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RESEARCH PAPER

Theoretical study for the inhibition ability of some bioactive imidazole derivatives against the Middle-East respiratory syndrome corona virus (MERS-Co)

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A B S T R A C T:
The Severe Acute Respiratory Syndrome (SARS) is a serious viral life-threatening and mortal respiratory illness caused by SARS-CoV. SARS-CoV plays an essential role in the viral replication cycle. It is considered a potential target for SARS inhibitor development. A series of twenty eight bioactive imidazole compounds as possible SARS-CoV inhibitors were designed and evaluated using computational calculations. Possible binding interaction modes were proposed by molecular docking studies. Among all studied compounds, compounds **5**, **15** and **22** showed most potent inhibitory activity against SARS-CoV. These results indicated that these inhibitors could be potentially developed into anti-SARS drugs.

KEY WORDS: SARS, Corona viruses, imidazole, docking.
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INTRODUCTION:

Coronaviruses are single stranded RNA viruses. They can infect humans and animals, such as bats, mice, birds, dogs, pigs, and cattle producing respiratory and enteric diseases (Perlman and Netland, 2009). It was believed that these viruses can only cause mild respiratory symptoms similar to common cold (Saif, 2004) however this idea changed after 2002-2003 outbreak of the severe acute respiratory syndrome (SARS) when it infected approximately 8000 and killed 774 people (Peiris et al., 2004).

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In the followed years hCoV-NL63 and hCoV-HKU1 human coronaviruses were discovered (van der Hoek et al., 2004, Woo et al., 2005). It is believed that bats are the major role in the process of interspecies transmission in all known human coronaviruses (To et al., 2013). The complete genome sequence of the coronavirus was obtained by different groups of scientists (van Boheemen et al., 2012), (de Groot et al., 2013).

The MERS-CoV common symptoms are fever followed by cough and shortness of breath then acute pneumonia and acute renal failure (Geng and Tan, 2013). Although the beginning of the MERS-CoV transmission to the human species was from an animal, the occurrence of new clusters suggests the human to human transmission.

Obviously, aerosol droplets, direct contact with biological secretions like stool, urine and blood are the main ways of virus transmission among humans (Danielsson and Catchpole, 2011).

To the moment, there are no effective anti-viral agents against human coronavirus (Chan et al., 2012) and the only available therapeutic interventions for SARS involve antibiotics, supportive care, antiviral agents and sometimes immunomodulatory therapy (Puzelli et al., 2013).
It is known that imidazole compounds are heterocyclic compounds or diazole compounds. There are a huge number of imidazole compounds that derived from natural product compounds like alkaloids or chemically synthesized. The imidazole ring compounds has a wide range of biological activity, such as certain antifungal drugs, the nitroimidazole series of antibiotics, and the sedative midazolam (Grimmett, 1997, Brown, 2012, Gilchrist, 1997, Rosemeyer, 2004, Katritzky et al., 1997) or available in biological molecules like histidine, and related hormone histamine. In addition imidazole represents an important part of many pharmaceuticals such as many fungicides and antifungal which includes ketoconazole, miconazole, and clotrimazole (Shargel and Swanson, 2004), antiprotozoal, and antihypertensive medications. Nevertheless, imidazole is found in tea leaves and coffee beans and in the anticancer medication mercaptopurine.

Computer-aided docking is one of the important tools for designing novel bioactive compounds and studying the binding interactions between a ligand (inhibitor) and its target receptor (protein or enzyme) (Anderson, 2003, Schneider, 2010). Computational methods are reliable methods that provide accurate results, cost-effective and time-saving technique for drug design and drug discovery process(Walters et al., 1998, Waszkowycz et al., 2001).

Recently, there are many articles targeting the inhibition of this virus theoretically (Maria, 2017, Radwan, 2018, Chafekar, 2018 and Kim 2018) however, to the best of our knowledge there is no theoretical of experimental study studying the inhibition activity of these compounds against corona virus.
In this study twenty eight bioactive imidazole compounds with known biological activity are studied theoretically to investigate the possibility of using these compounds against corona virus.

1. MATERIALS AND METHODS

A database of twenty eight bioactive imidazole compounds was built. The 2D and 3D structures of these imidazole derivatives were extracted from online databases, such as, PubChem, chemspider, drugbank websites. The structures were optimized to get the best 3D structure using Density Functional methods (DFT). The X-ray structure of SARS coronavirus 3CLpro was used as our initial protein model for docking as in Figure 1. Docking simulation of the fully optimized compounds was achieved using different computational methods. The protein structure was prepared using the Material Studio program. All water molecules were deleted from the protein structure before docking. The dimensions of the grid box are 60 x 60 x 60. The standard precision of Genetic Algorithm scoring functions was used to rank the binding pose and the limit of 100 conformations was used for each inhibitor.

2. RESULTS AND DISCUSSION

Twenty eight bioactive imidazole derivatives, some of them are pharmaceutical drugs and some are still under testing were extracted from literature. 2D structures for the bioactive imidazole compounds were downloaded from online databases such as, Chemspider, PubChem, and Zinc. The 2D structures of the studied bioactive imidazole derivatives are shown in Figure 2. The chemical formula, chemical names and the biological activity are listed in Table 1.

All the selected bioactive compounds have at least one imidazole ring however they have a variety of substituted groups. For example, in compound **1** the imidazole ring is connected with unsubstituted aromatic rings. Compounds **2**, **4**, and **6** have a substituted aromatic ring with chloride. Compounds **9**, **10**, **17** and **26** have no aromatic ring while compounds 10 and 25 have a nitro group. Compounds **9** and **21** have acidic properties with a carboxylic group while compounds **13** and **15** have a sulfur atom. In addition compounds **18**, **21** and **22** have a fluoride while others have amide or hydrophobic properties.

The protein crystal structure of the corona virus (SARS-CoV) was downloaded from protein data bank and the structure is viewed using discovery studio program. As shown in Figure 1, the secondary structure of the protein is composed mainly of beta sheets and helixes. The active site

of the protein is found by the same program discovery studio in which the inhibitor is located as shown in Figure 1. The active site of the protein is consist of the following amino acids; Asn142, Gly143, Leu141, Ser144, Cys145, Met49, Gln189, Met165, Arg188, Gln192 and Phe140.

In order to predict the best conformation of the studied compounds, rotation around the single bonds was allowed. Before docking, the crystalized ligand was re-docked at the active site of the protein of corona virus and the new conformation and binding site were compared. The same procedure was followed for the rest of the compounds. For each compound, 100 conformations were tested and the calculated binding free energies were ranked accordingly. Table 2, is showing the list of studied inhibitors (ligands) and the binding energies of the best three conformations. As shown in Table 2, the binding energies (E_{bind}) or docking free energy, of the best three conformations were listed and the repetition number for each confirmation was added between brackets. Generally, all the studied imidazole inhibitors have shown a good ability to bind to the active site of the protein where the binding energy between -4.57 and -9.64 kcal/mol.

In this study, we are going to focus on the best three inhibitors with the best docking score, namely, the inhibitors, **5**, **15** and **22** as highlighted in Table 2. The common characters between these inhibitors are aromatic ring, relatively big size (branched) and one or more of the following heteroatoms, O or F or N or Cl.

At the active site normally, there are different interactions between the ligand and the protein such as hydrogen bonds, pi-pi interactions, electrostatic interactions, hydrophobic and hydrophilic interactions. The more interactions with the active site the more binding forces made with the protein, the more stable complex formed between the protein and the inhibitor and the more inhibition ability. In addition, interactions made between the inhibitor and the active site depends on the substituted groups of the inhibitor, the characters of the amino acids at the active site and the size and conformation of the inhibitor.

Ligand **5**, is connected with the active site by two types of interactions, hydrogen bonds and pi-pi interaction. These interactions are due to the groups, OH and aromatic ring of the ligand. The calculated binding free energy is -9.16 kcal mol. Hydrogen bonds are made with Arg188, Leu141 and Ser144 with distances, 3.28, 2.97 and 3.25 Å, respectively, as shown in Figures 2 and 3. The hydrophobic group ($-(CH_2)_3-CH_3$) of the inhibitor seems to be not important in binding with the active site of the protein. In a similar manner, ligand **15** has an aromatic ring with Cl, O and S heteroatoms. The binding free energy is -9.64 kcal/mol. The most important interactions with the active site are the hydrogen bond formed with the amino acids Ser144 and Leu141 with a distances of 3.15 and 3.05 Å respectively and pi-pi interaction with the aromatic ring.

Ligand **22** has made a hydrogen bond interaction with the amino acids Ser144 and Leu141 with a distances of 3.09 and 3.20 Å respectively. The other interaction is pi-pi interaction in which two aromatic rings in parallel conformation is made between the aromatic ring of the ligand and the amino acid His41 at the active site of the protein of corona virus.

As shown from the previous results, the highest binding free energy or inhibition activity is due to the interaction with the amino acids at the active site. Hydrogen bonds and pi-pi interactions are the dominant interactions with the protein. For that we may conclude that the inhibition activity or the high binding free energy for those active compounds is due to the formations of strong interactions with the active site of the protein.

3. CONCLUSIONS

Twenty eight bioactive imidazole compounds with pharmaceutical application were docked against corona virus. Compounds **5**, **15** and **22** were found to be the best inhibitors against corona virus among the studied compounds. Interactions of the docked compounds were visualized and discussed. Hydrogen bonds and pi-pi interactions were the dominant interactions with the amino acids of the protein active site. The results of this study may help to develop novel drugs against corona virus.

Acknowledgements

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Conflict of Interest

There is no conflict of interest

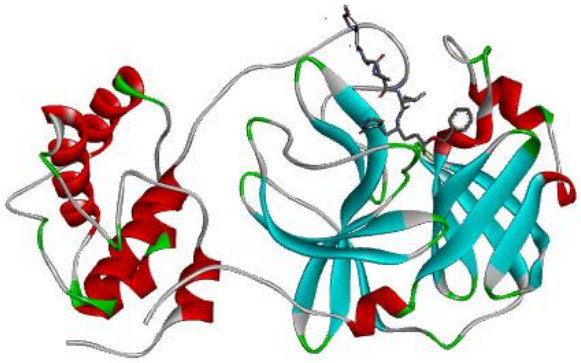


Figure (1) Crystal structure of SARS-CoV in complex with inhibitor

Table (2) The best three conformations and the values of binding energy in kcal/mol

Ligand	E_{Bind1}	E_{Bind2}	E_{Bind3}
1	**-8.57 (50)**	**-8.40 (15)**	**-8.05 (33)**
2	**-6.71 (48)**	**-6.65 (45)**	**-6.65 (6)**
3	**-8.12 (38)**	**-8.10 (9)**	**-7.80 (9)**
4	**-8.67 (3)**	**-8.00 (22)**	**-7.88 (31)**
5	**-9.16 (1)**	**-8.95 (5)**	**-8.81 (5)**
6	**-8.98 (4)**	**-8.93 (19)**	**-8.46 (14)**
7	**-8.12 (12)**	**-5.46 (20)**	**-4.59 (15)**
8	**-8.16 (12)**	**-7.99 (5)**	**-7.74 (20)**
9	**-4.66 (70)**	**-4.16 (11)**	**-3.91 (2)**
10	**-4.57 (44)**	**-4.35 (9)**	**-4.26 (3)**
11	**-6.08 (27)**	**-5.95 (24)**	**-5.93 (5)**
12	**-6.76 (27)**	**-5.99 (10)**	**-5.99 (2)**
13	**-8.55 (7)**	**-8.38 (17)**	**-8.09 (14)**
14	**-8.24 (19)**	**-8.16 (6)**	**-8.06 (51)**
15	**-9.64 (14)**	**-8.88 (33)**	**-8.72 (6)**
16	**-6.19 (18)**	**-6.18 (53)**	**-5.92 (28)**
17	**-7.46 (49)**	**-7.13 (19)**	**-6.89 (10)**
18	**-6.88 (31)**	**-6.06 (3)**	**-5.97 (20)**
19	**-8.59 (3)**	**-8.18 (5)**	**-8.15 (13)**
20	**-7.37 (6)**	**-7.12 (4)**	**-6.96 (6)**
21	**-5.40 (18)**	**-5.16 (11)**	**-5.01 (10)**
22	**-9.08 (78)**	**-8.95 (22)**	**-----**
23	**-6.08 (25)**	**-5.95 (19)**	**-5.89 (2)**
24	**-7.64 (8)**	**-7.64 (11)**	**-7.06 (18)**
25	**-6.67 (4)**	**-5.51 (9)**	**-5.48 (4)**
26	**-4.96 (3)**	**-4.86 (7)**	**-4.50 (9)**
27	**-5.52 (12)**	**-5.18 (9)**	**-4.95 (17)**
28	**-7.56 (32)**	**-7.26 (14)**	**-7.07 (6)**

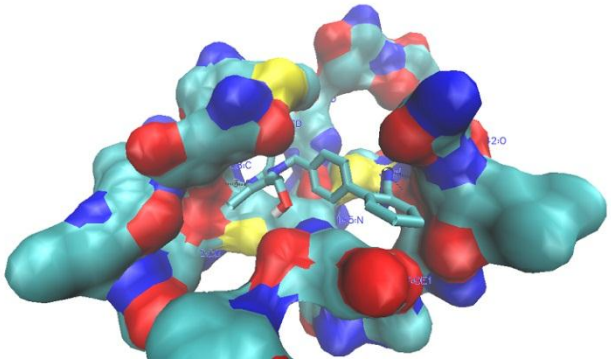


Figure (3) Ligand **5** at the active site of the protein.

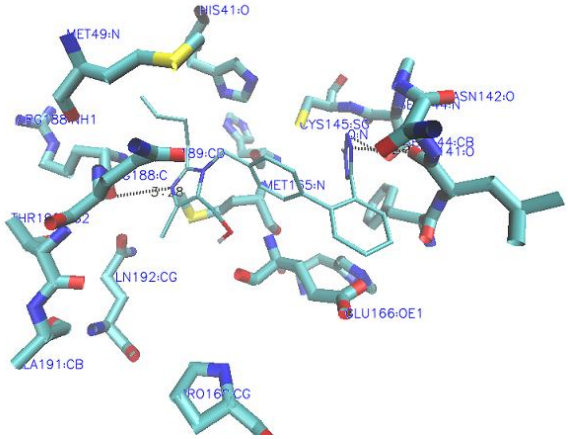


Figure (4) Ligand **5** at the active site of the protein making HB and pi-pi interactions.

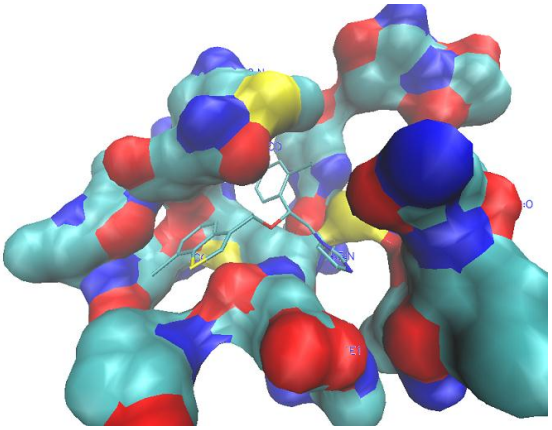


Figure (5) Ligand **15** at the active site of the protein

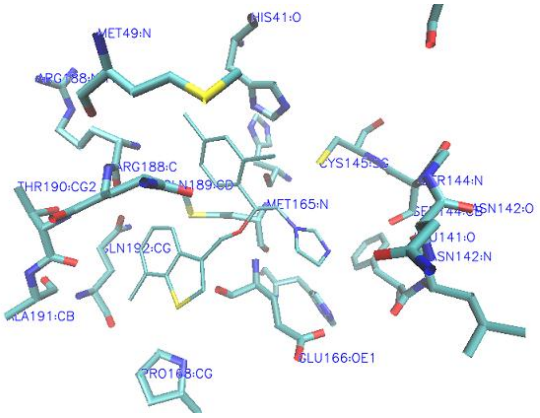


Figure (6) Interactions of ligand **15** at the active site of the protein.

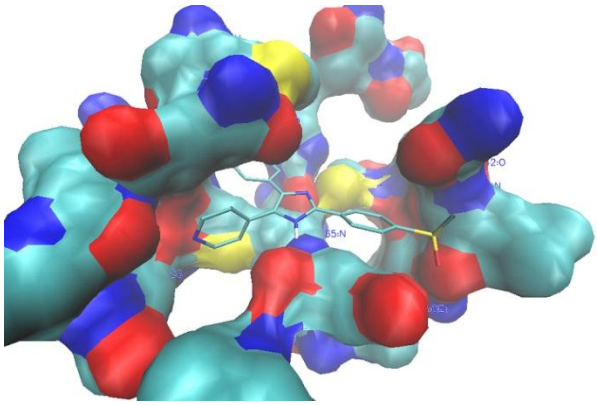


Figure (7) Ligand **22** at the active site of the protein.

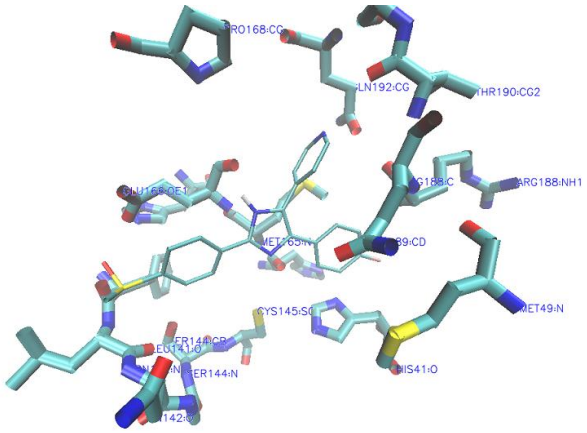
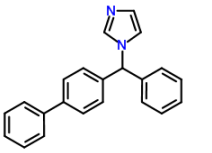


Figure (8) Interactions of ligand **22** at the active site of the protein.



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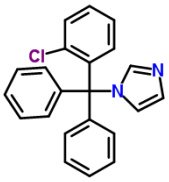

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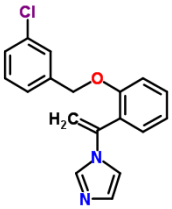

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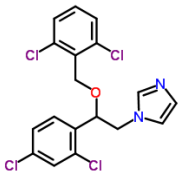

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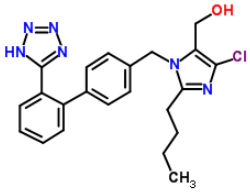

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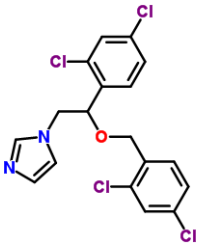

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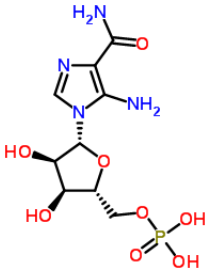

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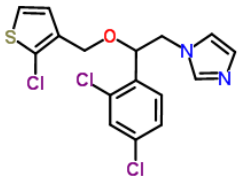

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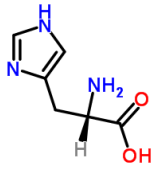

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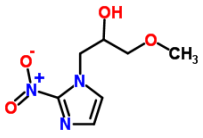

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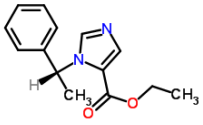

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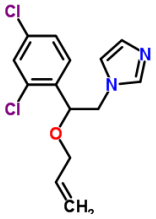

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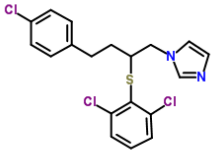

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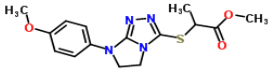

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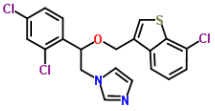

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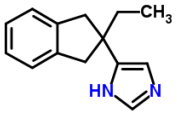

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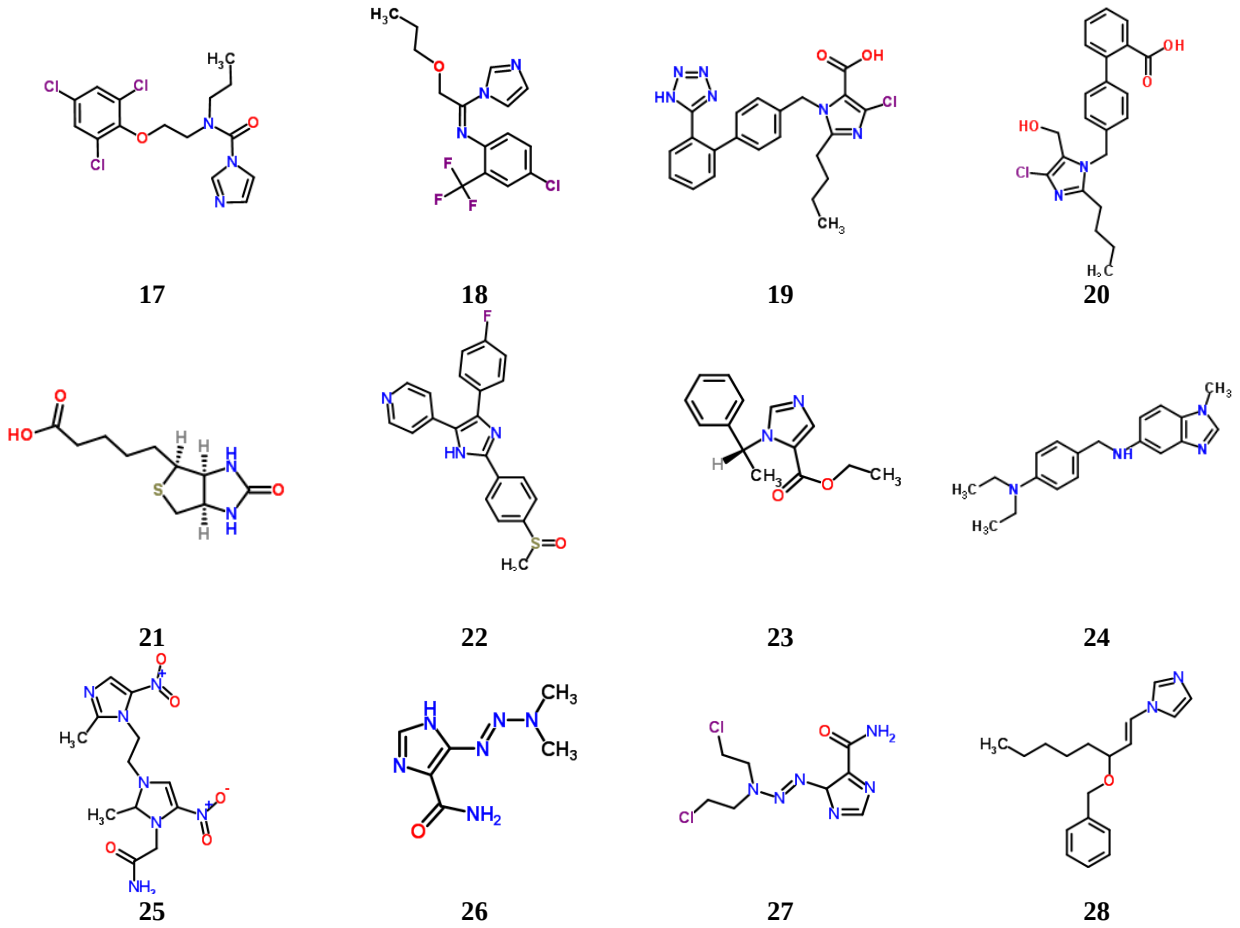

Figure (2) Structures of the bioactive imidazole compounds

Table (1) Molecular formula, chemical name and the biological activity of the studied bioactive imidazole compounds

Lig.	Molecular Formula	Chemical Names	biological activity
1	$C_{22}H_{18}N_2$	Bifonazole**,** Trifonazole	Inhibiting the production of ergosterol
2	$C_{22}H_{17}ClN_2$	Clotrimazole, Lotrimin, Mycosporin	Antimycotic activity, inhibits biosynthesis of the sterol ergostol.
3	$C_{18}H_{15}ClN_2O$	Croconazole, Croconazolum	no specific biological action
4	$C_{18}H_{14}Cl_4N_2O$	Isoconazole, Travogen, Fazol	Antifungal drug that has similar to clotrimazole in the treatment of foot and vaginal infections.
5	$C_{22}H_{23}ClN_6O$	Losartan, Lortaan, Cozaar, Hyzaar	Antagonist of angiotensin type 1 receptor with antihypertensive activity
6	$C_{18}H_{14}Cl_4N_2O$	Miconazole, Monistat, Daktarin IV, Minostate	An imidazole antifungal agent that is used topically and by intravenous infusion.
7	$C_9H_{15}N_4O_8P$	Amino imidazole carboxamide ribonucleotide,	Nucleotide transport and metabolism
8	$C_{16}H_{13}Cl_3N_2OS$	Tioconazole, Trosyd	Antifungal medication
9	$C_6H_9N_3O_2$	Histidine, Glyoxaline-5-alanine	L-histidine is an essential amino acid that is required for the production of Histamine.
10	$C_7H_{11}N_3O_4$	Misonidazole, Misonidazolum	Nitroimidazole that sensitizes normally radio-resistant hypoxic cells.
11	$C_{14}H_{16}N_2O_2$	Etomidate, Amidate, Radenarcon	Anesthetic and hypnotic with little effect on blood gases, ventilation.
12	$C_{14}H_{14}Cl_2N_2O$	Imazalil Chloramizol, Deccozil, Fungaflor	Brown solidified oil, non-corrosive, fungicide.
13	$C_{19}H_{17}Cl_3N_2S$	Butoconazole, Femstat	Imidazole antifungal used in gynecology.
14	$C_{15}H_{18}N_4O_3S$	Methyl 2-{[7-(4-methoxyphenyl)-6,7-dihydro-5H-imidazo[2,1-c][1,2,4]triazol-3-	no specific biological action

		yl]sulfanyl}propanoate	
15	$C_{20}H_{15}Cl_3N_2OS$	Sertaconazole, Sertaconazolum	Antifungal medication to treat skin infections
16	$C_{14}H_{16}N_2$	Atipamezole, Antisedan, 4-(2-ethyl-2,3-dihydro-1h-inden-2-yl)-1h-imidazole	no specific biological action
17	$C_{15}H_{16}Cl_3N_3O_2$	Prochloraz, Mirage, Octave	no specific biological action
18	$C_{15}H_{15}ClF_3N_3O$	Triflumizole, Procure	no specific biological action
19	$C_{22}H_{21}ClN_6O_2$	Losartan carboxylic acid	no specific biological action
20	$C_{22}H_{23}ClN_2O_3$	4'-{[2-Butyl-4-chloro-5-(hydroxymethyl)-1H-imidazol-1-yl]methyl}-2-biphenylcarboxylic acid	In vivo inhibitory activity against angiotensin II
21	$C_{10}H_{16}N_2O_3S$	Biotin, D-biotin, Vitamin H, Coenzyme R, Vitamin B7	Enzyme co-factor present in minute amounts in liver, kidney, pancreas, yeast, and milk.
22	$C_{21}H_{16}FN_3OS$	4-(4-(4-fluorophenyl)-2-(4-(methylsulfinyl)phenyl)-1H-imidazol-5-yl)pyridine	no specific biological action
23	$C_{14}H_{16}N_2O_2$	Etomidate, (R)-Ethyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate	Anesthetic and hypnotic with little effect on blood gases or the cardiovascular system.
24	$C_{19}H_{24}N_4$	N-[4-(Diethylamino)benzyl]-1-methyl-1H-benzimidazol-5-amine	no specific biological action
25	$C_{12}H_{17}N_7O_5$	2-{2-Methyl-3-[2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethyl]-5-nitro-2,3-dihydro-1H-imidazol-1-yl}acetamide	no specific biological action
26	$C_6H_{10}N_6O$	Dacarbazine, Imidazole carboxamide	Antineoplastic agent has significant activity against melanomas.
27	$C_8H_{12}Cl_2N_6O$	4-[(1E)-3,3-Bis(2-chloroethyl)-1-triazen-1-yl]-4H-imidazole-5-carboxamide	no specific biological action
28	$C_{18}H_{24}N_2O$	Midazogrel, Midazogrelum, (E)-1-(3-Benzyloxyocten-1-yl)imidazole	no specific biological action

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