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Advances in Bioresearch

ORIGINAL ARTICLE

Synthesis, Characterization, and Anticancer activity of some 1-(5-(4-chlorophenyl)-1,3,4-oxadiazol-3(2*H*)-yl)ethanoneDerivatives as Potential EGFR inhibitors

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ABSTRACT
In present study, 1-(5-(4-chlorophenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone derivatives were synthesized, characterized, and evaluated for anticancer activity. All of the synthetic compounds were put through a screening process to see whether or not they were able to inhibit EGFR kinase activity. Erlotinib was used as the reference molecule in this method.Sm9 and Sm12 compounds were found to be the most active EGFR inhibitor that exhibited 76.28 and 84.92% inhibition of AKT kinase activity at 10 µM.Compounds Sm9, and Sm12 showed excellent EGFR kinase inhibitory activity with GI_{50} of 4.17 ± 0.56, and 1.68 ± 0.13 µM respectively, whereas erlotinib displayed 0.43 ± 0.18 µM. The cytotoxicity of synthesized compounds was evaluated against three cancerous cell lines skin (A-431), kidney (A-498), and lung (A549) via SRB assay. Molecules displaying >50% inhibition at 10 µM.Sm9 displayed GI_{50} values of 12.85±0.027, 9.72±0.038, and 9.52±0.035µM, respectively against A-431, A-498, and A-549. Sm12 showed GI_{50} values of 11.14±0.017, 6.67±0.023, and 8.13±0.039µM respectively, against A-431, A-498, and A-549 whereas erlotinib showed 6.611±0.024, 5.71±0.033, and 7.20±0.041µM. Therefore, based on the results of this investigation, we came to the conclusion that Sm9 and Sm12 have the ability to be considered as possible lead compounds for the continued development of a powerful EGFR inhibitor as a potential anticancer drug.
Keywords: *Anticancer; EGFR inhibitors; A-431; A-498; A-549; cell lines*

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INTRODUCTION

EGFR (epidermal growth factor receptor) inhibitors are used in the treatment of cancer because they target a specific protein that plays a critical role in the growth and proliferation of cancer cells. The EGFR protein is found on the surface of cells and is involved in signaling pathways that promote cell growth and division[1]. In cancer cells, the EGFR protein can be overactive, leading to uncontrolled cell growth and proliferation. EGFR inhibitors work by binding to the EGFR protein and preventing it from activating downstream signaling pathways that promote cell growth and division. This can cause cancer cells to stop growing and eventually die. EGFR inhibitors are used to treat several types of cancer, including non-small cell lung cancer, colorectal cancer, and head and neck cancer. They are often used in combination with other cancer treatments, such as chemotherapy, radiation therapy, and surgery. They are also used as maintenance therapy to help prevent cancer recurrence[2–5].EGFR inhibitors have been found to be particularly useful in treating certain types of cancer that have specific mutations in the EGFR gene. For example, the presence of a specific mutation called the T790M mutation in the EGFR gene has been found to confer resistance to first- and second-generation EGFR inhibitors. Fourth-generation EGFR inhibitors have been developed to target these specific mutations and thus overcome resistance.In summary, EGFR inhibitors are used in the treatment of cancer because they target a specific protein that plays a critical role in the growth and proliferation of cancer cells, and they have been found to be particularly useful in treating certain types of cancer that have specific mutations in the EGFR gene[6–10]. Fourth-generation

EGFR inhibitors are a newer class of drugs that have been developed to target specific mutations in the EGFR protein that are known to confer resistance to first-, second-, and third-generation EGFR inhibitors[11–13].EGFR T790M mutation is the most common genetic alteration that leads to resistance to EGFR tyrosine kinase inhibitors (TKIs) such as erlotinib and gefitinib. Fourth-generation EGFR inhibitors are designed to specifically target the T790M mutation, allowing them to overcome resistance to earlier generations of EGFR inhibitors. Examples of fourth-generation EGFR inhibitors include osimertinib, rociletinib and lazertinib. Osimertinib is an oral, irreversible inhibitor of EGFR T790M, which has been FDA approved for the treatment of T790M-positive non-small cell lung cancer.Rociletinib is an oral, irreversible inhibitor of EGFR T790M and other resistance mutations, which has been FDA approved for the treatment of T790M-positive non-small cell lung cancer.Lazertinib is an oral, irreversible inhibitor of EGFR T790M, which is in phase III clinical trial for the treatment of T790M-positive non-small cell lung cancer[13–18]. Its todays need to discover some novel fourth generation EGFR inhibitors. In present study, we have designed some 1-(5-(4-chlorophenyl)-1,3,4-oxadiazol-3(2*H*)-yl)ethanone derivatives and the derivatives which possess most drug-likeness properties and favorable ADMET character were subjected for computational analysis which we already published in our previous article[19]. The most potent molecules observed in virtual screening were subjected for wet lab synthesis, characterization, and anticancer evaluation.

MATERIAL AND METHODS

Synthesis of 1-(5-(4-chlorophenyl)-1,3,4-oxadiazol-3(2*H*)-yl)ethanonederivatives

Throughout this study, we used chemicals and reagents acquired from Lab Trading Laboratories in Aurangabad, Maharashtra, India. Spots were viewed using UV light and iodine vapours in a technique called thin layer chromatography (TLC) to track the reaction's progress. The open capillary method was used to determine the melting points (uncorrected) of the compounds. Using a JEOL 500 MHz spectrometer, DMSO was used as the solvent, and tetramethylsilane (TMS) was used as the internal standard, yielding 1H and 13C NMR spectra. Singlet (*s*), doublet (*d*), triplet (*t*), and multiplet (*m*) coupling frequencies; ^{1}H-^{1}H coupling constants in *Hertz*; chemical shifts stated in units or ppm. The synthesized compounds' mass (m/z) spectra were collected using a Shimadzu LC-MS instrument. Using a Microlab IR Spectrophotometer from Agilent Technologies, we were able to capture the infrared spectra of the produced molecules. The detailed method of the synthesis is discussed in the section given below:

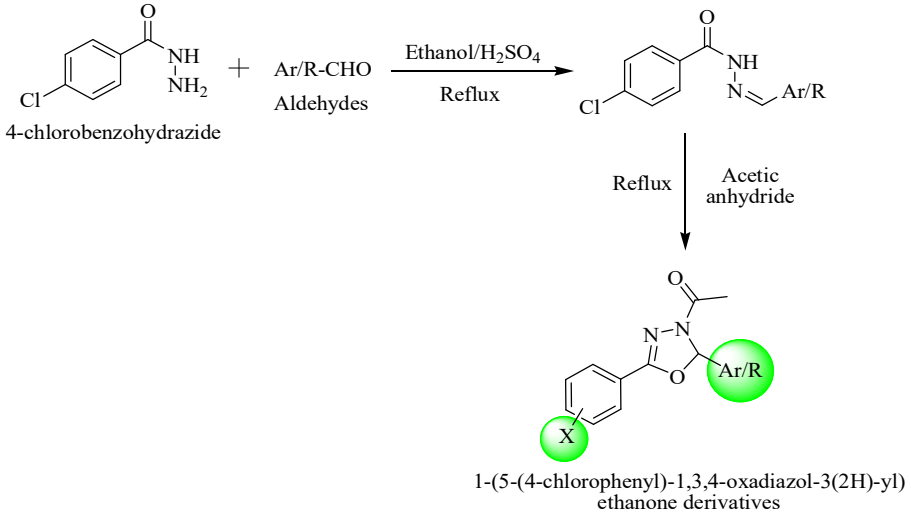


Figure 1. The reaction scheme for the synthesis of 1-(5-(4-chlorophenyl)-1, 3, 4-oxadiazol-3(2*H*)-yl) ethanone derivatives

Molecules Sm3, Sm8, Sm9, Sm10, Sm12, and Sm18 had formed either three or two conventional hydrogen bonds with EGFR enzyme and hence selected for synthesis which can be developed further to get more promising molecules for the treatment of cancer.0.01 mol of 4-chlorobenzohydrazide (1.7 gm) and respective substituted aldehydes (0.01 mol) were dissolved in 20 mL of ethanol and the reaction mixture was refluxed by adding few drops of sulphuric acid after getting cleared solution. After the reaction completion the contents were poured into the ice cold water to get solid crude product [(*Z*)-*N*'-benzylidene-4-chlorobenzohydrazide derivatives] and the product was washed with cold water three times and the obtained product was recrystallized with hot ethanol. (*Z*)-*N*'-benzylidene-4-chlorobenzohydrazide derivatives were refluxed with acetic anhydride to make internal cyclization to

afford final 1-(5-(4-chlorophenyl)-1, 3, 4-oxadiazol-3(2*H*)-yl) ethanone derivatives. The progression of reaction was monitored by TLC using benzene: ethanol: ethyl acetate (7:2:1). The detailed reaction scheme for the synthesis is depicted in Figure 1. The structures of synthesized derivatives are illustrated in Figure 2.

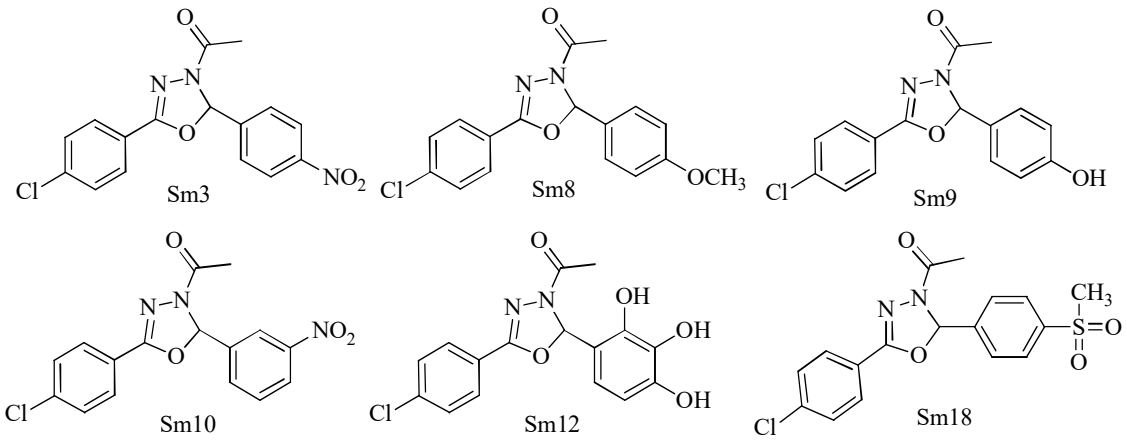


Figure 2. The structures of synthesized compounds

The physicochemical properties and spectral interpretations of synthesized compounds are depicted below:

Sm3*[1-(5-(4-chlorophenyl)-2-(4-nitrophenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone]*

Obtained as brownish solid, yield: 72%, molecular formula$C_{16}H_{12}ClN_3O_4$, melting point: 218-221^{0}C, Rf value: 0.68.Elemental analysis (*cal.*): C, 55.58; H, 3.50; Cl, 10.25; N, 12.15; O, 18.51. FT-IR (neat, cm^{-1}) ν_{max}: 3404.36 (NH stretch), 3053.32 (NH bend w), 2526.75 (Ar stretch), 1614.42 (C=O stretch), 1514.12 (N-O assy), 1382.96 (C-C stretch), 829.39 (C=O bend), 690.21 (C-Cl).^{1}H NMR (300 MHz, DMSO-d_6, chemical shift (ppm)); δ 2.201 (s, CH), 6.671 (s, diazole); 7.590, 7.602, 7.681, 7.689, 7.809, 7.819, 8.228, 8.248 (m, Ar-H). ^{13}C NMR ($CHCl_3$-d_6 400 MHz) δ ppm: MS m/z: 312.15, 313.67 (m+1), 315.21(m+2).

Sm8*[1-(5-(4-chlorophenyl)-2-(4-methoxyphenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone]*

Obtained as whitish solid, yield: 81%, molecular formula$C_{17}H_{15}ClN_2O_3$, melting point: 194-196^{0}C, Rf value: 0.74.Elemental analysis (*cal.*): C, 61.73; H, 4.57; Cl, 10.72; N, 8.47; O, 14.51. FT-IR (neat, cm^{-1}) ν_{max}: 3057.17 (NH stretch), 1961.61 (Ar stretch), 1822.73 (C=O stretch), 1440.83 (C-C stretch), 771.53 (C-Cl).^{1}H NMR (300 MHz, DMSO-d_6, chemical shift (ppm)); δ 2.218 (s, CH), 6.659 (s, diazole); 7.700, 7.789, 7.808, 7.952, 7.982, 8.001, 8.051, 8.091, 8.102 (m, Ar-H). ^{13}C NMR ($CHCl_3$-d_6 400 MHz) δ ppm: 24.00, 56.14, 84.02, 96.11, 100.04, 115.24, 128.47, 129.91, 131.09, 133.67, 137.18, 157.69, 159.12, 169.67. MS m/z: 298.01, 300.02 (m+1), 301.98 (m+2).

Sm9 *[1-(5-(4-chlorophenyl)-2-(4-hydroxyphenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone]*

Obtained as off-white solid, yield: 58%, molecular formula:$C_{16}H_{13}ClN_2O_3$, melting point: 183-185^{0}C, Rf value: 0.58.Elemental analysis (*cal.*): C, 60.67; H, 4.14; Cl, 11.19; N, 8.84; O, 15.15. FT-IR (neat, cm^{-1}) ν_{max}: 3456.44 (NH stretch), 2879.72 (NH bend w), 2358.94 (Ar stretch), 1894.10 (C=O stretch), 1446.61(C-C stretch), 773.46 (C=O bend), 658.90 (C-Cl).^{1}H NMR (300 MHz, DMSO-d_6, chemical shift (ppm)); δ 2.257 (s, CH), 6.410 (s, diazole); 7.306, 7.407, 7.421, 7.598, 7.610, 7.687, 7.710, 7.908, 8.003 (m, Ar-H). ^{13}C NMR ($CHCl_3$-d_6 400 MHz) δ ppm: 23.17, 83.23, 97.82, 101.21, 115.90, 128.10, 128.81, 130.78, 133.02, 136.89, 156.12, 156.67, 168.71. MS m/z: 283.34, 284.02 (m+1), 285.67 (m+2).

Sm10*[1-(5-(4-chlorophenyl)-2-(3-nitrophenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone]*

Obtained as brown solid, yield: 63%, molecular formula:$C_{16}H_{12}ClN_3O_4$, melting point: 204-206^{0}C, Rf value: 0.74.Elemental analysis (*cal.*): C, 55.58; H, 3.50; Cl, 10.25; N, 12.15; O, 18.51. FT-IR (neat, cm^{-1}) ν_{max}: 3404.36 (NH stretch), 3053.32 (NH bend w), 2526.75 (Ar stretch), 1614.42 (C=O stretch), 1514.12 (N-O assy), 1382.96 (C-C stretch), 829.39 (C=O bend), 740.67 (C-Cl).^{1}H NMR (300 MHz, DMSO-d_6, chemical shift (ppm)); δ 2.101 (s, CH), 6.328 (s, diazole); 7.302, 7.398, 7.412, 7.589, 7.602, 7.667, 7.700, 7.710, 7.911, 7.991, 8.099 (m, Ar-H). ^{13}C NMR ($CHCl_3$-d_6 400 MHz) δ ppm: 23.08, 82.19, 97.67, 116.20, 119.65, 121.68, 128.80, 130.00, 131.94, 132.02, 136.09, 140.08, 147.03, 156.17, 168.12. MS m/z: 312.02, 312.87, 313.38 (m+1).

Sm12*[1-(5-(4-chlorophenyl)-2-(2,3,4-trihydroxyphenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone]*

Obtained as slightly brown solid, yield: 78%, molecular formula:$C_{16}H_{13}ClN_2O_5$, melting point: 210-212^{0}C, Rf value: 0.80.Elemental analysis (*cal.*): C, 55.10; H, 3.76; Cl, 10.17; N, 8.03; O, 22.94. FT-IR (neat, cm^{-1})

ν_{max}: 3500 (-OH phenolic), 3100.01(NH stretch), 3049.46 (NH bend w), 2407.76 (Ar stretch), 1604.77 (C=O stretch), 1462.04 (N-O assy), 1371.39 (C-C stretch), 839.03 (C=O bend), 730.67 (C-Cl).^{1}H NMR (300 MHz, DMSO-d_6, chemical shift (ppm)); δ 2.078 (s, CH), 5.309, 5.317 (s, Ar-OH), 6.201, 6.219 (s, diazole); 6.509, 6.521, 6.534, 6.567, 6.589, 7.318, 7.352, 7.389, 7.419, 7.451 (m, Ar-H). ^{13}C NMR ($CHCl_3$-d_6 400 MHz) δ ppm: 22.77, 77.12, 97.65, 101.00, 107.06, 121.33, 122.19, 128.04, 129.89, 132.12, 136.03, 145.00, 147.07, 156.09, 168.12. MS m/z: 315.01, 316.17 (m+1), 318.02 (m+2).

Sm18*[1-(5-(4-chlorophenyl)-2-(4-(methylsulfonyl)phenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone]*

Obtained as whitish solid, yield: 59%, molecular formula:$C_{17}H_{15}ClN_2O_4S$, melting point: 235-237 ^{0}C, Rf value: 0.92.Elemental analysis (*cal.*): C, 53.90; H, 3.99; Cl, 9.36; N, 7.39; O, 16.89; S, 8.46. FT-IR (neat, cm^{-1}) ν_{max}: 3305.99 (NH stretch), 3120.08 (NH bend w), 2453.89 (Ar stretch), 1899.88 (C=O stretch), 1475.04 (N-O assy), 1315.45 (C-C stretch), 1030.87 (S=O) 835.18 (C=O bend), 744.52 (C-Cl). ^{1}H NMR (300 MHz, DMSO-d_6, chemical shift (ppm)); δ 2.289 (s, CH), 3.110 (methylsulfonamide), 6.388 (s, diazole); 7.314, 7.346, 7.503, 7.538, 7.601, 7.637, 7.661, 7.858, 7.921, 7.958 (m, Ar-H). ^{13}C NMR ($CHCl_3$-d_6 400 MHz) δ ppm: 23.78, 48.10, 83.71, 108.48, 111.10, 128.03, 129.02, 130.20, 136.90, 139.03, 145.08, 156.82, 169.05. MS m/z: 345.33, 346.21 (m+1), 347.23 (m+2).

EGFR Kinase Inhibitory Assay

All the reagent and working standards were prepared according to the Abbkine ELISA kit (product code: KTE62577, California, USA) for Human EGFR concentrations protocol by quantification in the cell culture supernatant of A-431. Stock solutions of Erlotinib, and synthesized compounds were prepared in dimethyl sulfoxide (DMSO) at a single concentration of 10 µM and stored at −20°C. When the cells were treated with synthesized compounds, Erlotinib was used as the positive control. The cell culture supernatant was prepared by centrifugation of the cell culture media at 1,500 rpm and 4°C for 10-15mins. Supernatants were assayed immediately or were stored at −80°C for use. The pre-absorbed plates were taken for sample incubation and a volume of 10 µl of each of the diluted sample solutions and control was pipetted to each empty well in duplicate. The plate was covered with an adhesive plastic and was incubated for 45 mins at room temperature. The contents were removed from the wells and washed five times with 250 µL wash buffer for 3-4 mins per each time. Flicked the plate and blotted it against clean paper. Then antibody incubation process was carried out with help of HRP-conjugated detection antibody. A volume of 50 µl of diluted antibody was added to the wells with control, standard and synthesized compounds samples. Plate was again covered with an adhesive plastic and was incubated for 30 mins at room temperature. The contents were again removed from the wells followed by earlier washing method for 3-4 mins per each time. A volume of 50µl of diluted Chromogen A solution and Chromogen B was added to the each well. Gently mixed and incubated for 15 mins at 37°C in the dark. Removed the content in the wells and washed them five times with 250 µl wash buffer for 3-4 mins per each time. Flicked the plate and patted the plate. After sufficient color development, 50 µl of stop solution was added to the wells. Optical density was recorded with a plate reader at 450 nm. Data was analyzed by preparing a standard curve using the diluted standard solutions (20–320 pg/ml) by plotting absorbance on the y-axis (linear) and concentration on the x-axis. The sample EGFR concentration (pg/ml) was interpreted from the standard curve. Results are presented as percentage enzyme inhibition and compared to erlotinib as a reference EGFR-TK inhibitor[20,21].

Cell line Studies

Cell lines and Reagents

All the cell lines: A-431 (Human Skin Epidermoid Carcinoma Epithelial Cells), A-498 (Human Renal Carcinoma cell line), A549 (lung adenocarcinoma) were purchased from National Centre for Cell Science (Pune, India). On receipt, the cell lines were passaged in our lab and the earliest passaged cells were cryopreserved in liquid nitrogen container for future use. The cell lines used in culture were passaged for fewer than 8 weeks and were carefully maintained as described. The cells were maintained in Dulbecco's modified Eagle medium (Cell clone genetix brand, Catalogue No.: CC3004) complete media with 10% fetal bovine serum (Cell clone genetix brand, Catalogue No.: CCS-500-SA-U3034)) and penicillin–streptomycin (50 U/ml, 50 mg/ml; HiMedia, Catalogue No.: A002) at 37°C, CO_2 (5%) and air (95%). Around 70–80% confluency of cultured cells was used for seeding during the assays. Dulbecco's phosphate-buffered saline (Cell clone genetix brand, Catalogue No.: CC3034), trypan blue (Bio-Rad, catalogue no: 1450013).

***In vitro* anticancer activity (SRB Assay)**

Cytotoxic activity of the compounds was evaluated by colorimetric SRB (Sulforhodamine B) assay on A-431 (Human Skin Epidermoid Carcinoma Epithelial Cells), A-498 (Human Renal Carcinoma cell line), A549 (lung adenocarcinoma) using Erlotinib as positive control. Briefly, logarithmically growing cells were seeded in 96-well plate (seeding density: A-431: 5,000 cells/well, A549: 5,000 cells/well, and A-498 (5000 cells/well) incubated for 24 hr in humidified condition (5% CO_2) at 37°C and then observed

under microscope. Test compounds with appropriate dilution were then added to the wells in triplicate along with DMSO as vehicle control. After 72hr of test compound exposure in 5% CO_2humidified condition at 37°C, at the end of incubation period each well was treated with 50 µl of ice-cold trichloroacetic acid (10% TCA) and it was further incubated for 1-2 hrat 4°C for cell fixation. The cells were washed using distilled water to remove excess TCA and allowed to dry in the air. After 2 h, 50 µl of SRB solution (0.045% w/v) was added to each well and allowed to stain at room temperature for 30 mins. The plate was washed with 1% v/v acetic acid to remove the unbound dye and was allowed to dry in the air. About 100µl of 10 mM unbufferedTris Base (pH 10.5) was added to each well and the plates were gently shaken for 5 mins on a shaker platform to extract the bound SRB. The absorbance was measured using an Epochmicroplate reader at a wavelength of 510nm[22].

Half Maximal Growth Inhibition (GI_{50}) Calculation

The molecules exhibited a convincingly potential cytotoxic effect in most of the tested cancer cell lines and were found to be active at less than 50 µM concentration. Compounds indicating 50 percent or more growth inhibition in tested cell lines were further screened at nine doses (i.e. 0.5 µM, 0.1, 5, 1, 5, 10, 30, 50 and 100 µM) and growth inhibition were calculated as GI_{50} values reflecting the concentration of drugs required to cause 50 percent cell growth inhibition[22–24].

RESULTS AND DISCUSSION

EGFR Kinase Inhibitory Assay

All the synthesized compounds were screened to evaluate their ability to inhibit EGFR kinase activity using Erlotinib as reference compound. *In vitro* kinase inhibition assay was performed at a testing dose of 10 µM by using the Abbkine ELISA kit (product code: KTE62577, California, USA).EGFR inhibitory activity of these compounds was analyzed and compared with the reference EGFR inhibitor. Amongst the tested compounds, two compounds(**Sm9** and **Sm12)**inhibited EGFR kinase by more than 50%. **Sm9** and **Sm12** compound was found to be the most active EGFR inhibitor that exhibited 76.28 and 84.92% inhibition of AKT kinase activity at 10 µM.They were selected and subjected to GI_{50} value determination. The % inhibition of compounds at 10 µM is tabulated in Table 1 and the graph illustrated in Figure 3.These potential moleculesfurther evaluated for GI_{50}value determination. Compounds **Sm9,** and **Sm12** showed excellent EGFR kinase inhibitory activity with GI_{50} 4.17 ± 0.56, and 1.68 ± 0.13 µM respectively, whereas erlotinib displayed EGFR kinase inhibitory activity at 0.43 ± 0.18 µM. Compound **Sm12**was found to be most potent EGFR inhibitor.

Table 1.The % enzyme inhibition of compounds at 10 µM

Sr. No.	Compoundcode	% enzyme inhibition at 10 µM
1	Sm3	34.06 ± 1.12
2	Sm8	46.11 ± 2.61
3	**Sm9**	**76.28 ± 3.16**
4	Sm10	44.29 ±1.01
5	**Sm12**	**84.92 ± 2.26**
6	Sm18	50.87 ± 0.72
7	Erlotinib	100±1.05

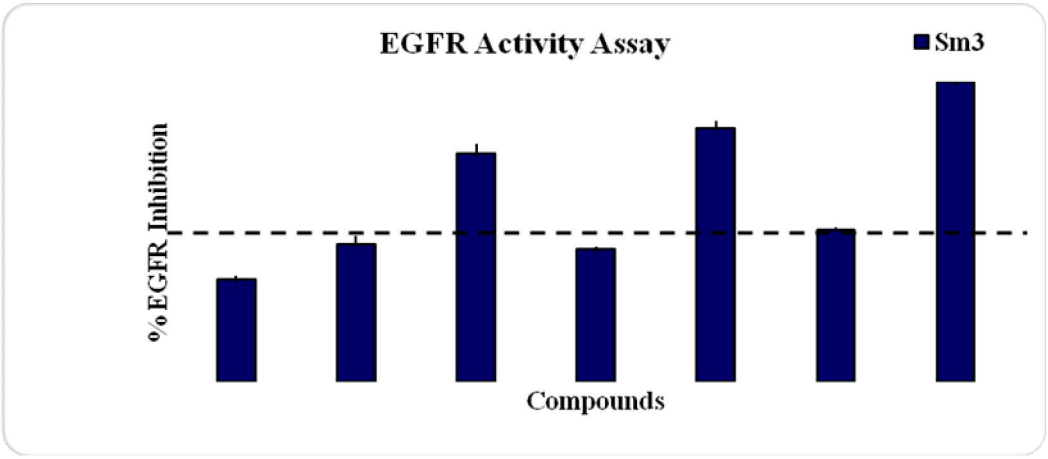


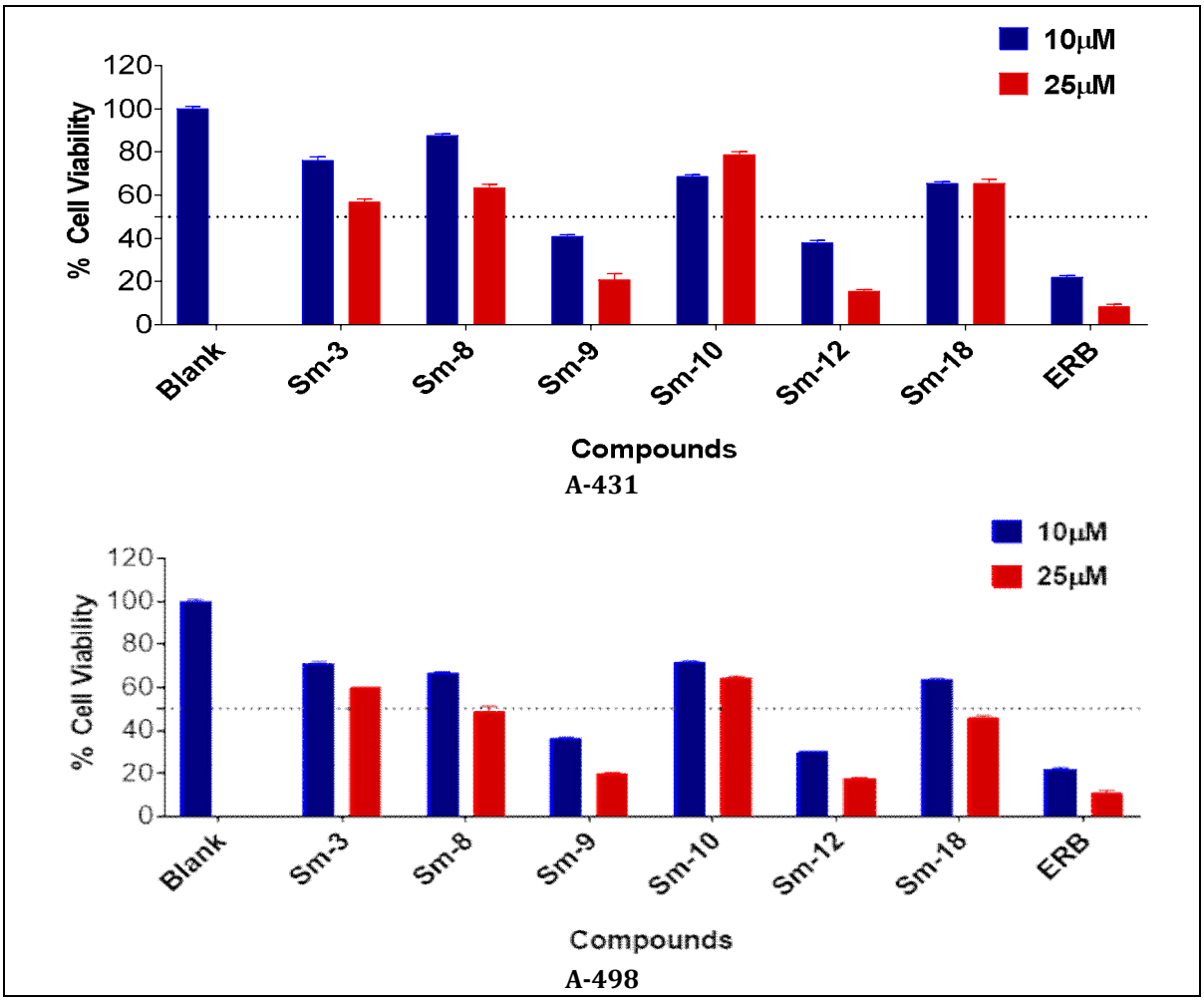
Figure 3. The % enzyme inhibition graph of compounds

***In vitro* Cell line Screening of Compounds**

The cytotoxicity of synthesized compounds was evaluated against three cancerous cell lines skin (A-431), kidney (A-498), and lung (A549) via SRB assay. Initially, two doses of each compound from synthesized series (10 µM and 25 µM) were evaluated for biological screening using SRB assay. Results obtained from biological screening data are presented in Table 2. Amongstall the compounds, it has been noticed that only two molecules (**Sm9** and **Sm12**)showed>50 % inhibition at 10 micromolar concentration against all the mentioned cell line. ErlotinibHCl (ERB) and the vehicle DMSOwere used as positive control and negative control, respectively. The % viability graphs of compounds are depicted in Figure 4.

Table 2. % viability of compounds on different cell lines

Sr. No.	Comp. code	% Viability at 10µM		
		A-431	A-498	A-549
1	Sm3	76.033	71.033	63.56
2	Sm8	87.467	66.467	67.467
3	**Sm9**	**40.966**	**35.966**	**36.514**
4	Sm10	68.62	71.62	85.62
5	**Sm12**	**38.04**	**30.04**	**33.04**
6	Sm18	65.337	63.337	78.337
7	Erlotinib	21.89	21.69	23.12



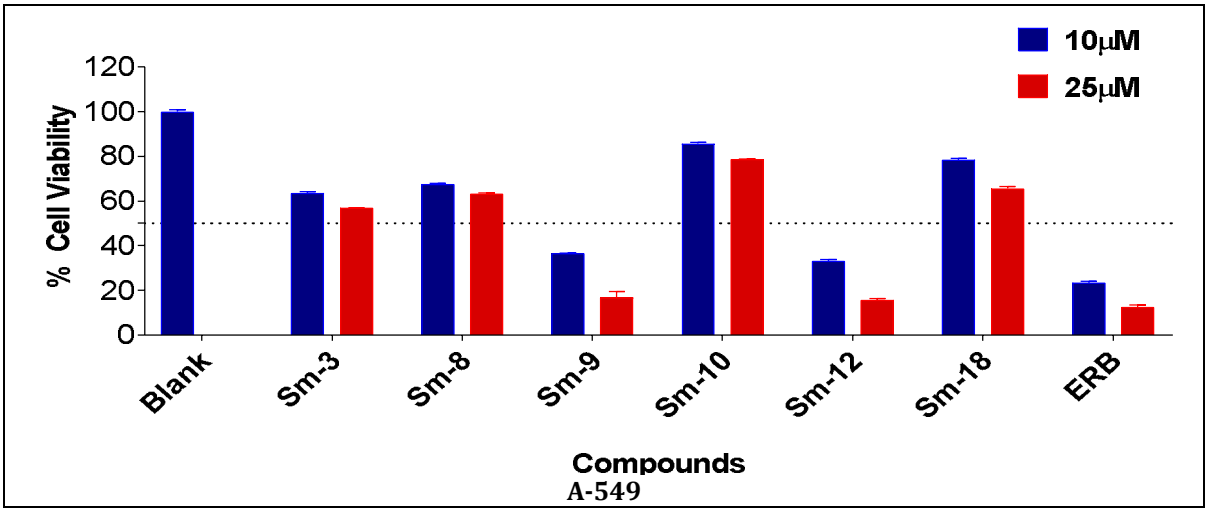
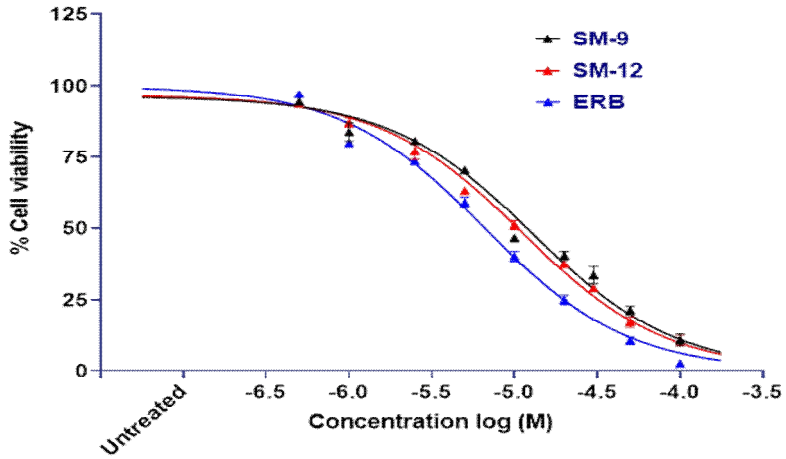


Figure 4.The % viability graphs of compounds

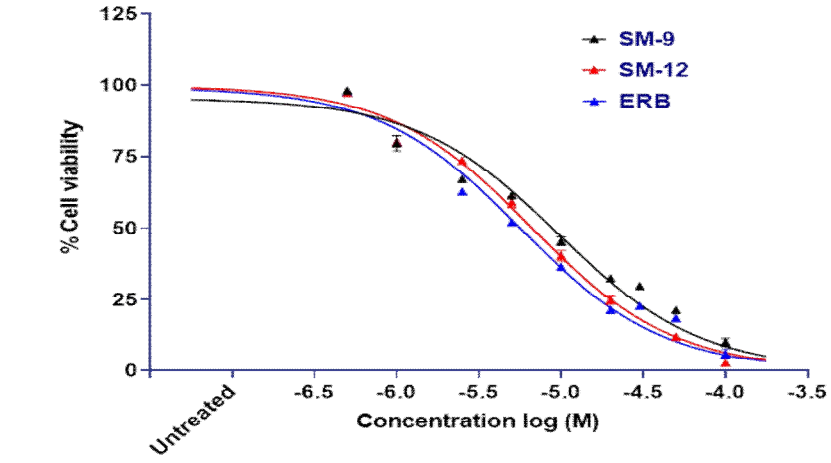
Molecules displaying >50% inhibition at 10 μM concentration were further taken up for GI_{50} determination.**Sm9** and **Sm12** displayed more than 50% inhibition and therefore GI_{50} was determined. The graphs of GI_{50} calculations are depicted in Figure 5. The values are tabulated in Table 3.Sm9 displayed GI_{50} values of 12.85±0.027, 9.72±0.038, and 9.52±0.035μM, respectively against A-431, A-498, and A-549. Sm12 showed GI_{50} values of 11.14±0.017, 6.67±0.023, and 8.13±0.039μMrespectively, against A-431, A-498, and A-549 whereas erlotinib showed 6.611±0.024, 5.71±0.033, and 7.20±0.041μM. Both the compounds displayed more potency against cell lines A-498 and A-549.

Table 3. GI_{50} values of selected compounds against different cell lines

Entry	Comp. code	GI_{50} ± SD (μM)		
		A-431	A-498	A-549
1	Sm9	12.85 ± 0.027	9.72 ±0.038	9.52 ± 0.035
2	Sm12	11.14 ± 0.017	6.67 ± 0.023	8.13 ± 0.039
3	Erlotinib	6.611± 0.024	5.71 ± 0.033	7.20 ± 0.041



A-431



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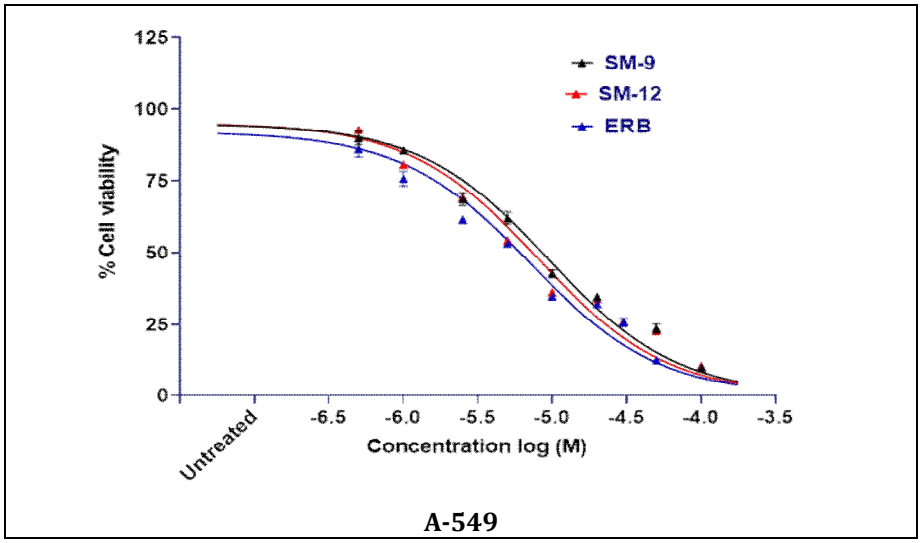

A-549

Figure 5. GI_{50} values of Sm9 and Sm12 against different cell lines

CONCLUSION

In present study, 1-(5-(4-chlorophenyl)-1,3,4-oxadiazol-3(2*H*)-yl)ethanonederivativeswere synthesized, characterized, and evaluated for anticancer activity. All the synthesized compounds were screened to evaluate their ability to inhibit EGFR kinase activity using Erlotinib as reference compound. *In vitro* kinase inhibition assay was performed at a testing dose of 10 µM.**Sm9** and **Sm12** compoundswere found to be the most active EGFR inhibitor that exhibited 76.28 and 84.92% inhibition of AKT kinase activity at 10 µM.They were selected and subjected to GI_{50} value determination. Compounds **Sm9,** and **Sm12** showed excellent EGFR kinase inhibitory activity with $GI_{50}$of 4.17 ± 0.56, and 1.68 ± 0.13 µM respectively, whereas erlotinib displayed 0.43 ± 0.18 µM.The cytotoxicity of synthesized compounds was evaluated against three cancerous cell lines skin (A-431), kidney (A-498), and lung (A549) via SRB assay.Molecules displaying >50% inhibition at 10 µM concentration were further taken up for GI_{50} determination.**Sm9** and **Sm12** displayed more than 50% inhibition and therefore GI_{50} was determined. **Sm9** displayed GI_{50} values of 12.85±0.027, 9.72±0.038, and 9.52±0.035µM, respectively against A-431, A-498, and A-549. **Sm12** showed GI_{50} values of 11.14±0.017, 6.67±0.023, and 8.13±0.039µMrespectively, against A-431, A-498, and A-549 whereas erlotinib showed 6.611±0.024, 5.71±0.033, and 7.20±0.041µM. Therefore from present study we concluded that **Sm9** and **Sm12** can be treated as potential lead compounds for the further development of potent EGFR inhibitor aspotential anticancer agents.

CONFLICTS OF INTERESTS

Authors declared that there is no conflicts of interest exists.

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REFERENCES

1. H. Neves, H.F. Kwok, (2015). Recent advances in the field of anti-cancer immunotherapy, BBA Clin. 3 280–288. https://doi.org/10.1016/j.bbacli.2015.04.001.
2. L. Huang, L. Fu, (2015). Mechanisms of resistance to EGFR tyrosine kinase inhibitors, Acta Pharm. Sin. B. 5 390–401. https://doi.org/10.1016/j.apsb.2015.07.001.
3. I. Sullivan, D. Planchard, (2016). Next-generation EGFR tyrosine kinase inhibitors for treating EGFR-mutant lung cancer beyond first line, Front. Med. 3. https://doi.org/10.3389/fmed.2016.00076.
4. H.M. Patel, I. Ahmad, R. Pawara, M. Shaikh, S. Surana, (2021). In silico search of triple mutant T790M/C797S allosteric inhibitors to conquer acquired resistance problem in non-small cell lung cancer (NSCLC): a combined approach of structure-based virtual screening and molecular dynamics simulation, J. Biomol. Struct. Dyn. 39 1491–1505. https://doi.org/10.1080/07391102.2020.1734092.
5. R. Roskoski, (2016). Cyclin-dependent protein kinase inhibitors including palbociclib as anticancer drugs, Pharmacol. Res. 107; 249–275. https://doi.org/10.1016/j.phrs.2016.03.012.
6. K. Miyazaki, T. Tamura, T. Kaburagi, K. Saito, M. Inagaki, T. Yamashita, H. Ichimura, T. Nawa, T. Endo, K. Hayashihara, M. Kimura, K. Kurishima, H. Nakamura, K. Furukawa, N. Kikuchi, H. Satoh, N. Hizawa, Real clinical practice of using afatinib therapy in NSCLC patients with an acquired EGFR T790M mutation, Anticancer Res. 38 (2018) 5409–5415. https://doi.org/10.21873/anticanres.12871.
7. S. Wan, R. Yan, Y. Jiang, Z. Li, J. Zhang, X. Wu, Insight into binding mechanisms of EGFR allosteric inhibitors using molecular dynamics simulations and free energy calculations, J. Biomol. Struct. Dyn. 37 (2019) 4384–4394. https://doi.org/10.1080/07391102.2018.1552197.
8. D.S.W. Tan, N.B. Leighl, G.J. Riely, J.C.H. Yang, L. V. Sequist, J. Wolf, T. Seto, E. Felip, S.P. Aix, M. Jonnaert, C. Pan, E.Y. Tan, J. Ko, S.E. Moody, D.W. Kim, Safety and efficacy of nazartinib (EGF816) in adults with EGFR-mutant non-small-cell lung carcinoma: a multicentre, open-label, phase 1 study, Lancet Respir. Med. 8 (2020) 561–572. https://doi.org/10.1016/S2213-2600(19)30267-X.
9. H. Cheng, S.K. Nair, B.W. Murray, C. Almaden, S. Bailey, S. Baxi, D. Behenna, S. Cho-Schultz, D. Dalvie, D.M. Dinh, M.P. Edwards, J.L. Feng, R.A. Ferre, K.S. Gajiwala, M.D. Hemkens, A. Jackson-Fisher, M. Jalaie, T.O. Johnson, R.S. Kania, S. Kephart, J. Lafontaine, B. Lunney, K.K.C. Liu, Z. Liu, J. Matthews, A. Nagata, S. Niessen, M.A. Ornelas, S.T.M. Orr, M. Pairish, S. Planken, S. Ren, D. Richter, K. Ryan, N. Sach, H. Shen, T. Smeal, J. Solowiej, S. Sutton, K. Tran, E. Tseng, W. Vernier, M. Walls, S. Wang, S.L. Weinrich, S. Xin, H. Xu, M.J. Yin, M. Zientek, R. Zhou, J.C. Kath,(2016). Discovery of 1-{(3R,4R)-3-[({5-Chloro-2-[(1-methyl-1H-pyrazol-4-yl)amino]-7H-pyrrolo[2,3-d]pyrimidin-4-yl}oxy)methyl] -4-methoxypyrrolidin-1-yl}prop-2-en-1-one (PF-06459988), a Potent, WT Sparing, Irreversible Inhibitor of T790M-Containing EGFR Mutants, J. Med. Chem. 59; 2005–2024. https://doi.org/10.1021/acs.jmedchem.5b01633.
10. Y. Zhang, J. Sheng, Y. Yang, W. Fang, S. Kang, Y. He, S. Hong, J. Zhan, Y. Zhao, C. Xue, Y. Ma, T. Zhou, S. Ma, F. Gao, T. Qin, Z. Hu, Y. Tian, X. Hou, Y. Huang, N. Zhou, H. Zhao, L. Zhang, (2016). Optimized selection of three major EGFR-TKIs in advanced EGFR-positive non-small cell lung cancer: A network meta-analysis, Oncotarget. 7; 20093–20108. https://doi.org/10.18632/oncotarget.7713.
11. Y. Sakuma, Y. Yamazaki, Y. Nakamura, M. Yoshihara, S. Matsukuma, H. Nakayama, T. Yokose, Y. Kameda, S. Koizume, Y. Miyagi, (2012). WZ4002, a third-generation EGFR inhibitor, can overcome anoikis resistance in EGFR-mutant lung adenocarcinomas more efficiently than Src inhibitors, Lab. Investig. 92; 371–383. https://doi.org/10.1038/labinvest.2011.187.
12. R.S.M. Ismail, N.S.M. Ismail, S. Abuserii, D.A. Abou El Ella, Recent advances in 4-aminoquinazoline based scaffold derivatives targeting EGFR kinases as anticancer agents, Futur. J. Pharm. Sci. 2 (2016) 9–19. https://doi.org/10.1016/j.fjps.2016.02.001.
13. J. Engel, A. Richters, M. Getlik, S. Tomassi, M. Keul, M. Termathe, J. Lategahn, C. Becker, S. Mayer-Wrangowski, C. Grütter, N. Uhlenbrock, J. Krüll, N. Schaumann, S. Eppmann, P. Kibies, F. Hoffgaard, J. Heil, S. Menninger, S. Ortiz-Cuaran, J.M. Heuckmann, V. Tinnefeld, R.P. Zahedi, M.L. Sos, C. Schultz-Fademrecht, R.K. Thomas, S.M. Kast, D. Rauh, Targeting Drug Resistance in EGFR with Covalent Inhibitors: A Structure-Based Design Approach, J. Med. Chem. 58 (2015) 6844–6863. https://doi.org/10.1021/acs.jmedchem.5b01082.
14. H. Patel, R. Pawara, A. Ansari, S. Surana, Recent updates on third generation EGFR inhibitors and emergence of fourth generation EGFR inhibitors to combat C797S resistance, Eur. J. Med. Chem. 142 (2017) 32–47. https://doi.org/10.1016/j.ejmech.2017.05.027.
15. J.C.H. Yang, Y.L. Wu, M. Schuler, M. Sebastian, S. Popat, N. Yamamoto, C. Zhou, C.P. Hu, K. O'Byrne, J. Feng, S. Lu, Y. Huang, S.L. Geater, K.Y. Lee, C.M. Tsai, V. Gorbunova, V. Hirsh, J. Bennouna, S. Orlov, T. Mok, M. Boyer, W.C. Su, K.H. Lee, T. Kato, D. Massey, M. Shahidi, V. Zazulina, L. V. Sequist, (2015). Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): Analysis of overall survival data from two randomised, phase 3 trials, Lancet Oncol. 16, 141–151. https://doi.org/10.1016/S1470-2045(14)71173-8.

16. R. Rosell, E. Carcereny, R. Gervais, A. Vergnenegre, B. Massuti, E. Felip, R. Palmero, R. Garcia-Gomez, C. Pallares, J.M. Sanchez, R. Porta, M. Cobo, P. Garrido, F. Longo, T. Moran, A. Insa, F. De Marinis, R. Corre, I. Bover, A. Illiano, E. Dansin, J. de Castro, M. Milella, N. Reguart, G. Altavilla, U. Jimenez, M. Provencio, M.A. Moreno, J. Terrasa, J. Muñoz-Langa, J. Valdivia, D. Isla, M. Domine, O. Molinier, J. Mazieres, N. Baize, R. Garcia-Campelo, G. Robinet, D. Rodriguez-Abreu, G. Lopez-Vivanco, V. Gebbia, L. Ferrera-Delgado, P. Bombaron, R. Bernabe, A. Bearz, A. Artal, E. Cortesi, C. Rolfo, M. Sanchez-Ronco, A. Drozdowskyj, C. Queralt, I. de Aguirre, J.L. Ramirez, J.J. Sanchez, M.A. Molina, M. Taron, L. Paz-Ares, (2012). Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial, Lancet Oncol. 13, 239–246. https://doi.org/10.1016/S1470-2045(11)70393-X.
17. Z. Song, Y. Ge, C. Wang, S. Huang, X. Shu, K. Liu, Y. Zhou, X. Ma,(2016). Challenges and perspectives on the development of small-molecule EGFR inhibitors against T790M-mediated resistance in non-small-cell lung cancer: Miniperspective, J. Med. Chem. 59; 6580–6594. https://doi.org/10.1021/acs.jmedchem.5b00840.
18. J.W. Carlisle, S.S. Ramalingam, (2019). Role of osimertinib in the treatment of EGFR-mutation positive non-small-cell lung cancer, Futur. Oncol. 15, 805–816. https://doi.org/10.2217/fon-2018-0626.
19. S.D. Magar, P.Y. Pawar, (2022). In silico ADMET screening & molecular docking of some 1-(5-(4-chlorophenyl)-1,3,4-oxadiazol-3(2H)-yl) ethanone derivatives to be developed as triple mutant T790M/C797S EGFR inhibitors, Int. J. Health Sci. (Qassim). 6 10432–10451. https://doi.org/10.53730/ijhs.v6ns3.9448.
20. T. Aiebchun, P. Mahalapbutr, A. Auepattanapong, O. Khaikate, S. Seetaha, L. Tabtimmai, C. Kuhakarn, K. Choowongkomon, T. Rungrotmongkol,(2021). Identification of vinyl sulfone derivatives as egfr tyrosine kinase inhibitor: In vitro and in silico studies, Molecules. 26; . https://doi.org/10.3390/molecules26082211.
21. M. Li, N. Xue, X. Liu, Q. Wang, H. Yan, Y. Liu, L. Wang, X. Shi, D. Cao, K. Zhang, Y. Zhang, (2021). Discovery of Potent EGFR Inhibitors With 6-Arylureido-4-anilinoquinazoline Derivatives, Front. Pharmacol. 12;. https://doi.org/10.3389/fphar.2021.647591.
22. S. Puri, K. Stefan, S.L. Khan, J. Pahnke, S.M. Stefan, K. Juvale, (2023). Indole Derivatives as New Structural Class of Potent and Antiproliferative Inhibitors of Monocarboxylate Transporter 1 (MCT1; SLC16A1), J. Med. Chem. 66 657–676. https://doi.org/10.1021/acs.jmedchem.2c01612.
23. P. Basu, C. Maier, (2018). Phytoestrogens and breast cancer: In vitro anticancer activities of isoflavones, lignans, coumestans, stilbenes and their analogs and derivatives, Biomed. Pharmacother. 107 (2018) 1648–1666. https://doi.org/10.1016/j.biopha. 08.100.
24. K.L. Ameta, N.S. Rathore, B. Kumar, (2012). Synthesis and in vitro anti-breast cancer activity of some novel 1,5-benzothiazepine derivatives, J. Serbian Chem. Soc. 77 (2012) 725–731. https://doi.org/10.2298/JSC110715219A.