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Quantum and Classical Evaluations of Carboxylic Acid Bioisosteres: From Capped Moieties to a Drug Molecule

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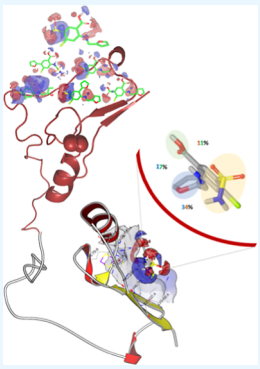
ABSTRACT: Using the Quantum Theory of Atoms in Molecules, the average electron density (AED) tool was developed and employed to quantitatively evaluate the similarities between bioisosteric moieties in drug design. Bioisosteric replacements are valuable in drug molecules to fine-tune their pharmacokinetic and pharmacodynamic properties while maintaining their biological activity. This study was performed on non-classical bioisosteres of carboxylic acid. It was found that the AED of a given bioisostere is generally transferable, within less than 5% difference, irrespective of its environment. It was shown that the AED tool succeeds at depicting not only the similarities of bioisosteric groups but also at highlighting, as counter examples, the differences in non-bioisosteric groups. For the first time, the AED was used to evaluate bioisosterism in an FDA-approved drug molecule, furosemide, and in five analogues of this medicine. In one of the analogues, non-bioisosteric moieties were exchanged, and in four of the analogues, carboxylic acid was replaced with either furan or sulfonamide, and vice versa. It was also found that irrespective of the pH, the AED tool consistently reproduced experimental predictions. The distinct power of the AED tool in quantitatively and precisely measuring the similarity among bioisosteric groups is contrasted with the relatively ambiguous bioisosteric evaluations through the classical qualitative electrostatic potential (ESP) maps. The ESP maps were demonstrated to fail, even qualitatively, in depicting the similarities, in some cases.



1. INTRODUCTION

Bioisosterism is a vital concept in drug design that leads to the production of more clinically effective medications.[1−3] Bioisosterism is the substitution of groups within a drug molecule while conserving its biological activity.[1−3] Bioisosteric replacements can be used to improve the pharmacodynamics and pharmacokinetics of drug candidates. For example, they enhance the absorption, distribution, metabolism, excretion, permeability, and solubility[3] or lower the toxicity.[4,5] For instance, carboxylic acid is one of the groups that happens to have many potential bioisosteric substitutions.[6] The bioisosteric replacement of carboxylic acid with tetrazole can either significantly enhance the plasma protein binding[7] or increase the lipophilicity of drugs to cross the blood−brain barrier in the treatment of neurodegenerative disorders.[8] In fact, carboxylic acid has been successfully substituted with tetrazole to develop many drugs such as cefamandole, ceftezole, tedizolid, letrozole, encequidar, oteseconazole, quilseconazole, losartan, valsartan, candesartan, and tomelukast.[9] In addition, carboxylic acid can be substituted with the chemically and enzymatically more stable sulfonamide bioisostere in order to increase the metabolic resistance of a drug.[10] In non-classical bioisosterism, the similarity in the biological activity is maintained, although the moieties may not share the same physical or chemical properties.[11]

Computer-aided drug design tools have been heavily involved in assessing bioisosterism. In particular, the biological similarity in non-classical bioisosteres has been previously explained using two major tools, the electrostatic potential (ESP) maps and the average electron densities (AED) tool. The ESP maps are used as a classical tool to qualitatively visualize molecular properties, while the AED is a quantum tool used to evaluate quantitatively atomic or, subsequently, group properties within a molecule. In the past, the ESP maps had been relied on, almost exclusively, to explain non-classical bioisosterism.[12,13] However, in the past decade, the AED tool was developed and referred to as a more robust quantitative tool for measuring the similarity among non-classical bioisosteres.[14−18] The AED tool is based on, first, generating the wavefunction of a system, and then, partitioning the molecule into atomic basins according to the Quantum Theory of Atoms in Molecules (QTAIM) theory. The property of a bioisosteric group is then calculated as the sum of the properties of the atoms constituting this group. For example, the AED of a bioisosteric group is given by $\rho_{\text{bioisostere}} = \sum N_i / \sum V_i$, where $\sum N_i$ is the sum of the electron populations and $\sum V_i$ is the sum of the volumes of all atoms (each atom denoted by i) in the bioisosteric moiety.

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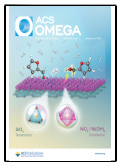

In this study, we investigate the similarities among three groups [carboxylic acid (R−COOH), sulfonamide (NH_2SO_2−R), and furan (R−C_4H_3O)] using both the ESP maps and the AED tool. The pairs to be compared in this study are carboxylic acid and sulfonamide (used in the literature as bioisosteres), carboxylic acid and furan (suggested in the literature as potential bioisosteres, according to the SwissBioisostere website and its algorithm of searching for bioisosteres, http://www.swissbioisostere.ch), and furan and sulfonamide (non-bioisosteres, based on the literature search and the SwissBioisostere search). Thus, one of the aims in this is to evaluate how the AED tool differentiates between confirmed bioisosteres, potential bioisosteres, and non-bioisosteres. The R group (see Figure 1) represents the different capping groups

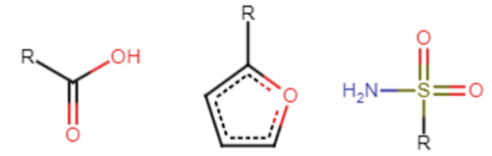

Figure 1. 2D structures of carboxylic acid, furan, and sulfonamide, capped with an R group (hydrogen, methyl, or chloro).

(methyl, chlorine, and hydrogen) used in this study. Different capping groups are used so that the transferability of the AED tool across different environments is tested, irrespective of the rest of the molecule. In other words, different environments surrounding the bioisosteric moiety in a drug molecule are mimicked by choosing three R groups that have different electronegativities. In addition, to depict a more realistic environment, a US Food and Drug Administration (FDA)-approved drug containing the three bioisosteres is also considered. This drug is furosemide. Furosemide has been recently highlighted in drug repurposing as a potential treatment, by targeting the mitochondria, for diseases such as cancer, inflammation, and metabolic and neurodegeneration disorders.[19,20] Furosemide was chosen in this study for the following reasons: (i) it is an FDA-approved drug molecule that contains the three bioisosteric moieties considered in this study (carboxylic acid, furan, and sulfonamide), (ii) all the three moieties in furosemide are terminal in such a way that they match the capped bioisosteric moieties studied here as shown in Figure 1, (iii) given that furosemide is being examined in drug repurposing, there are high chances that its pharmacokinetic and pharmacodynamic properties still need to be fine-tuned, which can be efficiently achieved through bioisosteric replacements, and (iv) the furosemide has been crystallized in its receptor, and the X-ray structure is readily available. Furosemide is a diuretic drug that binds to mitoNEET, which is a mitochondrial protein in the outer memberane,[19,21] with a K_i of 2.28 μM and an IC_{50} of 53.46 μM.

Therefore, in addition to evaluating the AED and generating the ESP maps of the capped carboxylic acid, furan, and sulfonamide moieties, the objective of this study is to investigate the bioisosterism of the same three moieties within the FDA-approved drug molecule.[19,20,22] which is a mitochondrial protein in the outer membrane. This would be the first time to test the AED tool in the evaluation of bioisosteric groups in an approved drug molecule. To account for the pH changes in the realistic model, it is possible to study the properties of furosemide in its anionic and neutral forms. The extent of the validity of the AED tool must be tested on the anionic furosemide as it has a diffuse electron density. Given that furosemide contains three bioisosteric groups, we will also attempt to substitute two of these groups (furan and sulfonamide) to test the effect of this replacement on the AEDs and the ESP maps within the drug molecule. Further substitutions will be performed where carboxylic acid is replaced with a furan or a sulfonamide group, and vice versa. The target is to highlight the differences and the complementarities between the ESP maps and the AED tool in realistic environments.

2. COMPUTATIONAL METHODS

To evaluate the AED tool in bioisosterism, three bioisosteric groups were considered: carboxylic acid, furan, and sulfonamide. The pairs that will be compared are the bioisosteres carboxylic acid and sulfonamide, the potential bioisosteres carboxylic acid and furan, and the non-bioisosteres furan and sulfonamide. To investigate the effect of the environment around the bioisosteric moieties, they were capped with various different capping groups (hydrogen, methyl, and chloro), denoted by R in Figure 1. The three chosen capping groups are commonly abundant in drug molecules, yet they have different electronegativities. According to the Pauling scale, the electronegativities of hydrogen, carbon, and chlorine are 2.1, 2.5, and 3.0, respectively.

Using the Gaussian16 package,[23] the bioisosteres capped with their various groups were optimized (disregarding the symmetry), in vacuum at the B3LYP/6−311++G(d,p)//B3LYP/6−311++G(d,p) level of theory. To confirm that the optimized geometries are not transition states and do not have imaginary frequencies, vibrational frequency analysis was completed. The ultrafine pruned (99,590) grids were used along with "tight" self-consistent field optimization criteria. The wavefunction files were obtained to complete the atomic partitioning according to QTAIM. AIMAll[24] was used for atomic integrations based on QTAIM.[25,26] While the interatomic basins are delimited by zero-flux surfaces, the outer limit of the atomic basins is delimited by different isodensity envelopes, namely, 0.004, 0.01, and 0.02 a.u. Unless otherwise specified (for testing purposes), a super-fine interatomic surface mesh was used for the AIMALL integrations, which are recommended for diffuse charge distributions such as anionic systems.

To evaluate the AED and ESP maps of the three bioisosteric groups in the realistic environment of an FDA-approved drug, the structure of furosemide was extracted in the same geometry as that found experimentally when complexed with its receptor, mitoNEET (PDB ID: 6DE9), and a single point calculation was performed on it at the same level of theory mentioned above. In order to account for the change in pH in the biological media, furosemide was considered in its neutral (protonated) and anionic (deprotonated) forms. The pK_a values of carboxylic acid in furosemide, furan as an isolated ring or in benzofuran, and sulfonamide in benzosulfonamide are 3.48,[27] ∼33−36,[28,29] and ∼9−10,[30] respectively. Therefore, under a physiological pH of ∼7.4, the carboxylic acid group will be deprotonated, while furan and sulfonamide will remain in their protonated state. To test the effect of group substitutions in a drug molecule, sulfonamide and furan were swapped in furosemide to form a furosemide analogue. The

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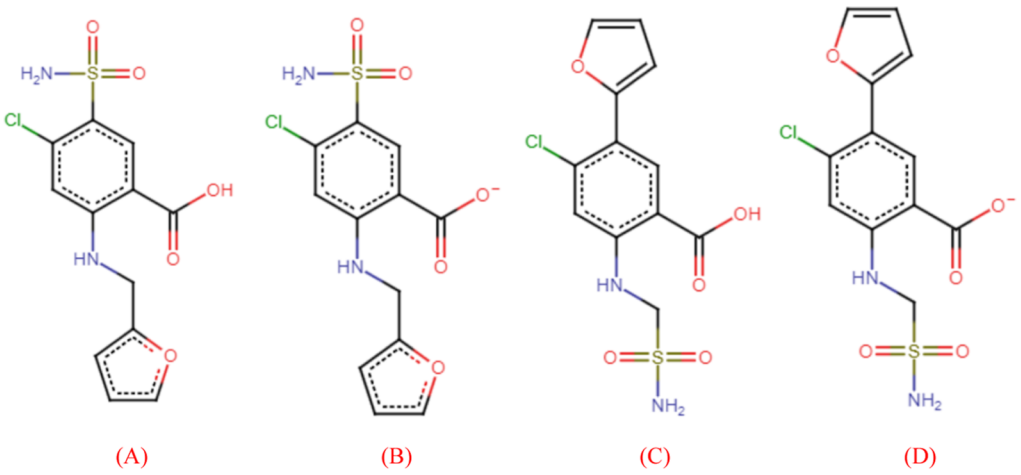

Figure 2. 2D structures of furosemide in its neutral (A) and anionic (B) forms and the furosemide analogue in its neutral (C) and anionic (D) forms.

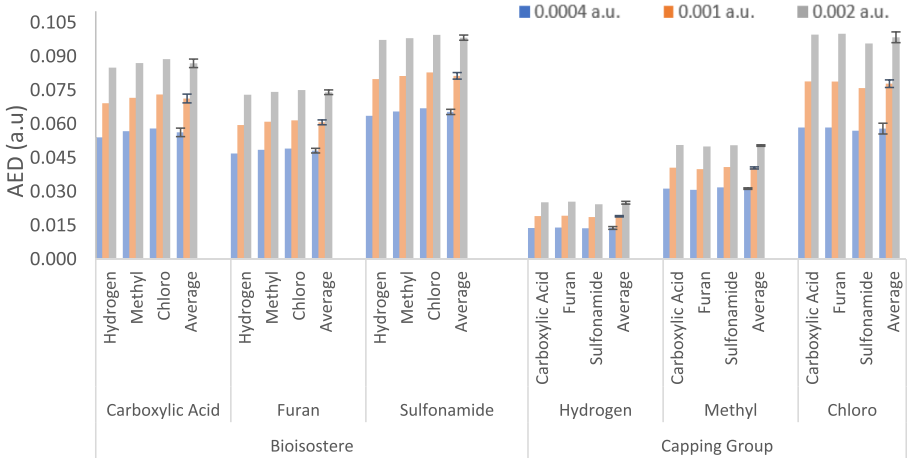

Figure 3. AEDs of the three bioisosteric moieties (carboxylic acid, furan, and sulfonamide), each capped with three different R groups (hydrogen, methyl, and chloro) (left). AEDs of each of the three capping groups with all three bioisosteric groups (right). All values are reported at the three isodensity values, 0.0004, 0.001, and 0.002 a.u.

structures of furosemide and its analogue in their neutral and anionic forms are depicted in Figure 2.

The carboxylic acid group in furosemide was then substituted with furan or sulfonamide, and vice versa (as shown in Table 4).

The ESP maps were generated using ChemCraft 1.8 (https://www.chemcraftprog.com).

3. RESULTS AND DISCUSSION

3.1. AED of the Capped Bioisosteric Groups. It is obvious from Figure 3 that the AED trends across the various bioisosteric and capping groups are reproducible, irrespective of the isodensity values, namely, 0.0004, 0.001, and 0.002 a.u. Therefore, unless otherwise specified, for the rest of the paper, the AED values will be reported exclusively at the isodensity of 0.001 a.u.

Figure 3 depicts the similarities in the AEDs of the bioisosteric (carboxylic acid and sulfonamide) and the potential bioisosteric (carboxylic acid and furan) pairs. These similarities are congruent with the similarities observed for other carboxylic acid bioisosteres from previous studies, which include tetrazole,[18] methylsquarate,[17] sulfonamide,[16] isoxazole, tetrazol-5-one, oxadiazole, thiazolidinedione, and oxazolidinedione.[15] To highlight the non-coincidence in this similarity of the bioisosteric or potential bioisosteric groups, the rather leveled-off AED values in these groups are contrasted with (1) the significant difference observed in the AEDs between the non-bioisosteric pair (furan and sulfonamide) and (2) the relatively varying AEDs of the capping groups (as shown in Figure 3). The AED difference, on average, between the carboxylic acid (0.0713 a.u.) and furan (0.0607 a.u.) or sulfonamide (0.0813 a.u.) does not exceed 0.0106 a.u. Furan, on average, has AEDs that are 15% smaller than those of carboxylic acid. On the other hand, sulfonamide (NH_2SO_2−R), on average, has AEDs that are 14% greater than those of carboxylic acid. This is close to half of the previously reported difference (26%) between carboxylic acid and a trifluoride derivative of sulfonamide (CF_3SO_2NH−R).[16] However, the AED difference between the capping groups, for example, between hydrogen (0.0190 a.u.) and chloro (0.0779 a.u.), reaches up to 0.0588 a.u. (i.e., up to 309% of the AED of hydrogen). This highlights the large fluctuations in the AED of the non-bioisosteric R groups (0.0588 a.u.); they were more than 5.5-fold higher than the minor variations among the AEDs of the bioisosteric groups (0.0106 a.u.). It is also noted that the AEDs of the capping groups increase as the

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electronegativity of the central atom (H, C, and Cl) increases. Overall, the AEDs of the bioisosteric moieties are much closer to each other than those of the capping groups. It is also worth noting that the average AEDs of sulfonamide are 34% higher than those of furan. The 14% AED difference between sulfonamide and carboxylic acid, and the 15% AED difference between furan and carboxylic acid are to be contrasted with the 34% AED difference between sulfonamide and furan. Based on this fact, it is suggests that, while furan and sulfonamide are independently bioisosteric (or suggested bioisosteric) groups of carboxylic acid[6,10,31] (http://www.swissbioisostere.ch/), they do not seem to be bioisosteres themselves of each other. This observation is supported by the results reported from the SwissBioisostere website (http://www.swissbioisostere.ch/), which provides potential bioisosteric replacements based on experimental measures collected from the literature. In fact, the SwissBioisostere website reported 265 matched molecular pairs (MMPs) for carboxylic acid and sulfonamide bioisosteres, and 27 MMPs for carboxylic acid and furan potential bioisosteres but 0 entries for furan and sulfonamide. In addition, with extensive searches in the literature, we were not able to find any publication, patent, or article referring to the bioisosteric substitution between furan and sulfonamide.

The similarity in the AED of a given bioisostere capped with three different groups is very obvious from the small standard deviations of 0.0019, 0.0011, and 0.0015 a.u. for carboxylic acid, furan, and sulfonamide, respectively (Figure 3). These standard deviations correspond to 2.7, 1.8, and 1.8% of the average AEDs of three bioisosteric moieties, respectively. This reflects the validity of the AED tool in depicting the similarity in the bioisosteric AEDs, irrespective of the capping group, that is, irrespective of the change in the environment around the bioisosteric moieties. This is aligned with the findings of the previous studies which reported 0.92−2.2% percent difference in the AED of bioisosteres (carboxylic acid, tetrazol-5-one, oxadiazole, oxazolidinedione, thiazolidinedione, and isoxazole) each capped with five different R groups.[14,16] This is a distinguished property for the bioisosteric groups given that the standard deviations of the monoatomic capping groups considered in this study (hydrogen and chloro) are within the same range, 1.6% and 2.2% of the respective average AEDs.

In summary, based on this part of the study, where three bioisosteric groups were capped with three different simple R groups, it was found that (i) the AEDs of the bioisosteric moieties (carboxylic acid and sulfonamide) or the potential bioisosteric moieties (carboxylic acid and furan), are off by 14−15%, and (ii) the AEDs fluctuate within up to ±2.7% (i.e., a full range of 2.7% × 2 = 5.4% maximum percent deviation upon the change in the environment). In addition, although furan and sulfonamide are independently bioisosteres of carboxylic acid, they do not appear to be bioisosteres of each other (they have a large percent difference in their AED values, 34%).

As explained above, the similarities in the AED of the bioisosteres are clearly not coincidental. This is despite the significant fluctuations in their charges as shown in Figure 4. It is obvious from this figure that the charges of the bioisosteric moieties span a large range from −0.15 to +0.24 a.u. The charges can be of the opposite sign even for the same given bioisosteric moiety. For example, carboxylic acid has a charge of −0.19 a.u. and +0.14 a.u. when capped with methyl and chloro, respectively. Similarly, furan has a charge of −0.11 and

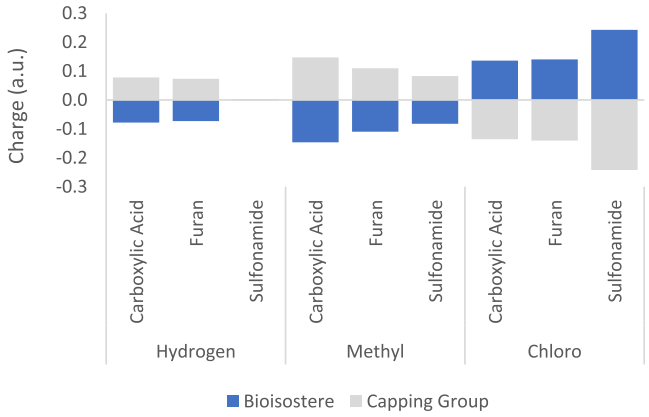

Figure 4. Charges of the three bioisosteric moieties (carboxylic acid, furan, and sulfonamide), each capped with three R groups (hydrogen, methyl, or chloro).

+0.14 a.u. when capped with methyl and chloro, respectively. It is noted that the charges of the capping groups and the bioisosteric moieties are of exact magnitude but of opposite charges. This, in fact, ensures the overall neutrality of the molecule.

Figure 5 (left) shows that the bioisosteric groups have different volumes and different electron populations, although they have similar AEDs (Figure 3). Carboxylic acid, furan, and sulfonamide have average volumes of 322 ± 10, 574 ± 12, and 502 ± 11 a.u., respectively, and average electron populations of 22.9 ± 0.1, 34.8 ± 0.1, and 40.8 ± 0.2 a.u., respectively. With respect to carboxylic acid, the volume of furan is off by 78%, and its electron population is off by 52%. Again, with respect to carboxylic acid, the volume of sulfonamide is off by 56%, and its electron population is off by 78%. Compared to carboxylic acid, furan has higher electron populations and higher volumes, and so does sulfonamide. The ratio of the volumes between furan and carboxylic acid is 1.8 while that of the electron populations is 1.5. These ratios are off by ∼15%, which is the same as the percent difference observed in the AEDs of furan and carboxylic acid (see Figure 3). However, it is noted that there is no proportionality between the variations in volumes and electron populations of furan and sulfonamide. The combination of higher electron populations and lower volumes of sulfonamide, with respect to furan, seems to be the reason behind the rather significant difference of 34% in the AEDs of these two moieties (see Figure 3). The variation of the capping groups does not make any significant difference in the volumes or electron populations of any of the three bioisosteres. In fact, the highest standard deviations in volumes and electron populations of the bioisosteres are 3.1 and 0.6%, respectively, while the corresponding values for the capping groups reach 6.3, and 4.7%, respectively.

We have shown so far that the bioisosteric pair (carboxylic acid and sulfonamide) and the potential bioisosteric pair (carboxylic acid and furan) share AED values that are off by only 14−15% despite the differences in their volumes (56 and 78%, respectively), electron populations (78 and 52%, respectively), and charges. To further highlight the importance of this similarity in AEDs of the bioisosteric moieties, Table 1 summarizes many of the obvious differences in the properties of each of these non-classical bioisosteric moieties. These differences are significant. For example, furan has double the number of atoms in carboxylic acid, it weighs ∼50% more, and

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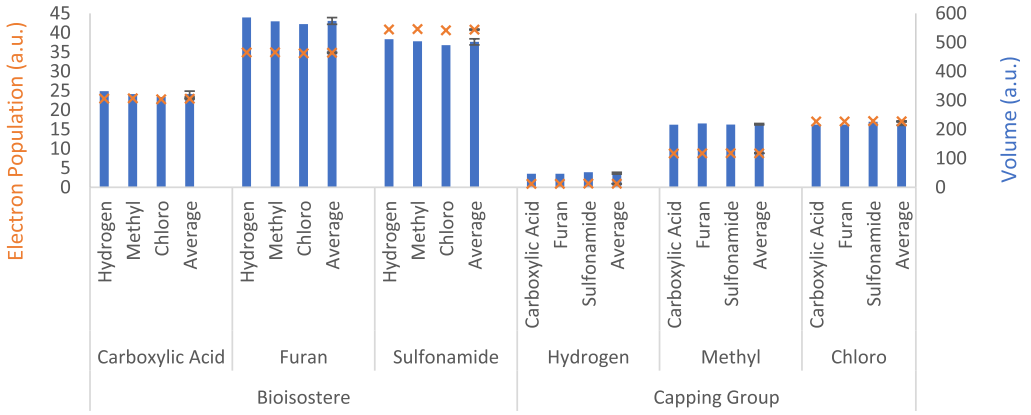

Figure 5. Electron populations (a.u.) and volumes (a.u.) of (left) the bioisosteric moieties capped with three R groups and (right) the capping groups with each of the three bioisosteres. The numbers are reported at the isodensity value of 0.001 a.u. The averages along with standard deviations are also included.

Table 1. Physio-Chemical Properties of Each of the Three Non-Classical Bioisosteric Moieties, Carboxylic Acid, Furan, and Sulfonamide

	carboxylic acid	furan	sulfonamide
number of atoms of the bioisosteric moiety	4	8	6
molecular shape	open	cyclic	open
molecular weight of the bioisosteric moiety (g mol^{-1})	43.99	67.06	80.06
number of hydrogen bond acceptors	2	1	3
number of hydrogen bond donors	1	0	1

its cyclic shape is different than the acyclic shape of carboxylic acid and sulfonamide. None of the three moieties has the same number of hydrogen bond acceptors.

3.2. AED of the Bioisosteres in Furosemide and Its Analogues. In order to account for a more realistic environment of the bioisosteric moieties considered in this study (carboxylic acid, furan, and sulfonamide), we evaluated their properties within the FDA-approved furosemide drug molecule. Furosemide was extracted as is from the furosemide−mitoNEET complex that was resolved experimentally (PDB ID: 6DE9). Carboxylic acid is more acidic than furan and sulfonamide; thus, under physiological conditions, it would deprotonate. Therefore, furosemide is considered in its fully protonated state and as anion where the carboxylic acid group is deprotonated to a carboxylate. Based on the results of the above-mentioned section and the literature search, the furan and sulfonamide moieties are not expected to be identified as bioisosteric groups of each other. To further test this hypothesis, a dual substitution was performed in furosemide to generate a structural analogue where furan is substituted with sulfonamide, and vice versa (see Figure 2). In the analogue, it is hypothesized that similarities in the AEDs of carboxylic acid with each of sulfonamide and furan are still observed.

Similar to the observations made in Figure 3 for the capped bioisosteres, the AED trends in furosemide and its analogue are reproduced at the three isodensity values (0.0004, 0.001, and 0.002 a.u.), and therefore, for clarity purposes, Figure 6 depicts the AEDs only at one isodensity, namely, 0.001 a.u. Figure 6 clearly shows that the bioisosteres maintain the same values, whether capped with R groups or taking part of a drug

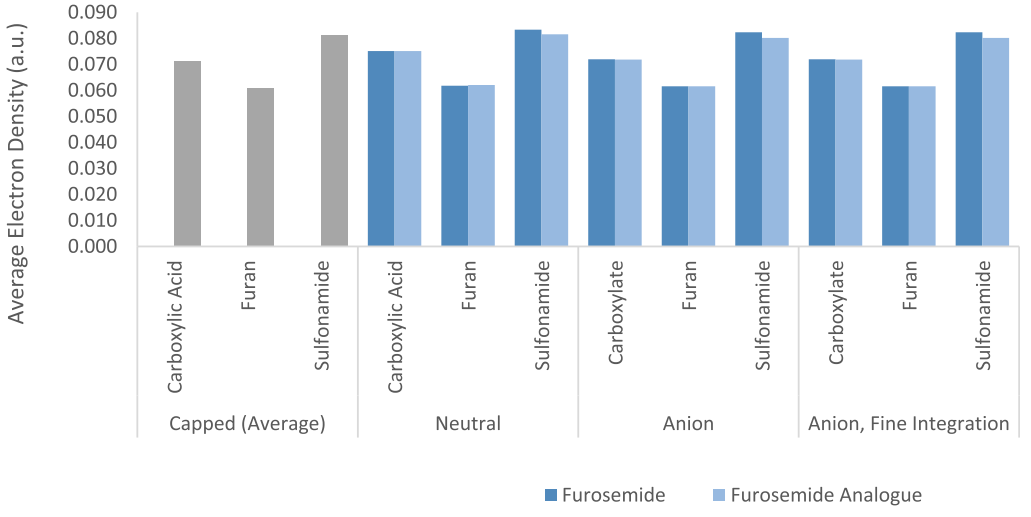

Figure 6. AEDs of the three bioisosteric groups (carboxylic acid, furan, and sulfonamide) in furosemide and its analogue, in their neutral and anionic forms, using superfine integrations. The anion values are also reported using fine integrations. The values are reported at the 0.001 a.u. isodensity.

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Table 2. Percent Differences in AEDs of Three Bioisosteric Moieties That Are Capped or in Real Environments, in Neutral or Anionic Forms, Evaluated Using Superfine and Fine Integrations[a]

Molecule	Bioisosteric Moiety	Δ (furosemide/analogue, capped) for each of the 3 bioisosteres wrt average capped bioisosteres (%)	Δ (furan, carboxylic acid) wrt carboxylic acid (%)	Δ (sulfonamide, carboxylic acid) wrt carboxylic acid (%)	Δ (sulfonamide, furan) wrt furan (%)	Δ (furosemide, analogue) wrt furosemide (%)	Δ (anion, neutral) wrt neutral (%)	Δ (fine, superfine) of anion wrt superfine of anion (%)
Furosemide Superfine	Carboxylic Acid Furan Sulfonamide	5.36 1.73 2.46	-17.78	10.96	34.95			
Furosemide Anion Superfine	Carboxylate Furan Sulfonamide	1.42 1.23			33.73		-0.30 -1.20	
Furosemide Anion Fine	Carboxylate Furan Sulfonamide							0.00 0.00 0.01
Furosemide Analogue Superfine	Carboxylic Acid Furan Sulfonamide	5.39 2.26 0.28	-17.37	8.57	31.39	0.02 0.52 -2.13		
Furosemide Analogue Anion Superfine	Carboxylate Furan Sulfonamide	1.36 -1.46			30.24	-0.14 -0.06 -2.67	-0.87 -1.74	
Furosemide Analogue Anion Fine	Carboxylate Furan Sulfonamide					-0.15 -0.06 -2.67		0.00 0.00 0.00

[a]The AEDs are considered at 0.001 a.u. isodensity. "wrt" stands for with respect to.

molecule, and whether in furosemide or its analogue. Therefore, the transferability of the AEDs of the bioisosteric moieties is applicable not only when varying the R groups but even when capping the moieties with more complex realistic environments as in furosemide and its analogue. The third column in Table 2 shows that the percent differences between the AEDs of the bioisosteric moieties in furosemide (or its analogue) versus the average AEDs of the same moieties capped with R groups do not exceed 5.4%. This largest percent difference corresponds to the carboxylic acid moiety. This difference is identical to the maximum percent difference observed for the same carboxylic acid capped with H versus Cl individual R groups (Figure 3). Carboxylic acid seems to be twice as much more affected by the change in the environment than furan or sulfonamide, which are sensitive to the change in the environment by a maximum of 2.5% (Table 2). This was also noticeable in the section mentioned above, where the standard deviations in the AEDs of carboxylic acid across the three R groups were ±2.7% versus ±1.8% for furan or sulfonamide.

In furosemide, the AED of furan is off by −17.8% compared to that of carboxylic acid. This difference corresponds to −17.4% in the furosemide analogue. The negative sign indicates that the AED of furan is smaller than that of carboxylic acid. This percent difference of 17−18% is for potential bioisosteres, and therefore, it is neither too small (as is the case for carboxylic acid and sulfonamide, 9−11%) nor too large (as is the case for furan and sulfonamide, 30−35%) (Table 2). Compared to that of carboxylic acid, the AED of sulfonamide is off by 11.0 and 8.6% in furosemide and its analogues, respectively. This suggests that in the realistic environment of a drug molecule, the AED tool differentiates between bioisosteres and potential bioisosteres, where the latter witness relatively higher differences in the AEDs compared to the former. Considering the +4.9% change in the AED exhibited by the change of the environment for carboxylic acid from being capped with methyl to being embedded in furosemide (or its analogue), these differences between the AED of sulfonamide and carboxylic acid would adjust to ca. 13.5−15.9%. These values are comparable to the percent AED differences observed for the same bioisosteric pairs when capped with a methyl group, that is, 14.5%.

Upon comparing the numbers in columns 4 and 5 of Table 2, it is obvious that the potential bioisosteric pair (furan and carboxylic acid) has larger AED differences (−17.4 to −17.8%) than the bioisosteric pair (carboxylic acid and sulfonamide) (8.6−11.0%). Assuming that the difference in AEDs between the bioisosteric moieties is proportional to the level of bioisosterism, it is thus suggested that compared to furan, sulfonamide is likely to be a better bioisostere of carboxylic acid. This observation is congruent with the fact that carboxylic acid and sulfonamide are bioisosteres, while carboxylic acid and furan are potential bioisosteres. This observation is also perfectly aligned with the bioactivity differences (Δactivity) reported on the SwissBioisostere website (http://www.swissbioisostere.ch/). These differences are computed using four standard activity types (IC_{50}, EC_{50}, K_i, and K_d),[32,33] which are experimental measures extracted from the ChEMBL database. The potential bioisosteric replacements reported in the SwissBioisostere database are primarily, although not solely, based on two types of experimental assays, the binding assays and the functional assays.[32,33] Binding assays are meant to measure the binding affinity and interactions between two molecules including ligand−receptor interactions.[36] On the other hand, functional assays are designed to assess the functional activity of molecular entries as part of biological processes.[37]

The information provided by the SwissBioisostere website (as of June 2022) about the carboxylic acid and furan replacements was exclusively (27 out of 27 replacement entries) based on binding assays. Similarly, for the search of carboxylic acid and sulfonamide, 94% of the data (248 out of 265 replacement entries) was based on binding assays, and only 6% was based on functional assays. Therefore, the

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reported occurrences mostly depended on the binding activity of the molecule with its target receptor.[32,33] As of June 2022, we collected from the SwissBioisostere website the bioactivity differences as a result of the replacement of carboxylic acid with sulfonamide or furan (Table 3). This website reports the

Table 3. Bioactivity Differences (ΔBioactivity) between Carboxylic Acid and Each of Sulfonamide and Furan[a]

		Range	Occurrences
Δ Bioactivity	Sulfonamide	3.28, -0.04	177
	Furan	1.91, -0.44	25
Δ IC_{50}	Sulfonamide	2.74, -0.49	128
	Furan	1.57, -0.44	17
Δ EC_{50}	Sulfonamide	2.16, 0.13	4
	Furan	1.120	1
Δ K_i	Sulfonamide	3.28, -0.48	91
	Furan	1.91, -0.09	7
Δ K_d	Sulfonamide	2.140	1
	Furan	N/A	N/A

[a]The range is collected from the experimental data summarized on the SwissBioisostere website as of June 2022.

occurrences in three categories: "improved" (Δactivity > 0.5 log units), "similar" (−0.5 log units < Δactivity < 0.5 log units), or "worsened" (Δactivity < −0.5 log units).[32] In Table 3, only the combined "improved" and "similar" categories are listed, as we were not interested in the substitutions that result in lower bioactivities. As captured in Table 3, the largest reported occurrences for each of the two replacements are based on the IC_{50} parameter, followed by K_i, EC_{50}, and then K_d. The Δbioactivity is inclusive of all four standard activity types. Larger Δactivity implies better improvement in the activity and better MMPs.[32,33] It is obvious from Table 3 that considering any of the four standard activity types (or the Δbioactivity), the replacement of carboxylic acid with sulfonamide can have differences in bioactivity which are at least 1 log unit greater than those of the replacement of carboxylic acid with furan. Therefore, the replacement of carboxylic acid with sulfonamide is likely to be more successful than its replacement with furan, just as noted with the AED tool (Table 2). This observation can be linked with some of the physio-chemical properties listed in Table 1: carboxylic acid and sulfonamide (the better bioisosteric pair) are both acyclic and share the same number of hydrogen-bond donors, while carboxylic acid and furan (the potential bioisosteric pair) have different shapes and a different number of hydrogen donors. Moreover, the Δbioactivity of the replacement of carboxylic acid with tetrazole (best bioisosteric replacement listed in the literature) can reach up to 4 log units, as opposed to a maximum of 3.28 log units with sulfonamide and 1.91 log units with furan. In addition, the number of occurrences of the replacements of carboxylic acid with tetrazole, sulfonamide, and furan is 521, 265, and 27 hits, respectively. This suggests that tetrazole is even a better replacement of carboxylic acid, and indeed, the AEDs of these two bioisosteric moieties were reported to be identical up to three decimal places, with only 0.2% difference.[18] In fact, tetrazole has been reported in the literature as one of the most common bioisosteres of carboxylic acid.[2,6,9,10,34,35]

In the furosemide analogue, and irrespective of its protonation state, the furan and the sulfonamide maintain their AED values as in furosemide. The difference in AEDs between these two moieties (furan and sulfonamide) is ∼34% in the furosemide drug and ∼31% in its analogue, irrespective of their protonation states (Table 2). These differences are analogous to the AED differences observed with the capped furan versus capped sulfonamide (i.e., 34%). This high difference is again aligned with the lack of any experimental examples in the literature of the bioisosterism between these two moieties. The swapping of these groups in furosemide to form its analogue led to differences that did not exceed 3% (Table 2), which is well within the ca. 5% difference attributed to the environmental change in the previous sections. In addition, these two moieties, furan and sulfonamide, maintained an AED difference of greater than 30%. The AED differences between furan and sulfonamide, each in the neutral versus the anionic forms of the drug (or its analogue), were less than 2% (Table 2). The protonation/deprotonation state in carboxylic acid/carboxylate caused a ca. 5% difference in the AEDs (although this comparison is not fully valid given that carboxylic acid and carboxylate do not have the same protonation state). In other words, changing the environment from neutral to anion does not affect the AED of the bioisosteric moieties beyond 5%. This value is to be contrasted with the average AEDs of 0.0722 and 0.0795 for carboxylic acid and carboxylate reported in ref 16. However, what is even more notable than the AED differences between carboxylic acid and carboxylate is the AED changes in the carboxylate anion itself as a result of environmental changes (Table 2, and the large standard deviation in the average AED of 0.0795 ± 0.0077 for carboxylate when capped with various R groups in ref 16). This difference may reach ∼10%, based on the results of this study. In fact, this difference may reach up to 13% when the AED of the carboxylate in the furosemide anion (0.0719 a.u. from this study) is compared to that of carboxylate capped with a methyl group (0.0816 a.u. from refs 16 and 17). Theoretically, this difference is likely to decrease not only if ultrafine grids are used but also if large maximum atomic integration radii, up to 30, are used in the AIMALL integration. However, practically, we found the difference in the AEDs when integrations were performed with fine and superfine grids to be very negligible; the change is only in the fifth decimal place of the AED, which corresponds to differences less than 0.01%.

Overall, studying the bioisosteres in real environments led to the same conclusions as those drawn from the capped bioisosteres. In terms of transferability, the AEDs of furan with capped groups versus furan in furosemide (or its analogue) do not exceed 2.5%, whether in the protonated or deprotonated forms. Similarly, these differences do not exceed 2.5% for the sulfonamide moiety. However, the AED of the carboxylic acid is off by 5.5% in furosemide (or its analogue) compared to that in the capped group. The swapping of sulfonamide and furan did not cause any substantial AED differences in the furosemide analogue compared to those observed in furosemide.

In order to test the effect of replacing bioisosteres and potential bioisosteric groups in furosemide, carboxylic acid was replaced with furan or sulfonamide, and vice versa, to form four new analogues as listed in Table 4.

The substitution of furan in furosemide with carboxylic acid, and vice versa, resulted in analogues 2 and 3, respectively. The

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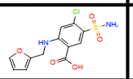
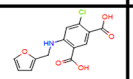
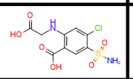
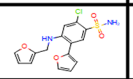
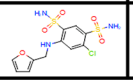
Table 4. AED Values of the Bioisosteric Moieties in Furosemide and Four of Its Analogues Where Carboxylic Acid Is Substituted with Furan or Sulfonamide and Vice Versa[a]

Drug molecule	Structure	Bioisosteric moiety	AED at 0.001 a.u. isodensity	% AED difference (change in environment)	% AED difference (change in bioisosteres)
Furosemide		carboxylic acid Furan Sulfonamide	0.0762 0.0627 0.0823	NA	NA
Analogue 1		carboxylic acid furan sulfonamide replaced with carboxylic acid	0.0760 0.0627 0.0741	-2.74	-9.91
Analogue 2		carboxylic acid furan replaced with carboxylic acid sulfonamide	0.0756 0.0727 0.0822	-4.59	15.92
Analogue 3		carboxylic acid replaced with furan furan sulfonamide	0.0633 0.0627 0.0822	1.01	-16.86
Analogue 4		carboxylic acid replaced with sulfonamide furan sulfonamide	0.0839 0.0631 0.0824	2.04	10.16

[a]The % AED difference (change in the environment) refers to the AED of the new bioisosteric group in the analogue with respect to the same group in furosemide. The % AED difference (change in bioisosteres) refers to the AED of the new bioisosteric group in the analogue with respect to the existing bioisosteric group in furosemide.

AEDs of furan are 16−17% smaller than those of carboxylic acid (Table 4). This is in full alignment with the values reported in Table 2. Similarly, as observed earlier, the AED values of the sulfonamide in analogues 1 and 4 are 10% higher than those of carboxylic acid. Table 4 also clearly shows that the change in the environment upon the replacements of the bioisosteric moieties alters the AED values within no more than 5%. This is fully congruent with the percentages reported for any change in the environment tested above.

Overall, it is found that the change in the environment causes consistent AED deviations that do not exceed 5%. It was also found that the substitutions of the bioisosteric or non-bioisosteric groups in furosemide generated reproducible values for all five analogues (Tables 2 and 4).

3.3. Electrostatic Potential Maps. The ESP maps are classically used to show the key and lock complementarity between a molecule and its receptor, and it is, therefore, used as a tool to explain bioisosterism. Figure 7 shows the ESP maps of all the molecules considered in this study. The first three rows show all three bioisosteric moieties capped with hydrogen (row 1), methyl (row 2), and chloro (row 3). From these first three rows, it is obvious that each of the three bioisosteric moieties shares the same topology, irrespective of the capping group. Carboxylic acid has two lobes of different sizes, one around each of the two oxygen atoms. Furan has three elliptical/elongated lobes, two on each side of the flat furan ring and one around the oxygen in the ring. Sulfonamide has two approximately symmetric lobes around the oxygen atoms and a mini lobe around the nitrogen. The same topology is reproduced for each bioisostere, irrespective of the capping group, with the exception of furan capped with a chloro group. In the latter, there are lobes around the chloro atom, and they show as an extension of the two lobes on both sides of the furan ring. The isodensity values of the hydrogen-capped bioisosteres are slightly smaller than those of the methyl-capped moieties. However, the isodensity values for the chloro-capped bioisosteres are the lowest, on a scale that is roughly half of that of the hydrogen- or methyl-capped moieties. The lobes of carboxylic acid and sulfonamide moieties appeared at comparable isodensity values; however, the lobes of furan appeared only at lower values. None of the topologies of the three bioisosteres are similar, and it is therefore very difficult to predict, based on ESP maps, the potential bioisosterism between carboxylic acid and furan or the bioisosterism between carboxylic acid and sulfonamide. This is to be contrasted with the precision of the AED tool, which not only shows the similarity between bioisosteres but also quantifies it within a 15% margin.

It was difficult to capture, using a single isodensity value, the hypothesized similarities between carboxylic acid and furan or carboxylic acid and sulfonamide bioisosteric groups within furosemide. Thus, to reproduce the ESP lobes observed in the capped bioisosteres for each of the three bioisosteric groups in furosemide (or its analogue), three screenshots were captured of the drug or its analogue (at fixed orientations) at different isodensities (see Figure 7). The three screenshots were captured at different isodensities in such a way to maximize the similarity between the ESP maps of each of the three bioisosteric moieties in furosemide and those of the corresponding capped bioisostere. Despite taking three screenshots, and despite all trials with the different isodensity values, it was difficult to always fully reproduce, in furosemide, the same lobes observed in the capped bioisosteres. For example, furan did not seem to always reproduce the two lobes on either side of the ring. In addition, a given group in different molecules does not necessarily show a similar topology at the same isodensity. For example, the three lobes of the sulfonamide group show at different isodensities, 0.055 and 0.033 a.u. in furosemide and its analogue, respectively. In furosemide, the carboxylic acid and sulfonamide share a similar big lobe in their ESP maps. They seem to share higher levels of similarity between each other compared to carboxylic acid and furan. This is, again, aligned with the fact that sulfonamide is a bioisostere of carboxylic acid, while furan is a potential bioisostere of carboxylic acid. This observation about the better ESP similarities between carboxylic acid and sulfonamide (compared to those between carboxylic acid and furan) is aligned with the smaller AED difference observed in the former

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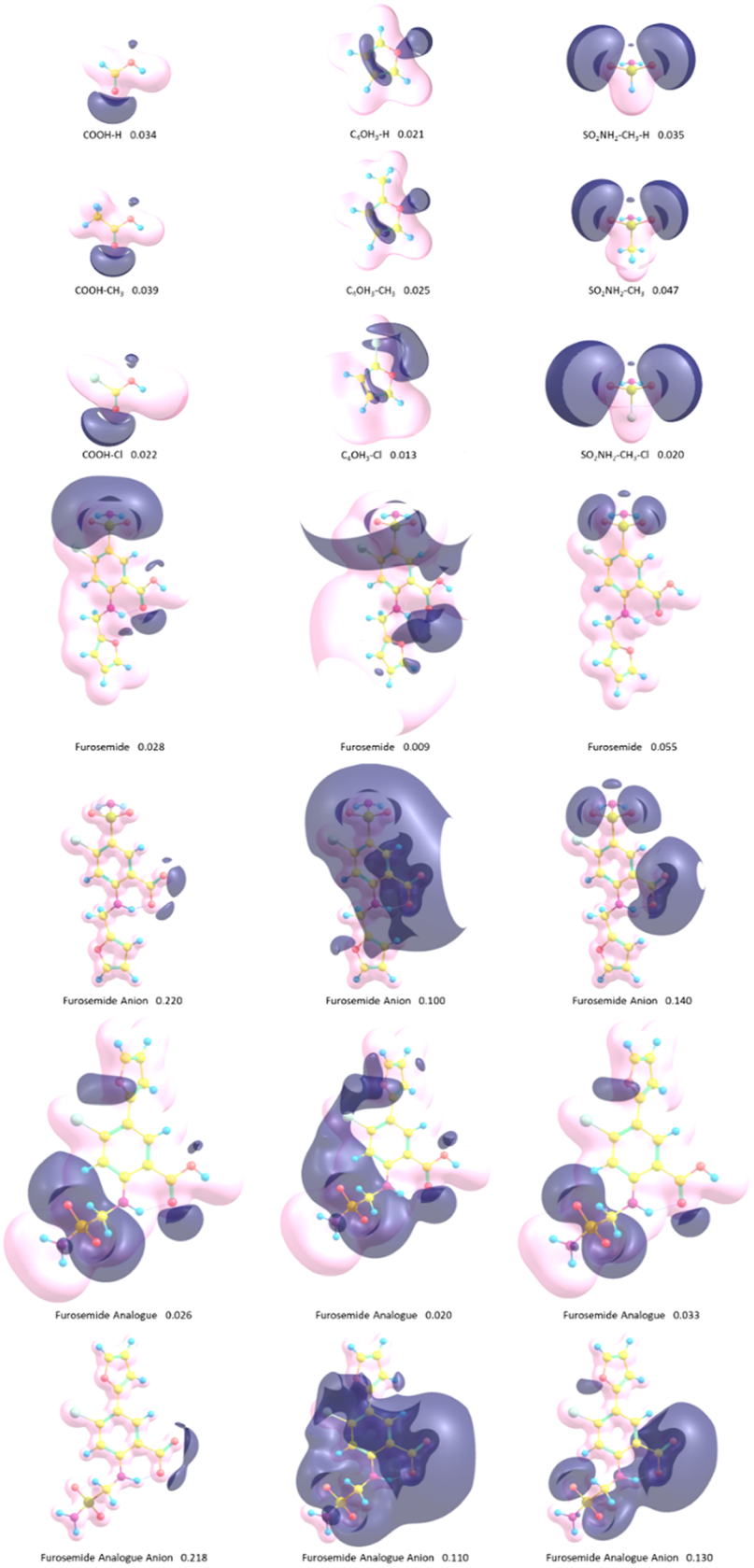

Figure 7. ESP maps of carboxylic acid, R−COOH (left), furan, R−C_4OH_3 (middle), and sulfonamide, R−SO_2NH_2 (right), capped with hydrogen (H), methyl (CH_3), and chloro (Cl) (rows 1−3), embedded in furosemide in its neutral and anion forms (rows 4−5), and embedded in the furosemide analogue (where furan and sulfonamide are swapped) in its neutral and anion forms (rows 6−7). The labels of the molecule and the molecular isodensity values are reported (in a.u., i.e., e^-/a_0^3) under each molecule. Color code of the atoms: yellow-carbon, blue-hydrogen, red-oxygen, pink-nitrogen, turquoise-chlorine, and gold-sulfur. Color code of the ESP maps: pink-positive and purple-negative.

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pair (11%) versus the latter (19%). Carboxylic acid shares the big lobe in its ESP with that of the sulfonamide and the elliptical elongated lobe with that of furan; however, sulfonamide and furan do not share anything obvious in common. This is likely related to the fact that furan and sulfonamide are not bioisosteres of each other despite the former being a potential bioisostere of carboxylic acid and the latter being a bioisostere of the same group (carboxylic acid). The isodensities of the anionic furosemide or its analogue are roughly 1 order of magnitude greater than those in the neutral molecules. While the carboxylic acid groups have only two lobes, the carboxylate groups, in the anionic molecules, have three of them. This is congruent with the ESP maps reported in ref 18 for carboxylate capped with a methyl group (except for the fourth missing lobe, which seems to be missing because of the presence of a hydrogen atom, in furosemide and its analogue anions, in the proximity of the position where this fourth lobe was supposed to show). For all the facts mentioned above, including the difficulty in the reproducibility of the lobes' topology, along with the need to consider multiple isodensity values to clearly observe the topology of separate bioisosteric groups or even the topology of the same group in different molecules, ESP maps are rather ambiguous to explain or reliably predict bioisosterism. On the contrary, the AED tool is a more robust (quantitative rather than qualitative) tool for evaluating the extent of bioisosterism among different moieties. The AED tool is also consistently reproducible within a 5% margin to account for the change in the environment.

This section highlights that, although they can visually reveal lots of biological and chemical insights, the ESP maps can be fraught with challenges. On the other hand, although it provides no visual intuitions, the evaluation of the AED is a much more straightforward tool to qualitatively assess bioisosterism. Overall, the ESP maps and the AED tool are complementary tools that, together, provide a full accurate assessment of the similarities among non-classical bioisosteres.

4. CONCLUSIONS

Using density functional theory and QTAIM, the AED tool is employed as a robust qualitative tool to explain the similarity among three bioisosteric moieties (carboxylic acid, furan, and sulfonamide), either as capped groups or even as embedded in an FDA-approved drug molecule, furosemide. The AED tool was clearly capable of distinguishing between the bioisosteric pair (carboxylic acid and sulfonamide, with a maximum AED difference of ∼11%), the potential bioisosteric pair (carboxylic acid and furan, with a higher AED difference of up to ∼17%), and the non-bioisosteric pair (furan and sulfonamide, with the highest AED difference of ∼34%). The trends observed with the AED tool for bioisosteres, potential bioisosteres, and non-bioisosteres are in full consistency with the experimental data.

The AED tool is not only reproducible but also transferable within a 5% margin depending on the environment of the bioisosteric group. The AED tool systematically reproduced all the differences between the bioisosteric moieties in all five analogues. The AED tool had better precision than the ESP maps. The ESP maps revealed only partial similarities between the bioisosteric pairs (carboxylic acid and sulfonamide) and the potential bioisosteric pair (carboxylic acid and furan).

Overall, the AED tool is a robust, transferable, and precise tool to evaluate the similarities in bioisosteric substitutions. The ESP maps could be used as a complementary tool to assist with visualizations of these similarities, although they were proven not to be consistently reliable. The power of our AED tool in qualitatively depicting the similarities among bioisosteres sets the stage for predicting bioisosterism. Therefore, the AED measures could be used as a feature in machine learning and artificial intelligence models, provided that large databases are available for training, validating, and testing purposes.

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Notes

The authors declare no competing financial interest.

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