

# New darbufelone derivatives as compounds with potential anticancer activity

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## INTRODUCTION

Cancers are a major problem in modern times. In 2020, they were the leading cause of death worldwide, making the search for new, effective, and safe drugs a priority for researchers globally. Darbufelone is a dual inhibitor of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX). Due to the presence of a 4-thiazolidinone core in its structure, darbufelone derivatives are highly likely to act on the peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) and consequently exhibit anticancer activity. These properties have spurred research into the anticancer activity of new darbufelone derivatives.

## METHODS

Preliminary cytotoxicity studies of the newly synthesized darbufelone derivatives were performed using the MTT assay. Tests were conducted on 11 new compounds using human colorectal adenocarcinoma cell lines DLD-1 and HT-29, gastric cancer cell line AGS, glioblastoma cell lines A172 and U87, and mouse astrocytes C8-D1A, with an incubation time of 24 hours. The reference compound used was doxorubicin. Molecular docking was performed for the four most active compounds against the HT-29 line. Calculations were carried out using the free software AMDock, using the PPAR $\gamma$  protein A-chain (PDB 4PRG), with CID 449532 as the reference ligand. The predicted pharmacokinetic properties were analyzed using the free online tool SwissADME. Selected 2D structures were prepared and exported to SMILES format, and predictions were made considering six pharmacokinetic properties: molecular weight (MW) [g/mol], topological polar surface area (TPSA) [ $\text{\AA}^2$ ], degree of saturation (fraction of  $\text{sp}^3$ -hybridized carbons), flexibility (number of rotatable bonds), lipophilicity (XLOGP3), and solubility (logS).

## CONCLUSION

Toxicity studies using the MTT assay revealed that darbufelone derivatives LES-5276, LES-6496, and LES-6497 exhibited the highest activity against the HT-29 cell line, with  $\text{IC}_{50}$  values of 2.98  $\mu\text{M}$ , 15.43  $\mu\text{M}$ , and 16.83  $\mu\text{M}$ , respectively. The HT-29 line, characterized by high PPAR $\gamma$  expression, is an appropriate model for further studies. Molecular docking suggests that these compounds can interact with PPAR $\gamma$ , achieving similar energy parameters and  $\text{K}_i$  inhibition constants as the reference ligand. Analysis using SwissADME indicates that most of the pharmacokinetic parameters tested are within the optimal range. The compounds are predicted to be strongly absorbed through the gastrointestinal tract but do not cross the blood-brain barrier. Additionally, they are characterized by potential interactions with CYP isoenzymes, which may lead to interactions with other drugs.

## RESULTS

Darbufelone derivatives	R	R1	MW
LES-5276			424,57
LES-6496			412,56
LES-6497			399,52

Table 1. Structural formulae of the three most active of the 11 darbufelone derivatives.

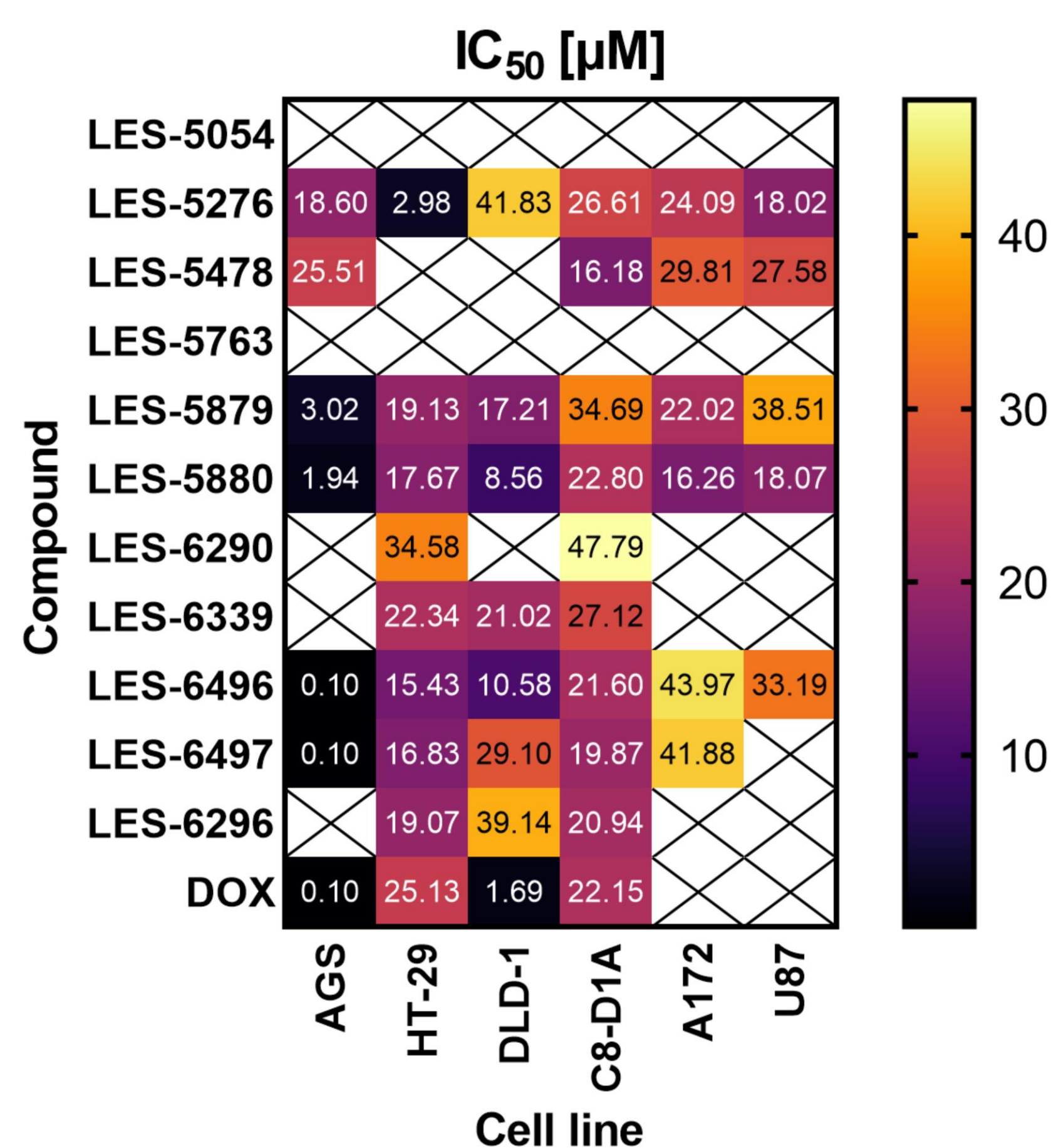


Figure 1: Heat map of  $\text{IC}_{50}$  values for darbufelone derivatives obtained from the MTT assay after 24 hours of incubation on AGS, HT-29, DLD-1, C8-D1A, A172, and U87 cell lines.  $\text{IC}_{50}$  values greater than 50  $\mu\text{M}$  are indicated by an "X".

Docked ligands	PPAR $\gamma$ (PDB 4PRG)	
	Binding Energy	$\text{K}_i$ , nM
LES-5276	-10,6	16,99
LES-5880	-8,7	419,63
LES-6496	-9,9	55,37
LES-6497	-9,4	128,75
CID 449532	-11,7	2,65

Table 2. Binding energy values obtained from docking analysis of PPAR $\gamma$  with LES-5276, LES-5880, LES-6496, LES-6497, and the reference compound CID 449532.

Pharmacokinetic property	LES-5276	LES-6496	LES-6497	Optimal range
MW (g/mol)	424,56	412,55	399,51	150–500
TPSA ( $\text{\AA}^2$ )	107,22	115,67	128,56	20–130
Fraction C-sp $^3$	0,33	0,41	0,40	$\geq 0,25$
RB	4	4	4	$\leq 9$
XLOGP3	5,92	1,87	1,87	-0,7 – +5,0
[ESOL] logS	-6,09	-3,01	-3,01	$\leq 6$

Table 3. Predicted pharmacokinetic values of selected compounds along with optimal values for each parameter.

Toxicity studies on tumor cell lines were performed using the MTT assay on newly synthesized darbufelone derivatives (Table 1). The cytotoxicity test identified the three most active compounds: LES-5276, LES-6496, and LES-6497 (Figure 1). The  $\text{IC}_{50}$  values for the HT-29 cell line were 2.98  $\mu\text{M}$ , 15.43  $\mu\text{M}$ , and 16.83  $\mu\text{M}$ , respectively. The HT-29 cell line shows the highest expression of PPAR $\gamma$ , which forms the basis for planning further *in vitro* studies on these selected compounds.

Molecular docking was performed for the four most cytotoxic compounds (LES-5276, LES-5880, LES-6496, LES-6497) to evaluate their affinity for PPAR $\gamma$  in the HT-29 cell line. The calculations suggest possible activity through interaction with PPAR $\gamma$ . The binding energy and  $\text{K}_i$  inhibition constant of the compound LES-5276 are similar to those of the reference ligand CID 449532 (Table 2). The molecule binds to the receptor through various types of  $\pi$  interactions.

The predicted parameter values obtained using SwissADME are mostly within the optimal range (Table 3). However, the lipophilicity and solubility values for the compound LES-5276 deviate slightly from the optimum. LES-5276, LES-6496, and LES-6497 are expected to be strongly absorbed through the gastrointestinal tract but do not have the ability to penetrate the blood-brain barrier. The tested derivatives are not substrates of P-glycoprotein. However, LES-5276 may act as an inhibitor of the CYP2C19, CYP2C9, and CYP3A4 enzymes. Compounds LES-6496 and LES-6497 may inhibit the CYP1A2 isoenzyme, suggesting potential effects on the metabolism of other substances.