Green Synthesis, Characterization and Photophysical Properties of Rhodamine Derivatives

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Abstract

Rhodamine 6G-chromone-derived compounds **RD1-RD4** have been synthesized by condensation of rhodamine 6G hydrazide and substituted chromone aldehydes in ethanol using microwave-assisted reaction. The structures of these synthesized rhodamine 6G derivatives were confirmed by proton nuclear magnetic resonance (^{1}H-NMR), carbon nuclear magnetic resonance (^{13}C-NMR), and high-resolution mass spectra data (HRMS). Colorimetric and photophysical studies show the synthesized compounds selectively detect copper (II) ion in aqueous acetonitrile solution.

Keywords

Rhodamine 6G, Microwave, Synthesis, Sensor, Absorbance, Fluorescence

1. Introduction

The development of green chemistry and environmentally friendly organic synthetic methods progressed rapidly in recent years. It has been known for many years that rhodamine derivatives are a widely used class of compounds due to their versatile applications in various fields [1-2]. Because of their strong fluorescence and photostability, rhodamine derivatives have been found to have applications in sensors [3-5], imaging [6], fluorescent markers [7], and in laser applications [8]. The rhodamine skeleton, which has excellent photophysical properties like long absorption and emission wavelength, high molar extinction coefficient, high florescence quantum yield, good solubility, and photostability, has attracted great interest [9-10]. These compounds have been well established as fluorescent sensors for cations due to their ability to trigger the change in structure between the spirocyclic and ring-open spirolactum forms, which result in different optical properties [11]. Several studies have shown that the type of the substituent group on the lactam ring has influence on the properties of rhodamine derivatives [12-13].

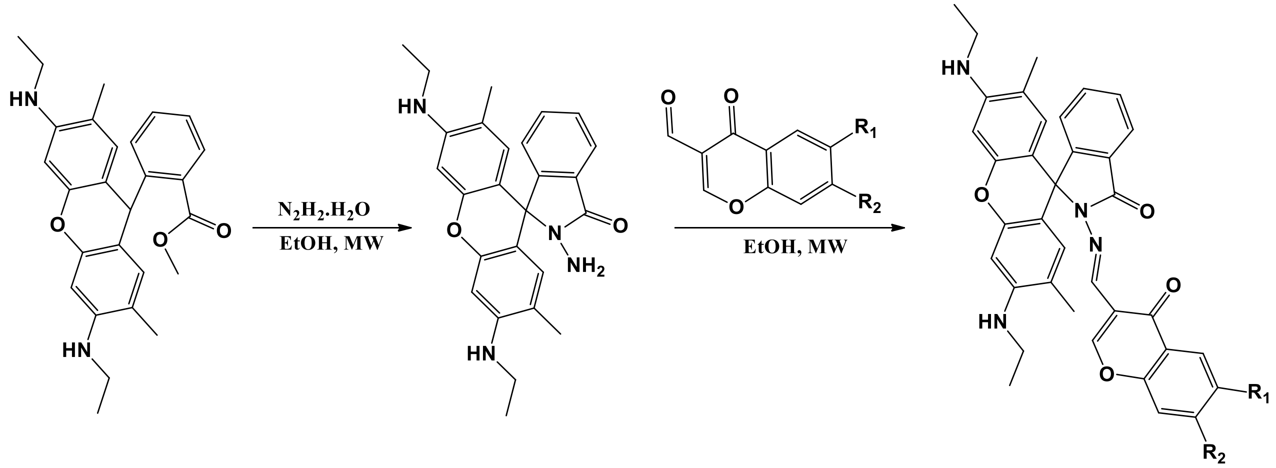
Microwave application in organic synthesis has had exponential growth over the last twenty years due to its easy experimental conditions, rapid turnaround, and easy workups. Moreover, it is considered environmentally friendly and typically offers high yields, along with simplified processing and handling compared to conventional synthesis methods [14-16]. Microwave irradiation technique possesses several green attributes such as increased energy efficiencies, less purification process, cleanliness, and use of green solvents. More efficient ways of synthesizing rhodamine derivatives are needed so that a variety of products with various chemical and spectral properties can be made available to thoroughly investigate their potential use as optical sensors. The power of microwave synthesis is utilized for speeding the reaction and providing an efficient and convenient way of obtaining these rhodamine variations or related compounds [17].

In this work, we present an efficient, clean, and straightforward synthetic procedure to prepare four rhodamine-derived compounds. These target compounds are synthesized in a two-step reaction (Scheme 1-4), using mild conditions and inexpensive reagents under green conditions with relatively high yields.

2. Experimental

2.1 Reagents and instruments

All chemicals used in this study were commercially purchased and used as received. Hydrazine monohydrate (79%), Rhodamine 6G, Chrome-3-carboxaldehyde, 3-Formyl-6-nitrochromone, 6-Chloro-3-formyl-7-methylchromone, 3-Formyl-6-methylchromone, acetonitrile, ethanol, DMSO (d_6), and nitrate salts were used in this experiment. Microwave irradiation reactions were carried out in a CEM reactor. The CEM microwave irradiation system is developed to enhance the capacity to perform reactions under controlled conditions. The reactions were performed safely at a maximum temperature of 100 °C. However, reactions can safely be performed at pressures up to 20 bar and temperatures ranging from 40 °C to 300 °C. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 400 MHz NMR on a Bruker Avance spectrometer with tetramethyl silane (TMS) as an internal standard and DMSO-d_6 as solvent. MALDI-HRMS analysis was recorded using Bruker 12T solarix FT-ICR-MS. All optical experiments were recorded using Agilent Cary 60 Ultraviolet -Visible (UV-Vis's) spectrometer and Varian Cary Eclipse fluorescence spectrophotometer respectively.



RD1 = **R1**=H; **R2**=H　　**RD3** = **R1**=Cl; **R2**=CH_3

RD2 = **R1**=NO_2; **R2**=H　　**RD4** = **R1**=CH_3; **R2**=H

Scheme 1: Microwave-assisted synthesis of compound **RD1-RD4**

2.2. Microwave-assisted synthesis of **RD1**

Rhodamine 6G hydrazide intermediate was synthesized according to Yang's method [18]. A mixture of Rhodamine hydrazide intermediate (100 mg, 0.219 mmol), Chrome-3-carboxaldehyde (22 mg, 0.0406 mmol) and 2 ml of ethanol was placed in a microwave vessel. The resulting mixture was stirred and placed in a reactor. The reaction vessel was then run under pressure and irradiation at a specific temperature and time shown in Table 1 and then the reaction vessel was cooled in ice bath. The reaction mixture was filtered out and washed three times with cold ethanol. After drying, the solid product was isolated, and obtained a higher yield ranging from 77% − 80%. ^{1}H-NMR (d_6-DMSO), δ (ppm): 8.59 (s, 1H), 8.41 (s, 1H), 8.00 (m, 1H), 7.90 (m, 1H), 7.71(m, 1H), 7.60 (m, 3H), 7.50 (m, 1H), 7.00 (d, 1H), 6.40 (s, 2H), 6.26 (s, 2H), 5.01 (s, 2H, -NH), 3.14 (q, 4H, NCH_2CH_3), 1.87 (s, 6H, $-CH_3$), 1.21 (t, 6H, NCH_2CH_3). ^{13}C-NMR (DMSO), δ (ppm): 165.23, 152.07, 151.33, 147.35, 132.31, 129.48, 128.00, 127.01, 123.43, 122.13, 117.79, 104.99, 95.85, 64.96, 55.99, 37.45, 18.53, 17.06, 14.20. HRMS (MALDI): m/z Calcd for **RD1** (M+1): 585.2496; Found: 585.2504.

Table 1: Microwave-assisted irradiation reaction method for the preparation of **RD1** (phase II).

Trials	Method/Solvent	Temperature (ºC)	Time (mins)	Yield (%)
T1	Microwave/Ethanol	100	30	78.5
T2	Microwave/Ethanol	100	20	80.5
T3	Microwave/Ethanol	80	30	77.7
T4	Microwave/Ethanol	80	20	82

2.3. Microwave-assisted synthesis of **RD2**

A mixture of Rhodamine hydrazide derivative (100 mg, 0.219 mmol), 3-Formyl-6-nitrochromone (22 mg, 0.0511mmol) and 2 ml of solvent was placed in a microwave vessel. The resulting mixture was stirred and placed in a reactor. The reaction vessel was then run under pressure and irradiation at a specific temperature and time as shown in Table 2. The reaction mixture was filtered out and washed three times with cold ethanol. After drying, the solid product was isolated, and obtained a higher yield ranging from 64% − 84%. ^{1}H-NMR (d$_6$-DMSO), δ (ppm): 8.68 (s, 1H), 8.50 (m, 2H), 7.90 (m, 2H), 7.62 (m, 2H), 7.00 (m, 2H), 6.27 (s, 2H), 6.10 (s, 2H), 5.11 (s, 2H, -NH), 3.14 (q, 4H, NCH_2CH_3), 1.86 (s, 6H, $-CH_3$), 1.22 (t, 6H, NCH_2CH_3). ^{13}C-NMR (DMSO), δ (ppm): 164, 153, 152, 145, 1321, 130, 127.02, 127.01, 122.43, 120.23, 116.79, 103.99, 94.85, 63.96, 54.99, 36.44, 18.43, 17.05, 14.20. HRMS (MALDI): m/z Calcd for **RD2** (M+1): 630.2347; Found: 630.2364.

Table 2: Microwave-assisted irradiation reaction method for the preparation of **RD2** (phase II).

Trials	Method/Solvent	Temperature (ºC)	Time (mins)	Yield (%)
T1	Microwave/Ethanol	100	30	65
T2	Microwave/Ethanol	100	20	64
T3	Microwave/Ethanol	80	30	84
T4	Microwave/Ethanol	80	20	84.6

2.4. Microwave-assisted synthesis of **RD3**

A mixture of Rhodamine hydrazide derivative (100 mg, 0.219 mmol), 6-Chloro-3-formyl-7-methylchromone (22 mg, 0.0519 mmol) and 2 ml of ethanol was placed in a microwave vessel. The resulting mixture was stirred and placed in a reactor. The reaction vessel was then run under pressure and irradiation at a specific temperature and time as shown in Table 3 The reaction mixture was filtered out and washed three times with cold ethanol. After drying, the solid product was isolated, and obtained a higher yield ranging from 78% – 84%. ^{1}H-NMR (d_6-DMSO), δ (ppm): 8.62 (s, 1H), 8.46 (s, 1H), 7.88 (m, 2H), 7.60 (s, 1H), 7.58 (m, 2H), 7.00 (m, 1H), 6.40 (s, 2H), 6.23 (s, 2H), 5.04 (s, 2H, -NH), 3.15 (q, 4H, NCH_2CH_3), 1.89 (s, 6H, $-CH_3$), 1.22 (t, 6H, NCH_2CH_3). ^{13}C-NMR (DMSO), δ (ppm): 165.23, 152.07, 151.33, 147.35, 132.31, 129.48, 128.00, 127.01, 123.43, 122.13, 117.79, 104.99, 95.85, 64.96, 55.99, 37.45, 18.53, 17.06, 14.20. HRMS (MALDI): m/z Calcd for **RD3** (M+1): 633.2263; Found: 633.2263.

Table 3: Microwave-assisted irradiation reaction method for the preparation of **RD3** (phase II).

Trials	Method/Solvent	Temperature (°C)	Time (mins)	Yield (%)
T1	Microwave/Ethanol	100	30	80
T2	Microwave/Ethanol	100	20	78
T3	Microwave/Ethanol	80	30	82
T4	Microwave/Ethanol	80	20	84

2.5. Microwave-assisted synthesis of **RD4**

A mixture of Rhodamine hydrazide derivative (100 mg, 0.219 mmol), 3-Formyl-6-methylchromone (22 mg, 0.0439 mmol) and 2 ml of solvent was placed in a vessel. The resulting mixture was stirred and placed in a reactor. The reaction vessel was then run under pressure and irradiation at a specific temperature and time as shown in Table 4. The reaction mixture was filtered out and washed three times with cold ethanol. After drying, the solid product was isolated, and obtained a higher yield ranging from 81% – 89%. ^{1}H-NMR (d_6-DMSO), δ (ppm): 8.62 (s, 1H), 8.40 (s, 1H), 7.88 (m, 1H), 7.70 (s, 1H), 7.56 (m, 4H), 7.02 (m, 2H), 6.37 (s, 2H), 6.20 (s, 2H), 5.08 (s, 2H, -NH), 3.20 (q, 4H, NCH_2CH_3), 2.40 (s, 3H, $-CH_3$), 1.86 (s, 6H, $-CH_3$), 1.21 (t, 6H, NCH_2CH_3). ^{13}C-NMR (DMSO), δ (ppm): 165.23, 152.07, 151.33, 147.35, 132.31, 129.48, 128.00, 127.01, 123.43, 122.13, 117.79, 104.99, 95.85, 64.96, 55.99, 37.45, 18.53, 17.06, 14.20. HRMS (MALDI): m/z Calcd for **RD4** (M+1): 599.2652; Found: 599.2655.

Table 4: Microwave-assisted irradiation reaction method for the preparation of **RD4** (phase II).

Trials	Method/Solvent	Temperature (° C)	Time (mins)	Yield (%)
T1	Microwave/Ethanol	100	30	86
T2	Microwave/Ethanol	100	20	81.6
T3	Microwave/Ethanol	80	30	83.3
T4	Microwave/Ethanol	80	20	89

3. Results and discussion

3.1. Microwave-assisted synthesis

Novel green microwave-assisted synthesis methods using ethanol as a solvent for the synthesis of rhodamine 6G-Chromone imines have been established. A total of four rhodamine 6G derived imine derivatives were synthesized using a controlled CEM microwave heating reactor under closed-vessel conditions. The microwave system is equipped with a magnetic stirrer as well as temperature and power controls. Microwave-assisted synthesis of Rhodamine hydrazide with various substituted chromone compounds such as Chrome-3-carboxyaldehyde, 3-formyl-6-Nitrochromone, 6-Chloro-3-formyl-7-methylchromone, 3-formyl-6-methylchromone, and 2-amino-3-formylchromone in ethanol results all target products in high yield, as illustrated in scheme 1. In the first phase, the intermediate compound was synthesized by microwave irradiation of Rhodamine 6G and excess hydrazine hydrate in ethanol, and in the second phase, the target products (**RD1-RD4)** were synthesized by microwave irradiation and condensation of the resulting rhodamine 6G hydrazone and corresponding aldehydes in 1:1 molar ratio in ethanol. The microwave-assisted synthesis results in a green and fast synthesis time, minimum solvent use, easy operation, and scalability. The reaction conditions for the microwave-assisted synthesis of rhodamine 6G imine derivatives **RD1-RD4** are summarized in Table 1 - 4. The optimized conditions for each rhodamine 6G-Chromone based imine derivatives were determined by conducting extensive temperature and time studies. It was observed that microwave-assisted reaction with temperature 80°C afforded the best result with 82% - 89% product yield for 20-30 minutes (Table 1-4, entry 3 and 4). The results indicate that microwave irradiation method accelerates synthesis of Rhodamine 6G-Chromone imine products in minutes. Due to our interest in green chemistry, minimum solvent conditions were mainly exploited. The target products required no rigorous purification, and pure solid products were isolated from the reaction mixture. Therefore, this irradiation method offers an easy and practical access for the synthesis of a series of rhodamine 6G-based imine products. Structural analysis of these rhodamine 6G-Chromone imine derivatives were performed with ^{1}H-NMR, ^{13}C-NMR, and high-resolution mass spectrometry, and all data are in accordance with the proposed structure (Figures **S1**-**S12**).

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3.2. Optical properties

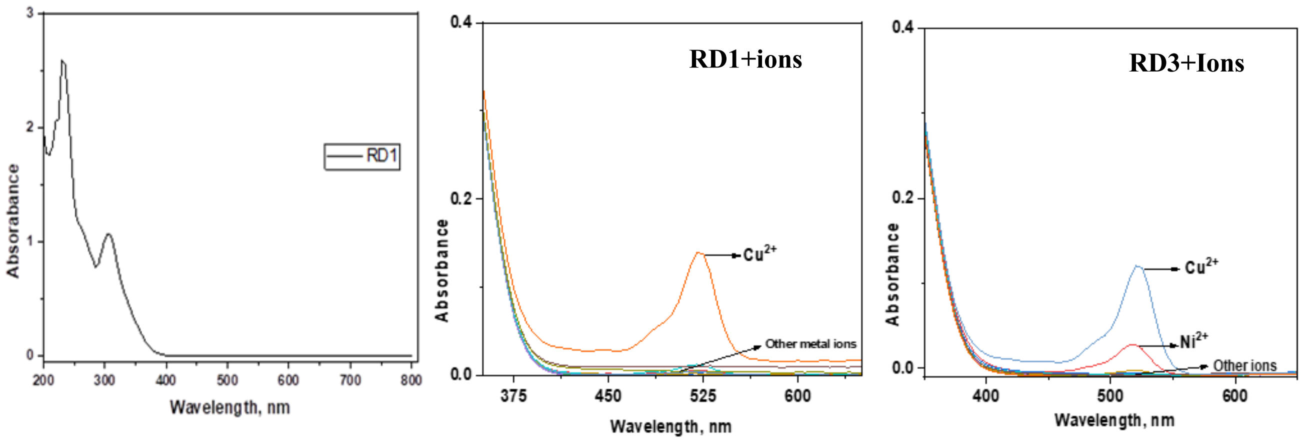


Figure 1. UV-Vis's spectra of 20 µM (a) free **RD1**, (b) **RD1**with 20 mM metal ions in CH_3CN/H_2O (7:3 v/v) HCl-tris buffer solution and (c) **RD3** with 20 mM metal ions in CH_3CN/H_2O (7:3 v/v) HCl-tris buffer solution.

The rhodamine 6G component has been demonstrated as an attractive sensor with emission turn on effect [18-19]. In this work, chrome-3-carboxaldehyde, 3-Formyl-6-nitrochromone, 6-Chloro-3-formyl-7-methylchromone, and 3-Formyl-6-methylchromone were used to modify rhodamine component, hoping to optimize a suitable structure for sensing application. All the photophysical studies were performed in aqueous acetonitrile in which rhodamine 6G-chromone compounds **RD1**-**RD4** formed colorless solutions. In addition, the absorption spectra showed no peak above 400 nm due to the predominant ring-closed spirolactum (Figure 1a). This was further confirmed by the ^{13}C NMR signal around δ 66 corresponding to bridging carbon, C-1 (Figure S2). Similarly, compounds were very weakly fluorescent at 565 nm (λ_{ex} = 488 nm) in the absence of any analyte due to the predominant ring-closed spirolactum. The fluorescence spectrum of target compounds showed a peak at 565 nm upon the addition of Cu^{2+} corresponding to the delocalization in the xanthene moiety of rhodamine 6G (Figure 2). Several studies show that substituents can affect the optical properties of compounds [20-25]. As shown in Figure 2a, Rhodamine 6G derivative **RD1** with no substituent on the chromone unit exhibits excellent selectivity and sensitivity towards Cu^{2+}. Rhodamine 6G derivatives **RD3** and **RD4** were designed to study the effect of an electron-donating group at the chromone ring. These compounds showed less fluorescence emission upon binding with copper (II) ion and some interference from Ni^{2+} and Pb^{2+} ions. The presence of a strong electron-withdrawing group on the chromone unit in compound **RD2** can significantly affect the solubility and optical properties. The results indicate different electronic distributions among the compound's structure have an influence on their optical sensing properties. Furthermore, Job's plot experiment was used to investigate the stoichiometry of rhodamine-copper (II) ion binding. In an aqueous acetonitrile solution, the rhodamine derivative **RD1** is coordinated to copper (II) ion with a 1:1 stoichiometry, Figure 3. As demonstrated in Scheme 2, a monomeric system forms a 1:1 complex, which is a reversible process.

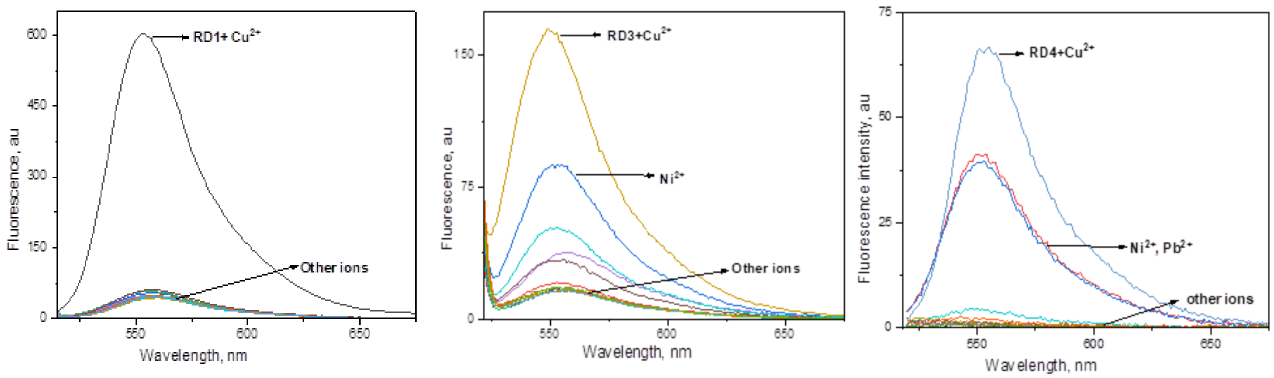


Figure 2. Fluorescence spectra of 20 µM (a) **RD1**, (b) **RD3** (c) **RD4** with 20 mM metal ions in CH_3CN/H_2O (7:3 v/v) HCl-tris buffer solution.

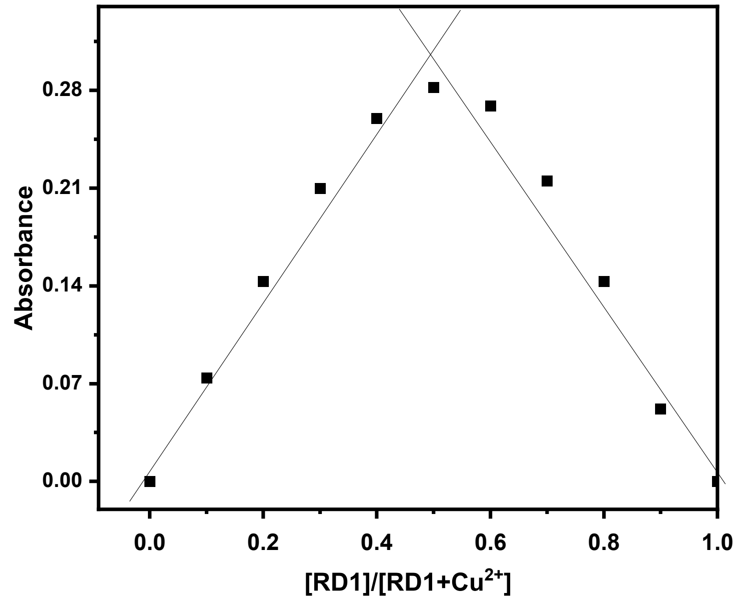
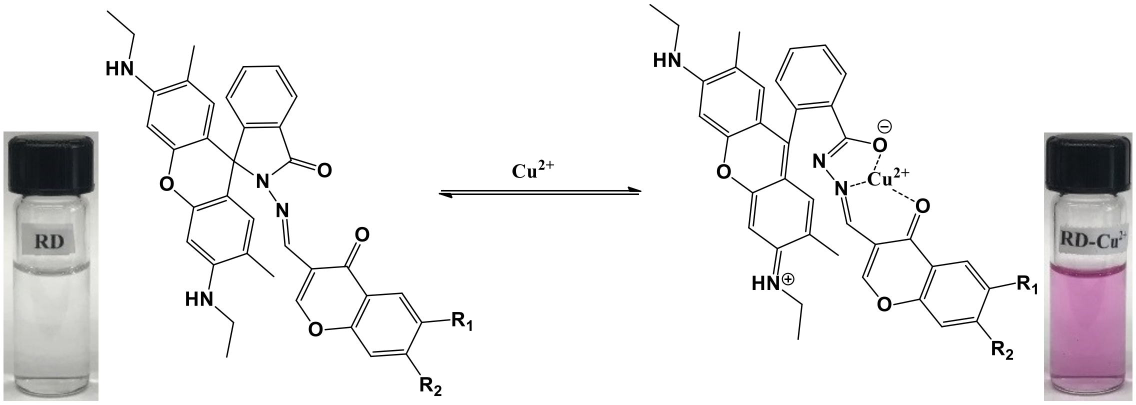


Figure 3. Binding stoichiometry and Job's plot experiment for compound **RD1** and Cu^{2+} in acetonitrile solution.



Scheme 2. A possible proposed binding mechanism of compounds **RD1-RD4** towards Cu^{2+}.

4. Conclusions

The microwave irradiation synthesis method described here is the most convenient way to synthesize the rhodamine 6G derivatives. The CEM single mode microwave irradiation system has provided substantially decreased reaction time, high yield, simple experimental procedure, and environmental friendliness. Upon binding, copper (II) ion triggers the formation of highly fluorescent ring-open spirolactam system, while other ions showed no significant change. The recognition ability of compounds **RD1-RD4** was investigated by absorbance and fluorescence spectroscopy.

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.

Acknowledgments

This research was funded by National Science Foundation's Division of Chemistry under grant [2100629] and National Institute of General Medical Sciences of the National Institutes of Health under [SC2GM125512] grants awarded to Morgan State University.

Reference

1. Beija, M.; Afonso, C.; Martinho, (2009) Synthesis and applications of rhodamine derivatives as fluorescent probes. J. Chem. Soc. Rev, **38**, 2410–2433.
2. Goncalves, M.; Dreyer, J.; Lupieri, P.; Patino, C.; Ippoliti, E.; Webb, M.; Carrie, J.; Carloni, P. (2013) Structural prediction of a rhodamine-based biosensor and comparison with biophysical data. Physical Chemistry Chemical Physics, **15**, 2177–2183.
3. Zhan, T.; Feng, X.; Tong, B.; Shi, J.; Chen, L.; Zhi, J.; Dong, Y. (2012) A novel "turn-on" fluorescent chemosensor for selective detection of Al^{3+} based on aggregation-induced emission. Chemical communication, **48**, 416–418.
4. Lohar, S.; Banerjee, A.; Sahana, A.; Banik, A.; Mukhopadhyay, S.; Das, D. (2013) A rhodamine-naphthalene conjugate as a FRET based sensor for Cr^{3+} and Fe^{3+} with cell staining application. Analytical Methods, **5**, 442–445.
5. Weerasinghe, A., Oyeamalu, A., Abebe, F.; Venter, A.; Sinn, E. (2016) Rhodamine based turn-on sensors for Ni^{2+} and Cr^{3+} in organic media: detecting CN^{-} via the metal displacement approach. Journal of Fluorescence, **26**, 891-898.
6. Rakeshwar, B.; Anca, P.; Aude, V.; Ann, K.; Friedhelm, S.; Kevin, B. (2006) Synthesis of a new water-soluble rhodamine derivative and application to protein labeling and intracellular imaging. Bioconjugate Chem. **17**, 1219–1225.
7. Hasegawa, T.; Kondo, Y.; Koizumib, Y.; Sugiyamab, T.; Takeda, A.; Ito S. (2009) A highly sensitive probe detecting low PH area of HeLa cells based on rhodamine B modified beta-cyclodextrins. Bioorganic and Medicinal Chemistry, **17**, 6015–6019.
8. Islam, M.; Chakraborty, C.; Pandya, P.; Masum, A.; Gupta, N.; Mukhopadhyay, S. (2013) Binding of DNA with rhodamine B: spectroscopic and molecular modeling studies. Dyes and Pigments, **99**, 412–422.
9. Abebe, F.; Gonzalez, J.; Dennis, K.M.; Shaw, R. (2020) A new bis (rhodamine)-based colorimetric chemosensor for Cu^{2+}. Inorganic chemistry Communications, **120**, 108154.
10. Abebe, F.; Perkins, P.; Shaw, R.; Tadesse, S. (2020) A rhodamine-based fluorescent sensor for selective detection of Cu^{2+} in aqueous media: synthesis and spectroscopic properties. Journal of molecular structure, **1205**, 127594.
11. Gao, Z.; Kan, C.; Liu, H.; Zhu, J.; Bao, X. (2019) A highly sensitive and selective fluorescent probe for Fe^{3+} containing two rhodamine B and thiocarbonyl moieties and its application to live cell imaging. Tetrahedron, 75, 1223-1230.
12. Weerasinghe, A.J.; Schmiesing, C.; Sinn, E. (2011) Synthesis, characterization, and evaluation of rhodamine-based sensors for nerve gas mimics. Tetrahedron, **67**, 2833-2838.
13. Du, W.; Cheng, Y.; Shu, W.; Wu, B.; Kong, Z.; Qi. Z. (2017) The influence of different substituents on spectral properties of rhodamine B based chemosensors for mercury ion and application in EC 109 cells. Canadian Journal of Chemistry, **95**, 751-757.
14. Abebe, F; Sutton, T; Perkins, P; Makins-Dennis, K; Winstead, A. (2018) Microwave-assisted synthesis of rhodamine derivatives. Green Chemistry Letters and Reviews, **11**, 237-245.
15. Oliveira, A.S.; Llanes, L.; Nunes, R.J.; Yunes, R.A.; Brighente, I. (2014) Use of ultrasound and microwave irradiation for clean and efficient synthesis of 3,3'-(Arylmethylene)bis (2-hydroxynaphthalene-1,4-dione) derivatives. Green and Sustainable Chemistry, **4**, 177-184.
16. Vahabi, V.; Hatamjafari. (2014) Microwave assisted convenient one-pot synthesis of coumarin derivatives via pechmann condensation catalyzed by FeF_3 under solvent-free conditions and antimicrobial activities of the products. Molecules, **19**, 13093-13103.

17. Pistara, V.; Rescifina, A.; Chiacchio, A.; Corsaro, A. (2014) Use of microwave heating in the synthesis of heterocycles from carbohydrates. Current Organic Chemistry, **18**, 417-445.
18. Aduroja, O.; Shaw, R.; Abebe, F. (2022) A bis(rhodamine 6G)-based fluorescent sensor for Hg^{2+}: microwave-assisted synthesis, photophysical; properties, and computational studies. Research on Chemical Intermediate, **48**, 1847-1861.
19. Zhai, Y., Huang, M., Jiang, L. and Liao, H. (2021) Ratiometric Fluorescence Detection of 6-Mercaptopurine Based on the Nanohybrid of Fluorescence Carbon Dots and Gold Nanoclusters. Journal of Sensor Technology, 11, 39-53.
20. Weerasinghe, A.; Schmiesing, C.; Sinn, E. (1011) Synthesis, characterization, and evaluation of rhodamine-based sensors for nerve gas mimics. Tetrahedron, **67**, 2833-2838.
21. Martynov, I.; Barachersky, V.; Ayt, A.; Kobeleva, O.; Valova, T.; Levchenko, K.; Yarovenko, V.; Krauvshkin, M. (2014) Fluorescence properties of light-sensitive chromone used in archival polymer recording media. Optical Materials, **37**, 488-492.
22. Czaplyski, W.; Purnell, G.; Roberts, C.; Allred, R.; Harbron, E. (2014) Substituent effects on the turn-on kinetics of rhodamine-based fluorescent pH probes. Organic and Biomolecular Chemistry, **12**, 526-533.
23. Stratton, S.; Taumoefolau, G.; Purnell, G.; Rasooly, M.; Czaplyski, W.; Harbron, E. (2017) Turning the pKa of fluorescent rhodamine pH probes through substituent effects. Chemistry A European Journal, **23**, 14064-14072.
24. Maedaa, H; Uenoa, R; Furuyamaa, T; Segia, M. (2020) Effects of substituents on absorption and fluorescence properties of trimethylsilylethynyl- and tert-butylethynyl-pyrenes. Journal of Photochemistry and Photobiology A: Chemistry, **392**, 112428.
25. Abou-Hatab, S; Spata, V; Matsika, S. (2017) Substituent effects on the absorption and fluorescence properties of anthracene. Journal of Physical Chemistry A, 121, 1213-1222.