Designing Model Imino Bifunctional Chelators for Radiopharmaceuticals – In vitro Antitumor Activity, Photoluminescence and Structural Analysis

Alice Brink[a], Robin.E. Kroon[b], Hendrik G. Visser[a], Connie EJ van Rensburg[c], Andreas Roodt[a]*

[a] Department of Chemistry, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa. Tel. no. +27 51 4017092, brinka@ufs.ac.za. [b] Department of Physics, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa. Tel. no. +27 51 4012884, Fax +2751 4013507, kroonre@ufs.ac.za. [c] Department of Pharmacology, University of Pretoria, Basic Medical Sciences Building; 6th floor, 9 Bophelo Road, Prinshof Campus, South Africa, Tel. no.: +27 12-8020926, Fax: +27 12 319 2411, profconniemedlen@gmail.com.

Supplementary Information

1. Introduction

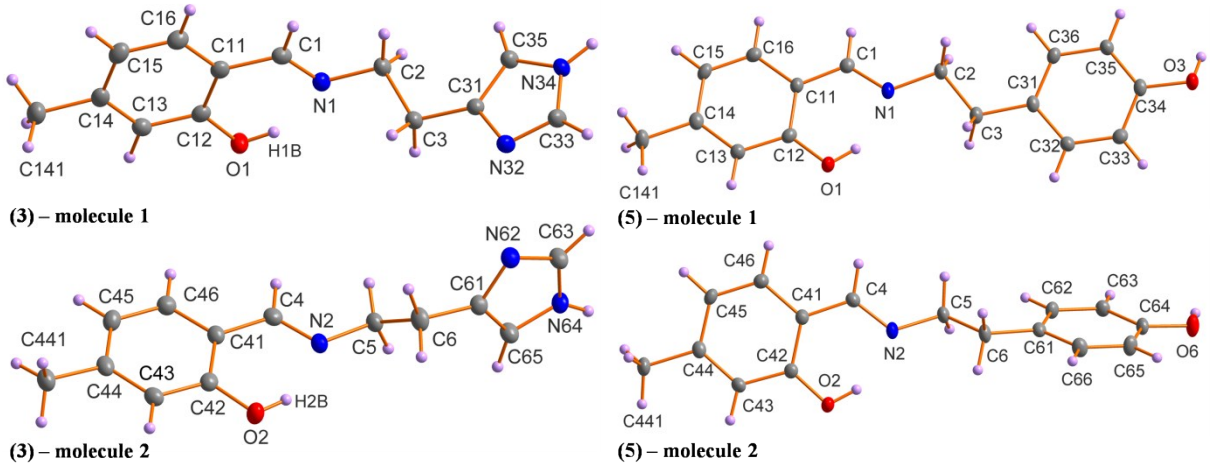


Fig. S1. Molecular structures of compounds **3** and **5** as found in the asymmetric unit indicating atom numbering scheme. Hydrogen atoms are drawn as spheres of arbitrary radius.

2. Experimental details

2.5 In vitro antitumor activity evaluation by SRB assays

The human cell lines TK10, UACC62 and MCF7 were obtained from National Cancer Institute (NCI) in the framework of a collaborative research program between Council for Scientific and Industrial Research (CSIR) and NCI. The three cell line panel is recommended by the National Cancer Institute for preliminary screens. The SRB Assay, for this study, was performed by the Council for Scientific and Industrial Research, South Africa[1,2] in accordance with the protocol of the Drug Evaluation Branch of the National Cancer Institute.

Cell lines were routinely maintained as monolayer cell cultures in RPMI containing 5% fetal bovine serum, 2 *m*M L-glutamine and 50μg/*m*l gentamicin. For the screening experiment, the cells (3-19 passages) were inoculated in 96-well microtiter plates at plating densities of 7-10 000 cells/well and were incubated for 24 h. After 24 h one plate was fixed with trichloroacetic acid (TCA) to represent a measurement of the cell population for each cell line at the time of drug addition (T_0). The other plates with cells were treated with the experimental compounds which were previously dissolved in DMSO as 10 *m*M stocks and diluted in medium to a final concentration 10 μM. Cells without compounds served as controls. Blank wells contained complete medium without cells. Emetine was used as a reference standard (concentration = 10 μM). The plates were incubated for 48 h after addition of the compounds. At the end of the incubation period, the cells were fixed to the bottom of each well with cold 50 % trichloroacetic acid, washed, dried and dyed with SRB. Unbound dye was removed and protein-bound dye was extracted with 10mM Tris base for optical density determination at a wavelength 540 *nm* using a multiwell spectrophotometer. Optical density measurements were used to calculate the net percentage cell growth:

The optical density of the test wells after 48 h period of exposure to test compound is T_i, the optical density at time zero is T_0, and the control (untreated cells) optical density is C.

Percentage cell growth was calculated as:

$[(T_i-T_0)/(C-T_0)]$ x 100 for concentrations at which $T_i \geq T_0$

$[(T_i-T_0)/T_0]$ x 100 for concentrations at which $T_i < T_0$.

The percentage (%) growth indicated in Table 4 is the total growth of cells in the treated wells relative to untreated controls over a forty-eight hour experimental period. Therefore a 100 % growth indicates there are the same amount of cells in treated wells as in untreated control wells, after 48 h. Zero percent growth indicates no increase in cell number as the treated wells contain the same number of cells as at the start of the incubation period, time zero (T_0). Negative one hundred percent growth indicates no cells remain after the 48 h incubation period.

3. Results and discussion

Additional discussion of results mentioned in primary manuscript.

3.2 Single crystal structure analysis

Compounds **3** and **5** contain two molecules in the asymmetric unit, with distinct variation with regards to their planarity as illustrated in Fig S1 and S2.

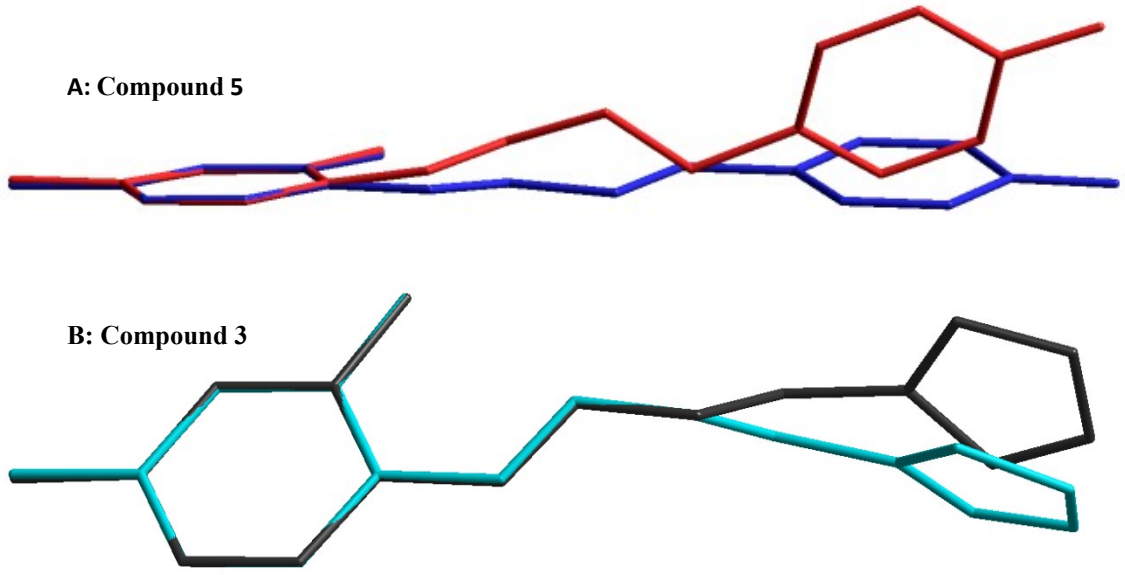


Fig. S2. An overlay of the two molecules found in the asymmetric unit of the crystal structures **3** and **5**. (A) Compound **5** (RMS = 1.172 x 10^{-2} Å) and (B) Compound **3** (RMS = 8.169 x 10^{-3} Å) to show variation of orientation. Overlay drawn only through atoms C11, C12, C13, C14, C15, C16 of the respective compounds to allow free

rotation of imine substituent, therefore low RMS values are indicated. (True overlay through all atoms yields an RMS value of 0.588 Å for **5** and 0.336 Å for **3**).

Table S1. Intra and intermolecular hydrogen bonds, C-H···π and N···N interactions for Compound **1** [Å and °].

Intra and Intermolecular hydrogen bonds				
D-H...A	d(D-H)	d(H···A)	d(D···A)	< (D-H···A)
O(1)-H(1B)···N(1)#1	0.84	1.86	2.606(3)	146.8
C(1)-H(1A)···N(25)#1	0.95	2.57	2.892(4)	99.9
C(16)-H(16)···N(23)#2	0.95	2.60	3.423(4)	145.8
C(24)-H(24)···O(1)#3	0.95	2.38	3.255(4)	152.1
Symmetry transformations used to generate equivalent atoms: #1 x,y,z #2 -x+3/2,y-1,z+1/2 #3 -x+3/2,y+1,z+1/2				
C-H···π Interaction				
C-H···Cg	**Centroid atom (Cg)**	**d(H···Cg)**	**d(C···Cg)**	**< (C-H···Cg)**
C141-H14C	Cg1 #1	2.7281(3)	3.568(3)	144.02(2)
Symmetry transformations: #1 x, -1+y, z Cg1 = centroid atom of C11, C12, C13, C14, C15, C16				
N···N interactions	**d(N···N)**			
N25···N22#1	2.910(3)			
N22···N25#2	2.910(3)			
Symmetry operation: #1 1.5-x, y, 0.5+z; #2 1.5-x, y, -0.5+z)				

Table S2. Intra and intermolecular hydrogen bonds, π - π and C-H···π interactions for Compound **2** [Å and °].

Intra and Intermolecular hydrogen bonds				
D-H···A	**d(D-H)**	**d(H···A)**	**d(D···A)**	**< (D-H···A)**
O(1)-H(1B)···N(1)#1	0.84	1.86	2.607(2)	146.7
C(41)-H(41A)···O(1)#2	0.99	2.47	3.407(2)	158.0
Symmetry transformations used to generate equivalent atoms: #1 x,y,z #2 -x+2,y+1/2,-z+1/2				
π-π Interactions				
Centroid atom (Cg)	Centroid atom (Cg)	Distance between centroid atoms (Å)	Interplanar angle (°)	
Cg2	Cg1' #1	4.5080(9)	2.69(4)	
Cg2	Cg3' #1	3.8145(9)	4.37(4)	
Cg1	Cg2" #2	4.5080(9)	2.69(4)	
Cg3	Cg2" #2	3.8145(9)	4.37(4)	
Symmetry transformations used to generate equivalent atoms: #1 -1+x, y, z; #2 1+x, y, z Cg1 = centroid atom of N2, C24, C25, C32, C31; Cg2 = centroid atom of C11, C12, C13, C14, C15, C16; Cg3 = C21, C22, C23, C24, C25, C26				
C-H···π Interaction				
C-H···Cg	**Centroid atom (Cg)**	**d(H···Cg) (Å)**	**d(C···Cg) (Å)**	**< (C-H···Cg) (°)**
C16-H16	Cg4' #1	2.682(2)	3.579(2)	157.6(1)
C36-H36	Cg4" #2	2.870(2)	3.751(2)	154.8(1)
C42-H42B	Cg2' #1	2.824(1)	3.625(2)	139.4(1)
Symmetry transformations:				

#1 2-x, 0.5+y, 0.5-z; #2 0.5+x, 1.5-y, 1-z
Cg2 = centroid atom of C11, C12, C13, C14, C15, C16; Cg4 = centroid atom of C31, C32, C33, C34, C35, C36

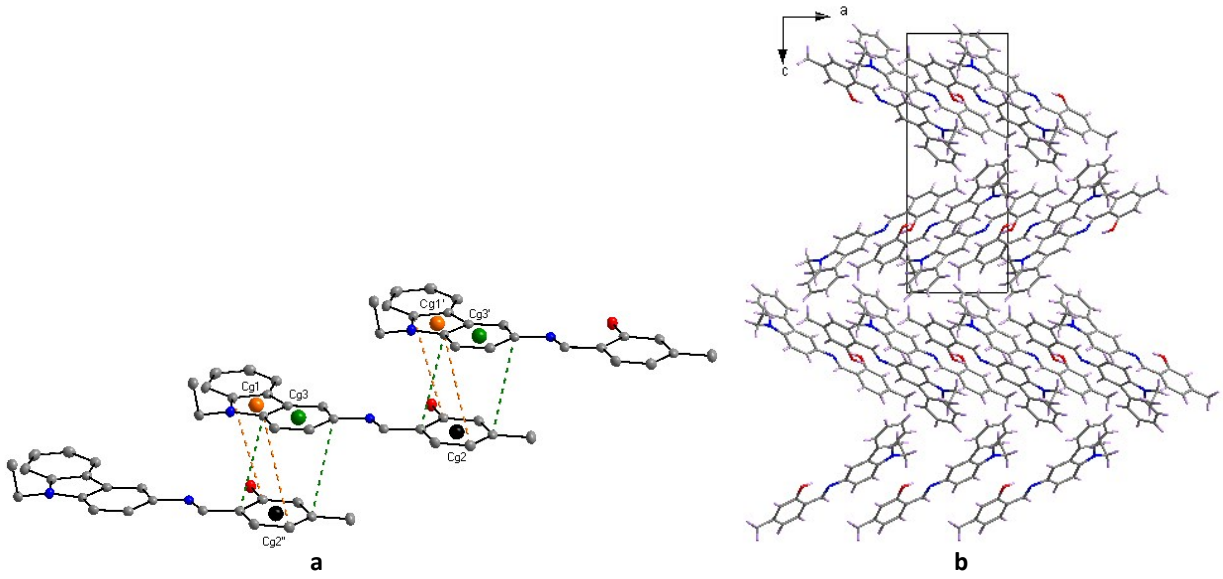


Fig. S3. (a) Graphical representation of π-π interactions found in Compound **2** and **(b)** packing of molecules on Compound **2** in the unit cell as viewed along the *b*-axis.

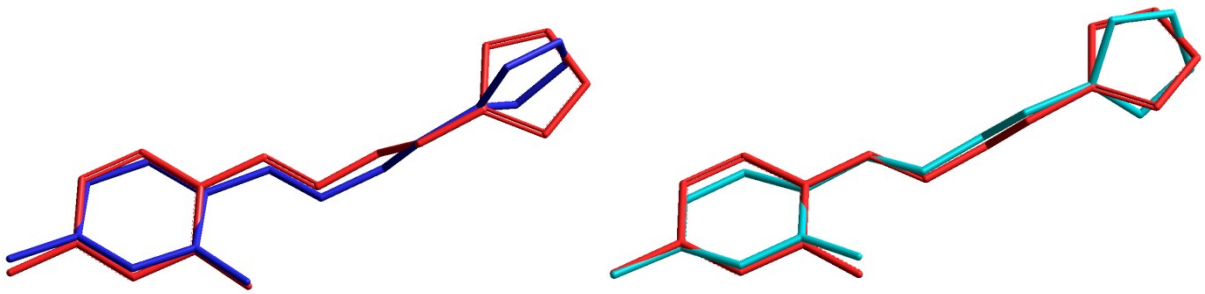
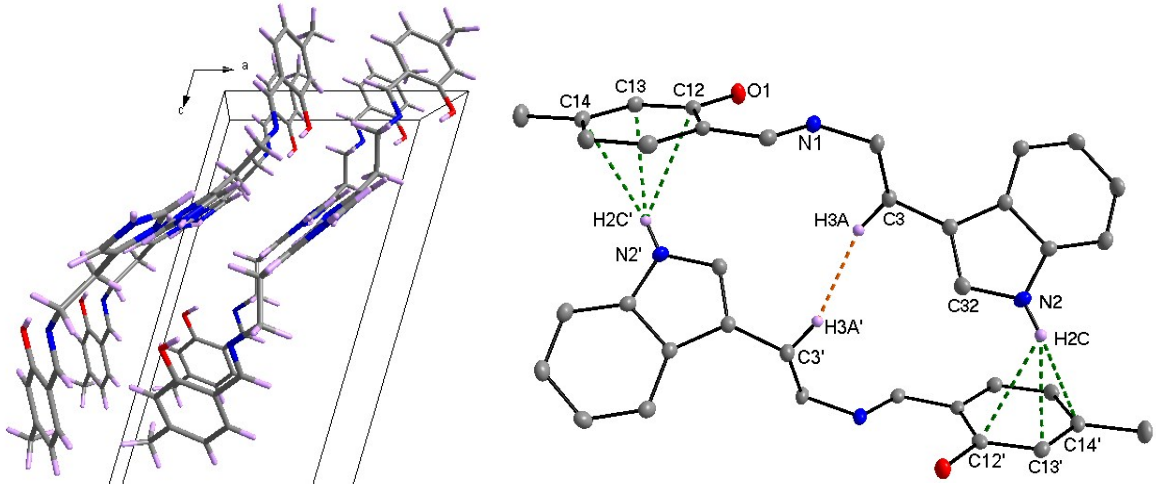


Fig. S4. Graphical representation of the overlay of the DFT optimized structure **3*** (red) with molecule 1 of Compound **3** (blue) (RMS value = 0.651 Å) and molecule 2 of Compound **3** (cyan) (RMS value = 0.495 Å). Overlay fit excludes all hydrogen atoms.

Fig. S5. Graphical representation of the overlay of the DFT optimized structure **4*** (red) with Compound **4** (blue) (RMS value = 0.419 Å). Overlay fit excludes all hydrogen atoms.



a **b**

Fig. S6. (a) Graphical representation of infinite one dimensional chains formed by the generation of symmetry related molecules along the [010] vector for Compound **3**. **(b)** Graphical representation of N-H…π interaction (green line) and C-H…H-C interaction (orange line) between related molecules of Compound **4**. Certain H atoms are omitted for clarity.

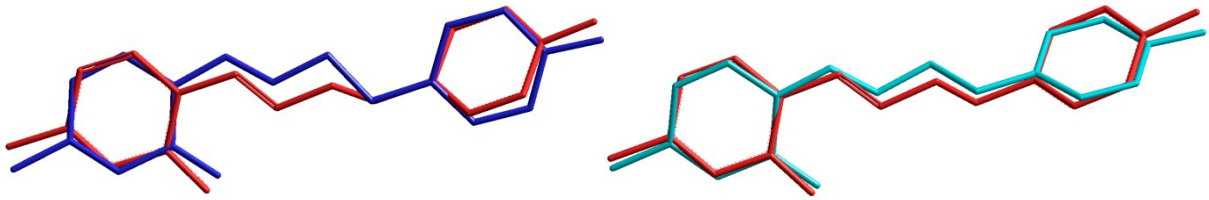


Fig. S7. Graphical representation of the overlay of the DFT optimized structure **5*** (red) with molecule 1 of Compound **5** (blue) (RMS value = 0.592 Å) and molecule 2 of Compound **5** (cyan) (RMS value = 0.521 Å). Overlay fit excludes all hydrogen atoms.

Table S3. Intra and intermolecular hydrogen bonds and C-H···π interactions for Compound **3** [Å and °].

Intra and Intermolecular hydrogen bonds				
D-H···A	**d(D-H)**	**d(H···A)**	**d(D···A)**	**< (D-H···A)**
O(1)-H(1B)···N(1)#1	0.84	1.86	2.602(2)	147.2
O(2)-H(2B)···N(2)#1	0.84	1.87	2.613(2)	147.3
N(34)-H(34)···N(32)#2	0.88	2.04	2.896(2)	163.3
N(64)-H(64)···N(62)#3	0.88	1.99	2.860(2)	167.8
Symmetry transformations used to generate equivalent atoms: #1 x,y,z #2 -x,y+1/2,-z+1/2 #3 -x+1,y+1/2,-z+1/2				
C-H···π Interaction				
C-H···Cg	**Centroid atom (Cg)**	**d(H···Cg)**	**d(C···Cg)**	**< (C-H···Cg)**
C2-H2D	Cg3#1	2.8417(1)	3.751(2)	153.2(9)
C15-H15	Cg1#2	2.9093(1)	3.707(1)	142.3(8)
C13-H13	Cg4#3	2.9067(1)	3.320(2)	107.6(9)
C2-H2C	Cg4#4	2.9417(1)	3.548(2)	120.5(1)
Symmetry transformations: #1 x, y, z; #2 –x, 1-y, -z; #3 1-x, -y, -z; #4 1-x, 1-y, -z Cg1 = centroid atom of C31, N32, C33, N34, C35; Cg2 = centroid atom of C11, C12, C13, C14, C15, C16 Cg3 = centroid atom of C61, N62, C63, N64, C65; Cg4 = centroid atom of C41, C42, C43, C44, C45, C46				

Table S4. Intra and intermolecular hydrogen bonds and C-H···π interactions for Compound **4** [Å and °].

Intra and Intermolecular hydrogen bonds				
D-H···A	**d(D-H)**	**d(H···A)**	**d(D···A)**	**< (D-H···A)**
O1-H1B···N1#1	0.84	1.83	2.576(1)	147.5
N2-H2C···C12#2	0.90(2)	2.890(1)	3.425(2)	120.8(8)
N2-H2C···C13#2	0.90(2)	2.650(1)	3.423(2)	147.2(8)
N2-H2C···C14#2	0.90(2)	2.801(1)	3.502(2)	137.7(8)
C-H···H-C	d(C-H)	d(H···H)	d(C···H)	< (D-H···H)
C3-H3A...H3A#2	0.99	2.308(1)	3.223(1)	153.3(7)
Symmetry transformations used to generate equivalent atoms: #1 x,y,z; #2 –x, -y, 1-z				
C-H···π Interaction				
C-H···Cg	**Centroid atom (Cg)**	**d(H···Cg)**	**d(C···Cg)**	**< (C-H···Cg)**
C1-H1A	Cg3' #1	2.8529(1)	3.569(1)	133.0(8)
C13-H13	Cg1 #2	2.9570(1)	3.792(1)	147.4(8)
C141-H14A	Cg3''' #3	2.9299(1)	3.529(2)	120.5(8)
C36-H36	Cg2 #4	2.8162(1)	3.483(1)	128.1(1)
Symmetry transformations: #1 –x, 1-y, 1-z; #2 1-x, -y, 1-z; #3 1-x, -y, 1-z ; #4 1-x, 1-y, 1-z Cg1 = centroid atom of C31, C32, N2, C33, C34; Cg2 = centroid atom of C11, C12, C13, C14, C15, C16 Cg3 = centroid atom of C33, C34, C35, C36, C37, C38				

Additional discussion on the hydrogen bonding of Compound 5 not mentioned in manuscript text.

Numerous intermolecular hydrogen bonding is observed by Compound **5** as experienced by the two molecules in the asymmetric unit primarily between O-H···N and C-H···O atoms as listed in Table S5. In addition, only one π-π interaction occurs between aromatic ring C1 (Cg1 = centroid atom of ring C11, C12, C13, C14, C15, C16) and aromatic ring of the tyramine substituent (Cg4' = centroid atom of ring C61, C62, C63, C64, C64, C65, C66; symmetry operation: 1+x, -1+y, -1+z) with a centroid to centroid distance of 4.002(1) Å and an interplanar angle of 10.76(5)°. The resultant packing of the unit cell forms rectangular tunnels when viewed along the *c*-axis.

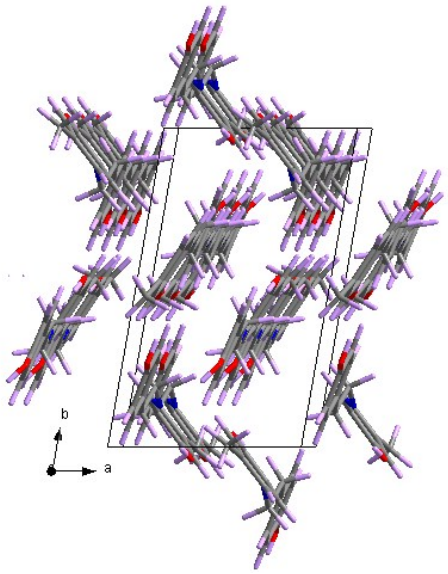


Figure S8. Rectangular packing of Compound **5** molecules in the unit cell as viewed along the *c*-axis.

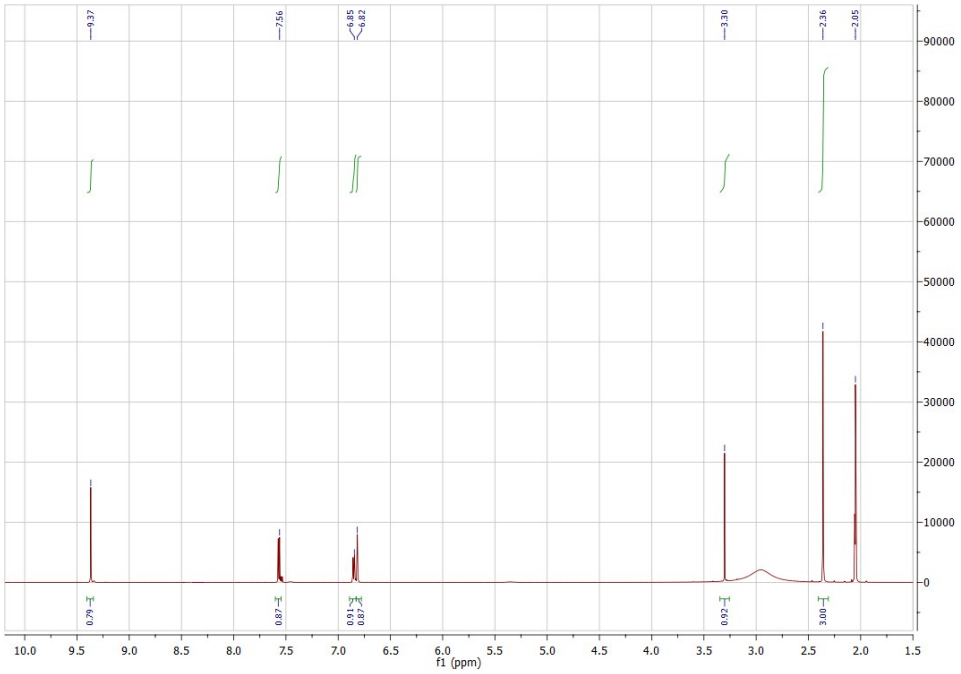
Table S5. Intra- and inter-molecular hydrogen bonds for Compound **5** [Å and °].

Intra and Intermolecular hydrogen bonds				
D-H···A	**d(D-H)**	**d(H···A)**	**d(D···A)**	**< (D-H···A)**
O(1)-H(1A)···N(1)#1	0.84	1.81	2.566(2)	149.1
O(2)-H(2)···N(2)#1	0.84	1.88	2.627(2)	146.7
O(3)-H(3)···O(2)#2	0.84	1.97	2.611(2)	132.4
O(6)-H(6C)···O(1)#3	0.84	1.74	2.578(2)	174.2
C(15)-H(15)···O(3)#4	0.95	2.44	3.387(2)	178.0
C(35)-H(35)···O(2)#5	0.95	2.60	3.514(2)	162.3
C(45)-H(45)···O(6)#4	0.95	2.53	3.408(2)	154.3
Symmetry transformations used to generate equivalent atoms: #1 x,y,z #2 -x+1,-y+1,-z+1 #3 -x+1,-y+2,-z+1 #4 x,y,z-1 #5 x,y-1,z				

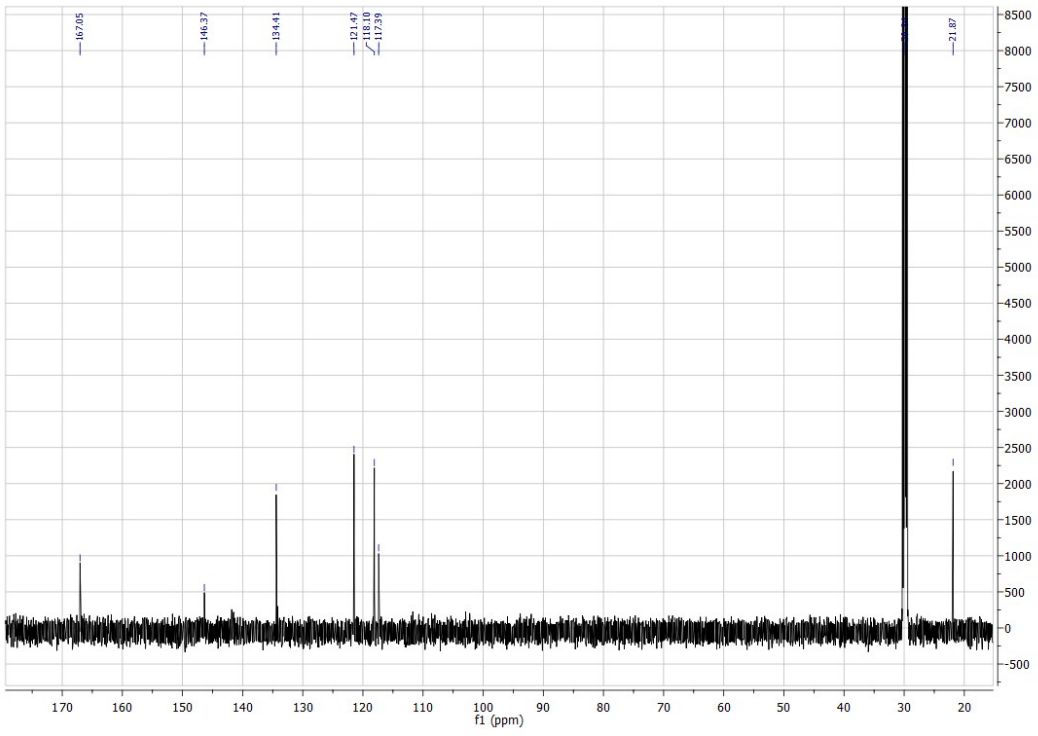
NMR Spectra

5-methyl-2-(1,2,4-triazol-3-yliminomethyl)phenol (1)

^{1}H NMR

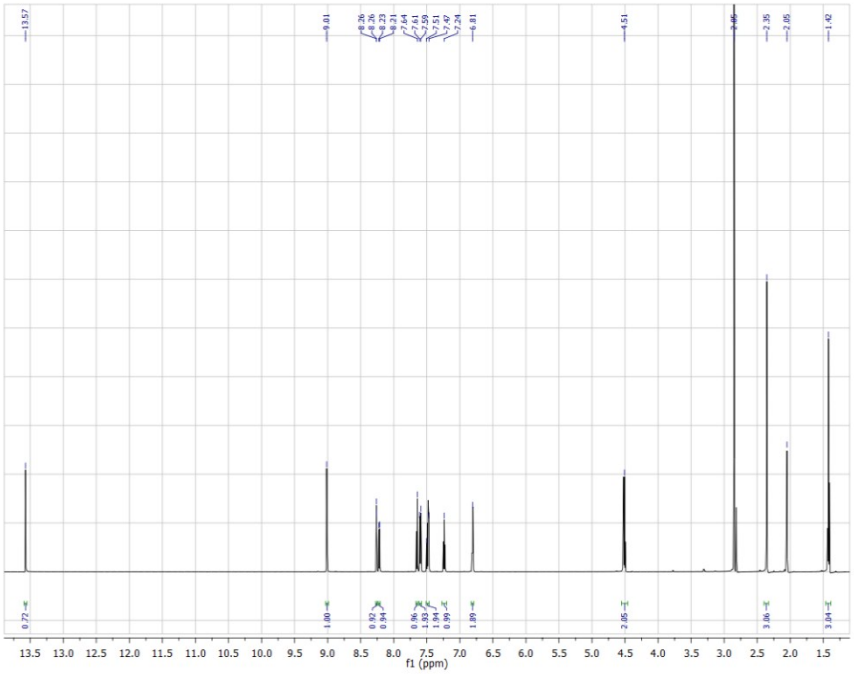


^{13}C NMR

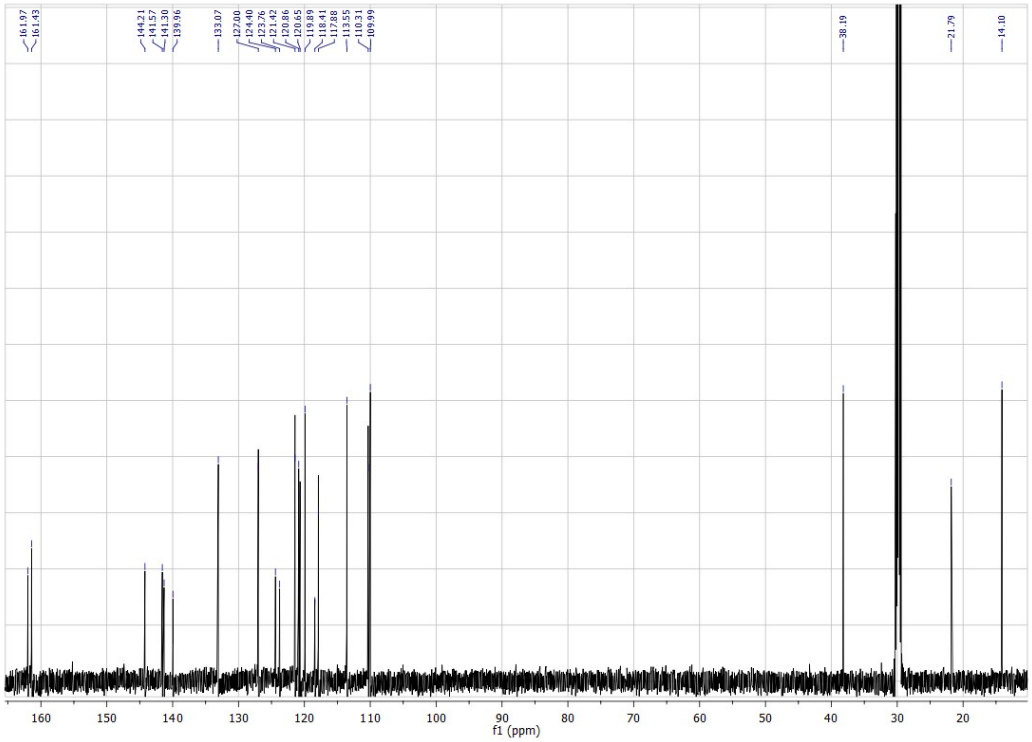


2-(9-ethylcarbazol-3-yliminomethyl)-5-methylphenol (2)

^{1}H NMR

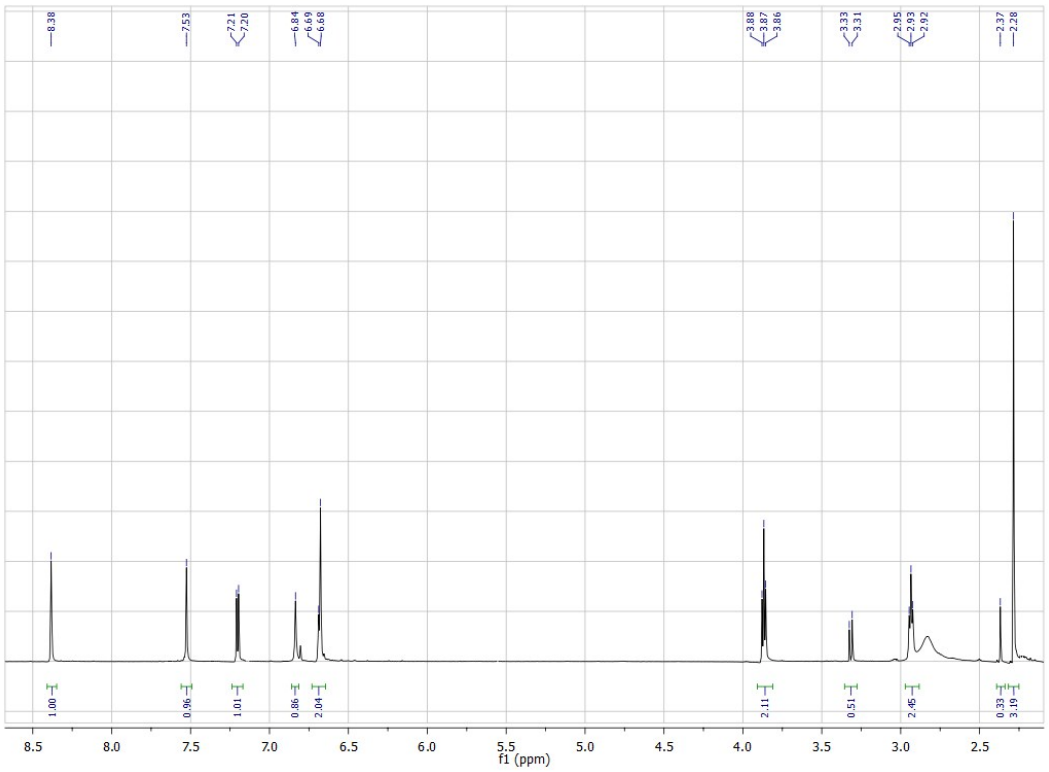


^{13}C NMR

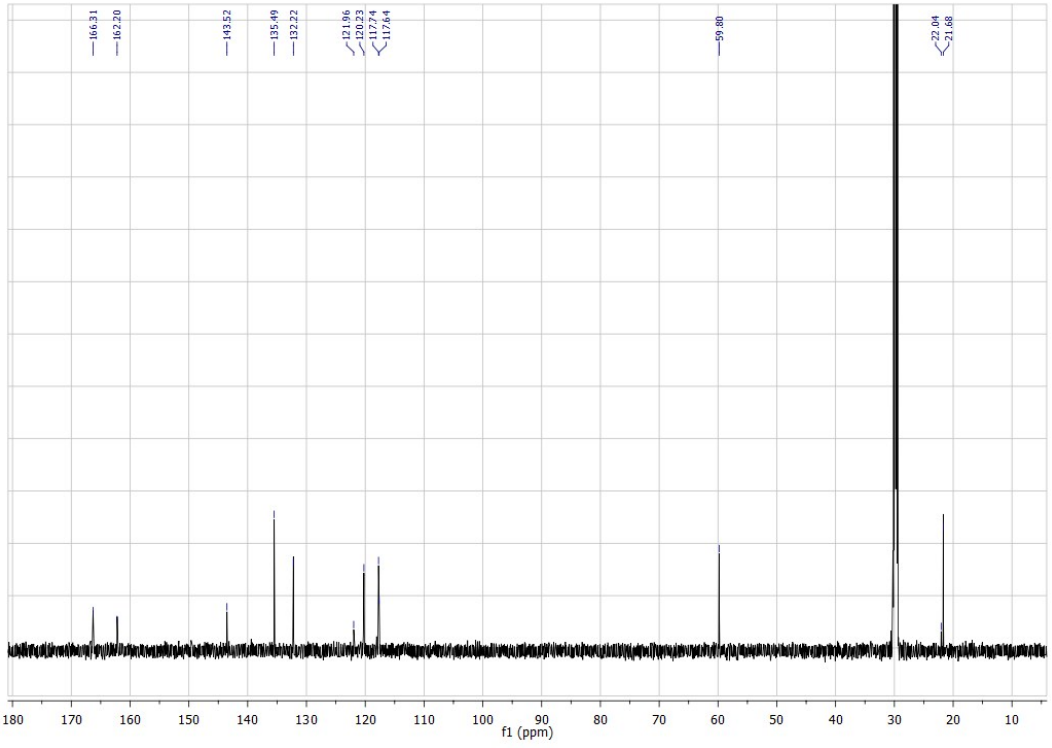


2-[(2-imidazol-4-yl)ethyliminomethyl]-5-methylphenol (3)

^{1}H NMR

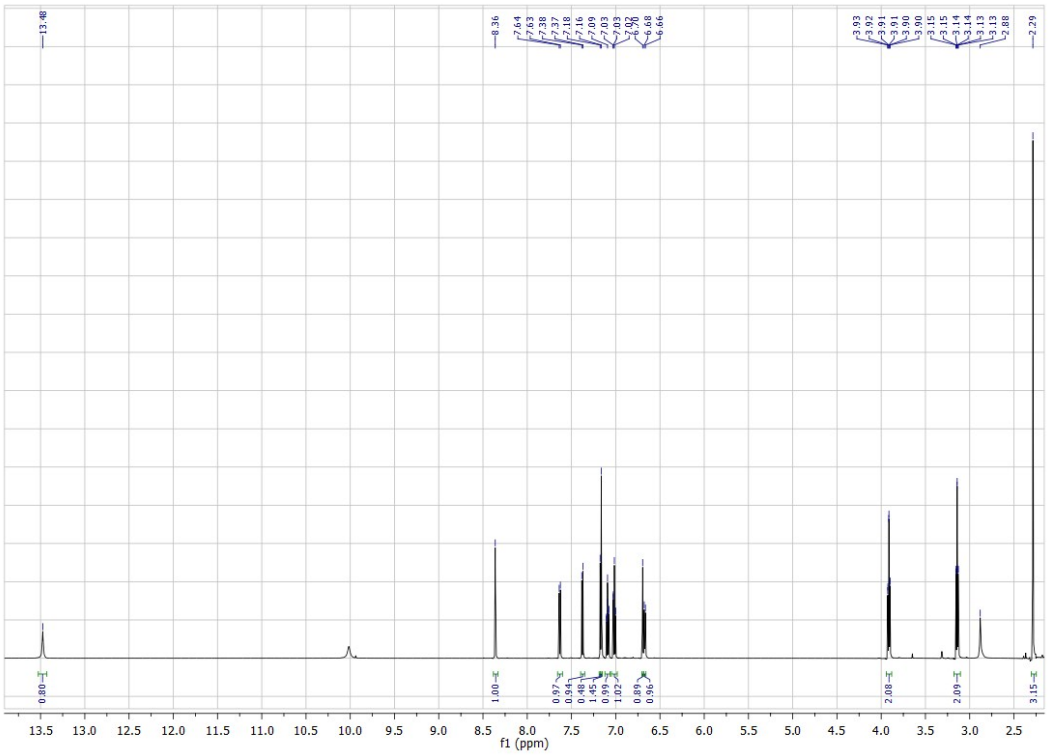


^{13}C NMR

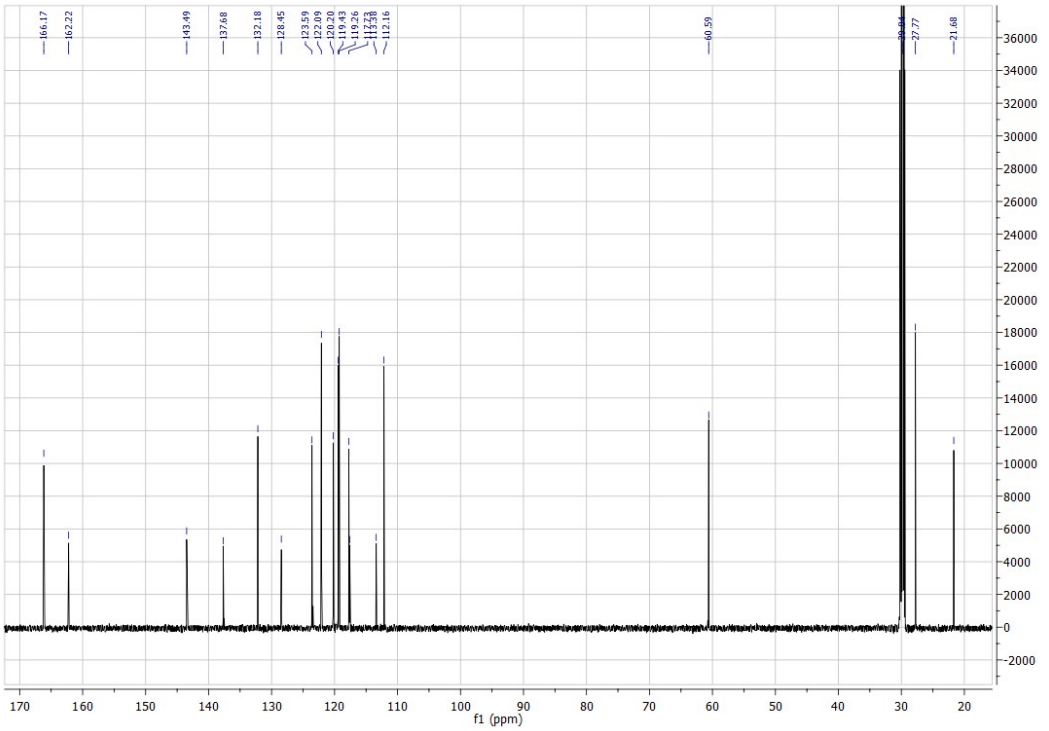


2-[(2-indol-3-yl-ethyl)iminomethyl]-5-methylphenol (4)

^{1}H NMR

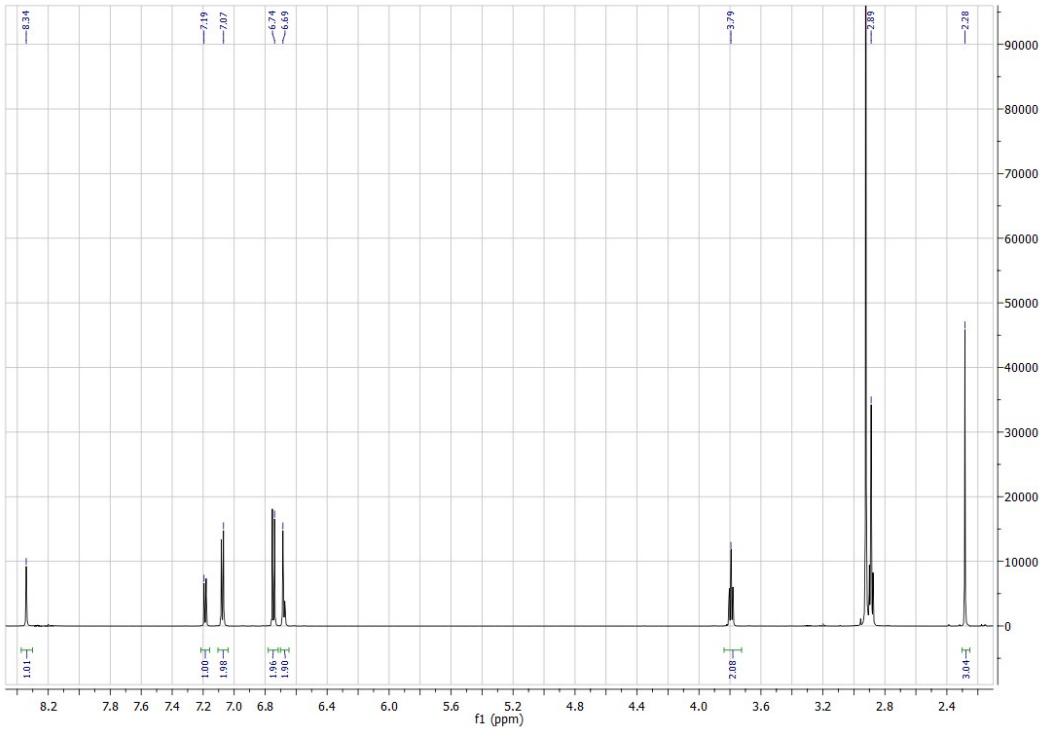


^{13}C NMR

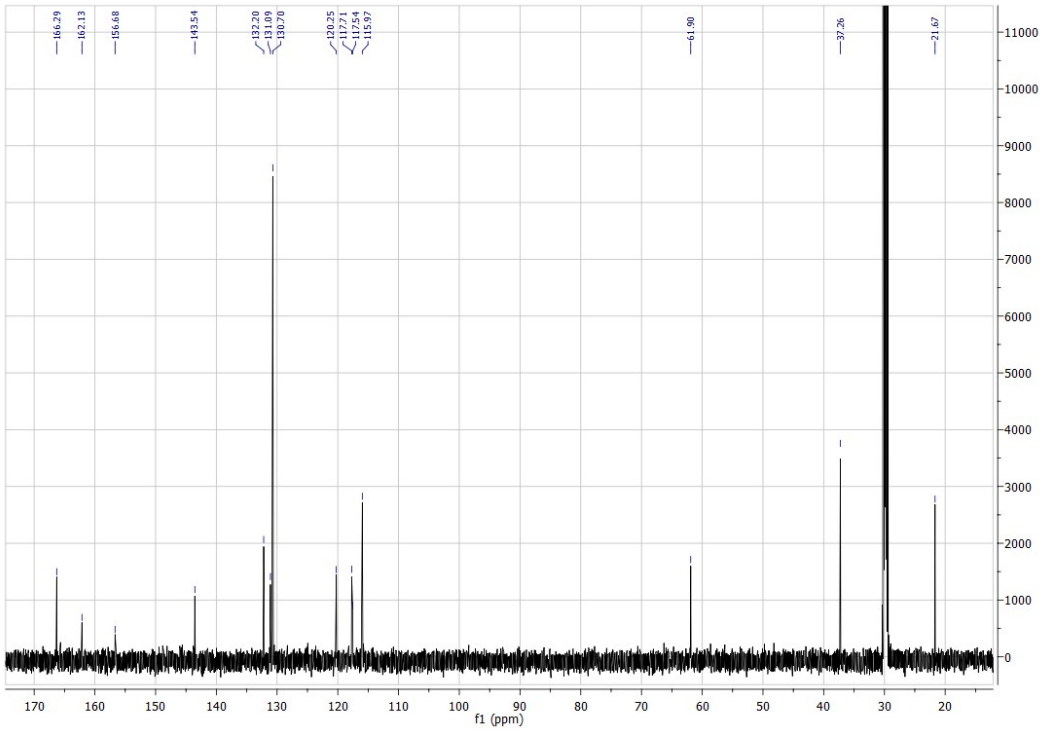


2-[2-(4-hydroxyphenyl)ethyliminomethyl]-5-methylphenol (5)

^{1}H NMR



^{13}C NMR



Notes and References

[1] N. Kolesnikova, H. Hoppe, CSIR Biosciences Pharmacology Group, *In Vitro Cancer Screening Report*, South Africa, reported 2011-01-17

[2] N. Kolesnikova, D. Koot, H. Hoppe, CSIR Biosciences Pharmacology Group, *In Vitro Cancer Screening Report*, South Africa, reported 2011-07-19