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NOVEL ANTI-FUNGAL AGENTS AS CLASSICAL ISOSTERES OF MICONAZOLE AND ECONAZOLE

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ABSTRACT

Fungal infections can be invasive and life threatening. Considering their substantial causation of morbidity and even mortality, the fact that only a limited number of therapeutic agents are available must be remedied. This study presents the consideration of five classical isosteres each of miconazole and econazole. Formation of classical isosteres of drugs is long established and successful approach to enhance the phamaceutical efficacy and increase the population of successful medicaments. Isosteres presented here utilize $-CH_3$, $-NH_2$, -OH, -Br, or -SH, to substitute the halogen atom (-Cl) on both miconazole and econazole. The molecular properties for all isosteres are presented and compared to the parent drugs miconazole and econazole. Analysis by non-metric multidimensional scaling clearly showed the distinction of the isosteres when compared to the parent drugs miconazole and econazole, by molecular properties. Neighbor joining cluster analysis indicated that the -SH isostere is most similar to the parent drug, for both miconazole and econazole. Both miconazole and econazole showed one violation of the Rule of 5, this violation being the Log P value for both. Interestingly, at least three isosteres for both parent drugs showed zero violations of the Rule of 5, indicating improved absorption and permeation compared to the parent drugs.

KEYWORDS: econazole, miconazole, anti-fungal, isosteres.

INTRODUCTION

Antifungal agents have gained in substantial importance in modern medicine.[1] Many life-threatening fungal infections afflict individuals with weakened immune systems.[1] Strains of common pathological fungi *Candida albicans* and *Candida glabrata* are developing resistance to utilized drugs, in addtion, species of infectious molds for which thtere are no suitable clinical treatment are appearing.[1] Invasive fungal infections are a considerable cause of morbidity and mortality, and can be life-threatening.[1] Clinically important fungal infections are viewed in two types: 1) Mycoses of skin, skin structures, as well as mucosa; 2) Invasive fungal infection, involving sterile areas of the physiology including central nervous system, bloodstream, liver, lung, kidneys, and other organs.[1] Presently, there are limited options for the clinical treatment of fungal infections and these drugs fall into only three classes of drugs based on molecular structures, which include: 1) Polyenes; 2) Azoles; and 3) Echinocandins.[1]

Life-threatening fungal infections can result from endemic fungi, *Aspergillus, Cryptococcus, and Pneumocystis*.[2] Routes of administration for antifungal agents include: 1) Ocular; 2) Intrathecal; 3) Vaginal; 4) Topical, 5) Oral; and 6) Intravenous.[3] Fungi resistance to therapeutic drugs can occur through acquired antifungal resistance as a result to the preventive use and regular use of antifungal drugs.[4] However, various fungal species are observed to have natural or intrinsic resistance to antifungal drugs.[4] The capacity of the fungi to cause harm is related as to whether it is an opportunistic infection (pathology occurring in individuals having a weakened immune system) or a primary infection (pathology occurring in an otherwise healthy individual).[5] Additional work and research is necessary for development of antifungal drugs, diagnostic tools, and vaccines.[6] Presently, causes of death caused by invasive fungi is surprisingly high and exceeds (or equal to) deaths incurred by malaria and drug resistant tuberculosis.[6] This study presents novel antifungal agents as classical isosteres of the proven drugs of miconazole and econazole. This study will demonstrate the properties of classical isosteres to be potentially new therapeutics for the clinical treatment of fungal infections.

MATERIALS AND METHODS

Assembly and visualization of all molecular structures, for analysis, examination, presentation, and various properties was accomplished utilizing ChemSketch 2024.2.0 C45E41 (www. acdlabs.com, copyright 1994-

2025). All statistical analysis, neighbor-joining cluster analysis, and non-metric multidimensional scaling was accomplished utilizing PAST version 2.15 (copyright Hammer and Harper 1999-2012, folk.uio.no/ohammer/past).[7]

Molecular properties presented for each isostere agent and parent compound were deterrmined utilizing Mcule online and freeware, by mcule.ocm/apps/property-calculator/ (Mcule, Inc. 535 Everett Ave #510 Palo Alto CA 94301 and Bartok Bela ut105-113 1115 Budapest Hungary).

RESULTS AND DISCUSSION

The use of isosteres has been shown to be amazingly useful and effective in modifying the molecular structure of pharmaceuticals, yet retaining and/or modifying their drug-like properties.[8] Classical isosteres are ions, atoms, or molecules having peripheral layes of electroms commonly considered to be highly similar to the original atom or group of atoms.[8] For this study, that applies classical isosteres, and focuses strictly on univalent atoms/groups, will encompass: $-CH_3$, $-NH_2$, -Cl, -SH, and -Br.[8] Non-classical bioisosteres produce similar biological activity but do not match the streric and electronic rules of classical isosteres.[8] In past studies, they are considered to be in either groups of classical isosteres (matching size, shape, outer electronic configuration) or non-classical isosteres (not matching number of atoms or steric and electronic rules of classical isosteres).[9] It is generally accepted that bioisosteres are different from isosteres.[10]

Non-metric multidimensional scaling (NMDS), is applied broadly in research for the examination of sets of numerical data, and provides a visual representation of the objects of the data in such a way as to enable identification of clusters and/or patterns.[11] NMDS reduces the dimensionality of the data, while preserving the rank order of their dissimilarities.[11] NMDS produces a plot where the most similar objects of the data are closer together (i.e. objects that are most dissimilar are further separated in the plot).[11] Neighbor-joining cluster analysis examines sets of data for the nearest neighbor amoung the data, pairing them, and is distance based.[12] Neighbor-joining cluster analysis is applied in a very wide range of scientific research, particularly bioinformatics and computational biology.[12]

Classical Isosteres of Miconazole

The mechanism of antifungal activity for miconazole is by inhibition of the fungal enzyme 14α-steroldemethylase, which results in a reduced production of ergosterol, a vital portion of the fungal cell membrane.[13] Miconazole can be applied externally for the treatment of ringworm, jock itch, and athlete's foot. Miconazole can be used internally for oral candidiasis and vaginally for vaginal thrush (yeast infection).[1,5,6] The application of classical isosteres (and bioisosteres), is to amend the ADME characteristics (absorption, distribution, metabolism, excretion) of a pharmaceutical, in order to maximize the efficacy of the drug and limit the effects of the initial metabolic response.

Presented in Fig. 1 are the molecular structures of miconazole and the classical isosteres, agents 1 to 5. The SMILES (Simplified Molecular Input Line Entry System) representation for each structure is presented.

Substituents of the aromatic rings of miconazole have been substituted; where -Cl is now $-CH_3$ (agent 1), $-NH_2$ (agent 2), -OH (agent 3), -Br (agent 4), and -SH (agent 5). Miconazole also has the imidazole ring covalently bonded in the structure. The substitution of ring substituents clearly incurs significant changes to various molecular properties that can effectuate the effectiveness for antifungal application. Alterations of properties of molecular weight, polar surface area, Log P, etc can be anticipated, whereas, the antifungal activity is expected to be retained to some extent due to the well established nature established for classical isosteres replacement.[6,8,9,10] Various approaches have been studied and verified to be effective in evaluating the drug-likeness of compounds, and one of the most effective is the Rule of 5.[14]

The Lipinski Rule of 5, states that poor absorption (permeability) occurs if more than one violation of the following guidelines is characteristic of a pharmacuetical: 1) The molecular weight is greater than 500 Daltons; 2) Greater than 5 hydrogen bond donors (nitrogen or oxygen atoms having hydrogen atoms attached); 3) Greater than 10 hydrogen bond acceptors (nitrogen or oxygen atoms that can accept hydrogen bonds); 4) A calculated Log P (also known as CLog P) greater than 5 (or MLog P greater than 4.15).[14]

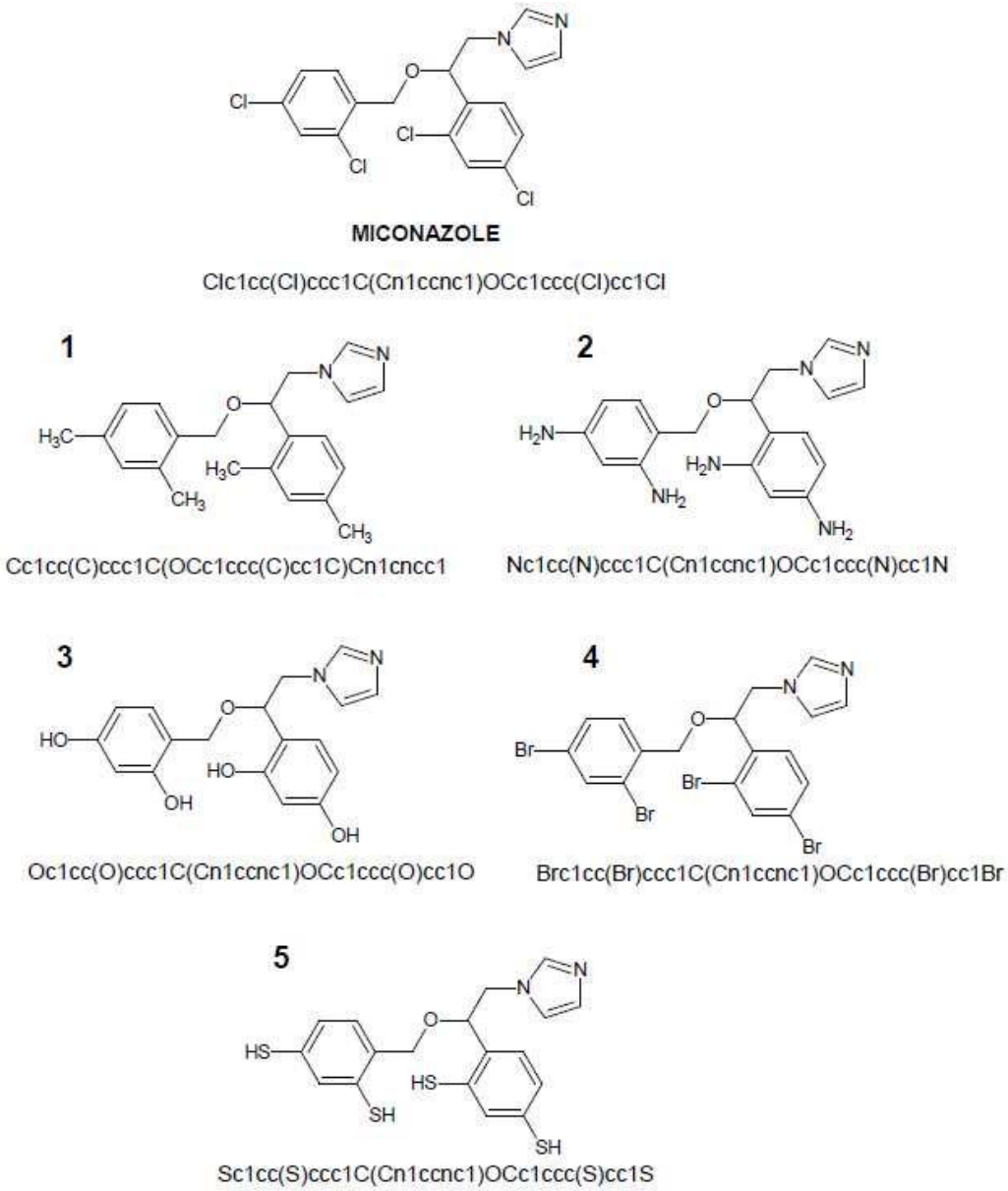

Figure 1: Molecular structures of miconazole and the classical isosteres agents 1 to 5. Note the substitution of the -Cl atoms on miconazole with classical isosteres -CH_3, -NH_2, -OH, -Br, and -SH.

Important molecular properties of miconazole and agents 1 to 5 are shown in Table 1. Notably, the molecular properties are significantly altered by just the use of classical isosteres substitution (see Fig. 1). Rotatable bonds are single bonds that can freely rotate around their axis, determining the molecule's conformational flexibility. The number of rotatable bonds in a drug molecule can effect the ADME properties of the drug. The number of rotatable bonds for miconazole and all agents 1 to 5, remains constant at six rotatable bonds.

The polar surface area (PSA) of drugs is important and a useful property to predict ADME characteristics of a drug, and is utilized early in the pathway of drug discovery.[14] PSA is the surface associated with heteroatoms (i.e. oxygen, nitrogen, and phosphorous atoms) along with polar hydrogen atoms.[8,9,10] PSA value of less than 140 Ångstroms² is often accepted as a good indicator of favorable oral bioavailability, and a PSA of less than 60 Ångstroms ² is desired for blood-brain-barrier (BBB) penetration.[14] Note that for miconazole, as well as agents 1, 2, 3, and 4; their PSA values are less than 140 Angstroms^2, and therefore will show good oral bioavailability.[14] In addition, for miconazole and agents 1 and 4, the PSA values will favor penetration of BBB. Only agent 4 has two violations of the Rule of 5, indicating all other agents will have good permeation (drug absorption). These findings are all favorable for the drug-likenss of this group of agents and their potential usage in clinical application.

A negative value for Log P indicates a higher affinity for the aqueous phase (more hydrophilic). In the case of Log P equal to zero, the compound is equally partitioned

between a lipid and aqueous phases; but a positive value for Log P indicates higher concentration in lipid phase and the drug is more lipophilic.[8,9,10] Miconazole, agent 1 and agent 4 has Log P values greater than 5, however, only in the case of agent 4 does this cause more than one violation of the Rule of 5 and hence a possible issue in drug-likeness. Overall, Log P ranges are 2.66 (agent 3) to 6.89 (agent 4).

Table 1: Molecular Properties of Miconazole and Classical Isosteres.

Agent	Molecular Mass (g/mole)	Log P	H-bond Acceptors	H-bond Donors	Rotatable Bonds	Polar Surface Area (Angstroms ^{2})	Violations of Rule of 5	Number of Atoms
miconazole	416.12	6.45	3	0	6	27.05	1	39
Agent 1	334.45	5.07	3	0	6	27.05	1	51
Agent 2	338.41	4.49	7	4	6	131.13	0	47
Agent 3	342.35	2.66	7	4	6	107.97	0	43
Agent 4	593.93	6.89	3	0	6	27.05	2	39
Agent 5	406.62	5.00	3	0	6	182.25	0	43

Non-metric multidimensional scaling, or NMDS, is a multivariate technique allowing a visualization a data set of complex relationship, into smaller dimensions making multivariate data relationships easier for interpretation.[15] NMDS intends to represent the pairwise dissimilarity between objects originally in a larger data space, into a lower-dimensional space.[15] NMDS is an analysis for which the rank order of dissimilarities is reproduced in a reduced number of dimensions, making possible the visualization of the level of similarity.[15]

Presented in Fig. 2 are results of NMDS analysis of molecular properties for miconazole and isosteres shown in Table 1. Spatial arangement of objects (drugs) shows relative similarity based on molecular properties, plotted Coordinate 1 versus Coordinate 2, utilizing Euclidean distance (measuring the straightest and shortest path between two points). From the 2-dimensional plot, it is ascertained that agents 2 and 3 are most similar to each other and distinct from miconazole and agent 5. Miconazole antifungal drug is less similar to agent 5, based on molecular properties, but more similar to agent 4 and agent 1.

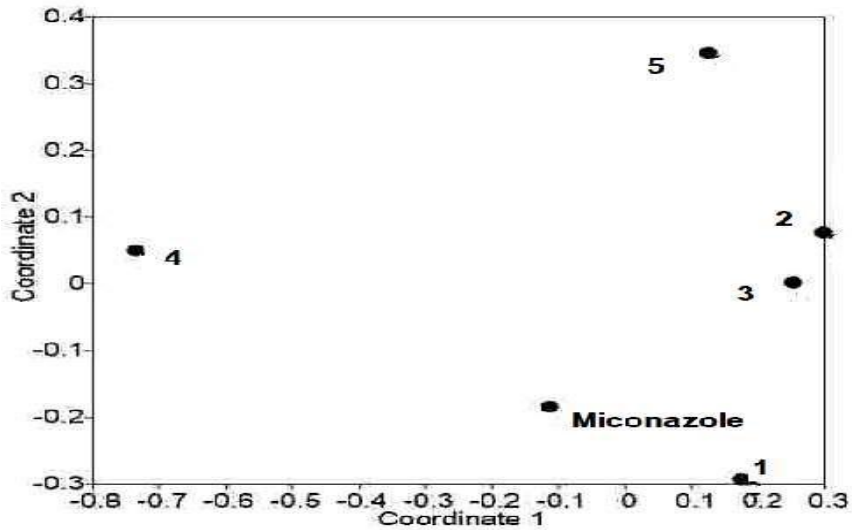

Figure 2: Results of non-metric multidimensional scaling of the molecular properties (see Table 1) for miconazole and it's classical isosteres is presented here. This analysis preserves the qualitative distances between objects (drugs) and demonstrates which have greater similarity by way of their proximity of agents to one another. Here, agents 2 and 3 appear to be most similar to each other, based on molecular properties (see Table 1). Overall, agents 1, 2, 3, and 4 appear most similar to the parent compound miconazole. However, the results can also be interpreted as showing the the overall distinctiveness of the isosteric substitutions as compared to the parent drug.

Neigbour-joining clustering is a specific method for hierarchical cluster analysis.[16] The method works by iteratively combining the most closely related pairs of objects based on their distance matrix and is found in a wide range of scientific research.[16] Shown in Fig. 3 are outcome for neighbor-joining cluster anlaysis for molecular properties presented in Table 1. Likewise, as for NMDS, these results show agents 2 and 3 to be most

similar to each other by way of their proximity in the neighbor-joining space. Also, it is seen that agents 1 and 4 are in proximity to miconazole and based on molecular properties, are anticipated to be most similar in activity (as are influenced by molecular properties).

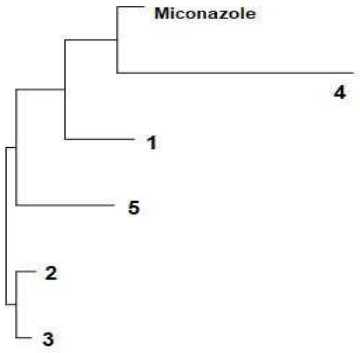

Figure 3: For neighbor-joining cluster analysis, the results from analysis of molecular properties (see Table 1), show agents 2 and 3 to have highest similarity to one another. Agents 4 and 1 have highest similarity to miconazole. Agent 5 is distinct from agents 2 and 3, as well as miconazole.

These types of analysis of multivariate data sets representing molecular properties of pharmaceuticals, allow the visualization of underlying relationships among the agents, and that accomplished respective of their molecular properties. Distinguishing similarity and dissimilarity of the agents in respect to their determined molecular properties is very important for identifying drug-likeness, which in turn can enable the recognition of compounds that may show highest level of the desirable activity relative to the successful parent drug.

Classical Isosteres of Econazole

The molecular structure of econazole is presented in Fig. 4, in addition to classical isosteres agents 1, 2, 3, 4, and agent 5. Substitutions of the halogen atoms (-Cl) located on the aromatic rings of econazole are accomplished for agent 1 (-CH_3), agent 2 (-NH_2), agent 3 (-OH), agent 4 (-Br), and agent 5 (-SH). Econazole also has the imidazole ring, which is not substituted. The SMILES designation is indicated with molecular structure for all compounds.

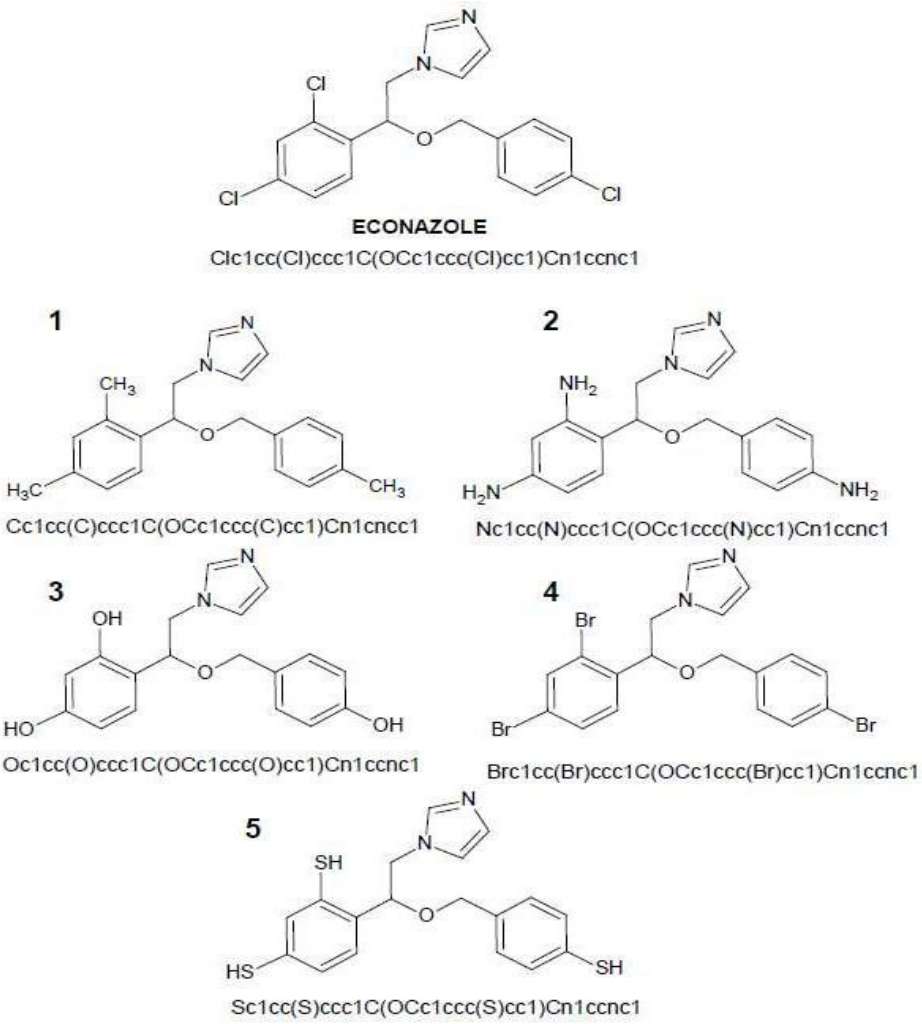

Figure 4: Molecular structures of econazole and with the classical isosteres substitutions for agents 1 to 5. Note the substitution of the -Cl atoms on econazole with classical isosteres -CH_3, -NH_2, -OH, -Br, and - SH.

The substitution of classical isosteres upon the structure of ecanozole, will result in noticeable alterations in the molecular properties that are indicative of drug-likeness. Molecular properties for econazole and agents 1 to 5 are shown in Table 2. Clearly notable, is the alterations in Log P values, where econazole with Log P of 5.80, will vary with classical isosteres substitution to range of 2.96 (agent 3) to 6.13 (agent 4). Likewise, a substantial alteration of PSA occurs, to begin with 27.05 Angstroms^2 for econazole, but ranging from 27.05 Angstroms^2 to 143.45 Angstroms^2 (agent 5). The number of rotatable bonds in a drug molecule can effect the ADME properties of the drug. The number of rotatable bonds remains constant for Econazole and agents 1 to 5, all have 6 rotatable bonds.

Econazole shows only one violation of the Rule of 5 (still favorable as drug-likeness), and only agent 4 showing two violations of the Rule of 5 and potentially issues in absorption.[14] However, agents 1, 2, 3, and 5 shown zero violations of the Rule of 5 and consequently, very favorable drug-likenss and favorable absorption/permeation.[14] These outcomes strongly support the drug-likeness potential of classical isosteres substitution upon the econazole molecular frame.

In addtion, with agent 5 as the only exception, agents 1, 2, 3, 4, and econazole have PSA values less than 140 Angstorms^2, which indicate these compounds will have favorable oral bioavailability.[14] PSA of less than 60 Ångstroms ^{2} is desired for blood-brain-barrier (BBB) penetration.[14] PSA for econazole, agent 1, and agent 4 have PSA values less than 60 Angstroms^2, values favorable for BBB penetration.

Table 2: Molecular Properties of Econazole and Classical Isosteres.

Agent	Molecular Mass (g/mole)	Log P	H-bond Acceptors	H-bond Donors	Rotatable Bonds	Polar Surface Area (Angstroms ^{2})	Violations of Rule of 5	Number of Atoms
econazole	381.68	5.80	3	0	6	27.05	1	39
Agent 1	320.43	4.77	3	0	6	27.05	0	48
Agent 2	32.39	4.33	6	3	6	105.11	0	45
Agent 3	326.35	2.96	6	3	6	87.74	0	42
Agent 4	515.04	6.13	3	0	6	27.05	2	39
Agent 5	374.55	4.70	3	0	6	143.45	0	42

NMDS intends to represent, the pairwise dissimilarity between objects in a larger data space into a low-dimensional space.[15] NMDS is an analysis for which the rank order of dissimilarities is reproduced in a reduced number of dimensions, making possible the visualization of the level of similarity.[15] Presented in Fig. 5 are results of NMDS analysis of molecular properties for econazole and isosteres, shown in Table 2.

Spatial arangement of objects (drugs) shows relative similarity based on their molecular properties, plotted as Coordinate 1 versus Coordinate 2, utilizing Euclidean distance (measuring the straightest and shortest path between two points). From the 2-dimensional plot, it is ascertained that agents 2 and 3 are most similar to each other and distinct from econazole and agent 5. Econazole antifungal drug is less similar to agent 5, based on molecular properties, but more similar to agent 4 and agent 1.

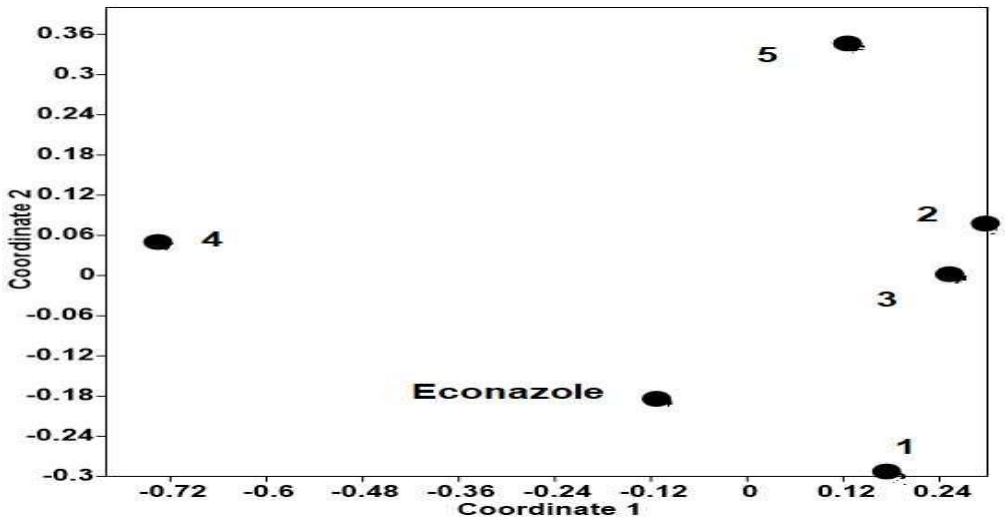

Figure 5: Non-metric multidimensional scaling of econazole and classical isosteres utilizing molecular properties presented in Table 2. This analysis preserves the qualitative distances between objects (drugs) and demonstrates

which have greater similarity by way of their proximity of agents to one another. Here, agents 2 and 3 appear to be most similar to each other, based on molecular properties (see Table 2).

Overall, agents 1, 2, 3, and 4 appear most similar to the parent compound econazole. However, the results can also be interpreted as showing the the overall distinctiveness of the isosteric substitutions as compared to the parent drug.

Neigbour joining clustering is a specific method for hierarchical cluster analysis.[16] The method works by iteratively combining the most closely related pairs of objects based on their distances determined from a data matrix (i.e. multivariate data table) and is found in a wide range of scientific research.[16] Shown in Fig. 6 are outcome for neighbor-joining cluster anlaysis for molecular properties presented in Table 2. Likewise, as for NMDS, these results show agents 2 and 3 to be most similar to each other by way of their proximity in the neighbor-joining space. Also, it is seen that agents 1 and 4 are in proximity to econazole and based on molecular properties, are anticipated to be most similar in activity (as influenced by molecular properties).

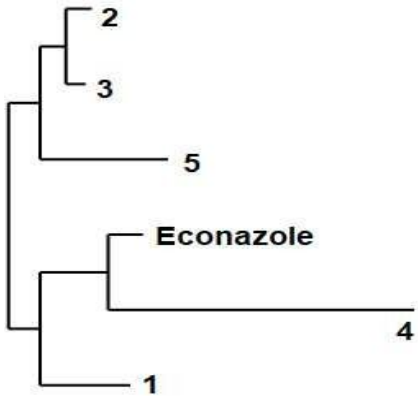

Figure 6: For neighbor-joining cluster analysis, the results from analysis of molecular properties (see Table 2), shows agents 2 and 3 to have highest similarity to one another. Agents 4 and 1 have highest similarity to econazole. Agent 5 is distinct from econazole and agents 1 and 4.

These approaches to analyze multivariate data sets representing molecular properties of pharmaceuticals, allow the visualization of underlying relationships among the agents and in respect to their molecular properties.

Distinguishing similarity and dissimilarity of the these agents, in respect to their determined molecular properties, provides important recognition of drug-likeness In addtion, it can enable the recognition of compounds that may show highest level of the desirable activity relative to the successful parent drug.

CONCLUSION

Fungal infections are a considerable medical threat and can be life threatening. Pathological fungi are gaining in drug resistance, so the study and development of novel antifungal agents is a vital goal in order to maintain an efficacious selection of clinical antifungals. This study has presented a total of 10 potential antifungal agents thar are classical isosteres of miconazole or econazole. The molecular properties for each set of classical isosteres were determined, compared to their parent compound, and analyzed for favorable dug-likeness and underlying similarities. Miconazole and its classical isostere agents 1, 2, 3, and 5, showed favorable drug-likeness by having 1 or fewer violations of the Rule of 5. Econazole and its classical isostere agents 1, 2, 3, and 5, showed favorable drug-likeness by having 1 or fewer violations of the Rule of 5. Miconazole and its classical isosteres 1, 2, 3, and 4 have polar surface area less than 140 Angstoms^2, which is another strong indicator of favorable drug-likenss. Econazole and its classical isosteres 1, 2, 3, and 4 have polar surface area less than 140 Angstoms^2, which is a strong indicator of favorable drug-likenss. Non-metric multidimensional scaling and neighbor-joining cluster analysis showed underlying similarities of these novel antifungal agents to their parent compound and each other. Further studies are needed to identify and develop new antifungal pharmaceuticals.

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REFERENCES

1. Roemer T, Krysan D. (Antifungal drug development: challenges unmet clinical needs, and new approaches). Cold Spring Harb Perspect Med, 2014; 4: 1-14.
2. Li Z, Lu G, Meng G. (Pathogenic fungal infection in the lung). Front Immunol, 2019; 3(10): 1524.
3. Fisher MC, Alastruey-Izquierdo A, Berman J, Bicanic T, Bichell EM, Bowyer P, et al. (Tackling the emerging threat of antifungal resistance to human health). Nature Reviews Microbiology, 2022; 20(9): 557-571.
4. Hossain CM, Ryan LK, Choudhuri S, Ali KA, Diamon G. (Antifungals and drug resistance). Encyclopedia, 2022; 2(4): 1722-1737.
5. McKeny PT, Nessel TA, Zito PM. Antifungal Antibiotics. NCBI Bookshelf National institutes of Health; Stat Pearls Publishing: 2025.
6. Calderone R, Sun N, Gay-Andrieu F, Groutas W, Weerawarna P, Prasad S, Alex D, Li D. (Antifungal drug discovery: the process and outcomes). Future Microbiol, 2014; 9(6): 791-805.
7. Hammer O, Harper DAT, Ryan PD. (PAST: paleontological statistics software package for education and data analysis). Palaentologia

Electronica, 2001; 4(1): 1-9.
8. Silverman R. The Organic Chemistry of Drug Design and Drug Action. San Diego; Academic Press, 1992.
9. King F. Medicinal Chemistry: Principles and Practice. Cambridge; The Royal Society of Chemistry, 2001.
10. Patrick G. An Introduction to Medicinal Chemistry. 2nd ed., New York; Oxford University Press, 2001.
11. Everitt BS, Dunn G. Applied Multivariate Data Analysis. New York; Wiley, 2001.
12. Bryant D. (On the uniqueness of the selction criterion in neighbor-joining). J Classif, 2005; 22: 3-15.
13. Becher R, Wirsel SG. (Fungal cytochrome P450 sterol 14α-demethylase (CYkP51) and azole resistance in plant and human pathgens). Applied Microbiology and Biotechnology, 2012; 95(4): 825-840.
14. Lipinski CA, Lombardo F, Dominyy BW, Feeney PJ. (Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings). Adv Drug Deliv Rev, 2001; 46(1-3): 3-26.
15. Lecture 8: Multidimensional scaling Advanced Applied Multivariate Analysis, Sungkyu Jung Department of Statistics University of Pittsburgh, http: //www.stat.pitt.edu/sungkyu/AAMA/.
16. Saitou N, Nei M. (The neighbor-joining method: a new method for reconstructing phylogenetic trees). Molecular Biology and Evolution, 1987; 4: 406-425.