2.0 CHAPTER – I

SYNTHESIS, CARDIAC EFFECTS AND ANTI BACTERIAL ACTIVITY OF 3, 4 - DIHYDROPYRIMIDIN - 2 - (1H) - ONE - 5 CARBOXYLATES

2.1 INTRODUCTION

Biginelli compounds or 3,4-Dihydropyrimidin-2(1H)-ones (DHPMs) possess interesting biological applications (Kappe, 1993; Snider and Shi, 1993; Overman et al., 1995; Kappe, 2000a; 2000b). The untapped potentials of DHPMs were explored because of their apparent structural similarities with clinically important dihydropyridines (DHPs) of nifedipine-type (Rovnyak et al., 1995). This reason accounts for the increased number of publications and patents in the DHPM synthesis. Synthesis, reactions and medicinal applications of DHPMs have been extensively reviewed (Kappe, 1993; 1998; 2000b; Kappe and Stadler, 2004). As early as 1930s, its simple derivative, 4-chlorophenyl-2-thio-DHPM was patented as an agent for the protection of wool against moths (Ertan et al., 1933). Later, the interest has focussed on the antiviral activity of Biginelli compounds, eventually leading to the excellent antiviral agent, nitractin (Hurst and Ann, 1962). The very same compound also exhibits modest antibacterial activity.

2.1.1 Synthetic Approaches

2.1.1.1 Biginelli Reaction

The Biginelli reaction is a multiple-component reaction invented by Pietro Biginelli in 1891 that creates 3,4-dihydropyrimidin-2(1H)-ones by the condensation of a dicarbonyl compound, an aryl aldehyde and urea (Biginelli, 1891; Biginelli, 1893; Zaugg and Martin, 1965; Kappe, 1993).
Ranu et al. (2000) have described the indium (III) chloride catalyzed synthesis of dihydropyrimidinones

Ma et al. (2000) have reported the lanthanide triflate catalyzed Biginelli reaction.

Lu and Ma (2000) have reported the iron (III)-catalyzed synthesis of dihydropyrimidinones.

Ananda Kumar et al. (2001) have reported the Mn (OAc)\(_3\) catalyzed synthesis of 3,4-dihydropyrimidin-2(1H)-ones by refluxing in acetonitrile for 2-4 h.
Sabitha et al. (2003) have reported the vanadium III chloride catalyzed Biginelli condensation.

Kappe and Falsone (1998) have reported the polyphosphate ester-mediated synthesis of dihydropyrimidines.

Salehi et al. (2003) have reported the silica sulfuric acid catalyzed synthesis of 3,4-dihydropyrimidin-2(1H)-ones by refluxing in ethanol for 6 h.
Rani et al. (2001) have reported the zeolite-catalyzed cyclocondensation reaction for the synthesis of 3,4-dihydropyrimidin-2(1H)-ones.

\[
\text{CHO} + \text{H}_2\text{N} - \text{NH}_2 + \text{RCHO} \xrightarrow{\text{Z} \text{e} \text{o} \text{l} \text{i} \text{t} \text{e}, \text{Toluene, Reflux}} \text{R} - \text{N} = \text{C} - \text{NH} - \text{EtO} \quad + \quad \text{EtO}
\]

Huang et al. (2005) have achieved the highly enantioselective synthesis of dihydropyrimidines by using a chiral ytterbium catalyst.

\[
\text{CHO} + \text{H}_2\text{N} - \text{NH}_2 + \text{RCHO} \xrightarrow{\text{Ligand, Ln(OTf)}_3} \text{R} - \text{N} = \text{C} - \text{NH} - \text{EtO} \quad + \quad \text{EtO}
\]

Peng and Deng (2001) have reported the ionic liquid mediated Biginelli synthesis of 3,4-dihydropyrimidin-2(1H)-ones at 100 ºC.

\[
\text{R} - \text{CHO} + \text{H}_2\text{N} - \text{NH}_2 + \text{RCHO} \xrightarrow{\text{Ionic liquid}} \text{R} - \text{N} = \text{C} - \text{NH} - \text{EtO}
\]
Debache et al. (2006) have reported the phenyl boronic acid catalyzed Biginelli reaction in acetonitrile under reflux condition.

\[
\begin{align*}
\text{CHO} + \text{NH}_2 \text{NH}_2 \text{O} + \text{OEt} \text{N} \text{NH} \text{O} \text{EtO} \\
\text{PhB(OH)}_2 \text{CH}_3\text{CN} \\
\end{align*}
\]

2.1.1.2 Micro wave-assisted organic reactions

The rapid heating associated with microwave technology has been applied in a number of disciplines such as the preparation of samples for analysis, application to waste treatment, polymer technology, drug release or targeting and hydrolysis of proteins and peptides. Its application in organic synthesis has not only reduced the reaction time in many folds but also has proven to give better yields.

The practical applications of the various methods under conventional thermal conditions suffer from the disadvantages such as the use of expensive or less readily available reagents, vigorous reaction conditions, prolonged reaction conditions and tedious manipulations in the isolation of the pure products. These necessitate a method for versatile, simple and environmentally friendly process whereby compounds may be obtained under microwave assisted milder conditions (Gohain et al., 2004).

2.1.1.3 Enantiomerically pure dihydropyrimidines

Dihydropyrimidines of the Biginelli type are inherently asymmetric molecules, and therefore are usually obtained as racemic mixtures. The influence of the absolute configuration at the stereogenic C-4 on biological activity is well documented (Atwal et al., 1990a, 1990b). Access to enantiomerically pure DHPMs is therefore of considerable interest and a prerequisite for the development of drugs in this field (Atwal et al., 1991).
In the absence of any known general asymmetric synthesis for this heterocyclic target system, resolution strategies have so far been the method of choice to obtain enantiomerically pure DHPMs.

Due to recent advances in preparative chromatographic enantioseparation techniques, enantioselective HPLC and related methods have gained importance in the synthesis of single-enantiomer drugs and intermediates. In a recent study by Kleidernigg and Kappe (1997), it is reported that the chromatographic enantioseparation of DHPM derivatives can be accomplished by using a variety of commercially available chiral stationary phases (CSPs) in normal and reversed phase analytical HPLC. Resolution of the enantiomers of DHPMs using semipreparative chiral HPLC, followed by attaching to monodisperse macroporous aminomethacrylate beads (Equation 1) provided the novel polymer-based CSP.

Such designer “CSPs” would prove extremely useful for the efficient separation of not only DHPMs but other structurally related compounds as well. The chiral separation of DHPMs by capillary electrophoresis (CE) using quarternary ammonium-β-cyclodextrin as chiral buffer additive has also been reported (Wang et al., 2000).

Biocatalytic strategy towards the preparation of enantiopure (R) - and (S) - 32926
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Equation 2: Biocatalytic strategy of synthesizing optically pure DHPM, (R)-32926, an antihypertensive agent via enzymatic resolution mediated by Thermomyces lanuginosus lipase.

has been developed. The key step in the synthesis is the enzymatic resolution of an N-3-acetoxy methyl-activated dihydropyrimidinone precursor by Thermomyces lanuginosus lipase. Readily available racemic DHPM was hydroxymethylated at N-3 with formaldehyde, followed by standard acetylation with acetyl chloride. The resulting N-3-acetoxy methyl-activated DHPM was then cleaved enantioselectively by Thermomyces lanuginosus lipase with excellent selectivity (Equation 2). Degradation of unreacted 3-hydroxy methylated (R)-DHPM with aqueous ammonia produced (R)-DHPM, which was converted into the desired target structure, (R)-32926, in one step by N-3 carbamoylation with trichloroacetyl isocyanate (Stadler and Kappe, 2000).

A critical point in every preparation of enantiomerically pure materials, regardless of the method, is the assignment of absolute configuration. For the DHPM series, a simple protocol for absolute configuration assignment based on the combination of enantioselective HPLC and circular dichroism (CD) spectroscopy has been developed.
By the comparison of the characteristic CD spectra of individual DHPM enantiomers with reference samples of known absolute configuration, the absolute configuration of 4-aryl DHPMs could be established. The enantiomers were obtained by semipreparative HPLC separation of racemic DHPMs on chiral stationary phases. The characteristic CD activity of the enamide chromophore around 300 nm allows the assignment of absolute configuration in this series of dihydropyrimidine derivatives (Krenn et al., 1999).

2.1.2 Biological activities

Biginelli compounds show a diverse range of biological activities. As early as 1930, these derivatives were patented as agents for the protection of wool against moths (Folkers and Johnson, 1933). In 1893, the synthesis of functionalized DHPMs was reported for the first time by Biginelli. This efficient approach to partly reduced pyrimidines was ignored in the following decades and therefore, the pharmacological properties of this interesting heterocyclic scaffolds remained unexplored. Since the early 1980s, however, interest in DHPMs has increased significantly (Kappe, 1993). This was originally due to the apparent structural similarity of DHPMs to the well known dihydropyridines calcium channel modulators of the Hantzsch type (Atwal et al., 1990a). It was soon established that DHPMs exhibit a similar pharmacological profile to DHP calcium channel modulators of the nifedipine type and much activity has been observed in this area (Cho et al., 1989; Schnell et al., 2000). They are also $\alpha_{1a}$ adrenoreceptor selective antagonists useful for the treatment of benign prosthetic hyperplasia (Wetzel et al., 1995). The recent identification of a DHPM analog as potential new anticancer lead that is involved in blocking mitosis by inhibition of a kinesin motor protein is another biological activity (Mayer et al., 1999).
2.1.2.1 Calcium channel modulators

DHPs e.g., nifedipine are the most studied class of organic calcium channel modulators. More than 25 years after the introduction of nifedipine, many DHP analogs have now been synthesised and numerous second-generation commercial products have appeared on the market e.g. nicardipine, amlodipine, felodipine, etc. (Bossert and Vater, 1989). These substances act by inhibiting the entry of $\text{Ca}^{2+}$ into

\[ \text{Fig. 1.1a-e Structures of DHPM calcium channel modulators} \]

the cells of cardiac and vascular muscle through the voltage-dependent calcium channels and decrease ventricular contractility by the same mechanism. The cardiovascular activity of DHPMs was first recognized by Khanina et al. (1978) who reported on $\beta$-aminoethyl esters of DHPMs that exhibit moderate hypotensive activity and coronary dilatory properties. Difluoromethoxy-substituted analogs of DHPMs also showed similar levels of activity (Kastron et al., 1987). DHPMs were shown to be potent calcium channel blockers; however most did not show significant antihypertensive activity $\text{in vivo}$.
(Atwal et al., 1990a). Further structural modifications on the dihydropyrimidine ring led to DHPMs bearing an ester group at N 3 (Atwal et al., 1990b) thereby more closely resembling nifedipine-type dihydropyrimidines (Fig. 1.1).

Among the most potent derivatives in a series of N (3) - substituted DHPMs, is the thiourea derivative (Fig. 1.1(e)) (Atwal et al., 1990b). These are calcium channel blockers devoid of antihypertensive activity in vivo. The lack of oral activity of these derivatives has been rationalized by their rapid metabolism. Further modification of the substituent at N (3) - subsequently led to the development of orally active long-lasting hypertensive agents such as SQ 32926 and SQ 32547 (Fig. 1.2). The improved oral bioavailability results from the increased chemical stability of the urea functionality. Both compounds have anti-ischaemic properties in animal models (Grover et al., 1995). Of critical importance for the biological activity of most of the DHPMs shown in Fig. 1.3 is the absolute stereochemistry at the C4 stereocenter. Pharmacologic studies with resolved enantiomers have demonstrated that, for example, for DHPM (Fig. 1.3(e)) a > 1000 –fold difference in potency can be observed (Atwal et al., 1990b). For the orally active antihypertensive DHPM derivatives SQ 32926 and SQ 32547, it was established that the desired antihypertensive effect resides solely in the (R)–enantiomer (Atwal et al., 1991; Rovnyak et al., 1992).

**Fig. 1.2** Structures of DHPM based calcium channel modulators
2.1.2.2 \( \alpha_{1a} \)-Adrenergic receptor antagonists

Benign prostatic hyperplasia (BPH) is a progressive enlargement of the prostate resulting in a number of obstructive and irritative symptoms. The incidence of BHP increases with advancing age, such that 70% of males, over 70 years old, manifest its symptoms. Non-selective \( \alpha_{1a} \)-adrenoreceptor antagonists, e.g. terazosin, are currently being approved pharmaceuticals for treating BPH (Nagarathnam et al., 1998). It is reported that the functional potency of a number of \( \alpha \) antagonists correlates well with the binding affinity for \( \alpha_{1a} \) subtype at the cloned human receptors. Thus the DHPM scaffold SNAP 6201 was developed with good binding affinity and excellent subtype selectivity for the \( \alpha_{1a} \) receptor with no cardiovascular effects (Fig. 1.3).

![SNAP 6201](image)

Fig. 1.3 \( \alpha_{1a} \) Subtype cloned human receptors.

2.1.2.3 Mitotic kinesin inhibitors

A common strategy for cancer therapy is the development of drugs that interrupt the cell cycle during the mitosis stage. Compounds that perturb microtubule shortening (depolarization) or lengthening (polymerization) cause arrest of the cell cycle in mitosis due to perturbation of the normal microtubule dynamics necessary for chromosome movement (Atwal and Moreland, 1991). A variety of such drugs that bind to tubulin and thus inhibit spindle assembly are currently used in cancer therapy e.g. paclitaxel and
docetaxel (Jordan et al., 1998). Mayer et al. (1999) have recently identified the structurally rather simple DHPM - termed monastrol - as a novel cell-permeable molecule, that blocks normal bipolar mitotic spindle assembly in mammalian cells and therefore, causes cell cycle arrest. Monastrol (Fig. 1.4) blocks mitosis by specifically inhibiting the motor activity of the mitotic kinesin Eg5, a motor protein required for spindle bipolarity. Monastrol is the only cell-permeable molecule currently known to specifically inhibit mitotic kinesin Eg5 and can therefore be considered as a lead for the development of new anticancer drugs.

**Fig. 1.4** Monastrol, the only cell-permeable molecule inhibiting mitotic kinesin Eg5

### 2.1.2.4 Miscellaneous biological effects

As early as the 1940s, DHPM type of compounds (Fig. 1.5) was shown to possess antiviral activity. Eventually, the nitrofuryl-substituted analog nitractin (Fig. 1.5a) was developed which displayed good activity against the viruses of the trachoma group, in addition to showing modest antibacterial activity. Other structurally simple DHPMs were screened as anti-tumor agents and found to be active against, for example, Walker carcinosarcoma in rats and mice. Pyrimidine-5-carboxamides type were claimed to have anticarcinogenic activity, while other derivatives were reported to have blood platelet aggregation inhibitory activity (Tozkoparan et al., 1995), or were shown to inhibit the uptake of adenosine by thrombocytes (Fig. 1.5b). Fused DHPMs, such as thiazolo [3,2-a] – pyrimidine (Fig. 1.5c) and pyrimido [2,1-b] [1,3] thiazine (Fig. 1.5d) were reported to
have anti-inflammatory activity. (Tozkoparan et al., 1999). Fungicidal activity towards *Aspergillus niger* and *A. ochraceus* was demonstrated for simple 2-thioxo DHPMs.

Not only synthetic DHPM derivatives, but also several natural products with interesting biological activities containing the dihydropyrimidine-5-carboxylate core, have been isolated from marine sources. Among these, the batzelladine alkaloids A and B, inhibits the binding of HIV envelop protein gp-120 to human CD4 cells and therefore, are potential new leads for AIDS therapy (Heys et al., 2000).

Extensive literature survey reveals that there are no reports on the cardiotonic and antibacterial activity of DHPMs. Therefore in the present study we describe the synthesis, cardiovascular and antibacterial activity of DHPMs.

Cardiovascular diseases have been the principal cause of death in many developing countries (WHO, 1986) and disability in industrialized nations and is among the syndromes most commonly encountered in clinical practice (Seth, 1999). The diagnosis of heart failure carries a risk of mortality comparable to that of the major malignancies (Henry and Colucci, 2001). Heart failure occurs when cardiac output is insufficient to meet the demands of tissue perfusion and may primarily be due to systolic or diastolic dysfunction (Tripathi, 2003). It is frequently, but not always, caused by a
defect in myocardial contraction (Braunwald, 1988). Myocardial contractility is largely
dependent upon the activity of the cardiac sympathetic nerves, but it can also be
increased by circulating catecholamines, tachycardia, and inotropic drugs (Julien et al.,
1998). These drugs induce changes in myoplasmic calcium and this may be responsible
for the cardioactive properties. Cardiac glycosides and catecholamines have been used as
the main therapeutic drugs in the treatment of congestive cardiac failure (Kitada et al.,
1987). However, the dangers of cardiac glycosides intoxication are well documented
(Beller et al., 1971) and doubts have been expressed about their long-term effectiveness.
The use of catecholamines is limited by their insufficient differentiation between positive
inotropic and chronotropic actions, their potential arrhythmogenic properties, and
tachyphylaxis due to receptor downregulation (Kitada et al., 1987).

3,4-Dihydropyrimidinones (DHPMs) also called Biginelli compounds possess
interesting biological applications. The apparent structural similarities of DHPMs to the
well-known Hantzsch-type dihydropyridines, calcium channel modulators, suggest a
good scope for this class of compounds in the field of medicinal chemistry (Rovnyak et
al., 1992 & 1995). Calcium channel blockers are used in the treatment of angina,
hypertension, cardiac arrhythmias (Jacob, 1992) and have a limited role in heart failure.
The dihydropyridines are the most potent Ca$^{2+}$ channel blockers. They have little effect
on the myocardium and conducting tissue. Used alone, they often cause a reflex
tachycardia which can be avoided by concomitant use of a β-blocker. More than 25 years
after the introduction of nifedipine, many DHP analogs and numerous second generation
products are available in the market (e.g., nicardipine, amlodipine, felodipine, etc.).
Advances in the knowledge of the biochemical and physiological changes during cardiac
failure as well as the development of new diagnostic and surgical procedures in cardiovascular medicines have been remarkable in the last few decades. Unfortunately no such claim could be made with respect to the development of new pharmacological agents with clinically useful positive inotropic properties (Vasavada et al., 1990). Nearly two centuries have passed since William Withering described the cardiovascular effects of *Digitalis purpurea* in 1785; however the basic treatment of congestive heart failure still depends on the cardiac glycosides. Although hundreds of cardiac glycosides have been investigated, not one has been found with a wide therapeutic index. This necessitates research for new drugs, which increase cardiac muscle contractility with a broad therapeutic index. It is well-established that only one particular enantiomer is responsible for calcium channel antagonist activity between the existing two enantiomeric DHPMs in the racemic mixture (Goldman et al., 1991; Kappe, 2000b). Herein, for the first time, the cardiotonic activity of DHPMs on an isolated perfused frog heart was demonstrated. The action may be attributed to the presence of the more potent agonistic enantiomer in the racemic mixture. Further in this study, synthesis, cardiovascular and antibacterial activity of DHPMs, which is vital for progress in the medical field were described.
2.2 OBJECTIVES

DHPM scaffolds are exhibiting a wide range of biological and medicinal activities, and synthetic procedures to prepare them are increasing tremendously. It shows a broad range of biological activity like calcium channel blockers, antihypertensive agents and \( \alpha_{1a} \)-antagonists, antiviral, antibacterial and anti-inflammatory activities.

The aim of the present work was to study

- Synthesis of structurally challenging DHPMs through improved protocols such as application of novel catalysts, microwave irradiation and neat synthesis.
- Evaluation of the possible cardiac and antibacterial activity of the synthesized and structurally characterized DHPMs.
2.3 EXPERIMENTAL

2.3.1 Equipments

Melting points were determined in open capillary tubes and are uncorrected. IR measurements were obtained as KBr pellets using Perkin-Elmer spectrum RXI FT-IR. The $^1$H NMR and $^{13}$C NMR spectra were recorded in CDCl$_3$ + DMSO-d$_6$ with JEOL 400 MHz (model GSX 400) high resolution NMR spectrometer with TMS as internal standard. Mass spectra were obtained using JEOL DX-303 in EI ionization mode at 70 eV. The reaction was carried out in a BPL—SANYO domestic microwave oven operating at 2.45 GHz. TLC was performed on precoated Polygram SIL G/UV254 sheets. The elemental analyses of the compounds were recorded using ThermoFinnigan FLASH EA 1112 CHNS analyzer. Column chromatography was carried out using 100-200 mesh silica gel. Ethylacetoacetate, methylacetoacetate, benzyl acetoacetate, urea, ortho-chloro benzaldehyde, ortho-nitro benzaldehyde, anisaldehyde, Meta-and para-hydroxy benzaldehydes were purchased from s.d.fine chemicals. Biphenyl carboxaldehyde and isobutyrylaldehyde was purchased from Aldrich Sigma Chemicals.

2.3.2 General Procedure for the Synthesis of 4-(substituted)-3,4-dihydropyrimidinones

A mixture of β-keto ester 1 (1 mmol), aldehyde 2 (1 mmol), and urea 3 (1 mmol) was irradiated in acetic acid medium with few drops of concentrated HCl. The solution was kept in an alumina bath and irradiated in a domestic microwave oven for 5–7 min with a pulse rate of 40 sec and 30% of power. The total consumption of aldehyde as monitored by TLC was an indication of completion of the reaction. After the reaction was over, the mixture was poured into 150 ml of ice-cold water and heated over a water bath.
for 30 min followed by stirring for 1 h at room temperature. The solid thus obtained was collected by filtration and column chromatographed with 1:3 petroleum ether and ethyl acetate mixture. Characterization of the compounds by IR, $^1$H NMR, $^{13}$C NMR, mass spectroscopy, elemental analyses, and melting point confirms the formation of products.

2.3.3 Cardiovascular activity

2.3.3.1 Animals

Common Indian adult frogs of Rana tigrina species were used in the present study. The great advantage of frog tissues is that they can function as isolated preparations for many hours when handled carefully and to maintain the tissue preparation, no extra supply of oxygen is needed as the frog muscles can directly imbibe oxygen from the atmosphere. The animals were maintained as per the norms of Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) and this experiment was cleared by CPCSEA and Institutional Animal Ethics Committee constituted for the purpose (IAEC/SRMC&RI/14.8.2003).

2.3.3.2 Introduction

Heart diseases are prime culprits in modern world for maximum number of deaths in Asian, American as well as European countries. Cardiac failure denotes the presence of one of the complexes of symptoms and signs associated with the congestion of tissues and organs or attributable to the inadequate perfusion of tissues and organs. Cardiovascular agents are the drugs which modify the functions of cardiovascular system.
2.3.3.3 Principle

Drugs may influence the rate (chronotropy) and force (inotropy) of contraction of the heart. An increase in the heart rate is called a ‘positive chronotropic’ response, while a ‘negative chronotropic’ response decrease in the heart rate. Similarly, an increase in the force of contraction is called a ‘positive inotropic’ response and a decrease in the force of contraction is called a ‘negative inotropic’ response.

2.3.3.4 Materials Requirement

Myograph board, dissection apparatus, starling heart lever, thread, pin, muscle/force transducer (INCO) and Student’s physiograph single channel (INCO). Frog ringer with the following composition was used as the perfusion fluid NaCl, 110.0; KCl, 1.90; CaCl₂, 1.10; NaH₂PO₄, 0.06; NaHCO₃, 2.40; dextrose, 11.10 l 1 µM made up to 1000 ml with distilled water and sodium carboxy methyl cellulose (Loba Chemie, Mumbai).

2.3.3.5 Preparation of drug solution

The marketed digoxin tablets (Lanoxin) were obtained from the local market and different dilutions were made with distilled water to get 25, 50 and 500 µg/ml. Adrenalin, verapamil (calaptin), metoprolol (Metocard) were obtained from the local market and diluted with frog ringers solution to get 5 µg/ml concentration.

2.3.3.6 Preparation of test compounds

The compounds 4(a-i) were made as an aqueous suspension in 1% sodium carboxy methyl cellulose to get 5, 50, 100, 500 and 1000 µg concentration.
2.3.3.7 Method

Common Indian frogs of *Rana tigrina* were divided into 10 groups comprising of standard and test compounds (4a-4i) of 6 animals each. Frogs were dissected from ventral surface to expose the heart and pericardium was removed. The frog heart was isolated and perfusion was done by Bulbring’s method as described by Burn (1952). The apex of ventricle was attached to the sterling heart lever, which in turn was attached by a thread to the muscle forced transducer and this is connected to a ‘physiograph’. Normal heart rate, contractile amplitude was recorded. The cardiac output from the isolated frog heart was collected every minute and measured. The various concentrations of compounds to be tested were prepared using 1% sodium carboxy methyl cellulose and administered to the ringer flowing through the cannula and the effects were recorded at different dose level (5 μg, 50 μg, 100 μg, 500 μg, and 1 mg/ml). Only one compound was tested in each preparation. The responses of the compounds were compared to those of digoxin. During the interaction studies verapamil (5 μg/ml) in frog ringer’s solution, metoprolol, a β-adrenergic blocker (5 μg/ml) in frog ringer’s solution and adrenaline (5 μg/ml) were administered to the ringer solution and recordings were noted. The data presented in figures are mean ± SEM. One-way ANOVA was used for statistical analysis. P values < 0.01 were considered to be statistically significant.

2.3.4 Antibacterial activity

2.3.4.1 Introduction

Antibacterial activity is measured *in vitro* in order to determine 1) the potency of an antibacterial agent in solution, 2) its concentration in body fluids or tissues, and 3) the sensitivity of a given microorganism to known concentrations of the drug. Determination
of the concentration may be undertaken by one of two principal methods viz. dilution or diffusion method.

2.3.4.2 Principle

Antibacterial study was carried out for the synthesized DHPMs (4a-i) by disc diffusion method against ATCC gram positive and gram negative bacterial strains (Cruickshank et al., 1975).

2.3.4.3 Materials requirement

- The gram positive organism used for this study was *Staphylococcus aureus* (ATCC 12600) and the gram negative organism was *Escherichia coli* (ATCC 11775) (The strains were received from Department of Veterinary Microbiology, Madras Veterinary College, Chennai-600 007)
- The medium Tryptose Soy Agar (TSA) powder (HiMedia, Mumbai) was used at 4 g /100 ml to prepare solid agar plates and was used for both Gram positive and Gram negative bacteria.

2.3.4.4 Method

1. The sterile discs (6 mm diameter, HiMedia) containing compounds were prepared by dissolving the compounds in DMSO at 10 mg/ml concentration. From this stock 100 μg concentration per disc of each compound were prepared and kept ready.

2. Overnight cultures of each organism in Tryptose Soy broth was prepared to use in this study.
3. The cultures were spread on the solid agar plates with a sterile cotton swab and allowed to dry. Then the compound loaded discs were placed with equal distance on the organism inoculated plates.

4. The plates were incubated at 37°C for 24 h and the readings were taken by measuring the diameter of the discs with zone of inhibition in the plates.

5. Student’s ‘t’ test was used for statistical analysis. P values < 0.001 and P<0.01 were considered to be statistically significant.
2.4 SPECTRAL DATA

2.4.1 Ethyl 4-(3-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (4a)
Yield: 84%. Mp: 164–166 °C. IR (KBr): 3518, 3351, 3243, 1722, 1639 cm⁻¹. \(^\text{1}H\) NMR \(\delta\): 1.07 (t, \(J = 6.9\) Hz, 3H), 2.27 (s, 3H), 3.95 (q, \(J = 6.9\) Hz, 2H), 5.03 (s, 1H, CH(4)), 6.58 (d, \(J = 8.6\) Hz, 1H), 6.63 (d, \(J = 7.45\) Hz, 2H), 7.04 (t, \(J = 8\) Hz, 1H), 7.66 (s, 1H, NH(3)), 9.13 (s, 1H, NH(1)), 9.34 (s, 1H, Ar-OH). \(^\text{13}C\) NMR \(\delta\): 14.6, 18.3, 54.3, 59.7, 99.9, 113.6, 114.7, 117.4, 129.8, 146.8, 148.6, 152.8, 157.9, 165.9 MS (EI, m/z): 276 (M⁺); Anal. Calcd for C\(_{14}\)H\(_{16}\)N\(_2\)O\(_4\): C, 60.86; H, 5.84; N, 10.14. Found: C, 60.81; H, 5.78; N, 10.06.

2.4.2 Ethyl 4-(isopropyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (4b)
Yield: 88%. Mp: 192–193 °C. IR(KBr): 3234, 3102, 1692, 1645 cm⁻¹. \(^\text{1}H\) NMR \(\delta\): 0.74 (d, \(J = 6.8\) Hz, 3H), 0.82 (d, \(J = 6.9\) Hz, 3H), 1.19 (t, \(J = 7.1\) Hz, 3H), 1.68 (m, 1H), 2.18 (s, 3H), 3.96 (t, \(J = 3.6\) Hz, 1H), 4.04 (m, 2H), 7.26 (s, 1H), 8.86 (s, 1H). \(^\text{13}C\) NMR \(\delta\): 14.2, 16.0, 17.7, 18.5, 34.6, 55.5, 58.1, 98.2, 148.4, 153.2, 165.8. MS (EI, m/z): 226 (M⁺); Anal. Calcd for C\(_{11}\)H\(_{18}\)N\(_2\)O\(_3\): C, 58.39; H, 8.02; N, 12.38. Found: C, 58.40; H, 8.00; N, 12.45.

2.4.3 Phenyl 4-(3-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (4c)
Yield: 85%. Mp: 170–172 °C. IR (KBr): 3365, 2937, 2369, 1716, 1573, 1508 cm⁻¹. \(^\text{1}H\) NMR \(\delta\): 2.28 (s, 3H), 5.22 (s, 1H), 6.79 (s, 1H), 6.85 (d, \(J = 8.6\) Hz, 1H), 6.88 (d, \(J = 7.2\) Hz, 1H), 6.96 (t, \(J = 7.6\) Hz, 1H), 7.02 (d, \(J = 8.2\) Hz, 1H), 7.1 (d,
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J = 6.8 Hz, 1H), 7.56 (s, 1H, NH(3)), 9.05 (s, 1H, NH(1)), 9.25 (s, 1H). $^{13}$C NMR δ: 14.5, 52.6, 113.5, 113.9, 116.5, 119.7, 121.8, 125.2, 127.6, 130.2, 134.1, 148.0, 153.5, 153.6, 158.2, 170.5. MS (EI, m/z): 324 (M$^+$); Anal. Calcd for C$_{18}$H$_{16}$N$_2$O$_4$: C, 66.66; H, 4.97; N, 8.64. Found: C, 66.82; H, 4.85; N, 8.56.

2.4.4 Ethyl 4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (4d)

Yield: 88%. Mp: 228–229 °C. IR (KBr): 3410, 3256, 2990, 1719, 1689 cm$^{-1}$. $^1$H NMR: δ: 1.12 (t, 3H, J = 7.5 Hz), 2.22 (s, 3H), 4.03 (q, 2H, J = 7.5 Hz), 5.12 (s, 1H), 6.70 (d, 2H, J = 9 Hz), 7.10 (d, 2H, J = 9.0 Hz), 7.5 (s, 1H, NH(3)), 9.01 (s, 1H, NH(1)), 9.20 (s, 1H, Ar-OH). $^{13}$C NMR δ: 15.04, 17.76, 53.54, 58.76, 99.73, 114.58, 127.19, 136.54, 147.25, 152.30, 156.19 and 165.24. MS (EI, m/z): 276 (M$^+$). Anal. Calcd for C$_{14}$H$_{16}$N$_2$O$_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.11; H, 5.80; N, 10.42.

2.4.5 Ethyl 4-(4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (4e)

Yield: 84%. Mp: 199–200 °C. IR (KBr): 3303, 3050, 1710, 1647 cm$^{-1}$. $^1$H NMR δ: 1.08 (t, 3H, J = 7.5 Hz), 2.24 (s, 3H), 3.71 (s, 3H, Ar-OMe), 3.97 (q, 2H, J = 7.5 Hz), 5.23 (d, 1H, J = 2.7 Hz), 6.88 (d, 2H, J = 8.58 Hz, Ar-H), 7.13 (d, 2H, J = 8.58 Hz, Ar-H), 7.67 (s, 1H, NH(3)), 9.15 (s, 1H, NH(1)). $^{13}$C NMR δ: 14.03, 18.32, 53.88, 55.61, 59.73, 100.13, 114.26, 127.96, 137.60, 148.58, 152.73, 159.00, 165.94. MS (EI, m/z): 290 (M$^+$). Anal. Calcd for C$_{15}$H$_{18}$N$_2$O$_4$: C, 62.08; H, 6.25; N, 9.65. Found: C, 61.93; H, 6.19; N, 13.87.
2.4.6 Ethyl 4-(4-(1,1’-biphenyl))-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (4f)

Yield: 70%. Mp: 212–214 °C. IR (KBr): 3224, 3105, 1692, 1639 cm⁻¹. ¹H NMR δ: 1.04 (t, J = 7.45 Hz, 3H), 2.21 (s, 3H), 3.82 (q, J = 7.45 Hz, 2H), 5.43 (d, 1H, J = 3.5 Hz), 7.26 (m, 3H), 7.38 (t, J = 7.45 Hz, 2H), 7.54 (t, J = 8.1 Hz, 4H), 7.71 (s, 1H, NH(3)), 9.12 (s, 1H, NH(1)). ¹³C NMR: δ 14.53, 18.27, 54.11, 60.05, 99.94, 127.09, 127.29, 127.41, 128.01, 129.52, 139.83, 140.19, 144.23, 148.88, 152.88, 166.06. MS (EI, m/z): 336 (M⁺). Anal. Calcd for C₂₀H₂₀N₂O₃: C, 71.41; H, 5.99; N, 8.33. Found: C, 71.33; H, 5.91; N, 8.37.

2.4.7 Ethyl 4-(2-nitrophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (4g)

Yield: 90%. Mp: 213–215 °C. IR (KBr): 3112, 1693, 1645, 1524, 1340 cm⁻¹. ¹H NMR δ: 0.93 (t, 3H, J = 7.6 Hz), 2.29 (s, 3H), 3.83 (q, 2H, J = 7.6 Hz), 5.83 (s, 1H), 7.48–7.50 (m, 2H), 7.66 (s, 1H, NH (3)), 7.70 (d, 2H, J = 9 Hz), 9.36 (s, 1H, NH (1)). ¹³C NMR δ: 13.58, 17.49, 49.20, 58.97, 97.92, 123.67, 128.45, 128.87, 133.85, 139.13, 146.62, 149.42, 150.60, 165.32. MS (EI, m/z): 305 (M⁺). Anal. Calcd for C₁₄H₁₅N₃O₅: C, 55.08; H, 4.95; N, 13.76. Found: C, 55.10; H, 4.91; N, 13.87.

2.4.8 Methyl 4-(2-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (4h)

Yield: 89%. Mp: 224–226 °C; IR: 3310, 3250, 1701, 1645 cm⁻¹. ¹H NMR (DMSO-d6) δ: 2.29 (s, 3H), 3.68 (s, 3H), 5.31 (s, 1H), 7.30–7.52 (m, 4H), 7.53 (s, 1H, NH(3)), 9.31 (s, 1H, NH(1)). ¹³C NMR δ: 17.85, 54.41, 59.23, 101.83, 126.13, 127.23, 127.51, 128.22,
140.11, 144.54, 150.21, 152.13 and 165.58. MS (EI, m/z): 280 (M⁺) 282 (M+2) Anal. Calcd for C₁₃H₁₃ClN₂O₃: C, 55.62; H, 4.67; N, 9.98. Found: C, 55.43; H 4.72; N, 9.97.

2.4.9 Ethyl 4-(2-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one-5-carboxylate (4i)

Yield: 91%. Mp: 213–215 °C. IR (KBr): 3363, 3225, 3100, 1690, 1650 cm⁻¹. ¹H NMR δ: 1.03 (t, 3H, J = 7.5 Hz), 2.45 (s, 3H), 3.98 (q, 2H, J = 7.5 Hz), 5.64 (s, 1H, NH(1)), 5.88 (d, 1H, J = 2.4 Hz), 7.20–7.24 (m, 3H), 7.36–7.39 (m, 1H), 7.87 (s, 1H, NH(1)).

¹³C NMR δ: 15.21, 17.88, 53.17, 58.03, 103.13, 127.23, 128.14, 129.32, 136.54, 140.11, 147.21, 152.37, 156.39 and 165.93. MS (EI, m/z): 294 (M⁺) 296 (M+2) Anal. Calcd for C₁₄H₁₄ClN₂O₃: C, 57.05; H, 5.13; N, 9.50. Found: C, 57.02; H, 5.10; N, 9.52.
2.5 RESULTS AND DISCUSSION

DHPMs scaffolds are exhibiting wide range of biological and medicinal activities. DHPMs have emerged as second generation (Rovnyak et al., 1995) substitute for the well-established calcium channel antagonists, Hantzsch dihydropyridines (DHPs). As early as 1930s, its simple derivative, 4-chlorophenyl-2-thio-DHPM was patented as an agent for the protection of wool against moths (Ertan et al., 1944). Later, the interest has focused on the antiviral activity of Biginelli compounds, eventually leading to the excellent antiviral agent, nitractin (Hurst and Ann, 1962). The very same compound also exhibits modest antibacterial activity.

2.5.1 Synthesis of 4-(substituted)-3,4-dihydropyrimidinones

The synthetic pathway employed in the preparation of 4-substituted-3,4-dihydropyrimidinones is outlined in Scheme 1. DHPMs were prepared readily by heating 1,3-dicarbonyl compounds 1, urea 3, and aromatic aldehydes 2 in acetic acid under microwave irradiation conditions. The formation of the products was monitored through TLC and irradiation was continued for appropriate time until completion of the reaction. The structures of the compounds 4a–i were confirmed by spectral (Spectrum 1.1 and 1.2), elemental analyses, and comparison with the available literature data (Ma et al., 2000; Shanmugam and Perumal, 2003; Shanmugam et al., 2003).
The formation of 4 was confirmed by $^1$H NMR spectra (Spectrum 1.1), which displays the CH (4) peaks at $\delta$ 5.0-6.0. IR spectra confirm the presence of the carbonyl peaks of amide and ester at 1650 and 1690 cm$^{-1}$ respectively and intense NH absorptions at 3400-3200 cm$^{-1}$. Mass spectra show the base peak corresponding to (M$^+$-Ar) at 183 (for ethyl acetoacetate series), and elemental analysis additionally corroborated the results. Tabulation of results are depicted below (Table 1.1).
Table 1.1 Synthesis of 3,4-Dihydropyrimidin-2(1H)-ones

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>R</th>
<th>R’</th>
<th>R”</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>Ethyl</td>
<td>3-hydroxy phenyl</td>
<td>Methyl</td>
<td>84</td>
</tr>
<tr>
<td>4b</td>
<td>Ethyl</td>
<td>Isopropyl</td>
<td>Methyl</td>
<td>88</td>
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<tr>
<td>4c</td>
<td>Phenyl</td>
<td>3-hydroxy phenyl</td>
<td>Methyl</td>
<td>85</td>
</tr>
<tr>
<td>4d</td>
<td>Ethyl</td>
<td>4-hydroxy phenyl</td>
<td>Methyl</td>
<td>88</td>
</tr>
<tr>
<td>4e</td>
<td>Ethyl</td>
<td>4-methoxy phenyl</td>
<td>Methyl</td>
<td>84</td>
</tr>
<tr>
<td>4f</td>
<td>Ethyl</td>
<td>Biphenyl</td>
<td>Methyl</td>
<td>70</td>
</tr>
<tr>
<td>4g</td>
<td>Ethyl</td>
<td>2-nitro phenyl</td>
<td>Methyl</td>
<td>90</td>
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<td>4h</td>
<td>Methyl</td>
<td>2-chloro phenyl</td>
<td>Methyl</td>
<td>89</td>
</tr>
<tr>
<td>4i</td>
<td>Ethyl</td>
<td>2-chloro phenyl</td>
<td>Methyl</td>
<td>91</td>
</tr>
</tbody>
</table>

2.5.2 Cardiovascular activity

Cardiovascular diseases refer to the class of diseases that involve the heart and/or blood vessels. Over the last several decades, clinical research on cardiovascular disease has advanced and improved tremendously. This has resulted in prevention as well as treatment of America’s number one killer of men and women of all races. The American Heart Association estimates that more than 50% of deaths are related to some form of cardiovascular diseases. (Ahluwalia and Madhu Chopra, 2008) The cardiovascular effects of DHPMs were evaluated on an isolated perfused frog heart at various dose levels and compared with the activity of digoxin under identical experimental conditions and the recordings were noted. To elucidate the mechanism of action, the interaction of selected compounds with β-blocker and calcium channel blocker was also investigated (Figs. 1.6–1.14) and the results are presented in the Table 1.2-1.4.
In the present investigation compounds 4a–d showed dose-dependent increase in force of contraction (positive inotropic action), decrease in rate of contraction (negative chronotropic action), and an increase in cardiac output. Among these compounds 4d was more potent than digoxin. This may be contributed by the more potent calcium channel agonistic S enantiomer (Kappe, 2000b) in the racemic mixture of R and S. Change of ‘ethyl’ (compound 4a) by ‘phenyl’ (compound 4c) substitution in the ester portion of the DHPM does not have marked effect on cardiovascular activity. Compound 4e showed negative chronotropic action, increase in cardiac output, positive inotropic action, and was not dose-dependent. Compound 4f at lower dose levels (at 5 and 50 μg/ml) did not show enhanced activity but at higher dose levels (at 500 μg/ml and 1 mg/ml) showed comparable positive inotropic action and an increase in cardiac output. Compound 4g showed no change in rate, force of contraction of the heart and cardiac output as expected (Kappe, 2000b). Compounds 4h and 4i were found to exhibit negative inotropic, chronotropic action, and decrease in cardiac output (Kappe, 2000b). Further 4h and 4i blocked the effect of adrenalin (5 μg/ml), thereby showing β-adrenergic blocking activity.

The positive inotropic action of the compounds (4a–f) was not blocked by metoprolol (a cardioselective β-adrenergic blocker) but significantly blocked by the calcium channel blocker—verapamil. Since verapamil blocks the cardiotonic action of the compounds (4a–f), these compounds might have produced their action by opening the voltage sensitive slow Ca^{2+} channel.
2.5.3 Antibacterial activity

Compounds 4a-i was tested against *Escherichia coli* (ATCC 11775) and *Staphylococcus aureus* (ATCC 12600) at 100 µg concentration. Compounds 4e, 4h and 4i showed significant antibacterial activity. Compounds 4a, 4b, 4c, 4d, 4f and 4g did not show any activity (Table 1.5 and Fig. 1.15).

2.6 SUMMARY
In summary, description of the present work on the synthesis, antibacterial and pharmacological effect of DHPMs on frog heart. The study deals with the synthesis of DHPMs by Biginelli reaction using aldehyde, β-keto ester and urea in the presence of acetic acid as catalyst. The results obtained clearly indicate that the compounds 4a–f discussed here showed good cardiotonic activity. Compounds 4h and 4i evinced β-adrenergic receptor antagonistic activity. Compound 4d appears to be the most interesting derivative in our series and more potent than the digoxin. It can be a better choice for the existing cardiotonic drugs in the treatment of congestive heart failure and to confirm this, further studies are to be carried out in other laboratory animals. Compounds 4e, 4h and 4i showed significant antibacterial activity. This observation may promote the synthesis of more active DHPMS in future. The development of methods for the enantioselective synthesis of chiral DHPMs using chiral catalysts and enzymes is underway since individual enantiomers of chiral DHPMs have opposing pharmacological effects and the use of enantiomerically pure compounds are a requirement for improving the efficacy of drugs of this type.
Chapter I

2.7 REFERENCES


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Cho, H., Ueda, M., Shima, K., Mizuno, A., Hayashimatsu, M., Ohnaka, Y., TakY., Hamguchi, M., Aisaka, K., Hidaka, T., Kawai, M., Takeda, M., Ishihara, T.,


Chapter I


Chapter I


Chapter I

Spectrum 1.1 $^1H$ NMR of DHPM (4e)
Chapter I

Spectrum 1.2  $^{13}$C NMR of DHPM (4e)
Table 1.2  The effects of dihydropyrimidinone derivatives (Force of Contraction) on frog heart

<table>
<thead>
<tr>
<th>Entry</th>
<th>Control$^a$</th>
<th>5 μg</th>
<th>50 μg</th>
<th>100 μg</th>
<th>500 μg</th>
<th>1 mg</th>
<th>Metoprolol</th>
<th>Verapamil</th>
</tr>
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<tbody>
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<td></td>
<td>FC (mm)</td>
<td>FC (mm)</td>
<td>FC (mm)</td>
<td>FC (mm)</td>
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<td>FC (mm)</td>
<td>FC (mm)</td>
</tr>
<tr>
<td>4a</td>
<td>13 ± 0.58</td>
<td>20 ± 0.58</td>
<td>25 ± 0.58</td>
<td>28 ± 0.58</td>
<td>30 ± 0.82</td>
<td>31 ± 0.58</td>
<td>31 ± 0.58</td>
<td>03 ± 0.58</td>
</tr>
<tr>
<td>4b</td>
<td>12 ± 0.57</td>
<td>24 ± 0.82</td>
<td>27 ± 0.58</td>
<td>29 ± 0.82</td>
<td>31 ± 0.82</td>
<td>33 ± 0.82</td>
<td>24 ± 0.82</td>
<td>03 ± 0.37</td>
</tr>
<tr>
<td>4c</td>
<td>11 ± 0.77</td>
<td>21 ± 0.82</td>
<td>24 ± 1.15</td>
<td>26 ± 1.63</td>
<td>33 ± 1.29</td>
<td>35 ± 1.39</td>
<td>33 ± 0.52</td>
<td>03 ± 0.26</td>
</tr>
<tr>
<td>4d</td>
<td>15 ± 1.31</td>
<td>51 ± 1.63</td>
<td>59 ± 1.96</td>
<td>63 ± 1.39</td>
<td>67 ± 1.03</td>
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<td>64 ± 1.63</td>
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<tr>
<td>4e</td>
<td>15 ± 0.82</td>
<td>24 ± 0.57</td>
<td>24 ± 1.15</td>
<td>24 ± 1.15</td>
<td>24 ± 0.58</td>
<td>24 ± 1.50</td>
<td>24 ± 0.77</td>
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<td>4f</td>
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<td>27 ± 0.58</td>
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<td>35 ± 1.39</td>
<td>35 ± 1.15</td>
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<td>4h</td>
<td>11 ± 0.58</td>
<td>04 ± 0.26</td>
<td>03 ± 0.37</td>
<td>03 ± 0.58</td>
<td>02 ± 0.26</td>
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<tr>
<td>4i</td>
<td>12 ± 0.58</td>
<td>05 ± 0.36</td>
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<td>01 ± 0.37</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Digoxin</td>
<td>11 ± 0.26</td>
<td>40 ± 1.39</td>
<td>47 ± 0.93</td>
<td>-</td>
<td>51 ± 1.03</td>
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$^a$ 1% Sodium carboxy methyl cellulose as control. n=6, values are mean± SEM. All compounds showed p< 0.01 when compared with control (one–way ANOVA) except entry 4g.
Fig. 1.6 The effects of dihydropyrimidinone derivatives (force of contraction) on frog heart
Table 1.3 The effect of dihydropyrimidinone derivatives (Heart Rate) on frog heart

<table>
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<tr>
<th>Entry</th>
<th>Control(^a)</th>
<th>5 µg</th>
<th>50 µg</th>
<th>100 µg</th>
<th>500 µg</th>
<th>1 mg</th>
<th>Metoprolol</th>
<th>Verapamil</th>
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<tr>
<td></td>
<td>HR (bpm)</td>
<td>HR (bpm)</td>
<td>HR (bpm)</td>
<td>HR (bpm)</td>
<td>HR (bpm)</td>
<td>HR (bpm)</td>
<td>HR (bpm)</td>
<td>HR (bpm)</td>
</tr>
<tr>
<td>4a</td>
<td>80 ± 0.58</td>
<td>70 ± 0.58</td>
<td>68 ± 0.42</td>
<td>68 ± 1.16</td>
<td>68 ± 1.12</td>
<td>68 ± 1.11</td>
<td>68 ± 0.37</td>
<td>17 ± 0.37</td>
</tr>
<tr>
<td>4b</td>
<td>78 ± 0.58</td>
<td>58 ± 0.57</td>
<td>56 ± 0.58</td>
<td>57 ± 0.58</td>
<td>56 ± 0.57</td>
<td>56 ± 0.58</td>
<td>58 ± 0.26</td>
<td>21 ± 0.26</td>
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<tr>
<td>4c</td>
<td>78 ± 1.39</td>
<td>65 ± 1.65</td>
<td>650 ± 2.69</td>
<td>650 ± 0.29</td>
<td>65 ± 1.15</td>
<td>65 ± 1.65</td>
<td>64 ± 0.82</td>
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<tr>
<td>4d</td>
<td>97 ± 1.39</td>
<td>87 ± 1.39</td>
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<td>77 ± 1.65</td>
<td>64 ± 0.82</td>
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<td>94 ± 0.75</td>
<td>94 ± 1.12</td>
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<td>94 ± 1.11</td>
<td>94 ± 0.75</td>
<td>94 ± 1.16</td>
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<td>4f</td>
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<td>93 ± 1.32</td>
<td>94 ± 0.52</td>
<td>95 ± 0.73</td>
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<td>4h</td>
<td>97 ± 2.88</td>
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<td>84 ± 0.91</td>
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<td>96 ± 2.06</td>
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<td>78 ± 0.93</td>
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<tr>
<td>Digoxin</td>
<td>97 ± 0.82</td>
<td>70 ± 0.76</td>
<td>68 ± 1.06</td>
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<td>68 ± 0.93</td>
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</table>

\(^a\) 1% Sodium carboxy methyl cellulose as control. \(n=6\), values are mean ± SEM. All compounds showed \(p < 0.01\) when compared with control (one-way ANOVA) except entry 4g.
Fig. 1.7 The effects of dihydropyrimidinone derivatives (heart rate) on frog heart
Table 1.4 The effects of Dihydropyrimidinone derivatives (cardiac output-CO) on frog heart

<table>
<thead>
<tr>
<th>Entry</th>
<th>Control(^a)</th>
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<th>50 µg CO(\text{ml}/\text{m})</th>
<th>100 µg CO(\text{ml}/\text{m})</th>
<th>500 µg CO(\text{ml}/\text{m})</th>
<th>1 mg CO(\text{ml}/\text{m})</th>
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<td>12 ± 0.032</td>
<td>11 ± 0.15</td>
<td>03 ± 0.037</td>
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<td>4b</td>
<td>15 ± 0.82</td>
<td>17 ± 0.021</td>
<td>17 ± 1.16</td>
<td>18 ± 0.82</td>
<td>18.4 ± 1.39</td>
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<td>17 ± 0.021</td>
<td>06 ± 0.58</td>
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<td>4c</td>
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<td>23.1 ± 0.021</td>
<td>24.2 ± 0.75</td>
<td>-</td>
<td>24± 0.82</td>
<td>07 ± 1.15</td>
</tr>
<tr>
<td>4e</td>
<td>15 ± 0.021</td>
<td>16 ± 1.16</td>
<td>16.1±0.037</td>
<td>16.8 ± 0.29</td>
<td>17 ± 1.12</td>
<td>17.1 ± 0.52</td>
<td>17 ± 0.58</td>
<td>07 ± 0.93</td>
</tr>
<tr>
<td>4f</td>
<td>19 ± 1.63</td>
<td>20 ± 1.11</td>
<td>22 ± 0.058</td>
<td>22 ± 0.021</td>
<td>23 ± 1.16</td>
<td>23 ± 0.58</td>
<td>23 ± 0.082</td>
<td>06 ± 0.037</td>
</tr>
<tr>
<td>4g</td>
<td>20 ± 0.058</td>
<td>20 ± 0.021</td>
<td>20 ± 0.58</td>
<td>20 ± 1.39</td>
<td>20 ± 0.91</td>
<td>20 ± 1.65</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4h</td>
<td>20 ± 0.58</td>
<td>11 ± 1.39</td>
<td>9.1 ± 0.93</td>
<td>8.8 ± 1.97</td>
<td>8.4 ± 0.021</td>
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</tr>
<tr>
<td>4i</td>
<td>18 ± 0.42</td>
<td>06 ± 0.76</td>
<td>4.5 ± 1.11</td>
<td>4.3 ± 0.021</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Digoxin</td>
<td>14 ± 1.15</td>
<td>18 ± 1.16</td>
<td>18.4 ± 0.58</td>
<td>-</td>
<td>19.1 ± 0.82</td>
<td>-</td>
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</table>

\(^a\) 1% Sodium carboxy methyl cellulose as control. n=6, values are mean\(\pm\) SEM. All compounds showed \(p < 0.01\) when compared with control (one –way ANOVA) except entry 4g.
Chapter I

Fig. 1.8 The effects of dihydropyrimidinone derivatives (cardiac output-CO) on frog heart

Fig. 1.9 Cardiogram of Frog for Digoxin and compound 4a
Chapter I

Fig. 1.10 Cardiogram of Frog for compound 4b

Fig. 1.11 Cardiogram of Frog for compounds 4c and 4d
Fig. 1.12 Cardiogram of Frog for compounds 4d and 4e

Fig. 1.13 Cardiogram of Frog for compounds 4f and 4g
Chapter I

Fig. 1.14  Cardiogram of Frog for compounds 4h and 4i
Chapter I
Table 1.5 Antibacterial activity of DHPMs

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Zone of inhibition (mm)</th>
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<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>4a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4d</td>
<td>-</td>
<td>-</td>
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<tr>
<td>4e</td>
<td>10.0**</td>
<td>10.5**</td>
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<tr>
<td>4f</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4g</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4h</td>
<td>13.0**</td>
<td>9.0*</td>
</tr>
<tr>
<td>4i</td>
<td>9.5**</td>
<td>9.0*</td>
</tr>
<tr>
<td>Control</td>
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<td>8.0</td>
</tr>
<tr>
<td>Standard</td>
<td>Ciprofloxacin-35** (5 μg)</td>
<td>Gentamicin-30** (10 μg)</td>
</tr>
</tbody>
</table>

* P<0.01, ** P<0.001 when compared with control (student’s ‘t’ – test)
Fig. 1.15 Antibacterial activity of DHPMs

Compounds and DMSO control tested against *Staphylococcus aureus*