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Synthesis of novel dihydropyrimidin-2(1H)-ones derivatives using lipase and their antimicrobial activity

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A B S T R A C T

A series of novel dihydropyrimidin-2(1H)-ones derivatives was synthesized by using *Rhizopus Oryzae* lipase biocatalyst in deep eutectic solvent. The reaction is characterized by high efficiency and selectivity, short reaction time, mild and environmentally friendly reaction conditions. The yields were found to be significantly higher and reuse of both the lipase and deep eutectic solvent was possible up to four consecutive cycles. The products are found to exhibit appreciable in vitro antibacterial activity against *Echerichia* coli, Pseudomonas *neumoniae* and in vitro antifungal activity against *Aspergillus niger* and *Candida albicans*.

1. Introduction

Dihydropyrimidin-2(1H)-ones (DHPMs) moieties are common in a variety of biologically important natural products and potent drugs including anti-hypertensive agents, anti-carcinogenic agents, anti-inflammatory, analgesic agents and calcium channel blockers[1-3]. DHPMs are also screened as neuropeptide antagonists agents in treating anxiety and recently, as anti-oxidants[4]. Barrow et al[5]. reported that the biologically active DHPMs derivatives exhibited α_{1a}-adrenoceptor antagonist properties and some of them such as monastrol showed anticancer activity against mitotic kinesin[6]. Tamaddon et al.[7] recently reported the synthesis of DHPMs using ammonium carbonate as base. However, in this method both Hantzsch and Biginelli products are formed.

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Debache et al.[8] investigated the use of triphenylphosphine as a Lewis base catalyst for the synthesis of DHPMs derivatives. It has been also reported by using imidazole based ionic liquids (ILs) such as water tolerant Lewis acid [bmim][$FeCl_4$][9], [bmim]BF_4 and [bmim]PF_6[10].

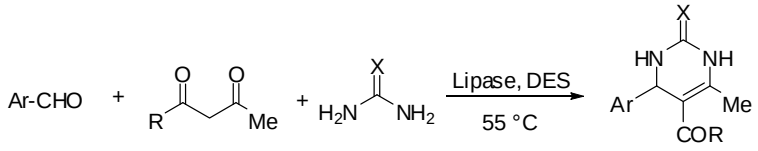
Use of the ILs as a reaction medium for biocatalysis has attracted great attention in recent years, as the processes are green and the ILs have several advantages compared to conventional reaction media[11-14]. However, *imidazole* base ILs containing anions $BF4^-$ and $PF6^-$ are not environmentally safe as they liberate hazardous HF gas and also their high costs and disposability problems make their use limited[15].

As an alternative approach to overcome these drawbacks, we used deep eutectic solvent (DES) which is nontoxic, cheap, easily available and also possesses biodegradability. The used DES was a mixture of choline chloride and urea, resulting in a very large depression of freezing point. The solvent and physical properties of DES are similar to ILs[16]. Both choline chloride and urea are naturally occurring biocompatible compounds that are environmentally safe if released back into nature as a choline or its DES[17].

Biocatalysis is an efficient and green tool for modern organic synthesis due to its high selectivity and mild conditions[18-19]. Biocatalytic promiscuities provide new tool for organic synthesis and thus expand largely the applications of enzymes. The lipase from *Rhizopus Oryzae* as a biocatalyst is well known in organic synthesis due to its easy availability, selectivity and stability[20]. Although it has found applications in ester hydrolysis[21], knoevenagel condensation[22] and mannich reaction[23], the application in synthesis of DHPMs derivatives has not been well explored.

In the last two decades, chemists have paid much attention to the clean synthesis leading to introduction of efficient and green methodology[24]. Earlier we have reported the synthesis of novel styryl colorants[22] and synthesis of trisubstituted alkenes[25] by using lipase as a biocatalyst. In continuation, we report herein for the first time environmentally-benign base catalyzed synthesis of novel DHPMs using lipase biocatalyst in DES and their antimicrobial activity.

2. Results and Discussion



Scheme 1: General Reaction Scheme for synthesis of DHPMs **4a-g**

The reaction of aromatic aldehydes **1a**, ethyl acetoacetate **2a** and urea **3a** was selected as a model reaction for optimizing the reaction parameters (**Scheme 1**) such as molar ratio, effects of solvents, catalysts study, catalysts amount, and reusability. Base catalyzed Biginelli reaction has been shown to be more effective in terms of reaction time, conversion and isolated yield compared to the acid catalyzed reaction[26-30]. Synthesis of DHPMs derivatives by using base catalyst has not been well explored. Therefore, we have employed base catalyzed synthesis of DHPMs derivatives.

As shown in **Table 1** (entries 1, 2) our attempt to perform the model reaction using conventional catalyst such as K_2CO_3 and t-BuOK, yielded the product **4a** in 65-79 % after reacting at 55 ^{0}C for 6-7 h. The model reaction using biocatalyst such as proline, L-histidine and lipase afforded the product **4a** in 80-95% yields after reacting at 55 ^{0}C for 4-5 h. The catalytic activity of the lipase biocatalyst was found to be more than that of the corresponding biocatalyst (**Table 1**, entries 3, 4, 5). Lipase biocatalysts were used in different organic transformation without need of additional coenzyme because of their high efficiency and selectivity[20, 22]. Different organic solvents were also screened to see their efficiency in the reaction. As shown in **Table 1** (entries 5-9) it seems that the reaction proceeds better in DES solvents than water, methanol, dioxane, and DMF. In the absence of the catalyst, it affords the desired product **4a** in 20 % yield after reacting at 55 ^{0}C for 7.5 h (**Table 1**, entry 10).

Table 1. Effect of catalysts and solvents on product yield **4a**

Entry[a]	Catalyst (5% w/w)	Solvent	Time(h)	Yield (%)[b]
1	K_2CO_3	DES	7	65
2	t-BuOK	DES	6	79
3	Proline	DES	5	80
4	L-Histidine	DES	5	88
5	Lipase	DES	4	95
6	Lipase	Water	8	50
7	Lipase	Methanol	5	80
8	Lipase	Dioxane	6	72
9	Lipase	DMF	5.5	78
10	No Catalyst	DES	7.5	20

Reaction Condition: [a]All reactions were carried out with aldehyde **1a** (2 mmol), ethyl acetoacetate (2.1 mmol), urea (2.2 mmol), catalyst (0.019g), solvent (3 ml), Temperature = 55 ^{0}C, [b]Isolated yields.

Use of *Rhizopus oryzae* lipase in DES reduced the reaction time to more than half coupled with the yield of the product as high as 95 % (**Table 1**, entry 5). For further reaction optimization, the lipase biocatalyst was used. To optimize the amount of the catalyst, the reaction was performed with different quantity of the lipase, and we observed that 5% w/w of lipase with respect to the aldehyde was found to be optimal (**Table 2**). Under the optimized conditions, various substituted aromatic aldehydes **1a-g** were reacted to obtain the corresponding products **4a-g** (**Table 3**).

Table 2. Optimization of amount of lipase

Entry[a]	Catalyst (% w/w)	Time(h)	Yield (%)[b]
A	3	6	80
B	5	4	95
C	7	4	95

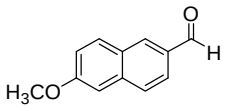
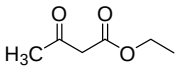
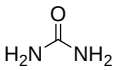
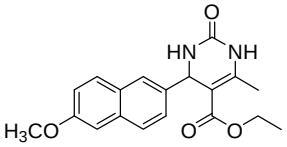
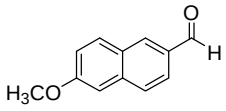
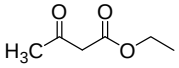
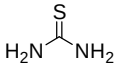
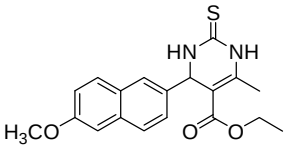
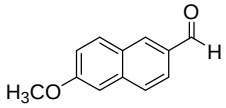
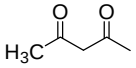
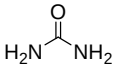
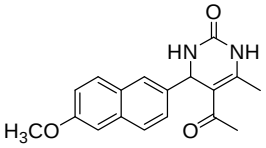
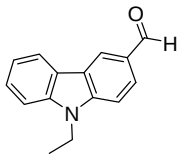
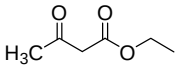
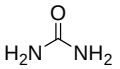
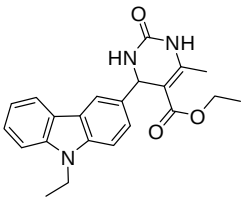
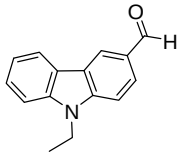
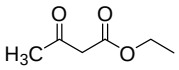
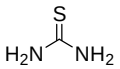
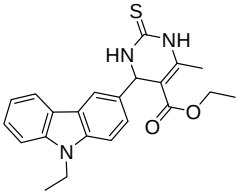
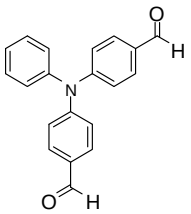
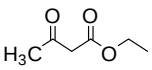
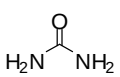
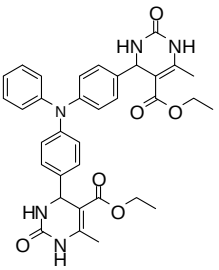
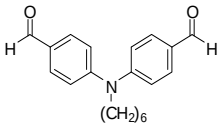
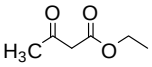
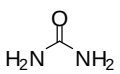
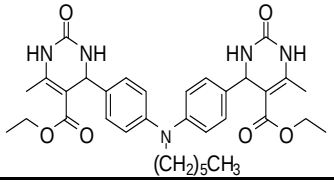
Reaction Condition: [a]All reactions were carried out with aldehyde **1a** (2 mmol), ethyl acetoacetate (2.1 mmol), urea (2.2 mmol), DES (3 ml), Temperature = 55 ^{0}C, [b]Isolated yields.

In all the synthesized compounds disappearance of aldehydic proton at around 9.8 δ in ^{1}H-NMR and C-H stretching frequency at around 2750 cm^{-1} in IR spectra gives the evidence of product formation. Thiourea has shown excellent reactivity to synthesis of 3,4-dihydropyrimidin-2-(1H)thione, which is also of much interest with respect to its biological activity[6]. In order to make the biocatalytic processes economical at large scale, the recyclability of both lipase and DES has to be taken into consideration.

Recycling experiments were conducted to find out the change in activity of the catalyst after the reaction. During this study, lipase and DES were recycled up to four times. No significant decrease in

the yield of the product was observed during the first recycle, whereas it continuously declined up to 75 % at the end of fourth cycle as shown in **Table 4**.

Table 3. Synthesis of dihydropyrimidin-2(1H)-ones (DHPMs) derivatives

Entry[a]	Aldehyde	AMG	Urea/ Thiourea	Product	Time(h)	Yield,%[b]
a					4	95
b					4	92
c					4	90
d					6	82
e					6	81
f					6	73
g					6	78

Reaction Conditions: [a]All reactions were carried out with aldehyde **1a-g** (2 mmol), AMG **2a-b** (2.1 mmol), urea/ thiourea (2.2 mmol), Lipase (5% w/w with respect to aldehyde), DES (3 ml), Temperature (55 ^{0}C). [b]Isolated yields.

Table 4. Recyclability of Lipase and DES

Entry[a]	**Recycle**	**Yield (%)[b]**
1	Fresh	95
2	First	93
3	Second	87
4	Third	81
5	Fourth	75

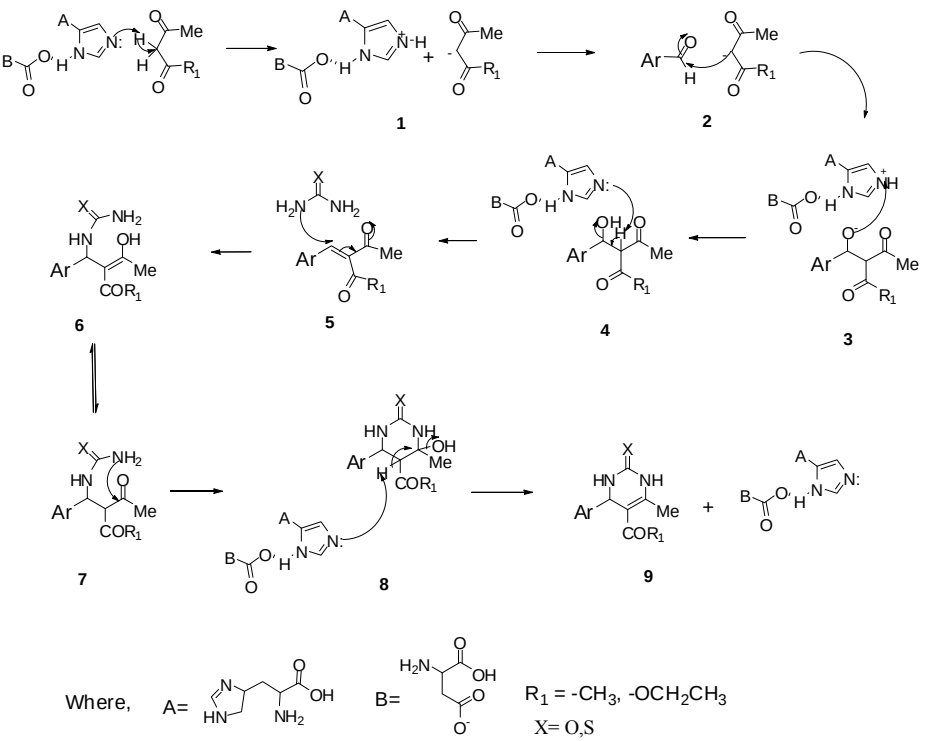
Reaction Condition: [a]All reactions were carried out with aldehyde **1a** (2 mmol), ethyl acetoacetate (2.1 mmol), urea (2.2 mmol), Lipase, DES, Time = 4h, Temperature = 55 ^{0}C, [b]Isolated yields.

All these novel DHPMs derivatives were evaluated for in vitro antibacterial activity against *Echerichia coli*, *Pseudomonas neumoniae*, *Micrococcus* and in vitro antifungal activity against *Aspergillus niger* and *Candida albicans*. Appreciable in vitro activity against the tested strains was exhibited by all the compounds. Minimal Inhibitory Concentrations (MIC) were determined by means of standard serial dilution method and summarized in **Table 5**. In all the cases, purity of the product was confirmed by elemental analysis. The structures of the pure products were confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR, elemental analysis and Mass spectral data.

Table 5. MIC (µg/mL) determination using the modified resazurin assay

Entry	Compound	Bacterial Strains		Fungal Strains	
		E. coli	***P. neumoniae***	***C. albicans***	***A. niger***
1	4a	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2
2	4b	2.5×10^2	5.0×10^2	2.5×10^2	2.5×10^2
3	4c	5.0×10^2	5.0×10^2	5.0×10^2	5.0×10^2
4	4d	5.0×10^2	5.0×10^2	2.5×10^2	2.5×10^2
5	4e	2.5×10^2	5.0×10^2	2.5×10^2	2.5×10^2
6	4f	5.0×10^2	5.0×10^2	2.5×10^2	5.0×10^2
7	4g	2.5×10^2	2.5×10^2	2.5×10^2	2.5×10^2

1. Antimicrobial activities were expressed in MIC (Minimal inhibitory concentration) values.
2. (-): Inactive.
3. Bacterial strain: E. coli NCIMB 8110, Pseudomonas *neumoniae*
4. Fungal Strain: *Candida albicans*; *Aspergillus niger*.
5. Solvent used: DMSO (*Dimethylsulphoxide*).
6. Standard: Bacterial strain: Streptomycin 125µg/mL, Fungal strains: *Fluconazole* 125µg/ mL



Scheme 2: Plausible Reaction Mechanism for synthesis of DHPMs **4a-g**

As shown in **Scheme 2** plausible reaction mechanisms were proposed for lipase catalyzed reaction, the condensation of the active methylene group and aromatic aldehyde gives intermediates (**5**) which further reacts with urea or thiourea to give DHPMs (**9**).

3. Conclusions

Environmentally benign synthesis of novel DHPMs in DES with lipase as a biocatalyst has been reported for the first time. It is a simple, convenient and effective method for the one pot synthesis of DHPMs. The lipase in DES gave high isolated yield of the DHPMs in a shorter reaction time and minimizes the use of harmful organic solvents and catalysts, which are the major environmental issues in the pharmaceutical research. Lipase is a heterogeneous catalyst, easily recoverable by simple filtration and reused in same reaction up to four cycles without major loss in its catalytic activity. All the synthesized compounds showed antibacterial and antifungal activity.

Acknowledgements

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4. Experimental

Materials and Methods

Lipase from *Rhizopus oryzae* having activity 41.6 U/ mg was procured from Zytex India Pvt. Ltd. All the solvents and chemicals were procured from S. D. Fine Chemicals (India) and were used without further purification. The reactions were monitored by TLC using 0.25 mm E- Merck silica gel 60 F_{254} precoated plates, which were visualized with UV light. FT-IR spectra were recorded on 8400S Fourier Transform Infrared Spectrophotometer Shimadzu. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on 300 MHz and 100 MHz on Varian Mercury Plus Spectrometer, respectively. Chemical shifts are expressed in δ ppm using TMS as an internal standard. Coupling constants are given in Hz. The following abbreviations are used to indicate the multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad signal. Elemental analysis was done on Harieus Rapid Analyzer. Mass spectral data were obtained with Micromass – Q– Tof (YA105) Spectrometer.

*General Procedures for the synthesis of DHPMs derivatives **4(a-g)**.*

A mixture of **1a-g** (2 mmol), active methylene group (AMG) **2a-b** (2.1 mmol), urea/ thiourea **3a-b** (2.2 mmol) and lipase (5% w/w with respect to aldehydes) was stirred in DES (3 ml) at 55 ^{0}C in 25 ml round bottom flask. The progress of a reaction was monitored by TLC. After completion of the reaction, ethyl acetate was added in reaction mass and Lipase was then filtered. The ethyl acetate and DES layers were separated, ethyl acetate layer dried by using sodium sulphate and then distilled out in high vacuum to afford final products which were recrystallized from methanol.

General Procedures for the synthesis of DES.

A DES solvent was prepared by previously reported simple method[17] with 100% atom economy. For DES preparation choline chloride (1 equiv.) was reacted with urea (2 equiv.) at 80 ^{0}C for 12h. The resulting molten salt was used directly in reactions without purification.

Recyclability study of Rhizopus Oryzae Lipase and DES

Once the reaction is over, since reaction mass is diluted with ethyl acetate, lipase was recovered by filtration and DES was immiscible with ethyl acetate, it was easily separated. Lipase and DES was dried under vacuum. The recovered lipase and DES was loaded in the same reactor, and the fresh reactant aldehyde **1a** (2 mmol), ethyl acetoacetate (2.1 mmol), and urea (2.2 mmol) were stirred at 55^{0}C for 4h to get final product (**4a**), the same procedure was repeated for consecutive recycling of the catalyst.

*Determination of Antimicrobial Activity **4a-g***

Incubator at 35 °C and 37 °C; pipettes of various sizes (Gilson); sterile tips, 100, 200, 500 and 1000 µL; sterile normal saline; sterile isosensitest agar (Southern Group Laboratory, SGL); antibiotic solutions (Sigma–Aldrich); sterile solution of 10 % (v/ v) DMSO in water (Sigma– Aldrich).

Medium

Isosensitest broth was used for this antimicrobial activity study. As recommended by NCCLS[31], Mueller Hinton medium was used for antimicrobial susceptibility testing, the most of the bacterial strains show comparable results in isosensitest medium[32, 33].

Preparation of the Plates

Plates were prepared under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 µL of test material in 10 % (v/ v) DMSO (usually a stock concentration of 4 mg/ ml) was pipetted into the first row of the plate. To all other wells, 50 µL of nutrient broth was added. Serial dilutions were performed using a multichannel pipette. Tips were discarded after use such that each well had 50 µL of the test material in serially descending concentrations. To each well, 10 µL of resazurin indicator solution was added. Using a pipette 30 µL of 3.3 × strength isosensitised broth was added to each well to ensure that the final volume was single strength of the nutrient broth. Finally, 10 µL of bacterial suspension (5 × 106 cfu/ mL) was added to each well to achieve a concentration of 5 × 105 cfu/ mL. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: a column with a broad-spectrum antibiotic as positive control, a column with all solutions with the exception of the test compound, and a column with all solutions with the exception of the bacterial solution adding 10 µL of nutrient broth instead. The plates were prepared in triplicate and placed in an incubator set at 37 °C for 18– 24 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the Minimum inhibitory concentration (MIC) value. The average of three values was calculated and that was the MIC for the test material and bacterial or fungal strain.[33]

Antimicrobial Activity

As shown in **Table 5** all the compounds were evaluated for their antifungal as well as antibacterial activity against the tested strains. The antibacterial activity of compounds **4a, 4b, 4e** and **4g** was found against *E. coli* and that of the compounds **4a** and **4g** against *pseudomonas neumoniae*. Further, the antifungal activity of the compounds **4a**, **4b**, **4d**-**4g** was found to be significant against *Candida albicans* and that of the compounds **4a**, **4b**, **4d**, **4e** and **4g** against *Aspergillus niger*. All the novel compounds have good antimicrobial activity against both bacteria and fungi.

Spectral Data 4(a-g)

Ethyl-4-(6-methoxynaphthalen-2-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate Table 3, entry 4a:
FT-IR (neat, cm^{-1}): 3358 (N-H), 1696 (C=O), 1093 (C-O).
^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.10 (t, 3H), 2.31 (s, 3H), 2.50 (q, 2H), 3.85 (s, 3H), 5.25 (d, *J* = 2.7 Hz, 1H), 7.12 (d, 1H), 7.19 (d, *J* = 6.5 Hz, 1H), 7.28 (d, *J* = 2.2 Hz, 1H), 7.35 (d, *J* = 6.5 Hz, 1H), 7.40 (d, *J* = 1.8 Hz, 1H), 7.79 (dd, *J* = 8.4 Hz, 1H), 7.58 (s, 1H, -NHgr), 9.20 (s, 1H, -NH gr).
^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 14.12, 17.88, 54.18, 55.18, 59.20, 99.22, 105.77, 118.80, 124.53, 125.38, 127.22, 128.05, 129.38, 133.64, 139.91, 148.40, 152.12, 157.26, 165.42.
Anal. Calcd. for $C_{19}H_{20}N_2O_4$: C, 67.05; H, 5.92; N, 8.23. Found: C, 67.12; H, 5.98; N, 8.32.
MS (EI) (m/z): 338.07 (M^-).

Ethyl-4-(6-methoxynaphthalen-2-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate Table 3, entry 4b:
FT-IR (neat, cm^{-1}): 3298 (N-H), 2985 (C-H), 1736 (C=O), 1663 (C=C), 1550(C=S), 1018(C-O).
^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.10 (t, 3H), 1.21 (q, 2H), 2.32 (s, 3H), 3.85 (s, 3H), 5.28 (s, *J* = 3.6 Hz, 1H), 7.12 (d, *J* = 2.5 Hz, 1H), 7.19 (d, *J* = 2.5 Hz, 1H), 7.28 (d, *J* = 2.5 Hz, 1H), 7.32 (d, *J* = 1.8 Hz, 1H), 7.36 (d, *J* = 6.9 Hz, 1H), 7.8 (d, *J* = 8.7 Hz, 1H), 9.85 (s, 1H, -NH gr), 10.20 (s, 1H, -NHgr).
^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 14.05, 17.25, 54.33, 55.21, 59.59, 100.62, 105.79, 118.97, 124.90, 125.31, 127.39, 128.01, 129.47, 133.81, 138.54, 145.11, 157.44, 165.20, 174.10.
Anal. Calcd. for $C_{19}H_{20}N_2O_3S$: C, 64.02; H, 5.66; N, 7.86; S, 9.00. Found: C, 64.12; H, 5.68; N, 7.96; S, 9.18.
MS (EI) (m/z): 357.2 (M^+).

5-acetyl-4-(6-methoxynaphthalen-2-yl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one Table 3, entry 4c:
FT-IR (neat, cm^{-1}): 3290 (N-H), 1670 (C=O), 1610 (C=C), 1083 (C-O), 1031(C-O).
^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.10 (s, 3H), 2.36 (s, 3H), 3.83 (s, 3H), 5.45 (d, *J* = 3 Hz, 1H), 7.15 (d, *J* = 2.1 Hz, 1H), 7.28 (d, *J* = 1.8 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 1H), 7. 60 (s, 1H), 7.80 (d, *J* = 8.7 Hz, 2H), 7.90 (s, 1H), 9.25 (s, 1H);
^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 19.02, 30.35, 54.04, 55.18, 105.74, 109.41, 118.81, 124.55, 125.60, 127.39, 128.10, 129.46, 133.68, 139.28, 148.26, 152.14, 157.29, 194.48;
Anal. Calcd. for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.71; H, 5. 92; N, 9.27.
MS (EI) (m/z): 310.93 (M^+).

Ethyl-4-(9-ethyl-9H-carbazol-3-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate Table 3, entry 4d:
FT-IR (neat, cm^{-1}): 3340 (NH), 2817 (C-H), 1651 (C=O), 1227, 1088 (C-O);
^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.10 (t, 3H), 1.30 (t, 3H), 2.42 (s, 3 H), 3.95 (q, 2H), 4.45 (q, 2H), 5.35 (d, *J* = 2.4 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 7.35-7.40 (dd, *J* = 8.4 Hz, 1.4 Hz, 2H), 7.54 (t, *J* = 6.9 Hz, 1 H), 7.58 (dd, *J* = 8.4 Hz, 1H), 8.02 (d, *J* = 8.7 Hz, 1H), 8.15 (d, *J* = 7.6 Hz, 1H), 9.20 (S, 1H, -NH gr), 10.24 (S, 1H, -NHgr);
^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 13.70, 14.13, 17.86, 36.99, 54.51, 59.15, 99.93, 109.19, 109.21, 118.04, 118.74, 120.11, 121.65, 122.05, 124.36, 125.73, 135.73, 138.86, 139.84, 147.86, 152.11, 165.52;
Anal. Calcd. for $C_{22}H_{23}N_3O_3$: C, 70.01; H, 6.14; N, 11.13. Found: C, 70.12; H, 6.20; N, 11.33.
MS (EI) (m/z): 376.07 (M^-).

Ethyl-4-(9-ethyl-9H-carbazol-3-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate Table 3, entry 4e:
FT-IR (neat, cm^{-1}): 3180 (N-H), 2998 (C-H), 1705 (C=O), 1590 (C=S), 1100 (C-O);
^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.10 (t, 3H), 1.30 (t, 3H), 2.40 (s, 3 H), 4.00 (q, 2H), 4.45 (q, 2H), 5.38 (d, *J* = 3.3 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 7.33 (dd, *J* = 7.9 Hz, 2H), 7.45 (t, *J* = 7.6 Hz, 1H), 7.60 (dd, *J* = 8.4 Hz, 1.4 Hz, 1 H), 7.92 (d, *J* = 1.4 Hz 1H), 8.13 (d, *J* = 7.6 Hz, 1H), 9.70(S, 1H, -NH gr), 10.24 (S, 1H, -NHgr);
^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 13.71, 14.05, 17.25, 37.01, 54.62, 59.54, 101.304, 109.236, 109.385, 118.29, 118.85, 120.16, 121.73, 121.99, 124.46, 125.87, 134.38, 139.03, 139.88, 144.63, 165.32, 173.81;
Anal. Calcd. for $C_{22}H_{23}N_3O_2S$: C, 67.15; H, 5.89; N, 10.68; S, 8.15; Found: C, 67.12; H, 5. 97; N, 10.92; S, 8.39.
MS (EI) (m/z): 393.07 (M^+).

Diethyl-4,4'-(4,4'-(phenylazanediyl)bis(4,1-phenylene))bis(6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate) Table 3, entry 4f:

FT-IR (neat, cm^{-1}): 3466 (N-H), 2983 (C-H), 1738 (C=O), 1153 (C-O), 1042 (C-O);
^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.15-1.20 (t, 6H), 2.25 (s, 6 H), 3.95-4.05 (q, 4H), 4.75 (s, 1H), 4.85 (s, 1H), 6.75 (d, *J* = 8.7 Hz, 4H), 7.05 (d, *J* = 8.4 Hz, 4H), 7.15 (dd, *J* = 7.3 Hz, 2H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.65 (d, *J* = 8.7 Hz, 2H), 8.80 (s, 2H), 9.70 (s, 2H);
^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 14.18, 14.29, 18.30, 21.67, 58.81, 59.04, 59.04, 91.78, 101.62, 101.62, 117.51, 125.51, 125.51, 126.06, 126.06, 126.52, 126.52, 128.20, 128.20, 129.01, 129.01, 129.01, 130.02, 130.02, 131.28, 131.28, 143.12, 145.51, 145.60, 152.92, 154.23, 156.83, 166.93, 168.31;
Anal. Calcd. for $C_{34}H_{35}N_5O_6$: C, 66.98; H, 5.79; N, 11.49; Found C, 66.57; H, 5.82; N, 11.82.
MS (EI) (m/z): 607.16 (M^-).

Diethyl-4,4'-(4,4'-(hexylazanediyl)bis(4,1-phenylene))bis(6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate) Table 3, entry 4g:
FT-IR (neat, cm^{-1}): 2982 (C-H), 1738 (C=O), 1666 (C=C), 1042 (C-O);
^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 0.88 (t, 3H), 1.15 (t, 6H), 1.10-1.35 (m, 8H), 2.25 (s, 6H), 2.50 (t ,2H), 4.15 (q, 4 H), 4.75 (s, 2H), 6.65 (d, *J* = 8.4, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 7.26 (d, *J* = 8.4 Hz, 2H), 7.60 (d, 2H), 8.85 (s, 2H), 9.60 (s, 2 H);
^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 13.83, 14.16, 14.27, 18.28, 21.67, 22.07, 25.84, 26.70, 29.08, 31.01, 51.79, 51.79, 58.80, 59.01, 91.70, 101.68, 112.77, 112.77, 125.67, 125.67, 126.69, 126.69, 129.23, 131.50, 142.88, 145.60, 146.36, 152.93, 154.23, 156.83, 166.92, 168.31, 189.82, 189.82;
Anal. Calcd. for $C_{34}H_{43}N_5O_6$: C, 66.1; H, 7.02; N, 11.34; Found C, 65.91; H, 6.97; N, 11.68.
MS (EI) (m/z): 617.19 (M^+).

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