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Water Mediated One Pot Synthesis of pyrazolo[1,5-a] Pyrimidine Derivatives and their Antimicrobial Activity

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ABSTRACT

In this paper we have described the water mediated synthesis of substituted triazolopyrimidine derivatives in moderate to good yield. The reaction of various substituted aldehydes with 1-(3,4-dimethoxyphenyl)-4,4,4-trifluorobutane-1,3-dione as active methylene compound and 5-amino-3-(methylthio)-1H-pyrazole-4-carbonitrile afforded the triazolo pyrimidine derivatives in the presence boric acid and water as a solvent. We have also optimized the reaction condition using the various solvent. We have confirmed the structure on the basis of spectroscopic technique. All the synthesized compounds were evaluated for their antimicrobial activity. The investigation of antimicrobial and antifungal screening data revealed that all the tested compounds showed moderate to significant activity.

Keywords: Pyrazolo[1,5-a]pyrimidine, Triazolo pyrimidine, Biological evaluation

I. INTRODUCTION

Biaryls and heterobiaryls have attracted significant attention from the scientific community because of their relevance in medicinal chemistry. Heterobiaryls frequently can be observed in numerous bioactive small molecules, and in particular, heterobiaryls fused with various heterocycles, such as pyrazole, pyridine, and pyrimidine, have been used as key pharmacophores.[1] Pyrazolo[1,5-*a*]pyrimidines have attracted considerable interest because of their biological activity. For instance, this heterocyclic system is found as purine analogues and has useful properties as antimetabolites in purine biochemical reactions.[2] Several compounds of this class display interesting antitrypanosomal[3] and antischistosomal activities.[4] They are used as HMG-CoA reductaseinhibitors,[5] COX-2 selective inhibitors,[6] 30,50-cyclic-AMP phosphodiesteraseinhibitors,[7] CRF_1 antagonists,[8a-d] selective peripheral benzodiazepine receptor ligands,[9a-c] potassium channel[10] and histamine-3 receptor ligands[11] and antianxiety agents.[12.] The pyrazolopyrimidine derivatives have considerable chemical and pharmacological importance because a broad range of biological activities have been displayed by these classes of molecules. As we demonstrated, the tremendous biological potential of pyrazolopyrimidine derivatives encouraged us to synthesize some new highly functionalized pyrazolopyrimidine derivatives. Various methodologies have been described for the synthesis of pyrazolopyrimidine derivatives. However, the existing methods are suffer with some drawbacks, such as; yield, time, product isolation, isomer formation. Various methodologies have been described for the synthesis of Pyrazolo[1,5-*a*]pyrimidines. Very well known method for this is one pot synthesis of ethylacetoacetate, 3-aminopyrazole and aldehyde through Biginelli reaction. In our work we introduced novel 1-(3,4-dimethoxyphenyl)-4,4,4-trifluorobutane-1,3-dione as active methylene compound and 5-amino-3-(methylthio)-1H-pyrazole-4-carbonitrile for the formation of pyrazolo[1,5-*a*]pyrimidine ring system. Current scenario of the research is application of green chemistry for the synthesis of molecules. By considering this water mediated, boric acid catalysed pyrazolo[1,5-*a*]pyrimidine analogues have been synthesized. The newly synthesized compounds were characterized by IR, Mass, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis. All the synthesized compounds were evaluated for their antimicrobial activity.

II. EXPERIMENTAL

All chemicals and solvents were purchased from Spectrochem Pvt Ltd., Mumbai of AR grade and were used without further purification. Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F^{254} (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on a FTIR-8400 spectrophotometer using DRS prob. ^{1}H (400 MHz), ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AVANCE II spectrometer in $CDCl_3$ and DMSO. Chemical shifts are expressed in δ *ppm* downfield from TMS as an internal standard. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu). Solvents were evaporated with a BUCHI rotary evaporator. Melting points were measured in open capillaries and are uncorrected. Physical constants of the synthesized compounds **MR-01 to MR-20** are shown in Table 1.

Synthesis of 2-(bis(methylthio) methylene) malononitrile (Int-2).

A 100mL conical flask equipped with magnetic stirrer and septum was charged with a solution of malononitril(**1**), (10 mmol) in DMF (10 mL). Dry K_2CO_3 (10 mmol) was added and the mixture was stirred at RT for 2 h. CS_2 (30 mmol) was added and the mixture was stirred for an additional 2 h at room temperature. Then, methyl iodide (20 mmol) was added at 0-5 ^{o}C and the mixture was stirred for 4 h at room temperature. The progress of the reaction was monitored by thin layer chromatography. After completion of the reaction, it was poured into 50ml cold water. The precipitated crude product was purified by filtration followed by crystallization from EtOH.

Synthesis of 5-amino-3-(methylthio)-1*H*-pyrazole-4-carbonitril (Int-3).

To the solution of 2-(bis(methylthio) methylene) malononitrile (**Int-02**) (0.1mol) in isopropyl alcohol (100mL), hydrazine hydrate (0.1mol) was added. The reaction mixture was stirr to 0^{o}C for 2 h. After completion of the reaction, it was poured into 50mL cold water. The precipitated crude product was purified by filtration followed by crystallization from EtOH.

1-(3,4-dimethoxyphenyl)-4,4,4-trifluorobutane-1,3-dione(INT-6)

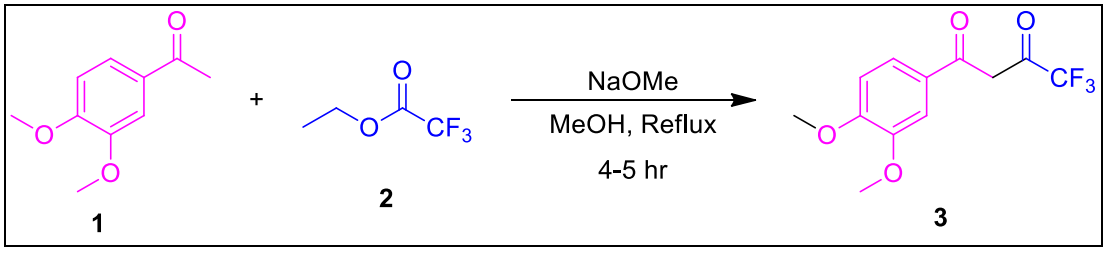
A mixture of 3,4 dimethoxy acetophenone(0.1mol), ethyl 2,2,2-trifluoroacetate (0.1 mol) and sodium Methoxide(0.2 mol) in MeOH, was stirred at Reflux temperature for 24 h. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under reduced pressure. The residue was poured in water and the precipitated crude product was purified by filtration followed by crystallization from EtOH.

General synthesis of pyrazolopyrimidine (MR-01 to 20).

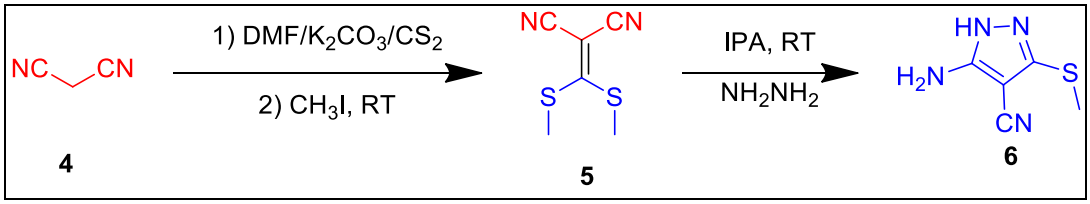
In 50ml RBF **Int-3**(2.5mmol), **Int-6**(2.5mmol) and substituted aldehyde were suspended in 20 ml water under stirring on magnetic stirrer. H_3BO_3(2.5 mmol) was added in to the reaction mixture and reaction mixture was refluxed for 10 to 12 h. The reaction was monitored by TLC. After the completion of reaction, water was decanted and solid residue was triturated with methanol to afford pure compound.

III. REACTION SCHEME

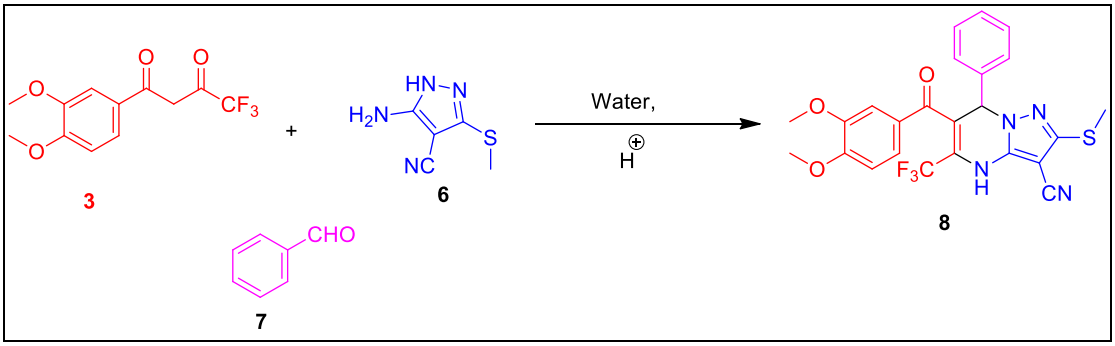
Scheme-1Synthesis of 1-(3,4-dimethoxyphenyl)-4,4,4-trifluorobutane-1,3-dione



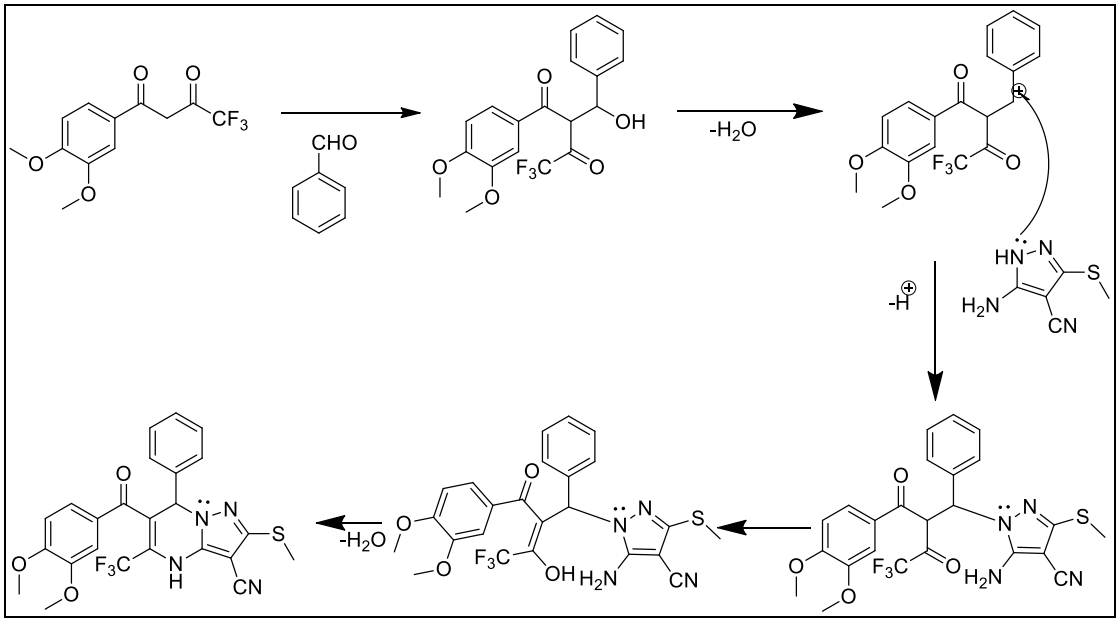
Scheme-2 Synthesis of 5-amino pyrazole derivative



Scheme-3 Synthesis of Pyarzolopyrimidine



Plausible mechanism for the formation of Pyrazolo[1,5-*a*]pyrimidine



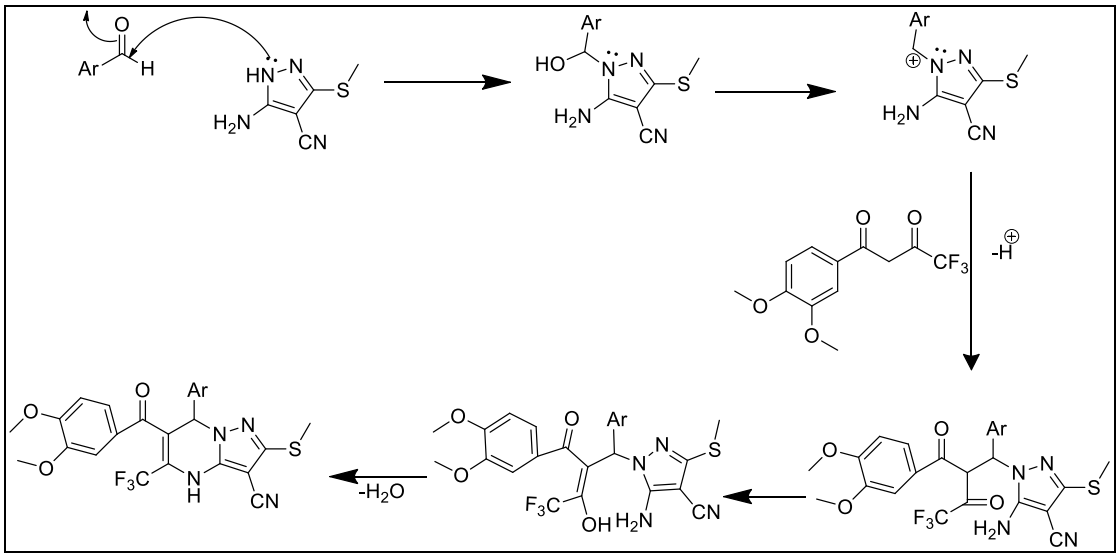


Figure-1. Proposed mechanism[39,40] for the formation of triazolopyrimidine

Table 1: Comparison of yield with different solvent and catalyst for MR-01

Solvent	catalyst	Yield %
Water	H_3BO_3	90
	Con HCl	76
	Acetic acid	56
Methanol	H_3BO_3	88
	Con HCl	70
	Acetic acid	65
DMF	H_3BO_3	84
	Con HCl	71
	Acetic acid	52
THF	H_3BO_3	77
	Con HCl	65
	Acetic acid	43
IPA	H_3BO_3	78
	Con HCl	68
	Acetic acid	32

Table-2: Physical Data of The synthesized Pyrazolopyrimidines derivatives

Entry	R	Time h	Yield %	Melting Range ^{0}C
MR-01	-H	12	90	200-202
MR-02	-4-Cl	12	88	214-216
MR-03	-4-Br	12	81	238-240
MR-04	-4(N,N-dimethylamino)	15	75	242-244
MR-05	-4-Me	13	92	220-222
MR-06	-4-F	12	87	228-230
MR-07	-2-Cl	12	84	212-214
MR-08	-2,4-di Cl	12	78	218-220
MR-09	-3,4-di OMe	14	93	204-206
MR-10	-3-OMe	14	91	218-220
MR-11	-2-OH	16	77	218-220
MR-12	-3-OH	16	83	216-218
MR-13	-2,5-di OMe	15	90	176-178
MR-14	-3-Cl	13	78	230-232
MR-15	-3-Br	12	79	230-232
MR-16	-4-OH	16	75	238-240
MR-17	-2-NO_2	18	85	200-202
MR-18	-3-NO_2	16	81	208-210
MR-19	Cinnamaldehyde	10	73	144-146
MR-20	Naphthaldehyde	11	78	178-180

Biological activity

***Microorganisms* used in the study**

The following microorganisms were used in this study: *Staphylococcus aureus* (ATCC29737), *Streptococcus pyogenes (*MTCC443), *Escherichia coli* (NCIM2931), *Pseudomonas aeruginosa (*MTCC 441), *Candida albicans (*MTCC 227), *Aspergillus Niger (*MTCC 282), all the strains were acquired from National chemical Laboratory (NCL), Pune, India. The micro organisms were maintained at 4ºC.

Antibiotics used in the study

All antibiotics were purchased from Hi-Media Laboratory Pvt. Ltd., (Mumbai, india) viz. Chloromphenicol, Ciprofloxacin, Nystatin, Greseofulvin were used for MIC.

Antimicrobial assay

The minimum inhibitory concentration (MIC) values of compounds (1-24) were determined by using the broth microdilution method with 96 micro test plate (Andrews, 2001). The samples were dissolved in DMSO at 1250 µg/mL, and were then diluted to achieve concentrations in the range 30 to 500 μg/ mL for each compound. Two fold dilutions of Chloromphenicol and Ciprafloxacin, Nystatin and Greseofulvin (1-32 µg/mL) were used for a positive control.

A 150 µL volume of Muller-Hinton broth was introduced into all the 96 wells and 20 µL of the varying concentration of the test compounds added in decreasing order along with 30 µL of the test organism suspension. A final volume of 200 µL was achieved in each well (150 µL Muller-Hinton suspension and 20 µL compounds/ antibiotic). Three control wells were maintained for each test batch. The positive control, sterility control and organism control. Plates were incubated at 37 ºC for 24 hours.

After incubation, 40 µL of 2-(4-Iodo phenyl)-3-(4-nitro phenyl)5-phenyltetrazolium chloride (I. N. T) solution (0.2 mg/ml) dissolved in sterile distilled water then added to each well. The plates were incubated for 30 mins and estimated visually for any change in color in to pink indicating reduction of the dye due to bacterial growth. The highest dilution (Lowest concentration) that retained clear corresponded to the MIC.

Table-3. Antimicrobial evaluation data

Compounds and standard drugs	**Antibacterial activity**				**Antifungal acitivity**	
	Minimum inhibitory concentration µg/ml				**Minimum inhibitory concentration µg/ml**	
	Gram +Ve Bacteria		**Gram -Ve Bacteria**			
	S. aureus	***S.pyogenes***	***E.coli***	***P.aeruginosa***	***C.albicans***	***A.niger***
Ciprofloxacin	7.8	7.8	15.625	15.625	-	-
Chloramphenicol	7.8	7.8	7.8	7.8	-	-
Nystatin	-	-	-	-	31.25	31.25
Greseofulvin					15.625	15.625
MR-1	31.25	31.25	**15.625**	**15.625**	**15.625**	**31.25**
MR-2	15.625	15.625	**15.625**	**7.81**	**31.25**	**31.25**
MR-3	31.25	62.5	31.25	31.25	**15.625**	62.5
MR-4	62.5	62.5	31.25	62.5	**15.625**	62.5
MR-5	15.625	31.25	15.625	62.5	**31.25**	62.5
MR-6	7.81	31.25	**7.81**	62.5	**31.25**	62.5
MR-7	15.625	15.625	**15.625**	62.5	**31.25**	62.5
MR-8	15.625	62.5	31.25	31.25	**31.25**	62.5
MR-9	**7.81**	31.25	**15.625**	**15.625**	**15.625**	**15.625**
MR-10	31.25	31.25	31.25	**15.625**	**15.625**	**15.625**
MR-11	31.25	15.625	31.25	31.25	**15.625**	62.5
MR-12	31.25	**7.8**	31.25	31.25	**15.625**	**31.25**
MR-13	31.25	31.25	31.25	**7.81**	**15.625**	**31.25**
MR-14	31.25	62.5	**15.625**	**7.81**	**31.25**	**31.25**

MR-15	15.625	15.625	**15.625**	**15.625**	**31.25**	**31.25**
MR-16	15.625	**7.81**	**15.625**	62.5	**31.25**	62.5
MR-17	**7.81**	15.625	**15.625**	62.5	62.5	**15.625**
MR-18	15.625	62.5	**15.625**	62.5	**31.25**	62.5
MR-19	31.25	62.5	62.5	31.25	**31.25**	62.5
MR-20	**7.81**	31.25	**7.8**	**15.625**	**15.625**	62.5

IV. CONCLUSION

In summary, we have described the water mediated synthesis of substituted triazolopyrimidine derivatives in moderate to good yield. The reaction of various substituted aldehydes with 1-(3,4-dimethoxyphenyl)-4,4,4-trifluorobutane-1,3-dione as active methylene compound and 5-amino-3-(methylthio)-1H-pyrazole-4-carbonitrile afforded the triazolo pyrimidine derivatives in the presence boric acid and water as a solvent. We have confirmed the structure on the basis of spectroscopic technique. All the synthesized compounds were evaluated for their antimicrobial activity. The investigation of antimicrobial and antifungal screening data revealed that all the tested compounds showed moderate to significant activity.

V. Spectral data of the synthesized compounds

6-(3,4-dimethoxybenzoyl)-2-(methylthio)-7-phenyl-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-01): IR (KBr): yellow solid; Melting range:200-202°C; R_f 0.40 (4:6 hexane-EtOAc); IR (KBr): 3335, 2931, 2851, 1626, 1591, 1577, 1537, 1488, 1433, 1312, 1285, 1250, 1172, 1123, 1059, 974, 831, 753 cm^{-1}; ^1H NMR(400 MHz, $CDCL_3$): *δppm* 2.39(s, 3H, -SCH3), 11.5(s, 1H, -NH), 3.5(s 1H,-OCH_3), 3.6(s,1H,-OCH_3). 7.3-7.5(d,1H,Ar-H),6.90-7.10(d,1H,Ar-H), 6.8-6.9(d,1H,Ar-H) 6.70-6.80(dd,2H,Ar-H), ^{13}C NMR (100 MHz, DMSO) *δppm*: 17.37, 24.49, 25.51, 33.88, 47.95, 60.69, 99.57, 123.72, 127.36, 127.84, 128.57, 128.90, 128.98, 130.16, 131.42, 132.30, 138.65, 139.44, 144.43, 162.32, 124.10, 132.72, 141.47; MS (*m/z*): 500 (M^+); Anal. Calcd for $C_{24}H_{19}F_3N_3O_3S$: C, 59.59; H, 5.00; N, 13.36; S, 6.12; Found: C, 59.52; H, 5.10; N, 13.29; S, 6.07.

7-(4-chlorophenyl)-6-(3,4-dimethoxybenzoyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-02): yellow solid; Melting range: 214-216°C; R_f 0.40 (4:6 hexane-EtOAc); IR (KBr): 3341, 2930, 2850, 1632, 1576, 1535, 1492, 1477, 1434, 1312, 1280, 1249, 1176, 1122, 1087, 1060, 975, 836, 774, 752 cm^{-1}; ^1H NMR(400 MHz, DMSO): *δppm;* 2.39(s, 3H, -SCH3), 11.5(s, 1H, -NH), 3.5(s 1H,-OCH_3), 3.6(s,1H,-OCH_3). 6.68(s, 1H, -CH), 7.24-7.44(m, 7H, Ar-H),; ^{13}C NMR (100 MHz, DMSO) *δppm*: 17.26, 25.49, 32.86, 48.00, 60.03, 99.70, 123.67, 127.70, 128.65, 128.90, 129.22, 130.21, 131.53, 132.09, 132.62, 134.91, 136.86, 137.17, 139.65, 141.83, 144.83, 162.23 ;MS (*m/z*): 534 (M^+); Anal. Calcd for $C_{24}H_{18}ClF_3N_3O_3S$: C, 55.92; H, 4.51; N, 12.54; S, 5.74; Found: C, 55.87; H, 4.47; N, 12.48; S, 5.67.

7-(4-bromophenyl)-5-(2-chlorophenyl)-*N*-cyclohexyl-2-(methylthio)-6-nitro-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide (MR-03): yellow solid; Melting range:238-240°C; R_f 0.40 (4:6 hexane-EtOAc); IR (KBr): 3338, 2930, 2850, 1632, 1575, 1535, 1477, 1434, 1312, 1280, 1250, 1176, 1124, 1065, 1009, 974, 835, 772, 752 cm^{-1}; MS (*m/z*): 578 (M^+); Anal. Calcd for $C_{24}H_{18}BrF_3N_3O_3S$: C, 51.79; H, 4.18; N, 11.62; S, 5.32 Found C, 51.73; H, 4.11; N, 11.72; S, 5.24.

6-(3,4-dimethoxybenzoyl)-7-(4-(dimethylamino)phenyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-04): yellow solid; Melting range:242-244°C; R_f 0.36 (4:6 hexane-EtOAc); IR (KBr): 3343, 2929, 2848, 1630, 1626, 1574, 1529, 1480, 1433, 1311, 1279, 1250, 1219, 1169, 1129, 1059, 975, 945, 837, 775 cm^{-1}; ^1H NMR(400 MHz, DMSO): *δppm* 2.85(s, 6H, -$N(CH_3)_2$), 2.39(s, 3H, -SCH3), 11.5(s, 1H, -NH), 3.5(s 1H,-OCH_3), 3.6(s,1H,-OCH_3). 6.68(s, 1H, -CH), 7.24-7.44(m, 7H, Ar-H),; ^{13}C NMR (100 MHz, DMSO) *δppm*: 17.52, 24.55, 25.52, 32.91, 40.37, 47.89, 60.38, 99.38, 112.02, 124.22, 126.00, 127.28, 128.20, 128.74, 129.95, 131.28, 132.09, 132.65, 139.40, 140.85, 144.00, 150.67, 162.46 ;MS (*m/z*): 543 (M^+);

Anal. Calcd for $C_{26}H_{24}F_3N_5O_3S$: C, 59.30; H, 5.51; N, 14.82; S, 5.65 Found: C, 59.23; H, 5.44; N, 14.77; S, 5.59.

6-(3,4-dimethoxybenzoyl)-2-(methylthio)-7-(p-tolyl)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-05): yellow solid; Melting range: 220-222°C; R_f 0.42 (4:6 hexane-EtOAc); IR (KBr): 758, 1058, 1124, 1174, 1223, 1250, 1282, 1312, 1434, 1476, 1536, 1576, 1630, 2931, 3343 cm^{-1}; ^1H NMR(400 MHz, DMSO): *δppm* 2.39(s, 3H, -SCH3), 11.5(s, 1H, -NH), 3.5(s 1H,-OCH_3), 3.6(s,1H,-OCH_3). 6.68(s, 1H, -CH),7.06-7.08(d, 2H, Ar-H, J=7.6Hz), 7.22-7.42(m, 5H, Ar-H),; ^{13}C NMR (100 MHz, DMSO) *δppm*:17.29, 21.25, 24.49, 25.52, 32.88, 47.94, 60.47, 99.51, 123.89, 127.24, 127.61, 128.59, 129.39, 129.97, 132.35, 132.05, 132.47, 135.44, 138.80, 139.44, 141.29, 144.33, 162.36 ;MS (*m/z*): 514 (M^+); Anal. Calcd for $C_{25}H_{21}F_3N_4O_3S$: C, 60.27; H, 5.25; N, 13.02; S, 5.96; Found: C, 60.21; H, 5.19; N, 13.12; S, 5.91.

6-(3,4-dimethoxybenzoyl)-7-(4-fluorophenyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-06): yellow solid; Melting range: 228-230°C; R_f 0.40 (4:6 hexane-EtOAc); IR (KBr): 3337, 2929, 2851, 1623, 1576, 1534, 1491, 1430, 1313, 1282, 1229, 1174, 1124, 1063, 975, 894, 841, 768 cm^{-1}; MS (*m/z*): 518 (M^+); Anal. Calcd for $C_{24}H_{18}F_3N_4O_3S$: C, 57.61; H, 4.65; N, 12.92; S, 5.92; Found: C, 57.52; H, 4.61; N, 12.86; S, 5.84.

7-(2-chlorophenyl)-6-(3,4-dimethoxybenzoyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-07): yellow solid; Melting range: 212-214°C; R_f 0.40 (4:6 hexane-EtOAc); IR (KBr): 3337, 2929, 2852, 1627, 1578, 1534, 1488, 1433, 1289, 1274, 1225, 1173, 1124, 1054, 972, 890, 833,754 cm^{-1}; MS (*m/z*): 534 (M^+); Anal. Calcd for $C_{24}H_{18}ClF_3N_4O_3S$: C, 55.92; H, 4.51; N, 12.54; S, 5.74; Found: C, 55.83; H, 4.43; N, 12.44; S, 5.68.

7-(2,4-dichlorophenyl)-6-(3,4-dimethoxybenzoyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-08): yellow solid; Melting range:218-220°C; R_f 0.38 (4:6 hexane-EtOAc); IR (KBr): 3333, 2929, 2850, 1626, 1578, 1535, 1489, 1438, 1397, 1315, 1288, 1253, 1226, 1175, 1104, 1057, 968, 860, 821 cm^{-1}; ^1H NMR(400 MHz, DMSO): *δppm* 2.40(s, 3H, -SCH_3),11.5(s, 1H, -NH), 3.5(s 1H,-OCH_3), 3.6(s,1H,-OCH_3). 6.68(s, 1H, -CH), 7.13-7.45(m, 6H, Ar-H),; ^{13}C NMR (100 MHz, DMSO) *δppm*:17.13, 25.50, 32.85, 48.02, 58.91, 99.29, 122.56, 127.42, 127.65, 128.56, 129.95, 130.04, 130.44, 131.55, 132.14, 132.60, 133.61, 134.51, 135.30, 139.53, 142.28, 144.86, 162.24; MS (*m/z*): 568 (M^+); Anal. Calcd for $C_{24}H_{17}Cl_2N_5O_3S$: C, 52.67; H, 4.08; N, 11.81; S, 5.41; Found: C, 52.62; H, 4.18; N, 11.73; S, 5.35.

6-(3,4-dimethoxybenzoyl)-7-(3,4-dimethoxyphenyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-9): yellow solid; Melting range:204-206°C; R_f 0.38 (4:6 hexane-EtOAc); IR (KBr): 3374, 2959, 2846, 1654, 1579, 1581, 1538, 1421, 1341, 1252, 1262, 1275, 1188, 1007, 915, 836 cm^{-1}; ^1H NMR(400 MHz, DMSO): *δppm* 2.41(s, 3H, -SCH_3), 3.5(s 1H,-OCH_3), 3.6(s,1H,-OCH_3). 3.76(s, 3H, -OCH_3), 3.78(s, 3H, -OCH3), 6.66(s, 1H, -CH), 7.04-7.43(m, 6H, Ar-H), 8.89(s, 1H. -NH); ^{13}C NMR (100 MHz, DMSO) *δppm*:17.34, 24.48, 25.49, 32.87, 47.93, 56.01, 60.48, 99.49, 110.39, 110.95, 111.23, 120.63, 123.67, 126.93, 127.33, 128.60, 129.92, 130.78, 131.40, 132.04, 132.43, 139.22, 141.25, 144.33, 149.05, 132.31; MS (*m/z*): 583 (M^+); Anal. Calcd for $C_{26}H2_3F_3N_4O_5S$: C, 57.58; H, 5.18; N, 11.99; S, 5.49; Found: C, 57.52; H, 5.11; N, 11.88; S, 5.42.

6-(3,4-dimethoxybenzoyl)-7-(3-methoxyphenyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-10): yellow solid; Melting range:218-220°C; R_f 0.40 (4:6 hexane-EtOAc); IR (KBr): 3383, 3336, 2929, 2850, 1627, 1586, 1580, 1533, 1587, 1435, 1311, 1278, 1249, 1224, 1171, 1143, 1045, 975, 886, 773 cm^{-1}; MS (*m/z*): 530 (M^+); Anal. Calcd for $C_{25}H_{21}ClF_3N_4O_4S$: C, 58.53; H, 5.09; N, 12.64; S, 5.79; Found: C, 58.47; H, 5.19; N, 12.58; S, 5.71.

6-(3,4-dimethoxybenzoyl)-7-(2-hydroxyphenyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-11): yellow solid; Melting range:218-220°C; R_f 0.30 (4:6 hexane-EtOAc); IR (KBr): 3369, 3289, 3185, 2941, 2851, 1629, 1580, 1553, 1489, 1432, 1369, 1319, 1284, 1250, 1223, 1173, 1123, 1059, 1033, 975, 874, 819, 754 cm^{-1}; ^1H NMR(400 MHz, DMSO): *δppm* 1.16-1.81(m, 10H, cyclohexane), 2.50(s, 3H, -SCH_3), 3.75(m, 1H, -CH, cyclohexane), 6.80-7.46(m, 10H, Ar-H, -CH, -NH), 8.75(s, 1H, -OH), 8.96(s, 1H. -NH); ^{13}C NMR (100 MHz, DMSO) *δppm*: 13.59, 24.50, 25.47, 32.80, 48.09, 55.33, 99.35, 118.79, 120.92, 121.86, 124.58, 127.10, 127.43, 128.69, 130.16, 130.72, 131.43, 132.00, 132.38, 139.40, 142.73, 144.65, 154.89, 161.96 ; MS (*m/z*): 539 (M^+); Anal. Calcd for

$C_{26}H_{26}ClN_5O_4S$: C, 57.83; H, 4.85; N, 12.97; S, 5.94; Found: C, 57.77; H, 4.72; N, 12.77; S, 5.87.

6-(3,4-dimethoxybenzoyl)-7-(4-hydroxyphenyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-12): yellow solid; Melting range: 216-218°C; R_f 0.36 (4:6 hexane-EtOAc); IR (KBr): 3323, 2931, 2851, 1620, 1579, 1538, 1469, 1453, 1312, 1279, 1249, 1226, 1174, 1062, 977, 795, 758 cm^{-1}; MS (*m/z*): 516 (M^+); Anal. Calcd for $C_{24}H_{19}F_3N_4O_4S$: C, 57.83; H, 4.85; N, 12.97; S, 5.94; Found: C, 57.75; H, 4.77; N, 12.87; S, 5.82.

6-(3,4-dimethoxybenzoyl)-7-(2,5-dimethoxyphenyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-13): yellow solid; Melting range:176-178°C; R_f 0.38 (4:6 hexane-EtOAc); IR (KBr): 3295, 2932, 2851, 1632, 1576, 1535, 1502, 1477, 1311, 1277, 1223, 1177, 1042, 797, 747 cm^{-1}; MS (*m/z*): 560 (M^+); Anal. Calcd for $C_{26}H_{23}F_3N_4O_5S$: C, 57.58; H, 5.18; N, 11.99; S, 5.49; Found: C, 57.49; H, 5.11; N, 11.81; S, 5.37.

7-(3-chlorophenyl)-6-(3,4-dimethoxybenzoyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-14): yellow solid; Melting range: 230-232°C; R_f 0.40 (4:6 hexane-EtOAc); IR (KBr): 3337, 2929, 2852, 1624, 1589, 1535, 1486, 1434, 1364, 1314, 1285, 1250, 1172, 1124, 1062, 975, 796, 771, 748 cm^{-1}; MS (*m/z*): 534 (M^+); Anal. Calcd for $C_{24}H_{18}ClF_3N_4O_3S$: C, 55.92; H, 4.51; N, 12.54; S, 5.74; Found: C, 55.84; H, 4.46; N, 12.44; S, 5.68.

7-(3-bromophenyl)-6-(3,4-dimethoxybenzoyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-15): yellow solid; Melting range:230-232°C; R_f 0.40 (4:6 hexane-EtOAc); IR (KBr): 3334, 2930, 2852, 1624, 1582, 1578, 1535, 1478, 1434, 1312, 1282, 1250, 1172, 1124, 1063, 973, 813, 769 cm^{-1}; MS (*m/z*): 579 (M^+); Anal. Calcd for $C_{24}H_{18}BrF_3N_4O_3S$: C, 51.79; H, 4.18; N, 11.62; S, 5.32; Found: C, 51.71; H, 4.13; N, 11.54; S, 5.23.

6-(3,4-dimethoxybenzoyl)-7-(4-hydroxyphenyl)-2-(methylthio)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-16): yellow solid; Melting range:238-240°C; R_f 0.32 (4:6 hexane-EtOAc); IR (KBr): 3324, 3160, 2930, 2852, 1621, 1573, 1543, 1480, 1434, 1367, 1314, 1280, 1248, 1225, 1174, 1064, 977, 828, 771, 749 cm^{-1}; MS (*m/z*): 516 (M^+); Anal. Calcd for $C_{24}H_{19}F3N_4O_4S$: C, 57.83; H, 4.85; N, 12.97; S, 5.94; Found: C, 57.76; H, 4.77; N, 12.89; S, 5.88.

6-(3,4-dimethoxybenzoyl)-2-(methylthio)-7-(2-nitrophenyl)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-17): yellow solid; Melting range:200-202°C; R_f 0.34 (4:6 hexane-EtOAc); IR (KBr): 3332, 2927, 2852, 1628, 1575, 1534, 1484, 1433, 1355, 1313, 1286, 1253, 1174, 1059, 978, 858, 823, 783, 750 cm^{-1}; MS (*m/z*): 545 (M^+); Anal. Calcd for $C_{24}H_{18}F_3N_5O_5S$: C, 54.88; H, 4.43; N, 14.77; S, 5.64; Found: C, 54.81; H, 4.37; N, 14.71; S, 5.58.

6-(3,4-dimethoxybenzoyl)-2-(methylthio)-7-(3-nitrophenyl)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-18): yellow solid; Melting range: 208-210°C; R_f 0.34 (4:6 hexane-EtOAc); IR (KBr): 3374, 3339, 2928, 2851, 1627, 1571, 1532, 1484, 1439, 1344, 1311, 1285, 1228, 1174, 1062, 978, 897, 812, 746, 723 cm^{-1}; ^1H NMR(400 MHz, DMSO): *δppm* 2.41(s, 3H, $-SCH_3$), 6.80(s, 1H, -CH), 7.30-8.13(m, 7H, Ar-H), 8.40(s, 1H, Ar-H), 9.06(s, 1H. -NH); ^{13}C NMR (100 MHz, DMSO) *δppm*: 17.04, 24.50, 25.48, 32.82, 48.05, 59.93, 99.89, 122.35, 122.37, 124.00, 127.53, 128.47, 129.77, 130.07, 131.68, 131.93, 132.52, 133.79, 139.17, 140.38, 142.47, 145.23, 148.51, 162.05 ;MS (*m/z*): 545 (M^+); Anal. Calcd for $C_{24}H_{18}F_3N_5O_5S$: C, 54.88; H, 4.43; N, 14.77; S, 5.64; Found: C, 54.79; H, 4.34; N, 14.70; S, 5.59.

(E)-6-(3,4-dimethoxybenzoyl)-2-(methylthio)-7-styryl-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-19): yellow solid; Melting range:144-146°C; R_f 0.40 (4:6 hexane-EtOAc); IR (KBr): 3345, 3032, 2956, 2824, 1610, 1565, 1588, 1546, 1472, 1428, 1311, 1290, 1120, 1066, 967, 852, 778 cm^{-1}; MS (*m/z*): 526 (M^+); Anal. Calcd for $C_{26}H_{21}F_3N_4O_3S$: C, 61.14; H, 5.13; N, 12.73; S, 5.83; Found: C, 61.09; H, 5.07; N, 12.67; S, 5.71.

6-(3,4-dimethoxybenzoyl)-2-(methylthio)-7-(naphthalen-1-yl)-5-(trifluoromethyl)-4,7-dihydropyrazolo[1,5-a]pyrimidine-3-carbonitrile (MR-20): yellow solid; Melting range:178-180°C; R_f 0.48 (4:6 hexane-EtOAc); IR (KBr): 3350, 2931, 2852, 1624, 1575, 1543, 1482, 1439, 1309, 1284, 1227, 1169, 1061, 969, 784, 752 cm^{-1}; MS (*m/z*): 550 (M^+); Anal. Calcd for $C_{28}H_{21}F_3N_4O_3S$: C, 62.76; H, 4.92; N, 12.20; S, 5.59; Found: C, 62.63; H, 4.85; N, 12.13; S, 5.52.

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