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Synthesis, characterization and *invitro* antimicrobial activity of some novel 3-substituted amino 2-mercapto 5,6,7,8-tetra hydro benzo(b)thieno-(2,3-d)-pyrimidine-4-(3h)-ones.

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ABSTRACT

An ecofriendly synthesis of 3-substituted amino 2-mercapto 5,6,7,8- tetrahydro -benzo (b) thieno-(2,3-d)-pyrimidine-4-(3H)-ones was carried out.Condensed quinazolines like thiadiazoloquinazolines & the corresponding bioisostere thiadiazolothienopyrimidines were found to be biologically active molecules, 2-substituted 1, 3, 4-thiadiazolo (2,3-b) quinazolin-4-ones was reported to possess activity from our laboratories. Therefore an attempt was made to utilize the concept of bio-isosterism for the synthesis of 3-substituted amino 2-mercapto-5,6,7,8-tetrahydro(b) benzo thieno(2,3-d) pyrimidin-4(3H)-ones for activity. The 3- amino 2-mercapto-5,6,7,8-tetrahydro benzo(b)thieno (2,3-d) pyrimidin-4(3H)-ones was further treated with various substituted aromatic aldehydes. The new synthesized compounds were characterized by MP, TLC, IR, ^{1}H NMR and Mass spectra. These synthesized compounds were subjected to anti-microbial studies using few Gram-positive, Gram-negative and fungal organisms. The standard drug used for anti-bacterial activity is Ampicillin and the standard drug used for anti-fungal activity is Miconazole nitrate. Among the compounds tested, three compounds exhibited significant antimicrobial activity.

Key words: Synthesis, Antimicrobial activity, Thienopyrimidinones, Eco-friendly, Bio-isosterism.

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INTRODUCTION

Thiephene and pyrimidine derivatives have a variety of pharmacological activities [1,2]. Thienopyrimidines have been shown to possess a variety of pharmacological activities like anti-microbial, anti-inflammatory and antimalarial activities [3-5]. Condensed Quinazoline and the corresponding bioisostere thieno pyrimidine were found to be biologically active molecules. There has been increasing interest in the chemistry of 4(3*H*)-Quinazolines [6] because of their biological significance. Many of them show antifungal, antibacterial, anticancer, anti-inflammatory activities. Therefore, it was thought of interest to utilize the concept of Bioisosterism for the synthesis of some Thienopyrimidin-4-ones. Thienopyrimidinones were synthesized as reported procedure [7,8]. The synthesized compounds were evaluated for their anti-microbial activity.

MATERIAL AND METHODS

Chemicals

All the chemicals were procured from S. D. Fine chem.Ltd, Bilaspur

Preparation of 2-amino-3-carbethoxy 4, 5,6,7-tetrahydrobenzo(b) thiophene [I]

A mixture of Cyclohexanone, Ethylcyanoacetate, Sulphur in 40ml of ethanol was warmed to a temperature between 40^0-50°C and then diethylamine 4.0ml was added dropwise till the sulphur dissolved into the solution. The stirring was continued for one hour till the solid separated. The reaction was cooled to room temperature and filtered. The product was recrystallized using ethanol. M. P of pure product : 112^0C

Preparation of Methyl N- [3-carbethoxy (4, 5, 6, 7-tetra hydro benzo) thienyl] dithiocarbamate [II]:

To a vigorously stirred solution of **[I]** (4.5 g, 0.02 mol) in dimethylsulphoxide (10 ml) at room temperature, carbondisulphide (1.6 ml;0. 26 mol) and aqueous sodium hydroxide (1.2 ml; 20 mol) were added dropwise. After 30 min dimethylsulphate (2.5 g; 0.25mol) was added dropwise under cooling in an ice bath. Stirring was continued for 3 h, and then the reaction mixture was poured into ice-water mixture. The precipitated solid was filtered, dried and recrystallized from ethanol-chloroform mixture to give pure product. M. P of pure product : 134^0C

Preparation of 3-amino-2-mercapto-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-d] pyrimidin -4(3H)-one[III]:

A Solution of **[II] (**3.2 g, 0.01 mol) in isopropanol (10 ml) was treated with hydrazine hydrate (99%; 4.3 g; 0. 1mol) and heated under reflux on water bath until the methylmercaptan evolution ceased. After cooling the solid obtained was filtered, dried and recrystallized from ethanol-chloroform mixture (1:1) to yield a white crystalline product. M. P. of pure product : 245^0C

All the products were subjected to analysis by different spectral methods like, IR, NMR and mass and the structural interpretation was carried out.

Antimicrobial studies

All the synthesized compounds were screened for their antibacterial and antifungal activity by agar diffusion method at a concentration of 50µg/ml against *Proteus vulgaris*, *Bacillus subtilis, Klebsiella pneumonia, Serratia Aspergillus niger* and *Candida albicans.* After 24h of drug addition, zone of inhibition was measured in mm and recorded. Ampicillin, Mecanazole nitrate at 50 µg/ml were used as standards in the experiment [9,10].

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RESULTS AND DISCUSSION

From the IR, ^{1}H NMR and Mass spectra obtained, characterization of data has been done and given in table 1, 2, 3, 4 and 5. The IR spectrum of the compound III showed distinct peaks at 3335 cm^{-1} , 3117 cm^{-1} (-NH^2),1684 cm^{-1} (C=O),1558 cm^{-1},1496 cm^{-1} (Ar-C=C). The difference in the TLC spots, confirm the formation of 3-amino-2-mercapto-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-*d*]pyrimidin-4(3*H*)-one(III). The next step was the synthesis of schiff bases carried out by the reaction of 3-amino-2-mercapto-5,6,7,8-tetrahydrobenzo(b)thieno[2,3-*d*] pyrimidin-4(3*H*)-one with substituted benzaldehydes in the presence of glacial acetic acid and ethanol is used as a solvent to yield ten schiff bases. Further the IR peaks confirmed the formation of schiff bases. The IR peaks are reported in table. Substantial proof for the formation of all these new title compounds has been provided by the difference in the melting point and R_f values (TLC).

Scheme

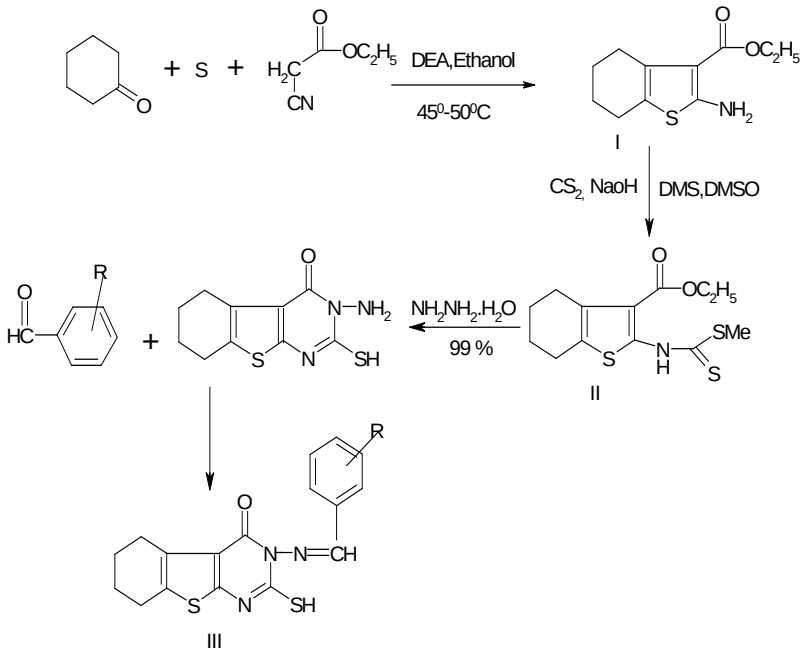

Table 1: Physical data of 3-amino-2-mercapto-5, 6,7,8-tetrahydrobenzo(b) thieno[2,3-*d*] pyrimidin-4(3*H*)-one (III)

Comp. Code	Mol. Formula	M.W(g)	Recrystalization Solvent	M.P (^{0}C)	% Yield	TLC Solvent System
III	$C_{10}H_{11}N_3OS_2$	253	Ethanol-chloroform mixture(1:1)	245	74.62	Benzene:Methanol (9:1)

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Table 2: Physical data of 3-(substituted- (phenylazo methine))2-mercapto-5,6, 7, 8-tetra hydro benzo (b) thieno[2,3-*d*] pyrimidin-4(3*H*)-one

Comp. Code	R	Mol. formula	M.W (g)	M.P (^{0}C)	% Yield	Recryatallization solvent	TLC Solvent System
IIIa	2-hydroxy	$C_{17}H_{15}N_3O_2S_2$	357	185	71.7	DMF and H_2O Mixture	C_2H_5OH: CH_3OH: H_2O (6:2:2)
IIIb	4-hydroxy	$C_{17}H_{15}N_3O_2S_2$	357	119	78.7	DMF and H_2O Mixture	C_2H_5OH: CH_3OH: H_2O (6:2:2)
IIIc	2-nitro	$C_{18}H_{17}N_3O_3S_2$	387	220	63.2	DMF and H_2O Mixture	C_2H_5OH: CH_3OH: H_2O (6:2:2)
IIId	3-nitro	$C_{17}H_{14}N_4O_3S_2$	386	229	69.4	DMF and H_2O Mixture	C_2H_5OH: CH_3OH: H_2O (6:2:2)
IIIe	4-methoxy	$C_{18}H_{17}N_3O_2S_2$	371	162	71.2	DMF and H_2O Mixture	C_2H_5OH: CH_3OH: H_2O (6:2:2)
IIIf	3-methoxy, 4-hydroxy	$C_{18}H_{17}N_3O_3S_2$	387	220	63.21	DMF and H_2O Mixture	C_2H_5OH: CH_3OH: H_2O (6:2:2)
IIIg	3,4,5 –Tri methoxy	$C_{17}H_{15}N_3O_2S_2$	357	119	78.7	DMF and H_2O Mixture	C_2H_5OH: CH_3OH: H_2O (6:2:2)
IIIh	2-chloro	$C_{17}H_{14}N_3OS_2Cl$	376	160	86.1	DMF and H_2O Mixture	C_2H_5OH: CH_3OH: H_2O (6:2:2)
IIIi	4-chloro	$C_{17}H_{14}N_3OS_2Cl$	376	140	76.5	DMF and H_2O Mixture	C_2H_5OH: CH_3OH: H_2O (6:2:2)
IIIj	4-Dimethyl amino	$C_{19}H_{20}N_4OS_2$	384	190	85.4	DMF and H_2O Mixture	C_2H_5OH: CH_3OH: H_2O (6:2:2)

Table 3: Spectral data of 3-amino-2-mercapto-5, 6,7,8-tetrahydrobenzo(b) thieno[2,3-*d*] pyrimidin-4(3*H*)-one (III)

Comp. Code	IR(KBr)(cm^{-1})
III	3335 cm^{-1} , 3117 cm^{-1} (-NH^2),1684 cm^{-1} (C=O),1558 cm^{-1},1496 cm^{-1} (Ar-C=C).

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Table 4: Spectral data of 3-(substituted- (phenylazo methine))2-mercapto-5,6, 7, 8-tetra hydro benzo (b) thieno[2,3-*d*] pyrimidin-4(3*H*)-one

Comp. Code	R	IR(KBr)(cm^{-1})	^{1}H NMR (DMSO)(δ)	MASS M^+
IIIa	2-hydroxy	3275 cm^{-1} (NH);1635 cm^{-1} (s)(C=O);1558 cm^{-1} (s)(N=CH);1522 cm^{-1}(ArC=); 3422 cm^{-1}(OH).	----	$[294]^+$
IIIb	4-hydroxy	3316 cm^{-1} (NH);1635 cm^{-1} (s) (C=O);3422 cm^{-1} (OH); 1522 cm^{-1}(Ar-C=)	----	----
IIIc	2-nitro	3422(N-H);1684(s),(C=O),1576, 1508(Ar-C=C) 1558(N=CH) 1520(NO_2)	----	----
IIId	3-nitro	1560 cm^{-1},1352 cm^{-1} (s) (Ar-NO_2); 1662 cm^{-1} (s),(C=O);3304 cm^{-1} (NH);1560 cm^{-1} (N=CH).	δ=1.62(d) (2H) (-CH_2) δ=1.62(t) (2H) (-CH_2) δ=2.55(t) (2H) (-CH_2) δ=2.55(d) (2H) (-CH_2) δ=1.55(s) (1H) (-SH) δ=7.64(d) (1H) (Ar-H) δ=7.14(t) (1H) (Ar-H) δ=6.89(d) (1H)(Ar-H) δ=7.12 (s) (1H) (Ar-H)	----
IIIe	4-methoxy	2926(NH);1670, 1635(C=O);1603, 1558(Ar-C=C);	δ=1.62 (d) (2H) (-CH_2) δ=1.62 (t) (2H) (-CH_2) δ=2.55 (t) (2H) (-CH_2) δ=2.55 (d) (2H) (-CH_2) δ=1.55(s) (1H) (-SH) δ=6.77(d) (1H) (Ar-H) δ=7.15(d) (1H) (Ar-H) δ=6.77(d) (1H)(Ar-H) δ=7.15 (d) (1H) (Ar-H)	----
IIIf	3-methoxy, 4-hydroxy	3275(NH);1635(s)(C=O); 1558(s)(N=CH);1522 (Ar-C=C);3422(OH)	----	----
IIIg	3,4,5 –Tri methoxy	3308(NH);1647(C=O); 1508(s)(Ar-C=C); 1595(s)(N=CH)	----	----

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IIIh	2-chloro	2851(C-H);3250(s) (NH);1647(s)(C=O); 1608(N=C-H)1496(s) (Ar-C=C),767(C=Cl)	----	----
IIIi	4-chloro	3337(N-H);3032(C-H),1647(C=O),1603(N=C-H);858(C-Cl),1522(Ar-C=C)	----	----
IIIj	4-Dimethyl amino	3265(NH);1662(s)(C=O);1489 , 1361(Ar-C=C); 1608(s)(N=CH)	----	----

Table 5: Antimicrobial activity of 3-(substituted- (phenylazo methine))2-mercapto-5,6, 7,8-tetra hydro benzo (b) thieno[2,3-*d*] pyrimidin-4(3*H*)-one

Comp. Code	**Zone of Inhibition in mm.**					
	P.Vulgaris	*B.subtilus*	*K.pneumonia*	*Serratia*	*A. niger*	*C. albicans*
IIIa	NA	8	7	10	NA	NA
IIIb	12	10	NA	9	9	10
IIIc	8	7	6	10	12	8
IIId	NA	NA	NA	NA	NA	NA
IIIe	NA	NA	NA	NA	10	7
IIIf	12	10	9	7	8	7
IIIg	9	10	12	NA	7	12
IIIh	NA	NA	NA	NA	12	10
IIIi	NA	NA	9	7	8	NA
IIIj	9	10	12	10	10	8
Ampicillin	20	15	20	20	----	----
Miconazole	----	----	----	----	20	15

NA = Not active.

CONCLUSION

10 new schiff bases were also synthesized as per the scheme. The IR spectra of all compounds, NMR and the mass spectra of compound were studied and ascertained. All the compounds were also screened for antibacterial and anti-fungal activities. From the screening results it was observed that, the compound with 2-nitrobenzaldehyde substituted schiff base (IIIc) showed moderate antibacterial activity against gram-positive, gram-negative organisms and fungi. The compound (IIIf) and (IIIj) with 3-methoxy,4-hydroxy & p-dimethylaminophenylbenzaldehyde substituted schiff bases substitution showed moderate activity against gram positive and gram negative organism and fungi. The compound with 2-chlorobenzaldehyde substituted schiff bases and (IIIh) substitution showed moderate activity against *Aspergillus niger* and *cladosporium* and low activity against gram positive and gram-negative microorganisms.

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