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2,6-Diphenyl-Imidazopyridine Derivatives as Novel Prototypes of Anticancer Agents Targeting Aldehyde Dehydrogenases.

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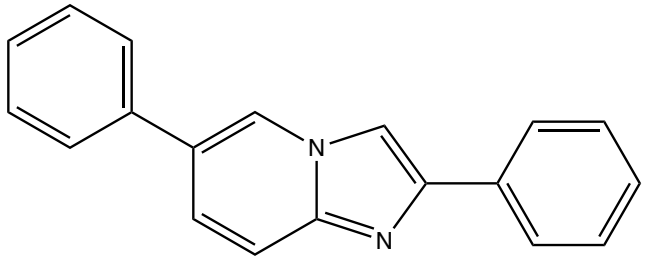
DIPARTIMENTO FARMACIA

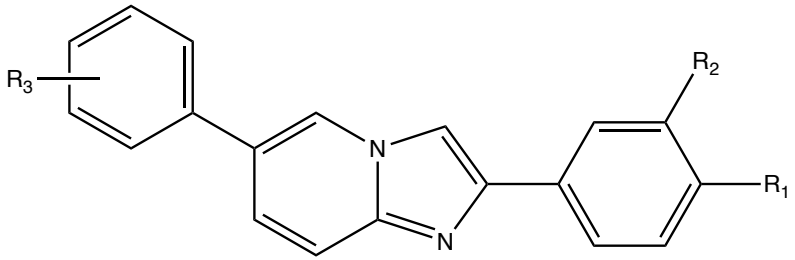
IN SUPREMÆ DIGNITATIS 1343

2,6-Diphenyl-Imidazopyridine Derivatives as Novel Prototypes of Anticancer Agents Targeting Aldehyde Dehydrogenases.

Graphical Abstract



GA11

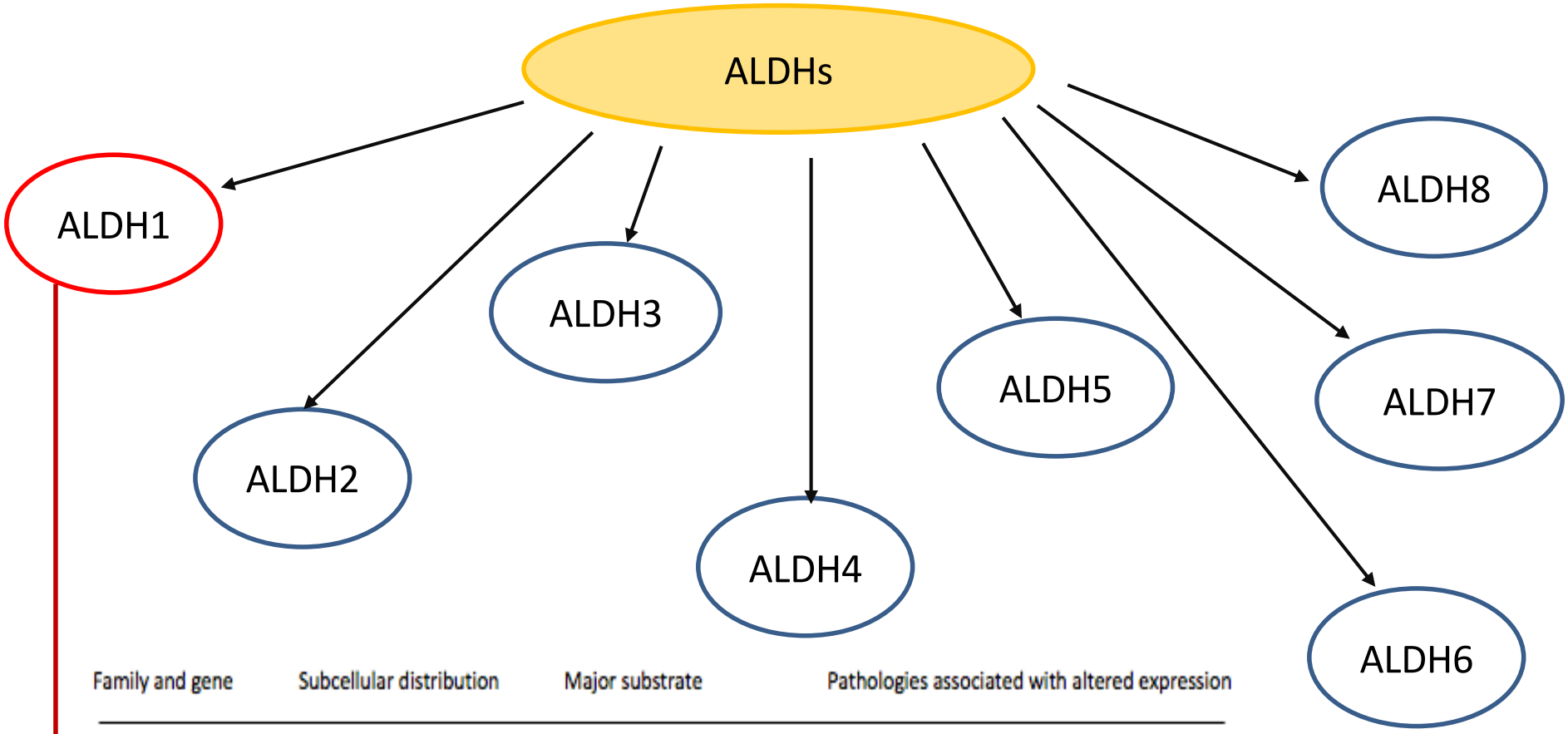


2,6-Diphenyl-Imidazopyridine Derivatives

Abstract:

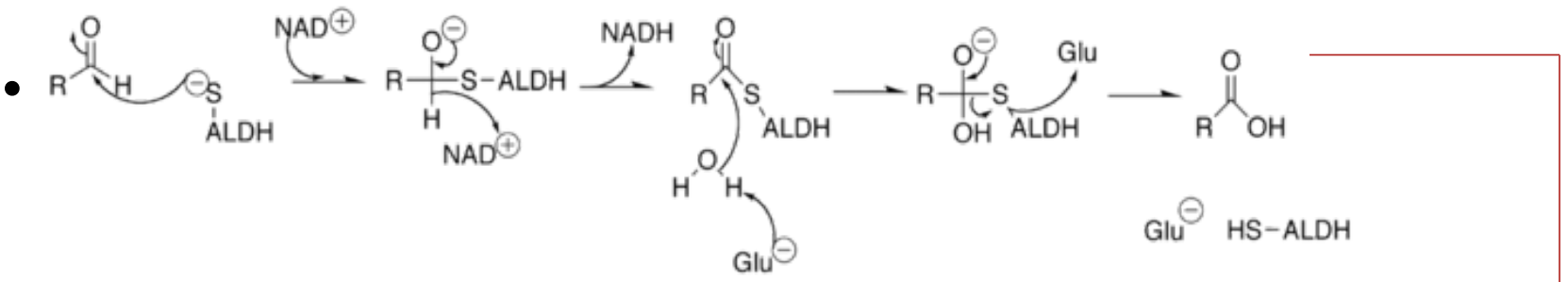
Aldehyde dehydrogenase (ALDH) superfamily comprises 19 different enzyme types located in specific subcellular districts, including cytosol and mitocondria. Their main function is to oxidize endogenous and exogenous aldehydes produced in human cells. In particular, isoforms 1A1, 1A2 and 1A3 catalyze the transformation of retinal into retinoic acid, which is a potent differentiation tissue factor for cellular development. Overexpression of these three isoforms in cancer stem cells (CSC), underlined in recent studies, is to date extremely important in cancer field, as it offers the chance to use these proteins both as prognostic marker and as novel targets in the fight against cancer. Here we present a novel series of 2,6-diphenyl-imidazol[1,2-*a*]pyridines, designed as aldehyde dehydrogenase inhibitors by means of a structured-based optimizations of a previously developed lead, GA11. The novel compounds were evaluated in vitro for their activity and selectivity against the three isoforms of the ALDH1A family, and investigated through crystallization and modeling studies for their ability to interact with the catalytic site of the 1A3 isoform. Tested *in vitro* on different populations of CSCs, obtained from glioma, colorectal and prostate tissue specimens, they exhibited a relevant anti-proliferative efficacy, thus paving the way for treating cancer by means of the still untapped aldehyde dehydrogenases.

Keywords: ALDH1A;ALDH1A3 subtype, ALDH1A3 inhibitors, cancer; Imidazo[1.2-a]pyridine.

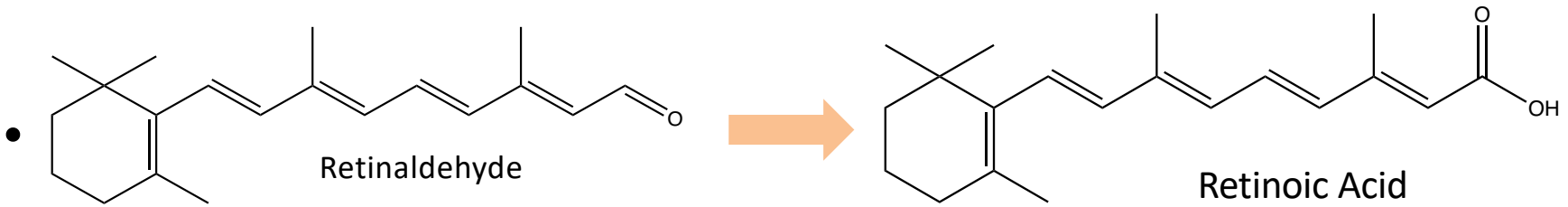


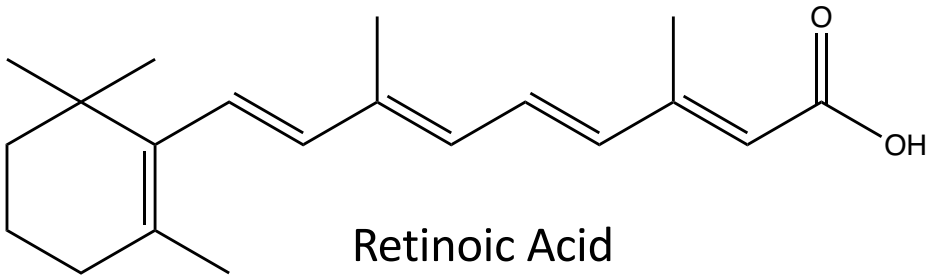
Family and gene		Subcellular distribution	Major substrate	Pathologies associated with altered expression
ALDH1	ALDH1A1	Cytosol	Retinal, aldophosphamide, acetaldehyde, lipid peroxidation-derived aldehydes.	Drug resistance, alcohol sensitivity
	ALDH1A2	Cytosol	Retinal	Tumors.
	ALDH1A3	Cytosol	Retinal	Perinatal lethality.
	ALDH1A7	Cytosol	Retinal	
	ALDH1B1	Mitochondria	Acetaldehyde, lipid peroxidation-derived aldehydes	
	ALDH1L1	Cytosol	10-Formyltetrahydrofolate	Tumors.
	ALDH1L2	Cytosol	unknown	

MAIN ALDHs FUNCTIONS



Reducing oxidative stress
ROS Production





Retinoic Acid

Two different pathways

Non-Target Tissue

CYP26

Degradation

Target Tissue

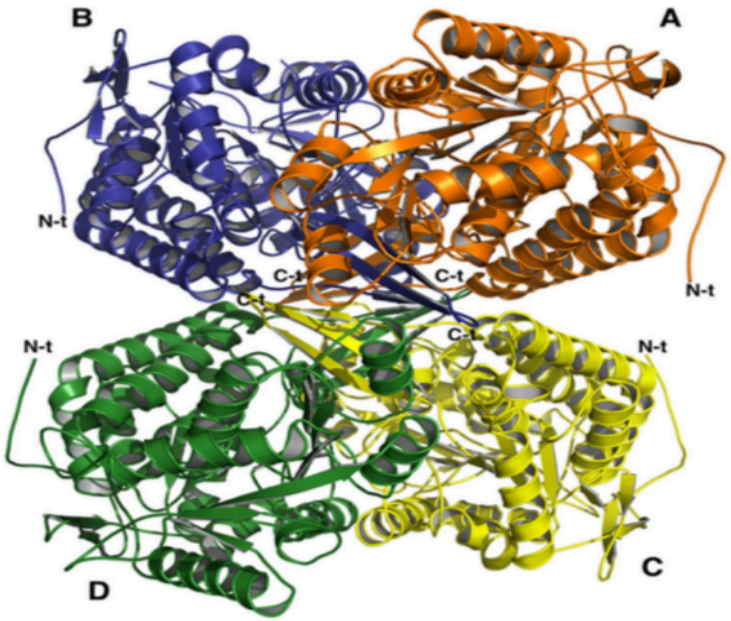
initiate the transcription of different kind of genes for differentiation, apoptosis.

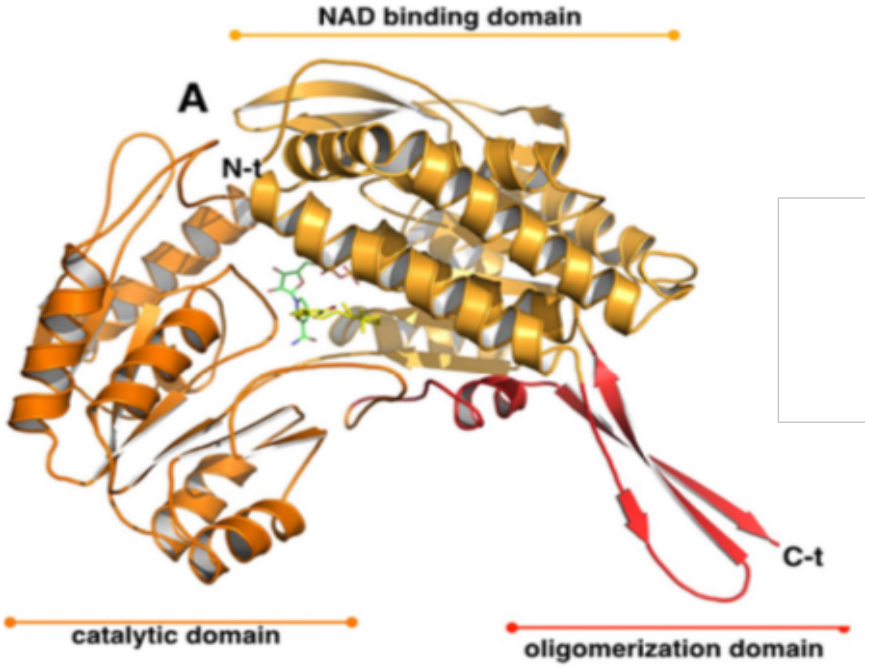
- Metabolism of Glucose / Lipids
- Activity linked with several pathological conditions and cancer
- Highly active in both normal and **cancer stem cells (CSC)**

ALDH1A overexpressed in CSC.

ALDH1A both as a prognostic marker and novel target against cancer

ALDH1A3



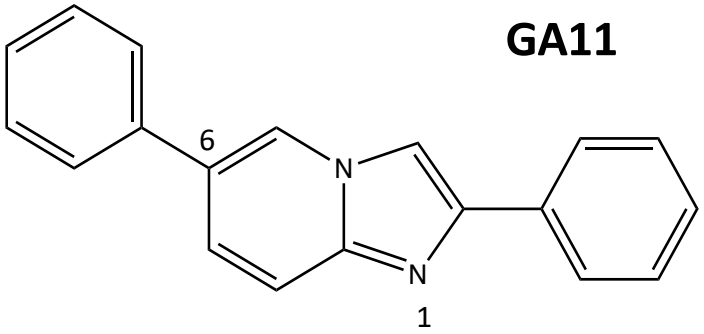


- molecular weight is around 224000 Da

- Despite this structure, each monomer is autonomously able to transform a single molecule of substrate in a molecule of product reducing NAD^+.

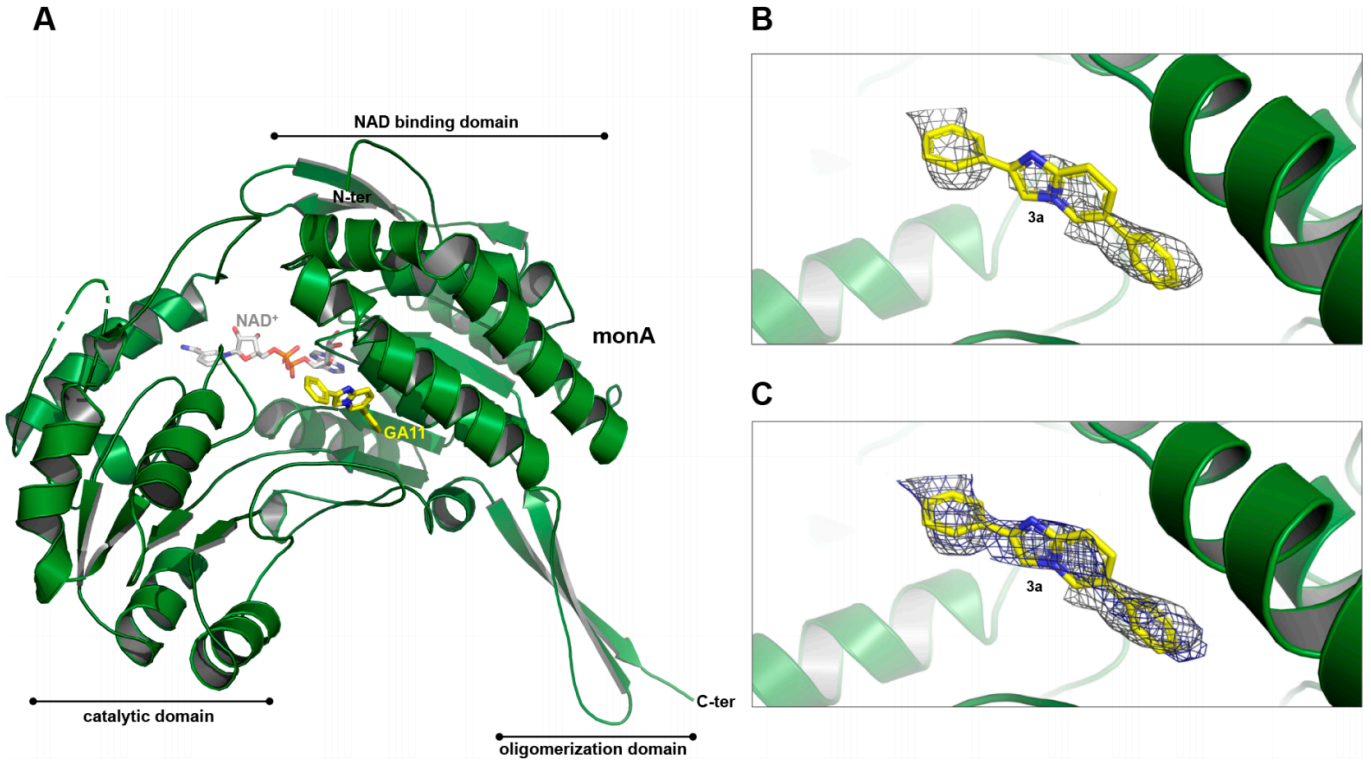
- **catalytic pocket:** goes from the protein surface to the catalytic cysteine
- To govern **substrate specificity**, ALDH1A3 uses special residues (G135, L471, T315) located in the tunnel

GA11



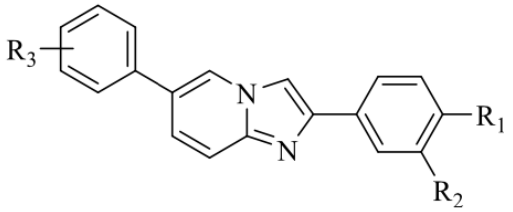
Anti-proliferativ **efficacy** on Glioma Stem Cells in the **nanomolar/picomolar range**

- **π–π stacking interaction with W189**
- **Van der Waals contacts with G136, L185 and L471**

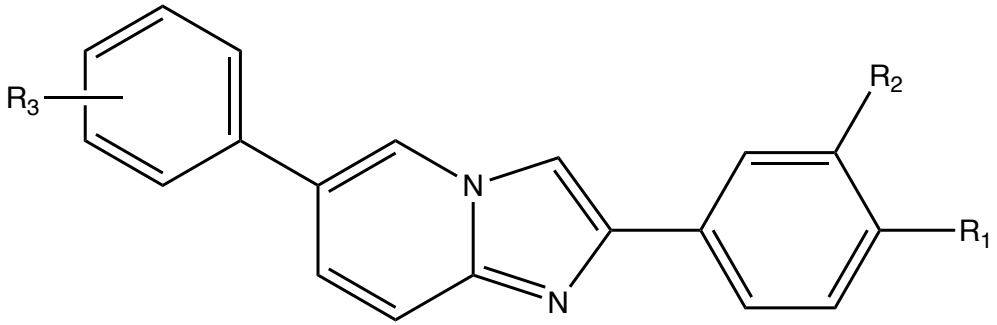


Quattrini et al. *J. Med. Chem*., 2020, 14, 4603

Table 2. ALDH Inhibitory Activity of Selected Imidazo[1,2-*a*]pyridine Derivatives 3a–v



				IC$_{50}$ (μM[a]) or % inhibition[b]		
N	R_1	R_2	R_3	ALDH1A1	ALDH1A2	ALDH1A3
3a	H	H	H	19.6 ± 1.6	54.6 ± 1.9	4.7 ± 1.7
3b	H	H	4-F	n.a.[c]	n.a.[c]	22.8 ± 1.6
3c	H	H	4-Cl	68.8 ± 1.5	n.a.[c]	45.8 ± 1.7
3d	H	H	4-SCH_3	n.a.[c]	n.a.[c]	27.1 ± 1.5
3e	H	H	4-OCH_3	n.a.[c]	n.a.[c]	17.8 ± 1.5
3f	H	H	4-$COOCH_3$	96.9 ± 2.2	n.a.[c]	27.4 ± 1.6
3h	H	H	3-CN	11.3%	n.a.[c]	5.3 ± 1.5
3i	H	H	4-CN	15.9%	n.a.[c]	5.2 ± 1.7
3j	CH_3	H	H	22.4%	n.a.[c]	11.3 ± 1.5
3m	Br	H	4-Cl	17.3 ± 1.6	n.a.[c]	n.a.[c]
3o	OCH_3	H	4-OCH_3	22.9%	n.a.[c]	11.4 ± 1.6
3p	OCH_3	H	3,4-diOCH_3	17.5%	n.a.[c]	8.3 ± 1.2
3q	OCH_3	H	3,5-diOCH_3	197.7 ± 3.0	n.a.[c]	3.5 ± 1.2
3r	CH_3	H	4-F	n.a.[c]	n.a.[c]	92.9 ± 1.8
3u	H	OCH_3	4-F	n.a.[c]	n.a.[c]	21.2 ± 1.7
3v	H	OCH_3	4-Cl	n.a.[c]	n.a.[c]	6.4 ± 1.3



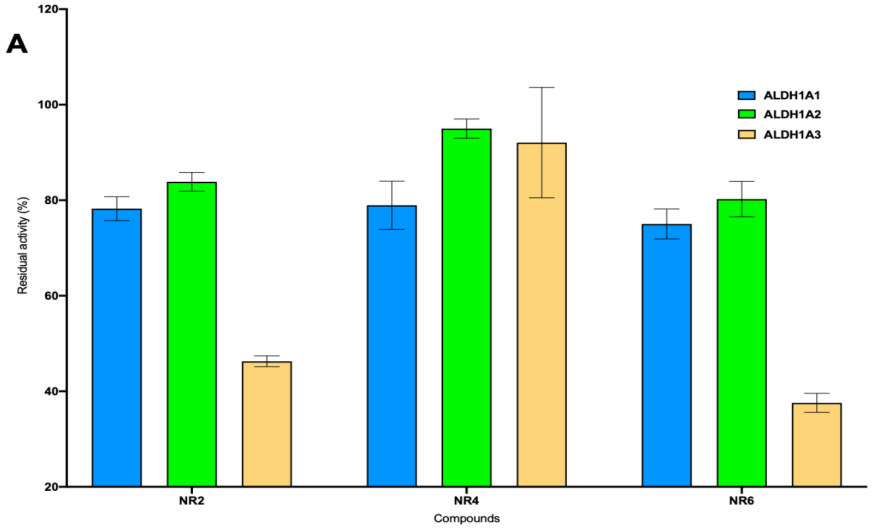
CYANIDE 2,6-DIPHENIL-IMIDAZO PYRIDINE

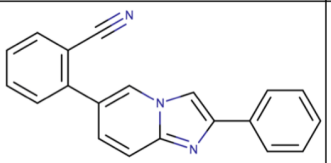
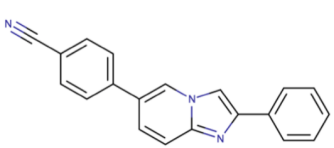
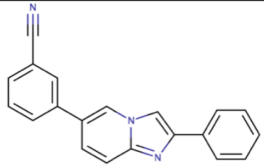
HALOGEN 2,6-DIPHENIL-IMIDAZO PYRIDINE

GLIOMA AND COLORECTAL CANCER CELL LINES

GLIOBLASTOMA AND PROSTATE CANCER CELL LINES

CYANIDE 2,6-DIPHENIL-IMIDAZO PYRIDINE



NR2	NR4	NR6
		
ALDH1A1= 78.2 ± 2.5 % ALDH1A2= 83.7 ± 2.0 % ALDH1A3= 46.3 ± 1.1 %	ALDH1A1= 79.0 ± 5.0 % ALDH1A2= 95.0 ± 2.0 % ALDH1A3= 92.1 ± 11.6 %	ALDH1A1= 75.0 ± 3.1 % ALDH1A2= 80.2 ± 3.7 % ALDH1A3= 37.6 ± 2.0 %

NR6, showed the best inhibitory activity at 25 µM; further investigation revealed a potent inhibitor activity with high specificity on isoform 1A3.

	ALDH1A1	ALDH1A2	ALDH1A3
K_i (µM)	262.2±76.4	257.6±26.4	3.7±0.4

- hU87MG (glioblastoma);
- hHCT116 (colorectal tumor).

ALDH1A3^{+}

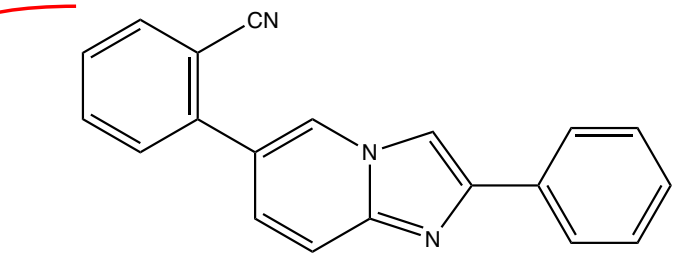
- hHEK293T (human embryonic kidney).

ALDH1A2^{+}

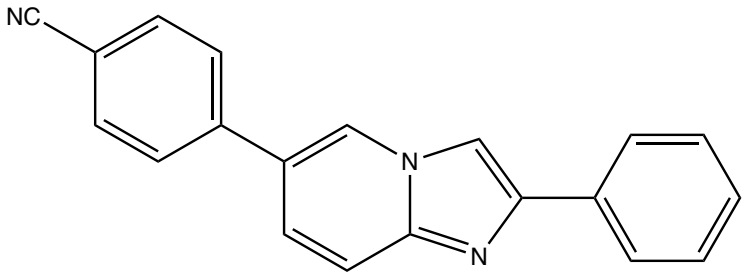
- hASTRO (foetal astrocytes).

ALDH1A1^{+}

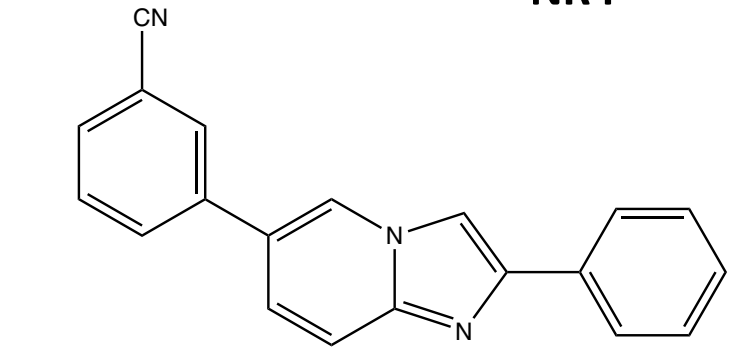
- h4T1 (mammary carcinoma).



NR2



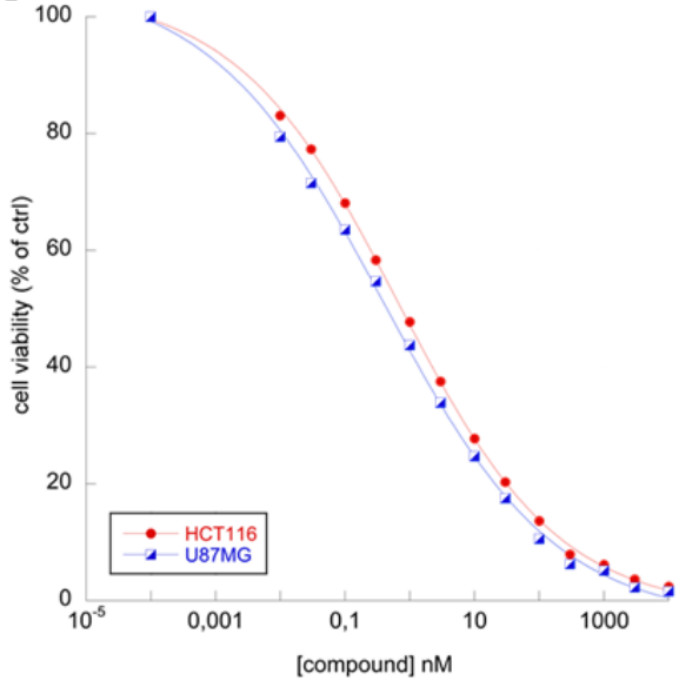
NR4



NR6

AFTER 72h TREATMENT

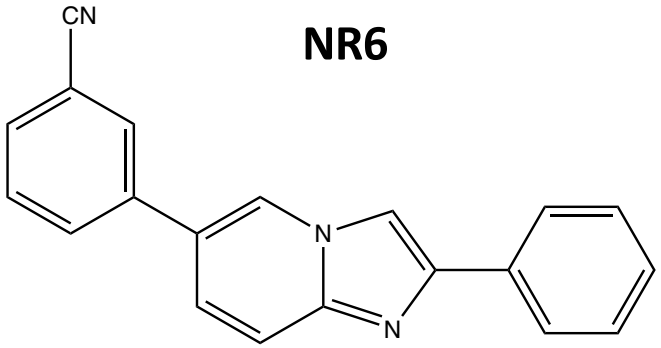
A



B

Cell line	EC_{50} (nM)
U87	0.378 ± 0.04
HCT116	0.648 ± 0.04
HEK293T	1600 ± 5.6
4T1	2000 ± 1.8
hASTRO	N.D. (75.6%)*

* - cell viability at maximum concentration tested (10 uM)

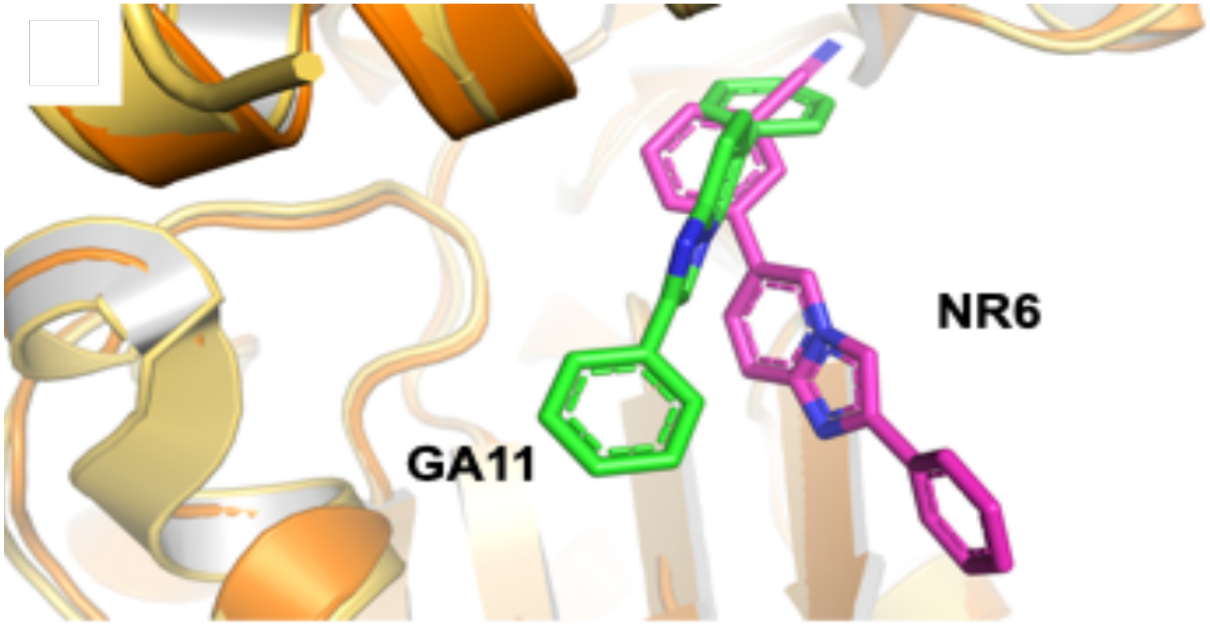


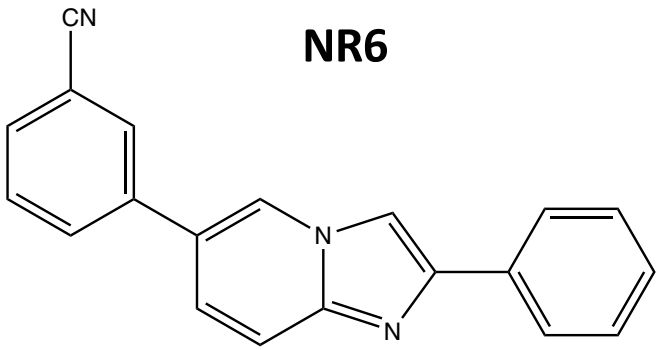
NR6

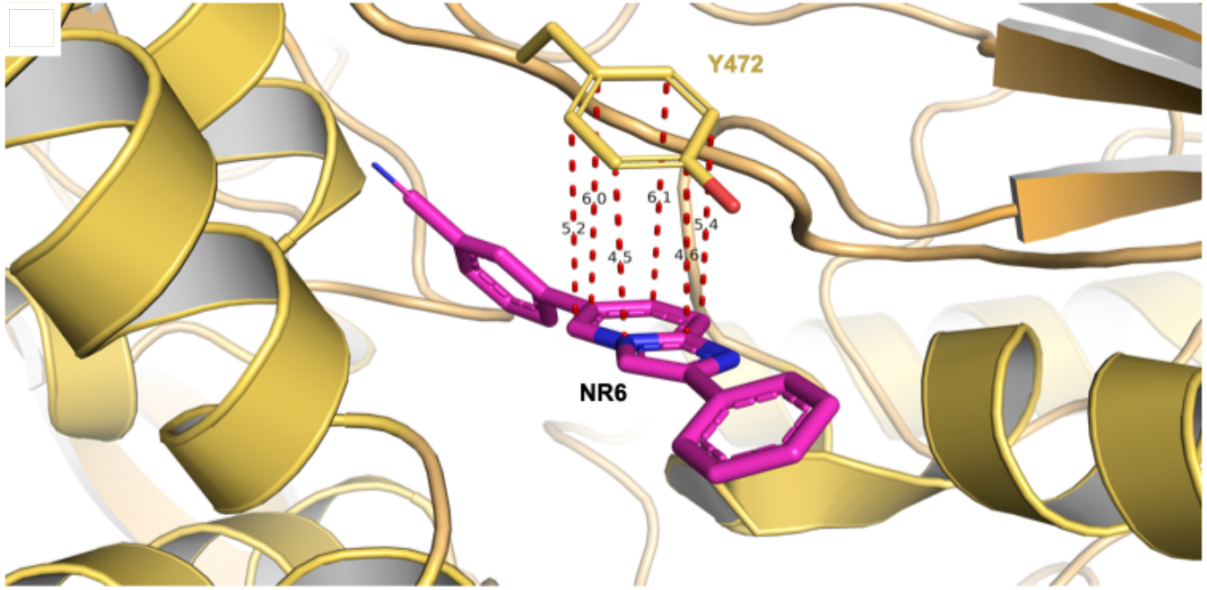
- NR6 not reaches the catalytic cysteine C314.

- The heterocyclic cores lie in the hydrophobic pocket at the entrance of the catalytic tunnel.

- the phenyl ring in 6 of the nucleus establishes Van der Waals contacts and hydrophobic interactions with the residues **G136, R139, W189, N469, A470, L471** and **Y472**







NOTABLE IMPORTANCE:

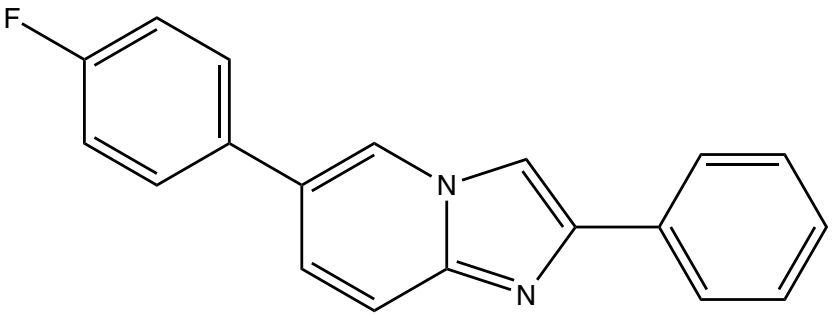
• Interaction of the pyridinc ring with Y472(edge-to face π-π stacking interaction) , for 1A3 specifity;

•Cyanide group that blocks the inhibitor in only one orientation toward Y472 (Difference with the symmetric structure of GA11).

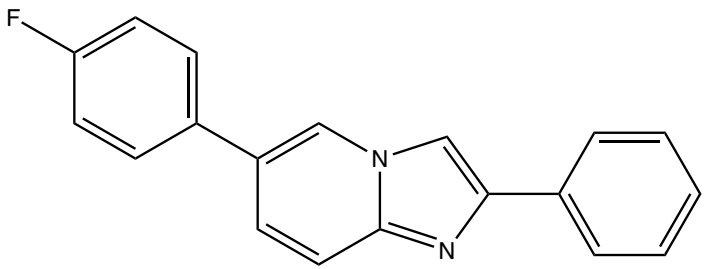
HALOGEN 2,6-DIPHENIL-IMIDAZO PYRIDINE

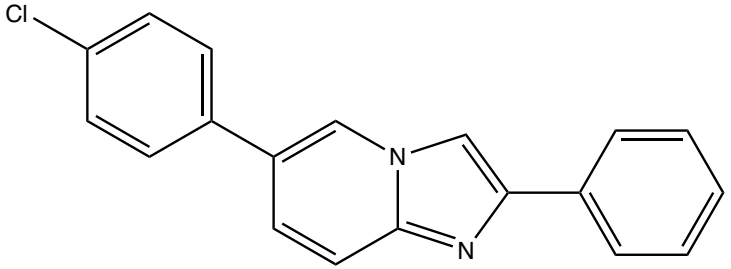
Test for antiproliferative efficacy against three different patient-derived glioma sphere samples; proneural (PN-157) and mesenchymal (MES-267 and MES-374). 72h EXPOSURE

	GSC lines		
N	157 (IC_{50}, nM[a])	267 (IC_{50}, nM[a])	374 (IC_{50}, nM[a])
3a	151.0	46.9	2.74
3b	25.2	63.4	0.00258
3c	53.1	21.5	0.0196
3e	2660	660	n.t.[b]
3f	822.0	823.0	n.t[b]
3u	1510	350	n.t.[b]
3v	29.6	71.2	13.5



The 6-(4-fluoro)phenyl derivative (3b), combining the best functional profile in terms of activity and selectivity against the ALDH1.





P4E6 (immortalised human prostate cell line)

PC-3 (human prostate cancer cell line)

LNCaP (androgen-sensitive human prostate adenocarcinoma cells)

PNT2-C2 (normal prostate epithelial cell line)

After 72 hours of exposure, all the compound exhibited antiproliferative activity in the nanomolar (nM) range in a dose-dependent manner.

Derivative **3b** turned out to be the most potent analogue against P4E6 and PC3, with a reduced but comparable activity between LNCaP and the normal epithelial PNT2-C2 cell line.

EC_{50} (nM)

	P4E6	PC-3	LNCaP	PNT2-C2
3b	4.038	70.92	240.0	217.0
3c	33.39	321.9	323.1	324.7

Conclusions

•Moving from the previously described hit GA11 and taking advantage of both crystallographic studies and molecular modeling investigations, we succeeded in customizing the selectivity profile of the compound by varying its substitution pattern on both the pendant phenyl rings.

•Studies in vitro on recombinant human isoforms of ALDH1A, and on cell lines highlights the importance of this novel group of compounds in the fight against cancer targeting CSC and the overexpression of ALDH1A.

•in particular, the cyanide derivatives showed high efficacy on glioma and colorectal tumor cell lines instead the fluoro and chlorine derivatives underlined their potent activity on glioblastoma and prostate cancer cell lines.

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