Supplementary information

Convenient synthesis and X-ray determination of 2-amino-6*H*-1,3,4-thiadiazine bromides endowed with antiproliferative activity

Hendawy N. Tawfeek[1,2], Alshaimaa Abdelmoez[3,4], Kholood A. Dahlous[5], Bahaa G. M. Youssif[3]*, Stefan Bräse[6]*, Kari Rissanen[7], Martin Nieger[8], Essmat M. El-Sherief[1]

[1]Chemistry Department, Faculty of Science, Minia University, El Minia, 61519 Egypt; [2] Unit of Occupational of Safety and Health, Administration Office of Minia University, El-Minia 61519, Egypt; [3]Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt; [4]Department of Neurology, Ulm University, Ulm, Germany; [5]Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; [6]Institute of Biological and Chemical Systems, IBCS-FMS, Karlsruhe Institute of Technology, 76131 Karlsruhe, Germany; [7]Department of Chemistry, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland; [8]Department of Chemistry, University of Helsinki, PO Box 55, A. I. Virtasen aukio 1, 00014 Helsinki, Finland.

**To whom correspondence should be addressed:*

Bahaa G. M. Youssif, Ph.D. Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt.

Tel.: (002)-01098294419

E-mail address: bgyoussif2@gmail.com, bahaa.youssif@pharm.aun.edu.eg

Stefan Bräse

Institute of Biological and Chemical Systems, IBCS-FMS, Karlsruhe Institute of Technology, 76131 Karlsruhe, Germany. E-mail: braese@kit.edu

Crystal X-ray structure determination of 9a and 9b

The single-crystal X-ray diffraction studies were carried out on a Rigaku XtaLAB Synergy R diffractometer with HyPix-Arc 100 detector at 133(2) K using Cu-Kα radiation (**9a**, λ = 1.54178 Å, Photon Jet R rotating anode generator) or a Bruker D8 Venture diffractometer with a Photon II detector at 173(2) K using Cu-Kα radiation (**9b**, λ = 1.54178 Å). Dual space methods (SHELXT) [G. M. Sheldrick, *Acta Crystallogr.* 2015, **A71**, 3-8] were used for structure solution and refinement was carried out using SHELXL-2014 (full-matrix least-squares on F^2) [G. M. Sheldrick, *Acta Crystallogr.* 2015, **C71**, 3-8]. Hydrogen atoms were localized by difference electron density determination and refined using a riding model. An extinction correction and a semi-empirical absorption correction were applied.

9a: colourless crystals, $C_{15}H_{14}N_3S \cdot Br$, M_r = 348.26, crystal size 0.18 × 0.14 × 0.13 mm, monoclinic, space group $P2_1/n$ (No. 14), a = 8.9077(1) Å, b = 9.1847(1) Å, c = 18.1367(2) Å, β = 93.364(1)°, V = 1481.29(3) Å^3, Z = 4, ρ = 1.562 Mg/m^{-3}, μ(Cu-K$_\alpha$) = 5.04 mm^{-1}, F(000) = 704, T = 133 K, $2\theta_{max}$ = 158.6°, 16484 reflections, of which 3177 were independent (R_{int} = 0.016), 188 parameters, 2 restraints, R_1 = 0.020 (for 3161 I > 2σ(I)), wR_2 = 0.051 (all data), S = 1.09, largest diff. peak / hole = 0.33 / -0.31 e Å^{-3}.

9b: yellow crystals, $C_{12}H_{14}N_3S \cdot Br$, M_r = 312.23, crystal size 0.14 × 0.25 × 0.20 mm, orthorhombic, space group *Pbca* (No. 61), a = 7.4414(3) Å, b = 18.2272(7) Å, c = 19.6573(7) Å, V = 2666.24(18) Å^3, Z = 8, ρ = 1.556 Mg/m^{-3}, μ(Cu-K$_\alpha$) = 5.51 mm^{-1}, F(000) = 1264, T = 173 K, $2\theta_{max}$ = 144.2°, 19426 reflections, of which 2613 were independent (R_{int} = 0.033), 161 parameters, 2 restraints, R_1 = 0.022 (for 2558 I > 2σ(I)), wR_2 = 0.056 (all data), S = 1.05, largest diff. peak / hole = 0.28 / -0.25 e Å^{-3}.

CCDC 2329624 (**9a**) and 2329625 (**9b**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

1.1.1. X-ray crystallographic data for compounds **9a**.

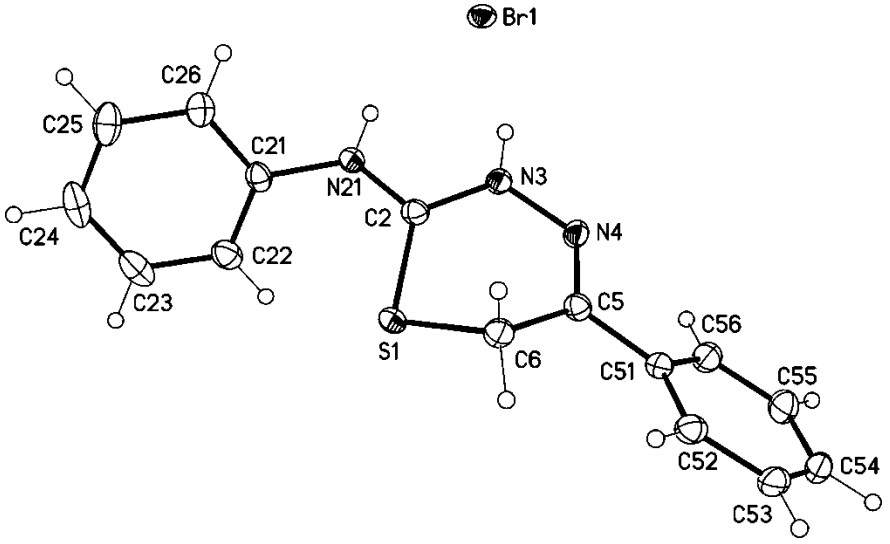

Figure S1. X-Ray crystallographic molecular structure of compound **9a (**5-phenyl-2-(phenylamino)-6H-1,3,4-thiadiazin-3-ium) (displacement parameters are drawn at 50% probability level)

Crystal data – sb1527_hy

$C_{15}H_{14}N_3S\cdot Br$	$F(000) = 704$
$M_r = 348.26$	$D_x = 1.562$ Mg m^{-3}
Monoclinic, $P2_1/n$ *(no.14)*	Cu $K\alpha$ radiation, $\lambda = 1.54184$ Å
$a = 8.9077$ (1) Å	Cell parameters from 14033 reflections
$b = 9.1847$ (1) Å	$\theta = 2.3–78.8°$
$c = 18.1367$ (2) Å	$\mu = 5.04$ mm^{-1}
$\beta = 93.364$ (1)°	$T = 133$ K
$V = 1481.29$ (3) Å^3	Blocks, colourless
$Z = 4$	0.18 × 0.14 × 0.13 mm

Data collection – sb1527_hy

XtaLAB Synergy R diffractometer, HyPix-Arc 100 detector	3177 independent reflections
Radiation source: Rotating-anode X-ray tube, PhotonJet R (Cu) X-ray Source	3161 reflections with $I > 2\sigma(I)$
Mirror monochromator	$R_{int} = 0.016$
Detector resolution: 10.0000 pixels mm^{-1}	$\theta_{max} = 79.3°$, $\theta_{min} = 5.4°$
ω scans, 0.5 deg.	$h = -10\rightarrow11$
Absorption correction: multi-scan *CrysAlis PRO* 1.171.42.89a (Rigaku Oxford Diffraction, 2023) Empirical absorption	$k = -11\rightarrow11$

correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.	
T_{min} = 0.763, T_{max} = 1.000	l = -23→18
16484 measured reflections	

Refinement – sb1527_hy

Refinement on F^2	Secondary atom site location: difference Fourier map
Least-squares matrix: full	Hydrogen site location: difference Fourier map
$R[F^2 > 2\sigma(F^2)]$ = 0.020	H atoms treated by a mixture of independent and constrained refinement
$wR(F^2)$ = 0.051	$w = 1/[\sigma^2(F_o^2) + (0.0219P)^2 + 1.127P]$ where $P = (F_o^2 + 2F_c^2)/3$
S = 1.09	$(\Delta/\sigma)_{max}$ = 0.001
3177 reflections	$\Delta\rangle_{max}$ = 0.33 e Å^{-3}
188 parameters	$\Delta\rangle_{min}$ = -0.31 e Å^{-3}
2 restraints	Extinction correction: *SHELXL2014*/7 (Sheldrick 2014), $Fc^*=kFc[1+0.001xFc^2\lambda^3/\sin(2\theta)]^{-1/4}$
Primary atom site location: dual	Extinction coefficient: 0.00182 (12)

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2) for (sb1527_hy)

	X	y	z	U_{iso}*/U_{eq}
Br1	0.25957 (2)	0.31608 (2)	0.44651 (2)	0.01901 (7)
S1	0.87653 (4)	0.32924 (4)	0.46049 (2)	0.01815 (9)
C2	0.68364 (16)	0.34164 (16)	0.46581 (8)	0.0162 (3)
N3	0.59958 (13)	0.40419 (14)	0.41130 (6)	0.0174 (2)
H3	0.5028 (16)	0.395 (2)	0.4142 (10)	0.021*
N4	0.64107 (13)	0.42265 (14)	0.33889 (6)	0.0173 (2)
C5	0.78026 (15)	0.45212 (16)	0.33009 (8)	0.0165 (3)
C6	0.89042 (16)	0.47704 (17)	0.39483 (8)	0.0188 (3)
H6A	0.8683	0.5708	0.4189	0.023*
H6B	0.9938	0.4817	0.3777	0.023*
N21	0.61397 (14)	0.28836 (14)	0.52253 (7)	0.0174 (2)
H21	0.5178 (17)	0.291 (2)	0.5179 (10)	0.021*
C21	0.68529 (16)	0.23158 (16)	0.58919 (8)	0.0179 (3)
C22	0.79148 (17)	0.12128 (17)	0.58685 (9)	0.0227 (3)
H22	0.8188	0.0830	0.5409	0.027*

C23	0.85738 (18)	0.06763 (19)	0.65280 (10)	0.0291 (4)
H23	0.9317	-0.0065	0.6520	0.035*
C24	0.81487 (19)	0.12212 (19)	0.71968 (9)	0.0295 (4)
H24	0.8608	0.0858	0.7646	0.035*
C25	0.7058 (2)	0.22914 (18)	0.72140 (9)	0.0269 (3)
H25	0.6756	0.2646	0.7675	0.032*
C26	0.64039 (18)	0.28487 (17)	0.65599 (8)	0.0208 (3)
H26	0.5658	0.3586	0.6570	0.025*
C51	0.82514 (16)	0.46556 (16)	0.25336 (8)	0.0174 (3)
C52	0.94752 (17)	0.55150 (17)	0.23639 (9)	0.0209 (3)
H52	1.0068	0.5978	0.2748	0.025*
C53	0.98283 (18)	0.56951 (18)	0.16333 (9)	0.0254 (3)
H53	1.0652	0.6293	0.1518	0.030*
C54	0.89789 (19)	0.50030 (19)	0.10735 (9)	0.0272 (3)
H54	0.9223	0.5129	0.0575	0.033*
C55	0.77736 (19)	0.41260 (19)	0.12366 (9)	0.0267 (3)
H55	0.7200	0.3646	0.0851	0.032*
C56	0.74090 (17)	0.39518 (18)	0.19642 (8)	0.0221 (3)
H56	0.6584	0.3352	0.2076	0.027*

Atomic displacement parameters (Å^2) for (sb1527_hy)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
Br1	0.01229 (9)	0.02543 (10)	0.01939 (9)	0.00061 (5)	0.00153 (6)	-0.00224 (5)
S1	0.01144 (16)	0.02432 (19)	0.01845 (17)	0.00033 (12)	-0.00117 (12)	0.00136 (13)
C2	0.0136 (6)	0.0181 (6)	0.0166 (6)	-0.0004 (5)	-0.0002 (5)	-0.0023 (5)
N3	0.0112 (5)	0.0264 (6)	0.0146 (6)	-0.0006 (5)	0.0004 (4)	0.0014 (5)
N4	0.0161 (6)	0.0225 (6)	0.0135 (5)	-0.0010 (5)	0.0004 (4)	0.0010 (5)
C5	0.0150 (6)	0.0170 (6)	0.0172 (7)	-0.0008 (5)	-0.0001 (5)	0.0001 (5)
C6	0.0149 (6)	0.0225 (7)	0.0190 (7)	-0.0033 (5)	0.0004 (5)	0.0000 (6)
N21	0.0119 (6)	0.0240 (6)	0.0160 (6)	0.0004 (5)	-0.0005 (4)	0.0015 (5)
C21	0.0152 (6)	0.0196 (7)	0.0184 (7)	-0.0030 (5)	-0.0026 (5)	0.0026 (5)
C22	0.0177 (7)	0.0235 (8)	0.0269 (8)	-0.0001 (6)	0.0010 (6)	0.0033 (6)
C23	0.0198 (7)	0.0251 (8)	0.0416 (10)	-0.0001 (6)	-0.0060 (7)	0.0099 (7)
C24	0.0308 (9)	0.0280 (8)	0.0280 (8)	-0.0091 (7)	-0.0135 (7)	0.0107 (7)
C25	0.0367 (9)	0.0238 (8)	0.0194 (7)	-0.0089 (7)	-0.0053 (6)	0.0014 (6)
C26	0.0249 (7)	0.0173 (7)	0.0198 (7)	-0.0022 (6)	-0.0017 (6)	0.0011 (6)
C51	0.0161 (6)	0.0179 (7)	0.0183 (7)	0.0022 (5)	0.0025 (5)	0.0015 (5)
C52	0.0183 (7)	0.0192 (7)	0.0256 (7)	0.0004 (6)	0.0041 (6)	0.0023 (6)
C53	0.0230 (8)	0.0219 (8)	0.0322 (8)	0.0042 (6)	0.0104 (6)	0.0097 (6)

C54	0.0314 (8)	0.0299 (8)	0.0214 (7)	0.0112 (7)	0.0099 (6)	0.0086 (6)
C55	0.0304 (8)	0.0313 (9)	0.0184 (7)	0.0044 (7)	0.0007 (6)	-0.0006 (6)
C56	0.0209 (7)	0.0256 (8)	0.0200 (7)	-0.0015 (6)	0.0015 (6)	0.0009 (6)

Geometric parameters (Å, º) for (sb1527_hy)

S1—C2	1.7304 (14)	C23—H23	0.9500
S1—C6	1.8148 (15)	C24—C25	1.384 (3)
C2—N21	1.3259 (19)	C24—H24	0.9500
C2—N3	1.3346 (18)	C25—C26	1.388 (2)
N3—N4	1.3954 (16)	C25—H25	0.9500
N3—H3	0.871 (14)	C26—H26	0.9500
N4—C5	1.2880 (18)	C51—C52	1.395 (2)
C5—C51	1.4753 (19)	C51—C56	1.399 (2)
C5—C6	1.5029 (19)	C52—C53	1.390 (2)
C6—H6A	0.9900	C52—H52	0.9500
C6—H6B	0.9900	C53—C54	1.385 (3)
N21—C21	1.4306 (18)	C53—H53	0.9500
N21—H21	0.857 (15)	C54—C55	1.388 (2)
C21—C26	1.387 (2)	C54—H54	0.9500
C21—C22	1.388 (2)	C55—C56	1.386 (2)
C22—C23	1.391 (2)	C55—H55	0.9500
C22—H22	0.9500	C56—H56	0.9500
C23—C24	1.385 (3)		
C2—S1—C6	95.36 (7)	C22—C23—H23	119.9
N21—C2—N3	117.77 (13)	C25—C24—C23	120.32 (15)
N21—C2—S1	122.04 (11)	C25—C24—H24	119.8
N3—C2—S1	120.19 (11)	C23—C24—H24	119.8
C2—N3—N4	125.55 (12)	C24—C25—C26	120.17 (16)
C2—N3—H3	115.5 (12)	C24—C25—H25	119.9
N4—N3—H3	112.7 (12)	C26—C25—H25	119.9
C5—N4—N3	116.88 (12)	C21—C26—C25	119.22 (15)
N4—C5—C51	116.78 (12)	C21—C26—H26	120.4
N4—C5—C6	121.62 (13)	C25—C26—H26	120.4
C51—C5—C6	121.57 (12)	C52—C51—C56	119.39 (14)
C5—C6—S1	109.41 (10)	C52—C51—C5	120.94 (13)
C5—C6—H6A	109.8	C56—C51—C5	119.63 (13)
S1—C6—H6A	109.8	C53—C52—C51	120.10 (15)
C5—C6—H6B	109.8	C53—C52—H52	120.0

S1—C6—H6B	109.8	C51—C52—H52	120.0
H6A—C6—H6B	108.2	C54—C53—C52	120.00 (15)
C2—N21—C21	125.83 (12)	C54—C53—H53	120.0
C2—N21—H21	115.1 (12)	C52—C53—H53	120.0
C21—N21—H21	119.0 (12)	C53—C54—C55	120.42 (14)
C26—C21—C22	121.06 (14)	C53—C54—H54	119.8
C26—C21—N21	118.22 (13)	C55—C54—H54	119.8
C22—C21—N21	120.65 (13)	C56—C55—C54	119.82 (15)
C21—C22—C23	119.08 (15)	C56—C55—H55	120.1
C21—C22—H22	120.5	C54—C55—H55	120.1
C23—C22—H22	120.5	C55—C56—C51	120.26 (15)
C24—C23—C22	120.11 (16)	C55—C56—H56	119.9
C24—C23—H23	119.9	C51—C56—H56	119.9
C6—S1—C2—N21	-159.15 (13)	C22—C23—C24—C25	0.6 (2)
C6—S1—C2—N3	21.95 (13)	C23—C24—C25—C26	-1.4 (2)
N21—C2—N3—N4	-157.72 (13)	C22—C21—C26—C25	1.6 (2)
S1—C2—N3—N4	21.2 (2)	N21—C21—C26—C25	178.65 (14)
C2—N3—N4—C5	-36.6 (2)	C24—C25—C26—C21	0.3 (2)
N3—N4—C5—C51	177.54 (12)	N4—C5—C51—C52	153.46 (14)
N3—N4—C5—C6	-4.6 (2)	C6—C5—C51—C52	-24.4 (2)
N4—C5—C6—S1	50.04 (17)	N4—C5—C51—C56	-24.4 (2)
C51—C5—C6—S1	-132.24 (12)	C6—C5—C51—C56	157.81 (14)
C2—S1—C6—C5	-51.66 (11)	C56—C51—C52—C53	1.5 (2)
N3—C2—N21—C21	-173.04 (14)	C5—C51—C52—C53	-176.31 (14)
S1—C2—N21—C21	8.0 (2)	C51—C52—C53—C54	-1.0 (2)
C2—N21—C21—C26	128.30 (16)	C52—C53—C54—C55	-0.1 (2)
C2—N21—C21—C22	-54.7 (2)	C53—C54—C55—C56	0.6 (2)
C26—C21—C22—C23	-2.4 (2)	C54—C55—C56—C51	0.0 (2)
N21—C21—C22—C23	-179.32 (14)	C52—C51—C56—C55	-1.0 (2)
C21—C22—C23—C24	1.3 (2)	C5—C51—C56—C55	176.84 (14)

Hydrogen-bond geometry (Å, º) for (sb1527_hy)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
N3—H3···Br1	0.87 (1)	2.39 (2)	3.2346 (12)	163 (2)
N21—H21···Br1	0.86 (2)	2.58 (2)	3.3795 (12)	155 (2)

Document origin: *publCIF* [Westrip, S. P. (2010). *J. Apply. Cryst.*, **43**, 920-925].

1.1.2. X-Ray crystallographic data for compound **9b**

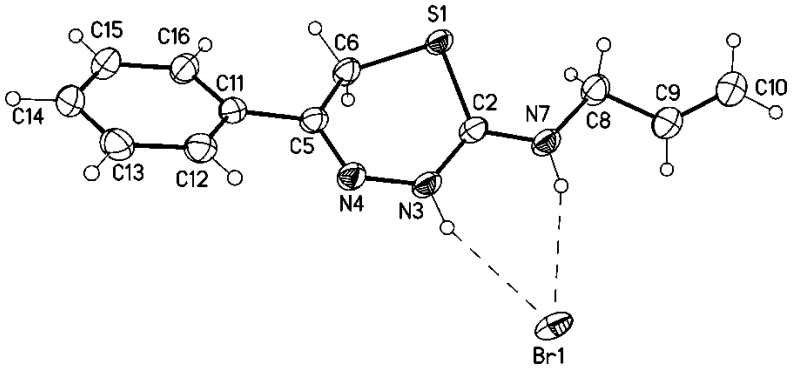

Figure 2. X-Ray crystallographic molecular structure of compound **9b (**Z)—N-(5-phenyl-3,6-dihydro-2H-1,3,4-thiadiazin-2-ylidene)prop-2-en-1-aminium bromide) (displacement parameters are drawn at 50% probability level)**– SB1532_HY**

Crystal data

$C_{12}H_{14}N_3S{\cdot}Br$	D_x = 1.556 Mg m^{-3}
M_r = 312.23	Cu $K\alpha$ radiation, λ = 1.54178 Å
Orthorhombic, *Pbca (no.61)*	Cell parameters from 9842 reflections
a = 7.4414 (3) Å	θ = 3.3–72.0°
b = 18.2272 (7) Å	μ = 5.51 mm^{-1}
c = 19.6573 (7) Å	T = 173 K
V = 2666.24 (18) Å^3	Blocks, yellow
Z = 8	0.40 × 0.25 × 0.20 mm
F(000) = 1264	

Data collection

Bruker D8 VENTURE diffractometer with PhotonII CPAD detector	2558 reflections with $I > 2\sigma(I)$
Radiation source: fine-focus sealed tube, IμS microfocus	R_{int} = 0.033
rotation in ϕ and ω, 1°, shutterless scans	θ_{max} = 72.1°, θ_{min} = 4.9°
Absorption correction: multi-scan SADABD (Sheldrick, 2014)	h = -9→8
T_{min} = 0.280, T_{max} = 0.424	k = -19→22
19426 measured reflections	l = -24→24
2613 independent reflections	

Refinement

Refinement on F^2	Secondary atom site location: difference Fourier map
Least-squares matrix: full	Hydrogen site location: difference Fourier map
$R[F^2 > 2\sigma(F^2)] = 0.022$	H atoms treated by a mixture of independent and constrained refinement
$wR(F^2) = 0.056$	$w = 1/[\sigma^2(F_o^2) + (0.0233P)^2 + 1.5642P]$ where $P = (F_o^2 + 2F_c^2)/3$
$S = 1.05$	$(\Delta/\sigma)_{max} = 0.002$
2613 reflections	$\Delta\rangle_{max} = 0.28$ e Å^{-3}
161 parameters	$\Delta\rangle_{min} = -0.25$ e Å^{-3}
2 restraints	Extinction correction: *SHELXL2014*/7 (Sheldrick 2014), $Fc^*=kFc[1+0.001xFc^2\lambda^3/\sin(2\theta)]^{-1/4}$
Primary atom site location: dual	Extinction coefficient: 0.00056 (8)

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2) for (sb1532_hy)

	X	*y*	*z*	U_{iso}*/U_{eq}
Br1	0.18731 (3)	0.61449 (2)	0.46365 (2)	0.03417 (9)
S1	0.28277 (6)	0.31472 (2)	0.47597 (2)	0.02802 (11)
C2	0.2441 (2)	0.40821 (8)	0.47334 (7)	0.0253 (3)
N3	0.17289 (19)	0.43874 (7)	0.41805 (7)	0.0295 (3)
H3	0.164 (3)	0.4853 (8)	0.4180 (10)	0.035*
N4	0.16045 (19)	0.40759 (7)	0.35338 (6)	0.0281 (3)
C5	0.1410 (2)	0.33723 (8)	0.35004 (7)	0.0249 (3)
C6	0.1191 (2)	0.29043 (8)	0.41218 (7)	0.0284 (3)
H6A	0.1338	0.2381	0.3997	0.034*
H6B	-0.0034	0.2970	0.4309	0.034*
N7	0.29116 (19)	0.44907 (7)	0.52562 (7)	0.0283 (3)
H7	0.281 (3)	0.4952 (9)	0.5193 (10)	0.034*
C8	0.3429 (2)	0.41764 (9)	0.59152 (8)	0.0323 (3)
H8A	0.4627	0.3945	0.5872	0.039*
H8B	0.2558	0.3789	0.6041	0.039*
C9	0.3489 (3)	0.47359 (10)	0.64633 (9)	0.0416 (4)
H9	0.2511	0.5068	0.6507	0.050*
C10	0.4840 (3)	0.47910 (11)	0.68900 (11)	0.0527 (5)
H10A	0.5831	0.4465	0.6855	0.063*

H10B	0.4826	0.5157	0.7234	0.063*
C11	0.1291 (2)	0.30449 (8)	0.28150 (7)	0.0255 (3)
C12	0.1823 (2)	0.34442 (9)	0.22445 (8)	0.0306 (3)
H12	0.2359	0.3913	0.2301	0.037*
C13	0.1575 (2)	0.31619 (10)	0.15953 (8)	0.0360 (4)
H13	0.1918	0.3442	0.1209	0.043*
C14	0.0827 (2)	0.24715 (10)	0.15112 (8)	0.0373 (4)
H14	0.0648	0.2280	0.1067	0.045*
C15	0.0342 (2)	0.20606 (9)	0.20728 (9)	0.0355 (4)
H15	-0.0146	0.1583	0.2014	0.043*
C16	0.0568 (2)	0.23439 (8)	0.27228 (8)	0.0308 (3)
H16	0.0229	0.2060	0.3107	0.037*

Atomic displacement parameters (Å^2) for (sb1532_hy)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
Br1	0.04537 (13)	0.01623 (11)	0.04092 (13)	0.00172 (6)	0.00761 (7)	0.00146 (6)
S1	0.0441 (2)	0.01723 (18)	0.02267 (18)	0.00509 (14)	-0.00190 (15)	-0.00002 (13)
C2	0.0327 (8)	0.0184 (7)	0.0248 (7)	0.0010 (6)	0.0036 (6)	-0.0001 (5)
N3	0.0482 (8)	0.0146 (6)	0.0258 (6)	0.0017 (5)	-0.0006 (5)	0.0005 (5)
N4	0.0415 (7)	0.0201 (6)	0.0227 (6)	0.0011 (5)	-0.0002 (5)	0.0012 (5)
C5	0.0318 (7)	0.0193 (7)	0.0236 (7)	0.0021 (6)	0.0009 (6)	0.0014 (5)
C6	0.0432 (9)	0.0180 (7)	0.0239 (7)	-0.0014 (6)	0.0002 (6)	0.0010 (5)
N7	0.0405 (8)	0.0165 (6)	0.0280 (6)	-0.0006 (5)	0.0000 (5)	-0.0006 (5)
C8	0.0457 (9)	0.0246 (7)	0.0266 (7)	-0.0032 (7)	-0.0011 (7)	-0.0002 (6)
C9	0.0524 (10)	0.0364 (9)	0.0359 (9)	0.0093 (8)	-0.0063 (8)	-0.0105 (7)
C10	0.0716 (14)	0.0362 (9)	0.0503 (11)	0.0063 (9)	-0.0225 (10)	-0.0109 (8)
C11	0.0294 (7)	0.0241 (7)	0.0231 (7)	0.0055 (6)	-0.0003 (6)	-0.0004 (6)
C12	0.0369 (8)	0.0271 (8)	0.0277 (7)	0.0047 (6)	0.0004 (6)	0.0025 (6)
C13	0.0439 (9)	0.0394 (9)	0.0246 (7)	0.0122 (7)	0.0008 (7)	0.0034 (7)
C14	0.0440 (9)	0.0409 (9)	0.0270 (8)	0.0157 (8)	-0.0057 (7)	-0.0090 (7)
C15	0.0410 (9)	0.0293 (8)	0.0363 (8)	0.0043 (7)	-0.0041 (7)	-0.0100 (7)
C16	0.0389 (8)	0.0252 (7)	0.0285 (7)	0.0008 (6)	0.0001 (6)	-0.0022 (6)

Geometric parameters (Å, º) for (sb1532_hy)

S1—C2	1.7290 (15)	C9—C10	1.313 (3)
S1—C6	1.8034 (16)	C9—H9	0.9500
C2—N7	1.316 (2)	C10—H10A	0.9500
C2—N3	1.331 (2)	C10—H10B	0.9500

N3—N4	1.3954 (18)	C11—C12	1.394 (2)
N3—H3	0.852 (15)	C11—C16	1.398 (2)
N4—C5	1.2923 (19)	C12—C13	1.388 (2)
C5—C11	1.4763 (19)	C12—H12	0.9500
C5—C6	1.4989 (19)	C13—C14	1.386 (3)
C6—H6A	0.9900	C13—H13	0.9500
C6—H6B	0.9900	C14—C15	1.382 (3)
N7—C8	1.468 (2)	C14—H14	0.9500
N7—H7	0.854 (15)	C15—C16	1.388 (2)
C8—C9	1.484 (2)	C15—H15	0.9500
C8—H8A	0.9900	C16—H16	0.9500
C8—H8B	0.9900		
C2—S1—C6	96.25 (7)	H8A—C8—H8B	107.9
N7—C2—N3	120.45 (14)	C10—C9—C8	122.64 (18)
N7—C2—S1	119.33 (12)	C10—C9—H9	118.7
N3—C2—S1	120.18 (11)	C8—C9—H9	118.7
C2—N3—N4	126.89 (13)	C9—C10—H10A	120.0
C2—N3—H3	116.6 (14)	C9—C10—H10B	120.0
N4—N3—H3	113.6 (13)	H10A—C10—H10B	120.0
C5—N4—N3	117.22 (12)	C12—C11—C16	118.82 (14)
N4—C5—C11	117.02 (13)	C12—C11—C5	120.39 (13)
N4—C5—C6	122.39 (13)	C16—C11—C5	120.72 (13)
C11—C5—C6	120.47 (12)	C13—C12—C11	120.55 (15)
C5—C6—S1	110.68 (11)	C13—C12—H12	119.7
C5—C6—H6A	109.5	C11—C12—H12	119.7
S1—C6—H6A	109.5	C14—C13—C12	119.97 (16)
C5—C6—H6B	109.5	C14—C13—H13	120.0
S1—C6—H6B	109.5	C12—C13—H13	120.0
H6A—C6—H6B	108.1	C15—C14—C13	120.10 (15)
C2—N7—C8	122.54 (13)	C15—C14—H14	119.9
C2—N7—H7	114.9 (13)	C13—C14—H14	119.9
C8—N7—H7	122.4 (13)	C14—C15—C16	120.14 (16)
N7—C8—C9	112.36 (14)	C14—C15—H15	119.9
N7—C8—H8A	109.1	C16—C15—H15	119.9
C9—C8—H8A	109.1	C15—C16—C11	120.38 (15)
N7—C8—H8B	109.1	C15—C16—H16	119.8
C9—C8—H8B	109.1	C11—C16—H16	119.8
C6—S1—C2—N7	158.81 (14)	N7—C8—C9—C10	-132.9 (2)

C6—S1—C2—N3	-23.40 (15)	N4—C5—C11—C12	16.0 (2)
N7—C2—N3—N4	161.73 (15)	C6—C5—C11—C12	-167.94 (15)
S1—C2—N3—N4	-16.0 (2)	N4—C5—C11—C16	-161.07 (15)
C2—N3—N4—C5	31.9 (2)	C6—C5—C11—C16	15.0 (2)
N3—N4—C5—C11	-179.62 (13)	C16—C11—C12—C13	2.4 (2)
N3—N4—C5—C6	4.4 (2)	C5—C11—C12—C13	-174.67 (15)
N4—C5—C6—S1	-46.52 (19)	C11—C12—C13—C14	-1.3 (2)
C11—C5—C6—S1	137.59 (12)	C12—C13—C14—C15	-0.6 (3)
C2—S1—C6—C5	49.37 (12)	C13—C14—C15—C16	1.3 (3)
N3—C2—N7—C8	169.97 (15)	C14—C15—C16—C11	-0.2 (3)
S1—C2—N7—C8	-12.2 (2)	C12—C11—C16—C15	-1.7 (2)
C2—N7—C8—C9	-166.69 (16)	C5—C11—C16—C15	175.41 (15)

Hydrogen-bond geometry (Å, °) for (sb1532_hy)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
N3—H3···Br1	0.85 (2)	2.53 (2)	3.3282 (13)	158 (2)
C6—H6*A*···Br1^{i}	0.99	2.90	3.6584 (15)	134
C6—H6*B*···Br1^{ii}	0.99	2.96	3.7628 (16)	139
N7—H7···Br1	0.85 (2)	2.53 (2)	3.3425 (14)	159 (2)
C8—H8*A*···Br1^{iii}	0.99	2.79	3.7067 (18)	153

Symmetry codes: (i) -*x*+1/2, *y*-1/2, *z*; (ii) -*x*, -*y*+1, -*z*+1; (iii) -*x*+1, -*y*+1, -*z*+1.

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2. Spectral Data

Spectra of compound (**9a**)

^{1}H NMR of compound **9a** ($CDCl_3$)

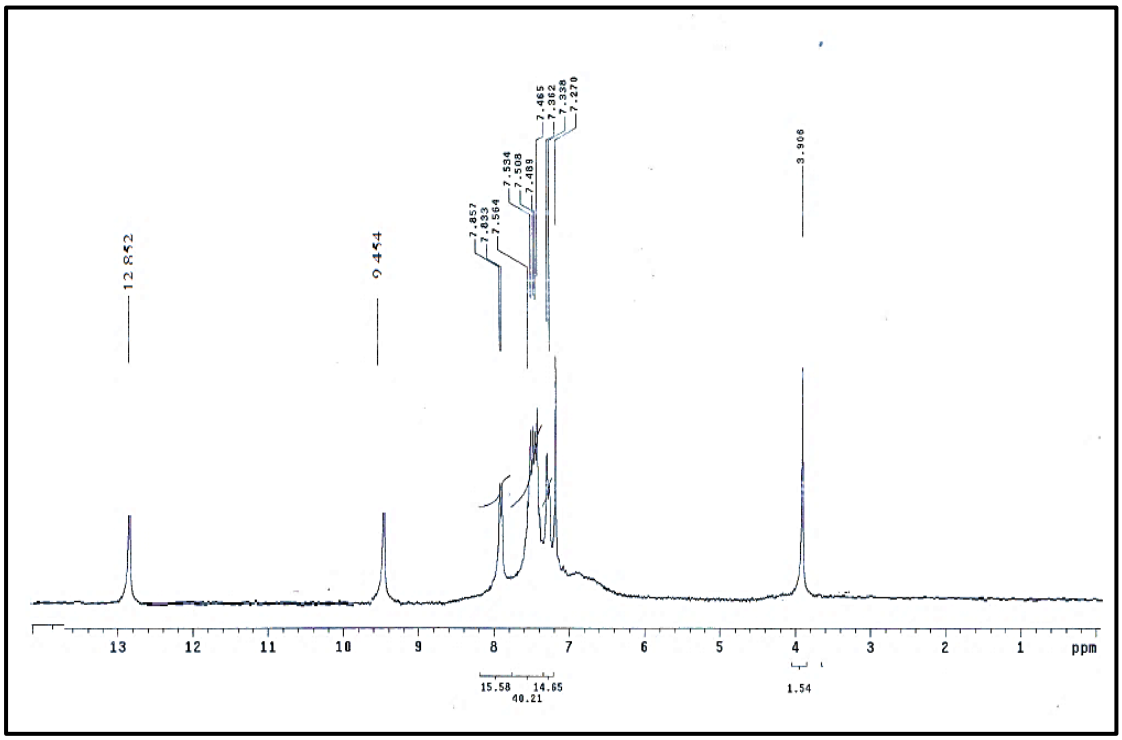


Figure 3

^{13}C NMR of compound **9a** (DMSO-d_6)

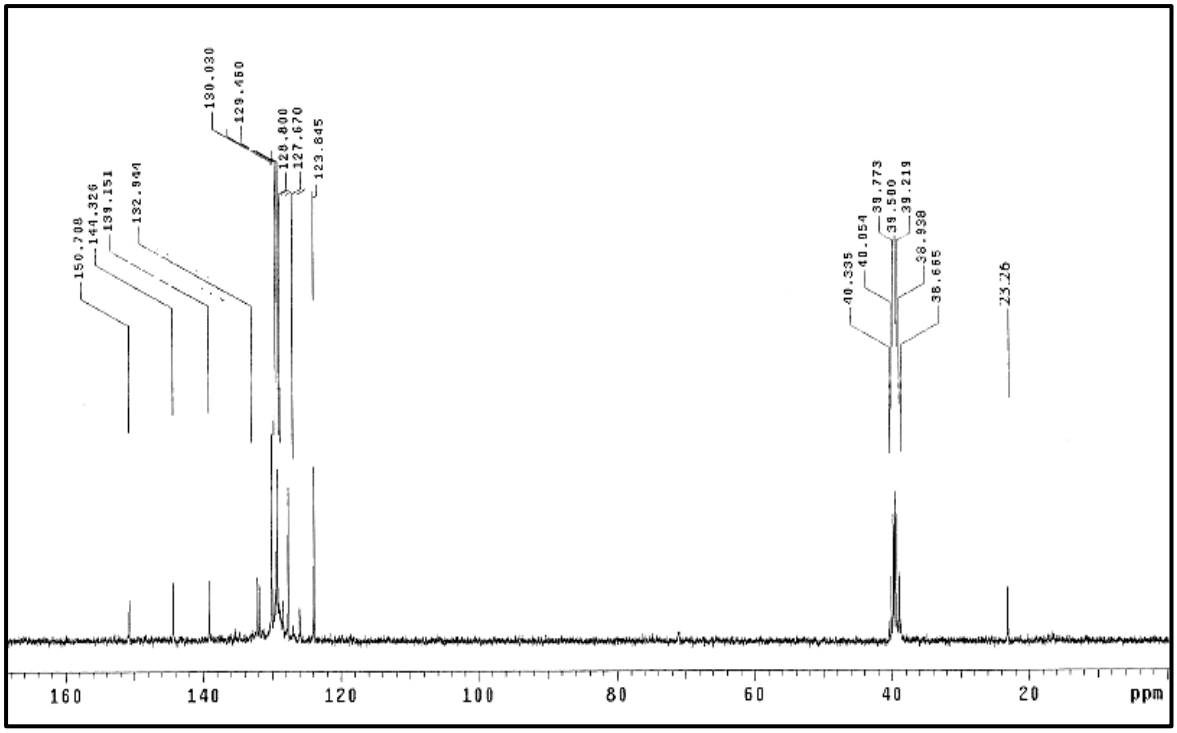


Figure 4

Spectra of compound **9b**

^{1}H NMR of compound **9b**

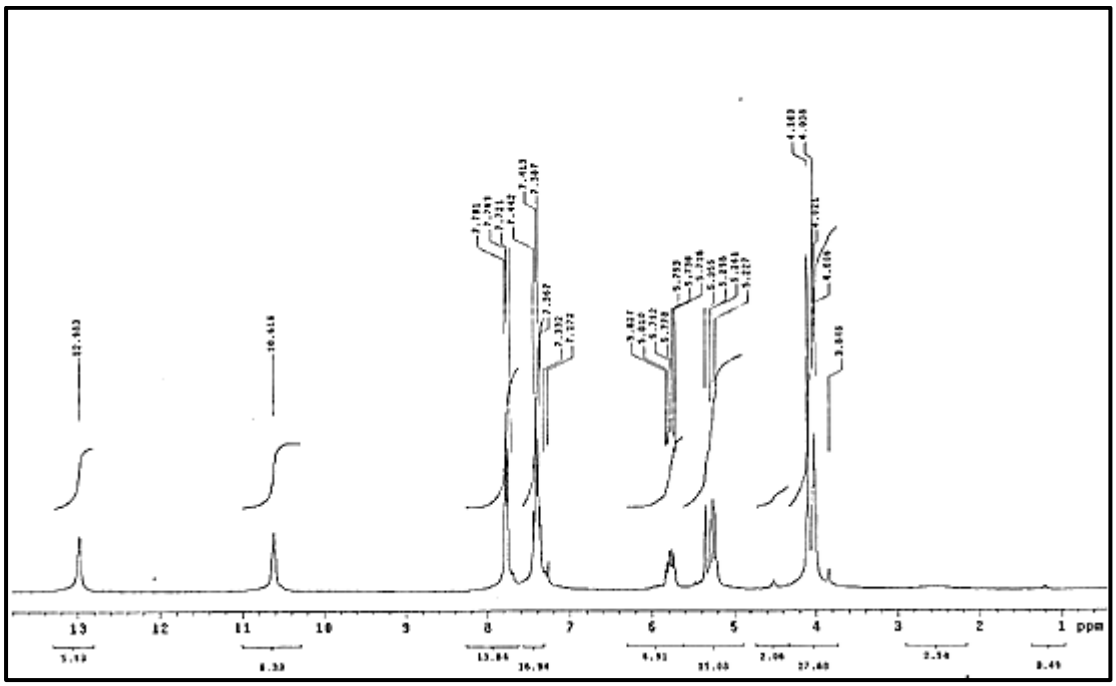


Figure 5

^{13}C NMR of compound **9b**

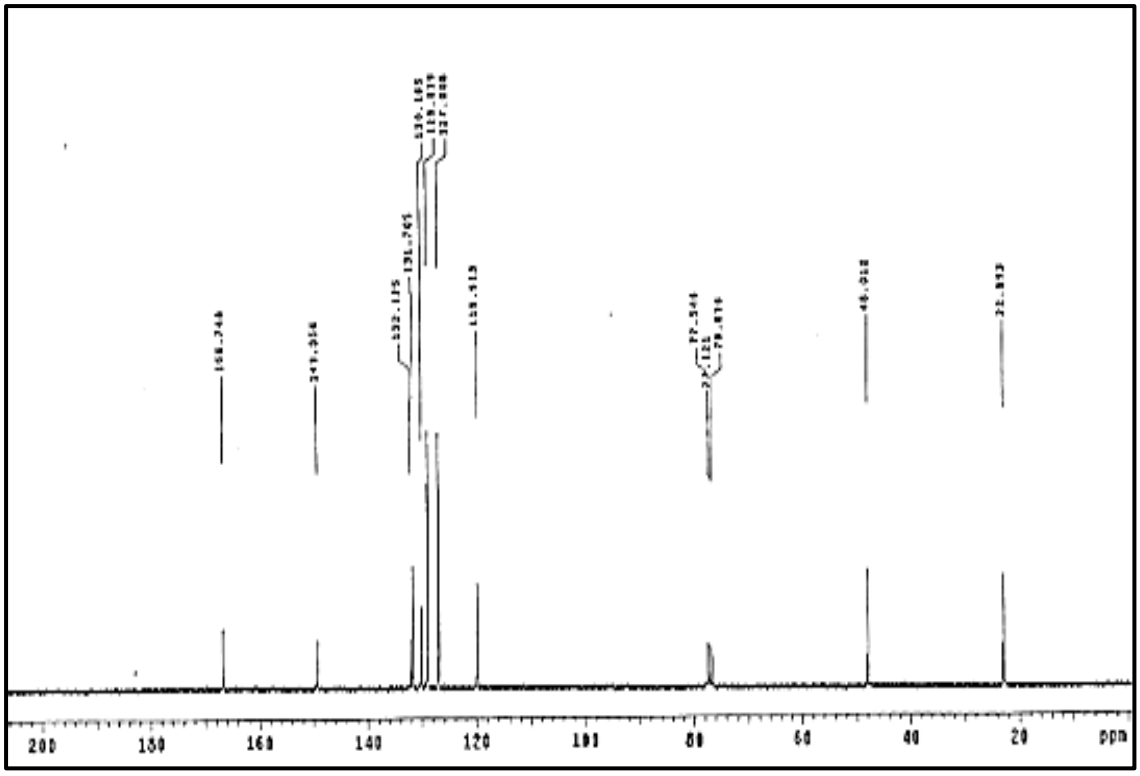


Figure 6

Spectra of compound **9c**

^{1}H NMR of **9c**

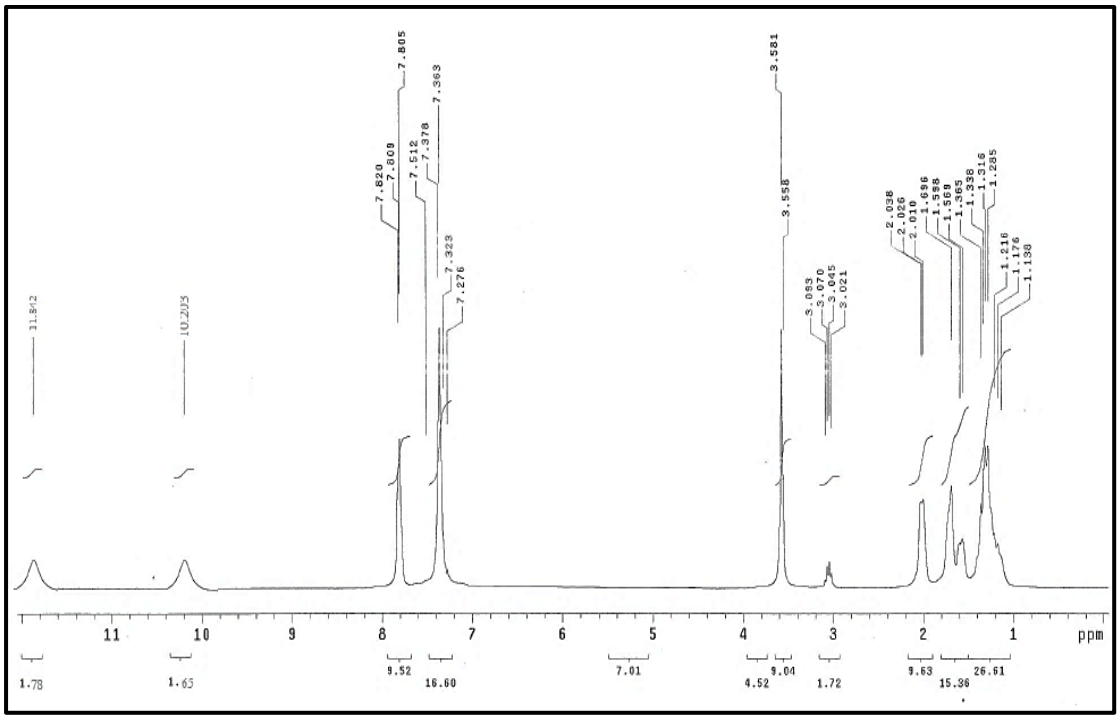


Figure 7

^{13}C NMR of compound **9c**

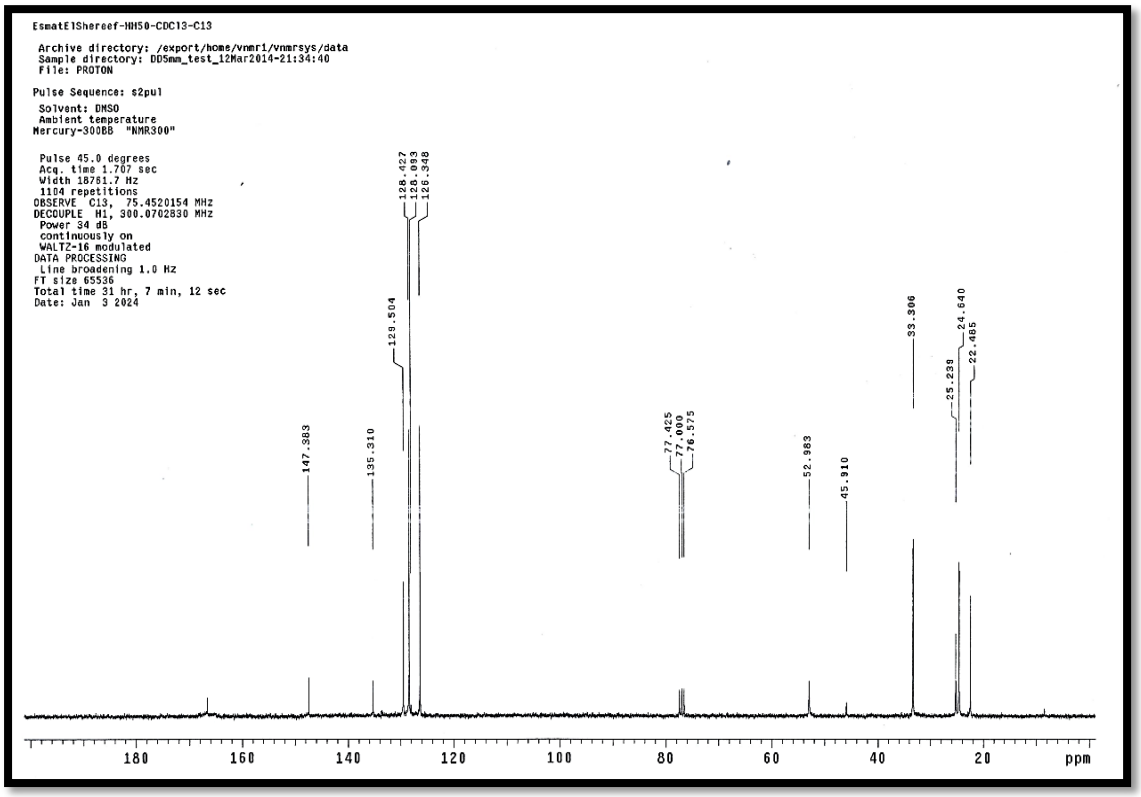


Figure 8

Spectral data of compound **9d**

^{1}H NMR of compound **9d**

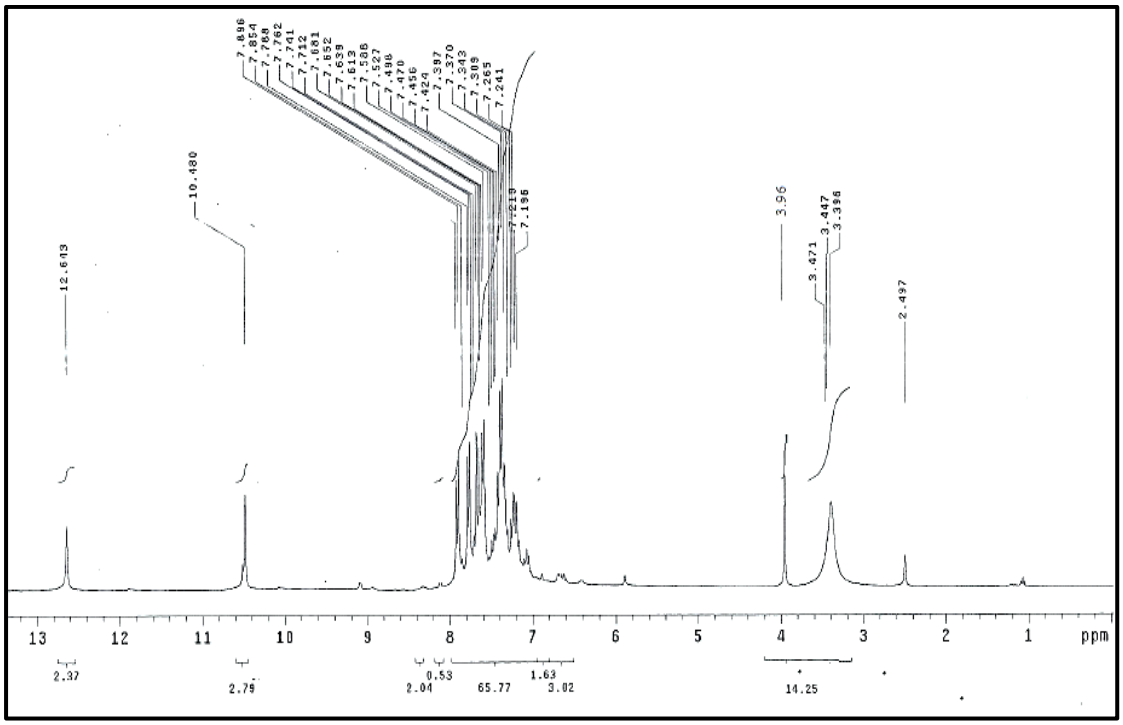


Figure 9

^{13}C NMR of compound **9d**

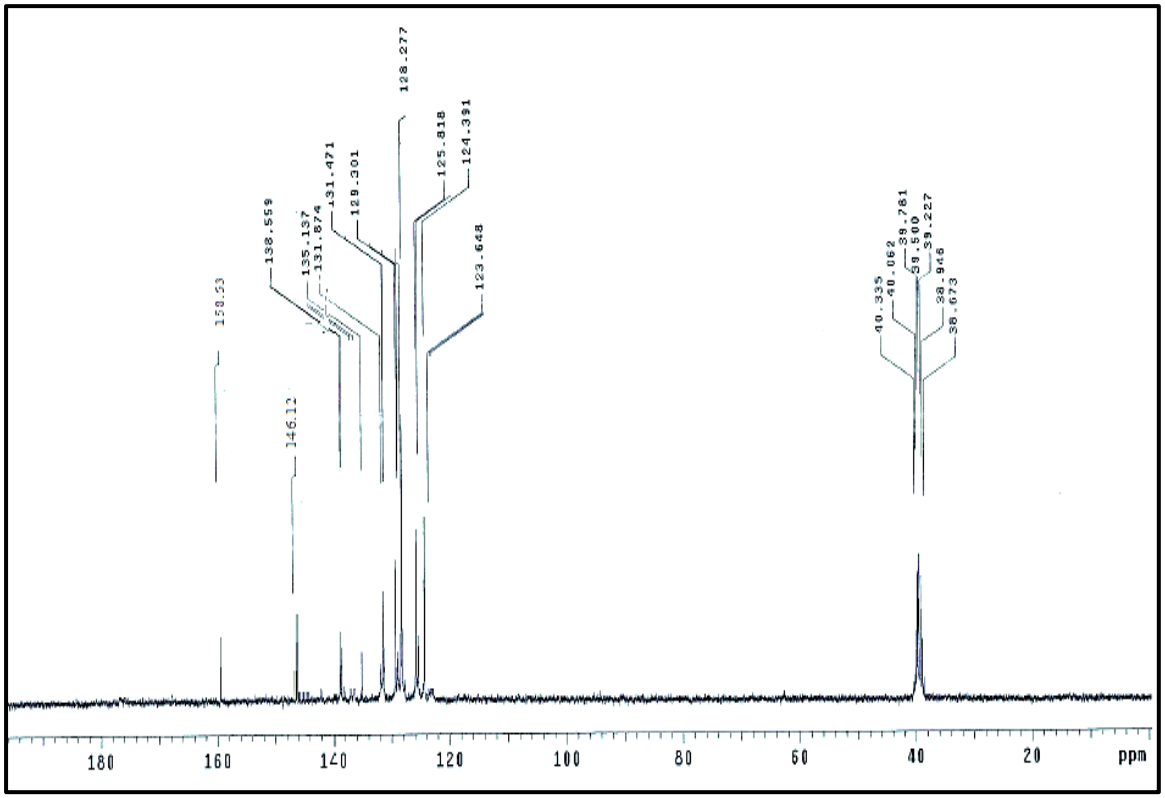


Figure 10

Spectral data of compound **9e**

^{1}H NMR of compound **9e**

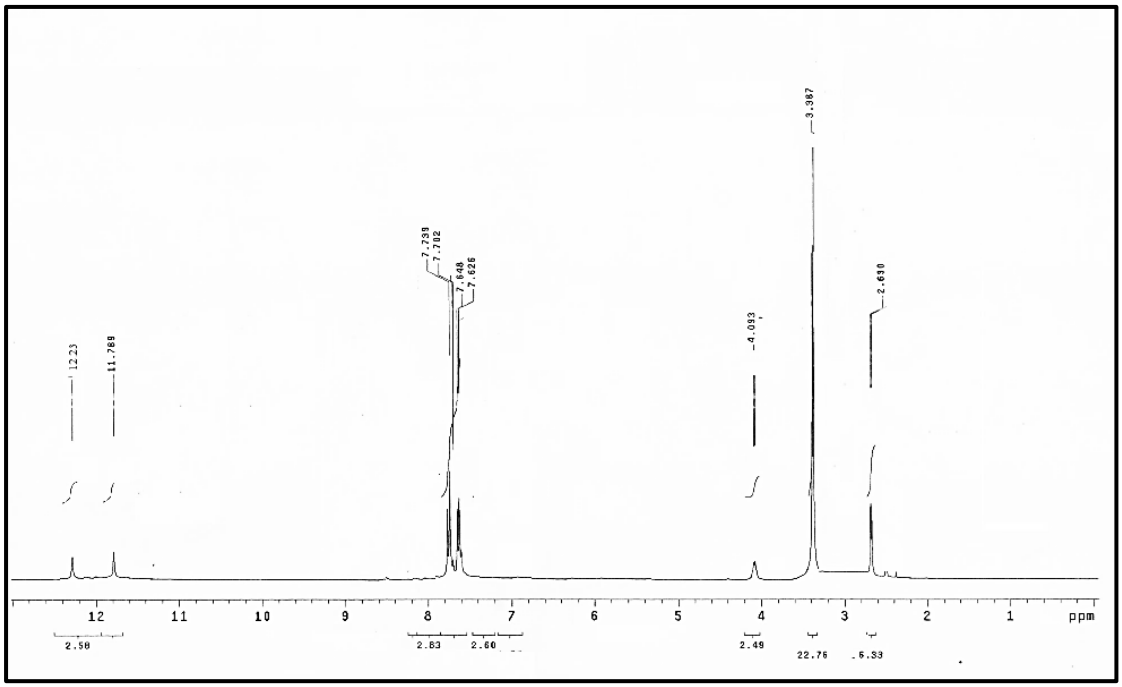


Figure 11

^{13}C NMR of compound **9e**

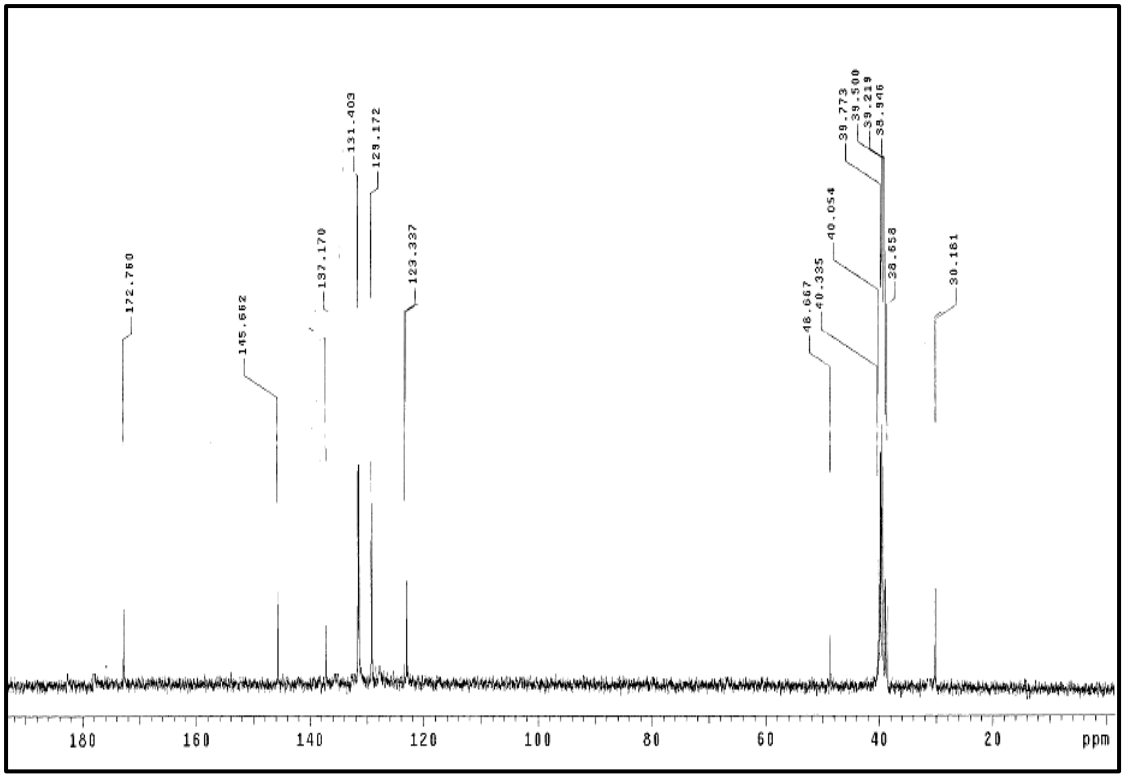


Figure 12

Spectral data of compound **9f**

^{1}H NMR of compound **9f**

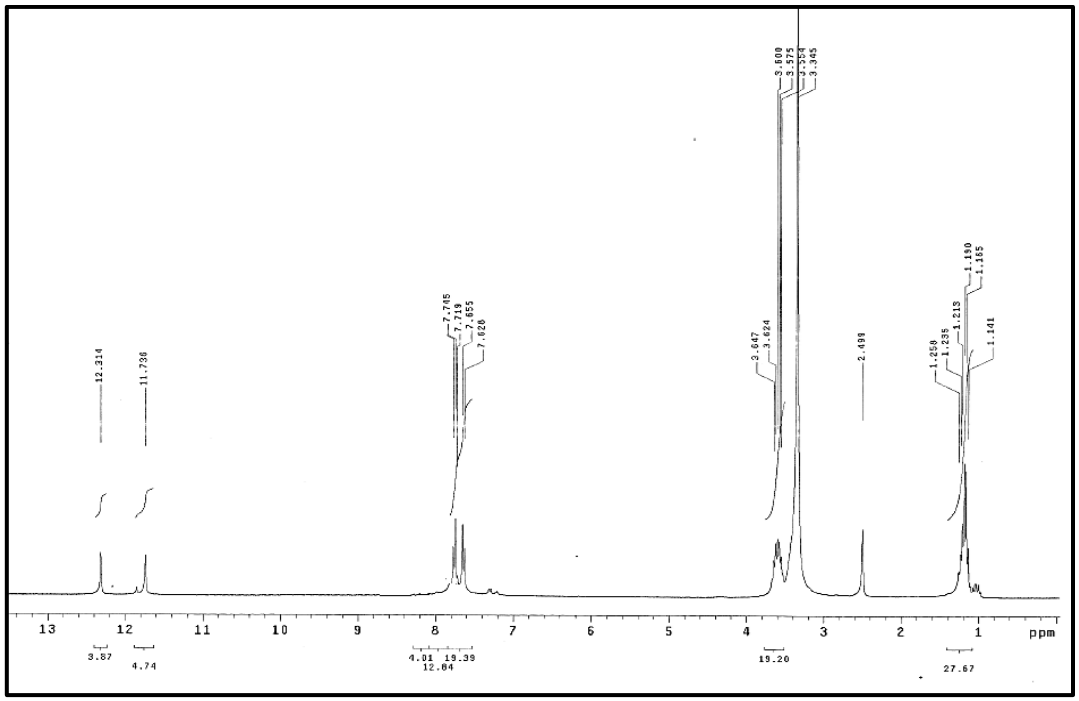


Figure 13

^{13}C NMR of compound **9f**

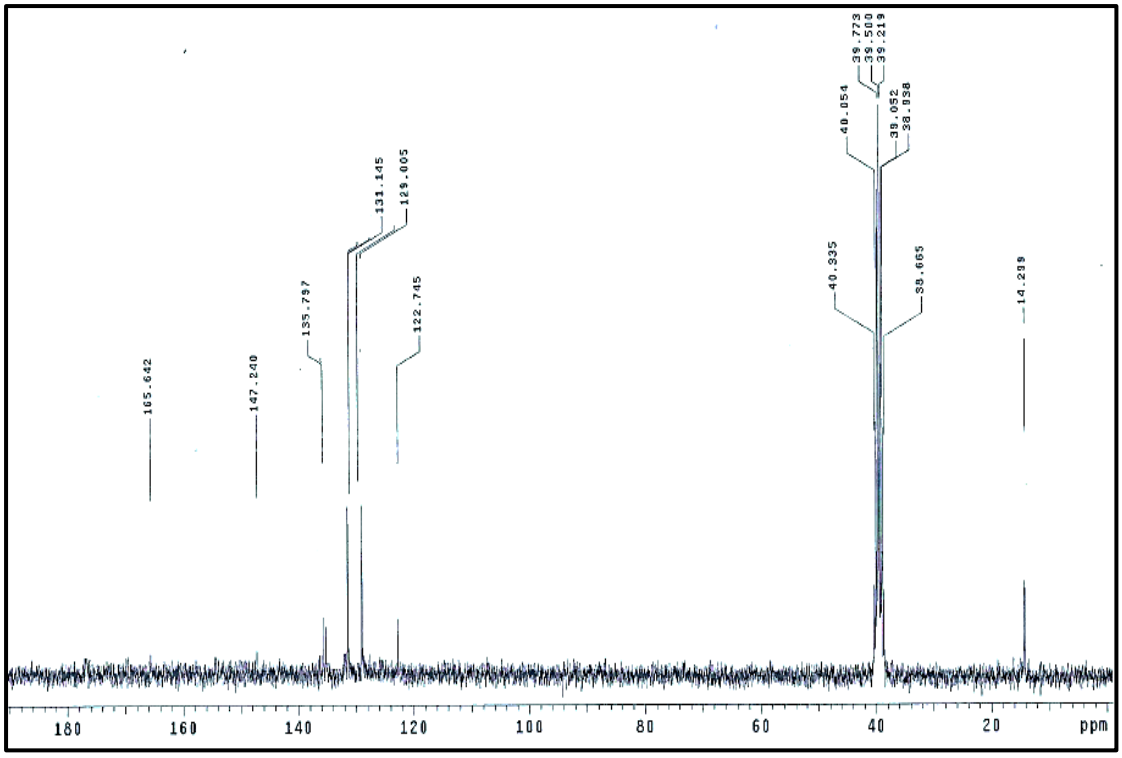


Figure 14

Spectra of compound **9g**

^{1}H NMR of compound **9g**

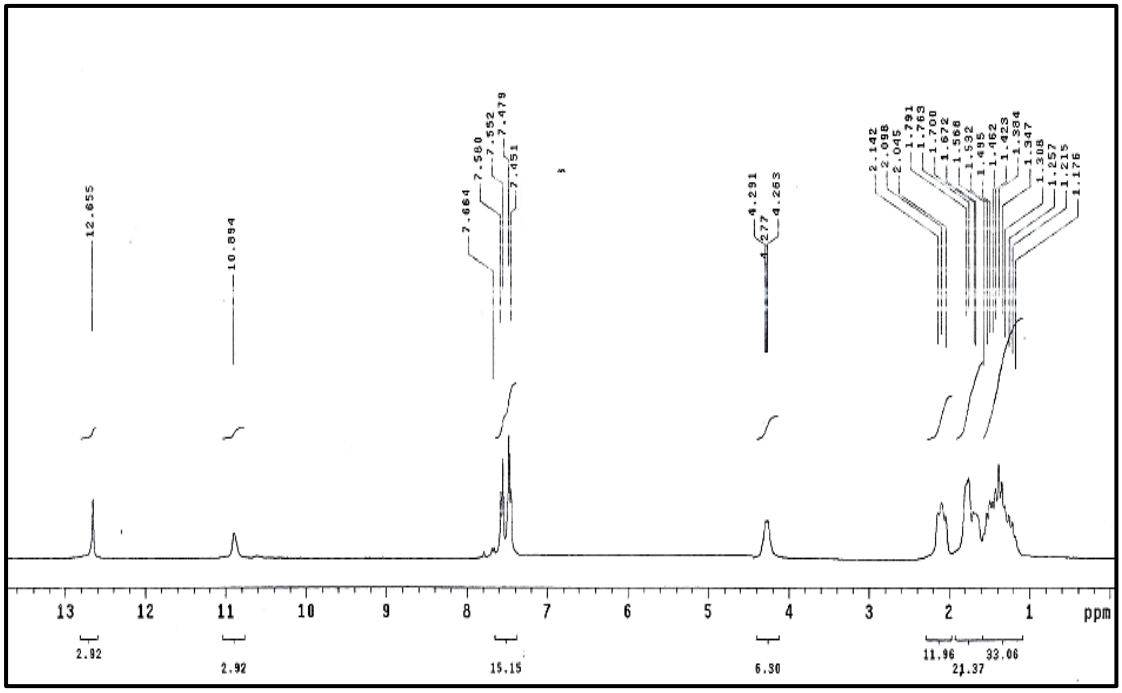


Figure 15

^{13}C NMR of compound **9g**

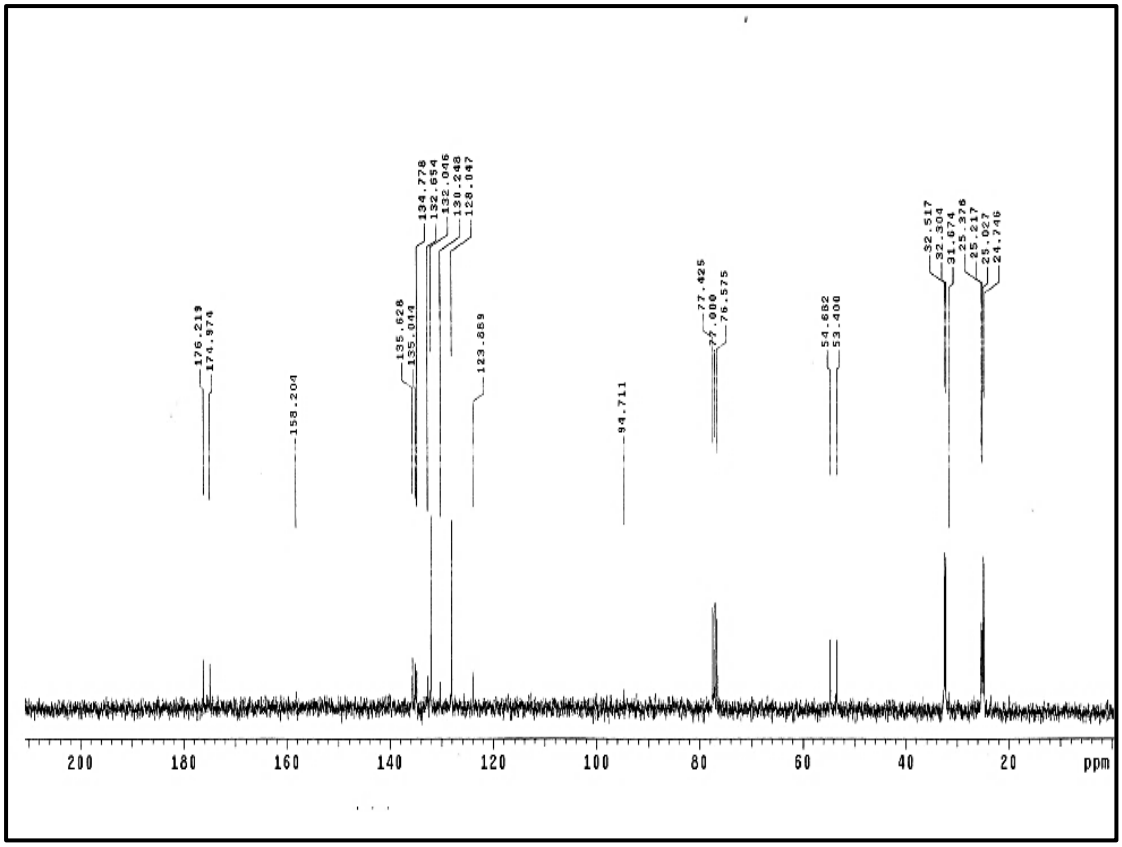

176.219
174.974
158.204
135.628
135.014
134.778
132.654
132.046
130.248
128.047
123.869
94.711
77.425
77.000
76.575
54.682
53.400
32.517
32.106
31.674
25.376
25.217
25.027
24.746
200
180
160
140
120
100
80
60
40
20
ppm

Figure 16

Appendix A

4.EXPERIMENTS

4.1. Chemistry

General details:

All reagents were used as purchased from Merck. The progress of all reactions was monitored with thin-layer chromatography (TLC) on Merck alumina-backed TLC plates and visualized under UV light. Spectra were measured in DMSO-d_6 on a Bruker AV-400 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) Microanalytical Center, Cairo University. Chemical shifts are expressed in δ (ppm) versus internal Tetramethylsilane (TMS) = 0 ppm for ^{1}H and ^{13}C. Chemical shifts (δ) are reported in parts per million (ppm) relative to Tetramethylsilane (TMS) as an internal standard, and the coupling constants (*J*) are reported in Hertz (Hz). Splitting patterns are denoted as follows: singlet (s), doublet (d), multiplet (m), triplet (t), quartet (q), doublet of doublets (dd), doublet of triplets (dt), triplet of doublets (td), and doublet of quartet (dq). Melting points (mp) were determined with a Stuart melting point instrument and are expressed in °C. Mass spectra were recorded on a Finnigan Fab 70 eV, and elemental analyses were carried out on a Perkin device at the Microanalytical Institute of Organic Chemistry, Karlsruhe Institute of Technology, Karlsruhe, Germany. X-ray crystallography was conducted at the Department of Chemistry, University of Helsinki, Finland.

4.2. Biological evaluation

4.2.1 Cell Viability assay (MTT assay)

MTT assay was performed to investigate the effect of the synthesized compounds on mammary epithelial cells (MCF-10A). The cells were propagated in medium consisting of Ham's F-12 medium/ Dulbecco's modified Eagle's medium (DMEM) (1:1) supplemented with 10% foetal calf serum, 2 mM glutamine, insulin (10 µg/mL), hydrocortisone (500 ng/mL) and epidermal growth factor (20 ng/mL). Trypsin ethylenediamine tetra acetic acid (EDTA) was used to passage the cells after every 2-3 days. 96-well flat-bottomed cell culture plates were used to seed the cells at a density of 104 cells mL^{-1}. The medium was aspirated from all the wells of culture plates after 24 h followed by the addition of synthesized compounds (in 200 µL medium to yield a final concentration of 0.1% (v/v) dimethyl sulfoxide) into individual wells of the plates. Four wells were designated to a single compound. The plates were allowed to incubate at 37°C for 96 h. Afterwards, the medium was aspirated and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (0.4 mg/mL) in medium was added to each well and subsequently incubated for 3 h. The medium was aspirated and 150 µL dimethyl sulfoxide (DMSO) was added to each well. The plates were vortexed followed by the measurement of absorbance at 540 nm on a microplate reader. The results were presented as inhibition (%) of proliferation in contrast to controls comprising 0.1% DMSO.

4.2.2. Assay for antiproliferative effect

To explore the antiproliferative potential of compounds propidium iodide fluorescence assay was performed using different cell lines such as Panc-1 (pancreas cancer cell line), MCF-7 (breast cancer cell line), HT-29 (colon cancer cell line) and A-549 (epithelial cancer cell line), respectively. To calculate the total nuclear DNA, a fluorescent dye (propidium iodide, PI) is used which can attach to the DNA, thus offering a quick and precise technique. PI cannot pass through the cell membrane and its signal intensity can be considered as directly proportional to quantity of cellular DNA. Cells whose cell membranes are damaged or have changed permeability are counted as dead ones. The assay was performed by seeding the cells of different cell lines at a density of 3000-7500 cells/well (in200µl medium) in culture plates followed by incubation for 24h at 37 °C in humidified 5%CO2/95% air atmospheric conditions. The medium was removed; the compounds were added to the plates at 10 µM concentrations (in 0.1% DMSO) in triplicates, followed by incubation for 48h. DMSO (0.1%) was used as control. After incubation, medium was removed followed by the addition of PI (25 µl, 50µg/mL in water/medium) to each well of the plates. At -80 °C, the plates were allowed to freeze for 24 h, followed by thawing at 25 ºC. A fluorometer (Polar-Star BMG Tech) was used to record the readings at excitation and emission wavelengths of 530 and 620 nm for each well. The percentage cytotoxicity of compounds was calculated using the following formula:

$$\% \text{ Cytotoxicity} = \frac{A_C - A_{TC}}{A_C} \times 100$$

Where A*TC*= Absorbance of treated cells and AC= Absorbance of control. Erlotinib was used as positive control in the assay.

4.2.3. EGFR inhibitory assay

EGFR-TK assay was performed to evaluate the inhibitory potency of the most potent compounds against EGFR. Baculoviral expression vectors including pBlueBacHis2B and pFASTBacHTc were used separately to clone 1.6 kb cDNA coding for EGFR cytoplasmic domain (EGFR-CD, amino acids 645–1186). 5′ upstream to the EGFR sequence comprised a sequence that encoded (His)6. Sf-9 cells were infected for 72h for protein expression. The pellets of Sf-9 cells were solubilized in a buffer containing sodium vanadate (100 µM), aprotinin (10 µg/mL), triton (1%), HEPES buffer (50mM), ammonium molybdate (10 µM), benzamidine HCl (16 µg/mL), NaCl (10 mM), leupeptin (10 µg/mL) and pepstatin (10 µg/mL) at 0°C for 20 min at pH 7.4, followed by centrifugation for 20 min. To eliminate the non-specifically bound material, a Ni-NTA super flow packed column was used to pass through and wash the crude extract supernatant first with 10 mM and then with 100 mM imidazole. Histidine-linked proteins were first eluted with 250 and then with 500 mM imidazole subsequent to dialysis against NaCl (50 mM), HEPES (20 mM), glycerol (10%) and 1 µg/mL each of aprotinin, leupeptin and pepstatin for 120 min. The purification was performed either at 4 °C or on ice. To record autophosphorylation level, EGFR kinase assay was carried out on the basis of DELFIA/Time-Resolved Fluorometry. The compounds were first dissolved in DMSO absolute, subsequent to dilution to appropriate concentration using HEPES (25 mM) at pH 7.4. Each compound (10 µL) was incubated with recombinant enzyme (10 µL, 5 ng for EGFR, 1:80 dilution in 100 mM HEPES) for 10 min at 25oC, subsequent to the addition of 5X buffer (10 µL, containing 2 mM MnCl2, 100 µM Na3VO4, 20 mM HEPES and 1 mM DTT) and ATP-MgCl2 (20 µL, containing 0.1 mM ATP and 50 mM MgCl2) and incubation for 1h. The negative and positive controls were included in each plate by the incubation of enzyme either with or without ATP-

MgCl2. The liquid was removed after incubation and the plates were washed thrice using wash buffer. Europium-tagged antiphosphotyrosine antibody (75 µL, 400 ng) was added to each well followed by incubation of 1h and then washing of the plates using buffer. The enhancement solution was added to each well and the signal was recorded at excitation and emission wavelengths of 340 at 615 nm. The autophosphorylation percentage inhibition by compounds was calculated using the following equation:

$$100\% - [(negative\ control)/(positive\ control) - (negative\ control)]$$

Using the curves of percentage inhibition of eight concentrations of each compound, IC50 was calculated. The majority of signals detected by antiphosphotyrosine antibody were from EGFR because the enzyme preparation contained low impurities.

4.2.4. BRAF kinase assay

V600E mutant BRAF kinase assay was performed to investigate the activity of tested compounds against BRAF. Mouse full-length GST-tagged BRAFV600E (7.5 ng, Invitrogen, PV3849) was pre-incubated with drug (1 µL) and assay dilution buffer (4 µL) for 60 min at 25oC. In assay dilution buffer, a solution (5 µL) containing MgCl2 (30 mM), ATP (200 µM), recombinant human full length (200 ng) and N-terminal His-tagged MEK1 (Invitrogen) was added to start the assay, subsequent to incubation for 25 min at 25oC. The assay was stopped using 5X protein denaturing buffer (LDS) solution (5 µL). To further denature the protein, heat (70° C) was applied for 5 min. 4-12% precast NuPage gel plates (Invitrogen) were used to carry out electrophoresis (at 200 V). 10 µL of each reaction was loaded into the precast plates and electrophoresis was allowed to proceed. After completion of electrophoresis, the front part of the precast gel plate (holding hot ATP) was cut and afterwards cast-off. The dried gel was developed using a phosphor screen. A reaction without active enzyme was used as

negative control while that containing no inhibitor served as positive control. To study the effect of compounds on cell-based pERK1/2 activity in cancer cells, commercially available ELISA kits (Invitrogen) were used according to manufacturer's instructions.

4.2.5. VEGFR-2 inhibitory assay

Prepare all the solutions using autoclaved, deionized water and analytical grade reagents. Prepare and store all the reagents at room temperature (unless indicated otherwise). EDTA solution (0.5 M, pH 8.0): add 95 mL ultra-pure water to 14.612 g EDTA, adjust the pH to 8.0 using NaOH solution, add 5 mL ultra-pure water; 1×Kinase Assay Buffer: add 25 mL HEPES solution (1 M), 190.175 mg EGTA, 5 mL MgCl2 solution (1 M), 1 mL DTT, 50 μL tween-20, 450 mL ultrapure water, adjust pH to 7.5 and constant volume to 500 mL using ultrapure water; 4×Stop solution (40 mM): Mix 0.8 mL of the foregoing EDTA solution, 1 mL 10×Detection Buffer and 8.2 mL ultra-pure water; 1×Detection Buffer: Mix 1 mL 10×Detection Buffer with 9 mL water; 4×VEGFR Kinase solution (1.74 nM, 521 times diluted, stored on ice): 1.2 μL VEGFR mother liquor (0.909 μM) was added to 624 μL 1×Kinase Assay Buffer and mixed; 4×ULight™-labeled JAK1 (substrate) (200 nM, 25 times diluted): 24 μL ULight™-labeled JAK1 (mother liquor concentration 5 μM) was added to 576 μL 1×Kinase Assay Buffer and mixed; 4×ATP Solution (40 μM, 250 times diluted): add 3 μL ATP solution (10 mM) to 747 μL 1×Kinase Assay Buffer and mixed; 4×Detection Mix (8 nM, 390.6 times diluted): 3 μL Europium- antiphospho-tyrosine antibody (PT66) (3.125 μM) was added to 1169 μL1×Detection Buffer and mixed; 2×substrate/ATP Mix: 560 μL foregoing 4×ULight™-labeled JAK1 and 560 μL 4×ATP solution and mixed (prepared before use). The assays used an ULight-labeled peptide substrate and a Europium-W1024-labeled antiphosphotyrosine antibody. The VEGFR-2 was purchased from Carna Biosciences, Inc. (New York, USA). The 384-well plates were obtained from

PerkinElmer. Compounds were dissolved in DMSO and diluted to 11 concentrations at a tripling rate from 2.500 μM to 0.042 nM and added 2.5 μL to 384-well plates. 5 μL 2×VEGFR-2 kinase solution (0.5 nM) was added to 384-well plates homogeneous mixing and pre-reaction at room temperature

for 30 min. Next, 2.5 μL 4×Ultra ULightTM-JAK-1(Tyr1023) Peptide (200 nM)/ATP (40 μM) was added to the corresponding wells of a 384-well plate. Negative control: 2.5 μL/well 4×substrate/ATP mixture and 7.5 μL 1×kinase assay buffer in 384-well plate well. Positive control: 2.5 μL/well 4×substrate/ATP mixture, 2.5 μL/well 1×kinase assay buffer with 16% DMSO, 5 μL/well 2×VEGFR-2 kinase solution was added to the 384-well plate, The final concentration of DMSO in the mixing system was 4%. After incubation at room temperature and dark for 60 min, 5 μL 4×stop solution was added to corresponding wells to react for 5 min and then 5 μL 4×detection mix was added to the corresponding wells of a 384-well plate. The mixture was centrifugally mixed and stayed for 60 min at room temperature for color development. The plate was read using an Envision plate reader. The inhibition rate (%) = (positive well reading-compound well reading)/(positive well reading-negative well reading)×100. The corresponding IC_{50} values were calculated using GraphPad Prism 5.0.

4.3. Western Blotting analysis

Gel electrophoresis and immuno-blot analysis of proteins (Western Blot) (Burnette, 1981; Sambrook et al., 1989).

Principle:

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is used to separate proteins based on their size. When coupled with western blotting (immunoblotting), both are typically used to determine the presence and/or relative abundance of a target protein in a sample containing a complex mixture of proteins. In this technique, total protein in each sample is loaded and electrophoretically separated by applying an electric current which allows the proteins to migrate through the gel matrix. In order for the proteins to migrate through the gel, they are first denatured and negatively charged by exposure to a detergent such as SDS. A molecular weight marker that produces bands of known size is used to help identifying proteins of interest. After the protein components have been sufficiently separated, they can be transferred to a polyvinylidene fluoride (PVDF) membrane by applying an electric current to the gel so that the proteins migrate out of the gel onto the membrane. For detection of a specific protein on the membrane, a primary antibody against that protein is added to form a protein-antibody complex followed by the addition of a secondary antibody that binds to the complex through its antibody side. The secondary antibody is typically linked to an enzyme that produces luminescence upon the reaction with its substrate. The amount of the luminescence, directly proportional to the amount of the protein that reacted with the antibody, is captured by Bio-Rad Imager

Reagents preparation:

- Lysis buffer: 10mM Tris, 100mM NaCl, 25mM ethylene diamine tetra acetic acid (EDTA), 25mM Ethylene glycol bis(2-aminoethyl) tetra acetic acid (EGTA),0.1 Sodium dodecyl sulfate (SDS), % 1% (v/v) Triton X-100, 2% (v/v) NP-40 (pH 7.4), with 1:200 protease inhibitor cocktail (Sigma) and 1:300 phosphatase inhibitor cocktail Tablet (Roche).
- Polyacrylamide gel prepared according to table
- Protein marker (Thermo scientific)
- SDS Loading buffer (6X): 750 mM Tris-HCl (pH 6.8); 600 mM dithiothreitol (DTT); 12 % SDS; 0.012 % Bromophenol blue; 60 % glycerol
- Tris-Glycine SDS running buffer: 25 mM Tris; 192 mM glycine (electrophoresis grade) (pH 8.3 -8.4); 0.1% SDS.
- Tris-Glycine transfer buffer: 25 mM Tris; 192 mM glycine; 0.05% SDS; 15% methanol. Methanol was immediately added before the transfer.
- Tris Buffered Saline Tween (TBS-T): 10 mM Tris-HCl (pH 8.0); 150 mM NaCl; 0.1% Tween-20
- 5 % non-fat dry milk in TBS-T
- Primary antibodies for the proteins to be detected .....
- Secondary antibodies for the proteins to be detected
- ECL^{TM} western blotting detection chemiluminescent substrate (PerkinElmer, USA).

Procedure:

- The experiment was terminated by lysing the cells in cold lysis buffer. The cells were then immediately frozen at −20 °C for 1 h for further lysis, and collected by cell scraper and sonicated 2×10s, followed by centrifugation at 4000 rpm for 10 min under cooling.

- Total protein concentrations were determined colorimetrically in the supernatant using Bradford method before proceeding to the western blotting.

- Western blotting

- Equal amounts (20 μg) of protein samples were mixed and boiled with SDS Loading buffer for 10 min, allowed to cool on ice and then loaded into SDS-polyacrylamide gel and separated by Cleaver electrophoresis unit (Cleaver, UK), transferred onto polyvinylidene fluoride (PVDF) membranes (BioRad) for 30 min using a Semi-dry Electroblotter (Biorad, USA) at 2.5 A and 25 V for 30 min.

- The membrane was blocked with 5% nonfat dry milk in TBS-T for two hours at RT, in order to reduce non-specific protein interactions between the membrane and the antibody.

- The membrane was incubated overnight at 4°C with primary antibodies (Cell Signaling Technology) and β-actin (Sigma). The blots were then washed for three times (10 min each) with TBS-T.

- The membrane was then incubated with the corresponding horse radish peroxidase (HRP)- linked secondary antibodies (Dako) for another hour at room temperature, followed by washing for three times (10 min each) with TBS-T

- The chemiluminescent Western ECL substrate (Perkin Elmer, Waltham, MA) was applied to the blot according to the manufacturer's recommendation. Briefly, the

membranes were incubated for 1 min with a mixture of equal volumes from ECL solution A and ECL solution B.

- The chemiluminescent signals were captured using a CCD camera-based imager (Chemi Doc imager, Biorad, USA), and the bands intensities were then measured by ImageLab (Biorad)

- Protein-sized markers were used in all gels to localize the gel transfer regions for specific proteins and determine the transfer efficiency.

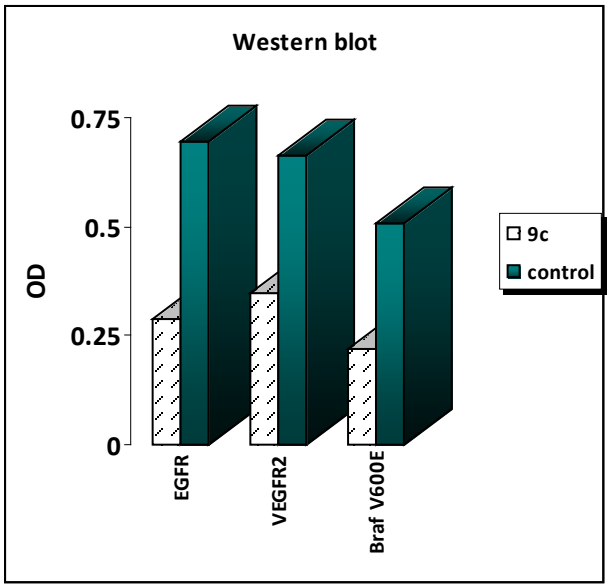
Table S : Solutions for preparing resolving and stacking gels for Tris-glycine SDS-Polyacrylamide gel electrophoresis

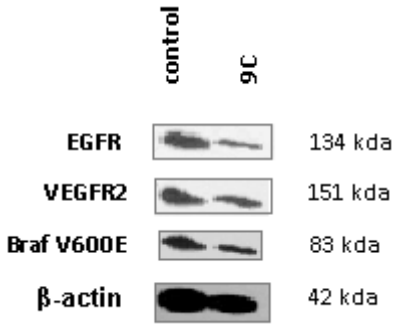
Stacking Gel	Resolving gel	Components (Used as ml)
	12%	
3.4	3.3	Water
0.83	4.0	30% acrylamide
---	2.5	1.5 M Tris (pH 8.8)
0.63	---	0.5 M Tris (pH 6.8)
0.05	0.1	10% SDS
0.05	0.1	10% Amm Persulfate
0.005	0.004	TEMED

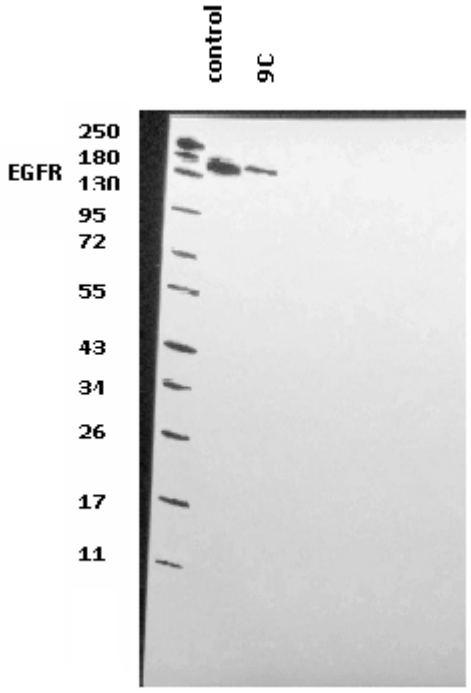
Lab Report

LPS induced raw 264.7 cells

	Code	MW	Cells	OD			
				EGFR	VEGFR-2	$BRAF^{V600E}$	
1	**9c**	354.31	A549	0.288±0.02	0.346±0.03	0.219±0.02	√
2	**control**	--	A549	0.695±0.05	0.662±0.05	0.507±0.04	√



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EGFR
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180
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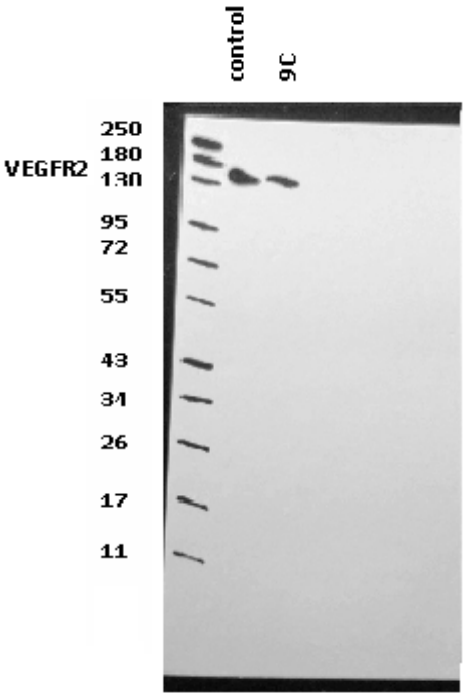
95
72
55
43
34
26
17
11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VEGFR2
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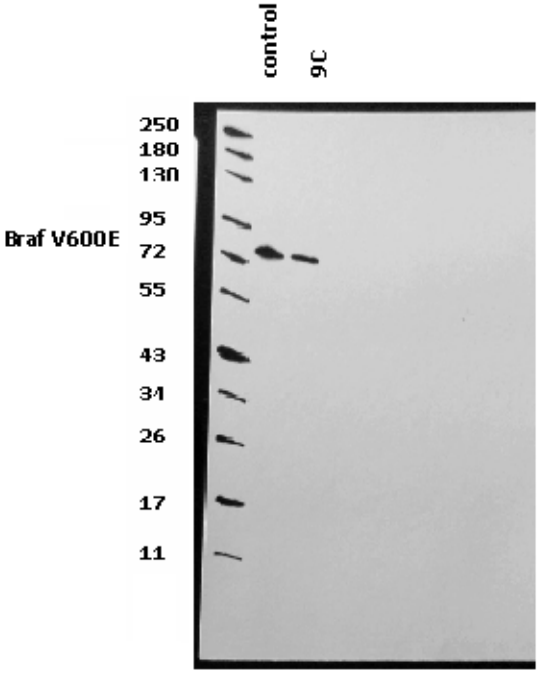
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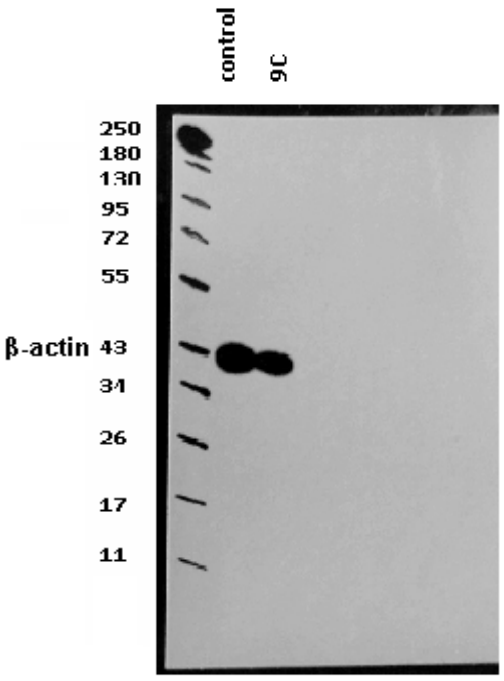
control
9C
β-actin
250
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95
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References:

Burnette, WN (1981) "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate--polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal Biochem* 112, 195-203.

Sambrook J, Fritsch EF and Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.

4.3. Statistical analysis

Computerized Prism 5 program was used to statistically analyzed data using one-way ANOVA test followed by Tukey's as post ANOVA for multiple comparison at $P \leq .05$. Data were presented as mean ± SEM.