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A novel class of potent influenza virus inhibitors: Polysubstituted acylthiourea and its fused heterocycle derivatives

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Abstract—A series of polysubstituted and fused heterocycle derivatives of acylthiourea was prepared and the biological activity against influenza virus was evaluated. Of the analogues that demonstrated IC_{50}s < 0.1 μM, acylthiourea derivatives **16** and **50** were further investigated as candidates with the most potential for future development. The SAR of these compounds are discussed and they represent a novel class of highly potent and selective inhibitors of influenza virus.

Influenza virus is the contagious etiologic agent that causes an acute respiratory infection followed by secondary bacterial infections, hence it has always been a major threat to human health worldwide. Each year in the USA alone, influenza epidemics cause morbidity and mortality. More than 200,000 patients are admitted to hospitals because of influenza and there are approximately 36,000 influenza-related deaths.

At present, effective therapy for influenza virus is limited due to newly discovered resistance[1] in mutant strains. In addition, current anti-viral agents, such as amantadine and rimantadine, provide only limited protection due to a narrow spectrum of activity.[2] Thus, research in the design and screening for more effective anti-viral drugs for the chemotherapy of influenza virus infection is a high priority.

Recently, thiourea derivatives have been reported to be effective against HIV[3,4] and to have bactericidal action.[5] Additionally, nitrogen heterocycles are known as versatile functional groups in active compounds such as fungicides.[6] Few studies have been done concerning evaluations of polysubstituted and fused heterocycle derivatives of acylthioureas for their anti-viral activity. In our early attempts to screen for activity against different viruses, acylthiourea **1** (Fig. 1) was revealed as a weak inhibitor of influenza virus. As part of the program to maximize this activity and to identify more effective inhibitors of influenza virus, we decided to combine these two parts into one molecule and investigate the resulting anti-viral activity. A new class of polysubstituted acylthioureas and their fused heterocycle derivatives (designated PAFHs, series **I**–**XII**) were prepared. A highly specific anti-influenza virus activity in cell culture was discovered and evaluated. Of these acylthiourea derivatives **16** and **50** inhibited virus in cultured MDCK cells at concentrations of 0.08 and 0.09 μM, respectively. These compounds were further investigated for their potential for future development.

Compounds were prepared using the routes shown in Schemes 1–3. The intermediates *N*′-(4,6-disubstituted pyrimidine-2-yl)-thiourea **II** and *N*′-(*tert*-butylamino-

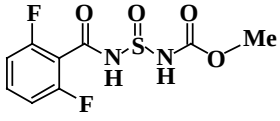

Figure 1. Initial influenza inhibitor, **1**.

Keywords: Influenza virus; Inhibitor; Acylthioureas; Heterocycle; Antiviral activity.
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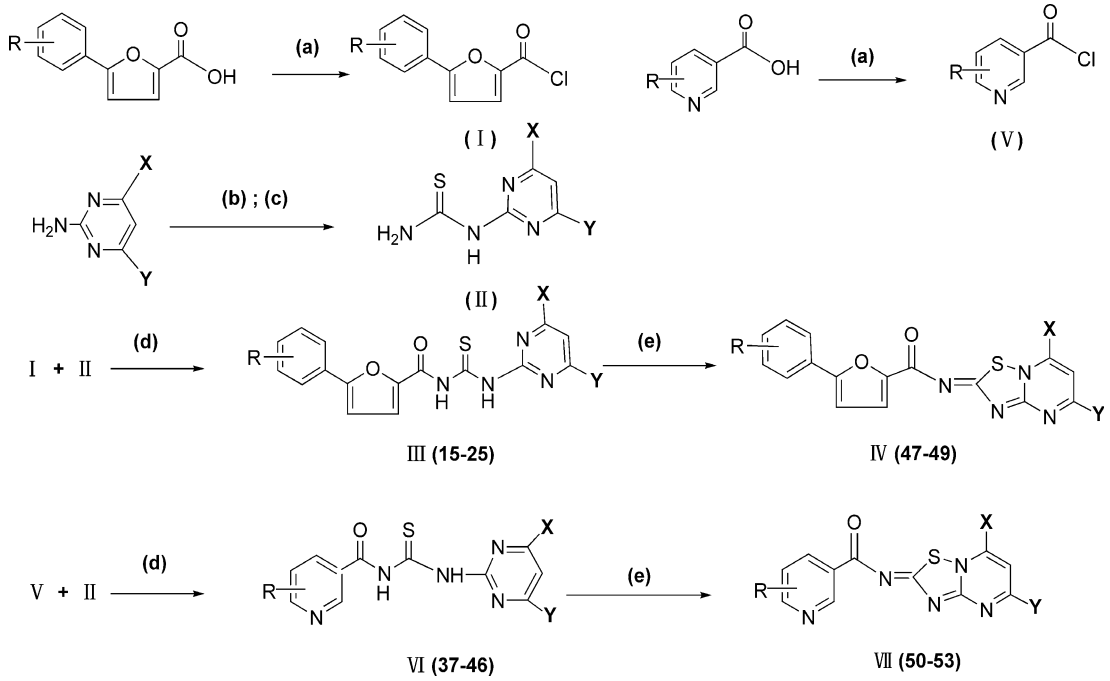
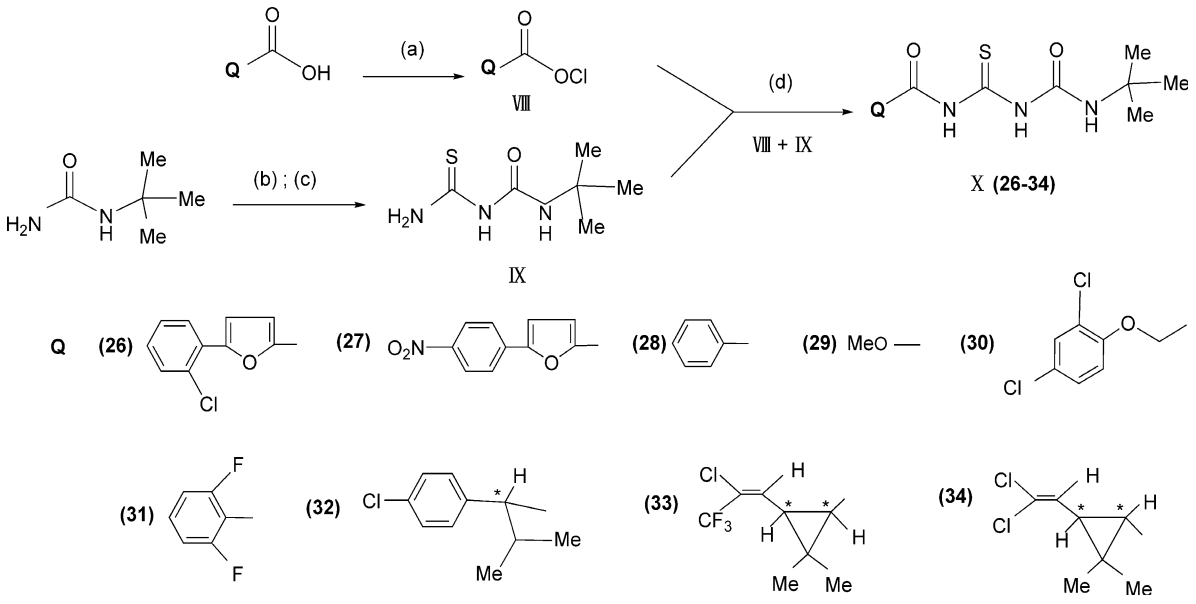


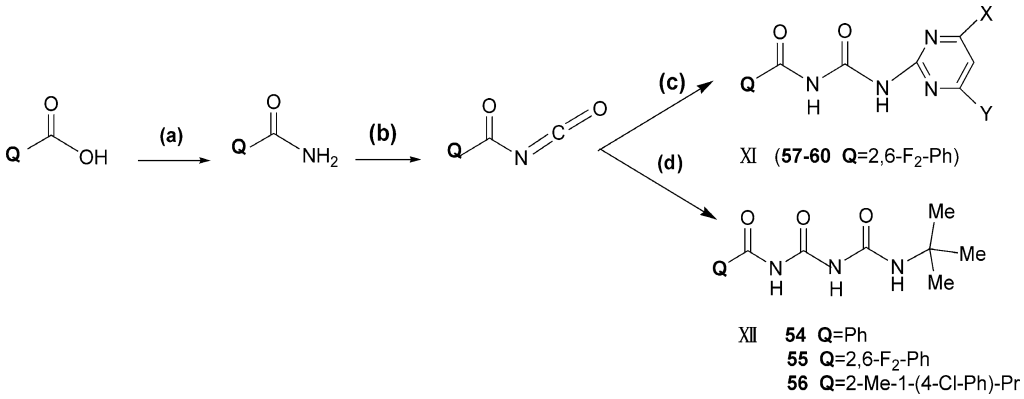
Scheme 1. Reagents: (a) $SOCl_2$; (b) HCl; (c) KSCN/CH_3CN; (d) Et_3N; (e) Br_2.



Scheme 2. Reagents: (a) $SOCl_2$; (b) HCl; (c) KSCN/CH_3CN; (d) Et_3N.

carbonyl)-thiourea **IX** (Scheme 2) were obtained by treatment with potassium thiocyanate (KSCN) and a hot aqueous solution of 2-amino-4,6-disubstituted pyrimidine or *tert*-butylurea, respectively. *N*′-(4,6-Di-substituted pyrimidine-2-yl)-*N*-(5-aryl-2-furoyl) thiourea **III** (Scheme 1) was prepared by the reaction of 5-aryl-2-furoic acid with $SOCl_2$ to form the intermediate 5-aryl-2-furoyl chloride. Subsequent treatment of the intermediate **II** gave the desired acylthiourea derivative. The preparation of *N*′-(4,6-disubstituted pyrimidine-2-yl)-*N*-substituted-β-pyridinecarbonyl thiourea **VI** (Scheme 1) was nearly identical to the procedure for the acylthiourea derivation **III** described above. Then acylthioureas **III** and **VI** were converted in a straightforward manner to thiadiazolo[2,3-*a*]pyrimidines **IV** and **VII** by oxidizing cyclization with the addition of drops of bromine as oxidant. Note that the intermediate substituted nicotinyl chloride **V**, substituted formyl chloride **VIII** were synthesized by treating the same acylating agent $SOCl_2$ with substituted nicotinic acid and substituted formic acid,[7,8] respectively.

The target adduct **X** was synthesized by the reaction of intermediates **VIII** with **IX** (Scheme 2) in a 1:1 molar ratio in acetonitrile, and the resulting precipitate was collected by filtration and recrystallized from CH_3CN to



Scheme 3. Reagents: (a) $NH_3(H_2O)$; (b) $(COCl)_2$; (c) 2-amino-4,6-disubstituted pyrimine; (d) $H_2NCONHC(CH_3)_3$.

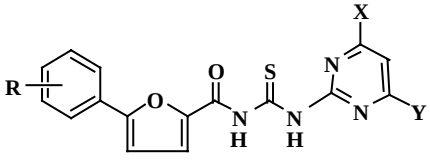
give the final compound **X**. Treatment of substituted acylisocyanates with 2-amino-4,6-substituted pyrimidines and *tert*-butylurea, respectively, yielded acylurea derivatives **XI** and **XII** (Scheme 3).

All compounds were assayed[11] against the influenza A_3/beijing/30/95(H1N1) virus, which were amplified and titrated in MDCK cells. Additionally, general cytotoxicity was measured using the MTS cellular toxicity assay[9,10] to distinguish between specific anti-viral activity and non-specific host cell toxicity. All the compounds reported herein have MTS IC_{50} values of >300 μM and are considered to be non-cytotoxic (these data are not included in the tables).

The SAR resulting from variation for the **X** and **Y** substituents in the pyrimidine ring are shown in Table 1. Different substituents at the 4- or 6-position in the pyrimidine ring had greatest influence on activity. In this series of 5-aryl-2-furoyl thioureas bearing a substituted pyrimidine ring, there was a large preference for electron-donating groups such as Me, OMe, OEt, and OH in this portion of the molecule (Table 1; **15**–**18**). Substitution by electron-withdrawing groups, such as Cl, (**19**–**21**) led to poor activity. For instance, the acylthiazole **16** was the most active compound in the series with an IC_{50} of 0.08 μM. However, the 4-nitrophenyl substitution did not offer any improvement over 2-chlorophenyl substituted analogues. A number of corresponding 4-nitrophenyl derivatives (**22**–**24**) had no appreciable activity against influenza virus when compared with their 2-chlorophenyl counterparts (**15** and **16**). Interestingly, the 4-nitrophenyl derivative **25** was an exception, which was found to inhibit the influenza virus in vitro at 0.36 μM and showed activity comparable to that of **17**. Nonetheless, substitutions on the pyrimidine ring profoundly affect the ability to inhibiting influenza virus in vitro.

With the right substituent **W** held constant as the *tert*-butylaminocarbonyl group, we also explored the SAR of substitutions at **Q** (Table 2). Introduction of a specific functional cycloalkyl, such as chrysanthemoyl (**33** and **34**), resulted in a significant increase in potency.

Table 1. SAR of polysubstituted pyrimidinyl acylthiourea analogues

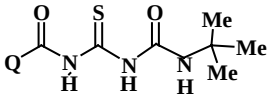


15-25

Compound	R	X	Y	IC_{50}[a]
15	2-Cl	OEt	Me	1.65
16	2-Cl	OEt	OEt	0.08
17	2-Cl	OH	Me	0.32
18	2-Cl	OMe	OMe	1.77
19	2-Cl	Cl	Cl	14.5
20	2-Cl	Cl	OEt	>20
21	2-Cl	OMe	Me	>20
22	4-NO_2	OMe	OMe	>20
23	4-NO_2	OEt	OEt	>20
24	4-NO_2	OEt	Me	11.3
25	4-NO_2	OH	Me	0.36

MTS IC_{50} > 300 μM for all compounds.
[a] IC_{50} of influenza (H1N1) virus, μM.

Table 2. *tert*-Butyl acylthiourea analogue SAR



26-34

Compound	Q	IC_{50}[d]
26	5-(2-Cl-Ph)-2-Furyl	1.42
27	5-(4-NO_2-Ph)-2-Furyl	1.30
28	Ph	1.79
29	OMe	1.83
30	(2,4-Cl_2-Ph)-OCH_2–	1.67
31	2,6-F_2-Ph	1.43
32[a] *S*-(+)	2-Me-1-(4-Cl-Ph)-Pr	1.35
33[a] Cis-(−)	*CFPC[b]	0.51
34[a] Trans-(−)	*DCPC[c]	0.26

[a] Compounds **32**–**34** are pure enantiomers as indicated.
[b] *CFPC, 3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethyl cyclopropyl.
[c] *DCPC, 3-(2,2-dichloro ethenyl)-2,2-dimethyl cyclopropyl.
[d] IC_{50} of influenza (H1N1) virus, μM.

Replacement of 5-aryl-2-furyl by groups, such as phenyl or methoxyl (**28** and **29**), resulted in a slight decrease in the potency (Table 2), while other substituents (**30** and **31**) showed activity that was equipotent to that of analogue **26**. With regard to the substituent **W**, introduction of the *tert*-butylaminocarbonyl group at this position afforded improved activity. Replacement of polysubstituted pyrimidine by *tert*-butylaminocarbonyl (**26** and **27**) further increased the potency 8- to 10-fold over analogue **19** and **24**. Analogue **34** was the most potent compound in this series and inhibited influenza virus in vitro at a concentration of 0.26 μM.

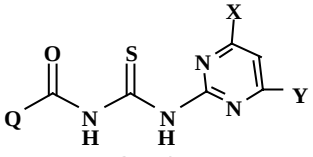
It was noted that these *tert*-butyl acylthiourea analogues have improved water solubility while retaining good lipophilicity. Although we can speculate that the decrease in log *P* values may contribute partially to the increase in anti-viral potency, the role of substituents such as *tert*-butylaminocarbonyl in improving the activity against influenza virus is unclear and this will be part of the subject of a forthcoming paper.

Heterocycles other than furan were synthesized and resulted in lowered potency (Table 3). This demonstrated a large advantage in activity of the existence of a five-membered ring (furan) over corresponding analogues without the furan ring in most instances, and this trend continued for other series of compounds. The compounds containing furan ring (**35** and **36**) were 15-fold more potent than the corresponding 6-chlorophenyl substituted pyridyl analogues (**37** and **38**), which had an IC_{50} of >20 μM. However, the only analogue with no furan ring that retained good activity was the test compound **44**, although it was 2-fold less potent than the corresponding furyl substituted analogue **36**. This result suggests that a furan ring at this position provides significantly improved activity and should be included in the rational design of new influenza virus inhibitors. Variation of the **X** and **Y** substituents in the pyrimidine ring (**39**–**43**) and other pyridyl analogues (**45** and **46**) was also examined. They did not achieve good efficiency against influenza virus. Additionally, substitution of chloro- at the 2- or 6-position in the pyridine ring was not a major determinant in activity against influenza virus (**39** vs **42**) in this series.

There was little correlation between the formation of thiadiazolo[2,3-*a*]pyrimidine ring and potency (Table 4). The former had a relatively small effect on potency against influenza virus (**47** vs **25**). However, compounds **49** and **50** in this series inhibited the virus with an IC_{50} of 0.35 and 0.09 μM, respectively. Compound **49** showed improved potency over the corresponding un-ringed analogues such as **15**. The high potency of **50** bearing the same diethyl groups as **16** is further support of a preference for electron-donating groups, such as ethoxy, in pyrimidine derivatives.

The necessity for a thiourea functionality (Table 5) was also investigated. The corresponding ureas (**54**–**60**) were found to be less effective than their thiourea counterparts. Replacement of the thiourea bridge with urea diminished or eliminated activity altogether in most

Table 3. SAR of heteroaryl **Q** groups

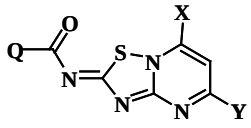


37-46

Compound	Q	X	Y	IC_{50}[a]
35	5-(4-NO_2-Ph)-2-Furyl	OMe	Me	1.22
36	5-(2-Cl-Ph)-2-Furyl	OMe	Cl	1.29
37	6-Cl-3-py	OMe	Me	>20
38	6-Cl-3-py	OMe	Cl	>20
39	6-Cl-3-py	OEt	OEt	>20
40	6-Cl-3-py	Me	OH	8.58
41	2-Cl-3-py	Me	Me	7.19
42	2-Cl-3-py	OEt	OEt	>20
43	2-Cl-3-py	OEt	Cl	>20
44	2-Cl-3-py	OMe	Cl	2.59
45	3-py	OMe	OMe	>20
46	5,6-Cl_2-3-py	OMe	OMe	18.5

[a] IC_{50} of influenza (H1N1) virus, μM.

Table 4. Effect of thiadiazolo[2,3-*a*]pyrimidines ring on inhibition of influenzavirus

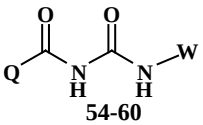


47-53

Compound	Q	X	Y	IC_{50}[a]
47	5-(4-NO_2-Ph)-2-Furyl	Me	OH	0.67
48	5-(4-NO_2-Ph)-2-Furyl	OEt	OEt	5.25
49	5-(2-Cl-Ph)-2-Furyl	OEt	Me	0.35
50	6-Cl-3-py	OEt	OEt	0.09
51	6-Cl-3-py	OMe	Cl	9.32
52	2-Cl-3-py	OMe	OMe	2.41
53	3-py	OMe	OMe	>20

[a] IC_{50} of influenza (H1N1) virus, μM.

Table 5. Thiourea versus urea comparison



54-60

Compound	Q	W	IC_{50}[a]
54	Ph	CONH(*t*-Bu)	>20
55	2,6-F_2-Ph	CONH(*t*-Bu)	14.1
56	2-Me-1-(4-Cl-Ph)-Pr	CONH(*t*-Bu)	15.5
57	2,6-F_2-Ph	4,6-$(Me)_2$-2-Pym[b]	>20
58	2,6-F_2-Ph	4,6-$(OMe)_2$-2-Pym	>20
59	2,6-F_2-Ph	4-Cl-6-(OMe)-2-Pym	15.9
60	2,6-F_2-Ph	4-Cl-6-Me-2-Pym	>20

[a] IC_{50} of influenza (H1N1) virus, μM.
[b] Pym, pyrimidinyl.

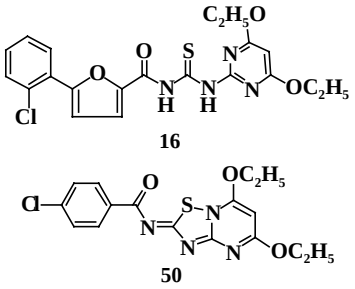


Figure 2. Advanced potential development candidates **16** and **50**.

cases. For instance, **55** and **56** had a 10-fold reduction in activity compared to **31** and **32**. In this respect, it follows that the thiourea bridge is an important contribution to inhibitory activity against influenza virus.

Although some of the PAFHs have a relative low solubility (<10 mg/mL at room temperature), their high lipophilicity is expected to lead to an efficient penetration of the PAFHs through cellular membranes and biological barriers. This property may be advantageous for this novel class of influenza inhibitors, as it may facilitate the uptake of the lipophilic PAFHs into cells or biological compartments where the virus accumulates.

In this paper, we have described the synthesis of a series of polysubstituted and fused heterocycle derivatives of acylthioureas. Evaluation of their in vitro anti-viral activity revealed compounds with high potent cellular activity against wild-type influenza virus in cultured MDCK cells. Analogues **16** and **50**[12] (Fig. 2) inhibited the influenza virus with an IC_{50} of 0.08 and 0.09 μM, respectively. These novel inhibitors were investigated as candidate compounds with the most potential for future drug development. This research has lead to a better understanding of SAR of influenza virus inhibitors and thereby provides some insight into the rational design of new anti-flu agents. Further studies are ongoing to assess the inhibitory activity against resistant influenza virus strains as well as searching for the specific influenza target(s) and mechanism-based drugs.

Acknowledgments

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References and notes

1. Hayden, F. G.; Belshe, R. B.; Glover, R. D. *New Engl. J. Med.* **1989**, *321*, 1696.
2. Erik, De Clercq *J. Clin. Virol.* **2001**, *22*, 73.
3. Venkatachalam, T. K.; Mao, C. *Bioorg. Med. Chem.* **2004**, *12*, 4275.
4. Zhang, H.; Vrang, L.; Backbro, K. *Antivir. Res.* **1995**, *28*, 331.
5. Sarkis, G. Y.; Faisal, E. D. *J. Heterocycl. Chem.* **1985**, *22*, 137.
6. Bessard, Y.; Crettaz, R. *Tetrahedron* **2000**, *56*, 4739.
7. Krutosikova, A.; Kovac, J.; Sykora, V. *Collect. Czech. Chem. Commun.* **1994**, *39*, 1892.
8. Oliveto, E. P. *Chem. Heterocycl. Compd.* **1962**, *14*, 178.
9. Barltrop, J. A.; Owen, T. C. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 611.
10. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
11. The MDCK cells were grown in 96-well microtiter plates with influenza virus at 50 plaque-forming units (PFU)/well. Each compound was serially diluted and tested to calculate its IC_{50} (Tables 1–5) and all values are means of at least three independent experiments on different days.
12. Physical data: *N*′-(4,6-diethoxylpyrimidin-2-yl)-*N*-[5-(2-chlorophenyl)-2-furoyl]thiourea (compound **16**). Yield: 86%, mp: 175–177 °C; IR (KBr plate, cm^{-1}): 1632 (C=O), 3195 (N=H), 1235 (C=S); ^1H NMR (DMSO-d_6): δH_{ppm} 7.24–7.86 (m, 6H, Ar-H), 6.15 (s, 1H, pyrimidine-5-H), 12.08 (s, 1H, N′-H), 12.46 (s, 1H, N′-H), 1.22 (t, 6H, CH_3), 4.08 (q, 4H, OCH_2). Anal. Calcd for $C_{20}H_{19}ClN_4O_4S$: C, 53.75; H, 4.26; N, 12.54. Found: C, 53.64; H, 4.35; N, 12.41.
5,7-Diethoxyl-2-(6-chloro-3-pyridinecarbonylimino)-2*H*-1,2,4-thiadiazolo[2,3-*a*]pyrimidine (compound **50**). Yield: 72%, mp: 176–178 °C; IR (KBr plate, cm^{-1}): 1690 (C=O), 1630 (C=N); ^1H NMR (DMSO-d_6): δH_{ppm} 7.60–8.95 (m, 3H, pyridine-H), 5.65 (s, 1H, pyrimidine-5-H), 1.16 (t, 3H, CH_3), 4.18 (q, 2H, OCH_2). Anal. Calcd for $C_{20}H_{17}ClN_4O_4S$: C, 47.43; H, 3.69; N, 18.45. Found: C, 47.51; H, 3.60; N, 18.36.