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Original article

Synthesis and pharmacological evaluations of novel 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one derivatives as a new class of anti-cancer agents

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ABSTRACT

The synthesis of novel 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one derivatives has been carried out using trifluoroacetic anhydride/phosphoric acid mediated C–C bond forming reaction as a key step. This method does not require the use of environmentally harmful $AlCl_3$ or moisture sensitive acid chloride. A number of compounds containing the benzooxazinone moiety attached to a five-membered central heterocyclic ring was synthesized and tested for their anti-cancer properties in vitro against three cell lines e.g. A549 (lung), DLD-1 (colorectal adenocarcinoma) and MV4-11 (acute myeloid leukemia). Some of them showed anti-cancer activities along with a number of reference compounds tested. Few of them showed promising anti-leukemic properties. A brief Structure–Activity-Relationship study within the series is presented. An imidazole derivative **9c** containing benzene ring with a *para*-CF_3 group at C-2 position was identified as a potent anti-leukemic agent.

1. Introduction

Triaryl substituted heterocyclic class of compounds represented by structure **A** (Fig. 1) have attracted considerable interest in the development of potential anti-cancer agents. For example, imidazole derivative SB-590885 possessing a 2,3-dihydro-1*H*-inden-1-one oxime substituent has been identified as a potent and extremely selective inhibitor of the B-Raf kinase [1]. B-Raf mutations have been identified in many types of cancer, especially in malignant melanomas and thyroid cancers [2], and several agents targeting B-Raf have been reported. 2*H*-Benzo[*b*][1,4]oxazin-3(4*H*)-one derivatives on the other hand have been reported to be useful chemical entities for treating and/or preventing various human diseases [3]. In our continuing effort to identify novel compounds of potential pharmacological interest [4] we became interested to build a library of small molecules incorporating the 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one moiety (e.g. compound **C**, Fig. 1) for assessing their anti-cancer properties in vitro. Herein, we report our initial findings on the synthesis and pharmacological evaluation of a series of compound **C** structurally related to **A** as potential anti-cancer agents.

A number of methods have been reported for the synthesis of diverse series of compounds related to **A**. One of the commonly utilized strategy for the synthesis of triaryl substituted heterocyclic compounds involved the use of appropriately functionalized 1,2-diaryl-1-ethanone **D** (Fig. 2) that could also be the precursor for compound **C**. These ketones are prepared *via* an array of methods including (a) synthesis from α-aminonitrile [5–7], (b) acylation in the presence of Lewis acids [8–10], or CF_3SO_3H [11], or palladium catalysts [12], (d) synthesis using Grignard reagent [13], (e) pinacol rearrangement catalyzed by $AlCl_3$ [14], (f) benzotriazole-assisted method [15], (g) self coupling of phenylacetic acid in the presence of PPA [16,17], (h) a multistep sequences consisting of Perkin condensation and Crutius rearrangement [18], and (i) synthesis *via* clay catalyzed rearrangement of phenyloxiranes [19]. However, the simplest one being the Friedel–Crafts acylation [18] of arenes using arylacetyl chloride or acid in the presence of stoichiometric amount of $AlCl_3$ or $ZnCl_2$–$POCl_3$ mixture. Since this process leads to the formation of environmentally harmful gaseous HCl thus a more convenient method was developed by reacting arylacetic acids with appropriate arenes in the presence of trifluoroacetic anhydride/

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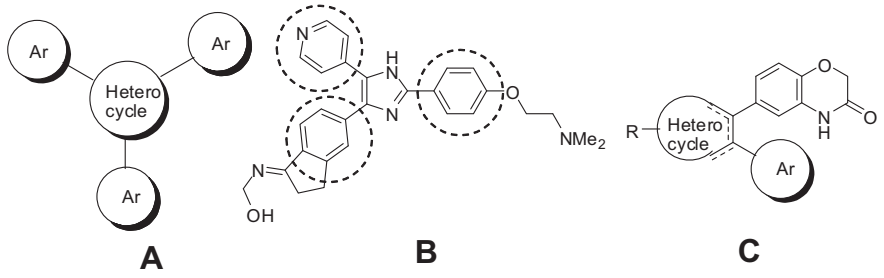


Fig. 1. Triaryl substituted heterocycles (A), anti-cancer agent SB-590885 (B) and the general structure of proposed small molecule library (C).

phosphoric acid [20]. In further continuation of our earlier work we became interested in the application of this C–C bond forming reaction as a key step to synthesize our target compounds. To the best of our knowledge this methodology has not been explored earlier for the preparation of compounds represented by **C**.

2. Results and discussion

2.1. Chemistry

The key starting material **3** required for our synthesis was readily prepared from 2-nitrophenol (**1**) *via* a two-step process (Scheme 1). Thus reaction of 2-nitrophenol (**1**) with ethyl bromoacetate followed by reduction and in situ cyclization of the resulting ether derivative **2** provided the desired benzooxazinone **3**. Subsequent reaction of compound **3** with phenylacetic acid in the presence of $H_3PO_4/(CF_3CO)_2O$ provided the ketone **4** in good yield. Notably, the present $H_3PO_4/(CF_3CO)_2O$ mediated C–C bond formation represents a straightforward, efficient and environmental friendly process and does not require the use of moisture sensitive acyl halides.

The compound **4** was then converted to the 3-(dimethylamino) acrylaldehyde derivative (**5**) which on treatment with appropriately substituted hydrazines provided triaryl substituted pyrazoles (**6**) (Scheme 2). In order to prepare a range of isoxazole derivatives the benzooxazinone **3** was reacted with an array of arylacetic acids to give the ketone **4a**–**e** (Scheme 3). These ketones were then converted to the compounds **5a**–**e** which along with **5** provided the desired compound **7a**–**f** when treated with hydroxylamine hydrochloride.

The imidazole derivatives were prepared (Scheme 4) from compound **5** that was initially oxidized to the 1,2-diketone **8**. Condensation of compound **8** with appropriate aldehydes in the presence of NH_4OAc afforded the desired products **9**.

2.2. Pharmacology

Having prepared a divers series of compounds based on 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one we then tested all these compounds for their potential anti-cancer properties in vitro. Cancer is the second leading cause of death [21] worldwide after cardiovascular diseases, according to WHO. Indeed, lung, breast, stomach, liver and colorectal cancers cause the most cancer deaths worldwide each year, and therefore discovery and development of suitable agents to treat various types of cancer is highly desirable. We have used A549 (NSCLC or Non small cell lung cancer), DLD-1 (colorectal adenocarcinoma) and MV4-11 (acute myeloid leukemia) cells obtained from ATCC (American Type Culture Collection) for our in vitro assay. The effect of test compounds on cell viability was measured using a colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay after 3 days of treatment in culture medium containing 5% FBS. The percentages of cell viability (calculated with respect to control cells grown in the presence of 0.1% v/v DMSO) for new and reference compounds at two concentrations (1.0 and 20 μM for test compounds whereas 1.0 and 10 μM for reference compounds) are presented in Table 1. The reference compounds used in our assay include Gefitinib [an epidermal growth factor receptor (EGFR) inhibitor], Imatinib (or Gleevec, an inhibitor of tyrosine kinase activities), Vandetanib [or Zactima, an antagonist of the vascular endothelial growth factor receptor (VEGFR) and the EGFR], Erlotinib (or Tarceva, a tyrosine kinase inhibitor which acts on the EGFR), Sunitinib [or Sutent, a multi-targeted receptor tyrosine kinase (RTK) inhibitor] and Sorafenib [or Nexavar, an inhibitor of several Tyrosine protein kinases (VEGFR and PDGFR) and Raf (Rapidly Accelerated Fibrosarcoma)]. All the experiments were performed twice with triplicate data points. It is evident from Table 1 that at the concentration of 20 μM compounds **9e**, **9d** and **9c** showed comparable activities to the reference compounds Vandetanib, Sunitinib and Sorafenib (all at 10 μM) against A549 cells. The other compounds that showed activities against A549 cells include **6g**, **6e**, **6a**, **6b** and **6c**. Similarly, all the compounds except **9b** and **6d** were found to be active against DLD-1 cells when tested at 20 μM. However, impressive results were obtained when all these compounds were tested against MV4-11 cells at 20 μM. Indeed a number of compounds e.g. **9a**, **9e**, **9d**, **9f** and **9c** showed significant anti-proliferative properties in this assay in compared to the reference compounds. A graphical presentation of percentage of cell viability after 3 days treatment of MV4-11 cells with most active compounds is shown in Chart 1. Overall, imidazole derivatives (**9**) were found to be superior to pyrazoles (**6**) in terms of their in vitro activities. Among the imidazole derivatives the compounds containing benzene ring with a *para*-substituent or no substituent at C-2 position was found to be superior to others. The CF_3 group was found to be best among all the *para*-substituents examined and compound **9c** was identified as a potent anti-leukemic agent. Notably, leukemia a cancer of blood-forming cells in the bone marrow affects approximately 3,00,000 people worldwide [22] and hence there is a need for the identification of new anti-leukemic agents. The compounds presented here therefore have medicinal value.

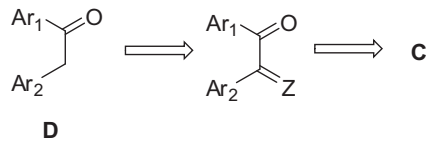
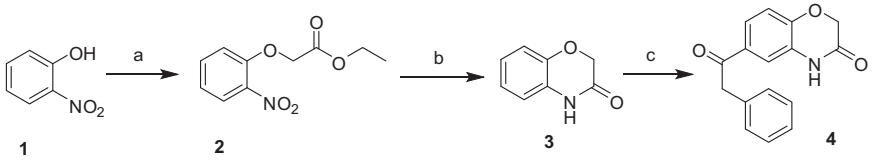


Fig. 2. Strategy for the synthesis of C from ketone D.

3. Conclusions

In conclusion, we have described the design and synthesis of a series of novel 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one derivatives as



Scheme 1. Reagents and condition: (a) K_2CO_3, $BrCH_2CO_2Et$, acetone, 50 °C, 1 h (92%); (b) Fe powder, CH_3CO_2H, 0 °C for 3 h, room temp for 2 h, reflux for 2 h (91%); (c) $C_6H_5CH_2CO_2H$, $H_3PO_4/(CF_3CO)_2O$, room temp, 1 h (85%).

potential anti-cancer agents. Multi-step synthesis of these compounds was carried out using trifluoroacetic anhydride/phosphoric acid mediated C–C bond forming reaction as a key step. This method does not require the use of environmentally harmful $AlCl_3$ or moisture sensitive acid chloride. Thus acylation of benzooxazinone ring under these conditions followed by appropriate reaction sequences afforded a range of tri and diaryl substituted heterocyclic compounds containing the benzooxazinone moiety attached to a five-membered central ring. Some of the compounds prepared were tested for their potential anti-cancer properties in vitro against A549 (lung), DLD-1 (colorectal adenocarcinoma) and MV4-11 (acute myeloid leukemia) cell lines. Few of them showed promising anti-proliferative properties especially against leukemia cells. An imidazole derivative containing benzene ring with a *para*-CF_3 group at C-2 position was identified as a potent anti-leukemic agent. Overall, the present series of heterocyclic compounds represent a potential scaffold for the development of potent anti-leukemic agents.

4. Experimental section

4.1. Chemistry

4.1.1. General methods

Unless stated otherwise, reactions were performed under nitrogen atmosphere. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (100–200 mesh) using distilled hexane, ethyl acetate, dichloromethane. ^{1}H NMR spectra were determined in $CDCl_3$ or DMSO-d_6 solution by using Varian 400 MHz spectrometer. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, $\delta = 0.00$) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on an FT-IR spectrometer (Perkin Elmer). Melting points were determined using melting point apparatus and are uncorrected. MS spectra were obtained on a mass spectrometer.

4.1.2. Synthesis of triaryl substituted pyrazoles (**6**)

*4.1.2.1. Synthesis of (2-nitrophenoxy) ethyl acetate (**2**).* To a stirred solution of 2-nitrophenol (10 g, 0.071 mol) in acetone (25 mL), K_2CO_3 (21.5 g, 0.156 mol) was added at room temperature and stirred for 15 min. Then ethyl bromoacetate (13.2 g, 0.079 mol) was added drop wise. After completion of addition, temperature was raised to 50 °C and stirred for 1 h. The reaction mixture was cooled and the solid separated out was filtered and washed with acetone. Filtrate was concentrated in reduced pressure. The crude was partitioned between methyl tertiary butyl ether and water. The organic layer was extracted with ethyl acetate (2 × 20 mL), washed with water (20 mL), dried over anhydrous sodium sulfate and concentrated to give the title compound (15.4 g, yield 92%); mp 39–40 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85 (1H, d, $J = 8.2$ Hz, ArH), 7.50 (1H, t, $J = 7.8$ Hz, ArH), 7.10 (2H, d, $J = 7.7$ Hz, ArH), 4.75 (2H, s, OCH_2), 4.30–4.20 (2H, m, OCH_2), 1.30 (3H, t, $J = 7.3$ Hz, CH_3); m/z (CI) 224 (M + 1, 100%).

4.1.3. Synthesis of 4H-benzo [1,4]oxazin-3-one (**3**)

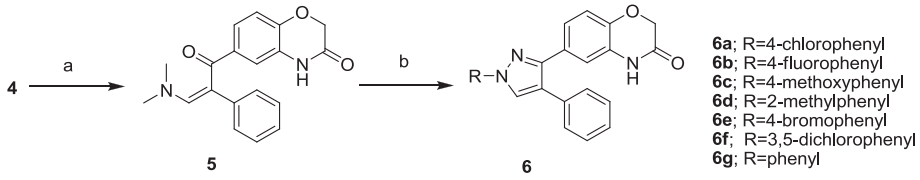
To a solution of **2** (15.4 g 0.068 mol) in acetic acid (75 mL), Fe powder (38.4 g, 0.68 mol) was added portion wise for 3 h at 0 °C. The reaction was exothermic. The reaction mixture was allowed to stir at room temperature for 2 h and heated to reflux for 2 h. After completion of the reaction, the reaction mixture was cooled to room temperature, filtered on celite and the cake was washed with acetic acid. Acetic acid was concentrated to ¼ of its volume and diluted with water (75 mL). The solid separated was filtered, washed with water (20 mL) and dried to give the title compound (7.5 g, yield 91%); mp 173–174 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.95 (1H, s, NH), 7.00–6.80 (4H, m, ArH), 4.60 (2H, s, OCH_2); m/z (CI) 150 (M + 1, 100%).

4.1.4. Synthesis 6-phenylacetyl-4H-benzo [1,4]-oxazin-3-one (**4**)

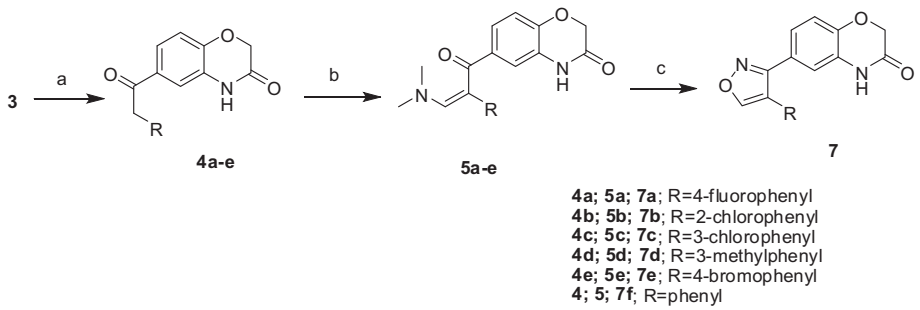
Phosphoric acid (3.94 g, 0.04 mol) and TFAA (18.6 mL, 0.132 mol) were added to the mixture of **3** (5 g, 0.033 mol) and phenylacetic acid (6.73 g, 0.04 mol), at 0 °C. After completion of addition, the mixture was allowed to warm to room temperature and stirred for 1 h. The reaction mixture was cooled to 0 °C and adjusted the pH to 7 by adding the saturated $NaHCO_3$ solution. The precipitated solid was filtered, washed with water and dried to furnish 7.5 g (85%) of the desired product, mp 188–189 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.85 (1H, bs, NH), 7.70 (1H, d, $J = 7.8$ Hz, ArH), 7.50 (1H, s, ArH), 7.40–7.20 (5H, m, ArH), 6.95 (1H, d, $J = 7.8$ Hz, ArH), 4.65 (2H, s, CH_2), 4.20 (2H, s, CH_2); m/z (CI) 266 (M-1, 100%).

4.1.5. Synthesis 6-(3-dimethylamino-2-phenyl-acrolyl)-4H-benzo [1,4]-oxazin-3-one (**5**)

DMF–DMA (1.07 g, 0.0089 mol) was added to a solution of 6-phenyl-4*H*-benzo [1,4]-oxazin-3-one (2 g, 0.0074 mol) in toluene (10 mL) and heated to reflux for 0.5 h. After sometime the solution



Scheme 2. Reagents and condition: (a) DMF–DMA, toluene, reflux, 0.5 h (76%); (b) $RNHNH_2 \cdot HCl$, EtOH, 40–50 °C, 15 min.



Scheme 3. Reagents and condition: (a) RCH_2CO_2H, $H_3PO_4/(CF_3CO)_2O$, room temp, 1 h; (b) DMF–DMA, toluene, reflux, 0.5 h; (c) $NH_2OH{\cdot}HCl$, EtOH, 40–50 °C, 15 min.

became clear and solid was precipitated out. The solid separated was filtered, washed with toluene (15 mL) and dried to give the title compound (1.75 g, yield 76%) as light yellow solid; mp 194–195 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.85 (1H, s, NH), 7.80 (1H, s, =CH), 7.65 (1H, s, ArH), 7.30–7.00 (5H, m, ArH), 6.60–6.45 (2H, m, ArH), 4.55 (2H, s, OCH_2), 2.80 (6H, s, $N(CH_3)_2$); *m/z* (CI) 321 (M-1, 100%).

*4.1.6. General procedure for the synthesis of triaryl pyrazoles (**6**)*

To a solution of 6-(3-dimethylamino-2-phenyl-acrolyl)-4*H*-benzo [1,4]-oxazin-3-one (0.00065 mol) in ethanol, substituted phenylhydrazine hydrochloride (0.00071 mol) in ethanol was added slowly. After completion of addition, the mixture was heated to 40–50 °C in a water bath. After 15 min, the solution became clear and solid was precipitated out. The solid separated was filtered, washed with ethanol and dried to give the expected product.

*4.1.6.1. 6-(1-(4-Chlorophenyl)-4-phenyl-1H-pyrazol-3-yl)-2H-benzo [b][1,4]oxazin-3(4H)-one (**6a**).* Yield 88%; white solid. mp > 270 °C; IR (KBr, cm^{-1}): 3480, 1702, 1494, 1044, 826, 769, 696; ^{1}H NMR (DMSO-d_6): δ 10.65 (1H, s, ArH), 8.05 (1H, s, ArH), 7.45 (2H, d, J = 8.2 Hz, ArH), 7.35–7.18 (7H, m, ArH), 6.95 (1H, d, J = 8.2 Hz, ArH), 6.80–6.60 (2H, m, ArH), 4.60 (2H, s, OCH_2); *m/z* (CI) 400 (M-1, 100%); HPLC 98.1%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: water, mobile phase B: acetonitrile, gradient (A/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 254 nm, retention time 12.7 min.

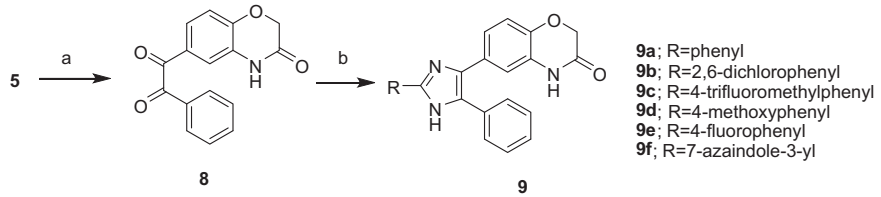
*4.1.6.2. 6-(1-(4-Fluorophenyl)-4-phenyl-1H-pyrazol-3-yl)-2H-benzo [b][1,4]oxazin-3(4H)-one (**6b**).* Yield 88%; white solid. mp > 270 °C; IR (KBr, cm^{-1}): 3195, 1703, 1588, 1219; ^{1}H NMR (DMSO-d_6): δ 10.65 (1H, s, ArH), 8.05 (1H, s, ArH), 7.38–7.10 (9H, m, ArH), 6.95 (1H, d, J = 8.2 Hz, ArH), 6.80–6.60 (2H, m, ArH), 4.60 (2H, s, OCH_2); *m/z* (CI) 286 (M + 1, 100%); HPLC 98.0%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: water, mobile phase B: acetonitrile, gradient (A/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 254 nm, retention time 11.9 min.

*4.1.6.3. 6-(1-(4-Methoxyphenyl)-4-phenyl-1H-pyrazol-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**6c**).* Yield 86%; white solid, mp 242–243 °C; IR (KBr, cm^{-1}): 3480, 1702, 1510, 1249, 1042, 830, 766, 695; ^{1}H NMR (DMSO-d_6): δ 10.65 (1H, s, –NH), 8.00 (1H, s, ArH), 7.35–7.10 (8H, m, ArH), 6.95 (2H, d, J = 8.4 Hz, ArH), 6.80–6.60 (2H, m, ArH), 4.60 (2H, s, OCH_2), 3.75 (3H, s, OCH_3); *m/z* (CI) 396 (M-1, 100%); HPLC 98.9%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: water, mobile phase B: acetonitrile, gradient (A/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 254 nm, retention time 11.6 min.

*4.1.6.4. 6-(4-Phenyl-1-o-tolyl-1H-pyrazol-3-yl)-2H-benzo[b][1,4] oxazin-3(4H)-one (**6d**).* Yield 83.3%; white solid, mp 144–146 °C; IR (KBr, cm^{-1}): 3208, 1701, 1491, 1372, 1039, 764, 697; ^{1}H NMR (DMSO-d_6): δ 10.70 (1H, s, NH), 8.10 (1H, s, ArH), 7.40–7.15 (9H, m, ArH), 6.85 (1H, d, J = 8.8 Hz, ArH), 6.75–6.68 (2H, m, ArH), 4.60 (2H, s, OCH_2), 2.00 (3H, s, CH_3); *m/z* (CI) 382 (M + 1, 97%); HPLC 98.9%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: water, mobile phase B: acetonitrile, gradient (A/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 254 nm, retention time 11.7 min.

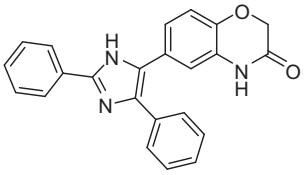
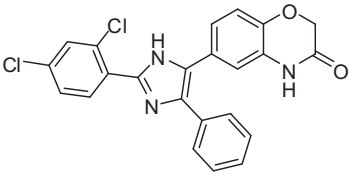
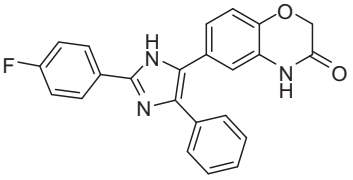
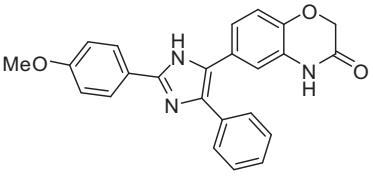
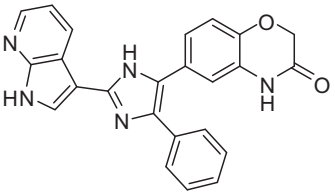
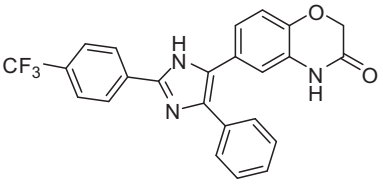
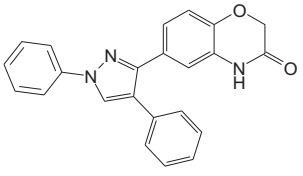
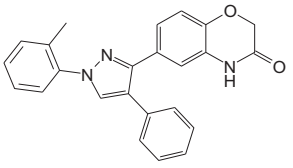
*4.1.6.5. 6-(1-(4-Bromophenyl)-4-phenyl-1H-pyrazol-3-yl)-2H-benzo [b][1,4]oxazin-3(4H)-one (**6e**).* Yield 86.2%; white solid, mp > 270 °C: IR (KBr, cm^{-1}): 3470, 1703, 1491, 1044, 823, 769, 697; ^{1}H NMR (DMSO-d_6): δ 10.65 (1H, s, NH), 8.10 (1H, s, ArH), 7.60 (2H, d, J = 8.7 Hz, ArH), 7.35–7.15 (7H, m, ArH), 6.95 (1H, d, J = 8.2 Hz, ArH), 6.88–6.70 (2H, m, ArH), 4.65 (2H, s, OCH_2); *m/z* (CI) 448 (M + 2, 100%); HPLC 97.0%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: water, mobile phase B: acetonitrile, gradient (A/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 254 nm, retention time 11.8 min.

*4.1.6.6. 6-(1-(3,5-Dichlorophenyl)-4-phenyl-1H-pyrazol-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**6f**).* Yield 85%; white solid, mp 189–190 °C; IR (KBr, cm^{-1}): 3447, 1705, 1583, 1046, 807, 765, 693; ^{1}H NMR (DMSO-d_6): δ 10.75 (1H, s, ArH), 8.10 (1H, s, ArH), 7.80 (1H, s, ArH), 7.30–7.10 (7H, m, ArH), 6.95 (1H, d, J = 8.1 Hz, ArH), 6.80–6.65 (2H, m, ArH), 4.60 (2H, s, OCH_2); *m/z* (CI) 436 (M + 1, 100%); HPLC 98.2%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: water, mobile phase B: acetonitrile, gradient (A/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 254 nm, retention time 14.0 min.



Scheme 4. Reagents and condition: (a) $NaIO_4$, THF–H_2O, room temp, 15 min; (b) RCHO, NH_4OAc, CH_3CO_2H, reflux, 2 h.

Table 1
The percentages of cell viability noted for 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one derivatives and reference compounds.

Entry	Compounds (**9** and **6**)		A549 (Lung)		DLD-1 (Colon)		MV4-11 (Leukemia)	
			1 μM	20 μM	1 μM	20 μM	1 μM	20 μM
1		**9a**	95.6	58.5	98.2	47.3	104.6	7.6
2		**9b**	97.4	68.3	97.8	61.9	108.7	41.1
3		**9e**	97.1	37.5	94.8	29.5	108.2	1.1
4		**9d**	100.8	38.1	96.0	19.1	108.1	1.2
5		**9f**	100.3	86.8	97.7	53.5	110.8	8.9
6		**9c**	102.8	21.7	95.0	12.1	110.8	0.6
7		**6g**	92.2	59.7	77.0	47.7	117.2	97.9
8		**6d**	94.8	85.3	92.2	62.9	99.4	54.2

(*continued on next page*)

Table 1 (*continued*)

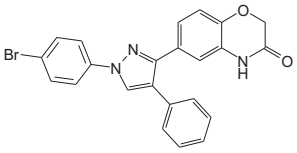
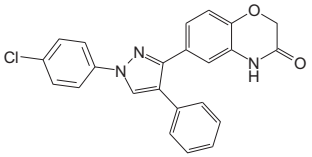
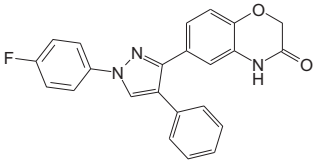
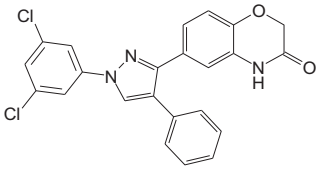
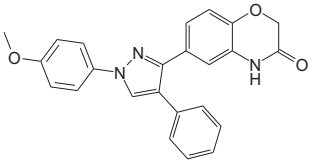
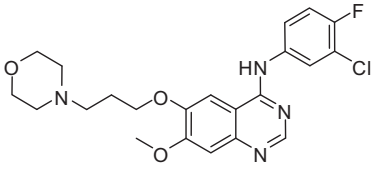
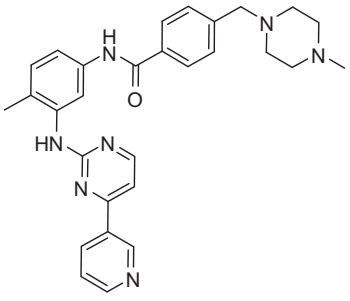
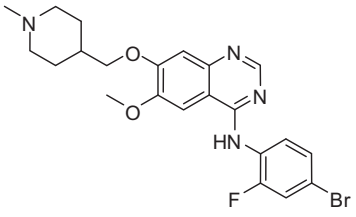
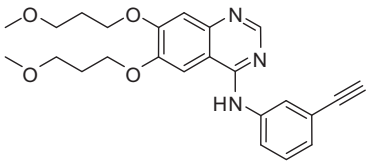
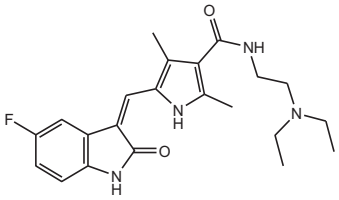
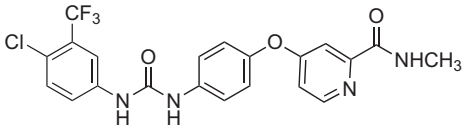
Entry	Compounds (**9** and **6**)		A549 (Lung)		DLD-1 (Colon)		MV4-11 (Leukemia)	
			1 μM	20 μM	1 μM	20 μM	1 μM	20 μM
9		**6e**	81.5	58.8	66.4	54.2	107.8	91.5
10		**6a**	84.4	52.4	61.9	47.1	107.8	91.9
11		**6b**	78.2	56.4	64.4	45.2	107.3	92.6
12		**6f**	93.1	70.2	90.3	52.2	105.1	50.5
13		**6c**	95.6	42.6	92.2	35.6	106.1	59.9
14		Gefitinib	84.4	61.5[a]	87.0	65.7[a]	92.4	7.0[a]
15		Imatinib	108.3	98.6[a]	89.9	91.5[a]	105.0	82.0[a]
16		Vandetanib	88.7	41.7[a]	88.6	37.9[a]	84.1	1.6[a]

Table 1 (*continued*)

Entry	Compounds (**9** and **6**)		A549 (Lung)		DLD-1 (Colon)		MV4-11 (Leukemia)	
			1 μM	20 μM	1 μM	20 μM	1 μM	20 μM
17		Erlotinib	83.0	68.5[a]	85.7	55.4[a]	98.3	16.8[a]
18		Sunitinib	102.5	37.4[a]	92.9	52.7[a]	1.4	0.5[a]
19	 Sorafenib	Sorafenib	103.2	28.6[a]	93.4	28.0[a]	1.6	0.7[a]

[a] The assay was carried out using the compound at 10 μM.

*4.1.7. Synthesis of isoxazole derivatives (**7**)*

4.1.7.1. Typical procedure for the synthesis 6-phenylacetyl-4H-benzo [1,4]-oxazin-3-one. Phosphoric acid (3.15 g, 0.032 mol) and TFAA (21.8 mL, 0.104 mol) were added to the mixture of 4*H*-Benzo [1,4] oxazin-3-one (4 g, 0.026 mol) and substituted phenylacetic acid (1.1eq), at 0 °C. After completion of addition, the mixture was allowed to warm to room temperature and stirred for 1 h. The reaction mixture was cooled to 0 °C and adjusted the pH to 7 by adding the saturated $NaHCO_3$ solution. The precipitated solid was filtered, washed with water and dried to give the desired product.

*4.1.7.2. 6-(2-(4-Fluorophenyl)acetyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**4a**).* Yield 80%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.85 (1H, s, NH), 7.74 (1H, d, J = 8.4 Hz, ArH), 7.52 (1H, s, ArH), 7.35–7.20 (2H, m, ArH), 7.18–7.05 (3H, m, ArH), 4.70 (2H, s, OCH_2), 4.30 (2H, m, =C–CH_2Ph); *m*/*z* (CI) 284 (M-1, 100%).

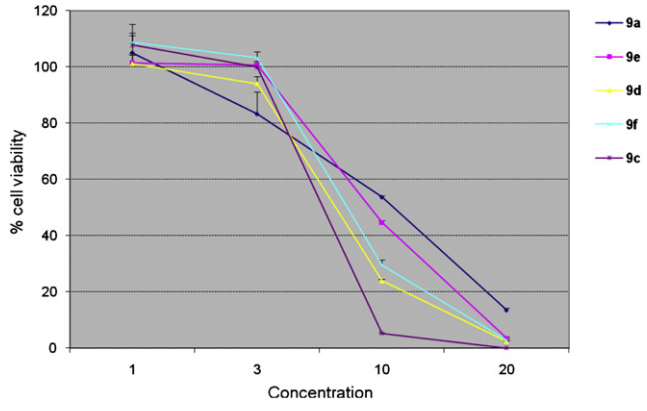


Chart 1. Three days treatment of MV4-11 cells with most active compounds.

*4.1.7.3. 6-(2-(2-Chlorophenyl)acetyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**4b**).* Yield 82%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.85 (1H, s, NH), 7.90–7.70 (1H, m, ArH), 7.53 (1H, s, ArH), 7.55–7.25 (4H, m, ArH), 7.05 (1H, d, J = 8.4 Hz, ArH), 4.69 (2H, s, OCH_2), 4.42 (2H, s, =C–CH_2–Ph); *m*/*z* (CI) 300 (M-1, 100%).

*4.1.7.4. 6-(2-(3-Chlorophenyl)acetyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**4c**).* Yield 82.3%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.75 (1H, s, NH), 7.75–7.65 (1H, m, ArH), 7.55 (1H, s, ArH), 7.40–7.25 (3H, m, ArH), 7.20 (1H, d, J = 7.2 Hz, ArH), 7.17 (1H, d, J = 7.2 Hz, ArH), 4.67 (2H, s, OCH_2), 4.32 (2H, s, =C–CH_2–Ph); *m*/*z* (CI) 300 (M-1, 100%).

*4.1.7.5. 6-(2-(3-Methylphenyl)acetyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**4d**).* Yield 80.6%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.85 (1H, s, NH), 7.72 (2H, d, J = 2.2 Hz, ArH), 7.55 (1H, s, ArH), 7.20–7.15 (1H, m, ArH), 7.15–7.00 (4H, m, ArH), 4.69 (2H, s, OCH_2), 4.16 (2H, s, =C–CH_2–Ph), 2.33 (3H, s, CH_3); *m*/*z* (CI) 280 (M-1, 100%).

*4.1.7.6. 6-(2-(4-Bromophenyl)acetyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**4e**).* Yield 81.5%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.85 (1H, s, NH), 7.74 (1H, d, J = 8.4 Hz, ArH), 7.41–7.57 (3H, m, ArH), 7.30–7.10 (2H, m, ArH), 7.05 (1H, d, J = 8.3 Hz, ArH), 4.69 (2H, s, OCH_2), 4.27 (2H, s, =C–CH_2–Ph); *m*/*z* (CI) 344 (M-2, 100%).

4.1.8. Typical procedure for the synthesis 6-(3-dimethylamino-2-phenyl-acrolyl)-4H-benzo [1,4]-oxazin-3-one

DMF–DMA (2 g, 0.0074 mol) was added to a solution of substituted 6-phenyl-4*H*-benzo [1,4]-oxazin-3-one (1.07 g, 0.0089 mol) in toluene and heated to reflux on an oil bath for 0.5 h.

After sometime the solution became clear and immediately solid was precipitated out. The solid was filtered, washed with toluene and dried to give the desired product.

*4.1.8.1. (Z)-6-(3-(Dimethylamino)-2-(4-fluorophenyl)acryloyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**5a**).* Yield 70%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.85 (1H, s, NH), 7.25 (1H, s, ArH), 7.15–7.05 (4H, m, ArH), 7.05 (1H, s, Me_2N–CH=), 6.95–6.80 (2H, m, ArH), 4.70 (2H, s, OCH_2), 2.70 (6H, s, $N(CH_3)_2$); *m/z* (CI) 291 (M + 1, 100%).

*4.1.8.2. (Z)-6-(3-(Dimethylamino)-2-(2-chlorophenyl)acryloyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**5b**).* Yield 77.9%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.85 (1H, s, NH), 7.40 (1H, d, J = 3.7 Hz, ArH), 7.35–7.15 (4H, m, ArH), 7.10 (1H, s, Me_2N–CH=), 7.00–6.85 (2H, m, ArH), 4.69 (2H, s, OCH_2), 2.70 (6H, s, $N(CH_3)_2$); *m/z* (CI) 355 (M-1, 100%).

*4.1.8.3. (Z)-6-(3-(Dimethylamino)-2-(3-chlorophenyl)acryloyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**5c**).* Yield 74%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.85 (1H, s, NH), 7.38–7.20 (3H, m, ArH), 7.17 (1H, s, Me_2N–CH=), 7.10–6.85 (4H, m, ArH), 4.60 (2H, s, OCH_2), 2.70 (6H, s, $N(CH_3)_2$); *m/z* (CI) 355 (M-1, 100%).

*4.1.8.4. (Z)-6-(3-(Dimethylamino)-2-(3-methylphenyl)acryloyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**5d**).* Yield 75%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.85 (1H, s, NH), 7.20–7.15 (2H, m, ArH), 7.00 (1H, s, Me_2N–CH=), 6.95–6.80 (5H, m, ArH), 4.69 (2H, s, OCH_2), 2.25 (3H, s, CH_3), 2.70 (6H, s, $N(CH_3)_2$); *m/z* (CI) 335 (M-1, 100%).

*4.1.8.5. (Z)-6-(3-(Dimethylamino)-2-(4-bromophenyl)acryloyl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**5e**).* Yield 70%; white solid; ^{1}H NMR (DMSO-d_6): δ 10.85 (1H, s, NH), 7.25 (1H, s, ArH), 7.15–7.05 (4H, m, ArH), 7.05 (1H, s, $(CH_3)_2$NCH=), 6.95–6.80 (2H, m, ArH), 4.70 (2H, s, OCH_2), 2.70 (6H, s, $N(CH_3)_2$); *m/z* (CI) 403 (M + 2, 100%).

4.1.9. Typical procedure for the synthesis of isoxazole

To a solution of 6-(3-dimethylamino-2-phenyl-acrolyl)-4*H*-benzo [1,4]-oxazin-3-one (0.00065 mol) in ethanol, hydroxylamine hydrochloride (0.00071 mol) in ethanol was added slowly. After completion of addition, the mixture was heated to 40–50 °C on water bath. After 15 min, the solution became clear and immediately solid precipitated out. The solid was filtered, washed with ethanol and dried.

*4.1.9.1. 6-(4-(4-Fluorophenyl)isoxazol-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**7a**).* Yield 90%; white solid; mp 176–177 °C; IR (KBr, cm^{-1}): 3464, 1694, 1498, 1398, 1221, 843, 810, 542; ^{1}H NMR (DMSO-d_6): δ 10.85 (1H, bs, –NH), 8.85 (1H, s, ArH), 7.55–7.40 (2H, m, ArH), 7.38–7.25 (2H, m, ArH), 7.10–7.00 (3H, m, ArH), 4.67 (2H, s, OCH_2); *m/z* (CI) 311 (M + 1, 98%); HPLC 97.0%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: acetonitrile, gradient (T/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 230 nm, retention time 11.1 min.

*4.1.9.2. 6-(4-(2-Chlorophenyl)isoxazol-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**7b**).* Yield 92%; White solid; mp 247–248 °C; IR (KBr cm^{-1}): 3447, 1654, 1508; ^{1}H NMR (DMSO-d_6): δ 10.80 (1H, bs, NH), 8.85 (1H, s, ArH), 7.63 (1H, d, J = 7.6 Hz, ArH), 7.55–7.38 (3H, m, ArH), 7.10 (1H, s, ArH), 7.10–6.85 (2H, m, ArH), 4.61 (2H, s, OCH_2); *m/z* (CI) 325 (M-1, 100%); HPLC 99.2%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: acetonitrile, gradient (T/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 254 nm, retention time 11.3 min.

*4.1.9.3. 6-(4-(3-Chlorophenyl)isoxazol-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**7c**).* Yield 92%; White solid; mp. 178–180 °C; IR (KBr, cm^{-1}): 3447, 1692, 1599, 1491, 1396, 780; ^{1}H NMR (DMSO-d_6): δ 10.80 (1H, bs, NH), 8.85 (1H, s, ArH), 7.58 (1H, s, ArH), 7.46 (2H, d, J = 5.0 Hz, ArH), 7.38 (1H, s, ArH), 7.20–7.00 (3H, m, ArH), 4.68 (2H, s, OCH_2); *m/z* (CI) 325 (M-1, 100%); HPLC 97.9%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: acetonitrile, gradient (T/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 215 nm, retention time 11.8 min.

*4.1.9.4. 6-(4-(3-Methylphenyl)isoxazol-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**7d**).* Yield 89%; White solid; mp 191–193 °C; IR (KBr, cm^{-1}): 3448, 1698, 1498, 1387, 1039, 699; ^{1}H NMR (DMSO-d_6): δ 10.80 (1H, bs, NH), 8.85 (1H, s, ArH), 7.40–7.30 (2H, m, ArH), 7.22 (3H, s, ArH), 7.10–6.98 (2H, m, ArH), 4.67 (2H, s, OCH_2), 2.33 (3H, s, CH_3); *m/z* (CI) 307 (M + 1, 100%); HPLC 97.4%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: acetonitrile, gradient (T/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 230 nm, retention time 11.7 min.

*4.1.9.5. 6-(4-(4-Bromophenyl)isoxazol-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**7e**).* Yield 91.3%; White solid; mp 205–206 °C; IR (KBr, cm^{-1}): 3448, 1690, 1490, 1388, 812; ^{1}H NMR (DMSO-d_6): δ 10.85 (1H, bs, –NH), 8.85 (1H, s, ArH), 7.64 (2H, d, J = 8.4 Hz, ArH), 7.39 (2H, d, J = 8.4 Hz, ArH), 7.10–7.00 (3H, m, ArH), 4.67 (2H, s, OCH_2); *m/z* (CI) 369 (M-2, 100%); HPLC 97.5%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: acetonitrile, gradient (T/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 230 nm, retention time 12.1 min.

*4.1.9.6. 6-(4-Phenylisoxazol-3-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**7f**).* Yield 87%; White solid; mp 166–167 °C; IR (KBr, cm^{-1}): 3134, 1700, 1382, 763, 689; ^{1}H NMR (DMSO-d_6): δ 10.85 (1H, bs, –NH), 8.90 (1H, s, ArH), 7.55–7.35 (5H, m, ArH), 7.20 (1H, s, ArH), 7.15–7.00 (2H, m, ArH), 4.70 (2H, s, OCH_2); *m/z* (CI) 291 (M-1, 100%); HPLC 99.2%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: acetonitrile, gradient (T/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 230 nm, retention time 10.9 min.

*4.1.10. Preparation of imidazole derivatives (**9**)*

*4.1.10.1. Synthesis of 1-(3-oxo-3,4-dihydro-2H-benzo [1,4]oxazin-6-yl)-2-phenyl-ethane-1,2-dione (**8**).* To a suspension of 6-(3-dimethyl amino-2-phenyl acronyl)-4H-benzo{1,4}-oxazine-3-one (1.5 g, 0.0046 mol) in a mixture of THF and H_2O (9:1) was added $NaIO_4$ (3.98 g, 0.018 mol) portion wise for a duration of 15 min at room temp. After completion of addition, the mixture was allowed to stir for 2 h. After completion of the reaction (indicated by TLC/1:1 EtOAc–hexane) the solid separated was filtered and washed with ethyl acetate (2 × 10 mL). The filtrate was collected and treated with cold water (15 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography using EtOAc–hexane (1:1) to give the desired product (yield 77%); ^{1}H NMR (DMSO-d_6): δ 10.80 (1H, bs, –NH), 7.93 (2H, d, J = 7.0 Hz, ArH), 7.85–7.75 (1H, m, ArH), 7.70–7.60 (3H, m, ArH), 7.50 (1H, d, J = 2.0 Hz, ArH), 7.15 (1H, d, J = 8.4 Hz, ArH), 4.86 (2H, s, OCH_2); *m/z* (CI) 280 (M-1, 100%).

4.1.11. Typical procedure for the synthesis of imidazole derivatives

To a solution of 1-(3-oxo-3,4-dihydro-2H-benzo [1,4]oxazin-6-yl)-2-phenyl-ethane-1,2-dione (0.1 g, 0.0003 mol) in acetic acid (10 mL) was added ammonium acetate (0.23 g, 0.003 mol) followed by an appropriate aldehyde (0.0003 mol) slowly. The mixture was heated to reflux for 2 h. After completion of the reaction the mixture was poured into crushed ice (20 g) and extracted with

CH_2Cl_2 (3 × 20 mL). The organic layers were collected, combined, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography using EtOAc–hexane (3:2) to give desired product.

*4.1.11.1. 6-(2,4-Diphenyl-1H-imidazol-5-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**9a**).* Yield 86%; white solid; mp 158–159 °C; IR (KBr, cm^{-1}): 3448, 1684, 1490, 1380, 775, 697; ^1H NMR (DMSO-d_6): δ 12.60 (1H, bs, NH), 10.70 (1H, bs, NH), 8.05 (2H, d, J = 7.2 Hz, ArH), 7.65–6.80 (11H, m, ArH), 4.60 (1H, s, OCH_2), 4.56 (1H, s, OCH_2); m/z (CI) 368 (M + 1, 100%); HPLC 98.4%, column: Intersil ODS-3 C-18 (150 × 4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: acetonitrile, gradient (T/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 230 nm, retention time 7.4 min.

*4.1.11.2. 6-(2-(2,4-Dichlorophenyl)-4-phenyl-1H-imidazol-5-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**9b**).* Yield 80%; white solid; mp 154–156 °C; IR (KBr, cm^{-1}): 3421, 2925, 1696, 1490, 1388, 787, 698; ^1H NMR (DMSO-d_6): δ 12.50 (bs, 1H, –NH), 10.80 (1H, bs, –NH), 7.25–7.75 (8H, m, ArH), 7.15 (1H, s, ArH), 6.90–7.10 (2H, m, ArH), 4.62 (2H, s, OCH_2); m/z (CI) 436 (M + 1, 100%); HPLC 97.6%, column: Intersil ODS C-18 (150 × 4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: acetonitrile, gradient (T/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 215 nm, retention time 7.7 min.

*4.1.11.3. 6-(4-Phenyl-2-(4-(trifluoromethyl)phenyl)-1H-imidazol-5-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**9c**).* Yield 85%; white solid; mp 164–166 °C; IR (KBr, cm^{-1}): 3255, 1685, 1327, 1124, 1066, 697; ^1H NMR (DMSO-d_6): δ 12.95 (1H, bs, NH), 10.75 (1H, d, NH), 8.30 (2H, d, J = 8.1 Hz, ArH), 7.85 (2H, d, J = 8.2 Hz, ArH), 7.65–7.20 (6H, m, ArH), 7.15–6.80 (2H, m, ArH), 4.65 (1H, s, OCH_2), 4.55 (1H, s, OCH_2); m/z (CI) 434 (M-1, 100%); HPLC 99.3%, column: Intersil ODS-3 C-18 (150 × 4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: acetonitrile, gradient (T/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 230 nm, retention time 8.7 min.

*4.1.11.4. 6-(2-(4-Methoxyphenyl)-4-phenyl-1H-imidazol-5-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**9d**).* Yield 85%; white solid; mp 134–135 °C; IR (KBr, cm^{-1}): 3447, 2923, 1654, 1458, 419; ^1H NMR (DMSO-d_6): δ 12.50 (bs, 1H, –NH), 10.75 (1H, bs, –NH), 8.00 (1H, d, J = 8.7 Hz, ArH), 7.65–7.20 (6H, m, ArH), 7.10–6.80 (4H, m, ArH), 4.60 (2H, s, OCH_2), 3.85 (3H, s, OCH_3); m/z (CI) 398 (M + 1, 100%); HPLC 97.4%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: acetonitrile, gradient (T/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 215 nm, retention time 7.5 min.

*4.1.11.5. 6-(2-(4-Fluorophenyl)-4-phenyl-1H-imidazol-5-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**9e**).* Yield 87%; white solid. mp 130–131 °C; IR (KBr, cm^{-1}): 3186, 1693, 1498, 1409, 692, 509. ^1H NMR (DMSO-d_6): δ 12.65 (1H, bs, NH), 10.70 (1H, bs, NH), 8.20–8.05 (2H, m, ArH), 7.65–7.20 (8H, m, ArH), 7.10–6.80 (2H, m, ArH), 4.65 (1H, s, OCH_2), 4.55 (1H, s, OCH_2); m/z (CI) 384 (M-1, 100%); HPLC 98.7%, column: Intersil ODS-3 C-18 (150 × 4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: acetonitrile, gradient (T/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 230 nm, retention time 7.6 min.

*4.1.11.6. 6-(4-Phenyl-2-(1H-pyrrolo [2,3-b]pyridin-3-yl)-1H-imidazol-5-yl)-2H-benzo[b][1,4]oxazin-3(4H)-one (**9f**).* Yield 83%; white solid; mp 201–202 °C; IR (KBr, cm^{-1}): 3198, 1695, 1492, 1405, 771, 698, 521; ^1H NMR (DMSO-d_6): δ 12.10 (1H, bs, –NH), 10.70 (1H, s, –NH), 8.78 (1H, d, J = 7.6 Hz, ArH), 8.40 (1H, bs, NH), 8.13 (1H, s, ArH), 7.57 (2H, d, J = 6.7 Hz, ArH), 7.45–6.90 (8H, m, ArH), 4.60 (2H, s, OCH_2); m/z (CI) 408 (M + 1, 100%); HPLC 98.5%, column: Intersil C-18 (150 × 4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: acetonitrile, gradient (T/%B): 90/10, 10/90, 10/90, 90/10, flow rate: 1.0 mL/min, UV 230 nm, retention time 7.4 min.

4.2. Pharmacology: In vitro cell proliferation assay

A549 (NSCLC), DLD-1 (colorectal adenocarcinoma) and MV4-11 (acute myeloid leukemia) cells were obtained from ATCC and grown as recommended. The effect of test compounds on cell viability was measured using a colorimetric MTT assay after 3 days of treatment in culture medium containing 5% FBS. A549 and DLD-1 cells were seeded in a 96 well plate at a density of 5000 cells per well (in 100 μl medium) the day before treatment. MV4-11 cells were seeded at 40,000 cells per well immediately before treatment. All the test compounds were prepared in DMSO as 20 mM stock solutions and diluted in RPMI as 20X solutions to achieve the desired final concentrations up to a maximum of 20 μM, with 0.1% v/v DMSO. At the end of treatment, cells were incubated with 0.5 mg/mL 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide for 4 h, followed by solubilization of the formazan crystals with 100 μl 10% SDS in 10 mM HCl. Absorbance at 595 nm was measured and the percentage of cell viability was calculated with respect to control cells grown in the presence of 0.1% v/v DMSO. Experiments were performed twice with triplicate data points.

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