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Communication

A Concise Synthesis towards Antimalarial Quinazolinedione TCMDC-125133 and Its Anti-Proliferative Activity against MCF-7

Sitthivut Charoensutthivarakul [1,2,3,*], Duangporn Lohawittayanan [1], Phongthon Kanjanasirirat [2], Kedchin Jearawuttanakul [2], Sawinee Seemakhan [2], Suparerk Borwornpinyo [2,4] and Matthew Phanchana [5]

1 Innovative Molecular Discovery Laboratory (iMoD), School of Bioinnovation and Bio-Based Product Intelligence, Faculty of Science, Mahidol University, Bangkok 10400, Thailand; duangporn.loha@gmail.com
2 Excellent Center for Drug Discovery (ECDD), Faculty of Science, Mahidol University, Bangkok 10400, Thailand; phongku1232@gmail.com (P.K.); o-nion@hotmail.com (K.J.); sawinee.ecdd@gmail.com (S.S.); bsuparerk@gmail.com (S.B.)
3 Center for Neuroscience, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
4 Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
5 Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand; matthew.pha@mahidol.edu
* Correspondence: sitthivut.cha@mahidol.ac.th; Tel.: +66-2201-5899

Abstract: Quinazolinedione is one of the most notable pharmacophores in drug discovery due to its broad spectrum of biological activities including antimalarial, anticancer, anti-inflammatory, and others. TCMDC-125133, whose structure features a quinazolinedione core, exhibits promising antimalarial activity and low toxicity as described in the GlaxoSmithKline (GSK) report. Herein, a concise four-step synthesis towards quinazolinedione TCMDC-125133 is described using low cost goods and greener alternatives where possible. All synthesized compounds were characterized using polarimetry, IR, NMR, and mass spectrometry. The in-house synthesized TCMDC-125133 was evaluated for its antimalarial activity against *P. falciparum* 3D7 and antiproliferative activity against MCF-7 cell line.

Keywords: quinazolinedione derivatives; antimalarial activity; antiproliferative activity

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1. Introduction

Quinazolinedione is a remarkable heterocycle which is widely used as a functional material for synthetic chemistry. It is also present in various active pharmaceutical molecules along with its diverse range of biological activities, including antimalarial, anticancer, antimicrobial, antihypertensive, antiviral, anti-inflammatory, and others [1–5]. The examples shown in Figure 1 are some representatives of commercial drugs and biologically active molecules with quinazolinedione moiety [6].

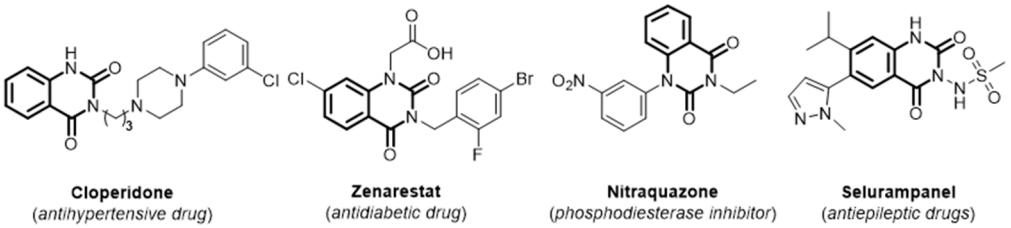
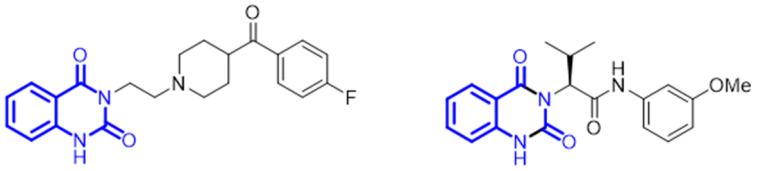


Figure 1. Examples of quinazolinedione-based drugs and its biological activity.

One of the interesting quinazolinedione-containing drugs is ketanserin, as displayed in Figure 2a. This drug is used clinically as an antihypertensive agent selectively targeting

at 5-HT_{2A} receptor, a subtype of the 5-HT_2 receptor that belongs to the serotonin receptor family (5-HT). Several studies have reported that serotonin was involved in the regulation of cell proliferation, survival, and metastasis [7]. Moreover, it is reported that 5-HT plays a mitogenic role and exerts its growth effect in human breast cancer cell line (MCF-7) [8]. Recently, Seyed H Hejazi and his group studied the pattern of serotonin receptor gene expression in MCF-7. Their result showed that the ketanserin had suppression effects on MCF-7 cell's proliferation of 72.36% at the ketanserin concentration of 25 μM [9]. In a preliminary drug repurposing study, ketanserin surprisingly possesses good antimalarial activity with the IC_{50} against multidrug-resistant *P. falciparum* K1 at around 10 μM [10].



(a) Ketanserin (b) TCMDC-125133

Figure 2. Chemical structures of (**a**) Ketanserin and (**b**) TCMDC-125133 with quinazolinedione core highlighted in blue.

In 2010, GlaxoSmithKline (GSK) published the Tres Cantos Antimalarial Set (TCAMS) containing 13,533 compounds that are the result of screening nearly 2 million compounds from the GSK corporate collection [11]. With the result openly available in the public domain, one of the compounds identified from the screen as a singleton is TCMDC-125133 whose structure featured a quinazolinedione core as displayed in Figure 2b. The preliminary in vitro antimalarial activity of this compound against asexual blood stage and gametocytes of *P. falciparum* 3D7 has shown excellent IC_{50}s at 0.27 and 0.54 μM, respectively [12]. This, combined with the drug-like property and low toxicity (cytotoxicity HG2; IC_{50} > 25 μM) of this compound, has made it an excellent target for further lead optimization.

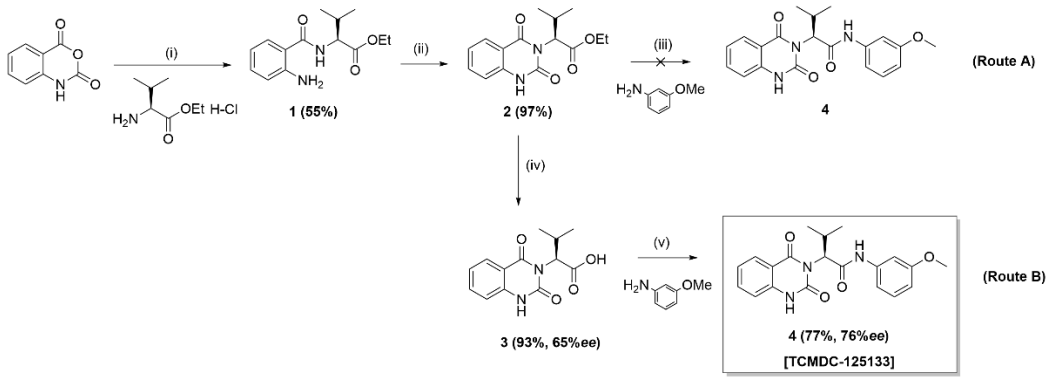
The main structural feature of these compounds is quinazolinedione; however, the synthesis of quinazolinedione core has been reported to achieve this in multiple steps using harsh conditions and tedious workup procedures with low yields [13,14], while there is no previous report on the synthesis towards TCMDC-125133. This present work, therefore, described a concise synthetic route towards the quinazolinedione TCMDC-125133 using commercially available starting materials with a low cost of goods. Several spectroscopic techniques were employed to confirm the structure of in-house synthesized TCMDC-125133 and the synthetic intermediates. The final compound was assayed for its antimalarial activity against 3D7 and for antiproliferative activity against MCF-7. The resulting synthetic route will be crucial for further structure-activity relationships (SARs) study in lead optimization and development of this class of compounds.

2. Results and Discussion

The synthesis towards the quinazolidinone TCMDC-125133 begins with the reaction between commercially available isatoic anhydride and corresponding L-valine ethyl ester hydrochloride in the presence of K_2CO_3 in CH_3CN to afford compound **1** (55%) [15]. This step involves the use of low-cost isatoic anhydride and a mild reaction condition with a capability to be performed at a multi-gram scale. Although the racemization of the α-carbon of the valine moiety could be of concern in this step, the working reagent and condition did not affect the stereochemistry of this optically active molecule as shown by its distinctive levorotatory property and a chiral HPLC trace (>99% *ee*). For the synthesis of compound **2**, the cyclocarbonylation reaction of compound **1** could be performed using various carbonylating reagents such as phosgene or its equivalent derivatives (i.e., ethyl chloroformate, diphosgene or triphosgene), however, those reagents are toxic and difficult to handle, and some require harsh conditions [6]. 1,1-carbonyldiimidazole (CDI) was chosen as a greener alternative for this reaction owing to its safety and ease of handling.

The resulting intermediate **1** was reacted with CDI in THF to yield the quinazolinedione **2** in 97% yield (>99% *ee*) [15].

As shown in Scheme 1 (Route A), it is proposed that the final product **4** (TCMDC-125133) could be made using a tert-butoxide-assisted amidation reaction [16], however, the reaction was not successful. An alternative synthesis was employed instead starting with hydrolysis of the ethyl ester followed by an amide formation as described in Scheme 1 (Route B). The ethyl ester **2** was subsequently hydrolyzed using LiOH in THF/H_2O mixture to give the carboxylic acid **3** without any purification (93%) [15]. It is worth noting that the levorotatory effect can still be observed in compound **3** suggesting the majority of product still retains its configuration (65% *ee* determined by chiral HPLC) (see Supplementary Materials). To generate the amide **4**, it is important to maintain that any harsh condition could affect the stereochemistry of the α-carbon of the valine moiety, hence, 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) is employed as it is one of the commonly used reagents in amide coupling chemistry. Although it is not 'low-cost' as originally proposed, HATU was chosen because of its mild condition, high coupling efficiencies, fast reaction rates, and its by-product can be easily removed [17]. In the final step, the corresponding acid **3** was then coupled to the m-anisidine side chain using HATU with the presence of triethylamine (Et_3N) in DMF [15] to afford the quinazolidinone **4** (TCMDC-125133) in 77% yield with 76% *ee* determined by chiral HPLC (see Supplementary Materials). All synthesized compounds described here were fully characterized using polarimetry, IR, NMR, and mass spectrometry. This newly discovered synthetic route will allow further structural modification around this pharmacophore core.



Scheme 1. Reagents and Conditions: (**i**) K_2CO_3, CH_3CN, 60 °C, 18 h; (**ii**) CDI, THF, 85 °C, 18 h; (**iii**) *m*-anisidine, *t*-BuOK, THF, room temperature, overnight; (**iv**) LiOH, THF/H_2O, 85 °C, 18 h; (**v**) *m*-anisidine, HATU, Et_3N, DMF, room temperature, 18 h.

Compound **4** derived from this synthesis was assayed for in vitro antimalarial activity against the blood stage *P. falciparum* 3D7 strain. The results show that the in-house synthesised compound **4** possesses a promising IC_{50} (3D7) of 219 nM (Figure 3) which is comparable to the primary screening result by TCAMS. Further lead optimisation is underway to enhance the antimalarial activity of this class of compound.

As previously mentioned, quinazolinedione containing molecules could potentially be repurposed as an anti-breast cancer agent. In this regard, compound **4** was assessed for in vitro antiproliferative activity against MCF-7 and additionally HCT-116 (human colorectal cancer) (Figure 4). These data show that compound **4** possesses a moderate anti-MCF-7 activity with an IC_{50} of 17.5 μM, but it also demonstrates a mild antiproliferative activity against HCT-116 (IC_{50} = 58.0 μM). The result shown here may suggest some selectivity within this class of compound over a specific type of cancer. Further research is ongoing to study the SARs around this pharmacophore.

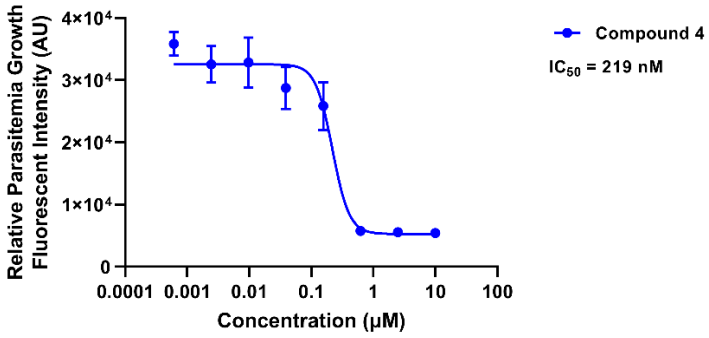


Figure 3. Dose-dependent antimalarial (3D7) activity of compound **4** (TCMDC-125133). The graph shows the reduction in parasitemia of blood stage 3D7 *P. falciparum* laboratory strain in human red blood cell at serial concentrations of compound **4**.

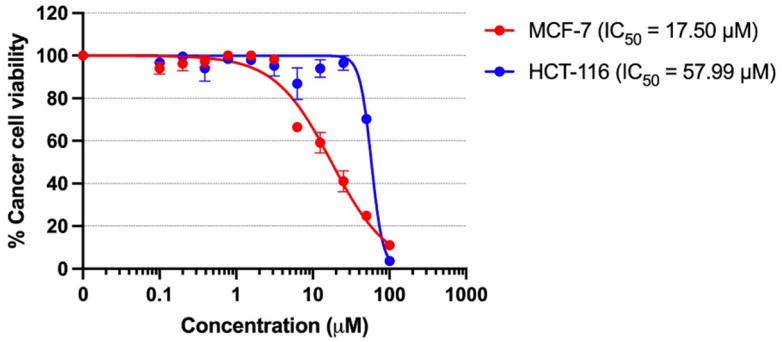


Figure 4. Dose-dependent anti-MCF-7 (human breast cancer; in red) and anti-HCT-116 (human colorectal cancer; in blue) activities of compound **4** (TCMDC-125133). The curves show the cell viability percentage of each cancer cell line at serial concentrations of compound **4**.

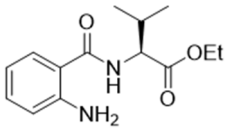
3. Materials and Methods

3.1. General

All reagents and solvents were purchased from commercial suppliers (Sigma-Aldrich, Merck, or Tokyo Chemical Industry (TCI)) and were used without further purification. NMR spectra were recorded on either a Bruker Avance AV400 or 500 (400/100 MHz $^{1}H/^{13}C$ and 500/125 MHz $^{1}H/^{13}C$) spectrometer (Bruker, Billerica, MA, USA) and chemical shifts (δ, ppm) were downfield from TMS. The chemical shifts are reported relative to residual the solvent signal in part per million (δ) (CD_3OD: ^{1}H: δ 3.31, ^{13}C: δ 49.1; DMSO-d_6: ^{1}H: δ 2.50, ^{13}C: δ 39.5; $CDCl_3$: ^{1}H: δ 7.26, ^{13}C: δ 77.23). For the ^{1}H-NMR spectrum, data are assumed to be first order with apparent singlet, doublet, triplet, quartets and multiplet reported as s, d, t, q, and m, respectively. Doublet of doublet was reported as dd, triplet of doublet was reported as td, and the resonance that appears broad was designated as br. High resolution mass spectral measurements were performed on a Thermo Scientific Orbitrap Q Exactive Focus mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). TLC was performed on TLC aluminium sheet coated with silica gel 60 F_{254} (Merck, Darmstadt, Germany). To visualize spots on the TLC sheet, UV lamps were used. Melting points were measured on a Buchi melting point M-565 (Büchi, Flawil, Switzerland). Fourier Transform Infrared (FTIR) spectroscopy was performed on a Bruker ALPHA FTIR model (Bruker, Billerica, MA, USA). Optical rotation was recorded on a Biobase Bk-P2s Digital Automatic Polarimeter (Jinan, China). The purification was performed on a Biotage® Selekt Automated flash column chromatography (Biotage, Uppsala, Sweden) where indicated. Chiral HPLC analysis was run on a Waters Alliance e2695 HPLC system with a Waters 2489 UV/Vis Detector (Waters, Milford, MA, USA) using a Chiralpak® ADH column (dimension (Ø) of 4.6 mm × 250 mm) (Daicel, Osaka, Japan).

3.2. Synthesis

3.2.1. (–)-Ethyl (2-aminobenzoyl)-L-valinate (**1**)

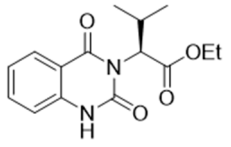


1

To a solution of acetonitrile (75 mL) in a round bottom flask, isatoic anhydride (1.6 g, 10 mmol, 1 eq), L-valine ethyl ester hydrochloride (1.8 g, 10 mmol, 1 eq), and potassium carbonate (3.4 g, 25 mmol, 2.5 eq) were added. The reaction was allowed stirred and heated to 60 °C for 18 h. After that the mixture was allowed to cool to room temperature and evaporated to remove the solvent. The resulting residue was then stirred in a 0.4 M Na_2CO_3 solution for an hour and the mixture was extracted with CH_2Cl_2. The organic phase was collected, dried with anhydrous $MgSO_4$, and evaporated to dryness by a rotary evaporator. Purification was performed using column chromatography (CC) over silica gel (30–50% EtOAc/Hexanes) to yield ethyl (2-aminobenzoyl)-L-valinate **1**. (1.39 g, 55% yield).

(–)-Ethyl (2-aminobenzoyl)-L-valinate (**1**): yellow oil, Enantiomeric excess (>99%) was determined by chiral HPLC analysis (Chiralpak® ADH), hexane:*i*-PrOH 90:10, 1.0 mL/min, t_r = 21.73 min, $[\alpha]_D = -53.1$ (c = 1.5, DMSO), Rf: 0.46 (30% EtOAc/Hexanes), FTIR (cm^{-1}): 3457.7 (NH-C=O), 3380.8 and 3353.5 (NH_2), 2967.7, 2925.7 and 2873.8 (=C-H aromatic), 1735.4 (O-C=O), and 1638.2 (O=C-NH), ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.40 (d, *J* = 7.8 Hz, 1H), 7.17 (td, *J* = 5.4, 7.7 Hz, 1H), 6.65–6.61 (m, 2H), 4.57 (dd, *J* = 5.0, 8.5, 1H), 4.26-4.13 (m, 2H), 2.27-2.19 (m, 1H), 1.27 (t, *J* = 7.2 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$); δ = 172.17, 168.99, 148.66, 132.39, 127.35, 117.18, 116.51, 115.62, 61.24, 57.01, 31.40, 18.91, 17.88, 14.13. ESI-HRMS (*m*/*z*): 265.1541 $[M + H]^+$ (calcd. for $C_{14}H_{21}N_2O_3^+$ 265.1547).

3.2.2. (–)-Ethyl (*S*)-2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-3-methylbutanoate (**2**)

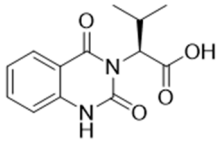


2

To a solution of compound **1** (1.39 g, 5.3 mmol, 1 eq) in THF (40 mL), CDI (1.71 g, 10.5 mmol, 2 eq) was added. The reaction was stirred for 18 h at 85 °C. When completed, the reaction was concentrated by a rotary evaporator. The resulting residue was then dissolved in EtOAc, washed with water, and dried over $MgSO_4$. The organic portion was filtered and concentrated to give a crude product. Purification was performed using CC over silica gel (30–50% EtOAc/Hexanes) to obtain the desired cyclized product **2** (1.49 g, 97% yield).

(–)-Ethyl (*S*)-2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-3-methylbutanoate (**2**): yellow oil, Enantiomeric excess (>99%) was determined by chiral HPLC analysis (Chiralpak® ADH), gradient hexane:*i*-PrOH 95:5 to 98:2, 1.0 mL/min, t_r = 61.92 min. $[\alpha]_D = -156.3$ (c = 1.5, DMSO), R_f: 0.26 (30% EtOAc/Hexanes), FTIR (cm^{-1}): 3253.5 (NH-C=O), 2965.5, 2928.6 and 2873.6 (=C-H aromatic), 1746.6 (O-C=O), 1716.9 (O=C-N-R), and 1656.4 (O=C-NH), ^{1}H-NMR (400 MHz, MeOD-d_4): δ = 8.02 (dd, *J* = 1.2, 8.0 Hz, 1H), 7.67 (td, *J* = 1.4, 7.8 Hz, 1H), 7.25 (t, *J* = 7.9 Hz, 1H), 7.19 (d, *J* = 8.2 Hz, 1H), 5.12 (d, *J* = 9.3 Hz, 1H), 4.21-4.07 (m, 2H), 2.77–2.68 (m, 1H), 1.67 (d, *J* = 6.5 Hz, 3H), 1.16 (t, *J* = 7.1 Hz, 3H), 0.76 (d, *J* = 6.9 Hz, 3H); ^{13}C-NMR (100 MHz, MeOD-d_4); δ = 171.22, 164.09, 152.11, 140.84, 136.79, 129.16, 124.43, 116.33, 114.95, 62.25, 60.12, 28.69, 22.51, 19.26, 14.45. ESI-HRMS: *m*/*z* calculated for $C_{15}H_{18}N_2O_4$ ($[M + H]^+$) 291.1339 found 291.1335.

3.2.3. (–)-(*S*)-2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-3-methylbutanoic acid (**3**)

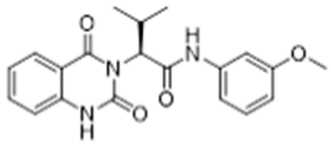


3

A solution of LiOH (0.3 g, 12.9 mmol, 2.5 eq) in water (6 mL) was added into a solution of compound **2** (1.49 g, 5.1 mmol, 1 eq) in THF (20 mL). The reaction mixture was heated and stirred at 85 °C for 18 h. After that the mixture was allowed to cool down to room temperature and was concentrated under a reduced pressure. The residue was dissolved in 10 mL of H_2O and acidified with 1 M HCl. The white precipitate was filtered off and washed successively with MeOH to afford the desired acid **3** without further purification (1.26 g, 93% yield).

(–)-(*S*)-2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-3-methylbutanoic acid (**3**): white solid, Enantiomeric excess (65%) was determined by chiral HPLC analysis (Chiralpak® ADH), hexane:*i*-PrOH 90:10, 1.0 mL/min, major enantiomer t_r = 20.22 min, minor enantiomer t_r = 31.99 min, $[\alpha]_D = -30.9$ (c = 1.4, DMSO), Rf: 0.08 (20% MeOH/EtOAc), m.p.: >305.4 °C (decomposed) FTIR (cm^{-1}): 3250.1 (NH-C=O), 3078.9 (OH) 2970.3, 2924.8 and 2850.6 (=C-H aromatic), 1751 (O=C-OH), 1682.4 (O=C-N-R), and 1640.6 (O=C-NH), ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.61 (br(-OH), 1H), 11.61 (s(-NH), 1H), 7.95 (d, *J* = 7.7 Hz, 1H), 7.70 (td, *J* = 1.1, 7.7 Hz, 1H), 7.25 (d, *J* = 7.6 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 4.96 (d, *J* = 9.3 Hz, 1H), 2.66–2.57 (m, 1H) 1.17 (d, *J* = 6.4 Hz, 3H), 0.68 (d, *J* = 6.9 Hz, 3H); ^{13}C-NMR (100 MHz, DMSO); δ = 172.21, 161.95, 150.22, 139.53, 135.19, 127.59, 122.64, 115,19, 113.48, 59.22, 26.93, 22.75, 19.28. ESI-HRMS (*m*/*z*): 263.1025 $[M + H]^+$ (calcd. for $C_{13}H_{15}N_2O_4^+$ 263.1026).

3.2.4. (–)-(*S*)-2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-*N*-(3-methoxyphenyl)-3-methyl-butanamide (**4**) [TCMDC-125133]



4
[TCMDC-125133]

To a solution of acid **3** (0.26 g, 1.0 mmol, 1 eq) in DMF (4 mL), triethylamine (TEA) (0.14 mL, 1 mmol, 1 eq) and HATU (0.38 g, 1 mmol, 1 eq) were added. The mixture was left stirring for 1 h at room temperature, after which *m*-anisidine (0.17 mL, 1.5 mmol, 1.5 eq) was added and the reaction was left stirring at room temperature for 18 h. After the reaction was completed, the solvent was removed under a reduced pressure. The residue was dissolved in EtOAc, and the solution was extracted with 0.4 M Na_2CO_3 solution and washed with water. The organic layer was collected, dried over $MgSO_4$, and evaporated under a reduced pressure. Purification was performed using automated flash column chromatography (Biotage®, gradient system of 10–50% EtOAc/Hexanes) to afford the desired quinazolinedione product **4** (TCMDC-125133) (0.29 g, 77% yield).

(–)-(*S*)-2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-N-(3-methoxyphenyl)-3-methylbutanamide (**4**) [TCMDC-125133]: light brown solid, Enantiomeric excess (76%) was determined by chiral HPLC analysis (Chiralpak® ADH), hexane:*i*-PrOH 90:10, 1.0 mL/min, major enantiomer t_r = 45.21 min, minor enantiomer t_r = 36.36 min, $[\alpha]_D = -57.8$ (c = 1.1, DMSO), Rf: 0.11 (30% EtOAc/Hexanes), m.p.: 72.8–73.4 °C, FTIR (cm^{-1}): 3206.5 and 3142.1 (NH-C=O), 2959.7, 2928.0 and 2872.3 (=C-H aromatic), 1715.7 and 1648.9 (O=C-N-H), ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 10.51 (s, 1H), 8.87 (s, 1H), 8.06 (d, *J* = 10.0 Hz, 1H), 7.55 (td,

J = 1.8, 9.5 Hz, 1H), 7.32 (s, 1H), 7.19 (td, *J* = 1.1, 9.6 Hz, 1H), 7.13 (t, *J* = 10.0 Hz, 2H), 6.98 (d, *J* = 10.0 Hz, 1H), 6.59 (dd, *J* = 2.34, 10.3 Hz, 1H), 5.30 (d, *J* = 13.4 Hz, 1H), 3.73 (s, 3H), 3.14-3.05 (m, 1H), 1.22 (d, *J* = 8.2 Hz, 3H), 0.85 (d, *J* = 8.4 Hz, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$); δ = 167.36, 163.56, 160.07, 152.11, 139.08, 138.57, 135.76, 129.56, 123.75, 115.42, 114.08, 112.22, 110.26, 105.69, 64.37, 55.28, 26.80, 21.00, 19.10. ESI-HRMS (*m*/*z*): 368.1602 $[M + H]^+$ (calcd. for $C_{20}H_{22}N_3O_4^+$ 368.1602).

3.3. Antimalarial Assay against P. falciparum 3D7

Plasmodium falciparum strain 3D7 was cultured in complete medium (RPMI-1640 supplemented with 10% Albumax II) using O Rh+ red blood cell in microaerobic environment (5% CO_2, 5% O_2, 90% N_2). IC50 assay plates were prepared by four-fold serially diluted test compounds in complete medium to a final volume of 50 µL. Artemisinin at 1 µM and complete medium were used as positive and negative controls, respectively. Then, 50 µL of parasite inoculum of 2% parasitemia of ring stage and 1% hematocrit was added to each well and incubated for 48 h in a microaerobic environment. The assay was terminated by freezing at −20 °C before growth measurement. Parasite growth was measured adding 100 µL of lysis buffer supplemented with 1X DNA fluorescent dye (UltraPower, Gellex, Tokyo, Japan) and fluorescent signal was measured at 495/530 nm. The IC_{50} value was calculated by GraphPad Prism 9.0 software (La Jolla, CA, USA) using the dose response (four parameter) function.

3.4. Antiproliferative Assay against MCF-7 and HCT-116

Human breast cancer cells (MCF-7) or human colorectal cancer cells (HCT-116 cells) purchased from ATCC were seeded at 2 × 10^3 cells/well on a 96-well plate and were cultured by DMEM (Dulbecco's Modified Eagle Medium) high glucose supplemented with 10% FBS and 1% penicillin/streptomycin. The culture was incubated at 37 °C, 5% CO_2 for 24 h. After the incubation period, TCMDC-125133 was added into the cell plate as a dose-response manner at: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.20, 0.10 (µM) and additional incubation for 72 h at 37 °C, 5% CO_2. After the 72-h incubation, the cultured medium containing compound was removed and the serum-free media containing MTT was added into the same well with additional incubation for 3 h at 37 °C, 5% CO_2. After 3 h incubation, the serum-free media containing MTT was removed and DMSO was added into the same well and the resulting-coloured solution was measured for its absorbance at 570 nm using a Multi-Mode Microplate Reader (ENVISION) (PerkinElmer, Waltham, MA, USA). The IC_{50} value was calculated using GraphPad. Doxorubicin at 10 µM was used as a positive control in both assays.

4. Conclusions

In conclusion, TCMDC-125133 can be prepared by employing the concise four-step synthesis reported here with good overall yields, low cost of goods, and mild reaction conditions. The in-house synthetic TCMDC-125133 was assayed against the *P. falciparum* 3D7 strain and its highly potent antimalarial activity corresponded to that of those previously published. The anti-proliferative activity of TCMDC-125133 was also performed and it exhibited moderate activity against the MCF-7 cell line. The result of this work confirmed the integrity of the TCMDC-125133 that was synthesized in-house. The presented synthesis would contribute to a future lead optimization campaign for this class of compounds as either antimalarial or antiproliferative agents.

Supplementary Materials: The following data are available online: ^{1}H-NMR, ^{13}C-NMR, IR spectra, mass spectra, and chiral HPLC traces of compound **1**–**4**.

Author Contributions: S.C. initiated the overall concept, designed the synthetic route, and secured funding. D.L., S.C. performed the chemical synthesis. D.L. carried out compound characterization. M.P. carried out antimalarial assay and its interpretation. P.K., K.J., S.S., S.B. carried out anti-

proliferative assay and its interpretation. S.C., D.L. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

1. Birhan, Y.S.; Bekhit, A.A.; Hymete, A. In Vivo antimalarial evaluation of some 2,3-disubstituted-4(3H)-quinazolinone derivatives. *BMC Res. Notes* **2015**, *8*, 589. [CrossRef] [PubMed]
2. Gouhar, R.S.; Kamel, M.M. Synthesis and Reactions of Some New Quinazoline Derivatives forIn VitroEvaluation as Anticancer and Antimicrobial Agents. *J. Heterocycl. Chem.* **2018**, *55*, 2082–2089. [CrossRef]
3. Tsuchihashi, H.; Nagatomo, T. Alpha-blocking potencies of antihypertensive agents (prazosin, terazosin, bunazosin, SGB-1534 and ketanserin) having with quinazoline or quinazolinedione as assessed by radioligand binding assay methods in rat brain. *J. Pharmacobiodyn.* **1989**, *12*, 170–174. [CrossRef] [PubMed]
4. Matharu, D.S.; Flaherty, D.P.; Simpson, D.S.; Schroeder, C.E.; Chung, D.; Yan, D.; Noah, J.W.; Jonsson, C.B.; White, E.L.; Aubé, J.; et al. Optimization of Potent and Selective Quinazolinediones: Inhibitors of Respiratory Syncytial Virus That Block RNA-Dependent RNA-Polymerase Complex Activity. *J. Med. Chem.* **2014**, *57*, 10314–10328. [CrossRef] [PubMed]
5. Baraka, M.M. Synthesis of novel 2,4 (1H, 3H)-quinazolinedione derivatives with analgesic and anti-inflammatory activities. *Boll. Chim. Farm.* **2001**, *140*, 90–96. [PubMed]
6. Zhang, X.; Ding, Q.; Wang, J.; Yang, J.; Fan, X.; Zhang, G. Pd(ii)-Catalyzed [4 + 1 + 1] cycloaddition of simple o-aminobenzoic acids, CO and amines: Direct and versatile synthesis of diverse N-substituted quinazoline-2,4(1H,3H)-diones. *Green Chem.* **2021**, *23*, 526–535. [CrossRef]
7. Banasr, M.; Hery, M.; Printemps, R.; Daszuta, A. Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone. *Neuropsychopharmacology* **2004**, *29*, 450–460. [CrossRef] [PubMed]
8. Sonier, B.; Arseneault, M.; Lavigne, C.; Ouellette, R.J.; Vaillancourt, C. The 5-HT2A serotoninergic receptor is expressed in the MCF-7 human breast cancer cell line and reveals a mitogenic effect of serotonin. *Biochem. Biophys. Res. Commun.* **2006**, *343*, 1053–1059. [CrossRef] [PubMed]
9. Hejazi, S.H.; Ahangari, G.; Deezagi, A. Alternative Viewpoint Against Breast Cancer Based on Selective Serotonin Receptors 5HTR3A and 5HTR2A Antagonists that can Mediate Apoptosis in MCF-7 Cell Line. *Curr. Drug Discov. Technol.* **2015**, *12*, 240–249. [CrossRef] [PubMed]
10. Matthews, H. *Accelerating Antimalarial Drug Discovery through Repositioning*; University of Salford: Salford, UK, 2015.
11. Calderon, F.; Barros, D.; Bueno, J.M.; Coteron, J.M.; Fernandez, E.; Gamo, F.J.; Lavandera, J.L.; Leon, M.L.; Macdonald, S.J.; Mallo, A.; et al. An Invitation to Open Innovation in Malaria Drug Discovery: 47 Quality Starting Points from the TCAMS. *ACS Med. Chem. Lett.* **2011**, *2*, 741–746. [CrossRef] [PubMed]
12. Almela, M.J.; Lozano, S.; Lelievre, J.; Colmenarejo, G.; Coteron, J.M.; Rodrigues, J.; Gonzalez, C.; Herreros, E. A New Set of Chemical Starting Points with Plasmodium falciparum Transmission-Blocking Potential for Antimalarial Drug Discovery. *PLoS ONE* **2015**, *10*, e0135139. [CrossRef] [PubMed]
13. Goto, S.; Tsuboi, H.; Kanoda, M.; Mukai, K.; Kagara, K. The Process Development of a Novel Aldose Reductase Inhibitor, FK366. Part 1. Improvement of Discovery Process and New Syntheses of 1-Substituted Quinazolinediones. *Org. Process Res. Dev.* **2003**, *7*, 700–706. [CrossRef]
14. Ismail, M.A.H.; Barker, S.; Abou El Ella, D.A.; Abouzid, K.A.M.; Toubar, R.A.; Todd, M.H. Design and Synthesis of New Tetrazolyl- and Carboxy-biphenylylmethyl-quinazolin-4-one Derivatives as Angiotensin II AT1 Receptor Antagonists. *J. Med. Chem.* **2006**, *49*, 1526–1535. [CrossRef] [PubMed]

15. Nencini, A.; Pratelli, C.; Quinn, J.M.; Salerno, M.; Tunici, P.; De Robertis, A.; Valensin, S.; Mennillo, F.; Rossi, M.; Bakker, A.; et al. Structure-activity relationship and properties optimization of a series of quinazoline-2,4-diones as inhibitors of the canonical Wnt pathway. *Eur. J. Med. Chem.* **2015**, *95*, 526–545. [CrossRef] [PubMed]
16. Yoon, Y.-J.; Park, J.; Kim, B.; Lee, H.-G.; Kang, S.-B.; Sung, G.; Kim, J.-J.; Lee, S.-G. tert-Butoxide-Assisted Amidation of Esters under Green Conditions. *Synthesis* **2011**, *44*, 42–50. [CrossRef]
17. Carpino, L.A.; Imazumi, H.; Foxman, B.M.; Vela, M.J.; Henklein, P.; El-Faham, A.; Klose, J.; Bienert, M. Comparison of the Effects of 5- and 6-HOAt on Model Peptide Coupling Reactions Relative to the Cases for the 4- and 7-Isomers. *Org. Lett.* **2000**, *2*, 2253–2256. [CrossRef] [PubMed]