


Article

Microwave-Assisted Expeditious Synthesis of 2-Alkyl-2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazolines Catalyzed by HgCl₂ under Solvent-Free Conditions as Potential Anti-HIV-1 Agents

Zhiping Che ^{*,†} , Yuee Tian [†], Shengming Liu, Jia Jiang, Mei Hu and Genqiang Chen

Laboratory of Pharmaceutical Design & Synthesis, Department of Plant Protection, College of Forestry, Henan University of Science and Technology, Luoyang 471003, China; tianyuee1985@163.com (Y.T.); zhenxiziji2005@126.com (S.L.); lxy_jiangjia@126.com (J.J.); lxy_humei@163.com (M.H.); genqiangchen@126.com (G.C.)

* Correspondence: zhipingche@163.com; Tel./Fax: +86-0379-64389603

† These authors contributed equally to this work.

Academic Editor: John Spencer

Received: 30 September 2018; Accepted: 9 November 2018; Published: 10 November 2018



Abstract: A series of 2-alkyl-2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazolines were expeditious prepared under microwave-assisted, catalyzed by HgCl₂ and solvent-free conditions. This method has the advantage of low catalyst loading and recovering catalyst, ease reaction and repaid reaction times, easy separation products and excellent yields, and more conducive to the large-scale synthesis products. Furthermore, compounds **3s**, **3y**, **3a'**, **3b'**, **3f'**, **3i'**, **3q'**, and **3r'** exhibited more potent anti-HIV-1 activity with EC₅₀ values of 3.35, 6.12, 3.63, 9.54, 1.79, 0.51, 3.00, and 4.01 µg/mL, and TI values of 32.66, >32.68, 31.22, 13.94, 24.27, 39.59, 26.01, and 24.51, respectively. Especially compound **3i'** displayed the highest anti-HIV-1 activity with TI values of 39.59.

Keywords: 1,3,4-oxadiazolines; microwave irradiation; HgCl₂ catalyst; anti-HIV-1 activity

1. Introduction

Acquired immunodeficiency syndrome (AIDS) is mainly caused by human immunodeficiency virus type 1 (HIV-1) infection and has remained one of the most difficult medical barriers for human health since it was first reported in 1981 [1]. The reverse transcriptase (RT) of the HIV-1 plays a significant role in the viral replication process, which makes it a pivotal target for anti-HIV-1 inhibitor discovery [2,3]. Although numerous RT inhibitors, including primarily the nucleoside/nucleotide RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs), have been developed, like other anti-HIV inhibitors, effectiveness of now approved NRTIs and NNRTIs have been hampered because of the fast development of resistance [4–7]. It is estimated that 36.9 million people (including 2.6 million children) were living with HIV infection in the year 2014 according to UNAIDS-2015 report, and 1.2 million people died due to HIV as well as related diseases [8]. To circumvent this challenge, it is urgent to discover and develop safe, green, efficient, selective, and novel anti-HIV inhibitors having significant potency against drug-resistant RT viral strains as well as less toxicity [8–11].

To the best of our knowledge, 1,3,4-oxadiazolines are excellent candidates for the research of anti-HIV-1 agents, antibacterial agents, antitumor agents, and chitin biosynthesis inhibitors [12–15]. As our ongoing work on research of anti-HIV-1 inhibitors, 2-alkyl-2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazoline derivatives were further prepared through a modified route.

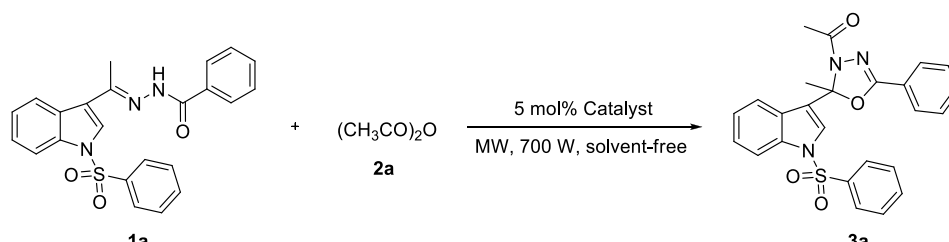
Generally, there are three ways to synthesize 1,3,4-oxadiazolines. The first kind is the traditional procedure, which is usually require excess of anhydride, and long reaction time [16–18]. The second is the ultrasonic irradiation assisted synthesis, it is with relatively mild reaction conditions and higher yield [19]. The third is the microwave irradiation assisted synthesis, which are under solvent-free conditions with short reaction time [15]. Although many advantages, there need to be further improved on the methods of the ultrasonic irradiation and microwave irradiation assisted synthesis of 1,3,4-oxadiazolines. On the other hand, since the first report of microwave irradiation assisted synthesis in 1986 [20,21], the technique has been accepted as a method for reducing reaction times and increasing yields of product compared to conventional procedure [22–26]. Especially, the use of microwave ovens as tools for synthetic chemistry is a fast growth area [27–32], so here we aimed to use this method to synthesize 2-alkyl-2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazolines catalyzed by HgCl₂ under solvent-free conditions.

2. Results and Discussion

2.1. Chemistry

To find out the most compatible reaction conditions for synthesizing 2-alkyl-2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazolines, the reaction of *N*-benzenesulfonyl-3-acetylindole benzoyl hydrazone (**1a**) with acetic anhydride (**2a**) under microwave irradiation and solvent-free conditions was investigated, and a wide range of reaction catalysts were also tested. As can be clearly seen in Table 1, the cyclization reaction catalyzed by MgCl₂·6H₂O as a Lewis acid catalyst was found to be sluggish at best condition, providing **3a** in 38% yield and recycling **1a** in 37% yield after 10 × 3 min following purification by preparative thin-layer chromatography (entry 1). The reaction was accelerated dramatically by the presence of ZnCl₂ providing the product **3a** in 48% yield and recycling the raw material **1a** in 23% yield after 10 × 3 min (entry 2). Although these experiments were not so efficient, the use of solvent-free reaction conditions does have some intrinsic ecological and chemical value. Inspired by these results, Lewis acid catalyst (entry 3) was found to be greatly enhancing the reaction in AlCl₃ catalyzed, which providing **3a** in 53% yield after 10 × 3 min following purification on silica. Similarly, reactions were improved by the presence of SnCl₂·2H₂O (entry 4) and FeCl₃ (entry 5) providing the product **3a** in 63% and 68% yield, respectively. However, the optimum conditions for this transformation employed HgCl₂ as a Lewis acid catalyst, providing **3a** in 91% yield after 10 × 3 min (entry 6).

Table 1. Screening of the catalyst for the cyclization reaction between **1a** and **2a**.

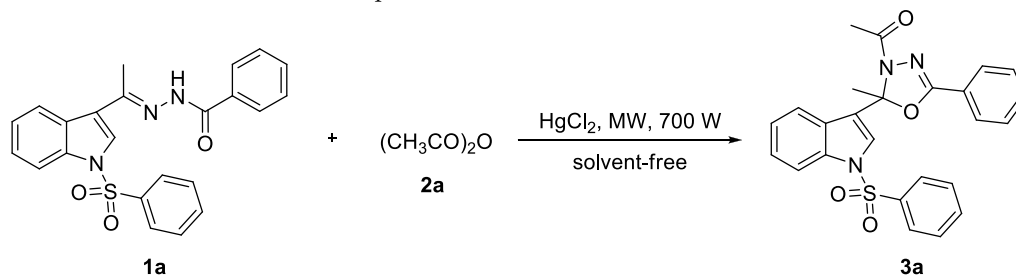


Entry	Amount of (mmol)		Catalyst (5 mol%)	<i>t</i> ^a (min)	Isolated Yield ^b (%)	
	1a	2a			3a	1a
1	0.5	1.5	MgCl ₂ ·6H ₂ O	10 × 3	38	37
2	0.5	1.5	ZnCl ₂	10 × 3	48	23
3	0.5	1.5	AlCl ₃	10 × 3	53	9
4	0.5	1.5	SnCl ₂ ·2H ₂ O	10 × 3	63	15
5	0.5	1.5	FeCl ₃	10 × 3	68	18
6	0.5	1.5	HgCl ₂	10 × 3	91	0

^a 10 × 3 means three times 10 min as reaction time, and the progress of the reaction was checked by TLC analysis at the end of each irradiation period. ^b Isolated yield (%) after preparative thin-layer chromatography.

In order to further optimize the reaction conditions, a wide range of reaction parameters were tested by altering the amount of **2a** and catalyst as well as the reaction time in a test reaction of **1a** and **2a** (Table 2).

Table 2. Optimization of the reaction conditions.



Entry	Amount of (mmol)		HgCl ₂ (mol%)	t ^a (min)	Isolated Yield ^b (%)	
	1a	2a			3a	1a
1	0.5	2.5	5	10 × 2	97	0
2	0.5	2.0	5	10 × 2	90	0
3	0.5	1.5	5	10 × 3	91	0
4	0.5	1.0	5	10 × 4	41	55
5	0.5	2.5	0	10 × 12	0	100
6	0.5	2.5	2.5	10 × 5	69	26
7	0.5	2.5	3	10 × 3	78	17
8	0.5	2.5	4	10 × 3	91	0
9	0.5	1.5	4	10 × 3	90	0

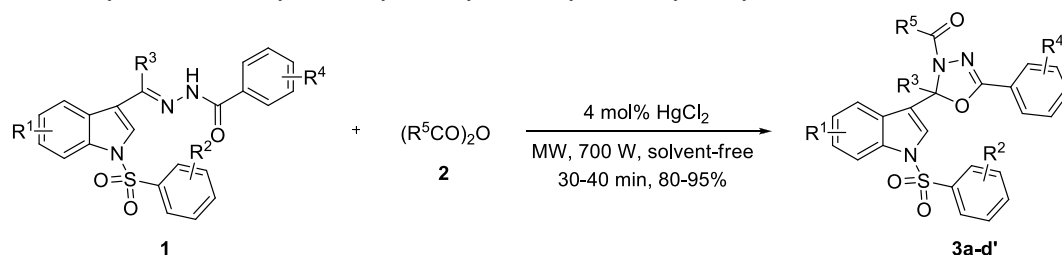
^a 10 × 2 means two times 10 min as reaction time, and the progress of the reaction was checked by TLC analysis at the end of each irradiation period. ^b Isolated yield (%) after preparative thin-layer chromatography.

As can be clearly seen in Table 2, when 0.5 mmol of **1a** and 2.5 mmol of **2a** reacted by HgCl₂ catalyst at the amount of 5 mol% under microwave irradiation, **3a** was obtained in a 97% yield after 10 × 2 min (entry 1). When the amount of **2a** reduced to 2.0 mmol, **3a** was obtained in a 90% yield for 10 × 2 min (entry 2). Moreover, when the amount of **2a** decreased to 1.5 mmol, **3a** was obtained in a 91% yield if the reaction time was prolonged to 10 × 3 min (entry 3). Nevertheless, if we keep reducing the amount of **2a** to 1.0 mmol, only providing **3a** in 41% yield and recycling **1a** in 55% yield after 10 × 4 min following purification by preparative thin-layer chromatography (entry 4). However, the cyclization reaction was terminated by the absence of HgCl₂ even if the reaction time was prolonged to 10 × 12 min and the amount of **2a** was increased to 2.5 mmol (entry 5). Inspired by these phenomena, we realized that the amount of HgCl₂ as a Lewis acid catalyst was significant for enhancing the cyclization reaction. For example, when 0.5 mmol of **1a** and 2.5 mmol of **2a** reacted by HgCl₂ catalyst at the amount of 2.5 mol% under microwave irradiation, **3a** was synthesized in a 69% yield after 10 × 5 min (entry 6). When the amount of HgCl₂ increased to 3.0 mol%, **3a** was prepared in a 78% yield for 10 × 3 min (entry 7). To our delight, when the amount of HgCl₂ increased to 4.0 mol%, the cyclization reaction was accelerated dramatically providing the product **3a** in 91% yield after 10 × 3 min (entry 8). We concluded that when 0.5 mmol of **1a** and 1.5 mmol of **2a** reacted by HgCl₂ catalyst at the amount of 4 mol% under microwave irradiation, **3a** was smoothly synthesized in a 90% yield after 10 × 3 min (entry 9).

Based upon the above results, a wide range of *N*-arylsulfonyl-3-acylindole arylcarbonyl hydrazones (**1**, R¹ = H, 6-Me, 5-CN or 5-NO₂; R² = H, *p*-Me, *p*-Cl, *m*-NO₂ or *p*-OMe; R³ = Me, Et or *n*-Pr; R⁴ = H or *m*-Me) with anhydrides (**2**, R⁵ = Me or Et) was investigated to explore the scope of the reaction. As outlined in Table 3, 2-alkyl-2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazolines (**3a-d'**) were prepared in 80–95% yields for 10 × 3–10 × 4 min (Of course, the reaction can also be carried out directly in one go for 30 or 40 min, with little impact on yield). The steric and electronic effects of substituents of **1** to the reaction were not very obvious.

We have previously developed an efficient method for the synthesis of 2-monosubstituted 3-*N*-acyl-5-phenyl-1,3,4-oxadiazolines **3e'–r'** under ultrasonic irradiation [19], however, under the above reaction conditions, 2,2-disubstituted 3-*N*-acyl-5-phenyl-1,3,4-oxadiazolines were not obtained at all even if the reaction time was prolonged. So we have previously described another a convenient, rapid, and high-yielding reaction for the synthesis of 2,2-disubstituted 3-*N*-acyl-5-phenyl-1,3,4-oxadiazolines **3a–w** under microwave irradiation and solvent-free conditions [15]. On the other hand, compared with the previous procedure, the present methodology has the advantages of low catalyst loading and recovering catalyst, short reaction and repaid reaction times, easy separation products, and increased reaction yields (except **3i**, **3j**, **3o**, **3r**, **3t**, **3u**, and **3w**), and more conducive to the large-scale synthesis products.

Table 3. Synthesis of 2-alkyl-2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazolines (**3a–d'**)^a.



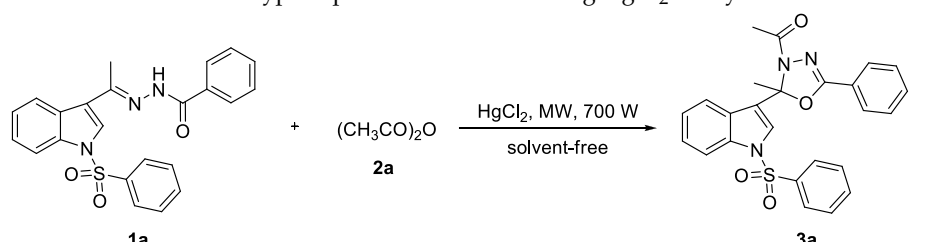
3a–d'	1				2	<i>t</i> ^b (min)	Yield ^c (%)
	R ¹	R ²	R ³	R ⁴	R ⁵		
3a	H	H	Me	H	Me	10 × 3	90
3b	H	<i>p</i> -Me	Me	H	Me	10 × 3	90
3c	6-Me	<i>p</i> -Me	Me	H	Me	10 × 3	95
3d	H	<i>p</i> -Cl	Me	H	Me	10 × 4	87
3e	6-Me	<i>p</i> -Cl	Me	H	Me	10 × 4	90
3f	H	<i>p</i> -Cl, <i>m</i> -NO ₂	Me	H	Me	10 × 4	90
3g	6-Me	<i>p</i> -Cl, <i>m</i> -NO ₂	Me	H	Me	10 × 4	89
3h	H	<i>m</i> -NO ₂	Me	H	Me	10 × 4	85
3i	6-Me	<i>m</i> -NO ₂	Me	H	Me	10 × 4	84
3j	5-CN	<i>m</i> -NO ₂	Me	H	Me	10 × 4	81
3k	5-CN	<i>p</i> -Me	Me	H	Me	10 × 4	84
3l	H	<i>p</i> -OMe	Me	H	Me	10 × 3	90
3m	6-Me	<i>p</i> -OMe	Me	H	Me	10 × 3	93
3n	H	<i>p</i> -Me	Et	H	Me	10 × 3	87
3o	H	H	<i>n</i> -Pr	H	Me	10 × 3	80
3p	H	<i>p</i> -Me	<i>n</i> -Pr	H	Me	10 × 3	86
3c	H	<i>p</i> -Cl	Et	H	Me	10 × 3	88
3r	H	H	Me	<i>m</i> -Me	Me	10 × 3	84
3s	H	<i>p</i> -Me	Me	<i>m</i> -Me	Me	10 × 3	87
3t	6-Me	<i>m</i> -NO ₂	Me	H	Et	10 × 3	81
3u	H	<i>p</i> -Me	Et	H	Et	10 × 3	88
3v	H	<i>p</i> -Me	<i>n</i> -Pr	H	Et	10 × 3	87
3w	H	H	Me	<i>m</i> -Me	Et	10 × 3	85
3s	5-NO ₂	H	Me	H	Me	10 × 4	90
3y	6-Me	H	Me	H	Me	10 × 3	81
3z	5-CN	H	Me	H	Me	10 × 4	83
3a'	5-CN	<i>p</i> -Cl	Me	H	Me	10 × 4	80
3b'	5-NO ₂	<i>p</i> -Me	Me	H	Me	10 × 4	87
3c'	5-NO ₂	<i>p</i> -Cl	Me	H	Me	10 × 4	88
3d'	H	H	Et	H	Me	10 × 3	82

^a A mixture of **1** (0.5 mmol), **2** (1.5 mmol), and HgCl₂ (0.02 mmol) was reacted under microwave heating at 700 W for 30–40 min. ^b 10 × 3 means three times 10 min as reaction time, and the progress of the reaction was checked by TLC analysis at the end of each irradiation period. ^c Isolated yield (%) after preparative thin-layer chromatography.

In order to further study the cyclization reaction, we have done the following study. As illustrated in Table 4, when the cyclization reaction of **1a** (3 mmol) with **2a** (9 mmol) was complete, the reaction mixture was cooled to room temperature. Then the mixture was filtered and the filter residue was washed with dichloromethane (3×10 mL). The HgCl_2 catalyst was reused directly in the next reaction. For examples, when 3 mmol of **1a** and 9 mmol of **2a** reacted by HgCl_2 catalyst at the amount of 4 mol% under microwave irradiation, **3a** was smoothly synthesized in 98% yield after 5 min for the first time (entry 1). When the reaction time prolonged to 6 min, **3a** was obtained in 98% yield for recovering HgCl_2 catalyst was reused directly in the reaction (entry 2). Entries 3 and 4, **3a** was obtained in 97% and 98% yield, respectively.

To our delight, in the case of reaction amplification, it was found that HgCl_2 had better catalytic effect on the reaction. It may be that HgCl_2 induces the cyclization reaction in which the turnover frequency is accelerated, and the yield of compound **3a** increased significantly.

Table 4. Typical procedure for recovering HgCl_2 catalyst.



Entry	Amount of (mmol)		HgCl_2 (mol%)	t (min)	Isolated Yield ^a (%)
	1a	2a			3a
1	3	9	4	5	98
2	3	9	4	6	98
3	3	9	4	8	97
4	3	9	4	9	98

^a Isolated yield (%) after preparative thin-layer chromatography.

2.2. Biological Activities

Fourteen compounds of 2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazolines (**3e'**–**r'**, Figure 1) were prepared using our previously published method, and characterized by ¹H-NMR, EI-MS or ESI-MS and HRMS [19]. Subsequently, 21 2-alkyl-2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazolines (**3s**–**r'**) were tested in vitro for their anti-HIV-1 activity, and 3'-azido-3'-deoxythymidine (AZT) was used as a positive control illustrated in Table 5.

Among these tested 2-alkyl-2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazolines, compounds **3s**, **3y**, **3a'**, **3b'**, **3f'**, **3i'**, **3q'**, and **3r'** exhibited more potent anti-HIV-1 activity with EC_{50} values of 3.35, 6.12, 3.63, 9.54, 1.79, 0.51, 3.00, and 4.01 $\mu\text{g}/\text{mL}$, and TI values of 32.66, >32.68, 31.22, 13.94, 24.27, 39.59, 26.01, and 24.51, respectively. Especially compound **3i'** displayed the highest anti-HIV-1 activity with TI values of 39.59. Meanwhile, a brief structure activity relationship (SAR) was determined. (1) When $\text{R}^1 = \text{H}$ or 6-Me, compound with $\text{R}^2 = m\text{-NO}_2$ was more vital for the anti-HIV-1 activities than $\text{R}^2 = m\text{-NO}_2$, $p\text{-Cl}$ or $p\text{-Cl}$ (**3f'** and **3i'** vs. **3h'** and **3j'**, EC_{50} values of 1.79, 0.51, 3.53, and 12.42 $\mu\text{g}/\text{mL}$, TI values of 24.27, 39.59, 1.36, and 0.18, respectively; that is, the TI value of **3f'** was close to 18 times of that of **3h'** and the TI value of **3i'** was close to 220 times of that of **3j'**. **3f'** and **3i'** vs. **3k'** and **3l'**; EC_{50} values of 1.79, 0.51, 19.37, and 57.49 $\mu\text{g}/\text{mL}$; TI values of 24.27, 39.59, 0.86, and 3.16, respectively; that is, the TI value of **3f'** was more than 28 times of that of **3k'** and the TI value of **3i'** was more than 12 times of that of **3l'**). (2) The length of the chain of substituent R^5 was significant for the anti-HIV-1 activity (**3q'** vs. **3e'**, EC_{50} values of 3.00 and 15.52 $\mu\text{g}/\text{mL}$, TI values of 26.01 and 0.97, respectively, especially the TI value of **3q'** was close to 27 times of that of **3e'**; **3r'** vs. **3o'**, EC_{50} values of 4.01 and 18.14 $\mu\text{g}/\text{mL}$, and TI values of 24.51 and 8.36, respectively). (3) When $\text{R}^1 = 5\text{-NO}_2$, compound

with $R^2 = H$ was more important for the anti-HIV-1 activities than $R^2 = p\text{-Me}$ or $p\text{-Cl}$ (**3s** vs. **3b'** and **3c'**, EC_{50} values of 3.35, 9.54 and 6.01 $\mu\text{g/mL}$, TI values of 32.66, 13.94 and 8.89, respectively; that is, the TI value of **3s** was more than two times of that of **3b'** and was more than three times of that of **3c'**). When $R^1 = 5\text{-CN}$, introduction of R^2 as the $p\text{-Cl}$ group could lead to the pronounced compound (**3a'** vs. **3z**, EC_{50} values of 3.63 and 17.57 $\mu\text{g/mL}$, TI values of 31.22 and 3.01, respectively, especially the TI value of **3a'** was more than 10 times of that of **3z**).

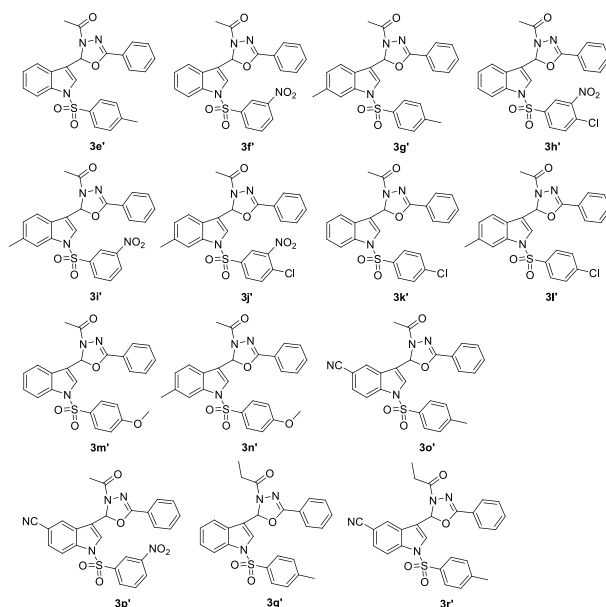


Figure 1. Chemical structures of 2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazolines **3e'–r'**.

Table 5. Anti-HIV-1 activity of 2-alkyl-2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazolines **3s–r'** in vitro ^a.

3s–r'	CC₅₀^b ($\mu\text{g/mL}$)	EC₅₀^c ($\mu\text{g/mL}$)	TI^d
3s	109.42	3.35	32.66
3y	>200	6.12	>32.68
3z	53.03	17.57	3.01
3a'	113	3.63	31.22
3b'	132.88	9.54	13.94
3c'	53.4	6.01	8.89
3d'	105.21	20.33	5.18
3e'	15.09	15.52	0.97
3f'	43.45	1.79	24.27
3g'	80.79	22.48	3.59
3h'	4.8	3.53	1.36
3i'	20.19	0.51	39.59
3j'	2.26	12.42	0.18
3k'	16.57	19.37	0.86
3l'	181.84	57.49	3.16
3m'	12.33	22.48	0.55
3n'	11.19	16.56	0.68
3o'	151.65	18.14	8.36
3p'	134.98	63.8	2.12
3q'	78.04	3.00	26.01
3r'	98.31	4.01	24.51
AZT^e	1373.17	0.00199	690,035

^a Values are means of two separate experiments. ^b CC_{50} (50% cytotoxic concentration), concentration of drug that causes 50% reduction in total C8166 cell number. ^c EC_{50} (50% effective concentration), concentration of drug that reduces syncytia formation by 50%. ^d In vitro therapeutic index (CC_{50} value/ EC_{50} value). ^e AZT was used as a positive control.

3. Experimental Section

3.1. General Information

All solvents and reagents were used as obtained from commercial sources without further purification. Analytical thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed with silica gel plates using silica gel 60 GF₂₅₄ (Qingdao Haiyang Chemical Co., Ltd., Shandong, China). Melting points are uncorrected. Nuclear magnetic resonance spectra (NMR) were recorded on a Bruker Avance DMX 400 MHz instrument (Bruker Daltonik, Bremen, Germany) in CDCl₃ (¹H at 400 MHz and ¹³C at 100 MHz) using TMS (tetramethylsilane) as the internal standard. Electrospray ion trap mass spectrometry (ESI-TRAP-MS) was carried out with a Bruker ESI-TRAP Esquire 6000 plus mass spectrometry instrument (Bruker, Germany) Microwave irradiation was performed in a Galanz microwave oven, WG700CTL20II-K6 (Galanz, Guangdong, China).

3.2. Preparation of 2-Alkyl-2-(N-arylsulfonylindol-3-yl)-3-N-acyl-5-aryl-1,3,4-oxadiazolines (3a–d')

A mixture of **1** (0.5 mmol), **2** (1.5 mmol), and HgCl₂ (0.02 mmol) was reacted under microwave heating at 700 W for 30–40 min. After allowing the mixture to cool to room temperature, the mixture was diluted with dichloromethane (30 mL) and filtered. The filtrate was washed with saturation sodium bicarbonate (2 × 20 mL), and brine (1 × 10 mL). Then the organic phase was dried over anhydrous Na₂SO₄, concentrated in vacuo and purified by preparative thin-layer chromatography (PTLC) to give the desired products **3a–d'** in 80–95% yields. Compounds **3a–w** were known compounds and characterized by comparison of the data as described in our previous paper [15]. The NMRs, MS (see Supplementary Materials) and typical spectral data of compounds **3s–d'** were as follows.

2-Methyl-2-(N-benzenesulfonyl-5-nitroindol-3-yl)-3-N-acetyl-5-phenyl-1,3,4-oxadiazolines (3s): Yellow solid, m.p. 186–188 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 8.42 (d, *J* = 2.0 Hz, 1H), 8.17–8.19 (m, 1H), 8.00–8.04 (m, 2H), 7.93–7.95 (m, 2H), 7.87–7.89 (m, 2H), 7.60–7.64 (m, 1H), 7.49–7.55 (m, 3H), 7.46 (t, *J* = 7.2 Hz, 2H), 2.36 (s, 3H), 2.33 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃) δ: 167.1, 154.0, 144.4, 137.9, 137.2, 134.7, 131.8, 129.7, 128.7, 128.4, 127.3, 127.0, 126.8, 124.0, 121.7, 120.1, 116.6, 113.9, 96.7, 23.6, 22.3. MS (ESI-TRAP), *m/z* (%): 527 ([M + Na]⁺, 100).

2-Methyl-2-(N-benzenesulfonyl-6-methylindol-3-yl)-3-N-acetyl-5-phenyl-1,3,4-oxadiazolines (3y): White solid, m.p. 142–144 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 7.90 (d, *J* = 7.2 Hz, 2H), 7.86 (d, *J* = 7.2 Hz, 2H), 7.78 (s, 1H), 7.71 (s, 1H), 7.40–7.57 (m, 6H), 7.25–7.28 (m, 1H), 6.99 (d, *J* = 7.6 Hz, 1H), 2.40 (s, 3H), 2.31 (s, 3H), 2.27 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃) δ: 166.6, 153.9, 138.1, 135.6, 135.1, 133.9, 131.4, 129.3, 128.6, 126.8, 125.3, 124.6, 120.8, 119.4, 113.7, 97.2, 23.3, 22.2, 21.7. MS (ESI-TRAP), *m/z* (%): 496 ([M + Na]⁺, 100).

2-Methyl-2-(N-benzenesulfonyl-5-cyanoindol-3-yl)-3-N-acetyl-5-phenyl-1,3,4-oxadiazolines (3z): White solid, m.p. 208–210 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 8.03 (d, *J* = 8.8 Hz, 1H), 7.97 (s, 1H), 7.94 (d, *J* = 7.6 Hz, 2H), 7.87 (d, *J* = 8.4 Hz, 2H), 7.75 (s, 1H), 7.60–7.64 (m, 1H), 7.51–7.56 (m, 4H), 7.43–7.47 (m, 2H), 2.31 (s, 6H). ¹³C-NMR (100 MHz, CDCl₃) δ: 167.0, 153.9, 137.4, 136.8, 134.6, 131.8, 129.7, 128.7, 127.9, 127.8, 127.5, 127.0, 126.8, 125.0, 124.0, 120.8, 119.1, 114.5, 107.5, 96.7, 23.3, 22.2. MS (ESI-TRAP), *m/z* (%): 507 ([M + Na]⁺, 100).

2-Methyl-2-(N-p-chlorobenzenesulfonyl-5-cyanoindol-3-yl)-3-N-acetyl-5-phenyl-1,3,4-oxadiazolines (3a'): White solid, m.p. 128–130 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 8.01 (d, *J* = 8.8 Hz, 1H), 7.93 (s, 1H), 7.84–7.87 (m, 4H), 7.75 (s, 1H), 7.44–7.57 (m, 6H), 2.31 (s, 6H). ¹³C-NMR (100 MHz, CDCl₃) δ: 167.0, 153.9, 141.5, 136.7, 135.7, 131.9, 130.0, 128.7, 128.3, 128.1, 127.7, 127.6, 126.8, 125.1, 123.9, 121.2, 118.9, 114.5, 107.7, 96.5, 23.3, 22.2. MS (ESI-TRAP), *m/z* (%): 541 ([M + Na]⁺, 100).

2-Methyl-2-(N-p-methylbenzenesulfonyl-5-nitroindol-3-yl)-3-N-acetyl-5-phenyl-1,3,4-oxadiazolines (3b'): Yellow solid, m.p. 110–112 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 8.41 (d, *J* = 2.0 Hz, 1H), 8.16–8.19 (m, 1H), 7.99–8.02 (m, 2H), 7.89 (d, *J* = 7.2 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.42–7.52 (m, 3H), 7.32 (d, *J* = 8.0 Hz, 2H), 2.37 (s, 3H), 2.35 (s, 3H), 2.33 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃) δ: 167.1, 154.0, 146.1,

144.3, 137.8, 134.2, 131.8, 130.3, 128.7, 128.4, 127.2, 127.1, 126.8, 124.0, 121.5, 120.0, 116.6, 113.8, 96.8, 23.6, 22.3, 21.6. MS (ESI-TRAP), m/z (%): 541 ($[M + Na]^+$, 100).

2-Methyl-2-(N-p-chlorobenzenesulfonyl-5-nitroindol-3-yl)-3-N-acetyl-5-phenyl-1,3,4-oxadiazolines (3c'): Yellow solid, m.p. 108–110 °C. 1H -NMR (400 MHz, $CDCl_3$) δ : 8.42 (d, $J = 1.6$ Hz, 1H), 8.18–8.21 (m, 1H), 7.97–8.02 (m, 2H), 7.85–7.89 (m, 4H), 7.43–7.52 (m, 5H), 2.35 (s, 3H), 2.33 (s, 3H). ^{13}C -NMR (100 MHz, $CDCl_3$) δ : 167.2, 154.0, 144.5, 141.6, 137.8, 135.6, 131.8, 130.1, 128.7, 128.4, 127.4, 126.9, 124.0, 122.1, 120.3, 116.8, 113.8, 96.6, 23.6, 22.3. MS (ESI-TRAP), m/z (%): 561 ($[M + Na]^+$, 78).

2-Ethyl-2-(N-benzenesulfonylindol-3-yl)-3-N-acetyl-5-phenyl-1,3,4-oxadiazolines (3d'): White solid, m.p. 224–226 °C. 1H -NMR (400 MHz, $CDCl_3$) δ : 7.85–7.93 (m, 6H), 7.41–7.56 (m, 7H), 7.25–7.29 (m, 1H), 7.13–7.17 (m, 1H), 3.05–3.11 (m, 1H), 2.54–2.59 (m, 1H), 2.31 (s, 3H), 1.03–1.07 (m, 3H). ^{13}C -NMR (100 MHz, $CDCl_3$) δ : 166.8, 154.5, 137.9, 135.1, 133.9, 131.5, 129.3, 128.6, 127.6, 126.9, 126.8, 125.8, 124.8, 124.3, 123.7, 120.9, 120.1, 113.7, 100.0, 28.4, 22.2, 6.5. MS (ESI-TRAP), m/z (%): 496 ($[M + Na]^+$, 100).

3.3. Anti-HIV-1 Activity Assay

3.3.1. Virus and Cells

Cell line (C8166) and the laboratory-derived virus (HIV-1_{IIIB}) were obtained from MRC, AIDS Reagent Project, London, UK. C8166 was maintained in RPMI-1640 supplemented with 10% heat-inactivated newborn calf serum (Gibco, Grand Island, NY, USA). The cells used in all experiments were in log-phase growth. The 50% HIV-1_{IIIB} tissue culture infectious dose (TCID₅₀) in C8166 cells was determined and calculated by the Reed and Muench method. Virus stocks were stored in small aliquots at -70 °C.

3.3.2. MTT-Based Cytotoxicity Assay

Cellular toxicity of 2-alkyl-2-(N-arylsulfonylindol-3-yl)-3-N-acyl-5-aryl-1,3,4-oxadiazolines **3s–r'** on C8166 cells was assessed by MTT method as described previously. Briefly, cells were seeded on 96-well microtiter plate in the absence or presence of various concentrations of 2-alkyl-2-(N-arylsulfonylindol-3-yl)-3-N-acyl-5-aryl-1,3,4-oxadiazolines in triplicate and incubated at 37 °C in a humid atmosphere of 5% CO₂ for 3 day. The supernatants were discarded and MTT reagent (5 mg/mL in PBS) was added to each wells, then incubated for 4 h, 100 μ L of 50% *N,N*-dimethylformamide (DMF)-20% SDS was added. After the formazan was dissolved completely, the plates were read on a Bio-TekElx800 ELISA reader (BioTek, Winooski, VT, USA) at 595/630 nm. The cytotoxic concentration that caused the reduction of viable C8166 cells by 50% (CC₅₀) was determined from dose–response curve.

3.3.3. Syncytia Assay

In the presence of 100 μ L various concentrations of 2-alkyl-2-(N-arylsulfonylindol-3-yl)-3-N-acyl-5-aryl-1,3,4-oxadiazolines, C8166 cells (4×10^5 /mL) were infected with virus HIV-1_{IIIB} at a multiplicity of infection (M.O.I) of 0.06. The final volume per well was 200 μ L. Control assays were performed without the testing compounds in HIV-1_{IIIB} infected and uninfected cultures. After 3 days of culture, the cytopathic effect (CPE) was measured by counting the number of syncytia. Percentage inhibition of syncytia formation was calculated and 50% effective concentration (EC₅₀) was calculated. AZT (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control. Therapeutic index (TI) = CC₅₀/EC₅₀.

4. Conclusions

Here we report a very superior method of the microwave-assisted expeditious synthesis of 2-alkyl-2-(N-arylsulfonylindol-3-yl)-3-N-acyl-5-aryl-1,3,4-oxadiazolines catalyzed by HgCl₂ under solvent-free conditions. This method has the advantages of low catalyst loading and recovering

catalyst, short reaction and repaid reaction times, easy separation products, excellent yields, and being more conducive to the large-scale synthesis products. Compound **3i'** especially displayed the highest anti-HIV-1 activity with TI values of 39.59. It implied that **3i'** might be regarded as the lead compound for further preparation of anti-HIV-1 agents.

Supplementary Materials: The following are available online. The NMRs and MS of the new compounds (**3s–d'**).

Author Contributions: Designed the experiments, synthesized the compounds, and analyzed the data: Z.C. Wrote the paper: Z.C., Y.T., S.L., J.J., M.H., and G.C. All authors approved the final manuscript.

Funding: The present research was supported by the National Natural Science Foundation of China (grant no. U1604105).

Acknowledgments: We would like to acknowledge the MRC AIDS Research Project and the NIH AIDS Research and Reference Reagent Program for providing cell lines and viruses.

Conflicts of Interest: The authors have reported no conflict of interest.

References

1. Gottlieb, M.S.; Schroff, R.; Shanker, H.M.; Weisman, J.D.; Fan, P.T.; Wolf, R.A.; Saxon, A. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: Evidence of a new acquired cellular immunodeficiency. *N. Engl. J. Med.* **1981**, *305*, 1425–1431. [[CrossRef](#)] [[PubMed](#)]
2. Jonckheere, H.; Anné, J.; De Clercq, E. The HIV-1 reverse transcription (RT) process as target for RT inhibitors. *Med. Res. Rev.* **2000**, *20*, 129–154. [[CrossRef](#)]
3. Yisma, E.; Dessalegn, B.; Astatkie, A.; Fesseha, N.; Benagiano, G.; Bastianelli, C.; Brusamento, S.; Elmoniry, H.; van Velthoven, M.; Pape, U. Global report: *UNAIDS* report on the global AIDS epidemic 2013. *Reprod. Health* **2014**, *10*, 23. [[CrossRef](#)] [[PubMed](#)]
4. Boone, L.R. Next-generation HIV-1 non-nucleoside reverse transcriptase inhibitors. *Curr. Opin. Investig. Drugs* **2006**, *7*, 128–135. [[PubMed](#)]
5. De Clercq, E. New developments in anti-HIV chemotherapy. *Biochim. Biophys. Acta* **2002**, *1587*, 258–275. [[CrossRef](#)]
6. Sluis-Cremer, N.; Wainberg, M.A.; Schinazi, R.F. Resistance to reverse transcriptase inhibitors used in the treatment and prevention of HIV-1 infection. *Future Microbiol.* **2015**, *10*, 1773–1782. [[CrossRef](#)] [[PubMed](#)]
7. Yu, M.Y.; Fan, E.K.; Wu, J.D.; Liu, X.Y. Recent advances in the DABOs family as potent HIV-1 non-nucleoside reverse transcriptase inhibitors. *Curr. Med. Chem.* **2011**, *18*, 2376–2385. [[CrossRef](#)] [[PubMed](#)]
8. Chander, S.; Wang, P.; Ashok, P.; Yang, L.M.; Zheng, Y.T.; Murugesan, S. Rational design, synthesis, anti-HIV-1 RT and antimicrobial activity of novel 3-(6-methoxy-3,4-dihydroquinolin-1(2H)-yl)-1-(piperazin-1-yl)propan-1-one derivatives. *Bioorg. Chem.* **2016**, *67*, 75–83. [[CrossRef](#)] [[PubMed](#)]
9. Huang, L.; Yu, D.L.; Ho, P.; Lee, K.H.; Chen, C.H. Synthesis and anti-HIV activity of bi-functional triterpene derivatives. *Lett. Drug Des. Discov.* **2007**, *4*, 471–478. [[CrossRef](#)]
10. Polanski, J.; Niedbala, H.; Musiol, R.; Podeszwa, B.; Tabak, D.; Palka, A.; Mencel, A.; Finster, J.; Mouscadet, J.F.; Le Bret, M. 5-Hydroxy-6-quinaldic acid as a novel molecular scaffold for HIV-1 integrase inhibitors. *Lett. Drug Des. Discov.* **2006**, *3*, 175–178. [[CrossRef](#)]
11. Safakish, M.; Hajimahdi, Z.; Zabihollahi, R.; Aghasadeghi, M.R.; Vahabpour, R.; Zarghi, A. Design, synthesis, and docking studies of new 2-benzoxazolinone derivatives as anti-HIV-1 agents. *Med. Chem. Res.* **2017**, *26*, 2718–2726. [[CrossRef](#)]
12. Ke, S.Y.; Liu, F.Y.; Wang, N.; Yang, Q.; Qian, X.H. 1,3,4-Oxadiazoline derivatives as novel potential inhibitors targeting chitin biosynthesis: Design, synthesis and biological evaluation. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 332–335. [[CrossRef](#)] [[PubMed](#)]
13. Coley, H.M.; Sarju, J.; Wagner, G. Synthesis and characterization of platinum(II) oxadiazoline complexes and their in vitro antitumor activity in platinum-sensitive and -resistant cancer cell lines. *J. Med. Chem.* **2008**, *51*, 135–141. [[CrossRef](#)] [[PubMed](#)]
14. El-Emam, A.A.; Al-Deeb, O.A.; Al-Omar, M.; Lehmann, J. Synthesis, antimicrobial, and anti-HIV-1 activity of certain 5-(1-adamantyl)-2-substituted thio-1,3,4-oxadiazoles and 5-(1-adamantyl)-3-substituted aminomethyl-1,3,4-oxadiazoline-2-thiones. *Bioorg. Med. Chem.* **2004**, *12*, 5107–5113. [[CrossRef](#)] [[PubMed](#)]

15. Che, Z.P.; Huang, N.; Yu, X.; Yang, L.M.; Ran, J.Q.; Zhi, X.Y.; Xu, H.; Zheng, Y.T. Microwave-assisted combinatorial synthesis of 2-alkyl-2-(*N*-arylsulfonylindol-3-yl)-3-*N*-acyl-5-aryl-1,3,4-oxadiazolines as anti-HIV-1 agents. *Comb. Chem. High Throughput Scr.* **2013**, *16*, 400–407. [CrossRef]
16. Joshi, S.D.; Vagdevi, H.M.; Vaidya, V.P.; Gadaginamath, G.S. Synthesis of new 4-pyrrol-1-yl benzoic acid hydrazide analogs and some derived oxadiazole, triazole and pyrrole ring systems: A novel class of potential antibacterial and antitubercular agents. *Eur. J. Med. Chem.* **2008**, *43*, 1989–1996. [CrossRef] [PubMed]
17. Cottineau, B.; Renaux, S.; Chenault, J.; Guillaumet, G. New synthesis of pyrazolyl-1,3,4-oxadiazole and 1,3,4-oxadiazoline derivatives. *Lett. Org. Chem.* **2005**, *2*, 599–601. [CrossRef]
18. Li, C.K.; Ma, Y.J.; Cao, L.H. Synthesis of novel 3-acetyl-2-aryl-5-(3-aryl-1-phenyl-pyrazol-4-yl)-2,3-dihydro-1,3,4-oxadiazoles. *J. Chin. Chem. Soc.* **2009**, *56*, 182–185. [CrossRef]
19. Xu, H.; Che, Z.P.; Wang, Q. An efficiently sonochemical synthesis of 2-(*N*-aryl-sulfonylindol-3-yl)-3-*N*-acyl-5-phenyl-1,3,4-oxadiazolines. *Heterocycles* **2011**, *42*, 825–832. [CrossRef]
20. Gedye, R.; Smith, F.; Westaway, K.; Humera, A.; Baldisera, L.; Laberge, L.; Rousell, L. The use of microwave ovens for rapid organic synthesis. *Tetrahedron Lett.* **1986**, *27*, 279–282. [CrossRef]
21. Giguere, R.J.; Bray, T.L.; Duncan, S.M.; Majetich, G. Application of commercial microwave ovens to organic synthesis. *Tetrahedron Lett.* **1986**, *27*, 4945–4948. [CrossRef]
22. Koziej, D.; Floryan, C.; Sperling, R.A.; Ehrlicher, A.J.; Issadore, D.; Westervelt, R.; Weitz, D.A. Microwave dielectric heating of non-aqueous droplets in a microfluidic device for nanoparticle synthesis. *Nanoscale* **2013**, *5*, 5468–5475. [CrossRef] [PubMed]
23. Pedersen, S.L.; Tofteng, A.P.; Malik, L.; Jensen, J. Microwave heating in solid-phase synthesis. *Chem. Soc. Rev.* **2012**, *41*, 1826–1844. [CrossRef] [PubMed]
24. Westman, J. An efficient combination of microwave dielectric heating and the use of solid-supported triphenylphosphine for Wittig reactions. *Org. Lett.* **2001**, *3*, 3745–3747. [CrossRef] [PubMed]
25. Mariappan, A.; Rajaguru, K.; Muthusubramanian, S.; Bhuvanesh, N. Microwave-assisted catalyst-free synthesis of tetrasubstituted pyrroles using dialkyl acetylenedicarboxylates and monophenacylanilines. *Syn. Commun.* **2016**, *47*, 805–812. [CrossRef]
26. Wang, S.L.; Cheng, C.; Wu, F.Y.; Jiang, B.; Shi, F.; Tu, S.J.; Rajale, T.; Li, G. Microwave-assisted multi-component reaction in water leading to highly regioselective formation of benzo[*f*]azulen-1-ones. *Tetrahedron* **2011**, *67*, 4485–4493. [CrossRef] [PubMed]
27. Vaddula, B.R.; Varma, R.S.; Leazer, J. Mixing with microwaves: Solvent-free and catalyst-free synthesis of pyrazoles and diazepines. *Tetrahedron Lett.* **2013**, *54*, 1538–1541. [CrossRef]
28. De-la-Torre, P.; Osorio, E.; Alzate-Morales, J.H.; Caballero, J.; Trilleras, J.; Astudillo-Saavedra, L.; Brito, L.; Cárdenas, A.; Quiroga, J.; Gutiérrez, M. Ultrasound-assisted phase-transfer catalysis method in an aqueous medium to promote the Knoevenagel reaction: Advantages over the conventional and microwave-assisted solvent-free/catalyst-free method. *Ultrason. Sonochem.* **2014**, *21*, 1666–1674. [CrossRef] [PubMed]
29. Driowya, M.; Saber, A.; Marzag, H.; Demange, L.; Bougrin, K.; Benhida, R. Microwave-assisted syntheses of bioactive seven-membered, macro-sized heterocycles and their fused derivatives. *Molecules* **2016**, *21*, 1032. [CrossRef] [PubMed]
30. Vera, G.; Diethelm, B.; Terraza, C.A.; Recabarren-Gajardo, G. Suzuki-type cross-coupling reaction of unprotected 3-iodoindazoles with pinacol vinyl boronate: An expeditive C-3 vinylation of indazoles under microwave irradiation. *Molecules* **2018**, *23*, 2051. [CrossRef] [PubMed]
31. Kantar, G.K.; Baltas, N.; Mentese, E.; Sasmaz, S. Microwave-assisted synthesis and investigation of xanthine oxidase inhibition of new phthalonitrile and phthalocyanines containing morpholino substituted 1,2,4-triazole-3-one. *J. Organomet. Chem.* **2015**, *787*, 8–13. [CrossRef]
32. Saber, A.; Marzag, H.; Benhida, R.; Bougrin, K. Microwave-assisted cycloaddition reactions in carbo- and heterocyclic chemistry. *Curr. Org. Chem.* **2014**, *18*, 2139–2180. [CrossRef]

Sample Availability: Samples of the compounds **3a–r'** are available from the authors.



© 2018 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).