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AN EXPEDITIOUS APPROACH TOWARDS SYNTHESIS OF PYRAZOLO[3,4-d][1,3]THIAZINE DERIVATIVES WITH THEIR ANTIMICROBIAL EVALUATION

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ABSTRACT: A series of novel heterocycles such as 3-amino-4-imino-6-phenyl-2N-(substituted)pyrazolo[3,4-*d*][1,3]thiazine derivatives (5a-j) have been synthesized by condensation of 6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile (3) with different derivatives of hydrazine (4a-j) by using anhydrous potassium carbonate as catalyst and solvent DMF. Compound (3) was prepared by reaction of benzothioamide (1) and bis(methylthio)methylene malononitrile (2) with same reaction condition which is used for title compounds. The newly synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral analysis. Furthermore, these synthesized compounds were tested for antimicrobial activity.

Key words: - Benzothioamide, anhydrous potassium carbonate, antimicrobial activity and bis(methylthio)methylene malononitrile.

INTRODUCTION :

The heterocyclic compound containing nitrogen and sulphur serve as the versatile moiety for drug designing which has potential pharmacological properties[1]. Among them thiazine is very useful moiety in the field of medicinal chemistry and have been reported to exhibit a variety of biological activities such as antioxidant[2], antiviral[3], blood platelet aggregation inhibitor[4], antimicrobial[5], antifungal[6]. Moreover, thiazine derivatives act as effective corrosion inhibitor[7-8] for carbon steel in acidic media due to the presence of hetero atoms (N and S) which has lone pairs and ring has plannar pi-electrons are two important structural features that determines the absorption of molecules on the surface of metal.

Pyrazolo thiazine and its derivatives shows fungicidal[9], herbicidal[10] and antibacterial activities[11]. Stefano Sabatini et al[12] synthesize pyrazolo[4,3-*c*][1,2]benzothiazines as a new class of staphylococcus aureus NorA efflux pump inhibitors. Auzzi G et al[13] synthesized different pyrazolo thiazines and used as gluconeogenesis and passive cutaneous anaphylaxis inhibition. In literature survey very few methods are available for the synthesis of pyrazolo[3,4-d][1,3]thiazines[14-19].

Keeping in view the long lasting interest of the synthetic community in thiazine and pyrazole as a potential drugs, in present investigation we planned to synthesize both the heterocyclic moieties in a single framework and study of their synergic effect which may result some biologically more potent molecules.

MATERIAL AND METHODS:

All compounds were purchased from SD-Fine, Spectrochem and Avra chemical companies and used without any additional purification. Melting points of synthesized compounds were determined by Electrothermal IA 9000 SERIES digital melting point apparatus and were uncorrected. Purity of compounds and completion of the reaction was monitored by thin layer chromatography (TLC) using ethyl acetate: hexane (3:7) as the mobile phase on

precoated sheets of silica gel-G (Merck, Germany) using iodine vapour for detection. FT-IR spectra were recorded in Nujol or as KBr pallets on infrared spectrophotometer. Brukner advance spectrophotometer 400 MHz was used to record ^{1}H-NMR and ^{13}C-NMR spectra using tetramethylsilane (TMS) as internal standard, Mass spectra were recorded on FT-VC-7070 H Mass spectrometer using the EI technique at 70 eV.

General procedure:

Synthesis of 6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile (3)

A mixture of benzothioamide (1) (0.01mol) and bis(methylthio)methylene malononitrile (2) (0.01mol) in 10 ml of DMF and anhydrous potassium carbonate (10mg) was refluxed for 5 hours. The reaction progress was monitored by thin layer chromatography (TLC) by using ethyl acetate:hexane (3:7) as irrigant. After completion of reaction, the reaction mixture was allow to cool at room temperature and transferred in to ice cold water. The separated solid product was filtered, washed with water and recrystallized from ethanol to give pure compound (3).

Synthesis of 3-amino-4-imino-6-phenyl-2N-(substituted)pyrazolo[3,4-d][1,3]thiazines (5a-j).

As per scheme-2, a mixture of 6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile (3) (0.001mol) and various derivatives of hydrazines (4a-j) (0.001mol) were independently refluxed in 10 ml of DMF and anhydrous K_2CO_3 (10mg) for 5-6 hours. After completion of reaction, the reaction mixture was allow to cool at room temperature and transferred in to ice cold water. The separated solid product was filtered, washed with water and recrystallized from ethanol to give pure compound (5a-j).

SPECTRAL ANALYSIS:

Synthesis of 6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile (3).

Yellow Solid, Yield 61%, M.P. 146 °C**,** IR (KBr/cm^{-1}) 2206 (CN), 3358 (-NH): ^{1}H-NMR (400 MHz,DMSO-d_6): δ 2.76 (s, 3H, SCH_3), 7.54-7.70 (m, 5H, Ar-H), 8.53 (s, 1H, =NH): ^{13}C-NMR (DMSO-d_6): δ 13.12 (SCH_3), 80.62 (C-CN), 114.34 (CN), 128.74-131.58 (aromatic C=C), 163.63 (C=NH), 165.13 (C-SCH_3), 172.12 (S-C=N), EI-MS(m/z: RA%): 259 (M^+), Anal. Calcd for $C_{12}H_9N_3S_2$ C 55.59; H 3.47; N 16.21. Found C 55.46; H 3.54; N 16.30.

2,4-dihydro-3-amino-4-imino-6-phenylpyrazolo[3,4-*d*][1,3] thiazine (5a).

Brown Solid, Yield 63 %, M.P. 138 °C, IR (KBr/cm^{-1}) 3287 (-NH), 3355 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 6.78 (s, 2H, NH_2), 7.48-7.91 (m, 5H, Ar-H), 8.27 (s, 1H, =NH): EI-MS(m/z: RA%): 243 (M^+), Anal. Calcd $C_{11}H_9N_5S$ C 54.32; H 3.70; N 28.80. Found C 54.50; H 3.82; N 28.64.

3-amino-4-imino-6-phenyl-2(4*H*)-(carbothioamide) pyrazolo[3,4-*d*][1,3] thiazine (5b)

Brown Solid, Yield 55 %, M.P. 154 °C, IR (KBr/cm^{-1}) 3222 (-NH), 3405 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 6.58 (s, 2H, NH_2), 7.23 (s, 2H, NH_2), 7.65-7.86 (m, 5H, Ar-H), 9.10 (s, 1H, =NH): EI-MS(m/z: RA%): 302 (M^+), Anal. Calcd $C_{12}H_{10}N_6S_2$ C 47.68; H 3.31; N 27.81. Found C 47.20; H 3.44; N 27.36.

2,4-dihydro-3-amino-4-imino-2,6-diphenylpyrazolo[3,4-*d*][1,3] thiazine (5c).

Faint Brown Solid, Yield 71 %, M.P. 143 °C, IR (KBr/cm^{-1}) 3224 (-NH), 3417 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 6.69 (s, 2H, NH_2), 7.30-7.68 (m, 10H, Ar-H), 8.81 (s, 1H, =NH): EI-MS(m/z: RA%): 319 (M^+), Anal. Calcd $C_{17}H_{13}N_5S$ C 63.94; H 4.07; N 21.94. Found C 63.71; H 4.25; N 21.80.

2,4-dihydro-3-amino-4-imino-6-phenyl-2-(*p*-tolyl)pyrazolo[3,4-*d*][1,3] thiazine (5d)

Faint Brown Solid, Yield 78 %, M.P. 162 °C, IR (KBr/cm^{-1}) 3264 (-NH), 3305 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 2.20 (s, 3H, Ar-CH_3), 6.92 (s,

2H, NH_2), 7.56-7.84 (m, 9H, Ar-H), 9.22 (s, 1H, =NH): EI-MS(m/z: RA%): 333 (M^+), Anal. Calcd $C_{18}H_{15}N_5S$ C 64.86; H 4.50; N 21.02. Found C 64.32; H 4.26; N 21.18.

2,4-dihydro-3-amino-4-imino-6-phenyl-2-(2,4-dinitrophenyl)pyrazolo[3,4-*d*][1,3]thiazine (5e)

Yellow Solid, Yield 66 %, M.P. 173 °C, IR (KBr/cm^{-1}) 3207 (-NH), 3311 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 6.92 (s, 2H, NH_2), 7.29-7.51 (m, 8H, Ar-H), 8.77 (s, 1H, =NH): EI-MS(m/z: RA%): 409 (M^+), Anal. Calcd $C_{17}H_{11}N_7O_4S$ C 49.87; H 2.68; N 23.96. Found C 49.48; H 2.52; N 23.77.

2,4-dihydro-3-amino-4-imino-6-phenyl-2-(2'-benzothiazolyl)pyrazolo[3,4-*d*][1,3]thiazine (5f)

Dark Brown Solid, Yield 75 %, M.P. 198 °C, IR (KBr/cm^{-1}) 3251 (-NH), 3422 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 7.12 (s, 2H, NH_2), 7.35-7.88 (m, 9H, Ar-H), 9.44 (s, 1H, =NH): EI-MS (m/z: RA%): 376 (M^+), Anal. Calcd $C_{18}H_{12}N_6S_2$ C 57.44; H 3.19; N 22.34. Found C 57.59; H 3.01; N 22.71.

2,4-dihydro-3-amino-4-imino-6-phenyl-2-(6'-methyl-2'-benzothiazolyl)pyrazolo[3,4-*d*][1,3] thiazine (5g).

Brown Solid, Yield 74 %, M.P. 211 °C, IR (KBr/cm^{-1}) 3236 (-NH), 3365 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 2.15 (s, 3H, Ar-CH_3), 7.06 (s, 2H, NH_2), 7.22-7.49 (m, 8H, Ar-H), 8.60 (s, 1H, =NH): EI-MS (m/z: RA%): 390 (M^+), Anal. Calcd $C_{19}H_{14}N_6S_2$ C 58.46; H 3.58; N 21.53. Found C 58.27; H 3.36; N 21.60.

2,4-dihydro-3-amino-4-imino-6-phenyl-2-(4,6'-dimethyl-2'-benzothiazolyl)pyrazolo[3,4-*d*][1,3]thiazine (5h).

Brown Solid, Yield 83 %, M.P. 194 °C, IR (KBr/cm^{-1}) 3210 (-NH), 3408 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 2.10 (s, 3H, Ar-CH_3), 2.26 (s, 3H, Ar-CH_3), 6.96 (s, 2H, NH_2), 7.38-7.88 (m, 7H, Ar-H), 8.44 (s, 1H, =NH): EI-MS (m/z: RA%): 404 (M^+), Anal. Calcd $C_{20}H_{16}N_6S_2$ C 59.40; H 3.96; N 20.79. Found C 59.55; H 3.78; N 20.90.

2,4-dihydro-3-amino-4-imino-6-phenyl-2-(6'-methoxy-2'-benzothiazolyl)pyrazolo[3,4-*d*][1,3]thiazine (5i).

Brown Solid, Yield 69 %, M.P. 184 °C, IR (KBr/cm^{-1}) 3293 (-NH), 3388 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 3.82 (s, 3H, -OCH_3), 6.66 (s, 2H, NH_2), 6.97-7.28 (m, 8H, Ar-H), 8.20 (s, 1H, =NH): EI-MS (m/z: RA%): 406 (M^+), Anal. Calcd $C_{19}H_{14}N_6OS_2$ C 56.15; H 3.44; N 20.68. Found C 56.29; H 3.61; N 20.85.

2,4-dihydro-3-amino-4-imino-6-phenyl-2-(6'-chloro-2'-benzothiazolyl)pyrazolo[3,4-*d*][1,3] thiazine (5j).

Yellow Solid, Yield 62 %, M.P. 197 °C, IR (KBr/cm^{-1}) 3256 (-NH), 3316 (=NH): ^{1}HNMR (400 MHz,DMSO-d_6): δ 6.81 (s, 2H, NH_2), 7.23-7.67 (m, 8H, Ar-H), 8.54 (s, 1H, =NH): EI-MS (m/z: RA%): 410 (M^+), Anal. Calcd $C_{18}H_{11}N_6S_2Cl$ C 52.68; H 2.68; N 20.48. Found C 52.94; H 2.72; N 20.75.

RESULT AND DISSCUSSION:

During the course of our ongoing interest to the synthesis of various heterocyclic compounds using 6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile (3), we observed that compound (3) is key intermediate for the synthesis of pyrazolothiazines. Thus in present view we have synthesized a series of 3-amino-4-imino-6-phenyl-2N-(substituted)pyrazolo[3,4-*d*][1,3] thiazines (5a-j). The key intermediate (3) was prepared by condensation of benzothioamide (1) and bis(methylthio) methylene malononitrile (2) in DMF and catalytic amount of anhydrous K_2CO_3 **Scheme-1**.

The compound (3) posses replaceable active thiomethyl group at 4th position and electron withdrawing nature of cyano group at 5th position. Due to presence of thiomethyl group and cyano group on compound (3) which has susceptibility for nucleophilic substitution-cyclization. When compound (3) was condensed independently with various hydrazine derivatives (4a-j) under similar experimental condition to

afford 3-amino-4-imino-6-phenyl-2N-(substituted) pyrazolo[3,4-*d*][1,3]thiazines (5a-j) **Scheme-2.**

The final compounds (5a-j) were characterized on the basis of physical and spectral (IR, ^{1}H-NMR and MS) data. Spectral analysis of these compounds were in agreement of the proposed structures.

ANTIMICROBIAL ACTIVITY :

All synthesized compounds were evaluated for their antimicrobial screening against different pathogenic micro-organisms such as *Bacillus subtilis, Staphylococcus aureus* (Gram +ve) *and Salmonella typhi, Escherichia coli* (Gram -ve). The technique used in this experiment was paper disk diffusion method. The cultures were diluted with 5% of autoclaved saline and the final volume was adjusted to a concentration of approximately 105-106 CFU ml^{-1}. All the compounds were diluted with dimethyl sulphoxide (100µg/ml in DMSO) for the antibacterial biological assay. The liquid formed of test compound was soaked on to a disc and allowed to air dry, such that the disc became completely saturated with the test compound. The saturated chemical discs were introduced onto the upper layer of medium evenly loaded with the bacteria. For bacterial growth incubation period was 24 hours at temperature 37°c. Activity of compounds were determined by measuring the diameter of zone of inhibition, values obtained was compared with the values produced from standard drugs like streptomycin and penicillin (100µg/ml).

From the screening studies (**Table 1**), it is evident that from all synthesized pyrazolo[3,5--*d*][1,3]thiazine derivatives **(5b)**, **(5h)** and **(5i)** showed good antibacterial activity against all the tested organisms whereas remaining derivatives showed comparative activity with standard drugs (streptomycin and penicillin). The newly synthesized compounds show zone of inhibition 05-26 mm in diameter whereas standard streptomycin exhibit zone of inhibition 26-28 mm in diameter.

CONCLUSION:

In summary, with the aim of good contribution in innovation of heterocyclic chemistry, we have demonstrated the preparation, characterization and antimicrobial activity of novel heterocyclic compounds such as 3-amino-4-imino-6-phenyl-2N-(substituted)pyrazolo[3,4-d][1,3]thiazine derivatives (5a-j) which were obtained by simple route with good product yield. The antimicrobial data revealed that all compounds showed good to moderate activity compared to standard drug. This protocol includes some important advantages such as mild reaction condition, easy work-up, purity of product and short reaction time.

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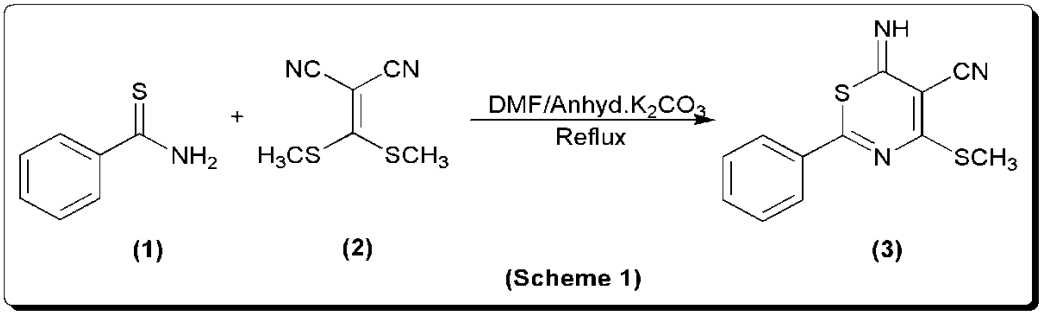
Table 1.Antimicrobial activity of compound (5a-5j)

Compounds	Gram positive		Gram negative	
	B. subtilis	***S. aureus***	***S. typhi***	***E. Coli***
5a	17	14	08	12
5b	24	19	23	21
5c	08	12	08	15
5d	12	15	09	05
5e	11	15	14	08
5f	14	09	18	11
5g	08	14	07	19
5h	25	21	17	24
5i	20	23	19	26
5j	12	08	11	13
Streptomycin	28	-	-	28
Penicillin	-	26	27	-

Scheme-1 Synthesis of 6-imino-4-(methylthio)-2-phenyl-6*H*-1,3-thiazine-5-carbonitrile (3).



Scheme-2. Synthesis of 3-amino-4-imino-6-phenyl-2N-(substituted)pyrazolo[3,4-*d*][1,3] thiazines (5a-j).