



Structure-based design of quadruplex-binding small molecule compounds: the essential role of water molecules

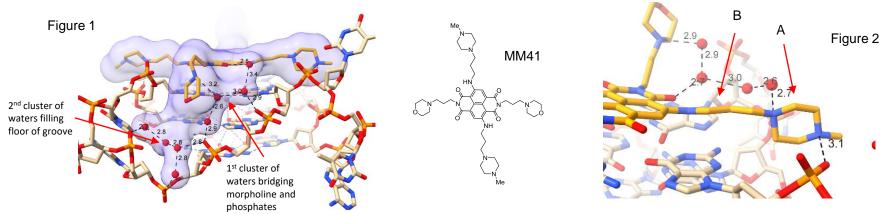
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Abstract # 2926

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Tetra-substituted naphthalene diimide (ND) derivatives have high affinity for human DNA quadruplexes (G4s). We present here the way in which knowledge of the water structure in co-crystal structures with a human G4 has been used in the design of the current lead ND compound, QN-302. This has single-digit 1-20 nM anti-proliferative activity in a panel of human pancreatic cancer (PDAC) cell lines (Marchetti *et al., J. Med. Chem,* 2018, **61**, 2500-2517: Ahmed *et al., ACS Med Chem Lett,* 2020, **11**, 1634-1644) and high anti-tumor activity in a xenograft model for PDAC as well as in PDX models (abstract #4069) and the more demanding KPC model.

A high-resolution crystal structure of a G4 complex with the earlier generation ND compound MM41 (PDB id 3UYH: *Micco et al., J. Med. Chem.*, 2013, **56**, 2959-2974) has been studied. Waters have been found in all four G4 grooves, associated directly or indirectly with the cationic side chains.



Surprisingly only one of the four cationic end groups has its charged nitrogen atom in direct H-bond contact with a phosphate group. Instead, there is in one groove a well-defined cluster of waters mediates between cationic nitrogen atom and phosphate group (1st cluster in Figure 1, around a morpholine group). The rest of the groove is filled by a 2nd water cluster, suggesting that larger substituents cannot readily be accommodated in this groove.

An adjacent groove has an N-methyl piperazine substituent (A), by contrast, oriented along the mouth of the groove and held in place by H bonds to an adjacent phosphate group and a water cluster (Figure 2). The linker group (B) lies on top of a guanine from a terminal G-quartet.

We hypothesized (Neidle, *Pharmaceuticals*, 2022, **15**, 7) that the water arrangements in the grooves, which are also mostly present in the native G4 structure, hold the ligand in position and that only conservative changes to it are possible without disrupting the water networks. Therefore, the extended linker and piperazine group (Figure 2) could be advantageously replaced by

- an aromatic group, enabling stacking on the guanine (A), thereby enhancing G4 affinity
- a suitable cationic group, H-bonding to an adjacent phosphate (B)

Molecular modeling has indicated that these changes are feasible without significant changes to the experimental ligand position. Subsequent SAR data is in accordance with this concept (Ahmed *et al.*, 2020).

One compound, QN-302, resulting from this approach, has enhanced quadruplex affinity, as predicted, together with superior biological potency

QN-302 is currently in pre-clinical development with Qualigen Therapeutics Inc