Can reaction with amino acid turn Dimefox or Fluoroacetamide to nontoxic derivative: in Silico Study

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Abstract

To answer the title question, two ways can be applied. The first way is the experimental methods through using multistep, various techniques, different chemicals, characterization instruments, time, cost, and environmental considerations, and in vitro – in vivo testing. The second way is in Silico calculation. In this path of working, all steps, instruments, testing, chemicals …etc. will be canceled and only evaluate the numerical results to qualify any chemical to be a drug. The above points encourage me to take a hypothetical reaction between two insecticides (Dimefox (D) and Fluoroacetamide (F)) and several amino acids (aspartic acid, glutamic acid, glycine, alanine, phenylalanine, valine, isoleucine, proline, and methionine). The resulted P-N or C-N derivatives were subjected to ADMET and Druglikeness predications. They showed various important notes like increasing water solubility, mutagen character of Ames test to all 20 compounds, non –inhibition predication to P-glycoprotein, non – inhibition character of CYP-2C19 and CYP-2C9 except F. Many of 20 compounds showed negative response to Mouse or Rat Carcinogenic test, TA100-10RLI, TA100-NA, TA1535-10RLI, and TA1535-NA beside low risk to hREG inhibition. The other calculated characters were varied with influence of polarity, surface area, hydrogen bonding, and molecular structure. So, if these 18 compounds, if they formed in any biological system or in lab, have a toxic character.
Keywords: in Silico, ADMET, Druglikeness, Dimefox, Fluoroacetamide insecticide, CNS, hypothetical derivatives, amino acid.

1. Introduction

Dimefox (D) and Fluoroacetamide (F) are Fluoro-organic compounds used as insecticides. With their destructive power against many organisms, they have lethal impact upon environment even at low concentration [1]. They vary in their half-life, bioaccumulation, transport mechanism, and chemical impact on water where both are freely soluble in water with LD₅₀ [2]. WHO identifies Fluoroacetamide as highly hazardous material and recommends Dimefox to be cut off using it as a pesticide [3].

Dimefox (D) is stable in neural or alkaline medium but its hydrolysis occurs in acidic medium [4] and it is considered as a high toxic cholinesterase. This dangerous side of (D) is a biological result of some enzymes found in blood plasma and liver causing hydrolysis and forming phosphonic (containing C-PO(OH) or C-PO(OR)₂) and phosphoric (H₃PO₄) acids. The action of D is not only in the Central Nerve System (CNS) but also has a cardiovascular action and at high concentration D causes fall in blood pressure [5]. According to Globally Harmonized System of Classification and Labelling of Chemicals (GHS), D is fatal in both oral and dermal conditions.

Fluoroacetamide (F) is toxic to human and other mammals because it is hydrolyzed to the toxic fluoroacetate [6] that inhibit transformation of citrate (in Citric acid cycle) to the corresponding isocitrate so poisoning increases in mammal CNS and heart.

Many research and review articles dealing with Quantitative Structure Activity Relationship (QSAR) showed the importance of these calculations [7, 8,9]. The high toxicity of D and F was the first reason to
our chemical model *in Silico* study with simple proposition. This proposition based upon substitution of fluorine atom by nitrogen of several essential amino acids. With this hypothetical substitution reaction, the numerical data of *Absorption, Distribution, Metabolism, Excretion*, and *Toxicity* (ADMET) and Druglikeness predictors become more necessary as a goal to evaluated safety and physicochemical properties of D, F, and their derivatives.

2. **Experimental parts**

2.1 **Part I:** Hypothetical reaction of Dimefox or Fluoroacetamide via fluorine atom with Nitrogen of amino acid.

Dimefox and Fluoroacetamide are known chemicals as insecticide. Both have fluorine atom in their structure which can be replaced (Scheme 1.). According to this our hypothetical derivatives (Figures 1. & 2.) can be formed by the replacement of fluorine atom with nitrogen atom from different amino acids. In this paper, aspartic acid, glutamic acid, glycine, alanine, phenylalanine, proline, valine, isoleucine, or methionine was chosen to be a reactant with Dimefox or Fluoroacetamide as shown in general reaction Scheme 1.

Scheme 1. Hypothetical reaction of Dimefox and Fluoroacetamide with amino acid.
Figure 1. Dimefox and its hypothetical products.
2.2 Part II: *in Silico* Calculations

MarvinSketch-Ver. 18.15 is program from www.ChemAxon.com website. It was selected to calculated several important characters such as Elemental analysis (chemical formula and Mol. Wt), Protonation (Isoelectric point (pI)), partitioning (log P, ChemAxon method, Cl¹, Na⁺, K⁺ electrolyte concentration under condition of calculation 0.1 mol./dm³), Hydrophilic – Lipophilic Balance (HLB, ChemAxon), geometry (Polar Surface Area (2D), PSA, without excluding Sulfur and Phosphors atoms, (Å)², and Hydrogen Bond Donor (HBD)/ Acceptor (HBA) without excluding of Sulfur and phosphors atoms at pH (0-14) as shown in Tables 1. & 2.
website was used to calculated ADMET and Druglikeness properties (Tables 3-6) which are BBB (Blood-Brain Barrier as \textit{in vivo} penetration, C. Brain / C. Blood), Buffer solubility of molecule (mg/L), Human colorectal carcinoma permeability (Caco2, \textit{in vitro}), \textit{in vitro} cytochrome P450 (CYP 2C19, CYP 2C9, and CYP 2D6 inhibitions), (CYP 2D6 and CYP 3A4, \textit{in vitro}) substrate, Human Intestinal Absorption (HIA, %), kidney cell permeability (Mandin Darby Canine Kidney, MDCK, \textit{in vitro}, nm/sec.), inhibition of P-glycoprotein (Pgp, \textit{in vitro}), Plasma Protein Binding (\textit{in vitro}, %), Pure Water Solubility (mg/L), transdermal logKp (Skin Permeability, \textit{in vitro}, cm/hr.), SK log D value (log D in pH 7.4), SK log P value (log P in pH 7.4), and SK log S (log S in pH 7.4 buffer system and pure water, mol./L).

To predict toxicity, algae –at (Acute algae toxicity), mutagenicity against histidine synthesis (Ames test), carcinogenicity with mouse (Carcino – Mouse) and rat (Carcino-Rat), Daphnia – at (acute \textit{Daphnia} toxicity), human ether –a-go-go inhibition (hERG, \textit{in vitro}), acute fish toxicity (medaka - at and minnow- at), beside TA100-10RLI, TA100 – NA, TA1535 -10RLI, and TA1535 - NA (\textit{in vitro}, Ames test, with (+S9) and without (-S9) metabolic activation in TA100 strain, rat liver) were calculated by \url{https://preadmet.bmdrc.kr} website (Tables 3,4).

Druglikeness predictors (Tables 4. and 5.) were calculated by \url{https://preadmet.bmdrc.kr} website involving: Comprehensive Medicinal Chemistry like Rule (CMC Like Rule, Violation Fields), Lead like Rule Violation, MDDR like Rule (Mid-Structure, Nondrug-, and drug- like, and their Violation fields), Lipinski 's Rule of Five, World Drug Index like Rule (WDI), and WDI Violation (molecular properties found in or out 90% cutoff in WDI).
Table 1. Physical properties of Dimefox and its hypothesized derivatives.

| Name (Symbol) | Molecular formula | M.Wt | pI | logP ChemAxon | HLB ChemAxon | PSA** | Hydrogen*** |
|---------------|-------------------|------|----|---------------|--------------|-------|-------------|
| Name (Symbol) | Molecular formula | M.Wt | pI | logP ChemAxon | HLB ChemAxon | PSA** | Hydrogen*** |
| Dimefox (D) | C₄H₂FN₂O₃P | 154.125 | * | -0.10 | 11.33 | 33.36 | 0 2 |
| D-Aspartic acid (D-AS) | C₄H₁₀N₂O₅P | 267.222 | 2.56 | -1.44 | 29.51 | 119.99 | 3 8 |
| D-Glutamic acid (D-GL) | C₄H₁₀N₂O₅P | 281.249 | 2.50 | -1.19 | 28.85 | 119.99 | 3 8 |
| D-Glycine (D-G) | C₄H₁₀N₂O₅P | 209.186 | 2.77 | -1.36 | 28.95 | 82.69 | 2 6 |
| D-Alanine (D-AL) | C₄H₁₀N₂O₅P | 223.213 | 2.79 | -0.82 | 28.13 | 82.69 | 2 6 |
| D-Phenylalanine (D-PA) | C₄H₁₀N₂O₅P | 299.311 | 2.78 | 0.86 | 24.52 | 82.69 | 2 6 |
| D-Valine (D-VA) | C₄H₁₀N₂O₅P | 251.267 | 2.88 | -0.69 | 26.73 | 82.69 | 2 6 |
| D-Isoleucine (D-IL) | C₄H₁₀N₂O₅P | 265.294 | 2.90 | 0.44 | 26.09 | 82.69 | 2 6 |
| D-Proline (D-P) | C₄H₁₀N₂O₅P | 249.251 | 2.88 | -0.59 | 27.20 | 73.90 | 1 6 |
| D-Methionine (D-ME) | C₄H₁₀N₂O₅P | 283.330 | 2.79 | -0.30 | 28.48 | 107.99 | 2 7 |

* no isoelectric point; ** for PSA calculation, P & S atoms were not excluded; *** For calculation of hydrogen acceptor, P & halogen atoms were not excluded.

Table 2. Physical properties of Fluoroacetamide and its hypothesized derivatives.

| Name (Symbol) | Molecular formula | M.Wt | pI | logP ChemAxon | HLB ChemAxon | PSA** | Hydrogen*** |
|---------------|-------------------|------|----|---------------|--------------|-------|-------------|

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| Compound                  | Formula    | Molar Mass | pKa 1   | pKa 2     | Count Sites | Count Sites |
|--------------------------|------------|------------|---------|-----------|-------------|-------------|
| Fluoroacetamide (F)      | C$_7$H$_4$FNO | 77.058    | -0.99  | 11.20     | 1           | 2           |
| F-Aspartic acid (F-AS)   | C$_5$H$_{10}$N$_2$O$_3$ | 190.155  | 3.05    | -2.07     | 4           | 6           |
| F-Glutamic acid (F-GL)   | C$_7$H$_{12}$N$_2$O$_5$ | 204.182  | 2.60    | -1.82     | 4           | 6           |
| F-Glycine (F-G)          | C$_7$H$_6$N$_2$O$_3$  | 132.119   | 5.23    | -1.99     | 3           | 4           |
| F-Alanine (F-AL)         | C$_9$H$_{10}$N$_2$O$_3$ | 146.146   | 5.33    | -1.45     | 3           | 4           |
| F-Phenylalanine (F-PA)   | C$_{11}$H$_{14}$N$_2$O$_3$ | 222.244  | 5.45    | 0.24     | 3           | 4           |
| F-Valine (F-VA)          | C$_{11}$H$_{14}$N$_2$O$_3$ | 174.200  | 5.58    | -0.58    | 3           | 4           |
| F-Isoleucine (F-IL)      | C$_{13}$H$_{16}$N$_2$O$_3$ | 188.227  | 6.26    | -0.18    | 3           | 4           |
| F-Proline (F-P)          | C$_{11}$H$_{14}$N$_2$O$_3$ | 172.184  | 4.11    | -1.10    | 2           | 3           |
| F-Methionine (F-ME)      | C$_{12}$H$_{14}$N$_2$O$_3$S | 206.260  | 5.40    | -0.92    | 3           | 4           |

* no isoelectric point; ** for PSA calculation, P & S atoms were not excluded; *** For calculation of hydrogen acceptor, P & halogen atoms were not excluded.
Table 3. ADMET calculations Dimefox and its hypothesized derivatives.

| Property                      | D       | D-AS  | D-GL  | D-G    | D-AL   | D-PA   | D-VA  | D-IL  | D-P    | D-ME  |
|-------------------------------|---------|-------|-------|--------|--------|--------|-------|-------|--------|-------|
| BBB                           | 0.575892| 0.0707597| 0.0800449| 0.0714621| 0.119902| 0.242295| 0.198189| 0.269072| 0.131561| 0.0893 |
| Buffer solubility, mg/L       | 177832  | 1.0321e+7 | 6.07071e+6 | 22233.8 | 941686 | 127060 | 418641 | 217537 | 871272 | 2.54785e+6 |
| Caco2                         | 21.7244 | 21.6356 | 21.6703 | 21.7143 | 21.7202 | 21.7228 | 21.7218 | 21.7225 | 21.7235 | 21.5709 |
| CYP-2C19 inhibition           | Non     | Non   | Non   | Non    | Non    | Non    | Non    | Non    | Non    | Non   |
| CYP-2C9 inhibition            | Non     | Non   | Non   | Non    | Non    | Non    | Non    | Non    | Non    | Non   |
| CYP-2D6 inhibition            | Non     | Non   | Non   | Non    | Non    | Non    | Non    | Non    | Non    | Non   |
| CY-2D6 substrate              | Non     | Non   | Non   | Non    | Non    | Non    | Non    | Non    | Non    | Non   |
| CYP-3A4 inhibition            | Non     | Non   | Non   | Non    | Non    | Non    | Non    | Non    | Non    | Non   |
| CYP-3A4 substrate             | Substrate | Weakly  | Weakly  | Substrate  | Substrate  | Substrate  | Substrate  | Substrate  | Substrate  | Substrate  |
| HIA                           | 96.65745 | 32.2454 | 35.66071 | 84.06577 | 68.22307 | 91.49626 | 75.05306 | 78.07312 | 83.91781 | 77.15494 |
| MDCK                          | 19.5647 | 0.537008 | 0.542565 | 0.537672 | 0.647451 | 0.894384 | 0.904618 | 1.16468 | 0.642132 | 0.551409 |
| Pgp inhibition                | Non     | Non   | Non   | Non    | Non    | Non    | Non    | Non    | Non    | Non   |
| Plasma Protein Binding        | 0.763602 | 60.55147 | 0.000000 | 1.211735 | 7.12492 | 40.10271 | 9.857798 | 11.03091 | 10.15772 | 21.87925 |
| Pure water solubility, mg/L   | 1.83349e+6 | 1.0603e+7 | 9.31235e+6 | 1.25016e+8 | 1.03809e+7 | 631044 | 994333 | 430045 | 9.71994e+6 | 1.9759e+6 |
| Skin Permeability             | -3.04478 | -4.71776 | -4.6366 | -4.94527 | -4.17294 | -4.03876 | -3.72136 | -3.4895 | -4.37169 | -3.81678 |
| SKlogD value                  | -0.17197 | -2.52179 | -2.20314 | -3.120950 | -1.98377 | -0.59076 | -1.19335 | -0.73938 | -1.71119 | -1.49386 |
| Property                  | D    | D-AS  | D-GL  | D-G   | D-AL  | D-PA  | D-VA  | D-IL  | D-P   | D-ME  |
|--------------------------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| SKlogP value             | -0.17197 | -1.27379 | -0.95514 | -1.872950 | -0.73577 | 0.657240 | 0.054650 | 0.508620 | -0.46319 | -0.24586 |
| SKlogS buffer            | 0.062140 | 1.586850 | 1.334150 | -0.969400 | 0.625190 | -0.372110 | 0.221710 | -0.086190 | 0.543520 | 0.953890 |
| SKlogS pure              | 1.07541 | 1.59856 | 1.51997 | 2.780550 | 1.66752 | 0.323940 | 0.597400 | 0.209790 | 1.59103 | 0.843480 |
| Algae- at                | 0.221001 | 0.319469 | 0.266093 | 0.353228 | 0.262968 | 0.182755 | 0.16039 | 0.123576 | 0.272584 | 0.212954 |
| Ames test                | Mutagen | Mutagen | Mutagen | Mutagen | Mutagen | Mutagen | Mutagen | Mutagen | Mutagen | Mutagen |
| Carcino- Mouse           | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative |
| Carcino- Rat             | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Negative |
| Daphnia- at              | 9.93788 | 16.5991 | 12.2348 | 19.1998 | 11.7284 | 1.17972 | 6.03248 | 2.79528 | 8.50088 | 4.39254 |
| hERG inhibition          | Low risk | Low risk | Low risk | Low risk | Low risk | Medium risk | Low risk | Low risk | Low risk | Low risk |
| Medaka- at               | 79.5255 | 267.708 | 150.255 | 324.825 | 126.958 | 1.81881 | 35.7214 | 8.32925 | 69.9577 | 23.2041 |
| Minnow- at               | 20.0572 | 64.1766 | 36.9778 | 86.2885 | 36.3818 | 2.3229 | 7.99027 | 3.48388 | 21.5967 | 12.8394 |
| TA100 10RLI              | Positive | Negative | Negative | Negative | Negative | Positive | Negative | Negative | Positive | Positive |
| TA100- NA                | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative |
| TA1535- 10RLI            | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive |
| TA1535 NA                | Negative | Positive | Negative | Negative | Negative | Positive | Negative | Positive | Negative | Positive |

Table 4. Druglikeness calculations of Dimefox and its hypothetical derivatives.
| Property                        | D     | D-AS   | D-GL   | D-G    | D-AL   | D-PA   | D-VA   | D-IL   | D-P    | D-ME   |
|--------------------------------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| CMC like Rule                  | Not qualified | Not qualified | Not qualified | Not qualified | Qualified | Not qualified | Qualified | Not qualified | Not qualified |
| CMC like Rule Violation Fields | AlopP98 value, Molecular weight, AMol Ref | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value |
| CMC like Rule Violations       | 3     | 1      | 1      | 1      | 1      | 0      | 1      | 0      | 1      | 1      |
| Lead-like Rule Violation Fields| AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value |
| Lead like Rule                 | Violated | Violated | Violated | Violated | Violated | Violated | Violated | Violated | Violated | Violated |
| Lead like Rule Violations      | 1     | 1      | 1      | 1      | 1      | 1      | 1      | 1      | 1      | 1      |
| MDDR like Rule                 | Mid-structure | Mid-structure | Mid-structure | Mid-structure | Mid-structure | Mid-structure | Mid-structure | Mid-structure | Mid-structure |
| MDDR like Rule Violation Fields| No Rings, No Rotatable bonds | No Rings, No Rotatable bonds | No Rings | No Rings, No Rotatable bonds | No Rings, No Rotatable bonds | No Rings | No Rings, No Rotatable bonds | No Rings | No Rings |
| MDDR like Rule Violations      | 2     | 2      | 1      | 2      | 2      | 1      | 2      | 1      | 2      | 1      |
| Rule of Five                   | Suitable | Suitable | Suitable | Suitable | Suitable | Suitable | Suitable | Suitable | Suitable | Suitable |
| Rule of Five Violation Fields  | 0     | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| WDI like Rule                  | Out of 90% cutoff | Out of 90% cutoff | Out of 90% cutoff | Out of 90% cutoff | Out of 90% cutoff | In 90% cutoff | Out of 90% cutoff | Out of 90% cutoff | Out of 90% cutoff | Out of 90% cutoff |
| WDI like Rule Violation Fields | Balaban index JX | Balaban index JX | Balaban index JX | Balaban index JX | Balaban index JX | Balaban index JX | Balaban index JX | Balaban index JX | Balaban index JX |
|-------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| WDI like Rule Violations      | 1             | 1             | 1             | 1             | 1             | 0             | 1             | 1             | 1             |

Table 5. ADMET calculations Fluoroacetamide and its hypothesized derivatives.

| Property                  | F       | F-AS     | F-GL     | F-G       | F-AL     | F-PA     | F-VA     | F-IL       | F-P       | F-ME       |
|---------------------------|---------|----------|----------|-----------|----------|----------|----------|------------|-----------|------------|
| BBB                       | 0.351222| 0.0855633| 0.101202 | 0.0938609 | 0.12029 | 0.221643 | 0.20317  | 0.275829   | 0.148418  | 0.083367   |
| Buffer solubility, mg/L   | 2500.68 | 3.8925e+6| 2.33581e+6| 556115   | 32673   | 50002.1  | 153825   | 81801.2    | 151866    | 983045     |
| Caco2                     | 11.9487 | 12.212   | 21.0082  | 10.0253  | 15.788  | 12.8352  | 19.4595  | 19.8815    | 19.8694   | 20.4934    |
| CYP-2C19 inhibition       | Inhibitor| Non      | Non      | Non       | Non      | Non      | Non      | Non        | Non       | Non        |
| CYP-2C9 inhibition        | Inhibitor| Non      | Non      | Non       | Non      | Non      | Non      | Non        | Non       | Non        |
| CYP-2D6 inhibition        | Inhibitor| Non      | Non      | Non       | Non      | Inhibitor| Non      | Inhibitor   | Non       | Inhibitor   |
| CY-2D6 substrate          | Weakly   | Non      | Non      | Weakly    | Non      | Non      | Non      | Non        | Non       | Non        |
| CYP-3A4 inhibition        | Inhibitor| Non      | Non      | Non       | Non      | Non      | Non      | Non        | Non       | Non        |
| CYP-3A4 substrate         | Non      | Non      | Non      | Non       | Non      | Non      | Non      | Non        | Non       | Non        |
| HIA                       | 66.01533 | 16.85704 | 18.16117 | 44.2286   | 46.2477  | 78.5917  | 51.44932 | 54.52027   | 68.03456  | 53.35744   |
| MDCK                      | 186.122  | 0.833095 | 1.70317  | 335.455   | 161.284  | 216.924  | 180.222  | 252.17     | 294.665   | 267.866    |
| Pgp inhibition            | Non      | Non      | Non      | Non       | Non      | Non      | Non      | Non        | Non       | Non        |
| Property                  | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 | Value 7 | Value 8 | Value 9 | Value 10 |
|--------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|-----------|
| Plasma Protein Binding   |         |         |         |         |         |         |         |         |         |           |
| Pure water solubility, mg/L |     |        |        |        |        |         |         |         |         |           |
| Skin Permeability        |         |         |         |         |         |         |         |         |         |           |
| SKlogD value             |         |         |         |         |         |         |         |         |         |           |
| SKlogP value             |         |         |         |         |         |         |         |         |         |           |
| SKlogS buffer            |         |         |         |         |         |         |         |         |         |           |
| SKlogS pure              |         |         |         |         |         |         |         |         |         |           |
| Toxicity                 |         |         |         |         |         |         |         |         |         |           |
| Algae- at                |         |         |         |         |         |         |         |         |         |           |
| Ames test                |         |         |         |         |         |         |         |         |         |           |
| Carino- Mouse            |         |         |         |         |         |         |         |         |         |           |
| Daphnia- at              |         |         |         |         |         |         |         |         |         |           |
| hERG inhibition          |         |         |         |         |         |         |         |         |         |           |
| Medaka- at               |         |         |         |         |         |         |         |         |         |           |
| Minnow- at               |         |         |         |         |         |         |         |         |         |           |
| TA100 10RLI              |         |         |         |         |         |         |         |         |         |           |
| TA100 106 NA             |         |         |         |         |         |         |         |         |         |           |
Table 6. Druglikeness calculations of Fluoroacetamide and its hypothetical derivatives.

| Property                      | F   | F-AS | F-GL | F-G | F-AL | F-PA | F-VA | F-IL | F-P  | F-ME |
|-------------------------------|-----|------|------|-----|------|------|------|------|------|------|
| CMC like Rule                 | Not qualified | Not qualified | Not qualified | Not qualified | Not qualified | Qualified | Not qualified | Qualified | Not qualified | Not qualified |
| CMC like Rule Violation Fields| AlopP98 value, Molecular weight, AMol Ref, No Total atoms | AlopP98 value, AMol Ref | AlopP98 value | AlopP98 value, Molecular weight, AMol Ref, No Total atoms | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value |
| CMC like Rule Violations      | 4   | 2    | 1    | 4   | 3    | 0    | 1    | 0    | 1    | 1    |
| Lead-like Rule Violation Fields| Molecular weight, AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value | AlopP98 value |
| Lead like Rule Violations     | Violated | Violated | Violated | Violated | Violated | Violated | Violated | Violated | Violated | Violated |
| MDDR like Rule                | Nondrug-like | Mid-structure | Mid-structure | Nondrug-like | Nondrug-like | Mid-structure | Mid-structure | Mid-structure | Mid-structure | Mid-structure |
| MDDR like Rule Violation Fields | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds | No Rings, No Rigid bonds, No Rotatable bonds |
|---------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| MDDR like Rule Violations       | 3                                               | 2                                               | 2                                               | 3                                               | 3                                               | 2                                               | 2                                               | 2                                               | 2                                               | 2                                               | 2                                               | 2                                               | 2                                               | 2                                               | 1                                               |
| Rule of Five                    | Suitable                                        | Suitable                                        | Suitable                                        | Suitable                                        | Suitable                                        | Suitable                                        | Suitable                                        | Suitable                                        | Suitable                                        | Suitable                                        | Suitable                                        | Suitable                                        | Suitable                                        | Suitable                                        | Suitable                                        |
| Rule of Five Violation Fields   | 0                                               | 0                                               | 0                                               | 0                                               | 0                                               | 0                                               | 0                                               | 0                                               | 0                                               | 0                                               | 0                                               | 0                                               | 0                                               | 0                                               | 0                                               |
| WDI like Rule                   | In 90% cutoff                                   | Out of 90% cutoff                               | Out of 90% cutoff                               | Out of 90% cutoff                               | Out of 90% cutoff                               | Out of 90% cutoff                               | In 90% cutoff                                   | Out of 90% cutoff                               | In 90% cutoff                                   | Out of 90% cutoff                               | In 90% cutoff                                   | Out of 90% cutoff                               | In 90% cutoff                                   | Out of 90% cutoff                               | Out of 90% cutoff                               |
| WDI like Rule Violation Fields  | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                | Balaban index JX                                |
| WDI like Rule Violations        | 0                                               | 1                                               | 1                                               | 1                                               | 1                                               | 0                                               | 1                                               | 1                                               | 1                                               | 0                                               | 1                                               | 1                                               | 0                                               | 1                                               | 1                                               |
4. Results and Discussion

Dimefox (D) and Fluoroacetamide (F) are toxic materials containing fluorine atom that can be substituted by nitrogen atom belong to an amino acid (Scheme 1.). With this hypothetical reaction, (D) and (F) formed a new P-N linkage and C-N linkage respectively with various amino acids (Scheme 1., Figures 1. & 2). These (D), (F), and their derivatives were subjected to mathematical model [MarvinSketch program and https://preadmet.bmdrc.kr website] to calculated ADMET and Druglikeness properties (Tables 1-6).

Physiochemical characters (Tables 1, 2) were chemical formula, molecular weight, isoelectric point, logP, HLB, and PSA:

- pI range of (D derivatives): 2.5-2.9, of (F derivatives): 2.6-6.26.
- logP (ChemAxon method) range of (D and its derivatives): -1.44 to 0.86, of (F and its derivatives): -0.2.07. Only D-PA, D-IL, and F-PA had a positive value of logP.
- HLB data (ChemAxon method) of (D and its derivatives): 11.33 – 29.51, of (F and its derivatives): 11.20 – 20.18.
- PSA data of (D and its derivatives): 33.36-119.99, of (F and its derivatives): 43.09 – 129.72.

To obtain low binding to hydrophilic protein, cytochrome P450 or hERG, logP have to be in its highest values so more toxicity may be occurred.

ADMET predications of all 20 compounds (Tables 3, 5) were as below:

- BBB range of D, F and their derivatives: 0.07076- 0.575892.
- Buffer solubility of D, F and their derivatives, mg/L: 2500.68 – 1.032e+7.
- Caco2 range of D, F and their derivatives: 10.0253 – 21.7244.
- All 20 compounds were non-inhibition character of CYP-2C19 and CYP-2C9 except F.
- D and its derivatives were with non-inhibition character of CYP-2D6 and CYP-3A4. Also, CY-2D6 substrate was with non-character of D and its derivatives.
- F and its derivatives showed a little difference from the above point. F, F-PA, and F-IL were only with inhibitor character of CYP-2D6. Only F and F-G were weakly reaction to CY-2D6 substrate. Only F showed inhibition character to CYP-3A4. Only F-PA was weakly toward CYP-3A4 inhibition.
- HIA range of D, F and their derivatives: 16.85704 – 96.65745.
- MDCK range of D, F and their derivatives: 0.537008 – 335.455.
- All 20 compounds were non-inhibitors towards Pgp.
- Plasma Protein Binding range of D, F and their derivatives: 0-60.55147.
- Pure water solubility of all 20 compounds ranged from 27821.4 mg/L to 1.25e+8 mg/L.
- Skin Permeability was -4.94527 to -3.04478 of D, F and their derivatives.
- SK log D value, SK logP value, SK logS buffer, and SK logS pure were (-4.38616 to -0.17197), (-1.8795 to 0.65724), (-1.48876 to 1.58685), (-0.8303 to 2.78055) respectively.
- Acute algae toxicity range of D, F and their derivatives: 0.123576 to 0.441248.
- According to Ames test, all 20 compounds were with mutagen character.
- D and its derivatives gave negative results with Carcinogenetic testing of mouse while only F, F-GL, F-PA, and F-ME gave the same negative results.
- Only D-ME, F-PA, and F-ME may causing carcinogenetic effect to rat.
- Acute *Daphnia* toxicity range of D, F and their derivatives: 1.17972 – 19.1998. In toxicity evaluation, Acute *Daphnia* toxicity is necessary to be considered because these small planktonic crustacean are aquatic toxicological indicators.
- Low risk of hERG inhibition of all 20 compounds except D-PA that was with medium risk.
- Acute Medaka toxicity range of D, F and their derivatives: 1.81881 to 324.825.
- Acute Minnow toxicity range of D, F and their derivatives: 2.0935 to 86.2885.
- Only D and D-PA showed positive test against TA100-10RLI.
- Only F showed positive test against TA100-NA.
- D and its derivatives beside F, F-AL, and F-P showed positive results against TA1535-10RLI.
- All F and its derivatives did not showed a negative result against TA1535-NA while Dimefox table showed that only D-AS, D-PA, D-IL, D-ME gave positivity to this test.

To candidate chemical to be a drug, this chemical must be at the maximum therapeutic capability at the necessary concentration in the target cell, tissue, or organ. This important goal may be *in Silico* be done through ADMET calculations. These quantifications are depending on polar-nonpolar molecular forces that control, for example, bonding to albumin or α-acidic glycoprotein.

It is a noticeable that all ADMET and Druglikeness calculations are related to these polar-nonpolar forces so all these calculations in Tables
(4-6) are in relevance to chemical structures in Figures (1.& 2.) and physicochemical properties in Tables (1& 2.).

Drug solubility especially in water is identity to measure its reaction with biological system. From this point of view, all 20 compounds were with high water (aqueous phase) more than oil (lipid or organic phase).

**Blood Brain Barrier (BBB)** is ADMET predictor specifies compound ability to penetrate this barrier according to its hydrogen bonding, lipophilic – lipophobic interaction, polar atom presence, molecular surface, ….etc. These factors among others determine the required energy for easily mechanism to take an action in the Central Nerve System (CNS). This system is a selective one specifies brain, liver, blood, and intestine action to drug transport to cell with the help of enzyme, ATP, P-glycoprotein, polarity force to form hydrogen bonding, and the power of BBB heterogeneity. Lipid bilayers cause lack of BBB homogeneity that obstruct or prevent polarized compound from crossing this barrier to access CNS.

From Figures 1. & 2. and Tables 3. & 5., D was with the highest BBB value while D-AS was with lowest. This is a normal evaluation because D contains P, O, and F atoms that perform lowest PSA and lower HLB beside forming P=O and P-F hydrogen bond acceptors with no hydrogen bond donor. Together these factors influence BBB predication which are matching with water solubility and toxicity [1].

Human body likes other mammals contains many proteins with various functions. One of these functions is acting as an enzyme. Cytochrome P450 may acting as oxidizer of C=C bond in unsaturated fatty acid to the corresponding epoxide. Many cytochromes P450 (CYPs) involve in oxidation - reduction process they need protein as poly amino acid units
to transport electron(s). CYP-2C19, CYP-2C9, CYP-2D6, and CYP-3A4 are enzymes in CYP family with various functions and locations in human or mammal body like CYP-2D6 in CNS and CYP-3A4 in liver and intestine.

ADMET predictions in this study showed that F had inhibition character towards CYP-2C19, CYP-2C9, and CYP-2D6. F-PA and F-IL had inhibition characterization towards CYP-2D6. All tested CYPs in this study were in resistance to the action of D and its 9 derivatives as inhibitors like most of F derivatives. The behavior of F with CYPs may be caused by hydrolysis of F in acidic medium to the more powerful Fluoroacetate compound [6].

HIA is another ADMET property specifies drug transporting towards target cell, tissue, or organ giving oral prediction in human starting from intestine. So, drug bioavailability that enters gastrointestinal tract then blood circle can be predicated with high accuracy [10]. In this paper, HIA range of all 20 compounds was 16.85704% to 96.65745%. Tables 3. & 5. reveal HIA superiority of D and its derivatives compared to F and its derivatives and this might be belong to the HLB and PSA effects that influence mechanisms of transporting and membrane permeating through diffusion and PgP contribution respectively.

MDCK is an effective tool to investigate drug permeability through membrane so human absorption of this drug [11]. This in vitro testing in ADMET predications ranged from 0.537008 nm/sec. to 335.455 nm/sec. MDCK as qualitative predication of drug absorption in intestine showed that F and its derivatives were very high in their values than D and its derivatives meaning more influence in drug interaction through three stages: Absorption –Transporting- Permeability processes [12].
MDCK is a mimic predictor resembles BBB in their behavior in drug molecule transport [13]. In MDCK and BBB predcitions, F was less than D but F derivatives were higher than the corresponding D derivatives (Tables 3. and 5.).

Multidrug Resistance Protein or Permeability Glycoprotein (P-gP) represents a remarkable component in membrane used in clinical studies of drug or toxin transportation. PgP has various functions such as ATPase activity, nucleotide or ATP binding, phospholipid or ceramide translocation, stem cell proliferation,…. etc. PgP controls cellular ADMET of chemicals having hydrophobic moieties. PgP can screen drug function through computational studies like done in this paper where PgP inhibition is important in several studies like cancer, Microbial treatment, Alzheimer, …and others [14, 15]. Non- inhibition character of PgP was the prediction of all 20 compounds (Tables 3. & 5.).

High water solubility influences species in aquatic environment and to evaluate this influence Daphnia is water organism predictor of drug solubility toward toxicity estimation. There is similarity sequence in Daphnia toxicity and HLB values as shown in Figure 3 and Tables 1.,2., 3., & 5. This figure of similarity sequence illustrates how increasing of chemical solubility in water (HLB value) raised toxicity of this planktonic crustacean organism. Increasing HLB values refer to increasing of chemical solubility in water and decreasing it in oil or lipid and this small animal (Daphnia) lives in water with maximum water contain in its body. Like acute Daphnia toxicity, acute Medaka and Minnow toxicities were in the same resemblance. Both Medaka and Minnow are fish used to identify drug safety in the aquatic environment. Numerical data of HLB,
The acute *Daphnia*, Medaka, and Minnow toxicities of D and its derivatives were higher than F and its derivatives.

The other predictions in ADMET tables 3. and 5. were TA1100 and TA1535. These predictors represent Salmonella mutagenicity caused by these 20 compounds. In general, TA1535 and TA100 are two strains of this bacterium having the base pair (hisG46) but TA100 has high sensitivity to mutagenic specification because it contain plasmid pKM101 that influence this specification [16,17].

According the above mentioned data in Tables 3. and 5., D, F, and their derivatives in general were mutagen to these Gram-negative bacteria. These bacteria contain a thin layer of peptidoglycan, lipopolysaccharide as its outer membrane, with absence of tecichoic and lipoteichoic acids so the mutagenic effect occurs when any compound pass through cell membrane with easy mechanism and less energy. The compound entrance enhances by compound solubility that is obvious in HLB character. D and D-PA had the lowest HLB values (more soluble in lipid or oil than in water compared to the others in Table 1.) so they gave positive result to TA100-10RLI. The same was in TA100-NA with positive result of F. Also, TA1535-10RLI with presence of D and its derivatives beside F, F-AL, and F-P. TA1535-NA mutagenic character presented in all F and its derivatives beside D-AS, D-PA, D-IL, and D-ME. When these

Druglikeness as a computer – aided Molecular Design predication was applied to evaluate all 20 compounds interaction with body according to specific rules: CMC like, Lead like, MDDR like, Five rules, and WDI like (Tables 4, 6).

Only D–PA, D-IL, F-PA, and F-IL were qualified to CMC like rules. Violation of other compounds based on AlogP98 value.
Three types of Lead-like Rule of any drug; Druglike, Leadlike, High affinity lead) are classified according to drug affinity to be binding, logP value, and molecular weight. The same violation in CMC like rule was found in Lead like rule of all 20 compounds.

All 20 compounds had MDDR like violations where D and its derivatives had Mid- structure violation but NonDrug-like was found in F, F-G, and F-AL compounds while other F derivatives were Mid-Structure. Also, all 20 compounds were suitable to Rule of Five with zero violation. D-PA, F, F-PA, and F-P were in 90% cutoff -zero violation to WDI like rule while others were out of 90% cutoff – one violation (Balaban index JX field).

Various factors influence Comprehensive Medicinal Chemistry (CMC) rule and its violations such as molecular weight, number of atoms, logP, and molecular reactivity. CMC rule limitations mainly depend upon drug classification to depressant, inflammatory, infective, …. etc. and its logP values. This lipophilic character (logP) is responsible important biological processes (Absorption, Solubility, and Metabolism) because it specifies partitioning in aqueous or organic phase. Tables 1. & 2. Showed that most of tested compounds had a negative logP value. So these negative logP compounds can be considered according to Ghose et al. as hypertensive, neoplastic, infective, and CMC clean drug classifications.

In human, there is hERG gene that combines with potassium ion (K⁺) in cardiac repolarization so heart beating. Hypokalemia (reduction of K⁺ in serum) and some drugs influence hREG action meaning serious heart problem may led to death. According to Tables 3. & 5., there was low risk of D, F, and their derivatives except F-PA and D-PA that had medium risk of hREG inhibition. The increasing in risk level may be attributed to the effect of phenyl ring presence and highest logP values.
Druglikeness predication has MDDR that has several limitations in its classification such as presence of reactive functional groups like carboxylic acid halide, sulfonic acid halide, number of ring, and bond with rigidity or rotating ability. According to these limitations, Modern Drug Data Report (MDDR) categorizes drug to non-drug, rug-like, and mid-structure. Most predication of all tested compounds in this paper was with Mid-structure specification. F, F-G, and F-AL were Non-drug – like predication suggestion the effect of molecular weight (lowest values of all 20 compounds) having number of rings, rigid bonds, and rotatable bonds as three MDDR violation fields.

Lipinski and co-workers set Five Rule which are logP (≤5), Molecular weight (≤ 500 Dalton), HBDs(≤5), and HBAs(≤10) and violation causes drug prohibiting from oral intake. All 20 compounds were suitable to be good in vivo drug absorption and penetration in biological system with zero violation to the Rule of Five (Tables 1, 2, 4. & 6.). Also, permeation of these 20 compounds agreed with the necessary PSA (range (33.36-129.72)Å²) depending on presence of polar atoms (N, O, P, and S) that formed P=O, C=O, NH, OH, C-N-C, and / or C-S-C as hydrogen bond acceptor(s) and NH and OH as hydrogen bond donors (Figures 1. and 2., Tables 1. and 2.).

World Drug Index (WDI) like Rule was violated in most of the 20 compounds under this in silico prediction as out of 90% cutoff but not D-PA, F, F-PA, or F-P having in 90% cutoff. These 4 non- violated compounds agreed with Brown and his group limitations depending upon various molecular descriptors, logP, HBAs, HBDs, …etc.

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