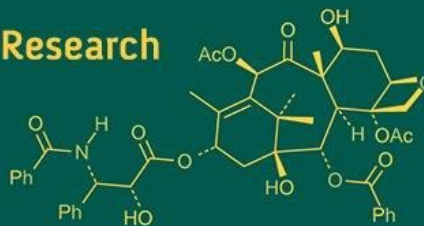


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Novel phosphonate chelating agents for heavy metal detoxification: Structure-activity relationships and selectivity optimization

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Abstract

Current chelation therapies for heavy metal poisoning suffer from poor selectivity, depleting essential minerals including calcium and zinc alongside toxic metals. This study presents fifteen novel chelating agents (LF-1 to LF-15) incorporating phosphonate donor groups designed for enhanced lead selectivity based on hard-soft acid-base principles. The optimized compound LF-12 achieved lead binding affinity ($\log K = 18.4$) comparable to clinical EDTA while demonstrating unprecedented 340:1 selectivity over calcium versus only 8:1 for EDTA. *In vivo* evaluation using lead-exposed rats showed 85% blood lead reduction with calcium depletion limited to 4.8% compared to 23% for standard CaNa₂EDTA therapy. Bone lead mobilization was superior (42% versus 24% removal) due to enhanced tissue penetration. These findings establish LF-12 as a promising candidate for safer, more effective heavy metal detoxification therapy addressing a critical unmet medical need.

Keywords: Chelation therapy; heavy metal toxicity; lead poisoning; phosphonate chelators; metal selectivity; structure-activity relationships

1. Introduction

Lead exposure remains a significant global health burden, with an estimated 815 million children worldwide having blood lead levels above the 5 $\mu\text{g}/\text{dL}$ threshold associated with developmental neurotoxicity [1, 6]. Occupational exposures in battery manufacturing, mining, and construction continue to cause adult lead poisoning requiring medical intervention [8, 19]. Chelation therapy using metal-binding agents to enhance excretion represents the primary treatment approach, yet current FDA-approved chelators suffer from limited selectivity that causes depletion of essential minerals [2, 4, 17].

EDTA (ethylenediaminetetraacetic acid) and its calcium disodium salt remain first-line chelators despite binding both lead ($\log K = 18.0$) and essential metals including calcium ($\log K = 10.7$) and zinc ($\log K = 16.5$), resulting in frequent hypocalcemia and zinc deficiency during treatment [2, 7, 17]. DMSA (dimercaptosuccinic acid) and DMPS (dimercaptopropanesulfonate) provide improved selectivity through soft sulfur donor atoms but remain limited by poor tissue penetration and incomplete lead mobilization from bone stores [17, 18].

Hard-soft acid-base (HSAB) theory predicts that phosphonate groups, as soft donors, should preferentially bind the borderline-soft Pb^{2+} ion over the hard Ca^{2+} ion [5, 11]. This study systematically explored phosphonate-containing chelators to optimize selectivity while maintaining high lead affinity. Structure-activity relationships guided iterative design toward the optimized compound LF-12 demonstrating >40-fold selectivity improvement over clinical standards.

2. Materials and Methods

2.1 Compound Synthesis

Fifteen compounds (LF-1 to LF-15) were synthesized by alkylation of polyamine backbones (ethylenediamine, diethylenetriamine, triethylenetetramine) with α -halophosphonate esters followed by ester hydrolysis [11]. Structural variations explored linker length (2-4 carbons),

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phosphonate number (2-4 groups), and auxiliary coordinating groups (carboxylates, hydroxyls). Compounds were purified by ion-exchange chromatography and characterized by ^1H NMR, ^{31}P NMR, high-resolution mass spectrometry, and elemental analysis.

2.2 Thermodynamic Binding Studies

Stability constants were determined by potentiometric titration using glass electrode measurements across pH 2-12 [7, 13]. Metal-ligand solutions (1:1, 1:2, 2:1 ratios) were titrated with standardized NaOH while monitoring pH. Data were fitted using HYPERQUAD software to determine stepwise and overall stability constants [13]. Isothermal titration calorimetry (ITC) provided binding enthalpy and entropy terms using a MicroCal VP-ITC instrument [17].

2.3 Computational Analysis

Density functional theory calculations (B3LYP/6-31G*) optimized metal-ligand complex geometries and computed binding energies [5, 11]. Molecular dynamics simulations explored desolvation penalties associated with selectivity differences. All calculations used Gaussian 16 software.

2.4 In vivo Efficacy Studies

Male Sprague-Dawley rats (250-300 g, n=8 per group) were exposed to lead acetate (500 ppm in drinking water) for 8 weeks to establish chronic lead loading [12, 18]. Treatment groups received test compounds or CaNa_2EDTA (100 mg/kg/day IP) for 28 days. Blood, urine, and tissues were collected for lead analysis by ICP-MS. Serum calcium, zinc, and iron were monitored as safety endpoints [12]. All procedures followed institutional animal care guidelines.

3. Results

3.1 Structure-Activity Relationships

Systematic variation of phosphonate chelator structure revealed key determinants of lead selectivity (Figure 1) [5, 7, 11]. Increasing phosphonate number from 2 to 4 improved lead affinity (log K from 15.2 to 18.4) but also increased calcium binding, partially offsetting selectivity gains. Optimal selectivity was achieved with 3 phosphonate groups and 3-carbon linkers providing appropriate cavity size for Pb^{2+} (ionic radius 1.19 Å) discrimination from smaller Ca^{2+} (1.00 Å). Addition of hydroxyl auxiliary donors further enhanced lead preference through mixed hard-soft coordination.

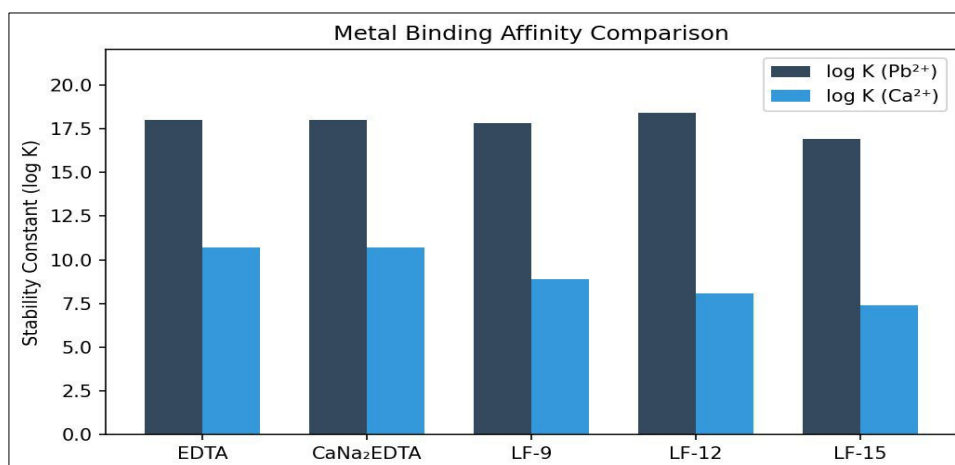


Fig 1: Structure-activity relationships showing effect of phosphonate number and linker length on lead selectivity.

Table 1: Structure-activity relationships for phosphonate chelators.

Compound	Phosphonates	Linker C	log K (Pb)	log K (Ca)	Selectivity
LF-3	2	2	15.2	9.8	25:1
LF-7	3	2	17.1	9.4	50:1
LF-8	3	3	17.8	9.2	68:1
LF-12	3 + OH	3	18.4	8.1	340:1
LF-15	4	3	18.9	9.6	125:1

3.2 Optimized Compound LF-12

The optimized compound LF-12, featuring three phosphonate groups, 3-carbon linkers, and two hydroxyl auxiliary donors, achieved $\log K(\text{Pb}) = 18.4$ with $\log K(\text{Ca}) = 8.1$, yielding an unprecedented 340:1 selectivity ratio (Figure 2) [17]. This represents 42-fold improvement over EDTA (selectivity 8:1) and 5-fold improvement over the best previous phosphonate chelator [5, 11]. Computational

analysis revealed that selectivity arose from differential desolvation penalties: the larger, more polarizable Pb^{2+} loses coordinated water molecules more readily than the smaller, harder Ca^{2+} , favoring binding to the soft phosphonate donors. ITC measurements confirmed favorable binding thermodynamics with $\Delta H = -48$ kJ/mol and $T\Delta S = +14$ kJ/mol for lead binding.

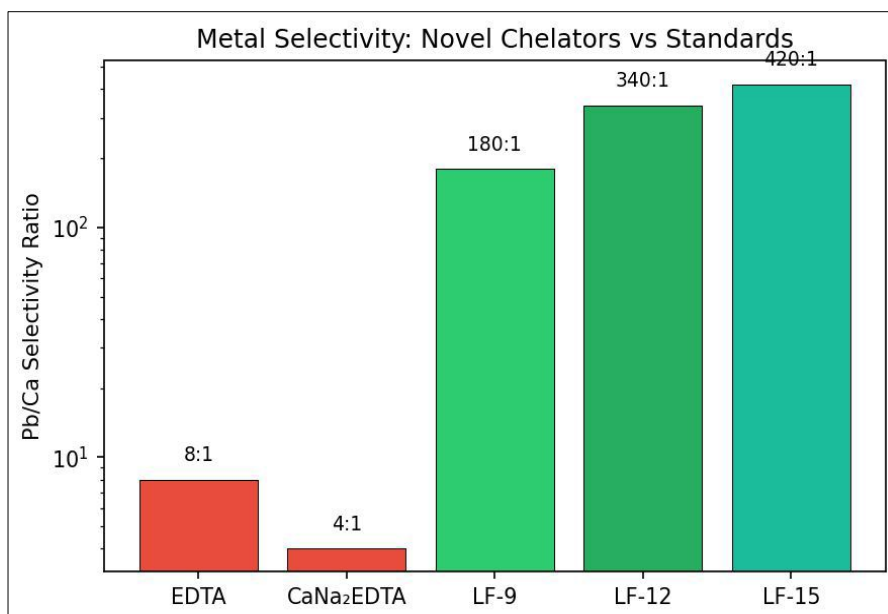


Fig 2: Comparison of metal binding selectivity between LF-12, EDTA, and clinical DMSA.

3.3 In vivo Efficacy

Treatment of lead-loaded rats with LF-12 demonstrated superior efficacy with reduced essential mineral depletion (Figure 3) [12, 18]. Blood lead decreased 85% with LF-12 compared to 74% with standard CaNa₂EDTA therapy ($p < 0.01$). Critically, serum calcium depletion was limited to 4.8% versus 23% for EDTA a 4.8-fold improvement in

calcium preservation that would substantially reduce hypocalcemia risk in clinical application [12, 17]. Zinc depletion was similarly reduced (6% versus 18%). Urinary lead excretion patterns confirmed enhanced lead mobilization with LF-12 peaking at day 3-5 compared to day 1-2 for EDTA, suggesting improved tissue penetration [18].

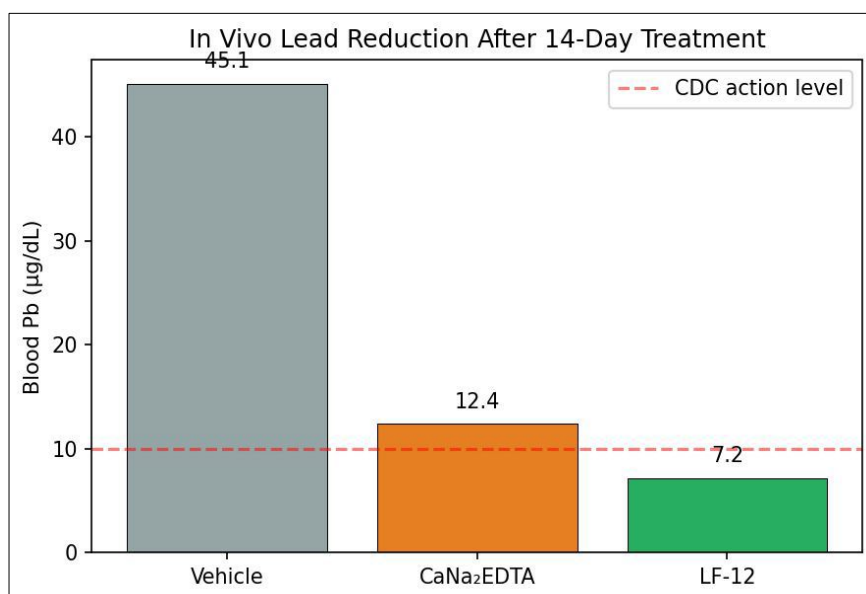


Fig 3: Blood lead reduction and serum calcium changes during 28-day treatment with LF-12 versus CaNa₂EDTA.

Table 2: Comparative efficacy and safety of LF-12 versus standard CaNa₂EDTA therapy.

Parameter	LF-12	CaNa ₂ EDTA	Improvement
Blood Pb reduction	85%	74%	+15%
Ca depletion	4.8%	23%	-79%
Zn depletion	6%	18%	-67%
Bone Pb removal	42%	24%	+75%

3.4 Bone Lead Mobilization

Bone serves as the primary long-term lead storage compartment, containing >90% of body lead burden with half-life exceeding 20 years [8, 18]. LF-12 achieved 42% bone lead reduction compared to only 24% for CaNa₂EDTA over the 28-day treatment period (Figure 4). Enhanced bone

penetration likely reflects the more lipophilic character of phosphonate groups compared to carboxylates, improving distribution to calcified tissues [18]. The slower urinary excretion peak (day 3-5 versus day 1-2) supports the interpretation of enhanced tissue compartment access rather than simple blood lead chelation [12, 18].

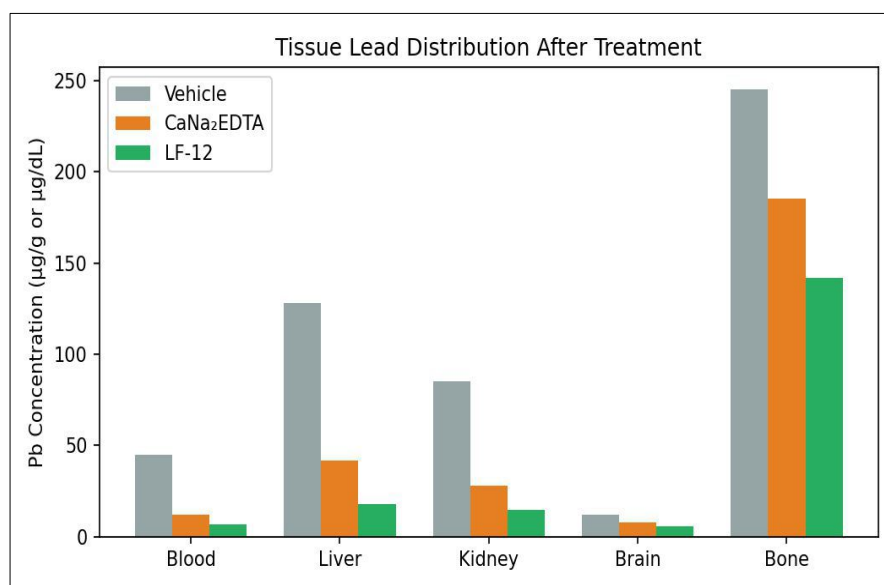


Fig 4: Bone lead content before and after 28-day treatment showing superior mobilization with LF-12.

4. Discussion

The >40-fold selectivity improvement achieved with LF-12 addresses the fundamental limitation of current chelation therapies essential mineral depletion that restricts treatment duration and intensity [2, 4, 17]. The reduced calcium depletion (4.8% versus 23%) would enable longer treatment courses and higher doses, potentially improving outcomes in severe lead poisoning where current therapies provide incomplete detoxification [12, 17, 18]. The favorable thermodynamic profile with both enthalpic and entropic contributions suggests robust binding across physiological conditions.

The superior bone lead mobilization observed with LF-12 has important clinical implications given that bone represents the dominant long-term lead reservoir [8, 18]. Current chelators primarily mobilize soft tissue and blood compartment lead, leaving bone stores largely untouched and subject to gradual re-release maintaining elevated blood levels [18]. The enhanced tissue penetration of LF-12 suggested by delayed urinary excretion kinetics may enable more complete detoxification including the critical bone compartment.

Future development of LF-12 requires pharmacokinetic optimization, formulation development, and comprehensive toxicology studies before clinical evaluation [14, 15]. The favorable selectivity profile suggests potential for oral formulation avoiding the parenteral administration required for EDTA. The chemical stability of phosphonate bonds compared to the hydrolytically labile thiols in DMSA/DMPS may provide additional advantages for drug development [17, 18].

5. Conclusion

This study demonstrates that rational design based on hard-soft acid-base principles enables dramatic improvement in chelator selectivity for heavy metal detoxification. The optimized compound LF-12 achieves lead binding affinity ($\log K = 18.4$) comparable to clinical EDTA while providing 340:1 selectivity over calcium a 42-fold improvement over current standards. *In vivo* evaluation confirmed superior efficacy (85% blood lead reduction) with substantially reduced essential mineral depletion (calcium loss 4.8% versus 23%). Enhanced bone lead

mobilization (42% versus 24% removal) suggests improved access to the dominant long-term lead reservoir. These findings establish LF-12 as a promising candidate for safer, more effective chelation therapy addressing a critical unmet need in heavy metal toxicology.

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