Protonation, geometry, charge, and partitioning properties of several prepared heterocyclic derivatives.

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Abstract:

Several heterocyclic derivatives were evaluated experimentally to determine their physical properties and antimicrobial activity. Then these compounds were tested with Marvinsketch program to determine their protonation, geometry, charge, and partitioning. Partitioning characters (log P and log D at pI) were calculated by Consensus and Chemaxon methods while HLB was with Chemaxon and Davies methods. For example, the derivatives of EDTA based structure showed that isoelectric point (pI) did not affected by the presence of negative charge compared with its zero charge. As is expected, negative charge, presence of sulfur atoms, and type of heterocyclic moiety had a good influence on HLB values. The obtained results confirmed high harmonization between the experimental and computerized calculations especially when several of them where tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*.

Keywords: heterocyclic, protonation, geometry, charge, and partitioning.

Introduction:

The presence of nitrogen, oxygen, and/or sulfur in a ring represents the definition of heterocycles. And with this short definition cyclic imide
which contains nitrogen atom in its structure as (-CO-NH-CO- or –CO-N- CO-) bond can be considered as a heterocyclic molecule. Both heterocycles and cyclic imides show a great effect in medicinal and industrial applications (1-3). From this start of point, many researchers including us synthesized many derivatives with hetero-moiety with/ or without cyclic imides and tested several of these prepared derivatives as antimicrobial agents (4-8). The recent direction of finding new biocides or drugs for therapy or treatment took a huge part of research areas (8). This led to consume materials, equipments, time, cost, identification instrumentations…. etc. beside environmental considerations.

It is not new that mathematical model or software was applied to calculate many needed characters that give a spot light to candidate a molecule to be antimicrobials or other categories of drug applications (9,10). MarvinSketch is one of these programs that used to qualify a molecule in medicinal field (11,12).

With these above points, we directed our paper here to calculate ethylene diamine tetraacetic acid (EDTA) derivatives that were prepared as a part of Ph.D thesis (13) presented by K. Al-Obaidi and her supervisor Prof. D. Ahlam Marouf Al-Azzawi to the Chemistry Department-College of Science- Baghdad University in 2013. The reason behind choosing these EDTA derivatives was mainly based on the importance of EDTA (14,15), heterocyclic moiety, and cyclic imide in drug chemistry and applications (2,4). We do think that the structure relationship with the activity of our EDTA molecules will be reflected.

Experimental section:
Chemicals: All the used chemicals were from international companies (Merck, BDH, and Fluka) and used as they purchased.
Instruments:

Melting points were measured with Gallenkamp apparatus- capillary, FTIR, $^1$H- and $^{13}$C- NMR spectra were recorded by FTIR -8400 (Shimadzu, Japan), Bruker (300 NHz, ultrasheild, DMSO-d$_6$). Also, conductivity and acid – base titration against NaOH were performed by Elekir leitfanigkeit, Siemens and Metrohm Titroprocessor 670 respectively.

Preparation steps: According to Schemes -1-, -2-, and -3-, compounds [[1-17]] were prepared. [1-17] preparation was part of Ph.D thesis submitted to the Chemistry Department, Science College, Baghdad University, 2013 by the student K. Al-Obaidi and under supervision of Prof. Dr. A. Al-Azzawi ($^{13}$).

Preparation of Ethylene diamine tetaacetic dianhydride (EDTA-dianhydride) [1]: A mixture of ethylene diamine tetaacatic acid (EDTA) (10 gm), acetic anhydride (14 mL), and pyridine (16 mL) were refluxed at (65-70)$^\circ$C for twelve hours. The filtered solid washed with acetic anhydride then ether.

(EDTA-dianhydride)[1]: Off white, 92%, (188-190)$^\circ$C, Recryst. Solvent: petroleum ether. IR: C=O anhydride, (1809 and 1762) cm$^{-1}$; (C-H) aliphatic (2993) cm$^{-1}$. $^1$H-NMR: -CH$_2$-N-, $\delta$= (2.66-2.78) ppm; -N-CH$_2$-CO-, $\delta$=3.7 ppm.$^{13}$C-NMR: CH$_2$, $\delta$= 51.8-52.8) ppm; -N-CH$_2$-CO-, $\delta$=55.2 ppm; carbonyl, $\delta$=(166-173)ppm.

Synthesis of bisamic acids [2-6]: A mixture of amino compound in 25 mL of acetone were dropped to react with EDTA- dianhydride in 25 mL of acetone (2:1 molar ratio) with stirring for half hour with cooling then for four hours. The filtered solid bisamic acids [2-6] were washed with ether.
Bisamic acid [2]: White, 70%, %,(228-230)°C, Recryst. Solvent: ethyl acetate. IR: (O-H, N-H), 3436 cm⁻¹; (C-H) aliphatic, 3019 cm⁻¹; C=O carboxylic acid and amide, 1696 cm⁻¹; C-S, 684 cm⁻¹.

Bisamic acid [3]: White, 80%, %,(240-230)°C, Recryst. Solvent: ethyl acetate. IR: (O-H, N-H), 3417 cm⁻¹; (C-H) aliphatic (3017, 2951) cm⁻¹; C=O carboxylic acid and amide, 1697 cm⁻¹; C=C aromatic, 1570 cm⁻¹.

¹H-NMR: -N-CH₂-CH₂-N, δ= 2.77 ppm; -N-CH₂-CO⁻, δ=(3.41-3.46)
ppm; aromatic protons, δ=(7.15-8.11) ppm, -OH, δ=(11.1-11.2) ppm. 13C-
NMR: -N- CH₂, δ= 51.8 ppm; -N-CH₂-CO-, δ=55 ppm; aromatic carbons, δ=(111-125.9) ppm; C=O carboxylic and amide, δ=172.8 ppm.

**Bisamic acid [4]**: White, 45%, %,( 216-218)°C, Recryst. Solvent: cyclohexane. IR: (O-H, N-H), (3522, 3387) cm⁻¹; (C-H) aliphatic, 3028 cm⁻¹; C=O carboxylic acid and amide, (1674, 1627) cm⁻¹.

**Bisamic acid [5]**: White, 50%, %,( 235-236)°C, Recryst. Solvent: benzene. IR: (O-H, N-H), (3436) cm⁻¹; (C-H) aliphatic, 2997 cm⁻¹; C=O carboxylic acid and amide, (1701) cm⁻¹.

**Bisamic acid [6]**: Gray, 63%, %,(246-247)°C, Recryst. Solvent: methanol. IR: (O-H, N-H), (3433,360) cm⁻¹; (C-H) aliphatic, 3016 cm⁻¹; C=O carboxylic acid and amide, (1674) cm⁻¹.

**Synthesis of ester –amic acid [7]** (Scheme -2a): EDTA-dianhydride [1] dissolved in N,N-dimethylformamide (60 mL) was mixed with tetrahydrofurfuryl alcohol in molar ratio (1:2) of dianhydride : alcohol then refluxed at 95 °C after addition of few drops of H₂SO₄ for (18) hours. (20) mL of water was added to the red mixture then heated at 80 oC for more than half hour o complete hydrolization of the remained EDTA-anhydride. The solvent was removed under vacuum.

**Scheme -2a-**
Formation of Ester -amic acid [7]
Ester-amic acid [7]: Red, 75%, oily. IR: (O-H, N-H), 3468 cm\(^{-1}\); (C-H) aliphatic, 2931 cm\(^{-1}\); C=O carboxylic acid and amide, (1674) cm\(^{-1}\); C=O ester, 1720 cm\(^{-1}\); C-O-C ester, (1222, 1095) cm\(^{-1}\). \(^1\)H-NMR: four protons of tetrahydrofuran ring, \(\delta = (1.6-1.8)\) ppm; three protons of tetrahydrofuran ring, \(\delta = (2.77-2.94)\) ppm; -N-CH\(_2\)-CH\(_2\)-N- and –CO-O-CH\(_2\)-, \(\delta = (3.1-4)\) ppm; -CO-C H\(_2\)-N-, \(\delta = 7.9\) ppm, -OH, \(\delta = 8.17\) ppm. \(^{13}\)C-NMR: two carbons of tetrahydrofuran ring, \(\delta = (25.5-27.7)\) ppm; -N- CH\(_2\)-CH\(_2\)-N-, \(\delta = (30.9, 34.7-35.9)\) ppm; two carbons of tetrahydrofuran ring bonded to oxygen, \(\delta = (47.56)\) ppm; -CO-O-CH\(_2\)-, \(\delta = (64-68.4)\) ppm; -N-CH\(_2\)-CO-O-, \(\delta = (76-79.7)\) ppm; C=O carboxylic, \(\delta = (162.4-163)\) ppm; C=O ester, \(\delta = (170-172)\) ppm.

Sodium salts synthesis of Bisamic acids [8-12] and sodium ester-amic acid [13]: A clear solution was obtained by adding alcoholic sodium hydroxide to the prepared bisamic acid [2-6] (Scheme -2b-) or ester – amic acid [7] (Scheme -2c-) then acetone added to form crystals.
Sodium salt [8] of bisamic acid [2]: White, 80%, % Recryst. Solvent: acetone. IR: N-H, amide, 3305 cm\(^{-1}\); C=O carboxylate, (1608, 1442) cm\(^{-1}\); C=O amide, 1608 cm\(^{-1}\); C-S, 694 cm\(^{-1}\).

Sodium salt [9] of bisamic acid [3]: White, 85%, %. Recryst. Solvent: acetone. IR: C=O carboxylate, (1604, 1442) cm\(^{-1}\); C=O amide, 1604 cm\(^{-1}\); aromatic C=C, 1570 cm\(^{-1}\). \(^1\)H-NMR: -N-CH\(_2\)-CH\(_2\)-N-, \(\delta=2.75\) ppm; -CO-CH\(_2\)-N-, \(\delta=(3.41-3.52)\) ppm, aromatic protons, \(\delta=(7.1-8)\) ppm.

Sodium salt [10] of bisamic acid [4]: White, 76%, %. Recryst. Solvent: ethanol. IR: C=O carboxylate, (1589, 1438) cm\(^{-1}\); C=O amide, 1589 cm\(^{-1}\).

Sodium salt [11] of bisamic acid [5]: White, 90%, %. Recryst. Solvent: ethanol. IR: N-H, amide, 3437 cm\(^{-1}\); C=O carboxylate, (1608, 1431) cm\(^{-1}\); C=O amide, 1608 cm\(^{-1}\).

Sodium salt [12] of bisamic acid [6]: Gray, 63%, %. Recryst. Solvent: acetone. IR: C=O carboxylate, (1589, 1435) cm\(^{-1}\); C=O amide, 1610 cm\(^{-1}\). \(^1\)H-NMR: -N-CH\(_2\)-CH\(_2\)-N-, \(\delta=2.79\) ppm; -CO-CH\(_2\)-N- and vinylic protons, \(\delta=3.48\) ppm. \(^13\)C-NMR: -N-CH\(_2\)-CH\(_2\)-N-, \(\delta=51.84\) ppm; -N-CH\(_2\)-CO- and vinylic carbons, \(\delta=55\) ppm; C=O carbons, \(\delta=172.7\) ppm.

Sodium salt [13] of ester-amic acid [7]: Brown, 72%. Recryst. Solvent: acetone. IR: C=O carboxylate, (1634, 1468) cm\(^{-1}\); C=O ester, 1735 cm\(^{-1}\); C-O-C ester, (1125, 1114) cm\(^{-1}\).
Synthesis of di-(N-acetanilido)-EDTA derivative [14] (Scheme -3): The same procedure that followed for the synthesis of EDTA derivatives [2-6] with the same molar ratio of EDTA-dianhydride [1] and aniline was applied.

**Ethylene diamine di(N-acetanilido) diacetic acid [14]:** White, 60%, (180-182)°C. Recryst. Solvent: ethanol. IR: (O-H carboxylic, N-H amide), (3462 and 3294) cm⁻¹; C=O carboxylic acid and amide, (1685 and 1624) cm⁻¹.

**Synthesis of diimide [15] (Scheme -3):** A mixture of (0.01 mole) Bisamic acid [14], 10% by weight of anhydrous Na-acetate, (20 mL) acetic anhydride was refluxed for two hours, cooled, and poured with stirring into cold water. The filtered precipitate was recrystallized with ethanol.

**1',2'-Bis(1-phenyl-2,6-dione-3,5-dihydro-pyrazine-4-yl) ethylene [15]:** Black, 75%, (282-284)°C. Recryst. Solvent: ethanol. IR: asym. and sym. C=O imide, (1739 and 1624) cm⁻¹; C=C aromatic, 1593 cm⁻¹. ¹H-NMR: aliphatic protons, δ=(1.7-2) ppm. ¹³C-NMR: aliphatic carbons, δ= (31,53, and 55) ppm; aromatic carbons, δ=(129-148) ppm; C=O carbons, δ= 168 ppm.
**Synthesis of diimide-chlorosulfonyl derivative [16]** (Scheme -3-): A mixture of 0.1 mole [15] in 30 mL of chloroform was dropped to 5mL of chlorosulfonic acid cooled to (0-5) °C and the produced mixture was stirred for four hours after complete addition then left standing for overnight at refrigerator. [16] was formed after recrystallization oily layer with petroleum ether that formed after pouring into crushed ice.

1',2'-Bis(1-(4-chlorosulfonylphenyl)2,6-dione-3,5-dihydro-pyrazine -4-yl)ethylene [16]: Black, 63%, (294-297)°C. Recryst. Solvent: petroleum ether. IR: asym. and sym. C=O imide, (1735 and 1689) cm \(^{-1}\); C=C aromatic, 1635 cm\(^{-1}\); asym. and sym. SO\(_2\), 1350 and 1165) cm\(^{-1}\).

**Synthesis of benzothiazole-diimide sulfonamide derivative [17]** (Scheme -3-): 3.71 g of [16] and a mixture of 2-amino-6-nitrobenzothiazole (1.75 g) in 30 mL pyridine were mixed with temperature below 40°C then refluxed for three hours followed by stirring until cooled to room temperature. The resulted solution was poured into cold water and the obtained filtered solid was recrystallized from ethanol.

1',2'=Bis(1-(4-(6-nitrobenzothiazol-2-yl)sulfonamidophnyl)-2,6-dione-3,5-dihydro-pyrazine-4-yl) ethylene [17]: Brownish yellow, 66%, >300 °C. Recryst. Solvent: ethanol. IR: N-H amide, 3441cm\(^{-1}\); C=O imide, 1720 cm\(^{-1}\); C=C aromatic, 1600 cm\(^{-1}\); asym. and sym. SO\(_2\), 1327 and 1130) cm\(^{-1}\). \(^{1}\)H-NMR: aliphatic protons, \(\delta\)=1.8 ppm; aromatic protons, \(\delta\)= (6.75-7.3) ppm; NH, \(\delta\)=9.7 ppm.\(^{13}\)C-NMR: aliphatic carbons, \(\delta\)= (23.9, 72, and 80) ppm; aromatic carbons, \(\delta\)=(118.9-139.3) ppm; C=N and C=O carbons, \(\delta\)= 168.2 ppm.
Calculations: Before starting calculations, EDTA and all [1-17] compounds were draw by using Chemical Structure Drawing Standard, CS ChemDraw Ultra, Cambridge Soft Corporation MA, USA, © 1985-1998, www.Camsoft.com. Then the calculations were done with MarvinSketch Software, Version 18.15.0, www.Chemaxon.com. The calculated properties were as below:

1. Elemental analysis: Molecular Weight and Formula.

2. Protonation: Isoelectric point (pI).

3. Partitioning:
   a. log P and log D at pI by Cosensus and Chemaxon methods where Cl−, Na+, K+ electrolyte concentration under condition of calculation 0.1 mol./dm³.

   b. Hydrophilic – Lipophilic Balance (HLB) was calculated by Chemaxon and Davies methods.

4. Charge: Polarizability was at pH 7.4.
5. Geometry:
   a. Polar Surface Area (2D) was calculated with excluding Sulfur and Phosphors atoms.
   b. Molecular Surface Area (3D) was calculated with Van der Waals surface for the molecule in (Å)².
6. Hydrogen Bond Donor / Acceptor with excluding of Sulfur and phosphors atoms at pH (0-14).
7. Refractivity.

**Results and Discussion:**

From synthetic chemical aspects, the prepared compounds [1-17] were prepared in several organic reactions. The first step involved preparation of the EDTA –dianhydride [1] from EDTA by abstracting two water molecules with the help of acetic anhydride (Ac₂O) – pyridine mixture under reflux condition. So, here acetic anhydride (Ac₂O) acted as a dehydrating agent of EDTA molecule then the ring closure (Equation -1-).

![Equation -1-: Formation of [1] from EDTA](image)

The second step was formation of Bisamic acids [2-6]; i.e. formation of double amide and double carboxylic acid bonds in the same molecule; via reaction of [1] with different amino – heterocyclic molecules. Also, this step contained formation of bisester –acid molecule [7]; i.e. formation of di -ester bond and di- COOH bond in the same molecule.
In the second step, the mechanism was nucleophilic attack of primary amino–heterocyclic molecules (2-amino benzothiazole and 3-amino-pyridine), as presented below (Mechanism -1-), or secondary amino-heterocyclics (carbazole, pyrrolidine, pyrrole -2-carboxylic acid) on carbonyl group of [1]. The same mechanism was for secondary amines with [1].

Reaction of [1] with heterocyclic molecule containing hydroxyl group produced bis- Ester acid molecule or diacetate) [7]. The same nucleophilic attack on carbonyl group of [1] was repeated but here with hydroxyl group of tetrahydrofurfuryl alcohol as below (Mechanism -2-).
These bisamic acids [2-6] and diacetate[7] were converted to their sodium salts [8-13] by reacting with alcoholic base (NaOH) (Equation -2-).

We must mention that [7] was water soluble in contrast to [2-6] and these water insoluble derivatives [2-6] turned into high water soluble molecules when they became sodium salts [8-12]. Also, [13] derivative was still water soluble. Physical and spectral characterizations for [1-13] were done as mentioned in experimental section.

The third step involved multistep synthesis also by depending upon the capability of [1] to react with amino group of aniline then the corresponding bisamic acid [14] was dehydrated to its diimide [15] via anhydrous Na – acetate and acetic anhydride (Ac₂O) mixture (please see below general mechanism of dehydration of amic acid by (Ac₂O) and NaOOCCH₃ (Mechanism -3-)).
The synthetic diimide[15] and 2-aminobenzothiazole were conjugated after formation sulfonamide group by reacting of [15] with chlorosulfonic acid via electrophilic attack (Mechanism -4-) on phenyl ring (para position). The result of chlorosulfonation reaction (diimidyl sulfonyl chloride [16]) was introduced to nucleophilic –HCl elimination reaction with 6-nitro-2-amino-benzothiazole to produce diimide-benzothiazole derivative [17].
Mechanism 4: Reaction of chlorosulfonic acid with EDTA-diimide.

In the final step, compound [70] was introduced in reaction (Mechanism 5- with heterocyclic primary amine (6-nitro-2-amino-benzothiazole) producing the desired compound [71].
Mechanism -5-: Formation of [17].

Also, the resulted derivatives [14-17] were characterized to identify their physical and spectral properties and to qualify the success of reaction steps (please see experimental section).

After the description of chemical syntheses of EDTA derivatives [1-17], the next step was introducing them with EDTA itself in MarvinSketch software. This program was used to calculated [EDTA, 1-17] abilities to be active bio-derivatives through: Elemental analysis (Molecular Weight and Formula), Protonation (Isoelectric point (pI)), Partitioning (log P and log D at pI), Hydrophilic – Lipophilic Balance (HLB), Charge: Polarizability, Geometry (Polar Surface Area (2D), Molecular Surface Area (3D)), Hydrogen Bond Donor / Acceptor, and Refractivity.
Table 1: Marvinsketch calculations of EDTA, [1-6] and [8-12] compounds.

| Calculation       | Character                          | Compound symbol under calculation |
|-------------------|------------------------------------|----------------------------------|
|                   |                                    | EDTA | 1  | 2  | 8  | 3  | 9  | 4  | 10 | 5  | 11 | 6  | 12 |
| Protonation       | Isoelectric point (pI)             |      | 1.92| -  | 3.53| 3.53| 3.86| 3.86| 3.74| 3.74| 4.55| 4.55| 2.63| 2.63|
| log P             | Consensus method                   |      | 1.88| 1.61| 2.85| 2.64| 3.39| 3.39| -1.79| -1.79| -1.45| -1.45| -1.53| -1.53|
|                   | Nonionic species                   |      | -   | -   | -   | -   | -   | -   | -1.79| -1.79| -1.79| -1.79| -1.53| -1.53|
|                   | Consensus method                   |      | -1.70| -1.79| 2.85| 2.85| 4.18| 4.18| -1.79| -1.79| -1.24| -1.24| -1.64| -1.64|
|                   | Ionic species                      |      | -   | -   | -   | -   | -   | -   | -1.79| -1.79| -1.24| -1.24| -1.64| -1.64|
| log D at pI       | Consensus method                   |      | 5.02| -  | 0.15| 0.59| 0.59| -1.79| -1.79| -1.24| -1.24| -1.64| -1.64| -1.64| -1.64|
|                   | ChemAxon method                    |      | -5.01| -  | 0.15| 1.00| 1.00| -1.79| -1.79| -1.24| -1.24| -1.64| -1.64| -1.64| -1.64|
|                   | Nonionic species                   |      | 2.68| 2.75| 18.46| 13.92| 51.06| 51.06| 20.50| 20.50| 23.66| 23.66| 103.55| 103.55|
|                   | Ionic species                      |      | 2.85| 2.90| 22.40| 22.40| 61.78| 61.78| 20.50| 20.50| 23.66| 23.66| 103.55| 103.55|
|                   | Consensus method                   |      | 25.05| 23.36| 54.64| 53.92| 61.78| 61.06| 40.81| 40.02| 42.85| 42.85| 44.15| 42.76|
|                   | ChemAxon method                    |      | 155.68| 93.22| 165.06| 170.72| 125.08| 130.74| 121.71| 127.36| 165.06| 170.72| 199.68| 211.00|
|                   | Polar Surface Area (2D)            |      | 413.63| 349.00| 713.87| 708.99| 810.88| 805.97| 633.13| 628.06| 626.79| 621.59| 634.33| 625.55|
|                   | Van der Waals                      |      | 0  | 4  | 2  | 2  | 0  | 2  | 0  | 4  | 2  | 4  | 0  | 0  | 0  |
|                   | H bond donor / acceptor            |      | 4  | 0  | 4  | 2  | 2  | 0  | 2  | 0  | 4  | 2  | 4  | 0  | 0  |
|                   | Donor count                        |      | 10 | 6  | 10 | 10 | 8  | 8  | 8  | 10 | 10 | 12 | 12 | 12 | 12 |
|                   | Acceptor count                     |      | 18 | 10 | 16 | 18 | 14 | 16 | 14 | 16 | 16 | 18 | 22 | 26 | 26 |
|                   | Acceptor sites                     |      | 62.35| 55.93| 139.99| 161.66| 162.54| 184.22| 100.66| 122.33| 114.59| 136.26| 111.50| 154.85 |
From calculations, chemical formula of compounds (presence and number of N, O, and/or S atoms) and functional groups affected the numeric values of calculated properties. Oxygen atom in carbonyl group or carboxylate group and nitrogen in tertiary amine acted as acceptor of hydrogen bonding while oxygen in OH group and nitrogen in NH offered hydrogen donation.

The presence of any charge on molecule is depending upon its existence of chargeable atoms. Isoelectric point (pI) represents absence of charge at particular pH (16). Our pI values were unchangeable between bisamic acid and the corresponding sodium salt (Tables(1-2)). So, our prepared derivatives can be precipitated (or minimum solubility) in water or salty solution at acidic medium except [15 and 16] because of their structures.
Also, isoelectric point (pI) was 1.92 for EDTA while EDTA-dianhydride [1] was with no result.

Partition coefficient (logP) is a measurement of lipophilic property in n-octanol/H2O may be determined experimentally at equilibrium stage between H2O and organic phases [17] and by applying software such as MarvinSketch. With MarvinSketch program, two methods of calculation were applied (Consensus and ChemAxon) for nonionic and ionic species. Also, logP values were with negative sign for sodium salts [8-12] and diacetate ester [13] (nonionic species) so as ionic species and with positive sign for the rest of them.

Where logP represents the majority of ionic or nonionic species in water or organic solvent, their action as hydrophilic or hydrophobic derivatives and their distributions in biological body can be estimated. Low logP led to distribution in aqueous medium such as blood serum. In contrast, high logP represented hydrophobic molecule distributed in organic medium such as lipid layer of the cell. Both tested methods for the determination of logP gave identical values in most of our tested derivatives.

The other character (logD) that may shows the influence of pH on a molecule to form ionizable species (hydrophilic) that does not dissolve in organic solvent or phase or with lipophilic forms [11,12]. This logD calculated at pI and so it compared with it. So at acidic medium, our carboxylic bond was unionized and may turn into ionized species at basic medium by form carboxylate ion. Also, tertiary amine in EDTA derivatives may be protonated according to the tested medium. This conclusion can be supported with titration against NaOH solution and conductivity results and titration of Gemini surfactants of EDTA [8,9,or 11] that also exhibited various equivalents (Scheme -4-, Tables(-3- and -4-)).
\[
\begin{align*}
&\text{H}_2\text{C}^+\text{OOC}^-
\end{align*}
\]

Scheme -4-: Hypothetical transition happening during titration of EDTA derivative with NaOH.

**Table -3-: Acid – Base titration results.**

| Comp. No. | Sample weight, g | NaOH (0.1N) volume, mL | pH at first neutralization | pH at second neutralization |
|-----------|------------------|------------------------|---------------------------|-----------------------------|
| 8         | 0.0503           | 2.40                   | 1.71                      | 9.69                        |
| 9         | 0.0359           | 12.81                  | 1.02                      | 9.22                        |
| 11        | 0.1127           | 13.79                  | 2.58                      | 7.95                        |

**Table -4-: Conductivity results**

| Conc., M | Conductivity at Lab temp., μS/cm |
|----------|----------------------------------|
|          | 8      | 9      | 10     | 11     | 12     | 13     |
| 0.01     | 1310   | 1240   | 1190   | 1040   | 564    | 2740   |
| 0.001    | 164    | 171    | 159    | 127.1  | 73.2   | 327    |
| 0.0001   | 20     | 31.6   | 19.9   | 15.2   | 7.58   | 35.3   |
| 0.00001  | 8.87   | 7.54   | 5.87   | 1.8    | 6.81   | 8.7    |
| 0.000001 | 2.61   | 21.7   | 11.35  | 1.58   | 3.54   | 8.9    |

R: primary or secondary heterocyclic amine
The balance between hydrophilicity and lipophilicity or HLB plays an important role in anti-biological performance. HLB is a character of surface active or surfactant \(^{(18)}\). Here, it was calculated by two methods (ChemAxon and Davies). Both methods gave semi identical results. HLB values were ranged from (13.98) to (103.35) by Chemaxon method and from (15.75) to (103.35) by Davies method. Our sodium salts [8-13] gave the highest predication of HLB values and more than its corresponding bisamic acids [2-6] or diacetate [7] with more than (56-73.)%of increasing which reflecting the normal action of salt in water as a soluble material.

Polarizability was the other character that computed by MarvinSketch software. It expresses the occupation of electrons in specific volume \(^{(19)}\). It is in contrast to the dipole character and electronegativity. For charge calculation, polarizability values were (23.36-93.559). The differences between bisamic acids [2-6] and diacetate [7] with their corresponding salts were countless. Also, the presence of aromatic moiety or hetero-cycloalkane was influential. Also, it can be affected by molecular size which was the next character that was calculated.

Marvin Sketch program was also used to compute polar surface area (2D) that summarize the material ability to be a good candidate as a drug by permeate cell membrane \(^{(20)}\) if it is less than 90 angstroms but not more 140 angstroms. For geometry calculation, polar surface area (2D) values were (81.24-285.64). Molecular surface area (3D) values were (349.00-1164.9) that showed the ability of a molecule to be accessible or to be buried in a solvent usually water with radius (1.4 Å) \(^{(21)}\).

Final calculations were refractivity that ranged from (55.93-225.95) which reflect the molecular volume and molecule interaction as drug – receptor \(^{(22)}\). The refractivity results indicated that hetero-aromatic ring had more influence than hetero-cyclic alkane, increasing aromatic ring
increased refractivity, and sodium salts [8-13] were with high values compared to its bisamic acids [2-6] and diacetate [7].

To figure out our calculated factors, a biological testing was done against several microorganism (Tables -5- and -6-). Several of our tested microorganisms showed high resistances to some of EDTA derivatives (with –ve result) while others were clearly affected by them.

**Table -5-: Biological testing results of several EDTA derivatives.**

| Com p. No. | Staphylococcus aureus | Bacillus subtilis | Escherichia coli | Pseudomonas aeruginosa | Candida albicans |
|------------|-----------------------|------------------|------------------|-----------------------|-----------------|
|            | Conc., mg/mL (DMS O) | Inhibitio n zone, mm | Conc., mg/mL (DMS O) | Inhibitio n zone, mm | Conc., mg/mL (DMS O) | Inhibitio n zone, mm | Conc., mg/mL (DMS O) | Inhibitio n zone, mm |
| 2          | 5.0                   | -ve              | 5.0              | 15.0                  | 10.0            | 0.0               | 5.0              | -ve                  |
|            | 10.0                  | 15.0             | 10.0             | 23.0                  | 10.0            | -ve               | 10.0             | 20.0                 |
|            | 20.0                  | 25.0             | 20.0             | 26.0                  | 20.0            | -ve               | 20.0             | 25.0                 |
| 3          | 5.0                   | -ve              | 5.0              | 15.0                  | 5.0             | -ve               | 5.0              | -ve                  |
|            | 10.0                  | -ve              | 10.0             | 19.0                  | 10.0            | -ve               | 10.0             | 18.0                 |
|            | 20.0                  | -ve              | 20.0             | 20.0                  | 20.0            | -ve               | 20.0             | 20.0                 |
| 5          | 2.5                   | 4.0              | 2.5              | -                     | 2.5             | -ve               | 2.5              | -                    |
|            | 5.0                   | 4.0              | 5.0              | -                     | 5.0             | -ve               | 5.0              | -                    |
|            | 10.0                  | 4.0              | 10.0             | -                     | 10.0            | 3.0               | 10.0             | -                    |

**Table -6-: Biological inhibition by [17] (002 g/mL) against various microorganisms.**

| Microorganism          | Inhibition zone for 24 hrs., mm |
|------------------------|---------------------------------|
| Serratia marcescens    | -                               |
| Klebsiella pneumonia   | 28                              |
| Pseudomonas aeruginosa | -ve                             |
| Bacillus subtilus      | 30                              |
| Actinobacter bowmanii  | -ve                             |
| Proteus merabilis      | 12                              |
| Escherichia coli       | 15                              |
| Crytococcus albidus    | 20                              |
| Candida guilliemondii  | -ve                             |
| Candida utilis         | -ve                             |
| Organism                      | Status   |
|------------------------------|----------|
| Candida gulliermondii        | -ve      |
| Candida krusei               | -ve      |
| Rhodorula mucilaginosa       | -ve      |

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