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QSAR STUDIES OF SOME PYRAZOLONES AS ANTIMICROBIAL AGENT

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ABSTRACT

In the present study Quantitative Structure Activity Relationship (QSAR) studies were performed on selected pyrazolone compounds as antimicrobial agent. The compoundshas been established on the basis of analytical methods and advanced spectroscopic techniques. Three compounds *viz*. 4-Amino antipyrine thiosemicarbazone (C-8), 1-(2-Chloro-5-sulfophenyl)-3-methyl-5-pyrazolone (C-9) and 1-(4-Sulfoamido phenyl)-3-methyl-5-pyrazolone (C-10) recorded significant antifungal activity against *Macrophomina phaseolina.*

KEY-WORDS: Antimicrobial activity, *Macrophomina phaseolina*, Pyrazolones, QSAR

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INTRODUCTION

The search for newer drug is an endless effort for which the researchers have always an interesting field open for the discovery of new more efficacious drugs with reduced toxicity profile. The synthesis of heterocyclic compounds has always drawn the attention of medicinal chemists over the years mainly because of their diverse biological properties [1, 2]. Pyrazolone derivatives are versatile nitrogen heterocyclic compounds which have long been known as a promising class of biologically active compounds [3] possessing wide variety of biological and pharmacological activities like antibacterial [4], anthelmintic [5], neuroleptic [6], anti-tubercular [7], anti-fungal [8], anti-cancer [9], anti-inflammatory [10] and anti-viral [11] etc. Quantitative Structure Activity Relationship (QSAR) is one of the most important area in medicinal chemistry, which gives information that is useful for drug design. QSAR models are mathematical equations relating chemical structure to a wide variety of physical, chemical and biological properties. The derived relationship between molecular descriptors and activity is used to estimate the property of other molecules and / or to find the parameters affecting the biological activity. Inspired by the above facts, we are hereby reporting the antimicrobial activity and QSAR studies of ten synthetic pyrazolone compounds against *Macrophomina phaseolina*.

EXPERIMENTAL

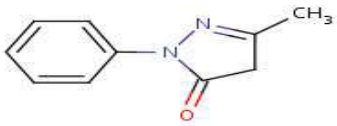
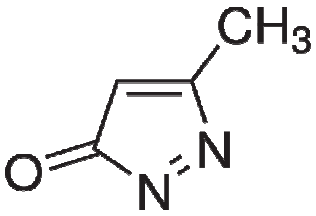
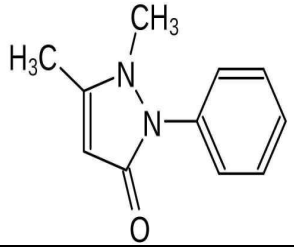
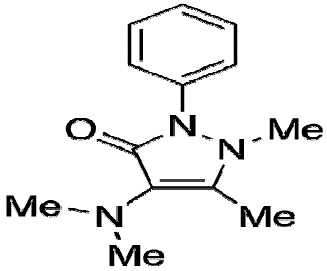
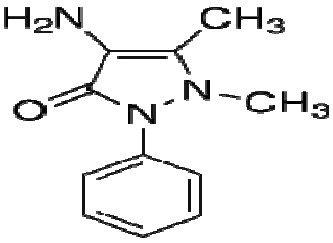
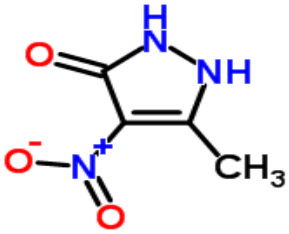
Synthetic Compounds

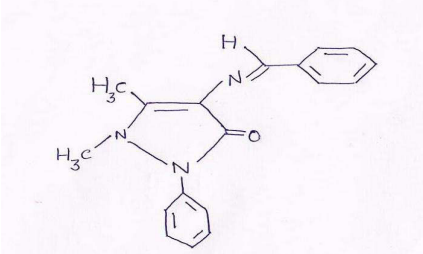
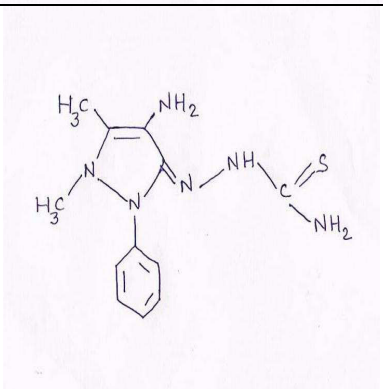
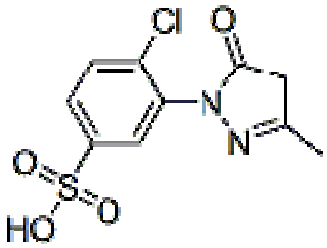
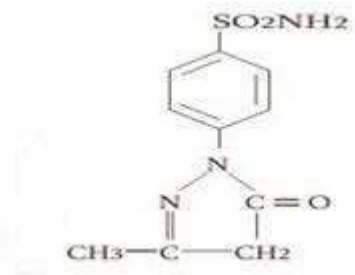
Total ten pyrazolone compounds were selected for study. Structures of pyrazolone compounds under study were constructed using Hyperchem 8.0 professional version software. Structures are shown in Table-1.Compounds were characterized by elemental analysis and spectral studies [12-13].

Test organism

The pathogen used for this study *viz*. *Macrophomina phaseolina* was procured from ITCC, Division of Plant Pathology, Indian Agriculture Research Institute (IARI), New Delhi. Fresh inoculation of fungi was done by taking a loop-full from IARI culture tube and streaking was done on Potato Dextrose Agar (PDA) plates and slants and incubated at 22-24^{o}c for *Macrophomina phaseolina*, for revival and sub-culturing purpose. Media were autoclaved at 121^{o}c for 15 lbs pressure for 15 min. Media were cooled and then plates were prepared by dispensing 15-20 ml medium per plate. Plates were kept in the same position for 0.5 h to solidify media and kept inverted in the incubator at 28^{o}c overnight for sterility checking. 100 µL of test organism was used to inoculate sterile Potato Dextrose Agar plate by spread plate swab method [14-20]. In swab method, the culture sample is applied to the agar plate with a special streaking technique, with the help of sterile cotton swab. Whatmann paper discs, loaded with 5 µL solution of compounds and dried in laminar air flow, were dispensed on inoculated plates at pre-designated positions. The plates were then incubated at 22-24^{o}c for 48 hrs.

Table-1
Code, Name, Molecular weight and Structures of Pyrazolone Compounds

Code	Name of Compound	Molecular weights	Structure
C-1	**3-Methyl-1-phenyl-5-pyrazolone**	174	
C-2	**3-Methyl-5-pyrazolone**	72	
C-3	**Antipyrine**	188	
C-4	**4-(Dimethyl amino) antipyrine**	231	
C-5	**4-Amino antipyrine**	203	
C-6	**3-Methyl-4-nitroso-5-pyrazolone**	143	

C-7	**4-Benzoyl amino antipyrine**	291	
C-8	**4-Amino antipyrine thiosemicarbazone**	276	
C-9	**1-(2-Chloro-5-sulfophenyl)-3-methyl-5-pyrazolone**	288	
C-10	**1-(4 -Sulfoamidophenyl) -3-methyl-5-pyrazolone**	253.28	

RESULTS AND DISCUSSION

Antifungal activity

Ten pyrazolone compounds (C-1 to C-10) as listed in Table-1, were tested for antifungal activity against *Macrophomina phaseolina*. Out of ten compounds, three compounds *viz*. 4-Amino antipyrine thiosemicarbazone (C-8), 1-(2-Chloro-5-sulfophenyl)-3-methyl-5-pyrazolone (C-9) and 1-(4-Sulfoamidophenyl)-3-methyl-5-pyrazolone (C-10) was found significant at the concentration of 1.5 to 5.0 mg against *Macrophomina phaseolina*.The results of MIC are presented in Table-2.

Table 2
Antifungal activity for pyrazolone compounds against Macrophomina phaseolina

Conc→ / ↓Compd	10ml DMF	0.5mg / 10ml	1.0mg / 10ml	1.5mg /10 ml	2.0mg /10 ml	2.5mg /10 ml	3.0mg /10 ml	3.5mg /10 ml	4.0mg /10 ml	4.5mg /10 ml	5.0mg/ 10 ml
C1	0.0	-	-	-	-	-	-	-	-	-	-
C2	0.0	-	-	-	-	-	-	-	-	-	-
C3	0.0	-	-	-	-	-	-	-	-	-	-
C4	0.0	-	-	-	-	-	1.0	1.0	1.0	1.0	1.0
C5	0.0	-	-	-	-	1.0	1.0	1.0	1.0	1.0	1.0
C6	0.0	-	-	-	-	-	1.0	1.0	1.5	1.5	1.5
C7	0.0	-	-	-	-	-	-	-	-	-	-
C8	0.0	-	-	-	-	1.5	1.5	2.0	2.5	2.5	2.5
C9	0.0	-	-	-	-	1.0	1.0	1.5	1.5	2.0	2.0
C10	0.0	-	-	2.0	2.0	2.0	2.0	2.5	2.5	3.0	3.0

Analytical studies of compounds

All the compounds were investigated by m. pt. determination, CHN analysis, Infrared and Mass spectral studies [21-22]. The preliminary investigation of the compounds viz. m. pt. determination was carried out in research laboratory, Dept. of Chemistry, Govt. K.R.G. Auto. P.G. College, Gwalior (MP). CHN analyses of these compounds were carried out on Elemental analyzer, Elemental Vario EL III and the results of m. pt. determination are included in Table-1.

Mass spectral studies of the compounds

Mass spectral studies of compounds were recorded on mass spectrometer, Jeol SX-102 (FAB) at SAIF, CDRI Lucknow. The study of parent ion peaks in mass spectra of any compound helps in the establishment of its molecular weight. Mass spectral studies of the compounds chosen for this study shows the parent ion peaks at 175.3, 99.08, 189.2, 232.1, 204.1, 145, 279, 276, 288.14, 254.10. These compounds show the m/e values where these are expected to come.

Infrared spectral studies of compounds

Infrared spectra of the compounds were recorded on Perkin-Elmer infrared spectrophotometer in the range of 4000 to 50 cm^{-1} at SAIF, CDRI Lucknow. Infrared absorption studies of pyrazolones have been assigned by a comparison of these spectra with those of pyrazole, five membered ring systems and the mono-substituted benzene ring system. The strong band has been assigned to the ring stretching of 5-membered ring in pyrazolone compounds. Five membered ring hetero atomic compounds are found to have two strong bands near 1590-1560 and 1450-1430 cm^{-1} which are considered to be characteristics of five .

QSAR Analysis

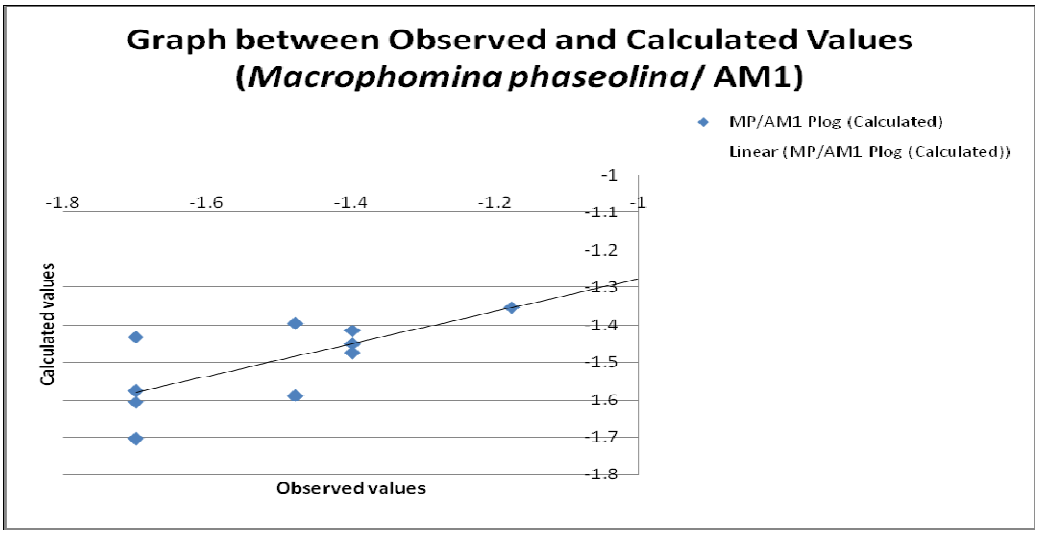
To established a Quantitative Structure Activity Relationship (QSAR) between their *in vitro* activity and descriptors such as Surface Area Approx (SAA), Surface Area Grid (SAG), Volume (VOL), Hydration Energy (HE), Log P (Log P), Refractivity (RF), Polarizability (POL),

Table 3: AM1 computed parameters for pyrazolone compounds

Compounds	SAA ($Å^2$)	SAG ($Å^2$)	VOL ($Å^3$)	HE (Kcal/mol)	Log P	RF ($Å^3$)	POL ($Å^3$)	Mass (amu)	TE (Kcal/mol)	EE (kcal/mol)	HF (kcal/mol)	DM (Debye)	ZPE (kcal/mol)
C-1	354.36	317.33	502.23	-4.16	0.12	46.39	17	168.15	-47433	-229739	307.92	1.96	73.28
C-2	237.23	241.26	334.2	-8.58	0.77	28.24	9.29	96.09	-29947	-105992	51.33	3.74	50.48
C-3	339.75	383.77	608.22	-1.84	0.4	61.23	21.43	188.23	-53153	-302313	63.63	4.41	135.45
C-4	419.06	439.75	732.69	-1.24	1.22	74.92	26.45	231.3	-65414	-426785	72.08	3.87	181.52
C-5	337	399.31	638.89	-6.51	-1.99	64.85	22.78	203.24	-58252	-343522	60.63	3.39	146.4
C-6	262.01	289	425.83	-21.96	-4.58	33.8	12.56	145.12	-50432	-220072	-9.58	3.39	82.74
C-7	442.46	523.41	888.29	-5.11	-0.57	98.38	34.28	293.27	-80807	-583434	94.24	3.71	216.95
C-8	328.97	520.54	853.25	-4.74	-1.34	86.56	31.41	276.36	-73459	-465295	236.13	4.43	174.31
C-9	372.62	422.1	670.98	-10.22	-0.95	70.44	25.16	2256.71	-69740	-379930	24.32	3.08	111.94
C-10	367.78	426.94	688.04	-9.88	-1.44	73.45	25.88	255.72	-67460	-378253	51.06	3.47	123.4

AM1/*Macrophomina phaseolina*/C-1 to C-10/3D-QSAR Equation

p(MIC) = - 0.008063 (POL) – 0.05267 (Log P) – 0.00028 (DM) – 1.73744
N = 10, SD = 0.182242, CC = 0.567886, F-test = 0.952004



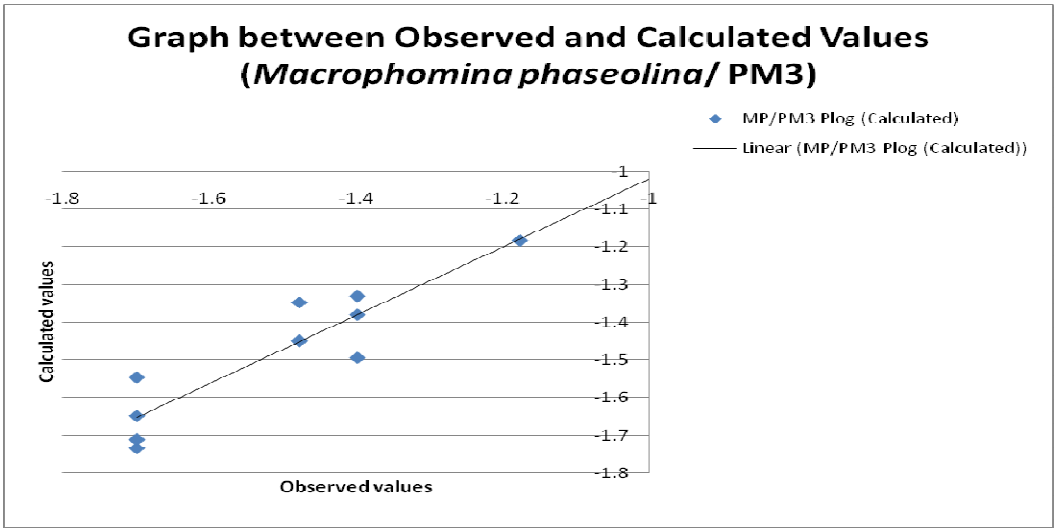
p(MIC) (Observed)	p(MIC) (Calculated)
-1.69897	-1.60724
-1.69897	-1.70414
-1.69897	-1.57695
-1.477121	-1.58951
-1.39794	-1.4499
-1.477121	-1.39589
-1.69897	-1.43206
-1.39794	-1.41484
-1.39794	-1.4754
-1.176091	-1.3539

Table 4: PM3 computed parameters for pyrazolone compounds

Compounds	SAA Å^2	SAG Å^2	VOL Å^3	HE Kcal/mol	Log P	RF Å^3	POL Å^3	Mass amu	TE Kcal/mol	EE kcal/mol	HF kcal/mol	DM Debye	ZPE kcal/mol
C-1	355.76	320.1	502.45	-4.18	0.12	46.39	17	168.15	-42727	-221869	274.63	1.43	71.68
C-2	237.14	238.8	333.28	-8.59	0.77	28.24	9.29	96.09	-26557	-101377	28.65	3.74	49.1
C-3	341.87	385.24	613.47	-1.85	0.4	61.23	21.43	188.23	-48543	-293434	22.75	4.04	130.46
C-4	422.9	443.56	724.65	-1.36	-1.22	74.92	26.45	231.3	-59530	-416062	23.36	4.17	176.43
C-5	343.8	390.57	629.1	-5.59	-1.99	64.85	22.78	203.24	-52645	-333790	22.07	3.7	142.23
C-6	275.65	281.66	409.68	-13.43	-1.74	33.14	11.65	143.1	-44165	-199607	-13.77	7.58	66.01
C-7	445.68	540.81	902.51	-5.11	-0.57	98.38	34.28	293.37	-73894	-573707	46.4	3.34	209.28
C-8	-41.32	397.64	659.91	3.7	-1.32	86.56	31.41	276.36	-65062	-462886	210.67	4.66	168.53
C-9	375.37	417.82	672.39	-11.64	-0.95	70.44	25.16	256.71	-63114	-369363	-17.9	4.92	111.27
C-10	371.32	435	693.47	-10.86	-1.44	73.45	25.88	255.72	-60446	-365609	19.35	4.5	119.43

PM3/*Macrophomina phaseolina*/C-1 to C-10/3D-QSAR Equation

p(MIC) = - 0.00471 (HE) – 0.15084 (Log P) – 0.00384 (DM) – 1.64337
N = 10, SD = 0.133334, CC = 0.798337, F-test = 3.514843



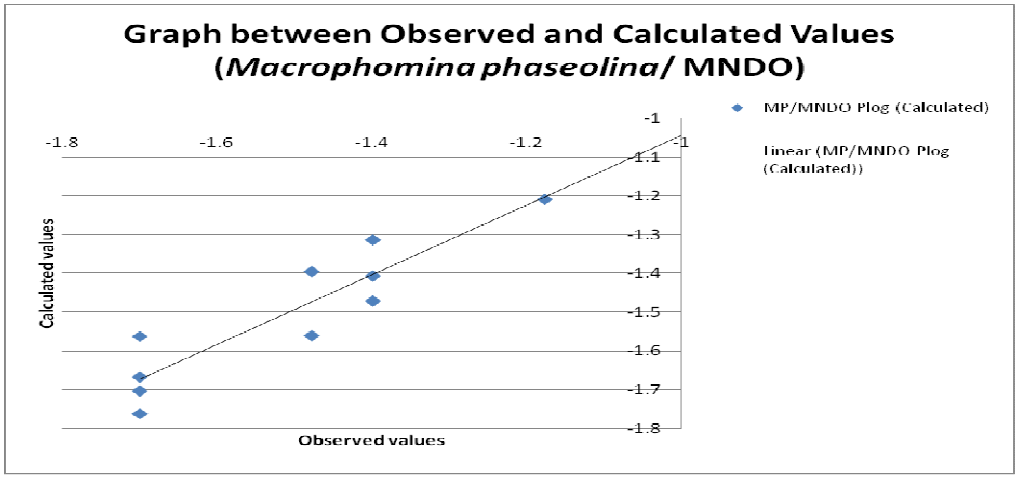
p(MIC) (Observed)	p(MIC) (Calculated)
-1.69897	-1.64727
-1.69897	-1.73342
-1.69897	-1.71051
-1.477121	-1.44895
-1.39794	-1.33108
-1.477121	-1.34676
-1.69897	-1.54615
-1.39794	-1.37958
-1.39794	-1.49414
-1.176091	-1.18229

Table 5: MNDO computed parameters/ SAR descriptors for pyrazolone compounds

Compounds	SAA Å^2	SAG Å^2	VOL Å^3	HE Kcal/mol	Log P	RF Å^3	POL Å^3	Mass amu	TE Kcal/mol	EE kcal/mol	HF kcal/mol	DM Debye	ZPE kcal/mol
C-1	356.68	321.7	506.12	-4.18	0.12	46.39	17	168.15	-47472	-228849	291.38	1.09	74.32
C-2	237.6	242.64	334.3	-8.56	0.77	28.24	9.29	96.09	-30047	-106208	17.61	3.6	52.65
C-3	340.55	392.42	620.67	-1.83	-0.4	61.23	21.43	188.23	-53297	-302001	28.81	3.71	139.85
C-4	423.24	461.04	759.23	-1.26	-1.22	74.92	26.45	231.3	-65604	-426490	39.68	3.34	187.01
C-5	339.34	415.13	658.53	-6.43	-1.99	64.85	22.78	203.24	-58408	-343088	28.77	2.87	151.05
C-6	278.99	288.33	416.15	-13.34	-1.74	33.14	11.65	143.1	-49945	-207139	10.5	6.5	71.32
C-7	440.29	534.89	917.75	-4.9	-0.57	98.38	34.28	293.37	-80986	-591131	59.57	2.58	223.36
C-8	-41.32	397.64	659.91	-3.7	-1.32	86.56	-31.44	276.36	-74378	-456409	230.21	2.8	181.98
C-9	390.91	442.86	694.31	-11.72	-0.95	70.44	25.16	256.71	-70251	-377400	-14.43	3.38	119.6
C-10	380.47	451.86	708.67	-10.74	-1.44	73.45	25.88	255.72	-67932	-376391	25.75	3.04	127.58

MNDO/*Macrophomina phaseolina*/C-1 to C-10/3D-QSAR Equation

p(MIC) = - 0.01926 (HE) – 0.16481 (Log P) – 0.04808 (DM) – 1.62619
N = 10, SD = 0.114839, CC = 0.854971, F-test = 5.43426



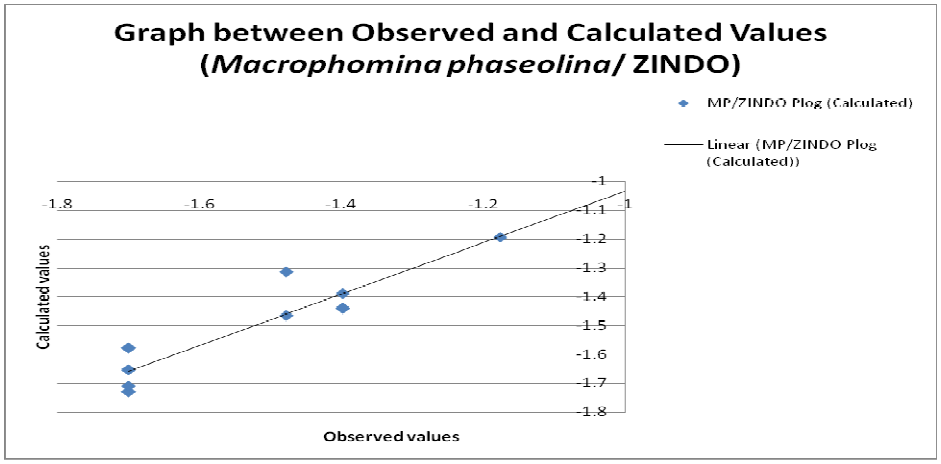
p(MIC) (Observed)	p(MIC) (Calculated)
-1.69897	-1.66787
-1.69897	-1.76132
-1.69897	-1.7034
-1.477121	-1.56144
-1.39794	-1.31237
-1.477121	-1.39501
-1.69897	-1.56192
-1.39794	-1.472
-1.39794	-1.4064
-1.176091	-1.20817

Table 6: ZINDO computed parameters/ SAR descriptors for pyrazolone compounds

Compounds	SAA Å^2	SAG Å^2	VOL Å^3	HE Kcal/mol	Log P	RF Å^3	POL Å^3	Mass amu	TE Kcal/mol	EE kcal/mol	HF kcal/mol	DM Debye	ZPE kcal/mol
C-1	339.01	322.6	492.53	0.35	0.12	46.39	17	168.15	-66169	-278099	-4133.4	6.17	100.03
C-2	235.87	238.28	330.45	-8.67	0.77	28.24	9.29	96.09	-41929	-129187	-2117.6	4.71	69.33
C-3	333.46	370.99	592.48	-1.86	-0.4	61.23	21.43	188.23	-74016	-354456	-5450.6	7.09	184.89
C-4	422.9	443.56	724.65	-1.36	-1.22	74.92	26.45	231.3	-91086	-493720	-6820	5.86	249.13
C-5	332.57	389.92	622.96	-6.59	-1.99	64.85	22.78	203.24	-81172	-400070	-5727	6.4	199.12
C-6	274.94	280.08	403.32	-12.94	-1.74	33.14	11.65	143.1	-70596	-249055	-2845.1	10.92	92.37
C-7	445.68	540.81	902.51	-5.11	-0.57	98.38	34.28	293.37	-112180	-662184	-8687.9	4.21	290.62
C-8	253.93	442.53	738.14	-9.06	-1.32	86.56	31.41	276.36	-102800	-609648	-7076.6	12.05	246.95
C-9	375.37	417.82	672.39	-11.64	-0.95	70.44	25.16	256.71	-95269	-441882	-5087.1	9.5	155.84
C-10	348.71	416.91	671.69	-10.31	-1.44	73.45	25.88	255.72	-91772	-437150	-5240.2	8.03	166.75

ZINDO/*Macrophomina phaseolina*/C-1 to C-10/3D-QSAR Equation

p(MIC) = - 0.01215 (HE) – 0.13079 (Log P) + 0.002321 (DM) – 1.72535
N = 10, SD = 0.128541, CC = 0.814214, F-test = 3.933753



p(MIC) (Observed)	p(MIC) (Calculated)
-1.69897	-1.73098
-1.69897	-1.7097
-1.69897	-1.65398
-1.477121	-1.46566
-1.39794	-1.39016
-1.477121	-1.31521
-1.69897	-1.57894
-1.39794	-1.441466
-1.39794	-1.43762
-1.176091	-1.19311

Mass (Mass), Total Energy (TE), Electronic Energy (EE), Heat of Formation (HF), Dipole Moment (DM), Zero Point Energy (ZPE), biological activity data determined as MIC values are first transformed into p (MIC) values and used as dependent variable in QSAR study. The computed values for these descriptors using AM1, PM3, MNDO and ZINDO methods are mentioned along with their QSAR equations (Table-3 to Table-6). Statistical parameters such as Correlation Coefficient (CC), Standard Error (SD) and Fischer-test (F-test) etc were considered to select best QSAR model. A set of parameters / descriptors were subjected to stepwise linear regression analysis in order to develop QSAR equation with different values of CC, SD and F-test. Correlation matrices of the parameters / descriptors used in the QSAR studies were also constructed using AM1, PM3, MNDO and ZINDO methods.The selected QSAR equations are mentioned here and graphs were also plotted between observed and predicted activities. The observed and predicted activities are mentioned along with graphs.

CONCLUSION

The results obtained from present investigation of *in vitro* antifungal activity against *Macrophomina phaseolina* indicate that the 4-Amino antipyrine thiosemicarbazone (C-8), 1-(2-Chloro-5-sulfophenyl)-3-methyl-5-pyrazolone (C-9) and 1-(4-Sulfoamidophenyl)-3-methyl-5-pyrazolone (C-10) are the most effective ones. Further, a general trend showed that the presence of electron withdrawing group (NO_2) leads to increase the activity in comparison to the presence of other group. The low value of standard error (SD) indicated in equations predict the ability of the developed QSAR models.

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