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Drug Analogs of COX-2 Selective Inhibitors Lumiracoxib and Valdecoxib Derived from in silico Search and Optimization

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Abstract:

The medicinal activity of COX-2 inhibitors are sufficiently beneficial to urge the search for new drug designs. This study presents 16 analogs of lumiracoxib and 10 analogs to valdecoxib having properties suitable as COX-2 inhibitors. For lumiracoxib analogs the mean Log P, polar surface area, and formula weight are 3.00, 70.46 Å², and 276.60, respectively. For valdecoxib analogs the mean Log P, polar surface area, and formula weight are 3.65, 68.46 Å², and 322.32, respectively. Grubb's test analysis of seven properties for seven known COX-2 selective inhibitors and those of 26 analog compounds indicated no outliers. The unpaired t-test compared Log P and polar surface area of seven known COX-2 inhibitors to all 26 analogs and found no difference. All 26 analogs showed no violation of the Rule of 5, this being an indicator of favorable bioavailability. Hierarchical cluster analysis by single linkage indicated lumiracoxib is most similar to analogs 2, 4, 5, 6, 7, 9, 10, 11, 14, 15, 16, and 17. Valdecoxib has highest similarity to analogs 8, 19, 21, 22, 23, 26, 27, and 28. Multiple regression analysis successfully produced equations for prediction of similar compounds to lumiracoxib and valdecoxib. Path analysis indicated that number of atoms, oxygen & nitrogen atoms, and Log P are the greatest determinants for formula weight for known COX-2 inhibitors. Criteria for molecular properties is established for identifying COX-2 inhibitors. These 26 analogs show much potential for active COX-2 inhibition.

Keywords:

Anti-inflammatory, COX-2, cyclooxygenase, lumiracoxib, pattern recognition, valdecoxib.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of drugs that provide analgesic (pain reducer), antipyretic (fever-reducing), and anti-inflammatory effects [1]. Clinical application of NSAIDs include the following: rheumatoid arthritis, osteoarthritis, gout, headache (migrane), pain due to inflammation and tissue injury, pyrexia, postoperative pain, pain associated with Parkinson's disease, and dysmenorrhea [1].

Selective COX-2 (cyclooxygenase-II enzyme) inhibitors avoid the complications and potentially fatal bleeding within the gastrointestinal track induced by COX-1 inhibitors [2-6].

Although the trial data varies substantially among COX-2 selective inhibitors on myocardial infarction (MI) events, an increased risk of MI exists shown in evidence based on patient-year exposure [7,8]. Desired beneficial medicinal activity maintains the strong interest in COX-2 selective inhibitors. A single daily dose of celecoxib has been shown to significantly reduce the occurrence of colorectal adenomas [9,10], and could be effective in preventing cutaneous squamous cell carcinomas and basal cell carcinomas in high risk individuals [11]. Studies focusing on the role of COX-2 in the tumorigenesis of breast cancer have strongly hinted to the potential of COX-2 specific inhibitors to prevent and treat breast cancer [12,13]. Likewise studies have suggested a similar application of COX-2 selective inhibitors for prostate cancer [14].

Previous studies have shown that inhibition of COX-2 enzyme will induce useful therapeutic effects on solid tumors and deter tumor cell growth [15].

Essentially the reduction of the activity of COX-2 enzyme or protein expression could inhibit cancer cell growth and NSAIDs can be employed for chemoprevention of cancer [16,17]. Use of COX-2 selective inhibitors parallel a doxorubicin regimen will inhibit growth of hepatocellular carcinoma cells which is the fourth leading cause of cancer related mortality worldwide [15]. However the available data clearly extends a cautionary flag concerning the risk of cardiovascular events or stroke with administration of COX-2 selective inhibitors [18-20].

Nevertheless the considerable potential benefits of COX2 selective inhibitors strongly supports the continued study and development of new drug designs. This study presents 26 drug structures analogous to known COX-2 selective inhibitors and possessing properties associated with successful drugs.

MATERIALS AND METHODS

Properties and Molecular Modeling

Numerical values of molecular properties and description of drug structures were accomplished by utilizing Molinspiration (Molinspiration Cheminformatics, Nova ulica 61, SK900 26 Slovensky Grob, Slovak Republic). In *silico* search for drug structures analogous to lumiracoxib was accomplished by Molinspiration 'search by similarity' with a success rate of less than 16% (Molinspiration Cheminformatics, Nova ulica 61, SK-900 26 Slovensky Grob, Slovak Republic, <http://www.molinspiration.com/>). In *silico* search for drug structures analogous to valdecoxib was accomplished by Molinspiration 'search by similarity' with a success rate of less than 10%. Additional elucidation of structure components was enhanced and enabled by utilizing ACD/ChemSketch Modeling v. 12.01 (Advanced Chemistry Development, 110 Yonge Street, Toronto Ontario, M5C 1T4 Canada, <http://www.molinspiration.com/services/search.html>).

Determination of drug-likeness scores for kinase inhibitor, protease inhibitor, and enzyme inhibitor was determined by Molinspiration Cheminformatics (<http://www.molinspiration.com/cgi-bin/properties>).

Pattern Recognition and Multivariate Statistical Analysis

To identify underlying associations and patterns within the numerical properties required the use of various pattern recognition techniques. Included in these analyses are hierarchical cluster analysis accomplished by KyPlot v. 2.0 Beta 15 (copyright Koichi Yoshioka 1997-2001). In addition, analysis of non-hierarchical K-means cluster analysis, oneway ANOVA, Kruskal-Wallis test, Mann-Whitney U Test, and 95% ellipses were performed by PAST v. 2.06 (copyright Oyvind Hammer, D.A.T. Harper 1999-2008). Path Analysis (the determination of path coefficients) was done by OpenStat statistics package (copyright W.G. Miller, November 2010; <http://openstat.software.informer.com/>).

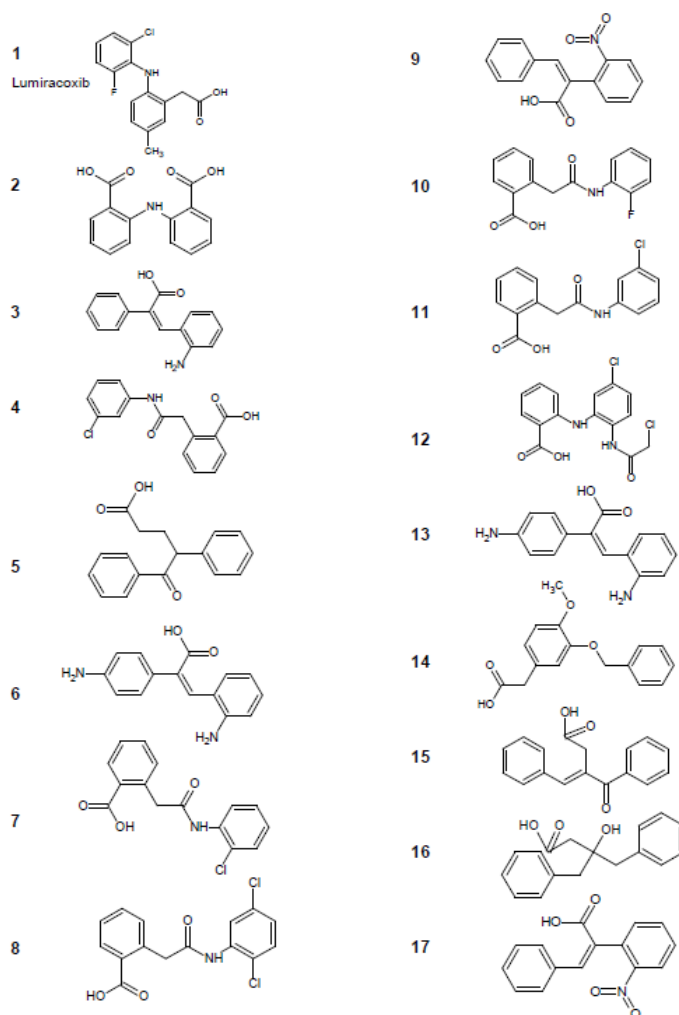


Fig. (1). Structures of drug analogs to lumiracoxib. Characteristic common to all drugs are: 1) Two aromatic rings; 2) At least two oxygen atoms; 3) At least one carboxyl group. Other atoms present but not universal are halogen atoms, nitrogen atoms, and amide groups.

Various Statistical Analysis Data

Statistical analysis of numerical data including Pearson r correlation and descriptive statistics were accomplished by Microsoft EXCEL v. 14.0.6112.5000 (EXCEL Professional plus 2010). Multiple regression analysis of molecular property values was accomplished by GraphPad InStat version 3.00 (GraphPad Software, Inc., San Diego California USA; www.graphpad.com). Determination of numerical outliers was done by Grubb's test (extreme studentized deviate), and t test to compare two means was done by (<http://www.graphpad.com/quickcalcs/>).

RESULTS

Utilizing lumiracoxib as the parent structure for an *in silico* search for similarity of scaffolding and substituents did identify a set of 16 analogs. The search utilized an in-situ library scrutiny and produced many structures. The count of suitable structures actually produced from the total number of generated compounds was less than 16% of the total. Shown in (Fig. 1), in addition to the parent compound lumiracoxib (drug 1), are 16 analogs that are examined for suitability as potential COX-2 selective inhibitors. Structures of drug analogs to lumiracoxib. Characteristic common to these analogs are: 1) Two aromatic rings; 2) At least two oxygen atoms; 3) At least one carboxyl group; 4) At least one –OH or –NHn group. Other atoms present but not universal are halogen atoms, nitrogen atoms, methoxy groups, alkene groups, and amide groups.

Utilizing valdecoxib as the parent structure for in silico search for similarity in scaffolding and substituents a set of 10 analogs were identified. The search by in situ library survey and scrutiny identified various complex structures. The number of suitable structures actually produced of the total number of generated compounds was less than 10% of the total. Shown in (Fig. 2), in addition to the parent compound valdecoxib (drug 1), are 10 analogs that are examined for suitability as potential COX-2 inhibitors. Structures of drug analogs to valdecoxib (sulfonamide based) have characteristic common to all: 1) Two aromatic rings; 2) At least two oxygen atoms; 3) One sulfonamide group; 4) At least one nitrogen atom. Other atoms present but not universal are chlorine atom, oxadiazole substituent, and benzimidazole groups.

Previous studies have determined and compared the properties of established COX-2 selective inhibitors [21]. Those results, inclusive of lumiracoxib and valdecoxib, are presented in Table 1 with statistical analysis shown in Table 2. Properties included are Log P, polar surface area (PSA), formula weight (FW), molecular volume (MV), etc, which will be analyzed to show that analog compounds in (Figs. 1 and 2) have the capacity to be COX-2 inhibitors. The mean values for properties of known selective COX-2 inhibitors (see summary statistics in Table 2) for Log P, PSA, FW, number of atoms, and MV are 2.939, 70.41 Å², 338.5, 23, and 282.7, respectively. The respective properties determined for lumiracoxib analogous compounds are compiled in Table 3, inclusive of Log P, PSA, FW, etc. These properties will be the focus of statistical and pattern recognition analysis to prove their potential as COX-2 inhibitors. Likewise the

respective properties determined for valdecoxib analogous compounds are compiled in Table 4, inclusive of Log P, PSA, FW, etc. Pearson r determinations for Table 3 and Table 4 showed that a strong positive correlation occurs ($r = 0.5298$) between Log P and number of atoms with a strong positive correlation ($r = 0.6342$) between Log P and formula weight. In addition, a very strong positive correlation occurs ($r = 0.7047$) between polar surface area and total oxygen and nitrogen atoms.

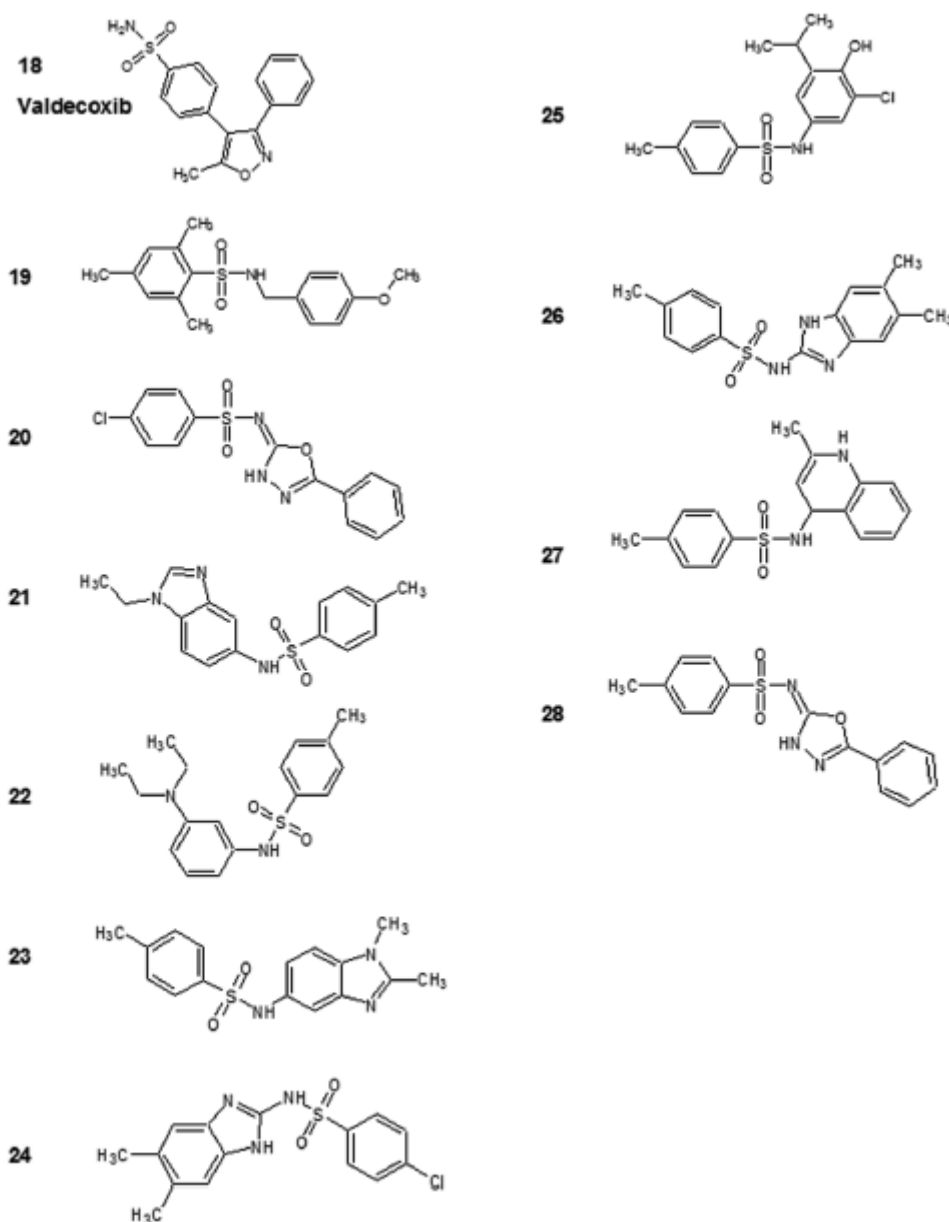


Fig. (2). Structures of drug analogs to valdecoxib. Characteristic common to all drugs are: 1) Two aromatic rings; 2) At least two oxygen atoms; 3) At least one sulfonamide group. Other structure present but not universal are chlorine atom, oxadiazole, and benzimidazole group.

The summary statistics for lumiracoxib based analogs (see Fig. 1) are presented in Table 5. These determinations will be compared to findings for known selective COX-2 inhibitors presented in Table 2. Likewise the summary statistics for all valdecoxib based analogs (see Fig. 2) are presented in Table 6. These determinations will be compared to numerical values for known COX-2 selective inhibitors presented in Table 2. The mean values of Log P, PSA, FW, number of atoms, and molecular volume shown in Table 2 (known COX-2 selective inhibitors) is shown to have no significant difference from mean values of the same properties for both lumiracoxib (Table 5) and valdecoxib (Table 6) analogs.

Analysis of variance (ANOVA) is a general method to test for significant differences between class means and enables all classes to be compared with each other simultaneously and not individually [22]. The one-way analysis of variance is used to test the claim that two or more population means are equal [22]. One-way ANOVA was applied to test that Log P, polar surface area, and number of rotatable bonds of seven known specific COX-2 inhibitors from Table 1 to the same properties of 26 analogs found in Table 3 and Table 4. The results showed the means of the three molecular properties (e.g. Log P, polar surface area, rotatable bonds) for 7 known specific COX-2 inhibitors and 26 analog compounds to be the same at $P > 0.05$ ($P = 0.09866$, $P = 0.925$, $P = 0.2202$, respectively).

Using the Kruskal-Wallis Test, we can decide whether the population distributions are identical [22]. If $P > 0.05$ then accept the null hypothesis that two or more populations originate from the identical distributions. Again the KruskalWallis test shows polar surface area and Log P of seven known selective COX-2 inhibitors of Table 1 to the same properties of 26 analogs shown in Table 3 and Table 4 are in identical distributions, $P > 0.05$ ($P = 0.989$, $P = 0.1718$, respectively).

Grubbs' test (or extreme studentized deviate test) is a statistical test used to detect numerical outliers [22]. Comparing known COX-2 inhibitors of Table 1 to all analogs of Tables 3 and 4 by Grubb's test showed no outliers in the following properties: polar surface area, number of atoms, molecular weight, number of rotatable bonds, number of oxygen or nitrogen or hydroxyl or amine groups, and molecular volume.

The unpaired t-test compares the means of two groups and can be used to decide if the averages of two samples are significantly different. If the P value is large, the data do not give you any reason to conclude that the overall means differ [22]. Again when applied to polar surface area and Log P for seven COX-2 inhibitors of Table 1 to all analogs of Tables 3 and 4 the $P = 0.8805$ and $P = 0.3543$ (i.e. $P > 0.05$) so no difference exists among the means of these drugs for properties tested.

Path analysis is used to describe the directed dependencies among a set of variables and is used for testing cause and effect with the aim to give estimates of the magnitude and significance of hypothesized causal connections between sets of variables [23,24]. Path Analysis for the formation of formula weight showed path coefficients (PC) for

descriptors Log P (PC = 0.824), number of atoms (PC = 2.024), number of oxygens & nitrogens (PC = 1.616), and rotatable bonds (PC = -0.397). This suggests that number of oxygen, nitrogen, and total number of atoms, as well as Log P important as well, plays the most prominent role in formula weight of drugs (rotatable bonds are nominal or extraneous).

Table 1. Properties of COX-2 selective inhibitors.

Drug	Log P	Polar Surface Area (Angstroms ²)	Number of Atoms	Molecular Weight	Number of Oxygen & Nitrogen	Number of -OH & -NH _n	Violations Of Rule 5	Number of Rotatable Bonds	Volume (Angstroms ³)
Celecoxib	3.611	77.991	26	381.379	5	2	0	4	298.65
Rofecoxib	0.706	60.447	22	314.362	4	0	0	3	264.79
Valdecoxib	2.734	86.197	22	314.366	5	2	0	3	263.553
Parecoxib	3.308	89.272	26	370.43	6	1	0	5	317.013
Lumiracoxib	4.477	49.326	20	293.75	3	2	0	4	246.686
Etoricoxib	3.01	59.926	24	358.85	4	0	0	3	296.637
Firocoxib	2.724	69.681	23	336.409	5	0	0	5	291.581

Table 2. Summary statistics for known COX-2 selective inhibitors.

Property	Mean	Minimum	Maximum	Median	Standard Deviation
Log P	2.939	0.706	4.477	3.01	1.157
Polar Surface Area (Angstroms ²)	70.41	49.33	89.27	69.68	14.81
Molecular Weight	338.5	293.8	381.4	336.4	32.77
Number of Atoms	23.29	20	26	23	2.215
Molecular Volume (Angstroms ³)	282.7	246.7	317	291.6	24.8

Table 3. Properties of lumiracoxib based COX-2 inhibitors.

Drug	Log P	Polar Surface Area (Å ²)	Number of Atoms	Formula Weight	Oxygens & Nitrogens	NH _n & -OH	Rule of Five	Rotatable Bounds	Volume (Å ³)
1, Lumiracoxib	4.477	49.326	20	293.75	3	2	0	4	246.686
2	4.016	86.625	19	257.245	5	3	0	4	221.857
3	2.501	63.322	18	239.274	3	3	0	3	221.159
4	3.098	66.397	20	289.718	4	2	0	4	244.177
5	3.315	54.37	20	268.312	3	1	0	6	251.868
6	1.577	89.345	19	254.289	4	5	0	3	232.447
7	3.074	66.397	20	289.718	4	2	0	4	244.177
8	3.728	66.397	21	324.163	4	2	0	4	247.713
9	3.157	83.123	20	269.256	5	1	0	4	233.204
10	2.560	66.397	20	273.263	4	2	0	4	235.572
11	3.098	66.397	20	289.718	4	2	0	4	244.177
12	4.363	78.424	22	339.178	5	3	0	5	270.115
13	1.577	89.345	19	254.289	4	5	0	3	232.447
14	2.604	55.767	20	272.300	4	1	0	6	250.587
15	3.266	54.370	20	266.296	3	1	0	5	245.656
16	2.863	57.527	20	270.328	3	2	0	6	257.380
17	3.157	83.123	20	268.256	5	1	0	4	233.204

Pattern recognition by cluster analysis (or clustering) has the goal of grouping a set of objects in such a way that objects in the same cluster or group are most similar to each other than to those in other clusters [23,24]. For obtaining a comparison of all analogs the molecular properties in Table 3 and Table 4 are combined, these include: Log P, polar surface area, number of atoms, number of oxygen-nitrogenhydroxide-amine groups, and formula weight. Hierarchical cluster analysis using Euclidean distance and single linkage was completed to resolve the 28 drugs into clusters of drugs having highest similarity (see Fig. 3). The dendrogram shown in (Fig. 3), inclusive of all compounds of (Figs. 1 and 2), clearly defines the 28 drugs as to the closest member based on molecular properties. Drug 1 (lumiracoxib) is bound to node B and within supercluster having 1, 10, 16, 14, 15, 5, 7, 11, 4, 17, 9, 13, 6, and 2. Drug 18 (valdecoxib) is joined to node C and within supercluster having 18, 20, 25, 24, 12, 28, 26, 27, 23, 21, 22, 19, and 8. Valdecoxib (drug 18) was found to be extremely close to drug 28 in similarity, both having two aromatic rings, one sulfonamide group, one methyl (-CH₃) substituent, and one heterocyclic ring.

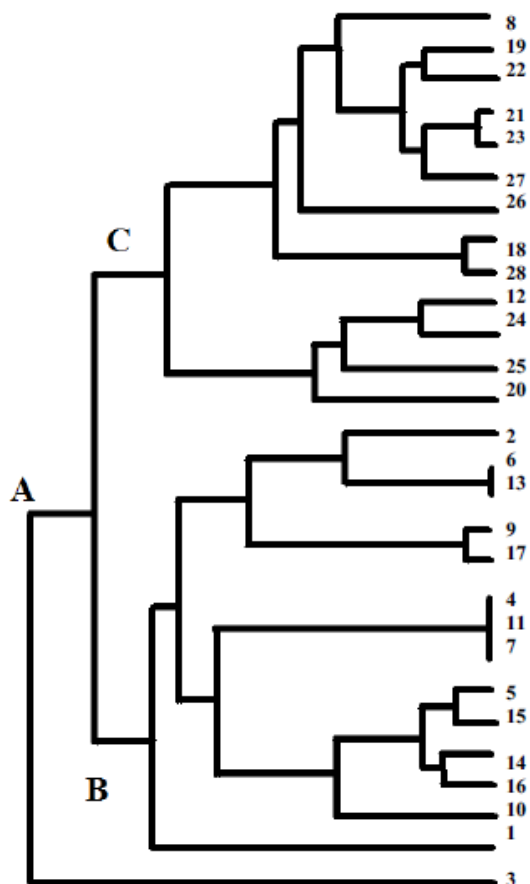


Fig. (3). Hierarchical cluster analysis by Euclidean distance and single linkage cluster rules. Beginning at origin node A the 28 compounds presented in Table 3 and Table 4 are grouped according to highest level of similarity according to properties. Drug 1 (lumiracoxib) is bound to node B and within supercluster having 1, 10, 16, 14, 15, 5, 7, 11, 4, 17, 9, 13, 6, and 2. Drug 18 (valdecoxib) is joined to node C and within supercluster having 18, 20, 25, 24, 12, 28, 26, 27, 23, 21, 22, 19, and 8.

Table 4. Properties of valdecoxib based COX-2 inhibitors.

Drug	Log P	Polar Surface Area (Å ²)	Number of Atoms	Formula Weight	Oxygens & Nitrogens	NH _a & -OH	Rule of Five	Rotatable Bounds	Volume (Å ³)
18, Valdecoxib	2.734	86.197	22	314.366	5	2	0	3	263.553
19	3.663	55.403	22	319.426	4	1	0	5	291.316
20	3.552	88.329	22	335.772	6	1	0	3	257.485
21	3.063	63.995	22	315.398	5	1	0	4	274.412
22	3.982	49.407	22	318.442	4	1	0	6	295.356
23	2.775	63.995	22	315.398	5	1	0	3	274.171
24	3.995	74.852	22	335.816	5	2	0	3	270.765
25	4.506	66.397	22	339.844	4	2	0	4	287.350
26	3.765	74.852	22	315.398	5	2	0	3	273.790
27	3.827	59.061	22	312.394	4	1	0	3	272.243
28	3.322	88.329	22	315.354	6	1	0	3	260.511

Table 5. Summary statistics for lumiracoxib analog COX-2 inhibitors.

Property	Mean	Minimum	Maximum	Median	Standard Deviation
Log P	3.00	1.577	4.363	3.10	0.75
Polar Surface Area (Angstroms ²)	70.76	54.37	89.35	66.40	12
Molecular Weight	276.6	239.3	339.2	269.8	25
Number of Atoms	20	18	22	20	0.89
Molecular Volume (Angstroms ³)	241.6	221.16	270.1	244.2	12

K-means analysis performs a non-hierarchical divisive cluster analysis on input data and is distinguished from the more common hierarchical clustering techniques [23,24]. Restraining the analysis to two clusters only, the resolution of drugs shown in (Figs. 1 and 2) into two groups for highest similarity closely resembles hierarchical cluster analysis (see Fig. 3). The clusters are determined as follows: cluster 1) drug 1 (lumiracoxib), 2, 3, 4, 5, 6, 7, 9, 10, 11, 13, 14, 15, 16, and 17; cluster 2) 18 (valdecoxib), 8, 12, 19, 20, 21, 22, 23, 24, 25, 26, 27, and 28.

The properties of polar surface area and molecular weight for all analogs and drugs of Table 3 and 4 are highly consistent and concordant in variation demonstrable in a 95% ellipses (see Fig. 4). Note that all 28 compounds of Table 3 and 4 are contained within a 95% ellipses. If an actual value of the parameter lies outside the 95% confidence interval, the parameter has a probability of 5% (or less) of happening by chance.

The purpose of multiple regression analysis is to predict a single variable from one or more independent variables and learn more about the relationship between independent or predictor variables [22]. The prediction of lumiracoxib analogous compounds (see Table 3) values of formula weight (FW), these from Log P, PSA,

number of atoms (nAtoms), MV, number of rotatable bonds (nRotB), number of oxygen/nitrogen (nON), and number of hydroxyl-amine groups (nOHNHn) is defined as follows: $FW = -22.57 + (4.750)(\text{Log } P) - (1.059)(\text{PSA}) + (21.248)(\text{nAtoms}) + (12.360)(\text{nON}) + (5.635)(\text{nOHNHn}) - (0.454)(\text{nRotB}) + (0.4957)(\text{MV})$. This model explains 96.74% of the variance within the model and has a R² of 0.9674. Similarly, the prediction of valdecoxib analogous compounds (see Table 4) using formula weight (FW), Log P, PSA, MV, nRotB, (nON), and (nOHNHn) for appears as follows: $FW = 156.47 + (13.308)(\text{Log } P) + (0.2402)(\text{PSA}) + (5.335)(\text{nON}) + (2.184)(\text{nOHNHn}) - (0.2935)(\text{nRotB}) + (0.2668)(\text{MV})$. This model explains 56.05% of the variance within the model and has a R² of 0.5605. By using these numerical models it is possible to predict additional compounds based on scaffolding for lumiracoxib and valdecoxib.

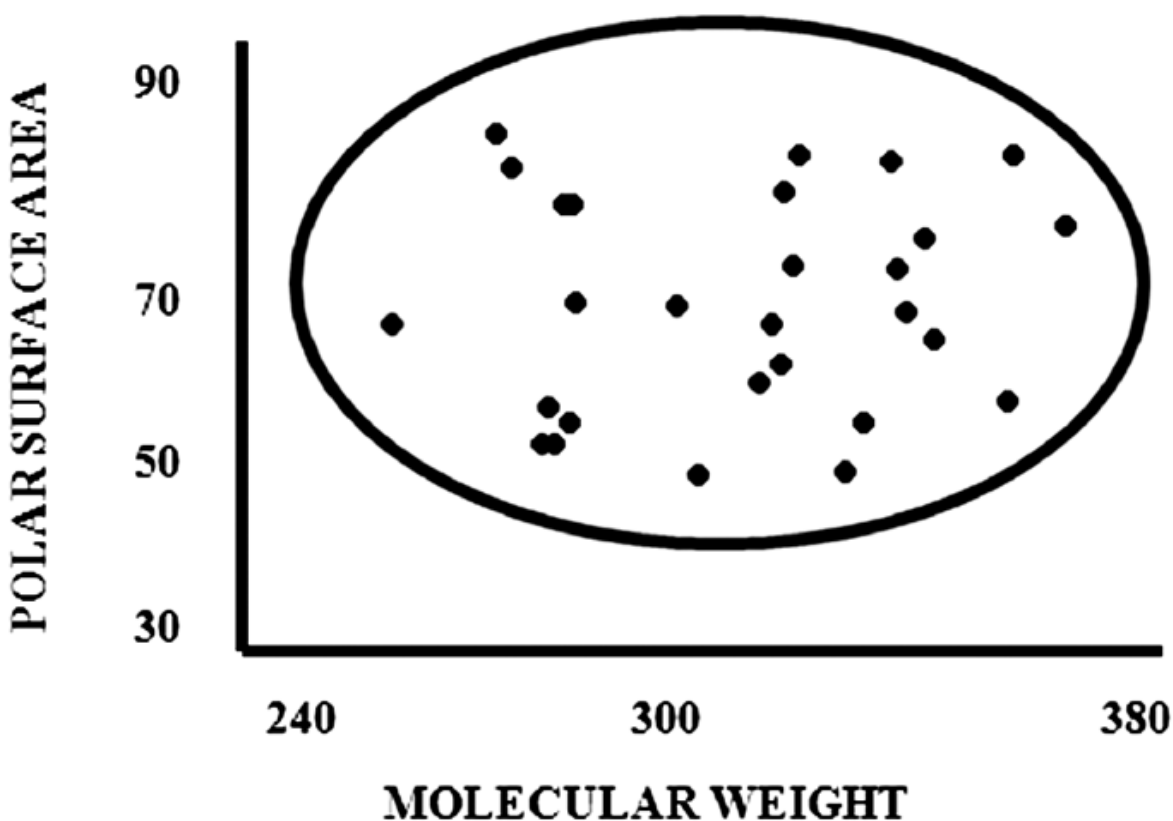


Fig. (4). 95% Ellipses plot. The relationship of molecular weight (independent variable) to polar surface area (dependent variable) shows that all drugs found in Table 3 and Table 4 fall within the 95% ellipses confidence interval. The ellipses is the region containing 95% of the uncertainty. The in silico search results are consistent when considering molecular weight and polar surface area.

Table 6. Summary statistics for valdecoxib analog COX-2 inhibitors.

Property	Mean	Minimum	Maximum	Median	Standard Deviation
Log P	3.645	2.775	4.506	3.714	0.50
Polar Surface Area (Angstroms ²)	68.46	49.41	88.33	65.20	13
Molecular Weight	322.3	312.4	339.8	316.9	10
Number of Atoms	22	22	22	22	0.0
Molecular Volume (Angstroms ³)	275.7	257.5	295.4	274.0	12

Further comparison of analogs to their parent compounds is achieved by determination of drug likeness scores for kinase inhibitor, protease inhibitor, and general enzyme inhibitor. Effective and useful scores for drug likeness of these categories determined by Molinspiration cheminformatics (see Materials and Methods) for kinase inhibitor fall within - 1.50 to 0.50 (90% confidence interval), protease inhibitor is - 1.50 to 0.4 (90% confidence interval), and enzyme inhibitor is -1.00 to 0.4 (90% confidence interval). Drug likeness scores of analogs are presented in Table 7 with all analogs falling well within the range of 90% confidence interval for kinase inhibitor, protease inhibitor, and general enzyme inhibitor. These results solidify the considerable potential for these analogs to perform as inhibitors of the COX-2 enzyme.

DISCUSSION

Previous studies have shown the COX-2 isozyme appears to play a role in a number of pathways to cancer and is induced by oncogenes and over expressed in premalignant and malignant tumors [25]. The COX-2 enzyme is known to be over expressed in numerous cancers such as [25]: gastric cancer, Barrett's esophagus, hepatocellular cancer, pancreatic malignancies, oral leukoplakia, head and neck cancer, nonsmall cell lung cancer, breast, prostate, bladder cancers, and colorectal carcinomas [26]. Because overexpression of COX2 inhibits apoptosis and allows cancer to grow, it is possible that COX-2 inhibitors can help ensure that cancer cells die and help target the earliest stages of carcinogenesis [25]. Consequently the search and design for new COX-2 inhibitors should have an immense benefit for many cancer patients.

Advances in chemo-informatics allow categorizing and screening by overall physicochemical properties of potential drug candidates based entirely on their molecular structures [27]. Studies have shown that drug candidates having PSA > 140 Å² are poorly absorbed and an increase of PSA decreases membrane permeability [27]. Other criteria of properties to enable good drug absorption are the Rule of 5 that states poor drug permeation is more likely with the following parameters [28]: 1) Molecular weight is over 500; 2) Log P is over 5; 3) More than 10 hydrogen bond acceptors (nitrogen and oxygen atoms); 4) More than 5 hydrogen bond donors (hydroxyl and amine groups). These criteria will assist the examination of the 26 novel COX-2 inhibitors presented in this study.

The structure of lumiracoxib (see Fig. 1) differs from other COX-2 inhibitors, it is an analogue of diclofenac, has one chlorine substituted by fluorine and the phenylacetic acid has a methyl group in meta position which makes it a member of the arylalkanoic acid class of NSAIDs. Lumiracoxib has the highest COX-2 selectivity of any NSAID [29]. The 16 lumiracoxib analogous compounds have pharmaceutical properties inherent to COX-2 inhibitors (see Fig. 1). Substituents common to all 17 drugs and establish criteria are: 1) Two aromatic rings; 2) At least two oxygen atoms; 3) At least one carboxyl group. Other atoms present but not general are halogen atoms, nitrogen atoms, and amide groups.

The success rate for in silico identification of lumiracoxib analogs was less than 16%. The 10 valdecoxib analogous compounds have pharmaceutical properties inherent to COX-2 inhibitors (see Fig. 2). Characteristic common to all 11 drugs are: 1) Two aromatic rings; 2) At least two oxygen atoms; 3) At least one sulfonamide group. Other atoms present but not general are chlorine atom, oxadiazole substituent, and benzimidazole groups. The success rate for in silico identification of valdecoxib analogs was less than 10%.

The variation of substituents will affect molecular properties of these analogs and allow comparison to those of known specific COX-2 inhibitors presented in Table 1 [21] and with summary statistics of Table 1 shown in Table 2 [21]. Comparing the maximum and minimum bounds of known COX-2 inhibitors (see Tables 1 and 2) to properties of lumiracoxib and valdecoxib based analogs (see Table 3 and Table 4) and their summary statistics (see Table 5 and Table 6), essentially all pertinent pharmaceutical descriptors fall within range. Those properties within numerical range of known COX-2 inhibitors include: number of oxygen atoms, nitrogen atoms, hydroxyl groups, amine groups; number of rotatable bonds, Log P, polar surface area, Rule of 5, and molecular volume (in the case of valdecoxib analogs).

Polar surface area of drug candidates have been shown to be indicative of the potential extent of intestinal absorption following oral administration [30]. Thusly set parameters can predict the expected level of intestinal absorption, which for lumiracoxib and valdecoxib analogs would be greater than 40% of drug present. Cluster analysis is a tool for exploratory data mining and a common technique for statistical data analysis [23,24]. Identifying groups of individuals or objects that are similar to each other but different from individuals in other groups can potentially resolve divergent clinical activities among alike drug categories. The vertical agglomerative dendrogram shown in (Fig. 3) differentiates lumiracoxib (drug 1) which joins at node B from valdecoxib (drug 18) which joins at node C. Rigorously considered the COX-2 inhibitor lumiracoxib is distinct from but most similar to drugs 10, 16, 14, 15, 5, 7, 11, 4, 17, 9, 13, 6, and 2. Likewise, the drug valdecoxib (drug 18) at highest resolution is highest alike to drug 28 but similar to drugs 20, 25, 24, 12, 26, 27, 23, 21, 22, 19, and 8. Drug 3 is joined to all other analogs at node A and counted distinct from other drugs.

Table 7. Drug-likeness and enzyme inhibition scores.

Drug	Kinase Inhibitor	Protease Inhibitor	Enzyme Inhibitor
1, Lumiracovib	0.05	-0.22	0.08
2	-0.01	-0.29	0.06
3	-0.29	-0.37	0.24
4	-0.20	-0.25	-0.10
5	-0.36	-0.14	0.19
6	-0.19	-0.25	0.28
7	-0.17	-0.28	-0.12
8	-0.14	-0.26	-0.12
9	-0.47	-0.53	-0.04
10	-0.14	-0.16	-0.05
11	-0.20	-0.25	-0.10
12	0.12	-0.54	-0.16
13	-0.19	-0.25	0.28
14	-0.19	0.01	0.15
15	-0.49	-0.30	0.14
16	-0.49	-0.30	0.14
17	-0.47	-0.53	-0.04
18, Valdecovib	0.19	-0.02	0.21
19	-0.42	-0.09	-0.25
20	-0.40	-0.44	-0.21
21	-0.03	-0.16	0.01
22	-0.27	-0.13	-0.21
23	-0.17	-0.16	-0.11
24	-0.01	-0.25	-0.11
25	-0.33	-0.21	-0.17
26	-0.02	-0.22	-0.10
27	-0.83	-0.50	-0.39
28	-0.43	-0.45	-0.24

Confidence Intervals for a population indicates a range of values that contains the estimated parameter a high proportion of the time [24]. It relates how stable an estimate is since a stable estimate is one that would be close to the same value if the survey were repeated. The 95% confidence interval is constructed so that 95% of such intervals will contain the parameter. A 95% ellipses outcome means that if this same population is sampled the resulting intervals would bracket the true population of values in 95% of the cases. The 95% ellipses shown in (Fig. 4) contains all 28 drugs of Table 3 and Table 4 that clearly indicates the in silico search produced drugs having very high consistency in descriptors of polar surface area and molecular weight.

Path analysis is closely related to multiple regression. This techniques allows the testing of theoretical propositions about cause and effect without manipulating variables. The causal modeling requires an assumption of the model rather than a property of the

output or consequence of the technique. Path coefficients provide estimates of the magnitude and significance of hypothesized causal connections between sets of properties. Considering properties of known COX-2 selective inhibitors (see Table 1) the path coefficients for determination of formula weight are very high for Log P (0.824), number of atoms (2.024), and number of oxygen & nitrogen atoms (1.616). Path analysis of Table 3 and Table 4 likewise showed very high path coefficient for number of atoms (0.863) and significant outcome for number of oxygen & nitrogen atoms (0.131), and Log P (0.231) in expression of formula weight. Emphasizing the importance of these properties for expression of formula weight and putatively for the design of novel effective COX-2 selective inhibitors.

Comparing COX-2 inhibitors of Table 1 to drugs of Table 3 and Table 4 by one-way ANOVA shows the means of properties Log P, polar surface area, and rotatable bonds are the same ($P = 0.09866$). In addition, all Log P and polar surface area values in Table 1, Table 3, and Table 4 are from identical populations based on results of Kruskal-Wallis test ($P = 0.172$ and $P = 0.989$, respectively). Therefore the analog drugs are found to be consistent and highly similar to previous known selective COX-2 inhibitors.

Comparison by unpaired t-test for Log P and polar surface area (two properties important for determination of membrane permeation [28]) of COX-2 inhibitors of Table 1 to all drugs in Table 3 and 4 indicate no difference among the groups (two-tailed $P = 0.3543$ and $P = 0.8805$, respectively). Therefore the analog drugs are found to be consistent and highly similar to previous known selective COX-2 inhibitors.

Cluster analysis aided resolution of novel drug designs for similarity using determined molecular properties has been shown to be effective distinguishing efficacious substituent substitution [31]. Therefore this study has shown that *in silico* substituent search via data library with numerical analysis, to include cluster analysis, can identify underlying relationships of analogous drug scaffolds and assist in identifying optimal substituent replacement for optimizing drug activity.

Drug likeness is characterized by various molecular properties and structure features which determine whether a molecule is similar to the known drugs. Molinspiration Chemoinformatics utilizes Bayesian statistics for comparing structures of active ligands with structures of inactive molecules in an effort to identify substructure features typical for active molecules. Because kinase inhibitors can be associated with the inflammatory mechanism [32], the determination of kinase inhibition scores assists the evaluation of these analogs. All analogs are also compared to protease inhibitors and general enzyme inhibitors since these compounds can have medicinal application [33]. Calculated drug likeness scores for all drugs are shown in Table 7 with values determined for every analog falling within the 90% confidence interval for potent drug likeness in the categories of kinase, protease, and general enzyme inhibitors. These results further substantiates their substantial potential for inhibition of COX-2 enzyme. The Grubbs' test for numerical outliers, indicated no outliers among the drug likeness

scores for all compounds in the cases of kinase, protease, or general enzyme inhibitors. All analogs are drug-like and comparable to known medicinal compounds lumiracoxib or valdecoxib.

For analogs of lumiracoxib (see Fig. 1) the substituents of amines, hydroxyl groups, carbonyl oxygens, and carboxyl groups remain polar as in the parent drug. Aromatic rings remain non-polar and with similar action as in the parent compound. For analogs of valdecoxib (see Fig. 2) the substituents of amines and sulfonamide remain polar as in the parent drug. Aromatic rings and methyl groups remain nonpolar and with similar action as in the parent compound. Examination of the structures for placement of polar groups (such as $-NH_n$ or $-OH$) relative to that of parent compounds show comparable placement of these moieties useful for binding and docking. In addition the molecular volumes (see Table 3 and 4) of analogs are statistically consistent to that of parent compounds. Altogether the drug likeness scores and physicochemical properties (i.e. molecular volume) indicates considerable likelihood for effective inhibitors of the COX-2 enzyme.

CONCLUSIONS

The beneficial medicinal action of COX-2 inhibitors affirms further study and development of new drug designs. In this study a total of 26 novel drug scaffolds focusing on COX-2 selective inhibition have been presented. These analogs of lumiracoxib and valdecoxib have molecular properties consistent with those of known specific COX-2 selective inhibitors. Following *in silico* search for substituent substitution a set of analogs were identified and provided criteria for structural features inherent for COX-2 inhibitors. For lumiracoxib set analogs, parameters required: 1) Two aromatic rings; 2) At least two oxygen atoms; 3) At least one carboxyl group. For valdecoxib analogous compounds (sulfonamide based) required pharmaceutical properties are: 1) Two aromatic rings; 2) At least two oxygen atoms; 3) At least one sulfonamide group. Valdecoxib analogs may include but not required multiple methyl ($-CH_3$) substituents, halogen atoms, and heterocyclic ring. The success rate for the *in silico* search outcome was less than 16% and less than 10% for lumiracoxib and valdecoxib analogs, respectively.

K-means cluster analysis indicated that drug 1 (lumiracoxib) is most similar to drugs 2, 3, 4, 5, 6, 7, 9, 10, 11, 13, 14, 15, 16, and 17 with drug 18 (valdecoxib), most similar to 8, 12, 19, 20, 21, 22, 23, 24, 25, 26, 27, and 28. Path analysis identified Log P, number of atoms, oxygens, and nitrogens to most influence formula weight of known COX-2 inhibitors. The unpaired t-test indicated that Log P and polar surface area of known COX-2 selective inhibitors is no different from those of 26 drug analogs presented here. A multiple regression model using vital drug-likeness properties will aid in the predicting additional analogs. The further search for novel COX-2 inhibitors is highly advisable due to their beneficial medicinal action. *In silico* optimization for substituent substitution is shown here to provide an effective tool in that regard.

CONFLICT OF INTEREST

The author confirms that this article content has no conflicts of interest.

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PATIENT CONSENT

Declared none.

ABBREVIATIONS

PSA = Polar surface area

FW = Formula weight

NO = Nitrogen and oxygen atoms

OH = Hydroxyl group

NHn = Amine group

COX-2 = Cyclooxygenase-II enzyme

NSAIDs = Non-steroidal anti-inflammatory drugs

MV = Molecular volume

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