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ORIGINAL ARTICLE

Synthesis and anticancer activity of long chain substituted 1,3,4-oxadiazol-2-thione, 1,2,4-triazol-3-thione and 1,2,4-triazolo [3,4-b]-1,3,4-thiadiazine derivatives

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Abstract In this paper, three novel series of 5-long chain alkenyl/hydorxyalkenyl-1,3,4-oxadiazol-2-thiones **2(a–d)**, 4-amino-5-long chain alkenyl/hydroxyalkenyl-1,2,4-triazol-3-thiones **3(a–d)** and 3-long chain alkenyl/hydroxyalkenyl-6-phenyl-(7H)-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines **4(a–d)** were synthesized with an aim to produce promising anticancer agents. We describe here the synthesis of compounds **2(a–d)**, **3(a–d)** and **4(a–d)** using long chain alkenyl/hydroxyalkenyl hydrazides **1(a–d)** as starting material. Our investigation shows that the thione tautomer of **2(a–d)** and **3(a–d)** dominates. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. After characterization, all compounds were tested for *in vitro* anticancer activity against PBMCs and three different human cancer cell lines. On the basis of SAR, it may be concluded that the potency of drugs depends on the nature of FA chain and the heterocyclic ring system. Among all the tested compounds, compounds having fused ring system

Abbreviations: PBMCs, peripheral blood mononuclear cells; FA, fatty acid; FAs, fatty acids; SAR, structure–activity relationship; FAH, fatty acid hydrazide; FAHs, fatty acid hydrazides; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide

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(triazolothiadiazine nucleus) and the hydroxyl group attached to the FA chain (**4c** and **4d**) were found to be the most promising anticancer agents.

1. Introduction

Nitrogen, oxygen and sulfur containing compounds are the most common heterocycles which serve as the core component of a large number of biochemical materials which are essential to life such as nucleic acids. Oxadiazoles are used as support on which pharmacophores are placed to provide potent and selective medicines (Somani and Shirodkar, 2009). During the past few years, considerable evidence has been accumulated that demonstrates the efficacy of 1,3,4-oxadiazoles including insecticidal, analgesic, diuretic, CNS depressant, antiviral, herbicidal, antihypertensive, pesticidal activities and these are cited in the literature (Deshmukh et al., 2011). Oxadiazoles also possess antitubercular (Rane et al., 2012), antimalarial (Mohan et al., 2004), antileishmanial (Rastogi et al., 2006) and anticancer (Bondock et al., 2012) activities. The 1,2,4-triazole derivatives possess a broad spectrum of activities including antimalarial, anticancer and antitubercular (Husain et al., 2012; Pattan et al., 2012) and also have a wide range of therapeutic properties like analgesic (Almasirad et al., 2011), insecticidal (Tirlapur and Tadmalle, 2011), hypoglycemic (Nath et al., 2011), antiparasitic, herbicidal (Wang et al., 2011) and plant growth activities (Aggarwal et al., 2011). The 1,2,4-triazole nucleus is extensively used in medicines (Aytaç et al., 2009). Due to ambidentate nucleophilic centers i.e. mercapto and amino groups, the 4-amino-1,2,4-triazol-3-thiones can be considered as excellent starting materials for the synthesis of triazolothiadiazines. The substituted 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine derivatives have been reported to possess antimicrobial activity mainly due to N–C–S linkage in the skeleton of triazolothiadiazine and also possess anticancer activity (Altintop et al., 2011; Badr and Barwa, 2011; Mohammad et al., 2012; Prakash et al., 2011). These biheterocyclic triazolothiadiazine derivatives also possess a broad spectrum of pharmacological activities (El-Shehry et al., 2010; Kumar et al., 2010). The literature survey (Lamani and Kotresh, 2010; Mohsen, 2012) reveals that there are many examples of triazole fused with pyridines, pyridazines, pyrimidines, pyrazines and triazines but triazolothiadiazines are not very common moieties.

Earlier works (Sharma et al., 2011; Shalini et al., 2011) showed that these heterocycles were synthesized from different substituted carboxylic acids and hydrazides (other than long chain alkenyl/hydroxyalkenyl hydrazides). Literature survey (Srivastava et al., 2010) also reveals that a minor change in the structure of 1,3,4-oxadiazoles, 1,2,4-triazoles and 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines can lead to quantitative and qualitative changes in biological activity. Recently (Jubie et al., 2012), stearic acid (a saturated FA) analogs having 1,3,4-oxadiazole, 1,2,4-triazole and 1,2,4-triazolo-[3,4-b]1,3,4-thiadiazole were reported as antidepressant and antimicrobial agents. Also, the usage of FAs shows an increasing trend in the treatment of neuropsychological disorders such as depression and schizophrenia (Sinclair et al., 2007; Yamima and Belmaker, 2009). Despite this, some of the FAs have been found to play a regulatory role in tumor growth progression and were reported as effective anticancer agents (Khan et al., 2011). Keeping in view the significance, the aforementioned facts of long chain alkenyl/hydroxyalkenyl carboxylic acids as potential pharmacophores and in continuation of earlier research work in our laboratory on the synthesis of novel series of biologically active heterocyclic derivatives of FAs (Farshori et al., 2011), we herein report the synthesis of three different novel series of biologically important heterocyclic FA analogs. And evaluate *in vitro* anticancer activity against three different human cancer cell lines and normal human cells (PBMCs). The salient features of the procedure described here are taking short reaction time, not requiring elevated temperatures, the use of cheap reagents and easily available starting materials.

2. Experimental protocol

2.1. Materials and methods

(Z)-octadec-9-enoic acid (purity, 97%) and undec-10-enoic acid (purity, 98%) were purchased from Fluka chemicals (Switzerland). (9Z, 12R)-12-hydroxyoctadec-9-enoic acid (Ricinolic) and (9R, 12Z)-9-hydroxyoctadec-12-enoic acid (Isoricinolic) were isolated from the natural sources i.e. from *Ricinus communis* and *Wrightia tinctoria* seed oils respectively, following Gunstone's partition procedure (Gunstone, 1954), general grade (GR) of solvents was used for the extraction purposes. Hydroxy FAs were purified by column chromatography and characterized by the spectral data. FA esters were prepared by refluxing the FAs in methanol in the presence of catalytic amount of concentrated sulfuric acid. Long chain alkenyl/hydroxyalkenyl hydrazide was used as starting material which was previously synthesized in our laboratory. All the products were purified by column chromatography. When required, solvents were dried and distilled before use. Carbon disulfide, potassium hydroxide, phenacyl bromide, hydrazine hydrate were purchased from Merck, Mumbai, India. Thin layer chromatography (TLC) was performed on glass plates (20×5 cm) with a layer of silica gel G (Merck, Mumbai, India, 0.55 mm thickness). Developing solvents used were the mixture of petroleum ether-diethyl ether-acetic acid (75:25:1; v/v). Silica gel (Merck, Mumbai, India, 60–120 mesh) was used for carrying column chromatography. IR spectra were obtained on Shimadzu 8201 PC FT-IR using KBr Pellet with absorption given in cm^{-1}. ^1H NMR spectra were recorded with Bruker DRX 400 Spectrometer (400 MHz) in $CDCl_3$, using TMS as internal standard. Chemical shifts (δ) are quoted in ppm and coupling constants (J) are given in Hz. ^{13}C NMR spectra were recorded with Bruker, DRX 400 Spectrometer in $CDCl_3$ (δ = 77.00). Mass spectra were recorded on JEOL-SX 102/DA-600 Mass Spectrometer.

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2.2. Chemistry

2.2.1. Synthesis of novel series of 5-long chain alkenyl/hydroxyalkenyl-1,3-4-oxadiazol-2-thiones, 2(a–d)

A mixture of 0.01 mol of long chain alkenyl/hydroxyalkenyl hydrazide (Rauf et al., 2007) **1(a–d)**, 0.01 mol of potassium hydroxide and 10 mL of carbon disulfide was refluxed (80–90 °C) in 50 mL ethanol for 8 h. The reaction mixture was concentrated on water bath, then cooled to room temperature, acidified with dil. HCl at 0 °C and the solid product was separated out. After that the product was filtered and washed with cold water. The solid products **2(a–d)** were then air dried. Further, the products **2(a–d)** were purified by silica gel column chromatography with petroleum ether: diethyl ether as eluent. The products were identified by spectral data.

*2.2.1.1. 5-(Dec-9-enyl)-(3H)-1,3,4-oxadiazol-2-thione, **2a**.* IR (KBr, cm^{-1}): 3215 (N–H stretching), 2921 (C–H stretching), 1613 (C=N stretching), 1165 (C=S stretching), 1054 (C–O–C stretching). ^1H NMR ($CDCl_3$, δ_H): 10.30 (1H, s, N*H*), 5.81 (1H, tdd, $J_{H-^8CH_2} = 6.48$ H_Z, $J_{H-H_Z} = 10.31$ H_Z, $J_{H-H_E} = 17.24$ H_Z, CH_2=C*H*), 5.01 (1H, dd, $J_{H_Z-H} = 10.31$ H_Z, $J_{H_Z-H_E} = 2.00$ H_Z, H_ZC=CH), 4.92 (1H, dd, $J_{H_E-H} = 16.91$ H_Z, $J_{H_E-H_Z} = 2.00$ H_Z, H_EC=CH), 2.69 (2H, t, J = 7.50 Hz, CH_2 α to ring), 2.01 (2H, m, CH_2=CH–CH_2), 1.74 (2H, m, CH_2 β to ring), 1.30 (10H, br.s, $(CH_2)_5$). ^{13}C NMR ($CDCl_3$, δ_C): 166.8, 165.4, 123.0, 122.3, 30.9, 30.8, 30.1, 29.9, 28.5, 27.1, 26.2, 24.2. MS (ESI): m/z = 262.880 found $[M+Na]^+$, calculated $[M+Na]^+$ = 263.146.

*2.2.1.2. 5-(Heptadec-8-enyl)-(3H)-1,3,4-oxadiazol-2-thione, **2b**.* IR (KBr, cm^{-1}): 3218 (N–H stretching), 2925 (C–H stretching), 1615 (C=N stretching), 1166 (C=S stretching) 1059 (C–O–C stretching). ^1H NMR ($CDCl_3$, δ_H): 10.85 (1H, s, N*H*), 5.35 (2H, m, C*H*=C*H*), 2.69 (2H, t, J = 7.58 H_Z, CH_2 α to ring), 2.01 (4H, m, CH_2CH=CHCH_2), 1.75 (2H, m, CH_2 β to ring), 1.31 (20H, br.s, $(CH_2)_{10}$), 0.88 (3H, dist.t, CH_3). ^{13}C NMR ($CDCl_3$, δ_C): 165.3, 163.2, 126.0, 125.8, 35.0, 33.9, 31.0, 29.4, 28.9, 28.7, 28.0, 27.3, 26.8, 25.8, 23.7, 23.4, 23.0, 22.0, 14.0. MS (ESI) m/z = 362.231 found $[M+Na]^+$, calculated $[M+Na]^+$ = 361.481.

*2.2.1.3. 5-[(8Z, 11R)-11-Hydroxyheptadec-8-enyl]-(3H)-1,3,4-oxadiazol-2-thione, **2c**.* IR (KBr, cm^{-1}): 3398 (O–H stretching), 3121 (N–H stretching), 2923 (C–H stretching), 1625 (C=N stretching), 1159 (C=S stretching), 1056 (C–O–C stretching). ^1H NMR ($CDCl_3$, δ_H): 12.34 (1H, s, N*H*), 5.47 (2H, m, C*H*=C*H*), 3.68 (1H, m, C*H*OH), 2.68 (2H, t, J = 7.49 H_Z, CH_2 α to ring), 2.28(1H, m, CHO*H*), 2.04 (4H, m, CH_2CH=CHCH_2), 1.72 (2H, m, CH_2 β to ring), 1.28 (18H, br.s, $(CH_2)_9$), 0.88 (3H, dist.t, CH_3). ^{13}C NMR ($CDCl_3$, δ_C): 168.3, 164.2, 133.2, 125.1, 72.0, 37.3, 36.6, 35.1, 31.8, 30.1, 29.7, 29.6, 29.1, 28.9, 27.2, 25.4, 24.0, 23.4, 14.2. MS (ESI) m/z = 376.689 found $[M+Na]^+$, calculated $[M+Na]^+$ = 377.420.

*2.2.1.4. 5-[(8R, 11Z)-8-Hydroxyheptadec-11-enyl]-(3H)-1,3,4-oxadiazol-2-thione, **2d**.* IR (KBr, cm^{-1}): 3353 (O–H stretching), 3159 (N–H stretching), 2921 (C–H stretching), 1619 (C=N stretching), 1152 (C=S stretching), 1051 (C–O–C stretching). ^1H NMR ($CDCl_3$, δ_H): 11.56 (1H, s, N*H*), 5.37 (2H, m, C*H*=C*H*), 3.59 (1H, m, C*H*OH), 2.70 (2H, t, J = 7.50 H_Z, CH_2 α to ring), 2.25 (1H, m, CHO*H*), 2.00 (4H, m, CH_2CH=CHCH_2), 1.72 (2H, m, CH_2 β to ring), 1.29 (18H, br.s, $(CH_2)_9$), 0.87 (3H, dist.t, CH_3). ^{13}C NMR ($CDCl_3$, δ_C): 167.5, 165.8, 133.0, 125.9, 71.9, 37.1, 36.4, 35.1, 32.8, 31.4, 30.9, 29.9, 28.2, 27.6, 26.9, 25.2, 24.6, 23.1, 14.0. MS (ESI) m/z = 376.450 found $[M+Na]^+$, calculated $[M+Na]^+$ = 377.420.

2.2.2. Synthesis of novel series of 4-amino-5-long chain alkenyl/hydroxyalkenyl-1,2,4-triazol-3-thiones, 3(a–d)

To a solution of 0.01 mol of potassium hydroxide in 50 mL absolute ethanol, 0.01 mol of long chain alkenyl/hydroxyalkenyl hydrazide, **1(a–d)** and 0.013 mol of carbon disulfide were added. The reaction mixture was stirred for 8 h at room temperature. The reaction mixture was then diluted with 30 mL ether and stirred for additional 1 h. The potassium salt without further purification was used for the next step. 0.02 Mol of hydrazine hydrate in 20 mL water was gradually added to the above potassium salt with constant stirring and then the reaction mixture was refluxed for 4 h. During refluxing, H_2S gas released and the reaction mixture color changed to light pink. The reaction mixture then cooled and acidified with conc. HCl. The white solid product was separated out which was then filtered and washed with water. The solid products **3(a–d)** were then air dried. The products **3(a–d)** were purified by silica gel column chromatography with petroleum ether:-diethyl ether as eluent. The products were identified by spectral data.

*2.2.2.1. 4-Amino-5-(dec-9-enyl)-1,2,4-triazol-3-thione, **3a**.* IR (KBr, cm^{-1}): 3228 (N–H stretching), 2924 (C–H stretching), 1599 (C=N stretching), 1186 (C=S stretching). ^1H NMR ($CDCl_3$, δ_H): 11.60 (1H, s, N*H*), 5.79 (1H, tdd, $J_{H-^8CH_2} = 6.70$ H_Z, $J_{H-H_Z} = 10.10$ H_Z, $J_{H-H_E} = 17.20$ H_Z, CH_2-=C*H*), 5.01 (1H, dd, $J_{H_Z-H} = 10.10$ H_Z, $J_{H_Z-H_E} = 1.30$ H_Z, H_Z-C=CH), 4.94 (1H, dd, $J_{H_E-H} = 17.20$ H_Z, $J_{H_E-H_Z} = 1.30$ H_Z, H_EC=CH), 4.57 (2H, br.s, NH_2), 2.74 (2H, t, J = 7.50 Hz, CH_2 α to ring), 2.02 (2H, m, CH_2=CH–CH_2), 1.70 (2H, m, CH_2 β to ring), 1.29 (10H, br.s, $(CH_2)_5$). ^{13}C NMR ($CDCl_3$, δ_C): 167.0, 160.5, 139.1, 121.2, 33.7, 31.8, 29.5, 29.4, 28.8, 27.2, 26.1, 24.8. MS (ESI): m/z = 276.830 found $[M+Na]^+$, calculated $[M+Na]^+$ = 277.347.

*2.2.2.2. 4-Amino-5-[(8Z)-heptadec-8-enyl]-1,2,4-triazol-3-thione, **3b**.* IR (KBr, cm^{-1}): 3224 (N–H stretching), 2924 (C–H stretching), 1595 (C=N stretching), 1179 (C=S stretching). ^1H NMR ($CDCl_3$, δ_H): 11.32 (1H, s, N*H*), 5.35 (2H, m, C*H*=C*H*), 4.55 (2H, br.s, NH_2), 2.75 (2H, t, J = 7.50 H_Z, CH_2 α to ring), 2.03 (4H, m, CH_2CH=CHCH_2), 1.68 (2H, m, CH_2 β to ring), 1.29 (20H, br.s, $(CH_2)_{10}$), 0.88 (3H, dist.t, CH_3). ^{13}C NMR ($CDCl_3$, δ_C): 167.3, 165.6, 134.6, 122.1, 36.5, 34.1, 30.9, 29.2, 29.0, 28.9, 28.6, 27.4, 26.0, 25.9, 24.6, 24.1, 23.2, 22.4, 14.2. MS (ESI) m/z = 374.912 found $[M+Na]^+$, calculated $[M+Na]^+$ = 375.508.

*2.2.2.3. 4-Amino-5-[(8Z, 11R)-11-hydroxyheptadec-8-enyl]-1,2,4-triazol-3-thione, **3c**.* IR (KBr, cm^{-1}): 3380 (O–H stretching), 3214 (N–H stretching), 2924 (C–H stretching), 1622 (C=N stretching), 1162 (C=S stretching). ^1H NMR ($CDCl_3$, δ_H): 12.17 (1H, s, N*H*), 5.41 (2H, m, C*H*=C*H*), 4.51 (2H, br.s,

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NH_2), 3.69 (1H, m, C*H*OH), 2.70 (2H, t, J = 7.51 H_Z, CH_2 α to ring), 2.26 (1H, m, CHO*H*), 2.02 (4H, m, CH_2-CH=CHCH_2), 1.67 (2H, m, CH_2 β to ring), 1.29 (18H, br.s, $(CH_2)_9$), 0.89 (3H, dist.t, CH_3). ^{13}C NMR ($CDCl_3$, δ_C): 168.0, 163.2, 135.1, 121.9, 71.9, 37.3, 36.2, 34.9, 32.8, 30.1, 29.9, 29.6, 29.1, 28.2, 28.0, 27.8, 25.4, 24.0, 13.9. MS (ESI) m/z = 392.014 found $[M+Na]^+$, calculated $[M+Na]^+$ = 391.507.

2.2.2.4. 4-Amino-5-[(8R, 11Z)-8-hydroxyheptadec-11-enyl]-1,2,4-triazol-3-thione, ***3d***. IR (KBr, cm^{-1}): 3376 (O–H stretching), 3217 (N–H stretching), 2921 (C–H stretching), 1619 (C=N stretching), 1164 (C=S stretching). ^{1}H NMR ($CDCl_3$, δ_H): 12.09 (1H, s, N*H*), 5.47 (2H, m, C*H*=C*H*), 4.56 (2H, br.s, NH_2), 3.68 (1H, m, C*H*OH), 2.72 (2H, t, J = 7.50 H_Z, CH_2 α to ring), 2.27 (1H, m, CHO*H*), 2.04 (4H, m, CH_2-CH=CHCH_2), 1.69 (2H, m, CH_2 β to ring), 1.28 (18H, br.s, $(CH_2)_9$), 0.87 (3H, dist.t, CH_3). ^{13}C NMR ($CDCl_3$, δ_C): 167.8, 164.4, 135.2, 122.5, 70.4, 37.2, 36.9, 34.0, 32.4, 30.1, 29.8, 29.5, 29.2, 28.7, 28.0, 27.6, 25.4, 23.5, 14.2. MS (ESI) m/z = 392.210 found $[M+Na]^+$, calculated $[M+Na]^+$ = 391.507.

2.2.3. Synthesis of novel series of 3-long chain alkenyl/hydroxyalkenyl-6-phenyl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines, ***4(a–d)***

To a solution of 0.0025 mol of 4-amino-5-long chain alkenyl/hydroxyalkenyl-1,2,4-triazol-3-thiones, **3(a–d)** in 15 mL absolute ethanol, 0.0025 mol of phenacyl bromide was added and the reaction mixture was refluxed at 90 °C for 12 h on oil bath. When all the triazole was consumed the reaction mixture was neutralized by ammonium hydroxide. The product was extracted with dichloromethane: water. The organic layer dried over anhydrous sodium sulfate. The solvent was evaporated on water bath from the oily product **4(a–d)**. Further, the products **4(a–d)** were purified by silica gel column chromatography with petroleum ether: diethyl ether as eluent. The products were identified by spectral data.

2.2.3.1. 3-(Dec-9-enyl)-6-phenyl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine, ***4a***. IR (KBr, cm^{-1}): 2923 (C–H stretching), 1604 (C=N stretching), 1125 (C–N stretching), 652 (C–S–C stretching). ^{1}H NMR ($CDCl_3$, δ_H): 7.55–7.39 (5H, m, Ar*H*), 5.78 (1H, tdd, $J_{H-^8CH_2}$ = 6.72 H_Z, J_{H-H_Z} = 10.00 H_Z, J_{H-H_E} = 17.24 H_Z, CH_2=C*H*), 5.01 (1H, dd, J_{H_Z-H} = 10.12 H_Z, $J_{H_Z-H_E}$ = 1.35 H_Z, H_ZC=CH), 4.94 (1H, dd, J_{H_E-H} = 17.23 H_Z, $J_{H_E-H_Z}$ = 1.34 H_Z, H_EC=CH), 4.12 (2H, s, CH_2 ring), 2.75 (2H, t, J = 7.50 Hz, CH_2 α to ring), 2.04 (2H, m, CH_2-=CH–CH_2), 1.69 (2H, m, CH_2 β to ring), 1.28 (10H, br.s, $(CH_2)_5$). ^{13}C NMR ($CDCl_3$, δ_C): 168.1, 165.4, 160.8, 134.8, 132.9, 132.5, 131.1, 130.5, 124.8, 122.5, 115.4, 36.2, 34.2, 33.9, 32.8, 32.1, 31.7, 30.9, 29.4, 25.0. MS (ESI): m/z = 378.041 found $[M+Na]^+$, calculated $[M+Na]^+$ = 377.459.

2.2.3.2. 3-[(8Z)-Heptadec-8-enyl]-6-phenyl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine, ***4b***. IR (KBr, cm^{-1}): 2926 (C–H stretching), 1599 (C=N stretching), 1120 (C–N stretching), 660 (C–S–C stretching). ^{1}H NMR ($CDCl_3$, δ_H): 7.62–7.40 (5H, m, Ar*H*), 5.35 (2H, m, C*H*=C*H*), 4.09 (2H, s, CH_2 ring), 2.67 (2H, t, J = 7.54 H_Z, CH_2 α to ring), 2.03 (4H, m, CH_2-CH=CHCH_2), 1.64 (2H, m, CH_2 β to ring), 1.25 (20H, br.s, $(CH_2)_{10}$), 0.87 (3H, dist.t, CH_3). ^{13}C NMR ($CDCl_3$, δ_C): 168.3, 165.6, 160.5, 134.5, 132.5, 132.0, 131.5, 130.8, 129.7, 125.0, 122.1, 35.5, 34.0, 32.5, 30.5, 29.0, 28.9, 28.4, 28.0, 27.2, 26.6, 25.5, 24.4, 24.0, 23.1, 21.4, 14.3. MS (ESI) m/z = 474.971 found $[M+Na]^+$, calculated $[M+Na]^+$ = 475.620.

2.2.3.3. 3-[(8Z, 11R)-11-Hydroxyheptadec-8-enyl]-6-phenyl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine, ***4c***. IR (KBr, cm^{-1}): 3360 (O–H stretching), 2922 (C–H stretching), 1613 (C=N stretching), 1115 (C–N stretching), 665 (C–S–C stretching). ^{1}H NMR ($CDCl_3$, δ_H): 7.60–7.35 (5H, m, Ar*H*), 5.40 (2H, m, C*H*=C*H*), 4.10 (2H, s, CH_2 ring), 3.62 (1H, m, C*H*OH), 2.72 (2H, t, J = 7.50 H_Z, CH_2 α to ring), 2.28 (1H, m, CHO*H*), 2.01 (4H, m, CH_2CH=CHCH_2), 1.68 (2H, m, CH_2 β to ring), 1.29 (18H, br.s, $(CH_2)_9$), 0.87 (3H, dist.t, CH_3). ^{13}C NMR ($CDCl_3$, δ_C): 167.0, 164.1, 163.8, 135.1, 132.7, 132.2, 131.4, 130.1, 123.4, 123.0, 121.9, 112.8, 36.3, 34.8, 33.9, 32.4, 30.3, 29.9, 29.5, 29.2, 28.7, 28.3, 27.8, 26.6, 24.7, 24.4, 14.2. MS (ESI) m/z = 492.106 found $[M+Na]^+$, calculated $[M+Na]^+$ = 491.619.

2.2.3.4. 3-[(8R, 11Z)-8-Hydroxyheptadec-11-enyl]-6-phenyl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine, ***4d***. IR (KBr, cm^{-1}): 3382 (O–H stretching), 2924 (C–H stretching), 1615 (C=N stretching), 1122 (C–N stretching), 667 (C–S–C stretching). ^{1}H NMR ($CDCl_3$, δ_H): 7.58–7.35 (5H, m, Ar*H*), 5.37 (2H, m, C*H*=C*H*), 4.19 (2H, s, CH_2 ring), 3.64 (1H, m, C*H*OH), 2.69 (2H, t, J = 7.50 H_Z, CH_2 α to ring), 2.28 (1H, m, CHO*H*), 2.04 (4H, m, CH_2CH=CHCH_2), 1.65 (2H, m, CH_2 β to ring), 1.28 (18H, br.s, $(CH_2)_9$), 0.87 (3H, dist.t, CH_3). ^{13}C NMR ($CDCl_3$, δ_C): 167.8, 166.2, 164.4, 133.2, 132.6, 131.9, 131.5, 130.6, 129.6, 123.1, 122.5, 70.2, 37.1, 36.5, 32.8, 32.6, 30.3, 29.8, 29.4, 29.1, 28.5, 28.0, 27.8, 27.2, 25.8, 23.9, 14.1. MS (ESI) m/z = 492.110 found $[M+Na]^+$, calculated $[M+Na]^+$ = 491.619.

2.3. In vitro anticancer activity

2.3.1. Blood peripheral mononuclear cell isolation

Fresh blood (20–15 mL) was kindly provided by blood bank, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh. The blood sample was diluted with the same volume of phosphate buffer saline (PBS). After that, the diluted blood sample was carefully layered on Ficoll-Histopaque (Sigma Aldrich, USA). The mixture was centrifuged at 400*g* for 30 min at 20–22 °C. The undisturbed lymphocyte layer was carefully transferred out. The lymphocyte was washed and pelleted down with three volumes of PBS twice and resuspended RPMI-1640 media (Sigma Aldrich, USA) with antibiotic and antimycotic solution (Sigma Aldrich, USA) 10%, v/v fetal calf serum (FCS) (Sigma Aldrich, USA). Cell counting was performed to determine the PBMC cell number with equal volume of trypan blue (Yeap et al., 2007; Yang et al., 1999).

2.3.2. MTT assay

The PBMCs/HeLa/Hep3 B/MCF 7 cell lines were maintained in RPMI 1640 (Sigma Aldrich) culture medium supplemented with 10% heat-inactivated fetal calf serum and antibiotic antimycotic solution (Sigma Aldrich). The cells were plated at a density of 5×10^3 cells per well in a 96-well plate, and cultured

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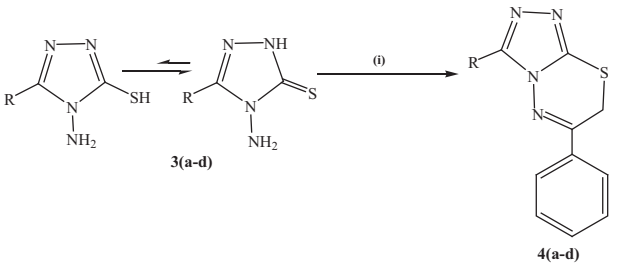
for 24 h at 37 °C. The cells were subsequently exposed to drugs. The plates were incubated for 48 h, and cell proliferation was measured by adding 20 μL of MTT (Sigma Aldrich) dye (5 mg/mL in phosphate-buffered saline) per well. The plates were incubated for a further 4 h at 37 °C in a humidified chamber containing 5% CO_2. Formazan crystals formed due to the reduction of dye by viable cells in each well were dissolved in 150 μL dimethyl sulfoxide, and absorbance was read at 570 nm. The absorption values were expressed as the cell viability (%), according to the control group as 100%. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software "Prism 3.0".

3. Result and discussion

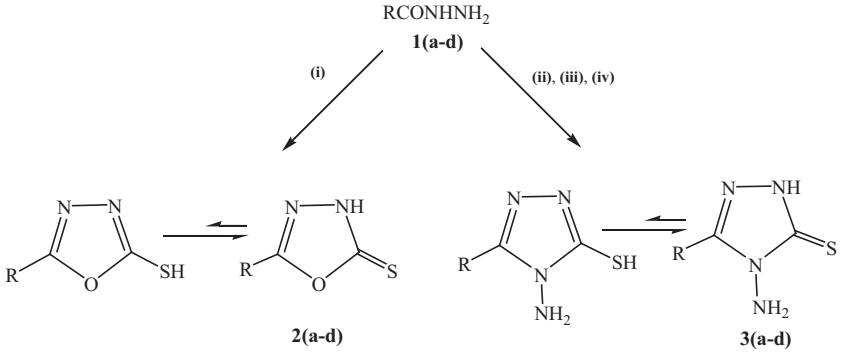
3.1. Chemistry

Earlier in our laboratory it has been reported that some other 1,3,4-oxadiazoles substituted with fatty acid chain possess antibacterial activity (Banday et al., 2010). Due to the biological importance of heterocyclic FA analogs, synthesis of target compounds **2(a–d)**, **3(a–d)**, **4(a–d)** and evaluation of anticancer activity were reported in this paper. All reactions are outlined in Schemes 1 and 2. The physicochemical parameters of all the newly synthesized compounds are tabulated in Table 1. Three novel series of oxadiazolthiones, **2(a–d)**; triazolthiones, **3(a–d)** and triazolothiadizines, **4(a–d)** were obtained from the corresponding unsaturated/hydroxy unsaturated FAHs, **1(a–d)**. These FAHs were used as starting material which was previously synthesized in our research laboratory from the corresponding FAs. The cyclization reaction of carbon disulfide with FAHs gives a novel series of 5-long chain alkenyl/hydroxyalkenyl-1,3,4-oxadiazol-2-thiones, **2(a–d)**. 4-Amino-5-long chain alkenyl/hydroxyalkenyl-1,2,4-triazol-3-thiones, **3(a–d)** were obtained by reaction of carbon disulfide and FAHs on treatment with hydrazine hydrate. Furthermore, biheterocyclic derivatives of FAs i.e. 4-amino-5-long chain alkenyl/hydroxy alkenyl-6-phenyl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines, **4(a–d)** were synthesized from corresponding compound **3(a–d)** and phenacyl bromide by ring closure reaction. The 1,3,4-oxadiazole-2-thiones and 1,2,4-triazol-3-thiones may exist in thiol–thione tautomeric forms, but in solid state thione form dominates. Such observations are also reported in the literature (Banday and Rauf, 2009). Structure of compound **2b** appeared in the literature also, (Toliwal et al., 2009) but without spectral data. All reactions were monitored by using thin layer chromatography (TLC) time by time. Products were purified by column chromatography. The structure of all the newly synthesized compounds was determined on the basis of their IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. Characteristic $[M+Na]^+$ ion peaks were observed for all the compounds under study. A detailed spectral description for compound **2a**, **3a** and **4a** is discussed below.



Scheme 2 Ring closure synthetic pathway of novel series of 3-long chain alkenyl/hydroxyalkenyl-6-phenyl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines, **4(a–d)**. Reagents and conditions: (i) $PhCOCH_2Br$, EtOH, reflux (90 °C), 12 h, neutralization with NH_4OH.

IR spectrum of compound 5-(dec-9-enyl)-(3H)-1,3,4-oxadiazol-2-thione, **2a** revealed characteristic bands at 3215 cm^{-1} for N–H stretching, 2921 cm^{-1} for C–H stretching and the detection of C=N stretching band at 1613 cm^{-1}, C=S stretching band at 1165 cm^{-1} and C–O–C stretching band at 1054 cm^{-1} for evidence of ring closure of 1,3,4-oxadiazol-2-thione ring. No peak was observed around 2600–2550 cm^{-1} for the thiol group, further confirmed the structure of compound **2a**. In the ^{1}H NMR spectrum of compound **2a**, characteristic peak was observed for N–H proton at δ 10.30 as singlet, in addition to peaks of FA chains. The ^{13}C NMR characteristic peaks for compound **2a** were observed at δ 166.8, 165.4, 123.0 and 122.3. Further evidence for the formation of **2a** was obtained by recording the mass spectrum which showed the $[M+Na]^+$ ion peak at m/z 262.880. Similarly, the structures of compounds **2(b–d)** were confirmed from their spectral data given in spectral section.



Scheme 1 Synthetic pathway for two novel series of 5-long chain alkenyl/hydroxyalkenyl-1,3-4-oxadiazol-2-thiones, **2(a–d)** and 4-amino-5-long chain alkenyl/hydroxyalkenyl-1,2,4-triazol-3-thiones, **3(a–d)** showing thiol-thione tautomerism. Reagents and conditions: (i) CS_2, KOH, EtOH, reflux (80–90 °C), 8 h, acidification with HCl up to pH 3. (ii) CS_2, KOH, EtOH, stir, room temperature, 8 h. (iii) Et_2O, stir, room temperature, 1 h. (iv) $N_2H_4.2H_2O$, reflux (90 °C), 4 h, acidification with HCl up to pH 3.

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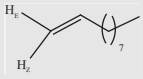
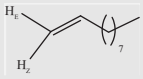
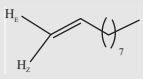
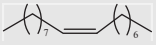
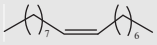
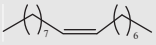
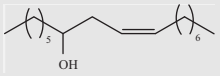
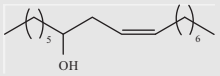
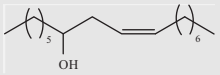
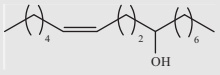
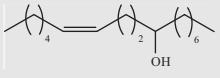
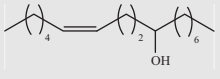
Table 1 Physico-chemical properties of all the newly synthesized compounds, **2(a–d)**, **3(a–d)** and **4(a–d)**.

S.No.	Compound code	R	Molecular formula	Physical state	M.P. (°C)	% Yield	Molec. wt.
1	**2a**		$C_{12}H_20N_2OS$	White powder	86–88	90	240.157
2	**3a**		$C_{12}H_{22}N_4S$	White powder	84–86	90	254.358
3	**4a**		$C_{20}H_{26}N_4S$	Brown sticky liquid	-	65	354.470
4	**2b**		$C_{19}H_{34}N_2OS$	White powder	87–89	80	338.492
5	**3b**		$C_{19}H_{36}N_4S$	White powder	76–77	85	352.519
6	**4b**		$C_{27}H_40N_4S$	Brown sticky liquid	-	62	452.631
7	**2c**		$C_{19}H_{34}N_2O_2S$	Yellow solid	76–78	80	354.431
8	**3c**		$C_{19}H_{36}N_4OS$	Yellow solid	62–64	78	368.518
9	**4c**		$C_{27}H_{40}N_4OS$	Brown sticky liquid	-	60	468.630
10	**2d**		$C_{19}H_{34}N_2O_2S$	Yellow solid	75–76	84	354.431
11	**3d**		$C_{19}H_{36}N_4OS$	Yellow solid	64–66	80	368.518
12	**4d**		$C_{27}H_{40}N_4OS$	Brown sticky liquid	-	60	468.630

M.P.: Melting point.

The structure of compound 4-amino-5-(dec-9-enyl)-1,2,4-triazol-3-thione, **3a** was confirmed by the appearance of absorption bands at 3228 cm^{-1} for N–H stretching, 2924 cm^{-1} for C–H stretching, 1599 cm^{-1} for C═N stretching and for C═S stretching, the absorption band appeared at 1186 cm^{-1}. No peak was observed around 2600–2550 cm^{-1} for the thiol group, further confirmed the thione structure of compound **3a**. The ^1H NMR characteristic peaks were observed at δ 11.60 as singlet for N–H proton, δ 4.57 as broad singlet for NH_2 protons. The ^{13}C NMR characteristic peaks for compound **3a** were observed at δ 167.0, 160.5, 139.1 and 121.2. In addition, evidence for the formation of **3a** was

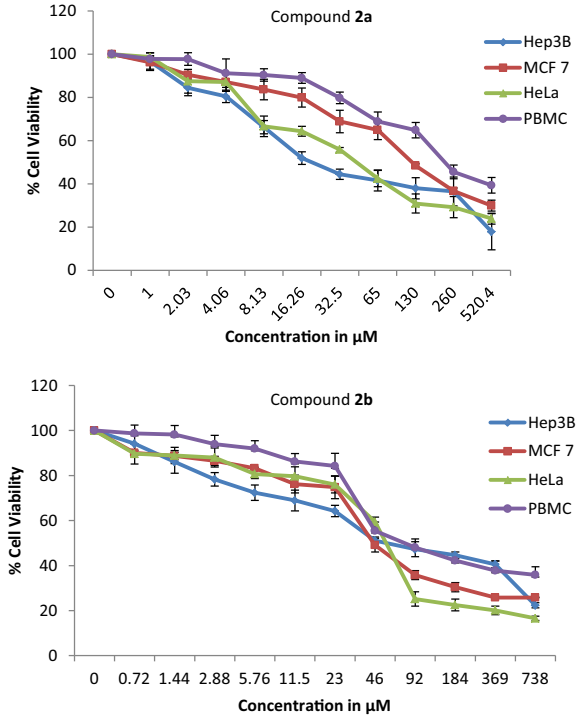


Figure 1 Dose–response effect of all the synthesized compounds **2(a–d)**, **3(a–d)** and **4(a–d)** on cell-viability of Hep3 B, MCF 7, HeLa and PBMCs. Data expressed here are mean ± standard deviation of three independent experiments.

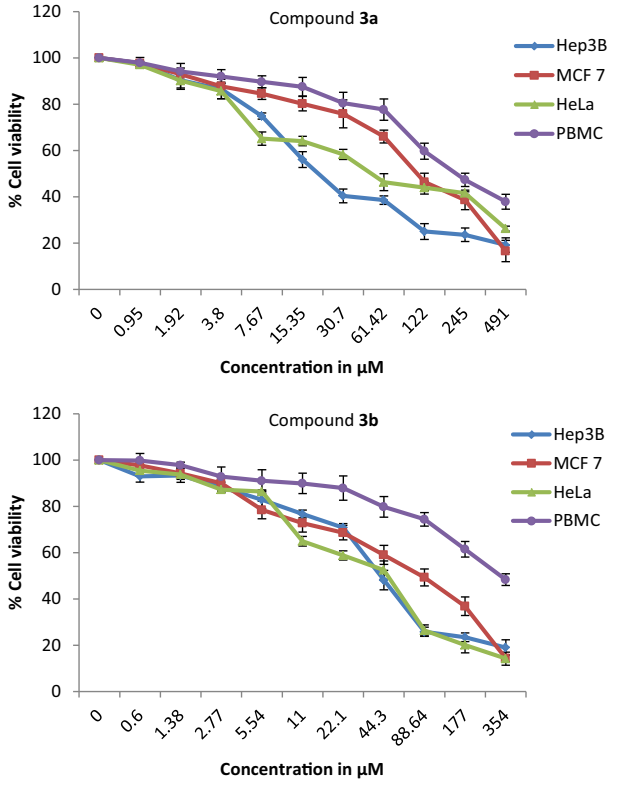


Fig. 1 (*continued*)

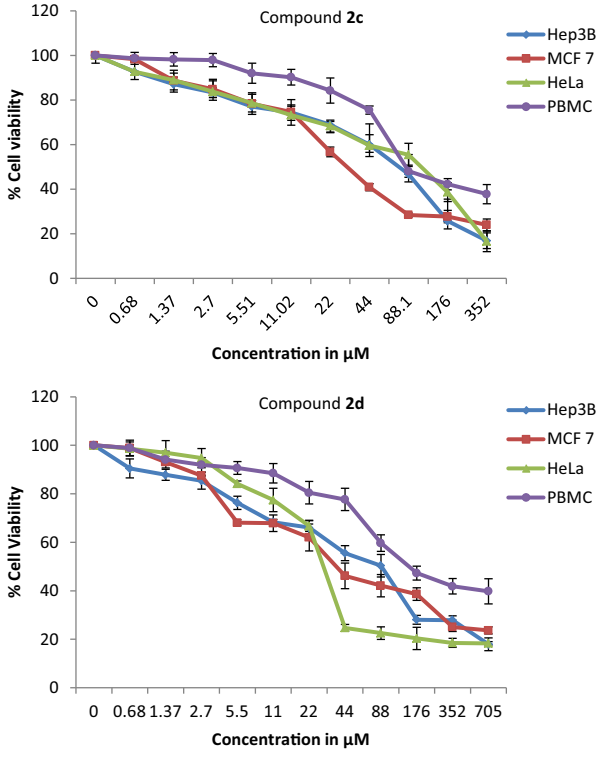


Fig. 1 (*continued*)

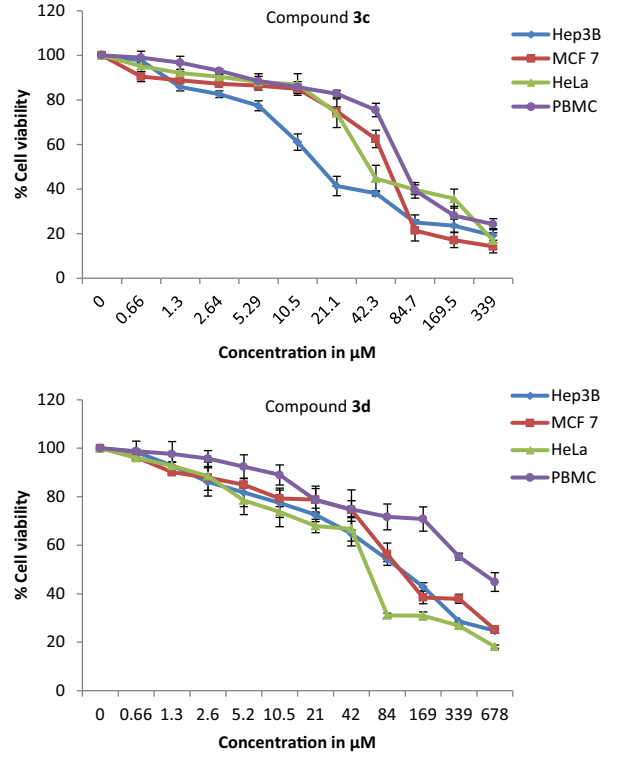


Fig. 1 (*continued*)

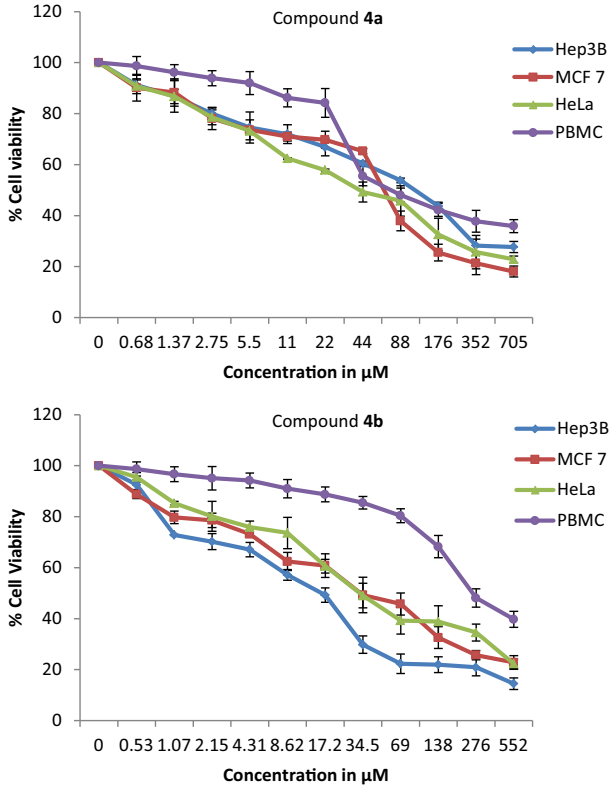


Fig. 1 (*continued*)

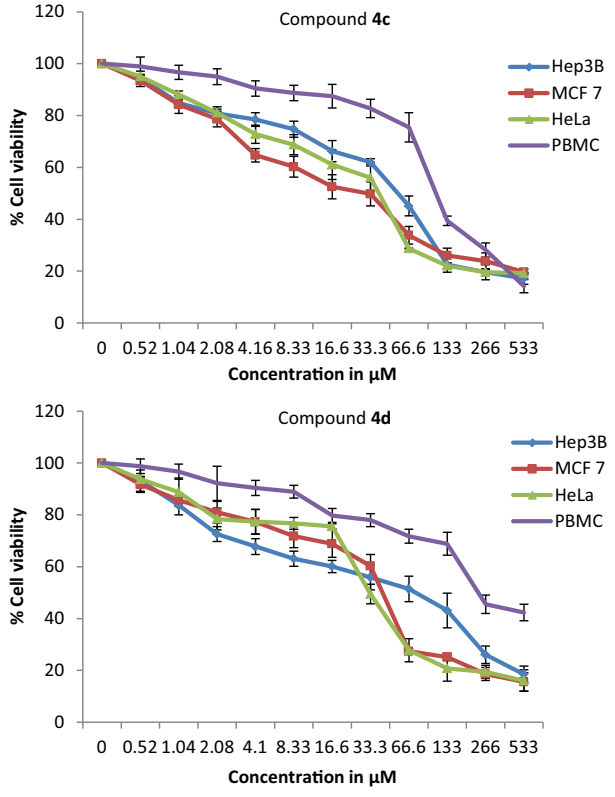


Fig. 1 (*continued*)

obtained by recording the mass spectrum which showed the $[M+Na]^+$ ion peak at m/z 276.830. Similarly, the structures of compounds **3(b–d)** were confirmed from their spectral data given in spectral section.

The structure of compound 3-(dec-9-enyl)-6-phenyl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine, **4a** was confirmed by the appearance of absorption bands at 2923 cm^{-1} for C–H stretching, 1604 cm^{-1} for C=N stretching, 1125 cm^{-1} for C–N stretching, 652 cm^{-1} for C–S–C stretching. The disappearance of absorption peaks for N–H and C=S stretching further confirmed the structure of compound **4a**. The ^1H NMR characteristic peaks were observed at δ 7.55–7.39 as multiplet for five aromatic protons, δ 4.12 as singlet for two ring protons. The disappearance of ^1H NMR peak for N–H proton further confirmed the structure of compound **4a**. The ^{13}C NMR characteristic peaks for compound **4a** were observed at δ 168.1, 165.4, 160.8, 134.8, 132.9, 132.5, 131.1, 130.5, 124.8, 122.5, 115.4 and 36.2. Confirmation for the formation of **4a** was also obtained by recording the mass spectrum which showed the $[M+Na]^+$ ion peak at m/z 378.041. Similarly, the structures of compounds **4(b–d)** were confirmed from their spectral data given in spectral section.

3.2. In vitro cytotoxic evaluation and structure–activity relationship

In vitro cytotoxicity of all the newly synthesized compounds (**2a–d**), (**3a–d**) and (**4a–d**) was measured by MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] assay against a panel of three different human cancer cell lines namely; human hepatocellular carcinoma (Hep3 B), human breast adenocarcinoma (MCF 7) and human cervical carcinoma (HeLa). These cell lines are procured from Cell Repository–National Centre for Cell Science, Pune (India). Normal human cells (PBMCs) were also used for the determination of cytotoxicity of synthesized compounds. The MTT assay is a colorimetric assay for measuring the cellular growth that reduces the tetrazolium yellow dye MTT, to its insoluble formazan (purple color) by mitochondrial dehydrogenases of living cells. MTT is used to determine the cytotoxicity of potential drugs and other toxic compounds. The insoluble purple formazan product is dissolved into a colored solution by the addition of a suitable solvent. At certain wavelength, the absorbance of this colored solution can be measured. The potency of the drug in causing cell death can be concluded through the production of dose–response curves when the purple formazan was produced by untreated control cells. Curves of dose-dependent effects of (**2a–d**), (**3a–d**) and (**4a–d**) on cell viability of different human cancer cell lines (Hep3 B, MCF 7, HeLa) and normal human cells (PBMCs) are displayed in Fig. 1. Doxorubicin and 5-fluorouracil were used as standard drugs. Experiment was performed in a triplicate. For each of the tested drug IC_{50} was calculated and the results are summarized in Table 2. Experiments revealed that there was a substantial increase in cytotoxicity in cancer cell lines with increasing exposure to drug concentration i.e. showing low IC_{50} values and our in-house synthesized drugs were not showing marked effects on normal human cells (PBMCs) i.e. showing high IC_{50} values. None of the synthesized compounds showed cytotoxicity to normal human cells (PBMCs). The present study showed that among the three human cancer cell

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Table 2 Showing anticancer data (IC_{50} values in μM) of all the synthesized drugs and standard drugs against three different human cancer cell lines and normal human cells.

S. No.	Compound codes	Hep3 B	MCF 7	HeLa	PBMC
1	**2a**	08.94 ± 2.5	13.60 ± 2.5	11.90 ± 1.3	35.32 ± 3.1
2	**2b**	16.42 ± 1.5	19.00 ± 2.5	17.60 ± 2.3	39.12 ± 1.7
3	**2c**	09.00 ± 1.6	15.90 ± 1.6	12.90 ± 1.3	45.42 ± 4.1
4	**2d**	10.10 ± 2.6	16.83 ± 2.6	13.30 ± 3.3	41.38 ± 2.5
5	**3a**	09.20 ± 0.8	14.00 ± 0.6	11.10 ± 2.1	39.12 ± 1.6
6	**3b**	16.60 ± 1.2	19.90 ± 0.7	18.80 ± 1.3	43.83 ± 1.9
7	**3c**	09.48 ± 1.4	13.20 ± 0.5	16.20 ± 2.4	33.57 ± 2.3
8	**3d**	09.96 ± 2.8	14.50 ± 1.6	15.82 ± 3.1	35.25 ± 1.9
9	**4a**	07.40 ± 2.2	10.30 ± 2.7	08.01 ± 1.3	32.78 ± 2.9
10	**4b**	09.49 ± 1.5	11.27 ± 2.4	10.03 ± 1.7	> 50
11	**4c**	06.50 ± 2.4	08.59 ± 1.5	08.83 ± 1.4	> 50
12	**4d**	07.36 ± 1.6	08.80 ± 2.1	06.00 ± 3.1	39.12 ± 3.1
	Doxo[a]	02.35 ± 1.2	03.12 ± 1.7	03.56 ± 2.7	09.23 ± 2.6
	5-Fu[b]	03.54 ± 2.1	04.12 ± 2.3	02.78 ± 2.6	08.91 ± 1.9

Standard drugs used for reference.
[a] Doxorubicin.
[b] 5-Fluorouracil.

lines tested, Hep3 B cells were found to be sensitive to all the tested compounds while HeLa and MCF 7 cells were found to be sensitive to some selected compounds. The obtained results revealed that compound **2a**, **2c**, **2d**, **3a**, **3c**, **3d**, **4a**, **4b**, **4c** and **4d** showed remarkable inhibitory activities against different human cancer cell lines and were also comparable to the standard drugs.

On the basis of structure–activity relationships, it could be concluded that 1,2,4-triazole fused with the 1,3,4-thiadiazine ring was found to have better antitumor activity than those of 1,2,4-triazoles and 1,3,4-oxadiazoles. The structural activity study also shows that anticancer activity is also dependent on the nature of alkenyl/hydroxyalkenyl FA chain. From IC_{50} values a number of correlations can be made. It is apparent from the IC_{50} values that, all the tested compounds show moderate to good cytotoxicity against different human cancer cell lines. For C_{10} terminal alkenyl FA chain residue which is substituted at 5-position of 1,3,4-oxadiazol-2-thione (compound, **2a**) and at 3-position of 1,2,4-triazol-3-thione (compound, **3a**) leads to a remarkable increase in potency against human hepatocellular carcinoma cells (IC_{50} value of 08.94 ± 2.5 and 09.20 ± 1.5 μM, respectively). Incorporation of the 1,3,4-thiadiazine ring with the 1,2,4-triazole ring having C_{10} terminal alkenyl FA chain residue at 3-position of 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazine (compound, **4a**) leads to an increase in cytotoxicity against all the three human cancer cell lines; IC_{50} values of 07.40 ± 2.2 μM (against Hep3 B cells), 10.30 ± 2.7 μM (against MCF 7 cells), 08.01 ± 1.3 μM (against HeLa cells). Increase in carbon chain length (C_{17}) of internal alkenyl FA chain residue at 5-position of 1,3,4-oxadiazol-2-thione (compound, **2b**) and at 3-position of 1,2,4-triazol-3-thione (compound, **3b**) leads to high IC_{50} values i.e. IC_{50} value was above 16.42 μM against all the three tested human cancer cells. In case of 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazine ring system, even the chain length of the internal alkenyl substituent at 3-position (compound, **4b**) was increased (C_{17}) still it displayed good antitumor activity against all the three cancer cell lines (IC_{50} value of 09.49 ± 1.5 μM against Hep3 B cells, 11.27 ± 2.4 μM against MCF 7 cells and 10.03 ± 1.7 μM against HeLa cells), this may be due to the fused ring system. Presence of the hydroxyl group on alkenyl FA chain residue which is attached to 1,3,4-oxadiazol-2-thione at 5-position (compound, **2c** and **2d**) was responsible for an increase in potency against hepatocellular carcinoma cells (IC_{50} values of 09.00 ± 1.6 and 10.10 ± 2.6 μM, respectively). Also, in case of 1,2,4-triazole, the presence of the hydroxyl group on the alkenyl FA chain residue at 3-position was responsible for increased cytotoxicity of compound **3c** and **3d** against Hep3 B cells (IC_{50} value of 09.48 ± 1.4 and 09.96 ± 2.8 μM, respectively). For compound **4c** and **4d**, there were two reasons for an increase in potency against all the three cancer cell lines: the presence of the hydroxyl group on the alkenyl fatty acid substituent and the other was the presence of fused ring system i.e. incorporation of thiadiazine with the triazole ring (IC_{50} values of 06.50 ± 2.4 μM against Hep3 B cells, 08.59 ± 1.5 μM against MCF 7 cells, 08.83 ± 1.4 μM against HeLa cells and 07.36 ± 1.6 μM against Hep3 B cells, 08.80 ± 2.1 μM against MCF 7 cells, 06.00 ± 3.1 μM against HeLa cells, respectively). These initial findings lead to further derivatization of heterocyclic FAs. Investigation of feasible mechanism of preventing cancer cell lines propagation is in progress.

4. Conclusion

We described here the synthesis of novel heterocyclic fatty acid analogs and *in vitro* anticancer activity evaluation of synthesized compounds against different human cancer cell lines and PBMCs by MTT assay. Anticancer activity results revealed that the synthesized compounds are non-toxic to the normal human cells and can be used safely for the treatment of cancer without damaging normal cells. The results of cytotoxic study also show that, all compounds possess moderate to good activity but compounds **4c** and **4d** were the most promising cytotoxic agent with IC_{50} values below 08.83 ± 1.4 μM against all the three tested human cancer cells (Hep3 B, MCF 7, HeLa cells) due to the presence of the hydroxyl group on FA chain and fused ring system (triazolothiadiazine

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nucleus). From this study, it can be concluded that the potency of drugs depends on the nature of FA chain and the heterocyclic ring system. From these studies, it is comprehensible that further derivatization of different heterocyclic analogs of fatty acids can serve as new templates for antitumor chemotherapy and could probably lead to more active molecules in the area of cancer chemotherapy.

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References

Aggarwal, N., Kumar, R., Dureja, P., Khurana, J.M., 2011. Synthesis, antimicrobial evaluation and QSAR analysis of novel nalidixic acid based 1,2,4-triazole derivatives Eur. J. Med. Chem. 46, 4089–4099.

Almasirad, A., Shafiee, A., Abdollahi, M., Noeparast, A., Shahrokhinejad, N., Vousooghi, N., Tabatabai, S.A., Khorasani, R., 2011. Synthesis and analgesic activity of new 1,3,4- oxadiazoles and 1,2,4-triazoles. Med. Chem. Res. 20, 435–442.

Altintop, M.D., Kaplancikli, Z.A., Turan-Zitouni, G., Ozdemir, A., Iscan, G., Akalin, G., Yildirım, S.U., 2011. Synthesis and anticandidal activity of new triazolothiadiazine derivatives. Eur. J. Med. Chem. 46, 5562–5566.

Aytaç, S.P., Tozkoparan, B., Kaynak, F.B., Aktay, G., Göktaş, Ö., Ünüvar, S., 2009. Synthesis of 3,6-disubstituted 7H–1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines as novel analgesic/anti inflammatory compounds. Eur. J. Med. Chem. 44, 4528–4538.

Badr, S.M.I., Barwa, R.M., 2011. Synthesis of some new [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines and [1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazoles starting from 5-nitro-2-furoic acid and evaluation of their antimicrobial activity. Bioorg. Med. Chem. 19, 4506–4512.

Banday, M.R., Rauf, A., 2009. Synthesis, characterization and antibacterial activity of 5- (alkenyl)-2-amino- and 2-(alkenyl)-5-phenyl-1,3,4-oxadiazoles. Ind. J. Chem. 48B, 97–102.

Banday, M.R., Mattoo, R.H., Rauf, A., 2010. Synthesis, characterization and antibacterial activity of 5-(alkenyl)-2-amino- and 2-(alkenyl)-5-phenyl-1,3,4-oxadiazoles. J. Chem. Sci. 122, 177–182.

Bondock, R., Adel, S., Etman, H.A., Badria, F.A., 2012. Synthesis and antitumor evaluation of some new 1,3,4-oxadiazole-based heterocycles. Eur. J. Med. Chem. 48, 192–199.

Deshmukh, R., Jha, A.K., Thakur, A.S., Dewangan, D., 2011. Synthesis and antibacterial activity of some 1, 3,4-oxadiazole derivatives and their thione analogues. Int. J. Res. Pharm. Biomed. Sci. 2, 215–219.

El-Shehry, M.F., Abu-Hashem, A.A., El-Telbani, E.M., 2010. Synthesis of 3-((2,4-dichlorophenoxy)methyl)-1,2,4-triazolo(thiadiazoles and thiadiazines) as anti inflammatory and molluscicidal agents. Eur. J. Med. Chem. 45, 1906–1911.

Farshori, N.N., Ahmad, A., Khan, A.U., Rauf, A., 2011. A facile, one-pot synthesis, characterization and antimicrobial activity of *o*-hydroxy anilide derivatives and 1- substituted-1,3-dicyclohexylurea analogs of long chain carboxylic acids. Eur. J. Med. Chem. 46, 1433–1438.

Gunstone, F.D., 1954. Fatty acids, part-II. The nature of the oxygenated acid present in *Vernonia anthelmintica* (Wild.) seed oil. J. Chem. Soc., 1611–1616.

Husain, A., Rashid, M., Mishra, R., Parveen, S., Shin, D., Kumar, D., 2012. Benzimidazole bearing oxadiazole and triazolo-thiadiazoles nucleus: design and synthesis as anticancer agents. Bioorg. Med. Chem. Lett. 22, 5438–5444.

Jubie, S., Ramesh, P.N., Dhanabal, P., Kalirajan, R., Muruganantham, N., Antony, A.S., 2012. Synthesis, antidepressant and antimicrobial activities of some novel stearic acid analogues. Eur. J. Med. Chem. 54, 931–935.

Khan, A.A., Alam, M., Tufail, S., Mustafa, J., Owais, M., 2011. Synthesis and characterization of novel PUFA esters exhibiting potential anticancer activities: an *in vitro* study. Eur. J. Med. Chem. 46, 4878–4886.

Kumar, G.V.S., Prasad, Y.R., Mallikarjun, B.P., Chandrashekhar, S.M., 2010. Synthesis and pharmacological evaluation of clubbed isopropylthiazole derived triazolothiadiazoles, triazolothiadiazines and mannich bases as potential antimicrobial and antitubercular agents. Eur. J. Med. Chem. 45, 5120–5129.

Lamani, K.S.S., Kotresh, O., 2010. Synthesis and biological activity of some novel 4-(5-mercapto-1,3,4-thiadiazol-2-yl)-2-phenyl-5-[2-phenylvinyl]-2,4-dihydro-3*H*-1,2,4- triazol-3-one. Eur. J. Chem. 7, 545–550.

Mohammad, R., Ali, S., Mahsa, R., Mehdi, B., 2012. Synthesis and antibacterial evaluation of new heterocyclic system: [1,2,4] triazolo-[3′,4′:6,1] pyridazino-[4, 3-*e*] [1,3,4] thiadiazine. Hetero. Commun. 18, 39–42.

Mohan, T.P., Vishalakshi, B., Bhat, K.S., Rao, K.S., Kendappa, G.N., 2004. Synthesis and insecticidal activity of some 1,3,4-oxadiazole derivatives containing phenoxyfluorophenyl group. Ind. J. Chem. 43B, 1798–1801.

Mohsen, U.A., 2012. Biological evaluation of some triazole and triazolothiadiazine. Marmara Pharm. J. 16, 229–234.

Nath, P.S., Ashish, P., Rupesh, M., 2011. Triazole: a potential bioactive agent (synthesis and biological activity). IJRAP 2, 1490–1494.

Pattan, S., Gadhave, P., Tambe, V., Dengale, S., Thakur, D., Hiremath, S.V., Shete, R.V., Deotarse, P., 2012. Synthesis and evaluation of some novel 1,2,4-triazole derivatives for antimicrobial, antitubercular and antiinflammatory activities. Ind. J. Chem. 41B, 297–301.

Prakash, O., Aneja, D.K., Hussain, K., Lohan, P., Ranjan, P., Arora, S., Sharma, C., Aneja, K.R., 2011. Synthesis and biological evaluation of dihydroindeno and indeno [1,2-e] [1,2,4]triazolo [3,4-b] [1,3,4]thiadiazines as antimicrobial agents. Eur. J. Med. Chem. 46, 5065–5073.

Rane, R.A., Gutte, S.D., Sahu, N.U., 2012. Synthesis and evaluation of novel 1,3,4-oxadiazole derivatives of marine bromopyrrole alkaloids as antimicrobial agent. Bioorg. Med. Chem. Lett. 22, 6429–6432.

Rastogi, N., Singh, V.R., Shukla, S., Sethi, R., 2006. Microwave mediated aminomethylation and antileishmanial activity of 2-{4'-(2″, 4″-dichlorobenzyl oxy)-phenyl}-1,3,4-oxadiazolin-5- thiones and 3-{4'-(2″,4″-dichlorobenzyloxy)-phenyl}-4-phenyl-1,2,4-triazolin-5-thiones. Ind. J. Hetero. Chem. 16, 5–8.

Rauf, A., Sharma, S., Gangal, S., 2007. Microwave assisted efficient one-pot synthesis of 3,5,6- trisubstituted-1,2,4-triazines from fatty acid hydrazides under solvent-free conditions and their antimicrobial activity. ARKIVOC xvi, 137–147.

Shalini, K., Kumar, N., Drabu, S., Sharma, P.K., 2011. Advances in synthetic approach to and antifungal activity of triazoles. Bleistein J. Org. Chem. 7, 668–677.

Sharma, V., Shrivastava, B., Bhatia, R., Bachwani, M., Khandelwal, R., Ameta, J., 2011. Exploring potential of 1,2,4-triazole: a brief review. Pharmacol. Online 1, 1192–1222.

Sinclair, A.J., Begg, D., Mathai, M., Weisinger, R.S., 2007. Omega 3 fatty acids and the brain: review of studies in depression. Asia Pac. J. Clin. Nutr. 16, 391–397.

Somani, R.R., Shirodkar, P.Y., 2009. Oxadiazole: a biologically important heterocycle. Der Pharma Chemica 1, 130–140.

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Srivastava, M., Singh, D., Kushwah, A.S., Gokulan, P.D., 2010. Synthesis and biological evaluation of some new 1-3-4-oxadizole derivatives. J. Curr. Pharm. Res. 4, 20–24.

Tirlapur, V.K., Tadmalle, T., 2011. Synthesis and insecticidal activity of 1,2,4-triazolo- thiazolidin-4-one. Der Pharm. Sin. 2, 135–141.

Toliwal, S., Jadav, K., Patel, K., 2009. Synthesis and biological evaluation of fatty hydrazides of byproducts of oil processing industry. Ind. J. Pharm. Sci. 71, 144–148.

Wang, B.L., Liu, X.H., Zhang, X.L., Zhang, J.F., Song, H.B., Li, Z.M., 2011. Synthesis, structure and biological activity of novel 1,2,4-triazole mannich bases containing a substituted benzylpiperazine moiety. Chem. Biol. Drug Des. 78, 42–49.

Yamima, O., Belmaker, R.H., 2009. Omega 3 fatty acids in depression: a review of three studies. CNS Neurosci. Ther. 15, 128–133.

Yang, N.S., Chou, C.J., Lin, L.C., Tsai, W.J., Kuo, Y.C., 1999. Evaluation of Chinese herbs that affect the cell-mediated immunity (III). J. Chin. Med. 10, 179–188.

Yeap, S.K., Alitheen, N.B., Ali, A.M., Omar, A.R., Raha, A.R., Suraini, A.A., Muhajir, A.H., 2007. Effect of Rhaphidophora korthalsii methanol extract on human peripheral blood mononuclear cell proliferation and cytolytic activity toward HepG2. J. Ethnopharmacol. 114, 406–411.

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