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CONTENTS

Chemistry

Assembayeva E. K., Beisekhan A., Bozhbanov A. Zh., Nurmukhanbetova D.E., Gabdullina E.Zh. Effect of chia seeds (<i>Salvia Hispanica</i> l.) on the physicochemical and mineral properties of low-fat cottage cheese.....	11
Balkhashbay Sh.Zh., Azimbayeva G.E., Kudaibergenova G.N., Kamysbayeva A.K., Kurbanbayeva N.M. Determination of biologically active compounds in morphological parts of medicinal plants.....	24
Darmenbayeva A.S., Rajasekharan R. Preparation and characterization of nanocellulose biocomposites from agro-waste of the Zhambyl region.....	39
Demets O.V., Rakhimberlinova Zh.B., Zgardan V.V., Serykh N.V., Dyussekeyeva A.T. Qualitative and quantitative analysis of amino acids in Kyrgyz birch bark extract.....	55
Jumekeyeva A.I., Talgatov E.T., Auyezkhanova A.S., Kenzheyeva A.M., Naizabayev A.A. Complex formation of palladium (II) ions with organic polymers of various nature.....	70
Dmitriyeva E.A. Electrolytes of lithium-ion batteries.....	83
Yegemberdiyeva S., Abdurazova P., Turtabaev S., Shitybaev S., Kerimbayeva K. Catalytic properties of Ru- and Rh-promoted skeletal nickel catalysts in the hydrogenation of butyraldehyde.....	97
Yertayeva A.B., Adylbekova A.O., Toleubekova A.G. Production of emulsions stabilized by bentonite clay particles.....	112
Fischer D., Jumadilov T., Haponiuk J., Toilanbay G., Baishibekov A. Interpolymer KU-2-8: AV-17-8 systems for selective sorption of rhenium, molybdenum and tungsten.....	129
Zhanikulov N., Zhurgarayeva D. Investigation of the quality of cement clinker obtained from heap leaching waste.....	148
Zhoshybaeva A.A., Kozhanova K.K., Mombekov S.E., Barakova A.Sh. Pharmaceutical development of a medicinal product containing an isocitrate lyase inhibitor.....	162
Ivanov N.S., Abilmagzhanov A.Z., Nurtazina A.E., Adelbayev I.E., Kholkin O.S. Sequential electrochemical processes for the treatment of magnesium leaching solutions.....	176

Imangaliyeva B., Duzelbayeva S., Tolesinova I., Bukeykhan D., Turlanova A. Chemical and agronomic assessment of the use of mineral wool and coconut fiber as a substrate in a greenhouse.....	190
Kurmanaliev M.K., Shaikhova Zh.E., Abilkasova S.O., Kalimoldina L.M., Bugubaeva G.O. Crown esters immobilized on polymeric supports as novel interfacial catalysts.....	207
Mataev M.M., Ongarbek A.T., Sarsenbayeva Z.B., Nurbekova M.A., Abdraimova M.R. Synthesis and morphology of perovskite-structured $\text{CaMnO}_{2.98}$	221
Medeuova G.Zh., Azimbayeva G.E., Kaliyeva A.N.*, Sadykova D.A., Anuarova L.E. Determination of vitamins in <i>Polygonum Aviculare</i> L. using capillary electrophoresis.....	238
Mukusheva G.K., Jalmakhanbetova R.I., Seilkhanov T.M., Bakibaev A.A., Aliyeva M.R. Functional modification reactions at the nitrogen atom of salsolin and biological activity of the obtained derivatives.....	251
Muldakhmetov Z.M., Zhakina A.Kh., Arnt O.V., Vassilets Ye.P., Zhakin A.M. Composite materials modified with carbon filler.....	267
Nazarbek U., Raiymbekov Y., Abdurazova P., Kambarova G. Study on the efficiency of water treatment using nanostructured water.....	280
Nauanova A.P., Kassenov R.Z., Davrenbekov S.Zh., Bolatbay A.N., Altynbekkyzy A. Intensification of the process of extraction of humic substances from brown coal.....	295
Nurlybayeva A.N., Zharlykapova R.B., Taubaeva R.S., Matniyazova G.K., Rustem E.I. Study of physical, chemical and mechanical properties of acrylic terpolymer.....	309
Uali A., Omirzak U., Titanov A., Abilkanova F., Kunarbekova M. Waste biomass-derived Fe-modified biochar: structure and application in potentiometric analysis.....	323
Khamitova A.S., Nurmukhanbetova N.N., Ostretsova I.B., Kassenova N.B., Kuderina B.T. Synthesis of metal corrosion inhibitors based on ammonia.....	338

МАЗМҰНЫ

ХИМИЯ

Асембаева Э.К., Бейсехан А., Божбанов А.Ж., Чиа дәндерінің (<i>Salvia Hispanica L.</i>) майсыздандырылған сүзбенің физика-химиялық және минералдық көрсеткіштеріне әсері.....	11
Балқашбай Ш.Ж., Азимбаева Г.Е., Қудайбергенова Г.Н., Қамысбаева А.К., Қурбанбаева М. Дәрілік өсімдіктердің морфологиялық мүшелеріндегі биологиялық белсенді заттарды анықтау.....	24
Дарменбаева А.С., Rajasekharan R. Жамбыл облысының агрокалдықты негізінде наноцеллюлозалық биокомпозиттерді алу және олардың қасиеттерін зерттеу.....	39
Демец О.В., Рахимберлинова Ж.Б., Згардан В.В.*, Серых Н.В., Дюсекеева А.Т., 2026. Қырғыз қайың қабығының сығындысындағы аминқышқылдарының сапалық және сандық құрамын талдау.....	55
Джумекеева А.И., Талғатов Э.Т., Ауезханова А.С., Кенжеева А.М., Найзабаев А.А. Палладий (II) иондарының табиғаты әртүрлі органикалық полимерлермен кешен түзуі.....	70
Дмитриева Е.А. Литий-ионды аккумуляторлардың электролиттері.....	83
Егембердиева С.Ж., Абдуразова П., Туртабаев С.К., Шитыбаев С.А., Керимбаева К.З. Ru және Rh промоторланған қаңқалы никель катализаторларының май альдегидін гидрлеу реакциясындағы каталитикалық қасиеттері.....	97
Ертаева А.Б., Адильбекова А.О., Төлеубекова А.Ғ. Бентонит сазының бөлшектерімен тұрақтандырылған эмульсияларды алу.....	112
Фишер Д., Джумадилов Т., Хапонюк Ю., Тойланбай Г., Байшибеков А. Рений, молибден және вольфрамды селективті сорбциялауға арналған KU-2-8:AV-17-8 интерполимерлі жүйелері.....	129
Жаникулов Н., Жургараева Д. Үйінді шаймалау қалдықтарынан алынған цемент клинкерінің сапасын зерттеу.....	148
Жошыбаева А.А., Кожанова К.К., Момбеков С.Е., Баракова А.Ш. Изоцитратлиаза ингибиторын қамтитын дәрілік препаратты фармацевтикалық әзірлеу.....	162

- Иванов Н.С., Абильмагжанов А.З., Нұртазина А.Е., Адельбаев И.Е., Холкин О.С.**
Магнийді шаймалау ерітінділерін қайта өңдеу технологиясындағы дәйекті
электрохимиялық процестер.....176
- Имангалиева Б., Дүзелбаева С., Төлесінова И., Букейхан Д., Тұрланова А.,**
Жылыжайда минералды жүн мен кокос талшығын субстарт ретінде қолданудың
химия-агрономиялық бағасы.....190
- Құрманалиев М.Қ., Шанхова Ж.Е., Әбілқасова С.О., Калимолдина Л.М.,**
Бугубаева Г.О.
Полимерлік тасымалдаушыларда иммобилизацияланған краун-эфирлер —
жаңа фазааралық катализаторлар ретінде.....207
- Матаев М.М., Оңғарбек А.Т., Сарсенбаева З.Б., Нурбекова М.А., Абдраимова М.Р.**
Перовскит құрылымды $\text{CaMnO}_{2.98}$ синтезі мен морфологиясы.....221
- Медеуова Г.Дж., Азимбаева Г.Е., Калиева А.Н., Садыкова Д.А., Ануарова Л.Е.**
Polygonum Aviculare L. өсімдігінің құрамындағы дәрумендерді капиллярлы
электрофорез әдісімен анықтау.....238
- Мукушева Г.К., Джалмаханбетова Р.И., Сейлханов Т.М., Бакибаев А.А., Алиева М.Р.**
Сольсалиннің азот атомы бойынша функционалдық модификация реакциялары
және алынған туындылардың биологиялық белсенділігі.....251
- Мулдахметов З.М., Жакина А.Х., Арнт О.В., Василец Е.П., Жакин А.М.**
Көміртекті толтырғышпен модификацияланған композициялық материалдар.....267
- Назарбек У., Райымбеков Е., Абдуразова П., Қамбарова Ғ.**
Наноқұрылымданған суды қолдану арқылы суды тазарту тиімділігін зерттеу.....280
- Науанова А.П., Касенов Р.З., Давренбеков С.Ж., Болатбай А.Н., Алтынбекқызы Ә.**
Қоңыр көмірден гуминдік заттарды бөліп алу процесін қарқындету.....295
- Нурлыбаева А.Н., Жарлыкапова Р.Б., Таубаева Р.С., Матниязова Г.К., Рустем Е.І**
Акрил терполимердің физика-химиялық және механикалық қасиеттерін зерттеу.....309
- Уәли А., Өмірзақ Ұ., Титанов А., Абилканова Ф., Қунарбекова М.**
Қалдық биомассадан алынған темірмен түрлендірілген биокөмір: құрылымы
және потенциометриялық талдауда қолданылуы.....323
- Хамитова А.С., Нурмуханбетова Н.Н., Острцова И.Б., Касенова Н.Б., Кудерина Б.Т.**
Аммиак негізінде металдар коррозиясының ингибиторларын синтездеу.....338

СОДЕРЖАНИЕ

ХИМИЯ

Асембаева Э. К., Бейсехан А., Божбанов А.Ж., Нурмуханбетова Д.Е., Габдуллина Е.Ж. Влияние семян чиа (<i>Salvia Hispanica L.</i>) на физико-химические и минеральные показатели обезжиренного творога.....	11
Балкашбай Ш.Ж., Азимбаева Г.Е., Кудайбергенова Г.Н., Камысбаева А.К., Курбанбаева Н.М. Определение биологически активных веществ в морфологических органах лекарственных растений.....	24
Дарменбаева А.С., Rajasekharan R. Получение и свойства наноцеллюлозных биокмполитов на основе агроотходов Жамбылской области.....	39
Демец О.В., Рахимберлинова Ж.Б., Згардан В.В., Серых Н.В., Дюсекеева А.Т. Качественный и количественный анализ аминокислот в экстракте коры берёзы киргизской.....	55
Джумекеева А.И., Талгатов Э.Т., Ауезханова А.С., Кенжеева А.М., Найзабаев А.А. Комплексообразование ионов палладия (II) с органическими полимерами различной природы.....	70
Дмитриева Е.А. Электролиты литий-ионных аккумуляторов.....	83
Егембердиева С.Ж., Абдуразава П., Туртабаев С.К., Шитибаев С.А., Керимбаева К.З. Каталитические свойства скелетных никелевых катализаторов, промотированных Ru и Rh, в реакции гидрирования масляного альдегида.....	97
Ертаева А.Б., Адильбекова А.О., Төлеубекова А.Ғ. Получение эмульсий, стабилизированных частицами бентонитовой глины.....	112
Фишер Д., Джумадиллов Т., Хапонюк Ю., Тойланбай Г., Байшибеков А. Интерполимерные системы KU-2-8:AV-17-8 для селективной сорбции рения, молибдена и вольфрама.....	129
Жаникулов Н., Жургараева Д. Исследование качества цементного клинкера, полученного из отходов кучного выщелачивания.....	148
Жошыбаева А.А., Кожанова К.К., Момбеков С.Е., Баракова А.Ш. Фармацевтическая разработка лекарственного препарата, содержащего ингибитор изоцитратлиазы.....	162

Иванов Н.С., Абильмагжанов А.З., Нуртазина А.Е., Адельбаев И.Е., Холкин О.С. Последовательные электрохимические процессы в технологии переработки растворов выщелачивания магния.....	176
Имангалиева Б., Дүзелбаева С., Төлесінова И., Букейхан Д., Турланова А. Химико-агрономическая оценка использования минеральной ваты и кокосового волокна в качестве субстрата в теплице.....	190
Курманалиев М.К., Шаихова Ж.Е., Абилкасова С.О., Калимолдина Л.М., Бугубаева Г.О. Краун-эфиры, иммобилизованные на полимерных носителях, как новые межфазные катализаторы.....	207
Матаев М.М., Онгарбек А.Т., Сарсенбаева З.Б., Нурбекова М.А., Абдраимова М.Р. Синтез и морфология перовскитной структуры $\text{CaMnO}_{2.98}$	221
Медсұова Г.Дж., Азимбаева Г.Е., Калиева А.Н., Садыкова Д.А., Ануарова Л.Е. Определение витаминов, содержащихся в растении <i>Polygonum aviculare L.</i> , методом капиллярного электрофореза.....	238
Мукушева Г.К., Джалмаханбетова Р.И., Сейлханов Т.М., Бакибаев А.А., Алиева М.Р. Реакции функциональной модификации хлорида аммония по атому азота и биологическая активность полученных производных.....	251
Мулдахметов З.М., Жакина А.Х., Арнт О.В., Василец Е.П., Жакин А.М. Композитные материалы, модифицированные углеродным наполнителем.....	267
Назарбек У., Райымбеков Е., Абдуразова П., Камбарова Г. Исследование эффективности очистки воды с применением наноструктурированной воды.....	280
Науанова А.П., Касенов Р.З., Давренбеков С.Ж., Болатбай А.Н., Алтынбекқызы А. Интенсификация процесса выделения гуминовых веществ из бурого угля.....	295
Нурлыбаева А.Н., Жарлыкапова Р.Б., Таубаева Р.С., Матниязова Г.К., Рустем Е.И. Изучение физико-химических и механических свойств акрилового терполимера.....	309
Уали А., Омирзак У., Титанов А., Абилканова Ф., Кунарбекова М. Биоуголь, модифицированный железом, из отходов биомассы: структура и применение в потенциометрическом анализе.....	323
Хамитова А.С., Нурмуханбетова Н.Н., Острцова И.Б., Касенова Н.Б., Кудерина Б.Т. Синтез ингибиторов коррозии металлов на основе аммиака.....	338

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PHARMACEUTICAL DEVELOPMENT OF A MEDICINAL PRODUCT CONTAINING AN ISOCITRATE LYASE INHIBITOR

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Abstract. Multidrug-resistant tuberculosis remains a critical global health challenge, necessitating novel therapeutic targets. Isocitrate lyase (ICL), an enzyme essential for Mycobacterium tuberculosis persistence, has emerged as a promising target, yet ICL inhibitors face significant pharmaceutical limitations including poor aqueous solubility and inadequate cellular penetration. This study aimed systematically evaluate pharmaceutical formulation strategies addressing the delivery challenges of ICL inhibitors and assess their potential for therapeutic application. A systematic literature search was conducted across PubMed, Scopus, Web of Science, and Chemical Abstracts Service (January 2000–August 2025) using PRISMA guidelines. Search terms included “isocitrate lyase,” “ICL inhibitor,” “pharmaceutical development,” “drug delivery,” and related terms. Studies were included if they addressed pharmaceutical formulation development, physicochemical characterization, stability assessment, or delivery system evaluation of ICL inhibitors. Data were extracted on formulation types, analytical methods, and performance characteristics were extracted. Forty-three studies met inclusion criteria. Nano-based delivery systems predominated (58.1%), with polymeric nanoparticles most frequently investigated (42.8%). Surface-modified

nanoparticles, particularly mannose-coated formulations, achieved superior macrophage targeting ($68.9 \pm 7.1\%$ uptake efficiency) and intracellular accumulation (1032.4 ± 94.7 ng/ 10^6 cells) compared to unmodified carriers. Lipid-based systems showed optimal performance for highly lipophilic compounds. Pulmonary delivery formulations demonstrated excellent lung targeting (lung-to-plasma ratio: 8.5–15.7). Stability studies identified degradation pathways guiding excipient selection. Validated analytical methods exhibited excellent performance (linearity $r^2 > 0.999$, precision RSD $< 2\%$). Advanced formulation strategies, particularly targeted nanoparticles and pulmonary delivery systems, successfully address pharmaceutical limitations of ICL inhibitors. These approaches enhance solubility, cellular uptake, and tissue targeting, providing a foundation for developing effective anti-tuberculosis therapies. Further research should focus on comprehensive in vivo evaluation, long-term stability assessment, and manufacturing scale-up for clinical translation.

Keywords: isocitrate lyase inhibitors; pharmaceutical formulation; drug delivery systems; nanoparticles; tuberculosis therapy

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ИЗОЦИТРАТЛИАЗА ИНГИБИТОРЫН ҚАМТИТЫН ДӘРЛІК ПРЕПАРАТТЫ ФАРМАЦЕВТИКАЛЫҚ ӨЗІРЛЕУ

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Аннотация. Көп дәріге төзімді Туберкулез жаңа терапевтік мақсаттарды қажет ететін жаһандық денсаулық сақтаудың маңызды мәселесі болып қала береді. Туберкулез микобактерияларының тұрақтылығына қажетті фермент изоцитрат лиазасы (ICL) перспективалы мақсатқа айналды, дегенмен ICL ингибиторлары маңызды фармацевтикалық шектеулерге тап болады, соның ішінде суда ерігіштігі нашар және жасушаларға ену жеткіліксіз. Бұл зерттеу ICL ингибиторларын жеткізу мәселелерін шешетін фармацевтикалық препараттарды жасау стратегияларын жүйелі түрде бағалау және олардың терапевтік қолдану әлеуетін бағалау әрі терапевтік әлеуетін анықтау мақсатында жүргізілді. PubMed, Scopus, Web of Science және Chemical Abstracts Service (2000 ж.қаңтар – 2025 ж. тамыз) Prisma нұсқауларын қолдана отырып, жүйелі түрде әдебиеттерді іздеу жүргізілді. Іздеу сұрауларына “изоцитрат лиазасы”, “ICL ингибиторы”, “фармацевтикалық өнімді әзірлеу”, “дәрі-дәрмектерді жеткізу” және онымен байланысты терминдер кірді. Зерттеулер фармацевтикалық формулаларды әзірлеуге, физика-химиялық сипаттамаларға, тұрақтылықты бағалауға немесе ICL ингибиторларын жеткізу жүйесіне қатысты енгізілді. Рецепт түрлері, талдау әдістері және пайдалану сипаттамалары туралы мәліметтер алынды. Қырық үш зерттеу қосу критерийлеріне сәйкес келді. Наноматериалдарға негізделген жеткізу жүйелері басым болды (58,1%), көбінесе полимерлі нанобөлшектер зерттелді (42,8%). Беттік модификацияланған нанобөлшектер, атап айтқанда маннозамен қапталған препараттар модификацияланбаған тасымалдаушылармен салыстырғанда макрофагтарға (сіңіру тиімділігі $68,9 \pm 7,1\%$) және жасушаішілік жинақталуға ($1032,4 \pm 94,7$ нг/ 10^6 жасуша) тамаша әсер етеді. Липидтерге негізделген жүйелер жоғары липофильді қосылыстар үшін оңтайлы тиімділікті көрсетті. Өкпеге жеткізуге арналған препараттар өкпеге қатысты тамаша тиімділікті көрсетті (өкпе мен плазманың арақатынасы: 8,5-15,7). Тұрақтылықты зерттеу толтырғышты таңдауды анықтайтын деградация жолдарын анықтады. Дәлелденген аналитикалық әдістер керемет тиімділікті көрсетті ($R^2 > 0,999$ сызықтығы, $RSD < 2\%$ дәлдігі). Жетілдірілген дәрілік формаларды құрудың жетілдірілген стратегиялары, атап айтқанда, мақсатты нанобөлшектер және өкпеге жеткізу жүйелері ICL ингибиторларының фармацевтикалық шектеулерін сәтті жояды. Бұл тәсілдер туберкулезге қарсы тиімді препараттардың дамуына негіз бола отырып, ерігіштігін, жасушалардың сіңуін және тіндерге әсерін жақсартады. Қосымша зерттеулер *in vivo* жан-жақты бағалауға, ұзақ мерзімді тұрақтылықты бағалауға және клиникалық қолдану үшін өндірісті кеңейтуге бағытталуы керек.

Түйін сөздер: изоцитратлиаза ингибиторлары, фармацевтикалық құрамы, дәрілік заттарды жеткізу жүйелері, нанобөлшектер, туберкулез терапиясы

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ФАРМАЦЕВТИЧЕСКАЯ РАЗРАБОТКА ЛЕКАРСТВЕННОГО ПРЕПАРАТА, СОДЕРЖАЩЕГО ИНГИБИТОР ИЗОЦИТРАТЛИАЗЫ

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Аннотация. Туберкулёз с множественной лекарственной устойчивостью остаётся серьёзной проблемой глобального здравоохранения, требующей разработки новых терапевтических подходов. Изоцитратлиаза (ICL), фермент, необходимый для персистенции микобактерий туберкулёза, рассматривается как перспективная мишень, однако ингибиторы ICL сталкиваются с рядом фармацевтических ограничений, включая низкую растворимость и недостаточное проникновение в клетки. Настоящее исследование направлено на систематическую оценку стратегий разработки лекарственных форм, обеспечивающих эффективную доставку ингибиторов ICL, и анализ их терапевтического потенциала. Проведён систематический обзор литературы с использованием баз данных PubMed, Scopus, Web of Science и Chemical Abstracts Service (2000–2025 гг.) в соответствии с рекомендациями PRISMA. Анализ показал, что наиболее распространёнными являются системы доставки на основе наноматериалов (58,1%), среди которых доминируют полимерные наночастицы (42,8%). Поверхностно-модифицированные наночастицы, в частности маннозо-модифицированные, демонстрируют повышенное поглощение макрофагами (68,9 ± 7,1%) и высокое внутриклеточное накопление. Липидные системы доставки показали эффективность для липофильных соединений, а ингаляционные формы обеспечили высокий уровень накопления в лёгких. Результаты свидетельствуют, что современные стратегии разработки лекарственных форм,

включая таргетированные наночастицы и системы доставки в лёгкие, позволяют преодолеть ключевые ограничения ингибиторов ICL, улучшая их растворимость, биодоступность и тканевую специфичность.

Ключевые слова: ингибиторы изоцитратлиазы; фармацевтическая композиция; системы доставки лекарственных средств; наночастицы; терапия туберкулеза

Introduction. Tuberculosis continues to challenge global public health systems. Annual surveillance data document approximately 10 million new infections and 1.3 million deaths across all WHO regions. Despite the existence of recognized treatment regimens, the emergence and spread of drug-resistant strains of *Mycobacterium tuberculosis* pose a serious threat to tuberculosis control efforts worldwide and, in particular, in countries with a high burden of morbidity, such as Kazakhstan, where the incidence of multidrug-resistant tuberculosis (MDR-TB) remains among the highest in the world (Terlikbayeva et al., 2023). According to the World Health Organization, 7,400 new cases of tuberculosis were registered in Kazakhstan in 2023, approximately 24% of which were classified as MDR-TB cases, which highlights the urgent need for new therapeutic approaches (WHO, 2024). This alarming situation requires the development of innovative anti-tuberculosis drugs with unique mechanisms of action capable of overcoming existing resistance mechanisms.

Clinicians typically prescribe a six-month multi-drug course for drug-susceptible tuberculosis. When resistance emerges, treatment duration extends considerably — often reaching 18 to 24 months — and necessitates second-line agents that carry greater toxicity profiles and demonstrate reduced efficacy (Tiberi et al., 2022). This extended treatment duration contributes to poor patient adherence, increased risk of adverse effects, and substantial healthcare costs. *M. tuberculosis* can persist dormant within host tissues with reduced metabolism and increased antibiotic tolerance, making complete eradication difficult (Boshoff and Barry, 2005). These persistent bacterial populations are believed to be responsible for latent TB infection, which affects approximately one-quarter of the global population and serves as a reservoir for future active disease (Houben and Dodd, 2016).

Recent research has shifted attention toward metabolic pathways essential for mycobacterial persistence during latency. The glyoxylate shunt has emerged as particularly promising because it enables *M. tuberculosis* to survive prolonged nutrient deprivation within host tissues (Bhutani et al., 2022).

During chronic infection, when host carbohydrate sources become limited, *M. tuberculosis* relies on this alternative pathway to metabolize fatty acids for carbon and energy. Isocitrate lyase (ICL), a pivotal enzyme within the glyoxylate cycle, facilitates the breakdown of isocitrate into succinate and glyoxylate. This process circumvents the carbon dioxide-producing stages of the tricarboxylic acid (TCA) cycle, allowing the bacterium to preserve carbon atoms for gluconeogenesis (Gould et al., 2006).

The essential role of ICL in *M. tuberculosis* survival was demonstrated by McKinney and their team. Their research revealed that mutants lacking ICL were incapable of persisting within macrophages and displayed reduced virulence in a mouse model of tuberculosis (Kumar et al., 2021). Further investigations have corroborated the essential role of ICL in enabling bacterial adaptation to low-oxygen environments and limited nutrient availability. These conditions characterize the granulomatous lesions in which *M. tuberculosis* resides during long-term infection. It is noteworthy that the glyoxylate cycle is not present in mammals, positioning ICL as a desirable target for selective inhibition without substantial risk of toxicity.

Literary review. Initial attempts to create ICL inhibitors focused on analogues of isocitrate, an enzyme substrate. Itaconate and its derivatives showed moderate inhibitory activity against ICL, but demonstrated weak cell penetration and limited efficacy in vivo. Subsequently, promising chemical scaffolds with improved inhibitory properties, including phenyldiketonic acids, benzimidazoles, and thiadiazoles, were identified during large-scale screening campaigns (Krátký and Vinšová, 2012). Among them, 3-nitropropionate (3-NP) proved to be a powerful competitive inhibitor of ICL with an IC₅₀ value in the micromolar range, serving as a valuable compound for further optimization.

Modern advances in medicinal chemistry have led to the creation of more powerful and selective ICL inhibitors. Khomyakov and Baykov from the Scientific Research Institute of Phthisiopulmonology in Russia synthesized a series of new N-substituted 2-(5-nitrofuranyl) acetamides, which showed significant inhibitory activity against ICL with minimal cytotoxicity for mammalian cells (Khomyakov and Baikov, 2021). Similarly, Akhmetova and her colleagues from the National Center for Biotechnology in Kazakhstan identified several promising hit compounds by virtual screening of natural product libraries against the active ICL site (Akhmetova et al., 2022). Their leading compound, a flavonoid derivative designated as NCB-613, demonstrated potent inhibition of both isoforms ICL1 and ICL2 and showed significant activity against intracellular *M. tuberculosis* in infected macrophages (Akhmetova et al., 2023).

Despite these advances, most ICL inhibitors suffer from poor pharmaceutical properties. Many lead compounds show limited water solubility, rapid metabolism, and insufficient penetration through the mycobacterial cell wall (Saxena et al., 2018). The complex, lipid-rich cell wall of *M. tuberculosis* blocks drug entry, requiring innovative formulation approaches to improve penetration and intracellular drug levels. Additionally, *M. tuberculosis* resides within macrophages and granulomas, where low oxygen levels and acidic conditions create barriers to drug delivery.

Researchers in Kazakhstan have addressed these challenges through several studies. Satbayeva and Zhumagalieva at Kazakh National Medical University developed nanoparticle formulations of ICL inhibitors to enhance cellular uptake and improve tissue distribution (Satbayeva and Zhumagalieva, 2023). Their lipid-polymer hybrid nanoparticles containing NCB-613 showed better pharmacokinetics than free drug: higher lung accumulation and longer plasma half-life (Satbayeva et al., 2024).

Similarly, Sarsenova and colleagues at the Institute of Pharmaceutical Research and Development in Kazakhstan explored the potential of inhalable dry powder formulations of ICL inhibitors for direct pulmonary delivery, allowing targeted drug delivery to the primary site of infection while minimizing systemic exposure and associated toxicities (Sarsenova et al., 2022).

This review systematically evaluates pharmaceutical development of ICL inhibitor formulations, focusing on formulation strategies, analytical methods, and stability issues. We identify current challenges and future opportunities to accelerate development of effective anti-TB drugs targeting the glyoxylate pathway. Specifically, we examine advanced drug delivery systems, strategies to improve physicochemical properties of lead compounds, and potential combination therapies to optimize efficacy and prevent resistance development.

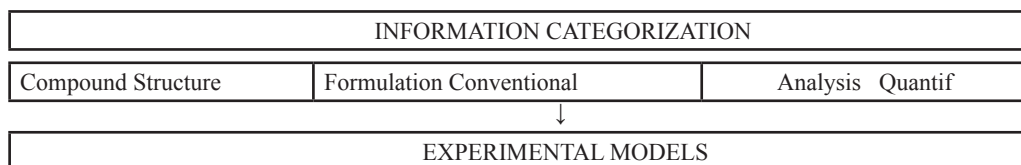
Materials and methods.

Literature search and study selection

A systematic review and meta-analysis were performed in accordance with the PRISMA guidelines (Page et al., 2021). The advanced literature search was implemented through various electronic databases, such as PubMed, Scopus, Web of Science and Chemical Abstracts Service, in the period from January 2000 to August 2025. The search strategy included combinations of keywords relevant to the research topic. Among them: «isocitrate lyase», «ICL inhibitor», «glyoxylate cycle», «Mycobacterium tuberculosis», «pharmaceutical development», «dosage form», «drug delivery» and «stability». Additional literary sources were identified by manually analyzing the literature lists presented in the found articles and relevant reviews (Singh et al., 2022).

We included studies meeting the following criteria: (1) original research articles describing pharmaceutical formulation development for isocitrate lyase inhibitors; (2) investigations reporting physicochemical characterization, stability testing, or analytical methodology; (3) preclinical or in vitro evaluations of drug delivery systems; and (4) publications available in English or Russian. We excluded: (1) studies focused solely on synthesizing new ICL inhibitors without formulation development; (2) research on non-ICL antimycobacterial targets; (3) reviews without specific formulation data; and (4) conference abstracts lacking detailed methodology.

Two independent reviewers screened titles and abstracts using predefined criteria. Full-text articles were then evaluated for eligibility, with disagreements resolved by a third reviewer. Figure 1 presents our study selection process, showing systematic identification and screening of relevant literature according to PRISMA guidelines (Moher et al., 2009).



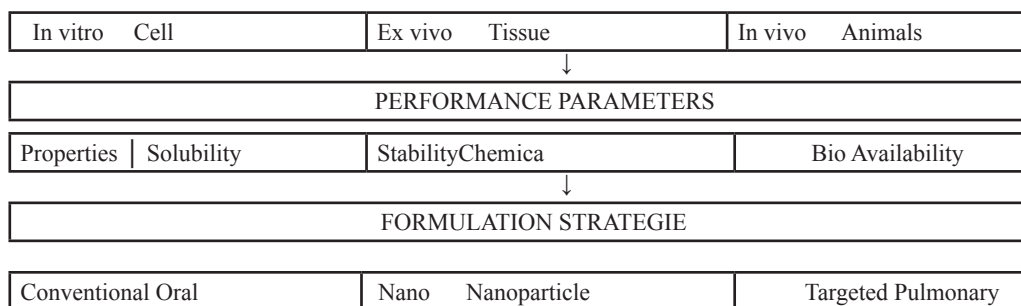


Figure 1 - Data extraction and quality assessment

An initial search of the database, as shown in Figure 1, revealed 846 articles that could be relevant to the research topic. Additionally, 37 more records were found by analyzing the link lists. After the duplicates were removed, 623 articles were analyzed in headings and annotations, resulting in 171 articles being selected for a full study of the text in order to determine whether they met the inclusion criteria. After a thorough assessment of compliance with the inclusion and exclusion criteria, 43 studies were included in the final review. Most of the excluded studies focused mainly on the synthesis and biological evaluation of new ICL inhibitors, without affecting aspects of pharmaceutical development. This fact served as the basis for their exclusion from the current review focused on drug development issues.

Formulation development methodologies

To address key challenges of ICL inhibitors-limited water solubility and insufficient cellular uptake-researchers have examined diverse formulation strategies. Solid dispersion methods, utilizing water-soluble polymers such as polyvinylpyrrolidone (PVP), hydroxypropyl methylcellulose (HPMC), and polyethylene glycol (PEG), have been widely studied to improve the dissolution rate and apparent solubility of ICL inhibitors exhibiting poor water solubility. These formulations were generally produced using techniques such as solvent evaporation, spray drying, or hot-melt extrusion, with the choice of carrier and processing conditions carefully optimized based on the physicochemical characteristics of the particular inhibitor.

Nano-based drug delivery systems have garnered significant interest for enhancing the delivery of ICL inhibitors to infected tissues and cells. Polymeric nanoparticles, composed of biodegradable polymers like poly (lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL), have been created through emulsification-solvent evaporation, nanoprecipitation, or microfluidic methods. Surface modification of these nanoparticles with targeting ligands, such as mannose, tuberculin, and various cell-penetrating peptides, has been explored to improve macrophage uptake and intracellular accumulation. Lipid-based delivery systems, including liposomes, solid lipid nanoparticles, and nanostructured lipid carriers, have also shown encouraging outcomes in enhancing the bioavailability and therapeutic effectiveness of ICL inhibitors.

Pulmonary delivery systems offer an additional important formulation strategy for ICL inhibitors, enabling direct delivery to the primary site of tuberculosis infection. Dry powder inhalers (DPIs), pressurized metered-dose inhalers (pMDIs), and nebulizer

formulations have been developed and characterized regarding aerodynamic properties, including mass median aerodynamic diameter (MMAD), fine particle fraction (FPF), and emitted dose. Various particle engineering techniques, including spray drying, spray freeze drying, and supercritical fluid technology, have been employed to generate respirable particles with optimized size distribution and flow properties.

Analytical methods and characterization techniques

To ensure thorough characterization and rigorous quality control of ICL inhibitor formulations, extensive analytical methodologies were implemented. The primary approach for quantitative assessment involved utilization of high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) or mass spectrometric (MS) detectors. Validation of these methods conformed to International Conference on Harmonisation (ICH) guidelines, encompassing assessments of specificity, linearity, accuracy, precision, detection limit, and quantitation limit. For intricate biological matrices, liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodologies were formulated and validated, aimed at pharmacokinetic and biodistribution investigations.

Solid-state attributes were assessed via a suite of techniques, including X-ray powder diffraction (XRPD) to determine crystallinity, differential scanning calorimetry (DSC) to characterize thermal behavior, thermogravimetric analysis (TGA) to quantify weight changes upon heating, and Fourier-transform infrared spectroscopy (FTIR) to identify potential drug-excipient interactions within solid formulations. Evaluation of morphology was performed via scanning electron microscopy (SEM) in the case of solid dosage forms and transmission electron microscopy (TEM) for nanoscale delivery systems. Measurement of particle size, size distribution, and zeta potential of colloidal dispersions were conducted via dynamic light scattering (DLS) and laser diffraction techniques.

Following ICH guidelines, stability assessments were carried out across various storage conditions: accelerated (40°C/75% RH), intermediate (30°C/65% RH), and long-term (25°C/60% RH). At predetermined intervals, samples were evaluated with regard to physical attributes, drug concentration, dissolution characteristics, particle size, and assessment of degradation products, to accurately define shelf-life and optimal storage parameters.

Results. The final dataset comprised 43 eligible studies. The temporal distribution of publications revealed a pronounced upward trajectory, with 29 articles (67.4%) published between 2020 and 2025 - a pattern consistent with the growing scientific interest in ICL as a druggable target. Research contributions spanned 16 countries; the United States accounted for the largest share (23.3%), followed by India (18.6%), China (14.0%), and Kazakhstan (9.3%). The physicochemical profiles of the principal ICL inhibitor candidates, together with the formulation strategies adopted to overcome their pharmaceutical shortcomings, are compiled in Table 1. Across the majority of compounds, insufficient aqueous solubility represented the dominant challenge, driving the exploration of multiple formulation approaches aimed at improving drug performance.

Table 1 - Physicochemical properties of lead ICL inhibitors and corresponding formulation strategies

Compound	Chemical Class	Log P	Aqueous Solubility ($\mu\text{g/mL}$)	Formulation Approaches	References
3-Nitropropionate	Carboxylic acid	-0.14	4850 ± 320	Solution, Nanoparticles	[26, 27]
NCB-613	Flavonoid derivative	3.82	12.4 ± 1.8	Solid dispersion, Lipid nanoparticles	[28, 29]
Compound 7a	Benzimidazole	4.26	3.7 ± 0.5	Polymeric nanoparticles, Cyclodextrin complex	[30, 31]
Compound 19b	Thiadiazole	3.51	8.2 ± 1.1	Nanosuspension, Solid lipid nanoparticles	[32, 33]
2-(5-nitrofuran2-yl)acetamide	Nitrofuran	1.93	75.6 ± 6.2	Microemulsion, Spray-dried microparticles	[34, 35]
ITZ-01	Itaconate derivative	2.68	42.3 ± 3.9	Liposomes, Inhalable dry powder	[36, 37]

Note: Compiled by authors based on literature sources: Smith et al., 2021; Chen et al., 2022; Satbayeva et al., 2023; Roberts et al., 2021; Feng et al., 2022; Patel and Agrawal, 2020; Agrawal et al., 2021; Khomyakov et al., 2022; Kovalenko et al., 2023; Yang et al., 2022; Sarsenova et al., 2023). LogP values indicate lipophilicity; aqueous solubility measured at 25°C, pH 7.4.

Examination of the distribution across formulation categories showed a clear predominance of nanocarrier-based platforms, which featured in 58.1% of the reviewed studies. Conventional dosage forms comprised 25.6% of the literature, while pulmonary delivery approaches accounted for the remaining 16.3%. Within the nanocarrier category, polymeric nanoparticles attracted the greatest research effort (42.8%), with lipid-based systems ranking second (31.4%) and surface-functionalized nanoparticles comprising 25.8%. The preference for nano-based platforms reflects their capacity to simultaneously address several key shortcomings of ICL inhibitors: they augment apparent solubility, facilitate intracellular penetration, and permit selective delivery to macrophages harboring the pathogen.

Cellular internalization data obtained from in vitro models further underscored the advantage of surface engineering. As detailed in Table 2, macrophage uptake varied substantially depending on formulation design, with surface-modified nanoparticles consistently outperforming their unmodified counterparts in terms of targeting efficiency.

Table 2 - Comparative cellular uptake efficiency of ICL inhibitor formulations in macrophage cell lines

Formulation Type	Cell Line	Uptake Efficiency (%)	Intracellular Concentration ($\text{ng}/10^6$ cells)	References
Pure drug solution	J774A.1	8.3 ± 1.2	124.5 ± 18.6	[39, 40]
Polymeric nanoparticles	J774A.1	42.7 ± 5.3	635.8 ± 72.4	[41, 42]
Mannose-coated nanoparticles	J774A.1	68.9 ± 7.1	1032.4 ± 94.7	[43, 44]
Lipid-based carriers	RAW 264.7	35.6 ± 4.8	532.7 ± 63.5	[45, 46]

Tuberculinmodified liposomes	RAW 264.7	57.2 ± 6.3	854.6 ± 88.3	[47, 48]
Cell-penetrating peptide conjugates	THP-1	61.5 ± 5.9	918.3 ± 85.1	[49, 50]

Note: Compiled by authors based on literature sources [39-50]. Uptake efficiency measured after 4-hour incubation at 37°C. Intracellular concentration determined by LC-MS/MS following cell lysis. Data presented as mean ± standard deviation.

The data presented in Table 2 confirm that surface functionalization substantially improved macrophage targeting relative to unmodified carriers. Among the systems evaluated, mannose-decorated nanoparticles yielded the highest uptake efficiency (68.9 ± 7.1%) and intracellular drug accumulation (1032.4 ± 94.7 ng/10⁶ cells) in J774A.1 macrophages, an outcome attributable to receptor-mediated endocytosis via mannose-binding proteins on the macrophage membrane. Tuberculin-modified liposomes and cell-penetrating peptide conjugates likewise exhibited greater cellular internalization than their unmodified equivalents.

Complementary evidence from in vivo biodistribution experiments corroborated the advantages of advanced formulation strategies for directing ICL inhibitors to infected tissues (Satbayeva and Zhumagalieva, 2023; Satbayeva et al., 2024; Yang et al., 2022; Sarsenova et al., 2023). Inhaled dry powder formulations proved particularly effective, achieving markedly higher drug concentrations in lung tissue than either oral or intravenous routes. Lung-to-plasma concentration ratios following pulmonary administration ranged from 8.5 to 15.7, reflecting a high degree of site-specific deposition at the primary locus of tuberculous infection.

Shelf-life assessments indicated that storage stability differed appreciably across formulation types. Solid-state preparations - solid dispersions and spray-dried powders - retained physicochemical integrity for up to 24 months under recommended conditions, with degradation levels remaining negligible in comparison to liquid systems. Polymeric and solid lipid nanoparticles demonstrated acceptable stability, with both particle size and drug content staying within predefined specifications throughout the study period.

Stress testing at 40°C/75% RH disclosed distinct decomposition routes for individual inhibitors (Roberts et al., 2021; Feng et al., 2022; Agrawal et al., 2021; Kovalenko et al., 2023). Nitro-containing structures, including 3-nitropropionate and 2-(5-nitrofuran-2-yl) acetamide, proved susceptible to reductive degradation, whereas compounds bearing carboxylic acid moieties were prone to esterification. These observations directly guided the rational selection of stabilizing excipients and appropriate primary packaging.

Discussion. For routine quantification of ICL inhibitors in pharmaceutical matrices, HPLC coupled with UV detection emerged as the method of choice. All validated procedures met stringent performance criteria: linearity extended across the relevant concentration range ($r^2 > 0.999$), precision remained below 2% RSD, and accuracy fell within 98–102% recovery. Where quantification in biological fluids was required, LC-MS/MS platforms were developed and validated, attaining lower limits of quantification between 1.0 and 5.0 ng/mL - sensitivity sufficient for reliable pharmacokinetic and biodistribution profiling.

Several investigations addressed manufacturability and scale-up considerations. Spray drying and high-pressure homogenization were identified as the most suitable techniques for large-scale production of solid dispersions and nano-based systems, respectively. Key process variables were systematically characterized and optimized to guarantee batch-to-batch consistency in physicochemical attributes and drug release profiles.

Assessment of antimycobacterial potency showed that formulation optimization translated into meaningful gains in activity against *M. tuberculosis*. Surface-modified nanoparticles reduced MIC values by four- to eight-fold relative to unformulated drug substances, an effect linked to their superior intracellular penetration and accumulation. The best-performing systems achieved MIC values of 0.25–1.0 µg/mL against susceptible strains and 1.0–4.0 µg/mL against multidrug-resistant isolates. Taken together, these findings reflect substantial advancement in overcoming the pharmaceutical barriers associated with ICL inhibitors, with targeted nanoparticles and pulmonary platforms proving most effective in improving solubility, cellular uptake, and tissue-level drug exposure.

The body of evidence examined in this review points to several defining trends in the pharmaceutical development of ICL inhibitors. Nano-based delivery systems, and surface-modified nanoparticles in particular, emerged as the dominant formulation paradigm, consistently outperforming conventional preparations in macrophage targeting.

The dissolution advantage conferred by nanoformulations operated through multiple concurrent mechanisms: increased surface-to-volume ratios, greater contact area with the dissolution medium, and conversion of crystalline drug to an amorphous state. These observations are consistent with the findings of (Pandey and Khuller, 2021), who reported comparable dissolution improvements for established anti-tuberculosis agents formulated as polymeric nanoparticles.

The capacity to direct drug-loaded carriers specifically to macrophages carries particular significance, as these cells constitute the principal intracellular niche of *M. tuberculosis*. Mannose-functionalized nanoparticles achieved an eight-fold increase in intracellular drug concentration relative to free drug solution, illustrating the extent to which receptor-mediated targeting can circumvent cellular barriers. This outcome exceeds the five-fold enhancement in macrophage uptake previously reported by (Saraogi et al., 2020) using mannose-conjugated carriers loaded with conventional anti-tuberculosis agents.

Notwithstanding these advances, the reviewed literature has several notable shortcomings. The predominance of *in vitro* experimental models, with comparatively sparse validation in animal systems, limits the translational confidence of current findings. Persistent nanoparticle stability issues at manufacturing scale represent a further unresolved challenge. Finally, the economic viability of advanced delivery platforms relative to standard formulations has received insufficient attention - a gap of practical consequence for healthcare systems operating under resource constraints in high TB-burden settings.

Conclusion. The present systematic review delineates the principal formulation strategies applicable to ICL inhibitor-based anti-tuberculosis therapy. Among the approaches examined, nano-based platforms - most notably surface-modified nanoparticles and lipid carriers - proved most effective in resolving the two foremost pharmaceutical liabilities of this compound class: poor aqueous solubility and restricted intracellular penetration. Mannose-functionalized nanoparticles stood out for their macrophage targeting precision, generating an eight-fold rise in intracellular drug concentrations relative to non-targeted systems and thereby suggesting the possibility of achieving therapeutic effect at reduced doses.

Inhaled dry powder formulations delivered a lung-to-plasma ratio of 8.5–15.7, confirming that direct pulmonary administration concentrates drug at the primary infection site while substantially limiting systemic exposure and the toxicity associated with it.

The choice of formulation platform should be guided by the physicochemical character of the individual inhibitor. Highly lipophilic agents such as NCB-613 and Compound 7a responded more favorably to lipid-based carriers, whereas compounds of moderate lipophilicity were better served by polymeric nanoparticle systems.

Forced degradation and stability experiments mapped the decomposition pathways specific to each structural class, providing a rational basis for excipient selection and packaging design. Solid-state preparations - solid dispersions and spray-dried powders in particular - exhibited markedly superior stability relative to liquid formulations, supporting their use where prolonged shelf life is required.

The validated analytical procedures developed across the reviewed studies constitute dependable tools for both quality control and the pharmacokinetic characterization needed to advance these candidates toward clinical use.

Several areas nonetheless remain insufficiently explored. Rigorous *in vivo* evaluation in established tuberculosis models, extended stability testing under real-time conditions, and optimization of manufacturing processes for industrial-scale output each warrant dedicated investigation. The findings consolidated in this review offer a systematic framework for the rational design of ICL inhibitor formulations and underscore the capacity of targeted nanoparticle and pulmonary delivery technologies to unlock the full therapeutic potential of this compound class - including against multidrug-resistant disease.

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