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N-(2-Bromophenyl)-2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamine, a New Selective Postemergent Herbicide for Weed Control in Winter Oilseed Rape

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N-(2-Bromophenyl)-2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamine is a highly active herbicide, which belongs to a novel class of chemistry. The compound is de novo synthesized in good yield, and the structure is confirmed by ^{1}H NMR, IR, MS, microanalysis, and X-ray. Its herbicidal activity is assessed under greenhouse conditions. It is effective against many grass weed species, as well as broadleaf weeds, under greenhouse conditions. Field trials indicate that it controls major weeds with a good tolerance on oilseed rape by postemergence application at rates of 15−90 g of active ingredient/ha. This compound possesses low mammalian toxicity and favorable environmental profile. These results suggest that the compound has potential as a new selective postemergent herbicide in winter oilseed rape.

KEYWORDS: Pyrimidinebenzylaniline; oilseed rape; toxicity; weed; herbicidal activity; *Alopecurus aequalis* Sobol.

INTRODUCTION

Oilseed rape is a major oil crop in the world. The occurrence of weeds in oilseed rape fields has been a serious problem, particularly due to their competitiveness with oilseed rape and their negative effects on yield. Herbicides, such as aryloxyphenoxypropionates, have been developed and used to control grasses, such as *Alopecurus aequalis* Sobol., the most troublesome weed in winter oilseed rape in China (*1*−*3*). There are several herbicides registered for broadleaf weed control. The occurrence of weed species in oilseed rape fields is becoming more and more complex, due to different factors including climate, area, soil condition, resistance, and usage of herbicides with a limited activity spectrum (*4*). Early control of these species is essential to prevent yield loss. There is a great demand for new effective herbicides that have different chemical structures and utilize novel action modes (*5*), which will allow growers to have more choices to control grasses and broadleaf weeds in oilseed rape.

Our study focuses on the chemical synthesis of pyrimidinebenzylamine derivatives, which belong to a novel chemical class, and the evaluation of their herbicidal activity (*6*). We find that *N*-(2-bromophenyl)-2-(4,6-dimethoxypyrimidin-2-yloxy)-benzylamine, compound **A**, exhibits an excellent herbicidal activity on *Alopecurus aequalis* Sobol. and good oilseed rape tolerance. This compound has been chosen for development of broad spectrum postemergence weed control in winter oilseed rape.

In this paper, we describe a de novo synthesis procedure for compound **A**, characterize its herbicidal activity, and evaluate its toxicological level.

MATERIALS AND METHODS

Proton NMR spectra were measured on a Bruker Avance DMX 500 instrument in $CDCl_3$. Chemical shifts were in parts per million downfield from TMS. Infrared spectra were recorded on a Nicolet Nexus 470 FT-IR spectrometer as potassium bromide tablets. Microanalyses were carried out with a Carlo Erba 1110 elemental analyzer. Mass spectra were recorded with an HP 5989B spectrometer using the EI method. Melting points were taken on an X-4 melting point apparatus and are uncorrected.

Synthesis. *N*-(2-Bromophenyl)-2-(4,6-dimethoxypyrimidin-2-yloxy)-benzylamine, compound **A**, was synthesized according to the procedure shown in **Figure 1**.

*2-Bromo-N-(2-hydroxybenzylidene)aniline (**1**).* Salicylaldehyde (14.3 g, 117 mmol) was added dropwise into a mixture of 2-bromoaniline (20.0 g, 116 mmol) in methanol (15 mL). After 10 min of stirring at room temperature, the resulting solid was filtered and dried in air to produce compound **1** (30.3 g, 95%) as a yellow solid.

*2-Bromo-N-(2-hydroxybenzyl)aniline (**2**).* After solid compound **1** (30.3 g, 133 mmol) had been dissolved in methanol (66 mL), potassium borohydride (2.4 g, 44.4 mmol) was added in a portion. The mixture was stirred in reduced pressure at room temperature for 30 min to remove methanol. Then water (30 mL) and ethyl acetate (30 mL) were added to the mixture. The resulting water layer was separated and

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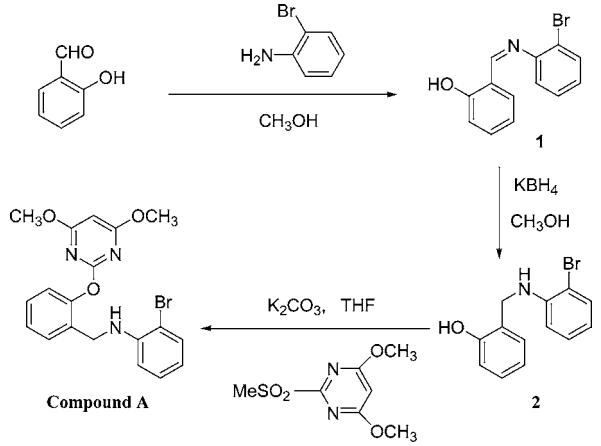

Figure 1. Chemical structures and synthesis scheme.

extracted with ethyl acetate (2 × 30 mL). The combined organic layer was dried by anhydride sodium sulfate and evaporated to give compound **2** (30.6 g, 100%).

*N-(2-Bromophenyl)-2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamine (**A**).* 2-Methanesulfonyl-4,6-dimethoxypyrimidine (26.5 g, 121.6 mmol) in tetrahydrofuran (THF) (280 mL) was added to the mixture of 2-bromo-*N*-(2-hydroxybenzyl)aniline (30.6 g, 110.1 mmol) and potassium carbonate (45.7 g, 330.5 mmol) in THF (204 mL). After the mixture had been stirred for 7 h with refluxing, the solid was filtered off, and the filtrate was evaporated to give compound **A** (34.5 g, 75%) as a white solid: mp 95−96 °C; ^{1}H NMR ($CDCl_3$) δ 3.79 (s, 6H, 2 × OCH_3), 4.41(s, 2H, CH_2), 4.66 (brs, 1H, NH), 5.77(s, 1H, CH), 6.50−6.56 (m, 2H, Ar), 7.04−7.42 (m, 6H, Ar); MS, *m*/*z* (abundance) 417/415 (M^+ + 2, 7), 334 (6), 245 (100), 180 (30), 157 (48); IR(KBr) 3410 (m), 1597(s),1571(s). Anal. Calcd for $C_{19}H_{18}BrN_3O_3$: C, 54.82; H, 4.36; N, 10.09. Found: C, 54.73; H, 4.42; N, 10.14.

Herbicidal Activity in the Greenhouse. Compound **A** was formulated as 50 g/L emulsified concentrates by using acetone as solvent and TW-80 as emulsification reagent. The concentrates were diluted with water to the required concentration and applied to pot-grown plants in a greenhouse. The rate of application [grams of active ingredient (ai) per hectare] was calculated by the total amount of active ingredient in the formulation divided by the surface area of the pot. Fifteen seeds of a testing plant were sown in a plastic pot (diameter = 9.5 cm) filled with soil with a composition of 1/1/1 m/m/m sand/silt/clay, an organic matter content of 2.5%, and a pH of 6.7. The pots were maintained at 20−25 °C in a greenhouse. The diluted formulation solutions of compound **A** were applied for pre-emergence treatment 24 h after weeds were sown. Dicotyledon weeds at the 2-leaf stage and monocotyledon weeds at the 2−2.5-leaf stage were treated for postemergence treatment. Compound **A** was sprayed at application rates up to 300 g of ai/ha. The herbicidal activity was evaluated visually on a scale of 0−100, where 0 indicates no visible effect and 100 indicates complete death of plants. Final evaluation was made 25 days after treatment.

Crop Selectivity. The conventional rice, soybean, and oilseed rape seeds were respectively planted in pots (diameter = 12 cm) containing test soil and grown in a greenhouse at 20−25 °C. After the plants had reached the 4-leaf stage, the spraying treatment was conducted at different dosages by diluting the formulation of compound **A** with water. The visual injury and growth state of the individual plant were observed at regular intervals. The final evaluation for crop safety of compound **A** was conducted by visual observation in 30 days after treatment on the 0−100 scale.

Application Windows for *A. aequalis* Sobol. *A. aequalis* Sobol. seeds were the sown staggered to allow application to all six growth stages on the same day. Compound **A** was sprayed at rates up to 45 g of ai/ha at each stage, and the treatments were replicated three times. After 30 days, the response of *A. aequalis* Sobol. was visually evaluated on a 0−100 scale, where 0 indicates no visible effect and 100 indicates complete death of plants.

Time Course for Effects on *A. aequalis* Sobol. *A. aequalis* Sobol. seeds were sown in pots as described above. After the weeds had germinated and grown to the 1−2-leaf stage, compound **A** was sprayed at a dosage of 15, 30, or 45 g of ai/ha. The weed control was visually determined on a 0−100 scale, where 0 indicates no visible effect and 100 indicates complete death of plants in 15 and 30 days post-treatments, respectively.

Table 1. Pre- and Post-emergence Herbicidal Activities of Compound **A** in the Greenhouse[a]

	pre-emergence						postemergence					
rate (g/ha)	EC	DS	EI	AR	PO	BJ	EC	DS	EI	AR	PO	BJ
300	50	50	50	90	90	30	100	90	98	100	90	90
150	50	50	50	80	80	20	100	80	95	100	85	70

[a] EC, *Echinochloa crus-galli* L.; DS, *Digitaria sanguinalis* (L.) Scop.; EI, *Eleusine indica* (L.) Gaertn.; AR, *Amaranthus retroflexus* L.; PO, *Portulaca oleracea* L.; BJ, *Brassica juncea* L. Final evaluation was made 25 days after treatment by visual rating scales of 0–100.

Field Trial. The field test results in **Table 5** were conducted in year 2005 at Shao Xin Academe of Agriculture Science, Zhejiang, People's Republic of China. The sandy loam soil at pH 6.8 contains 2.9% of organic matter content. The field was rotavated. Each individual plot was 2 m wide × 10 m long. The experimental design was a randomized complete block with four replicates. The product was formulated as 20% suspension concentrate and applied by postemergence application at dosages ranging from 15 to 90 g of ai/ha with a knapsack sprayer in 675 L of water/ha, when weed species had grown to the 2−3-leaf stage and oilseed rape had grown to the 3−4-leaf stage. Evaluation was made 90 days after treatment. Oilseed rape injury was evaluated visually on a 0−100 scale, where 0 indicates no visible effect and 100 indicates complete death of plants.

RESULTS AND DISCUSSION

Synthesis and Spectroscopic Properties. A new herbicide, compound **A**, is prepared as outlined in **Figure 1**. Salicylaldehyde reacts with 2-bromoaniline to produce Schiff base **1** in good yield. Then Schiff base **1** is reduced by potassium borohydride to give 2-bromo-*N*-(2-hydroxybenzyl)aniline **2**, which in turn reacts with 2-methylsulfonyl-4,6-dimethoxypyrimidine in the presence of potassium carbonate to generate compound **A**.

Elemental analysis indicates that compound **A** is has the molecular formula $C_{19}H_{18}BrN_3O_3$. In its IR spectral data, the band at 3410 cm^{-1} is originated from the stretching vibration of N−H, and the band at 1597 cm^{-1} is assigned to the C═N stretching vibration in the pyrimidine ring. ^{1}H data for compound **A** are described under Materials and Methods. The crystal structure of compound **A** is determined. There are three different planes in the molecule, each of which is conjugated. The dihedral angles between the pyrimidine plane and the planes of the two phenyl rings are 107.85 (4) and 77.38 (2)°, and the dihedral angle between the planes of the two phenyl rings is 103.15 (3)° (*7*).

Herbicidal Activity under Greenhouse Conditions. Compound **A** was evaluated in the greenhouse after pre- and postemergence application on several weed species. The preliminary screening results in **Table 1** indicate that compound **A** has strong postemergence herbicidal activity in the greenhouse. In a similar experiment, weak pre-emergence activity occurs. Weeds are controlled by compound A at 150 and 300 g of ai/ha.

The herbicidal activity of compound **A** on weeds was further elucidated by postemergence application at low rates (<90 g of ai/ha) in the greenhouse. Weed sensitivities are given in **Table 2**. Many important weeds including *Echinochloa crus-galli* L., *Alopecurus aequalis* Sobol., *Poa annua* L., *Beckmannia eru-*

Table 2. Weed Control Spectrum of Compound **A** in the Greenhouse

readily controlled, ≥80–100% (rate < 90 g of ai/ha)	partially controlled, 60–79% (rate < 90 g of ai/ha)	suppressed, <60% (rate < 90 g of ai/ha)
Echinochloa crus-galli L.	*Digitaria sanguinalis* (L.) Scop.	*Galium aparine* L.
Alopecurus aequalis Sobol.	*Eleusine indica* (L.) Gaertn.	
Poa annua L.	*Brassica juncea* L.	
Amaranthus retroflexus L.	*Chenopodium serotium* L.	
Cerastium viscosum L.		
Portulaca oleracea L.		
Polypogon fugax Nees ex Steud.		
Beckmannia erucaeformis Host		

Table 3. Activity of Compound **A** against *A. aequalis* Sobol. and Oilseed Rape in the Greenhouse

rate (g/ha)	oilseed rape[b] (%)[c]	*A. aequalis* Sobol.[a] 1–1.5-LS (%)	2–3-LS (%)	4–5-LS (%)	6-LS (%)[c]
15	0	100	98	95	90
30	0	100	100	100	95
45	0	100	100	100	95

[a] Herbicide solution was applied at each leaf stage (LS) of *A. aequalis* Sobol. [b] Pregerminated seeds and 4-leaf stage of oilseed rape were transplanted. [c] Data represent percentage growth inhibition as indicated by visual rating, where 0 indicates no visible effect and 100 indicates complete death of plants at 30 days after treatment.

caeformis Host., *Amaranthus retroflexus* L., *Polypogon fugax* Nees ex Steud., *Cerastium viscosum* L., and *Portulaca oleracea* L. are controlled at >80% at rates of <90 g of ai/ha. Weeds such as *A. aequalis* Sobol. and *P. annua* L. are highly sensitive to compound **A**, which can be controlled at rates as low as 30 g of ai/ha. *Digitaria sanguinalis* (L.) Scop., *Eleusine india* (L.) Gaertn., *Brassica juncea* (L.) Cosson., and *Chenopodium serotium* L. are slightly less sensitive. However, *Galium aparine* L. is only partially suppressed.

Crop Selectivity and Growth Stage Effects on *A. aequalis* Sobol. in the Greenhouse. Among crops, rice and soybean are susceptible, whereas 4-leaf stage oilseed rape exhibites excellent tolerance to compound **A** by postemergence application at the normal recommended dosages (**Table 3**).

A. aequalis Sobol. control efficacy at various stages was determined in the greenhouse (**Table 3**). *A. aequalis* Sobol. up to the 6-leaf stage is perfectly controlled at 15 g of ai/ha. The 1−1.5-leaf stage is most sensitive, followed by the 2−5-leaf stage and the 6-leaf stage. These results indicate that the optional time of compound **A** application is between the 1- and 5-leaf stages for *A. aequalis* Sobol. The application wnidow of compound **A** can also be extended up to the 6-leaf stage by increasing the application rate, which provides great flexibility for weed control in oilseed rape.

Time Effects on Susceptible *A. aequalis* Sobol. Table 4 describes the progression of herbicidal symptom development. In the first 15 days after application, few herbicidal symptoms are observed in the pre-emergence test, whereas during 30 days *A. aequalis* Sobol. is totally controlled. The results suggest that compound **A** appears to be a relatively slow-acting herbicide. Herbicidal symptoms in susceptible grasses include plant stunting followed by chlorosis and finally desiccation of the whole plant.

Compound **A** is highly effective against many weeds in oilseed rape including current problem weeds, such as *A. aequalis* Sobol. These results indicate that the compound may have great potential to be used as a selective postemergent herbicide for oilseed rape.

Table 4. Application Timing Effects of Compound **A** on Susceptible *A. aequalis* Sobol. in the Greenhouse[a]

	control[b] (%)					
	15 g of ai/ha		30 g of ai/ha		45 g of ai/ha	
application	15 days	30 days	15 days	30 days	15 days	30 days
pre-emergence	–[c]	95	–	98	–	98
postemergence	90	98	95	100	95	100

[a] Plants at the 2–3-leaf stage of *A. aequalis* Sobol. was transplanted. [b] Data represent percentage growth inhibition as indicated by visual rating, where 0 indicates no visible effect and 100 indicates complete death of plants at 30 days after treatment. [c] –, few herbicidal symptoms were observed.

Table 5. Herbicidal Efficacy and Oilseed Rape Injury of Compound **A** at 90 Days after Treatment in the Field[a]

treatment	rate (g/ha)	oilseed rape injury	fresh weight control value (%) AA	BS	SM	SA	CH	total
CoA	15	0	97.6	91.0	82.5	61.9	59.4	90.8
CoA	30	0	99.4	95.8	83.6	79.6	47.6	91.4
CoA	45	0	99.4	97.4	96.4	96.3	47.3	93.5
CoA	60	0	99.7	98.7	98.1	95.8	63.4	95.9
CoA	90	0	99.6	98.7	100	99.0	75.2	96.4
ETH	15	0	94.9	84.1	76.6	76.9	83.9	90.8
JHF	37.5	0	98.1	93.8	NT	NT	5.0	73.2

[a] AA, *Alopecurus aequalis* Sobol.; BS, *Beckmannia syzigachne* (Steud.) Fernald; SM, *Stellaria media* (L.) Cyrillo.; SA, *Stellaria alsine* Grimm; CH, *Cardamine hirsuta* L.; ETH, ethametsulfuron-methyl; JHF, quizalofop-*P*-ethyl; CoA, compound **A**. Final evaluation was made 90 days after treatment by visual rating scales of 0–100 for oilseed rape injury and measuring the fresh weight control value for weeds.

Field Performance. When it is applied postemergence in dosages ranging from 15 to 90 g of ai/ha in field trials, compound **A** shows excellent control efficacy against the important grass and broadleaf weeds in oilseed rape at 90 days after treatment (**Table 5**). *A. aequalis* Sobol. and *Beckmannia syzigachne* (Steud) Fernald, which are the most troublesome weeds in winter oilseed rape in China, are controlled (91−99%) very well at rates of 15−90 g of ai/ha, with the most favorable application timing at the 2−3-leaf stage. Broadleaf weeds, such as *Stellaria media* (L.) Cyrillo. and *Stellaria alsine* Grimm, are controlled (96%) by a rate of 45 g of ai/ha. Maximal control of weeds is achieved when compound **A** is applied at a favorable time of 2-leaf stage. Within 90 days after the application of compound **A**, it has the same control efficacy as the oilseed rape herbicide ethametsulfuron-methyl, which is applied at the rate of 15 g of ai/ha. However, compound **A** has better control efficacy than quizalofop-*P*-ethyl.

Oilseed rape tolerance was also evaluated in field trials. Compound **A**, which is applied as postemergence treatment at up to 90 g of ai/ha, exhibits good tolerance to the oilseed rape, provided the application was made after the oilseed rape had

Table 6. Mammalian Toxicity for Compound **A**

test (species or assay)	result
acute oral (rat)	LD_{50} > 5000 mg/kg
acute dermal (rat)	LD_{50} > 2000 mg/kg
eye irritation (rabbit)	slight to no irritation
skin irritation (rabbit)	not irritant
skin sensitization (guinea pig)	negative
mutagenicity (Ames)	negative
mutagenicity (mouse bone marrow micronucleus)	negative
mutagenicity (chromosome aberration)	negative

Table 7. Fish and Wildlife Toxicity for 20% Compound **A** Suspension Concentrate

species	result
quail	LD_{50} (7 days) > 1000 mg/kg
zebra fish	LC_{50} (96 h) > 100 mg/L
silkworm	LC_{50} (96 h) > 2000 mg/L
honeybee	LC_{50} (48 h) > 1005 mg/L

rreached the 4−5-leaf stage. These results also demonstrate that compound **A** can be used as a selective herbicide for transplanting oilseed rape. The dosage of the compound appears to be ≈30−60 g of ai/ha when it is applied alone.

Toxicity. Mammalian toxicity and fish and wildlife toxicity tests were performed according to the Chinese standard procedure. Compound **A** toxicity to mammals is summarized in **Table 6**. In all acute toxicological tests, compound **A** shows very low mammalian toxicity. No dermal sensitization is caused by compound **A**. Slight to no eye irritation was observed. No mutagenic effects were induced by compound **A**. The toxicity of 20% compound **A** suspension concentrate to fish and wildlife is summarized in **Table 7**. No adverse effects were observed on quail (*Coturnix coturnix japonica*), zerb fish (*Brachydanio rerio*), honeybees (*Apis mellifera* L.), and silkworm (*Bombyx mori*). These results indicate that toxicological and environmental profiles of compound **A** are favorable. There is no foreseeable risk if the product is applied according to label recommendation. Therefore, compound **A** can be registered in China according to these toxicological data.

Oilseed rape herbicides including aryloxyphenoxypropionates and cyclohexanediones have been introduced for selective control of grass in oilseed rape (*8*). However, applying these herbicides alone can hardly control broadleaf weeds. The sulfonylurea herbicide ethametsulfuron-methyl, which is used to control broadleaf weeds in oilseed rape, causes injury to rotational rice, due to its long residual activity (*9*, *10*). There is great demand for effective herbicides that have different structures and action modes in oilseed rape. Through our structural studies in pyrimidinebenzylamine derivatives, a new chemical class, and evaluation of their herbicidal activity in greenhouse and field trials, we have identified *N*-(2-bromophenyl)-2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamine, which has excellent efficacy on *A. aequalis* Sobol., wide application timing from the 1- to 6-leaf stages, and good crop compatibility to oilseed rape.

Compound **A** is a new postemergent herbicide in the pyrimidinebenzylamine class. Compound **A** provides selective control of various grass and broadleaf weeds in winter oilseed rape. When compound **A** is applied at low use rates of 15−90 g of ai/ha, its activity spectrum comprises major grass weeds, such as *A. aequalis* Sobol., various important broadleaf weeds including *B. syzigachne* (Steud.) Fernald, *S. media* (L.) Cyrillo., *S. alsine* Grimm, and *Cardamine hirsuta* L. In addition, compound **A** shows a high level of safety in oilseed rape and has a favorable toxicological and ecotoxicological profile. It also offers the flexibility of application timings in the control of *A. aequalis* Sobol., the most troublesome weed in winter oilseed rape.

In summary, compound **A** demonstrates its broad spectrum as a postemergent herbicide with a high level of selectivity in oilseed rape. It has a low use rate and a very flexible application time. Compound **A** is a safe herbicide with regard to human health. We believe that compound **A** can be a key herbicide to manage increasing problem weeds in winter oilseed rape. These chemical characteristics and its associated high unit activity give compound **A** excellent potential as a herbicide in oilseed rape.

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