The Reaction of Cyanoacetylhydrazine with ω-Bromo(4-methyl)acetophenone: Synthesis of Heterocyclic Derivatives with Antitumor Activity

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Received: 22 March 2010; in revised form: 14 April 2010 / Accepted: 21 April 2010 / Published: 17 May 2010

Abstract: New approaches for the synthesis of hydrazide-hydrazone derivatives were demonstrated as well as some heterocyclizations of such derivatives to afford 1,3,4-triazine, pyridine and 1,3,4-oxadiazine derivatives. The antitumor evaluation of the newly synthesized products against three cancer cell lines, namely breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) were recorded. Most of the synthesized compounds showed high inhibitory effects.

Keywords: hydrazide-hydrazone; 1,3,4-triazine; pyridine; pyridazine; antitumor

1. Introduction

Hydrazide-hyrazones are an important class of compounds that has gained much importance in recent years due to their diverse biological activities [1–10]. The therapeutic prominence of hydrazide-hydrazone derivatives is well established [11–12]. Hydrazide-hyrazones were also reported to elicit anticancer [13–20] and anti-HIV properties [21] and hence they have gained an important place in
medicinal chemistry. The discovery of the antineoplastic activity of the naturally occurring Schiff’s bases has stimulated considerable research efforts in the field of condensed systems [22]. With the aim of constructing such condensed systems with the hydrazide-hydrazone nucleus, we turned our attention to using such compounds as synthons for heterocyclic derivatives and their anitumor evaluation [23,24].

2. Results and Discussion

In this work we report the reaction of cyanoacetylhydrazine (1) with o-bromo-(4-methyl-acetophenone) (2) in 1,4-dioxane which gave the condensed product 3. The structure of compound 3 was confirmed based on analytical and spectral data. Thus, the $^1$H-NMR showed a singlet at $\delta$ 2.51 for the CH$_3$, two singlets at $\delta$ 4.31, 4.72 for the two CH$_2$ groups, a multiplet at $\delta$ 6.50–7.76 for the C$_6$H$_4$ group and a singlet at $\delta$ 11.46 (D$_2$O exchangeable) for the NH group. The reactivity of compound 3 towards different chemical reagents was studied. The reaction of 3 with either potassium cyanide or potassium thiocyanate gave the corresponding cyanide or the thiocyanate derivatives 4a and 4b, respectively (Scheme 1).

Scheme 1. Synthesis of the hydrazide-hydrazone 3 and 4a, b.

![Scheme 1](image)

The reaction of compound 3 with either hydrazine hydrate (5a) or phenylhydrazine (5b) gave the hydrazine derivative 6a or 6b, respectively. Analytical and spectral data of the reaction products are in agreement with the proposed structures (see Experimental section). The reaction of either 6a or 6b with benzaldehyde (7) gave the benzal derivative 8a or 8b, respectively (Scheme 2).

On the other hand, the reaction of either 6a or 6b with either benzenediazonium chloride (9a), 3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophene-2-diazonium chloride (9b) or ethyl 3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate-2-diazonium chloride (9c) gave the 3-(α-hydrazo-acetonitrilo)-1,2,4-triazine derivatives 10a–f, respectively (Scheme 3). The analytical and spectral data of the latter reaction products are all consistent with the proposed structures.
Next, we moved towards studying the reactivity of 6a and 6b with active methylene reagents. Thus, their reactions with either malononitrile (11a) or ethyl cyanoacetate (11b) gave the pyridine derivatives 12a–d, respectively (Scheme 4). The structures of the latter products were established on the basis of their analytical and spectral data. Thus, the $^1$H-NMR spectrum of 12c showed a singlet at $\delta$ 2.51 for the CH$_3$ group, a singlet at $\delta$ 3.38 for the CH$_2$ group, two singlets at $\delta$ 4.38, 4.79 for the two NH$_2$ groups and a multiplet at $\delta$ 6.48–8.19 for the pyridine H-3, C$_6$H$_5$ and C$_6$H$_4$, two singlets at $\delta$ 10.82, 11.20 for the two NH groups, respectively.
Scheme 4. Synthesis of 2-oxopyridines 12a–d.

On the other hand, the reaction of either 6a or 6b with either acetylacetone (13a) or ethyl acetoacetate (13b) gave the pyridine derivatives 14a–d, respectively (Scheme 5). The structures of the latter products were confirmed by their analytical and spectral data (see Experimental section).

On the other hand, the reaction of either 6a or 6b with either acetylacetone (13a) or ethyl acetoacetate (13b) gave the pyridine derivatives 14a–d, respectively (Scheme 5). The structures of the latter products were confirmed by their analytical and spectral data (see Experimental section).

Compound 3 underwent ready cyclization when heated in sodium ethoxide solution to give the 1,3,4-oxadiazine derivative 15, whose structure was established from its analytical and spectral data (see Experimental section). The oxadiazine derivatives 15 seemed to be an intermediate for many reactions between 3 and many chemical reagents. Thus, the reaction of 3 with benzaldehyde (7) gave the 2-(α-benzalacetonitrilo)-1,3,4-oxadiazine derivative 16. The analytical and spectral data of 16 were
in agreement with the proposed structure. Thus, the $^1$H-NMR spectrum showed a singlet at $\delta$ 2.51 for the CH$_3$ group, a singlet at $\delta$ 4.22 for the CH$_2$ group, a singlet at $\delta$ 5.16 for the (=CH) group and a multiplet at $\delta$ 7.35–8.02 for the C$_6$H$_5$ and C$_6$H$_4$ groups. The same product 16 was obtained through the reaction of compound 15 with benzaldehyde (7) (confirmed by m.p., mixed m.p. and fingerprint IR spectrum). On the other hand, the reaction of 15 with benzenediazonium chloride (9) gave the 2-(a-phenylhydrazo)-1,3,4-oxadiazine derivative 17 (Scheme 6).

**Scheme 6. Synthesis of 1,3,4-oxadiazines 15, 16 and 17.**

*Effect on the Growth of Human Tumor Cell Lines*

The effect of compounds 4–17 was evaluated on the *in vitro* growth of three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268), after a continuous exposure of 48 h. The results are summarized in Table 1. All the compounds were able to inhibit the growth of the tested human tumor cell lines in a dose-dependent manner (data not shown). The results showed that compound 14b, with its 2-hydroxyopyridine group, showed the highest inhibitory effect against all the three tumor cell lines. In addition compounds 10a, 10f, 12a, 12b and 14c showed high inhibitory effects. On the other hand, compounds 4b, 6a, 8b, 10b, 10d, 12c, 12d, 14a, 14c, 16 and 17 showed the lowest inhibitory effect.
towards adenocarcinoma (MCF-7). The rest of compounds showed the moderate growth inhibitory effects. Comparing compound 4a with 4b, it is obvious that the presence of the $\alpha$-SCN present in 4b showed lower inhibitory effect than 4a with it’s $\alpha$-CN group. Comparing 1,2,4-triazine derivatives 10a (with the 4-phenylamino group) and 10b (with the 4-thiophenoamino group), the first has a greater inhibitory effect than the second towards the three cell lines.

**Table 1.** Effect of the newly synthesized products on the growth of three human tumor cell lines.

| Compound | GI$_{50}$ (μM) |
|----------|----------------|
|          | MCF-7 | NCI-H460 | SF-268 |
| 4a       | 10.0 ± 0.6 | 12.9 ± 1.6 | 15 ± 1.1 |
| 4b       | 70.6 ± 15.3 | 38.1 ± 10.8 | 48.0 ± 9.1 |
| 6a       | 50.8 ± 18.5 | 20.2 ± 12.6 | 50.0 ± 8.7 |
| 6b       | 25.8 ± 12.5 | 22.4 ± 8.6  | 20.3 ± 8.6 |
| 8a       | 24.6*   | 77.9 ± 5.0  | 35.0*    |
| 8b       | 80.9 ± 10.2 | 70.9 ± 6.1  | 50.2 ± 2.2 |
| 10a      | 8.0 ± 0.6  | 11.7 ± 8.8  | 14.8 ± 1.6 |
| 10b      | 70.6 ± 8.6  | 55.6 ± 5.8  | 42.6 ± 8.6 |
| 10c      | 24.2 ± 0.6  | 8.4 ± 1.8   | 10.4 ± 8.4 |
| 10d      | 88.4 ± 4.3  | 66.8 ± 12.0 | 40.9 ± 2.6 |
| 10e      | 12.4 ± 2.6  | 6.8 ± 1.2   | 8.3 ± 9.2  |
| 10f      | 10.0 ± 0.4  | 14.9 ± 1.6  | 14 ± 1.1   |
| 12a      | 12.4 ± 1.6  | 10.8 ± 4.0  | 16 ± 6.0   |
| 12b      | 8.6 ± 2.4   | 10.4 ± 6.2  | 12.0 ± 4.2 |
| 12c      | 86.8 ± 6.0  | 48.5 ± 4.0  | 38.4 ± 2.6 |
| 12d      | 90.8 ± 2.4  | 78.2 ± 2.2  | 86.2 ± 1.8 |
| 14a      | 66.4 ± 6.0  | 42.4 ± 6.0  | 62.3 ± 6.0 |
| 14b      | 4.2 ± 2.8   | 10.2 ± 6.8  | 10.8 ± 5.0 |
| 14c      | 80.3 ± 4.0  | 64.8 ± 6.4  | 50.8 ± 6.4 |
| 16       | 40.5 ± 2.6  | 32.6 ± 4.0  | 26.8 ± 8.4 |
| 17       | 60.4 ± 6.0  | 77.8 ± 3.1  | 47.0 ± 6.4 |

Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI$_{50}$) after a continuous exposure of 48 h and show means ± SEM of three-independent experiments performed in duplicate. *Results from two-independent experiments performed in duplicate. Doxorubicin was used as positive control, GI$_{50}$: MCF-7 = 42.8 ± 8.2 nM, NCI-H460 = 94.0 ± 8.7 nM, and SF-268 = 94.0 ± 7.0 nM.

3. Experimental

3.1. General

Melting points were determined on an Electrothermal melting point apparatus (Electrothermal 9100) and are uncorrected. IR spectra were recorded for KBr discs on a Pye Unicam SP-1000 spectrophotometer. $^1$H-NMR & $^{13}$C-NMR spectra were measured on a Varian EM-390-200 MHz in CD$_3$SOCD$_3$ as solvent using TMS as internal standard, and chemical shifts are expressed as δ.
Analytical data were obtained from the Microanalytical Data Unit at Cairo University, Giza, Egypt. Antitumor evaluation for the newly synthesized products were performed by a research group at the National Research Center & the National Cancer Institute at Cairo University.

4-Methyl-ω-bromoacetophenone cyanoacetylhydrazone (3). To a solution of cyanoacetylhydrazine (1, 2.44 g, 0.02 mol) in 1,4-dioxane (20 mL), ω-bromo-(4-methylacetophenone) (5.24 g, 0.02 mol) was added. The reaction mixture was stirred at room temperature for 1 hr then poured onto a beaker containing an ice/water mixture. The solid product formed was collected by filtration and dried obtaining pale yellow crystals (from ethanol). Yield: 5.02 g (71%), m.p. 148 °C; IR (KBr) $\nu$/cm$^{-1}$: 3400–3378 (NH), 3105 (CH aromatic), 2956 (CH$_3$), 2259 (CN), 1681 (C=O), 1610 (C=C); $^1$H-NMR δ: 2.51 (s, 3H, CH$_3$), 4.31, 4.72 (2s, 4H, CH$_2$), 6.50–7.76 (m, 4H, C$_6$H$_4$), 11.46 (s, 1H, NH). MS: (m/z) 293 (M$^+$) (6%), 200 (28%), 117 (100%), 68 (69.9%); $^{13}$C-NMR: 22.6 (CH$_3$), 27.0, 58.7 (2CH$_2$), 118.5 (CN), 126.8, 128.4, 129.5, 138.8 (C$_6$H$_4$), 156.3 (C=N), 173.8 (C=O); Anal. Calcd. for C$_{12}$H$_{12}$N$_3$OBr (294.24): C, 48.00; H, 4.11; N, 14.29%. Found: C, 49.27; H, 4.45; N, 14.35%.

3.2. General Procedure for the Synthesis of 4a or 4b

To a solution of 3 (0.54 g, 1.83 × 10$^{-3}$ mol) in ethanol (25 mL) in a water bath at 60 °C, either potassium cyanide (0.11 g, 1.83 × 10$^{-3}$ mol) or potassium thiocyanate (0.17 g, 1.83 × 10$^{-3}$ mol) was added with continuous stirring. The reaction mixture was left in the water bath for 30 min at 60 °C then poured onto a beaker containing ice/water mixture and few drops of hydrochloric acid. The formed solid product was collected by filtration and dried.

4-Methyl-ω-cyanoacetophenonecyanoacetylhydrazone (4a). Pale brown crystals (from ethanol). Yield: 0.20 g (62%), m.p. 160 °C; IR (KBr) $\nu$/cm$^{-1}$: 3450–3205 (NH), 3031 (CH aromatic), 2260, 2209 (2CN), 1680 (C=O); $^1$H NMR δ: 2.39 (s, 3H, CH$_3$), 4.46, 5.06 (2s, 4H, 2CH$_2$), 7.18–7.84 (m, 4H, C$_6$H$_4$), 10.82 (s, 1H, NH); $^{13}$C NMR: 23.8 (CH$_3$), 27.3, 29.5 (2CH$_2$), 116.9, 117.7 (2CN), 127.3, 128.0, 129.8, 138.9 (C$_6$H$_4$), 155.8 (C=N), 173.8 (C=O); Anal. Calcd. for C$_{13}$H$_{12}$N$_4$O (240.26): C, 64.99; H, 5.03; N, 23.32%. Found: C, 64.79; H, 5.11; N, 22.99%.

4-Methyl-ω-thiocyanacetophenonecyanoacetylhydrazone (4b). Buff crystals (from ethanol). Yield: 0.33 g (67%), m.p. 130 °C; IR (KBr) $\nu$/cm$^{-1}$: 3445–3225 (NH), 3099 (CH aromatic), 3034 (CH$_3$), 2969, 2925 (2CH$_2$), 2264, 2225 (2CN), 1666 (C=O), 1606 (C=C); $^1$H-NMR δ: 2.49 (s, 3H, CH$_3$), 4.50, 5.08 (2s, 4H, 2CH$_2$), 7.06–7.93 (m, 4H, C$_6$H$_4$), 11.52 (s, 1H, NH); $^{13}$C-NMR: 23.8 (CH$_3$), 27.3, 29.4 (2CH$_2$), 116.7, 117.8 (2CN), 127.0, 128.3, 129.6, 138.6 (C$_6$H$_4$), 155.5 C=N), 173.9 (C=O); Anal. Calcd. for C$_{13}$H$_{12}$N$_4$OS (272.32): C, 57.33; H, 4.44; N, 20.57; S, 11.77%. Found: C, 57.38; H, 4.64; N, 20.68; S, 11.57%.

3.3. General Procedure for the Synthesis of 6a and 6b

To a solution of compound 3 (1.50 g, 5.09 × 10$^{-3}$ mol) in ethanol (35 mL) either hydrazine hydrate (0.25 g, 5.09 × 10$^{-3}$ mol) or phenylhydrazine (0.55 g, 5.09 × 10$^{-3}$ mol) was added. The reaction
mixture was heated under reflux for 3 hrs then poured onto a beaker containing an ice/water mixture and a few drops of hydrochloric acid. The formed solid product was collected by filtration and dried.

4-Methyl-ω-hydrazinoacetophenonecyanoacetylhydrazone (6a). Greenish brown crystals (from ethanol). Yield: 0.65 g (52%), m.p. 120 °C; IR (KBr) v/cm\(^{-1}\): 3400–3204 (NH\(_2\), 2NH), 3027 (CH aromatic), 2918 (CH\(_3\)), 2203 (CN), 1688 (C=O), 1607 (C=C); \(^1\)H-NMR δ: 2.51 (s, 3H, CH\(_3\)), 3.39, 4.23 (2s, 4H, 2CH\(_2\)), 4.82 (s, 2H, NH\(_2\)), 6.87–8.40 (m, 4H, C\(_6\)H\(_4\)), 9.26, 11.41 (2s, 2H, 2NH); \(^13\)C-NMR: 23.6 (CH\(_3\)), 27.0, 29.6 (2CH\(_2\)), 127.8, 128.3, 129.5, 138.6 (C\(_6\)H\(_4\)), 155.6, 164.2 (2C=N), 173.8 (C=O); Anal. Calcd. for C\(_{12}\)H\(_{15}\)N\(_5\)O (245.28): C, 58.76; H, 6.16; N, 28.55%. Found: C, 58.55; H, 5.96; N, 28.32%.

4-Methyl-ω-phenylhydrazinoacetophenonecyanoacetylhydrazone (6b). Brown crystals (from ethanol). Yield: 0.95 g (63%), m.p. 72 °C; IR (KBr) v/cm\(^{-1}\): 3500–3174 (3NH), 3026 (CH aromatic), 2969 (CH\(_3\)), 2205 (CN), 1687 (C=O), 1600 (C=C); \(^1\)H-NMR δ: 2.51 (s, 3H, CH\(_3\)), 3.39, 4.35 (2s, 4H, 2CH\(_2\)), 6.79–8.40 (m, 9H, C\(_6\)H\(_5\), C\(_6\)H\(_4\)), 10.82, 11.19, 12.63 (3s, 3H, 3NH); \(^13\)C-NMR: 23.6 (CH\(_3\)), 27.0, 29.9 (2CH\(_2\)), 120.3, 122.5, 127.3, 128.4, 129.3, 138.5, 140.1 (C\(_6\)H\(_4\), C\(_6\)H\(_5\)), 155.6, 164.2 (2C=N), 173.8 (C=O); Anal. Calcd. for C\(_{18}\)H\(_{19}\)N\(_5\)O (321.38): C, 67.27; H, 5.96; N, 21.79%. Found: C, 67.37; H, 5.84; N, 22.61%.

α-Benzal-4-methyl-ω-hydrazinoacetophenonecyanoacetylhydrazone (8a): To a solution of compound 6a (0.29 g, 1.18 × 10\(^{-3}\) mol) in ethanol (25 mL) containing piperidine (0.5 mL), benzaldehyde (0.11 g, 1.48 × 10\(^{-3}\) mol) was added. The reaction mixture was heated under reflux for 3hrs then poured onto a beaker containing an ice/water mixture and few drops of hydrochloric acid. The formed solid product was collected by filtration and dried obtaining deep yellowish brown crystals (from ethanol). Yield: 0.26 g (66%), m.p. 90 °C; IR (KBr) v/cm\(^{-1}\): 3500–3194 (NH\(_2\), 2NH), 3027 (CH aromatic), 2919 (CH\(_3\)), 2218 (CN), 1680 (C=O), 1608 (C=C); \(^1\)H-NMR δ: 2.51 (s, 3H, CH\(_3\)), 3.36 (s, 2H, CH\(_2\)), 4.77 (s, 2H, NH\(_2\)), 5.99 (s, 1H, =CH), 7.27–8.40 (m, 9H, C\(_6\)H\(_5\), C\(_6\)H\(_4\)), 8.81, 9.15 (2s, 2H, 2NH); \(^13\)C-NMR: 23.2 (CH\(_3\)), 27.4, 29.2 (2CH\(_2\)), 122.3, 122.8, 122.5, 126.0 128.3, 129.9, 138.3 (C\(_6\)H\(_4\), C\(_6\)H\(_5\)), 155.0, 164.3 (2C=N), 174.3 (C=O); Anal. Calcd. for C\(_{19}\)H\(_{19}\)N\(_5\)O (333.39): C, 68.45; H, 5.74; N, 21.01%. Found: C, 68.55; H, 5.54; N, 21.31%.

α-Benzal-4-methyl-ω-phenylhydrazinoacetophenonecyanoacetylhydrazone (8b). To a solution of compound 6b (0.36 g, 1.12 × 10\(^{-3}\) mol) in ethanol (25 mL) containing piperidine (0.5 mL), benzaldehyde (0.11 g, 1.12 × 10\(^{-3}\) mol) was added. The reaction mixture was heated under reflux for 3hrs then poured onto a beaker containing an ice/water mixture and a few drops of hydrochloric acid. The formed solid product was collected by filtration and dried obtaining yellowish brown crystals (from ethanol). Yield: 0.33 g (73%), m.p. 102 °C; IR (KBr) v/cm\(^{-1}\): 3300-3179 (3NH), 3026 (CH aromatic), 2920 (CH\(_3\)), 2210 (CN), 1688 (C=O), 1600 (C=C); \(^1\)H-NMR δ: 2.88 (s, 3H, CH\(_3\)), 3.37 (s, 2H, CH\(_2\)), 6.90–8.40 (m, 15H, =CH, 2CH\(_3\), C\(_6\)H\(_3\)), 9.20, 10.83, 12.62 (3s, 3H, 3NH); \(^13\)C-NMR: 23.2 (CH\(_3\)), 27.4, 29.2 (2CH\(_2\)), 120.9, 121.1, 122.3, 122.8, 123.4, 123.9, 122.5, 126.0 128.3, 129.5, 138.6 (2C\(_6\)H\(_5\), C\(_6\)H\(_4\)) 155.3, 164.0 (2C=N), 174.6 (C=O); Anal. Calcd. for C\(_{25}\)H\(_{21}\)N\(_3\)O (409.48): C, 73.33; H, 5.66; N, 17.10%. Found: C, 73.52; H, 5.97; N, 17.33%.
1-Amino-6-(4-methylphenyl)-3-(α-phenylhydrazoacetonitrilo)-1,2,4-triazine (10a). To a cold solution (0–5 °C) of compound 6a (0.40 g, 1.63 × 10^{-3} mol) in ethanol (50 mL) containing sodium hydroxide (10 mL, 10%) and a solution of benzenediazonium chloride (1.63 × 10^{-3} mol) [which was prepared by dissolving sodium nitrite (0.16 g, 2.44 × 10^{-3} mol) in water, 2 mL was added to a cold solution of aniline (0.15 g, 1.63 × 10^{-3} mol) containing the appropriate amount of hydrochloric acid and with continuous stirring] was added with continuous stirring. The formed solid product was collected by filtration and dried to give reddish brown crystals (from ethanol and few drops of dimethylformamide). Yield: 0.58 g (63%), m.p. 170 °C; IR (KBr) $\nu$/cm$^{-1}$: 3400–3307 (NH$_2$, NH), 3027 (CH aromatic), 2919, 2862 (CH$_3$, CH$_2$), 2211 (CN), 1600 (C=C); $^1$H-NMR $\delta$: 2.51 (s, 3H, CH$_3$), 3.37 (s, 2H, CH$_2$), 4.22 (s, 2H, NH$_2$), 6.50–8.20 (m, 9H, C$_6$H$_5$, C$_6$H$_4$), 11.64 (s, 1H, NH); $^{13}$C-NMR: 23.8 (CH$_3$), 51.9 (triazine CH$_2$), 115.8 (CN), 118.3, 118.9, 119.2, 121.6, 124.8, 133.0, 139.6 (C$_6$H$_5$, C$_6$H$_4$), 156.9, 163.5, 164.1 (3 C=N); Anal. Calcd. for C$_{18}$H$_{17}$N$_7$ (331.38): C, 65.24; H, 5.17; N, 29.58%. Found: C, 65.42; H, 5.27; N, 29.36%.

3.4. General Procedure for the Synthesis of 10b and 10c

To a cold solution (0–5 °C) of compound 6a (0.49 g, 1.99 × 10^{-3} mol) in ethanol (50 mL) containing sodium hydroxide solution (10 mL, 10%) was added with continuous stirring a solution of either 3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophene-2-diazonium chloride (9b) (1.99 × 10^{-3} mol) or ethyl 4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate-2-diazonium chloride (9c) (1.99 × 10^{-3} mol) [which was prepared by dissolving sodium nitrite (0.20 g, 2.99 × 10^{-3} mol) in water, 2 mL was added to a cold solution of either the 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophene (0.35 g, 1.99 × 10^{-3} mol) or ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (0.45 g, 1.99 × 10^{-3} mol) dissolved in acetic acid (50 mL) containing the appropriate amount of hydrochloric acid and with continuous stirring]. The solid product formed was collected by filtration and dried.

1-Amino-6-(4-methylphenyl)-3-[(α-(3-cyano-2-hydrazo-4,5,6,7-tetra-hydrobenzo[b]thiophene)acetonitrilo)-1,2,4-triazine (10b). Deep brown crystals (from ethanol). Yield: 0.67 g (75%), m.p. 210–214 °C; IR (KBr) $\nu$/cm$^{-1}$: 3600–3425 (NH$_2$, NH), 3030 (CH aromatic), 2930 (CH$_3$), 2250, 2217 (2CN), 1606 (C=C); $^1$H-NMR $\delta$: 1.63–2.34 (m, 8H, cyclohexene 4CH$_2$), 2.51 (s, 3H, CH$_3$), 3.60 (s, 2H, CH$_2$), 4.78 (s, 2H, NH$_2$), 6.97–8.32 (m, 4H, C$_6$H$_4$), 9.84 (s, 1H, NH); $^{13}$C-NMR: 19.4, 23.0, 25.1, 27.8 (cyclohexene 4CH$_2$), 156.0, 163.5 164.3 (3 C=N); Anal. Calcd. for C$_{21}$H$_{20}$N$_8$S (416.50): C, 60.56; H, 4.84; N, 26.90; S, 7.70%. Found: C, 60.28; H, 5.03; N, 26.61; S, 7.88%.

Ethyl 1-amino-6-(4-methylphenyl)-3-[(α(2-hydrazo-4,5,6,7-tetra-hydrobenzo[b]thiophene-3-carboxylate)acetonitrilo)-1,2,4-triazine (10c). Brown crystals (from ethanol) Yield: 0.53 g (57%), m.p. 90 °C; IR (KBr) $\nu$/cm$^{-1}$: 3400–3287 (NH$_2$, NH), 2976, 2934, 2860 (2CH$_3$, CH$_2$), 2213 (CN), 1711 (C=O); $^1$H-NMR $\delta$: 1.07–1.92 (m, 8H, cyclohexene 4CH$_2$), 2.51 (s, 3H, CH$_3$), 2.77 (t, 3H, J = 7.04 Hz, CH$_3$), 3.36 (s, 2H, CH$_2$), 4.24 (q, 2H, J = 7.04 Hz, CH$_2$), 4.99 (s, 2H, NH$_2$), 6.50–8.08 (m, 4H, C$_6$H$_4$), 9.88 (s, 1H, NH); $^{13}$C-NMR: 14.5, 24.6 (2 CH$_3$), 23.2, 23.6, 25.1, 27.6 (cyclohexene 4CH$_2$), 55.4 (triazine...
CH2), 60.8 (ester CH2), 128.7, 129.0, 129.7, 132.1, 136.5, 138.9, 140.8 (C6H5, thiophene C), 115.9, 116.7 (2CN), 155.2, 158.0, 163.1 (3 C=N), 165.6 (CO); Anal. Calcd. for C23H25N7O2S (463.56): C, 59.59; H, 5.44; N, 21.15; S, 6.93%. Found: C, 59.83; H, 5.46; N, 20.91; S, 7.05%.

1-Phenylamino-6-(4-methylphenyl)-3-(α-phenylhydrazoacetonitrilo)-1,2,4-triazine (10d). To a cold solution (0–5 °C) of compound 6b (0.53 g, 1.64 × 10⁻³ mol) in ethanol (50 mL) containing sodium hydroxide (10 mL, 10%) was added with continuous stirring a solution of benzenediazonium chloride (1.64 × 10⁻³ mol) [which was prepared by dissolving sodium nitrite (0.17 g, 2.47 × 10⁻³ mol) in water, 2 mL was added to a cold solution of aniline (0.15 g, 1.64 × 10⁻³ mol) containing the appropriate amount of hydrochloric acid and with continuous stirring]. The formed solid product was collected by filtration to give reddish brown crystals (from ethanol and few drops of dimethylformamide). Yield: 0.58 g (86%), m.p. 120 °C; IR (KBr) ν/cm⁻¹: 3500–3422 (2NH), 3057 (CH aromatic), 3028, 2921 (CH3, CH2), 2211 (CN), 1600 (C=C); 1H-NMR δ: 2.50 (s, 3H, CH3), 3.31 (s, 2H, CH2), 6.60–7.65 (m, 14H, 2C6H5, C6H4), 8.16, 9.05 (s, 2H, 2NH); 13C-NMR: 23.6 (CH3), 51.9 (triazine CH2), 115.6 (CN), 118.6, 118.7, 119.2, 120.1, 121.6, 124.8, 133.6, 134.8, 139.9 (2C6H5, C6H4), 156.6, 163.7, 164.0 (3 C=N); Anal. Calcd. for C24H21N7 (407.47): C, 70.74; H, 5.19; N, 24.06%. Found: C, 71.05; H, 5.38; N, 23.87%.

3.5. General Procedure for the Synthesis of 10e and 10f

To a cold solution (0–5 °C) of compound 6b (0.40 g, 1.24 × 10⁻³ mol) in ethanol (50 mL) containing sodium hydroxide solution (10 mL, 10%) was added with continuous stirring a solution of either 3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophene-2-diazonium chloride (9b) (1.24 × 10⁻³ mol) or ethyl 4,5,6,7-tetrahydrobenzo[b]thiophen-3-carboxylate-2-diazonium chloride (9c) (1.24 × 10⁻³ mol) [which was prepared by dissolving sodium nitrite (0.12 g, 1.86 × 10⁻³ mol) in water, 2 mL was added to a cold solution of either the 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophene (0.22 g, 1.99 × 10⁻³ mol) or ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (0.28 g, 1.99 × 10⁻³ mol) dissolved in acetic acid (50 mL) containing the appropriate amount of hydrochloric acid and with continuous stirring]. The solid product formed was collected by filtration and dried. 1-Phenylamino-6-(4-methylphenyl)-3-(α-(3-cyano-2-hydrazo-4,5,6,7-tetrahydrobenzo[b]thiophene)-acetonitrilo)-1,2,4-triazine (10e). Reddish brown crystals (from ethanol), yield: 0.34 g (55%), m.p. 190 °C; IR (KBr) ν/cm⁻¹: 3500–3425 (2NH), 3030 (CH aromatic), 2930 (CH3), 2250, 2217 (2CN), 1606 (C=C); 1H-NMR δ: 1.63–2.34 (m, 8H, cyclohexene), 2.51 (s, 3H, CH3), 3.60 (s, 2H, CH2), 6.97–8.32 (m, 9H, C6H5, C6H4), 9.84, 10.00 (s, 2H, 2NH); 13C-NMR: 19.1, 23.3, 25.4, 27.4 (cyclohexene 4CH2), 24.9 (CH3), 51.7 (triazine CH2), 115.6, 116.3 (2CN), 118.3, 118.9, 119.2, 121.6, 124.6, 133.2, 136.5, 136.9 139.4 (thiophene C, C6H5, C6H4), 156.1, 163. 3, 164.6 (3 C=N); Anal. Calcd. for C27H24N8S (492.60): C, 65.83; H, 4.91; N, 22.75; S, 6.51%. Found: C, 65.96; H, 5.24; N, 22.55; S, 6.79%.

Ethyl 1-phenylamino-6-(4-methylphenyl)-3-(α-(2-hydrazo-4,5,6,7-tetrahydrobenzo[b]thiophen-3-carboxylate)-acetonitrilo)-1,2,4-triazine (10f). Pale reddish brown crystals (from ethanol). Yield: 0.40 g (59%), m.p. 150–160 °C; IR (KBr) ν/cm⁻¹: 3400–3287 (2NH), 2976, 2934, 2860 (2CH3,
CH₂), 2213 (CN), 1711 (C=O); ¹H-NMR δ: 1.07–1.92 (m, 8H, cyclohexene), 2.51 (s, 3H, CH₃), 2.77 (t, 3H, J = 6.89 Hz, CH₃), 3.36 (s, 2H, CH₂), 4.24 (q, 2H, J = 6.89 Hz, CH₂), 6.50–8.08 (m, 9H, C₆H₅, C₆H₄), 9.88, 10.30 (2s, 2H, 2NH); ¹³C-NMR: 14.3, 24.8 (2 CH₃), 23.0, 23.3, 25.1, 27.8 (cyclohexene 4CH₂), 55.54 (triazine CH₂), 60.8 (ester CH₂), 128.7, 129.2, 129.7, 132.0, 136.5, 138.9, 140.8 (C₆H₅, thiophene C), 115.8, 116.5 (2CN), 155.2, 158.0, 163.3 (3 C=N), 165.6 (CO); Anal. Calcd. for C₂₉H₂₉N₇O₂S (539.65): C, 64.54; H, 5.42; N, 18.17; S, 5.94%. Found: C, 64.68; H, 5.23; N, 17.97; S, 6.20%.

3.6. General Procedure for the Synthesis of 12a and 12b

To a solution of compound 6a (0.47 g, 1.91 × 10⁻³ mol) in ethanol (20 mL) containing triethylamine (0.5 mL), either malononitrile (0.12 g, 1.91 × 10⁻³ mol) or ethyl cyanoacetate (0.21 g, 1.91 × 10⁻³ mol) was added. The reaction mixture was heated under reflux for 3 hrs then poured onto a beaker containing an ice/water mixture and a few drops of hydrochloric acid. The solid product formed was collected by filtration and dried.

3-Cyano-4,6-diamino-2-oxo-1-imino-(4-methyl-ω-hydrazinoaceto-phenonylidieno)pyridine (12a). Brown crystals (from ethanol). Yield: 0.38 g (64%), m.p. 158 °C; IR (KBr) υ/cm⁻¹: 3500–3227 (3NH₂, NH), 3028 (CH aromatic), 2974 (CH₃), 2919 (CH₂), 2201 (CN), 1685 (C=O), 1600 (C=C); ¹H-NMR δ: 2.51 (s, 3H, CH₃), 3.38 (s, 2H, CH₂), 4.38, 4.79, 5.30 (3s, 6H, 3NH₂), 7.05–8.19 (m, 5H, pyridine H-3, C₆H₄), 10.82 (s, 1H, NH); ¹³C-NMR: 23.8 (CH₃), 51.7 (CH₂), 80.9, 89.5, 114.8, 125.6 (pyridine C), 117.9 (CN), 126.2, 128.0, 129.3, 129.6 (C₆H₅), 163.5 (C=O), 170.9 (C=N); Anal. Calcd. for C₁₅H₁₇N₇O (311.34): C, 57.87; H, 5.50; N, 31.49%. Found: C, 57.96; H, 5.48; N, 31.68%.

4-Amino-3-cyano-6-hydroxy-2-oxo-1-imino(4-methyl-ω-hydrazinoacetophenonylidieno)pyridine (12b). Pale brown crystals (from ethanol). Yield: 0.66 g (111%), m.p. 140 °C; IR (KBr) υ/cm⁻¹: 3600–3227 (OH, 2NH₂, NH), 3028 (CH aromatic), 2974 (CH₃), 2919 (CH₂), 2201 (CN), 1685 (C=O), 1600 (C=C); ¹H-NMR δ: 2.50 (s, 3H, CH₃), 3.39 (s, 2H, CH₂), 4.34, 4.77, (2s, 4H, 2NH₂), 7.05–8.19 (m, 5H, pyridine H-3, C₆H₄), 10.84 (s, 1H, NH), 12.64 (s, 1H, OH); ¹³C-NMR: 23.8 (CH₃), 51.7 (CH₂), 82.9, 89.5, 116.8, 125.9 (pyridine C), 117.9 (CN), 126.4, 128.0, 129.3, 129.6 (C₆H₅), 170.9, 176.5 (2C=N), 164.3 (2C=O); Anal. Calcd. for C₁₅H₁₆N₆O₂ (312.33): C, 57.68; H, 5.16; N, 26.90%. Found: C, 57.42; H, 5.09; N, 26.76%.

3.7. General Procedure for the Synthesis of 12c and 12d

To a solution of compound 6b (0.60 g, 1.86 × 10⁻³ mol) in ethanol (20 mL) containing triethylamine (0.5 mL), either malononitrile (0.12 g, 1.86 × 10⁻³ mol) or ethyl cyanoacetate (0.21 g, 1.86 × 10⁻³ mol) was added. The reaction mixture was heated under reflux for 3 hrs then poured onto a beaker containing an ice/water mixture and a few drops of hydrochloric acid. The solid product formed was collected by filtration and dried.
3-Cyano-4,6-diamino-2-oxo-1-imino-(4-methyl-ω-phenylhydrazino-acetophenonylidieno)pyridine  (12c)  
Green crystals (from ethanol). Yield: 0.51 g (70%), m.p. 80 °C; IR (KBr) ν/cm⁻¹: 3480–3227 (2NH₂, 2NH), 3028 (CH aromatic), 2974 (CH₃), 2919 (CH₂), 2201 (CN), 1685 (C=O), 1600 (C=C); ¹H-NMR δ: 2.51 (s, 3H, CH₃), 3.38 (s, 2H, CH₂), 4.38, 4.79 (2s, 4H, 2NH₂), 6.48–8.19 (m, 10H, pyridine H-3, C₆H₅, C₆H₄), 10.82, 11.20 (2s, 2H, 2NH); ¹³C-NMR: 23.7 (CH₃), 51.9 (CH₂), 80.7, 89.3, 114.9, 125.3 (pyridine C), 118.3 (CN), 126.4, 128.0, 129.3, 129.6 (C₆H₅), 170.9 (C=N), 162.5 (C=O); Anal. Calcd. for C₂₁H₂₁N₇O (387.44): C, 65.10; H, 5.46; N, 25.30%. Found: C, 64.21; H, 5.36; N, 25.38%.

4-Amino-3-cyano-6-hydroxy-2-oxo-1-imino(4-methyl-ω-phenyl-hydrazinoacetophenonylidieno) pyridine  (12d). Yellowish green crystals (from ethanol). Yield: 0.58 g (80%). Mp 84–100 °C; IR (KBr) ν/cm⁻¹: 3600–3185 (OH, NH₂, 2NH), 3027 (CH aromatic), 2975 (CH₃), 2919 (CH₂), 2206 (CN), 1684 (C=O), 1600 (C=C); ¹H-NMR δ: 2.50 (s, 3H, CH₃), 3.39 (s, 2H, CH₂), 4.34 (s, 2H, NH₂), 6.21 (s, 1H, pyridine H-3), 7.33–8.70 (m, 9H, C₆H₅, C₆H₄), 10.84, 11.22 (2s, 2H, 2NH), 12.64 (s, 1H, OH); ¹³C-NMR: 23.6 (CH₃), 51.6 (CH₂), 80.2, 89.5, 114.5, 125.0 (pyridine C), 118.1 (CN), 126.2, 128.2, 129.5, 129.6 (C₆H₅), 164.8 (C=O), 170.9 (C=N); Anal. Calcd. for C₂₁H₂₀N₆O₂ (388.43): C, 64.93; H, 5.19; N, 21.63%. Found: C, 65.42; H, 5.69; N, 21.13%.

3.8. General Procedure for the Synthesis of 14a and 14b

To a solution of compound 6a (0.52 g, 2.12 × 10⁻³ mol) in ethanol (20 mL) containing piperidine (0.5 mL), either acetylacetone (0.21 g, 2.21 × 10⁻³ mol) or ethyl acetoacetate (0.27 g, 2.21 × 10⁻³ mol) was added. The reaction mixture was heated under reflux for 3 hrs then poured onto a beaker containing an ice/water mixture and a few drops of hydrochloric acid. The solid product formed was collected by filtration and dried.

3-Cyano-4,6-diamethyl-2-oxo-1-imino-(4-methyl-ω-hydrazinoaceto-phenonilidieno)pyridine  (14a) Brown crystals (from ethanol). Yield: 0.37 g (56%), m.p. 144 °C; IR (KBr) ν/cm⁻¹: 3433–3229 (NH₂, NH), 3027 (CH aromatic), 2221 (CN), 1685 (C=O), 1600 (C=C); ¹H-NMR δ: 2.34, 2.54, 3.04 (3s, 9H, 3CH₃), 3.39 (s, 2H, CH₂), 6.21 (s, 1H, pyridine H-3), 7.33–7.45 (m, 4H, C₆H₄), 10.84, 11.63 (s, 2H, 2NH); ¹³C-NMR: 16.0, 19.2, 24.3 (3CH₃), 51.2 (CH₂), 80.0, 88.3, 115.3, 123.9 (pyridine C), 116.8 (CN), 126.0, 127.9, 128.3, 129.1 (C₆H₅), 164.9 (C=O), 173.6 (C=N); Anal. Calcd. for C₁₇H₁₉N₅O (309.37): C, 66.00; H, 6.19; N, 22.63%; found: C, 66.29; H, 6.23; N, 23.53%.

3-Cyano-6-hydroxy-4-methyl-2-oxo-1-imino(4-methyl-ω-hydrazino-acetophenonylidieno)pyridine  (14b). Brown crystals (from ethanol). Yield: 0.38 g (57%), m.p. 136 °C; IR (KBr) ν/cm⁻¹: 3549–3321 (OH, NH₂, NH), 3027 (CH aromatic), 2221 (CN), 1680 (C=O), 1600 (C=C); ¹H-NMR δ: 2.53, 3.13 (3s, 9H, 3CH₃), 3.39 (s, 2H, CH₂), 6.22 (s, 1H, pyridine H-3), 7.33–7.39 (m, 4H, C₆H₄), 10.85, 11.33 (s, 2H, 2NH); ¹³C-NMR: 16.1, 19.0, 24.3 (3CH₃), 51.3 (CH₂), 80.0, 88.3, 115.3, 123.9 (pyridine C), 116.8 (CN), 126.3, 127.9, 128.3, 129.1 (C₆H₅), 164.9 (C=O), 173.6 (C=N); Anal. Calcd. for C₁₆H₁₇N₅O₂ (311.34): C, 61.44; H, 5.50; N, 22.49%; found: C, 61.23; H, 5.67; N, 22.30%. 

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3.9. General Procedure for the Synthesis of 14c and 14d

To a solution of compound 6b (0.60 g, $1.86 \times 10^{-3}$ mol) in ethanol (20 mL) containing piperidine (0.5 mL), either acetylacetone (0.18 g, $1.86 \times 10^{-3}$ mol) or ethyl acetoacetate (0.24 g, $1.86 \times 10^{-3}$ mol) was added. The reaction mixture was heated under reflux for 3 hrs then poured onto a beaker containing an ice/water mixture and a few drops of hydrochloric acid. The solid product formed was collected by filtration and dried.

3-Cyano-4,6-diamethyl-2-oxo-1-imino-(4-methyl-ω-phenylhydrazino-acetophenonyldieno)pyridine (14c). Yellowish brown crystals (from ethanol). Yield: 0.61 g (85%), m.p. 100 °C; IR (KBr) $\nu$/cm$^{-1}$: 3450–3229 (2NH), 3027 (CH aromatic), 2213 (CN), 1680 (C=O), 1600 (C=C); $^1$H-NMR $\delta$: 2.34, 2.51, 3.01 (3s, 9H, 3CH$_3$), 3.37 (s, 2H, CH$_2$), 6.21 (s, 1H, pyridine H-3), 6.50–8.41 (m, 9H, C$_6$H$_5$, C$_6$H$_4$), 10.82, 12.63 (s, 2H, 2NH); $^{13}$C-NMR: 16.3, 19.1, 24.3 (3CH$_3$), 51.5 (CH$_2$), 80.3, 88.6, 115.0, 127.7, 155.8 (pyridine C), 116.5 (CN), 118.7, 119.0, 120.8, 122.5, 126.0, 127.8, 128.7, 129.8 (C$_6$H$_5$, C$_6$H$_4$), , 166.2 (C=O), 173.6 (C=N); Anal. Calcd. for C$_{23}$H$_{23}$N$_5$O (385.47): C, 71.66; H, 6.01; N, 18.16%; found: C, 71.86; H, 5.98; N, 17.99%.

3-Cyano-6-hydroxy-4-methyl-2-oxo-1-imino(4-methyl-ω-phenyl-hydrazinoacetophenonyldieno)-pyridine (14d). Yellowish brown crystals (from ethanol). Yield: 0.48 g (66%). m.p. 96 °C; IR (KBr) $\nu$/cm$^{-1}$: 3600–3220 (OH, 2NH), 3027 (CH aromatic), 2211 (CN), 1682 (C=O), 1600 (C=C); $^1$H-NMR $\delta$: 2.51, 3.00 (2s, 6H, 2CH$_3$), 3.40 (s, 2H, CH$_2$), 6.54 (s, 1H, pyridine H-3), 7.34–8.40 (m, 9H, C$_6$H$_5$, C$_6$H$_4$), 9.62, 10.82 (2s, 2H, 2NH), 12.65 (s, 1H, OH); $^{13}$C-NMR: 16.3, 19.4, 24.6 (3CH$_3$), 51.5 (CH$_2$), 80.8, 88.4, 116.5, 155.4 (pyridine C), 116.5 (CN), 118.8, 119.0, 120.3, 121.7, 126.0, 127.8, 128.9, 129.0 (C$_6$H$_5$, C$_6$H$_4$), , 166.4 (C=O), 173.9 (C=N); Anal. Calcd. for C$_{22}$H$_{21}$N$_5$O$_2$ (387.44): C, 68.20; H, 5.46; N, 18.07%; Found: C, 68.49; H, 5.84; N, 17.79%.

2-Acetonitrilo-5-(4-methylphenyl)-1,3,4-oxadiazine (15). A solution of compound 3 (1.00 g, $3.39 \times 10^{-3}$ mol) in sodium ethoxide (50 mL) was heated under reflux for 3 hrs then poured onto a beaker containing an ice/water mixture and a few drops of hydrochloric acid. The formed solid product was collected by filtration and dried to give pale brown crystals (from ethanol). Yield 0.39 g (54%), m.p. 190–193 °C; IR (KBr) $\nu$/cm$^{-1}$: 3028 (CH aromatic), 2998 (CH$_3$), 2921 (CH$_2$), 2210 (CN), 1673 (C=N), 1609 (C=C); $^1$H-NMR $\delta$: 2.51 (s, 3H, CH$_3$), 3.35, 4.42 (2s, 4H, 2CH$_2$), 7.00–8.40 (m, 4H, C$_6$H$_4$); $^{13}$C-NMR: 24.4 (CH$_3$), 20.3 (CH$_2$), 64.3 (oxadiazine CH$_2$), 116.9 (CN), 126.0, 127.8, 128.9, 129.0, 133.1 (C$_6$H$_5$), 170.5 (C=N); Anal. Calcd. for C$_{12}$H$_{11}$N$_3$O (213.24): C, 67.59; H, 5.19; N, 19.70%; found: C, 67.70; H, 5.38; N, 19.88%.

2-(α-Benzalacetonitrilo)-5-(4-methylphenyl)-1,3,4-oxadiazine hydrochloride (16). To a solution of compound 3 (2.00 g, $6.79 \times 10^{-3}$ mol) in ethanol (30 mL) containing piperidine (0.5 mL), benzaldehyde (0.72 g, $6.79 \times 10^{-3}$ mol) was added. The reaction mixture was heated under reflux for 3 hrs then poured onto a beaker containing an ice/water mixture with a few drops of hydrochloric acid. The formed solid product was collected by filtration and dried to afford brown crystals (from ethanol). Yield: 1.35 g (58%), m.p. 100 °C; IR (KBr) $\nu$/cm$^{-1}$: 3050 (CH aromatic), 3029 (CH$_3$), 2921 (CH$_2$),
2830 (CH), 2216 (CN), 1604 (C=C); \(^1\)H-NMR \(\delta\): 2.51 (s, 3H, CH\(_3\)), 4.22 (s, 2H, CH\(_2\)), 5.16 (s, 1H, =CH), 7.35–8.02 (m, 9H, C\(_6\)H\(_5\), C\(_6\)H\(_4\)); \(^{13}\)C-NMR: 24.6 (CH\(_3\)), 63.8 (oxadiazine CH\(_2\)), 116.7 (CN), 118.2 (CH=CH), 120.4, 121.9, 124.5, 126.2, 127.4, 127.9, 129.2, 133.9 (C\(_6\)H\(_4\), C\(_6\)H\(_5\)), 144.2 (CH=CH), 171.5, 173.9 (2C=N); \textit{Anal.} Calcd. for C\(_{19}\)H\(_{15}\)N\(_3\)O (301.34): C, 75.73; H, 5.02; N, 13.94%; found: C, 75.52; H, 4.91; N, 14.31%.

2-(\(\alpha\)-Phenylhydrazoacetonitrilo)-5-(4-methylphenyl)1,3,4-oxadiazine (17). To a cold solution (0–5 °C) of compound 15 (1.00 g, 3.39 \(\times\) 10\(^{-3}\) mol), in ethanol (50 mL) was added with continuous stirring benzenediazonium chloride (3.39 \(\times\) 10\(^{-3}\) mol) [which was prepared by dissolving sodium nitrite (0.35 g, 5.09 \(\times\) 10\(^{-3}\) mol) in water, 2 mL was added to a cold solution of aniline (0.31 g, 3.39 \(\times\) 10\(^{-3}\) mol) containing the appropriate amount of hydrochloric acid and with continuous stirring]. The solid product formed after adjusting the pH 6 and stirring was collected by filtration and dried to give reddish brown crystals (from ethanol and dimethylformamide). Yield: 0.96 g (89%), m.p. 130 °C; IR (KBr) \(\nu/cm\)^\(-1\): 3300–3215 (NH), 3057 (CH aromatic), 3030 (CH\(_3\)), 2920 (CH\(_2\)), 2219 (CN), 1679 (C=N), 1603 (C=C); \(^1\)H-NMR \(\delta\): 2.52 (s, 3H, CH\(_3\)), 3.38 (s, 2H, CH\(_2\)), 7.21–8.82 (m, 9H, C\(_6\)H\(_5\), C\(_6\)H\(_4\)), 10.22 (s, 1H, NH). ). \(^{13}\)C-NMR: 24.3 (CH\(_3\)), 63.0 (oxadiazine CH\(_2\)), 116.5 (CN), 121.5, 121.9, 124.0, 123.5, 126.7, 127.0, 127.6, 129.2, 133.0 (C\(_6\)H\(_4\), C\(_6\)H\(_5\)), 166.9, 170.5, 173.9 (3C=N). \textit{Anal.} Calcd. for C\(_{18}\)H\(_{15}\)N\(_5\)O (317.35): C, 68.12; H, 4.76; N, 22.06%; found: C, 67.92; H, 4.91; N, 21.25%.

4. Conclusions

In this work cyanoacetyl hydrazine (1) reacted with \(\alpha\)-haloketone 2 to afford the \(\alpha\)-bromohydrazide-hydrazone derivative 3. The latter compound was used in a series of heterocyclic transformations to give compounds that were tested as antitumor agents. The 2-hydroxypyridine derivatives 14b showed the highest inhibitory activity.

Acknowledgments

R. M. Mohareb expressed his deepest thank to the Alexander von Humboldt Foundation in Bonn for its financial support through a fellowship in Erlangen University during Summer 2009 and for the completion of this work.

References and Notes

1. Bharti, S.K.; Nath, G.; Tilak, R.; Singh, S.K. Synthesis, anti-bacterial and anti-fungal activities of some novel Schiff bases containing 2,4-disubstituted thiazole ring. \textit{Eur. J. Med. Chem.} 2010, 45, 651–660.
2. Loncle, C.; Brunel, J.M.; Vidal, N.; Dherbomez, M.; Letourneux, Y. Synthesis and antifungal activity of cholesterol-hydrazone derivatives. \textit{Eur. J. Med. Chem.} 2004, 39, 1067–1071.
3. Garoufalias, S.P.; Pouli, N.; Marakos, P.; Ladas, A.C. Synthesis antimicrobial and antifungal activity of some new 3-substituted derivatives of 4-(2,4-dichlorophenyl)-5-adamantyl-1H-1,2,4-triazole. \textit{Farmaco} 2002, 57, 973–977.
4. Vicini, P.; Zani, F.; Cozzini P.; Doytchinova, I. Hydrazones of 1,2-benzisothiazole hydrazides: synthesis, antimicrobial activity and QSAR investigations. *Eur. J. Med. Chem.* 2002, 37, 553–567.

5. Popp, F.D. Potential anticonvulsant. XII. anticonvulsant activity of some aldehyde derivatives. *Eur. J. Med. Chem.* 1989, 24, 313–316.

6. Sridhar, S.K.; Pandeya, S.N.; Stables J.P.; Atmakuru, R. Anticonvulsant activity of hydrazones, Schiff and Mannich bases of isatin derivatives *Eur. J. Pharm. Sci.* 2002, 16, 129–132.

7. Küçükgüzel, S.G.; Mazi, A.; Sahin, F.; Öztürk, S.; Stables, J.P. Synthesis and biological activities of diflunisal hydrazide–hydrazones. *Eur. J. Med. Chem.* 2003, 38, 1005–1013.

8. Todeschini, A.R.; Miranda, A.L.P.; Silva, K.C.M.; Parrini, S.C.; Barreiro, E. Synthesis and evaluation of analgesic, antiinflammatory and antiplatelet properties of new 2-pyridylarylhydrazone derivatives. *Eur. J. Med. Chem.* 1998, 33, 189–199.

9. Gaston, M.A.; Dias, L.R.S.; Freitas, A.C.C.; Miranda, A.L.P.; Barreiro, E. Synthesis and Analgesic Properties of New 4-Arylhydrazone 1H-Pyrazolo[3,4-b] Pyridine Derivatives. *J. Pharm. Acta Helv.* 1996, 71, 213–219.

10. Melnyk, P.; Leroux, V.; Sergheraert, C.; Grellier, P. Design, synthesis and in vitro antimalarial of an acylhydrazone library. *Bioorg. Med. Chem. Lett.* 2006, 16, 31–35.

11. Küçükgüzel, S.G.; Rollas, S., Küçükgüzel, I.; Kiraz, M. Synthesis and antimycobacterial activity of some coupling products from 4-aminobenzoic acid hydrazones. *Eur. J. Med. Chem.* 1999, 34, 1093–1100.

12. Kaymakçıoğlu, B.K.; Rollas, S. Synthesis, characterization and evaluation of antituberculosis activity of some hydrazones. *Farmaco* 2002, 57, 595–599.

13. Terzioglu, N.; Gursoy, A. Synthesis and anticancer evaluation of some new hydrazone derivatives of 2,6-dimethylimidazo[2,1-b][1,3,4]thiadiazole-5-carbohydrazide. *Eur. J. Med. Chem.* 2003, 38, 781–786.

14. Sondhi, S.M.; Dinodia, M., Kumar, A. Synthesis, anti-inflammatory and analgesic activity evaluation of some amide and hydrazone derivatives *Bioorg. Med. Chem.* 2006, 14, 4657–4663.

15. Boga, C.; Fiume, L.; Baglioni, M.; Bertucci, C.; Farina, C.; Kratz, F.; Manerba, M.; Naldi, M.; Stefano, G. Characterisation of the conjugate of the (6-maleimidocaproyl)hydrazone derivative of doxorubicin with lactosaminated human albumin by 13C NMR spectroscopy. *Eur. J. Pharm. Sci.* 2009, 38, 262–269.

16. Garnett, M.C. Targeted drug conjugates: principles and progress. *Adv. Drug Deliv. Rev.* 2001, 53, 171–216.

17. Rodrigues, P.C.; Scheuermann, K.; Stockmar, C.; Maier, G.; Fiebig, H.; Unger, C.; Mühlaupt, R.; Kratz, F. Synthesis and *In vitro* efficacy of acid-Sensitive poly(ethylene glycol) paclitaxel conjugates. *Bioorg. Med. Chem. Lett.* 2003, 13, 355–360.

18. El-Sabbagh, O.I.; Rady, H.M. Synthesis of new acridines and hydrazones derived from cyclic β-diketone for cytotoxic and antiviral evaluation. *Eur. J. Med. Chem.* 2009, 44, 3680–3686.

19. Krakovicova, H.; Etrych, T.; Ulbrich, K. HPMA-based polymer conjugates with drug combination. *Eur. J. Pharm. Sci.* 2009, 37, 405–412.
20. Zhang, H.Z.; Drewe, J.; Tseng, B.; Kasibhatla, S.; Cai, S.X. Discovery and SAR of indole-2-carboxylic acid benzylidene-hydrazides as a new series of potent apoptosis inducers using a cell-based HTS assay. *Bioorg. Med. Chem.* **2004**, *12*, 3649–3655.

21. Vicini, P.; Incerti, M.I.; Colla, P.L.; Loddo, R. Anti-HIV evaluation of benzo[d]isothiazole hydrazones. *Eur. J. Med. Chem.* **2009**, *44*, 1801–1807.

22. Gürsoy, E.; Güzeldemirci, N.U. Synthesis and primary cytotoxicity evaluation of new imidazo[2,1-b]thiazole derivatives. *Eur. J. Med. Chem.* **2007**, *42*, 320–326.

23. Hao, J.J.; Xu, Y.; Geng, C.; Liu, W.Y.; Wang, E.; Gong, Z.; Ulbrich, N. Purification of α-Sarcin and an Antifungal Protein from Aspergillus giganteus by Blue Sepharose CL-6B Affinity Chromatography. *Protein Expr. Purif.* **1998**, *14*, 295–301.

24. Ke, S.Y.; Qian, X.H.; Liu, F.Y.; Wang, N.; Yang, Q.; Zhong, L. Novel 4H-1,3,4-oxadiazin-5(6H)-ones with hydrophobic and long alkyl chains: Design, synthesis, and bioactive diversity on inhibition of monoamine oxidase, chitin biosynthesis and tumor cell. *Eur. J. Med. Chem.* **2009**, *44*, 2113–2121.

*Sample Availability:* Samples of the compounds 4a–17 are available from the authors.

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