Molecules that inhibit fungal growth in Mycetoma

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Context

The most prominent agent in the fungal form of Mycetoma in vitro and vivo is *Madurella mycetomatis*. An open research group, MycetOS, has found that fenarimol compounds are so far the most potent compounds against *M. mycetomatis* in vitro cell-based assays.[1] MycetOS has already produced a large library of screened fenarimol compounds against *M. mycetomatis*, with a small portion of said compounds showing potency in vivo. This research seeks to expand this library by proposing chemical structures that should be further investigated and identifying which structures are inactive. This was done by using a similarity descriptor to analyse the dataset in DataWarrior in order to identify what chemical properties of fenarimol compounds showed the most effectiveness in inhibiting fungal growth. The possible drug targets to inhibit fungal growth were enzymes squalene cyclooxygenase and 14α-demethylase (CYP51).

This research's purpose is to use DataWarrior identify some novel compounds that will aid in the treatment of Mycetoma as well as identifying some compounds that are inactive towards *M. mycetomatis.* and hence do not need further investigation.

Methods

Firstly, calculations were done to compare the similarity between molecules using the PathFP descriptor based on the length/functionality of their carbon backbone. Following this, structural activity landscape index (SALI) plots were generated along with an activity cliff plot which allowed us to examine the structural similarity and biological activity of the molecules (measured in %), based on the concentrations of either 25 μM or 100 μM. Lastly, analysis of the data was then undertaken in order to confirm which functional groups produced the highest/lowest biological activity results from the given data. On inspection of the plots, the coordination of the colours indicated that the compounds which appeared bright green were the most active, whilst those that were dark blue were the most inactive.However, during this analysis, a large focus was placed on examining the data produced in the activity index, whilst the activity cliffs were more used as a secondary source.

Results

Below are examples of some common motifs, and a sampling of four molecules that contain them:

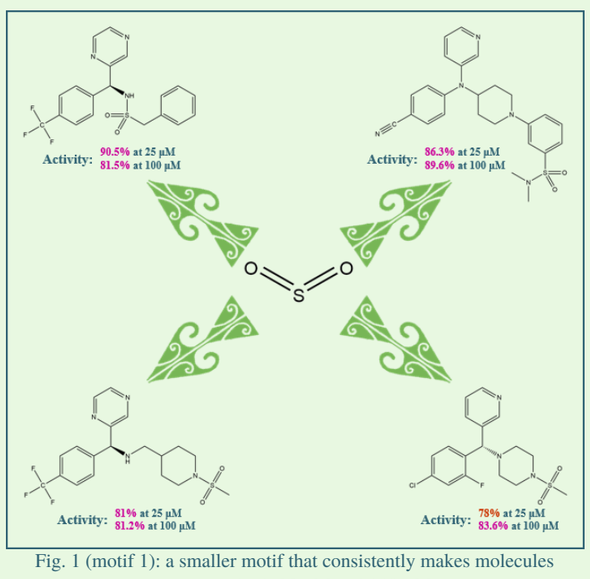


Fig. 1 (motif 1): a smaller motif that consistently makes molecules inactive at both 25 μM and 100 μM.

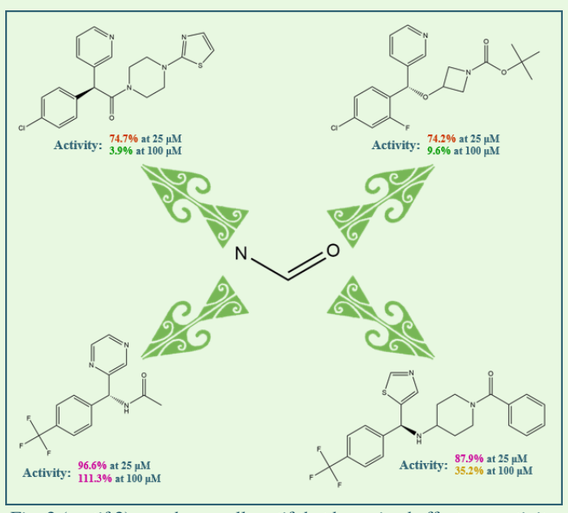


Fig. 2 (motif 2): another small motif that has mixed effects on activity at 100 μM, but is generally inactive at 25 μM.

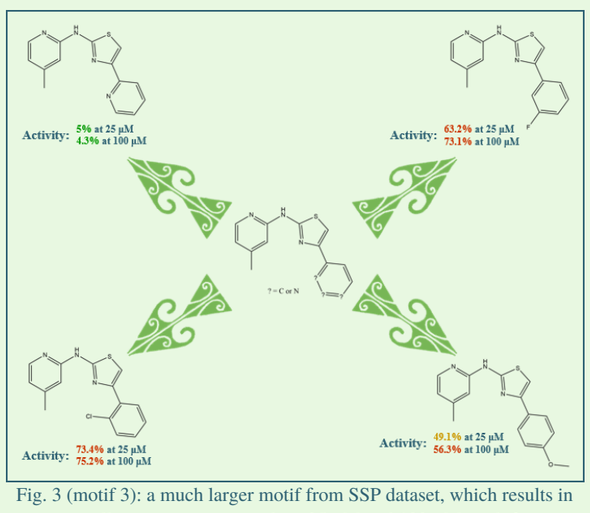


Fig. 3 (motif 3): a much larger motif from SSP dataset, which results in generally inactive compounds at 25 μM and 100 μM, with few exceptions.

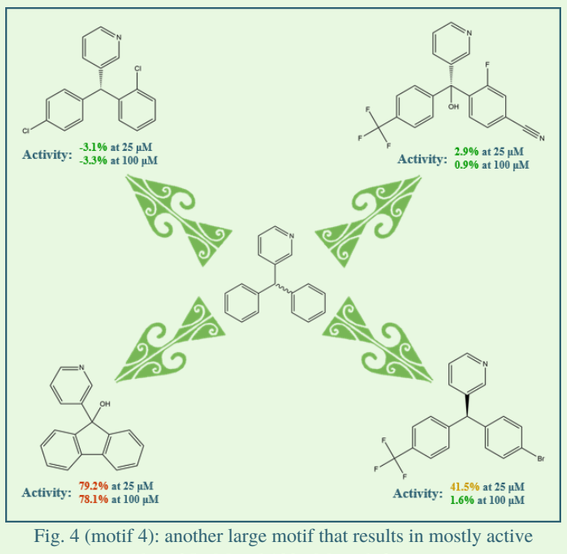


Fig. 4 (motif 4): another large motif that results in mostly active compounds at 25 μM and 100 μM, with few exceptions.

Discussion

The sulfone motif tends to make any novel compound become inactivated. This motif is active because the M. mycetomatis species tend to have resistance towards them. This resistance is implemented with the progressive incubation of their granules consisting of similar hyphae component motifs.[2] Molecules shown on figure 2 are unique chemical structures in the MycetomaOS data that consists of the sulfone motifs. Thus, chemical structures that consist of these sulfone motifs should be discontinued and not used in the drug discovery process.

In the MycetomaOS data, it seems that the amide motif (carbon atom double-bonded to an oxygen and an amine) can cause novel drug compounds to become inactivated or activated, which can cause variability in the data. This is because it could require an addition of an oxygen group onto the amide carbon position, which can assist in making novel compounds active, whereas adding a nitrogen group can make the novel compounds inactive. More research is require to determine the significance of the addition of these two different groups onto the amide carbon.

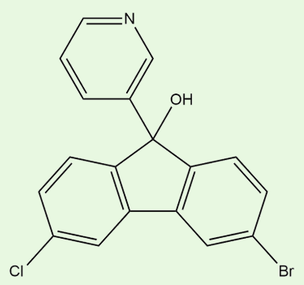
Another reason of variability in molecules with amide can be due to the amide motif being positioned incorrectly in the novel compound that tends to be inactive. Figure 2 shows that the amide motif tends to be positioned at one end of the molecules, which makes them inactive, whereas amide motifs placed between two large groups can result in the novel compound becoming active. It is possible to make these novel compounds more active if the amide motif was placed in the middle, as seen in an in-vitro cell-based test with the novel drug niclosamide having one of the lowest minimum inhibitory concentrations (MIC) when interacting with M. mycetomatis species.[3] Thus, the two molecules active at 100 μM can be retested in screening assays with the amide motif in the middle of the novel compounds. However, if there is still the presence of variability in the results, these amide motifs should be discontinued in the drug discovery process.

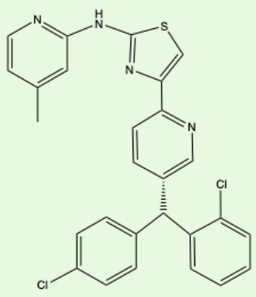
It seems that the 2-aminothaliomide and pyridine motifs in figure 3 and the phenyl and pyridine motifs in figure 4 tend to make novel compounds more active to inhibit fungal growth. These novel compounds usually target the enzymes squalene cyclooxygenase (SE) and 14α-demethylase (CYP51), which can inhibit ergosterol biosynthesis in M. mycetomatis species. The molecules that are active in figure 4 can use the phenyl group to interact with the SE enzyme in the proline307 and methionine333 amino acid residues, or the pyridine motif to interact with the heme group in the active site of CYP51; the one active molecule in figure 3 can also use its pyridine motif to interact with CYP51.[4] A compound that consists of a 2-aminothaliomide motif showed that it could inhibit Candida albicans fungal growth with a lower minimum inhibitory concentration, compared to the antifungal medication fluconazole.[5]

Figure 4 showed that a specific molecule with low activity on novel compounds bonds between two six-membered rings. This molecule can be a potential outlier in the DataWarrior dataset, but there is unknown information explaining this outlier's rationales. Thus, more future work is required to understand this.[6]

Future Works

Through the research, an outlier was discovered in molecules containing motif 4 that could be worth investigating. This can be investigated by adding various substituents to the motif and thus monitoring the change in activity. Therefore, the molecule that could be used in novel compounds that are needed for drug development testing is illustrated on the right hand side.





The research showed that the pyridine motifs, 2-aminothaliomide motifs and the various phenyl groups motifs appeared to make the molecules more active. By combing active motifs from figure 3 and 4, this can potentially make the new molecule more active than the parent molecules. Therefore, the molecule that can be used in novel compounds to be taken further for in-vivo assay experiments is illustrated on the left hand side.

References:

1. Lim, W.; Eadie, K.; Konings, M.; van de Sande, W. MycetOS – an open research model discover new drugs to treat one of the most neglected disease – mycetoma. International Journal for Infectious Diseases. [Online] 2020, 101 (1), 388. Science Direct. https://www.ijidonline.com/article/S1201-9712(20)31735-5/fulltext (accessed November 13, 2021).

2. Sahu, A., A.; Patri, B.; Agarwal, S.; Satpathy, K., P.; Mohanty, R. Eumycetoma - A rare case report in present era. International Journal of Health Sciences and Research. [Online] 2016, 6 (7), 432-434. https://www.ijhsr.org/IJHSR_Vol.6_Issue.7_July2016/69.pdf?fbclid=IwAR0qSLQXsdS9dm9BjLJzrweRE44U_npj-oyQlo7uoOk81E9mSkfTGjm9OzE (accessed November 13, 2021).

3. Mahmoud, A., B.; Abd Algaffar, S.; van de Sande, W.; Khalid, S.; Kaiser, M.; Mäser, P. Niclosamide is active in vitro against mycetoma pathogens. Molecules. [Online] 2021, 26, 4005-4011. https://doi.org/10.3390/molecules26134005 (accessed November 13, 2021).

4. Sun, B.; Dong, Y.; Lei, K.; Wang, J.; Zhao, L.; Liu, M. Design, synthesis and biological evaluation of amide-pyridine derivatives as novel dual-target (SE, CYP51) antifungal inhibitors. Bioorganic and Medicinal Chemistry. [Online] 2019, 27 (12), 2427-2437. https://doi-org.ezproxy.auckland.ac.nz/10.1016/j.bmc.2019.02.009 (accessed November 14, 2021).

5. Logu, A.; Saddi, M., Cardia, M. C.; Borgna, R.; Sanna, C.; Saddi, B.; Maccioni, E. In-vitro activiy of 2-cyclohexyildenhydrazo-4-phenyl-thiazole compared with those of amphotericin B and fluconazole against clinical isolates of Candida spp and fluconazole resistant Candida albicans. Journal of Antimicrobial Chemotherapy. [Online] 2005, 55 (5), 692-698. https://doi.org/10.1093/jac/dki084 (accessed November 14, 2021).

6. Carmer, J.; Sager, C. P.; Ernst, B. Hydroxyl groups in synthetic and natural-product-derived therapeutics: A perspective on a common functional group. Journal of Medicinal Chemistry. [Online] 2019, 62 (20), 8015-8930. https://doi.org/10.1021/acs.jmedchem.9b00179 (accessed November 14, 2021).