

Supplementary Material

Fluorochrome selection for imaging intraoperative ovarian cancer probes

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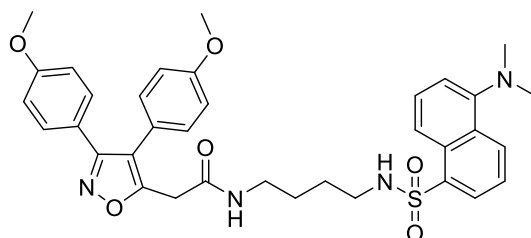
* Correspondence: antonio.scilimati@uniba.it (A.S.)

Content:

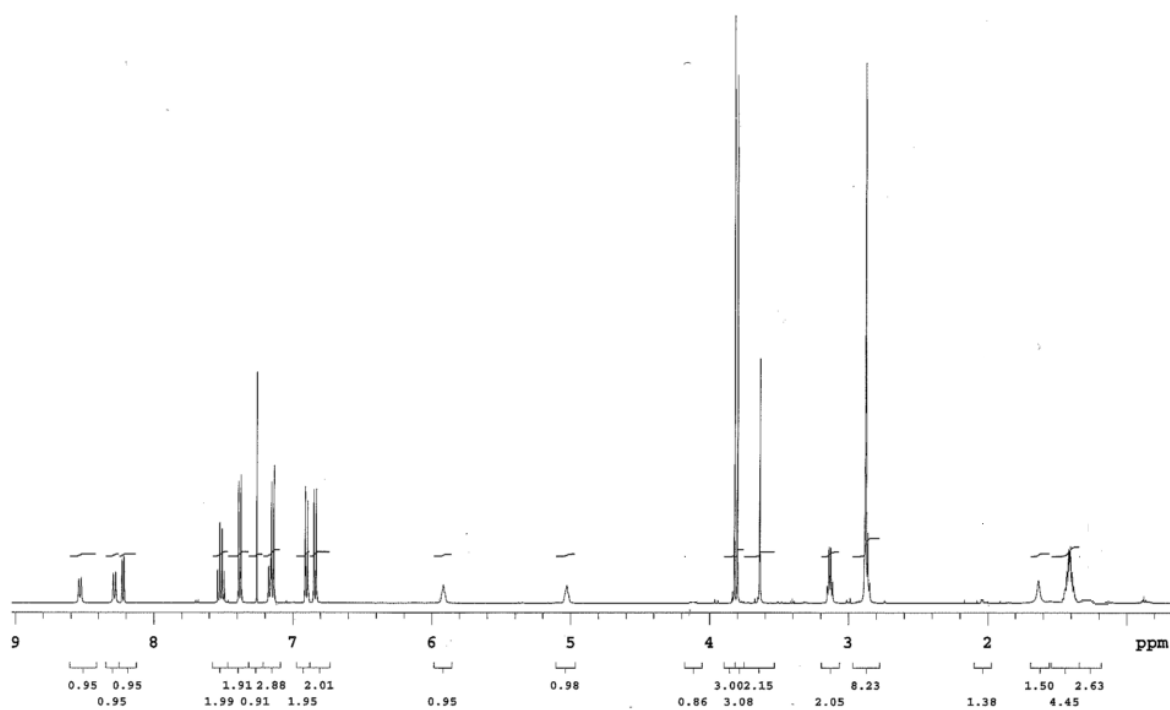
1. ¹H NMR and ¹³C NMR of the target compounds.
2. HPLC chromatograms of RR11 and 23 (MSA14).
3. FLAP S1-S7 figures of selected target compounds.

1. ^1H NMR and ^{13}C NMR of the target compounds.

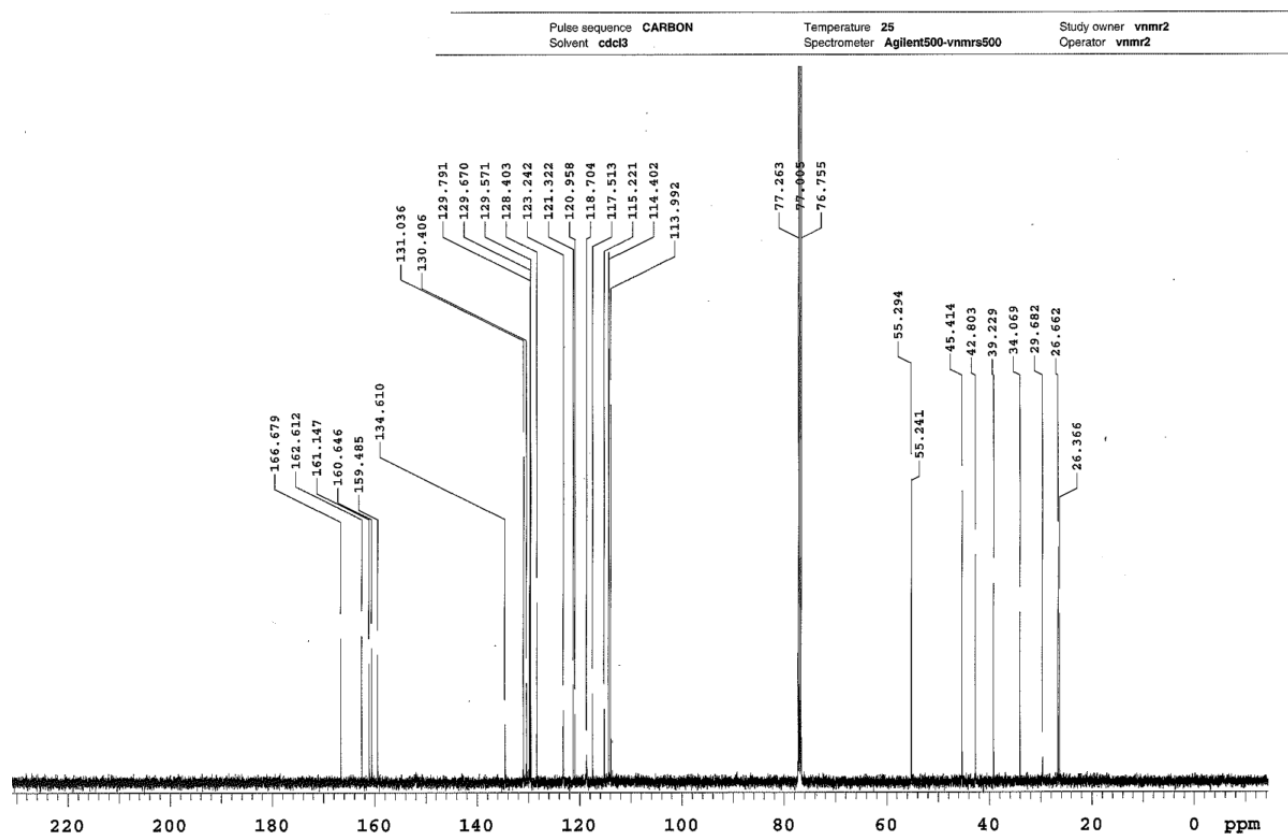
2-[3,4-Bis(4-methoxyphenyl)isoxazol-5-yl]-N-{4-[(5-dimethylaminonaphthalene)-1-sulfonamido]butyl}acetamide (3a)



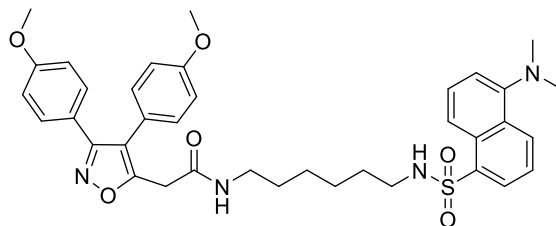
3a ^1H -NMR (500 MHz, CDCl_3)



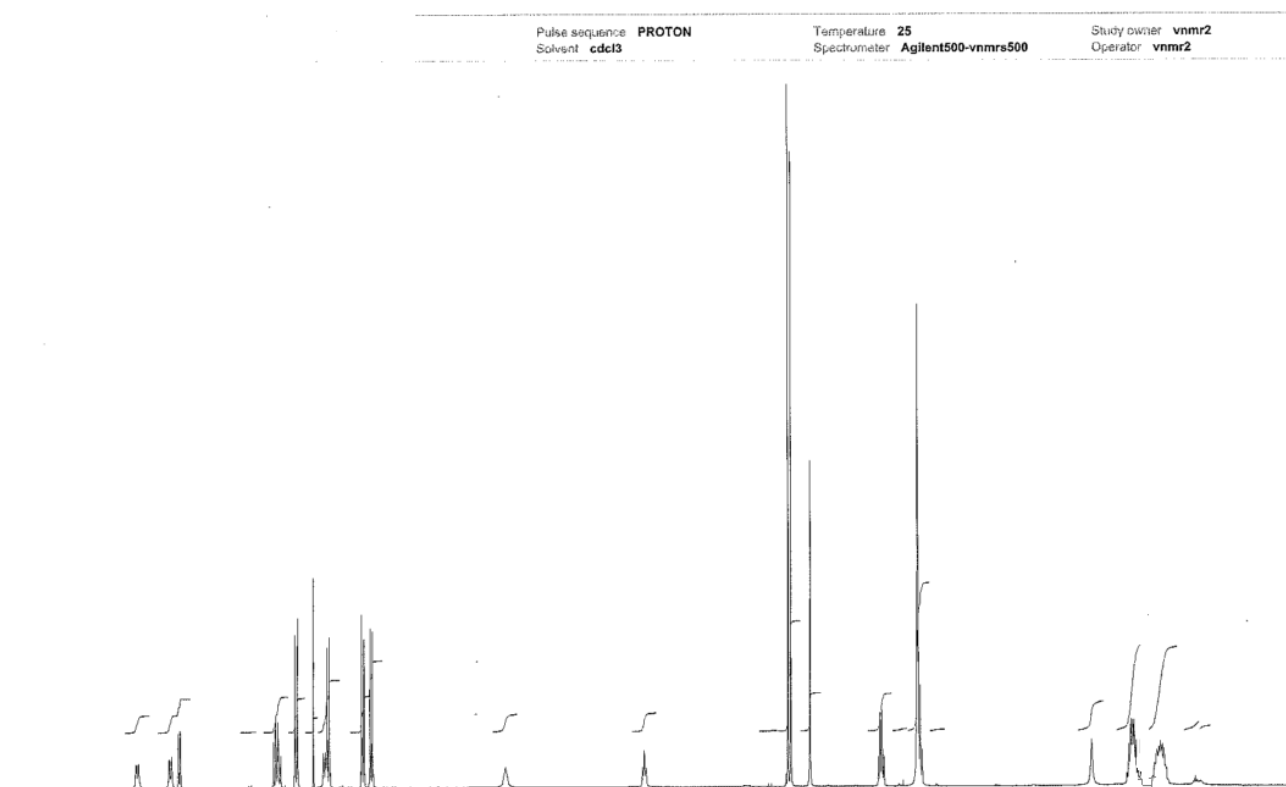
3a ^{13}C -NMR (125 MHz, CDCl_3)



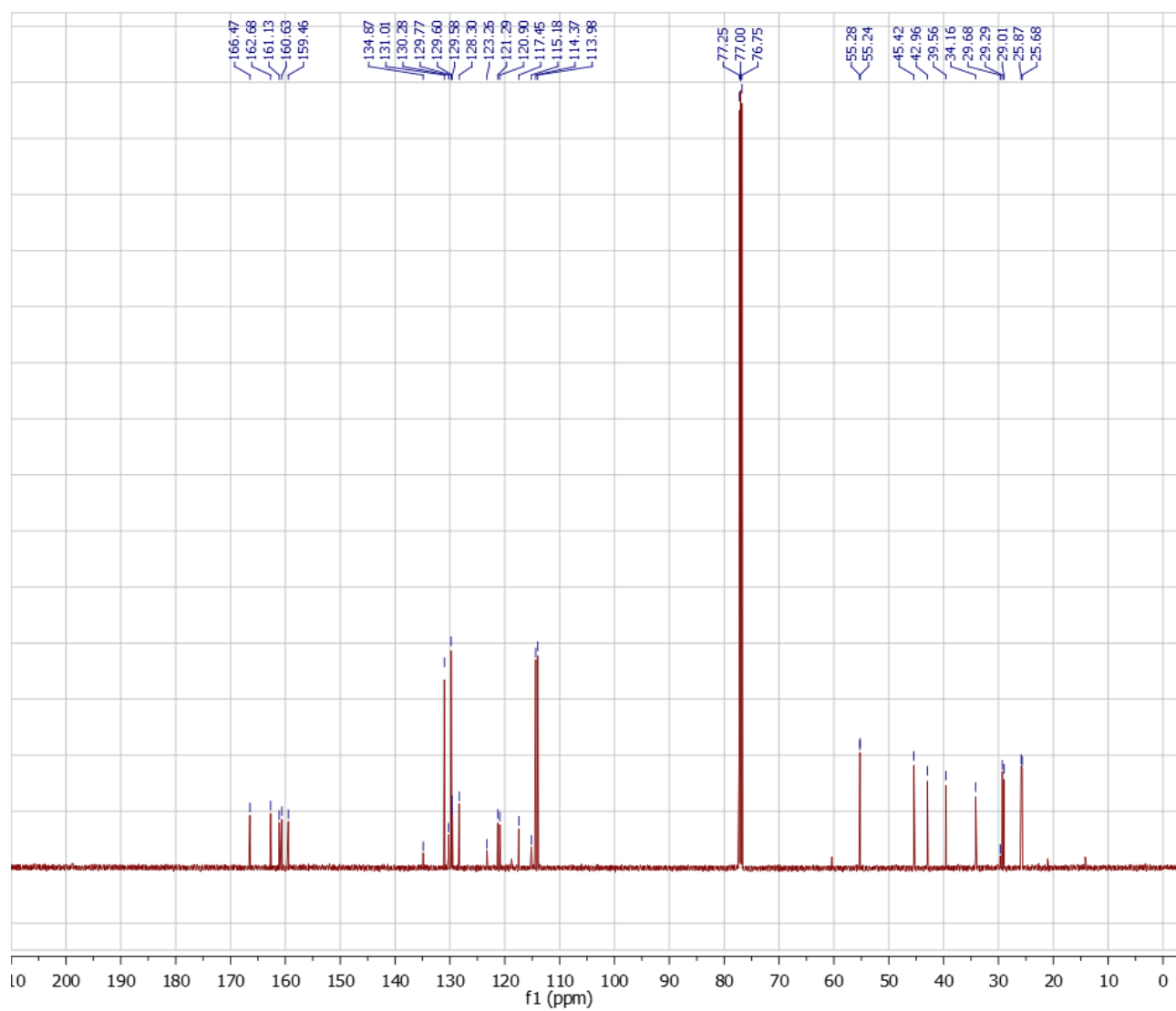
2-[3,4-Bis(4-methoxyphenyl)isoxazol-5-yl]-N-{4-[(5-dimethylaminonaphthalene)-1-sulfonamido]hexyl}-acetamide (6a)



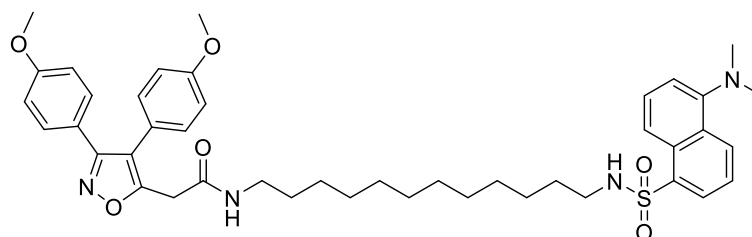
6a ¹H-NMR (500 MHz, CDCl₃)



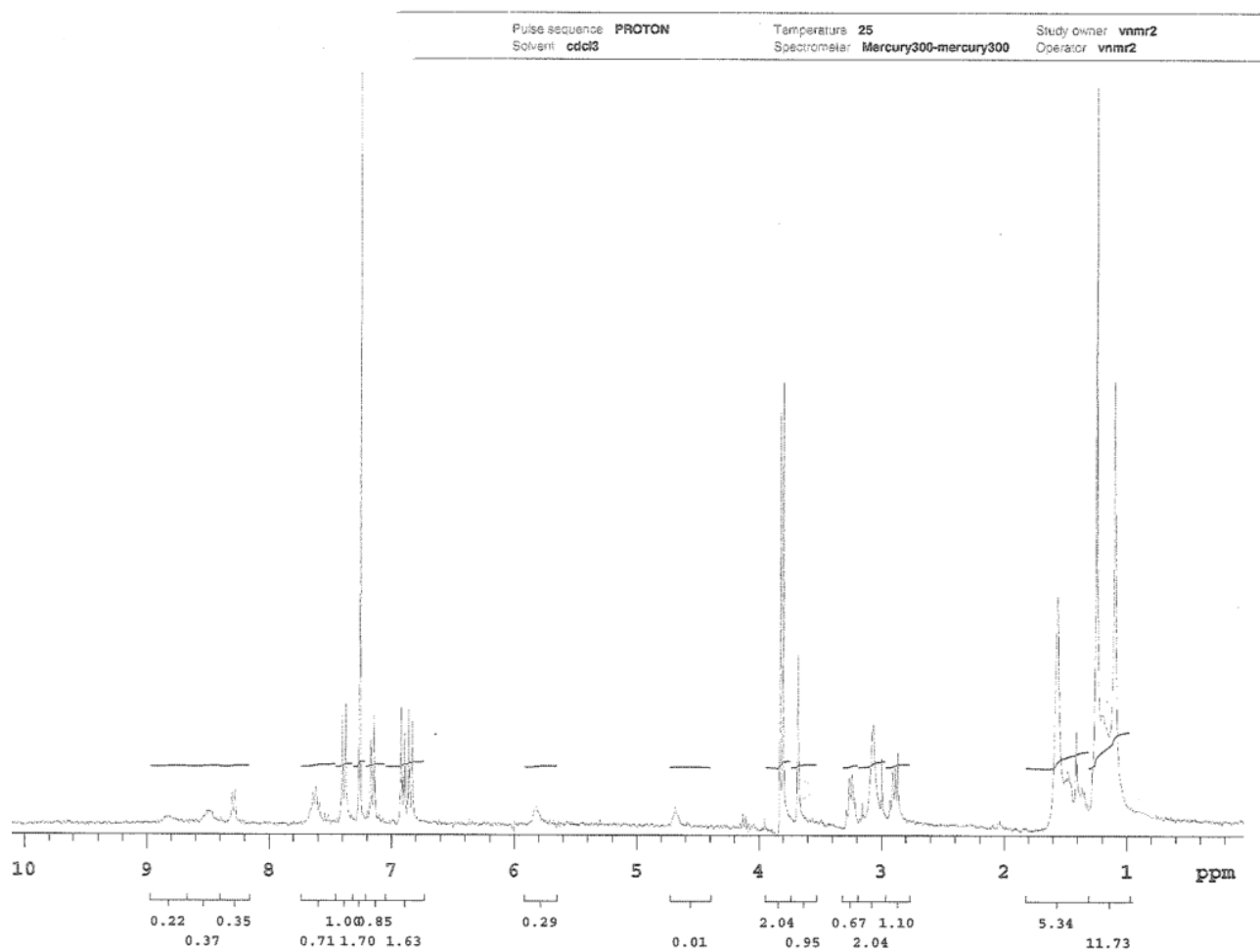
6a ^{13}C -NMR (125 MHz, CDCl_3)



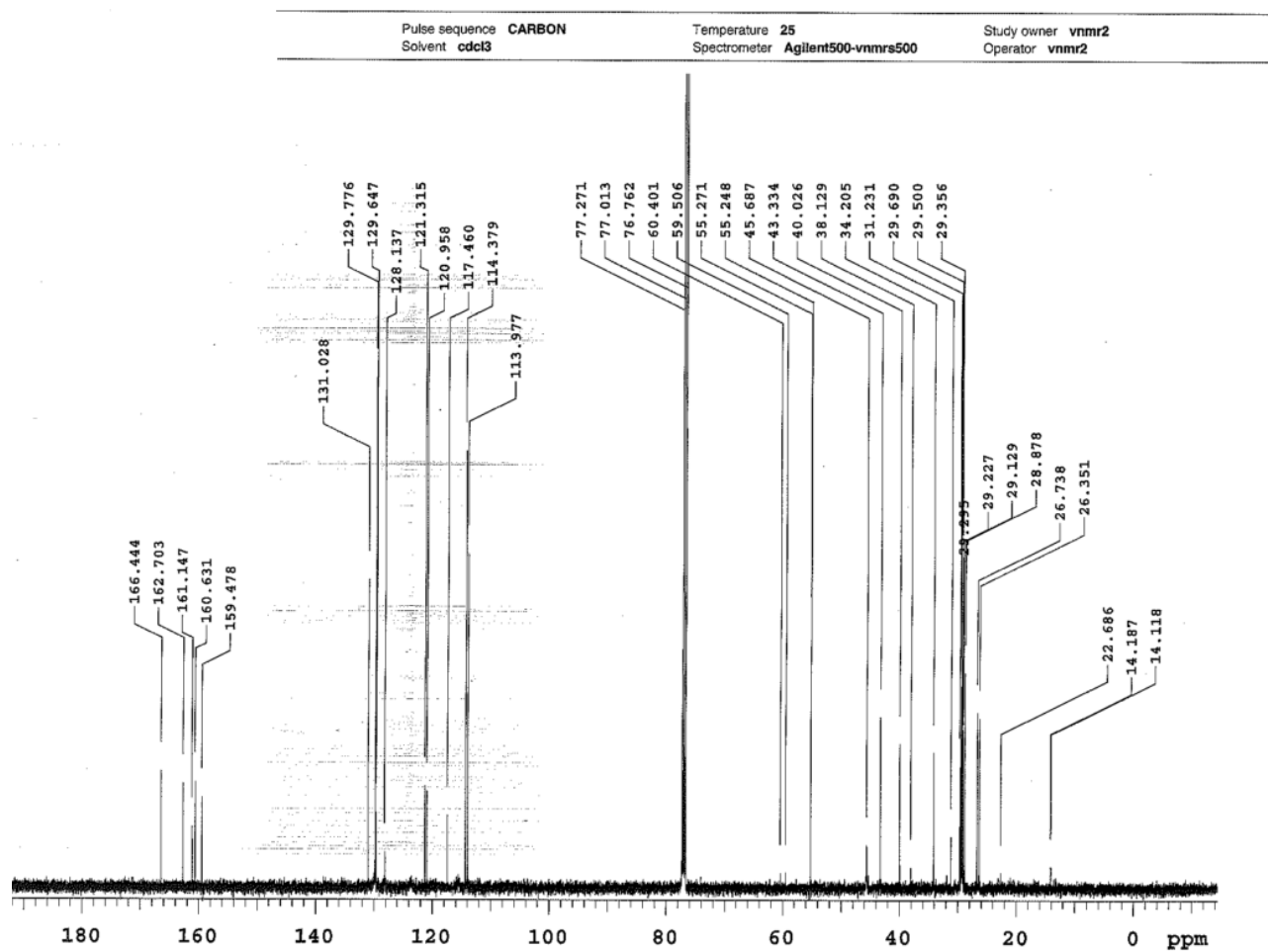
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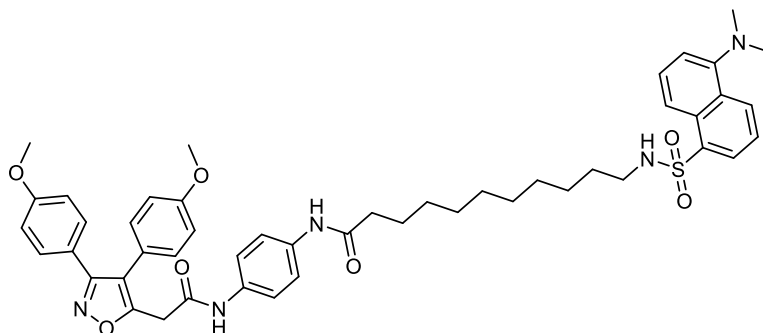
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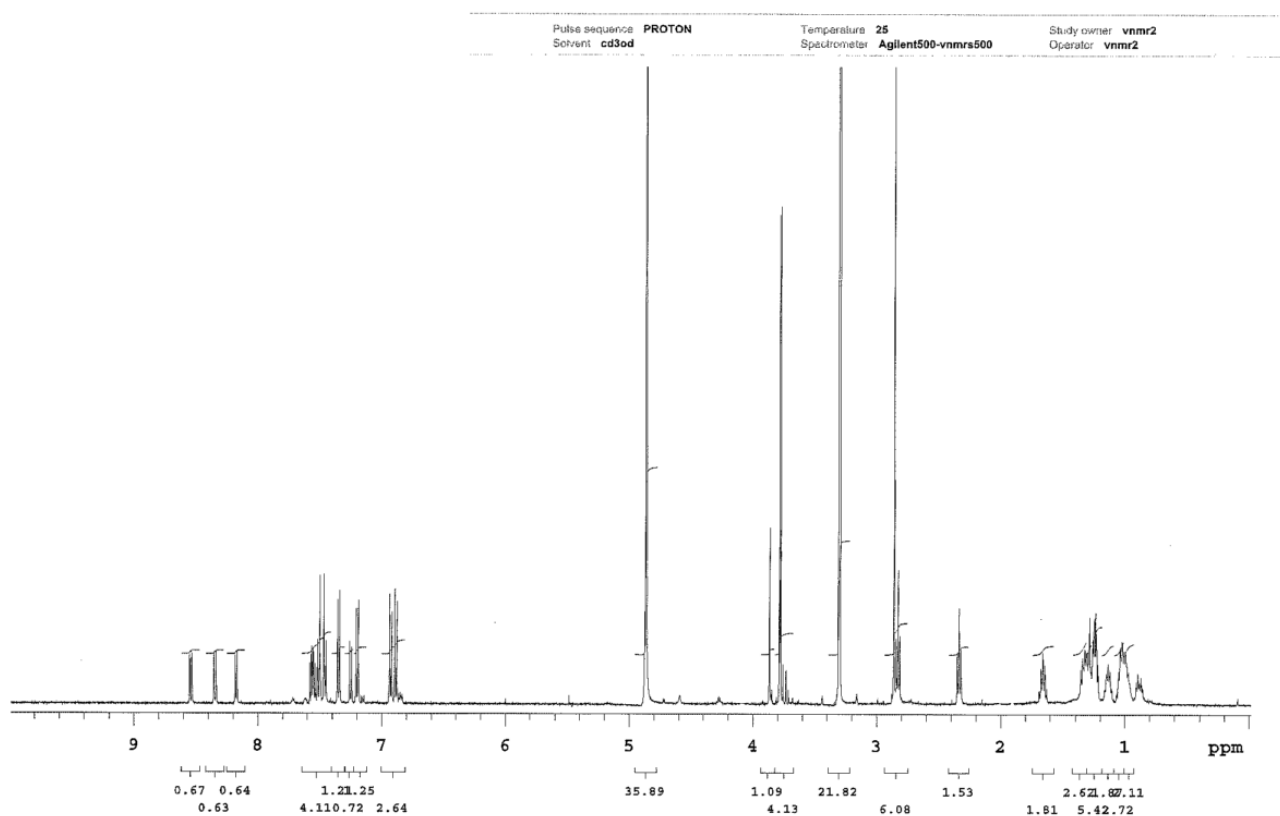
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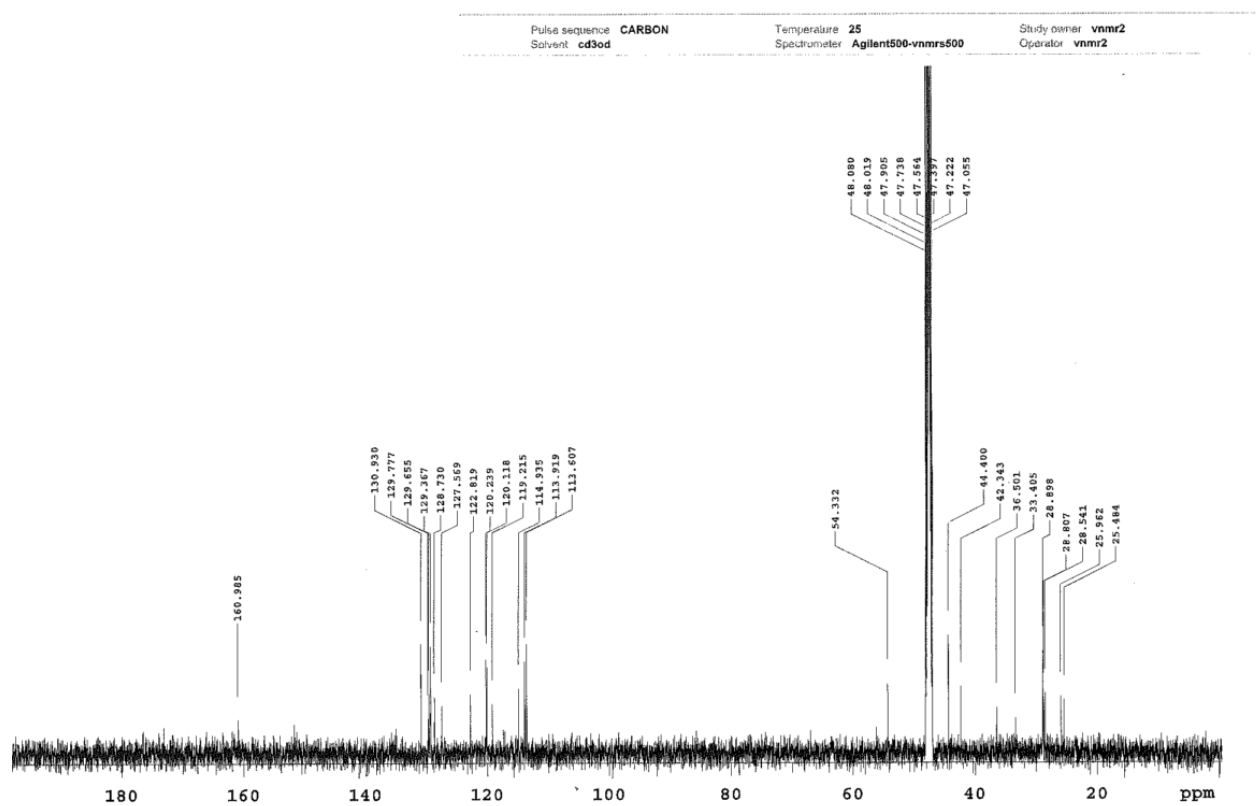
***N*-(4-(2-(3,4-Bis(4-methoxyphenyl)isoxazol-5-yl)acetamido)phenyl)-11-((5-dimethylamino)naphthalene)-1-sulfonamido)undecamide (9)**



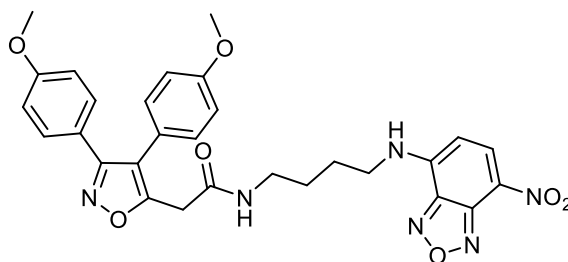
9 ¹H-NMR (500 MHz, CD₃OD)



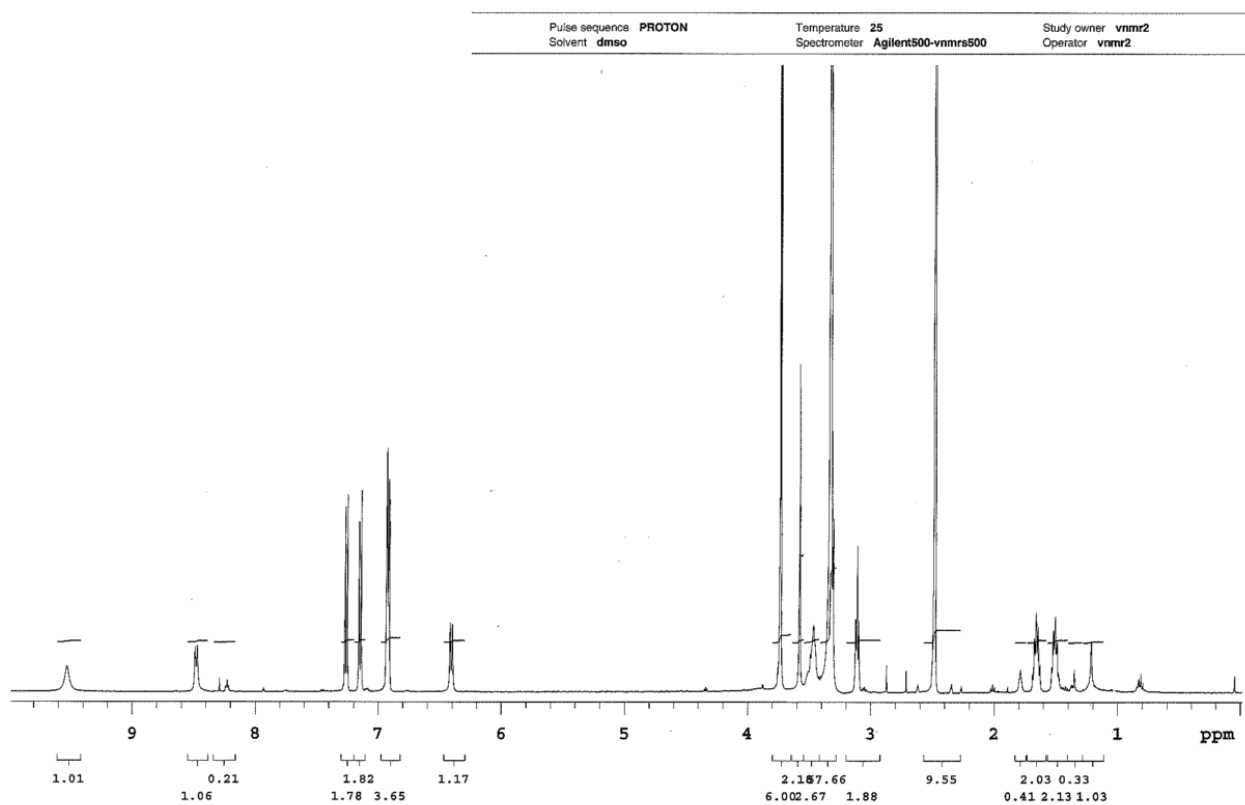
9 ¹³C-NMR (125 MHz, CD₃OD)



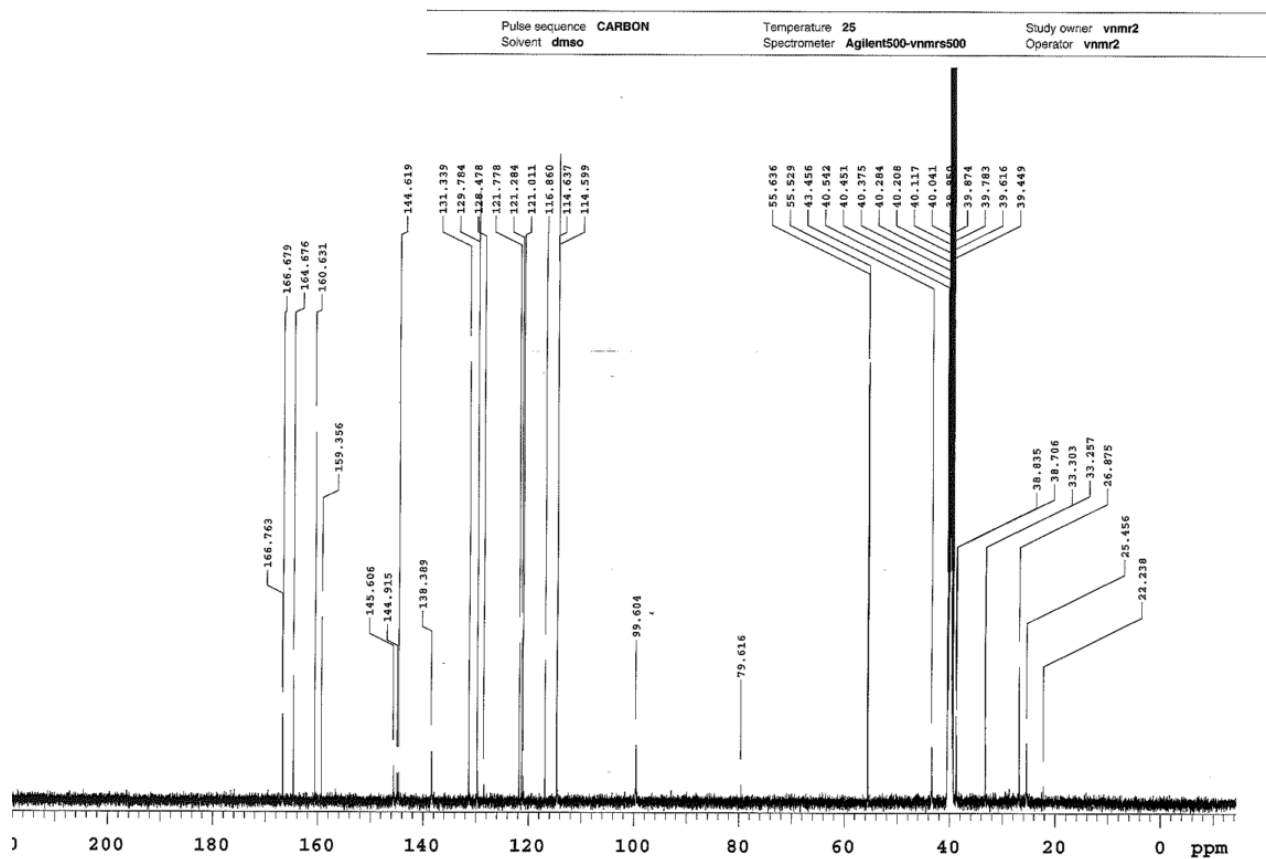
2-(3,4-Bis(4-methoxyphenyl)isoxazol-5-yl)-N-(4-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)butyl)acetamide (13a)



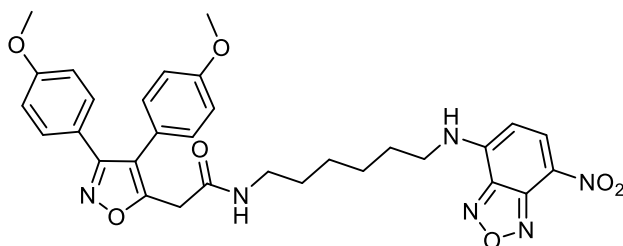
13a ¹H-NMR (500 MHz, DMSO-d₆)



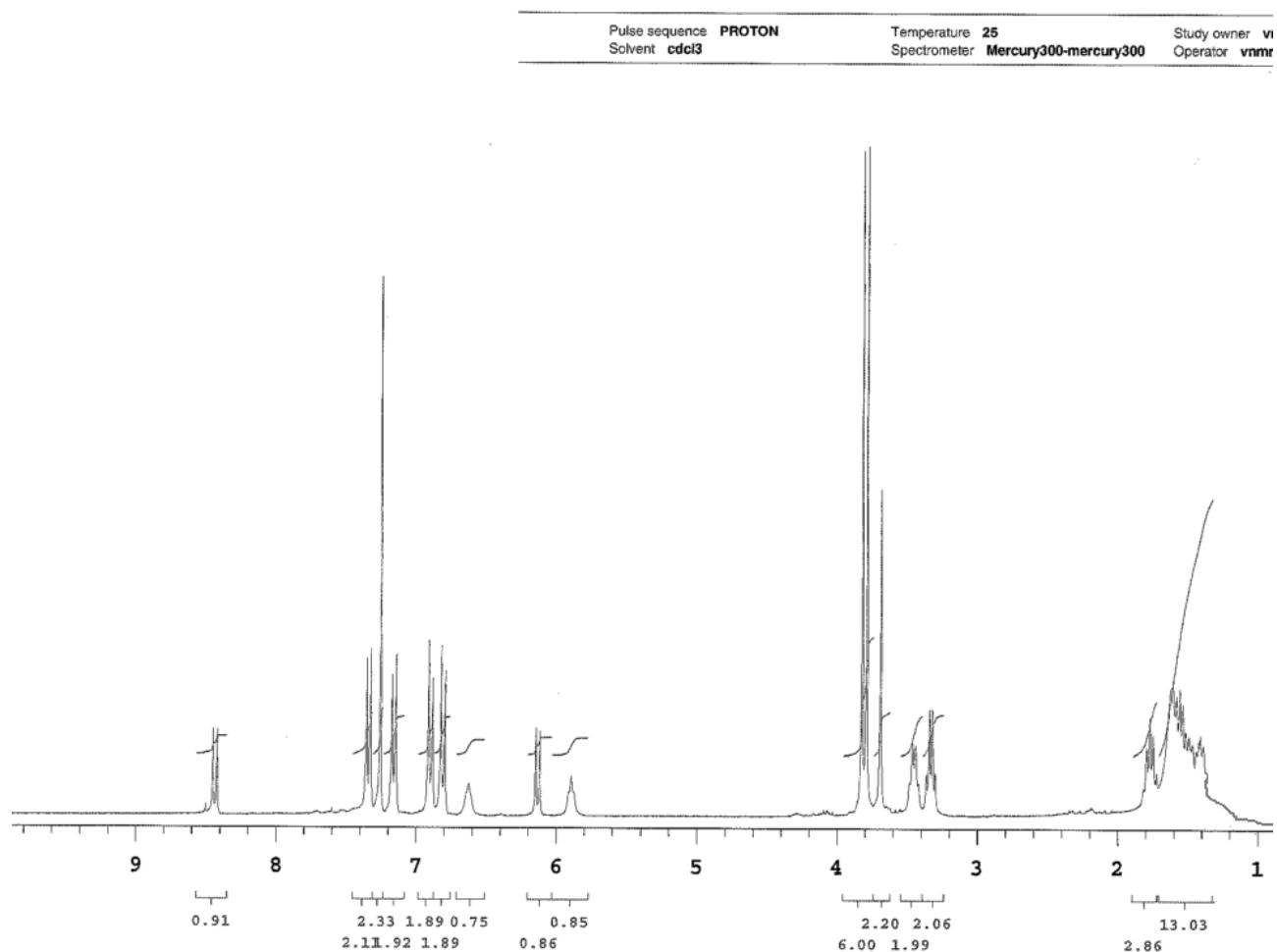
13a ^{13}C -NMR (125 MHz, DMSO- d_6)



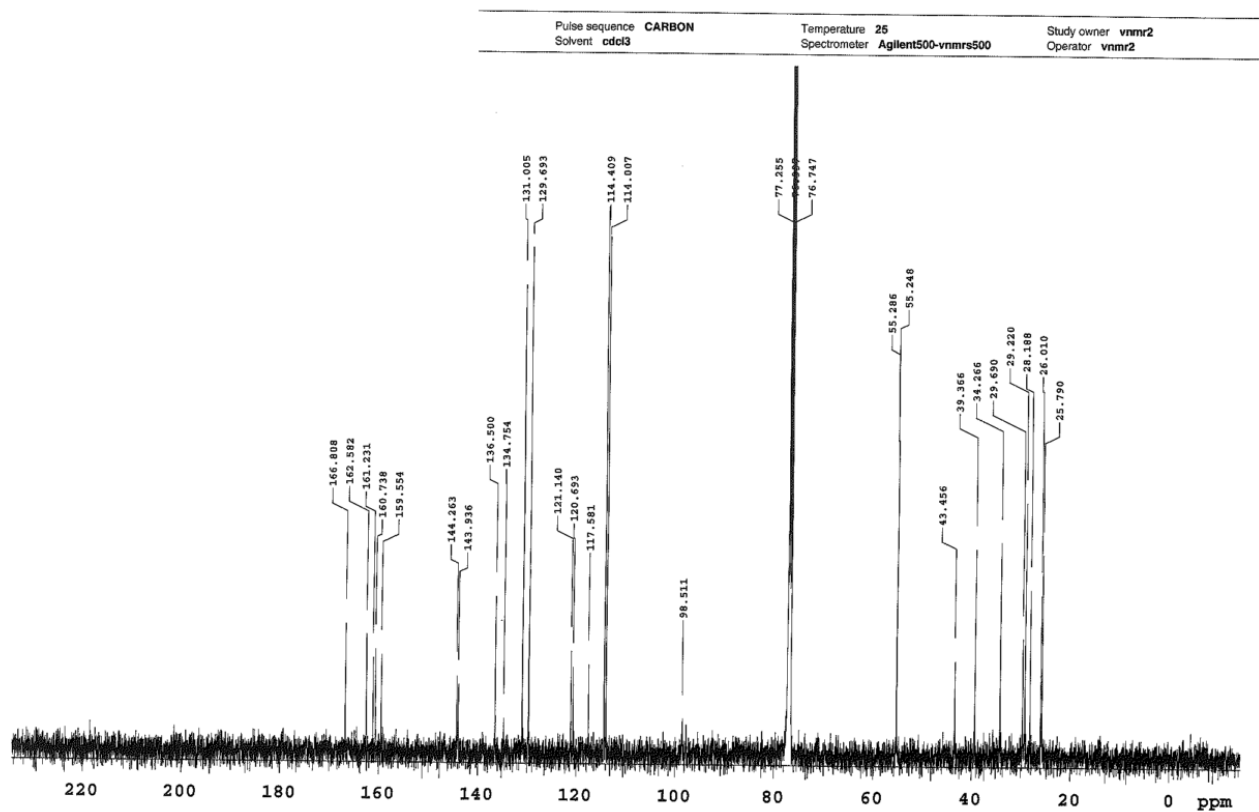
2-(3,4-Bis(4-methoxyphenyl)isoxazol-5-yl)-N-(6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hexyl)acetamide (13b)



13b ¹H-NMR (300 MHz, CDCl₃)



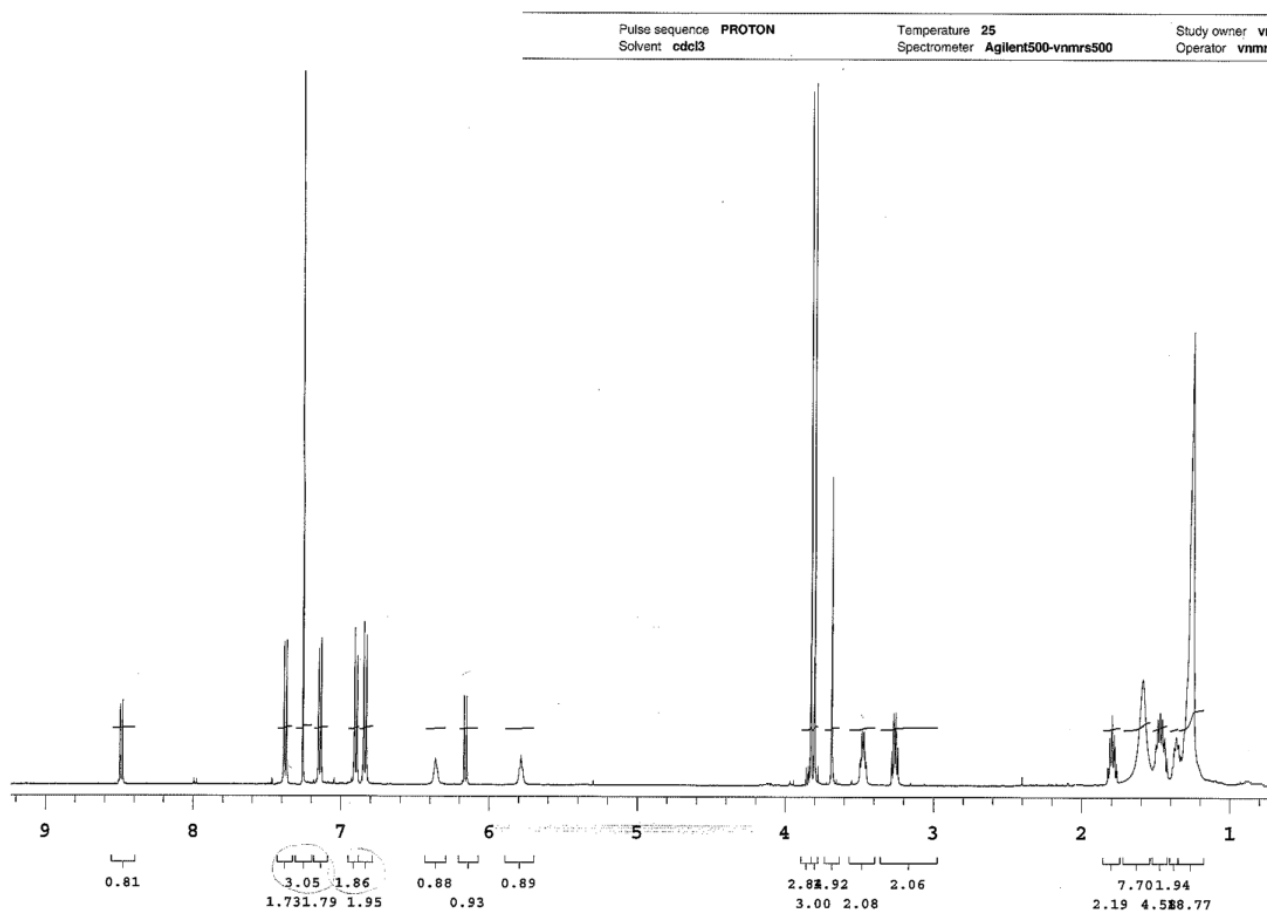
13b ^{13}C -NMR (125 MHz, CDCl_3)



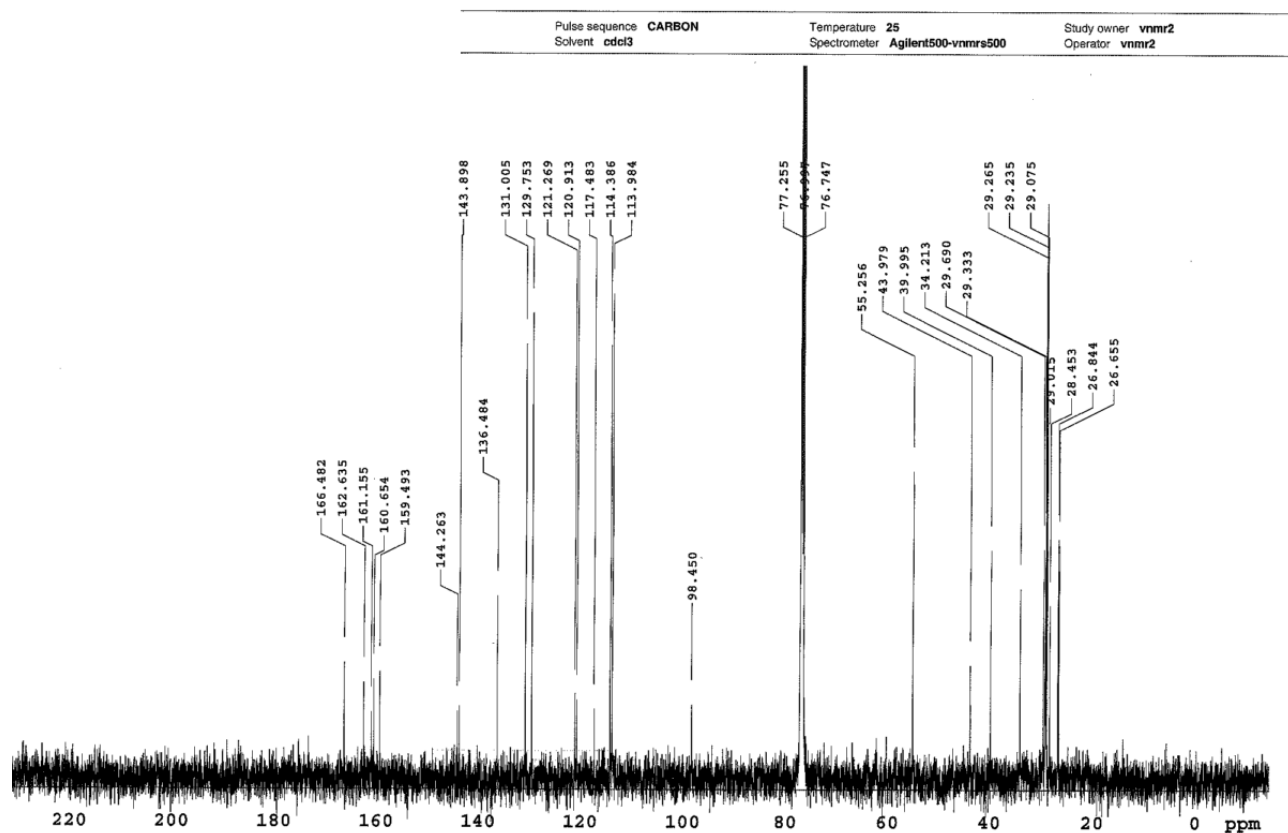
2-(3,4-Bis(4-methoxyphenyl)isoxazol-5-yl)-N-(12-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)dodecyl)acetamide (13c)



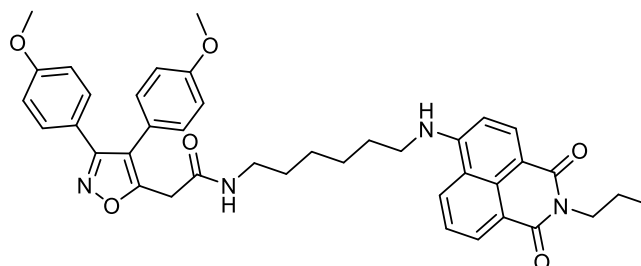
13c ¹H-NMR (500 MHz, CDCl₃)



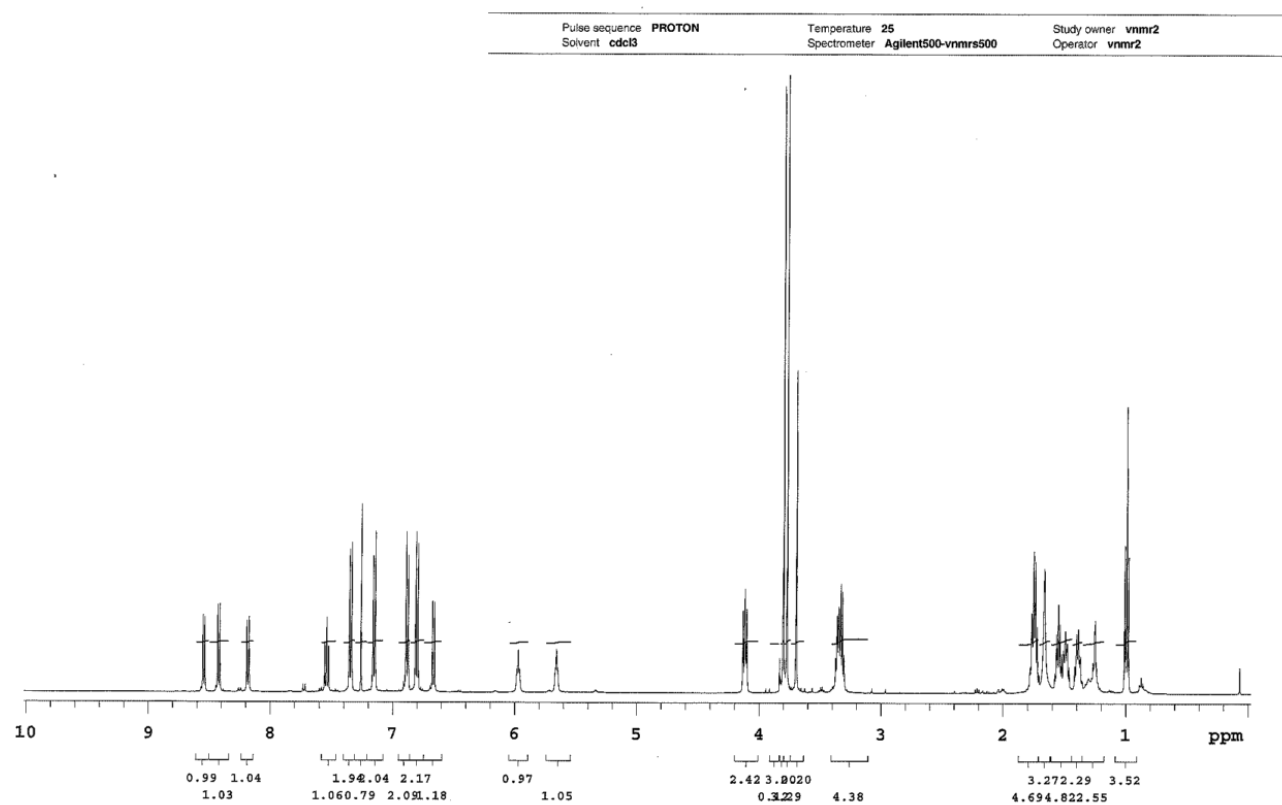
^{13}C ^{13}C -NMR (125 MHz, CDCl_3)



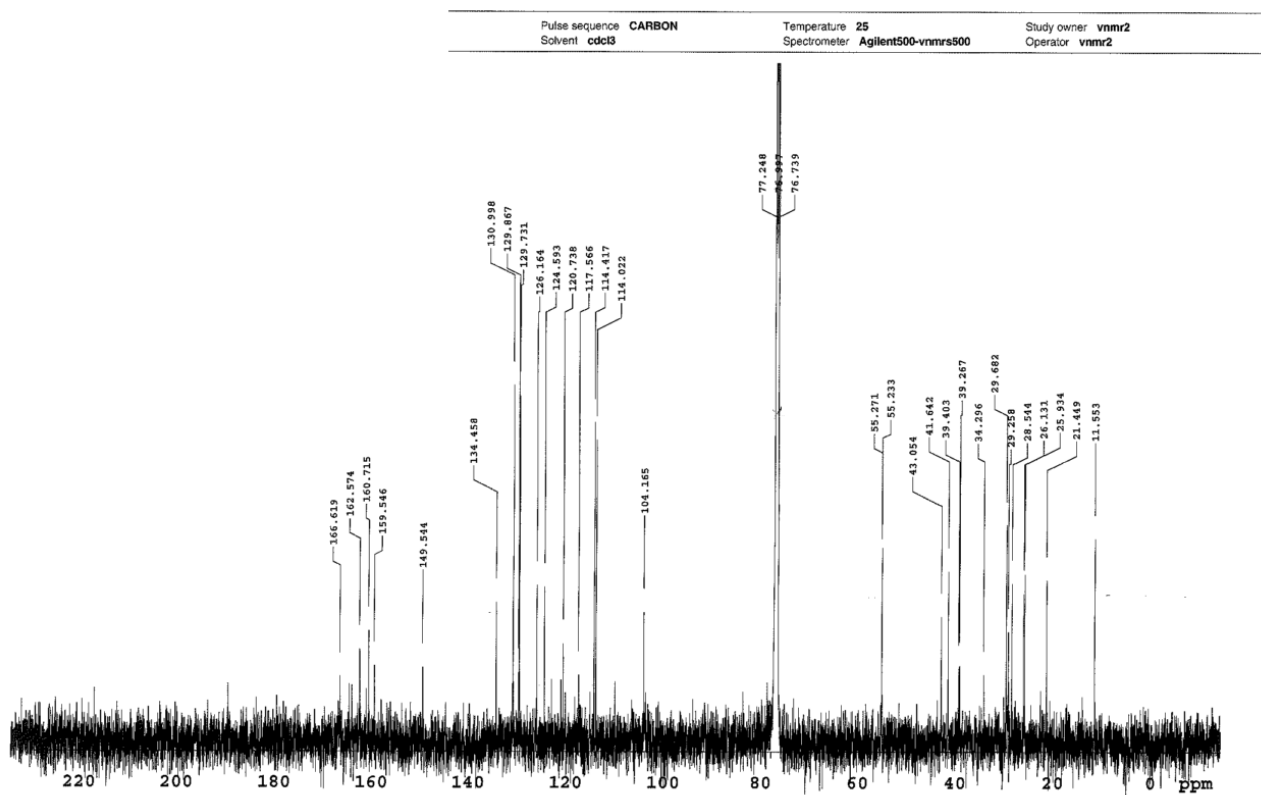
2-(3,4-Bis(4-methoxyphenyl)isoxazol-5-yl)-N-(6-((1,3-dioxo-2-propyl-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)amino)hexyl)acetamide (17a)



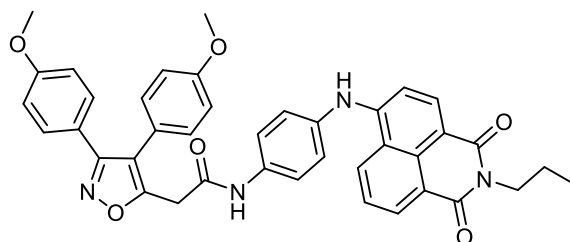
17a ¹H-NMR (500 MHz, CDCl₃)



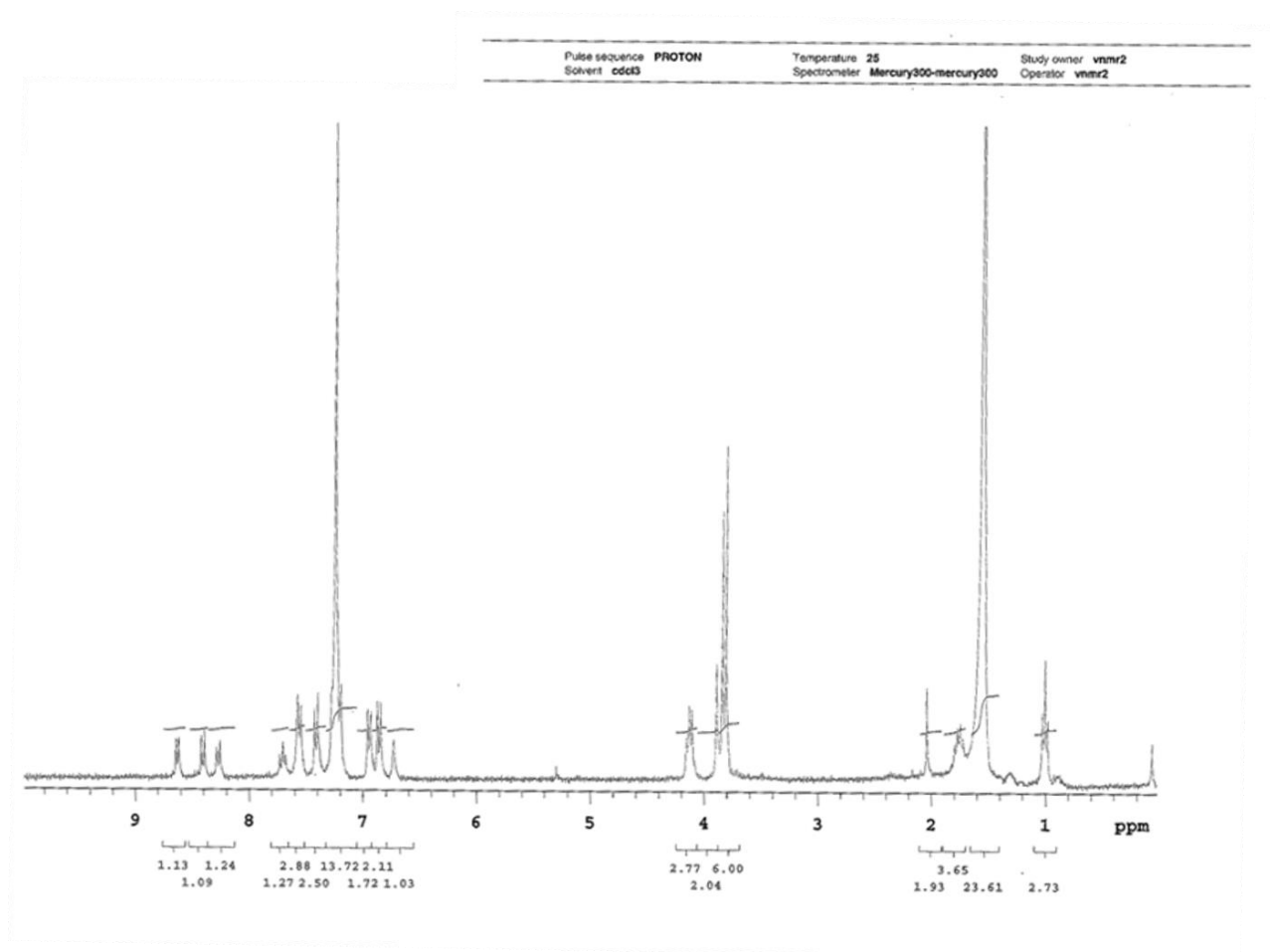
17a ^{13}C NMR (125 MHz, CDCl_3)



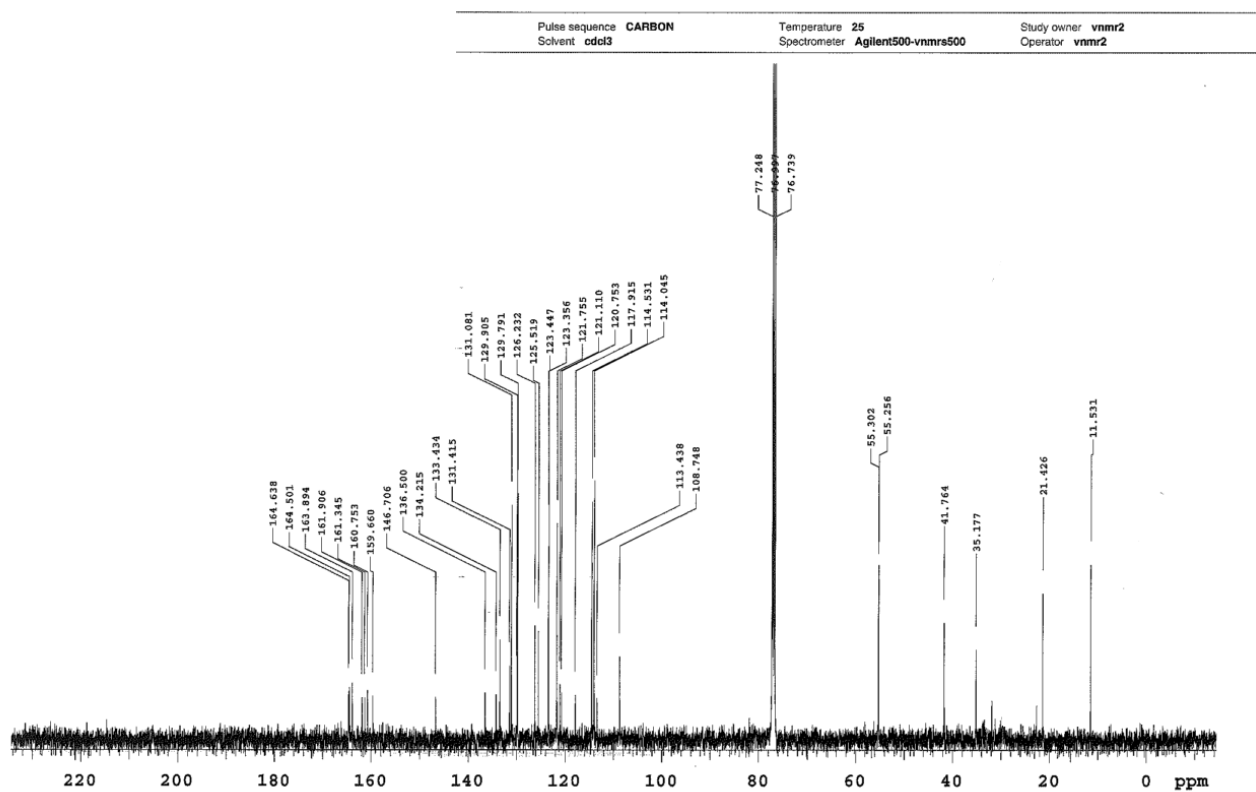
(3,4-Bis(4-methoxyphenyl)isoxazol-5-yl)-N-((1,3-dioxo-2-propyl-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)amino)phenyl)acetamide (17b)



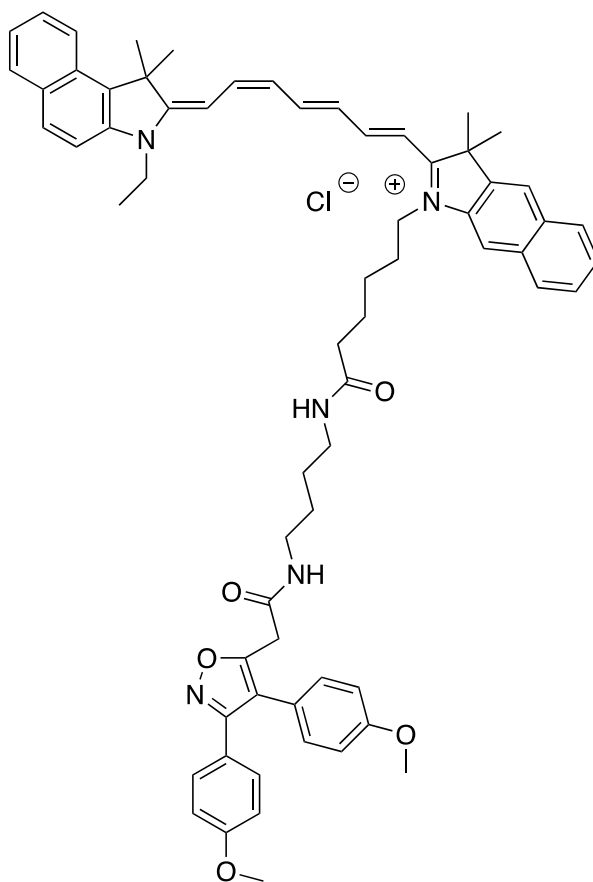
17b ^1H -NMR (300 MHz, CDCl_3)



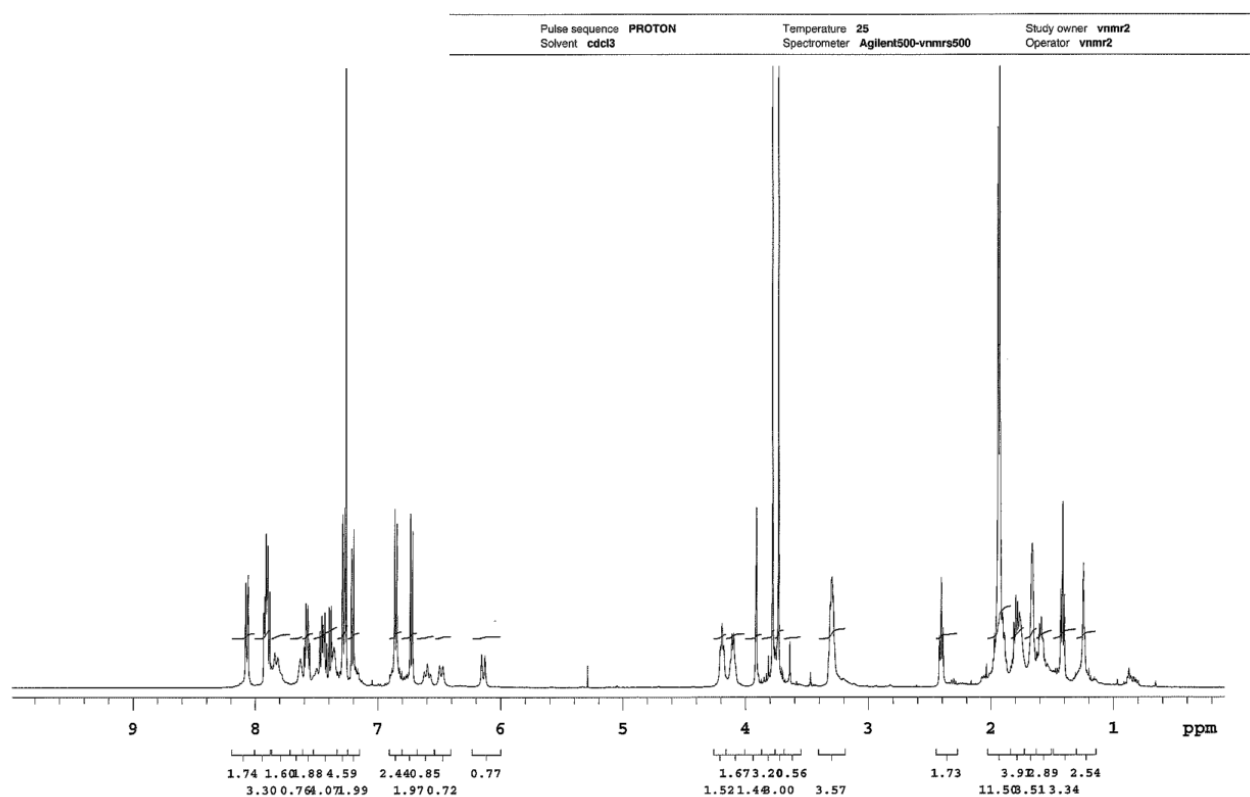
17b ^{13}C -NMR (125 MHz, CDCl_3)



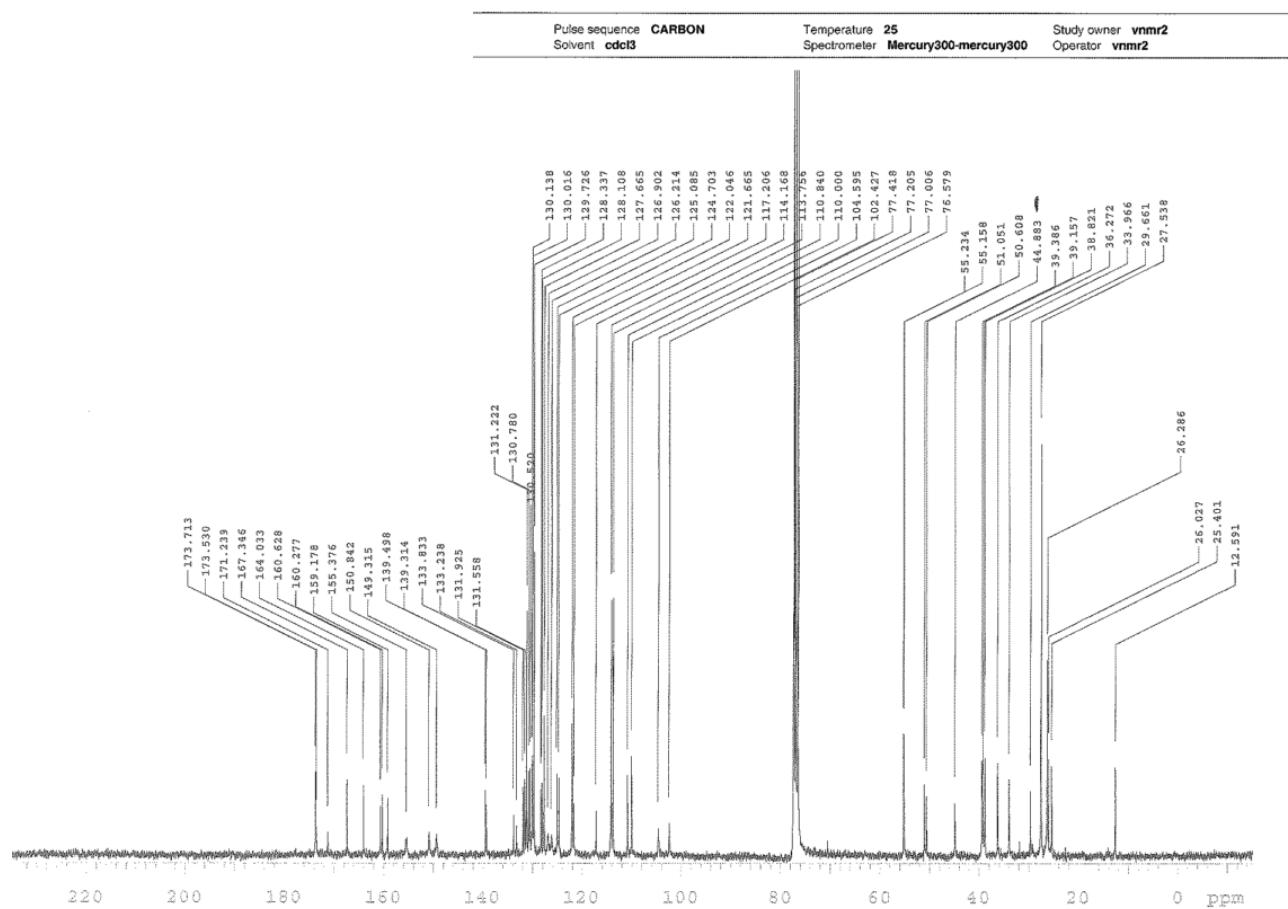
3-(6-(4-(2-(3,4-Bis(4-methoxyphenyl)isoxazole-5-yl)acetamido)butyl)amino-6-oxohexyl)-2-[7-(1,3-Dihydro-1,1-dimethyl-3-ethyl-2H-benz[e]indolin-2-yl-idene)-1,3,5-heptatrienyl]-1,1-dimethyl-3-(6-carboxilato-hexyl)-1H-benz[e]indolium chloride (23)



23 (MSA14) ^1H -NMR (500 MHz, CDCl_3)



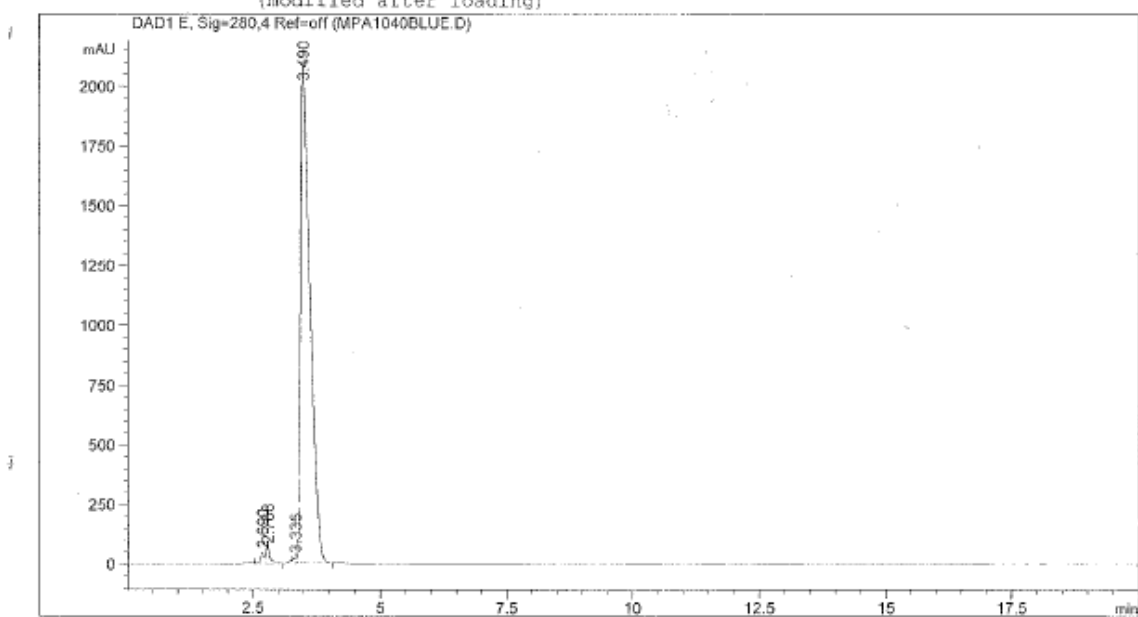
23 (MSA14) ^{13}C -NMR (75 MHz, CDCl_3)



2. HPLC chromatograms of RR11 and 23 (MSA14).

RR11 Rt =3.490 min. (mobile phase: 20 mM NH₄OAc (pH = 5.0)/CH₃CN: 20:80; stationary phase: ZORBAX ECLIPSE Plus C18, analytical 4.6x250mm, 5-Micron); rate = 1mL/min.

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Area Percent Report

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4	3.490	VB	0.1772	2.65661e4	2077.43945	97.5253

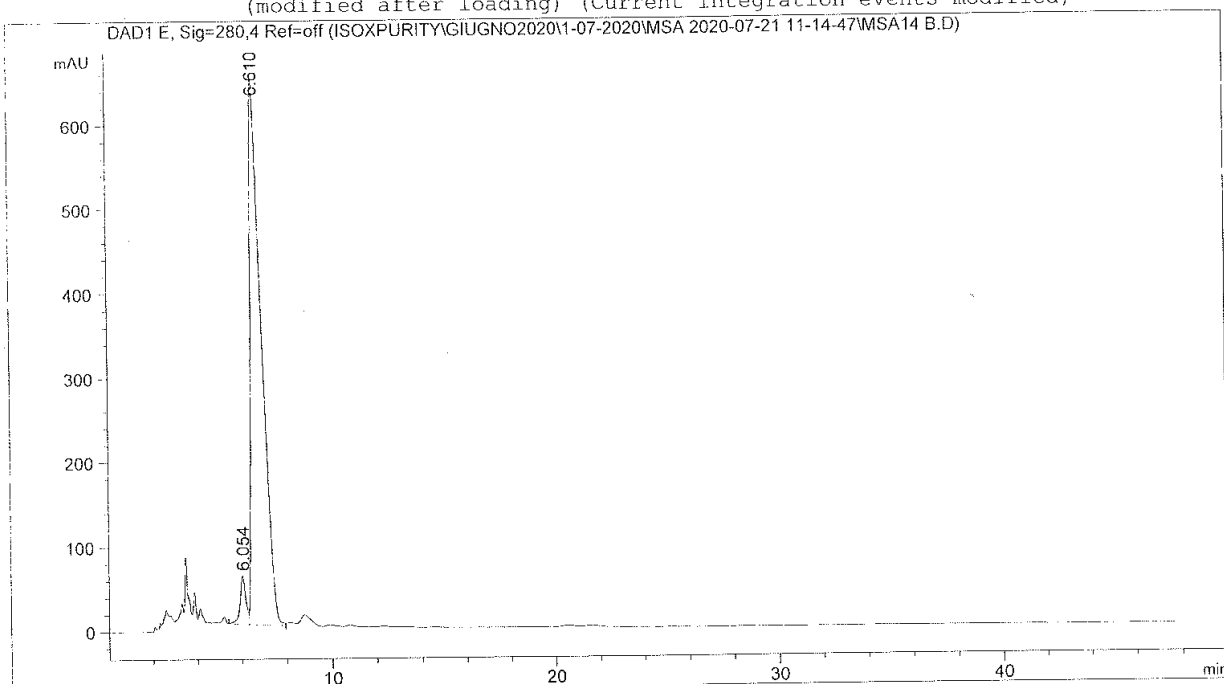
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*** End of Report ***

23 (MS14) Rt = 6.610 min. (mobile phase: 20 mM NH₄OAc (pH = 5.0)/CH₃CN: 10:90; stationary phase: ZORBAX ECLIPSE Plus C18, analytical 4.6x250mm, 5-Micron); rate = 1mL/min.

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Area Percent Report

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Use Multiplier & Dilution Factor with ISTDs
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Signal 1: DAD1 E, Sig=280,4 Ref=off

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2	6.610	VV	0.4933	2.22540e4	646.92200	95.5634

Totals : 2.32871e4 704.89908

3. FLAP S1-S7 figures of selected target compounds.

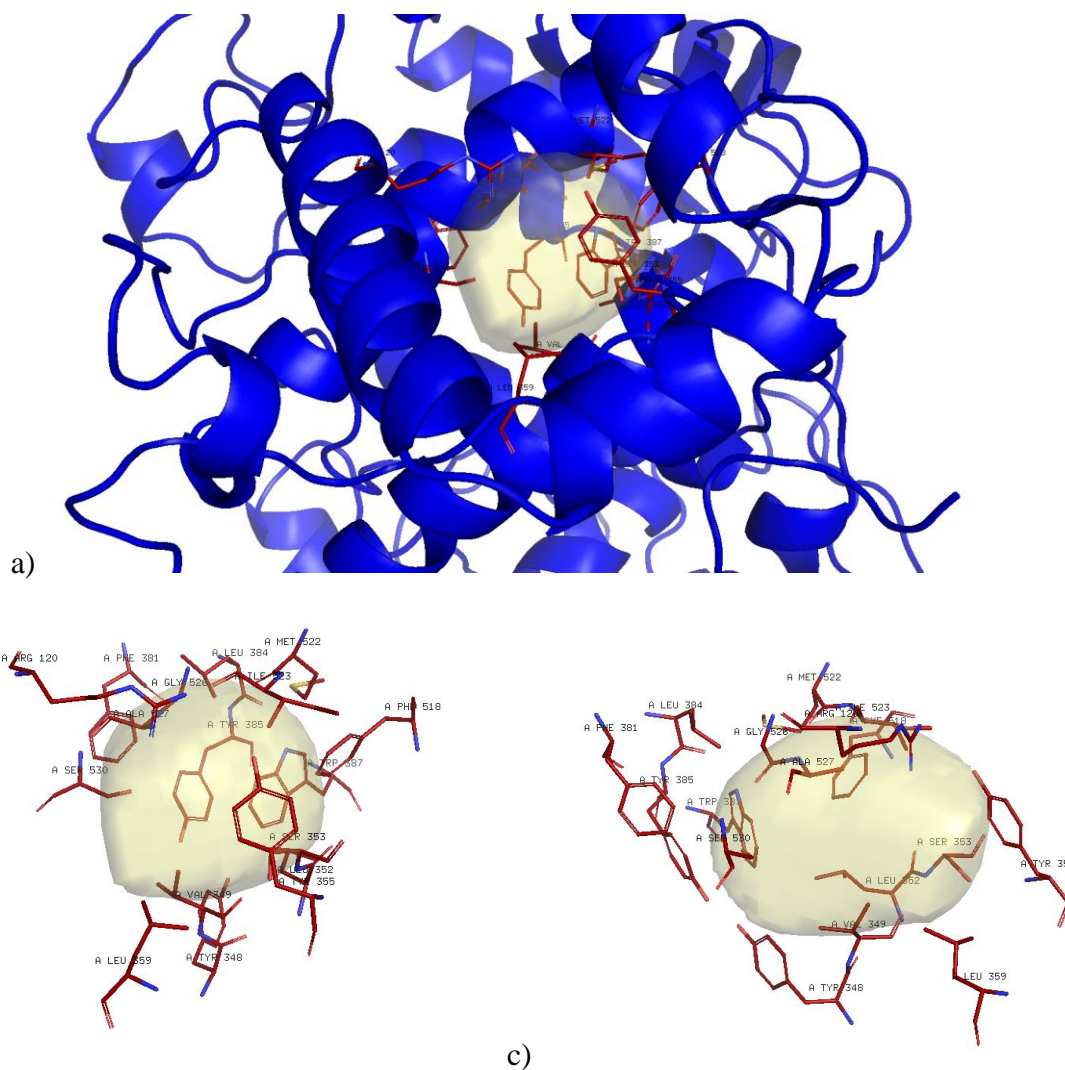


Figure S1. a) The top-ranked pocket (front view, P1) overlaid with the *h*COX-1 X-ray structures (PDB code 6Y3C); b), c) zoom of the substrate binding site and involved amino acids (R120 Y348 V349 L352 S353 Y355 L359 F381 L384 Y385 W387 F518 M522 I523 G526 A527 S530).

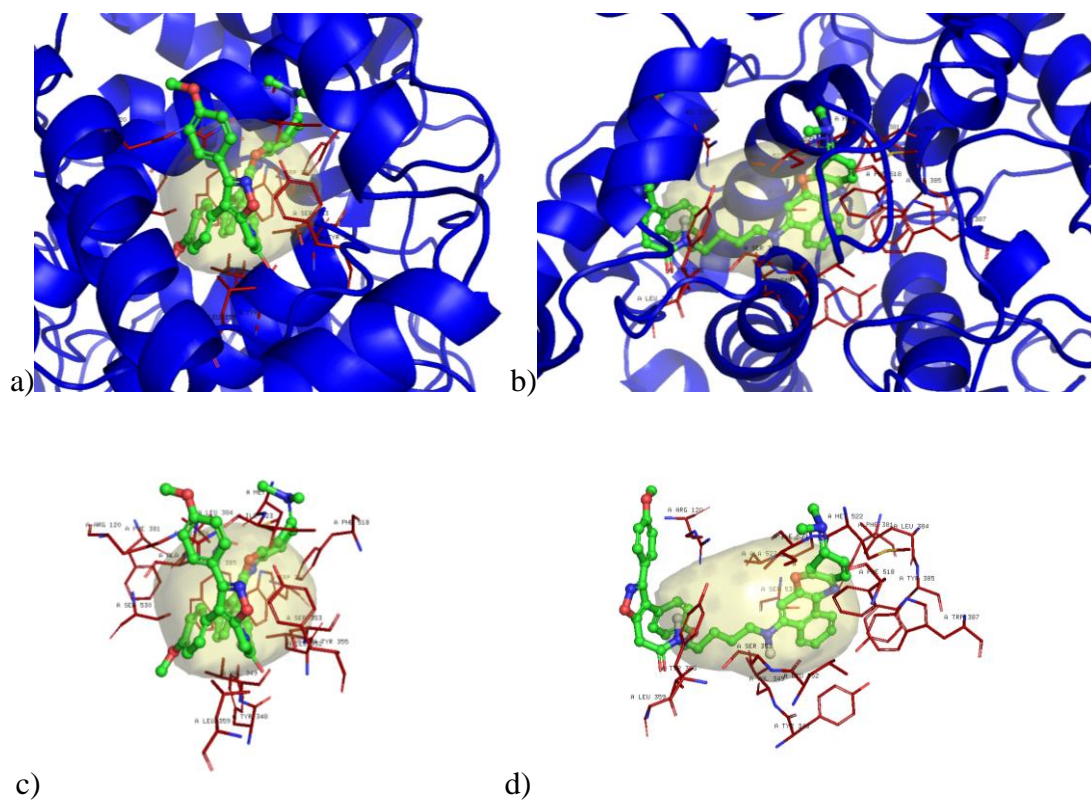


Figure S2. 3D binding mode of reference compound **RR11** inside the substrate binding site of *h*COX-1 (PDB code 6y3c) determined by FLAP analysis, front (a, c) and side view (b, d).

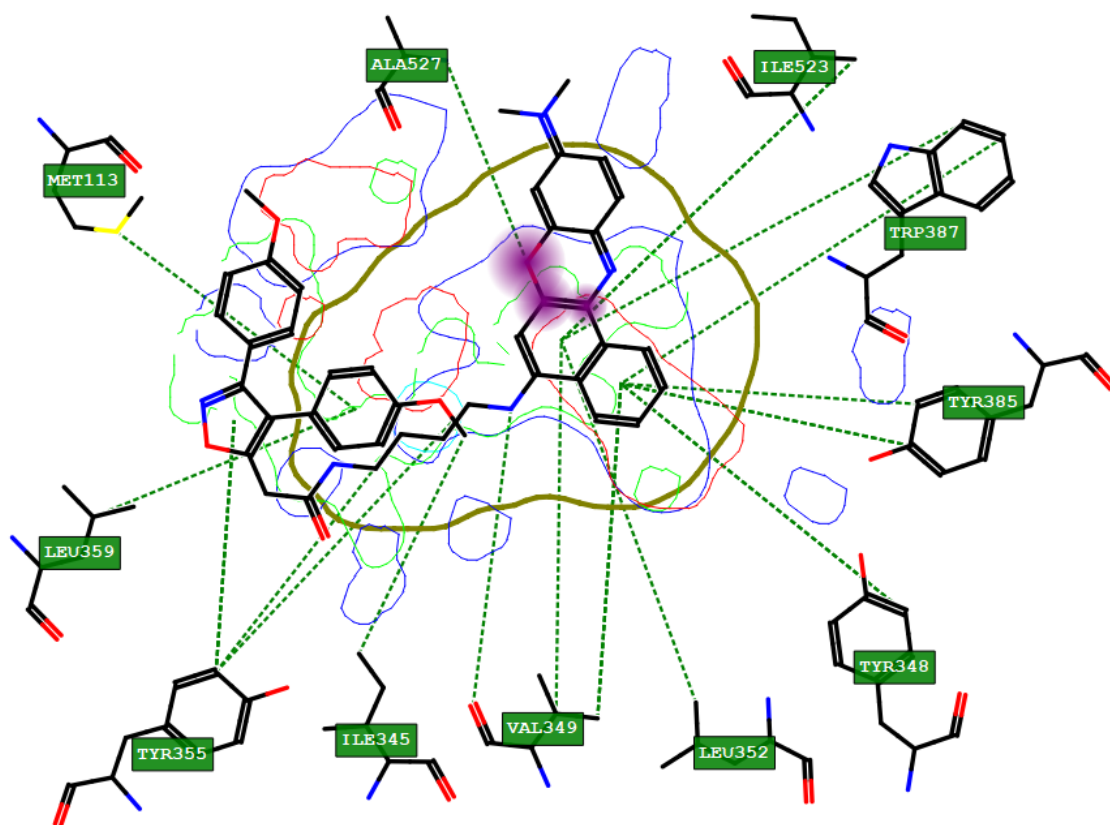


Figure S3. 2D FLAP binding poses of reference compound **RR11** inside the substrate binding site of *h*COX-1 (PDB code 6y3c). Residues located in the *h*COX-1 binding site are highlighted in stick-mode: the most important amino acids are shown together with their respective numbers. Purple regions indicate the strong interactions. Colored lines represent the area of hydrogen-bonding donor interactions (red), hydrogen-bonding acceptor interactions (blue), and the hydrophobic interactions (green).

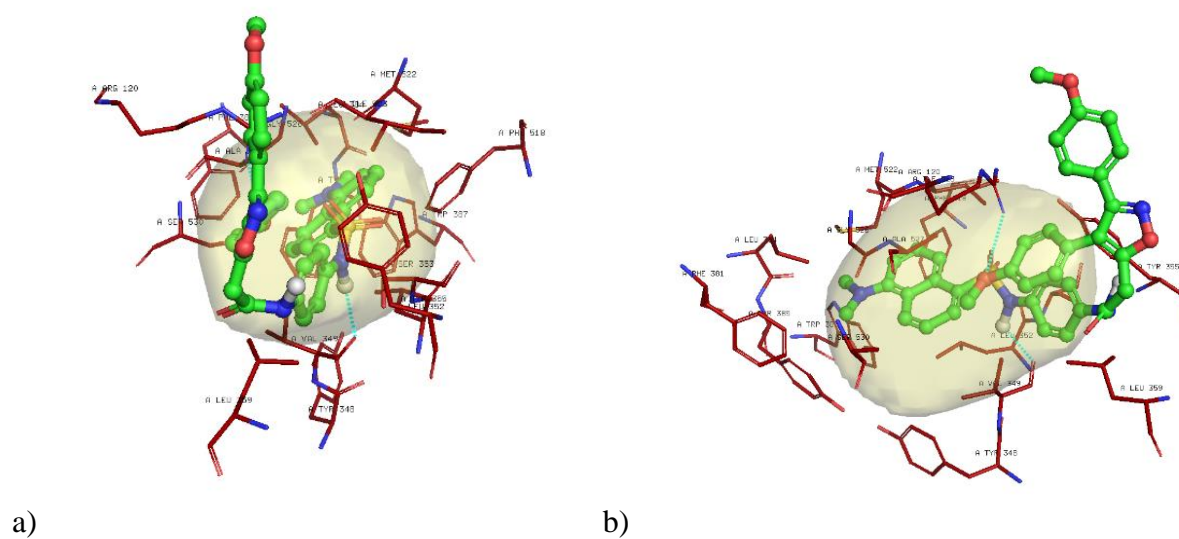


Figure S4. 3D binding mode of **3b** inside the substrate binding site of *h*COX-1 (PDB code 6y3c) determined by FLAP analysis, front (a) and side view (b).

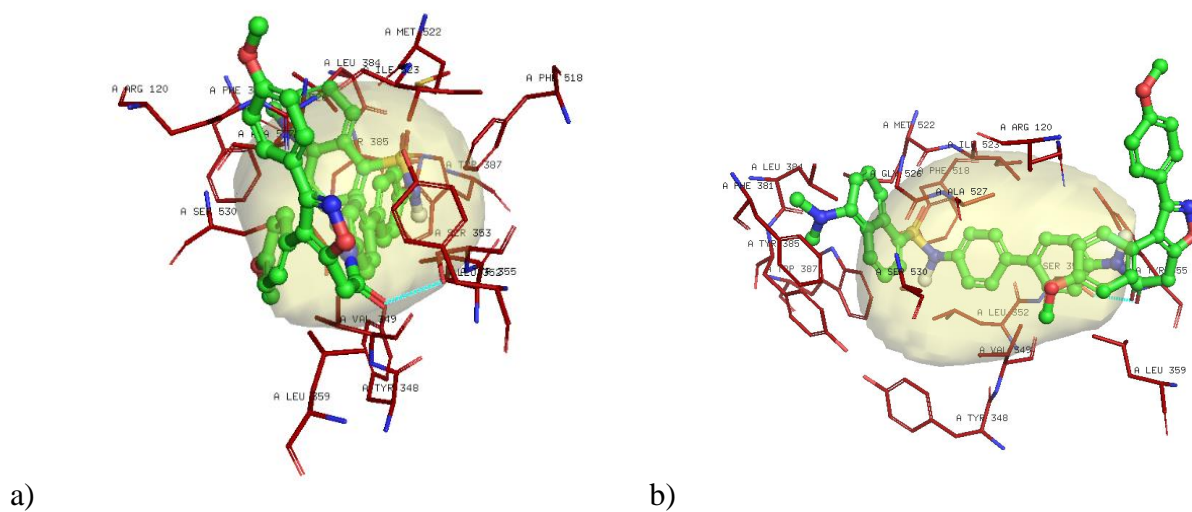


Figure S5. 3D binding mode of reference compound **3c** inside the substrate binding site of *h*COX-1 (PDB code 6y3c) determined by FLAP analysis, front (a) and side view (b).

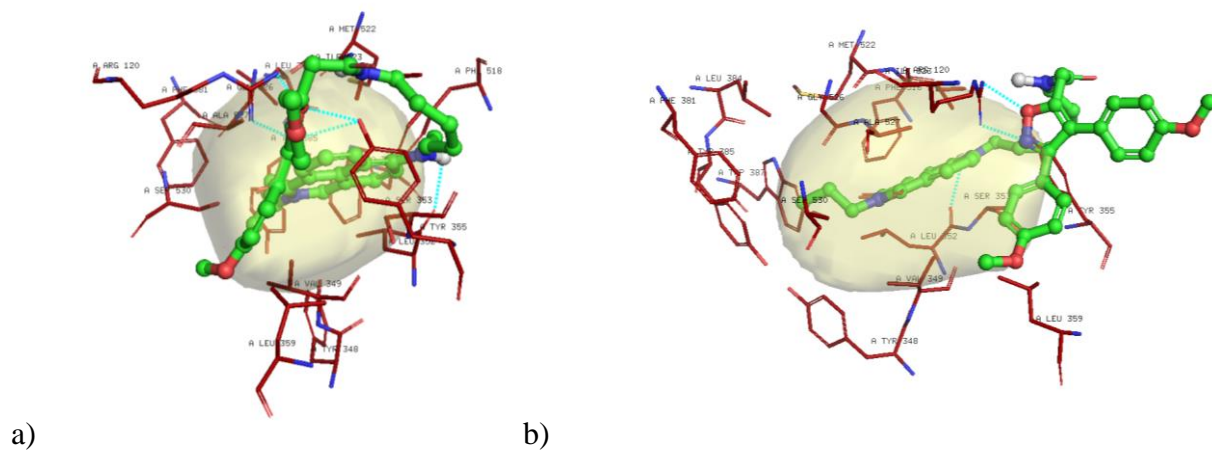


Figure S6. 3D binding mode of **17a** inside the substrate binding site of *h*COX-1 (PDB code 6y3c) determined by FLAP analysis, front (a) and side view (b).

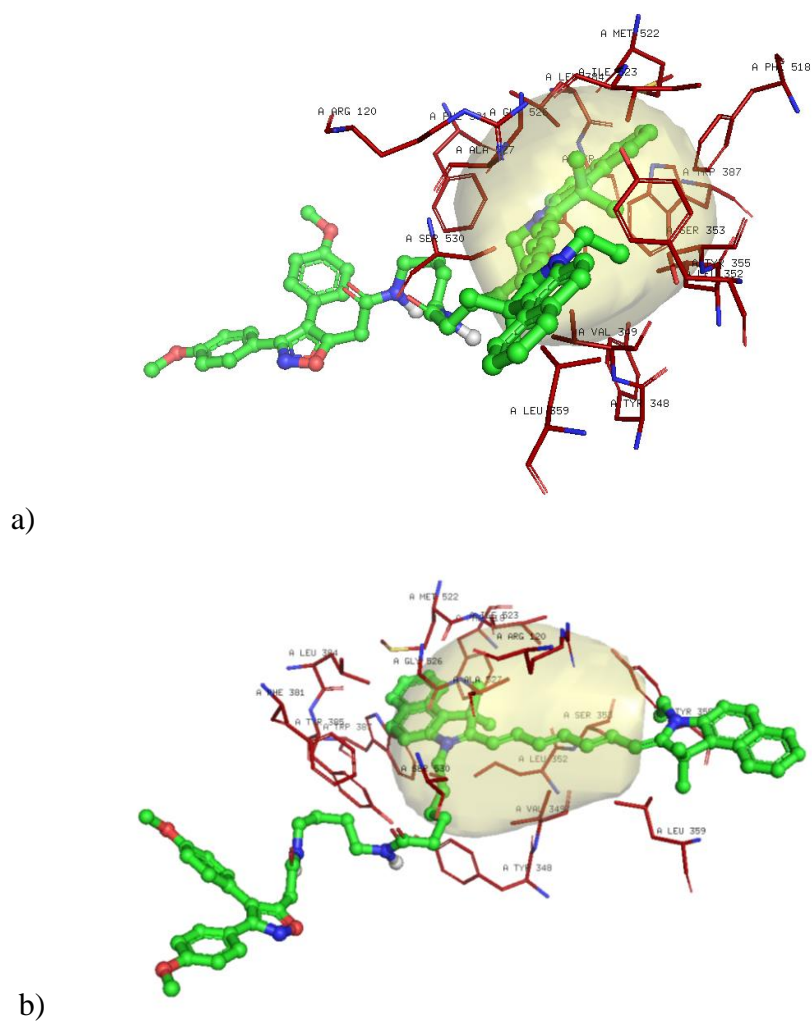


Figure S7. 3D binding mode of **23** inside the substrate binding site of *h*COX-1 (PDB code 6y3c) determined by FLAP analysis, front (a) and side view (b).