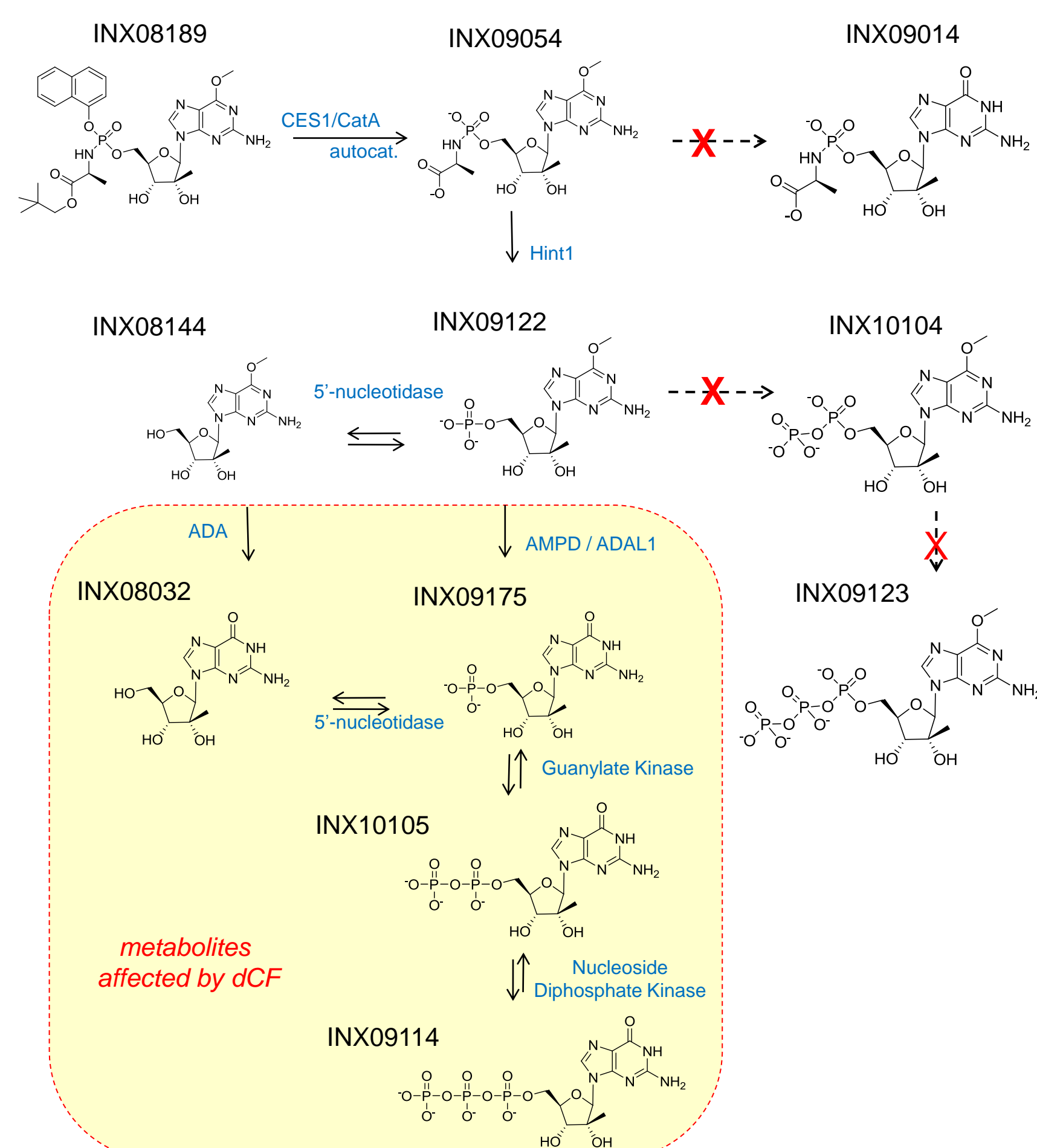


Introduction

INX08189 (INX-189) is a phosphoramidate of O⁶-Methyl-2'-C-methyl guanosine currently in Phase 2 clinical trials for the treatment of chronically infected HCV patients. INX-189 was designed to deliver the monophosphate form of the nucleoside analog 2'-C-MeG intracellularly, bypassing the inefficient first phosphorylation step. It has been proposed that the metabolic activation of INX-189 involves enzyme mediated hydrolysis of the C⁶-methoxy group of the purine base, presumably by adenosine monophosphate deaminase (AMPD) and/or by adenosine deaminase-like enzyme, ADAL1. ADAL1 was recently suggested as the primary hydrolyzing enzyme for O⁶- and N⁶-, but not the S⁶-substituted purine or 2-aminopurine nucleoside monophosphates (Murakami et al., 2011. *J. Med. Chem.* 54). We investigated anti-HCV activity of some C⁶-modified analogs of INX-189 and the effects of the ADA/AMPD/ADAL1 inhibitor 2'-deoxycofornycin (dCF) and of the ADA-specific inhibitor EHNA on anti-HCV activity of INX-189, its C⁶-modified analogs and the corresponding nucleosides. The difference in intracellular metabolite production in the presence and absence of dCF was also analyzed.

Figure 1: Proposed Metabolic Conversion of INX-189 Proside to the Active Triphosphate



Methods

The anti-HCV potency values were determined in genotype 1b replicon cells in the presence or absence of 40 μM dCF or 2 μM EHNA. Intracellular metabolites produced in replicon 1b cells were measured by LC-MS/MS (Muhammad et al. 59th ASMS Conference on Mass Spectrometry and Allied Topics, Denver, Colorado, June 5-9, 2011).

Phosphoramidate Structure

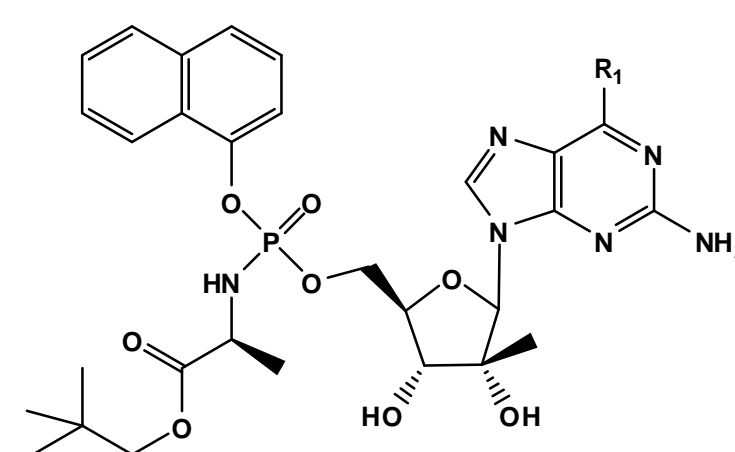


Table 1: Effect of dCF on Activity of Compounds

Compound	R ₁	Fold EC ₅₀ Increase in Presence of dCF
INX08189	MeO-	>8.4
INX08031	HO-	1.06
INX09012	EtO-	>27.6
INX09078	MeS-	>58.3
INX09079	MeNH-	>28.0
INX10006	Cl-	>21.1
INX08144	MeO- (nuc)	>4.4
INX08032	HO- (nuc)	1.03

Figure 3: Effect of dCF on intracellular formation of earlier stage metabolites in cells incubated with INX-189

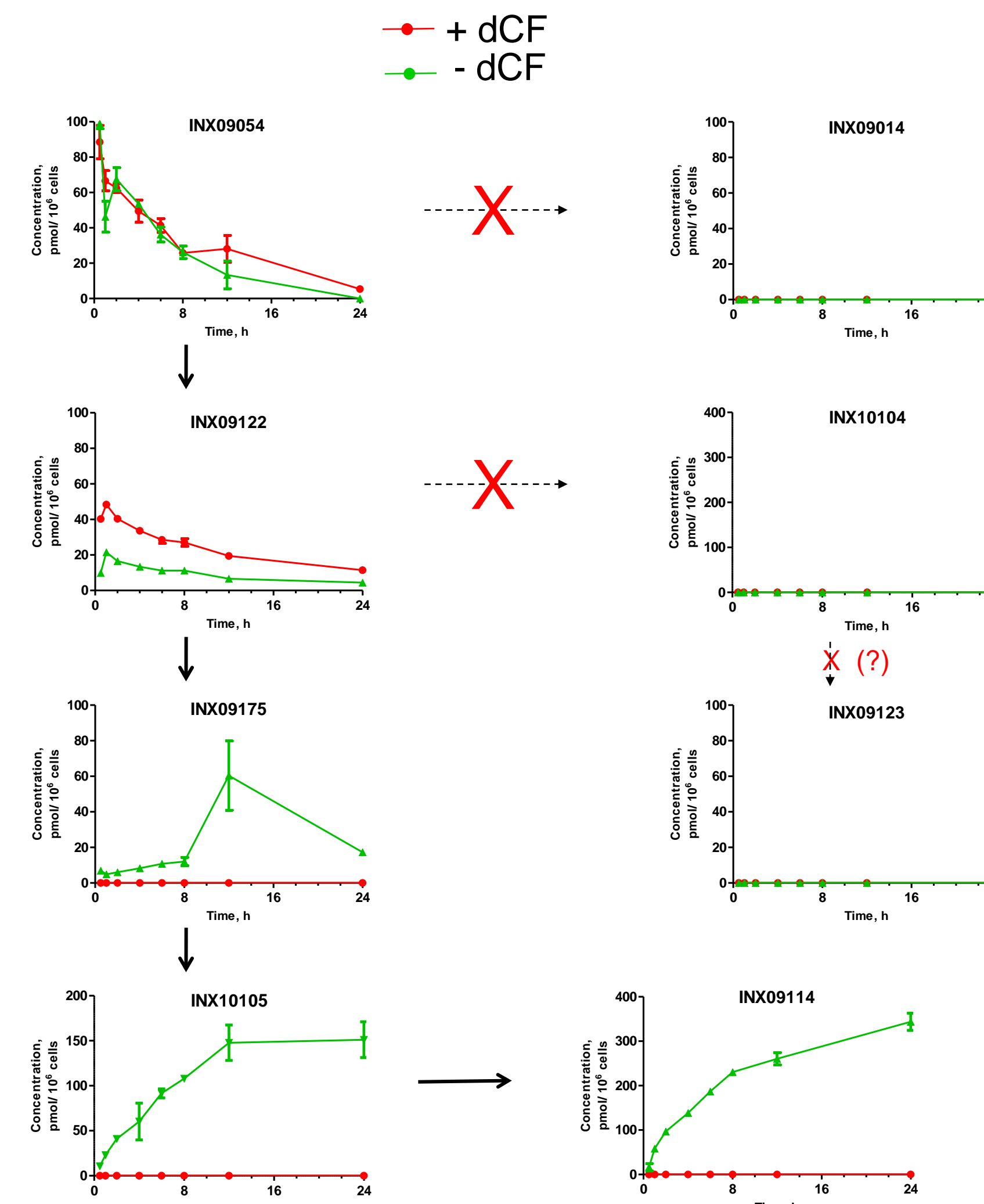


Figure 4: Effect of dCF on intracellular formation of metabolites in cells incubated with INX08031 (C⁶-oxo)

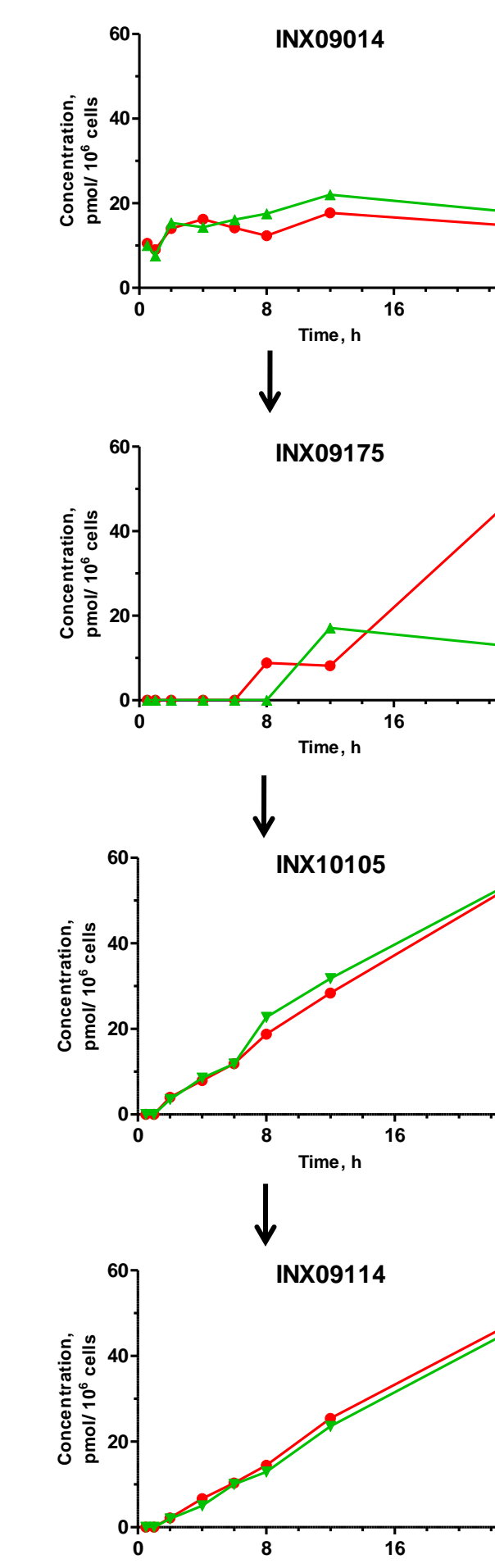


Figure 2: Effect of EHNA on Activity of INX-189 (A) Versus Nucleoside INX08144 (B).

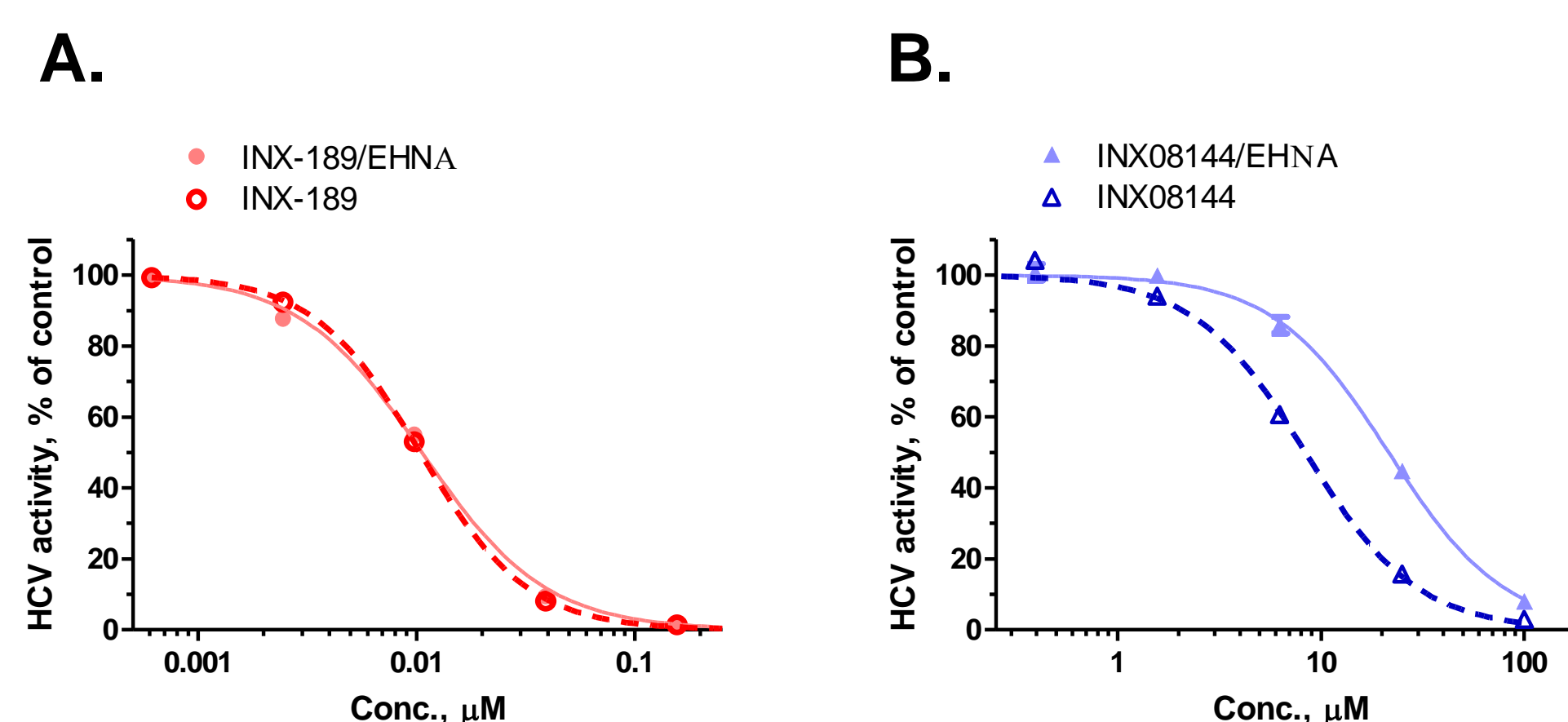


Table 2: Activity of C⁶-methylthio and C⁶-methylamino phosphoramidates

Compound	C ⁶ group (R ₁)	EC ₅₀ , μM	EC ₉₀ , μM	Repeats
INX09078	MeS-	0.041 ± 0.007	0.154 ± 0.013	n=3
INX09079	MeNH-	0.032 ± 0.008	0.102 ± 0.015	n=4

Conclusions

Hydrolysis of the purine C⁶ methoxy by a dCF-sensitive enzyme is required to process INX-189 to the diphosphate- and the therapeutically active triphosphate form, 2'-C-methyl GTP.

Data with ADA-specific inhibitor EHNA suggests that the primary route of activation of INX-189 involves direct conversion of the monophosphate to diphosphate and then triphosphate with negligible contribution of the nucleoside metabolite

C⁶-methylthio modified compounds have demonstrated replicon potency, suggesting that enzymes other than ADAL1 may play a role in the hydrolysis of these compounds.