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Synthesis of novel 1,4-benzoxazin-3-one derivatives as inhibitors against tyrosine kinases

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ABSTRACT

We designed and synthesized a novel 1,4-benzoxazin-3-one derivative **4** which would have inhibitory activities against tyrosine kinases. They could be synthesized easily from various carboxylic acids **10** and commercially available amines using TFP resin without purification. In this article, we will report the design and synthesis of a novel 1,4-benzoxazin-3-one chemical library **4** and the inhibitory activities against KDR and ABL which are closely related to chronic diseases such as cancer.

1. Introduction

Protein kinases play a very important role in the cell signaling process, affecting cell proliferation, differentiation, apoptosis and secretion.[1] Indolinone derivatives, SU5416 (semaxanib, **1**) and SU11248 (sunitinib malate, **2**) are reported to be potent inhibitors against vascular endothelial growth factor receptor 2 tyrosine kinase (KDR).[2,3] SU11248 shows especially potent antiangiogenic effects in vivo, by introducing a substituent group in R^2 to increase aqueous solubility. This compound inhibits other tyrosine kinases as well as the KDR, so the structure of indolinone is thought to be a good framework as a kinase inhibitor. On the other hand, 1,4-benzoxazin-3-one derivatives **3**, having a similar structure to these indolinone derivatives, are also reported in a patent to be anti-proliferative agents due to inhibition against tyrosine kinases.[4] However, 1,4-benzoxazin-3-one derivatives, which have a hydrophilic group like an amide moiety of SU11248 (**2**), have not been synthesized. Thus, we designed and synthesized novel 1,4-benzoxazin-3-one derivatives **4** which would have inhibitory activities against tyrosine kinases. They could be synthesized easily from various carboxylic acids **10** and commercially available amines **12** using TFP resin without purification[5] (Fig. 1).

2. Chemistry

Syntheses of novel 1,4-benzoxazin-3-one derivative chemical library **4** are illustrated in Scheme 1. The Knoevenagel reaction of 1,4-benzoxazin-3-one **5** with various haloarylcarboxamide **6a–i** afforded the 2-bromoarylyliden-1,4-benzoxazin-3-one **7a–i**. The palladium catalyzed C–C bond formation of various bromides **7a–i** with *tert*-butyl acrylate gave acrylate **8a–i** in good yields except in the case using **7b**. The acrylate **7b**, which was derived from bromides **6b**, did not react with *tert*-butyl acrylate in this condition. So in order to obtain the desired acrylate **8b**, the reaction of 1,4-benzoxazin-3-one **5** with aldehyde **13**, which was derived from *m*-bromobenzaldehyde **6b**, was investigated (Scheme 2). Fortunately, the desired acrylate **8b** was obtained, although in low yield. The N-alkylated acrylates **9** were prepared from various **8a–i** by usual methods (alkylhalide, NaH, DMF), and the *tert*-butyl group of acrylates **8** and **9** were removed by the hydrogen chloride solution of 1,4-dioxane. The loading TFP resin with acid **10a–i** were carried out following the reported procedure.[5] The reaction of the acrylated TFP resin **11a–i** with various amines **12a–m** afforded the desired amides in good to moderate yield without purification to achieve the preparation of the chemical library consisting of various 1,4-benzoxazin-3-one derivatives **4** (Fig. 2).

The double bond geometries of tri-substituted alkenes of various 1,4-benzoxazin-3-one derivatives **4** were determined by measurement of the coupling constants J_{C-H} between H1 and C1 on non-decoupling ^{13}C NMR of compounds **4a–d** and, **6f** and **6g**

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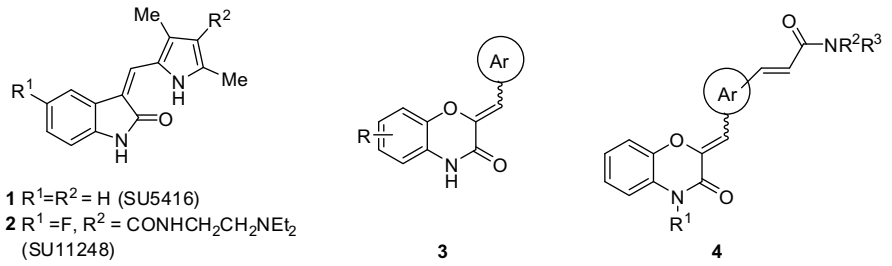
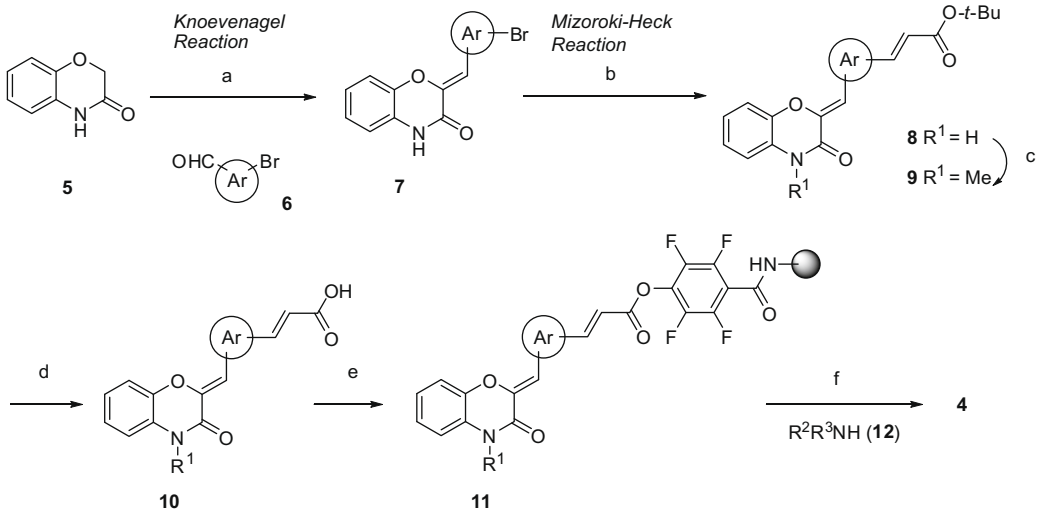
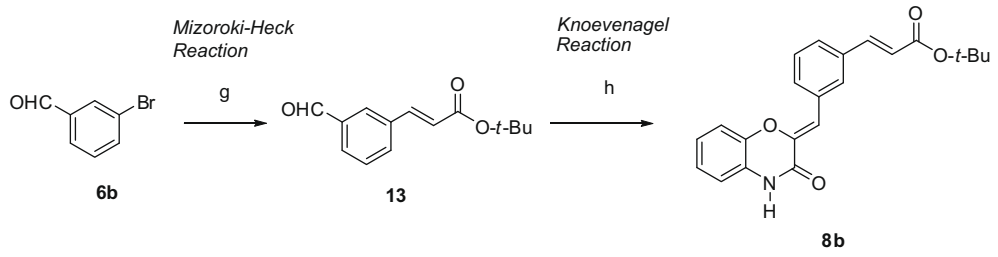


Figure 1. Structures of indolinone derivatives **1** (SU5416) and **2** (SU11248), and 1,4-benzoxazin-3-one derivatives **3** and **4**.



Scheme 1. Reagents and conditions: (a) Ac_2O/Et_3N (2/1), reflux, y. 7–30%; (b) *tert*-butyl acrylate (5 equiv), 10 mol % $Pd(OAc)_2$, 20 mol% P(*o*-tol)$_3$, *i*-Pr_2EtN (10 equiv), DMF, 100 °C, y. 43–94%; (c) R^1–X, NaH, DMF, rt, y. 59–88%; (d) 4 M HCl–1,4-dioxane, rt, y. 72–100%; (e) TFP resin, DIC, cat. DMAP, Et_3N, DMF, rt, percent loading 48–83%; (f) R^2R^3NH, DMF, rt–80°C, y. 9–98%.



Scheme 2. Reagents and conditions: (g) *tert*-butyl acrylate (5 equiv), 10 mol % $Pd(OAc)_2$, 20 mol % P(*o*-tol)$_3$, *i*-Pr_2EtN (10 equiv), 100 °C, y. 74% (h) Ac_2O/Et_3N (2/1), reflux, y. 18%.

(Fig. 3).[6] Based on these results, it was supposed that the tri-substituted alkenes of all compounds were *Z* configuration (Table 1). Moreover, X-ray crystal structure analysis of **4l** revealed that the tri-substituted alkene was of *Z* configuration in its structure, and the S–O distance (2.81 Å) were shorter than the sum of van der Waals radii of sulfur and oxygen (3.32 Å).[7] The result suggested that the intramolecular S–O non-bonded interaction was found in **4l** and it had a stable conformation with the pseudo 5-membered ring (Fig. 4).[8,9]

3. Results and discussion

The evaluation of kinase inhibitory activities of novel 1,4-benzoxazin-3-one derivative chemical library **4** was carried out at 4 μg/ml concentrations of compounds using commercially available kinase assay kits.[10] Their inhibitory activities against KDR and ABL are illustrated in Figure 5. The average of inhibitory activities of **4** against KDR was relatively high. In particular, the derivatives synthesized from carboxylic acid **10i** gave good results, including **4l** which had an IC_{50} value of 1.48 μM (Table 2). On the other hand, though the average of inhibitory activities against ABL of **4** was not so high, the derivatives synthesized from carboxylic acid **10e** and **10i** showed good efficacy for ABL (Table 2), including **4r** (IC_{50} 0.29 μM) which was the most potent ABL inhibitor of all, while **4l** showed potent inhibitory activity against not only KDR (IC_{50} 1.48 μM) but also ABL (IC_{50} 1.90 μM). The X-ray co-crystal structure of indolinone derivative SU5402 and FGFR1, which has high homology with KDR and ABL for amino acid sequences of the ATP binding site, revealed that the amide moiety of indolinone was a hinge binder confirming the potent inhibitory activity.[11] So the amide moiety of indolinone was thought to bind to the hinge region of KDR. However the results of inhibitory activities against

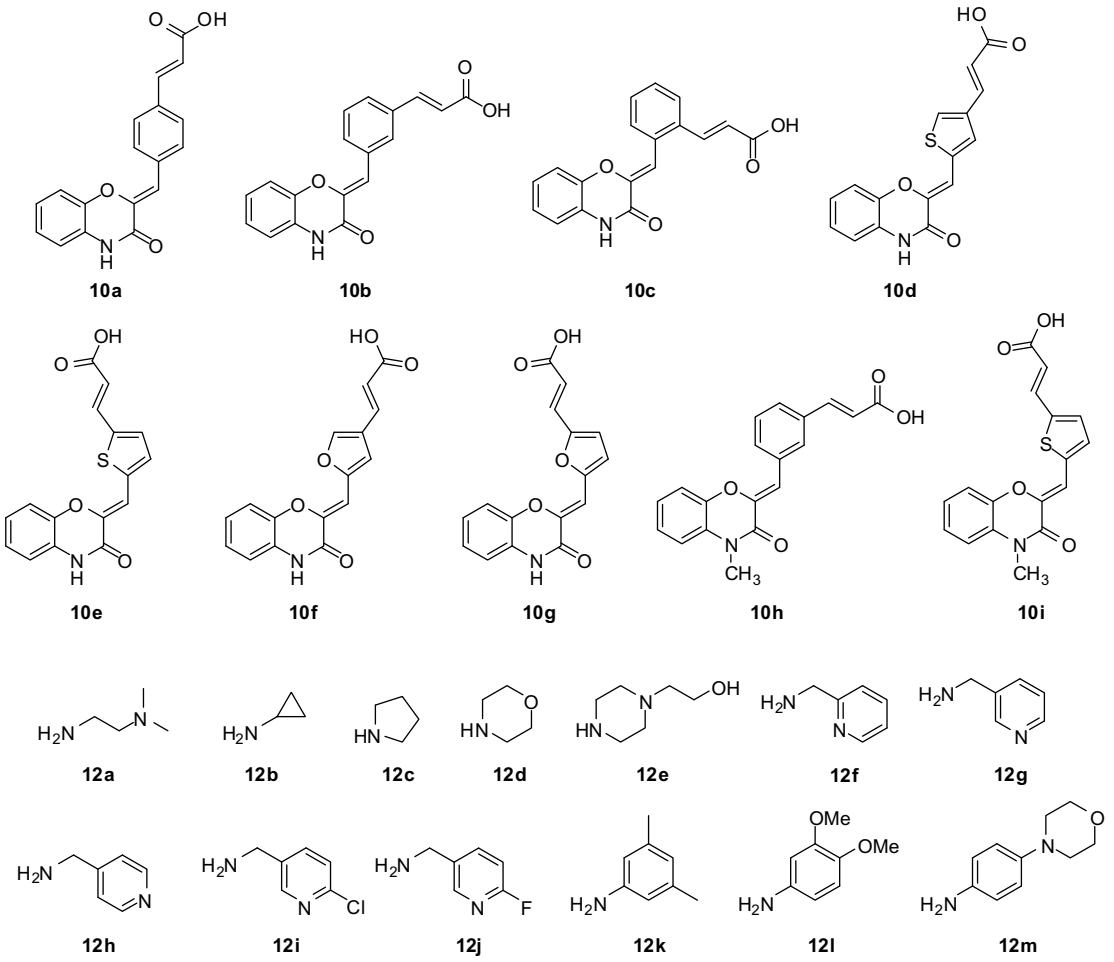


Figure 2. Structures of acid **10a–i** and amines **12a–m**.

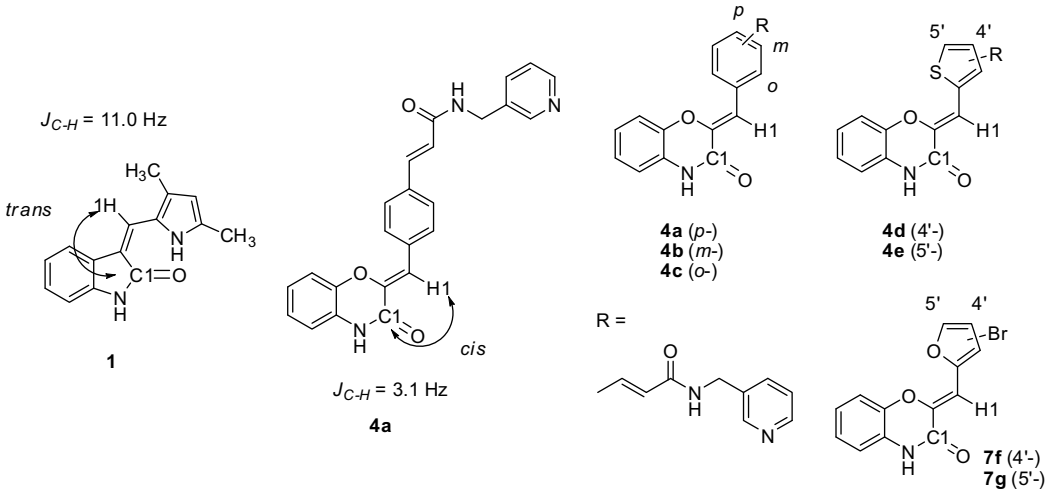


Figure 3. The coupling constants J_{C-H} between H1 and C1 of compounds **4a–d**, **7f** and **7g**.

KDR or ABL of **4l** or **4r**, which are *N*-methyl derivatives of the amide moiety of 1,4-benzoxazin-3-one, suggest that their interactions at the ATP binding site are different from that of indolinone derivatives. Figure 7 shows a docking model of compound **4r** at the ATP binding site of ABL.[12] In this proposed binding mode, 1,4-benzoxazin-3-one core of compound **4r** forms a hydrogen bond with residue Thr315 (Gatekeeper) (Table 3).

4. Conclusion

In summary, we synthesized 1,4-benzoxazin-3-one derivative chemical library **4** using TFP resin without purification and evaluated the inhibitory activities against KDR and ABL. The results supposed that a 1,4-benzoxazin-3-one framework was favorable structure for inhibiting KDR. Compound **4l** showed relatively

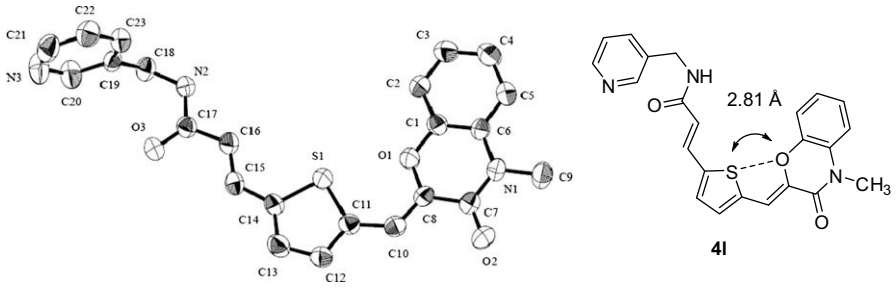


Figure 4. ORTEP drawing and structure of compound **4l**.

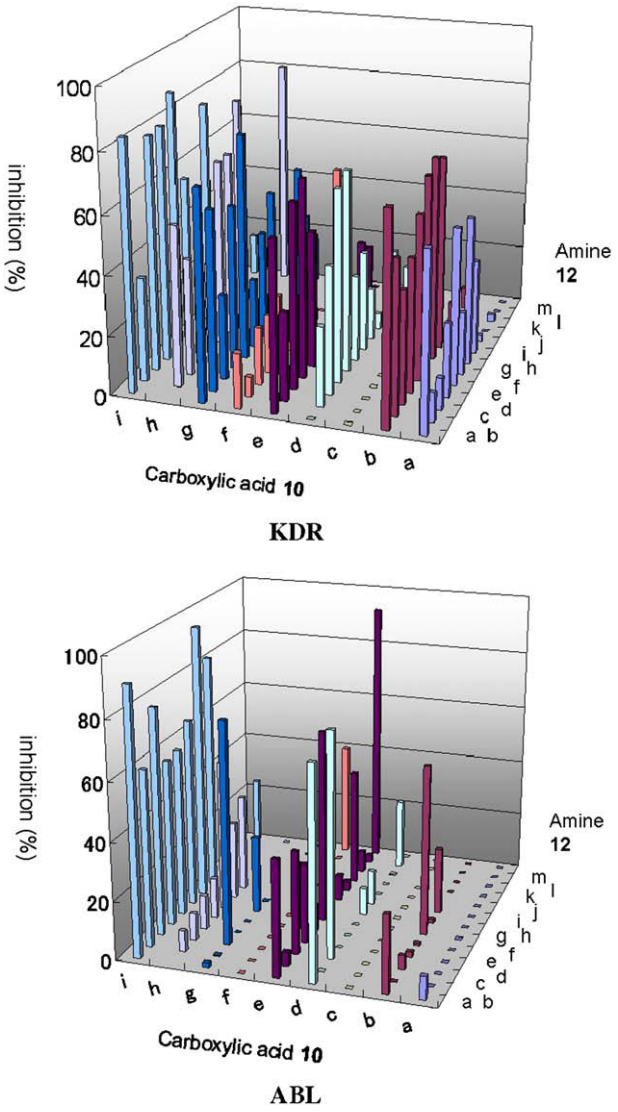


Figure 5. Kinase inhibitory activities of 1,4-benzoxazin-3-one chemical library **4**.

Table 1
The $J_{C\text{-}H}$ between H1 and C1 of compounds **4a**-**d**, **7f** and **7g**

Compd	$J_{C\text{–}H}$ (Hz)	*E*-/*Z*-
SU5416 (**1**)	11.0	*E*-
4a	3.1	*Z*-
4b	3.6	*Z*-
4c	3.0	*Z*-
4d	3.5	*Z*-
4e	3.0	*Z*-
7f	3.5	*Z*-
7g	3.3	*Z*-

Table 2
Inhibitory activities against KDR of selected compounds from library **4**[a]

Compd	Carboxylic acid	Amine	% Inhibition @ 4 g/ml	IC_{50} (M)
4f	**10b**	**12a**	70.8	N.D.[b]
4g	**10g**	**12e**	76.7	N.D.[b]
4h	**10h**	**12m**	79.9	N.D.[b]
4i	**10i**	**12a**	83.9	N.D.[b]
4j	**10i**	**12c**	79.2	N.D.[b]
4k	**10i**	**12e**	88.3	N.D.[b]
4l	**10i**	**12g**	76.7	1.48
4m	**10i**	**12h**	77.1	N.D.[b]

[a] Inhibitory activities were measured with a kinase assay development kit purchased from CARNA BIOSCIENCE Co. Ltd.
[b] IC_{50} value (M) of compound was more than 0.4 g/ml.

potent inhibitory activities against both KDR and ABL and the X-ray crystal structure analysis of **4l** revealed that the S–O non-bonded interaction was found in its structure and had the conformation stabilized by the psuedo 5-membered ring. On the other hand this framework is not so favorable as a ABL inhibitor, but the IC_{50} against ABL of compound **4r** was 0.29 μM, so it would be an ABL inhibitor as potent as Gleevec™ in in vitro assay (Fig. 6).[13]

5. Experimental

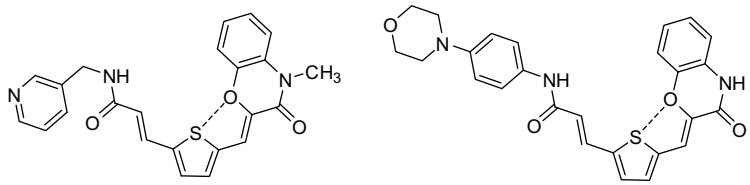
5.1. Synthesis

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. ^{1}H NMR spectra were recorded on a JEOL GSX400 or on a JEOL JMTC-300/54/SS spectrometer at ambient temperature. Chemical shifts are reported as ppm downfield from the tetramethylsilane (TMS). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants and number of protons.

5.1.1. (*Z*)-2-(4-Bromobenzylidene)-4*H*-benzo[*b*][1,4]oxazin-3-one (7a)

To a mixture of 2*H*-1,4-benzoxazin-3(4*H*)-one **5** (12.2 g, 82.1 mmol) and 4-bromobenzaldehyde **6a** (22.0 g, 120 mmol), acetic anhydride (50 ml) and triethylamine (25 ml) were added, and the reaction mixture was stirred at 110 °C for 5 h. The mixture was cooled to rt and stirred for an additional 12 h. The mixture was poured into aqueous 1.0 M NaOH solution with ice and then the precipitate was collected by filtration. The precipitate was washed with water, acetonitrile and AcOEt, and dried in vacuo to give the desired product (**7a**) (7.90 g, 24.9 mmol, y. 30 %).

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.18 (s, 1H), 7.85 (d, J = 8.8 Hz, 2H), 7.62 (d, J = 8.6 Hz, 2H), 7.31–7.28 (m, 1H), 7.07–7.03 (m, 2H), 7.01–6.98 (m, 1H), 6.76 (s, 1H); IR (cm^{-1}, KBr) 3025, 2978, 2895, 2864, 2767, 1687, 1638, 1598, 1586, 1394, 1286, 1267, 1234, 1112, 1072, 1008, 928, 815, 748; HRMS (EI) calcd for $C_{15}H_{10}BrNO_2$ 314.989, found 314.990.



4l **4r**

Figure 6. Structure of 1,4-benzoxazin-3-ones **4l** and **4r**.

Table 3
Inhibitory activities against ABL of selected compounds from library **4**[a]

Compd	Carboxylic acid	Amine	% Inhibition @ 4 μg/ml	IC_{50} (μM)
4i	**10i**	**12a**	90.8	0.82
4l	**10i**	**12g**	76.7	1.90
4m	**10i**	**12h**	82.6	1.76
4n	**10b**	**12f**	74.8	N.D.[b]
4o	**10d**	**12a**	72.3	N.D.[b]
4p	**10d**	**12c**	76.5	N.D.[b]
4q	**10e**	**12f**	90.7	N.D.[b]
4r	**10e**	**12m**	92.6	0.29
4s	**10g**	**12c**	81.1	N.D.[b]

[a] Inhibitory activities were measured with a kinase assay development kit purchased from CARNA BIOSCIENCE Co. Ltd.
[b] IC_{50} value (μM) of compound was more than 0.4 μg/ml.

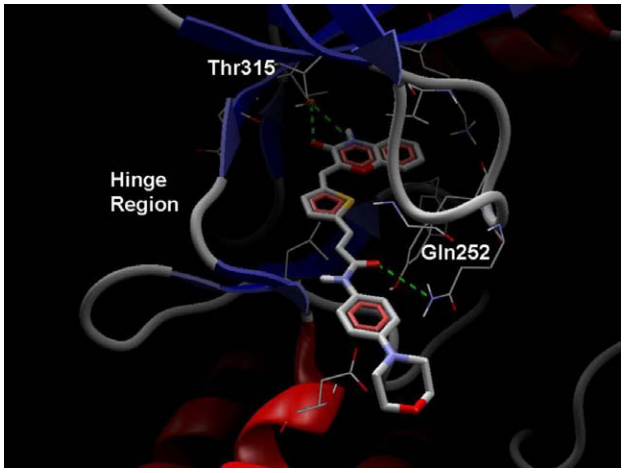


Figure 7. Compound **4r** docked at the ATP binding site of ABL. The protein structure is taken from Brookhaven Protein Data Bank, entry 1M52. Hydrogen bonds are shown as green dotted lines.

5.1.2. (*Z*)-2-(3-Bromobenzylidene)-4*H*-benzo[*b*][1,4]oxazin-3-one (7b)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.23 (s, 1H), 8.04 (s, 1H), 7.94 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 8.8 Hz, 1 H), 7.40 (t, J = 7.9 Hz, 1H), 7.26–7.23 (m, 1H), 7.08–7.04 (m, 2H), 7.01–6.98 (m, 1H), 6.77 (s, 1H); IR (cm^{-1}, KBr) 3180, 3148, 3084, 3054, 2977, 2907, 1694, 1640, 1501, 1389, 1236, 1111, 886, 781, 745; HRMS (EI) calcd for $C_{15}H_{10}BrNO_2$ 314.99, found 314.99.

5.1.3. (*Z*)-2-(2-Bromobenzylidene)-4*H*-benzo[*b*][1,4]oxazin-3-one (7c)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.31 (s, 1H), 8.29 (dd, J = 8.0, 1.6 Hz, 1H), 7.73 (dd, J = 8.0, 1.2 Hz, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.26 (dd, J = 14.9, 9.2 Hz, 2H), 7.07 (s, 1H), 7.06–6.98 (m, 3H); IR (cm^{-1}, KBr) 3032, 2977, 2896, 2858, 2769, 1688, 1638, 1501, 1466, 1437, 1397, 1268, 1232, 1113, 752; HRMS (EI) calcd for $C_{15}H_{10}BrNO_2$ 314.989, found 314.990.

5.1.4. (*Z*)-2-(4-Bromothiophen-2-ylmethylene)-4*H*-benzo[*b*][1,4]oxazin-3-one (7d)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.20 (s, 1H), 7.83 (s, 1H), 7.51 (s, 1H), 7.25–7.22 (m, 1H), 7.11 (s, 1H), 7.08–7.04 (m, 2H), 7.02–6.98 (m, 1H); IR (cm^{-1}, KBr) 3032, 2980, 2900, 1683, 1637, 1500, 1419, 1389, 1346, 1266, 1235, 1107, 871, 746; HRMS(EI) calcd for $C_{13}H_8BrNO_2S$ 320.946, found 320.945.

5.1.5. (*Z*)-2-(5-Bromothiophen-2-ylmethylene)-4*H*-benzo[*b*][1,4]oxazin-3-one (7e)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.16 (s, 1H), 7.32 (d, J = 3.9 Hz, 1H), 7.28 (d, J = 3.9 Hz, 1H), 7.10 (s, 1H), 7.07–7.03 (m, 2H), 7.01–6.97 (m, 1H); IR (cm^{-1}, KBr) 3036, 2980, 2909, 1682, 1635, 1501, 1427, 1234, 1335, 1266, 1234, 1104, 781, 742; HRMS (EI) calcd for $C_{13}H_8BrNO_2S$ 320.946, found 320.946.

5.1.6. (*Z*)-2-(4-Bromofuran-2-ylmethylene)-4*H*-benzo[*b*][1,4]oxazin-3-one (7f)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.25 (s, 1H), 8.03 (s, 1H), 7.47–7.44 (m, 1H), 7.24 (s, 1H), 7.07–7.03 (m, 2H), 7.01–6.97 (m, 1H), 6.64 (s, 1H); IR (cm^{-1}, KBr) 3032, 2981, 2898, 1686, 1636, 1501, 1389, 1350, 1268, 1230, 921, 746; HRMS (EI) calcd for $C_{13}H_8BrNO_3$ 304.9688, found 304.9688.

5.1.7. (*Z*)-2-(5-Bromofuran-2-ylmethylene)-4*H*-benzo[*b*][1,4]oxazin-3-one (7g)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.23 (s, 1H), 7.36–7.33 (m, 1H), 7.14 (d, J = 3.5 Hz, 1H), 7.06–7.02 (m, 2H), 7.00–6.96 (m, 1H), 6.80 (d, J = 3.5 Hz, 1H), 6.64 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 156.26, 140.95, 140.49, 138.15, 130.99, 130.88, 126.02, 124.30, 123.85, 116.33, 116.03, 115.02, 105.89; IR (cm^{-1}, KBr) 3032, 2978, 2909, 2864, 1685, 1632, 1500, 1479, 1387, 1266, 1233, 1212, 1189, 917, 788, 745; HRMS (EI) calcd for $C_{13}H_8BrNO_3$ 304.969, found 304.982.

5.1.8. *tert*-Butyl (*E*)-3-(4-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)phenyl) acrylate (8a)

To a mixture of **7a** (7.88 g, 24.9 mmol), palladium acetate (510 mg, 2.28 mmol) and tri(*o*-tryl)phosphine (1.36 g, 4.47 mmol), *N*,*N*-dimethylformamide (120 ml), diisopropylethylamine (36 ml, 207 mmol) and *tert*-butyl acrylate (15.0 ml, 102 mmol) were added under nitrogen atmosphere. The mixture was stirred at 100 °C over night. The reaction mixture was cooled to rt and filtered through Celite with AcOEt. The filtrate was washed with brine and dried over anhydrous sodium sulfate. After concentration in vacuo the resultant was collected by filtration with hexane–AcOEt (1:1) and washed with AcOEt to give the desired product (**8a**) (7.88 g, 21.7 mmol, y. 87%).

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.77 (s, 1H), 7.87 (d, J = 8.3 Hz, 2H), 7.60 (d, J = 15.2 Hz, 1H), 7.56 (d, J = 8.1 Hz, 2H), 7.26 (s, 1H), 7.21–7.19 (m, 1H), 7.09–7.04 (m, 2H), 6.95 (s, 1H), 6.91–6.88 (m,

1H), 6.41 (d, J = 15.8 Hz, 1H), 1.53 (s, 9H); IR (cm^{-1}, KBr) 3033, 2974, 2914, 2866, 2765, 1715, 1685, 1634, 1502, 1386, 1365, 1332, 1320, 1281, 1146, 1109, 870, 827, 751; HRMS (EI) calcd for $C_{22}H_{21}NO_4$ 363.147, found 363.146.

5.1.9. *tert*-Butyl (*E*)-3-(2-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)phenyl) acrylate (8c)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.13 (s, 1H), 7.93 (d, J = 6.1 Hz, 1H), 7.91 (d, J = 10.3 Hz, 1H), 7.61 (d, J = 6.8 Hz, 1H), 7.43 (t, J = 7.4 Hz, 1H), 7.34 (t, J = 7.3 Hz, 1H), 7.19 (s, 1H), 7.04–6.99 (m, 3H), 6.87–6.82 (m, 1H), 6.31 (d, J = 15.8 Hz, 1H), 1.49 (s, 9H); IR (cm^{-1}, KBr) 3438, 3047, 2980, 2920, 1702, 1686, 1632, 1502, 1389, 1292, 1268, 1237, 1161, 1112, 977, 763, 741; HRMS (EI) calcd for $C_{22}H_{21}NO_4$ 363.147, found 363.147.

5.1.10. *tert*-Butyl (*E*)-3-(5-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl) thiophen-3-yl)acrylate (8d)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.18 (s, 1H), 8.11 (s, 1H), 7.83 (s, 1H), 7.53 (d, J = 15.8 Hz, 1H), 7.27–7.24 (m, 1H), 7.09–7.04 (m, 2H), 7.05 (s, 1H), 7.02–6.98 (m, 1H), 6.37 (d, J = 15.8 Hz, 1H), 1.48 (s, 9H); IR (cm^{-1}, KBr) 3423, 3040, 2979, 2912, 2864, 1702, 1631, 1503, 1439, 1368, 1312, 1282, 1151, 979, 859, 746; HRMS (EI) calcd for $C_{20}H_{19}NO_4S$ 369.1035, found 369.1033.

5.1.11. *tert*-Butyl (*E*)-3-(5-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl) thiophen-2-yl) acrylate (8e)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.23 (s, 1H), 7.72 (d, J = 15.8 Hz, 1H), 7.52 (d, J = 3.9 Hz, 1H), 7.45 (d, J = 3.9 Hz, 1H), 7.45–7.43 (m, 1H), 7.12 (s, 1H), 7.09–7.05 (m, 2H), 7.03–6.99 (m, 1H), 6.33 (d, J = 15.6 Hz, 1H), 1.49 (s, 9H); IR (cm^{-1}, KBr) 3036, 2979, 2913, 2861, 1688, 1632, 1501, 1440, 1386, 1154, 967, 844, 745; HRMS (EI) calcd for $C_{20}H_{19}NO_4S$ 363.14706, found 369.10297.

5.1.12. *tert*-Butyl (*E*)-3-(5-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl)furan-3-yl)acrylate (8f)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.24 (s, 1H), 8.18 (s, 1H), 7.64–7.61 (m, 1H), 7.47 (d, J = 15.8 Hz, 1H), 7.46 (s, 1H), 7.08–6.97 (m, 3H), 6.63 (s, 1H), 6.55 (d, J = 16.0 Hz, 1H), 1.49 (s, 9H); IR (cm^{-1}, KBr) 3033, 2979, 2909, 2861, 1686, 1638, 1501, 1395, 1314, 1139, 975, 924, 815, 749; HRMS (EI) calcd for $C_{20}H_{19}NO_5$ 353.13, found 353.13.

5.1.13. *tert*-Butyl (*E*)-3-(5-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl) furan-2-yl)acrylate (8g)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.28 (s, 1H), 7.40–7.35 (m, 2H), 7.24 (d, J = 3.5 Hz, 1H), 7.13 (d, J = 3.5 Hz, 1H), 7.08–7.04 (m, 2H), 7.03–6.98 (m, 1H), 6.71 (s, 1H), 6.23 (d, J = 15.8 Hz, 1H), 1.48 (s, 9H); IR (cm^{-1}, KBr) 3036, 2979, 2915, 1685, 1631, 1503, 1387, 1280, 1231, 1157, 1099, 847, 797, 749; HRMS (EI) calcd for $C_{20}H_{19}NO_5$ 353.13, found 353.13.

5.1.14. *tert*-Butyl (*E*)-3-(3-formylphenyl)acrylate (13)

To a mixture of *m*-bromobenzaldehyde **6b** (35 ml, 30.0 mmol), palladium acetate (339 mg, 1.51 mmol) and tri(*o*-tolyl)phosphine (913 mg, 3.00 mmol), *N*,*N*-dimethylformamide (130 ml), diisopropylethylamine (60 ml, 345 mmol) and *tert*-butyl acrylate (20 ml, 137 mmol) were added under nitrogen atmosphere. The mixture was stirred at 110 °C over night. The reaction mixture was cooled to rt and filtered through Celite with AcOEt. The filtrate was washed with brine and dried over anhydrous sodium sulfate. After concentration in vacuo the resultant was collected by filtration with hexane–AcOEt (1:1) and washed with AcOEt to give the desired product (**13**) (5.17 g, 22.3 mmol, y. 87%).

^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.03 (s, 1H), 8.25 (s, 1H), 8.05 (d, J = 7.7 Hz, 1H), 7.92 (d, J = 7.7 Hz, 1H), 7.68–7.61 (m, 2 H), 6.67 (d, J = 16.0 Hz, 1H), 1.50 (s, 9H); IR (cm^{-1}, KBr) 3392, 2979, 2933, 2837, 2732, 1705, 1639, 1601, 1580, 1478, 1457, 1392, 1368, 1325, 1234, 1152, 980, 851, 797; HRMS (EI) calcd for $C_{14}H_{16}O_3$ 232.11, found 232.11.

5.1.15. *tert*-Butyl (*E*)-3-(3-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl)phenyl) acrylate (8b)

To a mixture of 2H-1,4-benzoxazin-3(4H)-one 5 (1.17 g, 7.91 mmol) and **13** (2.37 g, 10.2 mmol), acetic anhydride (4 ml) and triethylamine (2 ml) were added, and the reaction mixture was stirred at 120 °C for 5 h. The mixture was cooled to r.t. and stirred for an additional 12 h. The mixture was poured into aqueous 1.0 M NaOH solution with ice and then the precipitate was collected by filtration. The precipitate was washed with water, acetonitrile and AcOEt, and dried in vacuo to give the desired product (**8b**) (506 mg, 1.39 mmol, y. 18 %).

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.18 (s, 1H), 7.85 (d, J = 8.8 Hz, 2H), 7.62 (d, J = 8.6 Hz, 2H), 7.31–7.28 (m, 1H), 7.07–7.03 (m, 2H), 7.01–6.98 (m, 1H), 6.76 (s, 1H); IR (cm^{-1}, KBr) 3025, 2978, 2895, 2864, 2767, 1687, 1638, 1598, 1586, 1394, 1286, 1267, 1234, 1112, 1072, 1008, 928, 815, 748; HRMS (EI) calcd for $C_{15}H_{10}BrNO_2$ 314.989, found 314.990.

5.1.16. *tert*-Butyl (*E*)-3-((*Z*)-3-(4-methyl-3-oxo-3,4dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl) phenyl)acrylate (9h)

To a THF solution (3 ml) of **8b** (548 mg, 1.51 mmol), sodium hydride (60% oil dispersion, 90 mg, 2.25 mmol) was added and stirred rt for 15 min. methyl iodide (0.20 μl, 3.20 mmol) was added to the mixture at rt, and then the reaction mixture was stirred at rt for 1 h. The reaction mixture was diluted with AcOEt, washed with brine (2 times) and dried over anhydrous sodium sulfate. After evaporation, the residue was triturated with hexane–AcOEt (1:1), then the indissolved matter was collected by filtration and washed with AcOEt to give the desired product (**9h**) (447 mg, 1.33 mmol, y. 88%).

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.07 (s, 1H), 8.06 (d, J = 7.2 Hz, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.62 (d, J = 16.0 Hz, 1H), 7.49 (t, J = 8.1 Hz, 1H), 7.39–7.36 (m, 1H), 7.28–7.25 (m, 1H), 7.18–7.14 (m, 2H), 6.87 (s, 1H), 6.59 (d, J = 16.0 Hz, 1H), 3.42 (s, 3H), 1.50 (s, 9H); IR (cm^{-1}, KBr) 3051, 2984, 2941, 1708, 1673, 1640, 1593, 1505, 1473, 1422, 1384, 1323, 1292, 1450, 1154, 978, 857, 789, 741; HRMS (EI) calcd for $C_{23}H_{23}NO_4$ 377.16, found 377.16.

5.1.17. *tert*-Butyl (*E*)-3-(5-((*Z*)-4-methyl-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl) thiophen-2-yl)acrylate (9i)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.72 (d, J = 15.8 Hz, 1H), 7.52 (d, J = 3.9 Hz, 1H), 7.47 (d, J = 3.9 Hz, 1H), 7.29–7.25 (m, 1H), 7.21–7.13 (m, 3H), 6.34 (d, J = 15.6 Hz, 1H), 3.41 (s, 3H), 1.49 (s, 9H); IR (cm^{-1}, KBr) 1714, 1667, 1617, 1504, 1443, 1420, 1378, 1337, 1209, 1148, 1258, 1209, 1148, 962, 842, 788, 741; HRMS (EI) calcd for $C_{21}H_{21}NO_4S$ 383.12, found 383.12.

5.1.18. (*E*)-3-(4-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)phenyl)acrylic acid (10a)

To **8a** (6.80 g, 18.7 mmol), a dioxane solution of 4 M hydrogen chloride (150 ml, 600 mmol) and chloroform (50 ml) were added and stirred at rt over night. The reaction mixture was concentrated in vacuo to give the desired product (**10a**) (6.78 g, 18.7 mmol, y. quant.).

^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.43 (s, 1H), 11.21 (s, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.3 Hz, 2H), 7.61 (d, J = 16.0 Hz, 1H), 7.31 (q, J = 3.1 Hz, 1H), 7.09–7.04 (m, 2H), 7.01–6.99 (m, 1H), 6.81 (s, 1H), 6.57 (d, J = 16.0 Hz, 1H); IR (cm^{-1}, KBr) 3033, 2979, 2909, 2853, 1686, 1630, 1596, 1214, 1110, 747; HRMS (EI) calcd for $C_{18}H_{13}NO_4$ 307.300, found 307.085.

5.1.19. (*E*)-3-(3-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)phenyl)acrylic acid (10b)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.48 (s, 1H), 11.20 (s, 1H), 8.08 (s, 1H), 8.01 (d, J = 7.9 Hz, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.66 (d, J = 16.0 Hz, 1H), 7.50 (dd, J = 7.7, 7.7 Hz, 1H), 7.32 (t, J = 4.8 Hz, 1H), 7.07–7.04 (m, 2H), 7.02–6.97 (m, 1H), 6.82 (s, 1 H), 6.59 (d, J = 16.1 Hz, 1H); IR (cm^{-1}, KBr) 3040, 2979, 2905, 2861, 1688, 1632, 1503, 1437, 1390, 1281, 1227, 1110, 979, 742; HRMS (EI) calcd for $C_{18}H_{13}NO_4$ 307.08, found 307.09.

5.1.20. (*E*)-3-(2-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)phenyl)acrylic acid (10c)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.51 (s, 1H), 11.29 (s, 1H), 8.02 (d, J = 7.0 Hz, 1H), 7.85 (d, J = 15.8 Hz, 1H), 7.80 (d, J = 7.9 Hz, 1H), 7.51 (t, J = 7.1 Hz, 1H), 7.39 (t, J = 7.7 Hz, 1H), 7.12–6.98 (m, 4H), 6.98 (s, 1H), 6.46 (d, J = 15.8 Hz, 1H); IR (cm^{-1}, KBr) 3036, 2980, 2902, 1587, 1634, 1503, 1476, 1399, 1315, 1279, 1216, 1112, 746; HRMS (EI) calcd for $C_{18}H_{13}NO_4$ 307.300, found 307.085.

5.1.21. (*E*)-3-(5-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)thiophen-3-yl)acrylic acid (10d)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.17 (s, 1H), 8.09 (s, 1H), 7.82 (s, 1H), 7.56 (d, J = 16.0 Hz, 1H), 7.25–7.23 (m, 1H), 7.08–6.97 (m, 3H), 7.06 (s, 1H), 6.36 (d, J = 16.0 Hz, 1H); IR (cm^{-1}, KBr) 3033, 2979, 2913, 2861, 1685, 1632, 1502, 1387, 1281, 1233, 1104, 744; HRMS (EI) calcd for $C_{16}H_{11}NO_4S$ 313.0409, found 313.0424.

5.1.22. (*E*)-3-(5-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)thiophen-2-yl)acrylic acid (10e)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.40 (s, 1H), 11.23 (s, 1H), 7.76 (d, J = 15.6 Hz, 1H), 7.51 (d, J = 3.9 Hz, 1H), 7.46 (d, J = 3.9 Hz, 1H), 7.44–7.41 (m, 1H), 7.12 (s, 1H), 7.09–7.05 (m, 2H), 7.03–7.00 (m, 1H), 6.35 (d, J = 15.6 Hz, 1H); IR (cm^{-1}, KBr) 3021, 2470, 1680, 1631, 1502, 1403, 1351, 1301, 1258, 1232, 1110, 959, 930, 849, 804, 746; HRMS (EI) calcd for $C_{16}H_{11}NO_4S$ 313.04, found 313.04.

5.1.23. (*E*)-3-(5-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)furan-3-yl) acrylic acid (10f)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.32 (s, 1H), 11.25 (s, 1H), 8.18 (s, 1H), 7.62–7.59 (m, 1H), 7.51 (d, J = 16.0 Hz, 1H), 7.46 (s, 1H), 7.10–6.97 (m, 3H), 6.64 (s, 1H), 6.55 (d, J = 15.8 Hz, 1H); IR (cm^{-1}, KBr) 3143, 3084, 3027, 2909, 2853, 2624, 2519, 1683, 1640, 1504, 1407, 1280, 1260, 1218, 1150, 749; HRMS (EI) calcd for

5.1.24. (*E*)-3-(5-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)furan-2-yl) acrylic acid (10g)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.28 (s, 1H), 7.41 (d, J = 15.6 Hz, 1H), 7.38–7.35 (m, 1H), 7.22 (d, J = 3.7 Hz, 1H), 7.11 (d, J = 3.5 Hz, 1H), 7.08–7.05 (m, 2H), 7.02–6.98 (m, 1H), 6.71 (s, 1H), 6.26 (d, J = 15.6 Hz, 1H), 3.57 (s, 1H); IR (cm^{-1}, KBr) 3033, 2977, 2910, 2865, 2571, 1685, 1627, 1557, 1501, 1388, 1308, 1270, 1219, 1097, 1035, 965, 925, 849, 797; HRMS (EI) calcd for $C_{16}H_{11}NO_5$ 297.064, found 279.064.

5.1.25. (*E*)-3-((*Z*)-3-(4-Methyl-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl) phenyl)acrylic acid (10h)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.48 (s, 1H), 8.09 (s, 1H), 8.03 (d, J = 8.3 Hz, 1H), 7.68 (t, J = 3.9 Hz, 1H), 7.63 (s, 1H), 7.50 (t, J = 7.7 Hz, 1H), 7.39–7.36 (m, 1H), 7.28–7.25 (m, 1H), 7.21–7.12 (m, 2H), 6.87 (s, 1H), 6.59 (d, J = 16.0 Hz, 1H), 3.42 (s, 3H); IR (cm^{-1}, KBr) 3072, 2977, 2829, 2702, 2590, 1674, 1631, 1599, 1511, 1419, 1376, 1312, 1384, 978, 746; HRMS (EI) calcd for $C_{19}H_{15}NO_4$ 321.10, found 321.10.

5.1.26. (*E*)-3-(5-((*Z*)-4-Methyl-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl) thiophen-2-yl)acrylic acid (10i)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.32 (s, 1H), 7.76 (d, J = 15.8 Hz, 1H), 7.50 (dt, J = 9.8,3.1 Hz, 3H), 7.22 (tt, J = 16.2,5.4 Hz, 4H), 6.36 (d, J = 15.8 Hz, 1H), 3.41 (s, 3H); IR (cm^{-1}, KBr) 3068, 2925, 1675, 1629, 1610, 1504, 1418, 1383, 1215, 743; HRMS (EI) calcd for $C_{17}H_{13}NO_4S$ 327.06, found 327.06.

5.1.27. Polymeric TFP 4-((*E*)-3-(4-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl)phenyl) acryl)benzoate ester (11a)

Polymeric TFP ester **11c** was prepared by adding TFP resin (2.37 g, calcd 1.35 mmol/g) to a polypropylene reaction vessel at rt. The resin was swelled with *N,N*-dimethylformamide (30 ml) for 10 min with mild agitation. Acid **10a** (1.10 g, 3.50 mmol) was added to the resin mixture and agitated gently until all of the acid dissolved. 4-Dimethylaminopyridine (10 mg, 81.8 μmol) was added to the reaction. The reaction was agitated for 5 min, then *N,N*-diisopropyl carbodiimide (815 mg, 6.45 mmol) was added to the mixture. The polypropylene reaction vessel was capped and agitated over night. The resin was washed with *N,N*-dimethylformamide, THF and dichloromethane and dried in vacuo to give desired Polymeric TFP ester **11a** (3.11 g, calcd 1.01 mmol/g). Percent loading 75%.

IR (cm^{-1}, ATR) 3026, 2920, 1751, 1685, 1634, 1597, 1491, 1367, 1314, 1101, 992, 745, 698.

5.1.28. Polymeric TFP 4-((*E*)-3-(3-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl)phenyl)acryl)benzoate ester (11b)

Percent loading 83%.

5.1.29. Polymeric TFP 4-((*E*)-3-(2-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl)phenyl)acryl)benzoate ester (11c)

Percent loading 82%.

5.1.30. Polymeric TFP 4-((*E*)-3-(5-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl)thiophen-3-yl)acryl)benzoate ester (11d)

Percent loading 76%.

5.1.31. Polymeric TFP 4-((*E*)-3-(5-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl)thiophen-2-yl)acryl) benzoate ester (11e)

Percent loading 65%.

5.1.32. Polymeric TFP 4-((*E*)-3-(5-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl)furan-3-yl)acryl) benzoate ester (11f)

Percent loading 48%.

5.1.33. Polymeric TFP 4-((*E*)-3-(5-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidene-methyl)furan-2-yl)acryl) benzoate ester (11g)

Percent loading 65%.

5.1.34. Polymeric TFP 4-((*E*)-3-(3-((*Z*)-4-Methyl-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)phenyl)acryl) benzoate ester (11h)

Percent loading 74%.

5.1.35. (*E*)-3-(4-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)phenyl)-*N*-(pyridin-3-ylmethyl)acrylamide (4a)

The amide was prepared by adding TFP 4-benzoate ester **11a** (103 mg, 1.01 mmol/g) to a 5 ml polypropylene vessel. The resin was swelled with *N,N*-dimethylformamide (3 ml) for 10 min with

gentle agitation. 3-Aminomethylpyridine (16.8 mg, 155 μmol) was added, and the mixture was capped. The reaction was agitated over night. The mixture was filtered and washed with *N*,*N*-dimethylformamide and dichloromethane. The washes were collected and evaporated in vacuo. The product **4a** (45.3 mg, 114 μmol, y. 63%) was obtained as a solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.21 (s, 1H), 8.78 (t, J = 5.7 Hz, 1H), 8.58 (s, 1H), 8.52 (d, J = 4.8 Hz, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.80 (d, J = 8.3 Hz, 1H), 7.64 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 15.6 Hz, 1H), 7.46 (dd, J = 7.7, 5.1 Hz, 1H), 7.34–7.31 (m, 1H), 7.08–7.03 (m, 2H), 7.02–6.98 (m, 1H), 6.80 (s, 1H), 6.73 (d, J = 16.0 Hz, 1H), 4.46 (d, J = 5.9 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 165.19, 156.03, 147.66, 146.99, 142.36, 140.35, 128.58, 136.53, 135.57, 134.43, 134.31, 130.13, 127.86, 125.36, 123.94, 123.52, 123.14, 122.06, 115.60, 115.56, 109.79, 109.76; IR (cm^{-1}, KBr) 3285, 3192, 3140, 3052, 1678, 1647, 1322, 1591, 1551, 1503, 1422, 1378, 1336, 1319, 1223, 1110, 1033, 972, 763; HRMS (EI) calcd for $C_{24}H_{19}N_3O_3$ 397.14264, found 397.14249.

5.1.36. (*E*)-3-(3-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)phenyl)-*N*-(pyridin-3-ylmethyl)acrylamide (4b)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.20 (s, 1H), 8.77 (t, J = 6.0 Hz, 1H), 8.55 (d, J = 1.7 Hz, 1H), 8.47 (dd, J = 4.8,1.7 Hz, 1H), 8.05 (s, 1H), 7.91 (d, J = 7.5 Hz, 1H), 7.72 (d, J = 8.1 Hz, 1H), 7.58–7.47 (m, 3H), 7.40–7.32 (m, 2H), 7.07–7.04 (m, 2H), 7.02–6.98 (m, 1H), 6.80 (s, 1H), 6.74 (d, J = 15.8 Hz, 1H), 4.45 (d, J = 5.9 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.11, 156.00, 148.85, 148.07, 142.28, 140.30, 138.89, 135.19, 134.97, 133.85, 130.67, 129.27, 128.64, 127.08, 125.33, 123.48, 123.43, 123.19, 122.33, 115.74, 115.62, 109.77; IR (cm^{-1}, KBr) 3281, 3033, 2980, 2898, 2861, 1690, 1640, 1542, 1503, 1398, 1243, 1111, 1032, 966, 855, 783, 744; HRMS (EI) calcd for $C_{24}H_{19}N_3O_3$ 397.14, found 397.14.

5.1.37. (*E*)-3-(2-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)phenyl)-*N*-(pyridin-3-ylmethyl)acrylamide (4c)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.75 (t, J = 6.0 Hz, 1H), 8.52 (d, J = 1.8 Hz, 1H), 8.45 (dd, J = 4.8, 1.7 Hz, 1H), 8.04 (d, J = 7.7 Hz, 1H), 7.76 (d, J = 15.8 Hz, 1H), 7.68 (d, J = 7.9 Hz, 1H), 7.61 (d, J = 6.8 Hz, 1H), 7.45 (t, J = 7.1 Hz, 1H), 7.38–7.33 (m, 2H), 7.06 (d, J = 7.9 Hz, 1H), 6.98–6.92 (m, 3H), 6.93 (s, 1H), 6.59 (d, J = 15.6 Hz, 1H), 4.41 (d, J = 5.9 Hz, 2H); IR (cm^{-1}, KBr) 3282, 3066, 2977, 2894, 2845, 2762, 1696, 1652, 1546, 1503, 1428, 1376, 1265, 1228, 1110, 1033, 761, 743; HRMS (EI) calcd for $C_{24}H_{19}N_3O_3$ 397.14264, found 397.14243.

5.1.38. (*E*)-3-(5-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)thiophen-3-yl)-*N*-(pyridin-3-ylmethyl) acrylamide (4d)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.15 (s, 1H), 8.68 (t, J = 6.1 Hz, 1H), 8.52 (s, 1H), 8.46 (d, J = 4.2 Hz, 1H), 7.95 (s, 1H), 7.69 (t, J = 3.8 Hz, 2H), 7.45 (d, J = 15.8 Hz, 1H), 7.36 (dd, J = 7.9, 4.8 Hz, 1H), 7.25–7.22 (m, 1H), 7.11 (s, 1H), 7.07–7.05 (m, 2H), 7.01–6.97 (m, 1H), 6.50 (d, J = 15.6 Hz, 1H), 4.41 (d, J = 5.9 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ: 165.30, 155.76, 148.85, 148.12, 140.41, 140.37, 137.51, 136.79, 135.18, 132.99, 130.08, 127.19, 125.44, 123.68, 123.48, 123.26, 121.68, 115.75, 115.36, 105.16; IR (cm^{-1}, KBr) 3149, 3054, 2982, 2914, 1688, 1626, 1560, 1503, 1434, 1369, 1317, 1280, 1260, 1236, 1213, 1101, 967, 743; HRMS (EI) calcd for $C_{22}H_{17}N_3O_3S$ 403.0991, found 403.0988.

5.1.39. (*E*)-3-(5-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)thiophen-2-yl)-*N*-(pyridin-3-ylmethyl)acrylamide (4e)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.78 (s, 1H), 8.54 (d, J = 2.2 Hz, 1H), 8.48 (d, J = 4.8 Hz, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.64 (d, J = 15.4 Hz, 1H), 7.44 (d, J = 3.7 Hz, 1H), 7.40 (d, J = 3.3 Hz, 1H), 7.37 (dd, J = 7.7, 4.8 Hz, 1H), 7.25–7.21 (m, 1H), 7.10 (s, 1H), 7.08–7.01 (m, 3H), 6.57 (d, J = 15.6 Hz, 1H), 4.43 (d, J = 3.9 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 165.46, 156.31, 149.45, 148.70, 142.21, 141.13, 141.02, 137.87, 135.81, 135.47, 132.55, 131.48, 126.36, 124.33, 124.05, 123.71, 121.57, 116.60, 115.81, 105.87; IR (cm^{-1}, KBr) 3024, 2960, 2900, 2842, 2750, 2148, 1629, 1560, 1502, 1436, 1374, 1275, 1233, 1104, 849, 796, 746; HRMS (EI) calcd for $C_{22}H_{17}N_3O_3S$ 403.09906, found 403.09866.

5.1.40. (*E*)-*N*-((2-Dimethylamino)ethyl)-3-(3-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)-phenyl)acrylamide (4f)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.20 (s, 1H), 8.13 (s, 1H), 8.02 (s, 1H), 7.91 (d, J = 7.3 Hz, 1H), 7.56–7.45 (m, 3H), 7.34 (d, J = 9.2 Hz, 1H), 7.07–6.99 (m, 3H), 6.80 (s, 1H), 6.73 (d, J = 15.8 Hz, 1H), 3.33–3.31 (m, 4H), 2.24 (s, 6H).

5.1.41. (*Z*)-2-(5-((*E*)-3-(4-(2-Hydroxyethyl)piperazin-1-yl)-3-oxo-propenyl)fran-2-ylmethylene)-4*H*-benzo[*b*][1,4]oxazin-3-one (4g)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.23 (s, 1H), 7.38–7.33 (m, 2H), 7.22 (d, J = 3.7 Hz, 1H), 7.09–7.03 (m, 3H), 7.01–6.99 (m, 2H), 6.73 (s, 1H), 4.45 (t, J = 5.4 Hz, 1H), 3.65–3.50 (m, 8H), 3.31 (br s, 2H), 2.42 (t, J = 6.1 Hz, 2H).

5.1.42. (*E*)-*N*-(4-(Morpholin-4-yl)phenyl)-3-(3-((*Z*)-4-methyl-3-oxo-3,4-dihydrobenzo[*b*] [1,4]oxazin-2-ylidenemethyl)-phenyl)acrylamide (4h)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.07 (s, 1H), 8.07 (s, 1H), 7.95 (d, J = 7.5 Hz, 1H), 7.64–7.58 (m, 4H), 7.52 (t, J = 7.8 Hz, 1H), 7.42 (dd, J = 7.1, 2.5 Hz, 1H), 7.29–7.26 (m, 1H), 7.21–7.14 (m, 2H), 6.94 (d, J = 9.0 Hz, 2H), 6.87 (s, 1H), 6.86 (d, J = 15.6 Hz, 1H), 3.74 (t, J = 4.7 Hz, 4H), 3.43 (s, 3H), 3.07 (t, J = 4.7 Hz, 4H).

5.1.43. (*E*)-*N*-((2-Dimethylamino)ethyl)-3-(3-((*Z*)-4-methyl-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)-thiophen-2-yl)acrylamide (4i)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.05 (t, J = 5.5 Hz, 1H), 7.57 (d, J = 15.4 Hz, 1H), 7.46 (d, J = 3.9 Hz, 1H), 7.38 (d, J = 3.9 Hz, 1H), 7.32–7.26 (m, 2H), 7.22–7.17 (m, 3H), 6.56 (d, J = 15.6 Hz, 1H), 3.41 (s, 3H), 3.28 (q, J = 6.2 Hz, 2H), 2.35 (t, J = 6.4 Hz, 2H), 2.18 (s, 6H).

5.1.44. (*Z*)-2-(3-((*E*)-4-Methyl-3-oxo-3-(pyrrolidin-1-yl)propenyl)thiophen-2-ylmethylene)-4*H*-benzo[*b*][1,4]oxazin-3-one (4j)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.65 (d, J = 15.2 Hz, 1H), 7.49–7.47 (m, 2H), 7.46–7.43 (m, 1H), 7.29–7.25 (m, 1H), 7.22–7.14 (m, 3H), 6.78 (d, J = 15.2 Hz, 1H), 3.67 (t, J = 6.8 Hz, 2H), 3.41 (t, J = 6.6 Hz, 5H), 1.93 (dd, J = 12.9, 6.7 Hz, 2H), 1.83 (dd, J = 13.1, 6.3 Hz, 2H).

5.1.45. (*Z*)-4-Methyl-2-(3-((*E*)-3-(4-(2-hydroxyethyl)piperazin-1-yl)-3-oxo-propenyl)thiophen-2-ylmethylene)-4*H*-benzo[*b*][1,4]oxazin-3-one (4k)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.68 (d, J = 15.0 Hz, 1H), 7.50–7.46 (m, 3H), 7.29–7.25 (m, 1H), 7.22–7.17 (m, 3H), 7.05 (d, J = 15.2 Hz, 1H), 4.47 (t, J = 5.0 Hz, 1H), 3.69–3.50 (m, 8H), 3.41 (s, 3H), 2.47–2.40 (m, 4H).

5.1.46. (*E*)-3-(5-((*Z*)-4-Methyl-3-oxo-3,4-dihydrobenzo[*b*] [1,4]oxazin-2-ylidenemethyl)thiophen-2-yl)-*N*-(pyridin-3-ylmethyl)acrylamide (4l)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.59 (d, J = 2.0 Hz, 1H), 8.55 (dd, J = 4.9, 1.6 Hz, 1H), 7.80 (d, J = 15.2 Hz, 1H), 7.71 (td, J = 7.9, 2.0 Hz, 1H), 7.31–7.00 (m, 7H), 6.30 (d, J = 15.2 Hz, 1H), 6.01 (t,

J = 6.1 Hz, 1H), 4.61 (d, J = 5.9 Hz, 3H), 3.48 (d, J = 4.8 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 164.76, 155.30, 148.85, 148.13, 141.61, 140.89, 139.68, 137.21, 135.18, 130.70, 127.13, 123.80, 123.60, 123.44, 120.80, 115.33, 115.18, 106.03; IR (cm^{-1}, KBr) 3434, 3248, 3042, 1665, 1628, 1560, 1504, 1423, 1384, 1338, 1222, 745; HRMS (EI) calcd for $C_{23}H_{19}N_3O_3S$ 417.11, found 417.12.

5.1.47. (*E*)-3-(3-((*Z*)-4-Methyl-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)thiophen-2-yl)-*N*-(pyridin-4-ylmethyl)acrylamide (4m)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.75 (t, J = 6.1 Hz, 1H), 8.52 (dd, J = 4.5, 1.6 Hz, 2H), 7.65 (d, J = 15.4 Hz, 1H), 7.48 (d, J = 3.9 Hz, 1H), 7.42 (d, J = 3.9 Hz, 1H), 7.31–7.14 (m, 7H), 6.59 (d, J = 15.4 Hz, 1H), 4.44 (d, J = 6.1 Hz, 2H), 3.41 (s, 3H).

5.1.48. (*E*)-3-(3-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)phenyl)-*N*-(pyridin-2-ylmethyl)acrylamide (4n)

^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.20 (s, 1H), 8.80 (t, J = 5.9 Hz, 1H), 8.53 (d, J = 4.0 Hz, 1H), 8.07 (s, 1H), 7.91 (d, J = 8.1 Hz, 1H), 7.78 (td, J = 7.77 Hz, 1H), 7.57–7.47 (m, 3H), 7.37–7.26 (m, 3H), 7.07–6.98 (m, 3H), 6.82 (d, J = 15.6 Hz, 1H), 6.81 (s, 1H), 4.52 (d, J = 6.1 Hz, 2H).

5.1.49. (*E*)-*N*-((2-Dimethylamino)ethyl)-3-(5-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)thiophen-3-yl)acrylamide (4o)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.18 (s, 1 H), 8.01 (t, J = 5.7 Hz, 1H), 7.93 (s, 1H), 7.67 (d, J = 1.5 Hz, 1H), 7.39 (d, J = 15.6 Hz, 1H), 7.27–7.24 (m, 1H), 7.11 (s, 1H), 7.09–7.03 (m, 2H), 7.02–6.99 (m, 1H), 6.51 (d, J = 15.6 Hz, 1H), 3.26 (t, J = 6.1 Hz, 2H), 2.33 (t, J = 6.7 Hz, 2H), 2.16 (s, 6H).

5.1.50. (*Z*)-2-(4-((*E*)-3-Oxo-3-(pyrrolidin-1-yl)propenyl)-thiophen-2-ylmethylene)-4*H*-benzo[*b*][1,4]oxazin-3-one (4p)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.17 (s, 1H), 8.03 (s, 1H), 7.86 (s, 1H), 7.46 (d, J = 15.4 Hz, 1H), 7.27–7.24 (m, 1H), 7.09–7.04 (m, 2H), 7.03–6.99 (m, 1H), 6.84 (d, J = 15.4 Hz, 1H), 3.63 (t, J = 6.7 Hz, 2H), 3.39 (t, J = 6.8 Hz, 2H), 1.93 (tt, J = 6.8, 6.8 Hz, 2H), 1.81(tt, J = 6.8, 6.8 Hz, 2H).

5.1.51. (*E*)-3-(5-((*Z*)-3-Oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)thiophen-2-yl)-*N*-(pyridin-2-ylmethyl) acrylamide (4q)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.19 (s, 1H), 8.70 (t, J = 6.0 Hz, 1H), 8.53 (dq, J = 4.7, 0.9 Hz, 1H), 7.78 (td, J = 7.8, 1.8 Hz, 1H), 7.64 (d, J = 15.3 Hz, 1H), 7.46 (d, J = 3.7 Hz, 1H), 7.40 (d, J = 4.0 Hz, 1H), 7.33 (d, J = 7.9 Hz, 1H), 7.30–7.24 (m, 2H), 7.12 (s, 1H), 7.09–7.06 (m, 2H), 7.04–7.01 (m, 1H), 6.64 (d, J = 15.6 Hz, 1H), 4.50 (d, J = 5.5 Hz, 2H).

5.1.52. (*E*)-*N*-(4-(Morpholin-4-yl)phenyl)-3-(5-((*Z*)-3-oxo-3,4-dihydrobenzo[*b*][1,4]oxazin-2-ylidenemethyl)thiophen-2-yl)acrylamide (4r)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.20 (s, 1H), 10.02 (s, 1H), 7.71 (d, J = 15.3 Hz, 1H), 7.58 (d, J = 9.2 Hz, 2H), 7.48 (d, J = 3.7 Hz, 1H), 7.46–7.41 (m, 1H), 7.28–7.25 (m, 1H), 7.13 (s, 1H), 7.11–7.07 (m, 1H), 7.04–7.01 (m, 1H), 6.93 (d, J = 8.9 Hz, 2H), 6.66 (d, J = 15.3 Hz, 1H), 3.75–3.68 (m, 4H), 3.07–3.01 (m, 4H); IR (cm^{-1}, KBr) 3315, 3030, 2972, 2900, 2855, 1675, 1627, 1523, 1389, 1352, 1266, 1230, 1119; HRMS (EI) calcd for $C_{26}H_{23}N_3O_4S$ 473.14, found 473.15.

5.1.53. (*Z*)-2-(5-((*E*)-3-Oxo-3-(pyrrolidin-1-yl)propenyl)fran-2-ylmethylene)-4*H*-benzo[*b*][1,4]oxazin-3-one (4s)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.22 (s, 1H), 7.36–7.34 (m, 1H), 7.32 (d, J = 15.3 Hz, 1H), 7.20 (d, J = 3.4 Hz, 1H), 7.09–7.03 (m, 3H), 7.01–7.00 (m, 1H), 6.76 (d, J = 15.3 Hz, 1H), 6.73 (s, 1H), 3.64 (t, J = 6.9 Hz, 2H), 3.40 (t, J = 6.9 Hz, 2H), 1.93 (tt, J = 6.9, 6.9 Hz, 2H), 1.82 (tt, J = 6.9, 6.9 Hz, 2H).

5.2. X-ray crystallographic analysis

A yellow block crystal of $C_{23}H_{19}N_3O_3S$ having approximate dimensions of 0.66 × 0.10 × 0.09 mm was mounted on a glass fiber. All measurements were made on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated MoKα radiation.

5.2.1. Summary of X-ray crystallographic analysis of compound 4l

Formula: $C_{23}H_{19}N_3O_3S$; formula weight: 417.48; crystal color, habit: yellow, block; crystal system: monoclinic; space group: $P2_1/n$ (#14); lattice constants: a = 7.7010 Å, b = 22.3165(12) Å, c = 11.8450(6) Å, β = 95.4915(14)°, volume = 2026.33(18) Å^3; Z = 4; density (calcd): 1.368 g/cm^3; residual R_1 = 0.0344, wR_2 = 0.0684.

5.3. Kinase assay

KDR and ABL inhibitory activity assays were performed by the ELISA method by measuring the phosphorylation level of biotinylated peptide including Tyr in the sequence. Kinase reactions were carried out in a streptavidin-coated 96-well plate (Delfia® Streptavidin Microtitration Strips, PerkinElmer® 4009-0010) and consisted of baculovirus-expressed kinase (KDR or ABL), 250 nM substrate, 15 mM Tris–HCl (pH 7.5), 0.01% Tween 20, 2 nM DTT, 100 μM ATP and 40 mM Mg. Reactions were incubated for 1 h at rt and stopped by aspirating and washing them three times with 200 μL of wash buffer (50 mM Tris–HCl (pH 7.5) containing 150 mM NaCl and 0.02% Tween 20). Then 200 μM of blocking buffer (wash buffer containing 0.1% BSA) was added to each well in order to block the plate. After incubating the plate for 30 min, the blocking buffers were discarded and 100 μM of Detection Mixture (HRP-conjugated anti-phosphotyrosine (pY) antibody was added to each well. After incubating the plate for 30 min, detection mixtures were discarded and the plate was washed three times with 250 μL of the Wash Buffer. To each well 100 μL of Color Reagent (TMBE-1000; TMB Peroxidase substrate elisa, Moss, Inc.) was added and the plate was incubated for 5 min. To each well 100 μL of 0.1 M H_2SO_4 was then added and the plate was tapped gently to ensure thorough mixing. The absorbance of each well was measured immediately using a microplate reader set to 450 nM. Staurosporine was used as a positive control in both kinase assay.

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