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Synthesis and xanthine oxidase inhibitory activity of 7-methyl-2-(phenoxymethyl)-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidin-5-one derivatives

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ABSTRACT

An elevated level of blood uric acid (hyperuricemia) is the underlying cause of gout. Xanthine oxidase is the key enzyme that catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid. Allopurinol, a widely used xanthine oxidase inhibitor is the most commonly used drug to treat gout. However, a small but significant portion of the population suffers from adverse effects of allopurinol that includes gastrointestinal upset, skin rashes and hypersensitivity reactions. Moreover, an elevated level of uric acid is considered as an independent risk factor for cardiovascular diseases. Therefore use of allopurinol-like drugs with minimum side effects is the ideal drug of choice against gout. In this study, we report the synthesis of a series of pyrimidin-5-one analogues as effective and a new class of xanthine oxidase inhibitors. All the synthesized pyrimidin-5-one analogues are characterized by spectroscopic techniques and elemental analysis. Four (**6a**, **6b**, **6d** and **6f**) out of 20 synthesized molecules in this class showed good inhibition against three different sources of xanthine oxidase, which were more potent than allopurinol based on their respective IC_{50} values. Molecular modeling and docking studies revealed that the molecule **6a** has very good interactions with the Molybdenum–Oxygen–Sulfur (MOS) complex a key component in xanthine oxidase. These results highlight the identification of a new class of xanthine oxidase inhibitors that have potential to be more efficacious, than allopurinol, to treat gout and possibly against cardiovascular diseases.

1. Introduction

Xanthine oxidase (XO) is situated at the end of a catabolic sequence of the purine nucleotide metabolism in humans and a few other uricotelic species.[1] This enzyme catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid.[2] The overproduction of uric acid termed as hyperuricemia is the underlying cause of gout.[3] The increase in uric acid concentration eventually leads to the deposition of micro and macroscopic deposits of sodium hydrogen urate monohydrate crystals in joints of humans. This deposition of urate crystals initiates an inflammatory reaction at the joints that includes swelling, redness and pain, the hallmarks of inflammation.[4,5] XO also generates reactive oxygen species (ROS). ROS generated by XO have been implicated in a wide variety of pathophysiological processes including diabetes, post-ischemic reperfusion injury and chronic heart failure.[6]

Allopurinol is the widely used drug for inhibiting XO in gout.[7] Allopurinol blocks the synthesis of uric acid and it also prevents the formation of ROS, there by protecting against post-ischemic reperfusion injury.[8,9] More recently, allopurinol is also known to produce modest but beneficial adenosine A1 receptor-mediated anti-nociceptive effects in mice.[6] Thus action of XO inhibitors is beyond the mere inhibition of XO. However, a small but significant portion of the population suffers from adverse effects of allopurinol that includes gastrointestinal upset, skin rashes, and hypersensitivity reactions, liver dysfunction, exfoliative dermatitis, vasculitis, eosinophilia and acute interstitial nephritis.[8] Some of these symptoms are common in Stevens–Johnson syndrome and DRESS (Drug Rash with Eosinophylia and Systemic Symptoms).[10,11]

Synthetic pyrimidine derivatives are known to possess several beneficial biological activities that include antioxidant, immunotropic, anti-inflammatory and membrane stabilizing properties.[12–14]

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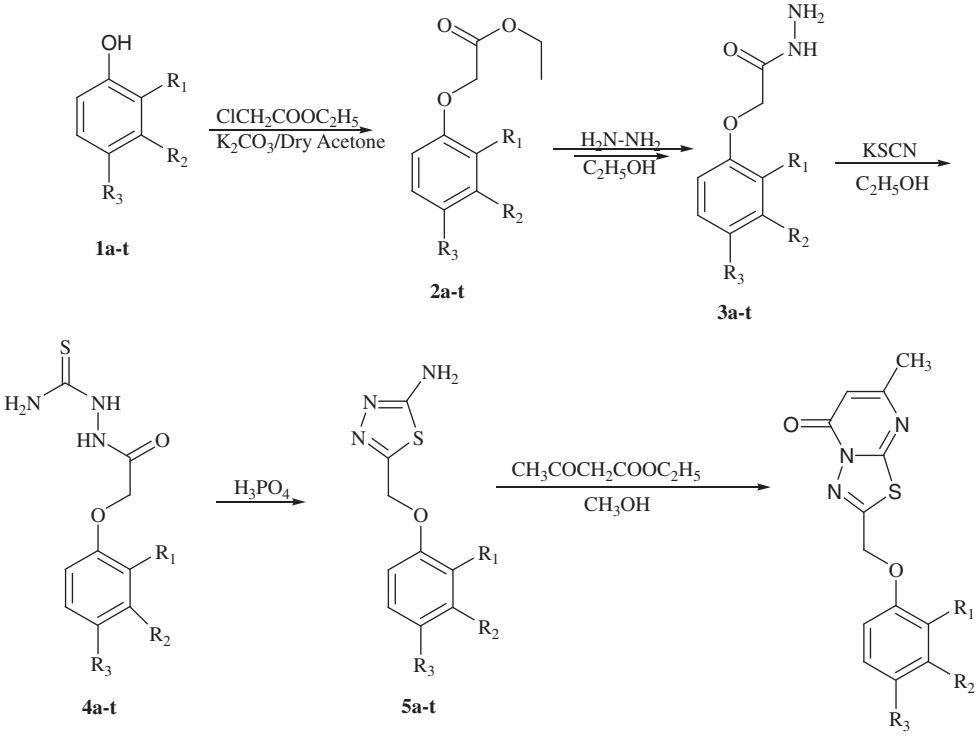
In this connection, several pyrimidine derivatives were synthesized with condensing various pharmacologically active alkyl groups by means of alkyl halides. The present study is undertaken with a view to find the effect of pyrimidine derivatives **6a–t** as XO inhibitors. Here, we report four pyrimidine analogues (**6a, 6b, 6d** and **6f**) that act as potent xanthine oxidase inhibitors.

2. Result and discussion

The novel 7-methyl-2-(phenoxymethyl)-5H-[1,3,4]thiadiazolo [3,2-*a*] pyrimidin-5-ones (**6a–t**) were synthesized by multiple step synthesis as depicted in Scheme 1. The effect of different doses of pyrimidine derivatives on XO in rat liver is shown in Table 1. Activity was reduced to 50% at a dose of 289 ± 5.7, 449 ± 10.3, 362 ± 11.1, 623 ± 23.1 nM for compounds **6a, 6b, 6d**, and **6f** respectively (Fig. 1). The significant inhibition was shown by **6a** with methoxy group at *para*-position, followed by **6d** with methyl group at ortho position, **6b** with chloride group at *para*-position and **6f** with methyl group at *meta*-position and chloro group at *para*-position. These compounds showed much more potent inhibitory activity than allopurinol (IC_{50}=753 ± 33.1 nM). This was true even with bovine milk, and microbial XO (Figs. 2 and 3). Compounds **6c**, **6e**, **6g**, **6h**, **6i**, **6j**, **6k**, **6l**, **6m**, **6n**, **6o**, **6p**, **6q**, **6r**, **6s** and **6t** showed inhibitory activity at much higher concentration and hence were not chosen further (Table 1). Compound **6a** with methoxy group at *para*-position is the most potent of all the inhibitory compounds tested.

The inhibitory activity of the newly synthesized compounds against XO was studied using the method of Kadam and Iyer[15] and Litwack et al.[16] Rate of formation of uric acid from the oxidation of xanthine in presence of these inhibitors against different sources of XO was studied. Pattern of XO inhibition, for all the three sources of XO is shown in Figures 1–3. The inhibitory activity of synthesized compounds was compared with the inhibitory activity shown by the standard inhibitor allopurinol. The different pyrimidine derivatives **6a–t** were tested for their ability to block the XO activity for the substrate xanthine. The order of potency is found to be **6a > 6d > 6b > 6f**. The other compounds screened failed to elicit inhibition of XO from rat liver homogenate at such a low concentration (Table 1).

Present study identifies a new class of XO inhibitor. It is possible that the pyrimidine binds to the active site and substituent containing heterocyclic rings bind to peripheral site of the enzyme and transfers electrons to the molybdenum center (a two electron acceptor from the MO site of XO). Accordingly, our docking studies revealed that all inhibitors bind between two phenylalanine residues, Phe649 and Phe1013, what is believed to promote the stabilization of the binding positions of the aromatic substrate and might be important for the substrate recognition.[17] The presence of these residues makes the region closer to the MOS1327 complex, which compromises the binding of larger substituted rings. This was observed with all docked inhibitors taken for our study, in which only phenyl ring was allowed to bind in that region. As shown in Figure 4, compound **6a** was expected to bind with XO



a: R_1=R_2=H, R_3=OCH_3 b: R_1=R_2=H, R_3=Cl
c: R_1=Br, R_2=R_3=H d: R_1= CH_3, R_2=R_3=H
e: R_1=Cl, R_2=R_3=H f:: R_1=H, R_2=CH_3, R_3=Cl
g: R_1=R_3=H, R_2=CH_3 h: R_1=R_2=H, R_3=Br
i: R_1=R_2=H, R_3=F j: R_1=NO_2, R_2= R_3=H
k: R_1=R_2=H, R_3=NO_2 l: R_1=R_3=CH_3, R_2=H
m: R_1=F, R_2=R_3=H n: R_1=R_3=H, R_2=NO_2
o: R_1=R_2=H, R_3=CH_3 p: R_1=R_3=H, R_2=Cl
q: R_1=R_3=H, R_2=Br r: R_1=R_3=H, R_2=F
s: R_1=R_2=R_3=H, t: R_1=R_2=CH_3, R_3=H

Scheme 1.

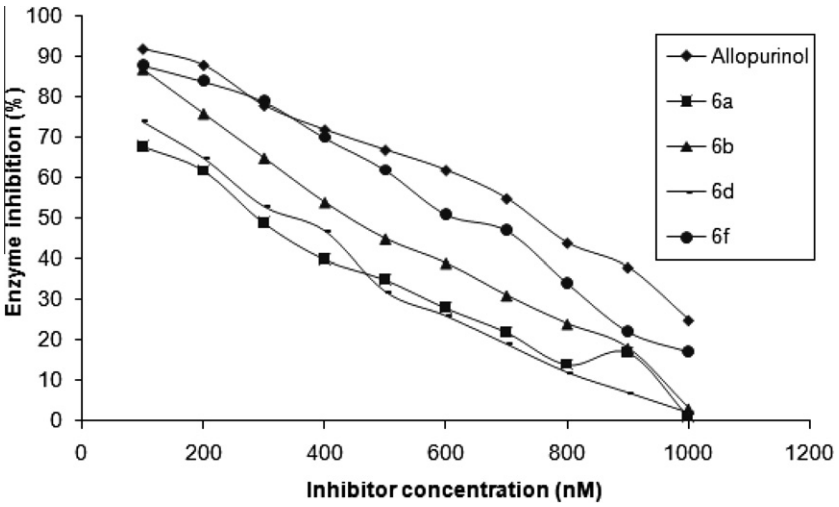


Figure 1. Percentage inhibition of rat liver Xanthine oxidase by pyrimidin-5-one derivatives.

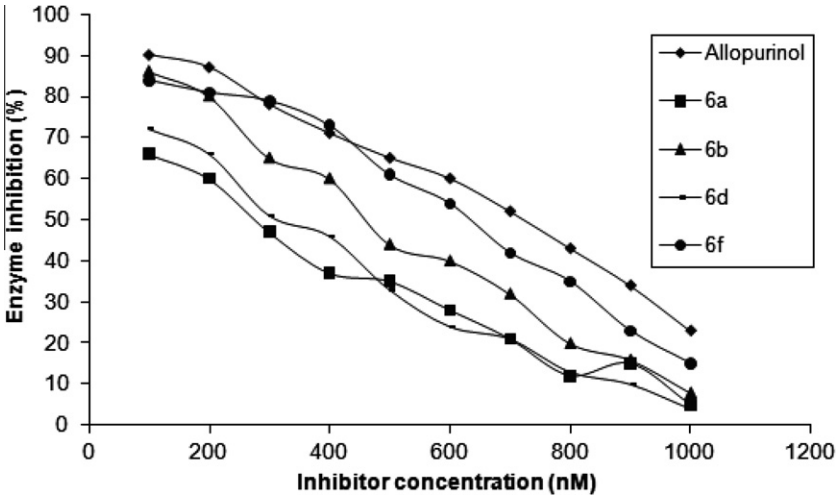


Figure 2. Percentage inhibition of bovine milk Xanthine oxidase by pyrimidin-5-one derivatives.

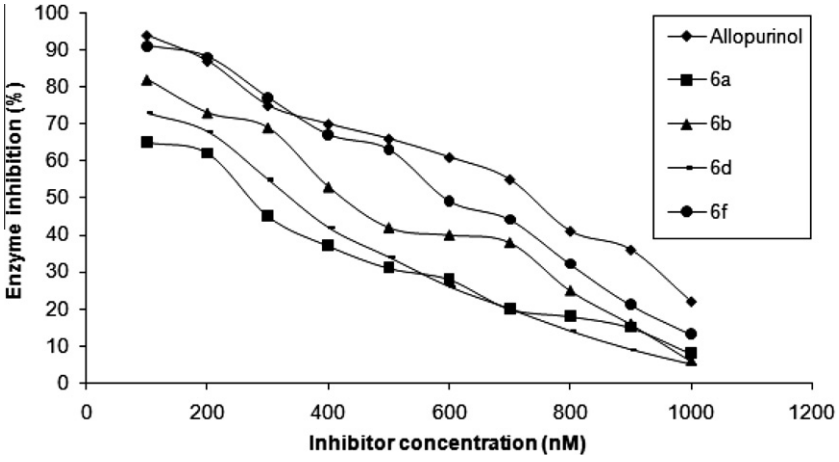


Figure 3. Percentage inhibition of microbial Xanthine oxidase by pyrimidin-5-one derivatives.

in a similar way. The compound **6a** was sandwiched between the active site aminoacids Phe 649 and Phe 1013, respectively. The ligand was surrounded by several hydrophobic amino acid residues and was positioned at the entrance of the active site-directed channel.[18] The reference compound lumazine (an allopurinol analogue) is also shown in Figure 4. Therefore the inhibitory

Table 1
Comparative inhibitory activities of pyrimidin-5-one derivatives (**6a–t**) against different sources of xanthine oxidase (IC_{50}, nM)

Compound	Rat liver	Bovine milk	Microbial source
6a	289 ± 5.7[a]	269 ± 21.1[a]	269 ± 20.7[a]
6b	449 ± 10.3[d]	461 ± 40.1[c]	423 ± 25.5[c]
6c	413 ± 10.1[c]	603 ± 45.5[e]	ND
6d	362 ± 11.1[b]	326 ± 23.4[b]	346 ± 30.1[b]
6e	1830 ± 27.7[j]	1650 ± 67.3	1821 ± 55.3[j]
6f	623 ± 23.1[f]	634 ± 37.7[d]	596 ± 57.3[d]
6g	1070 ± 32.1[h]	1030 ± 51.1[g]	1108 ± 73.1[g]
6h	ND	ND	ND
6i	ND	ND	2240 ± 87.8[k]
6j	ND	ND	ND
6k	2030 ± 55.7[k]	2501 ± 53.4[i]	ND
6l	1760 ± 27.3[i]	1800 ± 48.7[h]	1730 ± 73.6[ij]
6m	590 ± 21.3[ef]	ND	ND
6n	ND	ND	ND
6o	1020 ± 47.3[h]	ND	834 ± 55.1[f]
6p	6180 ± 100[m]	ND	ND
6q	2560 ± 76.1[l]	ND	ND
6r	1980 ± 57.8[k]	ND	1665 ± 73.3[h]
6s	555 ± 23.1[e]	ND	ND
6t	ND	ND	ND
Allopurinol	753 ± 33.1[g]	730 ± 37.4[f]	730 ± 40.7[e]

[*] Values are Mean ± SD of triplicate determinations.
[**] Values with different superscript letters a,b,c,d . . . differ significantly at $p \leqslant 0.001$.
[#] ND: not detected.

property of only pyrimidin-5-one derivatives **6a–t** was investigated in this work and we found that it effectively inhibited the enzymatic formation of uric acid from xanthine. The level of XO in the serum is significantly increased in several pathological statuses. Thus inhibition of this enzyme by compounds such as **6a**, **6b**, **6d** and **6f** may have properties that are of therapeutic interest.

3. Conclusion

From the results of the present study, it is concluded that, a series of novel pyrimidin-5-one derivatives **6a–t** were synthesized and their XO inhibitory activities from three different sources have been evaluated. Compounds **6a**, **6b**, **6d** and **6f** demonstrated potent inhibitory activities against XO. Further research in this area is in progress in our laboratory.

4. Experimental part

4.1. Chemistry

The synthesis of the hitherto unreported title compounds is as outlined in Scheme 1. Substituted phenols **1a–t** on reaction with ethyl chloroacetate affords substituted ethyl phenoxy acetates **2a–t** in excellent yield.[19,20] which on treatment with hydrazine hydrate yields corresponding phenoxy acetohydrazides **3a–t**.[21] Condensation of **3a–t** with potassium isothiocyanate in dry ethanol resulted in the formation of substituted phenoxy acetyl-*N*-hydrazine carbothioamides **4a–t**. Intramolecular cyclization of **4a–t** with anhydrous orthophosphoric acid afforded 5-phenoxy methyl-2-amino-1,3,4-thiadiazoles **5a–t**.[22] Finally compounds **5a–t** on condensation with ethylacetoacetate in presence of methanol afforded the title compounds 7-methyl-2-(phenoxymethyl)-5H-[1,3,4]thiadiazolo[3,2-*a*] pyrimidin-5-ones **6a–t**.

Chemicals were purchased from Sigma Aldrich Chemical Co. TLC was performed on aluminium-baked silica plates and visualized by UV-light. Melting points were determined on a Thomas Hoover capillary melting point apparatus with a digital thermometer. IR spectra were recorded in Nujol on FT-IR Shimadzu 8300 spectrophotometer, ^{1}H NMR spectra were recorded on a Bruker 300 MHz NMR spectrophotometer in $CDCl_3$ and chemical shift were recorded in parts per million down field from tetramethylsilane. Mass spectra were obtained with a VG70-70H spectrophotometer and important fragments are given with the relative intensities in the brackets. Elemental analysis results are within 0.5% of the calculated value.

4.1.1. Synthesis of ethyl-4-methoxy phenoxy acetate (2a)

A mixture of **1a** (5 g, 0.04 mol), ethyl chloroacetate (4.8 g, 0.04 mol) in dry acetone (50 mL) and anhydrous potassium carbonate (5.6 g, 0.04 mol) was refluxed for 8 h then cooled and the

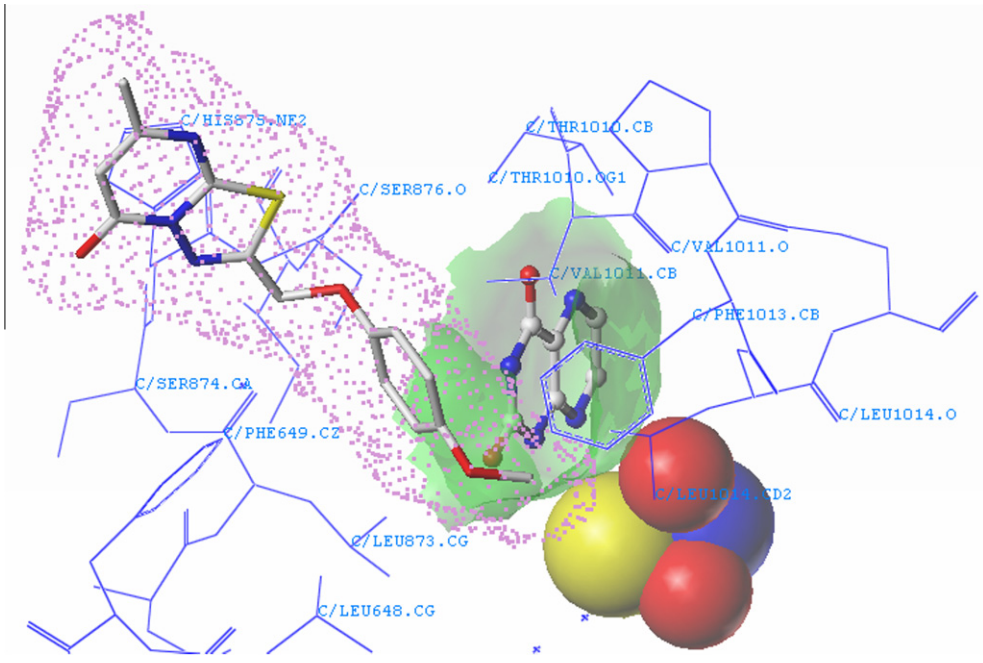


Figure 4. The active compound **6a** is shown in sticks (inside the dotted Connolly surface) in comparison with the allopurinol binding site (complexed with Lumazine, an allopurinol analogue inside the transparent green Connolly surface). The spherical balls closer to the compounds represent molybdenum (blue), Sulfur (yellow) and oxygens O1 and O2 (red).

solvent removed under reduced pressure. The residual mass was triturated with ice water to remove potassium carbonate and extracted with ether (3 × 50 mL) and the ether layer was washed with 10% sodium hydroxide solution (3 × 30 mL) followed by water (3 × 30 mL) and then dried over anhydrous sodium sulphate and evaporated to dryness to get crude solid, which on recrystallization with ethanol gave **2a** (yield 80%) as colorless paste mass.

Compound **2a**: IR (Nujol): 1735 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.22 (t, J = 7 Hz, 3H, $-CH_3$), 3.71 (s, 3H, $-OCH_3$), 4.22 (q, J = 6 Hz, 2H, $-CH_2-$), 4.45 (s, 2H, $-OCH_2-$), 6.92–7.40 (m, 4H, Ar-H). Anal. Calcd for $C_{11}H_{14}O_4$ (210): C, 62.85; H, 6.71. Found: C, 62.71; H, 6.41.

Compound **2b**: Yield (79%); IR (Nujol): 1715 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.25 (t, J = 7 Hz, 3H, $-CH_3$), 4.25 (q, J = 6 Hz, 2H, $-CH_2-$), 4.51 (s, 2H, $-OCH_2-$), 6.82–7.33 (m, 4H, Ar-H). Anal. Calcd for $C_{10}H_{11}ClO_3$ (214.5): C, 55.96; H, 5.17; Cl, 16.52. Found: C, 55.79; H, 5.29; Cl, 16.42.

Compound **2c**: Yield (76%); IR (Nujol): 1730 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.30 (t, J = 7 Hz, 3H, $-CH_3$), 4.31 (q, J = 6 Hz, 2H, $-CH_2-$), 4.55 (s, 2H, $-OCH_2-$), 6.95–7.45 (m, 4H, Ar-H). Anal. Calcd for $C_{10}H_{11}BrO_3$ (259): C, 46.36; H, 4.28; Br, 30.84. Found: C, 46.18; H, 4.44; Br, 30.71.

Compound **2d**: Yield (81%); IR (Nujol): 1740 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.25 (t, J = 7 Hz, 3H, $-CH_3$), 2.25 (s, 3H, Ar-CH_3), 4.29 (q, J = 6 Hz, 2H, $-CH_2-$), 4.49 (s, 2H, $-OCH_2-$), 6.99–7.35 (m, 4H, Ar-H). Anal. Calcd for $C_{11}H_{14}O_3$ (194): C, 68.02; H, 7.27. Found: C, 68.23; H, 7.42.

Compound **2e**: Yield (77%); IR (Nujol): 1720 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.24 (t, J = 7 Hz, 3H, $-CH_3$), 4.32 (q, J = 6 Hz, 2H, $-CH_2-$), 4.50 (s, 2H, $-OCH_2-$), 6.89–7.32 (m, 4H, Ar-H). Anal. Calcd for $C_{10}H_{11}ClO_3$ (214.5): C, 55.96; H, 5.17; Cl, 16.52. Found: C, 55.79; H, 5.29; Cl, 16.42.

Compound **2f**: Yield (75%); IR (Nujol): 1720 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.30 (t, J = 7 Hz, 3H, $-CH_3$), 2.38 (s, 3H, Ar-CH_3), 4.50 (q, J = 6 Hz, 2H, $-CH_2-$), 4.60 (s, 2H, $-OCH_2-$), 6.95–7.41 (m, 3H, Ar-H). Anal. Calcd for $C_{11}H_{13}ClO_3$ (228.5): C, 57.78; H, 5.73; Cl, 15.50. Found: C, 57.54; H, 5.21; Cl, 15.22.

Compound **2g**: Yield (80%); IR (Nujol): 1710 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.19 (t, J = 7 Hz, 3H, $-CH_3$), 2.22 (s, 3H, Ar-CH_3), 4.19 (q, J = 6 Hz, 2H, $-CH_2-$), 4.38 (s, 2H, $-OCH_2-$), 6.75–7.25 (m, 4H, Ar-H). Anal. Calcd for $C_{11}H_{14}O_3$ (194): C, 68.02; H, 7.27. Found: C, 68.25; H, 7.42.

Compound **2h**: Yield (78%); IR (Nujol): 1760 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.35 (t, J = 7 Hz, 3H, $-CH_3$), 4.30 (q, J = 6 Hz, 2H, $-CH_2-$), 4.60 (s, 2H, $-OCH_2-$), 7.00–7.45 (m, 4H, Ar-H). Anal. Calcd for $C_{10}H_{11}BrO_3$ (259): C, 46.36; H, 4.28; Br, 30.84. Found: C, 46.11; H, 4.43; Br, 30.66.

Compound **2i**: Yield (72%); IR (Nujol): 1780 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.32 (t, J = 7 Hz, 3H, $-CH_3$), 4.41 (q, J = 6 Hz, 2H, $-CH_2-$), 4.65 (s, 2H, $-OCH_2-$), 6.95–7.42 (m, 4H, Ar-H). Anal. Calcd for $C_{10}H_{11}FO_3$ (198): C, 60.60; H, 5.59; F, 9.59. Found: C, 60.49; H, 5.41; F, 9.39.

Compound **2j**: Yield (82%); IR (Nujol): 1700 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.15 (t, J = 7 Hz, 3H, $-CH_3$), 4.39 (q, J = 6 Hz, 2H, $-CH_2-$), 4.59 (s, 2H, $-OCH_2-$), 6.99–7.51 (m, 4H, Ar-H). Anal. Calcd for $C_{10}H_{11}NO_5$ (225): C, 53.33; H, 4.92; N, 6.22. Found: C, 53.55; H, 4.78; N, 6.41.

Compound **2k**: Yield (74%); IR (Nujol): 1715 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.29 (t, J = 7 Hz, 3H, $-CH_3$), 4.35 (q, J = 6 Hz, 2H, $-CH_2-$), 4.45 (s, 2H, $-OCH_2-$), 6.88–7.45 (m, 4H, Ar-H). Anal. Calcd for $C_{10}H_{11}NO_5$ (225): C, 53.33; H, 4.92; N, 6.22. Found: C, 53.11; H, 4.83; N, 6.41.

Compound **2l**: Yield (71%); IR (Nujol): 1725 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.29 (t, J = 7 Hz, 3H, $-CH_3$), 2.25 (s, 6H, Ar-$2CH_3$), 4.09 (q, J = 6 Hz, 2H, $-CH_2-$), 4.39 (s, 2H, $-OCH_2-$), 6.79–7.18 (m, 3H, Ar-H). Anal. Calcd for $C_{12}H_{16}O_3$ (208): C, 69.21; H, 7.74. Found: C, 61.21; H, 7.59.

Compound **2m**: Yield (79%); IR (Nujol): 1740 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.19 (t, J = 7 Hz, 3H, $-CH_3$), 4.30 (q, J = 6 Hz, 2H, $-CH_2-$), 4.51 (s, 2H, $-OCH_2-$), 6.65–7.09 (m, 4H, Ar-H). Anal. Calcd for $C_{10}H_{11}FO_3$ (198): C, 60.60; H, 5.59; F, 9.59. Found: C, 60.53; H, 5.72; F, 9.42.

Compound **2n**: Yield (76%); IR (Nujol): 1770 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.25 (t, J = 7 Hz, 3H, $-CH_3$), 4.41 (q, J = 6 Hz, 2H, $-CH_2-$), 4.53 (s, 2H, $-OCH_2-$), 6.90–7.31 (m, 4H, Ar-H). Anal. Calcd for $C_{10}H_{11}NO_5$ (225): C, 53.33; H, 4.92; N, 6.22. Found: C, 53.15; H, 4.88; N, 6.38.

Compound **2o**: Yield (80%); IR (Nujol): 1700 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.18 (t, J = 7 Hz, 3H, $-CH_3$), 2.15 (s, 3H, Ar-CH_3), 4.19 (q, J = 6 Hz, 2H, $-CH_2-$), 4.48 (s, 2H, $-OCH_2-$), 6.83–7.25 (m, 4H, Ar-H). Anal. Calcd for $C_{11}H_{14}O_3$ (194): C, 68.02; H, 7.27. Found: C, 68.19; H, 7.33.

Compound **2p**: Yield (79%); IR (Nujol): 1740 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.30 (t, J = 7 Hz, 3H, $-CH_3$), 4.18 (q, J = 6 Hz, 2H, $-CH_2-$), 4.50 (s, 2H, $-OCH_2-$), 6.91–7.15 (m, 4H, Ar-H). Anal. Calcd for $C_{10}H_{11}ClO_3$ (214.5): C, 55.96; H, 5.17; Cl, 16.52. Found: C, 55.83; H, 5.31; Cl, 16.65.

Compound **2q**: Yield (77%); IR (Nujol): 1780 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.32 (t, J = 7 Hz, 3H, $-CH_3$), 4.21 (q, J = 6 Hz, 2H, $-CH_2-$), 4.61 (s, 2H, $-OCH_2-$), 7.08–7.41 (m, 4H, Ar-H). Anal. Calcd for $C_{10}H_{11}BrO_3$ (259): C, 46.36; H, 4.28; Br, 30.84. Found: C, 46.18; H, 4.07; Br, 30.71.

Compound **2r**: Yield (70%); IR (Nujol): 1775 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.25 (t, J = 7 Hz, 3H, $-CH_3$), 4.30 (q, J = 6 Hz, 2H, $-CH_2-$), 4.62 (s, 2H, $-OCH_2-$), 6.99–7.42 (m, 4H, Ar-H). Anal. Calcd for $C_{10}H_{11}FO_3$ (198): C, 60.60; H, 5.59; F, 9.59. Found: C, 60.46; H, 5.39; F, 9.42.

Compound **2s**: Yield (72%); IR (Nujol): 1720 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.15 (t, J = 7 Hz, 3H, $-CH_3$), 4.22 (q, J = 6 Hz, 2H, $-CH_2-$), 4.45 (s, 2H, $-OCH_2-$), 6.81–7.25 (m, 5H, Ar-H). Anal. Calcd for $C_{10}H_{12}O_3$ (180): C, 66.65; H, 6.71. Found: C, 66.80; H, 6.56.

Compound **2t**: Yield (70%); IR (Nujol): 1735 cm^{-1} (ester, C=O); ^1H NMR ($CDCl_3$): δ 1.26 (t, J = 7 Hz, 3H, $-CH_3$), 2.32 (s, 6H, Ar-$2CH_3$), 4.18 (q, J = 6 Hz, 2H, $-CH_2-$), 4.44 (s, 2H, $-OCH_2-$), 6.80–7.15 (m, 3H, Ar-H). Anal. Calcd for $C_{12}H_{16}O_3$ (208): C, 69.21; H, 7.74. Found: C, 61.33; H, 7.32.

4.1.2. Synthesis of 4-methoxy phenoxy acetohydrazide (3a)

To **2a** (2 g, 9.5 mmol) in methanol (10 mL), 80% hydrazine hydrate (0.475 g, 9.5 mmol) was added in drops and stirred for 1 h at room temperature. A white solid separated, which on recrystallization with ethanol gave **3a** (75%) as white needles.

Compound **3a**: Mp 177–180 °C; IR (KBr): 1655 (amide, C=O), 3110–3215 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.71 (br s, 2H, $-NH_2$), 3.84 (s, 3H, $-OCH_3$), 4.52 (s, 2H, $-OCH_2-$), 6.91–7.43 (m, 4H, Ar-H), 9.25 (br s, 1H, –CO–NH–). Anal. Calcd for $C_9H_{12}N_2O_3$ (196): C, 55.09; H, 6.16; N, 14.28. Found: C, 55.21; H, 6.27; N, 14.43.

Compound **3b**: Yield (70%); mp 175–177 °C; IR (KBr): 1645 (amide, C=O), 3100–3205 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.55 (br s, 2H, $-NH_2$), 4.55 (s, 2H, $-OCH_2-$), 7.29–7.60 (m, 4H, Ar-H), 9.41 (br s, 1H, –CO–NH–). Anal. Calcd for $C_8H_9ClN_2O_2$ (200.5): C, 47.89; H, 4.52; Cl, 17.67; N, 13.96. Found: C, 47.77; H, 4.39; Cl, 17.51; N, 13.8.

Compound **3c**: Yield (69%); mp 185–187 °C; IR (Nujol): 1660 (amide, C=O), 3115–3220 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.72 (br s, 2H, $-NH_2$), 4.55 (s, 2H, $-OCH_2-$), 7.09–7.51 (m, 4H, Ar-H), 9.39 (br s, 1H, –CO–NH–). Anal. Calcd for $C_8H_9BrN_2O_2$ (245): C, 39.21; H, 3.70; Br, 32.60; N, 11.43. Found: C, 39.42; H, 3.82; Br, 32.75; N, 11.69.

Compound **3d**: Yield (71%); mp 179–181 °C; IR (Nujol): 1650 (amide, C=O), 3105–3210 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 2.23 (s, 3H, –CH_3), 3.55 (br s, 2H, –NH_2), 4.53 (s, 2H, –OCH_2–), 7.29–7.61 (m, 4H, Ar-H), 9.28 (br s, 1H, –CO–NH–). Anal. Calcd for $C_9H_{12}N_2O_2$ (180): C, 59.99; H, 6.71; N, 15.55. Found: C, 59.82; H, 6.62; N, 15.44.

Compound **3e**: Yield (68%); mp 167–169 °C; IR (KBr): 1615 (amide, C=O), 3140–3245 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.61 (br s, 2H, –NH_2), 4.61 (s, 2H, –OCH_2–), 6.92–7.35 (m, 4H, Ar-H), 9.41 (br s, 1H, –CO–NH–). Anal. Calcd for $C_8H_9ClN_2O_2$ (200.5): C, 47.89; H, 4.52; Cl, 17.67; N, 13.96. Found: C, 47.71; H, 4.33; Cl, 17.57; N, 13.82.

Compound **3f**: Yield (70%); mp 161–163 °C; IR (Nujol): 1620 (amide, C=O), 3115–3220 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 2.19 (s, 3H, –CH_3), 3.58 (br s, 2H, –NH_2), 4.49 (s, 2H, –OCH_2–), 6.91–7.35 (m, 3H, Ar-H), 9.19 (br s, 1H, –CO–NH–). Anal. Calcd for $C_9H_{11}ClN_2O_2$ (214.5): C, 50.36; H, 5.17; Cl, 16.52; N, 13.05. Found: C, 50.25; H, 5.28; Cl, 16.32; N, 13.15.

Compound **3g**: Yield (69%); mp 172–174 °C; IR (Nujol): 1640 (amide, C=O), 3105–3210 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 2.25 (s, 3H, –CH_3), 3.51 (br s, 2H, –NH_2), 4.55 (s, 2H, –OCH_2–), 7.17–7.45 (m, 4H, Ar-H), 9.06 (br s, 1H, –CO–NH–). Anal. Calcd for $C_9H_{12}N_2O_2$ (180): C, 59.99; H, 6.71; N, 15.55. Found: C, 59.77; H, 6.61; N, 15.49.

Compound **3h**: Yield (72%); mp 161–163 °C; IR (KBr): 1605 (amide, C=O), 3160–3255 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.55 (br s, 2H, –NH_2), 4.55 (s, 2H, –OCH_2–), 7.12–7.43 (m, 4H, Ar-H), 9.17 (br s, 1H, –CO–NH–). Anal. Calcd for $C_8H_9BrN_2O_2$ (245): C, 39.21; H, 3.70; Br, 32.60; N, 11.43. Found: C, 39.46; H, 3.56; Br, 32.49; N, 11.29.

Compound **3i**: Yield (68%); mp 155–157 °C; IR (KBr): 1635 (amide, C=O), 3150–3250 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.43 (br s, 2H, –NH_2), 4.61 (s, 2H, –OCH_2–), 6.95–7.35 (m, 4H, Ar-H), 9.15 (br s, 1H, –CO–NH–). Anal. Calcd for $C_8H_9FN_2O_2$ (184): C, 52.17; H, 4.93; F, 10.32; N, 15.21. Found: C, 52.29; H, 4.81; F, 10.19; N, 15.44.

Compound **3j**: Yield (71%); mp 180–182 °C; IR (KBr): 1640 (amide, C=O), 3130–3230 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.31 (br s, 2H, –NH_2), 4.53 (s, 2H, –OCH_2–), 6.92–7.33 (m, 4H, Ar-H), 9.16 (br s, 1H, –CO–NH–). Anal. Calcd for $C_8H_9FN_3O_4$ (211): C, 45.50; H, 4.30; N, 19.90. Found: C, 45.63; H, 4.41; N, 19.81.

Compound **3k**: Yield (67%); mp 187–189 °C; IR (KBr): 1630 (amide, C=O), 3140–3230 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.60 (br s, 2H, –NH_2), 4.62 (s, 2H, –OCH_2–), 6.82–7.25 (m, 4H, Ar-H), 9.23 (br s, 1H, –CO–NH–). Anal. Calcd for $C_8H_9FN_3O_4$ (211): C, 45.50; H, 4.30; N, 19.90. Found: C, 45.59; H, 4.46; N, 19.78.

Compound **3l**: Yield (71%); mp 166–168 °C; IR (Nujol): 1670 (amide, C=O), 3105–3210 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 2.39 (s, 6H, 2-CH_3), 3.51 (br s, 2H, –NH_2), 4.45 (s, 2H, –OCH_2–), 7.18–7.40 (m, 3H, Ar-H), 9.13 (br s, 1H, –CO–NH–). Anal. Calcd for $C_{10}H_{14}N_2O_2$ (194): C, 61.84; H, 7.27; N, 14.42. Found: C, 61.71; H, 7.42; N, 14.29.

Compound **3m**: Yield (72%); mp 150–152 °C; IR (KBr): 1645 (amide, C=O), 3110–3230 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.51 (br s, 2H, –NH_2), 4.65 (s, 2H, –OCH_2–), 7.09–7.45 (m, 4H, Ar-H), 9.23 (br s, 1H, –CO–NH–). Anal. Calcd for $C_8H_9FN_2O_2$ (184): C, 52.17; H, 4.93; F, 10.32; N, 15.21. Found: C, 52.22; H, 4.83; F, 10.22; N, 15.32.

Compound **3n**: Yield (69%); mp 187–189 °C; IR (KBr): 1655 (amide, C=O), 3110–3260 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.35 (br s, 2H, –NH_2), 4.55 (s, 2H, –OCH_2–), 6.95–7.35 (m, 4H, Ar-H), 9.32 (br s, 1H, –CO–NH–). Anal. Calcd for $C_8H_9FN_3O_4$ (211): C, 45.50; H, 4.30; N, 19.90. Found: C, 45.39; H, 4.44; N, 19.99.

Compound **3o**: Yield (71%); mp 170–172 °C; IR (Nujol): 1655 (amide, C=O), 3100–3200 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 2.19 (s, 3H, –CH_3), 3.41 (br s, 2H, –NH_2), 4.45 (s, 2H, –OCH_2–), 7.19–7.50 (m, 4H, Ar-H), 9.15 (br s, 1H, –CO–NH–). Anal. Calcd for $C_9H_{12}N_2O_2$ (180): C, 59.99; H, 6.71; N, 15.55. Found: C, 59.85; H, 6.58; N, 15.42.

Compound **3p**: Yield (66%); mp 179–181 °C; IR (KBr): 1665 (amide, C=O), 3160–3265 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.45 (br s, 2H, –NH_2), 4.5 (s, 2H, –OCH_2–), 7.19–7.55 (m, 4H, Ar-H), 9.31 (br s, 1H, –CO–NH–). Anal. Calcd for $C_8H_9ClN_2O_2$ (200.5): C, 47.89; H, 4.52; Cl, 17.67; N, 13.96. Found: C, 47.81; H, 4.42; Cl, 17.77; N, 13.85.

Compound **3q**: Yield (70%); mp 161–163 °C; IR (KBr): 1605 (amide, C=O), 3160–3255 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.55 (br s, 2H, –NH_2), 4.55 (s, 2H, –OCH_2–), 7.14–7.50 (m, 4H, Ar-H), 9.11 (br s, 1H, –CO–NH–). Anal. Calcd for $C_8H_9BrN_2O_2$ (245): C, 39.21; H, 3.70; Br, 32.60; N, 11.43. Found: C, 39.46; H, 3.56; Br, 32.49; N, 11.29.

Compound **3r**: Yield (69%); mp 147–149 °C; IR (KBr): 1675 (amide, C=O), 3120–3230 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.54 (br s, 2H, –NH_2), 4.55 (s, 2H, –OCH_2–), 7.08–7.55 (m, 4H, Ar-H), 9.21 (br s, 1H, –CO–NH–). Anal. Calcd for $C_8H_9FN_2O_2$ (184): C, 52.17; H, 4.93; F, 10.32; N, 15.21. Found: C, 52.26; H, 4.78; F, 10.22; N, 15.15.

Compound **3s**: Yield (70%); mp 149–151 °C; IR (Nujol): 1610 (amide, C=O), 3100–3200 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 3.31 (br s, 2H, –NH_2), 4.54 (s, 2H, –OCH_2–), 7.09–7.45 (m, 5H, Ar-H), 9.07 (br s, 1H, –CO–NH–). Anal. Calcd for $C_9H_{12}N_2O_2$ (166): C, 57.82; H, 6.07; N, 16.86. Found: C, 57.69; H, 6.18; N, 16.70.

Compound **3t**: Yield (72%); mp 186–188 °C; IR (Nujol): 1660 (amide, C=O), 3125–3220 cm^{-1} (NH–NH_2); ^1H NMR (CD_3COCD_3): δ 2.25 (s, 6H, –CH_3), 3.53 (br s, 2H, –NH_2), 4.53 (s, 2H, –OCH_2–), 7.05–7.55 (m, 3H, Ar-H), 9.13 (br s, 1H, –CO–NH–). Anal. Calcd for $C_{10}H_{14}N_2O_2$ (194): C, 61.84; H, 7.27; N, 14.42. Found: C, 61.70; H, 7.39; N, 14.30.

4.1.3. Synthesis of 4-methoxy phenoxy acetyl-*N*-hydrazine carbothioamides (4a)

A mixture of potassium isothiocyanate (0.44 g, 3.28 mmol) and **3a** (0.6 g, 3.18 mmol) in dry ethanol (20 ml) was refluxed with stirring for 4 h, the mixture was cooled, filtered, washed with ethanol, dried and recrystallized with ethanol to give **4a** (60%) as colorless needles.

Compound **4a**: Yield (58%); mp 210–213 °C; IR (Nujol): 1235 (C=S), 1700 (amide, C=O), 3200–3300 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 3.78 (s, 3H, –OCH_3), 4.62 (s, 2H, –OCH_2–), 7.01–7.55 (m, 4H, Ar-H), 9.52 (br s, 2H, 2-NH–), 10.11 (s, 2H, –NH_2). Anal. Calcd for $C_{10}H_{13}N_3O_3S$ (255): C, 47.05; H, 5.13; N, 16.46; S, 12.56. Found: C, 47.25; H, 5.23; N, 16.36; S, 12.36.

Compound **4b**: Yield (61%); mp 200–203 °C; IR (Nujol): 1230 (C=S), 1710 (amide, C=O), 3250–3350 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.56 (s, 2H, –OCH_2–), 6.99–7.51 (m, 4H, Ar-H), 9.48 (br s, 2H, 2-NH–), 10.08 (s, 2H, –NH_2). Anal. Calcd for $C_9H_{10}ClN_3O_2S$ (259.5): C, 41.62; H, 3.88; Cl, 13.65; N, 16.18; S, 12.35. Found: C, 41.51; H, 3.43; Cl, 13.49; N, 16.26; S, 12.24.

Compound **4c**: Yield (59%); mp 218–220 °C; IR (Nujol): 1225 (C=S), 1710 (amide, C=O), 3150–3360 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.55 (s, 2H, –OCH_2–), 6.95–7.41 (m, 4H, Ar-H), 9.37 (br s, 2H, 2-NH–), 10.21 (s, 2H, –NH_2). Anal. Calcd for $C_9H_{10}BrN_3O_2S$ (304): C, 35.54; H, 3.31; Br, 26.27; N, 13.81; S, 10.54. Found: C, 35.39; H, 3.48; Br, 26.18; N, 13.65; S, 10.41.

Compound **4d**: Yield (60%); mp 205–208 °C; IR (Nujol): 1205 (C=S), 1720 (amide, C=O), 3210–3325 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 2.18 (s, 3H, –CH_3), 4.65 (s, 2H, –OCH_2–), 6.95–7.52 (m, 4H, Ar-H), 9.46 (br s, 2H, 2-NH–), 10.15 (s, 2H, –NH_2). Anal. Calcd for $C_{10}H_{13}N_3O_2S$ (239): C, 50.19; H, 5.48; N, 17.56; S, 13.40. Found: C, 50.29; H, 5.31; N, 17.38; S, 13.32.

Compound **4e**: Yield (62%); mp 195–197 °C; IR (Nujol): 1240 (C=S), 1740 (amide, C=O), 3260–3360 cm^{-1} (NH–NH, NH); ^1H

NMR (CD_3COCD_3): δ 4.70 (s, 2H, $-OCH_2-$), 7.06–7.55 (m, 4H, Ar-H), 9.45 (br s, 2H, 2-NH–), 10.21 (s, 2H, $-NH_2$). Anal. Calcd for $C_9H_{10}ClN_3O_2S$ (259.5): C, 41.62; H, 3.88; Cl, 13.65; N, 16.18; S, 12.35. Found: C, 41.73; H, 3.61; Cl, 13.82; N, 16.28 S, 12.48.

Compound **4f**: Yield (60%); mp 220–222 °C; IR (Nujol): 1270 (C=S), 1760 (amide, C=O), 3260–3355 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.62 (s, 2H, $-OCH_2-$), 7.02–7.60 (m, 3H, Ar-H), 9.47 (br s, 2H, 2-NH–), 10.16 (s, 2H, $-NH_2$). Anal. Calcd for $C_{10}H_{12}ClN_3O_2S$ (273.5): C, 43.88; H, 4.42; Cl, 12.95; N, 15.35; S, 11.71. Found: C, 43.62; H, 4.21; Cl, 12.76; N, 15.51; S, 11.59.

Compound **4g**: Yield (56%); mp 215–217 °C; IR (Nujol): 1235 (C=S), 1735 (amide, C=O), 3230–3335 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 2.34 (s, 3H, $-CH_3$), 4.56 (s, 2H, $-OCH_2-$), 6.92–7.45 (m, 4H, Ar-H), 9.34 (br s, 2H, 2-NH–), 10.32 (s, 2H, $-NH_2$). Anal. Calcd for $C_{10}H_{13}N_3O_2S$ (239): C, 50.19; H, 5.48; N, 17.56; S, 13.40. Found: C, 50.31; H, 5.61; N, 17.41; S, 13.28.

Compound **4h**: Yield (63%); mp 228–230 °C; IR (Nujol): 1235 (C=S), 1730 (amide, C=O), 3160–3365 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.62 (s, 2H, $-OCH_2-$), 6.92–7.35 (m, 4H, Ar-H), 9.17 (br s, 2H, 2-NH–), 10.19 (s, 2H, $-NH_2$). Anal. Calcd for $C_9H_{10}BrN_3O_2S$ (304): C, 35.54; H, 3.31; Br, 26.27; N, 13.81; S, 10.54. Found: C, 35.41; H, 3.22; Br, 26.38; N, 13.70; S, 10.68.

Compound **4i**: Yield (61%); mp 190–192 °C; IR (Nujol): 1275 (C=S), 1750 (amide, C=O), 3260–3365 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.71 (s, 2H, $-OCH_2-$), 6.92–7.34 (m, 4H, Ar-H), 9.55 (br s, 2H, 2-NH–), 10.34 (s, 2H, $-NH_2$). Anal. Calcd for $C_9H_{10}FN_3O_2S$ (243): C, 44.44; H, 4.14; F, 7.81; N, 17.27; S, 13.18. Found: C, 44.58; H, 4.22; F, 7.69; N, 17.11; S, 13.30.

Compound **4j**: Yield (59%); mp 196–198 °C; IR (Nujol): 1280 (C=S), 1760 (amide, C=O), 3255–3365 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.75 (s, 2H, $-OCH_2-$), 6.81–7.23 (m, 4H, Ar-H), 9.42 (br s, 2H, 2-NH–), 10.21 (s, 2H, $-NH_2$). Anal. Calcd for $C_9H_{10}N_4O_4S$ (270): C, 40.00; H, 3.73; N, 20.73; S, 11.86. Found: C, 40.11; H, 3.88; N, 20.63; S, 11.77.

Compound **4k**: Yield (56%); mp 178–180 °C; IR (Nujol): 1285 (C=S), 1765(amide, C=O), 3265–3355 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.73 (s, 2H, $-OCH_2-$), 6.91–7.25 (m, 4H, Ar-H), 9.34 (br s, 2H, 2-NH–), 10.11 (s, 2H, $-NH_2$). Anal. Calcd for $C_9H_{10}N_4O_4S$ (270): C, 40.00; H, 3.73; N, 20.73; S, 11.86. Found: C, 40.17; H, 3.62; N, 20.66; S, 11.85.

Compound **4l**: Yield (61%); mp 161–163 °C; IR (Nujol): 1275 (C=S), 1740 (amide, C=O), 3230–3345 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 2.31 (s, 6H, 2-CH_3), 4.65 (s, 2H, $-OCH_2-$), 6.65–7.17 (m, 3H, Ar-H), 9.23 (br s, 2H, 2-NH–), 10.01 (s, 2H, $-NH_2$). Anal. Calcd for $C_{11}H_{15}N_3O_2S$ (253): C, 52.15; H, 5.97; N, 16.59; S, 12.66. Found: C, 52.28; H, 5.82; N, 16.49; S, 12.52.

Compound **4m**: Yield (55%); mp 230–132 °C; IR (Nujol): 1200 (C=S), 1720 (amide, C=O), 3230–3345 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.80 (s, 2H, $-OCH_2-$), 7.10–7.45 (m, 4H, Ar-H), 9.62 (br s, 2H, 2-NH–), 10.43 (s, 2H, $-NH_2$). Anal. Calcd for $C_9H_{10}FN_3O_2S$ (243): C, 44.44; H, 4.14; F, 7.81; N, 17.27; S, 13.18. Found: C, 44.61; H, 4.28; F, 7.73; N, 17.40; S, 13.28.

Compound **4n**: Yield 62%); mp 150–152 °C; IR (Nujol): 1235 (C=S), 1740 (amide, C=O), 3270–3385 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.65 (s, 2H, $-OCH_2-$), 6.95–7.43 (m, 4H, Ar-H), 9.42 (br s, 2H, 2-NH–), 10.15 (s, 2H, $-NH_2$). Anal. Calcd for $C_9H_{10}FN_3O_2S$ (243): C, 44.44; H, 4.14; F, 7.81; N, 17.27; S, 13.18. Found: C, 44.61; H, 4.27; F, 7.66; N, 17.38; S, 13.28.

Compound **4o**: Yield (59%); mp 240–242 °C; IR (Nujol): 1225 (C=S), 1725 (amide, C=O), 3240–3355 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 2.25 (s, 3H, $-CH_3$), 4.82 (s, 2H, $-OCH_2-$), 7.01–7.43 (m, 4H, Ar-H), 9.32 (br s, 2H, 2-NH–), 10.22 (s, 2H, $-NH_2$). Anal. Calcd for $C_{10}H_{13}N_3O_2S$ (239): C, 50.19; H, 5.48; N, 17.56; S, 13.40. Found: C, 50.30; H, 5.60; N, 17.05; S, 13.50.

Compound **4p**: Yield (55%); mp 253–255 °C; IR (Nujol): 1290 (C=S), 1740 (amide, C=O), 3280–3360 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.91 (s, 2H, $-OCH_2-$), 7.11–7.55 (m, 4H, Ar-H), 9.51(br s, 2H, 2-NH–), 10.30 (s, 2H, $-NH_2$). Anal. Calcd for $C_9H_{10}ClN_3O_2S$ (259.5): C, 41.62; H, 3.88; Cl, 13.65; N, 16.18; S, 12.35. Found: C, 41.76; H, 3.99; Cl, 13.88; N, 16.32; S, 12.48.

Compound **4q**: Yield (60%); mp 260–262 °C; IR (Nujol): 1245 (C=S), 1790 (amide, C=O), 3170–3385 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.77 (s, 2H, $-OCH_2-$), 7.09–7.42 (m, 4H, Ar-H), 9.52 (br s, 2H, 2-NH–), 10.51 (s, 2H, $-NH_2$). Anal. Calcd for $C_9H_{10}BrN_3O_2S$ (304): C, 35.54; H, 3.31; Br, 26.27; N, 13.81; S, 10.54. Found: C, 35.65; H, 3.43; Br, 26.19; N, 13.93; S, 10.43.

Compound **4r**: Yield (63%); mp 235–237 °C; IR (Nujol): 1240 (C=S), 1770 (amide, C=O), 3270–3385 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.75 (s, 2H, $-OCH_2-$), 7.02–7.41 (m, 4H, Ar-H), 9.65 (br s, 2H, 2-NH–), 10.51 (s, 2H, $-NH_2$). Anal. Calcd for $C_9H_{10}FN_3O_2S$ (243): C, 44.44; H, 4.14; F, 7.81; N, 17.27; S, 13.18. Found: C, 44.51; H, 4.04; F, 7.69; N, 17.10; S, 13.06.

Compound **4s**: Yield (61%); mp 183–185 °C; IR (Nujol): 1245 (C=S), 1720 (amide, C=O), 3200–3300 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 4.73 (s, 2H, $-OCH_2-$), 7.07–7.44 (m, 5H, Ar-H), 9.43 (br s, 2H, 2-NH–), 10.33 (s, 2H, $-NH_2$). Anal. Calcd for $C_9H_{11}N_3O_2S$ (225): C, 47.99; H, 4.92; N, 18.65; S, 14.23. Found: C, 47.83; H, 4.80; N, 18.51; S, 14.19.

Compound **4t**: Yield (58%); mp 155–157 °C; IR (Nujol): 1295 (C=S), 1780 (amide, C=O), 3240–3345 cm^{-1} (NH–NH, NH); ^1H NMR (CD_3COCD_3): δ 2.03 (s, 6H, 2-CH_3), 4.85 (s, 2H, $-OCH_2-$), 6.78–7.15 (m, 3H, Ar-H), 9.31 (br s, 2H, 2-NH–), 10.22 (s, 2H, $-NH_2$). Anal. Calcd for $C_{11}H_{15}N_3O_2S$ (253): C, 52.15; H, 5.97; N, 16.59; S, 12.66. Found: C, 52.22; H, 5.86; N, 16.47; S, 12.54.

4.4.4. Synthesis of 5(4-methoxy) phenoxy-2-amino-1,3,4-thiadiazoles (5a)

Compound **4a** (0.3 g, 0.89 mmol) was added gradually to anhydrous orthophosphoric acid (8 ml) in about 5 min. The reaction mixture was heated in an oil bath at 120 °C for 2 h. The slurry thus obtained was poured on crushed ice. The solid was filtered, washed with cold distilled water (3 × 15 ml), dried and recrystallized with ethanol to give **5a (**55%) as brown solid.

Compound **5a**: Yield (52%); mp 130–133 °C; IR (Nujol): 750 (C–S–C), 1620 (C=N), 3150 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 3.75 (s, 3H, $-OCH_3$), 4.68 (s, 2H, $-OCH_2-$), 4.85 (br s, 1H, –NH, D_2O exchangeable), 7.02–7.51 (m, 4H, Ar-H); EI-MS: *m*/*z* 237 (M^+, 45). Anal. Calcd for $C_{10}H_{11}ClN_3O_2S$ (237): C, 50.62; H, 4.67; N, 17.71; S, 13.51. Found: C, 50.47; H, 4.51; N, 17.85; S, 13.75.

Compound **5b**: Yield (50%); mp 128–130 °C; IR (Nujol): 750 (C–S–C), 1615 (C=N), 3140 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.52 (s, 2H, $-OCH_2-$), 4.85 (br s, 1H, –NH, D_2O exchangeable), 7.15–7.64 (m, 4H, Ar-H); EI-MS: *m*/*z* 241.5 (M^+, 40). Anal. Calcd for $C_9H_8ClN_3OS$ (241.5): C, 44.72; H, 3.34; Cl, 14.67; N, 17.39; S, 13.27. Found: C, 44.63; H, 3.41; Cl, 14.53; N, 17.49; S, 13.37.

Compound **5c**: Yield (54%); mp 135–137 °C; IR (Nujol): 745 (C–S–C), 1615 (C=N), 3145 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.59 (s, 2H, $-OCH_2-$), 4.82 (br s, 1H, –NH, D_2O exchangeable), 6.92–7.31 (m, 4H, Ar-H); EI-MS: *m*/*z* 286 (M^+, 43). Anal. Calcd for $C_9H_8BrN_3OS$ (286): C, 37.78; H, 2.82; Br, 27.92; N, 14.68; S, 11.21. Found: C, 37.88; H, 2.72; Br, 27.81; N, 14.76; S, 11.35.

Compound **5d**: Yield (50%); mp 125–127 °C; IR (Nujol): 755 (C–S–C), 1625 (C=N), 3155 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 2.18 (s, 3H, $-CH_3$), 4.62 (s, 2H, $-OCH_2-$), 4.91 (br s, 1H, –NH, D_2O exchangeable), 7.01–7.52 (m, 4H, Ar-H); EI-MS: *m*/*z* 207 (M^+, 47). Anal. Calcd for $C_{10}H_{11}N_3OS$ (221): C, 54.28; H, 5.01; N, 18.99; S, 14.49. Found: C, 54.38; H, 5.11; N, 18.80; S, 14.61.

Compound **5e**: Yield (55%); mp 120–122 °C; IR (Nujol): 755 (C–S–C), 1625 (C=N), 3155 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.62 (s, 2H, $-OCH_2-$), 4.86 (br s, 1H, –NH, D_2O exchangeable), 7.03–7.42 (m, 4H, Ar-H); EI-MS: *m*/*z* 241.5 (M^+, 40). Anal. Calcd

for $C_9H_8ClN_3OS$ (241.5): C, 44.72; H, 3.34; Cl, 14.67; N, 17.39; S, 13.27. Found: C, 44.65; H, 3.42; Cl, 14.51; N, 17.44; S, 13.41.

Compound **5f**: Yield (53%); mp 116–117 °C; IR (Nujol): 765 (C–S–C), 1645 (C=N), 3175 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 2.32 (s, 3H, $-CH_3$), 4.65 (s, 2H, $-OCH_2-$), 4.83 (br s, 1H, –NH, D_2O exchangeable), 7.01–7.35 (m, 3H, Ar-H); EI-MS: *m*/*z* 255.5 (M^+, 40). Anal. Calcd for $C_{10}H_{10}ClN_3OS$ (255.5): C, 46.97; H, 3.94; Cl, 13.86; N, 16.43; S, 12.54. Found: C, 46.83; H, 3.85; Cl, 13.73; N, 16.36; S, 12.67.

Compound **5g**: Yield (50%); mp 141–143 °C; IR (Nujol): 725 (C–S–C), 1655 (C=N), 3175 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 2.28 (s, 3H, $-CH_3$), 4.75 (s, 2H, $-OCH_2-$), 4.85 (br s, 1H, –NH, D_2O exchangeable), 7.13–7.55 (m, 4H, Ar-H); EI-MS: *m*/*z* 207 (M^+, 47). Anal. Calcd for $C_{10}H_{11}N_3OS$ (221): C, 54.28; H, 5.01; N, 18.99; S, 14.49. Found: C, 54.36; H, 5.13; N, 18.82; S, 14.64.

Compound **5h**: Yield (56%); mp 105–107 °C; IR (Nujol): 715 (C–S–C), 1655 (C=N), 3155 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.62 (s, 2H, $-OCH_2-$), 4.81 (br s, 1H, –NH, D_2O exchangeable), 6.95–7.34 (m, 4H, Ar-H); EI-MS: *m*/*z* 286 (M^+, 43). Anal. Calcd for $C_9H_8BrN_3OS$ (286): C, 37.78; H, 2.82; Br, 27.92; N, 14.68; S, 11.21. Found: C, 37.83; H, 2.75; Br, 27.84; N, 14.75; S, 11.34.

Compound **5i**: Yield (52%); mp 151–153 °C; IR (Nujol): 775 (C–S–C), 1660 (C=N), 3140 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.62 (s, 2H, $-OCH_2-$), 4.78 (br s, 1H, –NH, D_2O exchangeable), 6.92–7.34 (m, 4H, Ar-H); EI-MS: *m*/*z* 225 (M^+, 45). Anal. Calcd for $C_9H_8FN_3OS$ (225): C, 47.99; H, 3.58; F, 8.43; N, 18.66; S, 14.24. Found: C, 47.78; H, 3.67; F, 8.36; N, 18.52; S, 14.33.

Compound **5j**: Yield (51%); mp 136–138 °C; IR (Nujol): 795 (C–S–C), 1670 (C=N), 3160 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.63 (s, 2H, $-OCH_2-$), 4.75 (br s, 1H, –NH, D_2O exchangeable), 7.01–7.42 (m, 4H, Ar-H); EI-MS: *m*/*z* 252 (M^+, 40). Anal. Calcd for $C_9H_8N_4O_3S$ (252): C, 42.85; H, 3.20; N, 22.21; S, 12.71. Found: C, 42.97; H, 3.28; N, 22.33; S, 12.63.

Compound **5k**: Yield (58%); mp 160–162 °C; IR (Nujol): 750 (C–S–C), 1620 (C=N), 3140 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.71 (s, 2H, $-OCH_2-$), 4.82 (br s, 1H, –NH, D_2O exchangeable), 7.02–7.42 (m, 4H, Ar-H); EI-MS: *m*/*z* 252 (M^+, 40). Anal. Calcd for $C_9H_8N_4O_3S$ (252): C, 42.85; H, 3.20; N, 22.21; S, 12.71. Found: C, 42.98; H, 3.31; N, 22.34; S, 12.79.

Compound **5l**: Yield (54%); mp 169–171 °C; IR (Nujol): 730 (C–S–C), 1640 (C=N), 3150 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 2.25 (s, 6H, 2-CH_3), 4.73 (s, 2H, $-OCH_2-$), 4.92 (br s, 1H, –NH, D_2O exchangeable), 7.04–7.45 (m, 3H, Ar-H); EI-MS: *m*/*z* 235 (M^+, 43). Anal. Calcd for $C_{11}H_{13}N_3OS$ (235): C, 56.15; H, 5.57; N, 17.86; S, 13.63. Found: C, 56.23; H, 5.45; N, 17.74; S, 13.52.

Compound **5m**: Yield (50%); mp 110–112 °C; IR (Nujol): 780 (C–S–C), 1635 (C=N), 3185 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.68 (s, 2H, $-OCH_2-$), 4.76 (br s, 1H, –NH, D_2O exchangeable), 7.05–7.35 (m, 4H, Ar-H); EI-MS: *m*/*z* 225 (M^+, 45). Anal. Calcd for $C_9H_8FN_3OS$ (225): C, 47.99; H, 3.58; F, 8.43; N, 18.66; S, 14.24. Found: C, 47.80; H, 3.66; F, 8.55; N, 18.51; S, 14.16.

Compound **5n**: Yield (56%); mp 180–182 °C; IR (Nujol): 715 (C–S–C), 1615 (C=N), 3150 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.68 (s, 2H, $-OCH_2-$), 4.69 (br s, 1H, –NH, D_2O exchangeable), 7.05–7.35 (m, 4H, Ar-H); EI-MS: *m*/*z* 252 (M^+, 42). Anal. Calcd for $C_9H_8N_4O_3S$ (252): C, 42.85; H, 3.20; N, 22.21; S, 12.71. Found: C, 42.96; H, 3.25; N, 22.32; S, 12.61.

Compound **5o**: Yield (55%); mp 146–148 °C; IR (Nujol): 730 (C–S–C), 1670 (C=N), 3160 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 2.15 (s, 3H, $-CH_3$), 4.75 (s, 2H, $-OCH_2-$), 4.85 (br s, 1H, –NH, D_2O exchangeable), 7.05–7.45 (m, 4H, Ar-H); EI-MS: *m*/*z* 207 (M^+, 46). Anal. Calcd for $C_{10}H_{11}N_3OS$ (221): C, 54.28; H, 5.01; N, 18.99; S, 14.49. Found: C, 54.35; H, 5.15; N, 18.82; S, 14.36.

Compound **5p**: Yield (53%); mp 156–158 °C; IR (Nujol): 760 (C–S–C), 1605 (C=N), 3105 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.65 (s, 2H, $-OCH_2-$), 4.90 (br s, 1H, –NH, D_2O exchangeable), 6.95–7.32 (m, 4H, Ar-H); EI-MS: *m*/*z* 241.5 (M^+, 48). Anal. Calcd for $C_9H_8ClN_3OS$ (241.5): C, 44.72; H, 3.34; Cl, 14.67; N, 17.39; S, 13.27. Found: C, 44.84; H, 3.26; Cl, 14.77; N, 17.26; S, 13.21.

Compound **5q**: Yield (57%); mp 175–177 °C; IR (Nujol): 790 (C–S–C), 1620 (C=N), 3140 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.62 (s, 2H, $-OCH_2-$), 4.81 (br s, 1H, –NH, D_2O exchangeable), 6.95–7.33 (m, 4H, Ar-H); EI-MS: *m*/*z* 286 (M^+, 43). Anal. Calcd for $C_9H_8BrN_3OS$ (286): C, 37.78; H, 2.82; Br, 27.92; N, 14.68; S, 11.21. Found: C, 37.87; H, 2.71; Br, 27.99; N, 14.56; S, 11.09.

Compound **5r**: Yield (54%); mp 192–194 °C; IR (Nujol): 780 (C–S–C), 1650 (C=N), 3140 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.65 (s, 2H, $-OCH_2-$), 4.82 (br s, 1H, –NH, D_2O exchangeable), 6.95–7.34 (m, 4H, Ar-H); EI-MS: *m*/*z* 225 (M^+, 45). Anal. Calcd for $C_9H_8FN_3OS$ (225): C, 47.99; H, 3.58; F, 8.43; N, 18.66; S, 14.24. Found: C, 47.85; H, 3.44; F, 8.56; N, 18.76; S, 14.11.

Compound **5s**: Yield (51%); mp 100–102 °C; IR (Nujol): 735 (C–S–C), 1655 (C=N), 3145 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 4.63 (s, 2H, $-OCH_2-$), 4.75 (br s, 1H, –NH, D_2O exchangeable), 6.92–7.25 (m, 5H, Ar-H); EI-MS: *m*/*z* 207 (M^+, 50). Anal. Calcd for $C_9H_9N_3OS$ (207): C, 52.16; H, 4.38; N, 20.27; S, 15.47. Found: C, 52.28; H, 4.21; N, 20.39; S, 15.35.

Compound **5t**: Yield (49%); mp 198–200 °C; IR (Nujol): 755 (C–S–C), 1655 (C=N), 3155 cm^{-1} (NH); ^1H NMR ($CDCl_3$): δ 2.32 (s, 6H, 2-CH_3), 4.75 (s, 2H, $-OCH_2-$), 4.95 (br s, 1H, –NH, D_2O exchangeable), 7.01–7.43 (m, 3H, Ar-H); EI-MS: *m*/*z* 235 (M^+, 43). Anal. Calcd for $C_{11}H_{13}N_3OS$ (235): C, 56.15; H, 5.57; N, 17.86; S, 13.63. Found: C, 56.29; H, 5.69; N, 17.95; S, 13.51.

4.1.5. A typical procedure is described for the synthesis of 7-methyl-2(4-methoxy) phenoxymethyl-[1,3,4]thiadiazolo[3,2-*a*]pyrimidin-5-one (6a)

To a solution of ethyl acetoacetate (0.075 g, 0.58 mmol) in methanol (10 ml) **5a** (0.15 g, 0.58 mmol) was added and refluxed for 5 h and poured into cold water. The separated solid was filtered, dried and recrystallized form ethanol to afford **6a** (69%) as brown solid.

Compound **6a**: Yield (65%); mp 120–122 °C; IR (Nujol): 1670 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.71 (s, 3H, $-CH_3$), 3.73 (s, 3H, $-OCH_3$), 4.45 (s, 2H, $-OCH_2-$), 6.32 (s, 1H, –CH–), 6.94–7.32 (m, 4H, Ar-H); EI-MS: *m*/*z* 303 (M^+, 55). Anal. Calcd for $C_{14}H_{13}N_3O_3S$ (303): C, 55.43; H, 4.32; N, 13.85; S, 10.57. Found: C, 55.32; H, 4.47; N, 13.71; S, 10.69.

Compound **6b**: Yield (62%); mp 129–131 °C; IR (Nujol): 1660 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.72 (s, 3H, $-CH_3$), 4.44 (s, 2H, $-OCH_2-$), 6.31 (s, 1H, –CH–), 6.95–7.43 (m, 4H, Ar-H); EI-MS: *m*/*z* 307.5 (M^+, 53). Anal. Calcd for $C_{13}H_{10}ClN_3O_2S$ (307.5): C, 50.73; H, 3.28; Cl, 11.52; N, 13.65; S, 10.42. Found: C, 50.61; H, 3.38; Cl, 11.70; N, 13.79; S, 10.31.

Compound **6c**: Yield (61%); mp 135–137 °C; IR (Nujol): 1675 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.74 (s, 3H, $-CH_3$), 4.56 (s, 2H, $-OCH_2-$), 6.25 (s, 1H, –CH–), 7.06–7.44 (m, 4H, Ar-H); EI-MS: *m*/*z* 352 (M^+, 57). Anal. Calcd for $C_{13}H_{10}BrN_3O_2S$ (352): C, 44.33; H, 2.86; Br, 22.69; N, 11.93; S, 9.10. Found: C, 44.22; H, 2.73; Br, 22.79; N, 11.81; S, 9.19.

Compound **6d**: Yield (60%); mp 144–146 °C; IR (Nujol): 1680 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.75 (s, 3H, $-CH_3$), 2.11 (s, 3H, Ar-CH_3), 4.55 (s, 2H, $-OCH_2-$), 6.32 (s, 1H, –CH–), 7.05–7.43 (m, 4H, Ar-H); EI-MS: *m*/*z* 287 (M^+, 60). Anal. Calcd for $C_{14}H_{13}N_3O_2S$ (287): C, 58.52; H, 4.56; N, 14.62; S, 11.16. Found: C, 58.41; H, 4.69; N, 14.72; S, 11.28.

Compound **6e**: Yield (67%); mp 151–153 °C; IR (Nujol): 1680 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.69 (s, 3H, $-CH_3$), 4.53 (s, 2H, $-OCH_2-$), 6.25 (s, 1H, –CH–), 6.93–7.31 (m, 4H, Ar-H); EI-MS: *m*/*z* 307.5 (M^+, 55). Anal. Calcd for $C_{13}H_{10}ClN_3O_2S$ (307.5): C, 50.73; H, 3.28; Cl, 11.52; N, 13.65; S, 10.42. Found: C, 50.85; H, 3.17; Cl, 11.39; N, 13.78; S, 10.56.

Compound **6f**: Yield (60%); mp 160–162 °C; IR (Nujol): 1685 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.74 (s, 3H, $-CH_3$), 2.26 (s, 3H, Ar-CH_3), 4.64 (s, 2H, $-OCH_2-$), 6.35 (s, 1H, –CH–), 7.05–7.37 (m, 3H, Ar-H); EI-MS: *m/z* 321.5 (M^+, 52). Anal. Calcd for $C_{14}H_{12}ClN_3O_2S$ (321.5): C, 52.26; H, 3.76; Cl, 11.02; N, 13.06; S, 9.97. Found: C, 52.41; H, 3.64; Cl, 11.16; N, 13.19; S, 9.86.

Compound **6g**: Yield (65%); mp 178–180 °C; IR (Nujol): 1665 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.71 (s, 3H, $-CH_3$), 2.15 (s, 3H, Ar-CH_3), 4.52 (s, 2H, $-OCH_2-$), 6.29 (s, 1H, –CH–), 7.05–7.47 (m, 4H, Ar-H); EI-MS: *m/z* 287 (M^+, 58). Anal. Calcd for $C_{14}H_{13}N_3O_2S$ (287): C, 58.52; H, 4.56; N, 14.62; S, 11.16. Found: C, 58.65; H, 4.42; N, 14.51; S, 11.05.

Compound **6h**: Yield (62%); mp 165–167 °C; IR (Nujol): 1675 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.73 (s, 3H, $-CH_3$), 4.55 (s, 2H, $-OCH_2-$), 6.32 (s, 1H, –CH–), 6.95–7.31 (m, 4H, Ar-H); EI-MS: *m/z* 352 (M^+, 55). Anal. Calcd for $C_{13}H_{10}BrN_3O_2S$ (352): C, 44.33; H, 2.86; Br, 22.69; N, 11.93; S, 9.10. Found: C, 44.17; H, 2.97; Br, 22.58; N, 11.81; S, 9.22.

Compound **6i**: Yield (63%); mp 185–187 °C; IR (Nujol): 1690 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.75 (s, 3H, $-CH_3$), 4.54 (s, 2H, $-OCH_2-$), 6.31 (s, 1H, –CH–), 6.92–7.25 (m, 4H, Ar-H); EI-MS: *m/z* 291 (M^+, 51). Anal. Calcd for $C_{13}H_{10}FN_3O_2S$ (291): C, 53.60; H, 3.46; F, 6.52; N, 14.42; S, 11.01. Found: C, 53.69; H, 3.31; F, 6.67; N, 14.31; S, 11.19.

Compound **6j**: Yield (61%); mp 190–192 °C; IR (Nujol): 1670 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.72 (s, 3H, $-CH_3$), 4.54 (s, 2H, $-OCH_2-$), 6.35 (s, 1H, –CH–), 6.91–7.38 (m, 4H, Ar-H); EI-MS: *m/z* 318 (M^+, 54). Anal. Calcd for $C_{13}H_{10}N_4O_4S$ (318): C, 49.05; H, 3.17; N, 17.60; S, 10.07. Found: C, 49.15; H, 3.28; N, 17.48; S, 10.21.

Compound **6k**: Yield (61%); mp 111–113 °C; IR (Nujol): 1655 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.69 (s, 3H, $-CH_3$), 4.45 (s, 2H, $-OCH_2-$), 6.25 (s, 1H, –CH–), 7.05–7.35 (m, 4H, Ar-H); EI-MS: *m/z* 318 (M^+, 53). Anal. Calcd for $C_{13}H_{10}N_4O_4S$ (318): C, 49.05; H, 3.17; N, 17.60; S, 10.07. Found: C, 49.16; H, 3.06; N, 17.71; S, 10.15.

Compound **6l**: Yield (68%); mp 116–118 °C; IR (Nujol): 1690 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.72 (s, 3H, $-CH_3$), 2.34 (s, 6H, Ar-$2CH_3$), 4.65 (s, 2H, $-OCH_2-$), 6.35 (s, 1H, –CH–), 7.10–7.41 (m, 3H, Ar-H); EI-MS: *m/z* 301 (M^+, 60). Anal. Calcd for $C_{15}H_{15}N_3O_2S$ (301): C, 59.78; H, 5.02; N, 13.94; S, 10.64. Found: C, 59.87; H, 5.19; N, 13.83; S, 10.55.

Compound **6m**: Yield (60%); mp 198–200 °C; IR (Nujol): 1660 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.72 (s, 3H, $-CH_3$), 4.55 (s, 2H, $-OCH_2-$), 6.35 (s, 1H, –CH–), 6.91–7.32 (m, 4H, Ar-H); EI-MS: *m/z* 291 (M^+, 51). Anal. Calcd for $C_{13}H_{10}FN_3O_2S$ (291): C, 53.60; H, 3.46; F, 6.52; N, 14.42; S, 11.01. Found: C, 53.77; H, 3.59; F, 6.42; N, 14.56; S, 11.15.

Compound **6n**: Yield (69%); mp 102–104 °C; IR (Nujol): 1660 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.73 (s, 3H, $-CH_3$), 4.47 (s, 2H, $-OCH_2-$), 6.27 (s, 1H, –CH–), 7.05–7.42 (m, 4H, Ar-H); EI-MS: *m/z* 318 (M^+, 53). Anal. Calcd for $C_{13}H_{10}N_4O_4S$ (318): C, 49.05; H, 3.17; N, 17.60; S, 10.07. Found: C, 49.20; H, 3.31; N, 17.49; S, 10.19.

Compound **6o**: Yield (62%); mp 155–157 °C; IR (Nujol): 1655 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.75 (s, 3H, $-CH_3$), 2.32 (s, 3H, Ar-CH_3), 4.52 (s, 2H, $-OCH_2-$), 6.29 (s, 1H, –CH–), 7.01–7.39 (m, 4H, Ar-H); EI-MS: *m/z* 287 (M^+, 61). Anal. Calcd for $C_{14}H_{13}N_3O_2S$ (287): C, 58.52; H, 4.56; N, 14.62; S, 11.16. Found: C, 58.63; H, 4.43; N, 14.51; S, 11.29.

Compound **6p**: Yield (60%); mp 125–127 °C; IR (Nujol): 1670 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.73 (s, 3H, $-CH_3$), 4.55 (s, 2H, $-OCH_2-$), 6.29 (s, 1H, –CH–), 6.99–7.32 (m, 4H, Ar-H); EI-MS: *m/z* 307.5 (M^+, 51). Anal. Calcd for $C_{13}H_{10}ClN_3O_2S$ (307.5): C, 50.73; H, 3.28; Cl, 11.52; N, 13.65; S, 10.42. Found: C, 50.62; H, 3.39; Cl, 11.66; N, 13.55; S, 10.31.

Compound **6q**: Yield (66%); mp 201–203 °C; IR (Nujol): 1670 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.75 (s, 3H, $-CH_3$), 4.55 (s, 2H, $-OCH_2-$), 6.35 (s, 1H, –CH–), 6.95–7.34 (m, 4H, Ar-H); EI-MS: *m/z* 352 (M^+, 56). Anal. Calcd for $C_{13}H_{10}BrN_3O_2S$ (352): C, 44.33; H, 2.86; Br, 22.69; N, 11.93; S, 9.10. Found: C, 44.44; H, 2.76; Br, 22.55; N, 11.86; S, 9.19.

Compound **6r**: Yield (61%); mp 98–100 °C; IR (Nujol): 1670 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.73 (s, 3H, $-CH_3$), 4.57 (s, 2H, $-OCH_2-$), 6.31 (s, 1H, –CH–), 6.95–7.32 (m, 4H, Ar-H); EI-MS: *m/z* 291 (M^+, 54). Anal. Calcd for $C_{13}H_{10}FN_3O_2S$ (291): C, 53.60; H, 3.46; F, 6.52; N, 14.42; S, 11.01. Found: C, 53.77; H, 3.37; F, 6.43; N, 14.57; S, 11.18.

Compound **6s**: Yield (69%); mp 139–141 °C; IR (Nujol): 1680 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.75 (s, 3H, $-CH_3$), 4.53 (s, 2H, $-OCH_2-$), 6.25 (s, 1H, –CH–), 7.04–7.36 (m, 5H, Ar-H); EI-MS: *m/z* 273 (M^+, 59). Anal. Calcd for $C_{13}H_{11}N_3O_2S$ (273): C, 57.13; H, 4.06; N, 15.37; S, 11.73. Found: C, 57.24; H, 4.20; N, 15.48; S, 11.61.

Compound **6t**: Yield (65%); mp 194–196 °C; IR (Nujol): 1680 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.75 (s, 3H, $-CH_3$), 2.35 (s, 6H, Ar-$2CH_3$), 4.65 (s, 2H, $-OCH_2-$), 6.43 (s, 1H, –CH–), 7.05–7.45 (m, 3H, Ar-H); EI-MS: *m/z* 301 (M^+, 62). Anal. Calcd for $C_{15}H_{15}N_3O_2S$ (301): C, 59.78; H, 5.02; N, 13.94; S, 10.64. Found: C, 59.65; H, 5.22; N, 13.81; S, 10.73.

5. Biology

5.1. Assay of xanthine oxidase activity

The XO inhibitory activity was monitored spectrophotometrically following the absorbance of uric acid at 292 nm under aerobic condition. Rat liver was homogenized in 0.01 M Tris–HCl pH (8.0) containing 1 mM EDTA. The homogenate was centrifuged and the supernatant was used as a source of enzyme. It was stored at −80 °C until use.[15,16] Bovine milk crude XO was prepared according to Ball EG method.[23] Microbial XO was obtained from Sigma Laboratory and similar procedure was employed for the assay as that of rat liver XO. The protein content was determined by the Lowry's method,[24] using bovine serum albumin as standard.

Xanthine oxidase activity was recorded using a Shimadzu Spectrophotometer. The enzyme assay mixture consisted of 50 mM potassium phosphate buffer (pH 7.5) containing 0.3 mM EDTA and the enzyme source in a total volume of 2 ml. In dose-dependent inhibition studies, the reaction was initiated by the addition of xanthine (50 μM) as the substrate to the above assay mixture and the test compounds. The absorption rate at a wavelength of 292 nm indicates the formation of uric acid at 10 min intervals at ambient temperature. Duplicate assays were repeated three to four times. Allopurinol was used as positive control. The inhibitory activity of each test compound against XO was indicated by their IC_{50} values. The percentage inhibition of XO activity was calculated using the following formula.

$$\text{Xanthine oxidase Inhibition}(\%) = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Abs control = Absorbance of the control reaction (containing all reagents except the test compound).
Abs sample = Absorbance of the test compound.

5.2. Molecular modeling and docking studies

The crystal structure of XO from human milk was retrieved from the PDB database with the corresponding entry code 2CKJ (resolution of 3.59 Å). The protein was prepared by removing all water molecules and adding all hydrogen atoms using the Accelrys Discovery Studio ver 1.7 (Accelrys, Inc., San Diego.). The ligands, once built were docked into the active site using the molecular docking software SYBYL ver 7.3 (Tripos, L.P.) Surflex-Dock

(BioPharmics LLC) with the default parameters. Both of these proprietary software are licensed to Manipal Institute of Technology, Manipal University, India. Surflex-Dock is a program for calculating the docking modes of small molecules into protein-binding sites. In this study we have used ChemScore, a scoring function that is derived from regression against ligand-receptor binding free energies. In the docking process the active site was defined along with MOS1327 complex. For each ligand 20 conformations were generated and docked into XO.

5.3. Data analysis

Data was analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences and the correlations were calculated by Pearson's correlation using SPSS 14.0 software. IC_{50} values were calculated by Boltzmann's dose–response analysis using Origin 6.1 software.

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