

1 Using the drug-protein interactome to identify 2 anti-ageing compounds for humans

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16 Abstract

17 Advancing age is the dominant risk factor for most of the major killer diseases in
18 developed countries. Hence, ameliorating the effects of ageing may prevent multiple
19 diseases simultaneously. Drugs licensed for human use against specific diseases have
20 proved to be effective in extending lifespan and healthspan in animal models,
21 suggesting that there is scope for drug repurposing in humans. New bioinformatic
22 methods to identify and prioritise potential anti-ageing compounds for humans are
23 therefore of interest. In this study, we first used drug-protein interaction information, to
24 rank 1,147 drugs by their likelihood of targeting ageing-related gene products in

humans. Among 19 statistically significant drugs, 6 have already been shown to have pro-longevity properties in animal models ($p < 0.001$). Using the targets of each drug, we established its association with ageing at multiple levels of biological actions including pathways, functions and protein interactions. Finally, combining all the data, we calculated a comprehensive ranked list of drugs that predicted tanespimycin, an inhibitor of HSP-90, as the top-ranked novel anti-ageing candidate. We experimentally validated the pro-longevity effect of tanespimycin through its HSP-90 target in *Caenorhabditis elegans*.

keywords: human ageing, drug-protein interactions, drug repurposing, anti-ageing, longevity, lifespan, heat shock protein inhibitor, tanespimycin.

Author Summary

Human life expectancy is continuing to increase worldwide, as a result of successive improvements in living conditions and medical care. Although this trend is to be celebrated, advancing age is the major risk factor for multiple impairments and chronic diseases. As a result, the later years of life are often spent in poor health and lowered quality of life. However, these effects of ageing are not inevitable, because very long-lived people often suffer rather little ill-health at the end of their lives. Furthermore, laboratory experiments have shown that animals fed with specific drugs can live longer and with fewer age-related diseases than their untreated companions. We therefore need to identify drugs with anti-ageing properties for humans. We have therefore used computers to search for drugs that affect components and processes known to be important in human ageing. This approach worked, because it was able to re-discover several drugs known to increase lifespan in animal models, plus some new ones,

including one that we tested experimentally and validated in this study. These drugs are now a high priority for animal testing and for exploring effects on human ageing.

52

53 **Introduction**

54 Increasing life expectancy in developed countries is revealing advancing age as the
55 primary risk factor for numerous diseases [1]. Thus, identifying interventions that can
56 ameliorate the effects of ageing, and consequently delay, prevent or lessen the severity
57 of age-related conditions, are needed. Extensive research in laboratory animals has
58 demonstrated that the ageing process is malleable and that dietary, genetic and
59 pharmacological interventions can improve health during ageing, extend lifespan and
60 combat pathologies [2]. Furthermore, humans who lived to advanced ages show lower
61 late-life morbidity (disease burden) than those who die earlier, indicating that
62 compression of morbidity is achievable [3].

63

64 Although pharmacological interventions may prove to ameliorate the effects of ageing
65 in humans, development of new drugs for this purpose would present significant
66 difficulties, because of the need to treat healthy individuals in clinical trials over long
67 periods for multiple outcomes. For this reason, it is more feasible to repurpose drugs
68 already approved for specific diseases than to target ageing itself with new drugs [4,5].
69 With this goal in mind, researchers have begun to conduct human clinical trials to assess
70 the anti-ageing properties of drugs approved to treat human medical conditions, and that
71 extend lifespan and healthspan in animal models. Some examples include the anti-
72 diabetic drugs metformin (National Clinical Trial (NTC) number: NCT02432287) [6]
73 and acarbose (NCT02953093), the immunosuppressant sirolimus (NCT02874924) and
74 related compounds [7,8], and the natural compound resveratrol (NCT01842399). Two

75 natural metabolites, the NAD precursors nicotinamide riboside (NCT02950441) and
76 nicotinamide mononucleotide [9] are also being investigated. The development of
77 computational methods to complement and accelerate this approach, by prioritising
78 approved drugs that could ameliorate human ageing, is needed.

79

80 Several bioinformatic methods have been developed to identify potential geroprotective
81 drugs. For instance, caloric restriction (CR) mimetics have been identified, by
82 comparing genes differentially expressed in rat cells exposed to sera from CR rats and
83 rhesus monkeys with gene expression changes caused by drugs in cancer cell lines [10].
84 Structural and sequence information on ageing-related proteins have been combined
85 with experimental binding affinity and bioavailability data to rank chemicals by their
86 likelihood of modulating ageing in the worm *Caenorhabditis elegans* and the fruit fly
87 *Drosophila melanogaster* [11]. Drug-protein interaction information has also been used
88 to predict novel pro-longevity drugs for *C. elegans*, by implementing a label
89 propagation algorithm based on a set of effective and ineffective lifespan-extending
90 compounds and a list of ageing-related genes [12]. A similar approach used a random
91 forest algorithm and chemical descriptors of ageing-related compounds from the
92 DrugAge database [13] together with gene ontology (GO) terms related to the drug
93 targets [14]. Enrichment of drug targets has been assessed for a set of human
94 orthologues of genes modulating longevity in animal models to identify new anti-ageing
95 candidates [15].

96

97 Despite the increasing interest in drug-repurposing for human ageing, research has
98 tended to focus on predicting life-extending drugs for animal models. However, the
99 translation from non-mammalian species to humans is still a challenge, and certain

100 aspects of ageing may be human-specific. Only a few studies have focused on data from
 101 humans. For instance, Aliper et al. (2016) [16] applied the GeroScope algorithm [17] to
 102 identify drugs mimicking the signalome of young human subjects based on differential
 103 expression of genes in signalling pathways involved in the ageing process. Another
 104 study by Dönertas et al. (2018) [18] correlated a set of genes up- and down-regulated
 105 with age in the human brain with drug-mediated gene expression changes in cell lines
 106 from the Connectivity Map [19].

107

108 In the present study, we rank-ordered drugs according to their probability of affecting
 109 ageing, by measuring whether they targeted more genes related with human ageing than
 110 expected by chance, by calculating the statistical significance of the overlap between the
 111 targets of each drug and a list of human ageing-related genes using a Fisher's exact test
 112 [20]. Additionally, to enhance the power of the approach, we mapped the drugs' gene
 113 targets and ageing-related genes to pathways (KEGG, Reactome), gene ontology terms
 114 (biological processes, cellular components, molecular functions) and protein-protein
 115 interactions, and repeated the analysis. We found that, independently of the data source
 116 used, the analysis resulted in a list of drugs significantly enriched for compounds
 117 previously shown to extend lifespan in laboratory animals. We integrated the results of
 118 7 ranked lists of drugs, calculated using the different data sources, into a single list, and
 119 we experimentally validated the top compound, tanespimycin, an HSP-90 inhibitor, as a
 120 novel pro-longevity drug.

121 **Results**

122 **Defining a dataset of drug-protein interactions and ageing-related genes**

123 The drug-ageing association was inferred by comparing drug-gene interactions with
 124 gene-ageing associations. Fig 1 presents an overview of the procedure to prioritise the
 125 compounds. A dataset containing the interactions between drugs and proteins was built
 126 based on data from the STITCH database [21]. Only drugs targeting human proteins and
 127 successfully mapped to the DrugBank database [22] using the UniChem resource [23]
 128 were kept (Fig 1A). The dataset was composed of 18,393 interactions between 2,495
 129 drugs and 2,991 proteins. More than half of the drugs (51.1%) in the dataset are
 130 approved for human use, 18.6% are in some phase of the approval process and 28.4%
 131 have been shown to bind to disease targets in experiments.

132
 133 We obtained a set of ageing-related genes from the Aging Clusters resource [24]. A
 134 total of 1,216 ageing-related genes discovered in at least 2 among 4 categories of studies
 135 were selected. These 4 categories are human genes: i) changing expression with age or
 136 CR in different tissues ii) whose DNA methylation levels changes with age iii)
 137 associated with age-related diseases and iv) in manually curated databases of genes
 138 linked with longevity in genetic studies [25], associated with cellular senescence [26] or
 139 showing ageing-related effects in animal models in addition to evidence for a causative
 140 role in human ageing [27].

141 142 **Gene-based inference of drug-ageing associations**

143 We determined if there was evidence supporting an association between drugs and
 144 ageing-related genes by calculating the statistical significance of the overlap between
 145 the gene targets of each drug and the ageing-related genes (Fig 1B). From the 1,147

146 drugs analysed, 19 were statistically enriched for ageing-related targets after multiple
 147 testing correction (Table 1, S1 tables). To assess the capability of the method to
 148 prioritise pro-longevity compounds, we compared the list of top-ranked compounds
 149 with the DrugAge database [13]. Six out of the 19 drugs have already been reported to
 150 significantly extend the lifespan of at least one model organisms (S2 text), while only 1
 151 was expected by chance ($p < 0.001$). Additionally, using literature mining, we identified
 152 studies showing the association with longevity of cAMP analogues [28], selenium
 153 [29,30] and tanespimycin [31,32]. In contrast, we also found evidence for the DNA-
 154 mediated, pro-ageing (anti-longevity) effects of doxorubicin [33], cisplatin [34] and
 155 hydrogen peroxide [35]. We performed an interaction-based similarity analysis and
 156 found that the genotoxic compounds cluster separately from the other drugs, suggesting
 157 that they have a similar mechanism of action (S2 text). Similarities were also identified
 158 regarding the mechanisms of action of sorafenib and regorafenib, bexarotene and GW-
 159 501516, and sirolimus and ECGC, in agreement with previous studies [36].

160

161 Although drugs interact directly with proteins, proteins do not act alone and interact
 162 with other proteins within pathways to perform different functions. Anti-ageing effects
 163 are likely to be mediated through altered pathway activity and function, and we
 164 therefore investigated if we could enhance the prediction of pro-longevity drugs using
 165 other biological annotations as comparators. Therefore, we calculated the pathways and
 166 gene functions enriched in ageing-related genes, together with the proteins that interact
 167 with them. A total of 82 KEGG and 54 Reactome pathways were enriched in this set of
 168 genes, as well as 1,177 biological processes, 69 cellular components and 103 molecular
 169 functions. In addition, we calculated that 676 proteins interacted with the set of ageing-
 170 related genes. These terms, mapped at different biological levels, were defined as the set

171 of ageing-related terms (Fig 1C – left). Equivalently, drugs were then associated with
172 these terms through association with their targets using the list of genes defining each
173 term according to the DAVID knowledgebase [37] and the biological database network
174 [38]. This mapping procedure resulted in a set of terms from each data source related to
175 each drug (drug-related terms) (Fig 1C – right).

176

177 **Drug-ageing association based on protein-protein interactions, gene ontology and** 178 **pathways**

179 Analogously to the gene-based association analysis, we calculated for each level if the
180 overlap between ageing-related terms and drug-related terms was statistically significant
181 using a Fisher’s exact test. This procedure generated 6 lists of ranked compounds in
182 addition to the gene-based analysis (S1 tables). Notably, when we evaluated the
183 correlation between the ranking of compounds in the different lists (Fig 2A), we
184 observed a moderate correlation (Kendall’s coefficient of concordance $W = 0.58$, p-
185 value = $1.02E-266$). The highest correlations were observed between the results from
186 biological processes and cellular components (Kendall’s tau = 0.51 , p-value < $2.2E-16$),
187 while the lowest was observed between cellular components and genes (Kendall’s tau =
188 0.16 , p-value = $3.289E-11$).

189

190 Because in any enrichment analysis there is a potential for research bias, we performed
191 random permutations to simulate the enrichment of each drug for a different set of terms
192 on each level. None of the top-ranked drugs on each list ranked higher than in the
193 analysis in more than 1.7% of the simulations (Table A in S2 text). We also quantified
194 the capability of the strategy to prioritise pro-longevity compounds by calculating for
195 each list the fraction of known pro-longevity compounds (ranked by p-value) among the

fraction of drugs considered in each analysis (Fig 2B). The enrichment for pro-longevity compounds was quantified by calculating the area under the curve (AUC) generated by plotting these two variables. The maximum AUC was obtained when biological processes or molecular functions (AUC = 0.69) was used as the comparator (Table B in S2 text). The use of genes showed the lowest enrichment when non-statistically significant drugs are considered (AUC = 0.59), which suggests that the use of higher biological levels to calculate the inference improves the prediction capabilities, and that the use of genes leads to a loss power to rank drugs targeting a low proportion of ageing-related genes, which is observed in Fig 2B a loss of enrichment after 25% of the drugs were ranked. We evaluated if the AUCs were statistically significant by calculating the AUC from the simulations generated to quantify the research bias. The p-value for each curve was calculated by determining the number of simulated results with an AUC equal or higher than the analysis. All lists showed a higher enrichment than expected by chance (AUC > 0.5 and p-value < 0.05, Table B in S2 text). When we only considered the first 20 top-ranked drugs, we observed that using biological processes or cellular components to perform the comparison showed the highest proportion of pro-longevity drugs (45%), while only 2 pro-longevity drugs (10%) were found among the top 20 drugs when KEGG pathways are used.

Considering the lack of overlap between the ranked lists using the different data sources, we decided to integrate the results into a single list accounting for the complexity of multitiered effect of drugs by calculating their ranking average in the different analyses. The combination generated a list equally enriched as the maximum AUC obtained by the previous analysis (AUC = 0.69). Among the top 10 drugs with the best average ranking (Table 2, S1 tables), we found 3 drugs that have extended lifespan

221 in animal models (trichostatin [39], geldanamycin [10] and celecoxib[40]). Half of these
222 10 drugs are classified as kinase inhibitors, while 8 are indicated as anti-cancer drugs
223 and 7 are approved for human use.

224

225 **The HSP-90 inhibitor tanespimycin as a novel pro-longevity drug**

226 Leading the joint ranking was tanespimycin, also known as 17-AAG, a well-
227 characterized HSP-90 inhibitor that has been shown to activate the transcription factor
228 HSF-1 and induce a heat shock response in multiple model organisms [26]. As a proof-
229 of-principle, we decided to investigate whether tanespimycin could activate HSF-1 and
230 extend lifespan in the nematode worm *C. elegans*. To test the efficacy of tanespimycin
231 dosing in *C. elegans*, we grew worms expressing mCherry under the control of an HSF-
232 1 responsive promoter [41] on solid media plates containing various doses of
233 tanespimycin. Worms were exposed to tanespimycin continuously from the first larval
234 stage (L1) of development, or exclusively from the first day of adulthood. Worms
235 grown continuously on tanespimycin plates exhibited a dose-dependent activation of the
236 HSF-1 transcriptional reporter, starting at 25 μ M and peaking at 100 μ M (Fig 3A-B).
237 Similarly, exposure to tanespimycin plates exclusively in adulthood resulted in
238 significant activation of the HSF-1 reporter at 50 and 100 μ M concentrations. No
239 markers of toxicity were observed in any treatment groups, except for the 100 μ M larval
240 group, which were developmentally delayed by 24 hours and had a significantly
241 reduced brood size (data not shown), consistent with chronic HSP-90 inhibition [42].
242 Together, these data demonstrate that tanespimycin activates HSF-1 in *C. elegans* and
243 that treatment exclusively in adulthood is not associated with overt toxicity.

244

245 We next sought to determine whether tanespimycin treatment could extend lifespan in
 246 *C. elegans*. To circumvent potential longevity effects arising from delayed development
 247 and reproduction, we exposed worms to 100 μ M tanespimycin plates from the first day
 248 of adulthood. Tanespimycin treatment significantly extended median and maximal
 249 lifespan compared to vehicle-treated controls (Fig 3C). To determine whether the
 250 effects of tanespimycin on lifespan require *hsp-90*, we also exposed worms to
 251 tanespimycin treatment in the presence of *hsp-90(RNAi)*. Consistent with previous
 252 reports, *hsp-90(RNAi)* significantly shortened *C. elegans* lifespan [43]. Furthermore, in
 253 the absence of HSP-90, tanespimycin treatment no longer increased lifespan compared
 254 to vehicle controls. These data suggest that tanespimycin treatment extends lifespan in
 255 an *hsp-90* dependent manner, but that severe depletion of HSP-90 is toxic to animals,
 256 despite the activation of protective stress responses.

257 Discussion

258 This study was designed to infer and rank drugs matched to ageing at multiple levels of
 259 biological activity using a simple statistical test. In an initial gene-centric analysis, 19
 260 drugs were identified as candidates expected to modulate ageing in humans. A major
 261 finding was that 6 of the statistically significant drugs, resveratrol, genistein,
 262 simvastatin, epigallocatechin gallate, celecoxib and sirolimus, have already shown
 263 lifespan-extending properties in experimental studies in model organisms. This
 264 statistically significant enrichment suggests that, despite its simplicity, the method is
 265 able to prioritise pro-longevity compounds. We then expanded the analysis to higher
 266 levels of biological complexity, and again found a statistically significant enrichment
 267 for pro-longevity drugs in all cases. The results of the analysis at different levels
 268 showed a moderate correlation. Compounds ranked high on average included
 269 trichostatin, geldanamycin and celecoxib, 3 drugs with pro-longevity effects in animal
 270 models [10,39,40]. The compound ranked highest on average was tanespimycin, an
 271 HSP-90 inhibitor, shown to acts as a senolytic agent by killing human senescent cells
 272 without affecting the viability of healthy cells [31] and to ameliorate disease phenotypes
 273 in *Drosophila* models of Huntington's disease and spinocerebellar ataxia [32]. We
 274 found that tanespimycin treatment extended median (23%) and maximum (16%)
 275 lifespan in *C. elegans*, through its target HSP-90, possibly through the induction of
 276 cytoprotective pathways. Tanespimycin must act through more than one mechanism as
 277 a geroprotector, because cellular senescence has not been reported to occur in *C.*
 278 *elegans*.

279

280 Evidence from the literature supports the anti-ageing action of other drugs that we
 281 identified as potentially geroprotective. Dasatinib, a kinase inhibitor ranked 7th on

average, has been reported to induce apoptosis in senescent but not non-senescent primary human umbilical vein endothelial cells and preadipocytes [44]. Combination of dasatinib and quercetin, which also inhibits HSP-90, induced apoptosis specifically in senescent murine and human cells in vitro, improved cardiovascular function in aged mice, and decreased bone loss, neurological dysfunction and frailty in progeroid mice [45].

Three of the top 10 compounds from the combined ranked list have been previously proposed as anti-ageing candidates for humans using bioinformatic analysis. Specifically, tanespimycin, geldanamycin and trichostatin were among the 24 drugs predicted by Dönertas et al. (2018)[18] and Calvert et al. (2016)[10]. In contrast, we did not observe any overlap with the top results from Fernandes et al. (2016)[15] possibly due to the use of a different drug-protein interaction database (DGIdb [46]) or source of ageing data.

Similar enrichment-based methods that combine multiple levels of biological information have been used for drug-repurposing for Rheumatoid arthritis, Parkinson's disease and Alzheimer's disease [47,48], but not, to our knowledge, to identify anti-ageing drugs. Using annotated databases, our method evaluated the enrichment for pro-longevity of all compounds analysed, rather than only those with significant scores, and we observe that in all cases pro-longevity compounds are ranked higher than expected by chance. Although tanespimycin acts as a senolytic [31], and has been predicted to be geroprotective by two previous studies [10,18], we have demonstrated its effect on longevity experimentally.

307 A limitation of this study is that it is based on previous knowledge about drug-protein
 308 interactions, which for non-commonly studied drugs is incomplete. This may explain
 309 why we observed many anti-cancer and well-known drugs in our results. While we
 310 assessed this bias using permutations and we found no significant effect on our results,
 311 further research is needed to increase the drug-protein interactome data using, for
 312 example, high-throughput technologies like those currently available for kinases [49].
 313 While we combined the results from the different data sources using a strategy based on
 314 ranks, we hypothesise that the integration of these results using other methods may lead
 315 to a list with a higher enrichment for pro-longevity drugs. Additionally, further
 316 experimental testing is required on the lists produced in this study, particularly those
 317 generated by using gene ontology terms, which presented the higher enrichment for pro-
 318 longevity drugs. An inherent limitation of inferred associations is that they do not
 319 provide information about the directionality of the effect, which in this case means that
 320 it is unknown if the drugs will deaccelerate ageing or the opposite. While we indirectly
 321 assessed this using an interaction-based similarity analysis between the drugs, resulting
 322 in clusters or pairs of drugs with similar mechanism of action, experiments should be
 323 conducted to determine the effects of each drug on ageing. Finally, a practical limitation
 324 is that we validated the results of this study using experiments in animal models
 325 although we used human data to perform the analysis. Although testing the effects of
 326 drugs on human ageing is challenging, progress is starting to be made. A clinical trial
 327 conducted by Mannick et al. (2018)[8] showed that pharmacological inhibition of the
 328 mammalian target of rapamycin in humans by dactolisib plus everolimus reduces the
 329 rate of infections in elderly people. Moreover, a recent short-term clinical trial of
 330 sirolimus established its safety in healthy individuals [50]. Similarly, supplementation
 331 of nicotinamide ribose, identified as a possible CR mimetic, stimulated NAD+

332 metabolism in healthy individuals aged 55 to 79 years [51]. Some mechanisms of
333 ageing may be confined to humans and their near relatives, and ideally, the
334 bioinformatic findings should be evaluated in humans, initially through genetic
335 epidemiology and ultimately through clinical trials.

336 **Methods**

337 **Data sources**

338 **Drug-protein interaction dataset:** Chemical-protein interactions were extracted from
 339 the Search Tool for Interactions of Chemicals (STITCH) database 5.0 [21]. We chose
 340 this resource because it acts as a probabilistic network, by collecting interactions from
 341 multiple sources, including experiments, databases and a text-mining algorithm.
 342 Individual scores for each source are combined into an overall confidence score using a
 343 naive Bayesian formula defined as $Score = 1 - \prod_i (1 - S_i)$, where S_i represents the
 344 confidence score for the source i . Later, because the Bayesian combination of scores can
 345 overestimate the effect of small individual contributions, the score is corrected for the
 346 probability of observing an interaction by chance. The overall confidence score ranges
 347 from 0 to 1, where a value of 0.4 or greater is considered as medium confidence, and a
 348 score equal to or higher than 0.7 is regarded as high confidence. To obtain a reliable set
 349 of interactions, we removed all interactions with a confidence score lower than 0.7. The
 350 database also maps the direction of each interaction, i.e. whether chemical acts on the
 351 protein or if the protein modifies the chemical (e.g. transformation of the chemical
 352 during a catalytic reaction). To confine the analysis to the actions of chemicals on
 353 proteins, only the cases where the chemical activates or inhibits a protein were retained.
 354 To focus on drugs in development or approved for human use, we filtered the chemicals
 355 in STITCH by the drugs in DrugBank 5.0 [22] using UniChem [23]. The InChi key for
 356 each drug was retrieved from PubChem (<http://pubchemdocs.ncbi.nlm.nih.gov/pug-rest>)
 357 and used to obtain the DrugBank identifiers via UniChem
 358 (<https://www.ebi.ac.uk/unicem/info/webservices>). The names of the drugs were
 359 obtained from the DrugBank vocabulary file, and the development status was acquired
 360 using the structure external links file. Finally, we mapped the Ensembl identifiers for

each protein into HUGO Gene Nomenclature Committee (HGNC) approved gene names using Ensembl Biomart (version 91) [52]. All the chemicals included in this dataset will be referred as “drugs” or “compounds” throughout this article.

Drug-related terms: We mapped the targets of each drug in the drug-protein interaction dataset to multiple biological levels by using the information about the genes that define each level analysed. We downloaded the gene-centric definitions of *GO terms* and *Reactome pathways* from the DAVID knowledgebase [37]. Genes on each *KEGG pathways* were obtained using the biological database network (<https://biobdnet-abcc.ncicrf.gov/db/db2db.php>)[38]. *Protein-protein interactions* were mapped directly using the STRING database [53]. Only proteins interacting with the set of ageing-related genes with a confidence equal or higher than 0.9 were considered.

Ageing-related genes: Genes present in manually-curated databases are more susceptible to research and reporting bias than those found in objective searches. Instead of selecting a set of ageing-related genes from a particular study or database, we used genes linked with ageing from the Ageing Clusters resource (<https://gemex.eurac.edu/bioinf/age/>). This repository contains the results of a network-based meta-analysis of human ageing genes [24] that considered 35 different datasets. The author classified the genes into the following 4 categories: curated ageing-related genes from databases such as GenAge [27], LongevityMap [25] and CSGene [26]; genes differentially expressed with age, regimes of CR or healthy ageing; age-related changes in the methylation of cytosine guanine dinucleotides (CpGs) in the DNA; and genes associated with age-related diseases from databases such as the Human Gene Mutation [54] or the Human Phenotype Ontology [55]. To improve the reliability of the

386 set of ageing-related genes and reduce research bias we considered only the genes
387 present in at least two categories.

388

389 **Ageing-related terms:** Using the set of ageing-related genes we performed gene-based
390 enrichment analysis to infer the function and pathways associated with ageing. *Gene*
391 *Ontology (BP, CC, MF)* terms were calculated using the enrichGO function from the
392 clusterProfiler package [56], using the Benjamini and Yekutieli [57] method for
393 adjustment, a conservative correction that does not rely on the assumption that the test
394 statistics are independent. P-value and q-value cutoff were set to 0.5 and for biological
395 processes we consider the top 500 terms enriched. Enriched *KEGG pathways* were
396 determined using the enrichKEGG function from the clusterProfiler package, using the
397 same parameters used for the gene ontology enrichment. *Reactome pathways* were
398 calculated using the function enrichPathway from the ReactomePA package [58].
399 *Protein-protein interactions*, were obtained using STRING [53] database.

400

401 **Statistical analysis to rank the drugs**

402 Independently of the biological level, the drug-ageing associated was inferred by
403 calculating the statistical significance of the drug-related terms and ageing-related terms
404 using a Fisher's exact test. Drugs were associated with ageing at the following
405 biological levels: gene, pathways (KEGG, Reactome), functions (GO:BP, GO:CC,
406 GO:MF) and indirect protein interactions. The universe was defined as all the terms on
407 each level associated with at least one drug. Thus, drugs with a lower p-value modulate
408 a higher proportion of ageing-related terms than that expected by chance. To control for
409 the false discovery rate, we used the Benjamini and Yekutieli adjustment [57]. A p-
410 value lower than 0.05 after multiple testing correction was considered significant.

411

412 **Measuring the impact of research bias**

413 Some drugs have been more studied than others, which could bias the results towards
414 drugs with a higher proportion of discovered targets. To evaluate the impact of this
415 research bias, we randomly selected the same number of terms that were used as ageing-
416 related terms 1,000 times, and we repeated the statistical analysis. Then we counted the
417 times the statistically significant drugs appeared on the same or lower ranking. We
418 expected that drugs associated with many terms would rank higher independently of the
419 random set generated.

420

421 **Enrichment for pro-longevity drugs**

422 Each of the drug lists generated were ranked by the p-values obtained from the
423 statistical analysis. Then, we transformed the ranking of the drug into a value ranging
424 from 0 to 1. A set of 142 pro-longevity drugs present in the DrugAge and DrugBank
425 databases were used to determine the occurrence and ranking of pro-longevity
426 compounds in the lists. The ranking was then scaled into a value between 0 to 1. The
427 AUC between the variables describing the pro-longevity drugs and drugs analysed was
428 calculated using the function AUC from the DescTools package ([https://cran.r-](https://cran.r-project.org/package=DescTools)
429 [project.org/package=DescTools](https://cran.r-project.org/package=DescTools)). To measure its statistical significance, we calculated
430 the AUC of the lists previously generated to measure the research bias, and we counted
431 the number of simulations with an equal or higher AUC.

432

433 **Experimental procedure**

434 **Worm husbandry and lifespan**

435 N2 and TJ3002 (*zSi3002[hsp-16.2p::mCherry::unc-54; Cbr-unc-119(+)] II*)]
 436 hermaphrodite worms were maintained as previously described [59] at 20°C on 60 mm
 437 NGM plates. Plates were seeded with *Escherichia coli* (OP50) grown overnight in LB
 438 media. RNAi was essentially performed as previously described [60] with the slight
 439 modifications that bacterial cultures were induced with 5 mM IPTG for 3 hours
 440 following overnight growth in LB, and tetracycline was not included in plates or
 441 bacterial cultures.

442

443 **Tanespimycin dose-response test**

444 Tanespimycin (Fisher Scientific) was solubilized in DMSO to stock concentrations of 1,
 445 10, 25, 50, and 100 mM. 1 ml of DMSO or tanespimycin solutions were added to each
 446 litre of NGM media just prior to plate pouring to reach final concentrations of 1, 10, 25,
 447 50, and 100 µM in plates. Plates were kept away from light, stored at 4°C, and used
 448 within 2 weeks of pouring. TJ3002 reporter worms were synchronised by bleaching and
 449 added to 0.1% DMSO or tanespimycin plates as L1s or as day 1 adults. Worms were
 450 transferred to fresh plates every day and then imaged on day 6 of adulthood using a
 451 Zeiss Apotome fluorescent microscope and Hamamatsu Orca Flash 4.0 camera.
 452 Brightness and contrast were adjusted linearly, and equally, for all images, using Adobe
 453 Photoshop CS6. Fluorescence intensity was measured under different conditions using
 454 ImageJ. Significance testing of differences in fluorescence intensity were calculated by
 455 ONE-WAY ANOVA with Tukey pair-wise comparison of groups using GraphPad
 456 Prism.

457

458 **Lifespan assays**

Gravid N2 adults were bleached to release eggs, and L1 larvae were allowed to hatch overnight in M9 buffer without food. L1 worms were then added to plates seeded with bacteria expressing an RNAi control vector (L4440) and containing 0.1% DMSO. Worms were added to plates at a density of approximately 50 worms per plate. On the first day of adulthood (50h post plating L1s), worms were transferred to new 0.1% DMSO plates or 100 μ M tanespimycin plates, seeded with L4440 or bacteria expressing dsRNA against hsp-90 (hsp-90(RNAi)). Worms were transferred to fresh plates every day during the first 7 days of adulthood and every other day thereafter. Worms were scored for survival every two days by gently prodding animals repeatedly with a platinum wire. Animals that failed to exhibit signs of movement or pharyngeal pumping were scored as dead. Animals that displayed internal hatching of progeny (“bagging”) or prolapse of intestine through the vulva (“rupturing”) were censored from our analysis. Median lifespans and significance testing between lifespans of different treatment groups were performed in GraphPad Prism using a Log-rank (Mantel-Cox) test.

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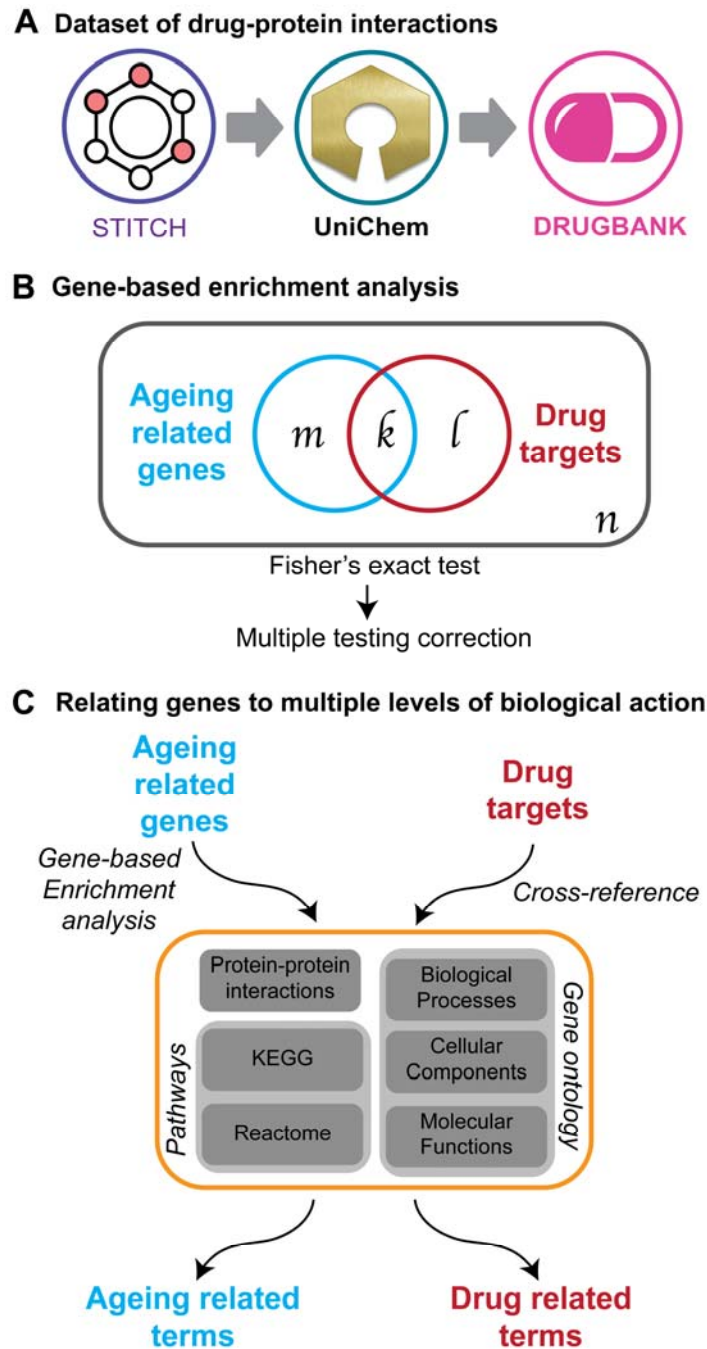
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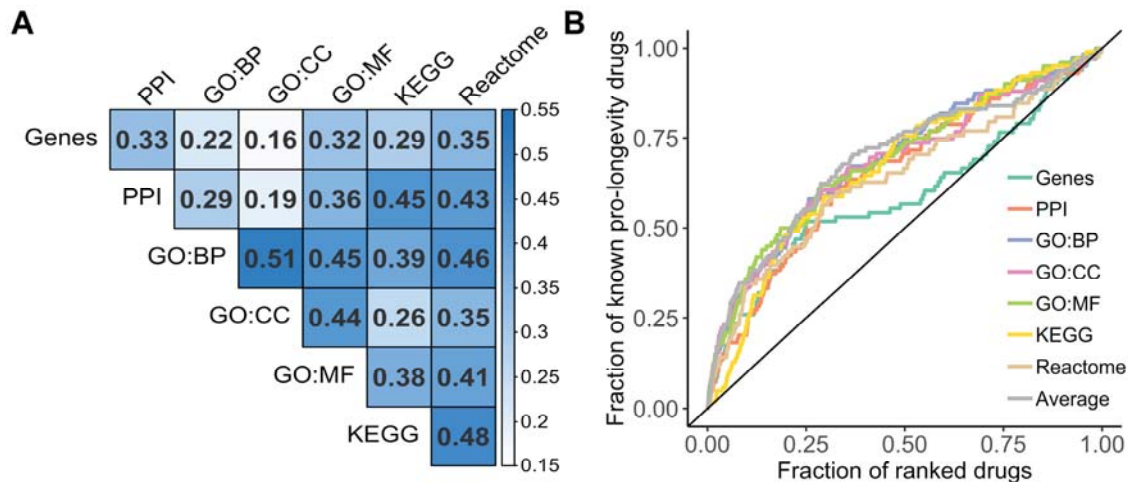
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676

677 **Figures**



678 **Fig 1. Overview of the methods used in this study to prioritise compounds likely to**
 679 **ameliorate ageing in humans.** A) STITCH chemicals were mapped into DrugBank
 680 drugs using the UniChem resource programmatically. B) The significance of the drug-
 681 ageing inference was calculated using a Fisher's exact test, which calculates the
 682 probability that the overlap between two samples (ageing-related genes and drug

683 targets) drawn from the same universe is due to chance. This comparison was made at
 684 different biological tiers. C) Diagram of the procedure to expand the “gene” information
 685 into multiple biological levels. Ageing-related genes were mapped to other levels using
 686 an enrichment analysis, while the drugs’ targets were cross-referenced with the list of
 687 genes defining each annotation.



688
 689 **Fig 2. Comparison between the results using different data sources.** A) Correlation
 690 between the ranked list of compounds. Boxes are coloured by the Kendall’s correlation
 691 coefficient. B) Enrichment curves for pro-longevity drugs. The results of each data
 692 source are displayed in lines with different colours. The enrichment expected by chance
 693 is shown as a diagonal line with AUC = 0.5.

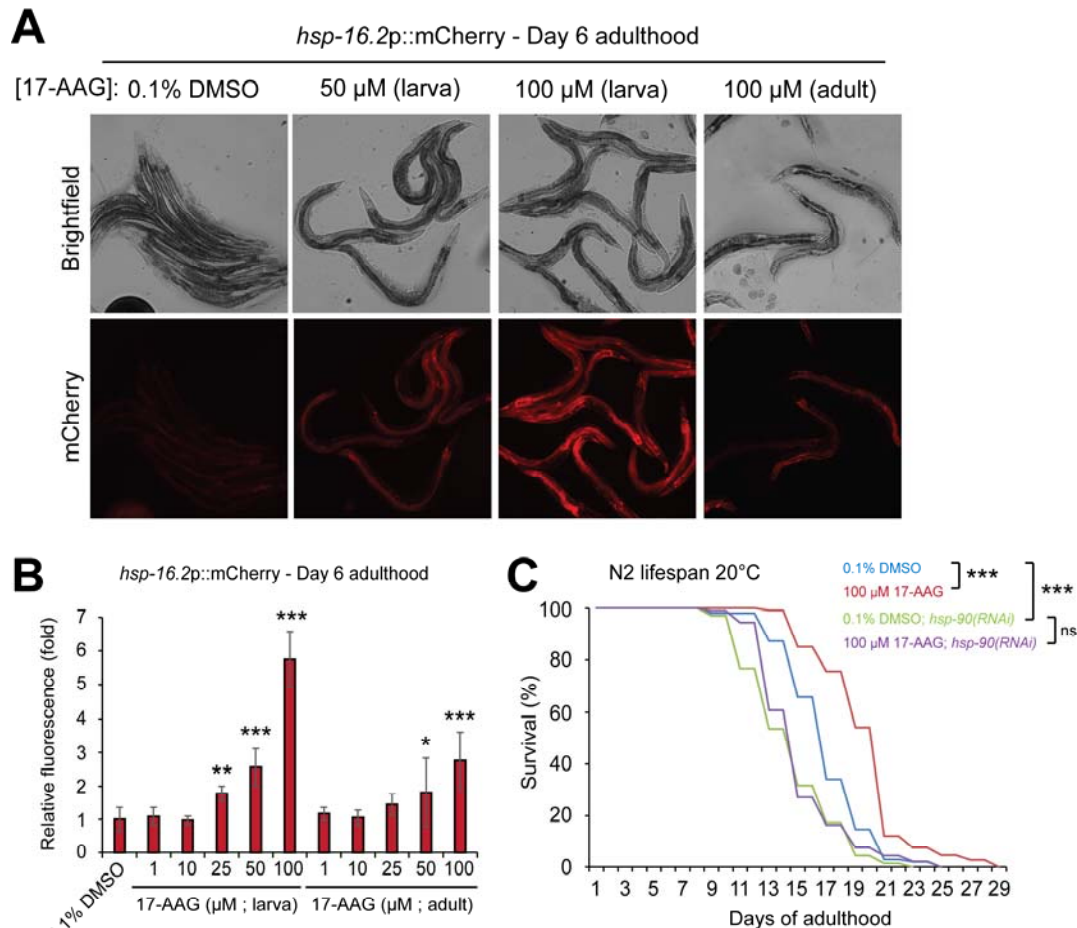


Fig 3. Pro-longevity effect of tanespimycin in *C. elegans*. A) Representative fluorescent images of day 6 adult, *hsp-16.2p::mCherry* transcriptional reporter worms, grown on plates containing 0.1% DMSO (vehicle) or different concentrations of tanespimycin (17-AAG) continuously from the first larval stage, or exclusively from the first day of adulthood onward. B) The relative fluorescent intensity of *hsp-16.2p::mCherry* worms grown on plates containing 0, 1, 10, 25, 50, or 100 μ M tanespimycin (17-AAG) continuously from the first larval stage or exclusively from the first day of adulthood onward. Values plotted are the mean of at least 5 animals, and error bars represent the standard deviation from the mean. Statistical significance relative to the DMSO control group was calculated by ONE-WAY ANOVA with Tukey post analysis pairwise comparison of groups. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. C) – Lifespan at 20 °C of N2 worms grown on plates containing 0.1% DMSO or

100 μ M Tanespimycin (17-AAG) from the first day of adulthood onward in the presence or absence of hsp-90(RNAi). Statistical significance was calculated by Log-rank (Mantel-Cox) test. *** = $p < 0.001$. Treatment groups: 0.1% DMSO (n = 102, 14 censored, median lifespan = 17 days), 100 μ M tanespimycin (n=107, 9 censored, median lifespan 21 days), 0.1% DMSO + hsp-90(RNAi) (n = 69, 30 censored, median lifespan = 15 days), 100 μ M tanespimycin + hsp-90(RNAi) (n = 92, 22 censored, median lifespan = 15 days).

713

714 Tables

715 **Table 1. Drugs significantly enriched for ageing-related targets.** The names of the
716 drugs previously shown to extend lifespan in animal models are in bold and genotoxic
717 molecules are in italic. The columns k(l) and m(n) are consistent with the diagram in
718 Fig 1B. OR stands for odd-ratios and adj.p-value is the p-value adjusted for multiple
719 testing.

Drug name	Status	k(l)	m(n)	OR	p-value	adj.p-value
Resveratrol	Approved	66(150)	388(2221)	2.52	2.09E-08	1.82E-04
Sunitinib	Approved	18(12)	436(2359)	8.11	4.92E-08	2.15E-04
Genistein	Investigational	41(80)	413(2291)	2.84	6.40E-07	1.86E-03
Simvastatin	Approved	39(77)	415(2294)	2.80	1.53E-06	3.35E-03
Tanespimycin	Investigational	15(12)	439(2359)	6.71	2.64E-06	4.62E-03
Regorafenib	Approved	12(7)	442(2364)	9.16	4.43E-06	6.45E-03
Epigallocatechin gallate	Investigational	42(93)	412(2278)	2.50	5.96E-06	7.44E-03
<i>Doxorubicin</i>	Approved	34(67)	420(2304)	2.78	7.20E-06	7.87E-03
Selenium	Approved	14(12)	440(2359)	6.25	9.44E-06	9.17E-03
Celecoxib	Approved	23(36)	431(2335)	3.46	1.58E-05	1.38E-02
Indole-3-carbinol	Investigational	13(11)	441(2360)	6.32	1.83E-05	1.46E-02
<i>Hydrogen peroxide</i>	Approved	59(165)	395(2206)	2.00	2.85E-05	2.07E-02
GW-501516	Investigational	9(5)	445(2366)	9.56	6.23E-05	3.82E-02
Bexarotene	Approved	10(7)	444(2364)	7.60	6.98E-05	3.82E-02
Dorsomorphin	Experimental	10(7)	444(2364)	7.60	6.98E-05	3.82E-02
Sorafenib	Approved	23(41)	431(2330)	3.03	7.25E-05	3.82E-02
Sirolimus	Approved	37(88)	417(2283)	2.30	7.42E-05	3.82E-02
<i>Cisplatin</i>	Approved	34(78)	420(2293)	2.38	8.39E-05	4.07E-02
cAMP	Experimental	36(86)	418(2285)	2.29	1.00E-04	4.60E-02

720

721 **Table 2. Top-ranked compounds using multiple levels of biological action.** The
 722 names of the drugs previously shown to extend lifespan in animal models are in bold.
 723 The numeric values represent the ranking of the drugs when different sources of data
 724 (columns) are used. The last column is the ranking average (Avg.) for each drug in the 7
 725 ranked lists.

Drug name	Status	Genes	PPI	Gene ontology			Pathways		Avg.
				BP	CC	MF	KEGG	Reactome	
Tanespimycin	Investigational	5	26	57	43	44	39	9	31.86
Imatinib	Approved	63	3	21	34	12	66	38	33.86
Sunitinib	Approved	2	1	59	31	31	56	63	34.71
Trichostatin	Experimental	83	41	19	54	13	41	52	43.29
Geldanamycin	Investigational	32	37	87	76	47	13	21	44.71
Sorafenib	Approved	16	68	11	15	8	155	42	45.00
Dasatinib	Approved	41	12	43	81	62	49	35	46.14
Erlotinib	Approved	27	6	93	85	71	64	7	50.43
Etoposide	Approved	23	11	20	90	32	120	67	51.86
Celecoxib	Approved	10	2	33	42	34	180	70	53.00

726