Synthesis and Antiproliferative Activity of 2,4,6,7-Tetrasubstituted-2\(^H\)-pyrazolo[4,3-c]pyridines

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1. Introduction

Despite being rarely found in nature, presumably due to the difficulty of forming N–N bonds in living organisms, naturally occurring pyrazoles are prominent in laboratories due to their vast variety of biological activities [1]. In current medicinal chemistry, the incorporation of a pyrazole nucleus is a common practice to develop new drug-like molecules with anti-cancer, anti-diabetic, anti-viral, anti-inflammatory, anti-bacterial, anti-fungal, anti-neurodegenerative, anti-tubercular, anthelmintic, antimalarial, and photosensitizing properties [2–10], among others, thus giving rise to a great number of approved therapeutics [11]. Besides numerous biological activities, pyrazoles have also been documented to possess dyeing and fluorescence properties [12–16], and some of them can be used as colorimetric or fluorescent probes for sensing small molecules, ions, or pH [17–28], which may have applications in in vivo imaging [29–31]. Pyrazolopyridines are among the most studied compounds that combine antiproliferative effects with the induction of cell death. Moreover, investigations of the fluorescence properties of the final compounds revealed that some pyrazolopyridines could be used as colorimetric or fluorescent pH indicators [1].

Abstract: A library of 2,4,6,7-tetrasubstituted-2\(^H\)-pyrazolo[4,3-c]pyridines was prepared from easily accessible 1-phenyl-3-(2-phenylethynyl)-1\(^H\)-pyrazole-4-carbaldehyde via an iodine-mediated electrophilic cyclization of intermediate 4-(azidomethyl)-1-phenyl-3-(phenylethynyl)-1\(^H\)-pyrazoles to 7-ido-2,6-diphenyl-2\(^H\)-pyrazolo[4,3-c]pyridines followed by Suzuki cross-couplings with various boronic acids and alkylation reactions. The compounds were evaluated for their antiproliferative activity against K562 and MCF-7 cancer cell lines. The most potent compounds displayed low micromolar GI\(_{50}\) values. 4-(2,6-Diphenyl-2\(^H\)-pyrazolo[4,3-c]pyridin-7-yl)phenol proved to be the most active, induced poly(ADP-ribose) polymerase 1 (PARP-1) cleavage, activated the initiator enzyme of apoptotic cascade caspase 9, induced a fragmentation of microtubule-associated protein 1 (MAP1B), and reduced the expression levels of proliferating cell nuclear antigen (PCNA). The obtained results suggest a complex action of these compounds. 4-(2,6-Diphenyl-2\(^H\)-pyrazolo[4,3-c]pyridin-7-yl)phenol that combines antiproliferative effects with the induction of cell death. Moreover, investigations of the fluorescence properties of the final compounds revealed that some pyrazolopyridines could be used as pH indicators that enable both fluorescence intensity-based and ratiometric pH sensing.

Keywords: antiproliferation; cell death; cross-coupling; cycloiodination; pyrazole; pyridine
6-(3,5-dimethoxyphenyl)-3-(4-fluorophenyl)-1H-pyrazolo[3,4-b]pyridine (APcK110) is an extensively researched Kit kinase inhibitor [32–34]. More recently, various 1H-pyrazolo[3,4-b]pyridine derivatives were reported as potent ALK-L1196M [35] and CDK8 inhibitors [36], PPARα agonists [37], and antimicrobial, anti-quorum-sensing, and anticancer agents [38], while 3-amino-1H-pyrazolo[3,4-b]pyridine core was identified as a novel scaffold for MELK kinase inhibitors [39]. 2-[[2-(1H-Pyrazolo[3,4-c]pyridin-3-yl)-6-(trifluoromethyl)pyrimidin-4-yl]amino]ethanol is a bacterial DNA ligase inhibitor [40], several compounds bearing 1H-pyrazolo[4,3-c]pyridin-3-amine scaffold act as positive allosteric modulators of the metabotropic glutamate receptor 4 (mGlu4) [41,42], 3-phenylpyrazolo[3,4-c]pyridines were reported to possess antiproliferative activity [43], and 1-(4-methoxybenzyl)-7-(4-methylpiperazin-1-yl)-N-[4-(4-methylpiperazin-1-yl)phenyl]-3-phenyl-1H-pyrazolo[3,4-c]pyridin-5-amine was suggested as potential angiogenesis inhibitor [44]. Among biologically active pyrazolo[4,3-c]pyridines, 3-amino-2-phenyl-2H-pyrazolo[4,3-c]pyridine-4,6-diol has shown inhibitory activity against p90 ribosomal S6 kinases 2 (RSK2) [45], while 3-aminopyrazolopyridinone derivatives were demonstrated to exhibit moderate inhibitory potency against CK1d, p38α, and aurora A kinases [46].

**Figure 1.** Selected examples of biologically relevant pyrazolopyridines.

In a continuation of our work devoted to the preparation and study of the properties of various condensed and aryl coupled pyrazole derivatives [47–55], we recently reported a structure–activity relationship study on 2,4,6-trisubstituted-2H-pyrazolo[4,3-c]pyridines, several of which displayed good anticancer activity in vitro through arresting cell cycle in mitosis and the induction of apoptosis [54]. Inspired by these results, in the current work, we prepared a library of 2,4,6,7-tetrasubstituted-2H-pyrazolo[4,3-c]pyridines and examined the influence of an additional substituent at the 7-position on the biological and optical properties of the compounds.

**2. Results and Discussion**

**2.1. Chemistry**

1-Phenyl-3-(2-phenylethynyl)-1H-pyrazole-4-carbaldehyde 2, which served as a starting material in this study, was prepared via a multi-step synthetic route from 1-phenyl-1H-pyrazol-3-ol 1 in accordance with a previously published procedure [56] (Scheme S1, Supplementary File). Then, primary alcohol 3 was obtained via the reduction of an alde-
hyde group [37] (Scheme 1). Sodium borohydride was chosen as a reducing agent, and the reaction was carried out in methanol at 0 °C under an argon atmosphere. The reaction mixture was protonated with an aqueous ammonium chloride solution to create primary alcohol 3 from the intermediate complex. For the synthesis of secondary alcohols 4–7, carbaldehyde 2 was dissolved in THF and reacted with an appropriate alkyl or arylmagnesium halogenide at room temperature by adopting a previously reported procedure [58]. The reaction was carried out under an argon atmosphere with a dry solvent due to the sensitivity of Grignard reagents to air and moisture [59]. Although it is known that this kind of secondary alcohol might be unstable [51], all of them were successfully purified by column chromatography, and their structures were determined with spectroscopic data.

The newly synthesized azides 8–12 were further used to form the pyrazolo[4,3-c]pyridine core with iodine in the 7-position by adopting electrophilic substitution reaction conditions that were previously used to obtain 1,3,4-trisubstituted isoquinolines from 2-alkynyl benzyl azides [66]. Namely, azides 8–12 were dissolved in DCM and treated with iodine and a proper base (Scheme 1). Five equivalents of K3PO4 were used for the primary azides 8 and 12, while one equivalent of NaHCO3 was used for the secondary azides 9–11. The reactions were carried out at room temperature in the dark for 12 h, furnishing compounds 13–17 in 70–88% yields. An attempt to make use of a weaker base NaHCO3 for the reaction with the primary azide 6 led to the formation of the dehalogenated side product 2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine, which resulted in a troublesome purification and a lower yield of the target product.

The obtained 7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridines 13–17 were further used in palladium-catalysed Suzuki–Miyaura cross-coupling reaction (Scheme 2) by adopting a previously reported procedure [67]. Namely, aromatic boronic acids were reacted with compounds 13–17 using palladium acetate as a catalyst and Cs2CO3 as a base in an aqueous ethanol solution under an argon atmosphere. To ensure a short reaction time, cross-coupling reactions were carried out under microwave irradiation, thus giving rise to compounds 18–37. Compounds 18 and 20–37 (Scheme 2) were obtained in fair to excell-
lent yields (48–96%), but the full cross-coupling conversion of 13 using 2-methoxyphenyl boronic acid could not be achieved, resulting in a lower yield of compound 19.

Scheme 2. Synthesis of compounds 18–39. Reagents and conditions: i: arylboronic acid, Pd(OAc)$_2$, Cs$_2$CO$_3$, EtOH/H$_2$O 3/1 and MW at 100 $^\circ$C for 0.5–1 h.

4-ethyl-7-(4-hydroxyphenyl)-2,6-diphenyl-2$H$-pyrazolo[4,3-c]pyridine 37 was further subjected to hydroxyl group alkylation reactions with ethyl, propyl, and isopropyl iodides. As a result, 7-(4-alkoxyphenyl)-4-ethyl-2,6-diphenyl-2$H$-pyrazolo[4,3-c]pyridines 40–42 were obtained in high yields (80–97%) (Scheme 3).

Scheme 3. Synthesis of compounds 40–42. Reagents and conditions: i: NaH, RI, and DMF at 70 $^\circ$C for 1 h.

2.2. Optical Properties

The fluorescence properties of all final compounds 18–42 were first investigated in THF, with the excitation wavelength $\lambda_{ex}$ being set to 350 nm (Table S1, Supplementary File). The emission maxima $\lambda_{em}$ of all the compounds were located in the 437–487 nm range, which corresponds to the blue part of the visible light spectrum. A polar 4-hydroxyphenyl substituent at the 7-position bearing compounds 23, 29, and 37, as well as derivatives 31–32, 38, 40–42 (all of which bear 4-alkoxyphenyl substituents at the 7-position and ethyl or isopropyl substituents at the 4-position), possessed the most pronounced fluorescence properties. Namely, the Stokes shifts for these compounds were in 199–205 nm range, and the quantum yield reached approximately 60–85%.

Intracellular pH plays an important role in many biological processes, and its changes from normal to abnormal levels can lead to cellular dysfunction, various diseases, and a decrease in physical performance [68]. pH-sensitive fluorescent indicators enable the precise measurement of intracellular pH, which consequently provides valuable information about ongoing physiological and pathological processes at the cellular and sub-cellular levels [69]. To assess whether the fluorescence properties of the prepared compounds are pH-dependent, they were all analysed in pH 5, 7, and 9 buffers with the excitation wavelength $\lambda_{ex}$ set to 360 nm (Table S2, Supplementary File). The quantum yield of compounds 18, 24, and 30, all of which bear phenyl substituent at the 7-position, increased at acidic pH without substantial shifts in emission maxima, which were observed to be in the 435–447 nm range. The further analysis of compound 18 in a range of pH 2–11 buffers revealed a gradual decrease in fluorescence intensity with the increase of pH (Figure S1A, Supplementary File). On the other hand, the quantum yield of 2-methoxyphenyl
or 4-methoxyphenyl substituent at the 7-position possessing compounds 19, 21, 25, 27, and 31 was higher in basic pH; moreover, in the case of 4-methoxyphenyl substituent at the 7-position bearing compounds 21, 27, and 31, an acidic pH caused the red shift of the emission maxima. For instance, in the case of compound 21, the emission spectrum was found to be composed of two partly overlapping bands (Figure S1B, Supplementary File). The short-wavelength part is pH-sensitive. It is dominant in the basic environment, and decreasing the pH from 11 to 6 caused a decrease in fluorescence intensity without a shift in emission maxima, which was maintained at 458 nm. After a further decrease of pH, it was the long-wavelength band that became more dominant, which was manifested as a gradual shift of emission maxima to 519 nm. Any other 4-alkoxyphenyl substituent at the 7-position bearing compounds only exhibited a drop of quantum yield at acidic pH without the shift of the emission maxima. A polar 4-hydroxyphenyl substituent at the 7-position bearing the 4-unsubstituted derivative 23 proved to be the most potent.

Subsequently, the effects of the most active compound 23, its less active 4-substituted analogues 29 and 37, and derivative 21 were studied on K562 leukemic cells. Asynchronously growing K562 cells were treated with 10 µM concentrations of selected compounds for 24, 48, and 72 h and analysed using immunoblotting and flow cytometry (Figure 2). Immunoblotting revealed that 48 h treatment with the most potent compound 23 was sufficient for the induction of poly(ADP-ribose) polymerase 1 (PARP-1) cleavage [71] and the activation of initiator enzyme of apoptotic cascade caspase 9 [72]. Interestingly, in addition to the clear pro-apoptotic effects, we also observed the time-dependent fragmentation of microtubule-associated protein 1-light chain 3 (LC3), which has appeared during autophagy [73]. Similar outcomes with lower efficiencies were observed in all tested compounds. In addition to cell-death-related proteins, the expression levels of proliferating cell nuclear antigen (PCNA), which plays a key role in DNA replication [74], were analysed. The results revealed that all studied compounds reduced the levels of PCNA time-dependently, with the most pronounced effect observed for compounds 23 and 29. To independently support this observation, immunoblotting was complemented with the flow cytometric analysis of bromodeoxyuridine (BrdU) incorporation, which allowed us to recognize replicating BrdU-positive cells in the population [75] (Figure 2B). In control samples the number of proliferating cells came up to 40%, but the 10 µM treatment with tested compounds 21, 23, 29, and 37 reduced the proportion of actively proliferating...
BrdU-positive cells in up to approximately 10% for the most active compounds 23 and 29. Overall, the obtained results suggest the complex action of the compounds, combining antiproliferative effects with the induction of cell death.

Table 1. In vitro antiproliferative activity of 2H-pyrazolo[4,3-c]pyridine derivatives 18–42.

| Structure | Compound | R¹ | R² | GI₅₀ ± SD, µM * |
|-----------|----------|----|----|-----------------|
| 18        | H        | Ph | 7.7 ± 2.6 | >10 | >10 |
| 19        | H        | 2-MeO-Ph | 6.5 ± 1.3 | 7.1 ± 2.9 | 6.3 ± 2.2 |
| 20        | H        | 3-MeO-Ph | 5.0 ± 1.8 | >10 | >10 |
| 21        | H        | 4-MeO-Ph | 3.5 ± 1.2 | 4.8 ± 1.5 | 7.3 ± 0.2 |
| 22        | H        | 3,4-di-MeO-Ph | 2.4 ± 1.3 | 6.0 ± 3.8 | 4.2 ± 0.9 |
| 23        | H        | 4-OH-Ph | 1.5 ± 0.7 | 2.4 ± 1.0 | 1.6 ± 0.2 |
| 24        | Me       | Ph  | >10 | >10 | >10 |
| 25        | Me       | 2-MeO-Ph | >10 | >10 | >10 |
| 26        | Me       | 3-MeO-Ph | >10 | >10 | >10 |
| 27        | Me       | 4-MeO-Ph | >10 | >10 | 8.4 ± 2.1 |
| 28        | Me       | 3,4-di-MeO-Ph | 7.6 ± 3.0 | >10 | >10 |
| 29        | Me       | 4-OH-Ph | 4.7 ± 2.7 | 3.9 ± 0.3 | 4.1 ± 0.4 |
| 30        | Et       | Ph  | >10 | >10 | >10 |
| 31        | Et       | 4-MeO-Ph | >10 | >10 | >10 |
| 32        | Et       | 2,4-di-MeO-Ph | >10 | >10 | >10 |
| 33        | Et       | 4-Me-Ph | >10 | >10 | >10 |
| 34        | Et       | 4-CF₃-Ph | >10 | >10 | >10 |
| 35        | Et       | 4-CF₂O-Ph | >10 | >10 | >10 |
| 36        | Et       | 4-Cl-Ph | >10 | >10 | >10 |
| 37        | Et       | 4-OH-Ph | 8.0 ± 3.1 | >10 | 3.9 ± 0.4 |
| 38        | iPr      | 4-MeO-Ph | 5.7 ± 1.4 | >10 | >10 |
| 39        | Ph       | 4-MeO-Ph | >10 | >10 | 7.9 ± 3.8 |
| 40        | Et       | 4-ETO-Ph | >10 | >10 | >10 |
| 41        | Et       | 4-PrO-Ph | >10 | >10 | >10 |
| 42        | Et       | 4-iPrO-Ph | >10 | >10 | >10 |

| | | | MV4-11 | K562 | MCF-7 |
|---|---|---|---|---|---|
| Flavopiridol | 0.2 ± 0.03 | 0.8 ± 0.1 | 0.2 ± 0.03 |

* Data are means of at least three independent measurements.

Figure 2. Effect of compounds 21, 23, 29, and 37 (10 µM) on K562 cell line after 24, 48, and 72 h treatment. (A) Immunoblotting of selected markers of cell death and proliferation. The actin level was detected to verify equal protein loading. (B) Analysis of BrdU incorporation. Results are representative of two independent experiments.

3. Materials and Methods

3.1. General

All chemicals and solvents were purchased from commercial suppliers and used without further purification unless otherwise specified. The ¹H, ¹³C, and ¹⁵N NMR spectra were recorded in CDCl₃ or DMSO-d₆ solutions at 25 °C on either a Bruker Avance III 700 (700 MHz for ¹H, 176 MHz for ¹³C, and 71 MHz for ¹⁵N) spectrometer equipped with a 5 mm TCI ¹H-¹³C/¹⁵N/D z-gradient cryoprobe or a Jeol ECA-500 (500 MHz for ¹H and 126 MHz for ¹³C) spectrometer equipped with a 5 mm Royal probe. The chemical...
shifts, expressed in ppm, were relative to tetramethylsilane (TMS). The $^{15}$N NMR spectra were referenced to neat, external nitromethane (coaxial capillary). The $^{19}$F NMR spectra (376 MHz) were obtained on a Bruker Avance III 400 instrument using $\text{C}_6\text{F}_6$ as an internal standard. FT-IR spectra were collected using the ATR method on a Bruker Vertex 70v spectrometer with an integrated Platinum ATR accessory or on a Bruker Tensor 27 spectrometer in KBr pellets. The melting points of crystalline compounds were determined in open capillary tubes with a Buchi M 565 apparatus (temperature gradient: 2 $^\circ$C/min) and are uncorrected. Mass spectra were recorded on Q-TOF MICRO spectrometer (Waters), analyses were performed in the positive (ESI$^+$) mode, and molecular ions were recorded in [M + H]$^+$ forms. High-resolution mass spectrometry (HRMS) spectra were obtained in the ESI mode on a Bruker MicrOTOF-Q III spectrometer. All reactions were performed in oven-dried flasks under an argon atmosphere with magnetic stirring. Reaction progress was monitored by TLC analysis on Macherey-Nagel™ ALUGRAM® Xtra SIL G/UV254 plates. TLC plates were visualized with UV light (wavelengths of 254 and 365 nm) or iodine vapour. Compounds were purified by flash chromatography in a glass column (stationary phase of silica gel, high-purity grade of 9385, pore size of 60 Å, particle size of 230–400 mesh, supplied by Sigma-Aldrich). $^1$H, $^{13}$C, and $^1$H-$^{15}$N HMBC NMR spectra, as well as the HRMS data of new compounds, are provided in Figures S2–S131 of the Supplementary Materials.

3.2. Chemistry

3.2.1. Procedure for the Synthesis of [1-Phenyl-3-(phenylethynyl)-1$^H$-pyrazol-4-yl]methanol 3

1-Phenyl-3-(2-phenylethynyl)-1$^H$-pyrazole-4-carbaldehyde 3 (560 mg, 2.06 mmol) was dissolved in MeOH (12 mL), and the solution was cooled to 0 $^\circ$C. Subsequently, NaBH$_4$ (156 mg, 4.12 mmol) was added under an argon atmosphere, and the mixture was stirred for 30 min. Upon completion (monitored by TLC), the reaction mixture was diluted with a saturated aqueous NH$_4$Cl solution (20 mL) and extracted with EtOAc (3 $\times$ 30 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 530 mg (94%), white crystalline solid, mp = 114–115 $^\circ$C, K$_r$ = 0.18 (EtOAc/Hex, 1:3 v/v). $^1$H NMR (700 MHz, CDCl$_3$): $\delta$ 2.12 (1H, s, OH), 4.77 (2H, d, $J$ = 7.0 Hz, C$_2$H$_2$OH), 7.28–7.32 (1H, m, NPh 4-H), 7.33–7.38 (3H, m, CPh 3,4,5), 7.42–7.46 (2H, m, NPh 3,5-H), 7.55–7.59 (2H, m, CPh 2,6-H), 7.66–7.72 (2H, m, NPh 2,6-H), 7.95 (1H, s, 5-H). $^{13}$C NMR (176 MHz, CDCl$_3$): $\delta$ 55.9 (C$_2$H$_2$OH), 80.3 (C≡CPh), 93.7 (C≡CPh), 119.4 (NPh C-2,6), 122.5 (C-4), 126.4 (C-5 and CPh C-1), 127.1 (NPh C-4), 128.5 (CPh C-3,5), 128.9 (CPh C-4), 129.6 (NPh C-3,5), 131.9 (CPh C-2,6), 135.6 (C-3), 139.7 (NPh C-1). $^{15}$N NMR (71 MHz, CDCl$_3$): $\delta$ −163.5 (N-1), N-2 not found. IR (v, cm$^{-1}$): 3373 (OH), 3126, 3066, 3056 (CH$_2$OH), 749, 686 (CH=CH of mono- and disubstituted benzenes). MS (ES$^+$): m/z (%): 275 ([M + H]$^+$), 100. HRMS (ESI) for C$_{18}$H$_{14}$N$_2$ONa ([M + Na]$^+$): requires 297.0998 and found 297.0988.

3.2.2. General Procedure (A) for the Synthesis of Alcohols 4–7

1-Phenyl-3-(2-phenylethynyl)-1$^H$-pyrazole-4-carbaldehyde 2 (1 equivalent) was dissolved in dry THF under an argon atmosphere. Subsequently, an appropriate Grignard reagent (1.2 equivalents) was added, and the mixture was stirred at room temperature for 10 min. Upon completion (monitored by TLC), the reaction mixture was diluted with water (20 mL) and extracted with EtOAc (3 $\times$ 20 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography.
1-[1-Phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]ethanol-1-ol 4

1-[1-Phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]ethanol-1-ol 4 was prepared in accordance with general procedure (A) from 1-phenyl-3-(2-phenylethynyl)-1H-pyrazole-4-carbaldehyde 2 (350 mg, 1.287 mmol) and MeMgCl (0.52 mL, 1.56 mmol) in THF (4 mL). The desired compound was purified by column chromatography (EtOAc/Hex, 1:4 v/v). Yield: 286 mg (70%), colourless liquid, Rf = 0.23 (EtOAc/Hex, 1:5 v/v). 1H NMR (700 MHz, CDCl3): δ 1.64 (3H, d, J = 6.5 Hz, CH3), 2.18 (1H, s, OH), 5.13 (1H, q, J = 6.5 Hz, CH3), 7.29–7.1 (1H, m, NPh 4-H), 7.34–7.38 (3H, m, CPh 3,4,5-H), 7.43–7.47 (2H, m, NPh 3,5-H), 7.55–7.59 (2H, m, CPh 2,6-H), 7.67–7.72 (2H, m, NPh 2,6-H), 7.92 (1H, s, 5-H). 13C NMR (176 MHz, CDCl3): δ 23.8 (CH3), 62.7 (CH) 80.6 (C=CPh), 93.8 (C=CPh), 119.1 (NPh C-2,6), 122.4 (CPh C-1), 124.4 (C-5), 126.9 (NPh C-4), 128.4 (CPh C-3,5), 128.7 (CPh C-4), 129.4 (NPh C-3,5), 131.5 (C-1), 131.7 (CPh C-2,6), 134.2 (C-2), 139.6 (NPh C-1). 15N NMR (71 MHz, CDCl3): δ –75.4 (N-2), –164.4 (N-1). MS (ES+): m/z (%): 275 ([M + Na]+), 96.

1-[1-Phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]propan-1-ol 5

1-[1-Phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]propan-1-ol 5 was prepared in accordance with general procedure (A) from 1-phenyl-3-(2-phenylethynyl)-1H-pyrazole-4-carbaldehyde 2 (100 mg, 0.37 mmol) and EtMgBr (0.15 mL, 0.44 mmol) in THF (2 mL). The desired compound was purified by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 100 mg (90%), yellowish crystalline solid, mp = 87–88 °C, Rf = 0.28 (EtOAc/Hex, 1:3 v/v). 1H NMR (700 MHz, CDCl3): δ 1.03 (3H, t, J = 7.7 Hz, CH3), 1.93–1.99 (2H, m, CH2), 2.22 (1H, br s, OH), 4.87 (1H, t, J = 6.5 Hz, CH), 7.29–7.32 (1H, m, NPh 4-H), 7.34–7.38 (3H, m, CPh 3,4,5-H), 7.43–7.46 (2H, m, NPh 3,5-H), 7.55–7.59 (2H, m, CPh 2,6-H), 7.68–7.72 (2H, m, NPh 2,6-H), 7.91 (1H, s, 5-H). 13C NMR (176 MHz, CDCl3): δ 10.0 (CH2), 30.8 (CH2), 68.1 (CHOH), 80.7 (C=CPh), 93.6 (C=CPh), 119.1 (NPh C-2,6), 122.5 (CPh C-4), 124.8 (C-5), 126.9 (NPh C-4), 128.4 (CPh C-3,5), 128.7 (CPh C-4), 129.4 (NPh C-3,5), 130.2 (C-4), 131.6 (CPh C-2,6), 134.5 (C-3), 139.6 (NPh C-1). 15N NMR (71 MHz, CDCl3): δ –75.8 (N-2), –164.1 (N-1). IR (KBr, cm–1): 3441 (OH), 3056 (CH=CH of mono- and disubstituted benzenes). MS (ES+): m/z (%): 303 ([M+H]+), 98. HRMS (ESI) for C20H18N2Ona ([M + Na]+): requires 325.1311 and found 325.1311.

2-Methyl-1-[1-phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]propan-1-ol 6

2-Methyl-1-[1-phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]propan-1-ol 6 was prepared in accordance with general procedure (A) from 1-phenyl-3-(2-phenylethynyl)-1H-pyrazole-4-carbaldehyde 2 (350 mg, 1.287 mmol) and iPrMgCl (0.97 mL, 1.93 mmol) in THF (6 mL). The desired compound was purified by column chromatography (EtOAc/Hex, 1:4 v/v). Yield: 286 mg (70%), yellow crystalline solid, mp = 78–79 °C, Rf = 0.23 (EtOAc/Hex, 1:5 v/v). 1H NMR (700 MHz, CDCl3): δ 0.98 (3H, d, J = 6.8 Hz, CH3), 1.05 (3H, d, J = 6.8 Hz, CH3), 2.12 (1H, s, OH), 2.15–2.12 (1H, m, CH=CH(CH3)2), 4.70 (1H, d, J = 6.3 Hz, CH=CH), 7.26–7.32 (1H, m, NPh 4-H), 7.34–7.39 (3H, m, CPh 3,4,5-H), 7.43–7.47 (2H, m, NPh 3,4-H), 7.55–7.59 (2H, m, CPh 2,6-H), 7.67–7.76 (2H, m, NPh 2,6-H), 7.91 (1H, s, 5-H). 13C NMR (176 MHz, CDCl3): δ 18.1 (CH3), 18.8 (CH3), 34.9 (CH=CH(CH3)2), 72.2 (CH=CH), 81.1 (C=CPh), 93.7(C=CPh), 119.2(NPh C-2,6), 122.7(CPh C-1), 125.3(C-5), 127.0(NPh C-4), 128.5(CPh C-3,5), 128.8(CPh C-4), 129.4(C-4), 129.6(NPh C-3,5), 131.8(CPh C-2,6), 134.9(C-3), 139.7(NPh C-1). 15N NMR (71 MHz, CDCl3): δ –163.8 (N-1), N-2 not found. IR (ν, cm–1): 3383 (OH), 3054 (CH=CH), 2959, 2872 (CH=CH), 1596, 1501, 1458, 1376, 1331, 1219 (C=C, C=C, C=N, C=N), 1056, 1033 (CH=CH), 963, 752, 688 (CH=CH of monosubstituted benzenes). MS (ES+): m/z (%): 317 ([M + H]+), 99. HRMS (ESI) for C21H20N2Ona ([M + Na]+): requires 339.1468 and found 339.1467.

Phenyl-[1-phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]methanol 7

Phenyl-[1-phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]methanol 7 was prepared in accordance with general procedure (A) from 1-phenyl-3-(2-phenylethynyl)-1H-pyrazole-4-
carbaldehyde 2 (100 mg, 0.37 mmol) and PhMgBr (0.15 mL, 0.44 mmol) in DCM (2 mL).

The desired compound was purified by column chromatography (EtOAc/Hex, 1:7 v/v).

Yield: 105 mg (81%), colourless liquid, R<sub>f</sub> = 0.35 (EtOAc/Hex, 1.3 v/v). <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>): δ 2.54 (1H, br s, OH), 6.06 (1H, s, CH), 7.27–7.31 (1H, m, NPh 4-H), 7.31–7.35 (1H, m, C4Ph 4-H), 7.33–7.36 (3H, m, C3-Ph 3,4,5-H), 7.37–7.40 (2H, m, C4Ph 3,5-H), 7.41–7.45 (2H, m, NPh 3,5-H), 7.48–7.51 (2H, m, C3-Ph 2,6-H), 7.52–7.54 (2H, m, C4Ph 2,6-H), 7.65–7.70 (2H, m, NPh 2,6-H), 7.80 (1H, s, 5-H). <sup>13</sup>C NMR (176 MHz, CDCl<sub>3</sub>): δ 68.8 (CH), 80.5 (C=CPh), 94.0 (C=CPh), 119.2 (NPh C-2,6), 122.4 (C3-Ph C-1), 125.5 (C-5), 126.4 (C4Ph C-2,6), 126.9 (NPh C-4), 127.9 (C4Ph C-1), 128.3 (C3-Ph C-3,5), 128.5 (C4Ph C-3,5), 128.7 (C3-Ph C-4), 129.4 (NPh C-3,5), 130.2 (C-4), 131.7 (C3-Ph C-2,6), 134.8 (C-3), 139.5 (NPh C-1), 142.6 (C4Ph C-1). <sup>15</sup>N NMR (71 MHz, CDCl<sub>3</sub>): δ −163.9 (N-1). MS (ES<sup>+</sup>): m/z (%): 351 ([M + H]<sup>+</sup>), 95. HRMS (ESI) for C<sub>24</sub>H<sub>18</sub>N<sub>2</sub>ONa ([M + Na]<sup>+</sup>): requires 373.1311 and found 373.1311.

3.2.3. General Procedure (B) for the Synthesis of Azide–Alkynes 8–12

To a solution of appropriate pyrazole alcohol 3–7 (1 equivalent) in dry DCM, TMSN<sub>3</sub> (1.5 equivalents) and BF<sub>3</sub>·Et<sub>2</sub>O (0.2 equivalents) were added dropwise. The reaction mixture was stirred for 10–60 min under an argon atmosphere at room temperature. Upon completion (monitored by TLC), the reaction mixture was diluted with an aqueous NaHCO<sub>3</sub> solution (10 mL) and extracted with DCM (3 × 25 mL). The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography.

4-(Azidomethyl)-1-phenyl-3-(phenylethynyl)-1H-pyrazole 8

4-(Azidomethyl)-1-phenyl-3-(phenylethynyl)-1H-pyrazole 8 was prepared in accordance with general procedure (B) from 1-[1-phenyl-3-(2-phenylethynyl)-1H-pyrazol-4-yl]methanol 3 (100 mg, 0.36 mmol), TMSN<sub>3</sub> (0.07 mL, 0.55 mmol), and BF<sub>3</sub>·Et<sub>2</sub>O (0.01 mL, 0.07 mmol) in DCM (1.5 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 54 mg (50%), light yellow liquid, R<sub>f</sub> = 0.71 (EtOAc/Hex, 1.3 v/v). <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>): δ 4.44 (2H, s, CH<sub>2</sub>N<sub>3</sub>), 7.31–7.35 (1H, m, NPh 4-H), 7.35–7.40 (3H, m, CPh 3,4,5-H), 7.45–7.49 (2H, m, NPh 3,5-H), 7.58–7.63 (2H, m, CPh 2,6-H), 7.69–7.74 (2H, m, NPh 2,6-H), 7.96 (1H, s, 5-H). <sup>13</sup>C NMR (176 MHz, CDCl<sub>3</sub>): δ 44.8 (CH<sub>2</sub>N<sub>3</sub>), 79.9 (C=S=CPh), 94.1 (C=S=CPh), 119.5 (NPh C-2,6), 120.9 (C-4), 122.4 (C4Ph C-1), 126.7 (C-5), 127.4 (NPh C-4), 128.5 (CPh C-3,5), 129.0 (CPh C-4), 129.7 (NPh C-3,5), 132.0 (CPh C-2,6), 136.5 (C-3), 139.6 (NPh C-1). <sup>15</sup>N NMR (71 MHz, CDCl<sub>3</sub>): δ −162.2 (N-1), −306.6 and −132.9 (N<sub>2</sub>, one not found), N-2 not found. IR (v, cm<sup>−1</sup>): 3050 (CH<sub>3</sub>arom), 2921 (CH<sub>3</sub>aliph), 2087 (N<sub>3</sub>), 1595, 1501, 1331, 1250 (C=C, C=N, C=N), 753, 688 (CH=CH of monosubstituted benzenes). MS (ES<sup>+</sup>): m/z (%): 300 ([M + H]<sup>+</sup>), 99. HRMS (ESI) for C<sub>18</sub>H<sub>13</sub>N<sub>3</sub>O ([M + Na]<sup>+</sup>): requires 322.1065 and found 322.1063.

4-(1-Azidoethyl)-1-phenyl-3-(phenylethynyl)-1H-pyrazole 9

4-(1-Azidoethyl)-1-phenyl-3-(phenylethynyl)-1H-pyrazole 9 was prepared in accordance with general procedure (B) from 1-[1-phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]ethan-1-ol 4 (205 mg, 0.71 mmol), TMSN<sub>3</sub> (0.14 mL, 1.07 mmol), and BF<sub>3</sub>·Et<sub>2</sub>O (0.02 mL, 0.14 mmol) in DCM (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:4 v/v). Yield: 160 mg (72%), colourless oil, R<sub>f</sub> = 0.72 (EtOAc/Hex, 1:3 v/v). <sup>1</sup>H NMR (700 MHz, CDCl<sub>3</sub>): δ 1.70 (3H, d, J = 7.0 Hz, CH<sub>3</sub>N), 7.34–7.36 (1H, m, NPh 4-H), 7.39–7.40 (3H, m, CPh 3,4,5-H), 7.48–7.51 (2H, m, NPh 3,5-H), 7.62–7.63 (2H, m, CPh 2,6-H), 7.74–7.75 (2H, m, NPh 2,6-H), 7.94 (1H, s, 5-H). <sup>13</sup>C NMR (176 MHz, CDCl<sub>3</sub>): δ 20.7 (CH<sub>3</sub>), 52.7 (CH<sub>3</sub>N), 80.2 (C=CPH), 94.0 (C=C=CPH), 119.3 (NPh C-2,6), 122.4 (CPh C-1), 124.9 (C-5), 126.7 (C-4), 127.2 (NPh C-4), 128.4 (CPh C-3,5), 128.9 (CPh C-4), 129.5 (NPh C-3,5), 131.8 (CPh C-2,6), 135.1 (C-3), 139.5 (NPh C-1). <sup>15</sup>N NMR (71 MHz, CDCl<sub>3</sub>): δ −294.3 (N<sub>3</sub>), −163.3 (N-1), −133.9 (N<sub>3</sub>), −73.6 (N-2). IR (KBr,
1502, 1328, 1215 (C=C, C=N, C–N), 959, 757, 689 (CH=CH of monosubstituted benzenes).

J (0.01 mL, 0.06 mmol) in DCM (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc:Hex, 1:12 v/v). Yield: 81 mg (75%), yellowish crystalline solid, mp = 74–75 °C, Rf = 0.73 (EtOAc/Hex, 1.3 v/v).

1H NMR (700 MHz, CDCl3): δ 1.06 (3H, t, J = 7.3 Hz, CH3), 1.99 (2H, p, J = 7.2 Hz, CH2), 4.63 (1H, t, J = 7.0 Hz, CH=N3), 7.31–7.35 (1H, m, NPh 4-H), 7.36–7.40 (3H, m, CPh 3,4,5-H), 7.44–7.50 (2H, m, NPh 3,5-H), 7.57–7.63 (2H, m, CPh 2,6-H), 7.69–7.77 (2H, m, NPh 2,6-H), 7.91 (1H, s, 5-H).

13C NMR (176 MHz, CDCl3): δ 10.7 (CH3), 28.4 (CH2), 58.9 (CH), 80.2 (C=CPh), 93.8 (C=CPh), 119.2 (NPh C-2,6), 122.3 (CPh C-1), 125.1 (C-5), 125.4 (C-4), 127.1 (NPh C-4), 128.4 (CPh C-3,5), 128.8 (CPh C-4), 129.5 (NPh C-3,5), 131.7 (CPh C-2,6), 135.4 (C-3), 139.4 (NPh C-1).

15N NMR (71 MHz, CDCl3): δ −163.4 (N-1), −134.5 (CH=N=N=N), −116.4 (CH=N=N=N), −74.4 (N-2). IR (KBr, ν cm−1): 3147 (CH=CH), 2967, 2934, 2870 (CH=CH), 1702 (N=O), 1598, 1502, 1328, 1215 (C=C, C=N, C=N), 959, 757, 689 (CH=CH of monosubstituted benzenes).

MS (ES†): m/z (%): 328 [M + H]+, 99. HRMS (ESI) for C20H16N5Na ([M + Na]+): requires 350.1376 and found 350.1376.

4-(1-Azidopropyl)-1-phenyl-3-(phenylethynyl)-1H-pyrazole 10

4-(1-Azidopropyl)-1-phenyl-3-(phenylethynyl)-1H-pyrazole 10 was prepared in accordance with general procedure (B) from 1-[1-phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]propan-1-ol 5 (100 mg, 0.33 mmol), TMSN3 (0.07 mL, 0.5 mmol), and BF3·Et2O (0.01 mL, 0.07 mmol) in DCM (1 mL). The desired compound was obtained after purification by column chromatography (EtOAc:Hex, 1:12 v/v). Yield: 75 mg (82%), colourless oil, Rf = 0.68 (EtOAc/Hex, 1:4 v/v).

1H NMR (700 MHz, CDCl3): δ 1.00 (3H, d, J = 6.8 Hz, CH3), 1.06 (3H, d, J = 6.7 Hz, CH3), 2.18–2.25 (1H, m, CH3C(6)H2), 4.52 (1H, d, J = 7.0 Hz, CH-N3), 7.31–7.35 (1H, m, NPh 4-H), 7.37–7.40 (3H, m, CPh 3,4,5-H), 7.45–7.50 (2H, m, NPh 3,5-H), 7.56–7.62 (2H, m, CPh 2,6-H), 7.71–7.79 (2H, m, NPh 2,6-H), 7.91 (1H, s, 5-H).

13C NMR (176 MHz, CDCl3): δ 19.0 (CH3), 19.4 (CH3), 33.7 (CH–C3H5), 64.2 (CH-N3), 80.5 (C=CPh), 93.9 (C=CPh), 119.4 (NPh C-2,6), 122.5 (CPh C-1), 124.5 (C-4), 125.6 (C-5), 127.3 (NPh C-4), 128.5 (CPh C-3,5), 129.0 (CPh C-4), 129.6 (NPh C-3,5), 131.9 (CPh C-2,6), 135.9 (C-3), 139.6 (NPh C-1).

15N NMR (71 MHz, CDCl3): δ −299.2 (N-2), −163.1 (N-1), −134.1 (N-3). IR (ν cm−1): 3060 (CH=CH), 2963, 2873 (CH=CH), 2093 (N=O), 1598, 1502, 1329, 1244 (C=C, C=N, C=N), 753, 687 (CH=CH of monosubstituted benzenes). MS (ES†): m/z (%): ([M + H]+, 99). HRMS (ESI) for C21H17N5Na ([M + Na]+): requires 364.1533 and found 364.1533.

4-(Azido-2-methylpropyl)-1-phenyl-3-(phenylethynyl)-1H-pyrazole 11

4-(Azido-2-methylpropyl)-1-phenyl-3-(phenylethynyl)-1H-pyrazole 11 was prepared in accordance with general procedure (B) from 2-methyl-1-[1-phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]propan-1-ol 6 (200 mg, 0.63 mmol), TMSN3 (0.1 mL, 0.76 mmol), and BF3·Et2O (0.02 mL, 0.13 mmol) in DCM (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc:Hex, 1:10 v/v). Yield: 177 mg (82%), white crystalline solid, mp = 86–87 °C, Rf = 0.73 (EtOAc/Hex, 1:3 v/v).

1H NMR (700 MHz, CDCl3): δ 5.90 (1H, s, CH), 7.30–7.33 (1H, m, NPh 4-H), 7.33–7.39 (4H, m, C4Ph 4-H and C3-P3H-3,4,5-H), 7.40–7.44 (2H, m, C4Ph 3,5-H), 7.44–7.48 (4H, m, C4Ph 2,6-H, NPh 3,5-H), 7.48–7.52 (2H, m, C3-P3H 2,6-H), 7.68–7.72 (2H, m, NPh 2,6-H), 7.82 (1H, s, 5-H).

13C NMR (176 MHz, CDCl3): δ 60.6 (CH), 80.0 (C=CPh), 94.3 (C=CPh), 119.2 (NPh C-2,6), 122.3 (C3-P3H C-1), 125.97 (C3-P3H C-1), 134.1 (N-2). IR (KBr, ν cm−1): 3147 (CH=CH), 2985, 2936 (CH=CH of monosubstituted benzenes). MS (ES†): m/z (%): 314 ([M + H]+, 100). HRMS (ESI) for C19H14N5 ([M + H]+): requires 314.1400 and found 314.1395.

4-[Azido(phenyl)methyl]-1-phenyl-3-(phenylethynyl)-1H-pyrazole 12

4-[Azido(phenyl)methyl]-1-phenyl-3-(phenylethynyl)-1H-pyrazole 12 was prepared in accordance with general procedure (B) from phenyl[1-phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]methanol 7 (105 mg, 0.3 mmol), TMSN3 (0.16 mL, 0.45 mmol), and BF3·Et2O (0.01 mL, 0.06 mmol) in DCM (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc:Hex, 1:7 v/v). Yield: 105 mg (93%), white crystalline solid, mp = 86–87 °C, Rf = 0.73 (EtOAc/Hex, 1:3 v/v).
3.2.4. General Procedure (C) for the Synthesis of 7-iodo-2H-pyrazolo[4,3-c]pyridines 13–17

To a solution of appropriate azide–alkyne 8–12 (1 equivalent) in DCM, the appropriate base K$_3$PO$_4$ (5 equivalents) or NaHCO$_3$ (1 equivalent) and I$_2$ (5 equivalents) were added. The reaction mixture was stirred at room temperature for 12 h. Upon completion (monitored by TLC), the reaction mixture was diluted with an aqueous Na$_2$SO$_4$ solution (20 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography.

7-Iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 13

7-Iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 13 was prepared in accordance with general procedure (C) from 4-(azidomethyl)-1-phenyl-3-(2-phenylethynyl)-1H-pyrazole 8 (276 mg, 0.92 mmol), K$_3$PO$_4$ (978 mg, 4.6 mmol), and I$_2$ (1.472 g, 4.6 mmol) in DCM (9.8 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:2 v/v). Yield: 299 mg (82%), light yellow crystalline solid, mp = 110–111 °C, R$_f$ = 0.13 (EtOAc/Hex, 1:3 v/v). $^1$H NMR (700 MHz, CDCl$_3$): δ 7.42–7.45 (1H, m, CPh 4-H), 7.46–7.52 (3H, m, CPh 3,5-H and NPh 4-H), 7.55–7.59 (2H, m, NPh 3,5-H), 7.68–7.74 (2H, m, CPh 2,6-H), 7.93–7.99 (2H, m, NPh 2,6-H), 8.77 (1H, s, 3-H), 9.13 (1H, s, 4-H). $^{13}$C NMR (176 MHz, CDCl$_3$): δ 78.2 (C-7), 118.5 (C-3a), 121.8 (NPh C-2,6), 123.3 (C-3), 128.1 (CPh C-3,5), 128.4 (CPh C-4), 129.3 (NPh C-4), 129.9 (NPh C-3,5), 130.1 (CPh C-2,6), 139.9 (NPh, C-1), 142.2 (CPh, C-1), 146.1 (C-4), 153.5 (C-7a), 155.7 (C-6). $^{15}$N NMR (71 MHz, CDCl$_3$): δ −146.1 (N-2), −90.6 (N-1), −78.4 (N-5). IR (ν, cm$^{-1}$): 3044, 3035 (CH=CH of monosubstituted benzenes). MS (ES$^+$): m/z (%): 398 ([M + H]$^+$, 100). HRMS (ESI) for C$_{18}$H$_{13}$N$_3$I ([M + Na]$^+$): requires 412.0304 and found 412.0303.

7-Iodo-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 14

7-Iodo-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 14 was prepared in accordance with general procedure (C) from 4-(1-azidoethyl)-1-phenyl-3-(2-phenylethynyl)-1H-pyrazole 9 (255 mg, 0.79 mmol), NaHCO$_3$ (69 mg, 0.82 mmol), and I$_2$ (1034 mg, 4.07 mmol) in DCM (8.1 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:2 v/v). Yield: 238 mg (72%), light yellow crystalline solid, mp = 186–189 °C, R$_f$ = 0.43 (EtOAc/Hex, 1:2 v/v). $^1$H NMR (700 MHz, CDCl$_3$): δ 2.83 (s, 3H, CH$_3$), 7.40–7.43 (1H, m, CPh 4-H), 7.46–7.49 (m, 3H, CPh 3,5-H and NPh 4-H), 7.55–7.57 (m, 2H, NPh 3,5-H), 7.67–7.69 (m, 2H, CPh 2,6-H), 7.95–7.96 (m, 2H, NPh 2,6-H), 8.72 (s, 1H, 3-H). $^{13}$C NMR (176 MHz, CDCl$_3$): δ 22.5 (CH$_3$), 79.0 (C-7), 119.0 (C-3a), 121.6 (NPh C-2,6), 123.0 (C-3), 128.0 (CPh C-3,5), 128.3 (CPh C-4), 129.0 (NPh C-4), 129.8 (NPh C-3,5), 130.0 (CPh C-2,6), 140.0 (NPh C-1), 142.5 (CPh C-1), 153.5 (C-7a), 155.3 (C-4), 155.6 (C-6). $^{15}$N NMR (71 MHz, CDCl$_3$): δ −147.5 (N-2), −88.5 (N-1), −80.5 (N-5). IR (ν, cm$^{-1}$): 3131, 3107 (CH$_{arom}$), 2956 (CH$_{aliph}$), 1586, 1504, 1370, 1205 (C=C, C=N, C=N), 798, 768, 750, 696 (CH=CH of monosubstituted benzenes). MS (ES$^+$): m/z (%): 412 ([M + H]$^+$, 100). HRMS (ESI) for C$_{19}$H$_{15}$N$_5$I ([M + H]$^+$): requires 412.0304 and found 412.0304.

4-Ethyl-7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 15

4-Ethyl-7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 15 was prepared in accordance with general procedure (C) from 4-(1-azidomethyl)-1-phenyl-3-(2-phenylethynyl)-1H-pyrazole 10 (329 mg, 1.01 mmol), NaHCO$_3$ (85 mg, 1.01 mmol), and I$_2$ (1278 mg, 5.03
molecules (26, 6747) a. 1.49 (6H, d, J = 89.9 (N-1), N-5 not found. IR (KBr, ν = 3297, 3286 (CH-arom), 2970, 2929 (CH-aliph), 1584, 1507, 1468, 1391, 1212 (C=C, C=N, C=N), 1106, 1023, 915, 763, 697 (CH=CH of monosubstituted benzenes). MS (ES+): m/z (%): 440 ([M + H]+, 99). HRMS (ESI) C20H17IN5 ([M + H]+): requires 426.0462 and found 426.0462.

7-Iodo-4-isopropyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 16

7-Iodo-4-isopropyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 16 was prepared in accordance with general procedure (C) from 4-azido-2-methylpropyl)-1-phenyl-(3-phenylethynyl)-1H-pyrazole 11 (177 mg, 0.52 mmol), NaHCO3 (44 mg, 0.52 mmol), and I2 (659 mg, 2.6 mmol) in DCM (5.2 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:6 v/v). Yield: 191 mg (84%), white crystalline solid, mp = 134–135 °C, Rf = 0.50 (EtOAc/Hex, 1:5 v/v). 1H NMR (300 MHz, CDCl3): δ 1.49 (6H, d, J = 7.0 Hz, CH-(CH3)2), 3.47 (1H, hept, J = 7.0 Hz, CH-(CH3)2), 3.79-7.44 (1H, m, CPh-H), 7.45-7.51 (3H, m, CPh 3,5-H and NPh 4-H), 7.54-7.59 (2H, m, NPh 3,5-H), 7.74-7.82 (2H, m, CPh 2,6-H), 7.93-7.99 (2H, m, NPh 2,6-H), 8.76 (1H, s, 3-H). 13C NMR (75 MHz, CDCl3): δ 12.1 (CH3(CH3)2), 35.6 (CH2(CH3)2), 78.8 (C-7), 117.0 (C-3a), 121.7 (NPh C-3,5), 122.5 (C-3), 127.8 (CPh C-3,5), 128.2 (CPh C-4), 129.0 (NPh C-4), 129.8 (NPh C-3,5), 130.4 (CPh C-2,6), 140.1 (NPh C-1), 142.5 (CPh C-1), 154.3 (C-7a), 154.9 (C-6), 163.8 (C-4). 15N NMR (71 MHz, CDCl3): δ −158.9 (N-2), −90.3 (N-1), −82.7 (N-5). IR (ν, cm−1): 3114, 3083, 3062 (CH-arom), 2970, 2928, 2868 (CH-aliph), 1584, 1507, 1468, 1391, 1212 (C=C, C=N, C=N), 1106, 1023, 915, 763, 697 (CH=CH of monosubstituted benzenes). MS (ES+): m/z (%): 440 ([M + H]+, 99.7). HRMS (ESI) for C21H10N3I ([M + H]+): requires 440.0618 and found 440.0618.

7-Iodo-2,4,6-triphenyl-2H-pyrazolo[4,3-c]pyridine 17

7-Iodo-2,4,6-triphenyl-2H-pyrazolo[4,3-c]pyridine 17 was prepared in accordance with general procedure (C) from 4-azido(phenyl)methyl)-1-phenyl-3-(phenylethynyl)-1H-pyrazole 12 (62 mg, 0.165 mmol), K3PO4 (210 mg, 0.83 mmol), and I2 (175 mg, 0.83 mmol) in DCM (1.7 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 69 mg (88%), white crystalline solid, mp = 93–94 °C, Rf = 0.59 (EtOAc/Hex, 1:3 v/v). 1H NMR (300 MHz, CDCl3): δ 7.43-7.49 (2H, m, C6Ph 4-H and NPh 4-H), 7.49-7.58 (7H, m, C6Ph, NPh and C4Ph 3,5-H; C4Ph 4-H), 7.80-7.87 (2H, m, C6Ph 2,6-H), 7.94-8.00 (2H, m, NPh 2,6-H), 8.04-8.12 (2H, m, C4Ph 2,6-H), 8.90 (1H, s, 3-H). 13C NMR (150 MHz, CDCl3): δ 80.3 (C-7), 116.7 (C-3a), 121.5 (NPh C-2,6), 123.7 (C-3), 127.8 (C6Ph C-3,5), 128.2 (C6Ph C-4), 128.4 (C4Ph C-2,6), 128.9 (C4Ph C-3,5), 129.0 (NPh C-4), 129.7 (NPh C-3,5), 129.9 (C4Ph C-4), 130.2 (C6Ph C-2,6), 138.4 (C4Ph C-1), 139.7 (NPh C-1), 142.3 C6Ph C-1), 154.5 (C-7a), 154.7 (C-4), 155.4 (C-6). 15N NMR (71 MHz, CDCl3): δ −146.4 (N-2), −90.3 (N-1), −82.7 (N-5) not found. IR (KBr, ν, cm−1): 3058 (CH-arom), 2922 (CH-aliph), 1570, 1505, 1464, 1371, 1212 (C=C, C=N, C=N), 969, 750, 698 (CH=CH of monosubstituted benzenes). MS (ES+): m/z (%): 474 ([M + H]+, 99). HRMS (ESI) C24H12IN3 ([M + H]+): requires 474.0462 and found 474.0462.

3.2.5. General Procedure (D) for the Synthesis of 7-Substituted Pyrazolo[4,3-c]pyridine derivatives 18–39 by Suzuki–Miyaura Cross-Coupling with Boronic acids

To a solution of appropriate7-ido-2H-pyrazolo[4,3-c]pyridine 13–17 (1 equivalent) in a mixture of EtOH and water (3:1, v/v), boronic acid (1.2 equivalents), Cs2CO3 (2 equiv-
alents), and Pd(OAc)$_2$ (0.07 equivalents) were added under argon atmosphere. The mixture was stirred at 100 °C under microwave irradiation (100 W and 300 Pa) for 0.5–1 h. Upon completion (monitored by TLC), the reaction mixture was cooled to room temperature and filtered through a pad of Celite, and the filter cake was washed with EtOAc (20 mL). The filtrate was diluted with water (20 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography.

2,6,7-Triphenyl-2H-pyrazolo[4,3-c]pyridine 18

2,6,7-Triphenyl-2H-pyrazolo[4,3-c]pyridine 18 was prepared in accordance with general procedure (D) from 7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 13 (60 mg, 0.15 mmol), phenylboronic acid (22 mg, 0.18 mmol), Cs$_2$CO$_3$ (98 mg, 0.3 mmol), Pd(OAc)$_2$ (2.4 mg, 0.01 mmol), EtOH (0.9 mL), and water (0.3 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:4 to 1:2 v/v). Yield: 50 mg (96%), brown crystalline solid, mp = 151–152 °C, R$_f$ = 0.08 (EtOAc/Hex, 1:3 v/v). $^1$H NMR (700 MHz, CDCl$_3$): δ 7.21–7.26 (3H, m, C6Ph 3,4,5-H), 7.28–7.31 (1H, m, C7Ph 4-H), 7.31–7.35 (2H, m, C7Ph 3,5-H), 7.42–7.46 (3H, m, C6Ph 2,6-H and NPh 4-H), 7.49–7.55 (4H, m, NPh, 3,5-H and C7Ph 2,6-H), 7.89–7.94 (2H, m NPh 2,6-H). 8.66 (1H, s, 3-H), 9.32 (1H, s, 4-H). $^{13}$C NMR (176 MHz, CDCl$_3$): δ 120 (C-3a), 121.4 (NPh C-2,6), 121.8 (C-3), 123.1 (C-6), 127.2 (C6Ph C-4), 127.3 (C7Ph C-4), 127.8 (C6Ph C-2,6), 128.0 (C7Ph C-3,5), 128.7 (NPh C-4), 129.6 (NPh C-3,5), 130.5 (C7Ph C-2,6), 131.1 (C7Ph C-2,6), 131.5 (C7Ph C-1), 140.0 (NPh C-1), 140.6 (C6Ph C-1), 145.7 (C-4), 149.3 (C-4), 151.2 (C-7a).

$^1$N NMR (71 MHz, CDCl$_3$): δ −144.9 (N-2), −96.8 (N-1), −80.5 (N-5). IR (v, cm$^{-1}$): 3502, 3081 (CH$_3$-pyr), 1663, 1610, 1592, 1504, 1203, 1180, 1127 (C=C, C=N, C=N), 761, 697, 688 (CH=CH of monosubstituted benzenes). MS (ES$^+$): $m/z$ (%): 348 [(M + H)$^+$], 98. HRMS (ESI) for C$_{25}$H$_{18}$N$_3$ [(M + H)$^+$]: requires 348.1495 and found 348.1495.

7-(2-Methoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 19

7-(2-Methoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 19 was prepared in accordance with general procedure (D) from 7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 13 (60 mg, 0.15 mmol), (2-methoxyphenyl)boronic acid (27 mg, 0.18 mmol), Cs$_2$CO$_3$ (98 mg, 0.3 mmol), Pd(OAc)$_2$ (2.4 mg, 0.01 mmol), EtOH (0.9 mL), and water (0.3 mL). The reaction was stopped after 1 h. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 23 mg (40%), light yellow crystalline solid, mp = 169–170 °C, R$_f$ = 0.08 (EtOAc/Hex, 1:3 v/v). $^1$H NMR (700 MHz, CDCl$_3$): δ 3.42 (3H, s, OCH$_3$), 6.82–6.87 (1H, m, C7Ph), 7.00–7.03 (1H, m, C7Ph), 7.17–7.21 (1H, m, C7Ph), 7.21–7.24 (2H, m, Ph), 7.30–7.35 (1H, m, Ph), 7.39–7.42 (1H, m, Ph), 7.43–7.46 (2H, m, Ph), 7.47–7.51 (3H, m, Ph), 7.81–7.92 (2H, m, NPh, 2,6-H), 8.61 (s, 1H, 3-H), 9.34 (s, 1H, 4-H).

$^{13}$C NMR (176 MHz, CDCl$_3$): δ 55.3, 111.6, 119.7, 120.1, 120.7, 121.6, 121.8, 125.1, 127.1, 127.6, 128.7, 129.3, 129.6, 129.7, 132.5, 140.3, 141.5, 145.9, 150.4, 151.6, 157.0. IR (v, cm$^{-1}$): 3062, 3019 (CH$_3$-pyr), 2922, 2852 (CH$_2$-pyr), 1600, 1590, 1501, 1478, 1435, 1242, 1233, 1203 (C=C, C=N, C=N), 1112, 1043, 1021 (C-O-C), 764, 750, 697, 666 (CH=CH of monosubstituted benzenes). MS (ES$^+$): $m/z$ (%): 378 [(M + H)$^+$], 99. HRMS (ESI) for C$_{25}$H$_{20}$N$_3$ [(M + H)$^+$]: requires 378.1601 and found 378.1601.

7-(3-Methoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 20

7-(3-Methoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 20 was prepared in accordance with general procedure (D) from 7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 13 (60 mg, 0.15 mmol), (3-methoxyphenyl)boronic acid (27 mg, 0.18 mmol), Cs$_2$CO$_3$ (98 mg, 0.3 mmol), Pd(OAc)$_2$ (2.4 mg, 0.01 mmol), EtOH (0.9 mL), and water (0.3 mL). The reaction was finished after 1 h. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 44 mg (78%), white crystalline solid, mp = 71–72 °C, R$_f$ = 0.08 (EtOAc/Hex, 1:3 v/v). $^1$H NMR (700 MHz, CDCl$_3$): δ 3.66 (3H, s,
Molecules 2021, 26, 6747

7-(4-Methoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 21

7-(4-Methoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 21 was prepared in accordance with general procedure (D) from 7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 13 (60 mg, 0.15 mmol), (4-methoxyphenyl)boronic acid (27 mg, 0.18 mmol), Cs$_2$CO$_3$ (98 mg, 0.3 mmol), Pd(OAc)$_2$ (2.4 mg, 0.01 mmol), EtOH (0.9 mL), and water (0.3 mL). The reaction was finished after 1 h. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 44 mg (78%), white crystalline solid, mp = 192–193 °C, $R_f = 0.08$ (EtOAc/Hex, 1:3 v/v). $^1$H NMR (700 MHz, CDCl$_3$): $\delta$ 3.82 (3H, s, OCH$_3$), 6.82–6.93 (2H, m, C7Ph 3,5-H), 7.21–7.25 (1H, m, C6Ph 4-H), 7.25–7.30 (2H, m, C6Ph 3,5-H), 7.41–7.49 (5H, m, NPh 2,6-H, C6Ph 2,6-H), 7.50–7.56 (2H, m, NPh 3,5-H), 7.84–7.97 (2H, m, NPh 2,6-H), 8.63 (1H, s, N-3-H). $^{13}$C NMR (176 MHz, CDCl$_3$): $\delta$ 55.2 (OCH$_3$), 113.5 (C7Ph 3,5), 120.1 (C-3a), 121.4 (NPh C-2,6), 121.7 (C-6), 122.6 (C-7), 127.0 (C6Ph C-4), 127.8 (C7Ph C-1), 127.9 (C6Ph C-3,5), 128.7 (NPh C-4), 129.6 (NPh C-3,5), 130.5 (C6Ph C-2,6), 132.4 (C7Ph C-2,6), 140.1 (NPh C-1), 140.9 (C6Ph C-1), 145.3 (C-4), 149.2 (C-6), 151.4 (C-7a), 158.9 (C7Ph C-4). $^{15}$N NMR (71 MHz, CDCl$_3$): $\delta$ $-145.4$ (N-2), $-97.0$ (N-1), $-79.1$ (N-5). IR (cm$^{-1}$): 3135, 3062, 3020 (CH$_2$), 2927, 2837 (CH$_{aliph}$), 1589, 1503, 1438, 1290, 1252 (C=C, C=N, C=N), 1178, 1031 (C-O-C), 763, 758, 689 (CH=CH of mono- and disubstituted benzenes). MS (ES$^+$): $m/z$ (%): 378 ([M + H]$^+$, 100). HRMS (ESI) for C$_{25}$H$_{20}$N$_3$O (M + H)$^+$: requires 378.1603 and found 378.1601.

7-(3,4-Dimethoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 22

7-(3,4-Dimethoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 22 was prepared in accordance with general procedure (D) from 7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 13 (60 mg, 0.15 mmol), (3,4-dimethoxyphenyl)boronic acid (33 mg, 0.18 mmol), Cs$_2$CO$_3$ (98 mg, 0.3 mmol), Pd(OAc)$_2$ (2.4 mg, 0.01 mmol), EtOH (0.9 mL), and water (0.3 mL). The reaction was finished after 1 h. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 44 mg (72%), white crystalline solid, mp = 163–164 °C, $R_f = 0.08$ (EtOAc/Hex, 1:3 v/v). $^1$H NMR (700 MHz, CDCl$_3$): $\delta$ 3.61 (3H, s, OCH$_3$), 3.90 (3H, s, 3-OCH$_3$), 6.85–6.90 (1H, m, C7Ph 5-H), 6.91–6.95 (1H, m, C7Ph 2-H), 7.21–7.30 (4H, m, C6Ph 3,4,5-H, C7Ph 6-H), 7.42–7.45 (1H, m, NPh 4H), 7.45–7.49 (2H, m, NPh 2,6-H), 7.50–7.56 (2H, m, NPh 3,5-H), 7.87–7.98 (2H, m, NPh 2,6-H), 8.65 (1H, s, N-3-H), 9.28 (1H, s, 4-H). $^{13}$C NMR (176 MHz, CDCl$_3$): $\delta$ 55.7 (3-OCH$_3$), 55.9 (4-OCH$_3$), 69.0 (C7Ph 2-H), 114.9 (C7Ph 2-C), 120.3 (C-3a), 121.5 (NPh C-2,6), 121.9 (C-3), 122.8 (C-7), 124.0 (C7Ph C-6), 127.2 (C6Ph C-4), 128.0 (C7Ph C-1), 128.1 (C6Ph C-3,5), 128.8 (NPh C-4), 129.8 (NPh C-3,5), 130.5 (C6Ph C-2,6), 134.0 (C7Ph C-2,6), 140.2 (NPh C-1), 141.2 (C6Ph C-1), 145.4 (C-4), 148.4 (C7Ph C-3,4), 149.4 (C-6), 151.4 (C-7a). $^{15}$N NMR (71 MHz, CDCl$_3$): $\delta$ $-145.4$ (N-2), $-97.1$ (N-1), $-79.2$ (N-5). IR (cm$^{-1}$): 3042, 3019 (CH$_{arom}$), 2967, 2919, 2850 (CH$_{aliph}$), 1592, 1507, 1468, 1253, 1225 (C=C, C=N, C=N), 1141, 1014 (C-O-C), 753, 699, 688 (CH=CH of mono- and disubstituted benzenes). MS (ES$^+$): $m/z$ (%): 408 ([M + H]$^+$, 100). HRMS (ESI) for C$_{26}$H$_{22}$N$_3$O$_2$ (M + H)$^+$: requires 408.1707 and found 408.1707.
4-(2,6-Diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol 23

4-(2,6-Diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol 23 was prepared in accordance with general procedure (D) from 7-ido-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 13 (80 mg, 0.2 mmol), (4-hydroxyphenyl)boronic acid (33 mg, 0.24 mmol), Cs₂CO₃ (131 mg, 0.4 mmol), Pd(OAc)₂ (3 mg, 0.014 mmol), EtOH (1.2 mL), and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:2 to 1:1 v/v). Yield: 44 mg (60%), yellowish crystalline solid, mp = 307–308 °C, Rₓ = 0.18 (EtOAc/Hex, 1:2 v/v). ¹H NMR (700 MHz, DMSO-d₆): δ 6.70–6.75 (2H, m, C7Ph 3,5-H), 7.20–7.28 (5H, m, C7Ph 2,6-H, C6Ph 3,4,5-H), 7.37–7.40 (2H, m, C6Ph 2,6-H), 7.49–7.52 (1H, m, NPh 4-H), 7.59–7.63 (2H, m, NPh 3,5-H), 8.04–8.09 (2H, m, NPh 2,6-H), 9.29 (1H, s, 4-H), 9.46 (1H, s, 3-H), 9.50 (1H, s, OH). ¹³C NMR (176 MHz, DMSO-d₆): δ 115.0 (C7Ph C-3,5), 119.7 (C-3a), 121.0 (NPh C-2,6), 122.3 (C-7), 124.2 (C-3), 125.8 (C7Ph C-1), 127.0 (C6Ph C-4), 127.6 (C6Ph C-3,5), 128.7 (NPh C-4), 129.8 (NPh C-3,5), 130.3 (C6Ph C-2,6), 132.0 (C7Ph C-2,6), 139.5 (NPh C-1), 140.6 (C6Ph C-1), 145.5 (C-4), 147.8 (C-6), 150.5 (C-7a), 156.7 (C7Ph C-4). ¹⁵N NMR (71 MHz, DMSO-d₆): δ = 144.8 (N-2), N-1 and N-5 not found. IR (KBr, cm⁻¹): 3449 (OH), 2924 (CH₃), 1607, 1592, 1505, 1440, 1273, 1172 (C=C, C=N, C=N), 767, 695 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): m/z (%): 364 ([M + H]⁺, 96). HRMS (ESI) for C₂₅H₁₈N₃O ([M + H]⁺): requires 364.1444 and found 364.1446.

4-Methyl-2,6,7-triphenyl-2H-pyrazolo[4,3-c]pyridine 24

4-Methyl-2,6,7-triphenyl-2H-pyrazolo[4,3-c]pyridine 24 was prepared in accordance with general procedure (D) from 7-ido-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 14 (50 mg, 0.12 mmol), phenylboronic acid (18 mg, 0.145 mmol), Cs₂CO₃ (79 mg, 0.24 mmol), Pd(OAc)₂ (1.9 mg, 0.008 mmol), EtOH (0.9 mL), and water (0.3 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:4 to 1:2 v/v). Yield: 116 mg (94%), mp = 202–205 °C, Rₓ = 0.17 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 2.93 (3H, s, CH₃), 7.19–7.24 (3H, m, C7Ph), 7.25–7.29 (1H, m, C6Ph 4-H), 7.32–7.33 (2H, m, C6Ph 3,5-H), 7.41–7.48 (5H, m, C6Ph 2,6-H, NPh 4-H and C7Ph), 7.49–7.54 (2H, m, NPh 3,5-H), 7.89–7.91 (2H, m, NPh 2,6-H), 8.61 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 22.8 (CH₃), 120.0 (C-3a), 121.1 (C-7), 121.3 (NPh C-2,6), 121.9 (C-3), 127.1 (C7Ph C-4), 127.2 (C6Ph C-4), 127.6 (C7Ph C-1), 127.8 (C7Ph), 127.9 (C6Ph C-3,5), 128.6 (NPh C-4), 129.6 (NPh C-3,5), 130.6 (C6Ph C-2,6), 132.0 (C7Ph C-2,6), 135.7 (C6Ph C-1), 140.0 (NPh C-1), 148.8 (C-6), 151.5 (C-7a), 154.5 (C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ = −145.9 (N-2), −95.0 (N-1), −88.2 (N-5). IR (KBr, cm⁻¹): 3137, 3061 (CH₃), 2916 (CH₃aliph), 1588, 1545, 1505, 1476, 1371 (C=C, C=N, C=N), 762, 700, 661 (CH=CH of monosubstituted benzenes). MS (ES⁺): m/z (%): 362 ([M + H]⁺, 100). HRMS (ESI) for C₂₅H₂₀N₃ ([M + H]⁺): requires 362.1652 and found 362.1650.

7-(2-Methoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 25

7-(2-Methoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 25 was prepared in accordance with general procedure (D) from 7-ido-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 14 (50 mg, 0.12 mmol), (2-methoxyphenyl)boronic acid (22 mg, 0.145 mmol), Cs₂CO₃ (79 mg, 0.24 mmol), Pd(OAc)₂ (1.9 mg, 0.008 mmol), EtOH (0.9 mL), and water (0.3 mL). The reaction was finished after 1 h. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 38 mg (80%), light yellow crystalline solid, mp = 159–160 °C, Rₓ = 0.13 (EtOAc/Hex, 1:3 v/v). ¹H NMR (500 MHz, CDCl₃): δ 2.95 (3H, s, CH₃), 3.42 (3H, s, OCH₃), 6.81–6.85 (1H, m, C7Ph 3-H), 6.97–7.01 (1H, m, C6Ph 4-H), 7.17–7.23 (3H, m, NPh 3,5-H and C6Ph 4-H), 7.28–7.32 (1H, m, C7Ph 4-H), 7.39–7.45 (4H, m, NPh 4-H, C6Ph 2,6-H and C7Ph 5-H), 7.47–7.51 (2H, m, C6Ph 3,5-H), 7.85–7.89 (2H, m, NPh, 2,6-H), 8.62 (1H, s, 3-H). ¹³C NMR (126 MHz, CDCl₃): δ 22.9 (CH₃), 55.4 (OCH₃), 111.5 (C7Ph C-3), 117.8 (C-7), 120.1 (C-3a), 120.6 (C7Ph C-5), 121.6 (NPh C-2,6), 122.2 (C-3), 125.0 (C7Ph C-1), 127.2 (C6Ph C-4), 127.6 (NPh C-3,5), 128.7 (NPh C-4), 129.2 (C7Ph C-4), 129.7 (C6Ph C-2,3,5,6), 132.6 (C7Ph C-5), 140.3 (NPh
Molecules, 26, 6747

C-1), 140.8 (C6Ph C-1), 149.8 (C-6), 151.8 (C-7a), 154.8 (C-4), 157.1 (C7Ph C-2). IR (v, cm⁻¹): 3143, 3069, 3019 (CH₃), 2955, 2922, 2853 (CH₂), 1599, 1586, 1578, 1552, 1504, 1495, 1479, 1434, 1372, 1240, 1217 (C=C, C=N, C–N), 1046, 1022 (C-O-C), 750, 698, 685 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): m/z (%): 392 ([M + H⁺], 100). HRMS (ESI) for C₁₉H₁₂N₃O ([M + H⁺]⁺): requires 392.1758 and found 392.1757.

7-(3-Methoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 26

7-(3-Methoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 26 was prepared in accordance with general procedure (D) from 7-iodo-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 14 (50 mg, 0.12 mmol), (3-methoxyphenyl)boronic acid (22 mg, 0.145 mmol), Cs₂CO₃ (79 mg, 0.24 mmol), Pd(OAc)₂ (1.9 mg, 0.008 mmol), EtOH (0.9 mL), and water (0.3 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 38 mg (80%), light yellow crystalline solid, mp = 190–191 °C, Rₓ = 0.18 (EtOAc/Hex, 1:3 v/v). ¹H NMR (500 MHz, CDCl₃): δ 2.97 (3H, s, CH₃), 3.65 (3H, s, OCH₃), 6.79–6.85 (1H, m, C7Ph 4-H), 6.96–7.03 (1H, m, C7Ph 2-H), 7.06–7.12 (1H, m, C7Ph 6-H), 7.17–7.32 (4H, m, C7Ph 5-H, C6Ph 3,4,5-H), 7.41–7.47 (1H, m, NPh 4-H), 7.50–7.55 (4H, m, NPh 3,5-H, C6Ph 2,6-H), 7.86–7.95 (2H, m, NPh 2,6-H), 8.67 (1H, s, 3-H). ¹³C NMR (126 MHz, CDCl₃): δ 23.1 (CH₃), 55.2 (OCH₃), 113.4 (C7Ph C-4), 116.6 (C7Ph C-2), 120.1 (C-3a), 120.8 (C-7), 121.3 (NPh C-2,6), 121.9 (C-3), 123.9 (C7Ph C-6), 127.2 (C6Ph C-4), 127.9 (C6Ph C-3,5), 128.6 (NPh C-4), 129.0 (C7Ph C-5), 129.7 (NPh C-3,5), 130.6 (C6Ph C-2,6), 137.2 (C7Ph C-1), 140.1 (NPh C-1), 140.8 (C6Ph C-1), 149.3 (C-6), 151.4 (C-7a), 154.6 (C-4), 159.2 (C7Ph C-3). IR (v, cm⁻¹): 3067, 3002 (CH₂), 2954, 2923, 2852 (CH₂), 1599, 1589, 1575, 1547, 1488, 1443, 1284, 1211 (C=C, C=N, C–N), 1160, 1049 (C=C, C–N), 753, 700, 686 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): m/z (%): 392 ([M + H⁺], 100). HRMS (ESI) for C₁₉H₁₂N₃O ([M + H⁺]⁺): requires 392.1757 and found 392.1757.

7-(4-Methoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 27

7-(4-Methoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 27 was prepared in accordance with general procedure (D) from 7-iodo-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 14 (50 mg, 0.12 mmol), (4-methoxyphenyl)boronic acid (22 mg, 0.145 mmol), Cs₂CO₃ (79 mg, 0.24 mmol), Pd(OAc)₂ (1.9 mg, 0.008 mmol), EtOH (0.9 mL), and water (0.3 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 118 mg (89%), mp = 157–161 °C, Rₓ = 0.15 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 2.92 (3H, s, CH₃), 3.84 (3H, s, OCH₃), 6.88–6.89 (2H, m, C7Ph 3,5-H), 7.23–7.25 (1H, m, C6Ph 4-H), 7.27–7.30 (2H, m, C6Ph 3,5-H), 7.42–7.45 (3H, m, NPh 4-H and C7Ph 2,6-H), 7.49–7.50 (2H, m, C6Ph 2,6-H), 7.52–7.54 (2H, m, NPh 3,5-H), 7.92–7.93 (2H, m, NPh 2,6-H), 8.61 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 23.2 (CH₃), 55.3 (OCH₃), 113.6 (C7Ph C-3,5), 120.2 (C-3a), 120.5 (C-7), 121.4 (NPh C-2,6), 121.7 (C-3), 127.0 (C6Ph C-4), 127.9 (C6Ph C-3,5), 128.3 (NPh C-4), 128.5 (C7Ph C-1), 129.7 (NPh C-3,5), 130.7 (C6Ph C-2,6), 132.5 (C7Ph C-2,6), 149.2 (C-6), 151.8 (C-7a), 154.0 (C-4), 158.7 (C7Ph C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ –146.8 (N-2), –95.7 (N-1), –81.6 (N-5). IR (KBr, v, cm⁻¹): 3056, 3012 (CH₂), 2951, 2834, 2903, 2831 (CH₂), 1607, 1598, 1588, 1507, 1247 (C=C, C=N, C–N), 1175, 1038 (C–O), 755, 728, 701, 685 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): m/z (%): 392 ([M + H⁺], 100). HRMS (ESI) for C₂₀H₁₄N₃O ([M + H⁺]⁺): requires 392.1757 and found 392.1757.

7-(3,4-Dimethoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 28

7-(3,4-Dimethoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 28 was prepared in accordance with general procedure (D) from 7-iodo-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 14 (50 mg, 0.12 mmol), (3,4-dimethoxyphenyl)boronic acid (26 mg, 0.145 mmol), Cs₂CO₃ (79 mg, 0.24 mmol), Pd(OAc)₂ (1.9 mg, 0.008 mmol), EtOH (0.9 mL), and water (0.3 mL). The reaction was finished after 1 h. The desired compound
was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 41 mg (67%), light yellow crystalline solid, mp = 180–181 °C, Rf = 0.12 (EtOAc/Hex, 1:3 v/v). 1H NMR (700 MHz, CDCl3): δ 2.91 (3H, s, CH3), 3.60 (3H, s, 3-OCH3), 3.89 (3H, s, 4-OCCH3), 6.85–6.90 (2H, m, C7Ph 2,5-H), 7.20–7.24 (2H, m, C7Ph 6-H, C6Ph 4-H), 7.33–7.44 (2H, m, CPh 2,6-H), 7.41–7.44 (1H, m, NPh 4-H). IR (KBr, ν cm⁻¹): 2920, 2857 (CH, aliph), 1702, 1608 (C=O, arom). HRMS (ESI) for C27H24N3O2 ([M + H]+): requires 422.1863 and found 422.1863.

4-(4-Methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine-7-yl)phenol 29

4-(4-Methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine-7-yl)phenol 29 was prepared in accordance with general procedure (D) from -iodo-4-methyl 2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 14 (80 mg, 0.2 mmol), (4-hydroxyphenyl)boronic acid (32 mg, 0.23 mmol), Cs2CO3 (127 mg, 0.59 mmol), Pd(OAc)2 (3 mg, 0.014 mmol), EtOH (1.2 mL), and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:4 to 1:2 v/v). Yield: 37 mg (50%), yellowish crystalline solid, mp = 269–270 °C, Rf = 0.20 (EtOAc/Hex, 1:2 v/v). 1H NMR (700 MHz, DMSO-d6): δ 2.80 (3H, s, CH3), 6.68–6.73 (2H, m, C7Ph 3,5-H), 7.16–7.19 (2H, m, C7Ph 2,6-H), 7.20–7.22 (1H, m, C6Ph 4-H), 7.22–7.26 (2H, m, C6Ph 3,5-H), 7.34–7.38 (2H, m, C6Ph 2,6-H), 7.46–7.49 (1H, m, NPh 4-H), 7.57–7.63 (2H, m, NPh 3,5-H), 8.04–8.08 (2H, m, NPh 2,6-H), 9.44 (1H, s, OH), 9.51 (1H, s, 3-H). 13C NMR (176 MHz, DMSO-d6): δ 22.6 (CH3), 114.9 (C7Ph 3,5), 119.7 (C-3a), 120.0 (C-7), 120.6 (NPh 2,6), 123.9 (C-3), 124.3 (C7Ph C-1), 126.7 (C6Ph C-4), 127.4 (C6Ph C-3,5), 128.4 (NPh C-4), 129.7 (NPh C-3,5), 130.7 (C6Ph C-2,6), 130.9 (C6Ph C-2), 132.0 (C7Ph C-2,6), 139.6 (NPh C-1), 141.1 (C6Ph C-1), 148.0 (C-6), 150.9 (80 mg, 0.19 mmol), phenylboronic acid (31 mg, 0.23 mmol), Cs2CO3 (123 mg, 0.38 mmol), Pd(OAc)2 (3 mg, 0.013 mmol), EtOH (1.2 mL), and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:5 v/v). Yield: 58 mg (81%), yellowish crystalline solid, mp = 179–180 °C, Rf = 0.39 (EtOAc/Hex, 1:3 v/v). 1H NMR (700 MHz, CDCl3): δ 1.54 (3H, t, J = 7.6 Hz, CH3), 3.23 (2H, q, J = 7.6 Hz, CH2), 7.19–7.23 (1H, m, C6Ph 4-H), 7.23–7.26 (2H, m, C6Ph 3,5-H), 7.26–7.29 (1H, m, C7Ph 4-H), 7.29–7.34 (2H, m, C7Ph 3,5-H), 7.40–7.44 (1H, m, NPh 4-H), 7.46–7.50 (4H, m, C6Ph 2,6-H), 7.50–7.53 (2H, m, NPh 3,5-H), 7.87–7.95 (2H, m, NPh 2,6-H), 8.62 (1H, s, 3-H). 13C NMR (176 MHz, CDCl3): δ 13.3 (CH3), 30.4 (CH2), 119.0 (C-3a), 120.7 (C-7), 121.2 (C-4), 121.3 (NPh C-2,6), 127.0 (C6Ph and C7Ph C-4), 127.7 (C6Ph C-3,5), 127.9 (C7Ph C-3,5), 128.4 (NPh C-4), 129.5 (NPh C-3,5), 130.7 (C6Ph C-2,6), 131.2 (C7Ph C-2,6), 136.2 (C7Ph C-1), 140.2 (NPh C-1), 141.0 (C6Ph C-1), 149.1 (C-6), 151.9 (C-7a), 159.3 (C-4). 15N NMR (71 MHz, CDCl3): δ −147.3 (N-2), N-1 not found, −83.4 (N-5). IR (KBr, ν cm⁻¹): 3059 (CHarom), 2970, 2930 (CHaliph), 1587, 1547, 1476, 1371, 1213
(C=C, C=N, C–N), 753, 697, 686 (CH=CH monosubstituted benzenes). MS (ES⁺): m/z (%): 375 ([M + H]⁺, 99). HRMS (ESI) for C₂₆H₂₂N₃ ([M + H]⁺): requires 376.1808 and found 376.1808.

4-Ethyl-7-(4-methoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 31

4-Ethyl-7-(4-methoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 31 was prepared in accordance with general procedure (D) from 7-iodo-4-methyl 2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 15 (80 mg, 0.19 mmol), (4-methoxyphenyl)boronic acid (34 mg, 0.23 mmol), Cs₂CO₃ (123 mg, 0.38 mmol), Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL), and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:5 v/v). Yield: 63 mg (82%), yellow crystalline solid, mp = 181–182 °C, Rf = 0.34 (EtOAc/Hex, 1:3 v/v). ³¹H NMR (700 MHz, CDCl₃): δ 1.53 (3H, t, J = 7.6 Hz, CH₃), 3.21 (2H, q, J = 7.6 Hz, CH₂), 3.82 (3H, s, OCH₃), 6.83–6.90 (2H, m, C7Ph 3,5-H), 7.19–7.23 (1H, m, C6Ph 4-H), 7.24–7.27 (2H, m), C6Ph 3,5-H), 7.40–7.44 (1H, m, C7Ph 3-H), 7.44–7.48 (1H, m, C7Ph 5-H), 7.58–7.59 (2H, m, NPh 3,5-H), 7.87–7.90 (2H, m, NPh 2,6-H). 13C NMR (150 MHz, CDCl₃): δ 13.3 (CH₃), 30.4 (CH₂), 55.3 (2-OCH₃), 80.3, 84.6 (N-5). IR (KBr, ν, cm⁻¹): 3134, 3058, 2986, 2934, 2889 (CH=CH monosubstituted benzenes). MS (ES⁺): m/z (%): 435 ([M + H]⁺, 99). HRMS (ESI) for C₂₆H₂₂N₃O ([M + H]⁺): requires 456.1914 and found 456.1914.

7-(2,4-Dimethoxyphenyl)-4-ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 32

7-(2,4-Dimethoxyphenyl)-4-ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 32 was prepared in accordance with general procedure (D) from 7-iodo-4-methyl 2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 15 (80 mg, 0.19 mmol), (2,4-dimethoxyphenyl)boronic acid (41 mg, 0.23 mmol), Cs₂CO₃ (123 mg, 0.38 mmol), Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL), and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:5 v/v). Yield: 40 mg (48%), yellow crystalline solid, mp = 202–203 °C, Rf = 0.24 (EtOAc/Hex, 1:3 v/v). ³¹H NMR (700 MHz, CDCl₃): δ 1.53 (3H, t, J = 7.6 Hz, CH₃), 3.21 (2H, q, J = 7.6 Hz, CH₂), 3.37 (3H, s), 2-OC₃H₃), 3.83 (3H, s, 4-OC₃H₃), 6.38–6.44 (1H, m, C7Ph 3-H), 6.53–6.59 (1H, m, C7Ph 5-H), 7.15–7.19 (1H, m, C6Ph 4-H), 7.19–7.25 (2H, m, C6Ph 3,5-H), 7.35–7.41 (2H, m, NPh 2,6-H) and C7Ph 6-H), 7.45–7.51 (4H, m, C6Ph 2,6-H and NPh 3,5-H), 7.84–7.90 (2H, m, NPh 2,6-H), 8.58 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 13.3 (CH₃), 30.4 (CH₂), 55.1 (2-OCH₃), 55.3 (4-OCH₃), 99.1 (C7Ph C-3), 104.6 (C7Ph C-5), 116.8 (C-7), 117.9 (C7Ph C-1), 119.0 (C-3a), 121.2 (C-3), 121.4 (NPh C-2,6), 126.7 (C6Ph C-4), 127.4 (C6Ph C-3,5), 128.3 (NPh C-4), 129.5 (NPh C-3,5 and C6Ph C-2,6), 132.8 (C7Ph C-6), 140.3 (NPh C-1), 141.9 (C6Ph C-1), 149.9 (C-6), 151.2 (C7Ph C-2), 157.9 (C7Ph C-2), 159.0 (C-4), 160.5 (C7Ph C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ −147.9 (N-2), −146.4 (N-1), −84.6 (N-5). IR (KBr, ν, cm⁻¹): 3134, 3058 (CH₃N), 2966, 2930, 2836 (CH₂N), 1606, 1588, 1547, 1505, 1462, 1305, 1204 (C=C, C=N, C=N), 1027 (C-O), 829, 764, 705, 691 (CH=CH of mono- and trisubstituted benzenes). MS (ES⁺): m/z (%): 435 ([M + H]⁺, 100). HRMS (ESI) for C₂₆H₂₃N₃O₂ ([M + H]⁺): requires 436.2020 and found 436.2020.

4-Ethyl-2,6-diphenyl-7-(p-tolyl)-2H-pyrazolo[4,3-c]pyridine 33

4-Ethyl-2,6-diphenyl-7-(p-tolyl)-2H-pyrazolo[4,3-c]pyridine 33 was prepared in accordance with general procedure (D) from 7-iodo-4-methyl 2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 15 (80 mg, 0.19 mmol), (4-methylphenyl)boronic acid (31 mg, 0.23 mmol), Cs₂CO₃ (123 mg, 0.38 mmol), Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL), and water...
Molecules 2021, 26, 6747

4-Ethyl-2,6-diphenyl-7-[4-(trifluoromethyl)phenyl]-2H-pyrazolo[4,3-c]pyridine 34

4-Ethyl-2,6-diphenyl-7-[4-(trifluoromethyl)phenyl]-2H-pyrazolo[4,3-c]pyridine 34 was prepared in accordance with general procedure (D) from 7-iodo-4-methyl 2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 15 (80 mg, 0.19 mmol), 4-(trifluoromethyl)phenylboronic acid (43 mg, 0.23 mmol), Cs₂CO₃ (123 mg, 0.38 mmol), Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL), and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 74 mg (92%), yellowish crystalline solid, mp = 179–180 °C, Rf = 0.41 (EtOAc/Hex, 1:3 v/v).

1H NMR (700 MHz, CDCl₃): δ 1.52 (3H, t, J = 7.6 Hz, CH₃C₂H₃), 2.34 (3H, s, Ph-CH₃), 3.19 (2H, q, J = 7.6 Hz, CH₂C₂H₃), 7.09–7.14 (2H, m, C7Ph 3,5-H), 7.19–7.22 (1H, m, C₆Ph 4-H), 7.23–7.25 (2H, m, C₆Ph 3,5-H), 7.34–7.38 (2H, m, C₆Ph 2,6-H), 7.38–7.41 (1H, m, NPh 4-H), 7.46–7.51 (4H, m, NPh 3,5-H and C₇Ph 2,6-H), 7.86–7.91 (2H, m, NPh 2,6-H), 8.59 (1H, s, 3-H).

13C NMR (176 MHz, CDCl₃): δ 13.3 (CH₂C₂H₃), 21.3 (Ph-CH₃), 30.4 (CH₂C₂H₃), 119.0 (C-3a), 120.7 (C-7), 121.2 (C-4), 121.3 (NPh C-2,6), 126.9 (C₆Ph C-4), 127.7 (C₆Ph C-3,5), 128.3 (NPh C-4), 128.7 (C₇Ph C-3), 129.5 (NPh C-3,5), 130.6 (C₇Ph C-2,6), 131.0 (C₆Ph C-2,6), 133.0 (C₇Ph C-1), 136.5 (C₇Ph C-4), 140.2 (NPh C-1), 141.2 (C₆Ph C-1), 148.9 (C-6), 152.0 (C-7a), 159.0 (C-4). 15N NMR (71 MHz, CDCl₃): δ −147.5 (N-2), −96.1 (N-1), −83.3 (N-5). IR (KBr, cm⁻¹): 3028 (CH₃), 2990, 2939 (CH₂), 1587, 1505, 1463, 1377, 1211, 1045 (C=C, C=N, C=N), 822, 753, 699, 686 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): m/z (%): 443 [(M + H)⁺, 98]. HRMS (ESI) for C₂₇H₂₃F₃N₃ [(M + H)⁺]: requires 444.1682 and found 444.1682.

4-Ethyl-2,6-diphenyl-7-[4-(trifluoromethoxy)phenyl]-2H-pyrazolo[4,3-c]pyridine 35

4-Ethyl-2,6-diphenyl-7-[4-(trifluoromethoxy)phenyl]-2H-pyrazolo[4,3-c]pyridine 35 was prepared in accordance with general procedure (D) from 7-iodo-4-methyl 2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 15 (80 mg, 0.19 mmol), 4-(trifluoromethoxy)phenylboronic acid (47 mg, 0.23 mmol), Cs₂CO₃ (123 mg, 0.38 mmol), Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL), and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 74 mg (85%), white crystalline solid, mp = 153–154 °C, Rf = 0.41 (EtOAc/Hex, 1:3 v/v).

1H NMR (700 MHz, CDCl₃): δ 1.54 (3H, t, J = 7.6 Hz, CH₃C₂H₃), 3.19 (2H, q, J = 7.6 Hz, CH₂C₂H₃), 7.22–7.28 (3H, m, C₆Ph 3,4,5-H), 7.41–7.46 (3H, m, C₆Ph 2,6-H, NPh 4-H), 7.50–7.54 (2H, m, NPh 3,5-H), 7.55–7.58 (2H, m, C₇Ph 3,5-H), 7.59–7.63 (2H, m, C₇Ph 2,6-H), 7.88–7.92 (2H, m, NPh 2,6-H), 8.64 (1H, s, 3-H).

13C NMR (176 MHz, CDCl₃): δ 13.3 (CH₂C₂H₃), 30.4 (CH₂C₂H₃), 119.0 (C-3a), 119.2 (C-7), 121.3 (NPh C-2,6), 124.3 (C₆Ph C-3,5), 127.4 (C₆Ph C-4), 129.7 (C₆Ph C-3,5), 128.6 (NPh C-4), 128.8 (C₇Ph C-4), 129.6 (NPh C-3,5), 130.7 (C₆Ph C-2,6), 131.5 (C₇Ph C-2,6), 140.0 (NPh C-1), 140.1 (C₇Ph C-1), 140.4 (C₆Ph C-1), 149.6 (C-6), 151.5 (C-7a), 160.1 (C-4). 15N NMR (71 MHz, CDCl₃): δ −146.9 (N-2), −97.4 (N-1), −83.5 (N-5). 19F NMR (376 MHz, CDCl₃): δ −65.59 (3F, s, CF₃). IR (KBr, cm⁻¹): 3028 (CH₃), 2990, 2939 (CH₂), 1585, 1484, 1327, 1130 (C=C, C=N, C=N, C-F), 759, 696 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): m/z (%): 443 [(M + H)⁺, 99]. HRMS (ESI) for C₂₇H₂₃F₃N₃ [(M + H)⁺]: requires 444.1682 and found 444.1682.
7-(4-Chlorophenyl)-4-ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 36

7-(4-Chlorophenyl)-4-ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 36 was prepared in accordance with general procedure (D) from 7-iodo-4-methyl 2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 15 (80 mg, 0.19 mmol), 4-chlorophenylboronic acid (36 mg, 0.23 mmol), Cs₂CO₃ (123 mg, 0.38 mmol), Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL), and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 65 mg (83%), yellow crystalline solid, mp = 226–227 °C, Rf = 0.49 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 1.52 (3H, t, J = 7.6 Hz, CH₃), 3.21 (2H, q, J = 7.6 Hz, CH₂), 7.22–7.30 (5H, m, C₆Ph 3,4,5-H; C₇Ph 3,5-H), 7.40–7.44 (3H, m, C₇Ph 2,6-H; NPh 4-H), 7.44–7.47 (2H, m, C₆Ph 2,6-H), 7.50–7.53 (2H, m, NPh 3,5-H), 7.86–7.93 (2H, m, NPh 2,6-H), 8.61 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 13.3 (CH₃), 30.4 (CH₂), 119.0 (C-3a), 119.4 (C-7), 121.3 (NPh C-1), 127.2 (C₆Ph C-4), 127.9 (C₆Ph C-3,5), 128.2 (C₇Ph C-3,5), 128.5 (NPh C-4), 129.6 (NPh C-3,5), 130.6 (C₆Ph C-2,6), 132.5 (C₇Ph C-2,6), 132.8 (C₇Ph C-4), 134.6 (C₇Ph C-2,6), 140.1 (NPh C-1), 140.6 (C₆Ph C-1), 149.3 (C₆Ph C-4), 151.6 (C₇Ph 1,7a), 159.7 (C-4). IR (KBr, ν cm⁻¹): 3057 (CH₃), 2991, 2939 (CH₂), 2972, 2961, 2932, 2873 (CH₃), 1589, 1584, 1481, 1267, 1209 (C=C, C=N, C=N), 1040 (C-O), 816, 760, 695 (C=CH of mono- and disubstituted benzenes). MS (ES⁺): m/z (%): 460 (M + H⁺, 49). HRMS (ESI) for C₂₈H₂₁F₃N₃O [(M + H)⁺]: requires 460.1636 and found 460.1631.

4-(Ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol 37

4-(Ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol 37 was prepared in accordance with general procedure (D) from 7-iodo-4-methyl 2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 15 (80 mg, 0.19 mmol), (4-hydroxyphenyl)boronic acid (31 mg, 0.23 mmol), Cs₂CO₃ (123 mg, 0.38 mmol), Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL), and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 50 mg (67%), yellow-brown crystalline solid, mp = 199–200 °C, Rf = 0.17 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 1.50 (3H, t, J = 7.6 Hz, CH₃), 2.05 (1H, s, OH), 3.21 (2H, q, J = 7.6 Hz, CH₂), 6.58–6.64 (2H, m, C₇Ph 3,5-H), 7.15–7.18 (1H, m, C₆Ph C-4), 7.19–7.24 (4H, m, C₆Ph 3,5-H and C₇Ph 2,6-H), 7.40–7.45 (3H, m, NPh 2,6-H and NPh 4-H), 7.49–7.53 (2H, m, NPh 3,5-H), 7.85–7.90 (2H, m, NPh 2,6-H), 8.60 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 13.6 (CH₃), 30.1 (CH₂), 115.3 (C₇Ph C-3,5), 118.9 (C-3a), 120.7 (C-7), 121.6 (NPh C-2,6), 122.0 (C-3), 126.9 (C₆Ph C-4), 127.3 (C₇Ph C-1), 127.7 (C₆Ph C-3,5), 128.6 (NPh C-4), 129.6 (NPh C-3,5), 130.6 (C₆Ph C-2,6), 132.1 (C₇Ph C-2,6), 140.0 (NPh C-1), 140.8 (C₇Ph C-1), 148.9 (C-6), 152.1 (C-7a), 155.3 (C₇Ph C-4), 159.0 (C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ −147.5 (N-2), −98.5 (N-1), −85.0 (N-5). IR (KBr, ν cm⁻¹): 3147 (OH), 3064 (CH₃), 2963, 2932, 2873 (CH₃), 1613, 1586, 1507, 1481, 1267, 1209 (C=C, C=N, C=N), 1040 (C-O), 816, 760, 695 (C=CH of mono- and disubstituted benzenes). MS (ES⁺): m/z (%): 391 [(M + H)⁺, 98]. HRMS (ESI) for C₂₆H₂₂N₂O [(M + H)⁺]: requires 392.1757 and found 392.1757.

4-Isopropyl-7-(4-methoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 38

4-Isopropyl-7-(4-methoxyphenyl)-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 38 was prepared in accordance with general procedure (D) from 7-iodo-4-isopropyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 16 (100 mg, 0.23 mmol), 4-methoxyphenylboronic acid (42 mg, 0.27 mmol), Cs₂CO₃ (148 mg, 0.46 mmol), Pd(OAc)₂ (4 mg, 0.015 mmol), EtOH (1.5 mL), and water (0.5 mL). The reaction was finished after 1 h. The desired compound...
was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 80 mg (83%), white crystalline solid, mp = 159–160 °C, Rf = 0.54 (EtOAc/Hex, 1:3 v/v).

1H NMR (700 MHz, CDC13): δ 1.55 (6H, d, J = 7.0 Hz, CH-(CH3)2), 3.53 (1H, p, J = 7.0 Hz, CH-(CH3)2), 3.83 (3H, s, OCH3), 6.86–6.91 (2H, m, C7Ph 3,5-H), 7.20–7.24 (1H, m, C6Ph 4-H), 7.24–7.28 (2H, m, C6Ph 3,5-H), 7.39–7.45 (3H, m, C7Ph 2,6-H and NPh 4-H), 7.50–7.55 (4H, m, C6Ph 2,6-H and NPh 3,5-H), 7.89–7.94 (2H, m, NPh 2,6-H), 8.63 (1H, s, 3-H).

13C NMR (176 MHz, CDCl3): δ 22.0 (CH-(C6H2)), 36.2 (CH-(CH3)2), 55.3 (OCH3), 113.7 (C7Ph C-3,5), 118.1 (C-3a), 120.3 (C-7), 121.2 (C-3), 121.5 (NPh C-2,6), 127.0 (C6Ph C-4), 127.8 (C6Ph C-3,5), 128.5 (NPh C-4), 128.6 (C7Ph C-1), 129.7 (NPh C-3,5), 131.0 (C6Ph C-2,6), 132.4 (C7Ph C-2,6), 140.4 (NPh C-1), 141.4 (C6Ph C-1), 148.5 (C-6a), 152.6 (C-7a), 158.7 (C7Ph C-1), 123.0 (C6Ph C-4), 0.8–8.20 (2H, m, C6Ph 2,6-H), 8.80 (1H, s, 3-H).

3.2.6. General Procedure (E) of 4-(4-Ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 39

4-(4-Ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol 37 Alkylation

4-(4-Ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol 37 (1 equivalent) was dissolved in DMF. Then, NaH (60% in mineral oil) (1.1 equivalents) was added at room temperature. Then, an appropriate amount of alkyl iodide (1.1 equivalents) was added at 70 °C and the mixture was stirred for 1 h. Upon completion (monitored by TLC), the reaction mixture was cooled to room temperature, diluted with water (20 mL), and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na2SO4, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography.

7-(4-Methoxyphenyl)-2,4,6-triphenyl-2H-pyrazolo[4,3-c]pyridine 39

7-(4-Methoxyphenyl)-2,4,6-triphenyl-2H-pyrazolo[4,3-c]pyridine 39 was prepared in accordance with general procedure (E) from (4-ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol 37 (60 mg, 0.15 mmol), NaH (60%) (7 mg, 0.17 mmol), ethyl iodide (0.014 mL, 0.17 mmol), and DMF (2 mL). The desired compound was obtained after
purification by column chromatography (EtOAc/Hex, 1:4 v/v). Yield: 62 mg (97%), yellow crystalline solid, mp = 140–141 °C, Rf = 0.38 (EtOAc/Hex, 1:3 v/v). 1H NMR (700 MHz, CDCl3): δ 1.42 (3H, t, J = 7.0 Hz, OCH2CH3), 1.52 (3H, t, J = 7.6 Hz, CH2CH3), 3.20 (2H, q, J = 7.6 Hz, CH2CH3), 4.04 (2H, q, OCH2CH3), 6.82–6.88 (2H, m, C7Ph 3,5-H), 7.19–7.23 (1H, m, C6Ph 4-H), 7.23–7.27 (2H, m, C6Ph 3,5-H), 7.37–7.43 (3H, m, NPh 4-H and C7Ph 2,6-H), 7.47–7.52 (4H, m, NPh 3,5-H and C7Ph 2,6-H), 7.88–7.93 (2H, m, NPh 2,6-H), 8.60 (1H, s, 3-H). 13C NMR (176 MHz, CDCl3): δ 13.4 (CH2CH3), 14.9 (OCH2CH3), 30.3 (CH2CH3), 63.2 (OCH2CH3), 114.0 (C7Ph C-3,5), 119.0 (C-3a), 120.4 (C-7), 121.2 (C-3), 121.3 (NPh C-2,6), 126.8 (C6Ph C-4), 127.7 (C6Ph C-3,5), 128.1 (C7Ph C-1), 128.4 (NPh C-4), 129.5 (NPh C-3,5), 130.6 (C6Ph C-2,6), 132.3 (C7Ph C-2,6), 140.1 (NPh C-1), 141.2 (C6Ph C-1), 148.8 (C-6), 152.0 (C-7a), 158.0 (C7Ph C-4), 158.8 (C-4). 15N NMR (71 MHz, CDCl3): δ −147.6 (N-2), −96.5 (N-1), −83.8 (N-5). IR (KBr, ν, cm−1): 3059 (CH3sym), 2983, 2930 (CH3asym), 1587, 1506, 1479, 1375, 1244, 1179 (C=C, C=N, C=N), 1047 (C=O), 826, 754, 670, 686 (CH=CH of mono- and disubstituted benzenes). MS (ESI+): m/z (%): 419 ([M + H]+, 95). HRMS (ESI) for C28H26N3O ([M + H]+): requires 434.2227 and found 434.2227.

4-Ethyl-2,6-diphenyl-7-(4-propoxyphenyl)-2H-pyrazolo[4,3-c]pyridine 41

4-Ethyl-2,6-diphenyl-7-(4-propoxyphenyl)-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol 37 (60 mg, 0.15 mmol), NaH (60%) (7 mg, 0.17 mmol), 1-iodo propane (0.016 mL, 0.17 mmol), and DMF (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:6 v/v). Yield: 64 mg (96%), yellow crystalline solid, mp = 117–118 °C, Rf = 0.41 (EtOAc/Hex, 1:3 v/v). 1H NMR (700 MHz, CDCl3): δ 1.04 (3H, t, J = 7.4 Hz, OCH2CH2CH3), 1.52 (3H, t, J = 7.6 Hz, CH2CH3), 1.81 (2H, hept, J = 7.1 Hz, OCH2CH2CH3), 3.20 (2H, q, J = 7.6 Hz, CH2CH3), 3.92 (2H, t, J = 6.6 Hz, OCH2CH2CH3), 6.83–6.87 (2H, m, C7Ph 3,5-H), 7.19–7.22 (1H, m, C6Ph 4-H), 7.23–7.27 (2H, m, C6Ph 3,5-H), 7.37–7.42 (3H, m, C7Ph 2,6-H and NPh 4-H), 7.47–7.51 (4H, m, NPh 3,5-H and C6Ph 2,6-H), 8.59 (1H, s, 3-H). 13C NMR (176 MHz, CDCl3): δ 10.5 (OCH2CH2CH3), 13.4 (CH2CH3), 22.6 (OCH2CH2CH3), 30.3 (CH2CH3), 69.3 (OCH2CH2CH3), 114.0 (C7Ph C-3,5), 119.0 (C-3a), 120.4 (C-7), 121.2 (C-3), 121.3 (NPh C-2,6), 126.8 (C6Ph C-4), 127.7 (C6Ph C-3,5), 128.0 (C7Ph C-1), 128.3 (NPh C-4), 129.5 (NPh C-3,5), 130.6 (C6Ph C-2,6), 132.3 (C7Ph C-2,6), 140.1 (NPh C-1), 141.2 (C6Ph C-1), 148.8 (C-6), 152.0 (C-7a), 158.2 (C7Ph C-4), 158.8 (C-4). 15N NMR (71 MHz, CDCl3): δ −147.5 (N-2), −96.2 (N-1), −83.5 (N-5). IR (KBr, ν, cm−1): 3045 (CH3sym), 2970, 2931, 2872 (CH3asym), 1609, 1588, 1508, 1250, 1242, 1176 (C=C, C=N, C=N), 1041 (C=O), 758, 697, 687 (CH=CH of mono- and disubstituted benzenes). MS (ESI+): m/z (%): 433 ([M + H]+, 95). HRMS (ESI) for C28H26N3O ([M + H]+): requires 434.2227 and found 434.2227.

4-Ethyl-7-(4-isopropoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine 42

4-Ethyl-7-(4-isopropoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol 37 (60 mg, 0.15 mmol), NaH (60%) (7 mg, 0.17 mmol), 1-iodo propane (0.016 mL, 0.17 mmol), and DMF (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:6 v/v). Yield: 52 mg (80%), yellow crystalline solid, mp = 139–140 °C, Rf = 0.41 (EtOAc/Hex, 1:3 v/v). 1H NMR (700 MHz, CDCl3): δ 1.35 (6H, d, J = 6.1 Hz, CH(CH3)2), 1.53 (3H, t, J = 7.6 Hz, CH2CH3), 3.21 (2H, q, J = 7.6 Hz, CH2CH3), 4.56 (1H, hept, J = 6.1 Hz, CH), 6.82–6.86 (2H, m, C7Ph 3,5-H), 7.20–7.23 (1H, m, C6Ph 4-H), 7.24–7.27 (2H, m, C6Ph 3,5-H), 7.38–7.43 (3H, m, NPh 4-H and C6Ph 2,6-H), 7.48–7.53 (4H, m, C6Ph 2,6-H and NPh 3,5-H), 7.89–7.94 (2H, m, NPh 2,6-H), 8.61 (1H, s, 3-H). 13C NMR (176 MHz, CDCl3): δ 13.4 (CH2CH3), 22.1 (CH2CH3), 30.3 (CH2CH3), 69.7 (OCH2), 115.3 (C7Ph C-3,5), 119.0 (C-3a), 120.4 (C-7), 121.25 (C-7), 121.29 (NPh C-2,6), 126.8 (C6Ph C-4), 127.7 (C6Ph C-3,5), 127.9 (C7Ph C-1), 128.4 (NPh C-4), 129.5 (NPh C-3,5), 130.6 (C6Ph C-2,6), 132.3 (C7Ph C-2,6), 140.2 (NPh C-1), 141.2 (C6Ph C-1), 148.8 (C-6), 152.0 (C-7a), 156.9 (C7Ph C-4), 158.8 (C-4). 15N NMR (71 MHz, CDCl3): δ...
−147.5 (N-2), −96.2 (N-1), −83.6 (N-5). IR (KBr, ν, cm−1): 3124, 3063 (CH\textsubscript{arom}), 2971, 2933 (CH\textsubscript{aliph}), 1608, 1588, 1507, 1280, 1238, 1182 (C=\(\text{C, C=N, C=N}\)), 1036 (C=O), 765, 702, 694 (CH=CH of mono- and disubstituted benzenes). MS (ES\textsuperscript{+}): \textit{m/z} (%): 433 ([M + H]\textsuperscript{+}, 97). HRMS (ESI) for \textit{C}_{29}\textit{H}_{28}\textit{N}_{3}\textit{O} ([M + H]\textsuperscript{+}): requires 434.2227 and found 434.2227.

3.3. Optical Properties

The UV–vis spectra of 10\textsuperscript{−4} mol solutions of the compounds in THF were recorded on a Shimadzu 2600 UV/vis spectrometer. The fluorescence spectra were recorded on an FL920 fluorescence spectrometer from Edinburgh Instruments. The PL quantum yields (\(\Phi_f\)) were measured from dilute THF solutions by an absolute method using the Edinburgh Instruments integrating sphere excited with a Xe lamp. The optical densities of the sample solutions were ensured to be below 0.1 to avoid reabsorption effects. All optical measurements were performed at room temperature under ambient conditions.

A Britton–Robinson buffer (a solution consisting of 0.04 M H\textsubscript{3}PO\textsubscript{4}, 0.04 M CH\textsubscript{3}COOH, and 0.04 M H\textsubscript{3}BO\textsubscript{3}) was used to evaluate the pH dependence of the spectral characteristics of the compounds. The final pH values of the solutions were adjusted by 0.2 M NaOH.

- Stock solutions (4 mM) of the compounds were prepared in DMSO and further diluted in a Britton–Robinson buffer to a final concentration of 2 \(\mu\)M for spectroscopic analyses. Absorption spectra at pH 5, 7, and 9 for all compounds and in the 2–11 pH range with 0.5 step for selected compounds were measured using a Specord 250 Plus spectrophotometer in appropriate Britton–Robinson buffers. The spectra were measured in the 240–450 nm interval with a step of 1 nm, a 1 nm bandpass, and an integration time of 0.5 s. The samples were placed into a quartz cuvette with an optical path of 1 cm. The baseline was measured for the cuvette containing the solvent only.

- The steady-state excitation and emission spectra of 2 \(\mu\)M solutions of all the compounds at pH 5, 7, and 9 and in the 2–11 pH range with a 0.5 step for selected compounds were recorded on a Fluorolog-3 fluorimeter in the quartz cuvette with the 1 cm optical path (both in excitation and emission). Bandpasses in both the excitation and emission monochromator were set to 2 nm, and the spectra were scanned with the 1 nm step and an integration time 0.2 s per data point at 22 °C. Emission spectra were recorded in a 370–700 nm range with excitation at 360 nm.

- The quantum yield was estimated via integration of the fluorescence intensity over a range of 370–700 nm, and a 2.5 \(\mu\)M quinine sulphate solution in 0.05 M H\textsubscript{2}SO\textsubscript{4} was used as a standard (\(\Phi_f = 60\%\)) [76].

3.4. Biology

3.4.1. Cell Cultures

Human cell lines were obtained from European Collection of Authenticated Cell Cultures (K562, MCF-7) or Cell Lines Service (MV4-11), and they were cultivated according to the provider’s instructions. Briefly, the MCF-7 and K562 cell lines were maintained in a DMEM medium (Sigma-Aldrich, St. Louis, MO, USA), and the MV4-11 cell line was maintained in an RPMI-1640 medium. All media were supplemented with 10% foetal bovine serum (Biowest, Nuaillé, France), penicillin (100 U/mL; Sigma-Aldrich, St. Louis, MO, USA), and streptomycin (100 mg/mL; Sigma-Aldrich, St. Louis, MO, USA), and cells were cultivated at 37 °C in 5% CO\textsubscript{2}.

3.4.2. Antiproliferative Activity Assay

Cells were treated in triplicate with six different doses of each compound for 72 h. After treatment, an MTT solution (Sigma-Aldrich, St. Louis, MO, USA) was added for 4 h, the formazan was subsequently dissolved by adding a 10% SDS solution (Sigma-Aldrich, St. Louis, USA), and absorbance was measured at 570 nm using a Tecan M200Pro microplate reader (Biotek, Winooski, VT, USA). The GI\textsubscript{50} value, the drug concentration lethal to 50% of the cells, was calculated from the dose–response curves. Flavopiridol (MedChemExpress, Monmouth Junction, NJ, USA) was used as a reference drug.
3.4.3. Immunoblotting

After the treatment of the K562 cells, lysates in a RIPA buffer were prepared and proteins were separated on SDS-polyacrylamide gels and electroblotted onto nitrocellulose membranes. After blocking, overnight incubation with specific primary antibodies, and incubation with peroxidase-conjugated secondary antibodies, the peroxidase activity was detected with SuperSignal West Pico reagents (Thermo Scientific, Waltham, MA, USA) using a CCD camera LAS-4000 (Fujifilm, Tokyo, Japan). All primary antibodies were diluted in TBS containing 4% BSA and 0.1% Tween 20. The specific antibodies were purchased from Cell Signalling (Danvers, MA, USA; anti-PARP-1, clone 46D11; anti-cleaved caspase 9, clone E5Z7N; HRP-linked secondary antibodies), Sigma-Aldrich (St. Louis, MO, USA; anti-LC3B), and Santa Cruz Biotechnology (Dallas, TX, USA; anti-β-Actin, clone C4), or they were kindly gifted by dr. B. Vojtěšek (Masaryk Memorial Cancer Institute, Brno, Czech Republic; anti-PCNA, clone PC-10).

3.4.4. Flow Cytometry

Asynchronously growing K562 cells were treated with a 10 µM concentration of test compounds for 24, 48, and 72 h, and 30 min before the end of incubation, the cells were labelled with 10 µM BrdU (Sigma-Aldrich, St. Louis, MO, USA) for 30 min. Subsequently, the cells were washed in PBS, fixed with ice-cold 70% ethanol, and denatured in 2 M HCl. After neutralization, the cells were stained with an anti-BrdU FITC-labelled antibody (eBioscience, San Diego, CA, USA) and propidium iodide (Sigma-Aldrich, St. Louis, MO, USA). Samples were then analysed by flow cytometry using a 488 nm laser (BD FACS Verse with software BD FACSuite™, version 1.0.6.; BD, Franklin Lakes, NJ, USA).

4. Conclusions

An efficient synthesis of 2,4,6,7-tetrasubstituted-2H-pyrazolo[4,3-c]pyridine derivatives was developed starting from easily accessible 1-phenyl-3-(2-phenylethynyl)-1H-pyrazole-4-carbaldehyde. The obtained compounds were evaluated for their antiproliferative activity against three cancer cell lines. Out of them, 4-(2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol 23 proved to be the most active, and further experiments revealed that it blocks proliferation and induces cell death in K562 cells. Moreover, the majority of the compounds were revealed to be pH-sensitive, and 7-(4-methoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine was found out to enable both fluorescence-intensity-based and ratiometric pH sensing.

Supplementary Materials: The following are available online. Scheme S1: Synthesis of 1-phenyl-3-(phenylethynyl)-1H-pyrazole-4-carbaldehyde (2) by previously published procedures. Table S1: Fundamental absorption and fluorescence characteristics of compounds 18–42 in THF (λ<sub>ex</sub> = 350 nm). Table S2: Fundamental absorption and fluorescence characteristics of compounds 18–42 in a Britton–Robinson buffer at pH 5, 7, and 9 (λ<sub>ex</sub> = 360 nm). Figure S1: Fluorescence spectra (λ<sub>ex</sub> = 360 nm) and titration profiles for compounds 18 and 21 in a Britton–Robinson buffer at pH 2-11. Figures S2-S147: <sup>1</sup>H, <sup>13</sup>C, NMR and HRMS (ESI-TOF) spectra of compounds 3–42, <sup>19</sup>F spectra of compounds 34 and 35, and <sup>1</sup>H–<sup>15</sup>N HMBC spectra of compounds 13, 15, 16, 18, 20–24, and 26–42.

Author Contributions: Conceptualization, AŠ. and VK.; methodology, EA.; formal analysis, BR., ER. and MK.; investigation, BR., ER., VD., RO. and MK.; resources, AŠ. E.A. and VK.; data curation, EA. and VK.; writing—original draft preparation, BR., ER., MK. and AZ.; writing—review and editing, AZ.; visualization, ER., RO. and AŽ.; supervision, AŽ., VK. and EA.; funding acquisition, V.K. and EA. All authors have read and agreed to the published version of the manuscript.

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