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Synthesis and cell imaging applications of fluorescent mono/di/tri-heterocyclyl-2,6-dicyanoanilines

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ABSTRACT

Synthesis of 3,4,5-triheterocyclyl-2,6-dicyanoanilines, starting from heterocyclic aldehydes and 1,2-diheterocycle-substituted ethanones, is described. 2,6-Dicyanoanilines with one or two heterocyclic substituents have also been synthesized. It was found that some of these molecules have selective cell-staining properties useful for cell imaging applications. The compounds **1**g, **10f** and **11** were found to stain cytoplasm of the cells in contact but not the nucleus while the compound **12** showed affinity to apoptotic cells resulting in blue fluorescence. The cell imaging results with compound **12** were similar to Annexin V-FITC, a known reagent containing recombinant Annexin V conjugated to green-fluorescent FITC dye, used for detection of apoptotic cells. These compounds were found to be non-cytotoxic and have potential application as cell imaging agents.

Substituted 2,6-dicyanoanilines are important compounds studied[1a] as such or as intermediates in diverse fields like optical materials, dyes, textile printing, heat resistant polymers, chiral stationary phases in chromatography *etc*. They also constitute molecular skeleton of a number of compounds, with an array of structural variations, some of which have been studied for potential medicinal use. The synthetic methods to prepare these moieties and their applications have been reviewed recently[1a] and the continued interest in these compounds has resulted in further publications.[1b,1c,2a–c] We desired to study the synthesis and optical/biological properties of dicyanoanilines with one, two or three heterocyclic substituents. The literature survey revealed that there are many references for 3-heterocyclyl-2,6-dicyanoanilines,[1a] a few references for 3,5-diheterocyclyl-2,6-dicyanoanilines[2] and a very few references for 4-heterocyclyl-2,6-dicyanoanilines[3] but there are no references for 3,4-diheterocyclyl-2,6-dicyanoanilines or 3,4,5-triheterocyclyl-2,6-dicyanoanilines. We report herein synthesis of various hitherto unknown 3,4-diheterocyclyl-2,6-dicyanoanilines and 3,4,5-triheterocyclyl-2,6-dicyanoanilines, their fluorescence properties and cell imaging applications. Though there are a large number of publications about cell imaging,[4] to our knowledge, this is the first report describing cell imaging potential of 2,6-dicyanoanilines and these preliminary results will lead to new applications of dicyanoanilines for cell imaging.

Various hitherto unknown 3,4,5-triheterocyclyl-2,6-dicyanoanilines were prepared from 1-benzyl-1,2,3[1*H*]-triazole-4- carboxaldehyde **8**, malononitrile and required 1,2-diheterocyclyl ethanone as exemplified by the synthesis of 2-amino-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-(4-oxo-6-propylthieno[2,3-*d*]pyrimidin-3(4*H*)-yl)-6-(thiophen-2-yl)isophthalonitrile (**1a**) shown in Scheme 1.

Initially, thienopyrimidinone was chosen as one of the heterocycles because thienopyrimidinones[5] with various substituents can be synthesized easily from substituted 2-aminothiophene-3-carboxylates which in turn can be prepared by Gewald synthesis.[6] Thus, ketone **6a** was prepared from 2-bromoacetylthiophene[7] **3a** and 6-propylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **5a**[5] in the presence of triethyl amine in ethyl acetate and reacted[8] with 1-benzyl-1,2,3[1*H*]-triazole-4-carboxaldehyde **8**[9] and malononitrile in DMF in the presence of morpholine to obtain the 3,4,5-triheterocyclyl-2,6-dicyanoaniline **1a** in 43% isolated yield. The 3,4,5-triheterocyclyl-2,6-dicyanoanilines and 3,4-diheterocyclyl-5-aryl-2,6-dicyanoanilines **1a–j** thus prepared, using aldehyde **8** and appropriate ketones, are shown in Table 1.

Structures of these compounds were assigned based on ^{1}H NMR, ^{13}C NMR, IR and HRMS data.[10] The structures of representative

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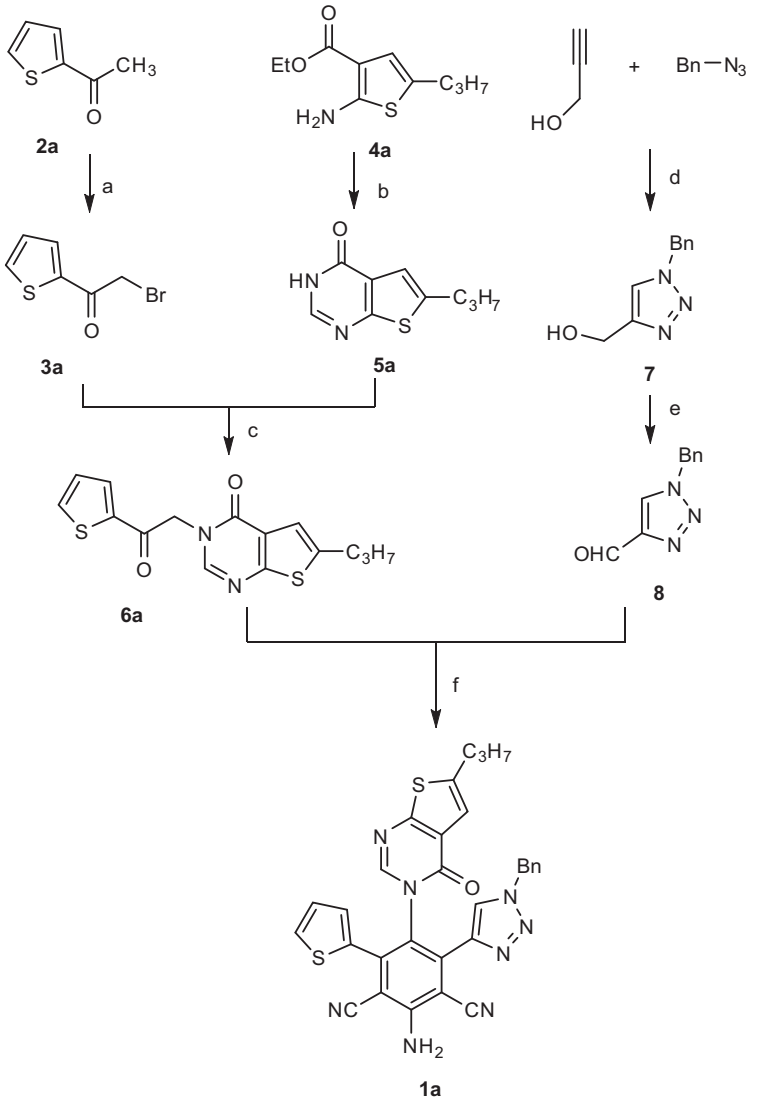

Scheme 1. Reagents and conditions: (a) Br_2, ether, 0 °C, 1.5 h, 72%; (b) Formamide, ammonium acetate, 145 °C, 10 h, 75%; (c) Triethyl amine, acetone, 0 °C, 1 h, RT, 3 h, 86%; (d) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, *t*-butanol-water, RT, 12 h, 94%; (e) MnO_2, ethyl acetate, RT, 2 h, 92%; (f) Malononitrile, morpholine, DMF, 80 °C, 12 h, 43%.

compounds **1c** and **1e** were further confirmed by X-ray crystallography.[10]

Using a similar strategy, various hitherto unknown 3-heterocyclyl-2,6-dicyanoanilines **9**, **10a–f**, **11** and **12** were prepared by reacting aldehyde **8** with appropriate aldehyde/ketone (Table 2). The dicyanoanilines **1e** and **1f** were methylated[2a] by subjecting them to NaH-MeI in THF to get the corresponding *N,N*-dimethylated compounds 5-(1-benzyl-1H-1,2,3-triazol-4-yl)-3-(dimethylamino)-2′,4′-difluoro-6-(1H-1,2,4-triazol-1-yl)-[1,1′-biphenyl]-2,4-dicarbonitrile (**13e**) and 5-(1-benzyl-1H-1,2,3-triazol-4-yl)-3-(dimethylamino)-4′-fluoro-6-(1H-1,2,4-triazol-1-yl)-[1,1′-biphenyl]-2,4-dicarbonitrile (**13f**).[10] Similarly, compounds **10a–f** were methylated to obtain the corresponding *N,N*-dimethylated compounds **14a–f** (Table 2).

There is a possibility to modify the structures of the compounds in the present work, in order to tune the optical properties, as there is a lot of flexibility in the synthetic schemes described.

These compounds were studied for their optical properties as they were colored in solution (Please see Supplementary data Table S2).

The UV–visible absorption spectra, of 2.5×10^{-5} M solutions in acetonitrile, for representative 2,6-dicyanoaniline compounds **1e**, **1g**, **10f**, **11** and **12** and N,*N*-dimethylated compounds **13e** and **14f** are shown in Fig. 1**A** while fluorescence spectra for these compounds are shown in Fig. 1**B**.

It was observed that there was a red shift in absorption maximum of the amino group after methylation due to enhanced electron-donating ability e.g. the λ_{max} value for amino compound **1e** was 367 nm while that for its corresponding *N,N*-dimethylated derivative **13e** was 395 nm. It was observed that the Stokes shift for the amino compound **1e** was 47 nm while the stokes shift for the corresponding *N,N*-dimethylated derivative **13e** was 74 nm indicating that the Stokes shift is increased after *N,N*-dimethylation of amino group. There was change in color of fluorescence in acetonitrile solutions of same concentration of these compounds. The compounds studied in the present work are soluble in DMSO, DMF, THF, chloroform, dichloromethane, ethyl acetate, acetone, acetonitrile, methanol and ethanol while they are insoluble in water and pet ether. The HOMO-LUMO gaps estimated from DFT calculations[11] were found to be in good agreement with the experimental values derived from uv-visible spectra (Please see Supplementary data).

The fluorescent molecules have a wide application in biological research where they have been developed as valuable tools to