Title:

2-Methylthio-1, 4-dihydropyrimidines: Design, Synthesis and Pharmacological Screening for Cardio protective and Analgesic Activity

Abstract:

A series of 2-methylthio-1,4-dihydropyrimidine derivatives (IIa–Il) were synthesized in good yields by alkylation of 1,2,3,4-tetrahydropyrimidines (Ia–l) 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 b subunit functional core and its complex with the a1 interaction domain i.e. AID-b 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 aneesthetized 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 pre-treatment of compounds **IIf and IIi.**

The compounds were also tested for analgesic activity by acetic acid induced writhing method. Compounds **IIb, IIe, IIk and IIl** showed excellent to good analgesic activity. Other compounds showed moderate analgesic activity. The observed analgesic activity is mainly because of inhibition of the peripheral pain mechanism by the title compounds.

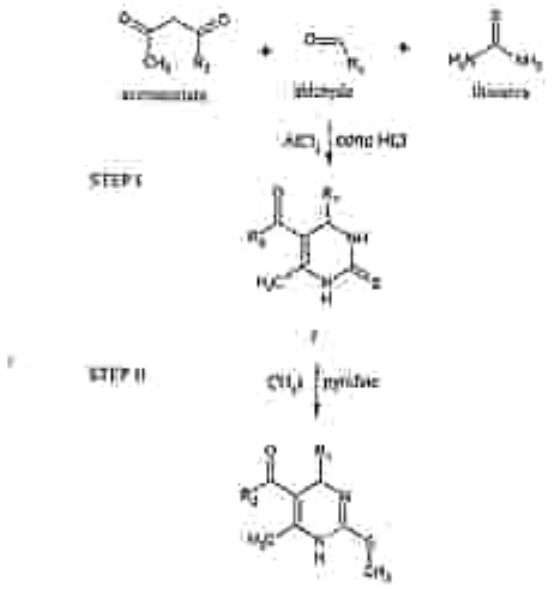
Introduction:

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 Ca^{2+} 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 Ca^{2+} 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.[1] 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 analgesics. Pain is defined as neuralgia, an unpleasant sensory experience associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause, or persist long after the precipitating injury has healed. Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory

response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids[2]. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells[3], acid sensing ion channels[4] and the prostaglandin pathways[5]. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for the treatment of pain and inflammation. However, longterm clinical usage of NSAIDs is associated with significant side effects of gastrointestinal lesions, bleeding, and nephrotoxicity[6]. Pyrimidine and condensed pyrimidine derivatives possessing anti-inflammatory and analgesic activities are well documented in the literature[7]. In the present work some novel analgesic 2-methylthio-1, 4-dihydropyrimidines are synthesized and structurally characterized.

General procedure for the synthesis of compounds (IIa-III)

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.



Scheme 1: Synthesis of 2-methylthio-1, 4-dihydropyrimidines

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 b-subunit in trafficking of a1 subunit to plasma membrane suggests that an inhibitor of this complex could have significant therapeutic potential. Therefore, AID-b 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-b 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 A° radius. Using biopredicta tool of software, open docking and then batch 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.

Biological Screening

a. **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[8].

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).

b. Analgesic activity

Experimental animals

Swiss albino mice of either sex weighing 25 to 30 g maintained in our college animal house were used for the study. The animals were divided into fourteen groups each containing six mice. Experiments reported in this study were carried out in accordance with local guidelines for the care of laboratory animals of PDVVPF's Medical College, Ahmednagar.

Writhing test method

Analgesic activity was carried out by acetic acid induced writhing method in Swiss albino mice (25-30 g). 0.1 mL of a 0.6 % aqueous acetic acid solution was injected intraperitoneally (IP) as writhing inducing agent. In each group six mice were kept. Mice were kept individually in test cage, before acetic acid injection. Screening of analgesic activity was performed after oral administration of test compounds at a dose of 50 mg/kg. All compounds were dissolved in sterile water for injection (SWF). Diclofenac was used as reference drug.

After 1 h of drug administration 0.10 mL of 0.6 % acetic acid solution was given to mice intraperitoneally. Stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted for 10 min of acetic acid injection. The analgesic activity was expressed in terms of percentage inhibition. Percentage analgesic activity was calculated as follows:

Analgesic activity (%inhibition) = (n-n' / n) ×100 Where, n = Mean number of writhes of control group. n' = Mean number of writhes of test group[9].

Statistical analysis

Values are expressed as mean ± SEM and data was analyzed by ANOVA followed by Dunnet's test. $p< 0.01$ was considered as significant.

Results and Discussion

a. **Cardioprotective Activity**
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 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. Pre-treatment with test compounds and adrenaline significantly prevented these alterations and restored ECG values to near control.

b. **Analgesic Activity**
The analgesic activity of the synthesized compound (IIa-III) was evaluated by acetic acid induced writhing test. The compound IIh bearing p-dimethylaminophenyl substituent at the fourth position of 1, 4-dihydropyrimidine exhibited maximum analgesic activity (70.32%). Whereas compounds IIk and III with unsubstituted fourth position of 1,4-dihydropyrimidine showed good analgesic activity (58.45 and 50.68 %). If p-chlorophenyl group is placed at the fourth position of 1,4-dihydropyrimidine compound IIe again showed good analgesic activity (57.08 %). The compound IIf with p-chlorophenyl group at the fourth position and methyl ester at fifth position of 1,4-dihydropyrimidine showed lowest analgesic activity in the present series.

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