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Synthesis, Characterisation and biological Evaluation of Novel Biginelli dihydropyrimidines

Karthikeyan Elumalai¹, Mohamed Ashraf Ali², Manogaren Elumalai³, Kalpana Eluri⁴, Sivaneswari. S¹, Srinivasan⁵, Sujit Kumar Mohanthy¹, Purnachander Kaleru¹, S. Durraivel¹

¹Department of Pharmaceutical Chemistry, Jayamukhi institute of pharmaceutical sciences, Warangal, India.

²Institute for Research in Molecular Medicine, University Sains Malaysia, Minden, Penang, Malaysia

³Faculty of pharmacy, UCSI University, Malaysia.

⁴Department of pharmacology, PSG College of pharmacy, Coimbatore, India.

⁵Department of Pharmacy Practice, C.L. Baidmetha college of Pharmacy, Chennai, India

ABSTRACT

We report a library consisting of some novel Biginelli dihydropyrimidines of biological interest as well as their Synthesis of 1,4-dihydropyrimidines by adopting Biginelli synthetic protocol involving one pot multicomponent reaction was performed by following steps as outlined in Scheme (Kappe and Stadler, 2004). In the first step, ethyl acetoacetate 1 and 4-fluoro aniline reacted under neat conditions resulting in the formation of N-(4-fluorophenyl)-3-oxobutanamide with the yield of 61%. The N-(4-fluorophenyl)-3-oxobutanamide was further taken for the Biginelli condensation reaction by reacting it with urea or thiourea and aryl or heteroaryl aldehyde in the presence of catalytic amount of p-toluenesulfonic acid (PTSA). synthesis and analysis by TLC, M.P, NMR, IR and Mass spectra. The important steps in the synthetic part were found to be biginelli multicomponent reactions. The synthesized compounds were screened for their in vitro antibacterial activity against two gram-positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis*. The title compounds did not exhibit significant antibacterial activity. Furthermore, compounds were subjected to in vitro cytotoxicity against Vero cells. Compounds exhibited weak, moderate, or high cytotoxicity. Compounds 1b, 1c, 1d, 1e, 1f, 1h, 1i, 1n, and 1o exhibited potential cytotoxicity.

Keywords: Dihydropyrimidines, Cytotoxicity, antibacterial activity, Verocells.

INTRODUCTION

Dihydropyrimidines (Kappe, 1993, 2003) represent important and extensively studied compounds belonging to the class of anticancer activity. Many researchers have attempted to determine the synthetic routes and various biological activities of these compounds. These developments led to the preparation and pharmacological evaluation of 3,4-dihydropyrimidines (DHPM). The discovery during the 1930s that a dihydropyridine (dihydropyridine derivative, NADH), "hydrogen transferring coenzyme" consequently became important in biological system, has generated numerous studies on the biochemical properties of dihydropyridines and their bioisosteres dihydropyrimidines (Bruce and Bencovic, 1966). The dihydropyrimidine-5-carboxylate core has been found in several marine natural products (Heys et al., 2000; Aron and Overman, 2004). DHPM derivatives are regarded as privileged structures in drug research (Horton et al., 2003; Kappe, 2003; Eynde and Watte, 2003; Shanumugam et al., 2003). In 1893, P. Biginelli reported the first synthesis of dihydropyrimidine by simple one pot condensation reaction of 1,3- dicarbonyl compound, aldehyde, and urea (Biginelli, 1893). During the past decades, the scope of the original cyclocondensation reaction was gradually extended by variation of all three building blocks, allowing access to a large number of structurally diverse multifunctionalized DHPMs. The present interest for Biginelli dihydropyrimidines is mainly due to their close structural relationship to similar drugs and compounds reported in the literature for their antitubercular (Desai et al., 2001; Prashantha Kumar et al., 2008), antimicrobial (Hooper et al.,

1982; Snider and Shi, 1993; Aron and Overman, 2004), and anticancer activities (Hattori *et al.*, 2003; Tsuji *et al.*, 2004).

MATERIALS AND METHODS

The entire chemicals were supplied by E. Merck (Germany) and S.D fine chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the solvent system ethanol, chloroform, ethylacetate (7:2:1), the spots were located under iodine vapors or UV light. IR spectrums were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr Pellets). ¹H-NMR spectra were recorded on a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO-*d*₆/CDCl₃. The chemical shifts were reported in δ ppm. Mass spectra were obtained using Shimadzu LCMS 2010A under ESI ionization technique.

RESULTS AND DISCUSSION

Chemistry

Synthesis of dihydropyrimidines by adopting Biginelli synthetic protocol involving one pot multicomponent reaction was performed by following steps as outlined in Scheme (Kappe and Stadler, 2004). In the first step, ethyl acetoacetate 1 and 4-fluoro aniline reacted under neat conditions resulting in the formation of *N*-(4-fluorophenyl)-3-oxobutanamide with the yield of 61%. The *N*-(4-fluorophenyl)-3-oxobutanamide was further taken for the Biginelli condensation reaction by reacting it with urea or thiourea and aryl or heteroaryl aldehyde in the presence of catalytic amount of *p*-toluenesulfonic acid (PTSA). The reaction times were found to be between 16 and 22 h. Fifteen 1a–o, various substituted-1,4-dihydropyrimidines, were synthesized with the yield ranging from 36 to 51% (Table 1).

Antibacterial activity

Dihydropyrimidines are known to possess antitubercular activity. All the synthesized compounds were screened for their potential antibacterial activity by cup plate method against two gram-positive bacteria, such as *Staphylococcus aureus* and *Bacillus subtilis*, because gram-positive bacteria share some common structural features with the *Mycobacterium tuberculosis*. However, none of the titled compounds exhibited significant antibacterial activity when tested in triplicate at 50 μ g concentration, as preliminary antibacterial study. Possibly, it seems that the *N*-(4-fluorophenyl)-3-oxobutanamide portion of structures would not have contributed toward their antibacterial activity to come out as candidate compounds to investigate further for the same.

In vitro cytotoxicity assay

The synthesized compounds were subjected to in vitro cytotoxicity assay against *Vero cells*. The motive for us to check the cytotoxicity for the synthesized compounds was that some reports in the past have claimed significant anticancer activity for similar kind of substructures (Kawase *et al.*, 2002). The assay was performed by the sulforhodamine B (SRB) method (Philip *et al.*, 1990). Almost all of the titled compounds exhibited weak, moderate, or high cytotoxicity. Compounds, 1b, 1c, 1d, 1e, 1f, 1h, 1i, 1n, and 1o exhibited significant cytotoxic activity with lesser CTC50 values (Table 1).

Experimental

General

Procedure for the preparation of *N*-(4-fluorophenyl)-3-oxobutanamide (1):

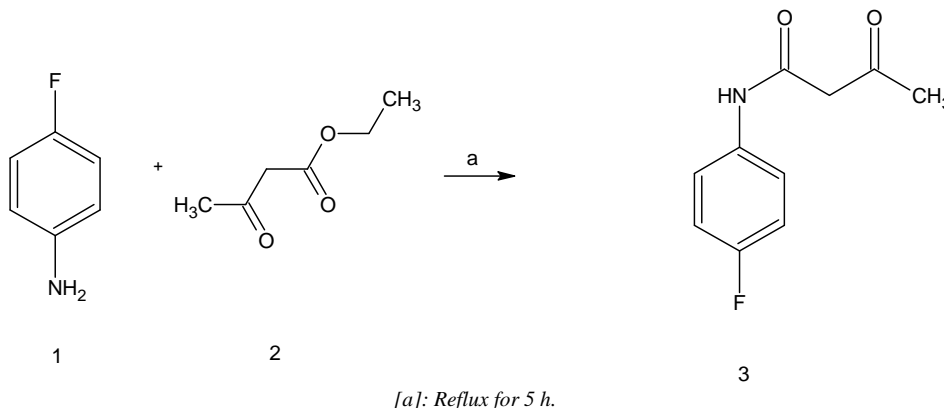
1 (0.01 M) and 2 (0.01 M) were mixed and refluxed for approximately 5 h. The colourless liquid formed was then heated on a water bath to remove the alcohol formed during the reaction. After allowing the reaction mixture to cool, crude crystals were obtained. Purification was performed by stirring crude crystals with cold diethyl ether for approximately 20 min using a mechanical stirrer. After allowing it to stand for 30 min, followed by filtration, gave 3 in a pure form of *N*-(4-fluorophenyl)-3-oxobutanamide (1).

General procedure for the preparation of dihydropyrimidines by one pot multicomponent, Biginelli synthesis (1a–o): The mixture of 3 (0.005 M), urea/thiourea (0.0075 M), and appropriate aldehyde (0.005 M) with catalytic amount of PTSA in 15 ml of ethanol was stirred for 19–26 h. The reactions were monitored through TLC using 30% ethyl acetate in pet ether as solvent system. After the reaction was complete, the reaction mixture was cooled in a refrigerator and filtered. The precipitate obtained was washed thoroughly with water to remove unreacted urea/thiourea and dried. The crude solid product was recrystallized with ethanol to give the pure compounds (1a–o). This reaction can be performed in a parallel synthetic way by taking above building blocks and varying the aldehydes for each reaction mixture. Biginelli 1,4-dihydropyrimidines, 1a–o, were synthesized relatively easily by using PTSA as an efficient catalyst compared with anhydrous AlCl₃ or HCl. The present protocol best describes the

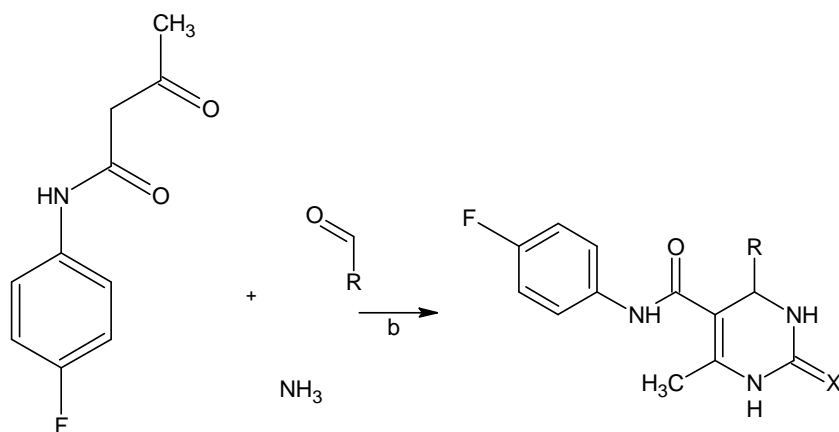
synthesis of Biginelli dihydropyrimidines. All the reported Biginelli dihydropyrimidines compounds were found to be novel and not reported elsewhere.

SCHEME : [Syntheses of dihydropyrimidines]

STEP-I: Synthesis of *N*-(4-fluorophenyl)-3-oxobutanamide



STEP-II One pot-multicomponent, Biginelli synthesis of 1,4-dihydropyrimidines using 3-oxo-*N*-(3-oxo-1,2-oxazolidin-4-yl)butanamide



Compound:

X=O or S

R=Aryl or Heteroaryl

[b]: PTSA, Ethanol and stir for 16 -22 hours at rt.

Fig:1 Scheme of work

Analytical data:

Colourless crystalline solid, mp 146–151 °C, Yield 30%, IR (KBr, cm⁻¹): 3246 (N–H), 3076 (Ht-ArC–H), 2982 (Alc–H), 1716 (C=O, ketone), 1662 (C=O, amide), 1558 (C=C), 1346 (C–N), 669 (C–F), 1H-NMR (DMSO-d₆) δ: 2.22 (s, 3H, CH₃), 3.57 (s, 2H, CH₂), 7.38 (d, 2H, ArH), 7.66 (d, 2H, ArH), 10.28 (s, 1H, NH), MS (m/z): M⁺ calculated 195.19, found, 194.86.

N-(4-fluorophenyl)-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1a)

Ash-colored solid, IR (KBr, cm⁻¹): 3274 (N–H), 3118(ArC–H), 2916 (Alc–H), 1688 (C=O, amide), 1523 (C=C), 1182 (O–C), 1H-NMR (DMSO-d₆) δ: 2.05 (s, 3H, CH₃), 3.69 (s, 3H, OCH₃), 5.32 (s, 1H, CH), 6.72–6.81(d, 2H, ArH), 7.20–7.37 (m, 5H, ArH), 7.44 (d, 2H, ArH), 7.57 (s, 1H, NH), 8.68 (s, 1H, NH), 9.43 (s, 1H, NH). MS (m/z): M⁺ calculated 325.34, found 325.16.

N-(4-fluorophenyl)-6-methyl-2-oxo-4-pyridin-4-yl-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1b)
Light-greenish solid, ¹H-NMR (DMSO-d₆) δ: 2.06 (s, 3H, CH₃), 3.74 (s, 3H, OCH₃), 5.42 (s, 1H, CH), 6.83 (d, 2H, ArH), 7.26–7.46 (m, 5H, ArH), 7.53 (d, 2H, ArH), 9.38 (s, 1H, NH), 9.63 (s, 1H, NH), 9.92 (s, 1H, NH), MS (m/z): M⁺ calculated 353, found 353.75. MS (m/z): M⁺ calculated 326.33, found 325.86.

N-(4-fluorophenyl)-6-methyl-4-pyridin-4-yl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1c)
Light-bluish colored solid, IR (KBr, cm⁻¹): 3332 (N–H), 3072 (ArC–H), 2958 (AliC–H), 1688 (C=O, amide), 1518 (C=C), 1287 (O–C), ¹H-NMR (DMSO-d₆) δ: 2.03 (s, 3H, CH₃), 3.76 (s, 3H, OCH₃), 5.38 (s, 1H, CH), 6.84 (d, 2H, ArH), 6.88–7.17 (m, 4H, ArH), 7.46 (d, 2H, ArH), 7.51 (s, 1H, NH), 8.66 (s, 1H, NH), 9.42 (s, 1H, NH). MS (m/z): M⁺ calculated 342.39, found 342.06.

N-(4-fluorophenyl)-4-(2-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1d)
Light-greenish solid, ¹H-NMR (DMSO-d₆): δ 2.06 (s, 3H, CH₃), 3.72 (s, 3H, OCH₃), 5.34 (s, 1H, CH), 6.84 (d, 2H, ArH), 6.92 (d, 2H, ArH), 7.18 (d, 2H, ArH), 7.48 (d, 2H, ArH), 9.42 (s, 1H, NH), 9.58 (s, 1H, NH), 9.98 (s, 1H, NH). MS (m/z): M⁺ calculated 341.34, found 341.08.

N-(4-fluorophenyl)-4-(2-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1e)
Dark-brownish solid, IR (KBr, cm⁻¹): 3286 (N–H), 3018 (ArC–H), 2921 (AliC–H), 1668 (C=O amide), 1522 (C=C), 1248 (C–O), ¹H-NMR (DMSO-d₆): δ 2.07 (s, 3H, CH₃), 3.72 (s, 3H, OCH₃), 5.6 (s, 1H, CH), 6.26 (d, 2H, ArH), 7.48 (m, 3H, ArH), 7.74 (d, 2H, ArH), 8.79 (s, 1H, NH), 9.43 (s, 1H, NH), 9.96 (s, 1H, NH). MS (m/z): M⁺ calculated 357.40, found 357.12.

N-(4-fluorophenyl)-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1f)
Ash-colored solid, ¹H-NMR (DMSO-d₆): δ 2.04 (s, 3H, CH₃), 3.78 (s, 3H, OCH₃), 5.38 (s, 1H, CH), 6.24 (d, 2H, ArH), 6.86 (d, 2H, ArH), 7.42–7.62 (m, 3H, ArH), 9.42 (s, 1H, NH), 9.52 (s, 1H, NH), 10.28 (s, 1H, NH), MS (m/z): M⁺ calculated 370.34, found 369.92.

N-(4-fluorophenyl)-6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1g)
Light-greenish solid, ¹H-NMR (DMSO-d₆): δ 2.06 (s, 3H, CH₃), 3.79 (s, 3H, OCH₃), 5.74 (s, 1H, CH), 6.76 (d, 2H, ArH), 7.52–7.60 (m, 4H, ArH), 8.78 (s, 1H, NH), 9.32 (s, 1H, NH), 9.84 (s, 1H, OH), 10.12 (s, 1H, NH). MS (m/z): M⁺ calculated 386.40, found 386.12.

4-(3-chlorophenyl)-*N*-(4-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1h)
Light-ash-colored solid, ¹H-NMR (DMSO-d₆): δ 1.68 (s, 3H, CH₃), 3.78 (s, 3H, OCH₃), 4.66 (s, 1H, CH), 6.85–7.46 (m, 8H, ArH), 9.01 (s, 1H, NH), 9.06 (s, 1H, NH), 9.08 (s, 1H, OH), 10.04 (s, 1H, NH). MS (m/z): M⁺ calculated 359.78, found 359.22.

4-(3-chlorophenyl)-*N*-(4-fluorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1i)
Light-greenish-colored solid, IR (KBr, cm⁻¹): 3352 (N–H), 3079 (ArC–H), 2962 (AliC–H), 1660 (C=O, amide), 1514 (ArC=C), 1622 (AliC=C), ¹H-NMR (DMSO-d₆): δ 2.05 (s, 3H, CH₃), 3.68 (s, 3H, OCH₃), 5.88 (s, 1H, CH), 6.21–6.56 (m, 4H, ArH), 7.22–7.59 (m, 4H, ArH), 9.86 (s, 1H, NH), 10.0 (s, 1H, NH), 10.14 (s, 1H, NH). MS (m/z): M⁺ calculated 375.85, found 375.42.

N-(4-fluorophenyl)-4-(2-furyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1j)
Light-bluish solid, ¹H-NMR (DMSO-d₆): δ 2.06 (s, 3H, CH₃), 3.76 (s, 3H, OCH₃), 5.44 (s, 1H, CH), 6.88 (d, 2H, ArH), 7.43 (d, 2H, ArH), 7.60–7.78 (m, 4H, ArH), 8.12 (s, 1H, NH), 8.74 (s, 1H, NH), 9.56 (s, 1H, NH). MS (m/z): M⁺ calculated 315.30, found 314.90.

N-(4-fluorophenyl)-4-(2-furyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1k)
Light-greenish solid, IR (KBr, cm⁻¹): 3386 (N–H), 3032 (ArC–H), 2982 (AliC–H), 1896 (C=S), 1684 (C=O, amide), 1568 (C=C), 1521 (NO₂), 1342 (NO₂), 1212 (C–O). ¹H-NMR (DMSO-d₆) δ: 2.04 (s, 3H, CH₃), 3.72 (s, 3H, OCH₃), 5.56 (s, 1H, CH), 7.87 (d, 2H, ArH), 7.62–8.18 (m, 6H, ArH), 9.62 (s, 1H, NH), 9.72 (s, 1H, NH), 10.22 (s, 1H, NH). MS (m/z): M⁺ calculated 331.36, found 331.05.

N-(4-fluorophenyl)-6-methyl-2-oxo-4-[(*E*)-2-phenylvinyl]-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1l)
Light-greenish solid, ¹H-NMR (DMSO-d₆) δ: 2.04 (s, 3H, CH₃), 3.58 (s, 3H, OCH₃), 5.32 (s, 1H, CH), 6.72–6.85 (m, 5H, ArH), 7.37 (d, 2H, ArH), 8.82 (s, 1H, NH), 9.34 (s, 1H, NH), 9.64 (s, 1H, OH), 9.92 (s, 1H, NH). MS (m/z): M⁺ calculated 351.38, found 351.10.

N-(4-fluorophenyl)-6-methyl-4-[(*E*)-2-phenylvinyl]-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide(1m)
Pale-yellowish solid, mp 187–189 °C, yield 37%, IR (KBr, cm⁻¹): 3372 (O–H), 3264 (N–H), 3072 (ArC–H), 2980 (AlC–H), 1664 (C=O, amide), 1978 (C=S), 1583 (ArC=C), 1166 (C–O), 1H-NMR (DMSO-d₆): δ 2.05 (s, 3H, CH₃), 3.21 (s, 3H, OCH₃), 4.66 (s, 1H, CH), 6.72–6.85 (m, 5H, ArH), 7.40 (d, 2H, ArH), 8.90 (s, 1H, NH), 8.96 (s, 1H, NH), 9.48 (s, 1H, OH), 9.52 (s, 1H, NH). MS (m/z): M⁺ calculated 366.44, found 366.20.

N-(4-fluorophenyl)-6-methyl-2-oxo-4-pyridin-3-yl-1,2,3,4-tetrahydropyrimidine-5-carboxamide (1n)
Light-bluish solid, mp 251–253 °C, yield 38%, 1H-NMR (DMSO-d₆) δ: 2.08 (s, 3H, CH₃), 3.54 (s, 3H, OCH₃), 5.38 (s, 1H, CH), 6.78 (t, 3H, ArH), 7.29–7.43 (m, 5H, ArH), 8.83 (s, 1H, NH), 9.52 (s, 1H, NH), 9.8 (s, 1H, NH). MS (m/z): M⁺ calculated 326.33, found 326.04.

N-(4-fluorophenyl)-6-methyl-4-pyridin-3-yl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide(1o)
Light-greenish solid, mp 205–207 °C, yield 41%, IR (KBr, cm⁻¹): 3378 (N–H), 3052 (ArC–H), 2968 (AlC–H), 1956 (C=S), 1690 (C=O, amide), 1557 (C=C), 1238 (C–O), 734 (C–Cl). 1H-NMR (DMSO-d₆) δ: 2.08 (s, 3H, CH₃), 3.84 (s, 3H, OCH₃), 5.40 (s, 1H, CH), 6.94 (d, 2H, ArH), 7.28 (d, 2H, ArH), 7.26–7.56 (m, 4H, ArH), 9.56 (s, 1H, NH), 9.74 (s, 1H, NH), 10.22 (s, 1H, NH). MS (m/z): M⁺ calculated 342.39, found 341.90.

Table1: Synthesized dihydropyrimidines physical parameter and its cytotoxicity

Compound no	R	X	Reaction time	Yield (%)	mp ^o C	CTC ₅₀ (μg/ml)	pCTC ₅₀ (μM)
1a	phenyl	O	23	51	224	235	0.166
1b	4-pyridyl	O	21	48	196	22	1.322
1c	4-pyridyl	S	26	44	206	20	1.243
1d	2-hydroxy phenyl	O	20	38	232	60	0.954
1e	2-hydroxy phenyl	S	19	42	247	53	0.834
1f	3-nitrophenyl	O	23	38	231	64	1.776
1g	3-nitrophenyl	S	21	41	228	72	0.892
1h	3-chlorophenyl	O	24	39	254	14	1.157
1i	3-chlorophenyl	S	21	42	268	12	1.287
1j	2-furyl	O	22	36	218	189	0.326
1k	2-furyl	S	20	39	227	152	0.435
1l	cinnamyl	O	19	43	189	156	0.765
1m	cinnamyl	S	23	43	199	162	0.645
1n	3-pyridyl	O	21	37	211	29	1.238
1o	3-pyridyl	S	20	41	219	22	1.315

CONCLUSION

A series of novel Biginelli dihydropyrimidines of biological interest were synthesized and analyzed for their structures. The Biginelli compounds were prepared by using PTSA as an efficient catalyst. Compounds, such as 1c, 1h, and 1i, exhibited potential cytotoxicity and are considered the candidates to investigate further for the same. The importance of substitution at the fourth position of dihydropyrimidines toward the cytotoxicity.

Antibacterial activity

All of the synthesized compounds were screened for their antibacterial activity against two gram-positive bacteria: *B. subtilis* and *S. aureus* (Frankel et al., 1970). The primary screening was performed by using the agar diffusion method using Muller-Hinton agar medium. The compounds were tested at the concentration of 50 μg per well. Sterile nutrient agar plates were prepared in petri dishes under aseptic conditions; 0.1 ml of each standardized test organism was spread onto agar plates and cavity was done by using a sterile borer of diameter 6 mm. Then, 50 μg per well of compounds or standard drug solution of streptomycin (10 μg) and DMSO solvent were placed in each cavity, separately. The plates were maintained at 37°C for 1 h to allow diffusion of solution into the medium; then the plates were incubated at 37°C for 24 h and observed for zone of inhibition.

In vitro cytotoxicity

Short-term in vitro cytotoxicity assay was performed using *Vero cells* according to the standard procedure (Moldeus et al., 1978). SRB is a bright-pink aminoxanthene dye with two sulfonic groups. Under mild acidic conditions, SRB binds to protein basic amino acid residues in trichloroacetic acid fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. After incubation, the solutions in the wells were flicked off and 100 μl of different concentrations (2–500 μg) of compounds were added to the cells and incubated at 37°C for 3 days in 5% CO₂ atmosphere. The microscopic examinations were performed and observations were recorded every 24 h. After, 72 h, 50% trichloroacetic acid (25 μl) was added to each well and the plates were incubated for 1 h at 4°C. The supernatant was then removed, and the cells were washed with water,

air-dried, and stained, each well with SRB for 30 min. The unbound dye was removed by washing with 1% acetic acid and the plates were airdried. Tris base (10 mM, 100 μ l) was added to wells to solubilize the dye. The plates were vigorously shaken for 5 min, and the absorbance was measured using microtiter plate reader at 540 nm. The mean absorbance of triplicate was recorded. Mean absorbance taken from cells grown in the absence of the test compound was taken as 100% cell survival (control). The percentage growth inhibition was calculated using the following formula:

$$\text{Growth inhibition \%} = 100 - (\text{sample absorbance} / \text{control absorbance}) \times 100$$

The percentage growth inhibition was plotted against concentration and the CTC50 (concentration required to reduce viability by 50%) value was calculated.

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