

THEORETICAL STUDY OF DECOMPOSITION OF
DIAZENIUMDIOLATES

by

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Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project

DEDICATION

I dedicate this thesis to my wonderful family. Particularly to my understanding and patient wife, Borelis, and to our precious kids Carmelo and Laura.

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ABSTRACT

Nitric oxide (NO) is of significant interest in biological research. NO is generated via the oxidation of L-arginine by NO synthase (NOS) and plays a key role in many bioregulatory systems, including smooth muscle relaxation, platelet inhibition, neurotransmission, and immune stimulation, primarily through the formation of cGMP. N-Diazeniumdiolates (NONOates) are an important class of NO donor, with potential biological or therapeutic value. The versatility of NONOates makes them ideal for studying NO in many different scenarios. Primary amine diazeniumdiolates such as produced from isopropyl amine (IPA/NO) can release HNO under physiological conditions.

Quantitative Structure Activity/Property Relationships (QSAR/QSPR) relate the structure of a compound, to a property/activity of interest (biological activity). QSAR/QSPR studies are of great importance in drug design. Models that predict the half-lives of NONOates were built to determine the influence of each variable on decomposition rate. External validation of this model will be made using a new set of NONOates to test the model.

CHAPTER 1

INTRODUCTION

1.1 Nitric Oxide

Nitric oxide (NO) has been studied for decades as a spectroscopic marker, and for its thermodynamic and quantum mechanical properties. With the discovery in the 1980s of physiological and pathophysiological effects, NO has become a molecule of interest in biological research. In 1992 *Sciencenamed* NO molecule of the year, as testimony to its importance in biological systems [9]. Furchgott, Ignarro and Murad were awarded the Nobel Prize in Physiology and Medicine in 1998 for their contributions and elucidation that NO is the molecule that endothelial cells secrete in response to stimuli such as acetylcholine.

Cellular NO is primarily generated via the oxidation of L-arginine (Figure 1-1 by NO synthase (NOS). Endogenous formation of NO plays a key role in many bioregulatory systems, including smooth muscle relaxation, platelet inhibition, neurotransmission, and immune stimulation, primarily through the formation of cGMP [50]. A deficiency in NO is linked to many diseases such as hypertension, atherosclerosis and cancer [39]. NO has a limited biological half-life and solubility in water (2 mM) making it difficult to introduce NO in a controlled way [1]. The potential Pharmacological action of NO coupled with the instability and inconvenient handling of aqueous solutions of NO brings an interest in synthesis of compounds capable of generating NO *in situ*. NO donors with potential biological or therapeutic value should have certain characteristics, such as controlled rate of decomposition, minimal side effects of by-products, and targeting of NO to specific tissues to induce physiological effects [44].

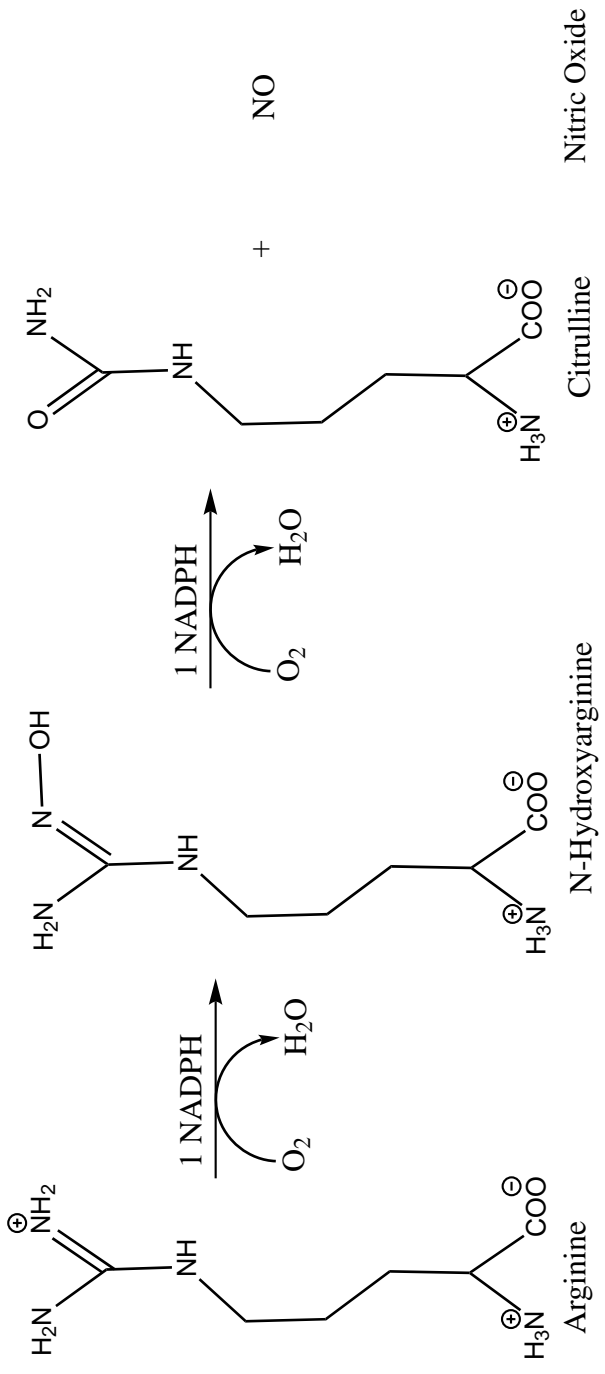


Figure 1.1: Endogenous synthesis of nitric oxide [7]

1.2 NO Donors

NO donors are a highly diverse group of compounds that decompose spontaneously or are metabolized in cells and tissues to generate NO. Only a few such compounds are used clinically today, and all were introduced as drugs prior to discovery of NO as a biological signaling molecule. There are three typical mechanisms for release of NO: donating NO spontaneously by decomposition, chemical reaction with metals, thiols or alkali, or metabolic activation of NOS. Some NO donors release NO by more than one route, and a new approach in the design of NO donors is to attach NO to an existing drug, producing synergistic effects. The most widely used NO donors are described below.

1.2.1 Organic Nitrates

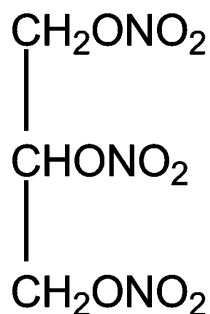


Figure 1.2: Glyceryl trinitrate (GTN)

Organic nitrates are esters of nitric acid and are synthesized by reaction with alcohols. This group of compounds includes the oldest pharmacological donor of NO and the most widely used cardiovascular therapeutic agents [23]. Glyceryl trinitrate (GTN) (Figure 1.2) is the prototypical organic donor of NO and is used for treatment of several forms of angina, congestive heart failure and myocardial infarction. Organic nitrates have potent short-term vascular and clinical effects, but a major limitation for chronic use is development of tolerance [51]. The mechanism of formation of NO from organic nitrates involves enzymatic activation, entry

into vascular muscle cells and conversion to NO (Figure 1.3)[23]. The hypothesis developed by Ignarro proposed that nitrate is transformed to nitrite, which under acidic conditions dimerize to provide the nitrosating species N_2O_3 . S-Nitrosation of low molecular weight thiols can lead nitrosation of sGC by transnitrosation, which presumably activates the enzyme.

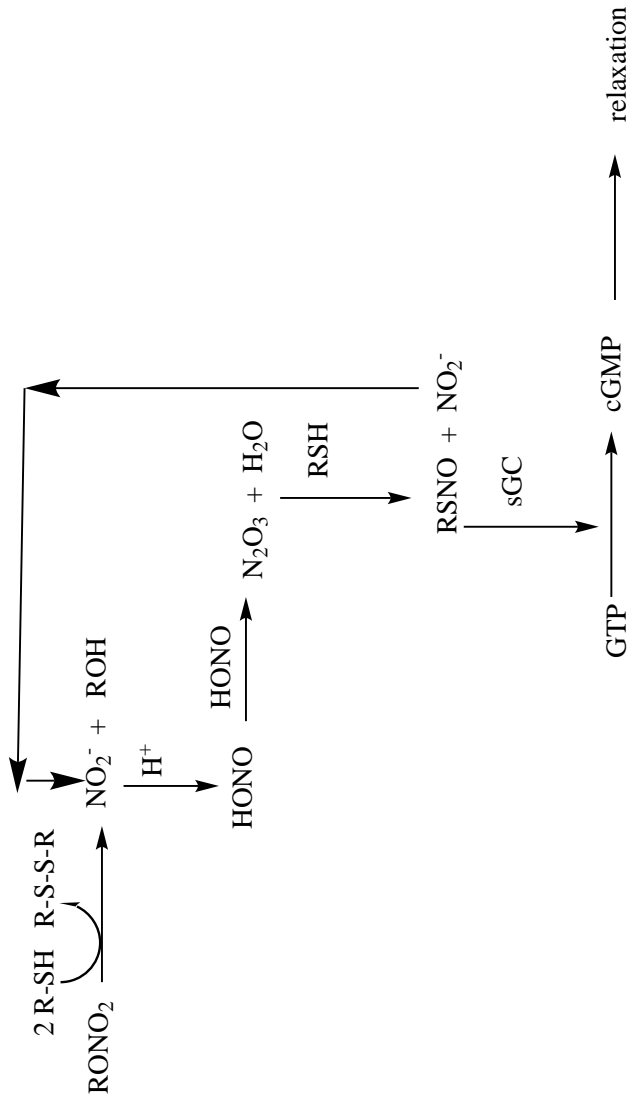


Figure 1.3: Mechanism of vasorelaxation by organic nitrates [24]

1.2.2 Nitrosothiols

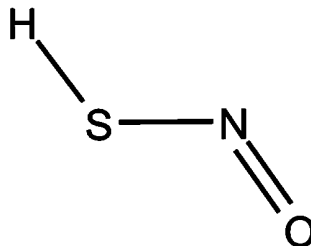
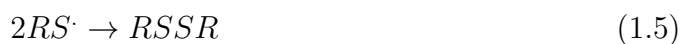
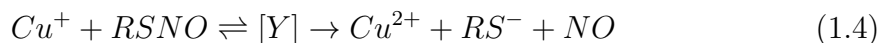
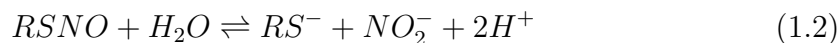


Figure 1.4: S-Nitrosothiols

S-Nitrosothiols contain an SNO moiety (Figure 1.4). Under physiological conditions, they are able to donate NO, but also nitrosonium and nitroxyl [4]. Visible light enhances the release of NO, and can be used for site-directed release. S-Nitrosothiols display potent vasodilating and platelet antiaggregatory activities [24, 16]. All known compounds of this class decompose in what appears to be a spontaneous manner as follows:

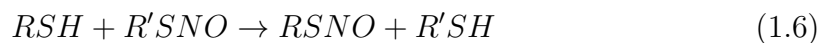


Copper increases the biological activity and rate of release of NO from S-nitrosothiols. Butler showed that Cu(I) is generated by reduction of Cu(II) by free thiolate or generated by hydrolysis of RSNO. Cu(I) can then catalyze RSNO decomposition through a complex intermediate Y [47].



There is not much free copper in organism, and it is concentrated in certain regions, which may specifically catalyze NO release [2]. RSNOs react with other

thiols, via transnitrosation, which is direct transfer of NO^+ from one thiol to another [21]. This reaction between thiols is suggested to transport NO under physiological conditions.



In summary, nitrosothiols are water soluble, produce non toxic by-products, and release NO by thermal decomposition or at an accelerated rate in the presence the copper. Nitrosothiols can be used as potent antiplatelet agents and vasodilators, but the nitrogen oxygen product can be NO, NO^+ , or NO^- , which complicates analysis, as does the dependence of decomposition rate on medium composition.

1.2.3 Metal Nitrosyl Complexes

The physiological effects of NO are mediated primarily through the enzyme soluble guanylyl cyclase (sGC), which contains a five-coordinated ferrous heme with a histidine as the axial ligand [46]. The affinity of NO for sGC is greater than that of CO [29]. During activation of sGC, NO binds to the sixth coordination position of the heme iron and leads to cleavage of the histidine-iron bond, yielding a five coordinated nitrosyl heme complex (Figure 1.5). The importance of nitrosylation in biology is echoed in use of metal nitrosyl pharmacologically.

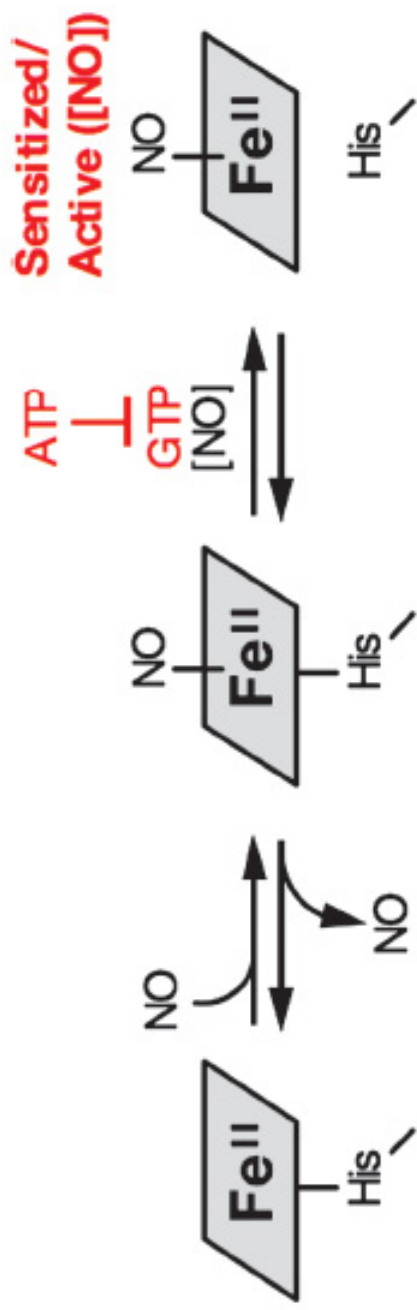


Figure 1.5: Nitrosylation of sGC [8]

Sodium nitroprusside (SNP) (Figure 1.6) was first synthesized in 1850 by Playfair [36]. Its hypotensive properties were demonstrated by Johnson in 1929 [26]. SNP is one of the most valuable vasodilators used in clinical practice for over 70 years to reduce blood pressure. The vasodilatory effect of SNP is through the formation of NO [31]. Its activity in vivo is short-lived, ending a few seconds after infusion is terminated, which makes it an ideal compound for controlled hypotension during hypertensive crises or during surgery [20]. SNP releases NO both spontaneously and photochemically, and its decomposition is accelerated by oxygen.

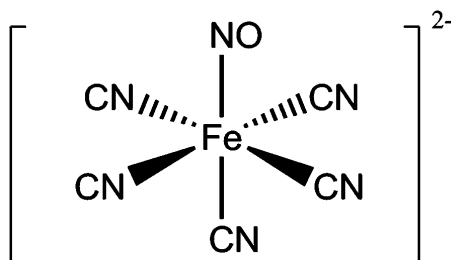


Figure 1.6: Sodium nitroprusside (SNP)

Absorption of heat and light induces electron transfer from the Fe^{2+} center to the NO^+ ligand, thus weakening the Fe-NO bond. In the presence of biological reductants such as vitamin C, release of NO is accelerated [30]. Decomposition of SNP can be accompanied by release of cyanide, which can be toxic [5]. In summary, SNP can pass through cell membranes and is an excellent vasodilator that acts quickly and is water-soluble. It is used clinically for treatment of advanced heart failure and hypertension, and also inhibits platelet aggregation [6]. However the potential for cyanide release limits its use.

Ruthenium nitrosyl complexes can be used as photosensitive donors of NO. Dissociation of the Ru-NO bond is regulated by a trans effect. Clarke and Gaul showed that modifying the trans ligand facilitates NO release from trans- $[NO(L)(NH_3)_4Ru]Cl_3$ (Figure 1.7) upon reduction. Bound NO exhibits strong ni-

trosonium (NO^+) character, but releases NO upon irradiation [19].

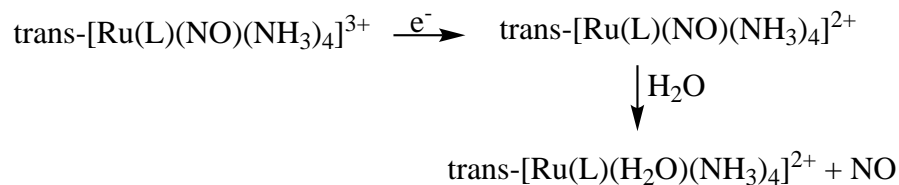


Figure 1.7: NO release upon irradiation

These species are able to effect a high hypotensive response by release NO [11]. The dual role of these ruthenium complexes as NO donor or scavenger may have promising applications in biological systems. However this donor type is still being developed.

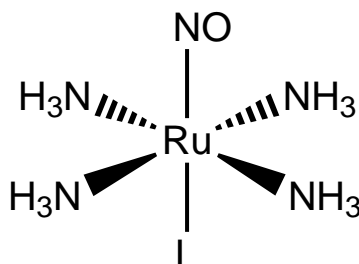


Figure 1.8: $Trans[\text{Ru}(\text{NH}_3)_4\text{L}(\text{NO})]^{3+}$

1.2.4 Diazeniumdiolates (NONOates)

N-Diazeniumdiolates are an interesting class of compound that can deliver NO specifically to a target site. Diazeniumdiolates are adducts of NO and nucleophiles and are capable of releasing two equivalents of NO spontaneously with first-order kinetics. The half-life of NONOates varies from a few seconds to many hours, depending on the amine structure and on the pH and temperature of the medium [22].

Drago reported the first synthesis of an N-bound diazeniumdiolate; Drago's complex is an NO adduct of diethylamine (Figure 1.8)[13].

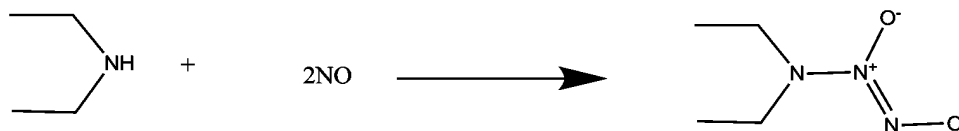


Figure 1.9: Synthesis of Drago Complex

Diazeniumdiolates are commonly referred to as NONOates. Synthesis of NONOates generally involves reaction of amines (primary, secondary and polyamines) in nonpolar solvents with high pressures of NO. Diversity of NONOates can be obtained by varying the organic moiety. The mechanism of NO release from NONOates, via thermal or photochemical pathways, has been investigated theoretically using density functional theory [15]. The mechanism of decomposition of NONOates (Figure 1.9) suggests protonation of the terminal oxygen followed by tautomerization to the amino nitrogen to leading to cleavage to RNH_2 and two equivalents of NO.

In summary, NONOates are water soluble and stable in the solid state and can provide continuous release of NO for periods beyond 24 h under physiological conditions.

The versatility of NONOates makes them ideal for studying NO in many different scenarios. For instance PROLI/NO ($t_{\frac{1}{2}} = 2s$) [37, 45] reverses spasm in the artery of monkeys or in disorder caused by the rupture of an intracranial aneurysm present in the brain [37, 38]. DETA/NO ($t_{\frac{1}{2}} = 72000s$) [22, 45] inhibited cerebral vasospasm in dogs when injected into the cerebrospinal [48] fluid during surgery where it is difficult to remove all of the excess blood from the cranium before closing the surgical opening. Residues of blood can predispose the affected

artery to undergo uncontrolled, spastic contractions. The resulting perturbations in cerebral blood flow can produce serious neurological deficit and death if not stopped immediately. With administration of NONOates into the carotid artery of an animal subject, a complete reversal of the spastic condition was immediately observed [37].

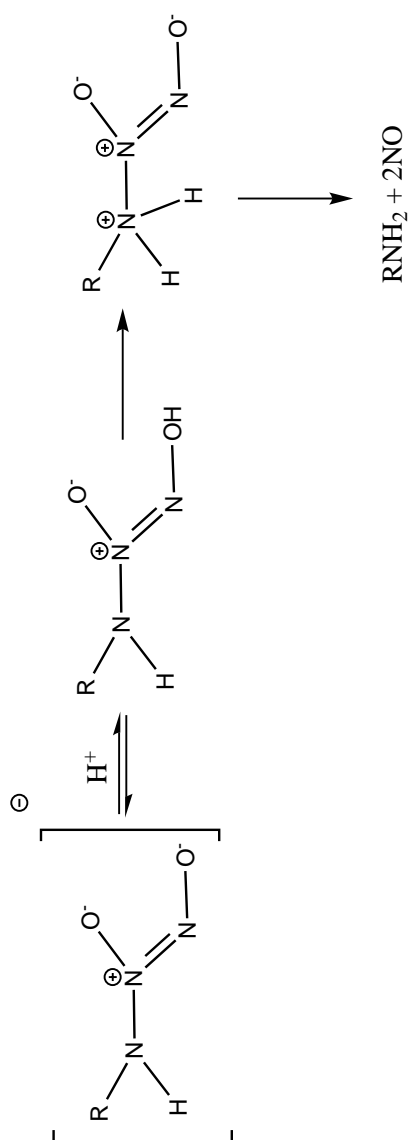


Figure 1.10: NONOate Decomposition Mechanism

Espadas-Torre determined that NONOates minimize thrombogenesis when extracorporeal devices such as biosensors or renal dialysis system are in contact with blood, which can cause unwanted clot formation [17]. To minimize this thrombogenic foreign-body response, antiplatelet agents such as heparin, coumadin, and aspirin are often used. However, systemic administration of such agents concomitantly increases the risk of uncontrolled bleeding in the body. NO donors offer an attractive way to eliminate this because NO is a potent inhibitor of platelet adhesion and aggregation.

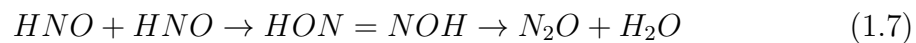
The chemically versatility of NONOates can be incorporated into polymers that generate NO with a very short half-life. Many NONOates are being examined as candidate prodrugs for chemotherapeutic treatment for drug resistant tumors, for reducing the risk of restenosis after coronary angioplasty, for killing intracellular parasites, and for inhibiting metastasis[14].

Target delivery is critical to the success of future of NO donors. However, many organs are difficult to access. In such cases research efforts are aimed at the development of prodrugs. NONOates are candidates for chemotherapeutic treatment for drug-resistant tumors and inhibiting metastasis [52]. The tunability of half-life, the ability to derivatize and the insensitivity of decomposition to medium (except pH and temperature) together with the direct release of NO are the major advantages to use of NONOates over other NO donor types.

1.2.5 HNO DONORS

Primary amine diazeniumdiolates such producer from isopropyl amine (IPA/NO) can release HNO under physiological conditions (Figure1.10). Numerous studies demonstrate potential biological activity associated with administration of HNO [32, 33]. For instance HNO is a potent vasorelaxant, possibly serving as a precursor to NO [49]. Nagasawa reported that HNO was a potent inhibitor of the enzyme aldehyde-dehydrogenase and had the potential to be developed as a treatment for al-

coholism [32, 35]. HNO dimerizes spontaneously producing hyponitrous acid, which decomposes to nitrous oxide N_2O and water H_2O .



HNO thus cannot be stored and HNO donor compounds are required. There are several classes of HNO donor molecules, but the most prevalent donor used for biological and chemical studies is Angeli's salt ($Na_2N_2O_3$), which was synthesized by Angeli in 1986 [3]. Angeli's salt produces HNO in aqueous solutions (pH 4-8) [34].

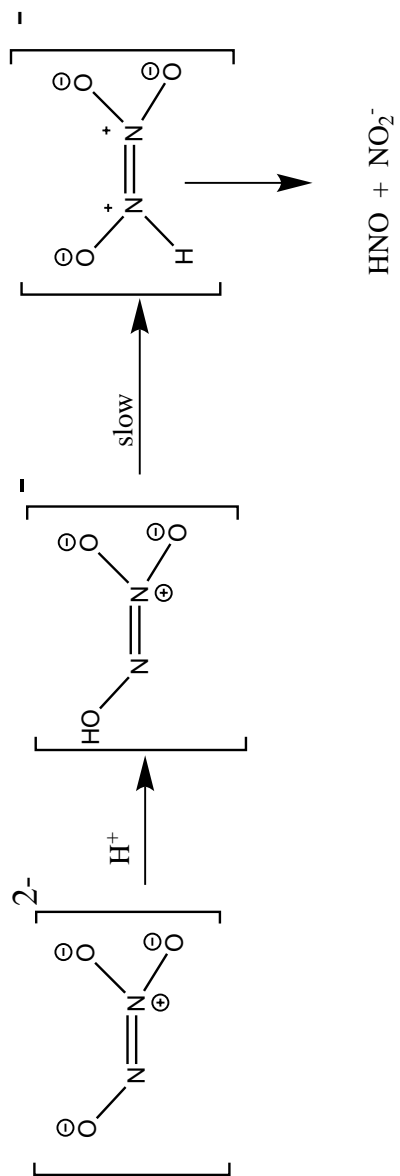


Figure 1.11: Decomposition mechanism of Angeli's salt

NONOates synthesized from secondary amines are typically NO donors. However, NONOates made from primary amines can release HNO spontaneously (Figure 1.11). IPA/NO is an HNO donor at pH ≤ 5 with a biological activity similar to Angeli's salt [35].

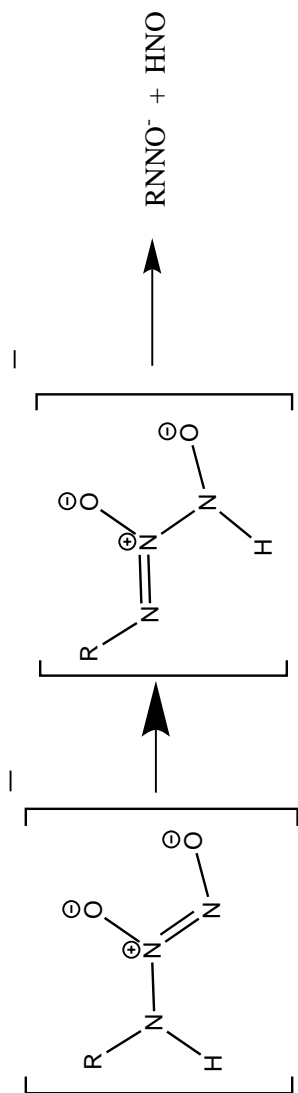


Figure 1.12: Mechanism of HNO from NONOates

The major sites of reactivity for HNO in biological systems are thiols and thiol proteins [32] as well as ferric hemoproteins, which are reductively nitrosylated by HNO [12].

1.3 QSAR

"There is no more basic enterprise in Chemistry than the determination of the geometrical structure of a molecule. Such a determination, when it is well done, ends all speculation as to the structure and provides us with the starting point for understanding of every physical, chemical and biological property of the molecule"
R. Hoffman

Molecular structures are important in drug design; each molecule has encoded information that can be read using quantum mechanics and mechanical statistic in stationary state [27].

Quantitative Structure Activity/Property Relationships (QSAR/QSPR) relate the structure of a compound, expressed in terms of descriptors that can be calculated directly from the structure (number of carbon of atoms, HOMO, etc), to a property/activity of interest (melting point, biological activity). If a correlation can be established, activity/property values can be predicted for other compounds with a certain degree of confidence.

QSAR/QSPR studies are of great importance in drug design. Chemical intuition is necessary to find the correlation between structure and activity, and the development of predictive statistical models is fundamental in QSAR/QSPR. A wide variety of statistical methods are used such as multilinear regression (MLR), principal component analysis (PCA), and partial least squares techniques (PLS). The MLR approach assumes that a property can be modeled as a linear function of several molecular descriptors. Building QSAR/QSPR models is necessary to have

reliable data (molecular descriptors, experimental properties).

There are a variety of molecular descriptors that can express the entire electronic, quantum and geometric properties of molecules and their interactions [28]. The definition of descriptors is the final result of a logical and mathematical procedure that transforms chemical information encoded within a symbolic representation of a molecule into a useful value.

Molecular descriptors [27] are divided into several classes, depending on their origin of calculation or on a structural item in the chemical structure (molecule, atom or bond). The main types of descriptors used in QSAR/QSPR are constitutional, quantum chemical, topological, thermodynamic, geometric, electrostatic and solvation descriptors. Several packages are available to calculate a wide variety of descriptors, for instance CODESSA, JOEL, and GAUSSIAN. The number and complexity of descriptors used is based on the chemical intuition of researchers since there are no rules for the selection of correct descriptors.

1.3.1 Descriptors

Constitutional Descriptors

Constitutional descriptors are attractive for their simplicity since they describe the chemical structure of the molecule, without any reference to electronic structure. Constitutional descriptors include the total number of atoms, total number of bonds, and absolute and relative numbers of bonds in a molecule.

Topological Descriptors

These descriptors show the atomic composition of a molecule and show the presence of chemical bonds, which atoms are connected to each other, and their distribution in space. Topological descriptors, also called topological indices, consider the molecule as a mathematical graph, and have been shown to be very useful in building

predictive models.

Geometric Descriptors

Geometric descriptors represent more advanced structural molecular descriptors and characterize the shape of the molecules in terms of their three-dimensional coordinates. These descriptors depend only on the atomic coordinates and masses. As a result, accurate coordinates are required to allow optimization before these descriptors can be calculated. Examples include molecular surface area, solvent-accessible molecular surface area, molecular volume, and principal moments of inertia of a molecule. In general these types of descriptors capture features related to molecular size and shape and which are generally physically interpretable. The drawback to these descriptors is that they require accurate molecular geometries and large sets of molecules such that the optimization step can become time consuming.

Electrostatic Descriptors

Chemical interactions are by nature either covalent or electrostatic (ionic). The electric charges in the molecule are the driving force for electrostatic interactions. These descriptors have been widely employed as chemical reactive indices or as measures of intermolecular interactions. Many electrostatic descriptors have been derived from electronic charge distributions in the molecule or from electronic densities on particular atoms. The principal electrostatic descriptors are Mullikan atomic partial charges, dipole moment and molecular polarizability.

Quantum Chemical Descriptors

These descriptors express the electronic environment and the characteristics of intermolecular interactions. The quantum chemical methodology is based on the solution of the time independent Schrödinger equation for the stationary states of molecular systems. The principal quantum descriptors are total energy of the molecule, standard heat of formation, highest occupied molecular orbital (HOMO) energy,

lowest unoccupied molecular orbital (LUMO) energy, absolute hardness and activation hardness.

Solvation Descriptors

Many intermolecular interactions are affected by the solvation of molecules, which produce changes in geometry and in electronic structure. It is necessary to construct a model that can describe these solvation phenomena. The most important descriptors are solute-solvent interaction energy, solute-solvent repulsion interaction energy, cavitations energy, and total nonelectrostatic energy.

1.4 Statistic Analysis

QSAR studies are divided into two types - regression and classification. The development of QSAR models essentially consists of the application of statistical methods to chemical data sets. The statistical literature provides a number of useful techniques, some of which are specifically designed to build models and others which carry out both classification and regression. QSAR/QSPR models are developed using these statistical methods, and multivariate methods are used to extract information from the data.

Selection of the method for statistical analysis among the many different modeling methods is crucial. MLR and OLS are readily interpretable and are commonly used for QSAR/QSPR. MLR employs a linear relationship between the predictor variables and the observed response to develop a predictive model. MLR expresses the relationships between descriptors (x variables) with experimental data (y property). In this thesis project this relationship is expressed in an equation that will be used to predict the half-life of new molecules. The equation obtained by regression of experimental data against a set of preselected descriptors gives the one-parameter (dependent variable) P and the set of descriptors x_k (independent variable), where α_k and a_k are the intercept of the regression model and the slope

of the regression. The quality of the multiple linear regression can be characterized by several statistical parameters. The square of the correlation (R) between the predicted and experimental half-life will be $R = 1$ with perfect correlation and $R = 0$ in the complete absence of correlation.

$$P = \alpha_o + \sum_k^n a_k \chi_k \quad (1.8)$$

The standard error of the multiple linear regression s is defined as:

$$s = \sqrt{\frac{\sum_{i=1}^n [P_{i\text{calc}} - P_{i\text{predict}}]^2}{n - p}} \quad (1.9)$$

Where p is the number of parameters estimated using the least-squares procedure and (n) is the number of data points. The difference $(n-p)$ represents the number of residual degrees of freedom. The value of (s) has been used as statistical criterion for rapid discrimination. The model with the smallest standard error is usually selected. The QSAR relationship is quantitative in the sense that it is used to account for an observed property. For compound i , the linear equation that relates molecular properties, x_1, x_2, \dots, x_n , to the desired activity, y , is: $y_i = x_1b_1 + x_2b_2 + \dots + x_nb_n + e_i$. In a typical QSAR study, a large number of descriptors can be used; however, attention must be paid to overfitting, because with enough parameters any model can be successfully correlated. The final QSAR equation uses the smallest number of descriptors that can adequately model the activity of the compounds in the study, and the maximum recommended ratio is a single independent variable to five compounds.

CHAPTER 2

RESULTS

2.1 QSAR/QSPR Protocol

This chapter describes the results of the development of QSAR/QSPR models using MLR to model the half-lives of NONOates to understand how structural variation affects decomposition rates and to build a predictive equation that can be used to predict the half-lives of new compounds. QSAR/QSPR involves several steps.

- Generation of the data set. High quality and reliable experimental data is required as the basis set. Compilation of information related to NONOates half-life and structure was carefully extracted from the literature.
- Molecular modeling of compounds. Once the compounds were selected, they were subjected to geometrical optimization of their structures, to obtain the correct shape and conformation. All structures of NONOates were constructed using Gauss view. The structures for NONOates are shown in Appendix A, with relevant information for each molecule. Geometry optimization for NONOates was performed using B3LYP density functional theory method with 6-311+ basis set implemented in Gaussian 03, the lowest energy was associated with the most stable structure. Aqueous solvation energies were calculated with B3LYP/6-311+ in the polarizable continuum model (PCM).
- Generation of molecular descriptors. In order to encode the compounds, descriptors were calculated using density functional theory (Tables 2.2-2.4). These include constitutional, topological, geometric electronic and quantum chemical descriptors. The aim at this stage is to show all descriptors calculated for NONOates and to identify any unusual observations or any unusual pat-

terns of observations that may cause problems for later analyses to be carried out on the data.

- Model building. This step involves the application of statistical analysis and correlation, dimension reduction, using MLR, and other parameter accounting for quality and to obtain reproducible models. Statistical plots were performed using commercial statistical packages such as MINITAB15.
- Interpretation and validation of the model. Interpretation of the model developed is an important aspect of any ASAR/QSPR study. Interpretation involves explanation of how each descriptor was selected and assessment of physicochemical meaning to descriptors. When the model is obtained, it is important to determine its reliability and statistical significance. Several procedures are available, including internal validation. This method predicts the property value for a compound from the data set, which is predicted using the regression equation from the data for all other compounds.
- Data analysis. The best model was chosen with the smallest standard error in prediction of half-life of the basis set. Once a model is chosen, variables with the highest weights were determined. The most important variables (descriptor) were assumed to have p-values ≤ 0.05 .
- Prediction. The QSAR/QSPR model was built to estimate the property of interest for an unknown or newly compound synthesized. The predicted accuracy of the model will be determined by synthesizing new NONOates and comparing the experimental and predicted half-life.

2.1.1 Selection of descriptors

Development of descriptors is the most important part of any QSAR/QSPR because the descriptors must contain enough information to permit the correct characterization of compounds. In this study, 17 descriptors were calculated, but not all

descriptors were used to develop each model. The set of descriptors with high significance were selected based on p-value and were incorporated into the QSAR/QSPR equation.

2.1.2 Model development using the MLR method

After the generation of descriptors, the statistical quality of the initial set can be evaluated using *Minitab*₁₅ to analyze its normal distribution of each descriptors. If the distribution does not correspond to a normal distribution, this descriptor must be discarded. The quality of MLR can be characterized by several statistical parameters. R_2 (the coefficient of determination), represents the fraction of the dependent variable described by the set of descriptors; a perfect correlation is indicated by $R_2 = 1$, while a complete absence of correlation between property and descriptors corresponds to $R_2 = 0$.

Model 1

The first model consisted of 17 descriptors, tabulated in Tables 1-3. The regression equation obtained is:

$$\begin{aligned} \text{Half - life}(s) = & 85666 + 2.12\text{totalenergy} + 22.5350\text{HOMO} + \\ & 45\text{totalnonelectronicenergy} - 173445\text{repulsionenergy} - 242829\text{dispersionenergy} - \\ & 239795\text{cavitationenergy} - 1009\text{polarizedsolutesolvent} - 5200\text{dipolemoment} + \\ & 3575\text{chargeonN} - 269\text{HOMO} - \text{LUMO} + 119\text{LUMO} + 954\text{number - of - atoms} - \\ & 42180\text{number - of - carbon} - 350\text{relative - number - of - carbons} - 420 - \text{area} + \\ & 5973 - \text{volume}. \end{aligned}$$

Standard error is measured in the units of the response variable and represents the standard distance values fall from the regression line. In Model 1 a standard error of 875 describes the variation in the observed response values that is explained by the predictor(s), R_2 of 0.70 indicates a poor model. A plot of experimental vs

experimental half-life is shown in Figure 2.1.

Analysis of a QSAR/QSPR model by MLR, allows the elimination of highly correlated variables, which contain redundant information. Multicollinearity causes problems in interpreting the results of a regression equation. A high correlation between HOMO-LUMO and HOMO-LUMO4 led to elimination of HOMO-LUMO4 as an important descriptor. Additionally, three descriptors (dipole moment, numbers of carbon and cavitations energy) that had $p - values > 0.5$ and thus were not sufficient to explain variation in response.

Model 1

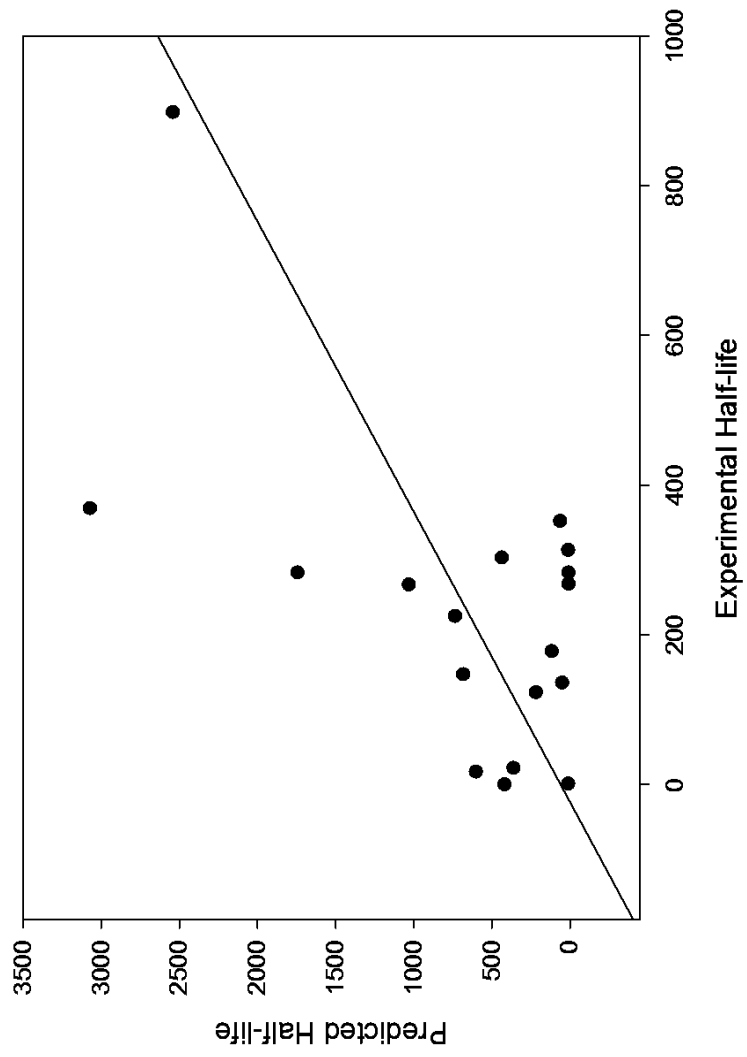


Figure 2.1: Plot of experimental vs. predicted half-life for Model 1

Model 2

In order to find a good model, the NONOates (PROLI/NO, PYRRO/NO, PIPERI/NO and 1-AMPIP/NO) with a high percent error in the predicted value (Table 4) were eliminated and Model 2 was developed with 13 descriptors and 17 NONOates in the range between 125-72000s, the regression equation model 2 is:

$$\begin{aligned} Half-life(s) = & -116187 - 3.78totalenergy - 12237totalnonelectronicenergy - \\ & 362157repulsionenergy - 22802dispersionenergy + 831polarizedsolutesolvent + \\ & 2922charge - on - Nitrogen + 1336HOMO + 1510LUMO + 26750number - of - \\ & atoms + 46872HOMO - LUMO + 235relativenumberofcarbon + 8272area - \\ & 20778volume. \end{aligned}$$

The standard error (475) and R_2 value (0.77) are improved from Model 1 (Figure 2.2), but the accuracy remains low.

Table 2.1: Descriptors 1

Compound	Half-life (s)	Total energy	HOMO	LUMO	LUMO4	HOMO-LUMO	Number of Atoms
PROLI/NO	2	-414182.25	-127.18	-21.03	12.51	-106.15	19
PYRRO/NO	3	-296135.87	-123.21	-16.38	10.67	-106.83	17
PIPERI/NO	19	-320801.11	-123.36	-18.8	9.24	-82.7	20
1-AMPIP/NO	24	-355446.15	-85.17	-2.47	10.22	-95.44	22
1-AMHA/NO	125	-370878.48	-123.53	-28.09	7.54	-95.1	28
IPA/NO	138	-272209.98	-122.50	-27.40	12.90	-103.05	16
DEA/NO	149	-296890.44	-116.58	-13.53	9.33	-148.72	19
4-AMPIP/NO	180	-355163.07	-163.30	-14.58	-2.74	-106.72	22
CPA/NO	227	-320812.23	-128.27	-21.55	41.17	-104.03	20
PIP/NO	269	-330852.75	-123.48	-19.45	9.63	141.63	19
2-AMPE/NO	270	-321573.90	-123.36	-18.27	9.15	-93.96	22
2-AMOA/NO	285	-395542.62	-120.82	-26.86	7.00	-96.08	31
2-AMHA/NO	285	-370879.05	-123.41	-27.33	7.35	-90.71	28
2-AMMHA/NO	305	-395544.54	-110.22	-19.51	13.39	-106.9	31
COCTA/NO	315	-394811.00	-127.51	-20.61	5.48	-107.18	29
CHEPTA/NO	354	-370149.02	-128.81	-21.63	7.23	-90.46	26
CHEXA/NO	371	-345438.70	-115.36	-24.90	8.05	-112.61	23
PAPA/NO	900	-381256.01	-163.92	-51.31	4.02	-112.61	28
DPTA/NO	10800	-415925.46	-123.05	-26.63	2.81	-96.42	30
DETA/NO	72000	-366299.67	-100.27	-1.85	8.56	-98.42	23

Table 2.2: Descriptors 2

Compound	Half-life	Dip moment	Charge on N	Area	Volume	No of C	Relative No of C
PROLI/NO	2	9.2	0.66	177.75	149.32	5	0.26
PYRRO/NO	3	15.6	0.25	150.07	122.36	4	0.24
PIPERI/NO	19	17.7	0.37	166.44	139.41	5	0.25
1-AMPIP/NO	24	19.4	-0.19	184.96	152.37	5	0.23
1-AMHA/NO	125	25.3	-0.16	229.44	187.71	7	0.25
IPA/NO	138	14.1	-0.28	147.82	114.36	3	0.19
DEA/NO	149	16.9	0.93	167.48	134.47	4	0.21
4-AMPIP/NO	180	2.3	0.53	180.01	150.22	5	0.23
CPA/NO	227	15.5	-0.16	170.12	139.68	5	0.25
PIP/NO	269	18.1	0.24	162.10	134.11	4	0.21
2-AMPE/NO	270	17.0	-0.05	189.02	151.23	5	0.23
2-AMOA/NO	285	22.7	-0.38	247.74	206.00	8	0.26
2-AMHA/NO	285	20.0	0.14	226.75	187.75	7	0.25
2-AMMHA/NO	305	19.9	-0.03	245.16	205.78	8	0.26
COCTA/NO	315	18.4	-0.33	217.93	191.41	8	0.28
CHEPTA/NO	354	18.9	-0.25	202.41	173.50	7	0.27
CHEXA/NO	371	18.6	-0.35	184.43	156.13	6	0.26
PAPA/NO	900	7.9	0.01	209.58	181.00	6	0.21
DPTA/NO	10800	29.1	0.86	222.43	191.68	6	0.20
DETA/NO	72000	13.5	-0.52	192.40	155.91	4	0.17

Table 2.3: Descriptors 3

Compound	Half-life(s)	Pol solute solv	Dispersion E	Cavitation E	Repulsion E	Total Nonelect E
PROLI/NO	2	23.31	-15.82	23.31	1.09	8.58
PYRRO/NO	3	19.75	-13.89	19.75	0.90	6.75
PIPERI/NO	19	21.64	-15.22	21.64	0.95	7.37
1-AMPIP/NO	24	23.43	-16.94	23.43	1.17	7.66
1-AMHA/NO	125	29.62	-19.65	29.62	1.20	11.17
IPA/NO	138	20.13	-12.17	20.13	0.73	8.69
DEA/NO	149	22.48	-13.22	22.48	0.68	9.94
4-AMPIP/NO	180	22.33	-17.14	22.33	1.22	6.41
CPA/NO	227	21.88	-15.40	21.88	1.09	7.57
PIP/NO	269	21.25	-15.23	21.25	1.11	7.13
2-AMPE/NO	270	25.24	-15.13	25.24	0.88	11.00
2-AMOA/NO	285	31.18	-20.12	31.18	1.17	12.22
2-AMHA/NO	285	29.68	-18.27	29.68	1.01	12.42
2-AMMHA/NO	305	31.49	-19.49	31.49	1.13	13.13
COCTA/NO	315	26.95	-18.99	26.95	1.20	9.15
CHEPTA/NO	354	25.28	-18.34	25.28	1.25	8.19
CHEXA/NO	371	23.26	-17.10	23.26	1.20	7.36
PAPA/NO	900	27.34	-18.02	27.34	1.02	2.34
DPTA/NO	10800	28.95	-2072	28.95	1.36	9.58
DETA/NO	72000	23.73	-16.82	23.73	1.18	8.09

Model 2

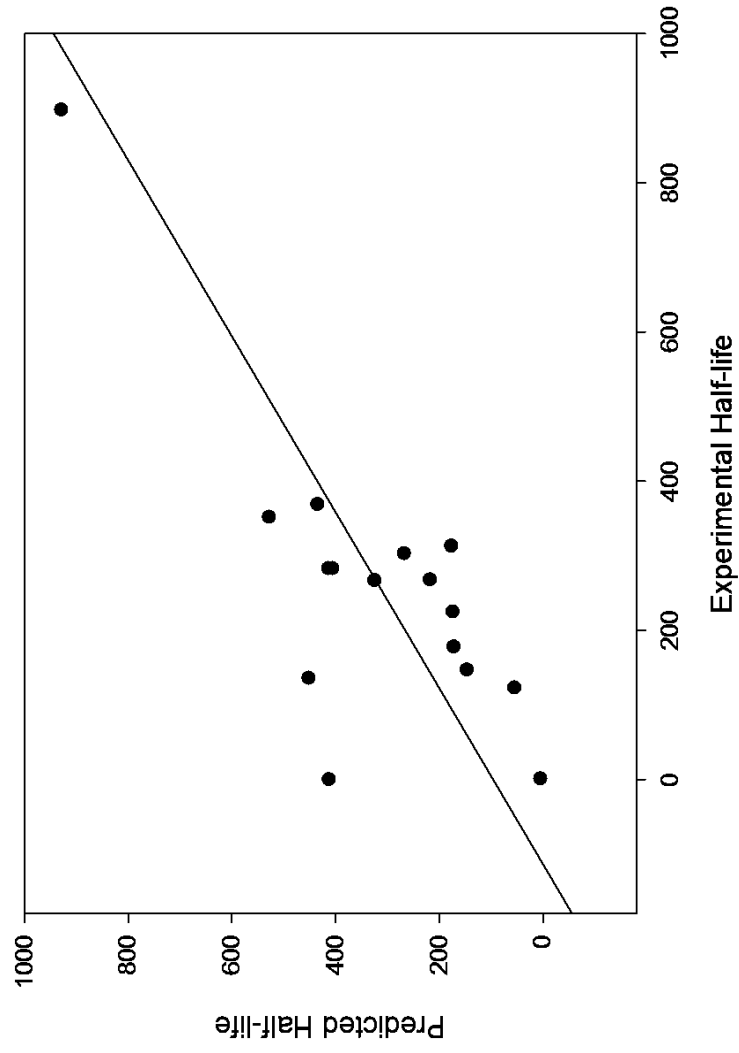


Figure 2.2: Plot of experimental vs. predicted half-life for Model 2

Model 3

Due to the inaccuracy of Models 1 and 2, it was necessary to increase the linear range. One method is to increase the numbers of molecules in the range between 900 - 72,000 s where there is a large gap. This method would require synthesis of new compounds, which is the goal of the project. Another method is to transform the dependent variable (half-life decomposition) to a more compact data set. Transformation was performed using the square root of the decomposition half-life.

The third model consisted of five descriptors and 17 NONOates in the range between 125 -72,000 s and gave the following regression equation:

$$\text{SquareHalf-life}(s) = 20.6 + 572\text{Charge-on-Nitrogen} + \text{Numberofatoms} + 2.513\text{HOMO} - 251\text{HOMO} - \text{LUMO} + 20.9 - \text{volume}$$

The standard error of 9 and an R_2 value of 0.99 indicates a good correlation between experimental and predicted half-life.

Table 2.4: Models 1 - 2

Compound	Half-life(s)	Model 1	% error	Model 2	error
PROLI/NO	2	411 20450	-	-	-
PYRRO/NO	3	3	0	-	-
PIPERI/NO	19	593	3021	-	-
1-AMPIP/NO	24	353	1370	-	-
1-AMHA/NO	125	210	68	53	57
IPA/NO	138	41	70	450	226
DEA/NO	149	676	353	145	3
4-AMPIP/NO	180	108	40	170	25
CPA/NO	227	728	220	172	24
PIP/NO	269	1023	280	323	88
2-AMPE/NO	270	1	99	216	20
2-AMOA/NO	285	1736	509	404	42
2-AMHA/NO	285	1	99	412	45
2-AMMHA/NO	305	428	40	266	13
COCTA/NO	315	3	99	175	44
CHEPTA/NO	354	55	84	526	49
CHEXA/NO	371	3064	725	433	17
PAPA/NO	900	2533	181	927	3
DPTA/NO	10800	8637	20	10722	1
DETA/NO	72000	62552	13	71804	3

Model 3

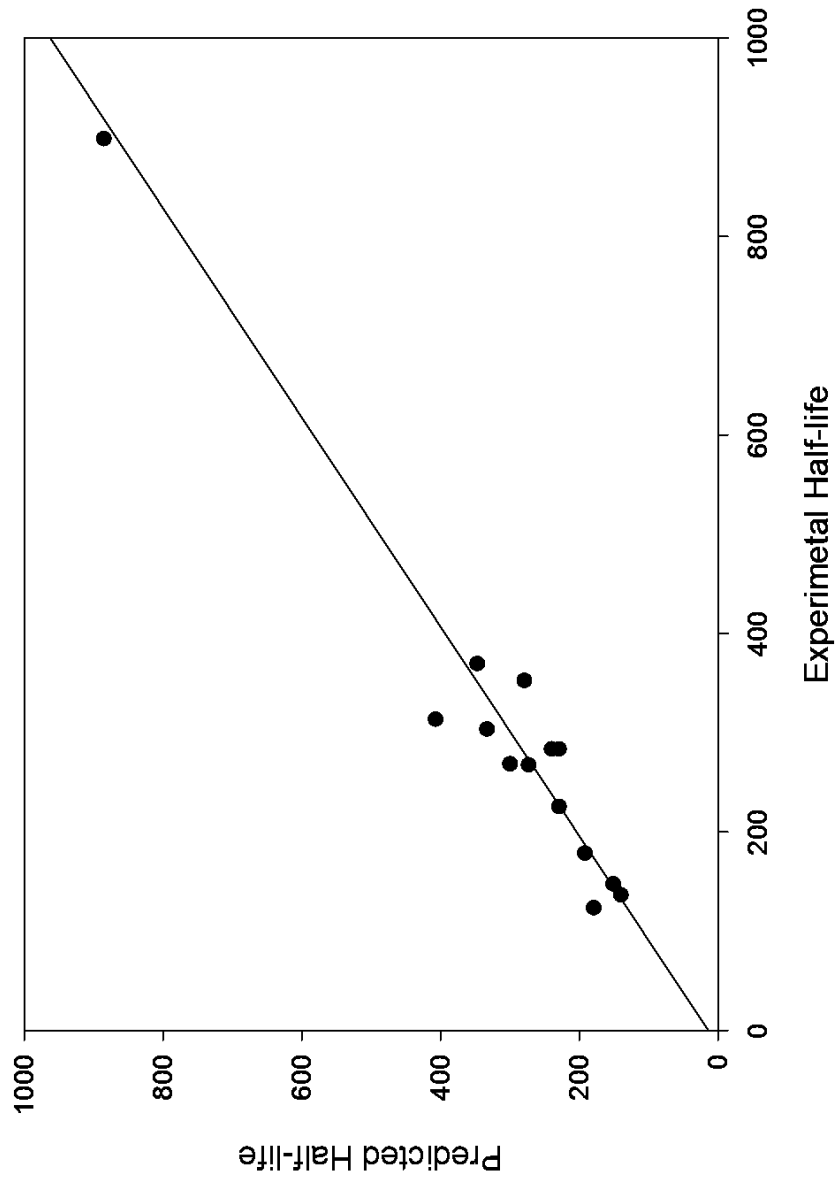


Figure 2.3: Plot of experimental vs. predicted half-life Model 3

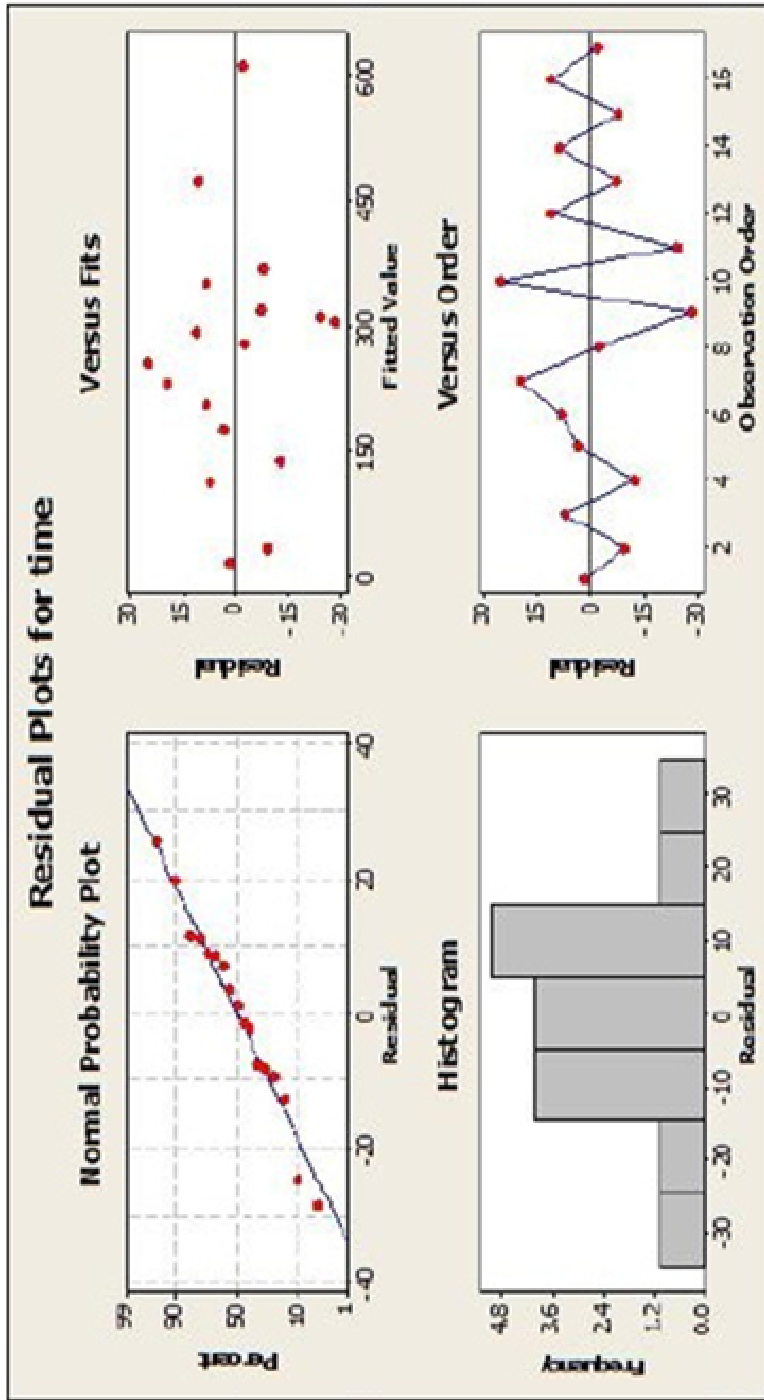


Figure 2.4: Residual plots of Model 3

The residuals are the difference between predicted and experimental half-lives. According to this plot the residuals were distributed, and there were no values considered to be outliers. In the normal probability plot, almost all residuals are in the range of 20% with a normal distribution.

Analysis of the variables

One application of this model is to predict the half-lives of NONOates but another is to understand or to interpret the influence of each variable on decomposition rate. In Table 6 the variables that have the largest influence on half-life are predicted based on $p < 0.05$.

Table 2.5: Variables

VARIABLE	p-value
HOMO	0.014
CHARGE on NITROGEN	0.029
TOTAL NUMBER of ATOMS	0.030
HOMO-LUMO	0.045

The HOMO energy in this equation is directly related to the increase of half-life. To understand the HOMO effect, three NONOates with different half-lives spacing the range (PROLI/NO (2 s), DEA/NO (149 s) and DETA/NO (72000 s)) were generated using Gaussian 03, (Figures 2-5, 6, 7). The bonding interaction shown in the HOMO orbital increases with the stability of the molecules. This interaction makes DETA/NO highly stable. On the other hand there is no bonding interaction in PROLI/NO, which has a 2 s half-life.

The HOMO-LUMO gap has been related to the chemical stability of compounds. HOMO-LUMO gaps are considered as indicators of higher stability. The influence of HOMO energies in the model higher energy gap is associated with molecular stability, and this information can be used to explain the different half-lives of NONOates.

The charge on the amine nitrogen is the second important variable in the model of NONOate half-life. Then the charge of the amine nitrogen will be analyzed for both primary and secondary amines. The energy required to break the N-N bond in NONOates can be correlated with bond lengths, bond force constants and the electron densities along the bond path. These properties can be changed for a variety of substituent. An alternative explanation is using the polar effect in NONOates, and to find if a correlation between nitrogen in the substituent and decomposition half-life exists.

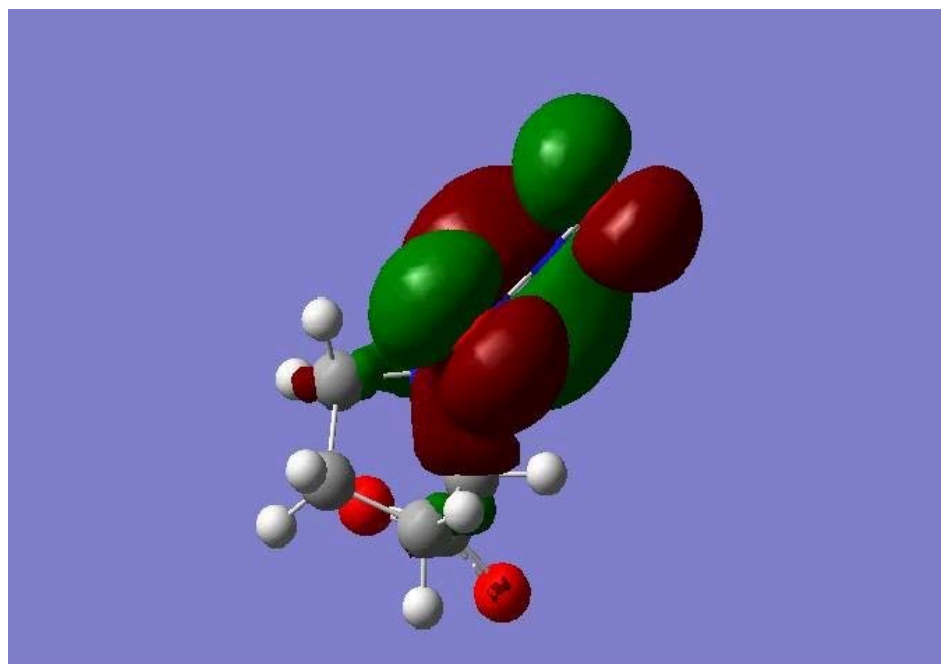


Figure 2.5: PROLI/NO

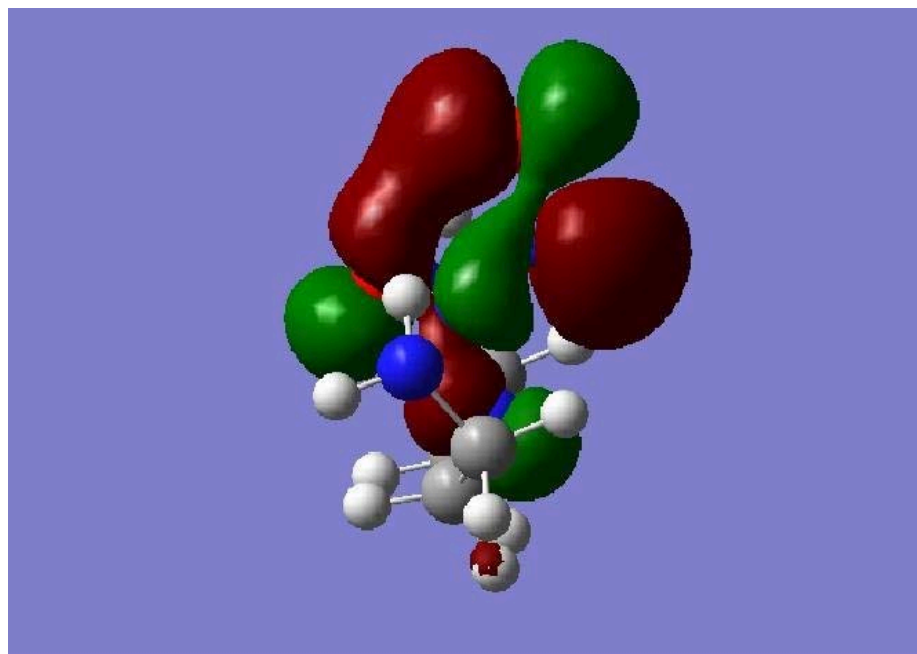


Figure 2.6: DEA/NO

2.1.3 Future Perspective

The conclusion, QSAR is good tool to predict the decomposition time of NONOates. Model 3 using square root of half-life gave the best model, and a high correlation between HOMO energy and half-life was found.

External validation of this model will be made using new set of NONOates to test Model 3. Perform specific studies for each groups of NONOates (primary and secondary amine). Future theoretical studies will be designed to calculate the transition state of NONOates of polyamine determine on basis for study the stability, and to examine the structure as bonding properties using a wide variety of computational methods.

Experiments will be designed that compare theoretical results to experimental methods such as photoelectron spectroscopy. Electrostatic potential can also provide

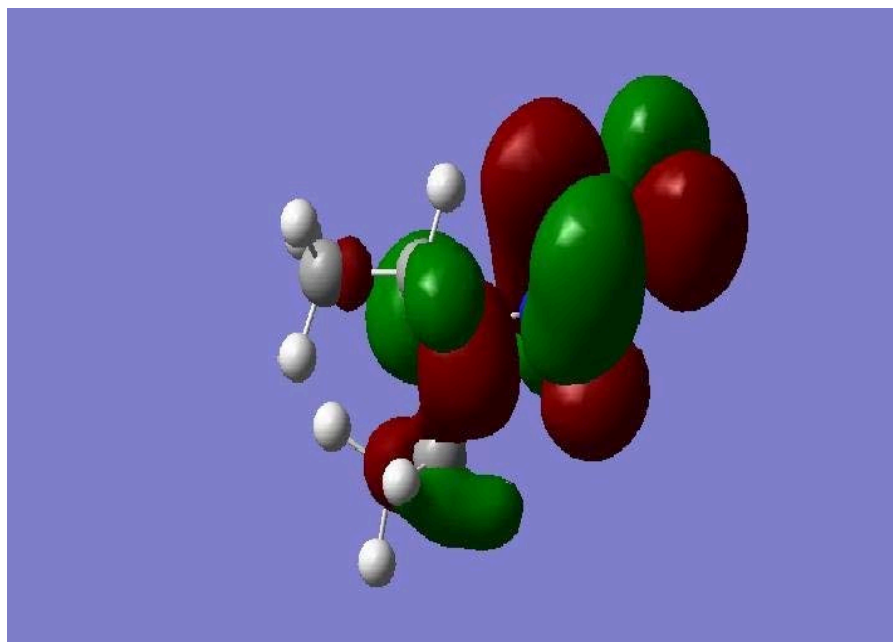


Figure 2.7: DETA/NO

direct insight to the effects the substituents have on the electronic structures of NONOates.

APPENDIX A

NONOATES

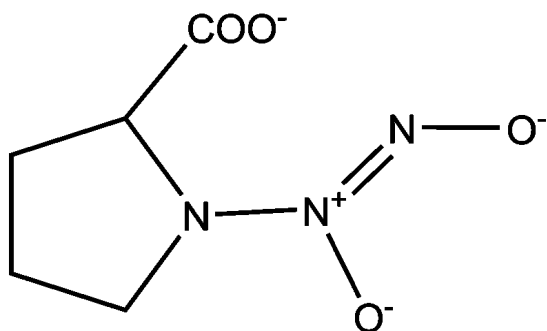


Figure A.1: PROLI/NO [37, 45]

Chemical name:

Disodium 1-[(2-Carboxylato)pyrrolidin-1-yl]diazene-1,1,2-diolate

Amine: Proline

Molecular Formula: $C_5H_7N_3Na_2O_4$

Half-life at pH 7.4 and 37°C: 2 s

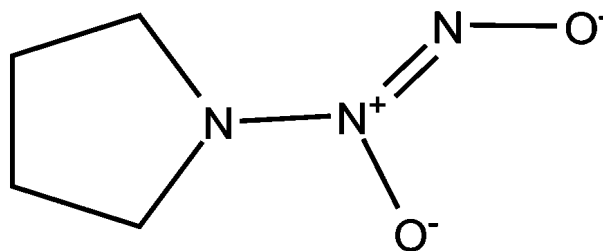


Figure A.2: PYRRO/NO [41, 25]

Chemical name:

Disodium 1-[N-(3-Ammoniopropyl)-N-(N-propyl)amino]diazene-1-ium-1,2-diolate

Amine: Pyrrolidine

Molecular Formula: $C_4H_8N_3O_2$

Half-life at pH 7.4 and 37°C: 3 s

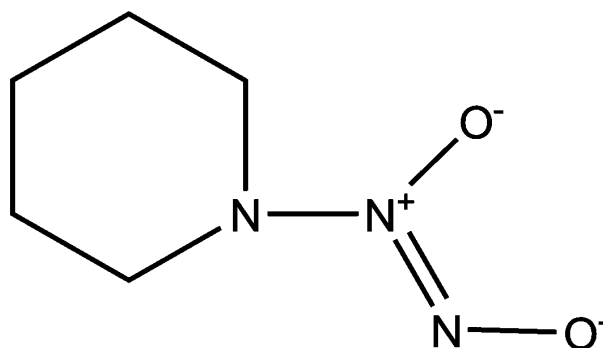


Figure A.3: PIPERI/NO [45]

Chemical name:

Disodium-1-(Piperidin-1-yl)diazen-1-ium-1,2-diolate

Amine: Piperidine

Molecular Formula: $C_5H_{10}N_3O_2$

Half-life at pH 7.4 and 37°C: 19 s

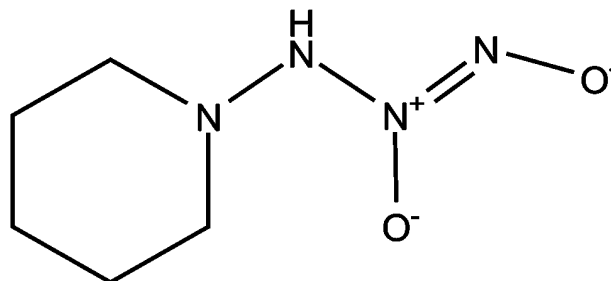


Figure A.4: 1-AMPIP/NO [42]

Chemical name:

Disodium-Methyl-1-(Cyclohexylamino)-diazene-1,2-diolate

Amine: 1-Aminopiperidine

Molecular Formula: $C_5H_{11}N_4O_2$

Half-life at pH 7.4 and $37^\circ C$: 24 s^{-1}

¹Unpublished, synthesized in Miranda Lab

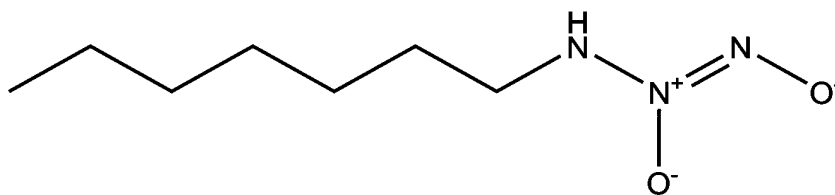


Figure A.5: 1-AMHA/NO

Chemical name:

Disodium-1-(heptylamino)-diazene-1,2-diolate

Amine: 1-Aminoheptamine

Molecular Formula: $C_7H_{15}N_3O_2$

Half-life at pH 7.4 and 37°C: 125 s²

²Unpublished, synthesized in Miranda Lab

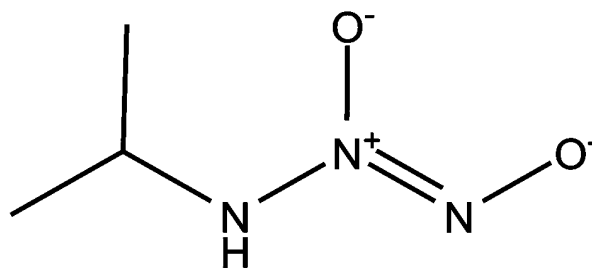


Figure A.6: IPA/NO [40]

Chemical name:

Disodium-1-Triazene, 1-hydroxy-3-(1-methylethyl)-,2-oxide

Amine: Isopropylamine

Molecular Formula: $C_3H_8N_3O_2$

Half-life at pH 7.4 and $37^\circ C$: 138 s

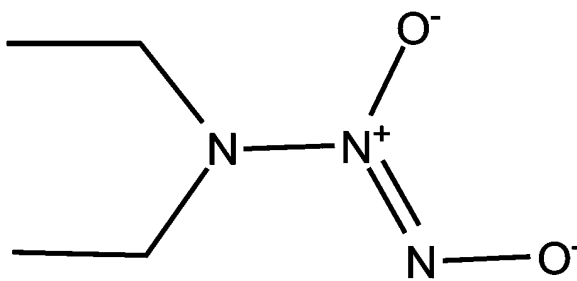


Figure A.7: DEA/NO [43, 18, 10]

Chemical name:

Disodium-(Z)-1-[N,N-Diethylamino]diazem-1-ium-1,2diolate

Amine: Diethylamine

Molecular Formula: $C_4H_{10}N_3O_2$

Half-life at pH 7.4 and 37°C: 149 s

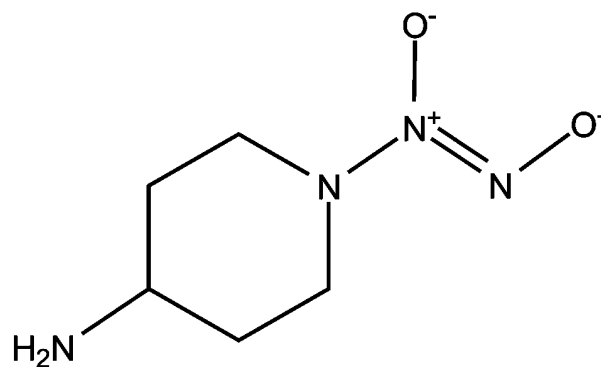


Figure A.8: 4-AMPIP/NO

Chemical name:

Disodium-1-(Piperazin-4-amin-yl)-diazene-1-ium-1,2-diolate

Amine: 4-Aminopiperidine

Molecular Formula: $C_5H_{11}N_3O_2$

Half-life at pH 7.4 and 37°C: 180 s³

³Unpublished, synthesized in Miranda Lab

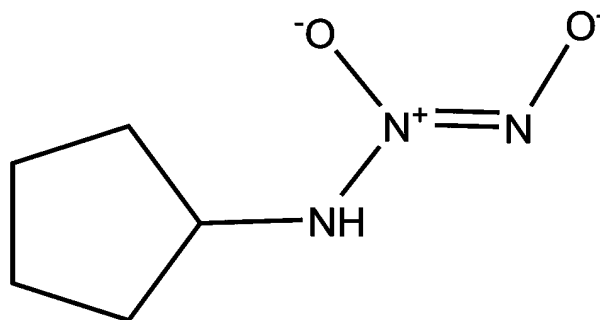


Figure A.9: CPA/NO

Chemical name:

Disodium-1-(Cyclopentamin)-diazene-1-ium-1,2-diolate

Amine: Cyclopentamine

Molecular Formula: $C_5HN_2O_2$

Half-life at pH 7.4 and 37 oC: 227 s ⁴

⁴Unpublished, synthesized in Miranda Lab

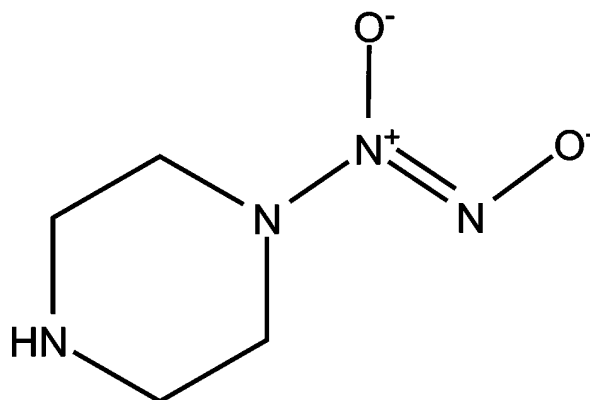


Figure A.10: PIP/NO [10]

Chemical name:

Disodium-1-[Piperazin-1-yl]-diazen-1-ium-1,2-diolate

Amine: Piperazidine

Molecular Formula: $C_4H_9N_4O_2$

Half-life at pH 7.4 and 37°C: 269 s

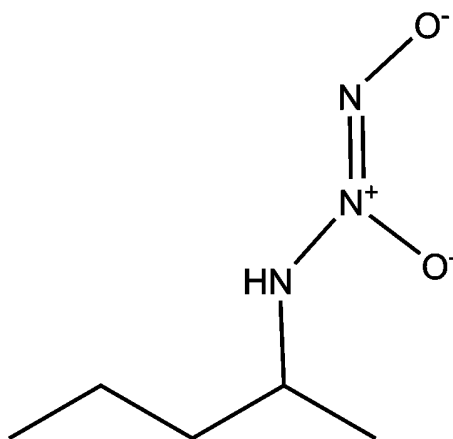


Figure A.11: 2-AMPEP/NO

Chemical name:

Disodium-Z-1-(N-2-pentyl-amino)-diazene-1-ium-1,2-diolate

Amine: 2-Aminopentamine

Molecular Formula: $C_5H_{11}N_3O_2$

Half-life at pH 7.4 and 37 oC: 270 s ⁵

⁵Unpublished, synthesized in Miranda Lab

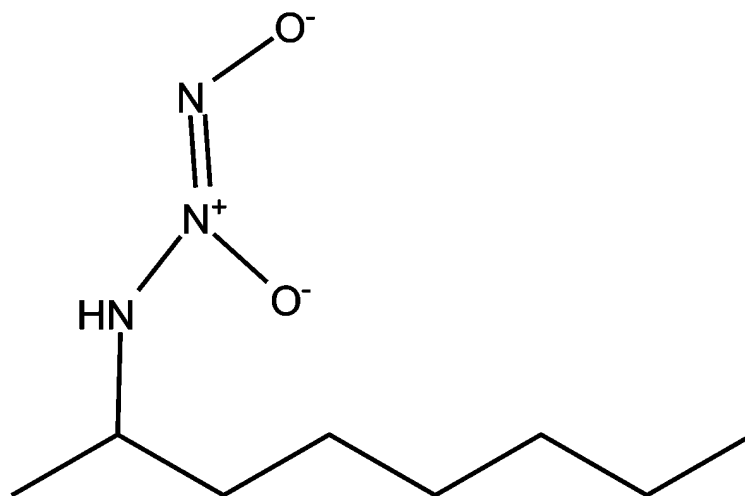


Figure A.12: 2-AMOA/NO

Chemical name:

Disodium-Z-1-(N-2-octyl-amino)-diazene-1,2-diolate

Amine: 2-Aminooctamine

Molecular Formula: $C_8H_{18}N_2O_2$

Half-life at pH 7.4 and 37°C: 285 s ⁶

⁶Unpublished, synthesized in Miranda Lab

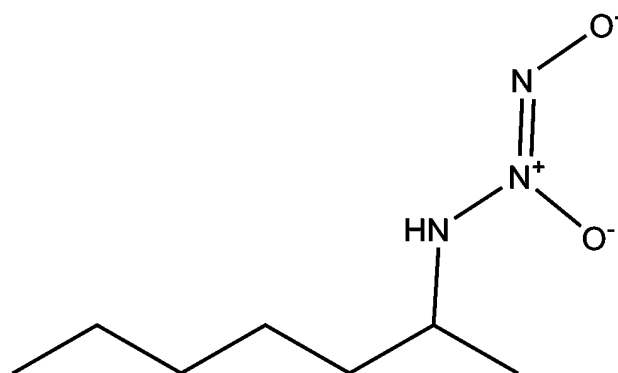


Figure A.13: 2-AMHA/NO

Chemical name:

Disodium-Z-1-(N-2-heptyl-amino)-diazene-1-ium-1,2-diolate

Amine: 2-Aminheptamine

Molecular Formula: $C_7H_{16}N_3O_2$

Half-life at pH 7.4 and 37°C: 285 s⁷

⁷Unpublished, synthesized in Miranda Lab

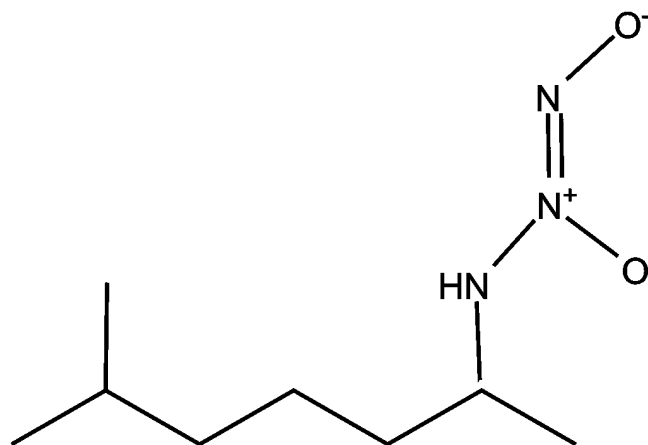


Figure A.14: AMMHA/NO

Chemical name:

Disodium-Z-1-(6-Methyl,N-2-heptyl-amino)-diazene-1,1,2-diolate

Amine: 2-Amino-6-methylheptamine

Molecular Formula: $C_8H_{18}N_3O_2$

Half-life at pH 7.4 and 37°C: 305 s ⁸

⁸Unpublished, synthesized in Miranda Lab

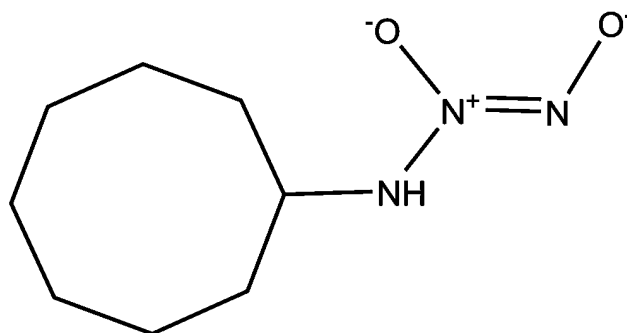


Figure A.15: COCTA/NO

Chemical name:

Disodium-N-(Cyclooctamin)-diazene-1,2-diolate

Amine: Cyclooctamine

Molecular Formula: $C_8H_{16}N_3O_2$

Half-life at pH 7.4 and 37°C: 315 s⁹

⁹Unpublished, synthesized in Miranda Lab

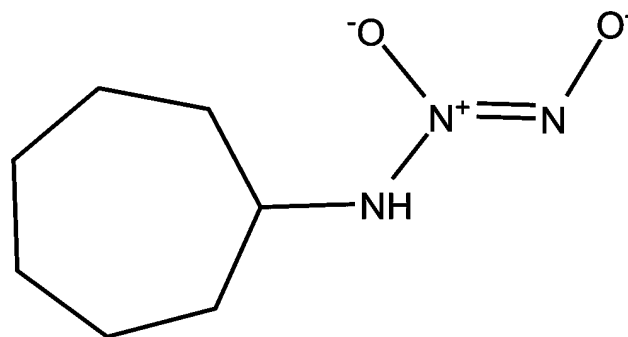


Figure A.16: CHEPTA/NO

Chemical name:

Disodium-N-(Cycloheptamin)-diazene-1,1,2-diolate

Amine: Cycloheptamine

Molecular Formula: $C_7H_{14}N_3O_2$

Half-life at pH 7.4 and 37°C: 354 s ¹⁰

¹⁰Unpublished, synthesized in Miranda Lab

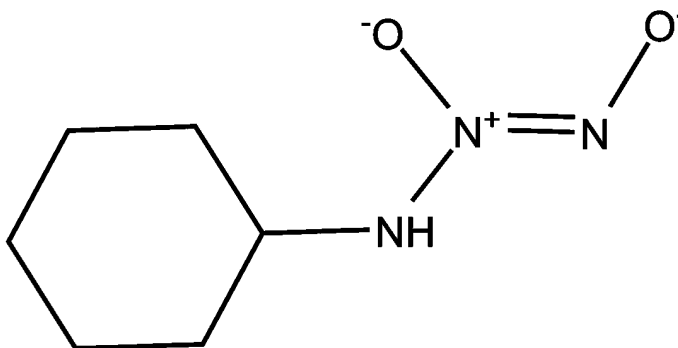


Figure A.17: CHEXA/NO

Chemical name:

Disodium-N-(Cyclohexamin)-diazene-1-ium-1,2-diolate

Amine: Cyclohexamine

Molecular Formula: $C_6H_{12}N_3O_2$

Half-life at pH 7.4 and 37°C: 371 s ¹¹

¹¹Unpublished, synthesized in Miranda Lab

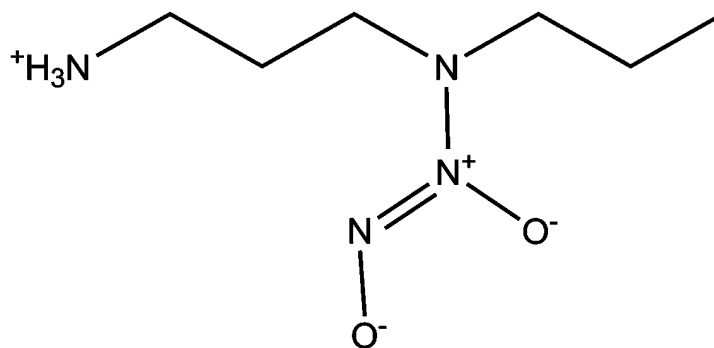


Figure A.18: PAPA/NO [45]

Chemical name:

Disodium-1-[N-(3-Ammoniopropyl)-N-(n-propyl)amino]diazene-1,2-diolate

Amine: 1-[N-(3-Ammoniopropyl)-N-(n-propyl)amino]

Molecular Formula: $C_6H_{16}N_4O_2$

Half-life at pH 7.4 and 37°C: 900 s

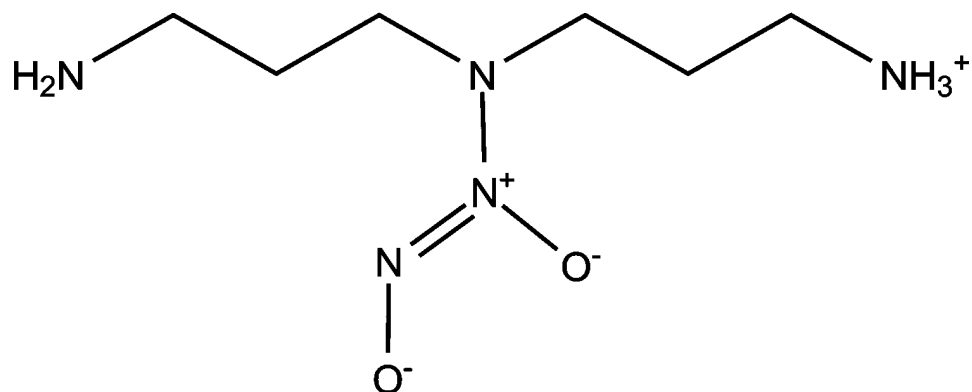


Figure A.19: DPTA/NO [22, 45]

Chemical name:

Disodium-1-[N-(3-Aminopropyl)-N-(3-ammoniopropyl)]diazene-1,2-diolate

Amine: 1-[N-(3-Ammoniopropyl)-N-(3-ammoniopropyl)]amino

Molecular Formula: $C_6H_{17}N_5O_2$

Half-life at pH 7.4 and 37°C: 10800 s

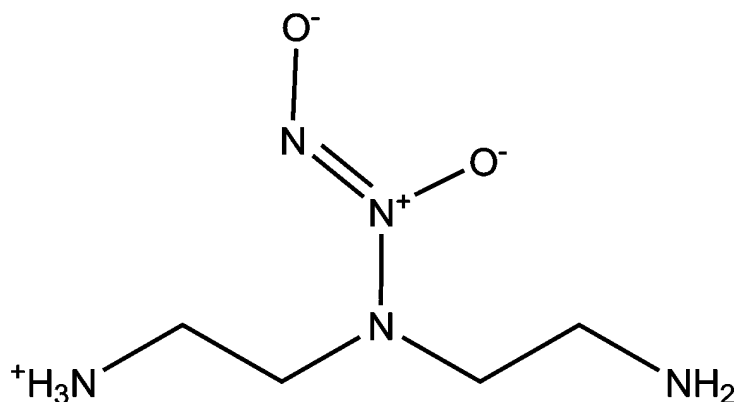


Figure A.20: DETA/NO [22, 45]

Chemical name:

Disodium-1-[N-(2-Aminoethyl)-N-(2-ammonioethyl)amino]-1-ium-1,2-diolate

Amine: 1-[N-(2-Aminoethyl)-N-(2-ammonioethyl)]amino

Molecular Formula: $C_4H_{13}N_5O_2$

Half-life at pH 7.4 and 37°C: 72000 s

REFERENCES

- [1] Al-Sa'doni, H, and Ferro, A. *Clinical Science*, 2000.
- [2] Al-Sa'doni, H, and Ferro, A. S-Nitrosothiols as Nitric Oxide-Donors: Chemistry, Biology and Possible Future Therapeutic Applications. *Current Medicinal Chemistry*, 11:2679–2690, 2004.
- [3] Angeli, A. *Chim. Ital*, 26:17, 1896.
- [4] Arnelle, D. R, and Stamler, J. S,. *Archives of Biochemistry and Biophysics*, 318:279–285, 1995.
- [5] Arnold, W. P and Epstein, R. M . *Anesthesiology*, 61:254–260, 1984.
- [6] Booth, B, and Ho-Leung . *Journal Cardiovascular Pharmacology*, 32:129, 1998.
- [7] Cary, S. P. L,Winger, J. A, and Marletta, M. A. *Trends in Biochemical Sciences*, 31:231–239, 2006.
- [8] Cary, Stephen P. L,Jonathan A,Marletta, and Michael A. *PNAS*, 102:13064–13069, 2005.
- [9] Christos A. Kontogiorgis. *Medicinal Research Reviews*, 102:385–418, 2002.
- [10] Davies, K. M,Saavedra, J. E, and Keefer, L. K. *Chemical Society 2001*, 123, 5473-5481., 123:5473–5481, 2001.
- [11] de Barros, B. F,Toledo, J. C, and Tfouni, E. *Nitric Oxide*, 7:50–56, 2002.
- [12] Doyle, M. P,Broene, R. D, and Guy, J. K. *Journal of the American Chemical Society*, 110:593–599, 1988.
- [13] Drago, R. S, and Paulik, F. E. *Journal of the American Chemical Society*, 82:96–98, 1960.
- [14] Keefer, L. K,Christodoulou, D, Dunams, T. M, and Wink, D. A. *Nitric Oxide*, 82:136–146, 1994.
- [15] Dutton, A. S,Suhrada, C. P,Miranda, K. M, Wink, D. A, Fukuto, J. M, and Houk, K. N. *Inorganic Chemistry*, 45:2448–2456, 2006.
- [16] Edwards, J,Ignarro, L,Hyman, A, and Kadowitz, P. *J Pharmacol Exp Ther*, 228:33–42, 1984.

- [17] Espadas-Torre, and Meyerhoff, M. E. *Journal of the American Chemical Society*, 119:2321–2322, 1997.
- [18] Fitzhugh, A. L, and Keefer, L. K. *Free Radical Biology and Medicine*, 28:1463–1469, 2000.
- [19] Gaul, M. J. . *Structure and Bonding*, 81:147, 1993.
- [20] Glidewell, A. *Chem. Soc. Rev*, 16:361–380, 1987.
- [21] Houk, K. N,Hietbrink, B. N, and Toone, E. J. *Journal of the American Chemical Society*, 125:6972–6976, 2003.
- [22] Hrabie, J. A,Klose, J. R, and Keefer, L. K. *The Journal of Organic Chemistry*, 58:1472–1476, 1993.
- [23] Huseyin Cavdar, N. S,. *European Journal of Organic Chemistry*, pages 4615–4621, 2008.
- [24] Ignarro, L,Lippton, H,Edwards, J,Baricos, W, Hyman, A, Kadowitz, P, and Gruetter,C . *J Pharmacol Exp Ther*, 218:739–749, 1981.
- [25] Joerg Konter,,Ali El-Emam, and Jochen Lehmann. *European Journal of Organic Chemistry*, pages 616–624, 2007.
- [26] Johnson, C. C. *Arch Int Pharmacodyn Ther*, 35:489, 1929.
- [27] Karelson, M,Lobanov, V. S, and Katritzky, A. R. *Chemical Reviews*, 96:1027–1043, 1996.
- [28] Katritzky, A. R,Lobanov, V. S, and Karelson, M. *Chemical Society Reviews*, 24:279, 1995.
- [29] Kharitonov, V. G,Sharma, V. S and Koesling, D. *Biochemistry*, 36:6814–6818, 1997.
- [30] Louis J. Ignarro,and Carl A. Gruette . *Febs Letters*, 110:275–278, 1980.
- [31] Marks GS, B. M., SL Jimmo, and K Nakatsu . *Drug Metabolism Disposition*, 23:1248, 1995.
- [32] Miranda, K. M,. *Coordination Chemistry Reviews*, 249:433–455, 2005.
- [33] Fukuto, J. M,Switzer, C. H, Miranda, K. M, and Wink, D. A. *Annual Review of Pharmacology and Toxicology*, 45:335–355, 2005.
- [34] Miranda, K. M,Dutton, A. S, and Houk, K. N . *Journal of the American Chemical Society*, 127:722–731, 2005.

- [35] Miranda, K. M, Nagasawa, H. T, and Toscano, J. P. *Current Topics in Medicinal Chemistry*, 5:647–664, 2005.
- [36] Playfair L. *Annalen*, 74:317, 1850.
- [37] Pluta, R.,. *Journal of Neurosurgery*, 90:373–376, 1999.
- [38] Pluta, R.,. *Neurological Research*, 28:665–671, 2006.
- [39] Pluta. R. *Current Neurovascular Research*, 4:121–129, 2007.
- [40] Saavedra, J. E, Smith, K. N, and Keefer, L. K. *Journal of the American Chemical Society* 2004, 126, 12880-12887., 126:12880–12887, 2004.
- [41] Saavedra, J. E, Billiar, T. R, and Keefer, L. K. *Journal of Medicinal Chemistry*, 40:1947–1954, 1997.
- [42] Saavedra J. E, Bohle D. S, Smith K. N, George C, Deschamps J. R, Citro M. L, and Keefer, L. K. *J. Am. Chem. Soc.*, 126, 2004.
- [43] Saavedra, J. E, Davies, K. M, and Keefer, L. K. *The Journal of Organic Chemistry*, 66:593–599, 2001.
- [44] Smith, D. J, Chakravarthy, D, and Keefer, L. K. *Journal of Medicinal Chemistry*, 39:1148–1156, 1996.
- [45] Srinivasan, A, and Toscano, J. P.,. *Journal of the American Chemical Society*, 123:5465–5472, 2001.
- [46] Stone, J. R, and Marletta, M. A. *Biochemistry*, 35:1093–1099, 1996.
- [47] Stuart C. Askew, John McAninly, and Lyn H. Williams. *J. Chem. Soc., Perkin Trans 2*, page 741, 1995.
- [48] Tierney, T. S., and Tamargo, R. J., *Neurosurgery*, 49:945–953, 2001.
- [49] Tocchetti, C. G, Katori, T, and Paolocci, N. *Nitric Oxide*, 82:156–166, 2004.
- [50] Xian, M.; Tang, X Wang, P. G and Janczuk, A. J. *Chemical Reviews* 2002, 102:1091–1134, July 2002.
- [51] Wong, P. S.-Y, and Fukuto, J. M. *Drug Metabolism and Disposition*, 27:502–509, 1999.
- [52] Zapol, W. M, and Hurford, W. E. *New Horizons*, 1:638–650, 1993.