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# Synthesis, characterization and biological study of some new substituted pyrazolo[3,4-d] thiazolo[3,2-a] pyrimidine derivatives

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**A B S T R A C T**

Fused pyrimidines play an imperative role in our life due to their biological importance in the struggle of microorganisms. A series of 4-aryl-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine **(4a-j)** were synthesized by cyclization of 3-methyl-1-phenyl-4-aryl-1,4,5,7-tetrahydro-6*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiones **(3a-j)** with 1,2-dibromoethane in presence of anhydrous potassium carbonate. Earlier, compounds **(3a-j)** were synthesized by the condensation of 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one **(2)**, different substituted benzaldehyde and thiourea with catalytic amount of con. HCl in methanol. Compound **2** was synthesized by condensation of ethyl acetoacetate **(1)** and phenylhydrezine in presence of a catalytic amount of acetic acid at reflux temperature. The constitution of the synthesized products has been characterized by using elemental analysis, Infrared, $^1$H-NMR spectroscopy and further supported by Mass spectroscopy. All the products have been screened for their *in-vitro* biological assay like antibacterial and antifungal activity at concentration of 500 µg/ml. It was exposed that most of the compounds displayed inspiring antibacterial and antifungal activity compared to the used reference standard.



## 1. Introduction

Pyrimidine derivatives are very significant in nature due to their various applications as bioactive agents used in agriculture and other fields[1-3]. Also, Due to enormous synthetic importance and wide-ranging bioactivities revealed by pyrimidines derivatives efforts have been made from time to time to generate libraries of these compounds[4,5]. Among these, Fused pyrimidines continue to attract sizable attention from researchers in different countries because of their great practical usefulness, primarily, due to a very wide spectrum of their biological activities[6,7]. Also, pyrazole systems are excellent precursors for the synthesis of condensed polyfunctionally substituted pyrimidine heterocycles[8,9]. Pyrazolo-annulated heterocycles such as pyrazolopyrimidines have attracted considerable interest because their derivatives display a wide range of pharmacological activities, e.g., antiproliferative agents[10], anti-inflammatories, analgesic[11] and anticancer agents[12]. Also, Pyrazolopyrimidines are well known for their pharmacological activities including antitumor, antipyretic, antimicrobial, antiviral, antileishmanial, and antihistaminic activities[13,14]. They also exhibit central nervous system (CNS) depressant, neuroleptic, and anti-tuberculostatic activities[15]. Additionally, Thiazole and pyrimidine nuclei are the active core of various bioactive molecules[16]. In general, thiazole encompassing a pyrimidine unit have found applications in a wide spectrum of biological and therapeutic areas[17-20]. Thus, the heterocyclic system resulting from annulation of a pyrimidine ring on the biologically versatile thiazole and pyrazole nucleus is an attractive scaffold to be utilized for exploiting chemical diversity.

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Continuing our efforts focused towards the straightforward preparation of biologically active target molecules, we performed the synthesis of some new 4-aryl-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo [3,4-*d*] thiazolo[3,2-*a*]pyrimidines **(4a-j)** to study their bipotentiality. An effort has been made to insert the thiazolo moiety in pyrazolopyrimidine derivatives for activity strengthening.

## 2. Results and Discussion

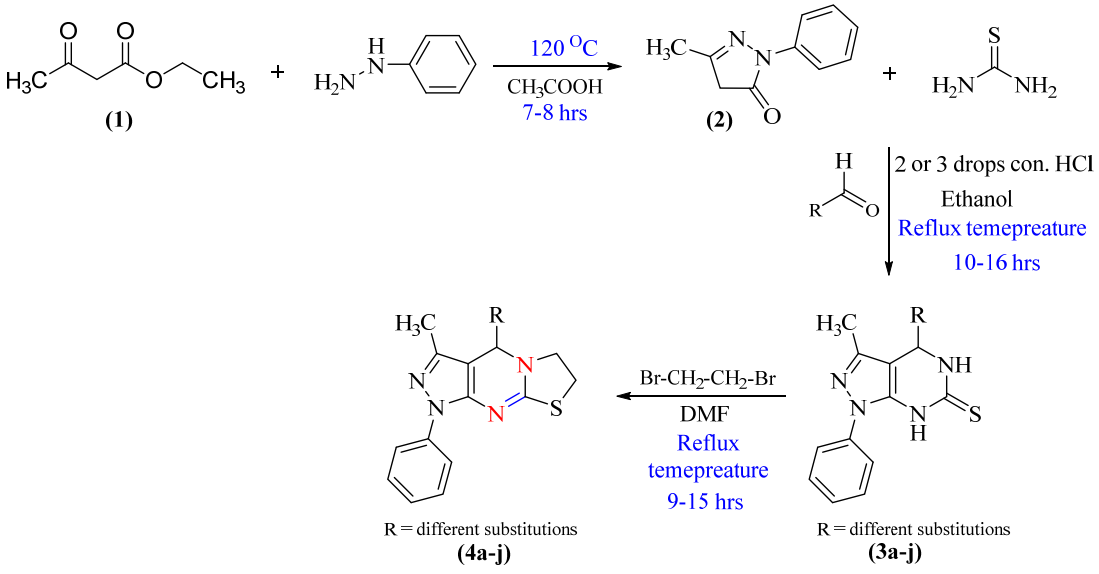
*2.1 Chemistry and Spectroscopic discussion*

The target compounds 4-aryl-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidines **(4a-j)** were synthesized as charted to as shown in reaction **Scheme 1**. The title compounds, 4-aryl-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidines **(4a-j)** were synthesized by a three-step procedure starting from ethyl acetoacetate **(1)**. Condensation reaction of ethyl acetoacetate **(1)** (0.04 mole) with phenylhydrazine (0.04 mole) in presence of catalytic amount of acetic acid gives 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one **(2)**. Later, The condensation reaction of 5-methyl-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one **(2)** with different substituted benzaldehyde and thiourea with catalytic amount of con. HCl in methanol solvent gives 3-methyl-1-phenyl-4-aryl-1,4,5,7-tetrahydro-6*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiones **(3a-j).** Finally, cyclization of 3-methyl-1-phenyl-4-aryl-1,4,5,7-tetrahydro-6H-pyrazolo[3,4-*d*]pyrimidine-6-thiones **(3a-j)** with 1,2-dibromoethane in the presence of anhydrous potassium carbonate in dimethyl formamide (DMF) solvent gives target compounds, 4-aryl-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidines **(4a-j)**.

The purity of all compounds has been checked by thin layer chromatography and their characterization is carried out by means of elemental analysis, Infrared, $^1$H-Nuclear Magnetic Resonance ($^1$H-NMR) spectroscopy and further supported by Mass spectroscopy.

Among all, the IR spectrum of compound **4e** showed bands at 2889 cm$^{-1}$ and 3030 cm$^{-1}$ which are due to aliphatic C–H stretching and aromatic C–H stretching vibration respectively. The bands observed at 1600 cm$^{-1}$ and 1296 cm$^{-1}$ is due to aromatic C=N and C–N stretching vibrations and bands at 1359 cm$^{-1}$ and 1200 cm$^{-1}$ is due to C-N-C and C-S-C stretching vibrations of Pyrazolo thiazolo pyrimidine moity, respectively. Band of C–H out of plane bending for p-disubstituted aromatic ring is observed at 831 cm$^{-1}$. Band of C-Cl bending vibration is observed at 665 cm$^{-1}$. The mass spectrum of compound **4e** showed base peak at 261 m/z and M-2 peak at 379 m/z. The $^1$H-NMR spectrum of compound **4e** (in $CDCl_3$) showed a singlet signal at 1.76 δ due to –$CH_3$ group present in the pyrazoline ring. The two triplet signals are at 3.12 δ due to the –N-$CH_2$– group and 3.89 δ due to the –S-$CH_2$– group present in the thiazole ring. The singlet signal at 5.11 δ integrating for one proton is due to chiral C–H of the pyrimidine ring. The remaining nine aromatic protons of both phenyl rings gives multiplate signal between 7.15 δ to 8.25 δ.

All the products have been screened for their *in-vitro* biological assay like antibacterial activity towards *Gram-positive bacteria* like *Staphytococcus aureus, Bacillus subtilis* and *Gram-negative bacteria* like *Escherichia coli*, *Salmonella paratyphi B* and antifungal activity towards *Aspergillus niger* and *Candida albicans* at concentration of 500 µg/ml.




**Scheme-1**

*2.2 biological activity*

All the synthesized compounds have been evaluated for antimicrobial activity.

*Antibacterial activity*

The purified products were screened for their antibacterial activity using cup-plate agar dilution method. The nutrient agar broth prepared by the usual method was inoculated aseptically with 0.5 ml of 24 hr., old subcultures of *Bacillus subtilis-ATCC 6633*, *Staphylococcus aureus-ATCC 25923, Escherichia coli-ATCC 25922, Salmonella paratyphi B* in separate conical flasks at 40-50 ºC and mixed well by gentle shaking. About 19 ml content of the flask was poured and evenly spreaded in a Petri dish (13 cm diameter) and allowed to set for 2 hr. The cups (10 mm diameter) were formed by the help of borer in agar medium and filled with 500 µg/ml of sample in DMF. The plates were incubated at 32 ºC for 24 hrs. and the control was also maintained with 0.04 ml of DMF in a similar manner and the zone of inhibition of the bacterial growth were measured in millimeters and recorded in **Table 1**.

*Antifungal activity*

*Aspergillus niger-MTCC 282* and *Candida albicans-MTCC 272* were employed for testing antifungal activity using the cup-plate method. The culture was maintained on *subouraud's agar slants*. Sterilized *sabouraud's agar* medium was inoculated with 72 hrs., old 0.5 ml suspension of fungal spores in a separate flask.

About 25 ml of inoculated medium was evenly spreaded in a Petri dish and allowed to sit for two hrs. The plates were incubated at 30 ºC for 48 hrs. After the completion of the incubation period, the zone of inhibition of growth in the form of diameter in *mm* was measured. Along the test solution in each Petri dish one cup was filled with solvent which act as control. Standard drugs like *Ciprofloxacin* and *Griseofulvin* were used for comparison purposes. The zones of inhibition are recorded in **Table 1**.

**Table 1**. Antimicrobial screening results of compounds **4a-j**

| ID | R | Zone of inhibition in *mm* | | | | | |
|---|---|---|---|---|---|---|---|
| | | Antibacterial activity (%) | | | | Antifungal activity (%) | |
| | | *B. subtilis* | *S. aureus* | *E. coli* | *S. parathypi* | *C. albicans* | *A. niger* |
| **4a** | 4-$NO_2$-$C_6H_4$- | 14 | 12 | **32** | **22** | 19 | 20 |
| **4b** | 4-$OCH_3$-$C_6H_4$- | 12 | 11 | 27 | 18 | **26** | **24** |
| **4c** | 4-OH-$C_6H_4$- | **24** | **24** | **31** | **23** | **28** | **25** |
| **4d** | 4-F-$C_6H_4$- | 15 | **26** | 21 | **25** | 27 | **28** |
| **4e** | 4-Cl-$C_6H_4$- | 16 | 13 | **30** | 16 | **30** | **30** |
| **4f** | 3-$NO_2$-$C_6H_4$- | **25** | **22** | **31** | **22** | 17 | **31** |
| **4g** | 3-Cl-$C_6H_4$- | **23** | **24** | **29** | **25** | **25** | **25** |
| **4h** | 2-$NO_2$-$C_6H_4$- | **22** | 11 | **28** | **27** | 24 | **26** |
| **4i** | 2-Cl-$C_6H_4$- | 19 | **24** | 21 | 14 | 19 | 20 |
| **4j** | 2-OH-$C_6H_4$- | **24** | **26** | **30** | **21** | **25** | **24** |
| Ciprofloxacin | - | **22** | **22** | **28** | **21** | - | - |
| Griseofulvin | - | - | - | - | - | **25** | **22** |

From activity data, it is observed that compounds **4c, 4f, 4g** and **4j** were found to be active which is attributed to the presence of 4-OH, 3-$NO_2$, 3-Cl and 2-OH-substituted benzene ring at $C_4$-position of pyrimidine nucleus, against *Bacillus subtilis* with reference to standard drug. Compounds **4c, 4d, 4g, 4i** and **4j** were found to be active due to the presence of 4-OH, 4-F, 3-Cl, 2-Cl and 2-OH substituted benzene ring at $C_4$-position of pyrimidine nucleus, against *Staphylococcus aureus.* Against *E. Coli* strain, compound **4a, 4c, 4e, 4f, 4g** and **4j** were found to be active which is attributed to the presence of 4-$NO_2$, 4-OH, 4-Cl, 3-$NO_2$, 3-Cl and 2-OH substituted benzene ring at $C_4$-position of pyrimidine nucleus. Against *S. parathypi,* compounds **4a, 4c, 4d, 4f, 4g** and **4h** are highly active due to the presence of 4-$NO_2$, 4-OH, 4-F, 3-$NO_2$, 3-Cl and 2-$NO_2$ substituted benzene ring at $C_4$-position of pyrimidine nucleus than others. Against *C. albicans strain,* compounds **4b, 4c** and **4e** were highly active as compared to standard drugs. Compounds **4b, 4c, 4d, 4e, 4f, 4g, 4h** and **4j** were highly active against *Aspergillus niger* as compared to standard drugs.

It was interesting to notice that the activity depends on the substituents rather than the basic skeleton of the molecule. It was noticed that electron donating groups -Cl, -F and -OH on the benzene ring at $C_4$-position of pyrimidine nucleus, enhanced their antimicrobial activity and are better antimicrobial agents, while due to the presence of strong electron withdrawing substituents on the aromatic ring to the pyrimidine nucleus decreases the antimicrobial activity.

## 3. Conclusions

To sum up, in this work, we have effectively synthesized a series of 4-aryl-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidines **(4a-j),** different spectral analysis techniques have utilized to confirm

the structures of the designed products. All compounds were *in vitro* investigated for their antibacterial as well as antifungal activities against different strains of bacteria and fungi showing moderate to high activity and inhibition zones for some synthesized derivatives were shown values close to the used reference drug as per comparison to the literature[21,22]. They were found to possess reasonably good antifungal activity and compounds **4c, 4d, 4e, 4f, 4g** and **4j** were found to be the most potent anti-microbial agents. It will be the topic of new research to substitute it with greener reagents and solvents, finding more effective anti-microbial agents. Also, In the future, we will focus on the development of different series of tetrahydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidines for the investigation of other pharmaceutical activities.

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## 4. Experimental

### *4.1. Materials and Methods*

Chemicals and solvents were purchased from Sigma-Aldrich Chemical Co., Merck chemical, Finar, and Spectrochem Ltd. Thin layer chromatography [silica gel G (E Merck) plates] was used to monitor the reactions and purity of newly synthesized compounds. Visualization was achieved with UV light (254 and 365 nm) or with iodine vapor. The melting point was determined by electrothermal apparatus using open capillary tubes and are uncorrected. IR spectra were recorded on a Shimadzu 8400 FTIR instrument in KBr disc and only significant absorbance levels (cm$^{-1}$) are listed. $^1$H (400 MHz) NMR spectra were recorded on a "Bruker AVANCE III spectrometer" in $CDCl_3$/ DMSO-$d_6$ solvent using TMS as internal standard. Chemical shift is given in ppm. Mass spectra were determined using a direct inlet probe on GCMS-QP2010 mass spectrometer (Shimadzu, Kyoto, Japan). Elemental analysis was performed on a Carlo Erba EA1108 elemental analyser.

### *4.2. General procedure*

#### *4.2.1 Procedure for the synthesis of 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one* **(2)**

A mixture of ethyl acetoacetate (5.2 g, 0.04M) and phenylhydrezine (4.3 g, 0.04M) was heated at 120 °C in oil bath for 7-8 hours. The reaction mixture was cooled and 15 ml diethyl ether was added. The mixture was stirred. So, solid product was obtained. It was filtered and wash with ether and crystallized from ethanol. Yield 82%, m.p.127 °C. IR(KBr): 3052 (C=C-H str.), 2990 (C-H str. of alkane), 1765 (C=O str. of ketone), 1490 (C=C str.), 1300 (C-N str. of aromatic-N-) cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): 1.92 (s, 3H, -$CH_3$), 3.12 (s, 2H, -$CH_2$-), 7.30 – 8.12 (m, 5H, Ar-H); EI-MS: m/z 174. ($C_{10}H_{10}N_2O$; required: C, 68.95; H, 5.79; N, 16.08; O, 9.18 %; found: C, 68.91; H, 5.74; N, 16.05; O, 9.17%).

#### *4.2.2 Procedure for the synthesis of 3-methyl-1-phenyl-4-aryl-1,4,5,7-tetrahydro-6H-pyrazolo[3,4-d]pyrimidine-6-thiones* **(3a-j)**

A mixture of 5-methyl-2-phenyl-2,4-dihydro-*3H*-pyrazol-3-one **(2)** (1.75g, 0.01M), various substituted benzaldehyde (0.01M), thiourea (0.1 g, 0.15 M) and 2-3 drops of con. HCl in methanol (15 ml) was refluxed for 10-16 hrs. The reaction mixture was kept at room temperature for 24 hrs. The product was isolated by filtration and crystallized from suitable solvent to give pure products.

*Spectral data of 4-(4-chlorophenyl)-3-methyl-1-phenyl-1,4,5,7-tetrahydro-6H-pyrazolo[3,4-d]pyrimidine-6-thione* **(3e)**

Yield 61%; m.p. 154°C; IR (KBr): 2887-3158 (two broad N-H str of pyrimidine ring), 3057 (C=C-H str.), 2987 (C-H str. of alkane), 1517 (C=C), 1307 (C-N str. of Ar-N-), 1230 (N-N def.), 1117 (C=S str.), 831 (C-H o.o.p. def. of p-disubstituted benzene), 624 (C-Cl str.) cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): 1.89 (s, 3H, -$CH_3$ of pyrazoline), 5.54 (s, 1H, chiral C-H of pyrimidine), 7.24 – 7.60 (m, 9H, aromatic ring), 9.65 (s, 1H, -NH-pyrimidine ring), 13.40 (s, 1H, -NH-pyrimidine ring); EI-MS: m/z 354. Anal. Calcd. for $C_{18}H_{15}ClN_4S$; required: C, 60.93; H, 4.26; Cl, 9.99; N, 15.79; S, 9.03%; found: C, 60.89; H, 4.22; Cl, 9.91; N, 15.74; S, 8.99 %).

#### *4.2.3 Procedure for the synthesis of* 4-aryl-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidines **(4a-j)**

A mixture of different *3-methyl-1-phenyl-4-aryl-1,4,5,7-tetrahydro-6H-pyrazolo[3,4-d]pyrimidine-6-thiones* **(3a-j)** (0.005M) and dibromoethane (1.87 g, 0.01 M) in DMF (15 ml) was refluxed for 9-15 hrs. The reaction mixture was poured

into ice cold water and neutralize by adding $NaHCO_3$. The product was so obtained. It was filtered dried and crystallized from DMF.

*4.3 Physical and Spectral Data*

*4.3.1 3-methyl-4-(4-nitrophenyl)-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine (4a)*

Yield 56 %; m.p. 157 °C; IR (KBr): 3033 (C=C-H str.), 2887 (C-H asym. str.), 1603 (C=N str.), 1537 (N-O asym. str.), 1358 (N-O sym. str.), 1492 (C=C str.), 1369 (C-N-C Str.), 1294 (C-N str.), 1228 (N-N def.), 1202 (C-S-C str.), 835 (C-H def. of p-disubstituted benzene) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): 1.72 (s, 3H, $-CH_3$), 3.27 (t, 2H, -N-$CH_2$-), 3.84 (t, 2H, -S-$CH_2$-), 5.18 (s, 1H, pyrimidine-H), 7.14-8.32 (m, 9H, Aromatic-H); EI-MS: m/z 391. Anal. Calcd. for $C_{20}H_{17}N_5O_2S$; required: C, 61.37; H, 4.38; N, 17.89; O, 8.17, S, 8.19 %; found: C, 61.33; H, 4.34; N, 17.84; O, 8.12, S, 8.17 %).

*4.3.2 4-(4-methoxyphenyl)-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine (4b)*

Yield 51%; m.p. 173 °C; IR (KBr): 3026 (C=C-H str.), 2870 (C-H asym. str.), 1570 (C=N str.), 1482 (C=C str.), 1351 (C-N-C Str.), 1283 (C-N str.), 1224 (N-N def.), 1204 (C-S-C str.), 1132 (C-O-C str.), 830 (C-H def. of p-disubstituted benzene) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): 1.71 (s, 3H, $-CH_3$), 4.12 (t, 2H, -N-$CH_2$-), 3.77 (s, 3H, $-OCH_3$), 3.17 (t, 2H, -S-$CH_2$-), 5.19 (s, 1H, pyrimidine-H), 7.14-8.07 (m, 9H, Aromatic-H); EI-MS: m/z 376. Anal. Calcd. for $C_{21}H_{20}N_4OS$; required: C, 67.00; H, 5.35; N, 14.88; O, 4.25; S, 8.52 %; found: C, 66.91; H, 5.27; N, 14.79; O, 4.21; S, 8.43 %).

*4.3.3 4-(4-hydroxyphenyl)-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine (4c)*

Yield 50%; m.p. 223 °C; IR (KBr): 3468 (O-H str.), 3031 (C=C-H str.), 2873 (C-H asym. str.), 1600 (C=N str.), 1490 (C=C str.), 1368 (C-N-C Str.), 1280 (C-N str.), 1224 (N-N def.), 1203 (C-S-C str.), 1037 (C-O str.), 814 (C-H def. of p-disubstituted benzene) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): 1.83 (s, 3H, $-CH_3$), 4.08 (t, 2H, -N-$CH_2$-), 3.13 (t, 2H, -S-$CH_2$-), 5.29 (s, 1H, pyrimidine-H), 6.72-8.12 (m, 9H, Aromatic-H), 9.12 (s, 1H, -OH); EI-MS: m/z 362. Anal. Calcd. for $C_{20}H_{18}N_4OS$; required: C, 66.28; H, 5.01; N, 15.46; O, 4.41; S, 8.85 %; found: C, 66.21; H, 4.95; N, 15.41; O, 4.39; S, 8.81 %).

*4.3.4 4-(4-fluorophenyl)-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine (4d)*

Yield 60%; m.p. 116 °C; IR (KBr): 3038 (C=C-H str.), 2895 (C-H asym. str.), 1604 (C=N str.), 1493 (C=C str.), 1357 (C-N-C Str.), 1294 (C-N str.), 1228 (N-N def.), 1207 (C-S-C str.), 839 (C-H def. of p-disubstituted benzene), 665 (C-F str.) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): 1.96 (s, 3H, $-CH_3$), 4.08 (t, 2H, -N-$CH_2$-), 3.15 (t, 2H, -S-$CH_2$-), 5.26 (s, 1H, pyrimidine-H), 7.18-8.12 (m, 9H, Aromatic-H); EI-MS: m/z 364. Anal. Calcd. for $C_{20}H_{17}FN_4S$; required: C, 65.91; H, 4.70; F, 5.21; N, 15.37; S, 8.80 %; found: C, 65.87; H, 4.65; F, 5.15; N, 15.34; S, 8.74 %).

*4.3.5 4-(4-chlorophenyl)-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine (4e)*

Yield 52 %; m.p. 178 °C; IR (KBr): 3030 (C=C-H str.), 2889 (C-H asym. str.), 1600 (C=N str.), 1498 (C=C str.), 1359 (C-N-C Str.), 1296 (C-N str.), 1230 (N-N def.), 1200 (C-S-C str.), 831 (C-H def. of p-disubstituted benzene), 665 (C-Cl str.) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): 1.76 (s, 3H, $-CH_3$), 3.12 (t, 2H, -N-$CH_2$-), 3.89 (t, 2H, -S-$CH_2$-), 5.11 (s, 1H, pyrimidine-H), 7.15-8.25 (m, 9H, Aromatic-H); EI-MS: m/z 380. Anal. Calcd. for $C_{20}H_{17}ClN_4S$; required: C, 63.07; H, 4.50; Cl, 9.31; N, 14.71; S, 8.42 %; found: C, 63.02; H, 4.54; Cl, 9.27; N, 14.44; S, 8.40 %).

*4.3.6 3-methyl-4-(3-nitrophenyl)-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine (4f)*

Yield 58%; m.p. 109 °C; IR (KBr): 3037 (C=C-H str.), 2890 (C-H asym. str.), 1588 (C=N str.), 1542 (N-O asym. str.), 1347 (N-O sym. str.), 1490 (C=C str.), 1367 (C-N-C Str.), 1285 (C-N str.), 1218 (N-N def.), 1200 (C-S-C str.), 783 & 715 (C-H def. of m-disubstituted benzene) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): 1.79 (s, 3H, $-CH_3$), 3.27 (t, 2H, -N-$CH_2$-), 3.93 (t, 2H, -S-$CH_2$-), 5.28 (s, 1H, pyrimidine-H), 7.54-8.28 (m, 9H, Aromatic-H); EI-MS: m/z 391. Anal. Calcd. for $C_{20}H_{17}N_5O_2S$; required: C, 61.37; H, 4.38; N, 17.89; O, 8.17, S, 8.19 %; found: C, 61.31; H, 4.32; N, 17.85; O, 8.10, S, 8.16 %).

*4.3.7 4-(3-chlorophenyl)-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine (4g)*

Yield 59 %; m.p. 201 °C; IR (KBr): 3030 (C=C-H str.), 2892 (C-H asym. str.), 1605 (C=N str.), 1490 (C=C str.), 1352 (C-N-C Str.), 1294 (C-N str.), 1231 (N-N def.), 1202 (C-S-C str.), 776 & 721 (C-H def. of m-disubstituted benzene), 674 (C-Cl str.) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): 1.86 (s, 3H, $-CH_3$), 3.12 (t, 2H, -N-$CH_2$-), 3.99 (t, 2H, -S-$CH_2$-), 5.24 (s, 1H, pyrimidine-H), 7.35-8.12 (m, 9H, Aromatic-H); EI-MS: m/z 380. Anal. Calcd. for $C_{20}H_{17}ClN_4S$; required: C, 63.07; H, 4.50; Cl, 9.31; N, 14.71; S, 8.42 %; found: C, 63.05; H, 4.52; Cl, 9.24; N, 14.64; S, 8.38 %).

*4.3.8 3-methyl-4-(2-nitrophenyl)-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine (4h)*

Yield 56%; m.p. 113 °C; IR (KBr): 3030 (C=C-H str.), 2884 (C-H asym. str.), 1590 (C=N str.), 1540 (N-O asym. str.), 1342 (N-O sym. str.), 1483 (C=C str.), 1362 (C-N-C Str.), 1282 (C-N str.), 1214 (N-N def.), 1207 (C-S-C str.), 748 (C-H def. of o-disubstituted benzene) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): 1.85 (s, 3H, $-CH_3$), 3.27 (t, 2H, $-N-CH_2-$), 4.03 (t, 2H, $-S-CH_2-$), 5.34 (s, 1H, pyrimidine-H), 7.57-8.10 (m, 9H, Aromatic-H); EI-MS: m/z 391. Anal. Calcd. for $C_{20}H_{17}N_5O_2S$; required: C, 61.37; H, 4.38; N, 17.89; O, 8.17, S, 8.19 %; found: C, 61.33; H, 4.34; N, 17.83; O, 8.13, S, 8.15 %).

*4.3.9 4-(2-chlorophenyl)-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine (4i)*

Yield 50 %; m.p. 87 °C; IR (KBr): 3027 (C=C-H str.), 2896 (C-H asym. str.), 1607 (C=N str.), 1488 (C=C str.), 1351 (C-N-C Str.), 1297 (C-N str.), 1228 (N-N def.), 1200 (C-S-C str.), 748 (C-H def. of o-disubstituted benzene), 680 (C-Cl str.) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): 1.89 (s, 3H, $-CH_3$), 3.10 (t, 2H, $-N-CH_2-$), 4.03 (t, 2H, $-S-CH_2-$), 5.23 (s, 1H, pyrimidine-H), 7.24-8.10 (m, 9H, Aromatic-H); EI-MS: m/z 380. Anal. Calcd. for $C_{20}H_{17}ClN_4S$; required: C, 63.07; H, 4.50; Cl, 9.31; N, 14.71; S, 8.42 %; found: C, 63.03; H, 4.53; Cl, 9.27; N, 14.67; S, 8.39 %).

*4.3.10 4-(2-hydroxyphenyl)-3-methyl-1-phenyl-1,4,6,7-tetrahydropyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine (4j)*

Yield 49 %; m.p. 165 °C; IR (KBr): 3445 (O-H str.), 3034 (C=C-H str.), 2878 (C-H asym. str.), 1604 (C=N str.), 1493 (C=C str.), 1369 (C-N-C Str.), 1285 (C-N str.), 1227 (N-N def.), 1200 (C-S-C str.), 1027 (C-O str.), 756 (C-H def. of o-disubstituted benzene),$cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$): 1.89 (s, 3H, $-CH_3$), 4.08 (t, 2H, $-N-CH_2-$), 3.17 (t, 2H, $-S-CH_2-$), 5.28 (s, 1H, pyrimidine-H), 6.74-8.10 (m, 9H, Aromatic-H), 9.62 (s, 1H, -OH); EI-MS: m/z 362. Anal. Calcd. for $C_{20}H_{18}N_4OS$; required: C, 66.28; H, 5.01; N, 15.46; O, 4.41; S, 8.85 %; found: C, 66.23; H, 4.96; N, 15.40; O, 4.37; S, 8.83 %).

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