86%. M.p.: 183-184 °C. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.88-7.85 (t, 1H, *J* = 6 Hz, NH), 7.70-7.68 (d, 2H, *J* = 8 Hz, Ar-H), 7.39-7.37 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.34-7.30 (m, 2H, Ar-H), 7.26-7.24 (m, 3H, Ar-H), 6.88-6.63 (m, 3H, Ar-H), 6.51 (t, 1H, *J* = 7.8 Hz, Ar-H), 4.48-4.37 (q, 2H, *J* = 24.8, 8.0 Hz, Ph-CH_2), 4.12 (m, 1H, CH_2-C*H*), 3.37 (m, 1H, N-CH_2), 3.17-3.12 (m, 1H, N-CH_2), 2.99 (t, 2H, *J* = 6 Hz, NH-CH_2), 2.37 (s, 3H, Ph-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 149.8, 145.5, 138.7, 135.1, 133.4, 132.8, 130, 129.8, 129.2, 127.2, 126.0, 124.9, 124.1, 123, 80.3, 60.34, 55.12, 43.18, 24.12. MS (EI, *m/z* (%)): 409 [M+H]. HPLC: Purity = 97.4 %, r.t. = 8.18 min.

N-((4-Benzyl-3, 4-dihydro-2H-benzo[b][1, 4]oxazin-2-yl) methyl)-4-chlorobenzenesulfonamide (**4c**): Color: White. Yield: 88%. M.p.: 207-208 °C. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.08 (br, 1H, NH), 7.82-7.80 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.67-7.44 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.34-7.24 (m, 5H, Ar-H), 6.70-6.60 (m, 3H, Ar-H), 6.51 (t, 1H, *J* = 7.2 Hz, Ar-H), 4.48-4.37 (q, 2H, *J* = 16.2, 11.2 Hz, Ph-CH_2), 4.12 (m, 1H, CH_2-CH), 3.37 (m, 1H, N-CH_2), 3.30-3.18 (m, 1H, N-CH_2), 3.04 (d, 2H, *J* = 5.2 Hz, NH-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 145.12, 143.11, 140.38, 137.68, 135.36, 129.2, 128.78, 127.66, 127.21, 127.11, 127.08, 126.12, 125.11, 125.18, 115.12, 115.10, 110.22, 110.24, 80.30, 60.34, 55.12, 43.14. MS (EI, *m/z* (%)): 429 [M+H]. HPLC: Purity = 95.4 %, r.t. = 8.18 min.

N-((4-Benzyl-3, 4-dihydro-2H-benzo[b][1, 4]oxazin-2-yl) methyl)-4-(trifluoromethyl)benzenesulfonamide (**4d**): Color: White. Yield: 89%. M.p.: 214-215 °C. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.08 (br, 1H, NH), 7.83-7.61 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.66-7.44 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.34-7.29 (m, 5H, Ar-H), 6.71-6.61 (m, 3H, Ar-H), 6.51 (m, 1H, Ar-H), 4.48-4.37 (q, 2H, *J* = 17.2, 11.0 Hz, Ph-CH_2), 4.10 (m, 1H, CH_2-CH), 3.37 (m, 1H, N-CH_2), 3.30-3.18 (m, 1H, N-CH_2), 3.06 (t, 2H, *J* = 5.4 Hz, NH-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 145.12, 143.11, 140.38, 135.36, 134.44, 129.24, 128.88, 128.12, 127.66, 127.11, 127.14, 127.01, 126.12, 125.11, 125.10, 115.12, 115.10, 110.22, 110.14, 80.30, 60.34, 55.22, 43.14. MS (EI, *m/z* (%)): 462 [M+H]. HPLC: Purity = 97.4 %, r.t. = 8.28 min.

N-((4-Benzyl-3, 4-dihydro-2H-benzo[b][1, 4]oxazin-2-yl) methyl)methanesulfonamide (**4e**): Color: Yellow. Yield: 81%. M.p.: 135-136 °C. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.35-7.23 (m, 6H, Ar-H), 6.74-6.67 (m, 3H, Ar-H), 6.54 (t, 1H, *J* = 6.4 Hz, NH), 4.53-4.42 (q, 2H, *J* = 15.6, 8.8 Hz, Ph-CH_2), 4.20 (m, 1H, CH_2-CH), 3.42-3.37 (m, 1H, N-CH_2), 3.21-3.16 (m, 3H, N-CH_2, NH-CH_2), 2.94 (s, 3H, S-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 145.12, 143.11, 140.38, 135.36, 128.78, 128.12, 127.66, 127.01, 126.12, 115.12, 115.10, 110.22, 110.14, 80.3, 60.34, 55.12, 43.15, 41.21. MS (EI, *m/z* (%)): 333 [M+H]. HPLC: Purity = 97.4 %, r.t. = 7.20 min.

2.2.5. Synthesis of 2-(chloromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazine (5)

To a stirred solution of 2-aminophenol (5 g, 45.8 mmol) was dissolved in toluene (50 mL) then added cesium carbonate (18.7 g, 57.4 mmol). Stirred reaction mixture at 60 °C for 0.5 h then added 2-chloromethylacrylo nitrile (5.79 g, 57.4 mmol) and heat reaction mixture at 110 °C for 4 h. After completion the raection, we evaporated reaction mixture under reduced pressure to obtain crude yellow gummy mass. Added H_2O (50 mL) and extracted it with EtOAc (2 × 50 mL). The organic layer was separated, washed with H_2O (25 mL) and brine (25 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuum. Purification of crude done by silica gel (100-200 mesh) column chromatography by using EtOAc:hexane (90:10, *v*:*v*) to obtain 3,4-dihydro-2*H*-benzo[b][1,4]oxazine-2-carbonitrile (**5**) (Scheme 2). Color: Yellow. Yield: 87 %. MS (EI, *m/z* (%)): 184 $[M+H]^+$. ^1H NMR (400 MHz, DMSO-d_6, δ, ppm):

6.80-6.71 (m, 2H, Ar-H), 6.60-6.54 (m, 2H, Ar-H), 5.31 (m, 1H, CH_2-CH), 4.01 (br, 1H, NH), 3.83-3.61 (m, 4H, CH-CH_2, Cl-CH_2).

2.2.6. Synthesis of 2-((benzylthio)methyl)-3,4-dihydro-2H-benzo[b][1,4]oxazine (6)

To a stirred solution of compound **5** (0.36 g, 2.00 mmol) in THF (10 mL) was added NaOH (0.08 g, 2.00 mmol) and mixture was stirred at room temperature for 15 min. The reaction mixture was cooled to 0 °C and phenylmethanethiol (0.29 g, 2.40 mmol) was added drop wise followed by stirring at 80 °C for 1 h. The reaction was monitored by TLC and LC-MS, after completion of reaction, we evaporated reaction mass under reduced pressure to obtain crude gummy material. The reaction mixture was diluted with H_2O (10 mL) and extracted with DCM (2 × 10 mL). The organic layer was washed with sat. $NaHCO_3$ solution (10 mL), and brine solution (10 mL), organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuum* to obtain 2-((benzylthio)methyl)-3,4-dihydro-2*H*-benzo[b][1,4]oxazine (**6**). The product was confirmed by LC-MS and used as such for next step (Scheme 2). Color: Yellow. Yield: 85 %. MS (EI, *m/z* (%)): 272 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.38-7.20 (m, 5H, Ar-H), 6.83-6.78 (m, 2H, Ar-H), 6.61-6.58 (m, 2H, Ar-H), 5.31 (m, 1H, CH_2-CH), 4.01 (br, 1H, NH), 3.81-3.60 (m, 4H, NH-CH_2, Ar-CH_2), 2.94-2.74 (m, 2H, S-CH_2).

2.2.7. General procedure for the synthesis of 2-((benzylthio) methyl)-3,4-dihydro-4-substituted-2H-benzo[b][1,4]oxazine (7a-7e)

To a stirred solution of compound **6** (1 equiv.) in DCM (10 times) was added pyridine (10 times) the mixture was stirred at room temperature for 15 min. Reaction mixture was cooled to 0 °C and substituted sulfonyl chloride (1.2 equiv.) was added drop wise followed by stirring at room temperature for 6 h. The reaction was monitored by TLC and LC-MS, after completion of reaction mass was diluted with cold 2 N HCl and stirred it for 30 min. The precipitation formed in reaction mixture. Filtered the obtained solid and washed it with excess of water and cold diethyl ether and cold pentane to obtain all compounds as white solids. For filtrate extracted with DCM twice. The organic layer was washed with brine solution; organic layer was evaporated under reduced pressure to get desired 2-((benzylthio)methyl)-3,4-dihydro-4-substituted-2*H*-benzo[b][1,4]oxazine derivatives (**7a-e**) (Scheme 2).

2-((Benzylthio)methyl)-4-(phenylsulfonyl)-3,4-dihydro-2H-benzo[b][1,4]oxazine (**7a**): Color: White. Yield: 95%. M.p.: 164-165 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.90 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.64-7.58 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.56-7.55 (m, 2H, Ar-H), 7.31-7.27 (m, 2H, *J* = 6.8 Hz, Ar-H), 7.24-7.20 (m, 3H, Ar-H), 6.68-6.60 (m, 2H, Ar-H), 6.62 (t, 1H, *J* = 8.0 Hz, Ar-H), 6.51 (t, 1H, *J* = 7.2 Hz, Ar-H), 4.12 (m, 2H, CH_2-CH, S-CH_2), 4.08 (q, 1H, S-CH_2), 3.32-3.29 (dd, 1H, N-CH_2), 3.12-3.09 (m, 1H, N-CH_2), 2.99 (d, 2H, *J*= 6 Hz, CH-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 141.12, 140.11, 139.38, 135.36, 129.2, 128.78, 128.12, 127.66, 127.11, 127.10, 127.01, 126.12, 125.11, 125.10, 115.12, 115.10, 110.22, 110.14, 78.30, 54.12, 38.15, 38.10. MS (EI, *m/z* (%)): 412 [M+H]. HPLC: Purity = 98.0 %, r.t. = 8.12 min.

2-((Benzylthio)methyl)-4-tosyl-3, 4-dihydro-2H-benzo[b] [1, 4]oxazine (**7b**): Color: White. Yield: 94%. M.p.: 169-170 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.72-7.68 (d, 4H, Ar-H), 7.39-7.37 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.34-7.30 (m, 2H, Ar-H), 7.26-7.24 (m, 1H, Ar-H), 6.88-6.63 (m, 3H, Ar-H), 6.51 (t, 1H, *J* = 6.2 Hz, Ar-H), 4.12 (m, 2H, CH_2-CH, S-CH_2), 4.06 (q, 1H, S-CH_2), 3.36-3.32 (dd, 1H, N-CH_2), 3.18-3.08 (m, 1H, N-CH_2), 2.98 (d, *J*=6 Hz, 2H, CH-CH_2), 2.37 (s, 3H, Ar-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 141.22, 140.11, 139.48, 135.36, 129.22, 128.22, 127.66, 127.11, 127.10, 127.01, 126.12, 125.11, 125.1, 115.12, 115.10, 110.22, 110.14, 78.31, 54.12, 38.14, 38.11, 23.64. MS (EI, *m/z* (%)): 426 [M+H]. HPLC: Purity = 98.4 %, r.t. = 7.17 min.

2-((Benzylthio)methyl)-4-((4-chlorophenyl)sulfonyl)-3, 4-dihydro-2H-benzo[b][1,4]oxazine (**7c**): Color: White. Yield: 87%. M.p.: 181-182 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.79-7.71 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.62-7.60 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.31-7.27 (m, 2H, Ar-H), 7.23-7.19 (m, 3H, Ar-H), 6.67-6.64 (m, 1H, Ar-H), 6.58 (d, 2H, *J* = 9.2 Hz, Ar-H), 6.49 (t, 1H, *J* = 7.2 Hz, Ar-H), 4.35 (m, 2H, CH_2-CH, S-CH_2), 4.08 (q, 1H, S-CH_2), 3.30-3.28 (dd, 1H, N-CH_2), 3.14-3.08 (m, 1H, N-CH_2), 3.03 (d, 2H, *J* = 4.4 Hz, CH-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 141.12, 140.21, 139.38, 135.36, 129.26, 128.78, 128.72, 127.66, 127.11, 127.10, 127.01, 126.12, 125.11, 125.10, 115.12, 115.10, 110.22, 110.14, 78.31, 54.12, 38.25, 38.11. MS (EI, *m/z* (%)): 447 [M+H]. HPLC: Purity = 97.4 %, r.t. = 8.12 min.

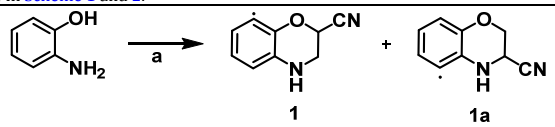
2-((Benzylthio)methyl)-4-((4-(trifluoromethyl)phenyl) sulfonyl)-3,4-dihydro-2H-benzo[b][1,4]oxazine (**7d**): Color: White. Yield: 86%. M.p.: 191-192 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.80-7.71 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.66-7.44 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.41-7.37 (m, 2H, Ar-H), 7.21-7.18 (m, 3H, Ar-H), 6.67-6.64 (m, 2H, Ar-H), 6.58 (d, 1H, *J* = 9.2 Hz, Ar-H), 6.49 (t, 1H, *J* = 7.2 Hz, Ar-H), 4.33 (m, 2H, CH_2-CH, S-CH_2), 4.06 (q, 1H, S-CH_2), 3.32-3.28 (dd, 1H, N-CH_2), 3.16-3.07 (m, 1H, N-CH_2), 3.02 (d, 2H, *J* = 4.4 Hz, CH-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 141.12, 140.10, 139.48, 135.26, 129.26, 128.78, 128.22, 127.66, 127.11, 127.10, 127.01, 126.12, 125.26, 125.11, 125.10, 115.12, 115.10, 110.22, 110.14, 78.31, 54.12, 38.15, 38.11. MS (EI, *m/z* (%)): 480 [M+H]. HPLC: Purity = 98.4 %, r.t. = 8.38 min.

2-((Benzylthio)methyl)-4-(methylsulfonyl)-3, 4-dihydro-2H-benzo[b][1,4]oxazine (**7e**): Color: Yellow. Yield: 81%. M.p.: 127-128 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.30-7.18 (br m, 5H, Ar-H), 6.74-6.67 (m, 3H, Ar-H), 6.54 (t, 1H, Ar-H), 4.46-4,32 (m, 2H, CH_2-CH, S-CH_2), 4.16 (q, 1H, S-CH_2), 3.36-3.33 (dd, 1H, N-CH_2), 3.19-3.13 (m, 3H, N-CH_2, CH-CH_2), 2.89 (s, 3H, S-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 141.12, 140.11, 139.38, 135.36, 129.20, 128.78, 128.12, 127.10, 127.01, 125.10, 115.12, 115.10, 110.22, 110.14, 78.3, 54.12, 38.15, 38.10, 37.28. MS (EI, *m/z* (%)): 350 [M+H]. HPLC: Purity = 98.4 %, r.t. = 7.64 min.

2.3. Anticancer activity

All the synthesized compounds were tested for their in vitro anticancer activity against A-549-Human lung cancer cell line, HeLa-Human cervical cancer cell line, MCF-7-Human breast cancer cell line, DU-145-Human prostate cancer cell line and HUVEC-Human umbilical vein endothelial cell line. The anticancer activity test is performed according to the procedure developed by the National Cancer Institute (NCI, USA) in the '*In vitro* Anticancer Drug Discovery Screen' that uses the protein-binding dye Sulforhodamine B (SRB) to assess cell growth [23,24]. Briefly, cells are grown in 96-well plates in suspension and then were exposed for 48 hours to four serial concentrations of 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-4} and 1×10^{-3} M of each compound. Following this, cells were fixed and stained with protein binding SRB stain. Excess stain is washed and bound stain was solubilized, and the absorbance was measured at 492 nm in a plate reader. Concentration of the compounds that inhibited 50% of the net cell growth, growth inhibition of 50% (GI_{50}), was calculated from the dose response curve obtained for each test compound and cell line. GI_{50} values were presented in micro molar (μM) concentration. 5-Flourouracil (5-Fu) was used as positive control for the comparison of cytotoxicity of synthesized compounds. Assays were performed in triplicate on three independent experiments and their mean values are taken as a final reading. All experiments were performed in duplicate and repeated three times.

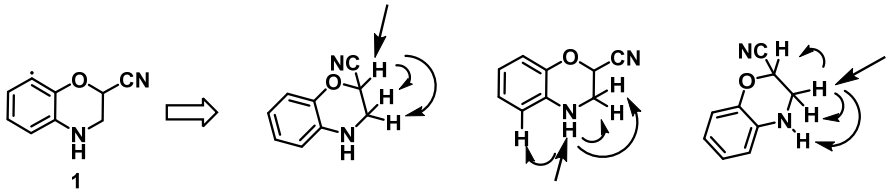
Table 1. Screening conditions for Step (a) in Scheme 1 and 2.



1 1a

Entry	Base (3 equiv.)	Solvent	Temperature (°C)	Time (h)	Yield (%) [a], Compound 1 and 1a
1	K_2CO_3	Acetone	70	12	35, 25
2	K_2CO_3	DMF	110	12	25, 32
3	K_2CO_3	Toluene	100	12	40, 36
4	Cs_2CO_3	Acetone	70	12	45, 25
5	Cs_2CO_3	DMF	110	12	40, 29
6	Cs_2CO_3	Toluene	110	12	70, 10
7	KOtBu	DMF	110	12	37, 25
8	KOtBu	Toluene	110	12	35, 38
9	Na_2CO_3	DMF	110	12	20, 45
10	Na_2CO_3	Toluene	110	12	25, 51
11	K_3PO_4	DMF	110	12	45, 30
12	K_3PO_4	Toluene	110	12	35, 31

[a] Isolated yield.



1

The ^{1}H NMR of compound **1** shows protons at 3.42 (dd, 1H, *J* = 12.8, 3.8 Hz, NH-CH_2), 3.58 (dd, 1H, *J* = 12.4, 3.6 Hz, NH-CH_2), 5.57 (s, 1H, CN-CH), 6.19 (s, 1H, NH), 6.58 (q, 1H, Ar-H), 6.64 (d, 1H, *J* = 8.82 Hz, Ar-H), 6.78 (q, 2H, Ar-H).

Scheme 3

3. Results and discussion

3.1. Synthesis

The synthetic methods adopted for the preparation of the title compounds **4a-e** and **7a-e** is depicted in the Scheme 1 and 2 presented below. An initial investigation of reaction conditions for Step (a) was done by using 2-aminophenol (1 equiv.) react with 2-chloroacrylonitrile (1.2 equiv.) and 2-chloromethylacrylonitrile (1.2 equiv.) by using different bases, solvents and temperature. The results are tabulated in Table 1.

We have screened K_2CO_3 in entry 1, 2 and 3 by taking solvents acetone DMF and toluene for 70, 110 and 100 °C, respectively for 12 h (For entry 1 and 4, we have used chilled water circulation). After column purification, we have obtained desired product in 35, 25 and 40%, respectively. In entry 4, 5 and 6, we have used Cs_2CO_3 in acetone DMF and toluene to obtain yields from 45, 40 and 70%, respectively. In conditions 7 to 12, we have used potassium *tert* butoxide (KOtBu), Na_2CO_3 and K_3PO_4 in DMF and toluene as solvents the yields are in the range of 20 to 45%. With K_3PO_4 the yield of compound **1** and **1a** is like 60 to 40%, respectively, so, it is not favorable and that of Na_2CO_3 the undesired compound **1a** is major isolated compound compared to desired isolated compound **1**, confirmed from entries 9 to 12.

In all conditions, there is formation of two merged spots among them one is product and one is side product which is confirmed by separating them. Both compounds are eluting at same polarity of EtOAc:hexane (10:90, *v:v*) from column chromatography. We have isolated both compounds by column chromatography by using silica gel (100-200 mesh) and confirmed the structure of compound **1** by the help of Nuclear Overhauser Effect (NOE) experiments there results are depicted in Scheme 3.

From first NOE experiment on compound **1**, we have irradiated proton at δ 5.57 ppm, both the protons of NH-CH_2 shows signals at δ 3.58 (weak) and 3.42 ppm (strong) because proton at δ 3.58 ppm is above the plane and proton of δ 3.42 ppm below the plane. In second NOE experiment, we have irradiated proton at δ 6.19 ppm (-NH), one aromatic proton at δ 6.64 ppm next to -NH shows signal along with δ 3.58 (strong) and 3.42 ppm (weak) as -NH and -NH-CH_2 protons are in different planes. In third NOE experiment, we have irradiated proton at δ 3.58 ppm of (-NH-CH_2) protons at δ 3.42 ppm shows signal along with δ 6.19 and 5.57 ppm as both of these are adjacent to (-NH-CH_2) protons. From above three NOE experiments confirms, compound **1** as desired product. In all optimization reactions major side product is compound **1a**, with the yields of 10 to 51%. In entry 6, when we use Cs_2CO_3 with toluene at 110 °C for 12 h, there is 70% formation of product **1** and 10% formation of compound **1a**.

Further, we have explored this condition to increase the yield of compound **1**. We have modified condition by preparing anion by heating 2-amino phenol with Cs_2CO_3 at 60 °C for 0.5 h then adding the 2-chloroacrylonitrile and further heating at 110 °C for 4 h there is 90% formation of compound **1** and 3% formation of compound **1a**. For Step (a) in Scheme 2, we treated 2-amino phenol with Cs_2CO_3 at 60 °C for 0.5 h then adding the 2-chloromethylacrylonitrile and further heating at 110 °C for 4 h, there is 87% formation of compound **5** and 5% formation of compound **1a**.

For Step (b), again, we have optimized the condition as mentioned in Table 2 by taking compound **1** and **5** (1 equiv.) and reacting it with benzyl bromide and phenylmethanethiol (1.2 equiv.) each.

We have optimized base, solvents, temperature and time for getting better yields for Step (b). All reactions were initially done from 0 °C to room temperature for 6 h (Table 2, Entries 1 to 5) on TLC, there is majority of starting remains as such then we applied heating them for remaining time at 70 °C the yields are in between 20-60%. But, in entries 6 and 7, we directly heated reaction mixture for 4 h the yield obtained of the product was 60 and 55%, respectively.

Table 2. Screening conditions for Step (b) in Scheme 1.

Entry	Base	Solvent	Temperature (°C)	Time (h)	Yield (%) [a], Compound 2 and 6
1	TEA	THF	0 to 70	12	30, 15
2	Pyridine	THF	0 to 70	8	25, NA
3	DIPEA	THF	0 to 70	12	20, NA
4	2,6-Lutidine	THF	0 to 70	12	25, 8
5	2,6-Lutidine	DMF	0 to 70	12	40, 15
6	2,6-Lutidine+DMAP	Acetone	0 to 70	4	60, 20
7	2,6-Lutidine+DMAP	Acetone	0 to 70	4	55, 16
8	2,6-Lutidine+DMAP	Acetone	0 to 80	1	85, 21

[a] Isolated yield.

Table 3. *In vitro* anticancer screening of the synthesized compounds against five cell lines.

Compound	A-549 [a]	SI[f]	HeLa [b]	SI [f]	MCF-7 [c]	SI [f]	DU-145 [d]	SI [f]	HUVEC [e]
4a	22.72±0.11	4.03	23.87±0.08	3.83	24.12±0.06	3.79	28.86±0.22	3.17	91.6±0.28
4b	33.81±0.11	2.94	24.32±0.04	4.09	25.32±0.06	3.93	23.73±0.12	4.19	99.6±0.14
4c	10.81±0.11	8.85	28.32±0.04	3.37	27.32±0.06	3.50	20.73±0.12	4.61	95.7±0.28
4d	10.65±0.11	8.78	10.79±0.22	8.67	12.86±0.12	7.27	10.82±0.11	8.65	93.6±0.28
4e	23.86±0.08	3.96	14.38±0.06	6.57	23.63±0.12	4.00	21.52±0.22	4.39	94.6±0.12
7a	25.72±0.11	3.71	26.87±0.08	3.55	24.12±0.06	3.96	28.86±0.22	3.31	95.6±0.28
7b	33.82±0.11	2.61	21.99±0.22	4.00	20.36±0.12	4.35	22.52±0.11	3.93	88.6±0.28
7c	10.78±0.14	9.23	10.78±0.08	9.23	13.82±0.08	7.20	14.72±0.06	6.76	96.6±0.19
7d	10.82±0.11	9.15	10.99±0.22	9.01	12.36±0.12	8.01	19.52±0.11	5.07	99.1±0.26
7e	24.13±0.12	3.71	25.16±0.08	3.56	26.12±0.12	3.43	21.62±0.11	4.14	89.6±0.22
5-FU	1.71±0.11	46.54	1.82±0.13	43.73	1.91±0.08	41.67	1.82±0.08	43.73	79.6±0.18

[a] A-549: Human lung cancer cell line.
[b] HeLa: Human cervical cancer cell line (ATCC CCL-2).
[c] MCF-7: Human breast cancer cell line.
[d] DU-145: Human prostate cancer cell line.
[e] HUVEC: Human umbilical vein endothelial cell line (ATCC CRL-1730).
[f] Selectivity Index (SI)= IC_{50} of pure compound in normal cell line/IC_{50} of same compound in cancer cell line; IC_{50}: The concentration required to inhibit 50% of cell population.

In entries 6 and 7, we have used DMAP as an additive in ratio of (For entry 6, 1 equiv. 2,6 leutidine : 1 equiv. DMAP; and for entry 7, 1 equiv. 2,6-leutidine : 0.5 equiv. DMAP) interestingly yield is increased. Further, we have modified the conditions in entry 8. In entry 8, we have used 2 equiv. of 2,6-lutidine and 0.2 equiv. of DMAP, the obtained yield was 85% after direct heating the reaction mixture at 80 °C for 1 h by using chilled water circulating condenser we obtain yield 85% in Scheme 1. In Scheme 2, for step b, all the conditions mentioned in Table 2 are failed to give good yields so need to modify the condition for synthesis of compound **6**.

We have modified conditions (Step (b), Scheme 2) for phenylmethanethiol by changing base as sodium hydroxide in THF and heating at 80 °C for 1 h to obtain the desired product in 87% yield which is used further for sulfonamide coupling reaction. We have modified the base and we got the yield 87% without purification which is used further for synthesis of compounds **7a-e**. During synthesis of compound **6** by using Table 2 conditions, we got sluggish reaction mixture which is difficult to purify multiple spots on TLC and LC-MS showing multiple unidentified masses so we modified the conditions b in Scheme 2 for synthesis of compound **6**.

For Step (c) (Scheme 1), we have used Raney Ni with hydrogen gas pressure of 60 psi in Parr shaker in saturated methanolic ammonia for 3 h at room temperature; it gives the best results and yield obtained is 85%. For Step (d) (Scheme 1 and 2), we have used 1:1 mixture of DCM and pyridine as a solvent and base to get yields ranging from 80-95% for all the compounds **4a-e** and **7a-e**.

3.2. Anticancer activity

All the newly synthesized compounds **4a-e** and **7a-e** were evaluated for their antiproliferative activities against a panel of four different human cancer cell lines. The IC_{50} for each synthesized compounds are calculated with respect to one human normal cell line Human umbilical vein endothelial cell line (ATCC CRL-1730) and results are summarized in Table 3. These values represent the concentration required to inhibit 50% cell population compared with the control cells treated with DMSO and positive control 5-fluorouracil under similar conditions. From compounds **4a-4e**, the IC_{50} value ranges from 10.65 to 33.81 μM all four cell lines. For cell line A-549 compound **4c** is most active with IC_{50} value of 10.65 μM and compound **4d** is also active with IC_{50} value of 10.81 μM. Compound **4b** is very less active with IC_{50} value of 33.81 μM. Similar results are obtained with remaining cell lines. Compound **4d** having IC_{50} values of 10.79, 12.86 and 10.82 μM in HeLa, MCF-7 and DU-145 cell lines, respectively. The compound **4c** is active in A-549 only, it is less active in HeLa, MCF-7 and DU-145 cell lines with IC_{50} values of 28.32, 27.32 and 20.73 μM, respectively. The compounds **7a-e** the IC_{50} value ranges from 10.78 to 33.82 μM. For cell line A-549, compound **7d** and **7c** are active with IC_{50} value of 10.82 and10.78 μM, respectively. Compound **7b** is less active with IC_{50} value of 33.82 μM. Similar results are obtained with remaining cell lines. Compound **7d** having IC_{50} values of 10.99, 12.36 and 19.52 μM in HeLa, MCF-7 and DU-145 cell lines, respectively. The compound **7c** is active in A-549 and also HeLa, MCF-7 and DU-145 cell lines with IC_{50} values of 10.78, 13.82 and 14.72 μM, respectively. In all the four cell lines the compounds having phenyl sulfonamide ring (Compound **4a** and **7a**) are moderately active with IC_{50} values from 22.72 to 28.86 μM, respectively. Compounds having para-methyl phenyl sulfonamide ring (compound **4b** and **7b**) are most inactive with IC_{50} value 20.36 to 33.82 μM, respectively. The compound having only methyl sulfonamide ring (compound **4e** and **7e**) is having moderate IC_{50} values from 14.38 to 23.86 μM, respectively. The compound **4e** is most active in HeLa cell line (ATCC CCL-2) with IC_{50} value of 14.38 μM, compare to it is inactive with remaining cell lines. Interestingly compounds having electron withdrawing group at *para* position of aromatic ring are most active on all four cell lines and that of electron donating groups are less active on all cell lines. If we replace aromatic ring with methane sulfonamide then also activity is less compared to aromatic substitutions. These are results from both series of compounds. There is not much difference in their inhibitions in all four cancer cell lines. Further we have studied the most active compounds **4d**, **7c** and **7d** on human kinases.

Table 4 Inhibitory activity of compound **4d**, **7c** and **7d** against panel of eight human kinases.

Kinase	% Inhibition (Compound 4d)	% Inhibition (Compound 7c)	% Inhibition (Compound 7d)
Aurora-A	33	34	31
Aurora-B	31	30	37
CDK2/cyclinA	28	37	43
CDK2/cyclinE	17	12	11
CDK5/P25	66	59	70
EGFR	4	21	15
mTOR	34	38	36
PDK1	13	21	9

The most active compounds **4d**, **7c** and **7d** were tested for its activity against a panel of eight human kinase at 10 μM concentrations. The results are summarized in Table 4. The compounds **4d** and **7d** having electron withdrawing group shows very promising activity against CDK5/P25 kinase with 66 and 70% inhibitions, respectively. Compound **7c** also shows promising activity of 59% inhibition. For remaining all the kinases Aurora-A, Aurora-B, mTOR and CDK/cyclinA shows moderate activity in the range of 28 to 43% inhibitions. For remaining kinase CDK/cyclinE, PDK1 and EGFR the activity is poor in the range of 4 to 21% inhibitions.

From all above IC_{50} values and the % inhibitions study, the SAR can be explained on the basis of substitutions on the R_1 position with aromatic or aliphatic substitution. The compounds containing electron withdrawing groups on R_1 phenyl ring show good to moderate activity (Compound **4c**, **4d**, **7c** and **7d**) when compared with the other simple substitution on R_1 phenyl ring. Very low activity is observed with the substitution of electron donating methyl group on the phenyl ring.

4. Conclusion

This reported work deals with the non-asymmetric synthesis of benzoxazine derivatives and their evaluation as antiproliferative agents against different cancer cell lines and in the growth of eight human kinases. The obtained yields were good to excellent with high purity. The compounds were synthesized on gram scale by using series of reactions having reduction and coupling reactions. We have optimized all the reaction steps for getting good yields. The compounds **4c**, **4d**, **7c** and **7d** were found to be most active on the treated cell lines, and compound **4d, 7c** and **7d** shows promising activity against CDK5/P25 kinase inhibitors.

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References

[1]. Alafeefy, A. M.; Ashour, A. E.; Prasad, O.; Sinha, L.; Pathak, S.; Alasmari, F. A.; Rishi, A. K.; Abdel-Aziz, H. A. *Eur. J. Med. Chem.* **2015**, *92*, 191-201.

[2]. Alberg, A. J.; Brock, M. V.; Samet, J. M. *J. Clin. Oncol.* **2005**, *23*, 3175-3185.

[3]. Zhou, X. W.; Ma, H. L.; Zhang, X.; Jing, S. Y.; Miao, J. Y.; Zhao, B. X. *Eur. J. Med. Chem.* **2014**, *79*, 95-101.

[4]. Lian, S.; Su, H.; Zhao, B. X.; Liu, W. Y.; Zheng, L.W.; Miao, J. Y. *Bioorg. Med. Chem.* **2009**, *17*, 7085-7092.

[5]. Wang, A.; Prouty, C. P.; Pelton, P. D.; Yong, M.; Demarest, K. T.; Murray, W. V.; Kuo, G. H. *Bioorg. Med. Chem.* **2010**, *20*, 1432-1435.

[6]. Matralis, A. N.; Katselou, M. G.; Nikitakis, A.; Kourounakis, A. P. *J. Med. Chem.* **2011**, *54*, 5583-5591.

[7]. Hasui, T.; Matsunaga, N.; Ora, T.; Ohyabu, N.; Nishigaki, N.; Imura, Y.; Igata, Y.; Matsui, H.; Motoyaji, T.; Tanaka, T.; Habuka, N.; Sogabe, S.; Ono, M.; Siedem, C.S.; Tang, T. P.; Gauthier, C.; De Meese, L. A.; Boyd, S. A.; Fukumoto, S. *J. Med. Chem.* **2011**, *54*, 8616-8631.

[8]. Filippou, P. S.; Koini, E. N.; Calogeropoulou, T.; Kalliakmani, P.; Panagiotidis, C. A.; Kyriakidis, D. A. *Bioorg. Med. Chem. Lett.* **2011**, *19*, 5061-5070.

[9]. Geng, B.; Comita-Prevoir, J.; Eyermann, C. J.; Reck, F.; Fisher, S. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5432-5435.

[10]. Borate, H. B.; Maujan, S. R.; Sawargave, S. P.; Chandavarkar, M. A.; Vaiude, S. R.; Joshi, V. A.; Wakharkar, R. D.; Iyer, R.; Kelkar, R. G.; Chavan, S. P.; Kunte, S. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 722-725.

[11]. Fringuelli, R.; Pietrella, D.; Schiaffella, F.; Guarraci, A.; Perito, S.; Bistoni, F.; Vecchiarelli, A. *Bioorg. Med. Chem.* **2002**, *10(6)*, 1681-1686.

[12]. Fringuelli, R.; Giacche, N.; Milanese, L.; Cenci, E.; Macchiarulo, A.; Vecchiarelli, A.; Costantino, G.; Schiaffella, F. *Bioorg. Med. Chem.* **2009**, *17*, 3838-3846.

[13]. Rajitha, C.; Dubey, P. K.; Sunku, V.; Piedrafita, F. J.; Veeramaneni, V. R.; Pal, M. *Eur. J. Med. Chem.* **2011**, *46*, 4887-4896.

[14]. Honda, T.; Terao, T.; Aono, H.; Ban, M. *Bioorg. Med. Chem.* **2009**, *17*, 699-708.

[15]. Hasui, T.; Ohra, T.; Ohyabu, N.; Asano, K.; Matsui, H.; Mizukami, A.; Habuka, N.; Sogabe, S.; Endo, S.; Siedem, C. S.; Tang, T. P.; Gauthier, C.; De Meese, L. A.; Boyd, S. A.; Fukumoto, S. *Bioorg. Med. Chem.* **2013**, *21*, 5983-5994.

[16]. Nguyen, K. T.; Li, F.; Poda, G.; Smil, D.; Vedadi, M.; Schapira, M. *J. Chem. Inf. Model.* **2013**, *53*, 681-691.

[17]. Konda, S.; Raparthi, S.; Bhaskar, K.; Munaganti, R. K.; Guguloth, V.; Nagarapu, L.; Akkewar, D. M. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1643-1646.

[18]. Chen, Z.; Wang, Z. C.; Yan, X. Q.; Wang, P. F.; Lu, X. Y.; Chen, L. W.; Zhu, H. L.; Zhang, H. W. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1947-1951.

[19]. Rathore, P.; Yaseen, S.; Ovais, S.; Bashir, R.; Yaseen, R.; Hameed, A. D.; Samim, M.; Gupta, R.; Hussain, F.; Javed, K. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1685-1691.

[20]. Darandale, S. N.; Mulla, N. A.; Pansare, D. N.; Sangshetti, J. N.; Shinde, D. B. *Eur. J. Med. Chem*. **2013**, *65*, 527-532.

[21]. Pawar, C. D.; Sarkate, A. P.; Karnik, K. S.; Bahekar, S. S.; Pansare, D. N.; Shelke, R. N.; Jawale, C. S.; Shinde, D. B. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 3525-3528.

[22]. Singh, S. N.; Jayaprakash, S.; Reddy, K. V.; Nakhi, A.; Pal, M. *RSC Adv*. **2015**, *5(103)*, 84889-84893.

[23]. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Jonathan, T. W.; Heidi, B.; Susan, K.; Michael, R. B. *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112.

[24]. Vichai, V.; Kirtikara, K. *Nat. Protoc.* **2006**, *1*, 1112-1116.