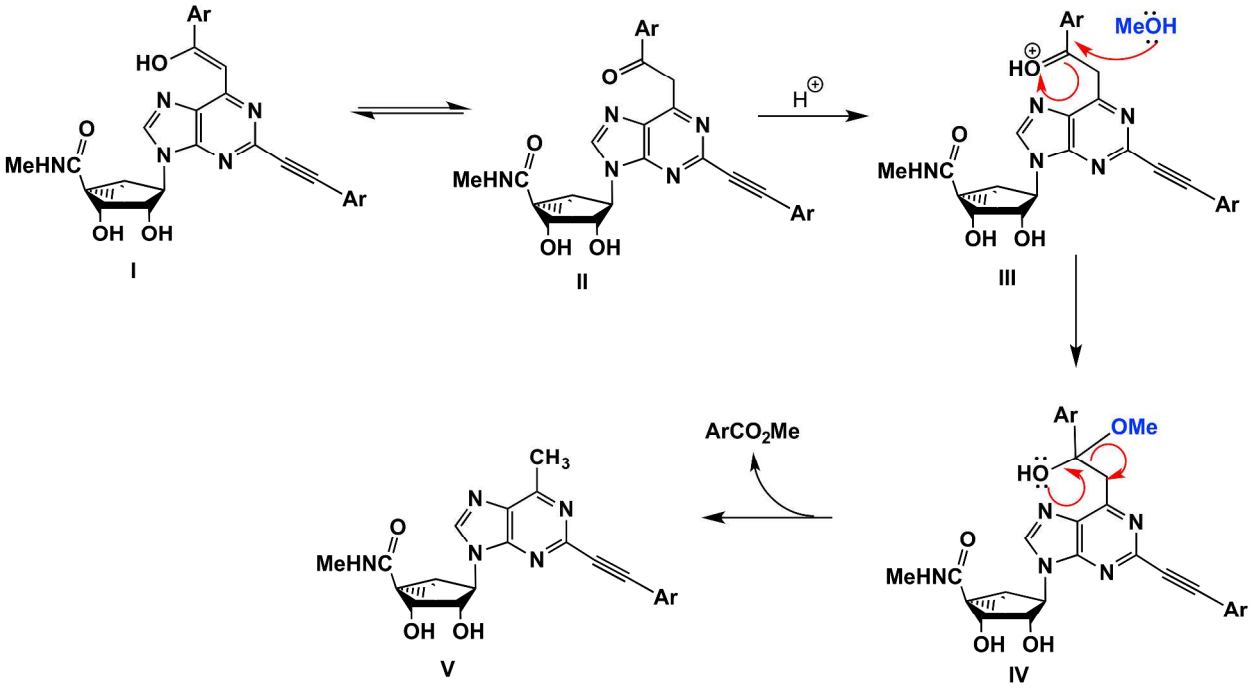
Supporting Information:

Purine (N)-Methanocarba Nucleoside Derivatives Lacking an Exocyclic Amine as Selective A_3 Adenosine Receptor Agonists

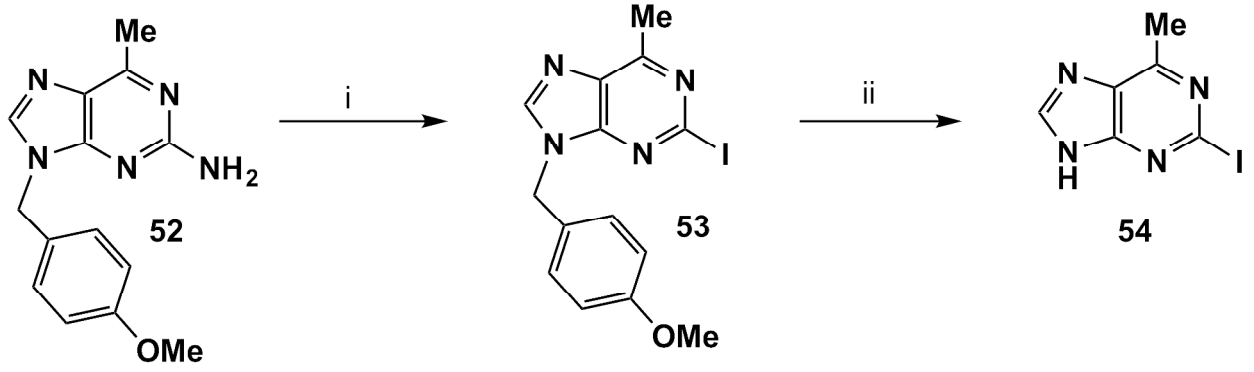
Dilip K. Tosh, Antonella Ciancetta, Eugene Warnick, Robert O'Connor, Zhoumou Chen, Elizabeth Gizewski, Steven Crane, Zhan-Guo Gao, John A. Auchampach, Daniela Salvemini, and Kenneth A. Jacobson

Contents: **Page**

Scheme S1. A proposed mechanism for the unexpected formation of 6-Me purine derivatives.



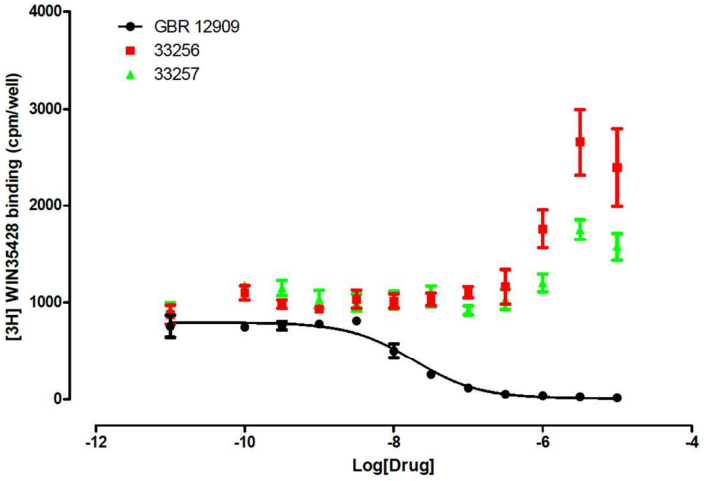
Scheme S2. Reagents and Conditions: (i) isoamyl nitrite, iodine, CH_2I_2, CuI, THF, reflux; (ii) TFA, reflux.

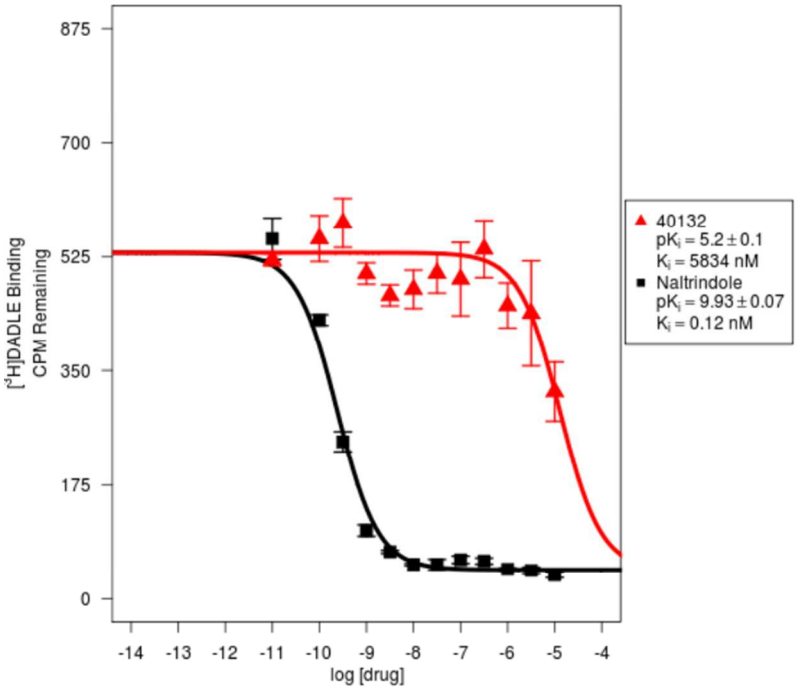


Binding to off-target sites

K_i determinations and binding profiles in a broad screen of receptors and channels were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda MD, USA. For experimental details please refer to the PDSP web site http://pdsp.med.unc.edu/ and click on "Binding Assay" or "Functional Assay" on the menu bar.

Figure S1. Radiologand binding at (A) the human dopamine transporter (hDAT); compound 33256 = Compound **15**; (B) the human d-opioid receptor (hDOR); compound 40132 = Compound **19**.





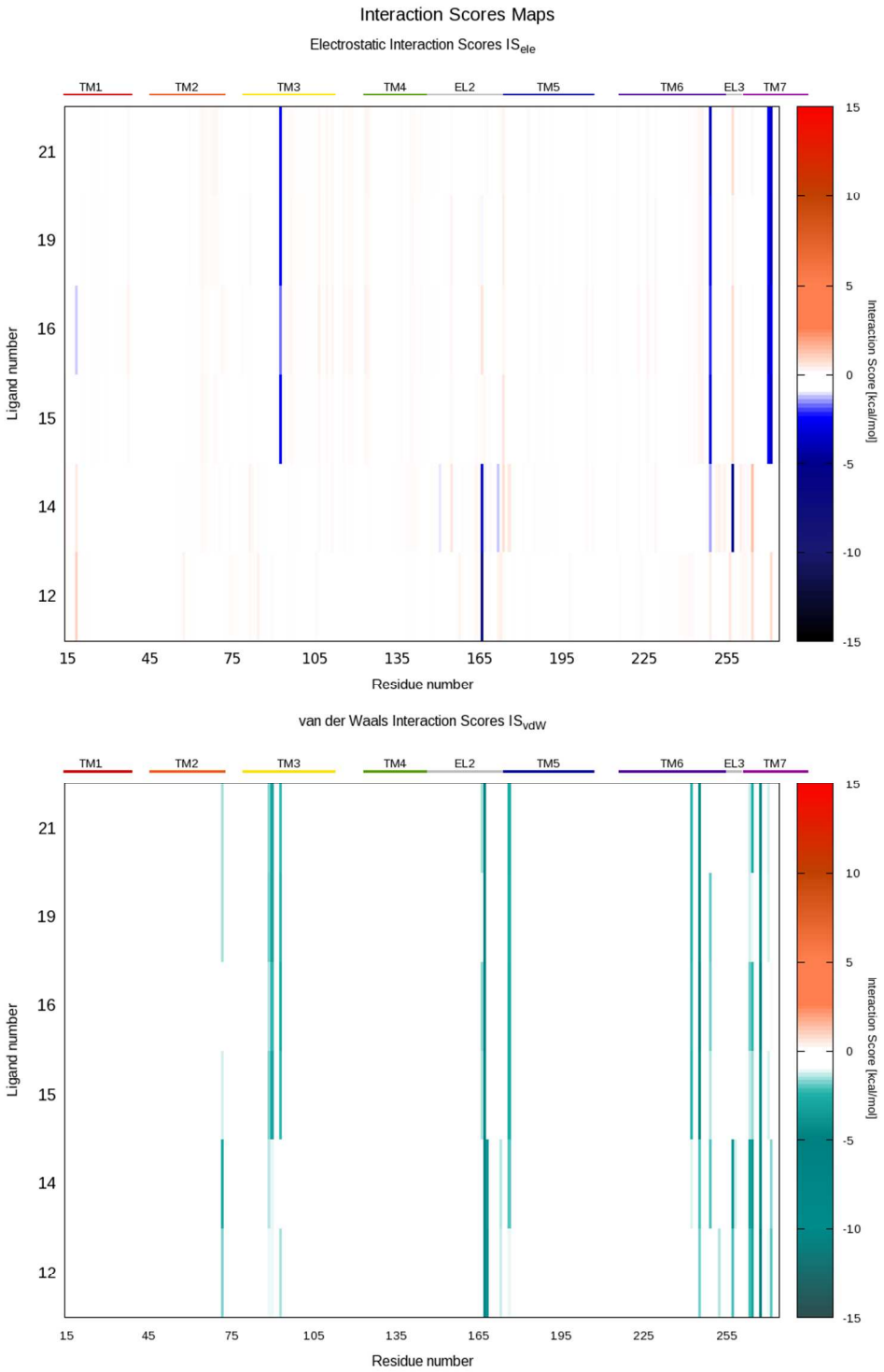

Interaction Scores Maps
Electrostatic Interaction Scores IS$_{ele}$
TM1
TM2
TM3
TM4
EL2
TM5
TM6
EL3
TM7
Ligand number
Residue number
Interaction Score [kcal/mol]
van der Waals Interaction Scores IS$_{vdW}$

Figure S2. Per residue IS_{ele} (panel A) and IS_{vdW} (panel B) maps for the most energetically favorable docking poses of compounds with K_i < 100 nM. The maps have been computed for selected poses of the considered compound inside the putative binding site of hA_3 AR. Values are expressed in kcal/mol.

Figure S3. Representative structure for compounds 32a, 12, and 15 (shift data given in Tables S1, S2 and S3).

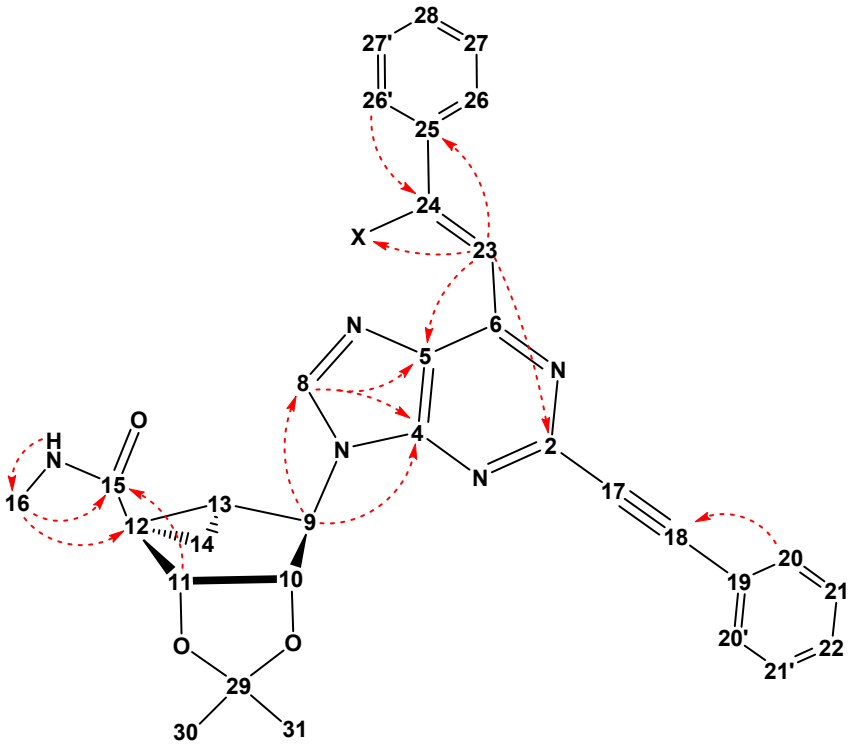


Table S1. Compound 32a, 298K, $CDCl_3$(δH =7.24, δC 77.2 ppm)

#	**δH,** multiplicity:*J* Hz	δC	**HMBC coupling to #**
2		158.0	
4		148.0	
5		128.2	
6		145.5	
8	7.82	141.5	2, 4, 5
9	4.82	61.8	4, 8, 10, 11, 12, 13, 14
10	4.85, dd:7.1:1.4	89.6	12, 13
11	5.78, dd:7.1:0.8	83.9	9, 12, 14, 15, 29
12		42.1	

13	1.97, ddd:9.4:5.1:1.5	35.6	10, 11, 15
14	1.66, ddd: 9.4:5.1:1.4 1.28, t:5.2	15.1	9, 11, 12, 13, 15 9, 11, 12, 13, 15
15	NH=7.37, q:5.1	171.2	15, 16
16	2.81, d:4.8	27.5	12, 15
17		89.7	
18		85.2	
19		122.2	
20	7.66	132.7	18, 20, 22
21	7.41	129.1	19, 20
22	7.42	129.9	20
23	5.77	89.1	X(CH_3), 2, 5, 6, 21, 25
24	NH=10.24, q:5.0 CH_3=3.0, d:5.4	163.6 32.6	23, 24(CH3), 25 24
25		136.9	
26	7.46	128.5	24, 26, 27, 28
27	7.42	128.8	25, 26
28	7.41	129.6	26
29		113.2	
30	1.28	24.8	29, 31
31	1.55	26.5	29, 30

Table S2. Compound 12, 298K, DMSO (δH =2.5, δC=39.5 ppm)

#	**δH,** multiplicity:*J* Hz	**δC(OH), δC(C=0)**	**HMBC coupling to #**
2		154.5, 156.0	
4		148.8, 150.4	
5		126.4, 132.4	
6		141.1, 144.6	
8	8.53	143.6, 145.3	2, 4, 5
9	4.85	61.4, 61.5	4, 8, 10, 11, 12, 13, 14
10	4.03, bt 0H=5.46	 76.3	9, 12, 13 9, 10, 11
11	5.01, t:6.3 OH=4.86	70.9	9, 12, 14, 15 10, 11, 12

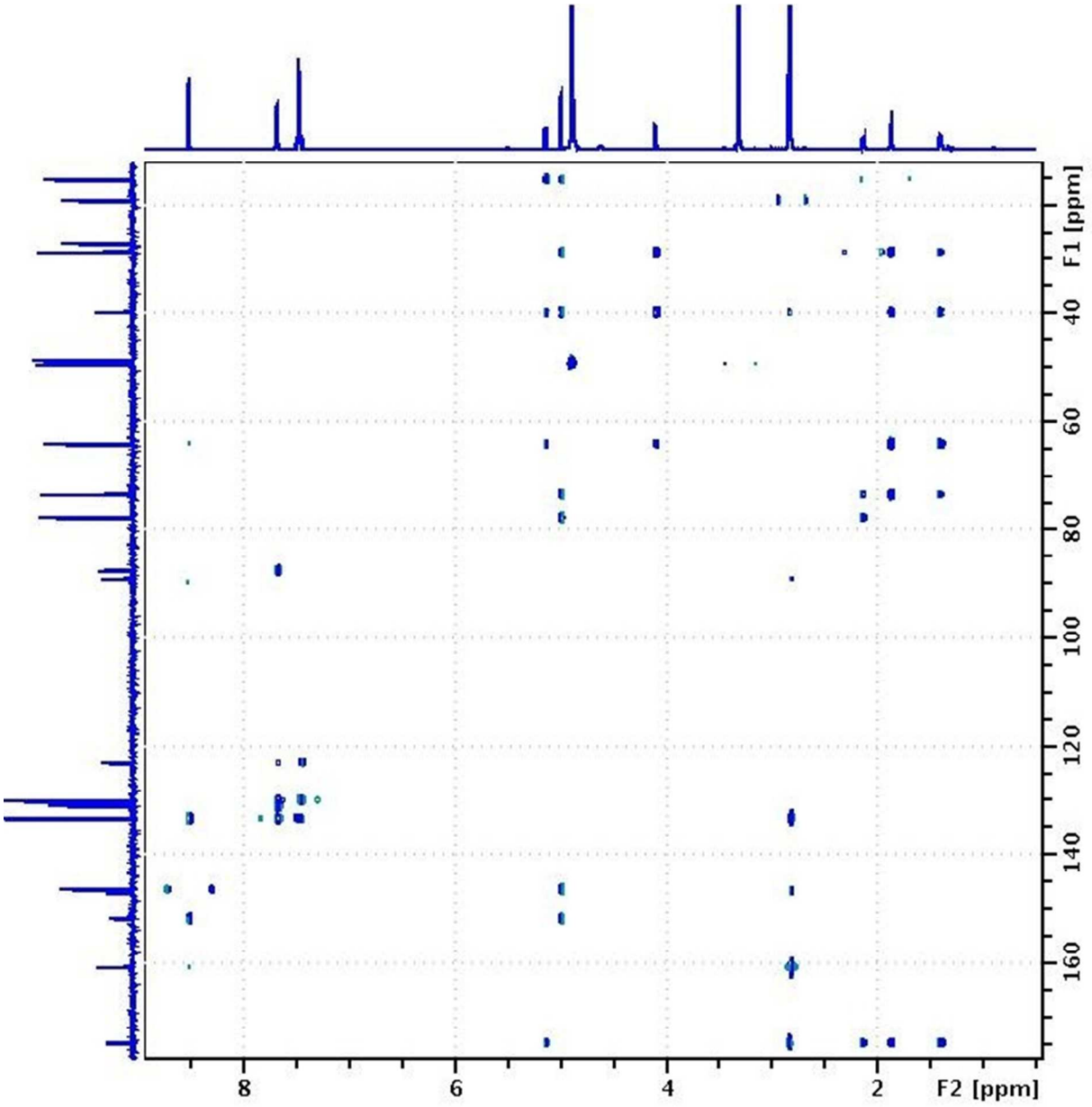
12		37.9	
13	1.94, dd:9.0:4.6	27.5	10, 11, 12, 14, 15
14	1.35, ddd: 9.0:4.6:1.5 1.65, t:4.6	13.9	9, 11, 12, 13, 14, 15 9, 11, 12, 13, 14, 15
15	NH=7.59	171.1	NH=15, 16
16	2.68	26.1	12, 15
17		86.89, 88.9	
18		86.94, 85.2	
19		120.2, 120.8	
20	7.76, dm:6.7	132.2, 132.0	18, 20, 21, 22
21	7.53	128.9	20, 21
22	7.55	130.2, 129.8	20, 22
23	6.76	88.0, 42.8	2, 6, 24, 25, 26
24	OH: 4.93	170.3, 194.5	OH: 2, 5, 24
25		135.0, 136.2	
26	7.99	126.0, 128.4	24, 25, 26, 27, 28
27	7.53, dm:7.4	128.8	
28	7.56	131.0, 133.6	

Table S3. Compound 15, 298K, MeOD (δH =3.31, δC=49.2 ppm)

#	**δH,** multiplicity:*J* Hz	**δC**	**HMBC coupling to #**
2		160.8	
4		151.8	
5		133.5	
6		147.2	
8	8.51	146.5	2, 4, 5, 6, 9, 17
9	4.99	64.2	4, 8, 10, 11, 12, 13, 14
10	4.10, dt:6.6:0.9	77.9	9, 12, 13, 14
11	5.14, dd:6.3:1.3	73.5	9, 10, 12, 14, 15
12		39.9	
13	2.14, ddd:9.0:4.9:1.3	28.9	10, 11, 12, 14, 15
14	1.40, ddd: 9.0:5.1:1.7 1.87, t:4.9	15.5	9, 11, 12, 13, 15 9, 11, 12, 13, 15

15		174.9	
16	2.84	27.3	12, 15
17		89.3	
18		87.6	
19		123.1	
20	7.68	133.5	18, 19, 20, 21, 22
21	7.46	130.1	19, 20, 21
22	7.47	131.1	20, 21
23	2.81	19.3	2, 6, 17, 20

Figure S4. HMBC spectrum of 15.



Most protonated carbon and ^{1}H-spin system assignments were relatively straightforward via HSQC and COSY/TOCSY, respectively, for **32a** and **15**, with ^{13}C-coupled and HMBC experiments providing additional information for overlapping resonances such as 15's #5 and #20 carbons. HMBC also provided the remaining pertinent information for the quaternary carbons. Notably, the purine #8 proton of **12** coupled to each ^{13}C in its ring and to that of the

#17 acetylene carbon. The structurally important couplings are shown with arrows in Figure S4, with all couplings listed in Tables S1-S3.

Compound **12** was a keto/enol mixture that was not exchanging on the chemical shift time scale. The ^{13}C-shift column in Table 2 shows the enol resonances first (major component, 60%) and then the keto resonances if they were assignable. Every aromatic ^{13}C and ^{1}H resonance as well as #9 shifted with tautomerization. Figures S5A and S5B show the ^{1}H and ^{13}C DMSO spectra of **12**, with examples of the enol/keto shifts in the insets.

Figure S5A. Compound 12 ^{1}H spectra in DMSO-*d*6

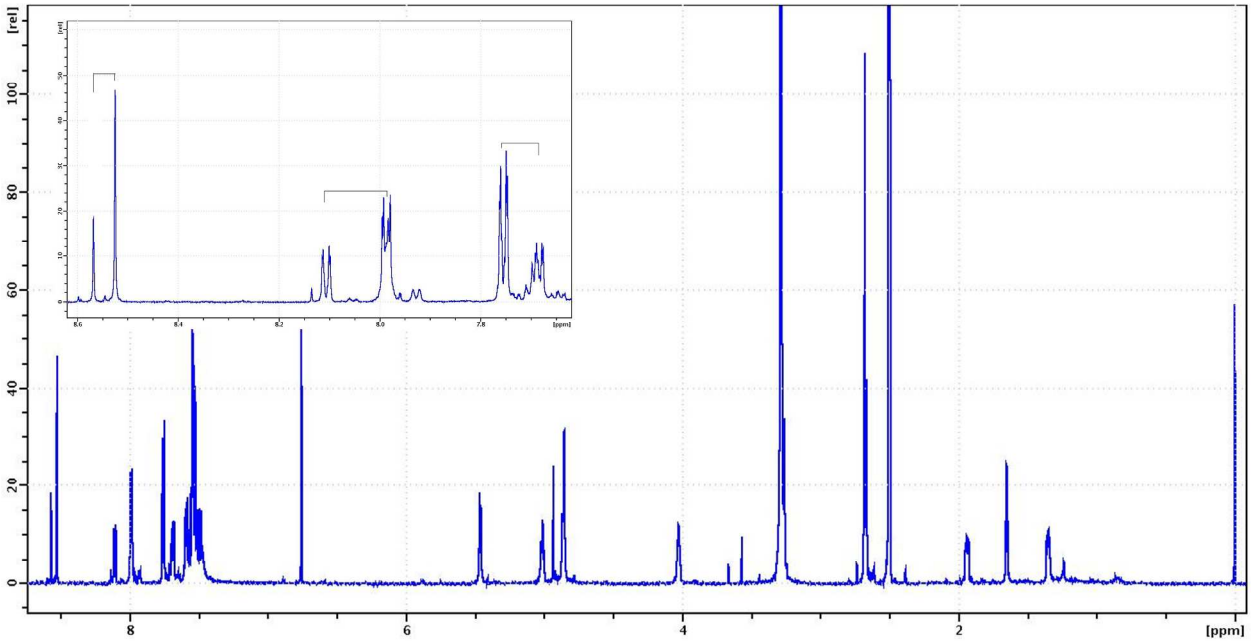
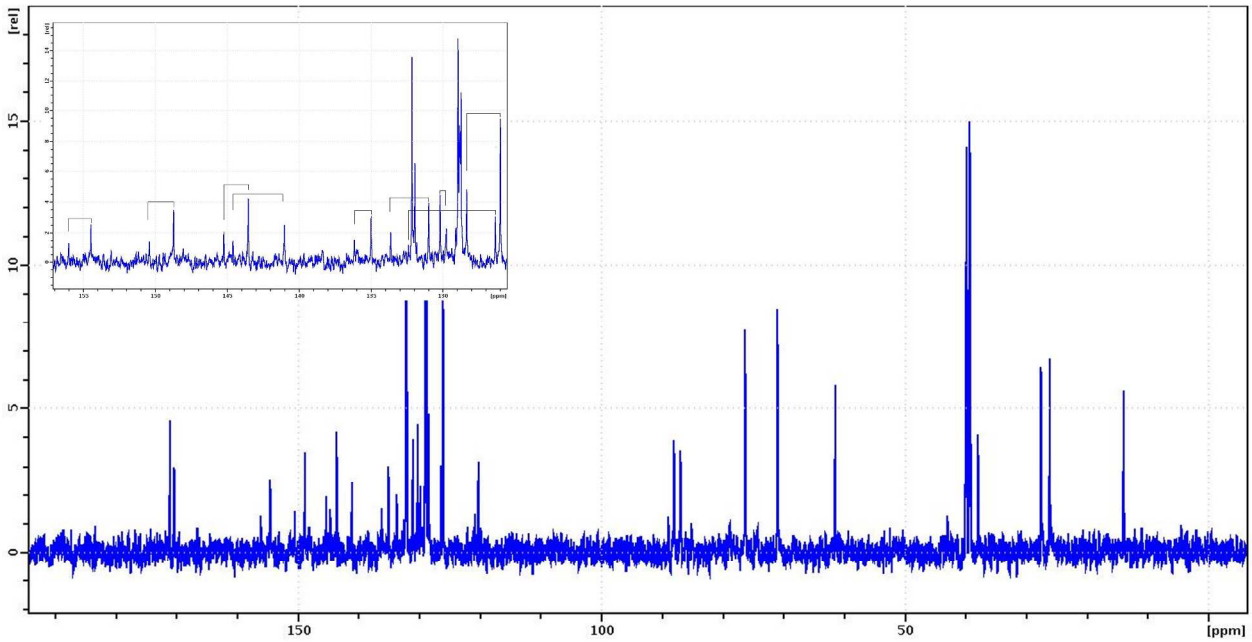
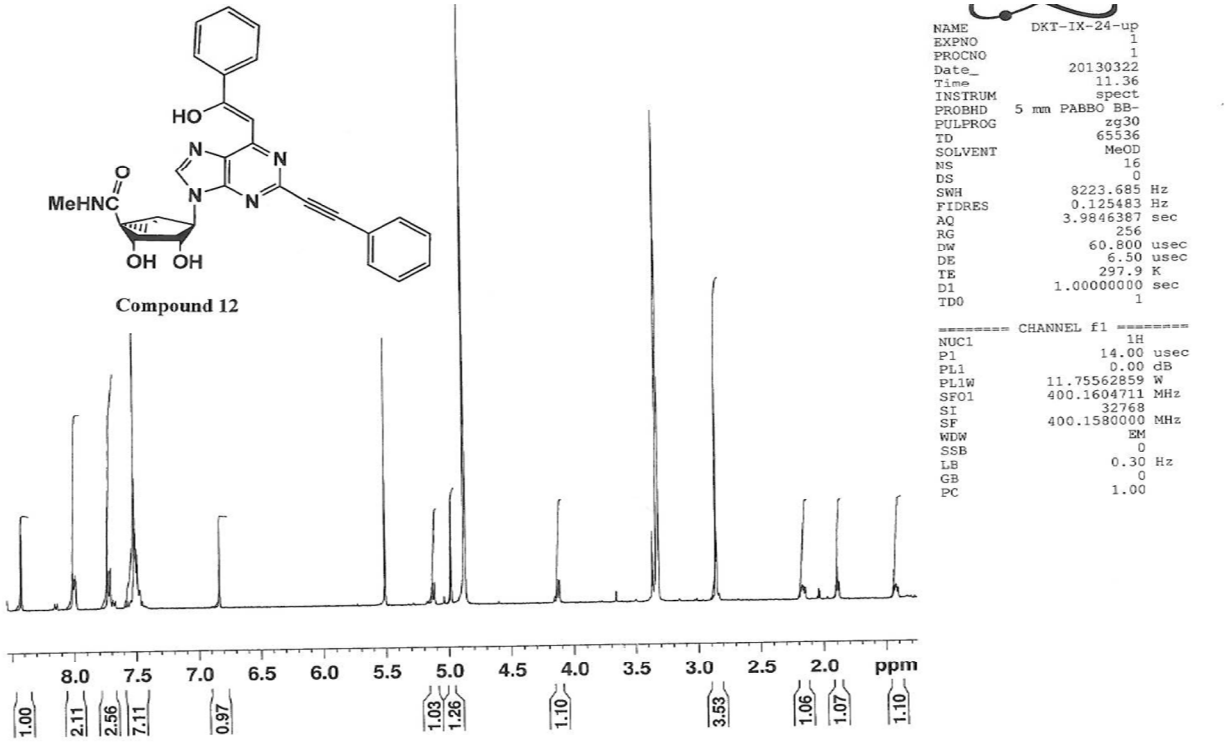


Figure S5B. Compound 12 ^{13}C spectra in DMSO-*d*6.



Selected NMR and mass spectra

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D1                      1.00000000 sec
TD0                            1

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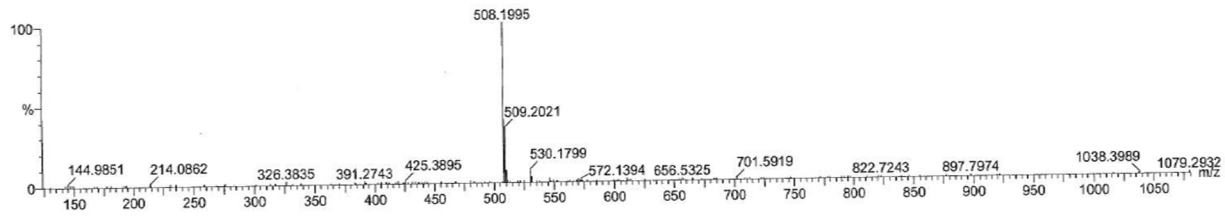

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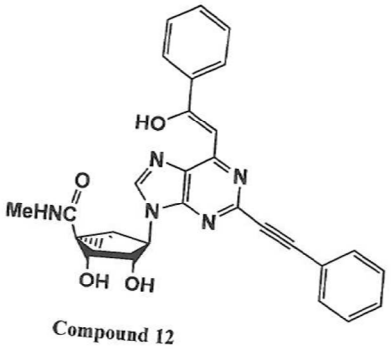
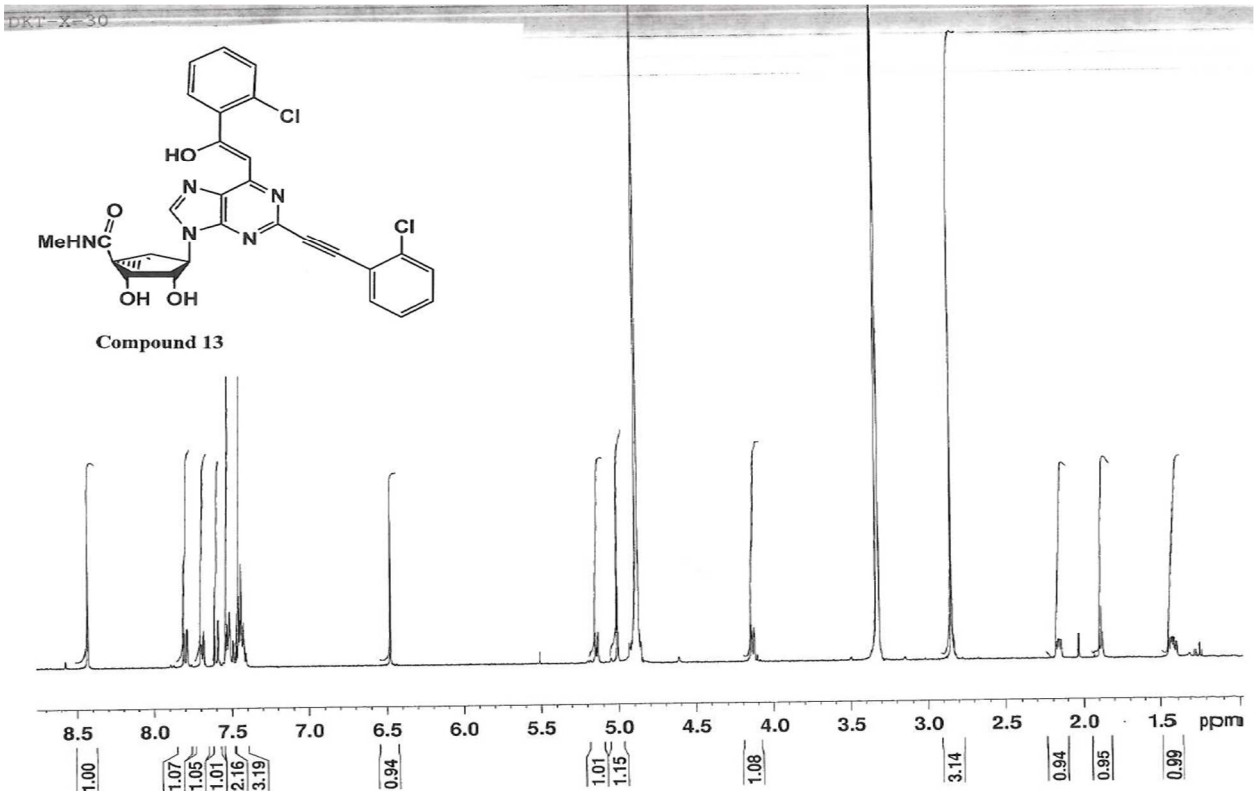
	508.1971	2.4	4.7	14.5	0.2	C28 H30 N O8
	508.2025	-3.0	-5.9	23.5	0.5	C34 H26 N3 O2
	508.2084	-8.9	-17.5	14.5	0.6	C27 H30 N3 O7
	508.1913	8.2	16.1	23.5	0.8	C35 H26 N O3
	508.2065	-7.0	-13.8	27.5	2.1	C39 H26 N



Compound 12



Compound 13

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13-Sep-2013
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TOF MS ES+
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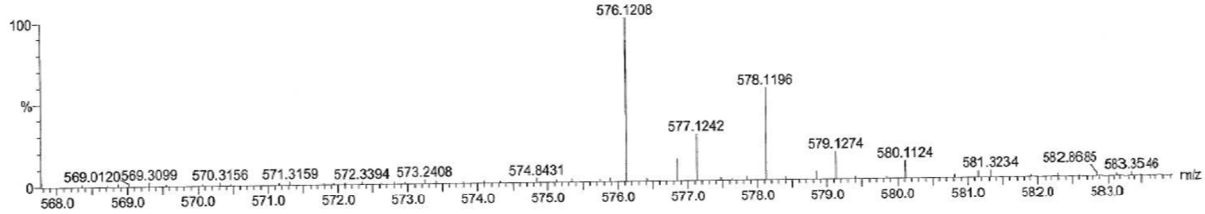

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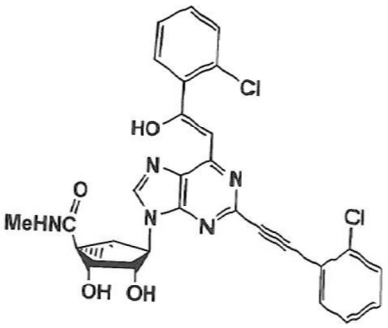
	576.1170	3.8	6.6	-2.5	5.5	C11 H32 N5 O17 Cl2
	576.1264	-5.6	-9.7	10.5	1.4	C22 H28 N5 O9 Cl2
	576.1112	9.6	16.7	6.5	2.3	C18 H28 N5 O12 Cl2



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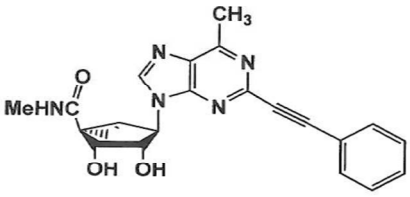

Compound 15

BRUKER

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FIDRES	0.125483 Hz
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RG	256
DW	60.800 usec
DE	6.50 usec
TE	297.6 K
D1	1.00000000 sec
TD0	1
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PL1W	11.75562859 W
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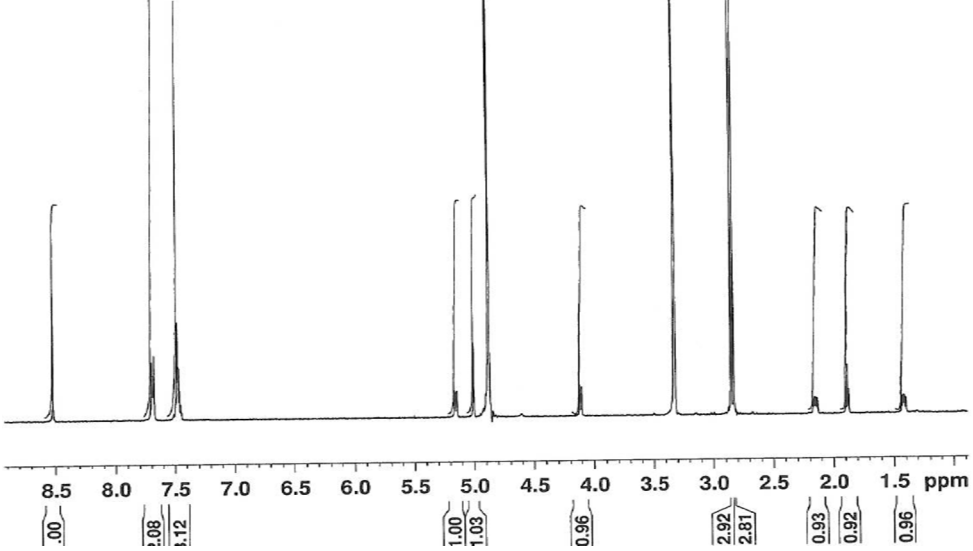
SF	400.1580000 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00



Monoisotopic Mass, Even Electron Ions
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22-Mar-2013 12:52:29
TOF MS ES+

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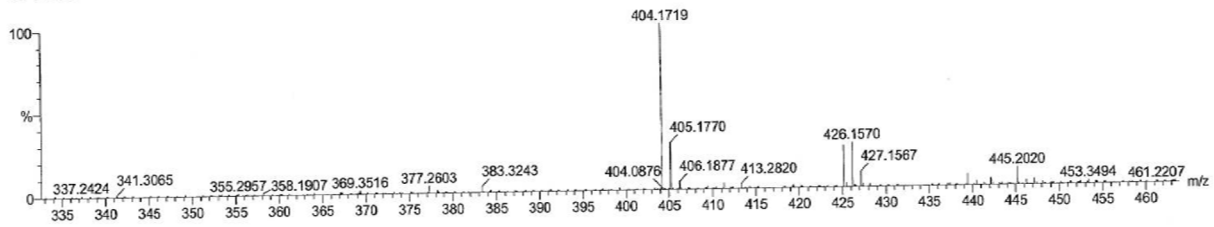

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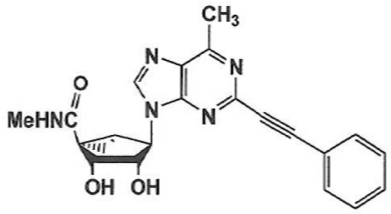
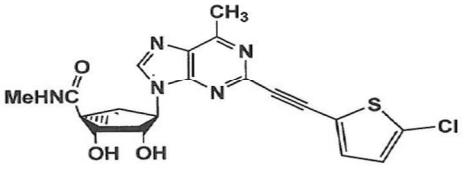
	404.1763	-4.4	-10.9	18.5	0.5	C27 H22 N3 O
	404.1651	6.8	16.8	18.5	0.8	C28 H22 N O2



Compound 15

DKT-XIII-18-1



Compound 19

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18-Jun-2015
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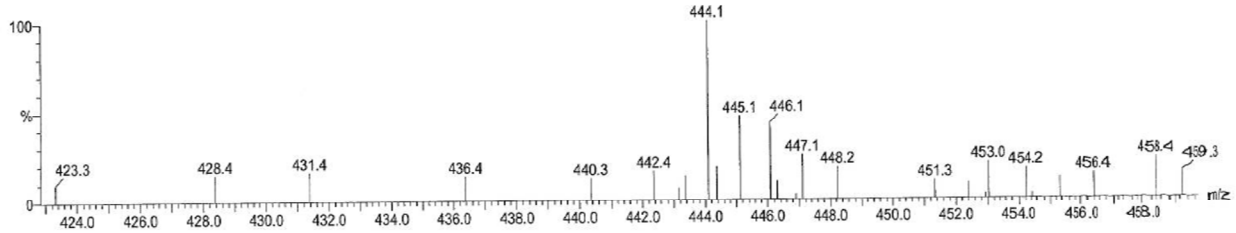

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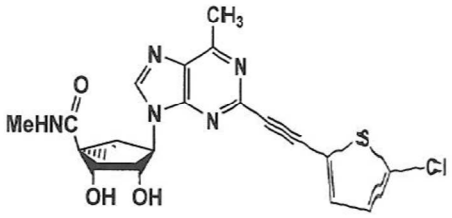
	444.0956	-5.7	-12.8	4.5	273.3	C13 H23 N5 O8 35Cl 32S
	444.0803	9.6	21.6	0.5	310.0	C9 H23 N5 O11 35Cl 32S
	444.1050	-15.1	-34.0	17.5	205.8	C24 H19 N5 35Cl 32S
	444.0745	15.4	34.7	9.5	261.5	C16 H19 N5 O6 35Cl 32S
	444.1108	-20.9	-47.1	8.5	252.3	C17 H23 N5 O5 35Cl 32S
	444.0686	21.3	48.0	18.5	224.5	C23 H15 N5 O 35Cl 32S



Compound 19

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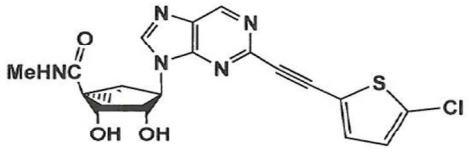

Compound 21

Monoisotopic Mass, Even Electron Ions
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13-Jul-2015
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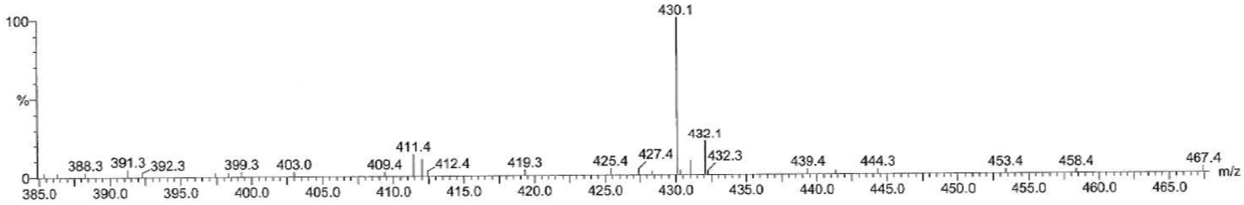
TOF MS ES+
2.66e+003



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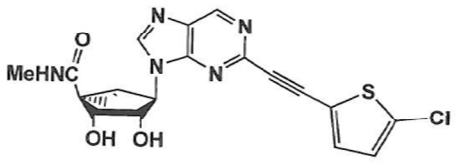
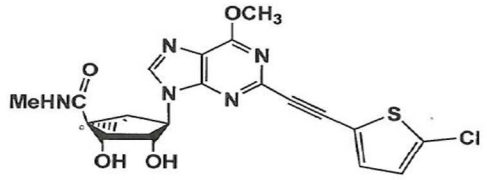
	430.0799	-6.5	-15.1	4.5	206.5	C12 H21 N5 O8 S 35Cl
	430.0647	8.7	20.2	0.5	146.0	C8 H21 N5 O11 S 35Cl
	430.0588	14.6	33.9	9.5	282.0	C15 H17 N5 O6 S 35Cl
	430.0893	-15.9	-37.0	17.5	470.0	C23 H17 N5 S 35Cl
	430.0529	20.5	47.7	18.5	459.3	C22 H13 N5 O S 35Cl
	430.0952	-21.8	-50.7	8.5	313.6	C16 H21 N5 O5 S 35Cl



Compound 21

DKT-XIII-58

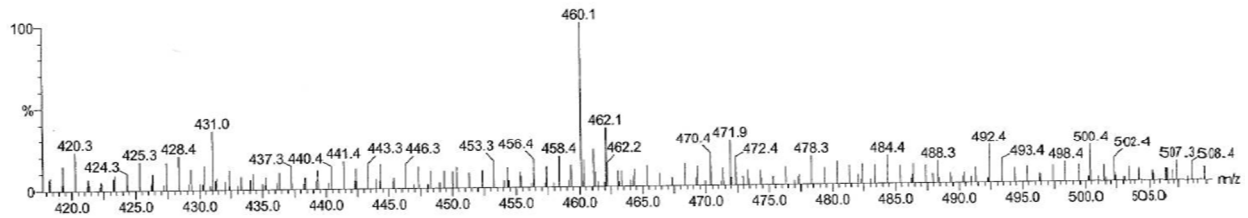


Compound 22

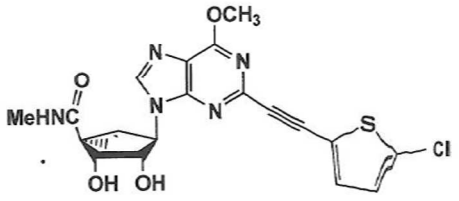
Monoisotopic Mass, Even Electron Ions
72 formula(e) evaluated with 3 results within limits (up to 19 closest results for each mass)
Elements Used:
C: 0-100 H: 0-200 N: 5-5 O: 0-40 32S: 1-1 35Cl: 1-1

14-Aug-2015
dkt-14aug15-xiii-58 67 (1.239) Cn (Cen,5, 50.00, Ar); Sm (SG, 1x2.00); Sb (12,5.00)

TOF MS ES+
1.86e+003



Minimum:				-2.0		
Maximum:		10.0	10.0	500.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
460.0852	460.0846	0.6	1.3	13.5	269.2	C20 H19 N5 O4 32S 35Cl
	460.0905	-5.3	-11.5	4.5	286.4	C13 H23 N5 O9 32S 35Cl
	460.0752	10.0	21.7	0.5	313.3	C9 H23 N5 O12 32S 35Cl



Compound 22