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Design, Synthesis, and Biological Activities of Arylmethylamine Substituted Chlorotriazine and Methylthiotriazine Compounds

Huaping Zhao,[†] Yuxiu Liu,[*,†] Zhipeng Cui,[†] David Beattie,[‡] Yucheng Gu,[*,‡] and Qingmin Wang[*,†]

[†]State Key Laboratory of Elemento-Organic Chemistry, Research Institute of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, People's Republic of China

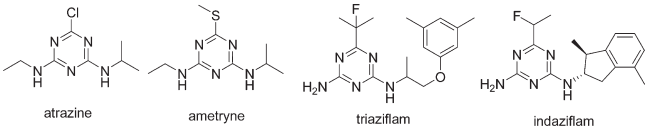
[‡]Syngenta Jealott's Hill International Research Centre, Bracknell, Berks, RG42 6EY, U.K.

ABSTRACT: Heterocyclic rings were introduced into the core structure of *s*-triazine to design and synthesize a series of novel triazines containing arylmethylamino moieties. These compounds were characterized by using spectroscopic methods and elemental analysis. Their herbicidal, insecticidal, fungicidal, and antitumor activities were evaluated. Most of these compounds exhibited good herbicidal activity, especially against the dicotyledonous weeds, and compound **F8** was almost at the same level as the control compound atrazine. Their structure−activity relationships were discussed. At the same time, some triazines had interesting fungicidal and insecticidal activities, of which **F4** exhibited 100% efficacy against *Puccinia triticina* even at 20 ppm, and **F5** showed Lepidopteran-specific activity in both leaf-piece and artificial diet assays. Moreover, these compounds showed antitumor activities against leukemia HL-60 cell line and lung adenocarcinoma A-549 cell line.

KEYWORDS: *s*-triazine, heterocyclic ring, herbicidal activity, insecticidal activity, fungicidal activity, antitumor activity, structure−activity relationships

INTRODUCTION

s-Triazines are a group of herbicides, with atrazine registered in 1958 as the biggest product of its class. They can displace plastoquinone from the Q_B binding niche of the D-1 protein of photosystem II in the photosynthetic electron transport chain of plants, therefore inhibiting electron transport in the photosynthesis of the plant and resulting in the wilt of leaves and then the death of the plant.[1] Due to their excellent herbicidal activities, tens of *s*-triazine herbicides were commercialized, and many of them are still in use. The simple alkyl-substituted *s*-triazines atrazine and ametryne are such representative pesticides. Nowadays, new *s*-triazines compounds are still in progress. In the late 1990s, triaziflam, a diaminotriazine herbicide bearing a dimethylphenoxyethyl group at one of the nitrogen atoms, was developed by a Japanese company, Idemitsu Kosan.[2] More recently, indaziflam, with a dihydroindene moiety, was developed by Bayer in 2008 and will be launched to the market in the next few months.[3]



Benzylaminotriazines and (α-substituted)benzylaminotriazines had also been synthesized and evaluated as herbicide in 1990s.[4] Some of these compounds exhibited potent phytotoxicity against weeds and high selectivity between rice and weeds, but had different phytotoxic properties to atrazine. However, none of the benzylaminotriazines had been commercialized due to the high dosage required to establish control in the field.

As PSII electron transport inhibitor and therefore binding with the same binding niche of the D1 protein, benzylamino-substituted cyanoacrylates and benzylaminotriazines have common benzylamino moieties which were assumed to bind to the very same site.[5,6] In our previous research on cyanoacrylates, when benzylamino group was alternated by different heterocyclic methylamino groups, their herbicidal activity changed to a large extent, and some of them exhibited enhanced herbicidal activity.[7] We wonder if a similar effect could be found in the triazine system. Literature review suggested that substituted benzylaminotriazines or heterocyclic methylamino substituted triazines had not been thoroughly investigated. Thus, some representative arylmethylamino-containing chlorotriazines and methylthiotriazines were synthesized to investigate the influence of heterocyclic methylamino moieties of triazines on their herbicidal activity (Figure 1).

Many herbicidal triazines are also reported to have antifungal activity.[8] In recent years, versatile bioactivities of *s*-triazine derivatives have been widely reported and aroused the study enthusiasm of scientists from all over the world.[9] Therefore, the synthesized triazine compounds were also investigated for their potential as fungicidal, insecticidal, or antitumor agents.

Herein we report the synthesis of arylmethylamino-containing chlorotriazines and methylthiotriazines (**F**) and their biological activities.

MATERIALS AND METHODS

Instruments. ^{1}H nuclear magnetic resonance (NMR) spectra were obtained at 400 MHz using a Bruker AV400 spectrometer in $CDCl_3$ or

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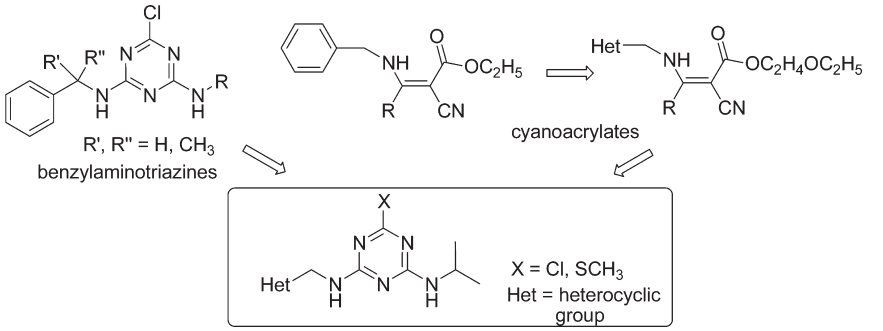
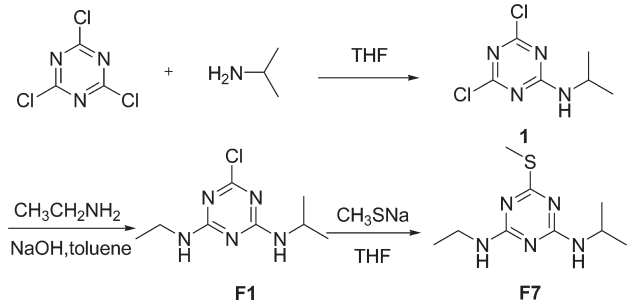


Figure 1. Design of arylmethylamino-containing chlorotriazines and methylthiotriazines.

Scheme 1



d_6-DMSO solution with tetramethylsilane as the internal standard. Chemical-shift values (δ) are given in parts per million (ppm). Elemental analyses were determined on a Yanaca CHN Corder MT-3 elemental analyzer. The melting points were determined on an X-4 binocular microscope melting point apparatus (Beijing Tech Instruments Co., Beijing, China) and are uncorrected. Yields were not optimized.

General Synthesis. The reagents were of analytical grade or chemically pure. All anhydrous solvents were dried and purified by standard techniques prior to use.

Synthetic Procedure for the Target Compounds F1−F11 (Schemes 1 and 2). *Synthesis of 2,4-Dichloro-6-isopropylamino-1,3,5-triazine (**1**).* A solution of cyanuric chloride (11.06 g, 60 mmol) in tetrahydrofuran (60 mL) was cooled to −10 °C by an ice−salt bath, and then isopropylamine (7.08 g, 120 mmol) in tetrahydrofuran (20 mL) was added slowly and the mixture was stirred at room temperature for 30 min. Then, the mixture was concentrated under reduced pressure, followed by addition of water (70 mL). The aqueous phase was extracted by ethyl acetate twice (70 mL × 2). The combined organic solution was washed with saturated brine (70 mL), then dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash-column chromatography on silica gel using a mixture of petroleum ether and ethyl acetate (10:1) as the eluent to afford **1** as a white solid (12.09 g, 97.4%); mp 42−44 °C [lit. 45 °C[10]]. ^{1}H NMR (400 M, $CDCl_3$) δ: 1.25 (d, $^3J_{HH}$ = 6.4 Hz, 6H), 4.16−4.28 (m, 1H), 5.70 (s, 1H).

*Synthesis of Atrazine (**F1**).* To a solution of **1** (1.92 g, 9.3 mmol) in toluene (4.96 g, 53.9 mmol) was added a solution of sodium hydroxide (0.37 g, 9.3 mmol) in water (1.5 mL). Then, the mixture was maintained at 15 °C, and ethylamine (0.42 g, 9.3 mmol) was added slowly. After the completion of addition, the mixture was stirred at room temperature for 15 min. Then, toluene was removed under reduced pressure, and water (40 mL) was added. The mixture was filtered to afford **F1** as a white solid (1.67 g, 83.5%). Further recrystallization from a mixture of petroleum ether (60−90 °C) and dichloromethane afforded a white solid; mp 179−180 °C [lit. 172.5−174 °C[11]]. ^{1}H NMR (400 M, $CDCl_3$) δ: 1.15−1.26 (m, 9H), 3.30−3.49 (m, 2H), 3.99−4.28 (m, 1H), 5.03−5.20 (m, 1H), 5.32−5.61 (m, 1H).

*Synthesis of 2-Chloro-4-isopropylamino-6-(p-fluorobenzylamino)-1,3,5-triazine (**F2**).* To a solution of **1** (2.07 g, 10 mmol) in toluene (3 mL) was added a solution of sodium hydroxide (0.40 g, 10 mmol) in water (2 mL). Then, the mixture was held constant at 15 °C, and a solution of *p*-fluorobenzyl amine (0.56 g, 10 mmol) in toluene (3 mL) was added slowly. After addition was completed, the mixture was stirred at room temperature for 5 h. Then, toluene was removed under reduced pressure, and water (50 mL) was added. The solid was filtered, washed with water abundantly, and recrystallized from methanol to afford **F2** as a white solid (2.27 g, 76.8%); mp 135−137 °C. ^{1}H NMR (400 M, $CDCl_3$) δ: 1.12−1.25 (m, 6H), 4.02−4.24 (m, 1H), 4.50−4.60 (m, 2H), 5.08−5.33 (m, 1H), 5.43−6.67 (m, 1H), 6.97−7.06 (m, 2H), 7.22−7.32 (m, 2H). Anal. Calcd for $C_{13}H_{15}ClFN_5$: C, 52.80; H, 5.11; N, 23.68. Found: C, 52.69; H, 4.97; N, 23.50.

The target compounds **F3**−**F6** were prepared by following the same procedure as for compound **F2**. The corresponding starting materials 2-tetrahydrofuranmethylamine, 2-chloro-5-pyridylmethylamine,[7c] 3-phenyl-1,2-oxazole-5-methylamine,[7g] and 2-bromo-5-thiazolylmethylamine[7b] were commercial available or prepared according to our previous paper.

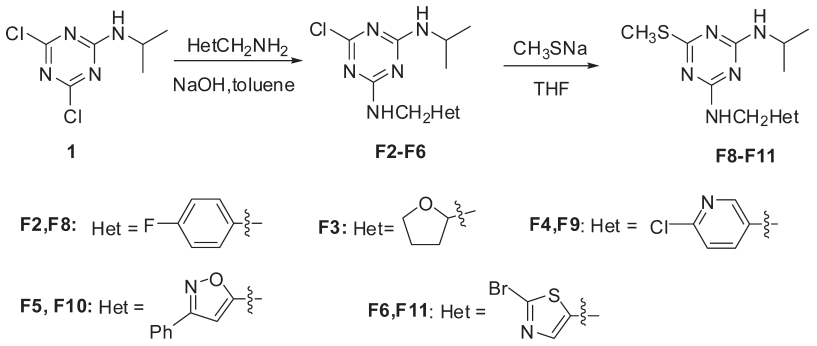
*2-Chloro-4-isopropylamino-6-(2-tetrahydrofuranmethylamino)-1,3,5-triazine (**F3**):* yield, 87.8%; an oil.[8b] ^{1}H NMR (400 M, $CDCl_3$) δ: 1.15−1.24 (m, 6H), 1.53−1.67 (m, 1H), 1.86−2.03 (m, 3H), 3.29−3.45 (m, 1H), 3.48−3.61 (m, 1H), 3.68−3.81 (m, 1H), 3.84−3.93 (m, 1H), 3.97−4.08 (m, 1H), 4.10−4.27 (m, 1H), 5.04−5.53 (m, 1H), 5.59−6.21 (m, 1H).

*2-Chloro-4-isopropylamino-6-(2-chloro-5-pyridylmethylamino)-1,3,5-triazine (**F4**):* yield, 76.6%; a white solid; mp 137−138 °C. ^{1}H NMR (400 M, $CDCl_3$) δ: 1.13−1.23 (m, 6H), 3.90−4.26 (m, 1H), 4.54−4.65 (m, 2H), 5.06−5.42 (m, 1H), 5.55−5.75 (m, 1H), 7.26−7.34 (m, 1H), 7.58−7.69 (m, 1H), 8.36−8.40 (m, 1H). Anal. Calcd for $C_{12}H_{14}Cl_2N_6$: C, 46.02; H, 4.51; N, 26.83. Found: C, 45.88; H, 4.43; N, 26.70.

*2-Chloro-4-isopropylamino-6-(3-phenyl-1,2-oxazole-5-methylamino)-1,3,5-triazine (**F5**):* yield, 80.8%; a white solid; mp 173−174 °C. ^{1}H NMR (400 M, $CDCl_3$) δ: 1.15−1.23 (m, 6H), 4.05−4.25 (m, 1H), 4.71−4.82 (m, 2H), 5.15−5.42 (m, 1H), 5.64−7.26 (m, 1H), 6.45−6.55 (m, 1H), 7.40−7.48 (m, 3H), 7.73−7.79 (m, 2H). Anal. Calcd for $C_{16}H_{17}ClN_6O$: C, 55.73; H, 4.97; N, 24.37. Found: C, 55.65; H, 4.90; N, 24.16. MS (ESI) for $C_{16}H_{18}ClN_6O$ (M+1): 345.

*2-Chloro-4-isopropylamino-6-(2-bromo-5-thiazolylmethylamino)-1,3,5-triazine (**F6**):* yield, 66.9%; a white solid; mp 201−202 °C. ^{1}H NMR (400 M, $CDCl_3$) δ: 1.06−1.15 (m, 6H), 3.91−4.18 (m, 1H), 4.49−4.58 (m, 2H), 7.55−7.61 (m, 1H), 7.66−8.00 (m, 1H), 8.06−8.44 (m, 1H). Anal. Calcd for $C_{10}H_{12}BrClN_6S$: C, 33.03; H, 3.33; N, 23.11. Found: C, 32.87; H, 3.29; N, 22.91.

Scheme 2



*Synthesis of Ametryne (**F7**).* To a solution of **F1** (0.68 g, 3 mmol) in tetrahydrofuran (12 mL) was added a solution of sodium methyl mercaptan (2.10 g, 30 mmol) in water (8.5 mL). Then, the mixture was refluxed for 24 h, and tetrahydrofuran was removed under reduced pressure. The residue was added water (50 mL) and extracted by dichloromethane (50 mL × 4). The combined organic extract was washed with saturated brine (50 mL), then dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by recrystallization from a mixture of methanol and water to afford **F7** as a white solid (0.45 g, 65.3%); mp 84−86 °C [lit. 86−88 °C[12]].

The target compounds **F8**−**F11** were prepared by following the same procedure as for compound **F7**.

*2-Isopropylamino-4-(p-fluorobenzylamino)-6-methylthio-1,3,5-triazine (**F8**):* yield, 66.5%; a white solid; mp 105−106 °C. ^{1}H NMR (400 M, $CDCl_3$) δ: 1.13−1.24 (m, 6H), 2.39−2.48 (m, 3H), 4.00−4.28 (m, 1H), 4.46−4.63 (s, 1H), 4.70−5.15 (m, 1H), 5.20−5.68 (m, 1H), 6.96−7.04 (m, 2H), 7.21−7.32 (m, 2H). Anal. Calcd for $C_{14}H_{18}FN_5S$: C, 54.70; H, 5.90; N, 22.78. Found: C, 54.51; H, 5.82; N, 22.72.

*2-Isopropylamino-4-(2-chloro-5-pyridylmethylamino)-6-methylthio-1,3,5-triazine (**F9**):* yield, 68.2%; a white solid; mp 163−164 °C. ^{1}H NMR (400 M, $CDCl_3$) δ: 1.06−1.28 (s, 6H), 2.37−2.46 (m, 3H), 3.95−4.25 (m, 1H), 4.50−4.60 (m, 2H), 4.70−5.10 (m, 1H), 5.45−6.79 (m, 1H), 7.23−7.29 (m, 1H), 7.57−7.67 (m, 1H), 8.36 (s, 1H). Anal. Calcd for $C_{13}H_{17}ClN_6S$: C, 48.07; H, 5.28; N, 25.87. Found: C, 48.12; H, 5.21; N, 25.74.

*2-Isopropylamino-4-(3-phenyl-1,2-oxazole-5-methylamino)-6-methylthio-1,3,5-triazine (**F10**):* yield, 77.6%; a white solid; mp 139−140 °C. ^{1}H NMR (400 M, $CDCl_3$) δ: 1.18 (s, 6H), 2.40−2.48 (m, 3H), 4.06−4.25 (m, 1H), 4.70−4.80 (m, 2H), 4.82−5.02 (m, 1H), 5.40−6.20 (m, 1H), 6.46 (s, 1H), 7.41−7.48 (m, 3H), 7.74−7.79 (m, 2H). Anal. Calcd for $C_{17}H_{20}N_6OS$: C, 57.28; H, 5.66; N, 23.58. Found: C, 57.23; H, 5.63; N, 23.44. MS (ESI) for $C_{17}H_{21}N_6OS$ (M+1): 357.

*2-Isopropylamino-4-(2-bromo-5-thiazolylmethylamino)-6-methylthio-1,3,5-triazine (**F11**):* yield, 61.6%; a yellow solid; mp 123−124 °C. ^{1}H NMR (400 M, $CDCl_3$) δ: 1.22 (s, 6H), 2.35−2.49 (m, 3H), 4.00−4.28 (m, 1H), 4.55−4.75 (m, 2H), 4.85−5.09 (m, 1H), 5.35−6.05 (m, 1H), 7.45−7.52 (m, 1H). Anal. Calcd for $C_{11}H_{15}BrN_6S_2$: C, 35.20; H, 4.03; N, 22.39. Found: C, 35.42; H, 4.24; N, 22.51.

Biological Assay. *Herbicide Screening Conducted at Nankai University.* The glasshouse herbicidal activities of compounds **F2**−**F11** and atrazine were evaluated using a previously reported procedure in Nankai University.[7a] One dicotyledonous crop, rape (*Brassica napus* L.), and one monocotyledonous weed, barnyard grass (*Echinochloa crus-galli*), were used to test the herbicidal activities of compounds. Purified compounds were dissolved in 100 μL of *N*,*N*-dimethylformamide with the addition of a little Tween 20 and then were sprayed using a laboratory belt sprayer delivering a 750 L/ha spray volume. The dosage (activity ingredient) for each compound corresponded to 1.5 kg/ha. Compounds were sprayed immediately after seed planting (preemergence treatment) or after the expansion of the first true leaf (postemergence treatment). The mixture of same amount of water, *N*,*N*-dimethylformamide, and Tween 20 was sprayed as the control. Each treatment was triplicated. The fresh weight of the above ground tissues was measured 10 days after treatment. The inhibition percent was used to describe the control efficiency of the compounds. The activity numbers in Table 1 represent the percent displaying herbicidal damage as compared to the control, where complete control of the target is 100 and no control is 0.

Table 1. Herbicidal Activities of Compounds F1−F11 (1.5 kg/ha, Percent Inhibition, %)

	preemergence treatment		postemergence treatment	
	rape	barnyard grass	rape	barnyard grass
F1 (atrazine)	100	100	100	100
F2	100	2.1	100	75.7
F3	100	46.4	100	41.3
F4	100	68.9	100	68.6
F5	20.1	4.3	42.4	17.7
F6	5.2	11.3	10.0	10.4
F7 (ametryne)	100	100	100	100
F8	100	21.4	100	100
F9	100	88.6	100	88.6
F10	33.1	37.6	100	14.3
F11	87.1	74.6	100	92.0

Screening Conducted at Syngenta. The compounds were screened for herbicidal, insecticidal, and fungicidal activity in a variety of laboratory and glasshouse-based assays.

Glasshouse Herbicide Screening. The glasshouse herbicidal screening performed by Syngenta followed similar methods to those at Nankai University, using the dicotyledonous weed species *Amaranthus retroflexus* and *Stellaria media* and the monocotyledonous species *Lolium perenne* and *Digitaria sanguinalis* as plant material. The compounds were formulated and applied at 1000 g/ha to preemergent and postemergent plants, which were then grown in the glasshouse for 12 days. Assessments were made of % herbicidal effects, and test samples were given a score between 0 and 100, with 100 as complete control of the target and 0 as no effect. Glyphosate was included in the test as a positive control compound. The results of these tests are presented in Table 2.

96-Well Plate Herbicide Screening. Compounds **F2**−**F11** and atrazine were tested for herbicidal activity against *Arabidopsis thaliana* at 10 ppm and *Poa annua* at 32 ppm, with two replicates of each treatment.

Table 2. Herbicidal Activities of Selected Compounds (Mean Scores/Percent Inhibition, %)[a]

	96-well plate tests		postemergence glasshouse				preemergence glasshouse			
	AT	PA	AR	LP	SM	DA	AR	LP	SM	DA
F1 (atrazine)	99	0	100	100	100	70	100	80	90	0
F2	99	0	100	50	90	0	100	30	90	70
F4	99	0	100	80	100	0	100	70	80	0
F7 (ametryne)	99	0	100	100	100	100	100	90	90	90
F8	99	0	100	80	100	80	100	50	90	0
F9	99	0	70	50	100	90	100	10	90	0
norflurazon	99	99	—[b]	—	—	—	—	—	—	—
glyphosate	—	—	90	80	80	90	90	0	0	0

[a] For 96-well plate tests, AT at 10 ppm and PA at 32 ppm, results are mean assessment scores. For glasshouse screening, 1.0 kg/ha. AT: *Arabidopsis thaliana*. PA: *Poa annua*. AR: *Amaranthus retroflexus*. LP: *Lolium perenne*. SM: *Stellaria media*. DA: *Digitaria sanguinalis*. Results are percent inhibition of growth. [b] "—" means not tested.

Table 3. Fungicidal Activities of Compounds F1—F10 (Mean Assessment Score across Replicates)

	Pythium dissimile[a,b]	*Alternaria solani*[a,b]	*Fusarium graminearum*[a,b]	*Botrytis cinerea*[a,b]	*Septoria tritici*[c,d]	*Phytophthora infestans*[c,e]	*Uromyces viciae-fabae*[c,d]
F1	0	0	0	0	18	0	NCH[f]
F2	0	0	0	99	99	0	99
F4	0	0	0	0	99	0	NCH
F5	0	0	0	49	69	0	77
F6	0	0	0	0	0	0	0
F7	0	0	0	27	0	0	NCH
F8	0	27	0	0	0	0	NCH
F9	0	0	0	55	0	0	NCH
F10	0	0	0	77	NCH	0	99

[a] Tests conducted in artificial media. [b] 2 ppm. [c] Tests conducted on leaf pieces. [d] 100 ppm. [e] 200 ppm. [f] "NCH" indicates that no assessment was possible due to herbicidal activity on the leaf piece.

Test plates were incubated for seven days in a controlled environment cabinet before assessment. The plates were then assessed, and each treatment replicate was scored as either 0 (for no observable effect) or 99 (where an herbicidal effect was observed). Norflurazon and glyphosate was tested alongside the compounds as a positive control. The data presented in Table 2 are the mean scores of the two replicates.

96-Well Plate Fungicide Screening. Compounds **F1—F11** were evaluated in mycelial growth tests in artificial media against *Pythium dissimile*, *Alternaria solani*, *Fusarium graminearum* (*Gibberella zeae*), and *Botrytis cinerea* (*Botryotinia fuckeliana*), at a rate of 2 ppm. The compounds were also evaluated in leaf-piece assays, at rates of 100 ppm for *Septoria tritici* on wheat and *Uromyces viciae-fabae* on bean, and 200 ppm and 60 ppm for *Phytophthora infestans* on tomato. Each assay contained two replicates for each rate, except the *Septoria tritici* assay, which contained three replicates. Chemicals were applied to leaf pieces prior to inoculation with spores of the pathogen, or incorporated into the growth medium for the artificial media assays. The plates were stored in controlled environment cabinets for between four and fourteen days, depending on the assay, after which mycelia growth or disease inhibition was assessed. Each well was scored using a three-banded system, with complete inhibition of mycelia growth or disease symptoms scored as 99, partial inhibition as 55, and no inhibition as 0. Azoxystrobin and prochloraz were included in the test as positive control compounds. The biological data presented in Table 3 are the mean scores for each treatment across replicates.

Additional Fungicide Screening. Compounds **F2**, **F4**, and **F10** were also screened on a broader range of fungal pathogens on leaf pieces, and against additional species in artificial media. The compounds were applied in a similar fashion to the 96-well tests, with the exception of a curative test against *Puccinia triticina* on wheat, in which the formulated compound was applied to leaf pieces after they had been inoculated with pathogen spores. The pathogens tested in this expanded screen were *Phytophthora infestans* on tomato, *Plasmopara viticola* on vine, *Puccinia triticina* on wheat (with both preventative and curative applications), *Septoria nodorum* (*Phaeosphaeria nodorum*) on wheat, *Pyrenophora teres* on barley, and *Alternaria solani* on tomato. The compounds were applied at rates of 200, 60, and 20 ppm. Assessments were made between three and nine days after inoculation, depending on the assay. Treatments were scored as percentage inhibition of disease development relative to untreated controls. The biological data presented in Table 4 are the mean scores for each treatment across replicates.

96-Well Plate Insecticide Screening. The compounds were tested for activity against an aphid species and the Lepidoptera *Heliothis virescens* in leaf-disk assays at 1000 ppm. The compounds were also evaluated at a rate of 500 ppm on *Plutella xylostella* in an artificial diet assay. Thiamethoxam and indoxacarb were included as positive control compounds. Each test contained three replicate treatments. The assay plates were stored in controlled environment cabinets for five to nine days (depending on the species). Mortality was then assessed relative to untreated control wells, with wells showing significant levels of mortality scored as 99, and wells without significant mortality scored as 0. The data presented in Table 5 are the mean scores of the three replicates.

Antitumor Activity Assays. The bioassay was tested at the National Center for Drug Screening (Shanghai, P. R. China). The antitumor activities of compounds **F2—11** on leukemia HL-60 cell line and lung adenocarcinoma A-549 cell line have been measured by using MTT

Table 4. Fungicidal Activities of Compounds F2, F4 and F10 (Percent Inhibition, %)[a]

	leaf-piece assays								
	test rates (ppm)	PI	PV	BG	PTP	PTC	SN	PT	AS
F2	200	0	50	70	100	100	70	0	0
	60	0	20	20	20	100	20	0	0
	20	0	0	k	0	20	0	0	0
	200	0	0	90	100	100	pt	0	0
F4	60	0	0	0	70	100	pt	0	0
	20	0	0	0	0	100	0	0	0
	200	0	0	70	100	100	pt	0	0
F10	60	0	0	70	70	100	70	0	0
	20	0	0	0	0	70	0	0	0

[a] pt: phytotoxic effect observed on leaf piece. k: test well not assessed due to contamination. PI: *Phytophthora infestans* (tomato-preventative). PV: *Plasmopara viticola* (grapevine-preventative). BG: *Blumeria graminis* f. sp. *tritici* (wheat-preventative). PTP: *Puccinia triticina* (wheat-preventative). PTC: *Puccinia triticina* (wheat-curative). SN: *Stagonospora nodorum* (wheat-preventative). PT: *Pyrenothora teres* (barley-preventative). AS: *Alternaria solani* (tomato-preventative).

Table 5. Insecticidal Activities of Compounds F1−F10 (Mean Assessment Scores)

	aphid species[a]	*Heliothis virescens*[a]	*Plutella xylostella*[b]
F1	33	0	0
F2	0	0	0
F4	0	0	0
F5	0	99	99
F6	0	0	0
F7	0	0	0
F8	0	0	0
F9	33	0	0
F10	0	0	0

[a] Leaf-disk assays. 1000 ppm. [b] Artificial diet assay. 500 ppm.

(methyl-thiazolyl-tetrazolium) and SRB (sulfur rhodamine B) methods respectively after incubation of cancer cell with various concentrations of the reagents for 72 h at 37 °C according to the reported method.[13] The antitumor activity data are presented in Table 6.

RESULTS AND DISCUSSION

Synthesis. Known compounds **1**, **F1** (atrazine), and **F7** (ametryne) were synthesized according to published procedures (Scheme 1). Because of the existance of both monomer and dimer of triazine, and because each has a different set of chemical shifts, there are three sets of proton signals in the ^{1}H NMR spectrum, and the overlap of part of the signals of the monomer and the dimer makes their spectrum complex. Similar phenomena were found and studied by Carper in 2002.[14] Compound **1** reacted with different aromatic or heterocyclic methylamines, for example, 4-fluorophenylmethylamine, 2-tetrahydrofuranmethylamine, 2-chloro-5-pyridylmethylamine, 3-phenyl-1,2-oxazole-5-methylamine, and 2-bromo-5-thiazolylmethylamine gave corresponding target molecules **F2**−**F6**, which were converted to corresponding methylthio-substituted triazines **F8**−**F11** by reacting with sodium thiomethoxide (Scheme 2). Just like atrazine, the ^{1}H NMR spectra of

Table 6. Antitumor Activities of Compounds F1−F10 (Percent Inhibition, %)

	lung adenocarcinoma A-549 cell		leukemia HL-60 cell	
	10^{-4} M	10^{-5} M	10^{-4} M	10^{-5} M
F1	40.6	2.8	55.5	6.4
F2	33.5	5.8	52.6	13.7
F4	55.4	17.0	70.2	1.7
F5	44.3	12.0	64.4	14.4
F6	35.7	11.5	56.5	10.5
F7	39.6	5.1	52.9	0
F8	27.3	2.7	58.8	6.6
F9	29.1	4.9	63.2	9.6
F10	27.0	2.3	57.2	14.1

F2−**F11** were also complex, but their structures and purity were definitely ensured by MS and elemental analysis.

Herbicidal Activities. The glasshouse screening of 4-fluorobenzylamino and representative arylmethylamino-substituted triazines (**F2**−**F6** and **F8**−**F11**) were evaluated and compared with known atrazine (**F1**) and ametryne (**F7**) (Table 1). As shown in the table, most of the compounds exhibited good herbicidal activities against dicotyledonous species (rape) both in post-emergence treatment and in preemergence treatment. From the activities against barnyard grass in both treatments we could obviously find that the compounds containing the methylthio moiety (**F7**−**F11**) gave higher herbicidal activities than the corresponding chloro analogues (**F1**−**F6**). Compounds with different aryl ring exhibited different level of inhibitory activity. In general, compounds bearing an isoxazole or thiazole ring, such as **F5** and **F6**, displayed lower activity on all of the four screening modes, while 4-fluorobenzylamino-substituted triazines **F2** and **F8** and 2-chloropyridyl-substituted compounds **F4** and **F9** gave relatively higher activities, but still lower than commercial compounds **F1** and **F7**, which gave 100% inhibition during all four screening modes.

Compounds **F2**, **F4**, **F8**, and **F9** were further screened at Syngenta in order to better characterize their herbicidal spectrum. The 96-well plate test and glasshouse screen were conducted, and the data are listed in Table 2. **F1**, **F7**, norflurazon, and glyphosate were added as control compounds. In the plate tests, the four compounds together with **F1** and **F7** were active only against the dicotyledonous species AT (*Arabidopsis thaliana*), but not the monocotyledonous species PA (*Poa annua*). In the glasshouse herbicide screening, a similar result could be concluded since almost all the compounds exhibited a little higher activity against dicotyledonous species (AR and SM) than monocotyledonous species (LP and DA). Overall, the herbicide screening data suggest that while the compounds are not inactive against monocot species, there is a degree of selectivity toward activity on dicots. The data also showed that all the compounds, including control compounds, exhibited the same or a little higher activity in postemergence treatment than in preemeergence treatment against the same species. Among the four compounds, **F8** gave a level of overall activity similar to the commercial herbicide atrazine and higher than glyphosate.

Fungicidal Activities. Compounds **F1**−**F10** were evaluated in a series of *in vitro* fungicidal tests, against a range of phytopathogenic species. The resulting data (Table 3) revealed that several compounds displayed a degree of fungicidal activity. Compounds

F2 and **F5** showed activity against *B. cinerea*, *S. tritici* and *U. viciae-fabae*, and compound **F10** was active against *B. cinerea* and *U. viciae-fabae*, but has high phytotoxicity to the leaf pieces in the *S. tritici* assay for a result to be recorded. Similarly, **F4** was active against *S. tritici*, but has high phytotoxicity on the leaf pieces of the *U. viciae-fabae* assay for a result to be recorded, as did several other compounds. No obvious structure−activity relationship was found.

Compounds **F2**, **F4**, and **F10** were also screened on more detailed *in vitro* fungicide tests against a broader range of species (Table 4). All three compounds showed activity on the leaf-piece assays, particularly against *Puccinia triticina* (both as preventative and curative applications) and *Blumeria graminis*. Compound **F4** gave 100% control of *P. triticina* at the lowest rate tested, but phytotoxic effects from this compound were recorded on leaf pieces in the *Septoria nodorum* assay, as well as for compound **F10** at the highest rate. None of the compounds displayed any activity on additional tests in artificial media (data not shown), and this, coupled with the activity from these compounds in the herbicide screens, suggests that the disease control observed is an artifact of phytotoxic activity rather than evidence of intrinsic fungicidal properties of the compounds (although this would not explain the better fungicidal control in the curative applications for *P. triticina* compared to the preventative).

Insecticidal Activities. Compounds **F1**−**F10** were tested for insecticidal activity against three pest species. The mean assessment scores are given in Table 5. Most of the compounds were inactive, but interestingly the oxazolemethylamino-substituted triazine **F5** was active against both Lepidopteran species *Heliothis virescens* and *Plutella xylostella*. The combination of activity in both leaf-piece and artificial diet assays may be evidence of this compound having intrinsic insecticidal qualities.

Antitumor Activities. The antitumor activities of compounds **F1**−**F10** on leukemia HL-60 cell line and lung adenocarcinoma A-549 cell line have been measured by using MTT (methyl-thiazolyl-tetrazolium) and SRB (sulfur rhodamine B) methods respectively. These compounds showed antitumor activities as shown in Table 6.

In summary, a series of novel triazines containing arylmethylamino moieties were synthesized, and the herbicidal, fungicidal, insecticidal, and antitumor activities of these triazines were evaluated. The results from both Nankai and Syngenta showed that most of these triazines exhibited good herbicidal activities, and these compounds gave certain selectivity toward dicotyledonous species in postemergence treatment. Of all the new compounds synthesized, 2-isopropylamino-4-(*p*-fluorobenzylamino)-6-methylthio-1,3,5-triazine (**F8**) was almost at the same level as the control compound atrazine. At the same time, some triazines had interesting fungicidal activity, of which **F4** exhibited 100% efficacy against *Puccinia triticina* even at 20 ppm. Although some of this activity may be attributed to phytotoxic effects from the compound inhibiting disease development, the disparity in activity between curative and preventative applications suggests that it may not be the only factor. On the insecticidal screening, **F5** showed Lepidopteran-specific activity in both leaf-piece and artificial diet assays. Moreover, these compounds showed antitumor activities against leukemia HL-60 cell line and lung adenocarcinoma A-549 cell line.

AUTHOR INFORMATION

Corresponding Author

*Tel: +86-(0)22-23499842. Fax: +86-(0)22-23499842. E-mail: wang98h@263.net; wangqm@nankai.edu.cn.

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