**Supplementary Figure S1:** Full multiple sequence alignment of the amino acid sequences of cystatins from animals and plants [human cystatin D (PDB:1RN7\_A); Stefin-1 (Uniprot:P35175), stefin-2 (Uniprot: P35174), stefin-3 (Uniprot: P35173) from Mouse, cystatin-A (Uniprot:P01039) from Rat; Oryzacystatin-I (PDB: 1EQK\_A) from *Oryza sativa*; Cystatin I (Uniprot:P31726) from *Zea mays*; Multicystatin (Uniprot: P37842), (PDB: 2W9P\_F) from *Solanum tuberosum*].

	10	20	30	40	50	) 60	
					• • •   • • • •	• • • •   • • • •	
sp P0103							
sp P3517							
sp P3517							
sp P3517							
pdb   1EOK							
splP3172				M	RKHRIVSLV		
ap   p2794	MA LUCCLUDUDI		FAUODVNOK	NDSSTRRKK	VINVKOOIV	ACIMVVITER	
sp113704	MAIVGGLVDVFI	BIRVERDDLAR	THVQD INQN	NDSSLEINN	VLIVING VI	AGIMITITE	
IRN/_AP							
Clustal							
	70	80	90	100	11	0 120	
sp P0103			<b>MD</b> P	GTTGIVGGV	SEAKPAT	PEIQEVADKV	
sp P3517			MSO	ENLKIKGGL	SEARPAT	PEIOMIADKV	
sp1P3517			MTR	VTRKIKGGI.	SEABPAT	SELOELADKY	
ap 1 D 2 5 1 7			MC_	TCCV	CPACDAT	DELOMIANKY	
spirssi/			Mag	December of	SEA-SRAI	PEIQMIANKV	
papitegk			MSS	DGGPVLGGV	EPV-GNEND	LHLVDLARFA	
sp P3172	AVSSTRSTQKES	s	VAD	NAGMLAGGI	KDVPANEND:	LQLQ <b>ELARFA</b>	
sp P3784	ATEGGNKKEYE!	KILLRKWEDLK	KVVGFKLVG	DDSTMPGGI	VNV-PNPNN	TKFQ <b>ELARFA</b>	
pdb 2W9P				GI	VNV-PNPNN	TKFQ <b>ELARFA</b>	
1RN7 A P			GSASA	QSRTLAGGI	HATDLND	KSVQRALDFA	
Clustal				*:			
	130	140	150	160	17	0 180	
en   P0103	KPOLEEKTN	- RKVRK FKVVRV	KSOWWAGOT	T.EMKVDVCN	CIDET HMKVT	PGLSCDD	
ap   D2517	DDITEROTH	BRIDRI ROVBI	KSQVVAOQ1	TELLIDUCN	COPT LIMENTE	DCLSCED	
sp[P3517	RPLLEBQIN	-EKIEKFEAVEI	KSQVVAGQN	LFIKIDVGN	GCFLIMKVF	RGLSGED	
sp[P3517	RPLLEEKTN	-EKYEKFKAIEY	KVQVVQGLN	YF IKMNVGR	GCYLHINVL	SGISSEN	
sp[P3517	RPQLEAKTN	-KKYEKFEAVEY	KTQVVAGEN	IFIKMDVGH	GCFIHIKVF	NGPTGKD	
pdb   1EQK	VTEHNKKAN	SLLEFEKLVSV	KQQVVAGTL	YYFTIEVKE	GD-AKKLYE	AKVWEKP	
sp P3172	VNEHNQKAN	-ALLGFEKLVKA	KTQVVAGTM	YYLTIEVKD	GE-VKKLYE	AKVWEKP	
sp P3784	IQDYNKKQN	AHLEFVENLNV	<b>KEQVVAGI</b> M	YYITLAATD	DAGKKKIYK	AKIWVKE	
pdb 2W9P	IQDYNKKQN	AHLEFVENLNV	<b>KEQVVAGIM</b>	YYITLAATD	DAGKKKIYK	AKIWVKE	
1RN7 AIP	ISEYNKVINKD	YYSRPLOVMAA	YOOIVGGVN	YYFNVKFGR	TTCTKSOPN	LDNCPFNDOP	
Clustal	: *		*:* *		:	~	
oraboar							
	190	200	210				
sp P0103	DLKLLDYQTNKTKNDELTDF						
sp P3517	DLKLKGYQTNK						
sp P3517	DLELTGYQTNK/	KNDELTYF					
sp P3517	NYELHGYQTDK	MDEELTYF					
pdb   1EQK	WMDFKELQEFK	VDASANA					
sp P3172	WENFKOLOEFKPVEEGASA						
sp1P3784	WEDFKKVVEFKLVG						
ndh 12W0P							
1DN7 ALD	KIKERECCEOINEUDMEDKICIINVKORV						
TKN/AP	KLKEEEFCSFQ.	INEVPWEDKISI	LINIKCRKV				
Clustal	. :						

Supplementary Figure S2: Predicted tertiary structure of the chimera inhibitor Mco-CPI.



MCOCPI GGVCQVVAGACRRDSDCPGACICRGNGYCGSG

**Supplementary Figure S3:** Construct design (in pET-28a(+) vector) for recombinant expression of Mco-CPI protein.



[C]

## Nt sequence

## Translation: Amino acid sequence

MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGR GGVCQVVAGACRRDSDCPGACICRGNGYCGSGSDNHV

**Supplementary Figure S4:** Kyte & Doolittle hydrophobocity plot of Mco-CPI. The more positive the value, the more hydrophobic are the amino acids located in that region of the protein.



## MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGR GGVCQVVAGACRRDSDCPGACICRGNGYCGSGSDNHV

**Supplementary Figure S5:** Calibration curve for standard SH (L-cysteine; 5-100  $\mu$ M) for free thiol content estimation by Ellman's assay.



**Supplementary Figure S6:** Construct design and characterization for McoTI-II protein. **A) & B)** Construct design and nucleotide sequence encoding McoTI-II. **C)** MALDI-TOF-MS spectrum of recombinant McoTI-II showing a peak of 7278.795 Da. Also, at a difference of 150 Da, a peak of 7126.421 is evident. This could be the result of terminal processing of an amino acid during expression. **D)** Ni-NTA purification of McoTI-II. Purified protein (~7.2 kDa) separated on 15% SDS-PAGE (tris-tricine) and stained with coomasie blue R250. **E)** Inhibition kinetics of McoTI-II against trypsin expressed as % trypsin inhibition. An increase in % trypsin inhibition is observed with increasing concentration of McoTI-II.



**Supplementary Table 1:** Statistics of the top 10 clusters generated for docking of McoCPI with Papain on HADDOCK server

Cluster No.	HADDOCK score	Cluster size	RMSD from overall lowest- energy structure	Van der Waals energy	Electrostatic energy	Desolvation energy	Buried surface area	Z- score
Cluster 3	-66.8 +/- 5.1	17	0.5 +/- 0.4	-43.5 +/- 4.3	-64.9 +/- 4.3	-10.9 +/- 2.3	1184.9 +/- 43.4	-1.5
Cluster 2	-65.5 +/- 3.2	36	7.2 +/- 0.2	-41.1 +/- 5.3	-119.7 +/- 9.1	-1.8 +/- 0.6	1207.3 +/- 45.5	-1.4
Cluster 1	-62.5 +/- 2.5	54	5.5 +/- 0.1	-35.9 +/- 1.7	-107.9 +/- 34.4	-6.3 +/- 3.1	1118.0 +/- 30.8	-1.1
Cluster 9	-55.6 +/- 6.4	5	5.6 +/- 0.2	-38.5 +/- 5.3	-72.0 +/- 24.7	-5.2 +/- 2.7	1105.1 +/- 64.4	-0.5
Cluster 11	-48.6 +/- 4.7	4	7.2 +/- 0.1	-34.8 +/- 3.0	-70.0 +/- 27.9	-3.9 +/- 1.5	940.1 +/- 30.3	0.2
Cluster 4	-48.5 +/- 0.7	16	3.9 +/- 0.2	-36.5 +/- 2.2	-41.0 +/- 8.9	-6.0 +/- 1.2	955.8 +/- 24.6	0.2
Cluster 7	-43.1 +/- 3.4	6	5.2 +/- 0.1	-32.1 +/- 2.3	-37.6 +/- 19.3	-9.1 +/- 3.9	1050.3 +/- 53.7	0.7
Cluster 5	-39.7 +/- 4.2	12	5.3 +/- 0.1	-28.4 +/- 3.0	-41.0 +/- 19.0	-6.3 +/- 5.3	932.5 +/- 26.6	1.0
Cluster 10	-38.0 +/- 14.7	4	4.0 +/- 0.8	-33.0 +/- 10.2	-30.7 +/- 7.7	-2.5 +/- 2.7	926.5 +/- 139.8	1.1
Cluster 8	-36.7 +/- 5.5	5	5.3 +/- 0.2	-27.8 +/- 3.3	-46.8 +/- 24.6	-1.8 +/- 2.0	867.8 +/- 66.7	1.3

**Supplementary Table 2:** Statistics of the top 10 clusters generated for docking of McoCPI with Cathepsin L on HADDOCK server

Cluster No.	HADDOCK score	Cluster size	RMSD from overall lowest- energy structure	Van der Waals energy	Electrostatic energy	Desolvation energy	Buried surface area	Z- score
Cluster 2	-80.6 +/- 0.8	36	4.9 +/- 0.1	-46.7 +/- 2.6	-147.5 +/- 13.6	-4.5 +/- 1.5	1324.9 +/- 51.7	-1.7
Cluster 1	-74.1 +/- 5.7	45	5.2 +/- 0.3	-49.0 +/- 5.8	-136.7 +/- 10.3	1.9 +/- 1.8	1231.4 +/- 29.5	-1.3
Cluster 3	-68.9 +/- 3.9	11	2.8 +/- 0.1	-44.9 +/- 1.7	-121.4 +/- 23.0	-0.2 +/- 1.1	1268.9 +/- 21.9	-0.9
Cluster 5	-63.5 +/- 5.0	10	4.9 +/- 0.0	-33.9 +/- 4.9	-164.7 +/- 10.9	3.0 +/- 1.4	1184.6 +/- 49.9	-0.5
Cluster 7	-54.2 +/- 1.8	8	5.0 +/- 0.1	-31.7 +/- 3.1	-85.5 +/- 15.7	-6.4 +/- 1.7	1036.5 +/- 54.9	0.1
Cluster 9	-52.9 +/- 7.8	6	5.0 +/- 0.5	-27.8 +/- 5.6	-136.9 +/- 42.0	1.3 +/- 2.8	1012.6 +/- 71.0	0.2
Cluster 6	-46.6 +/- 1.9	10	4.9 +/- 0.1	-35.8 +/- 0.5	-61.8 +/- 12.9	1.3 +/- 1.1	955.6 +/- 58.4	0.6
Cluster 4	-40.2 +/- 10.0	10	5.0 +/- 0.1	-17.8 +/- 3.8	-122.8 +/- 38.1	2.0 +/- 2.6	836.9 +/- 103.2	1.1
Cluster 10	-39.0 +/- 3.6	5	4.6 +/- 0.0	-22.2 +/- 2.2	-87.5 +/- 29.6	-0.7 +/- 1.1	1011.5 +/- 57.4	1.2
Cluster 11	-39.0 +/- 6.1	4	5.0 +/- 0.1	-23.0 +/- 2.7	-74.7 +/- 22.8	-1.2 +/- 2.7	924.4 +/- 144.9	1.2