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Q-Tube®-Assisted Alkylation and Arylation of Xanthines and Other N-H-Containing Heterocycles in Water

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Abstract: In this paper, a simple and clean process for the alkylation and arylation of nitrogen-containing heterocycles is reported. The reactions were conducted using the Q-tube®as a non-conventional technology, in water as a green solvent, at overboiling temperature. The developed strategy was used to improve two steps in the total synthesis of caffeine, as reported by Narayan, and then extended to the preparation of *N*-decorated xanthines. Finally, piperidine, methyl piperazine, and isatine were proven to be suitable substrates for the protocol proposed herein.

Keywords: alkylation; caffeine; Q-tube®; xanthines; alkylation; arylation

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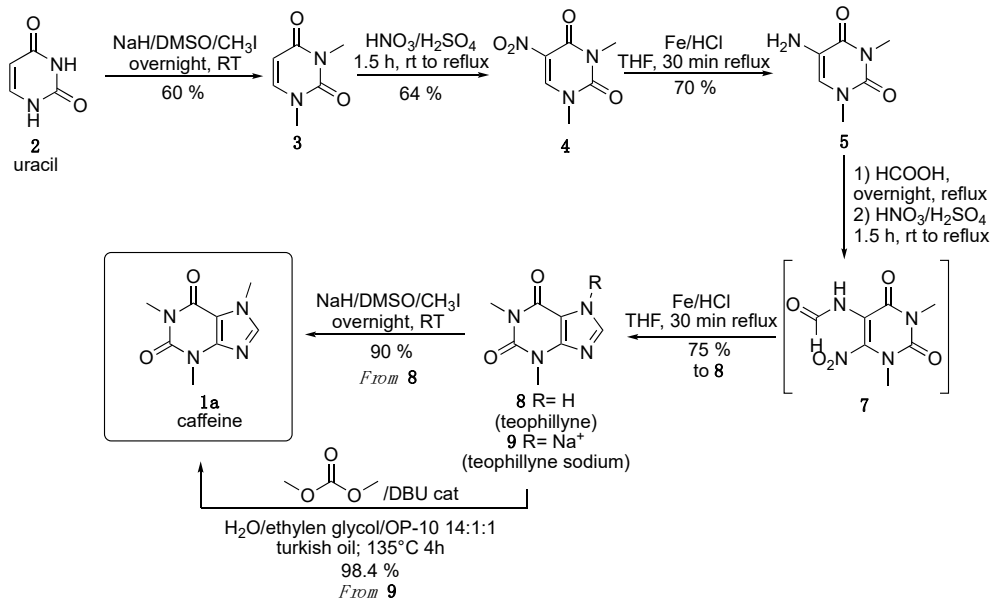
1. Introduction

Xanthines [3,9 dihydro-1*H*-purine-2,6-dione] are alkaloids discovered in the 19th century by the German chemist Emil Fisher, who synthesized all the naturally occurring ones together with some other structurally related compounds [1]. The most thoroughly studied xanthine derivative, caffeine (1,3,7-trimetyxanthine, compound **1a**, Scheme 1), is abundant in coffee, tea, kola nuts, mate leaves, guarana paste, cocoa beans, and other natural matrixes [2]. It is a known neuro-stimulatory and a neuro-protective compound able to alleviate drowsiness and promote mental alertness. Due to its presence in many energy drinks, caffeine is the most consumed psychoactive compound worldwide [3]. From a clinical standpoint, caffeine has been suggested for the treatment of neurodegenerative disorders [4], and its direct antimicrobial activity against *Staphylococcus aureus* has also been demonstrated [5]. Very recently, a comprehensive review article focusing on its beneficial effects on human health has been published [6].

Theophylline 1,3-dimetylxanthine, the second most investigated, naturally derived xanthine, has several pharmaceutical properties, such as diuretic, bronchodilator, cardiac, and CNS stimulator properties [7].

In general, xanthines are known as antagonists of the adenosine receptors, with concomitant anti-inflammatory activity, and as activators of histone deacetylases, enzymes essential for the control of inflammatory gene expression. Recently, they were also proven to inhibit the efflux of doxorubicin from tumor cells and to act as antioxidant compounds [4].

The widespread pharmaceutical application of xanthines prompted medicinal chemists to engage in drug discovery campaigns based on their synthetic modification. As examples, *N*-substituted theophylline derivatives were demonstrated to be biologically relevant as G2 checkpoint inhibitors [8] and anti-*Mycobacterium tubercolosis* agents [9], among other properties, as recently documented in a comprehensive report [4].



Scheme 1. Synthetic procedure for the preparation of caffeine.

Despite the attractive nature of the xanthine scaffold, a limited number of synthetic procedures for its preparation have been reported and none of them are aimed at supporting large-scale chemical diversity. As a result, the number of xanthine derivatives endowed with pharmacological properties is not as high as one might expect [4].

The very first report focused on the synthesis of caffeine was published by Fischer in 1895, starting from uric acid [1]. Later on, Traube synthetized the same compound starting from dimethyl urea [10].

Among the examples of caffeine's total synthesis, the report of Narayan and co-workers published in 2003 is worth mentioning (Scheme 1). In the first step, uracil **2** was methylated using 2.5 molar equivalents of methyl iodide, NaH, as a base, in DMSO as a solvent. The dimethyl analogue **3** was then nitrated and reduced, affording compound **5** in a 45% yield. The primary amine was successively formylated, nitrated, reduced, and cyclized, giving theophylline **8**, which was finally functionalized by adopting the same methylation procedure of the first step but using 5 molar equivalents of methyl iodide [11]. In 2020, Gao Yang et al. developed a greener approach for the preparation of caffeine, starting from theophylline sodium (compound **9**, Scheme 1), which is alkylated with dimethyl carbonate as a methylating reagent [12].

As part of our ongoing efforts to ameliorate synthetic procedures also using non-conventional technologies [13–18] to enable them to meet the principles of green chemistry, and intrigued by the manifold biological activities exerted by xanthines [4], we sought to develop a flexible synthetic procedure intended to improve caffeine synthesis from a green chemistry perspective and, at the same time, prepare a series of diversely *N*-7 substituted xanthine derivatives. With this aim, we chose to perform these reactions in the Q-tube®apparatus, which allows reactions to be performed under high pressure, overcoming the solvent boiling point and, as a result, increasing the reaction pressure and temperature, thus reducing the reaction time. Some of us recently highlighted the advantages of some representative reactions carried out in the Q-tube®apparatus [19].

The procedure herein developed was then extended to the alkylation and arylation of synthetically or biologically relevant amines and amides. All the reactions, in order to provide a general overview of the reactivity order, were performed using the conditions optimized for the synthesis of caffeine.

2. Materials and Methods

Chemistry: reactions were conducted in the Q-tube®(Sigma-Aldrich, St. Louis, MI, USA) and were stirred with a Teflon-coated magnetic stirring bar. Solvents and reagents were used as received, unless otherwise noted. All the starting materials are commercially available. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F254 pre-coated aluminum foil sheets and visualized by UV irradiation or by use of a $KMnO_4$ stain. Silica gel Kiesinger 60 (70–230 mesh) was used for column chromatography. NMR experiments were performed at 25 °C on a Bruker (Fällanden, Switzerland) DPX 200 spectrometer operating at 200 MHz for ^{1}H and 50.31 MHz for ^{13}C experiments, or in a Bruker (Fällanden, Switzerland) DRX spectrometer operating at 400 MHz for ^{1}H and 100.61 MHz for ^{13}C experiments. ^{1}H and ^{13}C chemical shifts (δ) are reported in parts per million (ppm) and they are relative to TMS (δ 0.0 ppm) or to the residual solvent peak of $CHCl_3$ at δ 7.27 and $CDCl_3$ (δ 77.00) ppm in ^{1}H and ^{13}C NMR (Supplementary Materials), respectively, and at δ 2.54 and δ 40.45 ppm in ^{1}H and ^{13}C NMR, respectively, for DMSO-d_6. Data are reported as follows: chemical shift (multiplicity, coupling constants where applicable, number of hydrogens). Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), sex (sextet), m (multiplet), brs (broad signal singlet). Coupling constant (J) quoted in Hertz (Hz) to the nearest 0.1 Hz.

GC-MS analyses were carried out with an HP-6890 gas chromatograph (dimethyl silicone column, 12.5 m) equipped with an HP-5973 mass-selective detector.

The accurate mass analysis was performed by LC (Dionex Ultimate 3000, San Jose, CA, USA) coupled with high-resolution mass spectrometry (Q Exactive, Thermo Scientific, San Jose, CA, USA). Chromatography was performed using a Luna Omega 1.6 µm Polar C18 100 × 2.1 mm with column guard. Mobile phases were water (A) and acetonitrile (B), both containing 0.1% of formic acid. The gradient was initiated with 0.5% eluent B at 0.6 mL/min. In 8 min, the eluent B increased to 95%, maintaining this condition for 2 min. In 0.5 min, the gradient returned to the initial condition, maintaining this condition for 2 min. The column temperature was set at 40 °C and tray temperature was kept at 16 °C. The injection volume was 5 µL. The mass analyzer was equipped with a heated electrospray ionization (HESI-II) source working in positive polarity. The ESI temperature was set at 320 °C, the capillary temperature at 300 °C, the electrospray voltage at 4.0 kV, and the S-Lens was set at 50 V. Sheath and auxiliary gas were 35 and 15 arbitrary units, respectively. The acquisition was performed setting the resolution at a value of 140,000 FWHM (@200 m/z), the AGC target at 1e6 ions, maximum injection time at 320 ms, and scan range was set from 250 m/z to 1200 m/z.

General procedure for the Q-tube®-assisted alkylation/arylation reaction. Compound **8** was ground for 5 min in a mortar with the proper amount of potassium carbonate. The resulting mixture was dissolved in water and transferred into the Q-tube®and allowed to react at 130 °C for 35 min in the presence of the alkylating/arylating reagent. Compounds **2**, **10**–**13**, and **20** were dispersed in the proper amount of water, together with potassium carbonate and the alkylating/arylating reagent. The resulting mixture was stirred in the Q-tube®at 130 °C for 35 min. Workup and purification procedures together with the physical and spectral data are reported below. Figures of NMR spectra and of the HRMS of the newly described compounds are reported in the SI.

Caffeine (1,3,7-trimethylxanthine) (**1a**) was prepared according to the general *procedure*, starting from 4.44 mmol (0.8 g) of theophylline **8**, 4.44 mmol (0.616 g) of potassium carbonate, and 8.88 mmol (0.55 mL) of iodomethane in 4 mL of deionized H_2O. The reaction mixture was filtered, giving 550 mg of **1a** as a white solid (64% yield). Moreover, **1a** also obtained starting from Theobromine **9,** 1.11 mmol (0.2 g), 1.11 mmol (0.154 g) of potassium carbonate, and 2.22 mmol (0.14 mL) of iodomethane in 1 mL of deionized H_2O. In the latter case, the reaction mixture was extracted with DCM (4 × 50 ml). The organic layers were collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure, giving 165 mg of **1a** as a white solid (76% yield) m.p.: 234–236 °C sublimate (Lit. 234–236.5 °C [11]). ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ 7.51 (s, 1H),

3.99 (s, 3H), 3.58 (s, 3H), 3.40 (s, 3H) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$, 298 K, TMS): δ 155.8, 152.1, 149.1, 141.8, 108.0, 34.0, 30.2, 28.3 ppm.

1,3-Dimethyl-7-propyl-1H-purine-2,6(3H,7H)-dione (**1b**) was prepared according to the general procedure, starting from 1.11 mmol (0.2 g) of theophylline **8**, 1.11 mmol (0.154 g) of potassium carbonate, and 2.22 mmol (0.216 mL) of propyliodide in 1.2 mL of H_2O. The reaction mixture was extracted with EtOAc (5 × 15 mL). The organic layers were collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure, giving 150 mg of **1b** as a white solid (60% yield), m.p.: 99–101 °C (Lit. 98–100 °C [20]). GC–MS: *m*/*z* (relative intensity): 222 (100) [M^+], 180 (100), 207 (30), 193 (29), 123 (29), 109 (23), 95 (42). ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ 7.53 (s, 1H), 4.24 (t, *J* = 7.1 Hz, 2H), 3.57 (s, 3H), 3.39 (s, 3H), 1.89 (sex, *J* = 7.3 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$, 298 K, TMS): δ 155.5, 152.1, 149.3, 141.2, 107.4, 49.2, 30.1, 28.4, 24.6, 11.2 ppm.

7-Benzyl-1,3-dimethylxanthine (**1c**) was prepared according to the general procedure, starting from 1.11 mmol (0.2 g) of theophylline **8**, 1.11 mmol (0.154 g) of potassium carbonate, and 2.22 mmol (0.264 mL) of benzylbromide in 1 mL of H_2O. The reaction mixture was filtered and purified by silica gel chromatography, using as eluent DCM/MeOH 98.5:1.5, giving 88 mg of **1c** as a white solid (30% yield), m.p.: 153–156 °C (Lit. 157–159 °C [20]). GC–MS: *m*/*z* (relative intensity): 270 (93) [M^+], 91 (100), 271(24). ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ 7.59 (s, 1H), 7.40–7.33 (m, 5H), 5.53 (s, 2H), 3.61 (s, 3H), 3.43 (s, 3H) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$, 298 K, TMS): δ 155.7, 152.1, 149.3, 141.2, 135.7, 129.5, 129.1, 128.4, 107.4, 50.7, 30.2, 28.4 ppm.

7-(3,5-Dinitrobenzyl)-1,3-dimethylxanthine (**1d**) was prepared according to the general procedure, starting from 1.11 mmol (0.2 g) of theophylline **8**, 1.11 mmol (0.154 g) of potassium carbonate, and 2.22 mmol (0.481 g) of 3,5-dinitrobenzylchloride in 1 mL of H_2O. The reaction mixture was extracted with DCM (6 × 12.5 mL). The organic layers were collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by silica gel chromatography, using as eluent DCM/MeOH 97:3, giving 153 mg of **1d** as a brown oil (42% yield). ^{1}H-NMR (400 MHz, DMSO-d6, 298 K, TMS): δ 8.75–8.74 (m, 1H), 8.69–8.65 (m, 2H), 8.38 (s, 1H), 5.71 (s, 2H), 3.40 (s, 3H), 3.17 (s, 3H) ppm. ^{13}C-NMR (50.31 MHz, $DMSO_{d6}$, 298 K, TMS): δ 155.1, 151.6, 149.3, 148.8, 143.4, 141.4, 129.4, 118.9, 106.4, 48.5, 30.1, 28.2 ppm. FT-IR (KBr) ν: 1562, 1549, 1350 cm^{-1}. HRMS calcd for $C_{14}H_{13}O_6N_6$ 361.0891, found 361.0890.

Ethyl 2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetate (**1e**) was prepared according to the general procedure, starting from 1.11 mmol (0.2 g) of theophylline **8**, 1.11 mmol (0.154 g) of potassium carbonate, and 2.22 mmol (0.246 g) of ethyl-bromoacetate in 1 mL of H_2O. The reaction mixture was extracted with EtOAc (5 × 12.5 mL). The organic layers were collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by silica gel chromatography, using as eluent DCM/MeOH 99:1, giving 57 mg of **1e** as a white solid (22% yield), m.p.: 140–142 °C (Lit. 143–144 °C [21]). GC–MS: *m*/*z* (relative intensity): 266 (100) [M^+], 220 (71), 194 (30), 193 (66), 109 (39), 81 (24). ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ 7.61 (s, 1H), 5.07 (s, 2H), 4.25 (q, *J* = 7.2 Hz, 2H), 3.57 (s, 3H) 3.35 (s, 3H), 1.29 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$, 298 K, TMS): δ 167.5, 155.7, 152.0, 148.9, 142.3, 107.5, 62.8, 47.7, 30.2, 28.3, 14.5 ppm.

3-(1,3-Dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)propanoic acid (**1f**) was prepared according to the general procedure, starting from 1.11 mmol (0.2 g) of theophylline **8**, 1.11 mmol (0.154 g) of potassium carbonate, and 2.22 mmol (0.2 mL) of methylacrylate in 1.5 mL of H_2O. The reaction mixture was acidified (HCl 10%); it was extracted with EtOAc (5 × 2 mL) and then with DCM/CH_3OH 2% (3 × 20 mL). The organic layers were collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure, giving 153 mg of **1f** as a white solid (55 % yield), m.p.: 210–211 °C (Lit. 204–205 °C [22]). ^{1}H-NMR (400 MHz, DMSOd6, 298 K, TMS): δ 12.47 (brs, 1H), 8.01 (s, 1H), 4.41 (t, *J* = 6.2 Hz, 2H), 3.39 (s, 3H), 3.21 (s, 3H), 2.85 (t, *J* = 6.2 Hz, 2H) ppm.

^{13}C-NMR (100.62 MHz, DMSO-d6, 298 K, TMS): δ 172.8, 155.2, 151.8, 149.3, 143.7, 106.7, 43.2, 35.4, 30.2, 28.4 ppm.

7.7′-(2-Hydroxypropane-1.3-diyl)bis(1.3-dimethylxanthine) (**1g**) was prepared according to the general procedure, starting from 1.11 mmol (0.2 g) of theophylline **8**, 1.11 mmol (0.154 g) of potassium carbonate, and 2.22 mmol (0.174 mL) of epichloridine in 1 mL of H_2O. The reaction mixture was extracted with EtOAc (3 × 12.5 mL) and then with DCM/CH_3OH 99/1 (4 × 12.5 mL). The organic layers were collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by silica gel chromatography, using as eluent DCM/MeOH 97:3, giving 72 mg of **1g** as a white solid (55% yield), m.p.: 290–295 °C (Lit. 283–285 °C [23]). ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ 7.73 (s, 2H), 4.58–4.54 (m, 2H), 4.31–4.24 (m, 3H), 3.57 (s, 6H), 3.37 (s, 6H), 2.43 (brs, OH) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$ + MeOD, 298 K, TMS): δ 156.2, 151.9, 149.4, 143.1, 107.4, 69.6, 50.4, 30.2, 28.5 ppm.

1,3-Dimethylpyrimidine-2,4(1H,3H)-dione (**3**) was prepared according to the general procedure, starting from 1.11 mmol (0.124 g) of uracil **2**, 2.22 mmol (0.308 g) of potassium carbonate, and 4.44 mmol (0.280 mL) of iodomethane in 1 mL of H_2O. The reaction mixture was extracted with DCM (3 × 12.5 mL) The organic layers were collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure, giving 128 mg of **3** as a white solid (90% yield), m.p.: 114–115 °C (Lit. 118–121 °C [24]). ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ 7.13 (d, *J* = 7.8 Hz, 1H), 5.71 (d, *J* = 7.8 Hz, 1H), 3.37 (s, 3H), 3.31 (s, 3H) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$, 298 K, TMS): δ 163.8, 152.3, 143.2, 101.7, 37.4, 28.1 ppm.

1-(4-Nitrophenyl)piperidine (**14**) was prepared according to the general procedure, starting from 2.35 mmol (0.23 mL) of piperidine **11**, 2.35 mmol (0.326 g) of potassium carbonate, and 4.7 mmol (0.74 g) of para-chloro-nitro-benzene in 1 mL of H_2O. The reaction mixture was extracted with EtOAc (5 × 12.5 mL). The organic layers were collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by silica gel chromatography using, as eluent, petroleum ether/EtOAc 95:5, giving 341 mg, 70% yield of **14** as a yellow solid, m.p.: 105–106 °C (Lit. 103–104 °C [25]). ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ 8.12 (d, *J* = 9.4 Hz, 2H), 6.82 (d, *J* = 9.4 Hz, 2H), 3.45–3.47 (m, 4H), 1.72–1.70 (m, 6H) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$, 298 K, TMS): δ 155.3, 137.8, 126.6, 112.7, 48.8, 25.7, 24.7 ppm.

1-Methyl-4-4(4-nitrophenyl)piperazine (**15**) was prepared according to the general procedure, starting from 2 mmol (0.22 mL) of 1-methyl-piperazine **12**, 2 mmol (0.277 g) of potassium carbonate, and 4 mmol (0.63 g) of para-chloro-nitro-benzene in 1 mL of H_2O. The reaction mixture was extracted with EtOAc (4 × 12.5 mL). The organic layers were collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by silica gel chromatography, using as eluent DCM/MeOH 95:5, giving 163 mg of **15** as a yellow solid (37% yield), m.p.: 107–108 °C (Lit. 105–106 °C [26]). ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ 8.12–8.09 (m, 2H), 6.82–6.79 (m, 2H), 3.44–3.41 (m, 4H), 2.56–2.53 (m, 4H), 2.34 (s, 3H) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$, 298 K, TMS): δ 155.2, 138.8, 126.4, 113.1, 54.9, 47.3, 46.5 ppm.

1-(5-Chloro-2.4-dinitrophenyl)-4-methylpiperazine (**16**) was prepared according to the general procedure, starting from 0.84 mmol (0.093 mL) of 1-methyl-piperazine **12**, 0.84 mmol (0.117 g) of potassium carbonate, and 0.84 mmol (0.2 g) of 1,3-dichloro-4,6-dinitrobenzene in 1.1 mL of H_2O. The reaction mixture was extracted with EtOAc (6 × 12.5 mL). The organic layers were collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by silica gel chromatography, using as eluent DCM/MeOH 98:2, giving 197 mg of **16** as a yellow solid (78% yield), m.p.: 156–157 °C. GC–MS: *m*/*z* (relative intensity): 300 (21) [M^+], 207 (100), 272 (20), 270 (59), 269 (34), 256 (27), 255 (22), 254 (35), 253 (45), 252 (59), 229 (30), 227 (92), 226 (21), 224 (23), 212 (25), 211 (25), 210 (59), 209 (56), 208 (24), 198 (21), 168 (26), 167 (21), 166 (49), 165 (32), 164 (30), 154 (22), 152 (48), 86 (24), 75 (28), 71 (23), 70 (34), 58 (51), 57 (69), 56 (33). ^{1}H-NMR (200 MHz, $CDCl_3$, 298 K, TMS): δ 8.64 (s, 1H), 7.13 (s, 1H), 3.32–3.27 (m, 4H), 2.62–2.57 (m, 4H), 2.39 (s, 3H)

ppm. ^{13}C-NMR (50.31 MHz, $CDCl_3$, 298 K, TMS): δ 147.9, 136.7, 136.3, 133.6, 126.4, 121.9, 54.1, 50.6, 45.9 ppm. HRMS calcd for $C_{11}H_{14}O_4N_4Cl$ 301,0698 found 301,0700.

4.4′-(4.6-Dinitro-1.3-phenylene)bis(1-methylpiperazine) (**17**) was prepared according to the general procedure, starting from 1.68 mmol (0.186 mL) of 1-methyl-piperazine **12**, 1.68 mmol (0.233 g) of potassium carbonate, and 0.84 mmol (0.2 g) of 1,3-dichloro-4,6-dinitrobenzene in 1.4 mL of H_2O. The reaction mixture was extracted with EtOAc (3 × 20 mL). The organic layers were collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by silica gel chromatography, using as eluent DCM/MeOH 9:1, giving 200 mg of **17** as a yellow solid (65% yield), m.p.: 199–200 °C. GC–MS: *m*/*z* (relative intensity): 364 (48) [M^+], 70 (100), 334 (32), 317 (49), 228 (27), 227 (26), 217 (22), 215 (32), 214 (27), 207 (32), 203 (22), 202 (22), 201 (73), 189 (20), 188 (29), 187 (27), 173 (45), 172 (32), 171 (23), 160 (20), 159 (31), 158 (24), 146 (26), 86 (76), 71 (36), 58 (73), 57 (41), 56 (29). ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ 8.71 (s, 1H), 6.30 (s, 1H), 3.24–3.22 (m, 8H), 2.65–2.62 (m, 8H), 2.40 (s, 6H) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$, 298 K, TMS): δ 150.7, 131.6, 130.1, 107.7, 54.7, 51.2, 46.4 ppm. HRMS calcd for $C_{16}H_{25}O_4N_6$ 365,1932 found 365,1933.

1-Benzyl-4-methylpiperazine (**18**) [27] was prepared according to the general procedure, starting from 2 mmol (0.2 g) of 1-methyl-piperazine **12**, 2 mmol (0.277 g) of potassium carbonate, and 4 mmol (0.48 mL) of benzylbromide in 1 mL of H_2O. The reaction mixture was extracted with EtOAc (5 × 12.5 mL). The organic layer was collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by silica gel chromatography, using as eluent DCM/MeOH 95:5, giving 45 mg of **18** as a yellow oil (12% yield).^{1}H-NMR (200 MHz, $CDCl_3$, 298 K, TMS): δ 7.33–7.28 (m, 5H), 3.55 (s, 2H), 2.62 (brs, 8H), 2.42 (s, 3H) ppm. ^{13}C-NMR (50.31 MHz, $CDCl_3$, 298 K, TMS): δ 137.5, 129.2, 128.3, 127.3, 62.7, 54.7, 52.1, 45.4 ppm.

1-Methylindoline-2,3-dione (**19**) was prepared according to the general procedure, starting from 2.04 mmol (0.3 g) of isatine **13**, 2.04 mmol (0.283 g) of potassium carbonate, and 4.08 mmol (0.25 mL) of iodomethane in 2 mL of H_2O. The reaction mixture was acidified (HCl 10%) and extracted with DCM (6 × 25 mL). The organic layers were collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by silica gel chromatography, using as eluent DCM/MeOH 95.5:0.5, giving 88 mg of **19** as an orange solid (26.8%), m.p.: 132–133 °C (Lit. 129–130 °C [28]). ^{1}H-NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ 7.65–7.61 (m, 2H), 7.15 (t, *J* = 7.6 Hz, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 3.28 (s, 3H) ppm. ^{13}C-NMR (100.62 MHz, $CDCl_3$, 298 K, TMS): δ 183.7, 158.6, 151.9, 138.8, 125.7, 124.2, 117.9, 110.3, 26.6 ppm.

N,N,N-trimethyl-1-phenylethanaminium iodide (**21**): was prepared according to the general procedure, starting from 1.65 mmol (0.213 mL) of alpha-methyl-benzyl-amine **20**, 3.33 mmol (0.462 g) of potassium carbonate, and 6.6 mmol (0.41 mL) of iodomethane in 1 mL of H_2O. The reaction mixture was basified (NaOH 10%) and extracted with DCM (3 × 12.5 mL). The organic layer was collected, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure, giving 185 mg of **21** as a yellow solid (39% yield), m.p.: 146–147 °C, (Lit. 146–147 °C [29]). ^{1}H-NMR (200 MHz, $CDCl_3$, 298 K, TMS): δ 7.64–7.40 (m, 5H), 5.37 (q, *J* = 7.0 Hz, 1H), 3.33 (s, 9H), 1.82 (d, *J* = 6.9 Hz, 3H) ppm. ^{13}C-NMR ($CDCl_3$, 298 K, TMS): δ 132.4, 130.8, 129.3, 73.2, 51.5, 15.5 ppm.

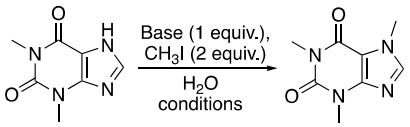
3. Results and Discussion

The reaction conditions were optimized for the conversion of theophylline (**8**) into caffeine **1**, exploring the effect of microwave irradiation (Method A) or using Q-tube®(Method B) as activation energy.

Different inorganic bases were tested under MW irradiation, confirming potassium carbonate as the best partner in promoting the nucleophilic substitution. Interestingly, carrying out the reaction in the Q-tube®, the NMR conversion was almost quantitative; considering that, with respect to the MW reactor, it is a simpler and cheaper apparatus [30], we evaluated the scope of explorations using the conditions reported in Table 1, entry 6.

In particular, theophylline (**8**) was ground in a mortar with one equivalent of base (K_2CO_3) for 5 min; then, the resulting potassium salt was dissolved in water to obtain a 1.11 M solution. The solution was stirred in the Q-tube®by setting the temperature to 130 °C for 35 min in the presence of two equivalents of iodomethane, affording a crude reaction mixture in which caffeine (**1**) was the unique compound. Intriguingly, while the mixture was cooling to room temperature, the desired product (**1**) crystallized spontaneously and was collected by filtration, giving compound **1** in a 64% yield and with 99% purity, as calculated by qNMR.

Table 1. Optimization of the reaction conditions.

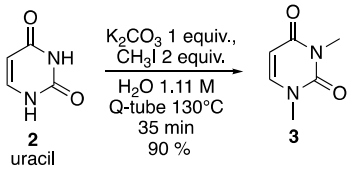


8 **1**

Entry	Base	Method [a]	Conversion [b] (Isolated Yield)
1	Cs_2CO_3	A	85%
2	K_2CO_3	A	91%
3	KOH	A	77%
4	NaOH	A	65%
5	LiOH	A	55%
6	K_2CO_3	B	99% (64%)

[a] Method (A) MW, 120 °C, 200 psi, 200W, 4 min; Method (B) Q-tube®, 130 °C, 35 min; [b] estimated by ^{1}H-NMR analysis.

The optimized conditions were then tested in the first step of the total synthesis of caffeine. In particular, uracil **2** was straight converted into its bis methylated derivative **3** in excellent chemical yield. Comparing this specific step with that reported by Narayan (Scheme 1) [11], not only was the reaction time shortened but the chemical yield was also improved (Scheme 2).



2 uracil **3**

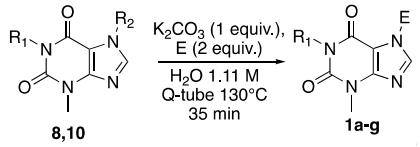
Scheme 2. Methylation of uracil **2**.

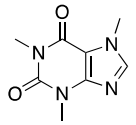
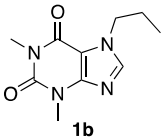
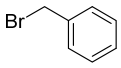
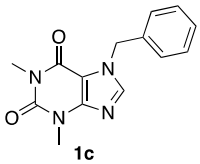
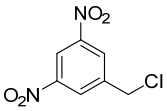
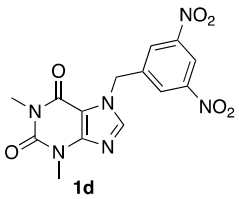
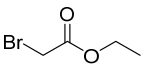
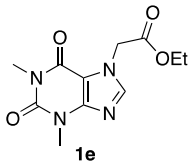
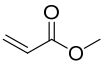
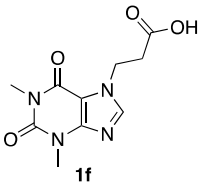
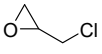
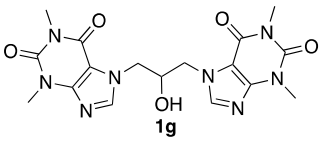
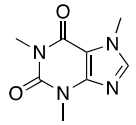
Following from these results, the synthesis of a small library of caffeine derivatives (compound **1b**–**g**) was attempted by changing the electrophilic partner (Table 2). All of the xanthine derivatives were obtained in yields ranging from moderate (64% in the case of **1a**) to poor (22% in the case of **1e**).

Propyl iodide proved to be a good electrophilic partner; indeed, compound **1b** was obtained in a 60% yield (Table 2, entry 2). Moderate conversion was observed for the benzylbromide affording derivative **1c** and the yield was slightly increased (**1d**) in the presence of electron-withdrawing substituents at the aromatic ring (Table 2, entries 3 and 4).

The Michael acceptor methylacrylate may be used as an alkylating agent, but the ester hydrolysis occurred concomitantly; thus, only the xanthine **1f** (isolated after acidification) was formed in a 55% yield. When the alkylating agent was ethylbromoacetate, the ester functionality was partially preserved and the corresponding derivative **1e** (obtained by the workup in basic conditions) was obtained in a 22% yield (Table 2, entries 5 and 6).

Table 2. Synthesis of caffeine analogues.



Entry	R_1	R_2	Electrophile (E)	Products (1a–g)	Isolated Yield
1	Me	H	MeI	 **1a**	64%
2	Me	H		 **1b**	60%
3	Me	H		 **1c**	30% [a]
4	Me	H		 **1d**	42%
5	Me	H		 **1e**	22% [b]
6	Me	H		 **1f**	55% [c]
7	Me	H		 **1g**	55%
8	H	Me	MeI	 **1a**	76%

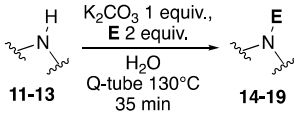
[a] NMR of the crude evidenced the presence of BzOH and BzBr largely arising from the excess of the electrophile; the yield estimated by the ^{1}H-NMR of the crude was 66%; [b] The ^{1}H-NMR of the crude showed resonances compatible with those of the presence of the hydrolyzed product that is reasonably lost during the workup under basic conditions. [c] Isolated after acidification of the aqueous layers.

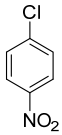
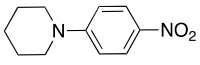
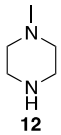
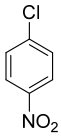
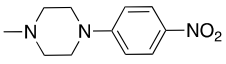
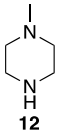
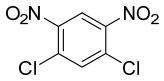
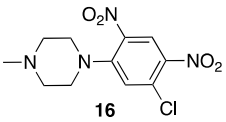
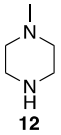
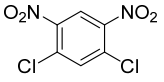
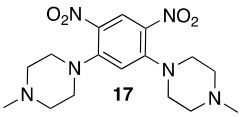
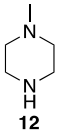
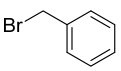
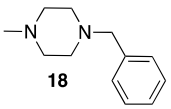
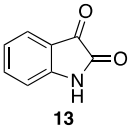
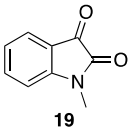
Furthermore, epichloridrine was used as a substrate; in this case, two electrophilic centers were present and the reaction afforded the 1-3,bis substituted propanol derivative **1g** in a good yield (Table 2, entry 7). It is known that when the reaction between the sodium salts of theophylline and epichloridrine is performed in DMF, the monosubstituted epoxide is the main reaction product, while, when the reaction is performed in neat conditions, it affords only the 1-3,bis substituted one [23]. Our results demonstrate that the protocol proposed here leads to the chemoselective formation of **1g** (the unique product present in the ^{1}H-NMR spectrum of the crude) even when using unfavorable stoichiometric conditions for its formation.

To investigate the general applicability of the method, the synthesis of caffeine **1a** was attempted also starting from theobromine, without major changes in either the conversion or the yield (Table 2, entry 1 vs. entry 8).

To extend the scope of our procedure, a small series of *N*-H-containing heterocycles (**11**–**13**) were functionalized with electron-poor arenes, methyl iodide and benzylbromide (Table 3).

Table 3. Scope of the *N*-arylation reaction.

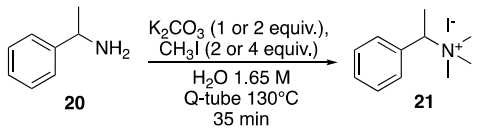


Entry	Heterocycles (11–13)	Electrophile (E)	Products (14–19)	Isolated Yield
1	 **11**		 **14**	70%
2	 **12**		 **15**	37% [b]
3	 **12**		 **16**	78%
4 [a]	 **12**		 **17**	65%
5	 **12**		 **18**	12% [c]
6	 **13**	MeI	 **19**	27% [d]

[a] **E** was used in 0.5 equiv; [b] ^{1}H-NMR of the crude evidenced the presence of starting material with a conversion yield of 44%; [c] Starting materials and other unidentified side products are evidenced by the ^{1}H-NMR of the crude; [d] Starting material can be partially recovered by acidification of the aqueous layers obtained after the first extraction.

Piperidine **11** and piperazine **12**, in consideration of their relevance as pharmacophoric structures [31,32], were reacted with *p*-NO_2-chlorobenzene, leading to the arylated derivatives **14** and **15** in 70% and 37% yields, respectively, in accordance with the different pKa of the N-H activated by the base (Table 3, entries 1 and 2). More electron-deficient arenes, 2,4 dichloro- 1,5 dinitro benzene, indeed afforded **16** in a 78% yield. The fine-tuning of the reagent stoichiometry (Table 3, entries 3 and 4) enabled the formation of double-substituted nitrobenzene derivative **17**. Benzylbromide as well as isatine **13** were demonstrated to unsuitable reactants in the proposed conditions, affording the desired products **18** and **19** in 12% and 27% yields, respectively (Table 3, entries 5 and 6).

Surprisingly, when we attempted the methylation of the primary amine **20,** the corresponding trimethyl ammonium salt **21** was obtained in poor yield (25%). This result is somewhat important since it is, to the best of our knowledge, the first example of the direct synthesis of this specific trimethyl ammonium salt starting from the primary amine, in one step. In addition, since compound **21** was chiral and amphiphilic, it could have potential for use as an auxiliary in phase transfer catalysis. Based on these considerations, we attempted to improve the chemical yield by doubling the amount of either the base or the methyl iodide. With these settings, the ammonium salt **21** was prepared in a 39% yield, probably affected by partial loss during the extraction step (Scheme 3).



Scheme 3. Synthesis of ammonium salt **21**.

4. Conclusions

Xanthines can be considered valuable natural products and, at the same time, privileged scaffolds for medicinal chemistry purposes. In this study, we developed a robust synthetic methodology intended for their structural modification. We employed, as a non-conventional technology, the Q-tube®apparatus, which allowed the reactions to be performed while overcoming the solvent boiling point. Water was used as a green solvent and the target compounds were achieved generally in good yields. The best conditions were then applied to other secondary cyclic amines or amides, highlighting the general applicability of the protocol in the *N*-decoration of nitrogenated heterocycles. The focus of the present investigation was to provide a general indication of the reactivity of the proposed protocol. Each synthesis (especially those reported in low–fair yields) can be further improved in efficiency using a longer reaction time or different bases.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/chemistry3040082/s1. Copies of ^{1}H and ^{13}C of all the synthesized compounds.

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References

1. Fischer, E.; Ach, L. Neue Synthese der Harnsäure und ihrer Methylderivate. *Berichte der Dtsch. Chem. Gesellschaft* **1895**, *28*, 2473–2480. [CrossRef]
2. Verster, J.C.; Koenig, J. Caffeine intake and its sources: A review of national representative studies. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 1250–1259. [CrossRef]
3. Lisko, J.G.; Lee, G.E.; Kimbrell, J.B.; Rybak, M.E.; Valentin-Blasini, L.; Watson, C.H. Caffeine Concentrations in Coffee, Tea, Chocolate, and Energy Drink Flavored E-liquids. *Nicotine Tob. Res.* **2016**, *19*, 484–492. [CrossRef] [PubMed]
4. Singh, N.; Shreshtha, A.K.; Thakur, M.S.; Patra, S. Xanthine scaffold: Scope and potential in drug development. *Heliyon* **2018**, *4*, e00829. [CrossRef] [PubMed]
5. Hosseinzadeh, H.; Bazzaz, B.S.F.; Sadati, M.M. In vitro Evaluation of Methylxanthines and Some Antibiotics: Interaction against Staphylococcus aureus and Pseudomonas aeruginosa. *Iran. Biomed. J.* **2006**, *10*, 163–167.
6. van Dam, R.M.; Hu, F.B.; Willett, W.C. Coffee, Caffeine, and Health. *N. Engl. J. Med.* **2020**, *383*, 369–378. [CrossRef] [PubMed]
7. Ma, Y.J.; Jiang, D.Q.; Meng, J.X.; Li, M.X.; Zhao, H.H.; Wang, Y.; Wang, L.Q. Theophylline: A review of population pharmacokinetic analyses. *J. Clin. Pharm. Ther.* **2016**, *41*, 594–601. [CrossRef]
8. Jiang, X.; Lim, L.Y.; Daly, J.W.; Li, A.H.; Jacobson, K.A.; Roberge, M. Structure-activity relationships for G2 checkpoint inhibition by caffeine analogs. *Int. J. Oncol.* **2000**, *16*, 971–979. [CrossRef]
9. Voynikov, Y.; Valcheva, V.; Momekov, G.; Peikov, P.; Stavrakov, G. Theophylline-7-acetic acid derivatives with amino acids as anti-tuberculosis agents. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3043–3045. [CrossRef]
10. Traube, W. Der synthetische Aufbau der Harnsäure, des Xanthins, Theobromins, Theophyllins und Caffeïns aus der Cyanessigsäure. *Berichte der Dtsch. Chem. Gesellschaft* **1900**, *33*, 3035–3056. [CrossRef]
11. Zajac, M.A.; Zakrzewski, A.G.; Kowal, M.; Narayan, S. A Novel Method of Caffeine Synthesis from Uracil. *Synth. Commun.* **2003**, *33*, 3291–3297. [CrossRef]
12. Yang, S.; Dong, Z.; Yin, C.; Yue, H.; Gao, W.; Yang, F. Green synthesis of caffeine based on methylating reagent dimethyl carbonate and environmental friendly separating method. *J. Chin. Chem. Soc.* **2020**, *67*, 1715–1720. [CrossRef]
13. Sancineto, L.; Tidei, C.; Bagnoli, L.; Marini, F.; Lenardão, E.; Santi, C. Selenium Catalyzed Oxidation of Aldehydes: Green Synthesis of Carboxylic Acids and Esters. *Molecules* **2015**, *20*, 10496–10510. [CrossRef]
14. Vieira, B.M.; Thurow, S.; Brito, J.S.; Perin, G.; Alves, D.; Jacob, R.G.; Santi, C.; Lenardão, E.J. Sonochemistry: An efficient alternative to the synthesis of 3-selanylindoles using CuI as catalyst. *Ultrason. Sonochem.* **2015**, *27*, 192–199. [CrossRef]
15. Krasowska, D.; Begini, F.; Santi, C.; Mangiavacchi, F.; Drabowicz, J.; Sancineto, L. Ultrasound-assisted synthesis of alkali metals diselenides (M2Se2) and their application for the gram-scale preparation of 2,2′-diselenobis(benzoic acid). *Arkivoc* **2019**, *2019*, 24–37. [CrossRef]
16. Begini, F.; Krasowska, D.; Jasiak, A.; Drabowicz, J.; Santi, C.; Sancineto, L. Continuous flow synthesis of 2,2′-diselenobis(benzoic acid) and derivatives. *React. Chem. Eng.* **2020**, *5*, 641–644. [CrossRef]
17. Mangiavacchi, F.; Crociani, L.; Sancineto, L.; Marini, F.; Santi, C. Continuous Bioinspired Oxidation of Sulfides. *Molecules* **2020**, *25*, 2711. [CrossRef]
18. Cerra, B.; Mangiavacchi, F.; Santi, C.; Lozza, A.M.; Gioiello, A. Selective continuous flow synthesis of hydroxy lactones from alkenoic acids. *React. Chem. Eng.* **2017**, *2*, 467–471. [CrossRef]
19. Nacca, F.G.; Merlino, O.; Mangiavacchi, F.; Krasowska, D.; Santi, C.; Sancineto, L. The Q-tube System, A Nonconventional Technology for Green Chemistry Practitioners. *Curr. Green Chem.* **2017**, *4*, 58–66. [CrossRef]
20. Malakar, C.C.; Schmidt, D.; Conrad, J.; Beifuss, U. Double C−H Activation: The Palladium-Catalyzed Direct C-Arylation of Xanthines with Arenes. *Org. Lett.* **2011**, *13*, 1378–1381. [CrossRef] [PubMed]
21. Ruddarraju, R.R.; Murugulla, A.C.; Kotla, R.; Tirumalasetty, M.C.B.; Wudayagiri, R.; Donthabakthuni, S.; Maroju, R.; Baburao, K.; Parasa, L.S. Design, synthesis, anticancer, antimicrobial activities and molecular docking studies of theophylline containing acetylenes and theophylline containing 1,2,3-triazoles with variant nucleoside derivatives. *Eur. J. Med. Chem.* **2016**, *123*, 379–396. [CrossRef] [PubMed]
22. Bhalla, H.L.; Vavia, P.R.; Samuel, G.; Sivaprasad, N. Development of radioimmunoassay. *J. Radioanal. Nucl. Chem.* **1997**, *220*, 73–76. [CrossRef]
23. Kondo, K.; Kuwata, K.; Takemoto, K. Functional monomers and polymers. IX. Synthesis of 2′,3′-epoxypropyl derivatives of adenine and theophylline. *Makromol. Chem.* **1972**, *160*, 341–346. [CrossRef]
24. Hartman, T.; Cibulka, R. Photocatalytic Systems with Flavinium Salts: From Photolyase Models to Synthetic Tool for Cyclobutane Ring Opening. *Org. Lett.* **2016**, *18*, 3710–3713. [CrossRef]
25. Toma, G.; Yamaguchi, R. Cobalt-Catalyzed C-N Bond-Forming Reaction between Chloronitrobenzenes and Secondary Amines. *European J. Org. Chem.* **2010**, *2010*, 6404–6408. [CrossRef]

26. Ibata, T.; Isogami, Y.; Toyoda, J. Aromatic Nucleophilic Substitution of Halobenzenes with Amines under High Pressure. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 42–49. [CrossRef]
27. Watson, A.J.A.; Maxwell, A.C.; Williams, J.M.J. Borrowing Hydrogen Methodology for Amine Synthesis under Solvent-Free Microwave Conditions. *J. Org. Chem.* **2011**, *76*, 2328–2331. [CrossRef]
28. Esmaeili, A.A.; Darbanian, M. Reaction between alkyl isocyanides and dialkyl acetylenedicarboxylates in the presence of N-alkyl isatins: Convenient synthesis of γ-spiro-iminolactones. *Tetrahedron* **2003**, *59*, 5545–5548. [CrossRef]
29. Lacour, J.; Vial, L.; Herse, C. Efficient NMR Enantiodifferentiation of Chiral Quats with BINPHAT Anion. *Org. Lett.* **2002**, *4*, 1351–1354. [CrossRef]
30. Sancineto, L.; Monti, B.; Merlino, O.; Rosati, O.; Santi, C. Q-Tube © assisted MCRs for the synthesis of 2,3-dihydroquinazolin-4(1H)-ones. *Arkivoc* **2018**, *2018*, 270–278. [CrossRef]
31. Mordini, A.; Reginato, G.; Calamante, M.; Zani, L. Stereoselective Synthesis of Polysubstituted Piperazines and Oxopiperazines. Useful Building Blocks in Medicinal Chemistry. *Curr. Top. Med. Chem.* **2014**, *14*, 1308–1316. [CrossRef] [PubMed]
32. Patel, R.; Park, S. An Evolving Role of Piperazine Moieties in Drug Design and Discovery. *Mini-Reviews Med. Chem.* **2013**, *13*, 1579–1601. [CrossRef] [PubMed]