

**Supporting Information for**

**Original article**

**Astaxanthine attenuates cisplatin ototoxicity *in vitro* and protects against cisplatin-induced hearing loss *in vivo***

**Benyu Nan<sup>a,b</sup>, Zirui Zhao<sup>c</sup>, Kanglun Jiang<sup>a</sup>, Xi Gu<sup>d,\*</sup>, Huawei Li<sup>e,f,g,h,\*</sup>, Xinsheng Huang<sup>a,\*</sup>**

<sup>a</sup>*Department of Otorhinolaryngology-Head and Neck Surgery, Zhongshan Hospital, Fudan University, Shanghai 200030, China*

<sup>b</sup>*Department of Otorhinolaryngology-Head and Neck Surgery, Wenzhou Medical University, Affiliated Hospital 2, Wenzhou 325000, China*

<sup>c</sup>*Department of Otolaryngology, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200437, China*

<sup>d</sup>*Department of Otolaryngology, the First Affiliated Hospital of Fujian Medical University, Fuzhou 350005, China*

<sup>e</sup>*ENT institute and Department of Otorhinolaryngology, Eye & ENT Hospital, State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, Fudan University, Shanghai 200031, China*

<sup>f</sup>*Institutes of Biomedical Sciences, Fudan University, Shanghai 200032, China*

<sup>g</sup>*NHC Key Laboratory of Hearing Medicine (Fudan University), Shanghai 200031, China*

<sup>h</sup>*The Institutes of Brain Science and the Collaborative Innovation Center for Brain Science, Fudan University, Shanghai 200032, China*

Received 11 March 2021; received in revised form 3 June 2021; accepted 16 June 2021

\*Corresponding authors. Tel.: +86 591 87982117, fax: +86 591 87982117 (Xi Gu); Tel.: +86 21 64377134 669, fax: +86 21 64377151 (Huawei Li); Tel.: +86 21 64041990, fax: +86 21 64041990 (Xinsheng Huang).

E-mail addresses: [harry-xixi@hotmail.com](mailto:harry-xixi@hotmail.com) (Xi Gu); [hwli@shmu.edu.cn](mailto:hwli@shmu.edu.cn) (Huawei Li), [huang.xinsheng@zs-hospital.sh.cn](mailto:huang.xinsheng@zs-hospital.sh.cn) (Xinsheng Huang).

Running title: Astaxanthine attenuates cisplatin ototoxicity and cisplatin-induced hearing loss

**Table S1**

**Table S2**

**Figure S1**

**Figure S2**

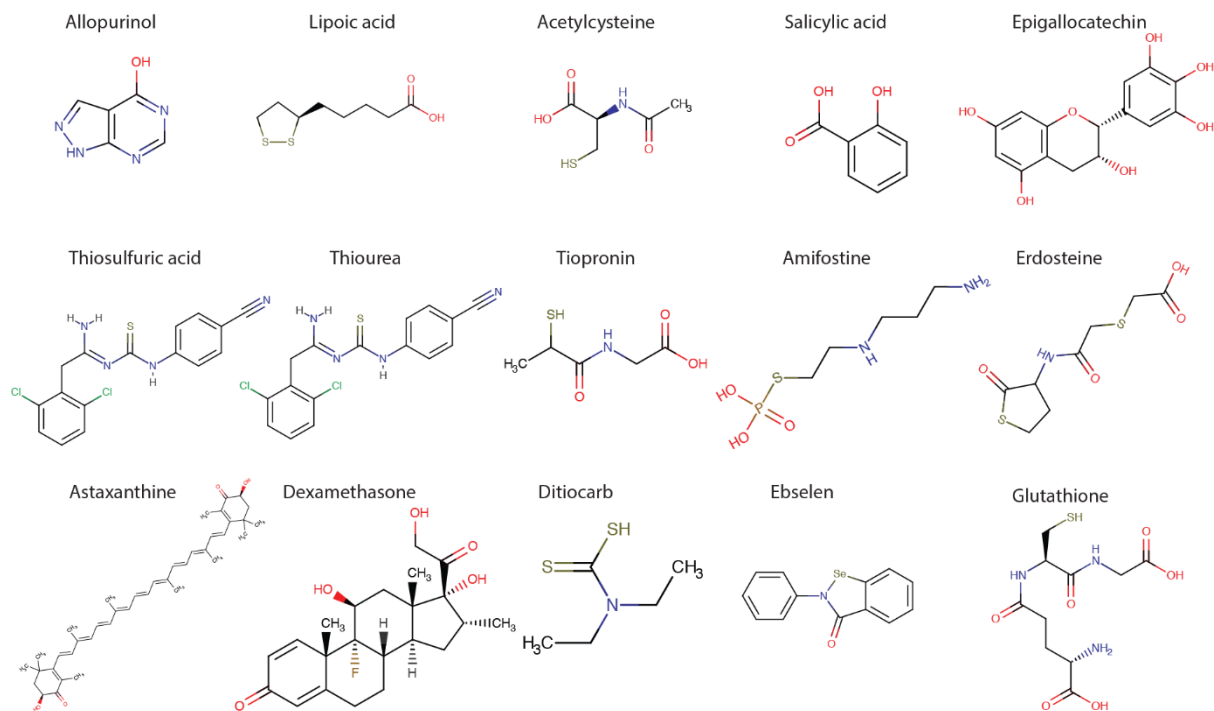
**Figure S3**

**Table S1** Primers used for quantitative PCR.

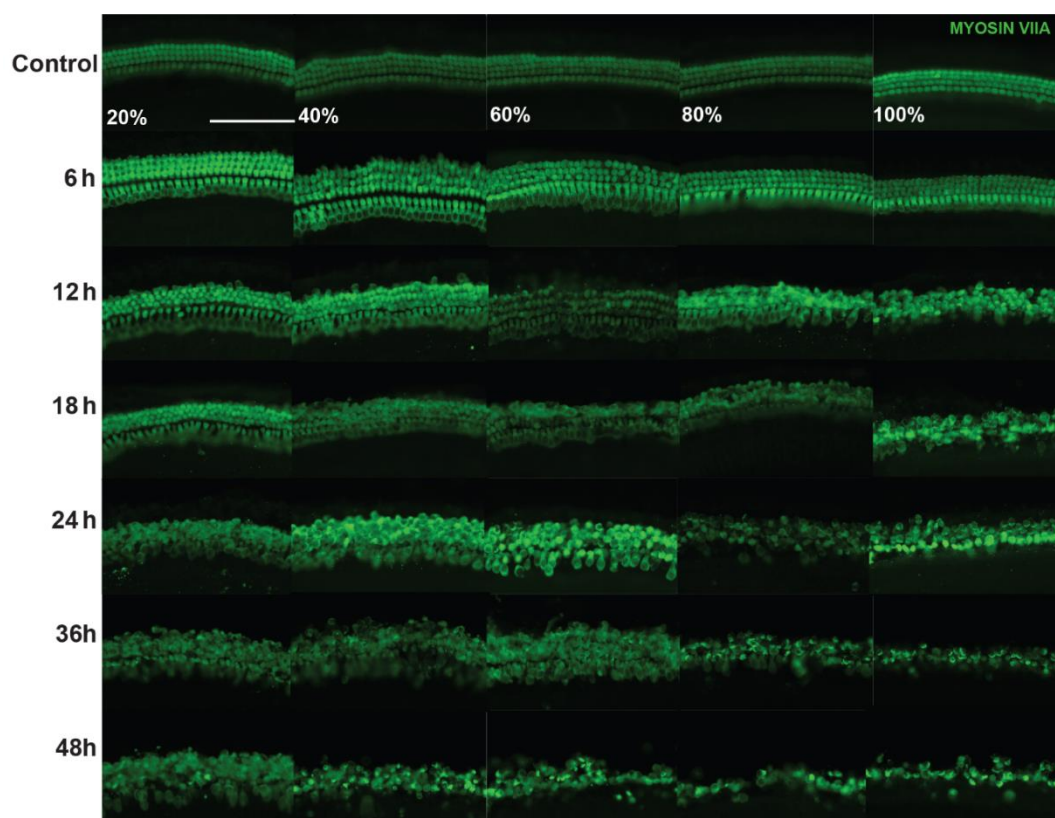
Name	Sequence
Caspase-3 forward	5'-GAGCTTGGAACGGTACGCTA-3'
Caspase-3 reverse	5'-GCGAGATGACATTCCAGTGC-3'
Caspase-8 forward	5'-TGCTTGGACTACATCCCACAC-3'
Caspase-8 reverse	5'-TGCAGTCTAGGAAGTTGACCA-3'
Caspase-9 forward	5'-TCCTGGTACATCGAGACCTTG-3'
Caspase-9 reverse	5'-AAGTCCCTTTTCGCAGAAACAG-3'
<i>Bax</i> forward	5'-AAACTGGTGCTCAAGGCC-3'
<i>Bax</i> reverse	5'-CTTGGATCCAGACAAGCAGC-3'
<i>Fadd</i> forward	5'-CTGCGCCGACACGATCTAC-3'
<i>Fadd</i> reverse	5'-CGGGCCAGTCTTTTCCAGT-3'
<i>Nrf2</i> forward	5'-TCCATTCCC GAATTACAGTGTC-3'
<i>Nrf2</i> reverse	5'-TCCAGCGAGGAGATCGATGA-3'
<i>Nqo1</i> forward	5'-GGTAGCGGCTCCATGTACTC-3'
<i>Nqo1</i> reverse	5'-CGCAGGATGCCACTCTGAAT-3'
<i>Hmox-1</i> forward	5'-CCAGAGAAGGCTTTAAGCTGGT-3'
<i>Hmox-1</i> reverse	5'-CACCTCGTGGAGACGCTTTA-3'
$\beta$ -Actin forward	5'-CACTGTCGAGTCGCGTCC-3'
$\beta$ -Actin reverse	5'-TCATCCATGGCGAACTGGTG-3'

**Table S2** List of interaction energy of molecular compounds docked with the KEAP1-NRF2 protein.

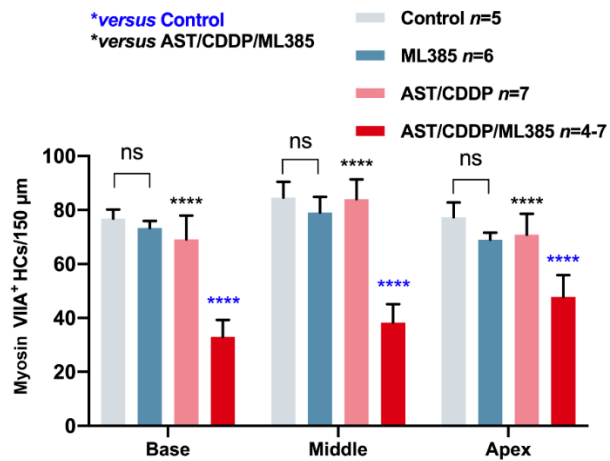
Molecular compounds	CDOCKER_INTERACTION_ENERGY (Kcal/mol)
Allopurinol	19.7815
Lipoic acid	38.5661
Acetylcysteine	23.3023
Salicylic acid	25.8723
Thiosulfuric acid	7.93491
Thiourea	8.47726
Tiopronin	26.3163
Amifostine	39.2029
Astaxanthine	70.6665
Dexamethasone	51.7312
Ditiocarb	16.629
Ebselen	27.2633
Epigallocatechin	63.0877
Erdosteine	43.123
Glutathione	52.3212



**Figure S1** 2D structures of molecular compounds from drug bank.



**Figure S2** Representative immunofluorescence image of HCs labelled with Myosin VIIA (green) in the cochlear explants treated with 50  $\mu\text{mol/L}$  cisplatin for 6, 12, 18, 24, 36, and 48 h. Scale bar = 100  $\mu\text{m}$ . The distance from the apex is presented as 20%, 40%, 60%, 80% and 100%. HC, hair cell.



**Figure S3** Quantitative analysis of the numbers of survival HCs stained with Myosin VIIA per 200  $\mu\text{m}$  in the various cochlear regions following the different treatments. The data is presented as means  $\pm$  SD, \*\*\*\* $P < 0.0001$  compared with AST/CDDP/ML385 by two-way ANOVA with Bonferroni correction. ns: not significant. AST, astaxanthine; CDDP, cisplatin; Base, basal turn; Middle, middle turn; Apex, apical turn.