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Synthesis, Characterization, and in vitro evaluation of the anticancer activity of new HA–based HDAC inhibitors containing amino acids and analides as a surface recognition moieties

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ABSTRACT

In the present study, two series of novel histone deacetylase [HDAC] inhibitors were designed, synthesized and their in vitro anticancer activity were evaluated. in the first series, we use the amino acids phenyl alanine, leucine and tyrosine as the surface recognition and capping groups, while in the second series, we use p- substituted a anilines as the surface recognition and capping groups, 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 using HeLa nuclear extract and normal embryonic fibroblasts cell lines. All the synthesized compounds show good anticancer activity, represented by their growth inhibition rate percent on Hela cell line and compound [IBD] show the best safety index[SI] that represented by its cytotoxic activity on cancer cell line while sparing the normal cell line.

Keywords: *HDACI, Amino acids, Analide, CAP groups.*

INTRODUCTION

The histone proteins play important roles in the control of gene expression through the modification of certain biochemical reactions such as acetylation of lysine residue in the N-terminal of histone core [1]. In most cases, histone acetylation increases the gene transcription while histone deacetylation represses gene transcription [2]. Histone acetylation is regulated by the opposing actions of two enzyme classes, histone acetyltransferases [HATs] and histone deacetylases [HDACs] [3]. The histone acetylation status was associated with regulation of expression of many genes that involved in regulation of many cellular processes such as cell cycle control, apoptosis, differentiation, and cell survival [4]. The inhibition of histone deacetylases [HDACs] causes the accumulation of acetylated histones, which bringing about a variety of cell type dependent responses such as induction of apoptosis, differentiation, and inhibition of cell proliferation [5]. There are 18 HDAC isozymes and they are divided into four classes: class I [HDAC 1-3, and 8], class II [HDAC 4, 5, 6, 7, 9, 10] and class IV [HDAC 11] are all zinc-dependent deacetylases, and class III isozymes including Sirt1 to Sirt7 are NAD+-dependent isozymes. The inhibitors can be classified as members of at least four classes of compounds: [i] hydroxamic acids, [ii] aliphatic acids, [iii] benzamides and [iv] cyclic tetrapeptides [6,7].

Most HDAC inhibitors fit a three-motif pharmacophoric model consisting of a zinc binding group [ZBG], a linker, and a surface recognition cap region [8]. Hydroxamic acid is by far the most common ZBG moiety in HDAC inhibitors owing to its ability to reliably chelate active-site zinc ions [9]. Several HDAC inhibitors such as vorinostat [SAHA] and panobinostat were approved by FDA for the treatment of cutaneous T cell lymphoma [CTCL] [10]. While HDACi shows promising antitumor effects, there are several draw backs which limit their use in clinic such as individual isoforms selectivity, low oral bioavailability, short half-life and bone marrow toxicity [11,12]. Therefore, there is considerable interest in developing compounds with selectivity and specificity towards individual family members of HDACs [13]. It has been shown that modification of cap and the linker shows promises of superior potency and isoform selectivity [14]. Therefore, the present study was undertaken to synthesize new hydroxamate –based HDACi having either amino acids or p-substituted aniline derivatives with suitable aliphatic linker with hope of obtaining inhibitors which are 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.

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 Hoover electric melting points apparatus and are uncorrected. FT-IR spectra [KBr] were recorded on Shimadzu FT-R-8400S spectrophotometer and 1HNMR spectra measured with400MHz, Advance III 400-Bruker, using tetramethylsilane as an internal standard. The chemical shifts are expressed in ppm δ scale. The percentage of carbon, hydrogen and nitrogen were obtained using a CHNS analyzer [Euro EA3000 elemental analyzer].

General Methods

The target compounds were synthesized by the following steps

General procedure for synthesis of compounds [1a-c] **[15].**

A suspension of the corresponding amino acids [50 mmol] [L-phenylalanine 8.25 g, l-leucine 6.6 g and l-tyrosine 9.05 g] in absolute methanol [150 ml] was cooled down to -15ºCin ice-bath, then thionyl chloride [4.8 ml, 65 mmol] was added drop wise, [the temperature should be kept below -10 ºC]. The reaction mixture was left at 40 ºC for 3 h, then refluxed for 3 h, and left at room temperature overnight, the solvent was evaporated to dryness under vacuum, redissolved in methanol and evaporated. This process was repeated several times and recrystallized from methanol / ethyl acetate [3:1], to give the desired product.

Synthesis of L-phenyl alanine methyl ester HCl, [Ia]

Off white powder, Yield 84%; m.p. 157-161°C; IR [υ= cm-1, KBr]:3200-2700 [NH_3 str.], 2958 and 2844[Asym and sym Str of CH_3 and CH_2, 1745 [C=O str of ester],

Synthesis of L-leucine methyl ester HCl, [Ib]

white powder Yield 82%; m.p. 146-150°C; IR [υ cm-1, KBr]:3200-2700 [NH_3 str.], 1738 [C=O str of ester], 1579 [NH_3 bending vib].

Synthesis of L-Tyrosine ethyl ester HCl, [Ic]

white powder; Yield 87%; m.p. 133-137°C; IR [υ= cm-1, KBr]: 3340 [OH str],3200-2700 [NH_3 str.], 1741 [C=O str of ester], 1608 [NH_3 bending]

General procedure for synthesis of compounds [2a-c] [16, 17].

A stirred solution of benzoic acid [2 gm, 0.013mole] and TEA [0.015 mole, 2.09 ml] in dry DCM [40 ml] was cooled down below -5ºC then ethyl chloroformate [0,013mole,1.24 ml] was added drop wise over a period of 20 minute with vigorous stirring. The stirring was continued for additional 30 minute and the temperature should have kept below 0 ºC. Compound 1a-c [0.015 mole] [1a3.23 g, 1b3.05, 1c 3.48 g] in dry DCM: DMF mixture [20ml] was then added together with two equivalent amount of TEA. Stirring was continued for 3hr at 5-10 0C, and the mixture was stirred overnight at ambient temperature. The solvent was evaporated to dryness under reduced pressure and the residue was washed twice with cooled [15ml] of 5%N HCl, 5% sodium bicarbonate and DW successively the product was filtered, collected, dried and then recrystallized from dilute ethanol 60%.

Synthesis of methyl-2-benzamido-3-phenylpropanoate[2a]

White viscous oil; Yield 71%; IR [υ= cm-1, KBr]: 3277 [NH str], 2949 and 2847 [A sym. and sym. C-H stretching vibration of CH_2] ,1737 [C=O str of ester], 1701 [C=O str. of amide.]

Synthesis of methyl-2-benzamido-4-methylpentanoate[2b]

White powder; Yield 68%; m.p. 76-79°C; IR [υ = cm-1, KBr]: 328, [NH str],3063 [C-H str.] 2955 and 2868 [A sym. and sym. C-H str. of $CH_{2]}$,1745 [C=O str of ester], 1799 [C=O str. of amide.]

Synthesis of Methyl-2-benzamido-3-[4-hydroxyphenyl]-2methylpropanoate[2c].

White powder; Yield 55%; m.p. 101-104 C; IR [υ= cm-1, KBr]: 3336[OH str.] 3284, [NH str], 3064 and 3024 [C-H str.] 2955 and 2850 [V as and Vs C-H str. of $CH_{2]}$,1712 [C=O str of ester], 1641 [C=O str of amide]

General procedure for synthesis of compounds [3a-c] [18]

Compound 2a-c [0.0063mole], [2a 2 g, 2b 1.69, 2c 2.14 g] was dissolved in 50ml of absolute ethanol in suitable round bottom flask, the solution was warmed to 40 ºC with stirring. To this mixture hydrazine hydrate [0,02mole, 1ml] was then added and the mixture was refluxed for 9-12 hrs, the mixture was cooled and left aside overnight , the product was precipitate out, filtered , collected . washed with ethanol, dried and recrystallized from diluted ethanol.

Synthesis of N-[1-hydrazinyl-2-methyl-1-oxo-3-phenylpropan-2-yl] benzamide [3a].

White powder; Yield 75%; m.p. 108-110°C; IR [υ= cm-1, KBr]: 3371 and3307, [NH_2 str], 3064 and 3024 [C-H str.] 2938 and 2813 [Sym as and asym Str of $CH_{2]}$,1656 [C=O str of amide],

Synthesis of [N- [1-hydrazinyl-2,4-dimethyl-1-oxopentan-2 yl] benzamide [3b]

White powder; Yield 78%; m.p. 89-91°C; IR [υ= cm-1, KBr]: 3369 and3269, [NH_2 str], 3066 [C-H str.] 2931 and 2870 [Sym as and a sym str of $CH_{2]}$,1691 [C=O str of amide],

Synthesis of N-[1-hydrazinyl-3-[4-hydroxyphenyl]-2-methyl-1-oxopropan-2-yl] benzamide [3c].

White powder; Yield 55%; m.p. 116-118°C; IR [υ= cm-1, KBr]: 3287 and 3198, [NH_2 str overlapped with OH str], 3067 [C-H str.] 2970 and 2864 [Sym and a sym str of $CH_{2]}$,1694 [C=O str of amide],

General procedure for synthesis of compounds [4a-c] [16] [17].

A stirred solution of adipic acid mono ethyl ester [1.74 gm, 0.01 mole] and TEA [0.012 mole, 1.675 ml] in dry DMF [15 ml] was cooled down below -5ºC. Ethylchloroformate [0.01mole,0.95 ml] was added drop wise over a period of 20 minute with vigorous stirring. The stirring was continued for additional 30 minute and the temperature should have kept below 0 ºC. Compound 3a-c [0.012 mole] [3a, 3.76 g, 3b 2.18, 3c 3.94 g] in dry DMF [15ml] was then added together with an equivalent amount of TEA. Stirring was continued for 3hr at 5-10 ºC, and the mixture was stirred overnight at ambient temperature. The solvent was evaporated to dryness under reduced pressure and the residue was washed twice with cooled [15ml] of 0.1N HCl, 5% sodium bicarbonate and DW successively. The product was filtered, collected, dried and then recrystallized from water: DMF mixture.

Synthesis of ethyl 6- [2-[2-benzamido-2-methyl-3-pheny lpropanoyl]hydrazinyl]-6-oxohexanoate[4a].

White powder; Yield 74%; m.p. 65-68 °C; IR [υ= cm-1, KBr]: 3238, [NH str], 3086 [C-H str.] 2977 and 2872 [Asym and sym. C-H str. of $CH_{2]}$,1734 [C=O str of ester], 1682 [C=O str. of amide.]

Synthesis of ethyl 6-[2-[2-benzamido-4methylpentanoyl] hydrazinyl]-6 oxohexanoate, [4b].

White powder; Yield 73%; m.p. 55-57°C; IR [υ= cm-1, KBr]: 3211, [NH str], 3043 [C-H str.] 2960and 2868 [Asym and sym. C-H str. of $CH_{2]}$,1733 [C=O str of ester], 1633 [C=O str. of amide.]

Synthesis of ethyl6 -[2-[2-benzamido-3-[4-hydroxyphenyl]-2-methyl propanoyl] hydrazinyl]-6-oxohexanoate, [4c].

White powder; Yield 64%; m.p. 98-101°C; IR [υ cm-1, KBr]: 3211, [OH and NH str], 3055 [C-H str.] 2955and 2871 [Asym. And sym. C-H str. of $CH_{2]}$,1730 [C=O str of ester], 1699[C=O str. of amide.]

General procedure for synthesis of compounds [5a-c] [19] [20].

A stirred solution of compounds 4a-c [0.0017 mol] [4a 0.75 gm ,4b 0.7 g, 4c 0.77g] in dry 1:3 DMF-methanol [20 ml] was cooled to 0 ºC in ice- bath, and hydroxylamine 50 % [1.048 ml, 0.017mole , 10 equivalent] was added followed by immediate addition of about 5 mg of KCN . The mixture was allowed to warm to room temperature and stirred for [25- 32] hrs. 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 10 ml of DW, 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, washed with 5% sodium bicarbonate [2× 15 ml] and DW and the product was filtered, collected and dried.

Synthesis of N-[1-[2-[6-[hydroxyamino]-6-oxohexanoyl] hydrazinyl]-1-oxo-3-phenylpropan-2-yl] benzamide[5a].

White powder; Yield 59%; m.p.133-136°C; IR [υ=cm-1, KBr]: 3240, [OH and NH str], 3053 [C-H str.] 2929and 2858 [Asym and sym. C-H str. of $CH_{2]}$,1673 [C=O str of amide]. HNMR[400MHz], DMSO-d_6, δ ppm]:12.1[s,1H, -O<u>H</u>],11.8[s,1H, CO-N<u>H</u>], 11.7[s,1H, CO-N<u>H</u>],11.3[s,1H, N<u>H</u>-OH],11.1[d, IH, CO-N<u>H</u>], 7-8. [m,10H, Ar-<u>H</u>],5.3 [q, 2H, C<u>H</u>$_2$-],3.6[t,4H, C<u>H</u>$_2$-CO] 3.2[d,2H, Ar-C<u>H</u>$_2$]2.9 [t, 4H, -C<u>H</u>$_2$-]; Elemental analysis Calcd. for $C_{22}H_{26}N_4O_5$: C, 61.96; H, 6.15; N, 13.14. Found: C, 60.07; H,6.33; N, 12.56.

Synthesis of N-[1-[2-[6-[hydroxyamino]-6-oxohexanoyl] hydrazinyl]-4-methyl-1-oxopentan-2-yl] benzamide [5b].

white oil Yield 70%; m.p. °C; IR [υ= cm-1, KBr]: 3240, [OH and NH str], 3049 [C-H str.] 2978and 2874 [Asym and sym C-H str. of CH_2] ,1692 [C=O str of amide]. 1HNMR[400MHz],DMSO-d6,δppm]:12.6[s,1H,-OH],11.5[s,1H,CO NH],11.4[s,1H,CO-NH],10.9.[s,1H,NH-OH],11.1[s,IH,CO-NH],6.9-7.5[m,5H,Ar-H], 3.4[t,4H,CH2-CO], 2.9[t,2H,-CH2-] ,2.7[t,4H,-CH2-],4.7[q,1H,-CH-],2.2[m,1H,-CH-], 1.2 [d,6H, -CH3]; Elemental analysis Calcd. For C19H28N4O: C, 58.15; H, 7.19; N, 14.28. Found: C, 59.11; H, 7.66; N, 14.41.

Synthesis of N-[1-[2-[6-[hydroxyamino]-6oxohexanoyl] hydrazinyl]-3-[4 hydroxyphenyl]-1-oxopropan-2-yl] benzamide, [5c].

White powder; Yield 55%; m.p. 88-90°C; IR [υ= cm-1, KBr]: 3219, [OH str], 3197 [NH str.] 3049 [C-H str.] 2920and 2872 [Asym and sym. C-H str. of CH2] ,1662 [C=O str of amide]. 1HNMR[400MHz], DMSO-d6, δppm]:12.8[s,1H, -OH],12.0[s, 1H, NH-OH],11.7[s,1H,NH-OH],11.3[s,1H,NH-CO],11.0[d,1H,NH-],10.8[d,1H,-NH]7-8[m,9H,Ar-H],5.7[q,1H,CH-CO], 3.4[t,4H,CO-CH2-], 3.1[d,2H,-Ar-CH2],2.3[t,4H,-CH2-] ;Elemental analysis Calcd. for C22H26N3O: C, 59.72; H, 5.92; N, 12.66. Found: C, 58.03; H, 5.22; N, 12.99.

General procedure for synthesis of compounds [6a-d] [21] [22].

Adipic acid monoethyl ester [1.742 gm, 0.01 mole] was placed in a dry-150 ml two-necked flask connected with a dropping funnel and reflex condenser, Thionyl chloride [1.089 ml, 0.015 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 C^0 for 5 hrs and the excess thionyl chloride was removed under reduced pressure. The clear pale-yellowish liquid was dissolved without further purification in 10 ml of dry DCM and added drop wise with stirring to an ice- cooled at 0 ºC mixture of TEA [1.67 ml, 0.012 mole] and [0.01 mole] of p-substituted aniline [p-chloroaniline 1.275 gm, p-nitro aniline1.38 g , p- bromo aniline 1.71 g and p- methoxy aniline 1.275 g] in 25 ml of dry DCM: DMf mixture. The resulted suspension was stirred at room temperature for 2-4 hrs and reflexed for additional 2-3 hrs. The solvent was evaporated and 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-[4-chlorophenylamino]-6-oxohexanoate[6a].

Khaki powder; Yield 77%; m.p. 85-88 °C; IR [υ =cm-1, KBr]: 3361 [NH str.] 3113 [C-H str.] 2947and 2875 [Asym and sym C-H str. of CH_2] ,1742 [C=O str. of ester], 1697 [C=O str of amide].

Synthesis of ethyl 6-[4-nitrophenylamino]-6-oxohexanoate [6b]

Yellow powder; Yield 72%; m.p. 108-111°C; IR [υ= cm-1, KBr]: 3339 [NH str.] 3109 [C-H str.] 2949and 2875 [Asym and sym. C-H str. of CH_2] ,1718 [C=O str. of ester], 1697[C=O str of amide].

Synthesis of ethyl6-[4-bromophenylamino]-6-oxohexanoate[6c].

White powder; Yield 68%; m.p. 77-79°C; IR [υ= cm-1, KBr]: 3218 [NH str.] 3111 [C-H str.] 2980 and 2875 [Asym and sym. C-H str. of CH_2] ,1716 [C=O str. of ester], 1698 [C=O str of amide].

Synthesis of ethyl6-[4-methoxyphenylamino]-6-oxohexanoate[6d].

White powder; Yield 69%; m.p. 95-98 °C; IR [υ= cm-1, KBr]: 3300 [NH str.] 3010 [C-H str.] 2964and 2868 [Asym and sym. C-H str. of CH_2] ,1720 [C=O str. of ester], 1660 [C=O str of amide].

General procedure for synthesis of compounds [7a-d] [19] [20].

A stirred solution of compound 6a-d [0.00176 mole] [6a 0.5 g, 6b0.5 g, 6c 0.58, 6d 0.52 g] in dry 1:3 DCM- methanol [20 ml] was cooled to 0 ºC in ice- bath, and hydroxylamine 50 % [1.08 ml, 0.0176, 10 equivalent] was added followed by immediate

addition of about 4-6 mg of KCN . The mixture was allowed to warm to room temperature and stirred for 30-32 hrs. 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 10 ml of DW, 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, washed with 5% sodium bicarbonate [2× 15 ml] and DW and the product was filtered, collected and dried.

Synthesis of N1-[4-chlorophenyl]-N6-hydroxyadipamide[7a].

White powder; Yield 70%; m.p. 65-67 °C; IR [υ= cm-1, KBr]: 3261 [OH str.] 3130 [NH str.] ,3067 [C-H str.], 2955and 2874 [Asym and sym. C-H str. of CH2] ,1673 [C=O str. of amide], 1638 [amide II]. 1HNMR[400MHz], DMSO-d6, δppm]:12.6[s,1H, -OH],11.8[s,1H, CO-NH],11.2[s,1H, OH-NH],7-8. [m,4H, Ar-H],3.4[t,4H, CH2-CO],2.1[t,4H, CH2-]; Elemental analysis Calcd for $C_{12}H_{15}ClN_2O_3$:C, 53. 24; H, 5.58; N, 10.35. found: C, 54.21; H, 5.29; N, 10.11.

Synthesis of N1-hydroxy-N6-[4-nitrophenyl] adipamide, [7b].

White powder; Yield 54%; m.p. 124-126°C; IR [υ =cm-1, KBr]: 3335 [OH str.] 3133 [NH str.] ,3081 [C-H str.], 2952and 2876 [Asym And sym C-H str. of CH_2] ,1696 [C=O str. of amide], 161 3 [amide II] ^{1}HNMR[400MHz], DMSO-d_6, δppm]:12.4[s,1H,-O<u>H</u>],11.9[s,1H,CO-N<u>H</u>],11.0[s,1H,OH-N<u>H</u>],7-8.[m,4H,Ar-<u>H</u>],3.3[t,4H,C<u>H</u>$_2$-CO],2.1[t,4H,C<u>H</u>$_2$-]; Elemental analysis Calcd. for $C_{12}H_{15}N_3O_5$:C, 51.24; H, 5.38; N, 14.94. found: C, 51. 76; H, 5.88; N, 14.09.

Synthesis of N1-[4-bromophenyl]-N6-hydroxyadipamide[7c].

White powder; Yield 59%; m.p. 97-99 °C; IR [υ =cm-1, KBr]: 3297 [OH str.] 3230 [NH str.], 3010 [C-H str.], 2989and 2842 [Asym And sym C-H str. of CH_2], 1686 [C=O str. of amide], 1624 [amideII].1HNMR[400MHz], DMSO-d6, δppm]:12.3[s,1 H, -OH],11.0[s,1H,CO-N<u>H</u>],11.3[s,1H,OH-N<u>H</u>],7-8.[m,4H,Ar-<u>H</u>],3.4[t,4H,C<u>H</u>$_2$ CO],2.6[t,4H,CH<u>2</u>-]; Elemental analysis Calcd. for C12H15BrN2O3:C, 45.73; H, 4.80; N, 8.89. found C, 45.07; H, 4.99; N, 8.33

SynthesisofN1-hydroxy-N6-[4-methoxyphenyl] adipamide, [7d].

White powder; Yield 66%; m.p. 116-118 °C; IR [υ = cm-1, KBr]: 3311 [OH str.] 3125 [NH str.] ,3057 [C-H str.], 2970and 2841 [Asym and sym C-H str. of CH_2] ,1698 [C=O str. of amide]. ^{1}HNMR[400MHz], DMSO-d_6, δppm]:12.3[s,1 H,-O<u>H</u>],11.7[s,1H, CO-N<u>H</u>],11.8[s,1H, OH-N<u>H</u>],7-8.[m,4H,Ar-<u>H</u>],3.5[t,4H,C<u>H</u>$_2$-CO],2.3[t,4H,C<u>H</u>$_2$-]; Elemental analysis Calcd. for $C_{13}H_{18}N_2O_4$; C,58.63; H,6.81; N,10.52 found C, 57.06; H, 6.11; N, 9.55.

Cytotoxicity assay [MTT assay] [23-25]

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 Drug 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 in triplicate.

RESULTS AND DISCUSSION

Chemistry

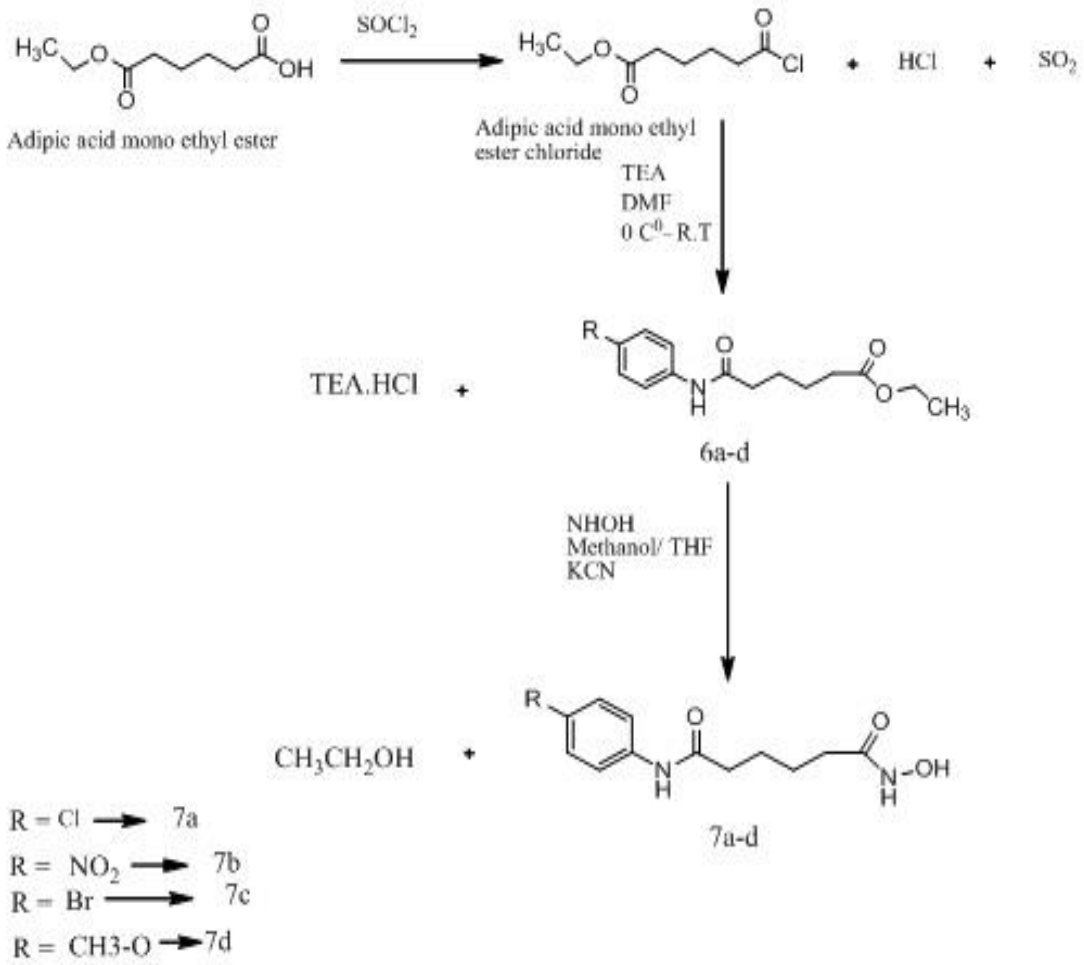
The synthesis of the title compounds [1a-c] to [5a-c] was accomplished and outlined in the scheme 1. While the synthesis of compounds 6a-d to 7a-d was outlined in the scheme 2. The scheme1 illustrated the reactions steps for all synthesized derivatives.in which the corresponding amino acids [l- phenyl alanine l- leucine and L- tyrosine] was esterified by reaction with thionyl chloride in methanolic solution to get compounds 1a-c in good yield. Compounds 2a-c were synthesized by reaction of compound 1a-c with benzoic acid in dry condition and in the presence of TEA and ethyl chloroformate [mixed anhydride mechanism].

Compounds 2a-c then reflexed with hydrazine hydrate in alcoholic solution to gets hydrazide derivatives of amino acids compound 3a-c, then hydrazide derivatives reacted with activated adipic acid mono ethyl ester by ethyl chloroformate through mixed anhydride mechanism to get compounds 4a-c. The final compounds 5a-c were obtained by reaction of compounds 4a-c with hydroxylamine in alcoholic solution and with the presence of KCN as catalyst. Compounds 6a-d were synthesized by activation of adipic acid mono ethyl ester with thionyl chloride to get acid chloride that react with [p- chloroaniline, p-nitro anile, p-bromo aniline and p-methoxy aniline] in the presence of TEA to get compounds 7a-d.

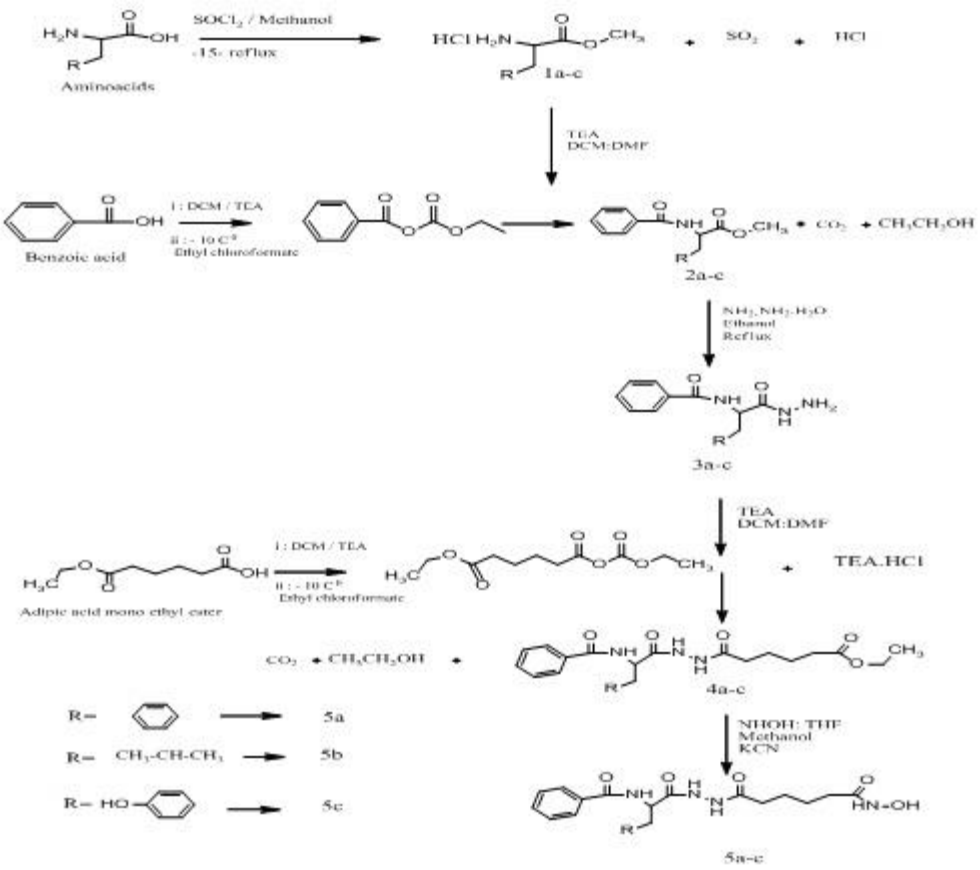
The IR spectra of the synthesized compounds[1a-c] show disappearance of the absorption bands of C=O and OH of COOH of the amino acids and appearance of new characteristics bands of the synthesized amino acid esters at [1745] cm-1 for compound 1a, [1738] cm-1 for compound 1b and at [1741]cm-1 for compound 1c,which represent the C=O stretching vibration of esters, while The IR spectra of compounds[2a-c] shows disappearance of asymmetric and symmetric stretching vibrations of NH_2 in the reactants and the appearance of new characteristic absorption bands which represents NH stretching vibrations of secondary amide, and C=O stretching vibrations of amide respectively at[3273,1641] for compound 2a, at [3281, 1699] for compound 2b and at [3284,1641] for compound 2c. The IR spectra of compounds[3a-c] shows the disappearance of C=O stretching vibrations of ester in the reactants and the appearance of new strong bands which represents the asymmetric and the symmetric stretching vibrations of NH2 at [3371. 3307] for compound 3a, at [3369, 3269] for compound 3b and at [3287, 3198] for compound 3c, while The IR spectra also shows the appearance of new strong bands in the spectra of compounds [4a-c], which represents the C=O stretching vibrations of ester group at [1734] for compound 4a, at [1733] for compound 4b and at [1730] for compound 4c. Also, The IR spectra of compounds[5a-c] shows the disappearance of C=O stretching vibrations of ester in the reactants and the appearance of new and relatively broad bands which results from hydroxyl and NH group stretching vibration of hydroxamic acid.

These bands appear at [3240 overlapped] for compound 5a, at [3240 overlapped] for compound 5b, at [3219 overlapped] for compound 5c. The IR spectrum of compounds[6a-d] shows disappearance of asymmetric and symmetric stretching vibrations of NH_2 in the reactants and the appearance of new strong bands which represents the C=O stretching vibration of ester at, at [1742] for compound 6a , at [1718] for compound 6b at [1,716] for compound 6c and at [1720] for compound 6d, The IR spectra of compounds [7a-d] shows the disappearance of C=O stretching vibrations of ester in the reactants and the appearance of new and

relatively broad bands which results from hydroxyl and NH group stretching vibration of hydroxamic acid . These bands appear at [3261 overlapped] for compound 7a, at [3335] for compound 7c and at [3311] for compound 7d



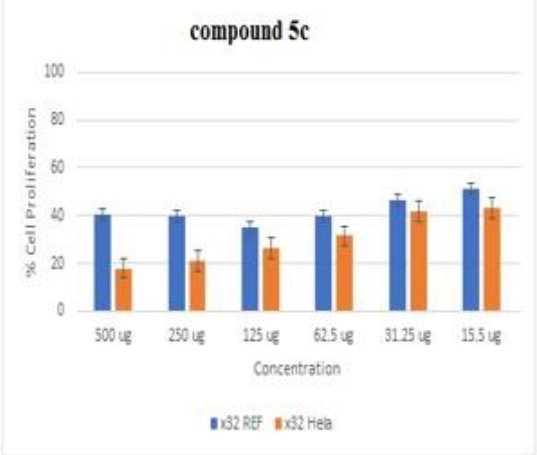
Scheme-1: synthesis of compounds 7a-d.



Scheme-2: synthesis of compounds 5a-c

Cytotoxicity evaluation

MTT cell viability assay was conducted on Hela nuclear extract and Normal Embryonic cells, to determine the preliminary cytotoxic activities and safety indexes for the synthesized compounds, the results show that the target compounds have good cytotoxic activity and selectivity represented by their high inhibition rate of cancel cells viability with low inhibition rate on normal cells, especially compounds 5a and 7a which have the highest safety index .as showing in figures bellow**.**



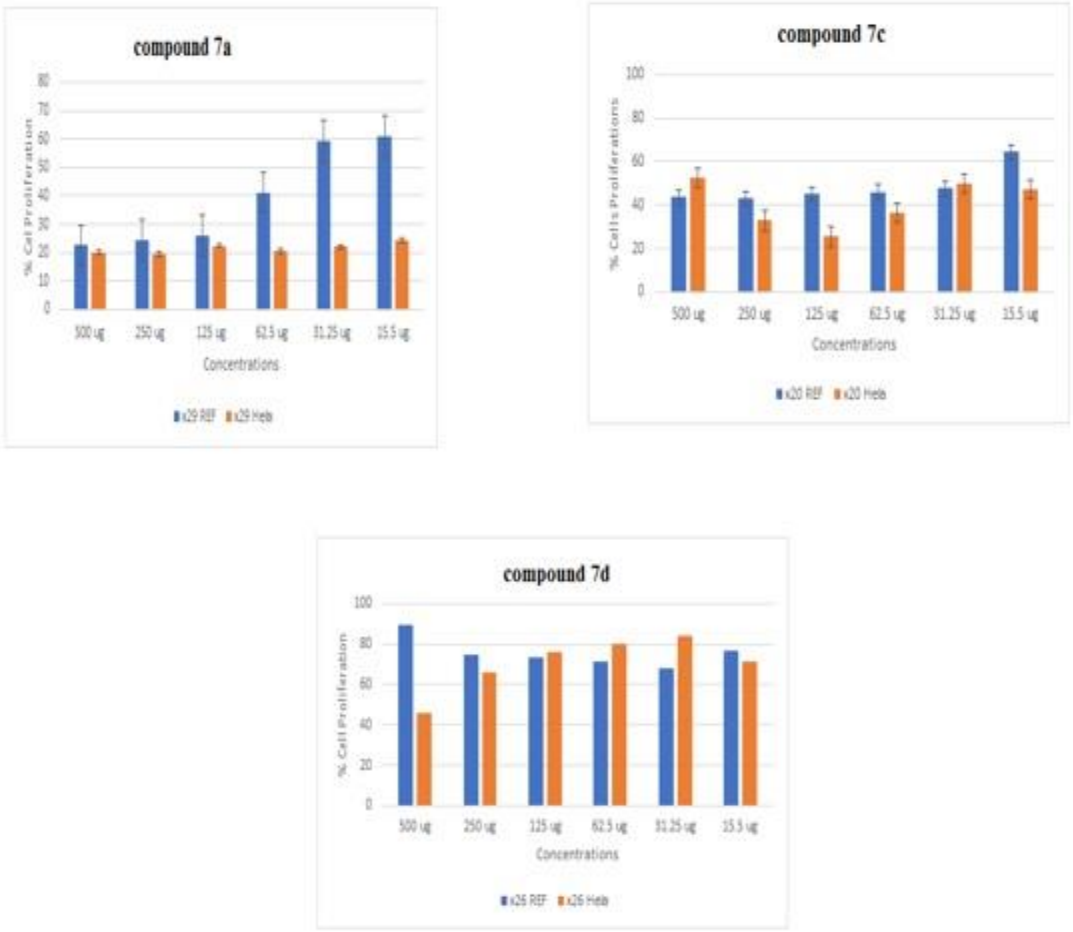


Figure-1: Comparative Study for the cytotoxic effect of compounds [5a ,5c ,7a,7c and 7d] on Hela and Normal embryonic cells for studying the safety index.

CONCLUSION

In this study, we report the synthesis of new derivatives of hydroxamic acid based- histone deacetylase inhibitors containing amino acids and analides as a surface recognition moieties, and evaluated for their antitumor activities against both HeLa nuclear extract cell lines and normal emberoinic fibroblast cells. Some of the tested compounds have potent antitumor activities, while have no or little effects on normal cells as demonstrated by high safety index especially compounds 5a and 7a that show the highest tumor cell selectivity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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