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ORIGINAL ARTICLE

Synthesis, X-ray and Fluorescence Characteristics of Pyrimido[5,4-*e*]thiazolo[3,2-*a*]pyrimidine as a Novel Heterocyclic System

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Abstract Derivatives of a new heterocyclic system, pyrimido[5,4-*e*]thiazolo[3,2-*a*]pyrimidine **3**, were prepared by sequential treatment of ethyl 4-chloro-2-(methylthio)pyrimidine-5-carboxylate **1** with 4,5-dihydrothiazol-2-amine **2** and various secondary amines. Single crystal X-ray analysis confirmed the structure of the regioisomer **3**. The photophysical characterization of these new compounds was performed by UV/VIS absorption and fluorescence emission spectroscopy. Out of six derivatives studied, only four products **4a–d** showed relatively strong fluorescence intensity. The relevant photophysical parameters for all derivatives in this series, including quantum yields and Stokes shifts for the best fluorophores are given.

Keyword Pyrimidothiazolopyrimidine · X-ray · Heterocyclization · Fluorescence spectroscopy

Introduction

Pyrimidopyrimidines as an important fused heterocyclic system have interested a great concern due to their wide range of biological activities [1–3]. The literature surveys revealed us they have been used as antitumor [4], antiviral [5], antifungal [6], antimicrobial [7] and antiplatelet [8] agents along with potent inhibitors of the tyrosine kinase domain of epidermal growth factor receptor [9] and 5-phosphoribosyl-1-pyrophosphate synthetase [10]. The [3 + 3] ring closure of 2-aminopyrimidines with a variety of bifunctional electrophiles [11, 12] as well as the treatment of 4,6-dichloro-5-formylpyrimidine with primary amines and aldehydes [13] are the most common synthetic pathways to pyrimidopyrimidines.

In addition, thaizolopyrimidines have been reported to display a range of significant biological properties such as calcium channel and CXCR2 receptor antagonists [14], antitumor, antimetastatic and antiinflammatory agents [15]. The synthesis of thaizolopyrimidines have been achieved via the reaction of 2-thiouracil with ethyl γ-chloroacetoacetate [16] or 1,2-dibromoethane [17] and intramolecular cyclization of 1-(2-hydroxyethyl)-thiouracil with mesyl chloride [18]. The other strategies involve the cyclization of 2-amino-2-thiazoline with ethoxymethylenemalonic esters [19], diketenes [20], acetylene dicarboxylic esters [21] and ethyl malonyl chlorides [22].

In conjunction with our previous work based on the synthesis of new heterocyclic compounds with interesting pharmacological and fluorescent properties [23–26], we wish to report the synthesis of several derivatives of pyrimido[5,4-*e*]thiazolo[3,2-*a*]pyrimidine as a novel heterocyclic system together with evaluation of their photophysical properties through their UV/Vis absorption and fluorescence emission spectra.

Experimental

Melting points were taken on an Electrothermal type 9200 melting point apparatus and are uncorrected. The IR spectra were obtained in KBr disks on an Avatar 370 FT-IR Thermo-

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Nicolet spectrometer (ν_{max} in cm^{-1}). The ^1H NMR spectra were recorded on a Bruker AC at 300 MHz using tetramethylsilane (TMS) as an internal reference. Chemical shift values were given in ppm downfield from TMS. The Mass spectra were obtained on a Varian Mat CH-7 instrument at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyzer. A hemisphere of X-ray data was collected on a Bruker D8 Venture Photon 100 diffractometer using ω scans under control of the *APEX2* [27] software package. Raw intensities were reduced to F^2 values with *SAINT* [27] which also performed a global refinement of unit cell parameters. Absorption corrections and merging of equivalent reflections was carried out with *SADABS* [27] and the structure solved by a combination of Patterson and direct methods (*SHELXT* [27]). The structure was refined by full-matrix, least-squares methods *(SHELX-2014* [28]) with hydrogen atoms attached to carbon placed in calculated positions and that attached to nitrogen placed in a location derived from a difference map and adjusted to give N–H = 0.91 Å. All hydrogen atoms were included as riding atoms with isotropic displacement parameters tied to their attached atoms.

2-(Methylthio)-7,8-dihydro-5H-pyrimido[5,4-e] thiazolo[3,2-a]pyrimidin-5-one (3)

To a solution of 2-aminothiazole **1** (1 mmol, 0.1 g) and Et_3N (1.5 mmol, 0.2 ml) in CH_3CN (10 mL), ethyl 4-chloro-2-(methylthio) pyrimidine-5-carboxylate **2** (1 mmol, 0.23 g) was added. The solution was stirred for 2 h at room temperature. The resulting precipitate was filtered off, washed with water (2 × 50 mL) and dried. Yield =80%, mp = 247–250 °C, ^1H NMR (300 MHz, DMSO-d_6) δ 2.57 (s, 3H, CH_3), 3.64 ppm (t, *J* = 7.8 Hz, 2H, CH_2), 4.58 ppm (t, *J* = 7.8 Hz, 2H, CH_2), 8.98 (s, 1H, CH-pyrimidine). ^{13}C NMR (75 MHz, DMSO) 14.4, 27.0, 49.1, 106.2. 155.3, 158.5, 166.4, 172.5, 176.3. IR (KBr disc, cm^{-1}) ν 3039, 2970, 2929, 1659, 1584. MS (*m/z)* 252 (M^+). Anal. Calcd. for $C_9H_8N_4OS_2$% C, 42.84; H, 3.20; N, 22.21; S, 25.41. Found C, 42.76; H, 3.35; N, 22.12; S, 25.59.

General Procedure for the Preparation of Compounds (4a–f)

The appropriate secondary amine (30 mmol) was added to a stirred mixture of compound **3** (1 mmol, 0.25 g) in acetonitril (10 mL), and the solution was heated under reflux for 3 h. The progress of the reaction was monitored by TLC using $CHCl_3$:MeOH (20:1). The solid obtained on cooling was filtered and recrystallized from ethanol.

2-(Pyrrolidin-1-yl)-7,8-dihydro-5H-pyrimido[5,4-e] thiazolo[3,2-a]pyrimidin-5-one (4a)

White powder, yield =55%, mp = 294–298 °C, ^1H NMR (300 MHz, DMSO-d_6) δ 1.96 (m, 4H, $2CH_2$), 3.48 (m, 6H, $2CH_2N$ and $2CH_2$), 4.47 (t, *J* = 7.2 Hz, 2H, $2CH_2$), 8.78 ppm (s, 1H, pyrimidine). ^{13}C NMR (75 MHz, DMSO) 26.7, 43.9, 46.1, 48.7, 100.8. 156.5, 160.6, 161.3, 166.6, 171.1. IR (KBr disc) ν 3132, 3088, 2958, 2873, 1677, 1586 cm^{-1}. MS (*m/z)* 275 (M^+). Anal. Calcd. for $C_{12}H_{13}N_5OS$ % C, 52.35; H, 4.76; N, 25.44; S, 11.64. Found C, 52.22; H, 4.89; N, 25.46; S, 11.94.

2-(Piperidin-1-yl)-7,8-dihydro-5H-pyrimido[5,4-e] thiazolo[3,2-a]pyrimidin-5-one (4b)

White powder, yield =73%, mp. = 147–150 °C, ^1H NMR (300 MHz, DMSO-d_6) δ 1.56 (m, 2H, CH_2), 1.67 (m, 4H, $2CH_2$), 3.57 (t, *J* = 7.5 Hz, 2H, $2CH_2$), 3.85 (m, 4H, $2CH_2N$), 4.47 (t, *J* = 7.2 Hz, 4H, $2CH_2$), 8.76 ppm (s, 1H, pyrimidine). ^{13}C NMR (75 MHz, DMSO) 22.4, 25.8, 26.7, 45.0, 48.7, 100.3, 156.5, 160.5, 166.4, 170.9, 172.5. IR (KBr disc) ν 3005, 2933, 2855, 1653 cm^{-1}. MS (*m/z)* 289 (M^+).

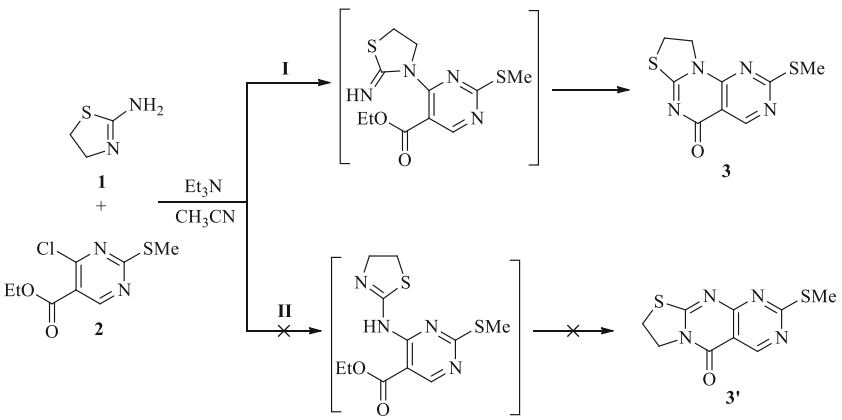

Fig. 1 Scheme for synthesis compound of 2-(methylthio)-8,9-dihydro-5H-pyrimido[5,4-*e*]thiazolo[3,2-*a*]pyrimidin-5-one

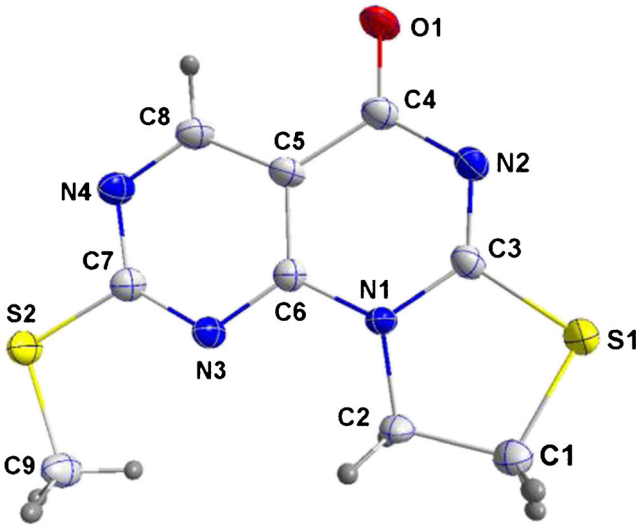

Fig. 2 ORTEP view of compound **3**. The displacement ellipsoids are shown at the 50% probability level

Anal. Calcd. for $C_{13}H_{15}N_5OS$ % C, 53.96; H, 5.23; N, 24.20; S, 11.08. Found C, 53.99; H, 5.42; N, 24.57; S, 11.20.

2-(4-Methylpiperidin-1-yl) -7,8-dihydro-5H-pyrimido[5,4-e]thiazolo[3,2-a] pyrimidin-5-one (4c)

White powder, yield =68%, mp. = 200–202 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.93 (d, J = 5.7 Hz 3H, CH_3), 1.08 (m, 2H, CH_2) 1.72 (m, 3H, CH_2 and CH), 2.98 (t, J = 12 Hz, 2H aqvatorial, CH_2), 3.57 (t, J = 7.5 Hz, 2H, CH_2), 4.46 (t, J = 7.2 Hz, 2H, CH_2), 4.76 (m, axial 2H, CH_2N), 8.75 ppm (s, 1H, pyrimidine). ^{13}C NMR (75 MHz, DMSO) 22.0, 26.7, 30.8, 34.0, 44.3, 48.7, 100.4, 156.5, 160.5, 161.2, 166.5, 170.9. IR (KBr disc) ν 3003, 2950, 2911, 2865, 1668, 1610 cm^{-1}. MS (*m/z*) 303 (M^+). Anal. Calcd. for $C_{14}H_{17}N_5OS$ % C, 55.43; H, 5.65; N, 23.08; S, 10.57 Found C, 55.59; H, 5.55; N, 23.34; S, 10.25.

2-Morpholino-7,8-dihydro-5H-pyrimido[5,4-e] thiazolo[3,2-a]pyrimidin-5-one (4d)

White powder, yield =79%, mp = 285–290 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.35 (t, J = 7.5, 2H, CH_2), 3.68 (t, 4H, J = 5.1 Hz, 2CH_2N), 3.86(m, 4H, 2CH_2O), 4.49 ppm (t, 2H, J = 7.5, CH_2); 8.81 ppm (s, 1H, pyrimidine). ^{13}C NMR (75 MHz, DMSO) 26.7, 44.5, 48.7, 66.3, 101.0, 151.4, 156.5, 160.6, 166.5, 171.2. IR (KBr disc) ν 3182, 3076, 2962, 2921, 2859, 1705, 1660 cm^{-1}. MS (*m/z*) 291 (M^+). Anal. Calcd. for $C_{12}H_{13}N_5O_2S$ % C, 49.47; H, 4.50; N, 24.04; S, 11.00. Found C, 49.41; H, 4.72; N, 24.26; S, 11.16.

2-(4-Methylpiperazin-1-yl) -7,8-dihydro-5H-pyrimido[5,4-e]thiazolo[3,2-a] pyrimidin-5-one (4e)

White powder, yield =68%, mp = 249–250 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.23 (s, 3H, CH_3), 2.39 (m, 4H, CH_2-N), 3.58 (t, J = 7.5 Hz, 2H, CH_2), 3.78 (m, 4H, 2CH_2N), 4.49 (t, J = 7.5 Hz, 2H, CH_2), 8.79 ppm (s, 1H, pyrimidine). ^{13}C NMR (75 MHz, DMSO) 26.5, 44.8, 48.7, 51.9, 52.5, 100.9, 156.5, 160.5, 161.3, 166.3, 171.0. IR (KBr disc) ν 3015, 2949, 2864, 2798, 1640, 1608 cm^{-1}. MS (*m/z*) 304 (M^+). Anal. Calcd. for $C_{13}H_{16}N_6OS$ % C, 51.30; H, 5.30; N, 27.61; S, 10.53 Found C, 51.63; H,5.50; N, 27.35; S, 10.37.

2-(4-Ethylpiperazin-1-yl)-7,8-dihydro-5H-pyrimido[5,4-e] thiazolo[3,2-a]pyrimidin-5-one (4f)

White powder; yield: 78%; mp = 246–248 °C; ^{1}H NMR (300 MHz, , DMSO-d_6): δ 1.04 (t, J = 7.2 Hz, 3H, CH_3),

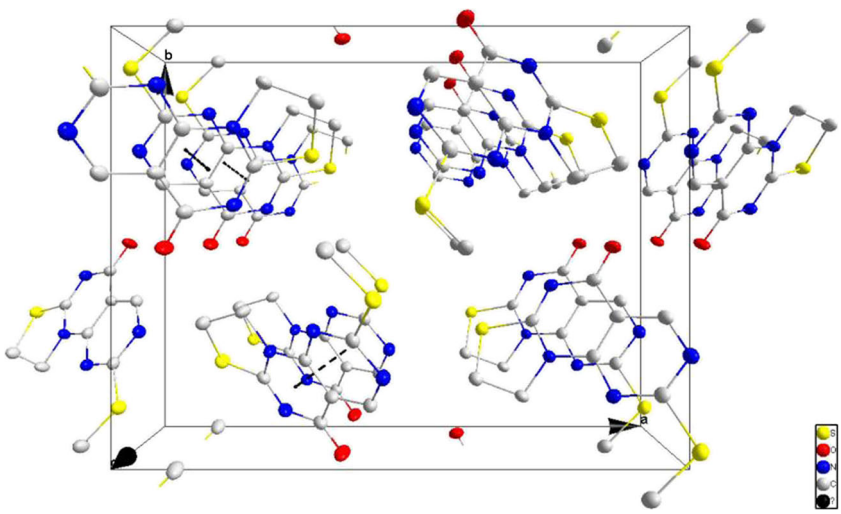

Fig. 3 Packing of compound **3** viewed down the c axis

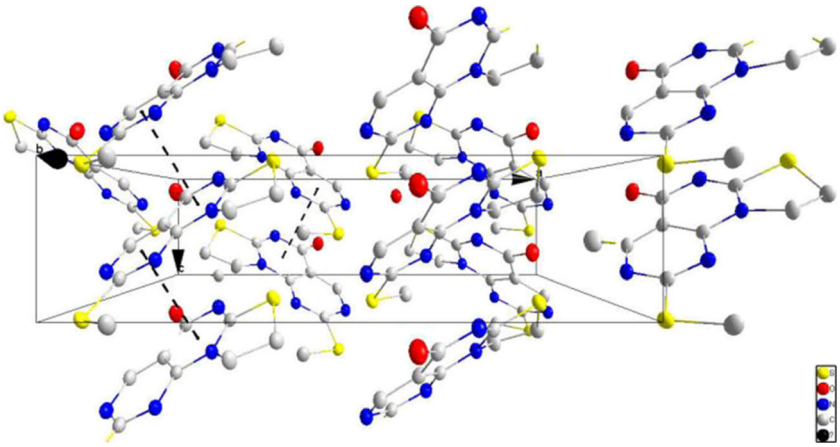

Fig. 4 Packing of compound **3** viewed approximately along (110)

2.37 (q, J = 7.2 Hz, 2H, CH_2), 2.45 (m, 4H, CH_2-N), 3.58 (t, 2H, J = 7.2 Hz, CH_2-N), 3.87 (m, 4H, CH_2-N), 4.49 (t, 2H, J = 7.2 Hz, CH_2),8.79 (s, 1H, pyrimidine).^{13}C NMR (75 MHz, DMSO) 12.3, 26.7, 44.1, 48.7, 51.9, 52.5, 100.8. 156.5, 160.5, 161.3, 166.5, 171.1. IR (KBr disc) ν 3019, 2966, 2937, 2831, 1652, 1609 cm^{-1}. MS (*m/z)* 318 (M^+). Anal. Calcd. for $C_{14}H_{18}N_6OS$ % C, 52.81; H, 5.70; N, 26.40; S, 10.07 Found C, 52.72; H, 5.75; N, 26.25; S, 10.12.

Results and Discussion

Chemistry

The presence of two electron-enriched positions in 2-aminothiazoline ring **1** was led to the reaction of this compound with electrophiles either at the exocyclic or endocyclic nitrogen atom depending on the electrophile and reaction conditions [29, 30]. Furthermore, ethyl 4-chloro-2-(methylthio)pyrimidine-5-carboxylate **2**, which was prepared according to the reported procedure [31], possesses two possible leaving groups, namely Cl and OEt, in which the chlorine atom has been reported to be more reactive than ethoxy moiety [32, 33].

Treatment of **1** with **2** in the presence of Et_3N in CH_3CN may lead to the synthesis of two possible regioisomers **3** and **3′** as depicted in Fig. 1. The experimental and spectral data showed the formation of only one product with no producing any isolable intermediate. The ^{1}H NMR spectrum of the product displayed a singlet signal at δ 2.51 ppm for the methyl group, two triplet signals at δ 3.64 and 4.58 for the hydrogens of thiazoline ring and a sharp singlet signal at 8.98 ppm belonging to the hydrogen of the pyrimidine moiety. The ^{13}C NMR spectrum also clearly showed nine resolved signals for the corresponding carbons of the compound. The IR spectrum of the product did not exhibit any bands of the starting materials (NH_2 and ester groups at 3389, 3419 and 1734, respectively) and the mass spectrum also showed the molecular ion peak at *m/z* 252 (M^+) indicating the molecular formula of $C_9H_8N_4OS_2$.

The ambiguity in the structure of the product can be clearly resolved through single crystal X-ray analysis (deposition no. CCDC 1442522) (Figs. 2, 3 and 4).

The single crystal X-ray crystallographic data clearly demonstrates the occurrence of nucleophilic substitution on precursor **2** with 4,5-dihydrothiazol-2-amine **1** through path **I** in Fig. 1 along with the subsequent cyclization reaction to give product **3**.

Upon on the regiochemistry of the product, it appears that the endocyclic amino group of 2-aminothiazole **1** is more nucleophilic than the other amino group and attacks the more electrophilic C-Cl bond on precursor **2** which is followed by an intramolecular acyl nucleophilic substitution on COOEt

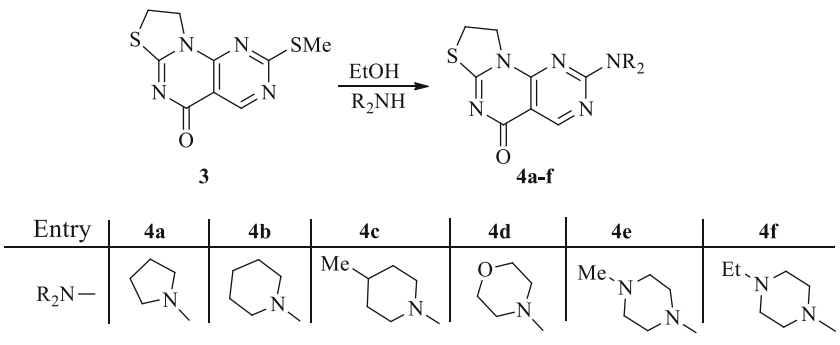

Fig. 5 Scheme for synthesis derivatives of 2-amino-8,9-dihydro-5H-pyrimido[5,4-*e*]thiazolo[3,2-*a*]pyrimidin-5-one

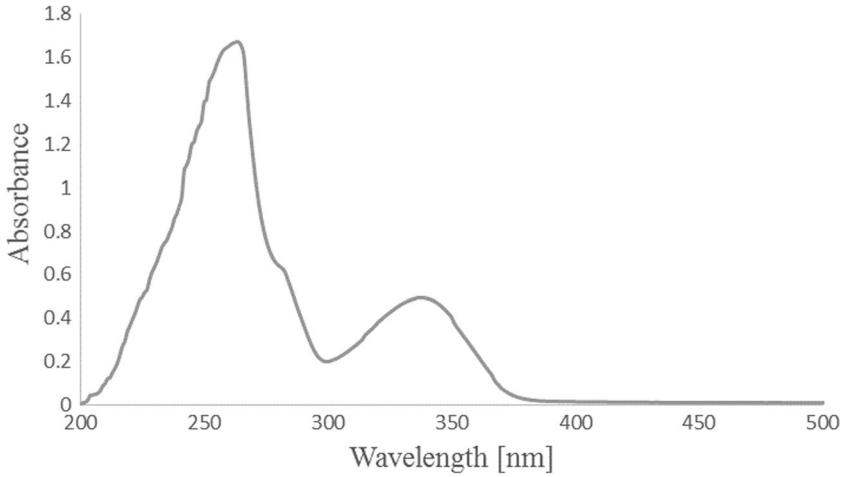

Fig. 6 UV/Vis Absorption spectrum of compound **4c** in dilute CH_3CN solution (2×10^{-5} M)

fragment to form the final product **3**. Then, the treatment of compound **3** with various cyclic secondary amines afforded the corresponding products **4a–f** (Fig. 5).

The structures of compounds **4a–f** have been confirmed by their spectral data. For example, the ^{1}H NMR spectrum of **4d** showed two triplet signals at δ 3.68 and 3.86 ppm corresponded to the hydrogens of the morpholine moiety, two triplet signals at δ 3.35 ppm and 4.49 ppm for the hydrogens of the thiazoline core and a sharp singlet peak at 8.81 ppm assigned to the hydrogen of the pyrimidine core. The ^{13}C NMR spectrum clearly showed four resolved signals at 26.8, 44.5, 48.7 and 66.3 ppm for the aliphatic carbons of compound **4d** and the other six signals at 101.0, 151.4, 156.5, 160.6, 166.5, 171.2 ppm were assigned to the aromatic carbons. The mass spectrum of compound **4d** also showed the molecular ion peak at *m/z* 291 (M^+) indicating the molecular formula of $C_{12}H_{13}N_5O_2S$.

Photophysical Study

The design and synthesis of selective and sensitive compounds containing the pyrimidine moiety with fluorescent probes are attractive research topics in the fields of optoelectronics [34], chemosensors and molecular imaging owing to their biological and environmental interest [35, 36]. Fluorescent probes are the important instrumentation in gaining insight into cellular and bacterial environments [37, 38], and fluorescence spectroscopy is one of the most useful techniques to probe the binding of liposomes to proteins, DNA, and other biomolecules in chemical biology [39, 40]. Herein, we describe the evaluation of these new fluorescent pyrimidine analogs and provide their structure-photophysical properties relationships.

The absorption spectrum of **4c** was scanned in CH_3CN (5×10^{-5} M) and shown in Fig. 6. The wavelengths of maximum absorption (λ_{max}) were in 275 and 340 nm but the highest emission was observed when the compound was excited in 340 nm.

All the absorption and emission spectra of compounds **4a–f** were recorded in CH_3CN, and their λ_{max} and λ_{flu} values were compiled in Table 1.

Values of the extinction coefficient (ε) were calculated as the slope of the plot of absorbance *vs.* concentration and had a precision on the order of 5%. The fluorescence quantum yield (Φ) which is the ratio of photons absorbed to photons emitted through fluorescence relative to fluorescein as the reference compound were calculated according to the literature [41]. (Table 1) Compounds **4a–d** exhibited moderately good fluorescence quantum yields. The highest value was obtained for compound **4c** (Φ = 0.80). The compounds under this study exhibited the stokes shifts ranging from 70 to 95 nm that was calculated from the difference between absorption and emission maxima in cm^{-1} [42].

Photophysical data in Table 1 demonstrated that all compounds were neutral, uncharged, highly fluorescent molecules that were absorbed with high extinction coefficients and emitted at around 410–425 nm. Compounds like biogenic amines are markers for many processes, e.g. recognition of cancer, so that their detection and determination are important [43, 44]. Amines which do not posses an aromatic system can not be detected directly by UV absorption or fluorescence. For

Table 1 Spectroscopic Properties of Products **4a–f** in CH_3CN

Compound	λ_{abs} [nm]	$\varepsilon \times 10^{-3}$ [M^{-1} cm^{-1}] [a]	λ_{flu}	Φ [b]	Stokes shift
4a	338	7.55	415	0.70	77
4b	332	8.00	415	0.59	83
4c	340	11.78	410	0.80	70
4d	330	7.85	425	0.52	95
4e	336	11.21	415	-	79
4f	336	7.83	425	-	89

[a] Extinction coefficient.

[b] Quantum yield of fluorescein in MeOH as standard (Φ = 0.95) [41].

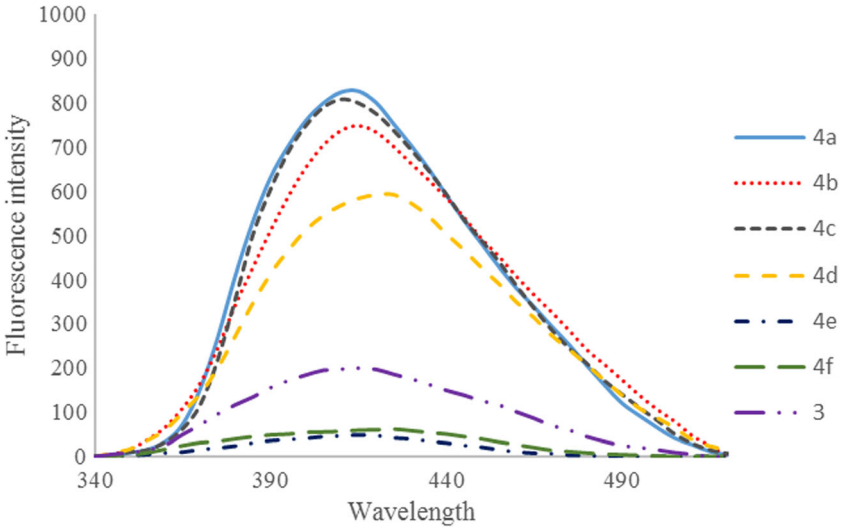

Fig. 7 Emission spectrum of compounds **4a–f** at λ_{ex} 330–340 nm in dilute CH_3CN solution (2×10^{-7} M)

determination of these compounds, especially at low concentrations, many fluorogenic reagents have been developed [45–47]. Moreover, the known fluorescence and pharmacological properties of pyrimidine [48, 49] and thiazole moieties [50–52] as well as the factors promoting proliferation in the biological and photophysical properties and possible relationship between these substances [53] were persuaded us to synthesize thiazolopyrimidine derivatives and study their fluorescence properties. For this purpose, the thiomethyl derivative **3** was converted into products **4a–f**, and speculates that the presence of amino group at the 2-position in pyrimidothiazolopyrimidine plays an important role in the aforementioned strong fluorescence. Compounds **4a–d**, which have morpholine, pyrrolidine, piperidine or 4-methylpiperidine group at the 2-position of the pyrimidine ring, showed stronger fluorescence intensity than did the precursor **3** with methylsulfanyl group. Compounds **4e** and **4f** showed very weak fluorescence intensities. (Fig. 7) This could mean that a new or extended planar system with enhanced delocalization of π-bonding has been formed.

The emission spectra of chemical compounds can be affected by the medium and they can bring about a change in the position, intensity, and shape of absorption bands [54]. Photophysical properties of **4c** have been studied in a number of organic solvents and H_2O. Fig. 8 shows the emission spectra of **4c** in $CHCl_3$, DMF, THF, MeCN, MeOH, EtOH, and H_2O. The fluorescence intensity maxima were found to vary with the nature of the solvent which can be attributed to solvatochromic properties [55]. As can be seen, aprotic solvents such as $CHCl_3$, DMF, THF and MeCN showed the higher emission value in comparison with the protic ones. The aprotic solvents do not have hydrogen bond with the solute and the spectrum of the solute closely approximates the spectrum that would be produced in the gaseous state, in which fine structure is often observed. In protic solvents, hydrogen bond forms a solute-solvent complex and the fine structure may disappear [56].

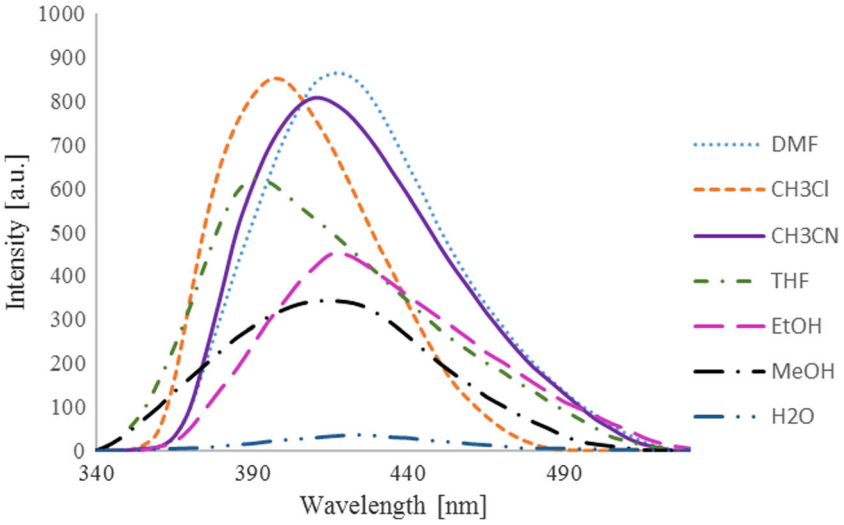

Fig. 8 Fluorescence intensity of a solution of compound **4c** (2×10^{-7} M) in the solvents DMF, $CHCl_3$, CH_3CN, THF, EtOH, MeOH and H_2O at $\lambda_{ex.}$ = 340 nm

Conclusion

In summary, we have described the synthesis of various derivatives of the new heterocyclic system, pyrimido[5,4-*e*]thiazolo[3,2-a]pyrimidin-5-one via sequential treatment of ethyl 4-chloro-2-(methylthio)pyrimidine-5-carboxylate **1** with 4,5-dihydrothiazol-2-amine **2** and subsequently with secondary amines to obtain products **4a–f**. An account on the single crystal X-ray analysis of the product **3** along with the photophysical properties of the corresponding substituted derivatives **4a–f** was given. Due to their interesting fluorescence properties, these new heterocyclic pyrimido[5,4-*e*]thiazolo[3,2-a]pyrimidine derivatives can be used as fluorescent markers and probes for studies in biochemistry and pharmacology.

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