

# Antileishmanial activity and in silico molecular docking studies of *Malachra alceifolia* Jacq. Fractions against *Leishmania mexicana* amastigotes

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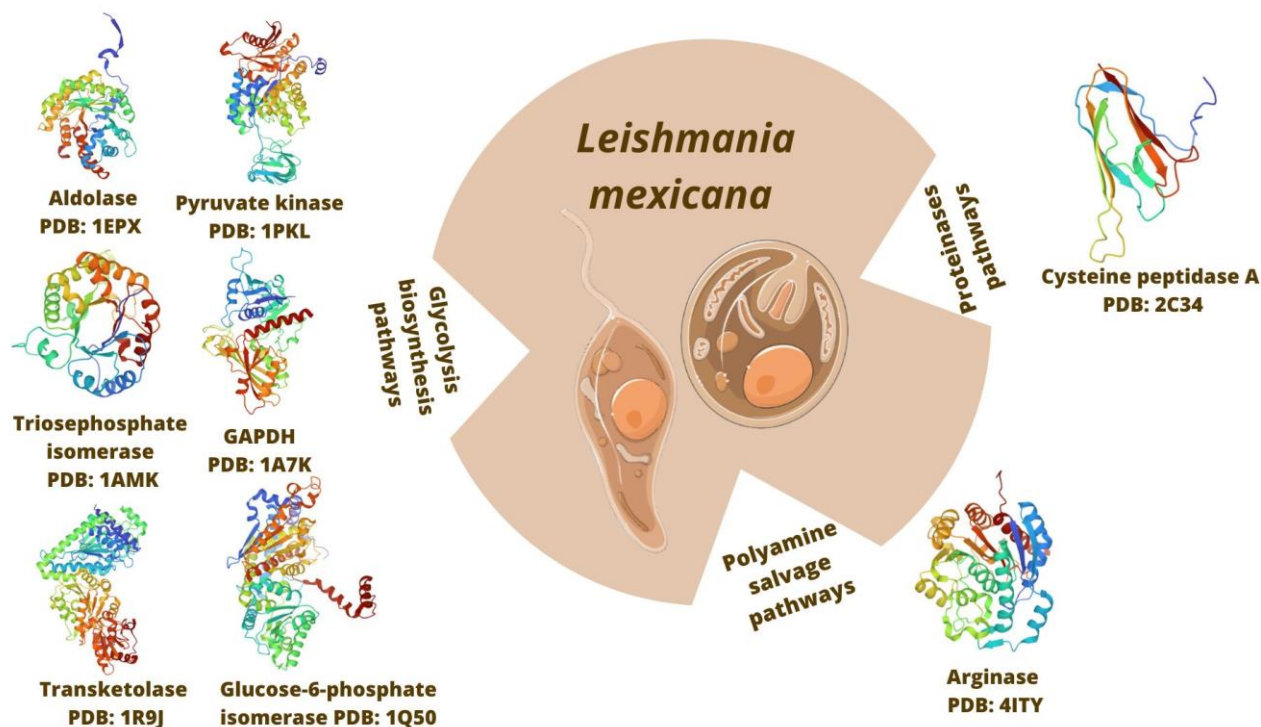
**Table S1.** Qualitative Phytochemical screening of *M. alceifolia* Jacq., extract and fractions

Extract/ fractions	Alc			Gc			Cu m	Flv		Tan		Ter/Est		Sap		Ant
	D	M	W	K	R	KK		Sh	Ci	Fe	Gs	LB	SW	Es	VS	BO
<b>MA-IEt</b>	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-
<b>MA-IH</b>	-	-	-	-	-	-	+	-	-	+	+	+	+	-	-	-
<b>MA-IC</b>	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-
<b>MA-IA</b>	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-
<b>MA-IM</b>	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-

Total ethanolic extract (MA-IEt); *n*-hexane fraction (MA-IH); chloroform fraction (MA-IC); ethyl acetate fraction (MA-IA) and Methanol fraction (MA-IM). Alc: Alkaloids, Gc: Cardiac Glycosides, Cum: Coumarins, Flv: Flavonoids, Tan: Tanins, Ter/est: Terpenoids/ Sterols, Sap: Saponins, Ant: Anthraquinones. D: Dragendorff, M: mayer, W: Wagner, B: Baljet, Fe: Ferric chloride, GS: Gelatin salt, K: Kedde, R: Raymond-Marthoud, KK: Keller-Kiliani, Sh: Shinoda, Ci: Citrobolic, Es: Foam test, LB: Liebermann-Buchard, SW: Salkowski, VS: Vanillin-sulfuric acid, BO: Bornträger. Presence (+); absence (-).

**Table S2.** Phytocomponents identified in the MA-24F fraction of leaves of *M. alceifolia* Jacq., by GC-MS

No.	RT (min)	Molecular Formula	[M] <sup>+</sup>	Compound	Type
1	14.297	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	238	Methyl 10,11-tetradecadienoate	Fatty acid derivative
2	15.418	C <sub>15</sub> H <sub>26</sub>	204	Santalane	sesquiterpenoids
3	19.946	C <sub>18</sub> H <sub>36</sub> O	268	2-Pentadecanone, 6,10,14-trimethyl	sesquiterpenoids
4	22.405	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	Methyl 9,10-octadecadienoate	Fatty acid derivative
5	22.559	C <sub>20</sub> H <sub>40</sub> O	296	Phytol	Diterpenoid
6	28.533	C <sub>29</sub> H <sub>50</sub> O <sub>4</sub>	462	α-Tocospiro A	Tocopherol
7	28.682	C <sub>29</sub> H <sub>50</sub> O <sub>4</sub>	462	α-Tocospiro B	Tocopherol
8	29.941	C <sub>28</sub> H <sub>48</sub> O <sub>2</sub>	416	γ-Tocopherol	Tocopherol
9	30.273	C <sub>31</sub> H <sub>52</sub> O <sub>2</sub>	456	β-Sitosterol acetate	Phytosterol
10	31.492	C <sub>29</sub> H <sub>48</sub> O	412	Stigmasterol	Phytosterol
11	32.001	C <sub>29</sub> H <sub>50</sub> O	414	(24R)-Stigmast-5-en-3-beta-ol	Phytosterol
12	32.390	C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>	456	Methyl commate A	Triterpene
13	32.842	C <sub>30</sub> H <sub>50</sub> O	426	Alpha Amyrin	Triterpene
14	33.317	C <sub>45</sub> H <sub>74</sub> O	631	Solanesol	Triterpene
15	33.620	C <sub>30</sub> H <sub>50</sub> O	426	epi-Swertenol	Triterpene



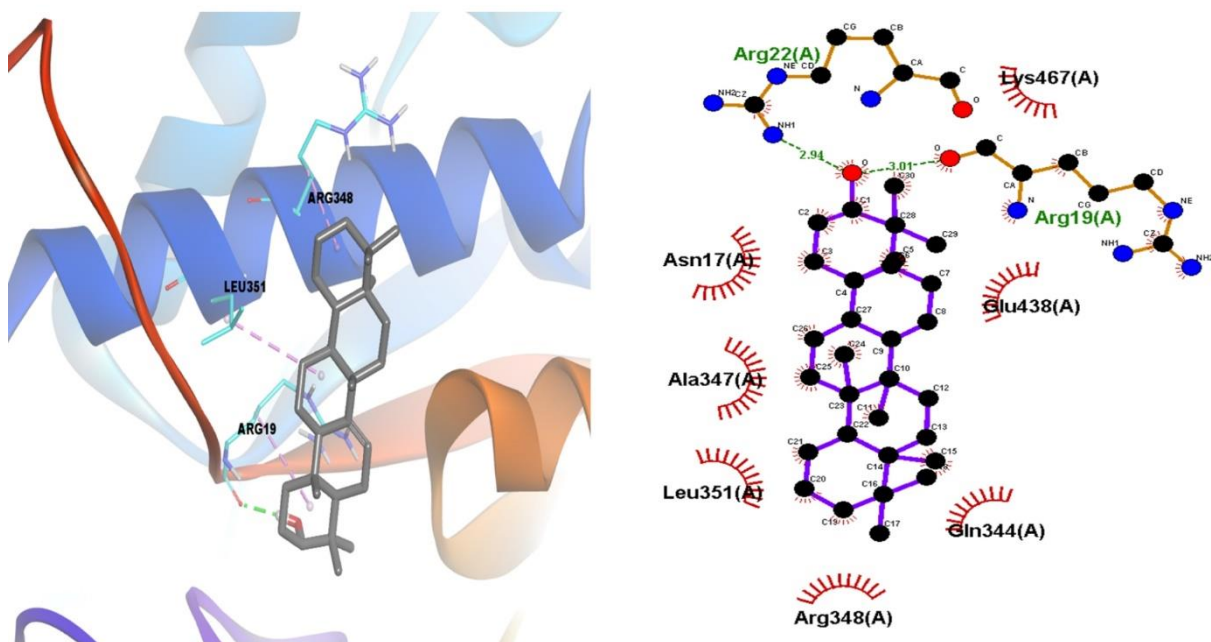
**Figure S1.** *Leishmania mexicana* protein targets. Protein Data Bank (PDB)

**Table S3.** Scoring Function estimation of binding affinity energy and molecular interactions of phytocomponents identified in the Ma-24F fraction of leaves of *M. alceifolia* by GC-MS upon docking in a potential allosteric sites of significant *L. mexicana* target proteins for AutoDock Vina program

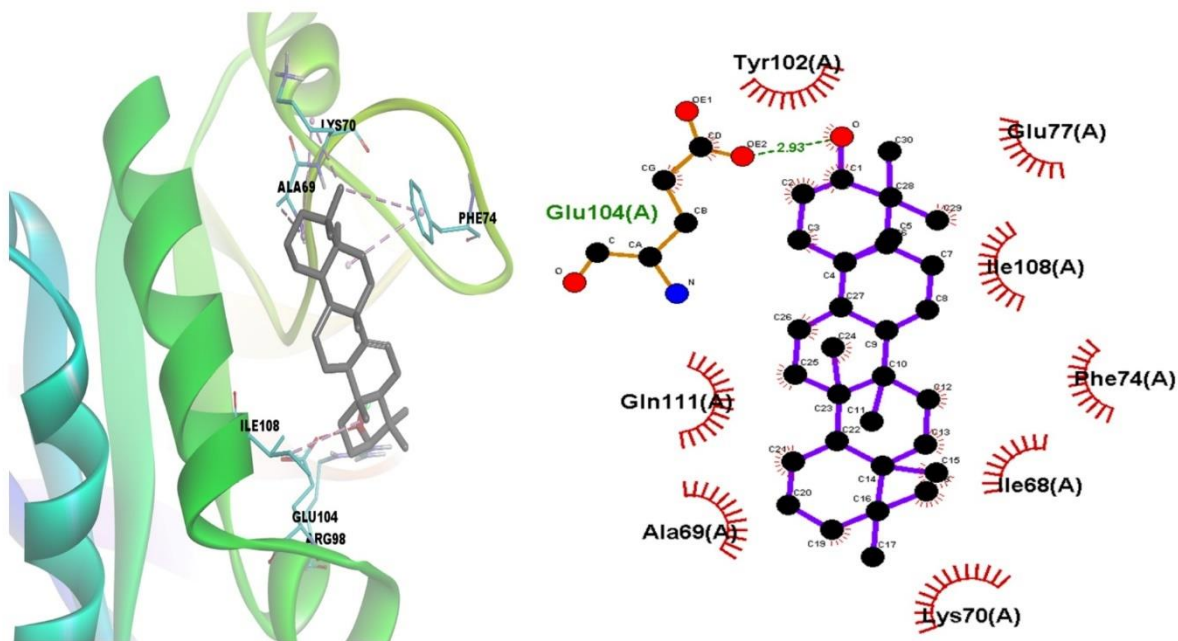
Compounds	Allosteric GScore (Binding energy kcal/mol <sup>-1</sup> ) *							
	1PKL	1A7K	1AMK	1EPX	1Q50	19RJ	4ITY	2C34
Amphotericin B	-9.1 ± 0.1	-9.3 ± 0.1	-6.9 ± 0.2	-8.6 ± 0.1	-9.0 ± 0.1	-9.6 ± 0.1	-7.4 ± 0.2	-7.6 ± 0.2
Miltefosine	-4.9 ± 0.2	-4.5 ± 0.2	-4.3 ± 0.2	-5.4 ± 0.1	-5.1 ± 0.1	-5.0 ± 0.1	-4.9 ± 0.2	-4.4 ± 0.2
Methyl 10,11-tetradecadienoate	-5.0 ± 0.1	-3.5 ± 0.1	-4.3 ± 0.2	-4.1 ± 0.1	-4.1 ± 0.1	-4.3 ± 0.2	-4.0 ± 0.2	-4.0 ± 0.1
Santalane	-6.9 ± 0.2	-5.5 ± 0.2	-6.0 ± 0.1	-5.5 ± 0.1	-5.9 ± 0.2	-5.5 ± 0.2	-5.4 ± 0.2	-5.3 ± 0.1
2-Pentadecanone, 6,10,14-trimethyl	-4.5 ± 0.1	-4.0 ± 0.1	-4.2 ± 0.1	-4.1 ± 0.1	-4.2 ± 0.1	-4.5 ± 0.2	-4.0 ± 0.1	-4.3 ± 0.2
Methyl 9,10-octadecadienoate	-4.3 ± 0.1	-4.1 ± 0.1	-3.6 ± 0.1	-4.0 ± 0.1	-3.7 ± 0.1	-3.2 ± 0.1	-4.1 ± 0.1	-3.4 ± 0.1
Phytol	-6.2 ± 0.1	-5.1 ± 0.1	-5.0 ± 0.1	-5.0 ± 0.1	-5.4 ± 0.1	-4.8 ± 0.1	-4.7 ± 0.1	-4.8 ± 0.1
α-Tocospiro A	-7.6 ± 0.2	-6.3 ± 0.2	-5.6 ± 0.1	-7.0 ± 0.1	-5.8 ± 0.1	-6.5 ± 0.1	-5.8 ± 0.1	-6.1 ± 0.2
α-Tocospiro B	-7.3 ± 0.1	-6.3 ± 0.1	-6.8 ± 0.1	-6.7 ± 0.1	-5.9 ± 0.1	-6.6 ± 0.1	-6.6 ± 0.1	-5.8 ± 0.1
γ-Tocopherol	-6.2 ± 0.1	-6.4 ± 0.1	-6.1 ± 0.2	-6.6 ± 0.2	-6.2 ± 0.2	-6.5 ± 0.1	-6.5 ± 0.2	-5.9 ± 0.1
β-Sitosterol acetate	-6.8 ± 0.1	-7.9 ± 0.1	-6.4 ± 0.1	-7.0 ± 0.1	-6.7 ± 0.1	-7.1 ± 0.1	-6.7 ± 0.1	-6.9 ± 0.1
Stigmasterol	-7.8 ± 0.1	-7.5 ± 0.1	-7.1 ± 0.1	-7.2 ± 0.1	-7.0 ± 0.1	-7.1 ± 0.1	-7.2 ± 0.1	-7.4 ± 0.1
(24R)-Stigmast-5-en-3-beta-ol	-7.9 ± 0.1	-7.5 ± 0.1	-7.2 ± 0.2	-7.6 ± 0.2	-7.8 ± 0.1	-7.0 ± 0.1	-7.5 ± 0.1	-6.9 ± 0.1
Methyl commate A	-8.3 ± 0.2	-7.9 ± 0.1	-7.4 ± 0.1	-8.3 ± 0.1	-7.8 ± 0.2	-8.6 ± 0.1	-7.5 ± 0.2	-8.1 ± 0.1 (-6.0) **
α- Amyrin	-9.9 ± 0.1 (-9.1) **	-8.6 ± 0.1	-7.9 ± 0.2	-8.6 ± 0.1	-7.5 ± 0.2	-8.2 ± 0.1	-8.5 ± 0.1	-7.8 ± 0.2
Solanesol	-5.7 ± 0.1	-6.0 ± 0.1	-5.3 ± 0.2	-5.9 ± 0.2	-5.7 ± 0.1	-5.7 ± 0.1	-5.2 ± 0.2	-5.1 ± 0.1
Episwertenol	-8.5 ± 0.1	-9.1 ± 0.1	-8.3 ± 0.2	-8.2 ± 0.2	-7.6 ± 0.1	-8.2 ± 0.1	-8.7 ± 0.1 (-7.3) **	-8.0 ± 0.1
<b>Glycolysis biosynthesis pathway proteins</b> Pyruvate kinase (PDB: 1PKL), Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) (PDB: 1A7K), triose phosphate isomerase (PDB: 1AMK), aldolase (PDB: 1EPX), phosphoglucose isomerase (PDB: 1Q50) transketolase (PDB: 1R9J)			<b>Polyamine salvage pathway protein</b> Arginase (PDB: 4ITY)			<b>Proteinase pathway protein</b> Cysteine peptidase A (PDB: 2C34)		

\*Each binding energy (kcal/mol) represents the mean ± standard error of the mean of six independent replicas kcal/mol<sup>-1</sup> n=6.

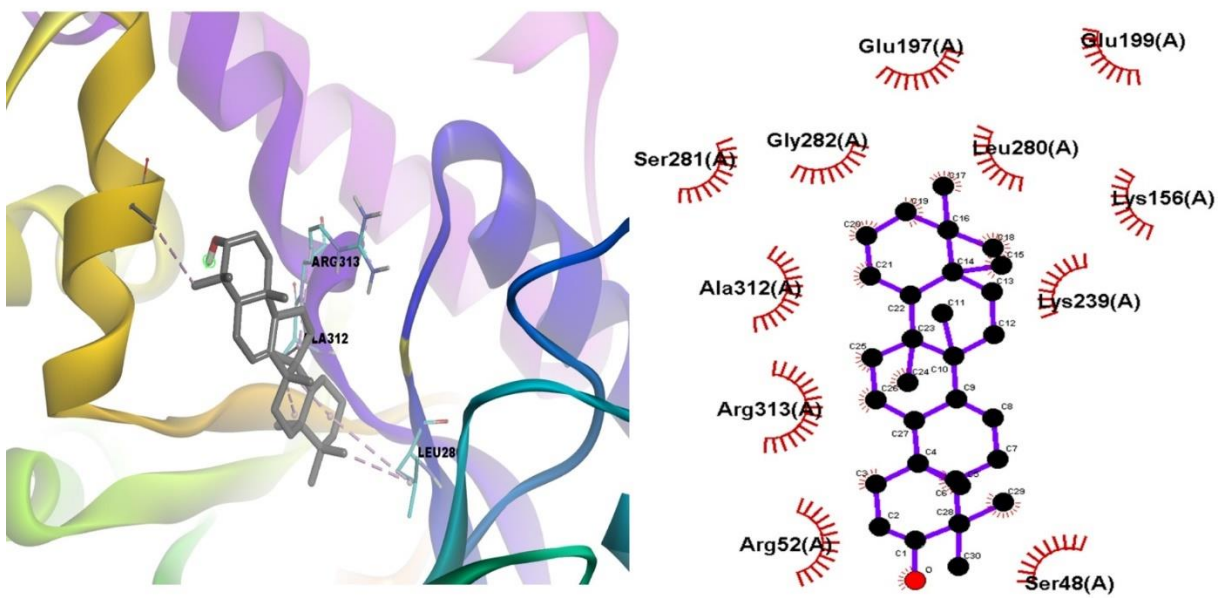
\*\* Validated values using the CB-Dock program.



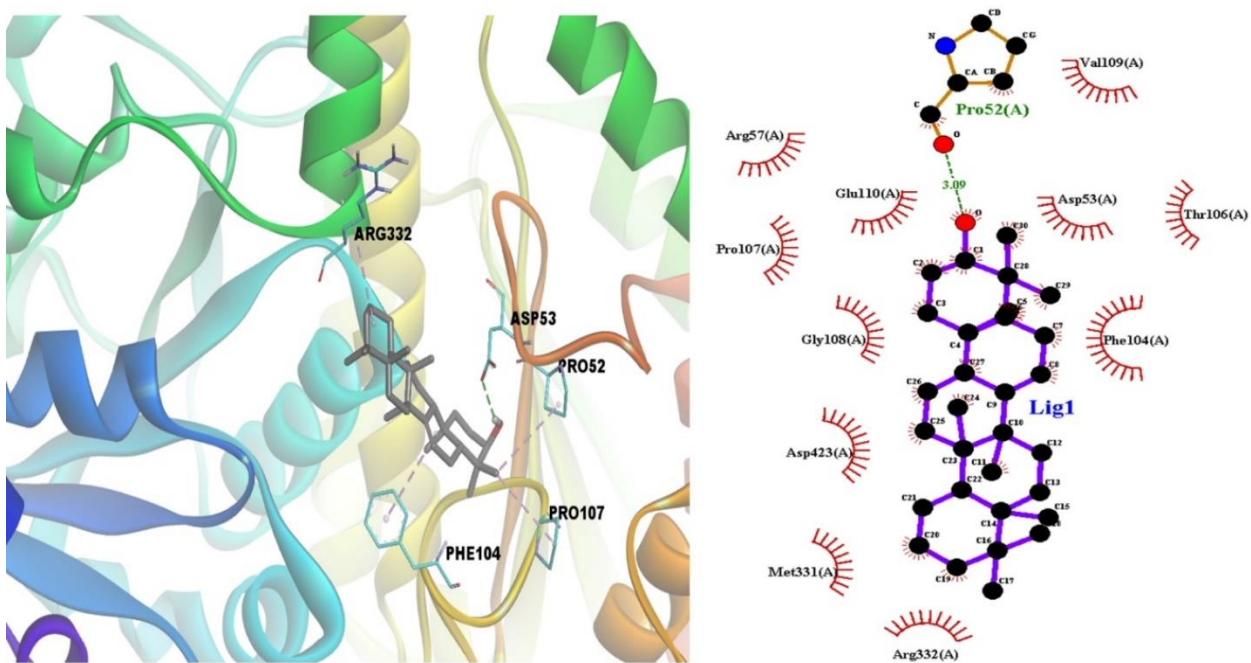
**Figure S2a.** Episwertenol compound interaction of allosteric binding sites of target proteins pyruvate kinase (PDB: 1PKL).



**Figure S2b.** Episwertenol compound interaction of allosteric binding sites of target triose phosphate isomerase (PDB: 1AMK).

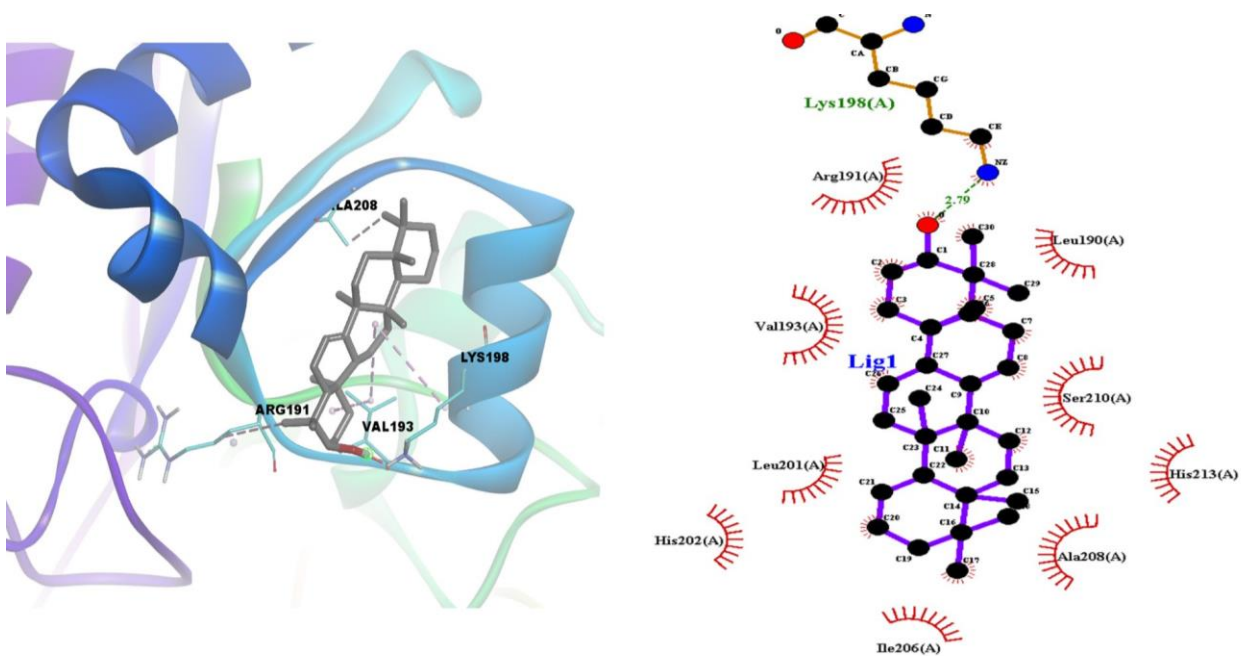


**Figure S2c.** Episwertenol compound interaction of allosteric binding sites of target aldolase (PDB: 1EPX).

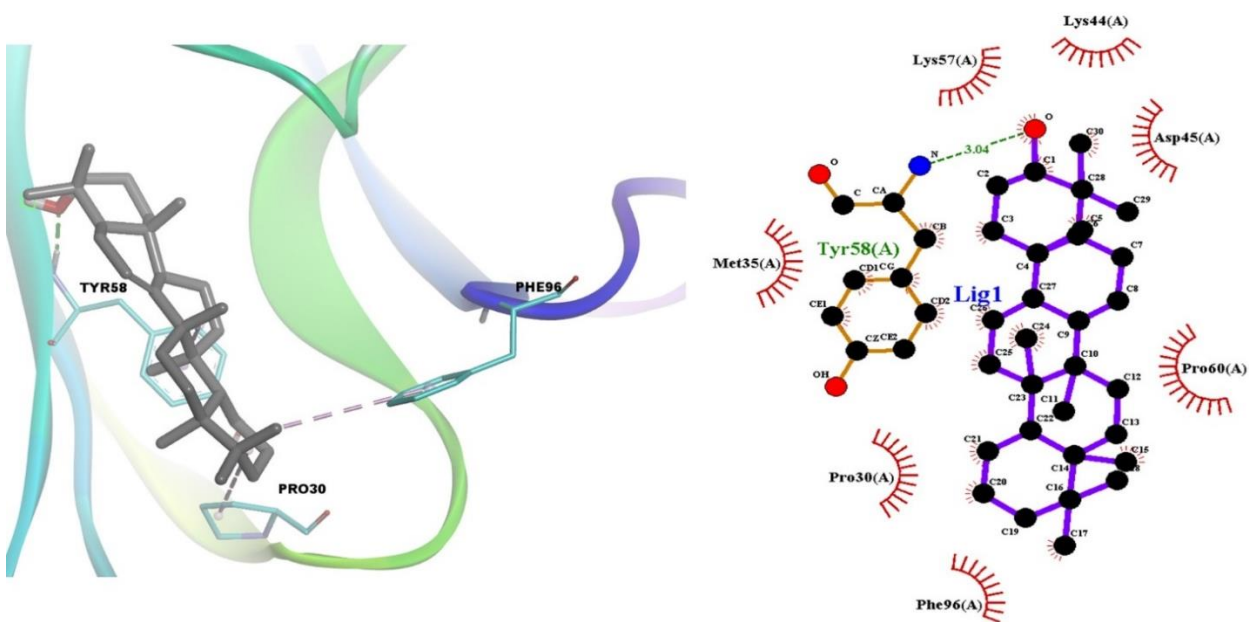


**Figure S2d.** Episwertenol compound interaction of allosteric binding sites of target transketolase (PDB: 1R9J).

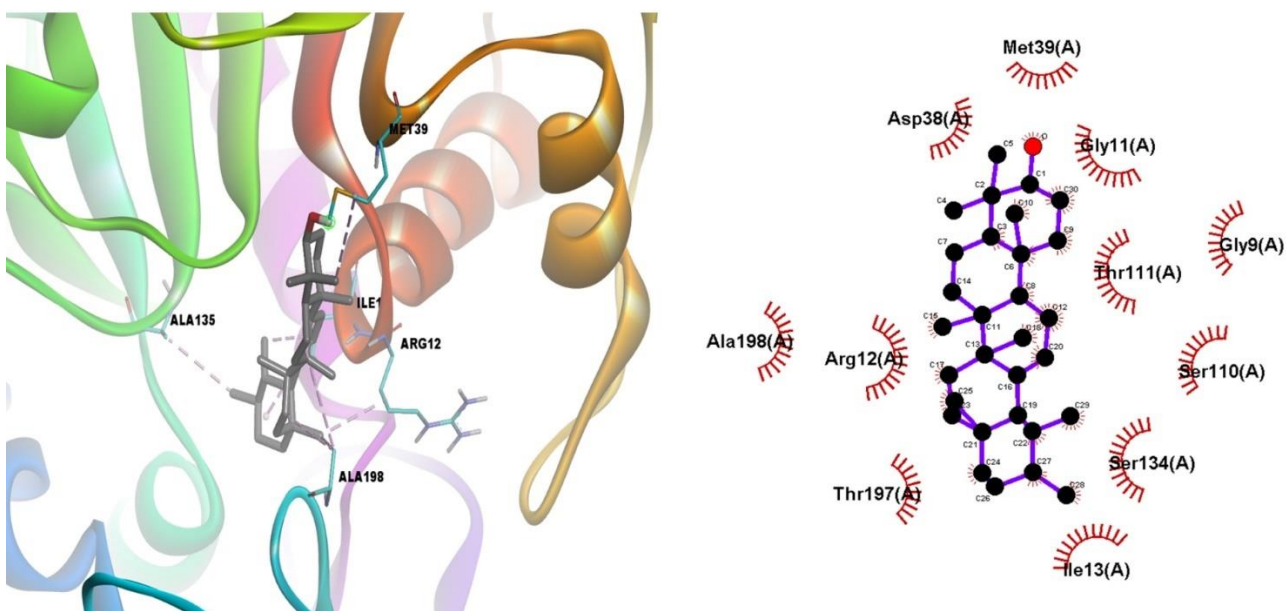




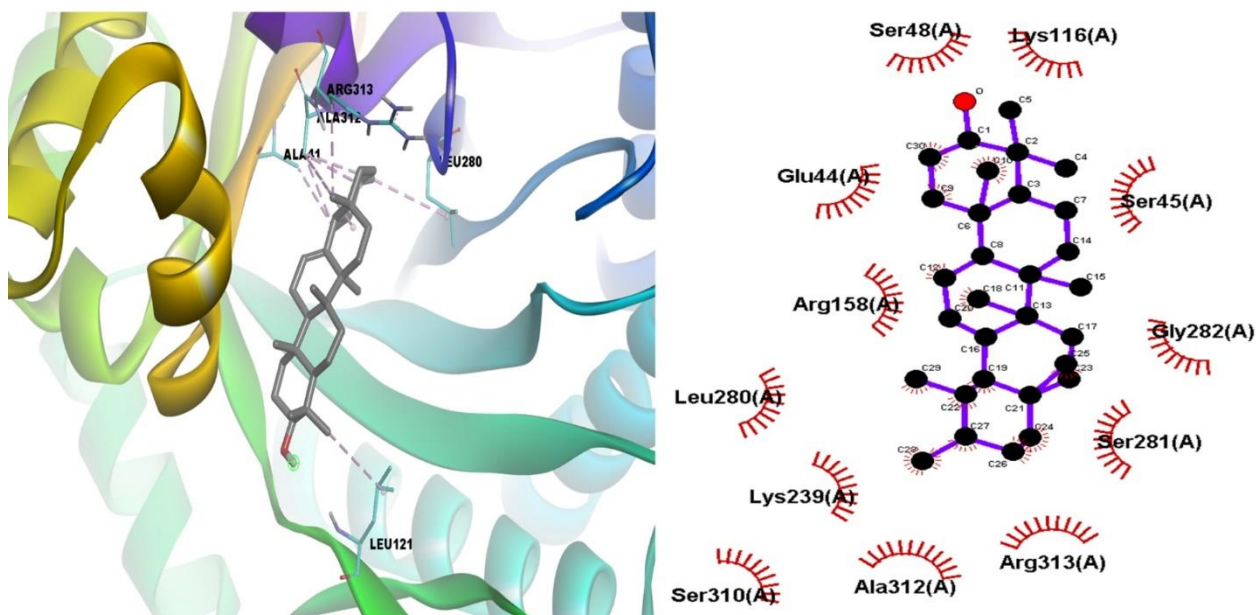
**Figure S2e.** Episwertenol compound interaction of allosteric binding sites of target arginase (PDB: 4ITY).



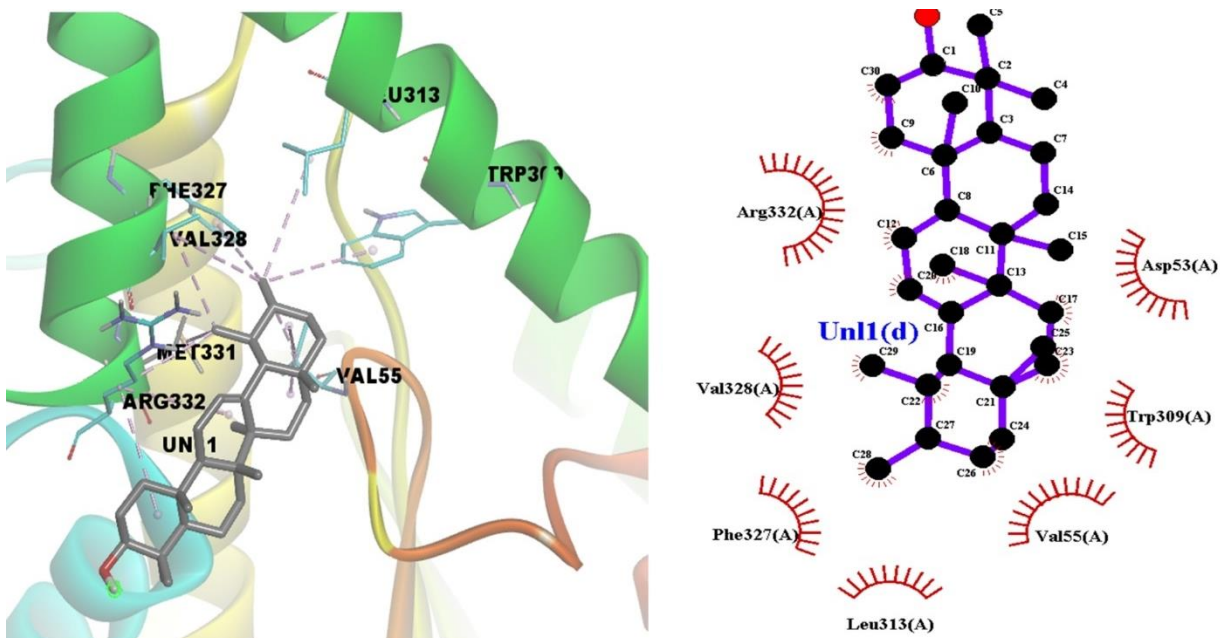
**Figure S2f.** Episwertenol compound interaction of allosteric binding sites of target Cysteine peptidases A (PDB: 2C34).



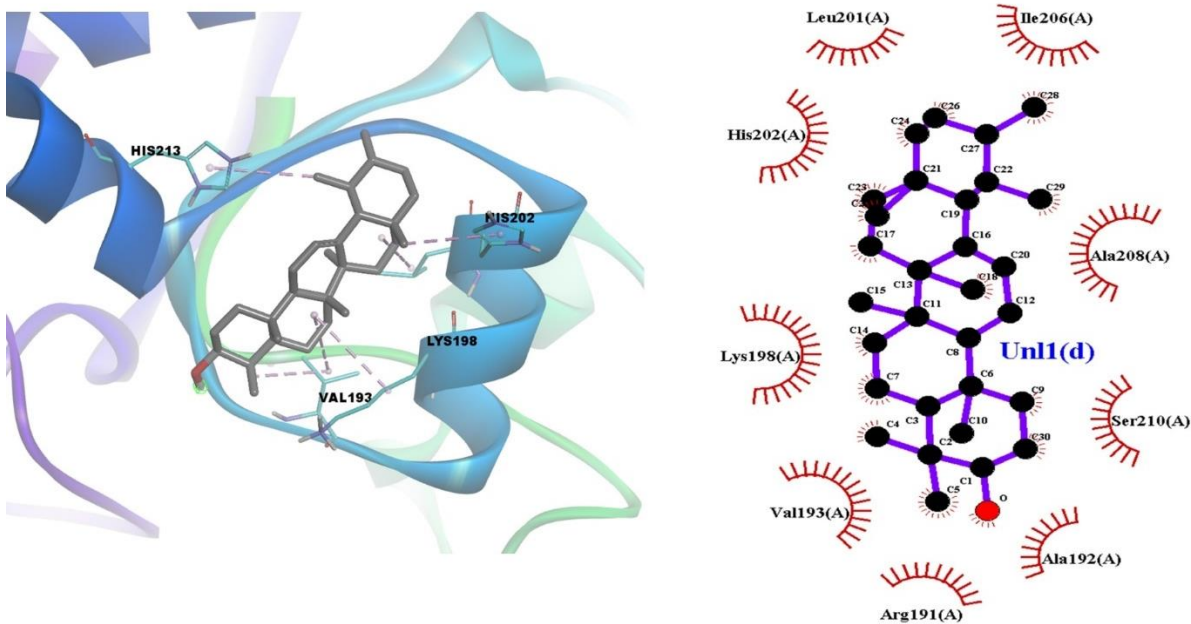
**Figure S3a.**  $\alpha$ -Amiryn compound interaction of allosteric binding sites of target GAPDH (PDB: 1A7K).



**Figure S3a.**  $\alpha$ -Amiryn compound interaction of allosteric binding sites of target aldolase (PDB:1EPX)

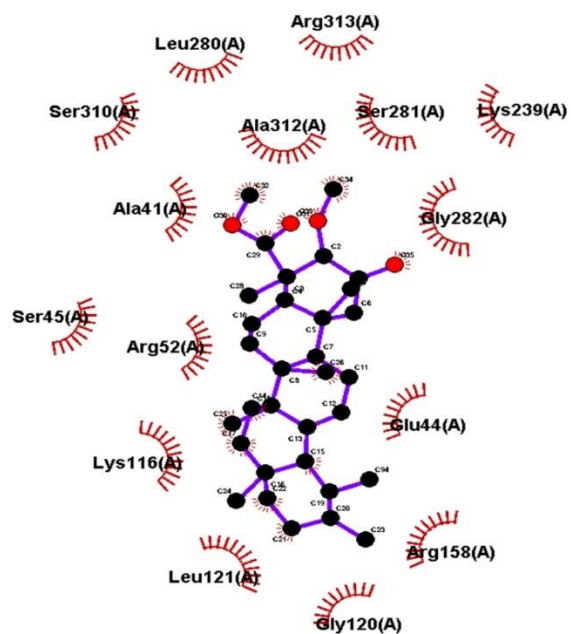
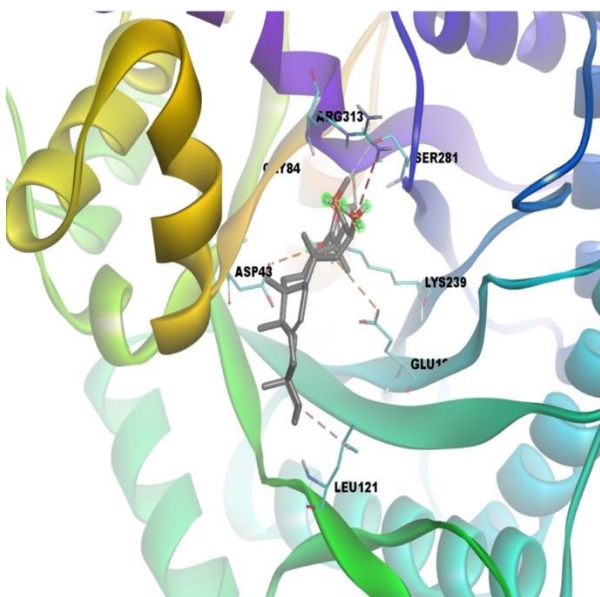


**Figure S3b.**  $\alpha$ -Amiryn compound interaction of allosteric binding sites of target transketolase (PDB: 1R9J).

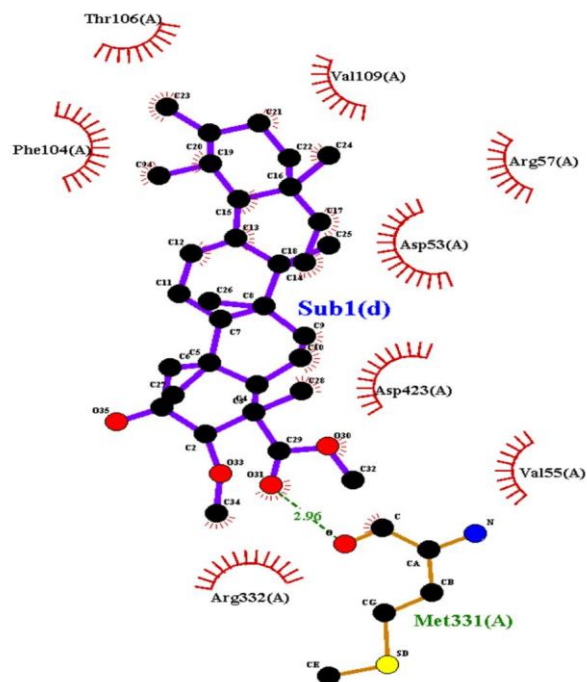
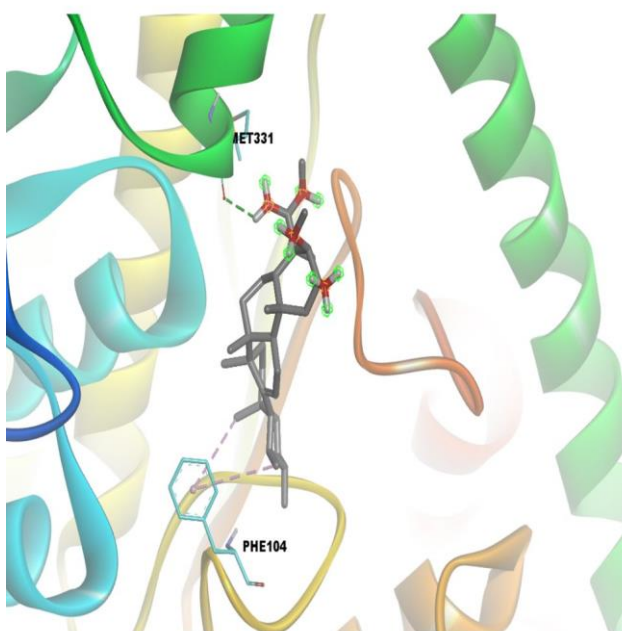


**Figure S3c.**  $\alpha$ -Amiryn compound interaction of allosteric binding sites of target arginase (PDB: 4ITY).

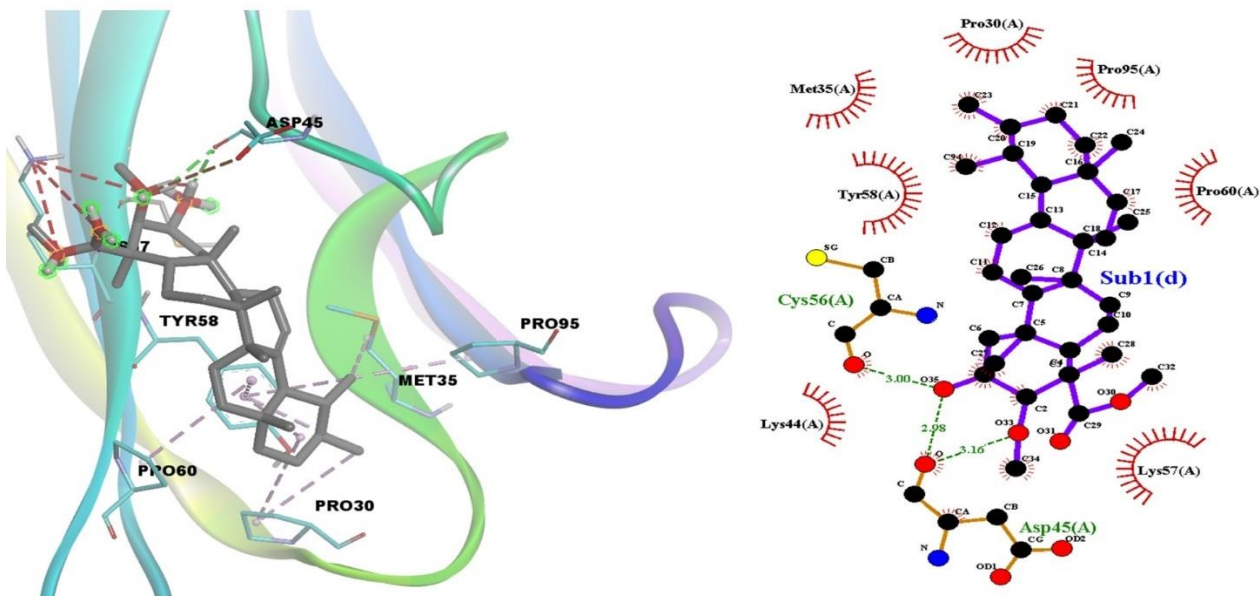




**Figure S4a.** Methyl commate A compound interaction of allosteric binding sites of target aldolase (PDB: 1EPX).



**Figure S4b.** Methyl commate A compound interaction of allosteric binding sites of target transketolase (PDB: 1R9J).



**Figure S4c.** Methyl commate A compound interaction of allosteric binding sites of target cysteine peptidases A (PDB: 2C34).

**Table S4.** Identification of amino acid residues in the allosteric site of proteins in *L. mexicana* selected for docking

Pathway	Proteins <i>L. mexicana</i>	Amino acid allosteric site residues	Size and shape descriptors	Surface descriptors
	GAPDH (PDB: 1A7K),	Arg12, Ile13, Gly11, Gly9, Phe10, Asp38, Met39, Thr111, Gly112, Leu113, Ser110, Ala135, Thr199, Ala198, Cys166, Thr197	Volume [Å <sup>3</sup> ]: 1166.66 Surface [Å <sup>2</sup> ]: 1497.66 Depth [Å]: 29.76	Apolar amino acid ratio 0.37 Polar amino acid ratio 0.47 Positive amino acid ratio 0.10 Negative amino acid ratio 0.07
Glycolysis biosynthesis pathway	Pyruvate kinase (PDB: 1PKL),	Gln40, Gly44, Ile41, Ser46, Leu74, Val76, Ile78, Asn77, Leu351, Cys428, Asn432, Phe463, Ser439, Glu438, Lys467	Volume [Å <sup>3</sup> ]: 1145.82 Surface [Å <sup>2</sup> ]: 1320.34 Depth [Å]: 20.04	Apolar amino acid ratio 0.44 Polar amino acid ratio 0.37 Positive amino acid ratio 0.11 Negative amino acid ratio 0.07
	Triose phosphate isomerase (PDB: 1AMK)	Ala69, Lys70, Ile68, Phe74, Glu77, Arg98, Tyr102, Lys112, Ile108, Glu104, Gln111, Glu115	Volume [Å <sup>3</sup> ]: 951.62 Surface [Å <sup>2</sup> ]: 1297.45 Depth [Å]: 24.92	Apolar amino acid ratio 0.37 Polar amino acid ratio 0.43 Positive amino acid ratio 0.09 Negative amino acid ratio 0.11
	Aldolase (PDB: 1EPX)	Ala41, Arg52, Glu44, Ser48, Ser45, Lys116, Gly120, Leu121, Lys156, Arg158, Leu280, Lys239, Ser281, Ser286, Gly282, Arg313, Ser310, Ala312, Tyr311	Volume [Å <sup>3</sup> ]: 1039.17 Surface [Å <sup>2</sup> ]: 1250.41 Depth [Å]: 25.51	Apolar amino acid ratio 0.46 Polar amino acid ratio 0.24 Positive amino acid ratio 0.17 Negative amino acid ratio 0.13
	Transketolase (PDB: 1R9J)	Pro52, Trp54, Asp53, Leu33, Pro52, Arg57, Arg103, Phe104, Thr106, Pro107, Gly108, Glu110, Met331, Arg332, Phe327, Trp309, Val55, Val328, Arg332, Asp423	Volume [Å <sup>3</sup> ]: 387.13 Surface [Å <sup>2</sup> ]: 316.33 Depth [Å]: 16.06	Apolar amino acid ratio 0.28 Polar amino acid ratio 0.50 Positive amino acid ratio 0.14 Negative amino acid ratio 0.06

Polyamine salvage pathway	Arginase (PDB: 4ITY)	Leu190, Arg19, Ala192, Val193, Lys198, Leu201, His202, Asn205, Ile206, Ala207, Ala208, Ser210, His212, His213, Val331, Arg332, Trp369	Volume [Å <sup>3</sup> ]: 548.16 Surface [Å <sup>2</sup> ]: 887.27 Depth [Å]: 16.94	Apolar amino acid ratio 0.58 Polar amino acid ratio 0.18 Positive amino acid ratio 0.12 Negative amino acid ratio 0.12
Proteinase pathway	Cysteine peptidase A (PDB: 2C34)	Thr31, Pro40, Met35, Gly43, Lys44, Asp45, Tyr58, Pro60, Val70, Phe96	Volume [Å <sup>3</sup> ]: 489.86 Surface [Å <sup>2</sup> ]: 1065.77 Depth [Å]: 14.07	Apolar amino acid ratio 0.40 Polar amino acid ratio 0.25 Positive amino acid ratio 0.15 Negative amino acid ratio 0.20

Count. Table S4.

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