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Research Article
Biological Evaluation of Some Imidazolidine-2,4-dione and 2-thioxoimidazolidin-4-one Derivatives as Anticoagulant Agents and Inhibition of MCF-7 Breast Cancer Cell Line

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Abstract

In this study, to investigate the synthesis and characterization of some imidazolidine-2,4-dione,2-thioxoimidazolidin-4-one derivatives and synthesized compounds were evaluated for anticoagulant and anticancer activities. Compounds 2a-2h, 3a-3h, 4a-4h and 5a-5h were prepared by cyclization method. The synthesized compounds were confirmed by Fourier transform infrared spectroscopy (IR), proton nuclear magnetic resonance (^{1}H-NMR), carbon nuclear magnetic resonance (^{13}C-NMR), Mass Spectrometry (MS) and elemental analyses. The synthesized compounds were screened for MCF-7 breast cancer cell line and anti-coagulant activities. Anticoagulant activity was determined by Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT) coagulation assays. Compound 3-(2,6-bis (4-methoxyphenyl)-1,3-dimethylpiperidin-4-ylideneamino)-2-thioxoimi dazolidin-4-one 5f (>1000 in APTT assays) was highly response in anticoagulant screening compared with the reference of heparin while the compound 3-{[-1,3-Dimethyl-2,6-di (4'-nitrophenyl) piperidin-4-ylidene]amino}imidazolidine-2,4-dione 3e (LD$_{50}$: 20.4 µg mL^{-1} was highly active against MCF-7 breast cancer cell line compared with the reference. Therefore, the compounds 5f and 3e are novel and beneficial for the development of anticoagulant and anticancer agents.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In recent years, anticoagulant and anticancer drugs development has been one of the most noticeable research areas (Park and Byun, 2016; Shim and Liu, 2014). The numbers of imidazolidine target molecules have been used for various biological application, particularly anticancer activity (Fig. 1).

Thrombosis-related diseases, such as myocardial infarction, deep vein thrombosis and unstable angina are major causes of mortality in the developed world. Current therapies, such as heparin and warfarin are based on indirect inhibition of thrombin. These therapies are limited by side effects, careful monitoring of drug level, slow onset of action and excessive bleeding (Hirsh and Poller, 1994). Therefore, safer and more active anticoagulant agents are required for clinical treatment. The discovered heparin and coumarins are widely used for anticoagulant therapy for more than 50 years (Shafer, 1998; Fan *et al.*, 2011).

These reasons are strongly inspired to find new anticoagulants and antithrombotic to replace the heparin and warfarin from natural sources. Previous study shows that the performance of anticoagulant activity in 1,4-dihydropyridine derivatives against APTT and PT assays (Kumar *et al.*, 2011) and antimicrobial activity of imidazolidine derivatives (Abdul Nasser *et al.*, 2008).

Present investigation is focus the piperidine connected with imidazolidine derivatives, basically piperidine derivatives have various pharmacological activities such as antitubercular (Aridoss *et al.*, 2008), cytotoxicity on HeLa cells (Parthiban *et al.*, 2011), antihyper tensive (Watanuki *et al.*, 2011), antitumor (Girgis, 2009), antimicrobial activities (Vinaya *et al.*, 2009; Peretto *et al.*, 2007; Kus *et al.*, 2009; Huang *et al.*, 2010; Sangshetti and Shinde, 2011), antimalarials (Misra *et al.*, 2009), coronary vasodilatation (Dabaeva *et al.*, 2008) and antiarrhythmic activities (Abdel-Aziz *et al.*, 2009). Imidazolidine-2,4-dione derivatives were found as pharmacological activity such as anxiolytic activity (Czopek *et al.*, 2010), antimalarial (Araujo *et al.*, 2005), piperidinyl connected with alkyl imidazoles for selective histamine H3 receptor agonist (Kitbunnadaj *et al.*, 2005). These references will serve as the main bases for the synthesis of piperidine with imidazolidine derivatives (3a-3h, 4a-4h and 5a-5h) (Fig. 2) and evaluate for anticoagulant and anticancer activities.

MATERIALS AND METHODS

Chemistry: Melting points were recorded in open capillary tubes and are uncorrected. Functional groups were confirmed by IR spectra on a shimadzu 8201pc (4000-400 cm^{-1}) instrument. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-300 MHz instrument. Elementer analyzer model (Varian EL III) instrument was used for analysis of (C, H and N) elements in synthesized compounds. The purity of compounds were confirmed by Thin Layer Chromatography (TLC) with silica gel plates.

Synthesis of 2,6-di-2-furyl-1,3-dimethylpiperidin-4-semicarbazone (2a): A reaction mixture was made up of

EGFR inhibitor (Carmi *et al.*, 2006)

Necroptosis inhibitors (Teng *et al.*, 2005)

Farnesyl tranferase inhibitor (Lee *et al.*, 2006)
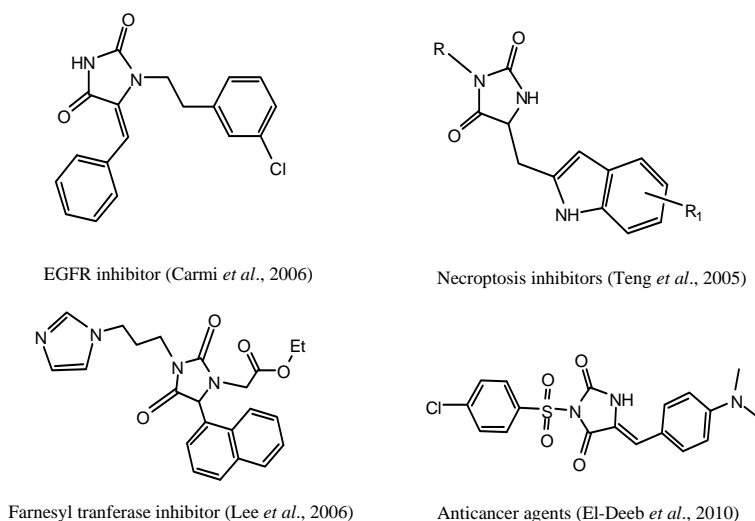
Anticancer agents (El-Deeb *et al.*, 2010)

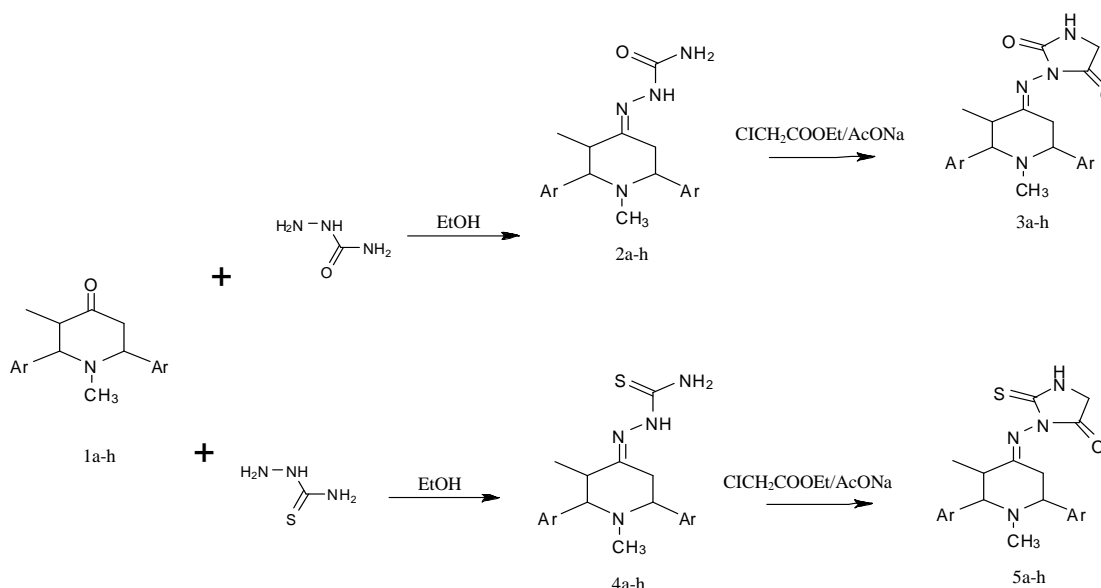Fig. 1: Structure of imidazolidine derivatives with anticancer activity

Fig. 2: Synthetic route of imidazolidine derivatives (3a-h) and (5a-h)

compound 1a (0.1 mol), semicarbazide hydrochloride (0.1 mol) in ethanol, it was refluxed for 5 h. The obtained solid was allowed to cool and then poured into ice-cold water. The precipitate was recrystallized in suitable solvents. Other compounds 2b-2h were synthesised by above mentioned procedure.

IR (KBr, cm^{-1}): 3424 (NH$_2$), 2974 (N-H), 1707 (C=O), 1623 (C=N), 727 (C-N-C); ^{1}H NMR (DMSO-d$_6$), δ (ppm): 11.05 (s, 1H, C=N-NH), 6.73 (s, 2H, NH$_2$), 6.34-6.23 (dd, 3H, furan), 3.74-3.68 (d, 1H, C2-H), 3.66-3.51 (dd, 1H, C6-H, J = 10.20 Hz), 3.41-3.36 (d, 1H, C5-Heq, J = 11.85 Hz), 2.16-2.12 (d, 1H, C5-Hax, J = 12.41 Hz), 2.10-1.92 (m, 1H, C3-H), 1.75 (s, 3H, -NCH$_3$), 0.76 (d, 3H, 3-CH$_3$), elemental analysis (C$_{16}$H$_{20}$N$_4$O$_3$): Calculated C, 60.75, H, 6.37, N, 17.71, found: C, 60.73, H, 6.34, N,17.67%.

2,6-bis(phenyl)-1,3-dimethyl piperidin-4-semicarbazone (2b): IR (KBr, cm^{-1}): 3445 (NH$_2$), 3060 (Ar-H), 2966 (N-H), 1717 (C=O), 1673 (C=N), 806 (ArH), 733 (C-N-C), ^{1}H NMR (DMSO-d$_6$), δ (ppm): 10.98 (s, 1H, C=N-NH), 7.45-7.29 (m, 4H, Ar-H), 6.78 (s, 2H, NH$_2$), 3.52-3.44 (d, 1H, C2-H, J = 10.20 Hz), 3.40-3.36 (dd, 1H, C6-H, J = 13.80 Hz), 3.15-3.09 (d, 1H, C5-Heq, J = 11.85 Hz), 2.81-2.72 (d, 1H, C5-Hax, J = 2.21 Hz), 2.37-2.26 (m, 1H, C3-H), 1.92 (s, 3H, -NCH$_3$), 0.57 (s, 3H, 3-CH$_3$), elemental analysis (C$_{20}$H$_{24}$N$_4$O): Calculated C, 71.40, H, 7.19, N, 16.65, found: C, 71.37, H, 7.16, N, 16.60%.

2,6-bis(4-chloroyphenyl)-1,3-dimethylpiperidin-4-semi carbazone (2c): IR (kBr, cm^{-1}): 3485 (NH$_2$), 3034 (Ar-H), 2947 (N-H), 1723 (C=O), 1656 (C=N), 837 (C-Cl), 802 (Ar-H), 752 (C-N-C), ^{1}H NMR (DMSO-d$_6$), δ (ppm): 10.12 (s, 1H, C=N-NH), 7.40-7.32 (dd, 4H, Ar-Cl), 6.58 (s, 2H, NH$_2$), 3.74-3.60 (d, 1H, C2-H, J = 10.20 Hz), 3.56-3.41 (dd, 1H, C6-H, J = 13.80 Hz), 3.22-3.14 (d, 1H, C5-Heq, J = 11.85 Hz), 2.79-2.87 (d, 1H, C5-Hax, J = 12.15 Hz), 2.30-2.21 (m, 1H, C3-H), 1.88 (s, 3H, -NCH$_3$), 0.62 (s, 3H, 3-CH$_3$), elemental analysis (C$_{20}$H$_{22}$Cl$_2$N$_4$O): Calculated C, 59.27, H, 5.47, N,13.82, found: C 59.18, H, 5.45, N, 13.83.

2,6-bis(4-hydroxyphenyl)-1,3-dimethylpiperidin-4-semicarbazone (2d): IR (kBr, cm^{-1}): 3412 (NH$_2$), 2908 (N-H), 1752 (C=O), 1684 (C=N), 1465 (OH), 804 (Ar-H), 745 (C-N-C), ^{1}H NMR (DMSO-d$_6$), δ (ppm): 10.33 (s, 1H, C=N-NH), 9.41(s, 1H, OH), 7.62-7.31 (dd, 4H, Ar-OH), 6.56 (s, 2H, NH$_2$), 3.61-3.55 (d, 1H, C2-H, J = 10.20 Hz), 3.52-3.47 (dd, 1H, C6-H, J = 13.80 Hz), 3.46-3.40 (d, 1H, C5-Heq, J = 11.85 Hz), 2.74-2.63 (d, 1H, C5-Hax, J = 2.21 Hz), 2.30-2.19 (m, 1H, C3-H), 1.84 (s, 3H, -NCH$_3$), 0.61 (s, 3H, 3-CH$_3$), elemental analysis (C$_{20}$H$_{24}$N$_4$O$_3$): Calculated C, 65.10, H, 6.57, N, 15.21, found: C, 65.12, H, 6.49, N, 15.18%.

2,6-bis(4-nitrophenyl)-1,3-dimethylpiperidin-4-semicarbazone (2e): IR (kBr, cm^{-1}): 3405 (NH$_2$), 3025 (Ar-H), 2912 (N H), 1710 (C=O), 1530 (NO$_2$), 1642 (C=N), 804 (Ar-H), 725 (C-N-C); ^{1}H NMR (DMSO-d$_6$), δ (ppm): 10.85 (s, 1H, C=N-NH), 7.36-7.45 (dd, 4H, Ar-NO$_2$), 6.91 (s, 2H, NH$_2$), 3.62-3.57 (d, 1H, 2-H, J = 10.00 Hz), 3.54-3.41 (dd, 6-H, 1H, J = 13.65 Hz), 3.21-3.16 (d, 1H, C5-Heq, J = 11.88 Hz), 2.76-2.60 (d, 1H,

C5-Hax, J = 12.11Hz), 2.41-2.38 (m, 1H, C3-H), 1.80 (s, 3H, -NCH$_3$), 0.56 (s, 3H, 3-CH$_3$), elemental analysis (C$_{20}$H$_{22}$N$_6$O$_5$): Calculatd C, 56.33, H, 5.20, N, 19.71, found: C, 56.22, H, 5.17, N, 19.72%.

2,6-bis(4-methoxyphenyl)-1,3-dimethylpiperidin-4-semicarbazone (2f): IR (kBr, cm^{-1}): 3442 (NH$_2$), 2914 (N-H), 1787 (C=O), 1603 (C=N), 810 (Ar-H), 797 (C-N-C), ^{1}H NMR (DMSO-d$_6$), δ (ppm): 10.79 (s, 1H, C=N-NH), 7.94-7.78 (dd, 4H, Ar), 6.64 (s, 2H, NH$_2$), 3.80 (s, 3H, OCH$_3$), 3.44-3.38 (d, 1H, C2-H, J = 10.20 Hz), 3.31-3.24 (dd, 1H, C6-H, J = 13.80 Hz), 3.17-3.11 (d, 1H, C5-Heq, J = 11.85 Hz), 2.78-2.66 (d, 1H, C5-Hax, J = 2.21 Hz), 2.33-2.26 (m, 1H, 3-H), 1.90 (s, 3H, -NCH$_3$), 0.68 (s, 3H, 3-CH$_3$), elemental analysis (C$_{22}$H$_{28}$N$_4$O$_3$): Calculated C, 66.64, H, 7.12, N, 14.13, found: C, 66.65 H, 7.11, N, 14.15%.

2,6-bis(4-methylphenyl)-1,3-dimethylpiperidin-4-semicarbazone (2g): IR (kBr, cm^{-1}): 3423 (NH$_2$), 2915 (N-H), 1728 (C=O), 1683 (C=N), 840 (Ar-H), 720 (C-N-C); ^{1}H NMR (DMSO-d$_6$), δ (ppm): 11.03 (s, 1H, C=N-NH), 7.32-7.15 (dd, 4H, Ar-CH$_3$), 6.64 (s, 2H, NH$_2$), 3.61-3.58 (dd, 1H, C6-H, J = 13.80 Hz), 3.56-3.50 (d, 1H, C5-Heq, J = 11.85 Hz), 3.39-3.31 (d, 1H, 2-H, J = 10.20 Hz), 2.98-2.91 (d, 1H, C5-Hax, J = 12.04 Hz), 2.49 (s, 3H, Ph-CH$_3$), 2.34-2.29 (m, 1H, C3-H), 1.84 (s, 3H,-NCH$_3$), 0.61 (s, 3H, 3-CH$_3$), elemental analysis (C$_{22}$H$_{28}$N$_4$O): Calculated C, 72.50, H, 7.74, N, 15.37, found: C, 72.47, H, 7.71, N, 15.31%.

2,6-bis(4-dimethylaminophenyl)-1,3-dimethylpiperidin-4-semi carbazone (2h): IR (kBr, cm^{-1}): 3478 (NH$_2$), 2945 (N-H), 1774 (C=O),1676 (C=N), 807 (Ar-H), 787 (C-N-C); ^{1}H NMR(DMSO-d$_6$), δ (ppm): 11.11 (s, 1H, C=N-NH), 7.45-7.35 (dd, 4H, Ar), 6.10 (s, 2H, NH$_2$), 3.91-3.89 (d, 1H, C2-H, J = 10.18 Hz), 3.65-3.59 (dd, 1H, C6-H, J = 13.78 Hz), 3.11-3.07 (d, 1H, C5-Heq, J = 11.81 Hz), 3.03 (s, 6H, -N(CH$_3$)$_2$), 2.71-2.67(d, 1H, C5-Hax, J = 2.20 Hz), 2.19 (m, 1H, C3-H), 2.05 (s, 3H, -NCH$_3$), 0.81 (s, 3H, 3-CH$_3$), elemental analysis (C$_{24}$H$_{34}$N$_6$O): Calculated C, 68.22, H, 8.11, N, 19.89, found: C, 68.13, H, 8.01, N, 19.85%.

Synthesis of 3-{[2,6-di-2-furyl-1,3-dimethylpiperidin-4-ylidene]amino}imidazolidine-2,4-dione (3a): A reaction mixture was made up of compound 2a (0.1 mol), ethyl chloroacetate (0.1 mol) and fused sodium acetate (0.03 mol) (4.1 g) in ethanol, it was heated and refluxed for 7 h. The reaction mixture was cooled to room temperature and then poured in to ice-cold water. The precipitate was recrystallized in suitable solvents. Other compounds (3b-3h) were synthesised by above mentioned procedure.

IR (kBr, cm^{-1}): 3084 (N-H), 1780 (C=O), 1623 (C=N), 722 (C-N-C); ^{1}H NMR (DMSO-d$_6$), δ (ppm): 11.15 (s, 1H, N-H),

6.34-6.24 (dd, 3H, furyl), 3.90 (s, 2H, CH$_2$N), 3.44 (dd, 1H, C6-H, J = 13.80 Hz), 3.15-3.09 (d, 1H, C5-Heq, J = 11.85 Hz), 2.60-2.51 (m, 1H, C3-H), 2.43-2.39 (d, 1H, C2-H, J = 10.20 Hz), 2.21-2.15 (d, 1H, C5-Hax, J = 12.43 Hz), 1.71 (s, 3H, -NCH$_3$), 0.76-0.71 (d, 3H, 3-CH$_3$); ^{13}C NMR(DMSO-d$_6$), δ (ppm): 161.1 (C=O), 158.1 (C=N), 170.7 (C=O), 110.2, 152.9, 141.8, 109.6 (Furyl ring), 54.5 (C2), 50.2 (C6), 46.9 (CH$_2$N), 36.7 (C3-CH$_3$), 35.8 (N-CH$_3$), 24.1 (C5), 13.3 (C3-CH$_3$); EI-Ms, m/z (Relative intensity percentage): 356.75 (M$^+$, 15%), 328.38 (5%), 196.20 (100%), 183.20 (11%), 155.19 (15%), 141.17 (12%), 113.16, 98.14, elemental analysis (C$_{18}$H$_{20}$N$_4$O$_4$): Calculated C, 60.66, H, 5.66, N, 15.72, found: C, 60.63, H, 5.65, N,15.77%.

3-{[1,3-dimethyl-2,6-diphenylpiperidin-4-ylidene]amino} imid azolidine-2,4-dione (3b): IR (kBr, cm^{-1}): 3031 (Ar-H), 3022 (N-H), 1750 (C=O), 1628 (C=N), 850 (Ar-H), 762 (C-N-C); ^{1}H-NMR (DMSO-d6), δ (ppm): 11.55 (s, 1H, N-H), 7.83-7.40 (m, 5H, Ar-H), 3.68-3.59 (d, 1H, C2-H, J = 10.20 Hz), 3.60 (s, 2H, CH$_2$N), 3.54-3.49 (dd, 1H, C6-H, J = 13.80 Hz), 3.45-3.40 (d, 1H, C5-Heq, J = 11.80 Hz), 2.31 (s, 3H, -NCH$_3$), 2.25-2.21 (m, 1H, C3-H), 2.14-2.10 (d, 1H, C5-Hax, J = 2.19 Hz), 0.77 (d, 3H, 3-CH$_3$); ^{13}C NMR (DMSO-d$_6$), δ (ppm): 172.0 (C=O), 167.9 (C=O), 157.6 (C=N), 142.7, 128.5, 127.9, 127.0, (Ph), 61.8 (C6), 56.5 (C2), 48.7 (CH$_2$N), 40.9 (N-CH$_3$), 26.8 (C5), 36.4 (C3-CH$_3$), 15.7 (C3-CH$_3$), EI-Ms, m/z (Relative intensity percentage): 376.56 (M$^+$, 5%), 348.33 (10%), 250.33 (100%), 237.33 (10%), 161.26 (10%), 85.41 (5%), elemental analysis (C$_{22}$H$_{24}$N$_4$O$_2$): Calculated C, 70.19, H, 6.43, N, 14.88, found: C, 70.10, H,6.35, N, 14.85%.

3-{[-1,3-dimethyl-2,6-di (4'-chlorophenyl)piperidin-4ylidene]amino}imidazolidine-2, 4-dione (3c): IR (kBr, cm^{-1}): 3087 (N-H), 3025 (Ar-H), 1746 (C=O), 1683 (C=N), 860 (Ar-H), 830 (C-Cl), 732 (C-N-C); ^{1}H NMR (DMSO-d$_6$), δ (ppm): 11.21 (s, 1H, N-H), 7.62-7.21 (d, 4H, Ar-Cl), 3.87 (s, 2H, CH$_2$N), 3.61-3.56 (d, 1H, C2-H, J = 10.14 Hz), 3.44-3.39 (d, 1H, C5-Heq, J = 11.27 Hz), 3.35-3.30 (dd, 1H, C6-H, J = 13.59 Hz), 2.29-2.15 (m, 1H, C3-H), 2.11(d, 1H, C5-Hax, J = 12.02 Hz), 0.73 (d, 3H, 3-CH$_3$); ^{13}C NMR (DMSO-d$_6$), δ (ppm): 171.8 (C=O), 166.1 (C=O), 156.2 (C=N), 142.4, 128.4, 128.10 (Ph), 131.8 (C-Cl), 67.6 (C2), 61.5 (C6), 48.1 (CH$_2$N), 41.2 (N-CH$_3$), 40.5 (Ph-N (CH$_3$)$_2$), 36.6 (C3), 23.1 (C5), 36.0 (C3-CH$_3$), 23.1 (C5), 14.1 (C3-CH$_3$); EI-Ms, m/z (Relative intensity percentage): 445.20 (M$^+$, 15%), 376.45 (19%), 348.39 (10%), 250.03 (100%), 237.85 (21%), 161.36 (8%), 161.71 (20%), 85.31 (11%), elemental analysis (C$_{22}$H$_{22}$Cl$_2$N$_4$O$_2$): Calculated C, 59.33, H, 4.98, N, 12.58, found: C, 59.31, H, 4.98, N, 12.51%.

3-{[-1,3-dimethyl-2,6-di(4'-hydroxyphenyl) piperidin 4ylidene]amino}imidazolidine-2,4-dione (3d): IR (kBr, cm^{-1}): 3048 (N-H), 1778 (C=O), 1642 (C=N),1450 (OH), 821 (Ar-H), 718 (C-N-C); ^{1}H NMR (DMSO-d$_6$), δ (ppm): 11.84 (s, 1H, N-H), 9.41 (s, 1H, Ph-OH), 7.84-7.41 (dd, 4H, Ar), 3.79 (s, 2H, CH$_2$N), 3.64-3.59 (d, 1H, C2-H, J = 10.33 Hz), 3.54-3.49 (d, 1H, C6-H, J = 13.18 Hz), 3.43 (d, 1H, C5-Heq, J = 11.89 Hz), 2.33 (s, 3H, -NCH$_3$), 2.34-2.19 (m, 1H, C3-H), 2.11 (d, 1H, C5-Hax, J = 12.17 Hz), 0.66 (d, 3H, 3-CH$_3$); ^{13}C NMR (DMSO-d$_6$), δ (ppm): 172.2 (C=O), 167.1 (C=O), 158.8 (C-OH), 157.8 (C=N), 132.5, 128.2, 116.2, (Ph), 54.0 (C2), 50.8 (C6), 46.3 (CH$_2$N), 40.2 (N-CH$_3$), 34.8 (C3-CH$_3$), 23.7 (C5), 13.5 (C3-CH$_3$), EI-Ms, m/z (Relative intensity percentage): 408.45 (M$^+$, 22%), 380.41 (11%), 348.77 (100%), 190.23, 183.20, 141.17, 113.16, 98.14, elemental analysis (C$_{22}$H$_{24}$N$_4$O$_4$): Calculated C, 64.69, H, 5.92, N, 13.72, found: C, 64.66, H, 5.91, N, 13.74%.

3-{[-1,3-dimethyl-2,6-di(4'-nitrophenyl)piperidin-4-ylidene]amino}imidazolidine-2,4-dione (3e): IR (kBr, cm^{-1}): 3074 (N-H), 3025 (Ar-H), 1710 (C=O), 1683 (C=N), 1530 (NO$_2$), 810 (Ar-H), 742 (C-N-C); ^{1}H-NMR (DMSO-d$_6$), δ (ppm): 11.36 (s, 1H, N-H), 7.61-7.51 (dd, 4H, Ar-NO$_2$), 3.74 (s, 2H, CH$_2$N), 3.66-3.60 (d, 1H, C2-H, J = 10.86 Hz), 3.50-3.48 (dd, 1H, C6-H, J = 13.77 Hz), 3.45-3.39 (d, 1H, C5-Heq, J = 11.68 Hz), 2.41-2.35 (m, 1H, C3-H), 2.24 (s, 3H, -NCH$_3$), 2.12-2.06 (d, 1H, C5-Hax, J = 2.14 Hz), 0.84(d, 3H, 3-CH$_3$); ^{13}C NMR (DMSO-d$_6$), δ (ppm): 174.5 (C=O), 167.1 (C=O), 157.7 (C=N), 146.0, 124.6, 124.0 (Ph), 145.2 (C-NO$_2$), 54.0 (C2), 51.6 (C6), 46.3 (CH$_2$N), 41.2 (N-CH$_3$), 34.0 (C3-CH$_3$), 24.6 (C5), 15.9 (C3-CH$_3$); EI-Ms, m/z (Relative intensity percentage): 466.44 (M$^+$, 5%), 376.45 (10%), 362.42 (19%), 348.39 (100%), 196.20, 183.20, 141.17, 131.16, 98.14, elemental analysis (C$_{22}$H$_{22}$N$_6$O$_6$): Calculated C, 56.65, H, 4.75, N, 18.02, found: C, 56.61, H, 4.76, N, 18.03%.

3-(2,6-bis (4-methoxyphenyl)-1,3-dimethylpiperidin-4-ylideneamino)imidazolidine-2,4-dione (3f): IR (kBr, cm^{-1}): 3082 (N-H), 1739 (C=O), 1673 (C=N), 805 (Ar-H), 725 (C-N-C); ^{1}H-NMR (DMSO-d$_6$), δ (ppm): 11.44 (s, 1H, N-H), 7.88-7.00 (dd, 4H, Ar-OCH$_3$), 3.89 (s, 2H, CH$_2$N), 3.68-3.63 (d, 1H, C2-H, J = 10.28 Hz), 3.76 (s, 3H, -OCH$_3$), 3.54-3.47 (dd, 1H, C6-H, J = 13.78 Hz), 3.49-3.40 (d, 1H, C5-Heq, J = 11.46 Hz), 2.35-2.21 (m, 1H, C3-H), 2.15 (s, 3H, -NCH$_3$), 2.16-2.10 (d, 1H, C5-Hax, J = 2.45 Hz), 0.77 (d, 3H, 3-CH$_3$); ^{13}C-NMR(DMSO-d$_6$), δ (ppm): 174.5 (C=O), 168.2 (C=O), 158.3 (C=N), 157.9 (C-OCH$_3$), 132.2, 127.8, 114.4 (Ph), 55.8 (C-OCH$_3$), 55.1 (C2), 50.1 (C6), 46.9 (CH$_2$N), 41.5 (N-CH$_3$), 34.7 (C3-CH$_3$), 25.0 (C5), 14.5 (C3-CH$_3$); EI-Ms, m/z (Relative intensity percentage): 436.50 (M$^+$, 16%),

422.47 (17%), 408.45 (10%), 396.43 (21%), 353.41 (19%), 325.40 (1%), 265.35 (100%), 250.33, 237.33, 161.24, 85.14, elemental analysis (C$_{24}$H$_{28}$N$_4$O$_4$): Calculated C, 65.97, H, 6.41, N, 12.82, found: C, 65.91, H, 6.35, N, 12.85%.

3-{[-1,3-dimethyl-2,6-di (4'-methylphenyl)piperidin-4-ylidene]amino}imidazolidine-2,4-dione (3g): IR (kBr, cm^{-1}): 3092 (N-H), 1760 (C=O), 1653 (C=N), 820 (Ar-H), 752 (C-N-C); ^{1}H-NMR (DMSO-d$_6$), δ (ppm): 11.41 (s, 1H, N-H), 7.74-7.63 (dd, 4H, Ar-CH$_3$), 3.88 (s, 2H, CH$_2$N), 3.71-3.64 (d, 1H, C2-H, J = 10.86 Hz), 3.47 (d, 1H, C5-Heq, J = 11.82 Hz), 3.42-3.36 (dd, 1H, C6-H, J = 13.66 Hz), 2.34 (s, 3H, -NCH$_3$), 2.32 (s, 3H, Ph-CH$_3$), 2.32-2.30 (m, 1H, C3-H), 2.10-2.04 (d, 1H, C5-Hax, J = 12.42 Hz), 0.91-0.76 (d, 3H, 3-CH$_3$); ^{13}C-NMR(DMSO-d$_6$), δ (ppm): 177.9 (C=O), 167.1 (C=O), 157.6 (C=N), 179.1, 136.5, 126.5, (Ph), 135.7 (C-CH$_3$), 67.8 (C2), 54.2 (C-CH$_3$), 51.2 (C6), 46.2 (CH$_2$N), 40.2 (N-CH$_3$), 35.8 (C3-CH$_3$), 26.2 (C5), 14.8 (C3-CH$_3$); EI-Ms, m/z (Relative intensity percentage): 404.50 (M$^+$, 12%), 376.66 (5%), 348.39 (100%), 335.39 (21%), 293.36 (10%), 265.65, 250.33, 237.33, 161.24, 85.14, elemental analysis (C$_{24}$H$_{28}$N$_4$O$_2$): Calculated C, 71.26, H, 6.98, N, 13.58, found: C, 71.23, H, 6.96, N, 13.88%.

3-(2,6-bis(4-(dimethylamino)phenyl)-1,3-dimethylpiperidin-4-ylideneamino)imidazolid ine-2,4-dione (3h): IR (KBr, cm^{-1}): 3041 (N-H), 1785 (C=O), 1621 (C=N), 809 (Ar-H), 728 (C-N-C); ^{1}H NMR (DMSO-d$_6$), δ (ppm): 11.49 (s, 1H, N-H), 7.88-7.00 (dd, 4H, Ar), 3.89 (s, 2H, CH$_2$N), 3.82 (s, 6H, -N(CH$_3$)$_2$), 3.68-3.61 (d, 1H, C2-H, J = 10.20 Hz), 3.54-3.46 (dd, 1H, C6-H, J = 13.80 Hz), 3.42-3.39 (d, 1H, C5-Heq, J = 11.85 Hz), 2.31 (s, 3H,-NCH$_3$), 2.29-2.21 (m, 1H, C3-H), 2.14-2.05 (d, 1H, C5-Hax, J = 12.29 Hz), 0.77-0.71 (d, 3H, 3-CH$_3$); ^{13}C NMR (DMSO-d$_6$), δ (ppm): 174.5 (C=O), 168.2 (C=O), 158.3 (C=N), 132.2, 130.2, 112.7, 149.4 (Ph), 54.6 (C2), 50.8 (C6), 47.1 (CH$_2$N), 41.2 (N-CH$_3$), 40.5 (Ph-N(CH$_3$)$_2$), 35.2 (C3-CH$_3$), 24.3 (C5), 14.1 (C3-CH$_3$); EI-Ms,m/z (Relative intensity percentage): 462.58 (M$^+$,22%), 434.53 (12%), 391.44 (9%), 348.41 (100%), 250.19, 237.21, 161.25, 85.13, elemental analysis (C$_{26}$H$_{34}$N$_6$O$_2$): Calculated C, 67.51, H, 7.41, N, 18.17, found: C, 67.48, H, 7.39, N, 18.11%.

2-(2,6-di(furan-2-yl)-1,3-dimethylpiperidin-4-ylidene) hydrazinecarbothioamide (4a): A reaction mixture was made up of compound 1a (0.1 mol), thiosemicarbazide (0.1 mol) in ethanol, it was refluxed for 5 h. The obtained solid was allowed to cool and then poured into ice-cold water. The precipitate was collected by filtration. The precipitate was recrystallized in suitable solvents. Other compounds 4b-4h were synthesised by above mentioned procedure.

IR (KBr, cm^{-1}): 3420 (NH$_2$), 2975 (N-H), 1701 (C=O), 1662 (C=S), 1629 (C=N), 718 (C-N-C); ^{1}H NMR (DMSO-d$_6$), δ (ppm): 11.09 (s, 1H, C=N-NH), 6.70 (s, 2H, NH$_2$), 6.34-6.20 (dd, 3H, furan), 3.74-3.61 (d, 1H, C2-H, J = 10.56 Hz), 3.66-3.59 (dd, 1H, C6-H, J = 10.20 Hz), 3.44-3.38 (d, 1H, C5-Heq, J = 11.85 Hz), 2.14-2.03 (m, 1H, C3-H), 2.00-1.97 (d, 1H, C5-Hax, J = 12.10 Hz), 1.75 (s, 3H, -NCH$_3$), 0.71-0.67 (d, 3H, 3-CH$_3$), elemental analysis (C$_{16}$H$_{20}$N$_4$O$_2$S): Calculated C, 57.81, H, 6.06, N, 16.85, S, 9.65, found: C, 57.86, H, 6.08, N, 16.82, S, 9.66%.

2-(1,3-dimethyl-2,6-diphenylpiperidin-4-ylidene) hydrazinecarbothioamide (4b): IR (KBr, cm^{-1}): 3441 (NH$_2$), 3064 (Ar-H), 2967 (N-H), 1718 (C=O), 1670 (C=N), 1652 (C=S), 802 (ArH), 736 (C-N-C); ^{1}H NMR (DMSO-d$_6$), δ (ppm): 10.92 (s, 1H, C=N-NH), 7.47-7.25 (m, 5H, Ar-H), 6.79 (s, 2H, NH$_2$), 3.18 (d, 1H, C5-Heq, J = 11.85 Hz), 3.52 (d, 1H, C2-H, J = 10.27 Hz), 3.45-3.39 (dd, 1H, C6-H, J = 13.66 Hz), 2.37-2.30 (m, 1H, 3-H), 2.88-2.81 (d, 1H, C5-Hax, J = 2.21 Hz), 1.94 (s, 3H, -NCH$_3$), 0.51-0.46 (d, 3H, C3-CH$_3$), elemental analysis (C$_{20}$H$_{24}$N$_4$S): Calculated C, 68.15, H, 6.86, N, 15.89, S, 9.10, found: C, 68.13, H, 6.85, N, 15.87, S, 9.12%.

3-(2,6-di(furan-2-yl)-1,3-dimethylpiperidin-4-ylideneamino) -2-thioxoimidazolidin-4-one (5a): A reaction mixture was made up of compound 4a (0.1 mol), ethyl chloroacetate (0.1 mol) and fused sodium acetate (0.03 mol) in ethanol, it was heated and refluxed for 7 h. The reaction mixture was cooled to room temperature and then poured in to ice-cold water. The precipitate was collected by filtration. The precipitate was recrystallized in suitable solvents. Other compounds 5b-5h were synthesised by above mentioned procedure.

IR (kBr, cm^{-1}): 3081 (N-H), 1786 (C=O), 1669 (C=S), 1623 (C=N), 727 (C-N-C), ^{1}H NMR (DMSO-d$_6$), δ (ppm): 11.16 (s, 1H, N-H), 6.34-6.27 (dd, 3H, furyl), 3.98 (s, 2H, CH$_2$N), 3.40-3.34 (dd, 1H, C6-H, J = 13.80 Hz), 3.11-3.07 (d, 1H, C5-Heq, J = 11.85 Hz), 2.62-2.56 (m, 1H, C3-H), 2.53-2.49 (d, 1H, C2-H, J = 10.20 Hz), 2.20-2.14 (d, 1H, C5-Hax, J = 2.21 Hz), 1.76 (s, 3H, -NCH$_3$), 0.78-0.73 (d, 3H, 3-CH$_3$); ^{13}C NMR (DMSO-d$_6$), δ (ppm): 170.2 (C=O), 181.2 C=S), 158.1 (C=N), 152.9, 141.8, 110.2, 109.6 (Furylring), 54.5 (C2), 50.2 (C6), 46.1 (CH$_2$N), 36.7 (C3-CH$_3$), 35.8 (N-CH$_3$), 13.3 (C3-CH$_3$), 24.1 (C5); EI-Ms; m/z (Relative intensity percentage): 372.40 (M$^+$, 24%), elemental analysis (C$_{18}$H$_{20}$N$_4$O$_3$S): Calculated C, 58.05, H, 5.41, N, 15.04, S, 8.61, found: C,58.07, H, 5.45, N, 15.01, S, 8.60%.

3-(1,3-dimethyl-2,6-diphenylpiperidin-4-ylideneamino)-2-thioxoimidazolidin-4-one (5b): IR (KBr, cm^{-1}): 3041 (N-H),

1785 (C=O), 1656 (C=S), 1621 (C=N), 802 (Ar-H), 724 (C-N-C); ^{1}H NMR (DMSO-d$_6$), δ (ppm): 11.44 (s, 1H, N-H), 7.81-7.03 (m, 4H, Ar-H), 3.82 (s, 2H, CH$_2$N), 3.63-3.59 (d, 1H, C2-H, J = 10.45 Hz), 3.55-3.48 (dd, 1H, C6-H, J = 13.65 Hz), 3.46-3.42 (d, 1H, C5-Heq, J = 11.85 Hz), 2.36 (s, 3H, -NCH$_3$), 2.32-2.21 (m, 1H, C3-H), 2.14-2.10 (d, 1H, C5-Hax, J = 11.75 Hz), 1.85 (s, 6H, -N(CH$_3$)$_2$), 0.72-0.68 (d, 3H, C3-CH$_3$); ^{13}C NMR (DMSO-d$_6$), δ (ppm): 187.8 (C=S), 174.5 (C=O), 157.1 (C=N), 146.1, 133.7, 132.1, 113.3 (Ph), 54.1 (C2), 50.2 (C6), 47.0 (CH$_2$N), 40.9 (Ph-N(CH$_3$)$_2$), 40.1 (N-CH$_3$), 35.3 (C3-CH$_3$), 24.1 (C5), 14.3 (C3-CH$_3$); EI-Ms, m/z (Relative intensity percentage): 392.59 (M$^+$, 21%), elemental analysis (C$_{22}$H$_{24}$N$_4$OS): Calculated C, 67.32, H, 6.16, N, 14.27, S, 8.17, found: C, 67.31, H, 6.18, N, 14.28, S, 8.19%.

Pharmacological activities
Anti-cancer activity
Drug preparation: The compound was dissolved in 10% dimethyl Sulfoxide to give a final concentration of dimethyl sulfoxide not more than 0.5% and did not affect cell survival. Cell viability test: The viability of cells was assessed by MTT assay (Mosmann, 1983) using MCF-7 cell lines.

Cytotoxic assay (MTT method): The cells were plated distinctly in 96 well plates at a concentration of 1×10^5 cells per well. After 24 h, cells were washed twice with 100 μL of serum-free medium and starved for an hour at 37°C. After starvation, cells were treated with the test material for 24 h.

At the end of the treatment period the medium was aspirated and serum free medium containing MTT (0.5 mg mL^{-1}) was added and incubated for 4 h at 37°C in a CO$_2$ incubator.

The MTT containing medium was then discarded and the cells were washed with PBS (200 μL). The crystals were then dissolved by adding 100 μL of DMSO and this was mixed properly by pipetting up and down. Spectrophotometric absorbance of the purple blue formazan dye was measured in a microplate reader at 570 nm (Biorad 80). Cytotoxicity was determined using graphpad prism 5 software.

Anticoagulant activity: The anticoagulant study was carried out according to the method described in previous literature (De Zoysa *et al.*, 2008; Idhayadhulla *et al.*, 2012; Kumar *et al.*, 2011).

RESULTS

Chemistry: The compounds 1a-1h and 2a-2h were synthesized by according to the method shown in the

literature (Sampath *et al.*, 2006; Balasubramanian *et al.*, 2002). Imidazolidin-2,4-dione derivatives 3a-3h were prepared by compounds 2a-2h react with ethyl chloroacetate and fused sodium acetate by cyclization method (Venkateswarlu and Vasireddy, 2005). The compounds 4a-4h and 5a-5h were prepared by according to literature method. Physicochemical data of compounds 2a-2h, 3a-3h, 4a-4h and 5a-5h are given in Table 1.

The IR spectrum of compound 2a shows an bands at 3424, 1707 and 1623 cm^{-1} corresponding to the NH_2, C=N and C=O groups respectively and ^{1}H NMR spectra of compounds 2a shows that the signals observed at δ 11.05 and δ 6.73 corresponding to C=N-NH and NH_2 protons. The IR spectrum of the compound 3a shows an absorption bands at 3084, 1623, 1780 and 722 cm^{-1} corresponding to the NH, C=N, C=O and C-N-C groups respectively and compound 3a showed that the signals observed at δ 11.15 corresponding to NH presented in imidazolidine-2,4-dione ring. The ^{13}C NMR spectra of compound 3a shows that important peak at d158.1 corresponding to C=N and 35.8 corresponding to N-CH$_3$

carbon, respectively. Mass spectrum of the compound 3a shows that molecular ion peak at m/z 376.56 corresponding to total molecular weight of the compound 3a.

The IR spectrum of compound 4a shows an bands at 3420, 1701 and 1662 cm^{-1} corresponding to the NH_2, C=O and C=S groups, respectively and ^{1}H NMR spectra of compounds 4a shows that the signals observed at δ 11.09 and δ 6.70 corresponding to C=N-NH and NH_2 protons. The IR spectrum of the compound 5a shows an absorption bands at 3081, 1669, 1786 and 727 cm^{-1} corresponding to the NH, C=N, C=S and C-N-C groups respectively and compound 5a showed that the signals observed at δ 11.16 corresponding to NH presented in imidazolidine-2,4-dione ring. The ^{13}C NMR spectra of compound 5a shows that important peak at d 158.1 corresponding to C=N and 35.8 corresponding to N-CH$_3$ carbon, respectively. Mass spectrum of the compound 5a shows that molecular ion peak at m/z 372.40 corresponding to total molecular weight of the compound 5a.

Compound 5b is used as an example for discuss the following conformation of other compounds (5c-h). Aromatic proton peaks appear in the region at 7.81-7.03 ppm as multiplets and N-CH$_3$ in piperidine ring resonate at 2.36 ppm as singlet and 2H and 6H positions in piperidine ring appear at 3.68-3.59 and 3.55-3.45 ppm resonate as a doublet which splitting with coupling constant value at J = 10.45 Hz and J = 13.65 Hz. Methyl group at equatorial position of carbon 3 shifts the axial proton of carbon 2 resonance towards up field with a magnitude of around 0.72-0.68 ppm.

The 3 position of carbon at axial protons absorb at 2.32-2.21 ppm, 5 position of axial protons absorb at 2.14-2.10 ppm and 5 position of equatorial protons absorb at 3.46-3.42 ppm, respectively.

The ^{13}C NMR spectra of compound 5b values are used as example to discuss the conformations of the C=N. Aromatic carbon of 5b is distinguished from other carbons by their characteristic absorption in the region of 113.3-146.7 ppm.

The carbons of imino groups (C=N) absorb in a downfield of 157.8 ppm, respectively, due to electronegativity of nitrogen and anisotropic effect. Thus the peak appearing in the downfield of the signals at 54.1 ppm is for carbon 2 and the upfield region at 50.2 ppm for carbon 6 position.

Deshielding magnitudes of carbon 3 position in piperdine ring at 35.3 ppm and carbon 2 position in piperdine ring at 54.1 ppm. The methyl carbons of carbon 3 resonate at unusual downfield region 14.3 ppm. Compound 5b adopted a normal chair conformation due to carbon 5 should be at around 24.1 ppm. All these interpretations propose the 5a-5h compounds adopts a normal chair conformation.

Table 1: Characterization data of compounds 2a-2h, 3a-3h, 4a-4h and 5a-h

Compounds	Ar	MW	Yield (%)	MP (°C)
2a	Furyl-	316.32	72	182
2b	4-H-Ph-	336.43	63	174
2c	4-Cl-Ph-	405.32	72	163
2d	4-HO-Ph-	368.48	86	170
2e	4-O$_2$N-Ph-	426.42	89	168
2f	4-CH$_3$O-Ph-	396.48	79	179
2g	4-CH$_3$-Ph-	364.48	71	175
2h	4-(CH$_3$)$_2$N-Ph-	422.56	60	160
3a	Furyl-	357.37	86	164
3b	4-H-Ph-	376.45	70	160
3c	4-Cl-Ph-	445.34	77	195
3d	4-HO-Ph-	408.45	73	156
3e	4-O$_2$N-Ph-	466.44	65	162
3f	4-CH$_3$O-Ph-	436.50	75	180
3g	4-CH$_3$-Ph-	404.50	88	162
3h	4-(CH$_3$)$_2$N-Ph	462.58	72	172
4a	Furyl-	332.13	62	198
4b	4-H- Ph-	352.17	73	187
4c	4-Cl-Ph-	421.39	62	195
4d	4-HO-Ph-	384.16	66	151
4e	4-O$_2$N-Ph-	442.14	79	165
4f	4-CH$_3$O-Ph-	412.55	69	182
4g	4-CH$_3$-Ph-	380.20	51	164
4h	4-(CH$_3$)$_2$N-Ph	438.63	56	176
5a	Furyl-	372.44	66	204
5b	4-H-Ph-	392.52	60	224
5c	4-Cl-Ph-	461.41	67	238
5d	4-HO-Ph-	424.52	83	243
5e	4-O$_2$N-Ph-	482.14	85	220
5f	4-CH$_3$O-Ph-	452.57	75	216
5g	4-CH$_3$-Ph-	420.57	88	208
5h	4-(CH$_3$)$_2$N-Ph	478.65	82	242

Ar: Aromatic compounds, MW: Molecular weight and MP: Melting point

Pharmacological screening

Anticoagulant activity: All of the synthesized compounds (2a-h), (3a-h), (4a-h) and (5a-h) were screened for their anticoagulant activity and the data were compared with heparin and warfarin. The anticoagulant evaluations data of the synthesised compounds were represented in Table 2. The *in vitro* anti-coagulant activity of APTT and PT in human plasma was examined. Various concentrations were required to achieve relative clotting times of compounds in human plasma *in vitro*. All imidazolidine derivatives (2a-h, 3a-3h, 4a-4h and 5a-5h) showed higher APPT and PT values than the vehicle control (6.65s). Among the synthesized compounds 3f and 5f was highly active against APTT and PT assays in anticoagulant screening. The APTT coagulation assay was performed at 60 mg mL^{-1} concentration.

Table 2: Anti-coagulant activity of compounds 2a-2h, 3a-3h, 4a-4h and 5a-5h with standard heparin

Compounds No.	Concentration (60 mg mL^{-1})			
	Clotting time (sec) (APTT)	APTT index	Clotting time (sec) (PT)	PT index
2a	24.4	0.67	16.5	0.83
2b	16.8	0.46	17.7	0.89
2c	59.6	1.63	41.4	2.09
2d	62.7	1.72	63.2	3.19
2e	52.4	1.43	46.8	2.36
2f	104.0	2.85	108.5	5.47
2g	34.7	0.95	28.8	1.45
2h	49.9	1.37	42.7	2.15
3a	32.4	0.89	24.3	1.22
3b	18.8	0.51	17.6	0.88
3c	63.6	1.74	51.5	2.60
3d	72.7	1.99	76.6	2.10
3e	66.9	1.83	53.8	2.71
3f	456.3	12.53	157.5	7.95
3g	32.7	0.89	27.2	1.37
3h	61.8	1.69	53.4	2.69
4a	33.4	0.91	27.5	1.38
4b	20.8	0.57	22.7	1.14
4c	66.6	1.82	52.4	2.64
4d	73.7	2.02	74.2	3.74
4e	65.4	1.79	57.8	2.91
4f	137.0	3.76	130.5	6.59
4g	45.7	1.25	39.8	2.01
4h	60.9	1.67	54.7	2.76
5a	40.4	1.10	36.3	1.83
5b	24.8	0.68	31.6	1.59
5c	76.6	2.10	65.5	3.30
5d	86.7	2.38	85.6	4.32
5e	76.9	2.11	66.8	3.37
5f	>1,000	27.47	922.5	46.50
5g	52.7	1.44	47.5	2.39
5h	79.8	2.19	63.4	3.20
Heparin	>1,000[a]	27.47	-	-
Warfarin	157.9	4.33	654.9	33.0
Control	36.4	1.0	19.8	1.0

ATTT: Activated partial thromboplastin time and PT: Prothrombin time

Compounds 2a-2h were low active compared to 3a-3h and compounds 4a-4h was low active compared with 5a-h against APTT and PT assays. Particularly, compound 2f shows low active (104.0s) in APTT assays compared with 3f (456.3s) and 2f has 108.5s low active in PT assays compared with 3f (157.5s) due to the presence of imidazolidine ring in compound 3f. Compound 2d (62.7s) and compound 3d (72.7s) was moderate active in APTT assays. Compound 2f was low active compared to with compound 4f (137.0s) in APTT assays and 2f was low active (108.5s) compared with 4f (130.5s) in PT assays. Compound 4f (137.0s) was low active compared with 5f (>1,000) in APTT assays and compound 4f (130.5 s) was low active compared to compound 5f (922.5s) in PT assays. The compounds 2d (62.7s), 3d (72.7s), 4d (73.7s) and 5d (86.7s) were low active in APTT assays were as compounds 2d (63.2s), 3d (76.6s), 4d (74.2s), 5d (85.6) were high active in PT assays.

Anticancer activity: All the synthesized compounds 2a-2h, 3a-3h, 4a-4h and 5a-5h were evaluated for their anticancer activity against a human cancer cell line such as MCF-7 (Human breast cancer) by employing MTT assay (Botta *et al.*, 2007). The results of cell viability data are summarized in Table 3.

The inhibitory concentration (IC$_{50}$) values, which are the drug concentration at which 50% of cells viable and calculated from the logarithmic trend line of the cytotoxicity graphs.

The *in vitro* screening results revealed that the compound 3e exhibited promising anticancer activity compared in MCF7 breast cancer cells (87.8% inhibition) were observed at concentration (100 µg mL^{-1}). This inhibition at the mentioned concentration indicates a greater potency of compound 3e with a strong lethal effect over the breast cancer (MCF-7) cell line.

Compounds 2a, 2b, 3a, 3b, 4a, 4b, 5a and 5b have low inhibitory activity against MCF7 cell line, whereas, compound 3e (NO$_2$) has (87.8 at 100 µg mL^{-1} and LD$_{50}$ range 20.4 µg mL^{-1}) highly active compared with compound 2e (NO$_2$) (53.3% at 100 µg mL^{-1} and LD$_{50}$ 89.2 µg mL^{-1}). The compound 4e (NO$_2$) has (48.8% inhibition at 100 µg mL^{-1} and LD$_{50}$ range at >100 µg mL^{-1}) highly active compared with compound 5e (NO$_2$) (77.8% inhibition at 100 µg mL^{-1} and LD$_{50}$ range at 27.6 µg mL^{-1}). Other remaining compounds have moderate activity such as compound 3d containing OH group (LD$_{50}$: 29.7 µg mL^{-1}), compound 5c containing Cl group (LD$_{50}$: 29.5 µg mL^{-1}), compound 5d containing OH group (LD$_{50}$: 26.2 µg mL^{-1}) and compound 5f containing CH$_3$O group (LD$_{50}$: 33.8 µg mL^{-1}), respectively.

Table 3: Anticancer activity of 2a-2h,3a-3h,4a-4h and 5a-5h

Compounds No.	Percentage inhibition of cell viability (µg mL^{-1})			LD$_{50}$ µg mL^{-1}
	25	50	100	
2a	-	-	10.6	>100
2b	-	-	17.9	>100
2c	22.4	37.9	42.4	>100
2d	27.9	39.9	48.7	>100
2e	28.9	42.4	53.3	89.2
2f	29.2	39.8	47.2	>100
2g	-	-	23.3	>100
2h	-	-	18.0	>100
3a	-	18.9	38.3	>100
3b	-	46.2	59.9	>100
3c	31.6	44.0	58.5	61.5
3d	42.6	59.9	82.6	29.7
3e	57.9	69.5	87.8	20.4
3f	50.4	56.6	70.2	24.9
3g	-	53.9	67.5	>100
3h	-	51.6	63.4	>100
4a	-	-	05.6	>100
4b	-	-	10.9	>100
4c	18.2	25.6	38.6	>100
4d	16.6	33.8	46.4	>100
4e	27.9	39.0	48.8	>100
4f	15.2	26.5	32.2	>100
4g	-	-	17.5	>100
4h	-	-	13.4	>100
5a	-	58.8	67.3	>100
5b	-	56.9	60.9	>100
5c	43.6	56.0	68.5	29.5
5d	48.6	64.7	72.6	26.2
5e	46.9	64.6	77.8	27.6
5f	38.6	52.1	61.2	33.8
5g	-	67.0	77.5	>100
5h	-	58.5	63.4	>100
Standard	52.2	65.8	84.2	24.2
Control	0	0	0	0

Highly significant index, [a]Clotting time >1,000, considered as to calculate the relative clotting factor, 1,3-bis (4-chlorobenzenesulfonyl) imidazolidine-2-one used as a standard. Standard data was taken from Abdel-Aziz *et al.* (2012), percentages of growth inhibition 84.2% at concentration 100 µg mL^{-1}

DISCUSSION

The *in vitro* anticoagulant activity was screened for piperidin connected imidazolidine derivatives. The heparin is caused by its complexing with antithrombin 111, which accelerates the formation of a stable 1:1 complex between antithrombin 111 and thrombin (Harpel and Rosenberg, 1976). Sulphur derivatives shows high anticoagulant activities with respect to activated partial thromboplastin time. These studies was propose that the synthetic sulphur containing derivatives work as anticoagulants in a mechanism same from that of heparin.

As a result, it was confirmed that the synthetic imidazolindine shows anticoagulant activity *in vitro* due to sulphur group of 2-thioxoimidazolidin-4-one series (5a-5h)

because an increase in anticoagulant activity in APTT and PT assays compared with compounds 4a-h. Compound 5f containing OCH$_3$ group with 2-thio-imidazolidin-4-one shows highly active among the synthesized compounds.

Anticancer assays were performed according to the US NCI protocol, which was described elsewhere (Monks *et al.*, 1991; Boyd and Paull, 1995; Boyd, 1997; Shoemaker, 2006).

The compounds were first evaluated at one dose primary anticancer assay towards MCF-7 cell lines (human breast cancer). Results for each test agent were reported as the percent growth of the treated cells when compared to the untreated control cells.

The induction of thiosemicarbazide interconversion by imidazolidin analogue has been suggested to be involved in the growth inhibitory effects of those compounds in MCF-7 cancer cell line (Davidson *et al.*, 1999; Kaminskyy *et al.*, 2009).

Recently, imidazolidin and related heterocycles were shown to be perspective as potential anticancer drug candidates (Rajic *et al.*, 2006; Alanazi *et al.*, 2013). The compounds (3a-3h) and (5a-5h) were active towards MCF-7 cell line and therefore screening towards about human cancers at five different concentrations (25-100 µg mL^{-1}). Significant effect of 3e and 5e on MCF-7 cancer cell lines was observed as well. It is interesting, that compounds low inhibitory effect on breast cancer cell lines, particularly 2e and 4e.

Thus compounds 4a-4h, 5a-h were characterized by strong and significant effect on MCF-7 cell lines, whereas compounds 4e and 5e showed distinctive selectivity and inhibit the growth of other cell lines.

Imidazolidine-2,4-dione was more biologically favored than 2-thioxoimidazolidin groups. However, without halogen group showed no activity towards MCF7 cell line.

This could be due to the sulphur bond connectivity of imidazolidine derivatives. The previous results are in concomitant with many trials for inhibiting cancer cells, recently the terminally acetylated polyamine analogue is most extensively studied and the most promising compounds are currently under investigation in clinical trials as anticancer agents (Casero and Woster, 2001).

It has recently shows that the growth and the inhibitory activity of some of these compounds might be mediated by stimulation of polyamine catabolism and polyamine oxidase (Acetylspermine) activity in particular (Wang *et al.*, 2001; Soror *et al.*, 2015).

Furthermore, great variations between 3e and 5e derivatives were observed in biological activity against MCF-7.

Hydantoin-carboxylic acids derivatives shows that imidazole moiety can inhibit Ras farnesyl transferase (Lee *et al.*, 2006). Ras protein plays an vital role in cell growth and needs a series of post-translational alterations including the farnesylation catalyzed by farnesyl transferase (Ftase), which is considered as potential anticancer agents (Mazieres *et al.*, 2004). Investigation on cell morphology was developed response to spermine analogue treatment in order to find out another evidence of inhibit Ras farnesyl transferase.

The structural activity relationship was demonstrated the following assumptions about the synthesised compounds.

In the present study, piperidine containing imidazolidine derivatives were synthesized and examine the anticancer and anticoagulant activities. It is interesting to point out that the imidazolidin moiety showed significant activity against both the anticancer and anticoagulant activities. The 4-substituted phenyl ring performances as a lipophilic domain, NH presenting in imidazolidin ring act as hydrogen bonding domain. Therefore, imidazolidin ring may be stated that vital pharmacophoric requirements for anticoagulant and anticancer activities.

The imidazolidine-2,4-dione and 2-thioxoimidazolidin-4-one rings was evaluated for biological activity and discuss the structure-activity relationships of changing the substitution pattern around that compounds.

Warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin] was performances low active than heparin against APTT assays where as warfarin has highly active than heparin against PT assays. Warfarin acts as an inhibitor of vitamin K epoxide reductase, preventing generation of the reduced form of vitamin K, which is a necessary cofactor for the hepatic synthesis (Zhou and Chan, 2003). Like warfarin, the 2-thioxoimidazolidin-4-one is competitive inhibitors of vitamin K in the biosynthesis of prothrombin and follow the same biochemical mechanism of action, it has been shown that the para substitution atoms pose a conformational restriction on the molecule such that the minimum energy is found for a non-perpendicular structure, which enables the imidazolidine moiety to be conjugated with the phenyl moiety (Meerman-Van Benthem *et al.*, 1975).

The compounds 2f, 3f, 4f and 5f containing para position CH$_3$O-atoms of phenyl ring shows that less potent inhibitors against APTT assays (clotting time values 104.0s, 456.3s, 137.6s and >1000s) and PT assays (clotting time values 108.5s, 157.5s, 130.5s and 922.5s).

The compound (5f) piperdine connected with 2-thioxo-imidazolidin-4-one moieties was responsible for a decreased potency with Clotting time (sec) values >1000s (APTT Index 27.47) and 922.5s (PT Index 46.50), compared with heparin and warfarin at concentration (60 mg mL^{-1}).

However, the 2-thioxoimidazolidin-4-one derivatives observed much longer activity compared with limidazolidine-2,4-dione against APTT and PT assays (Fig. 3).

This emphasizes that the hydrophobic and lipophilic domains in the molecules are responsible for the potent anticoagulant activity. In addition, the effect of electron donating groups on the substituted benzene with piperidine moiety.

The SAR study revealed that anticancer activity of compounds (2e, 3e, 4e and 5e) was significantly increase the activity imidazolidin-2,4-dione containing compounds (Fig. 4). These data are expressive with the high grade of possibility that the substitution of NO$_2$ group attached with piperidine derivatives leads to increase of anticancer activity. However,
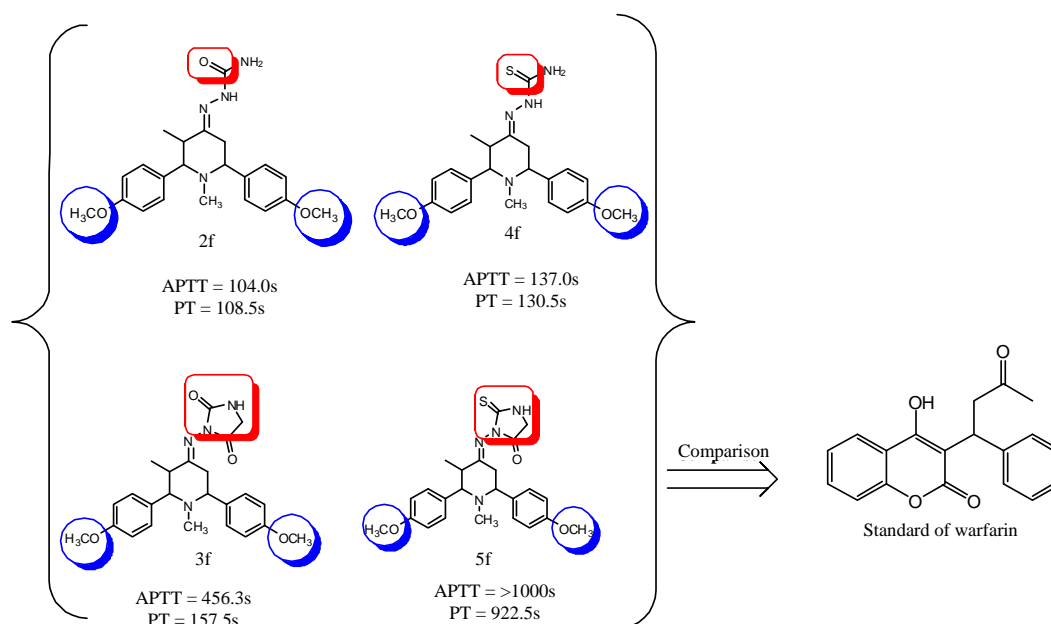
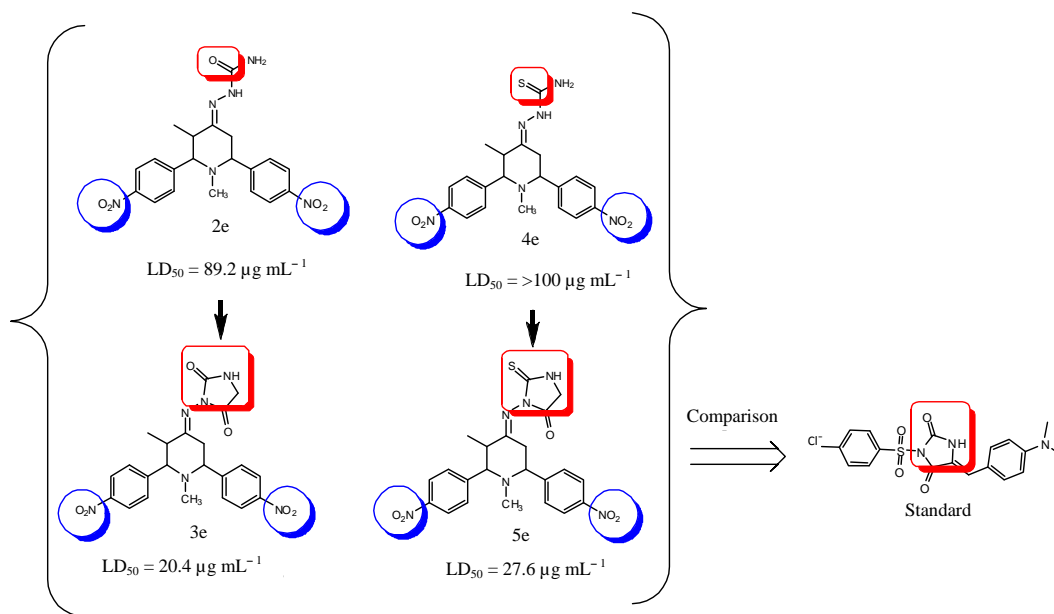Fig. 3: Comparison of anticoagulant activity of compounds 2f, 3f, 4f and 5f

Fig. 4: Comparison of anticancer activity of compounds 2e, 3e, 4e and 5e

the above-mentioned substitution was proposed as the possible route to modeling the substances for anticancer activity.

Recently, 1,5-disubstituted imidazolidine-2,4-diones shows that notable antitumor action and the inhibition of EGFR kinase activity (Zuliani *et al.*, 2009; Carmi *et al.*, 2006).

The compound 3e shows Ar-NO$_2$ group attached with imidazolidin-2,4-dione support the crucial role in anticancer activity compared with other compounds.

Imidazolidine-2,4-dione moiety 3e has highly active against anticancer activity compared with 2-thioxoimidazolidin-4-one 5e.

CONCLUSION

The objective of the study was synthesized a series of 2-thioxo-imidazolidin-4-one, imidazolidine-2,4-dione derivatives and screened for anticancer and anticoagulant activities. Compounds 3f and 5f was highly active compared with heparin in APTT assay and where as anticancer activity of the compounds 3e have highly active against MCF-7 breast cancer cell line.

These activities were comparable with the standards (Heparin, warfarin and 1,3-bis (4-chlorobenzenesulfonyl) imidazolidine-2-one) which are used for standard reference compounds for anticoagulant and MCF-7 cancer cell line. However, more studies must be undertaken to elucidate the exact mechanism of action and to evaluate its immunogenicity.

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