Small molecule based anti-virulence approaches against *Candida albicans* infections

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Abstract

Fungi are considered “silent killers” due to the difficulty of, and delays in diagnosis of infections and lack of effective antifungals. This challenge is compounded by the fact that being eukaryotes, fungi share several similarities with human cellular targets, creating obstacles to drug discovery. *Candida albicans* , a ubiquitous microbe in the human body is well known for its role as an opportunistic pathogen in immunosuppressed people. Significantly, *C. albicans* is resistant to all the three classes of antifungals that are currently clinically available. Over the past few years, a paradigm shift has been recommended in the management of *C. albicans* infections, wherein anti-virulence strategies are considered an alternative to discovery of new antimycotics. Small molecules, with a molecular weight less than 900 Daltons, can easily permeate the cell membrane and modulate the signal transduction pathways to elicit desired virulence inhibitory actions against pathogens. This review dissects in depth, the discoveries that have been made with small molecule anti-virulence approaches to tackle *C. albicans* infections.

30 **Introduction**

31 In recent years, tackling nosocomial and opportunistic infections has gained marked attention in
32 the scientific community, due to the continued emergence of multi-drug resistant pathogens[1,2].
33 Prominently, it has been estimated by the World Health Organization that approximately 10
34 million people will die due to antibiotic resistance by 2050[3]. Hence, a synergistic approach
35 involving several specialties, including microbiology, biotechnology, pharmacology, medicinal
36 chemistry, bioinformatics, and materials science has been taken to discover innovative
37 problems to this solution. While most such research focuses on bacterial infections, a kingdom
38 that is often less addressed, and yet notorious, are fungi.

39 The yeast *Candida albicans* exists as a commensal in the human microbiome in several
40 anatomical sites such as the oral cavity, gastrointestinal tract, skin, and genital organs[4,5]. The
41 unique phenotypic plasticity of *C. albicans* facilitates its survival in a variety of environmental
42 conditions with changes in pH, temperature, and oxygen levels[6]. Furthermore, the indiscriminate
43 use of antibiotics results in the depletion of commensal bacteria from these niches, allowing the
44 proliferation of yeasts. Immunosuppressed patients, including those with HIV, diabetes, those
45 on chronic steroid therapy, cancer chemotherapy, infants in the neonatal ICU, elderly, and
46 hospitalized patients are all at high risk of developing opportunistic fungal infections, which may
47 range from superficial (mucocutaneous) to life-threatening invasive fungemia[7]. Over 500 million
48 people in the world suffer from candidiasis[8] with an increasing mortality rate of 5 to 71 %[9].

49 *Candida* species use several strategies for their existence in the host as a normal commensal,
50 but when the equilibrium tilts in favor of the yeasts[6], they express several virulence factors
51 including a morphological switch from yeast to fungi[7,10], adhesion, invasion, secretion of
52 hydrolytic enzymes, thigmotropism, phenotypic switching, and biofilm formation[11–13]. There are
53 currently four classes of clinically used antifungal drugs for treating the *C. albicans* infections
54 such as polyenes, azoles, echinocandins and pyrimidine analogues[14,15]. Polyenes bind to cell
55 membrane ergosterol and cause cell lysis[16]. Due to increased toxicity, their clinical use is
56 limited. Azoles act by inhibiting the mechanism of ergosterol biosynthetic pathway, but
57 increased emergence of resistance, particularly the development of point mutations in the target
58 site of azoles or by the expression of efflux pumps in the *C. albicans* hampers its use in treating
59 fungal infections[17,18]. Echinocandins, the most recent of the antifungals, acts by inhibiting 1,3 -
60 beta glucans synthase[19] and help in cell wall biosynthesis inhibition, but mutation in the glucan
61 synthase gene *FKS1* has become a significant problem[20]. Pyrimidine analogue, 5-flucytosine is

62 coverted to 5-flurouracil by cytosine deaminase *FCY1,* which then inhibits the cellular division of
63 *C. albicans* upon incorporation of the toxic fluorinated pyrimidine analogue into DNA and RNA.
64 Emergence of resistance due to the overexpression of efflux pumps and mutation in the
65 cytosine deaminase *FCY1* gene has hindered its application[21] (Figure 1).

66 *Candida* species that are resistant to every clinical class of antifungal available today is a reality
67 and will continue to be a threat and a major healthcare burden, unless investments are made in
68 antifungal drug discovery[21,22]. However, as stated above antifungal drug discovery is
69 challenging since fungi share many common metabolic pathways with the host [14,23]. In some
70 pathogenic species such as *Candida*, the mechanisms that drive the development of antifungal
71 resistance are intimately linked to the same mechanisms that regulate growth and survival.
72 Therefore, anti-virulence strategies, where the key virulence aspects of *Candida* are inhibited,
73 without affecting growth/survival is considered an agreeable alternative to discover new
74 antimycotics[24].

75 **What are small molecules?**

76 With a molecular weight less than 900 Daltons, some molecules can easily permeate the cell
77 membrane and modulate the signal transduction pathways. These properties make small
78 molecules an ideal candidate for the development of drugs that control virulence in pathogenic
79 species[25]. They can potentially target virulence mechanisms of fungi, which harbors interesting
80 therapeutic properties. Moreover, they go hand in hand with the current therapeutic course of
81 antifungal drug development[26] and can also be seen as a remedy for the issues caused by the
82 existing antifungals.

83 In this review, we will review the progress made in the past decade on the discovery of small
84 molecule inhibitors against *C. albicans.* Over this decade a wide range of drug discovery
85 approaches have been used by the researchers for the anti-virulence therapy. In particular the
86 approaches for small molecule discovery include the i) use of existing drugs/pharmacologically
87 active compounds (drug repurposing), ii) screening of large library, chemically diverse
88 compounds (high throughput screening), iii) compounds derived from other organisms (e.g.,
89 plant/animal/microbe derived), iv) compounds with known targets i.e., compounds influencing a
90 specific phenotype of candida (e.g., Secreted aspartyl protease inhibitors, quorum sensing
91 inhibitors). The small molecules discussed here are categorized based on the approaches that
92 are used to discover/develop them for their anti-virulence effects.

93 **Old is gold: Repurposing drugs:**

94 Drug discovery is a tedious and inefficient process, considering the need for identification of
95 novel targets, safety issues, and the substantial associated costs. Therefore, drugs that have
96 been approved for other applications in humans (and therefore have a proven safety profile) can
97 be a quick route to the discovery of new antimicrobials. Drug repurposing has been developed
98 as an expedited approach for developing new therapies in many areas of the clinical research.
99 This type of the search i.e., identifying the drug for repurposing against a new disease happens
100 using either drug based phenotypic screening approach or using the high throughput library of
101 FDA approved drugs. Drug based phenotypic screening identifies successful molecules by
102 serendipitous or clinical observations without knowing about the specific biological targets[27].

103 It has been estimated that 10-16 years are required for the development of a drug using the
104 traditional approach while drug repurposing takes only 3-12 years, significantly reducing the
105 time and costs. Also, since the pharmacokinetic (PK), pharmacodynamic (PD), and toxicity
106 profile of drugs are already available, drug repurposing reduces the risk of failure of compounds
107 in clinical development making repurposing an ideal route for bench-to-bedside translation of
108 antifungal drugs. The schematic illustration (Figure 2) highlights the advantages of pursuing
109 drug repurposing over traditional drug discovery methods.

110 Quinacrine (QNC), an anti-malarial drug was found to be effective against the growth of
111 *Saccharomyces cereviseae* and *Cryptococcus neoformans*. Following this, Kulkarny *et al.,* 2014
112 explored the effect of QNC on *C. albicans* biofilms and filamentation. Interestingly, QNC
113 inhibited *C. albicans* biofilms at 256 µg/ml and prevented yeast-to-hyphal transition even in
114 echinocandin-resistant clinical isolates in neutral and alkaline pH by causing defects in
115 endocytosis by accumulating in the vacuoles of *C. albicans*[28]. However, in acidic pH, no effects
116 were observed due to the protonation of QNC, which prevented vacuolar accumulation. QNC
117 also disrupted the mature *C. albicans* biofilms by synergizing with sublethal doses of
118 amphotericin B and caspofungin. Finasteride, a drug used for treating benign prostatic
119 hyperplasia was found to have anti-biofilm and anti-filamentation effects against *C. albicans*
120 without affecting its growth. However, finasteride is broken down to inactive metabolites prior to
121 excretion and thus, the oral administration of finasteride for the treatment of urinary candidiasis
122 has limited use. An alternative approach would be to deliver it as a topical formulation at the site
123 of infection [29]. Iron acquisition plays a major role in *C. albicans* pathogenesis and adaptation to
124 the different host niches. Deferasirox, an FDA approved iron chelator reduced the invasion of *C.*

125 *albicans* in oral epithelial cells and significantly reduced fungal burdens in a murine model of
126 oropharyngeal candidiasis. [30]

127 The acetylcholine receptor agonist pilocarpine hydrochloride (PHCL), an anti-glaucoma drug
128 modulates the uncharacterized cholinergic receptor of *C. albicans* causing anti-biofilm and anti-
129 filamentation effects[31]. PHCL also reduced the pathogenesis of *C. albicans* in a *Galleria*
130 *mellonella* infection model. Azoles have belonged to the antifungal armamentarium for the last
131 four to five decades and yet only in 2019, an azole compound aripiprazole, which is used to
132 treat schizophrenia, was identified to have an antibiofilm and the anti-filamentation effect against
133 *C. albicans.* Aripiprazole significantly inhibited 90% of *C. albicans* biofilms at 50 µg/ml and
134 abolished the hyphal formation at 100 µg/ml. However, in a high dose (250 µg/ml) they inhibited
135 the growth of the *C. albicans* possibly by acting as an enzyme inhibitor thus inhibiting the
136 lanosterol 14α-demethylase enzyme in the sterol biosynthetic process[32]. As noted, aripiprazole
137 has found to have concentration dependent effects and a similar mechanism to the other
138 existing azole compounds, for which several clinical strains of *Candida sp.,* were found to
139 demonstrate resistance[32].

140 Combinatorial therapy of FDA-approved non-antifungal drugs and antifungal drugs have also
141 been shown to be effective against *C. albicans* biofilms. For instance, sub-inhibitory
142 concentrations of the off-patent drugs drospirenone (contraceptive), perhexiline maleate
143 (antianginal agent), and toremifene citrate (anticancer agent) were found to be "enhancers" by
144 increasing the activity of antifungal drugs amphotericin B and caspofungin synergistically
145 (FICI<0.5) against *C. albicans* biofilms both *in vitro* and *in vivo* (*Caenorhabditis elegans*) without
146 affecting the growth[33]. A group of sulfa antibacterial compounds has reversed the azole-
147 resistant strains, likely by inhibiting the folate pathway and not by inhibiting the efflux pumps,
148 which also mediate the development of resistance in *C. albicans*[34]. Even though drug
149 repurposing involves low risks, costs and time compared to the *de novo* development, one of
150 the major limitations in developing them for anti-virulence therapy is appropriate dosing
151 regimens. The ultimate goal is attainable only if the effective concentration of the drug for the
152 new therapy falls in the same range as those of the original therapy. But if the efficacy of the
153 drug is seen at an increased dosage compared to that of recommended doses specified in the
154 original PK profile, adverse side effects or toxicities might occur[35]. Despite this, such a
155 compound might provide a new lead chemical scaffold for new drug development. Some of the

156 compounds that have been discovered using the lead optimization approach for the anti-
157 virulence therapy against *C. albicans* have been discussed here.

158 ***Lead optimization approach***

159 The emerging and the promising repurposing methods for the identification of novel anti-virulent
160 small molecules, uses FDA approved drugs as a starting point/a scaffold for the development of
161 the derivatives which will exhibit the drug-like properties and have known pharmacological
162 profiles. This approach is termed as selective optimization of side activities (SOSA)[36]. Montoya
163 MC *et al.,* used the SOSA approach for the development of the antimalarial drug, melfoquine
164 (MEF) and its novel entities as antifungals. Sublethal concentrations of the drugs inhibited the
165 filamentation of the *C. albicans*. MEF has a long half-life and maintains the high serum levels
166 when administered orally making it as an ideal candidate for the treatment of disseminated
167 candidiasis[37]. Haloperidol, an antipsychotic drug was found to have antifungal effects and has
168 been used a scaffold/lead compound for the development of the novel compounds using the
169 scaffold hopping approach[38]. Several benzocyclane derivatives were designed and optimised
170 using haloperidol as a lead compound and tested for the anti-virulence properties. The lead
171 derivative named B10 were found to inhibit the morphological transition and biofilm formation in
172 FLC-resistant isolates[39]. Such novel chemical scaffold will require further testing of the
173 pharmacokinetic and pharmacodynamic as well as safety profiles.

174 **High-throughput screening (HTS)**

175 As discussed earlier, the antifungal pipeline is sparse due to the limited number of selective
176 drug targets that can be explored against *C. albicans* infections due to its eukaryotic nature.
177 Thus, targeting the key virulence aspects of *C. albicans* i.e., biofilm formation and filamentation,
178 without affecting growth/survival would be an excellent alternative option[40,41]. There are several
179 small molecules with "drug-like properties" that have been identified through HTS to be
180 exploited as a therapeutic option against *C. albicans* infections[42]. In HTS, large scale compound
181 libraries can be screened rapidly in a cost-effective way, which leads to the identification of
182 novel hit/lead compounds and desirable drug-like properties targeting the anti-virulence
183 approach of *C. albicans* (Table 1).

184 *C. albicans* biofilm adhesion, development, and maturation have been illustrated in the figure 3.
185 Once the yeast/planktonic form adhere to a substrate (adherence), they activate the multiple
186 core regulators of the filamentation pathway and undergo morphological transition to elongated
187 hyphal form (initiation and development). Then, these hyphal form cells elongate and proliferate

188 further forming a mature biofilm (biofilm maturation) (Figure 3)[23]. The compounds that have
189 been screened using the high-throughput approach target either one of these stages or all three
190 stages and are discussed here.

191 To thwart the adhesion of *C. albicans* to abiotic surfaces such as polystyrene, over 30,000 small
192 molecules were screened from DIVERSet library, Chembridge database[43]. This led to the
193 identification of antifungal compound that was christened filastatin, which showed remarkable
194 ability to prevent or reverse the adhesion of *C. albicans* to polystyrene surfaces, silicone
195 elastomers and human lung cells by affecting the transcriptional activation of *HWP1* (Hyphal
196 wall protein) promoter which in turn plays a major role in initial hyphal formation[43]. Filastatin also
197 prevented the formation of hyphae by affecting multiple signaling pathways depending on the
198 type of hyphae-inducing media: spider medium (cAMP/PKA pathway), serum (Ras1 pathway),
199 and GlcNAc medium (cph1, Efg1 pathway) which has direct influence on hyphal morphology.
200 Moreover, it reduced pathogenesis in both nematode and a mouse model of vulvo-vaginal
201 candidiasis[43]. As an important translational extension of their study, Fazly et al., have also
202 shown that filastatin prevents the adhesion of *C. albicans* to several biomaterials such as dental
203 resins, silicone elastomers and bioactive glasses. Due to the above mentioned properties,
204 filastatin appears to be an ideal candidate for biofilm-inhibitory coatings on prosthesis and
205 medical devices[43,44].

206 Filamentation is considered a high-value target, as it is a major virulence factor in *C. albicans.*
207 The yeast-to-hyphal switch facilitates colonization, biofilm formation, invasion, and inflammation.
208 Pierce *et al*., screened over 20,000 small molecules from the NOVACore library (Chembridge)
209 with "stringent drug like properties" and identified that diazaspiro-decane structural analogs
210 (Table 1) influenced both *C. albicans* biofilm formation and filamentation, without affecting
211 growth. Notably, it reduced virulence in mouse models of oral candidiasis and hematogenously
212 disseminated candidiasis. They also identified that *C. albicans* reference strain (SC5314) and a
213 clinical isolates from HIV^+ individuals could not develop resistance to the selected "lead"
214 compound [45].

215 Similarly, Romo *et al*. screened 20,000 small molecules from the DIVERSet library, Chembridge
216 and discovered two biaryl-amide derivatives (Table 1) that were able to inhibit *C. albicans*
217 filamentation at a very low micromolar concentration. Notably, these compounds arrested
218 hyphal development in most of the hyphae inducing signaling pathways including Efg1
219 (GlcNac), cAMP-PKA (spider), Cph2 and Tec1 (Lee's media), pH induced (RPMI-1640), Hog1
220 (DTPA induced), hydroxyurea induced filamentation and also reduced virulence in mouse

221 models of candidiasis[40,46]. Interestingly these compounds exhibited anti-biofilm efficacy against
222 *C. albicans* in the proliferation and maturation phases but not in the adhesion phase. Global
223 transcriptomic studies on one of these lead molecules showed significant downregulation of all
224 the core filamentation network genes[47].

225 Lohse *et al.* screened over 30,000 small molecules from the Chembridge small molecule
226 diversity library and found that compound CB17 ((1-[2-(2-methylphenoxy)-3-pyridinyl]-*N*-(3-
227 pyridinylmethyl)methanamine) inhibited biofilm adhesion and disrupted the mature biofilms but
228 didn't inhibit the biofilm development[48]. This study clearly demonstrated the need for testing the
229 effect of the compounds on different stages of biofilm formation, avoiding the risk of overlooking
230 compounds based on only one assessment criteria that may eventually lead to the loss of
231 promising compounds [49].

232 ***Compounds screened via HTS have synergistic interaction with existing antifungals***

233 The failure of conventional antifungals to eliminate/prevent biofilm development and virulence,
234 the need of high dosage of these antifungals to eliminate infections, and the resulting toxicities
235 and drug resistance, make combinatorial therapy an attractive strategy to explore. Drug
236 combinations will rule out the "single-drug single-target" concept, which helps in reducing the
237 required drug dosage, reducing toxicity, and potentially increases the efficacy of treatment due
238 to multiple targets[50]. For instance, the antifungal compound clotrimazole does not reduce the
239 metabolic activity of *C. albicans* biofilms, but when combined with the library of compounds
240 (~120,000) screened from Molecular Libraries Small Molecule Repository (MLSMR), 14
241 compounds were found to have anti-biofilm effects without exerting cytotoxicity. But the
242 resistance development assay and the *in vivo* efficacy of the compound has not been studied.
243 Further studies on these clotrimazole potentiators needs to be validated for development into
244 topical application for candidiasis and vaginitis[51].

245 Vila *et al*. screened the Pathogen Box chemical library (Medicines for Malaria Venture [MMV],
246 Switzerland) to identify inhibitors of *C. albicans* biofilm formation and identified six compounds[52].
247 Interestingly, out of these six, three compounds were able to reduce the metabolic activity of
248 preformed biofilms. The compound that had more potent antibiofilm activity was 2-Methyl-3-[(4-
249 methyl-1- piperazinyl)(2-thienyl)methyl]-1Hindole (MMV688768) (Table 2). Although this
250 compound had less activity against planktonic cells, its activity against preformed biofilms was
251 found to be profound. Hence, the authors safely concluded that this compound targets a
252 process (or processes) that plays a preponderant role in the survival of *C. albicans* cells within a

253 biofilm compared to their planktonic counterparts, but the targeted processes need to be
254 explored further. Moreover, *in vitro* assays against preformed biofilms proved that the
255 combination of this compound with fluconazole resulted in 89% reduction in cell viability, which
256 was significantly greater than the either drug alone. Thus indicating synergy and confirming the
257 potential for a combinatory approach using MMV688768 and fluconazole in the clinical setting[52].

258 ***Mitochondrial and vacuole inhibitors screened via HTS***

259 The fungal mitochondria are an attractive target for anti-virulence therapy; however, several *in*
260 *vitro* and *in vivo* studies should be conducted showing little or no harmful effects against human
261 mitochondria. Garcia et al., screened about 678 small molecules that attenuated invasive hyphal
262 growth in *C. albicans,* by affecting various filamentation pathways. The lead compound
263 halogenated salicylanilide (HAS), tri-chloro-salicylanilide and its analog niclosamide, (Table 3)
264 an FDA- approved anti-helminthic drug was found to have anti-filamentation and anti-biofilm
265 effects. Using transcriptomic analyses, they concluded that this effect is possibly due to binding
266 of HAS to Mge1 (a mitochondrial import complex) [53].

267 Screening a total of 50,240 small molecules, one study identified inhibitors of yeast-to-hyphal
268 transition. Small molecule 21 (SM-21) the lead compound (Table 3), inhibited hyphal
269 morphogenesis and biofilm adhesion to denture surfaces. It also showed fungal specificity,
270 relatively low toxicity to human oral keratinocytes, reduced tongue lesions in oral and improved
271 the survival rate in systemic candidiasis mouse models[54]. Wong *et al*., unraveled the
272 mechanism of action for SM-21 using *C. albicans* haploid strains, showing that the compound
273 acts on mitochondria by inhibiting ATP synthesis machinery specifically on fungal cytochrome
274 and ATPases which affects the ATP production and increases the reactive oxygen species
275 (ROS) production leading to cell death. As it affects growth, *C. albicans* haploid strains
276 developed resistance to SM-21 and the resistant strains shown increased SAP activity[55]. The
277 use of the haploid strains helps in uncovering both mechanism of action and *C. albicans'*
278 resistance mechanism against the small molecule, making this haploid model ideal for drug
279 development.

280 On the other hand, Vacuole Disrupting chemical Agents (VDA) were identified from a chemical
281 library, to targeting a particular role/function in *C. albicans* that is important for virulence. *C.*
282 *albicans* vacuoles play a predominant role in yeast-to-hyphae transition and vacuole deficient *C.*
283 *albicans* mutants are unable to tolerate stresses induced by host cells[56]. Thus, *Candida*
284 vacuoles mediate its survival within the host cells and the morphological transition which

285 facilitates invasion. Tournu *et al.,* screened for VDAs from three libraries such as Prestwick
286 Phytochemical Library, Prestwick Chemical Library, GreenPharma Natural Products Library.
287 Significantly, a wide range of compounds such as azoles, ionophore, statin and non-steroidal
288 anti-inflammatory drugs (NSAIDs) specifically cyclooxygenase inhibitors (Table 3) were
289 identified to have this property of vacuole disruption. These molecules caused physiological
290 stress to the fungal cells and inhibited their hyphal formation, one of the main virulence
291 characteristics[57].

292 ***Repurposing drugs screened via HTS***

293 The advantages of drug repurposing have been described in the previous sections. The bis-
294 biguanide alexidine dihydrochloride (AXD) was found to have potent antifungal, antihyphal and
295 antibiofilm activities against all the fungal pathogens tested including *C. albicans* reference
296 strain SC5314 as well as azole-resistant clinical isolates. In combination with existing
297 antifungals, AXD reduced the MIC of fluconazole and amphotericin B, clinically used first-line
298 antifungal drugs, ironically considered expendable for biofilm treatment, thereby emphasizing
299 their utility as an antibiofilm combination drugs. Furthermore, AXD displayed low toxicity to
300 mammalian cells and eradicated biofilms from mice central venous catheters, highlighting its
301 potential as a pan-antifungal drug, a drug that is effective against several fungal strains and
302 pathogens[58].

303 Piperazine-type phenothiazine derivatives, trifluoperazine (TFP, dopamine receptor agonist) and
304 CGS 1266B (serotonin 1β receptor agonist) have been shown to affect *C. albicans* hyphal
305 morphogenesis and biofilm formation by affecting the proteins of the fluid-endocytosis pathway
306 and vesicular transport (Vacuolar ATPase) without affecting cell growth[59]. The compound TFP
307 and CGS1266B (Table 4) particularly affected Rcy1, Vps15 proteins they are essential for
308 membrane protein recycling by endocytosis and protein transport to vacuole respectively.

309 Sileset *et al.* screened a small molecule library consisting of 1,200 off-patent drugs of the
310 Prestwick Chemical Library and they identified auranofin (antirheumatic), benzbromarone
311 (vasodilatory), pyrvinium pamoate (antiparasitic effects) drugs having novel anti-biofilm effects
312 (Table 4). These 3 novel antibiofilm drugs inhibited and prevented the biofilms of *C. albicans* [60].
313 Similarly, Lohse *et al.* screened Pharmakon 1600 repurposing library found that, nisoldipine and
314 nimodipine (a calcium channel inhibitor), paroxetine hydrochloride (serotonin uptake inhibitor),
315 and dexlansoprazole (a proton pump inhibitor) had significantly affected biofilm adhesion,
316 development and disrupted mature biofilms of *C. albicans* without affect the planktonic form[61].

317 Another fascinating approach in HTS is the use of animal models for screening potential
318 antifungal drugs[62]. Specifically, the similarity in the virulence factors involved in the infection of
319 several fungal pathogens in the *C. elegans* model is similar to virulence factors during
320 mammalian infection, the use of *C. elegans* for screening anti-microbial small molecules has
321 been a widely explored approach. Although some compounds could be potentially missed due
322 to their nematocidal activity, we cannot ignore the fact that this is an exciting approach to
323 identify molecules that would affect, modulate or even enhance the immune responses in the
324 host[62]. Over 80,000 repurposing drugs were screened using these *C. elegans* animal model for
325 its antibacterial effects and among one of the hit compounds BAY11-7085, an anti-infective drug
326 was further investigated for its effects against *C. albicans* biofilms[63]. Notable, BAY11-7085 had
327 significant effect on all the phases of biofilms (adhesion, development, and maturation) and
328 increased the survival rate of *C. albicans* infected *C. elegans* model. But the mechanism of
329 action of the BAY11 7085 for its effect on both bacteria and the fungi needs to be elucidated
330 further[64].

331 It is important to apply adequate screening methods to small-molecule compound libraries
332 because appropriate selection procedures are the key to successful screening. The HTS
333 procedure is more advantageous for screening the molecules that are already available in the
334 market which are known to have pharmacological effect and desirable drug like properties.
335 Additionally, the main effect of these small molecules on human cells are already known and
336 described in the database of the manufacture. Therefore, it may be easier to translate these
337 compounds to the clinic as they are expected to have fewer side effects. Even though several
338 groups have identified anti-virulence compounds which affect multiple targets/pathways through
339 high-throughput screening in the past decade, none of them have yet been translated to clinical
340 application. The major factors for this failure are poorly validated drug targets which causes
341 undesirable interactions with the host system, not considering physiological condition in
342 preliminary screening, a limited follow up, lack of using appropriate animal models, and
343 unpredictable toxicities. Once the hits/leads are identified by primary screening, chemical
344 synthesis approaches can be used to develop several analogues which may have superior
345 effects than the original scaffold.

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349 **Plant – derived small molecules**

350 Plant-derived bioactive natural products are known to be source of several medicinal products
351 and many molecules have been applied in therapeutic applications[65,66]. They are considered as
352 a "gold mine" in drug development, especially antibiotics owing to their minimal toxic effects and
353 increased efficacy[67]. Many researchers have explored the anti-candida activity of plant-derived
354 bioactive molecules which have also been reviewed elsewhere[65,67,68]. One of the major
355 advantages of plant derived bioactive molecules is that they have enormous scaffold diversity
356 and structural complexity. This natural product reservoir is enriched with unexplored bioactive
357 molecules which gives the researchers the broader area of chemical scaffolds compared with
358 the small molecule libraries. Initially, the bioactive molecules are screened by performing
359 biological screening for the "crude extracts" to identify the bioactive "lead" extract, which then
360 fractionated using several bioactivity guided fractionation/mass-spectrometry techniques to
361 isolate the bioactive molecules. The bioactive molecules, based on their structure are classified
362 further into terpenoids, alkaloids and flavonoids (Table 5, Figure 4). These specifically exert
363 anti-virulence effects by interacting with the specific downstream regulatory pathways of
364 candida that control hyphal morphogenesis, biofilm development and maturation and are
365 discussed here in this section.

366 ***a) Terpenoids***

367 Hinokitol (β-thujaplicin) isolated from *Chamacyparis taiwanesis* has been reported to possess a
368 wide range of antifungal properties. It has been shown to inhibit biofilm formation of both
369 fluconazole-susceptible and resistant strains of *C. albicans* , by down-regulation of adhesive
370 genes (*HWP1, ALS3)* and transcriptional regulators of cAMP pathway *(CYR1, RAS1*)[69]. Other
371 terpenoids such as thymol isolated from *Thymus vulgaris*, carvacrol isolated *Origanum vulgare*
372 and eugenol isolated from *Eugenia caryophyllata* which were found to be similar at a structural
373 level due to the presence of functionalized groups such as hydroxyl, alkyl and methyl groups
374 were found to have lower activities when compared with hinokitol. But, all these terpenoids,
375 were considered to have similar mechanism of action for affecting the biofilm formation that is
376 by disrupting the regulation of cAMP-PKA pathway[69].

377 Nepodin, a derivative of naphthalene isolated from roots of *Rumex crispus* displays structural
378 similarity to the above-mentioned compounds. Nepodin was found to inhibit the biofilms of FLU-
379 resistant *C. albicans* in polystyrene, and silicone catheter substrate. It also affected the hyphal
380 morphogenesis, mainly due to the 5-fold downregulation of genes such as *ECE1, HWP1, and*

381 *UME6* determined by RNA-seq. But surprisingly apart from having structural similarity to the
382 terpenoids the mechanism was found to be different, i.e., none of the downstream regulators of
383 c-AMP PKA pathway was affected which authors concluded as the complex mechanistic
384 process of nepodin[70].

385 ***b) Alkaloids***

386 An alkaloid, tetrandrine, inhibits *C. albicans* biofilm formation and morphogenesis without
387 affecting fungal growth. *In vivo*, it prolonged the survival of *C. albicans* infected *Caenorhabditis*
388 *elegans* and normal hyphal growth was restored with the addition of cAMP. This indicates that
389 tetrandrine inhibits hyphal morphogenesis through the Ras1p-cAMP-PKA pathway[71]. Lycorine
390 hydrochloride, an alkaloid isolated from the herb *Lycoris radiata* has proven anti-cancer
391 properties. Notably, it also demonstrates anti-biofilm, anti-filamentation, and anti-adhesive
392 properties against *C. albicans* interestingly by inhibiting the hyphal formation in varying media
393 conditions such as spider medium, GlcNAC medium, and in RPMI 1640[72]. Piperine, , was
394 shown to inhibit *C. albicans* biofilm formation, hyphal morphogenesis and disturbs mature
395 biofilms with no effect on *C. albicans* growth and metabolism. It was also found to inhibit the *in*
396 *vivo C. albicans* colonization and prolonged the survival rate of *C. albicans*-
397 infected *Caenorhabditis elegans* by affecting the various biofilm associated genes such as
398 *HWP1* and *ALS3*, drug resistance related genes such *CDR2* and *CDR4*. Furthermore, it showed
399 no toxicity to human buccal epithelial cells and diminished potential for drug resistance
400 development. With all these desirable properties, piperine can be considered a distinct anti-
401 virulence candidate for management of candida infections[73].

402 ***c) Flavonoids***

403 Quercetin, a flavonoid has been shown to inhibit biofilm development and filamentation of *C.*
404 *albicans*, in addition to increasing its susceptibility to FLC in FLC resistant clinical strains
405 isolated from Vulvovaginal Candidiasis patients. This synergism enhanced the property of both
406 drugs even in resistant strains and in murine models by preventing hyphae formation and
407 reduced fungal burdens in the vagina[74]. In another study, it was shown that Quercetin acts as a
408 sensitizer by increasing the susceptibility of FLC-to-FLC resistant isolates by increasing the
409 production of farnesol. Farnesol, a quorum sensing molecule at high concentrations inhibited
410 hyphal development and biofilm density of *C. albicans*. Quercetin-mediated suppression of
411 biofilm formation is reversed in the farnesol defective *ΔCzF1* mutant strain of *C. albicans*,
412 indicating that Quercetin acts by increasing the production of farnesol thus suppressing the

various virulence factors[75]. The effects of the farnesol on modulating the *C. albicans* virulence is discussed in later sections.

Catechins extracted from *Camella sinensis* (tea leaves) inhibited hyphal formation by reducing mRNA expression levels of hyphae specific genes such as *HWP1, ALS3, CPH1, SAP* 4-6 but interestingly *RAS1* was not affected,but disturbed the downstream effectors of *RAS1,* mainly Cek1 phosphorylation in the MAPK pathway and cAMP synthesis in CAMP-PKA pathway[76]. Morin, a compound found in various medicinal plants showed significant inhibition of *C. albicans* virulence factors [77]. It inhibited biofilm formation (up to 90%), yeast to hypha transition, phospholipase, protease, and exopolysaccharides production with no fatal effect on fungal cells. *In vivo* efficacy was also assessed using *C. albicans* infected zebrafish animal model where morin increased the survival rate with a significant reduction in fungal load compared with untreated animals[77]. Likewise, *Boesenbergia rotunda* extracts pinostrobin and pinocembrin inhibited *C. albicans* biofilm formation without altering the growth of planktonic cells. All stages of biofilm formation including adhesion, biofilm development and maturation were inhibited in a concentration-dependent manner with decreased *ALS3* mRNA level[78].

d) Anthraquinone and other plant derived products

Purpurin, an anthraquinone isolated from *Rubia tinctorum* L., affects the *C. albicans* biofilm development and filamentation. Purpurin significantly reduced the biofilm formation, but it was less effective in eradicating mature biofilms, due to the inherent diffusion limitation imposed by the biofilm architecture[79]. The genes *ALS3, HWP1* that are essential for adhesion of *C. albicans* to the substrate, arealso downregulated, indicating their effects on biofilm formation.

Similarly, other plant derived compounds such as essential oils, α-longipinene and linalool[80], 6-gingerol and 6-shogaol[81] and cedar leaf essential oil, camphor, and fenchone derivatives[82] were assessed for their anti-virulent potential. They were found to be effective in inhibiting *C. albicans* hyphal and biofilm formation without affecting the growth of planktonic cells. They also reduced *in vivo C. albicans* virulence in *Caenorhabditis elegans*.

4-hydroxycordoin, an isopentyloxychalcone also demonstrated antibiofilm properties by inhibiting *C. albicans* formation (by 85%) and yeast to hypha transition without affecting *C. albicans* growth[83]. Furthermore, plant derived oleic acid poses anti-virulence effects by inhibiting filamentous growth, biofilm formation, secreted aspartyl proteinases (SAPs) and lipase production of *Candida species*. It also reduced the ergosterol content without affecting

444 the viability and the growth of fungal cells. Gene expression and proteomic analysis of oleic acid
445 treated cells suggest that oleic acid treatment may results in oxidative stress and affects
446 glucose metabolism, lipase production, iron homeostasis and ergosterol and amino acids
447 biosynthesis[84]. Other compounds such as *trans*-cinnamaldehyde and coumarin also have been
448 reported to have antibiofilm effects against *C. albicans*[85,86].

449 ***e) Polyphenols***

450 Curcumin, a polyphenol isolated from the rhizome of *Curcuma longa* is one of the well- studied
451 compounds for its anti-adhesive, anti- biofilm[87], anti-filamentation[89], and anti-candidal[88] effects
452 against *C. albicans*. The anti-candida effects were observed at higher concentrations, possibly
453 by perturbing the cell wall integrity affecting the calcineurin and MAPK pathways[89]. Curcumin
454 prevented the adhesion and biofilm formation of *C. albicans* to Poly-methyl methacrylate
455 (PMMA), mostly used in denture materials, by affecting the major adhesins *ALS1* and *ALS3.*
456 Concentrations around 100 µg/mL and 200 µg/mL are needed to inhibit the growth and biofilms
457 of *C. albicans* respectively[87,88]. Despite the fact that curcumin demonstrates antifungal and anti-
458 biofilm properties through several mechanisms, their hydrophobicity precludes optimal
459 bioavailability, poor solubility and potential reduced efficacy *in vivo*[90]. Hence to overcome these
460 difficulties our group recently explored the curcumin-sophorolipid nanocomplex which improved
461 curcumin bioavailability, stability, and solubility. It also retained biofilm inhibitory effects and anti-
462 filamentation property but at very low concentration of 9.37 µg/mL[91]. Considering that these
463 compounds hold the "generally regarded as safe (GRAS)"category status by FDA, urgent
464 studies are needed to develop innovative delivery methods so that these molecules can be
465 rapidly translated to clinical applications.

466 Apart from the plant derived compounds, fungal metabolites, and by-products such as
467 mycotoxins are produced by some fungi and moulds to maintain equilibrium in the dynamic
468 ecosystem and considered as harmful or toxic to other microbes, these microbe-derived small
469 molecules were also found to have anti-virulence properties against *C. albicans*[92–97].Although
470 natural products have several advantages, the unmodified form may possess passable efficacy
471 or poor ADME (absorption, distribution, metabolism, excretion) properties. It is often required to
472 synthetically modify and optimize to achieve full efficacy. Also, translating the natural products
473 for the clinical application requires enough of the analogue. Fortunately, there are many recent
474 advances, in total chemical synthesis, Biosynthetic engineering (Exploiting the producing
475 organism at their biosynthetic pathway) etc., which ultimately leads to the development of
476 analogues with superior pharmacological activity[66].

477 **Insights into anti-virulence approaches**

478 Virulence is the ability of a pathogen to cause disease. Virulence determinants are defined as
479 fungal factors such as morphological transition, phenotypic switching, biofilm formation,
480 secretion of hydrolytic enzymes, adhesin expression, and invasion of the host cell surface, all of
481 which can actively cause damage to host tissues. Anti-virulence therapies are tailored at
482 disarming the pathogen by stripping of its virulence potential. For instance, impeding the
483 adherence of microbial cells will hamper their biofilm formation and force them to survive in the
484 planktonic state which will eventually make them vulnerable for clearance by the host immune
485 system and/or antimicrobial treatment[98]. By selectively targeting the virulence mechanisms
486 instead of employing fungicidal or fungistatic approaches, will create a weaker selective
487 pressure for the development of antimycotic resistance[99].

488 The development of anti-virulence approaches need intensive research on the mechanisms of
489 virulence and identification of virulence factors, which can reveal several potential targets that
490 may contribute to the developments of newer classes of antifungals. Moreover, this non-lethal
491 alternative is most suitable to the commensal *C. albicans* thus preserving the host
492 microbiome.[24,100]. While the approach has a certain advantage, there are potential challenges in
493 its clinical translation. One key challenge to this approach is that the timing of drug
494 administration must coincide with the time at which the targeted virulence factor actively
495 promotes disease. This poses a challenge in the clinical setting for the rapid and accurate
496 diagnosis prior the use of such antifungals.[101] Research in this area is still nascent and it
497 remains unknown if fungi will evolve strategies to resist the anti-virulence strategies.

498

499 **Targeting other virulence mechanisms:**

500 ***Proteases***

501 *C. albicans* possesses numerous virulence factors that enable the fungus to colonize, invade
502 the host, evade the host immune response and cause disease. One of the main virulence
503 attributes produced by *C. albicans*, is the secreted proteases enzymes, specifically secreted
504 aspartyl proteinases (SAPs), phospholipases (PLs) and lipases (LIPs). These are three families
505 of hydrolytic enzymes that are linked to virulence in *C. albicans*[6]. SAPs (SAP 1-10) are the best
506 characterized hydrolytic enzymes and of these, SAPs1-8 are secreted extracellularly into the
507 media while SAP9 and SAP10 remain bound to the fungal cell surface[102]. The PL family has
508 four classes (A, B, C and D). Only PL members of class B have been detected extracellularly

509 and contribute to virulence, with PLB1 constituting the major component of secreted PLBs[103].
510 LIP1-10 are lipases, which is are believed to facilitate penetration into host tissues in addition to
511 nutrient acquisition for fungal growth[104]. It has been shown that deletion mutants of *SAP1,*
512 *SAP2,* and *SAP3*[105,106]and *PLB1*[107] showed significantly attenuated virulence compared to the
513 wild type strains. Therefore, using therapeutics that target the secreted hydrolytic enzymes
514 would be of a great value in targeting *C. albicans* virulence and pathogenicity (Figure 5).

515 One notable application of this is the introduction of aspartyl protease inhibitor-type HIV (HIV-PI)
516 as a component of highly active retroviral therapy (HAART) in HIV+ patients. This category of
517 immunocompromised patients is highly predisposed to oropharyngeal candidiasis. Significantly,
518 the addition of the protease inhibitor resulted in a remarkable decline in opportunistic infections,
519 disease progression and HIV associated mortality[108]. The intended action of the HIV-PIs is to
520 inhibit the viral proteases essential to produce the mature infectious form of the virus. Although,
521 the associated reduction in candidiasis was mainly attributed to the improved host
522 immunological status[109], the direct antifungal effect of HIV-PIs against *C. albicans* SAPs was
523 also demonstrated *in vitro*[110–112]. This was further supported by the fact that *C. albicans* SAPs
524 and HIV aspartic protease belong to the same family[113]. In fact, HIV-PIs have been shown to
525 have a protective effect against Candida colonization in HIV infected patients[114].

526 By contrast, one recent study showed that HIV-PIs containing antiretroviral therapy was
527 significantly associated with oropharyngeal *Candida* colonization in HIV patients[115]. This
528 difference could be attributed to the fact that different types of HIV-PIs have variable effect on
529 *Candida* SAPs[116]. Despite the reported antifungal properties of HIV-PIs, their use as
530 therapeutics targeting non-HIV candidiasis may not be feasible owing to the serious side effects
531 associated with these drugs including toxicity, accumulation of intracellular free cholesterol, lipid
532 and insulin resistance[117]. Using the SPECS 3D database, a total of 28,700 small molecules
533 were screened by various virtual screening methods and 35 compounds were found to interact
534 with the SAP2 protease. From these small molecules, using a combination of *in vitro* screening
535 and Structure Activity Relationship (SAR), several structural analogues were synthesized.
536 Compound 23h (2,2'-((4-((4-oxo-3-(p-tolyl)-2-(p-tolylimino)thiazolidin-5-ylidene)methyl)-1,3-
537 phenylene)bis(oxy))diacetic acid) showed better activity and is inactive against human
538 proteases. Although this compound, when used alone, resulted in the survival of only 10-20%
539 fluconazole-resistant *Candida* infected mice, it resulted in 50% survival rate when combined
540 with fluconazole. This demonstrates for the first time that the combination therapy of SAP2
541 inhibitors and fluconazole is an effective strategy to combat the drug resistance[118.] Many small

542 molecules in the family of protease inhibitors have been shown to inhibit biofilm formation and
543 disturb mature biofilms alone and also in combination with subinhibitory concentration of
544 traditional antifungals[119]. Furthermore, it has been shown that not all *Candida* SAPs have the
545 same sensitivity to protease inhibitors. Aoki and colleagues[120] identified SAP7 as being
546 insensitive to Pepstatin A, a classical protease inhibitor. As a result, developing a potent
547 protease inhibitor capable of inhibiting all *Candida* SAPs would be highly advantageous.

548 ***Quorum sensing***

549 Quorum sensing (QS), a signaling mechanism used by *C. albicans* to communicate amongst
550 themselves, and is directed by soluble molecules that are secreted into the environment in a
551 density-dependent manner[121]. These molecules in the extracellular environment positively and
552 negatively influences both biofilm development and morphogenesis [122–124]. For instance,
553 Farnesol (3,7,11-trimethyl-2,6,10-dodecatriene-1-ol) the first QS molecule that has been studied
554 extensively in *Candida* was known to block the transition of yeast to fungi at higher cell densities
555 thereby inhibiting hyphae formation[121,125,126]. Farnesol inhibits the hyphal transition by targeting
556 the *RAS1* pathway, specifically affecting Cyr1 and cAMP signalling[127,128]. Some antifungal
557 molecules stimulate farnesol production through affecting the ergosterol biosynthesis in fungi.
558 For instance, naturally derived products such as *trans*-cinnamaldehyde, and a phenolic
559 compound bisbibenzyl was known to increase farnesol production by inducing the expression of
560 encoding phosphatase *DPP3,* which plays an essential role in farnesol synthesis by convertion
561 of farnesol pyrophosphate to farnesol[85,129]. Stimulation of farnesol production by these small
562 molecules then affected both biofilms and hyphal morphogenesis of *C. albicans.*

563 Apart from small molecules that induce the production of farnesol, a recent study demonstrated
564 the use of the medium chain fatty acid named nonanoic acid, which mimics the farnesol and has
565 anti-virulence properties[130]. However, farnesol may also induce the biofilm-related infections by
566 promoting the formation of the yeast cells within the mature biofilms, that are then easily
567 dispersed. These dispersed cells have been shown to promote the propagation of *C. albicans*
568 cells into the bloodstream[121,131].

569 The other quorum sensing molecule that is produced from *C. albicans* is Tyrosol (2-[4-
570 hydroxyphenyl] ethanol) a tyrosine derivative, that is known to regulate both biofilm formation
571 and hyphal development[132], conversely to the farnesol. Both quorum sensing molecules controls
572 their expression in a density-dependent manner, i.e., at low cell densities, tyrosol is expressed
573 and at high cell densities, farnesol is produced (Figure 6). Thus, the compounds modulating,

574 tyrosol production may help in eliminating the infection at low cell density state itself. Along with
575 these two major QSM the other molecules that have been identified in *Candida spp.* and are not
576 extensively studied are tryptophol and phenylethyl alcohol which were also found to inhibit cell
577 growth and germ tube formation similar to that of farnesol[133]. All these studies clearly
578 demonstrate that QSM either have a positive or negative regulation on the morphogenesis of *C.*
579 *albicans.*

580 **Future perspectives and conclusion**

581 Invasive and mucosal candidiasis affects approximately 500 million people worldwide with the
582 increasing mortality rate of 30-50% even when treated. The challenge posed for the
583 development of novel anti-fungals is mainly due to the eukaryotic nature of fungi, limiting drug
584 targets. Sparse availability of antifungals, toxicity, and emergence of resistance causes extreme
585 difficulties in treating these infections, clinically. Anti-virulence approaches provide a hope that
586 they may reduce/eliminate the selective pressures that has been imposed on the pathogens
587 which led to the failure of the conventional antifungals. Antifungals usually kill all the commensal
588 and beneficial fungi causing dysbiosis, a pernicious side effect of the antifungals which can be
589 bypassed using anti-virulence strategies. Finding specific targets for the fungal pathogens has
590 always been difficult, but an increased understanding of pathogenesis and host-microbe
591 interaction and the completion of the *C. albicans* genome project, has uncovered several targets
592 for the development of anti-virulence drugs.

593 One of the major questions to address here, is that even though several novel antifungal
594 molecules targeting anti-virulence approaches has been discovered in the last decade, as
595 discussed in this review in detail, why have none of the molecules reached the market? The
596 main answer for this question apart from time and budget constraints is that failure of these
597 molecules in preclinical/clinical trials. Thus, in the preliminary screening using the phenotypic
598 assays, incorporating more reliable physiological settings is essential. This will hopefully reduce
599 the likelihood of overlooking compounds. Of course, as anti-virulence therapy is yet to be
600 explored in the clinical setting, there is still no understanding of whether all this investment and
601 time will lead to anti-virulence therapies.

602 As there is a pressing need for the novel antifungal compounds, and to achieve rapid
603 translation into the clinical setting,"repurposed drugs" are an excellent candidate as their
604 pharmacological properties are established reducing time and cost. Also use of artificial
605 intelligence/machine learning approaches to discover anti-virulence drugs from the pool of

compounds/libraries available may reduce the time and lead to the discovery of novel chemical scaffolds. By integrating the efforts of biologists, clinicians, pharma companies, and funding agencies, it is possible the development of novel antifungals can be achieved affordably and lead to a reduction in the healthcare burden.

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913 **Legend to Figures and tables**
914
915 **Fig 1: Resistance mechanisms of *C. albicans* to conventional antifungals.** Azoles,
916 echinocandins, and polyenes act on the cell wall components of C. albicans causing cell lysis.
917 (Left) Azoles act by inhibiting the enzyme 14α – lanosterol demethylase is essential for the
918 synthesis of ergosterol causing loss of cell integrity. However, the development of efflux pumps
919 prevents the intake of the drug, mutation in the gene sequence of the target enzyme, and
920 overexpression of target reduces the efficacy of the azoles towards candida. (Center)
921 Echinocandin inhibits 1, 3 - beta glucans synthase (FKS1, FKS2) but mutation in these genes
922 leads to resistance development. (Right) Polyenes acts on ergosterol causing membrane
923 leakage. Mutation in ERG3 affects its susceptibility towards Candida infections. Flucytosine
924 affects the growth and development by converting to 5- Fluro UMP (down), this toxic pyrimidine
925 gets incorporated into DNA and RNA. Resistance to this occurs by the increased expression of
926 efflux pump and mutation to the genes involved in the conversion of flucytosine to 5-fluro UMP.
927
928 **Fig 2: Comparison of the traditional antifungal drug discovery process versus drug**
929 **repurposing.** The traditional way of drug discovery begins with a) identification of the effective
930 molecular targets and chemical synthesis of the compound, which involves optimizing the
931 stability, yield, and solubility; b) preclinical studies and animal studies with 50:50% success:
932 failure rates c) phase 1-4 clinical trials with just approximately 10% success rate. These
933 methods clearly lack efficiency as they are exorbitantly expensive, time-consuming, and have
934 an unpredictable success rate. However, drug repurposing holds the advantage due to its
935 proven lack of or reduced toxicity and pre-existing clinical trial data which will lead to the rapid
936 translation to the clinic when compared to the traditional approach. Anti-malarial, anti-cancer,
937 anti-glaucoma, and anti-bacterial drugs have been discovered for their ability to act as an anti-
938 fungal by themselves or in synergy with existing traditional antifungals.
939
940 **Fig 3: Multiple targets/phases of drug development identified via HTS method.** Planktonic
941 cells of *C. albicans* adhere to a substrate to initiate biofilm development and progresses to form
942 mature biofilms. HTS may be used to discover drugs that affect various phases of biofilm
943 development by targeting single and multiple signaling pathways.
944
945 **Fig 4: Target dependent action of plant-derived natural bioactive molecules:** Compounds
946 are characterized based on their class of bioactive compounds and listed. The advantages
947 (pros) of plants derived bioactive molecules include "GRAS" category recognized by FDA, ease
948 of consumption and less toxicity. Whereas the disadvantages (cons) in developing the plants'
949 natural products was purification, bioavailability, and solubility issues due to increased
950 hydrophobicity.
951
952 **Fig 5: Proteases in Candida and protease inhibitors.** (Top) cell surface associated Secreted
953 aspartyl proteases SAP1-3 mediate the epithelial cell adhesion. Fungal invades the epithelial
954 cells by endocytosis and active penetration, where SAPs play a role in mediating active
955 penetration. SAPs digests the molecules and helps in nutrient acquisition via the transporter
956 ZRT1. SAP4-6 plays an essential role in evading the host immune responses by cleavage of
957 complementary proteins. (Below) The developed protease inhibitors, acts by reducing the
958 adhesion, invasion and inducing the oxidative stress.
959
960 **Fig 6: Quorum sensing mechanism and inhibitors of *C. albicans*.** The QS molecules
961 produced in the high cell densities such as farnesol, phenylethyl alcohol, and tryptophol, inhibits
962 the biofilm formation and virulence. Farnesol inhibits the *CYR1* production in the RAS1 pathway

963 thus inhibited the filamentous growth. Conversely tyrosol, which is expressed in low cell
964 densities induces the morphogenesis for which mechanism is not fully understood.
965
966
967 **Table 1:** ***High throughput screening:*** *Table* represents the library and molecule screened via
968 the High-through put screening. The lead compound name with their chemical structure and
969 mechanism of action is reported.

970 **Table 2:** ***Details of compounds screened via HTS have synergistic interactions with***
971 ***existing antifungals.*** The lead compound name with their chemical structure and mechanism
972 of action is reported.

973 **Table 3*****: Details of mitochondrial and vacuole inhibitors screened via HTS.*** The lead
974 compound name with their chemical structure and mechanism of action is reported.

975 **Table 4*****: Details of repurposing drugs screened via HTS.*** The lead compound name with
976 their chemical structure and mechanism of action is reported.

977 **Table 5:** ***Plant and microbe derived drugs.*** The compound name, source, structure with their
978 inhibitory concentration range and mechanism of action is reported in the table.