Synthesis of 7-methyl-6-indolopterin and 7-methyl-6-indoloquinoxaline

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ABSTRACT

The first syntheses of indolopterin and indoloquinoxaline, two important and dissimilar diheterocycles linking C-2 of indole with C-6 of pterin (significant positions for showing biological activity), and quinoxaline, respectively, have been achieved based on two classical reactions. The introduction of a keto methyl group on to the 6-position of pterin and quinoxaline followed by Fischer indole synthesis led to these target diheterocycles. These indole-substituted diheterocycles will significantly increase the electron density on the pterin-5-N and quinoxazoline-2-N, which may change the redox properties of pterin and quinoxaline, and also the electron-withdrawing pterin or quinoxazoline should make the indole NH more acidic.

GRAPHICAL ABSTRACT

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Introduction

Pterin is one of the most important heterocyclic compounds containing a bicyclic ring system. The most well-known compounds, pterins and folates, are derived from pterin. Pterins are those compounds which possess the additional functional groups bonded to the pyrazine subring. On the other hand, the conjugated pterins containing p-aminobenzoic
acid and L-glutamates are called folates. These are the most significant compounds in a large number of natural and biological group transfer reactions.\cite{1–8} The fused heteroaromatic coenzymes originating from guanosine are called pterins, which are also ubiquitous and highly redox-active molecules.\cite{9} From the physiological point of view, pterins have correlations with several diseases, including hematological neoplasias\cite{10} and sudden infant death syndrome.\cite{11} Methotrexate, an important antileukemia drug, and folic acid, a significant nutrient and vitamin (vitamin B9), are also derived from pterins.

The existence of a pterin moiety\cite{12,13} in the molybdenum cofactor also gives us visible evidence of its importance in medicinal chemistry. Indole is also a hetero-bicyclic compound containing a six-membered benzene ring fused with a five-membered pyrrole ring. Indole is an essential constituent of fragrances and it can take part as the precursor of many pharmaceuticals.\cite{14} Indole-derived compounds are also of significance in natural product chemistry and pharmacology.\cite{15} In alkaloid chemistry, on the other hand, indole has a significant role and many indole alkaloids\cite{16} have importance in physiological activity and some of them are used in medicine. Consequently, the synthesis of indole derivatives is an essential area of research.

Now, in our laboratory, we are interested in synthesizing new pterin-derived compounds of potential medicinal interest. Pterin, indole, and quinoxaline are important heterocycles that have been utilized in many biologically active natural products that are used in a wide variety of medicines. Many designed synthetic medicines possess these heterocycles. Interestingly, most important pterin natural compounds are substituted at the 6-position. Since pterin and indole both are naturally and synthetically important, as they have strong relevance in medicinal chemistry, we wanted to synthesise pterin substituted by indole specifically at the 6-position (6-substitution has natural and synthetic significance). Quinoxaline and their derivatives are useful as antitumor antibiotics and potent bactericides. Though 2-(2-furyl) quinoxaline is known,\cite{17} to the best of our knowledge indoloquinoxaline or indolopterin are not known naturally or synthetically.

Results and discussion

Here we report the total synthesis of these combined heterocycles or conjugated heterocycles to be present not as fused system but as a distinct identity, with an intramolecular H bonding including a stable five-membered ring framework (Schemes 1f and 2d). In the retro-analysis, dissimilar targeted diheterocycles 1f and 2d originate from quick development of pterin-6-keto-methyl and quinoxaline-2-keto-methyl intermediates (1c and 2b), which should allow the construction of indole moiety by Fisher indole synthesis from the corresponding phenyl hydrazones of the corresponding keto methyl groups of pterin and quinoxaline (Scheme 1), respectively.

In continuation of our work on tri- and tetracarbonyl compounds\cite{18} for the synthesis of heterocycles, the formation of a 6-keto-methyl pterin ring can be conveniently achieved by the condensation of the stable tricarbonyl compound\cite{19} 1h with triamino-4-oxopyrimidine. The selenium dioxide oxidation (1 equivalent) of acetylacetone produces the desired stable tricarbonyl compound pentane-2,3,4-trione (1h). Thus, the total synthesis of 7-methyl-6-indolylpterin begins from 2,5,6-triamino-4-oxo-pyrimidine (1a) as shown in Scheme 2. The trione (1h) was allowed to react by Gabriel–Isay condensation\cite{20} with the pyrimidine 1a to give rise to 7-methyl-6-keto-methyl pterin (1c) in the crude form, which gave the soluble product 1d by
the action of pivalic anhydride in the presence of catalytic dimethylaminopyridine (DMAP). Compound 1d was then converted to the corresponding phenylhydrazone (1e), which underwent Fischer indolization to afford the yellow fluorescent target compound 1f in good yield. Thus, we have achieved the first synthesis of indolylpterin or pterinindole (1f).

We have also similarly synthesised another target indolylquinoxaline (2d) (Scheme 3). We have been able to develop suitable single crystals for the intermediates (2b and 2c) for x-ray studies. Crystallographic data are presented in Table S1 (S21). The asymmetric unit of the compound 2b [Fig. 1(a)] contains two independent molecules, molecules A and B, disordered over two sets of sites corresponding to a rotation of approximately 180°, with refined site occupancies of 0.557(4) and 0.443(4) for both molecules. Overall,

Scheme 1. Retro-synthetic route of indolopterin and indoloquinoxaline.

Scheme 2. Synthesis of N-[6-(1H-indole-2-yl)-7-methyl-4-oxo-3,4-dihydro-pteridin-2-yl]-2,2-dimethylpropionamide (1f). Reagents and conditions: (i) Na2SO3 and H2O, rt, 30 min; (ii) 1 h, H2O, rt, 8 h; (iii) pivalic anhydride, DMAP (cat.), 100 °C, 7 h; (iv) phenylhydrazine, EtOH, AcOH (few drops), 70–80 °C, 2 h; and (v) ZnCl2, AcOH, 150 °C, 1–2 h.
all molecules are close to being planar [r.m.s. deviation for all the non-H atoms = 0.0542 and 0.0638 Å (molecule A), 0.0591 and 0.0717 Å (molecule B), for the major and minor components, respectively]. Full molecule disorder has also been observed in 2c.

Scheme 3. Synthesis of 2-(1H-indole-2-yl)-3-methyl-quinoxalin (2d). Reagents and conditions: (i) 1 h, EtOH, rt, 1–2 h; (ii) phenylhydrazine, EtOH, AcOH (few drops), 70–80 °C, 2 h; (iii) ZnCl₂, AcOH, 150 °C, 1–2 h.

Figure 1. Molecular view of compounds (a) 2b and (b) 2c, with atomic numbering schemes. Both major (solid bonds) and minor (open bonds) components of the disorder are shown.

Figure 2. Crystal packing of the major component of compounds (a) 2b and (b) 2c, viewed along the (a) b axis and (b) a axis.
Fig. 1(b)). The molecule is disordered over two positions with occupancies of 0.615(5) and 0.385(5), exhibiting an inversion disorder. The benzene ring forms dihedral angles of 6.51 and 5.99° for the major and minor components, respectively, with the mean plane of the quinoxaline ring system. In the crystal structure, C1B—H1BD⋯N1A hydrogen bonds (Table S2 (S21)) in 2b bridge the molecules A and molecules B into pairs [Fig. 2(a), also shown in larger size in SI, S17] and these molecule pairs are further stacked down the c axis whereas the molecules in 2c are linked into a zigzag chain [Fig. 2(b), also shown in SI, S18] along [001] via N1—H1B⋯N4 hydrogen bonds.

**Photophysical properties**

We have studied here the fluorescence experiments of 1f (indolylpterin) and 2d (indolylquinoxaline) in several solvents. In nonpolar aprotic solvents (e.g., hexane, CCl₄, and toluene), they show deep blue fluorescence, including bathochromic shift (λ_max). Now the intensity of the blue fluorescence gradually decreases with increasing the polarity of the solvents (moderately polar aprotic). Here we also observe the compound 1f similar emission in CHCl₃, CH₂Cl₂, and tetrahydrofuran (THF), whereas the compound 2d gives different emission spectra with decreasing λ_max from CHCl₃ to THF. Among the polar aprotic solvents, acetone, dimethylformamide (DMF), and CH₃CN show the almost similar emission spectra (yellow fluorescence) but dimethylsulfoxide (DMSO) and ethyl acetate give a blue-shifted spectra of compound 1f (Fig. 3).

On the other hand, compound 2d shows the greater red-shifted emission spectra in DMSO and CH₃CN compared to other polar aprotic solvents (Fig. 4), indicating the fast ESIPT phenomenon.

In polar protic solvents such as methanol neither of the compounds show any fluorescence, probably because here methanol acts as a strong H-bond donor molecule and consequently excited state intramolecular proton transfer (ESIPT) is prevented due to possible intermolecular H-bonding interaction with methanol (Scheme 4).[21] The fluorescence quenching by MeOH may also be enhanced by proton-coupled depopulation of the excited state due to...
intermolecular hydrogen bonding. Furthermore, the positive solvochromicity accounts for a highly polar vibrationally relaxed excited singlet state. Therefore, ESIPT phenomena depend not only on solvent polarity but also on H-bond donor and acceptor ability of the solvents.[22]

If we compare the fluorescence properties of the target molecule \(1f\) with the indole-free \(1f\), i.e., the only 7-methyl pterin (\(1i\)) compound, the latter one exhibits only blue fluorescence in different solvents (Fig. 5). Again, if we compare the two molecules \(1f\) and \(1i\), it is clear that \(1f\) has no fluorescence in polar protic solvent methanol, whereas \(1i\) gives blue fluorescence in methanol, indicating the greater importance of indole moiety connected at the 6-position of pterin molecule. Therefore, the indole moiety of compound \(1f\) is the driving heterocyclic moiety onto pterin for solvatochromic ESIPT phenomena. On the other hand, eliminating indole moiety from compound \(2d\), i.e., only quinoxaline methyl moiety, has no naked-eye fluorescence property itself. Thus, the combination of the two dissimilar suitable heterocycles has a greater importance in modern ESIPT-based research.

**Figure 4.** Comparative fluorescence spectra of compound \(2d\) in different solvents at \(1 \times 10^{-5}\) M (top) and their naked-eye fluorescence change under handheld UV lamp (bottom).

**Scheme 4.** Mechanism of ESIPT.
Conclusion

Thus, we have achieved the synthesis of two important dissimilar diheterocycles, indolopterin (1f) and indoloquinoxaline (2d), using Gabriel–Isay synthesis for the pterin ring system followed by Fischer indole synthesis for the development of the substituted indole ring. The new system of two important heterocycles has the potential for new conjugated donor–acceptor moieties having interesting solvatochromic properties and for development of other possible fluorescence markers. Further studies are in progress in our laboratory for the synthesis of new arrays of heterocycles from pterins and quinoxalines for their possibility of new biological and sensor activities.

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