Characterization of 4-{[4-(2-fluorophenyl) piperazin-1-yl] methyl}-6-imino-N-(naphthalen-2-yl)-1,3,5-triazin-2-amine (FPMINT) as a novel ENT2-selective inhibitor.

Equilibrative nucleoside transporters (ENTs) facilitate nucleoside transport across plasma membrane. Among four ENTs subtypes, ENT1 and ENT2 are studied more extensively.AnENT1knockout animal model has demonstrated that ENT1 plays an important role in cardio protection [1]. However, the physiological functions of ENT2 remain obscured becauseENT2knockout animal is not available. In addition, pharmacological approach is not feasible since the currently available ENT inhibitors such as NBMPR, and dipyridamole are ENT1-selective.The aim of this study was to search forENT2-selective inhibitors.

The PK15NTD cells (nucleoside transporters-deficient pig kidney fibloblasts) stably transfected with ENT1 and ENT2 were incubated in Ringer solution containing [^{3}H]uridine (2µCi/ml, 10µM) for 5 minutes and the uridine uptake was measured [2]. To study the inhibitory effects on ENT1 and ENT2,tested compounds (0-25µM)were added with [^{3}H]uridine.The IC_{50}, K_i (inhibitor constant), mode of inhibition and binding reversibility were studied. Moreover, their effects on expression levels of ENT1 and ENT2were examined by western blotting. Data was expressed as mean±S.E.M. (n=4) and analysed by two-way ANOVA, as applicable.

A series of compounds were screened and the results showed that of 4-{[4-(2-fluorophenyl) piperazin-1-yl] methyl}-6-imino-N-(naphthalen-2-yl)-1,3,5-triazin-2-amine (FPMINT) was 10-fold more selective to ENT2 than ENT1 (IC_{50}: 18.04± 1.15/1.69± 1.12, n=4). Similar results were observed when inhibitor constants were calculated. FPMINT inhibited ENT1 and ENT2 in a non-competitive and irreversible manner (Table 1). It had no effect on the expression levels of ENT1 and ENT2.

Table 1. Effects of PFMINT on ENT1 and ENT2.

	IC_{50} (µM)	**Ki (µM)**	**Mode of inhibition**	**Expression level of ENTs**
ENT1	18.04± 1.15	5.151± 1.817	Non-competitive, irreversible	No significant effect
ENT2	1.69± 1.12	2.531± 0.606		

In summary, FPMINT is a selective, non-competitive and irreversible inhibitor of ENT2. It may be an important tool for the further study of physiological and pharmacological functions of ENT2.

1 Rose JB et al. (2010) Am J Physiol Heart CircPhysiol **298**(3): H771-7

2 Li RW et al (2009) Eur J Pharmacol **612**(1-3): 15-20.