A QSAR STUDY ON THE MUTAGENIC ACTIVITY OF AZO DYES AND AROMATIC AMINE DERIVATIVES

by

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ABSTRACT

A QSAR STUDY ON THE MUTAGENIC ACTIVITY OF AZO DYES AND AROMATIC AMINE DERIVATIVES

In the present study, the mutagenic activity of diverse structure of azo dyes and aromatic amine derivatives on TA98 *Salmonella typhimurium* bacterial strain with S9 activation was used to generate a quantitative structure – activity relationship (QSAR) model. The descriptors required for the model development were obtained by SPARTAN (v.10), DRAGON (v.7.0) software packages. The selection of descriptors was carried out by the tools implemented in QSARINS (v.2.2.3) software. Many division trials were performed on the dataset as training and test sets which comprise the 80% and 20% of the whole set, respectively. 6 descriptors (CIC2, Chi_D/Dt, L/Bw, TDB09p, Mor28s and piPC08) constitute the final model. The applicability domain (AD) of the generated QSAR model was defined by both the ranges of response and descriptors. The predictive ability of the final model was tested using an external dataset consisting of currently used 33 anionic water soluble textile dyes (eight anthraquinone and 25 azo dyes) with no experimental mutagenicity data. The proposed QSAR model had 70% structural coverage for the external set chemicals. The mutagenic activities of 22 current textile dyes belonging anthraquinone and azo dyes fell within the applicability domain of the proposed QSAR model which means that they were well predicted by the model. The order of 6 anthraquinone dyes which are mainly used for cotton, fiber dyeing and leather shading with the most mutagenic activity is: Acid Blue 62>Acid Blue 40>Acid Blue 45>Acid Blue 80>Acid Blue 230>Acid Blue 344. The least mutagenic azo dye is Direct Orange 34 which is mainly used for cotton, silk, wool and their blended fabric dyeing and printing, also can be used for leather and paper shading.

ÖZET

AZO BOYALARI VE AROMATİK AMİN TÜREVLERİNİN MUTAJENİK AKTİVİTESİ ÜZERİNE BİR KYAİ ÇALIŞMASI

Bu çalışmada, çeşitli azo boyaları ve aromatik amin türevlerinin S9 aktivasyonlu TA98 *Salmonella typhimurium* bakteri türü üzerindeki mutajenik aktivitesi kullanılarak kantitatif yapı - aktivite ilişkisi (KYAİ; (QSAR)) modeli geliştirilmiştir. Model geliştirme için gerekli tanımlayıcılar SPARTAN (v.10) ve DRAGON (v.7.0) yazılım programları ile elde edilmiştir. Tanımlayıcıların seçimi, QSARINS (v.2.2.3) yazılımında bulunan araçlar ile yapılmıştır. Tüm veri setinin % 80'i ve % 20'si sırası ile eğitim ve test setleri olacak şekilde farklı ayrımlar denenmiştir. 6 (CIC2, Chi_D/Dt, L/Bw, TDB09p, Mor28s and piPC08) tanımlayıcı ile nihai modeli oluşturulmuştur. Nihai modelin tahmin yeteneği, deneysel mutajenite verisi olmayan, halihazırda kullanılan 33 anyonik suda çözünür tekstil boyalarını (sekiz antrakinon ve 25 azo boyası) içeren harici bir veri seti kullanılarak test edilmiştr. Oluşturulan modelin uygulanabilirlik alanı (AD), hem aktivite hem de tanımlayıcıların oluşturduğu aralık gözönünde bulundurularak tanımlanmıştır. Önerilen KYAİ modelinin yapısal olarak dış setteki bileşiklerin %70 'ini kapsadığı görülmüştür. Halen kullanımda olan 22 adet antrakinon ve azo boyasının mutajenik aktiviteleri, önerilen QSAR modelinin uygulanabilirlik alanı içinde kalmıştır. Bu da mutajenik aktivitelerinin model tarafından iyi tahmin edildiklerini göstermiştir. En yüksek mutajenik aktivite gösteren, pamuk, elyaf boyama ve deri gölgemesi için kullanılmakta olan 6 antrakinon boyanın mutajenik aktivitelerinin sıralaması Asit Mavi 62>Asit Mavi 40>Asit Mavi 45>Asit Mavi 80>Asit Mavi 230>Asit Mavi 344 ‘tür. En düşük mutajenik aktiviteye sahip azo boya ise esas olarak pamuk, ipek, yün ve bunların karışımlı kumaş boyaması ve baskısı için kullanılmakta olan Direkt Turuncu 34’ tür. Bu boya ayrıca deri ve kağıt gölgelemesi için de kullanılmaktadır.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	**Explanation**	**Unit**
μ	Dipole Moment	
η	Hardness	
ω	Electrophilicity index	
σ	Softness	
E	Gas-phase Energy	eV
E_{aq}	Aqueous-phase Energy	eV
E_{HOMO}	Energy of the Highest Occupied Molecular Orbital	eV
E_{LUMO}	Energy of the Lowest Unoccupied Molecular Orbital	eV
R^2	Coefficient of Determination	
R^2_{adj}	Adjusted (for degrees of freedom) R^2	
Chi_D/Dt	Randic-like Index from Distance/Detour Matrix	
L/Bw	Length-to-breadth Ratio by WHIM	
CIC2	Complementary Information Content Index (neighborhood symmetry of 2-order)	
Mor28s	Signal 28 / weighted by I-state	
TDB09p	3D Topological distance based descriptors lag 9 weighted by polarizability	
piPC08	Molecular Multiple Path Count of Order 8	
WHIM	Weighted Holistic Invariant Molecular Descriptors	
*h**	Critical Hat Value	

Abbreviation	**Explanation**
AD	Applicability Domain
CAS	Chemical Abstracts Service
CCC	Concordance Correlation Coefficient
EC	European Commission

ECHA	European Chemicals Agency
EU	European Union
F	Fischer Statistics
Log*P*	Logarithm of *n*-Octanol/Water Partition Coefficient
MAE	Mean Absolute Error
MCDM	Multiple Criteria Decision Making
MLR	Multiple Linear Regression
OECD	Organization of Economic Co-operation Development
PM6	Parameterized Model 6
PRESS	Predicted Residual Sum of Squares
QSAR	Quantitative Structure-Activity/Toxicity Relationship
Q^2_{LOO}	Leave-One-Out Cross Validation
Q^2_{LMO}	Leave-Many-Out Cross Validation
QUIK	Q^2 Under Influence of *K*
RMSE	Root Mean Square Error

1. INTRODUCTION

Dyes have been applied in many industries for a long time, including the textile, printing, medical and energy industries. There are some properties of dyes which can be tested experimentally such as colour, brightness, solubility, fastness, mutagenicity, diffusion constant and so on (Kothari et al., 2013). Some of the dyes are toxic, carcinogenic, mutagenic or harmful to human health. The textile industry accounts for the largest consumption of dyestuffs at nearly 70 percent (Mathur et al., 2012). Textile dyes are mainly classified according to their uses in the dyeing process. Many functional classes of dyes, containing acid, basic, disperse, reactive and solvent dyes comprise azo compounds (Freeman, 2013). Azo dyes account for the largest proportion of all synthetic dyes and include approx. 70 % of all organic dyes, which are currently on the market and are manufactured mainly in China, India, Korea, Taiwan, and Argentina. Clothing textiles on the European market were mostly dyed in those Asian countries, and are then imported to Europe.

Aminoazo derivatives are important because of their widespread use in the textile industry (Stead, 1990). Some azo dyes are both mutagenic and carcinogenic. It has been proved that variety of 4-aminoazobenzene (AAB), *N*-methyl-4-aminoazobenzene (MAB) and *N*,*N*-dimethyl-4-aminoazobenzene (DAB) derivatives are mutagenic (Garg et al., 2002). It was known that benzidine is a mutagenic moiety of many azo dyes and can be generated from azo dyes through the reduction by intestinal and environmental microorganisms (Chung et al., 2006). Azo dyes also produce free aromatic amines that are significantly mutagenic and carcinogenic. In addition to the effects caused by exposure to contaminated water and food, workers who deal with these dyes can be exposed to them. Moreover, scientific researches have proven their adverse health effects. Myslak et al. (1991) observed that German painters developed bladder cancer after long- time exposure to azo dyes.

Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) is the Regulation (EC) with the No 1907/2006 of the European Parliament and of the Council of 18 December 2006. It came into force on 1 June 2007. The purpose of this regulation is to protect human health and the environment. The enforcement of REACH has a great impact on the textile and leather industry.

Textile and leather products belong to articles under REACH regulation. Those products might contain Substances of Very High Concern (SVHC) and REACH restricted substances regulated by

REACH regulation. Suppliers (manufacturers, importers or retailers) shall first identify all possible toxic chemicals in their products. If the products do not contain SVHC and REACH restricted substances above the threshold limits of REACH, the products are compliant with REACH.

If products contain any SVHC and restricted substances above the threshold limits, suppliers are responsible to identify these hazardous substances belong to (SVHC candidate list or REACH restriction list or Authorization list) and adopt the required measures to achieve REACH compliance.

Of the minimum required data for the assessment of environmental and human hazards under REACH regulation CMR (Carcinogenic, Mutagenic and Reproductive toxicity) properties are of major concern together with PBT (Persistence, Bioaccumulation and ecoToxicology) properties. However, this information is not available for the majority of the existing chemicals.

Mutagenicity is widely recognized as a valid surrogate of carcinogenicity. The term mutagenicity refers to the ability of a chemical to induce genetic damages that may occur by several mechanisms involving interactions with the DNA or with both the DNA and other cellular targets, e.g. proteins (i.e., chromosomal aberrations, and changes in the number of chromosomes) (Benigni et al., 2011).

Mutagenic activity is measured with Ames test which is known as the bacterial reverse mutation assay (Ames et al., 1973a; Ames et al., 1973b). It is a well-known and widely used assay to detect mutagenicity *in vitro* and is of crucial importance in development as an early alerting system for potential carcinogenicity and/or teratogenicity. Kaur (1993a and 1993b) screened textile azo dyes for mutagenicity with Ames/Salmonella assay with and without metabolic activation. Many of the amines were found to be mutagenic in tester strains TA98 and TA100 but require exogenous mammalian activation (S9) for activity (Chung, 2006).

The information concerning the mechanism of action of the molecules studied thanks to the various strains of *Salmonella typhimurium* available. In the Ames mutagenicity, histidine dependent strains of *Salmonella typhimurium* is used. When these strains are exposed to a mutagen, reverse mutations that restore the functional capability of the bacteria to synthesize histidine enable bacterial colony growth on a medium deficient in histidine (“revertants”). Knowing that many chemicals interact with genetic material only after metabolic activation by enzyme systems not available in the bacterial cell, the test compounds are in many cases additionally examined in the presence of a mammalian metabolizing system, which contains liver microsomes (rat liver S9 mix). TA98 bacterial strain with S9 activation is well known to detect frameshift mutagens (Carroll et al., 2002). Some of azo dyes such as dimethylamino-phenylazoenthiazole (6BT) are mutagen in the *Salmonella typhimurium* TA98 bacterial tester strain in the presence of an induced rat-liver S9 mix (TA98+S9) (Sztandera, 2003). Kojima et al. (1991) showed that the 3 methoxy-4 aminoazo-benzene (3-OMe-AAB) is a strong mutagen in *Escherichia coli* and *Salmonella typhimurium*, whereas 2-OMe-AAB is an extremely weak mutagen under the same condition.

The mutagenic potency of azo dyes is strongly dependent on the position of substituents with respect to both the aromatic rings and the amino nitrogen atom. This implies that there is a relationship between chemical structures and mutagenic activities. Mutagenicity data for most of the chemicals in use including dyes are scarce and should be known to understand their effects on the environment. However, conducting experiments for this purpose is time and energy consuming in addition to being costly. For this reason, quantitative structure-activity relationship (QSAR) method for predicting mutagenic activities without any experimental data may be desirable. Due to the lack of experimental data, there has been increasing use of theoretical structural descriptors in the hazard assessment of chemicals via QSAR models. QSAR studies are based on the idea that property/biological activity of a chemical can be predicted or characterized by its structure. Predicted property/biological activity values from valid QSAR models have a high potential to fill the data gaps. Another major goal of QSAR is the rational design of the new and more active compound starting from the known ones. One advantage is that this method needs no equipment and reagent. Researchers compute the molecular descriptors by computer using chemical structures and software packages. QSAR models have been used for decades and the usage of QSAR is recommended and supported by European Chemical Agency (ECHA, 2017).

In the literature, although there are some QSAR studies on the prediction of mutagenic activity of dyes (Garg et al., 2002; Bhat et al., 2005), they have some drawbacks. As such, Pasha et al. (2008) developed several QSAR models for the mutagenicity of aminoazobenzenes which do not carry out the principles set by Organization for Economic Co–operation and Development (OECD, 2007) and not tested with the up-to-date validation criteria. To increase the regulatory acceptance of (Q)SAR methods, the OECD is developing a QSAR Toolbox to make (Q)SAR technology readily accessible, transparent, and less demanding in terms of infrastructure costs in 2008. The Toolbox incorporates information and tools from various sources into a logical workflow. Devillers et al. (2010) evaluated the toolbox for estimating the mutagenicity of chemicals. Moreover, VEGA which is another ideal application for batch processing large dataset provides freely tens of QSAR models to predict the properties such as persistence, logP, bioconcentration factor (BCF), carcinogenicity, mutagenicity, skin sensitization of the compounds. Instituto Mario Negri is the coordinator of the VEGA project (www.vegahub.eu, last accessed June 2019).

Mombelli et al. (2016) studied the mutagenicity of the compounds using the different types of QSAR models such as VEGA QSAR. This application can be used in any system supported by JAVA. As it is mentioned above, the application of QSAR in filling the data gap is supported by the European Chemical Agency (ECHA). ECHA has reorganized its Practical Guide on How to use and report QSARs with further advice and examples on using QSARs for registering under REACH in Helsinki, 17 March 2016.

While the toxic effects of many of the chemicals now entering the public market place are being routinely examined, many thousands of new and untested compounds are being synthesized annually in thousands of laboratories and scientists are becoming increasingly concerned about the potential toxicity of the new chemicals they are being exposed to. Therefore, scientists focus on the design and development of non-toxic and environmentally friendly consumer goods using the principles of green chemistry (Clark, 2006). Hence, QSAR also promotes green chemistry. Not only due to their higher efficiency and lower cost, but they can also obtain a rapid assessment of the potential effects of chemicals on human health and also the environment. Therefore, the QSAR models have been developed as feedback to different legislation around the world (e.g. REACH) to help in decreasing animal testing and designing more greener compounds. Many reliable models have been produced by this method, and it was also employed in the present study. In an attempt to find an adaptable, well-performing and predictive model for mutagenicity of dyes, we applied QSAR modelling to predict the unknown mutagenic activity of chemicals (particularly current textile dyes) using the mutagenic activity of aminoazo dyes and aromatic amine derivatives. Such studies are useful in terms of identification and prioritization of dangerous chemicals as well as providing data for a wide range of chemicals with no experimental mutagenicity data.

1.1. Purpose of the Study

The aim of the present study is; (1) to compile mutagenicity data for azo dyes and aromatic amine derivatives from literature, (2) to develop valid linear QSAR models for the mutagenic activity for aminoazodyes and aromatic amine derivatives in the TA98 *Salmonella typhimurium* bacterial strain with S9 activation, (3) to evaluate the performance of the developed QSAR models using a test/validation set, in which data were not used in the generation of the model, and (4) to apply all the up-to-date internal and external validation criteria to the generated QSAR models, and (5) to test the predictive performance of the selected QSAR model for the unknown mutagenic activity of currently used 33 anionic water-soluble textile dyes (eight anthraquinone and 25 azo dyes).

2. THEORETICAL BACKGROUND

2.1. Dyes

Dyes are classified according to their application and chemical structure and are composed of a group of atoms known as chromophores, responsible for the dye color. These chromophore-containing centers are based on diverse functional groups, such as azo, anthraquinone, nitro, aryl-, carbonyl and others (Prasad, 2010). With respect to the number and production volumes, azo dyes are the largest group of colorants, constituting 60-70% of all organic dyes produced in the world (Bafana, 2011). An early theory of dyes first formulated by O. Witt provided a basis for understanding the relation between color and the structure of the compound. According to this theory, a dye consists of three components: one or more the fused benzene rings attached to the unsaturated groups called as chromophores (e.g., –N=N–, –NO_2, –C=O) and basic groups called as auxochromes (e.g., NH_2, OH groups). A chromophore is a chemical group that is responsible for the appearance of color in compounds where it is located. The colorants are sometimes also classified according to their main chromophore (e.g., azo dyes contain the chromophore –N=N–) (Iqbal, 2008).

Although dyes have an affinity to the substrates to which they are being applied (Pereira and Alves, 2012), they colored substances which are soluble or go into solution during the application process (Bafana et al., 2011). As such, up to 200000 tons of these dyes are lost to effluents every year during the dyeing and finishing operations, due to the inefficiency of the dyeing process (Maas and Chaudhari, 2005; Jin et al., 2007; Ogugbue et al., 2011; Saratale et al., 2011). Unfortunately, most of these dyes escape conventional wastewater treatment processes and are often found chemically unchanged in wastewater treatment plant effluents; contaminating raw water or become concentrated in the sludge, causing a disposal problem (Carneiro et al., 2010). Very small amounts of dyes in the water to cause a highly visible change in color. They can also affect the aquatic ecosystem, decreasing the passage of light penetration and gas dissolution in lakes, rivers and other bodies of water (Saranaik and Kanekar, 1995; Banat et al., 1996; Modi et al., 2010). Shaul et al. (1991) studied 18 azo dyes and found that 11 passed practically unchanged through the activated sludge system, 4 were adsorbed by the activated sludge and only 3 were biodegraded, resulting in the release of these substances into bodies of water. Textile effluents have been shown to be mutagenic (Claxton et al., 1998; Pinheiro et al., 2004; Sanchez et al., 1988), and mutagenic dyes have been detected in many rivers.

Dyeing is the treatment of fabric with a dye to impart color. Azo dyes are the most important groups. There are main types of dyes used for dyeing different kinds of fibers (Figure 2.1).

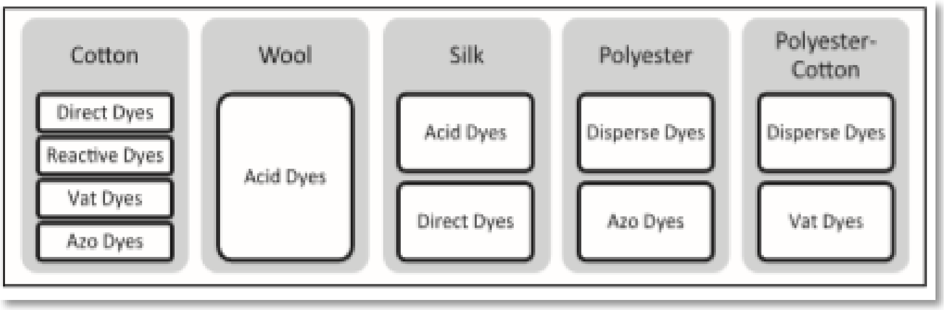


Figure 2.1. Dyes for different fibers.

Although the high colouring power of dyes gives rise to aesthetic damage and hence public complaint, several classes of dyes are considered as possible carcinogens or mutagens. It is also noteworthy that some dyes are highly toxic and limiting downstream beneficial uses such as recreation, drinking water and irrigation (Hubbe et al., 2012; Prztstas et al., 2012;). As these dyes are typically very stable in the aquatic environment, the presence of dyes in water bodies, especially those that will be used to produce drinking water, is also a health concern since the population can be exposed to these compounds from the ingestion of both contaminated food and water (Chequer, 2015).

Many dyes do not degrade easily due to their complex structure and textile dye effluent does not decolorize even if the effluent is treated by the municipal wastewater treatment systems (Shaul et al., 1991; Robinson et al., 2002; Forgacs et al., 2004). If textile wastewater, not properly treated is released into the environment, it can introduce metals (Cr and Cd) and organochlorine compounds which can bio-accumulate in fishes in receiving streams.

Before the discharge of textile wastewater into the water bodies, many treatment processes have been developed to treat the wastewater due to the various health risks of the azo dyes to human and aquatic organisms (Figure 2.2.). Of the physical, biological and oxidation methods, ozone treatment which is in the advanced oxidation category, has good decolorization performance (Holkar et. al., 2016). Flocculation is a useful method for the decolorization of wastewater containing disperse dyes although they have low decolorization efficiency for the wastewater having reactive and vat dyes. Contrarily, another physical method, adsorption, has significant decolorization efficiency for wastewater containing a variety of dyes. Oxidation methods are usually used for the degradation of dyes by chemical means due to its easiness of application. The biological methods have some advantages, such as eco-friendly, cost-competitive, less sludge production, giving non-hazardous metabolites and less water consumption compared to physical and oxidation methods (Hayat et al., 2015). However, textile wastewater parameters after biological treatment are not in compliance with the textile wastewater discharge standards. Therefore, the oxidation process should be applied before biological treatment. On the other hand, if the volume of textile effluent is small, physical and oxidation methods are effective for the removal of dyes in textile wastewater.

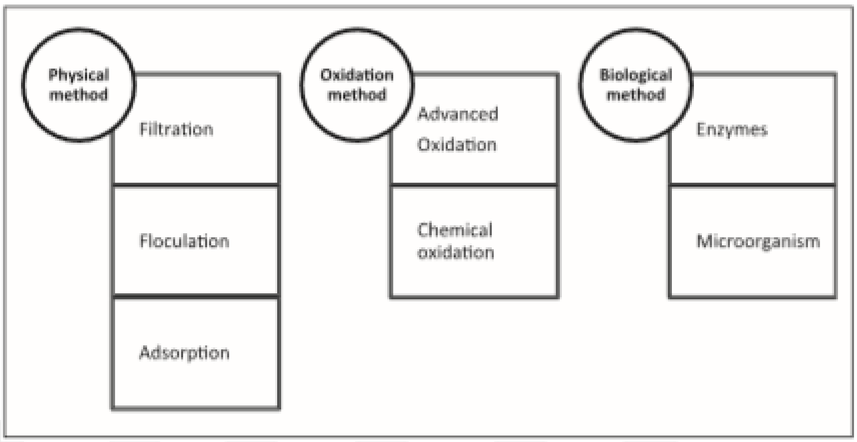


Figure 2.2. Treatment methods applied to textile wastewater.

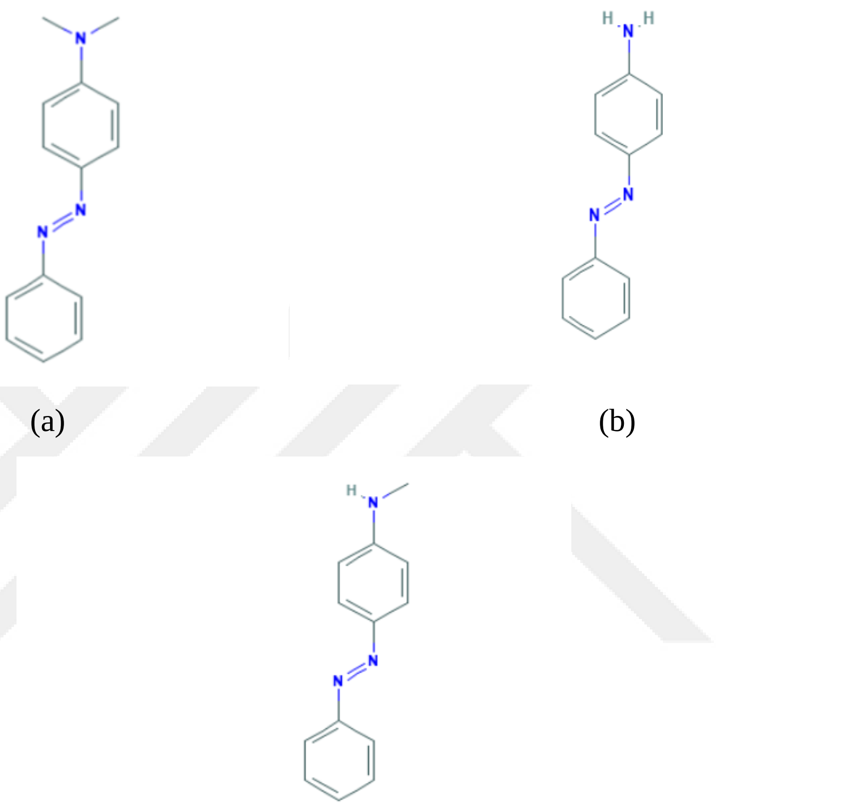
2.1.1. Aminoazobenzene dyes

Aminoazo derivatives are extremely important industrial colorants and widely used in the textile industry (Stead, 1990). As stated above, the azo dyes are carcinogenic, mutagenic or toxic, therefore, they are dangerous to human health as they might release aromatic amines, which in direct and prolonged contact with the human skin or oral cavity. Mathur et al. (2012) reviewed the mutagenicity of azo and non-azo dyes due to extensive recent data on the carcinogenicity and mutagenicity of this group of dyes.

Moreover, it has been proved that variety of 4-aminoazobenzene (AAB), *N*-methyl-4-aminoazobenzene (MAB) and *N*,*N*-dimethyl-4-aminoazobenzene (DAB) derivatives are mutagenic (Garg et al., 2002).

The main skeleton of aminoazobenzene (AAB) dye and examples of AAB are shown in Figure 2.3 and Figure 2.4, respectively. Freeman et al. (2013) have demonstrated that some small structural modifications of these aminoazobenzene derivatives can reduce or eliminate their mutagenic activity, while maintaining the physical and/or chemical properties that make them useful industrial chemicals. In Figure 2.3, the placement of an azo group between methyl groups produces a colorless compound, while a yellow-orange color is obtained when the azo group is placed between aromatic rings (IARC, 2010). In addition, changing the position of the methoxy group on the phenyl rings dramatically influences the carcinogenic behavior of the resulting compound. For example, 2-OMe-AAB is noncarcinogenic in rats, whereas 4¢-OMe-AAB is carcinogenic.

Representative chemical structures of some azo dyes and chromophore groups are given in Figure 2.3.



(a)

(b)

(c)

Figure 2.3. Example of aminoazobenzene dye: (a) 4-aminoazobenzene (AAB) and (b) *N*-methyl- 4-aminoazobenzene (MAB) and (c) *N*,*N*-Dimethyl-4-aminoazobenzene (DAB).

In the past years, several studies were conducted on human exposure to azo dye precursors. Since 70’s, intestinal cancer has been more widespread in highly industrialized societies, and thus there may be a relation between the rise in the number of cases of diseases and the use of azo dyes (Wolff and Oehme, 1974; Chung et al., 1978). Later, it has been proven that chronic exposure to aromatic amines would lead to bladder cancer (Bi et al., 1992; Carreon et al., 2010; Sorahan, 2008; You et al., 1990). If an azo compound is ingested orally it can be reduced by anaerobic intestinal microflora and possibly by mammalian azo reductases in the intestinal wall or the liver, to free aromatic amines. Additionally, Zeilmaker et al. (1999) concluded that the dermal exposure to a certain amount of aromatic amines from textiles may lead to cancer risk.

Chung and Cerniglia (1992) reviewed the mutagenicity of azo dyes and found that the MAA, p-phenylenediamine, is a mutagenic moiety of many azo dyes. Lin and Solodar (1988) have also previously pointed out that synthetic azo dyes, which contain a moiety that would be expected to be metabolized to p-phenylenediamine by liver microsomal enzymes, gave positive responses in the Salmonella/microsome assay. It was found that m-diaminobenzene, 2,4-diaminotoluene, 2,4-diaminoethylbenzene are mutagenic to TA98 and TA100 in the presence or absence of metabolic activation (Shahin, 1980). For instance, 2-methoxy-4-aminoazobenzene is an extremely weak mutagen whereas, under similar conditions, 3-methoxy-4-aminoazobenzene is a potent hepatocarcinogen in rats and a strong mutagen in *Escherichia coli* and *Salmonella typhimurium* (Hashimoto et al., 1977; Esancy et al., 1990; Garg et al., 2002; Umbuzeiro et al., 2005a). The potency of these azo dyes is dependent on the position of substituents with respect to both the aromatic rings and the amino nitrogen atom.

Ashby et al. (2006) showed that DAB was not mutagenic, but when pre-incubated with S9, it became mutagenic. They suggested that the mutagenicity of DAB may be attributed to its reductive product *N*,*N*-dimethylphenylenediamine (DMPD). Chung et al. (2006) demonstrated clearly that DMPD was mutagenic with Salmonella tester strain 1538 with S9 microsomal mix.

It should be noted that many of these monocyclic aromatic amines are mutagenic to humans and animals. Therefore, a complete evaluation of the safety of these dyes in the human environment must include an evaluation of their genotoxicity or mutagenicity. It should also be noted that azo dyes which are able to release specific aromatic amines are prohibited in most countries of the world (Chen et al., 2017). The European Community (EC) prevented the production and sale of consumer products dyed with azo derivatives, because azo dyes can be degraded into 22 aromatic amines in the following list in concentrations above 30 ppm in the finished articles or their dyed parts (Table 2.1). EC Directive 2002/61 restricts the use of only about 5% of azo dyes (Cox et al., 2002). Later, the European Commission performed a fast track consultation on a possible restriction of more hazardous substances (CMR 1A & 1B) in textile articles and clothing for consumer use.

Table 2.1. List of amines included in the EC Directive 2002/61

Aromatic Amine	**CAS number**
4-aminobiphenyl	92-67-1
Benzidine	92-87-5
4-chloro-o-toluidine	95-69-2
2-napthylamine	91-59-8
o-amino-azotoluene	97-56-3
5-nitro-o-toluidine	99-55-8
p-chloroaniline	106-47-8
4-methoxy-m-phenylenediamine	615-05-4
4,4-methylenedianiline	101-77-9
3,3-dichlorobenzidine	91-94-1
3,3-dimethoxybenzidine	119-90-4
3,3-dimethylbenzidine	119-93-7
4,4-methylenedi-o-toluidine	838-88-0
6-methoxy-m-toluidine	120-71-8
4,4-methyl bis -(2-chloro-aniline)	101-14-4
4,4-oxydianiline	101-80-4
4,4-thiodianiline	139-65-1
o-toluidine	95-53-4
4-methyl-m-phenylenediamine	95-80-7
2,4,5-trimethylaniline	137-17-7
o-anisidine	90-04-0
4-aminoazobenzene	60-09-3

2.1.2. Aromatic amine derivatives

Aromatic amines represent one of the most important classes of industrial and environmental chemicals. They have widely used in many industries such as agricultural chemicals, dyes etc. (Woo et al., 2001). Many aromatic amines have also been reported to be carcinogen and mutagen. They are a class of chemicals traditionally recognized as of high concern for human health. They find applications in several chemical industry manufacturing sectors such as oil refining, production of synthetic polymers, adhesives and rubbers, pharmaceuticals, pesticides and explosives (Synyderwine et al., 2002). The structure and numbering system for the parent aromatic amine derivatives used in the present study are given in Figure 2.4.

As early as the late nineteenth century, a doctor related the occurrence of urinary bladder cancer to the occupation of his patients, thus demonstrating concern about the exposure of humans to carcinogenic aromatic amines produced in the dye manufacturing industry. Laboratory investigations subsequently showed that rats and mice exposed to specific azo dye arylamines or their derivatives developed cancer, mainly in the liver (Weisburger, 1997, 2002). In addition, workers in textile dyeing, paper printing, and leather finishing industries, exposed to benzidine based dyes such as Direct Black 38, showed a higher incidence of urinary bladder cancer (Meal et al., 1981).

The large database of mutagenicity results for the aromatic amines has been studied with QSAR approaches (Benigni et al., 2003). Trieff et al. (1989) studied the *Salmonella typhimurium* mutagenicity of 19 aromatic amines tested in the strains TA98 and TA100 with the addition of S9 metabolizing fraction from Aroclor 1254-induced rat liver.

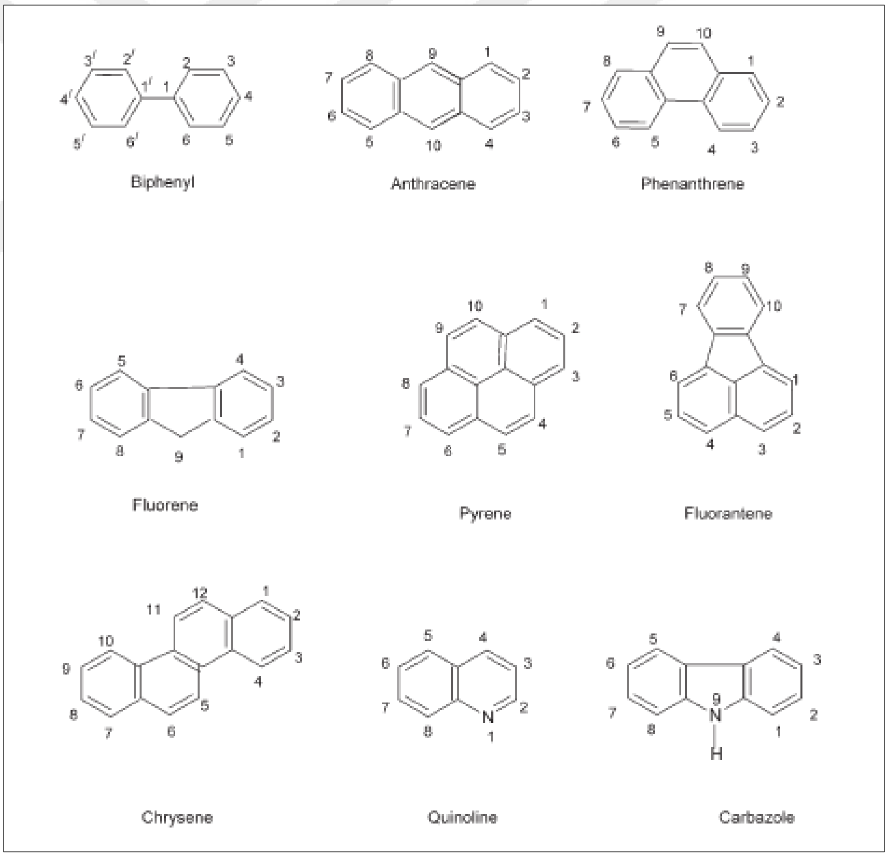


Figure 2.4. Structure and numbering system for the parent aromatic amine derivatives used in the present study.

Shahin et al. (1983) in a separate study on the mutagenicities of nitro-*p*-phenylenediamine derivatives, discovered that blockage of one amino group in nitro-*p*-phenylenediamine by two hydroxyalkyl groups or blockage of both amino groups, each by one hydroxyalkyl group, eliminated the mutagenic activity of the compound. In other words, while nitro-*p*-phenylenediamine is a strong mutagen, 3-nitro-4-amino-*N*,*N*-di-hydroxyethyl-aniline, 3- nitro-4-*N*-2-hydroxy-ethyl-amino-*N*-2-hydroxy-ethyl-aniline, 3-nitro-4-*N*-2-aminoethyl-amino-*N*,*N*-dihydroxyethyl-aniline, 3-nitro-4-*N*-methyl-amino-*N*,*N*-dihydroxyethyl-aniline, 3-nitro-4-2-*N*,*N*-diethylamino,ethylamino-*N*,*N*-dihydroxyethyl-aniline, and 3-nitro-4-*N*,*N*-di-ethylamino-propylamino-*N*,*N*-dihydroxyethyl-aniline are not mutagenic. It proves that both free amino groups in the para position and the nitro group are needed for the mutagenic activity of nitro-*p*-phenylenediamine. The mutagenic activity appears to be allied with the position of the amino, nitro, and hydroxy groups in their molecular structures (Shahin, 1985).

Depending on the individual compounds, many aromatic amine metabolites are considered to be non-biodegradable or only very slowly degradable (Saupe, 1999), showing a wide range of toxic effects on aquatic life and higher organisms (Weisburger, 2002; Pinheiro et al., 2004; Khalid et al., 2009).

2.2. Ames Test

The Ames test which is an inexpensive and a short-term bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage that leads to gene mutations. Moreover, since the assay is not a live animal model, it fits the 3R principles (Locke, 2006). This test is used worldwide as an initial screen to determine the mutagenic potential of new chemicals and drugs (Mortelmans et al., 2000). The Ames test is an *in vitro* method that commonly uses one of five strains of *Salmonella typhimurium.* In addition, the Ames test is by far the most commonly used, long-established *in vitro* test for chemical mutagenicity screening (OECD Test Guideline No.471, 1997).

The *Salmonella* mutagenicity test was specifically designed to detect chemically induced mutagenesis (Mortelmans et al., 2000). The *Salmonella*, or Ames test consists of a range of bacterial strains that together are sensitive to a large array of DNA-damaging agents (Ames, 1984; Zeiger, 1987). TA98 and TA1538 are sensitive to frameshift mutagens, TA100 and TA1535 are used to detect base-pair substitution mutation, TA97 and TA1537 are used for base-pair substitution and some frameshift mutations. TA102 detects mutagens that other strains cannot detect such as formaldehyde. All strains are histidine dependent by virtue of a mutation in the histidine operon (Mortelmans et al., 2000). Histidine is an essential component for growth. Therefore, the bacteria are unable to multiply unless a suitable mutagen causes the proper type of reverse mutation in the histidine gene.

The Ames test with the *Salmonella typhimurium* strains TA98 and TA100 was designed to be compatible with the procedure indicated in the OECD test guideline No. 471, with and without metabolic activation (S9 mix) (OECD Test Guideline No.471, 1997). Test No. 471 is a bacterial reverse mutation test. It uses amino-acid requiring at least five strains of *Salmonella typhimurium* and *Escherichia coli* to detect point mutations by base substitutions or frameshifts. It detects mutations which revert mutations present in the test strains and restore the functional capability of the bacteria to synthesize an essential amino acid (Ames et al., 1975). Harding et al. (2015) could demonstrate the significance of TA98 and TA100 for the detection of aromatic amines mutagenicity. It was noted that the TA98 strain of *Salmonella typhimurium* is sensitive to frameshift mutations (Garg, 2002).

2.3. Quantitative Structure-Activity Relationship/ Quantitative Structure-Property Relationship Studies on Dyes

Recently, computational methods have been used to predict the mutagenic potential of azo dyes instead of new experimental studies. Predictive models can be developed to predict the toxicity of azo compounds before their synthesis, based only on their chemical structure. QSAR methods are the most usual alternative for this purpose. They are mathematical models used to predict activities and properties from the physical characteristics (solubility, log Kow, etc.) and/or molecular structure.

In 2006, Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) was established by the European Council and the European Parliament. REACH states the need for the evaluation of chemicals that are imported or produced in quantities greater than 1 tonne per annum (tpa) for the assessment of toxic effects by 2018. The European REACH regulation was introduced with the main goal of protecting both human health and the environment. For the assessment of environmental and human hazards, PBT (Persistence, Bioaccumulation, and ecoToxicology) properties should be known as well as CMR (Carcinogenic, Mutagenic and Reproductive toxicity) properties. However, this information is not available for the majority of the existing chemicals.

It is well known that small changes in chemical structure can cause too deep differences in mutagenic activities (Shahin, 1987). The properties of dyes are determined by the structure of the compounds. The potency of azo dyes is strongly dependent on the nature and position of substituents with respect to both the aromatic rings and the amino nitrogen atom (Garg et al., 2002). Substitution of the amino group affects the mutagenicity. For example, 3-nitro-4-*N*-2 hydroxyethylaminoanisole is mutagenic, 3-nitro-4-*N*-2-hydroxyethyl-aminoaniline weakly mutagenic, but 3- nitro-4-*N*-b-hydroxyethyl-aminophenol is non-mutagenic. In addition, the colour of azobenzene compounds is dependent on the number of –N=N– bonds and the size of the conjugated system (Shahin, 1986). Therefore, studies on the structure-activity relationships may offer the prospect of identifying the critically important parameters in a molecular structure that influence their biological activities like mutagenicity.

Luan et al. (2013) reviewed recent advances and perspectives of QSAR/QSPR studies of dyes. They reported activity/property related research of dyes that have been published in the period from 1995 to 2012. Their emphasis was placed particularly on studies based on QSAR/QSPRs that have contributed to the theoretical design of new, potent and selective dyes. Other studies have concentrated on hazardous properties (such as mutagenicity) as some dyes are toxic, volatile, explosive or radioactive substances. Theoretical methods could provide a means of predicting such properties far more quickly and cheaply than carrying out formal laboratory test protocols. Garg et al. (2002) developed several linear and non-linear QSAR models for the observed mutagenic behaviour of aminoazobenzene derivatives with a variety of molecular descriptors. Three years later, Bhat et al. (2005) developed QSARs that correlated the observed mutagenic activity of 181 aromatic amine derivatives. Common features of the reported QSAR models in these studies are that they do not comply with the OECD principles. According to the Organisation for Economic Co-operation and Development (OECD), a valid QSAR model must have five features. These are (1) a defined endpoint, (2) an unambiguous algorithm, (3) a defined domain of applicability, (4) appropriate measures of goodness of fit, robustness and predictivity and (5) a mechanistic interpretation, if possible (OECD, 2007).

The main steps involved in the development and analysis of a QSAR model can be summarized as: (1) Preparation, analysis, and setup of the input dataset, (2) Model calculation based on selected descriptors, (3) Model exploration, validation, and selection (Roy et al., 2015) (Figure 2.5).

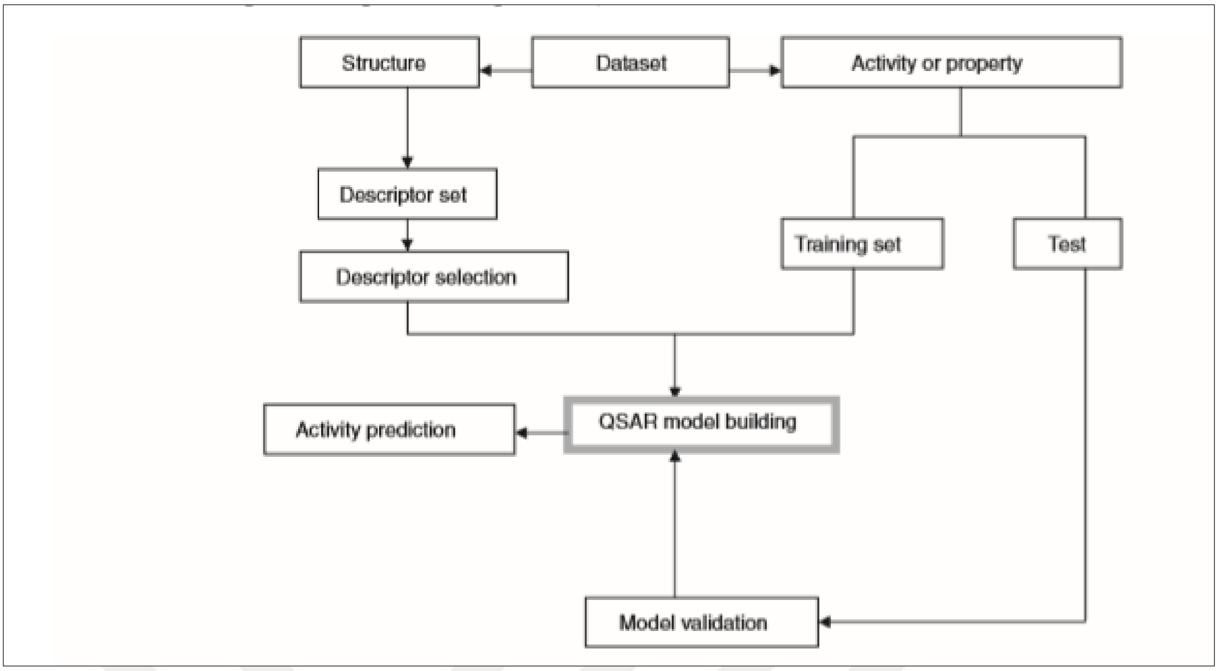


Figure 2.5. Flowchart of a general QSAR study.

3. MATERIALS AND METHODS

3.1. QSAR Workflow

A typical workflow of QSAR used in the present study is given in Figure 3.1. The first stage of the workflow is the selection of a dataset. QSAR modelling was done by following dataset compilation, dataset curation, geometry optimization, and descriptor calculation, dataset splitting, descriptor selection, model selection, and testing the generated QSAR models in terms of some internal and external validation parameters. Then, the best model was tested to achieve predictive ability by using external set compounds.

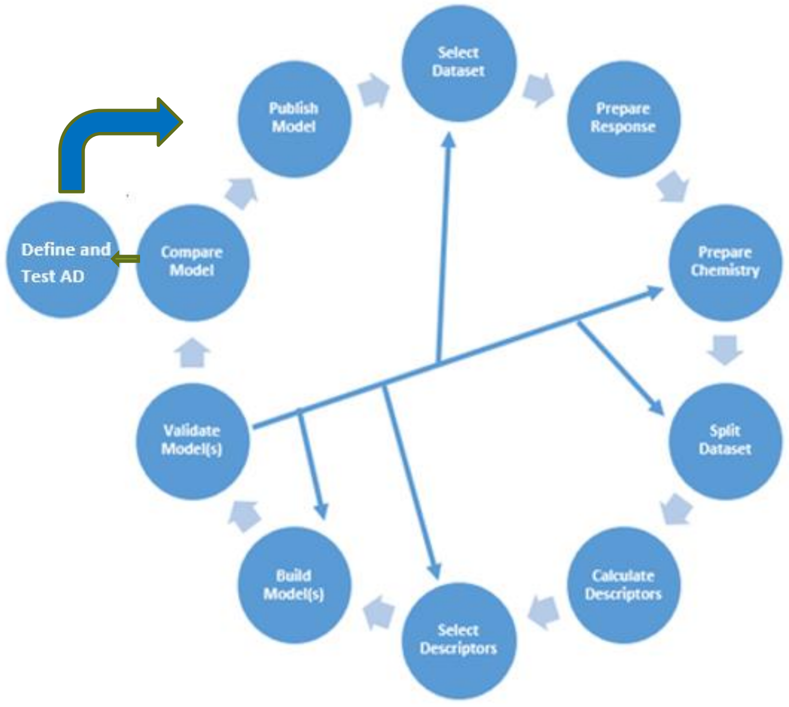


Figure 3.1. A typical QSAR Workflow.

3.2. Datasets

Two datasets were combined in the present study. The first dataset of mutagenicity data was taken from Garg et al. (2002). This data contains mutagenic activities (rev/nmol) of 43 aminoazobenzene dyes in the TA98 *Salmonella typhimurium* bacterial strain with S9 activation. The second dataset of mutagenic activity data of 181 aromatic amine derivatives was taken from Bhat et al. (2005). The compounds used in the present study include primary amines, diamines and derivatives of AAB, MAB and DAB. The common feature of this structurally diverse compounds is that they all contain at least one amino group bonded to an aromatic or heteroaromatic ring system (a simple ring or more than one ring forming a conjugated system, fused or nonfused). The 41 compounds of 181 amine derivatives are common in the two datasets. Additionally, the conformers for 6 molecules could not be calculated, because of their high number of conformers. Therefore, 177 compounds were used as a dataset in this study. The mutagenic activity values (rev/nmol) of all chemicals were converted into the logarithmic scale for modelling. As external set chemicals, currently used 33 anionic water-soluble textile dyes (eight anthraquinone and 25 azo dyes) with no experimental mutagenicity data were used. The structure of Acid Yellow 61 (AY61) which is a water-soluble textile dye is given in Figure 3.2.

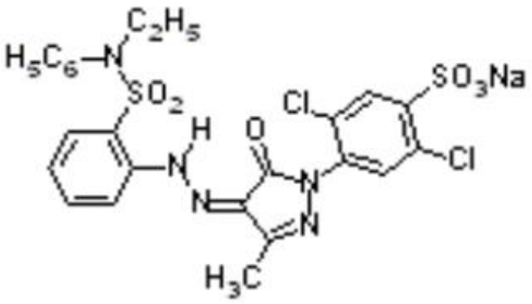


Figure 3.2. An example of a water-soluble textile dye: Acid Yellow 61 (AY61).

3.3. Descriptor Calculation

At first, the three-dimensional structure of each molecule was drawn with SPARTAN 10 (Wavefunction Inc., 2010) software. Besides structural drawings, conformational analyses and geometry optimizations were performed at the semi-empirical PM6 level using the same software. An example of the 3D structure of N-hydroxy-2-methoxy-AAB dye drawn in SPARTAN 10 was given in Figure 3.3. For potassium sulfonate ($-SO_3^-K^+$) salt of dye structures were drawn as sulfonic acid form by replacing potassium with hydrogen. The conformer which has the lowest aqueous energy (E_{aq}) was saved as .mol2 file. The number of examined conformers depends on number of rotable bonds and flexibility of the molecule. The calculation time for a molecule depends on the number of conformers. One of the conformers of a chemical in the dataset (*N*-hydroxy-2-methoxy-AAB) drawn in SPARTAN software is given in Figure 3.3. After that, these .mol2 files were used for the calculation of molecular descriptors using DRAGON 07 software (Kode, 2017). Type of descriptors calculated by DRAGON 7.0 software is given in Table 2.2.

Table 2.2. Descriptor blocks and types in DRAGON 7.0 software.

ID Block	**Block Description**	**Number of Descriptors**
1	Constitutional descriptors	43
2	Ring descriptors	32
3	Topological indices	75
4	Walk and path counts	46
5	Connectivity indices	37
6	Information indices	48
7	2D matrix-based descriptors	550
8	2D autocorrelations	213
9	Burden eigenvalues	96
10	P_VSA like descriptors	45
11	ETA indices	23
12	Edge adjacency indices	324
13	Geometrical descriptors	38
14	3D matrix-based descriptors	90
15	3D autocorrelations	80
ID Block	**Block Description**	**Number of Descriptors**
16	RDF descriptors	210
17	3D-MoRSE descriptors	224
18	WHIM descriptors	114
19	GATEWAY descriptors	273
29	Randic molecular profiles	41
21	Functional group counts	154
22	Atom-centered fragments	115
23	Atom-type E-state indices	170
ID Block	**Block Description**	**Number of Descriptors**
24	CATS 2D	150
25	2D Atom Pairs	1596
26	3D Atom Pairs	36
27	Charge descriptors	15
28	Molecular properties	20
29	Drug like indices	27
30	CATS 3D	300

The descriptors generated by the software SPARTAN are: energy of the lowest unoccupied molecular orbital (E_{LUMO}), the energy of the highest occupied molecular orbital (E_{HOMO}), the energy in the aqueous phase (E_{aq}), molecular weight, dipole moments, gas–phase energy (E), CPK volume and area (V and A, respectively). Additional descriptors such as (E_{HOMO}-E_{LUMO}) gap, hardness(η), softness (σ), chemical potential, electronegativity, and electrophilicity index (ω) were calculated from the energies obtained from SPARTAN 10 according to the equations given below (LoPachin et al., 2007).

$$\boldsymbol{I}(\text{ionization potential}) = -E_{HOMO} \tag{3.1}$$

$$\boldsymbol{A}(\text{electron affinity}) = -E_{LUMO} \tag{3.2}$$

$$\boldsymbol{X}(\text{electronegativity}) = \frac{I+A}{2} \tag{3.3}$$

$$\mu = -X \tag{3.4}$$

$$\boldsymbol{\omega}(\text{electrophilicity index}) = \frac{\mu^2}{2\eta} \tag{3.5}$$

$$\Delta\boldsymbol{E}\text{gap} = E_{HOMO} - \boldsymbol{E_{LUMO}} \tag{3.6}$$

$$\boldsymbol{\eta}(\text{hardness}) = \frac{\Delta Egap}{2} \tag{3.7}$$

$$\boldsymbol{\sigma}(\text{softness}) = \frac{1}{\eta} \tag{3.8}$$

The descriptor values derived from SPARTAN 10 were saved as .txt file to be imported into DRAGON 7.0. The software DRAGON 7.0 can calculate a total 5270 molecular descriptors. The output file of DRAGON software together with SPARTAN 10 derived descriptors were uploaded to QSARINS 2.2.3 (Gramatica et al., 2018) software to develop multiple linear regression (MLR) models for the mutagenic activity (log TA98) of aminoazo dyes (rev/nmol) in *Salmonella typhimurium* (TA98+S9).

SPARTAN is a useful tool to calculate the most stable conformation of compounds and to make geometry optimization. However, it might not be applicative for all chemicals. In this study, we came across some problems for some of the compounds during the calculation of conformer distribution. Thus, for some chemicals whose conformer calculation was not possible with the PM6 method, Molecular Mechanics (MMF) method was employed in SPARTAN 10. Then, we could not calculate aqueous phase energy (E_{aq}, whose value is used to determine the lowest energy conformation of a molecule) for some chemicals. At this stage, the gas-phase energy of the compounds was used to select the lowest energy conformer of the molecule. For some compounds, logP value could not be calculated via SPARTAN, because of the MMF method. Therefore, the calculated logP values from SPARTAN were not included in the descriptor pool.

The output of DRAGON software was saved as a .txt file which was then uploaded into QSARINS (v.2.2.3) software package together with the dependent variable (mutagenic activity) values. Descriptors calculated from SPARTAN 10 were added to this text file as well. Structure drawings, geometry optimization of external set chemicals have been carried out in a previous study (Tugcu et al., 2012). Descriptor values of external set chemicals/structures were calculated similarly.

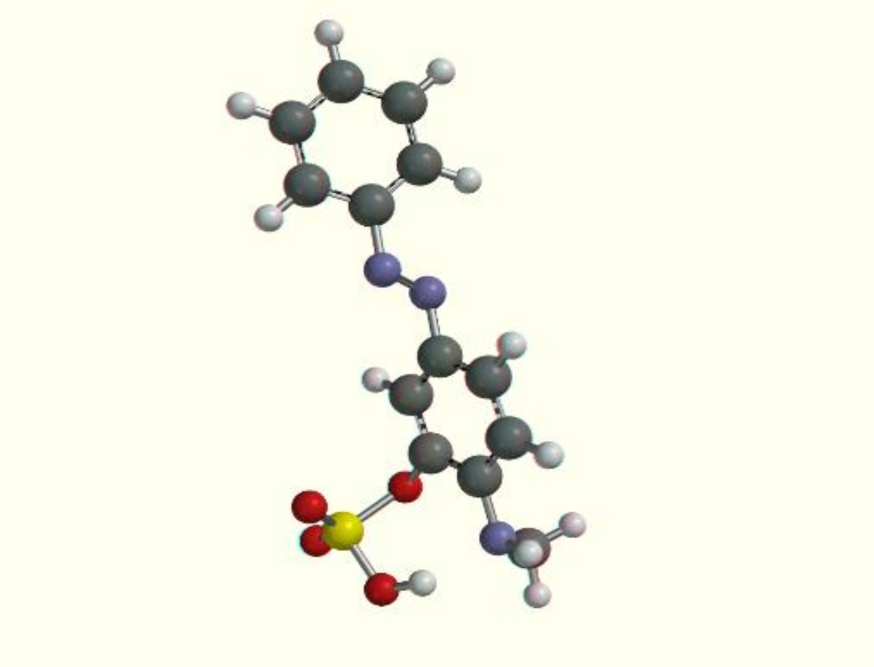
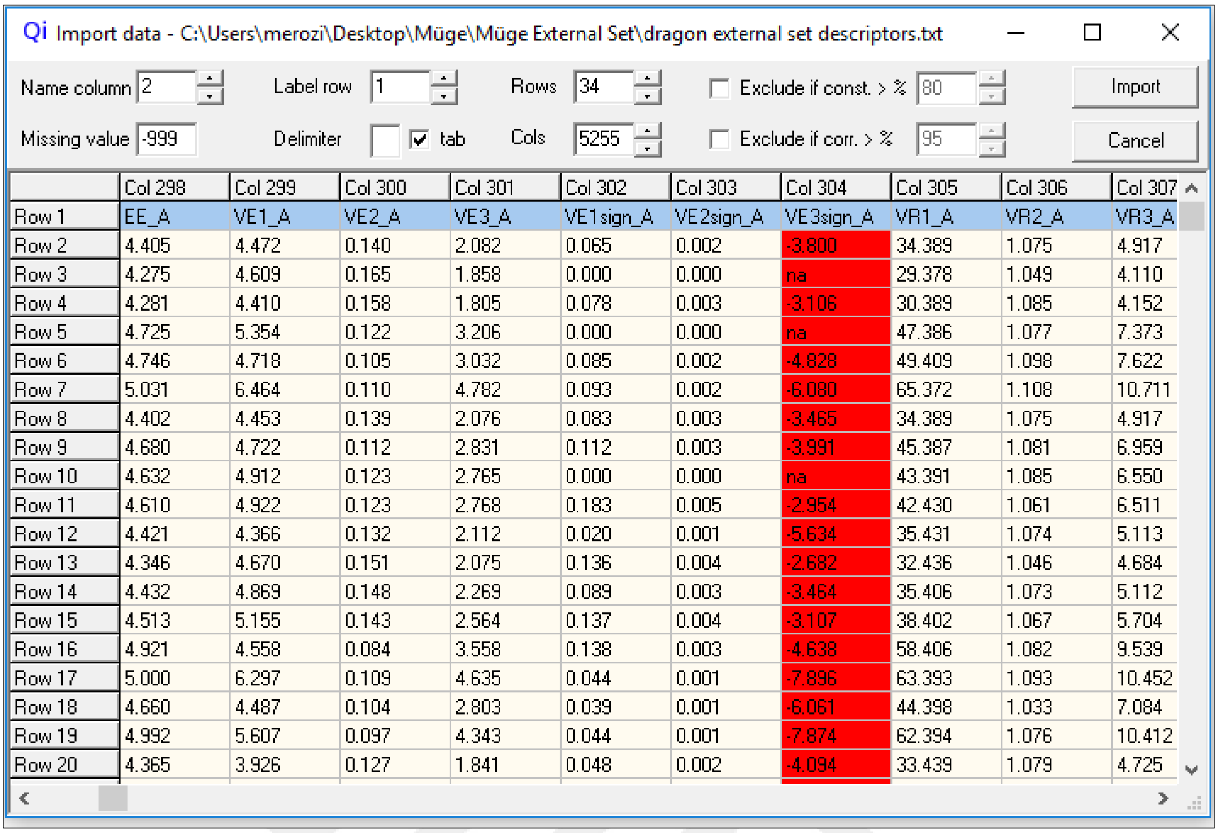


Figure 3.3. The conformer of *N*-hydroxy-2-methoxy-AAB drawn in SPARTAN 10 software.

3.4. Training/test set division, descriptor selection and model development

The appearance of some part of the imported data from DRAGON to QSARINS was given in Figure 3.4. Some of the DRAGON descriptors are not calculated for some chemicals, for those descriptor values “na” appears on the relevant column instead of a descriptor value. Therefore, before importing the data from DRAGON file to the software QSARINS, unacceptable descriptors were eliminated as highlighted by the software QSARINS (Figure 3.4).



Qi Import data - C:\Users\merozi\Desktop\Müge\Müge External Set\dragon external set descriptors.txt

Name column 2 | Label row 1 | Rows 34 | □ Exclude if const. > % 80 | Import

Missing value -999 | Delimiter ☑ tab | Cols 5255 | □ Exclude if corr. > % 95 | Cancel

	Col 298	Col 299	Col 300	Col 301	Col 302	Col 303	Col 304	Col 305	Col 306	Col 307
Row 1	EE_A	VE1_A	VE2_A	VE3_A	VE1sign_A	VE2sign_A	VE3sign_A	VR1_A	VR2_A	VR3_A
Row 2	4.405	4.472	0.140	2.082	0.065	0.002	-3.800	34.389	1.075	4.917
Row 3	4.275	4.609	0.165	1.858	0.000	0.000	na	29.378	1.049	4.110
Row 4	4.281	4.410	0.158	1.805	0.078	0.003	-3.106	30.389	1.085	4.152
Row 5	4.725	5.354	0.122	3.206	0.000	0.000	na	47.386	1.077	7.373
Row 6	4.746	4.718	0.105	3.032	0.085	0.002	-4.828	49.409	1.098	7.622
Row 7	5.031	6.464	0.110	4.782	0.093	0.002	-6.080	65.372	1.108	10.711
Row 8	4.402	4.453	0.139	2.076	0.083	0.003	-3.465	34.389	1.075	4.917
Row 9	4.680	4.722	0.112	2.831	0.112	0.003	-3.991	45.387	1.081	6.959
Row 10	4.632	4.912	0.123	2.765	0.000	0.000	na	43.391	1.085	6.550
Row 11	4.610	4.922	0.123	2.768	0.183	0.005	-2.954	42.430	1.061	6.511
Row 12	4.421	4.366	0.132	2.112	0.020	0.001	-5.634	35.431	1.074	5.113
Row 13	4.346	4.670	0.151	2.075	0.136	0.004	-2.682	32.436	1.046	4.684
Row 14	4.432	4.869	0.148	2.269	0.089	0.003	-3.464	35.406	1.073	5.112
Row 15	4.513	5.155	0.143	2.564	0.137	0.004	-3.107	38.402	1.067	5.704
Row 16	4.921	4.558	0.084	3.558	0.138	0.003	-4.638	58.406	1.082	9.539
Row 17	5.000	6.297	0.109	4.635	0.044	0.001	-7.896	63.393	1.093	10.452
Row 18	4.660	4.487	0.104	2.803	0.039	0.001	-6.061	44.398	1.033	7.084
Row 19	4.992	5.607	0.097	4.343	0.044	0.001	-7.874	62.394	1.076	10.412
Row 20	4.365	3.926	0.127	1.841	0.048	0.002	-4.094	33.439	1.079	4.725

Figure 3.4. Deletion of column/s with unacceptable data on QSARINs.

QSAR models were developed using the MLR method. MLR is a commonly used method in QSAR studies due to its simplicity, transparency, reproducibility, and easy interpretability. In order to obtain a validated and, therefore, predictive QSAR model, an available dataset should be divided into the training and test sets. Ideally, the division into the training and test sets must satisfy the following three conditions (Golbraikh et al., 2003): (i) All representative compound-points of the test set in the multidimensional descriptor space must be close to those of the training set. (ii) All representative points of the training set must be close to those of the test set. (iii) The representative points of the training set must be distributed within the whole area occupied by the entire dataset. There are many types of division in the literature, such as cluster analysis, periodical division and Kohonen networks (Papa et al., 2005). However, the division of the dataset can also be done randomly. In the present study, we considered three methods for the division of a dataset into training and test sets, (i) ordered by response, (ii) ordered based on the molecular structure, and (iii) random division. We divided the dataset into a training set and a test set comprising 80% and 20% of the whole dataset, respectively.

Response values were ordered in ascending order in both ordered by response and random divisons. These splittings should guarantee that the training set covers the entire range of the experimental responses leaving the minimum and maximum values of response variables (in the present study, mutagenic activity) in the training set. Divison in the case of ordered by structure, can be done regarding the molecular features of the chemicals. Test set chemicals were not considered in the calculation of the model. It was used only for the external validation to check the efficiency of the prediction of the selected model.

The selection of relevant descriptors, which relate the response variable (mutagenic activity/property/toxicity) to molecular structure, is an important step in the construction of a QSAR model. The most feasible descriptors were selected with “All Subset” and “Hold model add one more variable” tools which are implemented in QSARINS software.

The quality, robustness and predictive power of the model were estimated by different statistical parameters, such as the determination coefficient, either adjusted or not adjusted for the number of variables (R^2_{adj} or R^2), the standard deviation (s), the Fisher’s statistic (F), and the root-mean-square error of training set ($RMSE_{\text{Tr}}$). The use of $RMSE_{\text{Tr}}$ shows the error between the mean of the experimental values and predicted activities. However, excellent values of R^2 and $RMSE_{\text{Tr}}$ are not sufficient indicators of a QSAR model validity. Thus, alternative parameters must be provided to indicate the predictive ability of models.

3.5. Model Validation

A common method for internally validating a QSAR model is cross-validation (CV, Q^2). CV process repeats the regression many times on subsets of data. Usually, each molecule is removed from the dataset once (only), in turn, and the R is computed using the predicted values of the missing molecule. Sometimes more than one molecule (leave many out, LMO) is removed at a time. A cross-validated R^2 (R^2_{CV} or Q^2) is usually smaller than the overall R^2 for a QSAR equation. It is used as a diagnostic tool to evaluate the predictive power of an equation. Cross-validation is used to measure a model’s predictive ability and test the over-fitting. Over-fitting refers to the phenomenon in which a predictive model may well describe the relationship between predictors and response, but may subsequently fail to provide valid predictions for new compounds. Over-fitting of a linear QSAR model is usually suspected when the R^2 value from the original model is significantly larger (25%) than the Q^2 value (Difference between R^2 and Q^2 should not be more than 0.3) (Leach, 2001). Thus, Q^2 is considered a measure of goodness of prediction and not fit in the case of R^2. The process of CV begins with the removal of one or a group of compounds, which becomes a temporary test set, from the training set. In the leave-one-out (LOO) method of CV, the process of removing a molecule, and creating and validating the model against the individual molecules is performed for the entire training set. Once complete, the mean is taken of all the Q^2 values and reported. According to the rule-of-thumb, the ratio of the number of compounds in the training set over the number of variables (descriptors) should have a value of at least 5 which is called as Topliss ratio suggesting that at least 5 training compounds should be represented with one descriptor. For instance, a training set that has 25 training set compounds should not have more than 5 descriptors (Topliss and Edwards, 1979). Inter-correlation among descriptors was tested via the QUIK (Q Under Influence of K) rule (Todeschini et al., 2004). QUIK rule was set to 0.05 to minimize inter-correlation between descriptors.

The predictive power of the equation is poor when the observations are not sufficiently independent of each other. One way to test for this is by randomization of the dependent variable (response/activity). This procedure ensures that the model is not due to a chance. Activity values are shuffled randomly and the entire modelling procedure is repeated. This process is repeated many times. The significantly low coefficients of determination of new models indicate that the proposed model is not obtained by chance correlation. Therefore, the reliability of the models was tested by response randomization (*Y*-scrambling) procedure.

A developed QSAR model is accepted if it can satisfy the following criterion (The following values are the minimum recommended values for significant QSAR model (Veerasamy et al., 2011).

- If coefficient of determination $R^2 > 0.6$
- If the standard deviation *s* is not much larger than the standard deviation of the activity data.
- If its *F* value indicates that the overall significance level is better than 95%.
- If its confidence interval of all individual regression coefficients proves that they are justified at the 95% significance level.

Golbraikh and Tropsha (2003) identified some criteria for external prediction. If the following criteria are valid, the models are acceptable:

(a) If cross-validated R^2_{CV} (Q^2) > 0.5
(b) If R^2 for external/test set, $R^2_{\mathrm{Test}} > 0.6$
(c) $(R^2 – R^2_0)/R^2 < 0.1$ and $0.85 < k < 1.15$
(d) $(R^2 –R'^2_0) /R^2 < 0.1$ and $0.85 < k' < 1.15$ (for test set).
(e) r^2_{m} (overall) > 0.5 (or at least near 0.5); $\Delta r^2_{\mathrm{m}} < 0.2$.

where $R^2{}_0$ (predicted vs. observed) and $R'^2{}_0$ (observed vs. predicted) are the determination coefficients without intercept, *k* and *k'* are the slopes. The parameter $r^2{}_m$ (overall) penalizes a model for large differences between observed and predicted values of the compounds of the whole set (considering both training and test sets). $\Delta r^2{}_m$ estimated the closeness between the values of the predicted and the corresponding observed activity data.

In addition, the response data (mutagenic activity) should cover a range of at least two or even more logarithmic units. They should be well distributed over the whole distance.

An equation has to be rejected:

- If the above mentioned statistical measures are not satisfied
- If the number of the variables in the regression equation is unreasonably large.
- If the standard deviation is smaller than the error in the activity data.

Additional external validation criteria to be used in modelling are: $Q^2{}_{F1}$, $Q^2{}_{F2}$ (Shüürman et al., 2008) $Q^2{}_{F3}$ (Consonni et al., 2009, 2010), Concordance Correlation Coefficient (CCC) (Lin 1989, 1992), for training and test sets CCC_{Tr} and CCC_{Test} (Chirico and Gramatica 2011, 2012), respectively. $Q^2{}_{F1}$ shows the degree of correlation between the experimental and predicted activity of the dataset (Shi et al., 2001). The $Q^2{}_{F2}$ parameter was described by Schüürmann et al. (2008). The main difference between $Q^2{}_{F1}$ and $Q^2{}_{F2}$ is that the mean experimental activity is replaced in $Q^2{}_{F2}$ with the mean predicted activity. $Q^2{}_{F3}$ measures the model's predictivity it is sensitive to training set selection and it criticizes the dataset when they are very homogeneous. Concordance Correlation Coefficient (*CCC*) measures both the distance of observations to the fitting line and the distance which the regression line deviates from slope 1 passing through the origin. Thus, *CCC* value is often smaller than its ideal value of 1 (Chirico and Gramatica, 2011). The external validation ensures the predictability and applicability of the developed QSAR model for the prediction of untested molecules. In the present study, current textile dyes were used as an external set.

Multi-Criteria Decision Making (MCDM) implemented in the software QSARINS was used for the ranking of generated models. It consists of the summary of performances of a certain number of criteria associated with both the internal and external validation. Each validation criteria value ranges from 0 to 1 and the geometric average of all the values obtained from the desirability functions creates the MCDM value (QSARINS 2.2.3). The MCDM of fitting (maximizing R^2, $R^2{}_{adj}$, and CCC_{Tr} while minimizing $R^2 - R^2{}_{adj}$) cross-validation (maximizing $Q^2{}_{LOO}$, $Q^2{}_{LMO}$ and CCC_{CV}, while minimizing $R^2{}_{Y\text{-}SCR}$) and external validation (maximizing $Q^2{}_{F1}$, $Q^2{}_{F2}$, $Q^2{}_{F3}$, and CCC_{Test}) parameters are automatically calculated using all the corresponding criteria. The model with the best MCDM compromise among the selected validation criteria is sorted as the best. Additionally, for the model selection, the principle of parsimony was taken into account; the model with the highest statistical significance (both model and test set significance), but having as few parameters as possible was selected.

Besides the above-mentioned criteria, Mean Absolute Error (*MAE*)-based criteria proposed by Roy et al., (2016) was applied to test further the external validation of all models selected by MCDM. *MAE* provides equal weight to all the errors (Roy et al., 2016). Xternal Validation Plus tool developed by Roy et al. (2015) was used to check the presence of systematic errors in the model. It further computes all the required external validation parameters, while judges the performance of actual prediction quality of a QSAR model based on the mean absolute error. The mean absolute error (*MAE*) can be calculated with the following equation:

$$MAE= \frac{\sum_{i=1}^{\mathrm{n}_{ext}} |y_i - \hat{y}_i|}{\mathrm{n}_{ext}} \tag{3.9}$$

I. Good predictions:

From a general notation, an error of 10% of the training set range should be acceptable while an error value more than 20% of the training set should be a very high error. Thus, the criteria for good predictions should be the following:

MAE ≤ 0.1 x training set range and *MAE*+ 3σ ≤ 0.2 x training set range.

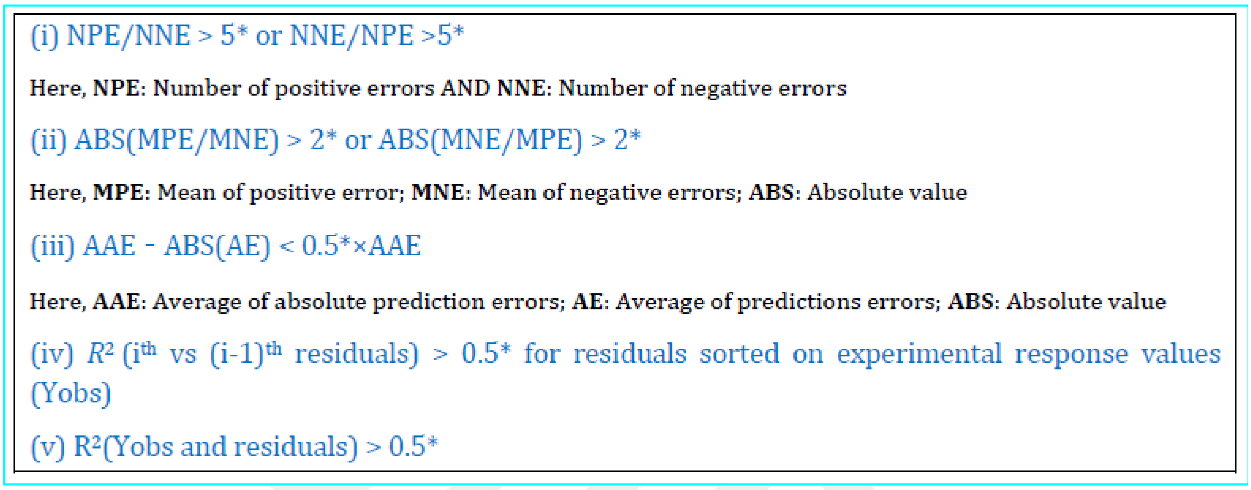
Where, the σ value refers to the standard deviation of the absolute error values for the test set data. Considering a normal distribution pattern, mean ± 3σ covers 99.7% of the data points.

II. Bad predictions:

A value of *MAE* more than 15% of the training set range should be high while an error of more than 25% of the training set is considered very high. Hence, the predictions could be considered very high. Hence, the predictions could be considered when:

MAE > 0.15 x training set range or *MAE* + 3σ > 0.25 x training set range.

The predictions which do not fall under either of the above two conditions may be considered as of moderate quality. The mentioned criteria should be used in cases where there are more than 10 data points in the test set (Roy et al., 2016). If anyone or more conditions stated in Figure 3.5. were met, a systematic error occurs. If the systematic error present in the output file, the model should be discarded.



(i) NPE/NNE > 5* or NNE/NPE >5*

Here, **NPE:** Number of positive errors AND **NNE:** Number of negative errors

(ii) ABS(MPE/MNE) > 2* or ABS(MNE/MPE) > 2*

Here, **MPE:** Mean of positive error; **MNE:** Mean of negative errors; **ABS:** Absolute value

(iii) AAE - ABS(AE) < 0.5*×AAE

Here, **AAE:** Average of absolute prediction errors; **AE:** Average of predictions errors; **ABS:** Absolute value

(iv) R^2 (i^{th} vs $(i\text{-}1)^{th}$ residuals) > 0.5* for residuals sorted on experimental response values (Yobs)

(v) R^2(Yobs and residuals) > 0.5*

Figure 3.5. Error types of Xternal validation plus tool.

“Prediction Reliability Indicator” was developed by Roy et al., (2018) to indicate or categorize the quality of predictions for the test set (known experimental response) or external (unknown experimental response) sets into three groups: good (with composite score 3), moderate (with composite score 2) and bad (with composite score 1). There is no clear discussion provided for the output of this program. For instance, it tabulates the prediction of the response variable of a chemical from the model as good, but it puts a mark for that chemical as out of the applicability domain (AD) of model. Therefore, we did not apply PRI to our selected models.

3.6. Applicability Domain of QSAR Model

The derivation of QSAR models is based primarily on training sets which are structurally limited and thus, their applicability to the query chemicals is limited (Dimitrov et al., 2005). Thus, their applicability towards reliable predictions is restricted in chemical space to some specific categories. The applicability domain (AD) is a theoretical region of the chemical space, defined by the model descriptors and modelled response and, thus, by the nature of the training set molecules. It is important to note that the AD of a model cannot be verified by studying only a few chemicals (even less than five), as in such cases, it could happen that extrapolated predictions are good, but probably only by chance, so it is impossible to rely on the possibility of obtaining general conclusions. AD is one of the five OECD criteria (OECD, 2007). It estimates the similarity of an individual compound's to the rest of the dataset.

The AD of a QSAR should be described in terms of the most relevant parameters i.e. usually those that are the range of descriptors and response.

In the present study, the AD of the generated MLR model was analyzed by Williams plot. To check whether a new chemical lies within the applicability domain, the leverage approach was used. A compound was considered outside the AD when the leverage value is higher than the warning limit of $3p/n$, where p is the number of model variables plus 1 and n is the number of objects used to develop the model. This indicates structural (*X*) outliers. Compounds with a residual higher than ± 3σ in the training set were considered as response (*Y*) outliers. Additionally, Insubria Graph was used to predict the unknown endpoints of chemicals from the model equation regarding the applicability domain of the generated QSAR model. In the present study, the predicted ability of all models was tested using an external dataset which comprises currently used 33 anionic water-soluble textile dyes (eight anthraquinone and 25 azo dyes) with no measured mutagenic activity. The chemical structures of these dyes are given in Appendix A.

4. RESULTS AND DISCUSSION

4.1. Model Development

The compiled experimental mutagenicity data from literature were given in Table 4.1 together with the molecular weight and logP values obtained from Garg et al., 2002 and Bhat et.al., 2005). The normality of the mutagenicity data was evaluated using the Kolmogorov-Smirnov test in SPSS 25 (IBM). The distribution of data was found to be normal ($p > 0.05$). Fortunately, the mutagenic activity of compounds ranges over nearly 7 orders of magnitude, providing a broad range of values for QSAR development.

The dataset contained 177 chemicals was divided into two groups as test and training sets. The training set was composed of 80% of the whole data with 142 compounds and the test set was composed of 20% of the whole data with 35 compounds. Test set compounds in each division and the ratio of the number of test set to the training set compounds in each division are given in Table 4.2.

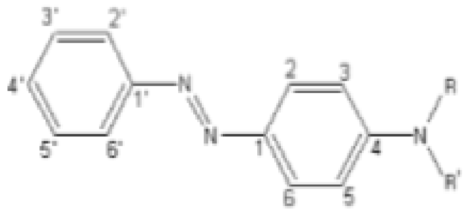
Numerous training/test divisions were tried and many different 3 to 6-descriptor QSAR models were generated using “AllSubset” and “Hold model add one more variable” tools of QSARINS (v.2.2.3) software. The generated models ranked by MCDM as well as the internal and external validation criteria. Fit, internal and external validation parameters of the best 3 to 6 descriptor models are given in Table 4.3 and 4.4, respectively. The generated models fulfilled all the fit, internal and external validation criteria, therefore they are subjected to further criteria to determine the best model. As such, all models were tested for their external prediction capacity regarding the highest number of compounds from external set chemicals which fell within the applicability domain. Models from each division were selected regarding the highest R^2 and Q^2 values, the minimum number of structural outliers and higher predictive performance of the external set chemicals and listed in Table 4.3.

The internal predictive power of each model is judged by the parameters like R^2 (the squared of determination coefficient) and Q^2_{LOO} (the leave-one-out cross-validation). For the 3-6-descriptor models, R^2 and Q^2_{LOO} values were very similar to each other which reveal the stability of all models (R^2 range is between 0.54 and 0.61, and Q^2_{LOO} range is between 0.51 and 0.57). The models’ reliability and robustness were checked by the Y-scrambling procedure. The mutagenicity values were shuffled randomly and new regression models were developed. The generated models' coefficients of determination were calculated. The procedure was repeated 2000 times. The average R^2 of shuffled models were significantly low (results were not shown here), revealing that there is no chance correlation in all models.

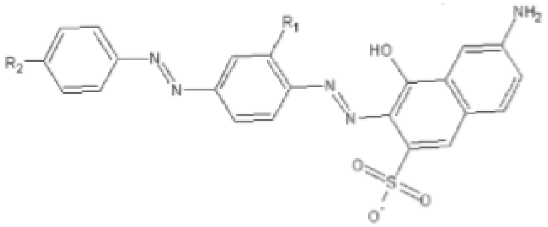
The high R^2_{Test} values (0.74 to 0.77) and low $RMSE_{\text{Test}}$ relative to the $RMSE_{\text{Tr}}$ proved to have good predictive power for all models. Additionally, all models passed the external validation criteria mentioned in section 3.5 and threshold values reported in the literature. All these results indicate that the developed models are robust, validated and predictive. For all chemicals in the training and the test sets, the standardized residuals are smaller than three standard deviation units (Williams plot). All models have no response outlier. The predicted vs. observed mutagenic activity and Williams plots of all models except model M2 are given in Figure B1 and Figure B2, respectively (Appendix B).

The structural coverage of all models for external set chemicals ranged from 58% to 70% (Table 4.5). Of the five models, all modelwere classified as GOOD regarding the *MAE*-based criteria.

Table 4.1. The name, LogP, molecular weight and mutagenic activity values of chemicals in the dataset.



Main Skeleton of Compond ID 1-42



Main Skeleton of Compound ID 162-177

ID	Compound Name	log P	Molecular Weight (g/mol)	Mutagenic activity in TA98+S9 (rev/nmol)	CAS Number
1	4'-NEt2-3-OMe-AAB	5.16	298.39	-2.15	**-
2	2-OMe-AAB	3.87	227.27	-2.00	80830-39-3
3	4'-OH-AAB	2.55	213.24	-1.28	6530-27-4
4	3'-Me-4'-OH-AAB	3.01	227.27	-2.23	-
5	4'-OH-2',3-diMe-AAB(4'-OH-OAT)	3.47	241.29	-0.95	-
6	AAB	3.13	197.24	-0.69	60-09-3
7	3'-Me-AAB	3.59	211.27	-0.62	-
8	3-OMe-4'-N(CH2CH2OH)2-AAB	2.58	330.39	-0.41	-
9	3'CH2OH-AAB	1.94	227.27	-0.22	-
10	3-OH-AAB	2.97	213.24	-0.16	14528-97-3
11	3-OCH2CH2OH-AAB	2.51	257.29	0.13	-
12	2'-CH2OH-3-Me-AAB	2.40	241.29	0.30	-
13	4-OMe-AAB	2.95	227.27	0.36	-
14	2',3-diMe-AAB	4.05	225.30	0.43	97-56-3
15	3-OBu-AAB	5.08	269.35	0.70	-
16	3-OEt-AAB	4.02	241.29	1.14	126335-27-1
17	3-OPr-AAB	4.55	255.32	1.28	-
18	3-OMe-AAB	3.48	227.27	1.89	-
19	3'-Me-4'-OH-MAB	3.67	241.29	-1.15	-
20	3'-COOH-MAB	3.52	255.28	-0.91	-
21	4'-OH-MAB	3.21	227.27	-0.85	-
22	MAB	3.79	211.27	-0.74	621-90-9
23	4'-Me-MAB	4.25	225.30	-0.55	28149-22-6
24	3'-Me-MAB	4.25	225.30	-0.35	2058-62-0
25	3'-CH2OH-MAB	2.60	241.29	-0.30	-
26	3'-Me-4'-OH-DAB	4.31	255.32	-0.96	-
27	p-(dimethylamino)azobenzene	4.43	225.30	-0.85	60-11-7
28	3'-COOH-DAB	4.17	269.30	-0.70	-
29	2-Me-DAB	4.89	239.32	-0.66	54-88-6

Table 4.1. Continued

ID	Compound Name	log P	Molecular Weight (g/mol)	Mutagenic activity in TA98+S9 (rev/nmol)	CAS Number
30	3'-Me-DAB	4.89	239.32	-0.45	55-80-1
31	3'-CHO-DAB	3.91	253.31	-0.42	-
32	3'-CH2-OAC-DAB	4.14	297.36	2.71	-
33	3'-CH2OH-DAB	3.25	255.32	-0.22	35282-68-9
34	3'-Me-AAB-N-Ac	3.73	253.31	-1.06	-
35	3'-Me-4'-OH-AAB-N-Ac	3.15	269.30	-1.05	-
36	N-OH-2-OMe-AAB	4.08	243.27	-0.96	-
37	3'-Me-MAB-N-Ac	3.03	267.33	-0.28	-
38	N-OH-MAB	2.74	227.27	-0.19	1910-36-7
39	N-OH-3'-Me-MAB	3.20	241.29	0.00	-
40	N-OH-AAB	2.98	213.24	0.01	6530-27-4
41	N-OH-4'-Me-MAB	3.20	227.27	0.05	-
42	N-OH-3-OMe-AAB	3.08	243.27	2.28	-
43	2-aminobiphenyl	2.68	169.23	*-1.49	90-41-5
44	4-aminobiphenyl	2.77	169.23	-0.14	92-67-1
45	2-2'-diaminobiphenyl	1.39	184.24	-1.52	1454-80-4
46	2,4'-diaminobiphenyl	1.47	184.24	-0.92	492-17-1
47	3,3'-diamnobiphenyl	1.41	184.24	-1.30	2050-89-7
48	3,4'-diaminobiphenyl	1.48	184.24	0.20	32316-90-8
49	2-amino-3'-nitrobiphenyl	2.21	216.24	-0.89	34862-87-8
50	2-amino-4'nitrobiphenyl	2.30	214.22	-0.62	6272-52-2
51	3-amino-2'nitrobiphenyl	2.24	214.22	-1.30	96187-18-7
52	3-amino-3'nitrobiphenyl	2.22	214.22	-0.55	31835-64-0
53	3-amino-4'nitrobiphenyl	2.31	214.22	0.69	53059-29-3
54	4-amino-2'nitrobiphenyl	2.32	214.22	-0.92	1140-28-9
55	4-amino-3'nitrobiphenyl	2.30	214.22	1.02	1141-29-3
56	4-amino-4'nitrobiphenyl	2.39	214.22	1.04	1211-40-1
57	1-aminoanthracene	3.40	193.25	1.18	610-49-1
58	2-aminoanthracene	3.40	193.25	2.62	613-13-8
59	9-aminoanthracene	3.40	193.25	0.87	779-03-3
60	1-aminophenanthrene	3.40	193.25	2.38	4176-53-8
61	2-aminophenanthrene	3.40	193.25	2.46	3366-65-2
62	3-aminophenanthrene	3.40	193.25	3.77	1892-54-2
63	9-aminophenanthrene	3.40	193.25	2.98	947-73-9
64	1-aminofluorene	2.88	181.24	0.43	6344-63-4
65	2-aminofluorene	2.88	181.24	1.93	153-78-6
66	3-aminofluorene	2.88	181.24	0.89	6344-66-7
67	4-aminofluorene	2.88	181.24	1.13	7083-63-8
68	2,7-diaminofluorene	1.60	196.25	0.48	525-64-4
69	2-amino-7-nitrofluorene	2.61	226.24	3.00	1214-32-0

Table 4.1. Continued

ID	Compound Name	log P	Molecular Weight (g/mol)	Mutagenic activity in TA98+S9 (rev/nmol)	CAS Number
70	2-bromo-7-aminofluorene	3.65	260.13	2.62	6638-60-4
71	2-hydroxy-7-aminofluorene	2.15	197.24	0.41	1953-38-4
72	2-amino-7-acetamidofluorene	1.75	238.29	1.18	6957-50-2
73	1-aminopyrene	4.31	217.27	1.43	1606-67-3
74	2-aminopyrene	3.81	217.27	3.50	1732-23-6
75	4-aminopyrene	3.89	217.27	3.16	17075-03-5
76	1-aminofluoranthene	3.89	217.27	3.35	13177-25-8
77	2-aminofluoranthene	3.89	217.27	3.23	13177-26-9
78	3-aminofluoranthene	3.89	217.27	3.31	2693-46-1
79	7-aminofluoranthene	3.89	217.27	2.88	5869-25-0
80	8-aminofluoranthene	3.89	217.27	3.80	2642-98-0
81	6-aminochrysene	4.63	243.31	1.83	580-17-6
82	3-aminoquinoline	1.49	144.18	-3.14	611-34-7
83	5-aminoquinoline	0.92	144.18	-2.00	580-15-4
84	6-aminoquinoline	1.26	144.18	-2.67	578-66-5
85	8-aminoquinoline	1.88	144.18	-1.14	18992-86-4
86	1-aminocarbazole	2.43	182.23	-1.04	4539-51-9
87	2-aminocarbazole	2.43	182.23	0.60	6377-12-4
88	3-aminocarbazole	2.44	182.23	-0.48	18992-64-8
89	4-aminocarbazole	2.44	182.23	-1.42	2876-22-4
90	1-aminophenazine	3.19	195.23	-0.01	2876-23-5
91	2-aminophenazine	2.13	195.23	0.55	16582-03-9
92	1,6-diaminophenazine	3.53	210.24	0.20	28124-29-0
93	1,7-diaminophenazine	3.53	210.24	0.75	102877-14-5
94	1,9-diaminophenazine	3.53	210.24	0.04	120209-97-4
95	2,7-diaminophenazine	1.64	210.24	3.97	7704-40-7
96	2,8-diaminophenazine	1.54	210.24	1.12	134-32-7
97	1-naphthylamine	2.17	143.19	-0.60	91-59-8
98	2-naphthylamine	2.17	143.19	-0.67	776-34-1
99	1-amino-4-nitronaphthalene	2.62	188.19	-1.77	606-57-5
100	2-amino-1-nitronaphthalene	3.06	188.19	-1.17	62-53-3
101	aniline	0.93	93.13	-1.51	106-40-1
102	*p*-bromoaniline	2.05	172.03	-2.70	95-51-2
103	*o*-chloroaniline	1.91	127.57	-3.00	106-47-8
104	*p*-chloroaniline	1.76	127.57	-2.52	371-40-4
105	*p*-fluoroaniline	1.15	111.12	-3.32	156-43-4
106	*p*-ethoxyaniline	1.28	137.18	-2.30	139-59-3
107	4-phenoxyaniline	2.77	185.23	0.38	123-30-8
108	4-hydroxyaniline	-0.30	109.13	-1.60	6373-50-8
109	4-cyclohexylaniline	3.46	175.28	-1.24	97-02-9

Table 4.1. Continued

ID	Compound Name	log P	Molecular Weight (g/mol)	Mutagenic activity in TA98+S9 (rev/nmol)	CAS Number
110	2,4-dinitroaniline	2.22	183.12	-2.00	95-68-1
111	2,4-dimethylaniline	1.86	121.18	-2.22	95-78-3
112	2,5-dimethylaniline	1.86	121.18	-2.40	367-25-9
113	2,4-difluoroaniline	1.49	129.11	-2.70	120-71-8
114	2-methoxy-5-methylaniline	1.55	137.18	-2.05	16452-01-0
115	3-methoxy-4-methylaniline	1.29	137.18	-1.96	102-50-1
116	4-methoxy-2-methylaniline	1.20	137.18	-3.00	89-63-4
117	4-chloro-2-nitroaniline	2.74	172.57	-2.22	137-17-7
118	2,4,5-trimethylanilne	2.32	135.21	-1.32	1817-73-8
119	2-bromo-4,6-dinitroaniline	3.09	262.02	-0.54	621-95-4
120	4,4'-ethylenebis(aniline)	2.27	212.30	-2.15	101-77-9
121	4,4'-methylenedianiline	1.64	198.27	-1.60	578-54-1
122	4,4'-methylenebis (O-ethylaniline)	2.32	121.18	-0.99	348-54-9
123	4,4'-methylenebis(o-fluoroaniline)	2.50	111.12	0.23	95-54-5
124	4,4'-methylenebis(o-isopropylaniline)	4.31	135.21	-1.77	108-45-2
125	1,2-Phenylenediamine	0.05	108.14	-0.75	106-50-3
126	1,3-benzenediamine	-0.82	108.14	-0.46	95-70-5
127	1,4-benzenediamine	-0.90	108.14	-0.89	5307-14-2
128	2-methyl-1,4-phenylenediamine	-0.45	122.17	-1.52	5307-02-8
129	2-nitro-1,4-phenylenediamine	0.75	153.14	-0.05	90-41-5
130	2-methoxy-1,4-phenylenediamine	-0.74	138.17	0.32	92-67-1
131	2-ethoxy-1,4-phenylenediamine	-0.21	152.20	-0.02	-
132	2-propoxy-1,4-phenylenediamine	0.29	166.22	-0.21	-
133	2-butyloxy-1,4-phenylenediamine	0.82	180.25	-3.00	-
134	4-chloro-1,2-phenylenediamine	1.15	142.59	-0.49	95-83-0
135	4-chloro-1,3-phenylenediamine	0.38	142.59	-0.77	5131-60-2
136	4-nitro-1,2-phenylenediamine	1.21	153.14	0.35	99-56-9
137	4-nitro-1,3-phenylenediamine	0.80	153.14	-2.40	5131-58-8
138	*N*-acetyl-1,4-phenylenediamine	0.08	150.18	-1.80	122-80-5
139	1,4-*N*Ac-phenylenediamine	-0.45	192.22	-1.43	140-50-1
140	2,6-dichloro-1,4-phenylenediamine	1.68	177.03	-0.69	609-20-1
141	*N*,*N*-diethyl-1,4-phenylenediamine	0.32	164.25	-2.15	93-05-0
142	*N*,*N*-dimethyl-1,4-phenylenediamine	-0.58	136.20	-0.87	99-98-9
143	*N*-methyl-1,4-phenylenediamine	-0.39	122.17	-0.38	623-09-6
144	2,4-diaminoethylbenzene	0.17	136.20	-0.87	1195-06-8
145	2,4-diaminotoluene	-0.36	122.17	-1.29	95-80-7
146	3,4-diaminotoluene	0.51	122.17	-1.42	496-72-0
147	3-amino-α,α,α-trifluorotoluene	2.30	161.13	-0.80	98-16-8

Table 4.1. Continued

ID	Compound Name	log P	Molecular Weight (g/mol)	Mutagenic activity in TA98+S9 (rev/nmol)	CAS Number
148	2,4-diamino-isopropylbenzene	0.52	150.23	-3.00	14235-45-1
149	2,4-diamino-*n*-butylbenzene	1.24	164.25	-2.70	63921-07-3
150	2-amino-4-chlorophenol	1.67	143.57	-3.00	95-85-2
151	2-amino-4-methylphenol	0.90	123.16	-2.10	95-84-1
152	2-amino-5-nitrophenol	1.61	154.13	-2.52	121-88-0
153	3-amino-6-methyl phenol	0.80	123.16	-1.40	2835-95-2
154	benzidine	1.56	184.24	-0.39	92-87-5
155	3,3'-diaminobenzidine	-0.21	214.27	-0.04	91-95-2
156	3,3'-dichlorobenzidine	3.45	253.13	0.81	91-94-1
157	4,4'-diamino-3,3'-dimethoxy-1,1'-biphenyl	1.58	244.29	0.15	119-90-4
158	3,3'-dimethyl-4,4'-bianiline	2.48	212.30	0.01	119-93-7
159	4-aminophenyl disulfide	1.65	248.37	-1.03	722-27-0
160	(4-aminophenyl)ether'	0.51	200.24	-1.14	287476-22-6
161	4-aminophenyl sulfide	3.07	216.31	0.31	139-65-1
162	3-OSO_3K-AAB	-1.00	293.30	-1.43	-
163	4'-OSO_3K-AAB	-1.29	293.30	-1.01	-
164	3-methoxy-4'-N,N-diethyl-AAB	5.16	313.41	-2.15	-
165	3-OSO_3K-MAB	-1.05	307.33	-1.47	-
166	4'-OSO_3K-MAB	-1.33	307.33	-0.38	-
167	3'-acetoxymethyl-DAB	4.14	297.36	-0.29	-
168	R1=OCH_2CH_2OH, R2=NEt2	0.12	562.65	-0.83	-
169	R1=OBu, R2=NEt2	2.69	574.71	-0.64	-
170	R1=OPr, R2=NEt2	2.16	560.68	-0.59	-
171	R1=OMe, R2=NEt2	1.09	532.63	-0.47	-
172	R1=OPr, R2=H	0.39	489.56	-0.40	-
173	R1=OEt, R2=NEt2	1.63	546.65	-0.24	-
174	R1=OBu, R2=H	0.92	503.58	-0.18	-
175	R1=OCH_2CH_2OH, R2= H	-1.65	475.53	0.13	-
176	R1=OMe, R2=H	-0.67	461.50	0.47	-
177	R1=OEt, R2=H	-0.14	475.53	0.64	-

*The mutagenic activity values of chemicals with ID values between 1-43 were taken from Garg et al., 2002 ; the others were taken from Bhat et al., 2005.
**data could not be found

Table 4.2. Test set compounds for three divisions and five models used in mutagenicity modelling.

Model	$n_{\text{Test}}/n_{\text{Tr}}$	Test Set Compounds*
		Division 1
M1	35/142	3,4,7,8,12,15,29,38,39,41,50,57,59,61,65,66,85,86,93,98, 101,108,114,116,126,134,138,139,148,150,154,155,156,160,174
M2	35/142	
		Division 2
M3	34/143	1,3,13,21,27,36,37,39,40,41,45,48,54,56,59,70,71,72,79,87,90,92, 100,109,114,115,118,119,124,127,141,146,153,159
		Division 3
M4	34/143	1,3,13,21,27,36,37,39,40,41,45,48,54,56,59,70,71,72,79,87,90,92, 100,109,114,115,118,119,124,128,141,146,153,159
M5	34/143	

* Compound numbers refer to the ID numbers given in Table 4.1

Table 4.3. The fit and internal parameters of the developed QSAR models for the mutagenic activity of textile dyes

Model No	**Number of Variables**	**Variables**	**Fitting Criteria and Internal Validation Parameters**						
			$\boldsymbol{R^2}$	$\boldsymbol{R^2_{\mathrm{adj}}}$	$\boldsymbol{Q^2_{\mathrm{LOO}}}$	$\boldsymbol{RMSE_{\mathrm{Tr}}}$	$\boldsymbol{S}$	$\boldsymbol{F}$	$\boldsymbol{CCC_{\mathrm{Tr}}}$
			Divison 1						
M1	6	piPC08 CIC1 Chi_D/Dt L/Bw TDB09p Mor28s	0.59	0.57	0.55	1.03	1.06	32.74	0.74
M2*	**6**	**CIC2 Chi_D/Dt L/Bw TDB09p Mor28s piPC08**	**0.60**	**0.58**	**0.56**	**1.02**	**1.04**	**33.71**	**0.75**
			Divison 2						
M3	6	WiA_Dt P_VSA_MR_2 Mor31e B09[C-O] MLOGP2 HATS1m	0.61	0.59	0.57	1.02	1.05	35.07	0.75
			Divison 3						
M4	3	WiA_Dt Mor31e MLOGP2	0.56	0.55	0.53	1.07	1.09	45.04	0.72
M5	6	WiA_Dt P_VSA_MR_2 Mor31e R1m+ B09[C-O] MLOGP2	0.59	0.56	0.56	1.03	1.06	40.14	0.74

*Selected Model.

Threshold values: $R^2 > 0.6$, $Q^2_{\mathrm{LOO}} > 0.5$

Table 4.4. The external parameters of the developed models for the mutagenic activity of textile dyes

Model No	**Number of Variables**	**Variables**	**Fitting Criteria and Internal Validation Parameters**						
			$\boldsymbol{R^2_{\mathrm{Test}}}$	$\boldsymbol{Q^2\text{-}_{\mathrm{F1}}}$	$\boldsymbol{Q^2\text{-}_{\mathrm{F2}}}$	$\boldsymbol{Q^2\text{-}_{\mathrm{F3}}}$	$\boldsymbol{CCC_{\mathrm{Test}}}$	$\boldsymbol{RMSE_{\mathrm{Test}}}$	$\boldsymbol{MAE_{\mathrm{Test}}}$
			Divison 1						
M1	6	piPC08 CIC1 Chi_D/Dt L/Bw TDB09p Mor28s	0.74	0.75	0.74	0.83	0.86	0.65	0.55
M2*	**6**	**CIC2 Chi_D/Dt L/Bw TDB09p Mor28s piPC08**	**0.76**	**0.75**	**0.74**	**0.84**	**0.85**	**0.65**	**0.53**
			Divison 2						
M3	6	WiA_Dt P_VSA_MR_2 Mor31e B09[C-O] MLOGP2 HATS1m	0.77	0.76	0.76	0.86	0.87	0.60	0.45
			Divison 3						
M4	4	WiA_Dt Mor31e MLOGP2 P_VSA_MR_2	0.74	0.73	0.73	0.85	0.85	0.64	0.52
M5	5	WiA_Dt Mor31e R1m+ B09[C-O] MLOGP2	0.62	0.75	0.74	0.85	0.86	0.62	0.45

*Selected Model.

Threshold values: Q^2_{F1} ,$Q^2_{\mathrm{F2,}}$ Q^2_{F3} > 0.70, CCC_{Test} >

Table 4.5. The structural coverage of all QSAR models for external set chemicals and the results of Xternal Validation Plus tool (*MAE*-based criteria).

Model Label	**Number of compounds within the AD of Selected Model (out of 33)**	**Structural Coverage (%)**	***MAE*-based Criteria**
	Division 1		
M1	22	66%	GOOD
M2*	**23**	**70%**	**GOOD**
	Division 2		
M3	22	66%	GOOD
	Division 3		
M4	19	58%	GOOD
M5	19	58%	GOOD

***Selected Model**

Of the generated models, the 6-descriptor MLR model labelled as M2 was highlighted regarding its structural coverage (70%) for the external set chemicals with no mutagenicity data. Model M2 resulted from random division. The model includes 6 descriptors from DRAGON 7.0. software. The change in R^2 and Q^2 of the selected model was given in Figure 4.1, indicating that the addition of each descriptor leads to an increase in R^2 more than 0.02. In other words, there is no redundant descriptor in model M2.

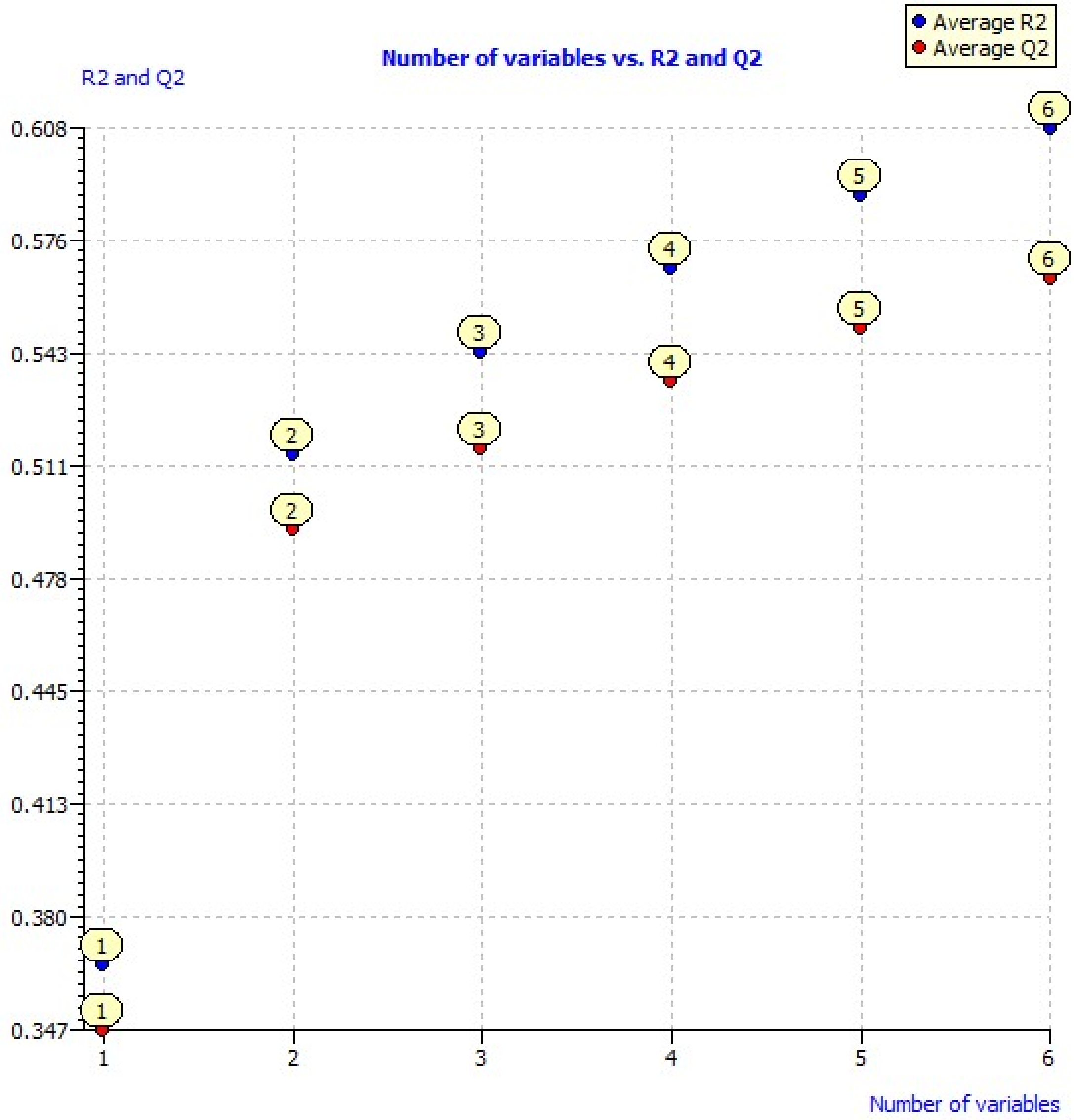


Figure 4.1. The change in R^2 and Q^2 along with the increase in the number of variables.

Model M2 gives the following equation (Eq. 4.1)_together with the descriptors involved and their regression coefficients and the 95% confidence intervals written in parenthesis.

Log (mutagenicty) **(TA98+S9 rev/nmol)** = -4.6629 (± 0.8191) + 0.9 (± 0.5918) **CIC2** + 1.0654 (± 0.2654)**Chi_D/Dt** – 0.0866 (±0.0558) **L/Bw** + 0.7161 (± 0.2631) **TDB09p** + 0.4636 (±0.2242) **Mor28s** – 0.1603 (±0.1405) **piPC08** (4.1)

n_{Tr} = 142, R^2= 0.60, R^2_{adj}=0.58, $RMSE_{\mathrm{Tr}}$ = 1.02, F = 33.71, CCC_{Tr} = 0.75, n_{Test} = 35, R^2_{Test} = 0.74, $RMSE_{\mathrm{Test}}$ = 0.65, Q^2_{F1} = 0.75, Q^2_{F2} = 0.74, Q^2_{F3} = 0.84, CCC_{Test} = 0.86

where the n_{Tr} and n_{Test} refer to the number of compounds in the training and test sets, respectively. The equations of all models except model M2 are given in Appendix C

Besides employed external validation, the prediction quality of the developed model was tested via *MAE*-based approach using Xternal Validation Plus tool developed by Roy et al. (2015). The output of this software for model M2 is given in Table 4.6, whereas the output of this software for the other models is given in Appendix D. Model M2 is regarded as **GOOD.** This criterion judges the performance of actual prediction quality of QSAR model based on the mean absolute error (*MAE*) showing that there was no systematic error in model M2.

Table 4.6. The results of Xternal Validation Plus tool for the selected model (M2).

User Input File Info.	FileName	*MAE*-test M2.xlsx
Model biasness test	Systematic Error Result	Absent
	nPE / nNE	0.7500
	nNE / nPE	1.3333
	|MPE / MNE|	0.9960
	|MNE / MPE|	1.0040
	AAE - |AE|	0.4559
	R^2 (Residuals; serial correlation)	0.0165
	R^2 (Residuals and Yobs values)	0.2080
Classical Metrics (for 100% data)	R^2_{Test}(100% data)	0.7431
	$R_0{}^2_{\text{Test}}$(100% data)	0.7385
	$R_0{}'^2_{\text{Test}}$(100% data)	0.7405
	Q^2_{F1}(100% data)	0.7537
	Q^2_{F2}(100% data)	0.7385
	Scaled Avg.$R_\text{m}{}^2$(100% data)	0.6455
	Scaled Delta $R_\text{m}{}^2$(100% data)	0.1930
	CCC(100% data)	0.8546
Classical Metric (after removing 5% data with high residuals)	R^2_{Test}(95% data)	0.8129
	$R_0{}^2_{\text{Test}}$ (95% data)	0.8128
	$R_0{}'^2_{\text{Test}}$ (95% data)	0.7426
	Q^2_{F1}((95% data)	0.8198
	Q^2_{F2} (95% data)	0.8127
	Scaled Avg $R_\text{m}{}^2$(95% data)	0.7389
	Scaled Delta $R_\text{m}{}^2$(95% data)	0.1317
	CCC(95% data)	0.8974
Error-based metrics (for 100% data)	$RMSE_\text{P}$(100% data)	0.6530
	SD(100% data)	0.3826
	SE(100% data)	0.0647
	MAE(100% data)	0.5330
Error-based metric (after removing 5% data with high residuals)	$RMSE_\text{P}$(95% data)	0.5542
	SD(95% data)	0.2940
	SE(95% data)	0.0512
	MAE(95% data)	0.4725
	MAE+3*SD(95% data)	1.3546

Table 4.6. Continued.

BASIC DATA STRUCTURE INFORMATION		
Number of test set compounds, Range and Mean (train and test)	NCompTest	35.0000
	Train range	7.2900
	TrainY_{Mean}	-0.2900
	Test range	5.4600
	Test Y_{Mean}	-0.6063
Distribution of observed response values of Test set around Test mean(in %)	%Y(+/-0.5)TestMean	31.4286
	%Y(+/-1.0)TestMean	62.8571
	%Y(+/-1.5)TestMean	80.0000
	%Y(+/-2.0)TestMean	85.7143
Distribution of observed response values of Test set around Train mean (in %)	%Y(+/-0.5)TrainMean	37.1429
	%Y(+/-1.0)TrainMean	54.2857
	%Y(+/-1.5)TrainMean	77.1429
	%Y(+/-2.0)TrainMean	85.7143
Distribution of prediction errors (in %)	%NComp>(0.1*TR)	25.7143
	%NComp>(0.15*TR)	8.5714
	%NComp>(0.2*TR)	2.8571
	%NComp>(0.25*TR)	0.0000
Threshold values utilized to judge the model predictions	(0.1*Training Set Range)	0.7290
	(0.15*Training Set Range)	1.0935
	(0.2*Training Set Range)	1.4580
	(0.25*Training Set Range)	1.8225
RESULT (*MAE*-based criteria applied on 95% data)	Prediction Quality	**GOOD**

From a general notation, an error of 10% of the training set range should be acceptable while an error value more than 20% of the training set should be a very high error. Thus, the criteria for good predictions should be the following:

$MAE \leq 0.1$ x training set range and $MAE + 3\sigma \leq 0.2$ x training set range.
0.4708 ≤ 0.1 x 7.29 and **1.3283 ≤ 0.2 x 7.29**

where, the σ value refers to the standard deviation of the absolute error values for the test set data. Considering a normal distribution pattern, mean ± 3σ covers 99.7% of the data points.

Predicted vs. observed mutagenicity values were plotted in Figure 4.2, together with y=x line. The training and test set compounds distributed homogeneously around the line (Figure 4.2.)

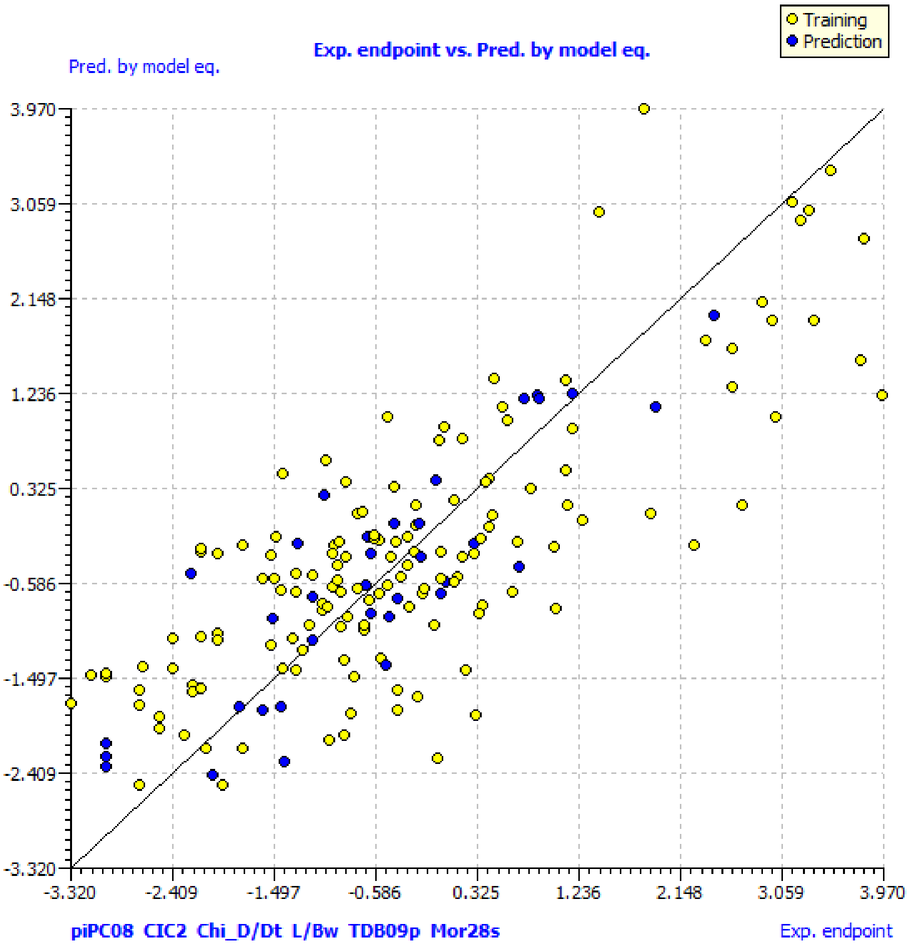


Figure 4.2. The plot of calculated/predicted vs. observed values of mutagenicity for the training/test set compounds by model equation (Eq.4.1), with yellow labeled training set compounds and blue labeled test set compounds.

The proposed model (M2) includes 6 descriptors from DRAGON 7.0 (Kode, 2017). The description of these 6 parameters is listed in Table 4.7. The description of descriptors appearing in the other four models are given in Table C1.

Regardless of their sign, the importance of 6 descriptors could be written and explained as below based on the magnitude of standardized coefficients:

Chi_D/Dt > CIC2 > TDB09p > Mor28s > piPC08 > L/Bw

Table 4.7. Descriptors appeared in model M2 and their descriptions.

Abbreviation of Descriptor	**Description**	**Block**
CIC2	Complementary Information Content Index (neighborhood symmetry of 2-order)	Information Indices
Chi_D/Dt	Randic-like index from distance/detour matrix	2D matrix-based descriptors
L/Bw	The length-to-breadth ratio by WHIM	Geometrical descriptors
TDB09p	3D Topological distance-based descriptors - lag 9 weighted by polarizability	3D autocorrelations
Mor28s	signal 28 / weighted by I-state	3D-MoRSE descriptor*
piPC08	molecular multiple path count of order 8	Walk and path counts

*MoRSE: Molecule Representation of Structure based on Electron diffraction.

The most important descriptor affecting the mutagenicity of chemicals is, Chi_D/Dt, Randic-like index from distance/detour matrix. Topochemical indices usually are two dimensional (2D) indices and encode information pertaining to both molecular topology and chemical nature of atoms and bonds in a molecule (Basak and Gute, 1997). Distance-based topological indices employ the distance matrix or the detour matrix to characterise molecular graphs. The distance matrix is based on topological distance, which is the number of edges in the shortest path between vertices v_i and v_j, whereas the detour matrix is based on the number of edges in the longest path between vertices v_i and v_j in a molecular graph G (Todeschini and Consonni, 2000). All these information reveal that as the distance between the atoms in 2D graph of a chemical increseas, mutagenicity increases.

The second important descriptor is CIC2 from information content block. The CIC2 index provides information about the abundance of rings in a molecule (Todeschini and Consonni, 2000). IC2 is the second-grade classification sphere near the atomic radius (Song et al., 2015). Both CIC2 and Chi_D/Dt values are positively correlated to the mutagenicity of chemicals in the dataset. Similar to our finding, Song et al. (2015) indicated that CIC2 index is positively correlated to the toxicity (LC_{50}) of nitrobenzene to *Paramecium caudatum*.

TDB09p and Mor28s are 3D descriptors and they are also positively correlated to mutagenicity. TDB09p which is the 3D topological distance-based descriptors -lag 9 weighted by polarizability has positive coefficient in the model. It is likely that chemicals with more negatively charged/ionizable group, lipophilic group and carbon-sulfur atom and with large 3D topological distance tended to have high mutagenic activity. TDB09 descriptor appeared in a QSAR model developed for predicting the binding affinity of endocrine disrupting chemicals to eight fish estrogen receptor (He et al., 2018). On the contrary, it has a negative coefficient in their QSAR model indicating that chemicals with more negatively/charged/ionizable group, lipophilic group and carbon-sulfur atom and with large 3D topological distance tended not to bind with estrogen receptor. This difference can be attributed to the different targets of chemicals in the two dataset.

The other 3D descriptor, Mor28s, is related to molecule representation of structures based on electron diffraction (MoRSE) weighted by electric state/I-state. It is based on the three-dimensional structure of molecules by a certain number of values and contains some molecular codes obtained by mathematical transformations used in electron diffraction (Schuur et al., 1996). In a modelling study of pharmaceuticals to fish, Tugcu et al. (2012) have used 3D-MoRSE descriptors and reported that 3D-MoRSE descriptors are related to fish toxicity because of its correlation with hydrophobicity. In addition, Caballero and Fernandez (2011) have also used a 3D-MoRSE descriptor for antifungal activity modeling.

The two descriptors L/Bw from WHIM descriptor group and piPC08 from walk and path counts group have inverse relationships with the mutagenicity. The negative coefficients of geometric descriptor (L/Bw) and molecular multiple path count of order 8 (piPC08) signify that as the value of these descriptors increases, mutagenicity decreases. The descriptor, piPC08, has a negative coefficient in a QSAR model equation in the study of Kusic et al. (2008) in which they modeled the rate constants for radical degradation of aromatic pollutants in water matrix. Taken together, these data suggest that descriptors relevant to the 2D and 3D topology, abundance of rings in a molecule, 3D geometry, polarizability which is reflected by the more negatively charged/ionizable group, lipophilic group and carbon-sulfur atom and with large 3D topological distance, and multiple path count of order 8 affected the mutagenic activity of the studied textile dyes.

4.2. Applicability Domain of the Selected Model

The ADs of linear models were defined by the boundaries of the descriptor and the response range (Table 4.8). Williams plot (Figure 4.3) shows the hat values of chemicals and their prediction accuracy. Errors are represented by standardized residuals. The vertical reference line refers to the critical hat value (h*=0. 0.148) and the horizontal reference lines are ±3σ, the cut-off values for the response outliers. While model M2 has no response outlier, it has only one structural outlier. Hat values of all chemicals were lower than the critical hat value (h* = 0.148), except 2-bromo-7-

aminofluorene Although this compound (2-bromo-7-aminofluorene) was out of the descriptor range, their predicted mutagenic activity value is good enough. Therefore we included it in our dataset, to increase the AD of the proposed model.

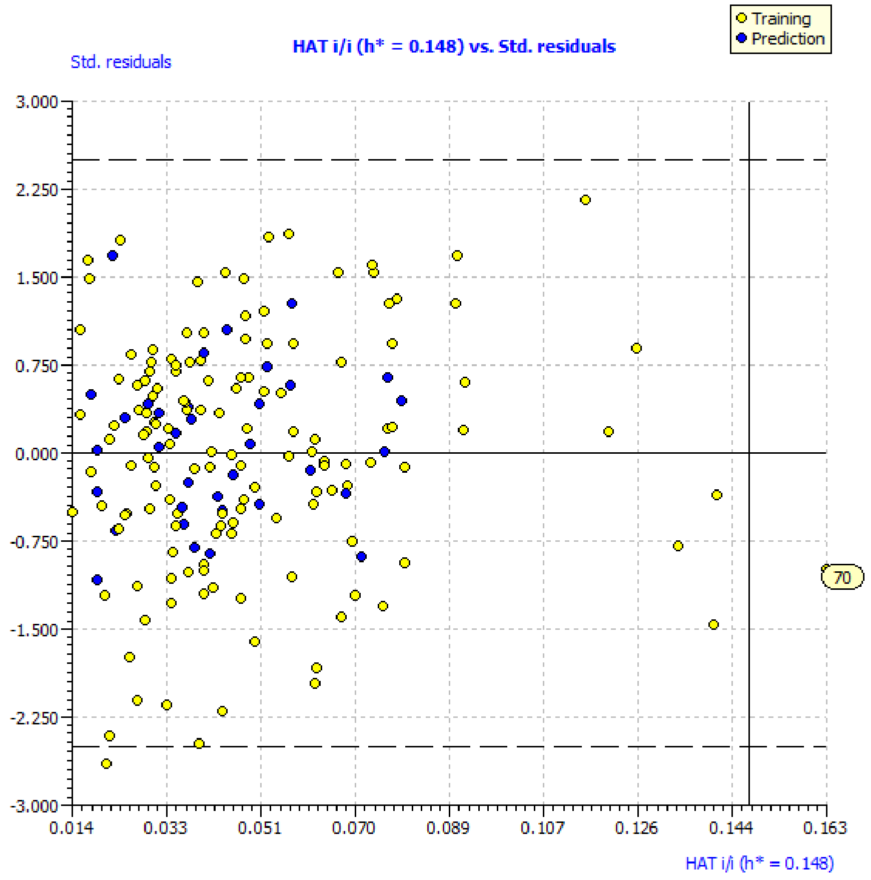


Figure 4.3. Williams plot for Model M2 with training set in yellow and test set in blue. ID number, 70 refers to 2-bromo-7-aminofluorene, respectively.

Table 4.8. Ranges of descriptors used in the model.

Variable	Minimum value	Maximum value
Chi_D/Dt	0.083	0.277
CIC2	0.450	2.236
TDB09p	0.000	4.612
Mor28s	-1.096	3.792
piPC08	0.000	8.230
L/Bw	1.210	23.36

Status of chemicals in the dataset, experimental and predicted mutagenicity values from model M2, descriptor and hat values were given in Table 4.9

Table 4.9. Chemicals that are used to model mutagenicity, their experimental and predicted values, descriptor and hat values.

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (h^*=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
1	4'-NEt2-3-OMe-AAB	Training	-1.77	-2.16	0.05	1.53	0.15	10.78	4.18	0.48	6.28
2	2-OMe-AAB	Training	3.31	3.00	0.07	1.41	0.17	6.15	2.92	1.04	6.00
3	4'-OH-AAB	Prediction	0.41	0.39	0.06	1.56	0.18	15.15	3.37	2.00	5.96
4	3'-Me-4'-OH-AAB	Prediction	0.60	0.99	0.04	1.24	0.17	11.04	3.29	1.47	6.03
5	4'-OH-2',3-diMe-AAB(4'-OH-OAT)	Training	-2.52	-1.86	0.05	1.18	0.17	8.23	3.24	1.71	6.13
6	AAB	Training	0.87	1.24	0.03	1.90	0.18	22.72	3.27	0.89	5.84
7	3'-Me-AAB	Prediction	3.00	1.02	0.06	1.43	0.17	15.64	3.26	0.97	5.93
8	3-OMe-4'-N(CH2CH2OH)2-AAB	Prediction	-1.52	-1.16	0.04	1.39	0.15	11.80	4.11	1.84	6.34
9	3'CH2OH-AAB	Training	3.23	2.90	0.06	1.29	0.17	8.35	3.31	1.78	5.99
10	3-OH-AAB	Training	0.48	1.39	0.03	1.46	0.18	10.69	3.17	0.53	5.93
11	3-OCH2CH2OH-AAB	Training	-1.51	-0.91	0.06	1.40	0.16	8.35	3.89	1.40	6.10
12	2'-CH2OH-3-Me-AAB	Prediction	-0.67	-0.59	0.05	1.04	0.16	5.26	3.12	1.57	6.08
13	4-OMe-AAB	Training	3.16	3.08	0.07	1.56	0.17	16.11	3.83	1.73	5.99
14	2',3-diMe-AAB	Training	-0.48	1.02	0.04	1.41	0.17	7.42	3.19	1.41	6.04
15	3-OBu-AAB	Prediction	-3.00	-1.48	0.05	1.39	0.15	6.96	3.16	1.04	6.12
16	3-OEt-AAB	Training	1.12	1.38	0.02	1.40	0.16	5.33	3.70	0.92	6.07
17	3-OPr-AAB	Training	-1.17	-0.97	0.06	1.34	0.16	3.56	3.46	0.88	6.10

Table 4.9. Continued.

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (h^*=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
18	3-OMe-AAB	Training	1.83	3.97	0.12	1.48	0.17	6.81	3.52	1.03	6.01
19	3'-Me-4'-OH-MAB	Training	3.97	1.23	0.02	1.21	0.17	12.64	3.62	1.40	6.06
20	3'-COOH-MAB	Training	-3.32	-1.74	0.04	1.24	0.16	12.39	3.91	1.63	6.13
21	4'-OH-MAB	Training	3.35	1.94	0.07	1.49	0.17	17.65	3.85	1.76	5.99
22	MAB	Training	2.46	2.00	0.04	1.79	0.18	14.02	3.78	1.30	5.88
23	4'-Me-MAB	Training	3.50	3.38	0.08	1.55	0.17	16.07	4.28	1.04	5.99
24	3'-Me-MAB	Training	1.18	0.90	0.07	1.38	0.17	12.15	3.58	0.87	5.96
25	3'-CH2OH-MAB	Training	-1.04	0.27	0.06	1.26	0.16	10.08	3.64	1.57	6.02
26	3'-Me-4'-OH-DAB	Training	-1.77	-0.21	0.07	1.46	0.16	13.29	3.89	1.19	6.09
27	*p(*dimethylamino)azobenzene	Training	0.89	1.19	0.04	2.03	0.17	14.82	4.18	1.17	5.92
28	3'-COOH-DAB	Training	0.38	-0.78	0.04	1.49	0.16	12.25	4.18	1.40	6.16
29	2-Me-DAB	Prediction	-3.14	-1.45	0.09	1.65	0.17	10.20	3.59	0.84	6.01
30	3'-Me-DAB	Training	2.88	2.13	0.07	1.63	0.17	13.00	3.86	0.65	6.00
31	3'-CHO-DAB	Training	-2.70	-2.51	0.12	1.54	0.16	10.71	4.14	1.54	6.11
32	3'-CH2-OAC-DAB	Training	-3.00	-2.24	0.05	1.44	0.14	6.81	3.90	1.28	6.20
33	3'-CH2OH-DAB	Training	-0.01	0.80	0.04	1.51	0.16	9.83	3.91	1.37	6.06
34	3'-Me-AAB-N-Ac	Training	-1.60	-1.79	0.05	1.48	0.16	15.58	3.65	0.84	6.16

Table 4.9. Continued.

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (*h**=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
35	3'-Me-4'-OH-AAB-N-Ac	Training	-1.60	-0.53	0.04	1.31	0.16	14.56	3.69	1.17	6.25
36	N-OH-2-OMe-AAB	Training	-1.42	0.47	0.05	1.30	0.17	7.30	2.97	0.85	6.04
37	3'-Me-MAB-N-Ac	Training	-2.05	-2.42	0.04	1.49	0.16	16.06	3.92	0.67	6.19
38	N-OH-MAB	Prediction	1.13	0.51	0.04	1.74	0.17	14.67	3.85	1.37	5.92
39	N-OH-3'-Me-MAB	Prediction	3.80	2.74	0.06	1.34	0.17	13.39	3.60	0.95	6.00
40	N-OH-AAB	Training	2.38	1.75	0.03	1.75	0.18	13.84	3.34	1.25	5.88
41	N-OH-4'-Me-MAB	Prediction	1.43	2.99	0.07	1.49	0.17	16.36	3.97	0.81	5.99
42	N-OH-3-OMe-AAB	Training	0.04	0.92	0.03	1.36	0.17	11.37	3.81	1.14	6.06
43	2-aminobiphenyl	Training	2.62	1.31	0.03	1.95	0.19	4.50	1.34	0.44	5.77
44	4-aminobiphenyl	Training	1.18	1.24	0.03	1.97	0.20	8.78	2.06	0.16	5.77
45	2-2'-diaminobiphenyl	Training	2.98	1.95	0.04	1.92	0.18	3.64	1.35	0.91	5.90
46	2,4'-diaminobiphenyl	Training	0.43	0.43	0.05	1.75	0.19	6.32	1.67	0.98	5.90
47	3,3'-diamnobiphenyl	Training	1.93	1.12	0.04	1.77	0.19	5.02	1.26	1.10	5.90
48	3,4'-diaminobiphenyl	Training	2.62	1.68	0.16	1.83	0.19	7.21	2.29	0.66	5.94
49	2-amino-3'-nitrobiphenyl	Training	-0.60	-0.14	0.04	1.38	0.17	5.21	1.53	1.13	6.07

Table 4.9. Continued.

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (*h**=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
50	2-amino-4'nitrobiphenyl	Prediction	-2.40	-1.40	0.05	1.44	0.18	8.48	2.44	1.98	6.19
51	3-amino-2'nitrobiphenyl	Training	-2.30	-2.03	0.03	1.41	0.17	2.61	1.31	1.93	6.14
52	3-amino-3'nitrobiphenyl	Training	-2.15	-0.24	0.06	1.46	0.18	4.78	1.42	1.89	6.23
53	3-amino-4'nitrobiphenyl	Training	-0.54	-1.30	0.13	1.52	0.18	10.38	2.63	2.23	6.22
54	4-amino-2'nitrobiphenyl	Training	-2.22	-1.61	0.09	1.52	0.17	3.21	1.74	1.44	6.21
55	4-amino-3'nitrobiphenyl	Training	-1.32	-1.11	0.08	1.52	0.18	6.48	2.40	1.64	6.22
56	4-amino-4'nitrobiphenyl	Training	-2.22	-1.55	0.05	1.77	0.18	14.39	2.37	1.58	6.19
57	1-aminoanthracene	Prediction	-2.10	-2.15	0.06	1.80	0.11	3.59	1.31	0.48	7.42
58	2-aminoanthracene	Training	-2.52	-1.97	0.05	1.82	0.11	5.71	2.04	0.10	7.45
59	9-aminoanthracene	Prediction	-3.00	-2.34	0.08	1.80	0.11	3.59	1.31	0.48	7.42
60	1-aminophenanthrene	Training	-1.42	-1.40	0.04	1.88	0.11	3.04	1.31	0.96	7.40
61	2-aminophenanthrene	Prediction	-3.00	-2.12	0.04	1.85	0.11	3.97	2.07	0.79	7.41

Table 4.9. Continued.

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (*h**=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
62	3-aminophenanthrene	Training	-0.80	-1.82	0.04	1.85	0.11	2.83	1.32	0.71	7.41
63	9-aminophenanthrene	Training	-0.87	-2.03	0.03	1.87	0.11	2.01	1.35	1.17	7.38
64	1-aminofluorene	Training	-3.00	-1.45	0.02	1.30	0.12	3.25	0.00	0.97	6.64
65	2-aminofluorene	Prediction	-0.49	-1.35	0.04	1.27	0.12	5.06	1.40	0.95	6.65
66	3-aminofluorene	Prediction	-0.77	-1.47	0.04	1.27	0.12	3.85	1.35	0.91	6.66
67	4-aminofluorene	Training	-0.21	-1.67	0.03	1.30	0.12	2.58	0.00	0.97	6.64
68	2,7-diaminofluorene	Training	-1.80	-1.76	0.02	1.46	0.12	6.58	1.89	1.39	6.76
69	2-amino-7-nitrofluorene	Training	-0.69	-0.98	0.05	1.17	0.11	8.85	2.10	2.62	7.00
70	2-bromo-7-aminofluorene	Training	-2.40	-1.11	0.08	1.02	0.12	15.46	4.20	1.01	6.76
71	2-hydroxy-7-aminofluorene	Training	0.35	-0.87	0.07	0.98	0.12	6.65	1.66	0.59	6.76
72	2-amino-7-acetamidofluorene	Training	-1.43	-1.76	0.02	1.07	0.11	10.52	3.82	0.79	6.97
73	1-aminopyrene	Training	-0.40	-0.17	0.05	2.03	0.10	2.02	1.31	1.25	8.21
74	2-aminopyrene	Training	-0.47	-0.59	0.03	2.07	0.10	2.51	2.06	1.13	8.21
75	4-aminopyrene	Training	-0.59	-0.12	0.04	1.96	0.10	1.41	1.34	1.34	8.20

Table 4.9. Continued.

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (*h**=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
76	1-aminofluoranthene	Training	-0.24	-0.27	0.03	1.85	0.10	1.50	0.00	1.09	8.22
77	2-aminofluoranthene	Training	0.47	0.07	0.03	1.90	0.10	1.82	1.36	1.17	8.23
78	3-aminofluoranthene	Training	0.64	-0.66	0.08	1.96	0.10	2.53	1.40	1.30	8.23
79	7-aminofluoranthene	Training	-0.18	-0.32	0.06	1.85	0.10	1.93	0.00	1.62	8.22
80	8-aminofluoranthene	Training	0.13	-0.57	0.05	1.86	0.10	2.71	1.39	1.16	8.23
81	6-aminochrysene	Training	-0.64	-0.73	0.06	2.24	0.08	3.47	2.89	1.38	8.18
82	3-aminoquinoline	Training	0.55	1.12	0.03	0.82	0.16	3.35	0.00	-0.06	6.06
83	5-aminoquinoline	Training	-2.67	-1.39	0.09	0.72	0.16	1.71	0.00	0.51	6.02
84	6-aminoquinoline	Training	0.20	0.81	0.03	0.72	0.16	3.37	0.00	0.31	6.06
85	8-aminoquinoline	Prediction	-2.00	-1.05	0.08	0.82	0.16	1.68	0.00	0.14	6.02
86	1-aminocarbazole	Prediction	-2.15	-1.58	0.05	1.27	0.12	3.22	0.00	0.95	7.37
87	2-aminocarbazole	Training	-0.38	-1.60	0.04	1.24	0.12	5.11	1.40	1.00	7.38
88	3-aminocarbazole	Training	-1.29	-1.41	0.04	1.24	0.12	3.96	1.36	0.88	7.39
89	4-aminocarbazole	Training	-0.87	-1.31	0.06	1.27	0.12	2.58	0.00	1.20	7.37

Table 4.9. Continued.

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (h^*=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
90	1-aminophenazine	Training	-2.70	-1.60	0.02	1.58	0.11	3.43	1.29	-0.01	7.42
91	2-aminophenazine	Training	-1.40	-2.28	0.07	1.60	0.11	5.50	2.01	0.14	7.45
92	1,6-diaminophenazine	Training	-0.39	-0.73	0.07	1.52	0.11	3.02	1.24	0.56	7.50
93	1,7-diaminophenazine	Prediction	0.81	0.33	0.03	1.40	0.11	4.42	2.20	0.66	7.55
94	1,9-diaminophenazine	Training	-0.04	0.41	0.08	1.52	0.11	2.77	1.27	0.70	7.51
95	2,7-diaminophenazine	Training	0.15	-0.51	0.02	1.67	0.11	6.74	2.15	0.88	7.56
96	2,8-diaminophenazine	Training	0.01	-0.53	0.02	1.67	0.11	6.19	2.38	0.71	7.56
97	1-naphthylamine	Training	-1.14	-1.12	0.08	1.56	0.16	1.73	0.00	0.76	6.02
98	2-naphthylamine	Prediction	0.75	1.19	0.03	1.52	0.16	3.41	0.00	0.39	6.06
99	1-amino-4-nitronaphthalene	Training	0.32	-1.85	0.03	1.25	0.15	1.70	0.00	2.83	6.47
100	2-amino-1-nitronaphthalene	Training	-0.02	-2.26	0.04	1.19	0.15	1.84	0.00	1.37	6.54
101	aniline	Prediction	-0.16	-0.67	0.01	1.46	0.28	2.14	0.00	0.34	0.00
102	*p*-bromoaniline	Training	0.13	0.22	0.03	1.00	0.27	14.02	0.00	0.45	0.00
103	*o*-chloroaniline	Training	0.43	-0.03	0.02	0.86	0.27	2.20	0.00	0.64	0.00
104	*p*-chloroaniline	Training	0.30	-0.20	0.04	1.00	0.27	6.85	0.00	0.52	0.00
105	*p*-fluoroaniline	Training	-0.22	-0.02	0.03	1.00	0.27	4.07	0.00	0.26	0.00

Table 4.9. Continued.

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (h*=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
106	*p*-ethoxyaniline	Training	-0.96	-0.62	0.02	0.99	0.25	5.53	1.07	-0.07	2.78
107	4-phenoxyaniline	Training	0.69	-0.18	0.03	1.89	0.19	9.10	2.33	-0.07	5.56
108	4-hydroxyaniline	Prediction	-0.62	-0.87	0.04	0.93	0.27	3.70	0.00	0.22	0.00
109	4-cyclohexylaniline	Training	0.20	-0.32	0.03	1.80	0.20	12.04	1.79	-0.21	4.47
110	2,4-dinitroaniline	Training	-0.62	-0.30	0.02	1.04	0.23	2.46	0.00	3.79	4.72
111	2,4-dimethylaniline	Training	-2.23	-0.48	0.02	1.21	0.26	2.23	0.00	0.06	0.00
112	2,5-dimethylaniline	Training	-0.69	-1.03	0.14	1.31	0.26	2.29	0.00	0.20	0.00
113	2,4-difluoroaniline	Training	-1.15	-0.51	0.03	0.63	0.26	2.14	0.00	0.82	0.00
114	2-methoxy-5-methylaniline	Prediction	2.71	0.18	0.04	0.78	0.25	2.96	0.00	0.23	2.15
115	3-methoxy-4-methylaniline	Training	-0.45	-0.32	0.02	0.78	0.25	1.62	0.00	-0.24	2.15
116	4-methoxy-2-methylaniline	Prediction	-1.28	-0.19	0.04	0.78	0.25	3.38	0.00	-0.01	0.00
117	4-chloro-2-nitroaniline	Training	0.01	-0.27	0.03	0.55	0.24	2.28	0.00	2.94	3.45
118	2,4,5-trimethylaniline	Training	-0.95	-0.22	0.03	1.71	0.25	2.09	0.00	0.16	0.00
119	2-bromo-4,6-dinitroaniline	Training	-0.89	-0.99	0.03	0.78	0.22	1.46	0.00	3.75	4.72
120	4,4'-ethylenebis(aniline)	Training	-1.30	-0.48	0.04	2.00	0.18	15.93	3.61	0.53	5.27

Table 4.9. Continued.

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (*h**=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
121	4,4'-methylenedianiline	Training	1.04	-0.81	0.06	1.93	0.18	9.21	2.39	0.57	5.67
122	4,4'-methylenebis (O-Ethylaniline)	Training	-0.42	0.35	0.03	0.94	0.25	1.96	0.00	0.23	2.15
123	4,4'-methylenebis(o-fluoroaniline)	Training	0.70	-0.41	0.02	0.86	0.27	1.42	0.00	0.65	0.00
124	4,4'-methylenebis(o-isopropylaniline)	Training	-1.05	-0.78	0.03	1.28	0.25	2.15	0.00	-0.15	2.78
125	1,2-phenylenediamine	Training	-0.74	0.10	0.03	1.50	0.27	1.44	0.00	1.03	0.00
126	1,3-benzenediamine	Prediction	-0.91	-0.19	0.03	1.38	0.27	2.03	0.00	0.88	0.00
127	1,4-benzenediamine	Training	-0.85	-0.32	0.06	1.75	0.27	3.69	0.00	0.98	0.00
128	2-methyl-1,4-phenylenediamine	Training	-0.35	-0.51	0.02	1.13	0.26	2.25	0.00	1.07	0.00
129	2-nitro-1,4-phenylenediamine	Training	2.28	-0.21	0.02	1.04	0.24	2.05	0.00	3.25	3.45
130	2-methoxy-1,4-phenylenediamine	Training	-0.22	0.17	0.03	1.08	0.25	1.59	0.00	0.58	2.15
131	2-ethoxy-1,4-phenylenediamine	Training	-0.19	-0.01	0.03	1.02	0.24	1.57	0.00	0.45	3.36
132	2-propoxy-1,4-phenylenediamine	Training	-1.49	-0.53	0.05	0.98	0.22	2.11	1.05	0.59	3.66

Table 4.9. Continued.

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (*h**=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
133	2-butyloxy-1,4-phenylenediamine	Training	-0.92	-0.55	0.03	1.09	0.21	4.47	1.51	0.85	3.87
134	4-chloro-1,2-phenylenediamine	Prediction	-0.85	0.40	0.05	1.05	0.26	4.11	0.00	1.18	0.00
135	4-chloro-1,3-phenylenediamine	Training	-0.55	-0.16	0.04	0.92	0.26	3.43	0.00	1.05	0.00
136	4-nitro-1,2-phenylenediamine	Training	-0.66	-0.13	0.02	1.15	0.25	3.73	0.00	2.46	0.00
137	4-nitro-1,3-phenylenediamine	Training	0.05	-0.56	0.04	1.04	0.25	3.10	0.00	3.20	3.45
138	*N*-acetyl-1,4-phenylenediamine	Prediction	-0.14	-0.62	0.05	1.08	0.24	6.28	1.30	0.70	3.84
139	1,4-*N*Ac-phenylenediamine	Prediction	-1.52	-0.31	0.05	1.08	0.24	6.28	1.30	0.70	3.84
140	2,6-dichloro-1,4-phenylenediamine	Training	-0.70	0.11	0.03	1.13	0.25	1.21	0.00	1.41	0.00
141	*N*,*N*-diethyl-1,4-phenylenediamine	Training	0.00	-0.67	0.02	1.63	0.25	3.75	1.11	-0.17	3.45
142	*N*,*N*-Dimethyl-1,4-phenylenediamine	Training	1.14	0.18	0.04	1.61	0.26	4.50	0.00	0.67	0.00
143	*N*-methyl-1,4-phenylenediamine	Training	1.28	0.03	0.05	1.20	0.26	4.95	0.00	0.70	0.00
144	2,4-diaminoethylbenzene	Training	-1.06	-0.84	0.03	0.98	0.25	3.06	0.00	0.68	2.15

Table 4.9. Continued.

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (*h**=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
145	2,4-diaminotoluene	Training	1.89	0.10	0.03	1.03	0.26	2.31	0.00	0.76	0.00
146	3,4-diaminotoluene	Training	0.36	-0.15	0.04	1.13	0.26	2.32	0.00	0.58	0.00
147	3-amino-α,α,α-trifluorotoluene	Training	-0.30	-0.13	0.03	0.87	0.26	3.95	0.00	1.11	0.00
148	2,4-diamino-isopropylbenzene	Prediction	-0.28	-0.81	0.04	1.29	0.25	3.16	0.00	0.31	2.78
149	2,4-diamino-n-butylbenzene	Training	-1.30	-0.66	0.04	1.16	0.23	7.17	1.85	0.49	3.88
150	2-amino-4-chlorophenol	Prediction	-0.41	0.00	0.04	0.45	0.26	3.91	0.00	0.23	0.00
151	2-amino-4-methylphenol	Training	-2.00	-0.28	0.02	0.64	0.26	2.41	0.00	-0.04	0.00
152	2-amino-5-nitrophenol	Training	-0.96	-0.29	0.02	0.63	0.25	3.66	0.00	1.14	0.00
153	3-amino-6-methyl phenol	Training	-2.15	-0.27	0.02	0.64	0.26	2.24	0.00	-0.35	0.00
154	benzidine	Prediction	3.77	1.56	0.03	2.08	0.19	11.44	2.17	0.35	5.90
155	3,3'-diaminobenzidine	Prediction	-2.70	-1.75	0.06	2.12	0.18	8.15	2.18	2.37	6.19
156	3,3'-dichlorobenzidine	Prediction	-2.00	-1.12	0.13	1.52	0.18	5.07	3.15	1.33	6.19
157	4,4'-diamino-3,3'-dimethoxy-1,1'-biphenyl	Training	-0.05	-0.98	0.08	1.68	0.17	6.28	2.54	0.64	6.35
158	3,3'-dimethyl-4,4'-bianiline	Training	-1.24	-1.22	0.06	1.72	0.18	7.23	2.18	0.98	6.19
159	4-aminophenyl disulfide	Training	-0.55	-0.67	0.04	2.00	0.18	2.66	2.92	0.93	5.27

. Table 4.9. Continued

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (*h**=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
160	(4-aminophenyl)ether'	Prediction	-0.92	-0.41	0.03	2.00	0.18	9.41	2.46	-0.04	5.67
161	4-aminophenyl Sulfide	Training	1.02	-0.22	0.02	2.00	0.18	5.92	2.43	0.26	5.67
162	3-OSO3K-AAB	Training	0.23	-1.41	0.05	1.25	0.16	7.06	4.27	-0.79	6.26
163	4'-OSO3K-AAB	Training	-0.75	-0.62	0.06	1.42	0.16	21.87	4.32	1.40	6.30
164	3-methoxy-4'-N,N-diethyl-AAB	Training	-0.99	-2.08	0.03	1.54	0.15	16.01	3.87	0.26	6.25
165	3-OSO3K-MAB	Training	-0.46	-0.89	0.05	1.30	0.16	6.07	4.61	-0.49	6.31
166	4'-OSO3K-MAB	Training	-0.89	-0.66	0.08	1.38	0.16	23.36	4.59	-0.71	6.32
167	3'-acetoxymethyl-DAB	Training	-1.96	-2.51	0.05	1.44	0.14	6.26	4.02	-0.26	6.20
168	R1=OCH2CH2OH, R2=NEt2	Training	-0.38	-1.80	0.14	1.41	0.09	7.89	4.15	-1.09	7.83
169	R1=OBu, R2=NEt2	Training	-0.83	-0.90	0.07	1.52	0.09	7.09	4.01	-0.85	7.84
170	R1=OPr, R2=NEt2	Training	-0.29	-0.40	0.05	1.50	0.09	7.46	4.16	0.31	7.83
171	R1=OMe, R2=NEt2	Training	-2.15	-1.09	0.04	1.45	0.09	10.97	4.05	0.18	7.81
172	R1=OPr, R2=H	Training	-1.43	-0.64	0.07	1.33	0.10	5.34	3.92	0.08	7.78
173	R1=OEt, R2=NEt2	Training	-1.47	-0.14	0.08	1.53	0.09	9.63	4.19	0.25	7.83
174	R1=OBu, R2=H	Prediction	-1.01	-0.80	0.09	1.36	0.09	4.14	3.80	-0.28	7.79

Table 4.9. Continued

ID	Name	Status	Exp. mutagenicity Log (rev/nmol)	Pred. mutagenicity by model M2	Hat value (h*=0.15)	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08
175	R1=OCH2CH2OH, R2= H	Training	0.31	-0.29	0.05	1.32	0.10	6.08	3.60	-0.16	7.78
176	R1=OMe, R2=H	Training	-1.03	0.60	0.07	1.41	0.10	6.39	3.84	0.67	7.76
177	R1=OEt, R2=H	Training	-1.14	-0.71	0.05	1.37	0.10	5.89	3.95	-1.05	7.78

4.3. Prediction of Mutagenicity Activity of External Set Chemicals with No Mutagenicity Data

The predicted ability of model M2 was tested on external set chemicals with no mutagenic activity data (Insubria graph, Figure 4.4). In this respect, 33 water-soluble textile dyes containing 27 acid and six direct dyes which were provided by Clariant, Huntsman and Dystar were used as an external dataset. The descriptors used in model M2 were calculated for each chemical in the external dataset. Regarding the Insubria graph (Figure 4.4.) the structural coverage of model M2 is 70%.

The chemicals which fall outside the applicability domain mostly belong to azo dyes. These are Direct Blue 78, Acid Red 154, Acid Red 134, Acid Red 138, Acid Red 145, Acid Orange 156, Acid Red 111, Direct Yellow 86, Direct Yellow 106 and one of them is anthraquinone dye (Acid Blue 127). There were many different chemical structures. Because of this heterogenicity of the external set, the 10 compounds were outside the AD. However, 23 compounds belonging both anthraquinone and azo dyes were well predicted by the proposed model (Eq.4.1). The predicted mutagenicity values from model M2 and the values of descriptors for the external set chemicals with no mutagenic activity data are given in Table 4.10.

When the individual descriptor range criterion is considered (Table 4.8.), the chemicals that were out of the descriptor ranges were removed from the list of chemicals given in Table 4.10. The new list of chemicals which fell into the AD of model M2 is given in Table 4.11. The order of the most mutagenic six dyes which fell into the AD of the selected model M2 is as follows:

Acid Blue 62 > Acid Blue 40 > Acid Blue 45> Acid Blue 80 > Acid Blue 230 > Acid Blue 344
These dyes are mainly used for cotton, fiber dyeing and leather shading.

The structures of these dyes are given in Figure 4.5. All these dyes belong to anthraquinone group and they have a substituent in the para position. The most mutagenic dye, Acid Blue 62, has a cyclohexyl group as a substituent. The structure of least mutagenic dye, Direct Orange 34, from the azo group is given in Figure 4.6. It is a synthetic dye that is mainly used for cotton, silk, wool and their blended fabric dyeing and printing, also can be used for leather and paper shading.

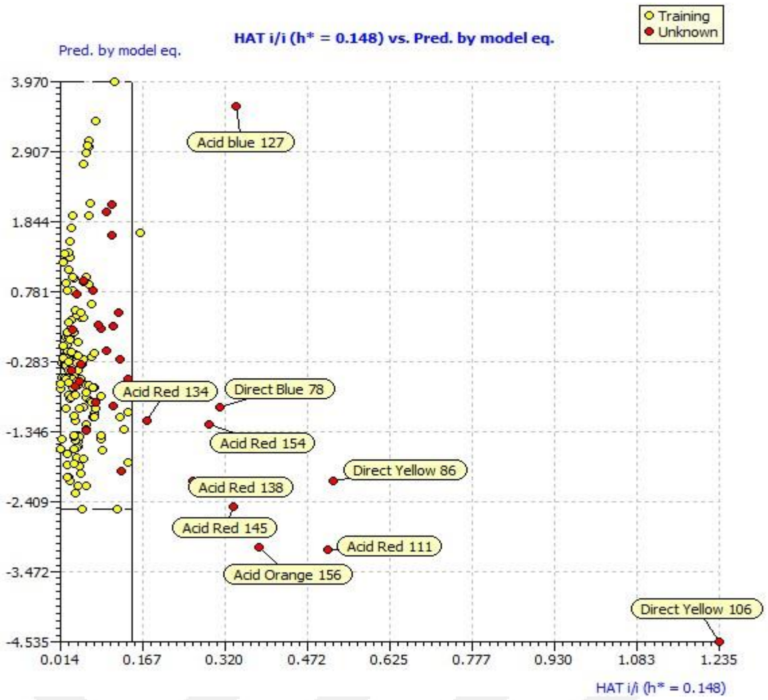


Figure 4.4. Insubria graph of model M2 training set in yellow and external set in red.

Table 4.10. The compounds that are in the AD of model M2, their predicted mutagenicity values.

Chemical Group	Name	CAS Number	CIC2	Chi_D/Dt	L/Bw	TDB09p	Mor28s	piPC08	Pred. by Eq.(4.1)
Anthraquinone	Acid Blue 40	6424-85-7	1.184	0.085	1.710	3.498	2.380	7.597	-1.212
Anthraquinone	Acid Blue 45	2861-02-1	1.211	0.096	5.940	2.736	2.614	7.520	-1.966
Anthraquinone	Acid Blue 62	4368-56-3	1.408	0.090	1.260	2.921	2.684	7.339	-1.187
Anthraquinone	Acid Blue 80	4474-24-2	2.170	0.074	7.000	3.934	-0.158	7.866	-1.659
***Azo Dye**	**Acid Blue 113**	3351-05-1	**2.310**	0.073	16.050	4.43	**-1.382**	**8.258**	-2.586
Anthraquinone	Acid Blue 230	12219-37-3	1.172	0.086	2.470	3.756	0.520	7.571	-1.962
Anthraquinone	Acid Blue 344	85153-93-1	1.569	0.081	4.510	3.936	0.554	7.784	-1.658
Anthraquinone	Acid Green 25	4403-90-1	1.783	0.075	2.060	4.49	0.960	7.765	-0.662
Azo Dye	**Acid Orange 67**	12220-06-3	1.630	0.100	19.300	4.098	**-1.123**	7.272	-3.441
Azo Dye	Acid Orange 127	12269-96-4	1.819	0.094	9.750	4.435	-0.223	7.612	-1.837
Azo Dye	Acid Red 42	6245-60-9	1.449	0.096	7.070	3.913	-0.717	7.729	-2.574
Azo Dye	Acid Red 57	12217-34-4	1.366	0.094	8.870	3.802	-0.454	7.730	-2.768
Azo Dye	Acid Red 151	6406-56-0	1.426	0.101	17.930	4.367	0.630	7.058	-2.474
Azo Dye	Acid Red 249	6416-66-6	1.425	0.086	3.040	4.403	-0.431	8.157	-1.844
Azo Dye	Acid Red 266	57741-47-6	0.760	0.106	4.650	3.423	2.776	7.573	-1.711
Azo Dye	Acid Red 337	67786-14-6	0.919	0.106	5.740	3.041	2.703	7.552	-1.959
Azo Dye	**Acid Yellow 17**	6359-98-4	0.936	0.127	15.840	**5.519**	-0.806	6.869	-2.538
Azo Dye	**Acid Yellow 49**	12239-15-5	0.979	0.136	9.370	**5.479**	-1.379	6.995	-2.242
Azo Dye	Acid Yellow 61	12217-38-8	1.199	0.102	6.870	4.235	-1.078	7.177	-2.635
Azo Dye	Acid Yellow 199	70865-20-2	1.163	0.121	8.980	3.718	1.434	6.975	-2.005
Azo Dye	**Direct Blue 71**	4399-55-7	2.201	0.056	10.740	**4.938**	**-1.683**	**8.857**	-2.119
Azo Dye	**Direct Green 26**	6388-26-7	1.912	0.056	4.720	4.267	-0.898	**8.849**	-1.986
Azo Dye	Direct Orange 34	12222-37-6	1.126	0.160	16.520	4.243	-0.200	6.192	-2.907

*Chemicals that are out of the descriptor ranges are written in bold.

Table 4.11. The compounds that are in the AD of model M2 remaining after the application of descriptor range criteria.

Chemical Group	**Compound Name**	**CIC2**	**Chi_D/Dt**	**L/Bw**	**TDB09p**	**Mor28s**	**piPC08**	**Pred. by Eq.(4.1)**
Anthraquinone*	**Acid Blue 40**	1.184	2.773	1.710	3.498	2.380	7.597	**1.706**
Anthraquinone	**Acid Blue 45**	1.211	2.830	5.940	2.736	2.614	7.52	**0.951**
Anthraquinone	**Acid Blue 62**	1.408	3.077	1.260	2.921	2.684	7.339	**2.060**
Anthraquinone	**Acid Blue 80**	2.170	2.521	7.000	3.934	-0.158	7.866	**0.918**
Anthraquinone	**Acid Blue 230**	1.172	2.678	2.470	3.756	0.520	7.571	**0.814**
Anthraquinone	**Acid Blue 344**	1.569	2.350	4.510	3.936	0.554	7.784	**0.754**
Azo Dye	Acid Red 151	1.426	1.885	17.930	4.367	0.630	7.058	-0.678
Azo Dye	Acid Red 266	0.760	2.154	4.650	3.423	2.776	7.573	0.484
Azo Dye	Acid Red 337	0.919	2.210	5.740	3.041	2.703	7.552	0.278
Azo Dye	Acid Yellow 199	1.163	1.617	8.980	3.718	1.434	6.975	-0.449
Azo Dye**	Direct Orange 34	1.126	1.649	16.520	4.243	-0.200	6.192	-1.425

*The most mutagenic 6 dyes are written in bold**;** ** The least mutagenic dye.

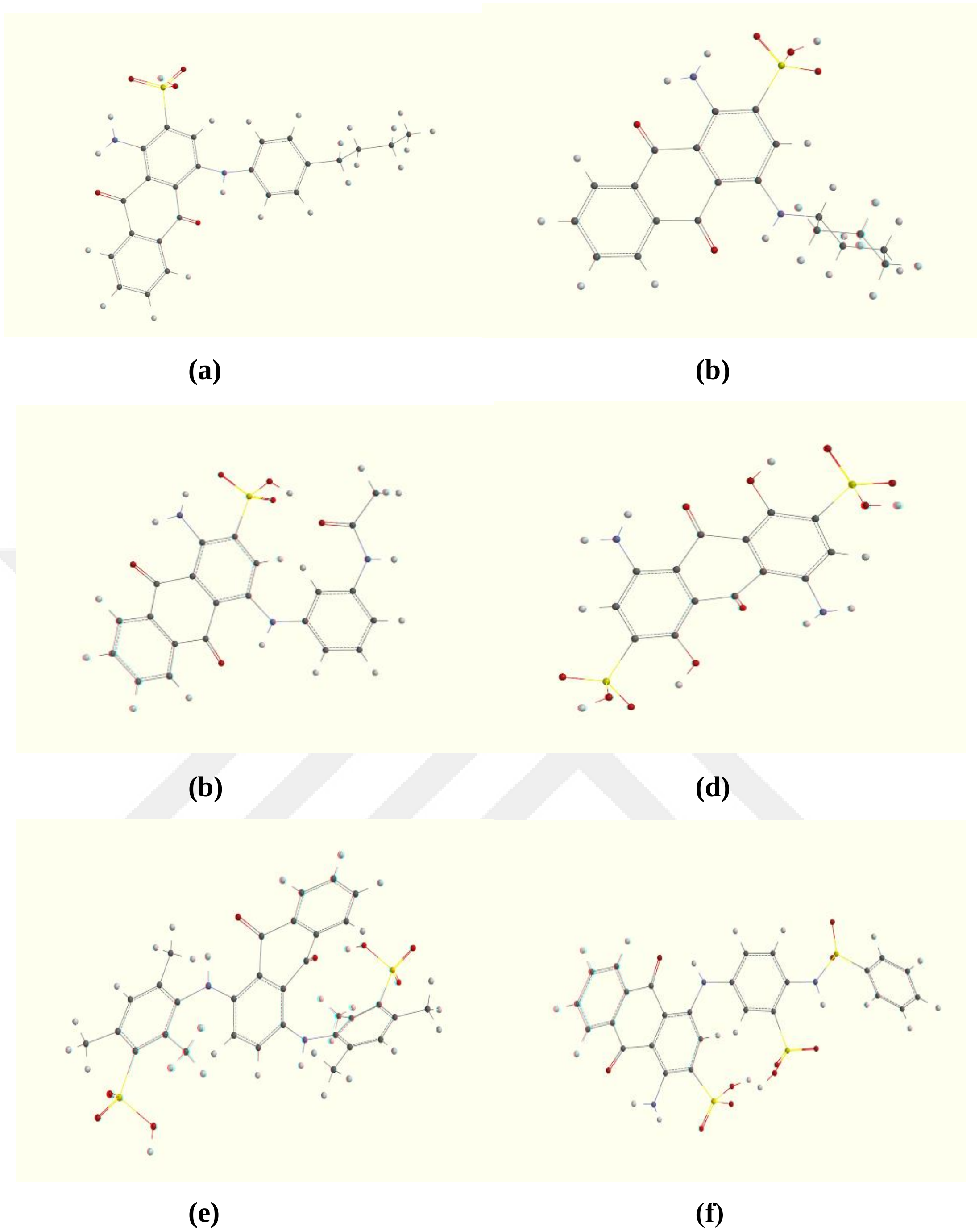


Figure 4.5. Conformers of (a) Acid Blue 230, (b) Acid Blue 62, (c) Acid Blue 40, (d) Acid Blue 45, (e) Acid Blue 80, (f) Acid Blue 344 drawn in SPARTAN 10 software.

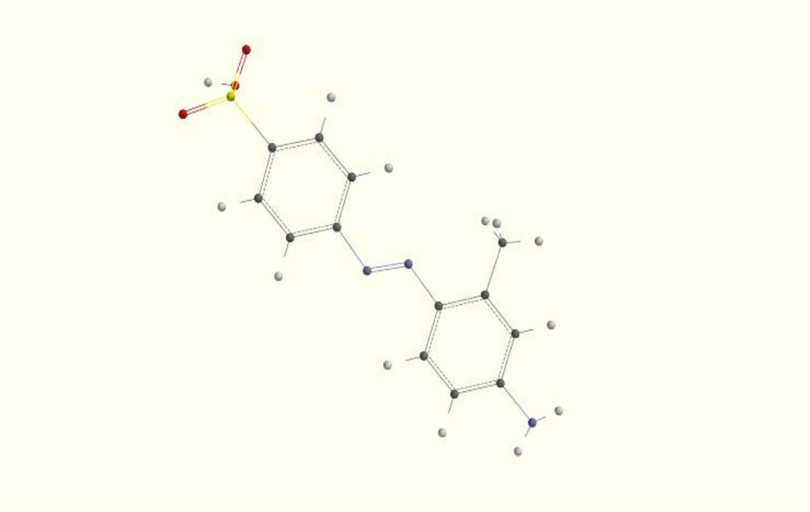


Figure 4.6. A conformer of Direct Orange 34 drawn in SPARTAN 10 software.

4.4. Comparison of the QSAR models from the present study with the previously published models

To compare the results of the current study with those of published studies in which mutagenic activity models were generated is of interest, since the comparison points out the strengths and weaknesses of the present study (Table 4.12). Even though an exact comparison is not possible, as each author use different software and a unique dataset with a different number of compounds. Moreover, the quality of prediction depends on various parameters.

Garg et al. (2002) generated several QSAR models on the mutagenicity of aminoazobenzene dyes and related structures and primarily used the best multilinear regression (BMLR) method implemented in CODESSA (CODESSA ^{TM} , 1992). Later, Bhat et al. (2005) developed several QSAR models on the mutagenicity of aromatic, heteroaromatic amines and related compounds primarily employing again BMLR method implemented in CODESSA (CODESSA ^{TM} , 1992). They stated that the rather large standard deviation for the test sets suggests the need for improvement in the model. However, they didn’t give detail for the standard deviation of test set.

Although R^2, Q^2, S and F values are provided, these previous works lack the necessary/up-to-date external validation parameters (Table 4.12) However, the fit parameters are not enough to assume that the model is valid and robust. There is no information on the model’s applicability domain. Division methods were not given in the previous study. Additionally, training and test set divisions are not clear. All these features reveal that the proposed model in the present study is superior that it passed all of the up-to-date internal and external validation criteria.

Additionally, in attempt to predict the mutagenicity of external chemicals having the most mutagenic activity (Table 4.11) using VEGA software program we uploaded smile code of each chemical to VEGA software. Unfortunately, the output of the program revealed no result for structures with SO_3H (Na) substituents. Therefore, the mutagenic activity values of the most mutagenic chemicals screened from our model could not be verified using VEGA software. Regarding that the VEGA software could not calculate the mutagenic activity of chemicals with sulphonate group, our model is superiror to VEGA software. On the other hand, the least mutagenic dye screened from our QSAR model, Direct Orange 34, was reported as non-mutagenic by VEGA software. This chemical do not contain -SO_3Na group. Therefore, its mutagenicity can be calculated by VEGA. This consistency supports the validation of our model as well. The output of VEGA software for Direct Orange 34 is given in Appendix E.

Table 4.12. Comparison of the statistical parameters of generated models to those of the previously published models.

Chemical groups	**Method**	***N****	***Number of descriptors***	R^2	Q^2_{LOO}	***S/F***	R^2_{Test}	$RMSE_{Test}$	**Reference**
Aminoazodyes	BMLR	43	5	0.62	0.68	0.13/40.40	N/A	N/A	**Garg et al., 2002**
Aromatic/heteroaromatic amine derivatives	BMLR	181	6	0.67	0.64	0.79/58.58	N/A	N/A	**Bhat et al., 2005**
Aromatic/heteroaromatic amine derivatives	MLR	177	6	0.61	0.56	1.04/33.71	0.740	0.650	**Present study**

* the number of chemicals in the datasets.

5. CONCLUSION

Aminoazobenzene derivatives and their related structures are really important industrial colorants. However, some of these dyes are shown to be mutagenic.

In the present study, in an attempt to determine the mutagenic activity of azo dyes and amine derivatives numerous QSAR models were generated for different training and test set divisions. All models were validated both internally and externally using recently reported validation parameters in the literature. Their applicability domains were defined by Williams plot and the range of descriptors . The best model from each division was selected via the Multi-Criteria Decision Making (MCDM) approach as implemented in QSARINS. We selected models with the best MCDM score, ensuring the statistical thresholds for fitting, internal and external validation, and with the least possible number of descriptors. The predictive performance of the 5 final models with 6 descriptors from DRAGON software was compared with an external set of 33 compounds mainly comprised of textile dyes. Of the five models, the one with the highest structural coverage (70%) for external set chemicals with no mutagenicity data was proposed as the best model. Descriptors appearing in the proposed model were based on 2D and 3D geometries of the molecule. Mutagenicity is found to be related directly to the abundance of rings in a molecule, polarizability and electric state of a molecule. It is also noteworthy that chemicals with more negatively charged/ionizable group, lipophilic group and carbon-sulfur atom and with large 3D topological distance tended to be more mutagenic. On the other hand, as the values of 3D geometrical descriptor and molecular multiple path count of order 8 increase mutagenic activity decreases.

The generated QSAR model was found to be superior to the previously published literature models regarding the up-to-date validation criteria and OECD principles.

Based on the predicted values, the most and least mutagenic chemicals were screened from the best model generated in the present study. The most mutagenic dyes contain anthraquinone as a common group and have a substituent in the para position. The order of the most mutagenic six dyes are found as Acid Blue 62 > Acid Blue 40 > Acid Blue 45> Acid Blue 80 > Acid Blue 230 > Acid Blue 344. These dyes are mainly used for cotton, fiber dyeing and leather shading. The most mutagenic dye, Acid Blue 62, has a cyclohexyl group as a substituent. The least mutagenic dye is found as Direct Orange 34 which is an azo dye. It was also classified as non-mutagenic by VEGA software. It is a synthetic dye that is mainly used for cotton, silk, wool and their blended fabric dyeing and printing, also can be used for leather and paper shading.

In the present study, the proposed model has the potential to notify the environmental scientists about the uncertain outcome of chemicals in the environment. The data gap in mutagenicity of currently used some textile dyes were filled. By using the proposed model the dyes manufacturer can have a chance to check the mutagenicity of the new chemicals which fell in the AD of our QSAR model. In this way, safer and environment-friendly textile dyes can be produced. By using this QSAR model, chemical consumption can be prevented and time can be saved. This is an economic gain in the scientific world, only prioritize chemicals (i.e the most mutagenic chemicals) can be tested, but non-mutagenic chemicals can no longer need to be tested in the laboratory.

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APPENDIX A: CHEMICAL STRUCTURES OF EXTERNAL SET COMPOUNDS

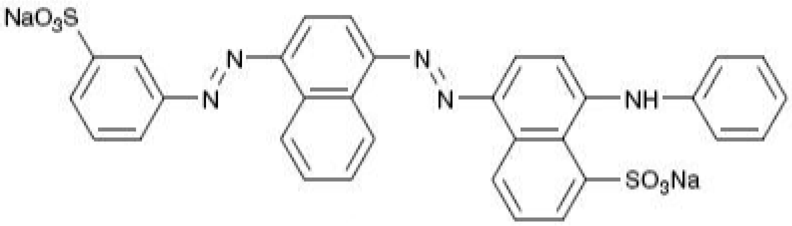


Figure A1. Acid Blue 113

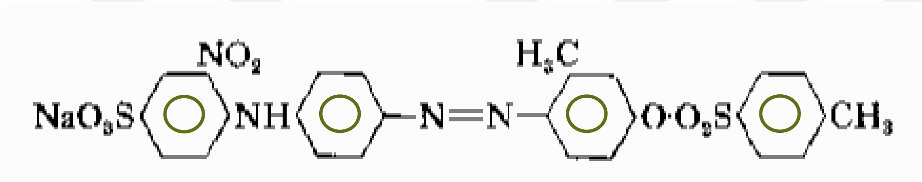


Figure A2. Acid Orange 67

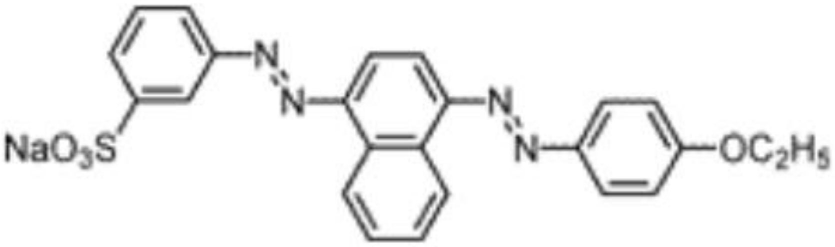


Figure A3. Acid Orange 127

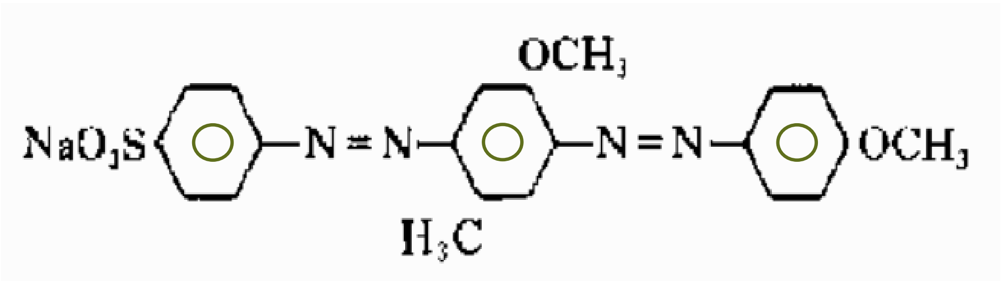


Figure A4. Acid Orange 156

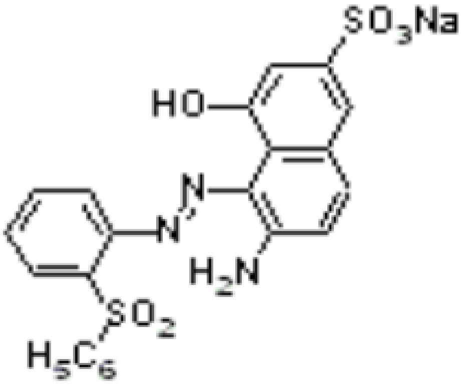
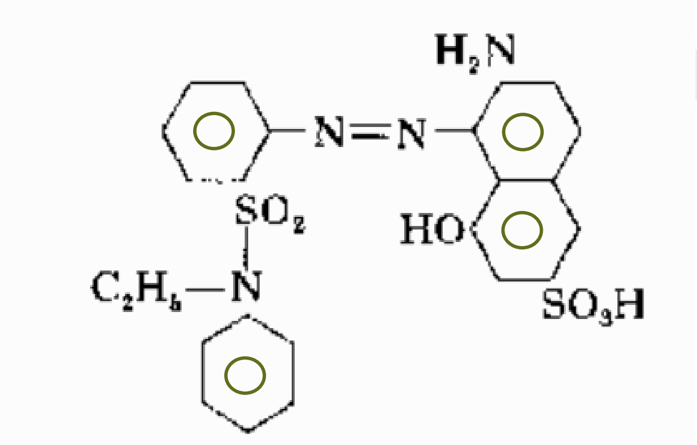


Figure A5. Acid Red 42



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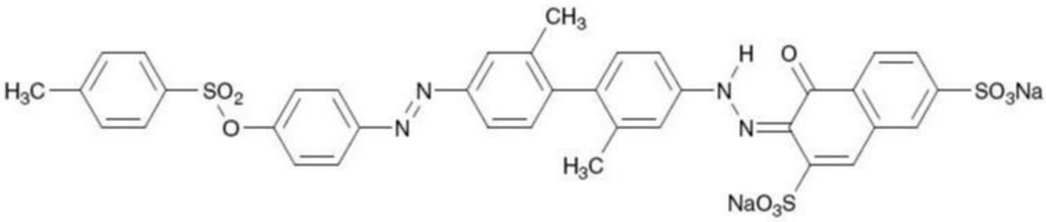

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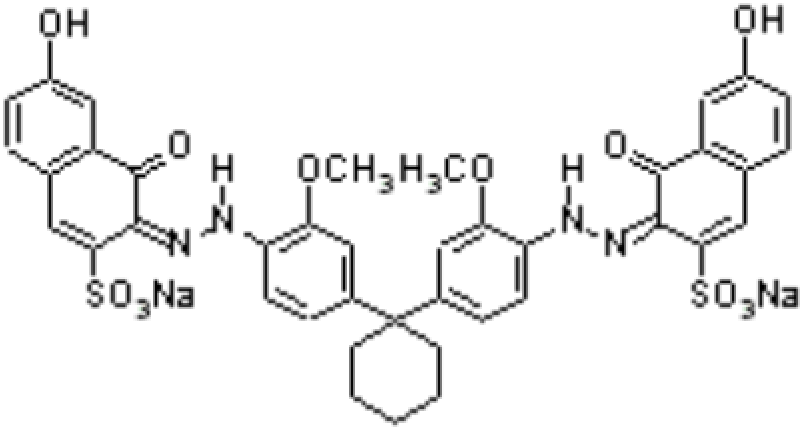
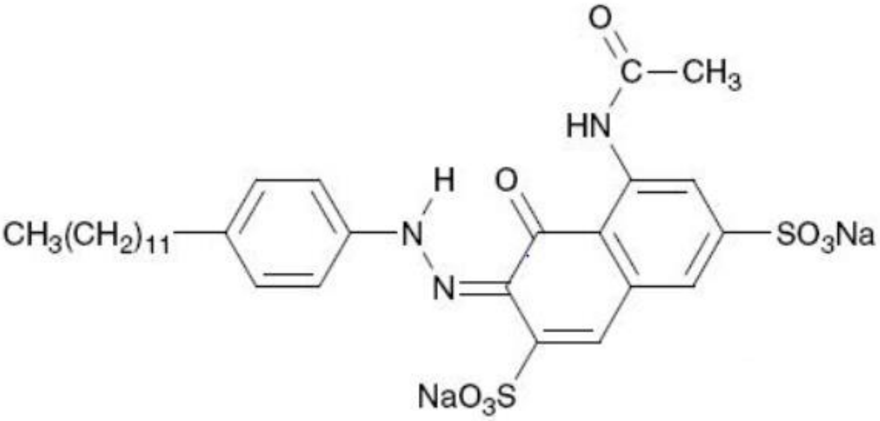


Figure A8. Acid Red 134



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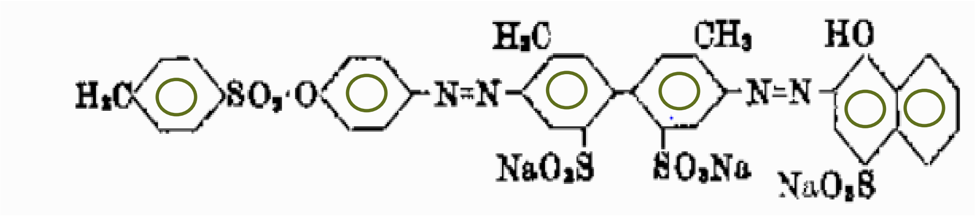

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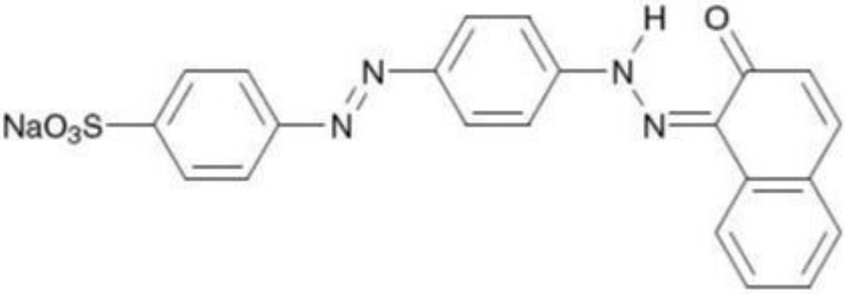

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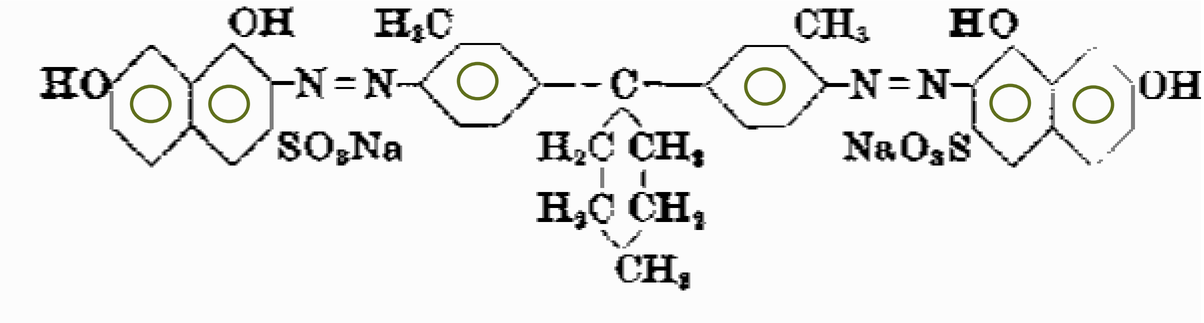

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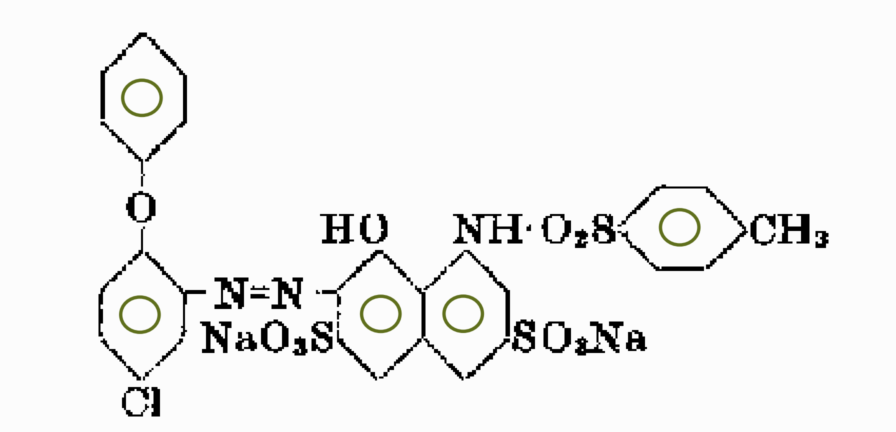

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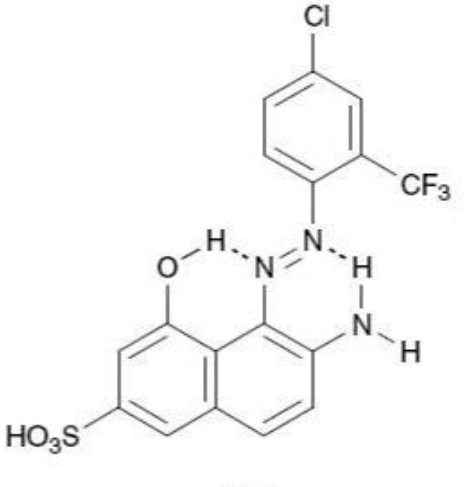

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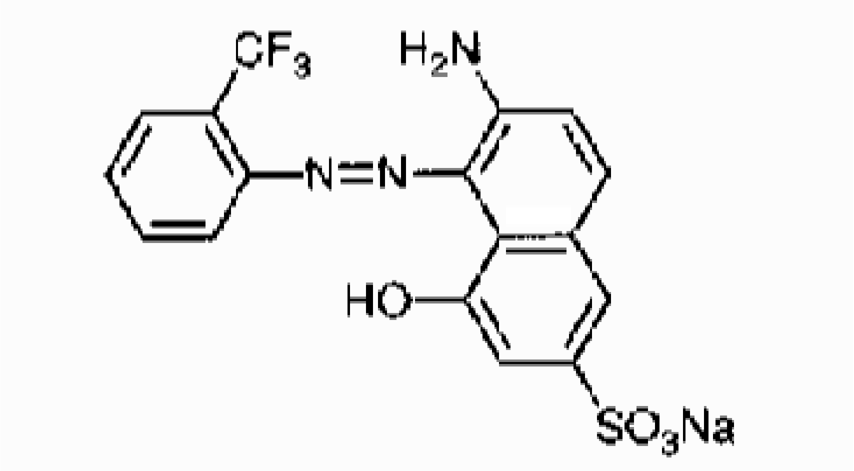

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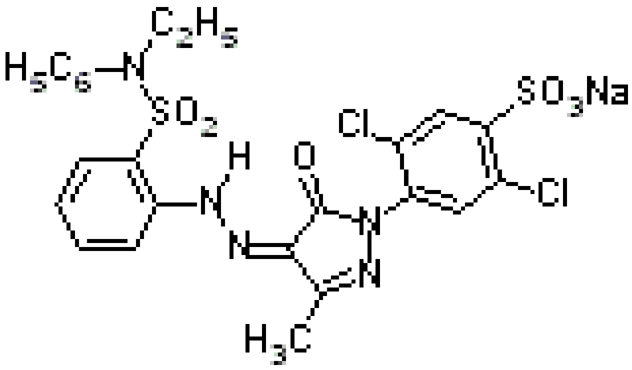


Figure A16. Acid Yellow 61

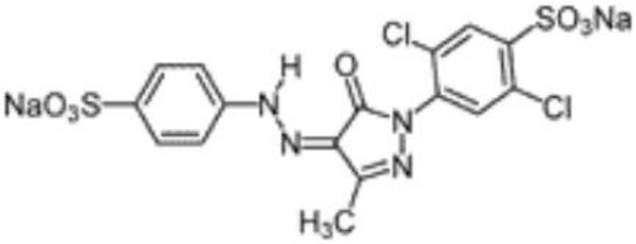
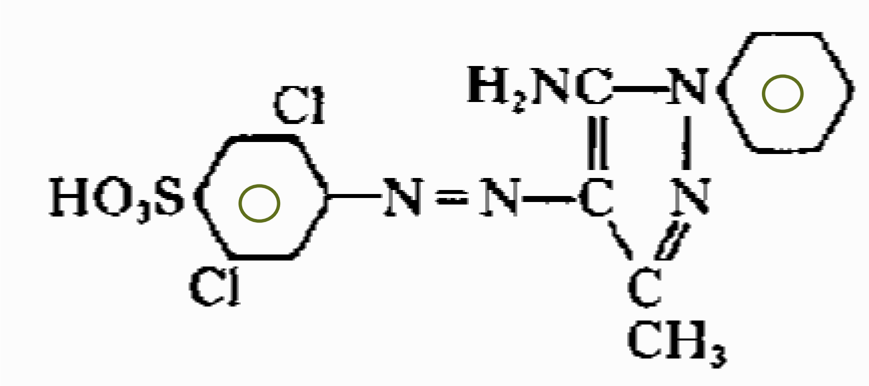


Figure A17. Acid Yellow 17



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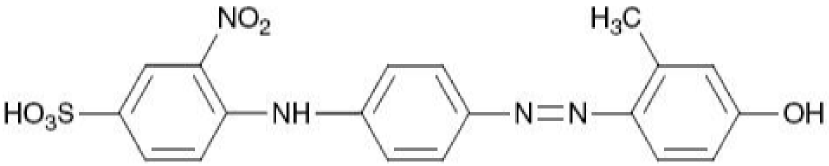


Figure A19. Acid Yellow 199

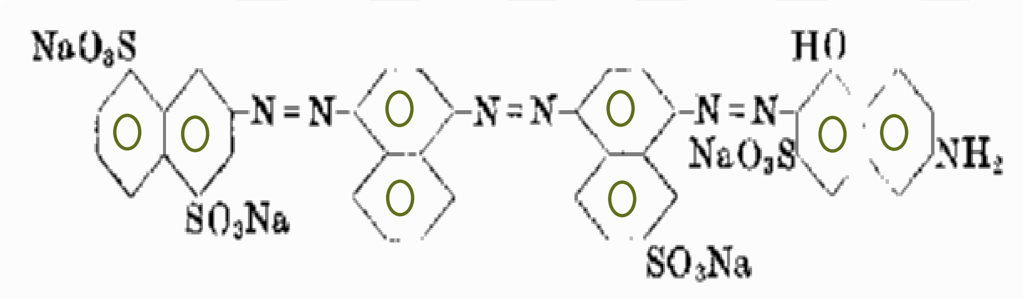


Figure A20. Direct Blue 71

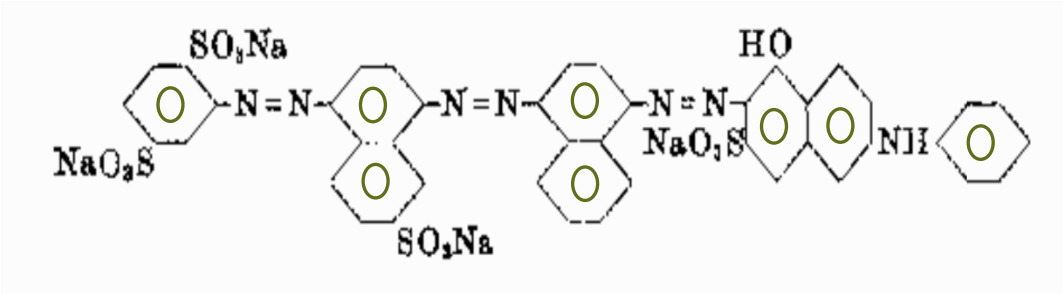


Figure A21. Direct Blue 78

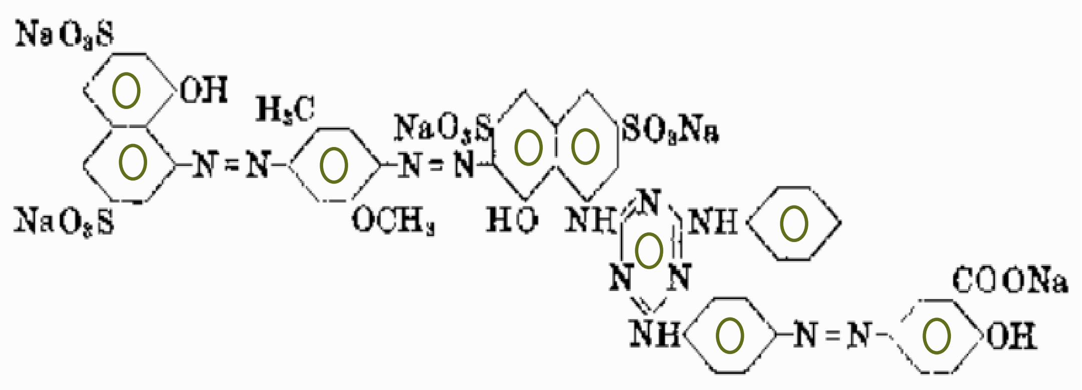


Figure A22. Direct Green 26

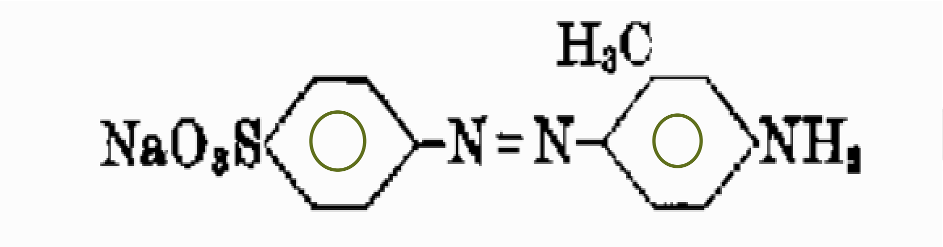


Figure A23. Direct Orange 34

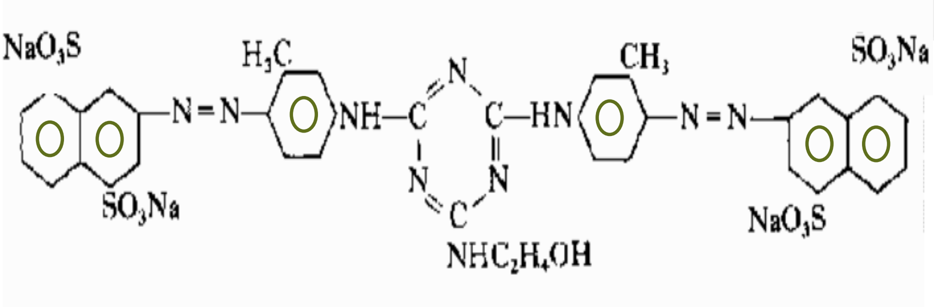
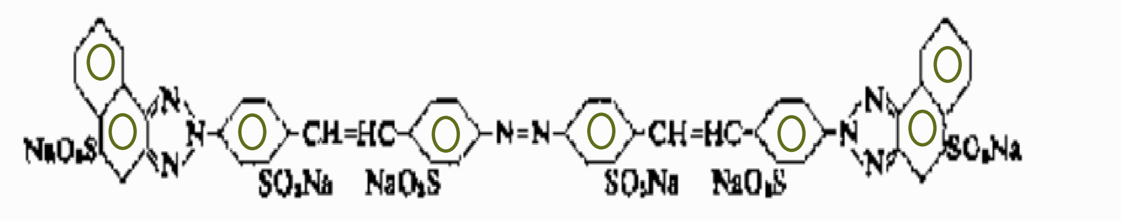


Figure A24. Direct Yellow 86



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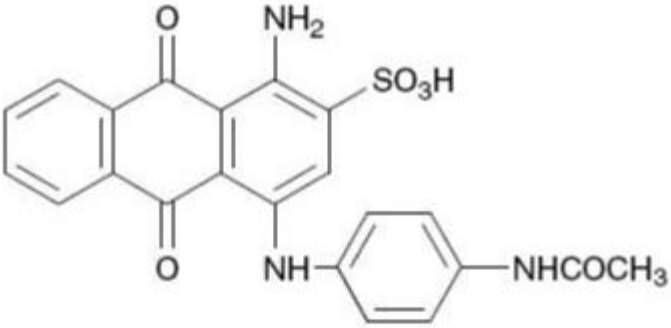

Figure A26. Acid Blue 40

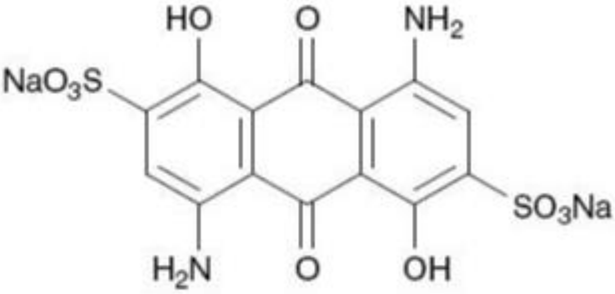

Figure A27. Acid Blue 45

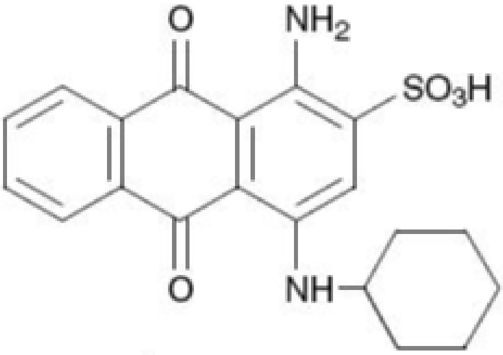

Figure A28. Acid Blue 62

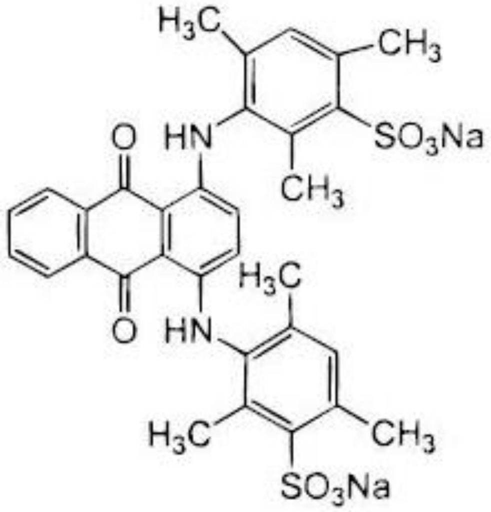

Figure A29. Acid Blue 80

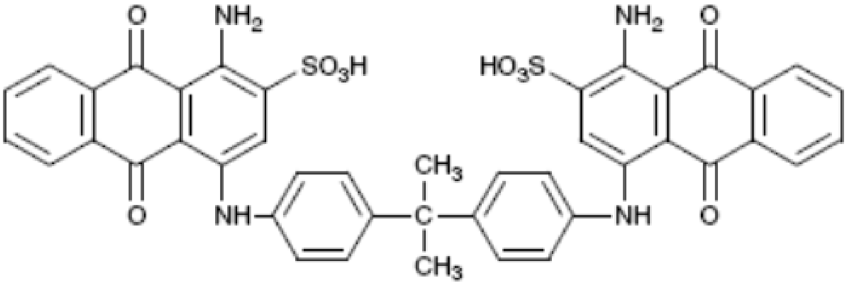

Figure A30. Acid Blue 127

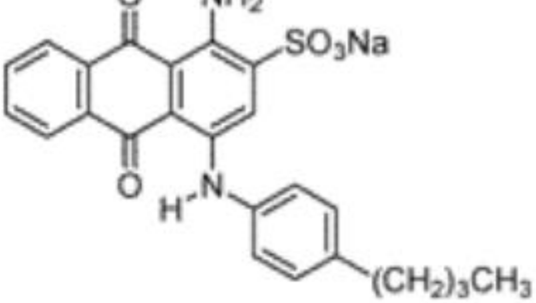

Figure A31. Acid Blue 230

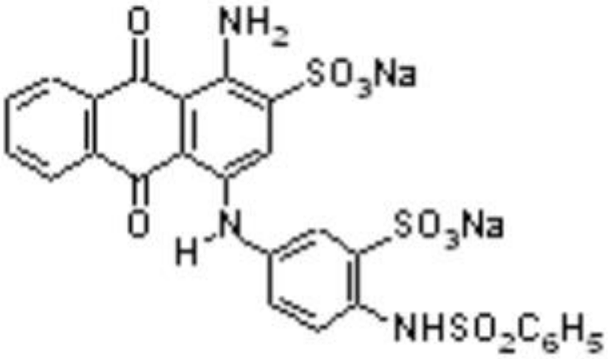

Figure A32. Acid Blue 344

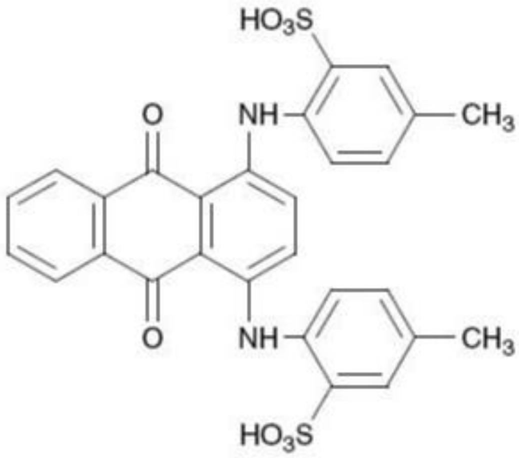

Figure A33. Acid Green 25

APPENDIX B : PREDICTED VS OBSERVED AND WILLIAMS PLOTS OF THE GENERATED QSAR MODELS

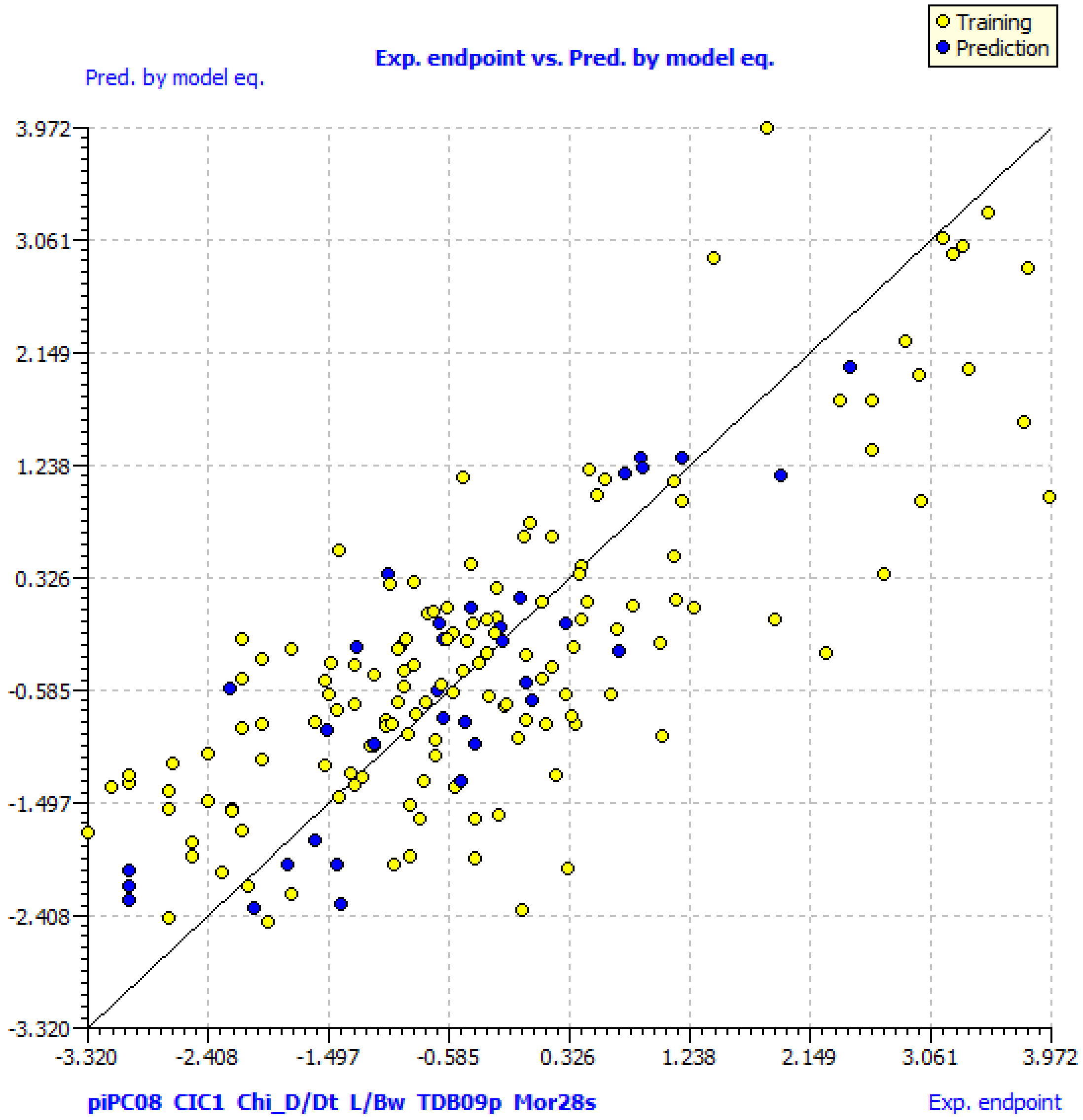

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Figure B1. Predicted vs Observed of Model M1

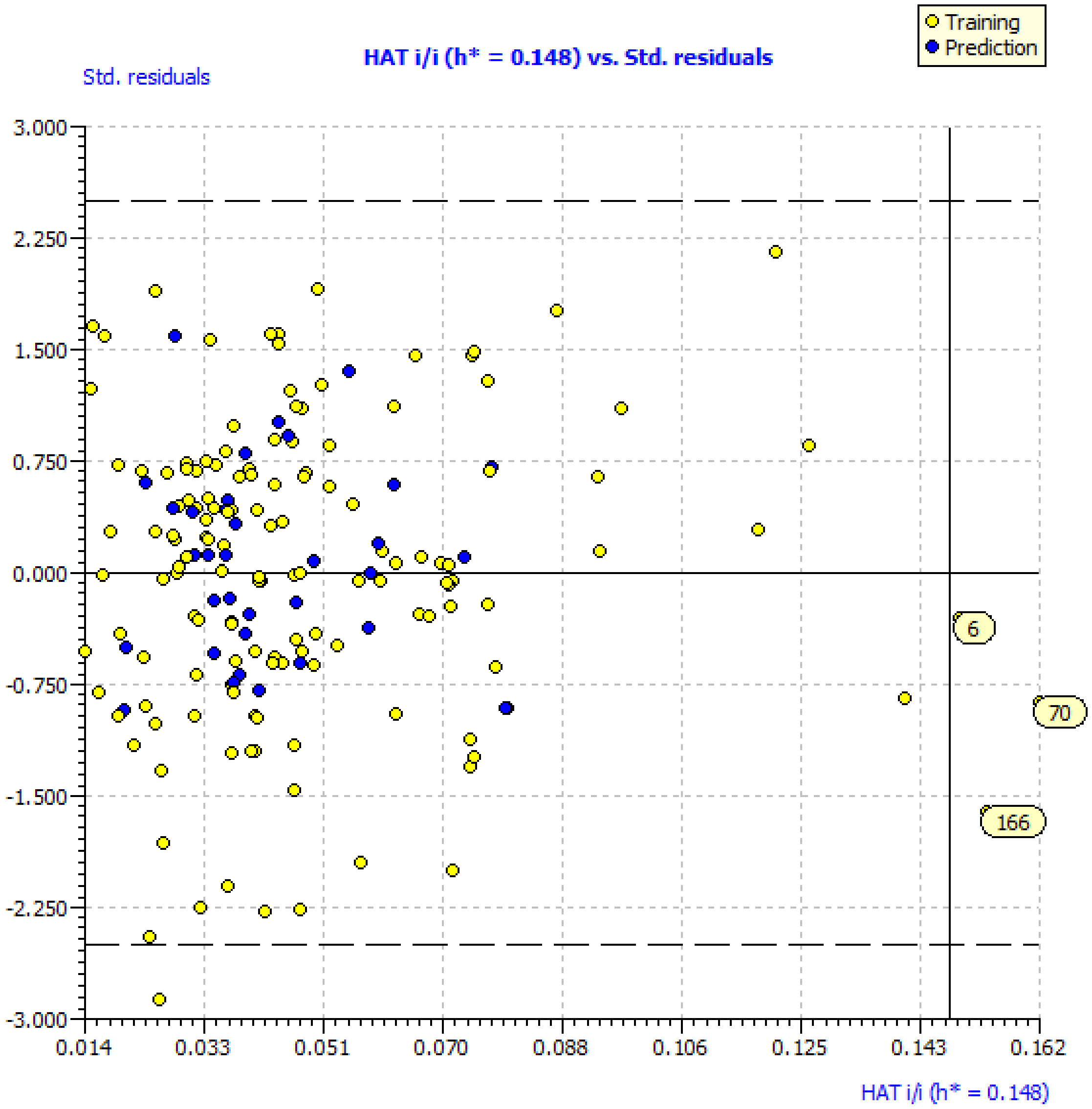


Figure B2. Williams plot of Model M1

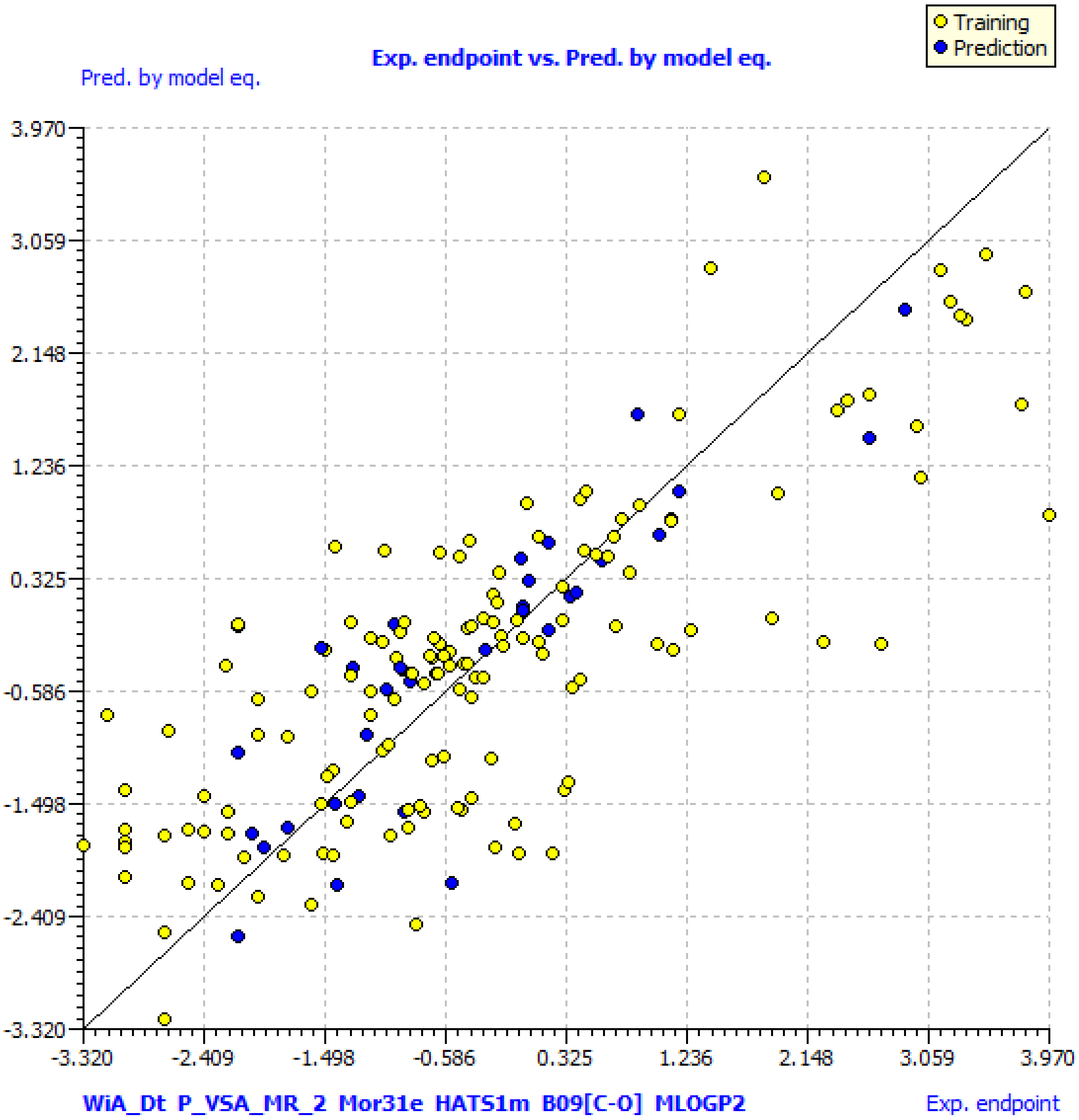


Figure B3. Predicted vs Observed of Model M3

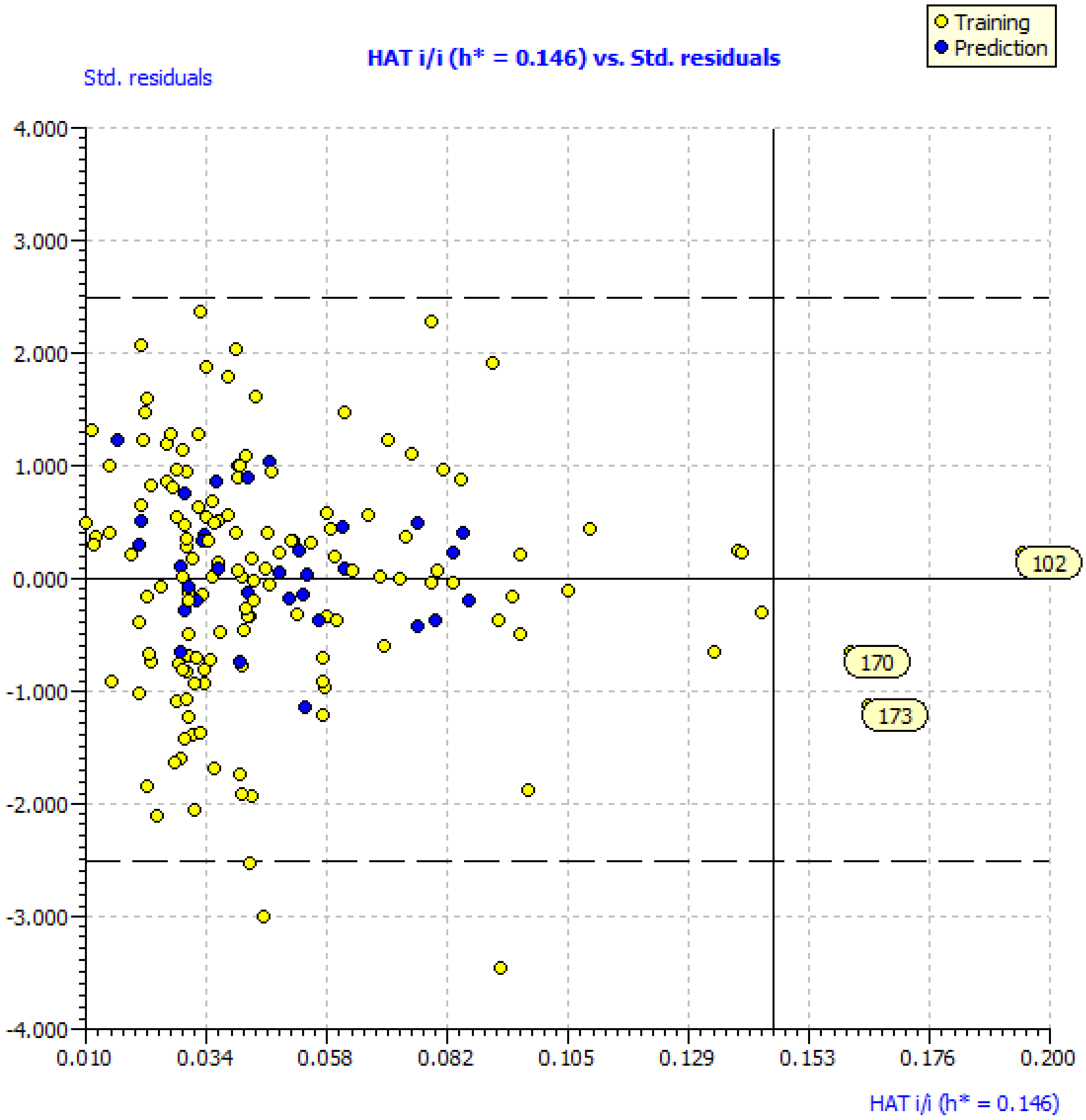


Figure B4. Williams plot of Model M3

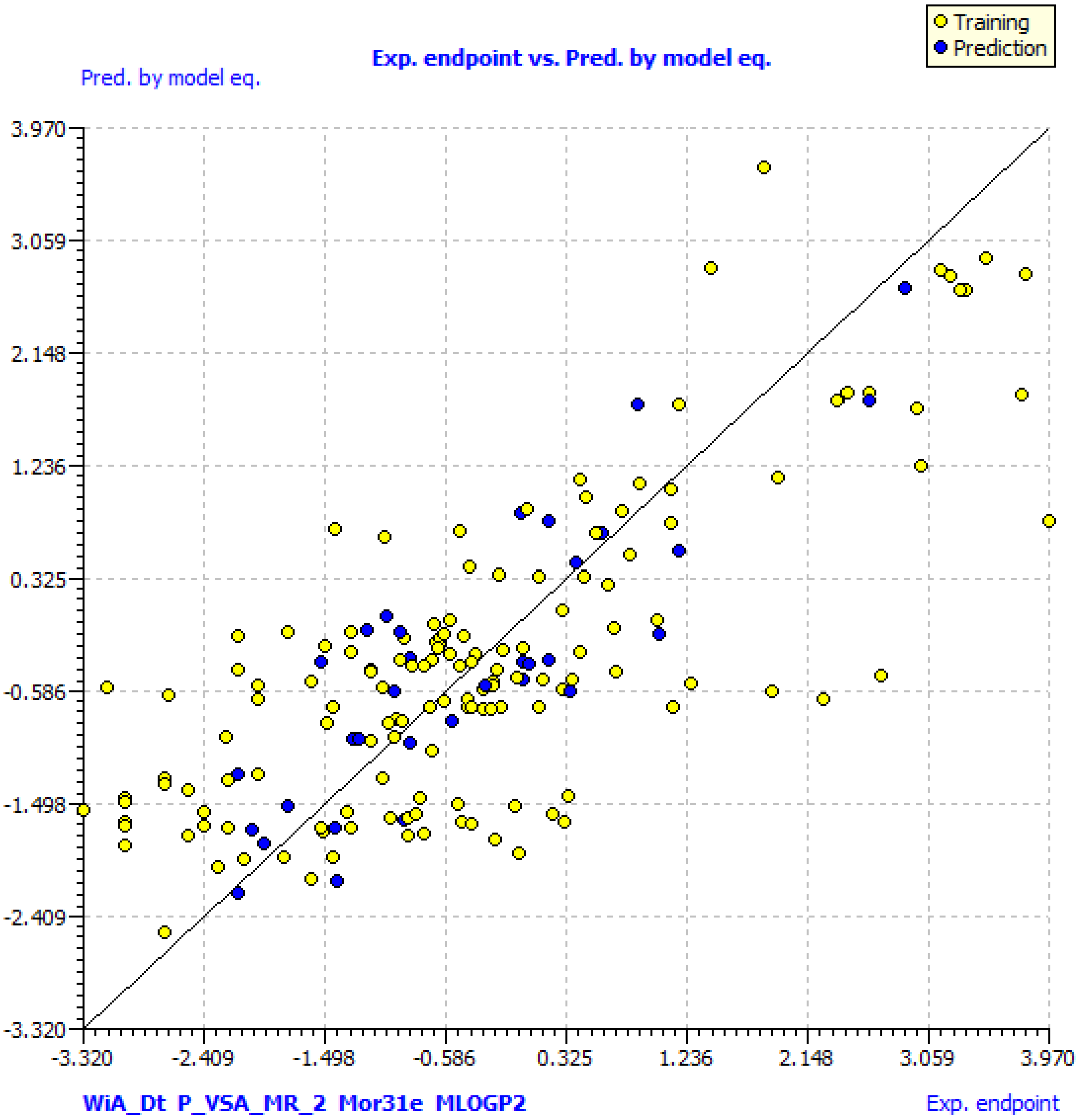


Figure B5. Predicted vs Observed of Model M4

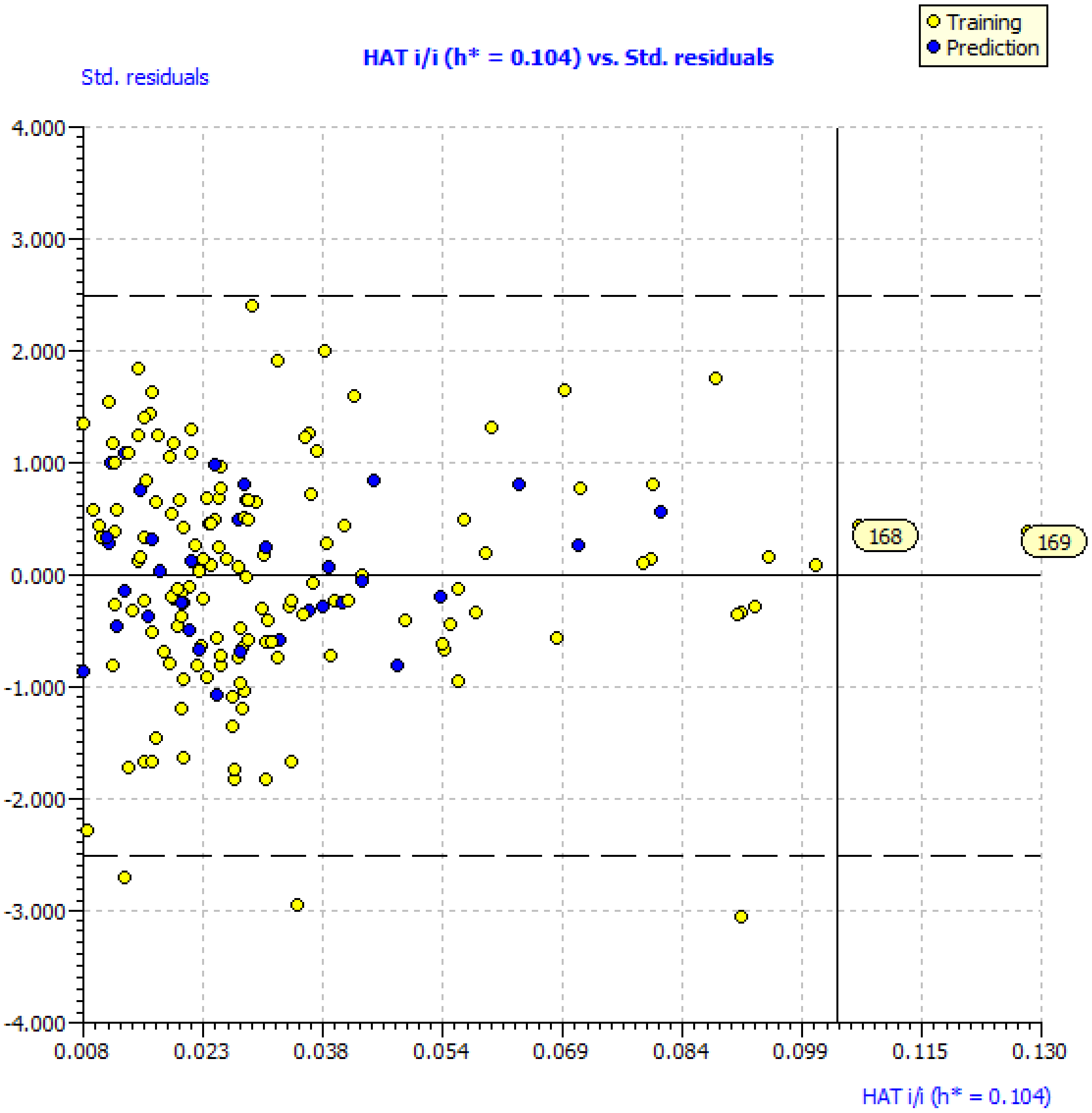


Figure B6. Williams plot of Model M4

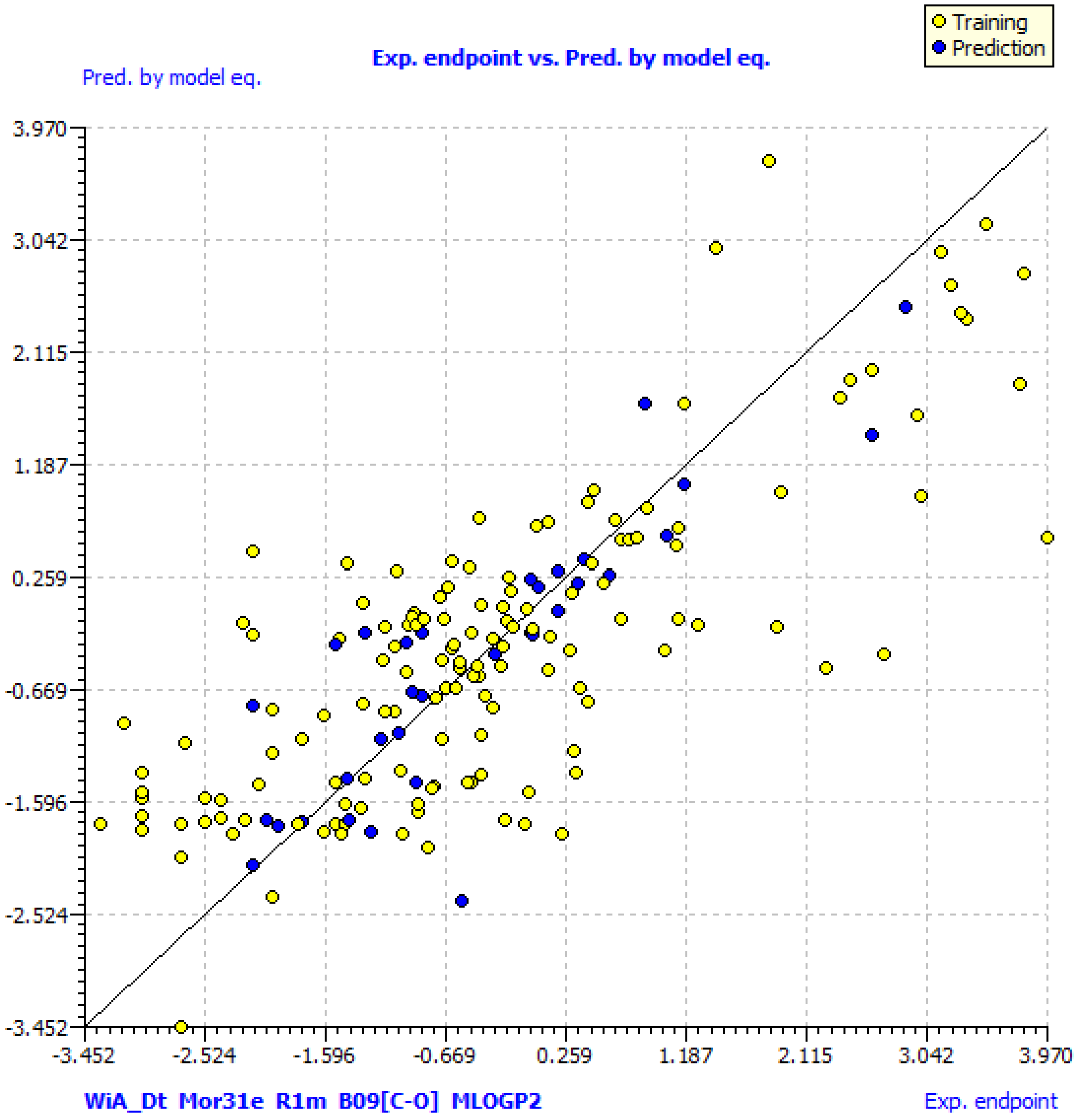


Figure B7. Predicted vs Observed of Model M5

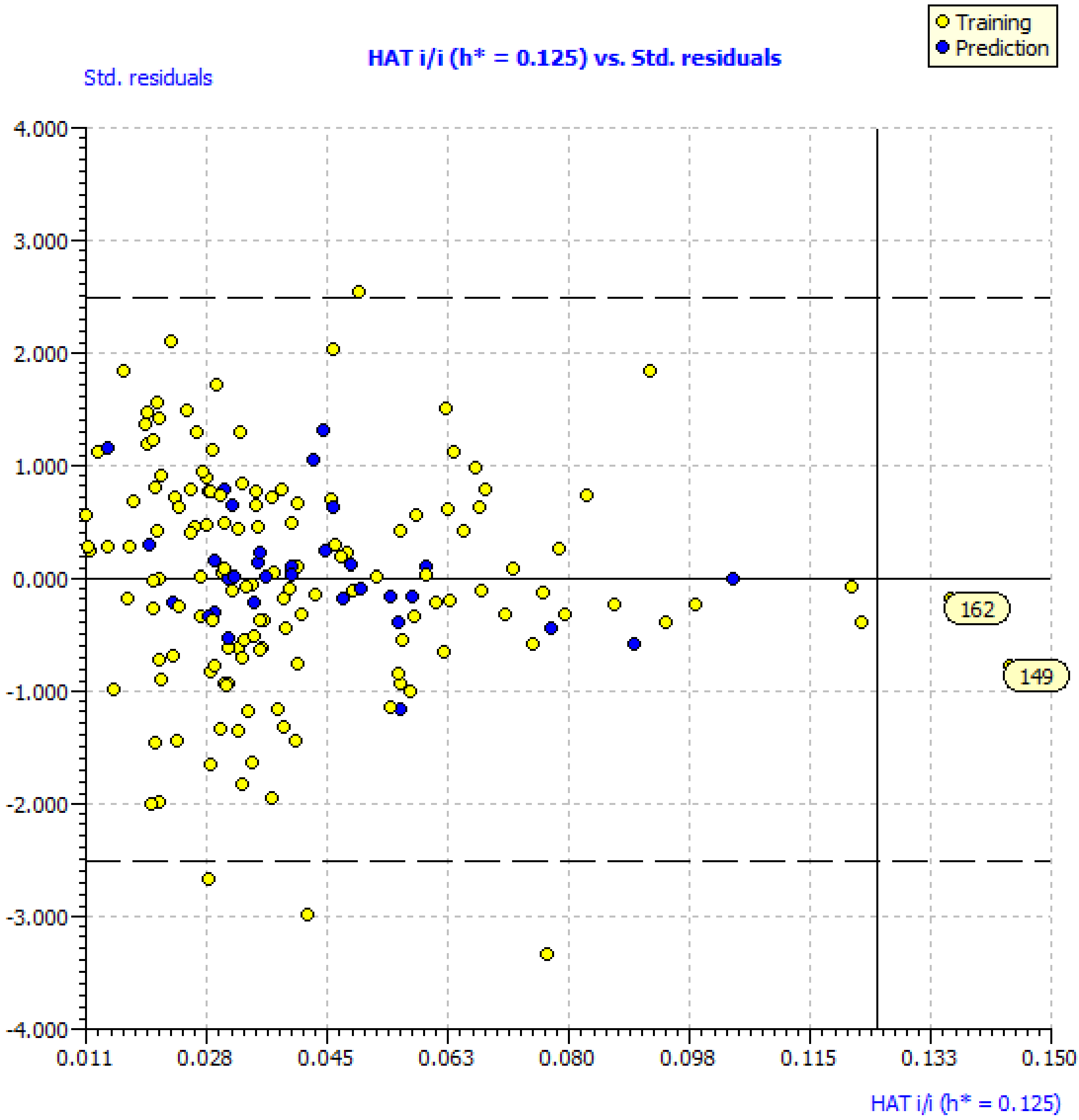


Figure B8. Williams plot of Model M5

APPENDIX C : THE EQUATIONS OF THE FOUR QSAR MODELS

95% Confidence intervals of descriptor coefficients were given in parentheses.

M1 **(Eq C.1)**

Log (mutagenicty) $_{\textbf{(TA98+S9 rev/nmol)}}$ = -4.9488 (±1.0301) -0.2142 (±0.1526) **piPC08** + 0.8727 (±0.6324)**CIC1** + 1.0999 (±0.2646) **Chi_D/Dt** – 0.0779 (±0.0562) **L/Bw** + 0.7257 (±0.2651) **TDB09p** + 0.5019 (±0.2297) **Mor28s**

M3 **(Eq C.2)**

Log (mutagenicty) $_{\textbf{(TA98+S9 rev/nmol)}}$ = -3.1225 (± 0.6239) + 0.2924 (±0.0948) **WiA_Dt** + 0.0102 (± 0.0065) **P_VSA_MR_2** – 1.8369 (±0.9872) **Mor31e** + 0.7406 (±0.5046) **B09[C-O]** + 0.1378 (±0.0657) **MLOGP2** – 4.6932 (±3.4316)**HATS1m**

M4 **(Eq C.3)**

Log (mutagenicty) $_{\textbf{(TA98+S9 rev/nmol)}}$ = -3.4908 (±0.5318) + 0.3286 (±0.0957)**WiA_Dt** – 0.9550(±0.8994) **Mor31e** + 0.1163 (± 0.0669) **MLOGP2** – 0.0078 (± 0.0059) **P_VSA_MR_2**

M5 **(Eq C.4)**

Log (mutagenicty) $_{\textbf{(TA98+S9 rev/nmol)}}$ = -2.6199 (±0.6621) + 0.2668 (±0.0804) **WiA_Dt** – 2.5458 (± 0.9550) **Mor31e** – 1.6902 (±0.8417) **R1m+** + 0.8092 (± 0.5323) **B09[C-O]** - 0.1852 (±0.0559) **MLOGP2**

Table C1. Descriptors appeared in the other four QSAR models and their descriptions.

Abbreviation of Descriptor	**Description**	**Block**
CIC1	Complementary Information Content index (neighborhood symmetry of 1-order)	Information Indices
WiA_Dt	average Wiener-like index from detour matrix	2D matrix-based descriptors
P_VSA_MR_2	P_VSA-like on Molar Refractivity, bin 2	P_VSA-like descriptors
Mor31e	signal 31 / weighted by Sanderson electronegativity	3D-MoRSE descriptors
B09[C-O]	Presence/absence of C - O at topological distance 9	2D Atom Pairs
MLOGP2	squared Moriguchi octanol-water partition coeff. (logP^2)	Molecular properties
HATS1m	leverage-weighted autocorrelation of lag 1 / weighted by mass	GETAWAY descriptors
R1m+	R maximal autocorrelation of lag 1 / weighted by mass	GETAWAY descriptors

APPENDIX D : OUTPUT of XTERNAL PLUS TOOL FOR QSAR MODELS

Table D1. The results of Xternal Validation Plus tool for model M1

User Input File Info.	FileName	*MAE*-test M1 .xlsx
Model biasness test	SystematicErrorResult	Absent
	nPE / nNE	0.7500
	nNE / nPE	1.3333
	|MPE / MNE|	0.9545
	|MNE / MPE|	1.0477
	AAE - |AE|	0.4456
	R^2 (Residuals; serial correlation)	0.0155
	R^2 (Residuals and Yobs values)	0.1826
Classical Metrics (for 100% data)	$R^2_{\text{TesT (}}$100% data)	0.7448
	$R_0{}^2{}_{TesT}$ (100% data)	0.7381
	$R_0'^2{}_{TesT}$ (100% data)	0.7437
	Q^2F_1(100% data)	0.7531
	Q^2F_2(100% data)	0.7380
	Scaled Avg.Rm^2(100% data)	0.6488
	Scaled DeltaRm^2(100% data)	0.1754
	CCC(100% data)	0.8567
Classical Metric (after removing 5% data with high residuals)	R^2Test(95% data)	0.8120
	$R_0{}^2Test$(95% data)	0.8119
	$R_0'^2Test$(95% data)	0.7550
	Q^2F1(95% data)	0.8186
	Q^2F2(95% data)	0.8115
	ScaledAvgRm^2(95% data)	0.7384
	ScaledDeltaRm^2(95% data)	0.1138
	CCC(95% data)	0.8983
Error-based metrics (for 100% data)	RMSEP(100% data)	0.6536
	SD(100% data)	0.3824
	SE(100% data)	0.0646
	MAE(100% data)	0.5340
Error-based metric (after removing 5% data with high residuals)	RMSEP(95% data)	0.5561
	SD(95% data)	0.2963
	SE(95% data)	0.0516
	MAE(95% data)	0.4734
	MAE+3*SD(95% data)	1.3622
BASIC DATA STRUCTURE INFORMATION		
Number of test set compounds, Range and Mean (train and test)	NCompTest	35
	Train range	7.2900
	TrainYMean	-0.2900
	Test range	5.4600
	TestYMean	-0.6063
Distribution of observed response values of Test set around Test mean(in %)	%Y(+/-0.5)TestMean	31.4286
	%Y(+/-1.0)TestMean	62.8571
	%Y(+/-1.5)TestMean	80.0000
	%Y(+/-2.0)TestMean	85.7143
Distribution of observed response values of Test set around Train mean (in %)	%Y(+/-0.5)TrainMean	37.1429
	%Y(+/-1.0)TrainMean	54.2857
	%Y(+/-1.5)TrainMean	77.1429
	%Y(+/-2.0)TrainMean	85.7143
	%NComp>(0.1*TR)	28.5714

Table D1. Continued.

Distribution of prediction	%NComp>(0.15*TR)	5.7143
errors (in %)	%NComp>(0.2*TR)	2.8571
	%NComp>(0.25*TR)	0.0000
Threshold values utilized to judge the model predictions	(0.1*TrainingSetRange)	0.7290
	(0.15*TrainingSetRange)	1.0935
	(0.2*TrainingSetRange)	1.4580
	(0.25*TrainingSetRange)	1.8225
RESULT (*MAE*-based criteria applied on 95% data)	Prediction Quality	GOOD

Table D2. The results of Xternal Validation Plus tool for model M3

User Input File Info.	FileName	*MAE*-test M3 .xlsx
Model biasness test	SystematicErrorResult	Absent
	nPE / nNE	0.7368
	nNE / nPE	1.3571
	|MPE / MNE|	0.9801
	|MNE / MPE|	1.0203
	AAE - |AE|	0.3888
	R^2 (Residuals; serial correlation)	0.0139
	R^2 (Residuals and Yobs values)	0.1263
Classical Metrics (for 100% data)	R^2_{TesT} (100% data)	0.7671
	$R_0^2{}_{TesT}$ (100% data)	0.7610
	$R_0'^2{}_{TesT}$ (100% data)	0.7671
	Q^2F_1(100% data)	0.7599
	Q^2F_2(100% data)	0.7588
	Scaled Avg.Rm^2(100% data)	0.6861
	Scaled DeltaRm^2(100% data)	0.1247
	CCC(100% data)	0.8727
Classical Metric (after removing 5% data with high residuals)	R^2Test(95% data)	0.8437
	R_0^2Test(95% data)	0.8389
	$R_0'^2Test$(95% data)	0.8230
	Q^2F1(95% data)	0.8388
	Q^2F2(95% data)	0.8386
	ScaledAvgRm^2(95% data)	0.7518
	ScaledDeltaRm^2(95% data)	0.1230
	CCC(95% data)	0.9129
Error-based metrics (for 100% data)	RMSEP(100% data)	0.6037
	SD(100% data)	0.3928
	SE(100% data)	0.0684
	MAE(100% data)	0.4635
Error-based metric (after removing 5% data with high residuals)	RMSEP(95% data)	0.5030
	SD(95% data)	0.3092
	SE(95% data)	0.0555
	MAE(95% data)	0.4006
	MAE+3*SD(95% data)	1.3283
BASIC DATA STRUCTURE INFORMATION		
Number of test set compounds, Range and Mean (train and test)	NCompTest	33
	Train range	7.2900
	TrainYMean	-0.3400
	Test range	5.0300
	TestYMean	-0.4248
Distribution of observed response values of Test set around Test mean(in %)	%Y(+/-0.5)TestMean	30.3030
	%Y(+/-1.0)TestMean	63.6364
	%Y(+/-1.5)TestMean	78.7879

Table D2. Continued.

	%Y(+/-2.0)TestMean	93.9394
Distribution of observed response values of Test set around Train mean (in %)	%Y(+/-0.5)TrainMean	18.1818
	%Y(+/-1.0)TrainMean	60.6061
	%Y(+/-1.5)TrainMean	78.7879
	%Y(+/-2.0)TrainMean	93.9394
Distribution of prediction errors (in %)	%NComp>(0.1*TR)	24.2424
	%NComp>(0.15*TR)	9.0909
	%NComp>(0.2*TR)	3.0303
	%NComp>(0.25*TR)	0.0000
Threshold values utilized to judge the model predictions	(0.1*TrainingSetRange)	0.7290
	(0.15*TrainingSetRange)	1.0935
	(0.2*TrainingSetRange)	1.4580
	(0.25*TrainingSetRange)	1.8225
RESULT (*MAE*-based criteria applied on 95% data)	Prediction Quality	GOOD

Table D3. The results of Xternal Validation Plus tool for model M4.

User Input File Info.	FileName	*MAE*-test M4.xlsx
Model biasness test	SystematicErrorResult	Absent
	nPE / nNE	0.9412
	nNE / nPE	1.0625
	|MPE / MNE|	0.8561
	|MNE / MPE|	1.1681
	AAE - |AE|	0.4801
	R^2 (Residuals; serial correlation)	0.0083
	R^2 (Residuals and Yobs values)	0.1796
Classical Metrics (for 100% data)	$R^2_{\mathrm{TesT}\ (}$100% data)	0.7376
	$R_0{}^2{}_{TesT}$ (100% data)	0.7339
	$R_0{}'^2{}_{TesT}$ (100% data)	0.7367
	Q^2F_1(100% data)	0.7340
	Q^2F_2(100% data)	0.7327
	Scaled Avg.Rm^2(100% data)	0.6477
	Scaled DeltaRm^2(100% data)	0.1524
	CCC(100% data)	0.8540
Classical Metric (after removing 5% data with high residuals)	$R^2 Test$(95% data)	0.7779
	$R_0{}^2 Test$(95% data)	0.7736
	$R_0{}'^2 Test$(95% data)	0.7694
	Q_2F1(95% data)	0.7733
	Q_2F2(95% data)	0.7719
	ScaledAvgRm2(95% data)	0.7001
	ScaledDeltaRm2(95% data)	0.1196
	CCC(95% data)	0.8793
Error-based metrics (for 100% data)	RMSEP(100% data)	0.6355
	SD(100% data)	0.3435
	SE(100% data)	0.0598
	MAE(100% data)	0.5380
Error-based metric (after removing 5% data with high residuals)	RMSEP(95% data)	0.5850
	SD(95% data)	0.3129
	SE(95% data)	0.0562
	MAE(95% data)	0.4975
	MAE+3*SD(95% data)	1.4362
BASIC DATA STRUCTURE INFORMATION		
Number of test set compounds, Range and Mean (train and test)	NCompTest	33
	Train range	7.2900
	TrainYMean	-0.3400
	Test range	5.0300
	TestYMean	-0.4248
Distribution of observed response values of Test set around Test mean(in %)	%Y(+/-0.5)TestMean	30.3030
	%Y(+/-1.0)TestMean	63.6364
	%Y(+/-1.5)TestMean	78.7879
	%Y(+/-2.0)TestMean	93.9394
Distribution of observed response values of Test set around Train mean (in %)	%Y(+/-0.5)TrainMean	18.1818
	%Y(+/-1.0)TrainMean	60.6061
	%Y(+/-1.5)TrainMean	78.7879
	%Y(+/-2.0)TrainMean	93.9394
Distribution of prediction errors (in %)	%NComp>(0.1*TR)	33.3333
	%NComp>(0.15*TR)	6.0606
	%NComp>(0.2*TR)	0.0000
	%NComp>(0.25*TR)	0.0000
	(0.1*TrainingSetRange)	0.7290

Table D3. Continued.

Threshold values utilized to judge the model predictions	(0.15*TrainingSetRange)	1.0935
	(0.2*TrainingSetRange)	1.4580
	(0.25*TrainingSetRange)	1.8225
RESULT (*MAE*-based criteria applied on 95% data)	Prediction Quality	GOOD

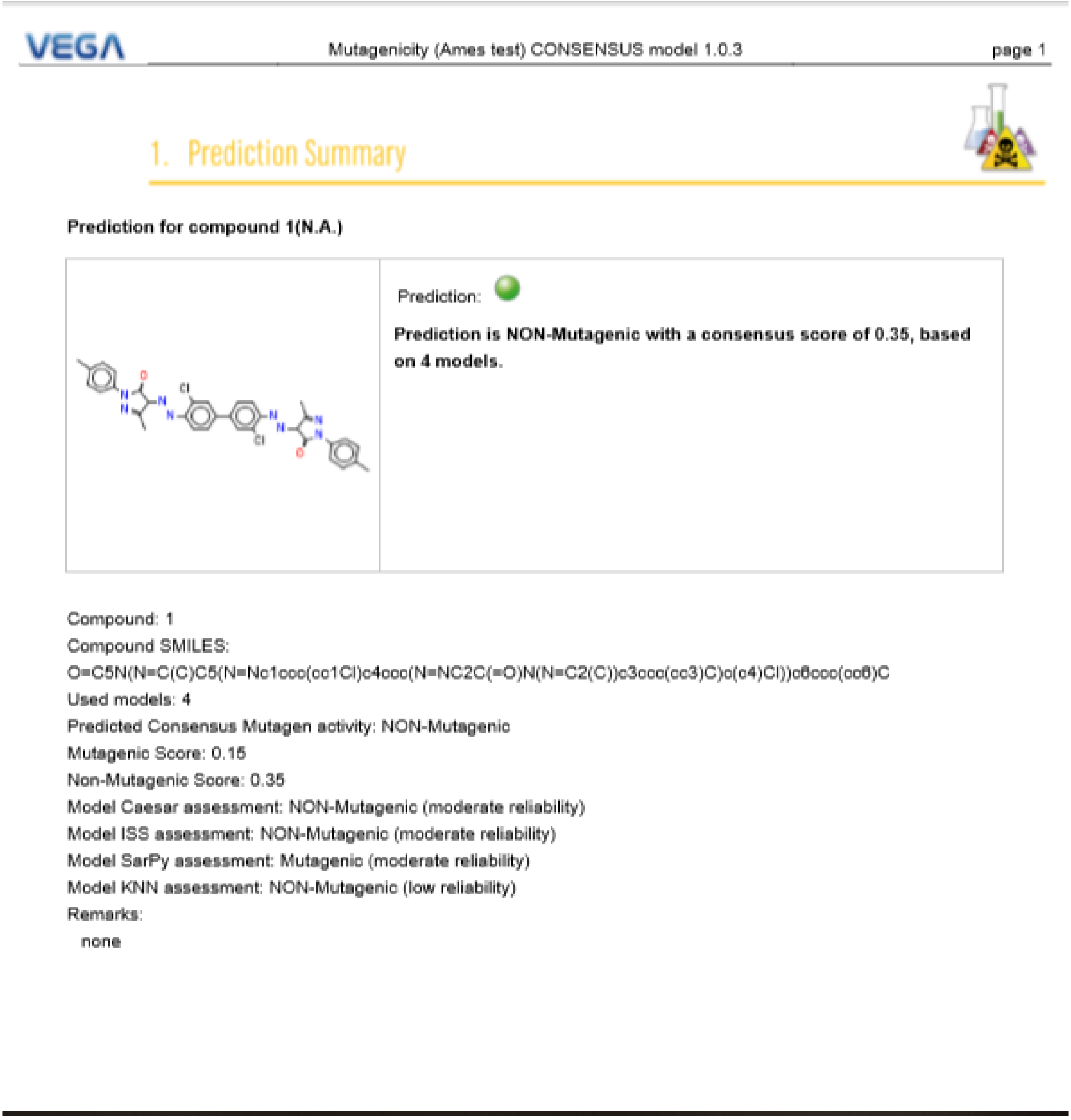
Table D4. The results of Xternal Validation Plus tool for model M5

User Input File Info.	FileName	*MAE*-test M5.xlsx
Model biasness test	SystematicErrorResult	Absent
	nPE / nNE	0.7368
	nNE / nPE	1.3571
	|MPE / MNE|	1.2017
	|MNE / MPE|	0.8321
	AAE - |AE|	0.4022
	R^2 (Residuals; serial correlation)	0.0120
	R^2 (Residuals and Yobs values)	0.1688
Classical Metrics (for 100% data)	R^2*Test*(100% data)	0.7471
	R_0^2*Test*(100% data)	0.7457
	$R_0'^2$*Test*(100% data)	0.7444
	Q_2*F1*(100% data)	0.7451
	Q_2*F2*(100% data)	0.7439
	Scaled Avg.Rm^2(100% data)	0.6610
	Scaled DeltaRm^2(100% data)	0.1236
	CCC(100% data)	0.8609
Classical Metric (after removing 5% data with high residuals)	R^2*Test*(95% data)	0.8434
	R_0^2*Test*(95% data)	0.8426
	$R_0'^2$*Test*(95% data)	0.8280
	Q_2*F1*(95% data)	0.8417
	Q_2*F2*(95% data)	0.8416
	ScaledAvgRm2(95% data)	0.7491
	ScaledDeltaRm2(95% data)	0.1248
	CCC(95% data)	0.9122
Error-based metrics (for 100% data)	RMSEP(100% data)	0.6220
	SD(100% data)	0.4582
	SE(100% data)	0.0798
	MAE(100% data)	0.4282
Error-based metric (after removing 5% data with high residuals)	RMSEP(95% data)	0.4888
	SD(95% data)	0.3454
	SE(95% data)	0.0620
	MAE(95% data)	0.3514
	MAE+3*SD(95% data)	1.3876
BASIC DATA STRUCTURE INFORMATION		
Number of test set compounds, Range and Mean (train and test)	NCompTest	33
	Train range	7.2900
	TrainYMean	-0.3400
	Test range	5.0300
	TestYMean	-0.4248
Distribution of observed response values of Test set around Test mean(in %)	%Y(+/-0.5)TestMean	30.3030
	%Y(+/-1.0)TestMean	63.6364
	%Y(+/-1.5)TestMean	78.7879
	%Y(+/-2.0)TestMean	93.9394

Table D4. Continued.

Distribution of observed response values of Test set around Train mean (in %)	%Y(+/-0.5)TrainMean	18.1818
	%Y(+/-1.0)TrainMean	60.6061
	%Y(+/-1.5)TrainMean	78.7879
	%Y(+/-2.0)TrainMean	93.9394
Distribution of prediction errors (in %)	%NComp>(0.1*TR)	18.1818
	%NComp>(0.15*TR)	15.1515
	%NComp>(0.2*TR)	3.0303
	%NComp>(0.25*TR)	3.0303
Threshold values utilized to judge the model predictions	(0.1*TrainingSetRange)	0.7290
	(0.15*TrainingSetRange)	1.0935
	(0.2*TrainingSetRange)	1.4580
	(0.25*TrainingSetRange)	1.8225
RESULT (*MAE*-based criteria applied on 95% data)	Prediction Quality	GOOD

APPENDIX E: THE OUTPUT OF VEGA SOFTWARE FOR DIRECT ORANGE 34



Mutagenicity (Ames test) CONSENSUS model 1.0.3 page 1

1. Prediction Summary

Prediction for compound 1(N.A.)

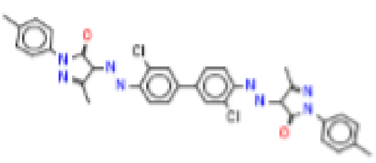
Prediction: ●

Prediction is NON-Mutagenic with a consensus score of 0.35, based on 4 models.

Compound: 1
Compound SMILES:
O=C5N(N=C(C)C5(N=Nc1ccc(cc1Cl)c4ccc(N=NC2C(=O)N(N=C2(C))c3ccc(cc3)C)c(c4)Cl))c6ccc(cc6)C
Used models: 4
Predicted Consensus Mutagen activity: NON-Mutagenic
Mutagenic Score: 0.15
Non-Mutagenic Score: 0.35
Model Caesar assessment: NON-Mutagenic (moderate reliability)
Model ISS assessment: NON-Mutagenic (moderate reliability)
Model SarPy assessment: Mutagenic (moderate reliability)
Model KNN assessment: NON-Mutagenic (low reliability)
Remarks:
none

1. Prediction Summary

Prediction for compound 1(N.A.)

 	Prediction: ● Reliability: ★★☆ **Prediction is NON-Mutagenic, but the result shows some critical aspects, which require to be checked:** **- only moderately similar compounds with known experimental value in the training set have been found** **- similar molecules found in the training set have experimental values that disagree with the predicted value**

Compound: 1

Compound SMILES:

O=C5N(N=C(C)C5(N=Nc1ccc(cc1Cl)c4ccc(N=NC2C(=O)N(N=C2(C))c3ccc(cc3)C)c(c4)Cl))c6ccc(cc6)C

Experimental value: -

Predicted Mutagen activity: NON-Mutagenic

Structural alerts: -

Reliability: the predicted compound could be out of the Applicability Domain of the model

Remarks:

none