

Benzodiazepine Receptor Antagonism: Mechanistic Roles of Flumazenil and BPC-157 in Reversing Benzodiazepine Tolerance

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Short Report

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Abstract

Introduction

Chronic benzodiazepine (BZD) pharmacotherapy induces adaptive uncoupling between the GABA and benzodiazepine binding sites of the GABA-A receptor, contributing to tolerance, dependence, and withdrawal.

Aim

To synthesize mechanistic evidence on flumazenil's receptor-level reversal of this uncoupling and to evaluate Body Protection Compound-157 (BPC-157) as a mechanistically complementary agent addressing the downstream biologic consequences of chronic BZD exposure that receptor antagonism alone does not resolve.

Method

A targeted narrative synthesis of preclinical and clinical literature (PubMed, Scopus, Google Scholar; January 1982-September 2026) on GABA-A receptor uncoupling, flumazenil pharmacology, endozepines, and BPC-157.

Results

Flumazenil competitively reverses receptor-level uncoupling and restores GABA/BZD coupling efficiency in preclinical models, and produces measurable clinical benefit in long-term BZD users, but its effects on tolerance are inconsistent and it does not address the neuroinflammation, endothelial dysfunction, or impaired tissue repair that can persist after chronic exposure. BPC-157 exerts angiogenic, nitric-oxide-dependent, and anti-inflammatory effects relevant to these downstream processes.

Conclusion

Flumazenil and BPC-157 act on distinct, complementary levels of BZD-related pathology; combined use warrants controlled clinical investigation.

Introduction

The GABA-A receptor is a pentameric, ligand-gated chloride channel and the principal mediator of fast inhibitory neurotransmission in the mammalian central nervous system (CNS) (Zhu et al. 2022). Benzodiazepines (BZDs) act as positive allosteric modulators at a site formed by the extracellular alpha-gamma subunit interface, distinct from the orthosteric GABA-binding sites, as resolved by high-resolution structural analyses (Zhu et al. 2018, 2022; Goldschen-Ohm 2022). Chronic BZD exposure produces a well-documented functional “uncoupling” between the GABA and BZD binding sites, characterized by reduced coupling efficiency, phosphorylation-mediated receptor internalization, and altered subunit composition, and is considered a principal molecular substrate of BZD tolerance,

dependence, and withdrawal (Hu and Ticku 1994; Gallager et al. 1984; Gutierrez et al. 2014a, b; Sieghart and Sperk 2002; Vinkers and Olivier 2012). Flumazenil, a competitive and largely neutral antagonist at the BZD site (behavior first characterized within a three-state receptor model in this journal; Polc et al. 1982), can acutely reverse this uncoupling in preclinical models and has shown clinical benefit in some long-term users, but its effects are inconsistent, ranging from partial reversal to precipitation of withdrawal (Ali and Olsen 2001; Savic et al. 1991; Hood et al. 2014; Gallo and Hulse 2021). This variability suggests that the BZD binding site operates within a dynamically regulated system rather than a fixed pharmacologic target, as underscored by endozepines, endogenous ligands derived from diazepam-binding inhibitor that modulate the same site under physiologic conditions (Farzampour et al. 2015; Tonon et al. 2020). Because flumazenil's mechanism is limited to receptor occupancy, it does not address the neuroinflammatory, vascular, and tissue-repair processes that may persist after chronic BZD exposure independent of receptor status. Body Protection Compound-157 (BPC-157), a pentadecapeptide with reported activity in nitric oxide signaling, angiogenesis, and cytoprotection, has emerged as a mechanistically distinct candidate for addressing these secondary processes (Sikiric et al. 2011; Jozwiak et al. 2025).

Aim

This Brief Report synthesizes mechanistic evidence on flumazenil's reversal of BZD-induced GABA-A receptor uncoupling and evaluates BPC-157 as a complementary agent targeting the downstream biologic consequences of chronic BZD exposure that receptor-level antagonism alone does not resolve.

Method

A targeted narrative literature search was conducted in PubMed, Scopus, and Google Scholar using combinations of "GABA-A receptor," "benzodiazepine binding site," "flumazenil," "GABA-A receptor uncoupling," "benzodiazepine tolerance/withdrawal," "endozepines," and "BPC-157." Eligible sources were peer-reviewed, English-language publications dated January 1, 1982, through September 6, 2026, comprising original research, structural and pharmacologic studies, and narrative or systematic reviews. Sources were selected for mechanistic convergence across GABA-A receptor uncoupling, flumazenil pharmacology, and BPC-157 biology rather than for quantitative synthesis, given the heterogeneity of preclinical designs and the paucity of controlled human data for BPC-157.

Ethics approval

Not applicable. This is a narrative synthesis of previously published literature; no new data involving human or animal subjects were generated, and no institutional review board approval or consent was required.

Results

Chronic benzodiazepine exposure produces GABA-A receptor uncoupling within hours to days. In cortical neurons, sustained flurazepam exposure reduced BZD-site E_{max} by approximately 50%, an effect linked to altered alpha-subunit expression and reversed by BZD-antagonist administration (Hu and Ticku 1994). Comparable uncoupling occurs after exposure as brief as 48 hours, mediated by receptor internalization and phosphorylation via protein kinases A and C rather than by transcriptional downregulation, alongside a measurable decline in postsynaptic GABA sensitivity (Gutierrez et al. 2014a, b; Gallager et al. 1984). Flumazenil reverses this uncoupling independent of direct phosphorylation, based on immunofluorescence evidence (Ali and Olsen 2001), and a 1.5-mg dose produced measurable receptor occupancy and clinical benefit in chronically exposed patients without provoking seizures (Savic et al. 1991). A systematic review of low-dose flumazenil regimens found consistent reduction in withdrawal symptoms when added to standard BZD tapering, across varying doses, frequencies, and routes of administration (Gallo and Hulse 2021; Hood et al. 2014). Notably, chronic flumazenil exposure itself alters GABA-A receptor subunit expression and channel function in neuronal culture, indicating pharmacologic effects beyond simple competitive displacement (Zheng et al. 1996). Clinically, low-dose outpatient flumazenil protocols, such as that used in the Rapid Benzodiazepine Detoxification program, have been used to mitigate withdrawal during rapid BZD tapering (Coleman Institute for Addiction Medicine 2025). Endozepines act at this same site under physiologic conditions (Farzampour et al. 2015; Tonon et al. 2020), reinforcing that flumazenil operates within a dynamically regulated system rather than a static target, which may explain the variability of its clinical effects in long-term BZD users.

Because flumazenil's mechanism is confined to receptor occupancy, it does not resolve the neuroinflammation, endothelial dysfunction, or impaired tissue repair that can persist after chronic BZD exposure independent of receptor status. BPC-157 has consistently demonstrated activity relevant to these processes: restoration of microvascular integrity and angiogenesis following endothelial injury (Sikiric et al. 2011, 2025; Jozwiak et al. 2025), reduced neuronal damage and glial activation with improved functional recovery in models of CNS injury, including hippocampal ischemia-reperfusion (Vukojevic et al. 2020, 2022), and enhanced fibroblast migration, collagen deposition, and biomechanical repair in musculoskeletal tissue (Brcic et al. 2009; McGuire et al. 2025; Vasireddi et al. 2025). BPC-157 has also shown anxiolytic-like activity distinct from BZD pharmacology and attenuated tolerance and physical dependence in a repeated-diazepam model without directly binding the BZD site (Sikiric et al. 2001; Jelovac et al. 1999). Table 1 summarizes the complementary features of flumazenil and BPC-157, and Fig. 1 depicts their convergence on CNS recovery following chronic benzodiazepine exposure.

Table 1

Comparison of flumazenil/receptor-uncoupling reversal and BPC-157 as complementary strategies in chronic benzodiazepine exposure.

Feature	Flumazenil / Uncoupling Reversal	BPC-157
Primary target	Benzodiazepine binding site on the GABA-A receptor	Downstream biologic pathways (nitric oxide signaling, angiogenesis, inflammation)
Mechanism	Competitive antagonism; reverses GABA/BZD site uncoupling	Modulates nitric oxide system activity, angiogenic signaling, and cytoprotection
Level of evidence	Preclinical (rodent, in vitro) plus limited human/clinical data	Predominantly preclinical (rodent); minimal controlled human data
Key strength	Rapid, targeted reversal of receptor-level tolerance	Addresses secondary injury processes not resolved by receptor antagonism alone
Key limitation	Effects inconsistent in long-term users; risk of precipitating withdrawal/seizures	Mechanism not fully defined in CNS; no controlled human trials

Relevant references. Flumazenil: Hu and Ticku 1994; Gutierrez et al. 2014a, b; Gallager et al. 1984; Sieghart and Sperk 2002; Vinkers and Olivier 2012; Ali and Olsen 2001; Savic et al. 1991; Hood et al. 2014; Gallo and Hulse 2021; Zheng et al. 1996. *BPC-157:* Sikiric et al. 2001, 2011, 2025; Jelovac et al. 1999; Brcic et al. 2009; Vukojevic et al. 2020, 2022; Jozwiak et al. 2025; McGuire et al. 2025; Vasireddi et al. 2025.

Conclusion

Chronic benzodiazepine exposure produces receptor-level uncoupling that flumazenil can reverse, but flumazenil's mechanism is confined to receptor occupancy and does not resolve the downstream neuroinflammatory, vascular, and tissue-repair consequences of prolonged exposure, nor does it reliably prevent withdrawal in long-term users. The presence of endozepines confirms that the benzodiazepine binding site is a dynamically regulated system rather than a fixed target, offering a plausible explanation for this clinical variability. BPC-157 represents a mechanistically distinct, complementary strategy, targeting angiogenesis, nitric oxide signaling, and inflammatory regulation rather than the GABA-A receptor itself. Because the evidence for BPC-157 remains largely preclinical, with minimal controlled human data and no established pharmacokinetic or dosing parameters, combining flumazenil-based receptor reversal with BPC-157-targeted downstream support should be evaluated in controlled translational studies before any claim of clinical benefit can be made.

Declarations

Clinical Trial Number

Not applicable.

Competing Interests:

The authors declare no conflicts of interest.

Use of Artificial Intelligence

Generative AI was used to assist with the preparation of tables and figures. All AI-assisted content was reviewed, fact-checked, and verified against primary sources by the authors, who take full responsibility for the accuracy, integrity, and originality of the final manuscript.

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Author Contribution

Marianne Koleng led manuscript preparation, coordinated the literature synthesis, and verified the reference list. Sarah Alvi and Gabby D. Mariblanca Garcia contributed to the analysis and discussion of flumazenil pharmacology. Bao L. Huynh and Ernesto Joubran contributed to the literature search and manuscript drafting. Carlos Menendez and Jason D. Nguyen contributed to the discussion of CNS recovery mechanisms. Scott C. Onyeke and Victoria A. Shaker contributed to the discussion of endozepine biology. Valerie J. Figueroa Thon and Gia Nghi Vo contributed to the discussion of BPC-157 pharmacology. Halford Warlick coordinated manuscript revision and resubmission and served as corresponding author. Kelley Davis, Vincent Gallicchio, and Jose Rey provided mentorship and critical revision. All authors reviewed and approved the condensed manuscript for resubmission as a Brief Report.

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Data Availability:

Not applicable. This is a narrative synthesis of previously published literature; no new data were generated or analyzed.

References

1. Ali NJ, Olsen RW (2001) Chronic benzodiazepine treatment of cells expressing recombinant GABA(A) receptors uncouples allosteric binding: studies on possible mechanisms. *J Neurochem* 79:1100–1108. <https://doi.org/10.1046/j.1471-4159.2001.00664.x>
2. Brcic L, Brcic I, Staresinic M, Novinscak T, Sikiric P, Seiwerth S (2009) Modulatory effect of gastric pentadecapeptide BPC 157 on angiogenesis in muscle and tendon healing. *J Physiol Pharmacol* 60(Suppl 7):191–196
3. Coleman Institute for Addiction Medicine (2025) Rapid Benzo Withdrawal Detox FAQs: The Coleman Institute. <https://www.thecolemaninstitute.com/about-addiction/faqs/rapid-benzodiazepine-detox-faqs/>. Accessed 6 September 2026
4. Farzampour Z, Reimer RJ, Huguenard J (2015) Endozepines. *Adv Pharmacol* 72:147–164. <https://doi.org/10.1016/bs.apha.2014.10.005>
5. Gallager DW, Lakoski JM, Gonsalves SF, Rauch SL (1984) Chronic benzodiazepine treatment decreases postsynaptic GABA sensitivity. *Nature* 308:74–77. <https://doi.org/10.1038/308074a0>
6. Gallo AT, Hulse G (2021) Pharmacological uses of flumazenil in benzodiazepine use disorders: a systematic review of limited data. *J Psychopharmacol* 35(3):211–220. <https://doi.org/10.1177/0269881120981390>
7. Goldschen-Ohm MP (2022) Benzodiazepine modulation of GABA-A receptors. *Biomolecules* 12(12):1784. <https://doi.org/10.3390/biom12121784>
8. Gutierrez ML, Ferreri MC, Farb DH, Gravielle MC (2014a) GABA-induced uncoupling of GABA/benzodiazepine site interactions is associated with increased phosphorylation of the GABA-A receptor. *J Neurosci Res* 92(8):1054–1061. <https://doi.org/10.1002/jnr.23387>
9. Gutierrez ML, Ferreri MC, Gravielle MC (2014b) GABA-induced uncoupling of GABA/benzodiazepine site interactions is mediated by increased GABAA receptor internalization and associated with a change in subunit composition. *Neuroscience* 257:119–129. <https://doi.org/10.1016/j.neuroscience.2013.10.077>
10. Hood SD, Norman A, Hince DA, Melichar JK, Hulse GK (2014) Benzodiazepine dependence and its treatment with low-dose flumazenil. *Br J Clin Pharmacol* 77(2):285–294. <https://doi.org/10.1111/bcp.12023>
11. Hu XJ, Ticku MK (1994) Chronic benzodiazepine agonist treatment produces functional uncoupling of the gamma-aminobutyric acid-benzodiazepine receptor ionophore complex in cortical neurons. *Mol Pharmacol* 45(4):618–625
12. Jelovac N, Sikiric P, Rucman R et al (1999) The effect of a novel pentadecapeptide BPC 157 on development of tolerance and physical dependence following repeated administration of diazepam. *Chin J Physiol* 42(3):171–179
13. Jozwiak M et al (2025) Multifunctionality and possible medical application of the BPC 157 peptide-literature and patent review. *Pharmaceuticals* 18:185. <https://doi.org/10.3390/ph18020185>

14. McGuire FP et al (2025) Regeneration or risk? A narrative review of BPC-157 for musculoskeletal healing. *Curr Rev Musculoskelet Med* 18:611–619. <https://doi.org/10.1007/s12178-025-09990-7>
15. Polc P, Bonetti EP, Schaffner R et al (1982) A three-state model of the benzodiazepine receptor explains the interactions between the benzodiazepine antagonist Ro 15-1788, benzodiazepine tranquilizers, beta-carbolines, and phenobarbitone. *Naunyn Schmiedebergs Arch Pharmacol* 321(4):260–264. <https://doi.org/10.1007/BF00498510>
16. Savic I, Widen L, Stone-Elander S (1991) Feasibility of reversing benzodiazepine tolerance with flumazenil. *Lancet* 337(8734):133–137. [https://doi.org/10.1016/0140-6736\(91\)90799-U](https://doi.org/10.1016/0140-6736(91)90799-U)
17. Sieghart W, Sperk G (2002) Subunit composition, distribution and function of GABA-A receptor subtypes. *Curr Top Med Chem* 2(8):795–816. <https://doi.org/10.2174/1568026023393507>
18. Sikiric P, Jelovac N, Jelovac-Gjeldum A et al (2001) Anxiolytic effect of BPC-157, a gastric pentadecapeptide: shock probe/burying test and light/dark test. *Acta Pharmacol Sin* 22(3):225–230
19. Sikiric P, Seiwerth S, Rucman R et al (2011) Stable gastric pentadecapeptide BPC 157: novel therapy in gastrointestinal tract. *Curr Pharm Des* 17(16):1612–1632. <https://doi.org/10.2174/138161211796196954>
20. Sikiric P et al (2025) BPC 157 therapy: targeting angiogenesis and nitric oxide's cytotoxic and damaging actions, but maintaining, promoting, or recovering their essential protective functions. Comment on Jozwiak et al. *Pharmaceuticals* 18(10):1450. <https://doi.org/10.3390/ph18101450>
21. Tonon MC, Vaudry H, Chuquet J et al (2020) Endozepines and their receptors: structure, functions and pathophysiological significance. *Pharmacol Ther* 208:107386. <https://doi.org/10.1016/j.pharmthera.2019.06.008>
22. Vasireddi N, Hahamyan H, Salata MJ, Karns M, Calcei JG, Voos JE, Apostolakos JM (2025) Emerging use of BPC-157 in orthopaedic sports medicine: a systematic review. *HSS J*. <https://doi.org/10.1177/15563316251355551>
23. Vinkers CH, Olivier B (2012) Mechanisms underlying tolerance after long-term benzodiazepine use: a future for subtype-selective GABA(A) receptor modulators? *Adv Pharmacol Sci* 2012:416864. <https://doi.org/10.1155/2012/416864>
24. Vukojevic J, Vrdoljak B, Malekinusic D et al (2020) The effect of pentadecapeptide BPC 157 on hippocampal ischemia/reperfusion injuries in rats. *Brain Behav* 10(8):e01726. <https://doi.org/10.1002/brb3.1726>
25. Vukojevic J, Milavic M, Perovic D et al (2022) Pentadecapeptide BPC 157 and the central nervous system. *Neural Regen Res* 17(3):482–487. <https://doi.org/10.4103/1673-5374.320969>
26. Zheng TM, Caruncho HJ, Zhu WJ, Vicini S, Ikonovic S, Grayson DR, Costa E (1996) Chronic flumazenil alters GABA(A) receptor subunit mRNA expression, translation product assembly and channel function in neuronal cultures. *J Pharmacol Exp Ther* 277(1):525–533
27. Zhu S, Noviello CM, Teng J, Walsh RM Jr, Kim JJ, Hibbs RE (2018) Structure of a human synaptic GABA(A) receptor. *Nature* 559(7712):67–72. <https://doi.org/10.1038/s41586-018-0255-3>

28. Zhu S, Sridhar AK, Teng J, Howard RJ, Lindahl E, Hibbs RE (2022) Structural and dynamic mechanisms of GABA-A receptor modulators with opposing activities. Nat Commun 13:4582. <https://doi.org/10.1038/s41467-022-32212-4>

Figures

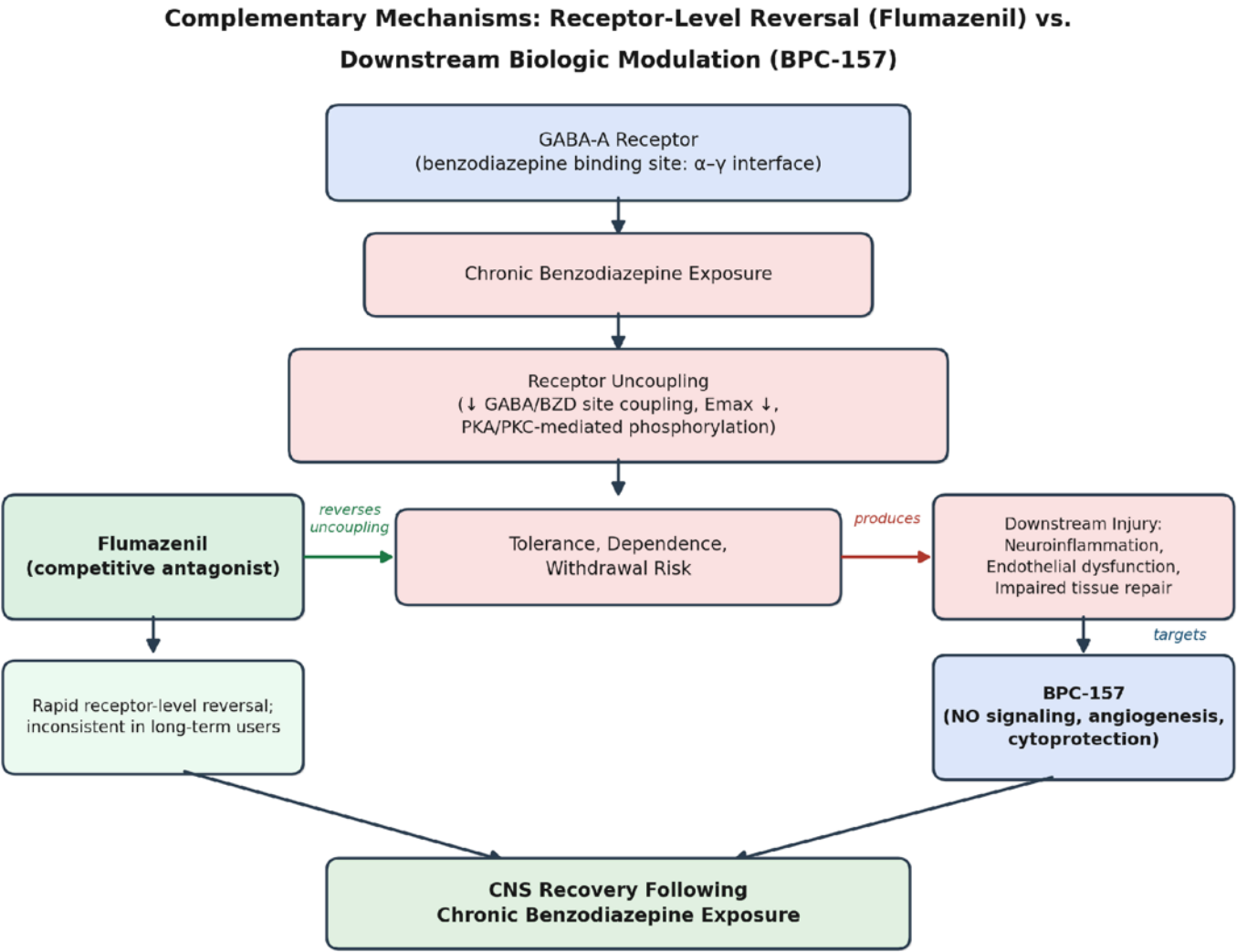


Figure 1

Conceptual schematic contrasting receptor-level reversal of GABA-A/benzodiazepine-site uncoupling by flumazenil with downstream biologic modulation by BPC-157, converging on CNS recovery following chronic benzodiazepine exposure. Adapted with permission (Jozwiak et al. 2025).