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Design of COX-1 Inhibitors Utilizing Class I Isosteres, Class II Isosteres, and Nonclassical Bioisosteres for Substituent Substitution on Proved Parent Structures

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aims: To identify isosteres and bioisosteres suitable for substitution on the molecular scaffold of meclofenamic acid and tolfenamic acid. The compounds will be studied to determine drug-likeness and other properties.
Study Design: Isosteres and bioisosteres were selected and emplaced on the scaffold of meclofenamic acid and tolfenamic acid to ascertain drug-likeness outcome. Drug candidates were selected based on favorable drug-likeness.
Place and Duration of Study: Chemistry Department, University of Nebraska at Omaha, Omaha Nebraska from March 2015 to May 2015.
Methodology: Two non-steroidal anti-inflammatory drugs, meclofenamic acid and tolfenamic acid, are selected based on versatile isosteres and bioisosteres substitution. Placement of class I isosteres, class II isosteres, and nonclassical bioisosteres was accomplished using molecular modeling software. Physicochemical properties were determined and compared by numerical

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analysis and by pattern recognition. This approach is evaluated for success in generating drug-like compounds.
Results: Utilizing meclofenamic acid as parent compound, a total of 13 class I isosteres, five class II isosteres, and four non-classical bioisosteres were identified. For this group the Log P and polar surface area values ranged from 2.534 to 6.268 and 49.326 Angstroms^2 to 101.372 Angstroms^2, respectively. Utilizing tolfenamic acid as parent compound, a total of ten class I isosteres, five class II isosteres, and four non-classical bioisosteres were identified. For this group the range of Log P and polar surface area values were 1.904 to 5.408, and 37.299 Angstroms^2 to 75.349 Angstroms^2, respectively. Multiple regression analysis of properties produced equations useful for prediction of similar drugs for both groups.
Conclusion: Variations of physicochemical properties by isosteres (class I and class II) and bioisosteres, successfully produced 22 of meclofenamic acid based drug designs and 19 of tolfenamic acid based drug designs. All compounds were evaluated for drug-likeness and similarities by cluster analysis. New drug designs are needed for COX-1 inhibition.

Keywords: COX-1; NSAIDs; cyclooxygenase; meclofenamic acid; tolfenamic acid.

ABBREVIATIONS

Term: PSA, polar surface area; MW, molecular weight; nON, number of oxygen and nitrogen atoms; nOHNH, number of hydroxyl and amine groups; nrotB, number of rotatable bonds; natoms, number of atoms; NSAIDs, non-steroidal anti-inflammatory drugs.

1. INTRODUCTION

Drugs identified as non-steroidal anti-inflammatory drugs (NSAIDs) are generally indicated for acute or chronic conditions in which inflammation and pain are present [1]. Conditions in which NSAIDs are indicated include the following [1]: metastatic bone pain, postoperative pain, pain due to tissue injury, fever, ileus, renal colic, headaches, and acute gout. In addition, aspirin is indicated for inhibiting platelet aggregation and is able to irreversibly inhibit COX-1 (isoenzyme cyclooxygenase-1). Two forms of cyclooxygenase enzyme exist and are referred to as COX-1 and COX-2, both being structurally distinct from each other [2]. The isoform COX-2 is induced within inflammatory cells, while COX-1 is known to be a constitutive component of normal cells [2].

Salicylic acid, an NSAID synthesized in 1860, has been used as an antipyretic, antiseptic, and antirheumatic [3]. Later, aspirin was developed as a more satisfactory alternative and preceded other drugs of similar action (hence aspirin-like drugs) and were termed as non-steroidal anti-inflammatory drugs [3]. The inhibition of cyclooxygenase accounts for both therapeutic effects and the side effects of cyclooxygenase inhibition [4]. The NSAID that selectively inhibits COX-2 will maximize anti-inflammatory activity with considerably less toxicity [4]. Undesired side effects, such as damaging of the gastric mucosa and kidneys, results from NSAID activity of inhibition of COX-1 [4].

Inflammation of tissue can originate from infectious and non-infectious mechanisms of irritation and chronic injury [5]. It is generally accepted that inflammation is a cancer risk, with various conditions of inflammation found to be a predisposition to cancer [5]. Aspirin, as well as other NSAIDs, have shown clear assurance as effective chemoprevention agents upon cancers of the stomach, colon, lung, and breast [5]. Previous studies have shown that NSAIDs will stimulate apoptosis (programmed cell death) and inhibit angiogenesis (formation of new blood vessels), both processes suppressing tumor growth and malignant transformation [6]. In particular, the selective inhibition of COX-2 shows promise as anticancer agents [6]. Daily administration of aspirin reduces the risk for colon cancer by 63%, for breast cancer by 39%, for lung cancer by 36%, for esophageal cancer by 73%, and for prostate cancer by 39% [7].

Studies have shown that long-term use of NSAIDs can reduce the risk of cancer of the breast, prostate, lung, skin, and colon [8,9,10, 11,12]. The routine intake of aspirin and other NSAIDs have been shown to reduce the risk of pancreatic cancer and other digestive cancers [13,14,15]. Studies have indicated that NSAID use among males confers what is measured as modest protection for lung cancer [16]. Whereas,

regular aspirin consumption could reduce risk of lung cancer among Asian women [17].

The design of new COX-1 inhibitors would enhance the prevention and clinical treatment of various inflammatory associated sickness, including cancers. This study demonstrates the efficacy of modifying substituents of COX-1 inhibitors meclofenamic acid and tolfenamic acid so that favorable drug-likeness properties remain. Both meclofenamic acid and tolfenamic acid are referred to as fenamic acid derivatives or fenamates. A systematic process that includes isosteric and nonclassical bioisosteric substitution is presented here, that demonstrates the effectiveness of this approach for the design of novel COX-1 inhibitors.

2. MATERIALS AND METHODS

2.1 Determination of Physicochemical Properties and Molecular Modeling

Construction of all compound structures for determination of molecular properties and visualization (2-Dimensional) was accomplished by utilizing the advanced molecular modeling software developed by ACD/Chem Sketch modeling version 10.00 (Advanced Chemistry Development, 110 Yonge Street, Toronto Ontario, M5C 1T4 Canada). Some physicochemical properties were determined by ACD/Chem Sketch (molecular weight) and primarily by Molinspiration (Molinspiration Chemiformatics, Nova ulica 61, SK-900 26 Slovensky Grob, Slovak Republic). Various properties such as polar surface area, violations of Rule of 5, molecular volume, number of oxygen, nitrogen, amines (-NHn) and hydroxyls (-OH), were determined using Molinspiration Properties Calculations module (Molinspiration Cheminformatics, Nova ulica 61, SK-900 26 Slovensky Grob, Slovak Republic). Potential biological activity of all compounds was determined by Molinspiration drug-likeness and bioactivity scoring, also by Molinspiration Cheminformatics (Molinspiration Chemin-formatics, Nova ulica 61, SK-900 26 Slovensky Grob, Slovak. Republic).

2.2 Analysis of Physicochemical Properties for Pattern Identification

For identification of complex and hidden underlying associations or patterns within the multivariate numerical data matrix of the physicochemical properties, then various pattern recognition techniques were implemented. This included an approach applying hierarchical cluster analysis, which was accomplished by KyPlot version 2.0 Beta 15 (copyright Koichi Yoshioka 1997-2001). Other pattern recognition elucidation by K-means nonhierarchical cluster analysis were performed by PAST version 1.80 (copyright Oyvind Hammer, D.A.T. Harper 1999-2008).

2.3 Numerical Analysis of Physico-chemical Properties

Descriptive statistical analysis, Pearson r, and coefficient of determination for all numerical data where indicated was performed by Windows 7 Microsoft Office Professional Plus 2013 EXCEL (EXCEL 2013). Screening for numerical outliers was done by Grubb's Test (extreme studentized deviate) by GraphPad Software **(**2236 Avenida de la Playa, La Jolla, CA 92037 USA). Multiple regression analysis was performed by GraphPad InStat version 3.0 for Windows 95 (HJ Motulsky, GraphPad InStat 3.0 GraphPad Software, Inc., San Diego California USA, www.graphpad.com). Analysis by ANOVA with F and P values was accomplished by PAST version 1.80 (copyright Oyvind Hammer, D.A.T. Harper 1999-2008).

3. RESULTS AND DISCUSSION

At variable rates, all NSAIDs will inhibit COX-1 and COX-2 [18]. Generally, the mechanisms by which inhibition takes place falls into three categories [18]: Category 1: rapid competitive reversible binding of COX-1 and COX-2; Category 2: rapid but lower affinity reversible binding followed by time-dependent, slowly reversible, but higher affinity binding of COX-1 and COX-2; Category 3: rapid reversible binding followed by covalent modification of COX-1 and COX-2. Exceptions to this include nimesulide (weak competitive inhibitor of COX-1, potent time-dependent inhibitor of COX-2) and celecoxib (slow competitive binding but irreversible binding at high concentrations) [18]. Therefore, generally COX inhibition is competitively reversible except for aspirin which incurs irreversible inhibition [19].

Groups of atoms that are isosteric groups are isoelectronic in their outermost electron shell [20]. The isosteres are classified by the number of electrons in the outer shell (valence) and include the following classes [20,21]:

Class I: $-CH_3$, $-NH_2$, -SH, -OH, and halogens (-Cl, -I, -Br, -F).
Class II: Oxygen atom, sulfur atom, Se, Te, -NH-, $-CH_2-$.

The application of isosteric replacement of atoms or groups from a drug molecule is broadly applied in the design of drugs, particularly in the design of antimetabolites (drugs altering mechanisms of metabolic processes) [20,21]. A large number of drugs have been discovered utilizing isosteres and bioisosteres replacement [21].

The use of nonclassical isosteres or bioisosteres is essentially the "classical" approach to modification of structure [20]. Bioisosteres are entities that do not have the same number of atoms or identical electron structure to substituents they are meant to replace [20]. However, bioisosteres have chemical similarities that produce highly similar biological activities. Bioisosteres have been highly successful as a lead modification useful for reducing toxicity, altering activity of a lead compound, and alteration of the metabolism of the lead compound [20,21]. Examples of nonclassical bioisosteres include the following [21]:

Nonclassical bioisosteres:

$-S(=O)_2OH$,
$-P(=O)(OH)NH_2$,
-C(=O)NHCN,
$-P(=O)(OH)OCH_2CH_3$

These groups of atoms produce a favorable outcome when covalently bonded to the molecular scaffolding of meclofenamic acid and tolfenamic acid.

3.1 Meclofenamic Acid Isosteres and Bioisosteres

Meclofenamic acid is a NSAID having antipyretic and antigranulation activity, which is administered clinically for treatment of joint and muscular pain, arthritis, and menstrual pain [22]. Studies have shown meclofenamic acid to be a potential antineoplastic agent for both androgen-dependent and androgen-independent prostate cancer, following demonstration of its highly cytotoxic action against neoplastic prostate cells [23].

The parent structure of the NSAID meclofenamic acid is presented in Fig. 1, showing the two aromatic rings connected by a secondary amine (-NH-) with a carboxyl group (-COOH), two chlorine atoms, and one methyl group ($-CH_3$). It is the two chlorine atoms and one methyl group that are amenable to class I isosteres replacement.

Systematic replacement of the original two chlorine atoms and methyl group by class I isosteres produces the derivatives of meclofenamic acid shown in Fig. 1. Note that the two aromatic rings, carboxyl group (-COOH), and the connecting secondary amine group remains. The immediate consequence in physicochemical properties such as Log P, polar surface area (measured in Angstroms^2 or A^2), number of atoms (natoms), molecular weight, number of oxygen/nitrogen/amine/hydroxyl moieties, and molecular volume are calculated with presentation in Table 1.

Property variations induced by class I isosteres replacement occurs for all 13 derivatives for all properties, save for number of atoms and number of rotatable bonds. Although there appears a broad range of Log P variation (from 3.244 to 6.268) and polar surface area (from 49.326 A^2 to 101.372 A^2), in fact, by the Grubb's test there are no outliers (alpha = 0.05) [24] within these two parameters even with meclofenamic acid inclusive (Log P = 5.633, PSA= 49.326 A^2).

Derivatives 1, 4, 5, 8, and 10 now show zero violations of the Rule of 5 [25], as opposed to meclofenamic acid showing one violation, along with derivatives 2, 3, 6, 7, 9, 11, 12, and 13. The Rule of 5 rule states that, in general, an orally active drug has no more than one violation of the following criteria [25]:

- No more than 5 hydrogen bond donors ($-NH_n$ and -OH)
- Not more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- A molecular weight less than 500 Daltons
- An octanol-water partition coefficient[] log *P* not greater than 5.

Essentially, all thirteen class I isosteres of meclofenamic acid show favorable properties for oral administration.

In the approach for class II isosteres replacement within meclofenamic acid, it is the connecting amine group (-NH-) that is replaced with $-CH_2-$, Te, Se, -S-, or –O- (see Fig. 2). Note that the two aromatic rings, two chlorine atoms, carboxyl

group, and methyl group remain intact within the derivative structures, see Fig. 2.

Variation of properties for class II isosteres is shown in Table 1, for comparison to the parent drug meclofenamic acid. In this case, by Grubb's test, there are no outliers among Log P, molecular weight, volume, number of atoms, number of rotatable bonds, and polar surface area (meclofenamic acid included). These class II isosteres show one violation of Rule of 5, as does meclofenamic acid, preserving favorable oral activity.

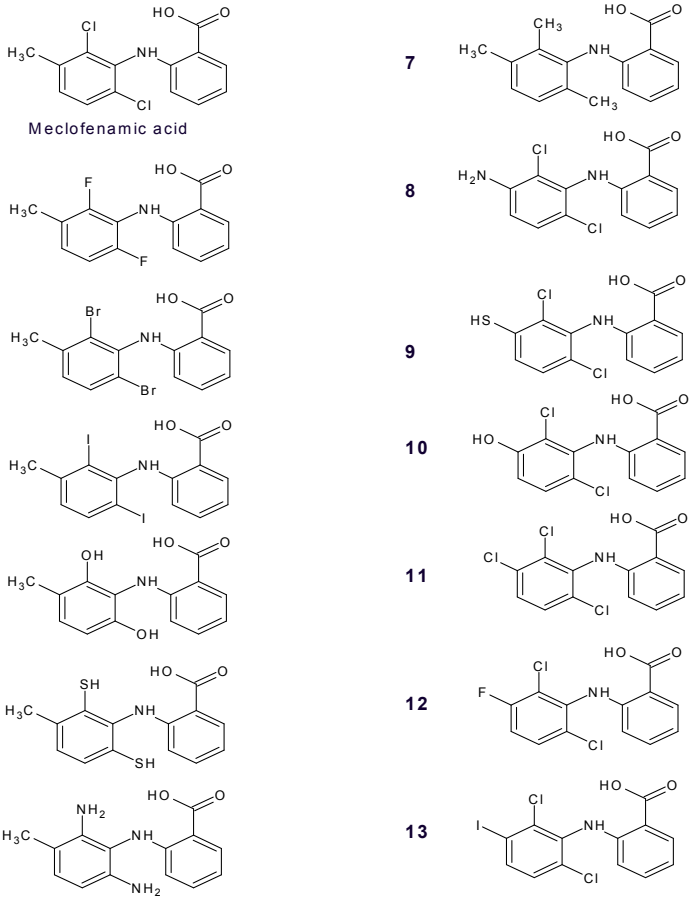

Fig. 1. Class I isosteres of meclofenamic acid

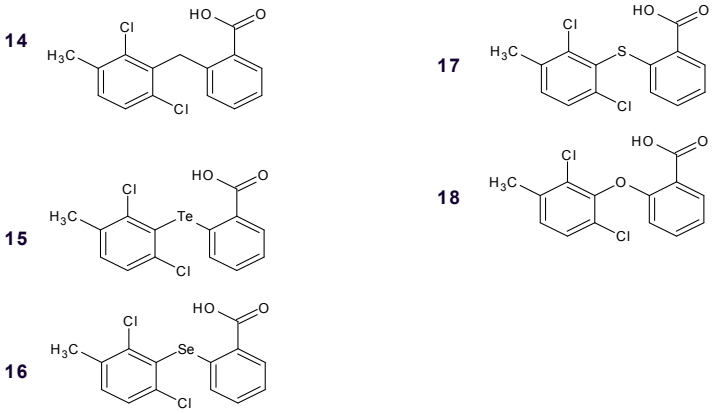

Fig. 2. Class II isosteres of meclofenamic acid

An interesting comparison is found when nonclassical bioisosteres are applied for substituent replacement within meclofenamic acid. These are presented in Fig. 3. The resulting changes in physicochemical properties are revealed in Table 1. Notable, it is seen that compounds 19, 20, and 21 show zero violations of the Rule of 5, a distinguishable improvement compared to the parent compound meclofenamic acid. The use of non-classical bioisosteres improves oral activity of this NSAID, with compound 22 have one violation as does the parent compound.

Likewise, there are no outliers among Log P, polar surface area, number of atoms, and molecular weight (including meclofenamic acid). This is of interest, because the nonclassical bioisosteres are of greater mass and volume than class I as well as class II isosteres.

The molecular properties for meclofenamic acid isosteres and nonclassical bioisosteres is reported in Table 1. For the derivative compounds 1, 4, 5, 8, 10, 19, 20, and 21 there is actually improvement in drug-likeness (e.g. zero violations of Rule of 5), compared to the parent compound meclofenamic acid which has one violation of Rule of 5.

3.1.1 Multiple regression for prediction and pattern recognition

There are two general applications of multiple regression, that being 1) prediction, and 2) explanation [24]. The value of R^2 (coefficient of determination) relates how well the model fits the data, explaining the relation of the independent variables (predictors) to the dependent variable (molecular weight in this study).

The result of multiple regression analysis of properties of Table 1 (meclofenamic acid and derivatives) produces the following relationship that account for 62.47% of the variance in molecular weight (R^2 = 0.6247). Where MW is molecular weight, natoms is number of atoms, nON is number of oxygen & nitrogen, nOHNH is number of hydroxyl & amines, nrotB is number of rotatable bonds, and volume is the molecular volume in Angstroms^3.

$$MW = 374.47 - 3.361\,(PSA) - 59.244\,(natoms) + 64.031\,(nON) + 2.701\,(nOHNH) - 31.614\,(nrotB) + 4.764\,(volume)$$

By assigning desired values for independent properties within the model, then it will be feasible to determine the expected molecular weight of the perspective drug compound. The result will be compounds analogous to the meclofenamic acid based group of COX-1 inhibitors.

The purpose of cluster analysis is to discover a system of organizing the compounds elucidated here into groups where members of each group share properties in common [24]. Hierarchical cluster analysis does not require preset knowledge of the number of groups. Two general methods of hierarchical clustering methods are: divisive and agglomerative [24]. The dendrogram presented in Fig. 4 is a vertical divisive outcome of Table 1 hierarchical cluster analysis, where meclofenamic acid (the parent compound) is represented as MA.

The divisive approach begins by assuming a single group encompassing all members to be analyzed (group A, see Fig. 4) and further partitions that group into subgroups. This is followed by further partitioning of these subgroups further into subgroups and continues until each object forms its own subgroup.

Conditions for this hierarchical cluster analysis is single linkage (i.e. the distance between two subgroups is the minimum distance between any two members of opposite groups) and Euclidean distance (the ordinary straight line distance between two points in Euclidean space) [24]. The initial group from which all members are divided begins at super node A (see Fig. 4).

The length of the branch indicates the distance between the subgroups when they are joined. By the dendrogram of Fig. 4, the parent compound meclofenamic acid (MA) is most closely similar to compounds 9, 11, 12, 18, and 7, in that corresponding order. The remaining compounds will likewise be clustered adjacent to compounds having greatest similarity based upon the physicochemical properties in Table 1.

All compounds can then be associated to the other compounds is such a manner as to ascertain the highest level of similarity based on molecular properties that are important in elucidating drug-likeness. The node B then indicates a group of compounds of highest similarity, which includes: compounds 1, 5, 4, 8, and 10 (see Table 1). The length of the branch indicates the distance between the subgroups when they are joined.

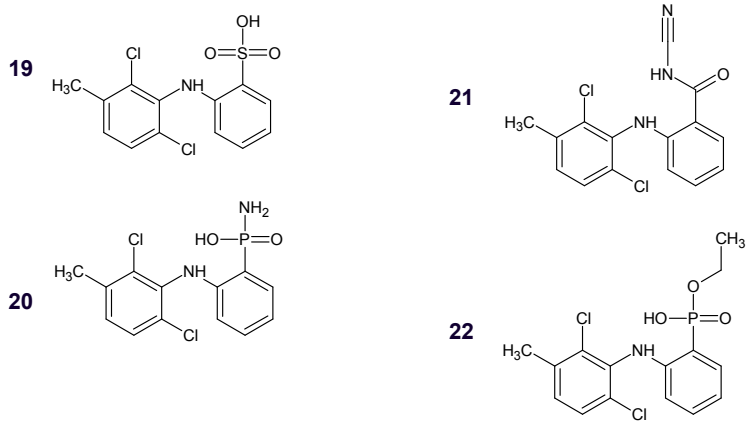

Fig. 3. Nonclassical bioisosteres of meclofenamic acid

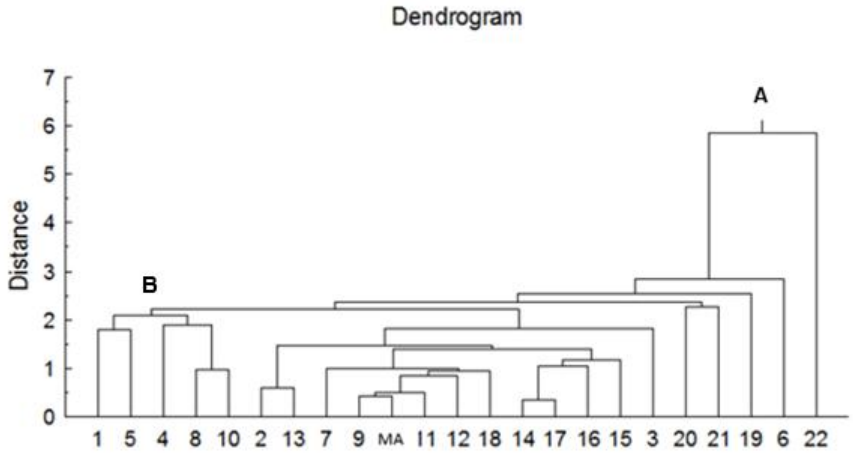

Fig. 4. Hierarchical cluster analysis of meclofenamic acid based compounds utilizing single linkage cluster conditions Single linkage (the distance between two subgroups as the minimum distance between any two members of opposite groups) with Euclidean distance. The parent structure is meclofenamic acid designated as MA

The applications of cluster analysis will suggest which compounds will have similarity in pharmacological activity, based on their physicochemical properties [20,21].

3.2 Tolfenamic Acid Isosteres and Bioisosteres

Tolfenamic acid is a fenamic acid derivative also referred to as fenamates. Previous studies have shown that tolfenamic acid is a successful anticancer agent that can significantly inhibit medulloblastoma, a common malignancy in children [26].

The parent structure of this NSAID is presented in Fig. 5, showing the two aromatic rings connected by a secondary amine (-NH-) group, a carboxyl group (-COOH), one chlorine atom, and one methyl group ($-CH_3$). It is the chlorine atom and methyl group that are amenable to class I isosteres replacement.

Systematic replacement of the original chlorine atom and methyl group by class I isosteres produces the derivatives of tolfenamic acid shown in Fig. 5. Note that the two aromatic rings, carboxyl group (-COOH), and connecting secondary amine group remains. The immediate consequence in physicochemical properties such as Log P, polar surface area (measured in Angstroms^2 or A^2), number of atoms (natoms), molecular weight, number of oxygen/nitrogen/ amine/hydroxyl moieties, and molecular volume are calculated with presentation in Table 2.

Table 1. Meclofenamic acid isosteres and nonclassical bioisosteres

Compound	Log P	Polar surface area (A^2)	Number of atoms	Molecular weight	Number of oxygens & nitrogens	Number of –OH and -NH_n	Violations of rule of 5	Number of rotatable bonds	Molecular volume (A^3)
1	4.605	49.326	19	263.243	3	2	0	3	221.279
2	5.895	49.326	19	385.055	3	2	1	3	247.188
3	5.491	49.326	19	479.055	3	2	1	3	259.397
4	3.839	89.782	19	259.261	5	4	0	3	227.452
5	4.736	49.326	19	291.397	3	2	0	3	246.738
6	3.244	101.372	19	257.293	5	6	1	3	233.994
7	5.174	49.326	19	255.317	3	2	1	3	244.539
8	4.668	75.349	19	297.141	4	4	0	3	233.216
9	5.414	49.326	19	314.193	3	2	1	3	239.588
10	4.966	69.554	19	298.125	4	3	0	3	229.945
11	5.863	49.326	19	316.571	3	2	1	3	235.463
12	5.348	49.326	19	300.116	3	2	1	3	226.859
13	6.268	49.326	19	408.022	3	2	1	3	245.918
14	5.083	37.299	19	295.165	2	1	1	3	242.888
15	5.035	37.299	19	408.738	2	1	1	3	257.464
16	5.534	37.299	19	360.098	2	1	1	3	249.577
17	5.089	37.299	19	313.205	2	1	1	3	244.215
18	5.296	46.533	19	297.137	3	1	1	3	235.071
19	2.534	66.397	20	332.208	4	2	0	3	250.936
20	4.059	75.349	20	331.139	4	4	0	3	259.14
21	4.507	64.917	21	320.179	4	2	0	3	259.732
22	5.008	58.56	22	360.177	4	2	1	5	290.199
Meclofenamic acid	5.633	49.326	19	296.153	3	2	1	3	238.488

A^2 = Angstroms^2, A^3 = Angstroms^3;

The following compounds have violations of Log P (not less than Log P = 5): compounds 2, 3, 7, 9, 11, 12, 13, 14, 15, 16, 17, 18, 22, and meclofenamic acid.

The following compounds have violations of the number of –OH and –NH_n (not less than 5 total): compound 6

Table 2. Tolfenamic acid isosteres and nonclassical bioisosteres

Compound	Log P	Polar surface area (A^2)	Number of Atoms	Molecular weight	Number of oxygens & nitrogens	Number of –OH & -NH	Violations of rule of 5	Number of rotatable bonds	Molecular volume (A^3)
1	4.489	49.326	18	245.253	3	2	0	3	216.348
2	5.134	49.326	18	306.159	3	2	1	3	229.302
3	5.408	49.326	18	353.159	3	2	1	3	235.407
4	4.314	69.554	18	243.262	4	3	0	3	219.435
5	4.555	49.326	18	259.33	3	2	0	3	229.077
6	3.808	75.349	18	242.278	4	4	0	3	222.705
7	4.774	49.326	18	241.29	3	2	0	3	227.978
8	4.038	75.349	18	262.696	4	4	0	3	219.68
9	4.784	49.326	18	279.748	3	2	0	3	226.052
10	4.336	69.554	18	263.68	4	3	0	3	216.409
11	4.453	37.299	18	260.72	2	1	0	3	229.352
12	4.405	37.299	18	374.293	2	1	0	3	243.928
13	4.904	37.299	18	325.653	2	1	0	3	236.041
14	4.459	37.299	18	278.76	2	1	0	3	230.679
15	4.667	46.533	18	262.692	3	1	0	3	221.535
16	1.904	66.397	19	297.763	4	2	0	3	237.401
17	3.429	75.349	19	296.694	4	4	0	3	245.604
18	3.877	64.917	20	285.734	4	2	0	3	246.197
19	4.378	58.56	21	325.732	4	2	0	5	276.663
Tolfenamic acid	5.003	49.326	18	261.708	3	2	1	3	224.953

A^2 = Angstroms^2, A^3 = Angstroms^3

The following compounds have violations of Log P (not less than Log P = 5): compounds 2, 3, and tolfenamic acid

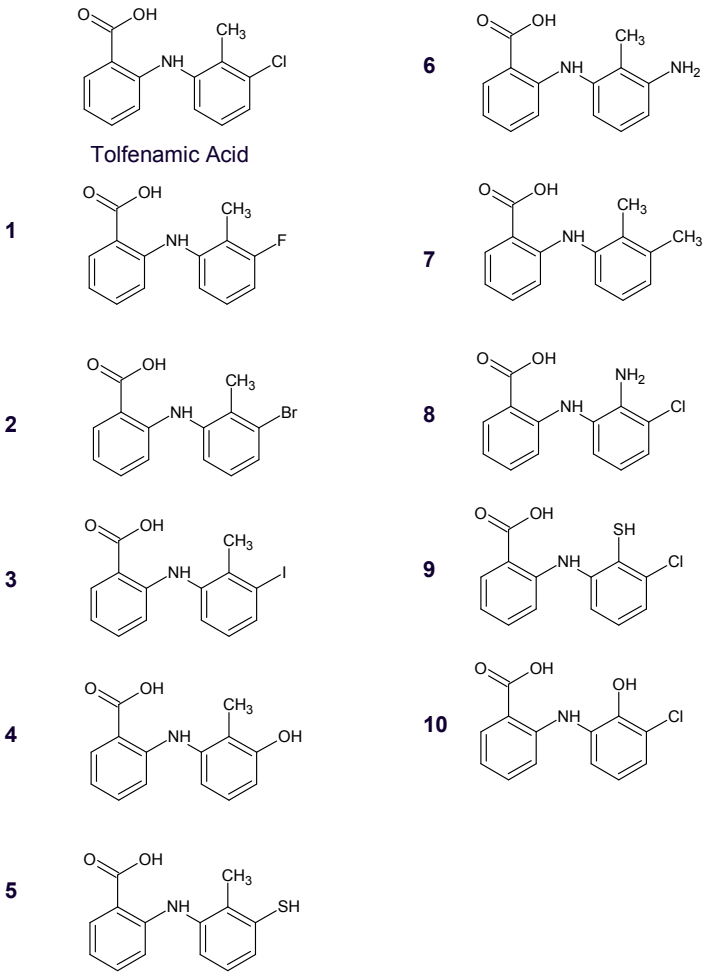

Fig. 5. Class I isosteres of tolfenamic acid

Property variations induced by class I isosteres replacement occurs for all 10 derivatives for all properties, save for number of atoms and number of rotatable bonds. Although there appears a range of Log P variation (from 3.808 to 5.408) and polar surface area (from 49.326 A^2 to 75.349 A^2), in fact, by the Grubb's test there are no outliers (alpha = 0.05) [24], within these two parameters even with tolfenamic acid inclusive (Log P = 5.003, PSA= 49.326 A^2).

Applying the Rule of 5 test to the ten isosteres of tolfenamic acid shows that only compound 2, 3, and tolfenamic acid have one violation. Hence, these class I isosteres show favorable properties for drug-likeness and oral administration.

In the approach for class II isosteres replacement within tolfenamic acid, it is the connecting amine group (-NH-) that is replaced with $–CH_2-$, Te, Se, -S-, or –O- (see Fig. 6). Note that the two aromatic rings, chlorine atom, carboxyl group, and methyl group remain intact within the derivative structures, see Fig. 6.

Variation of properties for class II isosteres is shown in Table 2, for comparison to the parent drug tolfenamic acid. In this case, by the Grubb's test, there are no outliers among Log P, molecular weight, number of atoms, number of rotatable bonds, and polar surface area (tolfenamic acid included). These class II isosteres show zero violations of Rule of 5, thus class II isosteres replacement preserves favorable drug-likeness and oral activity.

An interesting comparison is found when nonclassical bioisosteres are applied for substituent replacement within tolfenamic acid. These are presented in Fig. 7. The resulting

changes in physicochemical properties is revealed in Table 2. Notably, it is seen that all four compounds 16, 17, 18, and 19 show zero violations of the Rule of 5, a distinguishable improvement compared to the parent compound tolfenamic acid which shows only one violation of Rule of 5. The use of non-classical bioisosteres improves oral activity of this NSAID.

Likewise, there are no outliers among Log P, polar surface area, number of atoms, molecular volume, and molecular weight (including meclofenamic acid). This is of interest, because the nonclassical bioisosteres are of greater mass and volume than class I as well as class II isosteres.

The overall drug-likeness and oral activity of tolfenamic acid isosteres & bioisosteres is retained, with all instances (save for 2 and 3 which are equal to the parent compound tolfenamic acid) are actually improved in terms of drug-likeness (e.g. zero violations of Rule of 5).

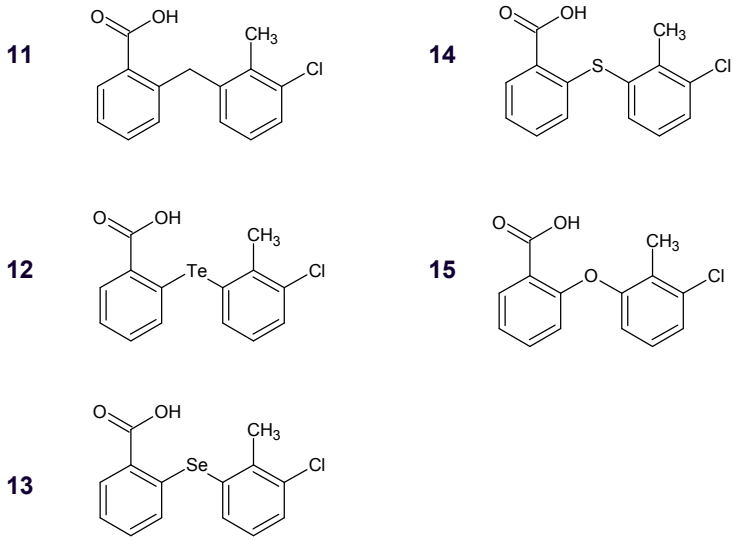

Fig. 6. Class II isosteres of tolfenamic acid

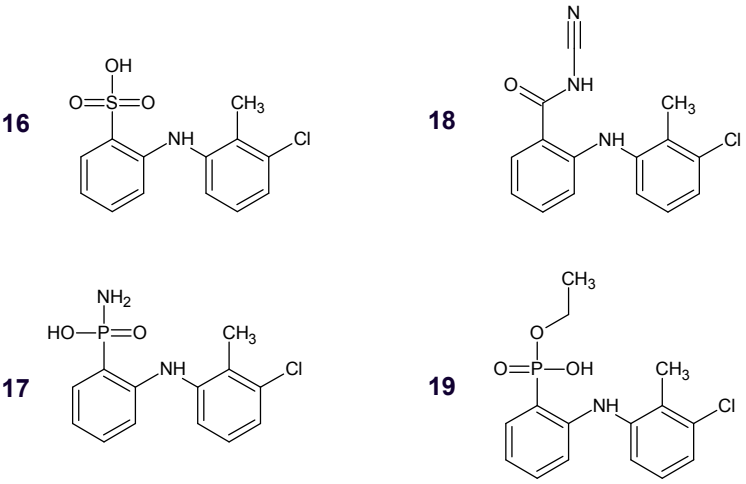

Fig. 7. Nonclassical bioisosteres of tolfenamic acid

By the t-test the means of Log P values for class I isosteres for both meclofenamic acid and tolfenamic acid derivatives are equal (*P=0.13*). By the t-test and one-way ANOVA, the Log P for nonclassical bioisosteres of both groups have equal means (P=0.44). However, this is not true for the class II isosteres of both groups. The means for Log P values for class II isosteres of meclofenamic acid compounds 14 to 18 compared to tolfenamic acid compounds 11 to 15 is 5.207 (standard deviation of 0.21) and 4.578 (standard deviation of 0.21), respectfully. Consequently, the substitution of class II isosteres (replaced with $–CH_2$-, Te, Se, -S-, or –O- groups) upon the tolfenamic acid structure having only one chlorine atom has substantial impact upon partition coefficient Log P.

3.2.1 Multiple regression for prediction and pattern recognition

The result of multiple regression analysis of properties of Table 2 produces the following relationship that account for 74.23% of the variance in molecular weight (R^2 = 0.7423). Where MW is molecular weight, natoms is number of atoms, nON is number of oxygen & nitrogen, nOHNH is number of hydroxyl & amines, nrotB is number of rotatable bonds, and volume is molecular volume in Angstroms^3:

MW = 338.75 + 1.131(PSA) – 72.025 (natoms) + 23.025 (nON) – 24.929 (nOHNH) - 17.483 (nrotB) + 5.344 (volume)

By assigning desired values for independent properties within the model, then it will be feasible to determine the expected molecular weight of the perspective drug compound. The result will be compounds analogous to the tolfenamic acid based group of COX-1 inhibitors.

The dendrogram presented in Fig. 8 is a vertical divisive outcome of Table 2 hierarchical cluster analysis, where tolfenamic acid (the parent compound) is represented as TA (see Fig. 8). Conditions for this hierarchical cluster analysis is single linkage (i.e. the distance between two subgroups is the minimum distance between any two members of opposite groups) and Euclidean distance (the ordinary straight line distance between two points in Euclidean space) [24]. The initial group from which all members are divided begins at super node A (see Fig.8).

The length of the branch indicates the distance between the subgroups when they are joined. By the dendrogram of Fig. 8, the parent compound tolfenamic acid (TA) is most closely similar to compounds 2 and 3, in that corresponding order. The remaining compounds will likewise be clustered adjacent to compounds having greatest similarity based upon the physicochemical properties in Table 2.

All compounds can then be associated to the other compounds is such a manner as to ascertain the highest level of similarity based on molecular properties that are important in elucidating drug-likeness. The node B then indicates a group of compounds of highest similarity, which includes: compounds 1, 5, 7, 9, 15, 11, 14, 13, and 12 (see Table 2 for molecular properties). The length of the branch indicates the distance between the subgroups when they are joined.

The applications of cluster analysis will suggest which compounds will have similarity in pharmacological activity, based on their physicochemical properties [20,21].

3.3 Comparison of Meclofenamic and Tolfenamic Acid Isosteres/ Bioisosteres

The replacement of substituents found on meclofenamic acid and tolfenamic acid results in significant changes of some properties from those of the parent compounds. However, the drug-likeness of the derivatives is generally improved (i.e. zero violations of the Rule of 5 or only one violation). The analysis of properties by cluster analysis is able to identify underlying relationships and divide the compounds into groups having the highest level of similarity. By this manner, the derivatives most similar to the parent compounds were identified.

The average values for the pharmacological properties important for consideration of drug-likeness are presented in Table 3.

The overall comparison for all of meclofenamic acid and tolfenamic acid based COX-1 inhibitors, their molecular properties, are presented in Table 3. By one-way ANOVA analysis it is determined that the two group means are equal (*P=0.99*). In addition, the Kruskal-Wallis test indicates the two groups have equal medians (*P=0.97*). The two groups have extremely strong positive correlation (Pearson's r = 0.9990).

The investigation and design of new COX-1 inhibitors is feasible and needed. Previous studies have shown that COX-1 expression is up-regulated in cancer such as human breast cancer and human prostate cancer [27]. COX-1 but not COX-2, is highly expressed in human epithelial ovarian cancers [27]. Up-regulation of COX-1 is found in squamous cell carcinoma and adenocarcinoma of the human cervix, suggesting that both COX-enzymes and/or their products may contribute to modulate the tumors genesis and the expression of factors responsible of the development of cervical cell neoplasia [27]. Clearly then, the investigation and design of novel COX-1 inhibitors is appropriate and imperative. This study affirms the efficacy of computer aided drug design in pursuit of COX-1 inhibitors. The numerous compounds presented in this study are based upon proven approaches to drug modification and lead design. Computer aided drug design will continue to be a powerful tool in the efforts to explore new drug designs as remedies for inflammatory based disease.

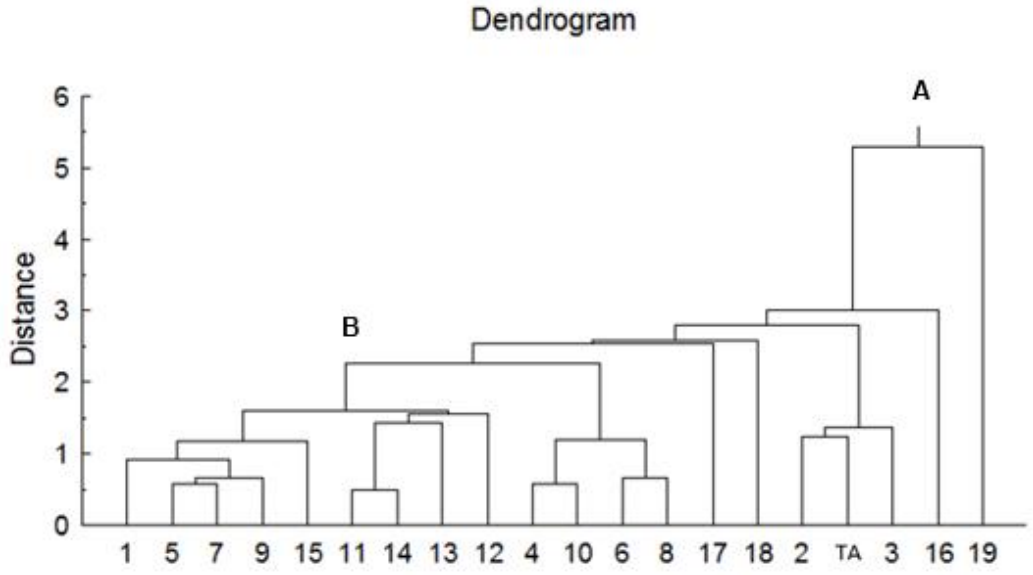

Fig. 8. Hierarchical cluster analysis of tolfenamic acid based compounds utilizing single linkage cluster conditions with Euclidean distance. The parent structure is tolfenamic acid designated as TA

Table 3. Comparison of properties of isosteres and non-classical bioisosteres of meclofenamic and tolfenamic

Property	Average values for all meclofenamic acid based cox-1 inhibitors	Average values for all tolfenamic acid based Cox-1 inhibitors
Log P	5.0±1.0	4.0±1.0
Polar surface area (A^2)	59.099±17	54.802±13
Number of atoms	19±1.0	18±1.0
Molecular weight	323.43±55	283.33±38
Number of –O and -N	3.0±1.0	3.0±1.0
Number of –OH and $–NH_n$	2.0±1.0	2.0±1.0
Violations of Rule of 5	1.0±0.0	0±0.0
Number of rotatable bonds	3.0±0.0	3.0±0.0
Molecular volume (A^3)	244.32±15	231.73±14

4. CONCLUSION

Both meclofenamic acid and tolfenamic acid express anticancer activity. That action and other aspects prove both these compounds to be desirable lead drugs for new compound structures. All derivatives of both drugs retain favorable drug-likeness as measured by the Rule of 5 (i.e. zero violations or only one). Comparison of average values for molecular properties indicated that the two populations have equal means, equal medians, and have extremely strong positive correlation. Cluster analysis revealed which derivatives were of highest similarity to their parent compound. Multiple regression analysis of all physicochemical properties produced models describing 62.47% of variance in meclofenamic acid compounds (R^2 = 0.6247) and 74.23% of variance in tolfenamic acid compounds (R^2 = 0.7423). The use of isosteres and nonclassical bioisosteres proved a successful approach for designing novel COX-1 inhibitors. Computer aided drug design is a powerful tool to explore new drug designs for treatment of inflammatory based disease.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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