Electronic Supplementary Material (ESI) for Food & Function.

Supplementary Information

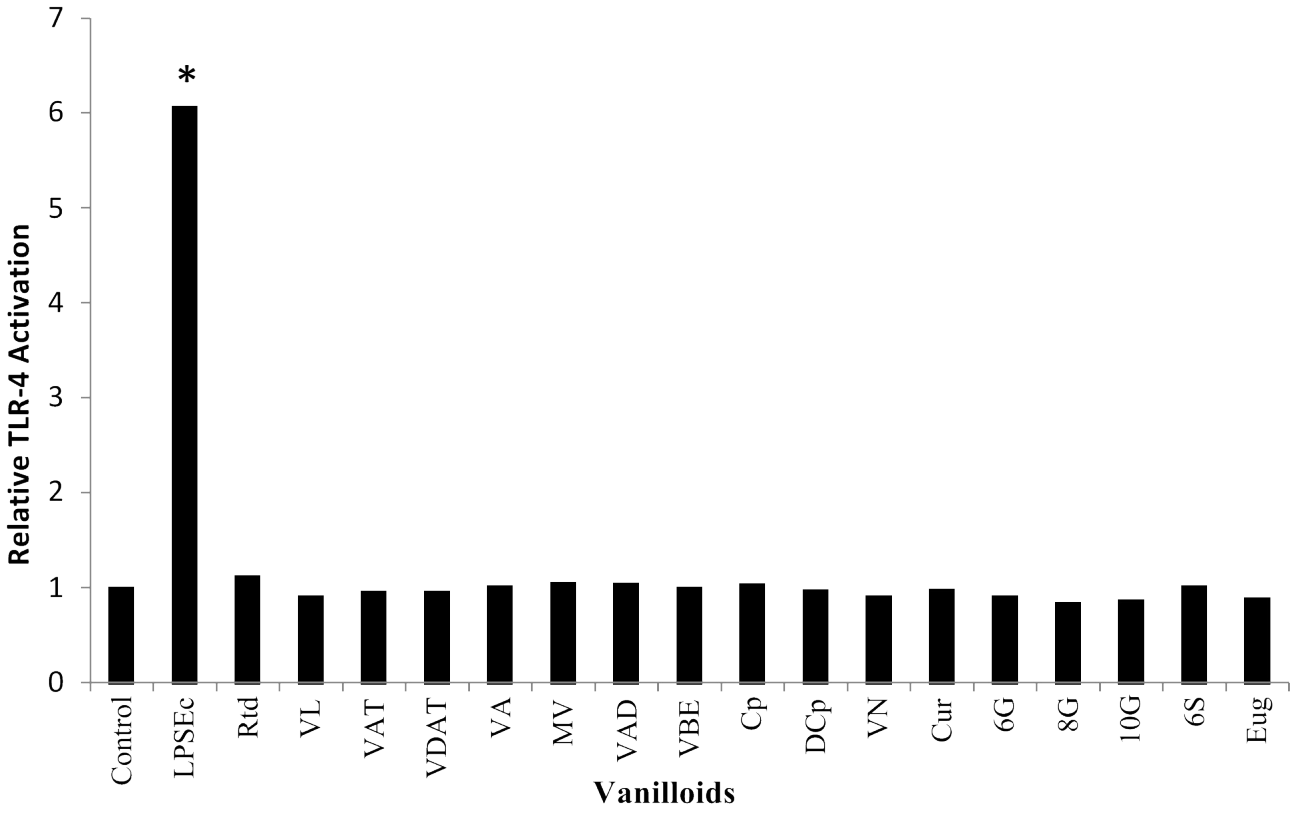


Figure 1: Vanilloids Do Not Induce TLR-4 Activation. All test compounds were tested at 100 µM except LPS (*Escherichia coli*) (LPSEc) which was 1 µg/mL. Results are expressed as mean percentage of TLR-4 activation ± SD of three independent experiments. Statistical significant different from the control, HEK-Blue^{TM}hTLR4 cells treated with 1% DMSO, are indicated as *p<0.05 by one-way ANOVA *post hoc* Dunnet's *t*-test.

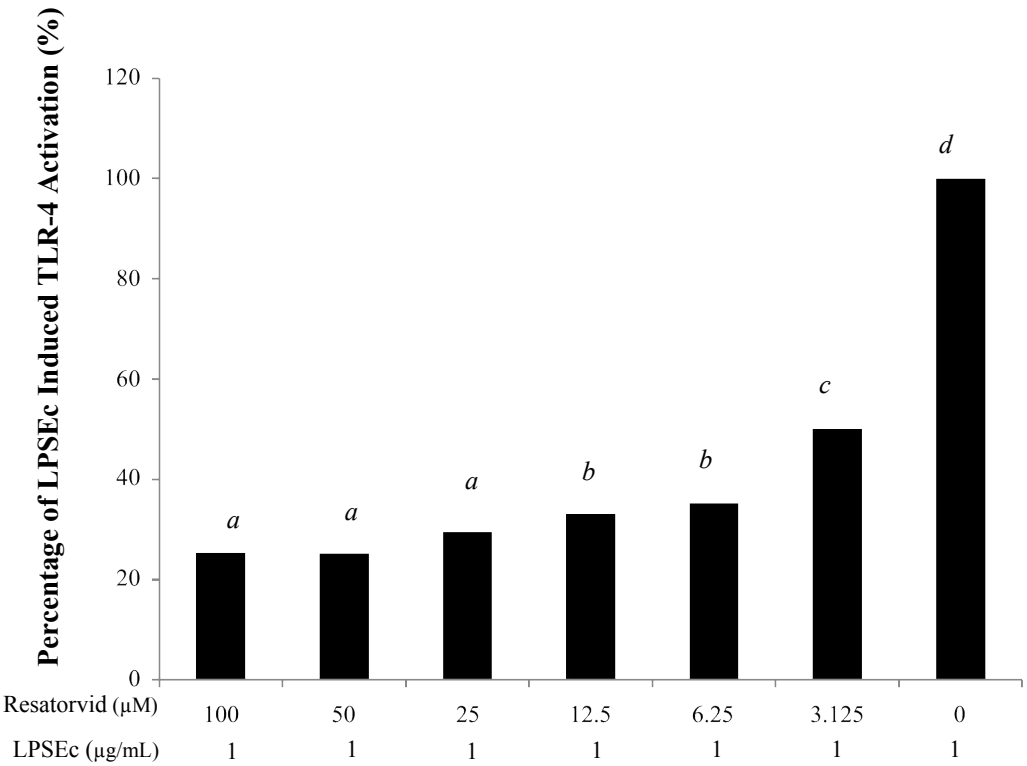


Figure 2: Resatorvid Inhibits LPSEc Induced TLR-4 Activation. Resatorvid was added at respective concentrations for 1 hour and followed up by 1 μg/mL (LPSEc) for 24 hours. Results are expressed as percentage of LPSEc induced TLR-4 activation ± SD as compared to cells with only LPSEc of at least three independent experiments. Bars with the different descriptors (*a, b, c, d*) are statistically significant ($p<0.05$) different by one-way ANOVA *post hoc* LSD.

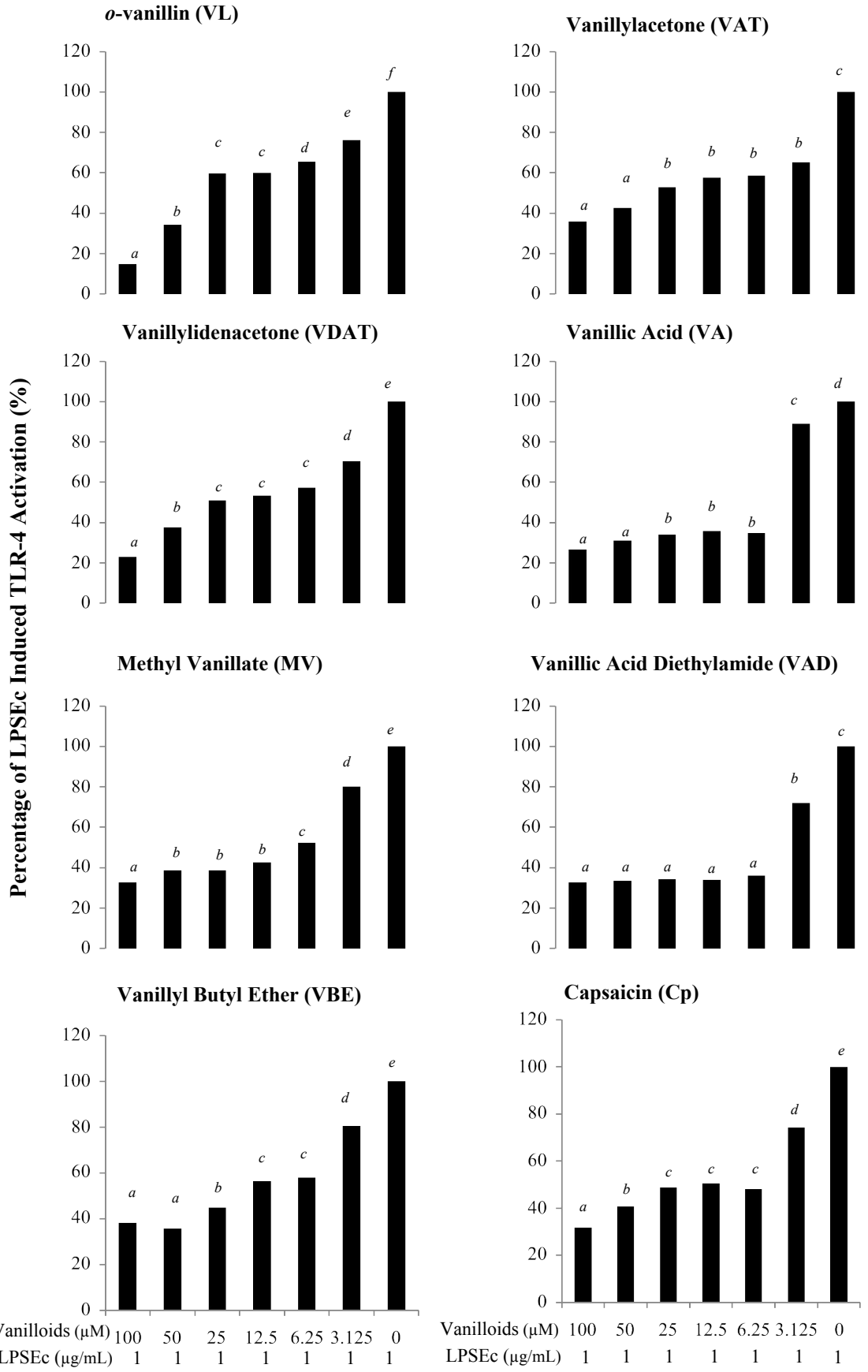


Figure 3: Vanilloids Inhibit LPSEc Induced TLR-4 Activation. All vanilloids were added at respective concentrations for 1 hour and followed up by 1 µg/mL (LPSEc) for 24 hours. Results are expressed as percentage of LPSEc induced TLR-4 activation ± SD as compared to cells with only LPSEc of at least three independent experiments. Bars with the different descriptors (*a, b, c, d, e, f, g, h*) are statistically significant (p<0.05) different by one-way ANOVA *post hoc* LSD.

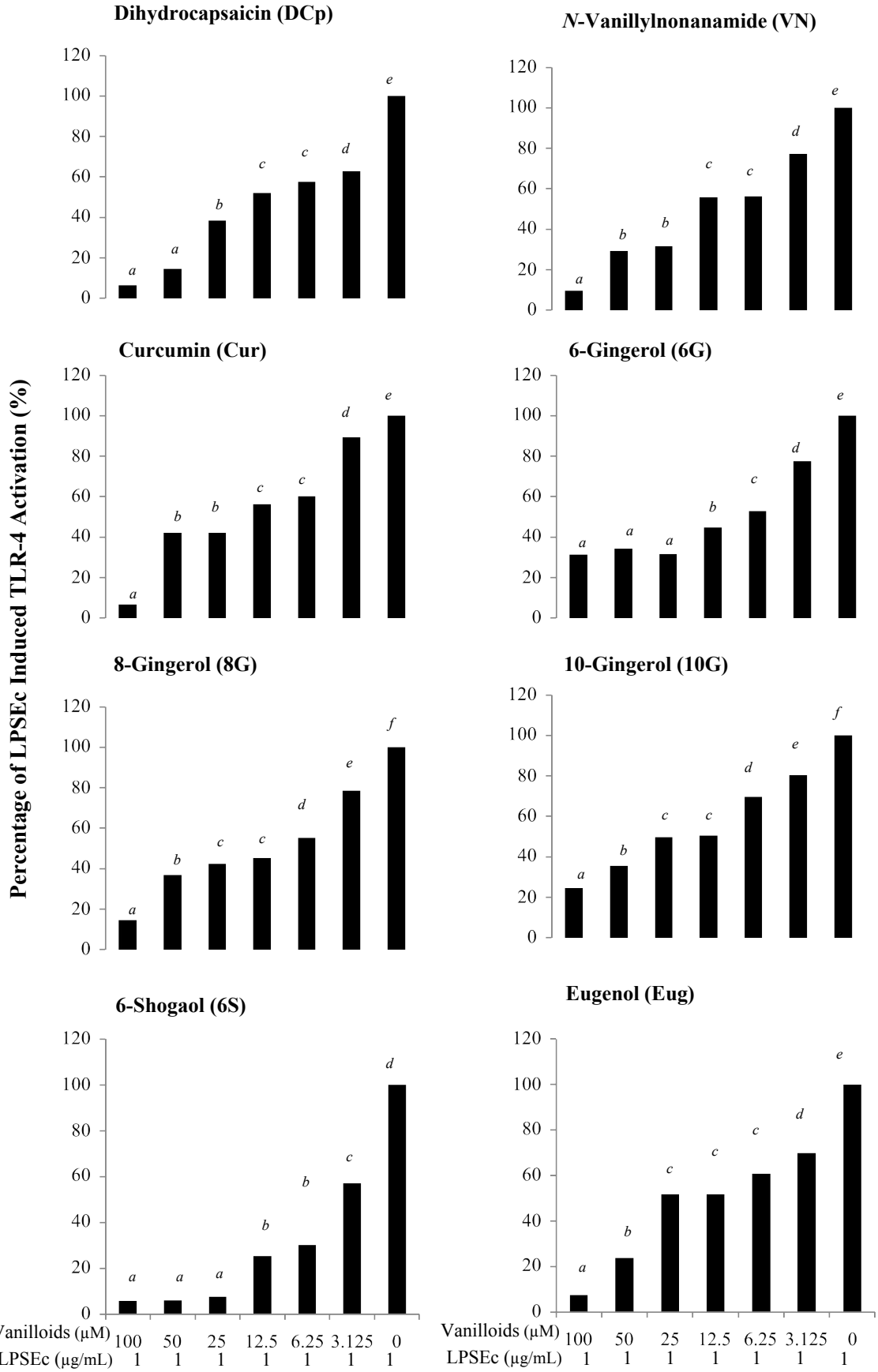


Figure 3 *(continued)*: Vanilloids Inhibit LPSEc Induced TLR-4 Activation. All vanilloids were added at respective concentrations for 1 hour and followed up by 1 μg/mL (LPSEc) for 24 hours. Results are expressed as percentage of LPSEc induced TLR-4 activation ± SD as compared to cells with only LPSEc of at least three independent experiments. Bars with the different descriptors (*a, b, c, d, e, f, g, h*) are statistically significant ($p<0.05$) different by one-way ANOVA *post hoc* LSD.

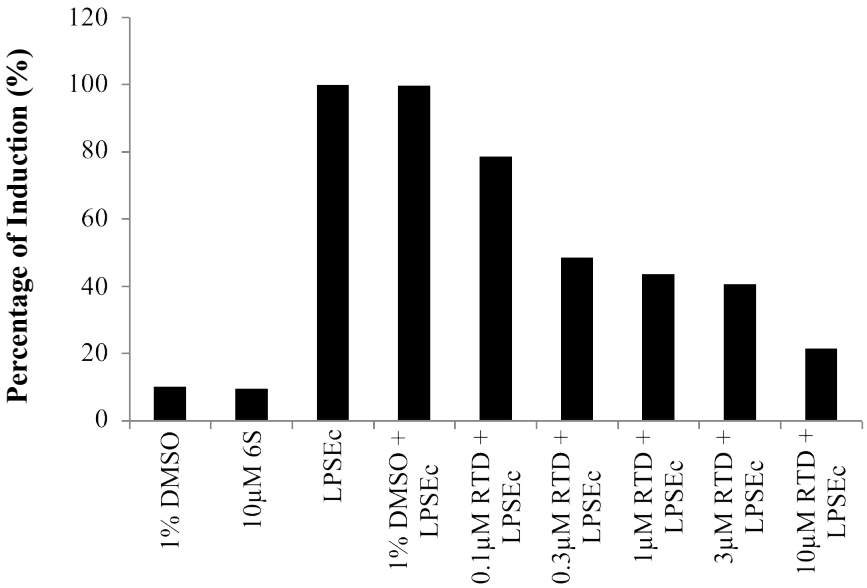


Figure 4: Resatorvid Inhibits LPSEc Induced Nitric Oxide Production. Resatorvid was added at respective concentrations for 1 hour and followed up by 1 µg/mL (LPSEc) for 18 hours. Results are expressed as percentage of LPSEc induced nitric oxide production ± SD as compared to cells with only LPSEc of at least three independent experiments. Bars with the symbo (*) are statistically significant ($p<0.05$) different by one-way ANOVA *post hoc* Dunnett's *t*-test.

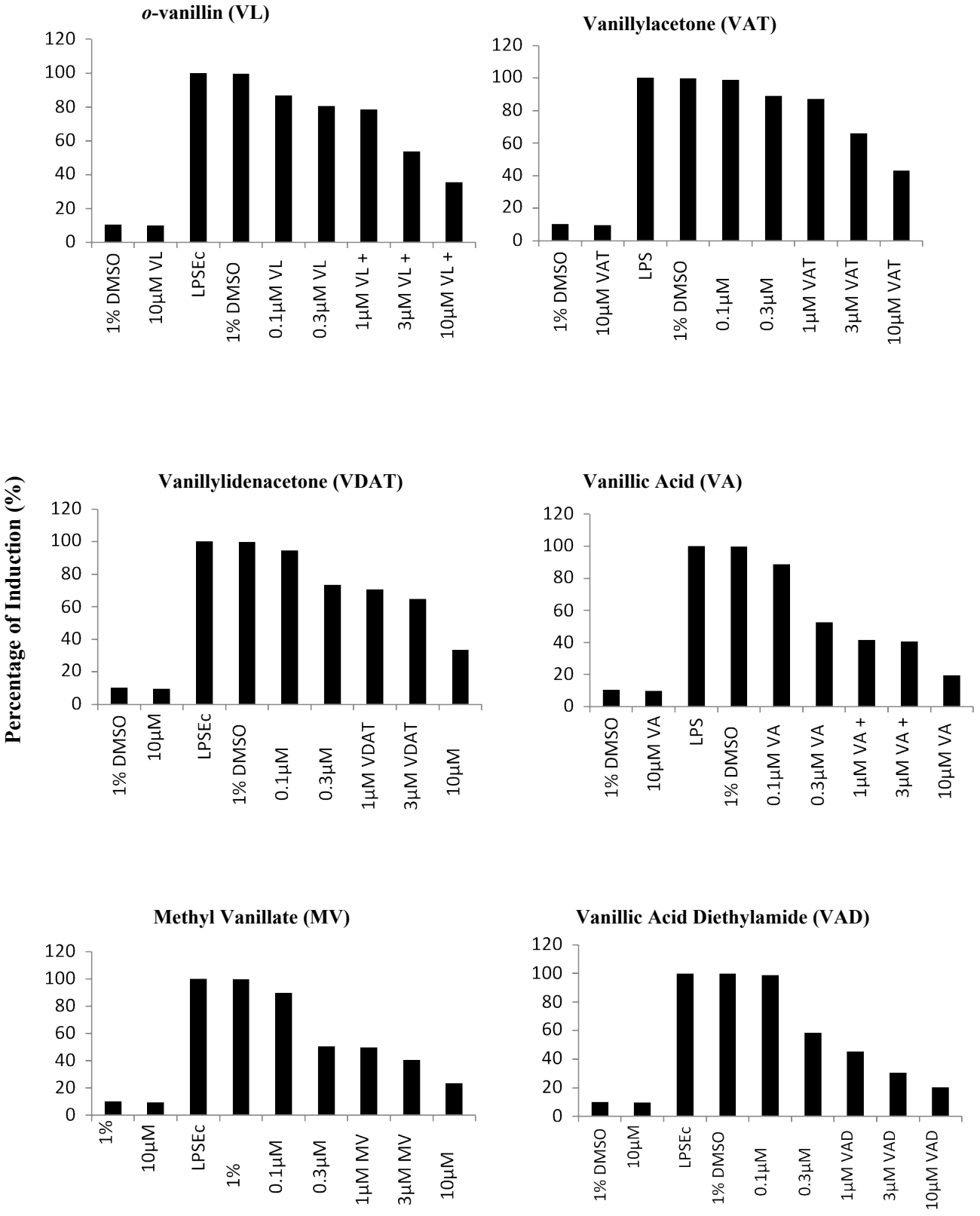


Figure 5: Vanilloids Inhibit LPSEc Induced Nitric Oxide Production. Resatorvid was added at respective concentrations for 1 hour and followed up by 1 µg/mL (LPSEc) for 18 hours. Results are expressed as percentage of LPSEc induced nitric oxide production ± SD as compared to cells with only LPSEc of at least three independent experiments. Bars with the symbo (*) are statistically significant (p<0.05) different by one-way ANOVA *post hoc* Dunnett's *t*-test.

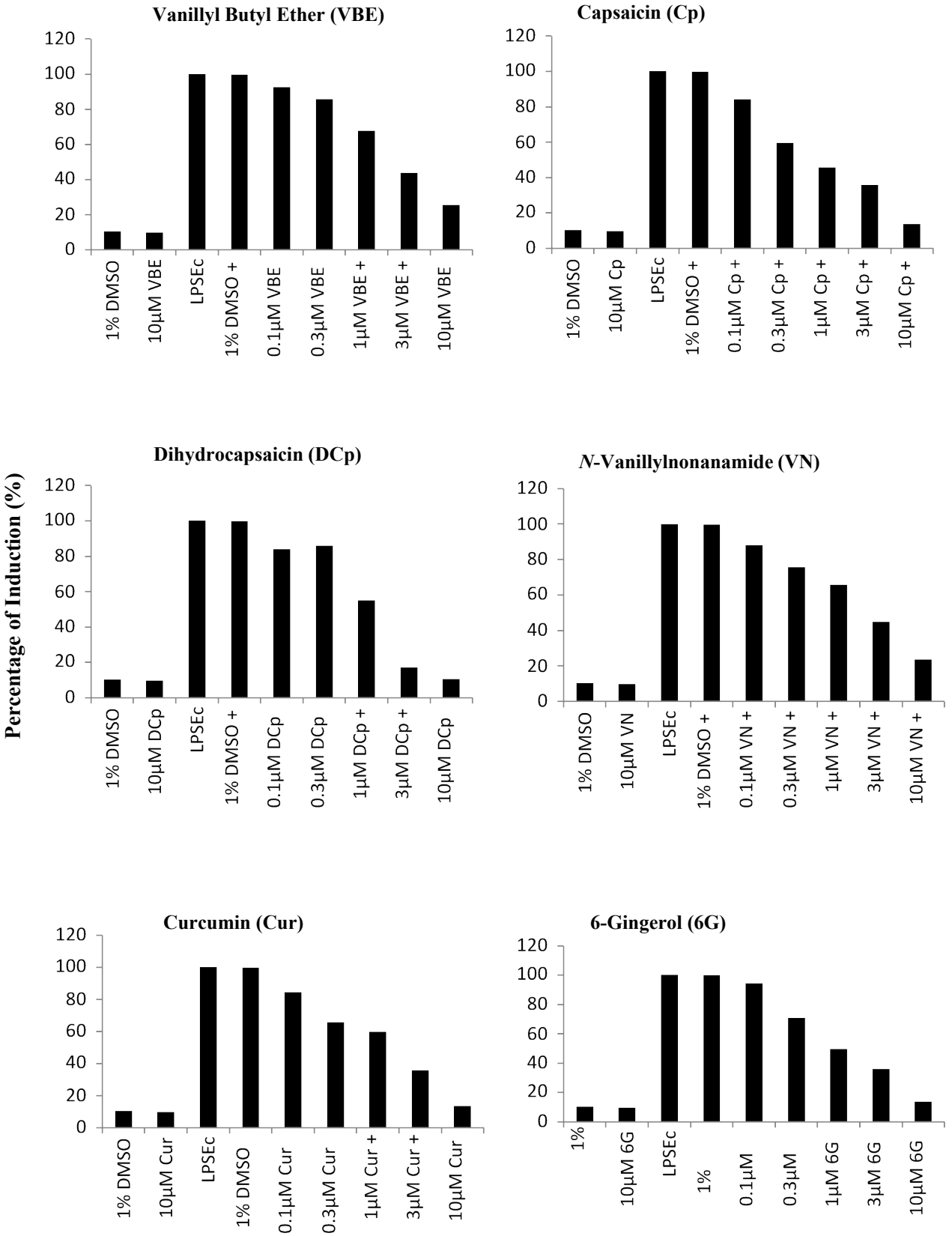


Figure 5 ***(continued)*****: Vanilloids Inhibit LPSEc Induced Nitric Oxide Production.** Resatorvid was added at respective concentrations for 1 hour and followed up by 1 µg/mL (LPSEc) for 18 hours. Results are expressed as percentage of LPSEc induced nitric oxide production ± SD as compared to cells with only LPSEc of at least three independent experiments. Bars with the symbo (*) are statistically significant ($p<0.05$) different by one-way ANOVA *post hoc* Dunnett's *t* -test.

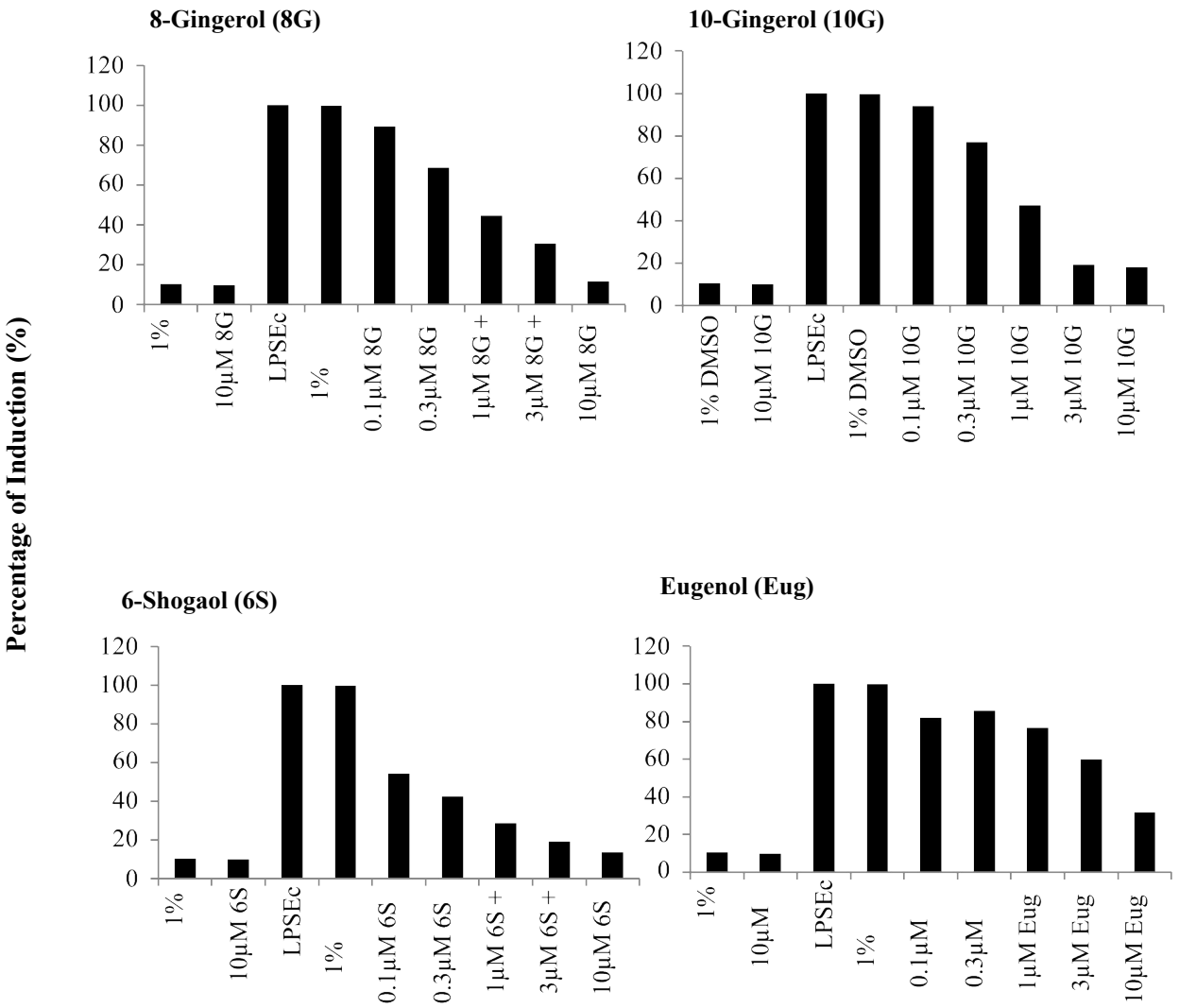
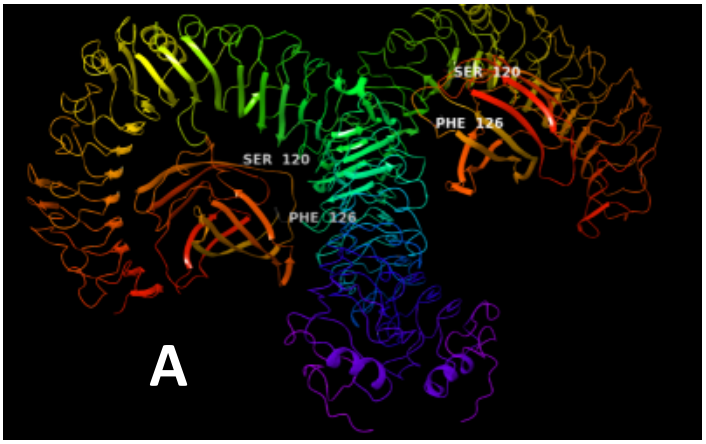
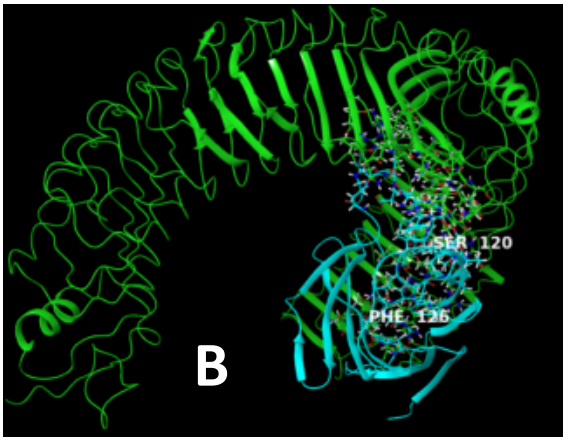
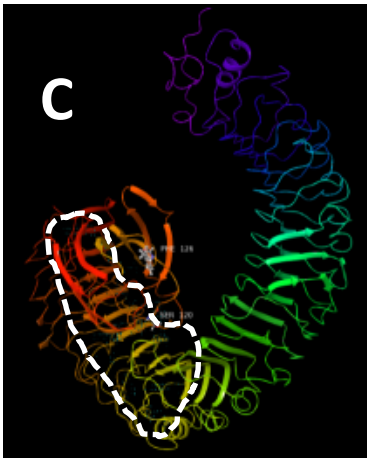
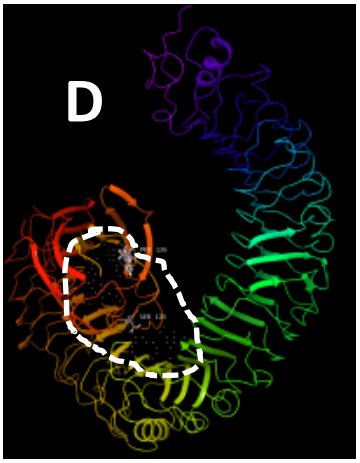


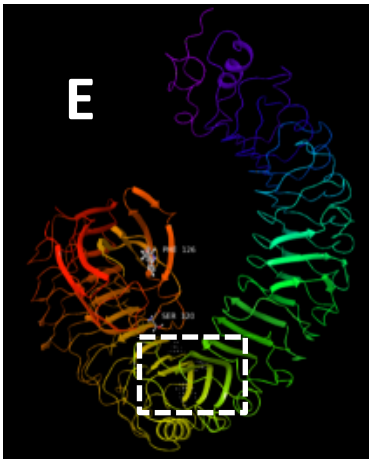
Figure 5 ***(continued)*** **: Vanilloids Inhibit LPSEc Induced Nitric Oxide Production.** Resatorvid was added at respective concentrations for 1 hour and followed up by 1 µg/mL (LPSEc) for 18 hours. Results are expressed as percentage of LPSEc induced nitric oxide production ± SD as compared to cells with only LPSEc of at least three independent experiments. Bars with the symbo (*) are statistically significant ($p<0.05$) different by one-way ANOVA *post hoc* Dunnett's *t* -test.

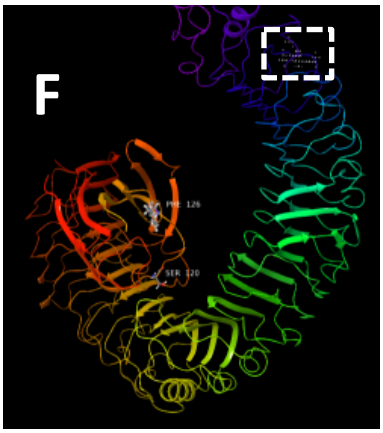












Site No.	Site Score	Size	D Score
Site 2	1.16	199	1.24
Site 1	1.02	349	1.05
Site 3	0.84	78	0.86
Site 4	0.82	61	0.82
Site 5	0.80	45	0.81

Figure 6: (A) TLR-4/MD-2 homodimer with key aminoacid residues (Phe 126, Ser 120); (B) Prepared TLR/MD-2 complex with key aminoacid residues at the interface; (C) Predicted binding site 1 represented in white dots; (D) Predicted binding site 2 represented in white dots; (E) Predicted binding site 3 represented in white dots; (F) Predicted binding site 4 represented in white dots; (G) Predicted binding site 5 represented in white dots.

The binding sites 1 and 2 are nearer to the key aminoacid residues (Ser 120, Phen 126). Table show the site score, size and D-scores of predicted binding sites. The binding site with high D-score (site 2) was chosen for further molecular docking studies.

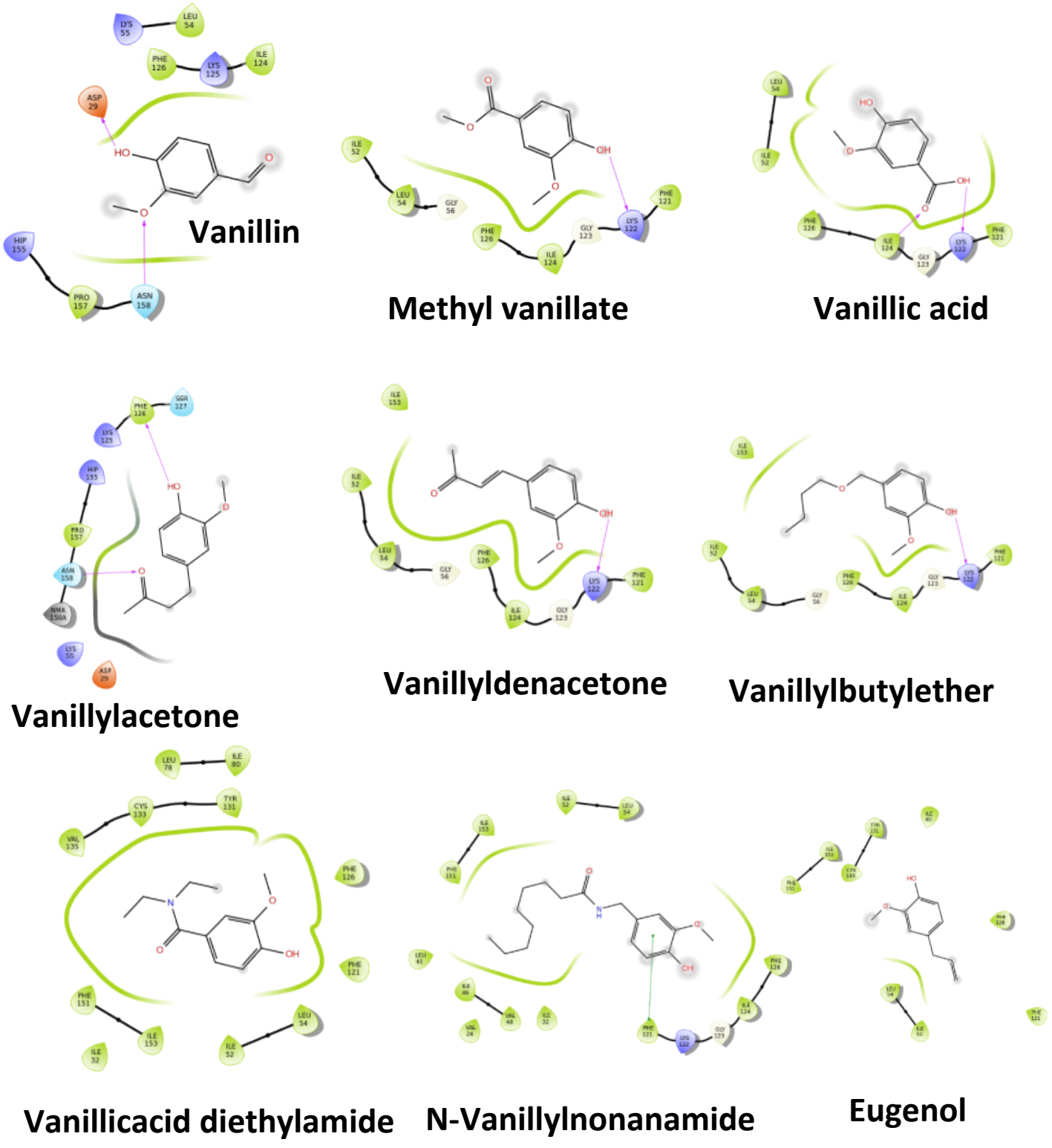
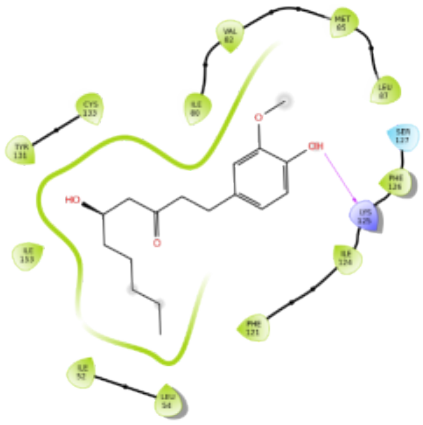
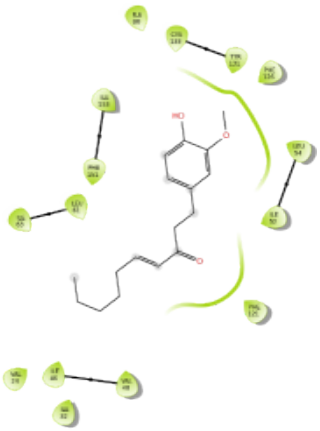


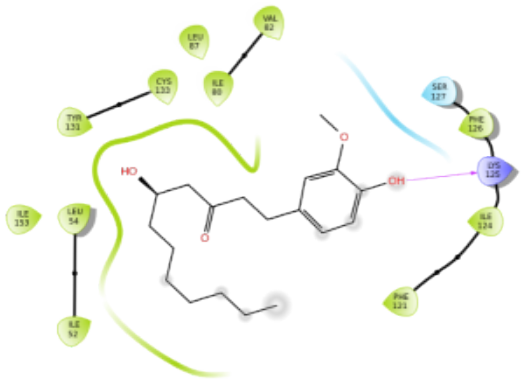
Figure 7: Two-dimensional diagrams of interaction between vanilloids and TLR-4/MD-2 receptor obtained from docked complexes using Glide-XP protocol.



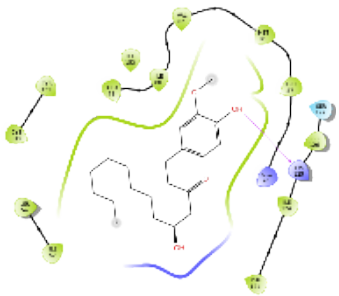
6-Gingerol



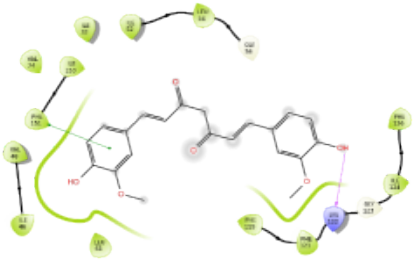
6-Shogaol



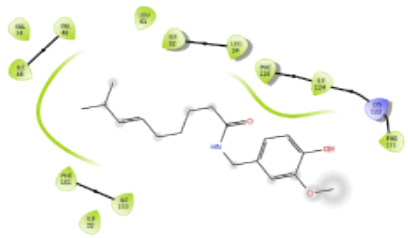
8-Gingerol



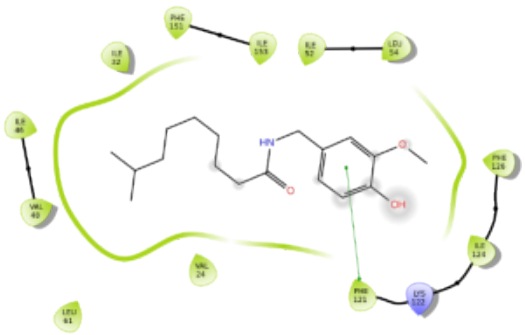
10-Gingerol



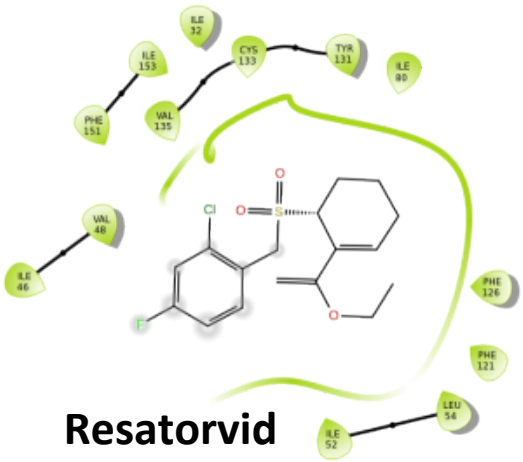
Curcumin



Capsaicin



Dihydrocapsaicin



Resatorvid

Figure 7 (Continued) : Two-dimensional diagrams of interaction between vanilloids and TLR-4/MD -2 receptor obtained from docked complexes using Glide-XP protocol.

Figure 8: Two-dimensional diagrams of interaction between vanilloids and TLR-4/MD-2 receptor obtained from docked complexes using Induced-Fit docking protocol.

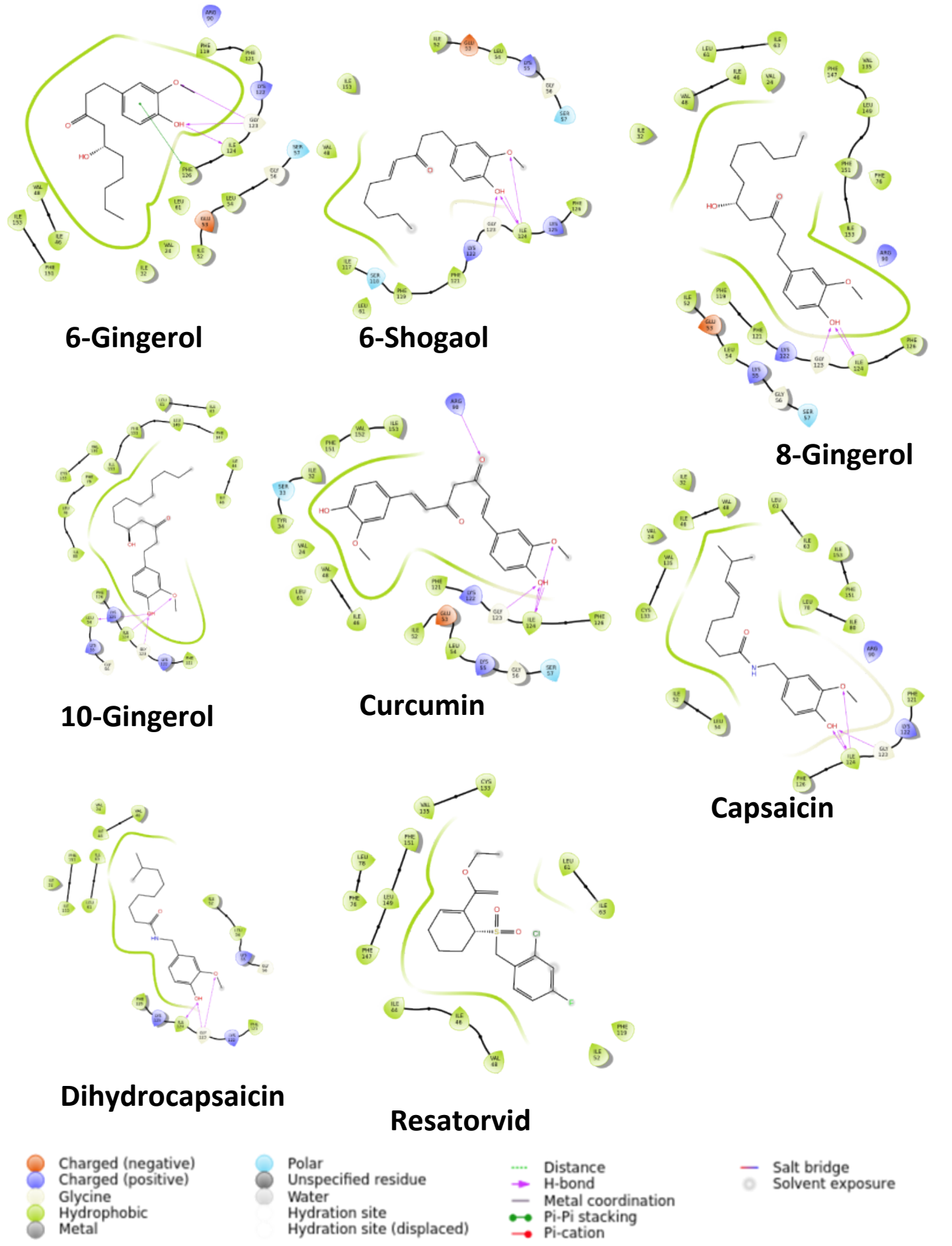


Figure 8 (Continued) : Two-dimensional diagrams of interaction between vanilloids and TLR-4/MD-2 receptor obtained from docked complexes using Induced-Fit Docking protocol.

Table 1: The predicted ADMET properties of vanilloids

Compound	HumanOralAbsorption	PercentHumanOralAbsorption	RuleOfFive	RuleOfThree	QPlogPo/w	QPlogS	QPlogHERG	QPPCaco	#metab	QPlogKhsa
Vanillin	3	81.712	0	0	0.973	-1.145	-3.476	551.652	2	-0.613
Vanillylacetone	3	91.186	0	0	1.772	-2.483	-4.172	1021.989	4	-0.271
Vanillyldenacetone	3	89.922	0	0	1.654	-2.331	-4.303	949.551	2	-0.324
Vanillic acid	3	66.335	0	0	1.031	-1.437	-1.716	73.024	2	-0.751
Methyl vanillate	3	89.487	0	0	1.755	-2.251	-4.065	832.046	2	-0.391
Vanillic acid diethylamide	3	95.065	0	0	1.983	-2.613	-4.15	1435.838	2	-0.248
Vanillyl butyl ether	3	100	0	0	2.707	-3.173	-4.652	2981.275	4	-0.077
N-vanillylnonanamide	3	100	0	0	3.276	-4.33	-4.149	760.282	4	0.027
Eugenol	3	100	0	0	2.65	-2.44	-4.103	2983.882	3	-0.113
6-Gingerol	3	100	0	0	3.596	-4.466	-5.226	573.74	6	0.262
6-Shogaol	3	100	0	0	3.92	-4.837	-5.289	1100.064	5	0.405
8-Gingerol	3	100	0	0	4.417	-5.559	-5.745	555.655	6	0.529
10-Gingerol	1	93.032	1	1	5.021	-5.81	-5.594	593.936	6	0.706
Curcumin	2	82.622	0	0	2.8	-4.592	-6.354	156.535	5	-0.008
Capsaicin	3	100	0	0	3.492	-4.737	-4.227	741.463	6	0.158
Dihydrocapsaicin	3	100	0	0	3.613	-4.798	-4.232	814.387	4	0.163
Resatorvid	3	100	0	0	4.4	-4.76	-5.014	4921.309	3	0.234