Convenient approach to novel tetracyclic-fused pyranoquinolinone compounds from 6-\(n\)-butyl-3-amino-4-hydroxypyrano[3,2-c]quinolinone

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Abstract

6-\(n\)-Butyl-3-aminopyrano[3,2-c]quinoline-2,5-dione has been synthesized and utilized to obtain various new heteroannulated pyranoquinolinones, containing pyrazine, oxazine, [1,2,4]triazine and oxadiazine in good yields. The newly synthesized compounds were characterized by spectral data and elemental analysis.

Keywords: Quinolinone, pyranoquinolinone, pyrazine, triazine, oxazine, oxadiazine
Introduction

Nitrogen containing heterocyclic compounds represent a notable type of anticancer drug applicants, which strongly activate cell apoptosis. Many quinoline containing compounds have been reported as potential antitumor agents. Quinoline skeleton perform an important aspect in anticancer drug improvement, as their derivatives show great results through different operations such as growth inhibitors by cell cycle arrest, apoptosis, inhibition of angiogenesis, disruption of cell migration, and modulation of nuclear receptor responsiveness. Alkaloids with pyrano[3,2-c]quinolone feature exhibit very wide range of biological activities. Additionally, 6-n-butylpyranoquinolone derivatives reveal highly forceful TOP2B inhibitors and disclose the highest cancer cell growth-inhibitory activity against different tumor cell lines. Addition a further heterocyclic ring to pyranoquinolinone scaffolds represent as a valuable performance for designing novel drugs by enhancing their biological effects and permit them to serve as antibacterial, anticoagulant, antitumor, and microtubule-targeting agents. Pyrazines are vital for our life due to their DNA strand-breakage activity and apoptosis efficiency. Synthetic pyrazine derivatives are useful as antiviral, anticancer, antibacterial, fungicidal, and herbicidal drugs. Otherwise, oxazine derivatives exhibit significant anticoagulant, antibacterial and antifungal activity. Additionally, compounds containing oxadiazine ring exhibit pronounced antibacterial, antifungal, antitumor and insecticidal activities. Likewise, the 1,2,4-triazine ring is a prominent structural core found in diverse biologically active systems and demonstrate an influential considerable group of antitumours. Heartened by exclusive facial characteristics of pyrazine, oxazine, triazine and oxadiazine, beyond their recorded promising bioactivity, it would be an attractive suggestion to merge the previous impressive heterocycles with 6-n-butylpyranoquinolinone moiety at face c in an individual molecular scaffolding. We pursue to devise innovative biological activity for the newly synthesized compounds.

Results and Discussion

The 3-aminopyranoquinolinone 1 was prepared by the method reported by Hassanin et al. N-Alkylated derivatives of amines are important synthetic intermediates in organic synthesis. Thus, reaction of compound 1 with bromoacetonitrile, in molar ratio (1:1) in boiling acetonitrile containing sodium hydride as a basic catalyst under dry nitrogen gas atmosphere was carried out (Scheme 1). The bearable products of this alkylation are either N-alkylated 2 or O-alkylated 3. In other previous studies, higher regioselectivity favoring N-alkylation was observed and the N-alkylated products were obtained in moderate to good isolated yields with only very small traces of O-alkylated products were detected.
Our product of alkylation gave a strong positive result with iron III chloride solution and it dissolved easily in diluted sodium hydroxide solution. These two observations indicate the presence of phenolic O-H. The IR spectrum of the product proved the non-changeable of OH band and nonappearance of the amino group, in addition to presence of two new stretching vibration bands at 3203 and 2188 cm$^{-1}$ due to NH and C≡N groups, respectively. $^1$H NMR spectrum showed a new distinguishable singlet signal at 4.29 ppm referred to CH$_2$-CN group and the presence of two deuterium-exchangeable protons, at 7.12 and 14.08 ppm, for the N-H and O-H protons, respectively. In addition, $^{13}$C NMR spectrum of the product revealed a new $sp^3$ hybridized carbon atom at 31.1 ppm attributed to the active methylene group (NH$_2$CH$_2$CN). Building on the above experimental and spectral analyses of the product, we assumed that a similar attitude of the literature was also observed and the N-alkylated compound 2 was the predominant regioisomer in moderate yield (45%). Also, the structure of compound 2 was supported by its mass spectrum which exhibited a molecular ion peak at $m/z$ 339 (M$^+$; 89%). Boiling acetonitrile derivative 2, in DMF containing sodium hydride as a basic catalyst, affected its intramolecular heterocyclization to produce intermediate compound 4 which subjected to Dimroth rearrangement under the reaction conditions to give the non-isolable intermediate compound 7. Therefore, a rearrangement can occur to yield the more stable pyrazinopyranoquinolinone compound 7 as described in scheme 1. The IR spectrum of compound 7 verified the absenteeism of the nitrile group. While the $^1$H NMR spectrum of 7 displayed the absence of the active methylene group and the presence of five aromatic signals in the region 7.42-8.05 ppm equivalent to the protons of the aryl functionality of the
quinolinone and the proton from pyrazine moiety. $^{13}$C-NMR spectrum confirmed the absence of methylene carbon signal at δ 31.14 ppm and appearance a new signal at δ 122.7 ppm attributed to \textit{sp}$^2$ hybridized carbon atom of pyrazine and other seventeen signals thus compatible with the number of carbon atoms in the molecular formula. The ESI-MS analysis of compound 7 showed an [M+H]$^+$ ion at m/z 340.4, and an abundant [M+Na]$^+$ ion at m/z 362.2. A [2M+Na]$^+$ was also observed at m/z 701.3.

In the continuation of our earlier work on the synthesis of oxazinopyranoquinolinones,\textsuperscript{28} the compound 2 was treated with triethyl orthoformate, under solvent free condition, to afford oxazinopyrano[3,2-c]quinolinone 8 (Scheme 1). The IR spectrum of compound 8 showed the absence of OH function. The $^1$H NMR spectrum showed singlet signal at 8.64 ppm distinguishable for oxazine proton and a chemical shift at 9.43 ppm due to NH, which disappeared on deuteration. \textsuperscript{13}C-NMR spectrum of compound 8 demonstrated the presence of fourteen aromatic carbons in the region 101-193 ppm due to the annulated tetracyclic system. The ESI-MS spectrum of compound 8 exhibited an [M+H]$^+$ ion at m/z 350.3, and an abundant [M+Na]$^+$ ion at m/z 372.1, in accordance with its structure.

We speculated that adding more amount of alkylating agent, would improve the yield of compound 2 which was formed in low yield (45%). Amazingly, we obtained a new product with alternative melting point. We supposed that the product may be N,O-dialkylated product 9 or the N,N-dialkylated product 10. The product gave a strong positive result with iron III chloride solution and it dissolved easily in diluted sodium hydroxide solution. The IR spectrum of the product proved the absence of the amino group and the appearance of broad band at 3347 cm$^{-1}$ due to OH group. The $^1$H NMR spectrum of the product displayed two characteristic singlet signals at 4.02 and 4.21 ppm. Also, \textsuperscript{13}C NMR spectrum revealed two carbon signals at 41.9, 42.1 ppm. Trusting on the above unexpected data, the N,O-dialkylated product 9 was excluded and we expected that further selectively alkylation on the amino group took place to produce the interesting diacetonitrile derivative 10 which contain two characteristic methylene groups (Scheme 2).

![Scheme 2](image-url)
polycrystalline nature as many sharp peaks were observed on its XRD chart. $^1$H NMR spectrum of compound 11 was marked by the existence of five aromatic signals in the region 7.51-8.71 ppm corresponding to the protons of the aryl functionality of the quinolinone and the proton from pyrazine moiety. $^{13}$C-NMR spectrum demonstrated the presence of methylene carbon signal at $\delta$ 40.9 and $\delta$ 117.5 ppm assigned to CN from the acetonitrile group, in addition to a new signal at $\delta$ 124.9 ppm discernible to $sp^2$ carbon atom of pyrazine and other 17 signals which are compatible with the number of carbon atoms in the molecular formula. The ESI-MS spectrum of compound 11 showed a [M+H]$^+$ ion at $m/z$ 379.2, [M+Na]$^+$ ion at $m/z$ 401.2 and the [2M+Na]$^+$ ion was also observed at $m/z$ 779.1.

We describe here the synthesis of the desired oxazinopyranoquinolinone, triazinopyranoquinolinones and oxadiazinopyranoquinolinone starting from the formamidine compound 12 which was prepared by treating amine 1 with dimethylformamide dimethylacetal (DMF-DMA). The compound 12 was allowed to react with bromoacetonitrile, in boiling tetrahydrofuran containing sodium hydride as a basic catalyst to obtain oxazinopyranoquinolinone 13 in high yield (67%) as outlined in scheme 3. The IR spectrum of compound 13 showed the absence of OH band and the appearance of two new stretching vibration bands at 3365 and 2203 cm$^{-1}$, due to the NH and C≡N groups, respectively. The $^1$H NMR spectrum showed singlet signal at 8.49 ppm distinguishable for oxazine proton and a deuterium-exchangeable singlet signal at 11.57 ppm assignable to NH proton. $^{13}$C-NMR spectrum of compound 13 exhibited the nonappearance of two $sp^3$ methyl carbons of formamidine 12 and the presence of fourteen aromatic carbons in the region 101-162 ppm due to the tetracyclic-fused system. Moreover, compound 13 showed a quasimolecular ion peak at $m/z$ 350.1 [M+H]$^+$ in the positive electrospray ionization-MS corresponding to C$_{19}$H$_{15}$N$_3$O$_4$. (Scheme 3)
The structure of formamidine 12 involves variable electron-deficient centers and it is expected to be relatively reactive towards nucleophilic reagents. Therefore, treatment of formamidine 12 with 1,2-binucleophiles such as hydrazine hydrate and phenyl hydrazine in DMF was studied to afford triazinopyranoquinolinones 14a and 14b as the main products in low yields (35-40%) (Scheme 3). Proof for the formation of compounds 14a and 14b attained from their 1H NMR spectra where there was a new singlet signal at 8.71 ppm in 14a and at 8.38 ppm in 14b assigned to the proton of triazine moiety. In addition, there are two deuterium-exchangeable signals at 9.10 ppm and 10.11 ppm due to 2NH protons in compound 14a and one (NH) at 9.40 ppm in compound 14b, also, nine signals of phenyl and benzo protons which were observed at 6.85 to 8.29 ppm in compound 14b. The 13C NMR spectra indicated the presence of seventeen singlet signals in 14a and twenty three singlet signals in 14b which were agreeable with the number of carbon atoms in their molecular formulas. Furthermore, the ESI-MS spectra of compounds 14a and 14b exhibited [M+Na]+ ions (m/z: 347.1 and 423.2, respectively) in accordance with their structures. Our attempts to study the reason of the low yield of products 14a and 14b by separating the side products from the mother liquor using chromatography technique were effective. We succeeded to isolate two side products from this reaction. The first one is the amine derivative 1 which probably resulted from thermal degradation of the aliphatic chain of compound 12 in a high boiling point polar solvent. The second side product was isolated in 25% yield with yellow crystals and distinctive melting point. Interesting observations were achieved from the spectra of this resulting side product. Its IR spectrum revealed a characteristic absorption band at 1679 cm⁻¹ due to new carbonyl group. Also, the 1H NMR spectrum showed the existence of more downfield chemical shift at 10.19 ppm, unchangeable with D₂O, which may belong to a formyl function. We think that this phenomenon is happen as a result for hydrolysis of the active formamidine derivative 12 by water content in the solvent to give formamide derivative 15. The 13C NMR spectrum proved the presence of a formyl group at 177.3 ppm. Moreover, the structure of compound 15 was confirmed by its ESI-MS spectrum which disclosed a quasimolecular ion peak at m/z 329.3 [M+H]+ and a sodiated molecular ion peak at m/z 351.2 [M+Na]+.

The compound 12 was heated with hydroxylamine hydrochloride in DMF at reflux, to obtain oxadiazinopyranoquinolinone 16. The 1H NMR spectrum of compound 16 showed a singlet signal at 9.41 ppm assignable for oxadiazine proton and a chemical shift at 12.36 ppm due to NH, which disappeared on deuteration. The 13C-NMR spectrum of compound 16 revealed the presence of four aliphatic carbon atoms due to the butyl group and thirteen sp² hybridized carbon atoms belonging to the aromatic carbon atoms of oxadiazinopyranoquinolinone system. ESI-MS spectrum of compound 16 showed a quasimolecular ion peak at m/z 326.3 [M+H]+ and a sodiated molecular ion peak at m/z 348.3 [M+Na]+. This reaction gave only a 43% yield of compound 16, as well as the two previous side products, the formamide derivative 15 in very low yield (20%) and the amine 1 in 20% yield.

Another oxadiazinopyranoquinolinone derivative 19 was synthesized by the coupling of bromoacetonitrile (methylene active compound) with diazonium chloride salt 17, in boiling pyridine. Firstly, The non-isolable intermediate compound 18 is formed by the coupling of diazonium salt 17 with quaternary salt which produced from the reaction of pyridine and bromoacetonitrile and then underwent intramolecular cyclization to furnish the desired tetracyclic system 19 (Scheme 4).
Scheme 4.

The IR spectrum of compound 19 displayed a new stretching vibration band at 2211 cm\(^{-1}\) due to C≡N group. The \(^1\)H NMR spectrum of product 19 was characterized by the existence of deuterium exchangeable singlet signal attributed to NH proton of oxadiazine ring at 11.22 ppm. The \(^{13}\)C-NMR of compound 19 had fourteen signals in the region 99-169 ppm belonging to the aromatic carbon atoms and the cyano group. The mass spectrum of the compound 19 represented the molecular ion peak as the base peak at \(m/z\) 350 (100%), which agree well with the proposed formula weight and confirm the suggested structures. ESI-MS spectrum of compound 19 showed a [M+H]\(^+\) ion at \(m/z\) 351.3 and a [2M+Na]\(^+\) ion at \(m/z\) 723.3.

**Experimental Section**

**General.** TLC analysis of the reaction mixtures was performed using Fluka analytical silica gel 60 F254 nm TLC plates. For column chromatography, Fluka analytical silica gel 60 0.063-0.2 mm (70–230 mesh ASTM) was used for the separation. Melting points were recorded on Sanyo Gallenkamp MPD 350-BM 3.5 Melting Point apparatus. A Thermo Nicolet Nexus 470 Ft-IR spectrophotometer was used for IR analyses. \(^1\)H-NMR (400 MHz) and \(^{13}\)C-NMR (101 MHz) measurements were performed using Varian-400 MHz spectrometer, and chemical shifts were expressed in \(\delta\) (ppm) relative to TMS (in CDCl\(_3\) or DMSO-\(d_6\) as solvent) as the internal standard. An EuroEA 3000 Elemental Analyzer (Italy) was used for elemental analyses. Mass spectra were performed using Finnigan 2000, Thermo Quest GC/MS (Italy). A triple-quadruple tandem mass spectrometer (Micromass W Quattro micro\textsuperscript{TM}, Waters Corp., Milford, MA, USA) equipped with electrospray ionization (ESI). X-ray diffraction patterns were carried out by XRD-6100 X-ray diffractometer, with CuK\(\alpha\) (\(\lambda = 1.5406\) Å) radiation in the 2\(\theta\) range from 5° to 90°. Compounds 1 and 12 were prepared according to the reported literature methods.\(^{25}\)

2-[(6-Butyl-4-hydroxy-2,5-dioxo-5,6-dihydro-2H-pyrano[3,2-c]quinolin-3-yl)amino] acetonitrile (2). A mixture of compound 1 (3 g, 10 mmol) and dry acetonitrile (50 mL) containing NaH (0.24 g, 10 mmol) was stirred at R.T for 15 minutes under nitrogen gas. During this time, one molar ratio (1.2 mL, 10 mmol) of bromoacetonitrile was added drop wise to previous mixture. The reaction mixture was heated at reflux for 24h and it was monitored by TLC, using ethyl acetate/hexane 7:3 as the eluent. At the end of the reaction, the reaction mixture was filtered on hot and the filtrate was poured on ice (100 g). The dark brown solid ppt. so obtained was filtered off, washed by water (3 x 10 mL), dried and crystallized from glacial AcOH to give compound 2. Yield 1.55 g (45%), brown crystals, mp 180-181 °C. IR (KBr, cm\(^{-1}\)): 3428 broad band (OH), 3203 (NH), 2958, 2928, 2876 (CH\(_{aliphatic}\)), 2188 (C≡N), 1738 (C=O\(_{pyrone}\)), 1676 (C=O\(_{quinolone}\)) and 1613 (C=C). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta_H\): 0.96 (t, 3H, \(J = 8.0\) Hz, C4\(^1\)), 1.39 - 1.48 (m, 2H, C3\(^3\)), 1.63 - 1.72 (m, 2H, C2\(^2\)), 4.29 (s,
2H, CH$_2$-CN), 4.36 (t, 2H, J 8.0 Hz, C1'), 7.12 (s, 1H, N-H, exchangeable in D$_2$O), 7.55 (t, 1H, J 8.0 Hz, C9-H), 7.83-7.94 (m, 2H, C7-H and C8-H), 8.17 (dd, 1H, J 8.0,1.6 Hz, C10-H), 14.08 (bs, 1H, OH, exchangeable in D$_2$O).$^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta_{C}$: 14.1 (s, 1C, 4'), 19.9 (s, 1C, 3'), 29.7 (s, 1C, 2'), 31.1 (s, 1C, CH$_2$CN), 42.3 (s, 1C, 1'), 99.9 (s, 1C, 3), 109.9 (s, 1C, 4a), 113.5 (s, 1C, 10a), 116.9 (s, 1C, C=NN), 117.5 (s, 1C, 7), 124.1 (s, 1C, 10), 124.9 (s, 1C, 9), 134.8 (s, 1C, 8), 138.1 (s, 1C, 6a), 156.9 (s, 1C, 10b), 159.5 (s, 1C, 2), 163.2 (s, 1C, 4), 163.8 (s, 1C, 5). Mass spectrum, $m/z$ (rel, %): 341 [M+2] (2), 340 [M+1] (21), 339 [M] (89), 300 (37), 299 (58), 245 (17), 244 (100), 189 (5), 188 (38). Anal. Calcd for C$_{18}$H$_{17}$N$_{3}$O$_4$ (339.35): C, 63.71; H, 5.05; N, 12.38%. Found: C, 63.78; H, 5.15; N, 12.31%.

6-n-Butyl-3-hydroxy-1H-pyrazino[2',3':4,5]pyrano[3,2-c]quinoline-5,12(4H,6H)-dione (7). A mixture of compound 2 (3.3 g, 10 mmol), DMF (50 mL) and NaH (0.24 g, 10 mmol) was heated at reflux for 24 h and monitored by TLC, using ethyl acetate/hexane 6:4 as the eluent. At the end of the reaction, the reaction mixture was filtered on hot and the filtrate was poured on ice (100 g). The brown solid precipitate so obtained was filtered off, washed by water (3 x 10 mL), dried and crystallized from ethanol to give compound 7. Yield 1.02 g (30.09%), brown crystals, mp 240-242 °C. IR (KBr, cm$^{-1}$): 3409 broad band (OH and NH's), 3077 (CH$_{aromatic}$), 2959, 2918, 2842 (CH$_{aliphatic}$), 1714 (C=O$_{pyrrole}$), 1674 (C=O$_{quinolone}$) and 1616 (C=O$_{caromatic}$). $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta_{H}$: 0.90 (t, J 8.0 Hz, 3H, C4'), 1.31 - 1.46 (m, 2H, C3'), 1.55 - 1.70 (m, 2H, C2'), 4.30 (t, J 8.0 Hz, 2H, C1'), 5.25 (s, 1H, NH$_{pyrazino}$, exchangeable in D$_2$O), 7.42 (t, J 8.0 Hz, 1H, C9-H), 7.48 (d, J 8.0 Hz, 1H, C7-H), 7.70 (t, J 8.0 Hz, 1H, C8-H), 7.76 (s, 1H, CH$_{pyrazino}$, un-exchangeable in D$_2$O), 8.05 (dd, J 8.0,1.2 Hz, 1H, C10-H), 13.14 (s, 1H, OH$_{pAryl}$, exchangeable in D$_2$O). $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta_{C}$: 13.8 (s, 1C, 4'), 20.3 (s, 1C, 3'), 29.4 (s, 1C, 2'), 42.3 (s, 1C, 1'), 106.4 (s, 1C, 7), 112.3 (s, 1C, 10a), 114.6 (s, 1C, 9), 119.0 (s, 1C, 10), 122.7 (s, 1C$_{pyrazin}$), 125.9 (s, 1C, 4a), 128.9 (s, 1C, 8), 129.7 (s, 1C, 3), 133.7 (s, 1C, 4), 135.6 (s, 1C, 6a), 141.3 (s, 1C OH$_{pyrazin}$), 156.9 (s, 1C, 10b), 157.7 (s, 1C, 2), 161.9 (s, 1C, 5). ESI-MS $m/z$: 340.4 [M+H]$^+$, 362.2 [M+Na]$^+$, 701.3 [2M+Na]$^+$. Anal. Calcd for C$_{18}$H$_{17}$N$_3$O$_4$ (339.35): C, 63.71; H, 5.05; N, 12.38. Found: C, 63.52; H, 5.13; N, 12.36%.

6-n-Butyl-5,12-dioxo-6H-[1,4]oxazino[2',3':4,5]pyrano[3,2-c]quinoline-2-carbonitrile (8). A mixture of compound 2 (3.39 g, 10 mmol) and triethylorthofomate (8 mL, 50 mmol) was heated for 12 h. The progress of the reaction was monitored by TLC, using ethyl acetate/hexane 6:4 as the eluent. The solid deposited after cooling was filtered off then washed with hot EtOH (2 x 10 mL) then crystallized from glacial AcOH to afford compound 8. Yield 1.92 g (55%), orange crystals, mp 280-282 °C. IR (KBr, cm$^{-1}$): 3356 (NH), 2954, 2930, 2864 (CH$_{aliphatic}$), 2197 (C=N), 1710 (C=O$_{pyrrole}$), 1677 (C=O$_{quinolone}$) and 1615 (C=O$_{caromatic}$). $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta_{H}$: 0.92 (t, J 8.0 Hz, 3H, C4'), 1.35 - 1.49 (m, 2H, C3'), 1.61 - 1.71 (m, 2H, C2'), 4.33 (t, J 8.0 Hz, 2H, C1'), 7.48 (t, J 8.0 Hz, 1H, C9-H), 7.72 (d, J 8.0 Hz, 1H, C7-H), 7.83 (t, J 8.0 Hz, 1H, C8-H), 8.26 (dd, J 8.0,1.2 Hz, 1H, C10-H), 8.64 (s, 1H, CH$_{oxazino}$, un-exchangeable in D$_2$O), 9.43 (s, 1H, NH$_{oxazino}$, exchangeable in D$_2$O). $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta_{C}$: 14.1 (s, 1C, 4'), 19.9 (s, 1C, 3'), 29.7 (s, 1C, 2'), 42.1 (s, 1C, 1'), 101.7, 114.0, 114.9, 116.4, 124.4, 124.9, 132.2, 134.9, 136.1, 144.6, 151.5, 159.0, 162.8, 163.5, 193.9. ESI-MS $m/z$: 350.3 [M+H]$^+$, 372.1 [M+Na]$^+$, 721.1 [2M+ Na]$^+$. Anal. Calcd for C$_{19}$H$_{15}$N$_3$O$_4$ (349.35): C, 65.32; H, 4.33; N, 12.03. Found: C, 65.52; H, 4.37; N, 12.11%.

2,2'-(6-n-Butyl-4-hydroxy-2,5-dioxo-5,6-dihydro-2H-pyrazo[3,2-c]quinolin-3-yl)azanediyl)diacetonitrile (10). A mixture of compound 1 (3 g, 10 mmol) and dry acetonitrile (50 mL) containing NaH (0.24 g, 10 mmol) was stirred at R.T for 30 minutes under nitrogen gas. During this time, two molar ratios (2.5 mL, 20 mmol) of bromoacetonitrile was added drop wise to previous mixture. The reaction mixture was heated at reflux for 24 h and it was monitored by TLC, a mixture of ethyl acetate and n-hexane was used as the mobile phase in ratio (6:4). At the end of the reaction time, the reaction mixture was filtered on hot and the filtrate was poured on
ice (100 g). The dark brown solid precipitate so obtained was filtered off, washed by water (3 x 10 mL), dried and crystallized from glacial AcOH to give compound 10. Yield 1.70 g (45%), brown crystals, mp 174-176 °C. IR (KBr, cm⁻¹): 3347 broad band (OH), 2959, 2926, 2850 (CH aliphatic), 2202 very stretching band attributed to (C=O)pyrazino[1,4]oxazino[2',3':4,5]pyrazino[3,2-c]quinolin-1-yl acetonitrile (11). A mixture of compound 10 (3.7 g, 10 mmol) and DMF (50 mL) with NaH (0.24 g, 10 mmol) was added carefully. The reaction mixture was heated at reflux for 24 h and it was monitored by TLC, a mixture of ethyl acetate and n-hexane was used as the mobile phase in ratio (7:3). At the end of the reaction time, the reaction mixture was filtered on hot and the filtrate was poured on ice (100 g). The brown solid precipitate so obtained was filtered, washed several times by water, dried and crystallized from ethanol to give compound 11. Yield 1.2 g (30.9%), brown crystals, mp 240-241 °C. IR (KBr, cm⁻¹): 3408 broad band (OH and NH), 2955, 2925, 2866 (CH aliphatic), 2210 (CN=), 1712 (C=O aliphatic), 1682 (C=O quinolone) and 1636 (C=C aromatic). ¹H NMR (400 MHz, CDCl₃) δH: 1.02 (t, J 8.0 Hz, 3 H, C₄'), 1.45 - 1.59 (m, 2 H, C₃'), 1.72 - 1.82 (m, 2 H, C₂'), 4.02 (s, 1 H, CH₂ acetonitrile), 4.21 (s, 2 H, CH₂ acetonitrile), 4.36 (t, J 8.0 Hz, 2 H, C₁'), 7.48 (t, J 8.0 Hz, 1 H, C₉-H), 7.53 (d, J 8.0 Hz, 1 H, C₇-H), 7.80 (t, J 8.0 Hz, 1 H, C₈-H), 8.32 (dd, J 8.0,1.6 Hz, 1 H, C₁₀-H). ¹³C NMR (101 MHz, DMSO-d₆) δC: 14.1 (s, 1 C 4'), 19.9 (s, 1 C 3'), 29.7 (s, 1 C 2'), 41.9 (s, 1 C - CH₂ acetonitrile), 42.1 (s, 1 C - CH₂ acetonitrile), 42.3 (s, 1 C 1'), 100.9 (s, 1 C 3), 113.1 (s, 1 C 4a), 113.5 (s, 1 C 10a), 113.9 (s, 1 C 7), 114.5 (s, 1 C, C≡N), 116.1 (s, 1 C, C≡N), 124.1 (s, 1 C 10), 128.9 (s, 1 C 9), 132.3 (s, 1 C 8) 136.1 (s, 1 C 6a), 149.0 (s, 1 C 10b), 159.4 (s, 1 C 2), 162.8 (s, 1 C 4), 163.0 (s, 1 C 5). Mass spectrum, m/z (Irel, %): 379 [M+1] (9), 378 [M⁺] (41), 339 (22), 338 [M⁺-CH₂CN] (76), 312 (22), 311 (100), 285 (20), 255 (38), 244 (99). Anal. Calcd for C₂₀H₁₈N₄O₄ (378.39): C, 63.49; H, 4.79; N, 14.81. Found: C, 63.42; H, 4.82; N, 14.74%.

6-butyl-5,12-dioxo-6H-[1,4]oxazino[2',3':4,5]pyrazino[3,2-c]quinoline-3-carbonitrile (13). A mixture of compound 12 (3.55 g, 10 mmol) and THF (50 mL) containing NaH (0.24 g, 10 mmol) was stirred at room temperature for 30 min. Then, one molar ratio (1.2 mol, 10 mmol) of bromoacetonitrile was added drop wise to previous mixture. The reaction mixture was heated under reflux for 12 h and it was monitored by TLC, using ethyl acetate/hexane 7:3 as the eluent. At the end of the reaction, the reaction mixture was filtered on hot and the filtrate was poured on ice (100 g). The orange solid ppt. so obtained was filtered off, washed by water (3 x 10 mL), dried and crystallized from glacial AcOH to give compound 13. Yield 2.3 g (67%), orange crystals, mp 250-252 °C. IR (KBr, cm⁻¹): 3365 (NH), 2922, 2852 (CH aliphatic), 2203 (CN=), 1713 (C=O aliphatic), 1683 (C=O quinolone) and 1567 (C=C aromatic). ¹H NMR (400 MHz, CDCl₃) δH: 0.90 (t, J 8.0 Hz, 3 H, C₄'), 1.25 - 1.45 (m, 2H, C₃'), 1.55 - 1.67 (m, 2H, C₂'), 4.19 (t, J 8.0 Hz, 2 H, C₁'), 7.34 (t, J 8.0 Hz, 1 H, C₉-H), 7.68 (d, J 8.0 Hz, 1 H, C₇-H), 7.92 (t, J 8.0 Hz, 1 H, C₈-H), 8.12 (dd, J 8.0,1.2 Hz, 1 H, C₁₀-H), 8.49 (s, 1 H, CH oxazino, un-exchangeable in D₂O), 11.57 (s, 1 H, NH oxazino, exchangeable in D₂O). ¹³C NMR (101 MHz, DMSO-d₆) δC: 14.1 (s, 1 C 4'), 19.9 (s, 1 C 3'), 29.7 (s, 1 C 2'), 42.1 (s, 1 C 1'), 101.7, 114.0, 115.0, 116.3, 122.7, 124.3, 132.10, 136.1, 138.8, 144.4,
148.8, 165.7, 158.9, 161.7, 162.8. ESI-MS m/z: 350.1 [M+H]^+, 721.1 [2M+ Na]^+. Anal. Calcd for C_{19}H_{15}N_{3}O_{4} (349.35): C, 65.32; H, 4.33; N, 12.03. Found: C, 65.38; H, 4.36; N, 12.10%.

6-n-Butyl-3,6-dihydro-4H-[1,2,4]triazine[6',5',4,5]pyrano[3,2-c]quinoline-5,12(4H)-dione (14a). A mixture of compound 12 (3.55 g, 10 mmol) and hydrazine hydrate (0.33 mL, 10 mmol) in (50 mL) DMF was heated at reflux for 6 h. The progress of the reaction was monitored by TLC, using ethyl acetate/hexane 6:4 as the eluent. The solid deposited after cooling was filtered off and washed with hot EtOH (3 x 10 mL). The targeted compound crystallized from AcOH to give compound 14a. Yield (1.15 g, 35.5%), pale yellow crystals, mp 235-237 °C. IR (KBr, cm⁻¹): 3318 (NH), 3200 (NH), 3054 (C=O), 2952, 2922, 2872 (C=H), 1712 (C=O), 1666 (C=O), 1619 (C=O), and 1593 (C=O). ¹H NMR (400 MHz, CDCl₃) δH: 1.02 (t, J 8.0 Hz, 3 H, C4), 1.44 - 1.59 (m, 2 H, C3'), 1.72 - 1.83 (m, 2 H, C2'), 4.36 (t, J 8.0 Hz, 2 H, C1'), 7.51 (t, J 8.0 Hz, 1 H, C9-H), 7.79 (d, J 8.0 Hz, 1 H, C7-H), 7.86 (t, J 8.0 Hz, 1 H, C8-H), 8.30 (dd, J 8.0,1.2 Hz, 1 H, C10-H), 8.71 (s, 1 H, CH₃), 9.10 (s, 1 H, NH, exchangeable in D₂O), 10.11 (s, 1 H, NH, exchangeable in D₂O). ¹³C NMR (101 MHz, CDCl₃) δC: 13.8 (s, 1 C 4'), 20.2 (s, 1 C), 29.5 (s, 1 C), 42.2 (s, 1 C), 106.3, 112.7, 114.7, 122.3, 122.8, 126.0, 130.9, 135.9, 141.4, 149.4, 157.8, 162.3, 178.5. ESI-MS m/z: 325.3 [M+H]^+, 347.1 [M+Na]^+, 671.1 [2M+ Na]^+. Anal. Calcd for C_{17}H_{15}N_{3}O_{3} (324.34): C, 62.95; H, 4.97; N, 17.27. Found: C, 62.85; H, 4.83; N, 17.33%.

6-n-Butyl-4-phenyl-3,6-dihydro-4H-[1,2,4]triazine[6',5',4,5]pyrano[3,2-c]quinoline-5,12(4H)-dione (14b). A mixture of compound 12 (3.55 g, 10 mmol) and phenyl hydrazine (1.08 mL, 10 mmol) in (50 mL) DMF was heated at reflux for 6 h. The progress of the reaction was monitored by TLC, using ethyl acetate/hexane 6:4 as the eluent. The solid deposited after cooling was filtered off and washed with hot EtOH (3 x 10 mL). The targeted compound crystallized from isopropanol to give compound 14b. Yield (1.6 g, 40%), yellow crystals, mp 243-244 °C. IR (KBr, cm⁻¹): 3392, 3135 (NH), 2959, 2918, 2872 (C=H), 1709 (C=O), 1679 (C=O), 1598 (C=N), 1582 (C=C). ¹H NMR (400 MHz, CDCl₃) δH: 1.01 (t, J 8.0 Hz, 3 H, C4'), 1.43 - 1.59 (m, 2 H, C3'), 1.70 - 1.82 (m, 2 H, C2'), 4.34 (t, J 8.0 Hz, 2 H, C1'), 6.85 - 7.21 (m, 5 H phenyl), 7.35 (t, J 8.0 Hz, 1 H, C9-H), 7.51 (d, J 8.0 Hz, 1 H, C7-H), 7.79 (t, J 8.0 Hz, 1 H, C8-H), 8.29 (dd, J 8.0,1.2 Hz, 1 H, C10-H), 8.38 (s, 1H, CH₃), 9.40 (s, 1 H, NH, exchangeable in D₂O). ¹³C NMR (101 MHz, CDCl₃) δC: 13.8 (s, 1 C 4'), 20.1 (s, 1 C, 2'), 29.5 (s, 1 C, 1'), 42.6 (s, 1 C, 1'), 101.6 (s, 1 C, 4a), 102.4 (s, 1 C, 3), 115.0 (s, 1 C 7), 115.5 (s, 1 C, 10a), 119.7 (s, 1 C, 10), 122.6 (s, 1 C, 9), 124.5 (s, 1 C, 2'), 124.8 (s, 1 C, 2'), 128.3 (s, 1 C, 3'), 129.3 (s, 1 C, 2), 134.9 (s, 1 C, 3), 134.7 (s, 1 C, 3'), 138.1 (s, 1 C, 6a), 143.9 (s, 1 C 10b), 145.6 (s, 1 C, 1H), 152.9 (s, 1 C 4), 162.7 (s, 1 C, 2), 163.3 (s, 1 C, 5). ESI-MS m/z: 401.4 [M+H]^+, 423.2 [M+Na]^+. Anal. Calcd for C_{23}H_{20}N_{4}O_{3} (400.44): C, 68.99; H, 5.03; N, 13.99. Found: C, 68.85; H, 4.93; N, 13.91%.

N-(6-n-Butyl-4-hydroxy-2,5-dioxo-6-dihydro-2H-pyrano[3,2-c]quinolin-3-yl)formamide (15). This compound was obtained as a side product of the previous reaction and was extracted from the mixture by column chromatography packed with (silica, ethyl acetate/hexane 7:3 as the eluent) as yellow crystals, mp 202-203 °C. IR (KBr, cm⁻¹): 3392 (OH), 3135 (NH), 2959, 2918, 2872 (C=H), 1709 (C=O), 1679 (C=O), 1656 (C=O), 1609 (C=O). ¹H NMR (400 MHz, DMSO-d₆) δH: 0.90 (t, J 8.0 Hz, 3 H, C4'), 1.31 - 1.44 (m, 2 H, C3'), 1.50 - 1.61 (m, 2 H, C2'), 4.19 (t, J 8.0 Hz, 2 H, C1'), 7.34 (t, J 7.6 Hz, 1 H, C9-H), 7.68 (d, J 8.0 Hz, 1 H, C7-H), 7.90 (t, J 8.0 Hz, 1 H, C8-H), 8.09 (d, J 8.0 Hz, 1 H, C10-H), 8.49 (s, 1 H, NH, exchangeable in D₂O), 10.19 (s, 1 H, CHO, partially exchangeable in D₂O), 11.57 (1H, S, C₄-OH, exchangeable in D₂O). ¹³C NMR (101 MHz, DMSO-d₆) δC: 14.2 (s, 1 C, 4'), 19.9 (s, 1 C, 3'), 29.7 (m, 1 C, 2'), 36.4 (s, 1 C, 1'), 97.3 (s, 1 C, 3), 107.9 (s, 1 C, 4a), 116.6 (s, 1 C, 10a), 119.3 (s, 1 C, 7), 123.9 (s, 1 C, 10), 124.9 (s, 1 C, 9), 128.8 (s, 1 C, 8), 132.4 (s, 1 C, 6a), 134.6 (s, 1 C, 10b), 149.1 (s, 1 C, 2), 158.2 (s, 1 C, 4), 163.4 (s, 1 C, 5), 177.3 (s, 1 C, CHO). ESI-MS m/z: 329.3 [M+H]^+, 351.2 [M+Na]^+, 675.3 [2M]+, 679.3 [2M+ Na]^+. Anal. Calcd for C_{17}H_{15}N_{2}O_{2} (328.33): C, 62.19; H, 4.91; N, 8.53. Found: C, 62.11; H, 4.95; N, 8.43%.
6-n-Butyl-3,6-dihydro-5H,12H-[1,2,4]oxadiazino[6'5':4,5]pyrano[3,2-c]quinoline-5,12-dione (16). A mixture of compound 12 (3.55 g, 10 mmol) and hydroxylamine hydrochloride (0.69 g, 10 mmol) in (50 mL) DMF was heated at reflux for 8 h. The progress of the reaction was monitored by TLC, a mixture of ethyl acetate and n-hexane was used as the mobile phase in ratio (7:3). The solid deposited after cooling was filtered off and washed with hot EtOH (3 x 10 mL). The targeted compound crystallized from AcOH to give compound 16. Yield (1.41 g, 43.3%), pale yellow crystals, mp 260-261°C. IR (KBr, cm⁻¹): 3208 (NH), 2955, 2919, 2872 (CHaliphatic), 1712 (C=O-π-pyrone), 1666 (C=O-quinolone), 1619 (C=N), and 1593 (C=Caromatic). ¹H NMR (400 MHz, CDCl₃) δH: 1.01 (t, J 8.00 Hz, 3H, C₄'), 1.49 - 1.58 (m, 2H, C₃'), 1.68 - 1.82 (m, 2H, C₂'), 4.35 (t, J 8.0 Hz, 2H, C₁'), 7.57 (t, J 8.0 Hz, 1H, C₉-H), 7.76 (d, J 8.0 Hz, 1H, C₇-H), 7.93 (t, J 8.0 Hz, 1H, C₈-H), 8.33 (dd, J 8.0,1.2 Hz, 1H, C₁₀-H), 9.41 (s, 1H, CHoxadiazine), 12.36 (s, 1H, NH, exchangeable in D₂O). ¹³C NMR (101 MHz, CDCl₃) δC: 13.8 (s, 1C 4'), 20.2 (s, 1C, 3'), 29.5 (s, 1C, 2'), 42.2 (s, 1C, 1'), 106.3, 112.7, 114.7, 122.8, 126.0, 130.9, 135.9, 141.4, 141.8, 149.4, 157.8, 162.3, 178.5. ESI-MS m/z: 326.3 [M+H]⁺, 348.3 [M+Na]⁺, 673.3 [2M+ Na]¹⁺. Anal. Calcd for C₁₇H₁₅N₃O₄ (325.33): C, 62.76; H, 4.65; N, 12.92. Found: C, 62.79; H, 4.73; N, 12.78%.

6-n-Butyl-5,12-dioxo-6,12-dihydro-1H,5H [1,3,4] oxadiazino[6'5':4,5]pyrano[3,2-c]quinoline-3-carbonitrile (19). Compound 1 (3 g, 10 mmol) was dissolved in conc. HCl (50 mL, 1M) and the solution was cooled in an ice bath to 0 °C and a solution of (0.69 g, 10 mmol) NaNO₂; 4.00 mL HCl was added in 0.5 mL portions for 1 h. The temperature of the mixture should not exceed 0 °C. Diazonium salt solution prepared above was added drop wise to a mixture of bromoacetanilide (1.1 mL, 10 mmol) with 50 mL of pyridine. The solution was cooled in an ice bath to 0 °C. After completion of addition, the reaction mixture stirred for 4h maintaining the temperature 0-5 °C. Then, the previous mixture was heated at reflux for 12 h. The solid deposited after cooling was filtered off, dried and crystallized from DMF to give compound 19. Yield (2.2 g, 62.85%), yellow crystals, mp 254-255 °C. IR (KBr, cm⁻¹): 3358 (NH), 2958, 2932, 2872 (CHaliphatic), 2211 (C=N), 1705 (C=O-π-pyrone), 1682 (C=O-quinolone) and 1613 (C=N) and 1570 (C=aromatic). ¹H NMR (400 MHz, CDCl₃) δH: 0.99 (t, J 8.0 Hz, 3H, C₄'), 1.40 - 1.49 (m, 2H, C₃'), 1.73 - 1.76 (m, 2H, C₂'), 4.30 (t, J 8.0 Hz, 2H, C₁'), 7.40 (t, J 8.0 Hz, 1H, C₉-H), 7.48 (d, J 8.0 Hz, 1H, C₇-H), 7.75 (t, J 12.0 Hz, 1H, C₈-H), 8.24 (dd, J 8.0,1.2 Hz, 1H, C₁₀-H), 11.22 (s, 1H, NHoxadiazine, exchangeable in D₂O). ¹³C NMR (101 MHz, DMSO-d₆) δC: 14.1 (s, 1C, 4'), 19.9 (s, 1C, 3'), 29.6 (s, 1C, 2'), 42.3 (s, 1C, 1'), 99.9, 102.8, 109.9, 113.4, 117.6, 123.2, 124.1, 124.9, 134.8, 148.9, 156.8, 159.5, 163.7, 169.1. ESI-MS m/z: 351.3 [M+H]⁺, 373.3 [M+Na]⁺, 701.1 [2M+H]¹⁺, 723.3 [2M+Na]¹⁺. Anal. Calcd for C₁₈H₁₄NaO₄ (350.34): C, 61.71; H, 4.03; N, 15.99. Found: C, 61.79; H, 4.12; N, 15.91%.

References


