Med Chem Res (2011) 20:1450–1454
DOI 10.1007/s00044-010-9378-2

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Identification of important structural features of thiazolo[4,5-*d*] pyrimidines required for potent antifungal activity

Mahesh Chhabria · Ishwarsinh Rathod · Khushbu Vala · Paulomi Patel

Received: 9 February 2010 / Accepted: 4 June 2010 / Published online: 27 June 2010

Abstract Pyrimidines, either mononuclear or condensed with other heterocycles have established its importance in medicinal chemistry. Variety of biological activities have been reported by thiazolo[4,5-*d*]pyrimidine derivatives. The present work describes the synthesis and antifungal activity of several 3-(substituted)-5-(substituted)phenylamino-6-(substituted)phenylthiazolo[4,5-*d*]pyrimidine-7(6*H*)-one-2(3*H*)-thiones. The target compounds were synthesized by cyclocondensation of 4-amino-5-carbethoxy-3-(substituted)thiazolo-2(3*H*)-thione and *S*-methyl di(substituted) phenylisothiourea. All synthesized compounds were tested for minimum inhibitory concentration against different fungal strains such as, *Aspergillus niger, A. clavatus and Candida albicans* and compared with fluconazole and nystatin as reference drug. Some of the compounds have exhibited potent inhibitory activity on all fungal strains, and were found more potent than reference standard. Some of the important structural features required for broad spectrum activity in this series have been derived.

Keywords Thiazolo[4,5-*d*]pyrimidine · Antifungal · MIC

Abbreviations
MIC Minimum inhibitory concentration.
SAR Structural activity relationship

M. Chhabria (✉) · I. Rathod · K. Vala · P. Patel
Department of Pharmaceutical Chemistry,
L. M. College of Pharmacy, Gujarat University,
Navrangpura, Ahmedabad 380 009, Gujarat, India
e-mail: mahesh.chhabria@rediffmail.com

Introduction

Invasive fungal infections, particularly in immunosuppressed patients, have continued to increase in incidence during the past 20 years and are now significant cause of morbidity and mortality (Andre, 2008; Russel, 2002; Beth, 1999). All current agents have some serious liabilities: such as inadequate spectrum, limited dosage forms, narrow therapeutic window and rapid emergence of resistance (Boucher *et al.*, 2004; William and Thomas 2001). The recent expansion of antifungal drug research has occurred because there is a critical need for new antifungal agents to treat these life-threatening invasive infections (Gallagher *et al.*, 2004; Georgopapadakou and Walsh 1994). Thus, intense efforts in antifungal drug discovery are still needed to develop more promising and effective antifungal agents.

It is well known that pyrimidine and condensed pyrimidine derivatives are of great biological interest, especially as antiviral, antitumor and antimicrobial agents. In continuation of our ongoing work on thiazolopyrimidines, it was planned to synthesize a series of 3-(substituted)-5-(substituted)phenylamino-6-(substituted)phenylthiazolo[4,5-*d*]pyrimidine-7(6*H*)-one-2(3*H*)-thiones as potential antifungal agent and it was also planned to derive important structural features required for antifungal activity.

Experimental

Chemistry

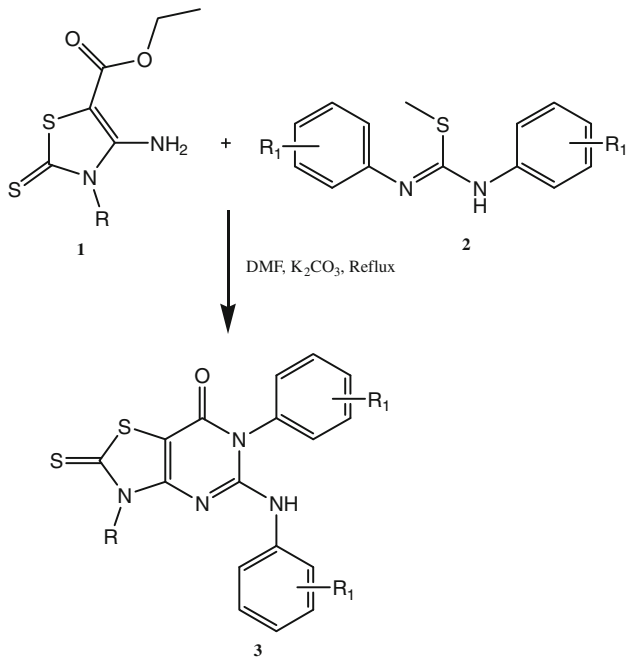
Earlier, thiazolo[4,5-*d*]pyrimidine was prepared by reaction of 4-amino-5-carbethoxy-3-(substituted)thiazolo-2(3*H*)-thione in the presence of triphenylphosphene, hexachloroethane and triethylamine to give iminophosphorane which on reaction with

aryl isocyanate afforded carbodiimide. Condensation of carbodiimides with alkylamine gave intermediate guanidines, which get cyclized to thiazolo[4,5-*d*]pyrimidine (Ying *et al.*, 2007) in the presence of sodium ethoxide.

In present study, we have eliminated the use of triphenylphosphene, hexachloroethane and arylisocyanate and cyclization was affected by weak base. The target compounds were synthesized by cyclocondensation of 4-amino-5-carbethoxy-3-(substituted)thiazolo-2(3*H*)-thione (Gewald, 2004) and *S*-methyldi(substituted)phenylisothiourea (Furniss and Hannaford 2006) in DMF in the presence of potassium carbonate (Scheme 1). All compounds were characterized by IR, ^{1}H-NMR and mass spectroscopy.

General method of synthesis of 3-(substituted)-5-(substituted)phenylamino-6-(substituted)phenylthiazolo [4,5-d]pyrimidine-7(6H)-one-2(3H)-thiones

A mixture of 4-amino-5-carbethoxy-3-(substituted)thiazolo-2(3*H*)-thione **1** (0.3 g, 0.01 mol) and appropriate symmetrical *S*-methyldi(substituted)phenylisothiourea **2** (0.33 g, 0.01 mol) was refluxed in dimethylformamide for 8–10 h in the presence of anhydrous K_2CO_3. The reaction mixture was allowed to cool and poured in ice-cold HCl solution (10% v/v). The product obtained was filtered, washed with water and dried. Recrystallization of the crude product in *n*-hexane-dichloromethane afforded 3-(substituted)-5-(substituted) phenylamino-6-(substituted)phenylthiazolo[4,5-*d*]pyrimidine-7(6*H*)-one-2(3*H*)-thiones **3** in moderate to good yield (Table 1).



Scheme 1 Synthetic scheme for 3-(substituted)-5-(substituted)phenylamino-6-(substituted)phenylthiazolo[4,5-*d*]pyrimidine-7(6*H*)-one-2(3*H*)-thiones

Spectral characteristics of synthesized compounds

3-(Ethyl)-5-(4-methoxyphenyl)amino-6-(4-methoxyphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3a)

IR (KBr, cm^{-1}): 3357 (N–H), 2860 (C–H), 1685 (C=O), 1247 (C=S). ^{1}H-NMR (δ ppm, DMSO-d$_6$): 1.2 (t, 3H,C$\underline{H}_3$), 2.3 (q, 2H, C$\underline{H}_2$), 3.7 (s, 3H, OC$\underline{H}_3$), 3.9 (s, 3H, OC$\underline{H}_3$), 6.7 (s, 1H, N$\underline{H}$), 6.70–7.95 (m, 8H, Ar–$\underline{H}$), MS: 441.0 (M+1), 439.1 (M−1).

3-(Phenyl)-5-(4-methoxyphenyl)amino-6-(4-methoxyphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3b)

IR (KBr, cm^{-1}): 3350 (N–H), 2860 (C–H), 1650 (C=O), 1250 (C=S). MS: 489.0 (M+1), 487.1 (M−1).

3-(Phenyl)-5-(2-methylphenyl)amino-6-(2-methoxyphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3c)

IR (KBr, cm^{-1}): 3357 (N–H), 2860 (C–H), 1685 (C=O), 1247 (C=S). ^{1}H-NMR (δ ppm, DMSO-d$_6$): 3.6 (s, 3H, C$\underline{H}_3$), 3.8 (s, 3H, OC$\underline{H}_3$), 6.5 (s, 1H, N$\underline{H}$, D$_2$O exchangeable), 6.90–7.80 (m, 13H, Ar–$\underline{H}$).

3-(Phenyl)-5-(4-methylphenyl)amino-6-(4-methylphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3d)

IR (KBr, cm^{-1}): 3374 (N–H), 1687 (C=O), 1250 (C=S). ^{1}H-NMR (δ ppm, DMSO-d$_6$): 2.2 (s, 6H, Ar–C$\underline{H}_3$), 6.6 (s,1H, N$\underline{H}$, D$_2$O exchangeable), 6.85–7.95 (m, 13H, Ar$\underline{H}$). MS: 456.9 (M+1), 455.0 (M−1).

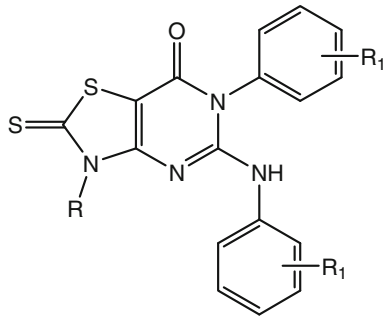
3-(Phenyl)-5-(2,6-dimethylphenyl)amino-6-(2,6-dimethylphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3e)

IR (KBr, cm^{-1}): 3374 (N–H), 1685 (C=O), 1250 (C=S). ^{1}H-NMR (δ ppm, DMSO-d$_6$): 2.3 (s, 12H, Ar–C$\underline{H}_3$), 6.7 (s, 1H, N$\underline{H}$, D$_2$O exchangeable), 6.9–7.69 (m, 11H, Ar–$\underline{H}$).

3-(Phenyl)-5-phenylamino-6-phenylthiazolo[4,5-d] pyrimidine-7(6H)-one-2(3H)-thione (3f)

IR (KBr, cm^{-1}): 3323 (N–H), 1683 (C=O), 1250 (C=S). ^{1}H-NMR (δ ppm, DMSO-d$_6$): 6.8 (s, 1H, N$\underline{H}$, D$_2$O exchangeable), 6.92–7.66 (m, 15H, Ar–$\underline{H}$).

Table 1 Physical characteristics of 3-(substituted)-5-(substituted)phenylamino-6-(substituted)phenylthiazolo[4,5-*d*]pyrimidine-7(6*H*)-one-2(3*H*)-thiones (3)



3

Compound no.	R	R_1	Molecular formula	Molecular weight	Melting point (°C)	Yield (%)
3a	C_2H_4	4-OCH_3	$C_{21}H_{20}N_4O_3S_2$	494.05	110–112	67.20
3b	C_6H_5	4-OCH_3	$C_{25}H_{20}N_4O_3S_2$	488.09	236–238	76.92
3c	C_6H_5	2-OCH_3	$C_{25}H_{20}N_4O_3S_2$	488.09	225–227	60.00
3d	C_6H_5	4-CH_3	$C_{25}H_{20}N_4OS_2$	456.12	282–284	62.50
3e	C_6H_5	2,6-diCH_3	$C_{27}H_{24}N_4OS_2$	484.10	278–280	44.11
3f	C_6H_5	C_6H_5	$C_{23}H_{16}N_4OS_2$	428.15	246–248	52.60
3g	4-CH_3O-C_6H_4	4-OCH_3	$C_{26}H_{22}N_4O_4S_2$	518.06	>300	66.66
3h	4-CH_3O-C_6H_4	4-F	$C_{24}H_{16}F_2N_4O_2S_2$	494.05	238–240	67.74
3i	4-CH_3O-C_6H_4	2-OCH_3	$C_{26}H_{22}N_4O_4S_2$	518.14	250–252	54.54
3j	4-CH_3O-C_6H_4	4-Br	$C_{24}H_{16}Br_2N_4O_2S_2$	616.04	290–295	48.71
3k	2-CH_3O-C_6H_4	4-OCH_3	$C_{26}H_{22}N_4O_4S_2$	428.15	250–252	54.54
3l	2-CH_3O-C_6H_4	2,6-diCH_3	$C_{28}H_{26}N_4O_2S_2$	518.06	270–272	48.48
3m	3-Cl,4-F-C_6H_3	4-OCH_3	$C_{25}H_{18}ClFN_4O_3S_2$	488.09	205–207	58.30
3n	3-Cl,4-F-C_6H_3	4-Br	$C_{23}H_{12}Br_2ClFN_4S_2$	456.12	202–204	31.50
3o	3,4-diCl-C_6H_3	4-OCH_3	$C_{25}H_{18}Cl_2N_4O_3S_2$	557.14	290–295	42.55
3p	3,4-diCl-C_6H_3	4-Br	$C_{26}H_{22}Cl_2Br_2\ N_4O_4S_2$	655.19	210–212	27.02
3q	4-Br-C_6H_4	4-OCH_3	$C_{25}H_{19}BrN_4O_3S_2$	484.10	245–247	54.80

3-(4-Methoxyphenyl)-5-(4-methoxyphenyl)amino-6-(4-methoxyphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3g)

IR (KBr, cm^{-1}): 3394 (N–H), 1689 (C=O), 1253 (C=S). ^{1}H-NMR (δ ppm, DMSO-d_6): 3.6 (s, 3H, $OC\underline{H}_3$), 3.8 (s, 3H, $OC\underline{H}_3$), 3.9 (s, 3H, $OC\underline{H}_3$), 6.7 (s, 1H, N<u>H</u>, D_2O exchangeable), 6.98-7.89 (m, 12H, Ar–<u>H</u>).

3-(4-Methoxyphenyl)-5-(4-fluorophenyl)amino-6-(4-fluorophenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3h)

IR (KBr, cm^{-1}): 3323 (N–H), 1683 (C=O), 1250 (C=S). ^{1}H-NMR (δ ppm, DMSO-d_6): 3.9 (s, 3H, $OC\underline{H}_3$), 6.5 (s, 1H, N<u>H</u>), 6.96-7.76 (m, 12H, Ar–<u>H</u>). MS: 494.9 (M+1), 493.1 (M−1).

3-(4-Methoxyphenyl)-5-(2-methoxyphenyl)amino-6-(2-methoxyphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3i)

IR (KBr, cm^{-1}): 3370 (N–H), 1689 (C=O), 1250 (C=S). ^{1}H-NMR (δ ppm, DMSO-d_6): 3.6 (s, 3H, $OC\underline{H}_3$), 3.8 (s, 3H, $OC\underline{H}_3$), 3.9 (s, 3H, $OC\underline{H}_3$), 6.7 (s, 1H, N<u>H</u>, D_2O exchangeable), 6.89–7.75 (m, 12H, Ar–<u>H</u>).

3-(4-Methoxyphenyl)-5-(4-bromophenyl)amino-6-(4-bromophenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3j)

IR (KBr, cm^{-1}): 3305 (N–H), 1677 (C=O), 1251 (C=S). ^{1}H-NMR (δ ppm, DMSO-d_6): 3.8 (s, 3H, $OC\underline{H}_3$), 6.7 (s, 1H, N<u>H</u>), 6.92-7.66 (m, 12H, Ar–<u>H</u>).

3-(2-Methoxyphenyl)-5-(4-methoxyphenyl)amino-6-(4-methoxyphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3k)

IR (KBr, cm^{-1}): 3375 (N–H), 1685 (C=O), 1251 (C=S). ^{1}H-NMR (δ ppm, DMSO-d_6): 3.6 (s, 3H, OCH_3), 3.7 (s, 3H, OCH_3), 3.9 (s, 3H, OCH_3), 6.7 (s, 1H, NH, D_2O exchangeable), 6.90–7.85 (m, 12H, Ar–H).

3-(2-Methoxyphenyl)-5-(2,6-dimethylphenyl)amino-6-(2,6-dimethylphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3l)

IR (KBr, cm^{-1}): 3371 (N–H), 1693 (C=O), 1253 (C=S). ^{1}H-NMR (δ ppm, DMSO-d_6):
2.3 (s, 12H, Ar–CH_3), 3.8 (s, 3H, OCH_3), 6.7 (s, 1H, NH, D_2O exchangeable), 6.80–7.80 (m, 10H, Ar–H).

3-(3-Chloro-4-fluorophenyl)-5-(4-methoxyphenyl)amino-6-(4-methoxyphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3m)

IR (KBr, cm^{-1}): 3350 (N–H), 1698 (C=O), 1248 (C=S). ^{1}H-NMR (δ ppm, DMSO-d_6): 3.8 (s, 3H, OCH_3), 3.9 (s, 3H, OCH_3), 6.7 (s, 1H, NH), 6.90–7.80 (m, 11H, Ar–H). MS: 542.9 (M+2), 539.1 (M−2).

3-(3-Chloro-4-fluorophenyl)-5-(4-bromophenyl)amino-6-(4-bromophenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3n)

IR (KBr, cm^{-1}): 3310 (N–H), 1683 (C=O), 1261 (C=S). ^{1}H-NMR (δ ppm, DMSO-d_6): 6.7 (s, 1H, NH), 6.90–7.65 (m, 11H, Ar–H). MS: 638.9 (M+2), 637.1 (M−2).

3-(3,4-Dichlorophenyl)-5-(4-methoxyphenyl)amino-6-(4-methoxyphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3o)

IR (KBr, cm^{-1}): 3350 (N–H), 1693 (C=O), 1251 (C=S). ^{1}H-NMR (δ ppm, DMSO-d_6): 3.7(s, 3H, OCH_3), 3.9 (s, 3H, OCH_3), 6.6 (s, 1H, NH), 6.92–7.70 (m, 11H, Ar–H). MS: 559.0 (M+2), 555.1 (M−2).

3-(3,4-Dichlorophenyl)-5-(4-bromophenyl)amino-6-(4-methoxyphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3p)

IR (KBr, cm^{-1}): 3312 (N–H), 1685 (C=O), 1249 (C=S). ^{1}H-NMR (δ ppm, DMSO-d_6): 6.6 (s, 1H, NH, D_2O exchangeable), 6.92–7.70 (m, 11H, Ar–H). MS: 657.0 (M+2).

3-(4-Bromophenyl)-5-(4-methoxyphenyl)amino-6-(4-methoxyphenyl)thiazolo[4,5-d]pyrimidine-7(6H)-one-2(3H)-thione (3q)

IR (KBr, cm^{-1}): 3289 (N–H), 1681 (C=O), 1251 (C=S). ^{1}H-NMR (δ ppm, DMSO-d_6): 3.7 (s, 3H, OCH_3), 3.9 (s, 3H,OCH_3), 6.7 (s, 1H, NH), 6.90–7.65 (m, 12H, Ar–H).

Antifungal screening

All synthesized compounds **3a–q** were tested in vitro against three fungal strains *Candida albicans, Aspergillus niger* and *A. clavatus* by broth dilution method using Sabouraud's nutrient media (Jennifer 2004) and MIC values were calculated (Table 2). Fluconazole and nystatin were taken as the standard drug.

Results and discussion

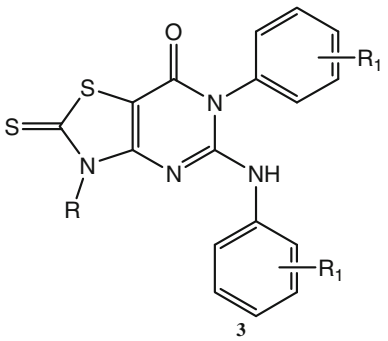
A series of 3-(substituted)-5-(substituted)phenylamino-6-(substituted)phenylthiazolo[4,5-*d*]pyrimidine-7(6*H*)-one-2(3*H*)-thiones **(3)** was synthesized (Scheme 1) to carry out SAR study. Compounds with various substituents at third, fourth and fifth position of thiazolopyrimidines were synthesized and screened. Phenyl ring was considered essential at all three positions as it imparts more lipophilicity and hence more penetration through membrane. All the tested compounds exhibited significant antifungal activity against all fungal strains with MIC value ranges from 0.2 to 25.6 μg/mL. Replacement of phenyl ring by alkyl group at the third position (compound **3a**) has also shown significant antifungal activity, but was found less potent than phenyl derivatives. Compound with 4-methoxyphenyl group at third position and 4-fluorophenyl group at fifth and sixth position exhibited potent activity.

Compound **3b** with phenyl ring on third position and 4-methoxyphenyl on fourth position was found to be the most potent compound against all strains of fungi. Compound **3i** and **q** have shown potent activity with MIC value of 0.4 μg/mL against *A. niger* and compound **3b, j, m** have shown most potent activity with MIC value of 0.2 μg/mL against *A. clavutus*.

Study reveals that substitution with electron donating group on phenyl ring retained potency on all strain. With electron withdrawing groups on phenyl ring potency on *A. niger* and *A. clavutus* reduced significantly.

So for broad spectrum antifungal thiazolopyrimidines, at third position either unsubstituted phenyl or phenyl with electron donating group is essential. Similarly at fourth and fifth position phenyl ring with electron donating group is required.

Table 2 Minimum inhibitory concentrations (μg/mL) of 3-(substituted)-5-(substituted) phenylamino-6(substituted)phenylthiazolo[4,5-*d*]pyrimidine-7(6*H*)-one-2(3*H*)-thiones



3

Compound no.	*C. albicans* MTCC 227	*A. niger* MTCC 282	*A. clavatus* MTCC 1323
Minimum inhibitory concentrations (μg/mL)			
3a	2	0.8	16
3b	0.5	0.8	0.2
3c	4	12.8	12.8
3d	0.8	12.8	0.8
3e	4	25.6	4
3f	4	25.6	25.6
3g	4	0.5	4
3h	0.4	0.5	0.4
3i	12.8	0.4	25.6
3j	0.8	4	0.2
3k	16	4	0.8
3l	4	4	4
3m	0.4	4	0.2
3n	4	4	4
3o	0.8	4	0.8
3p	0.8	4	16
3q	4	0.4	12.8
Fluconazole	0.5	16	16
Nystatin	8	7	7

References

Andre G (2008) The fight against fungi. Nature 3:452

Beth D (1999) Novel antifungal drugs. Curr Opin Microbiol 2(5):509–515

Boucher HW, Groll AH, Chiou CC, Wash TJ (2004) Newer systemic antifungal agents: pharmacokinetics, safety and efficacy. Drugs 64(18):1997–2020

Furniss BS, Hannaford AJ (2006) Phenyl isothiocyanate. Vogel's textbook of practical organic chemistry, p 966

Gallagher JC, Mac DC, Ashley ES (2004) Recent advances in antifungal pharmacotherapy for invasive fungal infections. Exp Rev Anti Infect Ther 2(2):253–268

Georgopapadakou NH, Walsh TJ (1994) Human mycoses: drugs and targets for emerging pathogens. Science 264(5157):371–373

Gewald K (2004) Heterocyclic aus CH-aciden Nitrilen. VI. Reaktion von methylenaktiven Nitrilen mit Senfölen und Schwefel. J Prakt Chem 32(1–2):26–30

Jennifer MA (2004) Determination of minimum inhibitory concentrations. J Antimicrob Chem 18:1–18

Russel EL (2002) Pharmacotherapy of *Candida* bloodstream infections: new treatment options, new era. Expert Opin Pharmacother 3(8):1039–1057

William JW, Thomas ER (2001) Progress with antifungal agents and approaches to combat fungal resistance. Annu Rep Med Chem 35:157–166

Ying L, Shuang F, Wenn YM, Hong WH (2007) A facile synthesis and herbicidal activities of novel fluorine-containing thiazolo[4,5-*d*]pyrimidin-7(6H)-ones. J Fluorine Chem 128:879–884