Supporting Information for:

Studying a Drug-Like, RNA-focused Small Molecule Library Identifies Compounds that Inhibit RNA-mediated Toxicity in Myotonic Dystrophy

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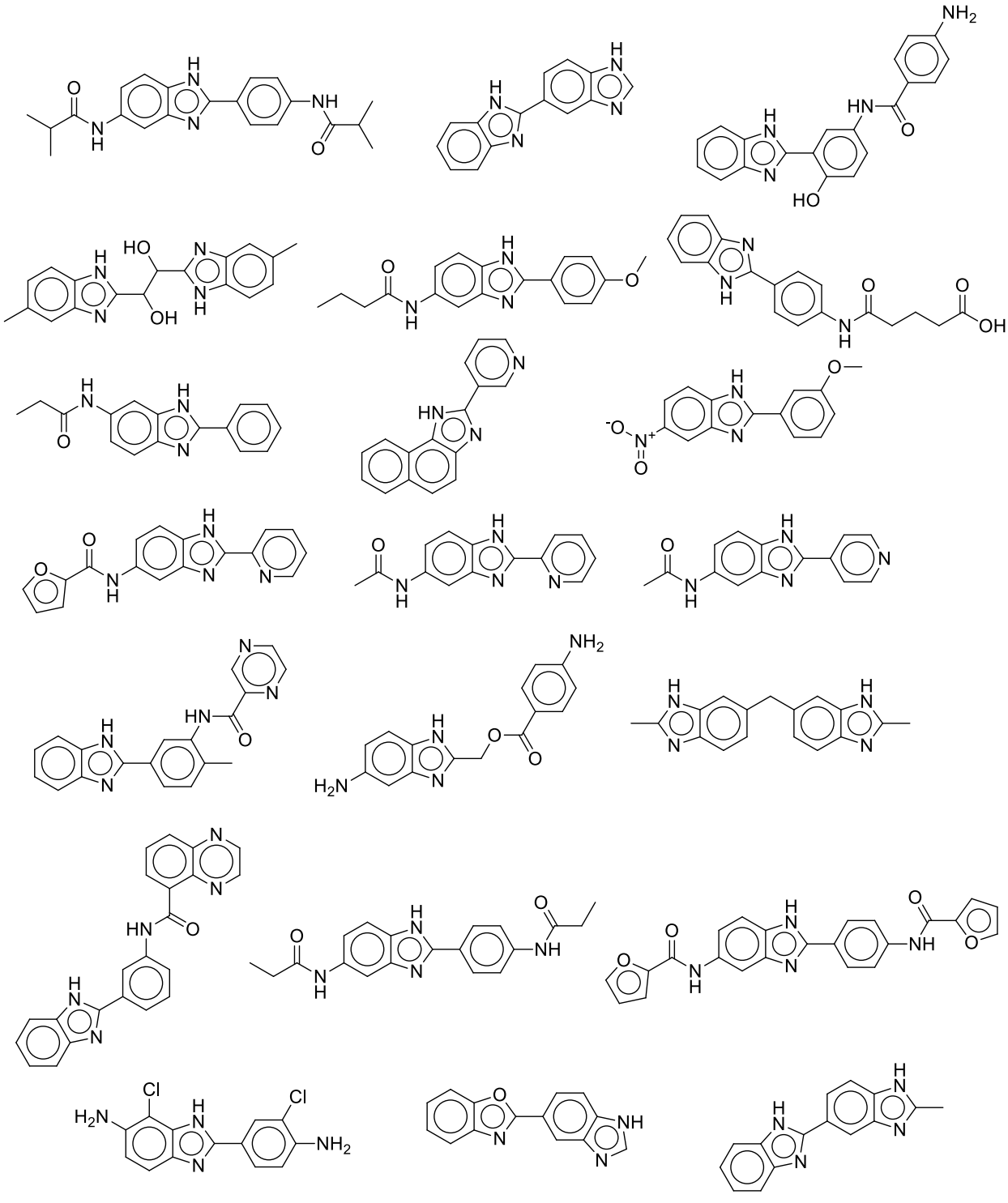
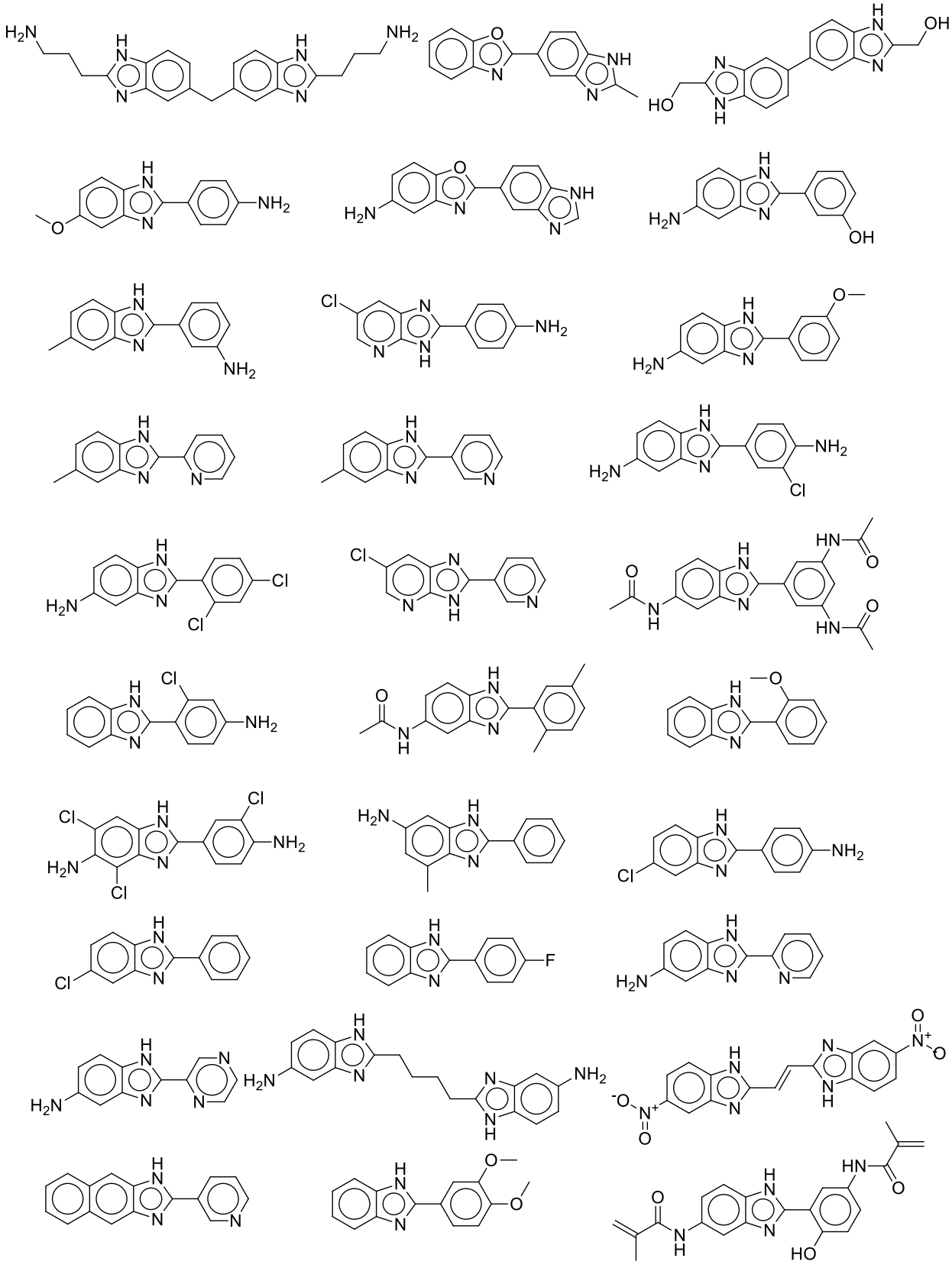
Contents

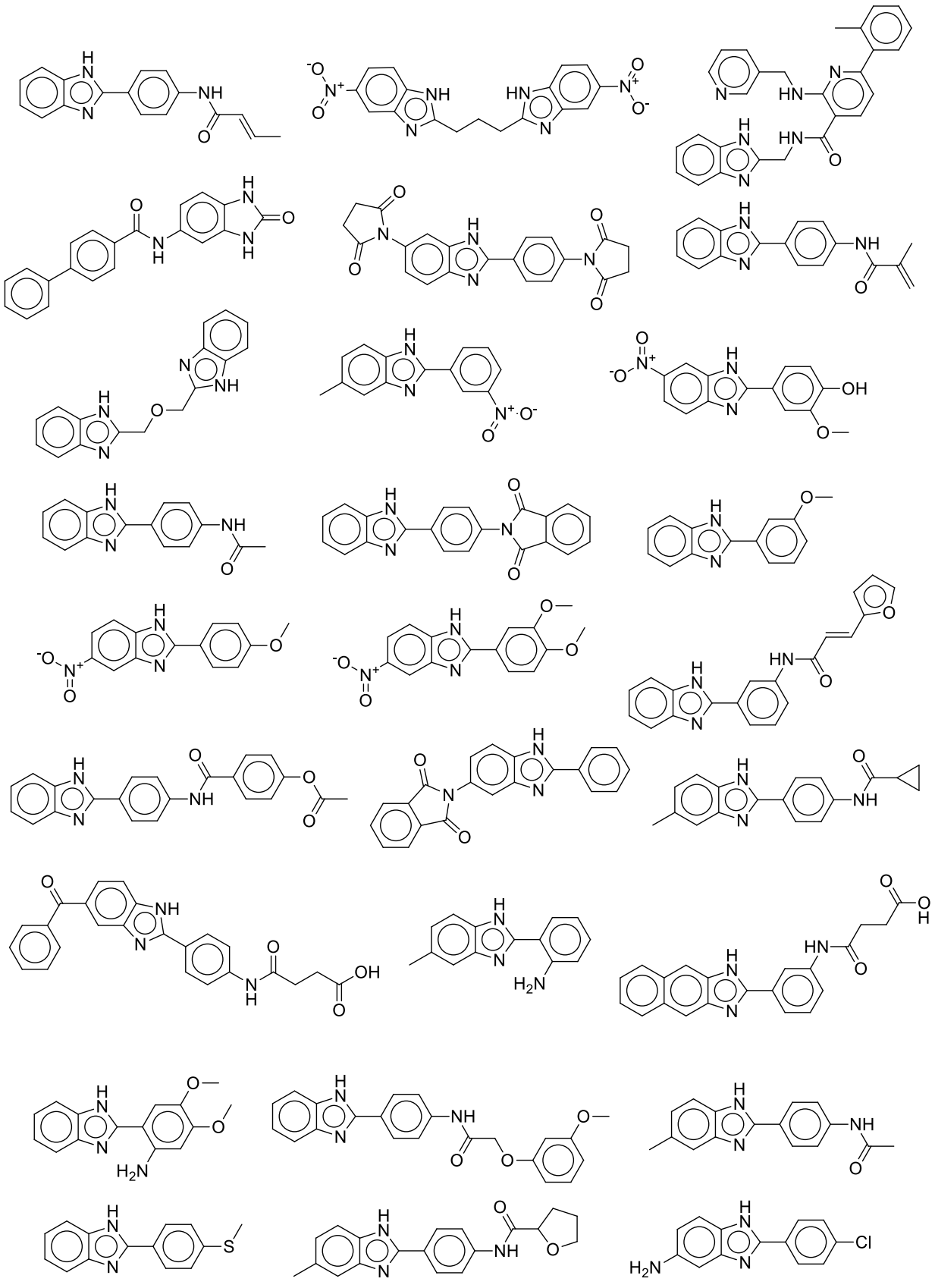
1. General Methods.

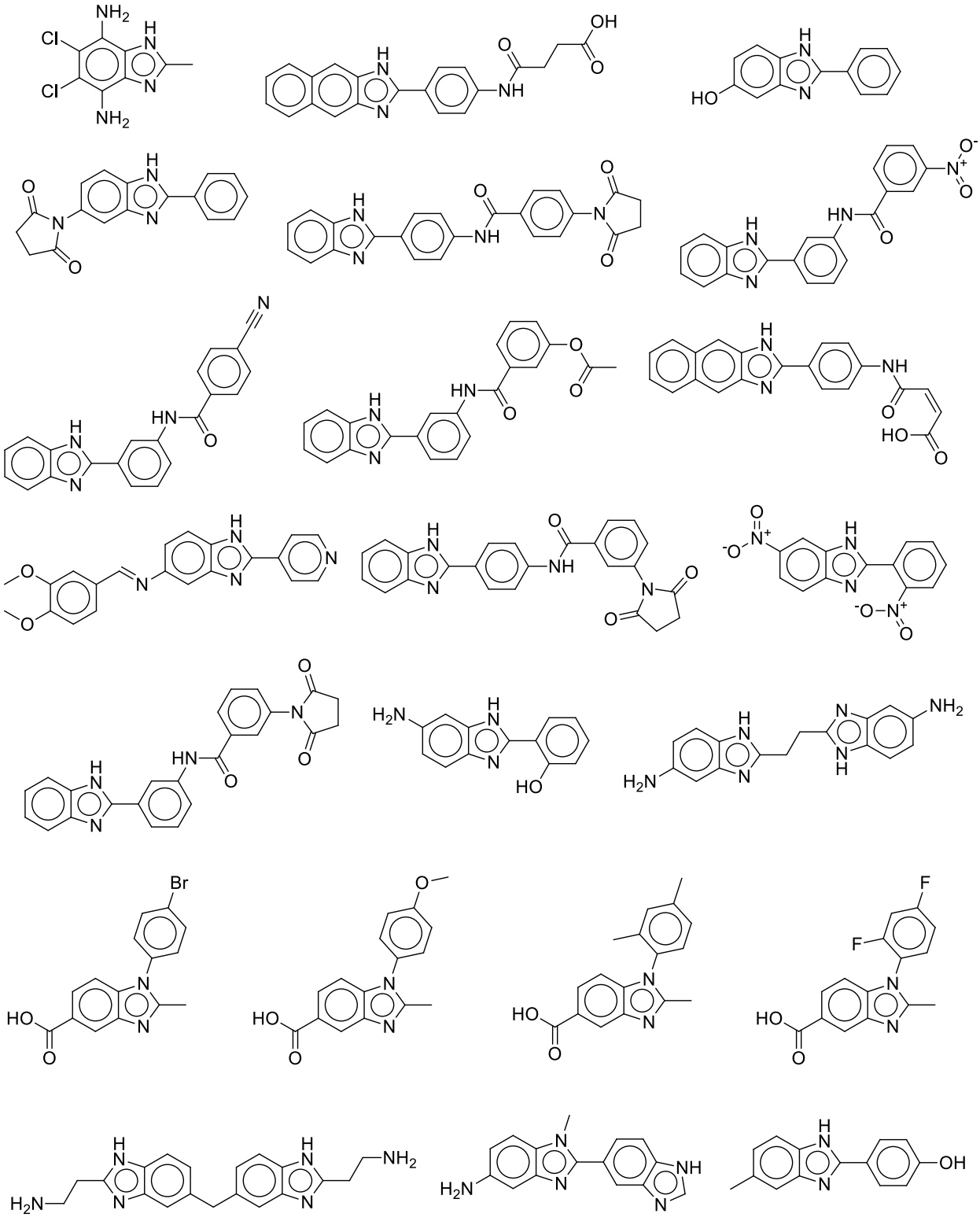
Small molecules evaluated in cellular assays. Compounds **1**, **7**, **11**, **16**, and **17** were purchased from Asinex Ltd. Small molecules **2**, **5**, **6**, **13**, **15**, **19**, **21** and **24** were obtained from Scientific Exchange, Inc. Compounds **3**, **9** and **26** were purchased from Chembridge. Compounds **8** and **25** were obtained from ChemDiv, Inc. Compounds **14**, **23**, and **27** were purchased from Sigma-Aldrich. Compound **20** was obtained from Ryan Scientific. Compound purity was assessed by analytical HPLC using a Waters Symmetry C18 5 μm 4.6 x 150 mm column. Compounds were analyzed using a gradient of 0-100% MeOH in H_2O with 0.1% TFA over 60 min. All compounds evaluated had ≥ 90% purity. The identities of compounds were confirmed by mass spectrometry using an an α-hydroxycinnamic acid matrix and an Applied Biosystems MALDI ToF/ToF Analyzer 4800 Plus.

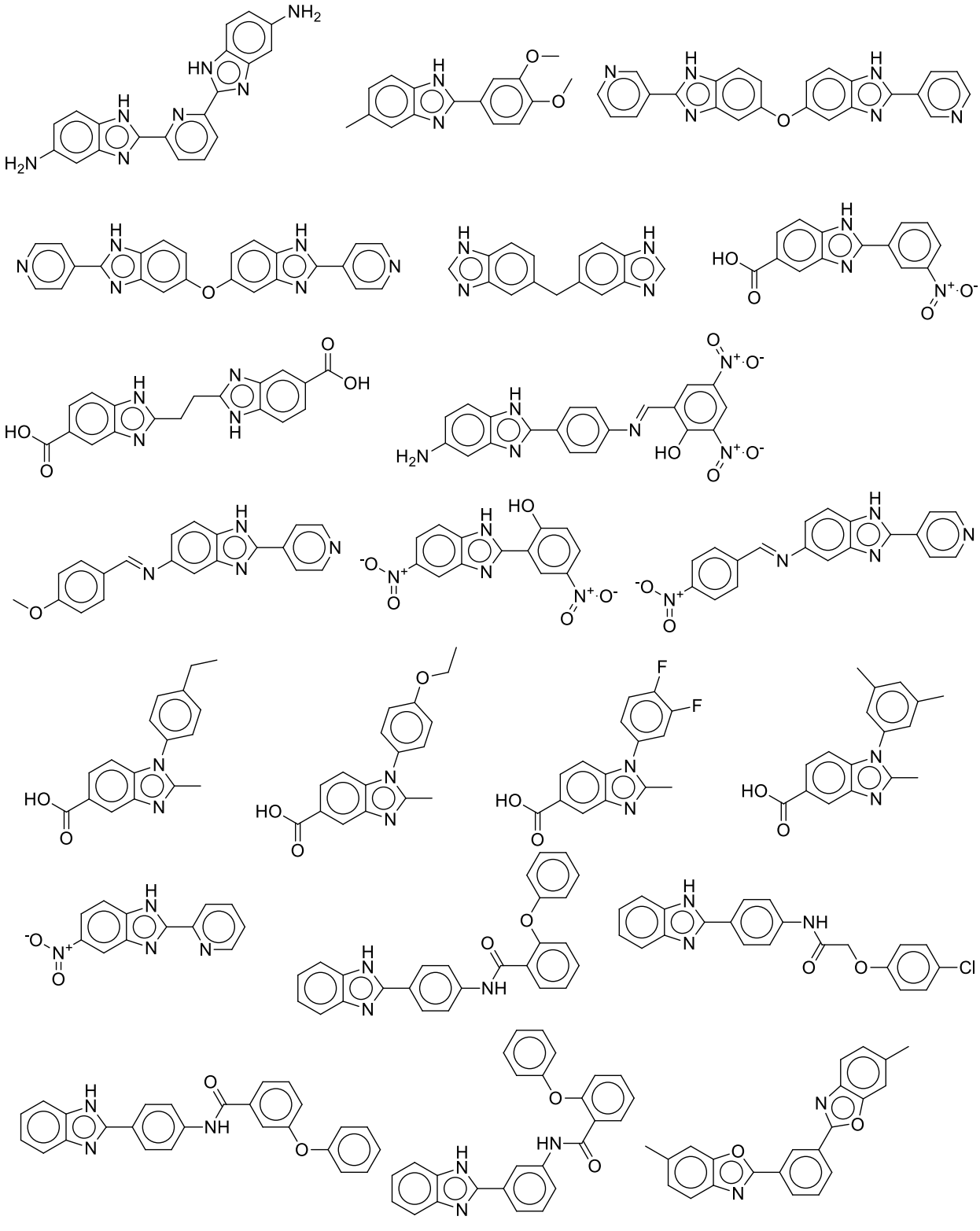
2. Chemical Structures of Library Compounds.

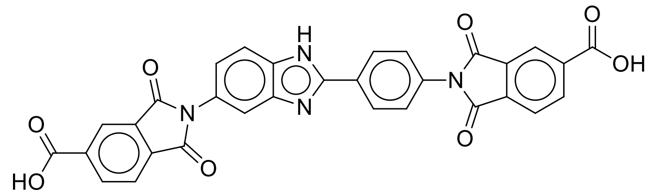
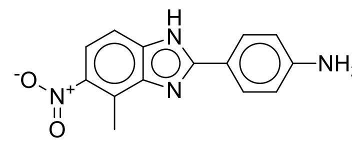
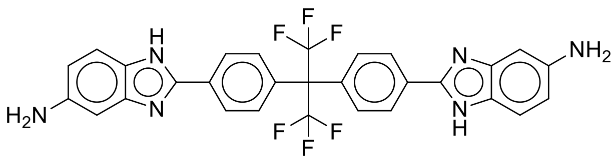
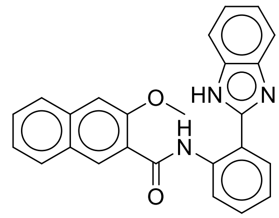
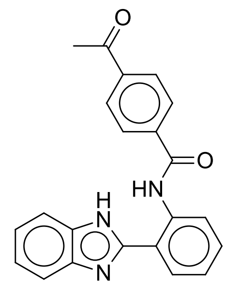
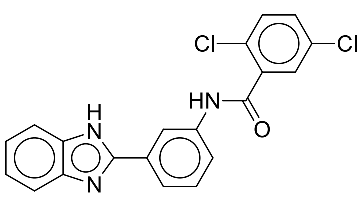
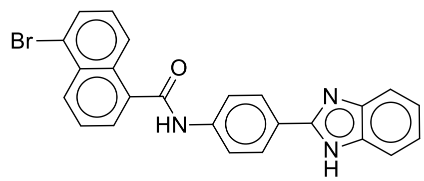
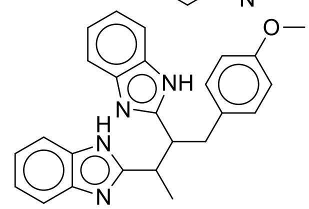
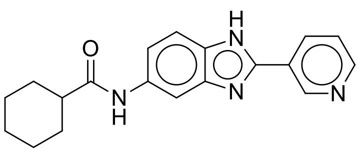
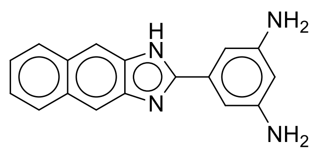
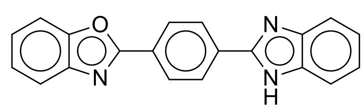
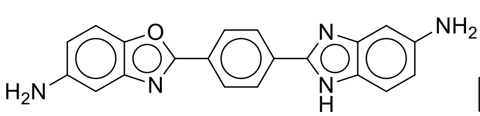
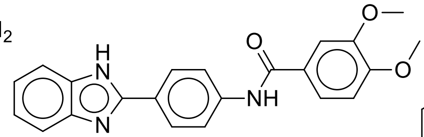
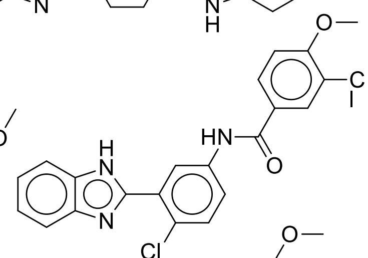
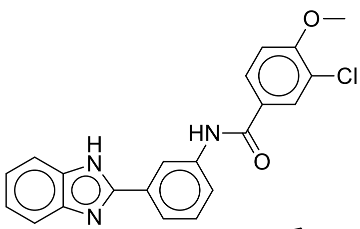
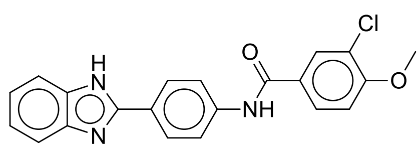
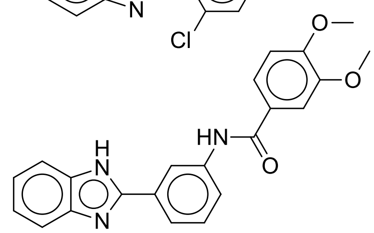
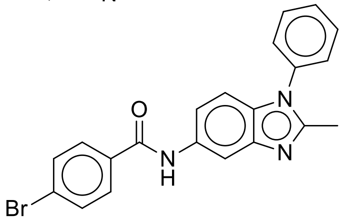
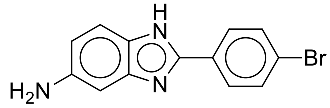
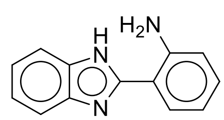
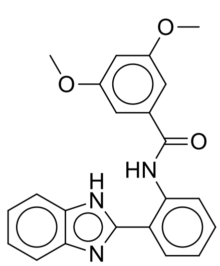
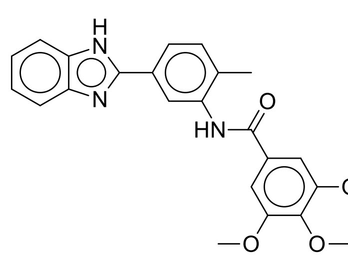
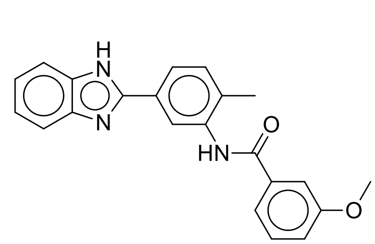


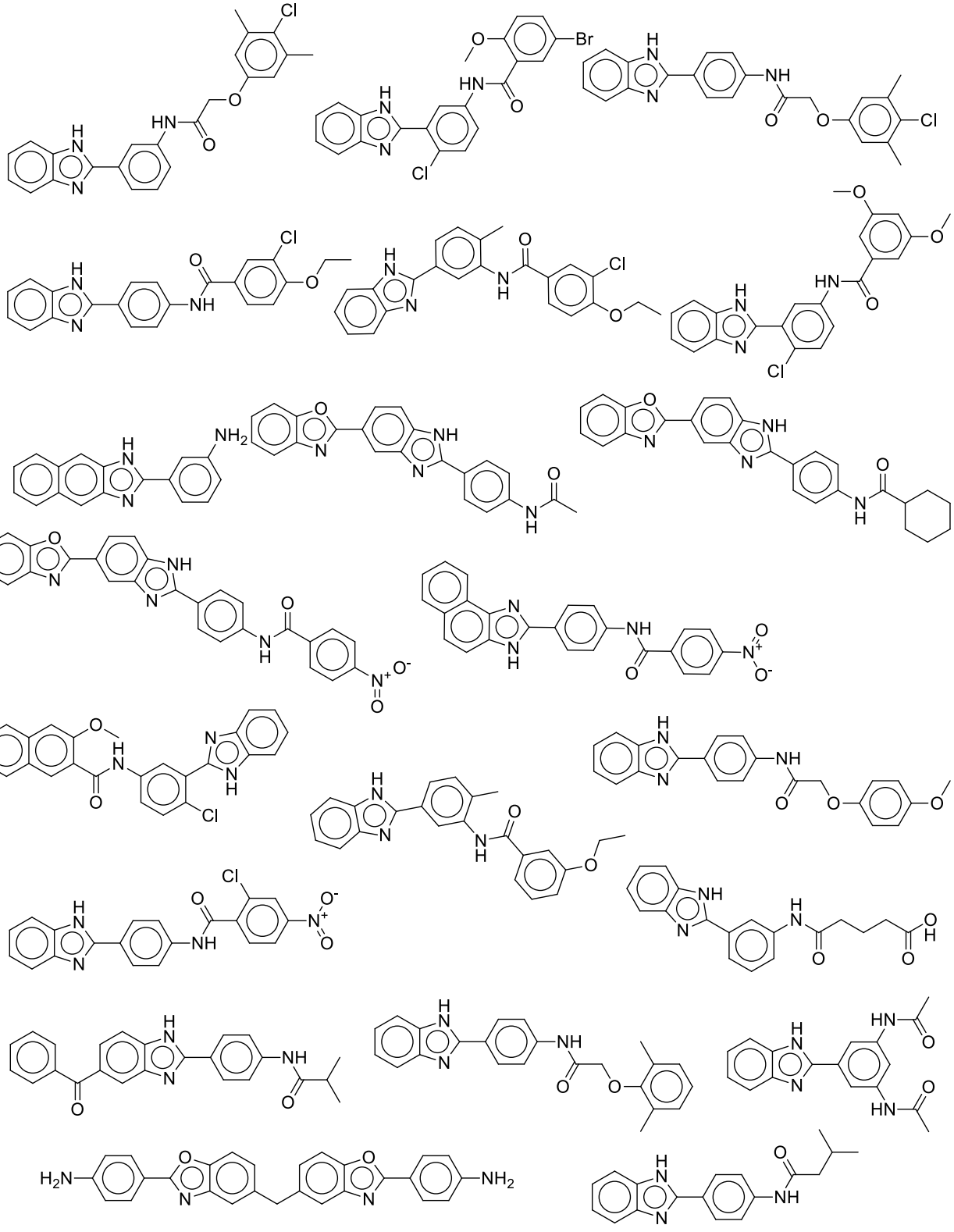


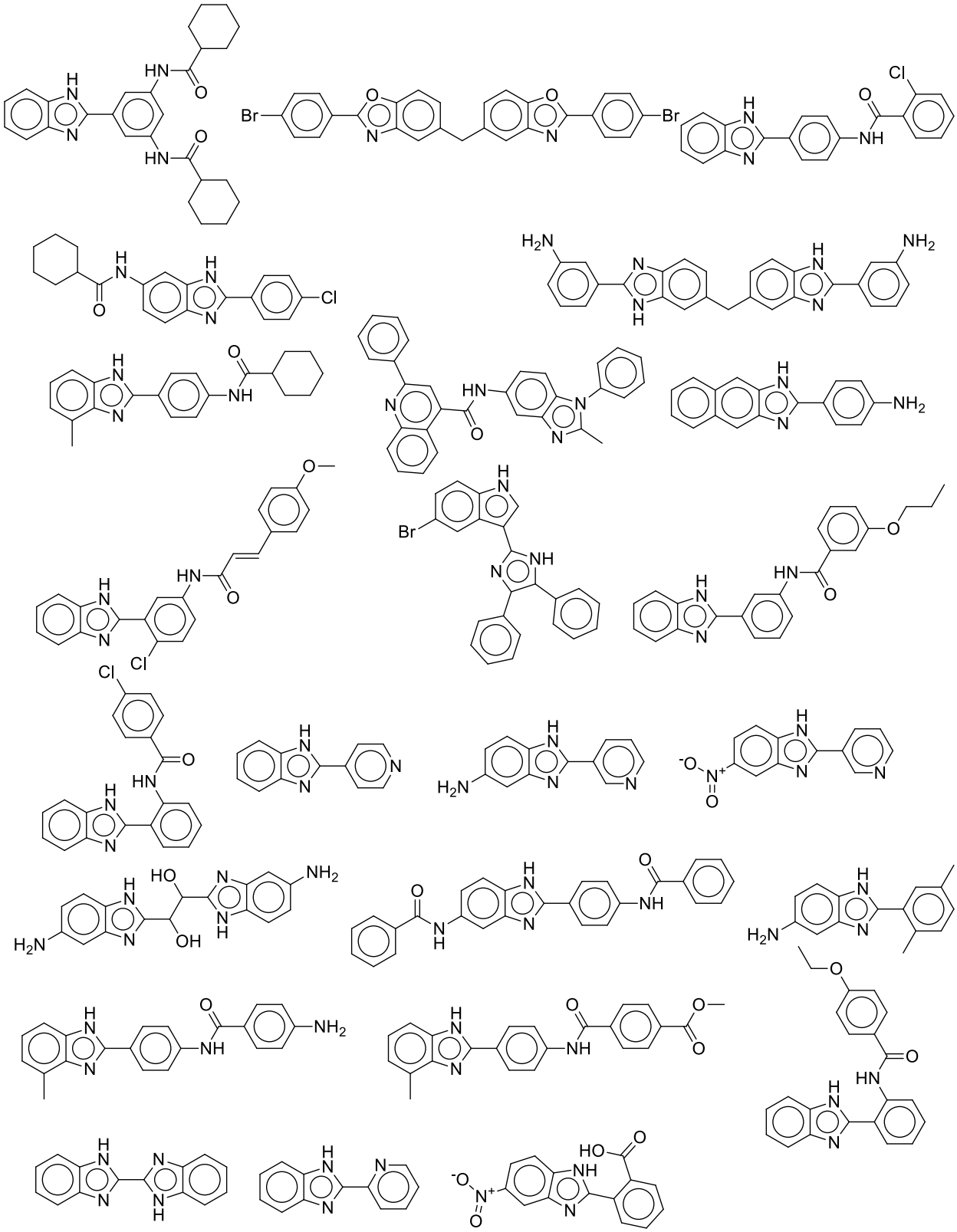


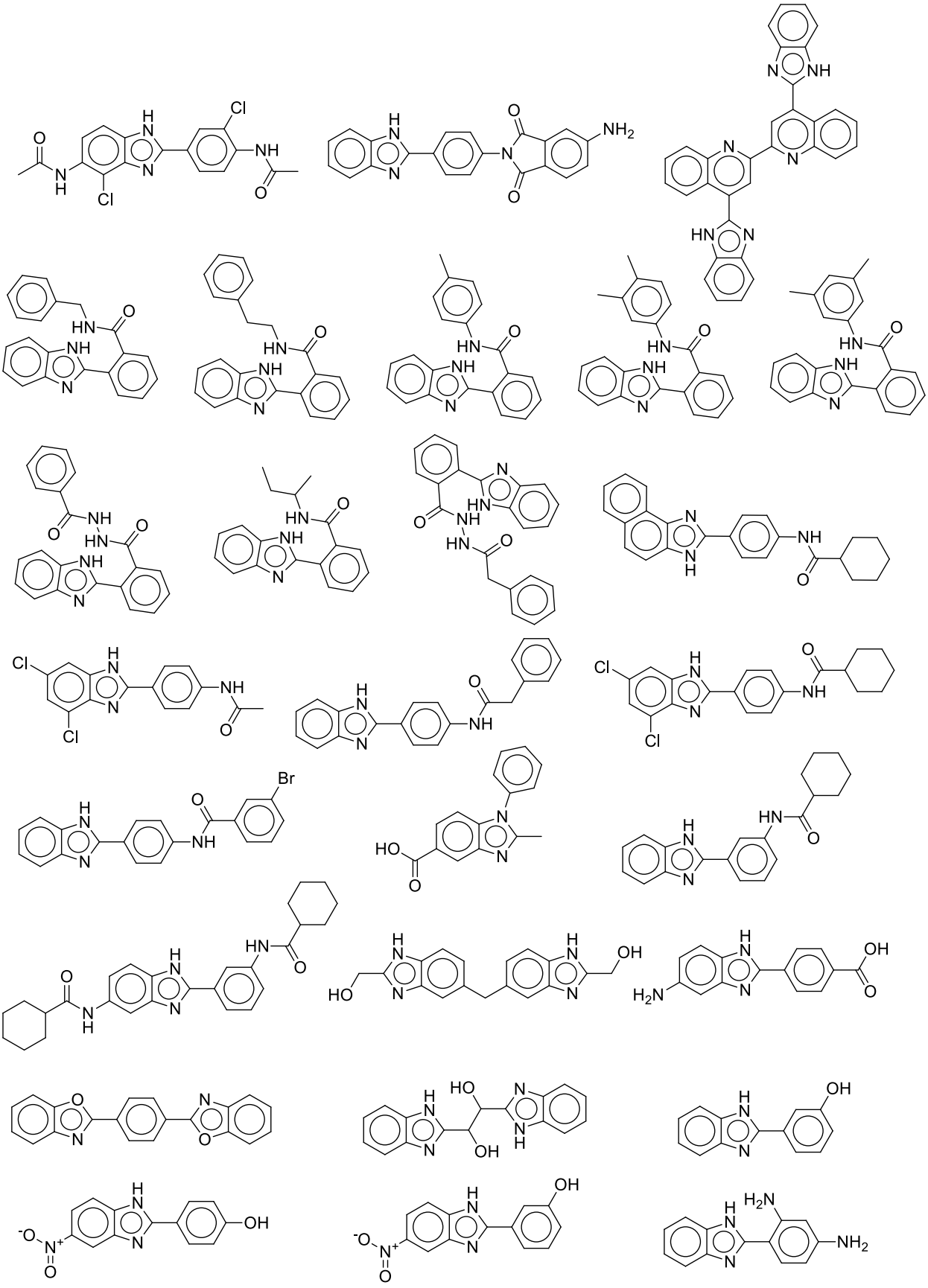


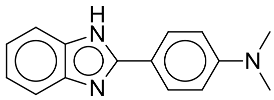










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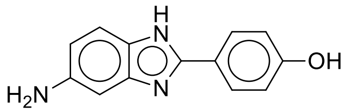

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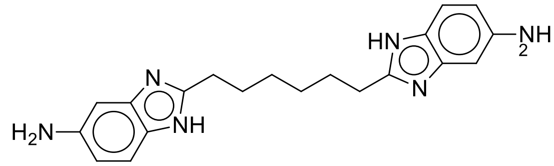

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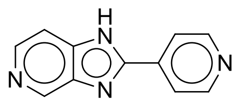

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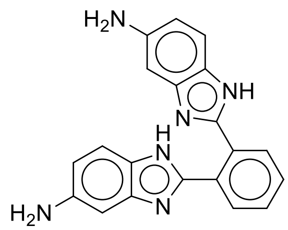

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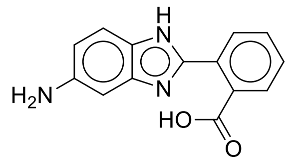

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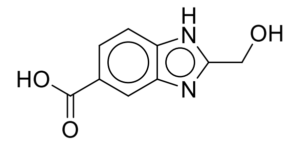

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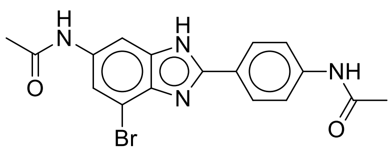

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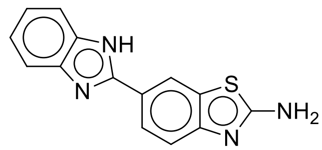

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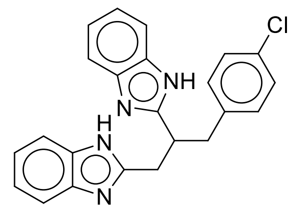

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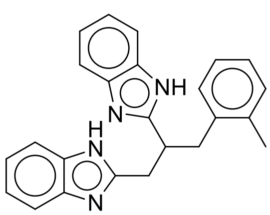

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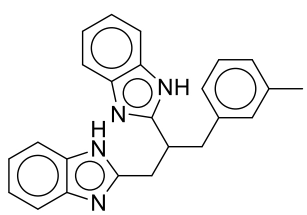

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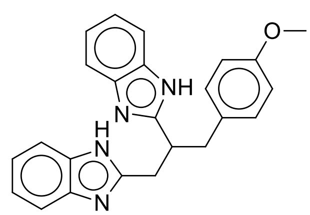

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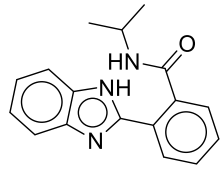

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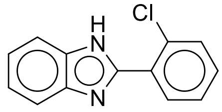

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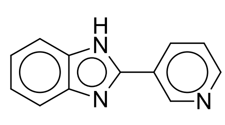

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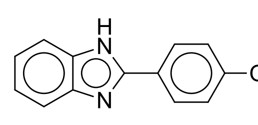

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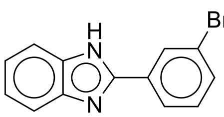

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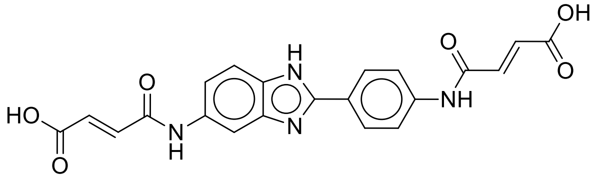

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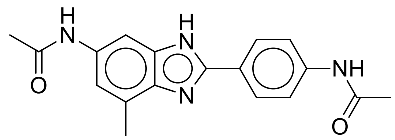

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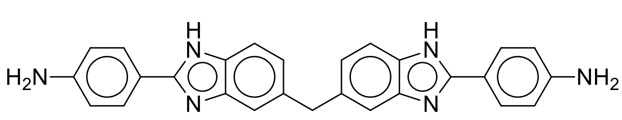

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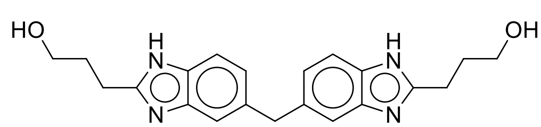

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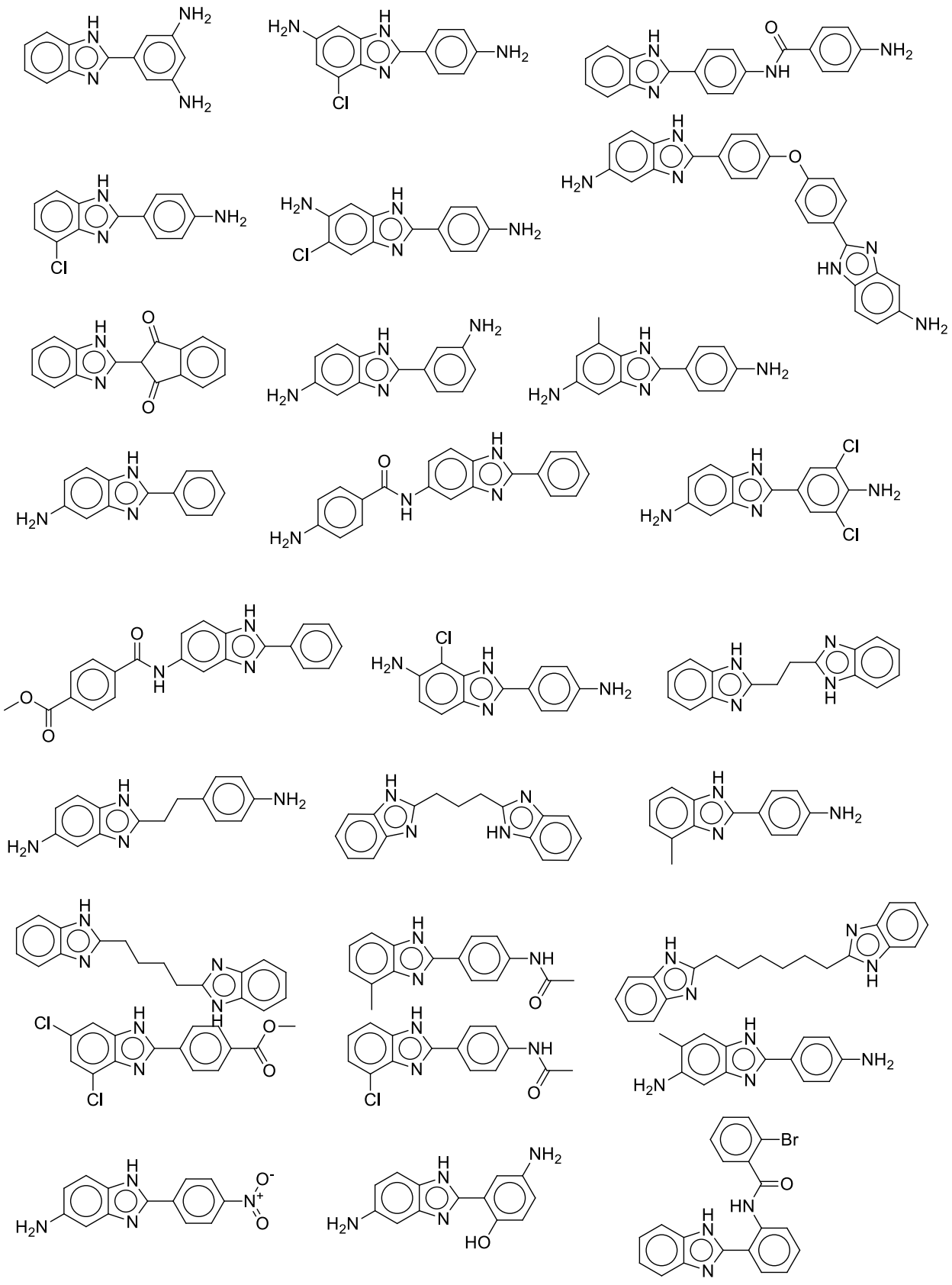
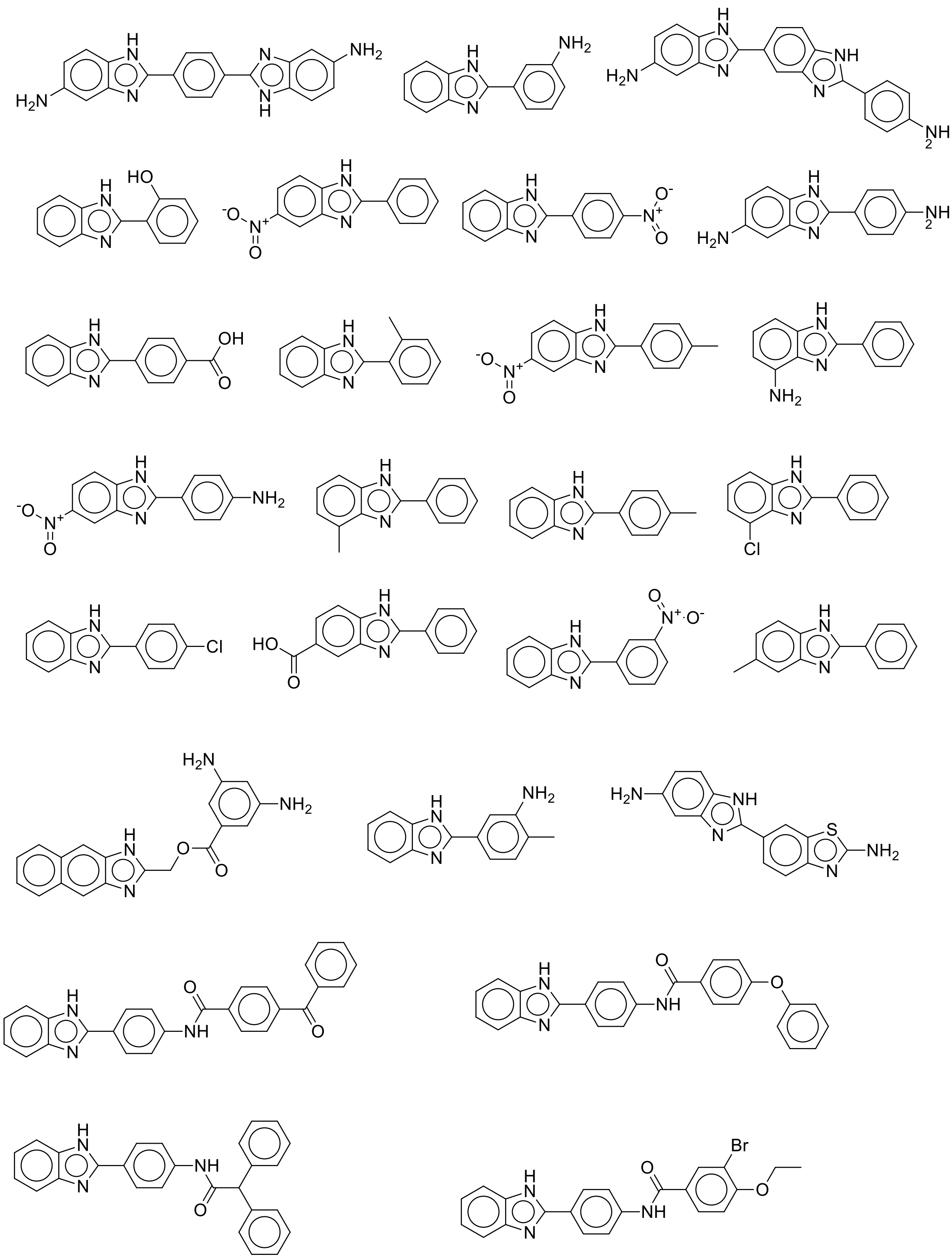
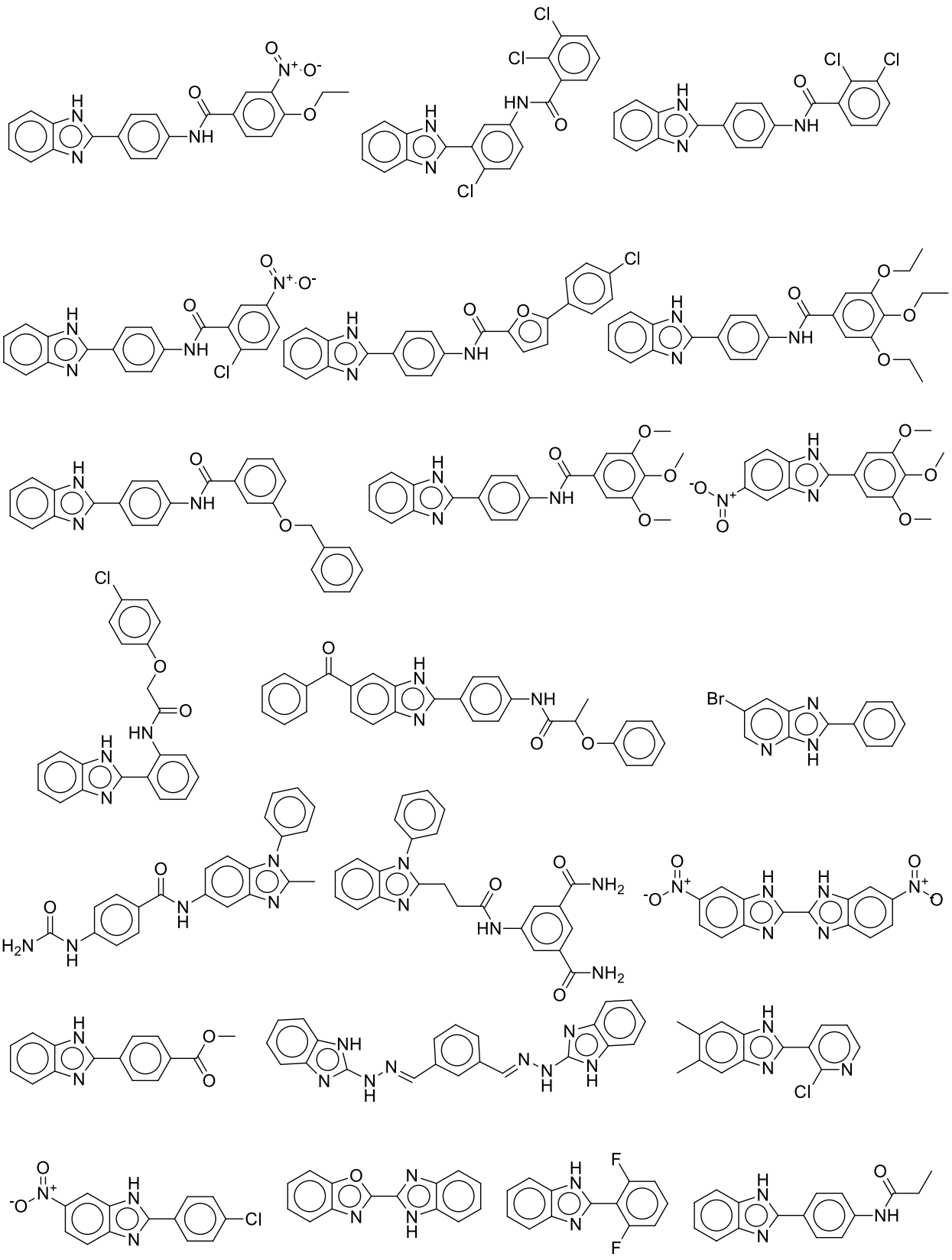






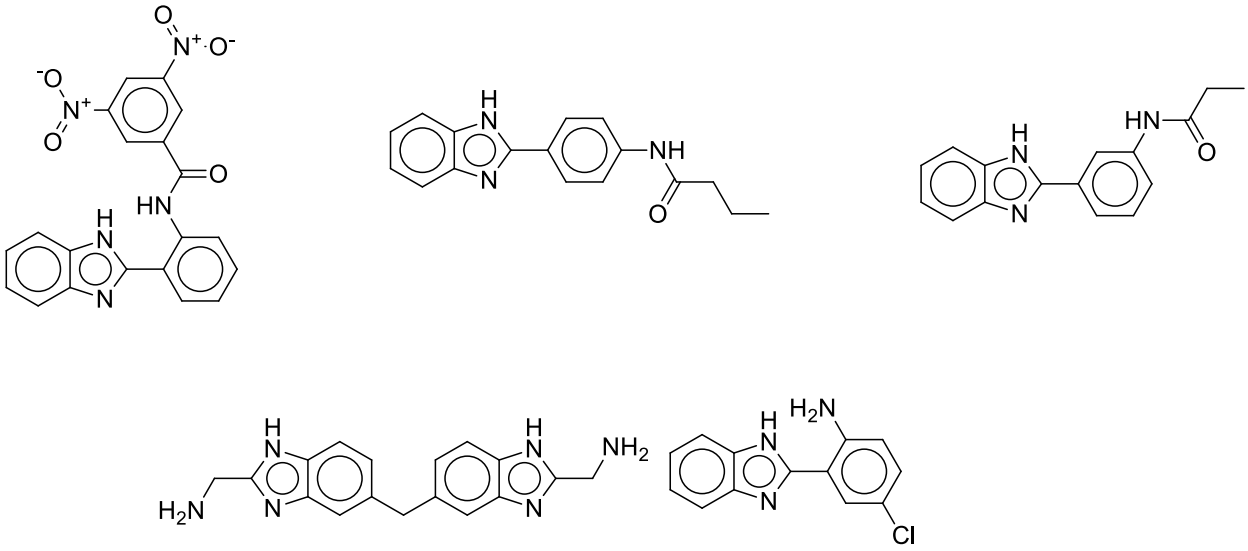


Figure S-1. Chemical structures of library compounds

3. Chemical Structures of Hit Compounds.

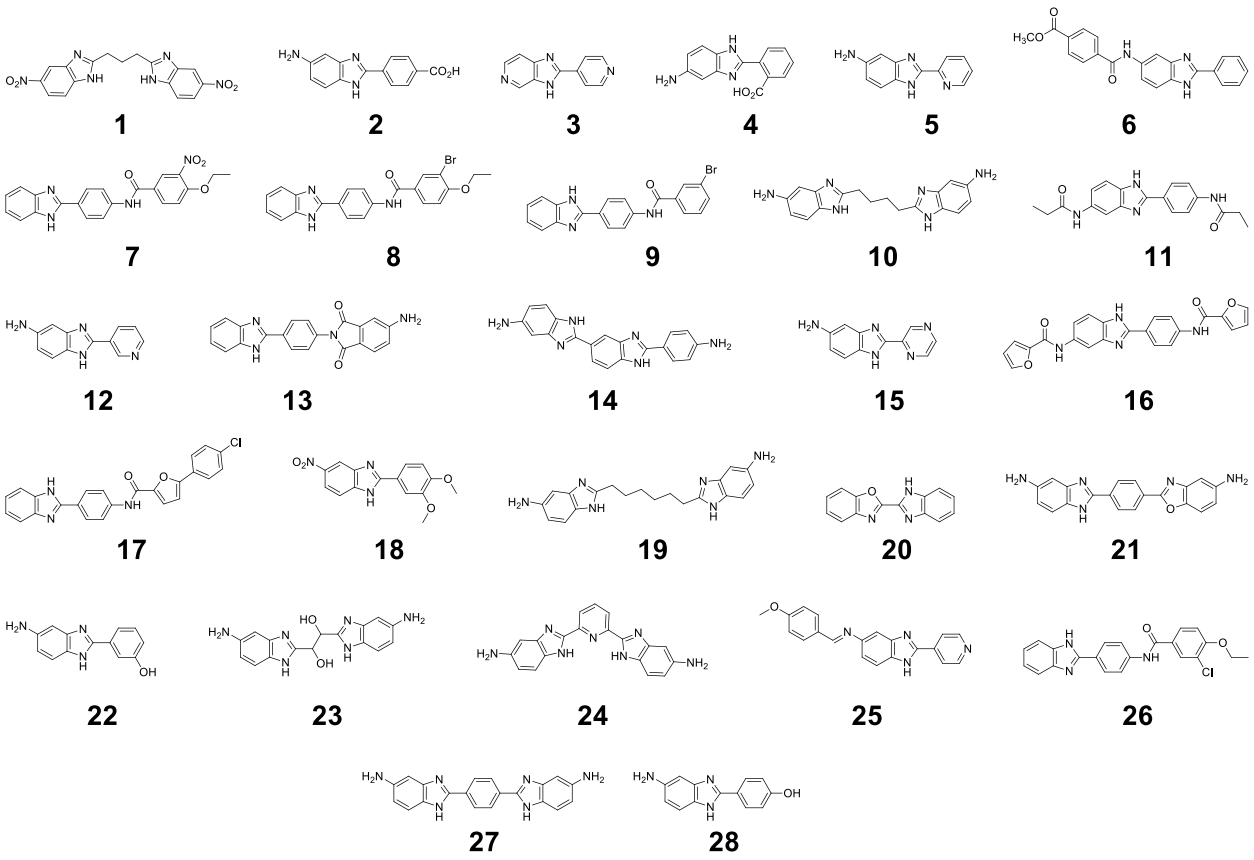


Figure S-2. Structures of hit compounds.

4. Evaluation of Fluorescence of Library Compounds.

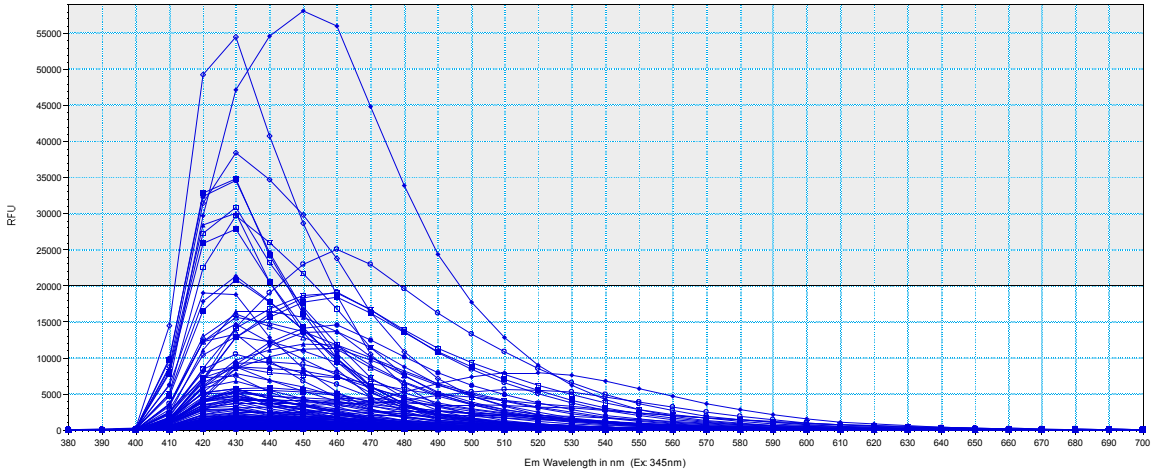


Figure S-3. Fluorescence emission scan of library compounds when excited at 345 nm. Two non-hit compounds have minor fluorescence at 545 nm and no compounds are fluorescent at 665 nm.

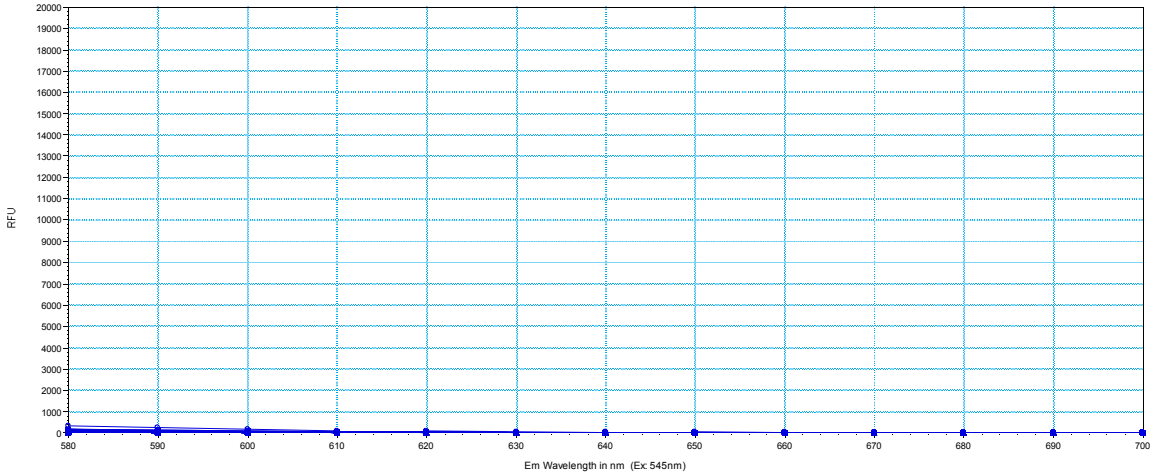


Figure S-4. Fluorescence emission scan of library compounds when excited at 545 nm. None of the library compounds are fluorescent at 665 nm.

5. Similarity Analysis of Scripps Drug Discovery Library and Hit Compounds.

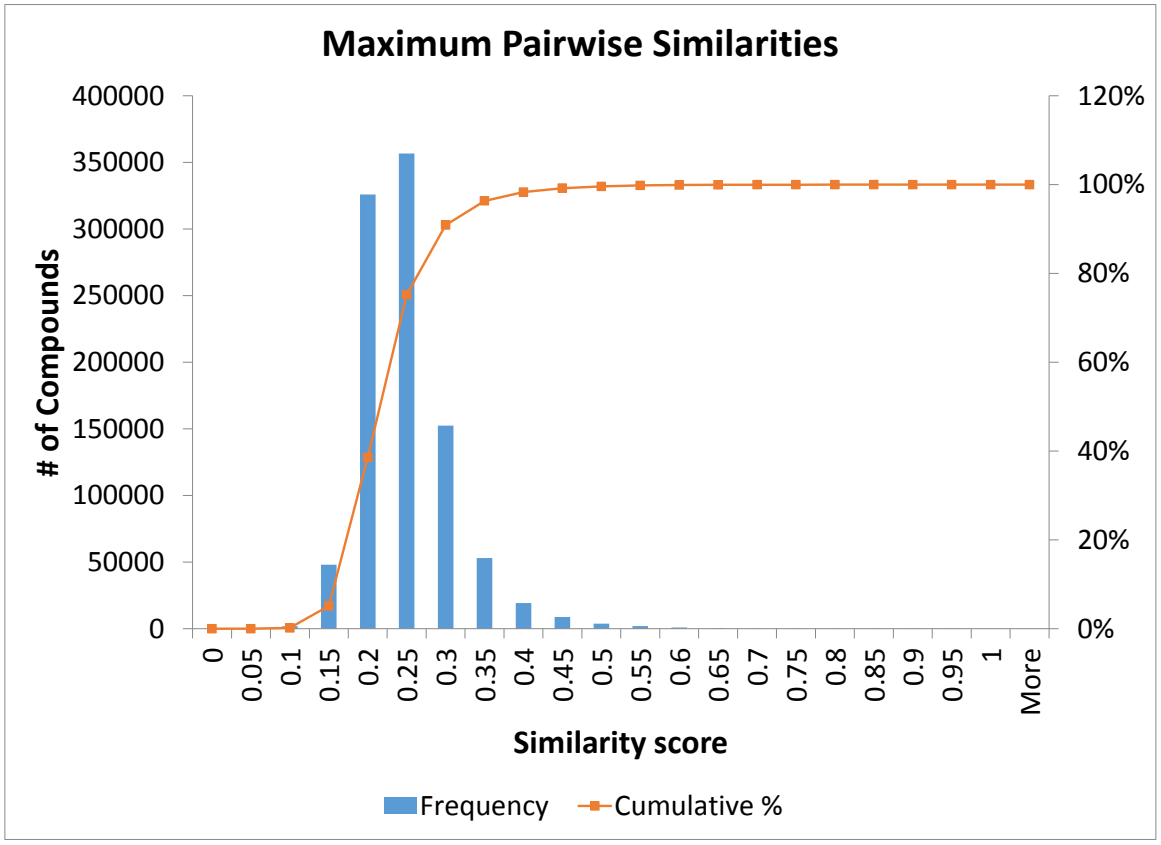
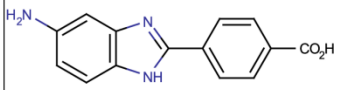
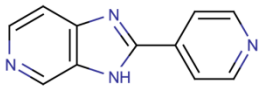
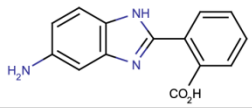
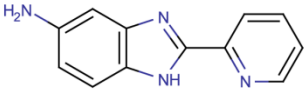
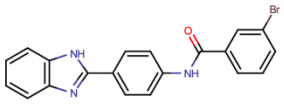
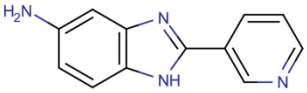
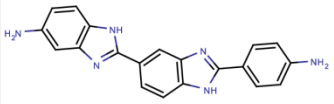
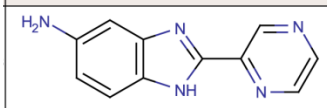
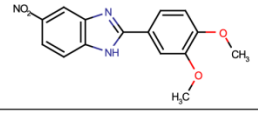
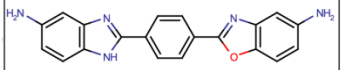
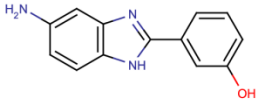
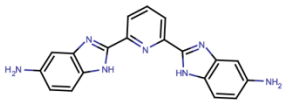
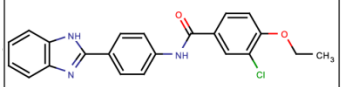
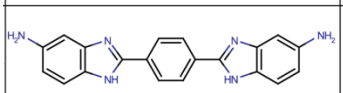
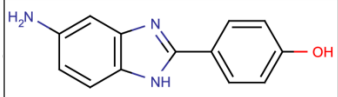
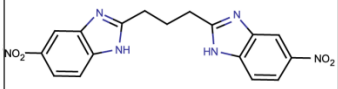
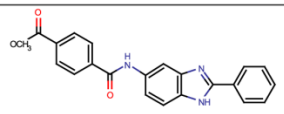
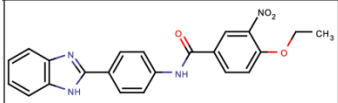
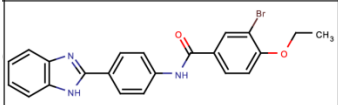
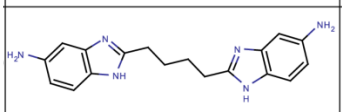
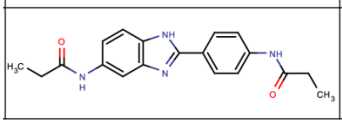


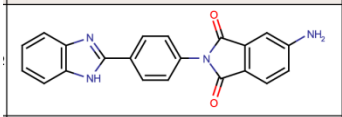
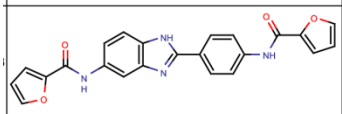
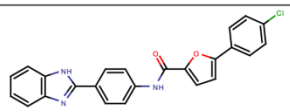
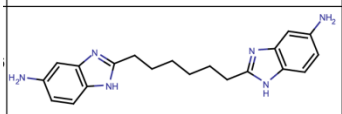
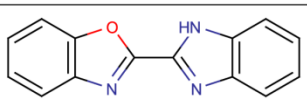
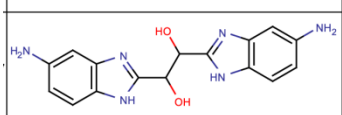
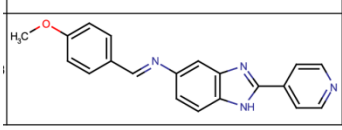
Figure S-5. Similarity analysis of Scripps Drug Discovery Library and hit compounds. Histogram of the closest pairwise similarities (via ECFP-6 fingerprints and the Tanimoto metric) between the Scripps Drug Discovery library and any of the active compounds.

6. Chemical Property Analysis of Hit Compounds

Structure	Mol Weight	Formula	LogP	LogD	H bond acceptors	H bond donors	Strongest acidic pKa	Strongest basic pKa	TPSA	Lipinski rule of 5 (3 of 4)	Lipinski rule of 5 (4 of 4)	Ring count	Rotatable bonds
	253.26	C14H11N3O2	0.49	-0.28	4	3	3.78	6.69	92.00	✓	✓	3	2
	196.21	C11H8N4	0.85	0.85	3	1	10.24	5.30	54.46	✓	✓	3	1
	253.26	C14H11N3O2	0.45	-0.35	4	3	3.03	6.63	92.00	✓	✓	3	2
	210.23	C12H10N4	1.62	1.62	3	2	11.44	3.80	67.59	✓	✓	3	1
	392.25	C20H14BrN3O	5.14	5.14	2	2	11.24	5.16	57.78	✓	☐	4	3
	210.23	C12H10N4	1.24	1.20	3	2	12.18	6.43	67.59	✓	✓	3	1
	340.38	C20H16N6	2.94	2.88	4	4	11.35	6.61	109.40	✓	✓	5	2

Structure	Mol Weight	Formula	LogP	LogD	H bond acceptors	H bond donors	Strongest acidic pKa	Strongest basic pKa	TPSA	Lipinski rule of 5 (3 of 4)	Lipinski rule of 5 (4 of 4)	Ring count	Rotatable bonds
	211.22	C11H9N5	0.41	0.41	4	2	11.14	3.59	80.48	✓	✓	3	1
	299.28	C15H13N3O4	2.91	2.91	5	1	10.55	3.62	92.96	✓	✓	3	4
	341.37	C20H15N5O	3.00	2.94	4	3	12.37	6.59	106.75	✓	✓	5	2
	225.25	C13H11N3O	2.15	2.09	3	3	9.53	6.60	74.93	✓	✓	3	1
	341.37	C19H15N7	2.49	2.49	5	4	11.10	4.08	122.29	✓	✓	5	2
	391.85	C22H18ClN3O2	5.18	5.18	3	2	11.31	5.16	67.01	✓	☐	4	5
	340.38	C20H16N6	2.94	2.81	4	4	12.10	6.91	109.40	✓	✓	5	2

Structure	Mol Weight	Formula	LogP	LogD	H bond acceptors	H bond donors	Strongest acidic pKa	Strongest basic pKa	TPSA	Lipinski rule of 5 (3 of 4)	Lipinski rule of 5 (4 of 4)	Ring count	Rotatable bonds
	225.25	C13H11N3O	2.15	2.07	3	3	9.64	6.71	74.93	✓	✓	3	1
	366.33	C17H14N6O4	3.14	3.14	6	2	11.28	4.93	149.00	✓	✓	4	6
	371.39	C22H17N3O3	4.38	4.38	3	2	10.40	5.13	84.08	✓	✓	4	5
	402.40	C22H18N4O4	4.52	4.51	5	2	11.21	5.16	112.83	✓	✓	4	6
	436.30	C22H18BrN3O2	5.34	5.34	3	2	11.32	5.16	67.01	✓	☐	4	5
	320.39	C18H20N6	2.04	1.18	4	4	13.20	7.98	109.40	✓	✓	4	5
	336.39	C19H20N4O2	3.16	3.16	3	3	11.55	5.16	86.88	✓	✓	3	5

Structure	Mol Weight	Formula	LogP	LogD	H bond acceptors	H bond donors	Strongest acidic pKa	Strongest basic pKa	TPSA	Lipinski rule of 5 (3 of 4)	Lipinski rule of 5 (4 of 4)	Ring count	Rotatable bonds
	354.36	C21H14N4O2	3.06	3.05	4	2	11.49	5.14	92.08	✓	✓	5	2
	412.40	C23H16N4O4	3.59	3.58	3	3	9.21	5.12	113.16	✓	✓	5	5
	413.86	C24H16ClN3O2	5.61	5.60	2	2	10.26	5.16	70.92	✓	☐	5	4
	348.44	C20H24N6	2.93	2.07	4	4	13.20	7.98	109.40	✓	✓	4	7
	235.24	C14H9N3O	3.01	2.99	2	1	8.82	2.99	54.71	✓	✓	4	1
	324.34	C16H16N6O2	-0.27	-0.32	6	6	11.55	6.44	149.86	✓	☐	4	3
	328.37	C20H16N4O	3.78	3.78	4	1	11.35	4.98	63.16	✓	✓	4	4

7. Toxicity Analysis of Bioactive Compounds.

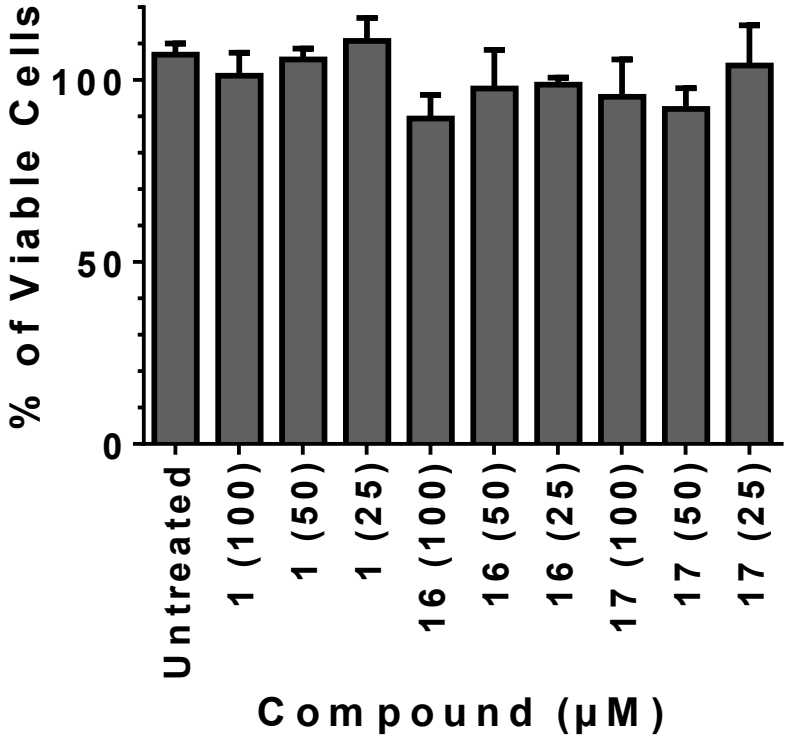


Figure S-6. Bioactive hit compounds were assessed for toxicity using WST-1 reagent. The compounds have little to no effect on cell viability in a cellular model of DM1.