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PREDICTING G-QUADRUPLEX INTERACTIONS OF RAPAMYCIN, METFORMIN, METHOTREXATE, AND CX–5461 WITH G4–QUADSCREEN

Introduction

G-quadruplexes (G4) are four-stranded secondary structures formed in guanine-rich DNA and RNA sequences. They are increasingly recognized as key regulators of critical biological processes, including transcription, replication, and genomic stability [1]. Consequently, G4 structures have emerged as promising therapeutic targets, particularly in oncology and, more recently, in the field of aging research [2, 3]. Several drugs, originally developed for other indications, are now being repurposed or investigated for their potential as geroprotectors. Among the leading contenders are the mTOR inhibitor Rapamycin and the AMPK activator Metformin [4, 5]. Other drugs like the antimetabolite Methotrexate and the experimental compound CX–5461, a known G4 stabilizer with potent anticancer activity [6], also present interesting cases for study. Understanding whether these compounds can directly interact with G4 structures could provide new insights into their mechanisms of action and their potential pleiotropic effects on gene expression related to aging and cancer. The G4–QuadScreen tool offers a rapid, computational method to predict the G4–binding potential of small molecules and has been successfully used to analyze other classes of compounds, such as green tea catechins [2, 7].

Goal

To conduct a comparative in silico analysis of the G–quadruplex binding potential of the anti-aging and anticancer compounds Rapamycin, Metformin, Methotrexate, and CX–5461 using the G4–QuadScreen prediction platform.

Material and methods of research

The SMILES strings for Rapamycin, Metformin, Methotrexate, and CX–5461 were obtained from PubChem [8], a resource of the National Center for Biotechnology Information [9]. These SMILES were used as input for the G4–QuadScreen computational tool (https://chemopredictionsuite.com/G4QuadScreenpredictions), a platform developed for predicting multi-target-directed anticancer leads against G-quadruplex DNA [7]. The tool employs machine learning models to predict activity across four screening panels:

1. Mt (Melting Temperature): Predicts the ligand's ability to stabilize a G4 structure, increasing its melting temperature.

2. Kd (Dissociation Constant): Predicts binding affinity to G4 DNA.

3. Mtt (MTT Assay): Predicts cytotoxicity in a panel of human cancer cell lines (HELA, A549, MCF7, A375, HCT116) at 48 and 72 hours.

4. Sel (Selectivity): Predicts binding selectivity for G4 structures over duplex DNA.

For each compound, the tool provides a predicted class (Class_Predicted: 1 for active/binder/toxic, 0 for inactive/non-binder/non-toxic) and an applicability domain (AD: "inside" for reliable prediction, "outside" for less reliable). The results for all four compounds were exported as separate .xlsx files and analyzed comparatively.

The results of the research and their discussion

The G4-QuadScreen predictions for the four compounds are summarized in Table 1. The analysis revealed stark differences in their predicted G4 interaction profiles and cytotoxicity.

Table 1 – Comparative G4–QuadScreen Prediction Results for Rapamycin, Metformin, Methotrexate and CX–5461

Screening Panel	Parameter	Rapamycin	Metformin	Methotrexate	CX–5461
Mt (G4 Stabilization)	LGR 5 / 10	0 / 0	0 / 0	0 / 0	1 (c-MYC, Kras) / 1
Kd (Binding Affinity)	Activity (Class=1)	0	0 (1 for one htelo condition)	1 (for one htelo condition)	1 (htelo, c-kit2)
Mtt (Cytotoxicity)	Active Cell Lines (48h)	None (0/5)	None (0/5)	None (0/5)	All 5 (HELA, A549, MCF7, A375, HCT116)
–	Active Cell Lines (72h)	None (0/5)	None (0/5)	None (0/5)	All 5
Sel (Selectivity over duplex)	Activity (Class=1)	1 (for 2/14 conditions)	1 (for 2/14 conditions)	All (14/14)	All (14/14)

Rapamycin and Metformin showed no predicted ability to stabilize G4 structures in the Mt assay (Class_Predicted=0 across all targets and LGR values) [4, 10]. Their predicted direct binding affinity (Kd) to G4s was also largely negative, with Metformin showing a single positive prediction for an htel sequence under a specific buffer condition. The MTT assay predictions were uniformly negative for cytotoxicity across all five cell lines at both 48 and 72 hours. However, in the Sel assay, both compounds showed sporadic positive predictions for selectivity (Class=1 in 2 out of 14 conditions each). This profile is consistent with their known mechanisms of action, which are primarily protein-mediated (AMPK/mTOR pathways) and suggests that direct G4 interaction is unlikely to be a primary mode of action for their geroprotective effects. The lack of predicted cytotoxicity aligns with their favorable safety profiles in humans, although it does not preclude other, non-cytotoxic effects on cell physiology.

Methotrexate also showed no predicted G4 stabilization (Mt assay) and no cytotoxicity (Mtt assay). It showed one positive binding prediction in the Kd assay for an htel sequence. Most strikingly, it was predicted to be highly selective for G4 structures over duplex DNA, with a Class_Predicted=1 for every single condition in the Sel assay (14/14). Methotrexate is an antimetabolite that inhibits dihydrofolate reductase (DHFR), and its primary mechanism is not thought to involve DNA binding. The strong, consistent prediction for G4 selectivity is a novel finding from this in silico analysis. It raises the question of whether some of methotrexate's well-known side effects, or perhaps even some of its immunomodulatory actions in diseases like rheumatoid arthritis, could be partly mediated by off-target binding to G4 structures in specific gene promoters [1].

CX–5461 exhibited the most pronounced and consistent G4–related activity. It was the only compound predicted to actively stabilize G4 structures in the Mt assay, showing activity against the c-MYC and Kras G4s. It also showed positive binding predictions in the Kd assay for htel and c-kit2. Crucially, CX–5461 was the only compound predicted to be potently cytotoxic, with Class_Predicted=1 for all five cancer cell lines at both time points in the Mtt assay. Furthermore, like Methotrexate, it showed a perfect selectivity score (14/14) in the Sel assay. This profile is in perfect agreement with the known pharmacology of CX–5461 as a first-in-class G4 stabilizer and potent anticancer agent currently in clinical trials. It serves as a positive control for the G4–QuadScreen tool, validating its ability to correctly identify a bona fide G4 binder [3].

Conclusions

The G4–QuadScreen analysis predicts that the primary geroprotective mechanisms of Rapamycin and Metformin are unlikely to involve direct stabilization of G-quadruplex DNA structures.

Methotrexate, despite its known mechanism as a DHFR inhibitor, is predicted to be a highly selective binder of G4 structures, a finding that warrants further experimental investigation.

CX–5461 is robustly predicted by G4–QuadScreen to be a potent, selective G4–stabilizer with broad-spectrum cytotoxicity, confirming its established mechanism of action and validating the computational tool's performance.

This comparative in silico study highlights the utility of computational screening tools like G4–QuadScreen for generating new hypotheses about drug mechanisms and identifying potential polypharmacology that may contribute to both therapeutic and adverse effects [7, 2, 1].

LITERATURE

1. Коваль, А. Н. Гуаниновые квадруплексы в ДНК: новые горизонты для диагностики и терапии рака / А. Н. Коваль, С. М. Сергеенко, Ч. А. Гельдимамедов // Наука и практика: актуальные вопросы, достижения и инновации : сборник статей II Международной научно-практической конференции, Пенза, 30 августа 2024 года. – Пенза : Наука и Просвещение, 2024. – С. 134–136.

2. Коваль, А. Н. Анализ катехинов зеленого чая как стабилизаторов g-квадруплексов ДНК с помощью онлайн-платформы G4–quadscreen / А. Н. Коваль, А. А. Литвин, О. С. Логвинович // Чай в историческом, культурном и медицинском аспектах : Материалы IV научно-теоретической конференции с международным участием, Курск, 15 декабря 2025 года. – Курск : Курский государственный медицинский университет, 2025. – С. 127–130.

3. Вторичные метаболиты растений и митохондриальные G-квадруплексы: терапевтический потенциал в лечении рака / А. Н. Коваль, А. В. Литвинчук, О. С. Логвинович [и др.]. // Современные достижения химико-биологических наук в профилактической и клинической медицине : сборник научных трудов 5-й Международной конференции, посвященной 155-летию со дня рождения профессора Е.С. Лондона, Санкт-Петербург, 05–06 декабря 2024 года. – СПб. : Северо-Западный государственный медицинский университет имени И. И. Мечникова, 2024. – С. 175–180.

4. Roark, K. M. Rapamycin for longevity: the pros, the cons, and future perspectives / K. M. Roark, P. H. Iffland // Frontiers in Aging. – 2025. – Vol. 6. – P. 1628187.

5. Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls / C. A. Bannister [et al.]. // Diabetes, Obesity and Metabolism. – 2014. – Vol. 16, № 11. – P. 1165–1173.

5. CX–5461 is a DNA G-quadruplex stabilizer with selective lethality in BRCA1/2 deficient tumours / H. Xu, M. Di Antonio, S. McKinney [et al.]. // Nature Communications. – 2017. – Vol. 8, № 1. – P. 14432.

6. G4–QuadScreen: a computational tool for identifying multi-target-directed anticancer leads against G-quadruplex DNA / J. Bhat-Ambure, P. Ambure, E. Serrano-Candelas [et al.]. // Cancers. – 2023. – Vol. 15, № 15. – P. 3817.

7. PubChem substance and compound databases / S. Kim [et al.]. // Nucleic acids research. – 2016. – Vol. 44, № D1. – P. D1202–D1213.

8. NCBI Resource Coordinators. Database resources of the National Center for Biotechnology Information // Nucleic Acids Research. – 2018. – Vol. 46, № D1. – P. D8–D13.

9. Topical rapamycin reduces markers of senescence and aging in human skin: an exploratory, prospective, randomized trial / C. L. Chung [et al.]. // Geroscience. – 2019. – Vol. 41, № 6. – P. 861–869.

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PHYSICO-CHEMICAL AND MECHANICAL CHARACTERISTICS OF FULLERENE-CONTAINING POLYMERIC MATERIALS

Introduction

Existing publications confirm that the use of ultrafine materials as fillers, among which carbon clusters – fullerenes – are increasingly playing a prominent role, can be considered as a basis for producing new high-tech materials and drugs for engineering and medicine [1, 2]. The introduction of fullerenes into the polymer matrix provides composites with new properties, such as antiviral and anticancer activity. Fullerenes also possess high magnetic and sorption properties.

Goal

To study the physico-chemical interaction of ultra-high molecular weight polyethylene (UHMWPE) and fullerene filler and evaluate the deformation and strength characteristics of the composites.