European Journal of Medicinal Chemistry 54 (2012) 223–231

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Regioselective N1-alkylation of 3,4-dihydropyrimidine-2(1*H*)-ones: Screening of their biological activities against Ca^{2+}-ATPase

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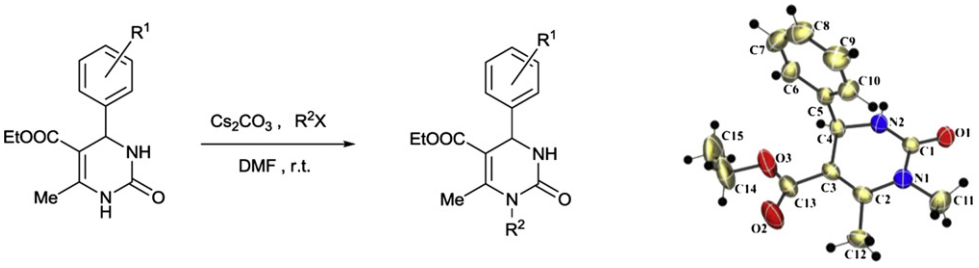
GRAPHICAL ABSTRACT



HIGHLIGHTS

- A Regioselective N1-Alkylation of 3, 4-dihydropyrimidine-2(1H)-ones has been done.
- Alkylation was done by using Cs_2CO_3 and alkyl halides at room temperature.
- Regioselectivity is excellent and the yields of the alkylated products are very good.
- Inhibitory action of 3, 4-DHPMs and their derivatives were tested on Ca^{2+}-ATPase.

ARTICLE INFO

Article history:
Received 6 March 2012
Received in revised form
28 April 2012
Accepted 30 April 2012
Available online 16 May 2012

Keywords:
N1-Alkylated dihydropyrimidones
Regioselective synthesis
Ca^{2+}-ATPase

ABSTRACT

A regioselective N1-alkylation of 3,4-dihydropyrimidin-2(1H)-ones using a very efficient mild base Cs_2CO_3 and alkyl halides at room temperature has been reported. The selectivity of this methodology is excellent and the yields of the alkylated products are very good. Furthermore inhibitory action of both the 3,4-dihydropyrimidin-2(1H)-ones and the N1-alkylated derivatives were tested on Ca^{2+}-ATPase, which revealed that the parent compounds can act as Ca^{2+}-ATPase inhibitors whereas the N1-alkylated derivatives are inefficient for this purpose.

1. Introduction

Dihydropyrimidones (DHPMs) have a wide range of synthetic importance due to the six diversity points around the heterocyclic core. DHPMs have been reported to possess diverse biological

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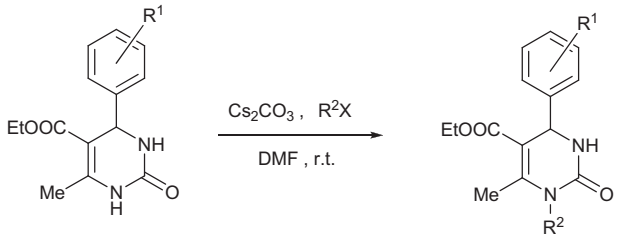
doi:10.1016/j.ejmech.2012.04.043



Scheme 1.

activities such as antiviral, antibacterial and antitumor effects and more recently DHPMs have emerged as the integral backbone of several calcium channel blockers [1–7]. In this report we have demonstrated the role of some synthetic compounds belonging to the DHPM group as inhibitors of the Ca^{2+}-ATPase.

Closely related to the calcium channels are the Ca^{2+}-ATPases - the conventional Mg^{2+}-dependent Ca^{2+}-ATPase, present in most mammalian tissues along with Mg^{2+}-independent Ca^{2+}-ATPase, enriched in the reproductive tissue [8–10]. They control Ca^{2+}-homeostasis in cells and maintain a resting cytoplasmic level of Ca^{2+}, necessary for the normal functioning of a cell. Additionally, they have been implicated in regulation of sperm motility and hence fertility [11,12].

Modification of DHPM moiety for the preparation of novel bioactive compounds encouraged us to develop an efficient methodology of N1-alkylation as it is least attempted, most probably due to its demands for regioselectivity. The achievement of regioselectivity is difficult because of the small difference in acidity of N1 and N3 hydrogens. There are only a few literature available describing N1-alkylation of DHPMs and they have their limitations in terms of yield, reagent, reaction conditions and selectivity.

Kappe and coworkers introduced several methods for the preparation of N1-monoalkyl derivatives through Biginelli cyclocondensation reaction by using N-monoalkylated urea [13–20]. The yields are not satisfactory in most of the cases and the commercial availability of substituted ureas are limited, thereby making variation in the alkyl group is often troublesome. Latter on Kappe et al. developed a good yielding method for monoalkylation of DHPM scaffold using Mitsunobu-type conditions [21], but the reagents used are reasonably expensive and hazardous. Another direct alkylation method had been demonstrated by Singh et al. [22], where strong aqueous alkali was used. The reaction condition is not suitable for most of the alkyl halides and a huge excess of alkyl halide is required. Thus a facile and good yielding method still has its importance.

In the present study not only a simple inexpensive and high yielding method for the synthesis of DHPM derivatives are described, their biological importance has also been studied.

2. Synthetic methodology and discussion

We have found that highly regioselective N1-alkylation of DHPMs could be achieved by using Cs_2CO_3 and alkyl halides (in some cases Bu_4NI is required for better yield) in DMF at room temperature (Scheme 1). The various non-alkylated DHPM moieties (**1**–**6**) used in this report are synthesized through Biginelli one pot condensation reaction following the reported method [23–25].

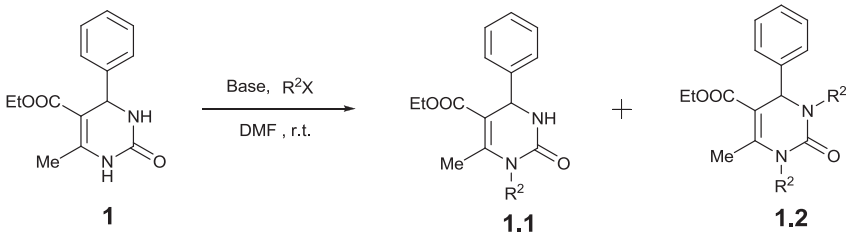
Cesium salts are well known for their unique physical and chemical properties, commonly called “cesium effect” [26–28]. In this transformation Cs_2CO_3 was chosen due to the following reasons-

i) Cs_2CO_3 is a moderately strong base which is found effective to capture a proton selectively from the N1 position in dihydropyrimidones.
ii) It is appreciably soluble in many organic solvents unlike other alkali metal carbonates.

Different mild bases like CsF, $LiOH.H_2O$, K_2CO_3 were tested (Table 1) but Cs_2CO_3 was found to be the most efficient in terms of yield and selectivity for different alkyl halides. Reaction using $LiOH.H_2O$ was very fast but it produced the dialkyl product from the very beginning of the reaction. It must be mentioned that DMSO is also a suitable solvent for this reaction whereas acetonitrile and acetone were not at all effective (Table 2).

The present method demonstrates a highly regioselective synthesis of N1-alkyl DHPMs by using 1.3 equiv. of Cs_2CO_3 and 1.5

Table 1
Optimization of the reaction conditions.



Entry	Electrophile R^2X	Mole Equivalent	Base (1.3 mol eq)	Additive	Reaction time (h)	Yield (%)	
						Mono alkyl (1.1)	Di alkyl (1.2)
1	$PhCH_2Br$	1.5	Cs_2CO_3	–	5	72	–
2	$PhCH_2Br$	1.5	CsF	–	5	65	10
3	$PhCH_2Br$	1.5	$LiOH.H_2O$	–	5	60	22
4	$PhCH_2Br$	1.5	K_2CO_3	–	5	Trace	–
5	n-$C_8H_{17}Br$	2	Cs_2CO_3	–	24	56	–
6	n-$C_8H_{17}Br$	2	Cs_2CO_3	–	24	60[a]	–
7	n-$C_8H_{17}Br$	2	Cs_2CO_3	Bu_4NI[b]	24	77	–
8	n-$C_8H_{17}Br$	2	CsF	Bu_4NI[b]	24	30	–

[a] Under elevated temperature (60 °C).
[b] 1eq. Bu_4NI was added before the addition of alkyl halide.

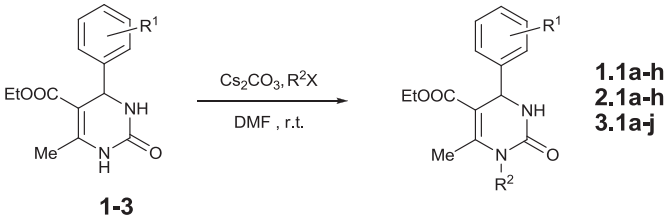
Table 2
Results of the alkylation of compound **1** in various solvents.

Entry	Electrophile R^2X	Solvent	Base	Reaction time (h)	Yield (%)
1	CH_3I	Anhydrous DMF	Cs_2CO_3	17	94
2	CH_3I	Anhydrous DMSO	Cs_2CO_3	17	93
3	CH_3I	Anhydrous Acetone	Cs_2CO_3	17	Trace
4	CH_3I	Anhydrous Acetonitrile	Cs_2CO_3	17	Trace

equiv. of alkyl halide in anhydrous DMF at room temperature. Most of the alkyl halides furnished N1-alkylated DHPMs with excellent yields under this condition until and unless mentioned (Table 3). DHPM **1**, **2** and **3** produced respectively 94%, 92% and 90% N-methyl derivative, no dialkylated or N2-alkylated product was isolated. For n-butyl bromide the yields were 83%, 73% and 73% respectively. Both Benzyl bromide and benzyl tosylate were found to be equally efficient electrophile for this reaction, 72% and 75% respective yields for compound **1.1f** were obtained.

Long chain alkyl halides like n-octyl, n-decyl and n-hexadecyl bromides were less reactive under the reaction condition and furnished N1-alkylated product with poor yield. A modified method was developed on using tetrabutylammonium iodide as additive, which leads to very good yield with selectivity for all the three

Table 3
Preparation of N1-alkylated DHPMs with different alkyl halides.



1-3 **1.1a-h** **2.1a-h** **3.1a-j**

Entry	DHPM	R^2X	Reaction time (h)	Additive	N1-alkyl DHPM	Yield (%)
1	**1** (R^1=H)	CH_3I	17	–	**1.1a**	94
2		n-C_4H_9Br	20	–	**1.1b**	83
3		n-$C_8H_{17}Br$	24	Bu_4NI	**1.1c**	77
4		n-$C_{10}H_{21}Br$	24	Bu_4NI	**1.1d**	78
5		n-$C_{16}H_{33}Br$	24	Bu_4NI	**1.1e**	74
6		$PhCH_2Br$	5	–	**1.1f**	72
7		$PhCH_2OTs$	4	–	**1.1f**	75
8		CH_2=$CHCH_2Br$	20 min	–	**1.1g**	82[a]
9		PhCH=$CHCH_2Cl$	17	–	**1.1h**	73
10	**2** (R^1 = p-Cl)	CH_3I	17	–	**2.1a**	92
11		n-C_4H_9Br	20	–	**2.1b**	73
12		n-$C_8H_{17}Br$	24	Bu_4NI	**2.1c**	73
13		n-$C_{10}H_{21}Br$	24	Bu_4NI	**2.1d**	76
14		n-$C_{16}H_{33}Br$	24	Bu_4NI	**2.1e**	73
15		$PhCH_2Br$	5	–	**2.1f**	69
16		$PhCH_2OTs$	4	–	**2.1f**	70
17		CH_2=$CHCH_2Br$	20 min	–	**2.1g**	78[a]
18		PhCH=$CHCH_2Cl$	17	–	**2.1h**	69
19	**3** (R^1=m-NO_2)	CH_3I	17	–	**3.1a**	90
20		n-C_4H_9Br	20	–	**3.1b**	73
21		n-$C_8H_{17}Br$	24	Bu_4NI	**3.1c**	72
22		n-$C_{10}H_{21}Br$	24	Bu_4NI	**3.1d**	71
23		n-$C_{16}H_{33}Br$	24	Bu_4NI	**3.1e**	68
24		$PhCH_2Br$	5	–	**3.1f**	68
25		$PhCH_2OTs$	4	–	**3.1f**	70
26		CH_2=$CHCH_2Br$	20 min	–	**3.1g**	76[a]
27		PhCH=$CHCH_2Cl$	17	–	**3.1h**	68
28		$PhCH_2CH_2Br$	24	–	**3.1i**	30
29		$PhCH_2CH_2CH_2Br$	24	–	**3.1j**	62

[a] Only 1.1 eq of Cs_2CO_3 was used and 5–8% dialkylated product was obtained.

DHPMs. Reactions with allyl bromide were very fast but a small amount of dialkyl derivatives (less than 8%) were formed for all the three cases. Cinnamyl chloride showed similar reactivity as benzyl bromide but it required longer time. Other two electrophiles $PhCH_2CH_2Br$ and $PhCH_2CH_2CH_2Br$ were also used in the reaction with DHPM **3**, the yields were 30% and 62% respectively.

All the N1-alkylated DHPMs were characterized by ^1H NMR, ^{13}C NMR, CHN elemental analysis and Mass spectroscopy. The position of alkylation was confirmed from the ^1H NMR splitting pattern of C-4 proton. In the alkylated product the C-4 proton appeared as doublet on coupling with adjacent N3-proton with coupling constant 3 Hz. Finally the structure of the compound **1.1a** was confirmed from X-ray crystallography analysis (Fig. 1). In the process of single crystal formation from the racemic mixture of the compound **1.1a** only the *R*-isomer was crystallized out (**CCDC 865427**). This phenomenon was reported by different workers as "chiral amnesia" [29,30].

3. Biological results and discussion

The inhibitory activities of the unsubstituted DHPMs and N1-alkyl DHPMs were examined on Mg^{2+}-dependent and independent Ca^{2+}-ATPase according to the previously established procedures [31]. The percentage of inhibition at different concentration of the inhibitors indicates the efficiency of the DHPMs (Fig. 2). The pre-incubation of the DHPM derivatives with the enzyme was done for about 5 min. However prolonging the incubation time did not significantly affect the percentage of inhibition. Increase in the concentration of the enzyme also did not produce any notable change in the percentage of inhibition. The I_{50} values of the DHPM derivatives were determined by measuring the enzyme activities at different concentrations of the compounds at a fixed concentration of the enzyme following the same above mentioned assay procedure.

Among all DHPM derivatives, compound **1** to **6** were found to be most potent in their inhibitory action. They differ only in the aromatic nucleus present at C-4 position of the heterocyclic core. The I_{50} values are listed in the Table 4. This is pertinent to mention that the N1-alkylated derivatives were found to be less potent or not at all effective when compared with their parent non-alkylated compounds (data not shown). These DHPM derivatives also showed an additive inhibitory effect in the presence of Verapamil, another potent Ca^{2+}-ATPase inhibitor (Fig. 3), clearly indicating that they possess different binding sites in the enzyme. These

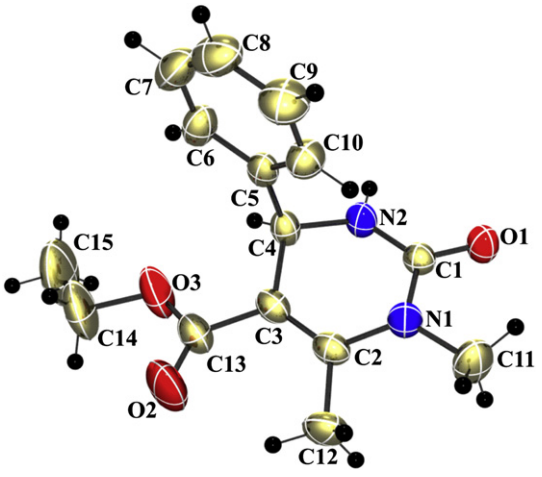


Fig. 1. Single crystal X-ray structure of compound **1.1a** (***R***-isomer).

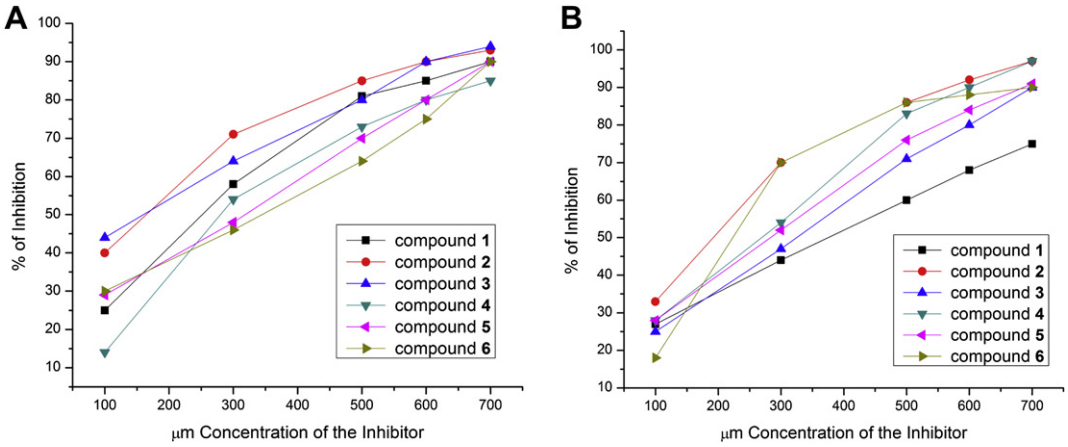


Fig. 2. Inhibitory activity of DHPMs on (A) Mg^{2+}-dependent Ca^{2+}-ATPase and (B) Mg^{2+}-independent Ca^{2+}-ATPase.

DHPM derivatives did not exhibit any significant effect on Na^+, K^+-ATPase.

4. Conclusion

It is worth remarking that by using this methodology it is possible to prepare variously substituted N1-alkyl DHPMs. In general the alkylation is highly efficient in terms of yield and selectivity. The reaction procedure is very simple, the reagents are not very costly and are easily available. Various alkyl halides can be used without any special precaution, which indicates the generality of this reaction. Moreover the study of inhibitory action reveals good potentiality of DHPM derivatives on both Mg^{2+}-dependent and Mg^{2+}-independent Ca^{2+}-ATPase activity which might be further investigated so as to develop compounds/drugs for a wide number of diseases. The novel N1-alkylated products are not good inhibitors and they have solubility problems in the assay condition.

5. Experimental

All reagents used were of analytical grades and obtained from recognized commercial suppliers and were used without further purification unless otherwise stated. Column chromatography was performed on SRL 100–200 mesh silica gel. Thin Layer chromatography was performed on Merck Silica gel 60 F_{254} plates. Melting points were determine on a LabX India, Digital Melting Point apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded at ambient temperature using Bruker 300 MHz (Bruker AVANCE 300 or Bruker DPX-300) and Bruker 500 MHz (Bruker Ultrashield Plus 500), FT NMR spectrometers (300 MHz and 500 MHz respectively, for ^1H and 75 MHz and 125 MHz respectively for ^{13}C). HRMS were performed on Waters Micromass Q-tof Micro mass spectrometer. CHN analysis was done on Perkin Elmer 2400 Series II CHN analyzer. Infrared spectra were recorded on Perkin Elmer Spectrum 100 FT-IR spectrometer in KBr pellets.

Table 4
[a]I_{50} values of different DHPM derivatives.

Entry	DHPM derivatives		I_{50} μM	
	R^1	Compound no.	Mg^{2+}-dependent	Mg^{2+}-independent
1.	H	**1**	222	226
2.	p-Cl	**2**	143	185
3.	m-NO_2	**3**	132	282
4.	m-Cl	**4**	242	258
5.	o-Cl	**5**	268	247
6.	p-OH	**6**	289	203

[a] I_{50} is the concentration (in μM) of inhibitors at which half the maximal inhibitory effect is achieved.

5.1. Typical N1-alkylation procedure for the synthesis of ethyl 1,6-dimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1.1a**) [21,22]

In a solution of compound **1** (250 mg, 0.96 mmol) in 3 ml anhydrous DMF, Cs_2CO_3 (407.28 mg, 1.25 mmol) was added. The mixture was stirred at room temperature for 1 h under anhydrous condition. Then methyl iodide (0.09 ml, 1.44 mmol) was added to the reaction mixture slowly and stirred at r.t. for 17 h. After completion of the reaction as indicated by TLC, saturated NaCl solution was added. The reaction mixture was extracted with ethyl acetate (3 × 25 ml). The combined organic layer was washed with water (2 × 50 ml), followed by brine solution (1 × 50 ml). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The crude mass was subjected to column chromatography using 100–200 mesh silica gel when the desired product was obtained by eluting the column with 30% EtOAc/60–80 °C Petrolium ether as solvent system. (94% yield).

White solid mp 176–177 °C (Ethyl acetate/n-Hexane).

^1H NMR (300 MHz, $CDCl_3$): δ 7.32–7.22 (m, 5H, ArH), 5.43 (brs, 1H, NH), 5.38 (d, 1H, $J = 3$ Hz, CH), 4.10 (q, 2H, $J = 7.2$ Hz, ester-CH_2), 3.24 (s, 3H, N–CH_3), 2.51 (s, 3H, CH_3), 1.18 (t, 3H, $J = 7.2$ Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.0, 154.1, 149.3, 143.3, 128.6, 127.6, 126.1, 104.1, 60.1, 53.7, 30.2, 16.5, 14.1.

IR (KBr, cm^{-1}): 3231, 3101, 2946, 1689, 1623, 1445, 1395, 1349, 1246, 1185, 1149, 1082, 1051.

Anal. Calcd for $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.61; N, 10.21. Found: C, 65.59; H, 6.65; N, 10.26.

HRMS (*m/z*) for $C_{15}H_{18}N_2O_3Na$ $[M + Na]^+$: Calculated 297.1215, found 297.1214.

5.2. Ethyl 1-butyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1.1b**) [22]

White solid (83% yield).

mp 106–107 °C (Ethyl acetate/n-Hexane).

^1H NMR (300 MHz, $CDCl_3$): δ 7.31–7.22 (m, 5H, ArH), 5.44 (brs, 1H, NH), 5.35 (d, 1H, $J = 2.7$ Hz, CH), 4.08 (q, 2H, $J = 7.2$ Hz, ester-CH_2), 3.95–3.85 (m, 1H, one H of N–CH_2–), 3.62–3.52 (m, 1H, one H of N–CH_2–), 2.51 (s, 3H, CH_3), 1.62–1.23 (m, 4H, two –CH_2- of n-

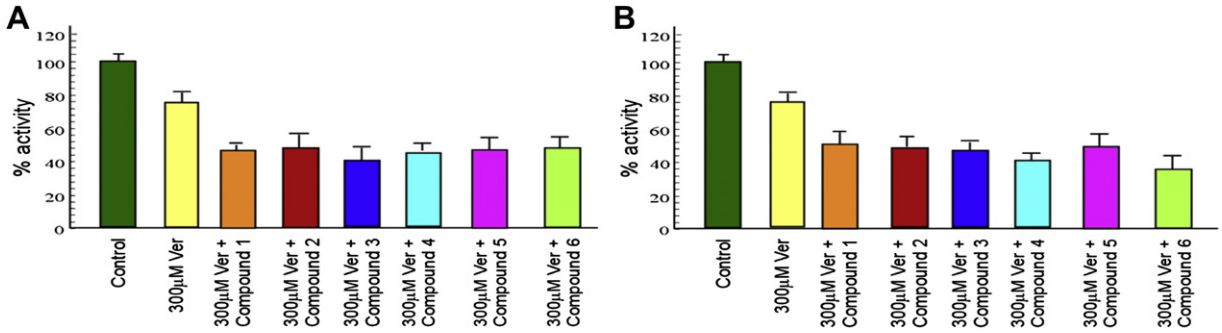


Fig. 3. Effect of verapamil on the ATPase activity in the absence and presence of the DHPM derivatives; (A) Mg^{2+}-dependent Ca^{2+}-ATPase and (B) Mg^{2+}-independent Ca^{2+}-ATPase.

butyl), 1.16 (t, 3H, $J = 7.2$ Hz, ester-CH_3), 0.97–0.88 (m, 3H, $-CH_3$ of n-butyl).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.2, 153.6, 148.8, 143.5, 128.5, 127.63, 126.2, 104.4, 60.1, 53.8, 42.4, 31.8, 19.9, 16.0, 14.1, 13.7.

IR (KBr, cm^{-1}): 3218, 3099, 2960, 1686, 1610, 1466, 1396, 1280, 1206, 1156, 1094.

Anal. Calcd for $C_{18}H_{24}N_2O_3$: C, 68.33; H, 7.65; N, 8.85. Found: C, 68.26; H, 7.62; N, 8.91.

5.3. *Ethyl 6-methyl-1-octyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1.1c**)*

White solid (77% yield).

mp 82–84 °C (Ethyl acetate/n-Hexane).

^1H NMR (300 MHz, $CDCl_3$): δ 7.32–7.24 (m, 5H, ArH), 5.60 (brs, 1H, NH), 5.37 (s, 1H, CH), 4.09 (q, 2H, $J = 7.1$ Hz, ester-CH_2), 3.93–3.85 (m, 1H, one H of N–CH_2–), 3.62–3.54 (m, 1H, one H of N–CH_2–), 2.52 (s, 3H, CH_3), 1.60–1.50 (m, 2H, one $-CH_2$- of n-octyl), 1.25–1.20 (m, 10H, five $-CH_2$- of n-octyl), 1.17 (t, 3H, $J = 7.1$ Hz, ester-CH_3), 0.90–0.86 (m, 3H, $-CH_3$ of n-octyl).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.2, 153.6, 148.8, 143.5, 128.5, 127.6, 126.2, 104.4, 60.1, 53.8, 42.6, 31.7, 29.8, 29.2, 29.2, 26.7, 22.6, 16.0, 14.1, 14.0.

IR (KBr, cm^{-1}): 3220, 3102, 2920, 2853, 1686, 1612, 1468, 1411, 1192, 1158, 1098.

Anal. Calcd for $C_{22}H_{32}N_2O_3$: C, 70.94; H, 8.66; N, 7.52. Found: C, 70.98; H, 8.60; N, 7.66.

5.4. *Ethyl 1-decyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1.1d**) [22]*

White solid (78% yield).

mp 104–105 °C (Ethyl acetate/n-Hexane).

^1H NMR (300 MHz, $CDCl_3$): δ 7.29–7.23 (m, 5H, ArH), 5.37–5.36 (m, 2H, NH & CH), 4.09 (q, 2H, $J = 7.2$ Hz, ester-CH_2), 3.95–3.85 (m, 1H, one H of N–CH_2–), 3.62–3.52 (m, 1H, one H of N–CH_2–), 2.52 (s, 3H, CH_3), 1.60–1.53 (m, 2H, one $-CH_2$- of n-decyl), 1.25–1.19 (m, 14H, seven $-CH_2$- of n-decyl), 1.17 (t, 3H, $J = 7.2$ Hz, ester-CH_3), 0.90–0.86 (t, 3H, $J = 6$ Hz, $-CH_3$ of n-decyl).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.2, 153.6, 148.8, 143.5, 128.5, 127.6, 126.2, 104.4, 60.1, 53.8, 42.6, 31.8, 29.8, 29.5, 29.3, 29.2, 26.7, 22.6, 16.0, 14.1, 14.1.

IR (KBr, cm^{-1}): 3220, 3099, 2923, 2854, 1690, 1611, 1466, 1409, 1357, 1279, 1195, 1160, 1095.

Anal. Calcd for $C_{24}H_{36}N_2O_3$: C, 71.96; H, 9.06; N, 6.99. Found: C, 72.04; H, 9.13; N, 6.91.

5.5. *Ethyl 1-hexadecyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1.1e**)*

White solid (74% yield).

mp 75–76 °C (Ethyl acetate/n-Hexane).

^1H NMR (300 MHz, $CDCl_3$): δ 7.25–7.17 (m, 5H, ArH), 5.52 (brs, 1H, NH), 5.31 (s, 1H, CH), 4.03 (q, 2H, $J = 7.1$ Hz, ester-CH_2), 3.99–3.78 (m, 1H, one H of N–CH_2–), 3.55–3.46 (m, 1H, one H of N–CH_2–), 2.45 (s, 3H, CH_3), 1.93 (m, 4H, two $-CH_2$- of n-hexadecyl), 1.54–1.44 (m, 2H, one $-CH_2$- of n-hexadecyl), 1.19–1.13 (m, 22H, eleven $-CH_2$- of n-hexadecyl), 1.10 (t, 3H, $J = 7.1$ Hz, ester-CH_3), 0.83–0.79 (m, 3H, $-CH_3$ of n-hexadecyl).

^{13}C NMR (125 MHz, $CDCl_3$): δ 166.3, 153.6, 148.9, 143.7, 128.8, 127.9, 126.4, 104.6, 60.3, 54.3, 42.9, 32.1, 30.0, 29.8, 29.8, 29.7, 29.5, 27.0, 22.8, 16.3, 14.3, 14.2.

IR (KBr, cm^{-1}): 3220, 3100, 2921, 2849, 1690, 1612, 1466, 1398, 1279, 1194, 1155, 1095.

Anal. Calcd for $C_{30}H_{48}N_2O_3$: C, 74.34; H, 9.98; N, 5.78. Found: C, 74.29; H, 9.85; N, 5.89.

5.6. *Ethyl 1-benzyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1.1f**) [16,21]*

White solid (72% yield).

mp 156–157 °C (Ethyl acetate/n-Hexane).

^1H NMR (300 MHz, $CDCl_3$): δ 7.30–7.23 (m, 8H, ArH), 7.14–7.12 (m, 2H, ArH), 5.64 (brs, 1H, NH), 5.44 (d, 1H, $J = 3$ Hz, CH), 5.19 (d, 1H, $J = 16.5$ Hz, one H of N–CH_2–), 4.89 (d, 1H, $J = 16.5$ Hz, one H of N–CH_2–), 4.09 (q, 2H, $J = 7.2$ Hz, ester-CH_2), 2.44 (s, 3H, CH_3), 1.15 (t, 3H, $J = 7.2$ Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.0, 154.1, 149.0, 143.2, 137.9, 128.6, 128.6, 127.7, 127.1, 126.4, 126.3, 104.8, 60.2, 53.7, 45.9, 16.4, 14.1.

IR (KBr, cm^{-1}): 3216, 3099, 2930, 1691, 1614, 1445, 1382, 1306, 1201, 1164, 1109.

Anal. Calcd for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 7.99. Found: C, 71.90; H, 6.21; N, 7.93.

5.7. *Ethyl 1-allyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1.1g**) [22]*

Pale yellow solid (82% yield).

mp 128–129 °C (Ethyl acetate/n-Hexane).

^1H NMR (300 MHz, $CDCl_3$): δ 7.32–7.23 (m, 5H, ArH), 5.90–5.81 (m, 1H, one H of N–CH_2–C<u>H</u>=), 5.70 (brs, 1H, NH), 5.41 (d, 1H, $J = 1.9$ Hz, CH), 5.18–5.08 (m, 2H, two H of N–CH_2–CH=C<u>H</u>$_2$), 4.47 (dd, 1H, $J_1 = 4.7$ Hz, $J_2 = 17.1$ Hz, one H of N–CH_2–), 4.35 (dd, 1H, $J_1 = 4.5$ Hz, $J_2 = 17.1$ Hz, one H of N–CH_2–), 4.09 (q, 2H, $J = 7.1$ Hz, ester-CH_2), 2.51 (s, 3H, CH_3), 1.17 (t, 3H, $J = 7.1$ Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 153.5, 148.9, 143.3, 133.9, 128.5, 127.6, 126.2, 115.9, 104.2, 60.1, 53.8, 44.7, 15.9, 14.0.

IR (KBr, cm^{-1}): 3210, 3084, 2987, 2938, 1687, 1386, 1270, 1214, 1088.

Anal. Calcd for $C_{17}H_{20}N_2O_3$: C, 67.98; H, 6.71; N, 9.33. Found: C, 68.09; H, 6.78; N, 9.20.

5.8. Ethyl 1-cinnamyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1.1h**)

Yellow solid (73% yield).

mp 122–123 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31–7.23 (m, 10H, ArH), 6.43 (d, 1H, J = 16.2 Hz, one H of N–CH_2–CH=C<u>H</u>-), 6.24–6.16 (m, 1H, one H of N–CH_2–C<u>H</u>=), 5.57 (brs, 1H, NH), 5.43 (d, 1H, J = 2.7 Hz, CH), 4.68 (dd, 1H, J_1 = 4.2 Hz, J_2 = 17.1 Hz, one H of N–CH_2–), 4.48 (dd, 1H, J_1 = 4.2 Hz, J_2 = 16.8 Hz, one H of N–CH_2–), 4.10 (q, 2H, J = 6.9 Hz, ester-CH_2), 2.56 (s, 3H, CH_3), 1.18 (t, 3H, J = 6.9 Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 153.6, 148.8, 143.3, 136.4, 131.6, 128.7, 128.5, 127.8, 127.7, 126.4, 126.3, 125.3, 118.1, 104.8, 60.2, 54.0, 53.4, 44.4, 16.2, 14.1.

IR (KBr, cm^{-1}): 3225, 3111, 2972, 1689, 1620, 1410, 1308, 1261, 1205, 1176, 1110.

Anal. Calcd for $C_{23}H_{24}N_2O_3$: C, 73.38; H, 6.43; N, 7.44. Found: C, 73.43; H, 6.49; N, 7.31.

5.9. Ethyl 4-(4-chlorophenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2.1a**)

White solid (92% yield).

mp 130–131 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33 (d, 2H, J = 8.7 Hz, ArH), 7.25 (d, 2H, J = 8.4 Hz, ArH), 5.65 (brs, 1H, NH), 5.43 (d, 1H, J = 3 Hz, CH), 4.17 (q, 2H, J = 6.9 Hz, ester-CH_2), 3.29 (s, 3H, N–CH_3), 2.58 (s, 3H, CH_3), 1.25 (t, 3H, J = 6.9 Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.0, 154.0, 149.7, 142.1, 133.6, 128.9, 127.8, 104.0, 60.4, 53.4, 30.5, 16.7, 14.3, 14.3.

IR (KBr, cm^{-1}): 3220, 3095, 2940, 1710, 1683, 1627, 1490, 1441, 1385, 1302, 1269, 1251, 1198, 1184, 1092, 1050.

Anal. Calcd for $C_{15}H_{17}ClN_2O_3$: C, 58.35; H, 5.55; N, 9.07. Found: C, 58.42; H, 5.48; N, 9.17.

5.10. Ethyl 1-butyl-4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2.1b**)

White solid (73% yield).

mp 123–124 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.20 (d, 2H, J = 9 Hz, ArH), 7.12 (d, 2H, J = 9 Hz, ArH), 5.50 (brs, 1H, NH), 5.27 (d, 1H, J = 3 Hz, CH), 4.03 (q, 2H, J = 7.2 Hz, ester-CH_2), 3.89–3.79 (m, 1H, one H of N–CH_2–), 3.55–3.45 (m, 1H, one H of N–CH_2–), 2.45 (s, 3H, CH_3), 1.54–1.38 (m, 2H, one –CH_2- of n-butyl), 1.30–1.17 (m, 2H, one –CH_2- of n-butyl), 1.15 (t, 3H, J = 7.2 Hz, ester-CH_3), 0.88 (t, 3H, J = 6 Hz, –CH_3 of n-butyl).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.0, 153.7, 149.1, 142.1, 133.3, 128.6, 127.6, 104.1, 60.2, 53.0, 42.3, 31.8, 19.9, 16.0, 14.1, 13.7.

IR (KBr, cm^{-1}): 3344, 3223, 3102, 2963, 1686, 1610, 1476, 1391, 1277, 1205, 1156, 1094.

Anal. Calcd for $C_{18}H_{23}ClN_2O_3$: C, 61.62; H, 6.61; N, 7.98. Found: C, 61.69; H, 6.72; N, 7.85.

5.11. Ethyl 4-(4-chlorophenyl)-6-methyl-1-octyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2.1c**)

White solid (73% yield).

mp 132–133 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.27 (d, 2H, J = 8.5 Hz, ArH), 7.20 (d, 2H, J = 8.5 Hz, ArH), 6.10 (brs, 1H, NH), 5.36 (d, 1H, J = 3.5 Hz, CH), 4.12 (q, 2H, J = 7 Hz, ester-CH_2), 3.95–3.89 (m, 1H, one H of N–CH_2–), 3.59–3.53 (m, 1H, one H of N–CH_2–), 2.54 (s, 3H, CH_3), 1.76–1.47 (m, 2H, one –CH_2- of n-octyl), 1.32–1.23 (m, 10H, five –CH_2- of n-octyl), 1.21 (t, 3H, J = 7 Hz, ester-CH_3), 0.90 (t, 3H, J = 7 Hz, –CH_3 of n-octyl).

^{13}C NMR (125 MHz, $CDCl_3$): δ 166.1, 153.6, 149.3, 142.2, 133.6, 128.9, 127.8, 104.3, 60.4, 53.4, 42.8, 31.9, 30.0, 29.4, 29.4, 26.9, 22.8, 16.3, 14.3, 14.2.

IR (KBr, cm^{-1}): 3352, 3211, 3094, 2928, 1687, 1613, 1479, 1390, 1196, 1158, 1096.

Anal. Calcd for $C_{22}H_{31}ClN_2O_3$: C, 64.93; H, 7.68; N, 6.88. Found: C, 64.98; H, 7.61; N, 6.85.

5.12. Ethyl 4-(4-chlorophenyl)-1-decyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2.1d**)

White solid (76% yield).

mp 103–104 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27 (d, 2H, J = 8.7 Hz, ArH), 7.18 (d, 2H, J = 8.4 Hz, ArH), 5.62 (brs, 1H, NH), 5.34 (d, 1H, J = 2.7 Hz, CH), 4.10 (q, 2H, J = 7.2 Hz, ester-CH_2), 3.95–3.85 (m, 1H, one H of N–CH_2–), 3.60–3.51 (m, 1H, one H of N–CH_2–), 2.52 (s, 3H, CH_3), 1.62–1.21 (m, 16H, eight –CH_2- of n-decyl), 1.19 (t, 3H, J = 7.2 Hz, ester-CH_3), 0.88 (t, 3H, J = 6 Hz, –CH_3 of n-decyl).

^{13}C NMR (125 MHz, $CDCl_3$): δ 166.1, 153.6, 149.3, 142.2, 133.7, 128.9, 127.8, 104.4, 60.4, 53.5, 42.9, 32.0, 30.0, 29.7, 29.7, 29.5, 29.4, 26.9, 22.8, 16.3, 14.3, 14.2.

IR (KBr, cm^{-1}): 3216, 3095, 2922, 2854, 1693, 1614, 1475, 1390, 1194, 1156, 1094.

Anal. Calcd for $C_{24}H_{35}ClN_2O_3$: C, 66.27; H, 8.11; N, 6.44. Found: C, 66.21; H, 8.19; N, 6.49.

5.13. Ethyl 4-(4-chlorophenyl)-1-hexadecyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2.1e**)

White solid (73% yield).

mp 110–111 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25 (d, 2H, J = 8.4 Hz, ArH), 7.16 (d, 2H, 8.4 Hz, ArH), 5.51 (d, 1H, J = 3 Hz, NH), 5.32 (d, 1H, J = 3 Hz, CH), 4.08 (q, 2H, J = 7.2 Hz, ester-CH_2), 3.98–3.82 (m, 1H, one H of N–CH_2–), 3.64–3.48 (m, 1H, one H of N–CH_2–), 2.50 (s, 3H, CH_3), 1.60–1.44 (m, 4H, two –CH_2- of n-hexadecyl), 1.44–1.19 (m, 24H, twelve –CH_2- of n-hexadecyl), 1.17 (t, 3H, J = 7.2 Hz, ester-CH_3), 0.88–0.84 (m, 3H, –CH_3 of n-hexadecyl).

^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 153.5, 149.3, 142.2, 133.7, 128.9, 127.8, 104.3, 60.4, 53.5, 42.9, 32.1, 30.0, 29.8, 29.7, 29.5, 26.9, 22.8, 16.3, 14.3.

IR (KBr, cm^{-1}): 3212, 3092, 2922, 2850, 1693, 1614, 1474, 1423, 1391, 1195, 1156, 1095.

Anal. Calcd for $C_{30}H_{47}ClN_2O_3$: C, 69.40; H, 9.12; N, 5.40. Found: C, 69.51; H, 9.17; N, 5.37.

5.14. Ethyl 1-benzyl-4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2.1f**)

White solid (69% yield).

mp 160–161 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23–7.01 (m, 9H, ArH), 5.60 (brs, 1H, NH), 5.31 (d, 1H, J = 3 Hz, CH), 5.10 (d, 1H, J = 16.8 Hz, one H of N–CH_2–), 4.78 (d, 1H, J = 16.2 Hz, one H of N–CH_2–), 3.99 (q, 2H, J = 7.2 Hz, ester-CH_2), 2.35 (s, 3H, CH_3), 1.07 (t, 3H, J = 7.2 Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.9, 154.1, 149.3, 141.7, 137.8, 133.4, 128.7, 127.8, 127.3, 126.4, 104.5, 60.3, 53.0, 45.8, 16.4, 14.1.

IR (KBr, cm^{-1}): 3216, 3096, 2982, 2940, 1692, 1618, 1420, 1390, 1318, 1205, 1163, 1107.

Anal. Calcd for $C_{21}H_{21}ClN_2O_3$: C, 65.54; H, 5.50; N, 7.28. Found: C, 65.47; H, 5.45; N, 7.39.

5.15. Ethyl 1-allyl-4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2.1g**)

White solid (78% yield).

mp 124–125 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.20 (d, 2H, J = 8.7 Hz, ArH), 7.13 (d, 2H, J = 8.7 Hz, ArH), 5.84–5.71 (m, 2H, NH & one H of N–CH_2–C<u>H</u>=), 5.32 (d, 1H, J = 3 Hz, CH), 5.13–5.00 (m, 2H, two H of N–CH_2–CH=C<u>H</u>$_2$), 4.40 (dd, 1H, J_1 = 6.3 Hz, J_2 = 18 Hz, one H of N–CH_2–), 4.27 (dd, 1H, J_1 = 6.3 Hz, J_2 = 16.2 Hz, one H of N–CH_2–), 4.04 (q, 2H, J = 7.2 Hz, ester-CH_2), 2.44 (s, 3H, CH_3), 1.11 (t, 3H, J = 7.2 Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.9, 153.5, 149.2, 141.9, 133.8, 133.3, 128.6, 127.7, 116.1, 103.8, 60.2, 53.1, 44.7, 16.0, 14.1.

IR (KBr, cm^{-1}): 3341, 3212, 3093, 2942, 1691, 1621, 1420, 1321, 1204, 1109.

Anal. Calcd for $C_{17}H_{19}ClN_2O_3$: C, 60.99; H, 5.72; N, 8.37. Found: C, 60.87; H, 5.79; N, 8.43.

5.16. Ethyl 4-(4-chlorophenyl)-1-cinnamyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**2.1h**)

Pale yellow solid (69% yield).

mp 116–117 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44–7.24 (m, 9H, ArH), 6.42 (d, 1H, J = 15.9 Hz, one H of N–CH_2–CH=C<u>H</u>-), 6.25–6.16 (m, 1H, one H of N–CH_2–C<u>H</u>=), 5.52 (brs, 1H, NH), 5.43 (d, 1H, J = 3 Hz, CH), 4.69 (dd, 1H, J_1 = 4.5 Hz, J_2 = 16.5 Hz, one H of N–CH_2–), 4.50 (dd, 1H, J_1 = 4.5 Hz, J_2 = 16.2 Hz, one H of N–CH_2–), 4.14 (q, 2H, J = 7.2 Hz, ester-CH_2), 2.58 (s, 3H, CH_3), 1.21 (t, 3H, J = 7.2 Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.9, 153.7, 149.2, 141.8, 136.1, 133.4, 131.5, 128.7, 128.6, 127.8, 127.7, 126.3, 125.0, 104.4, 60.3, 53.0, 44.3, 16.1, 14.1.

IR (KBr, cm^{-1}): 3221, 3102, 2980, 1690, 1619, 1399, 1310, 1267, 1203, 1179, 1104.

Anal. Calcd for $C_{23}H_{23}ClN_2O_3$: C, 67.23; H, 5.64; N, 6.82. Found: C, 67.31; H, 5.69; N, 6.79.

5.17. Ethyl 1,6-dimethyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3.1a**) [21]

White solid (90% yield).

mp 136–137 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13–8.10 (m, 2H, ArH), 7.59 (d, 1H, J = 7.5 Hz, ArH), 7.48 (t, 1H, J = 7.5 Hz, ArH), 5.97 (brs, 1H, NH), 5.50 (d, 1H, J = 3.3 Hz, CH), 4.13 (q, 2H, J = 7.2 Hz, ester-CH_2), 3.26 (s, 3H, N–CH_3), 2.56 (s, 3H, CH_3), 1.21 (t, 3H, J = 7.2 Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.6, 154.2, 150.8, 148.2, 145.4, 132.1, 129.5, 122.6, 121.6, 102.8, 60.4, 52.8, 30.4, 16.5, 14.1.

IR (KBr, cm^{-1}): 3236, 3104, 2982, 2936, 1683, 1620, 1527, 1385, 1347, 1305, 1172, 1075.

Anal. Calcd for $C_{15}H_{17}N_3O_5$: C, 56.42; H, 5.37; N, 13.16. Found: C, 56.47; H, 5.40; N, 13.15.

HRMS (m/z) for $C_{15}H_{17}N_3O_5Na$ $[M + Na]^+$: Calculated 342.1066, found 342.1065.

5.18. Ethyl 1-butyl-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3.1b**)

Pale yellow solid (73% yield).

mp 76–77 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14–8.11 (m, 2H, ArH), 7.60 (d, 1H, J = 7.5 Hz, ArH), 7.48 (t, 1H, J = 7.8 Hz, ArH), 6.01 (brs, 1H, NH), 5.47 (d, 1H, J = 3 Hz, CH), 4.12 (q, 2H, J = 7.2 Hz, ester-CH_2), 4.01–3.91 (m, 1H, one H of N–CH_2–), 3.63–3.53 (m, 1H, one H of N–CH_2–), 2.56 (s, 3H, CH_3), 1.64–1.41 (m, 2H, one –CH_2- of n-butyl), 1.36–1.26 (m, 2H, one –CH_2- of n-butyl), 1.20 (t, 3H, J = 7.2 Hz, ester-CH_3), 0.92 (t, 3H, J = 7.5 Hz, –CH_3 of n-butyl).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.6, 153.6, 150.1, 148.3, 145.7, 132.3, 129.5, 122.6, 121.5, 103.4, 60.4, 53.1, 42.4, 31.8, 19.9, 16.1, 14.1, 13.7.

IR (KBr, cm^{-1}): 3223, 3105, 2930, 1688, 1608, 1527, 1351, 1207, 1153, 1092.

Anal. Calcd for $C_{18}H_{23}N_3O_5$: C, 59.82; H, 6.41; N, 11.63. Found: C, 59.87; H, 6.45; N, 11.72.

5.19. Ethyl 6-methyl-4-(3-nitrophenyl)-1-octyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3.1c**)

White solid (72% yield).

mp 98–99 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.17–8.14 (m, 2H, ArH), 7.62 (d, 1H, J = 8 Hz, ArH), 7.50 (t, 1H, J = 8 Hz, ArH), 5.80 (brs, 1H, NH), 5.49 (d, 1H, J = 3 Hz, CH), 4.17–4.11 (m, 2H, ester-CH_2), 3.99–3.94 (m, 1H, one H of N–CH_2–), 3.62–3.56 (m, 1H, one H of N–CH_2–), 2.58 (s, 3H, CH_3), 1.58–1.54 (m, 2H, one –CH_2- of n-octyl), 1.29–1.26 (m, 10H, five –CH_2- of n-octyl), 1.23 (t, 3H, J = 7.5 Hz, ester-CH_3), 0.89 (t, 3H, J = 7 Hz, –CH_3 of n-octyl).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.8, 153.5, 150.3, 148.5, 145.9, 132.5, 129.7, 122.9, 121.7, 103.6, 60.6, 53.5, 42.9, 31.9, 30.0, 29.4, 26.9, 22.7, 16.3, 14.3, 14.2.

IR (KBr, cm^{-1}): 3216, 3093, 2925, 2855, 1691, 1617, 1530, 1344, 1158, 1098.

Anal. Calcd for $C_{22}H_{31}N_3O_5$: C, 63.29; H, 7.48; N, 10.06. Found: C, 63.22; H, 7.41; N, 9.94.

5.20. Ethyl 1-decyl-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3.1d**)

White solid (71% yield).

mp 84–85 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15–8.11(m, 2H, ArH), 7.61 (m, 1H, ArH), 7.51–7.49 (m, 1H, ArH), 5.81 (brs, 1H, NH), 5.48 (s, 1H, CH), 4.13 (q, 2H, J = 7.1 Hz, ester-CH_2), 3.95–3.93 (m, 1H, one H of N–CH_2–), 3.59–3.58 (m, 1H, one H of N–CH_2–), 2.56 (s, 3H, CH_3), 1.76 (m, 2H, one –CH_2- of n-decyl), 1.58 (m, 2H, one –CH_2- of n-decyl),1.25–1.08 (m, 15H, six –CH_2- of n-decyl & ester-CH_3), 0.88 (t, 3H, J = 6.9 Hz, –CH_3 of n-decyl).

^{13}C NMR (125 MHz, $CDCl_3$): δ 165.8, 153.4, 150.2, 148.6, 145.9, 132.5, 129.8, 122.9, 121.7, 103.6, 60.6, 53.6, 43.0, 32.0, 30.0, 29.7, 29.7, 29.4, 26.9, 22.8, 16.3, 14.3, 14.2.

IR (KBr, cm^{-1}): 3222, 3095, 2923, 2853, 1690, 1622, 1532, 1345, 1161, 1099.

Anal. Calcd for $C_{30}H_{47}N_3O_5$: C, 68.02; H, 8.94; N, 7.93. Found: C, 68.11; H, 8.82; N, 7.88.

*5.21. Ethyl 1-hexadecyl-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3.1e**)*

White solid (68% yield).

mp 86–87 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14–8.10 (m, 2H, ArH), 7.59 (d, 1H, J = 7.5 Hz, ArH), 7.47 (t, 1H, J = 8 Hz, ArH), 6.24 (brs, 1H, NH), 5.47 (d, 1H, J = 2.5 Hz, CH), 4.12 (q, 2H, J = 7.5 Hz, ester-CH_2), 3.97–3.91 (m, 1H, one H of N–CH_2–), 3.59–3.53 (m, 1H, one H of N–CH_2–), 2.55 (s, 3H, CH_3), 1.80–1.50 (m, 2H, one –CH_2- of n-hexadecyl), 1.31–1.25 (m, 26H, thirteen –CH_2- of n-hexadecyl), 1.19 (t, 3H, J = 7.5 Hz, ester-CH_3), 0.87 (t, 3H, J = 7 Hz, –CH_3 of n-hexadecyl).

^{13}C NMR (125 MHz, $CDCl_3$): δ 165.8, 153.4, 150.2, 148.6, 145.9, 132.5, 129.8, 122.9, 121.7, 103.7, 60.6, 53.6, 43.0, 32.1, 30.0, 29.8, 29.8, 29.7, 29.5, 29.4, 27.0, 22.8, 16.3, 14.3, 14.2.

IR (KBr, cm^{-1}): 3216, 3093, 2923, 2851, 1691, 1621, 1535, 1471, 1344, 1278, 1184, 1100.

Anal. Calcd for $C_{30}H_{47}N_3O_5$: C, 68.02; H, 8.94; N, 7.93. Found: C, 68.09; H, 8.90; N, 7.91.

HRMS (m/z) for $C_{30}H_{47}N_3O_5Na$ $[M + H]^+$: Calculated 530.3594, found 530.3584.

*5.22. Ethyl 1-benzyl-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3.1f**)*

Pale yellow solid (68% yield).

mp 138–139 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04–8.00 (m, 2H, ArH), 7.47 (d, 1H, J = 7.5 Hz, ArH), 7.34 (t, 1H, J = 7.8 Hz, ArH), 7.24–7.13 (m, 3H, ArH), 7.06–7.03 (m, 2H, ArH), 6.23 (d, 1H, J = 3 Hz, NH), 5.44 (d, 1H, J = 3 Hz, CH), 5.13 (d, 1H, J = 16.5 Hz, one H of N–CH_2–), 4.79 (d, 1H, J = 16.5 Hz, one H of N–CH_2–), 4.00 (q, 2H, J = 7.2 Hz, ester-CH_2), 2.39 (s, 3H, CH_3), 1.08 (t, 3H, J = 7.2 Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.5, 153.9, 150.3, 148.3, 145.5, 137.6, 132.5, 129.5, 128.8, 127.3, 126.2, 122.7, 121.6, 103.5, 60.5, 53.2, 46.0, 16.5, 14.1.

IR (KBr, cm^{-1}): 3223, 3107, 2945, 1688, 1628, 1531, 1390, 1351, 1276, 1207, 1175, 1115.

Anal. Calcd for $C_{21}H_{21}N_3O_5$: C, 63.79; H, 5.35; N, 10.63. Found: C, 63.72; H, 5.38; N, 10.66.

HRMS (m/z) for $C_{21}H_{21}N_3O_5Na$ $[M + Na]^+$: Calculated 418.1379, found 418.1377.

*5.23. Ethyl 1-allyl-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3.1g**)*

White solid (76% yield).

mp 96–97 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17–8.11 (m, 2H, ArH), 7.61 (d, 1H, J = 7.5 Hz, ArH), 7.48 (t, 1H, J = 7.8 Hz, ArH), 6.12 (brs, 1H, NH), 5.94–5.81 (m, 1H, one H of N–CH_2–C<u>H</u>=), 5.52 (d, 1H, J = 3.3 Hz, CH), 5.23–5.10 (m, 2H, two H of N–$\underline{CH_2}$–CH=C<u>H_2</u>), 4.52 (dd, 1H, J_1 = 4.8 Hz, J_2 = 17.1 Hz, one H of N–CH_2–), 4.36 (dd, 1H, J_1 = 4.8 Hz, J_2 = 17.1 Hz, one H of N–CH_2–), 4.12 (q, 2H, J = 7.2 Hz, ester-CH_2), 2.56 (s, 3H, CH_3), 1.20 (t, 3H, J = 7.2 Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.6, 153.4, 150.3, 148.2, 145.5, 133.6, 132.3, 129.5, 122.6, 121.6, 116.3, 103.0, 60.4, 53.1, 44.9, 16.0, 14.1.

IR (KBr, cm^{-1}): 3237, 3103, 2985, 2917, 1689, 1627, 1529, 1403, 1348, 1187, 1111.

Anal. Calcd for $C_{17}H_{19}N_3O_5$: C, 59.12; H, 5.55; N, 12.17. Found: C, 59.08; H, 5.53; N, 12.20.

HRMS (m/z) for $C_{17}H_{19}N_3O_5Na$ $[M + H]^+$: Calculated 346.1403, found 346.1394.

*5.24. Ethyl 1-cinnamyl-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3.1h**)*

White solid (68% yield).

mp 125–126 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19–8.11 (m, 2H, ArH), 7.64 (d, 1H, J = 7.8 Hz, ArH), 7.46 (t, 1H, J = 7.8 Hz, ArH), 7.33–7.26 (m, 5H, ArH), 6.47 (d, 1H, J = 16.2 Hz, one H of N–CH_2–CH=C<u>H</u>-), 6.27–6.19 (m, 1H, N–CH_2–C<u>H</u>=), 5.87 (d, 1H, J = 2.4 Hz, <u>NH</u>), 5.53 (d, 1H, J = 3.3 Hz, <u>CH</u>), 4.70 (dd, 1H, J_1 = 6 Hz, J_2 = 16.8 Hz, one H of N–CH_2–), 4.52 (dd, 1H, J_1 = 5.7 Hz, J_2 = 16.8 Hz, one H of N–CH_2–), 4.13 (q, 2H, J = 7.2 Hz, ester-CH_2), 2.61 (s, 3H, CH_3), 1.21 (t, 3H, J = 7.2 Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.6, 153.5, 150.1, 148.4, 145.6, 136.1, 132.3, 132.0, 129.6, 128.6, 127.9, 126.3, 124.9, 122.7, 121.6, 103.6, 60.5, 53.3, 44.7, 16.3, 14.1.

IR (KBr, cm^{-1}): 3222, 3105, 2981, 1690, 1617, 1527, 1344, 1207, 1171, 1109.

Anal. Calcd for $C_{23}H_{23}N_3O_5$: C, 65.55; H, 5.50; N, 9.97. Found: C, 65.60; H, 5.53; N, 9.92.

HRMS (m/z) for $C_{23}H_{23}N_3O_5$ $[M]^+$: Calculated 421.1638, found 421.1639.

*5.25. Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-1-phenethyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3.1i**)*

White solid (30% yield).

mp 122–123 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17–8.13 (m, 2H, ArH), 7.58 (d, 1H, J = 7.5 Hz, ArH), 7.49 (t, 1H, J = 7.8 Hz, ArH), 7.32–7.20 (m, 5H, ArH), 5.80 (brs, 1H, NH), 5.48 (d, 1H, J = 2.7 Hz, CH), 4.22–4.09 (m, 3H, ester-CH_2 & one H of N–CH_2–), 3.91–3.83 (m, 1H, one H of N–CH_2–), 2.95–2.88 (m, 2H, benzylic CH_2), 2.55 (s, 3H, CH_3), 1.21 (t, 3H, J = 7.2 Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.6, 153.3, 149.7, 148.3, 145.7, 138.0, 132.3, 129.6, 128.6, 128.6, 126.6, 122.7, 121.6, 103.3, 60.4, 53.3, 44.2, 35.9, 16.1, 14.1.

IR (KBr, cm^{-1}): 3265, 3099, 2975, 2933, 1684, 1610, 1527, 1351, 1157, 1098.

Anal. Calcd for $C_{22}H_{23}N_3O_5$: C, 64.54; H, 5.66; N, 10.26. Found: C, 64.58; H, 5.61; N, 10.30.

HRMS (m/z) for $C_{22}H_{23}N_3O_5Na$ $[M + Na]^+$: Calculated 432.1535, found 432.1534.

*5.26. Ethyl 6-methyl-4-(3-nitrophenyl)-2-oxo-1-(3-phenylpropyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3.1j**)*

White solid (62% yield).

mp 110–112 °C (Ethyl acetate/n-Hexane).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15–8.09 (m, 2H, ArH), 7.59 (d, 1H, J = 7.8 Hz, ArH), 7.45 (t, 1H, J = 7.8 Hz, ArH), 7.31–7.14 (m, 5H, ArH), 6.20 (brs, 1H, NH), 5.47 (d, 1H, J = 2.4 Hz, CH), 4.12 (q, 2H, J = 6.9 Hz, ester-CH_2), 3.96–3.91 (m, 1H, one H of N–CH_2–), 3.65–3.60 (m, 1H, one H of N–CH_2–), 2.62 (t, 2H, J = 7.5 Hz, benzylic CH_2), 2.44 (s, 3H, CH_3), 1.99–1.87 (m, 2H, two H of N–CH_2–C<u>H_2</u>-), 1.20 (t, 3H, J = 6.9 Hz, ester-CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 165.6, 153.6, 149.9, 148.3, 145.6, 140.8, 132.2, 129.6, 128.4, 128.2, 126.1, 122.6, 121.5, 103.4, 60.4, 53.1, 42.2, 32.8, 31.0, 16.0, 14.1.

IR (KBr, cm^{-1}): 3229, 3099, 2922, 1688, 1615, 1526, 1341, 1166, 1102.

Anal. Calcd for $C_{23}H_{25}N_3O_5$: C, 65.24; H, 5.95; N, 9.92. Found: C, 65.18; H, 5.99; N, 9.95.

HRMS (m/z) for $C_{23}H_{25}N_3O_5Na$ $[M + Na]^+$: Calculated 446.1692, found 446.1692.

5.27. Purification of Ca^{2+}-ATPase from microsomal membranes of goat spermatozoa

Ca^{2+}-ATPase was purified from the enzyme enriched microsomal membrane of goat spermatozoa as per previously reported literature [9]. The spermatozoa were collected from the caudal region of the goat epididymis in a buffer containing 25 mM imidazole, 0.25 mM sucrose, 1 mM EDTA and 1 mM 2-mercaptoethanol (pH-7.5). The sperm suspension was then homogenized in a Teflon homogenizer and spun at 12,000 rpm for 20 min at 4 °C. The supernatant was collected and centrifuged at 100,000*g* for 1 h. The pellet was re-suspended in the above mentioned buffer and was found to be enriched with Ca^{2+}-ATPase.

5.28. Assay of Mg^{2+}-dependent and independent Ca^{2+}-ATPase activities

For Mg^{2+}-dependent Ca^{2+}-ATPase activity assay, the assay mixture contained in a final volume of 0.25 ml, 50 mM histidine (pH 7.4) buffer in 25 mM sucrose, 0.5 mM EDTA, 1 mM 2-mercaptoethanol, 1 mM $MgCl_2$, 3 mM $CaCl_2$, 3 mM ATP, 5 μg enzyme, in absence and presence of the DHPM derivatives. The reaction mixture was pre-incubated for 5 min at 37 °C, followed by addition of ATP and incubation was continued for further 30 min. The reaction was then terminated with 6% cold TCA. The liberated inorganic phosphate (P_i) was estimated colorimetrically to determine the amount of enzymatically released P_i by the addition of a solution containing 1.75% ammonium molybdate and 2% ascorbic acid (6:1). The blue colour developed was measured at 820 nm using a spectrophotometer [28]. The values were expressed as the difference in activity in the presence of Mg^{2+} + Ca^{2+}, and Mg^{2+} alone. DHPMs inhibitors are soluble in DMSO. Same concentration of DMSO was used as a positive control for all the above experiments and the calculations have been done accordingly.

For Mg^{2+}-independent Ca^{2+}-ATPase activity assay, above procedure was used except that the reaction was done in imidazole buffer and the ATPase activity was determined as the difference in activity in presence and absence of Ca^{2+} and no Mg^{2+} was present in the mixture.

To determine the effect of verapamil on Mg^{2+}-dependent and independent Ca^{2+}-ATPase activity assay in the absence and presence of the DHPM derivatives, the I_{50} concentration of the DHPM derivatives were used at a fixed concentration (300 μM) of verapamil.

Acknowledgements

We gratefully acknowledge financial supports from University Grants Commission (UGC MRP No. PSW-053/10-11 to AC and UGC-JRF fellowship to SP), Bose Institute to PN and SC and Council of Scientific and Industrial Research to SG. We are grateful to Dr. Rina Ghosh (Jadavpur University), Dr. Dilip Kumar Maiti (University of Calcutta) and Mr. Saikat Bhattacharya for their various help in different stages of work.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2012.04.043.

References

[1] K. Kumsars, A. Velena, G. Duburs, J. Uldrikis, A. Zidermane, Biokhimiya 36 (1971) 1201–1209.
[2] M. Ashok, B.S. Holla, N.S. Kumari, Eur. J. Med. Chem. 42 (2007) 380–385.
[3] J.M. Blacquiere, O. Sicora, C.M. Vogels, M. Cuperlovic-Culf, A. decken, R.J. Ouellette, S.A. Westcott, Can. J. Chem. 83 (2005) 2052–2059.
[4] R. Kumar, S. Malik, R. Chandra, J. Chem. 48B (2009) 718–724.
[5] C.O. Kappe, Acc. Chem. Res. 33 (2000) 879–888.
[6] C.O. Kappe, Eur. J. Med. Chem. 35 (2000) 1043–1052.
[7] K. Singh, D. Arora, K. Singh, S. Singh, Mini-Reviews Med. Chem. 9 (2009) 95–106.
[8] E. Carafoli, M. Crompton, Curr. Top. Membr. Trans. 10 (1978) 151–216.
[9] R. Sikdar, U. Ganguly, P. Pal, B. Mazumder, P.C. Sen, Mol. Cell. Biochem. 103 (1991) 121–130.
[10] S. Vijayasarathy, S. Shivaji, P. Balaram, FEBS Lett. 114 (1980) 45–47.
[11] H. Breitbart, S. Rubinstein, L. Nass-Arden, J. Biol. Chem. 260 (1985) 11548–11553.
[12] H.T. Zeng, D.R.P. Tulsiani, J. Cell Sci. 116 (2003) 1981–1989.
[13] C.O. Kappe, G. Uray, P. Roschger, W. Linder, C. Kartky, W. Keller, Tetrahedron 48 (1992) 5473.
[14] C.O. Kappe, Tetrahedron 23 (1993) 360.
[15] P. Wipf, A. Cunningham, Tetrahedron Lett. 36 (1995) 7819.
[16] A. Stadler, C.O. Kappe, J. Comb. Chem. 3 (2001) 624–630.
[17] C.O. Kappe, Bioorg. Med. Chem. Lett. 10 (2000) 49.
[18] C.O. Kappe, QSAR -Comb. Sci. 22 (2003) 630–645.
[19] A. Dondoni, A. Massi, Mol. Divers. 6 (2003) 261–270.
[20] G. Aridoss, Y.T. Jeong, Bull. Korean Chem. Soc. 31 (2010) 863.
[21] D. Dallinger, C.O. Kappe, Synlett (2002) 1901–1903.
[22] K. Singh, D. Arora, E. Poremsky, J. Lowery, R.S. Moreland, Eur. J. Med. Chem. 44 (2009) 1997–2001.
[23] B.C. Ranu, A. Hajra, U. Jana, J. Org. Chem. 65 (2000) 6270.
[24] J. Lu, Y. Bai, Synthesis 4 (2002) 466–470.
[25] F.S. Falsone, C.O. Kappe, Arkivoc (2001) 122–134.
[26] A. Steinmetz, The Broad Scope of Cesium Salts in Organic Chemistry, Catalysts Cesium from Acros Organics, pp 3–11 and the references cited in the article.
[27] G. Dijkstra, W.H. Kruizinga, R.M. Kellogg, J. Org. Chem. 52 (1987) 4230–4234.
[28] R.N. Salvatore, A.S. Nagle, K.W. Jung, J. Org. Chem. 67 (2002) 674–683.
[29] C. Viedma, Astrobiology 7 (2007) 312–319.
[30] D.G. Blackmond, Chem.–Eur. J. 13 (2007) 3290–3295.
[31] S. Ghoshal, T. Sengupta, P.C. Sen, BioFactors 26 (2006) 259–271.