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A THEORITICAL STUDY OF BENZYL BENZOATES WITH AGARICUS BISPORUS TYROSINASE INHIBITORY PROPERTIES.

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ABSTRACT

Tyrosinase is a copper containing multifunctional oxidase that catalyzes both the hydroxylation of mono phenols to diphenols and the oxidation of o-diphenols to o-quinones. Tyrosinase is involved in neuromelanin formation in human brain and contribute to neurodegeneration associated with parkinson's disease. The benzyl benzoate analogs were found to inhibit tyrosinase enzyme. The biological activity of these analogs were correlated to different molecular properties. The AM1and PM3 semiempirical methods were used to estimate vertical ionization potentials(IPv's), electron affinity (EA) , electronegativity (χ), hardness (η), softness (S), electrophilic index (ω), partition coefficient (LogP),hydration energy(HE), ionization potential(IP) and charges. The different modeled equations were proposed by regression analysis. The leave-one-out cross-validation method was used to estimate the predictive power of final QSAR equations. The hydration energy (HE) and ionization potential (IP) were found to be indicative molecular properties by regression analysis. The high inhibitory nature of these analogs is found to have lower values of HE and IP. The lower values of HE and IP are responsible for binding ring A and ring B to the bi copper centre of tyrosinase. The inhibitory effect of benzyl benzoate analogs mainly depends on the position of the hydroxyl moieties instead of their quantity.

Keywords: AM1; PM3; Benzyl benzoates; Inhibitor; QSAR; Regression analysis.

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1. INTRODUCTION

Tyrosinase is an enzyme that catalyses the oxidation of phenols .It is also known as monophenol mono oxygenase. It is a copper containing enzyme present in plants, animal tissues and fungi that catalyses the production of melanin. Tyrosinase catalyses both the hydroxylation of monophenols to diphenols and the oxidation of o-di-phenols to o-quinones[1].

Quinones are highly reactive compounds, which can polymerize spontaneously to form high molecular weight compounds like melanin.

They also react with aminoacids and proteins which produce brown colour. However, recently it is found that alterations in melanin synthesis results in many skin effects like hyperpigmentation, melasma and lentigo [2]. Moreover, tyrosinase may involve in neuromelanin formation in human brain and contribute to neurodegeneration associated with parkinson's disease [3]. In plants, it causes undesired enzymatic browning such as bruised cut fruits and vegetables which leads to significant decrease in nutritional values [4].

As tyrosinase inhibitors have an increasing importance due to enormous application prospects in recent decades, the various tyrosinase inhibitors are extracted from natural sources and synthesized. Among which some are applicable to pharmaceutical and cosmetic fields [5]. The flavonoids were thought to be the most effective inhibitors which showed the IC_{50} and K_i value lower than 1μM against Agaricus bisporus (mushroom) tyrosinase [6]. Phlorizin (Benzyl benzoate analogs) (Table 1) is one of the flavonoids found in some fruits and vegetables such as apples and pears. The results suggested that phlorizin might act as competitive inhibitor to tyrosinase which is more effective than arbutin and kojic acid. However, their studies were insufficient and required further research. Later its analogs , which mainly distinguished by the alkyl chain between the two aryl rings, had been prepared and studied including N-benzylbenzamides (the α-C in the alkyl chain was replaced by a NH group)[7], chalcones (C–C single bond between α -C and β-C were changed into C=C double bond)[8] and phenethyl gallates (the α-C in the alkyl chain was replaced by an O atom, and the alkyl chain was lengthened with a CH_2 group)[9]. These analogs showed exceptional inhibitory to tyrosinase. On the other hand, it is pointed out that the inhibitory effect of benzylbenzoates mainly depended on the position of the hydroxyl moieties instead of their quantity. In present study Phlorizin analogs 2-15 (**Fig-1 and Table-1**) were synthesized recently [10] by varying different hydroxyl groups and their bioassay was carried against tyrosinase. The study helps in discovering and filtering effective compounds as tyrosinase inhibitors, which offer potential materials on food systems, cosmetic careers and other fields to inhibit enzymatic browning.

Figure 1: ***Structural skeleton of benzyl benzoate derivatives Agaricus bisporus tyrosinase activities.***

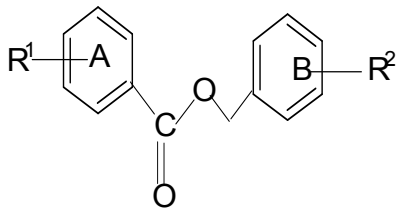


Table 1. Structural skeleton and Inhibition effect of benzyl benzoate derivatives Agaricus bisporus tyrosinase activities.

Compound	R^1	R^2	IC_{50}(μM)	Activity (3+log1/IC_{50})
2	3,5-OH	4'-OH	5.84 ± 0.96	2.233
3	2,5-OH	4'-OH	12.29 ± 0.20	1.909
4	2,4-OH	4'-OH	4.95 ± 0.38	2
5	3,4,5-OH	4'-OH	27.73 ± 0.36	1.58
6	2,4,6-OH	3'-OH	8.00 ± 0.41	2.097
7	3,5-OH	3'-OH	6.11 ± 0.71	2.213
8	2,5-OH	3'-OH	24.39 ± 0.27	1.613
9	2,4-OH	3'-OH	6.23 ± 0.85	2.205
10	3,4,5-OH	2'-OH	11.47 ± 0.69	1.94
11	3,5-OH	2'-OH	19.89 ± 0.24	1.699
12	2,5-OH	2'-OH	100	1
13	2,4-OH	2'-OH	66.23 ± 0.25	1.177
14	3,4,5-OH	2'-OH	100	1
15	2,4,6-OH	2' -OH	19.9 ± 40.18	1.699
kojic acid			20.99 ± 0.12	

The present study is to elucidate the QSAR study of phlorizin analogs as tyrosinase inhibitor using physicochemical parameters like ionization potentials, hydration energy, polarisability (Pol), LogP, etc… Recently Lien et. al [11] have reported on QSAR study of phenols with antioxidant activity by employing descriptors calculated by semi empirical methods AM1 and PM3 (Table 2, 3). This study was also made on quantitative basis in which 4 computational methods *viz.* density functional (DF), HF (Hartree-Fock) and AM1 and PM3 were employed to explore and determine various electronic descriptors with better accuracy to make the necessary improvement in the QSAR models. Vertical ionization potentials(IPv's) electron affinity (EA) , electronegativity (χ), hardness (η), softness (S), electrophilic index (ω), partition coefficient (Log P), charges and other properties were obtained for 41 phenolic compounds which have antioxidant activity.[11-14] (Table 4, 4(a), 5, 5(a)).

Table 2.
Values obtained for the PM3 computational method.

Compound	$-\varepsilon_{HOMO}$ (PM3)	IP	EA	EN	η	S	ω	Log P	HE	Pol(A^{o3})
2	-9.139441	-1.77	-7.79	-4.78	3.01	0.166	3.7928	-1.05	-24.11	26.40
3	-8.958868	-1.69	-7.75	-4.72	3.03	0.165	3.6759	-1.05	-23.26	26.40
4	-9.141992	-1.72	-7.79	-4.75	3.035	0.164	3.7003	-1.05	-23.15	26.40
5	-9.129124	-1.57	-7.85	-4.71	3.14	0.159	3.5273	-2.07	-28.28	27.03
6	-9.247292	-1.66	-7.96	-4.81	3.15	0.158	3.5630	-2.07	-27.84	27.03
7	-9.24489	-1.74	-8.17	-4.95	3.215	0.155	3.7979	-1.05	-23.78	26.40
8	-8.976559	-1.73	-7.66	-4.69	2.965	0.168	3.6953	-1.05	-22.84	26.40
9	-9.272421	-1.74	-7.82	-4.78	3.04	0.164	3.7471	-1.05	-22.73	26.40
10	-9.124651	-1.62	-7.84	-4.73	3.11	0.160	3.5797	-2.07	-27.94	27.03
11	-9.208811	-1.75	-7.80	-4.77	3.025	0.165	3.7540	-1.05	-19.05	26.40
12	-8.90451	-1.73	-7.62	-4.67	2.945	0.169	3.6857	-1.05	-18.00	25.76
13	-9.216689	-1.70	-7.81	-4.75	3.055	0.163	3.6777	-1.05	-17.94	26.40
14	-9.091803	-1.62	-7.76	-4.69	3.07	0.168	3.6953	-2.07	-23.22	27.03
15	-9.19454	-1.74	-7.85	-4.79	3.055	0.163	3.7399	-2.07	-22.24	27.03

Table 3. Values obtained for the AM1 computational method.

Compound	$-e_{HOMO(AM1)}$	IP	EA	EN	η	S	ω	LogP	HE	Pol (A^{o3})
2	-9.020069	-1.85	-12.77	-7.31	5.46	0.092	4.9161	-1.05	-24.20	26.40
3	-8.915448	-1.67	-10.21	-5.94	4.27	0.117	4.1282	-1.05	-23.31	26.40
4	-8.891648	-1.91	-10.24	-6.08	4.17	0.119	4.3990	-1.05	-23.23	26.40
5	-9.088475	-1.57	-10.28	-5.93	4.36	0.114	4.0088	-2.07	-28.29	27.03
6	-8.986326	-1.89	-10.23	-6.06	4.17	0.119	4.3701	-2.07	-27.99	27.03
7	-9.12661	-1.86	-10.35	-6.11	4.25	0.118	4.4052	-1.05	-23.79	26.40
8	-8.945368	-1.76	-10.09	-5.93	4.17	0.119	3.6391	-1.05	-22.82	26.40
9	-9.177288	-1.74	-10.60	-6.20	4.43	0.113	4.3437	-1.05	-22.72	26.40
10	-9.159107	-1.64	-10.59	-6.12	4.48	0.112	4.1949	-2.07	-27.87	27.03
11	-9.752435	-1.16	-11.05	-6.11	4.95	0.101	3.7705	-1.05	-18.60	26.40
12	-8.898338	-1.73	-10.17	-6.00	4.22	0.118	4.2480	-1.05	-17.45	25.76
13	-9.175468	-1.70	-10.58	-6.14	4.44	0.126	4.7501	-1.05	-17.40	26.40
14	-9.047676	-1.65	-10.31	-6.00	4.33	0.115	4.1400	-2.07	-22.70	27.03
15	-9.211817	-1.70	-10.51	-6.11	4.41	0.113	4.2185	-2.07	-22.82	27.03

Table 4. Correlation matrix between the selected variables, by using AM1 method.

		ACT	**IPv(AM1)**	*IP*	*EA*	*EN*	*η*	*S*	*EI*	*LogP*	*HE*	*Pol*
ACT	Pearson Correlation	1.000	-.002	-.357	-.300	-.374	.195	-.308	.243	.169	-.517	.077
	Sig. (2-tailed)	.	.995	.211	.297	.188	.504	.284	.402	.563	.059	.793
	N	14	14	14	14	14	14	14	14	14	14	14
IPv(AM1)	Pearson Correlation	-.002	1.000	-.387	.198	.084	-.291	.172	-.101	.273	-.017	-.393
	Sig. (2-tailed)	.995	.	.171	.498	.776	.312	.556	.731	.345	.955	.165
	N	14	14	14	14	14	14	14	14	14	14	14
IP	Pearson Correlation	-.357	-.387	1.000	-.047	.223	.308	-.330	-.554	-.050	.296	.060
	Sig. (2-tailed)	.211	.171	.	.873	.444	.285	.249	.040	.864	.304	.839
	N	14	14	14	14	14	14	14	14	14	14	14
EA	Pearson Correlation	-.300	.198	-.047	1.000	.962	-.965	.861	-.526	-.211	-.020	.096
	Sig. (2-tailed)	.297	.498	.873	.	.000	.000	.000	.054	.468	.945	.743
	N	14	14	14	14	14	14	14	14	14	14	14
EN	Pearson Correlation	-.374	.084	.223	.962	1.000	-.858	.754	-.663	-.228	.042	.131
	Sig. (2-tailed)	.188	.776	.444	.000	.	.000	.002	.010	.434	.886	.656
	N	14	14	14	14	14	14	14	14	14	14	14
η	Pearson Correlation	.195	-.291	.308	-.965	-.858	1.000	-.908	.353	.187	.095	-.075
	Sig. (2-tailed)	.504	.312	.285	.000	.000	.	.000	.215	.521	.747	.800
	N	14	14	14	14	14	14	14	14	14	14	14
S	Pearson Correlation	-.308	.172	-.330	.861	.754	-.908	1.000	-.108	-.055	.101	-.016
	Sig. (2-tailed)	.284	.556	.249	.000	.002	.000	.	.714	.851	.731	.957
	N	14	14	14	14	14	14	14	14	14	14	14
EI	Pearson Correlation	.243	-.101	-.554	-.526	-.663	.353	-.108	1.000	.152	.038	-.122
	Sig. (2-tailed)	.402	.731	.040	.054	.010	.215	.714	.	.603	.896	.679
	N	14	14	14	14	14	14	14	14	14	14	14
LogP	Pearson	.169	.273	-.050	-.211	-.228	.187	-.055	.152	1.000	.627	-.902

	Correlation											
	Sig. (2-tailed)	.563	.345	.864	.468	.434	.521	.851	.603	.	.016	.000
	N	14	14	14	14	14	14	14	14	14	14	14
HE	Pearson Correlation	-.517	-.017	.296	-.020	.042	.095	.101	.038	.627	1.000	-.712
	Sig. (2-tailed)	.059	.955	.304	.945	.886	.747	.731	.896	.016	.	.004
	N	14	14	14	14	14	14	14	14	14	14	14
Pol	Pearson Correlation	.077	-.393	.060	.096	.131	-.075	-.016	-.122	-.902	-.712	1.000
	Sig. (2-tailed)	.793	.165	.839	.743	.656	.800	.957	.679	.000	.004	.
	N	14	14	14	14	14	14	14	14	14	14	14

N = 14.

* *Correlation is significant at the 0.05 level (2-tailed).*

** *Correlation is significant at the 0.01 level (2-tailed).*

Table (4a).

Correlation matrix between the selected variables, by using AM1 method.

		ACT	IPv(AM1)	IP	EA	EN	η	S	EI	LogP	HE	Pol
ACT	Pearson Correlation	1.000	-.170	-.276	-.375	-.419	.294	-.397	.215	.106	-.546	.133
	Sig. (2-tailed)	.	.579	.362	.206	.154	.329	.179	.482	.729	.053	.666
	N	13	13	13	13	13	13	13	13	13	13	13
IPv(AM1)	Pearson Correlation	-.170	1.000	-.295	.155	.067	-.228	.113	-.170	.210	-.013	-.374
	Sig. (2-tailed)	.579	.	.328	.613	.828	.455	.712	.579	.490	.967	.208
	N	13	13	13	13	13	13	13	13	13	13	13
IP	Pearson Correlation	-.276	-.295	1.000	-.002	.254	.258	-.295	-.547	.017	.308	.018
	Sig. (2-tailed)	.362	.328	.	.994	.402	.395	.328	.053	.955	.305	.953
	N	13	13	13	13	13	13	13	13	13	13	13
EA	Pearson Correlation	-.375	.155	-.002	1.000	.966	-.967	.858	-.553	-.248	-.019	.117
	Sig. (2-tailed)	.206	.613	.994	.	.000	.000	.000	.050	.414	.951	.703
	N	13	13	13	13	13	13	13	13	13	13	13
EN	Pearson Correlation	-.419	.067	.254	.966	1.000	-.868	.757	-.677	-.245	.043	.140
	Sig. (2-tailed)	.154	.828	.402	.000	.	.000	.003	.011	.421	.889	.649
	N	13	13	13	13	13	13	13	13	13	13	13
η	Pearson Correlation	.294	-.228	.258	-.967	-.868	1.000	-.906	.393	.243	.095	-.107
	Sig. (2-tailed)	.329	.455	.395	.000	.000	.	.000	.184	.424	.759	.729
	N	13	13	13	13	13	13	13	13	13	13	13
S	Pearson Correlation	-.397	.113	-.295	.858	.757	-.906	1.000	-.132	-.094	.105	.007
	Sig. (2-tailed)	.179	.712	.328	.000	.003	.000	.	.666	.760	.733	.981
	N	13	13	13	13	13	13	13	13	13	13	13
EI	Pearson Correlation	.215	-.170	-.547	-.553	-.677	.393	-.132	1.000	.130	.040	-.107
	Sig. (2-tailed)	.482	.579	.053	.050	.011	.184	.666	.	.672	.896	.729
	N	13	13	13	13	13	13	13	13	13	13	13

LogP	Pearson Correlation	.106	.210	.017	-.248	-.245	.243	-.094	.130	1.000	.644	-.901
	Sig. (2-tailed)	.729	.490	.955	.414	.421	.424	.760	.672	.	.018	.000
	N	13	13	13	13	13	13	13	13	13	13	13
HE	Pearson Correlation	-.546	-.013	.308	-.019	.043	.095	.105	.040	.644	1.000	-.720
	Sig. (2-tailed)	.053	.967	.305	.951	.889	.759	.733	.896	.018	.	.005
	N	13	13	13	13	13	13	13	13	13	13	13
Pol	Pearson Correlation	.133	-.374	.018	.117	.140	-.107	.007	-.107	-.901	-.720	1.000
	Sig. (2-tailed)	.666	.208	.953	.703	.649	.729	.981	.729	.000	.005	.
	N	13	13	13	13	13	13	13	13	13	13	13

N = 13.

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed

Table 5.

Correlation matrix between the selected variables, by using PM3 method.

		ACT	IPv(PM3)	IP	EA	EN	η	S	EI	LogP	HE	Pol
ACT	Pearson Correlation	1.000	-.537	-.238	-.570	-.643	.415	-.541	.174	.108	-.459	.107
	Sig. (2-tailed)	.	.048	.412	.033	.013	.140	.046	.552	.714	.099	.715
	N	14	14	14	14	14	14	14	14	14	14	14
IPv(PM3)	Pearson Correlation	-.537	1.000	.073	.719	.705	-.618	.628	-.140	.172	.192	-.396
	Sig. (2-tailed)	.048	.	.804	.004	.005	.019	.016	.634	.556	.512	.162
	N	14	14	14	14	14	14	14	14	14	14	14
IP	Pearson Correlation	-.238	.073	1.000	-.049	.359	.459	-.294	-.854	-.737	-.613	.665
	Sig. (2-tailed)	.412	.804	.	.868	.207	.099	.308	.000	.003	.020	.009
	N	14	14	14	14	14	14	14	14	14	14	14
EA	Pearson Correlation	-.570	.719	-.049	1.000	.914	-.910	.907	-.076	.194	.403	-.352
	Sig. (2-tailed)	.033	.004	.868	.	.000	.000	.000	.797	.506	.153	.217
	N	14	14	14	14	14	14	14	14	14	14	14
EN	Pearson Correlation	-.643	.705	.359	.914	1.000	-.664	.726	-.418	-.116	.141	-.064
	Sig. (2-tailed)	.013	.005	.207	.000	.	.010	.003	.137	.694	.631	.828
	N	14	14	14	14	14	14	14	14	14	14	14
η	Pearson Correlation	.415	-.618	.459	-.910	-.664	1.000	-.929	-.288	-.483	-.611	.592
	Sig. (2-tailed)	.140	.019	.099	.000	.010	.	.000	.317	.080	.020	.026
	N	14	14	14	14	14	14	14	14	14	14	14
S	Pearson Correlation	-.541	.628	-.294	.907	.726	-.929	1.000	.291	.332	.571	-.445
	Sig. (2-tailed)	.046	.016	.308	.000	.003	.000	.	.313	.245	.033	.111
	N	14	14	14	14	14	14	14	14	14	14	14
EI	Pearson Correlation	.174	-.140	-.854	-.076	-.418	-.288	.291	1.000	.632	.575	-.508
	Sig. (2-tailed)	.552	.634	.000	.797	.137	.317	.313	.	.015	.031	.063
	N	14	14	14	14	14	14	14	14	14	14	14
LogP	Pearson Correlation	.108	.172	-.737	.194	-.116	-.483	.332	.632	1.000	.635	-.902
	Sig. (2-tailed)	.714	.556	.003	.506	.694	.080	.245	.015	.	.015	.000
	N	14	14	14	14	14	14	14	14	14	14	14

HE	Pearson Correlation	-.459	.192	-.613	.403	.141	-.611	.571	.575	.635	1.000	-.712
	Sig. (2-tailed)	.099	.512	.020	.153	.631	.020	.033	.031	.015	.	.004
	N	14	14	14	14	14	14	14	14	14	14	14
Pol	Pearson Correlation	.107	-.396	.665	-.352	-.064	.592	-.445	-.508	-.902	-.712	1.000
	Sig. (2-tailed)	.715	.162	.009	.217	.828	.026	.111	.063	.000	.004	.
	N	14	14	14	14	14	14	14	14	14	14	14

N = 14.

* *Correlation is significant at the 0.05 level (2-tailed).*

** *Correlation is significant at the 0.01 level (2-tailed)*

Table (5a).

Correlation matrix between the selected variables, by using PM3 method.

		ACT	IPv(PM3)	IP	EA	EN	η	S	EI	LogP	HE	Pol
ACT	Pearson Correlation	1.000	-.560	-.217	-.632	-.695	.480	-.594	.169	.040	-.489	.165
	Sig. (2-tailed)	.	.047	.476	.020	.008	.097	.032	.580	.897	.090	.591
	N	13	13	13	13	13	13	13	13	13	13	13
IPv(PM3)	Pearson Correlation	-.560	1.000	.070	.723	.707	-.624	.630	-.139	.183	.192	-.404
	Sig. (2-tailed)	.047	.	.820	.005	.007	.023	.021	.652	.549	.530	.171
	N	13	13	13	13	13	13	13	13	13	13	13
IP	Pearson Correlation	-.217	.070	1.000	-.042	.364	.453	-.291	-.855	-.736	-.616	.660
	Sig. (2-tailed)	.476	.820	.	.890	.222	.120	.334	.000	.004	.025	.014
	N	13	13	13	13	13	13	13	13	13	13	13
EA	Pearson Correlation	-.632	.723	-.042	1.000	.915	-.909	.906	-.079	.185	.404	-.347
	Sig. (2-tailed)	.020	.005	.890	.	.000	.000	.000	.798	.545	.171	.245
	N	13	13	13	13	13	13	13	13	13	13	13
EN	Pearson Correlation	-.695	.707	.364	.915	1.000	-.665	.726	-.419	-.124	.141	-.061
	Sig. (2-tailed)	.008	.007	.222	.000	.	.013	.005	.154	.687	.646	.843
	N	13	13	13	13	13	13	13	13	13	13	13
η	Pearson Correlation	.480	-.624	.453	-.909	-.665	1.000	-.930	-.286	-.476	-.614	.587
	Sig. (2-tailed)	.097	.023	.120	.000	.013	.	.000	.344	.101	.026	.035
	N	13	13	13	13	13	13	13	13	13	13	13
S	Pearson Correlation	-.594	.630	-.291	.906	.726	-.930	1.000	.289	.331	.572	-.443
	Sig. (2-tailed)	.032	.021	.334	.000	.005	.000	.	.338	.270	.041	.129
	N	13	13	13	13	13	13	13	13	13	13	13
EI	Pearson Correlation	.169	-.139	-.855	-.079	-.419	-.286	.289	1.000	.637	.575	-.508
	Sig. (2-tailed)	.580	.652	.000	.798	.154	.344	.338	.	.019	.040	.077
	N	13	13	13	13	13	13	13	13	13	13	13
LogP	Pearson Correlation	.040	.183	-.736	.185	-.124	-.476	.331	.637	1.000	.649	-.901
	Sig. (2-tailed)	.897	.549	.004	.545	.687	.101	.270	.019	.	.016	.000
	N	13	13	13	13	13	13	13	13	13	13	13
HE	Pearson Correlation	-.489	.192	-.616	.404	.141	-.614	.572	.575	.649	1.000	-.718
	Sig. (2-tailed)	.090	.530	.025	.171	.646	.026	.041	.040	.016	.	.006
	N	13	13	13	13	13	13	13	13	13	13	13
Pol	Pearson Correlation	.165	-.404	.660	-.347	-.061	.587	-.443	-.508	-.901	-.718	1.000
	Sig. (2-tailed)	.591	.171	.014	.245	.843	.035	.129	.077	.000	.006	.
	N	13	13	13	13	13	13	13	13	13	13	13

N = 13.

* *Correlation is significant at the 0.05 level (2-tailed).*

** *Correlation is significant at the 0.01 level (2-tailed).*

This prompted us to correlate the biological activity of benzyl benzoates analogs with ionization potentials, electron affinity ,electronegativity, hardness(η), partition coefficient (LogP), softness(S), hydration energy(HE) and Polarisability(Pol) from computational methods AM1 and PM3 (Table 6,7).

Table 6.
Observed activity and predicted activity values of benzyl benzoate analogs by using AM1 Eqs.

Compound	Observed	**Eq.3**	
		Predicted	Residual
2	2.233	1.8210	0.4120
3	1.909	1.7309	0.1791
4	2.302	-	-
5	1.58	2.0066	-0.4266
6	2.097	2.0527	0.0423
7	2.213	1.7986	0.4144
8	1.613	1.7202	-0.1072
9	2.205	1.7102	0.4948
10	1.94	1.9960	-0.056
11	1.699	1.3456	0.3534
12	1.00	1.3945	-0.3945
13	1.77	1.3855	0.3845
14	1.00	1.6905	-0.6905
15	1.699	1.7079	-0.0089

Table 7.
Observed activity and predicted activity values of benzyl benzoate analogs by using PM3 Eqs.

Compound	Observed	**Eq.5**	
		Predicted	Residual
2	2.233	1.7617	0.4713
3	1.909	1.6951	0.2139
4	2.302	-	-
5	1.58	1.9384	-0.3584
6	2.097	1.9371	0.1599
7	2.213	1.7362	0.4768
8	1.613	1.6823	-0.0693
9	2.2050	1.6789	0.5261
10	1.94	1.9325	0.0075
11	1.699	1.4809	0.2181
12	1.00	1.4186	-0.4186
13	1.77	1.4078	0.3622
14	1.00	1.6752	-0.6752
15	1.699	1.6522	-0.0468

2.Computational Calculations

2.1 Data Set

The physicochemical parameters ,such as vertical ionization potentials (IPv's) electron affinity (EA) , electronegativity (χ), hardness (η), softness (S), electrophilic index (ω), partition coefficient (LogP), charges, hydration energy(HE) and polarisability (Pol) were obtained for 14 Benzyl benzoates compounds which have mushroom inhibitory activity.

2.2. Molecular Structure Building

A series of compounds tested for inhibitory activity was selected for the present study and the program of window Hyperchem software inc [15] was used in modeling studies. The molecules were generated and the energy was minimized using

molecular modeling pro. The window version software SPSS10 [16] was used in the regression analysis.

2.3. Building of QSAR Models

QSAR technique was applied to the analogs of Benzyl benzoates that were varied at the $R^1$and R^2 position. The appropriate descriptors or parameters for the compounds, vertical ionization potentials(IP_v's), electron affinity (EA), electronegativity(χ), hardness(η), softness(S), electrophilic index (ω), partition coefficient (Log P) charges, polarisability(Pol) and hydration energy (HE) were used as indepedndent variables for desiding in Agaricus bisporus inhibitory activity.

2.4. CHEMICAL DESCRIPTORS

2.4.1. Calculated Properties

Quantum chemical calculations at the DFT/RB3LYP/631G* (restricted B3LYP), RHF/6-31G* (restricted Hartree-Fock) [17] and AM1 [18] and PM3 [19] semiempirical theory levels, are employed for full optimization of the selected neutral compounds. The geometrical structures of the radicals studied are optimized independently from the neutral molecules prior to the calculation of energies, treated as open shell systems. All calculations are performed by using the program of window Hyperchem software inc [15].

In this work, the more relevant electronic properties for phenolic compounds such as vertical ionization potential (IPv), electron affinity(EA), electronegativity (χ), hardness(η), softness(S), electrophilic index(ω), partition coefficient (Log P), charges (Mulliken's charges), hydration energy(HE) and polarisability (Pol) on some key atoms are calculated[11-14].

The calculated vertical ionization potenti als (IPv's) and electron affinity (EA) are corrected for zero-point energy, assuming a negligible error and thus saving computer-time. The IPv are calculated as the energy differences between a radical cation and the respective neutral molecule; IPv $(E_{cation} - E_{neutral})_{DFT}$ and Koopman's theorem ($IPv = -\varepsilon_{HOMO}$). The EA are computed as the energy differences between a neutral form and the anion molecule; $EA=E_{neutral} - E_{anion}$. The AM1 and PM3-based reactivity descriptors are obtained from Eqs. (1) – (4) [20-23].

2.5. Correlation Analysis

A relation between biological activity, expressed as $Log1/IC_{50,}$ and the physicochemical parameters and QSAR was analyzed statistically by fitting the data to correlation equations consisting of various combinations of these parameters. The statistical optimization was used to propose the best correlation model.

The matrix correlation uses the Pearson product moment correlation to measure the degree of linear relationship between two variables. The coefficient assumes a value between -1 and +1 .If one variable tends to increase the other decreases, the correlation coefficient is negative. Conversely, if the two variables tend to increase together the correlation coefficient is positive. We obtained the correlation matrix between inhibitory activity and respective calculated properties for 14 benzyl benzoate analogs. The more relevant regression models were selected following criteria: The correlation coefficient (R), the Fisher ratio values (F) and the standard deviations(s),standard error estimate (SEE), percentage of effective variable(%EV) and R^2adjusted(R^2_{adj}).

The best equation was also tested for their predictive power using a cross- validation procedure .The cross-validation is a practical and reliable method for testing this significance. In principle, the so-called "leave-one –out" approach consist in developing a number of models with one sample omitted at the time.

After developing each model ,the omitted data is predicted and the differences between actual and predicted reduction potential (y) values are calculated .The sum of squares of these differences is computed and finally the performance of the model (its predictive ability) is given by PRESS(Predictive Sum of Squares) and S_{PRESS} (Standard deviation of cross validation)[26].

The predictive ability of the model was also quantified in terms of the Q^2 [27].

3. RESULTS AND DISCUSSIONS

3.1. Simple linear regression model

The biological activity data and the physicochemical properties IPv, IP, EA, EI, EN, Hard, Soft, LogP, HE and Pol of the benzyl benzoate analogs are given in Tables 1-3. The data from these tables were subjected to regression analysis. The Correlation matrices were generated with 14 analogs(Tables 4,4a and 5,5a). The term close to 1 indicates high co-linearity, while the value below 0.5 indicates that no co-linearity exist between more than the two parameters.

The perusal of correlation matrix (Table 4a and Table 5a) indicates that HE and IP are the predicted parameters from AM1 method. In the initial stage, mono parametric QSAR equation was generated with HE. It is interesting to record that R^2_{ad} values take into account the adjustment of %EV.

Therefore, if a variable is added which does not contribute its fair share, then the R^2_{adj} value will actually decline [24]. It is observed that by the addition of IP to the model (Eq.1), R^2_{adj} increased which also supports the bivariant dependence of biological activity. Hence, multiple regressions has been sought.

The regression technique was applied through the origin using these explainable parameters. The resulted modeled equations explained the biological activity has a function of HE and IP.

Activity = -7.60×10^{-2} HE(0.004) ---------- (1)

N = 14; R = 0.979; R^2 = 0.957; R^2_{adj} = 0.954 ; %EV = 95.70;

SEE = 0.387569 ; F= 292.876; Q =2.5260

Activity = -5.19×10^{-2} HE(0.030) -0.332 IP(0.105) ---------- (2)

N = 14; R = 0 .980; R^2= 0.960; R^2_{adj} = 0.955 ; %EV = 96.0; SEE = 0.392528;
F = 143.098; Q = 2.4966;

In addition, the plot of observed activity versus predicted activity was not found to be satisfactory. Hence, the predictive ability of the model is not good. Eq.1 and 2 show that the values of %EV is less and to improve its value, outliers were sought and eliminated.

After the elimination of the outlier (compound 4), a third model was developed. Overall, there is an increase in R) and %EV(95.7-96.1) values, and a decrease in SEE(0.392-0.381).

Activity = -5.95×10^{-2}HE (0.029) – 0.206 IP (0.106) ---------- (3)

N = 13; R= 0.980; R^2= 0.961; R^2_{adj} = 0.963; %EV = 96.1; SEE = 0.381967;

F = 133.797; Q = 2.5657;

Eq.3 is an improved model since it explains the biological activity to the extent of (96.1%). In this way, the predictive molecular descriptors HE and IP were considered as variables.

From the correlation matrix, it reveals HE and IP are the explainable variables in PM3 method also. Here also the mono parametric QSAR equation with HE was generated. As the R^2_{adj} value was increased by the addition of IP, a biparametric regression was sought.

Activity = -5.39×10^{-2} HE(0.031) - 0.288 IP (0.118)----------(4)

N = 14; R = 0.974; R^2= 0.949; R^2_{adj} = 0.941; %EV = 94.90; SEE = 0.434834;

F = 112.621; Q = 2.2399;

Eq.4 shows that the values of %EV is less and to improve its value, outliers were sought and eliminated, In addition, the plot of observed activity versus predicted activity was not found to be satisfactory. Hence, the predictive ability of the model is not good. After the elimination of the outlier (compound 4), a second model was developed.

Activity = -0.253 IP (0.113) - 5.45×10^{-2} HE(0.030)----------(5)

N = 13; R = 0.975 ; R^2 = 0.951; R^2_{adj} = 0.942 ; %EV = 95.1 ; SEE = 0.418875;

F = 107.23; Q =2.3277;

In an attempt to investigate the predictive potential of proposed models, the cross-validation parameters (q^2_{cv} and PRESS) were calculated and used. The predictive power of the equations was

confirmed by leave-one-out (LOO) cross-validation method [25] where, compounds are deleted one after another and prediction of the activity of the deleted compound is made based on QSAR model. The cross-validation evaluates the validity of a model by how well it predicts the data rather than how well it fits the data. The cross-validation parameter, q^2_{cv}, is mentioned in the respective equations (Table6 and 7).

$$q^2_{cv} = \frac{(SD - PRESS)}{SD},$$

where the PRESS (predictive residual sum of squares) and SD(standard deviation) valves are obtained as

$PRESS = \sum (property_{observed} - property_{predicted})^2$,

$SD = \sum (property_{observed} - property_{mean})^2$.

Eq.3 and 5 of AM1 and PM3 methods respectively give a good q^2_{cv} values, which should be always smaller than %EV. A model is considered to be significant [26] when $q^2_{cv}>0.3$.

Another cross-validation parameter, PRESS which is the sum of the squared differences between the actual and that predicted when the compound is omitted from the fitting process, also supports the predictive ability of Eqs.3 and 6. Its value decreases from Eq.1 to Eq.5.

responsible for higher inhibitory activity.

In PM3 method Hydration energy (HE) and Ionization potential (IP) are also found to be physicochemical parameters for high inhibitory nature for tyrosinase enzyme. Lower values of HE and IP are responsible for higher inhibitory activity.

The quality factor Q ,[27],is defined as the ratio of regression constants (R) to the standard error estimation (SEE),that is , Q = R/SEE. This indicates that the higher the value of R, and the lower the value of SEE, the higher is the magnitude of Q and the better will be the correlation. In present case, Q increases from 2.4966 to 2.5657 and 2.23 to 2.32(Eq. 1 to 5).

4. CONCLUSION

The two aromatic rings in benzyl benzoates are asymmetric, therefore, different position of hydroxyl groups on ring A and B are responsible for the inhibitory effect on tyrosinase. The position of hydroxyl substitute on ring B remarkably effected the inhibition.Inhibitory activity is mainly determined by ring B. The 2',4'-hydroxyl moiety was essential on ring B for binding with active site of tyrosinase. Among these two positions 4'-OH was found to be more important to 2'-OH. If the 4'-OH on ring B is replaced by 2'-OH the inhibitory activity was unexpectedly decreased (Table 1). This infers that combination between 4'-OH and bicopper center of tyrosinase was stronger compared to 2'-OH. The Eq.3 from AM1, semi empirical calculation reveals both HE and IP cause the inhibitory activity. Lower values of HE and IP are

Therefore, HE and IP are responsible for binding the ring A and ring B to the bi copper centre of tyrosinase.

The linear dependence of inhibitory activity on hydration energy (HE) and ionization (IP) were evident from Figure 2 and 3.

Observed activity Vs Predicted activity
(AM1 method)
Activity= -5.45 x10^{-2} HE - 0.206 x IP **(Eq.3)**

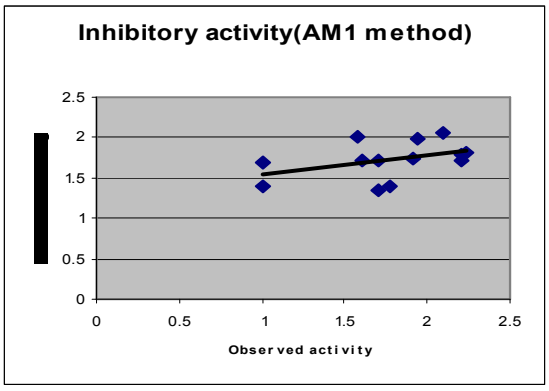


Figure. 2: ***Plot of Observed Versus Predicted Activity (AM1 Method)***

Observed activity Vs Predicted activity
(PM3 method)

Activity=-5.45 x10^{-2} HE - 0.253 x IP **(Eq.5)**

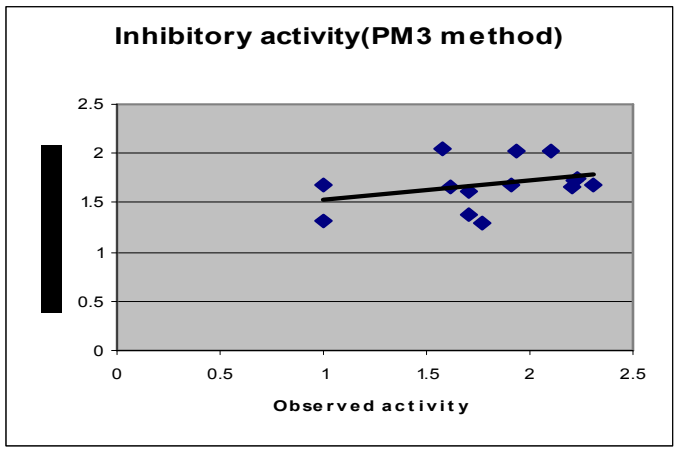
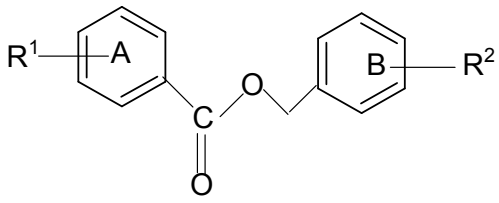


Figure. 3: ***Plot of Observed Versus Predicted Activity (PM3 Method)***



Benzyl benzoate analogs exhibit inhibitory activity for the Agaricus Bisporus Tyrosinase Enzyme. AM1, PM3 semiempirical computational methods are used for estimating physico-chemical parameters.

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