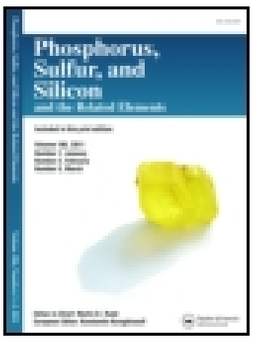


Taylor & Francis
Taylor & Francis Group

Phosphorus, Sulfur, and Silicon and the Related Elements

ISSN: 1042-6507 (Print) 1563-5325 (Online) Journal homepage: https://www.tandfonline.com/loi/gpss20

Synthesis, characterization and antitumor activities of some novel thiazinones and thiosemicarbazones derivatives

Selvam Athavan Alias Anand, Kiran George, Nisha Susan Thomas & Senthamaraikannan Kabilan

To cite this article: Selvam Athavan Alias Anand, Kiran George, Nisha Susan Thomas & Senthamaraikannan Kabilan (2020): Synthesis, characterization and antitumor activities of some novel thiazinones and thiosemicarbazones derivatives, Phosphorus, Sulfur, and Silicon and the Related Elements, DOI: 10.1080/10426507.2020.1757672

To link to this article: https://doi.org/10.1080/10426507.2020.1757672

PHOSPHORUS, SULFUR, AND SILICON AND THE RELATED ELEMENTS
https://doi.org/10.1080/10426507.2020.1757672

Synthesis, characterization and antitumor activities of some novel thiazinones and thiosemicarbazones derivatives

Selvam Athavan Alias Anand[a], Kiran George[b], Nisha Susan Thomas[c], and Senthamaraikannan Kabilan[a]

[a]Drug Discovery Lab, Department of Chemistry, Annamalai University, Annamalainagar, Tamilnadu, India; [b]Department of Biomedical Engineering, Chennai Institute of Technology, Chennai, Tamilnadu, India; [c]Department of Bio-Chemistry and Bio-Technology, Annamalai University, Annamalainagar, Tamilnadu, India

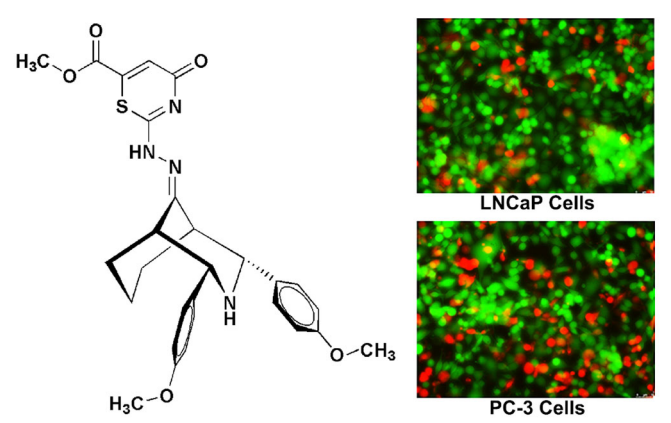
ABSTRACT

Two series of thiazinone and thiosemicarbazone derivatives (1-12) were synthesized using 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones (ABNs) and 3–alkyl–2,6–diarylpiperidin–4–ones as the starting materials. The structures of newly synthesized compounds were established on the basis of FT–IR, NMR spectroscopy and mass spectrometry. From the spectroscopic data, we identified that the cyclization reaction of thioamide with dialkyl acetylenedicarboxylate selectively gives six membered methyl 2-(2-(2,4-disubstituted-3-azabicyclo[3.3.1]nonan-9-ylidene)hydrazinyl)-4-oxo-4H-1,3-thiazine-6-carboxylates (1-6). In order to investigate the antitumor activities of the synthesized compounds, *in vitro* cytotoxicity studies were carried out using human prostate cancer cell lines. Tested compounds showed good/moderate activities against cancer cell lines and further investigation carried out by live/dead assay.

ARTICLE HISTORY
Received 4 November 2019
Accepted 15 April 2020

KEYWORDS
Thiazinone; thiosemicarbazone; piperidone; prostate cancer; apoptosis; live/dead assay

GRAPHICAL ABSTRACT



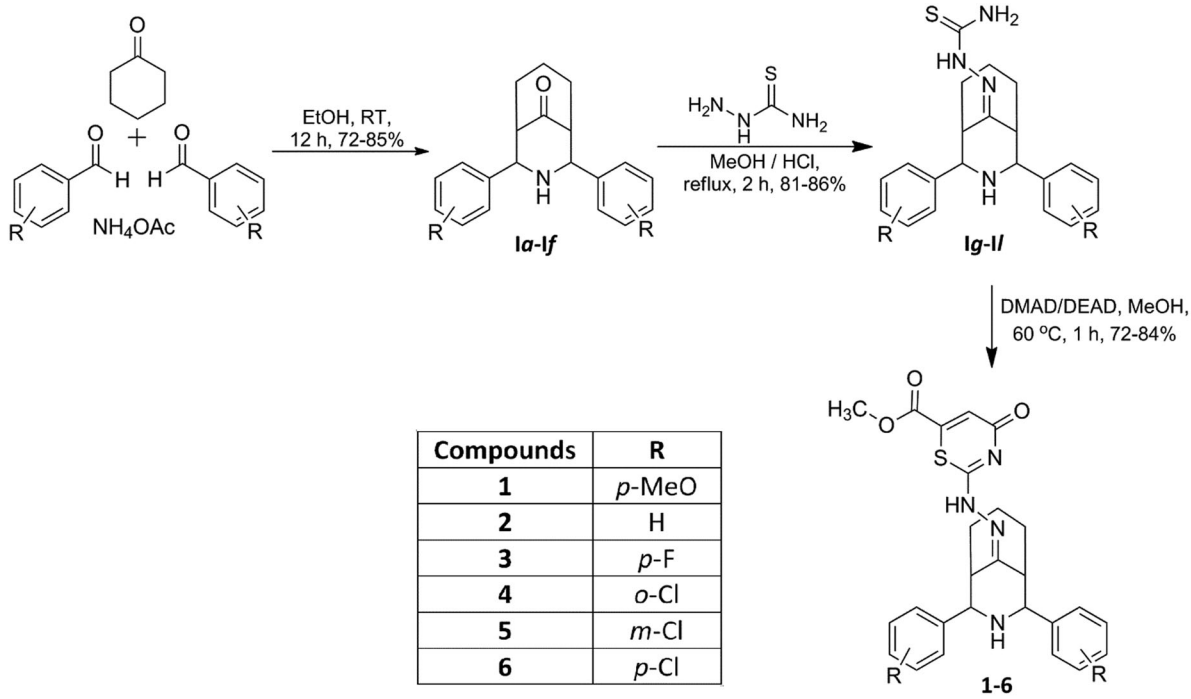
1. Introduction

Prostate cancer (PC) is the most common cancer in men, particularly in the western world and the majority of diagnosed patients are over the age of 50. PC has been reported as the second leading cause of cancer-related death among men worldwide and more than two million men are PC survivors in the United States of America.[1] In recent years, androgen receptor signal blockage is targeted for anti-prostate cancer drug development.[2,3] Some sulfur and nitrogen containing heterocyclic compounds such as apalutamide, casodex and enzalutamide are reported as anti-androgens to cure PC[4–6]. In continuation of our anti-cancer drug discovery research,[7–10] we synthesized few novel thiazinone, thiosemicarbazone derivatives of modified piperidones and their anti-prostate cancer activities were investigated using LNCaP (androgen sensitive) and PC-3 (androgen insensitive) cell lines.

Organosulfur compounds are frequently found in pharmacophores and play an important role in the medicinal chemistry field [11,12]. Among them, heterocyclic compounds with thiazinone and thiosemicarbazone scaffolds have been reported to furnish various biological activities. For examples thiazinone derivatives exhibit significant biological activities including antimalarial,[13] antifungal,[14] anticonvulsant[15] and anticancer properties.[16] On the other hand,

CONTACT Senthamaraikannan Kabilan profskabilanau@gmail.com Drug Discovery Lab, Department of Chemistry, Annamalai University, Annamalainagar, Tamilnadu 608002, India.

Supplemental data for this article is available online at https://doi.org/10.1080/10426507.2020.1757672.



Compounds	R
1	*p*-MeO
2	H
3	*p*-F
4	*o*-Cl
5	*m*-Cl
6	*p*-Cl

Scheme 1. Synthesis of Methyl 2-(2-(2,4-disubstituted-3-azabicyclo[3.3.1]nonan-9-ylidene)hydrazinyl)-4-oxo-4H-1,3-thiazine-6-carboxylates (1-6).

thiosemicarbazone moieties are also a class of small molecules that have been discovered for various pharmacological properties such as antibacterial,[17] antiviral,[18] antimalarial,[19] anti HIV,[20] anticancer,[21] antitubercular,[22] anticorrosion[23] and antileishmanicidal effects.[24] In addition, they also display significant inhibition against mushroom tyrosinase,[25] phenoloxidase,[26] *Trypanosoma Cruzi*,[27] and urease enzymes.[28] Considering the diverse biological properties, we synthesized a new series of thiazinone and thiosemicarbazone derivatives of piperidone and their anticancer activities were evaluated using human prostate cancer cell lines.

2. Results and discussion

2.1. Chemistry

The starting materials, 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones (ABNs) and 3–alkyl–2,6–diarylpiperidin–4–ones were prepared in good yields through consecutive steps as reported in the literature.[29–31] In Scheme 1, we have considered a reaction of 2,4-diaryl-3-azabicyclo[3.3.1] nonan-9-ones thiosemicarbazone derivatives with DMAD/DEAD (Dimethyl/Diethyl acetylenedicarboxylate) to examine the reactivity. To a warm solution of substituted 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-ones thiosemicarbazones in methanol, a methanolic solution of DMAD/DEAD was added dropwise with stirring. The reaction mixture was allowed to stir at 60 °C for 1 h to obtain the product.

The reaction between thiosemicarbazones and acetylene dicarboxylate starts with the conjugate addition of sulfur atom onto the triple bond of DMAD. Through the aminolysis of ester group and the elimination of an alcohol molecule, the intermediate undergoes intra-molecular condensation. The cyclization of this intermediate resulting in the formation of six-membered thiazinones regioselectively.[32] In addition, the NMR spectral data of the novel compounds were compared with our previous research work to confirm the reaction mechanism.[33] This unequivocally indicates that the compounds **1**-**6** involved in conjugate addition of the sulfur center and *N*-acylation furnishing a six-membered thiazinone ring but not a five membered thiazolidinone[34–37] as the final product (Figure 1).

In Scheme 2, the compound *N*-(4-chlorophenyl)hydrazinecarbothioamide (IIa) was obtained by reacting 4-chloroaniline with carbon disulfide in the presence of sodium hydroxide and then with hydrazine hydrate.[38] The 1,3-disubstituted-2,6-diarylpiperidin-4-ones were synthesized by an optimized successive Mannich condensation of corresponding ketones, substituted benzaldehydes and ammonium acetate or methylamine in 1:2:1 ratio in ethanol. The *N*-(4-chlorophenyl)hydrazinecarbothioamide was refluxed with the 1,3-disubstituted-2,6-diarylpiperidin-4-ones in the presence of the catalytic amount of concentrated hydrochloric acid to yield the compounds **7**-**12**.

The structures of newly synthesized compounds (1-12) were elucidated by spectral analyses such as FT–IR, Mass, and NMR spectra. The numbering pattern followed for the compounds **1** and **7** to explain the spectral data was given in Figure 2.

2.2. FT–IR and mass spectral analyses

In FT-IR spectrum of ABN derivative compound **1**, the ester carbonyl and amide carbonyl of showed sharp bands at 1728 cm^{-1} and 1703 cm^{-1} (Figure S 1 in the Supplemental Materials). The bands at 1642 cm^{-1} and 1609 cm^{-1} correspond to the imino groups of thiazinone and piperidine rings. The –*NH*- appeared at 3450 cm^{-1} and –*CH*- stretching at 2782-3157 cm^{-1}. In FT-IR spectra of compounds **7**-**12**, (Figures S 25, S 29, S 33, S 37, S 41 and S 45 in the Supplemental Materials) the bands appeared in the region of 3301-3440 cm^{-1} owing to the –NH- groups of the

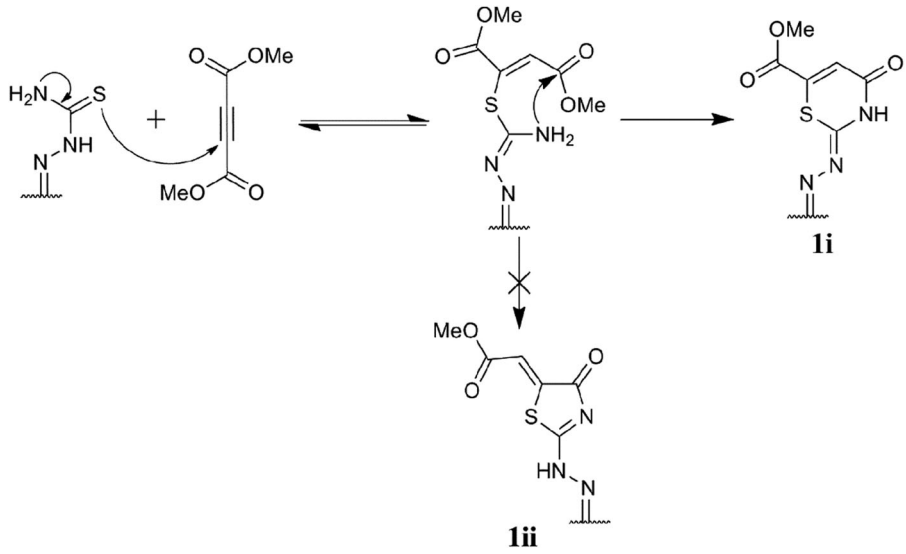
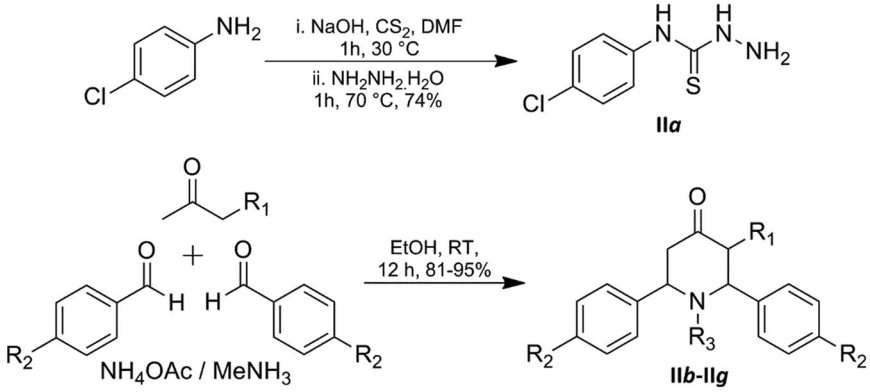


Figure 1. Formation of six membered thiazinone (1i) in the reaction of DMAD with thioamide.



Compounds	R_1	R_2	R_3
7	CH_3	H	CH_3
8	CH_2CH_3	H	H
9	CH_2CH_3	Cl	H
10	CH_3	H	H
11	CH_3	Cl	H
12	CH_2CH_3	H	CH_3

Scheme 2. Synthesize of N-(4-chlorophenyl)-2-(1,3-disubstituted-2,6-diarylpiperidin-4-ylidene)hydrazinecarbothioamides (7-12).

compounds. The aliphatic and the aromatic -CH stretching of the synthesized compounds showed absorptions in the region of 2776-3000 cm^{-1}. The presence of C = N stretching was confirmed by the absorption in the region of 1592-1528 cm^{-1}. There is a sharp band absorbed in the region of 1491-1509 cm^{-1} owing to the characteristic C = S stretching vibration of compounds **7**-**12**. The observed NH, C = S stretching vibrational bands and the absence of carbonyl group around 1700 cm^{-1} are supporting the formation of corresponding thiosemicarbazones **7**-**12**. Mass spectra were recorded for all the novel compounds and the observed peaks with mass values $(M + H)^+$ confirmed the purity of the products.

2.3 ^{1}H and ^{13}C NMR spectral analyses of compounds 1 and 7

In ^{1}H NMR of compound **1** (Figure S 2 in the Supplemental Materials), two singlets at 4.10 and 4.22 ppm with one

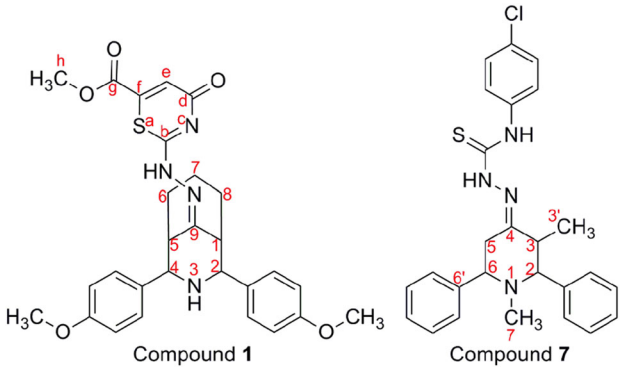


Figure 2. Numbering pattern of compounds 1 and 7 for the spectral data explanation.

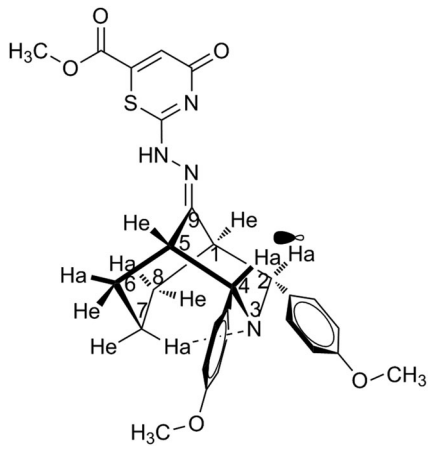


Figure 3. Interaction between H-7a proton and the nitrogen atom in compound 1 is shown by dotted line.

proton integral each are assigned to benzylic protons H-4 and H-2 respectively. The methine proton H-e in the thiazinone ring is observed at 6.65 ppm as a singlet. Also, the two singlets at 3.45 and 2.46 ppm are attributed to the bridgehead protons H-5 and H-1. The multiplets appeared at shielded regions 1.40 and 1.68 ppm with two protons integral each are assigned for H-6a & H-8a and H-6e & H-8e protons. Besides, the unassigned signals at 1.46 and 2.80 ppm are attributed to H-7e and H-7a protons. The chemical shift difference between H-7e and H-7a is about 1.34 ppm shows that H-7a is oriented toward piperidine nitrogen atom and hence there may be a weak interaction between the lone pair electrons of nitrogen and H-7a (Figure 3). As a result of this interaction, C-7 gets partial negative charge and H-7a gains a slight positive charge and thus the carbon signal is shielded and the proton signal is deshielded. The ester methoxy and phenyl substituted methoxy protons appeared at 3.78 and 3.76 ppm. The signals at deshielded regions 6.97, 7.42 and 7.51 ppm are assigned for aromatic protons.Similarly, in the ^{13}C NMR spectrum of compound **1** (Figure S 3 in the Supplemental Materials), the carbon resonance at 63.99 and 62.71 ppm are assigned to C-2 and C-4 carbons of the piperidine ring. The bridgehead carbons C-1 is observed at 45.98 ppm and C-5 peak might be merged with DMSO peaks. The cyclohexyl ring methylene carbons C6, C7 and C8 are observed at 27.18, 21.04 and 28.55 ppm. The methyl carbon C-e of thiazinone ring is observed at 113.82 ppm. The amide carbonyl in the thiazinone ring and the ester carbonyl carbons are assigned at 165.51 and 165.90 ppm in the deshielded region. Also, the imine carbon C-9 is found in the deshielded region at 176.71 ppm. The signal at 54.98 ppm is assigned for the methoxy carbons of phenyl ring attached to the 2nd and 4th positions of the piperidine ring.

The ^{1}H NMR spectrum of compound **7,** (Figure S 26 in the Supplemental Materials) a doublet observed at 0.91 ppm with three protons integral which is assignable to H-3a' methyl protons. Similarly, *N*-CH_3 protons (H7) are resonated as a singlet at 1.66 ppm with three protons integral. The H-5a proton of compound **7** appeared as a triplet at 2.48 ppm. In the aliphatic region, there are two signals as doublet at 2.90 ppm and doublet at 2.95 ppm with one proton integral each. Among the said two signals, the aliphatic signal 2.90 ppm is assigned as H-2a proton and relatively higher frequency signal 2.95 ppm is assigned as H-6a proton. The multiplet at 2.79 ppm with one proton integral is assigned for H-3a proton. The equatorial proton H-5e resonated as a doublet of doublet at 3.27 ppm. The signals appeared as doublet and multiplet in the region of 7.30-7.67 ppm is due to the phenyl protons present at the chlorophenyl thiosemicarbazone, C2 and C6. The two N-H protons of the thiosemicarbazone **7** appeared as a sharp singlet at 8.77 ppm and 9.34 ppm with one proton integral each. In the ^{13}C NMR of compound **7** (Figure S 27 in the Supplemental Materials), the signals in the aliphatic regions 12.9, 36.7, 41.4 and 45.0 ppm are assigned for C3', C5, C7 and C3 carbons of the piperidyl ring. The two peaks appeared at 69.3 ppm and 77.7 ppm is assigned for C6 and C2 carbons. For thiosemicarbazone, the C = S carbon appeared at 154.2 ppm whereas the *ipso* carbons displayed peaks at 142.4 and 143.1 ppm. The phenyl groups attached to the C2 and C6 positions of the piperidyl ring and the chlorophenyl carbons appeared in the aromatic region from 125.2 ppm to 128.9 ppm.

2.4. Anticancer studies

The anticancer activities of the synthesized compounds (1-12) were evaluated against LNCaP and PC-3 human prostate cancer cell lines. The concentrations of the compounds that inhibited 50% of cell growth (IC_{50}) in μM are calculated. With the intention of further anticancer investigation, the synthesized compounds were evaluated for the cell death studies using live/dead assay.

2.4.1. Evaluation of cytotoxicity

The anticancer activities of the synthesized compounds **1**-**12** were evaluated against LNCaP (androgen sensitive) and PC-3 (androgen insensitive) human prostate cancer cell lines (Table S 11 and Figure S 49 in the Supplemental Materials). The results clearly indicate that almost half of the synthesized compounds inhibit cell viability against prostate cancer cell lines at micromolar concentrations. Compounds **3** and **4** exhibited a high inhibition of cell viability against LNCaP cells, obtaining IC_{50} values 30.4 ± 4 and 35.2 ± 3μM respectively. The compounds **1**, **2**, **5**, and **6** accounted moderate

inhibition of LNCaP cell viability with the IC_{50} values 45.1 ± 3, 70.3 ± 3, 60.3 ± 2, and $67.4 \pm 2\,\mu M$ respectively. In PC-3 cells, the treatment with compounds **1**, **2**, and **4** exhibited good cytotoxicity with IC_{50} values 40.3 ± 3, 50.4 ± 2 and $45.6 \pm 3\,\mu M$ respectively and compounds **3**, **5**, and **6** showed moderate cytotoxicity. However, the compounds **7**-**12** exhibited no significant reduction in the viability of both the cells. The above results showed that the compounds (1-6) with thiazinone moiety exhibited better anticancer activities than the compounds (7-12) with thiosemicarbazone scaffolds.

2.4.2. Evaluation of apoptosis in LNCaP and PC-3 cells

To validate the results obtained from cytotoxicity studies, the compounds were further subjected to live/dead assay studies to evaluate the cell death percentage in LNCaP and PC-3 cells. Compounds **1**-**6** exhibited loss of membrane integrity in both the tested cells as a result of permitting EthD-1 to bind with nucleic acids of the cells with the damaged membrane and emits bright red fluorescence signal. Whereas the polyanionic dye calcein AM binds with live cells and produce uniform green fluorescent signal. The percentage of live and dead cells were quantified and depicted in Figures S 50, S 51 and S 52 in the Supplemental Materials. On comparing the results of compounds **1**-**6**, the compound **3** induced maximum ($60 \pm 3\%$) and compound 2 induced minimum ($34 \pm 1\%$) cell deaths and in LNCaP cells. Whereas in PC-3 cells, the treatment with compound **1** shows maximum ($55 \pm 3\%$) and compound **3** shows minimum ($34 \pm 2\%$) percentage of cell deaths. However, the thiosemicarbazone derivatives **7**-**12** showed low level of cell death in both LNCaP and PC-3 cells. The results of cytotoxicity and live/dead studies showed that the compounds with *methoxy*-substitution (**1**) and *fluoro*-substitution (**3**) exhibit better activities against PC-3 and LNCaP cells among the tested compounds and the thiosemicarbazone derivatives (7-12) showed poor activities against the prostate cancer cells.

3. Experimental

3.1. Instrumentation and general techniques

The melting points of the novel compounds were determined in open capillary tubes and are uncorrected. Infrared spectra were recorded on Thermo Nicolet FT–IR model *i*S5 spectrophotometer using KBr pellet. The NMR spectra were recorded at 400 MHz Bruker instruments using Tetramethylsilane (TMS) as an internal standard. Deuterated chloroform and deuterated dimethyl sulfoxide were used to record NMR spectra and the chemical shifts are reported in δ units (parts per million) relative to the standard. Mass spectra were recorded on Thermo Nicolet Exactive Plus mass spectrometers. All the chemical reactions were monitored by thin-layer chromatography using silica gel precoated aluminum sheets of Merck TLC 60 F254 and visualized in UV light chamber. All the chemical reactions were carried out using analytical grade solvents without further purification. The Supplemental Materials contains sample ^{1}H and ^{13}C NMR spectra and mass spectrometry scans for the products.(Figures S 1 – S 48)

3.2. Synthetic procedures

General procedures for the synthesis of substituted azabicyclo[3.3.1]nonan-9-ones (Ia-If) and piperidin-4-ones (IIb-IIg), azabicyclo[3.3.1]nonan-9-ones thiosemicarbazones (Ig-Il) and N-(4-chlorophenyl)hydrazinecarbothioamide (IIa) are given in the Supplemental Materials

3.2.1 General procedure for the synthesis of *methyl 2-(2-(2,4-disubstituted-3-azabicyclo[3.3.1]nonan-9-ylidene)hydrazinyl)-4-oxo-4H-1,3-thiazine-6-carboxylates* derivatives (1-6)

To a warm solution of substituted 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-one thiosemicarbazones (1.0 mmol) in methanol (15 mL), methanolic solution of DMAD/DEAD (1.0 mmol in 5 mL methanol) was added dropwise with stirring. The reaction mixture was allowed to stir at 60 °C for 1 h. The completion of the reaction was confirmed by TLC and the reaction mixture was cooled to room temperature. The yellow solid obtained was filtered and washed with warm methanol. The crude products were recrystallised from methanol.

3.2.2. General method for the synthesis of N-*(4-chlorophenyl)-2-(1,3-disubstituted-2,6-diarylpiperidin-4-ylidene)hydrazinecarbo thioamide (7-12)*

To the substituted 3–alkyl–2,6–diphenylpiperidin–4–one (1.0 mmol) in methanol (20 mL), concentrated hydrochloric acid (0.2 mL) and methanolic solution of *N*-(4-chlorophenyl)hydrazinecarbothioamide (1.0 mmol in 5 mL methanol) was added dropwise with stirring. The reaction mixture heated to reflux with water condenser for 2 h. After completion, the reaction mixture was cooled to room temperature and the solid separated was filtered. The crude products were recrystallised from methanol.

Methyl 2-(2-(2,4-bis(4-methoxyphenyl)-3-azabicyclo[3.3.1]-nonan-9-ylidene)hydrazinyl)-4-oxo-4H-1,3-thiazine-6-carboxylate (1)

Chemical formula: $C_{28}H_{30}N_4O_5S$; Yellow powder; Yield: 72%; mp: 174–176 °C; FTIR (KBr) (ν_{max}, cm^{-1}): 3430 (N-H), 3157, 2962, 2922 (C-H), 1728, 1703 (ester C = O), 1642 (amide C = O), 1609 (C = N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.40 (m, 2H, 6CH_2), 1.46 (m, 1H), 1.68 (m, 2H, 7CH_2), 2.46 (s, 1H, bridgehead 1CH), 2.80 (m, 1H), 3.45 (s, 1H, Bridgehead 5CH), 3.76 (s, 6H, phenyl methoxy CH_3), 3.78 (s, 3H, ester methoxy CH_3), 4.10 (s, 1H, Benzylic protons 4CH), 4.22 (s, 1H, Benzylic protons 2CH), 6.65 (s, 1H, thiazinone ring CH), 6.97 (m, 4H, Aromatic-H), 7.42 (d, $J = 8.4$ Hz, 2H, Aromatic-H), 7.51 (d, $J = 8$ Hz, 2H, Aromatic-H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.04 (CH_2, C-7), 27.18 (CH_2, C-6), 28.55(CH_2, C-8), 45.98 (CH, C-1), 52.37, 54.98 (C_6H_4-O-CH_3), 62.71 (CH, C-4), 63.99 (CH, C-2), 113.53, 113.57, 113.82 (CH in thazinone ring), 127.81, 127.86, 134.53, 143.18, 157.73, 158.18, 158.22, 165.51

(Amide C=O in thiazinone ring), 165.90 (ester C=O in thiazinone ring), 176.71 (C=N, C-9); HRMS (ESI/$(M+H)^+$) calculated 535.2015 found 535.2708.

Methyl 2-(2-(2,4-diphenyl-3-azabicyclo[3.3.1]nonan-9-ylidene)hydrazinyl)-4-oxo-4H-1,3-thiazine-6-carboxylate (2)

Chemical formula: $C_{26}H_{26}N_4O_3S$; Yellow powder; Yield: 81%; mp: 182–184 °C; FTIR (KBr) (ν_{max}, cm^{-1}): 3437 (N-H), 3059, 2958, 2782 (C-H), 1724, 1704 (ester C=O), 1651 (amide C=O), 1623 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.33 (m, 2H, 6CH_2), 1.41 (m, 2H), 1.61 (m, 2H, 7CH_2), 2.57 (s, 1H, bridgehead 1CH), 3.28 (s, 1H), 3.53 (s, 1H, Bridgehead 5CH), 3.78 (s, 3H, phenyl methoxy CH_3), 4.17 (s, 1H, Benzylic protons 4CH), 4.30 (s, 1H, Benzylic protons 2CH), 6.65 (s, 1H, thiazinone ring CH), 7.38-7.66 (m, aromatic H), 12.72 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.65 (CH_2, C-7), 27.32 (CH_2, C-6), 35.88 (CH_2, C-8), 41.57 (CH, C-1), 52.39, 60.05, 61.38 (CH, C-4), 115.12 (CH in thazinone ring), 118.63, 127.15, 131.43, 147.63, 154.43, 157.34, 165.92(ester C=O in thiazinone ring), 175.52 (C=N, C-9); HRMS (ESI/$(M+H)^+$) calculated 475.1804 found 475.1950.

Methyl 2-(2-(2,4-bis(4-fluorophenyl)-3-azabicyclo[3.3.1]nonan-9-ylidene)hydrazinyl)-4-oxo-4H-1,3-thiazine-6-carboxylate (3)

Chemical formula: $C_{26}H_{24}F_2N_4O_3S$; Yellow powder; Yield: 78%; mp: 171–173 °C; FTIR (KBr) (ν_{max}, cm^{-1}): 3423 (N-H), 3071, 2929, 2850, 2802, 2770 (C-H), 1734, 1706 (ester C=O), 1645 (amide C=O), 1605 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.47 (m, 2H, 6CH_2), 1.55 (m, 1H), 1.63 (m, 2H, 7CH_2), 2.72 (t, J = 6 Hz, 1H, bridgehead 1CH), 3.08 (s, 1H), 3.49 (s, 1H, Bridgehead 5CH), 3.78 (s, 3H, ester methoxy CH_3), 4.17 (s, 1H, Benzylic protons 4CH), 4.29 (s, 1H, Benzylic protons 2CH), 6.65 (s, 1H, thiazinone ring CH), 7.20 – 7.27 (m, 4H, Aromatic-H), 7.55 (t, J = 6.4 Hz, 2H, Aromatic-H), 7.66 (t, J = 6.4 Hz, 2H, Aromatic-H), 12.71 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.93 (CH_2, C-7), 27.11 (CH_2, C-6), 28.56 (CH_2, C-8), 45.62 (CH, C-1), 52.36, 62.37, 63.58 (CH, C-2), 113.91(CH in thazinone ring), 128.66, 128.74, 138.61, 143.10, 157.97, 159.98, 162.39, 165.47 (amide C=O in thiazinone ring), 165.88 (ester C=O in thiazinone ring), 176.07 (C=N, C-9); HRMS (ESI/$(M+H)^+$) calculated 511.1615 found 511.1273.

Methyl 2-(2-(2,4-bis(2-chlorophenyl)-3-azabicyclo[3.3.1]nonan-9-ylidene)hydrazinyl)-4-oxo-4H-1,3-thiazine-6-carboxylate (4)

Chemical formula: $C_{26}H_{24}Cl_2N_4O_3S$; Yellow powder; Yield: 74%; mp: 169–171 °C; FTIR (KBr) (ν_{max}, cm^{-1}): 3429 (N-H), 3182, 3061, 2943, 2765 (C-H), 1730, 1698 (ester C=O), 1643 (amide C=O), 1607 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.29 (bs, 1H), 1.42 (bs, 2H, 6CH_2), 1.52 (m, 2H, 7CH_2), 2.78 (s, 1H, bridgehead 1CH), 3.05 (s, 1H, Bridgehead 5CH), 3.78 (s, 3H, ester methoxy CH_3), 4.46 (s, 1H Benzylic proton 4CH), 4.59 (s, 1H, Benzylic proton 2CH), 6.65 (s, 1H, thiazinone ring CH), 7.34–8.10 (m, Aromatic-H), 8.75 (s, 1H, Aromatic-H), 12.70 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.47 (CH_2, C-7), 27.31 (CH_2, C-6), 28.57 (CH_2, C-8), 35.88, 41.56 (CH, C-1), 48.56, 52.40, 52.48, 60.06, 61.39(CH, C-4), 113.82 (CH in thazinone ring), 114.60, 127.05, 127.82, 129.48, 129.56, 130.67, 131.42, 133.92, 139.17, 142.62, 143.19, 154.38, 165.53 (Amide C=O in thiazinone ring), 165.88 (ester C=O in thiazinone ring), 175.34 (C=N, C-9); HRMS (ESI/$(M+H)^+$) calculated 543.1024 found 544.2947.

Methyl 2-(2-(2,4-bis(3-chlorophenyl)-3-azabicyclo[3.3.1]nonan-9-ylidene)hydrazinyl)-4-oxo-4H-1,3-thiazine-6-carboxylate (5)

Chemical formula: $C_{26}H_{24}Cl_2N_4O_3S$; Yellow powder; Yield: 79%; mp: 173–175 °C; FTIR (KBr) (ν_{max}, cm^{-1}): 3429 (N-H); 3105, 2921, 2854, 2794 (C-H); 1728 (ester C=O); 1651 (amide C=O); 1611 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.31 (m, 1H), 1.40 (m, 1H), 1.61 (m, 2H, 7CH_2), 2.58 (s, 1H, bridgehead 1CH), 2.66 (m, 1H), 3.28 (s, 1H, Bridgehead 5CH), 3.79 (s, 3H, ester methoxy CH_3), 4.18 (s, 1H, Benzylic proton 4CH), 4.30 (s, 1H, Benzylic proton 2CH), 6.66 (s, 1H, thiazinone ring CH), 7.36 - 7.67 (m, aromatic H), 12.70 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.94 (CH_2, C-7), 27.14 (CH_2, C-6), 28.62 (CH_2, C-8), 45.30 (CH, C-1), 52.39, 62.35 (CH, C-4), 63.54 (CH, C-2), 113.96 (CH in thazinone ring), 125.58, 126.66, 130.19, 133.00, 143.09, 144.96, 145.01, 165.49 (Amide C=O in thiazinone ring), 165.87 (ester C=O in thiazinone ring), 175.54 (C=N, C-9); HRMS (ESI/$(M+H)^+$) calculated 543.1024 found 544.2943.

Methyl 2-(2-(2,4-bis(4-chlorophenyl)-3-azabicyclo[3.3.1]nonan-9-ylidene)hydrazinyl)-4-oxo-4H-1,3-thiazine-6-carboxylate (6)

Chemical formula: $C_{26}H_{24}Cl_2N_4O_3S$; Yellow powder; Yield: 84%; mp: 164–166 °C; FTIR (KBr) (ν_{max}, cm^{-1}): 3425 (N-H); 3164, 3065, 2929, 2794 (C-H); 1716, 1704 (ester C=O); 1641 (amide C=O); 1621 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 1.48 (m, 2H, 6CH_2), 1.54 (m, 1H), 1.61 (m, 2H, 7CH_2), 2.69 (m, 1H, bridgehead 1CH), 3.17 (s, 1H), 3.50 (s, 1H, Bridgehead 5CH), 3.78 (s, 3H, ester methoxy CH_3), 4.16 (s, 1H, Benzylic proton 4CH), 4.28 (s, 1H, Benzylic proton 2CH), 6.65 (s, 1H, thiazinone ring CH), 7.45 – 7.85 (m, Aromatic-H), 12.77 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.92 (CH_2, C-7), 27.12 (CH_2, C-6), 28.56 (CH_2, C-8), 45.43 (CH, C-1), 52.39, 62.36 (CH, C-1), 63.54 (CH, C-2), 113.93 (CH in thazinone ring), 128.10, 128.66, 129.08, 129.59 131.36, 131.42, 132.58, 135.73, 141.47, 143.10, 157.42, 165.50 (Amide C=O in thiazinone ring), 165.88 (ester C=O in thiazinone ring), 175.74 (C=N, C-9); HRMS (ESI/$(M+H)^+$) calculated 543.1024 found 544.1170.

(E)-N-(4-chlorophenyl)-2-(1,3-dimethyl-2,6-diphenylpiperidin-4-ylidene)hydrazinecarbothioamide (7)

Chemical formula: $C_{26}H_{27}ClN_4S$; White powder; Yield: 81%; mp: 202–204 °C; FTIR (KBr) (ν_{max}, cm^{-1}): 3327 (NH), 3084, 3026, 2973, 2932, 2776 (CH), 1584, 1536 (C=N); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 0.91 (d, J = 6.8 Hz, 3H, 3'CH_3) , 1.66 (s, 3H, N-CH_3), 2.48 (t, J = 12.4 Hz, 1H, 5CH_a), 2.80 (m, 1H, 3CH), 2.90 (d, J = 2.8 Hz, 1H, 2CH), 2.95 (d, J = 10.4 Hz, 1H, 6CH), 3.27 (dd, J = 11.8 Hz, 1H, 5CH_e), 7.67-7.30 (m, 14H, Aromatic-H), 8.77 (s, 1H, NH), 9.34 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.97 (CH_3, C-3'), 36.73 (CH, C-5), 41.43 (N-CH_3, C-7), 45.06

(CH, C-3), 69.34 (CH, C-6), 77.71 (CH, C-2), 125.23, 127.77, 127.96, 128.09, 128.65, 128.87, 128.96 (Aromatic-CH), 131.21, 136.57, 142.44 (*ipso* carbon, C-2′), 143.12 (*ipso* carbon, C-6′), 154.27, 176.52; HRMS (ESI/$(M+H)^+$) calculated 463.1723 found 463.1641.

(E)-N-(4-chlorophenyl)-2-(3-ethyl-2,6-diphenylpiperidin-4-ylidene)hydrazinecarbothioamide (8)

Chemical formula: $C_{26}H_{27}ClN_4S$; White powder; Yield: 75%; mp: 218–220 °C; FTIR (KBr) (ν_{max}, cm^{-1}): 3372, 3305 (NH), 3058, 3041, 2967, 2877, 2677, 2545, 2427 (CH), 1586, 1528 (C = N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.81 (t, J = 7.2 Hz, 3H, 3'CH_3), 1.54 (m, 2H, 3'CH_2), 3.18 (t, J = 14 Hz, 1H, 5CH_a), 3.67 (d, J = 12.4 Hz, 1H, 3CH), 4.56 (d, J = 9.6 Hz, 1H, 2CH), 4.65 (d, J = 9.2 Hz, 1H, 6CH), 7.44 (m, 8H, Aromatic-H), 7.63 (d, J = 8.8 Hz, 2H, Aromatic-H), 7.84 (m, 4H, Aromatic-H) 9.89 (bs, 1H, NH), 10.47 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.36 (CH_3, C-3′), 18.25 (CH_2, C-3′), 31.88 (CH, C-5), 45.44 (CH, C-3), 59.34 (CH, C-6), 64.65 (CH, C-2), 126.15, 128.14, 128.33, 128.57, 128.64, 129.05, 129.32 (Aromatic-CH), 131.95, 134.85, 135.69, 137.77, 150.53, 177.04; HRMS (ESI/$(M+H)^+$) calculated 463.1723 found 463.1640.

(E)-2-(2,6-bis(4-chlorophenyl)-3-ethylpiperidin-4-ylidene)-N-(4-chlorophenyl)hydrazinecarbothioamide (9)

Chemical formula: $C_{26}H_{25}Cl_3N_4S$; White powder; Yield: 79%; mp: 188-190 °C; FTIR (KBr) (ν_{max}, cm^{-1}): 3382, 3274 (NH), 3028, 2973, 2935, 2874, 2635, 2546, 2442, 2390 (CH), 1589, 1540 (C = N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.80 (t, J = 7.2 Hz, 3H, 3'CH_3), 1.57 (m, 2H, 3'CH_2), 3.12 (s, 1H, 5CH_a), 3.65 (d, 1H, J = 12.8 Hz, 3CH), 4.58 (d, J = 10.4 Hz, 1H, 2CH), 4.66 (d, J = 11.2 Hz, 1H, 6CH), 7.41(d, J = 8.8 Hz, 2H, Aromatic-H), 7.54 (m, 4H, Aromatic-H), 7.62 (d, J = 8.8 Hz, 2H, Aromatic-H), 7.82 (d, J = 8.4 Hz, 2H, Aromatic-H), 7.88 (d, J = 8.4 Hz, 2H, Aromatic-H) 9.73 (bs, 1H, NH), 10.57 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.33 (CH_3, C-3′), 18.20 (CH_2, C-3′), 31.61 (CH, C-5), 45.29 (CH, C-3), 58.51 (CH, C-6), 63.74 (CH, C-2), 126.20, 128.13, 128.33, 128.54, 128.68, 130.52, 131.29, 133.67, 133.78, 134.04, 134.52 (Aromatic-CH), 137.75, 149.90, 177.07; HRMS (ESI/$(M+H)^+$) calculated 531.0943 found 531.0825.

(E)-N-(4-chlorophenyl)-2-(3-methyl-2,6-diphenylpiperidin-4-ylidene)hydrazinecarbothioamide (10)

Chemical formula: $C_{25}H_{25}ClN_4S$; White powder; Yield: 73%; mp: 194-196 °C; FTIR (KBr) (ν_{max}, cm^{-1}): 3440, 3295 (NH), 3085, 3028, 2963, 2928, 2871, 2783 (CH), 1586, 1533 (C = N); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 0.96 (d, J = 6.8 Hz, 3H, 3'CH_3), 2.30 (t, J = 12 Hz, 1H), 2.64 (m, 1H, 5CH_a), 2.94 (dd, J = 14 Hz, 1H, 3CH), 3.55 (d, J = 10 Hz, 1H, 2CH), 3.90 (dd, J = 11.6 Hz, 1H, 6CH), 7.63-7.26 (m, 14H, Aromatic-H), 8.80 (s, 1H, NH), 9.32 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.18 (CH_3, C-3′), 36.33 (CH, C-5), 45.20 (CH, C-3), 60.98 (CH, C-6), 69.23 (CH, C-2), 125.18, 126.67, 127.85, 128.12, 128.20, 128.60, 128.81, 128.86, (Aromatic CH), 131.18, 136.57, 142.11, 142.52, 155.00, 176.51; HRMS (ESI/$(M+H)^+$) calculated 449.1566 found 449.1487.

(E)-2-(2,6-bis(4-chlorophenyl)-3-methylpiperidin-4-ylidene)-N-(4-chlorophenyl)hydrazinecarbothioamide (11)

Chemical formula: $C_{25}H_{23}Cl_3N_4S$; White powder; Yield: 77%; mp: 206-208 °C; FTIR (KBr) (ν_{max}, cm^{-1}): 3272, 3207 (NH), 2969, 2930, 2802, 2625, 2551, 2498, 2430 (CH), 1589, 1540 (C = N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.88 (d, J = 6.4 Hz, 3H, 3'CH_3), 2.34 (bs, 1H, 5CH_a), 2.79 (bs, 1H, 3CH), 4.08 (bs, 1H, 6CH), 7.40-7.62 (m, 14H, Aromatic-H), 9.71 (s, 1H, NH), 11.06 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.62 (CH_3, C-3′), 12.13, 56.40 (CH, C-3), 58.89, 62.22 (CH, C-6), 66.96 (CH, C-2), 126.57, 127.87, 128.09, 128.29, 130.09, 132.28, 132.87, 137.87 (Aromatic CH), 176.95, 179.47; HRMS (ESI/$(M+H)^+$) calculated 517.0787 found 517.0701.

(E)-N-(4-chlorophenyl)-2-(3-ethyl-1-methyl-2,6-diphenylpiperidin-4-ylidene)hydrazinecarbothioamide (12)

Chemical formula: $C_{27}H_{29}ClN_4S$; White powder; Yield: 79%; mp: 212-214 °C; FTIR: (KBr) (ν_{max}, cm^{-1}): 3328, 3263, (NH), 2968, 2955, 2869, 2829, 2775, 2471 (CH), 1592, 1534 (C = N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 0.73 (bs, 3H, 3'CH_3), 1.09 (bs, 1H), 1.57 (s, 3H, N-CH_3), 1.66 (bs, 2H, 3'CH_2), 2.08 (s, 1H, 5CH_a), 2.32 (m, 1H, 3CH), 2.61 (bs, 1H, 2CH), 3.04 (d, J = 8.8 Hz, 1H, 6CH), 3.26 (d, J = 11.6 Hz, 1H, 5CH_e), 7.28-7.80 (m, 14H, Aromatic H), 9.66 (s, 1H, NH), 11.08 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 18.99 (CH_3, C-3′), 20.26 (CH_2, C-3′), 21.68, 29.68, 31.52 (CH, C-5), 48.55 (N-CH_3, C-7), 66.15 (CH, C-6), 72.05 (CH, C-2), 126.21, 128.05, 128.64, 129.01, 129.41, 129.63 (Aromatic CH), 134.22, 136.08, 137.70, 137.99 (*ipso* carbon, C-2′), 138.20 (*ipso* carbon, C-6′), 148.04, 156.32, 176.99; HRMS (ESI/$(M+H)^+$) calculated 477.1879 found 477.1795.

3.3. In vitro *biological studies*

3.3.1. Cell culture

PC-3 and LNCaP human prostate cancer cell lines were sourced from the National Center for Cell Science (NCCS, Pune, India). PC-3 and LNCaP cells were cultured by following standard procedures.[39] The PC-3 cells were cultured in F12-K medium (HiMedia laboratories, India) and LNCaP cells were cultured in RPMI-1640 medium (Gibco, Grand Island, USA). The media were supplemented with 10% fetal bovine serum (FBS) and with 1% antibiotics of 1X Streptomycin/Penicillin. Both the cells were grown in a humidified incubator at 37 °C supplemented with 5% carbon-di-oxide.

3.3.2. Cytotoxicity

The cytotoxic effect of the synthesized compounds 1–12 on prostate cancer cell lines (LNCaP and PC-3) was evaluated through 3(4,5-Dimethyl-thiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) assay.[40] The cells (1 × 106 cells/mL) were seeded into 96 well plates and treated with different concentrations of the compounds (1-12) ranging from 5, 25, 50, 75, 100, 125 and 150 μM obtained by appropriate dilution with dimethylsulfoxide (DMSO, Sigma, USA) and incubated for

24 h. Following 24 h incubation with various concentrations of the compounds, MTT (0.5 mg/mL in phosphate buffer saline) was added to each micro well and incubated for another 4 h. The supernatant was removed and replaced with DMSO (200 μL) and the optical density of each well was measured using multimode reader (Tecan, Austria).

3.3.3. Live/dead assay

Live/Dead assay (Invitrogen) was performed as described previously.[41] In brief, after 24 h of treatment with compounds, cells were washed twice with Dulbecco's phosphate-buffer saline (D-PBS) and were incubated with calcein AM (acetoxymethyl) and ethidium homodimer-1 (EthD-1) for 30 mins in dark at room temperature. Following incubation, the cells were washed with D-PBS and the images were captured under fluorescent microscopy Leica -MZ16FA (Leica Microsystems, Switzerland). The live and dead cells were quantified using ImageJ (1.49 u version) software.

4. Conclusion

Novel series of thiazinone and thiosemicarbazone derivatives of modified piperidones were synthesized in mild condition using methanol as a solvent. The structures of the novel compounds were elucidated using FTIR, NMR and mass spectral studies. It was determined that the compounds **1** and **3** showed better activity against the human prostate cancer cell lines. Moreover, *fluoro*-substituted compound **3** shows selective inhibition towards LNCaP cell lines and the *methoxy*-substituted compound **1** shows activity against PC-3 cell lines. When comparing the anticancer results, it is clearly seen that thiazinone derivatives (1-6) are more effective than thiosemicarbazone derivatives (7-12) against the prostate cancer cell lines. Further, the apoptotic characteristics of all the newly synthesized compounds were analyzed using live/dead assay studies. The live/dead assay studies showed that the compounds **1** and **3** caused maximum percentage of cell deaths in the tested cell lines and found to be better among the series.

Acknowledgements

We gratefully acknowledge the funding support from University Grants Commission (UGC) Grant No. 39–689–2010 SR New Delhi, India.

References

[1] Siegel, R. L.; Miller, K. D.; Jemal, A. Cancer Statistics, 2018. *CA Cancer J Clin.* **2018**, *68*, 7–30. DOI: 10.3322/caac.21442.

[2] Srinivasan, D.; Senbanjo, L.; Majumdar, S.; Franklin, R. B.; Chellaiah, M. A. Androgen Receptor Expression Reduces Stemness Characteristics of Prostate Cancer Cells (PC3) by Repression of CD44 and SOX2. *J. Cell. Biochem.* **2019**, *120*, 2413–2428. DOI: 10.1002/jcb.27573.

[3] Sternberg, C. N. Enzalutamide, an Oral Androgen Receptor Inhibitor for Treatment of Castration-Resistant Prostate Cancer. *Future Oncol* **2019**, *15*, 1437–1457. DOI: 10.2217/fon-2018-0940.

[4] Ito, Y.; Sadar, M. D. Enzalutamide and Blocking Androgen Receptor in Advanced Prostate Cancer: lessons Learnt from the History of Drug Development of Antiandrogens. *Rru.* **2018**, Vol *10*, 23–32. DOI: 10.2147/RRU.S157116.

[5] Ma, Y.; Ren, X.; Patel, N.; Xu, X.; Wu, P.; Liu, W.; Zhang, K.; Goodin, S.; Li, D.; Zheng, X. Nobiletin, a Citrus Polymethoxyflavone, Enhances the Effects of Bicalutamide on Prostate Cancer Cells *via* down Regulation of NF-κB. *RSC Adv.* **2020**, *10*, 10254–10262. DOI: 10.1039/C9RA10020B.

[6] Thomas, L.; Baratchian, M.; Sharifi, N. Supraphysiologic Testosterone Solutions for Enzalutamide-Resistant Prostate Cancer. *Eur. Urol* **2020**, *77*, 156–157. DOI: 10.1016/j.eururo.2019.07.037.

[7] Saravanan, K.; Elancheran, R.; Divakar, S.; Anand, S. A. A.; Ramanathan, M.; Kotoky, J.; Lokanath, N. K.; Kabilan, S. Design, Synthesis and Biological Evaluation of 2-(4-Phenylthiazol-2-yl) Isoindoline-1,3-Dione Derivatives as anti-Prostate Cancer Agents. *Bioorg. Med. Chem. Lett* **2017**, *27*, 1199–1204. DOI: 10.1016/j.bmcl.2017.01.065.

[8] Bhat, M.; Poojary, B.; Kalal, B. S.; Swamy, P. M. G.; Kabilan, S.; Kumar, V.; Shruthi, N.; Anand, S. A. A.; Pai, V. R. Synthesis and Evaluation of Thiazolidinone–Pyrazole Conjugates as Anticancer and Antimicrobial Agents. *Future Med. Chem* **2018**, *10*, 1017–1036. DOI: 10.4155/fmc-2017-0191.

[9] Thomas, N. S.; George, K.; Anand, S. A. A. Anticancer Mechanism of Troxerutin *via* Targeting Nrf2 and NF-κB Signalling Pathways in Hepatocarcinoma Cell Line. *Toxicol. In Vitro* **2019**, *54*, 317–329. DOI: 10.1016/j.tiv.2018.10.018.

[10] Thomas, N. S.; George, K.; Anand, S. A. A. Troxerutin Subdues Hepatic Tumorigenesis *via* Disrupting the MDM2–p53 Interaction. *Food Funct.* **2018**, *9*, 5336–5349. DOI: 10.1039/C8FO01111G.

[11] Ariga, T.; Seki, T. Antithrombotic and Anticancer Effects of Garlic-Derived Sulfur Compounds: A Review. *Biofactors* **2006**, *26*, 93–159. DOI: 10.1002/biof.5520260201.

[12] Vazquez-Prieto, M. A.; Miatello, R. M. Organosulfur Compounds and Cardiovascular Disease. *Mol. Aspects Med* **2010**, *31*, 540–545. DOI: 10.1016/j.mam.2010.09.009.

[13] Kouznetsov, V. V.; Barrio, A. G. Recent Developments in the Design and Synthesis of Hybrid Molecules Based on Aminoquinoline Ring and Their Antiplasmodial Evaluation. *Eur. J. Med. Chem* **2009**, *44*, 3091–3113. DOI: 10.1016/j.ejmech.2009.02.024.

[14] Yadav, L. D. S.; Misra, A. R.; Singh, H. Ring Transformation of Michael Adducts of 4-Benzylidene-5-Oxazolones and 3-Mercapto-s-Triazoles to 2,3-Dihydro-4H-s-Triazolo[3,4-b][1,3]Thiazin-4-Ones with Some Antifungal Activity. *J. Agric. Food Chem.* **1988**, *36*, 633–636. DOI: 10.1021/jf00081a056.

[15] Ohno, K.; Tsutsumi, R.; Matsumoto, N.; Yamashita, H.; Amada, Y.; Shishikura, J.; Yatsugi, H. I. S.; Okada, M.; Sakamoto, S.; Yamaguchi, T. Functional Characterization of YM928, a Novel Noncompetitive Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid (AMPA) Receptor Antagonist. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 66–72. DOI: 10.1124/jpet.103.049973.

[16] Rahman, V. J. M.; Mukhtar, S.; Ansari, W. H.; Lemiere, G. Synthesis, Stereochemistry and Biological Activity of Some Novel Long Alkyl Chain Substituted Thiazolidin-4-Ones and Thiazan-4-One from 10-Undecenoic Acid Hydrazide. *Eur. J. Med. Chem* **2005**, *40*, 173–184. DOI: 10.1016/j.ejmech.2004.10.003.

[17] Zhang, X. M.; Guo, H.; Li, Z.; Song, F.; Wang, W.; Dai, H.; Zhang, L.; Wang, J. Synthesis and Evaluation of Isatin-β-Thiosemicarbazones as Novel Agents against Antibiotic-Resistant Gram-Positive Bacterial Species. *Eur. J. Med. Chem* **2015**, *101*, 419–430. DOI: 10.1016/j.ejmech.2015.06.047.

[18] Kesel, A. J. Broad-Spectrum Antiviral Activity Including Human Immunodeficiency and Hepatitis C Viruses Mediated by a Novel Retinoid Thiosemicarbazone Derivative. *Eur. J. Med. Chem* **2011**, *46*, 1656–1664. DOI: 10.1016/j.ejmech.2011.02.014.

[19] Oliveira, R. B.; Fagundes, E. M. S.; Soares, R. P. P.; Andrade, A. A.; Krettli, A. U.; Zani, C. L. Synthesis and Antimalarial

Activity of Semicarbazone and Thiosemicarbazone Derivatives. *Eur. J. Med. Chem* **2008**, *43*, 1983–1988. DOI: 10.1016/j.ejmech.2007.11.012.

[20] Mishra, V.; Pandeya, S. N.; Pannecouque, C.; Witvrouw, M.; Clercq, E. D. Anti-HIV Activity of Thiosemicarbazone and Semicarbazone Derivatives of (±)-3-Menthone. *Arch. Pharm. Pharm. Med. Chem.* **2002**, *335*, 183–186. DOI: 10.1002/1521-4184(200205)335:5<183::AID-ARDP183>3.0.CO;2-U.

[21] Zhang, H.; Qian, Y.; Zhu, D.; Yang, X.; Zhu, H. Synthesis, Molecular Modeling and Biological Evaluation of Chalcone Thiosemicarbazide Derivatives as Novel Anticancer Agents. *Eur. J. Med. Chem* **2011**, *46*, 4702–4708. DOI: 10.1016/j.ejmech.2011.07.016.

[22] Sriram, D.; Yogeeswari, P.; Thirumurugan, R.; Pavana, R. K. Discovery of New Antitubercular Oxazolyl Thiosemicarbazones. *J. Med. Chem.* **2006**, *49*, 3448–3450. DOI: 10.1021/jm060339h.

[23] Goulart, C. M.; Souza, A. E.; Huitle, C. A. M.; Rodrigues, C. J. F.; Maciel, M. A. M.; Echevarria, A. Experimental and Theoretical Evaluation of Semicarbazones and Thiosemicarbazones as Organic Corrosion Inhibitors. *Corros. Sci* **2013**, *67*, 281–291. DOI: 10.1016/j.corsci.2012.10.029.

[24] de Melos, J. L. R.; Torres-Santos, E. C.; Faiões, V. d S.; de Nigris Del Cistia, C.; Sant'Anna, C. M. R.; Rodrigues-Santos, C. E.; Echevarria, A. Novel 3,4-Methylenedioxyde-6-X-Benzaldehyde-Thiosemicarbazones: Synthesis and Antileishmanial Effects against Leishmania Amazonensis. *Eur. J. Med. Chem* **2015**, *103*, 409–417. DOI: 10.1016/j.ejmech.2015.09.009.

[25] Yi, W.; Dubois, C.; Yahiaoui, S.; Haudecoeur, R.; Belle, C.; Song, H.; Hardre, R.; Reglier, M.; Boumendjel, A. Refinement of Arylthiosemicarbazone Pharmacophore in Inhibition of Mushroom Tyrosinase. *Eur. J. Med. Chem* **2011**, *46*, 4330–4335. DOI: 10.1016/j.ejmech.2011.07.003.

[26] Xue, C.; Zhang, L.; Luo, W.; Xie, X.; Jiang, L.; Xiao, T. 3D-QSAR and Molecular Docking Studies of Benzaldehyde Thiosemicarbazone, Benzaldehyde, Benzoic Acid, and Their Derivatives as Phenoloxidase Inhibitors. *Bioorg. Med. Chem* **2007**, *15*, 2006–2015. DOI: 10.1016/j.bmc.2006.12.038.

[27] Merlino, A.; Benitez, D.; Chavez, S.; Cunha, J. D.; Hernandez, P.; Tinoco, L. W.; Campillo, N. E.; Paez, J. A.; Cerecetto, H.; Gonzalez, M. Development of Second Generation Amidinohydrazones, Thio- and Semicarbazones as *Trypanosoma Cruzi* Inhibitors Bearing Benzofuroxan and Benzimidazole 1,3-Dioxide Core Scaffolds. *Med. Chem. Commun.* **2010**, *1*, 216–228. DOI: 10.1039/c0md00085j.

[28] Hameed, A.; Khan, K. M.; Zehra, S. T.; Ahmed, R.; Shafiq, Z.; Bakht, S. M.; Yaqub, M.; Hussain, M.; de la Vega de León, A.; Furtmann, N.; et al. Synthesis, Biological Evaluation and Molecular Docking of N-Phenyl Thiosemicarbazones as Urease Inhibitors. *Bioorg. Chem* **2015**, *61*, 51–57. DOI: 10.1016/j.bioorg.2015.06.004.

[29] Noller, C. R.; Baliah, V. The Preparation of Some Piperidine Derivatives by the Mannich Reaction. *J. Am. Chem. Soc.* **1948**, *70*, 3853–3855. DOI: 10.1021/ja01191a092.

[30] Alphonsa, A. T.; Loganathan, C.; Anand, S. A. A.; Kabilan, S. Molecular Structure, NMR, UV–Visible, Vibrational Spectroscopic and HOMO, LUMO Analysis of (E)-1-(2, 6-Bis (4-Methoxyphenyl)-3, 3-Dimethylpiperidine-4-Ylidene)-2-(3-(3, 5-Dimethyl-1H-Pyrazol-1-yl) Pyrazin-2-yl) Hydrazine by DFT Method. *J. Mol. Struct* **2016**, *1106*, 277–285. DOI: 10.1016/j.molstruc.2015.11.005.

[31] Alphonsa, A. T.; Loganathan, C.; Anand, S. A. A.; Kabilan, S. FT-IR, FT-Raman, UV, NMR Spectra and Molecular Structure Investigation of (E)-2-(3-Chloropyrazin-2-yl)-1-(3-Ethyl-2, 6-Diphenyl Piperidin-4-Ylidene) Hydrazine: A Combined Experimental and Theoretical Study. *J. Mol. Struct* **2015**, *1100*, 137–144. DOI: 10.1016/j.molstruc.2015.07.024.

[32] Anand, S. A. A.; Loganathan, C.; Thomas, N. S.; Saravanan, K.; Alphonsa, A. T.; Kabilan, S. Synthesis, Structure Prediction, Pharmacokinetic Properties, Molecular Docking and Antitumor Activities of Some Novel Thiazinone Derivatives. *New J. Chem.* **2015**, *39*, 7120–7129.

[33] Anand, S. A. A.; Loganathan, C.; Thomas, N. S.; Saravanan, K.; Alphonsa, A. T.; Kabilan, S. Synthesis of Novel 1,3-Thiazin-4-Ones by Acetylene Diester Cyclization and Their Anticancer Activities. *Phosphorus Sulfur Silicon Relat. Elem* **2016**, *191*, 1396–1401.

[34] Wadhwa, P.; Bagchi, S.; Sharma, A. A Regioselective Multicomponent Cascade to Access Thiosemicarbazone–Fused Thiazinones: Scope, Structure Elucidation and Gram Scale Synthesis. *ChemistrySelect* **2017**, *2*, 1386–1391.

[35] Wadhwa, P.; Hussen, A. S.; Sharma, A. A Multicomponent Strategy for the Regioselective Synthesis of [1,3]-Thiazinones from an Abundant Feedstock: Scope and Structural Elucidation. *Asian J. Org. Chem.* **2017**, *6*, 88–94.

[36] Britsun, V. N.; Lozinskii, M. O. Cycloacylation of Thioamides and Their Derivatives by Compounds Containing an Activated Multiple Bond (Review). *Chem. Heterocycl. Comp.* **2007**, *43*, 1083–1110.

[37] Acheson, R. M.; Wallis, J. D. Addition Reactions of Heterocyclic Compounds. Part 74. Products from Dimethyl Acetylenedicarboxylate with Thiourea, Thioamide, and Guanidine Derivatives. *J. Chem. Soc. Perkin. Trans* **1981**, *1*, 415–422. DOI: 10.1039/P19810000415.

[38] Tripathi, L.; Kumar, P.; Singh, R.; Stables, J. P. Design, Synthesis and Anticonvulsant Evaluation of Novel N-(4-Substituted Phenyl)-2-[4-(Substituted) Benzylidene]-Hydrazinecarbothio Amides. *Eur. J. Med. Chem* **2012**, *47*, 153–166.

[39] George, K.; Thomas, N. S.; Malathi, R. Modulatory Effect of Selected Dietary Phytochemicals on Delayed Rectifier K + Current in Human Prostate Cancer Cells. *J. Membrane Biol.* **2019**, *252*, 195–206.

[40] George, K.; Thomas, N. S. Malathi. R. 4,4'-Diisothiocyanatostilbene-2,2'-Disulfonate Modulates Voltage Gated K + Current and Influences Cell Cycle Arrest in Androgen Sensitive and Insensitive Human Prostate Cancer Cell Lines. *Toxicol. Mech. Methods* **2020**. DOI: 10.1080/15376516.2020.1745343.

[41] George, K.; Thomas, N. S.; Malathi, R. Piperine Blocks Voltage Gated K + Current and Inhibits Proliferation in Androgen Sensitive and Insensitive Human Prostate Cancer Cell Lines. *Arch. Biochem. Biophys* **2019**, *667*, 36–48.