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# Mononuclear silver(I) complexes with 1,7-phenanthroline as potent inhibitors of *Candida* growth

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## Abstract

Mononuclear silver(I) complexes with 1,7-phenanthroline (1,7-phen), [Ag($NO_3$-*O,O'*)(1,7-phen-*N7*)$_2$] (**1**) and [Ag(1,7-phen-*N7*)$_2$]X, X = $ClO_4^-$ (**2**), $CF_3SO_3^-$ (**3**), $BF_4^-$ (**4**) and $SbF_6^-$ (**5**) were synthesized and structurally characterized by NMR ($^1$H and $^{13}$C), IR and UV-Vis spectroscopy and ESI mass spectrometry. The crystal structures of **1, 3** and **4** were determined by single-crystal X-ray diffraction analysis. In all these complexes, 1,7-phen coordinates to the Ag(I) ion in a monodentate fashion *via* the less sterically hindered N7 nitrogen atom. The investigation of the solution stability of **1 – 5** in DMSO revealed that they are sufficiently stable in this solvent at room temperature. Complexes **1** – **5** showed selectivity towards *Candida* spp. in comparison to bacteria, effectively inhibiting the growth of four different *Candida* species with minimal inhibitory concentrations (MIC) between 1.2 and 11.3 μM. Based on the lowest MIC values and the lowest cytotoxicity against healthy human fibroblasts with selectivity index of more than 30, the antifungal potential was examined in detail for the complex **1**. It had the ability to attenuate *C. albicans* virulence and to reduce epithelial cell damage in the cell infection model. Induction of reactive oxygen species (ROS) response has been detected in *C. albicans*, with fungal DNA being one of the possible target biomolecules. The toxicity profile of **1** in the zebrafish model (*Danio rerio*) revealed improved safety and activity in comparison to that of clinically utilized silver(I) sulfadiazine.


*Keywords*: Silver(I) complexes; Antimicrobial activity; Cytotoxicity; *Candida*; *Danio rerio*

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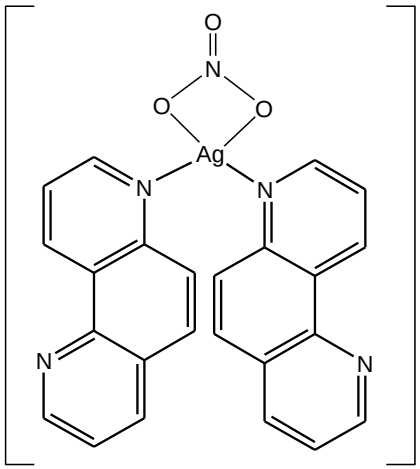
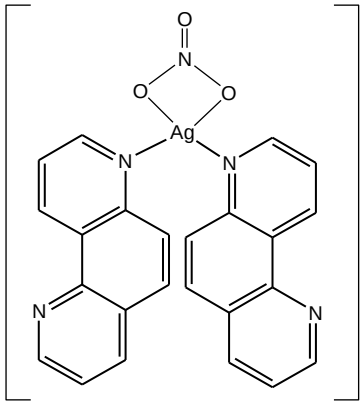
**$^1$H NMR (400 MHz, DMF-$d_7$)**



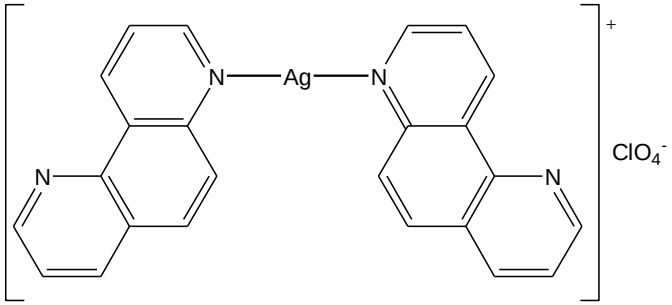

[Ag($NO_3$-*O,O'*)(1,7-phen-*N7*)$_2$] (**1**)

**$^{13}$C NMR (101 MHz, DMF-$d_7$)**



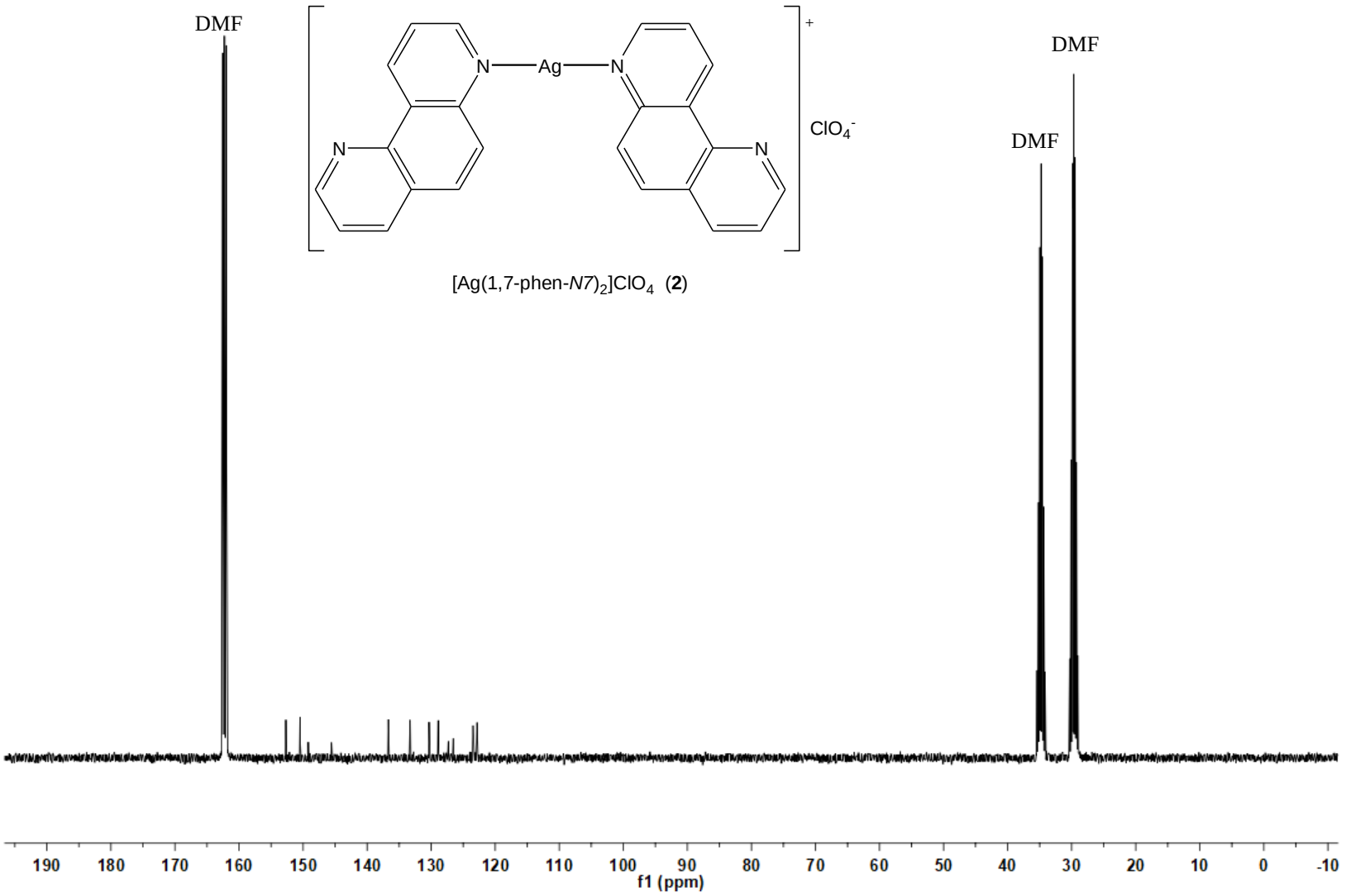

[Ag($NO_3$-*O*,*O*')(1,7-phen-*N7*)$_2$] (**1**)

**$^1$H NMR (400 MHz, DMF-$d_7$)**



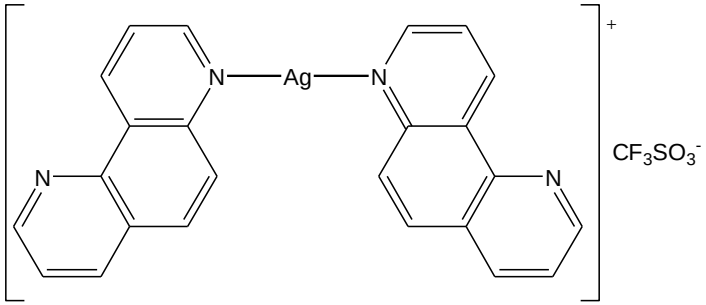

$[Ag(1,7\text{-phen-}N7)_2]ClO_4$ (**2**)

**$^{13}$C NMR (101 MHz, DMF-$d_7$)**



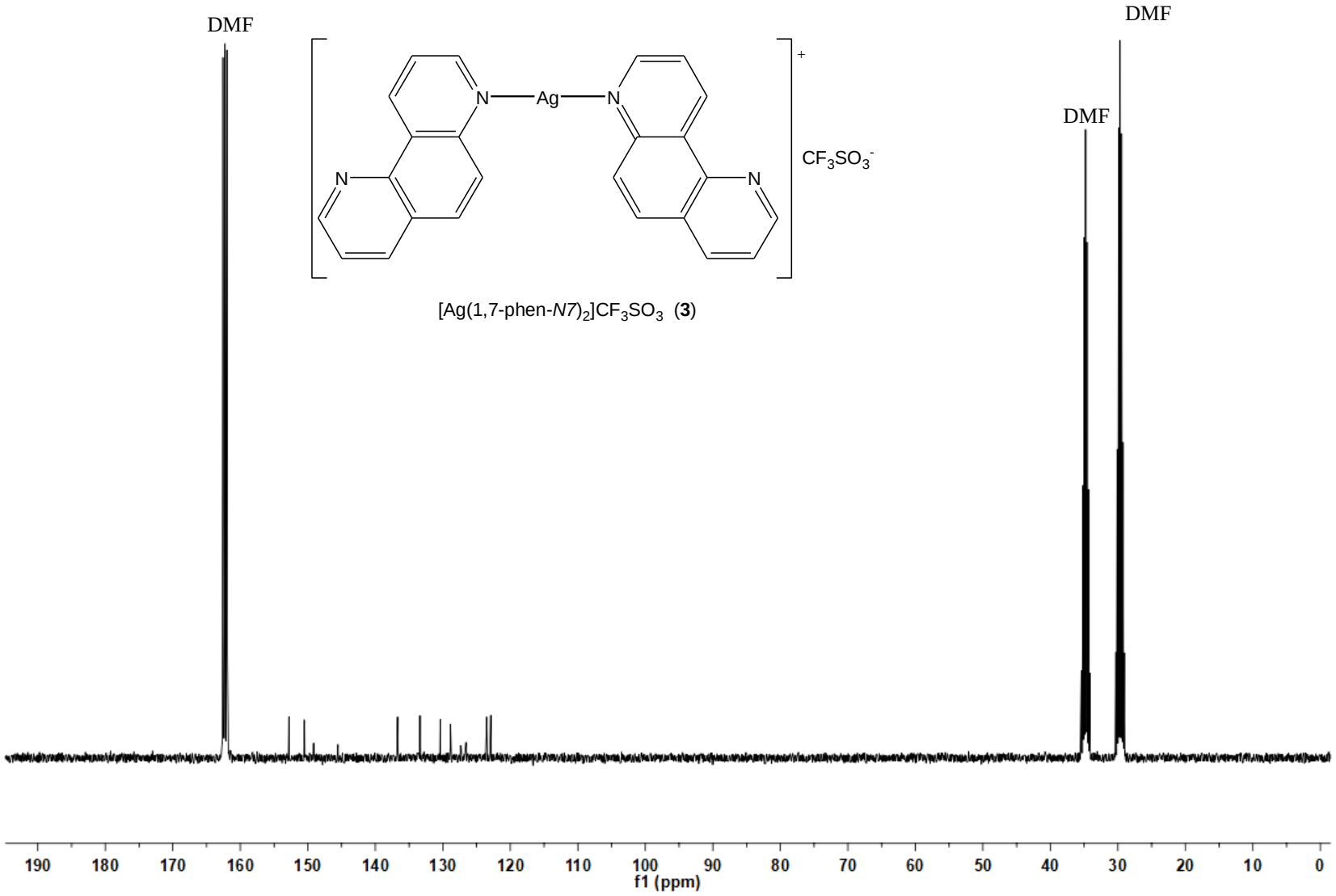

[Ag(1,7-phen-*N*7)$_2$]ClO$_4$ (**2**)

**$^1$H NMR (400 MHz, DMF-$d_7$)**



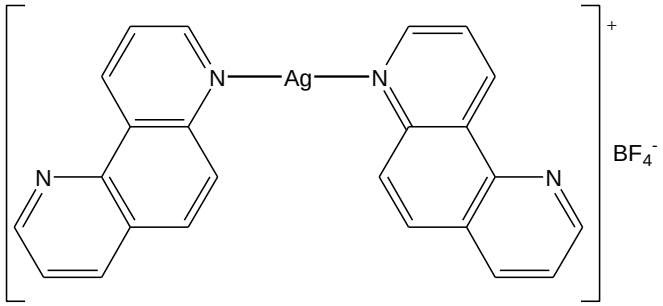

$[Ag(1,7\text{-phen-}N7)_2]CF_3SO_3$ (**3**)

**$^{13}$C NMR (101 MHz, DMF-$d_7$)**



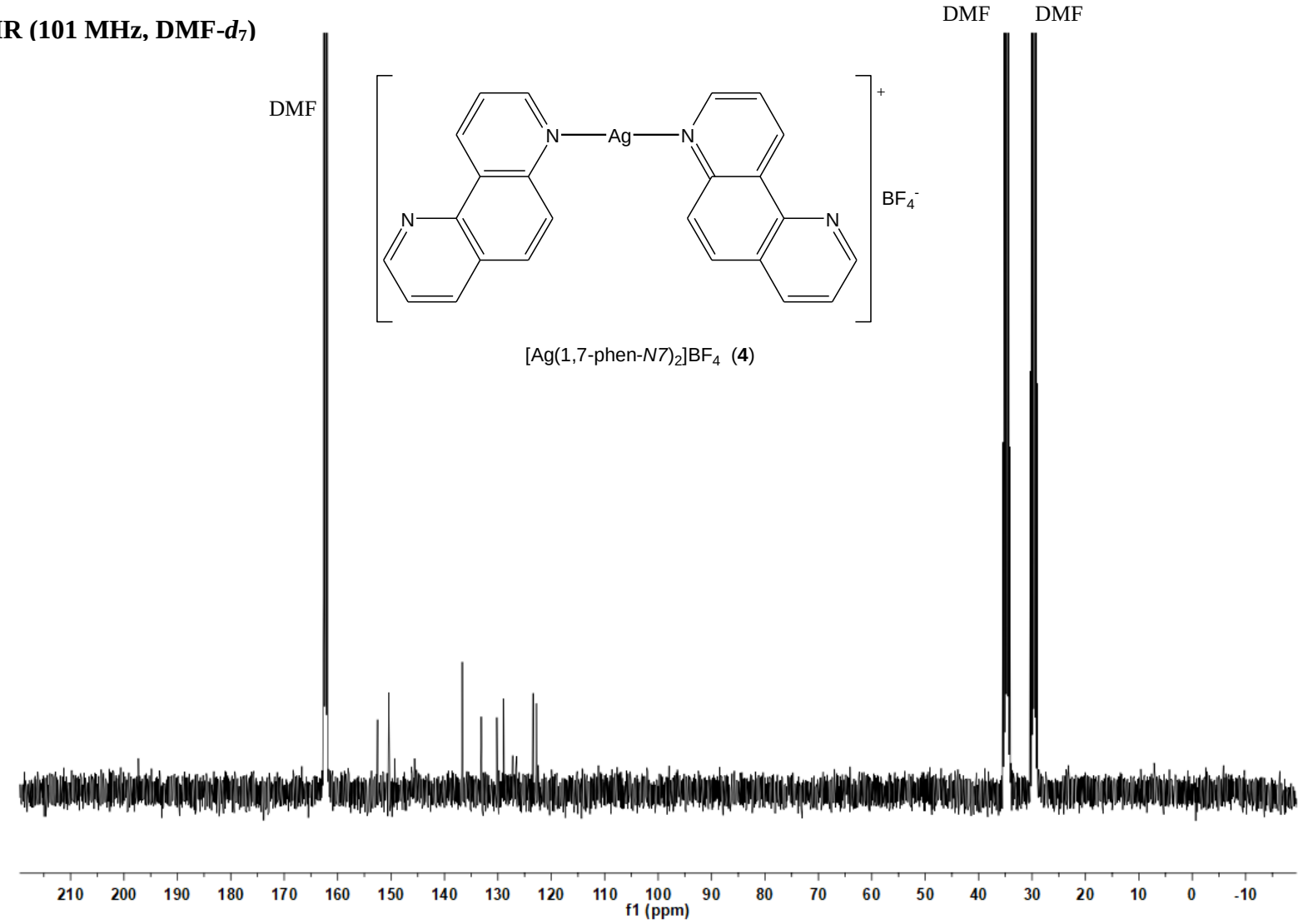

[Ag(1,7-phen-*N7*)$_2$]$CF_3SO_3$ (**3**)

**$^{1}$H NMR (400 MHz, DMF-$d_7$)**



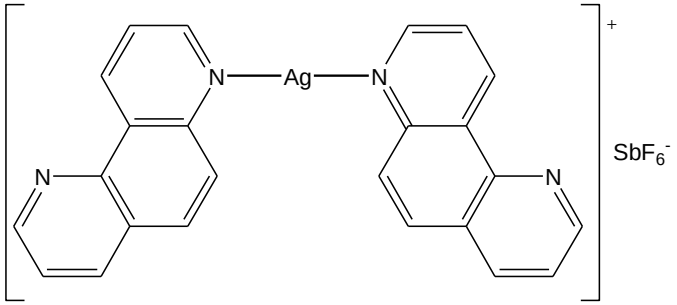

$[Ag(1,7\text{-phen-}N7)_2]BF_4$ (**4**)

**$^{13}$C NMR (101 MHz, DMF-$d_7$)**

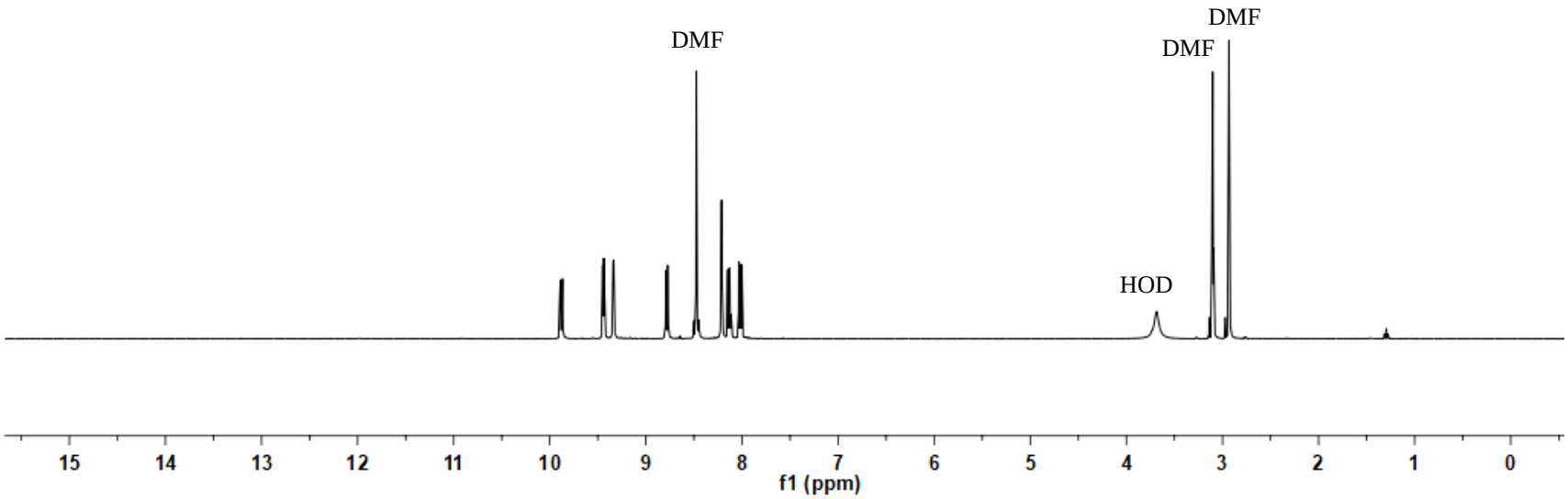



[Ag(1,7-phen-*N*7)$_2$]BF$_4$ (**4**)

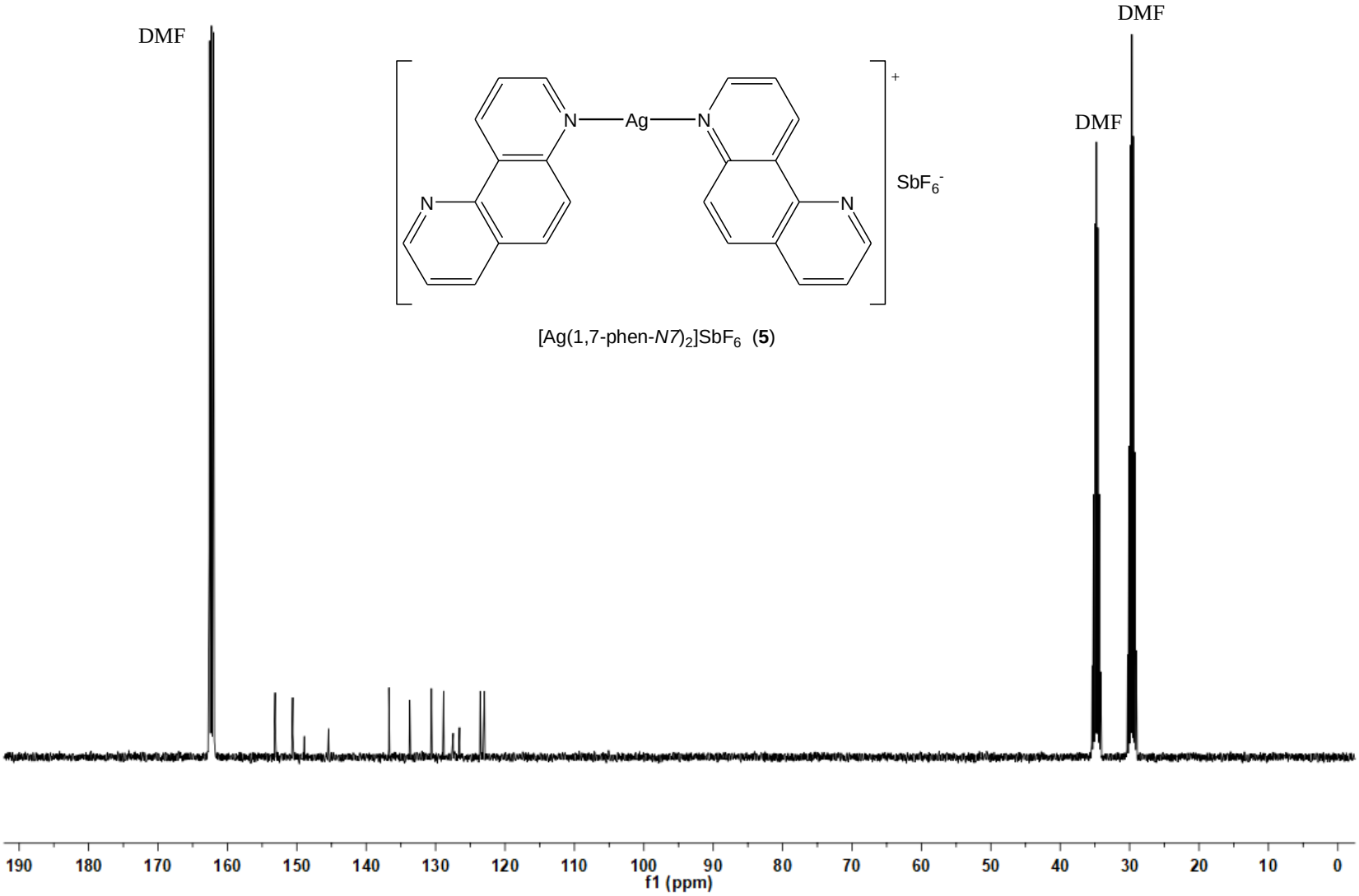
**$^1$H NMR (400 MHz, DMF-$d_7$)**




[Ag(1,7-phen-*N7*)$_2$]SbF$_6$ (**5**)



**$^{13}$C NMR (101 MHz, DMF-$d_7$)**




[Ag(1,7-phen-*N7*)$_2$]SbF$_6$ (**5**)

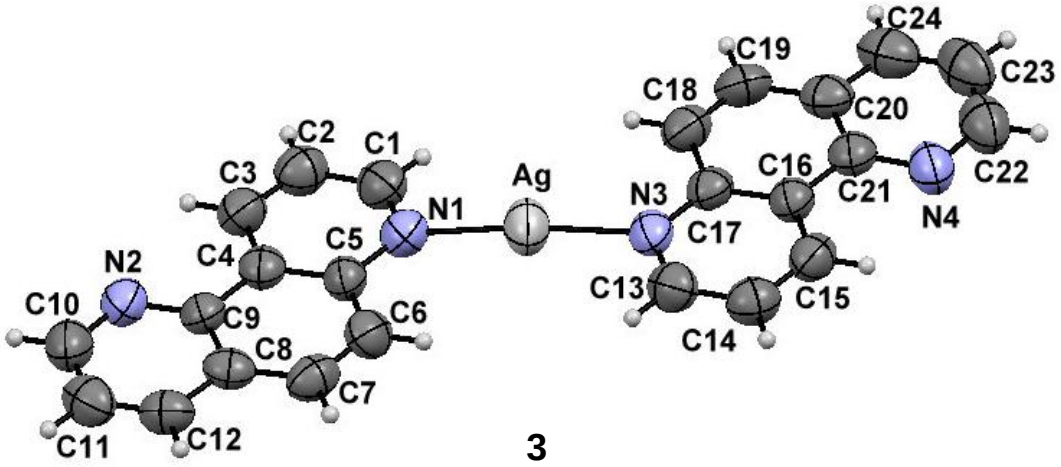



**Fig. S1.** Molecular structure of the silver(I) complex **3**. Non-coordinating triflate anion in **3** is omitted for clarity. Displacement ellipsoids are drawn at 50% probability level and H atoms are represented by spheres of arbitrary size.

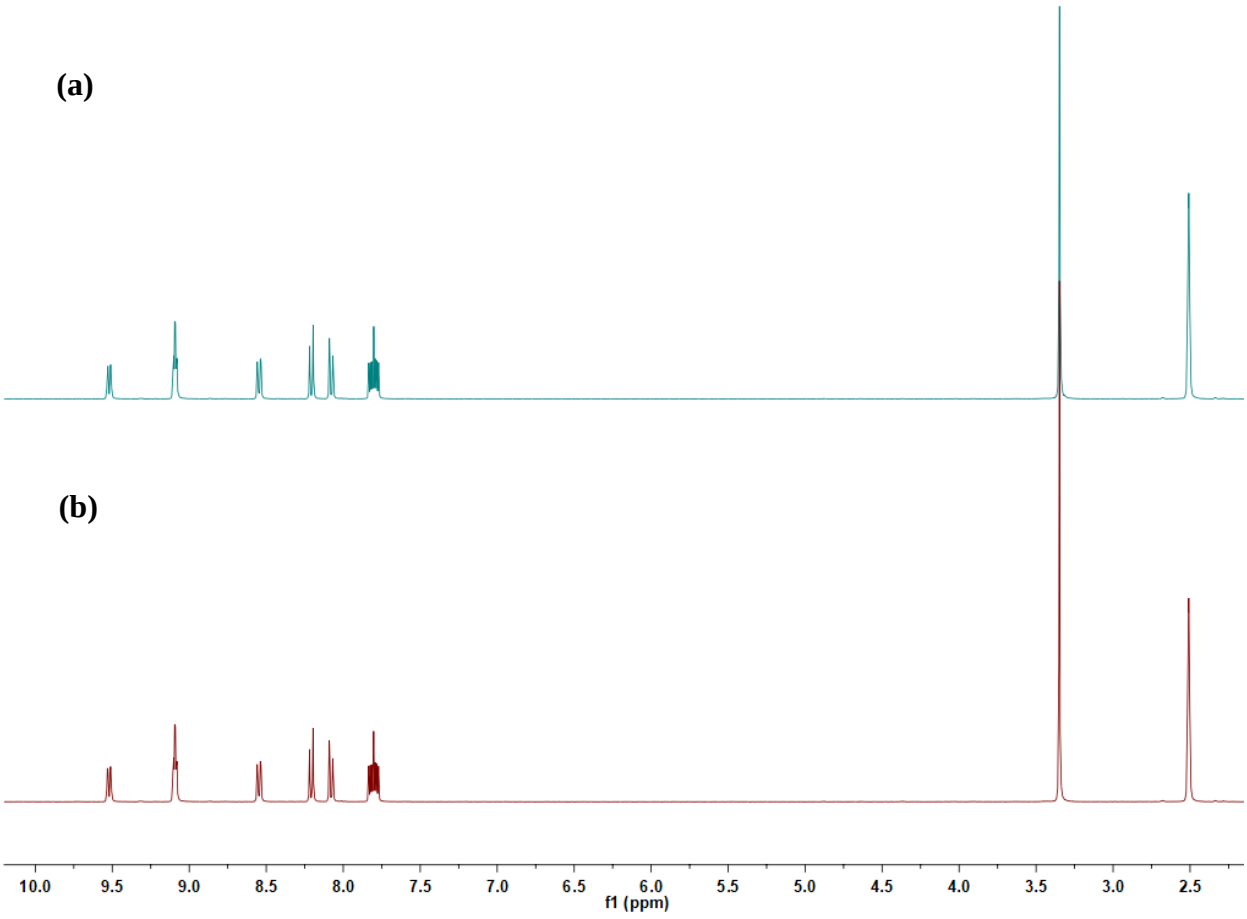



**Fig. S2.** Silver(I) complex **3** stability over time measured by $^{1}$H NMR spectroscopy. $^{1}$H NMR spectrum was measured immediately **(a)** and 48 h **(b)** after complex dissolution in DMSO-$d_6$.

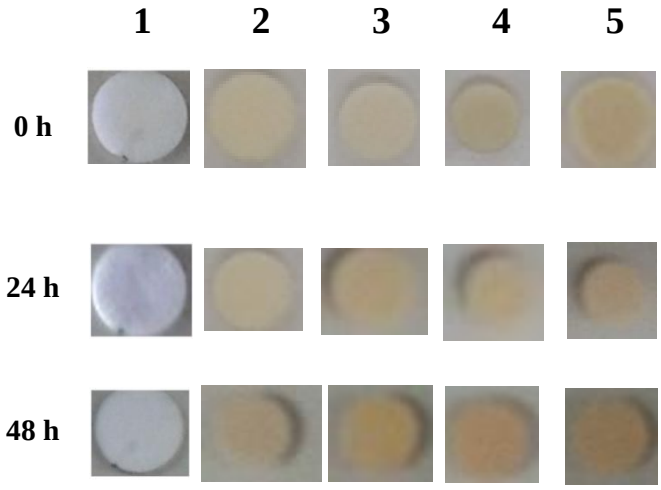
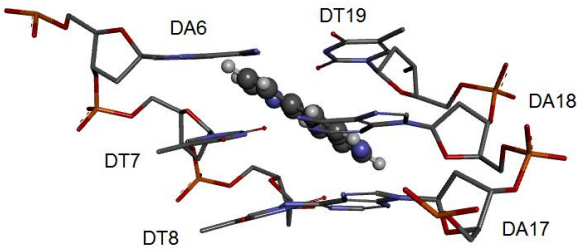
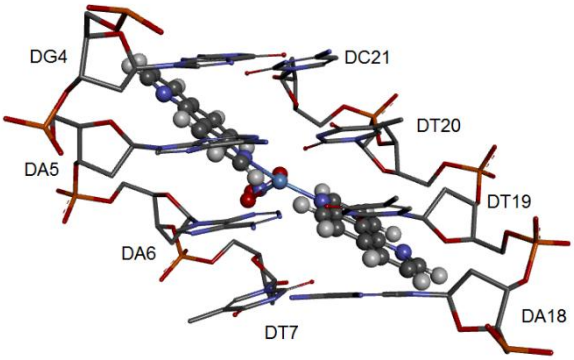



**Fig. S3.** Air/light stability of silver(I) complexes **1 – 5.**



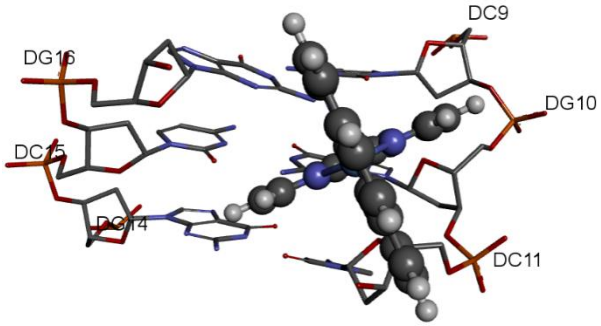

1,7-phen

-3.83 kcal/mol




complex **1**

-7.06 kcal/mol




$[Ag(1,10\text{-phen})_2]^+$

-4.80 kcal/mol

**Fig. S4.** The most stable binding of the tested compounds to base pairs of DNA and the corresponding binding energies, as assessed by molecular docking.

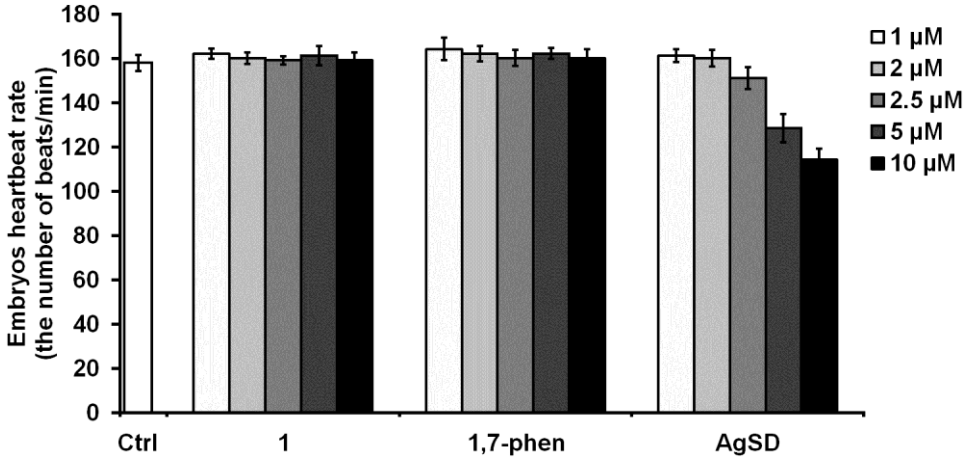



**Fig. S5**. The heartbeating rate of zebrafish embryos at 114 hpf upon different concentrations of complex **1**, 1,7-phen and silver(I) sulfadiazine (AgSD). Statistically significant differences between data of untreated control and treatments were denoted with asterisk (*P < 0.5, **P < 0.01; ***P < 0.001; Student t' test).

**Table S1**

Selected bond distances (Å) and valence angles ($^{o}$) in the silver(I) complexes **1, 3** and **4**.

| | **1** | **3** | **4** |
|---|---|---|---|
| Ag—N1 | 2.203(4) | 2.137(10) | 2.172(7) |
| Ag—N3 | 2.206(4) | 2.146(9) | 2.191(7) |
| Ag—O2 | 2.630(4) | / | / |
| Ag—O3 | 2.652(4) | / | / |
| | | | |
| N1—Ag—N3 | 154.78(15) | 174.2(3) | 166.6(3) |
| N1—Ag—O3 | 95.33(15) | / | / |
| N3—Ag—O2 | 93.83(15) | / | / |
| O2—Ag—O3 | 47.85(12) | / | / |
| N1—Ag—O2 | 110.83(15) | / | / |
| N3—Ag—O3 | 105.53(15) | / | / |
| N5—O3—Ag | 95.1(3) | / | / |
| N5—O2—Ag | 95.8(3) | / | / |
| C1—N1—Ag | 120.6(3) | 118.4(8) | 121.5(6) |
| C5—N1—Ag | 121.8(3) | 121.7(8) | 119.6(5) |
| C13—N3—Ag | 119.5(3) | 119.2(8) | 119.1(6) |
| C17—N3—Ag | 122.7(3) | 120.1(7) | 120.1(5) |

**Table S2**

Details of the crystal structure determinations of the silver(I) complexes **1, 3** and **4**.

| | **1** | **3** | **4** |
|---|---|---|---|
| Empirical formula | $C_{24}H_{16}AgN_5O_3$ | $C_{25}H_{16}AgF_3N_4O_3S$ | $C_{24}H_{16}AgBF_4N_4$ |
| CCDC number | CCDC-1829501 | CCDC-1829502 | CCDC-1829503 |
| Formula weight ($g.mol^{-1}$) | 530.29 | 617.35 | 555.09 |
| Crystal system, space group | triclinic, *P*ī | triclinic, *P*ī | triclinic, *P*ī |
| *a* (Å) | 7.1629(7) | 6.9800(5) | 7.1759(8) |
| *b* (Å) | 10.5142(9) | 13.6380(9) | 10.9209(12) |
| *c* (Å) | 14.5205(12) | 13.9579(9) | 14.5568(15) |
| $\alpha$ (°) | 78.215(7) | 109.122(5) | 83.120(9) |
| $\beta$ (°) | 81.410(7) | 103.327(5) | 86.671(9) |
| $\gamma$ (°) | 73.611(7) | 103.352(5) | 71.989(9) |
| *V* ($Å^3$) | 1022.11(16) | 1151.72(14) | 1076.8(2) |
| $F_{000}$ | 532 | 616 | 552 |
| *Z* | 2 | 2 | 2 |
| X-radiation, $\lambda$ /Å | Mo-$K_\alpha$ 0.71073 | Mo-$K_\alpha$ 0.71073 | Mo-$K_\alpha$ 0.71073 |
| data collect. temperat. /K | 298(2) | 298(2) | 250(2) |
| Calculated density (Mg $m^{-3}$) | 1.723 | 1.780 | 1.712 |
| Absorption coefficient ($mm^{-1}$) | 1.026 | 1.028 | 0.991 |
| Crystal size (mm) | 0.400 × 0.100 × 0.020 | 0.29 x 0.13 x 0.04 | 0.210 × 0.130 × 0.030 |
| $\theta$ range (°) | 1.4 to 25.2 | 1.6 to 25.1 | 1.4 to 25.3 |
| index ranges *h*, *k*, *l* | -8 ... 8, -12 ... 12, -17 ... 17 | -7 … 8, -16… 16, -16... 16 | -8 ... 8, -13 ... 13, -4 ... 17 |
| No. of collected and independent reflections | 13116, 3640 | 14162, 4106 | 3842, 3842 |
| $R_{int}$ | 0.1022 | 0.1034 | |
| Data / restraints / parameters | 3640 / 0 / 298 | 4106/151/334 | 3842 / 0 / 308 |
| Goodness-on-fit on $F^2$ | 0.990 | 0.993 | 0.915 |
| Final *R* indices | 0.0481, 0.1111 | 0.0868, 0.2212 | 0.0699, 0.1669 |
| $[F_o > 4\sigma(F_o)]$ $R(F)$, $wR(F^2)$ | | | |
| Final *R* indices | 0.0741, 0.1237 | 0.1638, 0.2838 | 0.1335, 0.2031 |
| (all data) $R(F)$, $wR(F^2)$ | | | |
| Difference density: max, min (e $Å^{-3}$) | 1.06, -0.62 | 2.03, -0.66 | 0.68, -0.65 |
| Twinned Data Refinement | / | / | 0.674(4), 0.326(4) |

**Table S3**

Lethal and teratogenic effects observed in zebrafish (*Danio rerio*) embryos at different hours post fertilization (hpf).

| Category | Developmental endpoints | Exposure time (hpf) | | | |
|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96/114 |
| Lethal effect | Egg coagulation[a] | ● | ● | ● | ● |
| | No somite formation | ● | ● | ● | ● |
| | Tail not detached | ● | ● | ● | ● |
| | No heartbeat | | ● | ● | ● |
| Teratogenic effect | Malformation of head | ● | ● | ● | ● |
| | Malformation of eyes[b] | ● | ● | ● | ● |
| | Malformation of sacculi/otoliths[c] | ● | ● | ● | ● |
| | Malformation of chorda | ● | ● | ● | ● |
| | Malformation of tail[d] | ● | ● | ● | ● |
| | Scoliosis | ● | ● | ● | ● |
| | Heartbeat frequency | | ● | ● | ● |
| | Blood circulation | | ● | ● | ● |
| | Pericardial edema | ● | ● | ● | ● |
| | Yolk edema | ● | ● | ● | ● |
| | Yolk absosrption | ● | ● | ● | ● |
| | Growth retardation[e] | ● | ● | ● | ● |

[a]No clear organs structure is recognized.
[b]Malformation of eyes was recorded for the retardation in eye development and abnormality in shape and size.
[c]Presence of no, one or more than two otoliths per sacculus, as well as reduction and enlargement of otoliths and/or sacculi (otic vesicles).
[d]Tail malformation was recorded when the tail was bent, twisted or shorter than to control embryos as assessed by optical comparation.
[e]Growth retardation was recorded by comparing with the control embryos in development or size (before hatching, at 24 and 48 hpf) or in a body length (after hatching, at and onwards 72 hpf) using by optical comparation using an inverted microscope (CKX41; Olympus, Tokyo, Japan).