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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NOVEL SUBSTITUTED THIENOPYRIMIDINE DERVATIVES AS ANTI-BACTERIAL AGENTS

Sameep Sonvane*[1], Maneesh Kumar Srivastva[2], Nitin Lonikar[3], Deshpande A.N[1] Riyaz Shaikh[1], Ajay Gadgul[1]

[1]Department of Pharmaceutical Chemistry, Dayanand College of Pharmacy, Latur.
[2]Department of Pharmaceutical chemistry, V.L College of Pharmacy, Raichur, Karnataka
[3]Department of Pharmaceutical chemistry, Shivlingeshwar College of Pharmacy, Almala

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ABSTRACT

Cyclization are 2-amino - 5, 6 - dihydro - 4*H*-cyclopenta[*b*]thiophene-3-carboxamide (1) with formamide yeilds 3,5,6,7-tertrahydro-4*H*-cyclopenta thieno [2,3-*d*] pyrimidine-4(3*H*)-one (2). The compound (2) on reaction with phosphorous oxychloride gives 4-chloro-3, 5, 6, 7-tetrahydro-4*H*-Cyclopenta thieno[2,3-*d*] Pyrimidine (3). Reaction of 4-chloro-3, 5, 6, 7-tetrahydro-4*H*-Cyclopenta thieno[2,3-*d*] Pyrimidine (3) with substituted secondary amines yeilded a series of new heterocyclic compound 4a-j. The structures of the compound were confirmed by spectral techniques like IR, NMR. Representative classes of compound were screened for Antibacterial activities against gram positive and gram negative bacteria by disc diffusion method. Some synthesized compound show moderate activities as well as few shows promising Antibacterial activity.

INTRODUCTION

Research is a continuous process for many reasons like: a) The pathogenic organisms are known to develop resistance gradually against particular drugs, hence the drugs, which are active today, may become inactive after several years. In order to overcome these problems, there is a need to replace old drugs by newer ones. b) Though some of the drugs are highly effective, they are associated with toxic side effects. Hence man is always in search of more and more potent, safer drugs than the existing ones. Investigation is mainly focused on the synthesis of Cyclopenta thieno [2, 3-d] Pyrimidine with thiophene as one of the heterocyclic partner to explore new pharmaceutical leads. The compounds have shown to posses various biological and pharmacological properties. In that series Cyclopenta thieno

Pyrimidine shows various kind of biological activity such as Antibacterial, Anti-inflammatory, Analgesic, Antihistaminic activity. Treating infectious (microbial) disease has become a major unresolved challenge as the microorganisms are developing resistance to current chemotherapeutic agents in several ways. So research is mainly focused on targeting the micro-organism which develops the resistance against chemotherapeutic agent.

EXPERIMENTAL:

All the reagent and compound used for the synthesis are Hi-media product and melting point are determined by open capillary method after the purification. IR spectra (V max in Cm-1) were recorded on a Shimadzu FTIR 8300 spectrophotometer using KBr pellets technique. ^{1}H NMR spectra were recorded using Bruker

WM-400 spectrophotometer using DMSO-d6 or CDCl3 as the solvent and TMS as the internal reference (Chemical Shifts in ppm). TLC using silica gel G60 (Merck, Germany) routinely checked the purity of the compounds and the spots were exposed in iodine vapor for visualization.

Compound 1: Preparation of 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxamide:

A mixture of cynoacetamide (0.1M), cyclopentanone (0.1M), ammonium acetate, glacial acetic acid is taken in dry alcohol and refluxed for 6 hours by using dean stark apparatus. After the completion of the reaction, excess solvent is removed by distillation and mixture is treated with sulphur (0.1M) and dimethylamine and again stirred for 90 min. After the completion of the reaction the mixture is poured into crushed ice with constant stirring. The product obtained was recrystallized from aq. Alcohol, Melting Point-182 ^{0}C.

IR Spectra: 3428 (N-H stretching), 3135 (Aromatic C-H Stretching), 1720 (C=O stretching), 1640 (C=N stretching), 570 (C-S stretching); ^{1}HNMR ($CDCl_3$): δ 0.9-1.0 (s, 2H of Cyclopentane ring), 2.3-2.7 (m, 4H of Cyclopentane ring), 5.4 (s, 2H of NH2), 6.3 (s, 2H of $CONH_2$); Mass Spectra (m/z): 182.

Compound 2: Preparation of 3,5,6,7-tertrahydro-4*H*-cyclopenta thieno [2,3-*d*] pyrimidine-4(3*H*)-one:

A mixture of 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxamide (1) and form amide (30 ml) is heated on an oil bath for 6 hours at 175°-180 °C. After completion of the reaction and cooling mixture is filtered and recrystallized from methanol-glacial acetic acid (1:1) Melting Point-233 ^{0}C.

IR Spectra: 3408 (N-H stretching), 3155 (Aromatic C-H Stretching), 1707 (C=O stretching), 1647 (C=N stretching), 576 (C-S stretching); ^{1}H NMR ($CDCl_3$): δ 0.9 (s, 2H of Cyclopentane ring), 2.4-2.7 (m, 4H, Cyclopentane ring), 7.2 (s, 1H of NH of Pyrimidine), 7.5 (s, 1H of CH of Pyrimidine); Mass Spectra (m/z): 193.

Compound 3: Preparation of 4-chloro-3,5,6,7-tetrahydro-4*H*-Cyclopenta thieno[2,3-*d*] Pyrimidine:

A mixture of 3, 5, 6, 7-tertrahydro-4*H*-cyclopena thieno[2,3-*d*]pyrimidine-4(3*H*)-one (2) and phosphorous oxy chloride is refluxed for 10 hours with Guard tube. After completion of the reaction, the mixture is poured on crushed ice with constant stirring. The product is filtered, dried and recrystallized from ethanol, Melting Point-132 ^{0}C. IR spectra: 3415 (N-H stretching), 3165 (Aromatic C-H Stretching), 1640 (C=N stretching), 570 (C-S stretching); ^{1} H NMR ($CDCl_3$): δ 1.1 (s, 2H of Cyclopentane ring), 2.9-3.2 (m, 4H of Cyclopentane ring), 8.5 (s, 1H of CH Pyrimidine); Mass Spectra (m/z): 212.

Compound 4a-j:-Preparation of N-benzyl-N-propyl-6, 7-dihydro-5H-Cyclopenta thieno[2,3-d]Pyrimidine-4-amine:

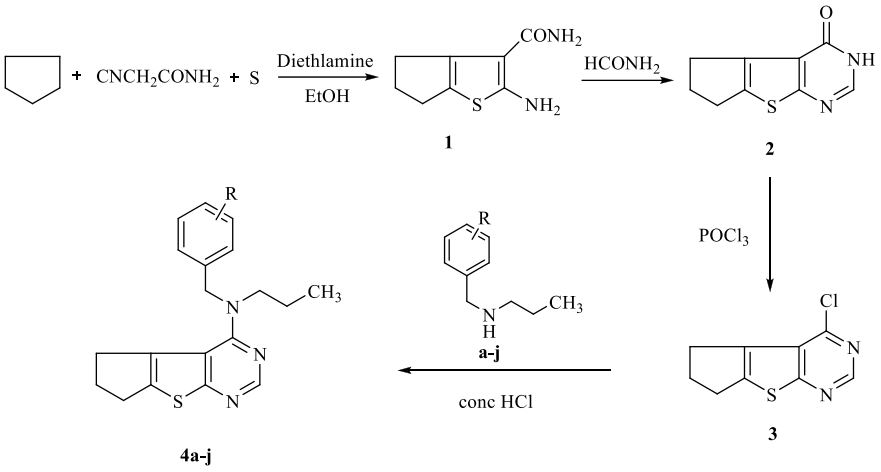
A mixture of 4-chloro-3,5,6,7-tetrahydro-4*H*-Cyclopenta thieno[2,3-*d*] Pyrimidine (3) and N(substituted Benzyl)propan-1-amine[6] (a-j) dissolved in 30 ml dried alcohol and refluxed for 18 hr. After completion of reaction the solution is poured in ice cold water and treated with 5% of dil HCl. the obtain solid filtered, washed with cold water, dried and recrystallise the solid from suitable solvent.

Compound 4a: IR Spectra: 3155 (Aromatic C-H Stretching), 1647 (C=N stretching), 576 (C-S stretching); ^{1}H NMR ($CDCl_3$): δ 0.9-1.0 (t, 3H of CH_3 of -CH_2-CH_2-CH_3), 1.8-1.9 (m, 2H of CH_2 of CH_2-CH_2-CH_3), 2.2 (s, 2H of Cyclopentane ring), 2.8-2.9 (m, 4H, Cyclopentane ring), 3.6-3.7 (t, 2H of CH_2 of N-CH_2-CH_2-CH_3), 5.0 (s, 2H of CH_2 of -N-CH_2-Ar), 7.2-7.9 (m, 5H of Ar-H); 8.1 (s, 1H of CH of Pyrimidine); Mass Spectra (m/z): 324.

Compound 4e: IR Spectra: 2949 (Aromatic C-H), 1599 (C=N Stretching) 779 (1,4 substituted benzene) 697 (C-S stretching); ^{1}H NMR ($CDCl_3$): δ 0.9-1.0 (t, 3H of CH_3 of -CH_2-CH_2-CH_3), 1.7-1.8 (m, 2H of CH_2 of CH_2-CH_2-CH_3), 2.3 (s, 2H of Cyclopentane ring), 2.8-2.9 (m, 4H, Cyclopentane ring), 3.7-3.8 (t, 2H of CH_2 of N-CH_2-CH_2-CH_3), 4.9 (s, 2H of CH_2 of -N-CH_2-Ar), 7.3-8.1 (m, 5H of Ar-H); 8.3 (s, 1H of CH of Pyrimidine); Mass Spectra (m/z):359

Compound 4h: IR Spectra: 3429 (N-H stretching), 2950 (Aromatic C-H), 1588 (C=N Stretching) , 765 (substituted benzene), 695 (C-S stretching); ^{1}H NMR ($CDCl_3$): δ 0.9-1.0 (t, 3H of CH_3 of -CH_2-CH_2-CH_3), 1.7-1.9 (m, 2H of CH_2 of CH_2-CH_2-CH_3), 2.4 (s, 2H of Cyclopentane ring), 2.7-2.8 (m, 4H, Cyclopentane ring), 3.6-3.7 (t, 2H of CH_2 of N-CH_2-CH_2-CH_3), 4.9 (s, 2H of CH_2 of -N-CH_2-Ar), 7.1-7.7 (m, 5H of Ar-H); 8.0 (s, 1H of CH of Pyrimidine), 10.1 (s, 1H of OH); Mass Spectra (m/z):340

Sceme - 1



R= H, 3-NO_2, 3-NO_2, 2-Cl, 4-Cl, 4-F, 2-OH, 3-OH, 4-CH_3, 4-OCH_3.

S. No	Compound Code	Substitute®	Molecular for-mula	Molecular weight	Yield %	Melting Point
1	4_a	Hydrogen	$C_{19}H_{21}N_3S$	323	79	202
2	4_b	2-Nitro	$C_{19}H_{20}N_4SO_2$	368	78	220
3	4_c	3-Nitro	$C_{19}H_{20}N_4SO_2$	368	75	178
4	4_d	2-Chloro	$C_{19}H_{20}N_3SCl$	357.5	56	172
5	4_e	4-Chloro	$C_{19}H_{20}N_3SCl$	357.5	63	168
6	4_f	4-Floro	$C_{19}H_{20}N_3SF$	341	68	173
7	4_g	2-Hydroxy	$C_{19}H_{21}N_3SO$	339	76	174
8	4_h	3-Hydroxy	$C_{19}H_{21}N_3SO$	339	89	176
9	4_i	4-Methyl	$C_{20}H_{23}N_3S$	337	43	178
10	4_j	4-Methoxy	$C_{20}H_{23}N_3SO$	353	66	158

Table 1: Physical Characterization of derivatives (4a-j)

S. No	**Sample code**	**Inhibition zone in diameter(µg)**							
		Bacillus subtilis		*staphylococcus aureus*		*Escherichia coli*		*salmonella typhi*	
		50	100	50	100	50	100	50	100
1	4a	10	13	11	12	10	13	11	13
2	4b	08	13	10	11	09	12	08	10
3	4c	07	14	08	11	12	13	10	11
4	4d	09	10	09	12	08	12	09	12
5	4e	08	10	09	11	09	13	08	11
6	4f	09	10	08	12	08	12	09	13
7	4g	11	13	12	14	13	14	12	14
8	4h	10	13	13	12	14	15	13	14
9	4i	13	15	13	15	12	14	13	15
10	4j	14	16	15	16	12	16	13	16
11	Ampicillin	18	20	19	22	22	24	16	21
12	DMSO	-	-	-	-	-	-	-	-

Table 2: Anti-bacterial activity of synthesized Compound 4a-j.

Compound 4j: IR Spectra: 2950 (Aromatic C-H stretching), 1673 (C=O stretching), 1599 (C=N stretching), 1359 (C-H in OCH3), 1178 (C=C in CH=CH), 688 (C-S stretching);
^{1}H NMR ($CDCl_3$): δ 0.9-1.0 (t, 3H of CH_3 of -CH_2-CH_2-CH_3), 1.6-1.8 (m, 2H of CH_2 of CH_2-CH_2-CH_3), 2.3 (s, 2H of Cyclopentane ring), 2.6-2.8 (m, 4H, Cyclopentane ring), 3.3 (s, 3H of CH_3 of O-CH_3), 3.5-3.7 (t, 2H of CH_2 of N-CH_2-CH_2-CH_3), 4.7 (s, 2H of CH_2 of -N-CH_2-Ar), 7.3-7.9 (m, 4H of Ar-H); 8.1 (s, 1H of CH of Pyrimidine); Mass Spectra (m/z): 354

ANTI-BACTERIAL ACTIVITY:

The synthesized compounds prepared during the present investigation were screened for Antibacterial activity. The antibacterial tests were conducted on common microorganisms such as *Bacillus subtilis, staphylococcus aureus, Escherichia coli* and *salmonella typhi*, which are the representative types of gram positive and gram-negative organisms respectively. The antibacterial activity of the compounds was assessed by disc diffusion method. Where 50 µg and 100 µg concentration of drug and Ampicillin was used as a standard.

RESULT AND DISCUSSION:

The synthesized compound were screened for Antibacterial activity at concentration of 50 µg/ml and 100µg/ml using DMSO as a control & Ampicillin is used as standard against two gram positive & gram negative bacteria by disc diffusion method on nutrient agar medium. The results of Antibacterial activity are summarized in Table 2. It was confirmed that compound 4g, 4h, 4i and 4j shows significant activity. The compound 4g, 4h contains hydroxy group (OH) at second and third position of aryl in substituted amine moreover 4i, 4j contain methyl & methoxy group at 4th position respectively. The above compound shows momentous action possibly due to the existence of electrophillic group. Whereas unsubstituted aryl amine (hydrogen) compound 4a shows moderate activity. Compound 4b, 4c, 4d, 4e & 4f containing electron withdrawing group (NO_2, F and Cl) shows comparatively poor anti bacterial activity.

CONCLUSION:

All the synthesized compound screened for in vitro Antibacterial activity against gram negative and gram positive bacteria. From this study, it is evident that some compound 4g, 4h, 4i and 4j shows a significant action when compared to standard Ampicillin towards inhibiting all bacterial strains. Especially compound 4j shows more promising action. The compound 4a shows moderate activity. Possible explanation for this result is may be due to the basic skeleton of a molecule as well as nature of substitute. So these Skelton of molecule can utilize for designing new lead molecule with different biological activity.

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