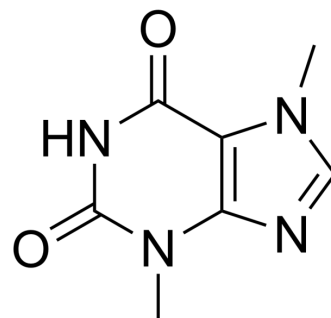


Theobromine

Cat. No.:	HY-N0138		
CAS No.:	83-67-0		
Molecular Formula:	C ₇ H ₈ N ₄ O ₂		
Molecular Weight:	180.16		
Target:	Adenosine Receptor; Endogenous Metabolite		
Pathway:	GPCR/G Protein; Metabolic Enzyme/Protease		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : 1.1 mg/mL (6.11 mM; Need ultrasonic)
 H₂O : < 0.1 mg/mL (insoluble)

Preparing Stock Solutions	Solvent		Mass		
	Concentration		1 mg	5 mg	10 mg
	1 mM		5.5506 mL	27.7531 mL	55.5062 mL
	5 mM		1.1101 mL	5.5506 mL	11.1012 mL
	10 mM		---	---	---

Please refer to the solubility information to select the appropriate solvent.

BIOLOGICAL ACTIVITY

Description	Theobromine is a methylxanthine found in cacao beans which can inhibit adenosine receptor A1 (AR1) signaling.
IC₅₀ & Target	AR1 ^[1]
In Vitro	Theobromine, at concentrations above 25 μM, decreases lipid accumulation in these cells. Cell viability is not affected by Theobromine. Theobromine, at concentrations above 25 μM, suppresses protein expression of PPARγ, C/EBPα and adipogenic genes. The mRNA levels of these genes are also decreased by Theobromine ^[1] . MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Body weights are lower in the Theobromine group than in the vehicle group. In addition, Theobromine suppresses gains in weight of epididymal and perirenal adipose tissues. The mean adipocyte area is smaller in the Theobromine group than in the vehicle group ^[1] . Theobromine group shows lower counts than the other groups when considering the number of bacteria per fecal weight (p=0.021 and p=0.055 compare to the reference (RF) and the cocoa (CC) groups, respectively). The Theobromine diet leads to higher pH values than those found after the RF and CC diets. Fecal concentrations of lactic acid

are not significantly affected by the experimental diets (4.26±1.54 mM in RF group; 1.96±0.41 mM in CC group; 2.69±0.73 mM in Theobromine group)^[2].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay ^[1]

3T3-L1 preadipocytes are pre-incubated with MG132 (10 µM) for 30 min, followed by incubation with IBMX in the presence or absence of Theobromine (25 µM) for 8 h. The cells are lysed in denaturing cell extraction buffer (50 mM Tris-HCl, pH 7.5, containing 70 mM β-mercaptoethanol and 2% SDS) at 95°C for 10 min. The cell lysates are diluted 20 fold with dilution buffer (20 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 1 mM EDTA, 1mM EGTA, 1% TritonX-100, 2.5 mM sodium pyrophosphate and protease inhibitor cocktail) and centrifuged at 20,000 g for 30 s. The supernatant is incubated with rabbit polyclonal anti-C/EBPβ IgG, anti-FLAG IgG or control IgG at 4°C overnight, followed by incubation with 30 µL protein G-Sepharose resin at 4°C for 1 h. The resin is washed with lysis buffer three times and proteins bound to the resin are separated by SDS-PAGE and analyzed by western blotting^[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Administration ^[2]

Lewis rats (3 week old) are used in this study. The rats are randomly distributed into three dietary groups (n=7 per group): the reference (RF) group ingested standard diet AIN-93M, the cocoa (CC) group ingested a standard diet with 10% of natural Forastero cocoa containing 0.25% Theobromine, and the Theobromine (TB) group ingested a standard diet including 0.25% of Theobromine, i.e. the content of Theobromine presents in the CC diet^[2].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Microbiol Immunol. 2023 Jul 6.

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REFERENCES

[1]. Mitani T, et al. Theobromine suppresses adipogenesis through enhancement of CCAAT-enhancer-binding protein β degradation by adenosine receptor A1.

[2]. Martín-Peláez S, et al. Effect of cocoa's theobromine on intestinal microbiota of rats. Mol Nutr Food Res. 2017 Oct;61(10).

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

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