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Article

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ABSTRACT:

Due to the key roles of auxins as master regulators of plant growth, there is considerable interest in the development of compounds with auxin-like properties for growth management and weed control applications. Herein, we describe the design and multi-step synthesis of ten compounds bearing combinations of functional groups commonly associated with auxin-type properties. Following synthesis, these compounds were tested against multiple weed species as well as sweet corn. In general, while these structures were not quite as active as commercial auxin mimic herbicides, multiple compounds exhibited broadleaf weed activity with concurrent selectivity in sweet corn. In addition, differential results were observed upon subtle changes to structure, providing insights into the structural properties required for activity.

KEYWORDS: auxins, weed science, synthesis, plant growth

INTRODUCTION

Auxins are an important class of plant growth substances called phytohormones that play essential roles in many growth and behavioral processes in a plant's life cycle. There are four natural auxins that are synthesized by plants (Figure 1): indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 4-chloroindole-3-acetic acid (4-Cl-IAA), and phenylacetic acid (PAA).[1] Since IAA influences almost every part of plant growth and development, it is believed to act as a "master hormone" in the network of interactions with other phytohormones.[2] In general, auxins regulate cell division, elongation, and developmental processes, which include vascular tissue and floral meristem differentiation, leaf initiation, phyllotaxy, senescence, apical dominance, and root formation.[3]

The stability and concentration of natural auxins is regulated by the plant through synthesis, conjugation, and degradation via multiple pathways.[4] At low auxin concentrations, growth and developmental activities are stimulated, while at high concentration, growth is interrupted, and the plant is lethally damaged. As a result, there has been considerable interest in the chemical manipulation of the auxin system via synthetic analogs in order to study auxin function.[5] In the 1940s, various derivatives of IAA were synthesized that exhibited auxin-like properties. These included 1-naphthalene acetic acid (1-NAA, Figure 1), 2-methyl-4-chlorophenoxyacetic acid (MCPA), and 2,4-dichlorophenoxyacetic acid (2,4-D).[6] They produce the same plant responses as IAA but are more durable and effective because they are not rendered inactive by the plant as rapidly as the endogenous auxins.[7]

Synthetic auxin mimics have found practical use not only as growth regulators for improving yields in agriculture and horticulture,[8] and as media components in tissue culture and plant micropropagation,[9] but also as herbicides to control weeds.[6] MCPA and 2,4-D launched a new era of weed control in modern agriculture after being introduced to the worldwide market after World War II. In fact, multiple classes of chemistry have been commercially produced as herbicides. These include the benzoic acids, the phenoxy-carboxylic acids, the pyridine-carboxylic acids, the pyrimidine-carboxylic acids, and the quinolone-carboxylic acids. To possess auxin activity, a chemical structure appears to need a strong negative charge on a carboxylic acid group that is separated by a distinct distance from a weaker positive charge on an aromatic ring.[10] When used as herbicides, synthesized auxins mimic the growth-inhibiting effects as those caused by IAA applied at high concentrations, which is also observed in transgenic, IAA-overproducing plants.[11] This occurrence has been described as an "auxin overdose," which is an effect of greater-than-optimal endogenous auxin concentrations, causing an imbalance in auxin homeostasis and interactions with other hormones in the tissue.[7]

More recent research in auxin mimic herbicides has uncovered more potent inhibitors with greater activity on more weed species at lower use rates. These newer herbicides contain a carboxylic acid and halogen (primarily chlorine and fluorine) substitutions on pyridine or pyrimidine like other auxin mimic herbicides, but these compounds are different due to a *para*-chlorophenyl substituent at the 4-position of the heterocyclic ring.[12-14] Currently, no research has been presented as to whether this

para-chlorophenol substituent would be efficacious if added to benzyl or indole ring structure. Therefore, the objectives of this research were to 1) synthesize benzyl and indole ring structures containing a carboxylic acid, at least one halogen, and a *para*-chlorophenol substituent and 2) to evaluate these new compounds for weed control and safety to sweet corn (*Zea mays* L. var. saccharum) in comparison to the commercial auxin standards of aminocyclopyrachlor, dicamba, and quinclorac.

MATERIALS AND METHODS

Chemicals

Dicamba (forumulated as Clarity) and quinoclorac (formulated as Drive) were obtained from the BASF Corporation and used as received. Aminocyclopyrachlor (DPX-MAT28, formulated as a 50% active granule) was obtained from DuPont Crop Protection. Starting materials and reagents for synthetic procedures were obtained from Fisher Scientific or Aldrich Chemical and used as received.

General experimental

a Pure solvent delivery system purchased from Innovative Technology, Inc. Column chromatography was performed using 230−400 mesh silica gel purchased from Sorbent Technologies. NMR spectra were obtained using Varian Mercury Vx 300 MHz or Bruker AC 250 MHz spectrometers.

Compound evaluation for weed control

Greenhouse trials were established at the University of Tennessee (35.98 N, 83.91W) to evaluate the herbicidal activity of the synthesized compounds. Herbicidal response of the compounds was evaluated on sweet corn, large crabgrass (*Digitaria*

sanguinalis), field bindweed (*Convolvulus arvensis*), barnyardgrass (*Echinochloa crus-gali*), velvetleaf (*Abutilon theophrasti*), and redroot pigweed (*Amaranthus retroflexus*). These specific weed species were selected in order to provide a mix of common small and large seeded monocot and dicot species. Four sweet corn seeds were planted in the middle of 23 cm diameter greenhouse pots (XAM09000, Dillen Products/Myers Industries Inc., Middlefield, OH) containing a potting media (Pro-Mix BX Mycorrhizae, Premier Tech Horticulture Inc., Quakertown, PA). Weed species were shallowly seeded around the margin of each greenhouse pot. Plants germinated and were allowed to grow for 12 days before application of experimental compounds. On the dates of application corn plants were approximately 15 cm in height. Weed species height ranged from 3 to 6 cm. Following these herbicide applications, plants were watered daily, fertilized as needed, and were augmented with artificial lights as needed to maintain adequate day length.

Compounds were dissolved in 3 mL of acetone before being added to 32 mL of deionized water and agitated by hand to form a spray solution. Crop oil concentrate was added to the spray solution to form a 1% v/v solution used for each treatment (Agridex, Helena Chemical Corp., Memphis, TN). Spray solutions were agitated again before application to the plant species using an enclosed sprayer chamber (Generation III track sprayer. DeVries Manufacturing, Hollandale, Minnesota) at 215 L ha^{-1} through an 8004 EVS nozzle (TeeJet, Wheaton, Illinois). All compounds were applied at a rate of 500 g ha^{-1}. Herbicidal activity was quantified by visually measuring injury to each plant species

10 days after treatment on a 0 (i.e., no injury) to 100 % (i.e., complete plant death) scale relative to a non-treated check.

RESULTS AND DISCUSSION

The goal of this research was to develop novel auxin mimic herbicides through the design and chemical synthesis of structures with similar properties to known auxin herbicides, followed by evaluation of herbicidal activities alongside three commercial herbicides, aminocyclopyrachlor,[15] dicamba,[16] and quinclorac[17] (Figure 2). Overall, ten compounds were synthesized and evaluated for auxin herbicidal activity (**1-7**).

The initial set of compound targets is shown in Figure 3A-C, each of which contains a *para*-chlorophenyl group fused to an aromatic unit bearing a carboxylic acid as well as different combinations of heteroatoms within the ring. These compounds were synthesized through a general scheme by converting commercially available carboxylic acids **8a-b**, **11a-b**, and **14a-b**, to methyl esters **9a-b, 12a-b**, and **13a-b**, respectively. This was followed by reaction with 4-chlorophenylboronic acid via a Suzuki coupling to attach a chlorophenyl group of **10a-b**, **13a-b**, and **16a-b**,[18] and then ester hydrolysis in base to restore the carboxylic acid functionality of target compounds **1a-b**, **2a-b**, and **3a-b**.

A similar process was used to access target compounds **4-5**, which also contain a *para*-chlorophenyl fused to different functionalized benzoic acid moieties (Figure 4A-C). To synthesize **4**, commercially available carboxylic acid **17** was first converted to methyl ester **18**. Next, the phenolic moiety of **18** was protected with a *para*-methoxybenzyl (PMB) group to produce intermediate **19**, which was coupled to 4-

chlorobronic acid to **20**, followed by PMB deprotection to **21** and ester hydrolysis to access **4**. For the synthesis of **5**, compound **22** was first subjected to nitration and esterification to form **23**, followed by reduction of the nitro group to the amine functionality of **24**. Finally Suzuki coupling to **27** and ester hydrolysis produced target compound **5**.

For the next set of compounds, we fused the *para*-chlorophenyl group that has been effective in prior auxin herbicides to the indole acid moiety of natural auxins (Figure 5). Here, compounds **6-7** were accessed through general schemes through the esterification of carboxylic acids **26** and **29** to **27** and **30**, Suzuki coupling to **28** and **31**, and ester hydrolysis to **6** and **7**, respectively.

Following the synthesis of the desired compounds, the herbicidal activities of these structures next evaluated in plant bioassays. The synthesized molecules were applied in the form of a spray solution to six different common weeds (redroot pigweed, velvetleaf, field bindweed, barnyardgrass, large crabgrass, and yellow nutsedge) as well as sweet corn. An ideal herbicide would be highly active against all the weeds but show little to no activity against crops such as corn. Most auxin herbicides typically have their greatest activity against dicot weeds as opposed to monocots. This differentiated selectivity has allowed auxin mimics to be used in monocot crops for the control of dicot weeds. However, quinoline-carboxylic acid auxin herbicides like quinclorac and the new 6-aryl-picolinate herbicides do maintain some herbicidal activity on key monocot grass and sedge species[12,19]. The commercial auxin mimic herbicides aminocyclopyrachlor,

dicamba, and quinclorac were tested alongside the newly synthesized compounds (Figure 2).

Out of the synthetic compounds, 2-chlorobenzoic acid **1a** provided the greatest level of activity, with about 90% control of pigweed, and only 2% injury to corn (Table 1). In addition, **1a** controlled velvetleaf 58%, however all other species were controlled by this analog 30% or less. Small substitution changes to **1a** changed weed control, especially on redroot pigweed. For example, introduction of the fluorine instead of a chlorine on the benzoic acid ring of analog **1b** diminished redroot pigweed and velvetleaf to 55 and 10%, respectively. A simple introduction of nitrogen into the 6-position of the benzoic acid ring in compound **2b** drastically reduced redroot pigweed to 53%, but velvetleaf control declined to only 43%. However, in compound **2a** the nitrogen placed in the ring at the 3-position reduced control of both redroot pigweed and velvetleaf to 3% or less. Curiously, the addition of an amine in the meta position relative to both the carboxylic acid and chlorophenyl groups in analog **5** reduced redroot pigweed and velvetleaf to 40% and 17%, respectively. Several potent commercial auxin mimic herbicides contain amine group substitutions in a similar position (e.g., aminopyralid, picloram, aminocylclopyrachlor, and halauxifen). In many instances these amine substituted compounds are more potent when compared to similar auxin mimics that lack this substitution.[12] The two indole acids, compounds **6** and **7,** displayed modest activity (<50% control) on all species evaluated with few differences between analogs. Symptoms expressed by these experimental analogs were primarily epinasty, which progressed to stunting, chlorosis and necrosis only on the most sensitive weed

166 species and only with the most potent analogs. These visual phenotypic responses
167 were similar to those associated with the auxin mimic herbicide standards included in
168 the trial. The only exception to this activity was the commercial standard quinclorac
169 activity on grass, which included some reddening symptoms on grasses consistent with
170 a secondary herbicide site of action.

171 Overall, these synthesized auxin mimics did not reach the performance level of
172 the commercial auxin mimic herbicides, with the exception of compound **1a**, which did
173 provided 90% control of redroot pigweed with adequate corn selectivity. However, this
174 compound did not provide broad-spectrum control of all weeds or even all broadleaf
175 weeds in these studies as observed with the commercial standards
176 aminocyclopyrachlor and dicamba (Table 1). These studies show that the 2-chloro
177 benzoic acid (**1a**) is significantly more herbicidal than the 2-fluoro benzoic acid version
178 (**1b**). Additionally, while the 2-chloro substitution increases herbicidal response there is
179 also evidence from evaluating compounds **3a** and **3b** that an additional 4-position
180 chlorine substitution on the benzoic acid ring may also be beneficial. While pyridine and
181 pyrimidine structures seem to be optimal for auxin mimic potency, our studies confirm
182 that the specific placement of the nitrogen in the pyridine rings is critical for maximizing
183 herbicidal performance (**2a** and **2b**). In addition, using a benzoic acid or indole acid
184 model for coupling with a *para*-chlorophenol moiety does not appear to be as active as
185 similar pyridine or pyrimidine versions reported in the literature.[12,13] Overall, these
186 results show that subtle modifications in the structure of molecular scaffolds can have
187 substantial effects on auxin herbicidal activity. In future work, it would be interesting to

understand these activities in the context of the structures of protein binding partners, particularly since the crystal structure of the auxin target TIR1 from *Arabidopsis* has been reported.[20] This provides a valuable model for understanding the properties of compounds that exhibit auxin-like properties and for predicting new compounds expected to be active.

ASSOCIATED CONTENT

Supporting Information: Procedures for synthesis and NMR spectra for characterization of synthetic compounds

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Figure Captions

Figure 1. Structures of natural and synthetic auxins

Figure 2. Commercial herbicides used as controls in testing auxin activities.

Figure 3. Generalized synthesis of initial targets compounds A. **1a-b**, B. **2a-b**, and C. **3a-b**.

Figure 4.. Synthesis of targets compounds A. **4**, and B. **5**

Figure 5. Synthesis of targets compounds A. **6**, and B. **7**

Table I. Activity of compounds **1-7** applied postemergence for control of key broadleaf and grass weeds with potential selectivity in sweet corn.

Figure 1

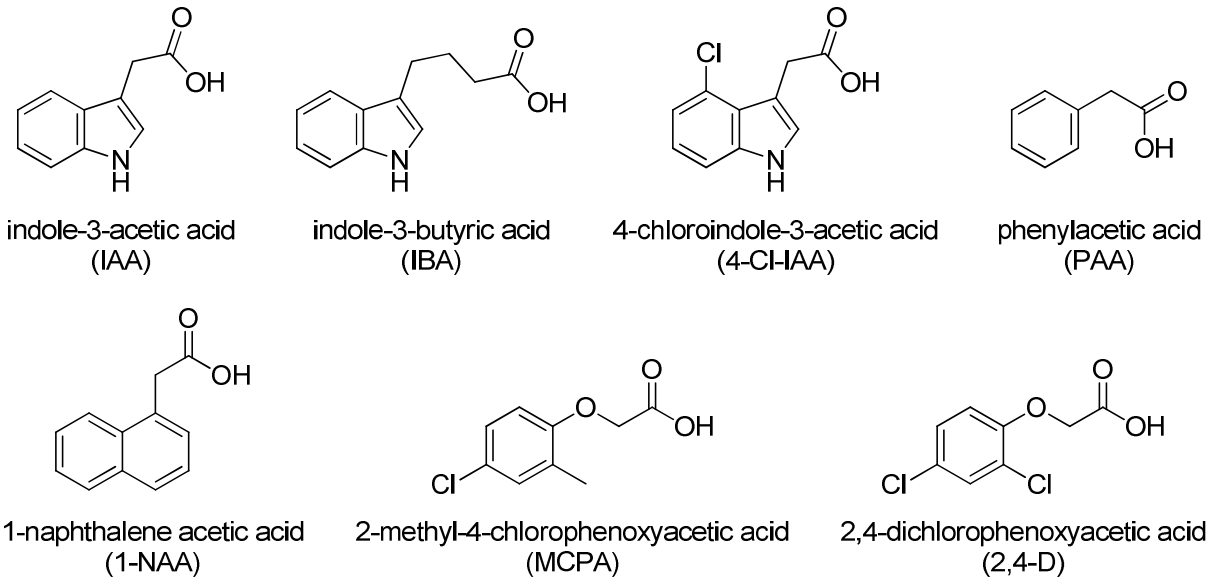
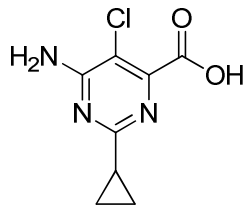
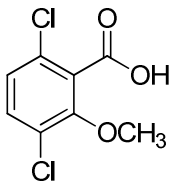

indole-3-acetic acid
(IAA)
indole-3-butyric acid
(IBA)
4-chloroindole-3-acetic acid
(4-Cl-IAA)
phenylacetic acid
(PAA)
1-naphthalene acetic acid
(1-NAA)
2-methyl-4-chlorophenoxyacetic acid
(MCPA)
2,4-dichlorophenoxyacetic acid
(2,4-D)

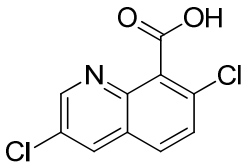
Figure 2



Aminocyclopyrachlor



Dicamba



Quinclorac

Figure 3

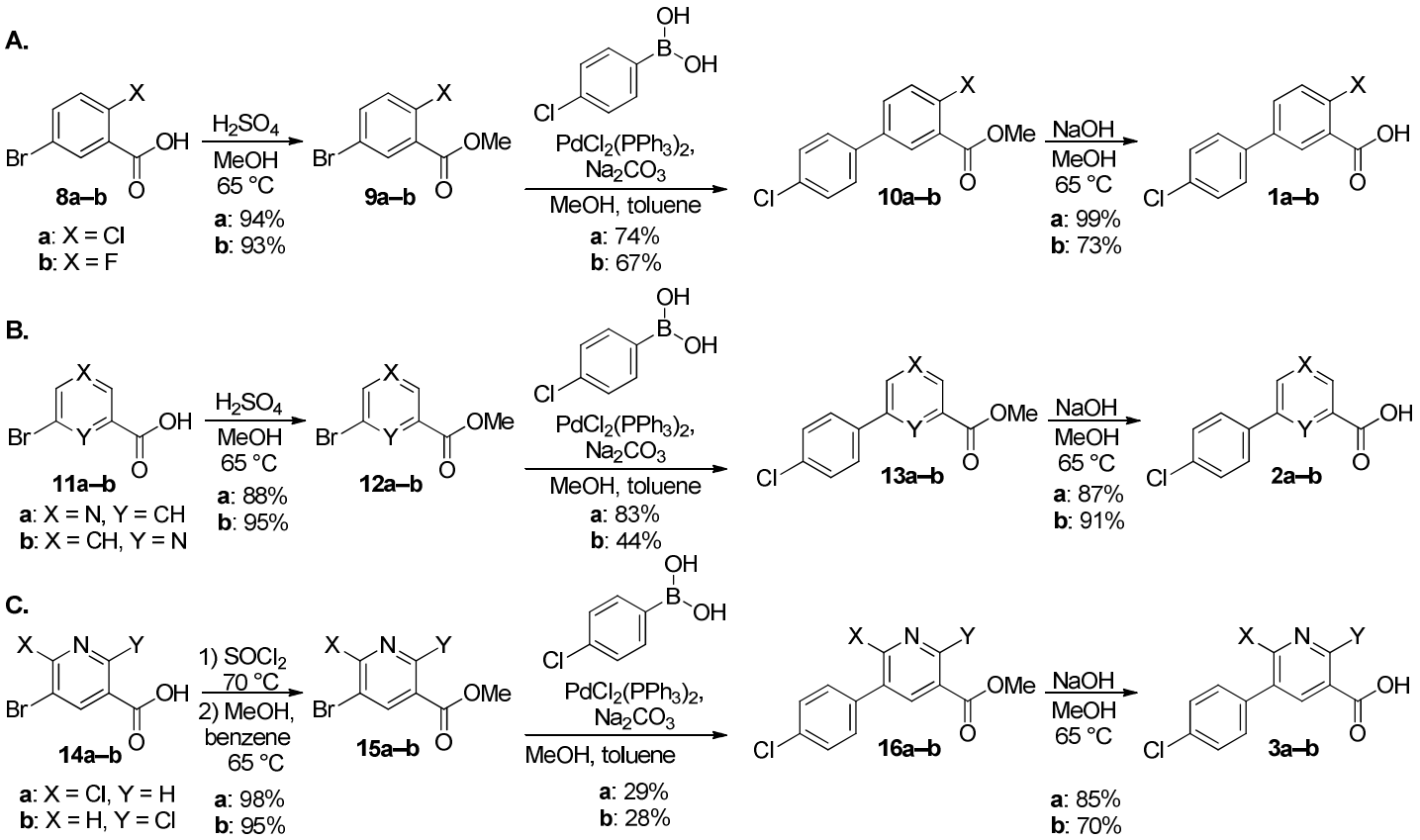

A.
8a–b
a: X = Cl
b: X = F
H2SO4
MeOH
65 °C
a: 94%
b: 93%
9a–b
PdCl2(PPh3)2,
Na2CO3
MeOH, toluene
a: 74%
b: 67%
10a–b
NaOH
MeOH
65 °C
a: 99%
b: 73%
1a–b
B.
11a–b
a: X = N, Y = CH
b: X = CH, Y = N
H2SO4
MeOH
65 °C
a: 88%
b: 95%
12a–b
PdCl2(PPh3)2,
Na2CO3
MeOH, toluene
a: 83%
b: 44%
13a–b
NaOH
MeOH
65 °C
a: 87%
b: 91%
2a–b
C.
14a–b
a: X = Cl, Y = H
b: X = H, Y = Cl
1) SOCl2
70 °C
2) MeOH,
benzene
65 °C
a: 98%
b: 95%
15a–b
PdCl2(PPh3)2,
Na2CO3
MeOH, toluene
a: 29%
b: 28%
16a–b
NaOH
MeOH
65 °C
a: 85%
b: 70%
3a–b

Figure 4

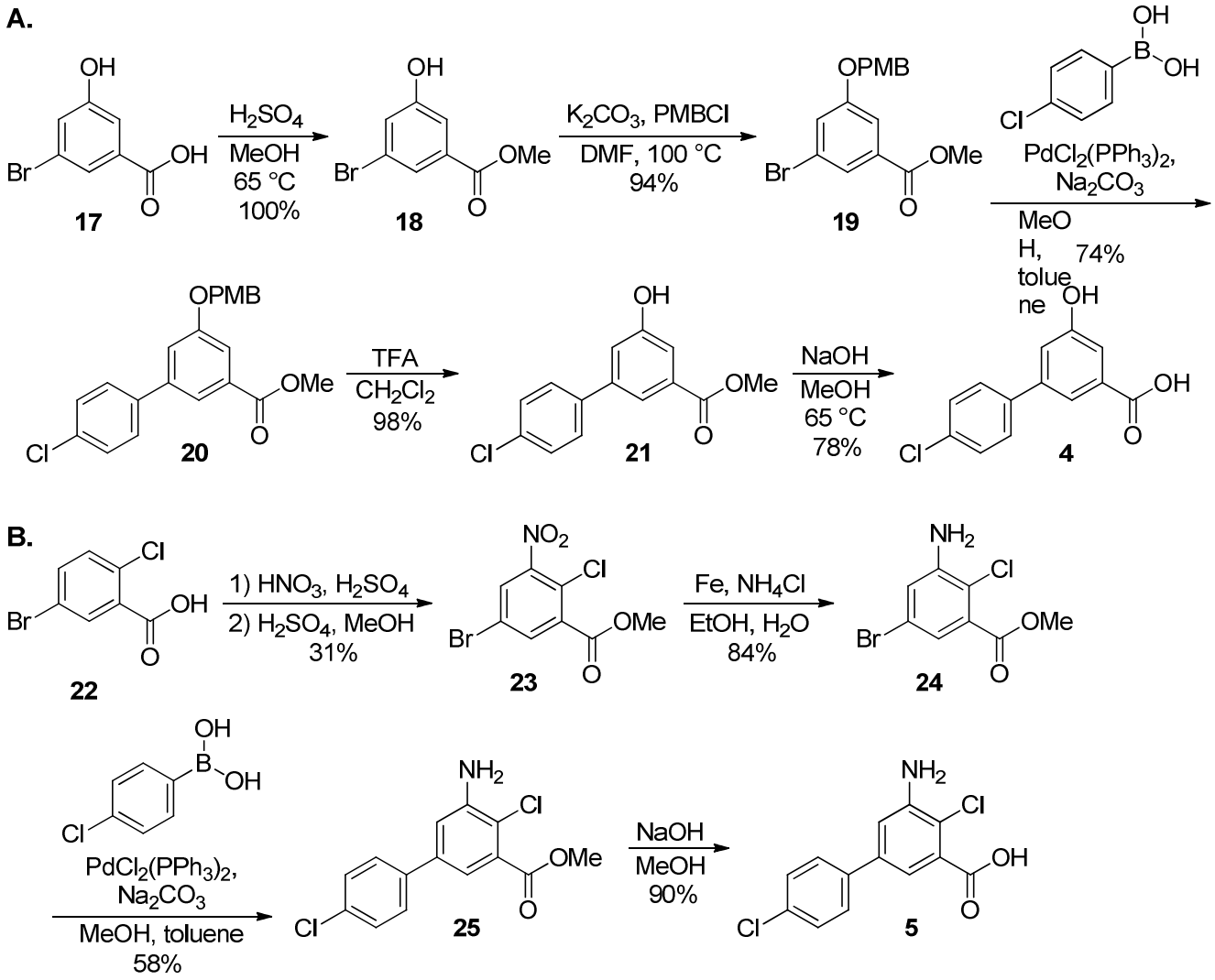

A.
17
18
19
20
21
4
H2SO4
MeOH
65 °C
100%
K2CO3, PMBCl
DMF, 100 °C
94%
PdCl2(PPh3)2,
Na2CO3
MeO
H,
tolue
ne
74%
TFA
CH2Cl2
98%
NaOH
MeOH
65 °C
78%
B.
22
23
24
25
5
1) HNO3, H2SO4
2) H2SO4, MeOH
31%
Fe, NH4Cl
EtOH, H2O
84%
PdCl2(PPh3)2,
Na2CO3
MeOH, toluene
58%
NaOH
MeOH
90%

Figure 5

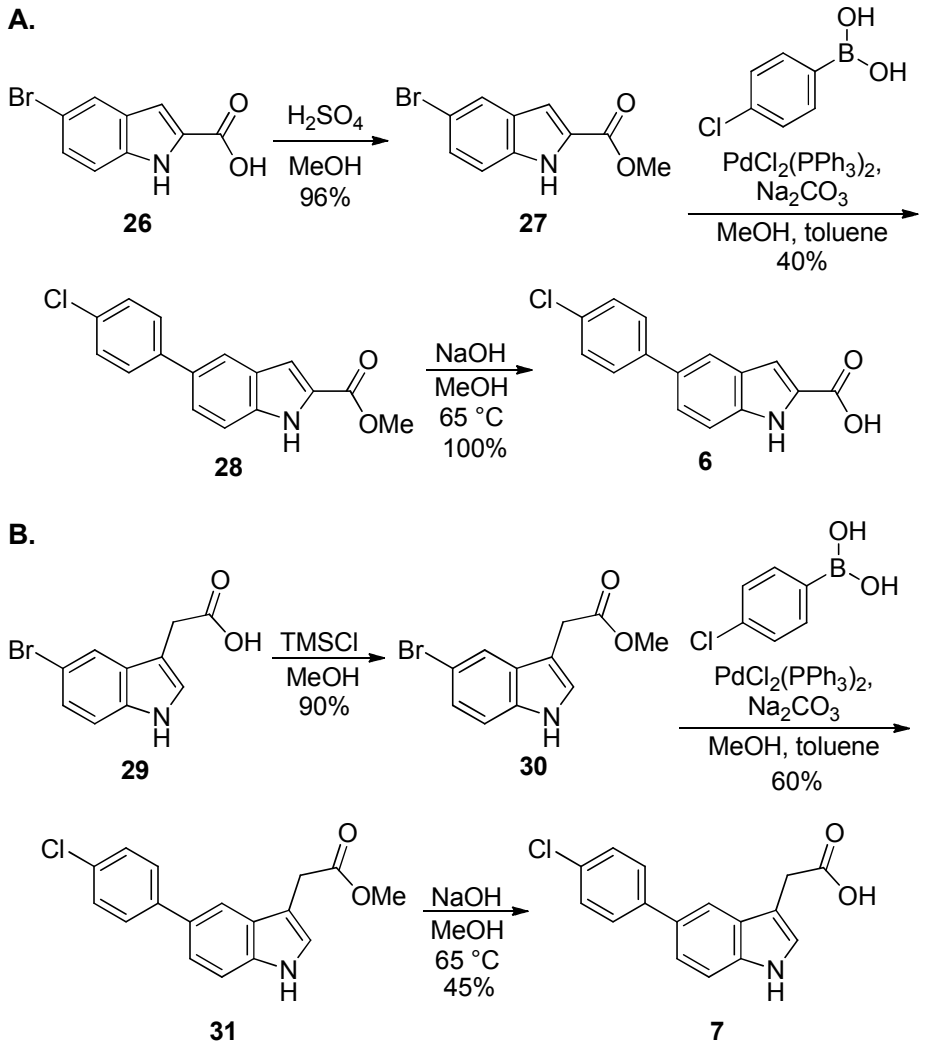

A.
Br
O
OH
N
H
26
H2SO4
MeOH
96%
Br
O
OMe
N
H
27
OH
B
OH
Cl
PdCl2(PPh3)2,
Na2CO3
MeOH, toluene
40%
Cl
O
OMe
N
H
28
NaOH
MeOH
65 °C
100%
Cl
O
OH
N
H
6
B.
Br
O
OH
N
H
29
TMSCl
MeOH
90%
Br
O
OMe
N
H
30
OH
B
OH
Cl
PdCl2(PPh3)2,
Na2CO3
MeOH, toluene
60%
Cl
O
OMe
N
H
31
NaOH
MeOH
65 °C
45%
Cl
O
OH
N
H
7

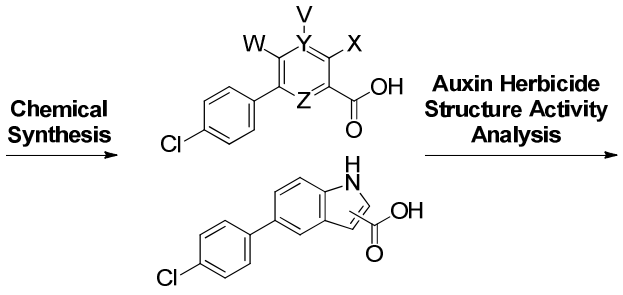
Table I. Activity of compounds **1-7** applied postemergence for control of key broadleaf and grass weeds with potential selectivity in sweet corn.

Herbicide analogue[a]	Rate	Redroot Pigweed[b]	Velvetleaf	Field Bindweed	Barnyardgrass	Large Crabgrass	Yellow Nutsedge	Sweet Corn
	g ai/ha	%						
1a	500	90	58	10	5	30	0	2
1b	500	55	10	3	15	8	0	2
2a	500	3	0	3	13	0	0	10
2b	500	53	43	25	3	12	10	7
3a	500	52	40	22	20	23	7	27
3b	500	28	20	13	20	30	3	27
4	500	37	30	22	28	35	10	52
5	500	40	17	37	10	5	0	0
6	500	49	17	25	3	7	7	3
7	500	30	12	25	10	12	5	13
Aminocyclopyrachlor	66	99	88	99	53	57	20	7
Dicamba	280	99	99	99	47	53	38	57
Quinclorac	840	80	73	99	99	93	33	63
Untreated Check	----	0	0	0	0	0	0	0

[a] All treatments were dissolved in acetone and water and were applied with 1% v/v crop oil concentrate.

[b] Redroot pigweed (*Amaranthus retroflexus*), Velvetleaf (*Abutilon theophrasti*), Field bindweed (*Convolvulus arvensis*), Barnyardgarss (*Echinochloa crus-galli*), Large crabgrass (*Digitaria sanguinalis*), Yellow nutsedge (*Cyperus esculentus*), sweet corn (*Zea mays* L. var. saccharata)

TOC Figure


Chemical Synthesis
V
W
Y
X
Z
OH
O
Cl
Auxin Herbicide Structure Activity Analysis
H
N
OH
O
Cl