Supporting Information

Discovery of a non-peptidic inhibitor of West Nile virus NS3 protease by high-throughput docking

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Contents

1 Structures and affinities of 37 known inhibitors

Table S-I: Peptide aldehyde inhibitors of WNV NS2B-NS3pro and binding affinity measured as in Knox et al., J. Med. Chem. **49**, 6585, 2006[a]

ID	Structure	no. of formal charges	IC_{50} (μM)	ΔG (kcal/mol)
1	Bz-Nle-Lys-Arg-Arg-H	3	4.1	-7.39
2	Bz-Nle-Lys-Lys(Z)-Arg-H	2	99.5	-5.49
3	Bz-Nle-Lys-Gln-Arg-H	2	1.7	-7.9
4	Bz-Nle-Lys-Lys-Arg-H	3	1.9	-7.86
5	Ac-Lys-Lys-Arg-H	3	**0.4**	-8.84
6	Bz-Lys-Arg-Arg-H	3	1.5	-7.99
7	Bz-Lys-Lys(Tos)-Arg-H	2	117.9	-5.39
8	Ac-Lys-Lys(Tos)-Arg-H	2	**463.4**	-4.58
9	Ac-Lys-Lys(Bz)-Arg-H	2	116.5	-5.4
10	Bz-Lys-(p-Me)Phe-Arg-H	2	194.3	-5.09
11	Bz-Lys-Lys(Bz)-Arg-H	2	68.1	-5.72
12	indole-Lys-Arg-Arg-H	3	2.4	-7.72
13	Bz-Lys-Asn-Arg-H	2	71.8	-5.69
14	Bz-Nle-Ala-Arg-Arg-H	2	3.8	-7.44
15	Bz-Ala-Lys-Arg-Arg-H	3	0.7	-8.45
16	Bz-Nle-Lys-Arg-Phe-H	2	109.8	-5.43
17	Bz-Nle-Lys-Phe-Arg-H	2	108	-5.44
18	Bz-Nle-Phe-Arg-Arg-H	2	4.2	-7.38
19	Bz-Phe-Lys-Arg-Arg-H	3	1.2	-8.14
20	Bz-Lys-Arg-Tyr-H	2	14.6	-6.64
21	Ac-Lys-Arg-Arg-H	3	0.5	-8.6
22	pyridine-Lys-Arg-Arg-H	3	0.8	-8.4
23	isoquinoline-Lys-Arg-Arg-H	3	0.6	-8.56
24	pyrazine-Lys-Arg-Arg-H	3	1.1	-8.18
25	3-pyridyl-Lys-Arg-Arg-H	3	1	-8.24
26	Bzl-Nle-Lys-Arg-(4-CN)-Phe-H	2	62	-5.77
27	Bzl-Nle-Lys-Arg-Trp-H	2	10	-6.86
28	Bz-Nle-Lys-Arg-Lys-H	3	57.7	-5.82
29	BZ-Nle-Lys-Arg-(4-guanidinyl)-Phe-H	3	11.8	-6.76
30	Bz-Nle-Lys-Arg-His-H	2	43.1	-5.99
31	Bz-Nle-Lys-Arg-Phg-H	2	90.9	-5.55
32	Bz-Arg-Arg-H	2	3.9	-7.42
33	Bz-Lys-Arg-H	2	1.4	-8.03
34	Bz-Arg-Lys-H	2	57.5	-5.82
35	Bz-Lys-Arg-Phe-H	2	71.1	-5.69
36	Bz-Lys-Arg-(P-Me)Phe-H	2	17.7	-6.52
37	Bz-Lys-Arg-Tyr(Bn)-H	2	11.8	-6.76

[a] Weakest and strongest affinities are in bold.

2 Binding studies by NMR spectroscopy

The binding of compounds to the WNV NS2B-NS3 protease (henceforth called protease) was assessed by NMR spectroscopy using 50 mM stock solutions of the compounds prepared by dissolution in DMSO-d_6. For each compound, the following NMR spectra were measured at 800 MHz and 25 °C.

1) A 1D ^{1}H NMR spectrum in D_2O to check the spectral appearance of the compound and to assess its aggregation state (narrow signals are expected for the monomeric state). The samples were prepared by adding 1 µl of stock solution to 200 µl D_2O, resulting in a compound concentration of about 0.25 mM.

2) A 1D ^{1}H NMR spectrum of ^{15}N-labeled protease in the presence of an about 3-fold excess of compound. The samples were prepared by adding 3 µl of stock solution to 200 µl of a 0.23 mM solution of protease in 90% H_2O/10% D_2O, 20 mM HEPES buffer, pH 7.0, 2 mM DTT. Any compound binding to the protease displayed broad signals because (i) transverse relaxation is faster in the complex with the macromolecule and (ii) chemical exchange between bound and free compound results in exchange broadening. In the limit of slow exchange between bound and free compound, narrow signals would be expected for the excess of free compound. This case was not observed in the present study, as compounds displaying narrow signals in the presence of the protease failed to change the ^{15}N-HSQC spectrum of the protease (see below).

3) A 2D ^{15}N-HSQC spectrum of the ^{15}N-labeled protease in the presence of compound using the sample described above. Binding of a compound was classified as non-specific, if the spectrum of the compound (spectrum 2) displayed line broadening but no chemical shift changes could be detected in the ^{15}N-HSQC spectrum. Binding of the compound was classified as very weak, if spectrum 2 displayed line broadening and any chemical shift changes in the ^{15}N-HSQC spectrum were small (<0.03 ppm in the ^{1}H dimension and <0.15 ppm in the ^{15}N dimension). Notably, some of these changes could have arisen from small pH changes caused by some to the compounds. Changes in pH were also evidenced by small chemical shift changes of the signals of small amounts of residual imidazol present in the samples that originated from the purification protocol of the His-tagged protease.

Only compounds **1** and **2** changed the appearance of the ^{15}N-HSQC spectrum substantially, indicating significant binding affinity and specific binding. The ^{15}N-HSQC cross peaks of the NS3 residues T52, T53, E101, and K104 (at 112.7/7.22, 105.8/6.50, 124.4/7.38, and 118.0/7.54 ppm, respectively) were well resolved in the spectrum of the apo-protein and their chemical shifts relatively sensitive to the presence of compounds.

Table S-II: Binding of compounds assessed by NMR spectroscopy of 22 compounds

Compound number	Broadening of the compound signals in spectrum 2	Chemical shift changes in the ^{15}N-HSQC spectrum	Binding
1	yes[1]	significant	yes
2	yes[1]	significant	yes
3	yes[1]	no	non-specific
4	yes	very small	very weak
5	no	no	no
6	yes[1]	no	non-specific
7	no	no	no
8	yes	very small	very weak
9	yes[1]	no	non-specific
10	no	very small[2]	no
11	no	no	no
12	no	no	no
13	no	no	no
14	no	no	no
15	no	no	no
16	no	very small[2]	no
17	no	no	no
18	yes	very small	very weak
19	yes[1]	significant but small	weak
20	yes[1]	no	non-specific
21[3]	yes[1]	no	at most very weak
22	yes[1]	no	non-specific

[1] broadened beyond detection

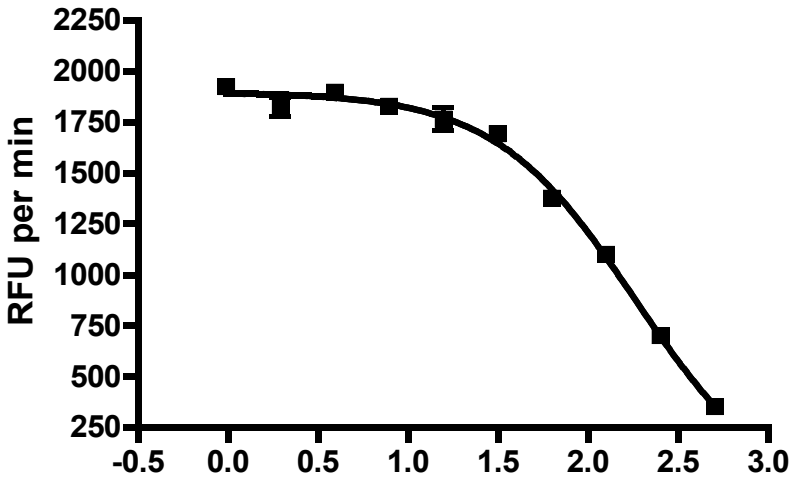
[2] possibly due to pH change

[3] barely soluble in water

Table S-III: NMR analysis of 12 compounds identified by substructure search

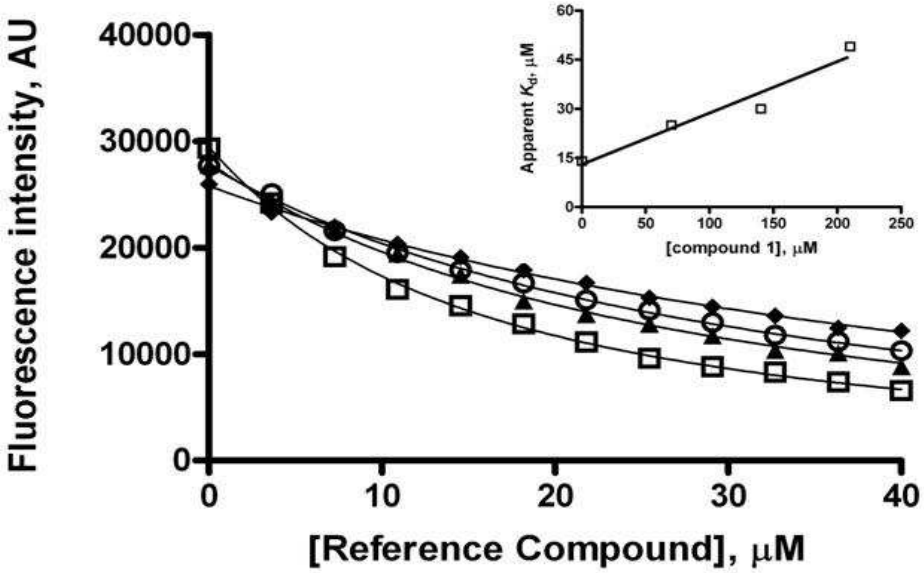
Compound number	Broadening of the compound signals in spectrum 2	Chemical shift changes in the ^{15}N-HSQC spectrum	Binding
23	yes	very small	very weak
24	yes	very small	very weak
25	no	no	no
26	yes	no	no
27	yes	no	no
28	yes	no	no
29	yes	no	no
30	yes	no	no
31	no	no	no
32	yes	very small	very week
33	yes	no	no
34	yes	very small	very weak

3 Enzymatic assay IC_{50} curve



IC_{50} of compound **1** was measured as described in Knox et al., J. Med. Chem. 49 **6585**,2006. The initial velocities in the enzyme reaction in the presence of increasing concentration of compound **1** (from 0 to 500 mM) against control wells without compounds were calculated and plotted. IC_{50} value was derived by fitting the calculated initial velocities to a non linear regression curve using GraphPad Prism software. Each point of the IC_{50} curve was carried out in duplicate during a single experiment.

4 Tryptophan fluorescence assay K_d curve



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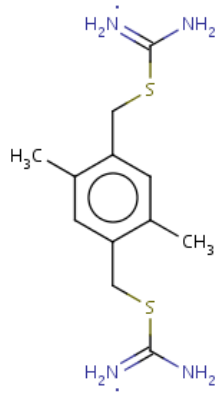
Binding affinity of compound **1** was measured by tryptophan fluorescence assay [Zhang, Y. L.; Zhang, Z. Y., *Anal Biochem* **1998,** 261, (2), 139-48.), Bodenreider C and et al., Manuscript to be submitted.]. Fluorescence titrations performed with a reference compound in presence of varying concentrations of compound **1** (0 μM (□), 70 μM (▲), 140 μM (○) and 240 μM (◆)). Solid lines show the fit of the data to a 1:1 binding equation. The resulting apparent K_d values of the reference compound are reported as a function of compound **1** concentration in the insert. This dependence yields K_d = 83 μM for binding of compound **1** to WNV protease. The reference compound is N,N-Bis-(4-(4-methyl-4,5-dihydro-1H-imidazol-2-yl-phenyl)-phthalazine-1,4-diamine.

5 Structures of 22 compounds from docking



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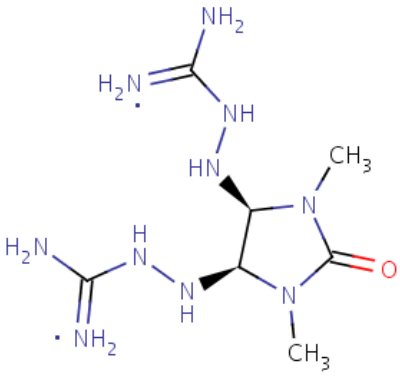

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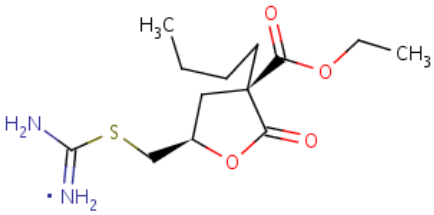

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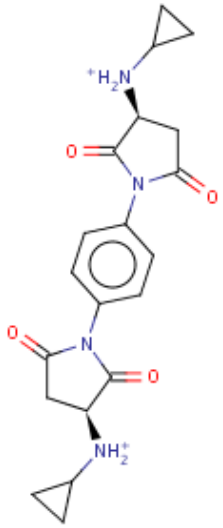

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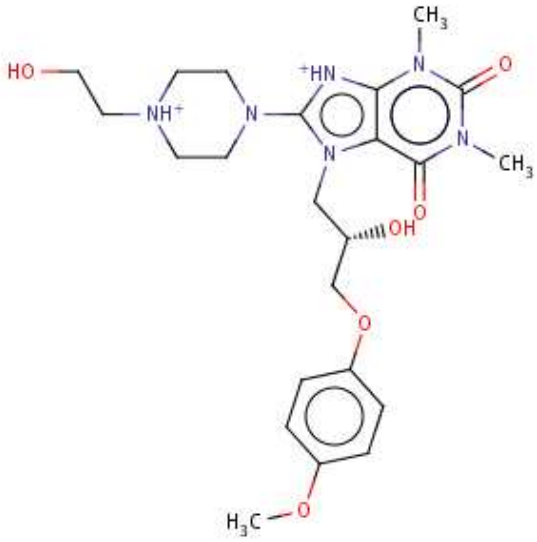

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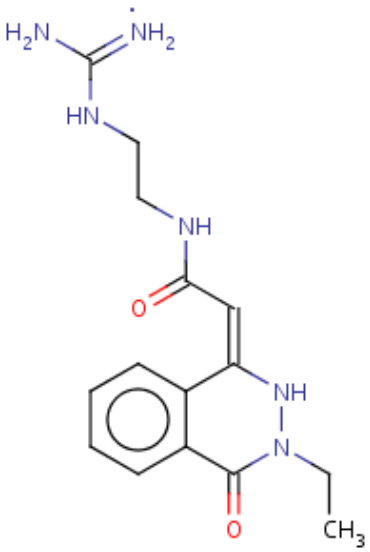

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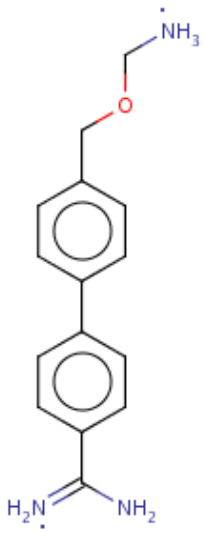

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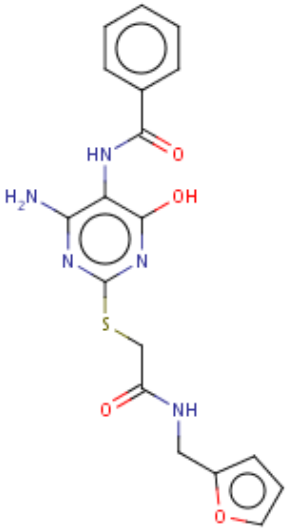

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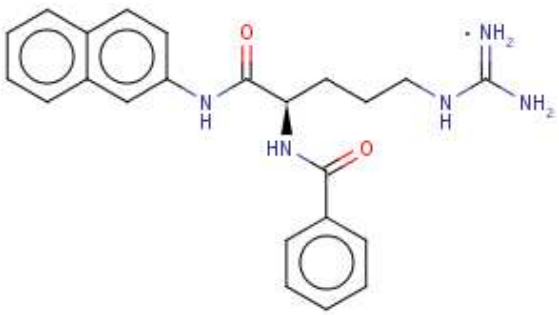

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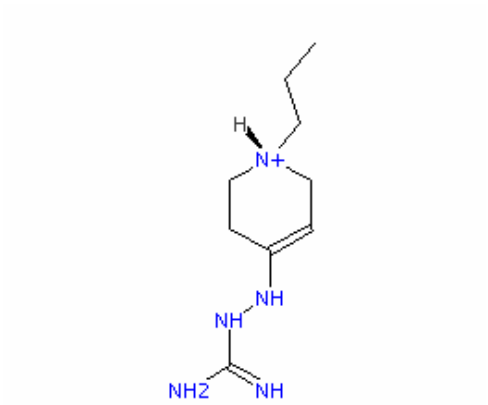

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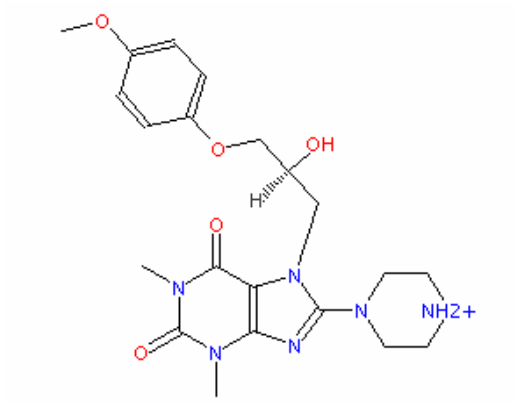

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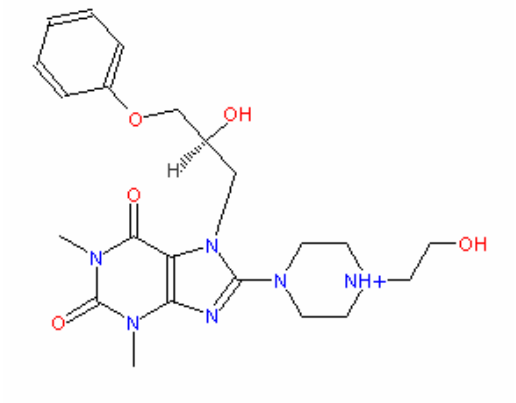

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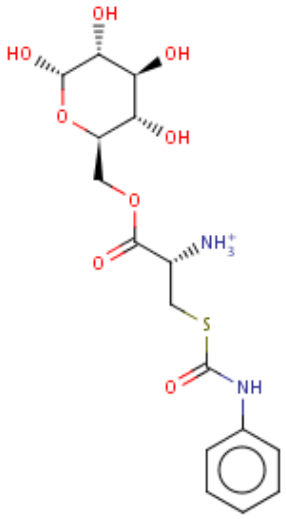
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OH
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OH
O
O
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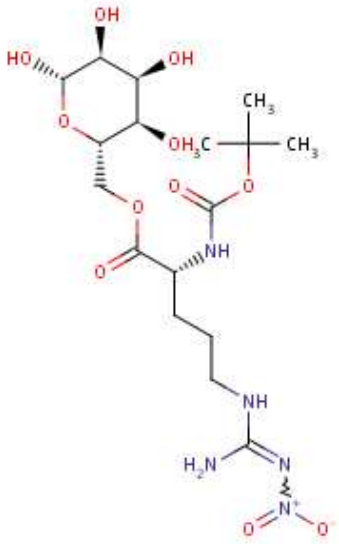
S
O
NH

13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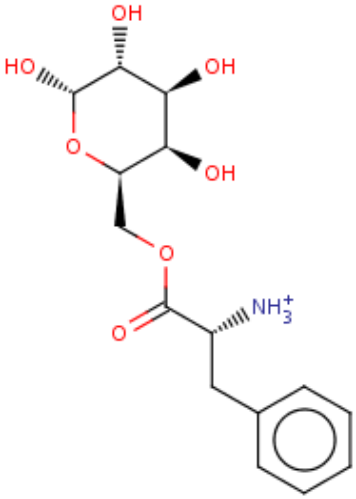
O-

14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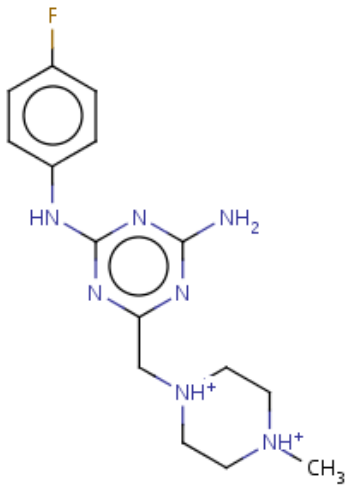
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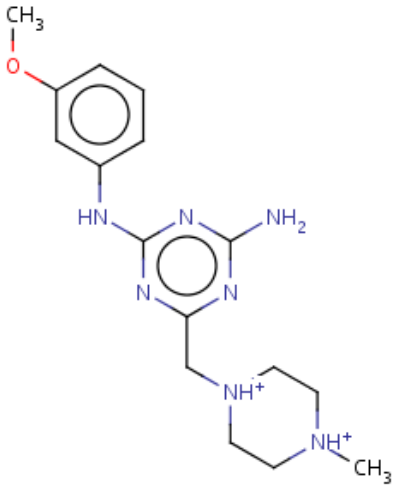
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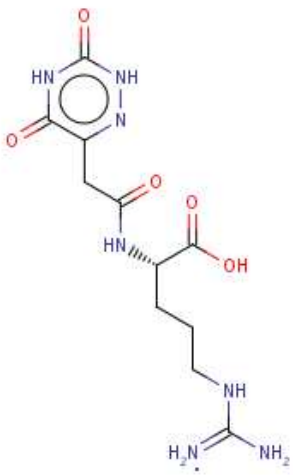
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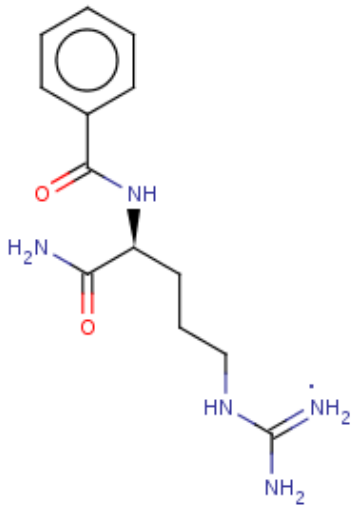
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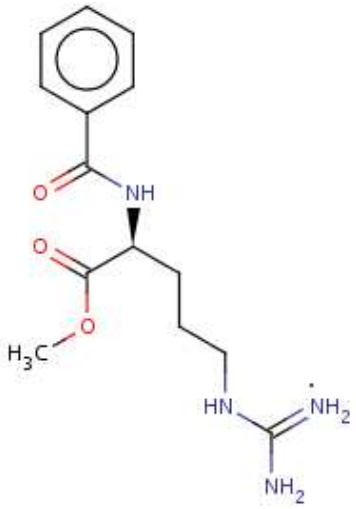
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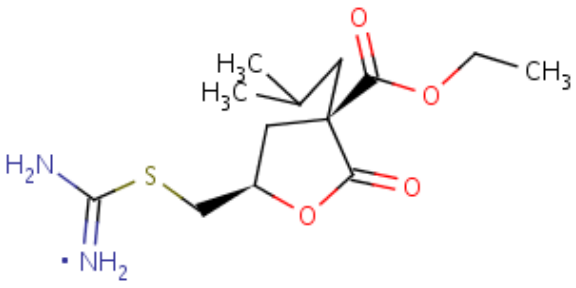
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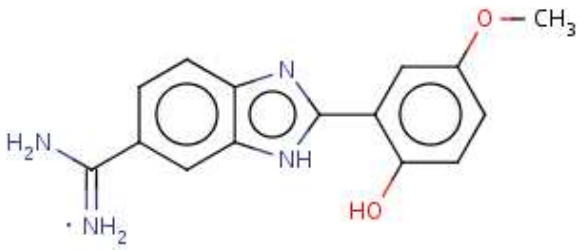

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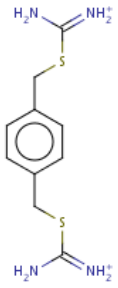
6 Structures of 12 compounds from substructural search



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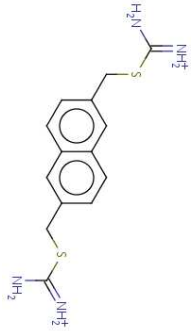

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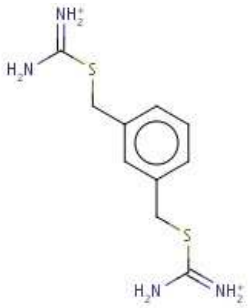

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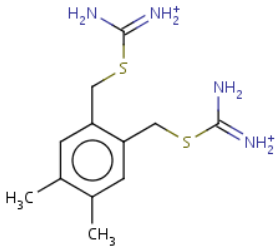

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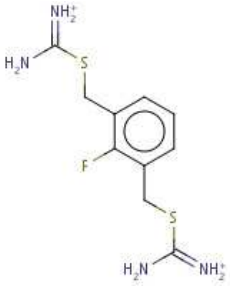

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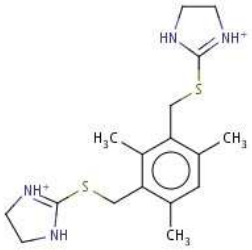

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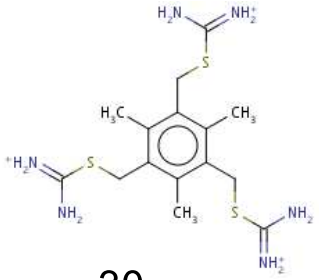

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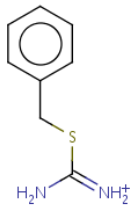

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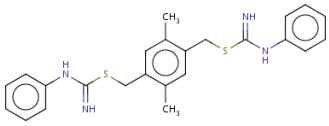

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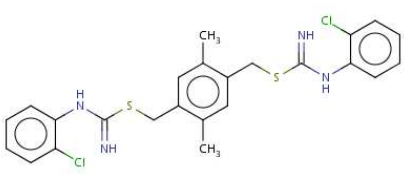

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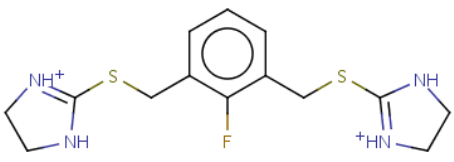

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7 Structures of 3 suggested compounds