A Synthesis and Pharmaceutical Evaluation of Pyrimidine Derivatives and Their Docking Studies

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Abstract:

A series of halogenated pyrimidine derivatives tetrahydropyrimidine; Ethyl-6-methyl-2-oxo-4-phenyl-1,3,4-tetrahydropyrimidine-5-carboxylate (10a), Ethyl-4-(3-Chlorophenyl)-6-methyl-2-oxo-1,3,4-tetrahydropyrimidine-5-carboxylate (10b) and Ethyl-4-(4-Chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (10c) were synthesized by using new strategy in "Grindstone protocol" to get high yield in short time. The synthesized compounds were characterized by ¹H-NMR, ¹³C-NMR and Mass spectral data and evaluated for their anticancer activity against human HepG2 Liver cell lines, for antifungal and insecticidal activities. All the synthesized compounds (10a-c) were studied to measure the interactions by docking study.

Keywords: Pyrimidine derivatives, MTT assays, grindstone, human HepG2 Liver cell Lines, NMR analysis, Mass spectroscopy

I. Introduction

Pyrimidine is the most important heterocyclic compound and its derivatives are used as essential pharmacophores and synthon in the field of medicine. Pyrimidine derivatives exhibit diverse biological activities such as antifungal, antimicrobial, antiviral, anticancer, anti-inflammatory and also used as hypnotics. To explore their pharmacological potential, the chemistry of pyrimidine is a blooming field. Pyrimidine derivatives are prepared by using different methods and provide enormous scope in the field of medicinal chemistry. Pyrimidine has remarkable biological significance. Pyrimidine (**1**) is core component of DNA bases (Kharb, Tyagi, & Sharma, 2014) and its nucleus (**1**) occurred in a wide variety of compounds of chemotherapeutic potentials, i.e. 5-fluorouracil (**2**) and tegafur (**3**), iodoxuridine (**4**), trifluoridine and zidovudine (**5**), flucytosine (**6**), pyrimethamine (antimalarial agent), and (sulfamerazine, sulfamethazines and sulfadiazines) (antibacterial agents) (Figure 1) (X Liu et al., 2016). Pyrimidine nucleus is building block in structure of naturally occurring biologically active compounds. Most of which belongs to alkaloids and alkaloids are the major constituents of pharmaceutical industry. Pyrimidine is the unit of RNA and DNA bases and play an important role in drug metabolism. Several derivatives of pyrimidines have already been synthesized (Cepanec, Litvić, Bartolinčić, & Lovrić, 2005; El-Sayed et al., 2015; Ghorab & Alsaid, 2015; Mohamed et al., 2015; Sonawane, 2014; Zahran & Keshta, 2014). Dihydro pyrimidine derivatives are well known for their broad-spectrum pharmaceutical applications/activities (antibacterial, anti-viral and antitumor agents). Recently, different research groups reported the synthesis and anticancer potential of 1, 4-dihydropyrimidine, 3,4-

dihydropyrimidine and amino thioxo substituted tetrahydro pyrimidine derivatives (Cepanec et al., 2005; Hazarkhani & Karimi, 2004; Rana, Arora, & Chawla, 2014) by using different methods; conventional, Biginelli, Grindstone. We synthesized the tetrahydro pyrimidine derivatives by using Grindstone method which has proved an excellent method due to less time consumption, solvent free, high product yield and low chance of side products. Grindstone protocol which has seen proved a very efficient and convenient method, give high yield of product in very short time. Moreover, avoid use of organic solvents whose toxic fumes pollute the environment.

Figure 1: Influence of Pyrimidine skeleton in pharmaceutical industry

II. Results and Discussion

Chemistry

Many synthetic methods for formulating dihydro-pyrimidine compounds have been designated including classical methods, microwave irradiation and by using Lewis acids as well as protic acids in recent years (XH Liu, Tan, & Weng, 2011; Lu & Bai, 2002; Ryabukhin, Plaskon, Ostapchuk, Volochnyuk, & Tolmachev, 2007; Schauble, Trauffer, Deshpande, & Evans, 2005; Wang et al., 2011). The sighting of a new and cheap catalyst for the preparation of dihydro pyrimidine in neutral and mild conditions with high yield is of prime importance. In solvent free organic synthesis reagents react together in the absence of any solvent have been look over as a fast-rising technology. A large number of methods have been designed to synthesize dihydro pyrimidine compound. But emergence of a new, cheap approach for preparation of dihydro pyrimidine in neutral and with high yield and solvent free method is of prime importance. In 2014, 3,4-dihydropyrimidine derivatives in high yield, by following the technique of Grindstone, one of green chemistry technique (El-Sayed et al., 2015). The newly synthesized derivatives (**10a-c**) were characterized by IR, but not reported their NMR (^1H & ^{13}C), mass spectrometry data and biological activities. In this protocol, reactions are started by grinding, with transfer of very small amount of energy through friction. This new approach consists of catalysts such as $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot \text{HCl}$, ZnCl_2 , CoCl_2 , $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ etc. Following these discoveries, it was our interest to synthesize some new tetrahydro pyrimidine derivatives using Grindstone protocol and evaluated them for their biological activity.

Pyrimidine derivatives **10 (a-c)** have been prepared using Grindstone procedure; a mixture of ethyl acetoacetate (**9**), secondary amine (**8**), substituted benzaldehyde (**7a-c**) and catalytic amount of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ was ground together for 2-5min at room temperature, left the mixture overnight. The products formed were washed with cold water, dried and re-crystallized with hot alcohol to achieved pure products. The progress of the reaction was examined with TLC and UV lamp (Nusrat, Sonia, Shagufta, & Basharat, 2019).

Biological Evaluation

All the synthesized compounds (**10a-c**) were screened for anticancer activity against human HepG2 Liver cell lines, anti-fungal and insecticidal activities.

Human Hep G2 Liver Cancer cell Inhibition:

Compounds **10a-c** were subjected to MTT assays to detect their activity. It was observed that compound **10a-c** showed excellent viability of cells against human Hep G2 liver cell lines (Table 1).

Out of synthesized compounds, only two compounds showed insecticidal activity while other was inactive. Our results of the insecticidal activity of compound

Table 1: Anti-cancer activity of synthesized compounds (10a-c)

Test Compounds	Anticancer (%age activity/ viability)		
Concentration	50 μ M	100 μ M	150 μ M
10a	73.64	127.28	170.92
10b	49.18	118.34	157.51
10c	45.01	114.02	148.02
Control value	100		

10b and **10c** have indicated moderate to high toxicity to *Spodoptera litura* second instar larvae depending upon the exposure time. Both the compounds, **10b** and **10c** showed very little activity at 24 h and moderate activity at 48 h post treatment with LD₅₀s value of 1035.49 and 473.16, respectively. The compound **10b** showed higher activity with LD₅₀ value of 114.86 compared to the LD₅₀ value of 235.32 of compound **10c** after 72 h post exposure to a treated artificial die (Table 2). As far as the dose response curve is concerned the compound **10c** showed more consistency with the increments in dose compared to **10b**. The r^2 values for **10b** remained at 0.75 and **10c** at 0.97 respectively (Figure 2 & 3). The response of both the compounds remained slow during early hours of exposure that may be due to the type of bioassay used which is normally recommended for stomach poisons.

Fig 2: Dose response of **10b** after 72 h post treatment against second instar larvae of *Spodopteralitura Fabricius* under laboratory condition

The pyrimidine derivatives have been in focus for the development of Agrochemicals owing to their biological activity as fungicides (Xing-Hai et al., 2017) herbicides (Huang, Zhang, Xu, & Zeng, 2005) and insecticides (Hüter, 2012). As an example, flucrypyrim has been reported with profound acaricidal activity against all life stages of red spider mite (J. Li, Wang, Wu, & Yang, 2015). Our findings about the insecticidal activity of pyrimidine derivatives is in line with the previously reported efficacy of pyrimidines on insects (Finney, 1971; T. Li et al., 2013).

Fig. 3; Dose response of **10c** after 72 h post treatment against second instar larvae of *Spodoptera litura* Fabricius under laboratory condition

Aryl substituted 3,4-dihydropyrimidine-2-(1H)-ones and their derivatives are an important class of substances in organic and medicinal chemistry. These are active as anti-bacterial, anti-fungal, anti-oxidant agents. The insecticidal study of the synthesized compounds revealed that these showed excellent potentials against armyworm. Out of the synthesized compounds, great potential of **10b** revealed that *m*-substitution is responsible for increase in activity. Both the compounds **10b** and **10c** in their potential as insecticidal agents showed the results in accordance with previously findings as reported.

Molecular Modelling Studies

Docking Study:

Molecular docking studies of synthesized compounds **10 a-c** were carried out by Molegro Virtual Docker 2010 to rationalize the biological activity results. In docking study, various interactions between ligand and protein was studied in active site. The crystal structure of 2BXE (pdb) were used for docking study.

From docking study with 2BXE following results were drawn. The synthesized compounds **10a-c** has Mol Dock Score -97.2311 to -71.2041. **10a** showed the following hydrogen bonding (Figure 4); The 1-NH in pyrimidine moiety showed interactions with Pro 147, Val 456, Asp 108, Lys 190, Ser 193 having hydrogen bonding of 3.50, 3.48, 3.07, 3.48, 2.66 Å° respectively in compounds **10a** and **10b** while 3-NH of pyrimidine moiety binds with Ser 192, Tyr 452, Ser 193, Val 455, Arg 145 having hydrogen bonding of 2.94, 2.53, 2.67, 2.92, 3.20, 2.97 Å° in compounds **10a-c**. The carbonyl of pyrimidine moiety showed binding with Tyr 184, Asn 458, Tyr 148, Tyr 452 having hydrogen bonding of 3.33, 2.95, 2.65, 2.72, 3.17, 3.16 Å° in compounds **10a** to **10c** while the carbonyl of ester chain showed interactions with Ser 193, Asn 429, Gln 459, His 146, Arg 145 with hydrogen bonding of 3.12, 3.26, 3.06, 3.10, 2.83 Å° in compounds **10 a-c**. Oxygen of ester chain showed bindings with Gln 459, Asn 109, Ser 193, His 146 having hydrogen bonding of 3.12, 3.05, 3.18, 3.04, 3.32 Å° in **10a, 10c**. Maximum interactions have been found in compound **10a-c** (Gul et al., 2021; Nusrat et al., 2019).

III. Experimental

Chemistry

All the chemicals and solvents used for synthesis were of analytical grade. The reactions were monitored by thin layer chromatography performed on silica gel 60 F₂₅₄ coated thin layer plates. The ¹H-NMR and ¹³C-NMR spectra were recorded on a 500 MHz instrument using CD₃OD as the solvent due to solubility of the compounds in methanol and TMS as internal standard. Mass spectra were recorded by using JEOL JMS-HX 100 at 70 eV.

General Procedure for synthesis of compound 10a-c:

1mmol (0.1ml) benzaldehyde (**7a**), 1mmol (0.06g) urea (**8**), ethylacetoacetate (**9**) (1mmol, 0.12ml) and catalytic amount of CuCl₂·2H₂O was collectively ground in a china dish for 2-5 minutes; solid mass formed was left overnight, washed with cold water, dried and re-crystallized with methanol at room temperature (Scheme 1 & 2). The products formed were washed with cold water, dried and re-crystallized with hot alcohol to achieved pure products. The progress of the reaction was examined with TLC and UV lamp (Nusrat et al., 2019).

Scheme 1: Synthesis of tetrahydropyrimidine derivatives 10 (a-c) by Grindstone method

Scheme 2: Reaction mechanism for synthesis of tetrahydropyrimidine derivatives 10 (a-c)

All the synthesized compounds were characterized by ¹H-NMR and ¹³C-NMR and mass spectra: data is as under;

Ethyl-6-methyl-2-oxo-4-phenyl-1,3,4-tetrahydropyrimidine-5-carboxylate(10a):

Physical Characteristics: White shiny crystals; ¹HNMR (500 MHz, CD₃OD): δ 9.20 (1H, s, NH), 7.65 (1H, s, NH), 7.32-7.21 (5H, m), 5.13 (1H, d, *J* = 3.0 Hz), 3.99 (2H, q, *J* = 6.0 Hz), 2.49 (3H, s, CH₃), 1.09 (3H, t, *J* = 6.0 Hz, CH₃). ¹³C-NMR (CD₃OD, 500 MHz): δ 165.2 (C), 152.0 (C), 148.3 (C), 144.9 (C), 128.3 (CH), 127.2 (CH), 126.2 (CH), 99.2 (C), 59.1 (CH₂), 53.9 (CH), 17.7 (CH₃), 14.0 (CH₃); EIMS-FAB⁺: *m/z* 261 [M+H]⁺ for C₁₄H₁₆N₂O₃ calculated 260; EIMS-FAB⁻: *m/z* 259 [M-H]⁻ for C₁₄H₁₆N₂O₃ calculated 260 (El-Sayed et al., 2015).

Table 2: Toxicity of newly synthesized compounds 10b and 10c against second instar larvae of *Spodoptera litura* Fabricius under laboratory condition

Chemical	Time	N	LD ₅₀	Slope (±SE)	χ^2	df	95% FL (ppm)
10b	48 h	120	1035.49	3.42 (±0.31)	0.30	3	268.56-4225.49
	72 h	120	114.86	3.10 (±0.38)	0.40	3	71.37-184.30
10c	48 h	120	473.16	2.84 (±0.31)	0.45	3	222.44-1028.34
	72 h	120	235.32	3.27 (±0.39)	0.33	3	127.13-440.68

Ethyl-4-(2-Chlorophenyl)-6-methyl-2-oxo-1,3,4-tetrahydropyrimidine-5-carboxylate (10b):
Physical Characteristics: White shiny crystals; ¹HNMR (500 MHz, CD₃OD): δ 9.75 (1H, *d*, NH), 7.75 (1H, *s*, NH), 7.35 (1H, H-2',*m*), 7.31 (1H, *m*, H-4'), 7.23 (1H, *m*, H-5'), 7.19 (1H, *m*, H-6'), 5.14 (1H, *d*, J=5.0 Hz, H-4), 4.0 (1H, *q*, J=10 Hz, *q*, CH₂), 2.25 (1H, *s*, CH₃), 1.20 (1H, *t*, CH₃); ¹³C-NMR (MeOD, 175 MHz): δ 165.2 (C-1'), 151.8 (C-2), 148.9 (C-6), 147.1 (C-1'), 132.8 (C-3'), 130.7 (C-5'), 129.0 (C-4'), 126.8 (C-2'), 126.7 (C-6'), 98.5 (C-5), 59.9 (C-3'), 17.69 (CH₃), 14.00 (C-4''); EIMS-FAB+: *m/z* 295 [M+H]⁺ for C₁₄H₁₅ClN₂O₃ calculated 294.1; EIMS-FAB-: *m/z* 293 [M-H]⁻ for C₁₄H₁₅ClN₂O₃ calculated 294.1.

Eethyl-4-(4-Chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (10c):

A mixture of 4-Chloro-benzaldehyde (7a) (4mmol, 0.56g), urea (8) (4mmol, 0.24g), ethylacetoacetate (9) (4mmol, 0.51ml) and catalytic amount of CuCl₂·2H₂O was ground, to obtain compound 10c in the same way as 10a and 10b. Physical Characteristics: White shiny crystals; ¹HNMR (500MHz, CD₃OD): δ 9.20 (1H, *s*, NH), 7.72 (1H, *s*, NH), 7.38-7.22 (4H, *m*), 5.13 (1H, *d*, J = 3.0 Hz,), 3.99 (2H, *q*, J = 6.0 Hz), 2.29 (3H, *s*, CH₃), 1.09 (3H, *t*, J = 6.0 Hz, CH₃); ¹³C-NMR (CD₃OD, 500 MHz): δ 165.0 (C), 152.5 (C), 148.3 (C), 144.4 (C), 132.4 (C), 129.0 (CH), 129.0 (CH), 128.8 (CH), 128.8 (CH), 99.5 (C), 59.9 (CH₂), 54.1 (CH), 17.4 (CH₃), 14.7 (CH₃); EIMS-FAB+: *m/z* 295 [M+H]⁺ for C₁₄H₁₅ClN₂O₃ calculated 294.1; EIMS-FAB-: *m/z* 293 [M-H]⁻ for C₁₄H₁₅ClN₂O₃ calculated 294.

IV. Biological Evaluation

Anti-cancer Bio-assays

Cell Culture and Treatment:

The human HepG2 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 µg/mL streptomycin and maintained at 37°C with 5% CO₂ in humidified atmosphere. Cells were treated with extracts/compound dissolved in DMSO with a final DMSO concentration of 0.05%. DMSO treated cells were used as control in all the experiments.

Determination of Cell Viability:

Cell viability was determined by MTT assay as described by us previously (Bauer, Kirby, Sherris, & Turck, 1996). Briefly HepG2 cells were treated with different concentrations of compounds for 48 h. Following treatment, the MTT reagent was added (500 µg/mL) and cells were further incubated at 37°C for 4 h. Subsequently 150 µL DMSO was added

to dissolve farmazan crystals and absorbance was measured at 490 nm in a microplate reader (Thermo Scientific). The percentage of cell viability was calculated.

Determination of insecticidal activity:

NAC TFs are linked to a number of processes, including several developmental programs, of secondary wall senescence and biotic and abiotic stress response. The genome-wide transcriptome analysis of a bioinformatics study shows 20 to 25 percent of NAC genes working at least in one stress response or another.

NAC Regulation And Activity

Maintenance of insect colony:

The susceptible population of *S. litura* was reared on semi artificial diet in an insectary without exposure to any insecticide on temperature, humidity and photoperiod maintained at 25± 2 °C, 45 5 and 12L: 12D. Freshly hatched neonates were carefully transferred collectively with the help of camel hair brush to the artificial diet consisting of chickpea powder 100 g, agar 12.8 g, sorbic acid 1 g, yeast 30 g, choline chloride 10% 7.20 ml, vitamin (ABCDE) 2 ml, ascorbic acid 3.20 g, streptomycin sulphate 0.04 g and a total of 800 ml of distilled water. Freshly emerged adults were transferred to glass container with cotton cloth in the base and on the top with a vial containing 10% honey solution.

Diet incorporation insect bioassay:

Newly synthesized compounds 10b and 10c both were serially diluted in analytical grade acetone and serially diluted before incorporation into the artificial diet. Serially diluted concentrations of 500, 250, 125, 62.5 and 31.25 ppm were introduced into the artificial diet and homogenized well (chickpea powder 100 g, agar 12.8 g, sorbic acid 1 g, yeast 30 g, choline chloride 10% 7.20 ml, vitamin (ABCDE) 2 ml, ascorbic acid 3.20 g, streptomycin sulphate 0.04 g and a total of 800 ml of distilled water) separately for both the compounds and kept on room temperature for 8 h to allow evaporation of acetone. Acetone alone was used as a control. Transparent plastic cups of 10 ml capacity with aeration net in the lid were dispensed with 2 g of the treated artificial diet. A total of 20 cups per concentration were prepared for each treatment and each cup was introduced with 5 second instar larvae. The mortalities among insects were observed 24, 48 and 72 h post treatment. The mortality data were analysed by POLO probit (Balouiri Sadiki & Ibnsouda).

Molecular Docking

Docking has been studied by using Molegro virtual docker version 2010. Protein has been downloaded from protein data bank online and saved as pdb. Then structures of synthesized compounds **10a-c** were drawn in BIOCHEM Draw, copy to 3D bio-chem draw and saved as Mol2 file. Open the software docking, import pdb protein and then prepare the template and then one by one all compounds were running

V. Conclusion

It has been concluded from the all results that the active moiety among the synthesized skeleton was pyrimidine ring with ester chain. As anti-cancer activity data showed that compounds 10a-c give more activity. Likewise, insecticidal data showed the activity of 10b and 10c. Thus conclusively, in all synthesized compounds, pyrimidine moiety is responsible for activity and provide active sites.

Figure 4: Different Interactions of 10a, 10b and 10 c with binding proteins by Mol. Docking (dotted lines showing interactions)

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