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Synthesis and Anti-Cancer Activity of Certain Novel Pyrazoline-Based 1,3-Oxathioles and 1,3,4-Thiadiazoles.

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ABSTRACT

New series of 1,3-oxathiole **6a-j/10a-b** and 1,3,4-thiadiazole **12/14a-e** were synthesized starting from thiols **2a, b**. The reaction of **2a, b** with 1-aryl-2-bromoethanones **3a-j** yielded 1,3-oxathioles **6a-j**. In similar manner, the treatment of **2a, b** with diones (**9a, b)** afforded the 1,3-oxathioles **10a, b.** Furthermore, compound **2a, b** were allowed to react with a series of hydrazonyl chloride to afford 1,3,4-thiadiazole derivatives **12** and **14a-f**. The newly synthesized compounds were screened for their cytotoxic activity against HepG-2 and MCF-7 using MTT assay cancer cells. Most of these compounds showed a significant anticancer activity,. The 1,3-oxathiole **10a** is the most potent compound against both two cell lines, whereas **6a**, **14e** showed potent anticancer activity against HepG-2 and MCF-7 cancer cells, respectively when compared with doxorubicin as reference drug.

Keywords: 1,3-Oxathiole, 1,3,4-Thiadiazole, Hydrazonyl chloride, Anticancer activity.

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INTRODUCTION

Cancer is one of the major health problems in the world from decades. Breast and liver cancer are the most frequent cancer in human. Pyrazole is versatile lead compound for designing potent anticancer compounds which it played a key role to the activity. Several recent studies suggest pyrazole derivatives as promising anticancer agents, indicating their use in the development of new anticancer agents [**1-7**]. For example, compound I, II, possess antitumor activity with IC_{50} 0.13 and 0.04 µM in addition to compound III displayed the most potent EGFR inhibitory activity with IC_{50} of 0.07 µM, which was comparable to the positive control erlotinib. The compound also showed significant antiproliferative activity against MCF-7 with IC_{50} of 0.08 µM and potent inhibitory activity in tumor growth inhibition [**8-10**].

1,3-Oxathiol represent a poorly studied class of heterocycles, although they hold promise as biologically active. For example, Benzoxathiole derivatives have many pharmacological properties such as anti-microbial, cytostatic, anti-psoriatic, anti-bacterial and anti-mycotic activities [**11,12**]. In addition to derivatives of compound **IV** demonstrate significant cytotoxic activity against various tumor cells, similarly as for its isomeric (**V**) [**13**], and a series of 6-(3-substitutedpropoxyl)benzo [d][1,3]oxathiol-2-ones as centrally acting antipsychotics [**14**].

The 1,3,4-thiadiazole scaffold was selected as a building block for the design and synthesis of new potent antitumor agents, 1,3,4-thiadiazoles were reported to possess antitumor activity against different human cell lines [**15–22**], in addition to antibacterial, antifungal, antituberculosis, anti-hepatitis B viral, antileishmanial, anti-inflammatory, analgesic, antioxidant, antidiabetic, molluscicidal, antihypertensive, diuretic, antimicrobial, antitubercular and anticonvulsant [**23–29**]. New synthesis of 1,3,4-thiadiazole such as compounds VI, VII and VIII showed good anticancer activities against different human cell lines [**15, 16, 30**,**31**].

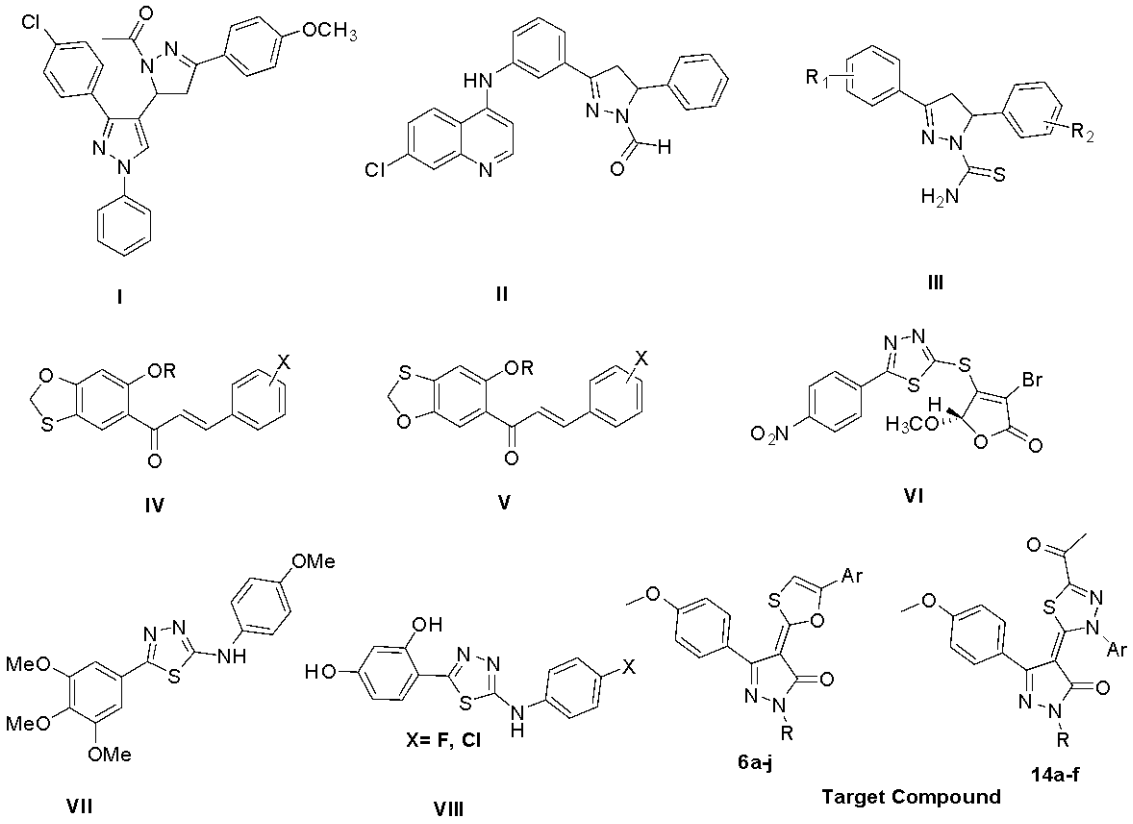

Fig. 1: The structure of compounds I-VIII, the newly synthesized 1,3-oxathiol 6a-j and 1,3,4-thiadizole derivatives 14a-f .

In this research, We are continuation of our interest work in the synthesis of new heterocycles to develop new potent anticancer agents [**32-34**], Thus, We have synthesized a new series of 1,3-oxathioles **6a-j, 10a-b** and 1,3,4-thiadiazole derivatives **12,14a-f** and screening of their activity as anticancer against human liver carcinoma (HepG-2) and human breast adenocarcinoma (MCF-7).

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EXPERIMENTAL

Chemistry

General

The melting points were uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Microanalytical data were performed by Vario El-Mentar apparatus (Shimadzu, Japan), National Research Centre, Cairo, Egypt. IR spectra were recorded as potassium bromide pellets using KBr disc technique on a Perkin-Elmer 1650 Spectrophotometer, National Research Centre, Cairo, Egypt. NMR experiments were determined on a Varian -300 MHz in deuterated dimethylsulphoxide (DMSO-d_6) and chemical shifts were expressed as parts per million; ppm (δ values) against TMS as an internal reference, Faculty of Science, Cairo University, Cairo, Egypt. Mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV, Cairo University, Cairo, Egypt.

Synthesis of pyrazol-3-ones 1a, b

These compounds were prepared according to the reported method [**41**] by the reaction of ethyl 3-(4-methoxyphenyl)-3-oxopropanoate (**3**) and hydrazine hydrate or phenyl hydrazine in absolute ethanol to yield 5-(4-methoxyphenyl)-2,4-dihydro-3*H*-pyrazol-3-one (**1a**) in 94% yield; m.p. 220 °C and 5-(4-methoxyphenyl)-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (**1b**) in 96% yield; m.p. 135 °C, respectively.

Synthesis of mercapto derivatives 2a, b

These compounds were prepared according to the reported method [**34**] by the reaction of compounds **1a**, **b** with PhNCS, in the presence of KOH, in DMF at room temperature followed by neutralization with HCl at 0-5°C to give 4-(mercapto(phenylamino)methylene)-5-(4-methoxyphenyl)-2,4-dihydro-3*H*-pyrazol-3-one (**2a**) in 83% yield; m.p. 125°C and 4-(mercapto(phenylamino)methylene)-5-(4-methoxyphenyl)-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (**2b**) in 77% yield; m.p. 160 °C, respectively.

Synthesis of 3-(4-methoxyphenyl)-4-(5-aryl-1,3-oxathiol-2-ylidene)-1(H/-phenyl)-pyrazol-5(4H)-ones 6a-j

To a mixture of 4-(mercapto(phenylamino)methylene)-3-(4-methoxyphenyl)-1*(H/*-phenyl)-pyrazol-5(4*H*)-one **2a,b** (1 mM) and bromo-1-phenylethanone dervatives **3a-e** (1 mM) in absolute ethanol (25 ml), few drops of triethylamine (TEA) were added. The reaction mixture was refluxed for 1 hr, and then cooled to room temperature. The formed precipitate was filtered, dried and washed with ethanol. The crude product was recrystallized from EtOH to afford compounds **6a-j**, respectively.

3-(4-Methoxyphenyl)-4-(5-phenyl-1,3-oxathiol-2-ylidene)-1H-pyrazol-5(4H)-one (6a)

Yield (71%); m.p. 260 °C; IR ν 3427 (NH), 1721 (C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 3.86 (s, 3H, -OCH_3), 7.07 (d, *J* = 9 Hz, 2H (*m*), OMe-$C_6\underline{H_4}$-), 7.13-7.44 (m, 3H (*m + p*), PhH), 7.73 (d, *J* = 9 Hz, 2H (*m*), OMe-$C_6\underline{H_4}$-), 7.84 (d, *J* = 9 Hz, 2H (*o*), PhH), 7.99 (s, H, -CH=), 11.53 (s, 1H, D_2O-exchangeable, NH); MS ($C_{19}H_{14}N_2O_3S$), *m/z* = 350 (M^+).

4-(5-(4-Chlorophenyl)-1,3-oxathiol-2-ylidene)-3-(4-methoxyphenyl)-1H-pyrazol-5(4H)-one (6b)

Yield (82%); m.p. > 300 °C; IR ν 3350 (NH), 1690 (C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 3.87 (s, 3H, -OCH_3), 7.07 (d, *J* = 9 Hz, 2H (*m*), OMe-$C_6\underline{H_4}$-), 7.54 (d, *J* = 9 Hz, 2H (*m*), Cl-$C_6\underline{H_4}$-), 7.62 (d, *J* = 9 Hz, 2H (*o*), OMe-$C_6\underline{H_4}$-), 7.72 (d, *J* = 9 Hz, 2H (*o*), Cl-$C_6\underline{H_4}$-), 7.85 (s, H, -CH=), 11.55 (s, 1H, D_2O-exchangeable, NH); MS ($C_{19}H_{13}ClN_2O_3S$), *m/z* = 384 (M^+).

4-(5-(4-Bromophenyl)-1,3-oxathiol-2-ylidene)-3-(4-methoxyphenyl)-1H-pyrazol-5(4H)-one (6c)

Yield (85%); m.p. 292 °C; IR ν 3432 (NH), 1658 (C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 3.87 (s, 3H, -OCH_3), 7.08 (d, *J* = 9 Hz, 2H (*m*), OMe-$C_6\underline{H_4}$-), 7.55 (d, *J* = 9 Hz, 2H (*m*), Br-$C_6\underline{H_4}$-), 7.69 (d, *J* = 9 Hz, 2H (*o*), OMe-$C_6\underline{H_4}$-),

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7.73 (d, *J* = 9 Hz, 2H (*o*), Br-C$_6$*H$_4$*-), 7.87 (s, H, -CH=), 11.55 (s, 1H, D$_2$O-exchangeable, NH); MS ($C_{19}H_{13}BrN_2O_3S$), *m/z* = 428 (M^+).

4-(5-(Benzofuran-2-yl)-1,3-oxathiol-2-ylidene)-3-(4-methoxyphenyl)-1H-pyrazol-5(4H)-one (6d)

Yield (85%); m.p. 298 °C; IR ν 3355 (NH), 1665 (C=O) cm^{-1}; ^{1}H NMR (DSMO-*d$_6$*) *δ* 3.90 (s, 3H, -OCH$_3$), 6.96 (d, *J* = 9 Hz, 2H (*m*), OMe-C$_6$*H$_4$*-), 7.13-7.91 (m, 7H, 5H of benzofuran + 2H (*o*) of OMe-C$_6$*H$_4$*-), 7.94 (s, H, -CH=), 11.61 (s, 1H, D$_2$O-exchangeable, NH); MS ($C_{21}H_{14}N_2O_4S$), *m/z* = 390 (M^+).

3-(4-Methoxyphenyl)-4-(5-(naphthalen-2-yl)-1,3-oxathiol-2-ylidene)-1H-pyrazol-5(4H)-one (6e)

Yield (85%); m.p. > 300 °C; IR ν 3427 (NH), 1693 (C=O) cm^{-1}; ^{1}H NMR (DSMO-*d$_6$*) *δ* 3.93 (s, 3H, -OCH$_3$), 7.16 (d, *J* = 9 Hz, 2H (*m*), OMe-C$_6$*H$_4$*-), 7.23-7.04 (m, 9H, 7H of *naphthalene* + 2H (*o*) of OMe-C$_6$*H$_4$*-), 8.10 (s, H, -CH=), 11.54 (s, 1H, D$_2$O-exchangeable, NH); MS ($C_{23}H_{16}N_2O_3S$), *m/z* = 400 (M^+).

3-(4-Methoxyphenyl)-1-phenyl-4-(5-phenyl-1,3-oxathiol-2-ylidene)-1H-pyrazol-5(4H)-one (6f)

Yield (78%); m.p. > 300 °C; IR ν 1682 (C=O) cm^{-1}; ^{1}H NMR (DSMO-*d$_6$*) *δ* 3.89 (s, 3H, -OCH$_3$), 7.13 (d, *J* = 9 Hz, 2H (*m*), OMe-C$_6$*H$_4$*-), 7.16-7.66 (m, 8H, 3H of C$_6$*H$_5$*-N< + 3H of PhH + 2H (*o*) of OMe-C$_6$*H$_4$*-), 7.86 (d, *J* = 9 Hz, 2H (*o*), C$_6$*H$_4$*-N<), 7.90 (s, H, -CH=), 8.05 (d, *J* = 9 Hz, 2H, *o*-PhH), MS ($C_{25}H_{18}N_2O_3S$), *m/z* = 426 (M^+).

4-(5-(4-Chlorophenyl)-1,3-oxathiol-2-ylidene)-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-5(4H)-one (6g)

Yield (84%); m.p. 264 °C; IR ν 1660 (C=O) cm^{-1}; ^{1}H NMR (DSMO-*d$_6$*) *δ* 3.89 (s, 3H, -OCH$_3$), 7.12 (d, *J* = 9 Hz, 2H (*m*), OMe-C$_6$*H$_4$*-),7.16 (t, *J* = 9 Hz, 1H (*p*), C$_6$*H$_5$*-N<), 7.41 (t, *J* = 9 Hz, 2H (*m*), C$_6$*H$_4$*-N<), 7.53 (d, *J* = 9 Hz, 2H (*o*), Cl-C$_6$*H$_4$*-), 7.62 (d, *J* = 9 Hz, 2H (*m*), Cl-C$_6$*H$_4$*-), 7.83 (d, *J* = 9 Hz, 2H (*o*), OMe-C$_6$*H$_4$*-), 7.93 (s, H, -CH=), 8.03 (d, *J* = 9 Hz, 2H (*o*), C$_6$*H$_5$*-N<), ^{13}C NMR 55.40 (CH$_3$), 98.98 (-CH=), 103.34 (C-4 of pyrazole), 113.67 (2C), 118, 02 (2C), 124.22, 124.64 (2C), 125.38, 126.61, 128.74 (2C), 129.05 (2C), 129.77(2C), 134.57 (C-Cl), 138.84 (C-N), 145.75 (C-5 of oxathiole), 150.63 (C-3 of pyrazole), 160.29 (C-4 of OMe-C$_6$*H$_4$*-), 164.57 (C=O), 174.97 (C-2 of oxathiole); MS ($C_{25}H_{17}ClN_2O_3S$), *m/z* = 462 (M+2).

4-(5-(4-Bromophenyl)-1,3-oxathiol-2-ylidene)-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-5(4H)-one (6h)

Yield (86%); m.p. 265 °C; IR ν 1656 (C=O) cm^{-1}; ^{1}H NMR (DSMO-*d$_6$*) *δ* 3.90 (s, 3H, -OCH$_3$), 7.13 (d, *J* = 9 Hz, 2H (*m*), OMe-C$_6$*H$_4$*-), 7.19 (t, *J* = 9 Hz, 1H (*p*), C$_6$*H$_5$*-N<), 7.43 (t, *J* = 9 Hz, 2H (*m*), C$_6$*H$_5$*-N<), 7.57 (d, *J* = 9 Hz, 2H (*o*), Br-C$_6$*H$_4$*-), 7.69 (d, *J* = 9 Hz, 2H (*m*), Br-C$_6$*H$_4$*-), 7.85 (d, *J* = 9 Hz, 2H (*o*), OMe-C$_6$*H$_4$*-), 7.96 (s, 1H, -CH=), 8.04 (d, *J* = 9 Hz, 2H (*o*), C$_6$*H$_5$*-N<), ^{13}C NMR 55.43 (CH$_3$), 100.05 (-CH=), 103.52 (C-4 of pyrazole), 113.72 (2C), 118, 06 (2C), 124.28, 124.81 (2C), 125.45, 126.85, 128.78 (2C), 129.80 (2C), 129.83 (2C), 132.02 (C-Br), 138.89 (C-N), 145.90 (C-5 of oxathiole), 151.03 (C-3 of pyrazole), 160.07 (C-4 of OMe-C$_6$*H$_4$*-), 164.81 (C=O), 175.02 (C-2 of oxathiole); MS ($C_{25}H_{17}BrN_2O_3S$), *m/z* = 504 (M^+).

4-(5-(Benzofuran-2-yl)-1,3-oxathiol-2-ylidene)-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-5(4H)-one (6i)

Yield (78%); m.p. 248 °C; IR ν 1670 (C=O) cm^{-1}; ^{1}H NMR (DSMO-*d$_6$*) *δ* 3.91 (s, 3H, -OCH$_3$), 7.01 (s, 1H -CH= of benzofuran), 7.20 (d, *J* = 9 Hz, 2H (*m*), OMe-C$_6$*H$_4$*-),7.35 (t, *J* = 9 Hz, 1H (*p*), C$_6$*H$_5$*-N<), 7.44-7.50 (m, 2H (*m*), C$_6$*H$_5$*-N< + 2H of benzofuran), 7.65 (d, *J* = 8 Hz, 1H of benzofuran), 7.77 (d, *J* = 8 Hz, 1H of benzofuran), 7.87 (s, 1H, -CH= of oxathiol), 7.91 (d, *J* = 9 Hz, 2H (*o*), OMe-Ar-), 8.05 (d, *J* = 9 Hz, 2H (*o*), C$_6$*H$_5$*-N<); MS ($C_{27}H_{18}N_2O_4S$), *m/z* = 466 (M^+).

3-(4-Methoxyphenyl)-4-(5-(naphthalen-2-yl)-1,3-oxathiol-2-ylidene)-1-phenyl-1H-pyrazol-5(4H)-one (6j)

Yield (78%); m.p. > 300 °C; IR ν 1680 (C=O) cm^{-1}; ^{1}H NMR (DSMO-*d$_6$*) *δ* 3.96 (s, 3H, -OCH$_3$), 7.20 (d, *J* = 9 Hz, 2H (*m*), OMe-C$_6$*H$_4$*-),7.24 (t, *J* = 9 Hz, 1H (*p*), C$_6$*H$_5$*-N<), 7.43 (t, *J* = 9 Hz, 2H (*m*), C$_6$*H$_5$*-N<), 7.59-7.98 (m, 9H, 2H (*o*) of C$_6$*H$_5$*-N< + 2H (*o*) of OMe-C$_6$*H$_4$* -+ 5H of *naphthalen-2-yl*), 8.01 (s, H, -CH= of oxathiole), 8.06 (d, *J* = 9 Hz, 2H, H-4, H-7, *naphthalen-2-yl*); MS ($C_{29}H_{20}N_2O_3S$), *m/z* = 476 (M^+).

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Synthesis of 1,3-oxathiole derivatives 10a, b

To a mixture of 4-(mercapto(phenylamino)methylene)-5-(4-methoxyphenyl)-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (**2b**) (1 mM) and 3-chloropentane-2,4-dione (**9a**) or ethyl 2-chloro-3-oxobutanoate (**9b**) (1 mM) in absolute ethanol (25 ml), few drops of triethylamine were added. The reaction mixture was refluxed for 1 hr, and then cooled to room temperature. The precipitate was filtered, dried and washed with ethanol. The crude product was recrystallized from EtOH to afford compounds **10a, b**, respectively.

4-(4-Acetyl-5-methyl-1,3-oxathiol-2-ylidene)-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-5(4H)-one (10a)

Yield (78%); m.p. 235 °C; IR ν 1661-1670 (2C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 2.22 (s, 3H, $\underline{CH_3}$), 2.49 (s, 3H, $COCH_3$), 3.80 (s, 3H, OCH_3), 6.84 (d, *J* = 9 Hz, 2H (*m*), OMe-$C_6\underline{H_4}$-), 6.98 (t, *J* = 7 Hz, 1H (*p*), Ph-N of pyrazole), 7.17 (t, *J* = 9 Hz, 2H (*m*), Ph-N of pyrazole), 7.80 (d, 2H (*o*), OMe-$C_6\underline{H_4}$-), 7.99 (d, *J* = 9 Hz, 2H (*o*), Ph-N of pyrazole); ^{13}C NMR 27.20 (C of acetyl $\underline{CH_3}$), 31.32 (CH_3 of C-5 oxathiole), 56.12 (C of OCH_3), 62.11 (C of O$\underline{CH_2}CH_3$), 98.87 (C-4 of pyrazole), 115.12 (C-4 of oxathiole), 115.14 (2C), 118.95 (2C), 127.57 (C), 128.82 (2C), 130.72 (3C), 140.15, 155.22 (C-5 oxathiole), 156.62 (C-3 of pyrazole), 163.34 (C-4 of OMe-C_6H_4-), 165.74 (C=O of C-5 of pyrazole), 168.40 (C-2 of oxathiole), 190.86 (C=O of acetyl); MS ($C_{22}H_{18}N_2O_4S$), *m/z* = 406 (M^+).

Ethyl2-(3-(4-methoxyphenyl)-5-oxo-1-phenyl-1H-pyrazol-4(5H)-ylidene)-5-methyl-1,3-oxathiole-4-carboxylate (10b)

Yield (85%); m.p. 190 °C; IR ν 1666 (C=O), 1702 (C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 1.30 (t, *J* = 7 Hz, 3H, $CH_2\underline{CH_3}$), 2.49 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.31 (q, *J* = 7 Hz, 2H, $\underline{CH_2}CH_3$), 7.06 (d, *J* = 9 Hz, 2H (*m*), OMe-$C_6\underline{H_4}$-), 7.19 (t, *J* = 7 Hz, 1H (*p*), Ph-N of pyrazole), 7.44 (t, *J* = 9 Hz, 2H (*m*), Ph-N of pyrazole), 7.80 (d, 2H (*o*), OMe-$C_6\underline{H_4}$-), 8.02 (d, *J* = 9 Hz, 2H (*o*), Ph-N of pyrazole); ^{13}C NMR 13.84 (C of $OCH_2\underline{CH_3}$), 38.73 (CH_3 of C-5 oxathiole), 55.22 (C of OCH_3), 62.11 (C of O$\underline{CH_2}CH_3$), 99.22 (C-4 of pyrazole), 102.28 (C-4 of oxathiole), 113.59 (2C), 117.99 (2C), 120.57 (C), 128.82 (2C), 129.62 (3C), 142.42, 156.06 (C-3 of pyrazole), 157.72 (CH_3 of C-5 oxathiole), 161.84 (C-4 of OMe-C_6H_4-), 165.63 (C=O of C-5 of pyrazole), 167.45 (2C, C-2 of oxathiole and C=O of acetyl); MS ($C_{23}H_{20}N_2O_5S$), *m/z* = 436 (M^+).

***Synthesis of 1,3,4-thiadiazole derivatives* 12 and 14a-f**

To a mixture of mercapto derivatives **2a, b** (1 mM) and hydrazonoyl chloride dervatives **11** or **13a-f** (1 mM) in absolute ethanol (25 ml), few drops of triethylamine (TEA) were added. The reaction mixture was refluxed for 1 hr, and then cooled to room temperature. The formed precipitate was filtered, dried and washed with ethanol. The crude product was recrystallized from EtOH to afford compounds **12** and **14a-f**, respectively.

4-(3,5-Diphenyl-1,3,4-thiadiazol-2(3H)-ylidene)-3-(4-methoxyphenyl)-1H-pyrazol-5(4H)-one (12)

Yield (72%); m.p. 250 °C; IR ν 3250 (NH), 1690 (C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 3.75 (s, 3H, -OCH_3), 6.65 (m, 3H (*o, p*), Ph-N), 7.09 (m, *J* = 9 Hz, 2H (*m*), OMe-$C_6\underline{H_4}$-), 7.42 (t, *J* = 9 Hz, 2H (*p*), Ph-N), 7.61-7.69 (m, 3H (*o, p*), Ph of thiadiazole), 7.74 (d, *J* = 9 Hz, 2H (*o*), Ph of thiadiazole), 8.01 (d, *J* = 9 Hz, 2H (*o*), (*m*), OMe-$C_6\underline{H_4}$-), 12.85 (s, 1H, D_2O-exchangeable, NH); MS ($C_{24}H_{18}N_4O_2S$), *m/z* = 428 (M^+).

***4-(5-Acetyl-3-phenyl-1,3,4-thiadiazol-2(3H)-ylidene)-3-(4-methoxyphenyl)-1H-pyrazol-5(4H)-one* (14a)**

Yield (64%); m.p. 235 °C; IR ν 3428 (NH), 1602 (C=O), 1706 (C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 2.61 (s, 3H, -CH_3), 3.75 (s, 3H, -OCH_3), 6.63 (m, 3H (*o, p*), Ph-N),7.04-7.44 (m, 4H, 2H (*m*) of OMe-$C_6\underline{H_4}$- + 2H (*m*), Ph-N), 7.60 (d, *J* = 9 Hz, 2H (*o*), OMe-$C_6\underline{H_4}$-), 11.56 (s, 1H, D_2O-exchangeable, NH); MS ($C_{20}H_{16}N_4O_3S$), m/z = 392 (M^+).

***4-(5-Acetyl-3-p-tolyl-1,3,4-thiadiazol-2(3H)-ylidene)-3-(4-methoxyphenyl)-1H-pyrazol-5(4H)-one* (14b)**

Yield (61%); m.p. 225 °C; IR ν 3350 (NH), 1630 (C=O), 1690 (C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 2.60 (s, 3H, -CH_3), 2.65 (s, 3H, -CH_3), 3.76 (s, 3H, -OCH_3), 6.57 (d, 2H (*o*), Ar-N), 6.64-7.44 (m, 4H, 2H (*m*), OMe-$C_6\underline{H_4}$- + 2H (*m*), Ph-N), 7.60 (d, *J* = 9 Hz, 2H (*o*), OMe-$C_6\underline{H_4}$-), 11.57 (s, 1H, D_2O-exchangeable, NH); MS ($C_{21}H_{18}N_4O_3S$), *m/z* = 406 (M^+).

***4-(5-Acetyl-3-(4-chlorophenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-3-(4-methoxyphenyl)-1H-pyrazol-5(4H)-one* (14c)**

Yield (84%); m.p. 270 °C; IR ν 3430 (NH), 1638 (C=O), 1680 (C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 2.49 (s, 3H, C=O-CH_3), 3.74 (s, 3H, -OCH_3), 6.58 (d, 2H (*o*), Ar-N), 6.91 (d, 2H (*m*), OMe-C_6H_4-), 7.08 (d, 2H (*m*), Ar-N), 7.27 (d, *J* = 9 Hz, 2H (*o*), OMe-C_6H_4-), 11.62 (s, 1H, D_2O-exchangeable, NH); MS ($C_{21}H_{15}ClN_4O_3S$), *m/z* = 426 (M^+).

***4-(5-Acetyl-3-phenyl-1,3,4-thiadiazol-2(3H)-ylidene)-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-5(4H)-one* (14d)**

Yield (85%); m.p. 210 °C; IR ν 1650 (C=O), 1692 (C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 2.49 (s, 3H, $COCH_3$), 3.74 (s, 3H, -OCH_3), 6.60 (d, 2H (*o*), Ar-N of thiadizole), 7.04-7.33 (m, 6H, 4H (*o, m*), OMe-C_6H_4- + 3H (*m, p*) Ar-N of thiadizole), 7.40 (t, *J = 9* Hz, 3H (*p*, *m*), Ph-N of pyrazole), 8.03 (d, *J* = 9 Hz, 2H (*o*), Ph-N of pyrazole), ^{13}C NMR 25.85 (C of CH_3 of acetyl), 55.10 (CH_3 of OMe-C_6H_4-), 93.25 (C4 of pyrazole), 113.39 (2C), 117.94 (2C), 124.09, 124.53 (2C), 126.56, 128.69 (2C), 128.74 (2C), 129.11 (2C), 139.06, 140.02, 146.23, 157.63, 158.91, 161.15, 164.37(C=O of C-5 of pyrazole), 189.94 (C=O of acetyl); MS ($C_{26}H_{20}N_4O_3S$), *m/z* = 469 (M+1).

***4-(5-Acetyl-3-p-tolyl-1,3,4-thiadiazol-2(3H)-ylidene)-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-5(4H)-one* (14e)**

Yield (86%); m.p. 255 °C; IR ν 1670 (C=O), 1705 (C=O)cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 2.49 (s, 3H, C=O-CH_3), 3.75 (s, 3H, -OCH_3), 6.61 (d, 2H (*o*), Ar-N of thiadizole), 6.85 (d, 2H (*m*), Ar-N of thiadizole), 6.99 (d, *J* = 9 Hz, 2H (*m*), OMe-C_6H_4-), 7.15 (d, *J* = 9 Hz, 2H (*o*), OMe-C_6H_4-), 7.40 (t, *J* = 9 Hz, 3H (*p*,*m*), Ph-N of pyrazole), 8.03 (d, *J* = 9 Hz, 2H (*o*), Ph-N of pyrazole), ^{13}C NMR 20.43 (C of Me-Ar), 25.83 (C of CH_3 of acetyl), 55.06 (CH_3 of OMe-C_6H_4-), 78.39, 93.19 (C4 of pyrazole), 112.99 (2C), 117.99 (2C), 120.55, 124.08 (2C), 124.49, 126.89 (2C), 128.70 (2C), 128.85 (2C), 129.52 (2C), 137.38, 138.99, 139.09 146.24, 158.93, 164.44(C=O of C-5 of pyrazole), 189.95 (C=O of acetyl); MS ($C_{27}H_{22}N_4O_3S$), *m/z* = 484 (M+2).

***4-(5-Acetyl-3-(4-chlorophenyl)-1,3,4-thiadiazol-2(3H)-ylidene)-3-(4-methoxyphenyl)-1-phenyl-1H-pyrazol-5(4H)-one* (14f)**

Yield (79%); m.p. 230 °C; IR ν 1660 (C=O), 1723 (C=O) cm^{-1}; ^{1}H NMR (DSMO-d_6) δ 2.49 (s, 3H, C=O-CH_3), 3.78 (s, 3H, -OCH_3), 6.65 (d, 2H (*o*), Ar-N of thiadizole), 7.05 (d, 2H (*m*), Ar-N of thiadizole), 7.12 (d, *J* = 9 Hz, 2H (*m*), OMe-C_6H_4-), 7.18 (d, *J* = 9 Hz, 2H (*o*), OMe-C_6H_4-), 7.41 (t, *J* = 9 Hz, 3H (*m*, *p*), Ph-N of pyrazole), 8.02 (d, *J* = 9 Hz, 2H (*o*), Ph-N of pyrazole); MS ($C_{26}H_{19}ClN_4O_3S$), *m/z* = 502 (M^+).

Biological Evaluation

In-vitro anticancer activity

Cell culture of HepG-2 (human liver carcinoma) and MCF-7 (human breast adenocarcinoma) cell lines were purchased from the American Type Culture Collection (Rockville, MD) and maintained in DMEM medium which was supplemented with 10% heat-inactivated FBS (fetal bovine serum), 100 U/ml penicillin and 100 U/ml streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO_2.

MTT cytotoxicity assay

The antitumor activity against HepG-2 and MCF-7 human cancer cell lines was estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells [**42-44**]. Cells were dispensed in a 96 well sterile microplate (5 x 104 cells/well), and incubated at 37°C with series of different concentrations, in DMSO, of each tested compound or Doxorubicin® (positive control) for 48 h in a serum free medium prior to the MTT assay. After incubation, media were carefully removed, 40 μL of MTT (2.5 mg/mL) were added to each well and then incubated for an additional 4 h. The purple formazan dye crystals were solubilized by the addition of 200 μL of DMSO. The absorbance was measured at 590 *n*m using a SpectraMax® Paradigm® Multi-Mode microplate reader. The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells.

Statistical analysis

All experiments were conducted in triplicate and repeated in three different days. All the values were represented as mean ± SD. IC_{50}s were determined by probit analysis using SPSS software program (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Chemistry

The present study based on the synthesis of certain novel 1,3-oxathiole and 1,3,4-thiadiazole derivatives to evaluate their anticancer activity against HepG-2 and MCF-7 cell lines. The synthetic strategies of the newly synthesized compounds are depicted in Schemes 1 and 2. The starting materials **2a, b** were prepared according to our previously reported method [**34**]. Next, compounds **2a. b** were reacted with a series of bromo-1-arylylethanone **3a-j** to deliver the functionalized 1,3-oxathiole derivatives **6a-j** of expected pharmacological interests. Where compounds **2a, b** were treated with **3a-j** in absolute EtOH, in the presence of TEA, to yield, in each case, a single product (TLC) ($CHCl_3$:EtOH 9:1)with two possible structures. There are two routes are suggested for the formation of the latter expected reaction products *via* losing aniline to produce 1,3-oxathiole derivatives **6a-j** (route a) [**35,36**] or *via* losing H_2O to form 1,3-thiazoles **8a-j** (route b) [**36-40**], respectively, these reactions afforded substituted 1,3-oxathioles in good yields as outlined in Scheme 1.

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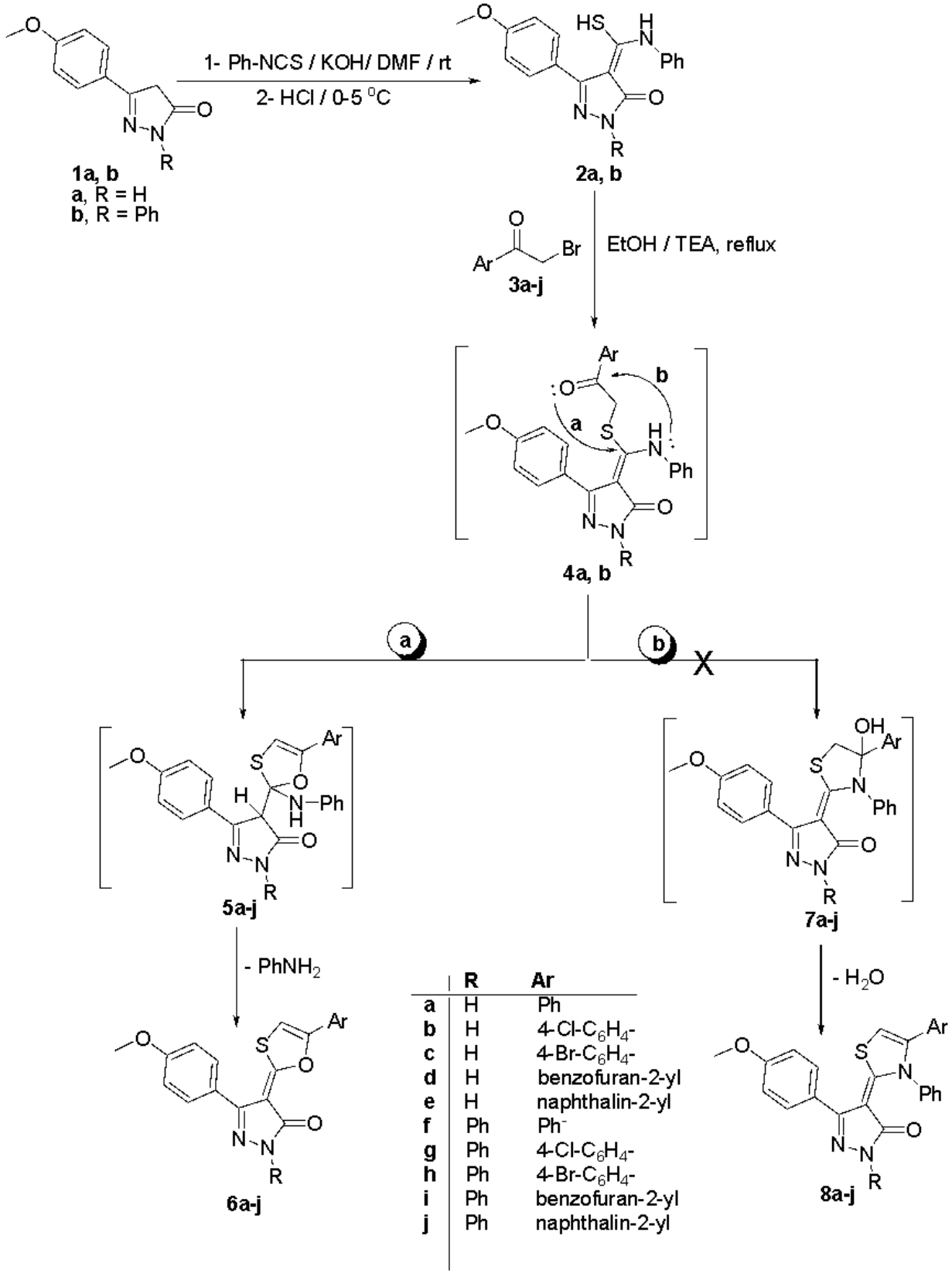

Scheme 1: Synthesis of 1,3-Oxathiole dervatives (6a-j)

The ^{13}C and ^{1}H-NMR spectra of the isolated compounds showed the disappearance of the characteristic signal of phenyl group. In addition, elemental analyses (Table 1) of some products were compatible with the 1,3-oxathioles structure **6a-j** and ruled out the formation of 1,3-thiazole **8a-j**. Therefore,

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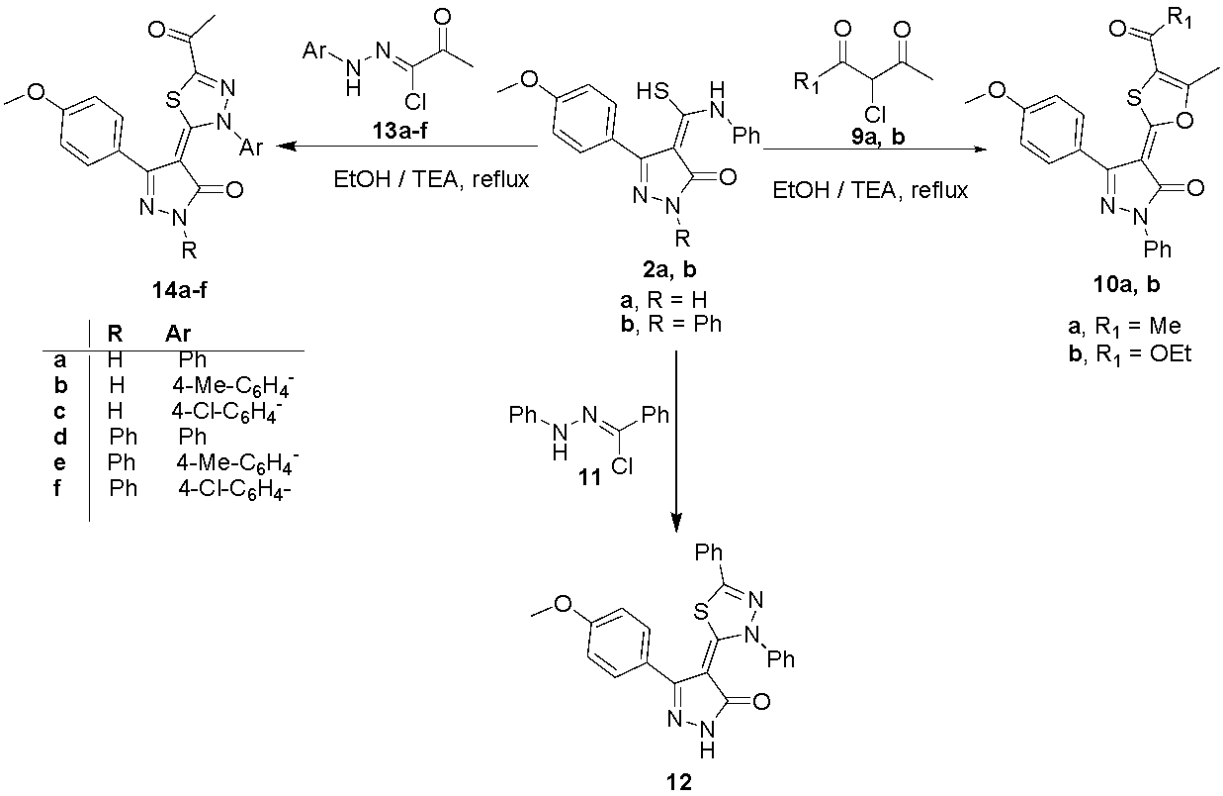
the previous reaction is proceeded through "route a" according to the proposed mechanism (**Scheme 1**). Furthermore, the treatment of compound **2b** with compounds **9a** or **9b** in absolute EtOH, in the presence of TEA, afforded the 1,3-oxathioles **10a, b** where the reactions were procceded through "route **a**".*via* losing of aniline molecule [**36**].



	R	Ar
a	H	Ph
b	H	4-Me-C_6H_4-
c	H	4-Cl-C_6H_4-
d	Ph	Ph
e	Ph	4-Me-C_6H_4-
f	Ph	4-Cl-C_6H_4-

Scheme 2: Synthesis of 1, 3-oxathiole and 1,3,4-thiadiazole derivatives

On the other hand, compound **2a** was allowed to react with N'-phenylbenzohydrazonoyl chloride **11** and 2-oxo-N'-phenylpropanehydrazonoyl chloride **13a-f** to afford 1,3,4-thiadiazoles **12** and **14a-f**, respectively. ^{1}H NMR of compound **12** showed the appearance of a D_2O-exchangeable singlet signal at δ 12.85 ppm due to NH proton and the multiplet signals of 14 protons of three aromatic moites. The IR spectra of compound **14a-f** exhibited, in each case, bands in the region 3250-3430 cm^{-1} corresponding to NH function (**14a-c**) in addition to the absorptions of 2 C=O in the region 1602–1723 cm^{-1}. These results indicate that the reaction of compound **2** with hydrazonyl chlorides **11** and **13a-f** was proceeded *via* loss of hydrogen chloride to form the nonisolable intermediate which cyclized by elimination of aniline molecule to form the final isolable 1,3,4-thiadiazole derivatives **12** and **14a-f,** respectively.

Table 1: Actual elemental analysis of compounds 6a,6c,6f,6h

Sample	C %	H %	Br %	N %	S %	O %
6a	64.25	4.50	---	7.93	8.94	14.38
6c	52.75	3.59	18.45	6.93	7.51	10.41
6f	68.90	4.10	---	6.56	7.76	12.68
6h	58.39	3.10	15.50	5.55	6.75	10.71

Biological activity

Anti-tumor activity

Twenty one compounds were tested *in vitro* for their anti-tumor activities against HepG-2 and MCF-7 human carcinoma cell lines using MTT assay. The percentage of the intact cells was measured and compared

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to the control (**Table 2**). The activities of these compounds against the two carcinoma cells were compared with that of Doxorubicin®..

From table 2 and figure **2** we can deduce that, at 100 µM, six compounds (**10a**, **6a**, **1b**, **12**, **6b** and **1a**, respectively) showed better anticancer activities against HepG-2 carcinoma cells when compared with the reference drug with IC_{50} = 72.5±3.1, 73.1±4.2, 73.9±1.9, 74.2±3.9, 75.5±2.9 and 76.9±3.1 μg/mL, respectively, whereas IC_{50} of doxorubicin = 80.9±2.1 μg/mL.

Seven compounds (**14c**, **6f**, **10b**, **6e**, **14a**, **14e** and **6j**, respectively) showed comparable results to the reference drug. The rest of the compounds showed moderate activities against HepG-2 cells. In addition, ten compounds (**10a**, **14e**, **14d**, **1a**, **14c**, **1b**, **6j**, **6d**, **12** and **14a**, respectively) showed better anticancer activities (IC_{50} = 62.7±2.9 - 64.3±2.5 μg/mL) against MCF-7 carcinoma cells when compared with the reference drug (IC_{50} = 65.6±4.2 μg/mL). The rest of the compounds showed comparable results to the reference drug. The IC_{50} values of all the investigated compounds are shown in Table 2.

Table 2: The anticancer IC_{50} values of the twenty one compounds using MTT assay against two cancer types.

Compound	HepG-2	MCF-7
	IC_{50} (µM)	
1a	76.9 ± 3.1	63.7 ± 2.1
1b	73.9 ± 1.9	63.9 ± 4.0
6a	73.1 ± 4.2	65.4 ± 3.9
6b	75.5 ± 2.9	66.0 ± 3.2
6c	87.4 ± 5.1	65.6 ± 1.9
6d	93.4 ± 6.0	64.3 ± 2.5
6e	82.8 ± 5.4	68.3 ± 3.7
6f	80.1 ± 3.9	67.3 ± 4.1
6g	87.4 ± 4.2	66.4 ± 5.0
6h	90.3 ± 5.9	67.1 ± 2.9
6i	89.8 ± 6.1	65.2 ± 3.4
6j	85.6 ± 4.1	64.2 ± 4.3
10a	72.5 ± 3.1	62.7 ± 2.9
10b	80.7 ± 4.9	65.1 ± 2.7
12	74.2 ± 3.9	64.3 ± 3.8
14a	82.9 ± 4.6	64.5 ± 3.9
14b	93.6 ± 2.6	66.1± 2.2
14c	79.4 ± 4.2	63.8 ± 2.7
14d	90.5 ± 7.0	63.6 ± 4.1
14e	84.9 ± 5.9	63.2 ± 2.9
14f	93.4 ± 5.5	65.6 ± 4.1
Doxorubicin	80.9 ± 2.1	65.6 ± 4.2

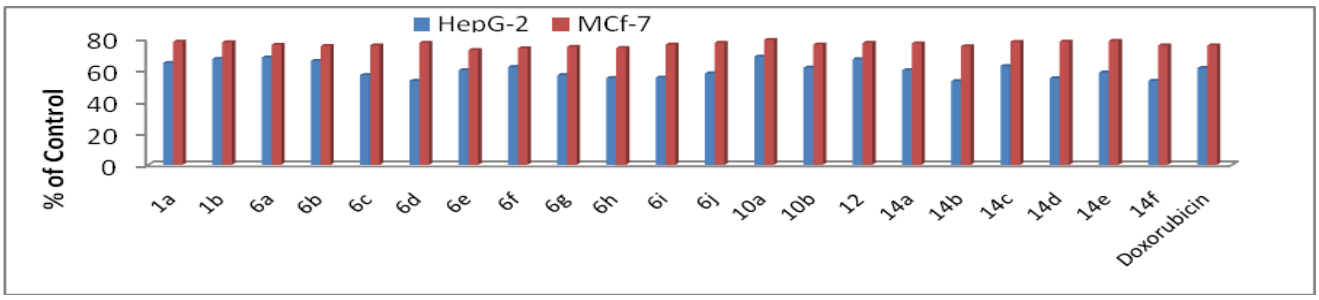

Fig 2: Anticancer activity of the twenty one compounds against two cancer types, using MTT assay at 100 ppm

In general, the response of MCF-7 cancer cells is higher than that of HepG-2 cancer cells against the newly synthesized compounds. From Structure Activity Relationship (SAR) point of view, the anticancer activities of 1,3,4-thiadiazole **12** and **14a-f** are higher than that of 1,3-oxathiole **6a-j**. In addition, the un-substituted C-4 of compound **6a** with IC_{50} = 73.1±4.2 µg/mL is the most potent compound against HepG-2 cancer cells whereas 1,3-oxathioles derivative, compound **10a** with IC_{50} = 72.5±3.1 and 62.7±2.9 μg/mL showed the highest anticancer activity against HepG-2 and MCF-7 cancer cells, respectively. The methyl substituted of hydrazonyl chloride, **14e** showed significant anticancer activity against MCF-7 with IC_{50} = 63.2±2.9 μg/mL more potent than doxorubicin (IC_{50} = 65.6±4.2 μg/mL).

CONCLUSION

Novel pyrazole-based 1,3-oxathiole and 1,3,4- thiadiazole derivatives were synthesized and evaluate their anticancer activity against HepG-2 and MCF-7. Several compounds revealed good anticancer activities against HepG-2 and MCF-7. The 1,3-Oxathiole **10a** is the most potent compound against HepG-2 and MCF-7 cancer cells, whereas **6a** and **14e** showed significancant anticancer activity against HepG-2 and MCF-7 cancer cells, respectively.

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