Synthesis and Anticancer Activity of Novel Hydrazone Linkage-Based Aryl Sulfonate Derivatives as Apoptosis Inducers

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Abstract

In the present study, the various 28 hybrid molecules containing hydrazone and sulfonate moiety were synthesized and characterized by FTIR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ spectroscopy and LC-MS spectrometry, besides elemental analysis. The compounds were evaluated for their antiproliferative effects against six cancer cell lines, namely A549 (non-small cell lung cancer), MCF-7 (breast cancer), HT-29 (colorectal adenocarcinoma cancer), PC-3 (androgen-independent prostate adenocarcinoma), Hep3B (hepatocellular carcinoma cancer), and HeLa (epitheloid cervix carcinoma cancer). Among all the target compounds, compounds **4g** and **4h** exhibited more promising effects on MCF-7 cell lines (IC_{50} =17.8 mM and 21.2 mM, respectively) with high selectivity. Further mechanistic studies proposed that compounds **4g** and **4h** induced apoptosis is mediated through the intrinsic apoptotic pathway with changes in mitochondrial membrane potential by finally activating caspase-9 and caspase-3. The results have been encouraging enough to merit further investigation.

Introduction

Hydrazone derivatives are known for their broad spectrum of biological activities [1]. A variety of hydrazones have been utilized as anticancer drugs. Representative members of this class are zorubicin and bisantrene which are widely used for treatment of different cancer types (Fig. 1).

As shown in Fig. 2, 2',4'-difluoro-4-hydroxy-*N*-(2-pyridyl methylidene)biphenyl-3-carbohydrazide has been reported as a hepatocellular carcinoma inhibitor [2]. Zhang et al. [3] studied a series of sulfonylhydrazone-substituted 8-ethoxy-3-nitro-2*H*-chromenes which were active against various cancer cell lines. Moreover, tolmetin hydrazide-hydrazones having 2,6-dichlorophenyl moiety exhibited significant cytotoxicity and induction of apoptosis in the HT-29 cancer line [4]. A very recent report deals with, the apoptosis-inducing effect and VEGFR-2 inhibition of (S)-2-(6-methoxynaphthalen-2-yl)-*N*-{(E)-[2-(trifluoromethoxy)phenyl]methylidene} propanehydrazide [5].

Sulfonates are widely used in medicine researches and have significant pharmacological applications such as antifungal [6], acaricidal [7] and antioxidant [8]. In particular, there have been many reports on the anticancer properties of compounds bearing aryl sulfonate moiety [9–11] (Fig. 2). Sulfonate-based compounds have a great affinity for lipid phases and can easily pass the membrane to attach to target sites due to their physicochemical features [7]. Also, the irreversible enzyme inhibitors usually possess reactive functional groups such as nitrogen mustards, aldehydes, alkenes and phenyl sulfonates [12]. These irreversible enzyme inhibitors may block certain enzymes that cancer cells.

On a pharmaceutical target, combining two or more effective pharmacophores of various potent bioactive agents can lead to potent new compounds. Based on the aforementioned compounds and in continuation of our work on hydrazones we were interested in studying the reaction of hydrazides with sulfonate aldehydes. Thus, we report here on the synthesis, characterization and anticancer evaluation of hydrazone derivatives with tosyl sulfonate moieties.

Materials And Methods

Experimental

All chemical compounds were purchased from Merck and Sigma Aldrich. Melting points were measured with a ThermoScientific 9300 melting point apparatus and were uncorrected. Infrared (IR) Spectra were recorded using Shimadzu FTIR 8400S spectrophotometer. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ experiments were carried out using BRUKER NMR

spectrometer, and chemical shifts (δ H) are reported relative to TMS as the internal standard. Mass spectra were recorded using Agilent LC/MSD and Xevo G2-XS QToF spectrometer. All reagents and solvents were dried and purified by the standard techniques. Chemical shift (δ) values of rotameric hydrogens whenever identified are presented within parentheses by assigning an asterisk (*) along with that of other forms [28]. Compounds **1a-n** and **2a-b** are already recorded in the literature [4, 13–21]. Compounds **1i** and its ester derivative, **3e** and **3j** have CAS Registry Numbers but no reference, analytical, or spectral data.

Synthesis

Methyl 4-[(thiophene-2-carbonyl)amino]benzoate

White cream solid; yield: 56%; m.p. 185–187°C; FTIR $\nu_{\max}$ (cm^{-1}): 3360 (N-H), 1666 (C=O), 1593 (N-H bending); $^1\text{H-NMR}$ (300 MHz), (DMSO- d_6 /TMS) δ ppm: 1.32 (t, 3H, $\text{CH}_2\text{-CH}_3$), 4.30 (q, 2H, $\text{CH}_2\text{-CH}_3$), 7.23–8.08 (m, 7H, Ar-H), 10.52 (s, 1H, CONH); Anal. calcd. for $\text{C}_{14}\text{H}_{13}\text{NO}_3\text{S} \cdot 1/2\text{C}_2\text{H}_5\text{OH}$: C: 60.38, H: 5.41, N: 4.69, S: 10.75. Found: C: 60.51, H: 5.19, N: 4.87, S: 10.51.

N-[4-(hydrazinecarbonyl)phenyl]thiophene-2-carboxamide (1i)

White cream solid; yield: 76%; m.p. 230–231°C; FTIR $\nu_{\max}$ (cm^{-1}): 3306 (N-H), 1633 (C=O), 1600 (N-H bending); $^1\text{H-NMR}$ (300 MHz), (DMSO- d_6 /TMS) δ ppm: 4.46 (s, 2H, CONHNH_2); 7.23–8.06 (m, 7H, Ar-H); 9.69 (s, 1H, CONH); 10.41 (s, 1H, CONH); Anal. calcd. for $\text{C}_{12}\text{H}_{11}\text{N}_3\text{O}_2\text{S} \cdot 1/5\text{H}_2\text{O}$: C: 54.41, H: 4.34, N: 15.86, S: 12.10. Found: C: 54.85, H: 4.45, N: 15.71, S: 12.34.

General procedure for the synthesis of target compounds

The mixture of aldehydes **2a-b** (1 mmol) and **1a-n** derivatives (1 mmol) was refluxed in ethanol (20 ml) in the presence of glacial acetic acid as the catalyst. After completion of the reaction (checked by TLC, t : 25°C, acetone/petroleum ether 50:50 (v/v)), ethanol was evaporated, and the solid compounds **3a-n** and **4a-n** were dried and recrystallized with ethanol.

Synthetic route and chemical structures of compounds **3a-n** and **4a-n** are presented in Scheme 1.

Scheme 1. Synthetic route to target compounds

4-((2-(2-(6-methoxynaphthalen-2-yl)propanoyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3a)

White cream solid; yield: 81%; m.p. 155–156 °C; FTIR $\nu_{\max}$ (cm^{-1}): 3185 (N-H), 1660 (C=O), 1600 (C=N), 1373&1174 (S=O); $^1\text{H-NMR}$ (300 MHz), (DMSO- d_6 /TMS) δ ppm: 1.46 (t, 3H, $J=7.2$ Hz, CH-CH_3); 2.43 (2.41*, s, 3H, Ar- CH_3); 3.85–4.74 (m, 4H, OCH_3 & CH-CH_3); 7.28–7.95 (m, 14H, Ar-H); 8.16 (7.85*, s, 1H, CH=N); 11.63 (11.34*, s, 1H, CONH); $^{13}\text{C-NMR}$ (75 MHz), (DMSO- d_6 /TMS) δ ppm: 18.9 (CH-CH_3), 21.6 (Ar- CH_3), 44.4 (CH-CH_3), 55.6 (O- CH_3), 106.1, 119.0, 119.1, 122.9, 123.3, 125.9, 126.1, 126.7, 127.1, 127.2, 127.3, 128.7, 128.8, 128.9, 129.4, 129.5, 130.7, 131.6, 131.7, 131.9, 133.5, 133.7, 133.9, 137.0, 137.5, 141.5, 145.5&146.3 (CH=N), 150.0&150.2 (C- OSO_2), 157.4&157.5 (C- OCH_3), 170.4&175.5 (C=O); Anal. calcd. for $\text{C}_{28}\text{H}_{26}\text{N}_2\text{O}_5\text{S} \cdot 1/3\text{H}_2\text{O}$: C: 66.03, H: 5.85, N: 5.33, S: 6.52. Found: C: 66.12, H: 5.28, N: 5.51, S: 6.30; LCMS (ES-API) m/z : 504 $[\text{M} + \text{H}]^+$, 527 $[\text{M} + \text{H} + \text{Na}]^+$.

4-((2-(2',4'-difluoro-4-hydroxy-[1,1'-biphenyl]-3-carbonyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3b)

White solid; yield: 79%; m.p. 218-219°C; FTIR $\nu_{\max}$ (cm⁻¹): 3185 (N-H), 1637 (C=O), 1595 (C=N), 1373&1166 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.42 (s, 3H, Ar-CH₃); 7.07-8.01 (m, 14H, Ar-H); 8.42 (s, 1H, CH=N); 11.92 (s, 2H, Ar-OH&CO-NH); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6 (Ar-CH₃), 104.9, 112.3, 116.8, 118.0, 123.1, 125.5, 128.7, 129.2, 129.4, 130.7, 131.6, 132.1, 133.7, 146.4, 147.7 (CH=N), 150.5 (C-OSO₂), 158.9, 157.7&161.0 (C-F, *J*=246 Hz), 160.2&163.5 (C-F, *J*=252 Hz), 164.8 (CONH); Anal. calcd. for C₂₇H₂₀F₂N₂O₅S.1/4 H₂O: C: 61.53, H: 3.92, N: 5.32, S: 6.08. Found: C: 61.88, H: 4.06, N: 5.39, S: 6.17; LCMS (ES-API) *m/z*: 523 [M + H]⁺, 546 [M + H + Na]⁺.

4-((2-(2-(2-fluoro-[1,1'-biphenyl]-4-yl)propanoyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3c)

White solid; yield: 87%; m.p. 146-147°C; FTIR $\nu_{\max}$ (cm⁻¹): 3228 (N-H), 1651 (C=O), 1599 (C=N), 1345&1170 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 1.06 (CH₃CH₂OH, ethanol); 1.43 (1.40*, d, 3H, *J*=6.9 Hz, CH-CH₃); 2.42 (s, 3H, Ar-CH₃); 3.46 (CH₃CH₂OH, ethanol); 4.36 (CH₃CH₂OH, ethanol); 4.74 (3.77*, q, 1H, *J*=6.9 Hz, CH-CH₃); 7.07-7.76 (m, 16H, Ar-H); 8.19 (7.92*, s, 1H, CH=N); 11.65 (11.44*, s, 1H, CO-NH); ¹³C-NMR (150 MHz), (DMSO-*d*₆/TMS) δ ppm: 18.6 (CH₃CH₂OH), 18.7&19.0 (CH-CH₃), 21.6 (Ar-CH₃), 43.9 (CH-CH₃), 56.5 (CH₃CH₂OH), 115.3, 115.4, 115.5, 115.7, 122.9, 123.0, 126.8, 126.9, 127.1, 127.2, 128.2, 128.2, 128.7, 128.7, 129.0, 129.0, 129.0, 129.1, 130.7, 131.08, 131.1, 131.1, 131.1, 131.6, 131.7, 133.8, 135.3, 142.0, 143.7, 143.8, 144.2, 144.2, 145.9, 146.3&146.4 (CH=N), 150.1&150.3 (C-OSO₂), 158.5&160.1 (C-F, *J*=245 Hz), 169.7&174.9 (CONH); Anal. calcd. for C₂₉H₂₅FN₂O₄S.1/3 C₂H₅OH: C: 66.98, H: 5.12, N: 5.27, S: 6.03. Found: C: 66.06, H: 4.86, N: 5.46, S: 6.18; LCMS (ES-API) *m/z*: 539 [M + Na]⁺.

4-((2-(2-(1-methyl-5-(4-methylbenzoyl)-1H-pyrrol-2-yl)acetyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3d)

White solid; yield: 80%; m.p. 183-184°C; FTIR $\nu_{\max}$ (cm⁻¹): 3352 (O-H), 3230 (N-H), 1651 (C=O), 1599 (C=N), 1373&1170 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.39 (s, 3H, ArCH₃); 2.42 (s, 3H, ArCH₃); 3.75-4.17 (m, 5H, N-CH₃ ve CH₂CO); 6.11 (d, 1H, *J*=3.9 Hz, CH₂, pyrrole); 6.57 (d, 1H, *J*= 3.9 Hz, pyrrole); 7.07-7.76 (m, 12H, Ar-H); 8.19 (8.00*, s, 1H, -CH=N); 11.64 (s, 1H, -CONH); ¹³C-NMR (150 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.5 (Ar-CH₃), 21.6 (Ar-CH₃), 31.1 (N-CH₃), 33.0&33.4 (CH₂CO), 109.7, 110.0, 122.1, 122.2, 123.0, 128.7, 128.8, 129.0, 129.1, 129.4, 129.4, 130.7, 131.6, 131.7, 133.7, 133.8, 137.3, 137.4, 137.5, 137.7, 141.9, 141.9, 142.3, 145.7, 146.4&146.4 (CH=N), 150.2&150.3 (C-OSO₂), 165.2&170.8 (CONH), 184.7&184.8 (C=O); Anal. calcd. for C₂₉H₂₇N₃O₅S.1/2 H₂O: C: 64.67, H: 5.24, N: 7.80, S: 5.95. Found: C: 64.50, H: 5.33, N: 7.91, S: 6.22; LCMS (ES-API) *m/z*: 553 [M + H + Na]⁺.

4-((2-(2-(4-isobutylphenyl)propanoyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3e)

White solid; yield: 82%; m.p. 99-101°C; FTIR $\nu_{\max}$ (cm⁻¹): 3230 (N-H), 1651 (C=O), 1599 (C=N), 1346, & 1170 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 0.81 (m, 6H, -CH(CH₃)₂); 1.36 (t, 3H, *J*=6.9 Hz, -CHCH₃); 1.72-1.83 (m, 1H, -CH(CH₃)₂); 2.36-2.42 (m, 5H, ArCH₃&CH₂CH(CH₃)₂); 4.60 (3.64*, q, 1H, *J*=6.9 Hz, CHCH₃); 7.04-7.76 (m, 12H, Ar-H); 8.16 (7.87*, s, 1H, -CH=N); 11.56 (11.04*, s, 1H, -CONH); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 18.8&18.9 (CHCH₃), 21.6 (Ar-CH₃), 22.6 (CH(CH₃)₂), 30.0 (CH(CH₃)₂), 44.0 (CH₂CH(CH₃)₂), 44.6 (CHCH₃), 122.9, 127.4, 127.7, 128.6, 128.7, 128.9, 129.3, 129.4, 130.7, 131.6, 131.7, 133.9, 139.1, 139.5, 139.6, 140.0, 141.5, 145.5, 146.3&146.4 (CH=N), 150.0&150.2 (C-OSO₂), 170.5&175.6 (C=O); Anal. calcd. for C₂₇H₃₀N₂O₄S: C: 67.76, H: 6.32, N: 5.85, S: 6.70. Found: C: 68.15, H: 5.96, N: 5.99, S: 6.73; LCMS (ES-API) *m/z*: 501 [M + Na]⁺.

4-((2-(2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3f)

Dark yellow solid; yield: 77%; m.p. 107-108°C; FTIR $\nu_{\max}$ (cm⁻¹): 3223 (N-H), 1651 (C=O), 1599 (C=N), 1346&1170 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 0.65 (0.57*, t, 3H, *J*=7.2 Hz, -CH₂-CH₃); 1.06 (t, ethanol); 1.24 (t, 3H, -CH₂-CH₃); 2.03-2.15 (m, 2H, CH₂-CH₃); 2.42 (s, 3H, Ar-CH₃); 2.58-2.68 (m, 2H, -CH₂-CH₃); 2.78-2.98 (m, 4H, -CH₂CONH & -CH₂); 3.45 (m, ethanol); 3.69-4.00 (m, 2H, -CH₂); 4.36 (t, ethanol); 6.86-7.75 (m, 11H, Ar-H); 8.19 (7.94*, s, 1H, N=CH); 10.51 (10.49*, s, 1H, indole N-H); 11.37 (11.24*, s, 1H, CO-NH); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 8.3 (pyran CH₂CH₃), 14.9 (indole CH₂CH₃), 21.6 (ArCH₃), 24.2 (pyran -CH₂CH₂O-), 22.3 (indole CH₂CH₃), 31.2 (pyran CH₂CH₃), 6.2&76.5 (-CH₂OCH-), 107.5, 107.7, 115.8, 119.1, 12.9, 126.5, 127.0, 128.6, 128.7, 128.9, 130.7, 131.7, 134.0, 134.9, 136.7, 137.0, 146.4 (CH=N), 150.0&150.2 (C-OSO₂), 166.1,&171.9 (C=O); Anal. calcd. for C₃₁H₃₃N₃O₅S.3/2 C₂H₅OH: C: 65.78, H: 6.32, N: 7.12, S: 5.43. Found: C: 65.16, H: 5.67, N: 7.14, S: 5.54; LCMS (ES-API) *m/z*: 582 [M + Na]⁺.

4-((2-(6-(2-(4-(tosyloxy)benzylidene)hydrazine-1-carbonyl)picolinoyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3g)

White solid; yield: 82%; m.p. 227-228°C; FTIR $\nu_{\max}$ (cm⁻¹): 3230, 3171 (N-H), 1668, 1651, 1599 (C=O & C=N), 1344, 1170 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.44 (s, 6H, CH₃), 7.16-7.84 (m, 16H, Ar-H), 8.26-8.38 (m, 3H, pyridine-H), 8.74 (s, 2H, CH=N), 12.37 (s, 2H, CONH-N=); ¹³C-NMR (150 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6 (2Ar-CH₃), 123.1, 126.1, 128.7, 129.3, 130.7, 131.7, 133.7, 140.5, 146.4, 148.6 (pyridine 2C-N), 149.0 (2CH=N), 150.6 (2C-OSO₂), 160.0 (2C=O); Anal. calcd. for C₃₅H₂₉N₅O₈S₂.4H₂O: C: 53.63, H: 4.76, N: 8.93, S: 8.18. Found: C: 53.99, H: 4.37, N: 9.21, S: 8.54; LCMS (ES-API) *m/z*: 734 [M + Na]⁺.

4-((2-(2-(2-(2,6-dichlorophenyl)amino)phenyl)acetyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3h)

White solid; yield: 88%; m.p. 228-229; FTIR $\nu_{\max}$ (cm⁻¹): 3317 (N-H), 1658 (C=O), 1614 (C=N), 1357&1178 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.43 (s, 3H, CH₃), 4.12 (3.70*, s, 2H, CH₂CO), 6.27-8.04 (m, 16H, Ar-H&CH=N), 8.20 (s, 1H, NH), 11.89 (11.68*, s, 1H, CONH-N=); ¹³C-NMR (150 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6&21.7 (Ar-CH₃), 35.8&36.0 (CH₂CO), 146.4&146.5 (CH=N), 150.2&150.4 (C-OSO₂), 168.3 (C=O); Anal. calcd. for C₂₈H₂₃Cl₂N₃O₄S₂.1/2H₂O: C: 58.24, H: 4.19, N: 7.28, S: 5.55. Found: C: 58.84, H: 4.29, N: 7.42, S: 5.80; LCMS (Q-TOF) *m/z*: 568 [M + H]⁺.

4-((2-(4-(thiophene-2-carboxamido)benzoyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3i)

White cream solid; yield: 76%; m.p. 281-283°C; FTIR $\nu_{\max}$ (cm⁻¹): 3354, 3292 (N-H), 1703, 1641 (C=O), 1599 (C=N), 1340&1172 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.43 (s, 3H, Ar-CH₃), 7.25-8.09 (m, 15H, Ar-H), 8.43 (s, 1H, CH=N), 10.49 (s, 1H, thiophene-CONH), 11.87 (s, 1H, CONH); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6 (Ar-CH₃), 119.9, 123.0, 128.4, 128.6, 128.7, 129.0, 130.0, 130.7, 131.6, 132.9, 134.0, 140.0, 142.4, 146.3, 146.4 (C-OSO₂), 150.3 (CH=N), 160.6 (C=O), 163.0 (C=O); Anal. calcd. for C₂₆H₂₁N₃O₅S₂: C: 60.10, H: 4.07, N: 8.09, S: 12.34. Found: C: 59.68, H: 4.16, N: 8.06, S: 12.91; LCMS (Q-TOF) m/z: 520 [M + H]⁺.

4-((2-(4-benzamidobenzoyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3j)

White solid; yield: 70%; m.p. 286°C; FTIR $\nu_{\max}$ (cm⁻¹): 3338, 3230 (N-H), 1668, 1651, 1599 (C=O & C=N), 1373&1172 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.43 (s, 3H, Ar-CH₃), 7.53-8.00 (m, 17H, Ar-H), 8.43 (s, 1H, CH=N), 10.53 (s, 1H, amide-CONH), 11.87 (s, 1H, CONH); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6 (Ar-CH₃), 119.9, 123.0, 128.2, 128.7, 128.9, 130.7, 131.6, 132.3, 134.0, 135.0, 146.4 (CH=N), 150.2 (C-OSO₂), 163.0 (C=O), 165.3 (C=O); Anal. calcd. for C₂₈H₂₃N₃O₅S₂·1/2H₂O: C: 64.35, H: 4.63, N: 8.04, S: 6.14. Found: C: 64.79, H: 4.13, N: 8.20, S: 6.11; LCMS (ES-API) m/z: 537 [M + H + Na]⁺.

4-((2-((5-(propylthio)-2,3-dihydro-1H-benzimidazol-2-yl)carbamoyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3k)

White solid; yield: 79%; m.p. 228-230°C; FTIR $\nu_{\max}$ (cm⁻¹): 3365, 3236 (N-H), 1699, 1626 (C=O & C=N), 1367&1174 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 0.95 (t, 3H, *J*=7.2 Hz, propyl CH₃); 1.48-1.60 (m, 2H, propyl CH₂); 2.43 (s, 3H, Ar-CH₃); 2.86 (t, 2H, *J*=7.2 Hz, propyl CH₂); 7.02-7.97 (m, 12H, Ar-H&CH=N); 10.53 (s, 1H, NHCONHN=); 11.28 (s, 1H, benzimidazole NH); 11.94 (s, 1H, NHCONHN=); ¹³C-NMR (150 MHz), (DMSO-*d*₆/TMS) δ ppm: 13.5 (propyl CH₃), 21.6 (Ar-CH₃), 22.5 (propyl CH₂), 37.2 (propyl CH₂), 122.7, 128.7, 129.2, 130.7, 131.7, 133.9, 141.5, 146.3 (CH=N), 150.0 (C-OSO₂), 159.1 (C=N, benzimidazole); Anal. calcd. for C₂₅H₂₅N₅O₄S₂: 57.34, H: 4.81, N: 13.37, S: 12.25. Found: C: 57.84, H: 5.03, N: 13.22, S: 12.69; LCMS (ES-API) m/z: 525 [M + 2H]⁺.

4-((2-((4-bromophenyl)sulfonyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3l)

Dark yellow solid; yield: 73%; m.p. 127-129°C; FTIR $\nu_{\max}$ (cm⁻¹): 3234 (N-H), 1599 (C=N), 1346&1177 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.42 (s, 3H, CH₃), 7.47-7.85 (m, 12H, Ar-H), 7.90 (s, 1H, CH=N), 11.72 (s, 1H, CONH-N=); ¹³C-NMR (150 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6 (Ar-CH₃), 128.0, 123.1, 123.5, 128.2, 128.7, 128.9, 129.6, 130.8, 131.6, 131.9, 132.8, 133.1, 138.5, 146.4, 146.6 (CH=N), 150.4 (C-OSO₂); Anal. calcd. for C₂₀H₁₇BrN₂O₅S₂·1/2H₂O: C: 46.34, H: 3.50, N: 5.40, S: 12.37. Found: C: 46.75, H: 3.95, N: 5.33, S: 12.77; LCMS (ES-API) m/z: 546 [M + K]⁺.

4-((2-((4-chlorophenyl)sulfonyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3m)

Dark yellow solid; yield: 73%; m.p. 127-129°C; FTIR $\nu_{\max}$ (cm⁻¹): 3223 (N-H), 1599 (C=N), 1346&1161 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.41 (s, 3H, CH₃), 7.47-7.91 (m, 12H, Ar-H), 8.66 (s, 1H, CH=N), 11.74 (s, 1H, CONH-N=); ¹³C-NMR (150 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6 (Ar-CH₃), 123.0, 123.1, 127.9, 128.1, 128.7, 129.6, 129.9, 130.5, 130.7, 131.6, 133.1, 133.2, 138.1, 138.3, 138.5, 146.4, 146.5, 146.6 (CH=N), 150.4 (C-OSO₂); Anal. calcd. for C₂₀H₁₇ClN₂O₅S₂ · 1/2C₂H₅OH: C: 51.69, H: 4.13, N: 5.74, S: 13.14. Found: C: 51.02, H: 4.54, N: 5.79, S: 13.29; LCMS (ES-API) m/z : 464 [M +2H]⁺.

4-((2-((4-nitrophenyl)sulfonyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (3n)

White cream solid; yield: 86%; m.p. 153-154°C; FTIR $\nu_{\max}$ (cm⁻¹): 3185 (N-H), 1599 (C=N), 1348&1174 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.42 (s, 3H, CH₃), 7.58-8.45 (m, 13H, Ar-H&CH=N), 11.98 (s, 1H, CONH-N=); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6 (Ar-CH₃), 123.0, 125.1, 123.6, 129.0, 129.2, 130.7, 131.6, 132.9, 144.6, 146.4, 147.2 (CH=N), 150.4 (C-NO₂), 150.5 (C-OSO₂); Anal. calcd. for C₂₀H₁₇N₃O₇S₂·H₂O: C: 48.67, H: 3.88, N: 8.51, S: 12.99. Found: C: 48.82, H: 3.81, N: 8.06, S: 13.08; LCMS (ES-API) m/z: 476 [M +H]⁺, 497 [M +Na]⁺.

2-methoxy-4-((2-(2-(6-methoxynapthalen-2-yl)propanoyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (4a)

Off-white solid; yield: 90%; m.p. 164-165°C; FTIR $\nu_{\max}$ (cm⁻¹): 3176 (N-H), 1660 (C=O), 1604 (C=N), 1373&1169 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 1.42-1.49 (m, 3H, CH-CH₃); 2.43 (s, 3H, Ar-CH₃); 3.51 (s, 3H, ArOCH₃); 3.57-4.76 (m, 4H, OCH₃ & CH-CH₃); 7.25-7.79 (m, 13H, Ar-H); 8.15 (7.81*, s, 1H, CH=N); 11.65 (11.39*, s, 1H, CONH); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 18.9&19.2 (CH-CH₃), 21.6 (Ar-CH₃), 44.3 (CH-CH₃), 55.6 (O-CH₃), 56.0&56.1 (ArOCH₃), 106.1, 110.6, 119.0, 119.1, 120.0, 120.6, 124.2, 125.9, 126.7, 127.3, 128.7, 128.8, 128.9, 129.3, 129.5, 130.2, 130.4, 132.4, 132.4, 133.5, 133.7, 134.9, 136.3, 137.0, 137.8, 138.7, 139.0, 141.5, 145.9 (C-OSO₂), 146.0&146.1 (CH=N), 151.9 (C-OCH₃), 157.4&157.5 (C-OCH₃), 170.4&175.5 (C=O); Anal. calcd. for C₂₉H₂₈N₂O₆S · 1/2H₂O: C: 64.31, H: 5.40, N: 5.17, S: 5.92. Found: C: 64.33, H: 5.62, N: 4.96, S: 6.10; LCMS (ES-API) m/z: 555 [M + Na]⁺.

2-methoxy-4-((2-(2',4'-difluoro-4-hydroxy-[1,1'-biphenyl]-3-carbonyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (4b)

White solid; yield: 72%; m.p. 221-222°C; FTIR $\nu_{\max}$ (cm⁻¹): 3244 (N-H), 1643 (C=O), 1606 (C=N), 1354&1169 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.43 (s, 3H, Ar-CH₃); 3.56 (s, 3H, ArOCH₃); 7.29-8.00 (m, 13H, Ar-H); 8.41 (s, 1H, CH=N); 11.94 (s, 2H, CONH&Ar-OH); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6 (Ar-CH₃), 56.1 (O-CH₃), 110.8, 117.0, 118.0, 121.0, 124.4, 125.5, 128.7, 129.5, 130.3, 132.4, 134.7, 139.3, 146.2 (C-OSO₂), 148.0 (CH=N), 157.6&161.0 (C-F, *J*=253 Hz), 158.8 (C-OH), 160.2&163.5 (C-F, *J*=243 Hz). 164.7 (C=O) Anal. calcd. for C₂₈H₂₂F₂N₂O₆S: C: 60.86, H: 4.01, N: 5.07, S: 5.80. Found: C: 60.59, H: 3.85, N: 5.09, S: 5.61; LCMS (ES-API) m/z: 574.9 [M +Na]⁺.

2-methoxy-4-((2-(2-(2-fluoro-[1,1'-biphenyl]-4-yl)propanoyl)hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (4c)

White solid; yield: 86%; m.p. 150-151°C; FTIR $\nu_{\max}$ (cm⁻¹): 3232 (N-H), 1651 (C=O), 1599 (C=N), 1373&1170 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 1.45 (1.40*, d, 3H, *J*=6.9 Hz, CH-CH₃); 2.42 (2.38*, s, 3H, Ar-CH₃); 3.57 (3.52*, s, 3H, ArOCH₃); 4.71 (3.77*, q, 1H, *J*=6.9Hz, CH-CH₃); 7.37-7.71 (m, 15H, Ar-H); 8.17 (7.86*, s, 1H, CH=N); 11.68 (11.48*, s, 1H, CO-NH); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 18.6&19.0 (CH-CH₃), 21.5&21.6 (Ar-CH₃), 43.9 (CH-CH₃), 56.0 (Ar-OCH₃), 110.5, 110.7, 115.5, 115.8, 120.2, 120.6, 124.2, 128.2, 128.7, 129.0, 129.0, 129.1, 130.2, 131.1, 132.4, 132.4, 134.9, 135.3, 138.8, 139.0, 142.0, 146.0 (ArOCH₃), 146.1 (CH=N), 152.0 (C-OSO₂), 157.6&160.9 (C-F, *J*=252 Hz), 169.7&174.9 (CONH); Anal. calcd. for C₃₀H₂₇FN₂O₅S: C: 65.92, H: 4.98, N: 5.12, S: 5.87. Found: C: 65.23, H: 4.68, N: 5.07, S: 5.56; LCMS (ES-API) *m/z*: 571 [M+H+Na]⁺.

2-methoxy-4-((2-(2-(1-methyl-5-(4-methylbenzoyl)-1 H -pyrrol-2-yl)acetyl) hydrazinylidene)methyl)phenyl 4-methylbenzenesulfonate (4d)

White solid; yield: 83%; m.p. 215-216°C; FTIR $\nu_{\max}$ (cm⁻¹): 3190 (N-H), 1662 (C=O), 1620 (C=N), 1365&1170 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.38 (s, 3H, ArCH₃); 2.42 (s, 3H, ArCH₃); 3.55 (s, 3H, ArOCH₃); 3.86 (s, 3H, N-CH₃), 4.18 (s, 2H, CH₂CO); 6.12 (d, 1H, *J*= 3.9 Hz, CH₂, pyrrole); 6.58 (d, 1H, *J*=3.9 Hz, C=O, pyrrole); 7.16-7.72 (m, 11H, Ar-H); 8.17 (7.97*, s, 1H, -CH=N); 11.66 (s, 1H, -CONH); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.5 (Ar-CH₃), 21.6 (Ar-CH₃), 31.2 (N-CH₃), 33.4 (CH₂CO), 56.1 (Ar-OCH₃), 110.0, 111.2, 120.0, 122.2, 124.2, 124.3, 128.7, 129.1, 129.4, 130.3, 130.6, 132.4, 132.4, 134.8, 137.3, 137.5, 137.7, 138.9, 139.1, 141.9, 142.5 (C-OCH₃), 146.1 (CH=N), 152.0 (C-OSO₂), 170.9 (CONH), 184.7 (C=O); Anal. calcd. for C₃₀H₂₉N₃O₆S: C: 64.39, H: 5.22, N: 7.51, S: 5.73. Found: C: 64.34, H: 5.21, N: 7.52, S: 5.55; LCMS (ES-API) *m/z*: 582 [M + Na]⁺.

4-((2-(2-(4-isobutylphenyl)propanoyl)hydrazinylidene)methyl)-2-methoxyphenyl 4- methylbenzenesulfonate (4e)

White solid; yield: 76%; m.p. 125-127°C; FTIR $\nu_{\max}$ (cm⁻¹): 3230 (N-H), 1668 (C=O), 1597 (C=N), 1375&1172 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 0.80-0.86 (m, 6H, -CH(CH₃)₂); 1.36 (q, 3H, *J*=7.2 Hz, -CHCH₃); 1.72-1.84 (m, 1H, -CH(CH₃)₂); 2.36-2.43 (m, 5H, ArCH₃&CH₂CH(CH₃)₂); 3.57 (3.52*, s, 3H, Ar-OCH₃); 4.57 (3.65*, q, 1H, *J*=6.9Hz, CHCH₃); 7.04-7.72 (m, 11H, Ar-H); 8.14 (7.82*, s, 1H, -CH=N); 11.58 (11.34*, s, 1H, -CONH); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 18.8&19.2 (CHCH₃), 21.6 (Ar-CH₃), 22.6 (CH(CH₃)₂), 30.0&30.0 (CH(CH₃)₂), 44.0 (CH₂CH(CH₃)₂), 44.6 (CHCH₃), 56.0 (Ar-OCH₃), 110.6, 120.6, 124.2, 127.4, 127.6, 128.7, 129.3, 130.2, 132.4, 132.5, 135.0, 138.7, 139.0, 139.1, 139.6, 139.8, 140.0, 141.4, 145.8 (C-OCH₃), 146.0&146.1 (CH=N), 151.9 (C-OSO₂), 170.4& 175.6 (C=O); Anal. calcd. for C₂₈H₃₂N₂O₅S.1/3 H₂O: C: 65.35, H: 6.40, N: 5.44, S: 6.03. Found: C: 65.31, H: 6.06, N: 5.45, S: 6.11; LCMS (ES-API) *m/z*: 509 [M+H]⁺, 531 [M + Na]⁺.

4-((2-(2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetyl) hydrazinylidene)methyl)-2-methoxyphenyl 4-methylbenzenesulfonate (4f)

Off-white solid; yield: 78%; m.p. 175-176°C; FTIR $\nu_{\max}$ (cm⁻¹): 3342 (O-H), 3223 (N-H), 1645 (C=O); 1599 (C=N); 1369&1175 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 0.66 (0.57*, t, 3H, *J*=7.2 Hz, -CH₂-CH₃); 1.25 (t, 3H, *J*=7.2 Hz, -CH₂-CH₃); 2.02-2.15 (m, 2H, CH₂-CH₃); 2.42 (s, 3H, Ar-CH₃); 2.63-2.69 (m, 2H, -CH₂-CH₃); 2.79-3.01 (m, 4H, -CH₂CONH & -CH₂); 3.48-4.03 (m, 5H, -CH₂ & -OCH₃); 6.87-7.71 (m, 10H, Ar-H); 8.17 (7.93*, s, 1H, N=CH); 10.51 (s, 1H, indole N-H); 11.43 (11.25*, s, 1H, CO-NH); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 8.3 (pyran CH₂CH₃), 14.9

(indole CH_2CH_3), 21.6 (Ar CH_3), 24.2 (pyran $-\text{CH}_2\text{CH}_2\text{O}-$), 22.3 (indole CH_2CH_3), 31.2 (pyran CH_2CH_3), 43.6 (CH_2CO), 55.9&56.0 (Ar OCH_3), 60.5&60.7 (pyran $-\text{CH}_2\text{CH}_2\text{O}-$), 76.2&76.5 ($-\text{CH}_2\text{OCH}-$), 107.5, 107.7, 115.8, 119.1, 12.9, 126.5, 127.0, 128.6, 128.7, 128.9, 130.7, 131.7, 134.0, 134.9, 136.7, 137.0, 146.4 ($\text{CH}=\text{N}$), 150.0& 150.2 (C-OSO_2), 166.1&171.9 ($\text{C}=\text{O}$); Anal. calcd. for $\text{C}_{32}\text{H}_{35}\text{N}_3\text{O}_6\text{S}_2 \cdot 1/2\text{H}_2\text{O}$: C: 64.20, H: 5.91, N: 7.05, S: 5.39. Found: C: 64.76, H: 6.06, N: 7.02, S: 5.36; LCMS (ES-API) m/z : 582 $[\text{M} + \text{Na}]^+$.

4-((2-(6-(2-(3-methoxy-4-(tosyloxy)benzylidene)hydrazine-1-carbonyl)picolinoyl)hydrazinylidene)methyl)-2-methoxyphenyl 4-methylbenzenesulfonate (4g)

White solid; yield: 88%; m.p. 263-264°C; FTIR ν_{max} (cm^{-1}): 3574 (O-H), 3379, 3244 (N-H), 1668, 1583 ($\text{C}=\text{O}$ & $\text{C}=\text{N}$), 1356&1165 ($\text{S}=\text{O}$); $^1\text{H-NMR}$ (300 MHz), ($\text{DMSO-}d_6/\text{TMS}$) δ ppm: 2.44 (s, 6H, CH_3), 3.61 (s, 6H, Ar OCH_3), 7.23-7.76 (m, 14H, Ar-H), 8.27-8.39 (m, 3H, pyridine-H), 8.75 (s, 2H, $\text{CH}=\text{N}$), 12.39 (s, 2H, CONH-N=); $^{13}\text{C-NMR}$ (150 MHz), ($\text{DMSO-}d_6/\text{TMS}$) δ ppm: 21.6 (2Ar- CH_3), 56.1 (2Ar OCH_3), 110.9, 121.0, 124.4, 126.1, 128.7, 130.3, 132.5, 134.8, 139.4, 140.5, 146.2 (pyridine 2C-N), 148.6 (2C- OCH_3), 149.3 (2 $\text{CH}=\text{N}$), 152.1 (2C- OSO_2), 160.0 (2C=O); Anal. calcd. for $\text{C}_{37}\text{H}_{33}\text{N}_5\text{O}_{10}\text{S}_2 \cdot 4\text{H}_2\text{O}$: C: 52.66, H: 4.90, N: 8.30, S: 7.60. Found: C: 52.98, H: 5.04, N: 8.54, S: 7.91; LCMS (ES-API) m/z : 794 $[\text{M} + \text{Na}]^+$.

4-((2-(2-(2-((2,6-dichlorophenyl)amino)phenyl)acetyl)hydrazinylidene)methyl)-2-methoxyphenyl 4-methylbenzenesulfonate (4h)

White solid; yield: 83%; m.p. 224-225°C; FTIR ν_{max} (cm^{-1}): 3329 (N-H), 1660 ($\text{C}=\text{O}$), 1600 ($\text{C}=\text{N}$), 1369&1182 ($\text{S}=\text{O}$); $^1\text{H-NMR}$ (300 MHz), ($\text{DMSO-}d_6/\text{TMS}$) δ ppm: 2.43 (s, 3H, CH_3), 3.54 (s, 3H, OCH_3), 4.13 (3.71*, s, 2H, CH_2CO), 6.27-8.19 (m, 16H, Ar-H& NH& $\text{CH}=\text{N}$), 11.89 (11.74*, s, 1H, CO-NH); $^{13}\text{C-NMR}$ (150 MHz), ($\text{DMSO-}d_6/\text{TMS}$) δ ppm: 21.6&21.6 (Ar- CH_3), 35.8&36.0 (CH_2CO), 56.0 (Ar- OCH_3), 111.1, 111.3, 116.1, 120.0, 120.7, 121.0, 121.3, 124.3, 124.4, 125.1, 125.7, 126.0, 127.8, 127.9, 128.6, 129.7, 130.0, 130.9, 131.6, 132.5, 134.7, 137.4, 138.9, 139.2, 143.1, 143.3, 143.5&146.1 ($\text{CH}=\text{N}$), 146.8, 152.0 (C- OSO_2), 168.3, 173.6 ($\text{C}=\text{O}$). Anal. calcd. for $\text{C}_{29}\text{H}_{25}\text{Cl}_2\text{N}_3\text{O}_5\text{S}$: C: 58.20, H: 4.21, N: 7.02, S: 5.36. Found: C: 58.20, H: 4.59, N: 6.96, S: 5.39.

4-((2-(4-(thiophene-2-carboxamido)benzoyl)hydrazinylidene)methyl)-2-methoxyphenyl 4-methylbenzenesulfonate (4i)

White solid; yield: 88%; m.p. 242-243°C; FTIR ν_{max} (cm^{-1}): 3315, 3234 (N-H), 1703, 1645 ($\text{C}=\text{O}$), 1608 ($\text{C}=\text{N}$), 1377&1174 ($\text{S}=\text{O}$); $^1\text{H-NMR}$ (300 MHz), ($\text{DMSO-}d_6/\text{TMS}$) δ ppm: 2.43 (s, 3H, Ar- CH_3), 3.57 (s, 3H, Ar- OCH_3), 7.26-8.09 (m, 14H, Ar-H), 8.41 (s, 1H, $\text{CH}=\text{N}$), 10.49 (s, 1H, thiophene-CONH), 11.89 (s, 1H, CONH); $^{13}\text{C-NMR}$ (75 MHz), ($\text{DMSO-}d_6/\text{TMS}$) δ ppm: 21.6 (Ar- CH_3), 56.0 (Ar OCH_3), 110.6, 120.7, 124.3, 128.6, 129.0, 130.3, 132.9, 135.1, 139.0, 140.0, 142.5, 146.1, 146.6 ($\text{CH}=\text{N}$), 152.0 (C- OSO_2), 160.6, 163.0 ($\text{C}=\text{O}$); Anal. calcd. for $\text{C}_{27}\text{H}_{23}\text{N}_3\text{O}_6\text{S}_2$: C: 59.00, H: 4.22, N: 7.65, S: 11.67. Found: C: 58.80, H: 4.26, N: 7.68, S: 11.94; LCMS (Q-TOF) m/z : 550 $[\text{M} + \text{H}]^+$.

4-((2-(4-benzamidobenzoyl)hydrazinylidene)methyl)-2-methoxyphenyl 4-methylbenzenesulfonate (4j)

White solid; yield: 79%; m.p. 276-278°C; FTIR $\nu_{\max}$ (cm⁻¹): 3336, 3273 (N-H), 1647 (C=O), 1585 (C=N), 1352&1178 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.43 (s, 3H, CH₃), 3.57 (s, 3H, Ar-OCH₃), 7.18-8.00 (m, 16H, Ar-H), 8.42 (s, 1H, CH=N), 10.53&11.88 (s, 2H, CONH); ¹³C-NMR (150 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6 (Ar-CH₃), 56.1 (Ar-OCH₃), 110.7, 120.0, 120.7, 124.3, 128.2, 128.4, 128.7, 128.9, 130.3, 132.3, 132.4, 135.1, 135.1, 139.1, 142.9, 146.1 (CH=N), 146.7 (C-OCH₃), 152.0 (C-OSO₂), 163.1 (C=O), 166.3 (C=O); Anal. calcd. for C₂₉H₂₅N₃O₆S·H₂O: C: 62.02, H: 4.85, N: 7.48, S: 5.71. Found: C: 62.70, H: 4.67, N: 7.77, S: 5.97; LCMS (ES-API) m/z: 567 [M + H + Na]⁺.

4-((2-((5-(propylthio)-2,3-dihydro-1H-benzo[d]imidazol-2-yl)carbamoyl)hydrazinylidene)methyl)-2-methoxyphenyl 4-methylbenzenesulfonate (4k)

White solid; yield: 72%; m.p. 182-184°C; FTIR $\nu_{\max}$ (cm⁻¹): 3398, 3252 (N-H); 1703 (C=O), 1627 (C=N); 1354&1172 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 0.95 (t, 3H, *J*= 7.2Hz, CH₃), 1.48-1.61 (m, 2H, CH₂), 2.43 (s, 3H, Ar-CH₃), 2.86 (t, 2H, *J*=7.2 Hz, CH₂), 3.60 (s, 3H, OCH₃), 7.09-7.75 (m, 10H, Ar-H); 7.96 (s, 1H, CH=N), 10.52 (s, 1H, NHCONHN=), 11.29 (s, 1H, benzimidazole NH), 11.96 (s, 1H, NHCONHN=); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 13.5 (propyl -CH₂-CH₂-CH₃), 21.5 (Ar-CH₃), 22.5 (propyl -CH₂-CH₂-CH₃), 37.1 (propyl -CH₂-CH₂-CH₃), 56.2 (OCH₃), 120.6, 123.9, 124.4, 128.7, 130.2, 132.4, 134.8, 138.7, 141.9, 146.0 (CH=N), 151.9 (C-OSO₂); Anal. calcd. for C₂₆H₂₇N₅O₅S₂: C: 56.40, H: 4.92, N: 12.65, S: 11.58. Found: C: 56.47, H: 5.01, N: 12.44, S: 11.76; LCMS (Q-TOF) m/z: 554 [M + H]⁺.

4-((2-((4-bromophenyl)sulfonyl)hydrazinylidene)methyl)-2-methoxyphenyl 4-methylbenzenesulfonate (4l)

Yellow solid; yield: 82%; m.p. 147-148°C; FTIR $\nu_{\max}$ (cm⁻¹): 3234 (N-H), 1599 (C=N), 1346&1151 (SO₂); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.42 (s, 3H, CH₃), 3.52 (s, 3H, Ar-OCH₃), 7.09-7.87 (m, 13H, Ar-H&CH=N), 11.73 (s, 1H, CONHN=); ¹³C-NMR (150 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6 (Ar-CH₃), 56.1 (Ar-OCH₃), 111.5, 119.7, 127.6, 128.2, 128.7, 129.7, 130.3, 130.4, 132.4, 132.8, 134.1, 138.5, 139.1, 146.1 (CH=N), 146.8 (C-OCH₃), 151.9 (C-OSO₂); Anal. calcd. for C₂₁H₁₉BrN₂O₆S₂: C: 46.76, H: 3.55, N: 5.19, S: 11.89. Found: C: 46.81, H: 3.45, N: 4.65, S: 11.31; LCMS (Q-TOF) m/z: 540 [M + 2H]⁺.

4-((2-((4-chlorophenyl)sulfonyl)hydrazinylidene)methyl)-2-methoxyphenyl 4-methyl benzene sulfonate (4m)

Dark yellow solid; yield: 87%; m.p. 136-137°C; FTIR $\nu_{\max}$ (cm⁻¹): 3252 (N-H); 1583 (C=N); 1357&1172 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.42 (s, 3H, CH₃), 3.51 (s, 3H, Ar-OCH₃), 7.10-7.89 (m, 12H, Ar-H&CH=N), 11.74 (s, 1H, CONHN=); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6 (Ar-CH₃); 56.0 (OCH₃), 111.5, 119.6, 124.3, 128.1, 128.7, 129.6, 129.9, 130.3, 132.3, 134.1, 138.5, 139.0, 146.5, 146.8 (CH=N), 151.9 (C-OSO₂); Anal. calcd. for C₂₁H₁₉ClN₂O₆S₂: C: 50.96, H: 3.84, N: 5.74, S: 11.91. Found: C: 49.82, H: 3.87, N: 5.66, S: 12.96. LCMS (ES-API) m/z: 495 [M + H]⁺.

4-((2-((4-nitrophenyl)sulfonyl)hydrazinylidene)methyl)-2-methoxyphenyl 4-methylbenzenesulfonate (4n)

Yellow solid; yield: 90%; m.p. 104-105°C; FTIR $\nu_{\max}$ (cm⁻¹): 3230 (N-H), 1668 (C=N), 1346&1170 (S=O); ¹H-NMR (300 MHz), (DMSO-*d*₆/TMS) δ ppm: 2.42 (s, 3H, CH₃), 3.53 (s, 3H, Ar-OCH₃), 7.09-8.46 (m, 12H, Ar-H&CH=N), 11.99 (s, 1H, CONHN=); ¹³C-NMR (75 MHz), (DMSO-*d*₆/TMS) δ ppm: 21.6 (Ar-CH₃), 56.1 (Ar-OCH₃), 111.6, 119.8, 124.3, 125.1,

128.6, 129.3, 130.3, 132.4, 133.9, 139.2, 144.5, 146.1 (**C**-OCH₃), 147.4 (**CH**=N), 150.4 (**C**-NO₂), 152.0 (**C**-OSO₂); Anal. calcd. for C₂₁H₁₉N₃O₈S₂·2H₂O: **C**: 46.57, **H**: 3.43, **N**: 7.76, **S**: 11.84. Found: **C**: 46.90, **H**: 3.44, **N**: 7.56, **S**: 11.86; LCMS (ES-API) m/z: 528 [M +Na]⁺.

Biological assay

Cell culture studies

Human lung adenocarcinoma cell line (A549), human breast cancer cell line (MCF-7), human colorectal adenocarcinoma cell line (HT-29), human prostate cell line (PC-3), human liver cancer cell line (Hep3B), human cervical cancer cell (HeLa) and nontumorigenic mouse embryonic fibroblast cell line (NIH 3T3) were purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were cultured in Dulbecco's modified eagle medium (DMEM) (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and maintained in a 37°C, 5% CO₂ incubator. Cell passage was conducted at 80-90% confluence.

Cell viability assay

Cell viability was determined by the MTT assay as described previously [29].

Apoptosis studies

Annexin V binding, caspase-3 and caspase-9 activation measurement and detection of the loss of mitochondrial membrane potential procedures have been previously described in detail [30].

Results And Discussion

Chemistry

The synthesis route of hydrazides (**1a-h** and **1l-n**) and semicarbazide (**1k**) is shown in Scheme 1. First, acid hydrazides (**1a-h**) were synthesized by refluxing corresponding esters and hydrazine hydrate in ethanol according to the reported method [4, 13–17]. The compounds (**1i-j**) were synthesized according to the previously reported procedure starting from benzocaine [18]. Methyl [(5-propylsulfanyl-3H-benzimidazol-2-yl)amino]formate (0.01 mol) (Albendazole) was refluxed hydrazine hydrate (80%, 0.2 mol) in methanol until the reaction was complete. After solvent evaporation and crystallization, compound **1k** was obtained [19]. Sulfonyl hydrazides (**1l-n**) were synthesized through the reaction between corresponding sulfonyl chlorides and hydrazine hydrate in THF [20].

Treatment of vanillin/p-hydroxybenzaldehyde with tosyl chloride and triethylamine in dimethylformamide gave the aldehyde derivatives (**2a-b**) [21]. In the final step of this study, hydrazone linkage-based aryl sulfonate derivatives were obtained by the reaction of compounds **1a-n** with related aldehydes (**2a-b**) in ethanol, as shown in Scheme 1.

The title compounds were characterized by FT-IR, ¹H-NMR, ¹³C-NMR and LC-MS spectroscopy, besides elemental analysis and melting point. All of the spectral data are listed in Supplementary Material.

The C=N and C=O groups of the synthesized compounds were confirmed by IR spectra. The IR spectral peaks of compounds were recognized for C=O of CONH from 1637 to 1703cm⁻¹; for C=N at 1583–1627 cm⁻¹.

In the ¹H-NMR spectrum of compounds, characteristic singlet signals in the range of 2.38-2.44 ppm are attributable to the tosyl groups. In agreement with the literature data, CH=N and CONH groups exhibited two separate singlet signals in the ¹H-NMR and ¹³C-NMR spectra of compounds **3a**, **3c-f**, **3h**, **4a**, **4c-f**, and **4h** due to the restricted rotation around

the C-N bond [22]. In the cases of aromatic aldehydes-condensed *N*-acylhydrazones, ^1H -NMR spectra in DMSO-d_6 showed two peaks of the $-\text{CH}_2\text{CO}$ and CH-CH_3 groups that be attributed to the presence of two possible syn-anti isomers of the amide bond or E-Z isomer. For all the final compounds, the protons of azomethine and amide resonated at the expected regions in the literature [13–15, 23].

The ^{13}C -NMR signals corresponding to the carbons from the azomethine and amide groups appear in the range 145.5–149.3 ppm and 160.6–184.8 ppm, respectively. MS spectra of the compounds displayed $[\text{M}+\text{H}]^+$, $[\text{M}+2\text{H}]^+$, and/or species exhibiting sodiated and potassiated adducts.

Biological evaluation

The *in vitro* antiproliferative activity of the title compounds was evaluated against A549 (non-small cell lung adenocarcinoma), MCF-7 (breast adenocarcinoma), HT-29 (colorectal adenocarcinoma), PC-3 (androgen-independent prostate adenocarcinoma), Hep3B (hepatocellular carcinoma), and HeLa (cervical carcinoma) cells by MTT method. The anticancer screening results at 10 μM concentration are summarized in Table 1. Also, whether or not compounds caused cytotoxic effects in noncancerogenic mouse embryonic fibroblast cell line (NIH3T3) was tested. Compounds showed no cytotoxicity effect on NIH3T3 cells.

Table 1

Percentage growth inhibition (GI %) of various cell lines at 10 μ M for compounds and IC₅₀ values (μ M) for compounds **4g** and **4h** for 24 h against MCF-7 cancer cell line

Compounds	GI%							IC ₅₀ (μ M)
	NIH3T3	HT-29	MCF-7	HEP3B	PC-3	HeLa	A549	(MCF-7)
3a	27.18	18.32	7.83	NA	26.11	10.29	NA	
3b	36.61	21.63	27.25	26.71	42.14	34.73	NA	
3c	-3.16	2.06	3.28	NA	34.22	7.63	1.92	
3d	0.11	7.91	NA	NA	28.21	1.54	NA	
3e	9.60	NA	8.80	NA	31.04	8.58	NA	
3f	20.92	16.21	19.49	NA	32.78	15.16	NA	
3g	10.86	7.72	8.42	2.34	11.98	15.41	3.43	
3h	-6.09	NA	7.42	NA	18.79	NA	25.14	
3i	38.56	37.07	22.18	19.95	17.14	23.98	33.93	
3j	24.66	35.25	30.97	1.88	38.02	11.87	32.26	
3k	-6.04	NA	30.27	19.42	9.69	NA	4.17	
3l	-0.28	31.13	NA	NA	NA	NA	1.59	
3m	-0.67	19.29	NA	8.49	5.75	NA	8.63	
3n	-5.30	23.99	4.15	NA	6.84	5.29	8.79	
4a	5.81	22.28	17.27	1.03	1.85	11.74	5.81	
4b	7.22	38.97	32.85	20.62	19.63	15.57	14.55	
4c	-19.02	10.71	NA	17.96	NA	NA	9.57	
4d	-18.17	NA	NA	23.43	NA	NA	8.30	
4e	-19.97	NA	NA	19.43	NA	NA	11.61	

NA: GI% $\leq$ zero

The structures of the compounds **3a-n** and **4a-n** differ from each other due to substituent on phenyl ring (-2OCH₃ or -H). Compounds showed strong antiproliferative activity against cancer cells. Compound **3b** exerted cytotoxic activity with GI more than 40% against prostate (PC-3) cell line. The compounds **4g** and **4h** were found to be the best anticancer activity against the MCF-7 cell line (50.37% and 53.98%, respectively) with no effect on normal cell lines. Compound **3b** was less potent on normal cells compared to cancer cells. So, two compounds (**4g** and **4h**) were selected for further studies because of their high anticancer activity and low toxicity to normal cells. The cell growth-inhibitory potencies, expressed as IC₅₀ values of these compounds, are listed in Table 1. Compounds **4g** and **4h** showed cytotoxicity with IC₅₀ values of 17.8 μ M and 21.2 μ M against MCF-7 cell line, respectively.

In this study, mitochondrial disfunction was determined based on the mitochondria membrane potential (MMP) index that was measured using JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) dye as a fluorescent probe. A loss of mitochondrial mass was characterized by a decrease in red fluorescence and an increase in the green fluorescent intensity [24]. As shown in Fig. 3, before the treatment of compounds, the untreated MCF-7 cancer cells exhibited red fluorescence intensity because of the JC-1 dye aggregation into the mitochondria. Compounds **4g** and **4h**-treated cells displayed a reduction in red emission intensity and an increase in green emission intensity when compared to control cells.

Compounds	GI%							IC ₅₀ (μ M) (MCF-7)
	NIH3T3	HT-29	MCF-7	HEP3B	PC-3	HeLa	A549	
4f	-26.38	NA	25.82	NA	NA	NA	14.06	
4g	-10.29	NA	50.37	NA	NA	NA	28.58	17.8
4h	-2.21	NA	53.98	NA	16.06	2.70	36.59	21.2
4i	-25.33	NA	28.81	NA	NA	3.42	11.04	
4j	-11.93	NA	38.57	1.75	31.88	3.33	28.95	
4k	-11.27	NA	20.74	16.06	NA	NA	NA	
4l	-23.04	16.22	18.32	9.20	NA	NA	9.57	
4m	-5.06	NA	15.90	NA	NA	10.17	11.90	
4n	-10.05	3.61	15.78	NA	31.89	10.39	34.30	
Imatinib	-10.21	-	-	-	34.52	30.63	26.10	
Etoposide	-2.6	3.64	-0.4	6.03	-	-	-	
NA: GI% $\leq$ zero								
The structures of the compounds 3a-n and 4a-n differ from each other due to substituent on phenyl ring (-2OCH ₃ or -H). Compounds showed strong antiproliferative activity against cancer cells. Compound 3b exerted cytotoxic activity with GI more than 40% against prostate (PC-3) cell line. The compounds 4g and 4h were found to be the best anticancer activity against the MCF-7 cell line (50.37% and 53.98%, respectively) with no effect on normal cell lines. Compound 3b was less potent on normal cells compared to cancer cells. So, two compounds (4g and 4h) were selected for further studies because of their high anticancer activity and low toxicity to normal cells. The cell growth-inhibitory potencies, expressed as IC ₅₀ values of these compounds, are listed in Table 1. Compounds 4g and 4h showed cytotoxicity with IC ₅₀ values of 17.8 μ M and 21.2 μ M against MCF-7 cell line, respectively.								
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The red/green fluorescence intensity ratio in the MCF-7 cells treated with different concentrations of compounds (10, 20, and 50 μ M) was displayed in Fig. 4. A clear concentration-dependent decrease in the ratio can be observed for both compounds. Therefore, one of the first steps by which the studied compounds trigger apoptosis is likely to be through changes in mitochondrial membrane potential.

Annexin V is a fluorescent probe that binds to translocated phosphatidylserine (PS) in apoptotic cells and is used to indicate early apoptotic cells [25]. To determine whether selected compounds undergo apoptosis, an annexin V assay was also performed. As shown in Fig. 5, compounds initiated early apoptotic processes in MCF-7 cells and were dependent on dose, especially for the compound **4h**.

Apoptosis can be an extrinsic pathway mediated via activation of death receptors or by an intrinsic mitochondria-mediated pathway. In the intrinsic pathway, proapoptotic and antiapoptotic proteins stabilize the outer membrane of the mitochondria and activate caspase-9 and caspase-3. Caspase-3 is activated in both extrinsic and intrinsic

apoptosis pathways, the initiator caspase-9 protein is only activated via the intrinsic pathway [26]. MMP depolarization results in the release of cytochrome into the cytosol, which activates caspase 9 via the formation of an apoptosome and the subsequent cleavage of caspase 3/7 effectors [27].

To determine whether caspases were involved in the apoptotic cell death induced by hybrid derivatives **4g** and **4h** in MCF-7 cells, we examined the effect of these compounds on caspase-3 and caspase-9 activation. It is important to underline that compounds significantly suppressed caspase-3 and caspase-9 activation (Fig. 6). These caspases were highly activated (~3.5-4-fold) with 50µM concentrations of **4g** and **4h** in MCF-7 cells, showing that apoptotic cell death takes place in a caspase-dependent pathway.

Conclusions

In conclusion, we designed two series of hybrid molecules (**3a-n** and **4a-n**) based on the biological significance of hydrazone and sulfonate moieties. All the derivatives were characterized and evaluated for in vitro antiproliferative activity. The numerous compounds displayed a wide variety of cytotoxicity against multiple cancer cell lines. The activity results showed that compounds **4g** and **4h** were found to be most effective against MCF-7 cell assayed with IC₅₀ values of 17.8 and 21.2 µM, respectively, besides their selectivity. Annexin V staining assay results showed that the selected compounds were induced apoptosis. This result is associated with depolarization of MMP and activation of caspase-3 and caspase-9. These compounds look like promising leads for further modification for the design of the new anticancer agents with high selectivity.

Declarations

Conflict of interest statement:

The authors declared no conflict of interest.

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Scheme

See the Supplemental Files section for Scheme 1.

Figures

Zorubicin

Bisantrene

Figure 1

Chemical structures of known hydrazone linkage-based anticancer drugs

Figure 2

Rationale for the design of the new compounds

Figure 3

Mitochondrial membrane potential ($\Delta\Psi_m$) of MCF-7 cancer cells after treated with compounds 4g and 4h was accessed via JC-1 staining assay.

Figure 4

Red/green fluorescence intensity for JC-1 staining of MCF-7 cells after treatment of compounds 4g and 4h at different concentrations (10, 20, and 50 μ M) for 24 hours.

Figure 5

Early apoptotic effects of compounds 4g and 4h on MCF-7 cells at different concentrations

Figure 6

Caspase-3 and caspase-9 activities induced by compounds 4g and 4h in MCF-7 cells.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Scheme1.jpg](#)
- [SupplementalMaterial.doc](#)

- [graphicalabstract2.jpg](#)