Chapter 5

Design and Synthesis of Some Novel pyrazolo[3,4-*d*]thiazolo [3,2-*a*]pyrimidine Derivatives

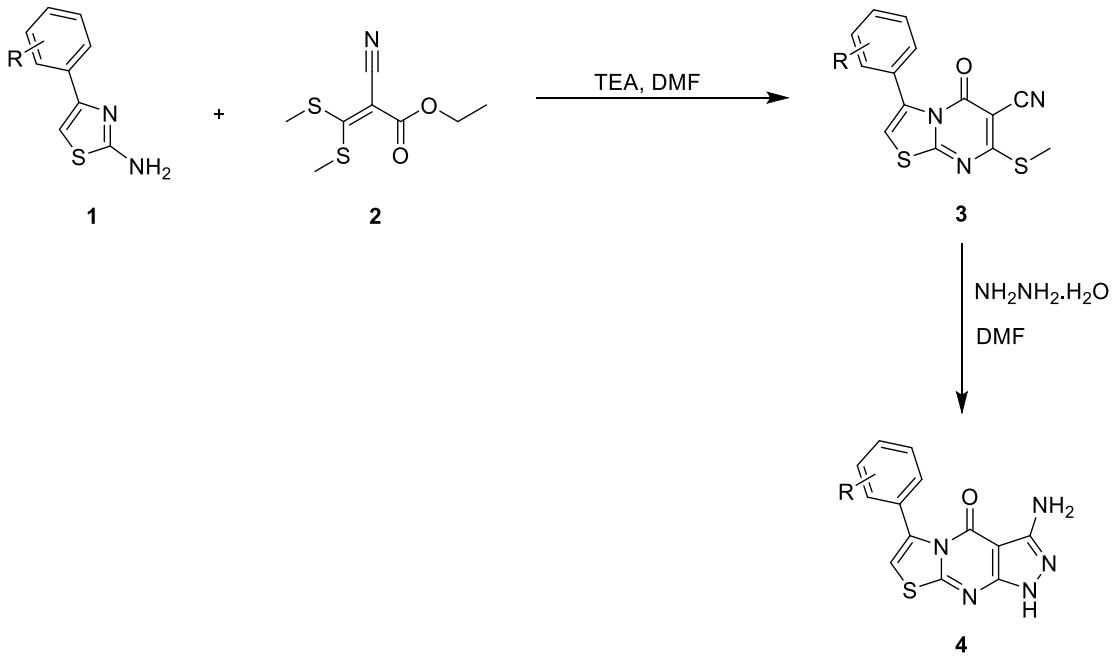
5.1 Introduction

Pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine is a fused heterocyclic compound that has gained considerable attention in medicinal chemistry due to its remarkable biological activities and potential as a lead molecule for drug discovery.[185,186] This heterocycle comprises a pyrazole, a thiazole and a pyrimidine ring, all of which possess diverse biological properties, such as anticancer, antimicrobial, anti-inflammatory and antidiabetic activities.[187-189] Pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine has been synthesized by various methods, including one-pot, multicomponent and click reactions,[190-192] making it an attractive scaffold for the design and development of new therapeutics. Thiazole derivatives are known to exhibit various biological activities, such as anticancer, antifungal, antiviral and antibacterial properties, which make them attractive targets for drug discovery.[193,194] Similarly, pyrazole derivatives have been extensively studied for their broad range of biological activities, including anti-inflammatory, antidiabetic, antiviral and anticancer properties.[195-197] The combination of these two rings, pyrazole and thiazole, with a pyrimidine ring in pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine provides an opportunity to discover new drugs with enhanced biological activities. The biological activities of pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine have been extensively investigated. For example, some studies have reported that this heterocycle exhibits significant anticancer activity against various cancer cell lines, including breast, lung and colon cancer.[198-200] Other studies have reported its antibacterial activities against Gram-positive and Gram-negative bacteria.[201,202] In addition, pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine has been reported to possess antifungal, anti-inflammatory and antidiabetic activities.[203-205] These promising biological activities have attracted considerable attention from medicinal chemists and various pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine derivatives have been designed and synthesized for further optimization.[206,207] In conclusion, pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine is an important heterocyclic scaffold that has shown remarkable biological activities and potential for drug discovery. With ongoing research, pyrazolo[3,4-*d*]thiazolo[3,2-

a]pyrimidine and its derivatives have the potential to become lead compounds for the development of novel drugs for the treatment of various diseases.

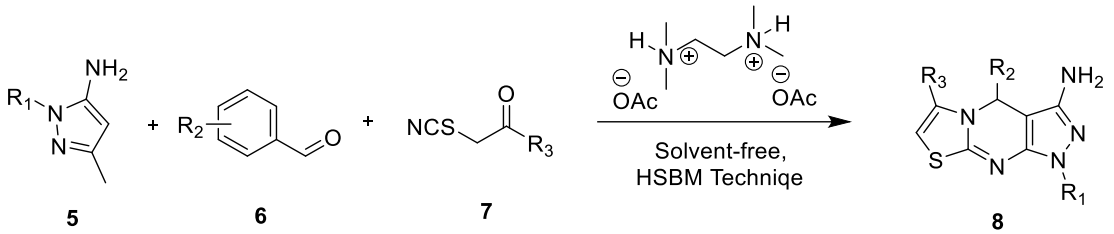
5.1.1 Synthetic approach for substituted pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine scaffold and its biological importance

C. Khobragade and co-workers[208] reported the synthesis of pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine via reacting substituted thiazole **1** with cyanoketene dithioacetal **2** to form thiazolo pyrimidine **3** molecule, which was reacted further with hydrazine hydrate to form molecule **4** in good yield. Furthermore, the synthesized molecules were subjected to molecular docking and xanthine oxidase inhibitory activity, in which it was reported that some of the synthesized molecules could be suitable to be the probable key to form potent xanthine oxidase inhibitors (**Scheme 5.1**).



Scheme 5.1

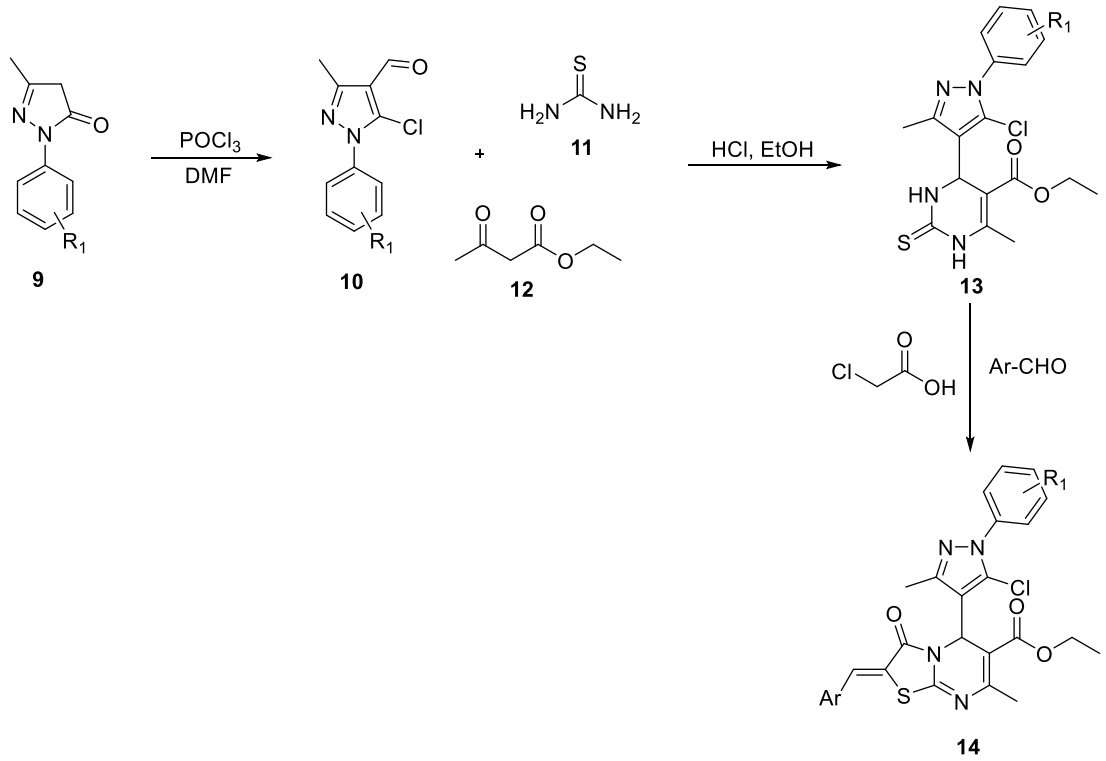
M. Ziyaadini *et al*[209] reported a multicomponent synthesis catalysed by ionic liquid of pyrazolo[3,4-d]thiazolo[3,2-a]pyrimidine under neat conditions. The reaction between amino pyrazole **5**, benzaldehyde **6** derivative and isocyanato ethane **7** derivative in presence of *N*',*N*',*N*",*N*"-tetramethylethane-1,2-diaminium acetate formed pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine **8** in 88% yield (**Scheme 5.2**).



5 6 7 8

Scheme 5.2

Wang[210] reported on the synthesis of pyrazole-thiazolopyrimidine molecules and evaluated them for antiproliferative activity. The phenyl pyrazole molecule **9** was converted to an aldehyde **10** molecule through a reaction with DMF and phosphorus oxychloride. Then molecule **10**, thiourea **11** and ethyl acetoacetate **12** was refluxed in ethanol containing catalytical amount of hydrochloric acid to form pyrimidine molecule **13** which was reacted with chloroacetic acid and aldehyde to form thiazolopyrimidine **14** molecule. Some of the synthesized molecules showed high potency and induced apoptosis in MGC-803 cells (**Scheme 5.3**).

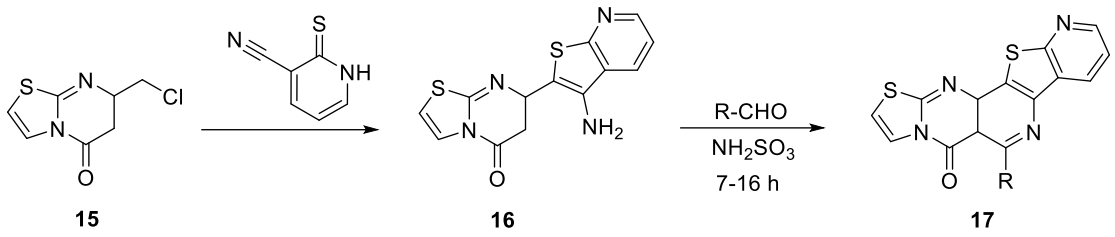


9 10 11 12 13 14

Scheme 5.3

H. Alzahrani[211] reported the synthesis of a fused thiazolopyrimidine molecule via reaction of chloro thiazolopyrimidine **15** molecule with dihydropyridine carbonitrile to form thienopyridine molecule **16** to which various substituted benzaldehydes were

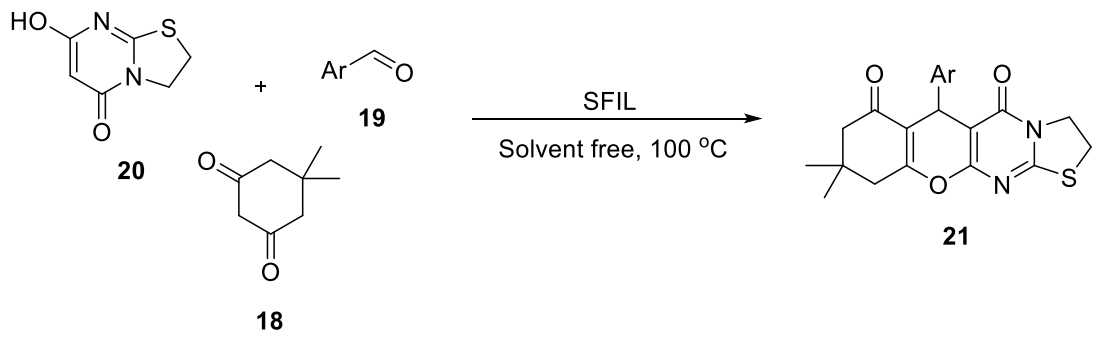
attached to form molecule **17** in moderate yield. The synthesized molecules showed good to excellent anticancer activity against MCF-7 cell line (**Scheme 5.4**).



15 **16** **17**

Scheme 5.4

M. Esmaeilinezhad[212] reported the synthesis of chroman fused thiazolopyrimidine molecule under solvent-free conditions using ionic liquid. The reaction between dimedone **18**, substituted benzaldehyde **19** and thiazolopyrimidine **20** heated with an acid catalyst in neat condition gave the fused chroman thiazolopyrimidine **21** molecule in good yields. The synthesized molecules were expected to be used of pharmacological interest (**Scheme 5.5**).

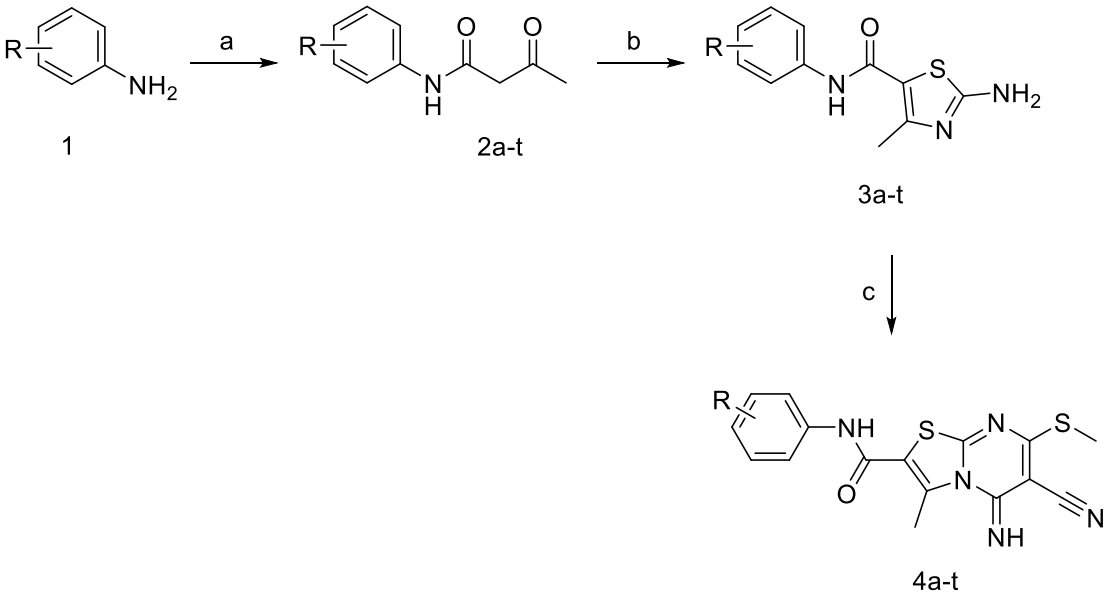


20 **19** **18** **21**

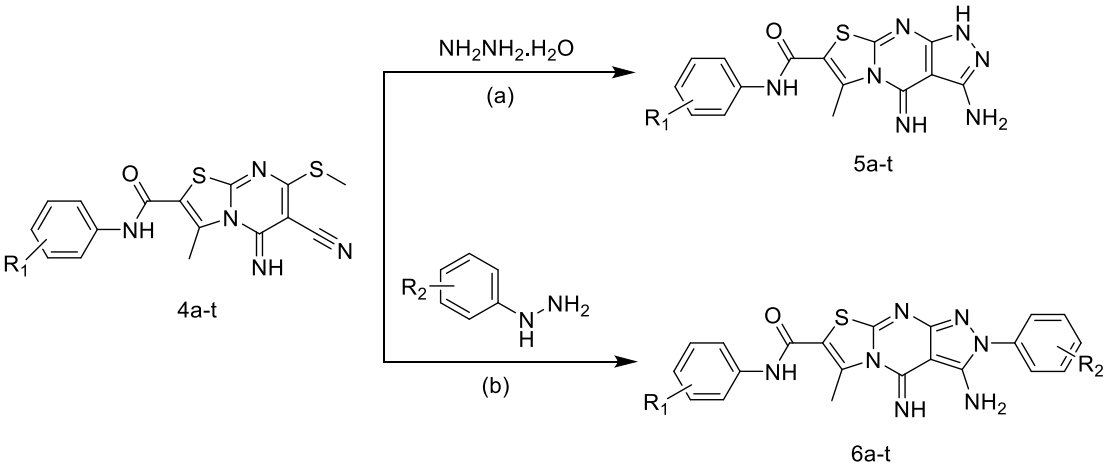
Scheme 5.5

5.2 Results and Discussion

To find a novel pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine molecule and the synthesis of its derivatives, here, we report twenty newly synthesized molecules with pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine in their main structure. The compounds **4a-t** were elucidated through inspecting their spectroscopic data like ^{1}H-NMR, NMR, FTIR and mass spectroscopy. In the first step, 3-oxo-*N*-arylbutanamide and *N*-bromosuccinimide reacted at ambient temperature to get 2-bromo-3-oxo-*N*-arylbutanamide. Then thiourea was added for ring closure to obtain 2-amino-*N*-aryl-4-methylthiazole-5-carboxamide **3a-t.**



Scheme 1: Reagents and Conditions: (a) Ethyl acetoacetate, KOH, Reflux, 24 h (b) NBS, Thiourea, MeOH, Reflux, 4 h (c) 2-bis(methylthio)methylene malononitrile, K_2CO_3, DMF, Reflux, 30 min.

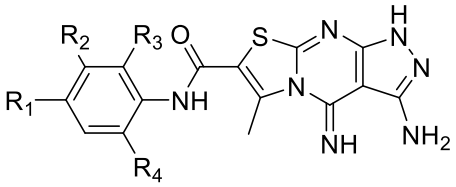


Scheme 2: Reagents and Conditions: (a) Reflux, 3 h (b) DMF, Reflux, 12 h.

Then, compound **3a-t** was reacted with 2-bis(methylthio)methylene malononitrile to obtain thiazolo[3,2-*a*]pyrimidine **4a-t** derivatives as appear in **Scheme 1**. The reaction with hydrazine hydrate in excess amount with molecule **4a-t** in neat condition afforded molecule **5a-t** and the reaction with phenyl hydrazine in DMF formed molecule **6a-t** in good yield. The ^{1}H-NMR graph of compounds **5a-t** presented that 4-methyl thiazole protons was detected at s 2.26 ppm (CH_3) as a singlet. The pyrazole amine peak was detected at s 6.41 ppm (NH_2) as a singlet The aromatic region was seen between 7.10-7.53 ppm. Imine hydrogen of pyrimidine ring was seen at s 7.98 ppm (=NH) as a singlet. Acetamide protons was observed at s 9.62 ppm (NH) as a singlet. The pyrazole secondary amine hydrogen was observed in downfield at s 11.58 (NH) as a singlet. The ^{1}H-NMR graph of compounds 6**a-j** presented that 4-methyl thiazole protons was detected at s 2.26 ppm (CH_3) as a singlet. The pyrazole amine peak was detected at s 6.78 ppm (NH_2) as a singlet The aromatic region was seen between 7.11-7.68 ppm. Acetamide protons was observed at s 9.95 ppm (NH) as a singlet. Pyrimidine imine hydrogen of ring was seen at s 12.34 ppm (=NH) as a singlet. Due to high nitrogen content, lipophobicity and poor solubility in a variety of solvents, the purification of the product was difficult. The one-pot reaction of **3a-t** with 2-bis(methylthio)methylene malononitrile followed by the addition of hydrazine hydrate or phenyl hydrazine required column chromatography purification.

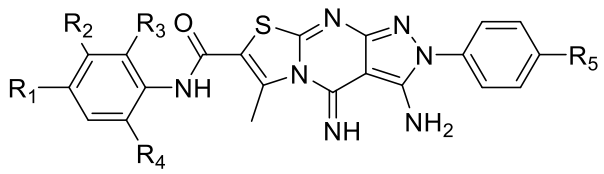
5.2.1 Physicochemical Properties

Table 1. Physicochemical Characteristics of the pyrazolo[3,4-*d*]thiazolo[3,2-*a*] pyrimidine 5a-t



Entry	R^1	R^2	R^3	R^4	Molecular weight	Molecular formula	Yield (%)	Melting point (°C)
CNTHNHZ-1	CH_3	H	H	H	353.40	$C_{16}H_{15}N_7OS$	90	254-256
CNTHNHZ-2	OCH_3	H	H	H	369.40	$C_{16}H_{15}N_7O_2S$	92	248-250
CNTHNHZ-3	H	H	CH_3	CH_3	367.43	$C_{17}H_{17}N_7OS$	91	236-238
CNTHNHZ-4	H	H	OCH_3	H	369.40	$C_{16}H_{15}N_7O_2S$	90	230-232
CNTHNHZ-5	H	CH_3	H	H	353.40	$C_{16}H_{15}N_7OS$	86	238-240
CNTHNHZ-6	CH_3	H	CH_3	H	367.43	$C_{17}H_{17}N_7OS$	89	229-231
CNTHNHZ-7	H	OCH_3	H	H	369.40	$C_{16}H_{15}N_7O_2S$	85	235-237
CNTHNHZ-8	CH_3	CH_3	H	H	367.43	$C_{17}H_{17}N_7OS$	88	226-228
CNTHNHZ-9	OCH_3	OCH_3	H	H	399.43	$C_{17}H_{17}N_7O_3S$	89	241-243
CNTHNHZ-10	Cl	H	H	H	373.82	$C_{15}H_{12}ClN_7OS$	80	239-241
CNTHNHZ-11	Br	H	H	H	418.27	$C_{15}H_{12}BrN_7OS$	86	245-247
CNTHNHZ-12	F	H	H	H	357.37	$C_{15}H_{12}FN_7OS$	71	226-228
CNTHNHZ-13	H	H	Cl	Cl	408.26	$C_{15}H_{11}Cl_2N_7OS$	75	230-232
CNTHNHZ-14	F	Cl	H	H	391.81	$C_{15}H_{11}ClFN_7OS$	68	235-237
CNTHNHZ-15	H	H	Br	Br	499.19	$C_{15}H_{13}Br_2N_7OS$	85	239-241
CNTHNHZ-16	H	F	H	H	359.38	$C_{15}H_{14}FN_7OS$	63	230-232
CNTHNHZ-17	H	H	F	H	357.37	$C_{15}H_{12}FN_7OS$	60	223-225
CNTHNHZ-18	H	Cl	H	H	373.82	$C_{15}H_{12}ClN_7OS$	65	226-228
CNTHNHZ-19	H	H	H	H	339.38	$C_{15}H_{13}N_7OS$	89	240-242
CNTHNHZ-20	H	Br	H	H	418.27	$C_{15}H_{12}BrN_7OS$	78	231-233

Table 2. Physicochemical Characteristics of the pyrazolo[3,4-*d*]thiazolo[3,2-*a*] pyrimidine 6a-t



Entry	R^1	R^2	R^3	R^4	R^5	Molecular weight	Molecular formula	Yield (%)	MP(°C)
CNTHNHPZ-1	CH_3	H	H	H	H	429.50	$C_{22}H_{19}N_7OS$	94	250-252
CNTHNHPZ-2	OCH_3	H	H	H	H	445.50	$C_{22}H_{19}N_7O_2S$	92	262-264
CNTHNHPZ-3	H	H	CH_3	CH_3	H	443.53	$C_{23}H_{21}N_7OS$	87	228-230
CNTHNHPZ-4	H	H	OCH_3	H	H	445.50	$C_{22}H_{19}N_7O_2S$	81	236-238
CNTHNHPZ-5	H	CH_3	H	H	H	429.50	$C_{22}H_{19}N_7OS$	85	243-245
CNTHNHPZ-6	CH_3	H	CH_3	H	H	443.53	$C_{23}H_{21}N_7OS$	82	258-260
CNTHNHPZ-7	H	OCH_3	H	H	H	445.50	$C_{22}H_{19}N_7O_2S$	80	252-254
CNTHNHPZ-8	CH_3	CH_3	H	H	H	443.53	$C_{23}H_{21}N_7OS$	82	255-257
CNTHNHPZ-9	OCH_3	OCH_3	H	H	H	475.53	$C_{23}H_{21}N_7O_3S$	87	260-262
CNTHNHPZ-10	Cl	H	H	H	H	449.92	$C_{21}H_{16}ClN_7OS$	79	243-245
CNTHNHPZ-11	Br	H	H	H	H	494.37	$C_{21}H_{16}BrN_7OS$	81	259-261
CNTHNHPZ-12	F	H	H	H	H	433.47	$C_{21}H_{16}FN_7OS$	61	224-226
CNTHNHPZ-13	H	H	Cl	Cl	H	484.36	$C_{21}H_{15}Cl_2N_7OS$	75	228-230
CNTHNHPZ-14	F	Cl	H	H	H	467.91	$C_{21}H_{15}ClFN_7OS$	73	256-258
CNTHNHPZ-15	H	H	Br	Br	H	573.27	$C_{21}H_{15}Br_2N_7OS$	81	262-264
CNTHNHPZ-16	H	F	H	H	H	433.47	$C_{21}H_{16}FN_7OS$	65	229-231
CNTHNHPZ-17	H	H	F	H	H	433.47	$C_{21}H_{16}FN_7OS$	62	231-233
CNTHNHPZ-18	H	Cl	H	H	H	449.92	$C_{21}H_{16}ClN_7OS$	76	255-257
CNTHNHPZ-19	H	H	H	H	H	415.48	$C_{21}H_{17}N_7OS$	85	251-253
CNTHNHPZ-20	H	Br	H	H	H	494.37	$C_{21}H_{16}BrN_7OS$	72	251-253

5.3 Conclusion

In conclusion, a series of novel pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine has been synthesized with excellent yields. The important features of this reaction technique are novel, easy, solvent free and less time consuming. The chemical structures of the molecules were characterized by their spectral data such as FTIR, ^{1}H NMR and MS. This procedure offers a good scope for the synthesis of a wide variety of pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine molecules with good purity and simple isolation of products.

5.4 Experimental Section

Melting points were determined on an electrothermal device using open capillaries and are uncorrected. Thin-layer chromatography was performed on precoated silica-gel 60 F254 (Merck), compounds were visualized with UV light at 254 nm and 365 nm or with iodine vapor. The IR spectra were recorded on a Shimadzu FT-IR spectrometer using the ATR technique. NMR spectra were recorded on a Bruker AVANCE III (400 MHz) spectrometer in DMSO-d_6 or $CDCl_3$. Chemical shifts are expressed in δ ppm downfield from Tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded using a direct inlet probe on Shimadzu GCMS QP2010 Ultra mass spectrometer. All reactions were carried out under an ambient atmosphere. All reagents were purchased from Loba, Molychem, SRL and CDH and used without further purification.

- **General process for the synthesis of acetoacetanilide (2a-t)**

Substituted amine (10 mmol) and ethyl acetoacetate containing catalytic amount of Potassium or sodium hydroxide lie (10%) in toluene was refluxed for approximately 24 h. On completion of the reaction, the mass was evaporated under vacuum and the residue was crystallized from methanol or ethanol to get pure acetoacetanilide.

- **General process for the synthesis of thiazoles (3a-t)**

To a stirred solution of compound acetoacetanilide (10 mmol) **(2a-t)** in MeOH, *N*-bromosuccinimide (15 mmol) was added and stirred at room temperature for 30 min. To this reaction mass thiourea (20 mmol) was slowly added and refluxed for 4-5 h. The reaction mixture was cooled to room temperature, poured into ice-cold water and neutralized with dil. HCl. The separated solid product was filtered, washed with water and dried at room temperature to get an analytically pure compound (**3a-t**), as a light brown solid. Yield: 89%.

- **General process for the synthesis of thiazolo[3,2-*a*]pyrimidine (4a-t)**

A mixture of **3a-t** (10 mmol) and 2-bis(methylthio)methylene malononitrile (10 mmol) in 10 mL of DMF and anhydrous potassium carbonate (10 mmol) was heated to reflux for 30 min. After the completion of the reaction, the reaction mixture was cooled to room temperature, poured into ice-cold water and neutralized with dil. HCl. The separated solid was filtered, washed with water and purified by recrystallization from DMF to afford crystals **(4a-t)**.

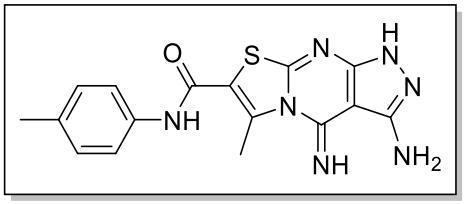
- **General process for the synthesis of pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine (5a-t)**

A mixture of **4a-t** (10 mmol) and hydrazine hydrate (100 mmol) was heated to reflux for 3 hr. After the completion of the reaction, the reaction mixture was cooled to room temperature, poured into ice-cold water and neutralized with dil. HCl. The separated solid was filtered, washed with methanol to get an analytically pure compound **(5a-t)**.

- **General process for the synthesis of pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine (6a-t)**

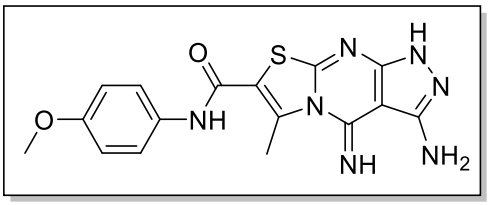
A mixture of **4a-t** (10 mmol) and phenyl hydrazine derivative (20 mmol) in DMF was heated to reflux for 12 hr. After the completion of the reaction, the reaction mixture was cooled to room temperature, poured into ice-cold water and neutralized with dil. HCl. The separated solid was filtered, washed with methanol to get an analytically pure compound **(6a-t)**.

3-amino-4-imino-6-methyl-*N*-(*p*-tolyl)-1,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*] pyrimidine-7-carboxamide (CNTHNHZ-1)



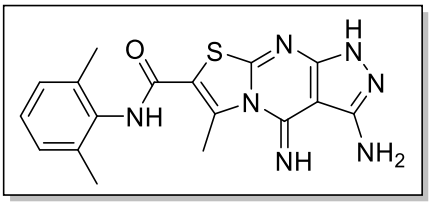
Brown solid, Yield: 90%, mp 254-256°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.58 (s, 1H), 9.62 (s, 1H), 7.98 (s, 1H), 7.52 (d, J = 8.1 Hz, 2H), 7.11 (d, J = 8.1 Hz, 2H), 6.41 (s, 2H), 2.44 (s, 3H), 2.26 (s, 3H); MS (m/z): 353 (M^+); Anal. Calcd. For $C_{16}H_{15}N_7OS$: C, 54.38; H, 4.28; N, 27.74; Found: C, 54.33; H, 4.27; N, 27.70.

3-amino-4-imino-*N*-(4-methoxyphenyl)-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo [3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-2)



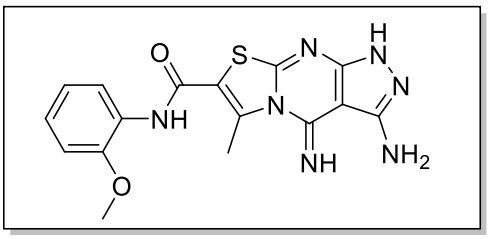
Brown solid, Yield: 92%, mp 248-250°C; ^{1}H NMR (400 MHz, DMSO-d_6) 11.58 (s, 1H), 9.62 (s, 1H), 7.98 (s, 1H), 7.53 (d, J = 8.1 Hz, 2H), 7.12 (d, J = 8.1 Hz, 2H), 6.41 (s, 2H), 3.76 (s, 3H), 2.26 (s, 3H); MS (*m*/*z*): 369 (M^+); Anal. Calcd. For $C_{16}H_{15}N_7O_2S$: C, 52.02; H, 4.09; N, 26.54; Found: C, 52.00; H, 4.03; N, 26.49.

3-amino-*N*-(2,6-dimethylphenyl)-4-imino-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo [3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-3)



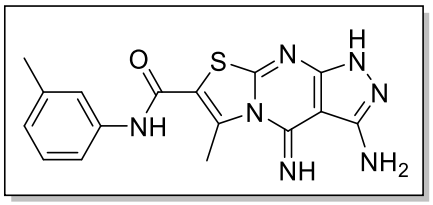
Brown solid, Yield: 91%, mp 236-238°C; MS (*m*/*z*): 367 (M^+); Anal. Calcd. For $C_{17}H_{17}N_7OS$: C, 55.57; H, 4.66; N, 26.69; Found: C, 55.61; H, 4.64; N, 26.72.

3-amino-4-imino-*N*-(2-methoxyphenyl)-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo [3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-4)



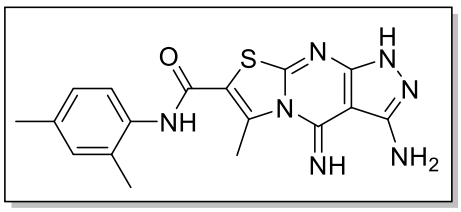
Brown solid, Yield: 90%, mp 230-232°C; MS (*m*/*z*): 369 (M^+); Anal. Calcd. For $C_{16}H_{15}N_7O_2S$: C, 52.02; H, 4.09; N, 26.54; Found: C, 52.05; H, 4.14; N, 26.59.

3-amino-4-imino-6-methyl-*N*-(*m*-tolyl)-1,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*] pyrimidine-7-carboxamide (CNTHNHZ-5)



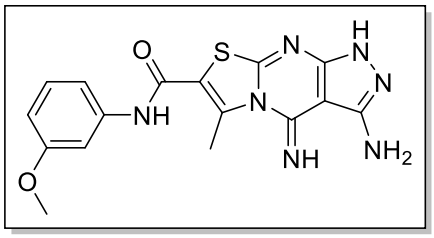
Brown solid, Yield: 86%, mp 238-240°C; MS (*m*/*z*): 353 (M^+); Anal. Calcd. For $C_{16}H_{15}N_7OS$: C, 54.38; H, 4.28; N, 27.74; Found: C, 54.33; H, 4.25; N, 27.71.

3-amino-*N*-(2,4-dimethylphenyl)-4-imino-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo [3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-6)



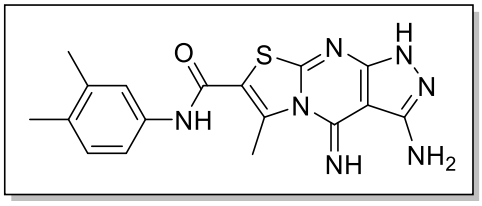
Brown solid, Yield: 89%, mp 229-231°C; MS (*m*/*z*): 367 (M^+); Anal. Calcd. For $C_{17}H_{17}N_7OS$: C, 55.57; H, 4.66; N, 26.69; Found: C, 55.51; H, 4.72; N, 26.65.

3-amino-4-imino-*N*-(3-methoxyphenyl)-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo [3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-7)



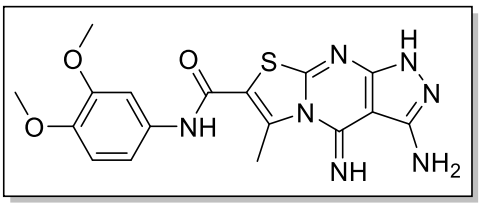
Brown solid, Yield: 85%, mp 235-237°C; MS (*m*/*z*): 369 (M^+); Anal. Calcd. For $C_{16}H_{15}N_7O_2S$: C, 52.02; H, 4.09; N, 26.54; Found: C, 52.05; H, 4.014; N, 26.57.

3-amino-*N*-(3,4-dimethylphenyl)-4-imino-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo [3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-8)



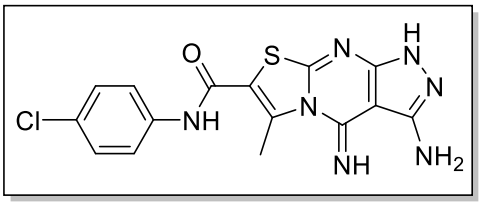
Brown solid, Yield: 88%, mp 226-228°C; MS (*m*/*z*): 367 (M^+); Anal. Calcd. For $C_{17}H_{17}N_7OS$: C, 55.57; H, 4.66; N, 26.69; Found: C, 55.62; H, 4.70; N, 26.74.

3-amino-*N*-(3,4-dimethoxyphenyl)-4-imino-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo [3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-9)



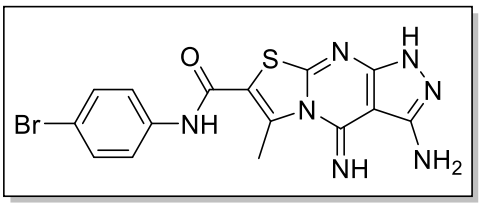
Brown solid, Yield: 89%, mp 241-243°C; MS (*m*/*z*): 399 (M^+); Anal. Calcd. For $C_{17}H_{17}N_7O_3S$: C, 55.57; H, 4.66; N, 26.69; Found: C, 55.49; H, 4.65; N, 26.73.

3-amino-*N*-(4-chlorophenyl)-4-imino-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-10)



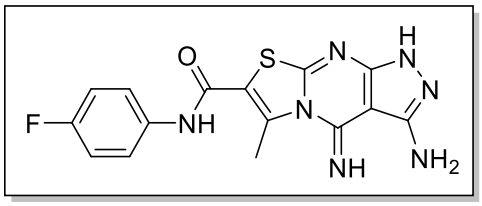
Brown solid, Yield: 80%, mp 239-241°C; MS (*m*/*z*): 373 (M^+); Anal. Calcd. For $C_{15}H_{12}ClN_7OS$: C, 48.20; H, 3.24; N, 26.23; Found: C, 48.25; H, 3.24; N, 26.26.

3-amino-*N*-(4-bromophenyl)-4-imino-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-11)



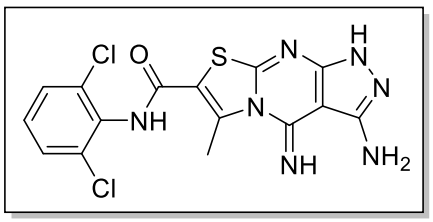
Brown solid, Yield: 80%, mp 245-247°C; MS (*m*/*z*): 418 (M^+); Anal. Calcd. For $C_{15}H_{12}BrN_7OS$: C, 43.07; H, 2.89; N, 23.44; Found: C, 43.05; H, 2.86; N, 23.39.

3-amino-*N*-(4-fluorophenyl)-4-imino-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*] pyrimidine-7-carboxamide (CNTHNHZ-12)



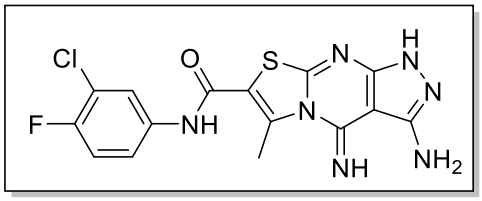
Brown solid, Yield: 71%, mp 226-228°C; MS (*m*/*z*): 357 (M^+); Anal. Calcd. For $C_{15}H_{12}FN_7OS$: C, 50.41; H, 3.38; N, 27.44; Found: C, 50.52; H, 3.44; N, 27.49.

3-amino-*N*-(2,6-dichlorophenyl)-4-imino-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo [3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-13)



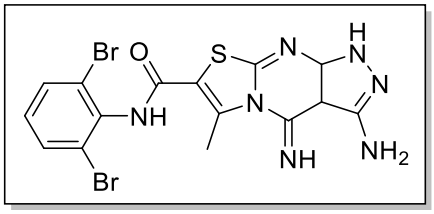
Brown solid, Yield: 75%, mp 230-232°C; MS (*m*/*z*): 408 (M^+); Anal. Calcd. For $C_{15}H_{11}Cl_2N_7OS$: C, 44.13; H, 2.72; N, 24.02; Found: C, 44.08; H, 2.67; N, 23.98.

3-amino-*N*-(3-chloro-4-fluorophenyl)-4-imino-6-methyl-1,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-14)



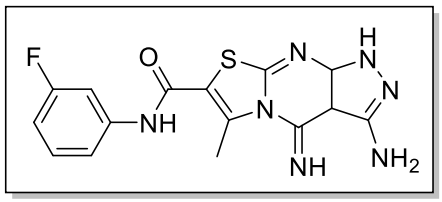
Brown solid, Yield: 68%, mp 235-237°C; MS (*m*/*z*): 391 (M^+); Anal. Calcd. For $C_{15}H_{11}ClFN_7OS$: C, 45.98; H, 2.83; N, 25.02; Found: C, 45.96; H, 2.84; N, 25.00.

3-amino-*N*-(2,6-dibromophenyl)-4-imino-6-methyl-1,3a,4,9a-tetrahydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-15)



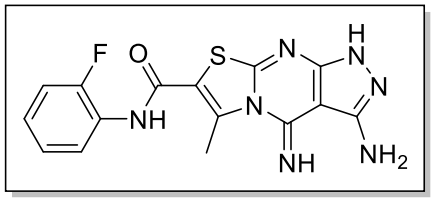
Brown solid, Yield: 85%, mp 239-241°C; MS (*m*/*z*): 499 (M^+); Anal. Calcd. For $C_{15}H_{13}Br_2N_7OS$: C, 36.09; H, 2.63; N, 19.64; Found: C, 36.02; H, 2.59; N, 19.65.

3-amino-*N*-(3-fluorophenyl)-4-imino-6-methyl-1,3a,4,9a-tetrahydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-16)



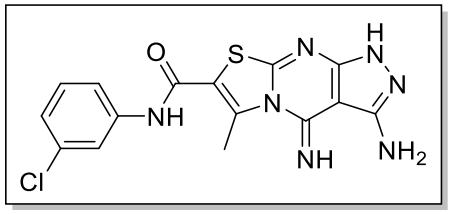
Brown solid, Yield: 63%, mp 230-232°C; MS (*m*/*z*): 359 (M^+); Anal. Calcd. For $C_{15}H_{14}FN_7OS$: C, 50.13; H, 3.93; N, 27.28; Found: C, 50.21; H, 3.96; N, 27.31.

3-amino-*N*-(2-fluorophenyl)-4-imino-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*] pyrimidine-7-carboxamide (CNTHNHZ-17)



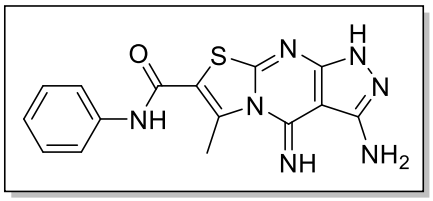
Brown solid, Yield: 60%, mp 223-225°C; MS (*m*/*z*): 357 (M^+); Anal. Calcd. For $C_{15}H_{12}FN_7OS$: C, 50.41; H, 3.38; N, 27.44; Found: C, 50.38; H, 3.40; N, 27.47.

3-amino-*N*-(3-chlorophenyl)-4-imino-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-18)



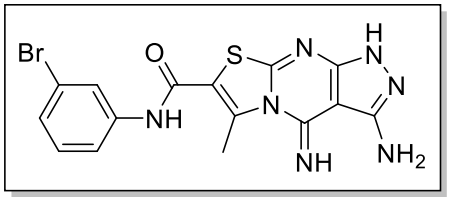
Brown solid, Yield: 65%, mp 226-228°C; MS (*m*/*z*): 373 (M^+); Anal. Calcd. For $C_{15}H_{12}ClN_7OS$: C, 48.20; H, 3.24; N, 26.23; Found: C, 48.25; H, 3.21; N, 26.26.

3-amino-4-imino-6-methyl-*N*-phenyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*] pyrimidine-7-carboxamide (CNTHNHZ-19)



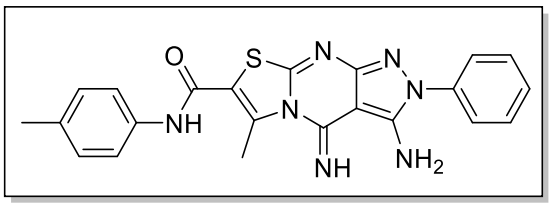
Brown solid, Yield: 89%, mp 240-242°C; MS (*m*/*z*): 339 (M^+); Anal. Calcd. For $C_{15}H_{13}N_7OS$: C, 53.09; H, 3.86; N, 28.89; Found: C, 53.12; H, 3.91; N, 28.95.

3-amino-*N*-(3-bromophenyl)-4-imino-6-methyl-1,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHZ-20)



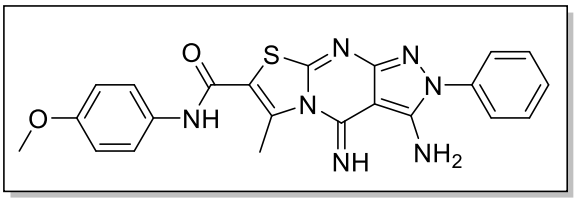
Brown solid, Yield: 78%, mp 231-233°C; MS (*m*/*z*): 418 (M^+); Anal. Calcd. For $C_{15}H_{12}BrN_7OS$: C, 43.07; H, 2.89; N, 23.44; Found: C, 43.01; H, 2.84; N, 23.38.

3-amino-4-imino-6-methyl-2-phenyl-*N*-(*p*-tolyl)-2,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-1)



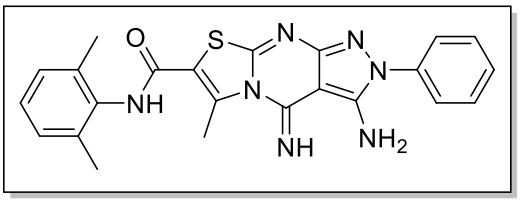
Brown solid, Yield: 94%, mp 250-252°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 9.95 (s, 1H), 7.68-7.11 (m, 9H), 6.78 (s, 2H), 2.43 (s, 3H), 2.26 (s, 3H); MS (*m*/*z*): 429 (M^+); Anal. Calcd. For $C_{22}H_{19}N_7OS$: C, 61.52; H, 4.46; N, 22.83; Found: C, 61.48; H, 4.51; N, 22.78.

3-amino-4-imino-*N*-(4-methoxyphenyl)-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-2)



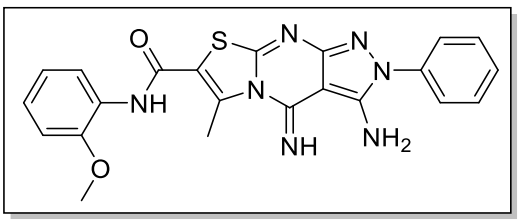
Brown solid, Yield: 92%, mp 262-264°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 9.95 (s, 1H), 7.68-7.11 (m, 9H), 6.78 (s, 2H), 3.76 (s, 3H), 2.26 (s, 3H); MS (*m*/*z*): 445 (M^+); Anal. Calcd. For $C_{22}H_{19}N_7O_2S$: C, 59.31; H, 4.30; N, 22.01; Found: C, 59.32; H, 4.35; N, 22.96.

3-amino-*N*-(2,6-dimethylphenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-3)



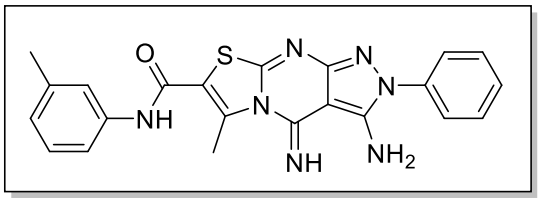
Brown solid, Yield: 87%, mp 228-230°C; MS (*m*/*z*): 443 (M^+); Anal. Calcd. For $C_{23}H_{21}N_7OS$: C, 62.29; H, 4.77; N, 22.11; Found: C, 62.32; H, 4.69; N, 22.15.

3-amino-4-imino-*N*-(2-methoxyphenyl)-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-4)



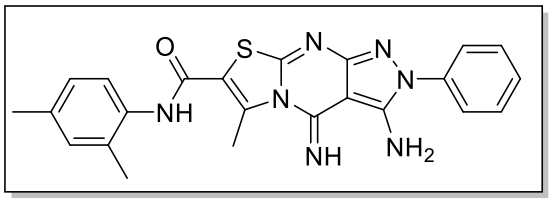
Brown solid, Yield: 81%, mp 236-238°C; MS (*m*/*z*): 445 (M^+); Anal. Calcd. For $C_{22}H_{19}N_7O_2S$: C, 59.31; H, 4.30; N, 22.01; Found: C, 59.28; H, 4.28; N, 21.96.

3-amino-4-imino-6-methyl-2-phenyl-*N*-(*m*-tolyl)-2,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-5)



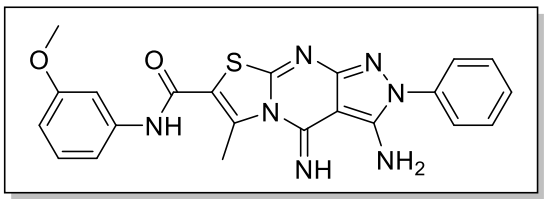
Brown solid, Yield: 85%, mp 243-245°C; MS (*m*/*z*): 429 (M^+); Anal. Calcd. For $C_{22}H_{19}N_7OS$: C, 61.52; H, 4.46; N, 22.83; Found: C, 61.58; H, 4.51; N, 22.85.

3-amino-*N*-(2,4-dimethylphenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-6)



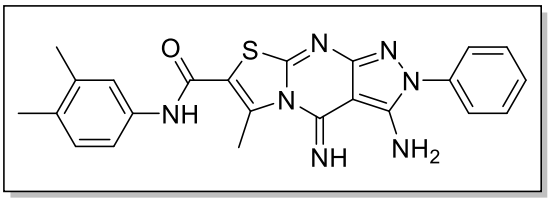
Brown solid, Yield: 82%, mp 258-260°C; MS (*m*/*z*): 443 (M^+); Anal. Calcd. For $C_{23}H_{21}N_7OS$: C, 62.29; H, 4.77; N, 22.11; Found: C, 62.32; H, 4.81; N, 22.12.

3-amino-4-imino-*N*-(3-methoxyphenyl)-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-7)



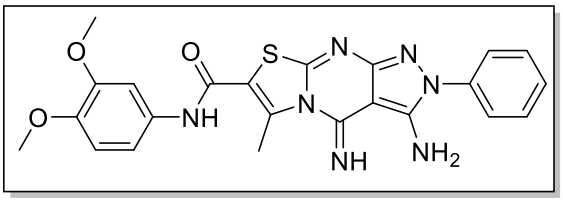
Brown solid, Yield: 80%, mp 252-254°C; MS (*m*/*z*): 445 (M^+); Anal. Calcd. For $C_{22}H_{19}N_7O_2S$: C, 59.31; H, 4.30; N, 22.01; Found: C, 59.29; H, 4.26; N, 21.99.

3-amino-*N*-(3,4-dimethylphenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-8)



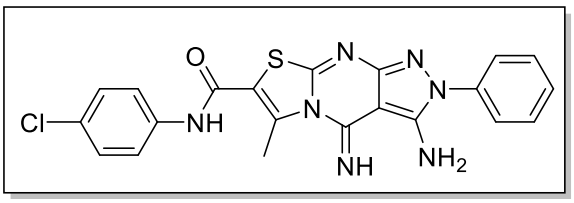
Brown solid, Yield: 82%, mp 255-257°C; MS (*m*/*z*): 443 (M^+); Anal. Calcd. For $C_{23}H_{21}N_7OS$: C, 62.29; H, 4.77; N, 22.11; Found: C, 62.26; H, 4.69; N, 22.15.

3-amino-*N*-(3,4-dimethoxyphenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-9)



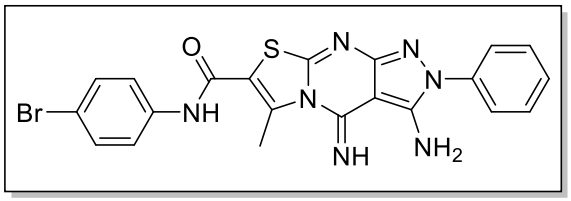
Brown solid, Yield: 87%, mp 260-262°C; MS (*m*/*z*): 475 (M^+); Anal. Calcd. For $C_{23}H_{21}N_7O_3S$: C, 58.09; H, 4.45; N, 20.62; Found: C, 58.04; H, 4.51; N, 20.67.

3-amino-*N*-(4-chlorophenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-10)



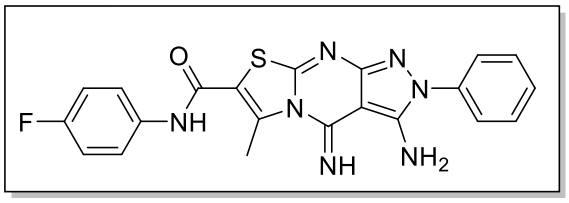
Brown solid, Yield: 79%, mp 243-245°C; MS (*m*/*z*): 449 (M^+); Anal. Calcd. For $C_{21}H_{16}ClN_7OS$: C, 56.06; H, 3.58; N, 21.79; Found: C, 56.01; H, 3.54; N, 21.75.

3-amino-*N*-(4-bromophenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-11)



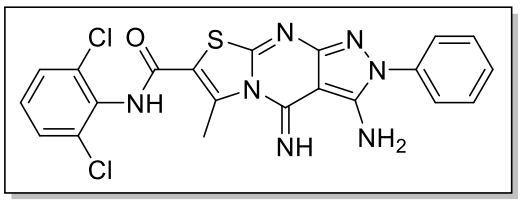
Brown solid, Yield: 81%, mp 259-161°C; MS (*m*/*z*): 494 (M^+); Anal. Calcd. For $C_{21}H_{16}BrN_7OS$: C, 51.02; H, 3.26; N, 19.83; Found: C, 51.00; H, 3.22; N, 19.81.

3-amino-*N*-(4-fluorophenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-12)



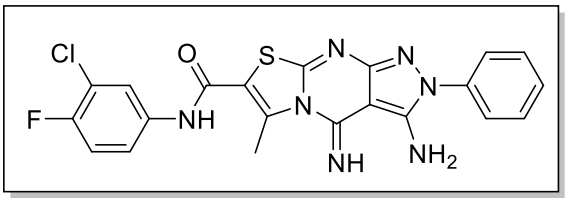
Brown solid, Yield: 61%, mp 224-226°C; MS (*m*/*z*): 433 (M^+); Anal. Calcd. For $C_{21}H_{16}FN_7OS$: C, 58.19; H, 3.72; N, 22.62; Found: C, 58.13; H, 3.77; N, 22.68.

3-amino-*N*-(2,6-dichlorophenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-13)



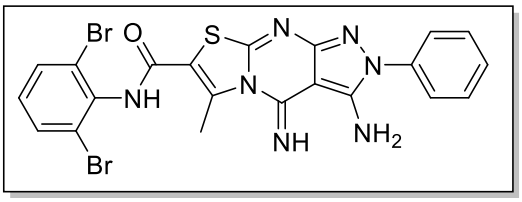
Brown solid, Yield: 75%, mp 228-230°C; MS (*m*/*z*): 484 (M^+); Anal. Calcd. For $C_{21}H_{15}Cl_2N_7OS$: C, 52.08; H, 3.12; N, 20.24; Found: C, 52.13; H, 3.07; N, 20.25.

3-amino-*N*-(3-chloro-4-fluorophenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo [3,4-*d*]thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-14)



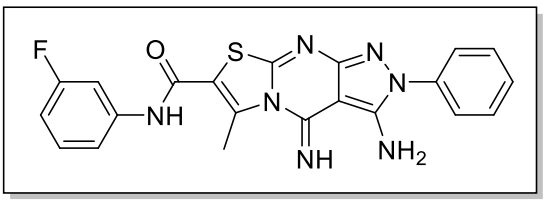
Brown solid, Yield: 73%, mp 256-258°C; MS (*m*/*z*): 467 (M^+); Anal. Calcd. For $C_{21}H_{15}ClFN_7OS$: C, 53.91; H, 3.23; N, 20.95; Found: C, 53.95; H, 3.22; N, 20.96.

3-amino-*N*-(2,6-dibromophenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-15)



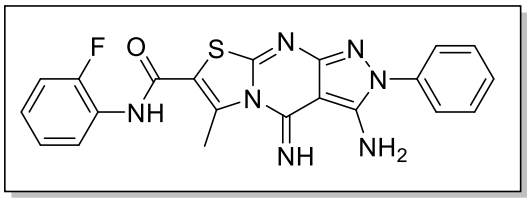
Brown solid, Yield: 81%, mp 262-264°C; MS (*m*/*z*): 573 (M^+); Anal. Calcd. For $C_{21}H_{15}Br_2N_7OS$: C, 44.00; H, 2.64; N, 17.10; Found: C, 44.03; H, 2.67; N, 17.14.

3-amino-*N*-(3-fluorophenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-16)



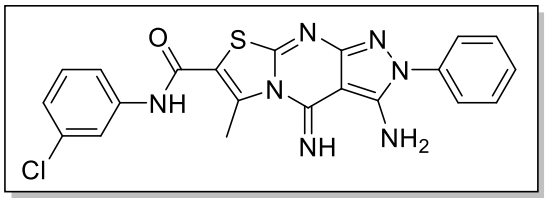
Brown solid, Yield: 65%, mp 229-231°C; MS (*m*/*z*): 433 (M^+); Anal. Calcd. For $C_{21}H_{16}FN_7OS$: C, 58.19; H, 3.72; N, 22.62; Found: C, 58.21; H, 3.76; N, 22.65.

3-amino-*N*-(2-fluorophenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-17)



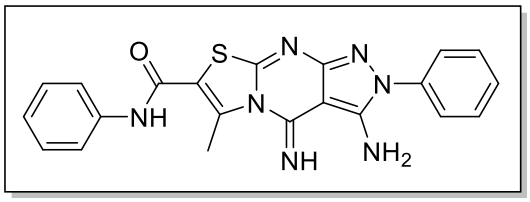
Brown solid, Yield: 62%, mp 231-233°C; MS (*m*/*z*): 433 (M^+); Anal. Calcd. For $C_{21}H_{16}FN_7OS$: C, 58.19; H, 3.72; N, 22.62; Found: C, 58.14; H, 3.65; N, 22.63.

3-amino-*N*-(3-chlorophenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-18)



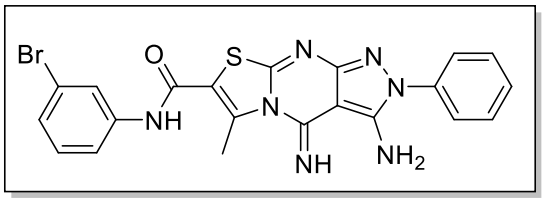
Brown solid, Yield: 90%, mp 255-257°C; MS (*m*/*z*): 449 (M^+); Anal. Calcd. For $C_{21}H_{16}ClN_7OS$: C, 56.06; H, 3.58; N, 21.79; Found: C, 56.02; H, 3.54; N, 21.76.

3-amino-4-imino-6-methyl-*N*,2-diphenyl-2,4-dihydropyrazolo[3,4-*d*]thiazolo[3,2-*a*] pyrimidine-7-carboxamide (CNTHNHPZ-19)



Brown solid, Yield: 90%, mp 251-253°C; MS (*m*/*z*): 415 (M^+); Anal. Calcd. For $C_{21}H_{17}N_7OS$: C, 60.71; H, 4.12; N, 23.60; Found: C, 60.70; H, 4.13; N, 23.57.

3-amino-*N*-(3-bromophenyl)-4-imino-6-methyl-2-phenyl-2,4-dihydropyrazolo[3,4-*d*] thiazolo[3,2-*a*]pyrimidine-7-carboxamide (CNTHNHPZ-20)



Brown solid, Yield: 90%, mp 251-253°C; MS (*m*/*z*): 494 (M^+); Anal. Calcd. For $C_{21}H_{16}BrN_7OS$: C, 51.02; H, 3.26; N, 19.83; Found: C, 51.00; H, 3.25; N, 19.80.

5.4 Spectral Data

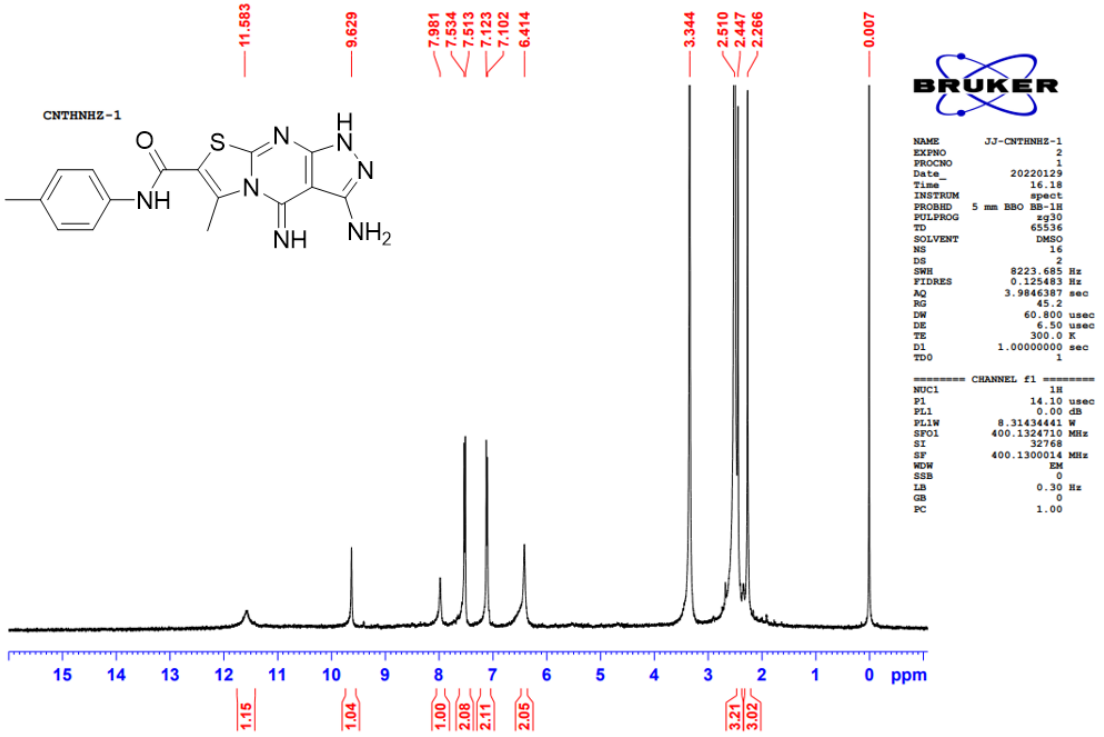


Fig. 1: Representative ^{1}H NMR spectrum of compound CNTHNHZ-1

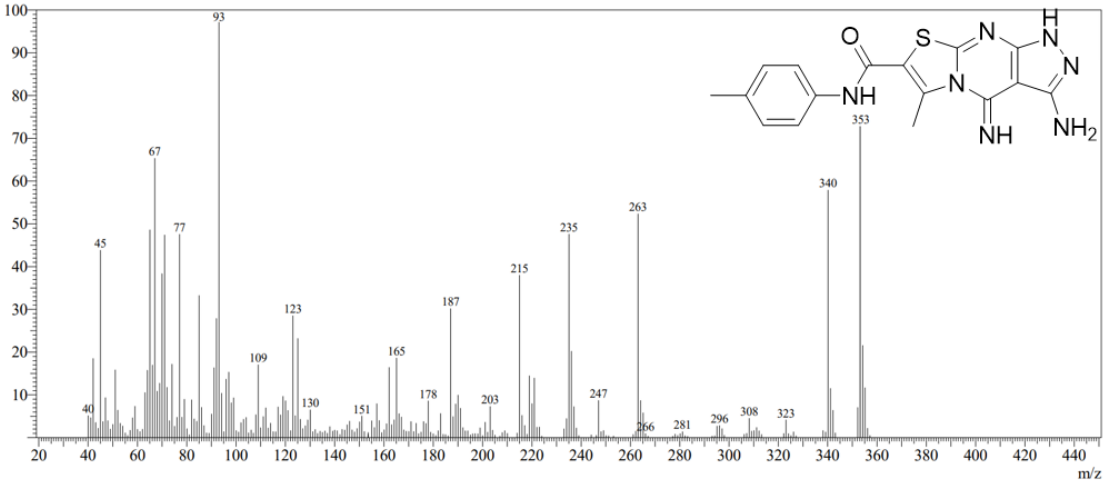


Fig. 2: Representative mass spectrum of compound CNTHNHZ-1

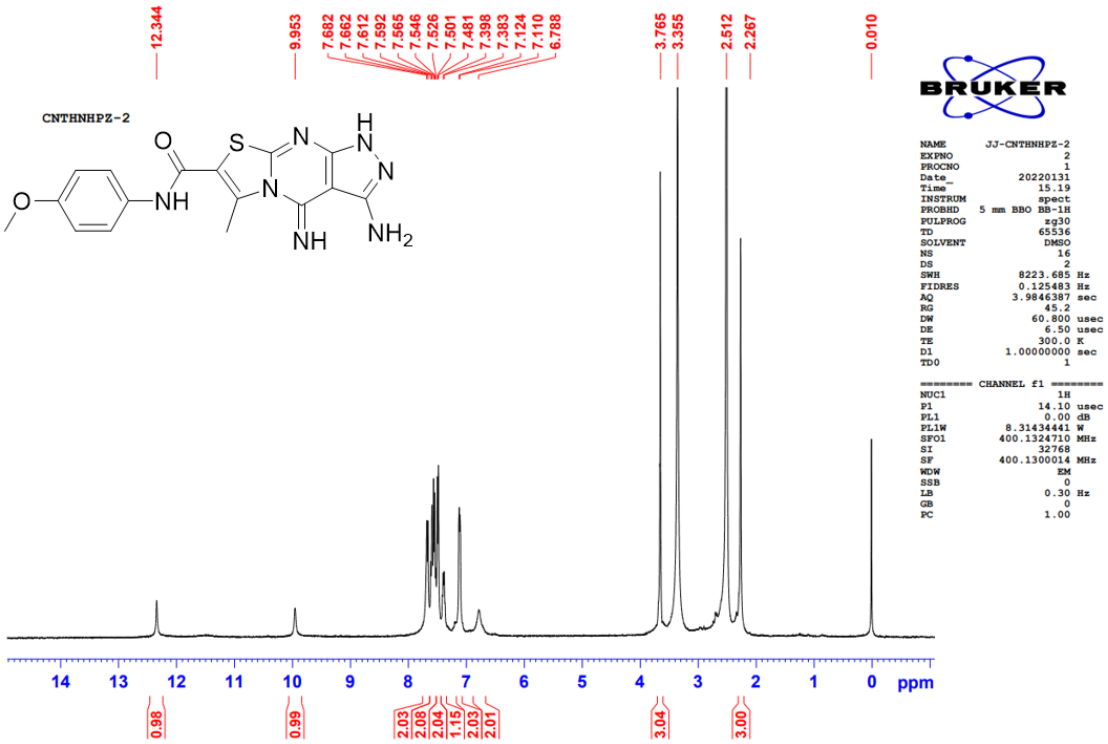


Fig. 3: Representative ^{1}H NMR spectrum of compound CNTHNHZ-2

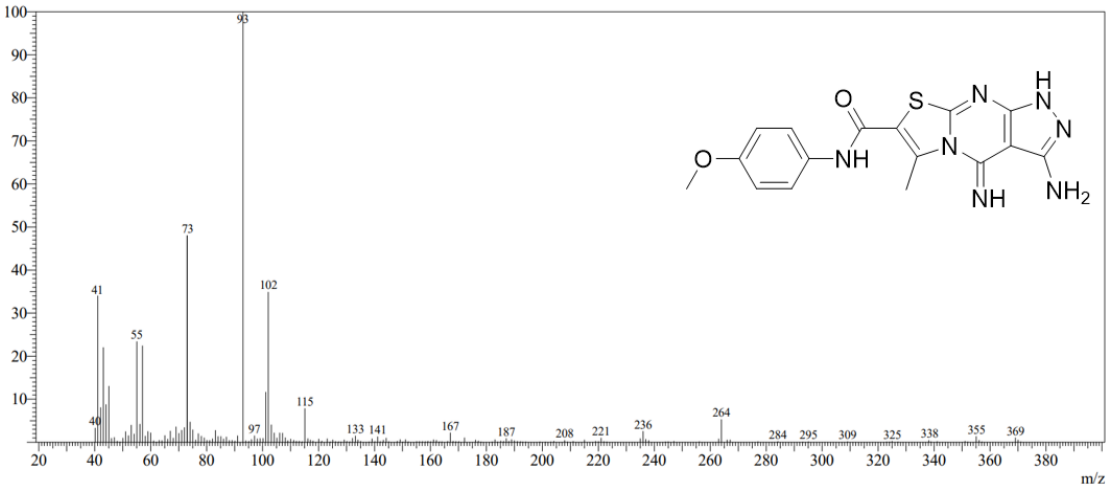


Fig. 4: Representative mass spectrum of compound CNTHNHZ-2

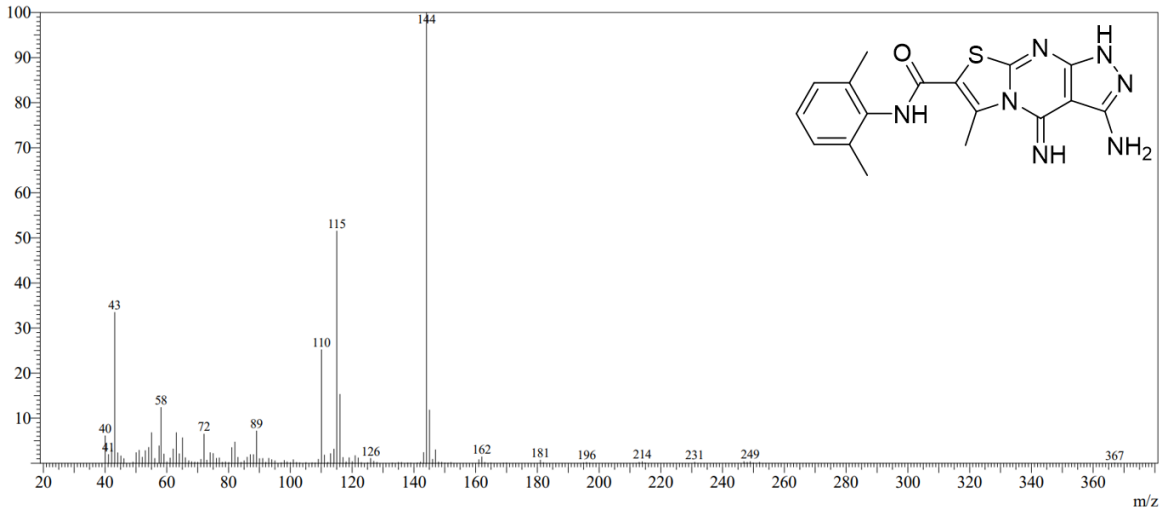


Fig. 5: Representative mass spectrum of compound CNTHNHZ-3

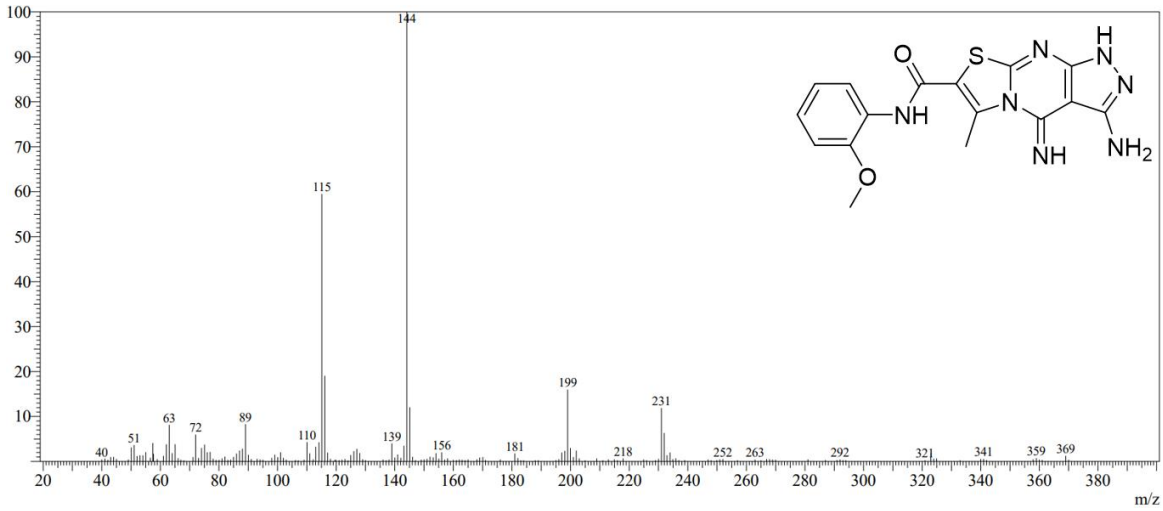


Fig. 6: Representative mass spectrum of compound CNTHNHZ-4

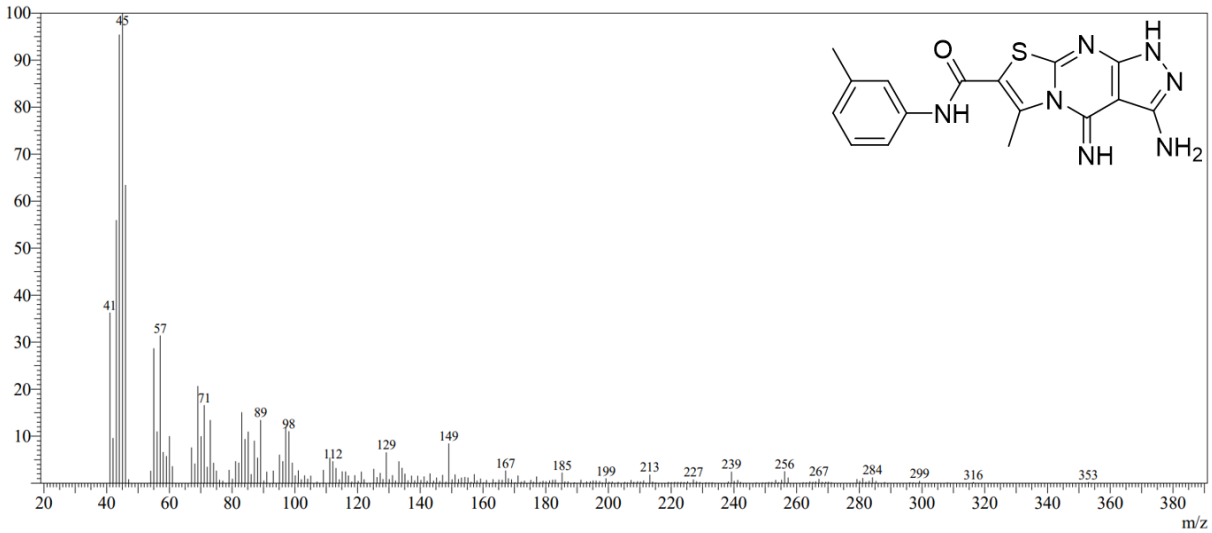


Fig. 7: Representative mass spectrum of compound CNTHNHZ-5

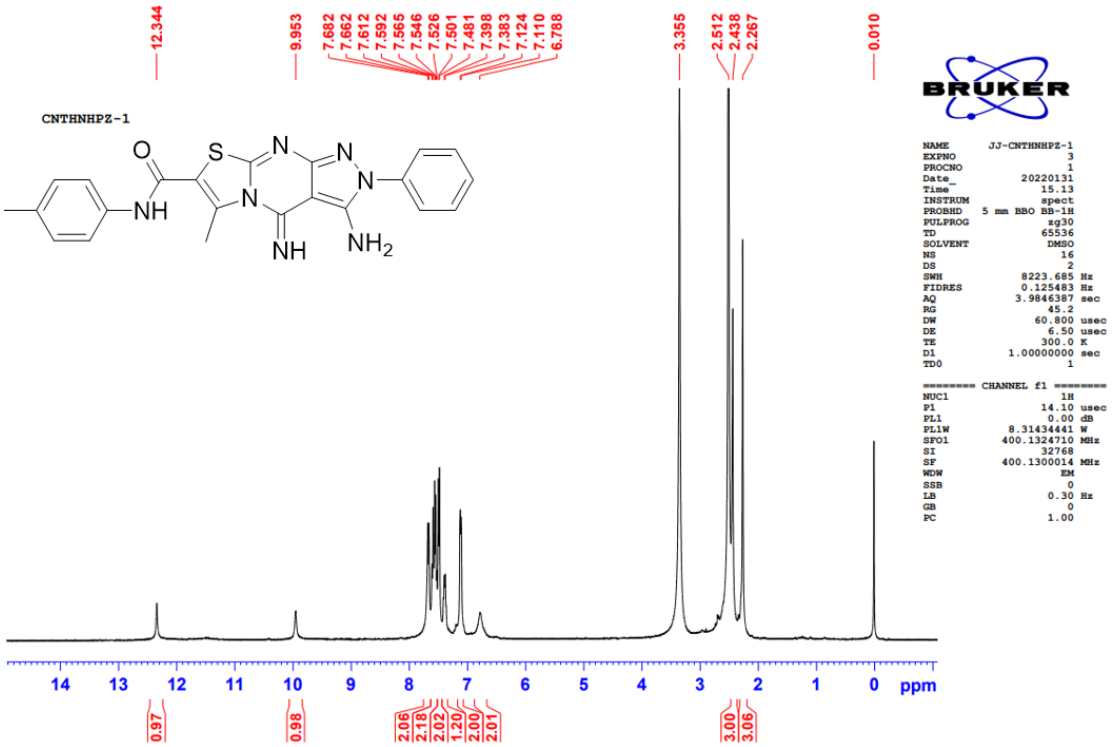


Fig. 8: Representative ^{1}H NMR spectrum of compound CNTHNHPZ-1

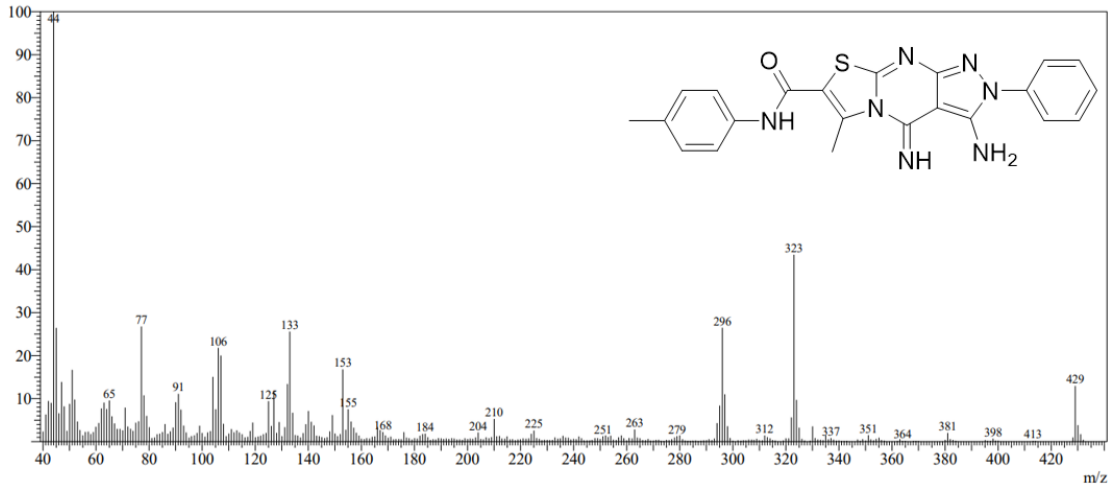


Fig. 9: Representative mass spectrum of compound CNTHNHPZ-1

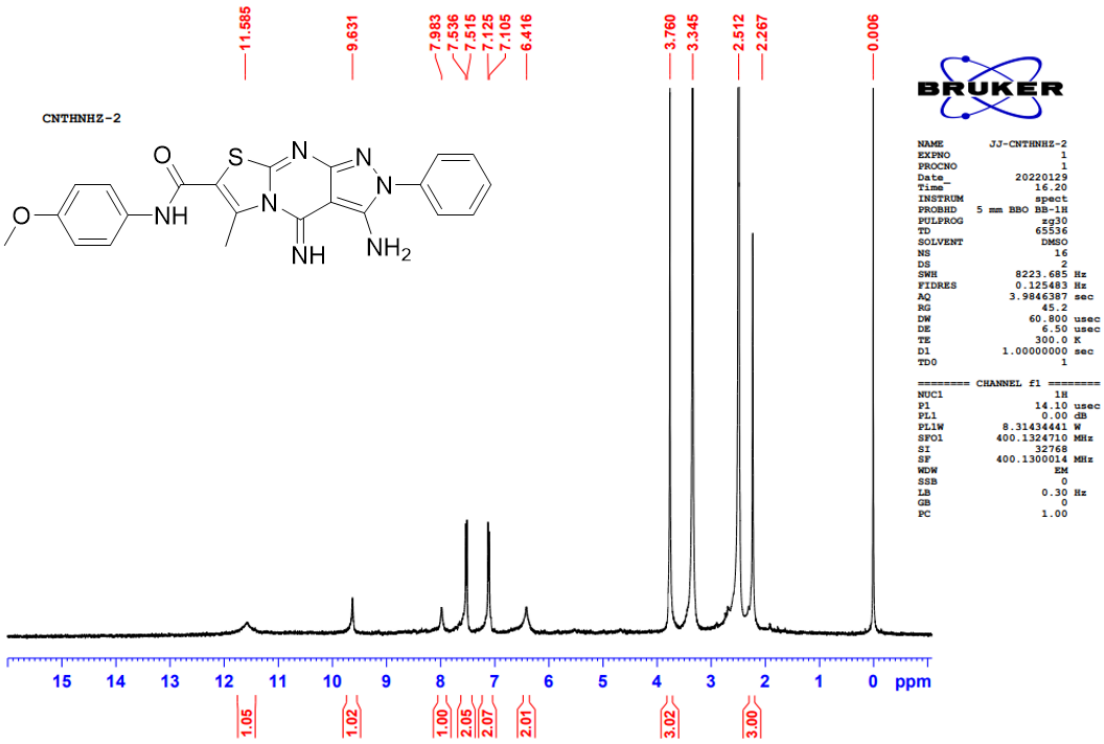


Fig. 10: Representative ^{1}H NMR spectrum of compound CNTHNHPZ-2

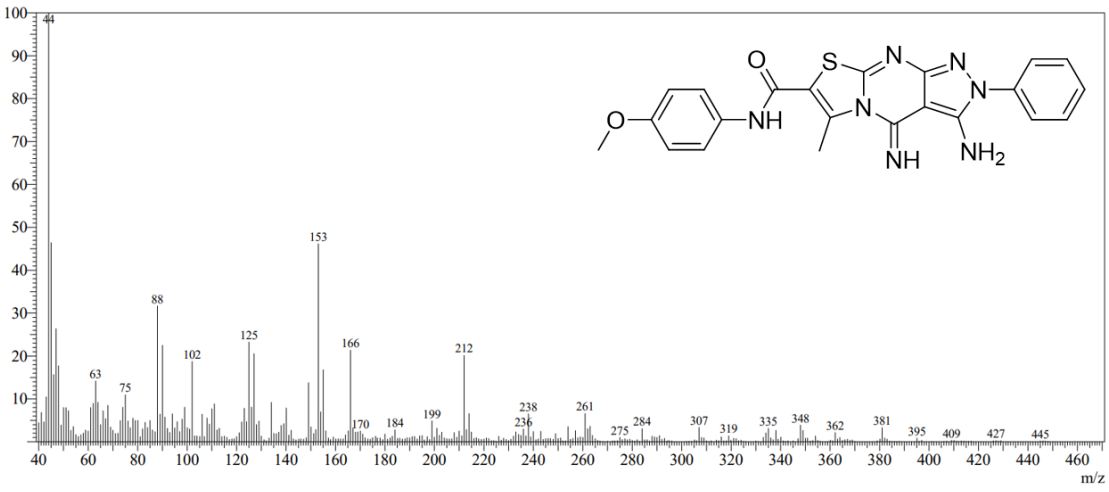


Fig. 11: Representative mass spectrum of compound CNTHNHPZ-1

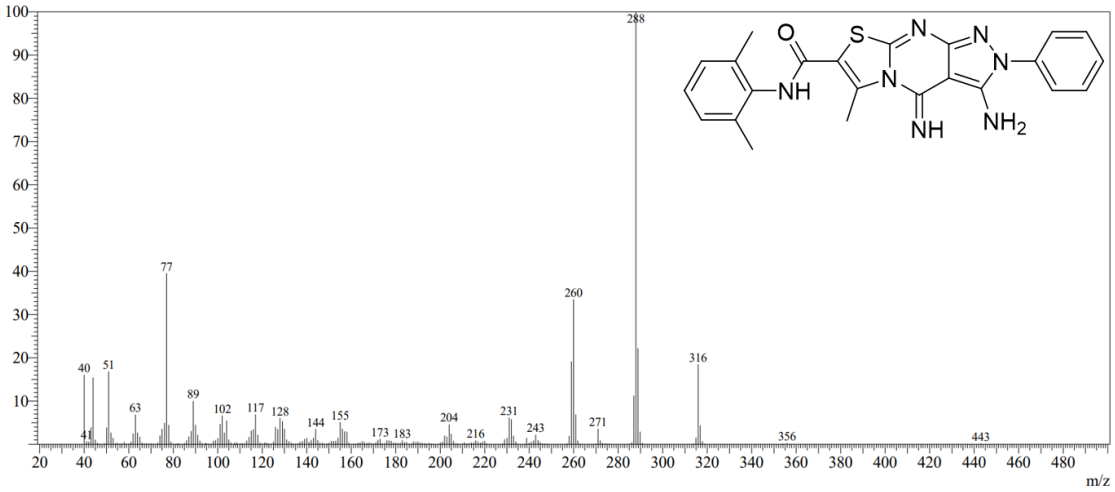


Fig. 12: Representative mass spectrum of compound CNTHNHPZ-3

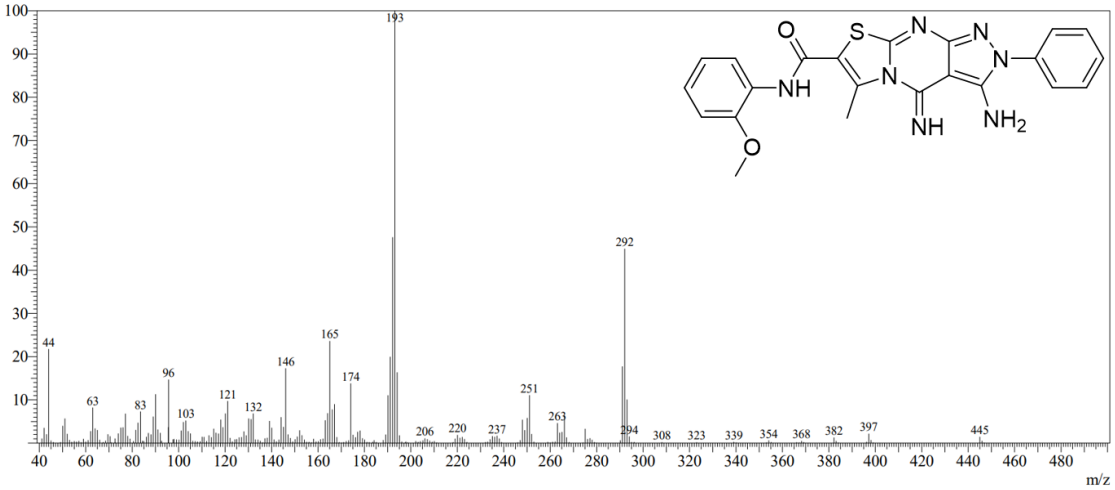


Fig. 13: Representative mass spectrum of compound CNTHNHPZ-4

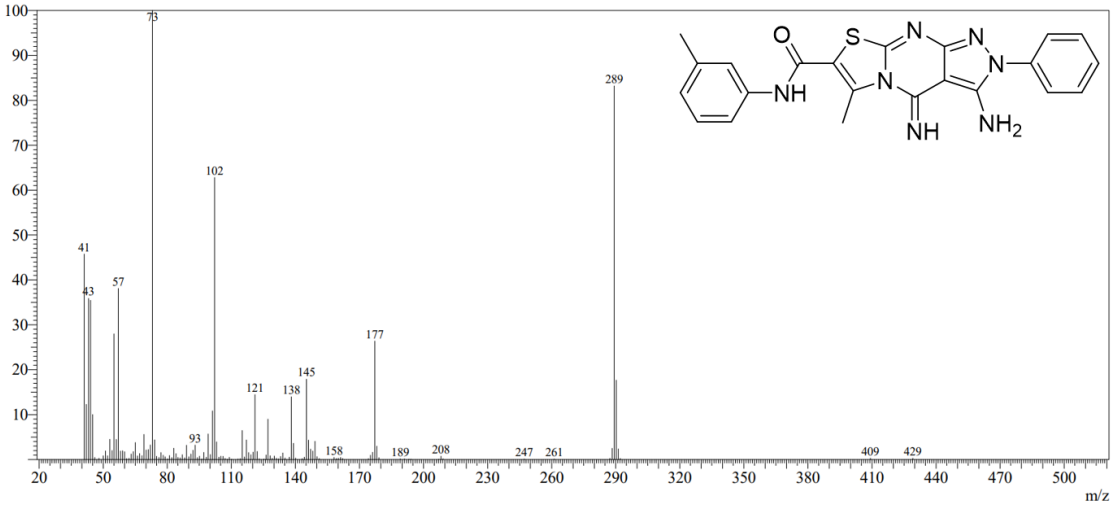


Fig. 14: Representative mass spectrum of compound CNTHNHPZ-4