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**Research Article**

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# Synthesis of 1,2,3,4-tetrahydro pyrimidine derivatives as an antimicrobial agent

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**ABSTRACT**

*The chemistry of pyrimidines is a blossoming field for the study of their pharmacological uses. Numerous methods for the synthesis of 1,2,3,4-tetrahydro pyrimidine as also their diverse reactions offer enormous scope in the field of medicinal chemistry. Keeping in mind various biomedical applications and with a view to further asseaa the pharmacological profile of these class of compounds, novel 1,2,3,4-tetrahydro-N-(substituted phenyl)- 6-methyl-2-oxo-[(4-(phenoxy)-methyl) phenyl] pyrimidine-5-carboxamide(AB101 to AB115) are synthesizes. The products were characterized by FT-IR, $^1$H NMR, Mass spectra and elemental analysis. The newly synthesized compounds were subjected to antimicrobial activities.*

**Key words** : 1,2,3,4-tetrahydro pyrimidine, Antimicrobial activity

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## INTRODUCTION

Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of acquired Immuno Deficiency syndrome(AIDS). The pyrimidine nucleus is embedded in a large number of alkaloids, drugs, antibiotics, agrochemicals and antimicrobial agents[1]. In the past decades, a broad range of biological effects, including antiviral, antitumor, antibacterial and anti-inflammatory activities, has been ascribed to partly reduced pyrimidine(DHPM) derivatives. More recently, appropriately functionalized DHPMs have emerged as eg. orally active antihypertensive agents[2,3,4]. A very recent highlight in this context, has been the identification of the structurally rather simple dihydropyrimidine(DHPM) monastrol as a novel cell-permeable molecule that blocks normal bipolar spindle assembly in mammalian cells and therefore causes cell cycle arrest[5]. M. D. Gavilan et al[6] reported the synthesis of (1,3,5-tetrahydro-4-,1-benzoxazepine-3-yl) pyrimidines and evaluated for anticancer activity, these compound showed significant antitumor activity(IC50=1.25-6.75µM on MCF-7cell.) Some other researchers [7,8,9] also prepared pyrimidine derivatives and tested their antitumor and anticancer activities. As a result of remarkable pharmacological activity of pyrimidine derivatives , in continuous of our earlier work[10] we have synthesized 1,2,3,4-tetrahydro pyrimidine derivatives and studied their antimicrobial activity.

## EXPERIMENTAL SECTION

Melting points were determined in open capillary tubes and are uncorrected. 0.5 mm thickness and spots were located by iodine. IR spectra were recorded on shimadzu FT-IR-8400 instrument using potassium bromide (KBr) pellet method. Mass spectra were recorded on shimadzu GC-MS-QP-2010 model using Direct Injection Probe

technique. $^1$H NMR was determined in DMSO-$d_6$ solution on a bruker Ac 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on elemental vario EL III Carlo erba 1108 model and the results are in agreements with the structures assigned.

## EXPERIMENTAL SECTION

**Synthesis of N-(substituted phenyl)-3-oxobutanamides**

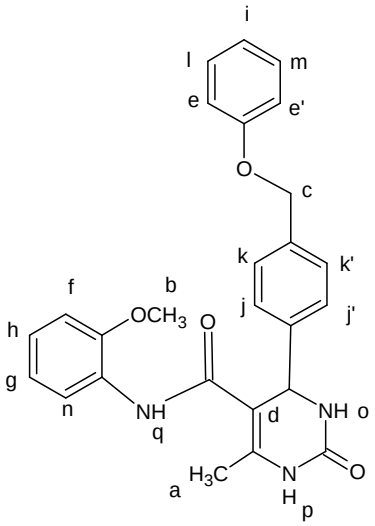
Syntheses of N-(substituted phenyl)-3-oxobutanamides were achieved using previously published methods[11,12].

**General procedure for the synthesis of 1,2,3,4-tetrahydro-N-(substitutedphenyl)-6-methyl-2-oxo-4-(4-(phenoxymethyl)phenyl)pyrimidine-5-carboxamide (AB-101 to115)**

A mixture of *N*-(substituted phenyl)-3-oxobutanamides(0.01M), 4-(phenoxy methyl)benzaldehydes (0.01 M), urea (0.015 M) and catalytic amount of conc. hydrochloric acid (HCl) in ethanol (30 ml) was heated under reflux condition for 8 to 10 hrs. The reaction mixture was kept at room temperature for 24 hrs. The crystalline product obtained and recrystallized from ethanol.

1,***1,2,3,4-tetrahydro-N-(2-methoxyphenyl)-6-methyl-2-oxo-4-(4-(phenoxymethyl)phenyl)pyrimidine-5-carboxami -de (AB-101)***

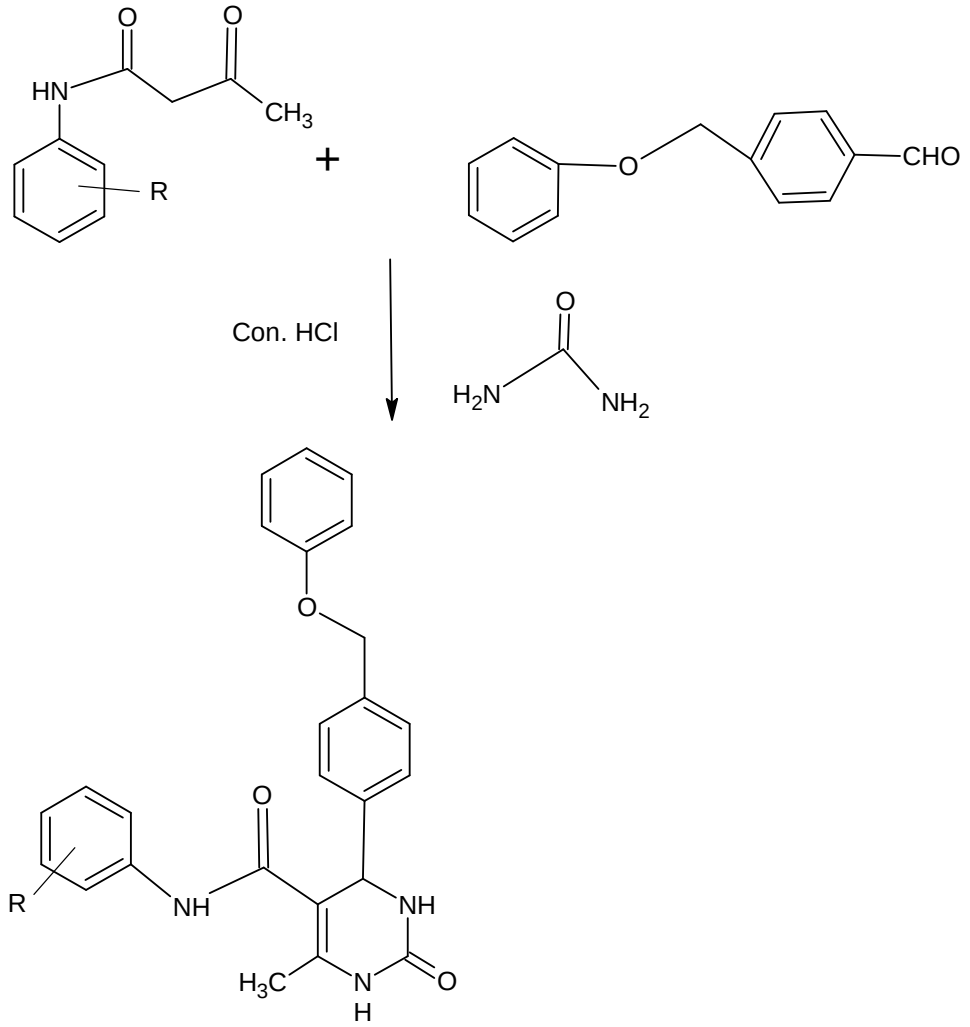
Yield: 63%; mp 207ºC; Anal. Calcd. for $C_{26}H_{25}N_3O_4$: C,70.41; H, 5.68; N, 9.47; O, 14.43; Found: C, 70.02; H,5.56; N, 9.23; O, 14.22%; IR (cm-1): 3410 (N-H stretching of amide), 3070 (C-H stretching of aromatic ring), 2935 (C-H asymmetrical stretching of $CH_3$ group), 2900 (C-H symmetrical stretching of $CH_3$ group), 1693 (C=O stretching of amide), 1693 (C=O stretching of cyclic) 1602 (N-H deformation of pyrimidine ring), 1525 (C=C stretching of aromatic ring), 1469 (C-H asymmetrical deformation of $CH_3$ group), 1344 (C-H symmetrical deformation of CH3 group), 1344 (C-N-C stretching vibration of pyrimidine ring), 1278 (C-N stretching), 1240 (C-O-C asymmetrical stretching $OCH_3$), 1074 (C-H in plane deformation of aromatic ring), 1030 (C-O-C symmetrical stretching $OCH_3$) 829 (para-substituted); MS: *m/z* 443; 1H NMR (DMSO-$d_6$) δ ppm: 2.02 (s, 3H, Ha), 3.32 (s, 3H, Hb), 5.18 (s, 2H, Hc) 5.43 (s, 1H, Hd), 6.96-6.99 (dd', 2H, Hee', *J* = 9.20 Hz), 7.10 (s, 1H, Hf), 7.14-7.15 (m, 1H, Hg), 7.26-7.28 (d, 2H, Hhi), 7.49-7.54 (m, 2H, Hjj'), 7.67 (m, 2H, Hkk'), 8.20-8.23 (m, 3H, Hl-n), 8.82 (s, 1H, Ho), 8.86 (d, 1H, Hp), 9.70 (s, 1H, Hq).




***2, N-(3-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-(4-(phenoxymethyl)phenyl)pyrimidine-5-carboxamide (AB-102)***

Yield: 68%; mp 217ºC; Anal. Calcd. for $C_{25}H_{22}ClN_3O_3$:C, 67.04; H, 4.95; Cl, 7.92; N, 9.38; O, 10.72; Found: C, 66.79; H, 4.24; Cl, 7.52; N, 9.01; O, 10.23%; MS: *m/z* 448.

**Reaction Scheme**




*AB-101 to AB-115,*

| | |
|---|---|
| *Where, R=* | *AB-108,R= 4-F* |
| *AB-101,R=2-OCH$_3$-* | *AB-109,R= 2-Cl* |
| *AB-102,R=3-Cl* | *AB-110,R= 3,4-DiCl* |
| *AB-103,R=2-F* | *AB-111,R= 3-OCH3* |
| *AB-104,R= 3-Cl 4-F* | *AB-112,R= 2,4-diCH3* |
| *AB-105,R= 4-OCH3* | *AB-113,R= 4-Br* |
| *AB-106,R= 4-Cl* | *AB-114,R= 3-Br* |
| *AB-107,R= 4-CH3* | *AB-115,R= H* |

***3, N-(2-fluorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-(4-(phenoxymethyl) phenyl)pyrimidine-5-carboxamide (AB-103)***

Yield: 57%; mp 202ºC; Anal. Calcd. for $C_{25}H_{22}FN_3O_3$:C, 69.59; H, 5.14; F, 4.40; N, 9.74; O, 11.12; Found: C,69.11; H, 5.00; F, 4.05; N, 9.23; O, 11.00%; MS: *m/z* 431.

***4, N-(3-chloro-4-fluorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-(4-(phenoxymethyl)phenyl)pyrimidine-5-carboxamide***
***(AB-104)***
Yield: 70%; mp 212ºC; Anal. Calcd. for $C_{25}H_{21}ClFN_3O_3$: C, 64.45; H, 4.54; Cl, 7.61; F, 4.08; N, 9.02; O, 10.30; Found: C, 64.08; H, 4.43; Cl, 7.12; F, 3.21; N, 8.39; O, 10.02%; MS: *m/z* 466.

***5,1,2,3,4-tetrahydro-N-(4-methoxyphenyl)-6-methyl-2-oxo-4-(4-(phenoxymethyl) phenyl)pyrimidine-5-carboxa - mide (AB-105)***
Yield: 63%; mp 205ºC; Anal. Calcd. for $C_{26}H_{25}N_3O_4$: C, 70.41; H, 5.68; N, 9.47; O, 14.43; Found: C, 70.04; H, 5.11; N, 9.13; O, 14.25%; MS: *m/z* 443.

***6, N-(4-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-(4-(phenoxymethyl) phenyl)pyrimidine-5-carboxa - mide (AB-106)***
Yield: 66%; mp 224ºC; Anal. Calcd. for $C_{25}H_{22}ClN_3O_3$: C, 67.04; H, 4.95; Cl, 7.92; N, 9.38; O, 10.72; Found: C, C, 66.82; H, 4.25; Cl, 7.45; N, 9.18; O, 10.23%; MS: *m/z* 448.

***7,1,2,3,4-tetrahydro-6-methyl-2-oxo-4-(4-(phenoxymethyl)phenyl)-N-p-tolyl -pyrimidine-5-carboxamide (AB-107)***
Yield: 69%; mp 201ºC; Anal. Calcd. for $C_{26}H_{25}N_3O_3$: C, 73.05; H, 5.89; N, 9.83; O, 11.23; Found: C, 72.46; H, 5.46; N, 9.42; O, 11.01%; MS: *m/z* 427.

***8, N-(4-flourophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-(4-(phenoxymeth -yl)phenyl)pyrimidine-5-carboxa - mide (AB-108)***
Yield: 59%; mp 195ºC; Anal. Calcd. for $C_{25}H_{22}FN_3O_3$: C, 69.59; H, 5.14; F, 4.40; N, 9.74; O, 11.12; Found: C, 69.12; H, 5.00; F, 4.20; N, 9.23; O, 11.00%; MS: *m/z* 431;

***9, N-(2-chlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-(4-(phenoxymethyl) phenyl)pyrimidine-5-carboxa - mide (AB-109)***
Yield: 75%; mp 185ºC; Anal. Calcd. for $C_{25}H_{22}ClN_3O_3$: C, 67.04; H, 4.95; Cl, 7.92; N, 9.38; O, 10.72; Found: C, 66.12; H, 4.58; Cl, 7.42; N, 9.20; O, 10.33%; MS: *m/z* 448.

***10, N-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-(4-(phenoxy-methyl)phenyl)pyrimidine-5-carboxamide (AB-110)***
Yield: 53%; mp 235ºC; Anal. Calcd. for $C_{25}H_{21}Cl_2N_3O_3$: C, 62.25; H, 4.39; Cl, 14.70; N, 8.71; O, 9.95; Found: C, 62.00; H, 4.01; Cl, 14.33; N, 8.42; O, 9.54%; MS: *m/z* 482.

***11, 1,2,3,4-tetrahydro-N-(3-methoxyphenyl)-6-methyl-2-oxo-4-(4-(phenoxy - methyl)phenyl)pyrimidine-5-carboxamide (AB-111)***
Yield: 59%; mp 200ºC; Anal. Calcd. for $C_{26}H_{25}N_3O_4$: C, 70.41; H, 5.68; N, 9.47; O, 14.43; Found: C, 70.10; H, 5.34; N, 9.12; O, 14.10%; MS: *m/z* 443.

***12, 1,2,3,4-tetrahydro-6-methyl-N-(2,4-dimethylphenyl)-2-oxo-4-(4-(phenoxy methyl)phenyl)pyrimidine-5-carbo - xamide (AB-112)***
Yield: 70%; mp 204ºC; Anal. Calcd. for $C_{27}H_{27}N_3O_3$: C, 73.45; H, 6.16; N, 9.52; O, 10.87; Found: C, 73.15; H, 6.01; N, 9.23; O, 10.41%; MS: *m/z* 441.

***13, 1,2,3,4-tetrahydro-6-methyl-N-(4-bromophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-(4-(phenoxymethyl) phenyl) pyrimidine-5-carboxamide (AB-113)***
Yield: 62%; mp 198ºC; Anal. Calcd. for $C_{25}H_{22}BrN_3O_3$: C, 60.98; H, 4.50; Br, 16.23; N, 8.53; O, 9.75; Found: C, 60.28; H, 4.20; Br, 16.04; N, 8.10; O, 9.25%; MS: *m/z* 492.

***14, N-(3-bromophenyl)-1,2,3,4-tetrahydro-6-methyl-2-oxo-4-(4-(phenoxymethyl)phenyl)pyrimidine-5-carboxa - mide (AB-114)***
Yield: 71%; mp 187ºC; Anal. Calcd. for $C_{25}H_{22}BrN_3O_3$: C, 60.98; H, 4.50; Br, 16.23; N, 8.53; O, 9.75; Found: C, 60.46; H, 4.21; Br, 16.01; N, 8.11; O, 9.22%; MS: *m/z* 492.

***15, 1,2,3,4-tetrahydro-6-methyl-2-oxo-4-(4-(phenoxymethyl)phenyl)-N-phenylpyrimidine- 5-carboxamide (AB-115)***
Yield: 75%; mp 214ºC; Anal. Calcd. for $C_{25}H_{23}N_3O_3$: C, 72.62; H, 5.61; N, 10.16; O, 11.61; Found: C, 72.10; H, 5.24; N, 9.78; O, 11.24%; MS: *m/z* 431.

**Biological evaluation**

**Antimicrobial evaluation**

All of the synthesized compounds **(AB- 101 to 115)** were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [13-15] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking gentamycin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin and gresiofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards[13].

***Minimal Inhibition Concentration [MIC]:-***

The main advantage of the 'Broth Dilution Method' for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at $37^0$ C overnight.
3. The MIC of the control organism is read to check the accuracy of the drug concentrations.
4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.
5. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

***Methods used for primary and secondary screening: -***

Each synthesized drug was diluted obtaining 2000 μg $mL^{-1}$ concentration, as a stock solution. Inoculum size for test strain was adjusted to $10^8$ cfu (colony forming unit) per milliliter by comparing the turbidity.

***Primary screen:*** **-** In primary screening 1000 μg $mL^{-1}$, 500 μg $mL^{-1}$and 250 μg $mL^{-1}$ concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

***Secondary screen:*** **-** The drugs found active in primary screening were similarly diluted to obtain 200 μg $mL^{-1}$, 100 μg $mL^{-1}$, 50 μg $mL^{-1}$, 25 μg $mL^{-1}$, 12.5 μg $mL^{-1}$, and 6.250 μg $mL^{-1}$ concentrations.

***Reading Result:*** **-** The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculums. The test mixture should contain $10^8$ organism/mL.

The results obtained from antimicrobial susceptibility testing are depicted in **Table 1**.

## RESULTS AND DISCUSSION

All the synthesized compounds have shown sharp melting points and melts clearly. Their elemental analysis result reveals that they are in well agreement with their structure. In spectral studies all the peaks are in the range of their value according to the structure assigned. The assignment of the infrared bands were made by comparing the spectra of the compounds with reported literature values on similar systems[16]. By the antimicrobial study of the compounds we conclude that most of compounds are good antimicrobial agents, very few of them are less or moderate active as compared to standard drugs. All the compounds possess better antifungal activity than antibacterial so most of the compounds are more toxic to fungi. Compounds AB-101 is active against all the strains

of bacteria and fungi where as compound AB-102 shows good activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*, but it is excellent against three fungal strains *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus.* Compounds AB-107 and AB-113 show good antibacterial activity and compounds AB-103, AB106 and AB-111 show very good activity against *Staphylococcus aureus.* Compounds AB- 106, AB-109 , AB-111 and compound AB-113 shows very good activity against *Streptococcus pyogenes.* Compounds AB-106, AB-109, AB-113 and AB-104 are also toxic for *Escherichia coli* and *Pseudomonas aeruginosa.* In general it is observed that compounds having functional group $-OCH_3$ and halogen are good antimicrobial agents.

**Table 1:- in vitro Antimicrobial Screening Results for AB-101 to AB-115**

| Code | Minimal inhibition concentration (µg $mL^{-1}$ ) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Gram-positive species | | Gram-negative species | | | Fungal species | |
| | *S. a.* | *S. p.* | *E. c.* | *P.a.* | *C.a.* | *N. a.* | *A. c.* |
| AB-101 | 500 | 500 | 500 | 500 | 250 | 1000 | 500 |
| AB-102 | 500 | 1000 | 1000 | 1000 | >1000 | >1000 | >1000 |
| AB-103 | 100 | 100 | 250 | 200 | 1000 | 500 | 500 |
| AB-104 | 1000 | 500 | 1000 | 1000 | 1000 | 500 | 1000 |
| AB-105 | 200 | 100 | 100 | 200 | 250 | 1000 | 1000 |
| AB-106 | 1000 | 1000 | 500 | 500 | 250 | 1000 | 1000 |
| AB-107 | 500 | 500 | 250 | 250 | 250 | 1000 | 1000 |
| AB-108 | 100 | 100 | 200 | 250 | 1000 | 500 | 1000 |
| AB-109 | 62.5 | 1000 | 200 | 1000 | 500 | >1000 | 1000 |
| AB-110 | 150 | 250 | 100 | 150 | 500 | 500 | 1000 |
| AB-111 | 1000 | 500 | 62.5 | 62.5 | >1000 | >1000 | >1000 |
| AB-112 | 200 | 200 | 100 | 100 | >1000 | 1000 | 500 |
| AB-113 | 500 | 1000 | 500 | 500 | 500 | >1000 | >1000 |
| AB-114 | 150 | 250 | 100 | 150 | 500 | 500 | 500 |
| AB-115 | 100 | 62.5 | 200 | 250 | 500 | 500 | 500 |
| Gentamycin | 0.25 | 0.50 | 0.05 | 1 | - | - | - |
| Ampicillin | 250 | 100 | 100 | 100 | - | - | - |
| Chloramphenicol | 50 | 50 | 50 | 50 | - | - | - |
| Iprofloxacin | 50 | 50 | 25 | 25 | - | - | - |
| Norfloxacin | 10 | 10 | 10 | 10 | - | - | - |
| Nystatin | - | - | - | - | 100 | 100 | 100 |
| Gresiofulvin | - | - | - | - | 500 | 100 | 100 |

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