*Cognitive Vitality Reports® are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.*

# HBX [2-(2-hydroxyphenyl)-benzoxazole]

**Evidence Summary**

HBX extends lifespan and ameliorates pathology in a mouse model of ALS. It is being investigated in the NIA ITP program. Safety of HBX is unknown as no studies have studied this compound in humans yet.

**Neuroprotective Benefit:** HBX chelates metals, binds to amyloid, and ameliorates pathology in a mouse model of ALS. No studies have examined its neuroprotective potential in humans.

**Aging and related health concerns:** HBX extended lifespan in a mouse model of ALS and in C. elegans, but no data exist for humans. HBX is being tested in the NIA ITP program to find out whether it can extend lifespan in mice.

**Safety:** Safety has not been established in humans; only a few preclinical studies exist to date.

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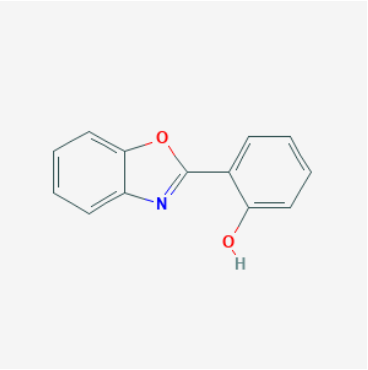
|---|---|---|
| **Availability**: Not available; research-grade only | **Dose**: Not established. The NIA-ITP study is testing a dose of 1 ppm in food to test whether it extends lifespan in mice. | **Chemical formula:** $C_{13}H_9NO_2$<br>**MW**: 211.22<br>  <br>Source: PubChem |
| **Half life**: Not established | **BBB**: Likely penetrant based on preclinical work | |
| **Clinical trials**: N/A | **Observational studies**: N/A | |

**What is it?** HBX [2-(2-hydroxyphenyl)-benzoxazole] is a structural analog of thioflavin T, a compound that binds protein fibrils and slows protein aggregation (Evans et al., 2016). Thioflavin is used in histopathology to stain amyloid in tissues. Unlike thioflavin T, the molecular structure of HBX allows it to cross the blood-brain barrier, making it amenable to *in vivo* studies. HBX is also known to have metal chelating properties.

HBX is one of the compounds being tested in the National Institute on Aging Interventions Testing Program (NIA ITP; see list of compounds here). The NIA ITP was designed to test compounds such as HBX that are purported to extend lifespan and/or delay onset of age-related diseases.

**Neuroprotective Benefit:** HBX chelates metals, binds to amyloid, and ameliorates pathology in a mouse model of ALS. No studies have examined its neuroprotective potential in humans.

*Types of evidence:*

- 2 laboratory studies

***Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function***:
None available.

***Human research to suggest benefits to patients with dementia***:
None available.

***Mechanisms of action for neuroprotection identified from laboratory and clinical research***:
No studies have tested whether HBX is neuroprotective in humans. Based on *in vitro* models, HBX is thought to cross the blood-brain barrier (Rodriguez-Rodriguez et al., 2015).

***Alzheimer's disease***: No studies have tested the efficacy of HBX in Alzheimer's patients or animal models of Alzheimer's. *In vitro* studies have reported that HBX interacts with Aβ fibrils from senile plaques present in human and transgenic mice AD models (Rodriguez-Rodriguez et al., 2015). However, fluorescent signals from binding to senile plaques were seen only at high concentrations of HBX (0.05-0.5 mg/mL). In *ex vivo* mouse models of AD as well as in human brain sections, HBX demonstrated binding to metal ions present in plaques [Cu(ii) and Zn(ii)], and dissociation of Aβ aggregates in the presence of these metal ions.

The authors of this study suggested that HBX and related compounds could be potential imaging and/or therapeutic agents (Rodriguez-Rodriguez et al., 2015). They also proposed the use of a glycosylated prodrug, GBX. GBX is more blood-brain barrier penetrant (via GLUT1), more soluble in solution, and less toxic than HBX, and can be activated into the active form by an enzyme.

***Amyotrophic lateral sclerosis***: ALS is a progressive and fatal neurological disease characterized by degeneration and death of motor neurons. Aberrant protein aggregation, increased levels of transition metals, and oxidative stress are implicated in its etiology. In a mouse model of ALS (G93ASOD1 mice), treatment with HBX (1 ppm added to control mouse chow) started at the age of 40 days significantly delayed disease onset (control, 19 days; vs HBX, 23 days) and increased maximum lifespan (Evans et al., 2016). However, no statistically significant treatment effects were found on overall survival distribution or median lifespan. While no differences were seen in average food consumption, HBX blunted the body weight loss between ages 90-146 days. ALS mice fed HBX also showed an increase in fat mass and a decrease in lean mass.

Disease progression was significantly slowed as measured by reduced neuromuscular denervation. Oxidative damage as measured by F2-isoprostanes (biomarkers that are increased in the urine of ALS patients), are elevated in the skeletal muscle from these ALS mice at onset and this increase is prevented with HBX treatment.

Aggregates of the SOD1 protein, specifically high molecular weight oligomers, are an early and widespread indicator of ALS and are found in both humans and animal models of disease. HBX treatment in ALS mice reduced mutant SOD1 protein aggregation in the spinal cord at disease onset.

In addition to the ability to bind to and prevent the formation of amyloid aggregates, HBX also possesses metal chelating properties. Copper was significantly elevated in ALS mice and HBX reduced this elevation. $Fe^{+2}$ was increased by 30% in gastrocnemius muscle in the ALS mice and HBX reduced this increase to levels comparable to controls.

HBX may be able to improve the degenerative symptoms of ALS through the prevention of oxidative damage and protein aggregation. Further studies are needed to determine the precise mechanisms of action of HBX in ameliorating ALS pathology and whether the benefits translate to humans.

***APOE4 interactions***:
Unknown.

**Aging and related health concerns:** HBX extended lifespan in a mouse model of ALS and in *C. elegans*, but no data exist for humans. HBX is being tested in the NIA ITP program to find out whether it can extend lifespan in mice.

*Types of evidence:*

- 3 laboratory studies

***Lifespan***: In a mouse model of amyotrophic lateral sclerosis (ALS; G93ASOD1 mice), treatment with HBX (1 ppm added to control mouse chow) started at the age of 40 days significantly increased maximum lifespan, though no significant differences were found for median lifespan or the overall survival distribution curves (Evans et al., 2016).

In a study in *C. elegans*, treatment with HBX (1, 10, 100 nM and 1 µM) throughout adult life extended lifespan by up to 40% (Alavez et al., 2011). HBX was effective at concentrations significantly lower (1 nM to 1 µM range) than its structural analog, thioflavin T. Thioflavin T was efficacious at doses ranging from 50 to 100 µM and increased maximal lifespan by 43-78%, increased median lifespan by 60%, reduced age-specific mortality at all ages, and slowed age-related decline in spontaneous movement. At higher doses (500 µM), thioflavin T was toxic and shortened lifespan. Other compounds with protein-

aggregate-binding properties, including curcumin and rifampicin, increased lifespan to a lesser extent (up to 45%). No additive effects were seen when thioflavin T was combined with curcumin. Beneficial effects of thioflavin T, HBX, and other related compounds on lifespan are mediated by the protein homeostasis network regulator heat shock factor 1 (HSF-1), the stress resistance and longevity transcription factor SKN-1, molecular chaperones, autophagy, and proteosomal functions. Because disruptions in protein homeostasis result in protein misfolding cascades and the accumulation of insoluble protein fibrils and aggregates (e.g., amyloid), preventing this disruption is suggested to increase overall health and longevity in worms.

HBX is one of the compounds being tested in the NIA-ITP Program (see list of compounds here). The NIA ITP was designed to test compounds such as HBX that are purported to extend lifespan and/or delay onset of age-related diseases. This collaborative program uses 1) parallel studies in male and female mice at 3 different sites, 2) genetically heterogeneous mice to guard against conclusions based on a single inbred genotype, and 3) enough samples to provide statistical power.

**Safety:** Safety has not been established in humans; only a few preclinical studies exist to date.

*Types of evidence:*

- A few laboratory studies

A study in *C. elegans* showed that HBX increases lifespan, suggesting it is safe in worms (Alavez et al., 2011). No studies have examined the safety of HBX in mammals, though the NIA ITP study is examining whether HBX treatment in mice extends lifespan.

Based on a cell viability assay (MTT assay), HBX and related compounds had high hydrophobicity which contributed to increased cell permeability and toxicity but HBX had lower toxicity compared to reference compounds (cisplatin and clioquinol) (Rodriguez-Rodriguez et al., 2015). HBX had an EC50 value of 73.28 µM.

***Drug interactions***: Not well studied.

**Sources and dosing:** HBX is not a commercial product and only a research grade product is available. Dosage has not been established. In the NIA ITP, ongoing studies are evaluating the effects of HBX treatment at a dose of 1 parts per million (ppm) in food (NIA-ITP).

**Research underway:** Under the NIA ITP initiative, ongoing studies are evaluating the effects of HBX treatment (1 ppm) started at the age of 15 months on lifespan in male mice (Gonzalez-Freire et al., 2020; NIA-ITP).

**Search terms:**

Pubmed, Google:

- HBX
- 2-(2-hydroxyphenyl)-benzoxazole

Websites visited for HBX, 2-(2-hydroxyphenyl)-benzoxazole:

- Clinicaltrials.gov (0)
- Examine.com (0)
- DrugAge (0)
- Geroprotectors (1)
- PubChem
- DrugBank.ca (0)
- Cafepharma (0)
- Pharmapro.com (0)

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*If you have suggestions for drugs, drugs-in-development, supplements, nutraceuticals, or food/drink with neuroprotective properties that warrant in-depth reviews by ADDF's Aging and Alzheimer's Prevention Program, please contact INFO@alzdiscovery.org. To view our official ratings, visit Cognitive Vitality's Rating page.*