Product Data Sheet

Product Name: Clemizole
Cat. No.: GC12444

Chemical Properties

Cas. No.	442-52-4		
Chemical Name	1-[(4-chlorophenyl)methyl]-2-(pyrrolidin-1-ylmethyl)benzimidazole		
SMILES	C1CCN(C1)CC2=NC3=CC=CC=C3N2CC4=CC=C(C=C4)Cl		
Formula	$C_{19}H_{20}ClN_3$	M.Wt	325.84
Solubility	≥ 16.2 mg/mL in DMSO, ≥ 95.8 mg/mL in EtOH	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure	Clemizole		

Protocol

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Cell experiment:

Huh7.5 cells are maintained in DMEM supplemented with 1% L-glutamine, 1% penicillin, 1% streptomycin, 1× nonessential amino acids and 10% FBS. Cell lines are passaged twice weekly after treatment with 0.05% trypsin-0.02% EDTA and seeding at a dilution of 1:5. Subconfluent Huh7.5 cells are trypsinized and collected by centrifugation at 700g for 5 min. The cells are then washed three times in ice-cold RNase-free PBS and resuspended at 1.5×107 cells/mL in PBS. Wild-type or mutant FL-J6/JFH-5′C19Rluc2AUbi RNA for electroporation is generated by transcription of XbaI linearized DNA templates using the T7 MEGAscript kit, followed by purification (RNA transcription and fluorescent labeling). We mixed 5 μg of RNA with 400 μL of washed Huh7.5 cells in a 2-mm-gap cuvette (BTX) and immediately pulsed (0.82 kV, five 99 μs pulses) with a BTX-830 electroporator. After a 10 min recovery at 25°C, pulsed cells are diluted into 10 mL of prewarmed growth medium. Cells from several electroporations are pooled to a common stock and seeded in 6-well plates (5×105 cells per well). After 24 h, medium is replaced and cells are grown in the presence of serial dilutions of the various inhibitory compounds (e.g., Clemizole hydrochloride) identified in the screen. Seventeen commercially available compounds, out of the 18 identified, are analyzed. Untreated cells are used as a negative control for water-soluble compounds. For compounds (e.g., Clemizole hydrochloride) solubilized in DMSO, untreated cells are grown in the presence of corresponding concentrations of the solvent as a negative control. Medium is changed daily. After 72 h of treatment cells are subjected to an Alamar Blue-based viability assay and luciferase assay. After 72 h of treatment cells are incubated for 3 h at 37°C in the presence of 10% Alamar Blue reagent. Plates are then scanned and fluorescence is detected by using FLEXstation II 384. Depending on the inhibitory compound’s solvent (e.g., Clemizole hydrochloride), water or DMSO, signal is normalized relatively to untreated samples or samples grown in the presence of DMSO, respectively[1].

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Animal experiment:

Mice[3]Eight control NOG mice and eight humanized TK-NOG mice are administered 25 mg/kg by mouth Clemizole, and blood samples are collected 30 minutes after administration. The C57BL/6J mice (3 per time point) are given 25 mg/kg by mouth clemizole, and blood samples are collected for analysis at 15 and 30 minutes and 1, 2, 4, and 6 hours after administration. For the DDI studies, eight humanized TK-NOG mice are given Clemizole (25 mg/kg by mouth) with or without Ritonavir (20 mg/kg by mouth), and blood samples are collected 30 minutes after administration. Six of these mice are also treated with Debrisoquine (10 mg/kg by mouth) in the presence or absence of Ritonavir (20 mg/kg by mouth), and plasma samples are obtained 2 hours later for analysis.

References:

[1]. Einav S, et al. Discovery of a hepatitis C target and its pharmacological inhibitors by microfluidic affinity analysis. Nat Biotechnol. 2008 Sep;26(9):1019-27
[2]. Richter JM, et al. Clemizole hydrochloride is a novel and potent inhibitor of transient receptor

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potential channel TRPC5. Mol Pharmacol. 2014 Nov;86(5):514-21
[3]. Nishimura T, et al. Using chimeric mice with humanized livers to predict human drug metabolism and a drug-drug interaction. J Pharmacol Exp Ther. 2013 Feb;344(2):388-96.

Background

The NS4B protein is a key player in HCV replication. Disrupting NS4B function thus represents an attractive new anti-HCV strategy. Combining clemizole with other anti-HCV agents could increase the antiviral effect achieved with 1 active drug alone and decrease emergence of viral resistance.

In vitro: Although significant, clemizole’s antiviral effect was moderate (50% effective concentration of 8 mM against a HCV genotype 2a clone). Clemizole’s antiviral effect was highly synergistic with the HCV protease inhibitors VX950 and SCH503034, without toxicity. In contrast, clemizole combinations with either interferon, ribavirin, or the nucleoside (NM283) and nonnucleoside (HCV796) HCV polymerase inhibitors were additive [1].

In vivo: Clemizole had an unexpectedly short plasma half-life; it was very rapidly biotransformed into a glucuronide (M14) and a dealkylated metabolite (M12) and into a

variety of lesser metabolites in C57BL/6J mice [2].

Clinical trial: The purpose of a study was to test the hypothesis that clemizole hydrochloride was safe and well tolerated when administered to subjects who were infected with hepatitis C virus and had not yet received treatment. This clinical study would also examine how the virus and body respond to clemizole hydrochloride.

References:
[1] Einav S, Sobol HD, Gehrig E, Glenn JS. The hepatitis C virus (HCV) NS4B RNA binding inhibitor clemizole is highly synergistic with HCV protease inhibitors. J Infect Dis. 2010;202(1):65-74.
[2] Nishimura T, Hu Y, Wu M, Pham E, Suemizu H, Elazar M, Liu M, Idilman R, Yurdaydin C, Angus P, Stedman C, Murphy B, Glenn J, Nakamura M, Nomura T, Chen Y, Zheng M, Fitch WL, Peltz G. Using chimeric mice with humanized livers to predict human drug metabolism and a drug-drug interaction. J Pharmacol Exp Ther. 2013;344(2):388-96. doi: 10.1124/jpet.112.198697. Epub 2012 Nov 8.