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Cluster and multi-linear regression analyses guided identification of molecular descriptors that account for cyclooxygenase activities

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ABSTRACT

Cluster analysis and multiple linear regression analysis were employed to determine the distinct group of molecular descriptors that largely account for the biological activity of known inhibitors against cyclooxygenase (COX). In 157 out of 3227 molecular descriptors the nonselective COX inhibitors and COX-2 selective inhibitors form two distinct clusters. Multiple linear regression analysis returned three equations accounting for the pIC_{50} of the inhibitors against COX. For the pIC_{50} of nonselective COX inhibitors against COX-1, the molecular descriptors with the highest importance include GGI1, GGI10, SM1_Dzm and Eta_alpha_A. For the pIC_{50} of nonselective COX inhibitors against COX-2, molecular descriptors Ts, GGI1, SpMax3_Bhv and GGI10 were key to the observed activity. The observed variation in pIC_{50} of COX-2selective inhibitors against COX-2 were attributed toSpMax3_Bhp, SpMax_AEAdm, VE2_Be, SM5_L, Eta_betaS, G2, Eig04_EAed, H_DzZ, SM4_L and VE3_Bp. The results of the study can be used to understand the nature of COX inhibitors and to further facilitate the development of COX-2 selective inhibitors.

Keywords: COX inhibitors, COX-1, COX-2, Cluster Analysis, Multiple Linear Regression Analysis, QSAR, NSAID, DRAGON® Descriptors

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INTRODUCTION

Cyclooxygenase (COX) is a rate-limiting enzyme that catalyzes the conversion of arachidonic acid, an essential fatty acid present in cell membrane phospholipids and liberated by phospholipase, into prostaglandins (PGs) and prostanoids[1]. It is recognized that cyclooxygenase (COX) has 2 isoenzymes: COX-1 and COX-2. COX-1is constitutively expressed in the gastrointestinal (GI) tract. COX-1 is the good COX because it makes essential hormones for the protection of the lining of the stomach and kidneys [1,2]. On the other hand, COX-2 promotes pain and inflammation, hence it is considered the bad COX[3]. COX-2 expression is normally low but is induced by inflammatory stimuli and cytokines. The anti-inflammatory actions of COX inhibitors are caused by the inhibition of COX-2, whereas the unwanted side effects, such as gastrointestinal and renal toxicity, are caused by the inhibition of COX-1 [4].Most non-steroidal anti-inflammatory drugs (NSAIDs) provide relief from inflammation and pain in the human body by blocking COX-2. Unfortunately, they also block COX-1, *i.e.* not selective forCOX-2.However, the intense efforts resulting in the synthesis of hundreds of COX-2 selective inhibitors [5] have sparked hope for further development of anti-inflammatory drugs without harmful side effects [6].

Inhibitors of COX activity include: (1) conventional non-selective non-steroidal anti-inflammatory drugs (ns-NSAIDs); (2) selective COX-2 inhibitors (COXIBs); and (3) COX-1 inhibitors. NSAIDs are one of the most commonly used classes of medication in the world. These drugs function by inhibiting both COX-1 and COX-2, relieving pain and inflammation but eliciting gastrointestinal (GI) toxicity [7]. Subsequently, COXIBs (*e.g.*

celecoxib, etoricoxib, lumiracoxib, rofecoxib and valdecoxib) were developed to reduce the incidence of serious upper GI adverse events associated with the administration of traditional NSAIDs [8]. However, the reduced incidence of adverse effects demonstrated by two selective COX-2 inhibitors (*i.e.* rofecoxib and lumiracoxib) has been countered by an increased incidence of myocardial infarction and stroke [9,10]. This prompted the withdrawal of blockbuster drug Rofecoxib (sold commercially as Vioxx) from the US market [11]. Thus, there is a need to further understand the characteristic of COX inhibitors in the hope of developing much safer pain relievers.

With the advent of modern technology, faster and less resource-consuming computational methods are now available to study the detailed characteristics of a compound. It has now become possible to extract molecular descriptors based on the structures of compounds that can subsequently be used to evaluate molecular structure-activity or structure-property relationships, as well as for similarity analysis and high throughput screening of molecule databases [12].A molecular descriptor is the final result of a logic and mathematical procedure, which transforms chemical information encoded within a symbolic representation of a molecule into a useful number or the result of some standardized experiment [13]. Recently, we have demonstrated the utility and application of molecular descriptors in predicting the biological activity of a set of compounds [14, 15].

This study aims to distinguish non-selective COX inhibitors from selective COX-2 inhibitors by cluster analysis of DRAGON®-derived molecular descriptors, and to identify the distinct set of molecular descriptors that influence the observed activity of the COX inhibitors by multiple linear regression analysis. The data obtained from this work can be utilized in further development of COX-2 selective inhibitors.

EXPERIMENTAL SECTION

A data set of 32 common nonselective COX inhibitors and 319 COX-2 selective inhibitors were obtained from existing literature [5, 16].The two-dimensional structures of the inhibitors were drawn using the Marvin Sketch software [17] running in Windows 7 OS. Each structure was geometrically optimized at semi-empirical Austin Method 1 (AM1) level using the HyperChem software [18]. The resulting structures were used as input in subsequent property calculations in the DRAGON® program [19]. Like in our previous study [15], DRAGON® was used to generate thousands of molecular descriptors (i.e. constitutional and ring descriptors, topological, connectivity, and information indices; walk and path, and functional group counts, etc.) for each molecule.

Prior to cluster analysis, the descriptors with a standard deviation of zero (*i.e.* invariant throughout the set) were not included in the analysis. The remaining molecular descriptors were made to undergo hierarchical cluster analysis using IBM SPSS Statistics software [20].The data was then analyzed to determine a set of molecular descriptors for which COX-2 selective inhibitors formed a distinct cluster.

Multiple linear regression analysis was done to determine which among the molecular descriptors identified in cluster analysis significantly account for the variation in biological activity exhibited by COX inhibitors. Three equations were generated: (1) for the pIC_{50} of nonselective COX inhibitors against COX-1, (2) for the pIC_{50} of nonselective COX inhibitors against COX-2, and (3) for the pIC_{50} of COX-2 selective inhibitors against COX-2. Bivariate correlation was also done to check for any correlations between molecular descriptors.

RESULTS AND DISCUSSION

Of the 319 COX-2 selective inhibitors, 305 belong to nine different families of compounds [5], namely, the derivatives of pyrrole (Family A), imidazole (Family B), cyclopentene (Family C), benzene (Family D), pyrazole (Family E), spiroheptene (Family F), spiroheptadiene (Family G), isoxazole (Family H), and thiophene (Family I). The remaining 14 COX-2 inhibitors obtained from chemical databases are diverse in structure and do not belong to any family in particular. A set of 35 common nonselective COX inhibitors, most of which are drugs presently available in the market, were also included in the study. The 2D structures of this data set of inhibitors were drawn and geometrically optimized at AM1 level before generating their molecular descriptors.

A total of 4885 molecular descriptors, grouped into 29 blocks, namely, constitutional indices (43), ring descriptors (32), topological indices (75), walk and path counts (46), connectivity indices (37), information indices (48), 2D matrix-based descriptors (550), 2D autocorrelations (21), Burden eigenvalues (96), P_VSA-like descriptors (45), ETA indices (23), edge adjacency indices (324), geometrical descriptors (38), 3D matrix-based descriptors (90), 3D autocorrelations (80), RDF descriptors (210), 3D-MoRSE descriptors (224), WHIM descriptors (114), GETAWAY descriptors (4), Randic molecular profiles (41), functional group counts (154), atom-centered fragments (115), atom-type E-state indices (170), CATS 2D (150), 2D atom pairs (1596), 3D atom pairs (36), molecular properties (36), and drug indices (27), were successfully computed and obtained.

The goal of cluster analysis is to identify the actual groups, with the objects within a group similar (or related) to one another and different (or unrelated to) the objects in other groups [21]. Among the various techniques, hierarchical clustering is one of the most straightforward methods of forming clusters [21]. From an initial 4885 molecular descriptors, the number of descriptors was subsequently narrowed down to 3227, after excluding those descriptors that do not vary throughout the set.

Cluster analysis of each of the 3227 molecular descriptors returned 157 descriptors for which COX-2 selective inhibitors form a distinct cluster from the nonselective COX inhibitors. However, some inhibitors we initially grouped under nonselective COX inhibitors almost always cluster with the COX-2 selective inhibitors. In contrast, some inhibitors we grouped under COX-2 selective inhibitors also cluster with nonselective COX inhibitors. Specifically, Lumiracoxib, etodolac, and N-tert-butyl-α –phenylnitrone are inhibitors initially classified under COX-2 selective inhibitors but were mostly found in cluster with nonselective COX-2 inhibitors (Figure 1.) Three more compounds grouped under COX-2 selective inhibitors were most often seen clustering with nonselective COX inhibitors (Table 1).

On the other hand, fifteen compounds initially grouped under nonselective COX inhibitors cluster with COX-2 selective inhibitors in many molecular descriptors, with lornoxicam, licofelone and talniflumate being the most frequent 'outlier' in most COX-2 selective inhibitor groups.Table 1 shows the complete list of inhibitors clustering with the group opposite their initial classification.The comparable activities of each inhibitor against COX-1 and COX-2seem to explain the observed incongruence.

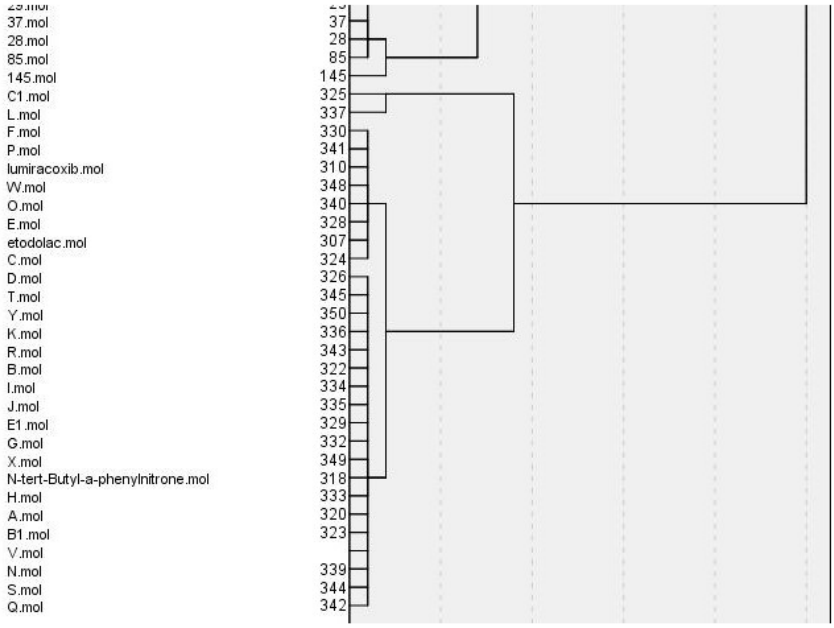
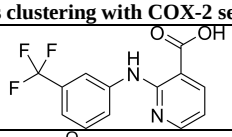
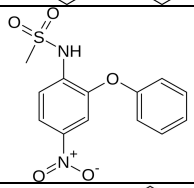
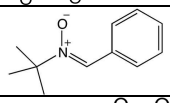
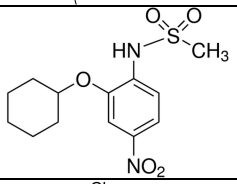
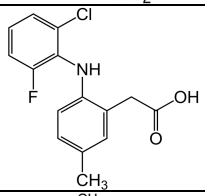
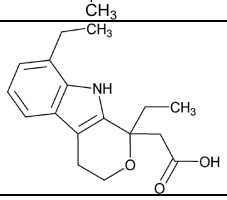
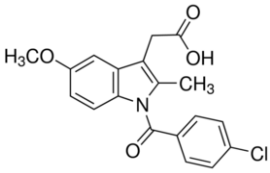
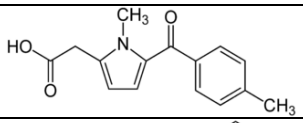
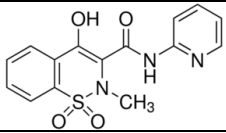
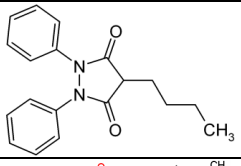
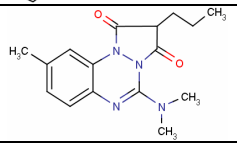
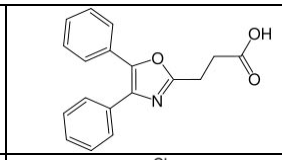
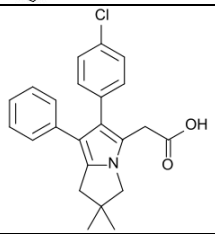
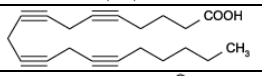
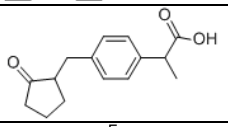
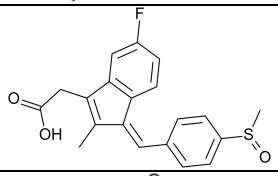
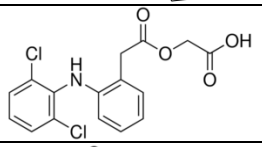
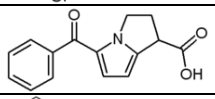
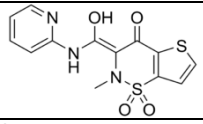
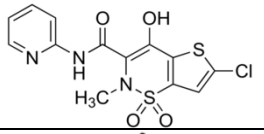
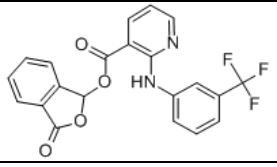


Figure 1. Portion of the dendrogram for molecular descriptor Eta_B which shows lumiracoxib, etodolac and N-tert-butyl- α – phenylnitrone clustering with nonselective COX inhibitors. The nonselective COX inhibitors were labeled with letters to easily distinguish from the COX-2 selective inhibitors, labeled with numbers

Table 1. The structures and COX activity[5,16] ofinhibitors often encountered as outliers in cluster analysis

Inhibitor	Structure	pIC_{50}	
		COX-1	COX-2
Nonselective COX inhibitors clustering with COX-2 selective inhibitors			
Niflumic acid		5.975	6.037
Nimesulide		4.155	5.896
N-tert-butyl-α-phenylnitrone		-	-
NS-398		5.161	6.456
Lumiracoxib		4.174	6.886
Etodolac		4.921	5.658
COX-2 selective inhibitors clustering with nonselective COX inhibitors			
Indomethacin		7.745	7.585
Tolmetin		6.237	5.842
Piroxicam		6.119	5.046
Phenylbutazone		5.523	5.421
Apazone		-	-

Oxaprozin		5.658	4.444
Licofelone		6.097	4.523
5,8,11,14-eicosatetraenoic acid		2.097	-
Loxoprofen		4.602	5.0
Sulindac		5.721	5.917
Aceclofenac		4.0	6.097
Ketorolac		4.502	4.218
Tenoxicam		4.75	4.433
Lornoxicam		8.301	8.097
Talniflumate		-	-

We generatedmulti-linear regression equations to determine which of the 157 descriptors significantly account for the variation of biological activity the inhibitors exhibit against COX. However, the outliers in the cluster analysis were not included from the linear regression analysis dataset, leaving us with 16 nonselective COX inhibitors and 313 COX-2 selective inhibitors.

Linear regression of the 157 descriptors for the nonselective COX inhibitors against pIC_{50} for COX-1 returned 15 descriptors, with an R^2 value of 1.0. This means that these 15 descriptors can fully account for the observed variation in pIC_{50} of the inhibitors against COX-1. However, 15 descriptors are too much for only 16 samples. The general rule of thumb is that to be able to detect reasonable size effects with reasonable power, 10-20 observations per parameter estimated are needed [22]. However, an "oversimplified guideline" allows the ratio of sample size to number of free parameters to go down to as low as 5:1 [23-25].

To narrow down the 15 molecular descriptors to four, dimension reduction via jackknifing was employed. In this method, one descriptor was left out of the regression analysis dataset at a time, and the resulting r-squared for the 14 remaining descriptors was computed. The lowest R^2 value corresponds to the largest change in R^2 value, which

means that the variable left out of the regression analysis for that particular R^2 value gives the highest contribution to the observed value of pIC_{50}. From 15 molecular descriptors, four descriptors were retained and subjected to linear regression analysis.For the pIC_{50} of the inhibitors against COX-1 the following equation was obtained:

pIC_{50} = 0.055(GGI1) + 21.923(GGI10) + 0.574(SM1_Dzm)+ 51.726(Eta_alpha_A) – 19.963
R^2 = 0.61 (equation 1)

The four molecular descriptors with the highest significant contribution to the pIC_{50} of the nonselective COX inhibitors against COX-1 are GGI1 (topological charge index of order 1), GGI10 (topological charge index of order 10), SM1_Dzm (spectral moment of order 1 from Barysz matrix weighted by mass), and Eta_alpha_A (ETA average core count). Bivarariate correlation analysis revealed that these four descriptors have no significant correlations with each other.

GGI1 and GGI10 are examples of topological charge indices. The ability of topological charge indices to describe molecular charge distribution has been established by correlating them with the dipole moment of a heterogeneous set of hydrocarbons [26].The extended topochemical atom (ETA) indices likeEta_alpha_A is related to size or bulk of the molecule [27]. SM1_Dzm is a 2D matrix descriptors derived from the Barysz matrix weighted by mass. The Barysz matrix is a symmetric weighted distance matrix accounting contemporarily for the presence of heteroatoms and multiple bonds in the molecule [28].

Apparently, the biological activity of nonselective COX inhibitors against COX-1 depends chiefly on the size or bulk of the molecule (Eta_alpha_A), the molecular charge distribution (GGI1 and GGI10) and the presence of heteroatoms and multiple bonds (SM1_Dzm). The positive coefficients of these predictors in the model equation imply that the pIC_{50} of the nonselective inhibitors against COX-1 will increase when these molecular properties are increased.

Likewise, the four molecular descriptors with the highest contribution to the pIC_{50} of the nonselective COX inhibitors against COX-2 are Ts (T total size index / weighted by I-state), GGI1 (topological charge index of order 1), SpMax3_Bhv (largest eigenvalue n. 3 of Burden matrix weighted by van der Waals volume), and GGI10 (topological charge index of order 1). Linear regression of these four descriptors against pIC_{50} for COX-2 gave the equation:

pIC_{50} = -0.017(Ts) + 0.889(GGI1) + 2.683(SpMax3_Bhv) -17.153(GGI10) – 6.167
R^2 = 0.29 (equation 2)

It appears that the 4-predictor model can account only 29% of the observed variability in pIC_{50} of nonselective COX inhibitors against COX-2. This means there are no remarkable predictors of COX-2 activity from nonselective COX inhibitors. Meanwhile, the Burden eigenvalues such as the SpMax3_Bhv in equation 2 have strong empirical relationship to electron distribution of the molecule as a whole and the calculated indices are able to predict the logP of molecules, with its predictive power being superior compared to connectivity indices [29].Ts is a part of a group of 3D structural descriptors called WHIM (Weighted Holistic Invariant Molecular) descriptors, which are built in such a way that they capture relevant 3D information regarding different features on molecular structure: size, shape, symmetry and atom distribution with respect to invariant reference frames [30]. The coefficients in the model equation imply that the bioactivitydecreases with the increase of Ts and GGI10 values, and increases with the increase of GGI1 and SpMax3_Bhv.

The regression analysis of descriptors derived from COX-2 selective inhibitors using stepwise method returned twelve models, with the first model yielding one descriptor, the seconds yielding two descriptors, and so on and so forth. Choosing the tenth model gives us a inhibitor-descriptor ratio of approximately 31:1, which is way above the minimum criterion [22-25]. The generated model 10 gives the equation:

pIC_{50} = 0.562(SpMax3_Bhp) – 1.173(SpMax_AEAdm) + 47.694(VE2_Be) – 29.781(SM5_L) – 0.563 (Eta_betaS) + 0.433(G2) – 0.335(Eig04_EAed) – 0.021(H_DzZ) + 48.924(SM4_L) – 2.970(VE3_Bp) – 96.497
R^2 = 0.60 (equation 3)

Thus, the top ten molecular descriptors which accounts for the variation in the pIC_{50} of the COX-2 selective inhibitors against COX-2 are SpMax3_Bhp (largest eigenvalue n. 3 of Burden matrix weighted by polarizability), SpMax_AEAdm (leading eigenvalue from augmented edge adjacency mat. weighted by dipole moment), VE2_Be (average coefficient of the last eigenvector from Burden matrix weighted by Sanderson electronegativity), SM5_L (spectral moment of order 5 from Laplace matrix), Eta_betaS (ETA sigma VEM count), G2 (gravitational index G2

(bond-restricted)), Eig04_EAed (eigenvalue n. 4 from edge adjacency mat. weighted by edge degree), H_DzZ (Harary-like index from Barysz matrix weighted by atomic number), SM4_L (spectral moment of order 4 from Laplace matrix), and VE3_Bp (logarithmic coefficient sum of the last eigenvector from Burden matrix weighted by polarizability). The model equation indicates that the pIC_{50} of COX-2 selective inhibitors has a direct relationship with SpMax3_Bhp, VE2_Be, G2 and SM4_L, and has an inverse relationship with SpMax_AEAdm, SM5_L, Eta_betaS, Eig04_EAed, H_DzZ and VE3_Bp.

Three of these descriptors are based on the Burden matrix, which correlates strongly with the electron distribution of a molecule, and is obtained from varied weighing schemes (Sanderson electronegativity for VE2_Be and polarizability for both SpMax3_Bhp and VE3_Bp). Descriptor H_DzZ is based on the Barysz matrix, and is therefore concerned with the presence of heteroatoms and multiple bonds in the molecule. Eta_betaS is an ETA index, and is sufficiently rich in chemical information [31,32]. Descriptors SpMax_AEAdm and Eig04_EAed are both based on the edge adjacency matrix, which deals with the position of atoms in a molecule with respect to each other [33]. Two descriptors, SM4_L and SM5_L, are based on the Laplace matrix, which gives the spanning tree number of a molecular graph. The number of spanning trees of a molecular graph is a measure of molecular complexity for polycyclic molecules; the higher the number of spanning trees, the higher the complexity of the molecular structure [28]. Lastly, the G2 molecular descriptor is a gravitational index, a geometrical descriptor reflecting the mass distribution in a molecule. The gravitational indices are related to the bulk cohesiveness of the molecules accounting, simultaneously, for both atomic masses (volumes) and their distribution within the molecular space. The G2 descriptor, however, is restricted to mass distribution in pairs of bonded atoms [34]. Furthermore, it is worthy of note that the equation for COX-2 selective inhibitors shares no descriptors with the equations for nonselective inhibitors. This an indication of the adeptness of our generated models in differentiating the two COX inhibitor groups from each other.

CONCLUSION

The cluster analysis of the molecular descriptors of nonselective COX and COX-2 selective inhibitors were done to determine which structure-based properties provide the distinction between the two groups and to furnish some insightsthat might prove helpful in the design of next generation COX-2 inhibitors. Here we have identified 157 molecular descriptors that differentiate COX-2 selective inhibitors from nonselective COX inhibitors. These descriptors were narrowed down to four descriptors accounting for the pIC_{50} of nonselective COX inhibitors against COX-1, another four descriptors accounting for the pIC_{50} of nonselective COX inhibitors against COX-2, and ten descriptors accounting for the pIC_{50} of COX-2 selective inhibitors against COX-2. The detailed understanding of these descriptors and their distinct influenceon COX activity may facilitate further development of new and improved COX-2 selective inhibitors.

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REFERENCES

[1] Z Radi;NK Khan, *Exp.Toxicol. Pathol.*,**2006**, 58, 163-173.
[2] P Patrignani;S Tacconelli;MG Sciulli;ML Capone,*Brain Res. Rev.*, **2005**, 48, 352-359.
[3] CJ Hawkey,*The Lancet*,**1999**, 353, 307-314.
[4] JR Vane;RM Botting,*Semin. Arthritis Rheum.*,1997, 26(6), 2-10.
[5] P Chavatte;S Yous;C Marot;N Baurin;D Lesieur, *J. Med. Chem.*, **2001**, 44(20), 3223-30.
[6] CJ Hawkey, *Best Pract. Res.Clin.Gastroenterol.*,**2001**, 15(5), 801-820.
[7] S Rahman; A Malcoun, *Prim.Care*, **2014**, 41(4), 803-821.
[8] ML Capone;S Tacconelli;L Di Francesco;A Sacchetti;MG Sciulli;PPatrignani,*Prostagl. Oth. Lipid Mediat.*,**2006**, 82, 85-94.
[9] PM Kearney;C Baigent;J Godwin;H Halls;J Emberson;C Patronoonal,*The BMJ*, **2006**, 332(7553),1302-1308.
[10] EM Antman;JS Bennett;A Daugherty;C Furberg;H Roberts; KA Taubert, *Circulation*,**2007**, 115, 1634-1642.
[11] CP Cannon;PJ Cannon,*Science*, **2012**, 336(6087), 1386-1387.
[12] A Mauri;V Consonni;M Pavan;R Todeschini,*MATCH Commun. Math.Co.*,**2006**, 56, 237-248.
[13] R Todeschini; V Consonni,*Handbook of Molecular Descriptors*. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, **2000**.
[14] AMM Reyes; JB Billones, *Kimika*,**2013**, 24(1), 8-17.
[15] LT Billones;JB Billones,*J. Chem. Pharm. Res.*, **2014**, 6(8), 209-217

[16] a) AbcampIc. (http://www.abcam.com), b) Cayman Chemical Co. (https://www.caymanchem.com), c) MedChem Express (http://www.medchemexpress.com), d) Santa Cruz Biotechnology Inc. (http://www.scbt.com), e) Selleck Chemicals (http://www.selleckchem.com),Sigma Aldrich Co. LLC (http://www.sigmaaldrich.com)
[17] ChemAxon Ltd. (https://www.chemaxon.com)
[18] Hypercube Inc. (http://www.hyper.com)
[19] Talete SRL (http://www.talete.mi.it)
[20] IBM Corporation (http://www.ibm.com)
[21] M Norusis, *SPSS 16.0 Statistical Procedures Companion* (2nd edition). Prentice Hall Press Upper Saddle River, NJ, USA, **2008**.
[22] FE Harrell,*Regression Modeling Strategies with Applications to Linear Models, Logistic Regression, and Survival Analysis.* New York: Springer **2001**.
[23] PM Bentler; CH Chou,*Socio.Meth.Res.*, **1987**, 16, 78-117.
[24] CE Lance;RJ Vandenberg,In *Statistical and Methodological Myths and Urban Legends*: *Doctrine, Verity and Fable in Organizational and Social Sciences*. Routledge, Taylor & Francis Group, NY, **2008**.
[25] E Vittinghoff; CE McCulloch, *Am. J.Epidemiol.*,**2006**, 165(6), 710-718.
[26] J Galvez;M Garcia;T Salabert;R Soler, *J. Chem. Inf. Comp.Sci.*,**1994**, 34, 520-525.
[27] K Roy;RN Das,*SAR QSAR Environ.Res.*, **2011**, 22(5-6), 451-472.
[28] R Todeschini;V Consonni, V, In *Molecular Descriptors for Chemoinformatics*. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, **2009**.
[29] FR Burden, *Quan.Struct. Act.Rel.*, **1997**, 16, 309-314.
[30] P Gramatica, *QSAR Comb.Sci.*,**2006**, 25(4), 327-332.
[31] K Roy; G Ghosh,*Internet Electron. J. Mol. Des.*,**2003**, 2, 599–620.
[32] K Roy;G Ghosh, *Curr. Pharm. Des.*,**2010**, 16(24), 2625-39.
[33] D Janezic;A Milicevic;S Nikolic;N Trinajstic,*Graph-Theoretical Matrices in Chemistry*. CRC Press, Taylor & Francis Group, LLC, **2015**.
[34] R Todeschini;V Consonni,*Descriptors from Molecular Geometry – Milano Chemometrics*(n.d).Retrieved from http://michem.disat.unimib.it/.