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Augmentation of steroidal β-formylenamide with pyrazolo and benzimidazo moieties: A tandem approach to highly fluorescent steroidal heterocycles

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ABSTRACT

A facile synthesis of pyrazolo[1,5-*a*]pyrimidine and benzimidazo[1,2-*a*]pyrimidine annulated steroids is described from the novel reaction of β-formyl enamides with amino pyrazoles, indazoles and benzimidazoles. Several of the products exhibited fluorescence properties with high quantum yields.

Introduction

Organic molecules that demonstrate strong fluorescence emissions have found its own niche amongst the elites of the scientific community [1,2]. In recent years, molecular fluorophores have been deeply introspected over a wide spectrum of research areas ranging from chemical, biological to material sciences [3,4]. Fluorescence properties of steroids find their applications in different diagnostic tools such as fluorescent immunoassay [5,6], membrane structure and function [7–9], steroid protein interaction [10] as well as steroid inclusion into cyclodextrins [11]. Nevertheless, the weak intrinsic fluorescence of steroids requires additional treatment like denaturation by strong acids [12,13] or attachment of different fluorescent fluorophores to steroids [14]. Consequently, discovery of steroidal single fluorophore [15] becomes a challenging task; and efforts are inevitable in this approach.

Recently, Pyrazolo[1,5-*a*]pyrimidines have emerged as attractive substrates with efficient synthetic methods [16–19] and fluorescence properties [20,21]. The combination of photophysical properties with biological activities allows the use of these compounds as lipid droplet biomarkers for HeLa cells (cancer cells) and L929 cells (normal cells) [22], demonstrating the interesting versatility of this heterocyclic core. Moreover, pyrazolo[1,5-*a*]pyrimidine and pyrazolo[3,4-*b*]pyridines exhibit underlying activities for COX-2 selective inhibitor [23], CK-2 kinase inhibitor [24], and HMG-CoA reductase inhibitor activities [25]. It has also been found to possess profound applications as selective peripheral benzodiazepine receptor ligands [26], antianxiety agents [27], histamine-3 receptor ligands [28], and as potassium channel ligands [29]. To the best of our knowledge, no literature report is available for the study of fluorescence potential of such steroidal heterocycles.

In 2014, our group first reported the synthesis of several A/D-ring annulated steroidal pyrazolo[1,5-*a*]pyrimidines from conjugated enones and substituted 3-amino-1*H*-pyrazoles in the presence of KO^{t}Bu under thermal condition (Scheme 1) [30,31].

Subsequently, another synthetic protocol involving β-bromovinyl aldehyde was developed for the synthesis of pyrazolo[1,5-*a*]pyrimidines and pyrazolo[3,4-*b*]pyridines under microwave irradiation using $Pd(OAc)_2$ as catalyst (Scheme 1) [32,33]. However, these studies were aimed specifically in developing new synthetic strategies for these class of steroidal heterocycles without any focus to explore their fluorescence potentials.

In continuation of our endeavor, herein, we report a novel application of steroidal β-formyl enamides [34] in the synthesis of new class of fluorescent steroids. Several of our synthesized steroidal pyrazolo[1,5-*a*]pyrimidines (**3a-d, 3f-h, 3j-k**) demonstrated strong fluorescence properties. It was also noted that steroidal benzimidazo[1,2-*a*]pyrimidine (**5a-b**) exhibited high fluorescence.

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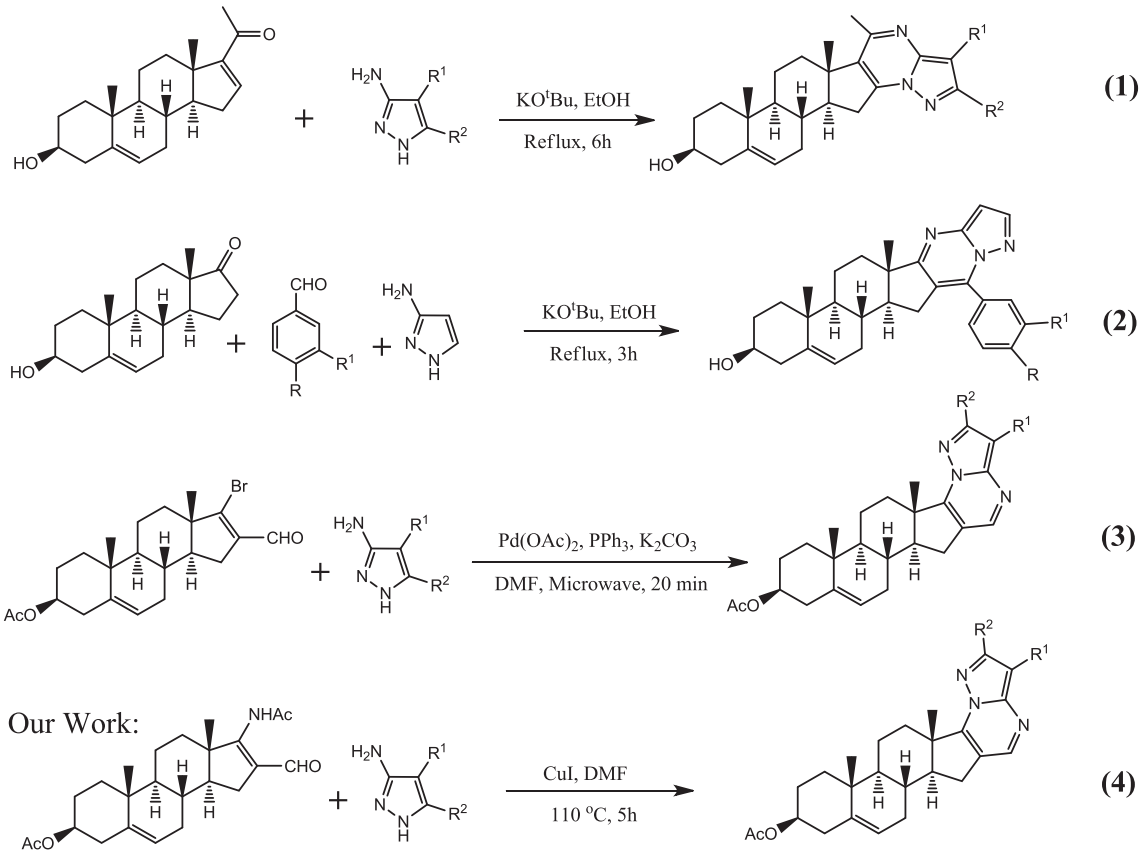


Scheme 1. Synthesis of steroidal pyrazolo[1,5-*a*]pyrimidines and pyrazolo[3,4-*b*]pyridines.

Result and discussion

β-Formyl enamide was found to be an excellent precursor for the preparation of pyrazolo(1,5-*a*)pyrimidines (**3a-k**) and benzimidazo(1,2-*a*)pyrimidines (**5a-c**). Initially, the reaction condition of β-formyl enamide **1a, (**1.0 mmol) with equimolar amount of 5-aminopyrazole **2a** (1.2 mmol) was optimized varying solvent, catalyst and reaction temperature.

The reaction of **1a** with **2a** in solvent-less condition did not proceed under thermal condition; and use of neat DMF as solvent at elevated temperatures afforded a poor yield of **3a**. The best yield of **3a** (87%) was achieved from reaction of **1a** and **2a** when the reaction was carried in DMF using 5 mol% of CuI at 110 ^{0}C. Neither change of solvent nor catalyst showed any improvement of yields under thermal conditions (Table 1). Reactions carried at higher temperature (above 110 °C) did not improve the yield of the product.

Using optimized condition, steroidal as well as non-steroidal analogs of pyrazolo[1,5-*a*]pyrimidines (**3b-k**) were synthesized (Scheme 2). The scope of the optimized protocol was further explored with other amino substituted heterocycles. It was found that 2-aminobenzamidazoles reacted with β-formyl enamide to afford benzimidazo[1,2-*a*]pyrimidines (**5a-c**) in high yields (Scheme 2). The single-crystal X-ray crystallography data (CCDC 2008212) confirmed the proposed structure of the synthesized compound **5a**.

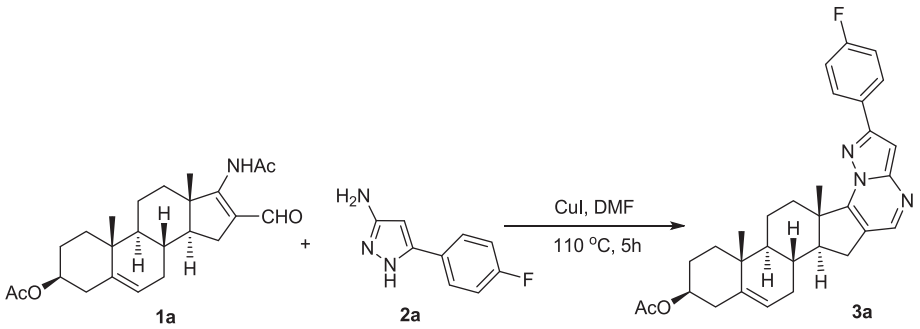
A probable mechanism for the ring formation reaction was proposed involving nucleophilic displacement of 17-acetamido group of **1a** during the cyclization process (Scheme 3). In the preliminary step, the amino group of the pyrazole (**2a**) condenses with the aldehyde group of the β-formyl enamide (**1a**) resulting Schiff base intermediate **A** [35]. The CuI mediated ring cyclization followed by elimination of acetamido group led to intermediate **B**. Finally, loss of proton from intermediate **B** afforded **3a**. The isolation of acetamide as a byproduct rendered support to our proposed mechanism.

In order to corroborate the proposed mechanistic pathway of the reaction, β-formyl enamide (**1a**) was reacted with benzamidine (**6a-b**) and a similar cyclization pattern was observed to yield pyrimidine fused product (**7a-c**) (Scheme 4).

Further, it was also observed that β-formyl enamide (**1a**) facilitated the formation of conjugated enone (**9a**) with concurrent loss of acetamido group (Scheme 4). The ^{1}H NMR of compound **9a** exhibited chemical shift for –NH proton at δ 10.75. This downfield shift can be attributed due to hydrogen bonding of –NH with C=O group; which favours Z isomer. The mechanistic pathway of this reaction probably proceeds in congruence to the formation of pyrazolo[1,5-*a*]pyrimidines (Scheme 3); however, due to the nucleophilic attack of water molecule the unstable pyrimido[1,6-*a*] pyridinium salt intermediate (**C**) opens up to give intermediate (**D**) followed by tautomerization to afford conjugated enone adduct (**9a**) (Scheme 5).

Several of the synthesized compounds were found to exhibit fluorescence activity which was ascertained from the strong fluorescent glow exuded by the compounds on exposure to long UV radiation (λ = 365 nm). All the steroidal as well as non-steroidal derivatives were dissolved in chloroform (2.0×10^{-3} M) and screened for fluorescence activity by illumination under long UV. It was found that the following compounds **3a-k** and **5a-b** demonstrated change in coloration from colorless to fluorescent white or light yellow on UV_{365} illumination (Fig. 1). Amongst the synthesized compounds, pyrazolo[1,5-a]pyrimidine **3k** and benzimidazo [1,2-*a*]pyrimidine **5b** demonstrated the highest fluorescence activities, accounting for fluorescence quantum yield of 88.5% and 90.2%

Table 1
Optimization of reaction condition.[a]



Entry	Solvent	Catalyst	T[b] (°C)	Yield[c] (%)
1	–	–	100	0
2	DMF	–	rt	0
3	DMF	–	80	10
4	DMF	–	110	40
5	DMF	–	120	42
6	DMF	CuI (10 mol %)	110	85
7	**DMF**	**CuI (5 mol %)**	**110**	**87**
8	DMF	$Cu(OAc)_2$ (10 mol %)	110	45
9	DMF	CuCl (5 mol %)	110	40
10	DMSO	CuI (5 mol %)	120	0
11	$CHCl_3$	CuI (5 mol %)	61	0
12	DMF	CuBr (5 mol %)	110	39
13	DMF	$ZnCl_2$ (5 mol %)	110	35
14	DMF	$FeCl_3$ (5 mol %)	110	28
15	DMF	L-proline	110	21
16	THF	CuI (5 mol %)	66	0
17	CH_2Cl_2	CuI (5 mol %)	40	trace
18	Acetone	CuI (5 mol %)	56	0
19	CH_3CN	CuI (5 mol %)	80	0
20	Methanol	CuI (5 mol %)	65	0
21	Ethanol	CuI (5 mol %)	78	0
22	H_2O	CuI (5 mol %)	100	0

[a] Reaction condition: **1a** (1 mmol), **2a** (1.2 mmol) in solvent (3 mL) for 5 h. [b]Oil bath temperature. [c]Isolated yields of **3a**.

respectively with respect to quinine hemi-sulfate as the reference at 2.0×10^{-5} M concentration (Fig. 2). Compounds **3a-d**, **3g-h**, **3j-k** and **5a-b** showed fluorescence quantum yields ranging 23.9–87.6%. However, products **3f** and **5c** exhibited weaker fluorescence and negligible quantum yields (4.1 and 5.7%).

Conclusion

In summary, herein, we report a novel application of β-formyl enamide towards synthesis of fluorescent pyrazolo[1,5-*a*]pyrimidine and benzimidazo[1,2-*a*]pyrimidine annulated steroidal and non-steroidal compounds. We demonstrate an interesting phenomenon of β-formyl enamides in nucleophilic reactions facilitating loss of 17-acetamido group, in contrast to earlier findings, wherein, acetamido group usually took part in cyclization process [36]. Furthermore, our studies revealed a novel synthesis of fluorescent steroidal aza heterocycles in high quantum yields (upto 90.2%). Because of inherent lipid properties of steroids, the fluorescent steroids could provide great scope as a valuable tool in cancer research and other biological studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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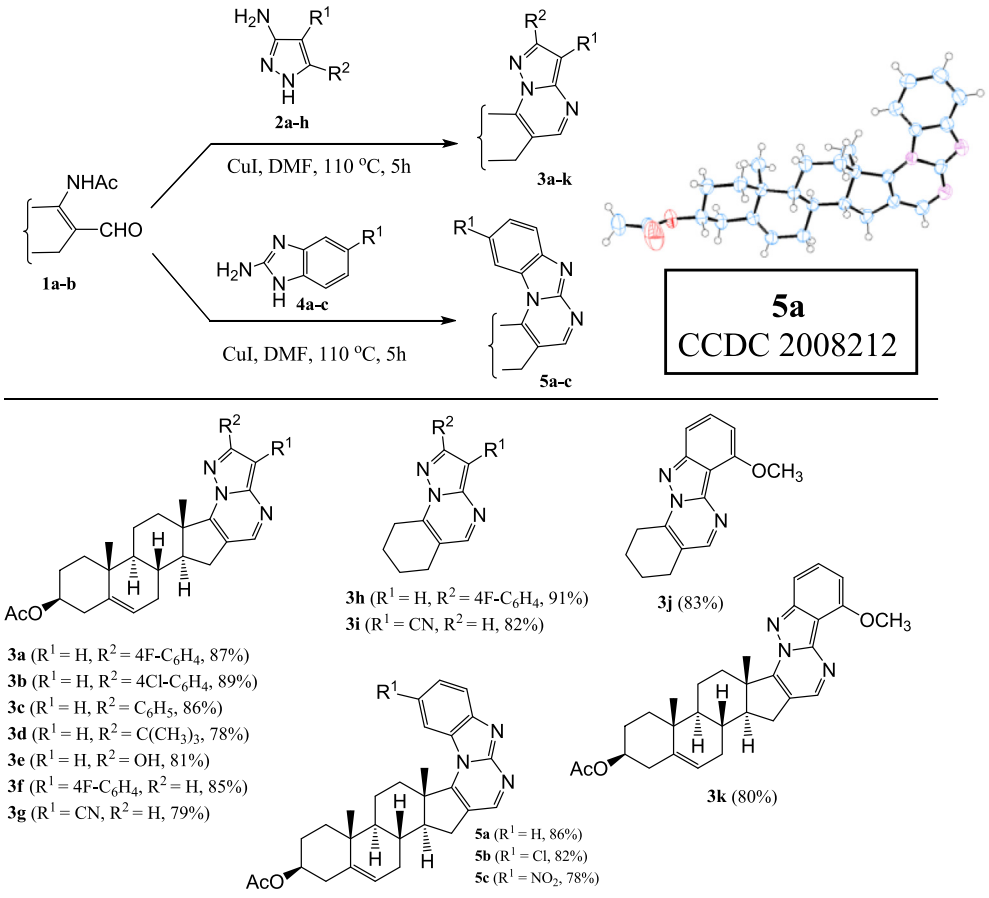
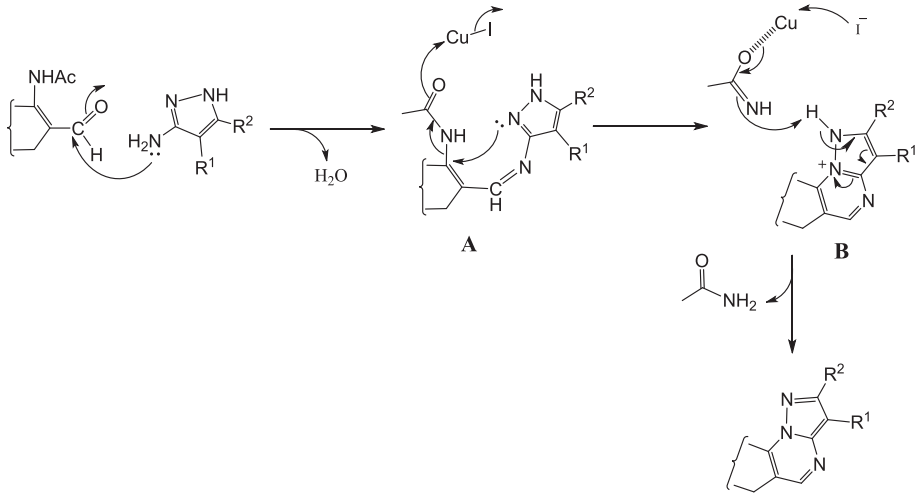
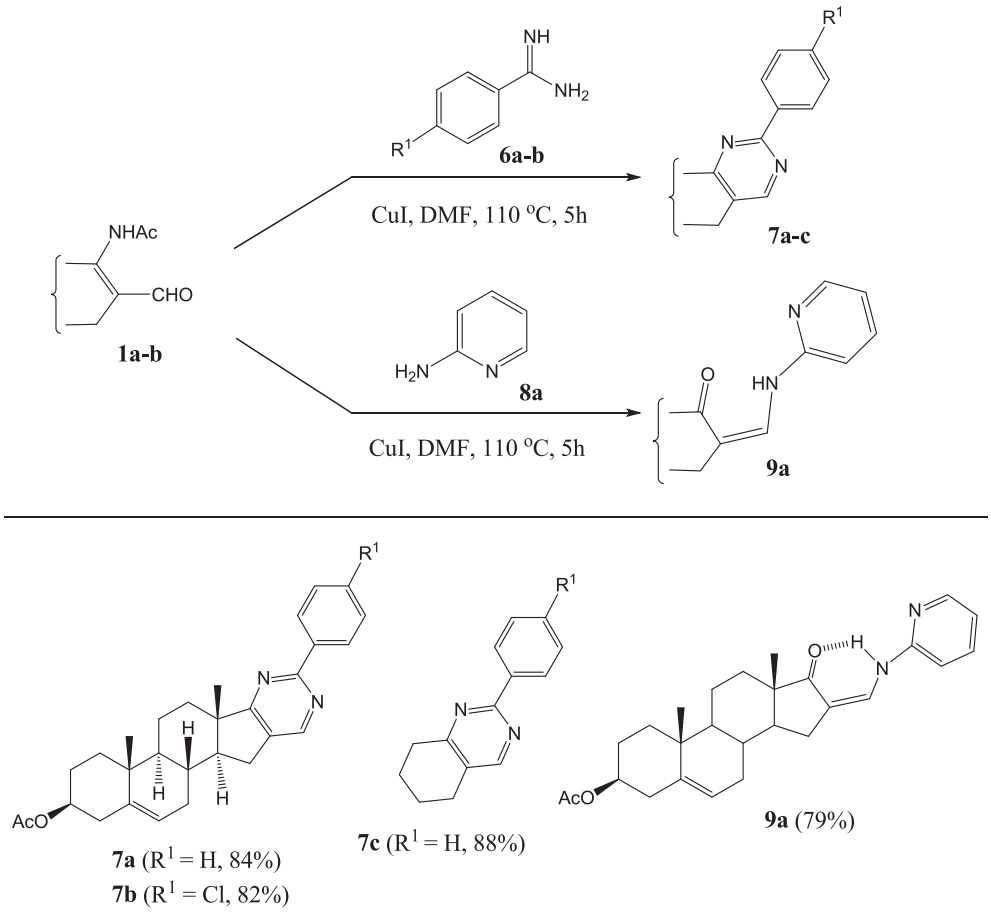


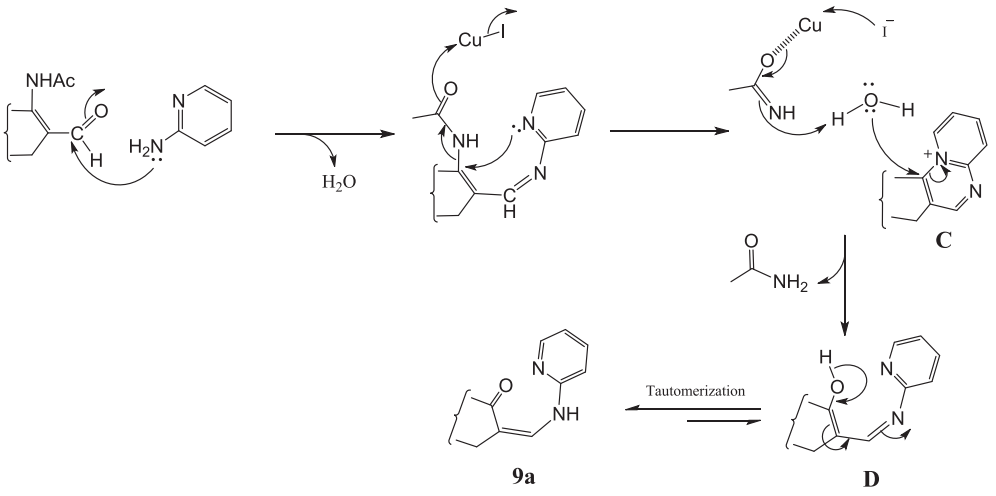
Scheme 2. Copper Iodide catalyzed synthesis of pyrazolo[1,5-*a*]pyrimidines (**3a-k**) and benzimidazo[1,2-*a*]pyrimidine (**5a-c**).



Scheme 3. Proposed mechanism for the synthesis of pyrazolo[1,5-*a*]pyrimidines.



Scheme 4. Copper Iodide catalyzed synthesis of pyrimidines (**7a-c**) and conjugated keto product (**9a**) from β-formyl enamide.



Scheme 5. Proposed mechanism for the synthesis of conjugated keto system from β-formyl enamide.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2021.152893.

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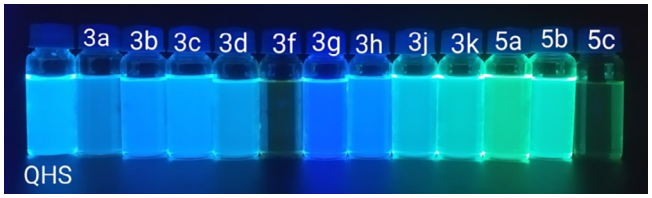

Fig. 1. Photographs of 2.0×10^{-3} M solutions of synthesized pyrazolo[1,5-*a*]pyrimidines (**3a-d**, **3f-h**, **3j-k**) and benzimidazo[1,2-*a*]pyrimidine (**5a-c**) in $CHCl_3$ under λ = 365 nm illumination.

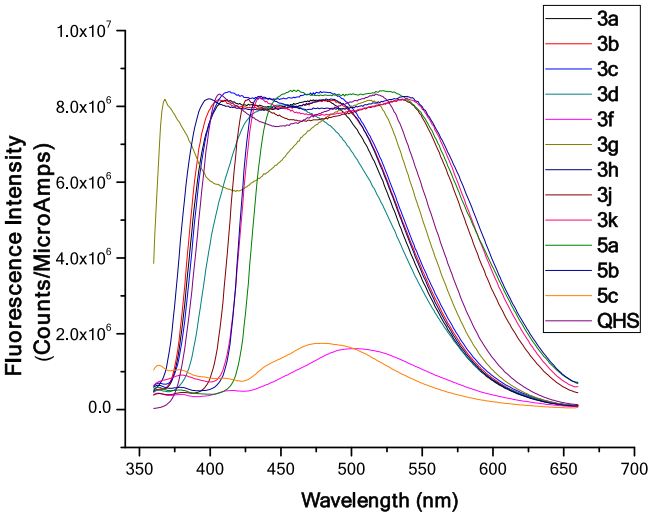

Fig. 2. Fluorescence (2.0×10^{-5} M, excitation at 340 nm) spectra of **3a-d**, **3f-h**, **3j-k** and **5a-c** in chloroform using quinine hemisulfate as the reference.

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