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Toxicity of a novel antifungal compound is modulated by ERAD components

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22 **Running title:** Endoplasmic reticulum-related antifungal toxicity

Abstract

In a search for new antifungal compounds, we screened a library of 4454 chemicals for toxicity against the human fungal pathogen *Aspergillus fumigatus*. We identified sr7575, a molecule that inhibits growth of the evolutionary distant fungi *A. fumigatus*, *Cryptococcus neoformans*, *Candida albicans*, and *Saccharomyces cerevisiae* but lacks acute toxicity for mammalian cells. To gain insight into the mode of inhibition, sr7575 was screened against 4885 *S. cerevisiae* mutants from the systematic collection of haploid deletion strains and 977 barcoded haploid DAMP strains in which the function of essential genes was perturbed by the introduction of a drug resistance cassette downstream of the coding sequence region. Comparisons with previously published chemogenomic screens revealed that the set of mutants conferring sensitivity to sr7575 was strikingly narrow, affecting components of the endoplasmic-associated protein degradation (ERAD) stress response and the ER membrane protein complex (EMC). ERAD-deficient mutants were hypersensitive to sr7575 in both *S. cerevisiae* and *A. fumigatus*, indicating a conserved mechanism of growth inhibition between yeast and filamentous fungi. Although the unfolded protein response (UPR) is linked to ERAD regulation, sr7575 did not trigger the UPR in *A. fumigatus* and UPR mutants showed no enhanced sensitivity to the compound. The data from this chemogenomic analysis demonstrate that sr7575 exerts its antifungal activity by disrupting ER protein quality control in a manner that requires ERAD intervention but bypasses the need for the canonical UPR. ER protein quality control is thus a specific vulnerability of fungal organisms that might be exploited for antifungal drug development.

44 **Introduction**

45 The burden of fungal infections in the human population is very high, with an estimated
46 1.5 million annual deaths worldwide, despite antifungal prophylaxis (1–3). The evolutionary
47 proximity between mammalian and fungal cells creates a challenge for the identification of
48 selective drug targets. Consequently, there are only a few mechanistically distinct classes of
49 antifungal agents. The major antifungal drugs in clinical use disrupt membrane homeostasis by
50 targeting ergosterol (4), impair cell wall integrity by inhibiting β -(1,3)-glucan synthase (5), or
51 perturb nucleic acid synthesis via a fluorinated nucleotide analogue (6). The limited number of
52 therapeutic options impedes effective management of invasive fungal infections, particularly
53 when resistance to a drug is either emerging or an intrinsic characteristic of the fungal
54 pathogen.

55 The identification of novel drugs and their targets can follow several strategies, ranging
56 from the inhibition of a known protein target with a panel of inhibitors to the analysis of
57 mutant strain sensitivity to toxic compounds (7). Chemical genomic screens analyze large
58 collections of genetically defined mutant strains for their sensitivity to chemical libraries in a
59 systematic manner. Data from these screens can provide insight into candidate targets for a
60 given drug, as well as the cellular pathways required to buffer drug toxicity (8–11). The
61 interpretation of chemogenomic screens depends on the type of mutant collection utilized for
62 the analysis. For example, the absence of a general dosage compensation mechanism in yeast
63 (12) allows heterozygous deletion strains to be used as tools to determine how a reduction in
64 the level of a gene product impacts drug sensitivity. However, since heterozygous deletion
65 strains retain some level of gene function, compensatory mechanisms could mask changes in

66 drug sensitivity. Haploid deletion strains can circumvent this problem and increase the
67 sensitivity of the screen. The resulting chemogenomic profile, or pattern of mutants that are
68 affected by a given compound, is predictive of the mechanism of action and has been
69 successfully applied to drug lead identification and target classification in yeast (13). Further
70 insight into pathways that are modulated by a compound can be obtained by developing *in*
71 *silico* comparison tools that link the results of a chemogenomic analysis to the data of other
72 published large-scale chemogenomic or genetic interaction screens (14, 15, for examples).

73 Most of the large chemogenomic data sets currently available have investigated non-
74 essential gene deletions in haploid strains (10, 16). Essential genes, representing about one
75 sixth of all *S. cerevisiae* genes, are more difficult to study in haploid or heterozygous deletion
76 strains, so an alternative approach is the use of Decreased Abundance by mRNA Perturbation
77 (DAmP) strains. DAmP strains contain a drug resistance marker inserted into the 3'
78 untranslated region (UTR) of a gene, resulting in defects in mRNA stability that can create
79 hypomorphic alleles for phenotypic analysis of essential gene function (17). These strains have
80 provided important insights into gene function, as well as the response of cells to stress (18,
81 for example).

82 In this study, we report the identification of sr7575, a small molecule with fungistatic
83 activity against *S. cerevisiae* and three genera of human fungal pathogens: *A. fumigatus*, *C.*
84 *neoformans*, and *C. albicans*. We employed a genome-wide approach to characterize the mode
85 of action of sr7575, using a systematic determination of *S. cerevisiae* deletion and DAmP
86 mutant sensitivity to the drug, combined with extensive *in silico* comparisons with large-scale
87 datasets from published chemogenomic and genetic interaction screens. The strategy led to the
88 conclusion that *S. cerevisiae* and *A. fumigatus* mutants that are deficient in ER-associated

89 degradation (ERAD), a degradative pathway that disposes of misfolded proteins that arise in
90 the ER membrane or lumen (19) are hypersensitive to sr7575. Collectively, these data
91 implicate ER protein quality control as the target of sr7575 toxicity in evolutionarily distant
92 fungi, and suggest that further analysis of compounds that disrupt ER homeostasis may
93 provide novel avenues for antifungal drug development.

94 **Materials and Methods**

95 **Screening procedure of the CERMN chemical library.**

96 All robotic steps were performed on a Tecan Freedom EVO platform. Compounds were
97 transferred from mother plates into clear, flat bottom, barcoded tissue culture 96-well plates
98 (Greiner Bio One): 1 μ L of a DMSO solution containing 3.3 mg/mL of each compound was
99 spiked into dry wells of daughter plates (80 compounds per plate). For each plate, columns 1
100 and 12 served as controls: 8 positive controls spiked with DMSO alone provided the reference
101 as 100% growth and 8 negative controls contained the antifungal drug amphotericin B at 15
102 μ g/mL to kill all cells. 130 μ L of a mixture containing 10 volumes of conidial suspension 10^5
103 conidia/mL (in RPMI with 0.1% Tween 20) and 3 volumes of resazurin 0.01% was added to
104 each well. After 48 hours of incubation at 37°C, the absorbance at 570 nm (measurement
105 wavelength) and 604 nm (reference wavelength) were measured on a Safire2 (Tecan)
106 microplate reader. The data were normalized using the following formula: % viability = 100 x
107 (sample value - average value of negative controls) / (average of positive controls - average of
108 negative controls).

109 For analysis of toxicity to human cells, compounds were added to HeLa cells at a
110 concentration of 10 μ M (2.8 μ g/mL for sr7575) and the release of cytoplasmic lactate

111 dehydrogenase was measured using the ELISA-based Cytotoxicity Detection Kit (Roche),
112 according to the manufacturer's recommendations. Mutagenic activity was tested in the
113 bacterial reverse mutation test, either in the presence or absence of a rat metabolizing system
114 (performed by CiToxLAB Safety and Health Research Laboratories). To determine acute
115 mouse toxicity, groups of four NMRI mice were given a single dose of sr7575 (100 mg/kg) by
116 i.p. injection, and mortality was monitored for 3 days.

117 **Yeast strains, growth, and media.**

118 All strains used in this study are described in Table S4. The pooled haploid deletion
119 library (MATa) contained deletions in 4885 non-essential genes along with DAMP
120 modifications of 977 essential genes (20, 21). Wild type (WT) *S. cerevisiae* strain BY4741
121 was routinely maintained on YPD agar (1% yeast extract, 2% peptone, 2% dextrose, 2% bacto
122 agar; YPDA).

123 For *S. cerevisiae* serial dilution spot assays, fresh colonies from plates were used to
124 inoculate overnight cultures in YPD. The next morning, cultures were washed once, diluted to
125 OD₆₀₀ 1 in PBS and serial ten-fold dilutions were carried out in a 96-well plate. 10 µL of each
126 dilution was spotted onto SC medium (containing 6.7 g/L yeast nitrogen base with ammonium
127 sulfate, BD Difco, with all amino acids, 2% dextrose and 2% bacto agar) lacking or
128 supplemented with 0.25 µg/mL sr7575. Plates were incubated at 30°C and growth was
129 monitored every 24 h over three days. Spot assays on SC supplemented with the analog sr7576
130 were conducted in a similar fashion.

131 The same serial dilution spot assay used to assess sensitivity in *S. cerevisiae* was used
132 for *C. albicans* and *C. neoformans*, with the exception that the plates were supplemented with

133 sr7575 between 1-8 $\mu\text{g}/\text{mL}$ and were incubated at 37°C.

134 **Verification of *S. cerevisiae* mutant strains.**

135 The following mutants were extracted from the gene deletion library maintained in the
136 96-well format(8): *YBR283C* (*SSH1*), *YCL045C* (*EMC1*), *YDL020C* (*RPN4*), *YDL226C*
137 (*GCS1*), *YER019C-A* (*SBH2*), *YER090W* (*TRP2*), *YKL126W* (*YPK1*), *YKL207W* (*EMC3*),
138 *YML105C* (*SEC65*; DAmP strain), *YMR022W* (*UBC7*), *YMR264W* (*CUE1*), *YOL013C*
139 (*HRD1*), *YOR008C* (*SLG1*), *YOR153W* (*PDR5*), *YPR060C* (*ARO7*), *YHR079C* (*IRE1*),
140 *YFL031W* (*HAC1*), *YBR201W* (*DER1*), *YLR207W* (*HRD3*), *YIL030C* (*SSM4/DOA10*),
141 *YDL190C* (*UFD2*), *YGL013C* (*PDR1*), *YNL181W* (DAmP strain), and *YML125C* (*PGA3*;
142 DAmP strain). Genomic DNA was extracted using phenol-chloroform followed by ethanol
143 precipitation. Mutants were verified by PCR amplification using a common forward primer
144 annealing to the KanMX cassette (KaniF) and gene specific reverse primers (oligonucleotides
145 listed in Table S5).

146 **Complementation tests in *S. cerevisiae*.**

147 Complementation tests were performed with plasmids from the Molecular Barcoded
148 Yeast ORF collection (MoBY-ORF) (22). *URA3* plasmids carrying ORFs corresponding to
149 genes *ARO7*, *CUE1*, *EMC1*, *EMC3*, *HRD1*, *RPN4*, *SSH1*, and *UBC7* (Table S4) were
150 recovered from *E.coli* grown in LB medium (1% tryptone, 0.5% yeast extract, 1% NaCl, 1.5%
151 bacto agar) supplemented with chloramphenicol (60 $\mu\text{g}/\text{ml}$) and kanamycin (50 $\mu\text{g}/\text{ml}$) (22).
152 500 ng of each plasmid was transformed into the appropriate yeast deletion parent following
153 the lithium acetate protocol (23) and *URA3*-expressing transformants were selected on SC
154 medium lacking uracil. The resulting transformants were purified by passaging onto fresh SC

155 (-URA) medium and four clones of each transformant-set were screened by colony PCR using
156 a gene-specific primer pair (Table S5), generating product sizes ranging between 500-1000 bp.
157 The deletion parent was always included as a negative control. Complemented strains were
158 screened in parallel with the parental deletion strains in spot assays.

159 **Overexpression tests in *S. cerevisiae*.**

160 2 micron-based *LEU2* plasmids from the systematic overexpression library (24)
161 corresponding to regions of the yeast genome that contain the ORFs *PDR1*, *PDR5*, *PDR12*,
162 and a control lacking intact genes (Table S4) were recovered from *E. coli* DH10B cultures
163 grown in LB medium supplemented with kanamycin (50 µg/mL), transformed into wild type
164 BY4741, and transformants selected on SC (-LEU) plates. Transformants were purified by
165 passaging onto fresh SC (-LEU) plates. Overexpressing strains were screened by serial
166 dilution spot assays on SC medium supplemented with increasing concentrations of sr7575.

167 **Chemogenomic profiling.**

168 Concentrations of sr7575 that inhibit WT growth by 10-20% in liquid culture were
169 determined using the haploid strain BY4741 (MATa; *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*).
170 Single colonies from fresh YPDA plates were inoculated into 10 ml YPD and incubated at
171 30°C for 14 h. Cultures were diluted to OD₆₀₀ of 0.01 and grown to an OD₆₀₀ of 0.05 prior to
172 the addition of increasing concentrations of sr7575 (0.0625 µg/mL to 0.5 µg/mL). DMSO was
173 used as a vehicle control but there was no observable difference in growth rate between the
174 no-vehicle and DMSO-treated cultures. Growth was monitored by measuring the OD₆₀₀ every
175 hour, starting from 0 h until 10 h (Fig. S2A).

176 Pooled 400 mL cultures of the haploid deletion library were grown for 12 generations in

177 the presence of sr7575 at 0.125 µg/mL or DMSO vehicle-control. Amplified TAG products
178 from the pooled cultures were hybridized to Agilent barcode-specific microarrays (platform
179 GPL18088, GEO) as previously described (14). Images obtained with a GenePix 4200AL
180 scanner were annotated by using GenePix Pro 7 (Molecular Devices, CA, USA). Gpr files
181 were normalized separately for the UP and DOWN barcodes and aggregated for each mutant.
182 Raw and normalized data were deposited in the GEO database under identifier GSE60934.
183 Only results for which data were obtained in two independent biological replicates were
184 further considered for analysis. A total of 4909 mutants, including both deletion and DAMP-
185 modified strains, showed consistent growth measurements (Table S6), with a Pearson
186 correlation coefficient between the two series of log-transformed values of 0.78.

187 **Gene set enrichment analysis and correlations.**

188 Over-representation of GO terms in the chemogenomic screen results was analyzed
189 using the web interface at <http://go.princeton.edu/cgi-bin/GOTermFinder> to the GO Term
190 finder program (25). This program identifies enriched GO terms by calculating the frequency
191 with which one expects to encounter a number of genes having the same annotation in a subset
192 of genes (hypergeometric distribution). Correlations with published large-scale datasets were
193 computed using the R project (<https://cran.r-project.org/>) function ‘cor.test’, using either
194 “*pearson*” or “*spearman*” as comparison methods. Treatments or gene deletion perturbations
195 were ranked in decreasing order of calculated correlation coefficients.

196 ***A. fumigatus* strains, growth, and media.**

197 WT *A. fumigatus* strain kuA and deletion mutants *derA*Δ, *hacA*Δ, *hrdA*Δ, and
198 *hrdA*Δ/*derA*Δ were maintained on malt slants (2% malt extract, 2% bacto agar) while strains

199 *ireA*Δ and *hacA*Δ/*derA*Δ were maintained on *Aspergillus* minimal medium (MM) with 5 mM
200 ammonium tartrate as the nitrogen source and osmotically stabilized with 1.2 M sorbitol (26).
201 G418 was obtained from Invitrogen and Sigma Aldrich was the source for ampicillin,
202 kanamycin, and chloramphenicol.

203 Conidia from *ireA*Δ and *hacA*Δ/*hrdA*Δ strains were recovered from *Aspergillus* MM +
204 1.2 M sorbitol slants, while that of the parental *kuA* strain and the remaining deletion strains
205 were recovered from 10-day old malt slants in 0.05% Tween. Conidia were diluted to 10⁷
206 conidia/mL and serial 10-fold dilutions were carried out in a 96-well plate prior to spotting 10
207 μL of each dilution onto MOPS-buffered RPMI-1640 pH 7.0 plates in the presence or absence
208 of 5 μg/mL sr7575. The analog sr7576 precipitated out of solution in RPMI-1640 media and
209 was therefore not included in the analysis. Plates were incubated at 37°C and growth was
210 monitored over four days.

211 **MIC determination.**

212 Determination of the minimal inhibitory concentration (MIC) for yeast strains was
213 carried out by the CLSI M27-A3 broth microdilution method (27). Growth inhibition of
214 *Aspergillus* strains was monitored using a colorimetric test described earlier (28). The MIC of
215 *A. fumigatus* strain that constitutively expresses DsRed fluorescent protein (29) was
216 determined following growth for 24 h at 37°C by measuring fluorescence using a Biotek
217 Synergy fluorescent microplate reader with an excitation wavelength of 254 nm and emission
218 filter set at 291 nm. The relative fluorescence units were plotted against the compound
219 concentrations to determine MIC.

220 **Measurement of fungistatic or fungicidal activity.**

221 For yeast, freshly growing YPD cultures were diluted to OD₆₀₀ 0.001. sr7575 was added
222 at 0.625 µg/mL, amphotericin B at 0.5 µg/mL, and DMSO was used as a vehicle control.
223 100 µL aliquots were recovered for plating on YPDA. Cultures were grown for 16 h, cells
224 were washed once in 1X PBS and 100 µL of serially diluted samples were plated. Colonies
225 were counted following 48 h and normalized to the OD₆₀₀.

226 For *A. fumigatus*, 50 mL RPMI cultures with a starting cell number of 1x10⁵ conidia/ml
227 were setup in the presence or absence of 5 µg/mL sr7575 (in duplicate). 100 µL aliquots were
228 recovered for enumeration of colony forming units. Following 16 h of growth, mycelia from
229 one pair of flasks were filtered and mycelial dry weight estimated. From the second pair,
230 100 µL from the drug-treated flask was serially diluted and plated to assess viability.

231 **qRT-PCR.**

232 *A. fumigatus* conidia were inoculated into YG medium (0.5% yeast extract, 2% glucose)
233 and incubated overnight at 37°C, 200 rpm. The mycelium was treated with the indicated
234 concentrations of sr7575 or dithiothreitol (DTT), along with appropriate vehicle controls, for
235 1 h. The mycelia were harvested by filtration and lysed by crushing in liquid nitrogen. RNA
236 was isolated using the TRIzol reagent, treated with DNase to remove traces of DNA, and
237 reverse-transcribed using M-MuLV reverse transcriptase (NEB) together with an oligo-d(T)
238 primer. Quantitation of *bipA* and *tigA* mRNA expression was performed by qRT-PCR, as
239 previously described (30).

240 **Results**

241 **Identification of a new inhibitor of fungal growth**

242 In a search for new antifungals, we tested the toxicity of 4454 chemicals from the
243 CERMN compound library against *A. fumigatus* using the strategy outlined in Fig. 1A. The
244 CERMN library is part of the French national collection of chemicals (31) and was built since
245 1998 to be used in the framework of partnerships with public research laboratories. The
246 dynamic range and degree of separation between positive and negative controls in the screen
247 was evaluated by computing the Z' score (32). The average Z' value was 0.92 ± 0.03 ,
248 indicating a robust and reliable assay. Data analysis identified 76 hits showing greater than
249 90% fungal growth inhibition, which were clustered into 7 chemical families and 29 singletons
250 (Table S1). Compounds with known effects on human physiology (33), or which showed
251 cytotoxicity for HeLa cells in a lactate dehydrogenase release assay, were eliminated from
252 further consideration. The compound sr1810 was active against *A. fumigatus* and was selected
253 for further analysis. Since sr1810 consisted of a mixture of two isomers, 75% of sr7575 (1)
254 (Fig. 1A) and 25% of sr7576 (2), we synthesized each isomer (Fig S1, A and B) and found that
255 it was only sr7575 that was responsible for the antifungal activity. The sr7575 compound
256 showed no mutagenic activity in the bacterial reverse mutation test, and no acute toxicity was
257 observed in mice at a dose of 100 mg/kg.

258 To gain insight into the structural basis for sr7575 antifungal activity, we prepared thirty
259 analogues using aniline derivatives with different substitutions in the first reaction
260 (compounds 3-32, Tables S2 and S3, synthesis detailed in Text S1). Growth inhibition tests
261 with these compounds showed that at least two features of sr7575 were required for its
262 antifungal potency: the chlorine at position 4 of the phenyl group and the positioning of the

263 nitro group in relation to the pyrrole moiety (Table S2).

264 In addition to its effects on *A. fumigatus*, sr7575 was active against *A. flavus* (Fig. 1B),
265 *C. neoformans*, *C. albicans* (Fig. 1C), and *S. cerevisiae* (Fig. 1D) on plates and in liquid
266 medium at inhibitory concentrations ranging from 0.6 to 10 $\mu\text{g}/\text{mL}$ (Fig. S2A, B, C, D). More
267 than 90% of either *S. cerevisiae* or *A. fumigatus* cells were able to resume growth after a 16 h
268 incubation in the presence of sr7575 (0.625 $\mu\text{g}/\text{mL}$ and 5 $\mu\text{g}/\text{mL}$, respectively) indicating that
269 the compound exerts a fungistatic effect.

270 **ERAD-deficient mutants of *S. cerevisiae* are hypersensitive to sr7575**

271 To gain insight into the mechanism by which sr7575 perturbs fungal physiology, the
272 effect of sr7575 was tested on the growth rate of each of 4885 haploid yeast deletion strains in
273 the systemic deletion collection (20). In addition, sr7575 activity was measured on 977 locus
274 tagged barcoded DAmP (17) mutants of essential genes that were previously generated in our
275 laboratory (21). This collection of gene knockout and DAmP strains contains molecular
276 barcodes to facilitate detection and quantitation of DNA by custom Agilent microarrays (34,
277 35). Following the strategy outlined in Fig. 1A, the normalized ratio of the hybridization
278 signal in the presence or absence of treatment was used as an estimate of relative growth rate
279 in pools of mutants. Only a fraction of mutant strains showed hypersensitivity to sr7575, as
280 indicated by the left tail of the distribution for sensitivity values (Fig. 2A). The strain that
281 showed the most dramatic increase in sr7575 sensitivity harbors a deletion of the *PDR1* gene,
282 encoding the main regulator of multidrug resistance in yeast (36). Pdr1 is a transcriptional
283 activator for xenobiotic efflux transporter genes, thereby governing resistance to numerous
284 toxic compounds. It is likely that the effect of *PDR1* deletion on sensitivity to sr7575 is

285 mediated through the plasma membrane ATP-binding cassette (ABC) transporter Pdr5, since
286 *PDR5* is a known target of Pdr1 (37) and the *pdr5* mutant was ranked 6th among deletion
287 strains that were most affected by *sr7575*.

288 To identify cellular pathways or protein complexes that allow cells to counteract *sr7575*
289 effects, we used a gene set enrichment analysis on 89 mutant strains that showed an average
290 increase in generation time of at least 10% relative to WT in the presence of *sr7575*. The most
291 over-represented pathway in the dataset was ER-associated protein degradation (ERAD),
292 specifically the GO term “ER-associated ubiquitin-dependent protein catabolic process”
293 (GO:0030433), with a p-value corrected for multiple hypotheses testing of 1.5×10^{-6} . This set
294 included *CUE1*, *UBC7*, *HRD1*, *HRD3*, *UFD2*, *UBX4*, *SSM4 (DOA10)*, *DSK2*, and *UBX2*,
295 encompassing one fifth of the total number of genes annotated to this term (Fig. 2B). The
296 second most over-represented GO term was “aromatic amino acid family biosynthetic
297 process” (GO:0009073). However, strains deficient in this pathway are known to exhibit a
298 multidrug response signature (9), so the study of the corresponding strains was not pursued
299 further.

300 Cellular component enrichment analysis was used to determine whether any of the 89
301 proteins selected in the screen were linked to the same protein complex or intracellular
302 location. The ER membrane protein complex (EMC) was the most over-represented group by
303 this analysis, with a p-value of 2×10^{-7} . In addition to gene deletions directly affecting *EMC1*,
304 *EMC3*, *EMC4* and *EMC5*, deletions affecting dubious ORFs which overlap with *EMC2*
305 (*YJR087W*) and *EMC1 (YCL046W)* that are distinct mutants of these genes, were also present
306 in this dataset. Members of the EMC complex are required for efficient protein folding in the
307 ER (38), potentially through roles in phospholipid metabolism at the ER membrane (39).

308 Other components of the ER membrane showed enrichment, including 10 of 58 genes
309 annotated as "intrinsic components of the ER membrane" (GO:0031227) and two DAmP-
310 modified essential genes of uncharacterized function (*YNL181W* and *PGA3*). In addition,
311 several genes encoding components of the signal recognition particle (SRP) involved in co-
312 translational targeting of proteins into the ER showed enrichment: *sec65*-DAmP, *srp21*-DAmP,
313 *shr3*-DAmP, *ssh1* Δ , and *sbh2* Δ . Taken together, these findings indicate that sensitivity to the
314 inhibitory effects of *sr7575* is exacerbated by defects in the ERAD stress response, as well as
315 by alterations in ER membrane composition that affect optimal ER protein translocation and
316 folding.

317 **The *sr7575* sensitivity profile suggests a UPR-independent stress response**

318 The effects of *sr7575* on haploid yeast deletion strains were compared to profiles
319 obtained from 1,824 different chemicals in a recently published large-scale chemogenomic
320 screen (16). The compound CMB4166 had the highest Spearman correlation coefficient in this
321 comparison (Fig. 2C, $r=0.44$) and showed a remarkably similar profile to that of *sr7575*
322 (Fig. 2D). Most of the strains showing sensitivity to *sr7575* were also sensitive to CMB4166
323 (Fig. 2E), suggesting that the two compounds trigger similar cellular responses. However,
324 CMB4166 is a macrolide (D. Hoepfner, personal communication) and shares no structural
325 homology to *sr7575*.

326 To acquire insights into the specificity of the response to *sr7575*, we compared its
327 sensitivity profile to published results on 3,356 other chemical compounds (10). The pattern of
328 *sr7575* sensitive mutants revealed little-to-no similarity to profiles obtained from the other
329 compounds in this comparison. For example, the maximum computed Pearson correlation

330 coefficient was 0.27 for the compound k048-0007 (screen SGTC_352, Fig. S3A). However,
331 this correlation was due to strains with deletions in *PDR1*, *RPN4*, or *GCSI*, which confer
332 sensitivity to multiple stresses (Fig. S3C). To avoid the typically large effect of outliers on the
333 Pearson correlation, we also tested correlation via the Spearman non-parametric test that uses
334 ranks rather than values. The maximum correlation by this approach was also low (0.18),
335 identifying the compound 4245-1575 used in screen SGTC_513 (Fig. S3B). Most of the
336 correlation in this case could be attributed to the hypersensitivity of the *ubc7Δ* and *cue1Δ*
337 ERAD mutants (Fig. S3D). Since this second compound was annotated as having an unfolded
338 protein response (UPR) signature (10), we also tested the correlation between the profile of
339 sr7575 and tunicamycin, a well-known and widely used inducer of the UPR (40, 41, for
340 review). However, no similarity was found (Fig. S3E). Collectively, these comparisons suggest
341 that while many sensitivity profiles are related and indicate the most frequent types of cellular
342 responses to chemical toxicity (10), the profile obtained for sr7575 was specific, with
343 similarity to only one compound out of over 5000 chemicals analyzed.

344 Large-scale chemical toxicity screens are complementary to synthetic genetic array
345 analyses (SGA), in which double deletion mutant strains are used to determine functional
346 interactions between genes. We compared the sensitivity profile of sr7575 with the results
347 from 1711 SGA screens (42). The closest hit was the profile shown by a strain harboring a
348 DAmP modification of the essential gene *PGA3* (17) (Fig. S4A). The correlation between the
349 sr7575 and *PGA3* profiles was robust, since it also ranked 5th when estimated using Spearman
350 correlation (Fig. S4B). Despite the low value of the correlation coefficient (0.23), several
351 strains containing gene deletions were affected by both sr7575 treatment and replacement of
352 *PGA3* with the *pga3*-DAmP allele, including the ERAD-associated genes *cue1Δ*, *ubc7Δ*,

353 *ufd2Δ*, *ssm4Δ*, and EMC complex components (Fig. S4C). A role in newly synthesized protein
354 trafficking has been proposed for Pga3 (43), raising the possibility that its presence in our
355 dataset is due to a function that impacts ER homeostasis.

356 The gene deletion that ranked second in terms of correlation with the *sr7575* profile
357 involved *CHO2*, encoding a phosphatidyl N-methyltransferase required for phosphatidyl-
358 choline synthesis. The absence of *CHO2* renders yeast cells dependent on the UPR for survival
359 (42, 44), suggesting that *CHO2* contributes to ER homeostasis. Consistent with this, *CHO2*
360 deletion shows an aggravating interaction with the loss of EMC genes in terms of yeast growth
361 (39).

362 A summary of the correlations between *sr7575* sensitivity profiles and those derived
363 from published chemogenomic screens is shown in Fig. 3. Since the numerical values reported
364 for genetic and chemogenomic screens are not readily comparable, the ranking of the different
365 mutants in each screen was used to generate a meaningful graphical display. The resulting
366 heatmap (Fig. 3A) highlights the unique ERAD signature of *sr7575* relative to currently
367 published screens. A schematic illustrating the ER membrane proteins involved in the ERAD
368 pathway is shown for perspective (Fig. 3B). Since ERAD is known to work in concert with the
369 UPR to relieve ER stress, it is interesting to note that hypersensitivity to *sr7575* was observed
370 for ERAD mutants, but not for the UPR-inactivated strains *ire1Δ* and *hac1Δ*. Taken together,
371 these data are consistent with a model in which *sr7575* toxicity is counteracted by a functional
372 ERAD machinery, independent of signaling through the UPR pathway.

373 **Specific ERAD deficiencies enhance *sr7575* toxicity in *S. cerevisiae***

374 Seventeen *S. cerevisiae* mutant strains were selected to validate the results of the

375 chemogenomic screen, encompassing strains with deletions in components of the ERAD and
376 proteasome pathways, the EMC, the Ssh1 co-translocase, the PDR network, aromatic acid
377 biosynthesis, and DAmP modifications of *YNL181W* and *PGA3*. Each strain was analyzed
378 individually for sr7575 susceptibility, using a subinhibitory concentration for WT (Fig. 4A and
379 Fig. S5). A strain deleted for aromatic amino acid biosynthesis (*aro7Δ*) showed increased
380 sr7575 sensitivity (Fig. 4A), consistent with the pleiotropic effects of this mutation on stress
381 response.

382 As predicted by the chemogenomic screen, mutants in the *PDR5* multidrug transporter
383 and its transcriptional activator *PDR1* were hypersensitive to sr7575 (Fig. S5). Conversely,
384 overexpression of *PDR1* and *PDR5*, but not *PDR12* rendered *S. cerevisiae* cells tolerant to
385 high concentrations of sr7575 (Fig. S6A). This phenotype was conserved across fungal
386 species, since clinical isolates and laboratory *C. albicans* strains that overexpress *CDR1*, the
387 ortholog of *S. cerevisiae* *PDR5*, were also tolerant to sr7575 (Fig. S6B).

388 Deletions of genes coding for EMC members, *EMC1* and *EMC3*, and the co-
389 translational translocase *SSH1* conferred increased sensitivity to sr7575, as suggested by the
390 chemogenomic screen. However, a mutant in *SBH2*, which functions in the Ssh1 translocase
391 complex (45), did not show increased sensitivity at least at this concentration (Fig. S5).
392 Hypersensitivity to sr7575 was confirmed for components of the ERAD complex, including
393 the Hrd1 E3 ubiquitin ligase, the E2 ubiquitin conjugating enzyme Ubc7, and the ER
394 membrane-resident recruiter Cue1 (46–48) (Fig. 4A). Although the ERAD component Der1
395 (49) was not identified in our chemogenomic screen, a mild increase in sr7575 sensitivity was
396 observed for this mutant (Fig. S5), consistent with ERAD involvement in sr7575 effects.
397 sr7575 hypersensitivity was also validated for a strain lacking *RPN4*, encoding a

398 transcriptional activator of proteasome genes (Fig. 4A). Since proteasomal degradation is the
399 final step in the disposal of misfolded proteins by ERAD, this finding is consistent with the
400 notion that sr7575 affects protein quality control in the ER. In conclusion, these findings
401 demonstrate that components of the ERAD pathway are necessary to protect yeast cells from
402 the toxic effects of sr7575 in *S. cerevisiae*, suggesting a mechanism of action that involves
403 perturbation of ER protein quality control.

404 **ERAD protects against sr7575 toxicity in *A. fumigatus*, but is UPR-independent**

405 The UPR is a stress response pathway that communicates information on ER
406 homeostasis to the nucleus (40, 41). The pathway is triggered by misfolded proteins, which
407 accumulate in the ER when the demand for secretion exceeds ER folding capacity, or when the
408 cell encounters adverse environmental conditions. Unfolded proteins are sensed by the ER-
409 transmembrane sensor Ire1, which triggers the synthesis of Hac1, a transcription factor. Hac1
410 translocates to the nucleus and upregulates the expression of chaperones, folding enzymes, and
411 other proteins that support ER function (44, 50). Since ERAD mutants are hypersensitive to
412 sr7575, and ERAD capacity can be regulated by the UPR, we were surprised to find that
413 neither *HAC1* nor *IRE1* were identified in the sr7575 chemogenomic screen. The UPR
414 independence of this response was confirmed by susceptibility testing: yeast *ire1Δ* and *hac1Δ*
415 mutants were not affected by sr7575 at concentrations of up to 0.5 μg/mL (Fig. 4C). These
416 findings suggest that ERAD protects against sr7575 toxicity through a mechanism that is
417 independent of the UPR in *S. cerevisiae*.

418 Consistent with the results obtained in *S. cerevisiae*, UPR mutants of *A. fumigatus* that
419 lack either the ER sensor IreA or the transcription factor HacA showed no hypersensitivity to

420 sr7575 (Fig. 5A). As in yeast, the *hrdA*Δ mutant, which lacks the ortholog of *S. cerevisiae*
421 *HRD1*, showed increased sensitivity to sr7575. A *derA*Δ mutant that is deficient in the DerA
422 component of the HrdA ERAD complex showed no increase in sr7575 sensitivity. However, a
423 double deletion mutant lacking both DerA and HrdA showed greater sensitivity to sr7575 than
424 a mutant lacking HrdA alone, underscoring the importance of the Hrd1 complex in the
425 response to sr7575 toxicity. We conclude that sr7575 action involves the inhibition of an
426 evolutionarily conserved target, necessitating the intervention of the ERAD complex
427 Hrd1/HrdA in both *S. cerevisiae* and *A. fumigatus*.

428 The ERAD-enriched signature for sr7575 suggested that some aspect of ER protein
429 quality control is adversely affected by this compound. However, since UPR deficient strains
430 of *S. cerevisiae* or *A. fumigatus* showed no increase in sr7575 sensitivity, the results suggest
431 that a UPR-independent mechanism of ERAD activity is involved in the sr7575 response. To
432 confirm UPR independence, qRT-PCR was used to measure mRNA levels for two well-known
433 UPR target genes: the ER chaperone *bipA* and the protein disulfide isomerase *tigA*. As
434 expected, the expression of both genes was strongly induced by treatment with a sub-
435 inhibitory concentration of dithiothreitol (DTT, 1 mM), a well-known inducer of the UPR
436 (Fig. 5B). By contrast, no increase in expression was observed following treatment with a sub-
437 inhibitory concentration of sr7575 (0.1 μg/mL). In addition, pretreatment with sr7575 for 1 h
438 prior to DTT exposure failed to block UPR activation. These results indicate that while
439 exposure to sr7575 does not trigger the UPR, it was also unable to prevent UPR activation by
440 DTT. We conclude that sr7575 is unlikely to target UPR signaling for its toxic effects in
441 *A. fumigatus* or *S. cerevisiae*, consistent with the UPR-independent response suggested by the
442 chemogenomic screen.

443 **Discussion**

444 In this study, we describe the identification of a novel antifungal compound, sr7575 that
445 was active against species from four fungal genera. Chemogenomic profiling in *S. cerevisiae*
446 demonstrated that the set of genes required for protection against sr7575 was markedly
447 narrow, involving components of the ERAD stress response and other components of the ER
448 membrane. The function of the ERAD pathway is to maintain protein quality control in the ER
449 by eliminating toxic unfolded proteins that may accumulate in the fungus during periods of
450 high secretory activity, or when the organism encounters adverse environmental conditions.
451 This disposal mechanism centers on a multi-protein complex in the ER membrane that
452 selectively identifies misfolded proteins in the ER lumen or membrane and transports them
453 back into the cytoplasm for degradation by the proteasome. The results from our
454 chemogenomic screen demonstrate that mutants of this complex, either in *S. cerevisiae* or
455 *A. fumigatus*, are hypersensitive to sr7575 inhibition, suggesting that the antifungal effects of
456 this compound involves a disruption of ER protein quality control.

457 ER protein quality control is also affected by the UPR, a signaling pathway that counters
458 the accumulation of unfolded proteins in the ER by increasing the expression of chaperones
459 and other proteins involved in protein folding when the demand for secretion exceeds the
460 folding capacity of the organelle. A tight coordination between the UPR and ERAD pathways
461 was demonstrated in yeast where UPR mutants have decreased ERAD activity whereas ERAD
462 mutants exhibit constitutive UPR upregulation (50). In addition, although ERAD is sufficient
463 to eliminate misfolded proteins that continually arise during normal growth, it requires the
464 UPR for optimal degradative capacity under conditions of severe ER stress (51). Basal ERAD
465 activity is thus sufficient to handle low levels of unfolded proteins and is UPR-independent.

466 However, upregulation of ERAD activity by the UPR is needed when the level of unfolded
467 proteins reaches a critical threshold of toxicity. The connection between these pathways is also
468 evident in *A. fumigatus*, where mutants deficient in both the UPR and ERAD are less fit than
469 those lacking the UPR or ERAD alone (52).

470 In view of the link between the UPR and ERAD pathways, we were surprised to find
471 that neither *ire1Δ* nor *hac1Δ* were among the strains most affected by sr7575 in our
472 chemogenomic screen, and that these strains showed no increase in sr7575 sensitivity when
473 tested individually. In addition, our experiments revealed that sr7575 did not trigger the UPR,
474 nor did it prevent the UPR from being activated by DTT, a strong inducer of unfolded
475 proteins. These observations suggest that sr7575 does not cause the widespread protein
476 unfolding that is typical of strong ER stress aggravators such as DTT and tunicamycin.
477 Specific ER stress can be induced, for example, by expressing topologically abnormal ERAD-
478 targeted integral membrane proteins without inducing the canonical UPR pathway in yeast
479 (53).

480 The ability of sr7575 to inhibit the growth of fungi but not human cells raises the
481 possibility that it targets a fungal-specific process. Our chemogenomic screen identified
482 *ynl181w*-DAmp as one of the top 10 strains most affected by sr7575 toxicity. *YNL181W*
483 encodes an essential ER-membrane protein, the function of which is currently unknown, but is
484 speculated to involve an oxido-reductase activity (54, 55). The Ynl181w protein is conserved
485 among fungi (Fig. 6A) and has no metazoan orthologue, as defined in the OrthoMCL database
486 (56). Since the protein is essential, a heterozygous *YNL181W/ynl181wΔ* deletion strain was
487 previously used to study its function in chemogenomic investigations (10, 16). We were
488 especially interested in the effects of chemical CMB4166 in these studies because our data

489 revealed that the strain sensitivity profile for that compound (16) most closely resembled that
490 of sr7575 (Fig. 2C, D, E). A striking finding from this comparison was that the heterozygous
491 deletion strain of *YNL181W* was among the strains most affected by CMB4166 (Fig. 3A),
492 indicating that the absence of Ynl181w sensitizes yeast to both sr7575 and CMB4166.

493 A DAmP modification of *YNL181W* has been previously combined with a large-scale
494 genetic screen to identify mutations that synergize with loss of *YNL181W* function (42). A
495 comparison of the sr7575 sensitivity profile with the results of this genetic screen identified
496 several ERAD mutants that were affected by both DAmP modification of *YNL181W* and by
497 treatment with sr7575, including *UBC7*, *CUE1*, and *RPN4* (Fig. 6B). The connection to *UBC7*
498 was particularly remarkable because a similar synergistic growth defect associated with
499 *ynl181w*-DAmP and *ubc7* Δ mutation was observed in two other large-scale studies (17, 57).
500 The correlation between Ynl181w mutation and sr7575 sensitivity obtained by
501 chemogenomics was confirmed on plates by showing that a *ynl181w*-DAmP mutant was
502 hypersensitive to sr7575 (Fig. 6C). We speculate that Ynl181w could be involved in processes
503 that are targeted by sr7575.

504 In conclusion, we report the identification of a novel compound that has activity against
505 both *S. cerevisiae* and *A. fumigatus*. The data are consistent with a model for sr7575 action in
506 which the compound disrupts the structure of one or more proteins in the ER lumen or
507 membrane, resulting in a situation that necessitates ERAD intervention to eliminate the
508 abnormal protein(s) but does not require UPR activation. These findings underscore the
509 importance of ER homeostasis to the growth fungi and suggest the presence of fungal-specific
510 ER processes that could represent new opportunities for antifungal intervention.

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723 **Figure legends**

724 **Fig. 1:** Identification of a compound with broad antifungal activity. **(A)** Selection of a
725 new antifungal, sr7575, through a chemical library screen of *A. fumigatus* growth inhibition
726 was followed by chemogenomic profiling in *S. cerevisiae* to identify a potential mechanism of
727 action. sr7575 inhibited growth of various fungi, including *A. flavus* (48 h, 37°C, RPMI
728 medium, 5 µg/mL) **(B)**; *C. albicans* and *C. neoformans* (24 h, 37°C, SC medium, 2 µg/mL)
729 **(C)**; *S. cerevisiae* (48 h, 30°C, SC medium, 1 µg/mL) **(D)**.

730 **Fig. 2:** Chemogenomic profiling reveals an ERAD-enriched signature for sr7575
731 toxicity. **(A)** Distribution of relative growth values for *S. cerevisiae* mutant strains grown in
732 the presence of sr7575. Colors indicate functional categories from the pooled library with
733 genes annotated as ERAD (violet), protein translocation (grey), ER membrane complex
734 (EMC; pink), and vesicular traffic (green) showing the most sensitivity to sr7575 when
735 mutated. Note: *YML012C-A** overlaps *UBX2* and “#” indicates a DAmP strain; **(B)**
736 Distribution of sensitivity values for deletion strains affected for genes annotated with the GO
737 term 0030433, ERAD; **(C)** Pearson correlation coefficients between results obtained with
738 sr7575 and a published large scale chemogenomics data set identifies chemical 4166 as having
739 a profile that is most similar to sr7575. Only the scores for the top 100 correlated treatments
740 are displayed; **(D)** Same as **(C)** but with the Spearman rank correlation; **(E)** Comparison of the
741 fitness defect scores between sr7575 and chemical 4166; gene names are color coded as in
742 **(A)**.

743 **Fig. 3:** Summary of mutations conferring increased susceptibility to sr7575. **(A)**
744 Heatmap showing the unique ERAD signature of sr7575 compared with published
745 chemogenomic and SGA growth defect profiles; **(B)** Model showing the two main pathways
746 responsible for ERAD in fungi- Doa10 pathway (green) for clearing misfolded proteins with
747 cytosolic lesions, and the Hrd1 pathway (violet), which degrades misfolded proteins with
748 luminal or transmembrane lesions. Shared components (Ubx2, Ubc7, Cue1, and the Cdc48
749 complex) are denoted in gray or black.

750 **Fig. 4:** Sensitivity to sr7575 depends on EMC and ERAD components. **(A)** Serial 10-
751 fold dilutions of the WT and selected haploid deletion mutants were grown on SC plates in the
752 absence or presence of 0.25 $\mu\text{g/mL}$ sr7575 for 48 h at 30°C; **(B)** Complementation of sr7575
753 sensitivity for the strains shown in panel **(A)** was tested by using single copy plasmids
754 carrying the corresponding genes; **(C)** Strains lacking core UPR components, *HAC1* and *IRE1*
755 were tested for sensitivity against sr7575 at 0.5 $\mu\text{g/mL}$.

756 **Fig. 5:** The hypersensitivity of ERAD mutants to sr7575 is conserved in *A. fumigatus*.
757 **(a)** Conidia from *A. fumigatus* WT and deletion mutants were recovered in 0.05% Tween-
758 water and serial dilutions were spotted onto sr7575-containing RPMI 1640, pH 7.0. Plates
759 were incubated at 37°C for 72 h; **(B)** Analysis of UPR target gene expression (*bipA* and *tigA*)
760 by qRT-PCR. Cultures were treated with sr7575, DTT, or sr7575 for 1 h followed by DTT.
761 RNA was extracted and analyzed by qRT-PCR, using *tubA* mRNA for normalization. The
762 results of treated vs untreated samples from three independent experiments are shown.

763 **Fig. 6:** Ynl181w is an ER protein conserved in fungi and involved in adaptation to
764 sr7575. **(A)** T-Coffee alignment of the conserved short chain dehydrogenase region within Sc
765 Ynl181w (PFAM 54-187) and its orthologs in pathogenic fungi. Gene annotations with

766 number range indicate the position of the PFAM domain: *A. fumigatus* (Afu5g10790; 54-205),
767 *C. albicans* (orf19.6233; 58-204), *C. glabrata* (XP_448202; 54-193); **(B)** Scatter plot showing
768 the correlation between sr7575 sensitivity values and the previously published SGA scores for
769 ynl181w-DAmP; **(C)** Spot assays showing the difference in sensitivity to sr7575 and UPR
770 inducer tunicamycin (TM) for *ynl181w*-DAmP as compared with a strain defective for UPR
771 (*hac1Δ*).

772 **Supplementary Data synopsis**

773 **Fig. S1:** Synthetic pathways for sr7575 and related compounds.

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775 cells in liquid medium in the presence of various concentrations of sr7575.

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777 chemogenomics dataset.

778 **Fig. S4:** Perturbation of PGA3 function shows similarities with the sensitivity profile for
779 sr7575.

780 **Fig. S5:** Susceptibility testing of yeast strains against sr7575.

781 **Fig. S6:** Susceptibility of multidrug resistant *S. cerevisiae* strains and azole resistant *C.*
782 *albicans* strains to sr7575.

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784 more growth inhibition of *A.fumigatus* at 25 µg/mL.

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791 **Text S1:** Synthesis of sr7575 and related compounds.

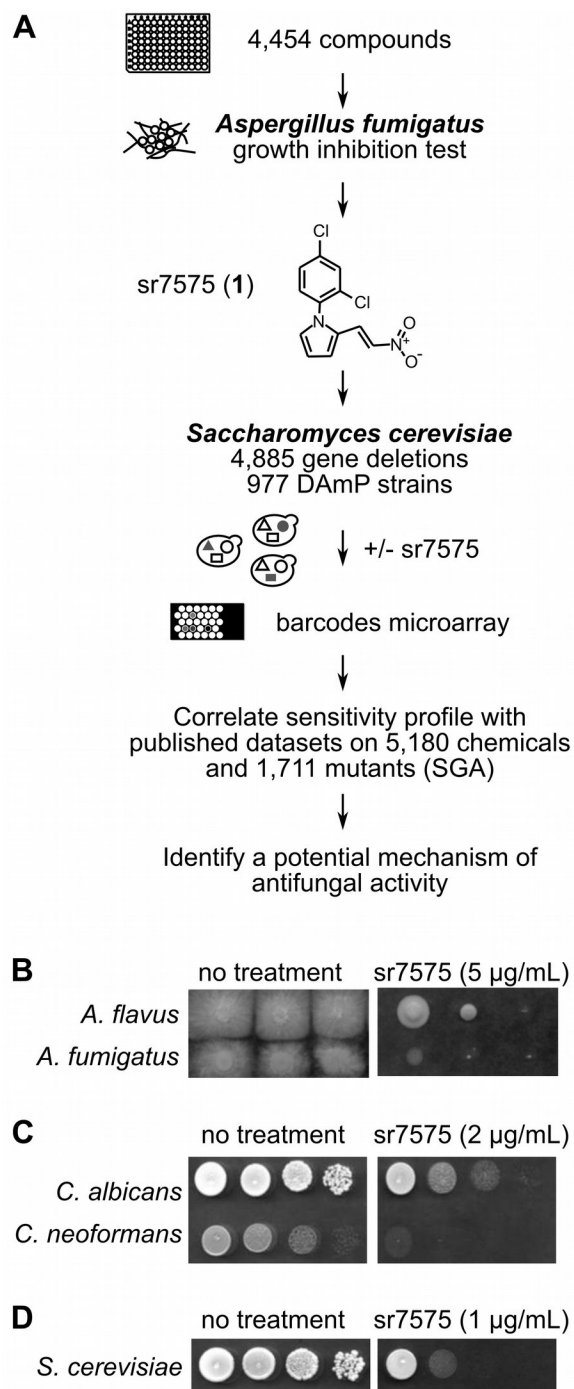


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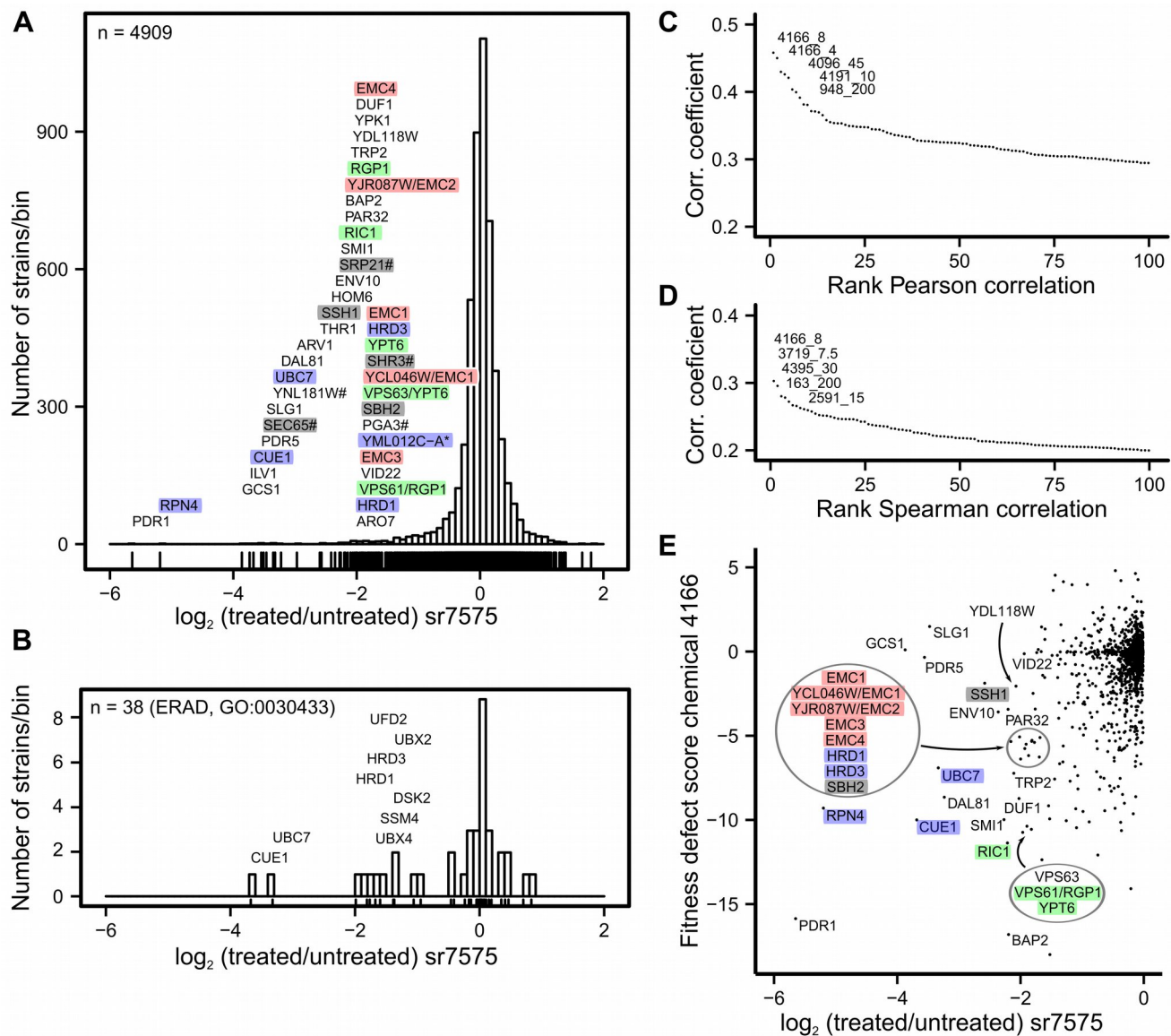


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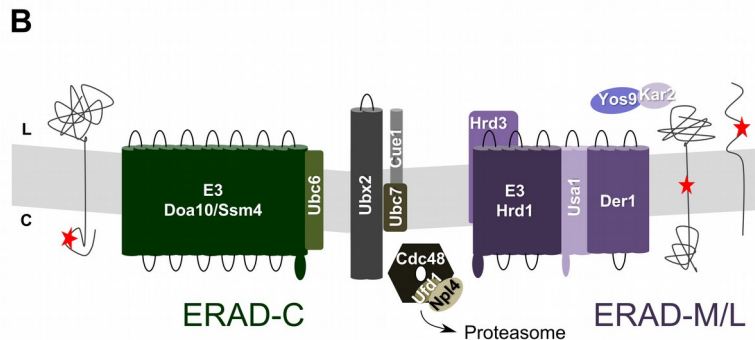
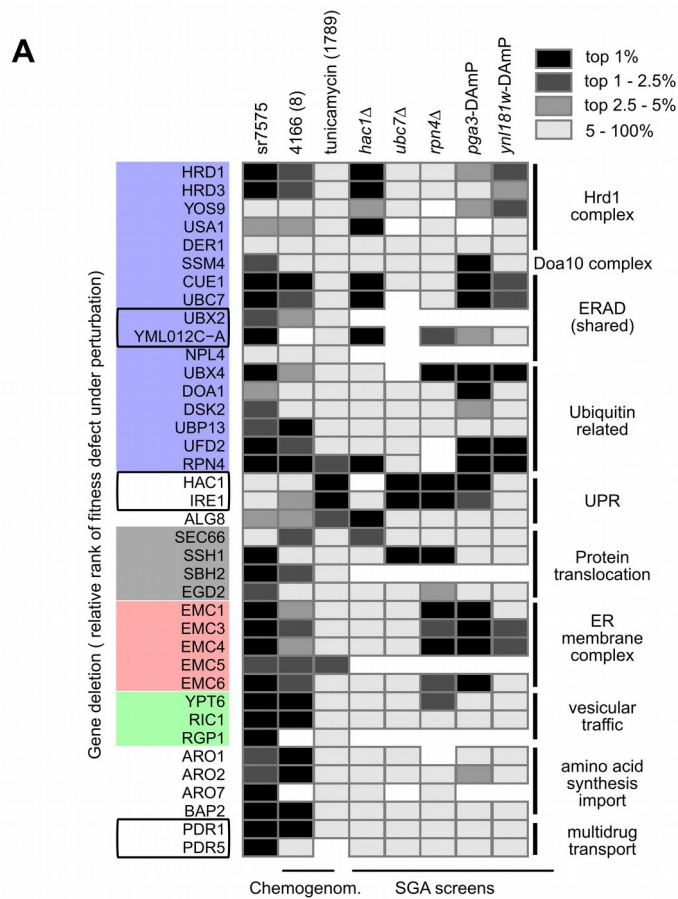


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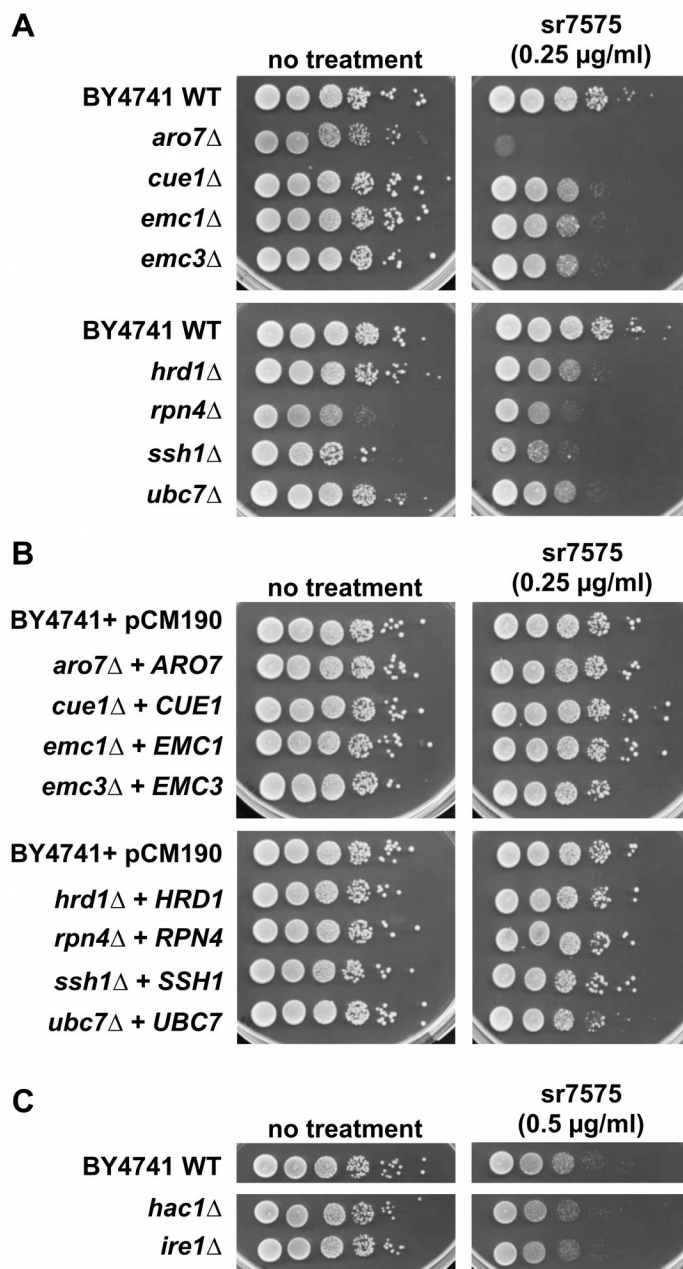


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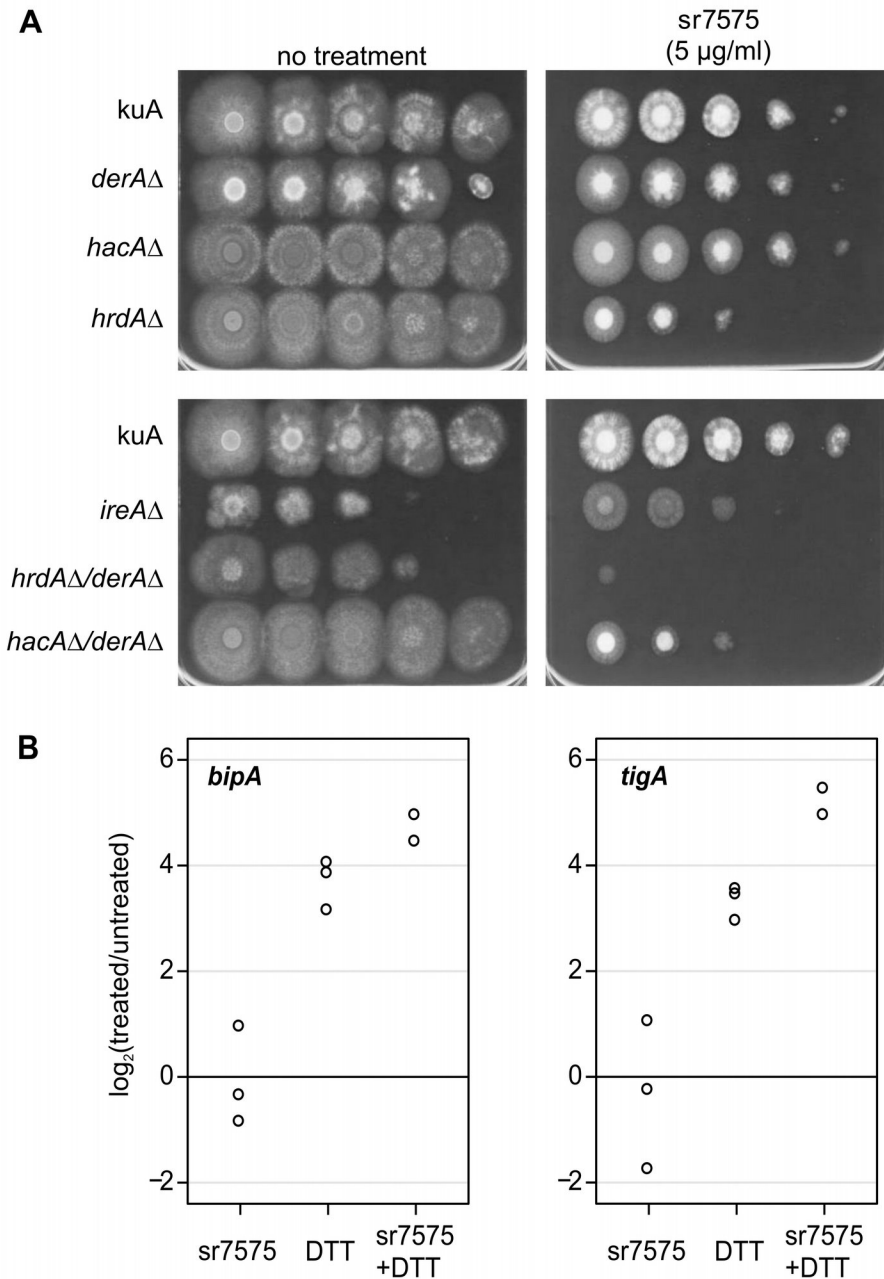


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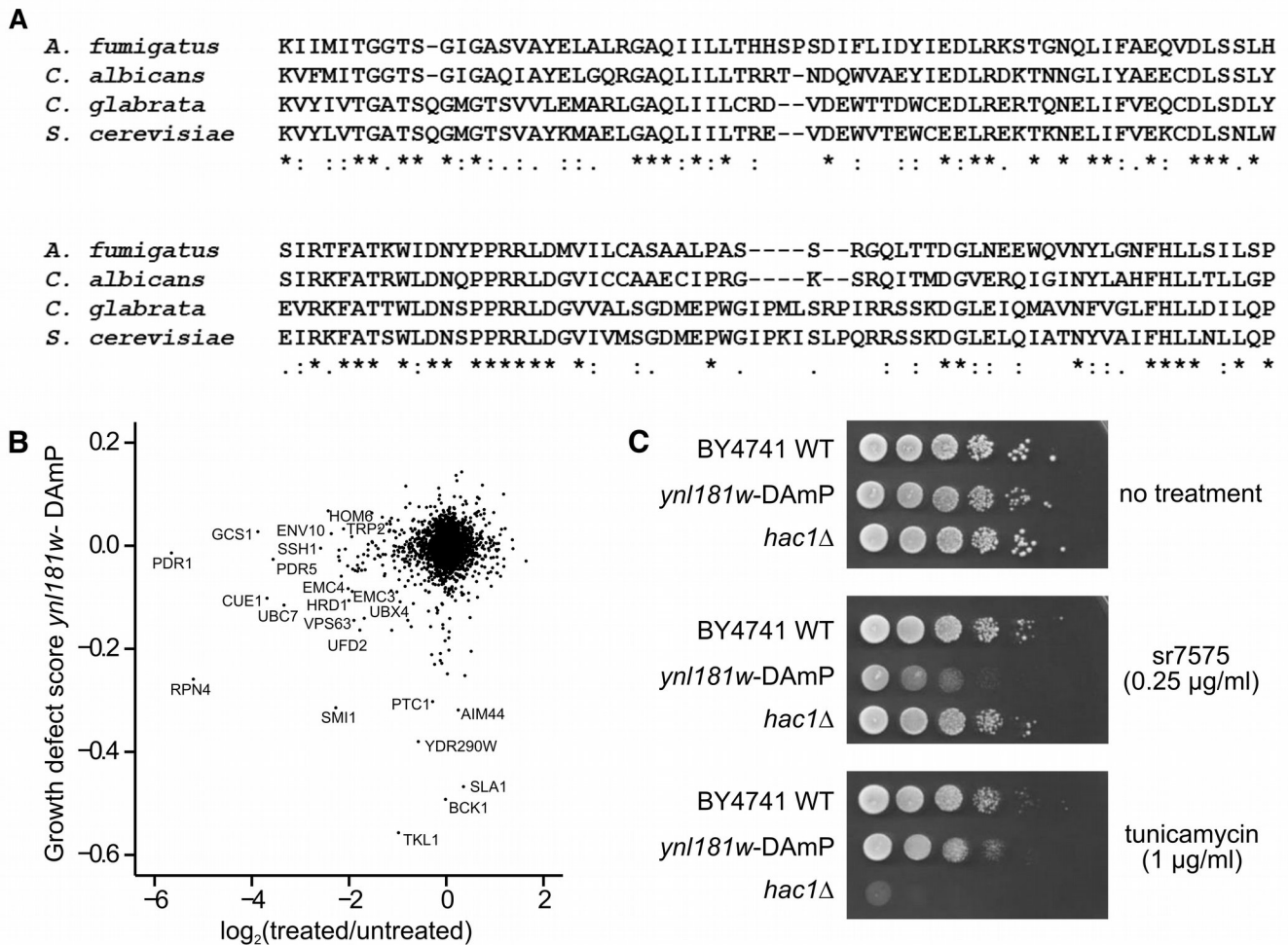


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Supplementary Material for Publication of manuscript "Toxicity of a novel antifungal compound is modulated by ERAD components", by Raj et al.

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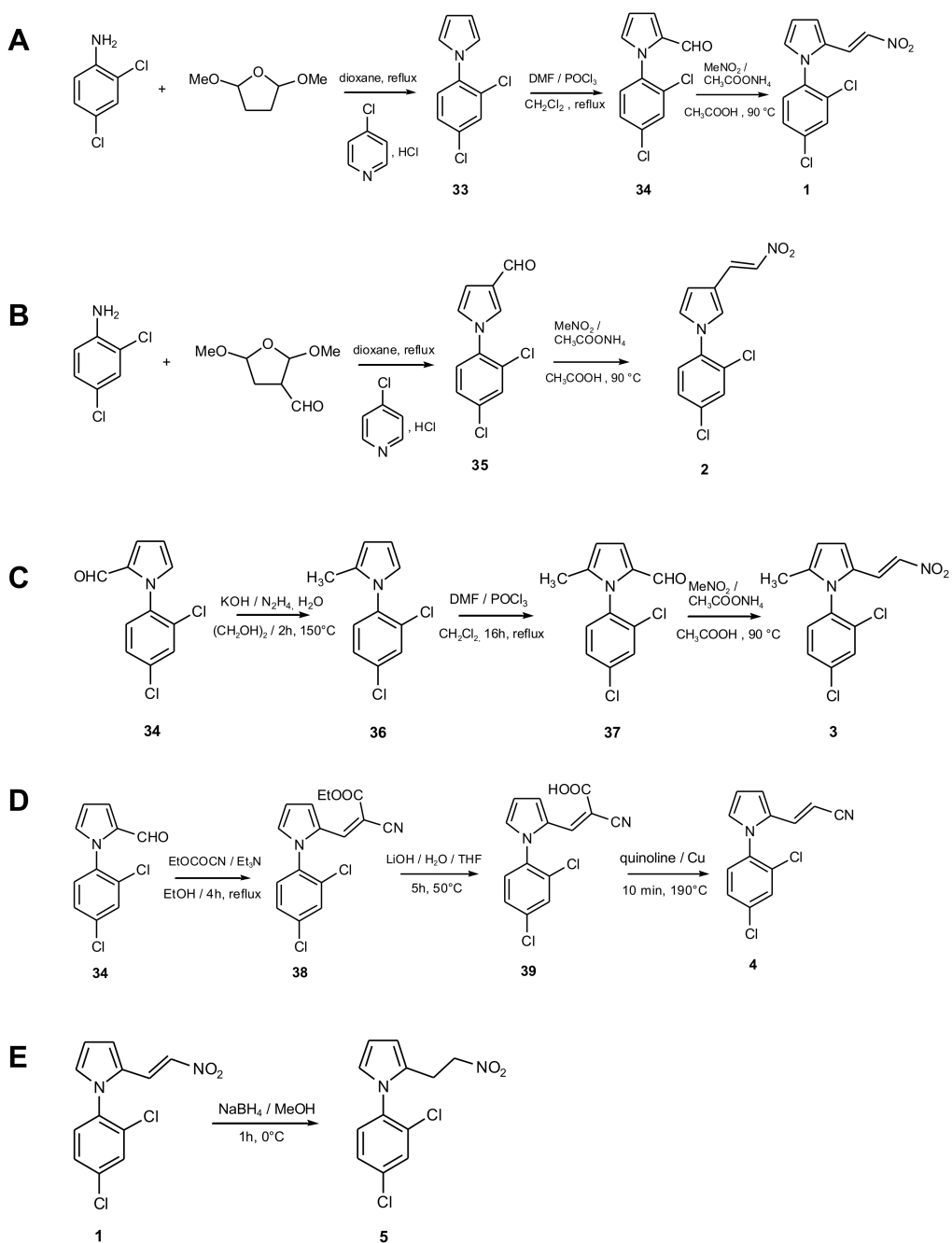


Figure S1. Synthetic pathways for sr7575 and related compounds. Pathways detailing the synthesis of sr7575 and analogues 2-5.

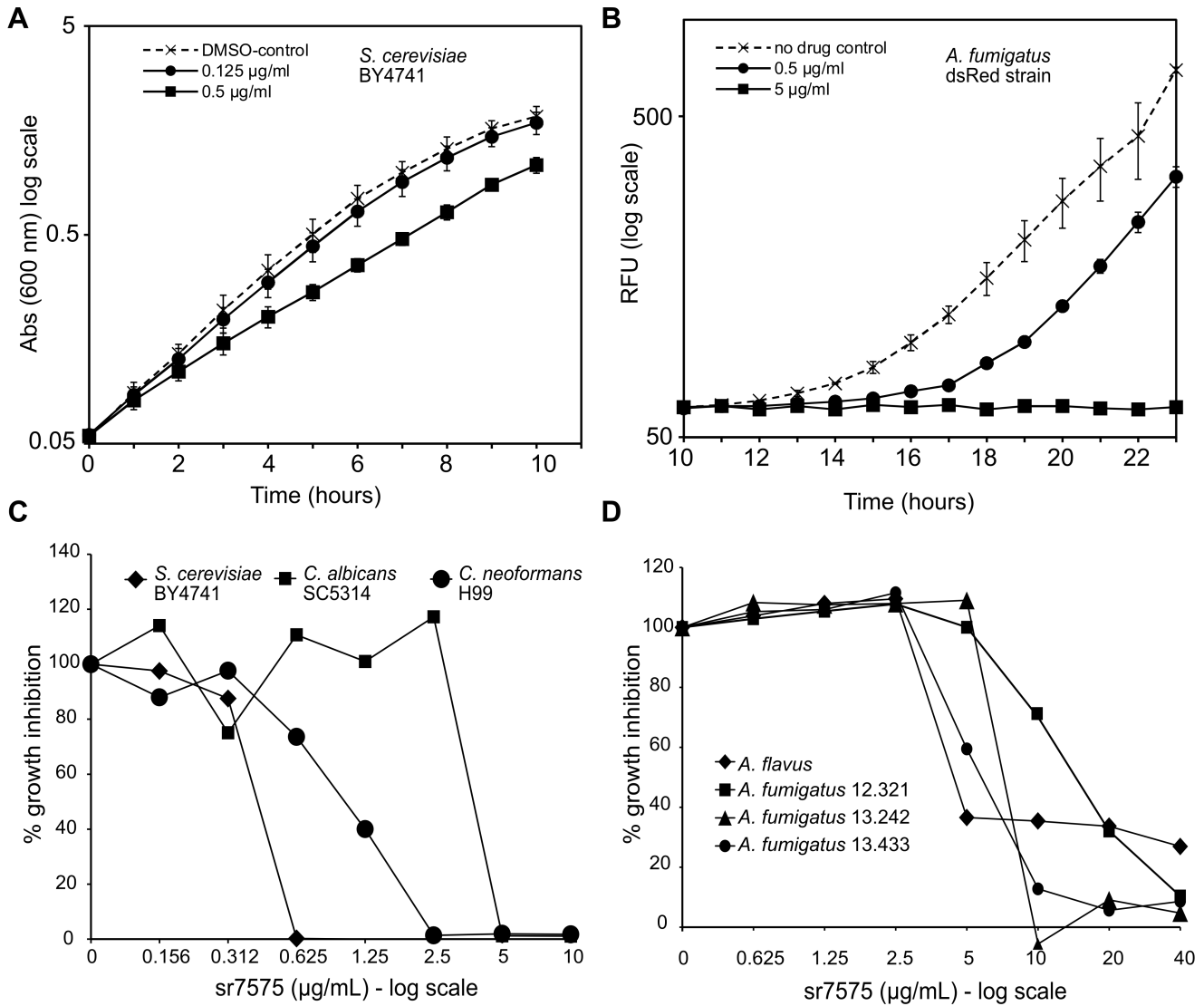


Figure S2. Growth of *A. fumigatus*, *A. flavus*, *S. cerevisiae*, *C. albicans* and *C. neoformans* cells in liquid medium in the presence of various concentrations of sr7575. (A) Log-phase cultures of *S. cerevisiae* WT strain BY4741 were grown in the presence of increasing concentrations of sr7575 with DMSO as vehicle control. The Abs₆₀₀ was determined every hour for 10 h. (B) *A. fumigatus* strain Af293-dsRed was grown for 23 h in RPMI-1640 medium in the presence of increasing concentrations of sr7575. Fluorescence (ex 254 nm/ em 291 nm) was measured and relative fluorescence units (RFU) plotted against time. (C) Growth inhibition estimates were obtained at various concentrations of sr7575 by measuring absorbance at 600 nm for *S. cerevisiae* (BY4741, YPD, 30°C, 48 h), *C. albicans* (SC5314, RPMI, 37°C, 48 h) and *C. neoformans* (H99, RPMI, 37°C, 72 h). (D) Growth inhibition estimates for *A. flavus* and three *A. fumigatus* clinical isolates (12.321, 13.242, 13.433) were obtained by the resazurin reduction assay in RPMI medium, 37°C, 39 h at concentrations of sr7575 up to 40 $\mu\text{g/ml}$.

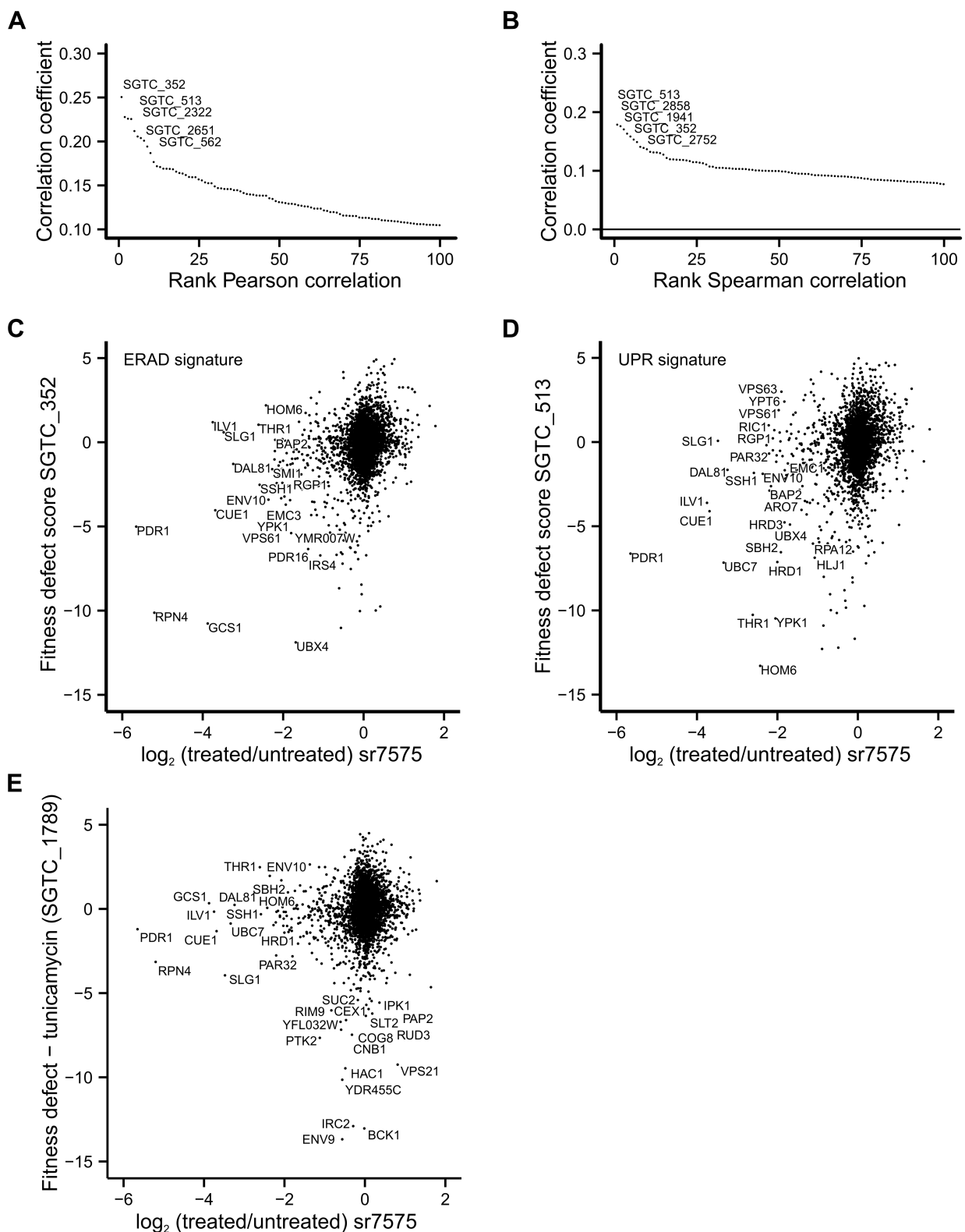


Figure S3. sr7575 profile shows little correlation with a previously published large-scale chemogenomics dataset. Computed Pearson (A) and Spearman (B) correlation coefficients between sr7575 values and previously published growth scores obtained with 3,356 compounds were ranked in descending order and the top 100 values are indicated. Among the highest correlations, we identified SGTC 352, a drug showing an ERAD signature (C) and SGTC 513, a compound with a UPR signature

(D) as being closest to the sr7575 profile. (E) The sr7575 profile showed no correlation with the one published for tunicamycin.

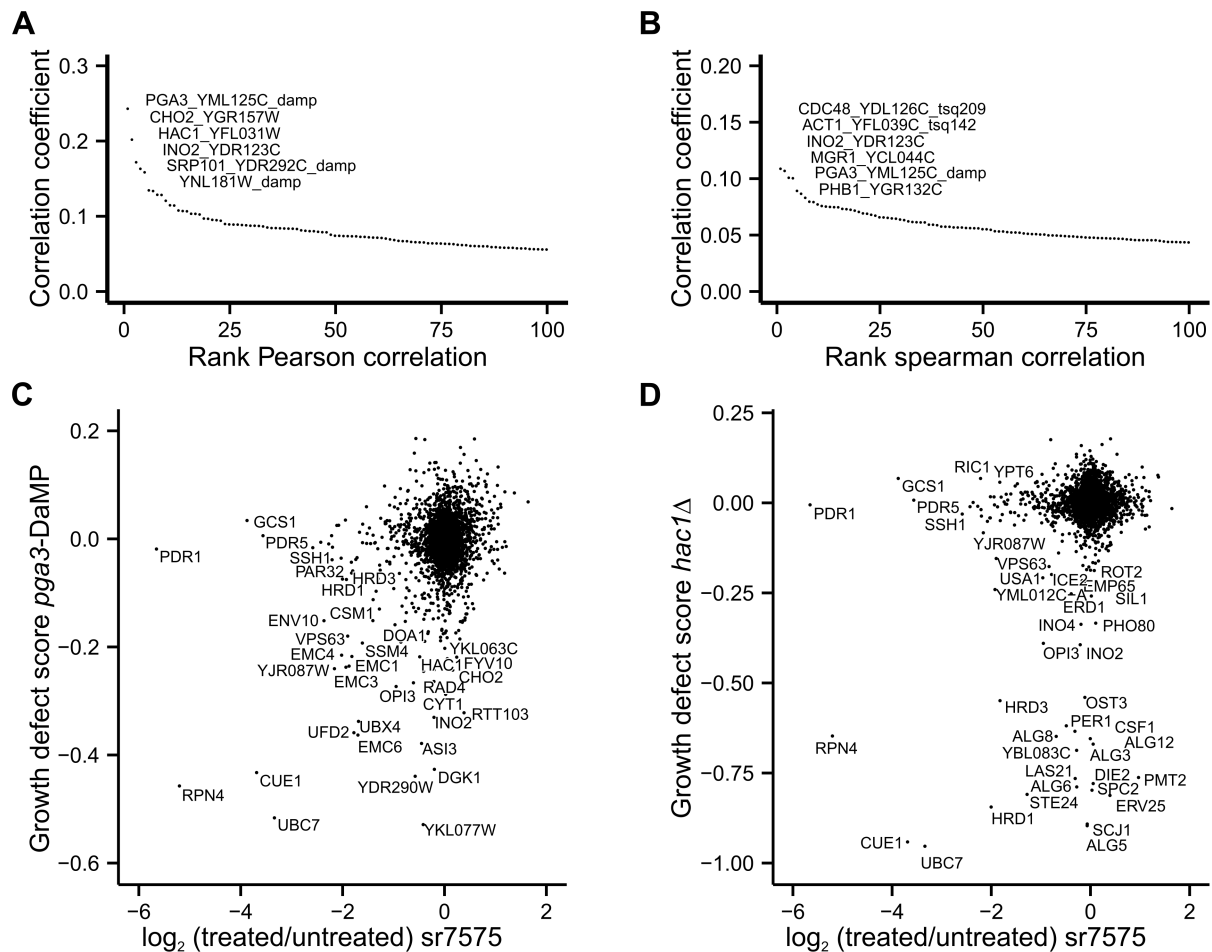


Figure S4. Perturbation of *PGA3* function shows similarities with the sensitivity profile for *sr7575*. Pearson (A) and Spearman (B) correlations between the *sr7575* profile and 1711 previously published SGA profiles. Fitness defect scores for DAMP modification of *PGA3* are shown in (C), while the interactions of *hac1* Δ and ERAD depleted strains are depicted in (D).

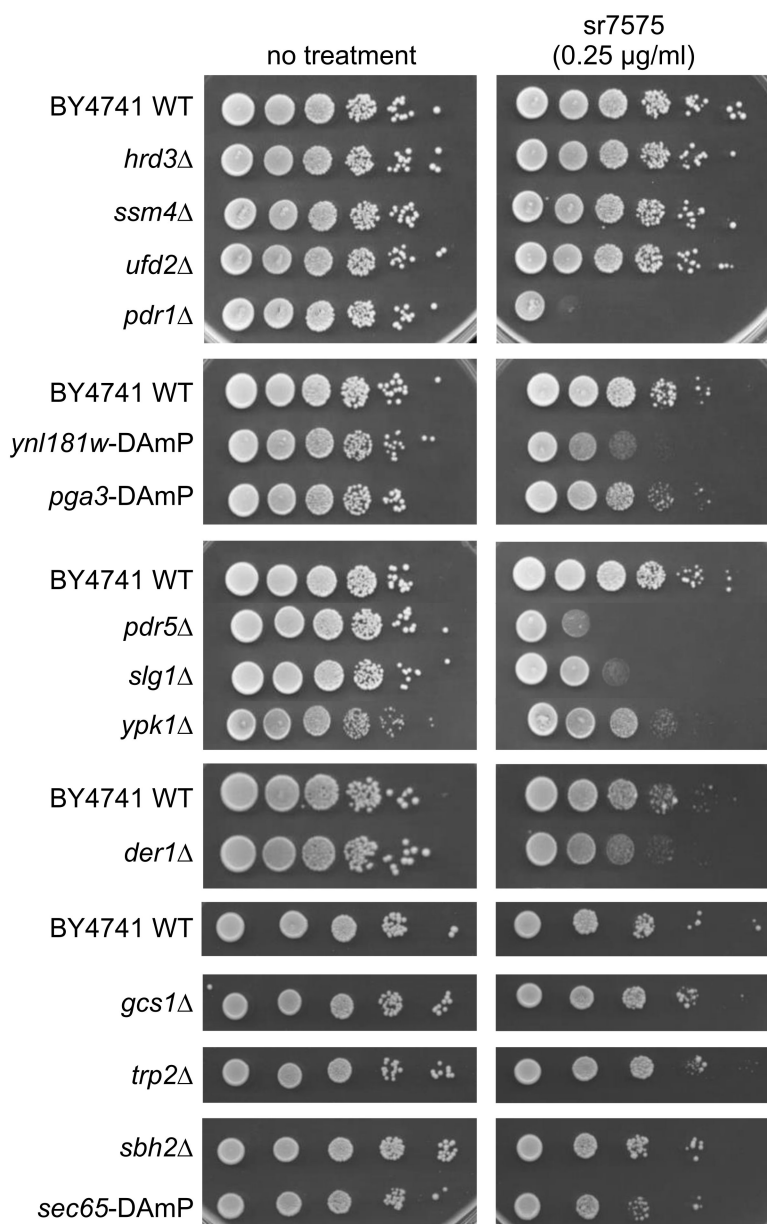


Figure S5. Susceptibility testing of yeast strains against sr7575. Mutants from the haploid deletion background were serially spotted onto SC plates supplemented with sr7575. Plates were incubated at 30°C for 48 h.

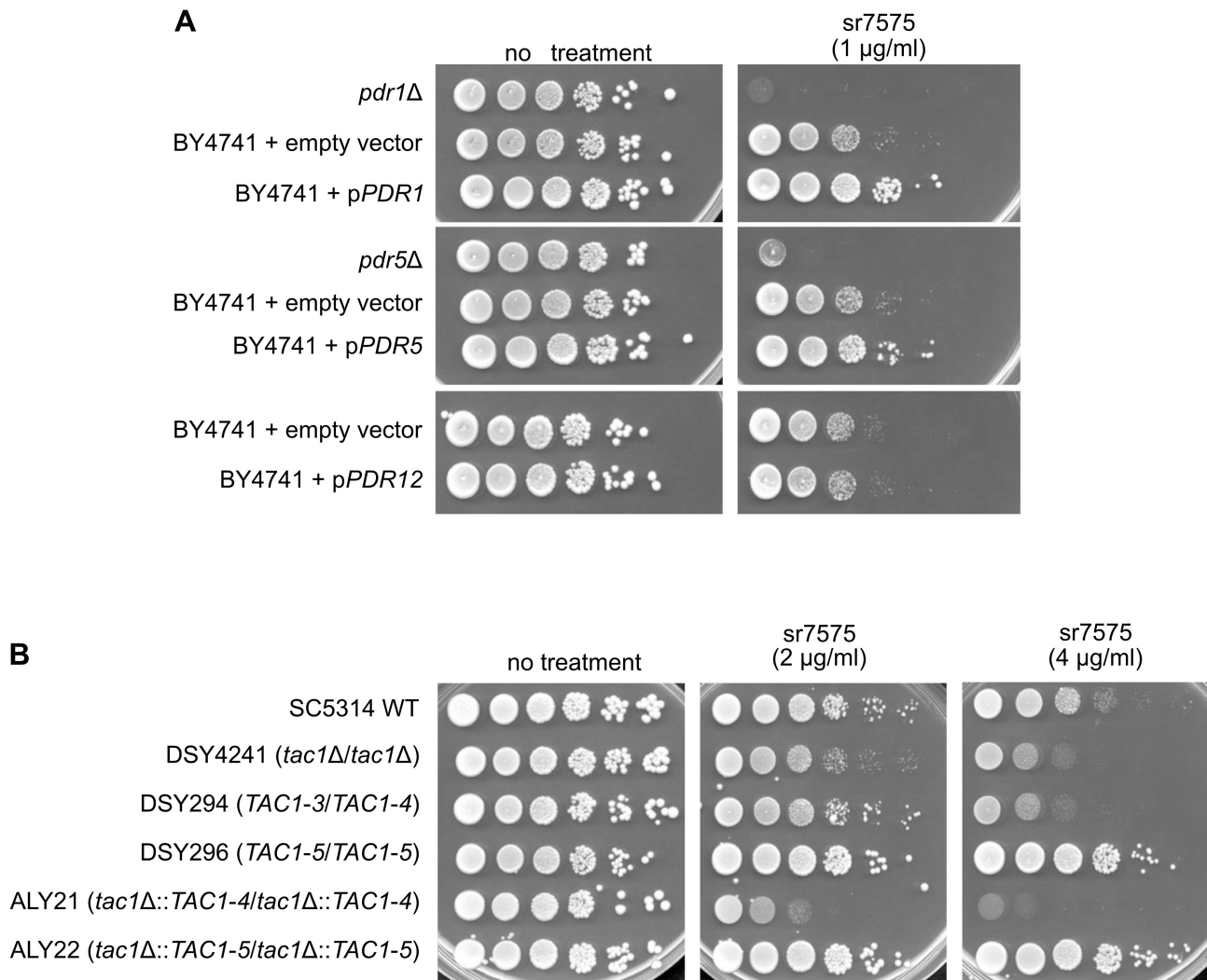


Figure S6. Susceptibility of multidrug resistant *S. cerevisiae* strains and azole resistant *C. albicans* strains to sr7575. (A) *S. cerevisiae* strains with deletions of or overexpressing *PDR1*, *PDR5* and *PDR12* were tested for susceptibility to sr7575 (1 µg/mL, SC medium, 30°C, 48 h). (B) Comparison of growth inhibition of *C. albicans* strains: WT (SC5314), *TAC1* transcription factor homozygous deletion strain (DSY4241), azole susceptible clinical isolate DSY294, azole resistant clinical isolate DSY296, azole susceptible laboratory generated strain ALY21 and azole resistant laboratory generated strain ALY22 (SC medium in the presence of 2 and 4 µg/mL sr7575, 37°C, 48 h).

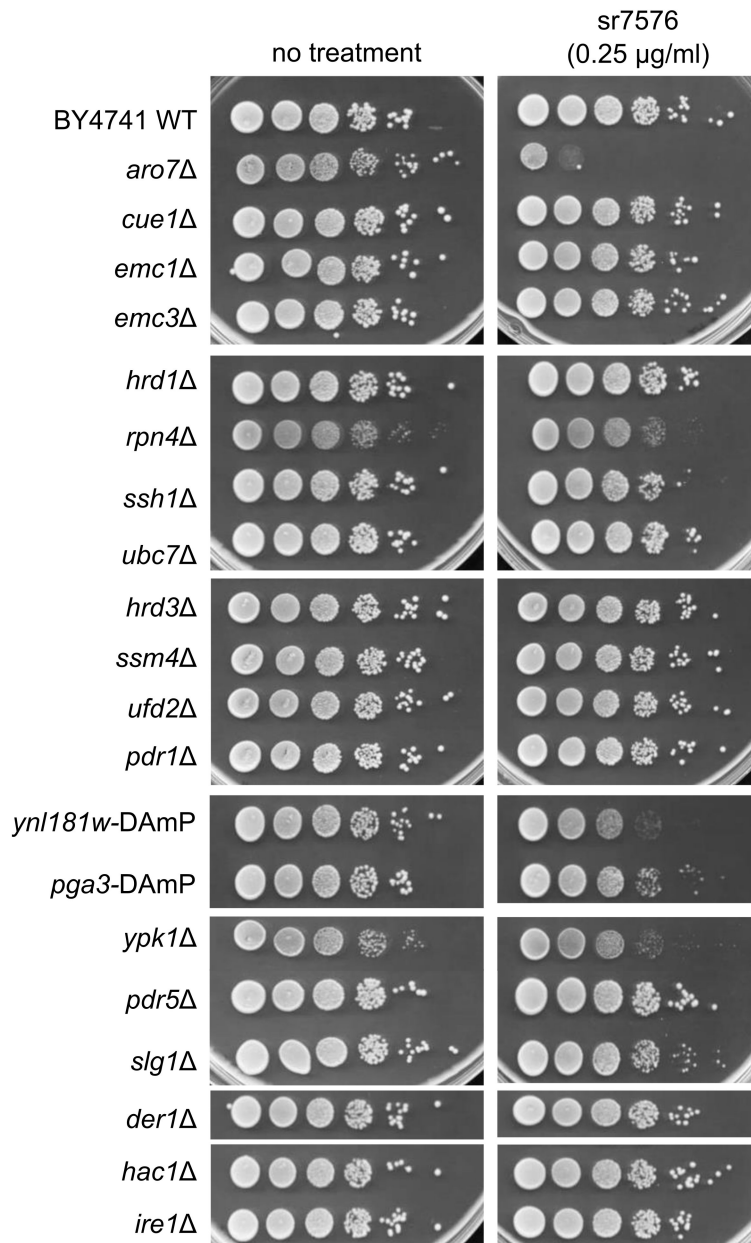


Figure S7. Response of yeast mutants against sr7576. Serial 10-fold dilutions of the WT and haploid deletion mutants were spotted onto SC plates supplemented with sr7576 (0.25 $\mu\text{g/ml}$). Plates were monitored for growth at 30°C for 48 h.

Supplementary Table S4 - Strains and plasmids used in this study

Strain	Genotype	Reference
BY4741	MATa;his3Δ 1;leu2Δ 0;met15Δ 0;ura3Δ 0	Brachmann et al, 1998
(Deletion mutants were generated in the Sc BY4741 background)		
<i>aro7</i> Δ	<i>aro7</i> ::KanMX4	Reference Giaever et al, 2002
<i>cue1</i> Δ	<i>cue1</i> ::KanMX4	
<i>der1</i> Δ	<i>der1</i> ::KanMX4	
<i>doa10</i> Δ	<i>doa10</i> ::KanMX4	
<i>emc1</i> Δ	<i>emc1</i> ::KanMX4	
<i>emc3</i> Δ	<i>emc3</i> ::KanMX4	
<i>gcs1</i> Δ	<i>gcs1</i> ::KanMX4	
<i>hac1</i> Δ	<i>hac1</i> ::KanMX4	
<i>hrd1</i> Δ	<i>hrd1</i> ::KanMX4	
<i>hrd3</i> Δ	<i>hrd3</i> ::KanMX4	
<i>ire1</i> Δ	<i>ire1</i> ::KanMX4	
<i>pdr1</i> Δ	<i>pdr1</i> ::KanMX4	
<i>pdr5</i> Δ	<i>pdr5</i> ::KanMX4	
<i>pga3</i> -DAmP	<i>pga3</i> -DAmP (KanMX4)	
<i>rpn4</i> Δ	<i>rpn4</i> ::KanMX4	
<i>sbh2</i> Δ	<i>sbh2</i> ::KanMX4	
<i>sec65</i> -DAMP	<i>sec65</i> -DAmP (KanMX4)	
<i>slg1</i> Δ	<i>slg1</i> ::KanMX4	
<i>ssh1</i> Δ	<i>ssh1</i> ::KanMX4	
<i>trp2</i> Δ	<i>trp2</i> ::KanMX4	
<i>ubc7</i> Δ	<i>ubc7</i> ::KanMX4	
<i>ufd2</i> Δ	<i>ufd2</i> ::KanMX4	
<i>ynl181w</i> -DAmP	<i>ynl181w</i> -DAmP (KanMX4)	
<i>ypk1</i> Δ	<i>ypk1</i> ::KanMX4	
A. fumigatus strains used in this study:		
Strain	Genotype	Reference
kuA	<i>akuA</i> :: <i>ptrA</i>	Krappmann et al, 2006
<i>derA</i> Δ	<i>akuA</i> :: <i>ptrA</i> , <i>derA</i> :: <i>hph</i>	Richie DL et al 2011
<i>hacA</i> Δ	<i>akuA</i> :: <i>ptrA</i> , <i>hacA</i> :: <i>hph</i>	Richie DL et al 2009
<i>hrdA</i> Δ	<i>akuA</i> :: <i>ptrA</i> , <i>hrdA</i> :: <i>hph</i>	Krishnan K et al 2013
<i>ireA</i> Δ	<i>akuA</i> :: <i>ptrA</i> , <i>ireA</i> :: <i>ble</i>	Jeng X et al 2011
<i>derA</i> Δ/ <i>hacA</i> Δ	<i>akuA</i> :: <i>ptrA</i> , <i>hacA</i> :: <i>hph</i> , <i>derA</i> :: <i>ble</i>	Richie DL et al 2011
<i>derA</i> Δ/ <i>hrdA</i> Δ	<i>akuA</i> :: <i>ptrA</i> , <i>hrdA</i> :: <i>hph</i> , <i>derA</i> :: <i>ble</i>	Krishnan K et al 2013
Other yeast strains used in this study:		
Strain	Genotype	Reference
<i>C. albicans</i> SC5314	wild type	Lohberger et al, 2014
DSY4241	<i>tac1</i> Δ::FRT/ <i>tac1</i> Δ::FRT	
DSY294	azole susceptible clinical isolate (<i>TAC1</i> -3/ <i>TAC1</i> -4)	
DSY296	azole resistant clinical isolate (<i>TAC1</i> -5/ <i>TAC1</i> -5 ; N977D mutation)	
ALY21	<i>tac1</i> Δ::TAC1-4-FRT/ <i>tac1</i> Δ::TAC1-4-FRT	
ALY22	<i>tac1</i> Δ::TAC1-5-FRT/ <i>tac1</i> Δ::TAC1-5-FRT	
<i>C. neoformans</i> H99	wild type	
MoBY plasmid (library v1.1) complemented S. cerevisiae strains:		
Strain	Genotype+ MoBY clone identifier	Reference
<i>aro7</i> Δ+ ARO7	<i>aro7</i> ::KanMX4+ YPR060C::29NP_C9	Ho et al, 2009
<i>cue1</i> Δ+ CUE1	<i>cue1</i> ::KanMX4+ YMR264W::33NP_H12	
<i>emc1</i> Δ+ EMC1	<i>emc1</i> ::KanMX4+ YCL045C::41NP_D8	
<i>emc3</i> Δ+ EMC3	<i>emc3</i> ::KanMX4+ YKL207W::8NP_A12	
<i>hrd1</i> Δ+ HRD1	<i>hrd1</i> ::KanMX4+ YOL013C::12NP_G12	
<i>rpn4</i> Δ+ RPN4	<i>rpn4</i> ::KanMX4+ YDL020C::30NP_F2	
<i>ssh1</i> Δ+ SSH1	<i>ssh1</i> ::KanMX4+ YBR283C::37NP_A11	
<i>ubc7</i> Δ+ UBC7	<i>ubc7</i> ::KanMX4+ YMR022W::36NP_G3	
YGPM systematic overexpression library in S. cerevisiae strains:		
Strain	Genotype+ YGPM clone identifier	Reference
Control	BY4741+ YGPM22k06 chrIII:151898...152647	Jones et al, 2008
PDR1	BY4741+ YGPM26h12 chrVII:466658...477209	
PDR5	BY4741+ YGPM33k24 chrXV:619141...631341	
PDR12	BY4741+ YGPM8p07 chrXVI:444386...454435	

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Supplementary Material for Publication of manuscript "Toxicity of a novel antifungal compound is modulated by ERAD components", by Raj et al.

Fig. S1: Synthetic pathways for sr7575 and related compounds.

Fig. S2: Growth of *A. fumigatus*, *A. flavus*, *S. cerevisiae*, *C. albicans* and *C. neoformans* cells in liquid medium in the presence of various concentrations of sr7575.

Fig. S3: sr7575 profile shows little correlation with a previously published large-scale chemogenomics dataset.

Fig. S4: Perturbation of PGA3 function shows similarities with the sensitivity profile for sr7575.

Fig. S5: Susceptibility testing of yeast strains against sr7575.

Fig. S6: Susceptibility of multidrug resistant *S. cerevisiae* strains and azole resistant *C. albicans* strains to sr7575.

Table S1: List of 76 compounds from the CERMN chemical library showing 90% or more growth inhibition of *A. fumigatus* at 25 µg/mL.

Table S2: Analogues of sr7575 and MIC₁₀₀ values against *A. fumigatus*.

Table S3: Analogues of sr7576 and MIC₁₀₀ values against *A. fumigatus*.

Table S4: List of strains and plasmids used in this study.

Table S5: List of oligonucleotides used in this study.

Table S6 (provided as a separate xls file): Sensitivity of *S. cerevisiae* deletion and DAmP strains to 0.125 µg/mL sr7575.

Text S1: Synthesis of sr7575 and related compounds.

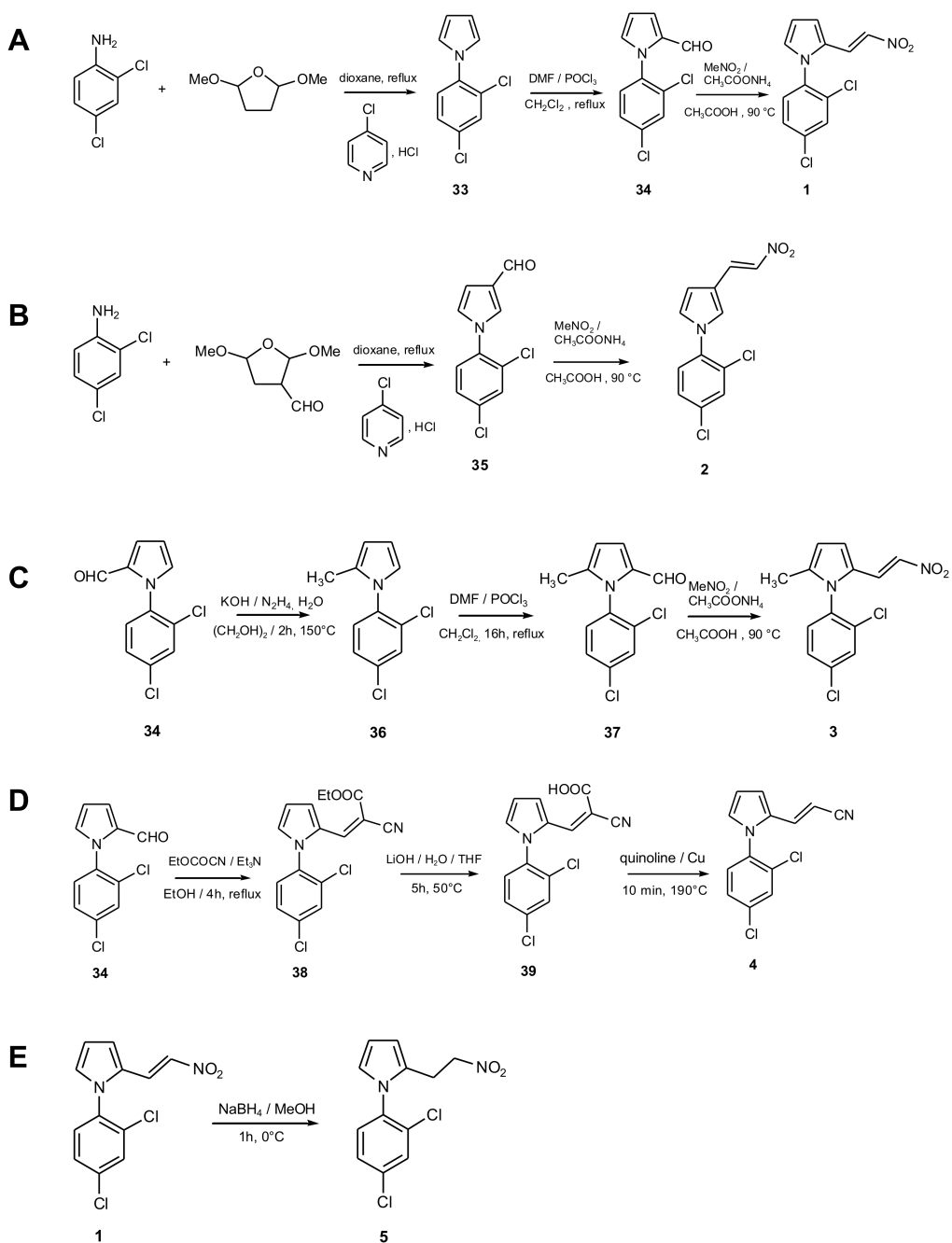


Figure S1. Synthetic pathways for sr7575 and related compounds. Pathways detailing the synthesis of sr7575 and analogues 2-5.

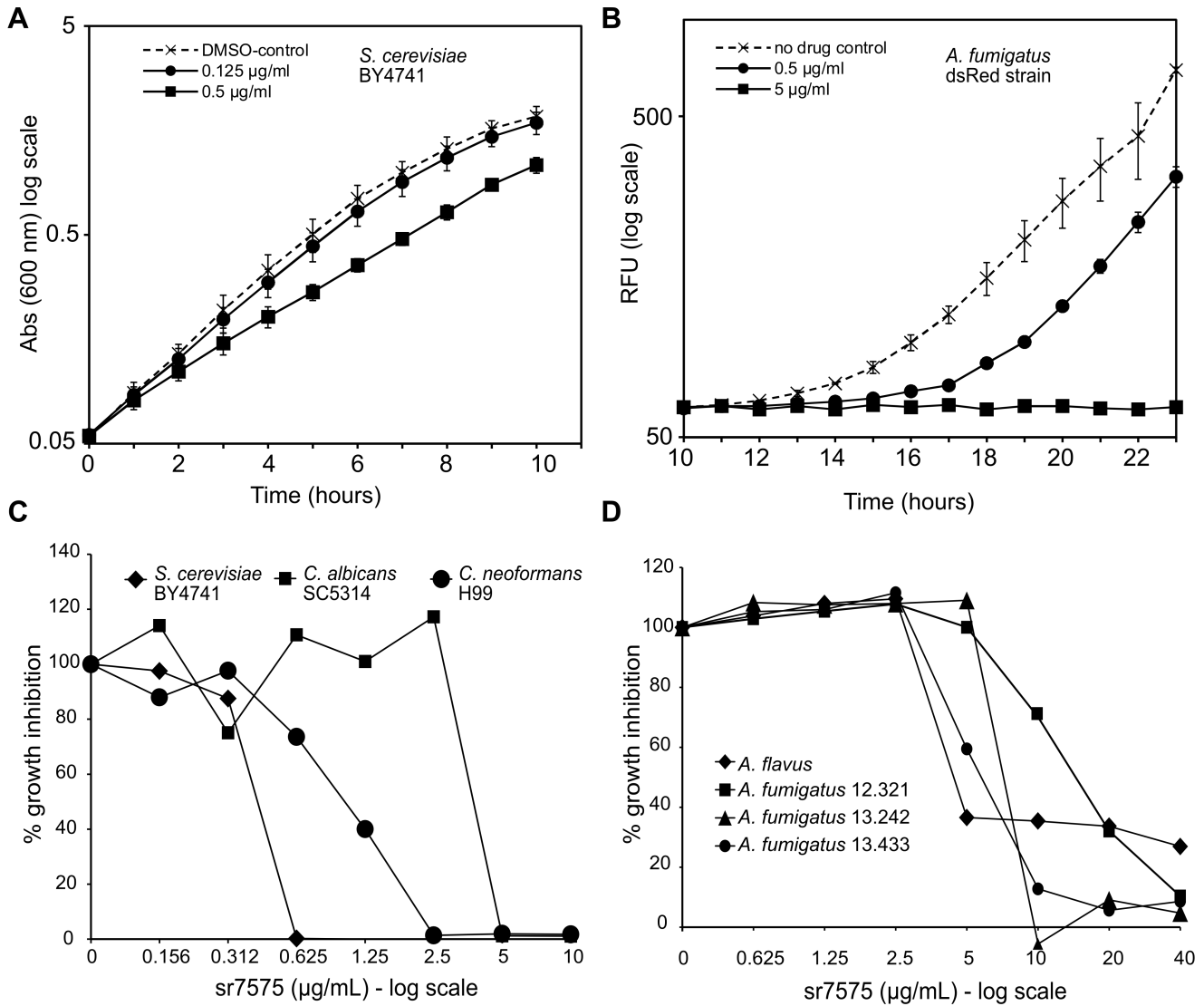


Figure S2. Growth of *A. fumigatus*, *A. flavus*, *S. cerevisiae*, *C. albicans* and *C. neoformans* cells in liquid medium in the presence of various concentrations of sr7575. (A) Log-phase cultures of *S. cerevisiae* WT strain BY4741 were grown in the presence of increasing concentrations of sr7575 with DMSO as vehicle control. The Abs₆₀₀ was determined every hour for 10 h. (B) *A. fumigatus* strain Af293-dsRed was grown for 23 h in RPMI-1640 medium in the presence of increasing concentrations of sr7575. Fluorescence (ex 254 nm/ em 291 nm) was measured and relative fluorescence units (RFU) plotted against time. (C) Growth inhibition estimates were obtained at various concentrations of sr7575 by measuring absorbance at 600 nm for *S. cerevisiae* (BY4741, YPD, 30°C, 48 h), *C. albicans* (SC5314, RPMI, 37°C, 48 h) and *C. neoformans* (H99, RPMI, 37°C, 72 h). (D) Growth inhibition estimates for *A. flavus* and three *A. fumigatus* clinical isolates (12.321, 13.242, 13.433) were obtained by the resazurin reduction assay in RPMI medium, 37°C, 39 h at concentrations of sr7575 up to 40 $\mu\text{g/ml}$.

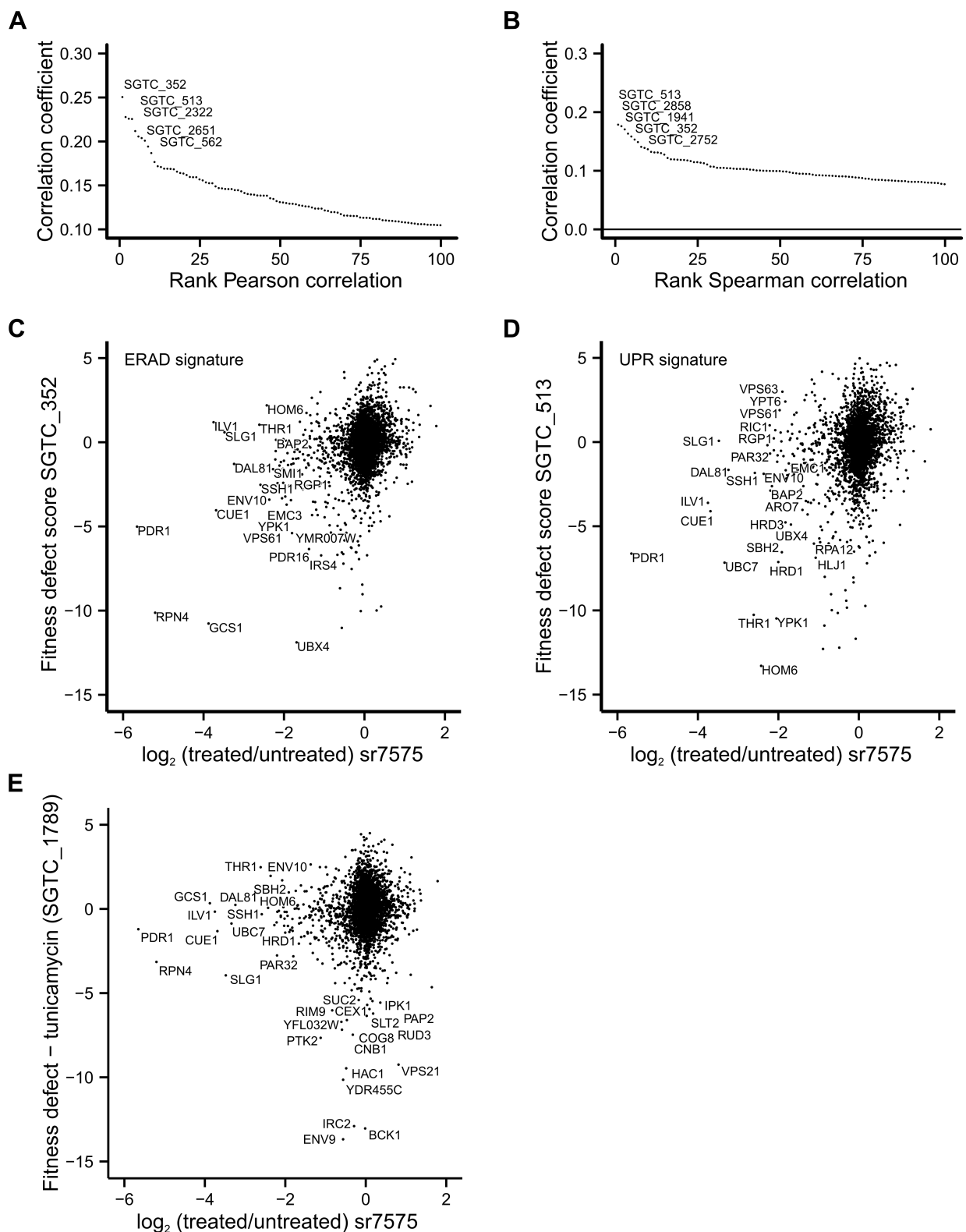


Figure S3. sr7575 profile shows little correlation with a previously published large-scale chemogenomics dataset. Computed Pearson (A) and Spearman (B) correlation coefficients between sr7575 values and previously published growth scores obtained with 3,356 compounds were ranked in descending order and the top 100 values are indicated. Among the highest correlations, we identified SGTC 352, a drug showing an ERAD signature (C) and SGTC 513, a compound with a UPR signature

(D) as being closest to the sr7575 profile. (E) The sr7575 profile showed no correlation with the one published for tunicamycin.

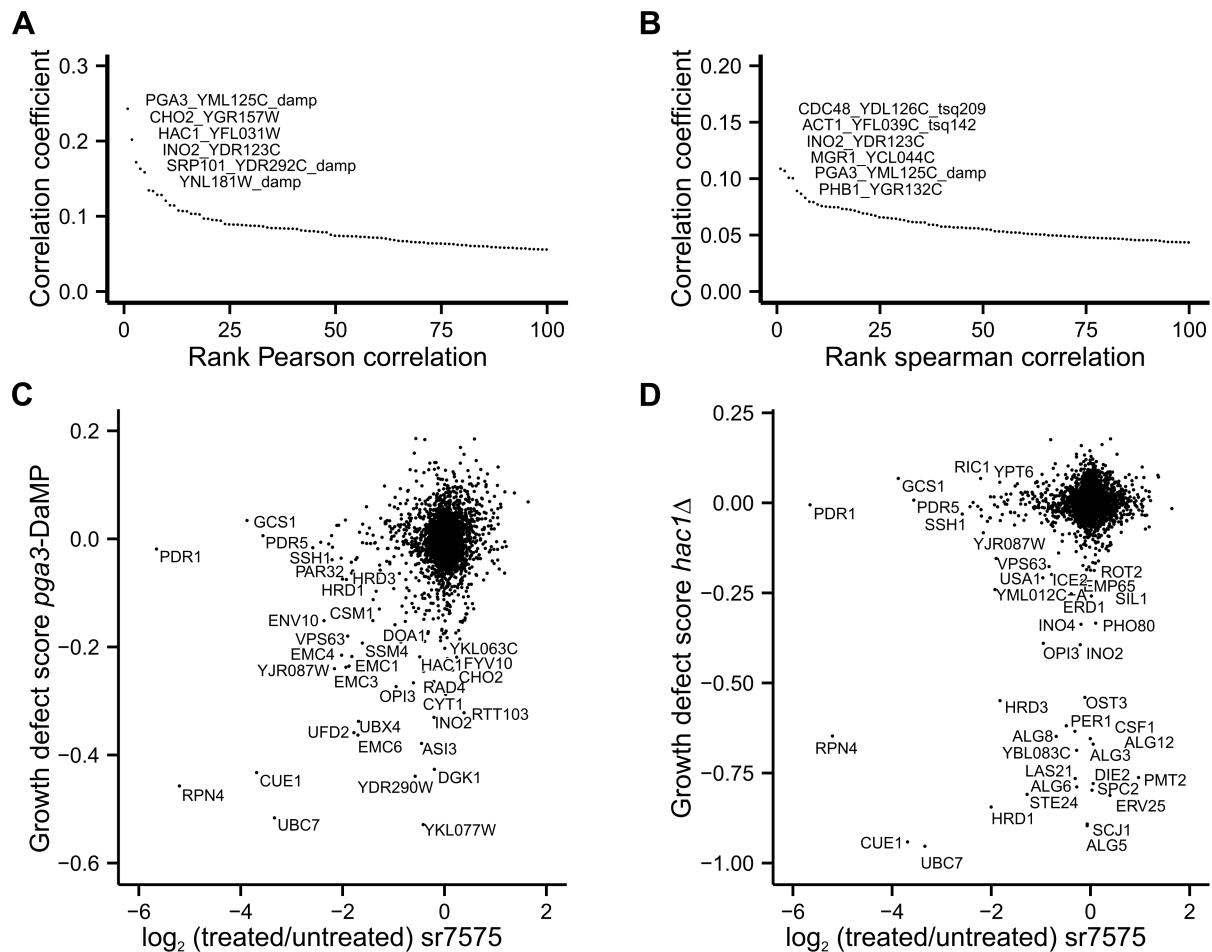


Figure S4. Perturbation of *PGA3* function shows similarities with the sensitivity profile for *sr7575*. Pearson (A) and Spearman (B) correlations between the *sr7575* profile and 1711 previously published SGA profiles. Fitness defect scores for DAMP modification of *PGA3* are shown in (C), while the interactions of *hac1* Δ and ERAD depleted strains are depicted in (D).

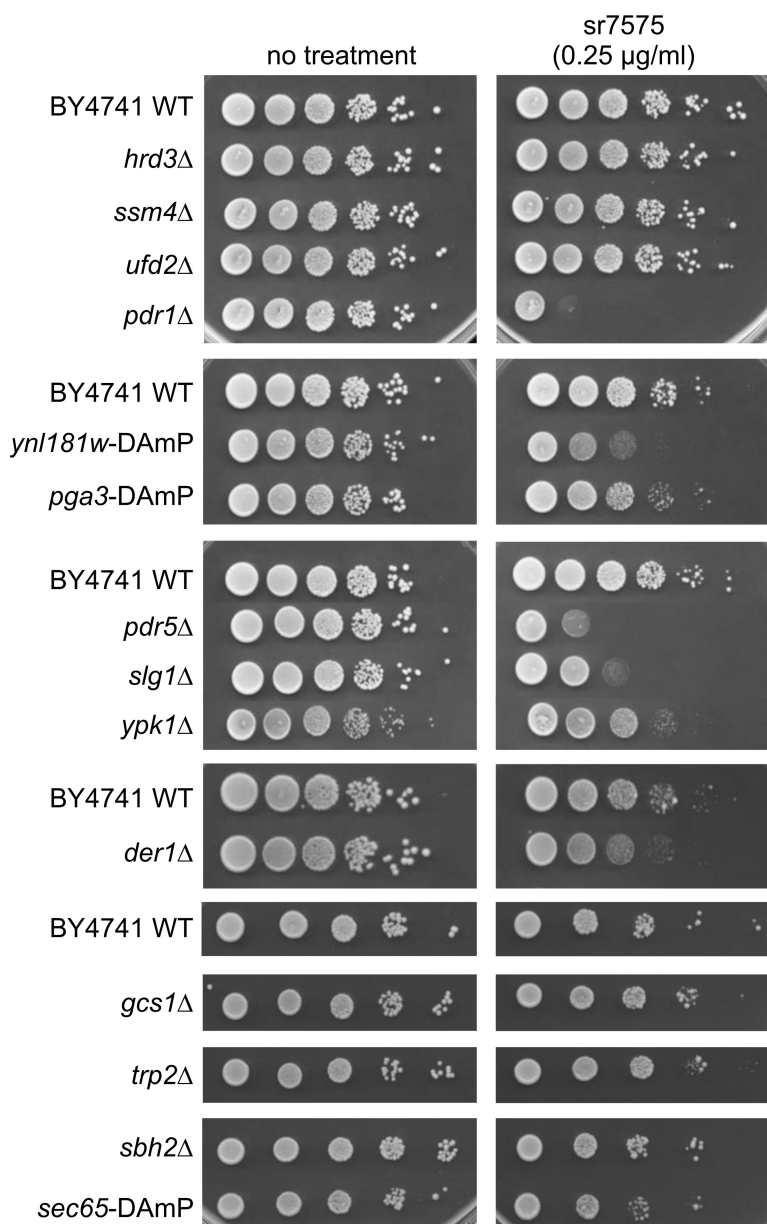


Figure S5. Susceptibility testing of yeast strains against sr7575. Mutants from the haploid deletion background were serially spotted onto SC plates supplemented with sr7575. Plates were incubated at 30°C for 48 h.

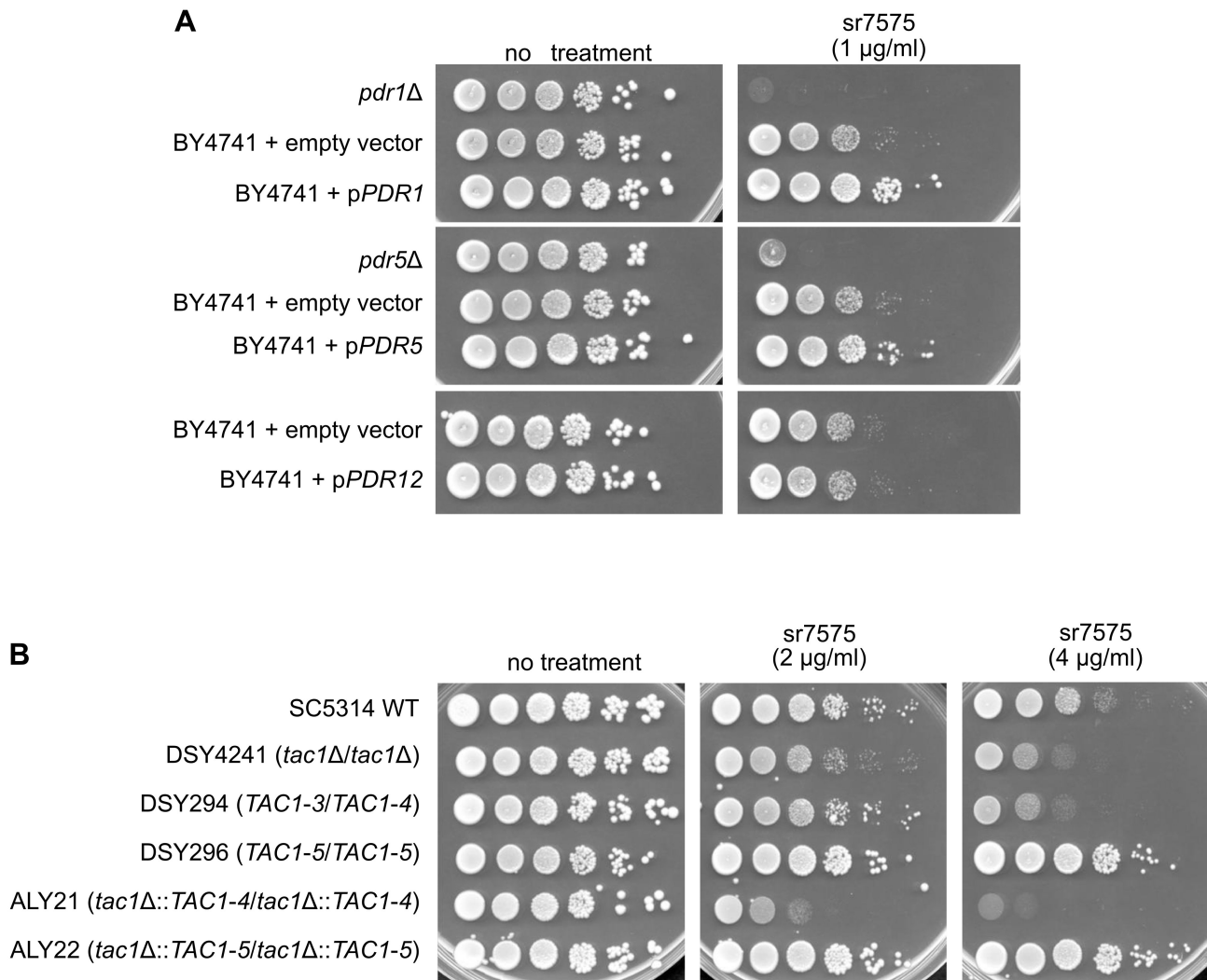
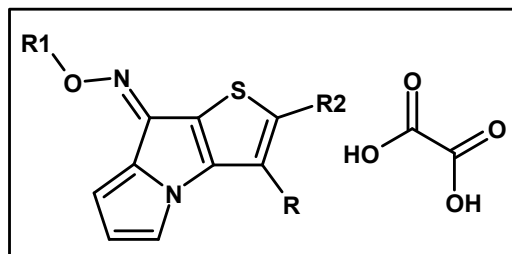


Figure S6. Susceptibility of multidrug resistant *S. cerevisiae* strains and azole resistant *C. albicans* strains to sr7575. (A) *S. cerevisiae* strains with deletions of or overexpressing *PDR1*, *PDR5* and *PDR12* were tested for susceptibility to sr7575 (1 µg/mL, SC medium, 30°C, 48 h). (B) Comparison of growth inhibition of *C. albicans* strains: WT (SC5314), *TAC1* transcription factor homozygous deletion strain (DSY4241), azole susceptible clinical isolate DSY294, azole resistant clinical isolate DSY296, azole susceptible laboratory generated strain ALY21 and azole resistant laboratory generated strain ALY22 (SC medium in the presence of 2 and 4 µg/mL sr7575, 37°C, 48 h).

Supplementary Table S1 - list of 76 compounds from the CERMN chemical library showing 90% or more growth inhibition of *Aspergillus fumigatus* at 25 µg/mL.

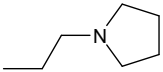
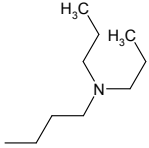
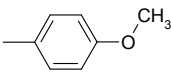

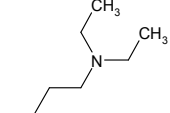
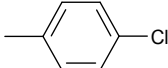
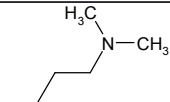
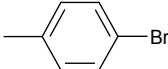
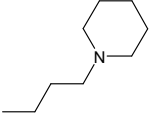
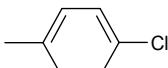
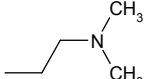
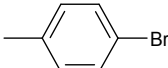
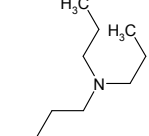
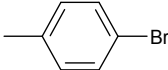
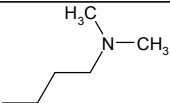
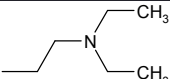
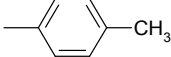
→ **Family A:**



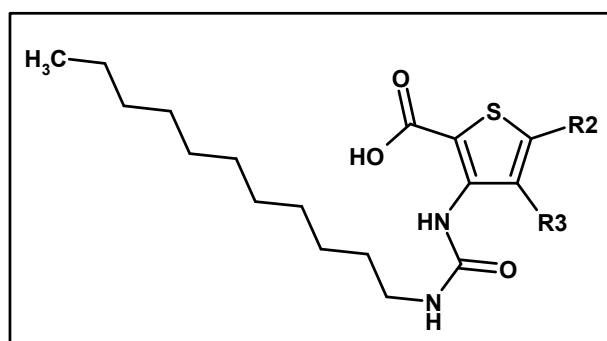
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sr1308 ²	-H			0
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sr3168 ³	-H			0
sr3169 ²	-H			0

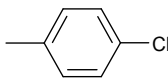
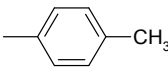
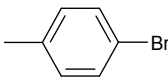
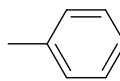
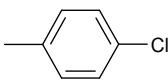
¹S. Rault, S. Lemaître, F. Dauphin, A. Kervabon, M. Boulouard, J.-C. Lancelot, PCT Int. Appl., WO2001014381, (2001).

²Dual Histamine H3R/Serotonin 5-HT4R Ligands with Antiamnesic Properties: Pharmacophore-Based Virtual Screening and Polypharmacology, Lepaillier, Alban; Freret, Thomas; Lemaître, Stéphane; Boulouard, Michel; Dauphin, François; Hinschberger, Antoine; Dulin, Fabienne; Lesnard, Aurelien; Bureau, Ronan; Rault, Sylvain; Journal of Chemical Information and Modeling (2014), 54(6), 1773-1784.

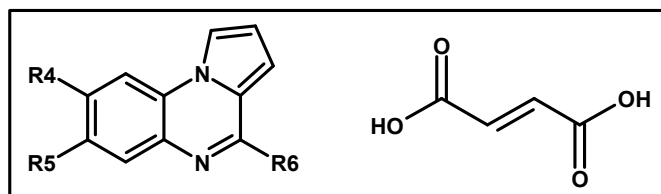
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sr3174 ³	-H			0
sr3179 ²			-H	0
sr3180 ²			-H	0
sr3181 ³			-H	0
sr3182 ²			-H	0
sr3185 ³			-H	0
sr3186 ¹			-H	0
sr3188 ²	-H			0

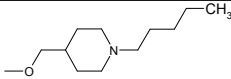
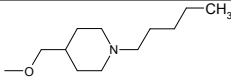
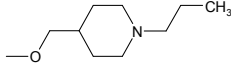
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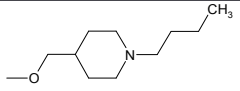
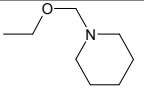
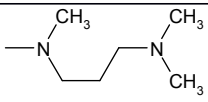
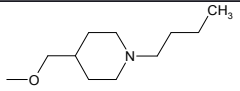
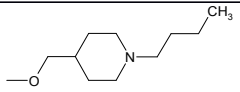
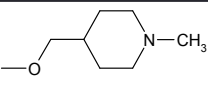
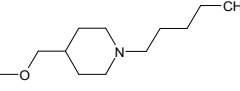

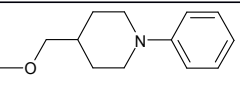
Compound	R2	R3	% viability
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sr4046 ¹		-H	0
sr4049 ¹		-H	0
sr4050 ³	-H		0
sr4051 ¹		-H	0
sr4052 ⁴	-H	-H	0

→ **Family C:** _____

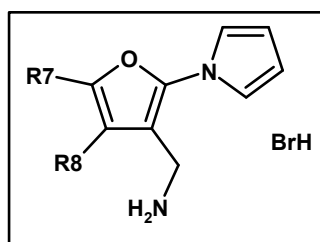


Compound	R4	R5	R6	% viability
mr22450 ²	-H	-CH ₃		0
mr22442 ²	-H	-Cl		0
mr22455 ²	-H	-Cl		0

³ Solution-phase parallel synthesis of a 1140-member ureidothiophene carboxylic acid library, Le Foulon, Francois-Xavier; Braud, Emmanuelle; Fabis, Frederic; Lancelot, Jean-Charles; Rault, Sylvain, Journal of Combinatorial Chemistry (2005), 7(2), 253-257.

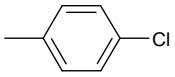
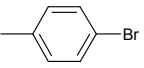
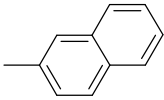
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mr18993 ⁴	-Cl	-Cl		4
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mr23270 ¹	-H	-F		0
mr24316 ¹	-H	-Cl		0
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sr1832 ⁵	-H	-H		0
sr2823 ¹	-H	-CH3		0

→ **Family D:**

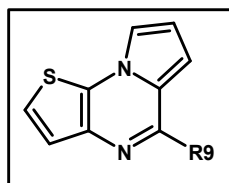



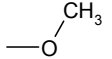
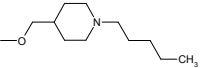
4 Synthesis of new pyrrolo[1,2-a]quinoxalines: potential non-peptide glucagon receptor antagonists, Guillon, Jean; Dallemagne, Patrick; Pfeiffer, Bruno; Renard, Pierre; Manechez, Dominique; Kervran, Alain; Rault, Sylvain, *European Journal of Medicinal Chemistry* (1998), 33(4), 293-308

5 Novel and Selective Partial Agonists of 5-HT₃ Receptors. 2. Synthesis and Biological Evaluation of Piperazinopyridopyrrolopyrazines, Piperazinopyrroloquinoxalines, and Piperazinopyridopyrroloquinoxalines Prunier, Herve; Rault, Sylvain; Lancelot, Jean-Charles; Robba, Max; Renard, Pierre; Delagrang, Philippe; Pfeiffer, Bruno; Caignard, Daniel-Henri; Misslin, Rene; Guardiola-Lemaitre, Beatrice; et al, *Journal of Medicinal Chemistry* (1997), 40(12), 1808-1819.

Compound	R7	R8	% viability
sr1457 ¹		-H	0
sr1460 ¹		-H	0
sr1462 ¹		-H	2

→ **Family E:**

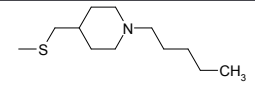
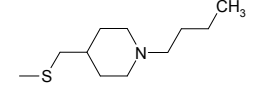


Compound	R9	% viability
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sr3584 ⁷		0
mr22410 ⁸		6

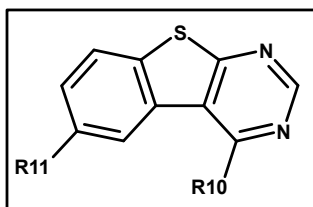
⁶ Novel Selective and Partial Agonists of 5-HT₃ Receptors. Part 1. Synthesis and Biological Evaluation of Piperazinopyrrolothienopyrazines, Rault, Sylvain; Lancelot, Jean-Charles; Prunier, Herve; Robba, Max; Renard, Pierre; Delagrang, Philippe; Pfeiffer, Bruno; Caignard, Daniel-Henri; Guardiola-Lemaitre, Beatrice; Hamon, Michel, *Journal of Medicinal Chemistry* (1996), 39(10), 2068-80.

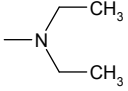
⁷ Pyrrolo[1,2-a]thieno[3,2-e]pyrazines, Rault, Sylvain; Cugnon de Sevracourt, Michel; Nguyen-Huy Dung; Robba, Max, *Journal of Heterocyclic Chemistry* (1981), 18(4), 739-42.

⁸ Novel antagonists of serotonin-4 receptors: Synthesis and biological evaluation of pyrrolothienopyrazines, Lemaitre, Stephane; Lepailleur, Alban; Bureau, Ronan; Butt-Gueulle, Sabrina; Lelong-Boulouard, Veronique; Duchatelle, Pascal; Boulouard, Michel; Dumuis, Aline; Daveu, Cyril; Lezoualc'h, Frank; et al, *Bioorganic & Medicinal Chemistry* (2009), 17(6), 2607-2622.

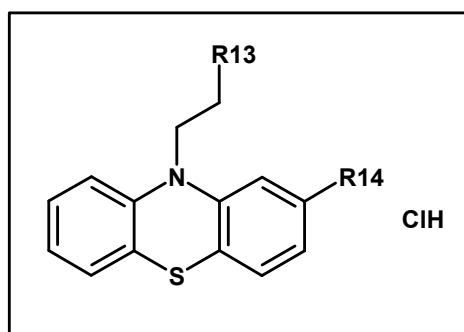
mr22422 ¹		0
mr24356 ¹		3

→ **Family F:**

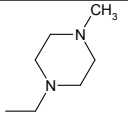
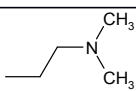


Compound	R10	R11	% viability
sr2205 ⁹		-H	3
sr2210 ¹	-NH ₂	-H	3
sr2634 ¹	-Cl	-NO ₂	0

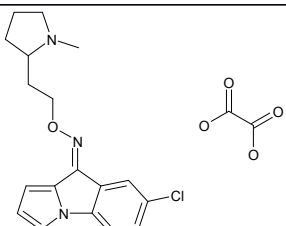
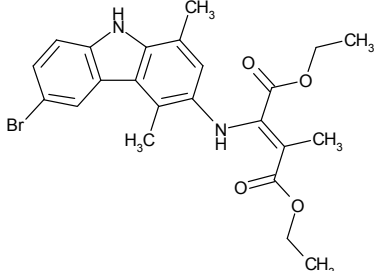
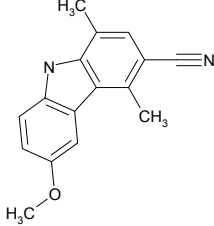
→ **Family G:**



Compound	R13	R14	% viability

pa2 ¹⁰		-CF ₃	0
pa18 ¹¹		-Cl	0

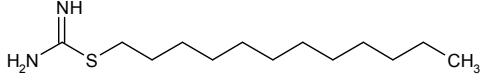
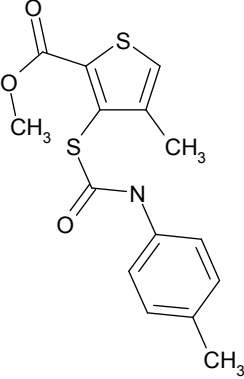
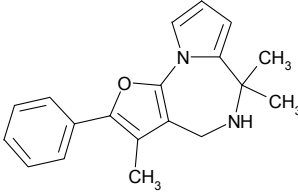
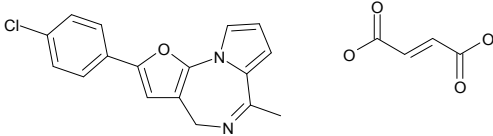
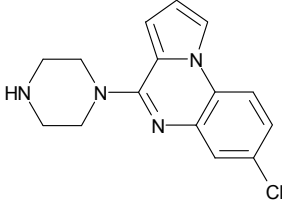
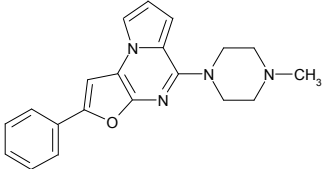
→ [Singletons:](#)

Compound	Structure	% viability
mr19807 ¹		0
mr15010a ¹		4
mr15059 ¹²		4

¹⁰Trifluoperazine (DCI) dihydrochloride; New (trifluoromethyl)phenothiazine derivatives, Craig, P. N.; Nodiff, E. A.; Lafferty, J. J.; Ulliyot, G. E., *Journal of Organic Chemistry* (1957), 22, 709-11.

¹¹Chlorpromazine (DCI) hydrochloride; Substituted 10-(dimethylaminopropyl)phenothiazines, Charpentier, Paul; Gailliot, Paul; Jacob, Robert; Gaudechon, Jacques; Buisson, Paul, *Compt. rend.* (1952), 235, 59-60.

¹²Anti-tumor heterocycles. Part 13. The syntheses of two new pyridocarbazoles (ellipticines) and some pyrrolocarbazole analogs, Chunchatprasert, Laddawan; Dharmasena, Priyanthi; Oliveira-Campos, Ana M. F.; Queiroz, Maria J. R. P.; Raposo, Maria M. M.; Shannon, Patrick V. R. *Journal of Chemical Research, Synopses* (1996), (2), 84-5.

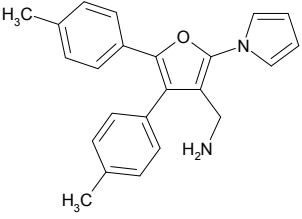
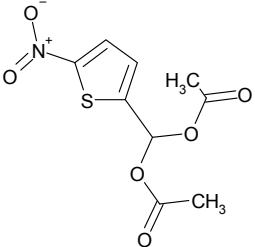
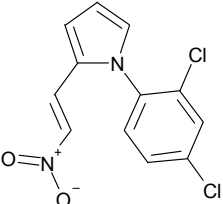
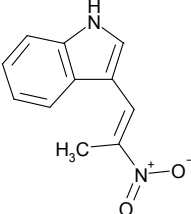
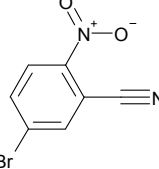
mr22633 ¹³		0
mr16310 ¹⁴		2
mr21034 ¹⁵		10
mr21018 ¹		8
mr18499 ¹⁶		0
sr1429 ¹		0

¹³ Comparative effect of a family of substituted thiopseudoureas on protein synthesis by rat liver and Walker carcinoma ribosomes, Carmona, Andres; Gonzalez-Cadavid, Nestor F., *Chemico-Biological Interactions* (1978), 22(2-3), 309-27.

¹⁴ Preparation of 3-mercapto-2-thiophenecarboxylic acid derivatives as intermediates for herbicides, Rault, Sylvain; Lancelot, Jean Charles; Letois, Bertrand; Robba, Max; Labat, Yves Fr. *Demande* (1993), FR 2689129 A1 19931001.

¹⁵ First synthesis of 5,6-dihydro-4H-furo[3,2-f]pyrrolo[1,2-a][1,4]diazepines, Feng, Xiao; Lancelot, Jean-Charles; Gillard, Alain-Claude; Landelle, Henriette; Rault, Sylvain, *Journal of Heterocyclic Chemistry* (1998), 35(6), 1313-1316.

¹⁶ Preparation of pyrrolopyrazines as 5-HT₃ ligands, Lancelot, Jean-Charles; Prunier, Herve; Robba, Max; Delagrance, Philippe; Renard, Pierre; Adam, Gerard, *Eur. Pat. Appl.* (1994), EP 623620 A1 19941109.

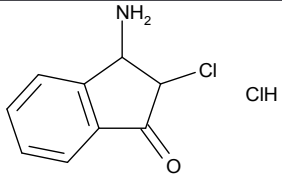
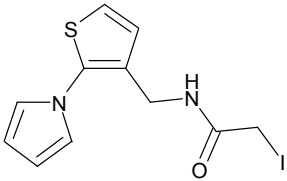
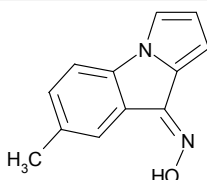
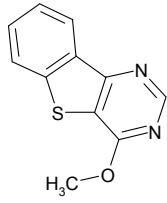
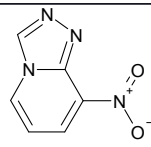
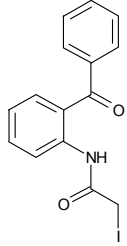
sr1461 ¹⁷		1
sr1475 ¹⁸		0
sr1810 ¹		9
sr1821 ¹⁹		0
sr1922 ²⁰		0

¹⁷ First synthesis of 4H-furo[3,2-f]pyrrolo[1,2-a][1,4]diazepines, Feng, Xiao; Lancelot, Jean-Charles; Prunier, Herve; Rault, Sylvain, *Journal of Heterocyclic Chemistry* (1996), 33(6), 2007-2011.

¹⁸ Synthesis and in vitro antibacterial evaluation of N-[5-(5-nitro-2-thienyl)-1,3,4-thiadiazol-2-yl]piperazinylquinolones, Foroumadi, Alireza; Mansouri, Shahla; Kiani, Zahra; Rahmani, Afsaneh, *European Journal of Medicinal Chemistry* (2003), 38(9), 851-854.

¹⁹ Alpha-ethyltryptamines as dual dopamine-serotonin releasers, Blough, Bruce E.; Landavazo, Antonio; Partilla, John S.; Decker, Ann M.; Page, Kevin M.; Baumann, Michael H.; Rothman, Richard B., *Bioorganic & Medicinal Chemistry Letters* (2014), 24(19), 4754-4758.

²⁰ Synthesis of nitrile and benzoyl substituted poly(biphenylene oxide)s via nitro displacement reaction, In, Insik; Kim, Sang Youl, *Polymer* (2006), 47(13), 4549-4556

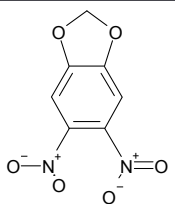
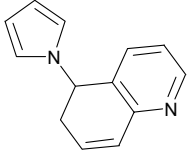
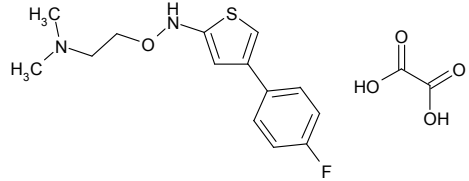
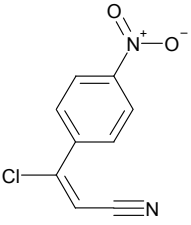
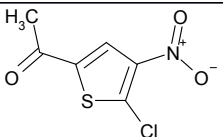
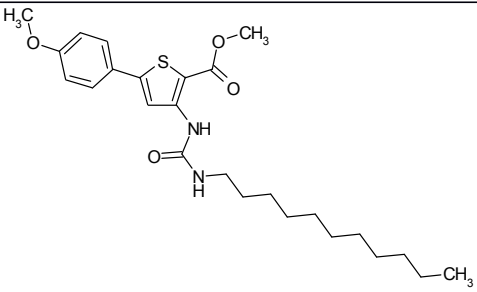
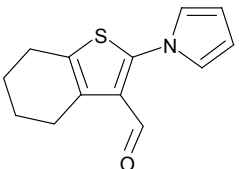
sr2223		8
sr2323 ¹		0
sr2565 ²¹		5
sr2627 ²²		1
sr2970 ²³		6
sr2809 ²⁴		0

²¹ First tricyclic oximino derivatives as 5-HT₃ ligands, Baglin, I.; Daveu, C.; Lancelot, J. C.; Bureau, R.; Dauphin, F.; Pfeiffer, B.; Renard, P.; Delagrangé, P.; Rault, S., *Bioorganic & Medicinal Chemistry Letters* (2001), 11(4), 453-457.

²² [1]Benzothienopyrimidines. I. Study of 3H-benzothieno[3,2-d]pyrimidin-4-one, Robba, Max; Touzot, Paulette; El-Kashef, Hussein, *Journal of Heterocyclic Chemistry* (1980), 17(5), 923-8.

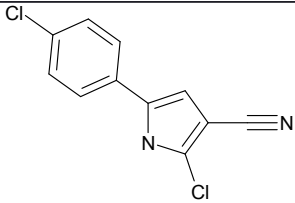
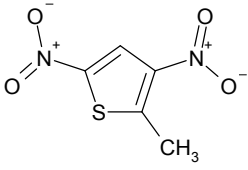
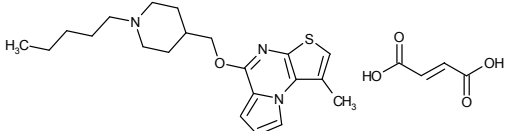
²³ Synthesis and physicochemical study of 1,2,4-triazolo[4,3-a]pyridines and of 1,2,4-triazolo[2,3-a]pyridines, Bouteau, Brigitte; Lancelot, Jean Charles; Robba, Max, *Journal of Heterocyclic Chemistry* (1990), 27(6), 1649-51.

²⁴ Method of producing 2-iodoacetylaminobenzophenones, Mazurov, A. A.; Andronati, S. A.; Yakubovskaya, L. N. U.S.S.R. (1991), SU 1622365 A1 19910123.

sr2967 ²⁵		3
mr12044 ¹		3
sr3184 ¹		0
sr3456 ²		0
sr3355 ²⁶		0
sr5443 ¹		6
sr4274 ¹		2

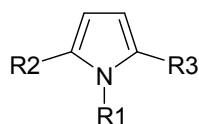
²⁵ Synthesis, in vitro cytotoxic and in vivo antitumor activities of new pyrrolo[2,1-c][1,4]benzodiazepines. Part I Foloppe, M. P.; Caballero, E.; Rault, S.; Robba, M., European Journal of Medicinal Chemistry (1992), 27(3), 291-5.

²⁶ Selective Dual Inhibitors of the Cancer-Related Deubiquitylating Proteases USP7 and USP47, Weinstock, Joseph; Wu, Jian; Cao, Ping; Kingsbury, William D.; McDermott, Jeffrey L.; Kodrasov, Matthew P.; McKelvey, Devin M.; Suresh Kumar, K. G.; Goldenberg, Seth J.; Mattern, Michael R.; et al, ACS Medicinal Chemistry Letters (2012), 3(10), 789-792.

sr4080 ²⁷		8
sr4211 ²⁸		8
mr24355 ¹⁰		0

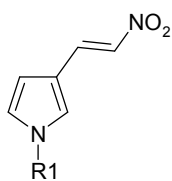
²⁷ Insecticidal action and mitochondrial uncoupling activity of AC-303,630 and related halogenated pyrroles, Black, Bruce C.; Hollingworth, Robert M.; Ahammadsahib, Kabeer I.; Kukel, Christine D.; Donovan, Stephen *Pesticide Biochemistry and Physiology* (1994), 50(2), 115-28.

²⁸ Synthesis of dinitro-substituted furans, thiophenes, and azoles, Katritzky, Alan R.; Vakulenko, Anatoliy V.; Sivapackiam, Jothilingam; Draghici, Bogdan; Damavarapu, Reddy *Synthesis* (2008), (5), 699-706.



Compound	R1	R2	R3	MIC ₁₀₀ (µg/ml)
sr7575 (1)	2,4-dichlorophenyl	H		2.4-4.8
3	2,4-dichlorophenyl	CH ₃		> 26-32
4	2,4-dichlorophenyl	H		> 26-32
5	2,4-dichlorophenyl	H		> 26-32
6	4-chlorophenyl	H		2.4-4.8
7	3-chlorophenyl	H		9.6
8	2-chlorophenyl	H		13 - 26
9	3,4-dichlorophenyl	H		9.6
10	2,3-dichlorophenyl	H		19.2
11	2,5-dichlorophenyl	H		> 38.5
12	3,5-dichlorophenyl	H		9.6
13	2,6-dichlorophenyl	H		19.2
14	2,4,5-trichlorophenyl	H		9.6
15	2,4,6-trichlorophenyl	H		26 - 32
16	2-fluoro-4-chlorophenyl	H		13 - 26
17	2-chloro-4-fluorophenyl	H		13
18	2,4-dibromophenyl	H		13 - 26
19	2-bromo-4-chlorophenyl	H		13 - 26
20	2-chloro-4-bromophenyl	H		13
21	2-iodo-4-chlorophenyl	H		26
22	2-chloro-4-iodophenyl	H		26

Supplementary Table S2: Analogues of sr7575 and MIC values against *A. fumigatus*.



Compound	R1	MIC₁₀₀ ($\mu\text{g/ml}$)
sr7576 (2)	2,4-dichlorophenyl	> 38.5
23	3-chlorophenyl	26 - 32
24	3,4-dichlorophenyl	26 - 32
25	3,5-dichlorophenyl	26 - 32
26	2,6-dichlorophenyl	38.5
27	2,4,6-trichlorophenyl	38.5
28	2-bromo-4-chlorophenyl	> 26 - 32
29	2-chloro-4-bromophenyl	> 26 - 32
30	2,4-dibromophenyl	> 26 - 32
31	2-iodo-4-chlorophenyl	> 26 - 32
32	2-chloro-4-iodophenyl	>26 - 32

Supplementary Table S3: Analogues of sr7576 and MIC values against *A. fumigatus*.

Supplementary Table S4 - Strains and plasmids used in this study

Strain	Genotype	Reference
BY4741	MATa;his3Δ 1;leu2Δ 0;met15Δ 0;ura3Δ 0	Brachmann et al, 1998
(Deletion mutants were generated in the Sc BY4741 background)		
<i>aro7</i> Δ	<i>aro7</i> ::KanMX4	Reference Giaever et al, 2002
<i>cue1</i> Δ	<i>cue1</i> ::KanMX4	
<i>der1</i> Δ	<i>der1</i> ::KanMX4	
<i>doa10</i> Δ	<i>doa10</i> ::KanMX4	
<i>emc1</i> Δ	<i>emc1</i> ::KanMX4	
<i>emc3</i> Δ	<i>emc3</i> ::KanMX4	
<i>gcs1</i> Δ	<i>gcs1</i> ::KanMX4	
<i>hac1</i> Δ	<i>hac1</i> ::KanMX4	
<i>hrd1</i> Δ	<i>hrd1</i> ::KanMX4	
<i>hrd3</i> Δ	<i>hrd3</i> ::KanMX4	
<i>ire1</i> Δ	<i>ire1</i> ::KanMX4	
<i>pdr1</i> Δ	<i>pdr1</i> ::KanMX4	
<i>pdr5</i> Δ	<i>pdr5</i> ::KanMX4	
<i>pga3</i> -DAmP	<i>pga3</i> -DAmP (KanMX4)	
<i>rpn4</i> Δ	<i>rpn4</i> ::KanMX4	
<i>sbh2</i> Δ	<i>sbh2</i> ::KanMX4	
<i>sec65</i> -DAMP	<i>sec65</i> -DAmP (KanMX4)	
<i>slg1</i> Δ	<i>slg1</i> ::KanMX4	
<i>ssh1</i> Δ	<i>ssh1</i> ::KanMX4	
<i>trp2</i> Δ	<i>trp2</i> ::KanMX4	
<i>ubc7</i> Δ	<i>ubc7</i> ::KanMX4	
<i>ufd2</i> Δ	<i>ufd2</i> ::KanMX4	
<i>ynl181w</i> -DAmP	<i>ynl181w</i> -DAmP (KanMX4)	
<i>ypk1</i> Δ	<i>ypk1</i> ::KanMX4	
A. fumigatus strains used in this study:		
Strain	Genotype	Reference
kuA	<i>akuA</i> :: <i>ptrA</i>	Krappmann et al, 2006
<i>derA</i> Δ	<i>akuA</i> :: <i>ptrA</i> , <i>derA</i> :: <i>hph</i>	Richie DL et al 2011
<i>hacA</i> Δ	<i>akuA</i> :: <i>ptrA</i> , <i>hacA</i> :: <i>hph</i>	Richie DL et al 2009
<i>hrdA</i> Δ	<i>akuA</i> :: <i>ptrA</i> , <i>hrdA</i> :: <i>hph</i>	Krishnan K et al 2013
<i>ireA</i> Δ	<i>akuA</i> :: <i>ptrA</i> , <i>ireA</i> :: <i>ble</i>	Jeng X et al 2011
<i>derA</i> Δ/ <i>hacA</i> Δ	<i>akuA</i> :: <i>ptrA</i> , <i>hacA</i> :: <i>hph</i> , <i>derA</i> :: <i>ble</i>	Richie DL et al 2011
<i>derA</i> Δ/ <i>hrdA</i> Δ	<i>akuA</i> :: <i>ptrA</i> , <i>hrdA</i> :: <i>hph</i> , <i>derA</i> :: <i>ble</i>	Krishnan K et al 2013
Other yeast strains used in this study:		
Strain	Genotype	Reference
<i>C. albicans</i> SC5314	wild type	Lohberger et al, 2014
DSY4241	<i>tac1</i> Δ::FRT/ <i>tac1</i> Δ::FRT	
DSY294	azole susceptible clinical isolate (<i>TAC1</i> -3/ <i>TAC1</i> -4)	
DSY296	azole resistant clinical isolate (<i>TAC1</i> -5/ <i>TAC1</i> -5 ; N977D mutation)	
ALY21	<i>tac1</i> Δ::TAC1-4-FRT/ <i>tac1</i> Δ::TAC1-4-FRT	
ALY22	<i>tac1</i> Δ::TAC1-5-FRT/ <i>tac1</i> Δ::TAC1-5-FRT	
<i>C. neoformans</i> H99	wild type	
Perfect et al, 1980		
MoBY plasmid (library v1.1) complemented S. cerevisiae strains:		
Strain	Genotype+ MoBY clone identifier	Reference
<i>aro7</i> Δ+ ARO7	<i>aro7</i> ::KanMX4+ YPR060C::29NP_C9	Ho et al, 2009
<i>cue1</i> Δ+ CUE1	<i>cue1</i> ::KanMX4+ YMR264W::33NP_H12	
<i>emc1</i> Δ+ EMC1	<i>emc1</i> ::KanMX4+ YCL045C::41NP_D8	
<i>emc3</i> Δ+ EMC3	<i>emc3</i> ::KanMX4+ YKL207W::8NP_A12	
<i>hrd1</i> Δ+ HRD1	<i>hrd1</i> ::KanMX4+ YOL013C::12NP_G12	
<i>rpn4</i> Δ+ RPN4	<i>rpn4</i> ::KanMX4+ YDL020C::30NP_F2	
<i>ssh1</i> Δ+ SSH1	<i>ssh1</i> ::KanMX4+ YBR283C::37NP_A11	
<i>ubc7</i> Δ+ UBC7	<i>ubc7</i> ::KanMX4+ YMR022W::36NP_G3	
YGPM systematic overexpression library in S. cerevisiae strains:		
Strain	Genotype+ YGPM clone identifier	Reference
Control	BY4741+ YGPM22k06 chrIII:151898...152647	Jones et al, 2008
PDR1	BY4741+ YGPM26h12 chrVII:466658...477209	
PDR5	BY4741+ YGPM33k24 chrXV:619141...631341	
PDR12	BY4741+ YGPM8p07 chrXVI:444386...454435	

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Supplementary Table S5 - list of oligonucleotides used in this study**List of oligonucleotides to screen deletion/DAmP mutants:**

Gene	Primer name	Sequence
Kanamycin	KANMX-FW	5'-AGATGCGAAGTTAAGTGCGC-3'
<i>ARO7</i>	ARO7dR	5'-GAGAGAAGGTCATGGATGTG-3'
<i>CUE1</i>	CUE1dR	5'-GTAAGGGGAGAAGAACGTTTC-3'
<i>DER1</i>	DER1dR	5'-TCTGCAAACGGACACCAAGT-3'
<i>EMC1</i>	EMC1dR	5'-GCACATCATTTCCAGACGAG-3'
<i>EMC3</i>	EMC3dR	5'-GCGAGGACTTTTTGCCATAC-3'
<i>GCS1</i>	GCS1dR	5'-GTGGTAGTTCTCTCTCCTTG-3'
<i>HAC1</i>	HAC1dR	5'-AGAGCCGTGAGAGTGAGAGT-3'
<i>HRD1</i>	HRD1dR	5'-TATGTCACCTTCCTATGCCG-3'
<i>HRD3</i>	HRD3dR	5'-ATGAACGGCAATTTGAGACC-3'
<i>IRE1</i>	IRE1dR	5'-TCTTGCACTTTTTCGCCATGC-3'
<i>PDR1</i>	PDR1dR	5'-TGGCAACTATGTGGTGCAAT-3'
<i>PDR5</i>	PDR5dR	5'-GCATCTTGCTCTTTCCTCTC-3'
<i>RPN4</i>	RPN4dR	5'-CTGGGTACGAATTCAAGGAG-3'
<i>SBH2</i>	SBH2dR	5'-CATGCACCCTTAACATCGTC-3'
<i>SEC65</i>	SEC65-DAmP	5'-GGAAGTTGTGAGTACTGACG-3'
<i>SLG1</i>	SLG1dR	5'-TATATCGTCTTTC AACGCGG-3'
<i>SSH1</i>	SSH1dR	5'-CCACGAAGCAAGGTAACAAG-3'
<i>SSM4</i>	SSM4dR	5'-GACGAGGGCTAAGCAGTTTG-3'
<i>TRP2</i>	TRP2dR	5'-CCAAACCACATTGGTCTAGG-3'
<i>UBC7</i>	UBC7dR	5'-TACTGTACGGCTTGGAAGAG-3'
<i>UFD2</i>	UFD2dR	5'-ACCGTCATCAACGAACAACA-3'
<i>YPK1</i>	YPK1dR	5'-CCGTTTCGTGGTTAAGGTAAG-3'

List of oligonucleotides to screen MoBY plasmids:

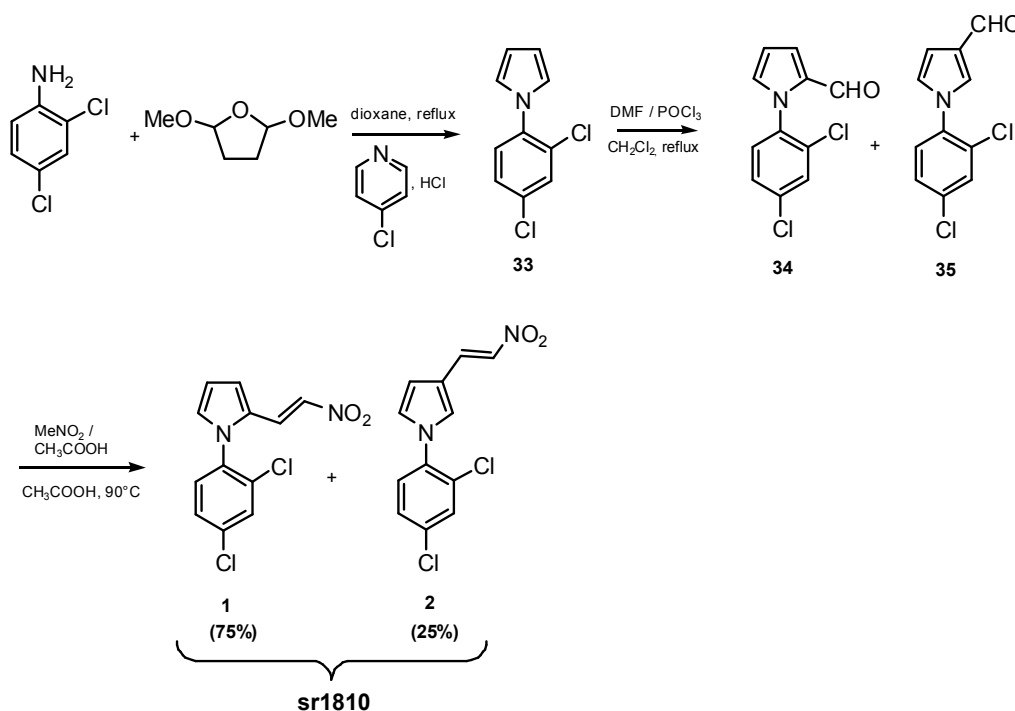
Gene	Primer name	Sequence
<i>ARO7</i>	ARO7iF	5'-TCGCCACATGTCCTTCAGTT-3'
	ARO7iR	5'-GCAAGTATTCCACCTCAACTTCC-3'
<i>CUE1</i>	CUE1iF	5'-ATGGAGGATTCGAGATTGCTT-3'
	CUE1iR	5'-CTGGCTTGCCAAACCAACAA-3'
<i>EMC1</i>	EMC1iF	5'-TGCCCCTTCTACGACCATTT-3'
	EMC1iR	5'-TGCCATTCGTGTCATGCTCT-3'
<i>EMC3</i>	EMC3iF	5'-ACCAGCTGAAGTATTGGGTCC-3'
	EMC3iR	5'-TATCCCGGCCTGAATACCCA-3'
<i>HRD1</i>	HRD1iF	5'-TGCGTGTATTTCAGCCACCAA-3'
	HRD1iR	5'-GCCAAGATATCCCACACCACA-3'
<i>RPN4</i>	RPN4iF	5'-GCGAAACCCATTGCAGAAG-3'
	RPN4iR	5'-TGGTGATGCAGTCGAAGGTT-3'
<i>SSH1</i>	SSH1iF	5'-TTGGTTCGGTGCTGGCATATT-3'
	SSH1iR	5'-GGATGCACCCGTAACAGCT-3'
<i>UBC7</i>	UBC7iF	5'-CGAAAACCGCTCAGAAACGT-3'
	UBC7iR	5'-GCATCAATGTTGGCACC ACT-3'

Supporting information (S1) - Synthesis of sr7575-related compounds.

General Methods

All chemical reagents and solvents were purchased from commercial sources and used without further purification. Thin-layer chromatography (TLC) was performed on silica gel plates. Silica gel 0.06–0.2 mm, 60 Å was used for all column chromatography. Melting points were determined on a Kofler melting point apparatus. NMR spectra were recorded on a BRUKER AVANCE III 400 MHz (^1H NMR at 399.8 MHz and ^{13}C NMR at 100 MHz) with the solvents indicated. Chemical shifts are reported in parts per million (ppm) on the δ scale and referenced to the appropriate solvent peak. High-resolution mass spectral (HRMS) were performed on a BRUKER maxis mass spectrometer by the “fédération de Recherche” ICOA / CBM (FR2708) platform. LC-MS Analysis was performed on a Waters alliance 2695 using the following gradient: A (95%)/B (5%) to A (5%)/B (95%) in 4 min. This ratio was held for 1.5 min before returning to initial conditions in 0.5 min. Initial conditions were then maintained for 2 min (A, H_2O ; B, MeCN; each containing 0.1% HCOOH; column, C18 Xbridge 4.6 x 50 mm / 2.5 μm). MS detection was performed with a SQDetector.

Compound sr1810: Mixture of (*E*) 1-(2,4-Dichlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole [75%] and (*E*) 1-(2,4-Dichlorophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole [25%].



1-(2,4-Dichlorophenyl)-1*H*-pyrrole (33).¹ 4-chloropyridine, hydrochloride (9.25 g, 0.0617 mol) and 2,5-dimethoxytetrahydrofuran (8.15 g, 0.0617 mol) were stirred in 150 mL of dioxane at room temperature for 30 min. 2,4-dichloroaniline (10 g, 0.0617 mol) was then added and the mixture was stirred at reflux for 4 h. The reaction was cooled down to room temperature and concentrated under vacuum. 150 mL of water were added to the residue, followed by 200 mL of Et₂O. The aqueous layer was extracted with Et₂O (2x 100 mL). The

¹ Azizi, N. *et al.* Iron-catalyzed inexpensive and practical synthesis of *N*-substituted pyrroles in water. *Synlett*, **14**, 2245-2248 (2009).

combined organic layers were washed with HCl 1N (200 mL) and water (2x 200 mL), dried over MgSO₄ and concentrated under vacuum. The resulting residue was purified by chromatography on silica gel using CH₂Cl₂ as eluant to give compound **33** as a brown oil (11.4 g, 87%). ¹H NMR (400 MHz, CDCl₃) δ 7.56 (d, *J* = 2.2 Hz, 1H), 7.35 (dd, *J* = 8.7 and 2.3 Hz, 1H), 7.31 (d, *J* = 8.6 Hz, 1H), 6.92 (t, *J* = 2.1 Hz, 2H), 6.39 (t, *J* = 1.9 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 137.5, 133.3, 130.5, 130.4, 128.5, 127.9, 122.1, 109.7.

Mixture of 1-(2,4-Dichlorophenyl)-1-*H*-pyrrole-2-carbaldehyde (**34**) and 1-(2,4-Dichlorophenyl)-1*H*-pyrrole-3-carbaldehyde (**35**). Dimethylformamide (2.20 mL, 0.0283 mol) was stirred at 0°C. Phosphorus oxychloride (2.7 mL, 0.0283 mol) was then added dropwise and the white solid obtained was stayed cold for 30 min. After this time, a solution of compound **33** (6 g, 0.0283 mol) in 120 mL of CH₂Cl₂ was slowly added dropwise to the reaction mixture. The reaction was refluxed for 20 h. After cooling, 120 mL of water were added and the mixture was stirred at room temperature for 30 min. Then, the layers were separated and the aqueous layer was alkalinized with 20% sodium hydroxide solution. This aqueous layer was extracted with Et₂O (2x 120 mL). The combined organic layers were dried over MgSO₄ and concentrated under vacuum. The mixture of compound **34** and **35** was engaged in the next step without further purification and was obtained as brown solid (4.4 g, 65%). LC-MS (ESI): *t*_R = 4.66 and 4.79 min; [M+H]⁺ 240.35. HRMS for C₁₁H₈Cl₂NO [M+H]⁺ calculated mass: 239.9977, measured: 239.9974.

Compound **34**: ¹H NMR (400 MHz, CDCl₃) δ 9.44 (s, 1H), 7.44 (d, *J* = 2.3 Hz, 1H), 7.26 (dd, *J* = 8.6 and 2.0 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 7.04 (dd, *J* = 1.4 and 4.0 Hz, 1H), 6.86 (m, 1H), 6.37 (dd, *J* = 4.0 and 2.8 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.5, 136.0, 135.2, 132.9, 132.9, 131.1, 130.0, 129.6, 127.7, 123.2, 111.2.

Compound **35**: ¹H NMR (400 MHz, CDCl₃) δ 9.79 (s, 1H), 7.50 (d, *J* = 2.3 Hz, 1H), 7.40 (t, *J* = 1.7 Hz, 1H), 7.31 (dd, *J* = 8.5 and 2.3 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 6.80 (t, *J* = 2.8 Hz, 1H), 6.73 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 185.4, 136.1, 135.0, 130.8, 130.7, 130.0, 128.4, 128.2, 127.8, 124.8, 108.8.

sr1810: Mixture of (*E*) 1-(2,4-Dichlorophenyl)-2-(2-nitrovinyl)-1-*H*-pyrrole (**1**) and (*E*) 1-(2,4-Dichlorophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (**2**). Nitromethane (54 mL, 1 mol) and ammonium acetate (15.4 g, 0.2 mol) were stirred in 100 mL of acetic acid at 30°C for 30 min. Then, the mixture of compounds **34** and **35** (12 g, 0.05 mol) was added and the solution was heated at 90°C for 24 h. Then, the reaction was concentrated under vacuum. A saturated solution of sodium hydrogenocarbonate (100 mL) was added to the residue. This aqueous layer was extracted with EtOAc (2x 100mL). The organic layers were washed with water (2x 100 mL), dried over MgSO₄ and concentrated under vacuum. The mixture of compound **1** and **2** was obtained as a yellow solid (8.7 g, 61%). Mp: 114 °C. LC-MS (ESI): *t*_R = 5.22 and 5.32 min; [M+H]⁺ 283.31. HRMS for C₁₂H₉Cl₂N₂O₂ [M+H]⁺ calculated mass: 283.0035, measured: 283.0035.

Compound **1**: ¹H NMR (400 MHz, CDCl₃) δ 7.62 (d, *J* = 2.3 Hz, 1H), 7.56 (d, *J* = 13.3 Hz, 1H), 7.45 (dd, *J* = 8.4 and 2.3 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.17 (d, *J* = 13.4 Hz, 1H), 7.00 (m, 1H), 6.95 (m, 1H), 6.49 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 136.4, 134.3, 133.4, 132.6, 130.8, 130.3, 130.0, 128.4, 127.4, 125.8, 116.7, 112.3.

Compound **2**: ¹H NMR (400 MHz, CDCl₃) δ 8.04 (d, *J* = 13.4 Hz, 1H), 7.58 (d, *J* = 2.3 Hz, 1H), 7.47 (d, *J* = 13.2 Hz, 1H), 7.39 (dd, *J* = 8.4 and 2.3 Hz, 1H), 7.30-7.26 (m, 2H), 6.92 (m, 1H), 6.56 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 136.0, 134.9, 134.2, 133.2, 130.9, 130.8, 128.3, 128.2, 127.9, 125.5, 116.8, 108.3.

(E) 1-(2,4-Dichlorophenyl)-2-(2-nitrovinyl)-1H-pyrrole (1 or sr7575). See **Supplementary Fig.1a**.

1-(2,4-Dichlorophenyl)-1H-pyrrole-2-carbaldehyde (**34**). Dimethylformamide (2.20 mL, 0.0283 mol) was stirred at 0°C. Phosphorus oxychloride (2.7 mL, 0.0283 mol) was then added dropwise and the white solid obtained was cooled for 30 min. After this time, a solution of compound **33** (6 g, 0.0283 mol) in 120 mL of CH₂Cl₂ was added dropwise to the reaction mixture. The reaction was refluxed for 20 h. After cooling to room temperature, 120 mL of water were added and the mixture was stirred at room temperature for 30 min. Then, the layers were separated and the aqueous layer was alkalinized with 20% sodium hydroxide solution. This aqueous layer was extracted with Et₂O (2x 120 mL) and the organic layers were dried over MgSO₄ and concentrated under vacuum. The residue was purified by chromatography on silica gel using cyclohexane and CH₂Cl₂ as eluants (50/50) to afford compound **34** as a beige solid (2.1 g, 30%). ¹H NMR (400 MHz, CDCl₃) δ 9.44 (s, 1H), 7.44 (d, *J* = 2.3 Hz, 1H), 7.26 (dd, *J* = 8.6 and 2.0 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 7.04 (dd, *J* = 4.0 and 1.4 Hz, 1H), 6.86 (m, 1H), 6.37 (dd, *J* = 4.0 and 2.8 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.5, 136.0, 135.2, 132.9, 132.9, 131.1, 130.0, 129.6, 127.7, 123.2, 111.2. LC-MS (ESI): *t*_R = 4.79 min; [M+H]⁺ 240.35. HRMS for C₁₁H₈Cl₂NO [M+H]⁺ calculated mass: 239.9977, measured: 239.9974.

(E) 1-(2,4-Dichlorophenyl)-2-(2-nitrovinyl)-1H-pyrrole (1). Nitromethane (54 mL, 1 mol) and ammonium acetate (15.4 g, 0.2 mol) were stirred in 100 mL of acetic acid at 30°C for 30 min. Then, 1-(2,4-dichlorophenyl)-1H-pyrrole-2-carbaldehyde (12 g, 0.05 mol) was added and the mixture was heated at 90°C for 24 h. Then, the reaction was concentrated under vacuum. A saturated solution of sodium hydrogenocarbonate (100 mL) was added to the residue. The aqueous layer was extracted with EtOAc (2x 100mL). The combined organic layers were washed with water (2x 100 mL), dried over MgSO₄ and concentrated under vacuum. The resulting residue was purified by chromatography on silica gel using cyclohexane and CH₂Cl₂ as eluant (50/50) to obtain compound **1** as a yellow solid (6.6 g, 47%). Mp: 118°C. ¹H NMR (400 MHz, CDCl₃) δ 7.62 (d, *J* = 2.3 Hz, 1H), 7.56 (d, *J* = 13.3 Hz, 1H), 7.45 (dd, *J* = 2.3 and 8.3 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 7.17 (d, *J* = 13.4 Hz, 1H), 7.00 (m, 1H), 6.95 (m, 1H), 6.49 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 136.4, 134.3, 133.4, 132.6, 130.8, 130.3, 130.0, 128.4, 127.4, 125.8, 116.7, 112.3. LC-MS (ESI): *t*_R = 5.20 min; [M+H]⁺ 283.44. HRMS for C₁₂H₉Cl₂N₂O₂ [M+H]⁺ calculated mass: 283.0035, measured: 283.0036.

(E) 1-(2,4-Dichlorophenyl)-3-(2-nitrovinyl)-1H-pyrrole (2 or sr7576). See **Supplementary Fig.1b**.

1-(2,4-Dichlorophenyl)-1H-pyrrole-3-carbaldehyde (**35**).² 4-chloropyridine, hydrochloride (9.88 g, 0.0617 mol) and 2,5-Dimethoxy-3-tetrahydrofuran-carboxaldehyde (8.15 g, 0.0617 mol) were stirred in 150 mL of dioxane at room temperature for 30 min. 2,4-dichloroaniline (10 g, 0.0617 mol) was then added and the mixture was stirred at reflux for 4 h. The reaction was cooled to room temperature and concentrated under vacuum. 150 mL of water were added to the residue, followed by 200 mL of Et₂O. The aqueous layer was extracted with Et₂O (2x 100 mL). The combined organic layers were washed with 1N HCl (200 mL) and water (2x 200 mL), dried over MgSO₄ and concentrated under vacuum. The resulting residue was purified by chromatography on silica gel using CH₂Cl₂ as eluant to afford compound **35** as a

² Dallemagne, P. *et al.* A convenient rearrangement of 1-phenylpyrrole-2-carboxaldehydes into their 3-isomers. *Synthetic Communications*. **13**, 1855-1857 (1994).

beige solid (5.2 g, 35%). Mp: 186°C. ¹H NMR (400 MHz, CDCl₃) δ 9.79 (s, 1H), 7.50 (d, *J* = 2.3 Hz, 1H), 7.40 (t, *J* = 1.7 Hz, 1H), 7.31 (dd, *J* = 8.5 and 2.3 Hz, 1H), 7.25 (d, *J* = 8.4 Hz, 1H), 6.80 (t, *J* = 2.8 Hz, 1H), 6.73 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 185.4, 136.1, 135.0, 130.8, 130.7, 130.0, 128.4, 128.2, 127.8, 124.8, 108.8. LC-MS (ESI): t_R = 4.66 min; [M+H]⁺ 240.35.

(*E*) 1-(2,4-Dichlorophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (**2**). Nitromethane (54 mL, 1 mol) and ammonium acetate (15.4 g, 0.2 mol) were stirred in 100 mL of acetic acid at 30°C for 30 minutes. Then, 1-(2,4-dichloro-phenyl)-1*H*-pyrrole-2-carbaldehyde (12 g, 0.05 mol) was added and the mixture was heated at 90°C for 24 h. The reaction was concentrated under vacuum. A saturated solution of sodium hydrogenocarbonate (100 mL) was added to the residue. This aqueous layer was extracted with EtOAc (2x 100 mL). The organic layers were washed with water (2x 100 mL), dried over MgSO₄ and concentrated under vacuum. The solid resulting residue was purified by chromatography on silica gel using cyclohexane and CH₂Cl₂ as eluant (50/50) to give compound **2** as a yellow solid (6.1 g, 43%). Mp: 186 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.11 (d, *J* = 13.3 Hz, 1H), 7.95 (d, *J* = 13.3 Hz, 1H), 7.90 (d, *J* = 2.3 Hz, 1H), 7.78 (m, 1H), 7.60 (dd, *J* = 6.4 and 8.5 Hz, 1H), 7.58 (d, *J* = 8.7 Hz, 1H), 7.19 (m, 1H), 6.86 (m, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 136.5, 134.8, 134.6, 134.0, 130.6, 130.2, 130.0, 129.7, 129.0, 126.3, 117.5, 109.3. LC-MS (ESI): t_R = 5.34 min; [M+H]⁺ 283.40. HRMS for C₁₂H₉Cl₂N₂O₂ [M+H]⁺ calculated mass: 283.0035, measured: 283.0036.

(*E*) 1-(2,4-dichlorophenyl)-2-methyl-5-(2-nitrovinyl)-1*H*-pyrrole (**3**). See **Supplementary Fig.1c**.

1-(2,4-Dichlorophenyl)-2-methyl-1*H*-pyrrole (**36**). A mixture of 1-(2,4-Dichlorophenyl)-1*H*-pyrrole-2-carbaldehyde (**34**) (1g, 0.02 mol), potassium hydroxide (0.7g, 0.06 mol) and hydrazine monohydrate (0.61 ml, 0.06 mol) in ethylene glycol (20 ml) was stirred at room temperature for 30 min, then slowly heated to 150°C and maintained for 2 h. The reaction mixture was allowed to cool to room temperature, poured into ice-water and extracted with Et₂O (2x 20 ml). The combined organic layers were washed with water, brine, dried over MgSO₄, filtered and concentrated under vacuum. The crude compound was purified by chromatography on silica gel using cyclohexane and CH₂Cl₂ as eluant (90/10) to afford compound **36** as an orange oil (0.78 g, 70%). ¹H NMR (400 MHz, CDCl₃) δ 7.53 (d, *J* = 2.2 Hz, 1H), 7.33 (dd, *J* = 8.5 and 2.3 Hz, 1H), 7.26 (d, *J* = 8.3 Hz, 1H), 6.60 (dd, *J* = 2.8 and 1.9 Hz, 1H), 6.22 (t, *J* = 3.0 Hz, 1H), 6.04 (m, 1H), 2.04 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 136.7, 134.6, 133.7, 130.5, 130.1, 130.0, 127.7, 121.3, 108.5, 107.4, 12.1. HRMS for C₁₁H₁₀Cl₂N [M+H]⁺ calculated mass: 226.0184, measured: 226.0185.

Synthetic procedure for the compound (**3**) is the similar as that described for the compound (**1**) and spectra data are shown below.

1-(2,4-Dichlorophenyl)-5-methyl-1*H*-pyrrole-2-carbaldehyde (**37**). Yellow oil (61%). ¹H NMR (400 MHz, CDCl₃) δ 9.37 (s, 1H), 7.55 (d, *J* = 2.4 Hz, 1H), 7.38 (dd, *J* = 8.5 and 2.4 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 7.03 (d, *J* = 3.9 Hz, 1H), 6.22 (d, *J* = 3.9 Hz, 1H), 2.04 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 177.6, 140.1, 135.4, 134.8, 133.7, 132.7, 130.4, 130.1, 128.0, 123.8, 110.4, 12.1. LC-MS (ESI): t_R = 4.52 min; [M+H]⁺ 254.37. HRMS for C₁₂H₁₀Cl₂NO [M+H]⁺ calculated mass: 254.0133, measured: 254.0133.

(*E*) 1-(2,4-Dichlorophenyl)-2-methyl-5-(2-nitrovinyl)-1*H*-pyrrole (**3**). Orange solid (50%). Mp: 90°C. ¹H NMR (400 MHz, CDCl₃) δ 7.65 (d, *J* = 2.4 Hz, 1H), 7.47 (m, 2H), 7.28 (m,

1H), 6.99 (d, $J = 13.4$ Hz, 1H), 6.90 (d, $J = 4.0$ Hz, 1H), 6.27 (d, $J = 4.0$ Hz, 1H), 2.06 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 139.8, 136.8, 134.5, 133.1, 131.1, 130.9, 130.6, 128.7, 127.7, 125.3, 118.0, 111.7, 12.8. LC-MS (ESI): $t_{\text{R}} = 5.35$ min; $[\text{M}+\text{H}]^+ 297.42$. HRMS for $\text{C}_{13}\text{H}_{11}\text{Cl}_2\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$ calculated mass: 297.0192, measured: 297.0193.

(E) 3-[1-(2,4-Dichlorophenyl)-1H-pyrrol-2-yl]-acrylonitrile (4). See Supplementary Fig.1d.

2-Cyano-3-(1-(2,4-dichlorophenyl)-1H-pyrrol-2-yl)-acrylic acid ethyl ester (**38**). To a solution of 1-(2,4-dichlorophenyl)-1H-pyrrole-2-carbaldehyde (**34**) (1g, 4.16 mmol) in ethanol (10 ml), were added ethylcyanoacetate (0.49 ml, 4.58 mmol) and triethylamine (0.58 ml, 4.16 mmol). The mixture was refluxed for 4 h. After removal of the solvent under vacuum, CH_2Cl_2 was added to the residue and the organic layer was washed with water, dried over MgSO_4 , filtered and concentrated in vacuo. The crude compound was purified by chromatography on silica gel using CH_2Cl_2 as eluant to obtain compound **34** as a yellow solid (1.2 g, 86%). Mp: 138 °C. ^1H NMR (400 MHz, CDCl_3) δ 7.86 (d, $J = 4.2$ Hz, 1H), 7.59 (d, $J = 1.6$ Hz, 1H), 7.52 (s, 1H), 7.41 (dd, $J = 8.3$ and 1.5 Hz, 1H), 7.28 (d, $J = 8.5$ Hz, 1H), 7.07 (m, 1H), 6.59 (m, 1H), 4.26 (q, $J = 7.1$ Hz, 2H), 1.32 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 163.5, 140.1, 136.5, 133.6, 133.5, 130.7, 130.7, 130.6, 128.3, 128.3, 119.4, 116.6, 113.3, 95.2, 62.2, 14.2. HRMS for $\text{C}_{16}\text{H}_{13}\text{Cl}_2\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$ calculated mass: 335.0348, measured: 335.0346.

2-Cyano-3-(1-(2,4-dichlorophenyl)-1H-pyrrol-2-yl)-acrylic acid (**39**). To a solution of lithium hydroxyde (340 mg, 0.014 mol) in water (50 ml) was added a solution of 2-cyano-3-(1-(2,4-dichlorophenyl)-1H-pyrrol-2-yl)-acrylic acid ethyl ester (**38**) (3.2 g, 9.54 mmol) in THF (50 ml) and the mixture was heated at 50°C for 5h. After removing THF under vacuum, the aqueous layer was acidified with 6N HCl and then extracted with EtOAc (2x 50 mL). The organic layers were washed with water (2x 50 mL), dried over MgSO_4 and concentrated in vacuo. The product was purified by recrystallization in CH_2Cl_2 to obtain compound **39** as a yellow solid (1.1 g, 37%). Mp: 110°C. ^1H NMR (400 MHz, CDCl_3) δ 7.95 (d, $J = 4.1$ Hz, 1H), 7.64 (d, $J = 2.2$ Hz, 1H), 7.56 (s, 1H), 7.46 (dd, $J = 8.3$ and 2.2 Hz, 1H), 7.31 (d, $J = 8.3$ Hz, 1H), 7.15 (m, 1H), 6.65 (m, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 168.8, 141.2, 136.7, 133.5, 133.2, 131.8, 130.8, 130.6, 128.4, 128.3, 120.7, 116.0, 113.8, 93.8. LC-MS (ESI): $t_{\text{R}} = 4.55$ min; $[\text{M}+\text{H}]^+ 307.32$. HRMS for $\text{C}_{14}\text{H}_9\text{Cl}_2\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$ calculated 307.0035, measured: 307.0034.

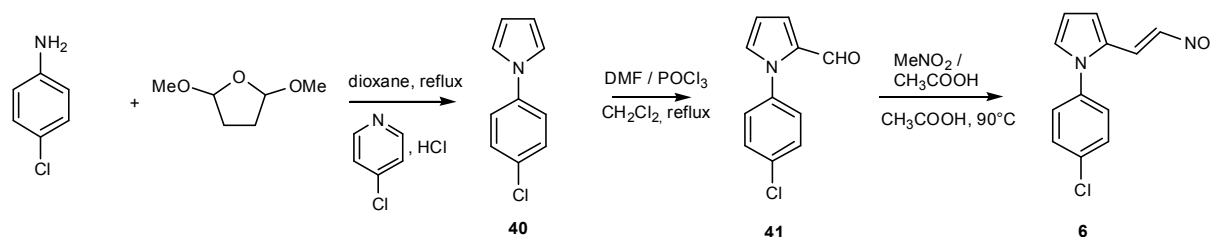
3-(1-(2,4-Dichlorophenyl)-1H-pyrrol-2-yl)-acrylonitrile (**4**). To a solution of copper (155 mg, 2.44 mmol) in quinoline (10 ml), heated at 190°C, was added 2-cyano-3-(1-(2,4-dichlorophenyl)-1H-pyrrol-2-yl)-acrylic acid (**39**) (500 mg, 1.62 mmol). The mixture was stirred vigorously. After the carbon dioxide evolution stopped and TLC indicated complete consumption of the starting material, the reaction was cooled to room temperature and 1N HCl (10 ml) was added. The aqueous layer was extracted with CH_2Cl_2 (2x 15 mL). The combined organic layers were washed with water (2x 20 mL), dried over MgSO_4 and concentrated under vacuum. The resulting residue was purified by chromatography on silica gel using cyclohexane and CH_2Cl_2 as eluant (50/50 to 30/70) to obtain compound **4** as a white solid (150 mg, 36%). Mp: 108 °C. ^1H NMR (400 MHz, CDCl_3) δ 7.58 (d, $J = 2.3$ Hz, 1H), 7.53 (m, 1H), 7.39 (dd, $J = 8.4$ and 2.3 Hz, 1H), 7.28 (d, $J = 8.5$ Hz, 1H), 6.86 (m, 1H), 6.49 (t, $J = 3.2$ Hz, 1H), 6.44 (d, $J = 12.1$ Hz, 1H), 5.01 (d, $J = 12.1$ Hz, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 135.8, 135.1, 134.5, 133.7, 130.8, 130.4, 129.3, 128.1, 126.4, 118.2, 114.3, 111.6,

88.7. LC-MS (ESI): $t_R = 5.18$ min; $[M+H]^+ 263.51$. HRMS for $C_{13}H_9Cl_2N_2$ $[M+H]^+$ calculated mass: 263.0137, measured: 263.0136.

1-(2,4-Dichlorophenyl)-2-(2-nitroethyl)-1H-pyrrole (5). See Supplementary Fig.1e.

1-(2,4-Dichlorophenyl)-2-(2-nitroethyl)-1H-pyrrole (5). To a solution of (*E*) 1-(2,4-Dichlorophenyl)-2-(2-nitrovinyl)-1H-pyrrole (1) (200 mg, 0.70 mmol) in methanol (7 ml) was added portionwise sodium borohydride (53 mg, 1.41 mmol) at 0°C. After the addition, the reaction mixture was stirred at 0°C for 1 h. Then a mixture of ice / water (10 ml) was added and the aqueous layer was extracted with EtOAc (2x 20 ml). The combined organic layers were washed with water (2x 30 ml), dried over $MgSO_4$, filtered and concentrated under vacuum to give compound 5 as a brown oil (0.1 g, 50%). 1H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, $J = 2.4$ Hz, 1H), 7.39 (dd, $J = 8.4$ and 2.3 Hz, 1H), 7.30 (d, $J = 8.5$ Hz, 1H), 6.64 (dd, $J = 2.9$ and 1.7 Hz, 1H), 6.27 (t, $J = 3.2$ Hz, 1H), 6.13 (m, 1H), 4.48 (dd, $J = 15.2$ and 7.7 Hz, 2H), 3.09 (dd, $J = 15.4$ and 7.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.7, 135.4, 133.7, 130.6, 130.3, 128.1, 127.6, 122.8, 109.2, 108.0, 74.1, 24.3. HRMS for $C_{12}H_{11}Cl_2N_2O_2$ $[M+H]^+$ calculated mass: 285.0192, measured: 285.0189.

(E) 1-(4-Chlorophenyl)-2-(2-nitrovinyl)-1H-pyrrole (6).



Synthetic procedure for compound (6) is similar as that described for compound (1) and spectra data are shown below.

1-(4-chlorophenyl)-1H-pyrrole (40).³ Beige solid (85%). Mp: 90 °C. 1H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, $J = 9.2$ Hz, 2H), 7.21 (d, $J = 9.1$ Hz, 2H), 6.94 (t, $J = 2.2$ Hz, 2H), 6.35 (t, $J = 2.2$ Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.4, 131.1, 129.8, 121.6, 119.3, 110.8.

1-(4-chlorophenyl)-1H-pyrrole-2-carbaldehyde (41).⁴ Orange solid (30%). Mp: 98°C. 1H NMR (400 MHz, $CDCl_3$) δ 9.49 (s, 1H), 7.35 (d, $J = 8.8$ Hz, 2H), 7.21 (d, $J = 8.7$ Hz, 2H), 7.07 (dd, $J = 4.0$ and 1.7 Hz, 1H), 6.97 (m, 1H), 6.34 (dd, $J = 4.1$ and 2.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.7, 137.5, 134.1, 132.4, 131.3, 129.2, 127.2, 123.7, 111.1. LC-MS (ESI): $t_R = 4.52$ min; $[M+H]^+ 206.39$.

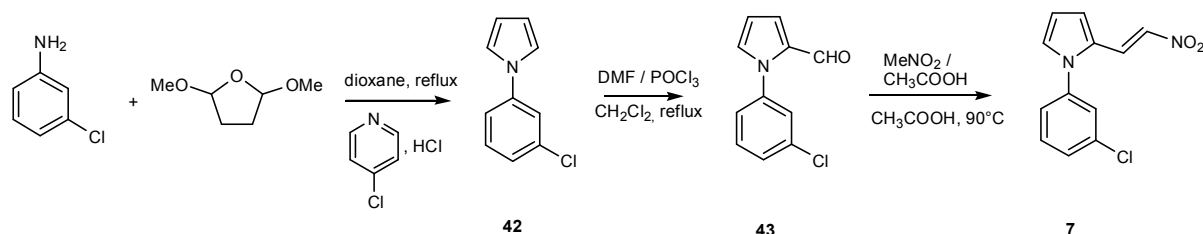
(*E*) 1-(4-chlorophenyl)-2-(2-nitrovinyl)-1H-pyrrole (6). Yellow solid (54%). Mp: 124 °C. 1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, $J = 13.3$ Hz, 1H), 7.49 (d, $J = 8.6$ Hz, 2H), 7.24 (d, $J =$

³ Das, B. *et al.* Novel approach for the synthesis of N-substituted pyrroles starting directly from nitro compounds in water. *Synthetic Communications*. **4**, 548-553 (2012)

⁴ Pina, M. *et al.* Synthesis and spectral data of 1-aryl-2-formylpyrroles. *Khimiya Geterotsiklicheskikh Soedinenii*. **2**, 180-184, (1989). *Khimiya*

13.4 Hz, 1H), 7.22 (d, $J = 8.6$ Hz, 2H), 7.07 (m, 1H), 6.91 (d, $J = 3.8$ Hz, 1H), 6.42 (t, $J = 3.6$ Hz, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 136.5, 134.9, 132.8, 130.0, 129.9, 127.8, 127.6, 125.2, 116.5, 112.1. LC-MS (ESI): $t_{\text{R}} = 5.07$ min; $[\text{M}+\text{H}]^+ 249.44$. HRMS for $\text{C}_{12}\text{H}_{10}\text{ClN}_2\text{O}_2$ $[\text{M}+\text{H}]^+$ calculated mass: 249.0425, measured: 249.0425.

(E) 1-(3-Chlorophenyl)-2-(2-nitrovinyl)-1H-pyrrole (7).



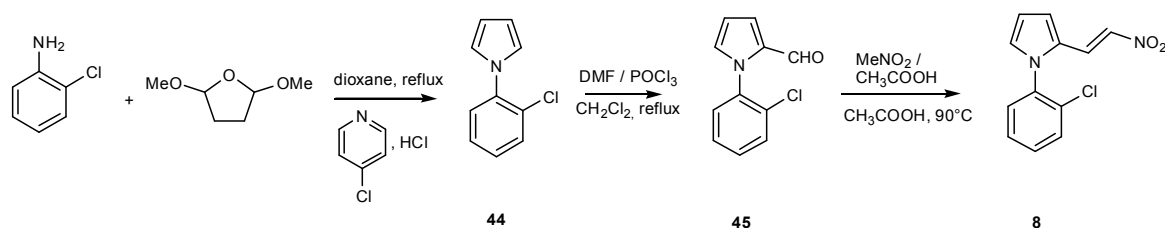
Synthetic procedure for compound (7) is similar as that described for compound (1) and spectra data are shown below.

1-(3-chlorophenyl)-1H-pyrrole (**42**).⁵ Brown solid (90%). Mp: 54 °C. ^1H NMR (400 MHz, CDCl_3) δ 7.30 (t, $J = 2.0$ Hz, 1H), 7.22 (d, $J = 7.9$ Hz, 1H), 7.18 (ddd, $J = 8.1, 2.1$ and 1.2 Hz, 1H), 7.11 (ddd, $J = 7.9, 2.0$ and 1.2 Hz, 1H), 6.97 (t, $J = 2.2$ Hz, 2H), 6.26 (t, $J = 2.2$ Hz, 2H). ^{13}C NMR (100 MHz, CDCl_3) δ 141.8, 135.2, 130.6, 125.6, 120.6, 119.2, 118.4, 111.1.

1-(3-chlorophenyl)-1H-pyrrole-2-carbaldehyde (**43**).⁴ Brown solid (50%). Mp: 68°C. ^1H NMR (400 MHz, CDCl_3) δ 9.49 (s, 1H), 7.31-7.27 (m, 3H), 7.18-7.15 (m, 1H), 7.06 (dd, $J = 4.0$ and 1.7 Hz, 1H), 6.97 (m, 1H), 6.33 (dd, $J = 3.9$ and 2.7 Hz, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 178.7, 140.0, 134.6, 132.4, 131.2, 130.0, 128.4, 126.3, 124.4, 123.5, 111.2. LC-MS (ESI): $t_{\text{R}} = 4.42$ min; $[\text{M}+\text{H}]^+ 206.34$.

(E) 1-(3-Chlorophenyl)-2-(2-nitrovinyl)-1H-pyrrole (**7**). Red oil (50%). ^1H NMR (400 MHz, CDCl_3) δ 7.78 (d, $J = 13.3$ Hz, 1H), 7.50-7.48 (m, 2H), 7.35 (m, 1H), 7.32 (d, $J = 13.3$ Hz, 1H), 7.20-7.23 (m, 1H), 7.12 (dd, $J = 2.6$ and 1.1 Hz, 1H), 6.95 (dd, $J = 4.0$ and 2.6 Hz, 1H), 6.45 (dd, $J = 3.8$ and 0.9 Hz, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ 139.1, 135.5, 132.9, 130.7, 129.8, 129.1, 127.7, 126.6, 125.2, 124.7, 116.5, 112.2. LC-MS (ESI): $t_{\text{R}} = 5.06$ min; $[\text{M}+\text{H}]^+ 249.40$. HRMS for $\text{C}_{12}\text{H}_{10}\text{ClN}_2\text{O}_2$ $[\text{M}+\text{H}]^+$ calculated mass: 249.0425, measured: 249.0425.

(E) 1-(2-Chlorophenyl)-2-(2-nitrovinyl)-1H-pyrrole (8).



⁵ Corsi, C. *et al.* Preparation of pyrrole derivatives as plant growth regulators. PCT Int. Appl., 2010069879, 24 Jun 2010.

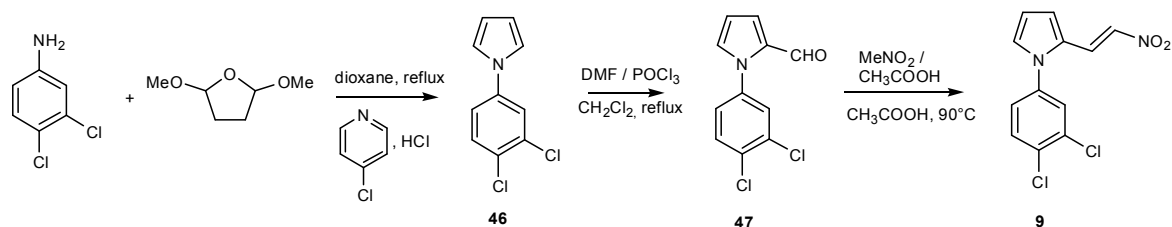
Synthetic procedure for compound (**8**) is similar as that described for compound (**1**) and spectra data are shown below.

1-(2-Chlorophenyl)-1*H*-pyrrole (**44**)¹. Brown oil (87%). ¹H NMR (400 MHz, CDCl₃) δ 7.47 (m, 1H), 7.32-7.22 (m, 3H), 6.88 (t, *J* = 2.2 Hz, 2H), 6.31 (t, *J* = 2.2 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 138.8, 130.7, 129.7, 128.3, 127.9, 127.6, 122.2, 109.3.

1-(2-Chlorophenyl)-1*H*-pyrrole-2-carbaldehyde (**45**)⁴. Beige solid (29%). Mp: 94°C. ¹H NMR (400 MHz, CDCl₃) δ 9.42 (d, *J* = 0.5 Hz, 1H), 7.44 (m, 1H), 7.35-7.27 (m, 2H), 7.05 (dd, *J* = 4.0 and 1.7 Hz, 1H), 7.07 (dd, *J* = 4.0 and 1.8 Hz, 1H), 6.89 (m, 1H), 6.37 (dd, *J* = 4.0 and 2.5 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.6, 137.1, 133.0, 132.0, 131.0, 130.2, 130.0, 129.0, 127.4, 122.2, 110.9. LC-MS (ESI): *t*_R = 4.38 min; [M+H]⁺ 206.39.

(*E*) 1-(2-Chlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**8**). Brown oil (50%). ¹H NMR (400 MHz, CDCl₃) δ 7.62-7.57 (m, 2H), 7.51 (dt, *J* = 7.5 and 1.7 Hz, 1H), 7.46 (dt, *J* = 7.6 and 1.5 Hz, 1H), 7.38 (dd, *J* = 7.6 and 1.7 Hz, 1H), 7.09 (d, *J* = 13.3 Hz, 1H), 7.04 (dd, *J* = 2.5 and 0.9 Hz, 1H), 6.95 (dd, *J* = 2.6 and 1.1 Hz, 1H), 6.49 (dd, *J* = 2.7 and 0.9 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 135.7, 132.5, 132.3, 131.0, 130.9, 130.3, 129.6, 128.0, 127.8, 125.8, 116.9, 112.0. LC-MS (ESI): *t*_R = 4.95 min; [M+H]⁺ 249.44. HRMS for C₁₂H₁₀ClN₂O₂ [M+H]⁺ calculated mass: 249.0425, measured: 249.0425.

(*E*) 1-(3,4-Dichlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**9**).



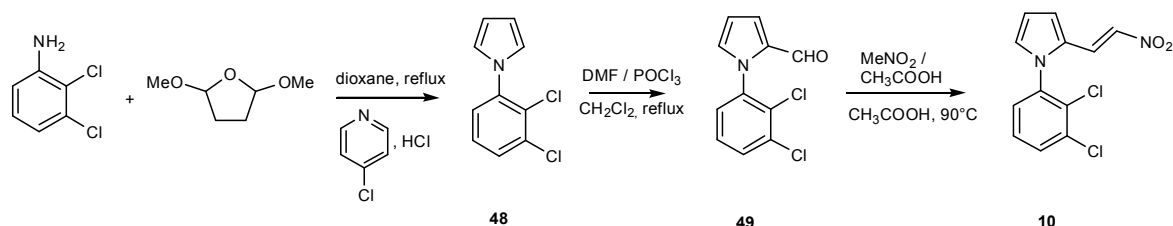
Synthetic procedure for compound (**9**) is similar as that described for compound (**1**) and spectra data are shown below.

1-(3,4-Dichlorophenyl)-1*H*-pyrrole (**46**). Brown solid (77%). Mp: 56 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.51-7.48 (m, 2H), 7.24 (dd, *J* = 8.7 and 2.6 Hz, 1H), 7.05 (t, *J* = 2.1 Hz, 2H), 6.39 (t, *J* = 2.2 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 140.0, 133.5, 131.2, 129.2, 122.1, 119.4, 119.2, 111.4.

1-(3,4-Dichlorophenyl)-1*H*-pyrrole-2-carbaldehyde (**47**). Brown solid (40%). Mp: 104°C. ¹H NMR (400 MHz, CDCl₃) δ 9.50 (d, *J* = 0.7 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 2.5 Hz, 1H), 7.14 (dd, *J* = 8.5 and 2.5 Hz, 1H), 7.07 (dd, *J* = 4.0 and 1.8 Hz, 1H), 6.97 (m, 1H), 6.35 (dd, *J* = 4.0 and 2.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.6, 138.3, 132.9, 132.5, 132.2, 131.6, 130.6, 127.8, 125.5, 124.6, 111.4. LC-MS (ESI): *t*_R = 4.81 min; [M+H]⁺ 240.30. HRMS for C₁₁H₈Cl₂NO [M+H]⁺ calculated mass: 239.9977, measured: 239.9975.

(*E*) 1-(3,4-Dichlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**9**): yellow solid (65%). Mp: 128 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.75 (d, *J* = 13.3 Hz, 1H), 7.64 (d, *J* = 8.5 Hz, 1H), 7.46 (d, *J* = 2.4 Hz, 1H), 7.34 (d, *J* = 13.3 Hz, 1H), 7.17 (dd, *J* = 8.5 and 2.4 Hz, 1H), 7.10 (dd, *J* = 2.6 and 1.1 Hz, 1H), 6.95 (dd, *J* = 4.0 and 1.3 Hz, 1H), 6.49 (dd, *J* = 3.9 and 2.8 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 135.7, 132.5, 132.3, 131.0, 130.9, 130.3, 129.6, 128.0, 127.8, 125.8, 116.9, 112.0. LC-MS (ESI): t_R = 5.29 min; [M+H]⁺ 283.44. HRMS for C₁₂H₉Cl₂N₂O₂ [M+H]⁺ calculated mass: 283.0035, measured: 283.0035.

(*E*) 1-(2,3-Dichlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (10**).**



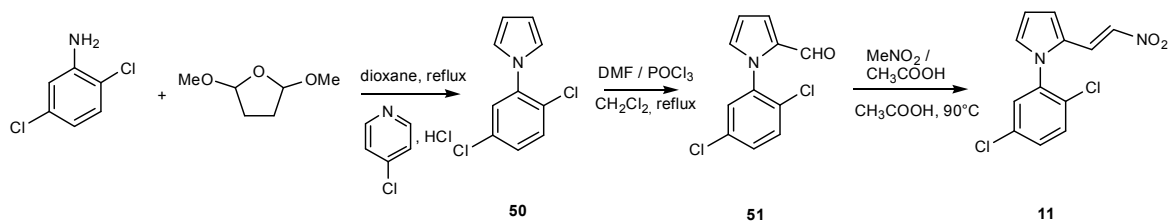
Synthetic procedure for compound (**10**) is similar as that described for compound (**1**) and spectra data are shown below.

1-(2,3-Dichlorophenyl)-1*H*-pyrrole (**48**).³ Pink oil (78%). ¹H NMR (400 MHz, CDCl₃) δ 7.49-7.47 (m, 1H), 7.27 (m, 2H), 6.90 (t, *J* = 2.1 Hz, 2H), 6.36 (t, *J* = 2.1 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 140.5, 134.4, 129.2, 129.0, 127.5, 126.2, 122.2, 109.6.

1-(2,3-Dichlorophenyl)-1*H*-pyrrole-2-carbaldehyde (**49**). Yellow solid (32%). Mp: 92°C. ¹H NMR (400 MHz, CDCl₃) δ 9.54 (s, 1H), 7.59 (dd, *J* = 7.6 and 2.1 Hz, 1H), 7.35-7.28 (m, 2H), 7.15 (dd, *J* = 4.0 and 1.6 Hz, 1H), 6.98 (m, 1H), 6.48 (dd, *J* = 4.1 and 2.5 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.5, 139.0, 134.0, 132.9, 131.3, 131.0, 130.8, 127.3, 127.2, 122.9, 111.1. LC-MS (ESI): t_R = 4.69 min; [M+H]⁺ 240.30. HRMS for C₁₁H₈Cl₂NO [M+H]⁺ calculated mass: 239.9977, measured: 239.9975.

(*E*) 1-(2,3-Dichlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**10**). Yellow solid (53%). Mp: 122 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.69 (dd, *J* = 8.1 and 1.5 Hz, 1H), 7.56 (d, *J* = 13.3 Hz, 1H), 7.40 (t, *J* = 8.0 Hz, 1H), 7.32 (dd, *J* = 7.9 and 1.5 Hz, 1H), 7.17 (d, *J* = 13.3 Hz, 1H), 7.03 (dd, *J* = 2.5 and 1.5 Hz, 1H), 6.96 (m, 1H), 6.50 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 137.3, 134.8, 132.6, 131.9, 131.8, 130.0, 128.0, 127.9, 127.4, 125.7, 116.7, 112.3. LC-MS (ESI): t_R = 5.11 min; [M+H]⁺ 283.44. HRMS for C₁₂H₉Cl₂N₂O₂ [M+H]⁺ calculated mass: 283.0035, measured: 283.0035.

(*E*) 1-(2,5-Dichlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (11**).**



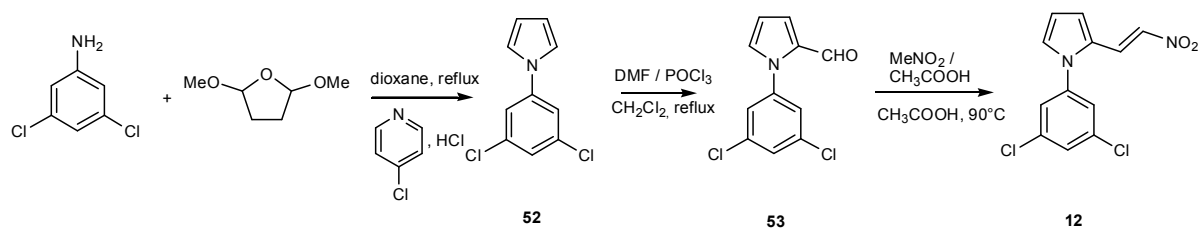
Synthetic procedure for compound (**11**) is similar as that described for compound (**1**) and spectra data are shown below.

1-(2,5-Dichlorophenyl)-1*H*-pyrrole (**50**). Brown oil (79%). ¹H NMR (400 MHz, CDCl₃) δ 7.35 (d, *J* = 8.4 Hz, 1H), 7.27 (d, *J* = 2.4 Hz, 1H), 7.17 (dd, *J* = 8.6 and 2.4 Hz, 1H), 6.82 (t, *J* = 2.2 Hz, 2H), 6.26 (t, *J* = 2.2 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 139.5, 133.1, 131.6, 128.1, 127.8, 127.7, 122.0, 109.9. HRMS for C₁₀H₉Cl₂N [M+H]⁺ calculated mass: 212.0028, measured: 212.0030.

1-(2,5-Dichlorophenyl)-1*H*-pyrrole-2-carbaldehyde (**51**). Orange solid (45%). Mp: 126°C. ¹H NMR (400 MHz, CDCl₃) δ 9.54 (s, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.40 (m, 1H), 7.37 (m, 1H), 7.13 (dd, *J* = 4.0 and 1.8 Hz, 1H), 6.95 (m, 1H), 6.46 (dd, *J* = 3.9 and 2.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.5, 138.2, 132.9, 132.8, 130.9, 130.8, 130.6, 130.0, 129.0, 123.1, 111.2. LC-MS (ESI): t_R = 4.72 min; [M+H]⁺ 240.35. HRMS for C₁₁H₈Cl₂NO [M+H]⁺ calculated mass: 239.9977, measured: 239.9972.

(*E*) 1-(2,5-Dichlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**11**) Yellow solid (59%). Mp: 124 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.55 (d, *J* = 13.2 Hz, 1H), 7.53 (s, 1H), 7.51 (d, *J* = 2.4 Hz, 1H), 7.41 (d, *J* = 2.3 Hz, 1H), 7.16 (d, *J* = 13.4 Hz, 1H), 7.01 (dd, *J* = 2.7 and 1.5 Hz, 1H), 6.95 (dd, *J* = 4.0 and 1.3 Hz, 1H), 6.50 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 136.6, 133.7, 132.8, 131.6, 131.2, 131.1, 129.9, 129.8, 127.4, 125.8, 116.8, 112.5. LC-MS (ESI): t_R = 5.13 min; [M+H]⁺ 283.49. HRMS for C₁₂H₉Cl₂N₂O₂ [M+H]⁺ calculated mass: 283.0035, measured: 283.0035.

(*E*) 1-(3,5-Dichlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**12**).



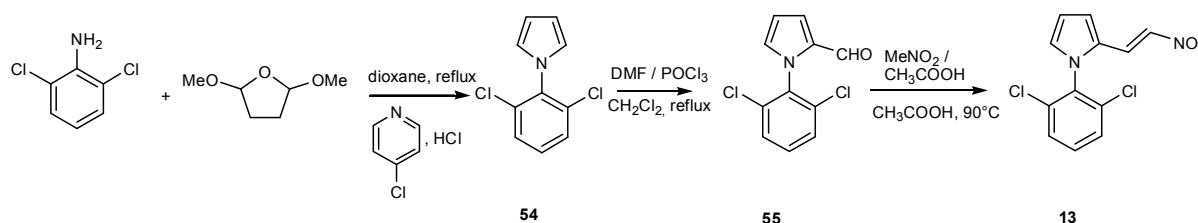
Synthetic procedure for compound (**12**) is similar as that described for compound (**1**) and spectra data are shown below.

1-(3,5-Dichlorophenyl)-1*H*-pyrrole (**52**). Brown solid (79%). Mp: 60 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.29 (d, *J* = 1.8 Hz, 2H), 7.23 (t, *J* = 1.8 Hz, 1H), 7.05 (t, *J* = 2.2 Hz, 2H), 6.38 (t, *J* = 2.2 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 142.2, 135.9, 125.4, 119.1, 118.7, 111.6. HRMS for C₁₀H₈Cl₂N [M+H]⁺ calculated mass: 212.0028, measured: 212.0032.

1-(3,5-Dichlorophenyl)-1*H*-pyrrole-2-carbaldehyde (**53**). Yellow solid (50%). Mp: 144°C. ¹H NMR (400 MHz, CDCl₃) δ 9.52 (s, 1H), 7.34 (t, *J* = 1.8 Hz, 1H), 7.20 (d, *J* = 1.8 Hz, 2H), 7.07 (dd, *J* = 3.9 and 1.6 Hz, 1H), 6.98 (m, 1H), 6.35 (dd, *J* = 3.9 and 2.7 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.5, 140.7, 135.1, 132.2, 131.3, 128.4, 124.8, 124.6, 111.5. LC-MS (ESI): *t*_R = 4.69 min; [M+H]⁺ 240.26. HRMS for C₁₁H₈Cl₂NO [M+H]⁺ calculated mass: 239.9975, measured: 239.9977.

(*E*) 1-(3,5-Dichlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**12**). Orange solid (65%). Mp: 120°C. ¹H NMR (400 MHz, CDCl₃) δ 7.76 (d, *J* = 13.3 Hz, 1H), 7.52 (t, *J* = 1.8 Hz, 1H), 7.46 (d, *J* = 2.4 Hz, 1H), 7.34 (d, *J* = 13.3 Hz, 1H), 7.25 (d, *J* = 1.8 Hz, 1H), 7.10 (dd, *J* = 2.6 and 1.5 Hz, 1H), 6.95 (dd, *J* = 4.0 and 1.3 Hz, 1H), 6.46 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 139.8, 136.2, 133.4, 129.6, 129.2, 127.2, 125.2, 125.1, 116.7, 112.5. LC-MS (ESI): *t*_R = 5.33 min; [M+H]⁺ 283.44. HRMS for C₁₂H₉Cl₂N₂O₂ [M+H]⁺ calculated mass: 283.0035, measured: 283.0036.

(*E*) 1-(2,6-Dichlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (13**)**



Synthetic procedure for compound (**13**) is similar as that described for compound (**1**) and spectra data are shown below.

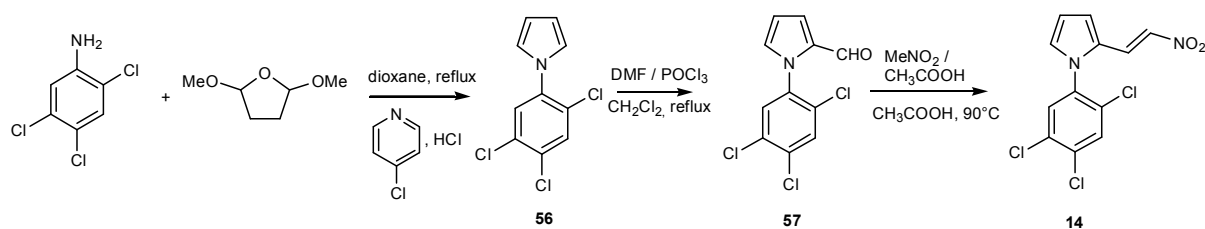
1-(2,6-Dichlorophenyl)-1*H*-pyrrole (**54**).⁶ Orange solid (83%). Mp: 90 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.47 (m, 2H), 7.33-7.29 (m, 1H), 6.76 (t, *J* = 2.3 Hz, 2H), 6.43 (t, *J* = 2.2 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 137.0, 134.5, 129.6, 128.7, 121.9, 109.4.

1-(2,6-Dichlorophenyl)-1*H*-pyrrole-2-carbaldehyde (**55**).⁶ Orange solid (45%). Mp: 94°C. ¹H NMR (400 MHz, CDCl₃) δ 9.53 (s, 1H), 7.45 (m, 2H), 7.34 (dd, *J* = 9.0 and 7.3 Hz, 1H), 7.16 (dd, *J* = 3.8 and 1.6 Hz, 1H), 6.92 (m, 1H), 6.52 (dd, *J* = 3.9 and 2.7 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.4, 135.6, 134.2, 132.3, 130.4, 130.2, 128.4, 123.1, 111.4. LC-MS (ESI): *t*_R = 4.61 min; [M+H]⁺ 240.35.

(*E*) 1-(2,6-Dichlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**13**). Orange solid (60%). Mp: 120°C. ¹H NMR (400 MHz, CDCl₃) δ 7.54 (m, 2H), 7.53 (d, *J* = 12.9 Hz, 1H), 7.46 (m, 1H), 7.03-7.01 (d, *J* = 13.1 Hz, 1H), 6.99 (dd, *J* = 4.0 and 1.4 Hz, 1H), 6.96 (dd, *J* = 2.7 and 1.4 Hz, 1H), 6.55 (dd, *J* = 3.9 and 2.8 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 135.1, 133.8, 132.3, 131.4, 129.5, 129.1, 127.2, 125.1, 117.5, 112.7. LC-MS (ESI): *t*_R = 5.05 min; [M+H]⁺ 283.40. HRMS for C₁₂H₉Cl₂N₂O₂ [M+H]⁺ calculated mass: 283.0035, measured: 283.0035.

(*E*) 1-(2,4,5-Trichlorophenyl)- 2-(2-nitrovinyl)-1*H*-pyrrole (14**).**

⁶ Ikegami, H. *et al.* Hydrazide compound and their preparation, formulation and pesticidal use. PCT Int. Appl., 2007043677, 19 Apr 2007



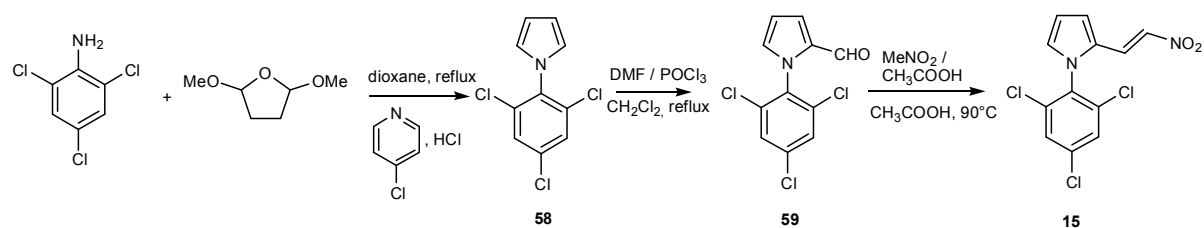
Synthetic procedure for compound (**14**) is similar as that described for compound (**1**) and spectra data are shown below.

1-(2,4,5-Trichlorophenyl)-1*H*-pyrrole (**56**).⁷ Brown solid (79%). Mp: 102 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.54 (s, 1H), 7.38 (s, 1H), 6.80 (t, *J* = 2.2 Hz, 2H), 6.27 (t, *J* = 2.2 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 138.0, 131.7, 131.7, 131.6, 128.8, 128.2, 122.0, 110.2.

1-(2,4,5-Trichlorophenyl)-1*H*-pyrrole-2-carbaldehyde (**57**). Beige solid (32%). Mp: 96°C. ¹H NMR (400 MHz, CDCl₃) δ 9.55 (s, 1H), 7.62 (s, 1H), 7.46 (s, 1H), 7.13 (dd, *J* = 4.0 and 1.5 Hz, 1H), 6.94 (m, 1H), 6.47 (dd, *J* = 3.9 and 2.5 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.5, 136.7, 133.7, 132.9, 131.4, 131.1, 131.0, 130.9, 130.0, 123.7, 111.4. LC-MS (ESI): *t*_R = 5.07 min; [M+H]⁺ 274.26. HRMS for C₁₁H₇Cl₃NO [M+H]⁺ calculated mass: 273.9587, measured: 273.9587.

(*E*) 1-(2,4,5-Trichlorophenyl)- 2-(2-nitrovinyl)-1*H*-pyrrole (**14**). Yellow solid (62%). Mp: 166 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.65 (s, 1H), 7.46-7.43 (m, 2H), 7.14 (d, *J* = 13.3 Hz, 1H), 6.91 (dd, *J* = 2.7 and 1.5 Hz, 1H), 6.88 (dd, *J* = 4.0 and 1.3 Hz 1H), 6.42 (dd, *J* = 3.8 and 2.8 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 135.1, 134.9, 133.0, 132.3, 131.8, 131.5, 130.8, 129.7, 127.1, 125.8, 116.6, 112.7. LC-MS (ESI): *t*_R = 5.42 min; [M+H]⁺ 317.27. HRMS for C₁₂H₈Cl₃N₂O₂ [M+H]⁺ calculated mass: 316.9645, measured: 316.9645.

(*E*) 1-(2,4,6-Trichlorophenyl)- 2-(2-nitrovinyl)-1*H*-pyrrole (15**).**



Synthetic procedure for compound (**15**) is similar as that described for compound (**1**) and spectra data are shown below.

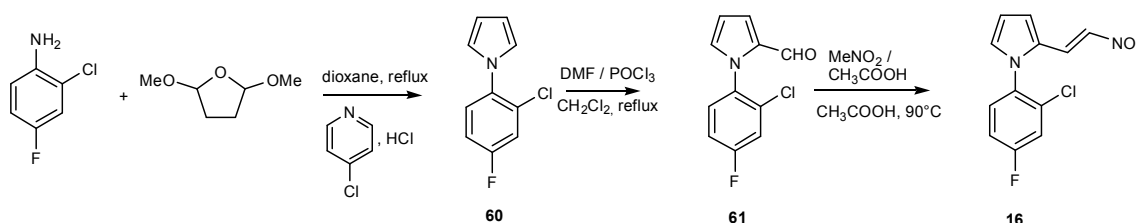
1-(2,4,6-Trichlorophenyl)-1*H*-pyrrole (**58**). Beige solid (88%). Mp: 90 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.37 (s, 2H), 6.60 (t, *J* = 2.2 Hz, 2H), 6.30 (t, *J* = 2.2 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 135.8, 135.1, 134.6, 128.6, 121.8, 109.7. HRMS for C₁₀H₇Cl₃N [M+H]⁺ calculated mass: 245.9638, measured: 245.9642.

⁷ Ma, F *et al.* A recyclable magnetic nanoparticles supported antimony catalyst for the synthesis of N-substituted pyrroles in water. *Applied Catalysis , A: General* , **457**, 34-41 (2013)

1-(2,4,6-Trichlorophenyl)-1*H*-pyrrole-2-carbaldehyde (**59**). Yellow solid (30%). Mp: 100 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.54 (s, 1H), 7.46 (s, 2H), 7.15 (dd, *J* = 4.0 and 1.5 Hz, 1H), 6.89 (m, 1H), 6.52 (dd, *J* = 4.0 and 2.8 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.4, 135.3, 134.8, 134.6, 132.2, 130.4, 128.5, 123.6, 111,7. LC-MS (ESI): t_R = 4.97 min; [M+H]⁺ 274.31. HRMS for C₁₁H₇Cl₃NO [M+H]⁺ calculated mass: 273.9587, measured: 273.9587.

(*E*) 1-(2,4,6-Trichlorophenyl)-2-(2-nitrovinyl)-1 *H*-pyrrole (**15**). Light brown solid (50%). Mp: 114 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.56 (s, 2H), 7.47 (d, *J* = 13.3 Hz, 1H), 7.10 (d, *J* = 13.3 Hz, 1H), 6.98 (dd, *J* = 4.0 and 1.3 Hz, 1H), 6.92 (dd, *J* = 2.7 and 1.4 Hz 1H), 6.55 (dd, *J* = 3.8 and 2.9 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 136.8, 135.7, 132.7, 132.6, 129.3, 129.2, 126.8, 125.1, 117.3, 113.0. LC-MS (ESI): t_R = 5.32 min; [M+H]⁺ 317.32. HRMS for C₁₂H₈Cl₃N₂O₂ [M+H]⁺ calculated mass: 316.9645, measured: 316.9645.

(*E*) 1-(2-Chloro-4-fluorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (16**).**



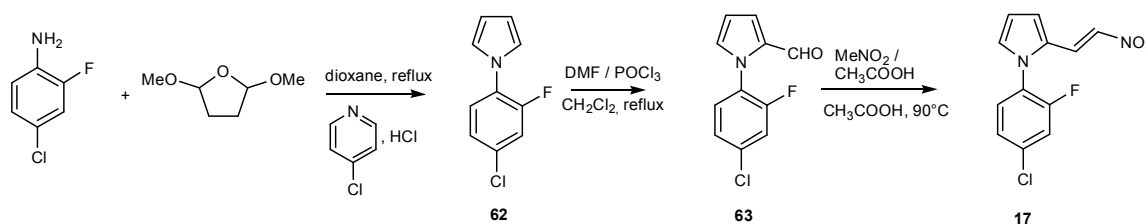
Synthetic procedure for compound (**16**) is similar as that described for compound (**1**) and spectra data are shown below.

1-(2-Chloro-4-fluorophenyl)-1*H*-pyrrole (**60**). Orange oil (80%). ¹H NMR (400 MHz, CDCl₃) δ 7.47 (d, *J* = 8.0 Hz, 2H), 7.31 (dd, *J* = 8.6 and 7.6 Hz, 1H), 6.76 (t, *J* = 2.3 Hz, 2H), 6.43 (t, *J* = 2.2 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 161.1 (d, *J* = 250.7 Hz), 135.3 (d, *J* = 3.9 Hz), 131.0 (d, *J* = 10.7 Hz), 129.0 (d, *J* = 10.7 Hz), 122.3 (s), 117.7 (d, *J* = 25.8 Hz), 114.6 (d, *J* = 21.9 Hz), 109.5 (s). HRMS for C₁₀H₈ClFN [M+H]⁺ calculated mass: 196.0323, measured: 196.0326.

1-(2-Chloro-4-fluorophenyl)-1*H*-pyrrole-2-carbaldehyde (**61**). White solid (61%). Mp: 104 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.43 (s, 1H), 7.25 (dd, *J* = 8.7 and 5.4 Hz, 1H), 7.18 (dd, *J* = 8.0 and 2.8 Hz, 1H), 7.04 (dd, *J* = 4.0 and 1.6 Hz, 1H), 7.02-6.97 (m, 1H), 6.86 (m, 1H), 6.37 (dd, *J* = 4.0 and 2.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.6 (s), 162.1 (d, *J* = 250.9 Hz), 133.6 (d, *J* = 3.7 Hz), 133.1 (d, *J* = 10.5 Hz), 133.0 (s), 131.2 (s), 129.9 (d, *J* = 9.2 Hz), 123.0 (bs), 117.5 (d, *J* = 25.9 Hz), 114,6 (d, *J* = 21.6 Hz), 111.0 (s). LC-MS (ESI): t_R = 4.49 min; [M+H]⁺ 224.40. HRMS for C₁₁H₈ClFNO [M+H]⁺ calculated mass: 224.0272, measured: 224.0272.

(*E*) 1-(2-Chloro-4-fluorophenyl)-2-(2-nitrovinyl)-1 *H*-pyrrole (**16**). Orange solid (55%). Mp: 102 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.54 (d, *J* = 13.4 Hz, 1H), 7.40-7.34 (m, 2H), 7.20-7.15 (m, 1H), 7.12 (d, *J* = 13.3 Hz, 1H), 7.01 (dd, *J* = 2.6 and 1.5 Hz, 1H), 6.95 (dd, *J* = 4.0 and 1.3 Hz, 1H), 6.49 (dd, *J* = 3.8 and 2.8 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 162.6 (d, *J* = 257.9 Hz), 133.9 (d, *J* = 10.9 Hz), 132.5 (s), 132.0 (d, *J* = 3.7 Hz), 130.8 (d, *J* = 9.5 Hz), 130.3 (s), 127.5 (s), 125.9 (s), 118.3 (d, *J* = 25.7 Hz), 116.8 (s), 115.4 (d, *J* = 22.3 Hz), 112.3 (s). LC-MS (ESI): t_R = 4.95 min; [M+H]⁺ 267.41. HRMS for C₁₂H₉ClFN₂O₂ [M+H]⁺ calculated mass: 267.0331, measured: 267.0329.

(E) 1-(4-Chloro-2-fluorophenyl)-2-(2-nitrovinyl)-1H-pyrrole (17).



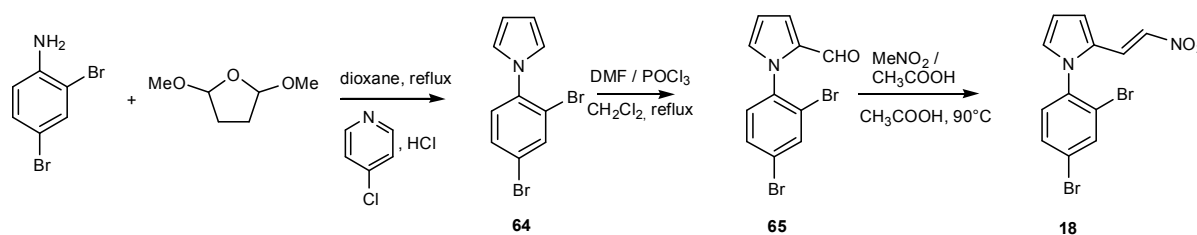
Synthetic procedure for compound (17) is similar as that described for compound (1) and spectra data are shown below.

1-(4-Chloro-2-fluorophenyl)-1H-pyrrole (**62**). Orange oil (75%). ¹H NMR (400 MHz, CDCl₃) δ 7.34 (t, *J* = 8.5 Hz, 1H), 7.27 (m, 1H), 7.21 (ddd, *J* = 8.4, 2.2 and 1.2 Hz, 1H), 7.03 (q, *J* = 2.1 Hz, 2H), 6.38 (t, *J* = 2.2 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 154.7 (d, *J* = 255.7 Hz), 131.9 (d, *J* = 9.8 Hz), 127.8 (d, *J* = 10.5 Hz), 125.5 (d, *J* = 2.0 Hz), 125.1 (d, *J* = 4.1 Hz), 121.2 (d, *J* = 4.8 Hz), 117.8 (d, *J* = 23.7 Hz), 110.3 (s). HRMS for C₁₀H₈ClFN [M+H]⁺ calculated mass: 196.0323, measured: 196.0329.

1-(4-Chloro-2-fluorophenyl)-1H-pyrrole-2-carbaldehyde (**63**). White solid (60%). Mp: 82 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.58 (s, 1H), 7.32-7.23 (m, 3H), 7.15 (m, 1H), 7.01 (m, 1H), 6.47 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.6 (s), 156.9 (d, *J* = 253.8 Hz), 135.0 (d, *J* = 9.2 Hz), 132.8 (s), 131.5 (s), 128.9 (s), 126.3 (d, *J* = 12.9 Hz), 124.7 (d, *J* = 3.7 Hz), 124.0 (bs), 117.2 (d, *J* = 22.8 Hz), 111.3 (s). LC-MS (ESI): t_R = 4.55 min; [M+H]⁺ 224.35. HRMS for C₁₁H₈ClFNO [M+H]⁺ calculated mass: 224.0272, measured: 224.0272.

(E) 1-(4-Chloro-2-fluorophenyl)-2-(2-nitrovinyl)-1H-pyrrole (**17**). Yellow solid (60%). Mp: 134 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.60 (dt, *J* = 13.4 and 0.6 Hz, 1H), 7.34 (dd, *J* = 1.9 and 0.5 Hz, 1H), 7.32 (m, 1H), 7.29 (m, 1H), 7.26-7.23 (m, 1H), 7.03 (m, 1H), 6.94 (dd, *J* = 4.0 and 1.4 Hz, 1H), 6.49 (dd, *J* = 3.8 and 2.8 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 156.9 (d, *J* = 257.9 Hz), 136.2 (d, *J* = 9.6 Hz), 133.0 (s), 130.2 (s), 129.7 (s), 127.3 (s), 125.8 (s), 125.7 (d, *J* = 3.7 Hz), 124.6 (d, *J* = 12.3 Hz), 118.1 (d, *J* = 22.7 Hz), 116.5 (s), 112.5 (s). LC-MS (ESI): t_R = 5.02 min; [M+H]⁺ 267.45. HRMS for C₁₂H₉ClFN₂O₂ [M+H]⁺ calculated mass: 267.0331, measured: 267.0330.

(E) 1-(2,4-Dibromophenyl)-2-(2-nitrovinyl)-1H-pyrrole (18).



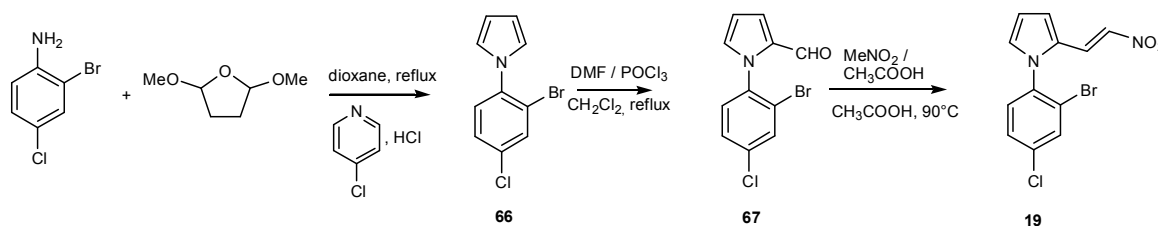
Synthetic procedure for compound (18) is similar as that described for compound (1) and spectra data are shown below.

1-(2,4-Dibromophenyl)-1*H*-pyrrole (**64**). Orange oil (94%). ¹H NMR (400 MHz, CDCl₃) δ 7.86 (d, *J* = 2.2 Hz, 1H), 7.52 (dd, *J* = 8.1 and 1.9 Hz, 1H), 7.21 (d, *J* = 8.3 Hz, 1H), 6.85 (t, *J* = 2.4 Hz, 2H), 6.35 (t, *J* = 2.3 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 139.6, 136.1, 131.3, 129.2, 122.1, 121.4, 120.6, 109.6. HRMS for C₁₀H₈Br₂N [M+H]⁺ calculated mass: 299.9018, measured: 299.9017.

1-(2,4-Dibromophenyl)-1*H*-pyrrole-2-carbaldehyde (**65**). White solid (30%). Mp: 88 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.52 (s, 1H), 7.55 (d, *J* = 2.3 Hz, 1H), 7.54 (dd, *J* = 8.4 and 2.3 Hz, 1H), 7.22 (d, *J* = 8.5 Hz, 1H), 7.12 (dd, *J* = 3.9 and 1.7 Hz, 1H), 6.93 (m, 1H), 6.45 (dd, *J* = 3.8 and 2.5 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.2, 138.1, 135.7, 132.7, 131.3, 130.9, 130.0, 123.2, 123.0, 122.9, 111.1. LC-MS (ESI): t_R = 4.92 min; [M+H]⁺ 328.25. HRMS for C₁₁H₈Br₂NO [M+H]⁺ calculated mass: 327.8967, measured: 327.8963.

(*E*) 1-(2,4-Dibromophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**18**). Orange solid (60%). Mp: 122 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.93 (d, *J* = 2.1 Hz, 1H), 7.63 (dd, *J* = 8.4 and 2.2 Hz, 1H), 7.53 (d, *J* = 13.4 Hz, 1H), 7.26 (d, *J* = 8.3 Hz, 1H), 7.13 (d, *J* = 13.5 Hz, 1H), 7.00 (dd, *J* = 2.7 and 1.5 Hz, 1H), 6.95 (dd, *J* = 4.0 and 1.4 Hz, 1H), 6.49 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 136.4, 134.3, 133.4, 132.6, 130.8, 130.3, 130.0, 128.4, 127.4, 125.8, 116.7, 112.3. LC-MS (ESI): t_R = 5.30 min; [M+H]⁺ 371.30. HRMS for C₁₂H₉Br₂N₂O₂ [M+H]⁺ calculated mass: 370.9025, measured: 370.9023.

(*E*) 1-(2-Bromo-4-chlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (19**).**



Synthetic procedure for compound (**19**) is similar as that described for compound (**1**) and spectra data are shown below.

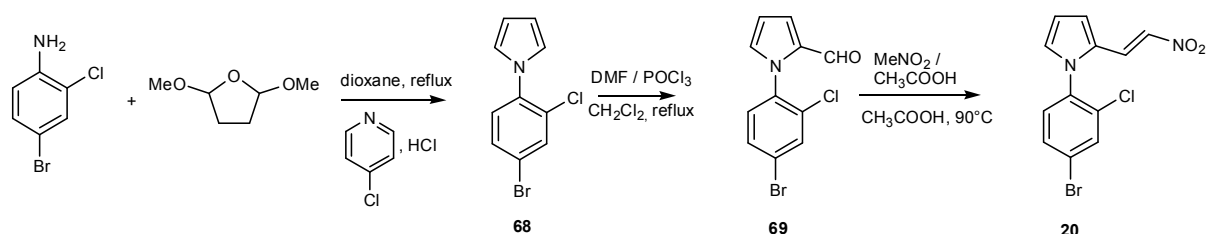
1-(2-Bromo-4-chlorophenyl)-1*H*-pyrrole (**66**).⁸ Orange oil (89%). ¹H NMR (400 MHz, CDCl₃) δ 7.60 (d, *J* = 2.3 Hz, 1H), 7.25 (dd, *J* = 8.4 and 2.3 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 6.74 (t, *J* = 2.2 Hz, 2H), 6.24 (t, *J* = 2.3 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 139.2, 134.6, 133.6, 128.9, 128.5, 122.2, 120.4, 109.6.

1-(2-Bromo-4-chlorophenyl)-1*H*-pyrrole-2-carbaldehyde (**67**). White solid (30%). Mp: 102 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.43 (s, 1H), 7.61 (d, *J* = 2.4 Hz, 1H), 7.31 (dd, *J* = 8.4 and 2.3 Hz, 1H), 7.20 (d, *J* = 8.3 Hz, 1H), 7.04 (dd, *J* = 4.1 and 1.7 Hz, 1H), 6.85 (m, 1H), 6.38 (dd, *J* = 3.9 and 2.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.5, 137.6, 135.4, 133.0, 132.8, 131.0, 129.6, 128.3, 123.0, 122.6, 111.1. LC-MS (ESI): t_R = 4.80 min; [M+H]⁺ 284.30. HRMS for C₁₁H₈BrClNO [M+H]⁺ calculated mass: 283.9472, measured: 283.9470.

⁸ Sugita, K *et al*. Preparation of tricyclic compounds such as pyrrolobenzoxazepine derivatives and analogs thereof for treatment of hypercholesteremia, hyperlipemia, and arteriosclerosis. Jpn. Kokai Tokkyo Koho, 2008291018, 04 Dec 2008

(*E*) 1-(2-Bromo-4-chlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**19**). Yellow solid (53%). Mp: 98 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.79 (d, *J* = 2.2 Hz, 1H), 7.53 (d, *J* = 13.3 Hz, 1H), 7.48 (dd, *J* = 8.3 and 2.3 Hz, 1H), 7.32 (d, *J* = 8.3 Hz, 1H), 7.12 (d, *J* = 13.3 Hz, 1H), 6.99 (dd, *J* = 2.5 and 1.5 Hz, 1H), 6.95 (dd, *J* = 4.0 and 1.3 Hz, 1H), 6.49 (dd, *J* = 3.8 and 2.7 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 136.6, 136.0, 133.7, 132.6, 130.4, 130.0, 129.0, 127.4, 125.7, 123.3, 116.9, 112.3. LC-MS (ESI): *t*_R = 5.23 min; [M+H]⁺ 327.35. HRMS for C₁₂H₉BrClN₂O₂ calculated mass: 326.9530, measured: 326.9529

(*E*) 1-(4-Bromo-2-chlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (20**).**



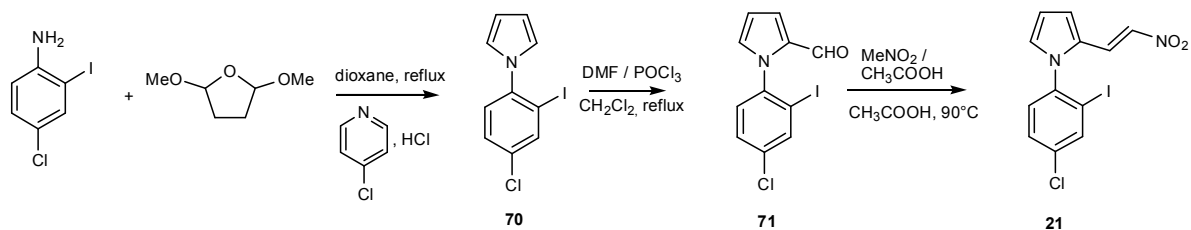
Synthetic procedure for compound (**20**) is similar as that described for compound (**1**) and spectra data are shown below.

1-(4-Bromo-2-chlorophenyl)-1*H*-pyrrole (**68**). Orange oil (90%). ¹H NMR (400 MHz, CDCl₃) δ 7.68 (d, *J* = 2.3 Hz, 1H), 7.47 (dd, *J* = 8.4 and 2.3 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 6.89 (t, *J* = 2.1 Hz, 2H), 6.35 (t, *J* = 2.2 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 137.9, 133.3, 130.8, 130.6, 128.8, 122.0, 120.8, 109.7. HRMS for C₁₀H₈BrClN [M+H]⁺ calculated mass: 255.9523, measured: 255.9523.

1-(4-Bromo-2-chlorophenyl)-1*H*-pyrrole-2-carbaldehyde (**69**). Beige solid (31%). Mp: 92 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.52 (s, 1H), 7.68 (d, *J* = 2.1 Hz, 1H), 7.50 (dd, *J* = 8.4 and 2.2 Hz, 1H), 7.22 (d, *J* = 8.3 Hz, 1H), 7.13 (dd, *J* = 4.0 and 1.7 Hz, 1H), 6.94 (m, 1H), 6.46 (dd, *J* = 3.9 and 2.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.5, 136.5, 133.1, 132.9, 132.8, 131.1, 130.7, 129.9, 123.2, 122.9, 111.2. LC-MS (ESI): *t*_R = 4.83 min; [M+H]⁺ 284.25. HRMS for C₁₁H₈BrClNO [M+H]⁺ calculated mass: 283.9472, measured: 283.9468.

(*E*) 1-(4-Bromo-2-chlorophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**20**). Orange solid (57%). Mp: 124 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.77 (d, *J* = 2.1 Hz, 1H), 7.59 (dd, *J* = 8.3 and 2.1 Hz, 1H), 7.54 (d, *J* = 13.3 Hz, 1H), 7.25 (d, *J* = 8.3 Hz, 1H), 7.16 (d, *J* = 13.3 Hz, 1H), 7.00 (dd, *J* = 2.7 and 1.4 Hz, 1H), 6.95 (dd, *J* = 4.0 and 1.4 Hz, 1H), 6.49 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 134.8, 133.6, 133.7, 132.6, 131.4, 130.6, 130.0, 127.4, 125.7, 124.1, 116.7, 112.4. LC-MS (ESI): *t*_R = 5.26 min; [M+H]⁺ 327.35. HRMS for C₁₂H₉BrClN₂O₂ calculated mass: 326.9530, measured: 326.9529.

(*E*) 1-(4-Chloro-2-iodophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (21**).**



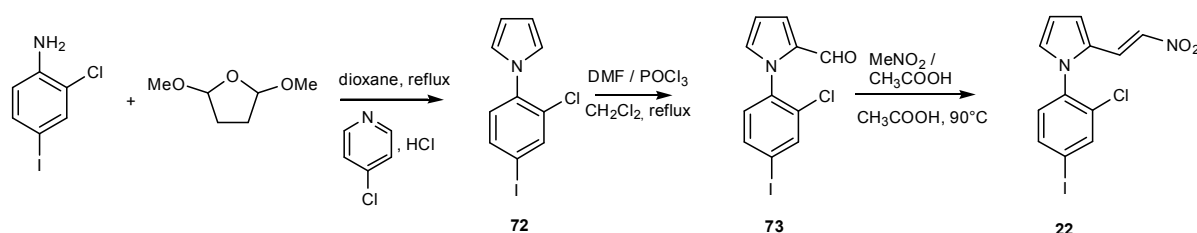
Synthetic procedure for compound (**21**) is similar as that described for compound (**1**) and spectra data are shown below.

1-(4-Chloro-2-iodophenyl)-1*H*-pyrrole (**70**).⁹ Yellow solid (88%). Mp: 72 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.84 (d, *J* = 2.3 Hz, 1H), 7.31 (dd, *J* = 8.4 and 2.2 Hz, 1H), 7.13 (d, *J* = 8.5 Hz, 1H), 6.74 (t, *J* = 2.2 Hz, 2H), 6.26 (t, *J* = 2.2 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 142.7, 139.2, 134.2, 129.1, 128.4, 122.1, 109.5, 96.0.

1-(4-Chloro-2-iodophenyl)-1*H*-pyrrole-2-carbaldehyde (**71**). Pink solid (30%). Mp: 62 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.51 (s, 1H), 7.91 (d, *J* = 2.3 Hz, 1H), 7.42 (dd, *J* = 8.3 and 2.2 Hz, 1H), 7.24 (d, *J* = 8.3 Hz, 1H), 7.12 (dd, *J* = 4.0 and 1.7 Hz, 1H), 6.90 (m, 1H), 6.46 (dd, *J* = 4.0 and 2.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 178.5, 141.2, 138.8, 135.3, 132.5, 130.8, 129.1, 128.8, 122.9, 111.2, 97.8. LC-MS (ESI): *t*_R = 4.86 min; [M+H]⁺ 332.23. HRMS for C₁₁H₈ClINO [M+H]⁺ calculated mass: 331.9333, measured: 331.9329.

(*E*) 1-(4-Chloro-2-iodophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**21**). Orange solid (57%). Mp: 100 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.99 (d, *J* = 2.0 Hz, 1H), 7.52 (m, 2H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.07 (d, *J* = 13.3 Hz, 1H), 6.94 (m, 2H), 6.49 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 139.6, 136.5, 132.6, 132.5, 129.9, 129.8, 129.5, 127.5, 125.4, 117.2, 112.4, 98.6. LC-MS (ESI): *t*_R = 5.35 min; [M+H]⁺ 375.29. HRMS for C₁₂H₉ClIN₂O₂ calculated mass: 374.9391, measured: 374.9391

(*E*) 1-(2-Chloro-4-iodophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**22**).



Synthetic procedure for compound (**22**) is similar as that described for compound (**1**) and spectra data are shown below.

1-(2-Chloro-4-iodophenyl)-1*H*-pyrrole (**72**). Orange oil (95%). ¹H NMR (400 MHz, CDCl₃) δ 7.78 (d, *J* = 2.0 Hz, 1H), 7.57 (dd, *J* = 8.3 and 1.9 Hz, 1H), 6.99 (d, *J* = 8.3 Hz, 1H), 6.80 (t, *J* = 2.3 Hz, 2H), 6.27 (t, *J* = 2.3 Hz, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 139.0, 138.6, 137.8,

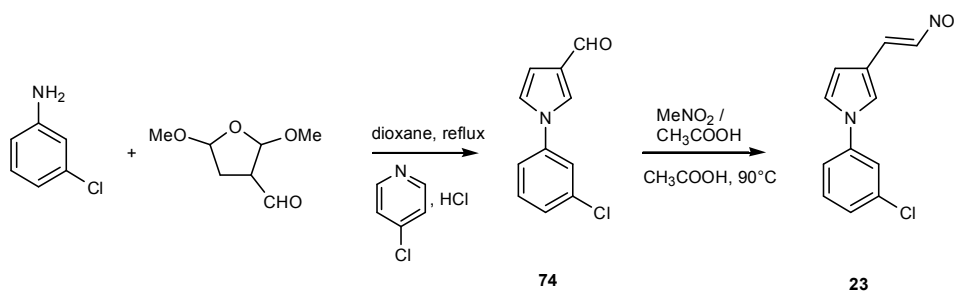
⁹ Chai, D. *et al*. Mechanistic Studies of Pd-Catalyzed Regioselective Aryl C-H Bond Functionalization with Strained Alkenes: Origin of Regioselectivity. *Chemistry - A European Journal*, **29**, 8175-8188, S8175/1-S8175/54 (2011).

130.5, 129.0, 122.0, 109.8, 91.6. HRMS for $C_{10}H_8ClIN$ $[M+H]^+$ calculated mass: 303.9384, measured: 303.9384.

1-(2-Chloro-4-iodophenyl)-1*H*-pyrrole-2-carbaldehyde (**73**). Yellow solid (32%). Mp: 92 °C. 1H NMR (400 MHz, $CDCl_3$) δ 9.52 (s, 1H), 7.87 (d, $J = 2.0$ Hz, 1H), 7.69 (dd, $J = 8.3$ and 2.0 Hz, 1H), 7.13 (dd, $J = 3.8$ and 1.6 Hz, 1H), 7.07 (d, $J = 8.3$ Hz, 1H), 6.94 (m, 1H), 6.46 (dd, $J = 3.9$ and 2.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.5, 138.5, 137.2, 136.6, 133.0, 132.8, 131.0, 130.1, 123.2, 111.2, 94.1. LC-MS (ESI): $t_R = 4.98$ min; $[M+H]^+$ 332.28. HRMS for $C_{11}H_8ClINO$ $[M+H]^+$ calculated mass: 331.9333, measured: 331.9329.

(*E*) 1-(2-Chloro-4-iodophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**22**). Orange solid (57%). Mp: 96 °C. 1H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, $J = 2.0$ Hz, 1H), 7.78 (dd, $J = 8.2$ and 1.9 Hz, 1H), 7.56 (d, $J = 13.4$ Hz, 1H), 7.17 (d, $J = 13.3$ Hz, 1H), 7.09 (d, $J = 8.3$ Hz, 1H), 7.00 (dd, $J = 2.1$ and 1.3 Hz, 1H), 6.95 (m, 1H), 6.49 (t, $J = 3.3$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.3, 137.3, 135.5, 133.4, 132.6, 130.8, 130.0, 127.4, 125.7, 116.8, 112.4, 95.4. LC-MS (ESI): $t_R = 5.35$ min; $[M+H]^+$ 375.29. HRMS for $C_{12}H_9ClIN_2O_2$ calculated mass: 374.9391, measured: 374.9391.

(*E*) 1-(3-Chlorophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (23**).**



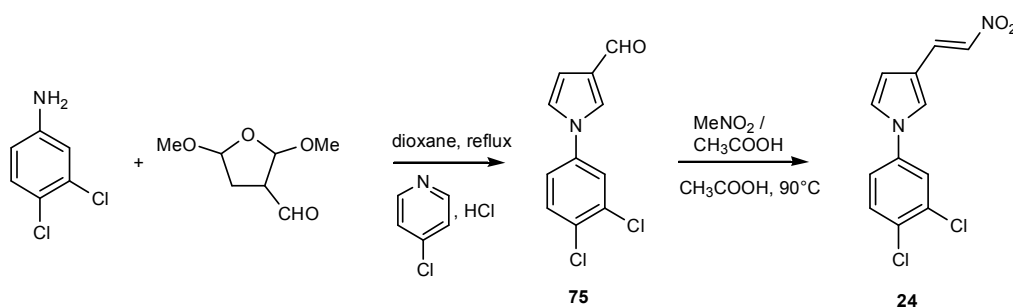
Synthetic procedure for compound (**23**) is similar as that described for compound (**2**) and spectra data are shown below.

1-(3-Chlorophenyl)-1*H*-pyrrole-3-carbaldehyde (**74**).¹⁰ Brown solid (50%). Mp: 68°C. 1H NMR (400 MHz, $CDCl_3$) δ 9.86 (s, 1H), 7.66 (t, $J = 2.0$ Hz, 1H), 7.44 (t, $J = 2.0$ Hz, 1H), 7.41 (d, $J = 8.0$ Hz, 1H), 7.37-7.31 (m, 2H), 7.07 (m, 1H), 6.81 (dd, $J = 3.1$ and 1.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.4, 140.5, 135.6, 131.0, 128.5, 127.4, 127.0, 122.2, 121.4, 119.2, 110.0. LC-MS (ESI): $t_R = 4.42$ min; $[M+H]^+$ 206.34.

(*E*) 1-(3-chlorophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (**23**). Brown solid (50%). Mp: 104 °C. 1H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, $J = 13.3$ Hz, 1H), 7.46 (d, $J = 13.3$ Hz, 1H), 7.43-7.40 (m, 3H), 7.33 (dt, $J = 8.1$ and 1.0 Hz, 1H), 7.39 (dt, $J = 8.0$ and 1.0 Hz, 1H), 7.11 (t, $J = 2.7$ Hz, 1H), 6.57 (dd, $J = 3.0$ and 1.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.4, 135.7, 134.3, 133.1, 131.0, 127.2, 124.9, 122.7, 121.1, 118.8, 118.2, 109.5. LC-MS (ESI): $t_R = 5.15$ min; $[M+H]^+$ 249.40. HRMS for $C_{12}H_{10}ClN_2O_2$ $[M+H]^+$ calculated mass: 249.0425, measured: 249.0424.

¹⁰ McInnes, Campbell and Liu, Shu. Cyclin based inhibitors of CDK2 and CDK4. U.S. Pat. Appl. Publ., 20130289240, 31 Oct 2013.

(E) 1-(3,4-Dichlorophenyl)-3-(2-nitrovinyl)-1H-pyrrole (24).

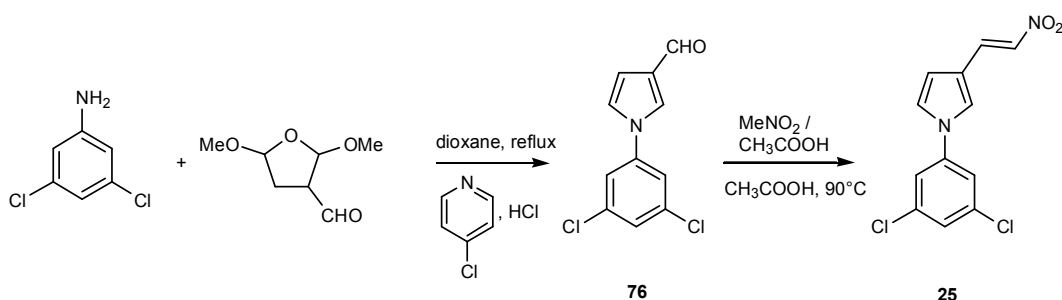


Synthetic procedure for compound (24) is similar as that described for compound (2) and spectra data are shown below.

1-(3,4-Dichlorophenyl)-1H-pyrrole-3-carbaldehyde (**75**).¹¹ Orange solid (30%). Mp: 112 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.78 (s, 1H), 7.56 (t, *J* = 1.9 Hz, 1H), 7.48 (m, 2H), 7.21 (dd, *J* = 8.6 and 2.6 Hz, 1H), 6.98 (t, *J* = 2.6 Hz, 1H), 6.74 (dd, *J* = 3.0 and 1.7 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 185.4, 138.8, 134.0, 131.6, 131.4, 128.7, 126.8, 123.0, 122.1, 120.2, 110.0. LC-MS (ESI): *t*_R = 4.92 min; [M+H]⁺ 240.26.

(*E*) 1-(3,4-Dichlorophenyl)-3-(2-nitrovinyl)-1H-pyrrole (**24**). Brown solid (40%). Mp: 128 °C. ¹H NMR (400 MHz, CDCl₃) δ 8.01 (d, *J* = 13.2 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 1H), 7.54 (d, *J* = 2.9 Hz, 1H), 7.47 (d, *J* = 13.4 Hz, 1H), 7.41 (t, *J* = 2.0 Hz, 1H), 7.27 (dd, *J* = 8.6 and 2.6 Hz, 1H), 7.10 (t, *J* = 2.5 Hz, 1H), 6.59 (dd, *J* = 3.1 and 1.8 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 138.6, 134.6, 134.0, 132.8, 131.6, 131.2, 124.7, 122.7, 122.6, 119.9, 118.4, 109.8. LC-MS (ESI): *t*_R = 5.37 min; [M+H]⁺ 283.44. HRMS for C₁₂H₉Cl₂N₂O₂ [M+H]⁺ calculated mass: 283.0035, measured: 283.0036.

(E) 1-(3,5-Dichlorophenyl)-3-(2-nitrovinyl)-1H-pyrrole (25).



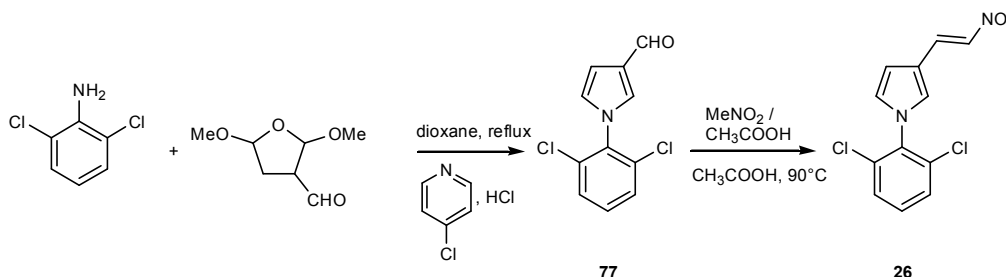
Synthetic procedure for compound (25) is similar as that described for compound (2) and spectra data are shown below.

1-(3,5-Dichlorophenyl)-1H-pyrrole-3-carbaldehyde (**76**). Brown solid (35%). Mp: 154 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.86 (s, 1H), 7.65 (t, *J* = 2.1 Hz, 1H), 7.36-7.34 (m, 3H), 7.07 (t, *J* = 2.8 Hz, 1H), 6.83 (dd, *J* = 3.0 and 1.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 185.3, 141.1, 136.3, 128.8, 127.3, 126.7, 122.0, 119.7, 110.4. LC-MS (ESI): *t*_R = 4.85 min; [M+H]⁺ 240.35. HRMS for C₁₁H₈Cl₂NO [M+H]⁺ calculated mass: 239.9975, measured: 239.9977.

¹¹ Halder, P. *et al.* Sodium borohydride-iodine mediated reduction of γ -lactam carboxylic acids followed by DDQ mediated oxidative aromatization: a simple approach towards N-aryl-formylpyrroles and 1,3-diaryl-formylpyrroles. *Tetrahedron*, **14**, 3049-3056 (2007)

(*E*) 1-(3,5-Dichlorophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (**25**). Brown solid (40%). Mp: 190°C. ¹H NMR (400 MHz, CDCl₃) δ 7.99 (d, *J* = 13.3 Hz, 1H), 7.45 (d, *J* = 13.4 Hz, 1H), 7.41 (t, *J* = 1.9 Hz, 1H), 7.34 (t, *J* = 1.7 Hz, 1H), 7.31 (d, *J* = 1.8 Hz, 2H), 7.10 (t, *J* = 2.7 Hz, 1H), 6.58 (dd, *J* = 3.0 and 1.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 140.9, 136.3, 134.7, 132.6, 127.1, 124.5, 122.6, 119.3, 118.6, 109.9. LC-MS (ESI): t_R = 5.49 min; [M+H]⁺ 283.40. HRMS for C₁₂H₉Cl₂N₂O₂ [M+H]⁺ calculated mass: 283.0035, measured: 283.0035.

(*E*) 1-(2,6-Dichlorophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (26**).**

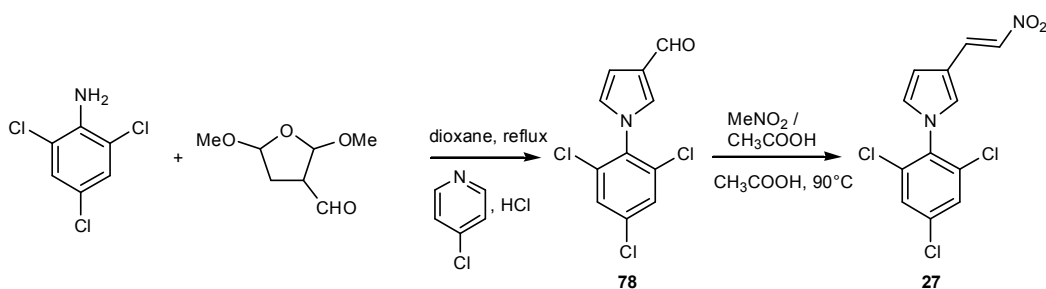


Synthetic procedure for compound (**26**) is similar as that described for compound (**2**) and spectra data are shown below.

1-(2,6-Dichlorophenyl)-1*H*-pyrrole-3-carbaldehyde (**77**).⁶ White solid (30%). Mp: 92°C. ¹H NMR (400 MHz, CDCl₃) δ 9.79 (s, 1H), 7.42-7.39 (m, 2H), 7.30 (m, 1H), 7.27 (dd, *J* = 3.7 and 1.1 Hz, 1H), 6.75 (dd, *J* = 3.1 and 1.5 Hz, 1H), 6.66 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 185.4, 135.5, 133.9, 130.6, 130.4, 128.9, 127.7, 124.7, 108.5. LC-MS (ESI): t_R = 4.85 min; [M+H]⁺ 240.39.

(*E*) 1-(2,6-Dichlorophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (**26**). Yellow solid (50%). Mp: 108°C. ¹H NMR (400 MHz, CDCl₃) δ 8.04 (d, *J* = 13.2 Hz, 1H), 7.49-7.46 (m, 3H), 7.37 (dd, *J* = 8.8 and 7.3 Hz, 1H), 7.12 (m, 1H), 6.77 (m, 1H), 6.61-6.58 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 134.1, 133.9, 133.4, 130.5, 129.9, 128.9, 128.1, 125.3, 117.1, 108.0. LC-MS (ESI): t_R = 5.08 min; [M+H]⁺ 283.40. HRMS for C₁₂H₉Cl₂N₂O₂ [M+H]⁺ calculated mass: 283.0035, measured: 283.0035.

(*E*) 3-(2-Nitrovinyl)-1-(2,4,6-trichlorophenyl)-1*H*-pyrrole (27**).**

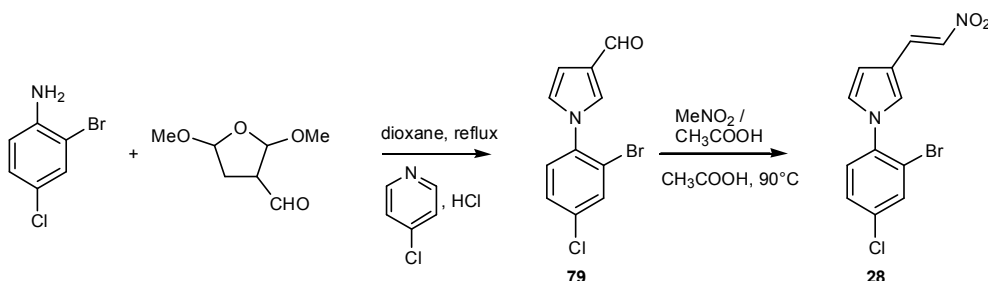


Synthetic procedure for compound (**27**) is similar as that described for compound (**2**) and spectra data are shown below.

1-(2,4,6-Trichlorophenyl)-1*H*-pyrrole-3-carbaldehyde (**78**). Beige solid (30%). Mp: 74°C. ¹H NMR (400 MHz, CDCl₃) δ 9.87 (s, 1H), 7.50 (s, 2H), 7.31 (t, *J* = 2.0 Hz, 1H), 6.83 (dd, *J* = 3.1 and 1.5 Hz, 1H), 6.71-6.69 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 185.3, 135.8, 134.6, 134.3, 130.1, 128.9, 127.9, 124.6, 108.8. LC-MS (ESI): t_R = 4.86 min; [M+H]⁺ 274.31. HRMS for C₁₁H₇Cl₃NO [M+H]⁺ calculated mass: 273.9587, measured: 273.9582.

(*E*) 3-(2-Nitrovinyl)-1-(2,4,6-trichlorophenyl)-1*H*-pyrrole (**27**). Yellow solid (50%). Mp: 90 °C. ¹H NMR (400 MHz, CDCl₃) δ 8.02 (d, *J* = 13.2 Hz, 1H), 7.50 (s, 2H), 7.47 (d, *J* = 13.3 Hz, 1H), 7.08 (t, *J* = 1.7 Hz, 1H), 6.73 (t, *J* = 6.7 Hz, 1H), 6.58 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 135.8, 134.5, 134.3, 134.2, 133.1, 128.9, 127.9, 125.2, 117.3, 108.3. LC-MS (ESI): t_R = 5.39 min; [M-H]⁻ 315.31. HRMS for C₁₂H₈Cl₃N₂O₂ [M+H]⁺ calculated mass: 316.9645, measured: 316.9645.

(E) 1-(2-Bromo-4-chlorophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (28**).**



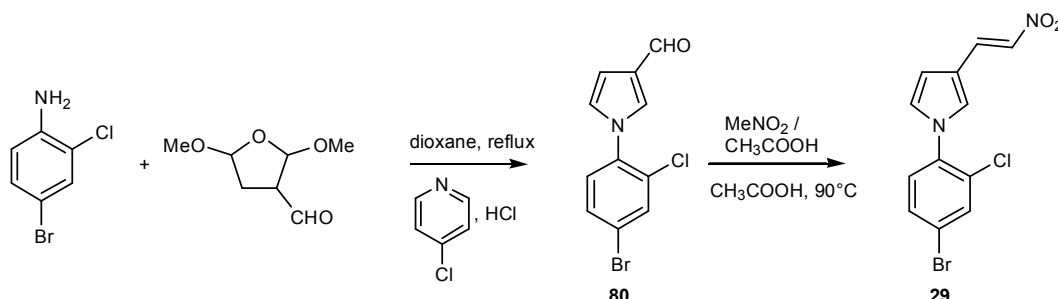
Synthetic procedure for compound (**28**) is similar as that described for compound (**2**) and spectra data are shown below.

1-(2-Bromo-4-chlorophenyl)-1*H*-pyrrole-3-carbaldehyde (**79**). Rose solid (32%). Mp: 86 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.85 (s, 1H), 7.74 (d, *J* = 2.3 Hz, 1H), 7.44 (t, *J* = 1.8 Hz, 1H), 7.42 (dd, *J* = 8.4 and 2.2 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 6.83 (m, 1H), 6.78 (dd, *J* = 3.0 and 1.7 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 185.4, 137.8, 135.3, 133.7, 130.1, 128.7, 128.7, 127.7, 124.9, 120.4, 108.7. LC-MS (ESI): t_R = 4.72 min; [M+H]⁺ 284.25. HRMS for C₁₁H₈BrClNO [M+H]⁺ calculated mass: 283.9472, measured: 283.9470.

(*E*) 1-(2-Bromo-4-chlorophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (**28**). Yellow solid (53%). ¹H NMR (400 MHz, CDCl₃) δ 8.08 (d, *J* = 13.2 Hz, 1H), 8.00 (d, *J* = 2.4 Hz, 1H), 7.93 (d, *J* = 13.1 Hz, 1H), 7.72 (t, *J* = 1.8 Hz, 1H), 7.61 (dd, *J* = 8.5 and 2.4 Hz, 1H), 7.53 (d, *J* = 8.6 Hz, 1H), 7.13 (t, *J* = 1.8 Hz, 1H), 6.82 (dd, *J* = 2.8 and 1.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃)

δ 138.2, 134.9, 134.5, 134.3, 133.3, 130.3, 130.0, 129.4, 126.4, 120.4, 117.4, 109.2. LC-MS (ESI): $t_R = 5.36$ min; $[M+H]^+$ 327.35. HRMS for $C_{12}H_9BrClN_2O_2$ $[M+H]^+$ calculated mass: 326.9530, measured: 326.9529.

(E) 1-(4-Bromo-2-chlorophenyl)-3-(2-nitrovinyl)-1H-pyrrole (29).

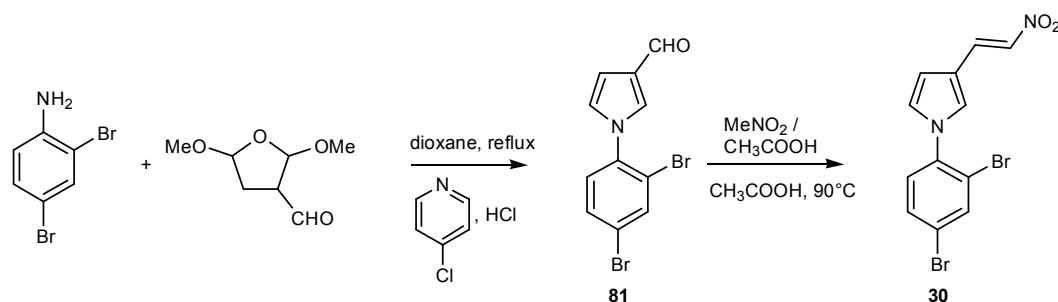


Synthetic procedure for compound (**29**) is similar as that described for compound (**2**) and spectra data are shown below.

1-(4-Bromo-2-chlorophenyl)-1H-pyrrole-3-carbaldehyde (**80**). Orange solid (34%). Mp: 100 °C. 1H NMR (400 MHz, $CDCl_3$) δ 9.88 (s, 1H), 7.75 (d, $J = 2.1$ Hz, 1H), 7.55 (dd, $J = 8.4$ and 2.1 Hz, 1H), 7.50 (t, $J = 1.7$ Hz, 1H), 7.28 (d, $J = 8.3$ Hz, 1H), 6.89 (t, $J = 2.7$ Hz, 1H), 6.82 (dd, $J = 2.9$ and 1.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.4, 136.6, 133.6, 131.2, 130.8, 130.0, 128.7, 127.8, 124.8, 122.5, 108.9. LC-MS (ESI): $t_R = 4.76$ min; $[M+H]^+$ 284.21. HRMS for $C_{11}H_8BrClNO$ $[M+H]^+$ calculated mass: 283.9472, measured: 283.9470.

(E) 1-(4-Bromo-2-chlorophenyl)-3-(2-nitrovinyl)-1H-pyrrole (**29**). Yellow solid (55%). Mp: 182 °C. 1H NMR (400 MHz, $DMSO-d_6$) δ 8.09 (d, $J = 13.3$ Hz, 1H), 8.00 (dd, $J = 2.1$ and 0.9 Hz, 1H), 7.93 (d, $J = 13.3$ Hz, 1H), 7.76 (bs, 1H), 7.71 (ddd, $J = 8.5$, 2.1, and 0.9 Hz, 1H), 7.49 (dd, $J = 8.4$ and 0.8 Hz, 1H), 7.17 (m, 1H), 6.83 (m, 2.8 and 1.6 Hz, 1H). ^{13}C NMR (100 MHz, $DMSO-d_6$) δ 136.9, 134.8, 134.6, 133.3, 132.0, 130.2, 130.1, 130.0, 126.3, 122.1, 117.6, 109.3. LC-MS (ESI): $t_R = 5.38$ min; $[M+H]^+$ 327.35. HRMS for $C_{12}H_9BrClN_2O_2$ $[M+H]^+$ calculated mass: 326.9530, measured: 326.9528.

(E) 1-(2,4-Dibromophenyl)-2-(2-nitrovinyl)-1H-pyrrole (30).

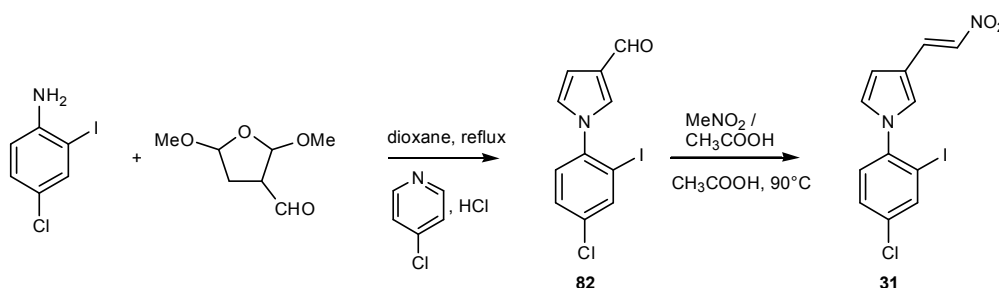


Synthetic procedure for compound (**30**) is similar as that described for compound (**2**) and spectra data are shown below.

1-(2,4-Dibromophenyl)-1*H*-pyrrole-3-carbaldehyde (**81**). Pink solid (32%). Mp: 86 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.85 (s, 1H), 7.89 (d, *J* = 2.1 Hz, 1H), 7.57 (dd, *J* = 8.5 and 2.1 Hz, 1H), 7.45 (t, *J* = 1.8 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 6.84 (m, 1H), 6.78 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 185.4, 138.2, 136.4, 131.7, 130.0, 129.1, 127.7, 124.8, 123.0, 120.6, 108.7. LC-MS (ESI): t_R = 4.81 min; [M+H]⁺ 328.25.

(*E*) 1-(2,4-Dibromophenyl)-2-(2-nitrovinyl)-1*H*-pyrrole (**30**). Yellow solid (52%). Mp: 190 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.10 (s, 1H), 8.08 (d, *J* = 13.2 Hz, 1H), 7.92 (d, *J* = 13.1 Hz, 1H), 7.73 (dd, *J* = 8.4 and 2.1 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.12 (m, 1H), 7.17 (m, 1H), 6.80 (dd, 3.0 and 1.6 Hz, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 138.6, 136.0, 134.9, 134.5, 132.4, 130.3, 130.2, 126.4, 122.6, 120.6, 117.4, 109.2. LC-MS (ESI): t_R = 5.44 min; [M+H]⁺ 369.37. HRMS for C₁₂H₉Br₂N₂O₂ [M+H]⁺ calculated mass: 370.9025, measured: 370.9023.

(*E*) 1-(4-Chloro-2-iodophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (31**).**

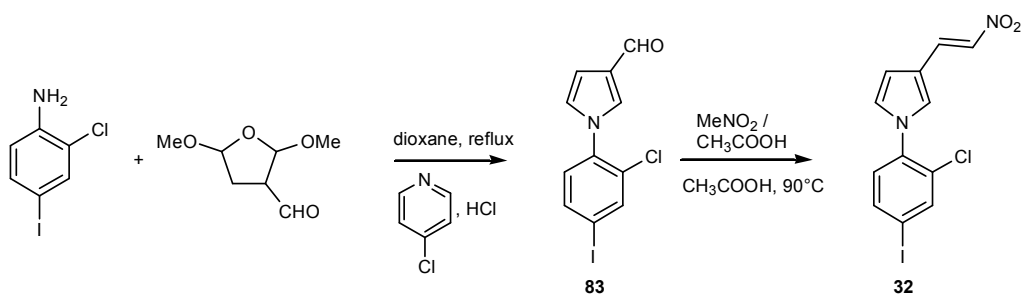


Synthetic procedure for compound (**31**) is similar as that described for compound (**2**) and spectra data are shown below.

1-(4-Chloro-2-iodophenyl)-1*H*-pyrrole-3-carbaldehyde (**82**). Yellow solid (31%). Mp: 102 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.79 (s, 1H), 7.89 (d, *J* = 2.4 Hz, 1H), 7.38 (dd, *J* = 8.4 and 2.3 Hz, 1H), 7.31 (t, *J* = 1.8 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 1H), 6.71 (m, 2H). ¹³C NMR (100 MHz, CDCl₃) δ 185.4, 141.3, 139.5, 135.5, 130.0, 129.5, 128.2, 127.7, 124.8, 108.8, 95.6. LC-MS (ESI): t_R = 4.81 min; [M+H]⁺ 332.23. HRMS for C₁₁H₈ClINO [M+H]⁺ calculated mass: 331.9333, measured: 331.9328.

(*E*) 1-(4-Chloro-2-iodophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (**31**). Yellow solid (53%). ¹H NMR (400 MHz, CDCl₃) δ 8.03 (d, *J* = 13.3 Hz, 1H), 7.96 (d, *J* = 2.3 Hz, 1H), 7.47 (d, *J* = 13.3 Hz, 1H), 7.45 (dd, *J* = 2.3 and 8.3 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.16 (t, *J* = 1.8 Hz, 1H), 6.82 (t, *J* = 2.8 Hz, 1H), 6.55 (dd, *J* = 2.9 and 1.6 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 139.3, 138.9, 137.2, 137.1, 134.2, 133.2, 130.5, 128.7, 127.8, 125.3, 117.3, 108.3. LC-MS (ESI): t_R = 5.40 min; [M-H]⁻ 373.32. HRMS for C₁₂H₉ClIN₂O₂ [M+H]⁺ calculated mass: 374.9391, measured: 374.9390.

(*E*) 1-(2-Chloro-4-iodophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (32**).**



Synthetic procedure for compound (**32**) is similar as that described for compound (**2**) and spectra data are shown below.

1-(4-Chloro-2-iodophenyl)-1*H*-pyrrole-3-carbaldehyde (**83**). Beige solid (35%). Mp: 118 °C. ¹H NMR (400 MHz, CDCl₃) δ 9.85 (s, 1H), 7.91 (d, *J* = 1.8 Hz, 1H), 7.72 (dd, *J* = 8.3 and 1.8 Hz, 1H), 7.48 (t, *J* = 1.8 Hz, 1H), 7.10 (d, *J* = 8.3 Hz, 1H), 6.87 (m, 1H), 6.79 (dd, *J* = 3.2 and 1.7 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 185.4, 139.3, 137.2, 137.1, 130.6, 130.0, 128.9, 127.8, 124.7, 108.8, 93.5. LC-MS (ESI): *t*_R = 4.87 min; [M+H]⁺ 332.28. HRMS for C₁₁H₈ClINO [M+H]⁺ calculated mass: 331.9333, measured: 331.9328.

(*E*) 1-(2-Chloro-4-iodophenyl)-3-(2-nitrovinyl)-1*H*-pyrrole (**32**). Yellow solid (53%). ¹H NMR (400 MHz, CDCl₃) δ 8.02 (d, *J* = 13.4 Hz, 1H), 7.91 (d, *J* = 1.9 Hz, 1H), 7.72 (dd, *J* = 8.2 and 2.0 Hz, 1H), 7.46 (d, *J* = 13.5 Hz, 1H), 7.25 (t, 1.9 Hz, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 6.91 (m, 1H), 6.55 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 139.3, 138.9, 137.2, 137.1, 134.2, 133.2, 130.5, 128.7, 127.8, 125.3, 117.3, 108.3. LC-MS (ESI): *t*_R = 5.47 min; [M-H]⁻ 373.27. HRMS for C₁₂H₉ClIN₂O₂ [M+H]⁺ calculated mass: 374.9391, measured: 374.9390.