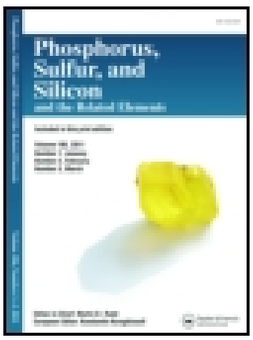

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Synthesis of some novel 5-substituted benzamido-6-arylamino-pyrazolo [3,4-*D*]pyrimidin-4-one derivatives for herbicidal activity

Jin Luo[a], Shu Li[b], Qiongwen Kang[b], Yi Sun[b], and Tao Wang[b]

[a]Analytical & Testing Center, Jiangxi Normal University, Nanchang, Jiangxi, China; [b]Jiangxi Province Key Laboratory of Chemical Biology, College of Chemistry and Chemical Engineering, Jiangxi Normal University, Nanchang, Jiangxi, China

ABSTRACT

Sixteen novel 3-methylthio-5-substituted benzamido-6-arylamino-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one derivatives (**4a–p**) were successfully synthesized from iminophosphoranes, aryl isocyanate, and substituted benzoylhydrazine. The structures of the title compounds were elucidated by FT-IR, ^{1}H NMR, ^{13}C NMR, and HRMS. Herbicidal activity of the compounds **4a–p** against *Brassica napus* (rape), *Echinochloa crusgalli* (barnyard grass), *Cucumis sativus* (cucumber), and *Triticum aestivum* (wheat) were determined. The results showed that 5-(2-chlorobenzamido)-6-phenylamino-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4c**) displayed remarkable inhibition activity against the stalk and root of rape with 100% inhibition rate at the dosages of 10 mg/L and 100 mg/L, and 5-(4-nitrobenzamido)-6-phenylamino-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4 (5*H*) -one (**4d**) exhibited excellent activity against the stalk and root of barnyard grass with 100% inhibition rate at the same dosages.

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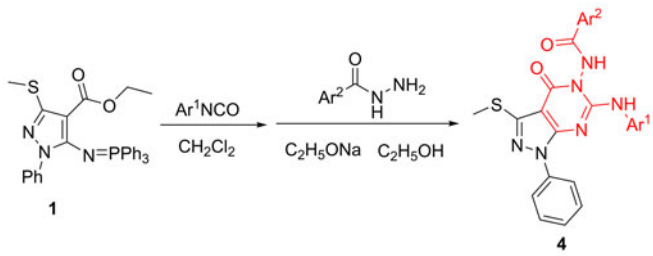
KEYWORDS
Pyrazolo[3,4-d]pyrimidin-4 (5H)-one derivatives; synthesis; herbicidal activity; inhibition rate

GRAPHICAL ABSTRACT



Introduction

Heterocyclic compounds with flexible structures, high-activities, and low- toxicity conform to requirements of the development of pesticide. The study of heterocyclic compounds inclines to complicated structural fused heterocyclic, bis-heterocyclic, multi-heterocyclic compounds.[1] Pyrazolo[3,4-*d*]pyrimidine, which represent a class of heterocyclic compound, is extensively used heterocyclic system in drug discovery and development. Pyrazolo[3,4-*d*]pyrimidine derivatives have received widespread attention due to their structural similarity with purine nucleus, and some of these derivatives exhibit remarkable biological activities such as antiviral,[2] antimicrobial,[3] anti-inflammatory,[4] antimicrobial,[5] antitubercular,[6] antifungal,[7] anticancer,[8,9] and herbicidal activity.[10]

Over the past thirty years, the aza-Wittig reactions of iminophosphoranes are an effective method for the synthesis of *N*-heterocyclic compounds.[11–18] The aza-Wittig reactions of iminophosphoranes can not only synthesize many known *N*-heterocyclic compounds with biological activity, but also prepare some new heterocyclic systems.[19] Recently, we have become interested in the synthesis of new bioactive fuze heterocycles, such as pyrazolo[3,4-*d*]pyrimidine-4-ones from various iminophosphoranes, with the aim of evaluating their biological activities. Continuing our ongoing program on the synthesis of herbicidal compounds,[20] and inspired by the excellent herbicidal properties of pyrazolo[3,4-*d*]pyrimidine-4-ones,[21] in the present study a new series of pyrazolo[3,4-*d*]pyrimidin-4-one compounds **4** has been synthesized from iminophosphoranes, aryl isocyanate, and substituted benzoylhydrazine via tandem aza-Wittig and annulation reactions. The subsequent herbicidal testing indicated that many target compounds showed good herbicidal activity against the root and stalk of *Brassica napus* (rape), *Echinochloa crusgalli* (barnyard grass), *Cucumis sativus* (cucumber), and *Triticum aestivum* (wheat).

CONTACT Jin Luo jinluo@jxnu.edu.cn Analytical & Testing Center, Jiangxi Normal University, Nanchang, Jiangxi, 330022, China; Tao Wang wangtao@jxnu.edu.cn Jiangxi Province Key Laboratory of Chemical Biology, College of Chemistry and Chemical Engineering, Jiangxi Normal University, Nanchang, Jiangxi, 330022, China.

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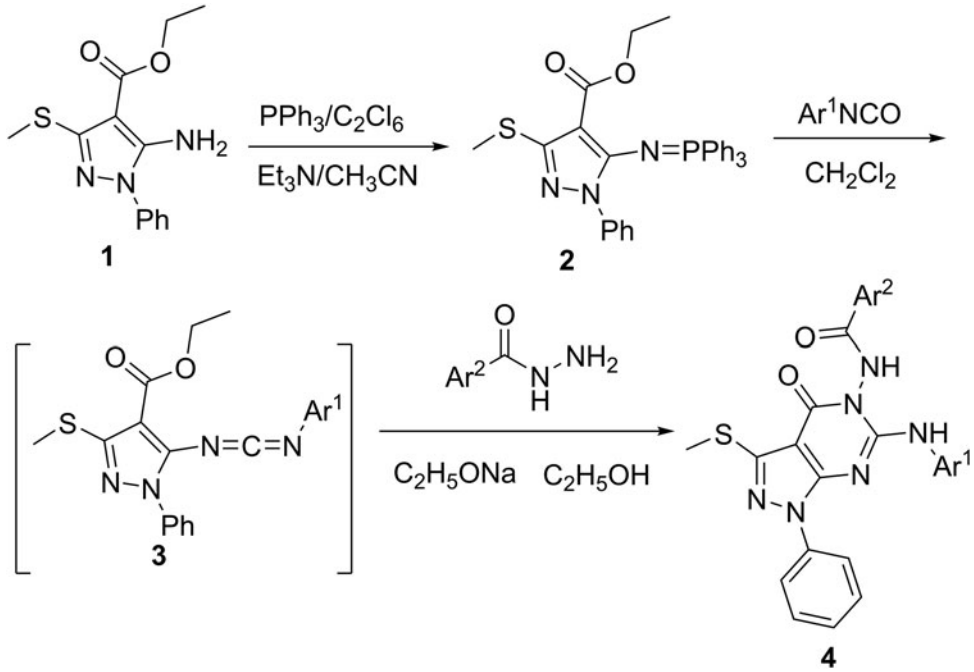

Scheme 1. The synthetic route of the compound **4**.

Results and discussion

Chemistry

Scheme 1 demonstrates the synthetic procedures of the intermediate and target compounds. Accordingly, the starting material 5-amino-3-methylthio-1-phenyl-1*H*-pyrazole-4-carboxylic acid ethyl ester **1** reacted with triphenylphosphine, hexachloroethane, and Et_3N in anhydrous acetonitrile to furnish iminophosphorane **2**. Iminophosphorane **2** treated with arylisocyanate in dry methylene chloride under nitrogen to give carbodiimides **3**. Then, the direct reaction of intermediate **3** and various benzoylhydrazine in the presence of a catalytic of sodium ethoxide at room temperature provided pyrazolo[3,4-*d*]pyrimidin-4-one compounds **4** (Figure 1).

All the compounds **4** were purified by recrystallization and characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and HRMS. The ^{1}H NMR spectra revealed that the single peak at about 2.6 ppm and 2.4 ppm were attributed to the methyl of SCH_3 and Ph-CH_3, respectively. The single peak at about 11.1 ppm and 9.8 ppm belonged to NH. In ^{13}C NMR, the carbon of SCH_3 appeared at 13.1 ppm, and the carbon of Ph-CH_3 appeared at 21.6 ppm. The characteristic carbon signal at about 167 ppm corresponded to the carbonyl group (C = O). In addition, the high-resolution mass spectra (HRMS) agreed with the calculated molecular weights of the title compounds **4**.

Herbicidal activity

The newly synthesized compounds **4a–p** were evaluated for their herbicidal activity against *Brassica napus* (rape), *Echinochloa crusgalli* (barnyard grass), *Cucumis sativus* (cucumber), and *Triticum aestivum* (wheat) at the dosages of 100 mg/L and 10 mg/L according to the literature procedure.[21] Table 1 shows the inhibition rates of these compounds. The obtained data illustrated that many of these compounds exhibited excellent herbicidal activity. For example, compounds **4c**, **4d**, **4e**, **4f**, **4g**, **4l**, **4m**, and **4p** showed 100% inhibition rate to root and stalk of rape at 100 mg/L. Compounds **4d**, **4m**, and **4p** displayed 100% inhibition rate to root and stalk of barnyard grass at 100 mg/L. Compounds **4d**, **4g**, **4m**, and **4p** indicated 100% inhibition rate to root and stalk of cucumber at 100 mg/L. Compounds **4d**, **4f**, **4g**, **4i**, **4l**, **4m**, and **4p** demonstrated 100% inhibition rate to root and stalk of wheat at 100 mg/L. Compound **4k** exhibited 100% inhibition rate to root and stalk of rape, barnyard grass, cucumber, and wheat at 10 mg/L. Comparing herbicidal activities among the title compounds in Table 1, it was found that the Ar^1 and Ar^2 groups have notable impact on the herbicidal activity. The title compounds with 4-Cl and NO_2 as Ar^1 and Ar^2 (**4d**, **4m**, and **4p**) showed higher inhibitory rate (100%) against the root and stalk of the tested plants at 100 mg/L.

Conclusions

In conclusion, various substituted pyrazolo[3,4-*d*]pyrimidine-4-one compounds **4a–p** were synthesized and confirmed by ^{1}H NMR, ^{13}C NMR, FT-IR, and HRMS. The herbicidal activity of the title compounds against the root and stalk of rape, cucumber, wheat, and barnyard grass was investigated. Some of the tested compounds revealed good herbicidal activity, in particular 5-(2-chlorobenzamido)-6-phenylamino-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4c**) and 5-(4-nitro benzamido)-6-phenylamino-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*]-pyrimidin-4 (5*H*)-one (**4d**). The studies of the novel pyrazolo[3,4-*d*]pyrimidine-4-one compounds with high herbicidal activity will provide useful information for the design and discovery of fuze heterocycles.

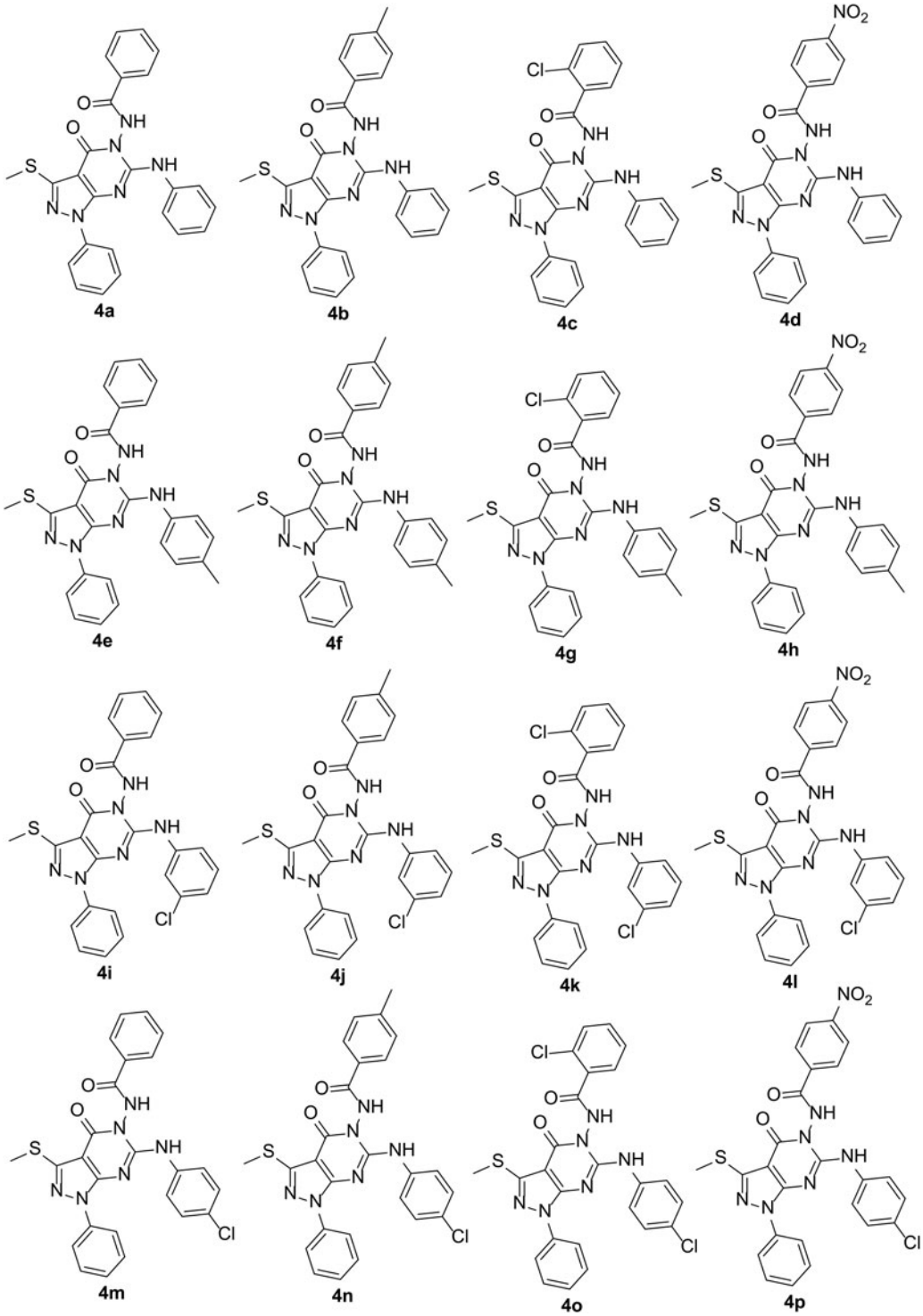

Figure 1. Structures of products **4a–p**.

Experimental

An electrothermal melting-point apparatus was used to record melting points. Infrared (IR) spectra were collected with a NicoQt 6700 spectrometer in KBr pellets. ^{1}H and ^{13}C NMR were recorded on Bruker 400 spectrometer with DMSO as the solvent at 400 MHz (^{1}H) and 100 MHz (^{13}C). High resolution mass spectra (HRMS) were acquired on a micro-TOF II Instrument. All the chemicals used were of AR grade. Methylene chloride, acetonitrile, and triethylamine were dried and distilled according to standard procedures. Iminophosphorane **2** was synthesized as reported.[21] The Supplemental Materials contains sample ^{1}H and ^{13}C NMR spectra of the products 4 (Figures S1–S32).

Synthesis of 5-substituted benzamido-6-arylamino-3-methylthio-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4 (5H)-one 4 (general procedure)

Iminophosphorane **2** (2 mmol) was dissolved in dry methylene chloride (25 mL) and arylisocyanate (2 mmol) was added

Table 1. The inhibition percentage (%) of compounds **4a–p** to barnyard grass, rape, cucumber, and wheat.

			Rape				Barnyard grass			
			Stalk		Root		Stalk		Root	
Compd.	Ar^1	Ar^2	10 mg/L	100 mg/L	10 mg/L	100 mg/L	10 mg/L	100 mg/L	10 mg/L	100 mg/L
4a	Ph	Ph	17	−6	44	32	−11	−42	−19	24
4b	Ph	4-CH_3Ph	−100	−40	28	39	−47	−28	−17	−13
4c	Ph	2-ClPh	100	100	100	100	−51	−30	6	45
4d	Ph	4-NO_2Ph	−100	100	79	100	100	100	100	100
4e	4-CH_3Ph	Ph	−54	100	86	100	100	−9	100	−12
4f	4-CH_3Ph	4-CH_3Ph	−100	100	71	100	−49	46	54	79
4g	4-CH_3Ph	2-ClPh	−100	100	52	100	−27	−30	54	75
4h	4-CH_3Ph	4-NO_2Ph	−54	−35	66	88	−63	−3	61	−24
4i	3-ClPh	Ph	−20	60	34	92	−13	8	70	67
4j	3-ClPh	4-CH_3Ph	−100	−48	86	91	−27	−55	49	35
4k	3-ClPh	2-ClPh	100	−37	100	33	100	−62	100	43
4l	3-ClPh	4-NO_2Ph	−62	100	63	100	−15	−20	8	59
4m	4-ClPh	Ph	−10	100	45	100	−23	100	−4	100
4n	4-ClPh	4-CH_3Ph	−69	−33	42	56	5	1.06	27	53
4o	4-ClPh	2-ClPh	−100	−100	68	87	−48	−41	33	59
4p	4-ClPh	4-NO_2Ph	−55	100	87	100	−13	100	66	100
2,4-D			87	100	96	100	5	100	90	100
			Cucumber				Wheat			
			Stalk		Root		Stalk		Root	
Compd.	Ar^1	Ar^2	10 mg/L	100 mg/L	10 mg/L	100 mg/L	10 mg/L	100 mg/L	10 mg/L	100 mg/L
4a	Ph	Ph	−15	23	5	6	4	31	−3	40
4b	Ph	4-CH_3Ph	−45	−1	24	−11	−2	15	28	39
4c	Ph	2-ClPh	−4	44	48	40	0	64	20	89
4d	Ph	4-NO_2Ph	−2	100	53	100	7	100	32	100
4e	4-CH_3Ph	Ph	−13	−24	35	−5	25	14	25	23
4f	4-CH_3Ph	4-CH_3Ph	−12	18	−21	83	12	100	16	100
4g	4-CH_3Ph	2-ClPh	13	100	52	100	−3	100	50	100
4h	4-CH_3Ph	4-NO_2Ph	−9	−15	47	54	−2	−2	58	61
4i	3-ClPh	Ph	−21	60	34	92	−17	100	7	100
4j	3-ClPh	4-CH_3Ph	−45	−9	43	34	−6	−13	57	56
4k	3-ClPh	2-ClPh	100	−37	100	33	100	1	100	26
4l	3-ClPh	4-NO_2Ph	−9	12	40	56	12	100	26	100
4m	4-ClPh	Ph	−37	100	15	100	−38	100	24	100
4n	4-ClPh	4-CH_3Ph	−61	−33	42	56	5	1	27	53
4o	4-ClPh	2-ClPh	−53	−13	60	50	−6	12	18	51
4p	4-ClPh	4-NO_2Ph	−9	100	66	100	26	100	61	100
2,4-D			40	100	92	100	10	95	88	92

under nitrogen at room temperature. After 6–8 h, the solid substituted benzoylhydrazine (2 mmol) was added to the solution. The mixture was stirred for additional 1 h. Then the solvent was evaporated, and anhydrous ethanol (25 mL) and cat. sodium ethoxide (1.5 mL, 3.0 mol/L) were added to the mixture. After stirring for 8–10 h at room temperature, the precipitate was isolated by filtration and recrystallized from DMSO to produce pure 5-substituted benzamido-6-arylamino-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4 (5 *H*)-one **4**.

5-benzamido-6-phenylamino-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidin-4 (5*H*) -one (**4a**). White crystals; yield: 81%; m.p 298-299 °C; IR (KBr, cm^{-1}): 3445, 1702 (C = O), 1686 (C = O), 1552;^{1}H NMR (400 MHz, DMSO) δ: 11.15 (s, 1H), 9.77 (s, 1H), 8.11 (d, J = 7.2 Hz, 2H), 8.04 (d, J = 7.7 Hz, 2H), 7.69 (t, J = 7.3 Hz, 1H), 7.61 (dd, J = 12.6, 6.1 Hz, 4H), 7.50 – 7.38 (m, 4H), 7.29 (t, J = 7.4 Hz, 1H), 7.21 (t, J = 7.4 Hz, 1H), 2.61 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 169.7 (C = O), 155.3, 152.2, 151.6, 145.4, 139.6, 139.5, 139.2, 129.4, 129.3, 129.1, 128.4, 127.7, 125.7, 123.4, 120.9, 120.1, 101.6, 13.1 (SCH_3); HRMS (ESI) calcd for $C_{25}H_{20}N_6O_2S^+$: 469.1447 $[M + H]^+$, found: 469.1441.

5-(4-methylbenzamido)-6-phenylamino-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4 (5*H*) -one (**4b**). White crystals; yield: 73%; m.p > 300 °C; IR (KBr, cm^{-1}): 3327, 1705 (C = O), 1674 (C = O), 1556; ^{1}H NMR (400 MHz, DMSO) δ: 11.07 (s, 1H), 9.77 (s, 1H), 8.03 (dd, J = 11.1, 8.1 Hz, 4H), 7.62 (d, J = 7.7 Hz, 2H), 7.50 – 7.38 (m, 6H), 7.29 (t, J = 7.4 Hz, 1H), 7.20 (t, J = 7.4 Hz, 1H), 2.61 (s, 3H), 2.42 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 167.3 (C = O), 154.9, 152.8, 152.6, 146.3, 143.2, 138.8, 137.9, 129.5, 129.4, 129.3,128.9, 128.8, 126.5, 125.4, 124.5, 120.8, 99.9, 21.6 ($PhCH_3$), 13.1 (SCH_3); HRMS (ESI) calcd for $C_{26}H_{22}N_6O_2S^+$: 483.1603 $[M + H]^+$, found: 483.1598.

5-(2-chlorobenzamido)-6-phenylamino-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4c**). White crystals; yield: 80%; m.p 290-292 °C; IR (KBr, cm^{-1}): 3294, 1704 (C = O), 1689 (C = O), 1561; ^{1}H NMR (400 MHz, DMSO) δ: 11.14 (s, 1H), 9.59 (s, 1H), 8.16 – 8.10 (m, 1H), 8.03 (d, J = 7.7 Hz, 2H), 7.69 – 7.53 (m, 5H), 7.46 (dt, J = 10.9, 8.0 Hz, 4H), 7.30 (t, J = 7.4 Hz, 1H), 7.22 (t, J = 7.4 Hz, 1H), 2.63 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 166.8 (C = O), 154.7, 152.6, 152.2, 146.3, 138.7, 137.8, 133.0, 132.7, 132.1, 131.1, 130.9, 129.4, 128.9, 127.4, 126.6, 125.4, 124.2, 120.9, 99.9, 13.1 (SCH_3); HRMS (ESI) calcd for $C_{25}H_{19}ClN_6O_2S^+$: 503.1057 $[M + H]^+$, found: 503.1051.

5-(4-nitrobenzamido)-6-phenylamino-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4 (5 *H*)-one (**4d**). Yellow crystals; yield: 74%; m.p > 300 °C; IR (KBr, cm^{-1}): 3319, 1714 (C = O), 1681 (C = O), 1551; ^{1}H NMR (400 MHz, DMSO) δ:

11.56 (s, 1H), 9.82 (s, 1H), 8.47 (d, $J = 8.7$ Hz, 2H), 8.33 (d, $J = 8.7$ Hz, 2H), 8.03 (d, $J = 7.9$ Hz, 2H), 7.61 (d, $J = 7.9$ Hz, 2H), 7.51 – 7.40 (m, 4H), 7.30 (t, $J = 7.4$ Hz, 1H), 7.22 (t, $J = 7.3$ Hz, 1H), 2.61 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 166.0 (C = O), 154.7, 152.7, 152.3, 150.3, 146.4, 138.7, 137.8, 137.7, 130.3, 129.4, 128.9, 126.6, 125.6, 124.5, 124.1, 120.9, 99.8, 13.1 (SCH_3); HRMS (ESI) calcd for $C_{25}H_{19}N_7O_4S^+$: 514.1297 $[M + H]^+$, found: 514.1292.

5-benzamido-6-(4-methylphenylamino)-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4e**). White crystals; yield: 79%; m.p > 300 °C; IR (KBr, cm^{-1}): 3345, 1703 (C = O), 1683 (C = O), 1560; ^{1}H NMR (400 MHz, DMSO) δ: 11.13 (s, 1H), 9.71 (s, 1H), 8.11 (d, $J = 7.4$ Hz, 2H), 8.05 (d, $J = 7.8$ Hz, 2H), 7.68 (t, $J = 7.3$ Hz, 1H), 7.60 (t, $J = 7.5$ Hz, 2H), 7.54 – 7.43 (m, 4H), 7.30 (t, $J = 7.4$ Hz, 1H), 7.22 (d, $J = 8.2$ Hz, 2H), 2.61 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 167.4 (C = O), 154.9, 152.9, 152.6, 146.3, 138.8, 135.3, 134.5, 133.0, 132.2, 129.4, 129.2, 128.8, 126.5, 124.3, 120.8, 99.8, 21.0 ($PhCH_3$), 13.1 (SCH_3); HRMS (ESI) calcd for $C_{26}H_{22}N_6O_2S^+$: 483.1603 $[M + H]^+$, found: 483.1598.

5-(4-methylbenzamido)-6-(4-methylphenylamino)-3-methylthio-1-phenyl-1*H*- pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4f**). White crystals; yield: 77%; m.p 279-281 °C; IR (KBr, cm^{-1}): 3304, 1705 (C = O), 1685 (C = O), 1543; ^{1}H NMR (400 MHz, DMSO) δ: 11.03 (s, 1H), 9.68 (s, 1H), 8.02 (dd, $J = 18.7, 7.9$ Hz, 4H), 7.48 (dd, $J = 15.7, 8.3$ Hz, 4H), 7.40 (d, $J = 8.0$ Hz, 2H), 7.30 (t, $J = 7.4$ Hz, 1H), 7.22 (d, $J = 8.3$ Hz, 2H), 2.60 (s, 3H), 2.42 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 167.3 (C = O), 154.9, 152.8, 152.6, 146.3, 143.1, 138.9, 135.3, 134.5, 129.5, 129.4, 129.3, 129.2, 128.9, 126.4, 124.2, 120.8, 99.8, 21.6 ($PhCH_3$), 21.0 ($PhCH_3$), 13.1 (SCH_3); HRMS (ESI) calcd for $C_{27}H_{24}N_6O_2S^+$: 497.1760 $[M + H]^+$, found: 497.1758.

5-(2-chlorobenzamido)-6-(4-methylphenylamino)-3-methylthio-1-phenyl-1*H*- pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4g**). White crystals; yield: 76%; m.p > 300 °C; IR (KBr, cm^{-1}): 3314, 1750 (C = O), 1701 (C = O), 1543; ^{1}H NMR (400 MHz, DMSO) δ: 11.11 (s, 1H), 9.51 (s, 1H), 8.18 – 8.10 (m, 1H), 8.03 (d, $J = 7.7$ Hz, 2H), 7.71 – 7.44 (m, 8H), 7.32 (dt, $J = 14.8, 7.4$ Hz, 1H), 7.24 (d, $J = 8.3$ Hz, 2H), 2.62 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 168.8 (C = O), 155.2, 152.4, 151.9, 145.8, 140.6, 139.2, 138.7, 129.4, 128.9, 128.6, 128.5, 126.0, 124.2, 122.6, 120.4, 100.8, 21.4 ($PhCH_3$), 13.1 (SCH_3); HRMS (ESI) calcd for $C_{26}H_{21}ClN_6O_2S^+$: 517.1213 $[M + H]^+$, found: 517.1208.

5-(4-nitrobenzamido)-6-(4-methylphenylamino)-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4h**). Yellow crystals; yield: 71%; m.p 260-266 °C; IR (KBr, cm^{-1}): 3319, 1695 (C = O), 1669 (C = O), 1573; ^{1}H NMR (400 MHz, DMSO) δ: 11.48 (s, 1H), 9.71 (s, 1H), 8.43 (d, $J = 8.3$ Hz, 2H), 8.32 (d, $J = 8.4$ Hz, 2H), 8.03 (d, $J = 7.8$ Hz, 2H), 7.48 (dd, $J = 13.5, 7.5$ Hz, 4H), 7.30 (t, $J = 7.1$ Hz, 1H), 7.22 (d, $J = 7.8$ Hz, 2H), 2.60 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 166.1 (C = O), 154.7, 152.8, 152.2, 150.1, 146.3, 138.8, 138.3, 135.2, 134.6, 130.3, 129.3, 126.5, 125.2, 124.1, 120.8, 99.8, 21.0 ($PhCH_3$), 13.0 (SCH_3); HRMS (ESI) calcd for $C_{26}H_{21}N_7O_4S^+$: 550.1273 $[M + Na]^+$, found: 550.1268.

5-benzamido-6-(3-chlorophenylamino)-3-methylthio-1-phenyl-1*H*- pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4i**). White crystals; yield: 80%; m.p 291-293 °C; IR (KBr, cm^{-1}): 3308, 1705 (C = O), 1679 (C = O), 1549; ^{1}H NMR (400 MHz, DMSO) δ: 11.20 (s, 1H), 9.86 (s, 1H), 8.13 (d, $J = 7.3$ Hz, 2H), 8.05 (d, $J = 7.8$ Hz, 2H), 7.99 (t, $J = 1.9$ Hz, 1H), 7.70 (t, $J = 7.3$ Hz, 1H), 7.65 – 7.48 (m, 5H), 7.42 (t, $J = 8.1$ Hz, 1H), 7.34 (t, $J = 7.4$ Hz, 1H), 7.25 (dd, $J = 7.9, 1.2$ Hz, 1H), 2.63 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 167.5 (C = O), 154.8, 152.3, 152.0, 146.4, 139.5, 138.7, 133.3, 133.1, 132.1, 130.4, 129.5, 128.9, 126.7, 124.8, 123.3, 122.1, 121.1, 100.2, 13.1 (SCH_3); HRMS (ESI) calcd for $C_{25}H_{19}ClN_6O_2S^+$: 503.1057 $[M + H]^+$, found: 503.1051.

5-(4-methylbenzamido)-6-(3-chlorophenylamino)-3-methylthio-1-phenyl-1*H*- pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4j**). White crystals; yield: 73%; m.p 298-299 °C; IR (KBr, cm^{-1}): 3303, 1701 (C = O), 1670 (C = O), 1551; ^{1}H NMR (400 MHz, DMSO) δ: 11.09 (s, 1H), 9.84 (s, 1H), 8.01 (ddd, $J = 12.1, 7.2, 4.9$ Hz, 5H), 7.60 – 7.48 (m, 5H), 7.42 (dt, $J = 7.9, 3.9$ Hz, 3H), 7.34 (t, $J = 7.4$ Hz, 1H), 7.24 (dd, $J = 7.9, 1.1$ Hz, 1H), 2.62 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 167.4 (C = O), 154.8, 152.3, 152.0, 146.4, 143.3, 139.5, 138.7, 133.3, 130.4, 129.5, 129.4, 129.3, 128.9, 126.7, 124.7, 123.3, 122.1, 121.1, 100.2, 21.6 ($PhCH_3$), 13.1 (SCH_3); HRMS (ESI) calcd for $C_{26}H_{21}ClN_6O_2S^+$: 517.1213 $[M + H]^+$, found: 517.1208.

5-(2-chlorobenzamido)-6-(3-chlorophenylamino)-3-methylthio-1-phenyl-1*H*- pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4k**). White crystals; yield: 72%; m.p > 300 °C; IR (KBr, cm^{-1}): 3292, 1704 (C = O), 1669 (C = O), 1557; ^{1}H NMR (400 MHz, DMSO) δ: 11.16 (s, 1H), 9.72 (s, 1H), 8.12 (d, $J = 7.0$ Hz, 1H), 8.07 – 8.00 (m, 3H), 7.66 – 7.59 (m, 2H), 7.59 – 7.49 (m, 4H), 7.44 (t, $J = 8.1$ Hz, 1H), 7.35 (t, $J = 7.4$ Hz, 1H), 7.26 (dd, $J = 7.9, 1.0$ Hz, 1H), 2.63 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 166.8 (C = O), 154.6, 152.2, 151.7, 146.4, 139.5, 138.6, 133.3, 133.1, 132.6, 132.2, 131.1, 131.0, 130.5, 129.5, 127.4, 126.8, 124.8, 123.1, 122.0, 121.2, 100.2, 13.1 (SCH_3); HRMS (ESI) calcd for $C_{25}H_{18}Cl_2N_6O_2S^+$: 537.0667 $[M + H]^+$, found: 537.0667.

5-(4-nitrobenzamido)-6-(3-chlorophenylamino)-3-methylthio-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4l**). Yellow crystals; yield: 73%; m.p > 300 °C; IR (KBr, cm^{-1}): 3324, 1703 (C = O), 1683 (C = O), 1565; ^{1}H NMR (400 MHz, DMSO) δ: 11.59 (s, 1H), 9.87 (s, 1H), 8.48 (d, $J = 8.7$ Hz, 2H), 8.34 (d, $J = 8.7$ Hz, 2H), 8.04 (d, $J = 7.9$ Hz, 2H), 7.98 (s, 1H), 7.52 (t, $J = 7.9$ Hz, 3H), 7.43 (t, $J = 8.1$ Hz, 1H), 7.35 (t, $J = 7.4$ Hz, 1H), 7.26 (d, $J = 7.9$ Hz, 1H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 166.1 (C = O), 154.6, 152.3, 151.7, 150.3, 146.4, 139.4, 138.6, 137.7, 133.3, 130.5, 130.4, 129.5, 126.8, 124.9, 124.1, 123.4, 122.2, 121.2, 100.1, 13.1 (SCH_3); HRMS (ESI) calcd for $C_{25}H_{18}ClN_7O_4S^+$: 548.0908 $[M + H]^+$, found: 548.0902.

5-benzamido-6-(4-chlorophenylamino)-3-methylthio-1-phenyl-1*H*- pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4m**). White crystals; yield: 83%; m.p 282-284 °C; IR (KBr, cm^{-1}): 3303, 1707 (C = O), 1682 (C = O), 1551; ^{1}H NMR (400 MHz, DMSO) δ: 11.17 (s, 1H), 9.82 (s, 1H), 8.11 (d, $J = 7.4$ Hz, 2H), 8.01 (d, $J = 7.8$ Hz, 2H), 7.73 – 7.64 (m, 3H), 7.61 (t, $J = 7.5$ Hz, 2H), 7.50 (dd, $J = 16.4, 8.3$ Hz, 4H), 7.32 (t, $J = 7.4$ Hz, 1H), 2.61 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 167.4 (C = O), 154.8, 152.5, 152.3, 146.3, 138.7, 136.9, 133.1, 132.1, 129.5, 129.2, 128.9, 128.8, 128.7, 126.7, 125.9, 121.1, 100.0, 13.1 (SCH_3);

HRMS (ESI) calcd for $C_{25}H_{19}ClN_6O_2S^+$: 503.1057 $[M+H]^+$, found: 503.1051.

5-(4-methylbenzamido)-6-(4-chlorophenylamino)-3-methylthio-1-phenyl-1*H*- pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4n**). White crystals; yield: 77%; m.p > 300 °C; IR (KBr, cm^{-1}): 3306, 1696 (C = O), 1678 (C = O), 1552; ^1H NMR (400 MHz, DMSO) δ: 8.03 (t, J = 9.4 Hz, 2H), 7.89 (d, J = 8.0 Hz, 2H), 7.70 – 7.64 (m, 2H), 7.51 (t, J = 8.0 Hz, 2H), 7.39 (d, J = 8.9 Hz, 2H), 7.29 (dd, J = 13.5, 6.2 Hz, 1H), 7.12 (d, J = 8.0 Hz, 2H), 2.58 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 168.7 (C = O), 155.7, 152.8, 151.9, 145.5, 140.6, 139.6, 139.5, 134.9, 129.4, 128.7, 128.5, 128.4, 126.2, 125.5, 123.4, 120.1, 100.8, 21.4 ($PhCH_3$), 13.0 (SCH_3); HRMS (ESI) calcd for $C_{26}H_{21}ClN_6O_2S^+$: 517.1213 $[M+H]^+$, found: 517.1208.

5-(2-chlorobenzamido)-6-(4-chlorophenylamino)-3-methylthio-1-phenyl-1*H*- pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4o**). White crystals; yield: 73%; m.p > 300 °C; IR (KBr, cm^{-1}): 3311, 1708 (C = O), 1693 (C = O), 1556; ^1H NMR (400 MHz, DMSO) δ: 11.15 (s, 1H), 9.69 (s, 1H), 8.16 – 8.10 (m, 1H), 8.00 (d, J = 7.7 Hz, 2H), 7.73 – 7.67 (m, 2H), 7.66 – 7.46 (m, 7H), 7.33 (t, J = 7.4 Hz, 1H), 2.63 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ: 166.8 (C = O), 154.7, 152.4, 152.0, 146.4, 138.6, 136.9, 133.1, 132.6, 132.2, 131.1, 131.0, 129.5, 129.2, 128.8, 127.4, 126.8, 125.7, 121.1, 100.0, 13.1 (SCH_3); HRMS (ESI) calcd for $C_{25}H_{18}Cl_2N_6O_2S^+$: 537.0667 $[M+H]^+$, found: 537.0662.

5-(4-nitrobenzamido)-6-(4-chlorophenylamino)-3-methylthio-1-phenyl-1*H*- pyrazolo[3,4-*d*]pyrimidin-4 (5*H*)-one (**4p**). Yellow crystals; yield: 75%; m.p > 300 °C; IR (KBr, cm^{-1}): 3311, 1704 (C = O), 1684 (C = O), 1558; ^1H NMR (400 MHz, DMSO) δ: 11.59 (s, 1H), 9.86 (s, 1H), 8.48 (d, J = 8.2 Hz, 2H), 8.32 (d, J = 8.3 Hz, 2H), 8.00 (d, J = 7.7 Hz, 2H), 7.66 (d, J = 8.3 Hz, 2H), 7.49 (d, J = 8.4 Hz, 4H), 7.33 (t, J = 6.9 Hz, 1H), 2.61 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ: 166.0 (C = O), 154.6, 152.5, 152.0, 150.3, 146.4, 138.6, 137.9, 136.8, 130.3, 129.5, 129.3, 128.8, 126.8, 125.9, 124.1, 121.1, 99.9, 13.1 (SCH_3); HRMS (ESI) calcd for $C_{25}H_{18}ClN_7O_4S^+$: 548.0908 $[M+H]^+$, found: 548.0902.

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References

[1] Wu, M. H.; Hu, J. H.; Guo, H. B.; Li, Y. A Facile and Efficient One-Pot Three-Step Protocol for Synthesis of 6-aryl-7-oxo-5-aryoxy/alkoxy-1-(p-tolyl)-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidine-3-carbonitrile. *Arkivoc* **2012**, *6*, 295–303.

[2] Rashad, A. E.; Hegab, M. I.; Abdel-Megeid, R. E.; Micky, J. A.; Abdel-Megeid, F. M. E. M. Synthesis and Antiviral Evaluation of Some New Pyrazole and Fused Pyrazolopyrimidine Derivatives. *Bioorg. Med. Chem.* **2008**, *16*, 7102–7106. DOI: 10.1016/j.bmc.2008.06.054.

[3] Holla, B. S.; Mahalinga, M.; Karthikeyan, M. S.; Akberali, P. M.; Shetty, N. S. Synthesis of Some Novel Pyrazolo[3,4-d]Pyrimidine Derivatives as Potential Antimicrobial Agents. *Bioorg. Med. Chem.* **2006**, *14*, 2040–2047. DOI: 10.1016/j.bmc.2005.10.053.

[4] Tageldin, G. N.; Fahmy, S. M.; Ashour, H. M.; Khalil, M. A.; Nassra, R. A.; Labouta, I. M. Design, Synthesis and Evaluation of Some Pyrazolo[3,4-d]Pyrimidines as Anti-Inflammatory Agents. *Bioorg. Chem.* **2018**, *78*, 358–371. DOI: 10.1016/j.bioorg.2018.03.030.

[5] Khobragade, C. N.; Bodade, R. G.; Konda, S. G.; Dawane, B. S.; Manwar, A. V. Synthesis and Antimicrobial Activity of Novel Pyrazolo[3,4-d]Pyrimidin Derivatives. *Eur. J. Med. Chem.* **2010**, *45*, 1635–1638.

[6] Moukha-Chafiq, O.; Taha, M. L.; Lazrek, B. H.; Barascut, J. L.; Imbach, J. L. Synthesis of Some Acyclonucleosides with the Alkylating Chain of Acyclovir. *C. R. Acad. Sci. IIc: Chem.* **2000**, *3*, 639–641. DOI: 10.1016/S1387-1609(00)01157-9.

[7] Chauhan, M.; Kumar, R. Medicinal Attributes of Pyrazolo[3,4-d]Pyrimidines: A Review. *Bioorg. Med. Chem.* **2013**, *21*, 5657–5668. DOI: 10.1016/j.bmc.2013.07.027.

[8] Cherukupalli, S.; Chandrasekaran, B.; Kryštof, V.; Aleti, R. R.; Sayyad, N.; Merugu, S. R.; Kushwaha, N. D.; Karpoormath, R. Synthesis, Anticancer Evaluation, and Molecular Docking Studies of Some Novel 4,6-Disubstituted Pyrazolo[3,4-d]Pyrimidines as Cyclin-Dependent Kinase 2 (CDK2) Inhibitors. *Bioorg. Chem.* **2018**, *79*, 46–49. DOI: 10.1016/j.bioorg.2018.02.030.

[9] Abd El Razik, H. A.; Mroueh, M.; Faour, W. H.; Shebaby, W. N.; Daher, C. F.; Ashour, H. M. A.; Ragab, H. M. Synthesis of New Pyrazolo[3,4-d]Pyrimidine Derivatives and Evaluation of Their Anti-Inflammatory and Anticancer Activities. *Chem. Biol. Drug Des.* **2017**, *90*, 83–96. DOI: 10.1111/cbdd.12929.

[10] Liu, H.; Wang, H. Q.; Ding, M. W.; Liu, Z. J.; Xiao, W. J. A Novel Synthesis and Herbicidal Activity of Fluorine-Containing Pyrazolo[3,4-d]Pyrimidin-4-One Derivatives. *J. Fluor. Chem.* **2006**, *127*, 1584–1590. DOI: 10.1016/j.jfluchem.2006.08.008.

[11] Nie, Y. B.; Wang, L.; Ding, M. W. Synthesis of 1,2,4,5-Tetrasubstituted Imidazoles by a Sequential aza-Wittig/Michael/Isomerization Reaction. *J. Org. Chem.* **2012**, *77*, 696–700. DOI: 10.1021/jo201846w.

[12] Wang, T.; Ke, X. X.; Zhou, S. T.; Chen, H. Z. New Efficient Synthesis of 5,6-Disubstituted-3-Phenyl-1,2,3-Triazolo[4,5- d]Pyrimidin-7-Ones via a Tandem aza-Wittig Reaction. *Synth. Commun* **2012**, *42*, 1393–1400. DOI: 10.1080/00397911.2010.535940.

[13] Wang, H. Q.; Zhou, W. P.; Wang, Y. Y.; Lin, C. R. Synthesis and Antifungal Activities of Novel 5-Amino-6-Arylamino-1H-Pyrazolo[3,4-d]Pyrimidin-4(5H)-One Derivatives. *J. Agric. Food Chem.* **2008**, *56*, 7321–7325. DOI: 10.1021/jf801359f.

[14] Yang, Y. Y.; Shou, W. G.; Chen, Z. B.; Hong, D.; Wang, Y. G. A Tandem Approach to Isoquinolines from 2-Azido-3-Arylacrylates and Alpha-Diazocarbonyl Compounds. *J. Org. Chem.* **2008**, *73*, 3928–3930. DOI: 10.1021/jo8003259.

[15] Zhao, J. F.; Xie, C.; Xu, S. Z.; Ding, M. W.; Xiao, W. Iminophosphorane-Mediated Efficient Synthesis of New Tricyclic 3,5-Dihydro-1,2,3-Triazolo[4,5-d]-1,2,4-Triazolo[1,5-a]Pyrimidin-9-Ones. *Org. Biomol. Chem.* **2006**, *4*, 130–134. DOI: 10.1039/b513715b.

[16] Bonini, C.; Auria, M. D.; Funicello, M.; Romaniello, G. Novel N-(2-Benzo[b]Thienyl)Iminophosphoranes and Their Use in the Synthesis of Benzo[b]Thieno[2,3-b]Pyridines. *Tetrahedron* **2002**, *58*, 3507–3512. DOI: 10.1016/S0040-4020(02)00324-1.

[17] Wang, T.; Xiong, J.; Wang, W. J.; Li, R.; Tang, X. L.; Xiong, F. Tunable Regioselective Synthesis of Pyrazolo[3,4-d]Pyrimidine Derivatives via aza-Wittig Cyclization and Dimroth-Type Rearrangement. *RSC Adv.* **2015**, *5*, 19830–19837. DOI: 10.1039/C4RA15777J.

[18] Chen, C. N.; Lv, L. L.; Ji, F. Q.; Chen, Q.; Xu, H.; Niu, C. W.; Xi, Z.; Yang, G. F. Design and Synthesis of N-2,6-Difluorophenyl-5-Methoxyl-1,2,4-Triazolo[1,5-a]-Pyrimidine-2-Sulfonamide as

Acetohydroxyacid Synthase Inhibitor. *Bioorg. Med. Chem.* **2009**, *17*, 3011–3017. DOI: 10.1016/j.bmc.2009.03.018.

[19] Molina, P.; Vilaplana, M. Iminophosphoranes: Useful Building Blocks for the Preparation of Nitrogen-Containing Heterocycles. *Synthesis* **1994**, *12*, 1197–1218. DOI: 10.1055/s-1994-25672.

[20] Wang, T.; Luo, J.; Zheng, C. H. Synthesis and Herbicidal Activity of 3,6-Dialkylthio-1-phenyl-pyrazolo[3,4-d]pyrimidin-4-ones. *Chin. J. Chem.* **2010**, *30*, 1749–1753. DOI: 10.1002/cjoc.201290000.

[21] Luo, J.; Zhao, A. L.; Zheng, C. H.; Wang, T. Synthesis and Herbicidal Activity of Novel 6-Arylthio-3-Methylthio-1 H-Pyrazolo[3,4-d]Pyrimidin-4(5 H)-Ones. *Phosphorus Sulfur Silicon Relat. Elem.* **2017**, *192*, 1124–1126. DOI: 10.1080/10426507.2017.1333508.