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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, molecular docking, and cardioprotective activity of 2-methylthio-1,4-dihydropyrimidines

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Abstract A series of 2-methylthio-1,4-dihydropyrimidine derivatives (**IIa–III**) were synthesized in good yields by alkylation of 1,2,3,4-tetrahydropyrimidines (**Ia–Il**) with methyl iodide in the presence of pyridine. Their structures were confirmed by elemental analysis, IR, and 1H NMR spectra. Molecular docking of title compounds was done using VLife MDS 3.5 on voltage-dependent calcium channel β subunit functional core and its complex with the α1 interaction domain i.e. AID-β complex (PDB code 1T3L) to identify potential candidates with minimum dock score for cardioprotective activity. Biological screening of the potential candidates (**IIf** and **IIi**) was done for cardioprotective activity. Adult Sprague–dawley rats were pretreated with test compounds **IIf** and **IIi**. After the treatment period, adrenaline was subcutaneously injected to rats at an interval of 24 h for 2 days to induce myocardial injury. After 48 h, rats were anaesthetized and electrocardiographic (ECG) observations were performed. Potential compounds **IIf** and **IIi** showed significant cardioprotective activity against adrenaline-induced myocardial infarction in rats. Adrenaline-induced ECG alterations such as reduced R–R interval, increased heart rate, reduced P duration, and ST-segment elevation were brought to the near normal values by pretreatment of compounds **IIf** and **IIi**.

Keywords 2-Methylthio-1 · 4-Dihydropyrimidine · Biginelli reaction · Synthesis · Molecular docking · Cardioprotective activity

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Introduction

The term cardioprotection refers to the techniques used to prevent or delay the development of myocardial injury, particularly during ischemia. Ischemia and reperfusion produce profound effects on the function of molecules involved in the control of calcium homeostasis, leading to increased free cystolic Ca2+ concentration. Calcium overload is one of the most crucial alterations responsible for ischemia and reperfusion injury. Calcium overload can trigger several injurious mechanisms. Many ATP-consuming enzymes require Ca2+ for activity, so that calcium overload increases ATP consumption and exacerbates the unbalance between energy supply and energy demand, which is the metabolic hallmark of ischemia (Zucchi *et al.*, 2001).

From a quantitative point of view, under physiological conditions, free calcium concentration is on the order of 100 nm during diastole, and it increases up to 0.5–1.5 μm during systole. After a few minutes of ischemia, time-averaged calcium concentrations in the range of 1–10 μm have been reported. With the onset of reperfusion, calcium concentration may show either further massive increase, which is invariably associated with cell death (Zucchi *et al.*, 2001). Literature survey showed that calcium channel blockers are cardioprotective, for e.g., nicorandil, amlodipine, labedipinedilol A, and diltiazem (Sathish *et al.*, 2003; Liang *et al.*, 2006; Malhotra *et al.*, 1997).

The computational process of searching for a ligand that is able to fit both geometrically and energetically into the binding site of a protein is called molecular docking. Molecular docking is an efficient tool for investigating receptor-ligand interactions and for virtual screening, which plays a key role in rational drug design, especially when the crystal structure of a receptor or enzyme is available (Teodoro *et al.*, 2001).

In recent years, acid-catalyzed cyclocondensation of acetoacetate with aldehydes and (thio)ureas, known as the Biginelli reaction, has attracted significant attention (Mohammad and Zahra, 2009; Ezzat and Hadi, 2006; Kotharkar *et al.*, 2006; Ahmad and Abbas, 2005; Shinde *et al.*, 2008; Arfan *et al.*, 2007; Chen-Jiang and Ji-De, 2009; Yang *et al.*, 2007; Fang *et al.*, 2007). The resulting dihydropyrimidines (DHPMs) have been reported to have antibacterial (Chitra *et al.*, 2010), antiviral (Kappe, 2000), anti-inflammatory (Mohammad *et al.*, 2008), analgesic (Chikhale *et al.*, 2009), antihypertensive as well as calcium channel blocker (Inci *et al.*, 2006; Rovnyak *et al.*, 1992), and antioxidant (Ismaili *et al.*, 2008) activities. Recently, structurally simple DHPM derivative monastrol has emerged as a mitotic kinesin Eg5 motor protein inhibitor for the development of anticancer drugs (Bose *et al.*, 2005). Furthermore, the biological activity of several recently isolated marine alkaloids has also been attributed to the dihydropyrimidinone moiety in the structure. Among them the batzelladine alkaloids A and B which inhibit the binding of HIV envelope protein gp-120 to human CD4 cells are potential compounds in AIDS therapy (Snider and Chen, 1998).

In the present work, we propose to synthesize a series of 2-methylthio-1,4-dihydropyrimidines by alkylation of 1,2,3,4-tetrahydropyrimidines, confirm their structures by spectral analysis, molecular docking studies of the title compounds to ascertain their calcium channel blocking activity and will try to correlate the same with cardioprotective activity.

Experimental section

Melting points were determined in open capillaries and are uncorrected. All compounds were characterized by elemental analysis, IR, and 1H NMR spectra. The IR spectra were recorded on a JASCO FT-IR 4100 spectrometer, using KBr discs. The 1H NMR spectra were obtained on a Varian-NMR-mercury 300 spectrometer in DMSO-d6 as solvent and TMS as internal standard, chemical shifts are given in ppm.

General procedure for the synthesis of compounds (IIa–IIl)

A mixture of appropriate aldehyde (0.02 mol), acetoacetate (0.02 mol), thiourea (0.03 mol), catalyst aluminum chloride (0.01 mol) in methanol (10 ml), and concentrated hydrochloric acid (2 drops) was placed in round bottom flask. The mixture was stirred well and then refluxed. The completion of reaction was monitored by thin layer chromatography. After cooling, precipitate was formed which was filtered and washed with cold methanol (I).

Compound I (0.01 mol), methyl iodide (0.011 mol) in methanol (20 mL) was placed in round bottom flask and refluxed for 2 h. Pyridine (0.037 mol) was then added and refluxed again for 10 min. After cooling, the reaction mixture was poured onto crushed ice (approx. 200 g) and stirred for 5 min. Compound **II** obtained was filtered.

Ethyl 6-methyl-2-(methylthio)-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (**IIa**)

Yield: 82.10%. mp 160–162°C. IR (KBr), ν, cm^{-1}: 3328.53 (NH), 2974 (Ar C–H), 1665.23 (C=O), 1576.52 (C=N). ^1H NMR (300 MHz, DMSO, TMS):δ 9.18 (s, 1H, NH), 7.75–7.20 (m, 5H, C_6H_5), 5.988 (S, 1H, CH), 3.98 (q, 2H, OCH_2CH_3), 2.35 (s, 1H, S-CH_3), 2.193 (s, 3H, CH_3), 1.11 (t, 3H, OCH_2CH_3).

Methyl 6-methyl-2-(methylthio)-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (**IIb**)

Yield: 67.51%. mp 100–102°C. IR (KBr), ν, cm^{-1}: 3328.53 (NH), 2948.63 (Ar C–H), 1712.48 (C=O), 1641.13 (C=N). ^1H NMR (300 MHz, DMSO, TMS): δ 9.876 (s, 1H, NH), 7.751–7.201 (m, 5H, C_6H_5), 5.988 (s, 1H, CH), 3.71 (s, 3H, OCH_3), 2.351 (s, 1H, S-CH_3), 2.193 (s, 3H, CH_3).

Ethyl 4-(4-methoxyphenyl)-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**IIc**)

Yield: 78.94%. mp 116–117°C. IR (KBr), ν, cm^{-1}: 3304.43 (NH), 2961.16 (Ar C–H), 1652.7 (C=0), 1267.97 (C–O–C). ^1H NMR (300 MHz, DMSO, TMS): δ 9.876 (s, 1H, NH), 7.68–7.069 (m, 4H, C_6H_4), 5.988 (s, 1H, CH), 3.98 (q, 2H, OCH_2CH_3), 3.605 (s, 3H, OCH_3), 2.351 (s, 3H, S-CH_3), 2.193 (s, 3H, CH_3), 1.11 (t, 3H, OCH_2CH_3).

Methyl 4-(4-methoxyphenyl)-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**IId**)

Yield: 73.68%. mp 146–147°C. IR (KBr), ν, cm^{-1}: 3316.96 (NH), 2940.91 (Ar C–H), 1665.23 (C=O), 1244.83 (C–O–C). ^1H NMR (300 MHz, DMSO, TMS): δ 9.87 (s, 1H, NH), 7.68–7.069 (m, 4H, C_6H_4), 5.988 (s, 1H, CH), 3.708 (s, 3H, OCH_3), 3.605 (s, 3H, OCH_3), 2.351 (s, 3H, S-CH_3), 2.193 (s, 3H, CH_3).

Ethyl 4-(4-chlorophenyl)-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**IIe**)

Yield: 92.98%. mp 138–139°C. IR (KBr), ν, cm^{-1}: 3326.61 (NH), 2982.37 (Ar C–H), 1671.02 (C=O), 1569.77 (C=N),

748.245 (C–Cl). ^{1}H NMR (300 MHz, DMSO, TMS): δ 9.876 (s, 1H, NH), 7.778–7.396 (m, 4H, C_6H_4), 5.988 (s, 1H, CH), 3.98 (q, 2H, OCH_2CH_3), 2.351 (s, 3H, S-CH_3), 2.193 (s, 3H, CH_3), 1.11 (t, 3H, OCH_2CH_3).

Methyl 4-(4-chlorophenyl)-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**IIf**)

Yield: 57.89%. mp 130–132°C. IR (KBr), ν, cm^{-1}: 3310.21 (NH), 2945.73 (Ar C–H), 1665.23 (C=O), 1475.28 (C=N), 777.172 (C–Cl). ^{1}H NMR (300 MHz, DMSO, TMS): δ 9.876 (s, 1H, NH), 7.778–7.396 (m, 4H, C_6H_4), 5.988 (s, 1H, CH), 3.708 (s, 3H, OCH_3), 2.351 (s, 3H, S-CH_3), 2.193 (s, 3H, CH_3).

Ethyl 4-[4-(dimethylamino)phenyl]-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**IIg**)

Yield: 62.93%. mp 124–126°C. IR (KBr), ν, cm^{-1}: 3319.86 (NH), 2975.62 (Ar C–H), 1653.66 (C=O), 1515.78 (C=N). ^{1}H NMR (300 MHz, DMSO, TMS): δ 9.876 (s, 1H, NH), 7.532–6.577 (m, 4H, C_6H_4), 5.988 (s, 1H, CH), 3.98 (q, 2H, OCH_2CH_3), 2.831 (s, 6H, $N(CH_3)_2$), 2.351 (s, 3H, S-CH_3), 2.193 (s, 3H, CH_3), 1.11 (t, 3H, OCH_2CH_3).

Methyl 4-[4-(dimethylamino)phenyl]-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**IIh**)

Yield: 75.75%. mp 214–216°C. IR (KBr), ν, cm^{-1}: 3319.86 (NH), 2809.78 (Ar C–H), 1671.02 (C=O), 1557.24 (C=N). ^{1}H NMR (300 MHz, DMSO, TMS): δ 9.876 (s, 1H, NH), 7.532–6.577 (m, 4H, C_6H_4), 5.988 (s, 1H, CH), 3.708 (s, 3H, OCH_3), 2.831 (s, 6H, $N(CH_3)_2$), 2.351 (s, 3H, S-CH_3), 2.193 (s, 3H, CH_3).

Ethyl 6-methyl-2-(methylthio)-4-(4-nitrophenyl)-1,4-dihydropyrimidine-5-carboxylate (**IIi**)

Yield: 83.68%. mp 180–182°C. IR (KBr), ν, cm^{-1}: 3227.29 (NH), 2980.45 (Ar C–H), 1694.16 (C=O), 1522.52 (Asy Ar-NO_2), 1345.11 (Sym Ar-NO_2), 847.561 (C–N). ^{1}H NMR (300 MHz, DMSO, TMS): δ 9.876 (s, 1H, NH), 7.89–8.10 (m, 4H, C_6H_4), 5.988 (s, 1H, CH), 3.98 (q, 2H, OCH_2CH_3), 2.351 (s, 3H, S-CH_3), 2.193 (s, 3H, CH_3), 1.11 (t, 3H, OCH_2CH_3).

Ethyl 6-methyl-2-(methylthio)-4-(3,4,5-trimethoxyphenyl)-1,4-dihydropyrimidine-5-carboxylate (**IIj**)

Yield: 75.14%. mp 206–208°C. IR (KBr), ν, cm^{-1}: 3298.64 (NH), 2980.45 (Ar C–H), 1659.45 (C=O), 1576.52 (C=N),1185.04, 1132.01 (C–O–C). ^{1}H NMR (300 MHz, DMSO, TMS): δ 9.87 (s, 1H, NH), 5.874 (s, 1H, CH), 3.98 (q, 2H, OCH_2CH_3), 3.83 (s, 9H, OCH_3), 2.351 (s, 3H, S-CH_3), 2.193 (s, 3H, CH_3), 1.11 (t, 3H, OCH_2CH_3).

Ethyl 6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**IIk**)

Yield: 65.47%. mp 210–211°C. IR (KBr), ν, cm^{-1}: 3204.15 (NH), 2992.45 (Ar C–H), 1659.45 (C=O), 1505.17 (C=N). ^{1}H NMR (300 MHz, DMSO, TMS):δ 9.179 (s, 1H, NH), 4.723 (S, 2H, CH_2), 4.089 (q, 2H, OCH_2CH_3), 2.402 (s, 3H, S-CH_3), 2.173 (s, 3H, CH_3), 1.222 (t, 3H, OCH_2CH_3).

Methyl 6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**III**)

Yield: 54.05%. mp 198–200°C. IR (KBr), ν, cm^{-1}: 3314.07 (NH), 3008.41 (Ar C–H), 1665.23 (C=O),1587.13 (C=N). ^{1}H NMR (300 MHz, DMSO, TMS):δ 9.17 (s, 1H, NH), 4.723 (S, 2H, CH_2), 3.705 (s, 3H, OCH_3), 2.402 (s, 3H, S-CH_3), 2.173 (s, 3H, CH_3).

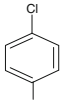
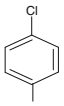
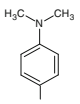
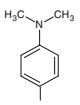
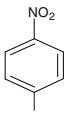
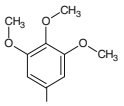
Docking studies

Docking studies of the title compounds was done on VLife MDS 3.5 using grid-based docking method to ascertain calcium channel blocking activity. The involvement of β-subunit in trafficking of α_1 subunit to plasma membrane suggests that an inhibitor of this complex could have significant therapeutic potential. Therefore, AID-β complex of L-type calcium channel is selected as a biological target for carrying out the docking study of title compounds. The crystal structure of AID-β complex was obtained from protein data bank, opened in MDS sheet, saved by removing water molecule and used further for docking purpose.

The 2D structures of the compounds were built and then converted into the 3D with the help of VLife MDS 3.5 software. The 3D structures were then energetically minimized up to the rms gradient of 0.01 using Merck Molecular Force Field (MMFF).

By using cavity determination option of software, cavities of enzyme were determined. The cavities in the receptor were mapped to assign an appropriate active site, the basic feature used to map the cavities was the surface mapping of the receptor and identifying the geometric voids as well as scaling the void for its hydrophobic characteristics. Hence, all the cavities that are present in receptor are identified and ranked based on their size and hydrophobic surface area. Cavity no. 1 is selected for docking. The active site for docking was defined as all atoms within 5 Å radius. Using biopredicta tool of software, open docking and then batch

Table 1 Chemical structures and physicochemical characteristic of title compounds (**IIa–III**)

Compound	R_1	R_2	Molecular formula	Molecular weight	Melting point (°C)	Yield (%)	R_f value
IIa		OC_2H_5	$C_{15}H_{18}N_2O_2S$	290.38	160–162	82.1	0.56
IIb		OCH_3	$C_{14}H_{16}N_2O_2S$	276.35	100–102	67.51	0.64
IIc		OC_2H_5	$C_{16}H_{20}N_2O_3S$	320.4	116–117	78.94	0.56
IId		OCH_3	$C_{15}H_{18}N_2O_3S$	320.4	146–147	73.68	0.51
IIe		OC_2H_5	$C_{15}H_{17}ClN_2O_2S$	324.82	138–139	92.98	0.72
IIf		OCH_3	$C_{14}H_{15}ClN_2O_2S$	310.79	130–132	57.89	0.69
IIg		OC_2H_5	$C_{17}H_{23}N_3O_2S$	333.44	124–126	62.93	0.56
IIh		OCH_3	$C_{16}H_{21}N_3O_2S$	319.42	214–216	75.75	0.48
IIi		OC_2H_5	$C_{15}H_{17}N_3O_4S$	335.37	180–182	83.68	0.46
IIj		OC_2H_5	$C_{18}H_{24}N_2O_5S$	380.45	206–208	75.14	0.33
IIk	H	OC_2H_5	$C_9H_{14}N_2O_2S$	214.28	210–211	65.47	0.3
III	H	OCH_3	$C_8H_{12}N_2O_2S$	200.25	198–200	54.05	0.72

grid docking. Batch docking shows browsing of receptor, ligand (molecule), and the result generated was saved in output file. Molecules saved in output file as a docked ligand format with proper conformation and further used to check binding interactions. Result generated was saved as log file in output folder.

For checking binding interaction, first receptor structure was opened in MDS followed by compound which was saved as ligand dock file. From tool option clicked on merge molecule so that compound and receptor is merged together. From biopredicta tool edited this complex and selected ligand and receptor structure to check their interactions.

Cardioprotective activity

Chemical and reagents

Adrenaline (Sigma-Aldrich) was purchased from market and 2-methylthio-1,4-diydropyrimidines synthesized on lab scale (**IIf** and **IIi**).

Animals

Adult rats of Sprague–dawley strain were aged between 2 and 3 months of both sexes, weighing between 180 and 220 g. All the animals were obtained from animal house.

They were kept in medium-sized plastic cages. They were allowed to live at room temperature, fed on standard pellets of rat's food and allowed to drink water ad libitum.

All the protocols of animal experiments were approved by the Institutional Animal Ethics Committee in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India, New Delhi.

Experimental design

Adrenaline-induced cardiac hypertrophy and cardiotoxicity

The experimental rats were divided into six groups ($n = 6$ in each group) and treated as follows:

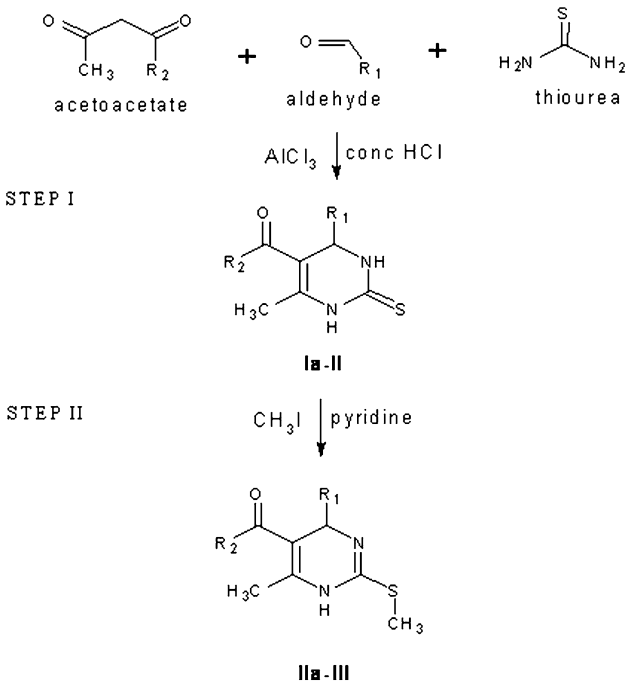
Group 1: Normal control rats treated with distilled water.
Group 2: Rats treated with adrenaline (2 mg/kg for 2 consecutive days, s.c.).
Group 3: Rats treated with test compounds (5 mg/kg body weight/day orally for 15 days).
Group 4: Rats pretreated with test compounds (5 mg/kg body weight/day orally for 15 days) and adrenaline (2 mg/kg for 2 days, s.c. on 14th and 15th day).
Group 5: Rats treated with nifedipine (5 mg/kg body weight/day orally for 15 days).
Group 6: Rats pretreated with nifedipine (5 mg/kg body weight/day orally 15 days) and adrenaline (2 mg/kg for 2 days, s.c. on 14th and 15th day).
Group 5 and Group 6 are positive control.

Measurement of ECG

At the end of experimental period (after 24 h of second adrenaline injection), the rats were anesthetized with urethane and ECGs were recorded using computerized data acquisition system (Power Lab AD instrument).

Results and discussion

The 1,2,3,4-tetrahydropyrimidine-2-thione derivatives were prepared by Biginelli three-component reaction of appropriate aldehydes, acetoacetate, and thiourea. Alkylation reaction was used for the formation of C-2 modified DHPM derivatives of type **II**. The title compounds, 2-methylthio-1,4-dihydropyrimidine derivatives (Table 1) were synthesized from the respective tetrahydropyrimidine-2-thiones by reaction with methyl iodide in the presence of pyridine (Scheme 1).



Scheme 1

The purity of the synthesized compounds was monitored by TLC using silica gel G and visualization was done using iodine vapor. Their structures were confirmed by elemental analysis, IR, and ^{1}H NMR spectra. The amount of carbon, hydrogen, and nitrogen found by elemental analysis is in good agreement with calculated.

VLife MDS 3.5 was used for docking studies of the title compounds to ascertain calcium channel blocking activity and hence cardioprotection using AID-β complex of L-type calcium channel as a target obtained from PDB with code 1T3L (Fig. 1). The docking of the title compounds yielded fitness scores ranging from −3.952602 to −5.262574 (Table 2). The docking study revealed that the title compounds have good interaction with AID-β complex, and compounds **IIf** and **IIi** are potential candidates as a cardioprotective agent because of the highest negative dock score (−4.978000 of compounds **IIf** and −5.262574 of compounds **IIi**). Compound **IIf** bind with AID-β complex

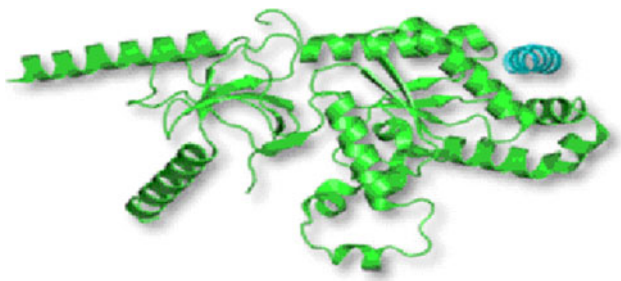


Fig. 1 AID-β complex of L-type calcium channel (PDB code 1T3L)

Table 2 Docking score of title compounds (**IIa–III**)

Sl. no.	Compound	Dock score (kcal/mol)
1	**IIa**	−4.506683
2	**IIb**	−4.902032
3	**IIc**	−4.452957
4	**IId**	−4.523580
5	**IIe**	−4.693726
6	**IIf**	−4.978000
7	**IIg**	−4.625088
8	**IIh**	−4.713718
9	**IIi**	−5.262574
10	**IIj**	−3.952602
11	**IIk**	−4.691329
12	**III**	−4.769786
13	Nifedipine	−4.564425

of L-type calcium channel by forming hydrogen bond interaction with amino acid residue THR58, hydrophobic interaction with amino acid residue THR58, LYS59, PRO60, VAL61, LYS97, LEU314, SER415, and van der Waals interaction with amino acid residue THR58, LYS59, PRO60, VAL61, LYS97, GLN315, SER415 (Figs. 2, 3). Compound **IIi** binds with AID-β complex of L-type calcium channel by forming hydrogen bond interaction with amino acid residue THR313, hydrophobic interaction with amino acid residue PRO60, VAL61, THR313, LEU314, SER415, and van der Waals interaction with amino acid residue LYS57, THR58, PRO60, VAL61, LYS97, ARG312, THR313, HIS411, SER414 (Figs. 4, 5). Compounds **IIf** and **IIi** showed significant cardioprotective activity on adrenaline-induced myocardial infarction in rats (Table 3). Electrocardiographic (ECG) abnormalities are the main criteria generally used for the diagnosis of myocardial infarction. Significant alterations in ECG patterns were observed in adrenaline-administered rats as compared to normal control rats. Adrenaline showed significant

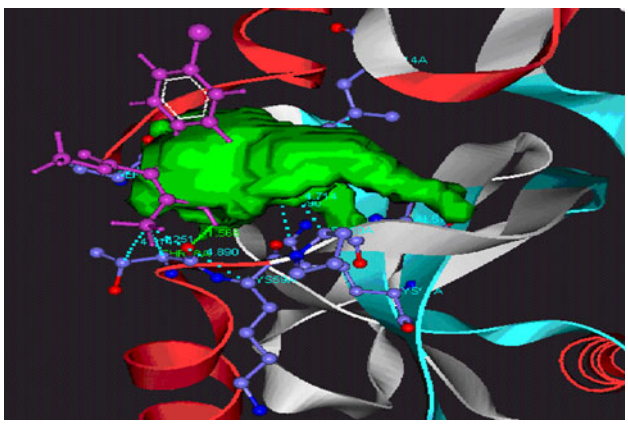


Fig. 2 Compound **IIf** docked in cavity no 1of AID-β complex of L-type Ca2+ channel

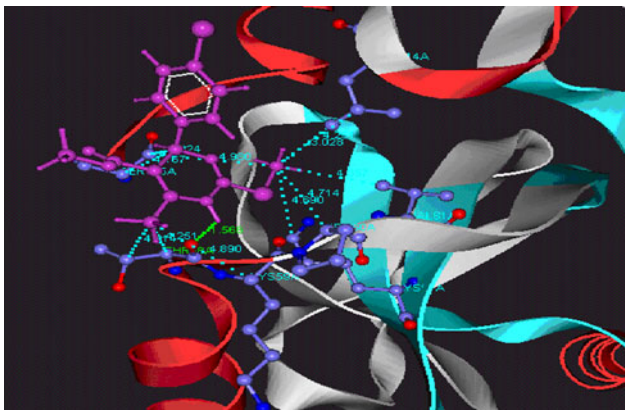


Fig. 3 Interaction of compound **IIf** with AID-β complex of L-type Ca2+ channel

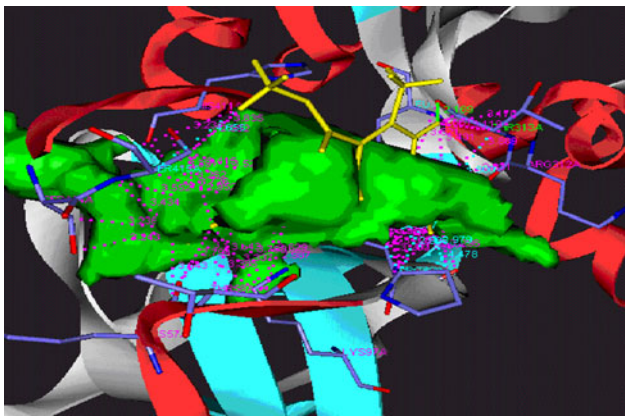


Fig. 4 Compound **IIi** docked in cavity no 1 of AID-β complex of L-type Ca2+ channel

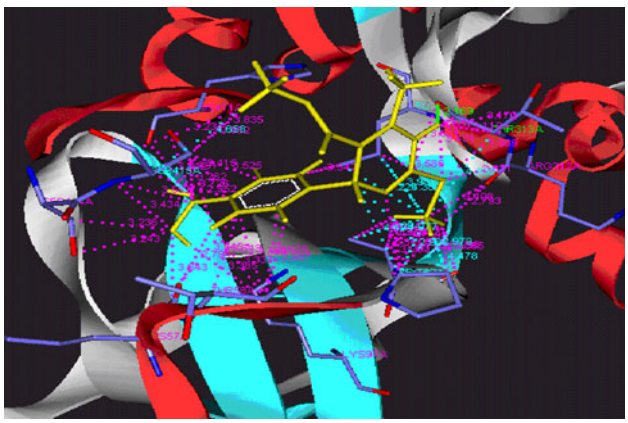


Fig. 5 Interaction of compound **IIi** with AID-β complex of L-type Ca2+ channel

elevation in ST interval, reduction in R–R interval, and P duration. In addition, there was increase in heart rate and prolongation of QT interval (Fig. 6). Pretreatment with test compounds and adrenaline significantly prevented these

Table 3 Cardioprotective activity of compounds **IIf** and **IIi** against adrenaline-induced myocardial infarction in rats

Compound	R–R interval (s)	Heart rate (BPM)	ST interval (s)	P-duration (s)	QT interval (s)
Control	0.1812 ± 0.0189	338.2 ± 6.541	0.04677 ± 0.0084	0.01657 ± 0.0029	0.06466 ± 0.0017
Adrenaline	0.135 ± 0.0176$^{\#\#}$	422.76 ± 6.158$^{\#\#}$	0.0829 ± 0.0024$^{\#\#}$	0.01136 ± 0.0044$^{\#\#}$	0.08205 ± 0.0023$^{\#\#}$
IIf + Adrenaline	0.1533 ± 0.0367**	382.03 ± 8.760**	0.0545 ± 0.0062**	0.01358 ± 0.0016**	0.03879 ± 0.0024**
IIi + Adrenaline	0.1852 ± 0.0294**	315.766 ± 5.121**	0.05879 ± 0.0026**	0.01660 ± 0.0048**	0.06664 ± 0.0054**
Nifedipine	0.2146 ± 0.0294**	278.5 ± 7.143**	0.0455 ± 0.0013**	0.01815 ± 0.0011**	0.06101 ± 0.0040**

Values are expressed as Mean ± SEM. Statistical comparisons were performed by one-way ANOVA followed by Dunnett's test

The ECG parameters are expressed in seconds (s) and the heart rate as Beats Per Minute (BPM)

$^{\#\#}$ $P < 0.01$ was considered significant when compared with control

** $P < 0.01$ was considered significant when compared with adrenaline

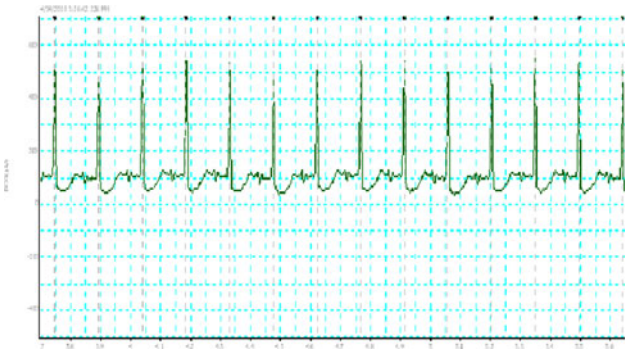


Fig. 6 ECG after administration of adrenaline

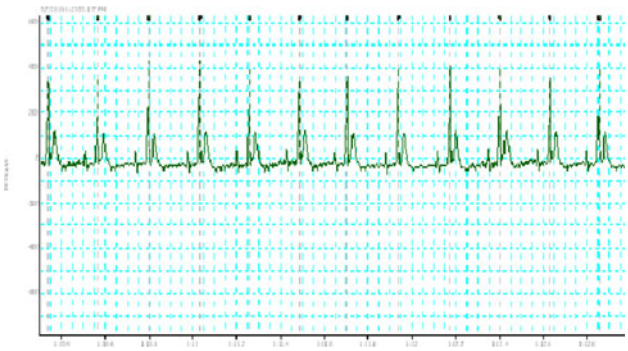


Fig. 7 ECG of control

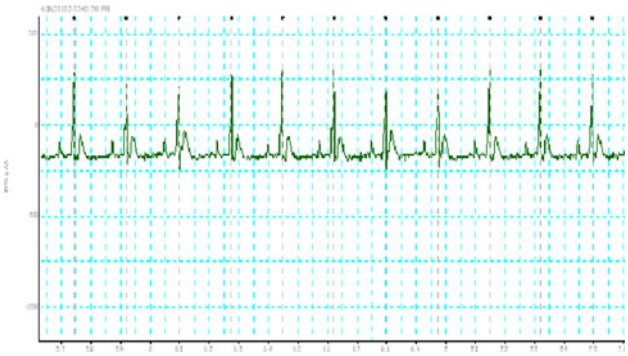


Fig. 8 ECG after administration of compound **IIf** + adrenaline

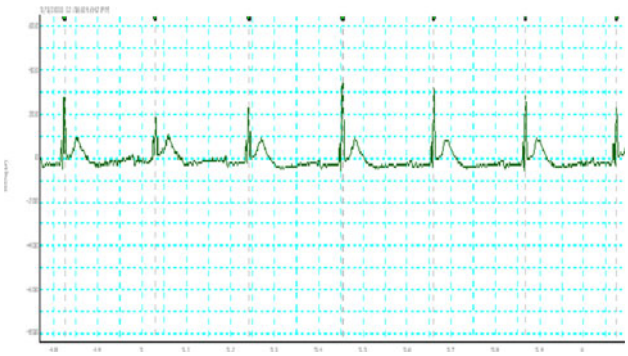


Fig. 9 ECG after administration of compound **IIi** + adrenaline

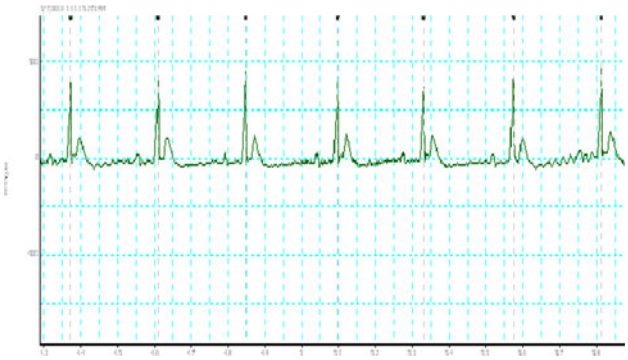


Fig. 10 ECG after administration of nifedipine + adrenaline

alterations and restored ECG values to near control (Figs. 7, 8, 9, 10).

Conclusion

The docking of 2-methylthio-1,4-dihydropyrimidines with AID-β complex of L-type calcium channel and subsequent evaluation of shortlisted compounds, methyl 4-(4-chlorophenyl)-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**IIf**) and ethyl 6-methyl-2-(methylthio)-4-(4-nitrophenyl)-1,4-dihydropyrimidine-5-carboxylate (**IIi**), for

cardioprotective activity reveals that the observed cardio protection may be because of inhibition of AID-β complex of L-type calcium channel and thus reducing calcium influx.

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