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Anti-tumor and anti-leishmanial evaluations of 1,3,4-oxadiazine, pyran derivatives derived from cross-coupling reactions of β-bromo-6*H*-1,3,4-oxadiazine derivatives

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ABSTRACT

Cyanoacetylhydrazine reacted with the ω-bromoacetophenones **2a,b** to give hydrazide-hydrazone derivatives **3a,b**. The latter products were cyclized to the 1,3,4-oxadiazine derivatives **4a,b**. Bromination of the latter products gave the 6-bromo-6*H*-1,3,4-oxadiazine derivatives **5a,b** which underwent a series of cross-coupling reactions. The antitumor evaluation of the newly synthesized products against the three cancer cells namely breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) showed that some of them have high inhibitory effect towards three cell lines which is higher than the standard. Moreover, the anti-leishmanial activity of the newly synthesized product was tested on *Leishmania* donovani amastigotes showed that some compounds have high activity.

1. Introduction

A broad range of synthetic applications demonstrates that 1,3,4-oxadiazine derivatives constitute a versatile class of N, O heterocycles.[1–15] Considerable attention has been paid to their cross-coupling and cycloaddition reactions.[16–18] Such group of compounds are useful intermediates for the synthesis of heterocyclic compounds that can not be obtained by any other route.[19–22] Although cross-coupling on oxadiazine systems have been reported in the literature,[23,24] these papers with few exceptions are usually target-oriented and systematic investigations are still needed and of great interest in order to establish a reactivity platform of the various positions and methods in oxadiazine cross-coupling chemistry. The compounds obtained through cross-coupling pathway are important subunits in a multitude of synthetically and medicinally relevant compounds due to their key role in interestingly biological activities, such as antiestrogen,[25] analgesic,[26] antiallergy,[27] cyclooxygenase (COX)-1 inhibitors[28] neuroleptic[29] 5-HT_6 receptor antagonists,[30] FTase inhibitors (FTIs),[31] and anti HIV-1 activities.[32] The aim of this study was to study the synthesis of 1,3,4-oxadiazine derivatives followed by their bromination and the use of the resulting products as precursors in palladium-catalyzed cross-coupling reactions. Furthermore, we aimed at evaluating of the newly synthesized products against three human cancer cell lines, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) together with their evaluation through anti-leishmanial activity using *Leishmania* donovani amastigotes.

2. Results and discussion

Not much is known about brominated 1,3,4-oxadiazine and only a few mostly inefficient procedures are described for some oxazines.[33,34] This prompted us to investigate a more practical access to 6-brominated-1,3,4-oxadiazines. Recently our research group were involved through a comprehensive diagram to synthesize hydrazide-hydrazone derivatives together with their uses in heterocyclic synthesis.[35,36] In continuation to this synthetic route we demonstrate in this work the uses of hydrazide-hydrazone to synthesize 1,3,4-oxadiazine derivatives. Thus the reaction of the cyanoacetylhydrazine with ω-bromoacetophenone derivatives **2a,b** in 1,4-dioxan gave the hydrazide-hydrazone derivatives **3a,b** in reasonable to good yields. The structures of compounds **3a and 3b** were based on analytical and spectral data (see Section 5). Compounds **3a and 3b** underwent ready cyclization in sodium ethoxide solution to give the 2-(5-(4-bromoaryl)-6*H*-1,3,4-oxadiazin-2-yl)acetonitrile derivatives **4a** and **4b**, respectively

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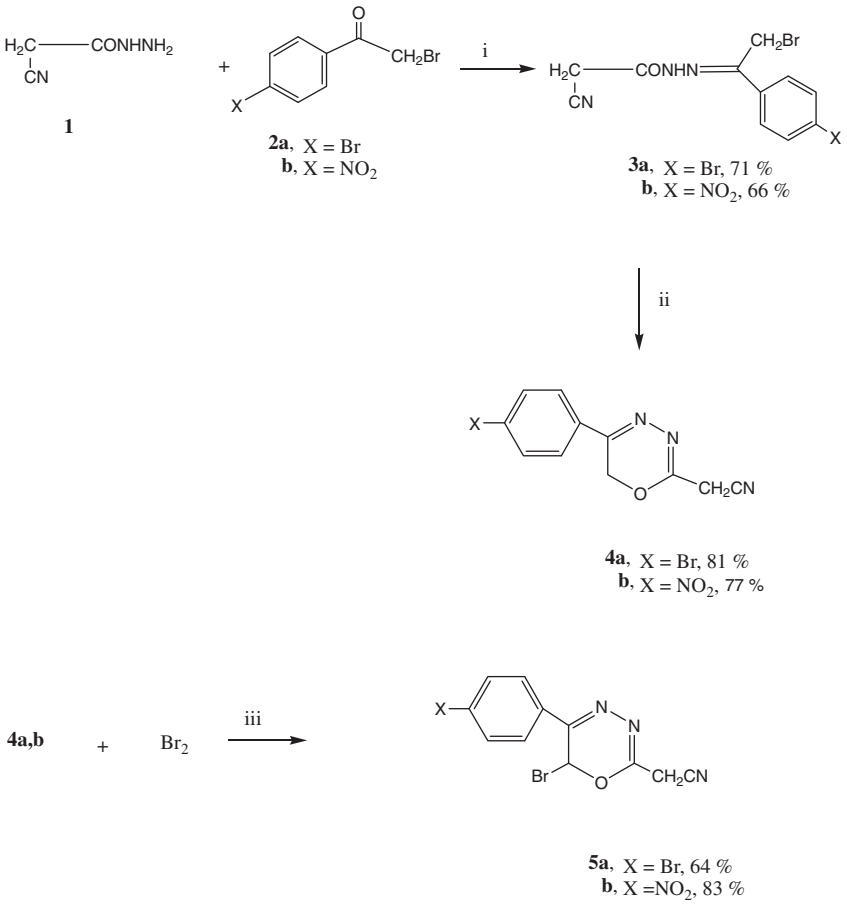
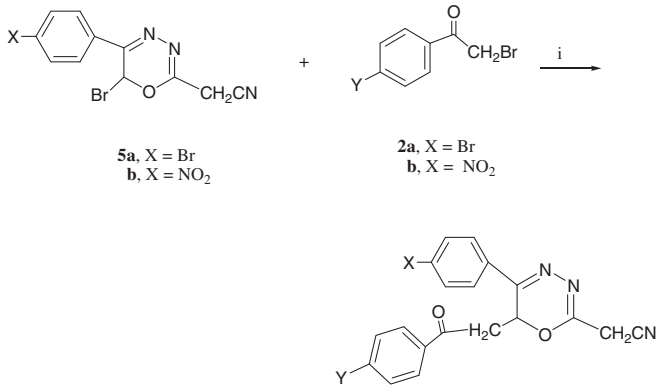


Scheme 1. Reagents and conditions: (i) 1,4-dioxan, reflux 2 h; (ii) NaOEt/EtOH, heat in water bath for 4 h, HCl till pH 6; (iii) AcOH, stirring at 60 °C, ice/water.

(Scheme 1). The analytical data of the latter products are in agreement with their proposed structures. Herein, we describe our results dealing with the halogenation of the 6*H*-1,3,4-oxadiazines **4a,b** and the use of the resulting products as precursors in palladium-catalyzed cross-coupling reactions. Gratifyingly, the desired 6-bromosubstituted 6*H*-1,3,4-oxadiazines **5a,b** could be prepared in a one pot procedure by bromine addition to **4a,b** and HBr elimination in acetic acid solution. With the 6-bromo-6*H*-1,3,4-oxadiazines **5a,b** in hand, palladium-catalyzed cross-couplings offer an efficient and useful approach for the synthesis of novel functionalized 6*H*-1,3,4-oxadiazine. The Suzuki-coupling of the 6-bromo-6*H*-1,3,4-oxadiazine **5a,b** with ω-bromoacetophenones **2a,b** in the presence of $Pd(PPh_3)_4$ and sodium carbonate at 80 °C in toluene gave the expected 6-aryl-oxoethyl-6*H*-1,3,4-oxadiazines **6a–d** in 65–86% yields (Scheme 2). The structures of compounds **6a–d** were established on the basis of analytical data. Thus, the ^{13}C NMR spectrum of **6a** (as an example) showed δ: 17.3 (CH_2), 40.7 (CH_2), 116.5 (CN), 122.9, 130.6, 131.0, 131.5, 132.3, 133.5, 134.2, 135.9, 136.2 ($2C_6H_4$), 164.3, 164.6 (2C=N), 189.9 (CO). Compounds **6a–d** underwent [4+2] cycloaddition reactions upon the reaction with acrylonitrile **7** to give the pyran derivatives **9a–d** (Scheme 3). The reactions took place through the intermediate formation of **8a–d** followed by nitrogen molecule elimination together with autodehydrogenation. The structures of the latter products were based on analytical data (see experimental data). The [4+2] cycloaddition between **6a–d** and acrylonitrile **7** took place in analogy of the reported literature in this respect.[37,38]



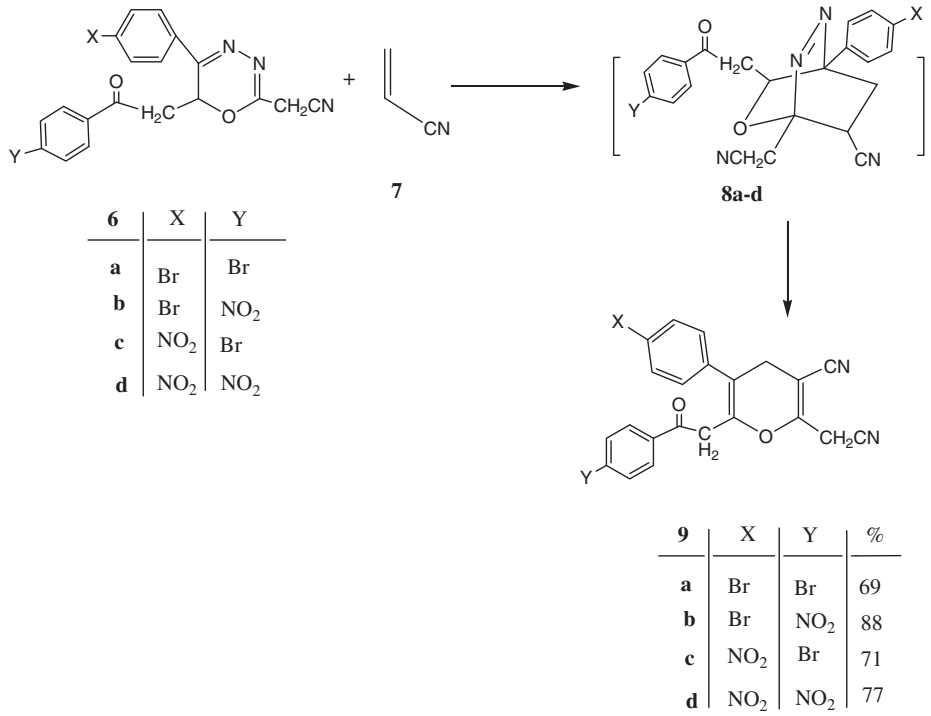
6	X	Y	%
a	Br	Br	86
b	Br	NO_2	55
c	NO_2	Br	74
d	NO_2	NO_2	59

Scheme 2. Reagents and conditions: (i) $Pd(PPh_3)_4$, toluene, MeOH, Na_2CO_3, reflux 15 h at 80 °C, column chromatography (SiO_2, hexan, EtOAc 9:1, then 4:1).

3. Biological evaluation

3.1. Materials and methods

Material, methods and reagents: Fetal bovine serum (FBS) and L-glutamine, were from Gibco Invitrogen Co. (Scotland, UK).



6	X	Y
a	Br	Br
b	Br	NO_2
c	NO_2	Br
d	NO_2	NO_2

9	X	Y	%
a	Br	Br	69
b	Br	NO_2	88
c	NO_2	Br	71
d	NO_2	NO_2	77

Scheme 3. Reagents and conditions: 1,4-dioxan, AcOH, reflux 6 h, ice/water.

RPMI-1640 medium was from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, USA). Samples: Stock solutions of compounds **3a–9d** were prepared in DMSO and kept at −20 °C. Appropriate dilutions of the compounds were freshly prepared just prior the assays. Final concentrations of DMSO did not interfere with the cell growth.

Cell cultures: Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) were used. MCF-7 was obtained from the European Collection of Cell cultures (ECACC, Salisbury, UK) and NCI-H460 and SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 μg/mL), at 37 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for MCF-7 and SF-268 and 0.75×10^4 cells/mL for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

3.1.1. Anti-tumor activity

3.1.1.1. Effect of the synthesized compounds on the growth of human tumor cell lines. All the synthesized compounds were 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 tested compounds were able to inhibit the growth of the human tumor cell lines in a dose-dependent manner (data not shown). Compounds **4a** (2-(5-(4-bromophenyl)-6*H*-1,3,4-oxadiazin-2-yl)acetonitrile) and **4b** (2-(5-(4-nitro-phenyl)-6*H*-1,3,4-oxadiazin-2-yl)acetonitrile) are among the compounds that showed the highest inhibitor effect of the tested compounds. It is obvious that substituting the 4-bromophenyly group in **4a** by the 4-nitrophenyl group in **4b** showed marked increase of the inhibitor effects towards the three cell lines. The 1,3,4-oxadiazine derivative **6d** and the pyran derivatives **9b** and **9d** are also among the compounds that showed high inhibitory effect of the tested compounds, exhibiting an equivalent potency in all the three tumor cell lines. Comparing the inhibitory effect of **9b** with the substituted with bromine and nitro groups is less than the inhibitory effect of **9d** substituted with the two nitro groups. It is of great value to notice that compound **9d** showed the maximum inhibitory effect towards the three cell lines and its reactivity is higher than doxorubicin. While compounds **3b**, **5b**, **9a** and **9c** showed moderated growth inhibitory effect relative to compounds **4a**, **4b**, **6d** and **9d**. On the other hand, compounds **3a**, **5a**, **6a** and **6c** showed the lowest inhibitory effect relative to the tested compounds. It is convenient to observe that the 1,3,4-oxadiazine derivative **4b** with its 4-*nitro* group showed the maximum inhibitory effect through MCF-7 and NCI-H460 cell lines. Comparing the activities of compounds **9a**, **9b**, **9c** and **9d** it is observed that the two 4-*nitrophenyl* groups present in **9d** showed higher inhibitory effect relative to **9a**, **9b** and **9c** although the results in MCF-7 cell line are comparable. Compound **9b** with the 4-bromophenyl and the 4-nitrophenyl showed the highest inhibitory effect towards NCI-H460 which is much higher than the reference standard doxorubicin (Table 1).

3.1.2. Anti-leishmanial activity

Anti-leishmanial activity was tested on *L. donovani* amastigotes growing in macrophages at concentrations, which showed less than 40% cytotoxicity for the macrophage cell line THP-1. The compound **6c, 6d**, **9b**, **9c** and **9d** showed high activity on *L. donovani* amastigotes growing in macrophages similar to that seen with axenic amastigotes at 50 μM, 73%, 88%, 90%, 82% and 98%, respectively. It is obvious that the 2-(4-nitroacetophenon-ω-yl)-3-(4-nitrophenyl)-5-cyano-6-cyano-methylpyran **9d** showed that maximum average inhibition which is higher than the positive control (Table 2). On the other hand, compounds **3a**, **3b** and **4a** showed the lowest activity. In most cases, one can say that the substitution by

Table 1
Effect of compounds **3a–9d** on the growth of three human tumor cell lines

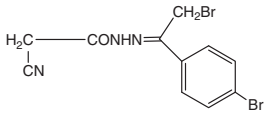
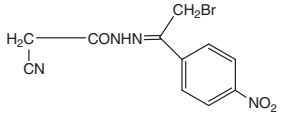
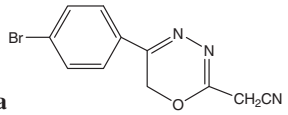
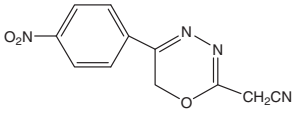
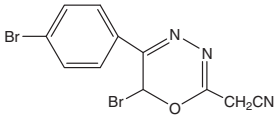
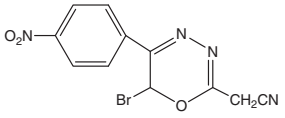
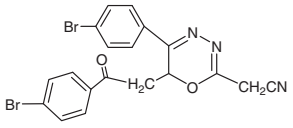
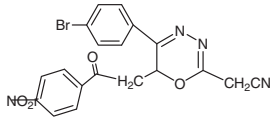
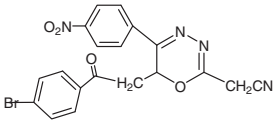
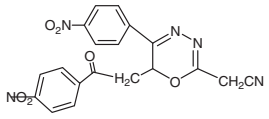
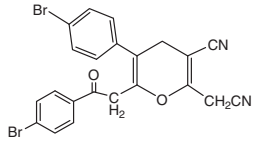
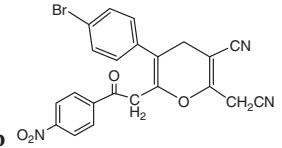
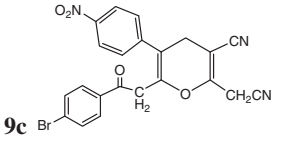
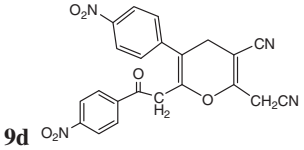
Compound no.	GI_{50} (μM)		
	MCF-7	NCI-H460	SF-268
3a 	40.6 ± 10.2	10 ± 6.2	12.8 ± 3.0
3b 	18.4 ± 0.6	10.3 ± 0.6	14.0 ± 0.2
4a 	1.5 ± 0.6	0.9 ± 1.1	10.3 ± 2.51
4b 	0.001 ± 0.005	0.002 ± 0.002	1.8 ± 0.002
5a 	70.7 ± 16.5	38.2 ± 10.8	32.0 ± 4.1
5b 	10.6 ± 0.4	8.5 ± 0.4	6.7 ± 1.4
6a 	30.4 ± 10.2	22.1 ± 0.8	16.9 ± 6.8
6b 	32.0 ± 8.6	23.0 ± 1.9	18.5 ± 1.2
6c 	36.0 ± 1.8	40.0 ± 0.6	16.5 ± 1.4
6d 	1.6 ± 0.6	2.6 ± 1.0	2.0 ± 0.2
9a 	12.0 ± 0.6	10.4 ± 4.0	8.2 ± 1.2
9b 	0.09 ± 0.02	0.01 ± 0.06	0.003 ± 0.5

Table 1 (*continued*)

Compound no.	GI_{50} (μM)		
	MCF-7	NCI-H460	SF-268
9c 	4.9 ± 0.6	3.6 ± 1.6	2.8 ± 0.6
9d 	0.03 ± 0.006	0.08 ± 0.006	0.005 ± 0.005
Doxorubicin	0.0428 ± 0.008	0.0940 ± 0.008	0.0940 ± 0.007

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.

nitro group enhance the anti-leishmanial activity, this can cleared by comparing the activity **3a**, **4a** and **5a** (all are bromo-substituted) with average inhibitions 20%, 26% and 40%, respectively, and **3b**, **4b** and **5b** with average inhibitions 35%, 45% and 58%, respectively (all with nitro-substituted).

4. Conclusion

In summary, we have developed a new simple and versatile strategy for cyclization of hydrazide-hydrazone derivatives **3a,b** to the 1,3,4-oxadiazine derivatives **4a,b**. The 6-bromo-1,3,4-oxadiazine derivatives underwent cross-coupling reactions yielding products capable for [4+2] cycloaddtion reactions. The method offers several advantages including moderate to high yields of products and an easy experimental work-up procedure. These new structures broaden the Suzuki-coupling reactions and many of them represent interesting anti-tumor agents. Moreover, the anti-leishmanial activity of the newly synthesized products against against *L. donovani* axenic amastigotes indicating that some of them showed high activity. It is convenient to notice that compound **9b** has the maximum inhibitor effect towards the breast cancer cells and *L. donovani* axenic amastigotes cells.

Table 2
Anti-leishmanial activity of compounds **3a**–**9d** at 50 μM against *L. donovani* axenic amastigotes

Compound	X	Y	Average inhibition (%)	GI_{50}[a] (μM)
3a	Br	—	20	
3b	NO_2	—	35	
4a	Br	—	26	
4b	NO_2	—	44	
5a	Br	—	40	
5b	NO_2	—	58	22
6a	Br	Br	48	
6b	Br	NO_2	60	20
6c	NO_2	Br	73	
6d	NO_2	NO_2	88	33
9a	Br	Br	60	
9b	Br	NO_2	90	12.9
9c	NO_2	Br	82	26
9d	NO_2	NO_2	98	10.2
Positive control[b]			95	
Negative control[c]			0	

[a] GI_{50} = concentration for 50% growth inhibition.
[b] Amphotericin B (1 μM).
[c] Culture medium and DMSO.

5. Experimental section

5.1. General information

All melting points were obtained on a Buchi Melting Point B-540 apparatus (Buchi Labortechnik, Flawil, Switzerland) and are uncorrected. IR spectra were measured using KBr discs on a Pye Unicam SP-1000 spectrophotometer (Pye Unicam Ltd, Cambridge, England). ^{1}H-NMR and ^{13}C-NMR spectra was measured on a Varian EM390-300 MHz instrument (Varian Inc., Palo Alto, CA, USA) in CD_3SOCD_3 as solvent using TMS as internal standard, and chemical shifts are expressed as δ ppm. Elemental analyzes were determined on a Yanaco CHN Corder elemental analyzer (Japan). Mass spectra were obtained on an Agilent Micro Q-TOF mass spectrometer

5.1.1. Cyanoacetylhydrazido-*N*-(4-substitutedphenyl-α-bromo aceto)-hydrazone (3a,b)

General procedure: To a solution of cyanoacetylhydrazide (**1**) (1.0 g, 0.01 mol) in 1,4-dioxan (40 mL), either the ω-bromo-4-bromoacetophene (**3a**) (2.77 g, 0.01 mol) or ω-bromo-4-nitroacetophene (**3b**) (2.44 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then left to cool. The solid product formed, in each case, was collected by filtration.

5.1.2. Cyanoacetylhydrazido-*N*-(4-bromophenyl-α-bromoaceto) -hydrazone (3a)

Crystallized from 1,4-dioxan, white crystals. Mp: 165–169 °C in 71%. Analysis for $C_{11}H_9Br_2N_3O$, M. Wt. (359.02). Calcd: C, 36.80; H, 2.53; Br, 44.51; N, 11.70. Found: C, 36.77; H, 2.81; Br, 44.60; N, 11.94. IR (υ cm^{-1}): 3450–3267 (NH), 3055 (CH aromatic), 2893 (CH_2), 2258 (CN), 1689 (CO), 1658 (C=N), 1630 (C=C). ^{1}H NMR (DMSO) δ: 4.89, 5.41 (2s, 4H, $2CH_2$), 7.26–7.39 (m, 4H, C_6H_4), 8.30 (s, 1H, NH). ^{13}C NMR, δ: 25.8 (CH_2), 62.0 (CH_2), 116.9 (CN), 125.0, 130.6, 131.9, 133.5, 134.2, 138.9 (C_6H_4), 157.8 (C=N), 162.9 (C=O). *m/z* (EI, 70 eV): 358 (100%, M^+), 360 (42%), 359 (10%), 357 (5%).

5.1.3. Cyanoacetylhydrazido-*N*-(4-nitrophenyl-α-bromoaceto)-hydrazone (3b)

Crystallized from 1,4-dioxan, white crystals. Mp: 180–82 °C in 66%. Analysis for $C_{11}H_9BrN_4O_3$, M. Wt. (325.12). Calcd: C, 40.64; H, 2.79; Br, 24.58; N, 17.23. Found: C, 40.91; H, 2.84; Br, 24.85; N, 17.01. IR (υ cm^{-1}): 3489–3321 (NH), 3058 (CH aromatic), 2890 (CH_2), 2258 (CN), 1705 (CO), 1656 (C=N), 1632 (C=C). ^{1}H NMR (DMSO) δ: 4.93, 5.48 (2s, 4H, $2CH_2$), 7.32–7.42 (m, 4H, C_6H_4),

8.26 (s, 1H, NH). ^{13}C NMR, δ: 26.3 (CH_2), 61.9 (CH_2), 116.6 (CN), 124.2, 131.3, 131.9, 133.9, 135.8, 139.2 (C_6H_4), 157.8 (C═N), 162.9 (C═O). *m*/*z* (EI, 70 eV): 325 (98%, M^+), 327 (10%).

5.1.4. 2-(5-(4-Sbstituted-phenyl)-6*H*-1,3,4-oxadiazin-2-yl)aceto nitrile (4a,b)

General procedure: A suspension of either **3a** (3.59 g, 0.01 mol) or 3b (3.25 g, 0.01 mol) in sodium ethoxide solution [prepared by adding elemental sodium (0.23 g, 0.01 mol) in absolute ethanol (50 mL)] was heated under reflux for 4 h in a boiling water bath then left to cool. The solid product formed, in each case, upon pouring onto ice/water containing few drops of hydrochloric acid (till pH 6) was collected by filtration.

5.1.5. 2-(5-(4-Bromophenyl)-6*H*-1,3,4-oxadiazin-2-yl)acetoni trile (4a)

Crystallized from ethanol, yellow crystals. Mp: 210–213 °C in 81% yield. Analysis for $C_{11}H_8BrN_3O$, M. Wt. (278.10). Calcd: C, 47.51; H, 2.90; Br, 28.73; N, 15.11. Found: C, 47.72; H, 2.85; Br, 29.05; N, 15.32. IR (υ cm^{-1}): 3053 (CH aromatic), 2898 (CH_2), 2220 (CN), 1646 (C═N), 1632 (C═C). ^{1}H NMR (DMSO) δ: 4.72, 6.17 (2s, 4H, $2CH_2$), 7.29–7.36 (m, 4H, C_6H_4). ^{13}C NMR, δ: 18.5 (CH_2), 64.8 (CH_2), 116.6 (CN), 123.1, 130.3, 130.9, 132.8, 134.6, 139.4 (C_6H_4), 163.8, 164.7 (2C═N). *m*/*z* (EI, 70 eV): 278 (89%, M^+), 279 (10%), 276 (100%).

5.1.6. 2-(5-(4-Nitrophenyl)-6*H*-1,3,4-oxadiazin-2-yl)acetonitrile (4b)

Crystallized from ethanol, yellow crystals. Mp: 177–180 °C in 77% yield. Analysis for $C_{11}H_8N_4O_3$, M. Wt. (244.21). Calcd: C, 54.10; H, 3.30; N, 22.94. Found: C, 54.39; H, 3.52; N, 23.04. 3050 (CH aromatic), 2896 (CH_2), 2222 (CN), 1644 (C═N), 1632 (C═C). ^{1}H NMR (DMSO) δ: 4.81 (s, 2H, CH_2), 6.99 (s, 1H, oxadiazine H-6), 7.31–7.42 (m, 4H, C_6H_4). ^{13}C NMR, δ: 18.7 (CH_2), 64.9 (CH_2), 116.8 (CN), 123.0, 131.5, 131.8, 133.3, 136.9, 144.3 (C_6H_4), 163.9, 164.9 (2C═N). *m*/*z* (EI, 70 eV): 244 (100%, M^+), 245 (11%).

5.1.7. 2-(5-(4-Substituted-phenyl)-6-bromo-6*H*-1,3,4-oxadiazin-2-yl)acetonitrile (5a,b)

General procedure: To a solution of either **4a** (2.78 g, 0.01 mol) or **4b** (2.44 g, 0.01 mol) in acetic acid (50 mL), bromine (1.60 g 0.01 mol) in acetic acid (15 mL) was added drop-wise with continuous stirring. The reaction mixture was stirred for an additional 30 min at 60 °C then poured onto ice/water mixture and the formed solid product was collected by filtration.

5.1.8. 2-(5-(4-bromophenyl)-6-bromo-6*H*-1,3,4-oxadiazin-2-yl) acetonitrile (5a)

Crystallized from acetic acid, orange crystals. Mp: 145 °C in 64% yield. Analysis for $C_{11}H_7Br_2N_3O$, M. Wt. (357.0). Calcd: C, 37.01; H, 1.98; Br, 44.76; N, 11, 77. Found: C, 37.30; H, 2.25; Br, 44.81; N, 11.63. IR (υ cm^{-1}): 3058 (CH aromatic), 2894 (CH_2), 2221 (CN), 1649 (C═N), 1638 (C═C). ^{1}H NMR (DMSO) δ: 4.89 (s, 2H, CH_2), 6.99 (s, 1H, oxadiazine H-6), 7.31–7.38 (m, 4H, C_6H_4). ^{13}C NMR, δ: 17.8 (CH_2), 74.8 (CH), 116.9 (CN), 123.3, 131.0, 131.5, 132.9, 136.4 (C_6H_4), 164.0, 164.9 (2C═N). *m*/*z* (EI, 70 eV): 356 (100%), 357 (8%, M^+).

5.1.9. 2-(5-(4-Nitroophenyl)-6-bromo-6*H*-1,3,4-oxadiazin-2-yl) acetonitrile (5b)

Crystallized from acetic acid, orange crystals. Mp: 190–192 °C in 83% yield. Analysis for $C_{11}H_7BrN_4O_3$, M. Wt. (323.10). Calcd: C, 40.89; H, 2.18; Br, 24.73; N, 17.34. Found: C, 40.62; H, 2.09; Br, 24.92; N, 17.47. IR (υ cm^{-1}): 3053 (CH aromatic), 2892 (CH_2), 2220 (CN), 1648 (C═N), 1635 (C═C). ^{1}H NMR (DMSO) δ: 4.82 (s, 2H, CH_2), 6.93 (s, 1H oxadiazine H-6), 7.28–7.38 (m, 4H, C_6H_4). ^{13}C NMR, δ: 18.5 (CH_2), 74.6 (CH), 116.4 (CN), 123.2, 131.8, 132.6, 133.7, 136.4, 144.0 (C_6H_4), 163.9, 164.5 (2C═N). *m*/*z* (EI, 70 eV): 323 (100%, M^+), 325 (3%), 321 (95%),

5.1.10. 2-(5-(4-Subtituted-phenyl)-6-(4-substtuted-phenylacet-ω-yl)-6*H*-1,3,4-oxadiazin-2-yl)acetonitrile (6a–d)

General procedure: Either of the 6-bromo-6*H*-1,3,4-oxadiazine derivatives **5a** (3.57 g, 0.01 mol) or **5b** (3.23 g, 0.01 mol) and $Pd(PPh_3)_4$ (0.23 g, 0.027 mmol) were dissolved in a mixture of toluene/MeOH (3 mL/0.75 mL) in a heat-gun-dried and argon-flushed flask. A 2 M Na_2CO_3 solution (1.5 mL) was finally added and the reaction mixture was heated for 15 h at 80 °C. Then, the reaction mixture was cooled to room temperature and washed with 2 M Na_2CO_3 (with 1% NH_3) solution. After separation of the phases, the aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL) and the combined organic phases were dried with Na_2SO_4. The crude product was purified by column chromatography (SiO_2, hexan, EtOAc 9:1, then 4:1) to afford the corresponding product **6a–d**.

5.1.11. 2-(5-(4-Bromophenyl)-6-(*p*-bromophenylacet-ω-yl)-6*H*-1,3,4-oxadiazin-2-yl)acetonitrile (6a)

Crystallized from 1,4-dioxan, pale yellow crystals. Mp: 210–213 °C in 86% yield. Analysis for $C_{19}H_{13}Br_2N_3O_2$, M. Wt. (475.13). Calcd: C, 48.03; H, 2.76; Br, 33.63; N, 8.87. Found: C, 48.11; H, 3.01; Br, 33.72; N, 8.63. IR (υ cm^{-1}): 3060 (CH aromatic), 2886 (CH_2), 2220 (CN), 1689 (CO), 1649 (C═N), 1635 (C═C). ^{1}H NMR (DMSO) δ: 2.99 (s, 2H, CH_2), 3.80 (s, 2H, CH_2), 7.20 (m, 1H, oxadiazine H-6), 7.26–7.42 (m, 8H, $2C_6H_4$). ^{13}C NMR, δ: 17.3 (CH_2), 40.7 (CH_2), 62.8 (oxadiazine C-6), 116.5 (CN), 122.9, 130.6, 131.0, 131.5, 132.3, 133.5, 134.2, 135.9, 136.2 ($2C_6H_4$), 164.3, 164.6 (2C═N), 189.9 (CO). *m*/*z* (EI, 70 eV): 375 (20%, M^+), 477 (10%, M+2), 374 (100%).

5.1.12. 2-(5-(4-Bromophenyl)-6-(*p*-nitrophenylacet-ω-yl)-6*H*-1,3,4-oxadiazin-2-yl)acetonitrile (6b)

Crystallized from 1,4-dioxan, orange crystals. Mp: 166 °C in 55% yield. Analysis for $C_{19}H_{13}BrN_4O_4$, M. Wt. (441.23). Calcd: C, 51.72; H, 2.97; Br, 18.11; N, 12.70. Found: C, 51.63; H, 2.84; Br, 17.95; N, 12.53. IR (υ cm^{-1}): 3062 (CH aromatic), 2890 (CH_2), 2223 (CN), 1644 (C═N), 1636 (C═C). ^{1}H NMR (DMSO) δ: 2.98 (s, 2H, CH_2), 4.85 (s, 2H, CH_2), 7.21 (s, 1H, oxadiazine H-6), 7.23–7.40 (m, 8H, $2C_6H_4$). ^{13}C NMR, δ: 17.6 (CH_2), 40.5 (CH_2), 62.6 (oxadiazine C-6), 116.9 (CN), 121.6, 129.3, 131.4, 131.8, 132.6, 133.8, 134.0, 134.9, 138.0 ($2C_6H_4$), 164.0, 164.4 (2C═N), 189.8 (CO). *m*/*z* (EI, 70 eV): 441 (5%, M^+), 443 (20%, M+2).

5.1.13. 2-(5-(4-Nitrophenyl)-6-(*p*-bromophenylacet-ω-yl)-6*H*-1,3,4-oxadiazin-2-yl)acetonitrile (6c)

Crystallized from 1,4-dioxan, deep orange crystals. Mp: 192–194 °C in 74% yield. Analysis for $C_{19}H_{13}BrN_4O_4$, M. Wt. (441.23). Calcd: C, 51.72; H, 2.97; Br, 18.11; N, 12.70. Found: C, 51.59; H, 2.77; Br, 17.88; N, 12.44. IR (υ cm^{-1}): 3055 (CH aromatic), 2891 (CH_2), 2223 (CN), 1644 (C═N), 1636 (C═C). ^{1}H NMR (DMSO) δ: 2.96 (s, 2H, CH_2), 4.85 (s, 2H, CH_2), 7.24 (s, 1H, oxadiazine H-6), 7.7.26–7.36 (m, 8H, $2C_6H_4$). ^{13}C NMR, δ: 17.2 (CH_2), 40.3 (CH_2), 61.6 (oxadiazine C-6), 116.6 (CN), 121.9, 129.3, 130.6, 131.6, 133.4, 134.5, 135.0, 135.6, 138.2 ($2C_6H_4$), 164.3, 164.6 (2C═N), 189.6 (CO). *m*/*z* (EI, 70 eV): 440 (100%, M^+), 442 (6%, M+2).

5.1.14. 2-(5-(4-Nitrophenyl)-6-(*p*-nitrophenylacet-ω-yl)-6*H*-1,3,4-oxadiazin-2-yl)acetonitrile (6d)

Crystallized from 1,4-dioxan, red crystals. Mp: 255–258 °C in 59% yield. Analysis for $C_{19}H_{13}N_5O_6$ M. Wt. 407.34. Calcd: C, 56.02; H, 3.22; N, 17.19. Found: C, 55.85; H, 3.08; N, 17.01. IR (υ cm^{-1}): 3050 (CH aromatic), 2889 (CH_2), 2221 (CN), 1640 (C═N), 1634 (C═C). ^{1}H NMR (DMSO) δ: 2.98 (s, 2H, CH_2), 4.89 (s, 2H, CH_2), 7.21 (m, 1H, oxadiazine H-6), 7.23–7.40 (m, 8H, $2C_6H_4$).

^{13}C NMR, δ: 17.4 (CH_2), 40.0 (CH_2), 61.4 (oxadiazine C-6), 116.8 (CN), 120.9, 129.3, 130.3, 131.9, 132.7, 134.8, 135.5, 135.6, 138.0 ($2C_6H_4$), 164.3, 164.3 (2C=N), 189.4 (CO). *m/z* (EI, 70 eV): 407 (100%, M^+), 408 (20%).

5.1.15. 2-(4-Substitutedcetophenon-ω-yl)-3-(4-substituted-phenyl)-5-cyano-6-cyanomethylpyran (9a–d)

General procedure: To a solution of either **6a** (4.75 g, 0.01 mol), **6b** (4.41 g, 0.01 mol), **6c** (4.41 g, 0.01 mol) or **6d** (4.07 g, 0.01 mol) in 1,4-dioxan (50 mL) and acetic acid (10 mL), acrylonitrile (0.53 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 6 h, reaction was monitored by TLC, then poured onto ice/water and the formed solid product, in each case, was collected by filtration.

5.1.16. 2-(4-Bromoacetophenon-ω-yl)-3-(4-bromophenyl)-5-cyano-6-cyanomethylpyran (9a)

Crystallized from methanol, yellow crystals. Mp: 180–183 °C in 69% yield. Analysis for $C_{22}H_{14}Br_2N_2O_2$ M. Wt. 498.17. Calcd: C, 53.04; H, 2.83; Br, 32.08; N, 5.62. Found: C, 53.29; H, 3.11; Br, 31.87; N, 5.72. IR (υ cm^{-1}): 3055 (CH aromatic), 2893 (CH_2), 2223, 2220 (2 CN), 1693 (CO), 1631 (C=C). ^{1}H NMR (DMSO) δ: 2.93 (s, 2H, CH_2), 3.21 (s, 2H, pyran CH_2), 3.92 (s, 2H, CH_2), 7.28–7.39 (m, 8H, $2C_6H_4$). ^{13}C NMR, δ: 20.8 (CH_2), 40.9 (CH_2), 116.0, 116.8 (2CN), 38.6, 78.8, 108.0, 138.8, 162.0 (pyran C), 120.4, 124.4, 126.9, 130.0, 131.3, 131.4, 132.2, 135.5 ($2C_6H_4$), 189.6 (CO). *m/z* (EI, 70 eV): 498 (8%, M^+), 500 (17%, M+2), 479 (100%).

5.1.17. 2-(4-Bromoacetophenon-ω-yl)-3-(4-nitrophenyl)-5-cyano-6-cyanomethylpyran (9b)

Crystallized from 1,4-dioxan, orange crystals. Mp: 210–214 °C in 88% yield. Analysis for $C_{22}H_{14}BrN_3O_4$, M. Wt. (464.27). Calcd: C, 56.91; H, 3.04; Br, 17.21; N, 9.05. Found: C, 57.29; H, 3.11; Br, 31.87; N, 8.92. IR (υ cm^{-1}): 3058 (CH aromatic), 2889 (CH_2), 2223, 2220 (2 CN), 1688 (CO), 1634 (C=C). ^{1}H NMR (DMSO) δ: 2.82 (s, 2H, CH_2), 3.24 (s, 2H, pyran CH_2), 3.93 (s, 2H, CH_2), 7.31–7.49 (m, 8H, $2C_6H_4$). ^{13}C NMR, δ: 20.4 (CH_2), 41.3 (CH_2), 116.2, 116.6 (2CN), 38.2, 78.9, 108.2, 138.5, 158.7 (pyran C), 120.9, 123.2, 124.8, 129.6, 130.9, 131.7, 132.0, 134.6 ($2C_6H_4$), 188.9 (CO). *m/z* (EI, 70 eV): 463 (100%), 464 (30%, M^+), 466 (3%, M+2).

5.1.18. 2-(4-Nitroacetophenon-ω-yl)-3-(4-bromophenyl)-5-cyano-6-cyanomethylpyran (9c)

Crystallized from 1,4-dioxan, orange crystals. Mp: 180–182 °C in 71% yield. Analysis for $C_{22}H_{14}BrN_3O_4$, (464.27). C, 56.91; H, 3.04; Br, 17.21; N, 9.05. Found: C, 53.29; H, 3.11; Br, 17.33; N, 9.11. IR (υ cm^{-1}): 3054 (CH aromatic), 2884 (CH_2), 2227, 2221 (2 CN), 1687 (CO), 1638 (C=C). ^{1}H NMR (DMSO) δ: 2.80 (s, 2H, CH_2), 3.24 (s, 2H, pyran CH_2), 4.91 (s, 2H, CH_2), 7.26–7.43 (m, 8H, $2C_6H_4$). ^{13}C NMR, δ: 20.2 (CH_2), 41.0 (CH_2), 116.7, 116.6 (2CN), 38.5, 78.2, 108.0, 138.4, 158.6 (pyran C), 120.8, 121.3, 122.9, 123.0, 124.6, 129.6, 132.0, 134.6 ($2C_6H_4$), 188.9 (CO). *m/z* (EI, 70 eV): 464 (30%, M^+), 466 (5%, M+2).

5.1.19. 2-(4-Nitroacetophenon-ω-yl)-3-(4-nitrophenyl)-5-cyano-6-cyanomethylpyran (9d)

Crystallized from dilute DMF, deep red crystals. Mp: 169–172 °C in 77% yield. Analysis for $C_{22}H_{14}N_4O_6$ (430.37). C, 61.40; H, 3.28; N, 13.02. Found: C, 61.62; H, 3.38; N, 12.87. IR (υ cm^{-1}): 3044 (CH aromatic), 2893(CH_2), 2218 (CN), 1638 (C=N), 1623 (C=C). ^{1}H NMR (DMSO) δ: 2.81 (s, 2H, CH_2), 4.27 (s, 2H, pyran CH_2), 4.90 (s, 2H, CH_2), 7.22–7.39 (m, 8H, $2C_6H_4$). ^{13}C NMR, δ: 20.1 (CH_2), 41.2 (CH_2), 116.2, 116.9 (2CN), 38.6, 78.1, 108.3, 138.9, 158.4 (pyran C), 120.2, 121.8, 122.4, 122.9, 123.4, 125.2, 128.3, 130.2 ($2C_6H_4$), 187.3 (CO). 430 (100%, M^+), 432 (6%, M+2).

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