Does electron-correlation has any role in the quantitative structure–activity relationships?

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ABSTRACT

For developing quantitative structure–activity relationships (QSARs), quantum-mechanical molecular descriptors based on the state-of-the-art quantum-mechanical methods such as Hartree–Fock (HF) method and density-functional theory (DFT), are now routinely employed. The validity of these quantum-mechanical methods, however, rests on the accurate estimation of electron-correlation energy. This work analyses the role of electron-correlation, using correlation energy as a molecular descriptor, in the QSARs. In particular, QSAR models, for the mutagenic activity of a set of nitrated polycyclic aromatic hydrocarbons (nitro-PAHs), are examined for the role of electron-correlation through state-of-the-art external validation parameters such as concordance correlation coefficient and recently proposed predictive squared correlation coefficients, namely, Q_{F1}^2, Q_{F2}^2, and Q_{F3}^2 etc. The electron-correlation contribution to the highest occupied and lowest unoccupied molecular orbital (HOMO/LUMO) energies is also analyzed. QSAR models based on the semi-empirical quantum-mechanical methods like PM6 and RM1 are also compared. It is found that the models, developed using electron-correlation contribution of the quantum-mechanical descriptors, are not only robust but also relatively more predictive than those developed with the HF and DFT descriptors. The latter are found to be even less reliable than PM6 and RM1 descriptors based models, which show comparable robustness and predictivity with those developed using electron correlation based descriptors. The external predictivity of model based on semi-empirical descriptors can be improved if electron-correlation contribution of the quantum-mechanical descriptors is explicitly included in the model. This work reports the first-ever use of electron-correlation energy and its contribution to the HOMO/LUMO energies as molecular descriptors.

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1. Introduction

Electron-correlation [1,2] describes the instantaneous correlated motion of electrons in a molecule. It is one of the crucial quantities that decides the molecular geometry and hence, the molecular properties. For example, electron-correlation energy amounts to ~1 kcal/mol, which is a fraction of the total energy of a molecule, but it is vital for the existence of a chemical bond, and its contribution towards the bond formation can be as much as 100% of the chemical bonding. This becomes more important since the biological activity of a molecule can be related to its geometrical structure and changes in the electronic environment. All the advanced quantum-mechanical approaches attempt for the accurate estimation of the electron-correlation in order to be an acceptable method. The *ab initio* wave-function (Ψ) based approaches such as Hartree–Fock (HF) method [3] has now replaced semiempirical (SE) quantum-mechanical methods

for the computations of molecular descriptors for quantitative structure–activity relationships (QSAR) analysis. The SE methods [4] such as AM1, PM3, MINDO etc., widely used earlier, can be highly unreliable as they may yield inaccurate orbital energies. However, the newly developed SE methods, namely, RM1 [5] and PM6 [6], may perform even better [7,8] than the *ab initio* methods. The HF method is being deployed nowadays in most of the drug-designing and QSAR analysis software for purpose of calculation of molecular descriptors. However, HF method is not only computationally uneconomical, but also neglects a significant part of the electron-correlation. HF method does not take into account the instantaneous electrostatic interactions between the electrons; it only considers the electron-electron interactions in an average way. An alternative to the HF method is the density functional theory (DFT) [9,10] that includes electron-correlation through the exchange-correlation (XC) energy density functional. Since DFT, for a time-independent non-relativistic *N*-electron system being based on three-dimensional electron-density (ρ) rather than 3*N* dimensional wave function, is computationally less extensive, quantum-mechanical molecular descriptors based on the DFT are now most sought after for developing QSARs [11–14]. During

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recent years [15,16], there has been a spurt in the use of DFT based molecular descriptors that include chemical potential, chemical hardness, softness and electrophilicity index to name a few. In any case, the deciding constituent remains the accurate computation of electron-correlation by the chosen quantum mechanical method. In this preliminary work, we address an important question: Up to what extent the contribution of electron-correlation is significant in the quantum mechanical descriptors used for developing QSARs?

Conventionally, electron-correlation is described through the correlation energy [2],

$$E_{\mathrm{CORR}} = E_{\mathrm{exact}} - E_{\mathrm{HF\text{-}Limit}}, \tag{1}$$

where E_{exact} is the exact non-relativistic energy of an atomic/molecular species, and $E_{\mathrm{HF\text{-}Limit}}$ is the HF limiting energy which is the lowest energy possible if a complete basis set (functions used to represent orbitals) is employed in the HF calculations. The HF method includes only the exchange interactions exactly, however, neglects electron-correlation entirely, whereas the DFT incorporates electron-correlation along with the exchange interactions through the XC energy density functional. Following this, if E_{exact} is computed through the DFT, then the electron-correlation energy, to an extent of an approximation, can be formulated as,

$$E_{\mathrm{CORR}} \approx E_{\mathrm{DFT}} - E_{\mathrm{HF\text{-}Limit}}. \tag{2}$$

This is a reasonably valid approximation, since the sum of the HF energy with the exact correlation energy approximately equates to the DFT energy (see the next section). This is in fact true if the XC energy functional is exact. However, exact XC functional is still unknown, and one has to rely on an approximate XC functional. Moreover, most of the electron-correlation can be estimated if highly correlated approaches such as many-body perturbation theories (MBPT), coupled-cluster methods [3], full-configuration interaction (CI) method [17] or other advance quantum mechanical methods are chosen. Such approaches, however, have their own inherent problems, and are practically not realizable for large molecules. Furthermore, since a complete basis set requires an infinite number of functions, only a finite basis set has to be employed in the HF calculations for computation of $E_{\mathrm{HF\text{-}Limit}}$. This, however, results in a basis set truncation error. The latter can be minimized by a clever choice of the basis set such as a Gaussian basis set (see next section). Moreover, since DFT can yield energies even lower than the true value, Eq. (2) may result in the overestimation of electron-correlation. However, Eq. (2) remains the simplest route available for estimation of electron-correlation, though it yields only an approximate correlation energy.

To the best of our knowledge, the electron-correlation energy as a molecular descriptor is yet to be deployed for developing QSARs, and there has been no implementation of it in any of the available software for drug-designing and QSAR analysis. This may be due to the reason that the computation of electron-correlation energy is not only difficult but also computationally quite expensive. In this work, the electron-correlation energy is used as a molecular descriptor to develop QSARs for the mutagenic activity of a set of nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) acting on TA98 stain of *Salmonella typhimurium* bacteria [18]. The robustness and predictive ability of the developed QSARs is tested using both internal validation and the state-of-the-art external validation procedures employing an external prediction set. The QSARs developed with E_{CORR} are compared with those developed using the total energy of the molecule (E) and energies of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) as molecular descriptors calculated through PM6, RM1, HF and DFT methods. Through these QSARs, our primary aim

is to check whether the contribution of electron-correlation is significant in the QSARs as far as internal validation and external predictability of the model is concerned. The paper is organized as follows: Section 2 outlines the theoretical and computational methodology for calculating the electron-correlation energy and other molecular descriptors. Section 3 explains the methodology of model development and validation, namely, splitting of the data set into training set and external prediction set, employment of internal and external validation parameters to test robustness and predictive ability of the model. This is followed by results and discussion in Section 4 that compares the robustness and external predictive capability of developed QSAR models to see any role of electron-correlation and the descriptors based on it. Finally, the last section makes a few concluding remarks.

2. Theoretical and computational methodology

In the HF method, the total energy of a system is described as [1–3,9,10],

$$E_{\mathrm{HF}}[\Psi] = V_{\mathrm{NN}} + T_{\mathrm{e}} + V_{\mathrm{Ne}} + J_{\mathrm{ee}} - K_{\mathrm{ee}} \tag{3}$$

where V_{NN} is the potential energy resulting due to the nuclear-nuclear Coulombic repulsion, T_{e} is the electronic kinetic energy, and V_{Ne} is the potential energy due to the nuclear-electron Coulombic attraction. The last two terms on the RHS of Eq. (3) correspond to the classical coulomb repulsion of the electrons (J_{ee}), and non-classical exchange energy (K_{ee}) due to the quantum nature of the electrons. The difference between these two terms accounts for the electron-electron Coulombic repulsion, $V_{\mathrm{ee}} = J_{\mathrm{ee}} - K_{\mathrm{ee}}$. In the ground-state DFT of Kohn and Sham (KS) [9,10], the exact HF exchange is replaced by the XC energy density functional that accounts both for the exchange and correlation energy. In the KS formulation of DFT, the total energy of the system is described as,

$$E_{\mathrm{DFT}}[\rho] = V_{\mathrm{NN}} + T_{\mathrm{e}} + V_{\mathrm{Ne}} + J_{\mathrm{ee}} + E_{\mathrm{X}}[\rho] + E_{\mathrm{CORR}}[\rho] \tag{4}$$

where $E_{\mathrm{X}}[\rho]$ is the exchange energy density functional of electron-density ρ, and $E_{\mathrm{CORR}}[\rho]$ is the correlation energy density functional. The exact exchange- and correlation energy density functionals, however, are yet unknown. There are various approximate XC energy density functionals available, one of the widely used being a hybrid XC energy density functional: B3LYP [19]. Further, comparing Eqs. (3) and (4), it can be seen that in a special case when $E_{\mathrm{CORR}} = 0$ and $E_{\mathrm{X}}[\rho] = -K_{\mathrm{ee}}$, the DFT and HF energies are equivalent. Therefore, the electron-correlation contribution to the energy or any molecular descriptors can be determined, for example, with respect to density functional theory as,

$$D_{\mathrm{CORR}} = D_{\mathrm{DFT}} - D_{\mathrm{HF}} \tag{5}$$

where D is one of the descriptors such as E, E_{LUMO}, E_{HOMO} etc., computed with HF and DFT methods at the same level of theory employing same basis set. Using such electron-correlation based molecular descriptors, QSAR models were developed for the mutagenic activity of a set of 51 nitro-PAHs (listed in Table 1) acting on TA98 stain of *S. typhimurium*. The geometry of each of the compound studied was optimized using 6-311G(d,p) basis set with both the HF and DFT methods. For the latter, a hybrid B3LYP XC functional was employed. Each geometry optimization was followed by the frequency analysis at the same level of the theory and using the same basis set to check if the optimized geometry corresponds to the true minima. All the SE calculations using RM1 and PM6 methods were performed using MOPAC2012 [20] while HF and DFT computations were performed using Gaussian 03 [21] suite of quantum-chemistry software package. Further, various molecular descriptors such as E, E_{LUMO}, E_{HOMO} were obtained through the DFT and HF method as well as using RM1 and PM6 semi-empirical methods. From the DFT and HF based descriptors, electron-correlation

Table 1

Quantum-mechanical descriptors (all in a.u., 1 a.u. = 27.21165 eV) calculated with PM6, RM1, HF/6-311G(d,p) and DFT/B3LYP/6-311G(d,p) level of the theory for different nitro-PAHs along with the mutagenicity expressed as log TA98 (in log revertants/nmol).

C	Name of compound	LOG TA98[a]	$-E_{PM6}$	$-E_{RM1}$	$-E_{HF}$	$-E_{DFT}$	$-E_{CORR}$	$-E_{PM6}^{HOMO}$	$-E_{RM1}^{HOMO}$	$-E_{HF}^{HOMO}$	$-E_{DFT}^{HOMO}$	$-E_{CORR}^{HOMO}$	$-E_{PM6}^{LUMO}$	$-E_{RM1}^{LUMO}$	E_{HF}^{LUMO}	$-E_{DFT}^{LUMO}$	$-E_{CORR}^{LUMO}$
1	2,3,4-Trichloronitrobenzene	−2.94	83.947	101.807	1811.006	1815.700	4.694	0.377	0.379	0.380	0.287	0.093	0.072	0.053	0.043	0.108	0.151
2	2-Nitroanisole	−2.70	78.317	78.317	548.174	551.406	3.232	0.369	0.369	0.355	0.254	0.101	0.029	0.029	0.061	0.091	0.152
3	4-Nitroanisole	−2.70	73.604	78.322	548.189	551.421	3.232	0.361	0.357	0.346	0.256	0.090	0.043	0.029	0.067	0.086	0.153
4	2-Nitrobenzaldehyde	−1.92	72.579	77.260	547.022	550.035	3.013	0.389	0.387	0.379	0.277	0.102	0.070	0.042	0.038	0.113	0.151
5	2,4-Dinitroanisole	−1.89	101.007	108.640	751.695	755.962	4.267	0.388	0.382	0.375	0.279	0.096	0.066	0.052	0.042	0.106	0.148
6	3,4-Dinitro-1-fluorobenzene	−1.84	101.592	108.741	736.483	740.539	4.057	0.416	0.406	0.313	0.224	0.089	0.069	0.053	−0.009	0.132	0.123
7	2-Chloronitrobenzene	−1.72	66.243	74.633	893.182	896.472	3.290	0.377	0.373	0.367	0.279	0.088	0.057	0.034	0.059	0.097	0.156
8	2,4-Difluoronitrobenzene	−1.66	91.013	95.820	632.019	635.380	3.361	0.405	0.390	0.386	0.291	0.095	0.064	0.050	0.052	0.102	0.154
9	2,5-Dichloronitrobenzene	−1.54	75.099	88.223	1352.099	1356.090	3.991	0.372	0.372	0.367	0.278	0.089	0.066	0.046	0.047	0.106	0.153
10	2,3-Dichloronitrobenzene	−1.51	75.095	88.221	1352.094	1356.086	3.992	0.372	0.372	0.371	0.281	0.090	0.065	0.043	0.052	0.102	0.154
11	3-Chloro-4-fluoronitrobenzene	−1.21	83.058	92.023	992.068	995.743	3.675	0.383	0.380	0.378	0.287	0.091	0.067	0.051	0.043	0.108	0.151
12	2,5-Difluoronitrobenzene	−0.79	91.008	95.820	632.015	635.377	3.363	0.394	0.390	0.375	0.282	0.093	0.069	0.050	0.043	0.106	0.149
13	1-Methyl-2-nitronaphthalene	−0.70	81.894	86.143	625.994	629.861	3.867	0.347	0.336	0.314	0.241	0.073	0.051	0.037	0.057	0.091	0.148
14	1-Nitronapthalene	−0.61	76.377	80.481	586.949	590.535	3.586	0.353	0.341	0.319	0.246	0.073	0.059	0.035	0.049	0.097	0.146
15	2-Nitronapthalene	−0.30	76.380	80.485	586.957	590.542	3.584	0.354	0.341	0.319	0.247	0.072	0.057	0.039	0.045	0.098	0.143
16	4-Fluoronitrobenzene	−0.23	74.204	78.437	533.154	536.128	2.974	0.398	0.383	0.380	0.288	0.092	0.058	0.041	0.053	0.100	0.153
17	1,3-Dinitronapthalene	−0.05	103.778	110.797	790.467	795.087	4.620	0.376	0.363	0.344	0.268	0.076	0.072	0.055	0.017	0.121	0.138
18	1,3-Dinitrobenzene	0.03	84.797	91.368	637.793	641.415	3.622	0.423	0.410	0.404	0.316	0.088	0.080	0.061	0.023	0.121	0.144
19	1,4-Dinitrobenzene	0.15	84.796	91.368	637.792	641.415	3.623	0.418	0.407	0.399	0.314	0.085	0.092	0.070	0.007	0.134	0.141
20	2,4-Dinitro-1-chlorobenzene	0.30	93.641	104.948	1096.697	1101.022	4.325	0.402	0.400	0.401	0.306	0.095	0.070	0.055	0.017	0.125	0.142
21	1,3,8-Trinitronapthalene	0.35	131.177	141.113	993.966	999.622	5.656	0.399	0.385	0.372	0.287	0.085	0.102	0.083	0.000	0.134	0.134
22	1,5-Dinitronapthalene	0.52	103.779	110.797	790.460	795.081	4.621	0.378	0.366	0.347	0.268	0.079	0.072	0.054	0.022	0.119	0.141
23	1,8-Dinitronapthalene	0.90	103.767	110.790	790.452	795.073	4.621	0.375	0.363	0.347	0.266	0.081	0.071	0.052	0.029	0.108	0.137
24	2,4-Dinitro-1-fluorobenzene	1.20	101.606	108.752	736.658	740.667	4.010	0.426	0.410	0.410	0.313	0.097	0.085	0.069	0.024	0.122	0.146
25	2-Nitrofluorene	1.43	90.345	94.772	701.758	706.098	4.340	0.336	0.324	0.314	0.245	0.069	0.056	0.037	0.046	0.095	0.141
26	2,3,5-Trinitronapthalene	1.51	131.171	141.106	993.964	999.621	5.657	0.396	0.384	0.372	0.288	0.084	0.094	0.076	0.004	0.133	0.137
27	10-Hydroxy-1-nitropyrene	1.89	115.081	120.753	890.276	895.695	5.420	0.322	0.311	0.285	0.215	0.070	0.064	0.049	0.029	0.087	0.116
28	2-Nitro-9,10-dihydrophenanthrene	1.99	96.396	100.998	740.804	745.425	4.620	0.343	0.337	0.315	0.244	0.071	0.053	0.037	0.046	0.095	0.141
29	2-Nitrophenanthrene	2.11	95.374	99.925	739.640	744.220	4.580	0.344	0.332	0.308	0.240	0.068	0.057	0.040	0.043	0.097	0.140
30	9-Nitrophenanthrene	2.25	95.370	99.921	739.670	744.212	4.542	0.346	0.335	0.313	0.242	0.071	0.060	0.037	0.050	0.095	0.145
31	1-Nitrofluoranthene	2.74	104.348	109.090	815.381	820.439	5.059	0.343	0.330	0.305	0.237	0.068	0.068	0.052	0.024	0.108	0.132
32	1-Nitropyrene	2.78	104.373	109.117	815.402	820.461	5.060	0.331	0.316	0.287	0.226	0.061	0.071	0.054	0.031	0.102	0.133
33	2-Nitroanthracene	2.95	95.366	99.916	739.630	744.212	4.583	0.330	0.316	0.285	0.222	0.063	0.066	0.049	0.030	0.103	0.133
34	2-Nitro-4,5-dihydropyrene	3.27	105.397	110.198	816.573	820.467	3.894	0.339	0.328	0.303	0.224	0.079	0.053	0.038	0.046	0.095	0.141
35	2-Nitropyrene	3.35	104.369	109.120	815.409	820.460	5.051	0.327	0.314	0.284	0.223	0.061	0.058	0.043	0.045	0.099	0.144
36	4-Nitropyrene	3.39	104.368	109.119	815.401	820.460	5.059	0.327	0.314	0.284	0.223	0.061	0.060	0.044	0.037	0.099	0.136
37	1-Hydroxy-3-nitropyrene	3.87	115.083	120.752	890.280	895.705	5.424	0.319	0.307	0.277	0.215	0.062	0.061	0.045	0.033	0.102	0.135
38	2,7-Dinitropyrene	4.58	131.776	139.440	1020.094	1025.021	4.927	0.347	0.333	0.304	0.242	0.062	0.077	0.061	0.015	0.117	0.132
39	1,3,6,8-Tetranitropyrene	4.99	186.571	200.062	1425.925	1434.094	8.169	0.385	0.371	0.352	0.277	0.075	0.122	0.104	−0.038	0.161	0.123
40	1,3,6-Trinitropyrene	4.99	159.174	169.751	1222.421	1229.553	7.132	0.368	0.353	0.331	0.261	0.070	0.104	0.086	−0.017	0.143	0.126
41	1,3-Dinitropyrene	5.04	131.780	139.430	1018.912	1025.006	6.094	0.352	0.334	0.308	0.244	0.064	0.094	0.078	0.004	0.124	0.128
42	1,6-Dinitropyrene	5.06	131.778	139.435	1018.913	1025.008	6.095	0.352	0.336	0.305	0.242	0.063	0.091	0.073	0.017	0.117	0.134
43	1,8-Dinitropyrene	5.39	131.777	139.435	1018.913	1025.009	6.096	0.352	0.336	0.307	0.244	0.063	0.091	0.073	0.012	0.124	0.136
44	Nitrobenzene	na	57.390	61.048	434.277	436.865	2.588	0.390	0.379	0.369	0.287	0.082	0.051	0.032	0.055	0.096	0.151
45	2-Fluoronitrobenzene	na	74.199	78.433	533.142	536.117	2.975	0.393	0.379	0.372	0.284	0.088	0.057	0.041	0.055	0.098	0.153
46	3-Fluoronitrobenzene	na	74.201	78.434	533.152	536.127	2.975	0.393	0.379	0.372	0.284	0.088	0.062	0.044	0.044	0.104	0.148
47	3-Chloronitrobenzene	na	66.246	74.638	893.195	896.484	3.288	0.379	0.375	0.369	0.282	0.087	0.061	0.043	0.043	0.106	0.149
48	4-Chloronitrobenze	na	66.248	74.639	893.197	896.485	3.288	0.385	0.380	0.376	0.285	0.091	0.062	0.045	0.044	0.105	0.149
49	2,4-Dichloronitrobenzene	na	75.101	88.223	1352.100	1356.091	3.991	0.385	0.380	0.378	0.286	0.092	0.666	0.047	0.047	0.105	0.152
50	3,4-Dichloronitrobenzene	na	75.099	88.226	1352.109	1356.099	3.990	0.378	0.377	0.377	0.286	0.091	0.069	0.053	0.034	0.112	0.146
51	1,2-Dinitrobenzene	na	84.784	91.362	637.608	641.278	3.670	0.415	0.407	0.306	0.219	0.087	0.075	0.057	−0.003	0.127	0.124

na, inactive compounds.

[a] Ref. [18] and references therein.

contribution towards each of the descriptors was calculated, and the electron-correlation based descriptors were formulated using Eq. (5).

Employing these descriptors, various QSAR models were developed and validated through the linear and multilinear regression (MLR), using QSARINS [22] software for QSAR MLR model development and validation, as described in the next section. The experimental values of the mutagenicity, reported as logTA98 (in log revertants/nmol), were taken from the existing literature [18]. It should be noted that more than 200 nitro-PAHs are reported in Ref. [18], however, in the present work we have considered mainly those compounds which do not have any heteroatom in the ring. It should be further noted that the computations using HF and DFT methods are still expensive; therefore, compounds falling mainly into the category of homogeneous-ring congeners were only selected for the present study. These congeners form a major class of environmental pollutants particularly in automobile emissions. The selection of the compounds is made such that the entire range of mutagenic activities is covered, and the effect of different isomers is incorporated so that the present selection is representative of the data set of Debnath et al. [18].

3. Model development and validation

The data set of 51 nitro-PAHs (listed in Table 1) is arranged in the increasing order of response (activity), and is divided into active (C1–43) and inactive (C44–51) compounds. The compounds with two or more fused benzene rings such as naphthalene, anthracene, phenenthrene, pyrene show significant activity. In fact, the fused ring systems are known to be mutagenic. A few of the compounds, namely, 1,3,6,8-tetranitropyrene, 1,3,6-trinitropyrene, 2-nitro-4,5-dihydropyrene, 2,3,4-trichloronitrobenzene, are identified as either high leverage (structural outlier) or response outlier, observed through the Williams plot of hat values vs standardized residuals, in most of the models with different splitting methods [23,24]. The compounds having cross-validated standardized residuals of more than three standard deviation units are generally taken as response outlier that can also be identified from the Williams plot. Such compounds are generally avoided in the model development as they can be very influential in the determination of model parameters and hence, can be highly unreliable. This also ensures a proper quantitative measure of the model applicability domain. It should be, however, noted that since this can reduce the data set drastically in particular when different models are being compared, therefore, compounds occurring as response outlier or with high leverage in most of the models are excluded. The data set of active compounds is further split into training and prediction set using random splitting through activity sampling. The training set is chosen such that it includes the most active and the least active compound. The training and prediction set is constituted taking alternate compounds, when arranged according to the ordered response. Such splitting ensures that the prediction set spans over whole observed activities. In addition, different random splitting of the data set was also performed with prediction set constituting 35–50% of the data set (see supporting information Tables S1–S5). The splitting of the data set, and further model development and validation were performed through QSARINS [22] software. The regression models developed for the training set are first internally validated on the basis of simple statistical parameters such as coefficient of determination (R^2), adjusted R^2, mean absolute error (MAE) and cross-validated R^2 using leave-one-out method (Q_{LOO}^2 or Q_{CV}^2), which gives an estimate of model robustness. The robustness of the model was further confirmed through cross-validated leave-many-out, Q_{LMO}^2 (with 5000 iterations and randomly leaving 30% of the compounds from the training set at a time), and through

Y scrambling (Q_{Yscr}^2) and randomization procedures (Q_{Yrand}^2) with 1000 iterations. The later procedure should yield Q_{Yscr}^2 values significantly lower than the proposed model to ensure the model's validity and avoid any chance-correlation. Further, QUIK rule (Q Under Influence of K) [25] was also applied to check descriptor collinearity through permissible values of ΔK. Besides this, statistical significance of the regression models is also tested using p-values.

However, to access the real predictive power of a QSAR model, the latter should be tested on entirely external chemicals not used in model building. For a more rigorous validation [23–28], external-validation using the external prediction set was performed. The external validation parameters analyzed include predictive squared correlation coefficient Q_{F1}^2 of Shi et al. [29,30], Q_{F2}^2 of Schüürmann et al. [31], and Q_{F3}^2 of Consonni et al. [27], having following formulation, respectively,

$$Q_{F1}^2 = 1 - \frac{\sum_{i=1}^{n_{EXT}}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n_{EXT}}(y_i - \bar{y}_{TR})^2}, \quad Q_{F2}^2 = 1 - \frac{\sum_{i=1}^{n_{EXT}}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n_{EXT}}(y_i - \bar{y}_{EXT})^2}, \quad \text{and}$$

$$Q_{F3}^2 = 1 - \frac{\left[\sum_{i=1}^{n_{EXT}}(y_i - \hat{y}_i)^2\right]/n_{EXT}}{\left[\sum_{i=1}^{n_{TR}}(y_i - \bar{y}_{TR})^2\right]/n_{TR}} \tag{6}$$

where y_i is the experimental response value in the corresponding training (TR) and external prediction (EXT) set with the mean values being $\bar{y}_{TR}$ and $\bar{y}_{EXT}$, respectively, whereas $\hat{y}_i$ is the computed value from the model. n_{TR} and n_{EXT} corresponds to the total number of objects in the training and external prediction data set, respectively. It should be noted that the range of R^2 is between 0 and 1, whereas Q^2 can be negative. In fact, the value range $-\infty \leq Q^2 \leq 1$ holds true. The negative value may results when the predictive residual sum of squares (PRESS) takes infinite values as the predicted values ($\hat{y}_i$) approaches infinity [31], however, it depends on the relative magnitude of PRESS and total sum of squares (TSS):

$$\frac{PRESS}{TSS} = \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}; \quad Q^2 = 1 - \frac{PRESS}{TSS}, \tag{7}$$

where PRESS and TSS refer to the same data set. Another simple validation parameter for model validation is the root-mean-square error (RMSE) determined through cross-validated LOO method ($RMSE_{CV}$) in the training set or in the external prediction set ($RMSE_{EXT}$) [32]:

$$RMSE_{CV} = \sqrt{\frac{\sum_{i=1}^{n_{TR}}(y_i - \hat{y}_{i,LOO})^2}{n_{TR}}}, \quad RMSE_{EXT} = \sqrt{\frac{\sum_{i=1}^{n_{EXT}}(y_i - \hat{y}_i)^2}{n_{EXT}}}. \tag{8}$$

However, Chirico and Gramatica [28,33] had recently proposed a most restrictive external validation parameter known as concordance correlation coefficient (CCC):

$$CCC_{EXT} = \frac{2\sum_{i=1}^{n_{EXT}}(y_i - \bar{y})(\hat{y}_i - \bar{\hat{y}})}{\sum_{i=1}^{n_{EXT}}(y_i - \bar{y})^2 + \sum_{i=1}^{n_{EXT}}(\hat{y}_i - \bar{\hat{y}})^2 + n_{EXT}(\bar{y} - \bar{\hat{y}})^2}, \tag{9}$$

where $\bar{\hat{y}}$ is the mean of the predicted values. Moreover, CCC for the training set (CCC_{TR}), and test set using LOO cross validation (CCC_{CV}) can also be determined. For the present work, thresholds values for the external validation parameters were chosen as 0.70 for each of Q_{F1}^2, Q_{F2}^2, Q_{F3}^2 and 0.85 for CCC_{EXT}, however, all of these can have a maximum value of 1. Another important external validation parameters based on r_m^2 metrics [34] utilized in this work are $\overline{r_m^2}$ and Δr_m^2, where former can attain maximum value of 1 whereas the latter has to be <0.2 for an acceptable model. Furthermore, as recently suggested by Chirico and Gramatica [35],

a scatter plot of experimental vs predicted external data always needs to be examined when external predictivity of a model is to be assured.

It should be noted that in the present study, models were developed using classical linear and multiple linear regression (MLR) since linear models are simple to model and interpret. However, these modelling techniques are not state-of-the-art such as regression based on artificial neural networks (ANN), principal component analysis (PCA), k-nearest neighbour (kNN) and machine learning methods being developed nowadays [36]. MLR can be unreliable to model biological activities, which are affected by multiple mechanisms. Moreover, the collinear descriptors in MLR may lead to erroneous values, for the coefficients, resulting in inaccurate interpretations. In the present study, the use of highly collinear descriptors in the model development is avoided (see next section). The other modelling techniques, however, may differ significantly particularly in internal validation, but relative external predictivity of different models is unlikely to change considering state-of-the-art external validation measures employed in the present study.

4. Results and discussion

Various quantum-mechanical molecular descriptors, namely, E, E_{LUMO}, E_{HOMO}, computed through the PM6, RM1, HF, and DFT methods using B3LYP XC energy density functional and 6-311G(d,p) basis set, are listed in Table 1 for the 51 nitro-PAHs. The table also lists the correlation energy (E_{CORR}) and the descriptors obtained from the electron-correlation contribution to the HOMO and LUMO energies as described in Eq. (5). It should be noted that for most of the compounds, LUMO energies computed with RM1 and PM6 SE methods as well as with the DFT have negative sign whereas those calculated with the HF have positive values. A negative value of the LUMO energy is generally associated with the increased activity of the molecule since electrons can be easily promoted from

Table 2
Optimum value of internal and external validation parameters of QSAR models developed using PM6, RM1, HF, DFT and electron-correlation (CORR) based descriptors. *RMSE* values represent maximum root mean square error observed (see also the supporting information Tables S1–S5).

Model	Method	Descriptors employed	R^2	Q^2_{LOO}	Q^2_{F1}	Q^2_{F2}	Q^2_{F3}	CCC_{CV}	CCC_{EXT}	$\overline{r^2_m}$	Δr^2_m	$RMSE_{CV}$	$RMSE_{EXT}$	p-Value
I	PM6	E_{PM6}	0.700	0.655	0.852	0.825	0.770	0.796	0.890	0.828	0.074	1.808	1.570	0.000
	RM1	E_{RM1}	0.684	0.636	0.808	0.773	0.702	0.782	0.842	0.797	0.080	1.901	1.682	0.000
	HF	E_{HF}	0.221	−0.084	0.111	0.056	0.019	0.099	0.202	0.074	0.624	3.202	2.785	0.042
	DFT	E_{DFT}	0.229	−0.080	0.113	0.059	0.021	0.104	0.206	0.076	0.616	3.189	2.783	0.041
	CORR	E_{CORR}	0.781	0.743	0.889	0.883	0.860	0.856	0.934	0.856	0.059	1.508	1.182	0.000
II	PM6 + CORR	E_{PM6}, E_{CORR}	0.799	0.386	0.889	0.882	0.849	0.839	0.934	0.856	0.057	2.026	1.178	0.000
	RM1 + CORR	E_{RM1}, E_{CORR}	0.792	0.693	0.891	0.884	0.841	0.827	0.935	0.854	0.057	1.838	1.128	0.000
	HF + CORR	E_{HF}, E_{CORR}	0.790	0.563	0.900	0.894	0.887	0.732	0.945	0.852	0.047	1.841	1.069	0.000
III	PM6	E^{HOMO}_{PM6}	0.496	0.405	0.449	0.434	0.404	0.609	0.560	0.284	0.272	2.182	2.320	0.000
	RM1	E^{HOMO}_{RM1}	0.580	0.495	0.521	0.508	0.486	0.686	0.655	0.426	0.265	1.993	2.134	0.000
	HF	E^{HOMO}_{HF}	0.392	0.346	0.579	0.568	0.578	0.562	0.691	0.506	0.224	2.150	1.984	0.000
	DFT	E^{HOMO}_{DFT}	0.253	0.104	0.415	0.399	0.477	0.302	0.469	0.305	0.345	2.441	2.316	0.013
	CORR	E^{HOMO}_{CORR}	0.831	0.789	0.742	0.726	0.729	0.885	0.840	0.727	0.129	1.382	1.451	0.000
IV	PM6 + CORR	$E^{HOMO}_{PM6}, E^{HOMO}_{CORR}$	0.835	0.775	0.768	0.754	0.779	0.877	0.861	0.745	0.114	1.412	1.386	0.000
	RM1 + CORR	$E^{HOMO}_{RM1}, E^{HOMO}_{CORR}$	0.836	0.776	0.764	0.750	0.770	0.878	0.861	0.743	0.113	1.413	1.380	0.000
	HF + CORR	$E^{HOMO}_{HF}, E^{HOMO}_{CORR}$	0.861	0.810	0.555	0.547	0.641	0.462	0.630	0.748	0.079	2.739	2.423	0.000
V	PM6	E^{LUMO}_{PM6}	0.250	0.087	0.263	0.193	0.356	0.269	0.327	0.205	0.626	2.347	2.460	0.027
	RM1	E^{LUMO}_{RM1}	0.325	0.159	0.303	0.223	0.333	0.363	0.388	0.227	0.660	2.360	2.413	0.011
	HF	E^{LUMO}_{HF}	0.158	−0.098	0.295	0.282	0.432	0.050	0.388	0.518	5.740	2.715	2.525	0.092
	DFT	E^{LUMO}_{DFT}	0.021	−0.149	0.053	0.036	0.237	−0.128	0.058	−0.042	2.018	2.736	2.861	0.462
	CORR	E^{LUMO}_{CORR}	0.341	0.080	0.625	0.555	0.646	0.360	0.673	0.735	0.098	2.430	1.967	0.001
VI	PM6 + CORR	$E^{LUMO}_{PM6}, E^{LUMO}_{CORR}$	0.436	0.179	0.664	0.601	0.585	0.463	0.701	0.475	0.087	2.582	2.037	0.006
	RM1 + CORR	$E^{LUMO}_{RM1}, E^{LUMO}_{CORR}$	0.468	0.220	0.661	0.597	0.525	0.487	0.704	0.488	0.076	2.591	2.151	0.006
	HF + CORR	$E^{LUMO}_{HF}, E^{LUMO}_{CORR}$	0.441	0.176	0.555	0.547	0.641	0.462	0.630	0.748	0.079	2.739	2.423	0.001
VII	PM6	E_{PM6}, E^{HOMO}_{PM6}	0.803	0.711	0.864	0.855	0.841	0.841	0.911	0.862	0.052	1.427	1.128	0.000
	RM1	E_{RM1}, E^{HOMO}_{RM1}	0.824	0.744	0.874	0.867	0.851	0.860	0.921	0.865	0.055	1.351	1.122	0.000
	HF	E_{HF}, E^{HOMO}_{HF}	0.680	0.500	0.647	0.637	0.607	0.716	0.764	0.627	0.184	2.973	2.270	0.000
	DFT	E_{DFT}, E^{HOMO}_{DFT}	0.516	0.168	0.504	0.491	0.449	0.514	0.610	0.478	0.175	3.211	2.543	0.002
	CORR	$E_{CORR}, E^{HOMO}_{CORR}$	0.893	0.856	0.884	0.884	0.908	0.924	0.933	0.849	0.060	1.188	0.961	0.000
VIII	PM6	E_{PM6}, E^{LUMO}_{PM6}	0.705	0.637	0.851	0.823	0.768	0.783	0.889	0.812	0.074	1.967	1.562	0.000
	RM1	E_{RM1}, E^{LUMO}_{RM1}	0.684	0.600	0.794	0.755	0.704	0.766	0.826	0.763	0.076	1.995	1.600	0.000
	HF	$E_{HF}, E_{LUMO,HF}$	0.322	−0.241	0.284	0.240	0.227	0.043	0.375	0.266	0.409	3.692	3.020	0.045
	DFT	$E^{HOMO}_{DFT}, E^{LUMO}_{DFT}$	0.286	−0.093	0.107	0.052	0.031	0.223	0.192	0.094	0.558	3.346	3.361	0.051
	CORR	$E_{CORR}, E^{LUMO}_{CORR}$	0.788	0.685	0.884	0.864	0.839	0.826	0.924	0.844	0.073	1.810	1.181	0.000
IX	PM6	$E^{LUMO}_{PM6}, E^{HOMO}_{PM6}$	0.839	0.794	0.852	0.843	0.799	0.889	0.894	0.771	0.079	1.324	1.348	0.000
	RM1	$E^{LUMO}_{RM1}, E^{HOMO}_{RM1}$	0.925	0.895	0.915	0.910	0.851	0.946	0.949	0.821	0.054	1.253	1.153	0.000
	HF	$E^{LUMO}_{HF}, E^{HOMO}_{HF}$	0.519	0.262	0.803	0.798	0.801	0.541	0.870	0.759	0.088	2.351	1.540	0.000
	DFT	$E^{LUMO}_{DFT}, E^{HOMO}_{DFT}$	0.318	−0.170	0.673	0.665	0.698	0.245	0.739	0.658	0.192	2.854	1.915	0.010
	CORR	$E^{LUMO}_{CORR}, E^{HOMO}_{CORR}$	0.831	0.695	0.765	0.751	0.719	0.831	0.857	0.723	0.123	1.482	1.446	0.000
X	PM6	$E^{HOMO-LUMO}_{PM6}$	0.804	0.753	0.762	0.748	0.717	0.864	0.847	0.613	0.140	1.510	1.400	0.000
	RM1	$E^{HOMO-LUMO}_{RM1}$	0.784	0.725	0.832	0.822	0.774	0.846	0.885	0.746	0.107	1.337	1.354	0.000
	HF	$E^{HOMO-LUMO}_{HF}$	0.519	0.394	0.799	0.793	0.789	0.608	0.867	0.726	0.089	2.052	1.487	0.000
	DFT	$E^{HOMO-LUMO}_{DFT}$	0.316	0.057	0.658	0.649	0.672	0.334	0.725	0.576	0.148	2.523	1.850	0.002
	CORR	$E^{HOMO-LUMO}_{CORR}$	0.754	0.720	0.844	0.840	0.852	0.840	0.907	0.814	0.059	1.614	1.448	0.000

Table 3
Range of various internal validation parameters of QSAR models obtained using different types of splitting (see also the supporting information Tables S1–S5).

Model	Method	Descriptors employed	R^2	Q^2_{LOO}	Q^2_{LMO}	Q^2_{Yscr}	Q^2_{Yrand}	CCC_{TR}	MAE_{TR}	ΔK
I	PM6	E_{PM6}	0.398–0.700	0.279–0.655	13.962–48.420	−17.799 to −12.639	−19.028 to −12.539	0.570–0.824	1.095–1.420	0.631–0.837
	RM1	E_{RM1}	0.341–0.684	0.208–0.636	3.823–45.058	−18.886 to −12.692	−18.809 to −12.768	0.509–0.812	1.119–1.526	0.584–0.827
	HF	E_{HF}	0.025–0.221	−0.698 to −0.084	−56.169 to −18.154	−20.416 to −12.842	−21.937 to −13.086	0.049–0.362	1.707–1.747	0.158–0.470
	DFT	E_{DFT}	0.026–0.229	−0.684 to −0.080	−53.852 to −19.930	−20.996 to −12.884	−19.581 to −12.917	0.050–0.365	1.706–1.745	0.160–0.472
	CORR	E_{CORR}	0.550–0.781	0.453–0.743	41.153–62.056	−18.209 to −12.371	−18.384 to −12.611	0.709–0.877	0.951–1.168	0.741–0.884
II	PM6 + CORR	E_{PM6}, E_{CORR}	0.550–0.799	0.101–0.386	35.709–61.723	−27.095 to −17.827	−26.589 to −17.756	0.710–0.888	0.934–1.160	−0.119 to −0.009
	RM1 + CORR	E_{RM1}, E_{CORR}	0.565–0.792	0.261–0.693	40.120–60.456	−26.418 to −17.400	−26.893 to −16.648	0.722–0.884	0.880–1.093	−0.136 to −0.015
	HF + CORR	E_{HF}, E_{CORR}	0.590–0.790	0.438–0.563	30.910–47.982	−68.488 to −19.306	−68.609 to −18.969	0.742–0.883	0.890–1.060	0.018–0.050
III	PM6	E^{HOMO}_{PM6}	0.290–0.496	0.175–0.405	0.192–36.343	−18.818 to −12.130	−18.402 to −12.214	0.450–0.663	1.076–1.578	0.539–0.705
	RM1	E^{HOMO}_{RM1}	0.410–0.580	0.298–0.495	11.948–45.169	−18.596 to −11.733	−18.349 to −12.356	0.581–0.734	1.010–1.458	0.640–0.762
	HF	E^{HOMO}_{HF}	0.364–0.392	0.234–0.346	2.797–30.855	−18.492 to −12.235	−18.447 to −12.148	0.533–0.629	1.130–1.493	0.603–0.678
	DFT	E^{HOMO}_{DFT}	0.163–0.253	0.008–0.104	−22.706 to 6.953	−18.675 to −12.367	−18.813 to −12.130	0.281–0.403	1.345–1.819	0.404–0.503
	CORR	E^{HOMO}_{CORR}	0.748–0.831	0.684–0.789	55.315–73.748	−18.420 to −12.047	−18.321 to −12.525	0.856–0.908	0.761–0.926	0.865–0.912
IV	PM6 + CORR	$E^{HOMO}_{PM6}, E^{HOMO}_{CORR}$	0.765–0.835	0.670–0.775	54.677–73.149	−26.532 to −18.065	−26.449 to −17.804	0.867–0.910	0.660–0.911	−0.046 to 0.008
	RM1 + CORR	$E^{HOMO}_{RM1}, E^{HOMO}_{CORR}$	0.760–0.836	0.669–0.776	56.293–73.025	−27.690 to −17.829	−28.399 to −17.580	0.863–0.911	0.664–0.902	−0.064 to −0.025
	HF + CORR	$E^{HOMO}_{HF}, E^{HOMO}_{CORR}$	0.786–0.861	0.701–0.810	60.679–77.788	−27.029 to −17.919	−27.888 to −17.508	0.878–0.926	0.638–0.880	−0.070 to −0.032
V	PM6	E^{LUMO}_{PM6}	0.055–0.250	−0.195 to 0.087	−28.941 to −14.177	−19.862 to −12.299	−20.169 to −12.707	0.103–0.400	1.723–1.953	0.233–0.500
	RM1	E^{LUMO}_{RM1}	0.043–0.325	−0.220 to 0.159	−31.260 to −14.622	−18.893 to −12.397	−18.991 to −12.093	0.082–0.490	1.723–1.869	0.207–0.570
	HF	E^{LUMO}_{HF}	0.004–0.158	−0.352 to −0.098	−38.267 to −15.307	−18.355 to −12.409	−18.958 to −12.547	0.008–0.273	1.701–1.945	0.065–0.398
	DFT	E^{LUMO}_{DFT}	0.001–0.021	−0.240 to −0.149	−51.912 to −18.867	−18.743 to −12.220	−18.921 to −12.485	0.002–0.041	1.747–2.187	0.033–0.144
	CORR	E^{LUMO}_{CORR}	0.191–0.341	−0.293 to 0.080	−18.954 to 15.350	−19.163 to −12.556	−18.775 to −12.804	0.320–0.509	1.236–1.654	0.437–0.584
VI	PM6 + CORR	$E^{LUMO}_{PM6}, E^{LUMO}_{CORR}$	0.208–0.436	−0.460 to 0.179	−22.472 to 11.021	−28.877 to −17.471	−28.204 to −17.487	0.345–0.607	1.215–1.567	−0.040 to 0.191
	RM1 + CORR	$E^{LUMO}_{RM1}, E^{LUMO}_{CORR}$	0.194–0.468	−0.469 to 0.220	−24.623 to 14.067	−25.516 to −17.374	−27.334 to −18.053	0.325–0.638	1.184–1.538	−0.063 to 0.163
	HF + CORR	$E^{LUMO}_{HF}, E^{LUMO}_{CORR}$	0.274–0.441	−0.243 to 0.176	−20.585 to 26.828	−29.049 to −17.443	−31.489 to −17.229	0.430–0.612	1.083–1.652	−0.130 to −0.283
VII	PM6	E_{PM6}, E^{HOMO}_{PM6}	0.678–0.803	0.578–0.711	49.083–63.686	−25.108 to −16.778	−26.218 to −17.194	0.808–0.891	0.750–0.995	0.252–0.348
	RM1	E_{RM1}, E^{HOMO}_{RM1}	0.712–0.824	0.623–0.744	51.782–69.850	−28.988 to −17.329	−24.974 to −18.361	0.832–0.903	0.713–0.952	0.234–0.362
	HF	E_{HF}, E^{HOMO}_{HF}	0.442–0.680	−0.464 to 0.500	−19.799 to 49.226	−30.688 to −17.637	−31.832 to −18.114	0.613–0.809	0.852–1.401	0.181–0.344
	DFT	E_{DFT}, E^{HOMO}_{DFT}	0.272–0.516	−0.708 to 0.168	−45.798 to 22.721	−29.751 to −17.930	−30.969 to −17.933	0.427–0.680	1.123–1.621	0.147–0.207
	CORR	$E_{CORR}, E^{HOMO}_{CORR}$	0.788–0.893	0.685–0.856	67.408–83.257	−25.761 to −17.496	−26.430 to −18.110	0.907–0.943	0.523–0.810	0.027–0.134
VIII	PM6	E_{PM6}, E^{LUMO}_{PM6}	0.422–0.705	0.153–0.637	6.943–47.514	−27.986 to −18.024	−28.584 to −17.900	0.593–0.827	1.047–1.417	−0.083 to 0.102
	RM1	E_{RM1}, E^{LUMO}_{RM1}	0.397–0.684	0.129–0.600	7.325–44.212	−28.497 to −18.684	−28.155 to −18.106	0.569–0.813	1.128–1.481	−0.190 to −0.007
	HF	$E_{HF}, E_{LUMO,HF}$	0.077–0.322	−1.258 to −0.241	−76.827 to −27.356	−31.754 to −18.040	−33.256 to −17.941	0.143–0.487	1.645–1.758	−0.069 to 0.174
	DFT	$E^{HOMO}_{DFT}, E^{LUMO}_{DFT}$	0.078–0.286	−0.855 to −0.093	−60.967 to −5.835	−27.920 to −18.315	−28.790 to −17.978	0.051–0.444	1.453–1.757	−0.132 to 0.171
	CORR	$E_{CORR}, E^{LUMO}_{CORR}$	0.561–0.788	0.213–0.685	34.143–59.501	−27.778 to −18.700	−26.894 to −19.581	0.719–0.881	0.944–1.125	−0.013 to 0.095
IX	PM6	$E^{LUMO}_{PM6}, E^{HOMO}_{PM6}$	0.685–0.839	0.607–0.794	54.839–74.392	−28.675 to −17.468	−27.720 to −17.573	0.813–0.913	0.677–1.015	−0.019 to 0.198
	RM1	$E^{LUMO}_{RM1}, E^{HOMO}_{RM1}$	0.729–0.925	0.643–0.895	62.557–85.931	−25.827 to −17.480	−25.449 to −17.427	0.843–0.961	0.512–0.919	0.033–0.338
	HF	$E^{LUMO}_{HF}, E^{HOMO}_{HF}$	0.468–0.519	0.084–0.262	2.775–35.187	−26.478 to −17.426	−27.350 to −16.584	0.637–0.683	1.063–1.306	0.192–0.345
	DFT	$E^{LUMO}_{DFT}, E^{HOMO}_{DFT}$	0.272–0.318	−0.525 to −0.170	−30.287 to 3.573	−27.233 to −17.871	−29.018 to −18.562	0.427–0.482	1.321–1.617	−0.143 to −0.053
	CORR	$E^{LUMO}_{CORR}, E^{HOMO}_{CORR}$	0.748–0.831	0.636–0.695	52.676–70.332	−28.284 to −18.366	−28.854 to −17.510	0.856–0.908	0.696–0.924	0.049–0.155
X	PM6	$E^{HOMO-LUMO}_{PM6}$	0.641–0.804	0.586–0.753	45.412–70.561	−18.571 to −12.372	−17.309 to −12.620	0.781–0.891	0.763–1.157	0.801–0.897
	RM1	$E^{HOMO-LUMO}_{RM1}$	0.686–0.784	0.634–0.725	51.871–66.813	−18.567 to −12.043	−18.372 to −12.282	0.814–0.879	0.815–1.056	0.829–0.885
	HF	$E^{HOMO-LUMO}_{HF}$	0.467–0.519	0.299–0.394	16.648–38.017	−18.038 to −12.421	−17.643 to −12.151	0.637–0.683	1.043–1.316	0.683–0.720
	DFT	$E^{HOMO-LUMO}_{DFT}$	0.259–0.316	−0.144 to 0.057	−15.351 to 10.336	−18.830 to −12.486	−18.815 to −12.693	0.411–0.481	1.344–1.624	0.509–0.562
	CORR	$E^{HOMO-LUMO}_{CORR}$	0.647–0.754	0.569–0.720	42.140–69.203	−17.933 to −11.944	−18.611 to −12.753	0.786–0.860	0.722–1.038	0.804–0.868

HOMO to LUMO. Further from the table, it is clearly evident that the magnitude of the electron-correlation energy is much smaller than the total energy of the molecule. For example, the value of E_{CORR} is less than 1% of the total energy of the molecule. Furthermore, Table 2 compares the internal validation and external predictive capability of different QSAR models developed with one- and two-descriptors computed using the PM6, RM1, HF and DFT methods, and from the electron-correlation contribution (CORR) of the corresponding descriptor. It should be noted that Table 2 lists the optimum values of the internal and external validation parameters obtained among different types of splitting procedures (see also supporting information Tables S1–S5). For most of the regression models, the p-value is not only <0.001 but is also found to be closer to zero, in particular for all the models having R^2 and $Q^2_{LOO} > 0.6$, indicating different models being compared to be statistically significant. However, a few of the models based on LUMO descriptors are statistically insignificant as also indicated by other internal validation parameters. The range of the internal validation parameters obtained in different splitting procedures is further listed in Table 3. It should be further noted that a few of the descriptors calculated with different quantum mechanical methods such as PM6, RM1, HF and DFT, are not used together in the model development. Such descriptors, in fact, are also found to be strongly correlated pairwise, with correlation even greater than 0.98 in a few cases. Such paired descriptors are not employed for the model development to avoid any chance correlation.

It is quite evident from Table 2 that the robustness and external predictivity of QSAR models developed using the electron-correlation energy is far better than the corresponding HF and DFT descriptors based QSARs, though latter show worst internal validation even compared to the models based on PM6 and RM1 descriptors. For example in the single-descriptor model I, R^2 value of PM6, RM1, HF and DFT based models is 0.700, 0.684, 0.221, 0.229, respectively, compared to a value of 0.781 in case of CORR based model. Similarly, cross validated Q^2_{CV} or Q^2_{LOO} value is 0.655, 0.636, −0.084, −0.080 for PM6, RM1, HF and DFT based models, respectively, compared to a value of 0.743 for CORR based model. Similar trend is observed in the external validation parameters. However, if electron-correlation contribution (CORR) is included in the HF descriptor based model, the robustness of the model is considerable enhanced. For example, HF energy (E_{HF}) based model I has R^2 value of 0.221, which is greatly enhanced to 0.790 when the correlation energy contribution (E_{CORR}) is included as evident in model II. Not only this, robustness of PM6 and RM1 based models is also found to be enhanced on the inclusion of corresponding CORR descriptor. For example, comparing models III and IV based on E_{HOMO}, Q^2_{LOO} value of PM6 based model is enhanced from 0.405 to 0.775 on the inclusion of electron-correlation contribution to the HOMO energy. It should be noted that addition of another descriptor most often improves, as a consequence of statistical effect, the robustness of the model. However, inclusion of the electron-correlation contribution into the model, in particular based on the HF method, not only makes a model more robust but also more predictive. For example comparing models III, IV and VII based on the total energy and energy of HOMO, Q^2_{LOO} value in models based on E^{HOMO}_{HF} increases from 0.346 (as in model III) to 0.500 (as in model VII) when E_{HF} is included, while it increases to 0.810 (as in model IV) when electron-correlation contribution is included. Similarly, comparing the external validation, Q^2_{F3} value increases from 0.578 (as in model III) to 0.607 (as in model VII) when E_{HF} is included whereas it increases to 0.641 (as in model IV) when electron-correlation contribution is included. However, comparing SE PM6 and RM1 based models I–IV and VII with those of CORR based model, it is clearly evident that the addition of another SE descriptor to the model increases external predictivity of the SE based model though the CORR based models are relatively more robust. For example, Q^2_{LOO}

value in models based on E^{HOMO}_{PM6} increases from 0.405 (as in model III) to 0.711 (as in model VII) when E_{PM6} is included, compared to 0.775 (as in model IV) when correlation contribution (E^{HOMO}_{CORR}) is included. On the other hand, the value of external validation parameter such as Q^2_{F3} increases from 0.404 (as in model III) to 0.841 (as in model VII) when E_{PM6} is included, which is significantly better compared to 0.779 (of model IV) when correlation contribution (E^{HOMO}_{CORR}) is included, though, a model based purely on electron-correlation contribution i.e., E_{CORR} and E^{HOMO}_{CORR} has more reliable Q^2_{F3} value of 0.908 (as in model VII). However, this is not always the case as evident in models IX and X (see later). In general, the robustness and external predictive ability of the models based on the electron-correlation based descriptors, other than E_{CORR}, is also found to be far more significant and reliable than any other HF or DFT descriptor based QSAR models. It should be further noted that the HF and DFT based models can achieve high internal validation using reduced data set, however, even than the models show highly unacceptable external validation. For example, for HF based model I, exclusion of 2,3-dichloronitrobenzene and 2,5-dichloronitrobenzene in activity sampling splitting method, results in a value of 0.778 and 0.742 for R^2 and Q^2_{LOO}, respectively, however, an unacceptable value of −0.118 for Q^2_{F3} along with highly unreliable other external validation parameters. However, using other splitting methods, HF based model still show unacceptable internal and external validation. Similar behaviour is observed for the DFT based model, whereas CORR based model exhibits acceptable internal as well as external validation parameters.

In the above discussion, we have preferably chosen Q^2_{F3} as external validation parameter, from Tables 2 and 3, for comparison of external predictivity of various models. The other external validation parameter Q^2_{F2}, though often used, has a drawback [28] that it does not take into account the model training set, whereas Q^2_{F1} and Q^2_{F3} refer to the training set through $\bar{y}_{TR}$. The parameters Q^2_{F1} and Q^2_{F2} differ in the denominator for calculation of TSS, see Eqs. (6) and (7). In Q^2_{F1}, TSS is calculated using the training set mean ($\bar{y}_{TR}$) whereas in Q^2_{F2}, external set mean ($\bar{y}_{EXT}$) is utilized. In fact, Q^2_{F1} and Q^2_{F2} are also known to be biased depending on the data distribution. Moreover, as the size of prediction set increases, Q^2_{F2} converges to Q^2_{F1}. On the contrary, Q^2_{F1} which can be even greater than R^2, is known to give wrong conclusion of high predictivity of the model. This risk can be minimized if the predictive ability of the model is tested using Q^2_{F3}. The latter also has an advantage that it remains invariant to the measured sampling. Moreover, as the prediction set size increases, Q^2_{F1} and Q^2_{F2} are known to converge to Q^2_{F3} [28]. Therefore, Q^2_{F3} can be a good choice for comparison of external predictivity of the models. Beside this, other validation parameter of high importance is CCC_{EXT}, which is a very good measure of external predictivity. The external predictive ability of the CORR based models can also be seen from the value of CCC_{EXT} parameter (in Table 2), which is found to be far more significant and reliable than that in PM6, RM1, HF or DFT descriptor based QSAR models. However, to ensure external predictivity, scatter plots should always be examined. From the scatter plots (Figs. S1 and S2 in supporting information), of experimental vs predicted activity of the training set and prediction set, it is clearly evident that in the case of electron-correlation based models (with $CCC_{EXT} > 0.80$), the degree of data scattering (relative to the regression line passing through the origin) is much less than that in the case of PM6, RM1, HF and DFT descriptors based models. However, PM6 and RM1 based models exhibits reliable scatter plots not only compared to those from the HF and DFT descriptors but can also show scatter plots that are comparable to those of CORR based models as evident in models IX and X. Furthermore, another interesting comparison of the external predictivity of these quantum mechanical methods can

be seen from the root mean square error in Table 2. For most of the models, irrespective of the quantum mechanical method used, the maximum value of $RMSE_{CV}$ and $RMSE_{EXT}$, is more than 1, and is highly unacceptable for the HF and DFT descriptors based models, where the value of $RMSE_{ext}$ can be even greater than three in few of the models. However, validation on the basis of $RMSE$ is meaningless if different models have to be compared [28]. Besides this, another important external validation parameters based on r_m^2 metrics, namely, $\overline{r_m^2}$ and Δr_m^2, are also found to be acceptable for the CORR based models. In fact, for most of the CORR based models Δr_m^2 is <0.1, which is a highly stringent criterion for model acceptability. However, different validation parameters need to be used cautiously.

Further, in Table 2, models IX and X based on HOMO/LUMO energies and HOMO–LUMO gap, respectively, clearly indicate that the PM6 and RM1 based models can not only be more robust but can also be relatively more predictive (as in model IX) than the CORR based model, though the latter is still robust and predictive (as in model X). For example, Q_{LOO}^2 value in RM1 HOMO/LUMO energies based model is 0.895 (as in model IX) compared to 0.695 in case of CORR based model, whereas, the value of Q_{F3}^2 and CCC_{EXT} is 0.851 and 0.949, respectively, which is significantly better compared to 0.719 and 0.857, respectively, of CORR based model. However, in case of model X based on HOMO–LUMO gap, CORR based models show similar robustness and predictivity as of PM6 and RM1. In fact, the HOMO–LUMO gap is observed to be the most widely used quantum mechanical descriptor for robust and predictive QSAR models since HOMO–LUMO gap is a very good measure of chemical reactivity. It should be, however, noted that the models V and VI, based on LUMO energies, are not reliable even when electron-correlation contribution is added though their predictivity increases considerably. The models become more reliable and predictive when the total energy $E_{PM6/RM1}$ is included as evident in model VIII, however, still relatively less robust and predictive than that observed in the case of purely electron-correlation based model. It should be noted further that the coefficients associated with HOMO and LUMO energies in the models, based on HOMO and LUMO energies together, have opposite sign. This indicates that some cancellation of errors may be occurring since HOMO and LUMO descriptors based models, when not taken together, are relatively less robust and less predictive than CORR based models. Furthermore, HF and DFT descriptors based models are not only less reliable compared to CORR models but are also unreliable even compared to PM6 and RM1 based models as evident in all the models, I–X, studied in this work. It should be noted that in these advanced SE methods, all the empirical parameters are optimized, and parameterization properties include dipole moment, heat of formation, ionization potential, bond lengths and angles of a set of training compounds, that are representative of organic and biochemical origin constituting mainly of atoms: C, H, N, O, P, S, F, Cl, Br, and I [5,6]. Therefore, for the nitro-PAHs considered in this work, SE methods turn out to be more reliable than HF and DFT since former are already optimized using linear scaling. This is important since HF and DFT methods are computationally far expensive than the SE methods.

Furthermore, QSAR models developed using HF based descriptors are found to be relatively more reliable than the corresponding DFT based descriptor models, though, not as reliable when the correlation-contribution is also included as a descriptor. In fact, in model IX based on HOMO/LUMO energies, the Q_{F3}^2 value of HF based model is not only reliable than DFT based model but also relatively more reliable than PM6 and CORR based models, though, the HF based model is relatively less robust compared to PM6, RM1 and CORR based models. It should be noted that the present work employs a hybrid B3LYP XC

energy density functional which has the following formulation [19,35],

$$E_{XC}^{B3LYP} = E_X^{LDA} + 0.20(E_X^{HF} - E_X^{LDA}) + 0.72\Delta E_X^{B88} \\ + E_{CORR}^{VWN} + 0.81(E_{CORR}^{LYP} - E_{CORR}^{VWN}), \tag{10}$$

where E_X^{LDA}, and E_X^{HF} represents Slater's exchange energy density functional in the local density approximation (LDA), and HF exchange-energy, respectively. The third term on the right hand side of Eq. (10) represents Becke's gradient correction to the LDA exchange functional. The correlation interactions are included through a local correlation functional due to Vosko, Wilk, and Nusair (VWN), and a non-local correction due to the Lee–Yang–Parr (LYP) [37]. The XC energy density functional of B3LYP includes more exchange interactions than those are in the HF, to be exact, 20% of HF in B3LYP. It is, therefore, evident that the exchange interactions do not contribute significantly to the robustness and predictivity of a model. The exchange energy arises due to interactions between electrons with same spin. The latter tend to avoid each other resulting in an exchange hole known as Fermi hole [9,10], whereby the probability of finding the two electrons (with parallel spin) in the same infinitesimal region of space exhibits a dip. The self-interaction correction, which is consequence of the fact that an electron cannot interact with itself, also contributes to the exchange hole. In fact, electrons with parallel spin cannot occupy same point in the space even if they belong to the different orbitals. On the other hand, correlation energy results from the Coulombic repulsions that keep any two electrons away from each other in the space. The latter gives rise to a correlation hole, also known as Coulomb hole [9,10]. The instantaneous interactions between all the electrons in a molecule account for the correlation energy. Any change in the correlation energy is significant and crucial whenever bond formation and dissociation occurs. Therefore, it is clear that the electron-correlation, which plays a vital role in the determination of the equilibrium geometry of a molecule, can be a key predictor of the activity of molecules.

Though the DFT based quantum mechanical descriptors which are being used for developing QSARs include electron-correlation through the XC functional, but as evident from the present results, the electron-correlation contribution of these descriptors when included explicitly in the model, can alone be far more reliable than the descriptor itself. In fact, as clear from the above discussions, HF descriptors based model are relatively more reliable than the DFT based descriptor model. However, from the comparison of models based on the various quantum-mechanical methods, and their external validation parameters, it is clearly evident that the models based on the electron-correlation contribution of the descriptor have relatively better robustness and external prediction capability than those based on the descriptor itself. The predictability of electron-correlation based descriptors is readily evident from Table 4 in which the observed mutagenicity is compared with the predicted mutagenicity (using model VII) for compounds which are either inactive or show insignificant mutagenicity. The models based on PM6, RM1 and CORR descriptors are able to make better prediction, however, the HF and DFT based descriptor models are highly unpredictive compared to not only those of correlation based descriptors but also to semi-empirical descriptors based models [18], in particular, for compounds with low activity. For example, HF and DFT based models predict activity of inactive 2,4-dichloronitrobenzene to be >10^3 revertants/nmol. Therefore, it is evident that the electron-correlation is an important predictor of changes in the electronic environment, and it may be included explicitly if an acceptable external predictivity of a QSAR model is desired.

Table 4
Range of predicted mutagenic activity, log TA98, of inactive compounds (using model VII in different splitting methods).

Name of the compound	Observed	Predicted					
		PM6	RM1	HF	DFT	CORR	Ref. [18]
Nitrobenzene	na	−3.678 to −2.850	−3.466 to −2.713	−3.043 to −1.166	−3.257 to −1.121	−1.864 to −1.287	−1.45
2-Fluoronitrobenzene	na	−2.136 to −1.785	−1.955 to −1.611	−2.441 to −1.071	−2.318 to −0.807	−1.763 to −1.594	−1.24
3-Fluoronitrobenzene	na	−2.123 to −1.775	−1.961 to −1.619	−2.441 to −1.071	−2.318 to −0.807	−1.764 to −1.594	−1.04
3-Chloronitrobenzene	na	−2.512 to −1.841	−2.176 to −1.721	−0.249 to 0.331	0.020–0.691	−1.310 to 1.172	−0.79
4-Chloronitrobenzene	na	−2.672 to −2.052	−2.315 to −1.889	−0.488 to 0.101	−0.0856 to 0.599	−1.864 to −1.287	−0.73
2,4-Dichloronitrobenzene	na	−1.814 to −1.447	−1.282 to −1.087	0.363–**3.299**	0.844–**4.234**	−1.109 to −0.541	−0.30
3,4-Dichloronitrobenzene	na	−1.622 to −1.195	−1.124 to −0.956	0.397–**3.338**	0.844–**4.234**	−0.997 to −0.470	−0.02
1,2-Dinitrobenzene	na	−2.305 to −1.694	−2.259 to −1.721	0.954–**1.428**	1.349–**1.706**	−0.894 to −0.645	−0.60

na, inactive compounds [18]. Bold values represent high activity.

5. Conclusions

In this work, we have compared QSAR models based on quantum-mechanical molecular descriptors computed through the state-of-the-art quantum-mechanical methods such as PM6, RM1, HF and DFT methods to those based on the electron-correlation energy and its contribution (CORR) to the quantum mechanical descriptors such as HOMO/LUMO energies. The robustness and external prediction capability of the different QSAR models, developed for the mutagenic activity of a set of nitro-PAHs acting on TA98 stain of *S. typhimurium* bacteria, reveal some surprising facts about the descriptors computed with the HF and DFT methods. From a comparison of robustness and the external predictivity of PM6, RM1, HF, DFT and electron-correlation based QSAR models, following important conclusions are evident:

 (i) The electron-correlation descriptor based models are not only reliable, but are also far more predictive than the corresponding HF and DFT descriptors based models, in particular, when the real external predictivity matters the most.
 (ii) The electron-correlation contribution of the quantum-mechanical descriptor can alone be far more reliable and predictive than the corresponding quantum-mechanical descriptor itself.
(iii) QSAR models based on the HOMO and LUMO energies, widely used quantum mechanical descriptors, may show high robustness with reliable internal validation and external predictivity.
(iv) The models based on descriptors computed using advanced semi-empirical methods like RM1 and PM6 can be far more reliable than those computed using advanced quantum mechanical methods like HF and DFT. The robustness of SE based models is comparable to those of CORR based models, particularly, when HOMO and LUMO energies are considered as descriptors in the model.

Therefore, an acceptable internal and external validation of a QSAR model based on the quantum-mechanical molecular descriptors can be achieved if electron-correlation contribution of the quantum-mechanical descriptor is explicitly included in the model. In this work, however, we have computed the electron-correlation from the HF and DFT methods through Eq. (5). Though this is the simplest, yet still expensive, way of computation of the electron-correlation, it is only an approximate route, and overestimation of electron-correlation can occur. More accurate computation of the correlation-energy, however, would require the use of MBPT, CC, and CI methods at the highest level of the theory, which is computationally highly expensive. Moreover, for large molecules, these methods demand huge computational resources even at the lowest level of the theory. The electron-correlation, however, may have to be included if the external predictivity of a QSAR model, based on the quantum mechanical molecular descriptors, is to be tested on a chemical containing an atom not generally present in the molecules

of organic and biological origin, for which SE methods may not be reliable. Further testing of this hypothesis will be focus of our future studies. To capture a fair amount of electron-correlation [38], advance quantum mechanical approaches being developed nowadays, in particular, based on random phase approximation and quantum Monte Carlo methods hold a good promise.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jmgm.2013.02.005.

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