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Rapid, Microwave-Assisted Organic Synthesis of Selective ^{V600E}BRAF Inhibitors for Preclinical Cancer Research

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Abstract

We report a dramatically improved total synthesis of two highly selective ^{V600E}BRAF inhibitors, PLX4720 and PLX4032, that leverages microwave-assisted organic synthesis (MAOS). Compared with previously reported approaches, our novel MAOS method significantly reduces overall reaction time without compromising yield. In addition to providing a gram-scale route to these compounds for preclinical oncology research, we anticipate this approach could accelerate the synthesis of azaindoles in high-throughput, library-based formats.

Keywords

PLX4720; PLX4032; BRAF; MAOS; Melanoma

As mutations in vital genes accrue, normal programs of cell proliferation, differentiation, and death are recast, forming the basis of cancer. Of the known protein kinases, the BRAF paralog of the *ra*pidly growing *f*ibrosarcoma (RAF) family of proteins is the most frequently mutated in human cancer[1]. Activating somatic mutations in BRAF occur in malignant melanomas (50%), ovarian cancer (30%), thyroid cancer (30%), colorectal cancer (CRC) (15%), and less frequently in other cancer types[2,3]. While several mutations in BRAF have been reported, the most common mutation substitutes valine for glutamic acid at codon 600 (^{V600E}BRAF) in the activation segment of the kinase. This particular mutation accounts for greater than 90% of BRAF mutations in cancer [2]. ^{V600E}BRAF is constitutively activated, as

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Supplementary Material

Supplementary data associated with this article can be found in the online version at

are its associated downstream effectors within the mitogen-activated protein kinase (MAPK) pathway[4]. Clinically, tumour expression of *V600EBRAF* correlates with elevated proliferation, aggressiveness, and poor prognosis[5]. Since growth and proliferation of tumours expressing ^{V600E}BRAF tend to rely upon MAPK pathway activity, pharmacological inhibition of ^{V600E}BRAF represents an attractive therapeutic approach in oncology[6].

Small-molecule inhibition of BRAF in oncology has been historically approached using pan-kinase inhibitors[7,8], such as sorafenib (Nexavar) (Figure 1). However, for a variety of postulated reasons[9], this approach has led to disappointing outcomes in ^{V600E}BRAF-dependent tumours such as melanoma[10,11].

An encouraging alternative approach to pan-kinase inhibitors that has shown recent success has been the clinical development of ^{V600E}BRAF-selective small-molecule inhibitors. Currently, two of the most promising selective inhibitors include PLX4720[12] and its clinically used analogue, PLX4032 (vemurafinib)[13]. Uniquely, these drugs selectively inhibit ^{V600E}BRAF kinase at low nanomolar concentrations and, accordingly, attenuate associated MAPK pathway activity in *V600EBRAF* tumours[12-17]. Recently, PLX4032 has been approved by the FDA for treatment of late-stage *V600EBRAF*-positive melanoma[18], and continues to be evaluated in other single-agent and combination settings[19-21].

Our laboratory's interest in developing and validating predictive imaging biomarkers to reflect tumour response to ^{V600E}BRAF inhibition required milligram- to gram-scale quantities of PLX4032 and PLX4720 suitable for preclinical *in vivo* studies. We found published production-scale syntheses of these compounds, yet these methods were inappropriate for typical academic research laboratories, as well as being time and labour intensive[12,13]. These compounds could also be purchased commercially, but only at great expense given the scale required for our research activities. To circumvent these limitations, we developed a rapid, in-house approach to synthesize PLX4720 and PLX4032 that capitalized upon the advantages of microwave-assisted organic synthesis (MAOS). MAOS employs non-classical heating *via* microwaves *in lieu* of traditional thermal convection or conduction. Commonly, MAOS reaction times are dramatically reduced, reaction efficiencies are increased, and material and labour costs are reduced[22,23].

In this study, MAOS was successfully adapted to each of the traditional syntheses reported by Tsai *et al.*[12] and Bollag *et al.*[13] (Table 1), with MAOS offering significant advantages in all steps required to synthesize PLX4720 and four of the six steps required to synthesize PLX4032. We herein report dramatically reduced reaction times required for synthesis of the drugs while achieving comparable, or in most cases, improved yields. The divergent synthesis developed within for this study is shown in Scheme 1. In Table 1, we report conditions, reaction times, and yields described in the original literature (refs. a,b) and MAOS application. Assuming overnight to be 16 hours, for PLX4720, MAOS resulted in a 91% reduction in overall reaction time (87 hours to 6 hours). For PLX4032, four of the six steps were amenable to MAOS and resulted in a 33% reduction in reaction time (141 hours to 94 hours). Successful gram-scale MAOS was carried out for select intermediates (**3, 6b**) to ensure scalability of the developed method. Full synthetic methodology and characterization data can be found in Supplementary Data.

1. Synthesis of PLX4720 (8a)

1.1 MAOS of *N*-(3,5-difluorophenyl)propane-1-sulfonamide (3)

The divergent synthesis begins with formation of **3** by reaction of 2,4-difluoroaniline (**1**) and propane-1-sulfonyl chloride (**2**) in anhydrous methylene chloride, dimethyaminopyridine (DMAP), and pyridine. Previous studies carried out this reaction at room temperature

overnight with a quantitative yield (*entry 1*, Table 1)[12]. Microwave irradiation at 100 °C reduced the reaction time to 30 minutes. Subsequent flash chromatography on silica gel gave **3** in a comparable yield of 89% (*entry 2*, Table 1).

1.2 MAOS of *N*-(3-5-difluoro-4-formylphenyl)propane-1-sulfonamide (5)

Synthesis of **5** features a two-step formylation of **3** with morpholine-4-carbaldehyde (**4**). Previously[12], *in situ* generation of lithium diisopropylamide (LDA) using *n*-butyllithium in THF and diisoproplyamine was carried out at 0 °C for one hour. This was followed by deprotonation of **3** with this LDA solution for four hours. Subsequent formylation with morpholine-4-carbaldehyde (**4**) at -78 °C for four hours, and a further 16 hours at room temperature gave **5** in 51% yield (*entry 1*, **Table 2**). In this study, given the temperature requirements of the deprotonation, we chose not to pursue MAOS. Moreover, we attained comparable yields using commercially available lithium bis(trimethylsilyl)amide (LHMDS) in place of LDA. The subsequent formylation, however, was adaptable to MAOS *via* microwave irradiation at 100 °C for one hour, with a comparable yield of 56% (*entry 4*, Table 1).

1.3 MAOS of *N*-(3-((5-chloro-1H-pyrrolo[2,3-*b*]pyridine-3-yl)(hydroxyl)methyl)2,4-difluorophenyl)propane-1-sulfonamide (7a)

The PLX4720 intermediate **7a** was originally synthesized in 88% from the reaction of **5** with the azaindole core 5-chloro-1*H*-pyrrolo[2,3-*b*]pyridine (**6a**) in methanol:water (1:1) for 48 hours at room temperature (*entry 7*, Table 1)[12]. Optimization with microwave irradiation resulted in final reaction conditions of 130 °C in the same solvent system for only a fraction of the original time (30 minutes). Purification by flash chromatography on silica gel gave **7a** in a matching yield of 88% (*entry 8*, Table 1).

1.4 MAOS of *N*-(5-chloro-1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonoamide (8a, PLX4720)

From compound **7a**, reported oxidation to PLX4720 (**8a**) utilized 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in a mixture of water and 1,4-dioxane at room temperature for two hours, with a yield of 90% (*entry 13*, Table 1)[12]. Under MAOS, an optimized reaction temperature of 100 °C was achieved with a reduction in reaction time to 10 minutes, a factor of 12, while still achieving a comparable yield of 87% (*entry 14*, Table 1).

2 . Synthesis of PLX4320 (8b)

2.1 MAOS of 5-(4-chlorophenyl)-1H-pyrrolo[2,3-*b*]pyridine (6b)

Suzuki coupling of 5-bromo-1*H*-pyrrolo[2,3-*b*]pyridine (**6e**) with 4-(chlorophenyl)boronic acid (**6f**) in the presence of K_2CO_3 and $Pd(PPh_3)_2Cl_2$ in 1,2-dimethoxyethane (DME) for 30 minutes at 130 °C secured the necessary PLX4032 azaindole core (**6b**) (*entry 6*, Table 1). Previous synthesis of this intermediate required overnight reflux, with a yield of 81% (*entry 5*, Table 1)[13], thus underscoring the effectiveness of MAOS in reducing overall reaction time.

2.2 Synthesis of *N*-(3-((5-(4-chlorophenyl)-1H-pyrrolo[2,3-*b*]pyridin-3-yl)(hydroxy)methyl)-2,4-difluorophenyl)propane-1-sulfonamide (7b)

Coupling of the respective azaindole cores of PLX4032 (**6b**) and PLX4720 (**6a**) with intermediate (**5**) is where the synthesis diverges. For PLX4032, unlike PLX4720, application of MAOS of **6b** with **5** in the presence of KOH and methanol proved less advantageous (*entry 11*, Table 1), frequently yielding mixed by-products. Moreover, deprotection of the

methyl ether intermediate (structure not shown) with aqueous hydrogen bromide and acetic acid to the final product (**7b**) (*entry 12*, Table 1) was also problematic under microwave irradiation, primarily yielding by-products. Modification of both reaction conditions marginally affected applicability of microwave irradiation. Accordingly, MAOS was not pursued further, in favour of the published methodology of Bollag *et al*.[13]. Nevertheless, the combined yield of these two steps in our hands was 44%, compared to Bollag's 45%, which also included the final synthetic step (*entry 15*, Table 1).

2.3 MAOS of *N*-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide (8b, PLX4032)

Bollag *et al*.[13] reported a final oxidation of precursor **7b** to PLX4032 (**8b**) analogous to Tsai *et al*.[12]. The exact yield of this step is unknown as it was reported in combination with the two preceding reactions (*entry 15*, Table 1). Nonetheless, the MAOS conditions developed for the final-step oxidation of PLX4720 (**8a**) were also employed towards PLX4032 (**8b**). Irradiation of **7b** at 100 °C for 10 minutes gave a 92% yield of the final PLX4032 (**8b**) (*entry 16*, Table 1).

In summary, we report optimized, gram-scale syntheses of PLX4720 and PLX4032 that leverage MAOS. Where applicable, the MAOS protocol reported herein significantly improves overall reaction times while maintaining or even improving synthetic yields. We envision this methodology could potentially be extended not only to the synthesis of PLX4032, PLX4720, but to other novel azaindoles as well.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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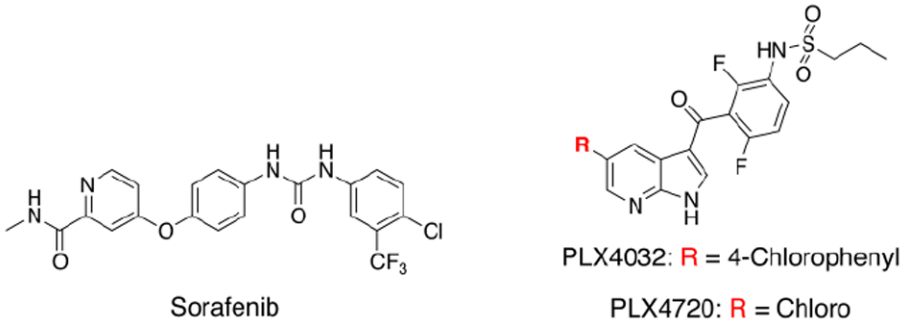
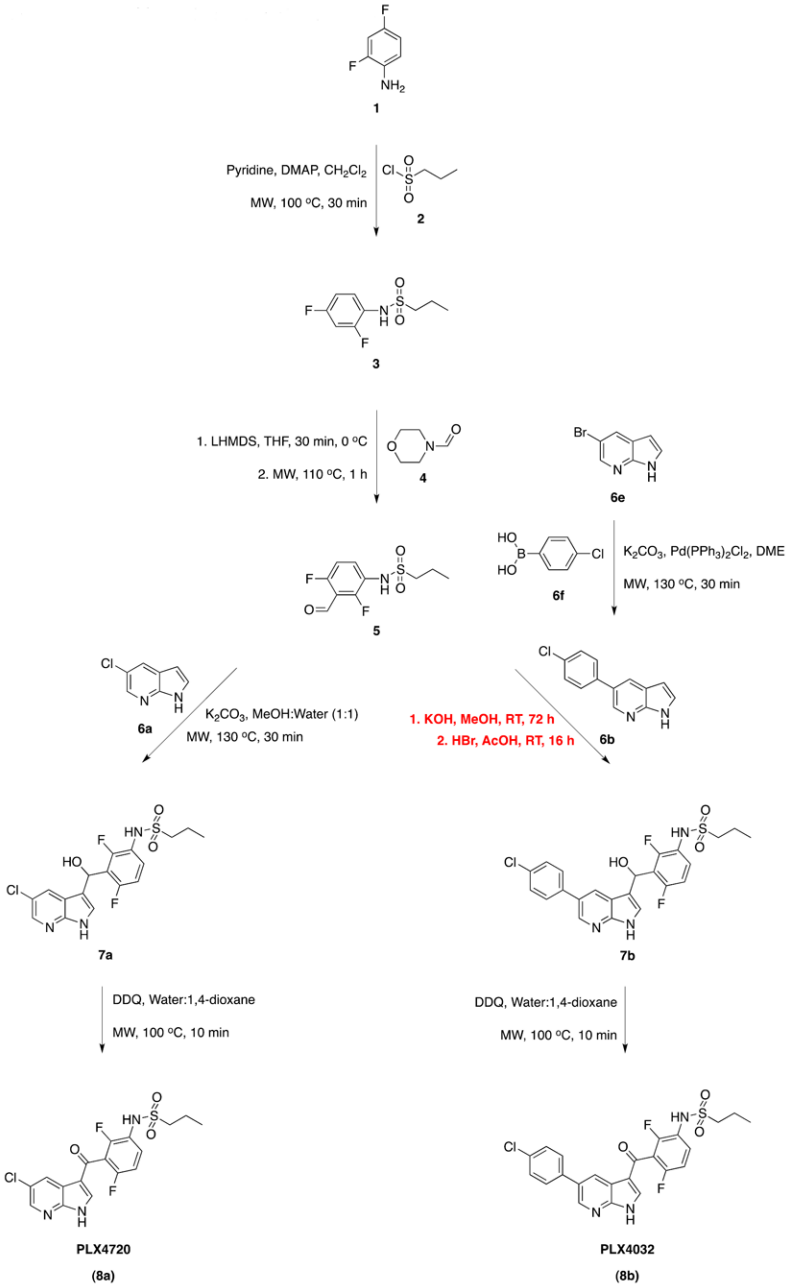


Figure 1.
Chemical structures of select BRAF inhibitors.



Scheme 1.
MAOS of PLX4720 and PLX4032 (MW = microwaves).

Table 1

MAOS of *N*-(3,5-difluorophenyl)propane-1-sulfonamide (**3**).

Entry	MAOS	Reaction Conditions	Temperature	Time	Yield
1	No	Pyridine, DMAP, CH_2Cl_2	Reflux	16 h	Quant.[a]
2	Yes	Pyridine, DMAP, CH_2Cl_2	100 °C	30 min	89%

MAOS of *N*-(3-5-difluoro-4-formylphenyl)propane-1-sulfonamide (**5**).

Entry	MAOS	Reaction Conditions	Temperature	Time	Yield
3	No	LDA, THF	-78 °C/RT	5/16 h	51%[a]
4	Yes	LHMDS, THF	0/110 °C	30 min/1 h	56%

MAOS of 5-(4-chlorophenyl)-1H-pyrrolo[2,3-*b*]pyridine (**6b**).

Entry	MAOS	Reaction Conditions	Temperature	Time	Yield
5	No	K_2CO_3, $Pd(PPh_3)_2Cl_2$, DME	Reflux	16 h	81%[b]
6	Yes	K_2CO_3, $Pd(PPh_3)_2Cl_2$, DME	130 °C	30 min	76%

MAOS of *N*-(3-((5-chloro-1H-pyrrolo[2,3-*b*]pyridine-3-yl)(hydroxyl)methyl)2,4-difluorophenyl)propane-1-sulfonamide (**7a**).

Entry	MAOS	Reaction Conditions	Temperature	Time	Yield
7	No	K_2CO_3, MeOH:Water	RT	48 h	88%[a]
8	Yes	K_2CO_3, MeOH:Water	130 °C	30 min	88%

Synthesis of *N*-(3-((5-(4-chlorophenyl)-1H-pyrrolo[2,3-*b*]pyridin-3-yl)(hydroxy)methyl)-2,4-difluorophenyl)propane-1-sulfonamide (**7b**).

Entry	MAOS	Reaction Conditions	Temperature	Time	Yield
9	No	KOH, MeOH	RT	72 h	NA[c]
10	No	HBr (aq), AcOH	RT	16 h	NA[c]
11	No	KOH, MeOH	RT	72 h	NA[d]
12	No	HBr (aq), AcOH	RT	16 h	44%[e]

MAOS of *N*-(5-chloro-1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonoamide (**8a, PLX4720**).

Entry	MAOS	Reaction Conditions	Temperature	Time	Yield
13	No	DDQ, H_2O:1,4-Dioxane	RT	2 h	90%[a]
14	Yes	DDQ, H_2O:1,4-Dioxane	100 °C	10 min	87%

MAOS of *N*-(3-(5-(4-chlorophenyl)-1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)-2,4-difluorophenyl)propane-1-sulfonamide (**8b, PLX4032**).

Entry	MAOS	Reaction Conditions	Temperature	Time	Yield
15	No	DDQ, H_2O:1,4-Dioxane	RT	2 h	45%[f]
16	Yes	DDQ, H_2O:1,4-Dioxane	100 °C	10 min	92%

[a] See Tsai *et al.* 2008.

[b] See Bollag *et al.* 2010.

[c] See Bollag *et al.* 2010, combined yield with entry *15*.

[d] Combined yield with entry *12*.

[e] Combined yield with entry *11*.

[f] See Bollag *et al.* 2010, combined yield with entries *9* & *10*.