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Original article

Synthesis and evaluation of ^{18}F-labeled ATP competitive inhibitors of topoisomerase II as probes for imaging topoisomerase II expression

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ABSTRACT

Type II topoisomerase (Topo-II) is an ATP-dependent enzyme that is essential in the transcription, replication, and chromosome segregation processes and, as such, represents an attractive target for cancer therapy. Numerous studies indicate that the response to treatment with Topo-II inhibitors is highly dependent on both the levels and the activity of the enzyme. Consequently, a non-invasive assay to measure tumoral Topo-II levels has the potential to differentiate responders from non-responders. With the ultimate goal of developing a radiofluorinated tracer for positron emission tomography (PET) imaging, we have designed, synthesized, and evaluated a set of fluorinated compounds based on the structure of the ATP-competitive Topo-II inhibitor QAP1. Compounds **18** and **19b** showed inhibition of Topo-II in *in vitro* assays and exhibited moderate, Topo-II level dependent cytotoxicity in SK-BR-3 and MCF-7 cell lines. Based on these results, ^{18}F-labeled analogs of these two compounds were synthesized and evaluated as PET probes for imaging Topo-II overexpression in mice bearing SK-BR-3 xenografts. [^{18}F]-**18** and [^{18}F]-**19b** were synthesized from their corresponding protected tosylated derivatives by fluorination and subsequent deprotection. Small animal PET imaging studies indicated that both compounds do not accumulate in tumors and exhibit poor pharmacokinetics, clearing from the blood pool very rapidly and getting metabolized over. The insights gained from the current study will surely aid in the design and construction of future generations of PET agents for the non-invasive delineation of Topo-II expression.

1. Introduction

Type II topoisomerase (Topo-II) is an ATPase that unravels DNA supercoiling in a very precise fashion through transient double-strand DNA cleavage followed by re-ligation. The enzyme is essential in the transcription, replication, and chromosome segregation processes, and its accurate functioning is a prerequisite for the development of normal mitosis [1–3]. In mammals, two isoforms of Topo-II exist: Topo-IIα (170 kDa) and Topo-IIβ (180 kDa) [4]. Despite a high degree of homology, recent studies have suggested that the two isoforms might play different roles in cellular processes and are regulated very differently. Topo-IIα is expressed in proliferating cells only. This isoform overexpression, a specific and sensitive marker for cell proliferation, has been observed in many types of cancer [5,6] and presents an attractive target for cancer therapy. Topo-IIβ, on the other hand, is expressed at lower levels at steady state levels during all stages of cell cycle.

Overall, inhibitors of Topo-II can be divided into two broad classes: poisons and catalytic inhibitors. Members of the first class interfere with Topo-II function by binding to and stabilizing the DNA–Topo-II complex, ultimately promoting the formation of extremely toxic double-strand breaks. The discovery of topoisomerase poisons has been tapped effectively in the development of several clinically successful chemotherapeutics [4,7–9].

Abbreviations: DAST, diethylaminosulfur trifluoride; DCM, dichloromethane; DHP, dihydroyrane; DMF, dimethylformamide; DMSO, dimethylsulfoxide; ESI, electrospray ionization; GI, gastrointestinal; MS, mass spectrometry; NMR, nuclear magnetic resonance; PET, positron emission tomography; p.i, post-injection; PTSA, para-toluenesulfonic acid; QAP1, quinoline aminopurine 1; RP-HPLC, reversed phase high-performance liquid chromatography; RT, room temperature; SARs, structure–activity relationships; SNAr, aromatic nucleophilic substitution; TBAF, tetra-n-butylammonium fluoride; TFA, trifluoroacetic acid; THF, tetrahydrofurane; THP, tetrahydropyrane; TLC, thin layer chromatography; Topo-II, type II topoisomerase; UV, ultra-violet.

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Anthracyclines, for example, represent one of the most successful classes of topoisomerase poisons and are widely used in the treatment of breast cancer. Catalytic inhibitors of Topo-II, on the other hand, are a heterogeneous group of compounds with antineoplastic activity that act on various steps in the catalytic cycle [10]. These compounds might interfere with the binding of Topo-II to DNA, stabilize non-covalent DNA–topoisomerase II complexes, or prevent the catalytic turnover by inhibiting ATP binding [10,11]. The currently used Topo-II-targeted anticancer drugs are not specific to an isoform and inhibit both Topo-IIα and Topo-IIβ.

While inhibitors of Topo-II — both poisons and catalytic inhibitors — have proven to be effective anti-neoplastic drugs, several independent studies have established that the sensitivity of tumors to Topo-II inhibitors is highly dependent on both the expression and activity levels of the enzyme, and that lower Topo-II levels in tumors minimizes drug efficacy [5,12–17]. Currently, however, this information can be obtained only through biopsies. Therefore, it follows that there is an urgent need to develop non-invasive imaging methods that can provide this vital information without the risk, discomfort, and unreliability of invasive procedures. Indeed, in this regard, positron emission tomography (PET) represents an extremely attractive route. Over the past twenty years, PET and single photon emission computer tomography (SPECT) have revolutionized the field of diagnostic imaging by allowing clinicians to visualize biomarkers that provide functional information about tumor biology. In the case at hand, a PET radiotracer capable of the non-invasive delineation of the levels of Topo-II expression or activity in a tumor would be a powerful tool as a prognostic indicator of response to the Topo-II inhibitor therapy.

We have been interested in evaluating the efficacy of topoisomerase II targeted probes as non-invasive markers for imaging Topo-II expression levels. Previously, we investigated the potential of ^{64}Cu-radiolabeled thiosemicarbazone complexes as Topo-II expression probes and provided proof-of-concept that Topo-II-rich tumors could be visualized via PET using these radiotracers [18]. To date, this remains the only successful report of the use of PET agents for Topo-II delineation in tumor. However, the pharmacokinetic properties of the lipophilic copper complexes were suboptimal and led to high non-target tissue uptake. Additionally the exact mechanism of these complexes in targeting the Topo-II enzyme remains unclear and therefore complicates the interpretation of images.

More recently, as part of our continuing efforts toward the noninvasive delineation of Topo-II expression *in vivo*, we reasoned that a radiolabeled agent that is rationally designed to specifically bind to the ATP-binding pocket of Topo-II has potential to provide a direct measurement of Topo-II expression levels in the tumor. Following the identification of substituted purine derivatives as a novel structural class of catalytic topoisomerase II inhibitors, Furet et al. recently used a structure-based approach to rationally design a purine scaffold that acts as a ATP competitive inhibitor of Topo-II [11,19,20]. Quinoline aminopurine 1 (QAP1, Fig. 1) and its benzothiazole derivatives were described as first representatives of this new class of ATP competitive inhibitors. QAP1 was reported to have micromolar affinity for the enzyme with a fairly good selectivity over various protein kinases, and represents a promising starting point for lead optimization campaigns and further drug discovery efforts [20]. QAP1 inhibits topoisomerase ATPase activity as well as the decatenation reaction and targets both alpha and beta isoforms in cell-free assays. The ability of QAP1 to antagonize doxorubicin induced DNA damage and aberrant chromosome segregation further provides evidence that QAP1 is a catalytic inhibitor of Topo-II rather than a poison. [20,21]

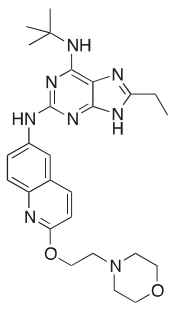
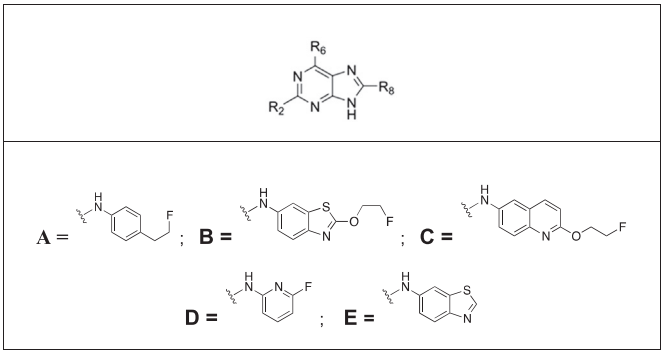


Fig. 1. The structure of QAP1.

Table 1
Structures of fluorinated purine derivatives.



Compound	R_2	R_6	R_8
18	A	*t*BuNH–	H
19b	B	*t*BuNH–	H
20	B	*t*BuNH–	Et
21b	B	CYNH–	H
22b	C	*t*BuNH–	H
23b	C		Et
24b	D	*t*BuNH–	H
25b	E	$F(CH_2)_2O$–	H

The aim of this work is to identify fluorinated purine analogues that function as potent Topo-II inhibitors and have potential to be developed as radiotracers for the non-invasive assessment of Topo-IIα overexpression levels in the tumors. In addition, these probes have the potential to provide information on the pharmacokinetics and tissue biodistribution of these new Topo-II catalytic inhibitors. Herein, we describe the radiosynthesis and *in vitro* studies on fluorinated purine derivatives as Topo-II ATP competitive inhibitors and their *in vivo* evaluation as PET tracers for imaging Topo-II expression.

2. Results and discussion

2.1. Design of the compounds

The 2,6-diaminopurine moiety is critical for optimal binding of the molecule to the ATP binding site of Topo-II, whereas the aryl moiety sits outside the binding pocket and is amenable for modification [11]. Therefore this aryl group was chosen for derivatization and installation of fluorine substituent [20]. The aryl group is ideal for substitution because it does not disrupt the binding pocket interactions and is oriented away from important amino acid residues