Supporting Information

Natural Products Targeting Amino Acid Metabolism: From Discovery to Synthetic Development

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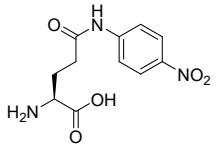
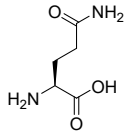
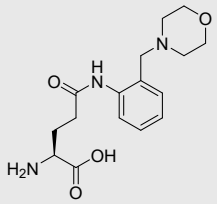
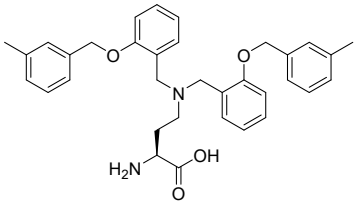
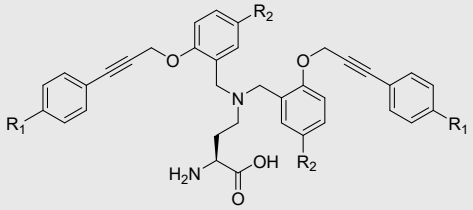
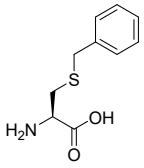
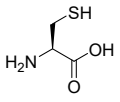
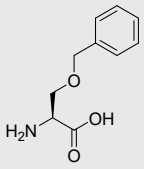
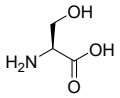
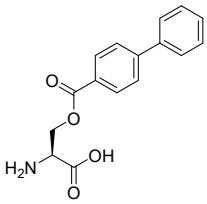
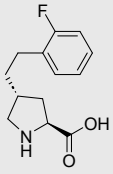
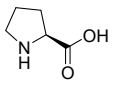
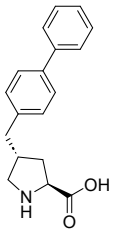
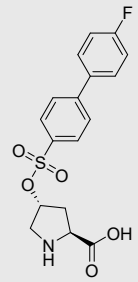
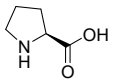
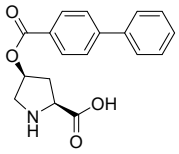
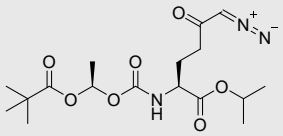
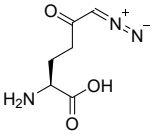
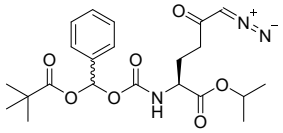
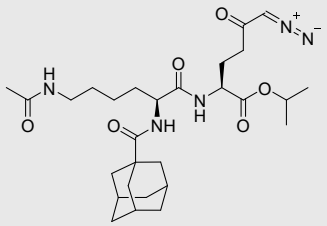
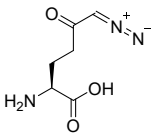
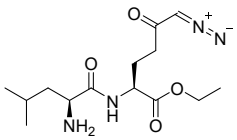
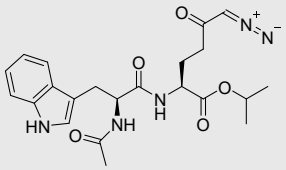
Table S1. Natural Products-Based Inhibitors Modulating Glutamine Metabolism

Enzyme	Structure and Name	Natural Source	Chemical development	Indication	Ref.
ASCT2	 **2** L-γ-Glutamyl-*p*-nitroanilide (GPNA)	 **1** L-Glutamine	Decreasing pKa of the amide proton; Enhancing binding affinity	C6 rat glioma cells (in-vitro)	88
	 **3** *N*-(2-(Morpholinomethyl)phenyl)-L-glutamine		Improving inhibition of glutamine uptake (3-fold than GPNA)	HEK293 cells (in-vitro)	89
	 **4** 2-Amino-4-*bis*(2-((3-methylbenzyl)oxy)benzyl) aminobutanoic acid (V-9302)		Improving inhibition of glutamine uptake (100-fold than GPNA)	Colorectal cancer (in vivo)	90-92
	 **5a**: R_1 = Cl, R_2 = H **5b**: R_1 = H, R_2 = *tert*-Bu 2-Amino-4-*bis*(2-((3-(4-chlorophenyl)prop-2-yn-1-yl)oxy)benzyl)aminobutanoic acid (**5a**) 2-Amino-4-*bis*(5-(*tert*-butyl)-2-((3-phenylprop-2-yn-1-yl)oxy)benzyl)aminobutanoic acid (**5b**)		Enhancing selectivity for ASCT2; Improving inhibition of glutamine uptake, microsomal stability, and bioavailability; Suppressing tumor growth in an A549 xenograft model, with higher tumor growth inhibition (TGI)	Non-small-cell lung cancer (NSCLC) (in vivo)	93

ASCT2	 **8** *S*-Benzyl-L-cysteine	 **6** L-Cysteine	First ASCT2 inhibitor	HEK293 cells (in-vitro)	94
	 **9** *O*-Benzyl-L-serine	 **7** L-Serine	First ASCT2 inhibitor	MCF-7 cells (in-vitro)	94, 95
	 **10** *O*-(4-Phenylbenzoyl)-L-serine		Enhancing apparent affinity (K_m = 30 μM)	HEK293 cells (in-vitro)	96
	 **12** (2*S*,4*R*)-4-(2-Fluorophenethyl)pyrrolidine-2-carboxylic acid (γ-FBP)	 **11** L-Proline	First proline analogue ASCT2 inhibitor; Enhancing apparent affinity (K_m = 87 μM)	C8161 melanoma cells (in-vitro)	97
	 **13** (*R*)-γ-(4-Biphenylmethyl)-L-proline		Enhancing apparent binding affinity (K_i = 3 μM)	HEK293 cells (in-vitro)	98

ASCT2	 **14** (2*S*,4*R*)-4-(((4'-Fluoro-[1,1'-biphenyl]-4-yl) sulfonyl)oxy)pyrrolidine-2-carboxylic acid	 **11** L-Proline	Enhancing apparent binding affinity (K_i = 8.07 μM)	HEK293 cells (in-vitro)	99
	 **15** L-*cis*-Hydroxyproline biphenyl-4-carboxylate ester (L*c*-BPE)		Stereospecific inhibition; Enhancing apparent binding affinity (K_i = 0.86 μM)	HEK293 cells (in-vitro)	100
GLS	 **17** Methyl-POM-DON-isopropyl-ester	 **16** (*S*)-6-Diazo-5-oxo-L-nor leucine (DON)	Improving chemical and metabolic stability; Enhancing cerebrospinal fluid (CSF)-to-plasma ratio (10-fold than DON)	Glioblastoma multiforme (GBM) (in vivo)	101
	 **18** Isopropyl 6-Diazo-5-oxo-2-(((phenyl(pivaloyloxy) methoxy)carbonyl)amino)hexanoate		Enhancing CSF-to-plasma ratio and brain-to-plasma ratio (15-fold and 9-fold than DON)	HIV-associated neurocognitive disorders (HAND) (in vivo)	102

GLS	 **19** Isopropyl 2-(6-Acetamido-2-(2-acetyl-1,2,3,4-tetrahydroisoquinoline-3-carboxamido)hexanamido)-6-diazo-5-oxohexanoate	 **16** (*S*)-6-Diazo-5-oxo-L-nor leucine (DON)	Enhancing tumor cell-to-plasma ratio (55-fold than DON); Enhancing tumor exposure in plasma and GI (5-fold and 11-fold than DON)	Lymphoma (in vivo)	103
	 **20** Ethyl 2-(2-Amino-4-methylpentanamido)-6-diazo-5-oxohexanoate (JHU083)		Orally bioavailable Reducing the generation of MDSCs	MYC-expressing medulloblastoma, IDH1 mutation glioma, Thyroid cancer (in vivo)	104-107
	 **21** Isopropyl (*S*)-2-((*S*)-2-acetamido-3-(1*H*-indol-3-yl) propanamido)-6-diazo-5-oxohexanoate (DRP-104)		Enhancing tumor exposure in plasma and GI (6-fold and 11-fold than DON)	Pancreatic ductal adenocarcinoma (PDAC), castration-resistant prostate cancer (CRPC), lung cancer (in vivo) Clinical trials; NCT 06027086 (Recruiting)	108-111

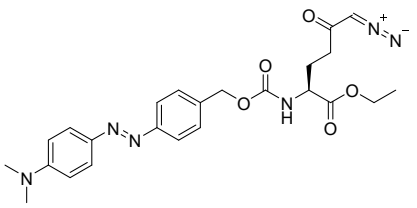
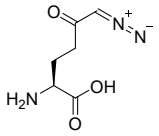
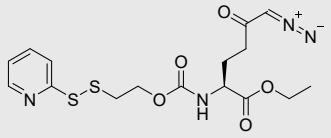
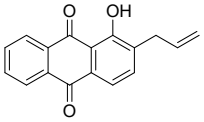
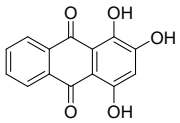
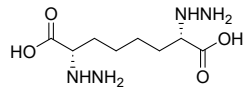
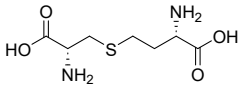
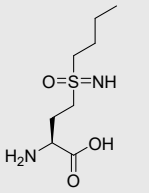
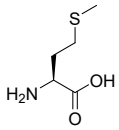
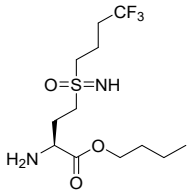
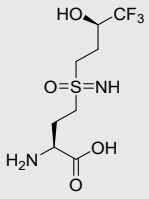
GLS	 **22** Ethyl (*E*)-6-diazo-2-((((4-((4-(dimethylamino) phenyl)diazenyl)benzyl)oxy)carbonyl)amino)-5-oxohexanoate (Azo-DON)	 **16** (*S*)-6-Diazo-5-oxo-L-nor leucine (DON)	Hypoxic-activated prodrug of DON; TSR of 84.2 % in hepatoma cancer (monotherapy); TSR of 96.6 % in colon cancer (combination with CBP)	Hepatoma cancer, colon cancer (in vivo)	112
	 **23** Ethyl (*S*)-6-diazo-5-oxo-2-(((2-(pyridin-2-yl disulfaneyl)ethoxy)carbonyl)amino)hexanoate (redox-DON)		Redox-responsive prodrug of DON; Improved safety profile than JHU083	Colon cancer (in vivo)	113
GLUD1	 **25** 2-Allyl-1-hydroxyanthracene-9,10-dione (R162)	 **24** Purpurin	Improving potency and specificity for GLUD1 inhibition; Enhancing the cell-permeability; Attenuating cancer cell proliferation and tumor metastasis	Lung cancer, breast cancer, LKB1-deficient lung cancer Non-small-cell lung cancer (NSCLC) (in vivo)	38, 114, 115

Table S2. Natural Products-Based Inhibitors Modulating Cysteine/Cystine Metabolism

Enzyme	Structure and Name	Natural Source	Chemical development	Indication	Ref.
CBS	 **27** α-(L,L)-bis-hydrazino acid (6S)	 **26** (L,L)-Cystathionine	Enhancing apparent binding affinity (K_i = 48 μM); Reduction of infarct volume with an 83% at 30 min prior-stroke treatment and a 66% reduction at 1 h post-stroke treatment	transient middle cerebral artery occlusion (tMCAO) for ischemic stroke (In vivo)	158
GCL	 **29** L-Buthionine sulfoximine (BSO)	 **28** L-Methionine	Enhancing inhibiting potency (100-fold than MSO); Decreasing renal GSH levels in mice (<20% of the control level)	melanoma, multiple myeloma (MM), neuroblastoma (In vivo)	159-164
	 **30** (KOJ-1)		Enhancing binding, cellular potency and effectively inducing ferroptosis *in vitro*	canine cancer cell lines (In vivo)	165
	 **31** (KOJ-2)		Ester removal allowed enhanced GCL inhibition, potent ferroptosis induction in cells, and improved bioavailability upon oral administration	HT1080 xenograft-nude mice model	165

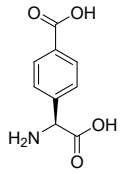
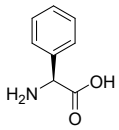
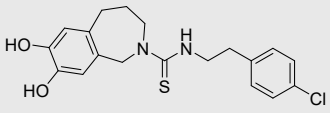
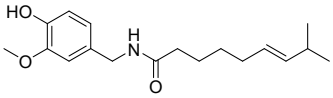
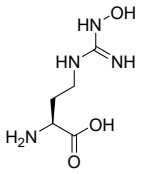
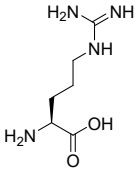
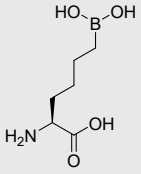
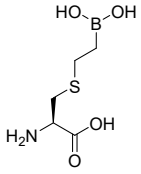
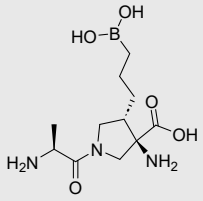
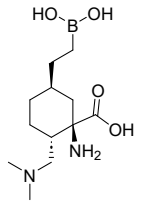
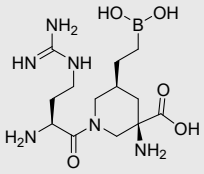
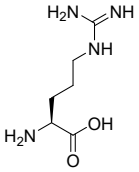
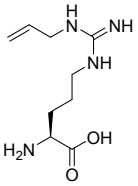
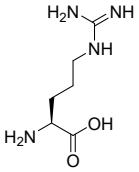
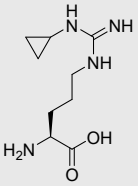
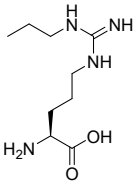
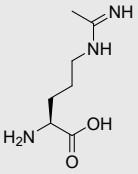
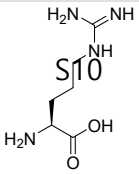
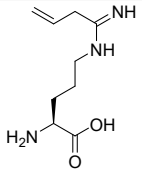
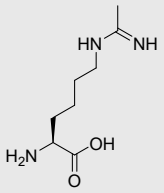
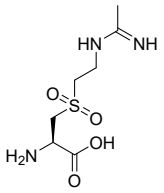
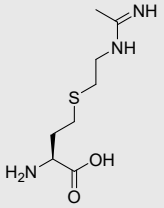
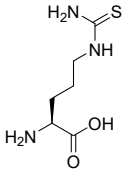
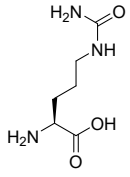
system X_c^- (xCT)	 **33** (*S*)-4-Carboxylphenylglycine	 **32** L-Phenylglycine	A competitive non-substrate inhibitor; Enhancing apparent binding affinity (K_i = 5 μM)	LRM55 and SNB-19 glioma cells (in-vitro)	166
	 **35** Capsazepine (CPZ)	 **34** Capsaicin	Inhibition of cysteine uptake (IC_{50} = ~ 3 μM); Increasing ROS levels	Cancer-induced bone pain (CIBP) (In vivo)	167

Table S3. Natural Products-Based Inhibitors Modulating Arginine Metabolism

Enzyme	Structure and Name	Natural Source	Chemical development	Ref.
ARG1/2	 **37** *N*ω-Hydroxy-nor-L-arginine (Nor-NOHA)	 **36** L-Arginine	Inhibition of ARG (IC_{50} = 2 μM for rARG, 50 μM for mARG); Enhancing binding affinity (K_i = 0.5 μM, 20-fold than NOHA); Anti-leukemic activity in hypoxia	212-214
	 **38** 2(*S*)-Amino-6-boronohexanoic Acid (ABH)		First boronic acid-based ARG inhibitor; High-affinity inhibition (IC_{50} = 0.8 μM for rARG, K_d = 0.11 μM); Nonadrenergic, noncholinergic (NANC) nerve-mediated cavernosal smooth muscle relaxation	215-217
	 **39** *S*-(2-Boronoethyl)-L-cysteine (BEC)		High-affinity inhibition (K_i = 0.4-0.6 μM, K_d = 2.22 μM); NO-mediated cavernosal smooth muscle relaxation	218
	 **40** Numidargistat/CB-1588		Dual ARG1/2 inhibitor; Enhancing ARG inhibition (IC_{50} = 20 nM for hARG1, 39 nM for hARG2); Antitumor activity for glioma, melanoma, leukemia, ovarian, lung, and colon cancer; First-in-human (FIH) Phase I trial in advanced/metastatic solid tumors	219-222
	 **41** OATD-02		Potent ARG inhibition (IC_{50} = 86 nM for ARG1, 296 nM for ARG2); Antitumor activity for lung, colon, and breast cancer; Phase 1/2 trials evaluating monotherapy and combination with immune checkpoint inhibitors in advanced solid tumors	223

ARG1/2	 **42** (3*R*,5*S*)-3-amino-1-((*S*)-2-amino-4-guanidino butanoyl)-5-(2-boronoethyl)piperidine-3-carboxylic acid (A17)	 **36** L-Arginine	First boronic acid-based piperidine analogue ARG inhibitor; Low cellular permeability; Selective targeting extracellular ARG; High oral bioavailability; No potential cardiotoxicity	224
NOS	 **43** *N*ω-Allyl-L-arginin (L-ALA)	 **36** L-Arginine	Both reversible (K_i = 2.1 μM) and irreversible inhibition (K_i = 3.4 μM) for iNOS	225, 226
	 **44** *N*ω-Cyclopropyl-L-arginine (L-CPA)		Reversible inhibitor for iNOS (K_i = 7.7 μM)	225
	 **45** *N*ω-Propyl-L-arginine		Remarkable selectivity for nNOS (K_i = 57 nM)	226
	 **46** L-*N*5-(1-iminoethy)ornithine (L-NIO)		Potent inhibition for eNOS	227-229
NOS		 **36** L-Arginine	Strong inhibitory selectivity	230

	 **47** N^5-(1-Imino-3-butenyl)-L-ornithine (Vinyl-L-NIO)		for nNOS (K_i of 100 nM)	
	 **48** L-N^6-(1-1minoethyl)lysine (L–NIL)		Potent selective inhibitor for iNOS (IC_{50} = 3.3 μM for iNOS, 92 μM for nNOS)	231
	 **49** GW273629		Enhancing iNOS selectivity (IC_{50} = 8.0 μM for iNOS, 125-fold selectivity over eNOS)	232
	 **50** GW274150		Enhancing iNOS selectivity (IC_{50} = 1.4 μM for iNOS, 333-fold selectivity over eNOS)	232
	 **52** L-Thiocitrulline	 **51** L-Citrulline	First citrulline analogue NOS inhibitor; Inhibition of bNOS (91%) and iNOS(87%) at 100 μM	233

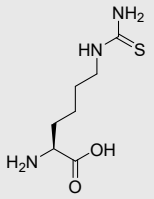
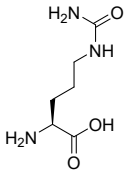
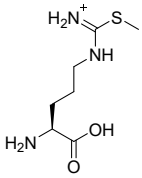
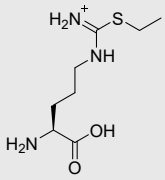
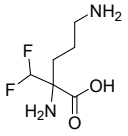
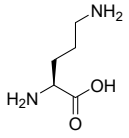
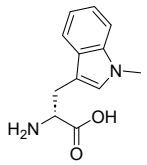
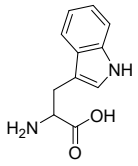
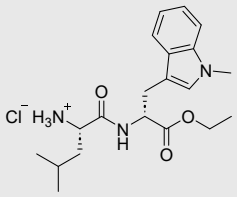
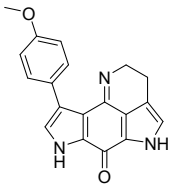
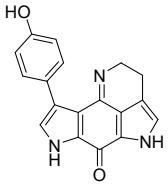
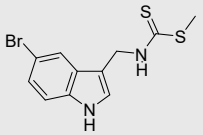
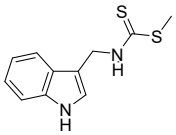
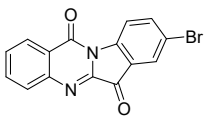
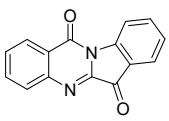
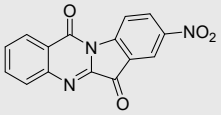
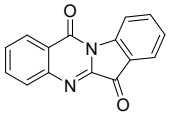
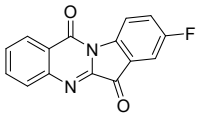
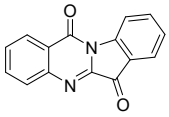
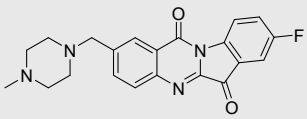
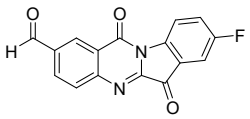
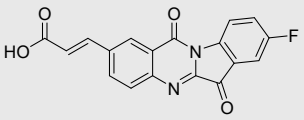
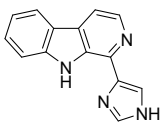
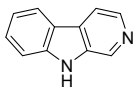
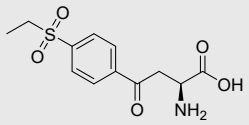
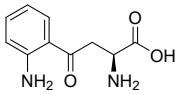
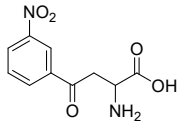
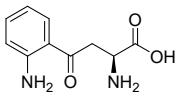
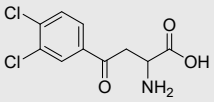
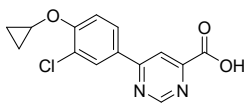
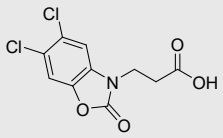
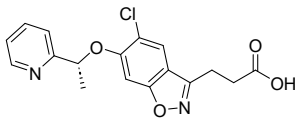
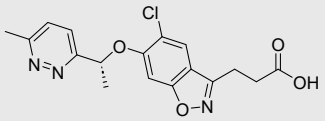
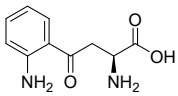
NOS	 **53** L-Homothiocitrulline	 **51** L-Citrulline	First citrulline analogue NOS inhibitor; Inhibition of bNOS (93%) and iNOS(75%) at 100 μM	233
	 **54** *S*-Methyl-L-thiocitrullin (S-MTC)		First citrulline analogue NOS inhibitor; Inhibition of bNOS (96%) and iNOS(87%) at 10 μM; Potent and selective inhibitor for nNOS (K_i = 1.2 nM for nNOS and 11 nM for eNOS); Potent inhibitory activity *in vivo* (rat model) (IC_{50} values: 0.047 μM for nNOS (brain cytosol), 0.31 μM for nNOS (brain slices), 5.4 μM for eNOS (aortic rings))	233, 234
	 **55** *S*-Ethyl-L-thiocitrullin (S-ETC)		Potent and selective inhibitor for nNOS (K_i = 0.5 nM for nNOS and 24 nM for eNOS); Potent inhibitory activity *in vivo* (rat model) (IC_{50} values: 0.073 μM for nNOS (brain cytosol), 1.2 μM for nNOS (brain slices), 7 μM for eNOS (aortic rings)	234
ODC	 **57** α-Difluoromethylornithine (DFMO/Eflornithine)	 **56** L-Ornithine	Clinical trials in anaplastic glioma and colon cancer; Inhibition of tumor progression to pancreatic ductal adenocarcinoma (PDAC); Approved for the treatment of late-stage *Trypanosoma brucei gambiense* infections in 1990; Approved as an oral maintenance therapy for adult and pediatric patients with high-risk neuroblastoma (HRNB)	235- 244

Table S4. Natural Products-Based Inhibitors Modulating Tryptophan Metabolism>

Enzyme	Structure and Name	Natural Source	Chemical development	Ref.
IDO1	 **59** 1-Methyl-D-tryptophan (D-1-MT/Indoximod/ NLG8189)	 **58** D/L-Tryptophan	Potent immunomodulator via non-enzymatic mTORC1 pathway activation ($IC_{50} \approx 70$ nM); Antitumor activity in HER2-driven breast cancer, prostate cancer, metastatic melanoma, pediatric brain tumor (DIPG); Synergistic effects with chemotherapy and checkpoint inhibitors demonstrated in Phase I/II trials	299-303
	 **60** Ethyl leucyl-1-methyl-D-tryptophanate (NLG-802)		Optimized prodrug of indoximod for enhanced oral bioavailability (5-fold than indoximod); Enhanced C_{max} and AUC; Favorable toxicology profile (NOAEL 120 mg/kg BID in non-human primates); Superior antitumor efficacy in murine melanoma models via enhanced T cell-mediated responses	304
	 **62** Tsitsikammamine A analogue	 **61** Tsitsikammamine A	Potent IDO1 enzymatic inhibition ($IC_{50} = 0.9$ μM)	305
	 **64** 5-Br-brassinin	 **63** Brassinin	Competitive IDO1 inhibitor ($K_i = 24.5$ μM); Orally bioavailable with superior pharmacokinetics ($T_{max} = 526.3$ min and $AUC_{\infty} = 1485$ μg·min/mL); Selective for IDO over TDO2; Enhancing tumor regression in combination with paclitaxel	306
TDO	 **66** Tryptanthrin derivatives	 **65** Tryptanthrin	Potent uncompetitive TDO inhibitor ($IC_{50} = 0.937 \pm 0.215$ μM; $K_i = 0.356 \pm 0.078$ μM); Rosbust cellular inhibition ($IC_{50} = 0.054$ μM in U87 MG cells; 0.053 μM in HEK293-hTDO cells)	307

	 **67** Tryptanthrin derivatives	 **65** Tryptanthrin	Potent uncompetitive TDO inhibitor (IC_{50} = 0.101 ± 0.062 μM; K_i = 0.215 ± 0.020 μM); Robust cellular inhibition (IC_{50} = 0.040 μM in U87 MG cells; 0.061 μM in HEK293-hTDO cells)	307
IDO/ TDO dual	 **68** Tryptanthrin derivative	 **65** Tryptanthrin	Potent IDO1 inhibition (IC_{50} = 0.534 μM for rhIDO1, 0.023 μM in HEK293-IDO1 cells); Moderate TDO inhibition (IC_{50} = 0.937 ± 0.148 μM); Enhanced T cell proliferation and antitumor activity in LLC (Lewis lung cancer) tumor model; 62% tumor volume reduction and Treg depletion *in vivo*.	283, 285, 307
	 **69** Tryptanthrin derivative		Potent IDO1/TDO dual inhibition (IDO1 enzymatic IC_{50} = 0.50 μM; cellular IC_{50} = 0.02 μM / TDO enzymatic IC_{50} = 0.76 μM; cellular IC_{50} = 0.09 μM); Uncompetitive inhibition with K_i = 2.64 μM (IDO1); 0.31 μM (TDO); Enhanced T cell proliferation and superior efficacy over L-1-MT; Tumor volume reduction (56.2% in LLC, 47.3% in H22 models)	308
	 **70** Tryptanthrin derivative		Potent IDO1/TDO dual inhibition (IDO1 IC_{50} = 0.46 μM; TDO IC_{50} = 0.06 μM); IDO1 inhibitory activity in HeLa cells (IC_{50} = 0.16 μM)	285
	 **71** Tryptanthrin derivative		Potent IDO1/TDO dual inhibition (IDO1 IC_{50} = 0.12 μM; TDO IC_{50} = 0.03 μM); IDO1 inhibitory activity in HeLa cells (IC_{50} = 0.06 μM)	285
	 **73** 1-(1*H*-Imidazole-5-yl)-9*H*-pyrido[3,4-*b*]indole	 **72** Norharmane	Potent IDO1/TDO dual inhibition (IDO1 IC_{50} = 3.53 ± 0.81 μM; TDO IC_{50} = 1.15 ± 0.09 μM); Anti-inflammatory effects in LPS-induced BV2 microglial cells; High plasma exposure ($AUC_{0-\infty}$ = 4464.9 h·ng/mL); Moderate oral bioavailability (F = 52.55%); Amelioration of depressive-like behaviors in LPS-induced mouse model	309

KATII	 **75** (*S*)-4-(Ethylsulfonyl) benzoylalanine (*S*-ESBA)	 **74** L-Kynurenine	First synthetic selective KAT II inhibitor (IC_{50} = 6.1 μM); Selective inhibition without affecting KAT I, KMO, or KYNU; Decreased extracellular KYNA levels in rat hippocampus	310
KMO	 **76** *m*-Nitrobenzoylalanine (m-NBA)	 **74** L-Kynurenine	Potent KMO inhibition (IC_{50} = 0.9 ± 0.1 μM); High selectivity over KYNU (IC_{50} = 100 ± 12 μM); In vivo elevation of KYN (13-fold) and KYNA (up to 5-fold) in rat brain, liver, and blood; Increased hippocampal extracellular KYNA; Dose-dependent sedative and anticonvulsant effects in rats and DBA-2 mice	311
	 **77** (*R*,*S*)-3,4-dichlorobenzoylalanine (FCE 28833A)		Potent KMO inhibition (IC_{50} = 0.2 ± 0.02 μM); 15-fold greater potency than m-NBA; Sustained 10- to 80-fold elevation of hippocampal extracellular KYNA	312
	 **78** CHDI-340246		Subnanomolar KMO inhibition (IC_{50} = 0.5-0.6 nM); Excellent selectivity over KATs and KYNU; Favorable pharmacokinetics (oral bioavailability ~64%, low plasma clearance 291 mL/h/kg)	313
	 **79** GSK180		Nanomolar KMO inhibition (IC_{50} ≈ 6 nM); Protection against lung, kidney, and liver injury in AP-MODS rodent models	314, 315
	 **80** GSK065		Potent KMO inhibition (pIC_{50} = 8.3; cellular pIC_{50} = 8.5); Picomolar affinity (Ki ≈ 50 pM) with slow dissociation kinetics ($T_{1/2}$ ≈ 10 h); Favorable PK (low clearance, high exposure); Protective effects in rat model of acute pancreatitis (AP)	315, 316

	 **81** GSK366	 **74** L-Kynurenine	Potent KMO inhibition (IC_{50} = 2.3 nM for hKMO; 0.7 nM for Pf-KMO); High affinity ($K_i \approx 12$ pM) with a prolonged dissociation half-life ($T_{1/2} \approx 12$ h)	315, 316