

## **Supplementary Material**

### **Naphtoquinone as a new chemical scaffold for leishmanicidal inhibitors of *Leishmania GSK-3***

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<sup>&</sup>Both authors contribute equally to this work

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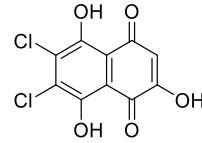
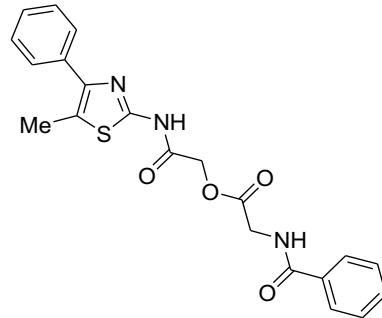
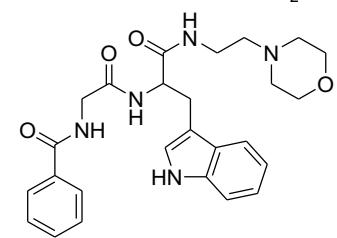
**Page S2:** Sequence alignment of the GSK-3 structures of *Leishmania major* (Q4QE15) and *Homo sapiens* (P49841).

**Page S3:** Chemical structures of the 24 compounds from the MBC library selected by virtual screening and their biological evaluation.

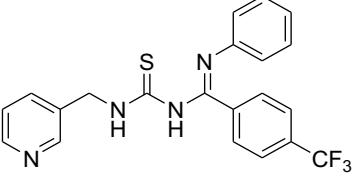
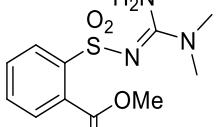
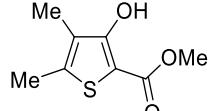
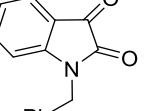
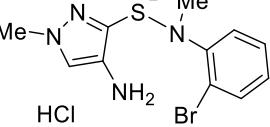
GSK3B_HUMAN	MSGRPRTTSFAESCKPVQQPSAFGSMKVS RDKDGSKVTTVVATPGQGPDRPQEVS	<span style="color: blue;">Y</span>	TDTK	60								
Q4QE15_ LEIMA	-----	-----	MSLNAAAADERSRKEMDR	<span style="color: red;">F</span> QVER	24							
		.	.	*	.	*	:	:	:			
GSK3B_HUMAN	VIGNGS	<span style="color: green;">FGVVY</span>	QAKLCDSG	<span style="color: red;">ELVAI</span>	<span style="color: blue;">KKVL</span>	<span style="color: green;">QDKRFKNRE</span>	LQIMRKL	---	DHCNIVRLRYFFY	117		
Q4QE15_ LEIMA	MAGQGT	<span style="color: green;">FGTVQ</span>	LGKEKSTG	<span style="color: red;">MSVAI</span>	<span style="color: blue;">KKVI</span>	<span style="color: green;">QDPRFRNR</span>	ELQIMQDLAVLHHPNIVQLQSYFY			84		
	.	*	:	*	.	*	*****:	**	**:	*****:	*	
GSK3B_HUMAN	SSGEK-KDEVYLNLV	<span style="color: red;">LDYV</span>	PETVYRVARHYSRAKQTLPV	IYVKLYMYQLFRSLAYI	<span style="color: blue;">H</span>	--S				174		
Q4QE15_ LEIMA	TLGERDRDIYLNVV	<span style="color: green;">MEYV</span>	PDTLHRCRNYRRQVAPP	PILIKVFLFQLIRSIGCL	<span style="color: blue;">HLPS</span>					144		
	.	*	:	:	*	:	*	*	:	**:	*	
GSK3B_HUMAN	FGICH	<span style="color: red;">RDIKPQN</span>	LLLDPDTA	<span style="color: blue;">VL</span>	<span style="color: blue;">KLCD</span>	<span style="color: green;">FGSA</span>	<span style="color: green;">QLVRGE</span>	PNSY	<span style="color: blue;">ICSRYY</span>	RAPELIFGAT	<span style="color: red;">DY</span>	234
Q4QE15_ LEIMA	VNVCH	<span style="color: red;">RDIKPHNV</span>	LVNEADG	<span style="color: blue;">TL</span>	<span style="color: blue;">KLCD</span>	<span style="color: green;">FGSA</span>	<span style="color: green;">KKLSP</span>	SEPNV	<span style="color: blue;">AYICSRYY</span>	RAPELIFGN	<span style="color: red;">QHY</span>	204
	.	**:	**:	**:	**:	**:	**:	**:	**:	*****:	*****:	*
GSK3B_HUMAN	TSSIDVWSAGCVLAELL	LLGQP	IFPGDGSVDQLVE	IIKVLGTP	REQIREMNP	NYTEFKFP						294
Q4QE15_ LEIMA	TTAVDIWSVGCI	FAEMMLGE	PIFRGDNSAGQLHE	IVRVLGCP	SREVLRKL	NPSHTDV	DLY					264
	.	**:	**:	**:	**:	**:	**:	**:	**:	**:	**:	:
GSK3B_HUMAN	QIKAHPWT	KVFRPRT	---	PPEAIALCSR	LLEYPTPTARLT	<span style="color: purple;">PLEACAH</span>	SFFDEL	RDPNV	KLP		351	
Q4QE15_ LEIMA	NGRDTPA	-LFNF	TTQELSSNPLATI	LIPPHARI	QAAASTPTNATAAS	DANTGDRG	QTNN				410	
	.	*	*	*	*	*	*	*	*	*	*	355
GSK3B_HUMAN	-----	AASASASN	T420									
Q4QE15_ LEIMA	-----	-----	355									

**Figure S1.** Sequence alignment of the GSK-3 structures of *Leishmania major* (Q4QE15) and *Homo sapiens* (P49841). Some of the most important residues of the ATP binding site (Pocket 0) and substrate binding site are depicted in blue and green respectively; in red (Pocket 1), light blue (Pocket 4) and purple (Pocket 6 and 9).

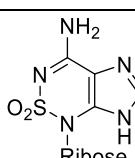
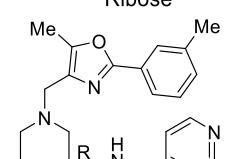
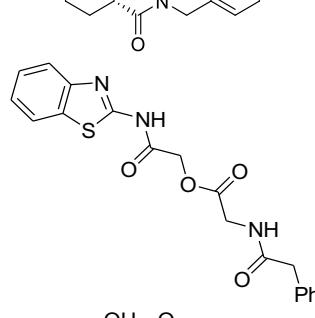
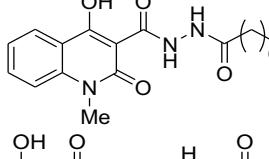
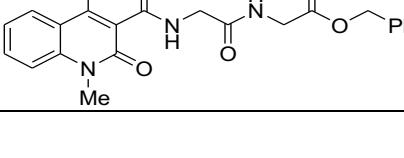
**Table S1.** Enzymatic and biological characterization of the 24 compounds from the MBC library selected by virtual screening.

Compound	Chemical formula	LdGSK-3s IC <sub>50</sub> (μM)	<i>L. infantum</i> promastigotes IC <sub>50</sub> (μM)	<i>L. pifanoi</i> axenic amastigotes IC <sub>50</sub> (μM)	Murine peritoneal macrophages IC <sub>50</sub> (μM)	Selectivity index (SI)
MBC-10		~10*	10.5 ± 1.2	11.2 ± 2.5	ND	ND
MBC-49		>10	>50	>50	-	-
MBC-61		>10	>50	>50	-	-
MBC-85		>10	>50	>50	-	-

\* MBC-10 at 10 μM causes 45.7 ± 7.6 % inhibition of LdGSK-3s activity.

Compound	Chemical formula	LdGSK-3s IC <sub>50</sub> (μM)	<i>L. infantum</i> promastigotes IC <sub>50</sub> (μM)	<i>L. pifanoi</i> axenic amastigotes IC <sub>50</sub> (μM)	Murine peritoneal macrophages IC <sub>50</sub> (μM)	Selectivity index (SI)
MBC-11		>10	>25	>25	-	-
MBC-188		>10	>50	>50	-	-
MBC-189		>10	>50	>50	-	-
MBC-190		>10	>50	>50	-	-
MBC-191		>10	>50	>50	-	-

Compound	Chemical formula	LdGSK-3s IC <sub>50</sub> (μM)	<i>L. infantum</i> promastigotes IC <sub>50</sub> (μM)	<i>L. pifanoi</i> axenic amastigotes IC <sub>50</sub> (μM)	Murine peritoneal macrophages IC <sub>50</sub> (μM)	Selectivity index (SI)
<b>MBC-192</b>		>10	>50	>50	-	-
<b>MBC-193</b>		>10	>50	>50	-	-
<b>MBC-194</b>		>10	>50	>50	-	-
<b>MBC-195</b>		>10	>50	>50	-	-
<b>MBC-196</b>		>10	>25	>50	-	-

Compound	Chemical formula	LdGSK-3s IC <sub>50</sub> (μM)	<i>L. infantum</i> promastigotes IC <sub>50</sub> (μM)	<i>L. pifanoi</i> axenic amastigotes IC <sub>50</sub> (μM)	Murine peritoneal macrophages IC <sub>50</sub> (μM)	Selectivity index (SI)
<b>MBC-197</b>		>10	>50	>50	-	-
<b>MBC-198</b>		>10	>50	>50	-	-
<b>MBC-199</b>		>10	>50	>50	-	-
<b>MBC-200</b>		>10	>50	20.8 ± 0.0	>25	>1.2
<b>MBC-201</b>		>10	>50	>50	-	-

Compound	Chemical formula	LdGSK-3s IC <sub>50</sub> (μM)	<i>L. infantum</i> promastigotes IC <sub>50</sub> (μM)	<i>L. pifanoi</i> axenic amastigotes IC <sub>50</sub> (μM)	Murine peritoneal macrophages IC <sub>50</sub> (μM)	Selectivity index (SI)
<b>MBC-202</b>		>10	>50	>50	-	-
<b>MBC-203</b>		>10	>50	>50	-	-
<b>MBC-204</b>		>10	>50	>50	-	-
<b>MBC-205</b>		>10	>50	>50	-	-
<b>MBC-206</b>		>10	>50	>50	-	-