Type of the Paper (Article)

Synthesis of four steroidal carbamates with antitumor activity against mouse colon carcinoma CT26WT cells: *in vitro* and *in silico* evidence

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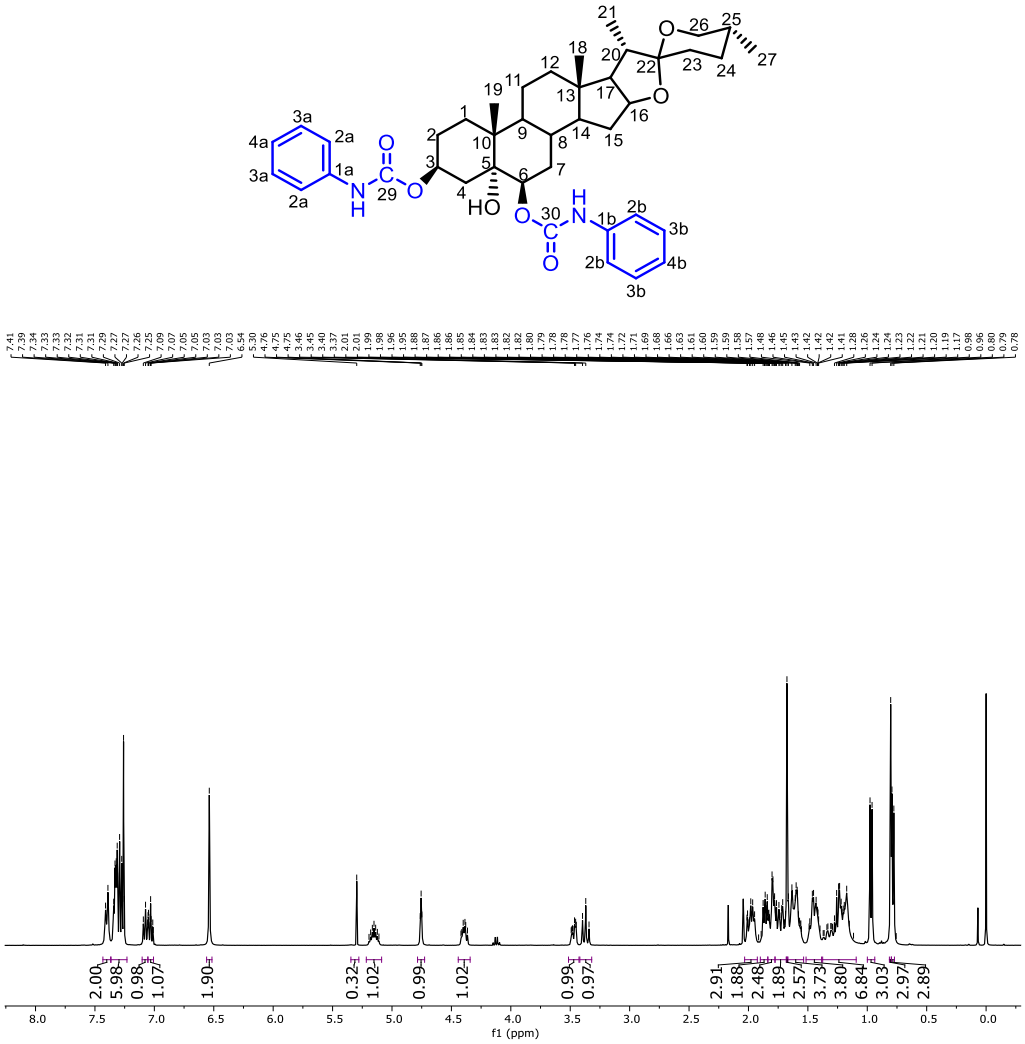
Supplementary Materials: **Figure S1**: NMR spectra of (25R)-5α-hydroxy-Spirostan--3β, 6 β-yl phenylcarbamate (**5**); **Figure S2**: NMR spectra of (25R)- 5α-hydroxy-6-oxo-spirostan-3β-yl phenylcarbamate (**6**); **Figure S3**: NMR spectra of 4-en-androst-17β-yl phenylcarbamate (**7**); **Table S1:** Receptor-ligand interactions found by BINANA; **Figure S4:** Interaction pattern of A) **4**(1), B) **4**(2), C) **5**(1), D) **5**(2) E) **6**(1), F) **6**(2), G) **7**(1) and H) **7**(3) within the active site of EP4. Hydrogen bonds are highlighted with yellow dashed lines. Residues with van der Waals interactions are displayed as green sticks. All distances are given in Å. **Table S2:** Abbreviations used in this work.

Figure S1: NMR spectra of mixture (25R)-5α-hydroxy-Spirostan-3β,6β-yl phenylcarbamate (**5**).

^{1}H-NMR



^{13}C-NMR

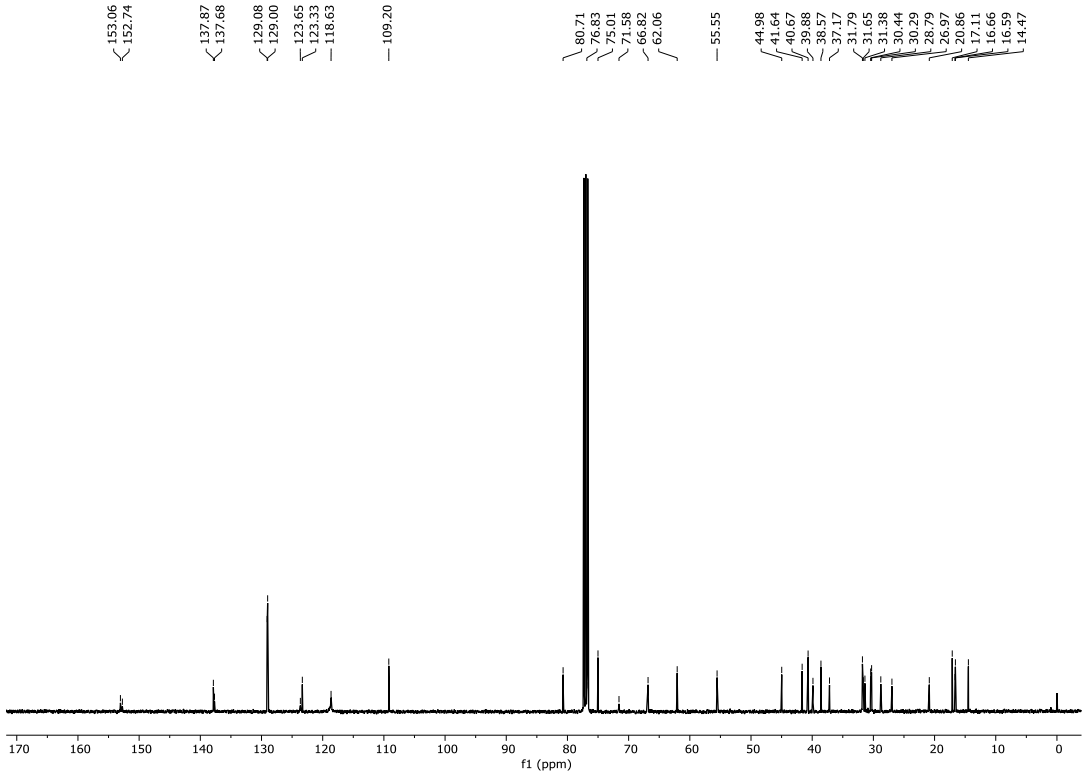
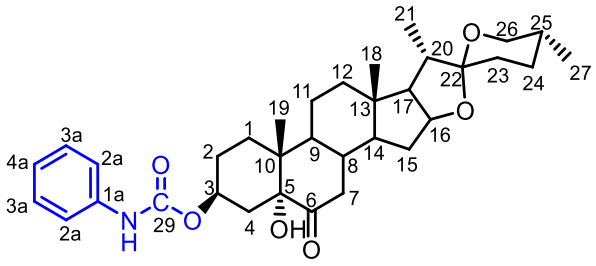
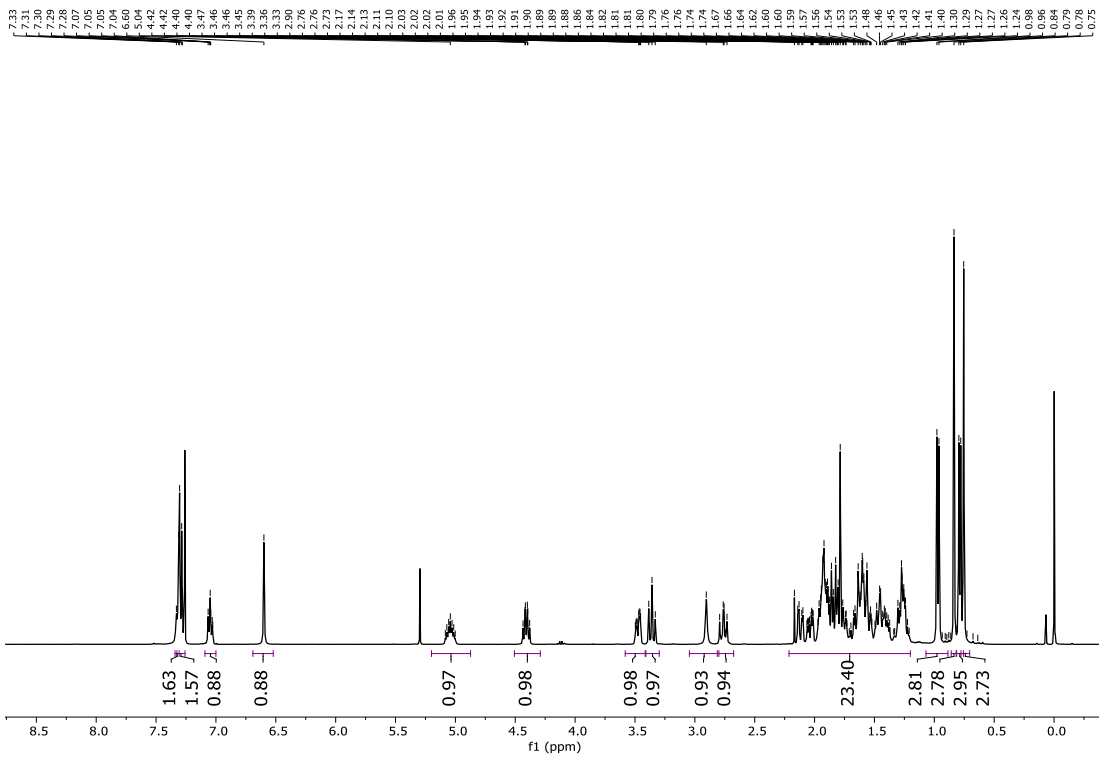


Figure S2: NMR spectra of (25R)- 5α-hydroxy-6-oxo-spirostan-3β-yl phenylcarbamate (**6**).

^{1}H- NMR




1.63
1.57
0.88
0.88
0.97
0.98
0.98
0.97
0.93
0.94
23.40
2.81
2.78
2.95
2.73
f1 (ppm)

^{13}C-NMR

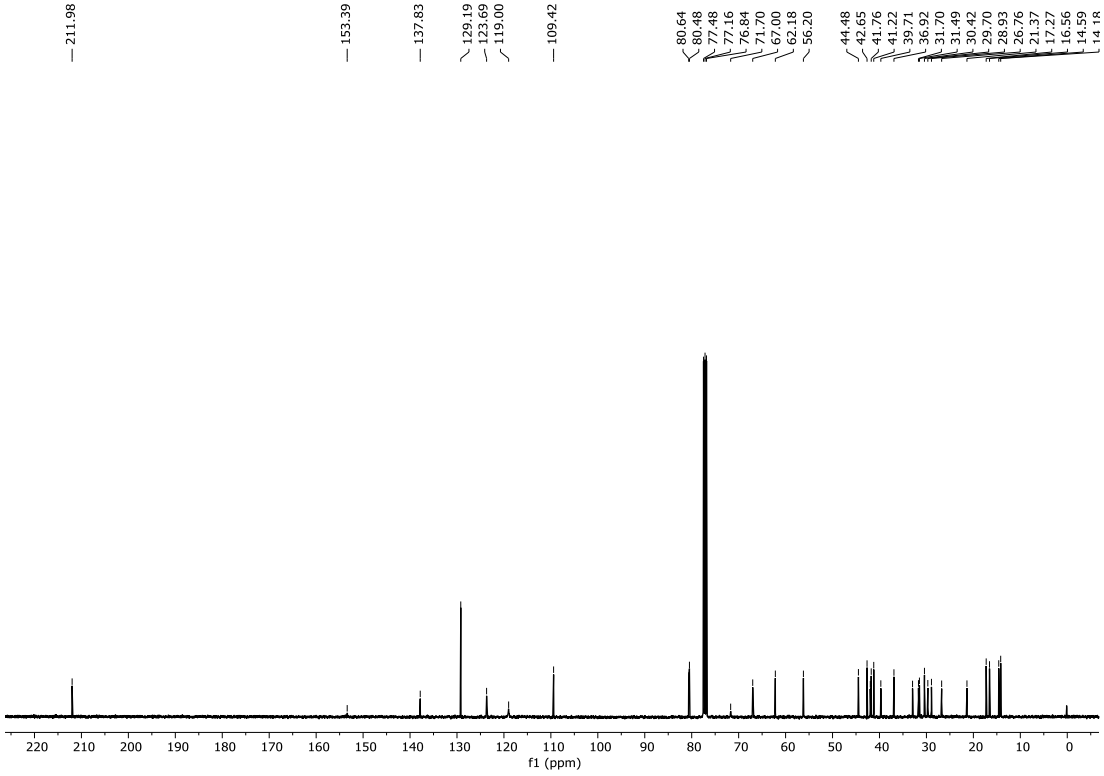
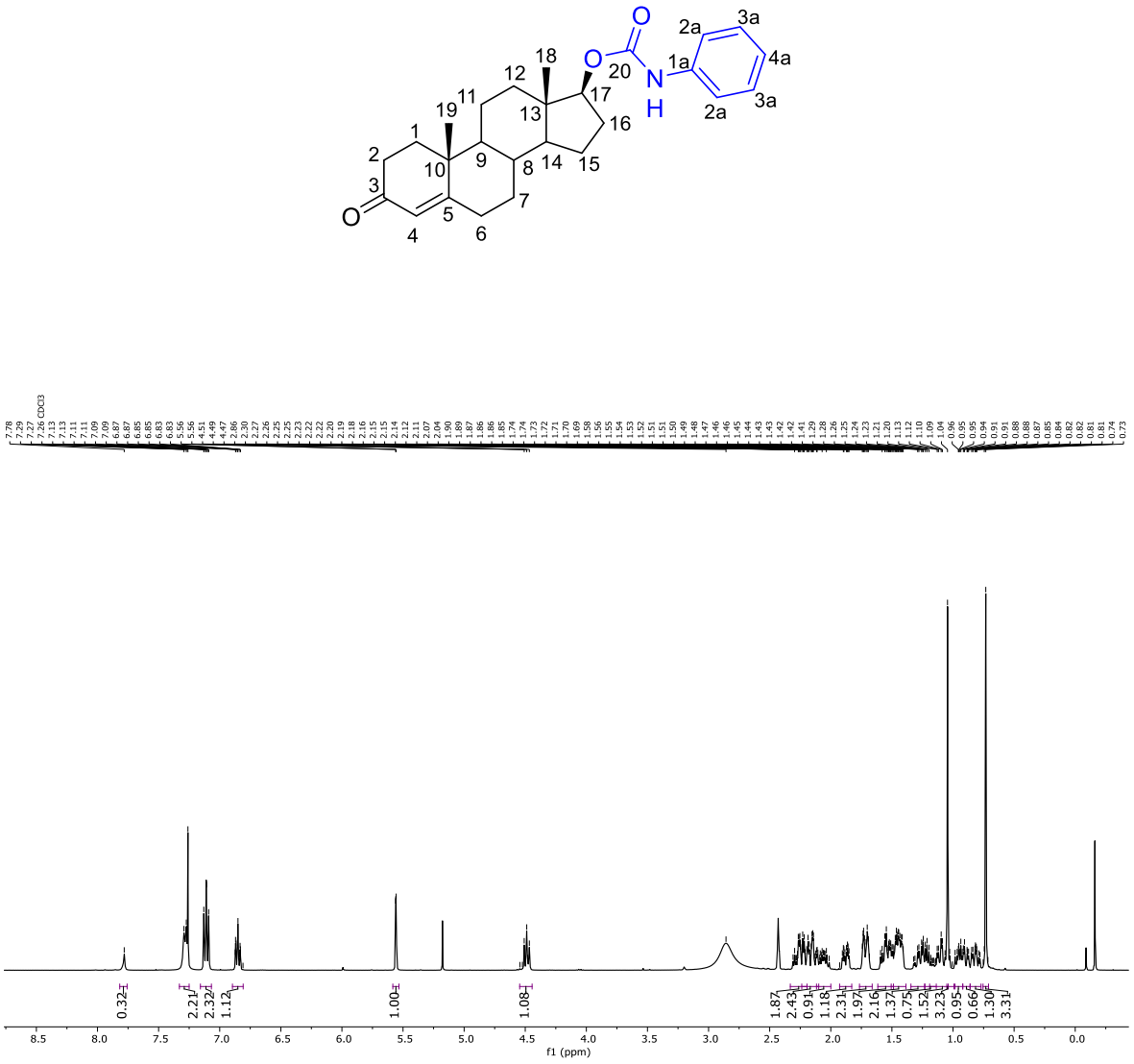

211.98
153.39
137.83
129.19
123.69
119.00
109.42
80.64
80.48
77.48
77.16
76.84
71.70
67.00
62.18
56.20
44.48
42.65
41.76
41.22
39.71
36.92
31.70
31.49
30.42
29.70
28.93
26.76
21.37
17.27
16.56
14.59
f1 (ppm)

Figure S3: NMR spectra of 4-en-androst-17β-yl phenylcarbamate (**7**).

^{1}H- NMR



^{13}C-NMR

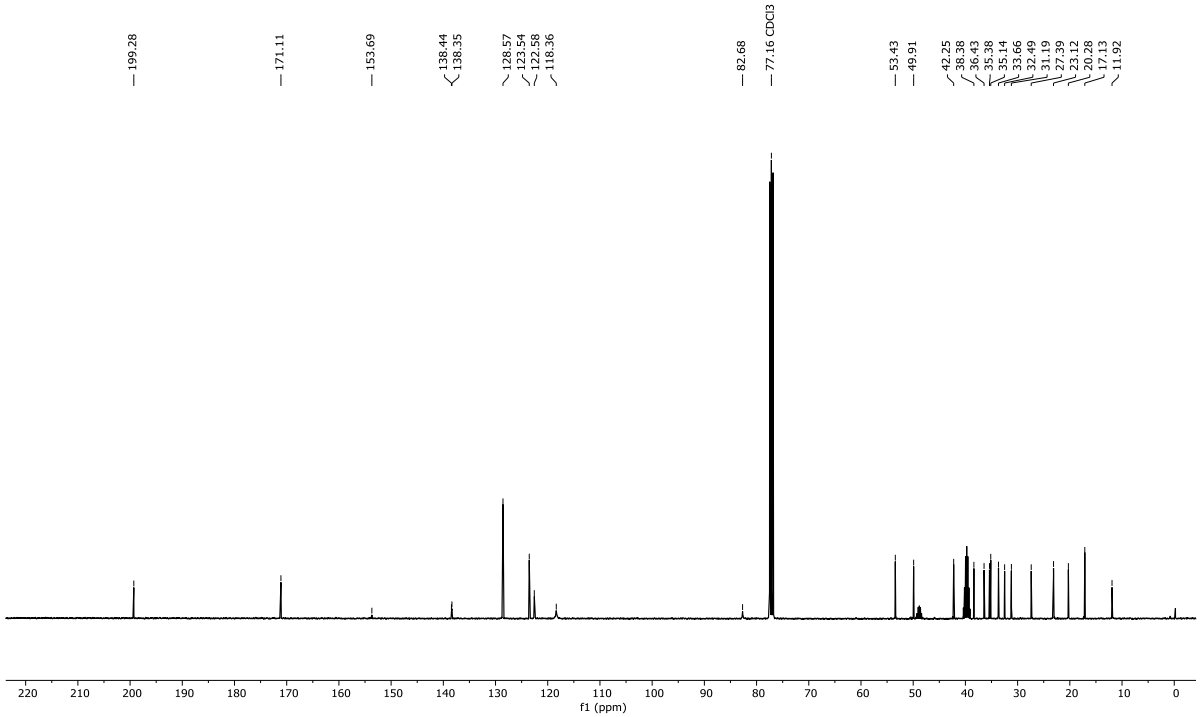
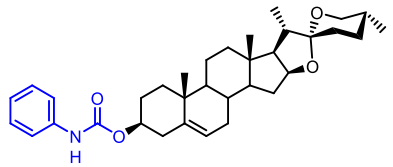
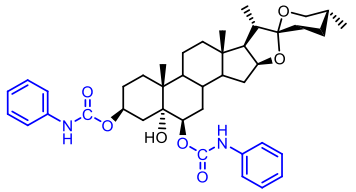
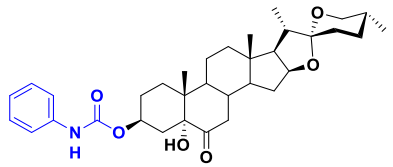

199.28
171.11
153.69
138.44
138.35
128.57
123.54
122.58
118.36
82.68
77.16 CDCl3
53.43
49.91
42.25
38.38
36.43
35.38
35.14
33.66
32.49
31.19
27.39
23.12
20.28
17.13
11.92
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

Table S1: Receptor-ligand interactions found by BINANA.

Ligand	Group	Hydrogen Bond	Van der Waals	Short distance interactions	# Similar Interactions	Structure
4	1	-	ILE(23), PRO(24), MET(27), VAL(75), THR(76), TYR(80), LEU(99), TRP(169), GLN(313), ARG(316), ILE(317), SER(319)		7	
	2	THR(76)	ILE(23), MET(27), VAL(72), THR(76), TYR(80), LEU(99), TRP(169), GLN(313), ILE(315), ARG(316), ILE(317), VAL(320)		8	
5	1		PRO(24), THR(76), TYR(80), ARG(316), VAL(320)		5	
	2		THR(76), TYR(80), ARG(316), VAL(320)	ARG(316) 2.4 Å	4	
6	1	TYR (80)	ILE(23), MET(27), VAL(72), VAL(75), THR(76), TYR (80), LEU(99), ILE(315), ARG(316), SER(319), VAL(320)	ARG(316) 2.2 and 2.4 Å	8	
	2	-	ILE(23), MET(27), VAL(72), THR(76),		5	

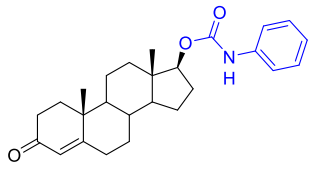
			GLN(313), ARG(316), ILE(317), SER(319), VAL(320)			
7	1	THR(76)	ILE(23), MET(27), VAL(75), THR(76), TYR(80), LEU(99), TRP(169), ILE(315), ARG(316), SER(319), VAL(320).		8	
	2	-	ILE(23), PRO(24), MET(27), THR(69), THR(76), TYR(80), ARG(316), SER(319), VAL(320), PRO(322)		6	

Figure S4: Interaction pattern of A) 4(1), B) 4(2), C) 5(1), D) 5(2) E) 6(1), F) 6(2), G) 7(1) and H) 7(3) within the active site of EP4. Hydrogen bonds are highlighted with yellow dashed lines. Residues with Van der Waals interactions are displayed as green sticks. All distances are given in Å.

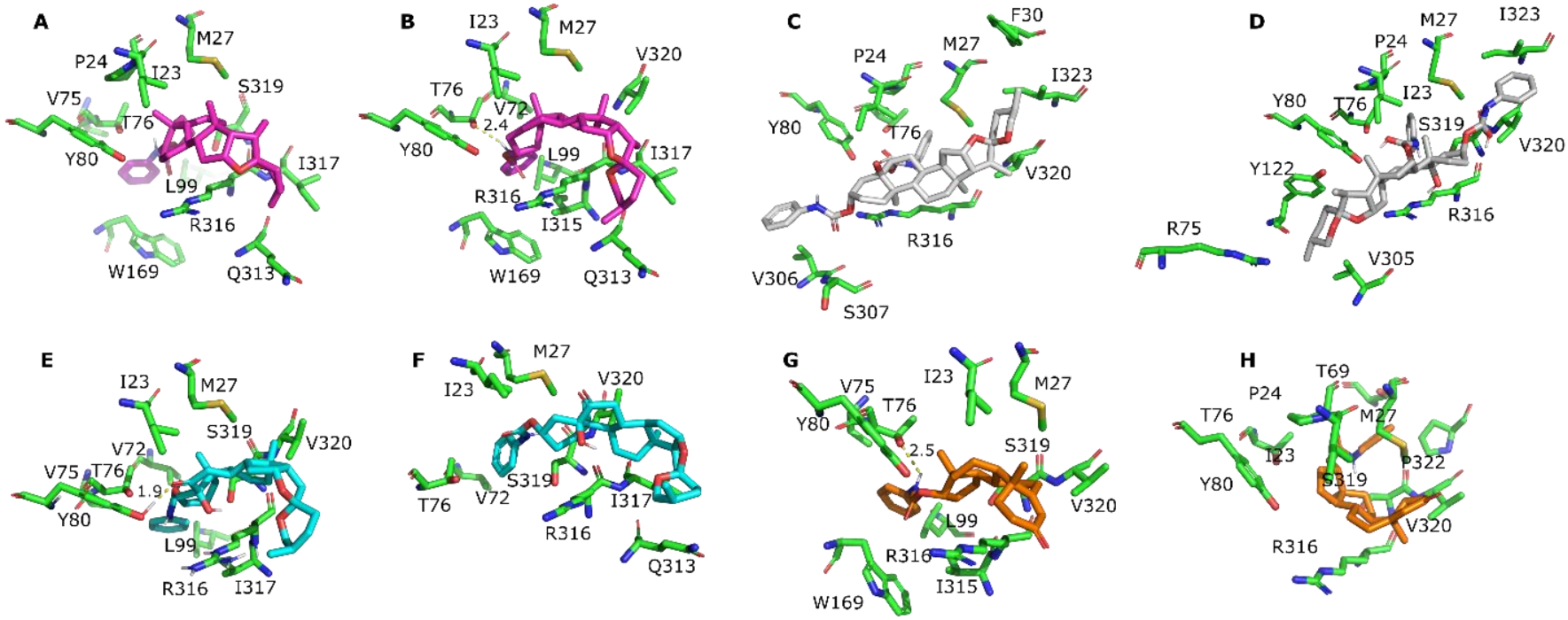


Table S2: Abbreviations. The following abbreviations are used in this manuscript.

h	hours
J	coupling constants
δ	chemical shifts
s	singlet
d	doublet
t	triplet
q	quartet
dt	doublet triplet
dq	doublet quartet
tt	triple triplet
m	multiplet
ax	axial
eq	equatorial
PhNCO	phenyl isocyanate
ppm	parts per millions
rt.	room temperature
TLC	thin-layer chromatography
MTT	[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]