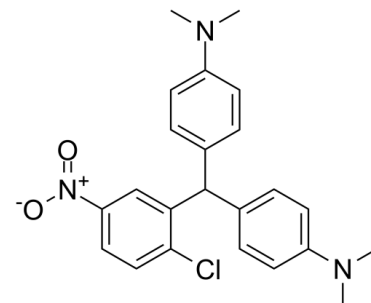


## Data Sheet

Product Name:	AL 082D06
Cat. No.:	CS-3964
CAS No.:	256925-03-8
Molecular Formula:	C <sub>23</sub> H <sub>24</sub> ClN <sub>3</sub> O <sub>2</sub>
Molecular Weight:	409.91
Target:	Glucocorticoid Receptor
Pathway:	GPCR/G Protein
Solubility:	DMSO : 7.5 mg/mL (18.30 mM; Need ultrasonic and warming)



### BIOLOGICAL ACTIVITY:

AL 082D06 is a selective, nonsteroidal glucocorticoid receptor (GR) antagonist with  $K_i$  of 210 nM. IC<sub>50</sub> & Target:  $K_i$ : 210 nM (GR)<sup>[1]</sup> **In Vitro:** AL 082D06 (D06) binds specifically to GR with nanomolar affinity. Addition of AL 082D06 causes a dose-dependent decrease in transcriptional activation from the MMTV:Luc reporter stimulated with half-maximal DEX concentrations. AL 082D06 acts to antagonize reporter activity using several glucocorticoid-responsive promoter-reporter systems including the 3-kb tyrosine amino transferase (TAT) promoter and less complex promoters comprised of isolated glucocorticoid response element (GRE) sequences. AL 082D06 competes with <sup>3</sup>H-Dex for baculovirus-expressed GR with nanomolar affinity. Other intracellular receptors (AR, ER, PR, and MR) have no affinity for AL 082D06 in a similarly structured binding assay with the appropriate receptor and tritiated ligand (>2500 nM). AL 082D06 has no activation efficacy on the progesterone, androgen, mineralocorticoid, retinoid, glucocorticoid, or estrogen receptors. AL 082D06 is very efficacious at antagonizing GR activity but exhibits much weaker efficacy when tested against the other steroid receptors in contrast to the reference antagonists used as controls<sup>[1]</sup>.

### PROTOCOL (Extracted from published papers and Only for reference)

**Kinase Assay:** <sup>[1]</sup>The extract and binding assay buffer consists of 25 mM sodium phosphate, 10 mM potassium fluoride, 10 mM sodium molybdate, 10% glycerol, 1.5 mM EDTA, 2 mM dithiothreitol, 2 mM CHAPS, and 1 mM phenylmethylsulfonyl fluoride (pH 7.4), at room temperature. Intracellular receptors produced in this fashion exhibit reproducible interaction with known ligands at the published affinity. These preparations are subjected to extensive quality control experiments before the assays, covering receptor response, specificity, size, and reference ligand affinity. Receptor assays are performed with a final volume of 250  $\mu$ L containing from 50-75  $\mu$ g of extract protein, plus 1-2 nM [<sup>3</sup>H]Dex at 84 Ci/mmol and varying concentrations of competing ligand (0 to 10  $\mu$ M). Assays are set up using a 96-well minitube system, and incubations are carried out at 4°C for 18 h. Equilibrium under these conditions of buffer and temperature is achieved by 6-8 h. Nonspecific binding is defined as that binding remaining in the presence of 1000 nM unlabeled Dex. At the end of the incubation period, 200  $\mu$ L of 6.25% hydroxyapatite are added in wash buffer (binding buffer in the absence of dithiothreitol and phenylmethylsulfonyl fluoride). Specific ligand binding to receptor is determined by a hydroxyapatite-binding assay. Hydroxyapatite absorbs the receptor-ligand complex, allowing for the separation of bound from free radiolabeled ligand. The mixture is vortexed and incubated for 10 min at 4°C and centrifuged, and the supernatant is removed. The hydroxyapatite pellet is washed two times in wash buffer. The amount of receptor-ligand complex is determined by liquid scintillation counting of the hydroxyapatite pellet after the addition of 0.5 mM EcoScint A scintillation cocktail from National Diagnostics<sup>[1]</sup>.

### References:

[1]. Miner JN, et al. A nonsteroidal glucocorticoid receptor antagonist. Mol Endocrinol. 2003 Jan;17(1):117-27.

**CAIndexNames:**

Benzenamine, 4,4'-[(2-chloro-5-nitrophenyl)methylene]bis[N,N-dimethyl-

**SMILES:**

O=[N+](C1=CC=C(Cl)C(C(C2=CC=C(N(C)C)C=C2)C3=CC=C(N(C)C)C=C3)=C1)[O-]

**Caution: Product has not been fully validated for medical applications. For research use only.**

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