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RESEARCH ARTICLE

Novel 1,3,4-Thiadiazole Linked Amide Derivatives of Pteridone: Synthesis and Study of Anticancer Activities

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Abstract: Cancer is a second leading cause of death after heart attack, in developing as well as undeveloped countries. It is caused by unregulated growth and metastasis of the abnormal cancer cells. Cancer can be cured by radiation, immunotherapy and chemotherapy, among them; chemotherapy is a good treatment for cancer, in which chemotherapeutic drug is used. The anticancer activity of newly compounds (**13a-j**) was carried out on four different types of human cancer cell lines like MCF-7 (breast), A549 (lung), Colo-205 (colon) and A2780 (ovarian) by the MTT method, and compared to etoposide used as a positive control. Among them, compound **13g** with electron-withdrawing (3,5-dinitro) group, exhibited more promising activity in all cell lines (MCF-7 = 0.10±0.076 μM, A549 = 0.17±0.039 μM, Colo-205= 0.13±0.022 μM and A2780 = 0.87±0.027μM). This compound may act as lead drug in cancer chemotherapy. In future, this compound can be examined for clinical studies.

Keywords: Pteridone, Pralatrexate, Triamterene, Thiamethoxam and anticancer activity.

1. INTRODUCTION

Cancer is a second leading cause of death after heart attack, in developing as well as undeveloped countries [1]. It is caused by unregulated growth and metastasis of the abnormal cancer cells. Cancer can be cured by radiation, immunotherapy and chemotherapy, among them; chemotherapy is the good treatment for cancer, in which chemotherapeutic drug is used. Many researchers are focusing to develop new anticancer agents and medicinal chemistry is the best source for developing, discovery and identification of biologically significant anticancer compounds [2-17].

Pteridines are nitrogen class of heterocyclic compounds present in a wide range of synthetic and natural origin and they also have the pyrazino [2,3-d] pyrimidine nucleus structure. These derivatives displayed a wide range of biological activities including anticancer [18], hepatitis [19], anti-inflammatory [20], antimicrobial [21], antimycobacterial [22], antiradical [23], antifungal [24], antibacterial [25] and immunosuppressive activity [26]. Pralatrexate (**1**, Fig. **1**) is one of the DHFR inhibitors with anticancer properties and approved in 2009 by Food and Drug Administration for the treatment of relapsed peripheral T-cell lymphoma [27].

Triamterene (**2**, Fig. **1**) is used in combination with hydrochlorothiazide as a hypertensive drug since their effects are synergetic [28].

On the other hand, 1,3-thiazole derivatives are five-membered heterocyclics and very useful intermediates in medicinal chemistry. A variety of pharmacological properties such as anticancer [29], antimicrobial [30], anti-allergies [31], hypnotics [32], renal hypertension [33], anti-inflammation [34], anti-HIV infections [35], antifungal [36], antitubercular [37], anti-pseudomonal [38] and CNS are processed [39]. Thiamethoxam (**3**) [40] is a thiazole skeleton unit containing moiety used for agrochemicals.

Many researcher have synthesized various 1,3,4-thiadizole derivatives and reported for various medical uses, those are antitumor [41], antihelicobacter pylori [42], antituberculosis [43], antidepressant [44], antimicrobial [45], antibacterial [46], antifungal [47], anti-inflammatory [48] and antiviral [49].

In view of biological information of both pteridone and thiazole moieties, and continuous of efforts, we have de-

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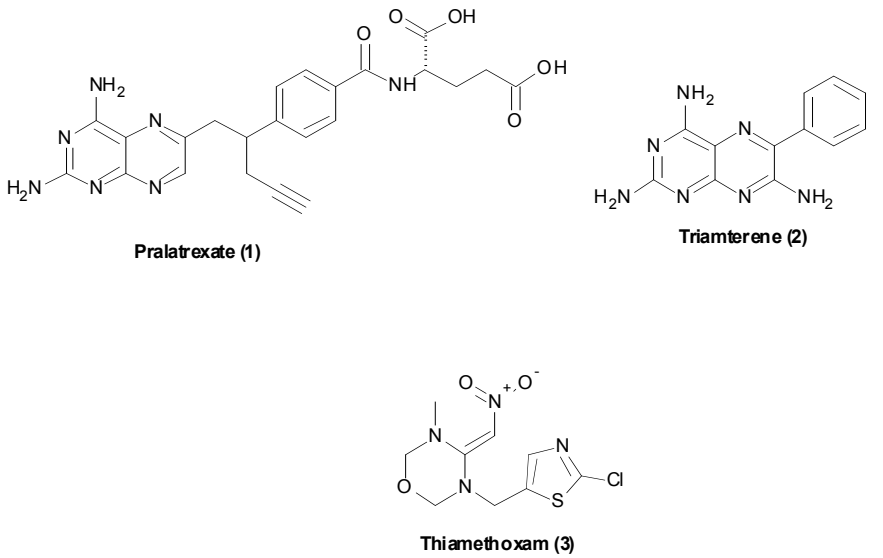


Fig. (1). Structures of Pralatrexate, Triamterene and Thiamethoxam.

signed and synthesized a library of amide derivatives (**13a-j**) of pteridones and their structures were confirmed by ^{1}HNMR, ^{13}CNMR and mass spectral analysis. Further, these derivatives were tested for their anticancer activity on human cancer cell lines.

2. RESULTS AND DISCUSSION

2.1. Chemistry

These newly synthesized compounds were described in Scheme **1**. The reaction of compound 3-aminopyrazine-2-carboxylic acid (**4**) with formaldehyde (**5**) and 2-aminothiazole-5-carboxylic acid (**6**) was dissolved in ethanol and reaction mixture stirred at reflux for 6 hours to afford pure intermediate **7**. Compound **7** was coupled with acid hydrazide (**8**) in the presence of EDCI (*N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride), HOBt (hydroxybenzotriazole) in dry THF and was stirred at room temperature for 6 hours to afford pure intermediate **9**. Then, intermediate **9** underwent cyclization in the presence of lawesson's reagent in anhydrous THF and was stirred at reflux for 4 hours to afford pure compound **10** with good yield. Further, intermediate **10** underwent reduction with iron in ammonium chloride in ethanol and reaction mixture was stirred at reflux for 2 hours to afford pure amine intermediate **11**. Finally, amine intermediate **11** was coupled with different substituted aromatic acid chlorides (**12a-j**) in the presence of triethyl amine in anhydrous THF and was stirred at room temperature for 12 hours to afford pure compounds **13a-j**.

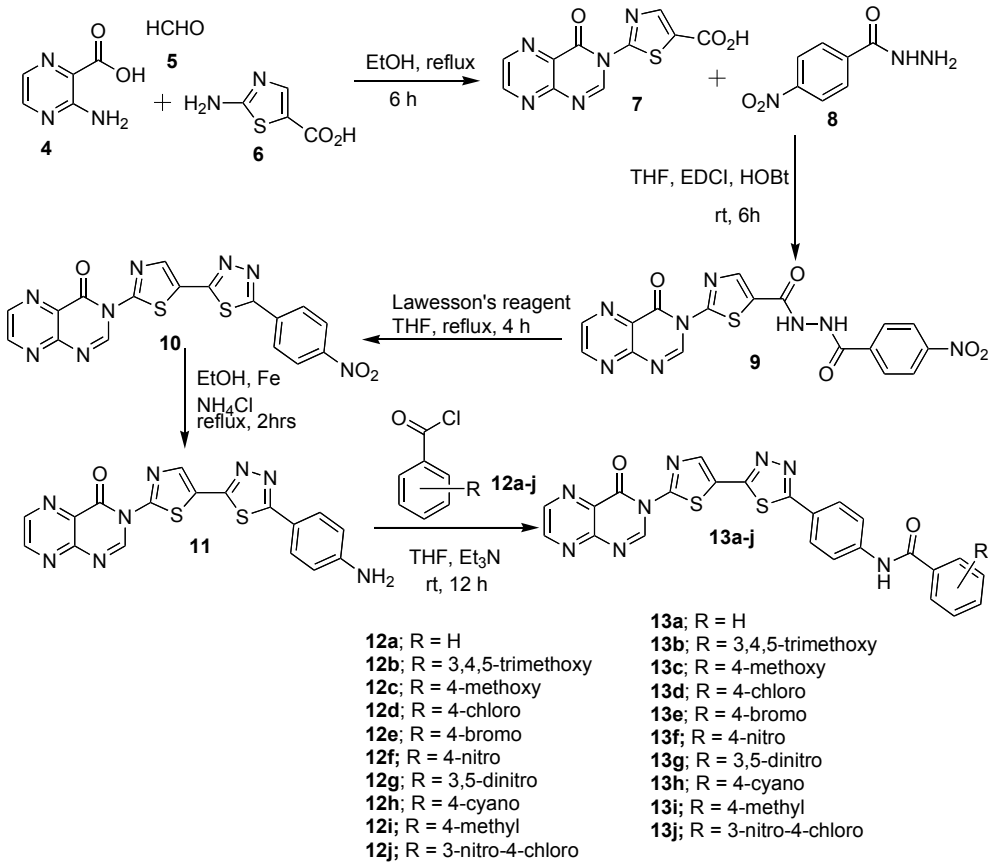
2.2. Biological Activity

2.2.1. In vitro Cytotoxicity

The anticancer activity of the newly compounds (**13a-j**) were carried out on four different types of human cancer cell lines, like MCF-7 (breast), A549 (lung), Colo-205 (colon) and A2780 (ovarian) by the MTT method, and etoposide was used as a positive control. These abstained results are summarized in Table **1**. Most of the synthesized derivatives showed good anticancer activity. Among them, compounds **13b**, **13c**, **13f**, **13g** and **13j** exhibited more potent activity than the positive control. Moreover, all these compounds were examined for their structure-activity relationship (SAR) analysis, which indicated that, compound **13b** with electron-donating (3,4,5-trimethoxy) group on phenyl ring, showed good activity with IC_{50} values ranging from MCF-7 = 0.98±0.091μM, A549 = 0.21±0.043 μM, Colo-205= 1.40±0.82 μM and A2780 = 0.66±0.015 μM. Compound **13c** having only 4-methoxy substitution showed lower activity (MCF-7 = 1.39±0.48 μM, A549 = 2.66±1.23 μM, Colo-205= 2.03±1.56 μM and A2780 = 1.99±0.55 μM) than **13b**. Interestingly, compound (**13g**) with electron-withdrawing (3,5-dinitro) group, exhibited more promising activity in all cell lines (MCF-7 = 0.10±0.076 μM, A549 = 0.17±0.039 μM, Colo-205= 0.13±0.022 μM and A2780 = 0.87±0.027μM). Where compound **13f** with 4-nitro substitution on the phenyl ring, have shown decreased activity (MCF-7 = 0.19±0.014 μM, A549 = 0.15±0.066 μM, Colo-205= 1.22±0.87 μM and A2780 = 1.87±0.44 μM) compared to **13g**. Similarly, compound **13j** having 3-nitro-4-chloro group, have displayed good anticancer activity (MCF-7 = 1.45±0.37 μM, A549 = 0.72±0.061 μM, Colo-205= 1.22±0.45 μM and A2780 = 1.39±0.87 μM), respectively. Whereas, compounds (**13d**, **13e** and **13h**) with 4-chloro, 4-bromo and 4-cyano substitution on the phenyl ring showed moderate activity.

3. EXPERIMENTAL SECTION

All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. ^{1}H and ^{13}C NMR spectra were recorded on Bruker, Bruker UXNMR/XWIN-NMR (400 MHz) instrument. Chemical shifts (d) were reported in ppm downfield from internal TMS standard. ESI+ spectra were recorded on Micro mass, Quattro LC using ESI+ software with



Scheme (1). Novel 1,3,4-Thiadiazole linked amide derivatives of pteridones.

Table 1. Anticancer activity data of newly synthesized compounds 13a-j with IC_{50} µM[a].

Compound	[c]MCF-7	[d]A549	[e]Colo-205	[f]A2780
13a	2.77±1.89	3.42±2.44	16.2±6.43	7.34±3.11
13b	0.98±0.091	0.21±0.043	1.40±0.82	0.66±0.015
13c	1.39±0.48	2.66±1.23	2.03±1.56	1.99±0.55
13d	8.34±5.22	-	11.2±5.23	-
13e	5.22±3.76	10.8±4.56	2.88±1.99	9.66±4.11
13f	0.19±0.014	0.15±0.066	1.22±0.87	1.87±0.44
13g	0.10±0.076	0.17±0.039	0.13±0.022	0.87±0.027
13h	2.18±1.44	3.99±2.09	-	-
13i	13.9±5.34	5.29±3.05	8.66±5.32	-
13j	1.45±0.37	0.72±0.061	1.22±0.45	1.39±0.87
Etoposide	2.11 ± 0.024	3.08 ± 0.135	0.13 ± 0.017	1.31 ± 0.27

"-" = Not active.

[a]Each data represents as mean ±S.D values. From three different experiments performed in triplicates.[b] [c]MCF-7: human breast cancer cell line. [d]A549: human lung cancer cell line. [e]Colo-205: human colon cancer cell line. [f]A2780: human ovarian cancer cell line.

capillary voltage 3.98 kV and ESI mode positive ion trap detector a. For compound 7, MS was recorded on MS (FAB) instrument. Melting points were determined with an electro-thermal melting point apparatus, and are uncorrected.

3.1. 2-(4-Oxopteridin-3(4H)-yl)thiazole-5-carboxylic acid (7)

The equimolar amounts of 0.01 moles each of 3-aminopyrazine-2-carboxylic acid (**4**), (20 g, 0.143 mmol),

formaldehyde (**5**) (4 ml, 0.143 mmol) and 2-aminothiazole-5-carboxylic acid (**6**) (20.6 g, 0.143 mmol), were mixed together and dissolved in 60 mL of ethanol in round bottomed flask. The resulted mixture was stirred for 10 minutes and refluxed for 6 hours. The progress of the reaction was monitored by thin layer chromatography (TLC). After the completion of the reaction, the flask content was poured into in 150 mL of cold water to afford pure compound (**7**) 17.3 g, 44% yield. The products were filtered, washed and dried over anhydrous calcium chloride followed by recrystallisation with ethanol. pale yellow solid; Mp: 228-230^{o}C; ^{1}H NMR (300 MHz, DMSO-d6): δ 7.36 (d, 1H, J = 8.34 Hz), 7.55 (s, 1H), 8.14 (s, 1H), 8.25 (d, 1H, J = 8.34 Hz), 12.5 (brs, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 1293.3, 130.6, 140.3, 143.3, 150.7, 152.2, 156.6, 157.1, 158.2, 160.1; MS (FAB): 275 [M+].

3.2. *N*'5-(4-Nitrobenzoyl)-2-(4-oxo-3,4-dihydro-3-pteridinyl)-1,3-thiazole-5-carbohydrazide (9)

A mixture of 2-(4-oxopteridin-3(4H)-yl)thiazole-5-carboxylic acid (**7**) (15 g, 0.0543 mmol), EDCI (10.4 g, 0.0543 mmol) and HOBt (0.073 mg, 0.000543 mmol) in dry THF (50 mL) was stirred at room temperature for 15 minutes. To this reaction mixture, compound 4-nitrobenzohydrazide (**8**) (9.8 g, 0.0543 mmol) was added and continued stirring at room temperature for 6 hours. The reaction contents were concentrated at reduced pressure to afford pure compound (**9**) 21.2 g, with 89% yield. Pale yellow solid; Mp: 233-236^{o}C; ^{1}H NMR (300 MHz, DMSO-d6): δ 7.37 (d, 1H, J = 8.33 Hz), 7.63 (s, 1H), 7.69 (d, 2H, J = 7.28 Hz), 7.78 (d, 2H, J = 7.28 Hz), 8.17 (s, 1H), 8.25 (d, 1H, J = 8.33 Hz), 9.65 (brs, 1H), 9.81 (brs, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 123.6, 128.8, 130.1, 137.3, 139.5, 140.6, 142.7, 150.1, 151.2, 154.6, 158.3, 158.8, 159.1, 160.4, 167.1; MS (ESI): 439 $[M+H]^{+}$.

3.3. 3-5-[5-(4-Nitrophenyl)-1,3,4-thiadiazol-2-yl]-1,3-thiazol-2-yl-3,4-dihydro-4-pteridinone (10)

A mixture of compound (**9**) (20 g, 0.0456 mmol) and Lawesson's reagent (16.6 g, 0.041 mmol) in tetrahydrofuran (50 mL) was stirred at reflux for 4 hours. After completion of the reaction as monitored by TLC, the crude product was adsorbed over silica gel and purified by column chromatography using ethyl acetate: hexane (7:3) as eluent to afford pure compound **10**, 14.4 g, with 73% yield. pale yellow solid; Mp: 227-229^{o}C; ^{1}H NMR (300 MHz, DMSO-d6): δ 7.36 (d, 1H, J = 8.34 Hz), 7.58 (s, 1H), 7.70 (d, 2H, J = 7.30 Hz), 7.83 (d, 2H, J = 7.30 Hz), 8.16 (s, 1H), 8.24 (d, 1H, J = 8.34 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ 122.4, 124.3, 131.6, 132.7, 137.9, 140.1, 146.3, 148.9, 150.6, 151.7, 158.3, 159.1, 160.2; MS (ESI): 437 $[M+H]^{+}$.

3.4. 3-5-[5-(4-Aminophenyl)-1,3,4-thiadiazol-2-yl]-1,3-thiazol-2-yl-3,4-dihydro-4-pteridinone (11)

To a round bottomed flask was added iron powder (3.3 g, 0.0596 mmol) and ammonium chloride solution (2.4 g, 0.0447 mmol) in 10 mL of distilled water. Compound (**10**) (13 g, 0.0298 mmol) was dissolved in ethanol (50 ml) solvent and the reaction mixture was stirred at reflux for 2 hours. After completion of reaction was filtered followed by hot ethanol was of the inorganic residue and the combined washings to afford pure amine compound **11**, 9.6 g with 79% yield. Mp: 267–270 ^{o}C; pale yellow solid; ^{1}HNMR (400 MHz, DMSO-d6): δ 5.46 (brs, 2H), 7.37 (d, 1H, J = 8.32 Hz), 7.56 (s, 1H), 7.67 (d, 2H, J = 7.28 Hz), 7.71 (d, 2H, J = 7.28 Hz), 8.16 (s, 1H), 8.25 (d, 1H, J = 8.32 Hz); ^{13}C NMR (75 MHz, DMSO-d6): δ 113.8, 122.2, 127.1, 131.3, 133.7, 140.2, 146.1, 150.0, 151.9, 152.6, 158.8, 159.3, 160.0; MS (ESI): 407 $[M+H]^{+}$.

3.5. *N*1-(4-5-[2-(4-Oxo-3,4-dihydro-3-pteridinyl)-1,3-thiazol-5-yl]-1,3,4-thiadiazol-2-ylphe nyl)benzamide (13a)

The compound (**11**) (500 mg, 0.00122 mmol) was dissolved in 10 mL of dry THF, followed by addition of benzoyl chloride (**12a**) (0.14 ml, 0.00122 mmol), and Et_3N (0.34 mL, 0.00244 mmol). The reaction mixture was stirred at room temperature for 12 hours, till the completion of the reaction as monitored by TLC. The reaction mixture was washed with water and extracted with dichloromethane, dried over anhydrous Na_2SO_4 and the crude product was purified by column chromatography with ethyl acetate/ hexane (7:3) to obtain pure compound **13a** in 310.8 mg; pale yellow solid; 50% yield. Mp: 265-267^{o}C, ^{1}H NMR (300 MHz, DMSO-d6): δ 7.36 (d, 1H, J = 8.33 Hz), 7.58 (s, 1H), 7.65 (d, 2H, J = 7.29 Hz), 7.69 (t, 1H), 7.73 (d, 2H, J = 7.29 Hz), 7.78-7.85 (m, 2H), 7.93 (d, 2H, J = 7.36 Hz), 8.15 (s, 1H), 8.26 (d, 1H, J = 8.31 Hz), 8.66 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 123.5, 126.3, 128.6, 129.7, 130.2, 131.2, 132.6, 133.5, 136.8, 140.4, 141.2, 146.7, 150.5, 151.3, 152.9, 156.4, 158.5, 159.3, 160.5, 162.4; MS (ESI): 511 $[M+H]^{+}$; Anal. Calcd for $C_{24}H_{16}N_8O_2S_2$: C, 56.24; H, 3.15; N, 21.86. Found: C, 56.46, H, 3.21; N, 21.94.

3.6. *N*1-(4-5-[2-(4-Oxo-3,4-dihydro-3-pteridinyl)-1,3-thiazol-5-yl]-1,3,4-thiadiazol-2-ylphe nyl)-3,4,5-trimethoxybenzamide (13b)

This compound **13b** was prepared following the method described for the preparation of the compound **13a**, employing (**11**) (500 mg, 0.00122 mmol) with 3,4,5-trimethoxybenzoyl chloride (**12b**) (281 mg, 0.00122 mmol), Et_3N (0.34 mL, 0.00244 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (8:2) to afford pure compound **13b**, 408.4 mg in 55% yield. pale yellow solid; Mp: 278-280^{o}C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.86 (s, 6H), 3.92 (s, 3H), 7.35 (d, 1H, J = 8.29 Hz), 7.44 (s, 2H), 7.57 (s, 1H), 7.66 (d, 2H, J = 7.30 Hz), 7.75 (d, 2H, J = 7.30 Hz), 8.17 (s, 1H), 8.25 (d, 1H, J = 8.29 Hz), 8.65 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.3, 61.8, 107.4, 123.6, 126.8, 129.5, 130.2, 132.3, 133.7, 140.4, 141.3, 143.7, 146.5, 150.6, 151.2, 157.8, 158.4, 158.9, 159.3, 160.4, 162.5; MS (ESI): 602 $[M+H]^{+}$; Anal. Calcd for $C_{27}H_{22}N_8O_5S_2$: C, 53.81; H, 3.68; N, 18.59. Found: C, 53.72; H, 3.79; N, 18.74.

3.7. *N*1-(4-5-[2-(4-Oxo-3,4-dihydro-3-pteridinyl)-1,3-thiazol-5-yl]-1,3,4-thiadiazol-2-ylphe nyl)-4-methoxybenzamide (13c)

This compound **13c** was prepared following the method described for the preparation of the compound **13a**, employing **11** (500 mg, 0.00122 mmol) with 4-methoxybenzoyl chloride (**12c**) (0.165 ml, 0.00122 mmol), Et_3N (0.34 mL,

0.00244 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (8:2) to afford pure compound **13c**, 376.4 mg in 57% yield. pale yellow solid; Mp: 268-270°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 3.89 (s, 3H), 7.36 (d, 1H, *J* = 8.30 Hz), 7.56 (s, 1H), 7.67 (d, 2H, *J* = 7.31 Hz), 7.74 (d, 2H, *J* = 7.31 Hz), 7.79 (d, 2H, *J* = 7.25 Hz), 7.84 (d, 2H, *J* = 7.25 Hz), 8.16 (s, 1H), 8.26 (d, 1H, *J* = 8.30 Hz), 8.66 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 57.3, 115.2, 123.7, 126.5, 128.4, 129.5, 130.7, 132.4, 133.6, 140.6, 141.2, 146.5, 150.8, 151.2, 152.7, 156.7, 158.3, 158.9, 160.4, 161.2, 162.7; MS (ESI): 542 $[M+H]^+$; Anal. Calcd for $C_{25}H_{18}N_8O_3S_2$: C, 55.34; H, 3.34; N, 20.65. Found: C, 55.23; H, 3.23; N, 20.79.

3.8. *N*1-(4-5-[2-(4-Oxo-3,4-dihydro-3-pteridinyl)-1,3-thiazol-5-yl]-1,3,4-thiadiazol-2-ylphe nyl)-4-chlorobenzamide (13d)

This compound **13d** was prepared following the method described for the preparation of the compound **13a**, employing **11** (500 mg, 0.00122 mmol) with 4-chlorobenzoyl chloride (**12d**) (0.156 ml, 0.00122 mmol), Et_3N (0.34 mL, 0.00244 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (7:3) to afford pure compound **13d**, 412.6 mg in 62% yield. pale yellow solid; Mp: 280-282°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 7.36 (d, 1H, *J* = 8.30 Hz), 7.57 (s, 1H), 7.63-7.75 (m, 4H), 7.80 (d, 2H, *J* = 7.32 Hz), 7.85 (d, 2H, *J* = 7.26 Hz), 8.17 (s, 1H), 8.26 (d, 1H, *J* = 8.30 Hz), 8.65 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 123.6, 126.5, 129.3, 130.5, 132.4, 133.6, 134.5, 135.7, 140.6, 141.3, 142.7, 146.5, 150.6, 151.4, 152.3, 156.4, 158.3, 159.3, 160.5, 162.9; MS (ESI): 546 $[M+H]^+$; Anal. Calcd for $C_{24}H_{15}ClN_8O_2S_2$: C, 52.70; H, 2.76; N, 20.48. Found: C, 52.79; H, 2.64; N, 20.57.

3.9. *N*1-(4-5-[2-(4-Oxo-3,4-dihydro-3-pteridinyl)-1,3-thiazol-5-yl]-1,3,4-thiadiazol-2-ylphe nyl)-4-bromobenzamide (13e)

This compound **13e** was prepared following the method described for the preparation of the compound **13a**, employing **11** (500 mg, 0.00122 mmol) with 4-bromobenzoyl chloride (**12e**) (268 mg, 0.00122 mmol), Et_3N (0.34 mL, 0.00244 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (7:3) to afford pure compound **13e**, 430.5 mg in 60% yield. pale yellow solid; Mp: 284-286°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 7.37 (d, 1H, *J* = 8.31 Hz), 7.56 (s, 1H), 7.66 (d, 2H, *J* = 7.36 Hz), 7.76 (d, 2H, *J* = 7.36 Hz), 7.84 (d, 2H, *J* = 7.28 Hz), 7.87 (d, 2H, *J* = 7.28 Hz), 8.16 (s, 1H), 8.27 (d, 1H, *J* = 8.31 Hz), 8.67 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 123.5, 124.7, 126.7, 129.5, 130.6, 132.4, 133.5, 134.2, 135.5, 140.4, 141.6, 146.5, 150.6, 151.3, 152.6, 156.4, 158.5, 159.3, 160.5, 163.5; MS (ESI): 589 $[M+H]^+$; Anal. Calcd for $C_{24}H_{15}BrN_8O_2S_2$: C, 48.74; H, 2.56; N, 18.95. Found: C, 48.81; H, 2.49; N, 19.02.

3.10. *N*1-(4-5-[2-(4-Oxo-3,4-dihydro-3-pteridinyl)-1,3-thiazol-5-yl]-1,3,4-thiadiazol-2-ylphe nyl)-4-nitrobenzamide (13f)

This compound **13f** was prepared following the method described for the preparation of the compound **13a**, employing **11** (500 mg, 0.00122 mmol) with 4-nitrobenzoyl chloride (**12f**) (226 mg, 0.00122 mmol), Et_3N (0.34 mL, 0.00244 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (7:3) to afford pure compound **13f**, 427.2 mg in 63% yield. pale yellow solid; Mp: 288-290°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 7.37 (d, 1H, *J* = 8.30 Hz), 7.57 (s, 1H), 7.67 (d, 2H, *J* = 7.36 Hz), 7.75 (d, 2H, *J* = 7.36 Hz), 7.89-7.98 (m, 4H), 8.17 (s, 1H), 8.26 (d, 1H, *J* = 8.30 Hz), 8.67 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 123.8, 125.8, 126.8, 129.4, 131.2, 132.7, 133.7, 136.8, 140.6, 141.5, 146.7, 150.6, 151.6, 151.6, 152.8, 156.9, 158.7, 159.8, 161.3, 163.7; MS (ESI): 556 $[M+H]^+$; Anal. Calcd for $C_{24}H_{15}N_9O_4S_2$: C, 51.70; H, 2.71; N, 22.61. Found: C, 51.62; H, 2.83; N, 22.53.

3.11. *N*1-(4-5-[2-(4-Oxo-3,4-dihydro-3-pteridinyl)-1,3-thiazol-5-yl]-1,3,4-thiadiazol-2-ylphe nyl)-3,5-dinitrobenzamide (13g)

This compound **13g** was prepared following the method described for the preparation of the compound **13a**, employing **11** (500 mg, 0.00122 mmol) with 3,5-dinitrobenzoyl chloride (**12g**) (281 mg, 0.00122 mmol), Et_3N (0.34 mL, 0.00244 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (7:3) to afford pure compound **13g**, 456.7 mg in 62% yield. pale yellow solid; Mp: 300-302°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 7.36 (d, 1H, *J* = 8.31 Hz), 7.56 (s, 1H), 7.66 (d, 2H, *J* = 7.35 Hz), 7.73 (d, 2H, *J* = 7.35 Hz), 8.16 (s, 1H), 8.26 (d, 1H, *J* = 8.31 Hz), 8.36 (s, 2H), 8.44 (s, 1H), 8.66 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 123.8, 124.7, 126.5, 128.7, 129.5, 132.6, 133.8, 134.7, 136.8, 140.7, 146.7, 148.7, 150.6, 151.5, 152.8, 156.8, 158.9, 159.6, 161.7, 163.8; MS (ESI): 602 $[M+H]^+$; Anal. Calcd for $C_{24}H_{14}N_{10}O_6S_2$: C, 47.84; H, 2.34; N, 23.25. Found: C, 47.92; H, 2.44; N, 23.42.

3.12. *N*1-(4-5-[2-(4-Oxo-3,4-dihydro-3-pteridinyl)-1,3-thiazol-5-yl]-1,3,4-thiadiazol-2-ylphe nyl)-4-cyanobenzamide (13h)

This compound **13h** was prepared following the method described for the preparation of the compound **12a**, employing **11** (500 mg, 0.00122 mmol) with 4-cyanobenzoyl chloride (**12h**) (202 mg, 0.00122 mmol), Et_3N (0.34 mL, 0.00244 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (7:3) to afford pure compound **13h**, 443.8 mg in 67% yield. pale yellow solid; Mp: 290-292°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 7.37 (d, 1H, *J* = 8.30 Hz), 7.57 (s, 1H), 7.65 (d, 2H, *J* = 7.36 Hz), 7.74 (d, 2H, *J* = 7.36 Hz), 7.80 (d, 2H, *J* = 7.29 Hz), 7.86 (d, 2H, *J* = 7.29 Hz), 8.16 (s, 1H), 8.27 (d, 1H, *J* = 8.30 Hz), 8.67 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 114.7, 119.7, 123.8, 126.8, 129.7, 131.7, 132.3, 133.7, 134.8, 139.7, 140.6, 141.7, 146.5, 150.6, 151.7, 152.8, 156.7, 158.5, 159.4, 161.5, 162.9; MS (ESI): 537 $[M+H]^+$; Anal. Calcd for $C_{25}H_{15}N_9O_2S_2$: C, 55.86; H, 2.81; N, 23.45. Found: C, 55.98; H, 2.73; N, 23.61.

3.13. *N*1-(4-5-[2-(4-Oxo-3,4-dihydro-3-pteridinyl)-1,3-thiazol-5-yl]-1,3,4-thiadiazol-2-ylphe nyl)-4-methylbenzamide (13i)

This compound **13i** was prepared following the method described for the preparation of the compound **13a**, employ-

ing **11** (500 mg, 0.00122 mmol) with 4-methylbenzoyl chloride (**12i**) (188 mg, 0.00122 mmol), Et_3N (0.34 mL, 0.00244 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (7:3) to afford pure compound **13i**, 308.6 mg in 48% yield. pale yellow solid; Mp: 267-269°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 2.38 (s, 3H), 7.35 (d, 1H, *J* = 8.30 Hz), 7.54 (s, 1H), 7.58 (d, 2H, *J* = 7.29 Hz), 7.64 (d, 2H, *J* = 7.35 Hz), 7.70-7.78 (m, 4H), 8.16 (s, 1H), 8.26 (d, 1H, *J* = 8.30 Hz), 8.66 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 23.7, 123.6, 126.5, 128.6, 129.5, 130.6, 132.6, 133.7, 134.7, 140.3, 141.5, 143.6, 146.7, 150.7, 151.3, 152.7, 156.5, 158.4, 158.9, 160.8, 162.8; MS (ESI): 526 $[M+H]^+$; Anal. Calcd for $C_{25}H_{18}N_8O_2S_2$: C, 57.02; H, 3.45; N, 21.28. Found: C, 57.17; H, 3.37; N, 21.43.

3.14. *N*1-(4-5-[2-(4-Oxo-3,4-dihydro-3-pteridinyl)-1,3-thiazol-5-yl]-1,3,4-thiadiazol-2-ylphe nyl)-4-chloro-3-nitrobenzamide (13j)

This compound **13j** was prepared following the method described for the preparation of the compound **13a**, employing **11** (500 mg, 0.00122 mmol) with 4-chloro-3-nitrobenzoyl chloride (**12j**) (268 mg, 0.00122 mmol), Et_3N (0.34 mL, 0.00244 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (7:3) to afford pure compound **13j**, 461.4 mg in 64% yield. pale yellow solid; Mp: 302-304°C, ^{1}H NMR (300 MHz, DMSO-d6): δ 7.36 (d, 1H, *J* = 8.31 Hz), 7.55 (s, 1H), 7.65 (d, 2H, *J* = 7.36 Hz), 7.73 (d, 2H, *J* = 7.36 Hz), 7.80 (d, 1H, *J* = 7.26 Hz), 7.86 (d, 1H, *J* = 7.26 Hz), 7.95 (d, 1H, *J* = 7.11 Hz), 8.16 (s, 1H), 8.25 (d, 1H, *J* = 8.31 Hz), 8.66 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 123.8, 126.8, 128.7, 129.5, 132.5, 133.5, 133.8, 134.6, 134.9, 137.6, 138.6, 140.8, 146.8, 150.6, 151.4, 151.8, 152.8, 156.7, 158.9, 159.8, 161.7, 163.2; MS (ESI): 591 $[M+H]^+$; Anal. Calcd for $C_{24}H_{14}ClN_9O_4S_2$: C, 48.69; H, 2.38; N, 21.29. Found: C, 48.82; H, 2.51; N, 21.12.

3.15. MTT Assay

Individual wells of a 96-well tissue culture microtiter plate were inoculated with 100 µL of complete medium containing 1×10^4 cells. The plates were incubated at 37 °C in a humidified 5% CO_2 incubator for 18 hours prior to the experiment. After medium removal, 100 µL of fresh medium containing the test compounds and etoposide at different concentrations, such as 0.5, 1, and 2 µM were added to each well and incubated at 37°C for 24 hours. Then the medium was discarded and replaced with 10 µL MTT dye. Plates were incubated at 37°C for 2 hours. The resulting formazan crystals were solubilized in 100 µL extraction buffer. The optical density (O.D) was read at 570 nm with micro plate reader (Multi-mode Varioskan Instrument-Themo Scientific). The percentage of DMSO in the medium never exceeded 0.25%.

CONCLUSION

In conclusion, we have designed and synthesized a series of (**13a-j**) amide derivatives of pteridones were prepared and their structures confirmed by ^{1}HNMR, ^{13}CNMR and mass spectral analysis. Further, these derivatives were tested for their anticancer activity on four human cancer cell lines including MCF-7 (breast), A549 (lung), Colo-205 (colon) and A2780 (ovarian) and etoposide used as a positive control. Among them, five compounds **13b**, **13c**, **13f**, **13g** and **13j** showed the most promising activity than the etoposide.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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