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Novel fluorene/fluorenone DNA and RNA binders as efficient non-toxic ds-RNA selective fluorescent probes

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ABSTRACT

A series of structurally similar 1-substitued heteroaryl fluorene derivatives were prepared in a simple single step reaction, oxidized to fluorenones and then both, fluorenes and fluorenones, were methylated to enhance the solubility and increase the affinity to DNA/RNA. Interactions of both, fluorene and fluorenone analogues with various ds-DNA, ds-RNA revealed strong ds-DNA/RNA binding, and various thermal stabilization effects. Most intriguingly, some fluorene derivatives showed opposite fluorescence change (increase for ds-RNA and decrease for ds-DNA), which was not previously reported for any fluorene analogue. CD experiments along with other methods support ds-DNA minor groove binding and major groove ds-RNA binding. All compounds showed negligible interaction with G-quadruplex DNA. Very low cell cytotoxicity of studied compounds combined with very efficient cellular uptake makes these fluorescent dyes safe for laboratory applications. Moreover, especially compounds which show opposite fluorescence response to ds-DNA and ds-RNA, are promising lead compounds for further studies aimed toward ds-RNA-specific fluorescence markers.

1. Introduction

Molecular visualization and recognition of DNA and RNA are important areas of ongoing research in medicine and molecular biology. However, many small molecular probes are also used as therapeutics (so called Theragnostic Molecular Tools); for instance as effective anticancer, antibiotic and antiviral therapeutic agents.[1] Aside from naturally abundant ds-DNA (double stranded DNA), many other DNA structures were discovered (i-motif, triplex and G-quadruplex (G4) structures), becoming novel targets for drugs, for instance in cancer chemotherapy.[2] The G-quadruplex DNA structures have generated a huge research interest because there is a large number of guanine rich sequences with potential to form G-quadruplex structures at critical locations in the human genome: for instance telomeres and oncogenic promoter regions.[3] Further on, ds-RNA-small molecule interactions were, for a long time, under-investigated. However lately these interactions are becoming popular in therapeutic applications due to the involvement in many genetic pathways and human diseases.[4] Recently, the fluorene/fluorenone-containing compounds have gained some attention due to their potential application as chemotherapeutic agents in cancer therapy.[5] For instance, the commercial fluorenone-derivative Tilorone (Fig. 1, **A**) binds to DNA by intercalation but also induces interferon excretion.[6] Its close analogues are currently the focus of intensive research and biological evaluation.[7] Fluorenone-alkyl amines were also tested for their antimicrobial activity.[8] In respect to specific DNA sequences, some fluorenone derivatives showed intriguing activity as inhibitors of human telomerase.[9] Also several different series of fluorenones showed applications in G-quadruplex binding.[10] As close analogues to fluorenones, fluorene derivatives (Fig. 1, **B**) were also tested for biological impact; for instance as minor groove binders in certain DNA sequences.[11] A series of bis-guanidine and *N*-alkyl bis-guanidine fluorene derivatives acted as DNA minor groove binders and showed intriguing in vitro and in vivo activity.[12] Some very similar fluorene analogues showed intriguing ability to bind to the minor groove of certain DNA sequences and intercalate to others.[11] Very recently, fluorene

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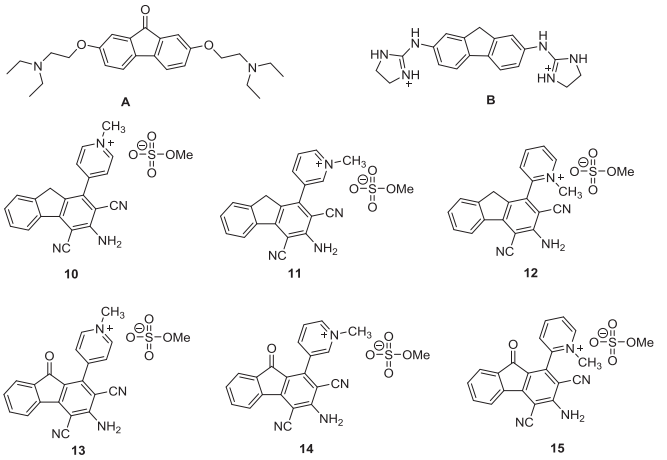


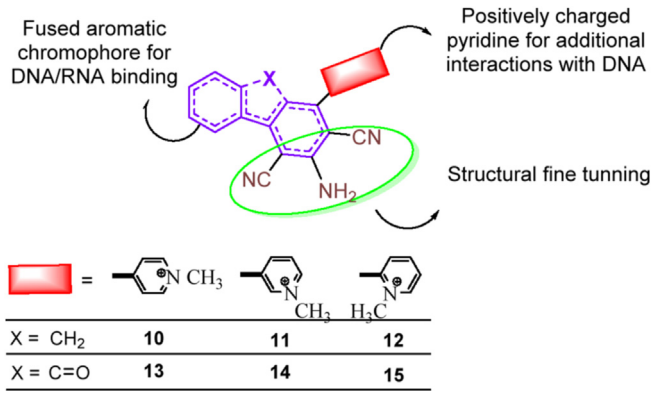
Fig. 1. Structures of known DNA binders with fluorene/fluorenone ring: **A** Tilorone (DNA Intercalator) **B** Minor groove binder. Here studied cationic fluorene/fluorenone derivatives **10**–**15**.

derivatives were reported to be inhibitors of RNA Polymerase I, with promising properties as cancer therapeutics.[13] Fluorene/fluorenone derivatives are also used as fluorescent labels for various DNA constructs.[14]

Within the last two decades we have studied many different aromatic moieties for their interactions with DNA/RNA and possible applications in DNA/RNA recognition, spectrophotometric sensing of various sequences and also for in vitro biological activity. Our research also showed intriguing photochemical approach to DNA-binding bioactive fluorenes over DNA-inactive precursors.[15]

To explore in more detail their structural features, we decided to design and prepare a series of novel compounds based on a simple one-pot synthesis approach.[16] We have applied this approach to 1-heteroaryl substituted fluorene moiety, decorated by a variety of functional groups with potential impact on DNA/RNA binding and spectrophotometric sensing (Scheme 1; -NH_2, -CN, pyridinium). Furthermore, the fluorene derivatives were oxidized to related fluorenone compounds, thus yielding a series of close analogues with altered spectrophotometric and also DNA/RNA binding properties (keto-group offering additional H-bonding acceptor). The solubility in water was ensured by methylation of pyridine heterocyclic nitrogen, which as a permanent positive charge, also served as an additional binding possibility for negatively charged DNA/RNA backbone. Cyano-substituents were chosen for several reasons: As strong electron withdrawing groups they influence the chromophore absorption and emission properties by modulating the fluorescence response. Cyano-groups (nitrile) are pharmacophores and moreover could in future development be easily converted into other chromogenic and pharmacogenic groups (amines, amidines etc.). Contrary to the cyano-group, the amino group is strongly electron-donating, thus in one aromatic moiety we have a D-A-D effect, also modulating spectroscopic properties.

We have investigated the spectroscopic response of new compounds for structurally different double-stranded DNA and RNA under biologically relevant conditions. For these studies, we have chosen synthetic polynucleotides to explore interactions between novel fluorene/fluorenone derivatives (**10**–**15**, Fig. 1) and various, well defined DNA or RNA secondary structures. This also included the G-quadruplex forming F21T (fluorescein labelled) sequence.

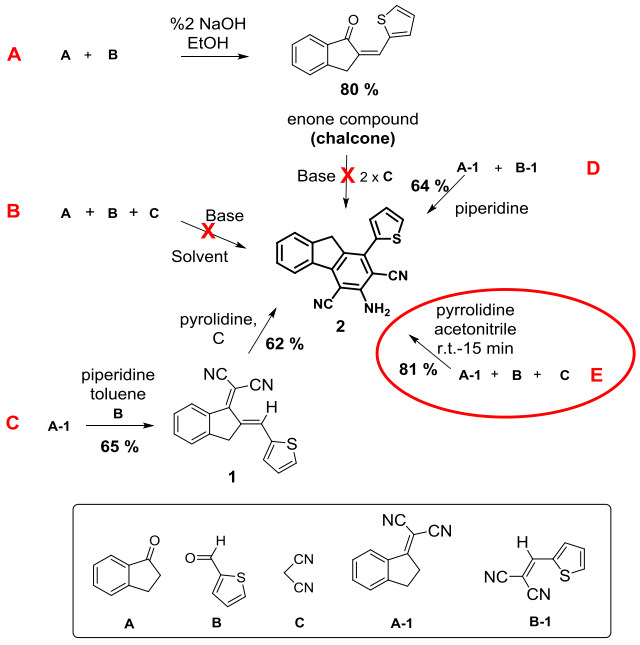


Scheme 1. The structural features of synthesized fluorene/fluorenone derivatives.

2. Results and discussion

2.1. Synthesis

Various synthetic methods for the preparation of fluorene/fluorenone have been reported.[17] Many of them couldn't be applied for our targeted compounds. Therefore, a new reaction pathway for the targeted 3-Amino-1-heteroaryl-2,4-dicyano-9(*H*)-fluorene compounds (**2**–**5**) was tested (Schemes 2 and 3). Obtained fluorene compounds (**2**–**5**) were oxidized to fluorenone analogues (**6**–**9**) by means of microwave irradiation of the BuOH solution catalysed by KOH. Finally, the fluorene (**2**–**5**) and fluorenone derivatives (**6**–**9**) were methylated on the pyridine heterocyclic nitrogen. Initially, we tested several reaction conditions to synthesize 3-Amino-1-(heteroaryl)-2,4-dicyano-9(*H*)-fluorenes compared to known literature reactions like the effects of reaction time, solvent, base, different intermediates, reagents etc. The synthesis of 3-Amino-1-(2-thienyl)-2,4-dicyano-9(*H*)-fluorene (**2**) is chosen as a model reaction by using different intermediates via A, B, C, D and E reaction pathways (Scheme 2). It was found that the best reaction conditions for compound **2** were: a mixture of 1-dicyanomethyleneindane (1 eq.), appropriate aldehyde (1.5 eq. 2-thiophencarboxaldehyde), malononitrile (1.5 eq) and pyrrolidine (1.2 eq) in acetonitrile was



Scheme 2. Possible synthetic routes to obtain fluorene **2**.