

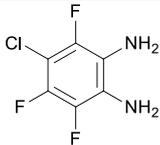
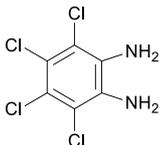
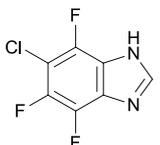
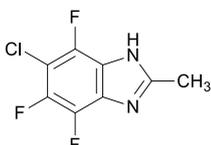
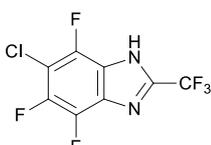
Halogenated (F, Cl) 1,3-benzodiazoles, 1,2,3-benzotriazoles, 2,1,3-benzothia(selena)diazoles and 1,4-benzodiazines inducing Hep2 cell apoptosis

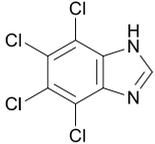
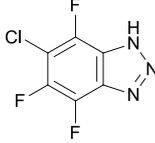
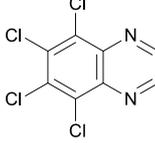
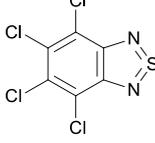
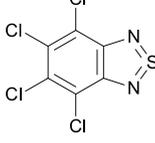
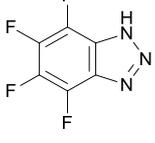
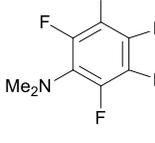
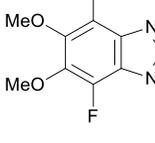
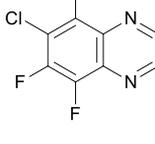
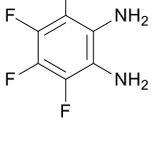
Darya O. Prima, Elena V. Vorontsova, Arkady G. Makarov, Alexander Yu. Makarov, Irina Yu. Bagryanskaya, Tatiana F. Mikhailovskaya, Yuri G. Slizhov and Andrey V. Zibarev

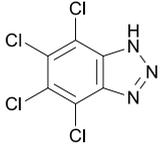
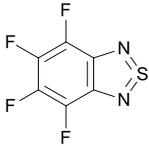
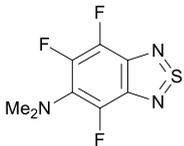
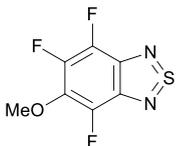
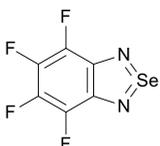
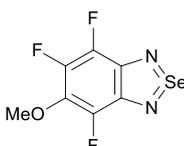
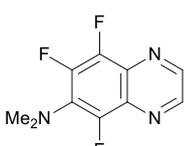
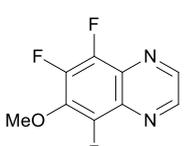
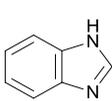
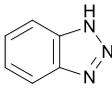
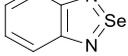
Content

- §1. Structures and chemical names of compounds
- §2. Instrumental methods (including XRD structure determination)
- §3. Cytotoxicity and apoptotic activity

§1. Structures and chemical names of compounds

Compound	Name
1 	1,2-diamino-4-chloro-3,5,6-trifluorobenzene
2 	1,2-diamino-3,4,5,6-tetrachlorobenzene
3 	6-chloro-4,5,7-trifluoro-1,3-benzodiazole
4 	6-chloro-4,5,7-trifluoro-1,3-benzodiazole-2-methyl
5 	6-chloro-4,5,7-trifluoro-1,3-benzodiazole-2-trifluoromethyl

- 6  4,5,6,7-tetrachloro-1,3-benzodiazole
- 7  6-chloro-4,5,7-trifluoro-1,2,3-benzotriazole
- 8  5,6,7,8-tetrachloro-1,4-benzodiazine
- 9  4,5,6,7-tetrachloro-2,1,3-benzothiadiazole
- 10  4,5,6,7-tetrachloro-2,1,3-benzoselenadiazole
- 11  4,5,6,7-tetrafluoro-1,2,3-benzotriazole
- 12  4,5,6,7-tetrafluoro-1,3-benzodiazole
- 13  5-dimethylamino-4,6,7-trifluoro-2,1,3-benzoselenadiazole
- 14  4,7-difluoro-5,6-dimethoxy-2,1,3-benzoselenadiazole
- 15  6-chloro-5,7,8-trifluoro-1,4-benzodiazine
- 16  1,2-diamino-3,4,5,6-tetrafluorobenzene

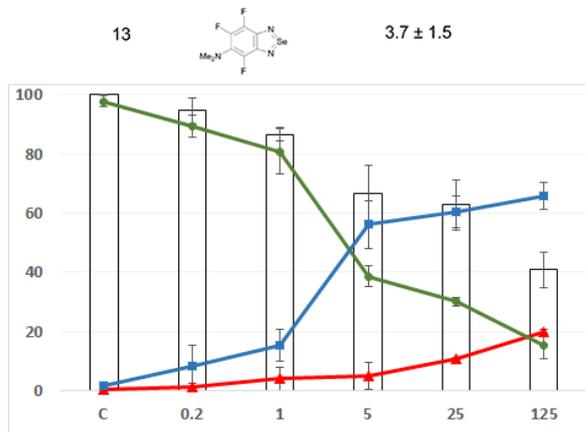
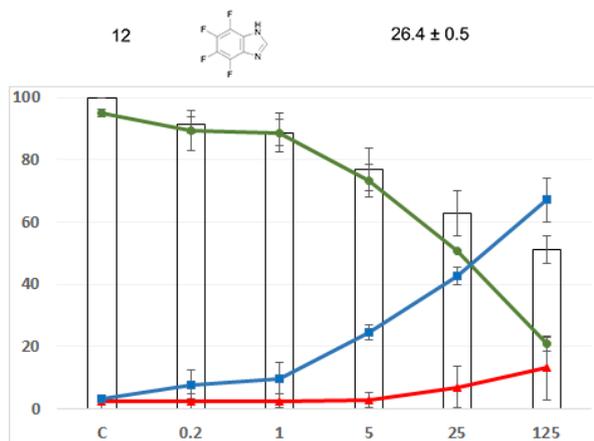
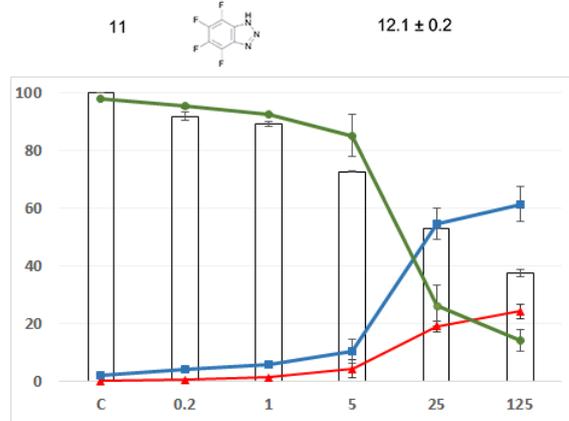
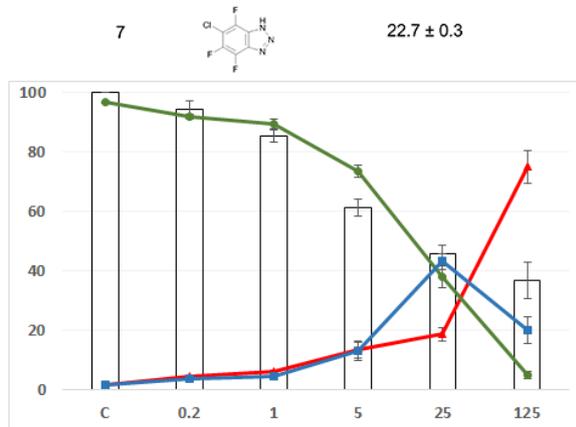
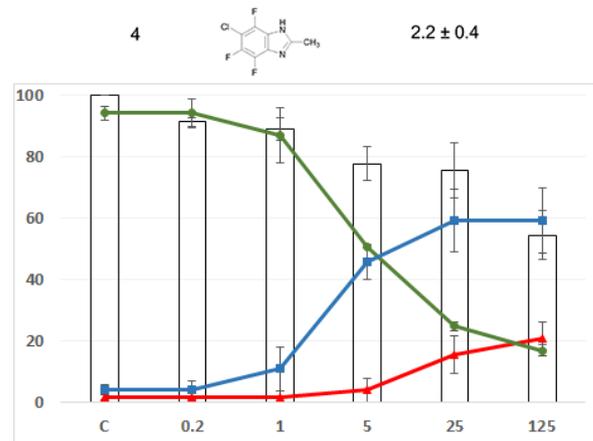
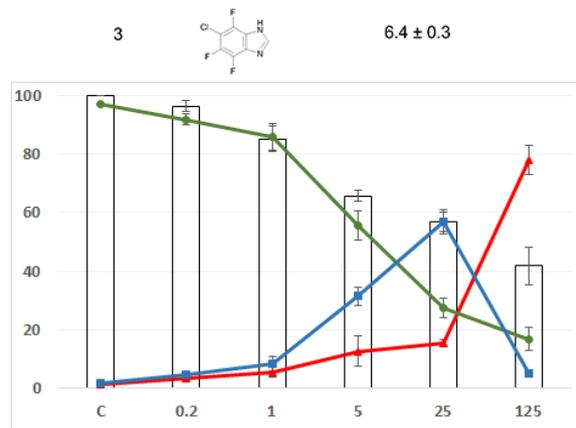
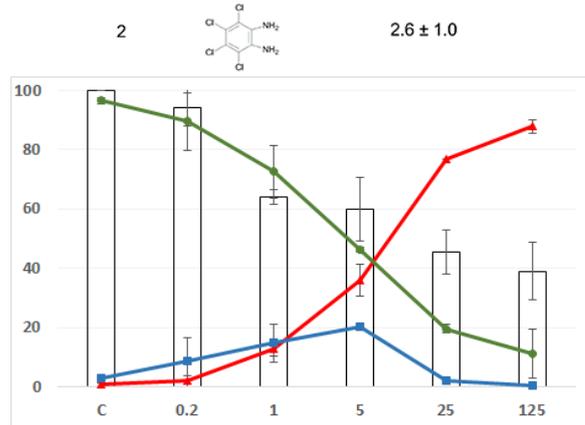
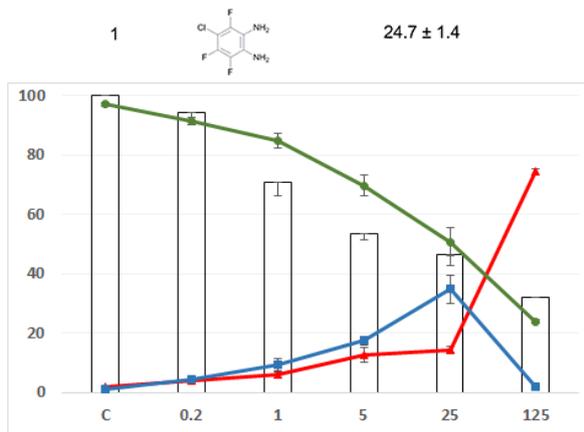
- 17  4,5,6,7-tetrachloro-1,2,3-benzotriazole
- 18  4,5,6,7-tetrafluoro-2,1,3-benzothiadiazole
- 19  5-dimethylamino-4,6,7-trifluoro-2,1,3-benzothiadiazole
- 20  4,5,7-trifluoro-5-methoxy-2,1,3-benzothiadiazole
- 21  4,5,6,7-tetrafluoro-2,1,3-benzoselenadiazole
- 22  4,5,7-trifluoro-5-methoxy-2,1,3-benzoselenadiazole
- 23  5,6,7,8-tetrafluoro-1,4-benzodiazine
- 24  6-dimethylamino-5,7,8-trifluoro-1,4-benzodiazine
- 25  5,6,8-trifluoro-6-methoxy-1,4-benzodiazine
- 26  1,3-benzodiazole
- 27  1,2,3-benzotriazole
- 28  2,1,3-benzoselenadiazole
-

§2. Instrumental methods

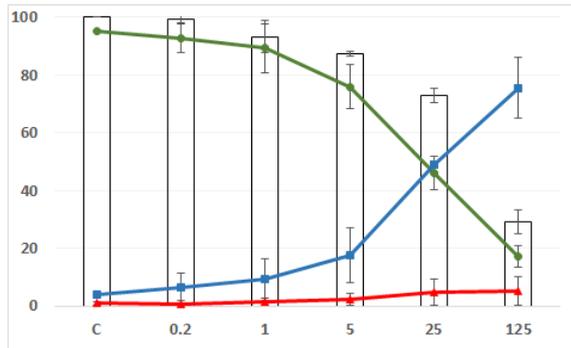
General. NMR spectra were measured with Bruker AV-300 (^1H , 300.1; ^{13}C , 100.6; ^{19}F , 282.4 MHz) and Bruker AV-600 (^{77}Se , 114.5 MHz) spectrometers. The standards were TMS (^1H , ^{13}C), C_6F_6 (^{19}F) and Me_2Se (^{77}Se); for C_6F_6 , $\delta^{19}\text{F} = -162.9$ with respect to CFCl_3 . High-resolution mass-spectra (EI, 70 eV) were collected with a Thermo Electron Corporation DFS mass-spectrometer. Gas-chromatography – mass-spectrometry (GC-MS) experiments were performed with Hewlett-Packard G1800A instrument combining HP 5890 Series II gas chromatograph (equipped with capillary column HP-5) and HP 5971 mass-selective detector (EI, 70 eV), with helium, 1 ml min^{-1} , as carrier gas. UV-Vis and fluorescence (FL) spectra were taken with Varian Cary 5000 and Varian Cary Eclipse spectrophotometers, respectively.

X-ray structure determination. Selected bond lengths (Å) and angles ($^\circ$): **2** (CCDC 1524477): C1–C2 1.413(2), C2–C3 1.393(2), C3–C4 1.396(2), C4–C5 1.389(2), C5–C6 1.398(2), C6–C1 1.390(3), C1–N1 1.402(2), C2–N2 1.395(3), C3–Cl1 1.730(1), C4–Cl2 1.724(1), C5–Cl3 1.717(2), C6–Cl4 1.728(1); the bond angles not involving H atoms are close to 120° . **3** (CCDC 1510245): N1–C2 1.333(4), C2–N3 1.325(4), N3–C3a 1.370(4), C3a–C7a 1.401(4), C7a–N1 1.372(4); C7a–N1–C2 104.7(2), N1–C2–N3 114.5(3), C2–N3–C3a 105.1(3), N3–C3a–C7a 107.7(3), C3a–C7a–N1 107.9(3); the molecule is planar within $\pm 0.019 \text{ \AA}$. **9** (CCDC 1524234): N1–S2 1.621(2), S2–N3 1.615(2), N3–C3a 1.349(3), C3a–C7a 1.426(3), C7a–N1 1.340(3); C7a–N1–S2 105.9(2), N1–S2–N3 101.0(1), S2–N3–C3a 106.1(2), N3–C3a–C7a 113.1(2), C3a–C7a–N1 113.9(2). **10** (CCDC 1487294): N1–Se2 1.792(3), Se2–N3 1.779(3), N3–C3a 1.326(4), C3a–C7a 1.449(4), C7a–N1 1.326(4); C7a–N1–Se2 106.3(2), N1–Se2–N3 94.34(12), Se2–N3–C3a 107.0(2), N3–C3a–C7a 115.9(3), C3a–C7a–N1 116.4(3); the molecule is planar within $\pm 0.029 \text{ \AA}$. **11** (CCDC 1524233; average of two crystallographically independent molecules): N1–N2 1.339(2), N2–N3 1.309(2), N3–C3a 1.369(2), C3a–C7a 1.393(2), C7a–N1 1.353(2); C7a–N1–N2 110.7(1), N1–N2–N3 108.6(1), N2–N3–C3a 108.4(1), N3–C3a–C7a 108.1(1), C3a–C7a–N1 104.3(1).

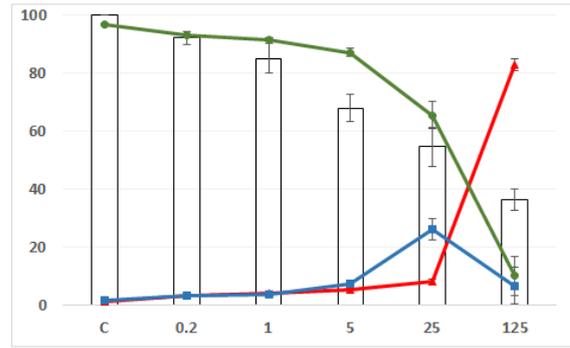
§3. Cytotoxicity and apoptotic activity



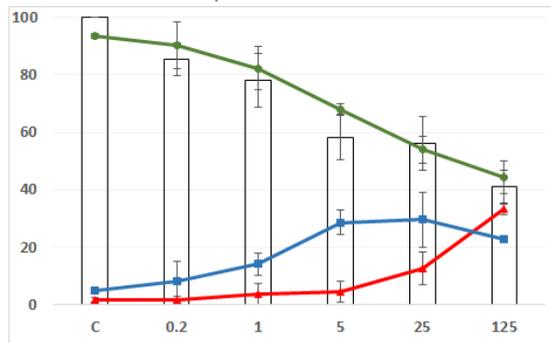
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 17.4 ± 2.2 

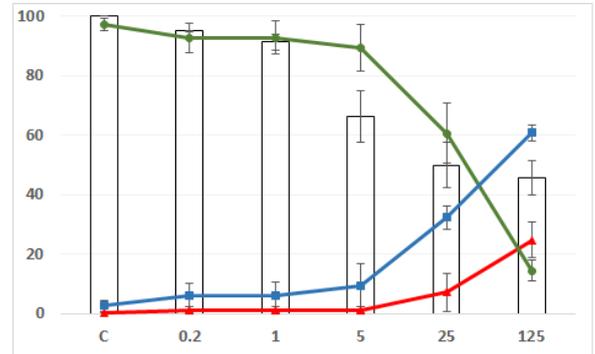
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 43.6 ± 1.9 

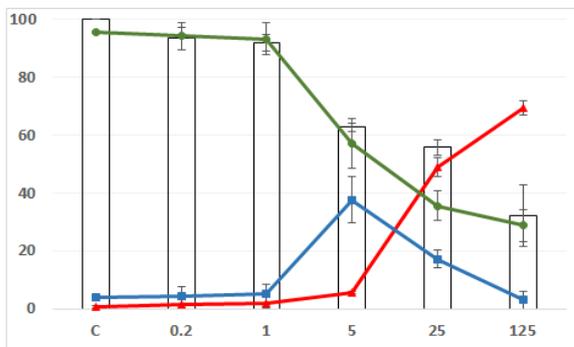
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 10.1 ± 0.8 

16

 37.2 ± 1.6 

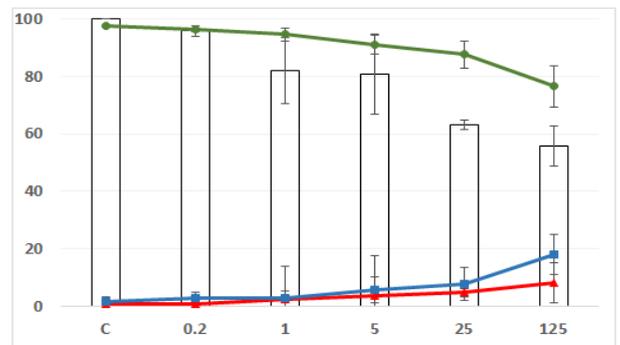
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 8.1 ± 1.5 

18



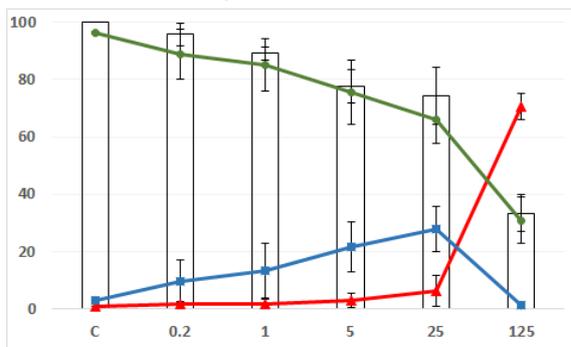
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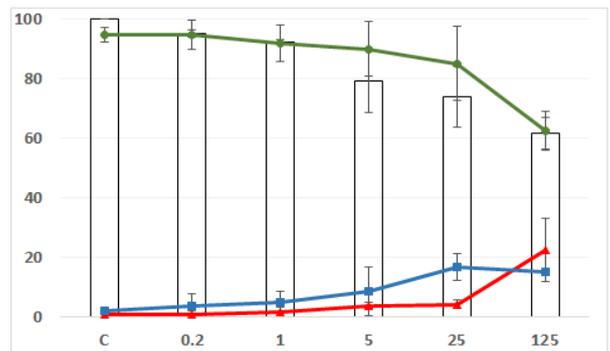
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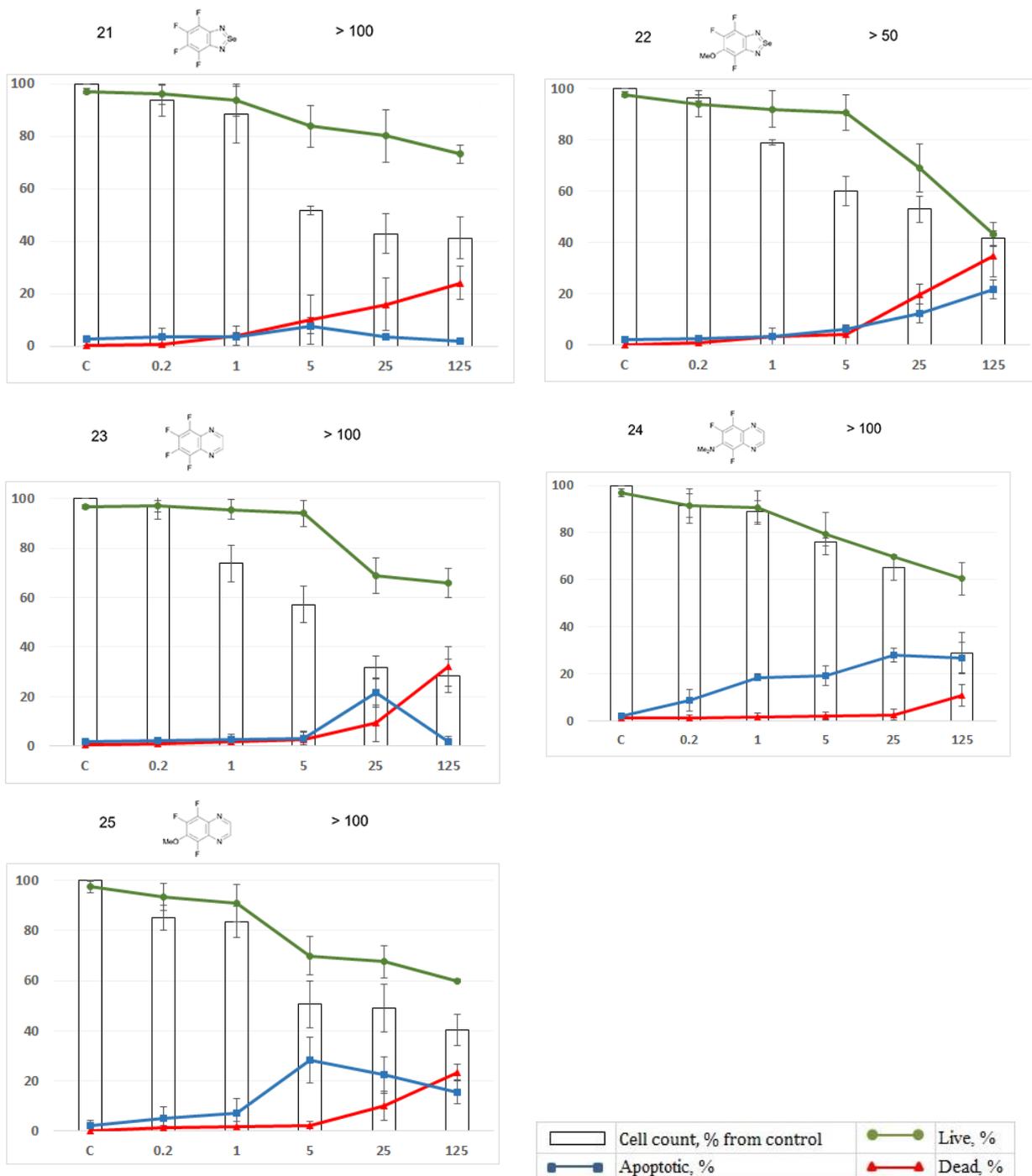


Figure S1 The medium inhibitory concentrations (IC_{50} , μM) and cytotoxic effects of compounds on the Hep2 cells (other polychlorinated derivatives revealed low apoptotic activity in preliminary tests were not studied in detail). The axis y: cells, %; the axis x: compound concentration in 0.2-125 μM range, C – vehicle control. Experiment duration 48 h, the data represent the mean \pm S.D. of three independent experiments.

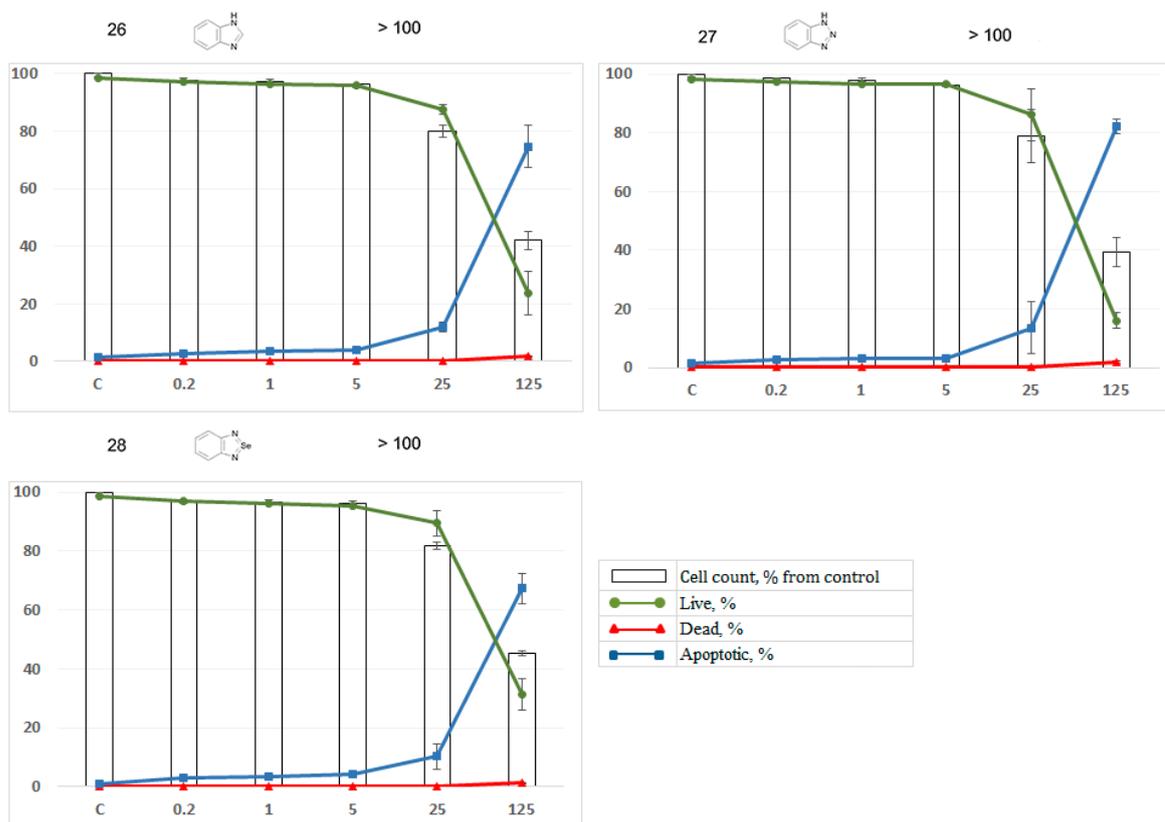


Figure S2 The medium inhibitory concentrations (IC_{50} , μM) and cytotoxic effects of the archetypal diazole, triazole and selenadiazole on the Hep2 cells. The axis y: cells, %; the axis x: compound concentration in 0.2-125 μM range, C – vehicle control. Experiment duration 48 h, the data represent the mean \pm S.D. of three independent experiments.

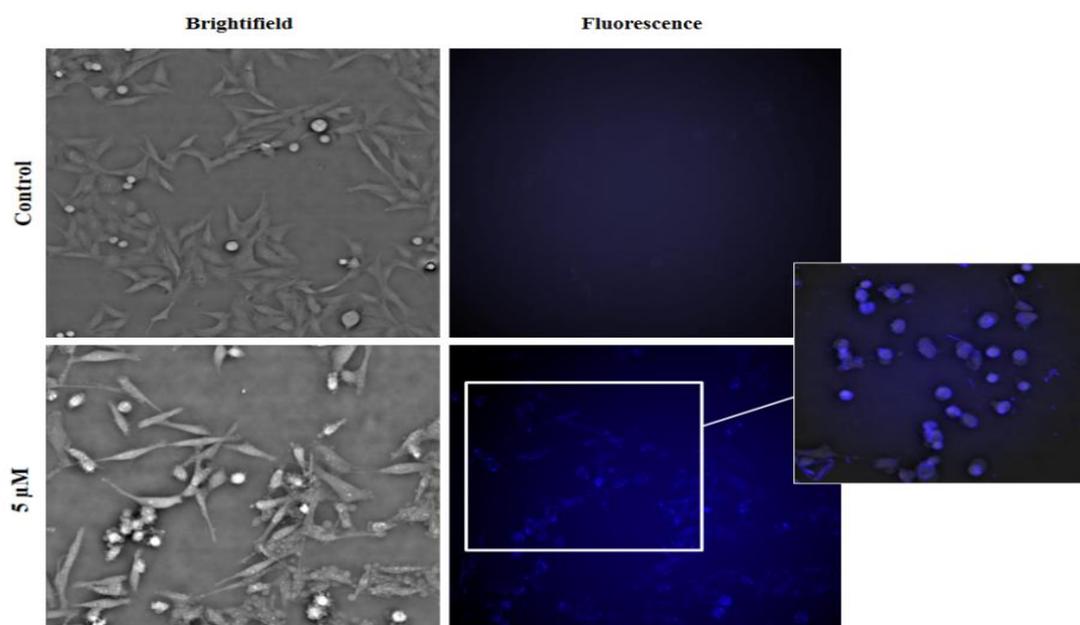


Figure S3 Fluorescence of diazine 24 in Hep2 cancer cells at concentration of 5 μM after 48 h, $\lambda_{\text{exc}} = 390 \text{ nm}$.

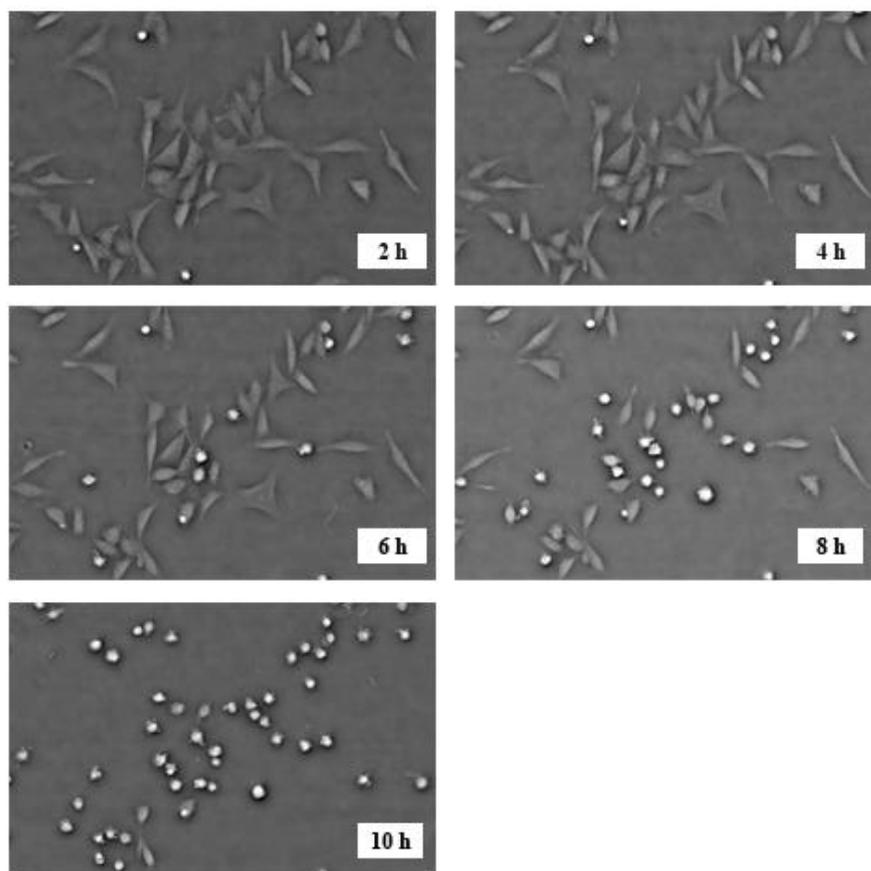


Figure S4 Dynamics of Hep2 cells death with selenadiazole **13** at concentration of 25 μM in the first 10 h after the treatment. Apoptotic cells appeared in the first hours, morphological changes and vesicles were clearly visible after 8 h.

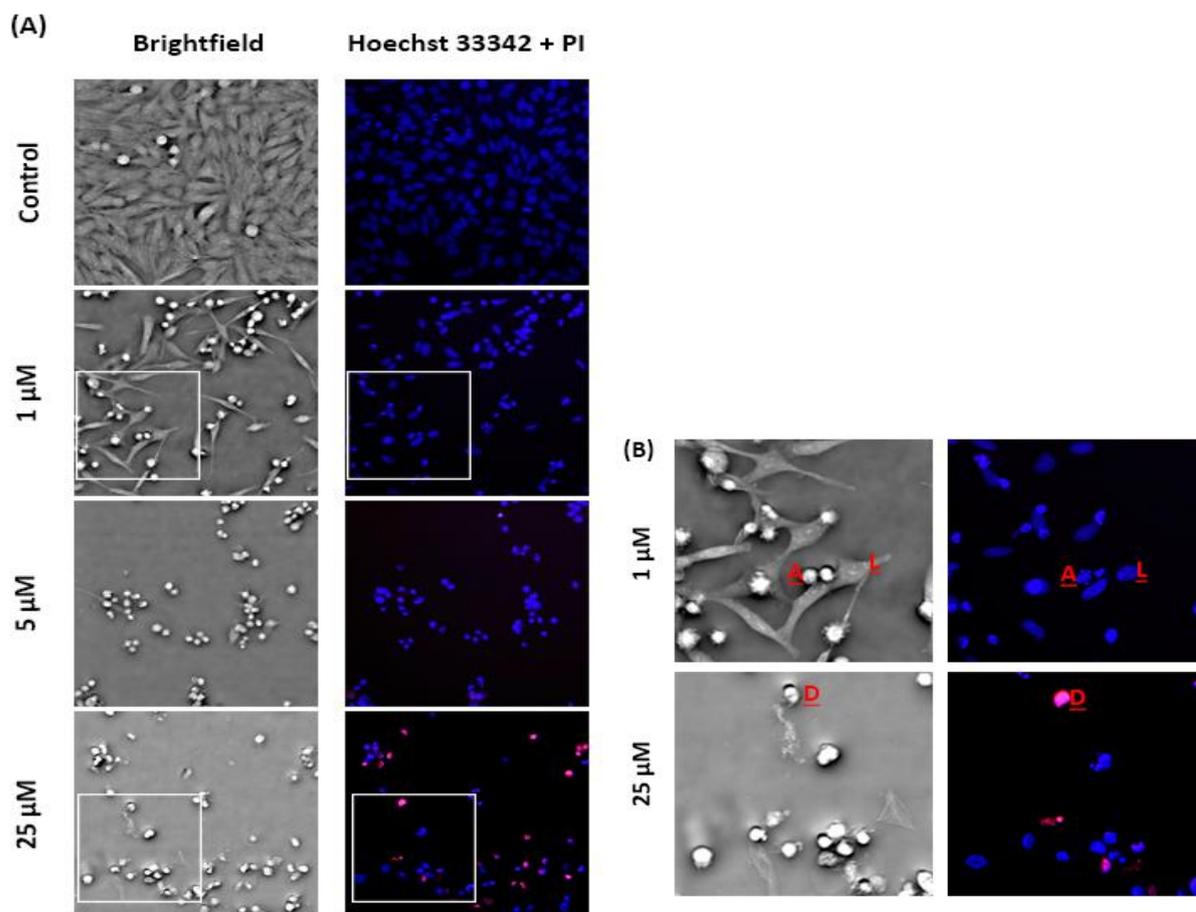


Figure S5 A: Toxicity of diazole **12** towards the Hep2 cells at concentrations of 1, 5 and 25 μ M after 48 h. Color code: pink – dead cells, bright blue – apoptotic cells, blue – live cells. B: Toxicity of **12** towards the cells at concentrations of 1 and 25 μ M after 48 h with magnified images of the squares which are shown in part A. Letter code: A – apoptotic cells, L – live cells, D – dead cells.