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ANTIPROLIFERATIVE AND APOPTOSIS EVALUATION OF NOVEL SYNTHESIZED CHALCONE-SULPHONAMIDE HYBRIDS

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Abstract:

Chalcone and sulphonamide both pharmacophores have been identified as fascinating compounds with cytotoxic effect. In the current study, chalcone-sulphonamide hybrids were synthesized by tethering sulphonamide pharmacophore with substituted chalcones and evaluated for anticancer activity. The structures of the compounds were substantiated by IR, NMR, and HRMS. The data asserted that compounds 3b, 3e, 4a, 4e, 4f, 5a, 5e, 5f, 6a, and 6f showed in vitro cytotoxicity against selected five cancer cell lines as compared to standard doxorubicin drug among all the twenty-four tested compounds and also confirmed to be non-toxic to normal cell line. To evaluate whether more potent hybrids induced cell death via an apoptotic or necrotic pathway, they were examined for DNA fragmentation and the result showed that 4a, 4f, 5a, 5f, 6a, and 6f markedly induced DNA fragmentation and apoptosis in selected cancer cells. The molecular docking was performed to predict the binding mode of the most promising compounds 4a, 4f, 5a and 6f with the active site of relevant amino acids in the binding pocket on the basis of standard bond length and docking score.

Key Words: Chalcones, Sulphonamides, Antiproliferative, Apoptosis, Docking.

Introduction

An affliction of cancer is raised world widely, with the changes in the living environment and also one of the most dominant causes of the morbidity and mortality. World widely deaths related to cancer are estimated to elongate 12 million in the year 2015[1]. Uncontrollably cell replication and rapid proliferation are the most important mechanism that causes cancer [2]. During proliferation, microtubules or tubulins are most important molecular targets for cancer chemotherapeutic agents since they play a vital role and involve in cellular functions [3]. The new generation of anticancer drugs affect the signals that promote or regulate cell cycle, growth factor, pathway

affecting DNA repair and apoptosis.

Chalcone is chemically 1,3-diaryl-2-propen-1-one in which two aryl rings are joined by α,β-unsaturated carbonyl system. There are numerous reviews of the anticancer activity exhibited by chalcone [4-7]. Chalcones have been reported for a wide range of pharmacological activities including anticancer activity [8-11]. Chalcones display a variety of biological activities such as anti-inflammatory [12], antileishmanial [13], antimitotic [14], antitubercular [15], cardiovascular [16] and antihyperglycemic [17]. Previous literature revealed that anticancer activity of chalcone might be due to molecular alteration such as tubulin inhibition, kinase inhibition, apoptosis, DNA and mitochondrial damage, inhibition of angiogenesis and also drug efflux protein activities.

Many sulphonamide compounds exhibited their anticancer activity by inhibiting tubulin to form microtubule [18]. The synthesized sulphonamides selectively inhibit proliferation, block the cell cycle and induce apoptosis in human cancer cells but not in normal cells [19,20]. Chalcones containing a sulphonyl or sulphonamide group for cytotoxic effect on cells of many types of cancer. These compounds also showed in vivo anticancer activity and reduce tumor size in rats without showing any significant toxic effect [21].

In continuation of our drug formatting program on anticancer agents, we synthesized novel chalcone sulphonamide hybrids through molecular hybridization strategy. Molecular hybridization is a rationally attractive approach involving the integration of two or more pharmacophoric units having a different mechanism of action. These combined pharmacophores probably offer some advantages in their biological potency. In this article, the synthetic approach has been designed as follows

(i) Use 1,3-diphenyl propenone (ring-A and B) as a core structure
(ii) Very methoxy and/or hydroxyl groups on ring-A
(iii) Vary groups/heterocycle attached with sulphonamide linkage.

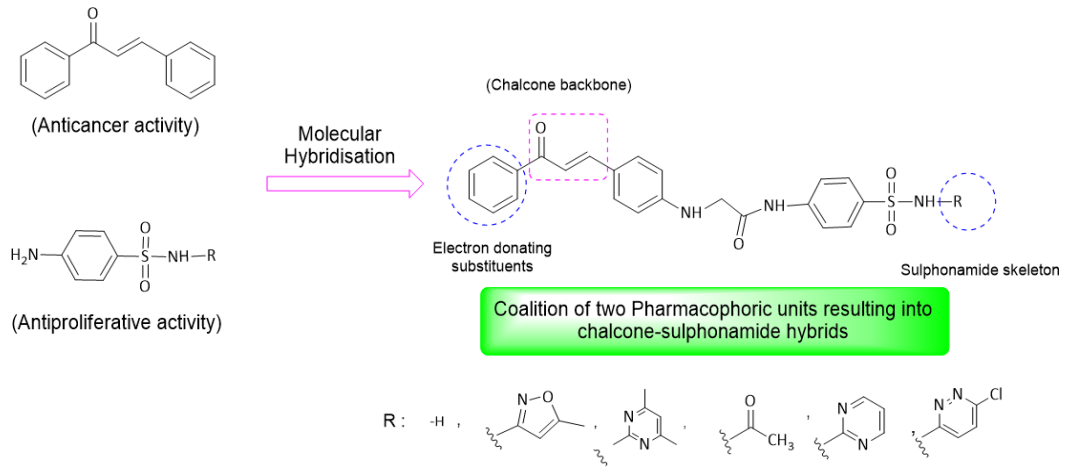


Figure-1 Demonstration of the design stretagy for novel chalcon-sulphonamide hybrid

In sum, a panel of twenty-four novel chalcone-sulphonamide hybrids was synthesized and their structures were investigated through IR, NMR, and HR-MS. In vitro antiproliferative activity of all synthesized compound were then screened against five selected cancer cell lines using MTT assay method.

Experimental

2.1 General Information

The starting materials and solvents were purchased from Sigma-Aldrich and SD Fine and are used without any further purification. Melting points were determined by the conventional method and then by electrocapillary apparatus and were uncorrected. All the synthesized compounds were inspected by thin layer chromatography (ethyl acetate and n-hexane in 1:3ratio) with precoated Aluminium sheets on silica gel (E-Merck) and the spots were visualized by UV lamp. The IR spectra of the compounds were recorded on Shimadzu FT-IR spectrometer. 1HNMR and 13CNMR spectra are recorded using a Bruker in DMSO at 500 MHz. IR, 1HNMR and 13CNMR spectra were performed at Centre of Excellence Saurashtra University and Q-Exactive plus Biopharma-High Resolution Orbitrap Liquid Chromatography-Mass Spectra were performed at the SAIF Indian Institution of Technology. Aminochalcone compounds 1a-1c are synthesized as shown in scheme-1. Commercially available sulphonamides 2a-2e are treated with chloroacetyl chloride to provide chlorosulfonyl acetamide as shown in scheme 2. The general route for the synthesis of the target sulphonamide-chalcone hybrids is depicted in scheme 3. The structures of targeted compounds are characterized using spectral methods. All spectral data are fascinated with assumed structures.

2.2 Synthesis

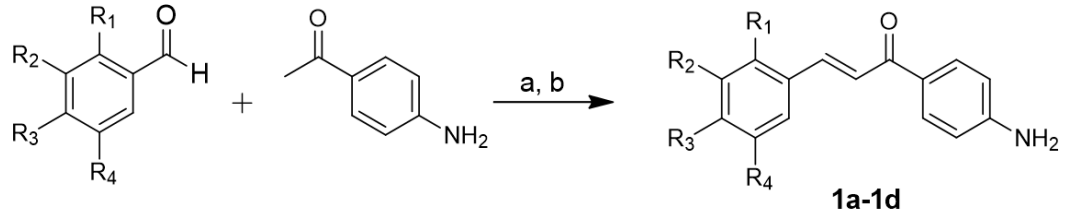
3.2.1 General method for preparation of (E)-1-(4-aminophenyl)-3-(substituted-phenyl) prop-2-en-1-one (1a-1d):

4-Aminoacetophenone (5 mmol) and methanol (10 mL) were stirred and catalyzed by NaOH (5%, 5 mL). Then substituted benzaldehyde (5 mmol) was added and the reaction mixture was stirred for 4 hours at room temperature. The reaction was monitored by thin layer chromatography. It was kept for 18-24 hours. Then cold aquadest was added on it and pH was neutralized with 5% HCl solution. The solid layer was separated and recrystallized from ethyl acetate.

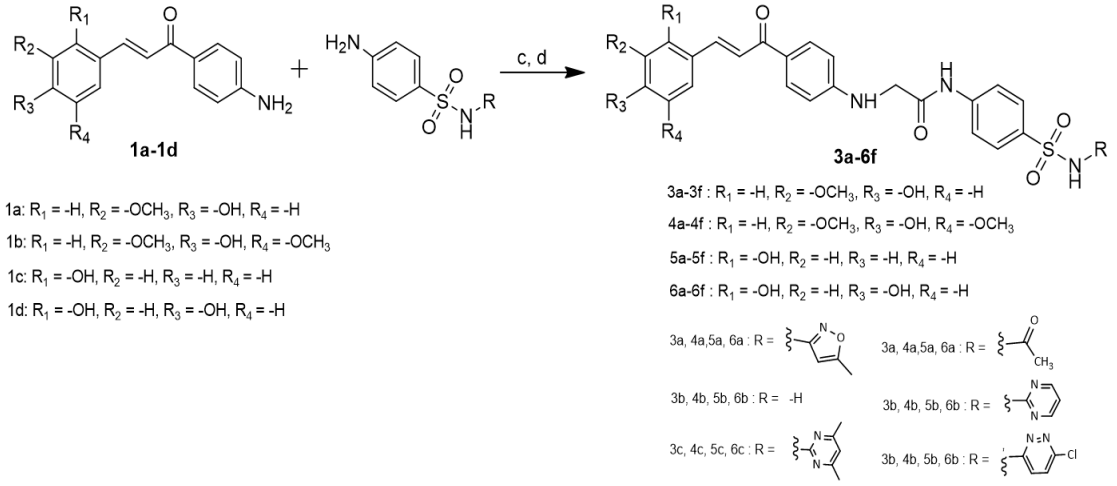
3.2.2 General method for preparation of Chalcone-sulphonamide hybrids (3a-6f):

To a stirred solution of sulphonamide (2mmol), chloroacetylchloride (2mL) and try ethylamine (0.1 mL) in dry dimethylformamide at 0-5 ^{o}C, aminochalcone 1a-1c (2 mmol) was added and stirred at room temperature for 3-4 hours by a magnetic stirrer. The stirred reaction mixture was then refluxed for 8-9 hours. The reaction was monitored by TLC.

After the completion of the reaction, the reaction mixture on hot was poured to crushed ice afforded precipitates of chalcone-sulphonamide hybrids 3a-6f. Precipitates then washed with cold aqueous sodium carbonate and the crude product was recrystallized in acetone.



Scheme-1: Synthesis of chalcone compounds 1a-1d. Reagent and condition: (a) 20% NaOH, CH3OH, 25-30 ^{o}C, overnight (b) 25% HCl, ethyl acetate.



Scheme-2: Synthesis of targeted Chalcone-sulphonamide hybrids 3a-6f. Reagent and condition: (c) DMF, chloroacetylchloride, triethylamine, 0-5˚C (d) Reflux, 8-9 h.

(E)-2-((4-(3-(4-hydroxy-3-methylphenyl) acryloyl)phenyl)amino)-N-(4-(N-(5-methylisoxazole-3-yl) sulfamoyl)phenyl) acetamide (3a)

Yellow solid, M.p 90-95^{o}C, Yield 60.6%, Rf 0.52. FT-IR (ν, cm-1): 3741(-OH), 3591, 3360,3331(3-NH-), 3064-3100(Ar C-H), 2974 (Aliphatic –CH), 1739 (-CONH-), 1678 (-CO), 1608 (-C=N), 1591 (-HC=CH-), 1454(C-O), 1398, 1157 (-SO2-) 952 (S-N), 833(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.20 (s,1H, CONH-), 8.10(s,1H,-OH), 7.65(d,1Hβ, J 12.2, -CH=CH-), 7.56(s,1H,-SO2NH-) , 6.45(d,1Hα, J 8.0, -CH=CH-), 6.02-8.02(m,10H, Ar-H), 6.51(t, 1H, -NH-), 4.63(s,1H, CH=C isoxazole), 3.36 (d,2H,-CH2-), 2.67 (s,3H,-CH3), 2.27(s,3H,-OCH3). 13CNMR (500 MHz, DMSO-d6, ppm): 195.17, 167.08, 159.56, 154.09, 151.03, 141.66, 144.34, 138.80, 130.80, 127.02, 126.00, 125.54, 122.95, 119.07, 119.07, 112.40, 40.80, 39.67, 39.46, 39.07, 38.83, 27.03, 25.92, 12.11. HR-MS (ESI): Calculated for C28H26N4O7S [M+H+] 546.16, found

546.157. Molecular formula: Calculated C28H26N4O7S, found C28H26N4O7S.

(E)-2-((4-(3-(4-hydroxy-3-methylphenyl) acryloyl)phenyl)amino)-N-(4-sulfamoylphenyl)acetamide (3b)

Brown solid, M.p 145-148°C, Yield 50.5%, Rf 0.56. FT-IR (ν, cm-1): 3741(-OH), 3591, 3566, 3365,3280(2-NH, -NH2), 3005(Ar C-H), 2976 (Aliphatic –CH), 1734 (-CONH-), 1683 (-CO), 1639 (-C=N), 1595 (-HC=CH-), 1452(C-O), 1311, 1149 (-SO2-), 962 (S-N), 829(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.20 (s,1H, CONH-), 8.10(s,1H,-OH), 7.65(d,1Hβ, J 12.0, -CH=CH-), 7.56(s,2H,-SO2NH2) , 6.45(d,1Hα, J 8.1, -CH=CH-), 6.02-8.02(m,10H, Ar-H), 6.51(t, 1H, -NH-), 3.13(s ,2H,-CH2-), 2.67 (s,3H,-OCH3), 2.40(s,3H,-CH3).13CNMR (500 MHz, DMSO-d6, ppm): 195.17, 169.08, 159.56, 154.08, 141.66, 138.80, 130.54, 129.62, 127.02, 126.70, 125.54, 122.95, 117.17, 46.32, 40.09, 39.83, 39.09, 38.83, 27.03, 25.92. HR-MS (ESI) Calculated for C24H23N3O6S [M+H+] 465.135, found 465.136. Molecular formula: Calculated C24H23N3O6S, found C24H23N3O6S.

(E)-N-(4-(N-(4,6-dimethylpyrimidin-2-yl) sulfamoyl)phenyl)-2-((4-(3-(4-hydroxy-3-methylphenyl) acryloyl)phenyl)amino) acetamide (3c)

Brown solid, M.p 106-110°C, Yield 62.0%, Rf 0.52. FT-IR (ν, cm-1): 3741(-OH), 3591, 3360,3253(3-NH-), 3000-3100(Ar C-H), 2974, 2883 (Aliphatic –CH), 1739 (-CONH-), 1678 (-CO), 1647 (-C=N), 1593 (-HC=CH-), 1456(C-O), 1396, 1157 (-SO2-) 952 (S-N), 835(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.31 (s,1H, CONH-), 8.30(s,1H,-OH), 7.65(d,1Hβ, J 12.6, -CH=CH-), 7.56(s,1H,-SO2NH-) , 6.55(d,1Hα, J8.7, -CH=CH-), 6.09-8.02(m,10H, Ar-H), 6.45(1H,s,-CH=CH(pyrimidine)), 6.41(t, 1H, -NH-), 3.36 (d,2H,-CH2-), 2.56(s,3H,-CH3), 2.54(s,3H,-OCH3), 2.31(s,3H,-CH3).13CNMR (500 MHz, DMSO-d6, ppm): 195.17, 167.08, 159.56, 154.09, 154.09, 141.66, 138.80, 130.80, 129.24, 127.02, 127.02, 126.00, 125.54, 122.95, 112.40, 119.02, 119.12, 114.40, 114.40, 40.80, 39.67, 39.46, 39.07, 38.83, 27.03, 12.11. HR-MS (ESI) Calculated for C30H29N5O6S [M+H+] 571.18, found 571.211. Molecular formula: Calculated C30H29N5O6S, found C30H29N5O6S.

(E)-N-(4-(N-acetylsulfamoyl)phenyl)-2-((4-(3-(4-hydroxy-3-methylphenyl) acryloyl)phenyl) amino)acetamide (3d)

Red solid, M.p 152-150°C, Yield 58%, Rf 0.70. FT-IR (ν, cm-1): 3741(-OH), 3568, 3360,3246 (3-NH-), 3000(Ar C-H), 2976,2889 (Aliphatic –CH), 1734 (-CONH-), 1678 (-CO), 1647 (-C=N), 1597 (-HC=CH-), 1456(C-O), 1363, 1155 (-SO2-), 958 (S-N), 839(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.20 (s,1H, CONH-), 8.10(s,1H,-OH), 7.84(d,1Hβ, J 14.0, -CH=CH-), 7.76(s,1H,-SO2NH-) , 6.45(d,1Hα, J 8.2, -CH=CH-), 6.02-8.02(m,10H, Ar-H), 6.51(t, 1H, -NH-), 3.35 (d,2H,-CH2-), 2.67 (s,3H,-CH3), 2.40(s,3H,-OCH3).13CNMR (500 MHz, DMSO-d6, ppm): 190.17, 170.20, 167.08, 159.56, 154.09, 141.66, 138.80,

130.80, 129.06, 129.06, 127.02, 122.95, 119.08, 119.12, 114.40, 44.80, 39.67, 39.46, 39.07, 38.83, 21.03, 16.11. HR-MS (ESI) Calculated for C26H25N3O7S [M+H+] 507.14, found 507.23. Molecular formula: Calculated C26H25N3O7S, found C26H25N3O7S.

(E)-2-((4-(3-(4-hydroxy-3-methylphenyl) acryloyl)phenyl)amino)-N-(4-(N-(pyrimidin-2-yl) sulfamoyl) phenyl) acetamide(3e)

Red Brick solid, M.p 185-190°C, Yield 63.6%, Rf 0.41. FT-IR (ν, cm-1): 3743(-OH), 3589, 3360,3153(3-NH-), 3000-3100(Ar C-H), 2972, 2918 (Aliphatic –CH), 1738 (-CONH-), 1674 (-CO), 1649 (-C=N), 1590 (-HC=CH-), 1463(C-O), 1399, 1155 (-SO2-), 954 (S-N), 835(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.50 (s,1H, CONH-), 8.20(s,1H,-OH), 7.77(d,1Hβ, J 12.2 -CH=CH-), 7.65(s,1H,-SO2NH-) , 6.54(d,1Hα,J 8.0, -CH=CH-), 6.09-8.05(m,10H, Ar-H), 6.65(1H,s,-CH=CH(pyrimidine)), 6.41(t, 1H, -NH-), 3.35(d,2H,-CH2-), 2.38(s,3H,-OCH3). 13CNMR(500MHz, DMSO-d6,ppm): 194.87, 167.08, 159.56, 154.09, 153.58, 138.80, 130.80, 129.24, 127.02, 124.78, 122.95, 112.40, 119.02, 119.12, 114.40, 114.40, 40.80, 39.67, 39.46, 39.25, 39.07, 38.83, 27.03, 25.11. HR-MS (ESI) Calculated for C28H25N5O6S [M+H+] 543.157, found 543.159. Molecular formula: Calculated C28H25N5O6S, found C28H25N5O6S. Molecular formula: Calculated C28H25N5O6S, found C28H25N5O6S.

(E)-N-(4-(N-(6-chloropyridazin-3-yl) sulfamoyl)phenyl)-2-((4-(3-(4-hydroxy-3methoxy phenyl)acryloyl)phenyl)amino) acetamide(3f)

Black solid, M.p 186-185°C, Yield 60.0%, Rf 0.61. FT-IR (ν, cm-1): 3750(-OH), 3585, 3356,3143(3-NH-), 3000-3100(Ar C-H), 2972, 2918 (Aliphatic –CH), 1738 (-CONH-), 1674 (-CO), 1649 (-C=N), 1590 (-HC=CH-), 1463(C-O), 1399, 1155 (-SO2-), 954 (S-N), 835(C-S), 595(C-Cl). 1H NMR (500 MHz, DMSO-d6, ppm): 8.50 (s,1H, CONH-), 8.20(s,1H,-OH), 7.77(d,1Hβ, J 12.2, -CH=CH-), 7.65(s,1H,-SO2NH-) , 6.54(d,1Hα, J 8.8, -CH=CH-), 6.09-8.05(m,10H, Ar-H), 6.65(1H,s,-CH=CH(pyrimidine)), 6.41(t, 1H, -NH-), 3.35(d,2H,-CH2-), 2.38(s,3H,-OCH3). 13CNMR (500 MHz, DMSO-d6, ppm): 194.87, 167.08, 159.56, 154.09, 153.58, 146.52, 138.80, 130.80, 129.24, 127.02, 124.78, 122.95, 112.40, 119.02, 119.12, 114.40, 114.40, 40.80, 39.67, 39.46, 39.25, 39.07, 38.83, 27.03, 25.11. HR-MS (ESI) Calculated for C28H24ClN5O6S [M+H+] 593.118, found 593.113. Molecular formula: Calculated C28H24ClN5O6S, found C28H24ClN5O6S.

(E)-2-((4-(3-(4-hydroxy-3,5-dimethylphenyl)acryloyl)phenyl) amino)-N-(4-(N-(5-methylisoxazol-3-yl) sulfamoyl)phenyl)acetamide (4a)

Red solid, M.p 135-140°C, Yield 61.1%, Rf 0.51. FT-IR (ν, cm-1): 3741(-OH), 3566, 3360,3331(3-NH-), 3064-3100(Ar C-H), 2974,2883 (Aliphatic –CH), 1772 (-CONH-), 1678 (-CO), 1653 (-C=N),

1591 (-HC=CH-), 1456(C-O), 1398, 1159 (-SO2-) 952 (S-N), 831(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.20 (s,1H, CONH-), 8.10(s,1H,-OH), 7.65(d,1Hβ -CH=CH-), 7.56(s,1H,-SO2NH-) , 6.45(d,1Hα -CH=CH-), 6.02-8.02(m,10H, Ar-H), 6.51(t, 1H, -NH-), 4.63(s,1H, CH=C isoxazole), 3.36 (d,2H,-CH2-), 2.67 (s,3H,-CH3), 2.50(s,3H,-OCH3), 2.27(s,3H,-OCH3).13CNMR (500 MHz, DMSO-d6, ppm): 195.17, 167.08, 159.56, 154.09, 151.03, 141.66, 144.34, 138.80, 130.80, 127.02, 126.00, 125.54, 122.95, 119.07, 119.07, 112.40, 40.80, 39.67, 39.46, 39.07, 38.83, 27.03, 25.92, 12.11, 12.03. HR-MS (ESI): Calculated for C29H28N4O8S [M+H+] 560.173, found 560.175. Molecular formula: Calculated C29H28N4O8S, found C29H28N4O8S.

(E)-2-((4-(3-(4-hydroxy-3,5-dimethylphenyl)acryloyl)phenyl)amino)-N-(4-sulfamoyl phenyl)acetamide (4b)

Pale yellow solid, M.p 114-116°C, Yield 40%, Rf 0.69. FT-IR (ν, cm-1): 3741(-OH), 3591, 3566, 3365,3280(2-NH, -NH2), 3005(Ar C-H), 2976 (Aliphatic –CH), 1734 (-CONH-), 1683 (-CO), 1639 (-C=N), 1595 (-HC=CH-), 1452(C-O), 1311, 1149 (-SO2-), 962 (S-N), 829(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.20 (s,1H, CONH-), 8.10(s,1H,-OH), 7.65(d,1Hβ -CH=CH-), 7.56(s,2H,-SO2NH2) , 6.45(d,1Hα -CH=CH-), 6.02-8.02(m,10H, Ar-H), 6.51(t, 1H, -NH-), 3.13(s ,2H,-CH2-), 2.67 (s,3H,-CH3), 2.50(s,3H,-OCH3), 2.40(s,3H,-OCH3).13CNMR (500 MHz, DMSO-d6, ppm): 195.17, 169.08, 159.56, 154.08, 141.66, 138.80, 130.54, 129.62, 127.02, 126.70, 125.54, 122.95,117.17, 46.32, 40.09, 39.83,39.09, 38.83, 27.03, 25.92,12.54. HR-MS (ESI): Calculated for C25H25N3O7S [M+H+] 479.52, found 479.53. Molecular formula: Calculated C25H25N3O7S, found C25H25N3O7S.

(E)-N-(4-(N-(4,6-dimethylpyrimidin-2-yl) sulfamoyl)phenyl)-2-((4-(3-(4-hydroxy-3,5-dimethyl phenyl)acryloyl)phenyl) amino)acetamide (4c)

Black solid, M.p 110-112°C, Yield 52.8%, Rf 0.57. FT-IR (ν, cm-1): 3741(-OH), 3591, 3560,3329(3-NH-), 3000(Ar C-H), 2974, 2883 (Aliphatic –CH), 1739 (-CONH-), 1678 (-CO), 1645 (-C=N), 1593 (-HC=CH-), 1456(C-O), 1396, 1157 (-SO2-) 952 (S-N), 835(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.31 (s,1H, CONH-), 8.30(s,1H,-OH), 7.65(d,1Hβ -CH=CH-), 7.56(s,1H,-SO2NH-) , 6.55(d,1Hα -CH=CH-), 6.09-8.02(m,10H, Ar-H), 6.45(1H,s,-CH=CH(pyrimidine)), 6.41(t, 1H, -NH-), 3.36 (d,2H,-CH2-), 2.56(s,3H,-CH3), 2.54(s,3H,-OCH3), 2.31(s,3H,-OCH3).13CNMR (500 MHz, DMSO-d6, ppm): 195.17, 167.08, 159.56, 154.09,154.09 141.66, 138.80, 130.80, 129.24, 127.02,127.02, 126.00, 125.54, 122.95, 112.40, 119.02, 119.12, 114.40, 114.40, 40.80, 39.67, 39.46, 39.07, 38.83, 27.03, 12.78,12.10. HR-MS (ESI) Calculated for C31H31N5O7S [M+H+] 585.20, found 585.201. Molecular formula: Calculated C31H31N5O7S, found C31H31N5O7S.

(E)-N-(4-(N-acetylsulfamoyl)phenyl)-2-

((4-(3-(4-hydroxy-3,5-dimethylphenyl) acryloyl)phenyl) amino)acetamide (4d)

Brown solid, M.p 120-123°C, Yield 58.8%, Rf 0.80. FT-IR (ν, cm-1): 3741(-OH), 3591, 3360,3255 (3-NH-), 3000-3100(Ar C-H), 2974,2883 (Aliphatic –CH), 1734 (-CONH-), 1678 (-CO), 1653 (-C=N), 1591 (-HC=CH-), 1456(C-O), 1363, 1153 (-SO2-), 952 (S-N), 829(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.20 (s,1H, CONH-), 8.10(s,1H,-OH), 7.84(d,1Hβ -CH=CH-), 7.76(s,1H,-SO2NH-) , 6.45(d,1Hα -CH=CH-), 6.02-8.02(m,10H, Ar-H), 6.51(t, 1H, -NH-), 3.35 (d,2H,-CH2-), 2.67 (s,3H,-CH3), 2.50(s,3H,-OCH3), 2.40(s,3H,-OCH3).13CNMR (500 MHz, DMSO-d6, ppm): 190.17,170.20, 167.08, 159.56, 154.09, 141.66, 138.80, 130.80,129.06,129.06, 127.02, 122.95, 119.08, 119.12, 114.40, 44.80, 39.67, 39.46, 39.07, 38.83, 21.03, 16.11, 12.23. HR-MS (ESI) Calculated for C27H27N3O8S [M+H+] 521.16, found 521.25. Molecular formula: Calculated C27H27N3O8S, found C27H27N3O8S.

(E)-2-((4-(3-(4-hydroxy-3,5-dimethylphenyl)acryloyl)phenyl)amino)-sulfamoyl)phenyl)-2-((4-(3-(4-hydroxy-3,5-dimethoxy phenyl)acryloyl)phenyl) amino)acetamide (4f)

Brown solid, M.p 140-142°C, Yield 72.1%, Rf 0.65 FT-IR (ν, cm-1): 3745(-OH), 3638, 3560,3355(3-NH-), 3000-3100(Ar C-H), 2974, (Aliphatic –CH), 1738 (-CONH-), 1674 (-CO), 1687 (-C=N), 1587 (-HC=CH-), 1463(C-O), 1363, 1153 (-SO2-), 950 (S-N), 829(C-S), 590.22(C-Cl). 1H NMR (500 MHz,

N-(4-(N-(pyrimidin-2-yl)sulfamoyl) phenyl)acetamide (4e)

Red solid, M.p 134-136°C, Yield 63.6%, Rf 0.61 FT-IR (ν, cm-1): 3743(-OH), 3649, 3564,3358(3-NH-), 3000-3100(Ar C-H), 2974, (Aliphatic –CH), 1738 (-CONH-), 1674 (-CO), 1687 (-C=N), 1587 (-HC=CH-), 1463(C-O), 1363, 1153 (-SO2-), 950 (S-N), 829(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.50 (s,1H, CONH-), 8.20(s,1H,-OH), 7.77(d,1Hβ -CH=CH-), 7.65(s,1H,-SO2NH-), 6.54(d,1Hα -CH=CH-), 6.09-8.05(m,10H, Ar-H), 6.65(1H,s,-CH=CH(pyrimidine)), 6.41(t, 1H, -NH-), 3.35(d,2H,-CH2-), 2.50(s,3H,-CH3), 2.38(s,3H,-OCH3).13CNMR (500 MHz, DMSO-d6, ppm): 194.87, 167.08, 159.56, 154.09, 153.58, 138.80, 130.80, 129.24, 127.02, 124.78, 122.95, 112.40, 119.02, 119.12, 114.40, 114.40, 40.80, 39.67, 39.46, 39.25, 39.07, 38.83, 27.03, 25.81, 16.12. HR-MS (ESI) Calculated for C29H27N5O7S [M+H+] 557.173, found 557.173. Molecular formula: Calculated C29H27N5O7S, found C29H27N5O7S.

(E)-N-(4-(N-(6-chloropyridazin-3-yl)

DMSO-d6, ppm): 8.50 (s,1H, CONH-), 8.20(s,1H,-OH), 7.77(d,1Hβ -CH=CH-), 7.65(s,1H,-SO2NH-) , 6.54(d,1Hα -CH=CH-), 6.09-8.05(m,10H, Ar-H), 6.65(1H,s,-CH=CH(pyrimidine)), 6.41(t, 1H, -NH-), 3.35(d,2H,-CH2-), 2.50(s,3H,-CH3), 2.38(s,3H,-OCH3).13CNMR (500 MHz, DMSO-d6, ppm): 194.87, 167.08, 159.56, 154.09, 153.58, 144.56, 138.80, 130.80, 129.24, 127.02, 124.78, 122.95,11 2.40,119.02,119.12,114.40, 114.40, 40.80,

39.67, 39.46,39.25, 39.07, 38.83, 27.03, 25.81,16.12. HR-MS (ESI) Calculated for C29H26ClN5O7S [M+H+] 623.140, found 623.124. Molecular formula: Calculated C29H26ClN5O7S, found C29H26ClN5O7S.

(E)-2-((4-(3-(2-hydroxyphenyl) acryloyl)phenyl)amino)-N-(4-(N-(5-methylisoxazol-3-yl) sulfamoyl)phenyl) acetamide (5a)

Red solid, M.p 100-102°C, Yield 82.2%, Rf 0.68. FT-IR (ν, cm-1): 3741(-OH), 3672, 3568,3360,(3-NH-), 3064-3100(Ar C-H), 2974,2883 (Aliphatic –CH), 1734 (-CONH-), 1678 (-CO), 1608 (-C=N), 1593 (-HC=CH-), 1454(C-O), 1396, 1153 (-SO2-) 954 (S-N), 831(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.20 (s,1H, CONH-), 8.10(s,1H,-OH), 7.65(d,1Hβ -CH=CH-), 7.56(s,1H,-SO2NH-) , 6.45(d,1Hα -CH=CH-), 6.02-8.02(m,10H, Ar-H), 6.51(t, 1H, -NH-), 4.63(s,1H, CH=C isoxazole), 3.36 (d,2H,-CH2-), 2.67 (s,3H,-CH3). 13CNMR (500 MHz, DMSO-d6, ppm): 197.61, 195.18, 162.45, 154.17, 153.67, 138.87, 136.44, 132.62 130.64, 129.82, 129.48, 124.96, 123.02, 122.54, 117.28, 116.66, 112.58, 109.52, 96.60, 95.52, 56.41, 48.04, 45.00, 40.41, 39.08, 35.87, 30.86. HR-MS (ESI) Calculated for C27H24N4O6S [M+H+] 532.14, found 532.14. Molecular formula: Calculated C27H24N4O6S, found C27H24N4O6S.

(E)-2-((4-(3-(2-hydroxyphenyl)acryloyl) phenyl)amino)-N-(4-sulfamoylphenyl) acetamide (5b)

Red solid, M.p 124-125°C, Yield 70%, Rf 0.56. FT-IR (ν, cm-1): 3741(-OH), 3591, 3566, 3365, 3280 (2-NH, -NH2), 3005 (Ar C-H), 2976 (Aliphatic –CH), 1734 (-CONH-), 1683 (-CO), 1595 (-HC=CH-), 1452 (C-O), 1311, 1149 (-SO2-), 962 (S-N), 829 (C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.20 (s, 1H, CONH-), 8.10 (s, 1H,-OH), 7.65 (d,1Hβ -CH=CH-), 7.56(s,2H,-SO2NH2) , 6.45(d,1Hα -CH=CH-), 6.02-8.02(m,10H, Ar-H), 6.51(t, 1H, -NH-), 3.13(s ,2H,-CH2-). 13CNMR (500 MHz, DMSO-d6, ppm): 197.48, 195.17, 169.08, 162.45, 159.56, 154.08, 141.66, 138.80, 130.54, 129.62, 127.02, 126.70, 125.54, 122.95, 119.27, 117.17, 46.32, 40.09, 39.83, 38.83, 30.72. HR-MS (ESI) Calculated for C23H21N3O5S [M+H+] 451.120, found 451.117. Molecular formula: Calculated C23H21N3O5S, found C23H21N3O5S.

(E)-N-(4-(N-(4,6-dimethylpyrimidin-2-yl) sulfamoyl)phenyl)-2-((4-(3-(2-hydroxy phenyl)acryloyl) phenyl)amino)acetamide (5c)

Red solid, M.p 135-136°C, Yield 68%, Rf 0.77. FT-IR (ν, cm-1): 3741(-OH), 3672,3591, 3360(3-NH-), 3000-3100(Ar C-H), 2974, 2883 (Aliphatic –CH), 1739 (-CONH-), 1678 (-CO), 1645 (-C=N), 1593 (-HC=CH-), 1454 (C-O), 1396, 1155 (-SO2-), 954 (S-N), 835(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.31 (s,1H, CONH-), 8.30 (s,1H,-OH), 7.65 (d,1Hβ -CH=CH-), 7.56 (s,1H,-SO2NH-) , 6.55 (d,1Hα -CH=CH-), 6.09-8.02 (m,10H, Ar-H), 6.45 (1H,s,-CH=CH(pyrimidine)), 6.41 (t, 1H, -NH-), 3.36 (d,2H,-CH2-),

2.56 (s,3H,-CH3), 2.31 (s,3H,-CH3). 13CNMR (500 MHz, DMSO-d6, ppm): 197.57, 195.18, 167.17, 164.85, 160.41, 154.09, 153.66, 138.86, 136.51, 130.80, 129.67, 129.48, 124.02, 123.03, 121.65, 119.02, 119.12, 118.40, 116.40, 112.59, 47.90, 44.03, 40.41, 39.09, 35.88, 30.86, 27.03, 26.76. HR-MS (ESI) Calculated for C29H27N5O5S [M+H+] 557.62, found 557.62. Molecular formula: Calculated C29H27N5O5S, found C29H27N5O5S.

(E)-N-(4-(N-acetylsulfamoyl)phenyl)-2-((4-(3-(2-hydroxyphenyl)acryloyl)phenyl) amino)acetamide (5d)

Red solid, M.p 120-122°C, Yield 78.4%, Rf 0.70. FT-IR (ν, cm-1): 3741(-OH), 3672,3568, 3360, (3-NH-), 3000-3100(Ar C-H), 2972 (Aliphatic –CH), 1739 (-CONH-), 1678 (-CO), 1647 (-C=N), 1593 (-HC=CH-), 1456(C-O), 1396, 1155 (-SO2-), 952 (S-N), 835(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.20 (s,1H, CONH-), 8.10(s,1H,-OH), 7.84(d,1Hβ -CH=CH-), 7.76(s,1H,-SO2NH-) , 6.45(d,1Hα -CH=CH-), 6.02-8.02(m,10H, Ar-H), 6.51(t, 1H, -NH-), 3.35 (d,2H,-CH2-), 2.40(s,3H,-CH3).13CNMR (500 MHz, DMSO-d6, ppm): 195.47, 192.04, 164.81, 163.37, 162.44, 160.44, 147.96, 136.49, 133.44, 132.66, 130.36, 129.88, 128.69, 127.87, 125.38, 123.77, 122.99, 121.63, 119.56, 118.99, 116.34, 112.65, 47.65, 40.03, 35.84, 30.17. HR-MS (ESI) Calculated for C25H23N3O6S [M+H+] 493.13, found 493.18. Molecular formula: Calculated C25H23N3O6S, found C25H23N3O6S.

(E)-2-((4-(3-(2-hydroxyphenyl)acryloyl) phenyl)amino)-N-(4-(N-(pyrimidin-2-yl) sulfamoyl)phenyl)acetamide (5e)

Red Brick solid, M.p 110-111°C, Yield 73.3%, Rf 0.53 FT-IR (ν, cm-1): 3743(-OH), 3672,3589, 3358(3-NH-), 3000-3100(Ar C-H), 2972 (Aliphatic –CH), 1739 (-CONH-), 1678 (-CO), 1649 (-C=N), 1593 (-HC=CH-), 1456(C-O), 1369, 1157 (-SO2-), 952 (S-N), 833(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.50 (s,1H, CONH-), 8.20(s,1H,-OH), 7.77(d,1Hβ -CH=CH-), 7.65(s,1H,-SO2NH-) , 6.54(d,1Hα -CH=CH-), 6.09-8.05(m,10H, Ar-H), 6.65(1H,s,-CH=CH(pyrimidine)), 6.41(t, 1H, -NH-), 3.41(d,2H,-CH2-).13CNMR (500 MHz, DMSO-d6, ppm): 195.87, 192.12, 162.08, 160.56, 158.38, 154.09, 153.58, 138.80, 130.80, 129.24, 128.02, 124.78, 122.95, 112.40, 119.02, 118.12, 116.40, 112.40, 68.64 55.85, 40.15, 39.67, 39.46, 37.83, 35.88, 30.73. HR-MS (ESI) Calculated for C27H23N5O5S [M+H+] 529.14, found 529.21. Molecular formula: Calculated C27H23N5O5S, found C27H23N5O5S.

(E)-N-(4-(N-(6-chloropyridazin-3-yl)sulfamoyl)phenyl)-2-((4-(3-(2-hydroxyphenyl) acryloyl)phenyl)amino) acetamide (5f)

Red Brick solid, M.p 140-142°C, Yield 80%, Rf 0.60 FT-IR (ν, cm-1): 3745(-OH), 3670, 3578, 3358(3-NH-), 3000-3100(Ar C-H), 2972(Aliphatic –CH), 1739(-CONH-), 1678(-CO), 1649 (-C=N), 1593 (-HC=CH-), 1456(C-O), 1369, 1157 (-SO2-), 952 (S-N), 833(C-S), 597.93(C-Cl). 1H NMR (500 MHz,

DMSO-d6, ppm): 8.50 (s,1H, CONH-), 8.20(s,1H,-OH), 7.77(d,1Hβ -CH=CH-), 7.65(s,1H,-SO2NH-) , 6.54(d,1Hα -CH=CH-), 6.09-8.05(m,10H, Ar-H), 6.65(1H,s,-CH=CH(pyrimidine)), 6.41(t, 1H, -NH-), 3.41(d,2H,-CH2-). 13CNMR (500MHz,DMSO-d6,ppm): 195.22, 192.23, 164.08, 162.45, 156.09, 155.53, 152.55, 136.51, 132.72, 130.49, 129.59, 127.01, 125.79, 124.62, 122.28, 121.38, 119.33, 118.31, 116.71, 112.65, 62.02, 46.52, 40.95, 39.07, 37.33, 35.86, 30.86. HR-MS (ESI) Calculated for C27H22ClN5O5S [M+H+] 563.098, found 563.103. Molecular formula: Calculated C27H22ClN5O5S, found C27H22ClN5O5S.

(E)-2-((4-(3-(2,4-dihydroxyphenyl) acryloyl)phenyl)amino)-N-(4-(N-(5-methylisoxazol-3-yl)sulfamoyl)phenyl) acetamide (6a)

Red solid, M.p 121-124°C Yield 84.4%, Rf 0.62. FT-IR (ν, cm-1): 3741(-OH), 3672(-OH), 3649, 3568,3360,(3-NH-), 3064-3100(Ar C-H), 2974,2883 (Aliphatic –CH), 1734(-CONH-), 1678 (-CO), 1608 (-C=N), 1593 (-HC=CH-), 1454(C-O), 1396, 1153 (-SO2-) 954 (S-N), 831(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.48(s,1H,-OH), 8.49(s,1H,-OH), 7.95(s,1H, CONH-), 7.70(s,1H,-SO2NH-), 7.65(d,1Hβ -CH=CH-), 6.54(d,1Hα -CH=CH-), 6.02-7.93(m,10H, Ar-H), 6.01(t, 1H, -NH-), 3.53(s,1H,CH=Cisoxazole), 2.88(d,2H,-CH2-), 2.73(s,3H,-CH3). 13CNMR (500MHz, DMSO-d6, ppm): 195.17, 167.08, 153.09, 151.03, 144.34, 138.80, 130.54, 127.02, 126.00, 125.54, 122.95, 119.07, 119.07, 112.40, 40.80, 39.67, 39.46, 39.07, 38.83, 30.73, 25.82. HR-MS (ESI) Calculated for C27H24N4O7S [M+H+] 548.146, found 548.136. Molecular formula: Calculated C27H24N4O7S, found C27H24N4O7S.

(E)-2-((4-(3-(2,4-dihydroxyphenyl) acryloyl)phenyl)amino)-N-(4-sulfamoylphenyl) acetamide (6b)

Brick red solid, M.p 118-120°C, 70.7%, Rf 0.66. FT-IR (ν, cm-1): 3741(-OH), 3672(-OH), 3591, 3566, 3365, 3280(2-NH, -NH2), 3101(Ar C-H), 2974(Aliphatic –CH), 1739(-CONH-), 1678 (-CO), 1593(-HC=CH-), 1456(C-O), 1315,1153(-SO2-), 952 (S-N), 833(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.10(s,1H,-OH), 7.95 (s,1H, CONH-), 7.71(s,2H,-SO2NH2), 7.64(d,1Hβ -CH=CH-), 6.56(d,1Hα -CH=CH-), 6.02-7.86(m,10H, Ar-H), 6.54(t, 1H, -NH-), 3.13(s ,2H,-CH2-). 13CNMR (500 MHz, DMSO-d6, ppm): 195.34, 169.88, 162.48, 159.63, 153.67, 141.60, 138.61, 137.27, 130.64, 130.44, 127.07, 126.75, 119.09, 118.89, 112.57, 111.37, 60.91, 46.44, 42.85, 40.95, 40.02, 35.89, 30.87. HR-MS (ESI) Calculated for C23H21N3O6S [M+H+] 467.116, found 467.115. Molecular formula: Calculated C23H21N3O6S, found C23H21N3O6S.

(E)-2-((4-(3-(2,4-dihydroxyphenyl) acryloyl)phenyl)amino)-N-(4-(N-(4,6-dimethylpyrimidin-2-yl) sulfamoyl) phenyl)acetamide (6c)

Brick red solid, M.p 130-133°C, Yield

74.5%, Rf 0.57. FT-IR (ν, cm-1): 3736(-OH), 3597(-OH), 3360, 3344,3261(3-NH-), 2929(Ar C-H), 2846(Aliphatic –CH), 1739 (-CONH-), 1651 (-CO), 1645 (-C=N), 1521 (-HC=CH-), 1400(C-O), 1396, 1153(-SO2-), 960(S-N), 827(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.31 (s,1H, CONH-), 8.30(s,1H,-OH), 7.65(d,1Hβ -CH=CH-), 7.56(s,1H,-SO2NH-) , 6.55(d,1Hα -CH=CH-), 6.09-8.02(m,10H, Ar-H), 6.45(1H,s,-CH=CH(pyrimidine))6.41(t, 1H, -NH-), 3.36 (d,2H,-CH2-), 2.56(s,3H,-CH3), 2.31(s,3H,-CH3).13CNMR (500 MHz, DMSO-d6, ppm): 195.17, 167.08, 159.56, 154.09, 154.09 141.66, 138.80, 130.80, 129.24, 127.02,127.02, 126.00, 125.54, 122.95, 112.40, 119.02,119.12,114.40,114.40, 40.80, 39.67, 39.46,39.07, 38.83, 27.03. HR-MS (ESI) Calculated for C29H27N5O6S [M+H+] 573.148, found 573.168. Molecular formula: Calculated C29H27N5O6S, found C29H27N5O6S.

(E)-N-(4-(N-acetylsulfamoyl)phenyl)-2-((4-(3-(2,4-dihydroxyphenyl)acryloyl) phenyl)amino) acetamide (6d)

Black solid, M.p 128-130°C, Yield 59.90%, Rf 0.56. FT-IR (ν, cm-1): 3741, 3672(-OH), 3647, 3591, 3360, (3-NH-), 3000-3120(Ar C-H), 2974(Aliphatic –CH), 1734(-CONH-), 1678(-CO), 1591(-HC=CH-), 1456(C-O), 1363, 1153(-SO2-), 952(S-N), 829(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.10(s,1H,-OH), 7.95(s,1H, CONH-), 7.67(d,1Hβ -CH=CH-), 7.64(s,1H,-SO2NH-) , 6.64(d,1Hα -CH=CH-), 6.04-7.95(m,10H, Ar-H), 6.54(t, 1H, -NH-), 3.35 (d,2H,-CH2-), 2.73(s,3H,-CH3). 13CNMR (500 MHz, DMSO-d6, ppm): 195.67, 162.94, 159.56, 154.09, 141.66, 138.80, 131.09, 129.06, 129.06, 125.02, 122.95, 119.08, 119.12, 113.02, 40.46, 39.67, 39.46, 39.07, 38.83, 36.34, 31.33, 26.33. HR-MS (ESI) Calculated for C25H23N3O7S [M+H+] 509.122, found 509.125. Molecular formula: Calculated C25H23N3O7S, found C25H23N3O7S.

(E)-2-((4-(3-(2,4-dihydroxyphenyl) acryloyl)phenyl)amino)-N-(4-(N-(pyrimidin-2-yl)sulfamoyl) phenyl) acetamide (6e)

Red Brick solid, M.p 142-144°C, Yield 66.4%, Rf 0.60 FT-IR (ν, cm-1): 3743 (-OH), 3672,3589, 3358(3-NH-), 3000-3100(Ar C-H), 2972 (Aliphatic-CH), 1739(-CONH-), 1678 (-CO), 1649 (-C=N), 1593 (-HC=CH-), 1456(C-O), 1369, 1157 (-SO2-), 952 (S-N), 833(C-S). 1H NMR (500 MHz, DMSO-d6, ppm): 8.49 (s,1H,-OH), 8.48 (s,1H, CONH-), 7.76 (d,1Hβ -CH=CH-), 7.65 (s,1H,-SO2NH-), 6.65(d,1Hα -CH=CH-), 6.09-7.95(m,10H, Ar-H), 6.01 (1H,s,-CH=CH (pyrimidine)), 6.54 (t,1H, -NH-), 3.35 (d,2H,-CH2-). 13CNMR (500 MHz, DMSO-d6, ppm): 194.87, 167.08, 159.56, 154.09, 153.58, 138.80, 130.80, 129.24, 127.02, 124.78, 122.95, 112.40, 119.02, 119.12, 114.40, 114.40, 40.80, 39.67, 39.46, 39.25, 39.07, 38.83, 29.03. HR-MS (ESI) Calculated for C27H23N5O6S [M+H+] 545.1363, found 545.1369. Molecular formula: Calculated C27H23N5O6S, found C27H23N5O6S.

(E)-N-(4-(N-(6-chloropyridazin-3-

yl)sulfamoyl)phenyl)-2-((4-(3-(2,4-dihydroxyphenyl)acryloyl) phenyl) amino) acetamide (6f)

Red solid, M.p 134-137°C, Yield 65%, Rf 0.60 FT-IR (ν, cm-1): 3745(-OH), 3670,3578, 3358(3-NH-), 3000-3100(Ar C-H), 2972 (Aliphatic –CH), 1739 (-CONH-), 1678 (-CO), 1649(-C=N), 1593(-HC=CH-), 1456(C-O), 1363,1172(-SO2-), 937(S-N), 833(C-S), 597.93(C-Cl). 1H NMR (500 MHz, DMSO-d6, ppm): 8.20 (s,1H,-OH), 7.95(s,1H, CONH-), 7.77 (d,1Hβ,-CH=CH-), 7.65 (s,1H,-SO2NH-), 6.54(d,1Hα -CH=CH-), 6.09-7.84(m,10H, Ar-H), 6.53(1H,s,-CH=CH(pyrimidine)), 6.46 (t,1H,-NH-), 3.40(d,2H,-CH2-). 13CNMR(500 MHz, DMSOd6,ppm): 195.49, 195.26, 164.45, 162.51, 153.58, 152.59, 151.92, 141.10 140.36, 137.52, 134.23, 130.47, 129.86, 128.31, 126.97, 124.98, 119.02, 118.30, 112.63, 111.33, 108.78, 61.98, 60.25, 45.85, 40.40, 35.91, 30.90. HR-MS (ESI) Calculated for C27H22ClN5O6S [M+H+] 579.0912, found 579.0979. Molecular formula: Calculated C27H22ClN5O6S, found C27H22ClN5O6S.

Determination of Anticancer Activity

Anticancer activity of all the synthesized chalcone-sulphonamide hybrids was assayed against selected human cancer cell lines MCF-7(Breast cancer), DU-145(Human prostate carcinoma), HCT-15 (Colon cancer), NCIH-522 (stage 2, adenocarcinoma; non-small cell lung cancer) and HT-3 (Human cervical cancer) cell lines at different concentration using MTT assay method. The assay is based on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble substrate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue formazan product which is insoluble in water. The amount of formazan produced is directly proportional to the cell number in range of cell lines. These compounds were also evaluated for standard anticancer drug Doxorubicin. Dose Response Curve (DRC) against all cell lines was plotted with 10 analysis point i.e. with 10 different drug concentrations [22]. The concentration causing 50% cell growth inhibition (IC50) was determined from DRC using Graph Pad Prism software (Ver. 5.04) (Graph Pad Software, Inc., USA) and Microsoft Excel 2007 (Microsoft Corporation, USA) application. IC50 values were obtained from regression lines with coefficient factors between R2 = 0.52 and 0.99. All the results of antimicrobial tests are listed in Table-1.

DNA Fragmentation Assay

The DNA Fragmentation Assay allows determining the amount of DNA that is degraded upon treatment of cells with certain agents, e.g. with TNF-alpha or anti-Fas antibody (IPO-4). Apoptosis is characterized by the activation of endogenous endonucleases with subsequent cleavage of chromatin DNA into internucleosomal fragments of 180 BP and multiples thereof. DNA cleavage during apoptosis occurs at sites between nucleosomes, protein-containing structures that occur in chromatin at ~200-

BP intervals. This DNA fragmentation is often analyzed using agarose gel electrophoresis to demonstrate a "ladder" pattern at ~200-BP intervals. Photograph the gel directly on UV trans-illuminator or first stain gel with ethidium bromide 10 to 30 min, distains at 30 min in water if necessary[23].

Preparation of docking

Default docking parameters and flexible space were validated by docking parameters and biological activities of small molecule compound with respect to low energetic molecules. Docking study was performed on a single machine equipped with Intel Core i5 processor, operating system windows 8.0 using glide protein which was prepared on protein preparation wizard. Glide searches for favorable interactions between synthesized hybrids and protein receptor using a grid-based method. Protein was downloaded from protein databank.

Result and discussion

3.1 Chemistry

The synthetic approaches utilized for the synthesis of the targeted chalcone-sulphonamides hybrids are outlined in scheme-1 and scheme-2. Hydroxy and methoxy substitution on benzene ring-A of chalcone scaffold could play a vital role in imparting significant cytotoxicity [24]. Hence, to develop biological efficacy, we chose a synthetic approach based on substitution on ring-A of chalcone, the first outline is the reaction of a substituted aldehyde with 4-aminoacetophenone to give chalcones 1a- 1d in good yield using base-catalyzed Claisen-Schmidt condensation (scheme-1) [25]. The structural investigation to synthesized compounds was based on their spectroscopic (IR, NMR, MS) data. IR spectrum of compound 1a revealed characteristic strong intensity bands at 3340, 3219 cm-1, for carbonyl at 1647-1680, -CH=CH- at 1590-1610 and for –OH at 3570-3395 cm-1. The 1HNMR spectra of compounds 1a-1d displayed downfield doublets at 8.06 and 7.5 ppm assigned for CH=CO and CH=CH respectively. Broad singlet in upfield at δ 3.47-3.50 ppm appeared for –NH2 group and singlet of methoxy proton in 1a and 1b appeared about at δ 3.87 ppm.

The further pathways adapted to design and synthesize novel chalcone-sulphonamide hybrids which are considered an important class of anticancer agents [26]. Thus, the treatment of chalcone 1a-1d with sulphonamide in DMF afforded the desired hybrids 3a-6f (Scheme-2). The key reaction observed is the formation of a C-N bond between the nitrogen of chalcone and carbon of sulphonamide derivatives. Structures of all the synthesized hybrids 3a-6f are further supported by IR, NMR, and HRMS. IR spectra of 3a-6f displayed -NH- absorption band at 3330-3360 cm-1 and stretching band of amide carbonyl at 1734-1739 cm-1. The broad singlet in 1HNMR of 3a-6f has disappeared which implies the absence of free –NH2 group of chalcone moiety and further resulted in the formation of a C-N bond between two

pharmacophores. In the 1HNMR spectra, methylene protons present between -NH- and -CO- appear as a doublet at δ 3.36 ppm. 13CNMR spectrum of 3a revealed different characteristic signals at δ 44.54ppm for methylene, 56.79ppm for methoxy, 167.97ppm for amide carbonyl and 190.97ppm for vinyl carbonyl also reinforce the proposed structure. High-resolution mass spectroscopy reveals that hybrid 3a showed molecular ion (M+H+) peak at 546.157 corresponding to the molecular formula of C28H26N4O6S.

3.2 In vitro antiproliferative evaluation against a panel of five human cancer cell lines

Chemically synthesized all chalcone-sulphonamide hybrids 3a-6f were screened for their antiproliferative activity by using MTT assay method against MCF-7(Breast cancer), DU-145(Human prostate carcinoma), HCT-15 (Colon cancer), NCIH-522 (stage 2, adenocarcinoma; non-small cell lung cancer) and HT-3 (Human cervical cancer) cell line mentioned in Table-1. From the result in table-1, it was found that compounds 3b, 3e, 4a, 4e, 4f, 5a, 5e, 5f, 6a, and 6f were most potent compounds in the study with IC50 ranges 3-12 µg/ml and exhibited higher cytotoxicity when compared with control and the reference drug doxorubicin. Compound 3a showed equipotent activity than that of the reference drug. Data in table-1 showed that compound 5a and 5f were slightly toxic towards normal non-cancerous Vero cells as their IC50 values were less than 100 µg/ml.

A closure looks into the structural activity relationship (SAR) indicates that the introduction of isoxazole heterocycle in sulphonamide pharmacophore was proved to be successful in case of compounds 3a, 4a, 5a and 6a which showed an increased activity. The efficiency of introducing pyrimidine ring was proved in compounds 3e, 4e, 4f, 5e, 5f and 6f whose activities interestingly improved.

The synthesis of chalcone-sulphonamide hybrids 3a-6f indicated that the most potent hybrid among six novel compounds was 6f against breast cancer cells. This finding encouraged us to synthesize more derivatives by introducing different substituents in chalcone moiety, aiming to obtain more potent anticancer agents but unfortunately, the resulting compounds were with low activities and these results proved the importance of α,β-unsaturated ketone system in chalcones as anticancer pharmacophore without any substitution.

Table-1: In vitro inhibitory activity of chalcone-sulphonamide hybrids 3a-6f and doxorubicin as reference drug.

Hybrids	(Cell lines / IC_{50}* values (µg/ml					
	MCF-7	HCT-15	DU-145	NCIH-522	HT-3	Vero

3a	16.72	20.41	79.86	8.486	8.723	-
3b	25.54	100<	76.99	7.763	32.77	100<
3c	100<	64.37	51.36	34.44	8.298	-
3d	68.24	100<	10.07	57.89	80.42	-
3e	68.24	100<	5.092	16.52	29.93	100<
3f	19.76	27.69	11.15	30.54	18.49	-
4a	5.527	63.98	33.83	14.17	110.2	100<
4b	100<	32.24	36.48	12.44	34.16	-
4c	17.11	20.13	9.193	34.44	19.47	-
4d	100<	18.02	100<	10.35	13.921	-
4e	36.26	10.61	4.810	39.81	100.53	100<
4f	43.27	10.67	9.248	5.252	9.783	100<
5a	67.16	5.828	22.07	6.045	132.5	90.97
5b	82.3	25.88	20.82	41.20	62.48	-
5c	100<	100<	33.83	11.61	105.2	-
5d	61.10	41.37	21.23	100	43.53	-
5e	11.32	100<	19.41	100	88.10	-
5f	100<	29.11	3.321	10.99	76.40	85.44
6a	100<	55.20	14.40	6.53	54.36	57.22
6b	100<	51.92	99.05	11.81	17.90	-
6c	68.52	53.70	22.50	10.08	14.48	-
6d	36.53	49.57	24.82	100<	17.89	-
6e	100<	16.24	19.26	12.49	116.4	-
6f	3.774	15.09	16.90	18.87	12.10	100<
STD	**12.19**	**8.772**	**4.152**	**7.784**	**3.740**	**100<**

*IC50 value represents the concentration of the compound required to produce 50% inhibition of cells which is the mean value of two determinations where the deviation from the mean is < 10% of the mean value. STD-doxorubicin.

3.3 *DNA fragmentation*

To further validate that whether most potent compounds 4a, 4f, 5a, and 6f induces apoptosis in relevant cells, DNA fragmentation was determined by selecting dose as per the IC50 value of the various effective concentration against various cancer cells. The compounds 4a and 6f found to be highly effective in inducing apoptosis in MCF-7 cells, while compounds 4f and 5a exhibited excellent DNA fragmentation pattern which confirms the apoptosis in lung cancer NCIH-522 cells. From the results of fragmentation, it was found that almost all test samples were exhibited excellent DNA fragmentation pattern, which confirms the apoptosis rather necrosis when compared with standard DNA Ladder. While in the case of Vero cell line fragmentation pattern was not that much clear whereas it also not gives clear single band. So it indicates that compound having less effect on the normal cell line, which revealed that hybrids exhibited apoptosis effect.

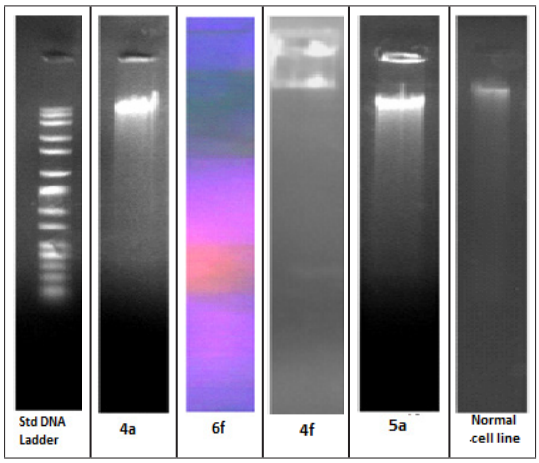


Figure-2: Photograph of UV-illuminated DNA ladder harvested MCF-7 cells, NCIH-522 cells and normal Vero cells resolved on 1% agarose gel by electrophoresis.

3.4 *Molecular docking*

With the aim to understand the protein-drug interaction and structure binding to the active site of the relevant enzymatic system, a molecular modeling study was performed. Molecular docking of synthesized potent compounds 4a, 4f, 5a and 6f into a relevant enzyme was carried out using the MOE (Molecular operating environment) 10.4 modeling software [27]. The docking results of hybrids 4a, 4f, 5a, and 6f are presented in Table-4. The docking score of standard reference doxorubicin drug is -10.00 kcal mol-1. While methoxy group, hydroxyl group and –NH- group of 5a are bound in the adjacent pocket of Gly166 and Tyr165, Lys168, Asn95, and Hid130 with the hydrogen bond distance range of 1.94-2.70 A^{o}, such interactions are almost essential for an enzymatic inhibitory activity (figure-3). Furthermore, -NH- group of compound 6f forms a hydrogen bond with the amino acids namely Asn91 having a distance of 1.93 A^{o}, also a hydroxy group of 6f showed binding with Asn163, Asn158, and Ser148 (figure-4).

Compound 4f forms six hydrogen bonds with the amino acids present in the protein. One of the oxygen of sulphonamide groups forms a hydrogen bond with Lys168 and the nitrogen atom of –NH- group forms a hydrogen bond with Asn91 and Lys168 respectively (figure-5). While compound 5a forms five hydrogen bonds with protein receptor in which both the oxygen atom of sulphonamide forms a hydrogen bond

with –NH- of Ala167, Asn91, and Lys168. Among these hydrogen bonds, one oxygen of sulphonamide bind at the least distance 1.98 A˚. The further oxygen atom of a hydroxyl group, nitrogen of –NH- group and nitrogen atom isoxazole forms a hydrogen bond with Hid130, Lys168, and Gly164 respectively (figure-6).

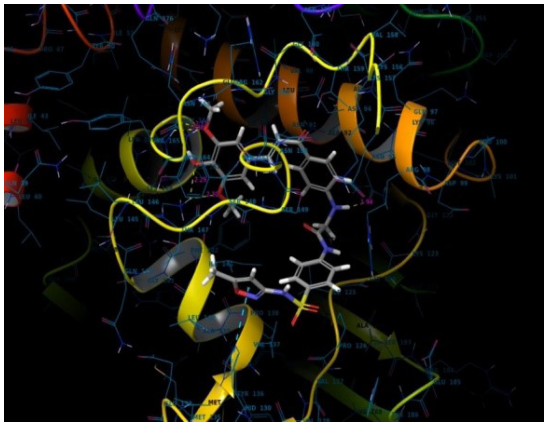


Figure-3. The orientation and docking pose of 4a in the active site of amino acid.

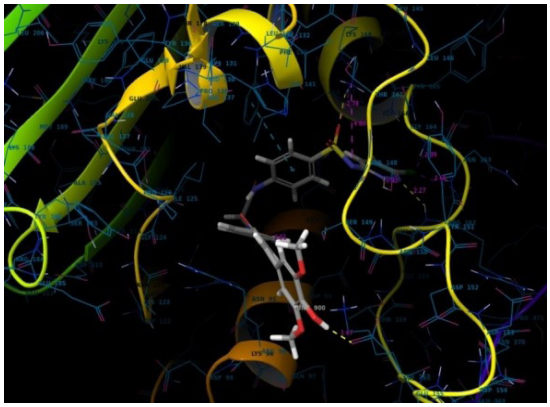


Figure-4. The orientation and docking pose of 4f at the active site of amino acid.

Figure-5. The orientation and docking pose of 5a in the active site of amino acid.

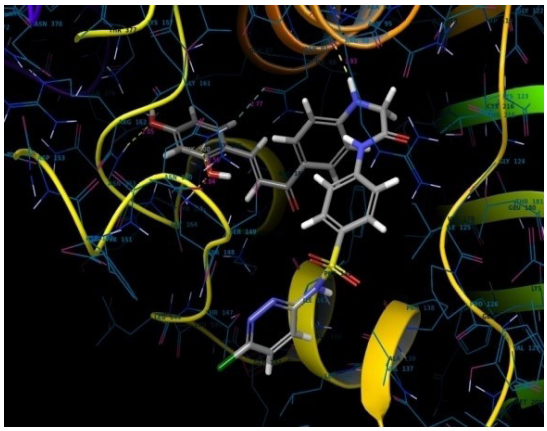


Figure-6. The orientation and docking pose of 6f in the active site of amino acid

The molecular simulation and hydrogen bond interaction analysis of four hybrids 4a, 4f, 5a and 6f suggested that they bound to the receptor in such a way to profound their interaction with the binding site. As seen in table-2, compounds 4a, 4f, 5a and 6f exhibited higher binding affinity with docking score of -8.114, -8.996, -7.906 and -9.364 kcal mol-1 respectively which is close to that of doxorubicin (docking score -10.00 kcal mol-1). The hydrogen bonding efficacy of sulphonamide group and heterocyclic ring and π-π interaction of chalcone linkage with various amino acids play a crucial role in the inhibitory effect. The dominant anticancer activity of 4a, 4f, 5a, and 6f which have superior inhibitory value than doxorubicin, corroborated them to be enlisted as lead compounds as significant anticancer agents.

Table-2: Interaction of Chalcone-sulphonamide hybrids 4a, 4f, 5a and 6f with amino acids and hydrogen bond distance based on docking results

Hybrids	Docking score	Interacting amino acids	Groups of compounds interacted	H-bond (˚distance(A
4a	-8.114	GLY 166	OCH_3-	2.70
		TYR 165	OCH_3-	2.38
		LYS 168	OH, -OCH_3-	2.30 ,2.29
		ASN 95	-NH-	1.94
4f	-8.996	GLU 155	OH-	1.67
		ASN 91	-NH-	2.78
		LYS 168	-O=S=O, -NH	4.80 ,2.78
		SER 148	(N (Pyrimidine-	2.42
		ARG-167	(N (Pyrimidine-	2.27
5a	-7.906	HID 130	OH-	2.18
		LYS 168	-O=S=O, -NH	4.70 ,2.15
		ASN 91	O=S=O	1.98
		ALA 167	O=S=O	2.79
		GLY 164	(N (Isoxazole-	2.10
6f	-9.364	ASN 163	OH-	2.05
		ASN 158	OH-	2.56
		SER 148	OH-	1.84
		ASN 91	-NH-	1.93
.Doxo	-10.00	ASN 150	OH-	-
		PHE 142	O=C	
		GLY 92	OCH_3-	
		ALA 139	(O(pyrane-	
		ARG 98	OH-	

Conclusion

In sum, a novel series of chalcone-sulphonamide hybrids were designed, synthesized and evaluated in vitro for their anticancer activity against five selected human cancer cell lines MCF-7, DU-145, HCT-15, NCIH-522, HT-3 and normal Vero cells. Biological assay demonstrates that compound 4a, 4f, 5a, and 6f are found a promising anticancer agent as their IC50 values less than that of reference doxorubicin drug. DNA fragmentation study of these four potent compounds indicates that apoptosis process induced in MCF-7 cells by 4a and 6f whereas in NCIH-522 by 4f and 5a, which is associated with the dysregulation of mitochondria. Molecular docking study and binding mode of 4a, 4f, 5a, and 6f make them versatile lead candidates for further optimization and development of potent and safer anticancer agents.

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Conflict of interest

The authors reveal no conflict of interest

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