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Structure-based design rules for potent quadruplex-binding compounds based on the naphthalene diimide core

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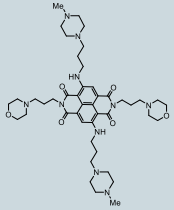
Abstract #3098

G4s are higher-order four-stranded DNA and RNA structures that can be formed by the folding of several G-tracts. G4s comprise a central core of stacked G-quartets held together by loops of variable length and sequence. G4s are over-represented in many cancer genes and can be stabilized by small molecule compounds, resulting in down-regulation of the expression of these genes and ultimately anti-cancer activity. > 3,000 G4 ligands have been reported to date, of which < 5% have in vivo activity. A central dogma of effective G4 ligand binding is a requirement for an extended planar aromatic or heteroaromatic chromophore to stack onto a G-quartet, and cationic side-chains to interact with G4 phosphate groups

We have developed several series of G4 ligands based on the naphthalene diimide (ND) chemotype. Recent lead compounds have high cellular potency and G4 affinity, with anti-cancer activity in several models of human cancers. Crystallographic studies have also been reported by us on eight ND-telomeric G4 complexes. The crystal structures includes several NDs with distinct side chain end groups, all with intramolecular telomeric parallel G4s. End-groups include N-methyl piperazine, hydroxyl, morpholino and N-dimethyl.

We present here a comparison of ND binding modes from 3 of these and from modelling studies on G4-duplex systems, leading to ND design rules & the successful design of the clinical candidate ND compound QN-302 (Ahmed et al., ACS Med Chem Lett, 2020)



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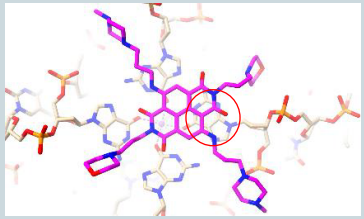

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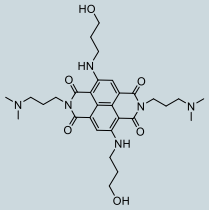

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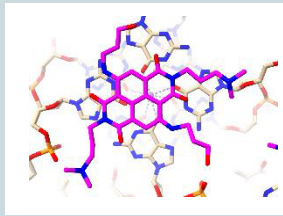

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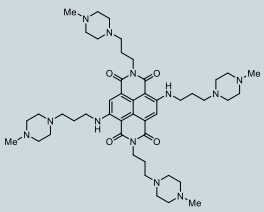

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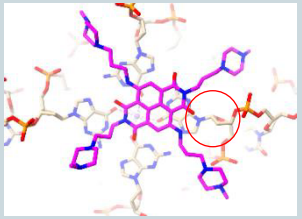


PDB id 4DAQ

The crystal structures show distinct orientations for the ND core. In all of them overlap with the G-quartet is minimal (shown by red circles) and involves 1 out of 4 ND rings at most with 2.5 Å^2 overlap area.

Whole-genome RNA-seq transcriptome analysis of CM03 and QN-302 dosed MIA-Paca2 cells shows that numerous promoter G4s are targeted. It is not known which G4s are critical for biological potency, so G4 binding data on individual G4s is a qualitative indication of cellular G4 response. Note: BMSG-FC-24 has only been tested to A549 cells.

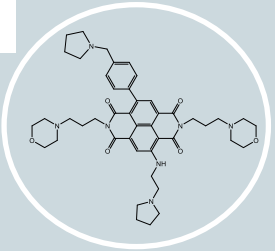
Cell growth inhibition (96 hr SRB, MIA-PaCa2 cells) and G4 telomere binding data for ND compounds. CM03 is a trisubstituted ND

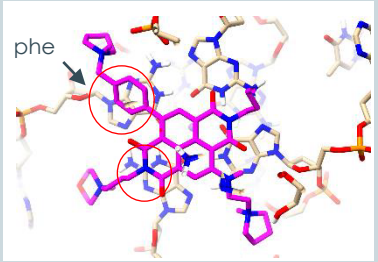
Ligand	IC_{50}, nM	K_d, hTEL nM	ΔTm, G4 hTEL, °C
BMSG-FC-24*	1700	-	23.7
BMSG-SH-3	111	-	23.8
MM41	10	5	26.6
QN-302	**1.3**	**28**	**24.2**
CM03	9.0	82	12.0

The available data indicates that G4 affinity is necessary but insufficient for cellular activity.
The ND core contributes little to G4 affinity. Charged side-chains are important for strong G4 binding **and** cellular activity,
>2 cationic side chains are required for both G4 binding and activity.
Four highly cationic side chains reduce cellular activity but enhance G4 affinity - less basic morpholine groups enhances it.

Molecular modelling suggests that enhancing the ND core with a planar hydrophobic group could increase G4 affinity and enhance cellular uptake
Four basic side chains, two of which are morpholino, are used to optimize the series without excess basicity.
Compound QN-302 is the result.
Docking QN-302 into the G4-duplex structure (using MOLSOFT) indicates that the phenyl substituent is well stacked on a G of the quartet, as predicted.

QN-302





QN-302 docked into PDB 5DWX

QN-302 has high cellular potency, targets G4 sequences in the promoter regions of cancer genes, high anti-tumor activity in xenograft & genetic (KPC) models of PDAC.

QN-302 is bio-available and well tolerated at therapeutic doses in animal models. It is being developed for clinical evaluation by Qualigen Therapeutics Inc. It is currently undergoing GLP toxicity evaluation prior to IND submission. QN-302 was granted Orphan Drug status for PDAC by the FDA in January 2023.