Research Square

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Design, Synthesis and Biological Evaluation of Amide derivatives of 1,3,4-oxadiazole-isoxazol-pyridine-benzimidazole as Anticancer Agents.

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Abstract

We have designed and synthesized a series of novel amide derivatives of 1,3,4-oxadiazole-isoxazol-pyridine-benzimidazole (**10a-j**), and their structures are characterized by ^{1}HNMR, ^{13}CNMR and mass spectral data. The preliminary anticancer applications of these compounds are screened towards four types of human cancer cell lines including PC3 (prostate), A549 (lung), MCF-7 (breast) and DU-145 (prostate). The assay results revealed that many of the target compounds displayed remarkable anticancer activity. Among them, the compounds **10f, 10g, 10h** and **10j** are found to be more potent than rest of the compounds. In particularly, one compound **10f** displayed most promising anticancer activity.

Introduction

Generally, heterocyclic compounds are considered as heart of medicinal filed due to its utility as a crucial core intermediates for discovery and development of novel chemotherapeutic entities. Specifically, nitrogen contained heterocyclic motifs is a part of many therapeutic agents [1–3]. In addition to that, nitrogen atoms bearing heterocyclic like benzimidazoles are one of the most promising hetero-aromatic scaffolds having a significant consideration in the field of modern medicinal chemistry [4, 5]. These derivatives have demonstrated a wide variety of biological applications such as DNA topoisomerase-I [6], human cytomegalovirus (HCMV) [7], antiulcer [8], anticancer [9], antimicrobial [10], antihelminthic [11], anti-inflammatory [12], anticoagulant [13] and antifungal [14]. Among the numerous benzimidazole derivatives, one of the derivative namely Nocodazole (**1**, Fig. 1) was a proved as a very good anticancer agent in the inhibition of tubulin polymerization [15]. In addition, 1,2-isoxazole derivatives are well established compounds with potential medicinal activity [16]. They possessed a broad range of biological activities including anti-HIV [17], antinociceptive [18], anticancer [19], antituberculosis [20], analgesic [21], anti-inflammatory [22], antimicrobial [23], anticonvulsant [24] and anthelmintic [25]. One of the compound with 1,2-isoxazole isooxazole moiety, which is approved by US Food and Drug Administration as anticancer drug candidate is Luminespib (**2**, Fig. 1), it was developed by pharmaceutical company Vernalis [26].

In view of numerous biological findings of both benzimidazole and 1,2-isoxazole moieties and as continuation of efforts, we have designed and developed a new bunch of amide derivatives of 1,3,4-oxadiazole-isoxazol-pyridine-benzimidazole (**10a-j**). Further, all compounds were characterized by ^{1}HNMR, ^{13}CNMR and mass spectral data. Furthermore, compounds were evaluated for their preliminary anticancer applications against four human cancer cell lines including PC3 (prostate), A549 (lung), MCF-7 (breast) and DU-145 (prostate).

Results And Discussion

Chemistry

The synthetic route for the amide derivatives contained 1,3,4-oxadiazole-isoxazol-pyridine-benzimidazole (**10a-j**) was illustrated in Scheme 1. The key intermediate 5 was developed from intermediate **3** underwent Claisen condensation reaction with 5-bromo-1H-benzo[d]imidazole-2-carbaldehyde **4** by using of aq. NaOH in ethanol at room temperature for 12 hours. Further, this chalcone **5** is allowed to react with $NH_2OH.HCl$ and pyridine in isopropyl alcohol with continuous stirring at reflux for 6 hours to give pure isoxazole intermediate **6.** Furthermore, this intermediate **6** undergone Suzuki coupling reaction with pyridin-4-yl-4-boronic acid **7** in presence of $Pd(PPh_3)_4$ catalyst and triethyl amine in 1,4-dioxane at reflux for 6 hours to afford intermediate **8.** Afterwords, intermediate **8** coupled with various substituted benzoyl chlorides (**9a-j**) by using of Cs_2CO_3 in acetonitrile and reaction mixture is stirred at room temperature for 5 hours to provide pure target compounds **10a-j.** The structure of compound **10f** was confirmed by ^{1}HNMR spectrum values indicated that the methoxy protons appeared two sets at 3.83 (s, 6H), 3.90 (s, 3H) ppm, two aryl proton observed at 7.32 (s, 2H) ppm, and the isoxazole contained proton at 7.39 (s, 1H) ppm, 1,3,4-oxadiazole proton observed at 8.29 (s, 1H) ppm. The benzimidazole and pyridine contained protons appeared at 7.70 (d, 1H, *J* = 8.2 Hz), 7.76–7.80 (m, 3H), 8.12 (d, 1H, *J* = 8.2 Hz), 8.48 (d, 2H, *J* = 6.1 Hz). The mass spectrm value MS (ESI): m/z 525 $[M + H]^+$.

Biological Evaluation

In vitro **cytotoxicity**

In vitro anticancer effects of the newly developed compounds **10a-j** was examined against a panel of four human cancer cell lines such as PC3 (prostate), A549 (lung), MCF-7 (breast) and DU-145 (prostate) by using of MTT assay and results were summarized in Table 1, which compared with etoposide as the positive control. Among the all compounds **10f, 10g, 10h** and **10j** were found to be more potent than rest of the compounds. In particularly, one compound **10f** displayed most promising anticancer activity. Further, the structure activity relationships (SARs) study of the compounds indicated that the presence of electron-rich group contained compound **10f** (3,4,5-trimethoxy) displayed highest anticancer activity with IC_{50} values (PC3 = 0.26 ± 0.084μM; A549 = 0.11 ± 0.071μM; MCF-7 = 0.87 ± 0.096μM and DU-145 = 0.55 ± 0.067μM), respectively. The effect was observed in compound **10g** with 3,5-dimethoxy substituent on the phenyl moiety showed slightly lower activity (PC3 = 0.98 ± 0.082μM; A549 = 1.02 ± 0.45μM; MCF-7 = 1.29 ± 0.66μM and DU-145 = 1.74 ± 0.89μM) than with **10f.** Similarly, 4-methoxy group having compound **10h** showed lesser activity (PC3 = 1.90 ± 0.49μM; A549 = 2.12 ± 1.37μM; MCF-7 = 2.45 ± 1.60μM and DU-145 = 1.88 ± 2.12μM) than with both **10f** and **10g.** Where, the weaker electron-donating group contained compound **10i** (4-dimethylamino) demonstrated decreased activity. Whereas, compound **10j** with 4-methyl group and showed increased activity (PC3 = 2.78 ± 1.56μM; A549 = 2.98 ± 1.48μM; MCF-7 = 2.13 ± 1.43μM and DU-145 = 2.44 ± 1.77μM) than with **10i.** The antiviral activity of the compounds were weakened when an electron-withdrawing groups at phenyl ring, this effect was observed in compounds **10a** (4-nitro), **10b** (3,5-dinitro), **10c** (4-chloro), **10d** (4-bromo) and **10e** (4-cyano) have shown very poor activity.

Table 1

In vitro cytotoxicity of newly developed compounds**10a-j** with IC_{50} in μM.[a]

Compound	[c]PC3	[d]A549	[e]MCF-7	[f]DU-145
10a	ND	7.45 ± 4.32	4.91 ± 3.12	5.23 ± 3.61
10b	9.45 ± 6.87	8.55 ± 6.22	ND	ND
10c	ND	10.3 ± 7.79	7.53 ± 6.02	8.12 ± 5.63
10d	11.20 ± 7.12	ND	ND	3.69 ± 2.58
10e	ND	4.60 ± 3.27	6.33 ± 2.90	11.98 ± 6.85
10f	0.26 ± 0.084	0.11 ± 0.071	0.87 ± 0.096	0.55 ± 0.067
10g	0.98 ± 0.082	1.02 ± 0.45	1.29 ± 0.66	1.74 ± 0.89
10h	1.90 ± 0.49	2.12 ± 1.37	2.45 ± 1.60	1.88 ± 2.12
10i	3.40 ± 2.42	3.09 ± 2.16	4.26 ± 2.96	6.44 ± 3.10
10j	2.78 ± 1.56	2.98 ± 1.48	2.13 ± 1.43	2.44 ± 1.77
Etoposide	2.39 ± 1.56	3.08 ± 0.135	2.11 ± 0.024	1.97 ± 0.45

ND = Not determine.

[a]Each data represents as mean ± S.D values. From three different experiments performed in triplicates.[bc]PC3: human prostate cancer cell line. [d]A549: human lung cancer cell line. [e]MCF-7: human breast cancer cell line. [f]DU-145: human prostate cancer cell line.

Conclusion

In summary, a series of novel amide derivatives (**10a-j)** contained 1,3,4-oxadiazole-isoxazol-pyridine-benzimidazole skeleton were designed and synthesized. The newly synthesized compounds were characterized by ^{1}HNMR, ^{13}CNMR and mass spectral data. The preliminary anticancer applications of these compounds were screened towards four types of human cancer cell lines including PC3 (prostate), A549 (lung), MCF-7 (breast) and DU-145 (prostate). The assay results revealed that many of the target compounds displayed remarkable anticancer activity. Among them, the compounds **10f, 10g, 10h** and **10j** were found to be more potent than rest of the compounds. In particularly, one compound **10f** displayed most promising anticancer activity.

Experimental

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and

visualization on TLC was achieved by UV light or iodine indicator. ^{1}H and ^{13}C NMR spectra were recorded on BRUKER NMR (300 MHz, 400 MHz) instrument. Chemical shifts (d) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, QuattroLC using ESI + software with capillary voltage 3.98 kV and ESI modepositive ion trap detector. Melting points were determined with an electro thermal melting point apparatus, and are uncorrected.

(E)-3-(5-Bromo-1H-benzo[d]imidazol-2-yl)-1-(1,3,4-oxadiazol-2-yl)prop-2-en-1-one (5):The compound **4** (8 g, 0.035 mmol) is dissolved in 50 mL of ethanol, followed by addition of 1-(1,3,4-oxadiazol-2-yl) ethanone (**3)** (3.3 ml, 0.035 mmol) and 30%NaOH (10 ml). The reaction mixture is stirred at room temperature for 12 hours. After completion of reaction, solvent is evaporated and acidified with 2M HCl. Then compound was extracted in ethyl acetate and dried over with sodium sulphate, the crude product is recrystallized with methanol to give pure compound **5**, dark yellow solid (9.6 g, 85% yield); mp 156–158 ^{o}C; ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 6.91 (d, 1H, J = 15.2 Hz), 7.50 (d, 1H, J = 8.1 Hz), 7.61–7.68 (m, 2H), 8.10 (s, 1H), 8.44 (s, 1H), 8.73 (s, 1H). ^{13}C NMR (DMSO-*d6*, 100 MHz,) δ 111.4, 117.3, 118.6, 121.7, 123.5, 132.2, 139.1, 142.6, 151.6, 152.3, 162.5, 190.5. ESI (MS) : m/z 318 $(M)^{+}$.

2-(3-(1,3,4-Oxadiazol-2-yl)isoxazol-5-yl)-5-bromo-1H-benzo[d]imidazole (6): A mixture of compound **5** (9 g, 0.028 mmol) and $NH_2OH.HCl$ (4 g, 0.056 mmol) is dissolved in 60 ml of 2-propanol, then 1 ml of pyridine is added and reaction mixture is stirred at reflux for 6 hours. After completion of reaction by TLC and the solvent is evaporated under reduced pressure. The precipitated product is washed with water (3×20 ml) and crude product is purified by column chromatography with ethyl acetate/hexane (4:6) to afford pure compound **6**, white solid (8.3 g, 88% yield). mp 222–224 ^{o}C; ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 7.43 (s, 1H), 7.66 (d, 1H, J = 7.9 Hz), 7.77–7.83 (m, 2H), 8.31 (s, 1H), 8.81 (s, 1H). ^{13}C NMR (DMSO-*d6*, 100 MHz,) δ 101.3, 117.6, 118.3, 121.4, 132.5, 139.4, 142.2, 151.5, 152.6, 152.9, 153.5, 168.4. ESI (MS): m/z 331 $[M]^{+}$.

2-(3-(1,3,4-Oxadiazol-2-yl)isoxazol-5-yl)-5-(pyridin-4-yl)-1H-benzo[d]imidazole (8): A mixture of compound **6** (7 g, 0.022 mmol), $Pd(PPh_3)_4$ (2.5 g, 0.0022 mmol) in 1,4-dioxane (90 mL), pyridin-4-yl-4-boronic acid (**7**) (2.2ml, 0.022 mmol) and Et_3N (3.1 ml, 0.108 mmol) is stirred at reflux for 6 hours. The reaction mixture is cooled to room temperature and the solvent is evaporated through vacuum. The residue is dissolved in ethyl acetate (100 mL) and water (80 mL) is added. The organic phase is separated and dried over anhydrous sodium sulfate. The crude compound is purified by column chromatography by using ethyl acetate/hexane (1:1) to afford compound **8**, off white solid (5.9 g, 82% of yield); mp 240–242 ^{o}C; ^{1}H NMR (DMSO-*d6*, 400 MHz) δ 7.45 (s, 1H), 7.68 (d, 1H, J = 8.3 Hz), 7.79–7.86 (m, 3H), 8.30 (s, 1H), 8.51 (s, 1H), 8.63 (d, 2H, J = 6.5 Hz), 8.81 (s, 1H). ^{13}C NMR (DMSO-*d6*, 100 MHz,) δ 101.4, 115.4, 120.4, 123.5, 128.3, 137.4, 138.5, 139.4, 145.6, 150.2, 151.4, 152.3, 152.7, 153.4, 168.6. MS (ESI): m/z 331 $[M + H]^{+}$.

(2-(3-(1,3,4-Oxadiazol-2-yl)isoxazol-5-yl)-5-(pyridin-4-yl)-1H-benzo[d]imidazol-1-yl)(4-nitrophenyl)methanone (10a): The compound **8** (200 mg, 0.60 mmol) is dissolved in 10 mL of acetonitrile, followed by addition of 4-nitrobenzoyl chloride (**9a**) (112 mg, 0.60 mmol), and Cs_2CO_3 (320

mg, 1.2 mmol). The reaction mixture is stirred at room temperature for 5 hours, till the completion of the reaction and it is monitored by TLC. The reaction mixture is washed with water and extracted with dichloromethane, dried over anhydrous Na_2SO_4 and the crude product was purified by column chromatography with ethyl acetate/hexane (6:4) to obtain pure compound **10a**, white solid (196.2 mg, 68% yield); mp 263–265 ^oC; ^1H NMR (DMSO-*d6*, 300 MHz) δ 7.40 (s, 1H), 7.68 (d, 1H, J = 8.3 Hz), 7.84–7.90 (m, 5H), 8.10–8.21 (m, 3H), 8.27 (s, 1H), 8.49 (d, 2H, J = 6.3 Hz). ^{13}C NMR (DMSO-*d6*, 100 MHz,) δ 101.3, 111.5, 120.3, 121.5, 123.4, 128.2, 130.3, 132.5, 137.4, 138.4, 142.2, 145.1, 150.5, 151.2, 152.3, 152.6, 153.4, 165.4, 165.8, 168.5. MS (ESI): *m/z* 480 $[M + H]^+$.

(2-(3-(1,3,4-Oxadiazol-2-yl)isoxazol-5-yl)-5-(pyridin-4-yl)-1H-benzo[d]imidazol-1-yl)(3,5-dinitrophenyl)methanone (10b): This compound **10b** is prepared by following the method described for the preparation of the compound **10a**, employing **8** (200 mg,0.60 mmol) with 3,5-dinitrobenzoyl chloride (**9b**) (138 mg, 0.60 mmol), Cs_2CO_3 (320 mg, 1.2 mmol) and the crude product is purified by column chromatography with ethyl acetate/hexane (6:4) to afford pure compound **10b**, off white solid (216.8 mg, 68% yield); mp 310–312 ^oC; ^1H NMR (DMSO-*d6*, 300 MHz) δ 7.39 (s, 1H), 7.69 (d, 1H, J = 8.2 Hz), 7.81–7.89 (m, 3H), 8.12 (s, 1H), 8.29 (s, 1H), 8.50 (d, 2H, J = 6.5 Hz), 8.59 (s, 1H), 8.76 (s, 2H). ^{13}C NMR (DMSO-*d6*, 100 MHz,) δ 101.3, 111.4, 116.2, 120.4, 120.7, 123.5, 128.3, 132.5, 135.4, 138.6, 142.2, 145.6, 150.3, 151.4, 152.3, 152.6, 153.4, 165.2, 165.6, 168.5. MS (ESI): *m/z* 525 $[M + H]^+$.

(2-(3-(1,3,4-Oxadiazol-2-yl)isoxazol-5-yl)-5-(pyridin-4-yl)-1H-benzo[d]imidazol-1-yl)(4-chlorophenyl)methanone (10c): This compound **10c** is prepared by following the method described for the preparation of the compound **10a**, employing **8** (200 mg,0.60 mmol) with 4-chlorobenzoyl chloride (**9c**) (0.08 ml, 0.60 mmol), Cs_2CO_3 (320 mg, 1.2 mmol) and the crude product is purified by column chromatography with ethyl acetate/hexane (6:4) to afford pure compound **10c**, off white solid (220.6 mg, 78% yield); mp 270–272 ^oC; ^1H NMR (DMSO-*d6*, 300 MHz) δ 7.41 (s, 1H), 7.49 (d, 2H, J = 8.4 Hz), 7.67 (d, 1H, J = 8.0 Hz), 7.85–7.96 (m, 5H), 8.16 (d, 1H, J = 8.0 Hz), 8.30 (s, 1H), 8.49 (d, 2H, J = 6.3 Hz. ^{13}C NMR (DMSO-*d6*, 100 MHz,) δ 101.2, 111.4, 120.5, 123.4, 128.2, 129.3, 129.9, 132.5, 134.2, 137.4, 138.4, 142.2, 145.5, 150.2, 151.5, 152.6, 152.6, 153.2, 165.6, 168.6. MS (ESI): *m/z* 469 $[M]^+$.

(2-(3-(1,3,4-Oxadiazol-2-yl)isoxazol-5-yl)-5-(pyridin-4-yl)-1H-benzo[d]imidazol-1-yl)(4-bromophenyl)methanone (10d): This compound **10d** is prepared by following the method described for the preparation of the compound **10a**, employing **8** (200 mg,0.60 mmol) with 4-bromobenzoyl chloride (**9d**) (132 mg, 0.60 mmol), Cs_2CO_3 (320 mg, 1.2 mmol) and the crude product was purified by column chromatography with ethyl acetate/hexane (6:4) to afford pure compound **10d**, off white solid, (201.7 mg, 65% yield); mp: 276–278 ^oC; ^1H NMR (DMSO-*d6*, 300 MHz) δ 7.40 (s, 1H), 7.55 (d, 2H, J = 8.5 Hz), 7.68 (d, 1H, J = 8.1 Hz), 7.86–7.98 (m, 5H), 8.17 (d, 1H, J = 8.1 Hz), 8.31 (s, 1H), 8.50 (d, 2H, J = 6.4 Hz). ^{13}C NMR (DMSO-*d6*, 100 MHz) δ 101.2, 111.3, 120.4, 123.4, 123.5, 128.3, 130.5, 132.3, 132.6, 137.2, 138.4, 143.4, 145.6, 150.2, 151.4, 152.5, 152.8, 153.5, 165.3, 168.5. MS (ESI): *m/z* 514 $[M + 2H]^+$.

(2-(3-(1,3,4-Oxadiazol-2-yl)isoxazol-5-yl)-5-(pyridin-4-yl)-1H-benzo[d]imidazol-1-yl)(4-cyanophenyl)methanone (10e): This compound **10e** is prepared by following the method described for the preparation of the compound **10a**, employing **8** (200 mg,0.60 mmol) with 4-cyanobenzoyl chloride (**9e**) (99 mg, 0.60 mmol), Cs_2CO_3 (320 mg, 1.2 mmol) and the crude product is purified by column chromatography with ethyl acetate/hexane (6:4) to afford pure compound **10e**, off white solid, (286.2 mg, 69% yield); mp 280–282 °C; ^{1}H NMR (DMSO-*d6*, 300 MHz) δ 7.40 (s, 1H), 7.70 (d, 1H, J = 8.2 Hz), 7.78–7.99 (m, 7H), 8.18 (d, 1H, J = 8.2 Hz), 8.30 (s, 1H), 8.51 (d, 2H, J = 6.3 Hz). ^{13}C NMR (DMSO-*d6*, 100 MHz,) δ 101.3, 111.3, 111.6, 119.4, 120.5, 123.4, 128.3, 129.2, 132.5, 133.2, 137.4, 138.4, 142.2, 145.5, 150.3, 151.4, 152.3, 152.6, 153.5, 165.2, 168.5. MS (ESI): *m/z* 460 $[M + H]^+$.

(2-(3-(1,3,4-Oxadiazol-2-yl)isoxazol-5-yl)-5-(pyridin-4-yl)-1H-benzo[d]imidazol-1-yl)(3,4,5-trimethoxyphenyl)methanone (10f): This compound **10f** is prepared by following the method described for the preparation of the compound **10a**, employing **8** (200 mg,0.60 mmol) with 3,4,5-trimethoxybenzoyl chloride (**9f**) (138 mg, 0.60 mmol), Cs_2CO_3 (320 mg, 1.2 mmol) and the crude product is purified by column chromatography with ethyl acetate/hexane (6:4) to afford pure compound **10e**, white solid (210.3 mg, 66% yield); mp 300–302 °C; ^{1}H NMR (DMSO-*d6*, 300 MHz) δ 3.83 (s, 6H), 3.90 (s, 3H), 7.32 (s, 2H), 7.39 (s, 1H), 7.70 (d, 1H, J = 8.2 Hz), 7.76–7.80 (m, 3H), 8.12 (d, 1H, J = 8.2 Hz), 8.29 (s, 1H), 8.48 (d, 2H, J = 6.1 Hz). ^{13}C NMR (DMSO-*d6*, 100 MHz,) δ 57.4, 57.8, 101.3, 105.5, 111.5, 120.6, 123.2, 128.3, 132.4, 135.4, 138.2, 140.3, 142.2, 145.6, 150.3, 151.4, 152.3, 152.7, 153.5, 154.6, 165.5, 168.6. MS (ESI): 525 $[M + H]^+$.

(2-(3-(1,3,4-Oxadiazol-2-yl)isoxazol-5-yl)-5-(pyridin-4-yl)-1H-benzo[d]imidazol-1-yl)(3,5-dimethoxyphenyl)methanone (10g): This compound **10g** is prepared by following the method described for the preparation of the compound **10a**, employing **8** (200 mg,0.60 mmol) with 3,5-dimethoxybenzoyl chloride (**9g**) (120 mg, 0.60 mmol), Cs_2CO_3 (320 mg, 1.2 mmol) and the crude product is purified by column chromatography with ethyl acetate/hexane (6:4) to afford pure compound **10g**, white solid (176.5 mg, 59% yield); mp 304–306 °C; ^{1}H NMR (DMSO-*d6*, 300 MHz) δ 3.80 (s, 6H), 6.62 (s, 1H), 7.12 (s, 2H), 7.40 (s, 1H), 7.69 (d, 1H, J = 8.3 Hz), 7.75–7.79 (m, 3H), 8.11 (d, 1H, J = 8.3 Hz), 8.30 (s, 1H), 8.49 (d, 2H, J = 6.2 Hz. ^{13}C NMR (DMSO-*d6*, 100 MHz,) δ 57.2, 101.4, 102.5, 111.5, 113.4, 120.4, 123.5, 128.2, 132.5, 135.3, 138.4, 142.2, 145.3, 150.5, 151.5, 152.6, 152.8, 153.5, 161.3, 165.4, 168.7. MS (ESI): *m/z* 495 $[M + H]^+$.

(2-(3-(1,3,4-Oxadiazol-2-yl)isoxazol-5-yl)-5-(pyridin-4-yl)-1H-benzo[d]imidazol-1-yl)(4-methoxyphenyl)methanone (10h): This compound **10h** is prepared by following the method described for the preparation of the compound **10a**, employing **8** (200 mg,0.60 mmol) with 4-methoxybenzoyl chloride (**9h**) (0.085 ml, 0.60 mmol), Cs_2CO_3 (320 mg, 1.2 mmol) and the crude product is purified by column chromatography with ethyl acetate/hexane (6:4) to afford pure compound **10h**, white solid (188.3 mg, 67% yield); mp 290–292 °C; ^{1}H NMR (DMSO-*d6*, 300 MHz) δ 3.89 (s, 3H), 7.40 (s, 1H), 7.49 (d, 2H, J = 7.8 Hz), 7.70 (d, 1H, J = 8.2 Hz), 7.77–7.86 (m, 5H), 8.10 (d, 1H, J = 8.2 Hz), 8.30 (s, 1H), 8.50 (d, 2H, J = 6.1

Hz). ^{13}C NMR (DMSO-*d6*, 100 MHz,) δ 57.4, 101.3, 111.5, 115.4, 120.5, 122.7, 128.2, 130.6, 132.4, 137.4, 138.5, 142.3, 145.5, 150.4, 151.3, 152.5, 152.7, 153.5, 160.3, 165.4, 168.6. MS (ESI): *m/z* 465 $[M + H]^+$.

(2-(3-(1,3,4-Oxadiazol-2-yl)isoxazol-5-yl)-5-(pyridin-4-yl)-1H-benzo[d]imidazol-1-yl)(4-(dimethylamino)phenyl)methanone (10i): This compound **10i** is prepared by following the method described for the preparation of the compound **10a**, employing **8** (200 mg,0.60 mmol) with 4-(dimethylamino)benzoyl chloride (**9i**) (110 mg, 0.60 mmol), Cs_2CO_3 (320 mg, 1.2 mmol) and the crude product is purified by column chromatography with ethyl acetate/hexane (6:4) to afford pure compound **10i**, off white solid (205.3 mg, 71% yield); mp: 294–296 °C; ^{1}H NMR (DMSO-*d6*, 300 MHz) δ 2.90 (s, 6H), 7.41 (s, 1H), 7.51 (d, 2H, *J* = 7.9 Hz), 7.69 (d, 1H, *J* = 8.1 Hz), 7.75–7.84 (m, 5H), 8.11 (d, 1H, *J* = 8.1 Hz), 8.31 (s, 1H), 8.51 (d, 2H, *J* = 6.2 Hz). ^{13}C NMR (DMSO-*d6*, 100 MHz,) δ 43.5, 101.5, 111.5, 113.2, 120.2, 123.5, 128.5, 130.4, 132.5, 137.2, 138.4, 141.2, 145.5, 150.2, 151.4, 151.7, 152.3, 152.6, 153.5, 165.6, 168.5. MS (ESI): *m/z* 478 $[M + H]^+$.

(2-(3-(1,3,4-Oxadiazol-2-yl)isoxazol-5-yl)-5-(pyridin-4-yl)-1H-benzo[d]imidazol-1-yl)(p-tolyl)methanone (10j):This compound **10j** is prepared by following the method described for the preparation of the compound **10a**, employing **8** (200 mg,0.60 mmol) with 4-methylbenzoyl chloride (**9j**) (0.06 ml, 0.60 mmol), Cs_2CO_3 (320 mg, 1.2 mmol) and the crude product is purified by column chromatography with ethyl acetate/hexane (6:4) to afford pure compound **10j**, off white solid (200.5 mg, 74% yield); mp 281–283 °C; ^{1}HNMR (DMSO-*d6*, 300 MHz) δ 2.41 (s, 3H), 7.39 (d, 2H, *J* = 7.7 Hz), 7.41 (s, 1H), 7.70 (d, 1H, *J* = 8.0 Hz), 7.74–7.86 (m, 5H), 8.10 (d, 1H, *J* = 8.0 Hz), 8.30 (s, 1H), 8.50 (d, 2H, *J* = 5.9 Hz). ^{13}C NMR (DMSO-*d6*, 100 MHz,) δ 27.2, 101.3, 111.5, 120.4, 123.4, 128.5, 129.1, 130.4, 132.5, 137.4, 138.4, 142.2, 142.6, 145.3, 150.3, 151.4, 152.5, 152.8, 153.5, 165.5, 168.6. MS (ESI): *m/z* 449 $[M + H]^+$.

MTT assay:

Individual wells of a 96-well tissue culture micro titer plate are inoculated with 100 µL of complete medium containing 1×10^4 cells. The plates are incubated at 37 °C in a humidified 5% CO_2 incubator for 18 hours prior to the experiment. After medium removal, 100 µL of fresh medium containing the test compounds and etoposide at different concentrations such as 0.5, 1, and 2 µM are added to each well and incubated at 37 °C for 24 hours. Then the medium is discarded and replaced with 10 µL MTT dye. Plates are incubated at 37 °C for 2 hours. The resulting formazan crystals were solubilized in 100 µL extraction buffer. The optical density (O.D) is read at 570 nm with micro plate reader (Multi-mode Varioskan Instrument-Themo Scientific). The percentage of DMSO in the medium never exceeded 0.25%.

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Scheme

Schemes 1 is available in the Supplementary Files section.

Figures

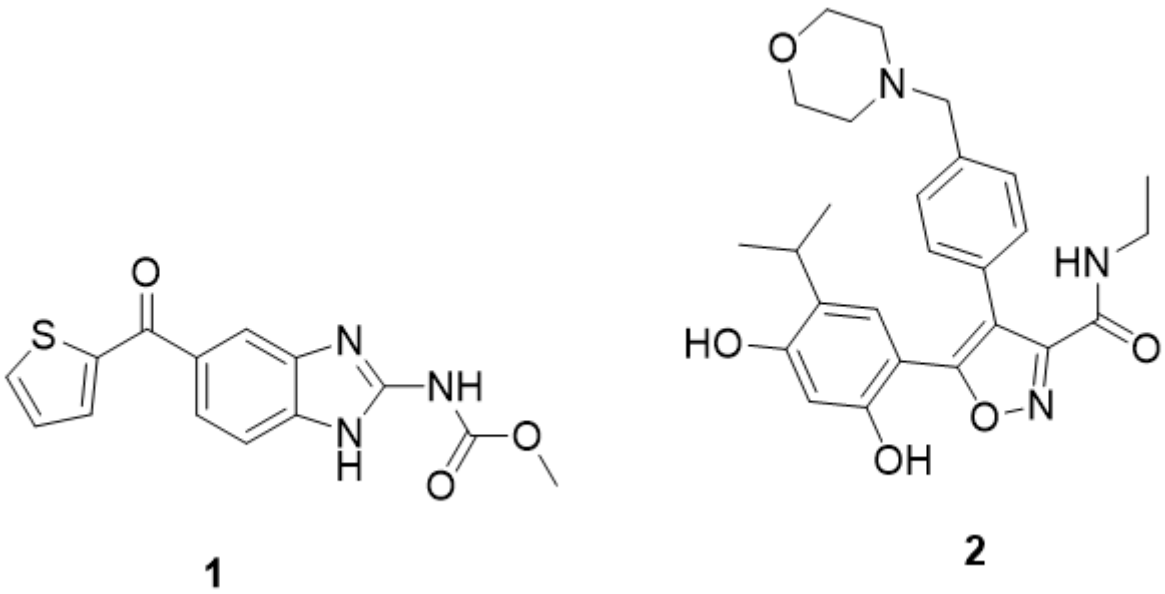

Figure 1

Structures of Nocodazole **1** and Luminespib **2**

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