Structure-based Design of Potent Bcl-2/Bcl-xL Inhibitors with Strong *in vivo* Antitumor Activity

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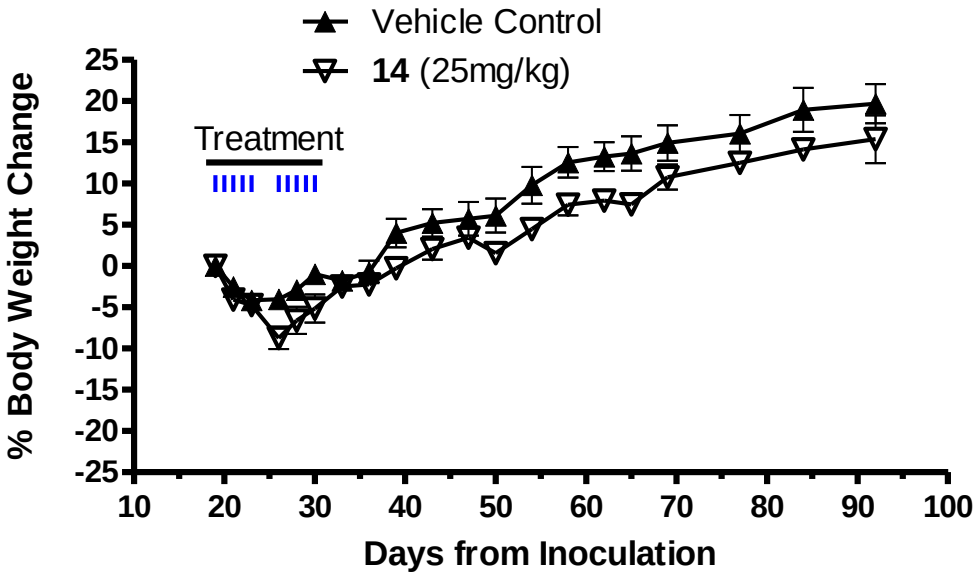


Figure S1. Animal weight change for each group as compared to the animal weights before the start of the treatment.

Table S1: Data Collection and Refinement Statistics

Data Collection	**Bcl-xL-12**
Space Group	$P2_12_12_1$
Unit Cell a, b, c (Å)	41.627, 54.891, 79.569
Wavelength (Å)	0.97857
Resolution (Å)[1]	1.4 (1.42 – 1.4)
Rsym (%)[2]	5.3 (45.8)
<I/sI>[3]	20 (5)
Completeness (%)[4]	94.5 (91.2)
Redundancy	7.0 (7.0)
Refinement	
Resolution (Å)	1.4
R-Factor (%)[5]	0.1641
Rfree (%)[6]	0.1847
Protein atoms	1271
Water Molecules	175
Unique Reflections	34821
R.m.s.d.[7]	
Bonds	0.01
Angles	0.97
MolProbity Score[8]	4.97
Clash Score[8]	1.4
RSR of compound[9]	0.061
RSCC of compound[9]	0.972
PDB ID	3SP7

[1]Statistics for highest resolution bin of reflections in parentheses.
[2]$R_{sym} = \Sigma_h\Sigma_j \, l \, I_{hj} - \langle I_h \rangle \, l \, / \Sigma_h\Sigma_j I_{hj}$, where I_{hj} is the intensity of observation j of reflection h and $\langle I_h \rangle$ is the mean intensity for multiple recorded reflections.
[3]Intensity signal-to-noise ratio.
[4]Completeness of the unique diffraction data.
[5]R-factor = $\Sigma_h \, I \, IF_oI - IF_cI \, I \, / \, \Sigma_h IF_oI$, where F_o and F_c are the observed and calculated structure factor amplitudes for reflection h.
[6]R_{free} is calculated against a 10% random sampling of the reflections that were removed before structure refinement.
[7]Root mean square deviation of bond lengths and bond angles.
[8]Molprobity Server.[1]
[9]Predeposition Electron Density Server.[2]

Table S2: Purity data of all the biologically evaluated compounds.

Compound	Retention time, t_R (min)	% purity
8	3.64	> 99
9	4.40	97
10	4.18	98.5
11	5.05	97.7
12	5.05	> 99
13	6.01	> 99
14	5.26	> 99
15	5.41	> 99
16	5.32	> 99

The purity of the biologically evaluated compounds was determined by Waters ACQUITY UPLC. The conditions of the UPLC were as follow:

Eluents: solvent A (0.1% of TFA in water) and solvent B (0.1% of TFA in CH_3CN).

Column: ACQUTIY UPLC BEH C18 column (2.1X50 mm, 1.7 µm).

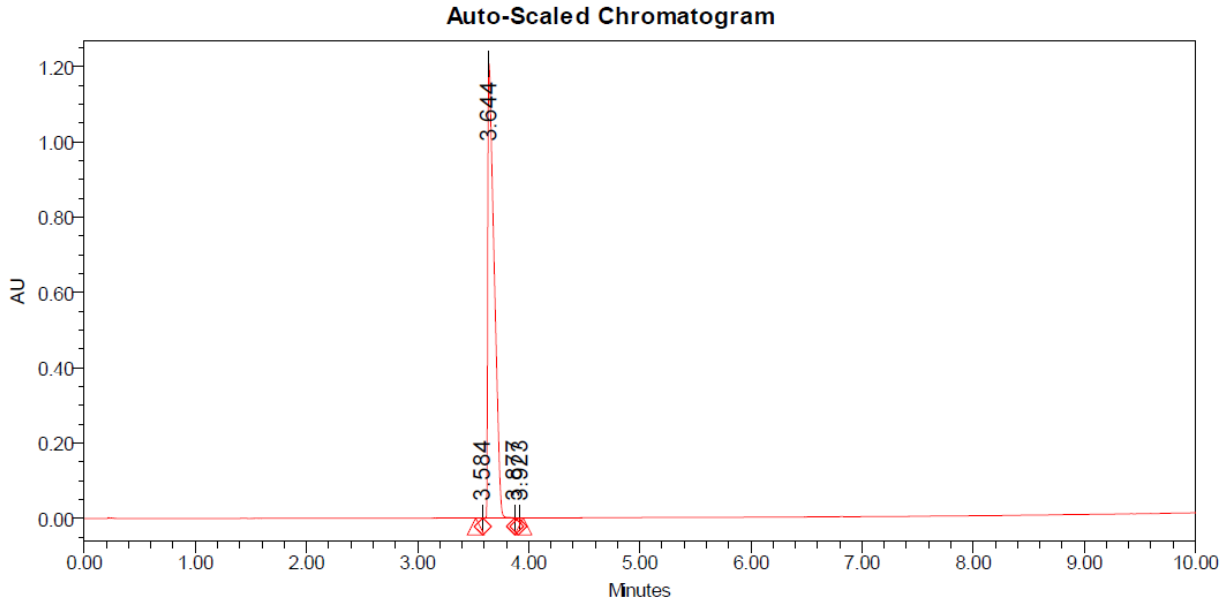
Method: 10% B to 100% B in 10 min.

Flow rate: 0.61 mL/min.

Detector: UV 254 nm

Run time: 10 min

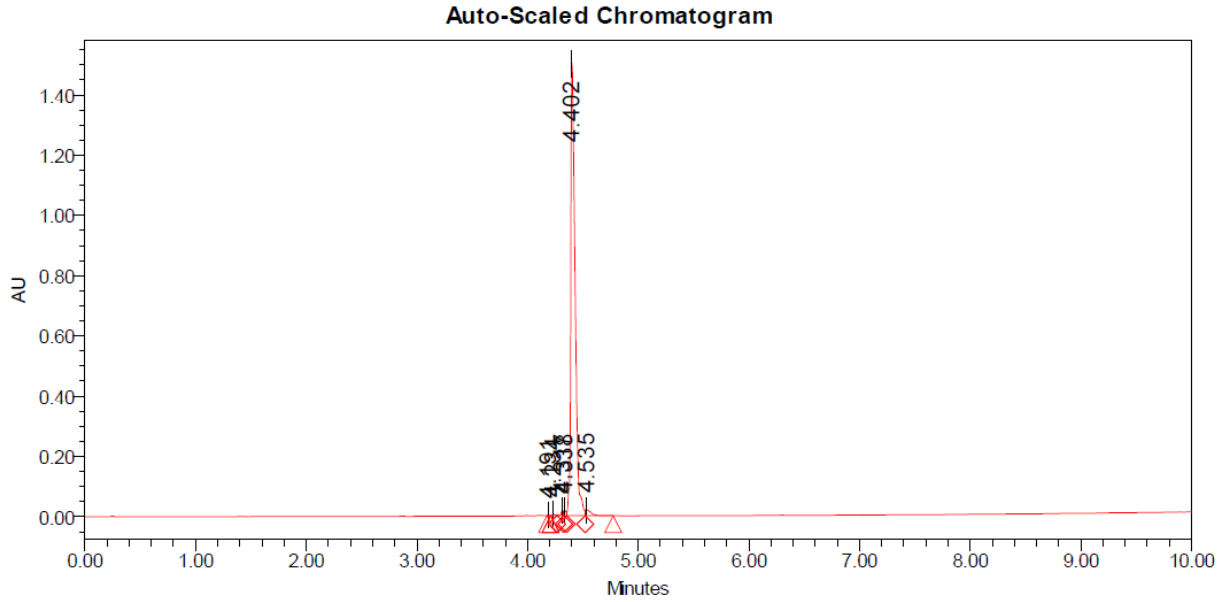
Figure S2. UPLC analysis of compound **8**.



Processed Channel: PDA 254.0 nm

	Retention Time (min)	Area	% Area
1	3.584	256	0.01
2	3.644	4815500	99.98
3	3.877	344	0.01
4	3.923	248	0.01

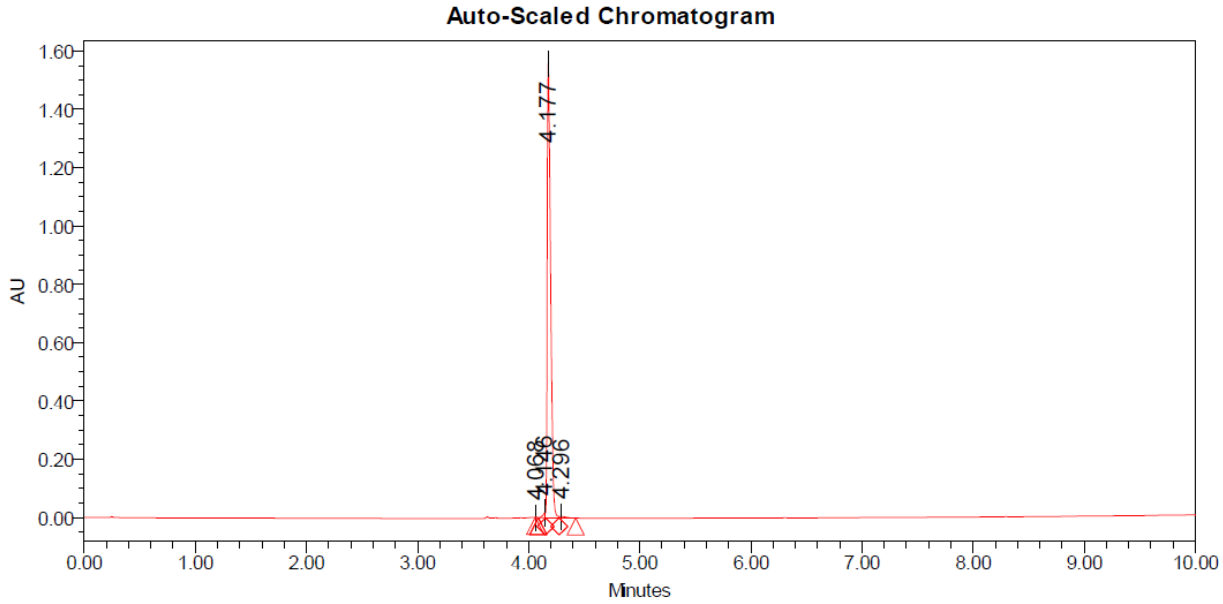
Figure S3. UPLC analysis of compound **9**.



Processed Channel: PDA 254.0 nm

	Retention Time (min)	Area	% Area
1	4.191	588	0.01
2	4.234	6838	0.16
3	4.317	25745	0.62
4	4.338	21326	0.51
5	4.402	4045704	97.00
6	4.535	70465	1.69

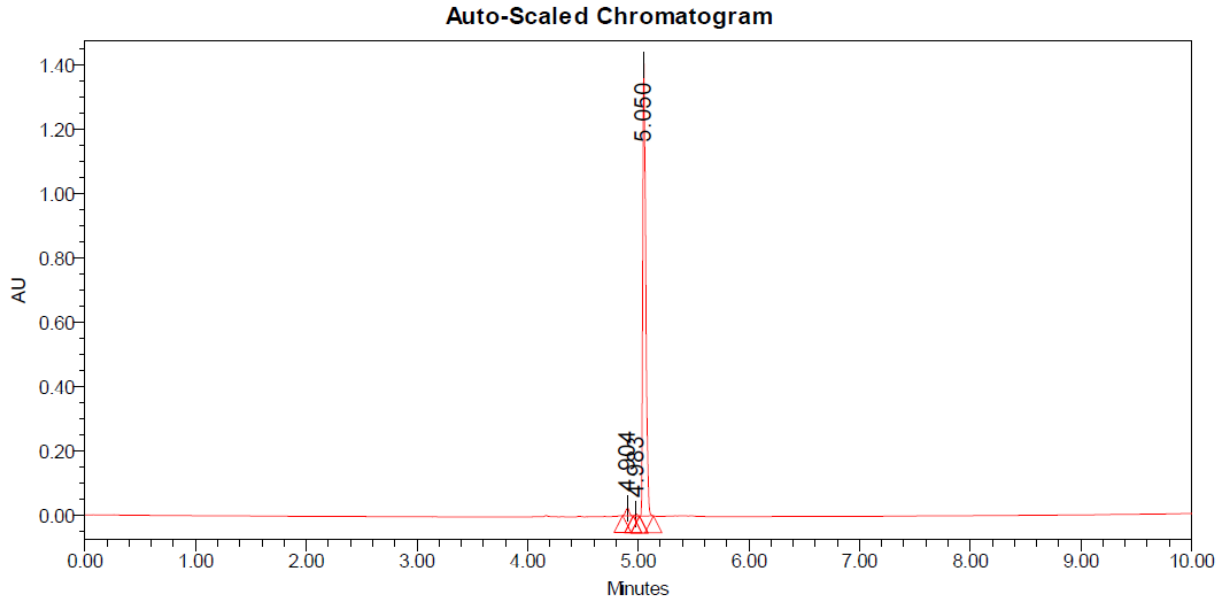
Figure S4. UPLC analysis of compound **10**.



Processed Channel: PDA 254.0 nm

	Retention Time (min)	Area	% Area
1	4.068	215	0.01
2	4.146	27480	0.81
3	4.177	3325686	98.54
4	4.296	21716	0.64

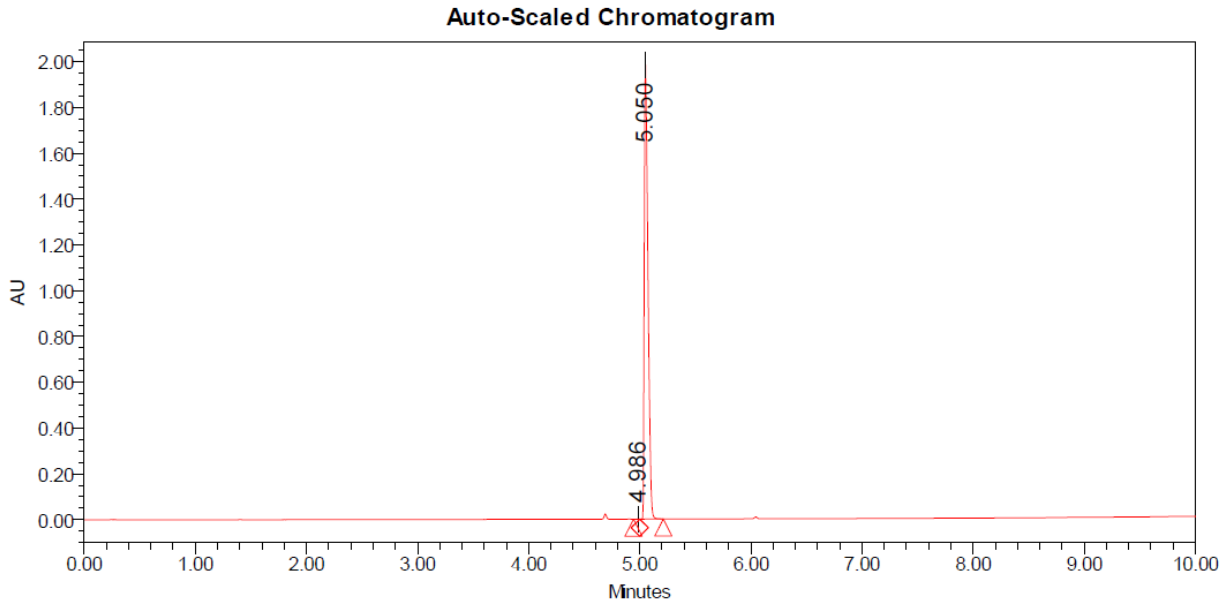
Figure S5. UPLC analysis of compound **11**.



Processed Channel: PDA 254.0 nm

	Retention Time (min)	Area	% Area
1	4.904	53922	1.97
2	4.983	8661	0.32
3	5.050	2670582	97.71

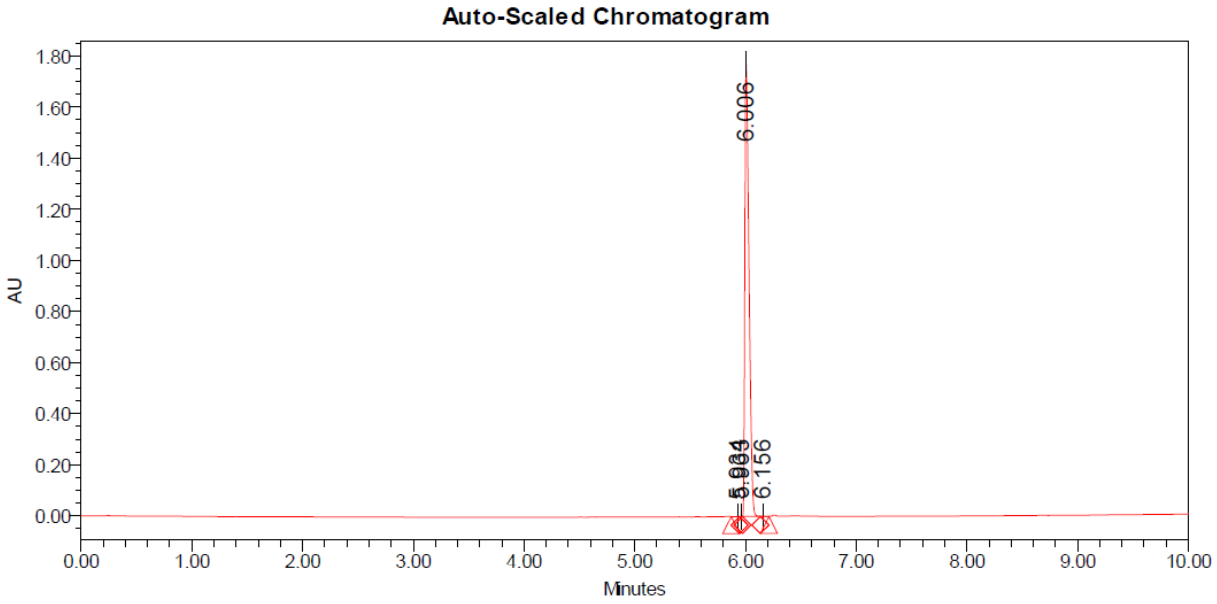
Figure S6. UPLC analysis of compound **12**.



Processed Channel: PDA 254.0 nm

	Retention Time (min)	Area	% Area
1	4.986	780	0.02
2	5.050	4613662	99.98

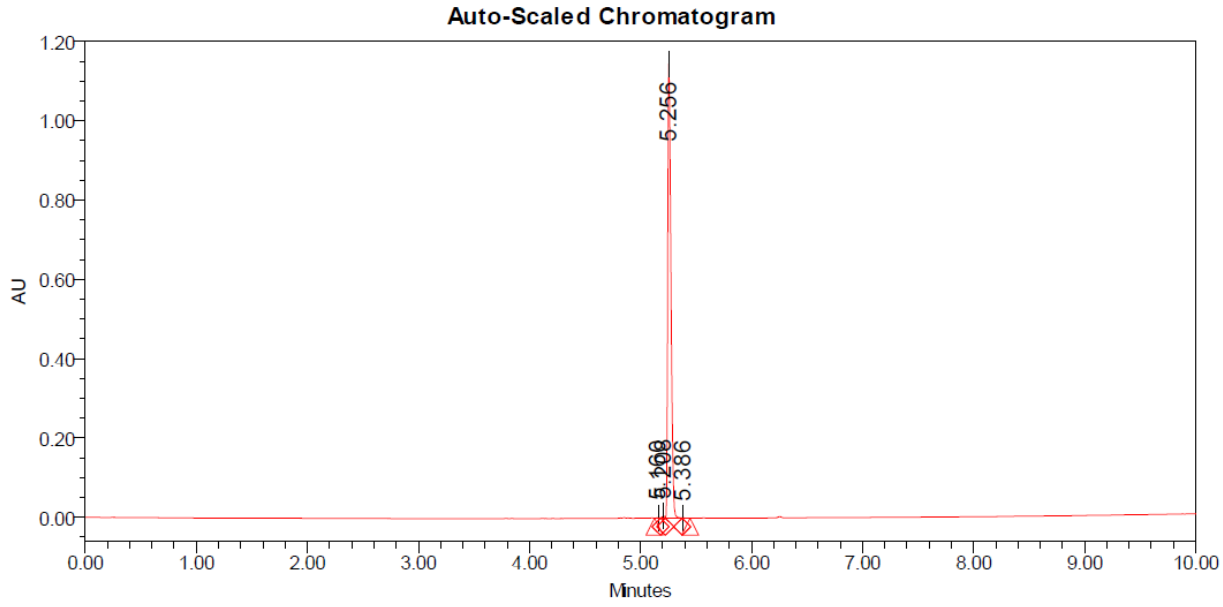
Figure S7. UPLC analysis of compound **13**.



Processed Channel: PDA 254.0 nm

	Retention Time (min)	Area	% Area
1	5.934	1846	0.04
2	5.963	1192	0.03
3	6.006	4592906	99.91
4	6.156	1158	0.03

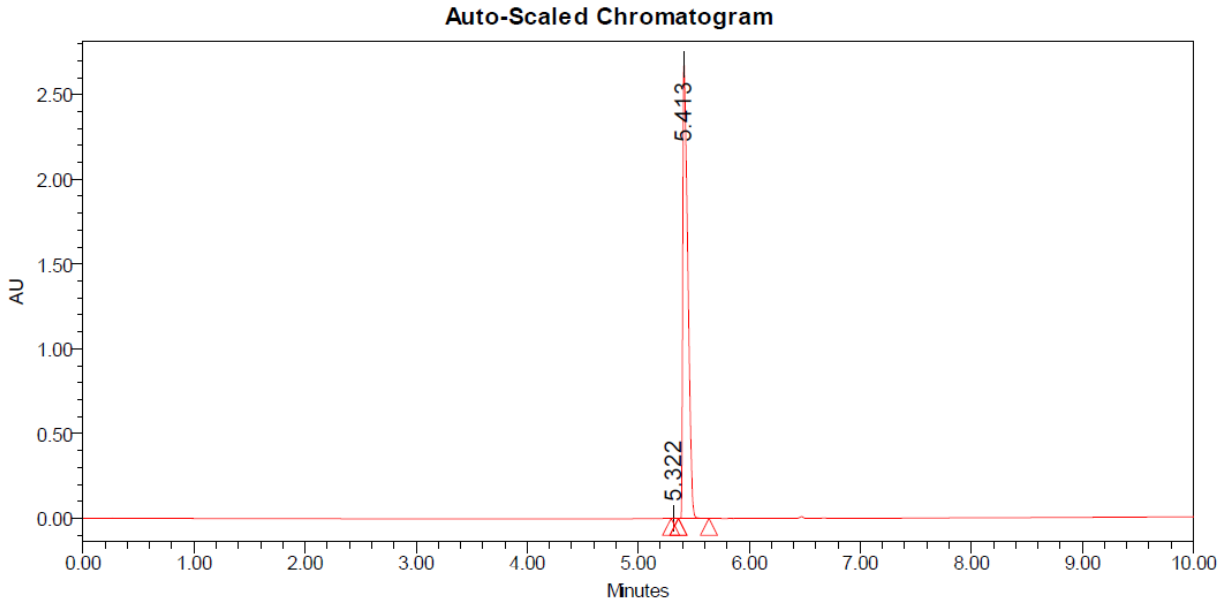
Figure S8. UPLC analysis of compound **14**.



Processed Channel: PDA 254.0 nm

	Retention Time (min)	Area	% Area
1	5.160	2135	0.09
2	5.208	8207	0.36
3	5.256	2253195	99.49
4	5.386	1258	0.06

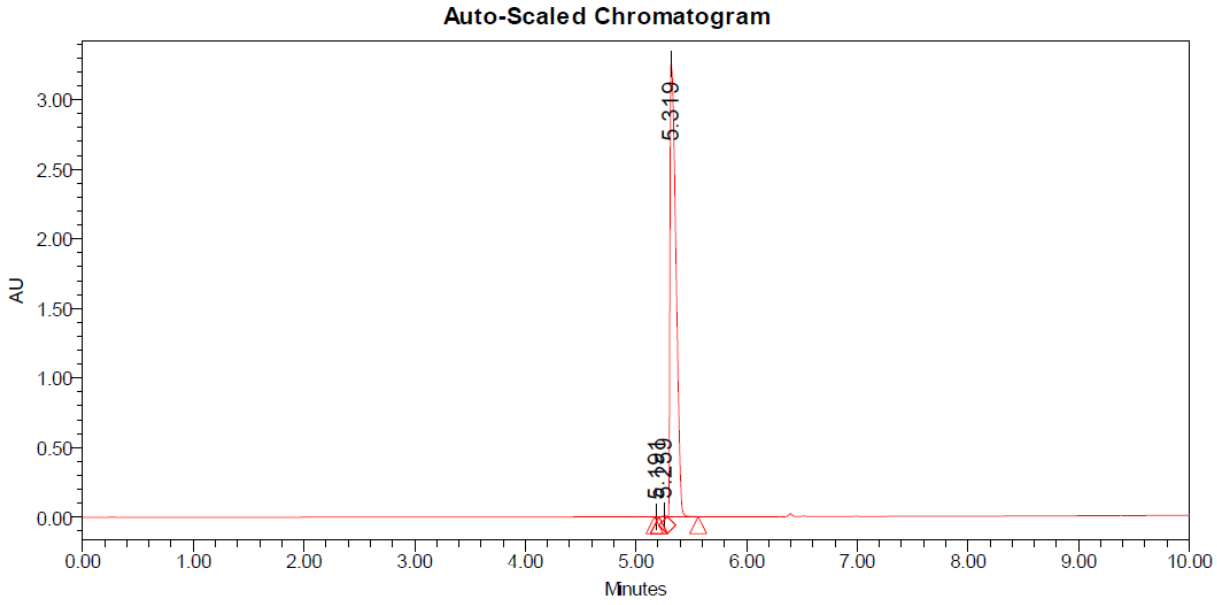
Figure S9. UPLC analysis of compound **15**.



Processed Channel: PDA 254.0 nm

	Retention Time (min)	Area	% Area
1	5.322	3412	0.04
2	5.413	8442254	99.96

Figure S10. UPLC analysis of compound **16**.



Processed Channel: PDA 254.0 nm

	Retention Time (min)	Area	% Area
1	5.191	224	0.00
2	5.259	11709	0.10
3	5.319	12105593	99.90

References:

1. Davis, I. W.; Leaver-Fay, A.; Chen, V. B.; Block, J. N.; Kapral, G. J.; Wang, X.; Murray, L. W.; Arendall, W. B., 3rd; Snoeyink, J.; Richardson, J. S.; Richardson, D. C. MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. *Nucleic Acids Res* **2007,** 35, W375-83.
2. Kleywegt, G. J.; Harris, M. R.; Zou, J. Y.; Taylor, T. C.; Wahlby, A.; Jones, T. A. The Uppsala Electron-Density Server. *Acta Crystallogr D Biol Crystallogr* **2004,** 60, 2240-9.