ISSN 2321-807X
Volume 13 Number10
Journal of Advances in chemistry

Synthesis, Characterization and in vitro evaluation of anticancer activity of new hydroxamicacid basedHDACis containingsubstitutedThaidiazole as a cap linking moiety.

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ABSTRACT

The present study was undertaken to synthesize, characterize and evaluate the anticancer activity of new derivatives of hydroxamate –based HDACi having S-subistituted-5-amino1,3,4thadiazole as a cap linking moiety ,with suitable aliphatic linker.The structures and purity of the targeted compounds were confirmed by TLC , FTIR ,H-NMR and mass spectroscopy and their anticancer activity were evaluated by comparative cytotoxic study , Using HeLa nuclear extract and normal embryonic fibroblasts cell lines. All the synthesized compounds shows good anticancer activity, represented by their high rate of growth inhibition on Hela cell line and low cytotoxic effect on normal cell line . Compound (VAb) show the best safety index(SI) that represented by its selective cytotoxic activity on HeLa cell line with low cytotoxic effect on normal embryonic cell line.

KEYWORDS:HDACis ,5-amino 1,3,4thiadiazole-2-thiol . new CAP groups.

1.INTRODUCTION

Epigenetic regulation of specific gene expression is mediated by several mechanisms, among which is post-translational acetylation of the side-chain amino groups of specific histone lysine residues.(1). The acetylation status of histones is modulated by histone acetyltransferases (HAT) and histone deacetylases (HDAC).(HAT) is generally considered as a transcriptional activator, and HDAC is considered as a transcriptional inhibitor(2). Eighteen mammalian HDACs have been identified and categorized into four structural and functionally distinct classes(3).

Many recent studies have shown that inhibition of HDAC elicits anticancer effects in several lines of tumor cells by inhibiting cell growth and inducing apoptosis(4).Natural and synthetic HDAC inhibitors have been studied extensively , and suberoylanilidehydroxamic acid (SAHA)has been approved by the FDA for once-daily oral treatment of advanced cutaneous T-cell lymphoma (CTCL), (5).

. Most of the HDAC inhibitors have three common features: cap group, zinc binding group (ZBG) and hydrophobic spacer(6). The hydroxamic acid moiety has been widely used as zinc binding group., the cap group is by far the most common moiety to modify in order to obtain isoform selectivity(7). The hydrophobic linker is also relatively versatile, although it often takes the form of a lengthy aliphatic chain. The zinc-binding group is a requirement for HDAC inhibition and takes the form of hydroxamic acids(8) .Inhibition of HDAC activity has emerged as a promising option for reversing the abnormal epigenetic status that associated with cancer as well as other chronic diseases, however, Most HDACi are global, non-selective inhibitors of various HDAC isoform, so they does not differentiate the relevant HDACs that regulate proliferation, apoptosis or angiogenesis(9). In addition, low oral bioavailability, short half-life time, bone marrow toxicity, and cardio toxicity are limiting the clinical use of some current HDACi(10). Therefore, there is considerable interest in developing compounds with great selectivity towards individual family members of HDACs, and with improved pharmacokinetic and pharmacodynamics profiles(11).

The present study was undertaken to synthesize and evaluate the anticancer activity of new hydroxamate –based HDACi having S-subistituted-1-3,4thadiazole derivatives ,with suitable aliphatic linker with hope of obtaining inhibitors which more selective , potent and with improved pharmacokinetic properties All the new compounds were characterized by elemental and spectral analysis and screened for their invitro, antitumor activity

2.MATERIALS AND METHODS

EXPERIMENTAL

All the newly synthesized compounds gave good to moderate yields and their structures were ascertained by thin layer chromatography (TLC) on silica gel G (Merck) coated plates by using different solvent system. The visualization of (TLC) spots was done by using Iodine chamber and UV lamp. The chemicals and solvents were purchased from Fluka, BDH, and Thomas Baker companies. Melting points were determined on Thomas Hooverelectric melting points apparatus and are uncorrected. FT-IR spectra (KBr) were recorded on Shimadzu FT-R-8400S spectrophotometer and ^{1}HNMR spectra measured with400MHz,Avance III 400-Bruker, using tetramethylsilane as an internal standard ..The percentage of carbon,hydrogen and nitrogen were obtained using a CHN analyzer (Euro EA3000 elemental analyzer.

GENERAL METHODS

The target compounds were synthesized by the following steps

2.1Synthesis of 5-amino-1, 3, 4-thiadiazole-2-thiol, compound (1)[12]

Thiosemicarbazide (9.11 g, 100 mmol), was dissolved in absolute ethanol (75 ml), anhydrous sodium carbonate (5.3 g, 50 mmol), and carbon disulfide (6 ml, 100 mmol) were then added and the reaction mixture was refluxed with stirring for five hours. The reaction mixture then allows cooling at room temperature, then filtered. The filtrate was evaporated to dryness under vacuum, and the residue was dissolved in distilled water (200 ml), and acidified to pH 6 with 2 N, HCl to give greenish-yellow precipitate. The crude product was filtered, washed excessively with D.W and crystallized from hot water .to give compound 1 as greenish-yellow crystals.

yellow powder , Yield 57%; m.p. 130°C ; **IR (υ= cm-1,KBr):**3396, 3277 (Asym. And sym. Str. of NH_2 3091(NH str.)2773(SH str,)1600(NH bending) 1533 and 1496(C=N str.)1363(C=S str.)746(NH_2 wagging).

2.2General procedure for synthesis of compounds (2a-f)(13)

A stirred solution that containing 0.028mole (3 ml benzylamine ,2.6 g 2-aminopyridine , 3.88g p-nitroaniline ,3.61 g p-chloroaniline ,4.94 g p-bromoaniline ,3.5 g p-methoxyaniline) in dry benzene(30 ml),TEA (4 ml, 0.029 mole) was added drop wise, and the solution was cooled to 0 ^{o}C in ice bath,(2.147 ml ,0.028 mole) of CAC in 10 ml of dry benzene was added drop wise over a period 30 minute .The resulted mixture was stirred at room temperature for 2-4hr and reflexed for 1-3 hr. then the solvent was evaporated under vacuum and the residue was washed twice with 15 of cold 5% potassium carbonate solution, and D.W successively filtered , dried and the obtained powder was recrystallized from ethanol

Synthesis of compound N-benzyl-2-chloroacetamide, compound (2a).

white powder , Yield 87%; m.p. 94-95°C ;**FT IR (υ= cm-1,KBr):**3278 (NH str.) , 30062and 3005(Ar-H str.), 2947 , 2877 (Asym.and sym. Str. of CH_2), 1650 (C=O str.amide), 1550(NH bending), 1427,1480(C=C str.)

Synthesis of 2-chloro-N-(pyridin-2-yl)acetamide, compound (2b).

Gray powder , Yield 85%; m.p. 121-124°C; **FT IR (υ= cm-1,KBr):**3251 (NH str.) ,3078 (Ar-H str.), 2947 , 2870(Asym.and sym. Str. of CH_2), 1681 (C=O str.amide),1581(C=N str.),1543(NHbending)1462,1438(C=C str.)

Synthesis of 2-chloro-N-(4-nitrophenyl)acetamide, compound (2c).

yellow powder , Yield 70%; m.p. 182-185°C;**FT IR (υ= cm-1,KBr):**3228 (NH str.) , 3107 ,3070 (Ar-H str.), 2941 , 2829 (Asym.and sym. Str. of CH_2), 1685 (C=O str.amide),1624(NO_2 str.),1599(NH bending) 1568,1408 (C=C str.)

Synthesis2-chloro-N-(4-chlorophenyl)acetamide,compound(2d).

Brawn powder , Yield 78%; m.p. 175-177°C;**FT IR (υ= cm-1,KBr):**3282 (NH str.) , 3101 (Ar-H str.), 2935 , 2829 (Asym.and sym. Str. of CH_2), 1660 (C=O str.amide),1591(NH bending) 1539,1491 (C=C str.)

Synthesis of N-(4-bromophenyl)-2-chloroacetamide,compound (2e)

Brawn powder , Yield 72%; m.p. 172-173°C;**FT IR (υ= cm-1,KBr):**3263 (NH str.) ,3078 , 3101 (Ar-H str.), 2953 , 2885(Asym.and sym. Str. of CH_2), 1670 (C=O str.amide),1608(NH bending) 1548,1459(C=C str.)

Synthesisof2-chloro-N(4-methoxyphenyl)acetamide, compound (2f).

Gray crystals , Yield 81%; m.p. 119-120; FT IR (υ= cm-1,KBr):3294 (NH str.) ,3072 , 3009 (Ar-H str.), 2956 , 2835 (Asym.and sym. Str. of CH_2), 1670 (C=O str.amide),1606(NH bending) 1550,1462(C=C str.)

2.3 General procedure for synthesis of compounds (3a-g).[14].

To a suspension of compound 1 (2 gm , 0.015 mole) in 30 ml of D.W placed in a suitable flask ,TEA (2.123 ml , 0.017 mole) was added gradually at room temperature with continuous stirring, the resulted pale – yellow solution was filtered to remove the remaining insoluble starting material, then 0.013 mole of (benzyl chloride 1.5 ml ;2a,2.38g ; 2b ,2.21 g ;2c ,2.78 g ;2d , 2.65 g ;2e ,1.8g ;2f ,2.6g) was added In several divided portions to the clear yellowish filtrate over a period of 20-30 minute. Stirring was continued at room temperature for 3 hrs ,the progression of the reaction was followed by TLC. The resulted precipitate was filtered , washed with 20 ml of D.W several times ,dried and recrystallized from aqueous ethanol.

Synthesis of 5-(benzylthio)-1,3,4-thiadiazol-2-amine, (3a)

white crystals , Yield 92%; m.p. 113-116**; FT IR (υ= cm-1,KBr):** 3294,3107 (NH_2str.) , 2955 , 2835 (Asym.and sym. Str. of CH_2), 1616 (C=O str.amide),1518(NH bending) 1452,1423(C=N str.), 1375(CH_2 bending).

Synthesis of 2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N benzylacetamide (3b)

white crystals , Yield 88%; m.p. 161-163**;FT IR (υ= cm-1,KBr)**: 3394 and 331 (NH_2 str.),3118(NH str.), 3082(Ar-H str.) , 2931 , 2900 (Asym.and sym. Str. of CH_2), 1651 (C=O str.amide),1597 (NH bending) 1524(C=N str.).

Synthesisof2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(pyridin-2-yl)acetamide, (3c).

Khaki crystals , Yield 83%; m.p. 123-126**;FT IR (υ= cm-1,KBr)**: 3358 and 3275(NH_2 str.),3167(NH str.), 3095, 3005 (Ar-H str.) , 2970 , 2837 (Asym.and sym. Str. of CH_2), 1676 (C=O str.amide),1608 (NH bending) 1583(C=N str.).

Synthesisof2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-nitrophenyl)acetamide, (3d).

Yellow crystals , Yield 81%; m.p**.** 155-157**;FT IR (υ= cm-1,KBr)**: 3338 and 3231 (NH_2 str.), 3172 (NH str.), 3111 (Ar-H str.) , 2939 , 2837 (Asym.and sym. Str. of CH_2), 1684 (C=O str.amide),1625 (NH bending) 1570(C=N str.),

Synthesis of 2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-chlorophenyl)acetamide, (3e).

white crystals , Yield 84%; m.p. 144-147;FT IR (υ= cm-1,KBr): 3285 and 3199 (NH_2 str.), 3132 (NH str.), 3084 ,3005 (Ar-H str.) , 2953 , 2889 (Asym.and sym. Str. of CH_2), 1670 (C=O str.amide),1614 (NH bending) 1597 (C=N str.).

Synthesisof2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-bromophenyl)acetamide (3f).

white crystals , Yield 83%; m.p. 161-164;FT IR (υ= cm-1,KBr): 3300 ,3197 (NH_2 str.), 3163 (NH str.), 3020 (Ar-H str.) , 2875 (Asym.and sym. Str. of CH_2), 1662 (C=O str.amide),1618 (NH bending) 1565 (C=N str.).

Synthesisof2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4methoxyphenyl)acetamide, (3g).

white crystals , Yield 83%; m.p. 105-107;FT IR (υ= cm-1,KBr): 3337 3275 (NH_2 str.), 3136 (NH str.), 3070 (Ar-H str.) , 2949 , 2837 (Asym.and sym. Str. of CH2), 1653 (C=O str.amide), 1608 (NH bending) 1541 (C=N str.).

2.4General procedure for synthesis of compounds (4a-g)[15].

Adipic acid monoethyl ester (4 gm , 0.022 mole) was placed in a dry-100 ml two-necked flask connected with a dropping funnel and reflex condenser , Thionyl chloride (3.26 ml , 0.045 mole) was added drop wise through the dropping funnel at room temperature over a period of 30 minute with gentle stirring . Stirring was continued after completion of addition for additional 1 hr till the evolution of gases was stopped . The solution was heated under reflex at 40 – 45 oC for 4 hrs and the excess thionyl chloride was removed under reduced pressure. The clear liquid was dissolved without further purification in 5 ml of dry DMF and added drop wise with stirring to an ice- cooled at 0 ^oC mixture of TEA (4.2ml , 0.03 mole) and 0.022mole of compounds 3a-g (3a ,4.9 g ;3b ,6.08g ;3c,5.8 g ;3d, 6.2 g; 3e,6.6 g; 3f , 7.5 g ; 3g ,6.6 g) in 25 ml of dry DMF. The resulted suspension was stirred at room temperature for 4 hrs and reflexed for additional 2 hrs. the solvent was evaporated, the residue was washed with cooled solution (3×20 ml) of 5 % HCl ,5 % sodium bicarbonate and D.W successively. The product was filtered ,collected ,dried and recrystallized from aqueous ethanol. .

Synthesis of ethyl 6-(5-(benzylthio)-1,3,4-thiadiazol-2-ylamino)-6-oxohexanoate (4a).

white crystals , Yield 74%; m.p. 90-93;**FT IR (υ= cm-1,KBr):** 3273 (NH str. of sec .amide), 3157 (NH str.), 3053 (Ar-H str.) , 2931 and 2870 (Asym.and sym. Str. of CH_2), 1733(C=O str. of ester),1691(C=O str.amide), 1651(NH bending) 1558 (C=N str.).

Synthesisofethyl6-(5-(2-(benzylamino)-2-oxoethylthio)-1,3,4-thiadiazol-2 ylamino)-6-oxohexanoate(4b).

white crystals , Yield 76%; m.p. 111-113;**FT IR (υ= cm-1,KBr)**: 3325 (NH str. of sec .amide), 3163 (NH str.), 3034 (Ar-H str.) , 2939 , 2877 (Asym.and sym. Str. of CH_2), 1722 (C=O str. of ester),1695 (C=O str.amide), 1643 (NH bending) 1568 (C=N str.).

Synthesisofethyl6-oxo-6-(5-(2-oxo-2-(pyridin-2ylamino)ethylthio)-1,3,4-thiadiazol-2-ylamino)hexanoate,(4c).

white crystals , Yield 65%; m.p. 104-106;**FT IR (υ= cm-1,KBr)**: 3172 (NH str. of sec .amide), 3122 (NH str.), 3032 (Ar-H str.) , 2978 ,2873 (Asym.and sym. Str. of CH2), 1734 (C=O str. of ester), 1691 (C=O str.amide), 1575 (NH bending) 1550 (C=N str.).

Synthesis of ethyl 6-(5-(2-(4-nitrophenylamino)-2-oxoethylthio)-1,3,4-thiadiazol-2-ylamino)-6-oxohexanoate,(4d).

yellow crystals , Yield 64%; m.p. 121-124;**FT IR (υ= cm-1,KBr)**: 3266 (NH str. of sec .amide), 3161 (NH str.), 3109 (Ar-H str.) , 2941 and 2873 (Asym.and sym. Str. of CH2), 1728 (C=O str. of ester), 1693 (C=O str.amide), 1618 (NH bending) 1597 (C=N str.).

Synthesis of ethyl 6-(5-(2-(4-chlorophenylamino)-2-oxoethylthio)-1,3,4-thiadiazol-2-ylamino)-6-oxohexanoate, . (4e).

white crystals , Yield 69%; m.p. 117-119;**FT IR (υ= cm-1,KBr):** 3265 (NH str. of sec .amide), 3132 (NH str.), 3084 (Ar-H str.) , 2939 and 2844(Asym.and sym. Str. of CH2), 1732 (C=O str. of ester), 1693 (C=O str.amide), 1668 (NH bending) 1668 (C=N str.).

Synthesis of ethyl 6-(5-(2-(4-bromophenylamino)-2-oxoethylthio)-1,3,4-thiadiazol-2-ylamino)-6-oxohexanoate, (4f).

white crystals , Yield 66%; m.p. 87-91;**FT IR (υ= cm-1,KBr):** 3388 (NH str. of sec .amide), 3132 (NH str.), 3084 (Ar-H str.) , 2938 , 2875 (Asym.and sym. Str. of CH2), 1732 (C=O str. of ester), 1691 (C=O str.amide), 1654 (NH bending) 1598 (C=N str.).

Synthesis of ethyl 6-(5-(2-(4-methoxyphenylamino)-2-oxoethylthio)-1,3,4-thiadiazol-2-ylamino)-6-oxohexanoate, (4g).

white crystals , Yield 58%; m.p. 72-74;**FT IR (υ= cm-1,KBr)**: 3308 (NH str. of sec .amide), 3140 (NH str.), 3051 (Ar-H str.) , 2931 , 2833 (Asym.and sym. Str. of CH2), 1718 (C=O str. of ester), 1691 (C=O str.amide), 1656 (NH bending) 1602 (C=N str.).

2.5 General procedure for synthesis of compounds (5a-g)[16].

A stirred solution of compounds 4a-g (0.0013 mole) (4a 0.5g , 4b,0.57g ;4c, 0.576g ;4d,0.65 g ;4e, 0.6g ; 4f,0.7 g ;4g,0.58g) in dry 1:1 THF- methanol (15 ml) was cooled to 0^{o}Cin ice-bath, and aqueous hydroxylamine 50 % (0.85 ml , 0.013 mole, 10 equivalent) was added followed by immediate addition of 3-6 mg of KCN . The mixture was allowed to warm to room temperature and stirred for 24-32hrs .Then, to the resulted yellow mixture , sodium hydroxide 10 equivalent was added and stirring continued for additional 1 hr. The solvent was removed under reduced pressure, and the obtained solid was dissolved in 15 ml of D.W ,filtered, and the clear filtrate was cooled and acidified with 0.1 N aqueous solution of HCl to pH 7. The resulted precipitate was filtered ,dried and recrystallized from methanol: n- hexane.

SynthesisofN1-(5-(benzylthio)-1,3,4-thiadiazol-2-yl)-N6hydroxyadipamide,compound (5a).

white crystals , Yield 72%; m.p. 81-83;**FT IR (υ= cm-1,KBr):** 3335 (OH str.), 3252 (NH str.) , 2924 (Asym.and sym. Str. of CH_2), 1606 (C=O str. of amide, 1555 (C=N str.).**^{1}HNMR(400MHz),(DMSO-d6,δppm):**12.1(s,1H,-OH),11.7(s,1H,CO-NH), 11.3(s,1H,OH-NH), 7-8. (m,5H,Ar-H),4.4 (s, 2H,CH_2-S),3.7(t,4H,CH_2-CO) 2.8(t,4H,-CH_2-) ; Elemental analysis Calcd. for $C_{15}H_{18}N_4O_3S_2$: C, 49.16; H, 4.95; N, 15.29.Found: C, 49.55; H,4.656.33; N, 12.56.,N,14.05.

Synthesis of N1-(5-(2-(benzylamino)-2-oxoethylthio)-1,3,4-thiadiazol-2-yl)-N6-hydroxyadipamide, (5b).

white crystals , Yield 60%; m.p. 122-124;**FT IR (υ= cm-1,KBr):** 3292 (OH str.), 3252 (NH str.) , 2929 , 2871 (Asym.and sym. Str. of CH_2), 1692 (C=O str. of amide), 1643(amide II band),1551 (C=N str.). **^{1}HNMR(400MHz),(DMSO-d6,δppm):**12.4(s,1H,-OH),12.(s,1H,CO-NH), 11.5(s,1H,OH-NH),11.2(t,1H,CO-NH), 7-8. (m,5H,Ar-H),4.9(s,2H,CH_2-S),4.1 (s, 2H,CH_2-Ar),3.4(t,4H,CH_2-CO) 2.2(t,4H,-CH_2-);Elemental analysis Calcd. for $C_{17}H_{21}N_5O_4S_2$: C, 48.21; H, 5.00; N, 16.54. Found: C, 49.07; H, 4.66; N,16.87 .

SynthesisofN1-hydroxy-N6-(5-(2-oxo-2-(pyridin-2-ylamino)ethylthio)-1,3,4-thiadiazol-2-yl)adipamide,(5c).

white crystals , Yield 66%; m.p. 132-135**;FT IR (υ= cm-1,KBr**): 3280 (OH str.), 3252 (NH str.) ,3086(Ar-H str.) 2979 and 2874 (Asym.and sym. Str. of CH_2), 1697 (C=O str. of amide), 1573 (C=N str.).

^{1}HNMR(400MHz),(DMSO-d6,δppm):11.9(s,1H,-OH),11.8 (s,1H,CO-NH-Ar), 11.4(s,1H,CO-NH),11 (s,1H,OH-NH), 7-8. (m,4H,Ar-H),4.1(s,2H,CH_2-S),3.8 (t, 2H,CH_2-CO),2.7(t,4H,-CH_2-) ; -);Elemental analysis Calcd. for $C_{15}H_{18}N_6O_4S$: C, 43.89; H, 4.42; N, 20.47. Found: C, 43.22; H, 4.23; N, 20.78.

Synthesis of N1-hydroxy-N6-(5-(2-(4-nitrophenylamino)-2-oxoethylthio)-1,3,4-thiadiazol-2-yl)adipamide,(5d).

white crystals , Yield 59%; m.p. 150-153;**FT IR (υ= cm-1,KBr)**: 3241 (OH str.), 3165 (NH str.) , 3061 (Ar-H str.) 2951 and 2873 (Asym.and sym. Str. of CH2), 1693 (C=O str. of amide), 1619 (C=N str.).

^{1}HNMR(400MHz),(DMSO-d6,δppm):12.1(s,1H,-OH),11.7 (s,1H,CO-NH-Ar), 11.1(s,1H,CO-NH),10.9 (s,1H,OH-NH), 7-8. (m,4H,Ar-H),4.3(s,2H,CH_2-S),3.6 (t, 2H,CH_2-CO),2.4(t,4H,-CH_2-) ;Elemental analysis Calcd for $C_{16}H_{18}N_6O_6SC$, 42.28; H, 3.99; N, 18.49. Found: C, 41.60; H, 3.08; N, 17.88.

Synthesis of N1-(5-(2-(4-chlorophenylamino)-2-oxoethylthio)-1,3,4-thiadiazol-2-yl)-N6-hydroxyadipamide,(5e).

white crystals , Yield 46%; m.p. 147-150;**FT IR (υ= cm-1,KBr):** 3209 (OH str.), 3132 (NH str.) , 3084 (Ar-H str.) 2939 and 2880 (Asym.and sym. Str. of CH_2), 1685 (C=O str. of amide), 1616 (C=N str.).

[1]HNMR(400MHz),(DMSO-d6,δppm):12.4(s,1H,-OH),11.7 (s,1H,CO-NH-Ar), 11.3(s,1H,CO-NH),11.1 (s,1H,OH-NH), 7-8.(m,4H,Ar-H),4 (s,2H,CH_2-S),3.4 (t, 2H,CH_2-CO),2.4(t,4H,-CH_2-) ; Elemental analysis Calcd for $C_{16}H_{18}ClN_5O_4S_2$:C, 43.29; H, 4.09; N, 15.78. Found: C, 44.07; H, 4.18; N, 15.44

Synthesis of N1-(5-(2-(4-bromophenylamino)-2-oxoethylthio)-1,3,4-thiadiazol-2-yl)-N6-hydroxyadipamide,compound (5f).

white crystals , Yield 54%; m.p. 113-115;**FT IR (υ= cm-1,KBr):** 3314 (OH str.), 3188 (NH str.) , 3117(NH str.) , 3077 (Ar-H str.) 2920 and 2872 (Asym.and sym. Str. of CH_2), 1680 (C=O str. of amide), 1596 (C=N str.). **[1]HNMR(400MHz),(DMSO-d6,δppm):**12 (s,1H,-OH),11.8(s,1H,CO-NH-Ar), 11.4(s,1H,CO-NH),11.1 (s,1H,OH-NH),7-8.(m,4H,Ar-H),4.2(s,2H,CH_2S),3.6(t,2H,CH_2-CO),2.7(t,4H,-CH_2-) ; Elemental analysis Calcd for $C_{16}H_{18}BrN_5O_4S_2$:C, 39.35; H, 3.71; N, 14.34. Found: C, 39.87; H, 3.45; N, 14.09

Synthesis of N1-hydroxy-N6-(5-(2-(4-methoxyphenylamino)-2-oxoethylthio)-1,3,4-thiadiazol-2-yl)adipamide ,compound (5g).

white crystals , Yield 65%; m.p. 133-136;**FT IR (υ= cm-1,KBr)**: 3292 (OH str.), 3140 (NH str.) , 3045 (Ar-H str.) 2935 and 2875 (Asym.and sym. Str. of CH_2), 1691 (C=O str. of amide), 1604 (C=N str.). **[1]HNMR(400MHz),(DMSO-d6,δppm):**11.8 (s,1H,-OH),11.4 (s,1H,CO-NH-Ar), 11.1(s,1H,CO-NH),10.7 (s,1H,OH-NH), 7-8. (m,4H,Ar-H),4.3(s,2H,CH_2-S),3.8 (t, 2H,CH_2-CO),3.4(s,3H,CH_3-O),2.3(t,4H,-CH_2-) ; Elemental analysis Calcd for $C_{17}H_{21}N_5O_5S_2$:C, 46.46; H, 3.71; N, 15.93. Found: C, 39.87; H, 3.45; N, 14.09

CYTOTOXICITY ASSAY (MTT ASSAY)[17][18][19].

MTT cell viability assay was conducted on 96-well plates (Santacruz Biotechnology, USA), Hela and Normal Embryonic cells were seeded at 10000 cells/well, 200 µl of cells in growth medium were added to each well of a sterile 96-well microtitration plate. The plates were sealed with a self-adhesive film, lid placed on and incubated at 37°C.After 24hr or confluent monolayer is achieved, when the cells were in exponential growth, the medium was removed and serial dilutions of the compounds(5a,5c,5e and 5f) were added to the wells. triplicates were used for each. Control cells treated with Serum Free Media only as well as positive control treated with the solvent (DMSO) in the same concentration used to solve the chemical compounds. Afterwards, the plates were re-incubated at 37°C for 72 hrs.

Cell viability was measured after 72 hrs of exposure by removing the medium, adding 28 µl of 2 mg/ml solution of MTT (Bio-World, USA) and incubating for 1.5h at 37°C. After removing the MTT solution, the crystals remaining in the wells were solubilised by the addition of 130 µl of DMSO (Dimethyl Sulphoxide) (Santacruz Biotechnology, USA) followed by 37°C incubation for 15 min with shaking.

The absorbency was determined on a microplate reader (Biochrom, UK) at 584 nm (test wavelength); the assay was performed intriplicate.

3.RESULTS AND DISCUSSION

3.1 Chemistry

The synthesis of the title compounds (1), (2a-f) and (3a-g) to(5a-g) was accomplished anddepicted in the scheme 1,The scheme1 illustrated the reactions steps for all synthesized derivatives.in which compound 1 was synthesized from the reaction of thiosemicarbazide with CS_2 in the presence of sodium carbonate.while each of compounds 2a-f were synthesized from the reaction of CAC with benzyl amine , 2-aminopyridine ,(p-nitro,p-chloro ,p-bromo and p-methoxy anile).

The synthesis of compounds 3a-g were done by alkylation of thiolate ion of compound 1 in aqueous media(green reactions).Compounds 4a-g were synthesized by activation of carboxyl group of adipic acid mono ethyl ester by thionyl chloride and reaction of the produced acid chloride with amine in dry conditions with presence of TEA to neutralizes the acid produced during the reaction.

The compounds 5a-g were synthesized by aminolysis of ester group in compounds 4a-g by hydroxyl amine in alcoholic solution with the presence of KCN as a catalyst.

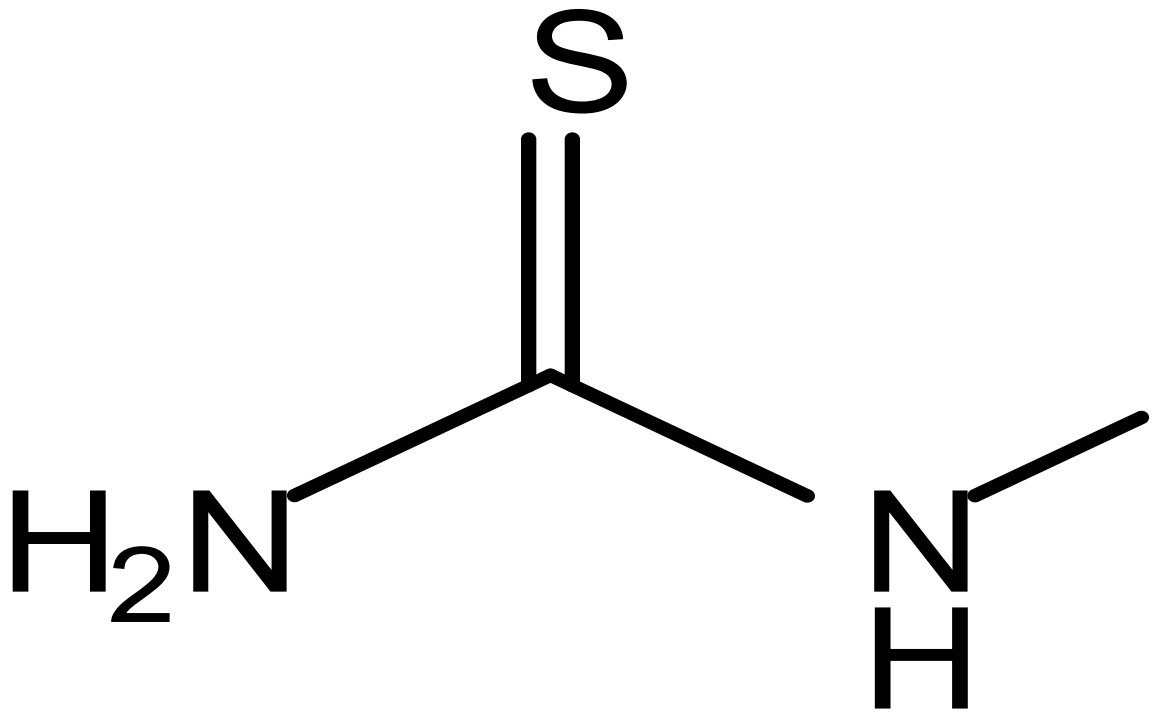
The IR spectrum of compound 1 shows the appearance of characteristics bands such as asymmetric and symmetric stretching of amine and also the appearance of absorption band in about 2500-2700 which represent SH stretching. The IR spectrum of (2a-f) show disappearance of asymmetric and symmetric stretching for primary aromatic NH_2 in the starting compounds and the appearance of new absorption bands which represent the NH stretching of secondary amide and C=O stretching vibration of amide (amide I Band) respectively , at (2278 ,1651) for compound 2a , at (3251 ,1681) for 2b, at (3228 , 1685) for 2c , at (3282 ,1652) for 2d at (3263 ,1668) for 2e and at (3294 , 1669) for 2f.

The IR spectra of(3a-g) show disappearance of the absorption band of SH group in compound 1 at 2773 and the appearance of weak bands in some of the synthesized compound which represents C-S-C stretching vibration that don't has structural diagnostic value., in addition to the appearance of strong bands of asymmetric and symmetric stretching vibration of NH_2 of thiazole ring and the strong bands of C=O stretching of amide bands in the synthesized compounds .

ISSN 2321-807X
Volume 13 Number10
Journal of Advances in chemistry

The IR spectrum (4a-g) shows disappearance of asymmetric and symmetric stretching of NH_2 in the reactants and the appearance of new strong bands that represents the C=O stretching vibration of ester at (1733) for 4a , at (1722) for 4b , at (1734) for 4c , at (1728) for 4d, at(1732) for 4e ,at (1731) for 4f and at (1718) for 4g respectively .

The IR spectra of (5a-g) shows the disappearance of C=O stretching vibrations of ester in the reactants and the appearance of new and relatively broad bands whichresults from hydroxyl group and NH group stretching vibration of hydroxamic acid respectively . These bands appears at (3338,3252) 5a , at (3292 overlapped) for 5b , at (3280 overlapped) for 5c , at (3241 , 3165) for 5d , at (3209 , 3132) for 5e , at (3314 ,3271) for 5f and at (3292 overlapped) for 5g.



Thiosemicarbazide

Scheme(1): Synthesis of compounds (1),(2a-f) and(3a-g) to (5a-g).

3.2 Cytotoxicity evaluation

MTT cell viability assay was performed on both Hela nuclear extract and Normal Embryonic cells, to determine the preliminary antitumor activities and safety indexes for some of the synthesized compounds, the results of the tested compounds 5a,5c,5e and 5fshows that , these compounds have good cytotoxic activity and also good selectivityfor cancer cellswhich represented by their high inhibition rate of cancer cells viability with low inhibition rate on normal cells, especially compounds 5a and 5e which have the highest safety index .as showing in figure 1 bellow.

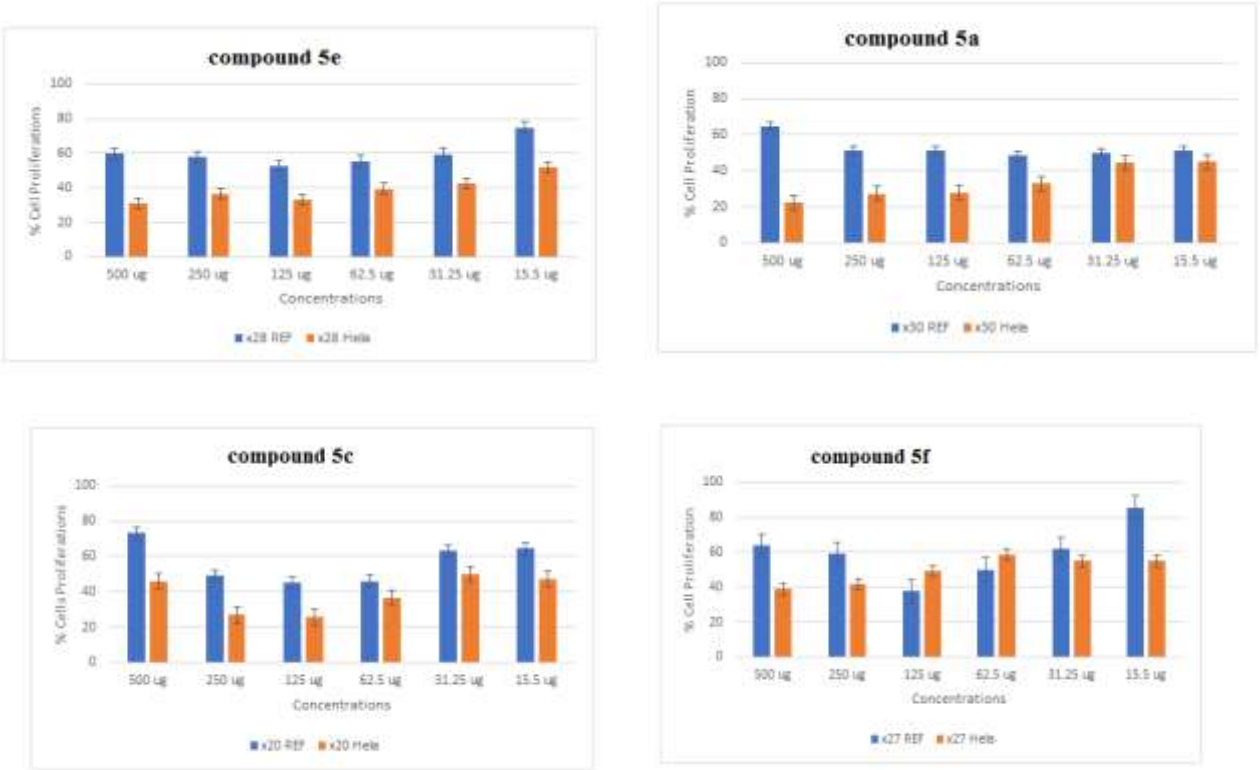


Figure 1 :Comparative Study for the cytotoxic effect of compounds (5a ,5c ,5e,5f) on HeLa and Normal embryonic cells for studying the safety index. .

CONCLUSION

In this work, we report the synthesis of new derivatives of hydroxamic acid based- HDACis containing substituted 1,3,4 thiadiazole as a surface recognition moieties with aliphatic linker, and evaluated for their antitumor activities against both HeLa nuclear extract cell lines and normal emberoinic fibroblast cells. Some of the tested compounds have good antitumor activities represented by inhibition of cell viability, while have no or little effects on normal cells as demonstrated by high safety index especially compounds 5a and 5e that show the highest tumor cell selectivity.

ACKNOWLEDGEMENT

The authors would like to thank the college of pharmacy/Dept.of pharmaceutical chemistry-Baghdad for support and carrying out this research , also a great appreciation and gratitude to Dr, Ahmmad M. Al shimmery for performing the antitumor work. .

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