sustainability

MDPI

Article

From Technical Efficiency to Economic Efficiency: Development of Aza-Friedel–Crafts Reaction Using Phosphoric Acid Immobilized in Glycerol as a Sustainable Approach

Lan Tan [1,*] and Abdul Rahman [2]

[1] Business School, Zhejiang University City College, Hangzhou 310015, China
[2] Faculty of Science, Zhejiang University, Hangzhou 310027, China; a.rahman.chemist@gmail.com
* Correspondence: tanl@zucc.edu.cn; Tel.: +86-571-8828-4363

Received: 13 June 2017; Accepted: 3 July 2017; Published: 5 July 2017

Abstract: The search for sustainable and practical synthetic methodology with high levels of technical efficiency is a highly topical subject that would contribute to developing recycling economy and saving resources. Green synthetic science has been firmly established and has provided essential design criteria for the development of a sustainable approach to high added value molecules and drug discovery, and the further development of sustainable manufacturing processes of medicines with new optimality principles for economic efficiency. In this study, a green atom economical aza-Friedel–Crafts reaction catalyzed by phosphoric acid immobilized in glycerol has been developed. This protocol provides a sustainable approach for the preparation of pyrrolyl and trifluoromethyl dihydrobenzoxazinones in excellent yields with remarkable features, such as bio-renewable glycerol as a cheap, safe and green solvent, easy product separation and catalytic system recycling under mild conditions. Furthermore, the preliminary biological activity of these products was evaluated in glioma (C6) and melanoma (B16BL6) tumor cell lines by using adriamycin as a positive control with the thiazoyl blue tetrazolium bromide (MTT) assay. The result suggests that product 5n shows promising cancer growth inhibition of glioma and melanoma, and is a promising lead compound for further investigation as anti-glioma and anti-melanoma agents.

Keywords: technical efficiency; recycling economy; catalysis; economic efficiency; aza-Friedel–Crafts; glioma; melanoma

1. Introduction

The concept of atom economy as a green criterion was introduced by Barry M. Trost of Stanford University (USA) in 1991, for which he received the Presidential Green Chemistry Challenge Award in 1998 [1]. It is an efficient method of expressing how efficiently a particular reaction makes use of the reactant atoms. Atom economy is a simple yet useful tool to guide reaction selection, and one of 12 principles of green and sustainable synthesis [2]. Thus, an efficient strategy towards a sustainable synthesis approach is to achieve the high incorporation of the starting materials into the final product, which guarantees a minimal formation of waste by-products. The aza-Friedel–Crafts reaction [3–5] has superior 100% atom economy, and represent an elementary example in this regard. The aza-Friedel–Crafts reaction catalyzed by Brønsted acid or Lewis acid is an important reaction for new carbon–carbon bond formation to construct a variety of nitrogen-containing aromatic compounds. After over a century of development, it still attracts much research interest in both academia and industry.

Since sustainability, by definition, is a multivariable optimization exercise, the key challenge in synthetic science is to select the few metrics that would drive the right reactivity toward more

sustainable, greener practical methodologies. Solvents are perhaps the most active area of research in green synthetic science, not only because they make up by far the greatest proportion of waste, but also make up a significant part of the hazard issues and energy intensity of a reaction process [6]. Additionally, the choice of reaction medium is often critical in synthetic reaction, as it may influence the course of a reaction, its rate, and selectivity to a remarkable extent. The development of a new generation of greener solvent is a primary concern due to the ultimate goal of solving these environmental problems. In this sense, biomass-derived solvents are emerging as very promising alternatives, such as 2-methyl-THF and glycerol. In particularly, glycerol is drawing increasing interest of the scientific community as a promising cheap and environmentally friendly sustainable reaction medium [7–11]. Indeed, bio-renewable glycerol is highly hydrophilic, safe, non-toxic, non-flammable, low volatile, cheap (0.5$ Kg^{-1}), and available on a large scale from biomass (hydrolysis of vegetable oils, production of glycerol = 2.0 Mt in 2016). In additional, glycerol is recyclable, biodegradable, and compatible with most of the organic and inorganic compounds, and it also does not require special handling or storage with high boiling point. Hence, glycerol fully exhibits its promise as a sustainable solvent for green organic reactions with new optimality principles for economic efficiency.

On the other hand, the crucial points in realizing a catalytic green synthetic process involve the following: (i) atom economy should be maximized; (ii) process with energy efficiency, especially synthetic methods should be conducted at ambient temperature; (iii) reactions should proceed in high yields with selective and specific processes; (iv) the isolation of the resulting products should be straightforward, and; (v) the catalyst immobilized in green solvent system should be recycled. Such a catalytic green synthetic process would be a sustainable approach with high levels of technical efficiency. Currently, the search for sustainable and practical organic synthesis methodology is a highly topical subject which would contribute to develop recycling economy and save resources [12–15].

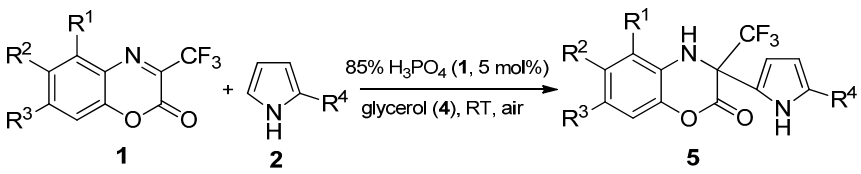
In this paper, we report a highly efficient and green aza-Friedel–Crafts reaction catalyzed by cheap phosphoric acid immobilized in bio-renewable glycerol for the synthesis of pyrrolyl and trifluoromethyl dihydrobenzoxazinones, as well as their biological evaluations. The following features of this practical methodology are remarkable: (i) this is the first example of an aza-Friedel–Crafts reaction performed in sustainable glycerol; (ii) the reactions proceed at room temperature under air atmosphere and metal-free conditions; (iii) 100% atom economy, easy product separation and catalyst recycling; (iv) some products exhibit impressive antitumor activity.

2. Materials and Methods

2.1. General Information

All the reagents used were of analytical grade without further purification. ^{1}H NMR (Nuclear Magnetic Resonance), and ^{13}C NMR spectra were measured at 400, 100 MHz spectrometer, respectively. The shifts were reported relative to internal standard Tetramethylsilane (TMS, 0) in $CDCl_3$. Data are reported as: multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant in hertz (Hz) and signal area integration in natural numbers. High Resolution Mass Spectrometer (HRMS) were obtained using EI ionization. Benzoxazinone derivatives and 2-arylpyrroles were obtained following the literatures [16,17].

2.2. General Sustainable Procedure for Environmentally Friendly and Atom Economical Synthesis of Dihydrobenzoxazinones



To a solution of cheap catalyst **3** (phosphoric acid 85%, 0.1 mmol) in sustainable biomass-derived solvent **4** (glycerol, 4 mL) was added benzoxazinone **1** (2 mmol) and pyrrole **2** (2 mmol). The resulting mixture was stirred at room temperature under air atmosphere for 24 h (complete consumption of starting materials was observed by Thin Layer chromatography (TLC)). The reaction mixture was extracted with a biomass-derived solvent (2-methyltetrahydrofuran, 3 × 4 mL) and the retained glycerol phase with phosphoric acid was reused. The organic solvent (2-methyltetrahydrofuran) was recovered by distillation to give the solid residue. The resulting solid was washed with cold water (5 mL), filtered, and dried under vacuum to give the crude product **3** which is reasonably pure (>95% purity by ^{1}H NMR). However, silica gel column chromatography (petroleum ether/ethyl acetate = 10:1) provided the analytically pure isolated product. To the retained glycerol layer, the substrates were again added, and the mixture was stirred under the same conditions for the required time. This procedure was repeated up to 10 consecutive times. Characterization data of products is listed below:

3-(1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5a**): 553 mg isolated product (558 mg crude product), m.p. 104–105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.76 (s, 1H), 7.14–7.04 (m, 1H), 6.99 (d, *J* = 7.6 Hz, 1H), 6.94 (dd, *J* = 7.9, 1.1 Hz, 1H), 6.91–6.83 (m, 2H), 6.28 (m, 1H), 6.16 (dd, *J* = 6.1, 2.8 Hz, 1H), 4.74 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.01, 139.33, 128.84, 125.95, 122.92 (q, *J* = 285.1 Hz), 121.32, 121.08, 120.88, 116.85, 114.92, 109.55, 108.94, 63.14 (q, *J* = 29.2 Hz); HRMS (EI-TOF): calculated for $C_{13}H_9F_3N_2O_2$ 282.0616, found 282.0614.

6-Fluoro-3-(1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5b**): 582 mg isolated product (582 mg crude product), m.p. 123–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.77 (s, 1H), 6.97–6.92 (m, 1H), 6.91–6.86 (m, 1H), 6.72–6.63 (m, 1H), 6.62–6.52 (m, 1H), 6.33–6.26 (m, 1H), 6.21–6.15 (m, 1H), 4.87 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.09 (d, *J* = 243 Hz), 159.65,135.38 (d, *J* = 3 Hz), 129.95 (*J* = 11 Hz), 122.79 (q, *J* = 285.0 Hz), 121.57, 120.50, 117.92 (d, *J* = 10.1 Hz), 110.01, 108.99, 107.43 (d, *J* = 24.0 Hz), 102.13 (d, *J* = 28.1 Hz), 62.51 (q, *J* = 29.2 Hz); HRMS (EI-TOF): calculated for $C_{13}H_8F_4N_2O_2$ 300.0522, found 300.0525.

6-Chloro-3-(1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5c**): 608 mg isolated product (614 mg crude product), m.p. 131–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.76 (s, 1H), 6.95 (d, *J* = 4 Hz, 1H), 6.94–6.87 (m, 2H), 6.88–6.83 (m, 1H), 6.32–6.25 (m, 1H), 6.23–6.14 (m, 1H), 4.82 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ= 159.43, 137.82, 131.01, 129.84, 122.76 (q, *J* = 285.2 Hz), 121.60, 120.94, 120.42, 117.90, 114.78, 110.07, 109.04, 62.55 (q, *J* = 296.1 Hz); HRMS (EI-TOF): calculated for $C_{13}H_8ClF_3N_2O_2$ 316.0226, found 316.0225.

7-Bromo-3-(1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5d**): 671 mg isolated product (686 mg crude product), m.p. 127–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.72 (s, 1H), 7.24–7.17 (m, 1H), 7.14 (d, *J* = 2.0 Hz, 1H), 6.93–6.86 (m, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 6.29–6.23 (m, 1H), 6.21–6.14 (m, 1H), 4.74 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 159.31, 139.67, 128.78, 128.13, 122.74 (q, *J* = 285 Hz), 121.60, 120.38, 119.98, 116.08, 112.37, 110.07, 109.05, 62.68 (q, *J* = 28.9 Hz); HRMS (EI-TOF): calculated for $C_{13}H_8BrF_3N_2O_2$ 359.9721, found 359.9721.

6-Methyl-3-(1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5e**): 574 mg isolated product (586 mg crude product), m.p. 114–115 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.76 (s, 1H), 6.90–6.84 (m, 2H), 6.74 (d, *J* = 1.2 Hz, 1H), 6.70–6.64 (m, 1H), 6.33–6.25 (m, 1H), 6.19–6.13 (m, 1H), 4.66 (s, 1H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 160.16, 137.33, 135.94, 128.43, 122.94 (q, *J* = 285 Hz), 121.64, 121.24, 121.01, 116.49, 115.33, 109.89, 108.90, 62.82 (q, *J* = 29 Hz), 20.97; HRMS (EI-TOF): calculated for $C_{14}H_{11}F_3N_2O_2$ 296.0773, found 296.0774.

7-Methyl-3-(1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5f**): 550 mg isolated product (562 mg crude product), m.p. 133–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.73 (s, 1H), 6.93–6.76 (m, 4H), 6.31–6.25 (m, 1H), 6.21–6.11 (m, 1H), 4.59 (s, 1H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 160.25, 139.32, 131.16, 126.42, 126.25, 122.94 (q, *J* = 285.1 Hz), 121.20, 120.93, 117.19,

114.89, 109.94, 108.93, 62.94 (q, *J* = 29.3 Hz), 20.55; HRMS (EI-TOF): calculated for $C_{14}H_{11}F_3N_2O_2$ 296.0773, found 296.0774.

5-Methyl-3-(1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5g**): 556 mg isolated product (556 mg crude product), m.p. 97–99 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.76 (s, 1H), 6.96 (d, *J* = 8 Hz, 1H), 6.91–6.84 (m, 2H), 6.83–6.76 (m, 1H), 6.22–6.16 (m, 1H), 6.18–6.14 (m, 1H), 4.52 (s, 1H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 160.09, 139.31, 126.11 (q, *J* = 248.2 Hz), 127.12, 126.94, 123.05, 121.43, 120.96, 120.51, 114.71, 109.39, 108.93, 62.90 (q, *J* = 291.0 Hz), 16.42; HRMS (EI-TOF): calculated for $C_{14}H_{11}F_3N_2O_2$ 296.0773, found 296.0773.

3-(5-Phenyl-1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5h**): 673 mg isolated product (687 mg crude product), m.p. 111–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.84 (s, 1H), 7.49–7.41 (m, 2H), 7.39–7.36 (m 2H), 7.29–7.22 (m, 1H), 7.14–7.06 (m, 1H), 7.05–6.98 (m, 1H), 6.98–6.94 (m, 1H), 6.93–6.85 (m, 1H), 6.43–6.38 (m, 1H), 6.36–6.31 (m, 1H), 4.74 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 159.98, 139.34, 135.66, 131.54, 128.98, 128.77, 127.29, 126.03, 124.30, 122.69 (q, *J* = 244.1 Hz), 121.51, 121.18, 116.92, 114.94, 111.59, 106.27, 62.90 (q, *J* = 29.4 Hz); HRMS (EI-TOF): calculated for $C_{19}H_{13}F_3N_2O_2$ 358.0929, found 358.0929.

6-Fluoro-3-(5-phenyl-1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5i**): 699 mg isolated product (714 mg crude product), m.p. 132–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.85 (s, 1H), 7.46 (d, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.7 Hz, 2H), 7.31–7.23 (m, 1H), 6.96 (dd, *J* = 8.9, 4.9 Hz, 1H), 6.69 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.65–6.53 (m, 1H), 6.45–6.39 (m, 1H), 6.37–6.31 (m, 1H), 4.85 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 160.32, 159.56, 158.90, 135.90, 135.39 (d, *J* = 2 Hz), 131.46, 129.85 (d, *J* = 109 Hz), 129.00, 127.38, 124.34, 121.12, 118.00 (d, *J* = 9.9 Hz), 111.61, 107.54 (d, *J* = 24.1 Hz), 106.30, 102.14 (d, *J* = 27.6 Hz), 62.51 (q, *J* = 29.2 Hz); HRMS (EI-TOF): calculated for $C_{19}H_{12}F_4N_2O_2$ 376.0835, found 376.0830.

6-Chloro-3-(5-phenyl-1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5j**): 746 mg isolated product (754 mg crude product), m.p. 159–160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.88 (s, 1H), 7.46 (dd, *J* = 8.2, 1.1 Hz, 2H), 7.42–7.35 (m, 2H), 7.29–7.23 (m, 1H), 6.99–6.92 (m, 2H), 6.85 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.42 (dd, *J* = 3.7, 2.8 Hz, 1H), 6.33 (dd, *J* = 3.7, 2.8 Hz, 1H), 4.89 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 159.40, 137.84, 135.94, 131.46, 131.06, 129.83, 128.99, 127.38, 124.34, 122.76 (q, *J* = 285.1 Hz), 121.02, 120.98, 117.95, 114.79, 111.71, 106.32, 62.62 (q, *J* = 29.1 Hz); HRMS (EI-TOF): calculated for $C_{19}H_{12}ClF_3N_2O_2$ 392.0539, found 392.0536.

6-Methyl-3-(5-phenyl-1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5k**): 714 mg isolated product (723 mg crude product), m.p. 125–126 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.88 (s, 1H), 7.45 (d, *J* = 7.4 Hz, 2H), 7.37 (d, *J* = 7.7 Hz, 2H), 7.29–7.23 (m, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.75 (s, 1H), 6.68 (d, *J* = 8.2 Hz, 1H), 6.44–6.38 (m, 1H), 6.37–6.31 (m, 1H), 4.71 (s, 1H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 160.17, 137.40, 136.04, 135.62, 131.63, 128.99, 128.44, 127.27, 124.33, 122.98 (q, *J* = 285.1 Hz), 121.76, 121.71, 116.59, 115.38, 111.59, 106.29, 62.93 (q, *J* = 29.2 Hz), 21.02; HRMS (EI-TOF): calculated for $C_{20}H_{15}F_3N_2O_2$ 372.1086, found 372.1083.

7-Methyl-3-(5-phenyl-1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5l**): 707 mg isolated product (722 mg crude product), m.p. 137–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.84 (s, 1H), 7.48–7.41 (m, 2H), 7.41–7.34 (m, 2H), 7.29–7.23 (m, 1H), 6.94–6.79 (m, 3H), 6.40 (t, *J* = 3.2 Hz, 1H), 6.32 (t, *J* = 3.2 Hz, 1H), 4.64 (s, 1H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 160.22,139.36,135.53, 131.58, 131.28, 128.97, 127.25, 126.48, 126.18, 124.29, 122.77 (q, *J* = 254.5 Hz), 121.59, 117.27, 114.90, 111.56, 106.29, 62.98 (q, *J* = 29.5 Hz), 20.58; HRMS (EI-TOF): calculated for $C_{20}H_{15}F_3N_2O_2$ 372.1086, found 372.1089.

3-(5-(4-Chlorophenyl)-1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5m**): 761 mg isolated product (769 mg crude product), m.p. 139–140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.82 (s, 1H), 7.40–7.32 (m, 4H), 7.14–7.06 (m, 1H), 7.01 (dd, *J* = 8.1, 1.2 Hz, 1H), 6.96 (dd, *J* = 7.9,

1.4 Hz, 1H), 6.94–6.87 (m, 1H), 6.42–6.37 (m, 1H), 6.36–6.31 (m, 1H), 4.73 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 159.96, 139.33, 134.51, 132.98, 130.07, 129.15, 128.68, 126.07, 125.52, 124.70 (q, *J* = 81.2 Hz), 122.00, 121.22, 116.93, 114.93, 111.70, 106.71, 62.87 (q, *J* = 29.3 Hz); HRMS (EI-TOF): calculated for $C_{19}H_{12}ClF_3N_2O_2$ 392.0539, found 392.0535.

3-(5-(4-Chlorophenyl)-1H-pyrrol-2-yl)-6-fluoro-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5n**): 763 mg isolated product (788 mg crude product), m.p. 171–172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.84 (s, 1H), 7.40–7.33 (m, 4H), 7.00–6.93 (m, 1H), 6.73–6.67 (m, 1H), 6.63–6.55 (m, 1H), 6.40 (dd, *J* = 3.7, 2.8 Hz, 1H), 6.34 (dd, *J* = 3.8, 2.7 Hz, 1H), 4.86 (s, 1H); ^{13}C NMR(100 MHz, $CDCl_3$) δ = 160.14 (d, *J* = 242.7 Hz), 159.58, 135.37, 134.76, 133.06, 129.96, 129.75 (d, *J* = 11.3 Hz), 129.16, 125.54, 124.64 (q, *J* = 92 Hz), 121.56, 118.02 (d, *J* = 91 Hz), 111.73, 107.60 (d, *J* = 23.6 Hz), 106.72, 102.14 (d, *J* = 27.9 Hz), 62.49 (q, *J* = 29.3 Hz); HRMS (EI-TOF): calculated for $C_{19}H_{11}ClF_4N_2O_2$ 410.0445, found 410.0447.

3-(5-(4-Chlorophenyl)-1H-pyrrol-2-yl)-6-methyl-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5o**): 764 mg isolated product (773 mg crude product), m.p.106–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.87 (s, 1H), 7.41–7.31 (m, 4H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.76 (d, *J* = 1.2 Hz, 1H), 6.68 (dd, *J* = 8.2, 1.2 Hz, 1H), 6.43–6.37 (m, 1H), 6.34 (dd, *J* = 3.7, 2.7 Hz, 1H), 4.67 (d, *J* = 24.0 Hz, 1H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 160.16, 137.36, 136.10, 134.48, 132.95, 130.14, 129.16, 128.35, 125.55, 123.66 (q, *J* = 295 Hz), 121.81, 116.60, 115.36, 111.70, 106.97, 106.72, 62.89 (q, *J* = 29 Hz), 21.03; HRMS (EI-TOF): calculated for $C_{20}H_{14}ClF_3N_2O_2$ 406.0696, found 406.0701.

3-(5-(4-Chlorophenyl)-1H-pyrrol-2-yl)-7-methyl-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5p**): 764 mg isolated product (772 mg crude product), m.p.145–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.83 (s, 1H), 7.41–7.33 (m, 4H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.87–6.80 (m, 2H), 6.41–6.38 (m, 1H), 6.35–6.31 (m, 1H), 4.63 (s, 1H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 160.23, 139.36, 134.41, 132.95, 131.37, 130.14, 129.16, 126.56, 126.12, 125.54, 122.11, 122.93 (q, *J* = 285.1 Hz), 117.29, 114.92, 111.72, 106.75, 62.99 (q, *J* = 29.2 Hz), 20.60; HRMS (EI-TOF): calculated for $C_{20}H_{14}ClF_3N_2O_2$ 406.0696, found 406.0696.

3-(5-(p-Tolyl)-1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5q**): 714 mg isolated product (744 mg crude product), m.p. 127–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.80 (s, 1H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.18 (d, *J* = 8.0 Hz, 2H), 7.13–7.07 (m, 1H), 7.00 (dd, *J* = 8.1, 1.2 Hz, 1H), 6.95 (dd, *J* = 7.9, 1.3 Hz, 1H), 6.92–6.86 (m, 1H), 6.37–6.34 (m, 1H), 6.34–6.29 (m, 1H), 4.75 (s, 1H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 160.01, 139.34, 137.14, 135.84, 129.62, 128.84, 128.80, 125.99, 124.25, 122.90 (q, *J* = 285.1 Hz), 121.13, 121.05, 116.89, 114.93, 111.54, 105.76, 61.42 (q, *J* = 29.4 Hz), 21.15; HRMS (EI-TOF): calculated for $C_{20}H_{15}F_3N_2O_2$ 372.1086, found 372.1085.

6-Fluoro-3-(5-(p-tolyl)-1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5r**): 764 mg isolated product (772 mg crude product), m.p.177–178 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.79 (s, 1H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.19 (d, *J* = 7.9 Hz, 2H), 6.99–6.94 (m, 1H), 6.69 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.63–6.55 (m, 1H), 6.40–6.35 (m, 1H), 6.34–6.28 (m, 1H), 4.83 (s, 1H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 160.11 (d, *J* = 242.8 Hz), 159.58, 137.27, 136.08, 135.38, 129.89 (d, *J* = 11.3 Hz), 129.66, 128.70, 124.28, 122.62 (q, *J* = 253.3 Hz), 120.65, 118.00 (d, *J* = 9.9 Hz), 111.53, 107.51 (d, *J* = 23.8 Hz), 105.78, 102.13 (d, *J* = 27.5 Hz), 62.52 (q, *J* = 29.6 Hz), 21.16; HRMS (EI-TOF): calculated for $C_{20}H_{14}F_4N_2O_2$ 390.0991, found 390.0995.

7-Methyl-3-(5-(p-tolyl)-1H-pyrrol-2-yl)-3-(trifluoromethyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-one (**5s**): 749 mg isolated product (773 mg crude product), m.p.150–151 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ =8.78 (s, 1H), 7.36–7.32 (m, 2H), 7.18 (d, *J* = 7.9 Hz, 2H), 6.93–6.78 (m, 3H), 6.38–6.32 (m, 1H), 6.32–6.28 (m, 1H), 4.64 (s, 1H), 2.35 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 160.25, 139.33, 137.09, 135.70, 131.21, 129.62, 128.83, 126.46, 126.25, 124.22, 122.93 (q, *J* = 284.5 Hz), 121.11, 117.23, 114.90, 111.53, 105.77, 63.01 (q, *J* = 29.1 Hz), 21.14, 20.56; HRMS (EI-TOF): calculated for $C_{21}H_{17}F_3N_2O_2$ 386.1242, found 386.1241.

2.3. Method for Biological Activity Study

The sodium MMT was purified at Ningcheng, Inner Mongolia, China. Metronidazole (MTZ). Cell Culture: All cell lines used in this study were purchased from ATCC. Rat glioma cell line C6 was cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS). Murine melanoma cell line B16BL6 was cultures in RPMI 1640 culture medium supplemented with 10% fetal bovine serum (FBS). Cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2.

Compound Treatment and MTT assay: The cytotoxicity of the compound was evaluated by MTT assay using C6 and B16BL5 cells. Generally, the cells were seeded in a 96-well tissue culture plate at a density of 1×10^4 cells/well in 0.2 mL 1640 or DMEM medium containing 10% serum for 18 h, separately. The medium was replaced with 0.2 mL serum-free one containing 1% DMSO and serial dilutions of compound solutions for 24 h. Then, the solutions were replaced with 0.1 mL serum-free media containing 0.5 mg/mL MTT and incubated for another 4 h. Finally, each well was replaced with 0.1 mL DMSO and measured spectrophotometrically in an ELISA plate reader (Model 550, Bio-Rad) at a wavelength 570 nm. The relative cell growth (%) related to control cells cultured in media without compound was calculated by the formula below:

$$V\% = ([A]_{experimental} - [A]_{blank})/([A]_{control} - [A]_{blank}) \times 100\%$$

3. Results and Discussion

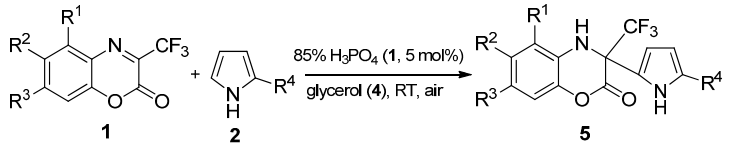
3.1. Sustainable and Practical Methodology

We designed phosphoric acid immobilized in glycerol as a catalytic system for aza-Friedel–Crafts reactions. The reaction was performed in the presence of 5 mol% phosphoric acid in sustainable biomass-derived solvent (glycerol), using benzoxazinone 1a and pyrrole 2a as substrates. The reaction was completed at room temperature under air atmosphere for 12 h, which was observed by TLC. The reaction mixture was extracted with a biomass-derived solvent (2-methyltetrahydrofuran), and 2-methyltetrahydrofuran was recovered by distillation to give the desired product 3a with simple workup and quantitative yield. The product 5a is confirmed by ^{1}H NMR, ^{13}C NMR and HRMS.

As summarized in Table 1, we explored the efficiency and generality of our methodology to various substrates. We first investigated the effect of the substituents on the benzoxazinone 1 by examining their reactions with pyrrole (2a). The reaction works well with a range of trifluoromethyl benzoxazinones (1a–g), containing both electron-withdrawing groups (F, Cl and Br) and electron donating (Me) to provide the corresponding products (5b–g) in excellent yields, showing no sensitivity to electronic effects (entries 2–7, 93–97% yield; Table 1).

Next, we examined the reactions of trifluoromethyl benzoxazinone 1 with 2-phenylpyrrole (2b). In all cases, the corresponding pyrrolyl and trifluoromethyl dihydrobenzoxazinones (5h–l) were obtained in high yields (entries 8–12, 93–96% yield; Table 1). In addition, we found that 2-arylpyrroles (2c and 2d) bearing either an electron-withdrawing group (Cl) or electron-donating group (Me) were also well tolerated to give the desired products with satisfactory yields (entries 13–19, 92–97% yield; Table 1).

Table 1. Sustainable approach for synthesis of dihydrobenzoxazinones in glycerol.[a]



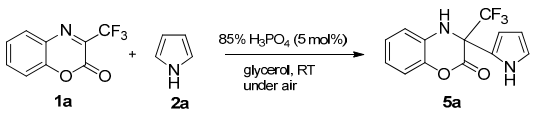
Entry	1	R^1	R^2	R^3	2	R^4	Product	Crude Yield (%)	Isolated Yield (%)
1	1a	H	H	H	2a	H	5a	99	98
2	1b	H	F	H	2a	H	5b	97	97
3	1c	H	Cl	H	2a	H	5c	97	96
4	1d	H	H	Br	2a	H	5d	95	93
5	1e	H	Me	H	2a	H	5e	99	97
6	1f	H	H	Me	2a	H	5f	95	93
7	1g	Me	H	H	2a	H	5g	94	94
8	1a	H	H	H	2b	Ph	5h	96	94
9	1b	H	F	H	2b	Ph	5i	95	93
10	1c	H	Cl	H	2b	Ph	5j	96	95
11	1e	H	Me	H	2b	Ph	5k	97	96
12	1f	H	H	Me	2b	Ph	5l	97	95
13	1a	H	H	H	2c	*p*-ClC_6H_4	5m	98	95
14	1b	H	F	H	2c	*p*-ClC_6H_4	5n	96	93
15	1e	H	Me	H	2c	*p*-ClC_6H_4	5o	95	94
16	1f	H	H	Me	2c	*p*-ClC_6H_4	5p	95	92
17	1a	H	H	H	2d	*p*-MeC_6H_4	5q	99	95
18	1b	H	F	H	2d	*p*-MeC_6H_4	5r	98	97
19	1f	H	H	Me	2d	*p*-MeC_6H_4	5s	99	96

[a] Reactions were performed with 1 (2 mmol), 2 (2 mmol) and phosphoric acid 85% (0.1 mmol) in glycerol (4 mL) under air at room temperature (RT) for 24 h.

3.2. The Catalytic System Recycling

As is well known, some very important advantages in metal-catalyzed organic reactions with the use of glycerol as a solvent are the separation of the product by a simple extraction with solvents, the possibility of recycling the catalytic system, the lifetime, and its level of reusability of the catalytic system [18]. We designed phosphoric acid immobilized in glycerol as a recoverable and reusable catalytic system for aza-Friedel–Crafts reactions. After the reaction was completed, the reaction mixture was extracted with a biomass-derived solvent (2-methyltetrahydrofuran) and the retained glycerol phase with phosphoric acid was reused. To the retained glycerol layer, the substrates were again added, and the mixture was stirred under the same conditions for the required time. We have found that this procedure could be repeated up to 10 consecutive times, as shown in Table 2. Thus, no loss of activity occurs in the glycerol during the 10 consecutive runs. Quantitative conversion and excellent yield with high efficiency of the catalytic system were achieved after the tenth cycle.

Table 2. Catalytic system recycling.[a]



Cycle	Time (h)	Crude yield	Cycle	Time (h)	Crude Yield
1	24	99	6	24	97
2	24	98	7	24	98
3	24	99	8	24	99
4	24	99	9	24	98
5	24	98	10	24	99 (96) [b]

[a] Reactions were performed under air at room temperature, using phosphoric acid 85% (0.1 mmol) in glycerol (4 mL), and 2 mmol of 1a and 2a was always employed. [b] Data in parentheses was isolated yield.

3.3. Biological Activity Study

A variety of dihydrocoumarins is a class of pharmacologically important compounds and has frequently been found in natural sources, among which there are inhibitors of cancer [19]. The pyrrole structure is present in clinical trial drugs, such as Atorvastatin [20]. The trifluoromethylated target molecules have shown a great diversity of superior biological properties, mainly due to improved chemical and metabolic stability, lipophilicity, and the membrane permeability of the parent molecules [21]. Thus, dihydrobenzoxazinone derivatives with pyrrolyl and trifluoromethyl groups are interesting targets for medicinal chemistry research. We were intrigued by the possible potential biological activity of these pyrrolyl and trifluoromethyl dihydrobenzoxazinones, which may be considered as 4-aza dihydrocoumarins. To the best of our knowledge, efficient and sustainable synthesis of these molecules has only been rarely reported [22], and no biological activity test is investigated.

Glioblastoma is one type of tumor with a high proliferative potential and poor prognosis [23]. The development of new treatment strategies is essential for the treatment of this disease. Malignant melanoma is the most dangerous form of skin cancer due to the rising incidence and lack of effective treatments [24], and the search for novel melanoma-specific agents is urgently needed. Therefore, we evaluated the preliminary biological activity of our products as anti-glioma or anti- melanoma agents. The cytotoxicities of pyrrolyl and trifluoromethyl dihydrobenzoxazinones (5a–s) in glioma (C6) and melanoma (B16BL6) tumor cells were tested by using adriamycin as a positive control with the MTT assay. The results are summarized in Table 3. Interestingly, these compounds showed different effectivities in inhibiting the proliferation of the tumor cells. Compounds 5a and 5e displayed high selectivity for inhibition of B16BL6 and C6, respectively. The antiproliferative activity against C6 and B16BL6 tumor cell lines of compounds (5m–p) was clearly increased when compared to adriamycin, and 5s showed similar cytotoxicity in both tumor cells to adriamycin. Compounds (5m–p) possess the same 2-(4-chlorophenyl) pyrrole scaffold structure which thus appears to be an important structural determinant for antiproliferative activity. Notably, compound 5n showed impressive cytotoxicity in both tumor cells with IC_{50} values in the low micromolar range. The cell viability upon treatment with 5n at different concentration using adriamycin as a positive control is shown in Figure 1. The results suggest that compound 5n is a promising lead compound for further design and synthesis of inhibitor of glioma and melanoma growth.

Table 3. Cytotoxicities in glioma (C6) and melanoma (B16BL6) cells.

Compound	IC_{50} (μM) [a]		Compound	IC_{50} (μM) [a]	
	C6	B16BL6		C6	B16BL6
5a	n.a.	92.4	5k	46.4	58.1
5b	169.1	154.1	5l	39.5	41.6
5c	115.6	n.a.	5m	15.4	63.2
5d	232.5	n.a.	5n	8.9	29.8
5e	20.9	n.a.	5o	18.7	24.2
5f	81.0	100.2	5p	19.4	25.7
5g	149.2	70.9	5q	42.3	68.4
5h	127.7	193.3	5r	44.2	37.4
5i	58.3	75.7	5s	19.6	90.9
5j	42.2	94.3	adriamycin	18.8	96.7

[a] IC_{50} values are reported as a mean value of three determinations. n.a. = not active up to a high concentration.

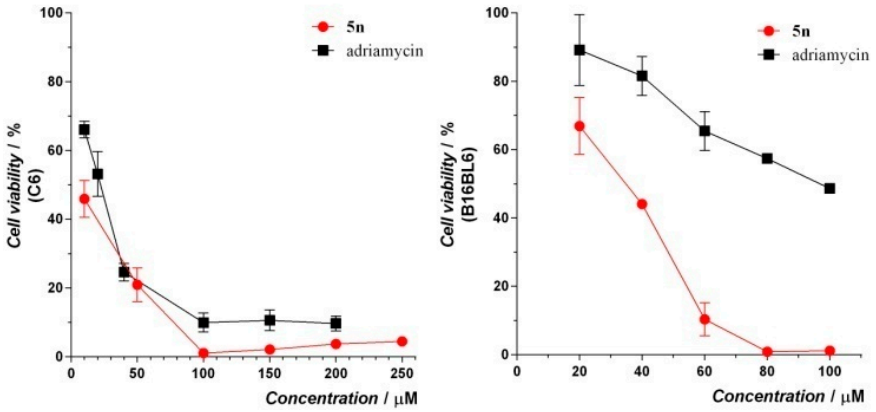


Figure 1. Viability of C6 (**left**) and B16BL6 (**right**) cells upon treatment with 5n using adriamycin as a positive control.

4. Conclusions

A highly efficient and green atom economical aza-Friedel–Crafts reaction catalyzed by phosphoric acid immobilized in glycerol has been developed and shows high levels of technical efficiency. The features of this protocol are the following: glycerol is a cheap, safe and sustainable solvent; there is easy product separation and catalytic system recycling under mild conditions, and it provides a sustainable approach for preparation of pyrrolyl and trifluoromethyl dihydrobenzoxazinones with excellent yields; and it exhibits new optimality principles for economic efficiency. Significantly, the dihydrobenzoxazinone products exhibit impressive cytotoxicity against C6 and B16BL6 tumor cell lines, and product 5n is a promising lead compound for further design and synthesis of inhibitor of glioma and melanoma growth.

Acknowledgments: This work is supported by Zhejiang Social Science Planning Zhi Jiang Youth Project. We thank H. Lou for contributed starting materials and analysis methods, E. Jin for assistance with the cytotoxicity assays, and X. Lin for assistance with designing the research, analyzing the data and performing the experiments.

Author Contributions: Lan Tan conceived and directed the project; Abdul Rahman performed the experiments and the characterizations. Both authors co-wrote the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Trost, B.M. The atom economy—A search for synthetic efficiency. *Science* **1991**, *254*, 1471–1477. [CrossRef] [PubMed]
2. Tang, S.L.Y.; Smith, R.L.; Poliakoff, M. Principles of green chemistry: Productively. *Green Chem.* **2005**, *7*, 761–762. [CrossRef]
3. Bandini, M.; Melloni, A.; Umani-Ronchi, A. New catalytic approaches in the stereoselective Friedel–Crafts alkylation reaction. *Angew. Chem. Int. Ed.* **2004**, *43*, 550–556. [CrossRef] [PubMed]
4. Poulsen, T.B.; Jorgensen, K.A. Catalytic asymmetric Friedel−Crafts alkylation reactions—Copper showed the way. *Chem. Rev.* **2008**, *108*, 2903–2915. [CrossRef] [PubMed]
5. You, S.L.; Cai, Q.; Zeng, M. Chiral Brønsted acid catalyzed Friedel–Crafts alkylation reactions. *Chem. Soc. Rev.* **2009**, *38*, 2190–2201. [CrossRef] [PubMed]
6. Cioc, R.C.; Ruijter, E.; Orru, R.V.A. Multicomponent reactions: advanced tools for sustainable organic synthesis. *Green Chem.* **2014**, *16*, 2958–2975. [CrossRef]
7. Gu, Y.; Jérôme, F. Glycerol as a sustainable solvent for green chemistry. *Green Chem.* **2010**, *12*, 1127–1138. [CrossRef]
8. Gu, Y.; Barrault, J.; Jérôme, F. Glycerol as an efficient promoting medium for organic reactions. *Adv. Synth. Catal.* **2008**, *350*, 2007–2012. [CrossRef]

9. Tagliapietra, S.; Orio, L.; Palmisano, G.; Penoni, A.; Gravotto, G. Catalysis in glycerol: a survey of recent advances. *Chem. Pap.* **2015**, *69*, 1519–1531. [CrossRef]
10. Wolfson, A.; Dlugy, C.; Shotland, Y. Glycerol as a green solvent for high product yields and selectivities. *Env. Chem. Lett.* **2007**, *5*, 67–71. [CrossRef]
11. Ying, A.; Zhang, Q.; Li, H.; Shen, G.; Gong, W.; He, M. An environmentally benign protocol: Catalyst-free Michael addition of aromatic amines to α,β-unsaturated ketones in glycerol. *Res. Chem. Inter.* **2013**, *39*, 517–525. [CrossRef]
12. Anastas, P.T.; Warner, J.C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, NY, USA, 1998.
13. Laird, T. Green chemistry is good process chemistry. *Org. Process. Res. Dev.* **2012**, *16*, 1–2. [CrossRef]
14. Dunn, P.J. The importance of green chemistry in process research and development. *Chem. Soc. Rev.* **2012**, *41*, 1452–1461. [CrossRef] [PubMed]
15. Augé, J.; Scherrmann, M.C. Determination of the global material economy (GME) of synthesis sequences—A green chemistry metric to evaluate the greenness of products. *New J. Chem.* **2012**, *36*, 1091–1098. [CrossRef]
16. Mohamed El-said, M.; Akio, T.; Nobuo, I. Trifluoropyruvic acid hydrate in heterocyclic synthesis, part II: Synthesis of trifluomethylated benzoxazine, benzothiazine, and benzoxazole derivatives. *Heterocycles* **1986**, *24*, 593–599.
17. Wen, J.; Zhang, R.; Chen, S.; Zhang, J.; Yu, X. Direct arylation of arene and N-heteroarenes with diaryliodonium salts without the use of transition metal catalyst. *J. Org. Chem.* **2012**, *77*, 766–771. [CrossRef] [PubMed]
18. Vidal, C.; García-Álvarez, J. Glycerol: a biorenewable solvent for base-free Cu(I)-catalyzed 1,3-dipolar cycloaddition of azides with terminal and 1-iodoalkynes. Highly efficient transformations and catalyst recycling. *Green Chem.* **2014**, *16*, 3515–3521. [CrossRef]
19. Wu, P.L.; Hsu, Y.L.; Zao, C.W.; Damu, A.G.; Wu, T.S. Constituents of vittaria anguste-elongata and their biological activities. *J. Nat. Prod.* **2005**, *68*, 1180–1184. [CrossRef] [PubMed]
20. Bellina, F.; Rossi, R. Synthesis and biological activity of pyrrole, pyrroline and pyrrolidine derivatives with two aryl groups on adjacent positions. *Tetrahedron* **2006**, *62*, 7213–7256. [CrossRef]
21. Múüller, K.; Faeh, C.; Diederich, F. Fluorine in pharmaceuticals: Looking beyond intuition. *Science* **2007**, *317*, 1881–1886. [CrossRef] [PubMed]
22. Lou, H.; Wang, Y.; Jin, E.; Lin, X. Organocatalytic asymmetric synthesis of dihydrobenzoxazinones bearing trifluoromethylated quaternary stereocenters. *J. Org. Chem.* **2016**, *81*, 2019–2026. [CrossRef] [PubMed]
23. Rohle, D.; Popovici-Muller, J.; Palaskas, N.; Turcan, S.; Grommes, C.; Campos, C.; Tsoi, J.; Clark, O.; Oldrini, B.; Komisopoulou, E.; et al. An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science* **2013**, *340*, 626–630. [CrossRef] [PubMed]
24. García-ÁA, I.; Corrales, G.; Doncel-Pérez, E.; Muñoz, A.; Nieto-Sampedro, M.; Fernández-Mayoralas, A. Design and synthesis of glycoside inhibitors of glioma and melanoma growth. *J. Med. Chem.* **2007**, *50*, 364–373.