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Divergent Synthesis and Evaluation of the in vitro Cytotoxicity Profiles of 3,4-Ethylenedioxythiophenyl-2-propen-1-one Analogues

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A new series of 3,4-ethylenedioxythiophene (EDOT)-appended propenones were prepared by condensation reaction and their in vitro cytotoxicity effects were evaluated against five human cancer cell lines. Preliminary structure–activity relationships of EDOT-incorporated 2-propenone derivatives were also established. The EDOT-appended enones demonstrated significant cytotoxicity against human cancer cell lines. The most active analogue, (*E*)-3-(2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**3p**, $GI_{50} = 110$ nM), severely inhibited the clonogenic potential of cancer cells, and induced cell-cycle arrest in the G2/M phase and caused an accumulation of HCT116 colon cancer cells with >4N DNA content. Also, **3p** exhibited weak inhibition of the enzymatic activity of human topoisomerase I. Molecular docking studies indicated preferential binding of the compounds to the ATP-binding pocket of the human checkpoint 2 kinase (Chk2) catalytic domain, thus, identifying a novel diaryl 2-propenone chemotype for the development of potent inhibitors of Chk2.

Introduction

For many years, diaryl chalcone derivatives have been extensively studied for their diverse biological and pharmacological properties.[1] The chalcones have a basic skeleton similar to flavonoids and are also known as key intermediates in the synthesis of various bioactive heterocycles.[2] In recent decades, diaryl 2-propenones have been investigated for their biological activities, including as antimalarial,[3] antibacterial,[4] antitumor,[5] antioxidant,[6] antihyperglycemic,[7] and anti-HIV agents.[8] Recently, Guo and co-workers developed an efficient synthesis of α,β-unsaturated carbonyl compounds by Fe^{II}-mediated decarboxylative cross-coupling reactions,[9] whereas Correa et al. established the synthesis of the γ-butenolide natural product framework with epoxidation of 2-propenone as a crucial step.[10] Owing to their biological importance, nitrogen-containing six-membered pyrimidines[11] and five-membered isoxazoles were synthesized from chalcones by cyclocondensation reactions. Pyrimidines are basic components of nucleic acids, and substituted pyrimidines have exhibited excellent biological activity against various human cancer cell lines.[12] Isoxazoles are also versatile building blocks for constructing numerous natural products.[13] Substituted isoxazoles are well-known for their promising and potent biological activities such as analgesic,[14] antiviral,[15] antimicrobial,[16] hypoglycemic,[17] and anticancer properties.[15,18] Attar and co-workers reported that organic chalcones displayed better biological activity than organometallic chalcones.[19]

Notably, the 3,4-ethylenedioxythiophene (EDOT) unit plays a vital role in the field of material sciences[20] owing to the noncovalent interaction between oxygen and a sulfur atom. However, reports describing the biological activity of EDOT derivatives are few in number.[21] The direct C–H functionalization of EDOT has led to the synthesis of wide variety of EDOT analogues.[22] In general, several thiophene-based chalcone analogues exert cytotoxic activities through a variety of molecular mechanisms involving disruption of the cell cycle, inhibition of angiogenesis, tubulin polymerization and induction of apoptosis.[23] Also, several chalcones were reported to inhibit the activity of kinases associated with growth factor receptors essential for the survival and proliferation of cancer cells, such as vascu-

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lar endothelial growth factor receptor-2 (VEGFR-2) and epidermal growth factor receptor (EGFR).[24] Recently, several chalcone inhibitors of human checkpoint kinase 2 (Chk2) were reported to exhibit antitumor activity.[25] A series of 2-aminopyridines containing thiophene dioxole rings were reported as Chk2 inhibitors through interactions within the ATP-binding pocket of Chk2 kinase.[26] The unrelated PV1019 exerted antiproliferative activity in cancer cells expressing high levels of Chk2 kinase, whereas selective inhibition of Chk2 in p53-deficient cells sensitizes them to DNA-damaging agents and radiation.[27]

Based on the above findings, we planned the synthesis of new types of EDOT-based chalcone analogues and investigated their in vitro cytotoxicity potential against human cancer cell lines. Using an available crystal structure of Chk2 bound with a small molecule, an attempt was made to determine the binding modes of the EDOT enones reported here using molecular docking studies.

Results and Discussion

Synthesis of novel thiophene-based derivatives 3a–u, 10, and 12

As a typical example, the EDOT-based enone **3a** can be smoothly prepared by a condensation reaction between 2-acetyl-EDOT (**1**) and veratraldehyde (**2a**) using 10% aqueous NaOH solution heated at reflux for 1 h. Using similar procedures, various EDOT-2-propenone derivatives **3b–f** were also synthesized. Next, condensation of **1** with 2,6-dichlorobenzaldehyde, thiophene-2-carboxaldehyde (**2h**), and EDOT-2-carboxaldehyde (**5a**) furnished respective enones **3g–i** (Scheme 1).

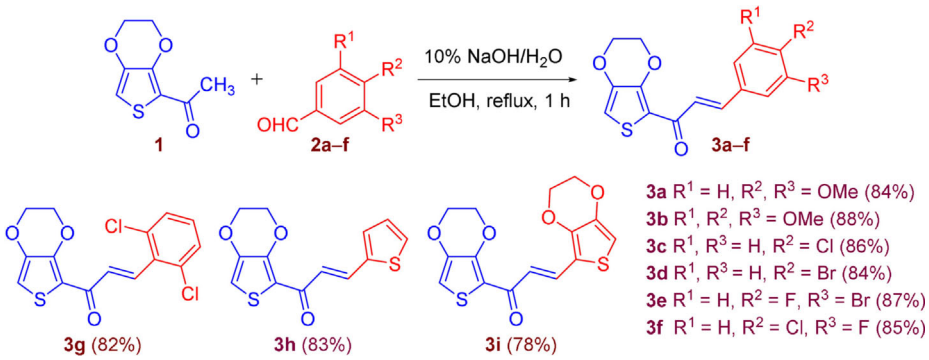
A detailed literature survey revealed that the biological activity of enone derivatives could be varied by changing either group or individual atoms, that is, the positions of the group play an essential role in the activity of diaryl-2-propenone analogues.[19,28] Hence, to better understand the structure–activity relationships of EDOT enones, derivatives lacking the EDOT unit were synthesized. As expected, 2-acetylthiophene (**4a**) underwent a similar type of base-mediated condensation reaction with substituted aromatic aldehydes **2a**, **2c**, and **2e** to afford the corresponding 1-(2-thienyl)-3-aryl-propen-2-ones **3j–l**, respectively, in good yields (Scheme 2). Similarly, syntheses of isomeric 2-thiophenyl, 3-methyl-2-thiophenyl and 2,2′-bithiophenyl-appended enones **3m--o** were achieved by base-mediated condensation of the respective thiophene aldehydes with trimethoxyacetophenone (**6a**) in 84–88% yields (Scheme 2).

Next, base-mediated condensation of trisubstituted acetophenone **6a** and disubstituted **6b** with EDOT-2-carboxaldehyde (**5a**) led to the formation of the corresponding propenones **3p** and **3q** (Scheme 3). Furthermore, the condensation of 3,4,5-trimethoxyacetophenone with 5-bromo-EDOT-2-carboxaldehyde as and EDOT-2,5-dicarboxaldehyde afforded derivatives **3r** and **3s**, respectively. As a representative case, to understand the significance of the vinyl spacer, trimethoxybenzoyl EDOT **3t** was also prepared. As expected, Suzuki coupling of 5-bromo-EDOT-2-carboxaldehyde (**7**) with 3,4-dimethoxyphenylboronic acid (**8**) in the presence of zero-valent Pd in DMF heated at reflux furnished the 3,4-dimethoxyphenyl-substituted EDOT aldehyde, which upon condensation with **6a** using 10% NaOH solution heated at reflux for 1 h afforded 3,4-dimethoxyphenyl-appended enone **3u** in 82% yield (Scheme 3).

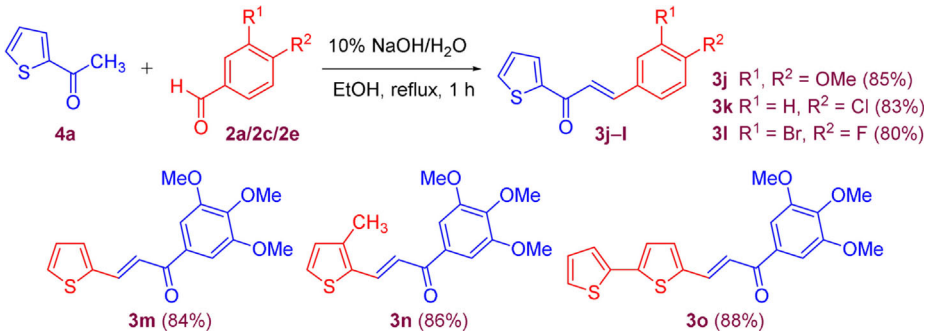
Finally, to investigate the importance of the enone, EDOT compounds with masked enone moieties were synthesized. Accordingly, the reaction of EDOT enone **3a** with hydroxylamine hydrochloride (**9**) in the presence of K_2CO_3 furnished the isoxazole-containing EDOT **10** in 70% yield. Next, as an illustrative case, the EDOT enone **3d** reacted with guanidine hydrochloride (**11**) in 10% NaOH solution heated at reflux for 2 h to afford EDOT-substituted pyrimidine **12** in a moderate yield (Scheme 4).

Cytotoxicity screening

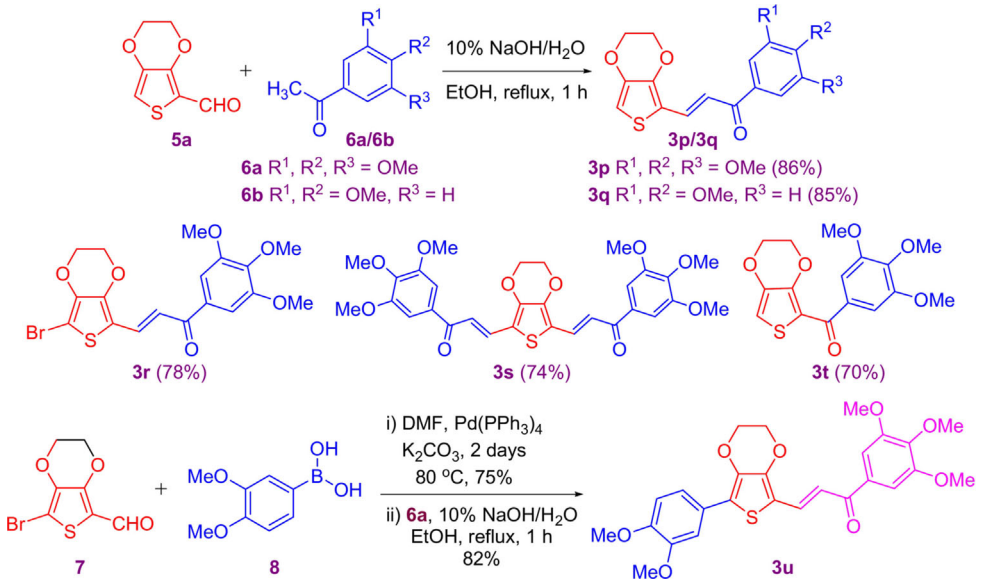
The synthesized compounds **3a–u** were evaluated for their cytotoxic potential using a sulforhodamine B assay against five different human cancer cell lines of diverse tumor origin: HCT116 (colon), NCI-H460 (lung), U251 (glioma), MCF7 (breast), and HeLa (cervical). The in vitro concentration for 50% growth inhibition (GI_{50}) values of test compounds against individual cell lines are presented in Table 1. Detailed structure–activity



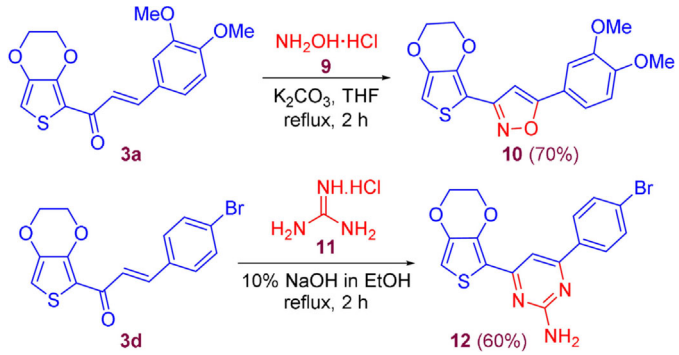
Scheme 1. Synthesis of EDOT-based 2-propenones **3a–i**.



Scheme 2. Synthesis of thiophene-based 2-propenones **3j–o**.



Scheme 3. Synthesis of EDOT-based ketones **3p–u**.



Scheme 4. Synthesis of masked-enone EDOT derivatives **10** and **12**.

relationships were examined by varying the position of the ketone functional group in the linker and by changing the substituents on the thiophene and phenyl groups. Firstly, among the 2-propen-1-ones containing an EDOT moiety, varying the di- or trisubstitution on the 3′-phenyl ring resulted in promising cytotoxic profiles for compounds **3a–g**, with GI_{50} values in the range of 2–20 μM, except for **3g** having two chlorine atoms in *ortho* positions to the vinyl ketone spacer (GI_{50} > 8 μM). However, in the case of **3e** (Figure 1A), having *para*-fluoro and *meta*-bromo substitution on the phenyl unit with respect to the 2-propen-1-one spacer, the average GI_{50} value across the five cell lines was below 2.5 μM.

The growth inhibition of **3a** was moderately compromised upon introduction of a thiophene group (**3h**) but was completely abolished if a second EDOT moiety was introduced at the 3-position (**3i**). Also, if the EDOT unit was replaced with thiophene (**3j–m**), the activity was marginally decreased (GI_{50} > 4 μM). However, a similar replacement of the EDOT unit with 3-methylthiophene resulted in **3n** exhibiting potent cytotoxic activity (average GI_{50} < 2.5 μM). Again, the activity was decreased for the compound containing bis-thiophene and 3,4,5-trimethoxyphenyl moieties (**3o**).

Interestingly, changing the position of the vinyl ketone function in the spacer, with a trimethoxyphenyl group attached to the 1-position of 2-propen-1-one (**3p**), resulted in superior cytotoxicity relative to the other compounds tested, with GI_{50} values between 40 and 110 nM (Figure 1B and Table 1). Similar results were obtained with **3r** (Figure 1C and Table 1), which, with a bromine substituent on the EDOT ring, has both its 2-

Table 1. In vitro cytotoxicity data for EDOT-based enones.

Compound	GI_{50} [μM][a]				
	HCT116 (colon)	NCI-H460 (lung)	U251 (glioma)	HeLa (cervix)	MCF7 (breast)
3a	5.0 ± 1.0	5.7 ± 0.58	4.2 ± 1.35	3.7 ± 0.76	2.1 ± 0.14
3b	20.0 ± 0.0	5.5 ± 0.71	7.0 ± 1.41	3.4 ± 1.52	7.5 ± 0.71
3c	4.9 ± 0.42	6.0 ± 1.0	2.4 ± 0.56	3.0 ± 0.0	1.0 ± 0.14
3d	5.0 ± 1.80	5.5 ± 0.71	2.9 ± 1.27	3.23 ± 1.63	1.5 ± 0.46
3e	2.1 ± 0.33	5.0 ± 1.0	1.8 ± 0.24	2.1 ± 0.32	1.3 ± 0.35
3f	5.0 ± 0.71	6.3 ± 1.06	2.4 ± 0.85	4.3 ± 1.13	1.7 ± 0.14
3g	> 20	> 20	8.0 ± 0.0	11.0 ± 1.41	11.0 ± 1.41
3h	9.0 ± 1.41	8.5 ± 0.71	7.5 ± 0.71	5.6 ± 0.64	3.5 ± 0.42
3i	> 20	> 20	> 20	20.0	5.0 ± 0.0
3j	7.7 ± 0.58	7.5 ± 0.71	6.4 ± 1.48	6.5 ± 0.71	3.2 ± 0.42
3k	5.8 ± 0.35	7.3 ± 0.35	4.6 ± 0.64	5.0 ± 0.0	4.2 ± 0.58
3l	4.6 ± 0.57	6.5 ± 0.71	2.8 ± 0.28	5.3 ± 1.06	2.8 ± 0.35
3m	9.5 ± 2.12	7.0 ± 0.0	6.0 ± 1.41	7.0 ± 1.41	3.5 ± 0.71
3n	2.8 ± 1.06	2.1 ± 0.14	3.5 ± 2.12	1.6 ± 0.07	1.5 ± 0.35
3o	9.5 ± 0.71	1.8 ± 0.35	12.5 ± 0.71	9.5 ± 0.71	4.0 ± 1.41
3p	0.07 ± 0.0	0.10 ± 0.021	0.09 ± 0.21	0.04 ± 0.008	0.11 ± 0.014
3q	> 20	> 20	> 20	> 20	> 20
3r	0.3 ± 0.20	0.5 ± 0.07	0.5 ± 0.21	0.31 ± 0.01	0.1 ± 0.01
3s	1.3 ± 0.14	1.9 ± 0.0	2.3 ± 0.35	1.0 ± 0.07	ND
3t	> 20	> 20	> 20	> 20	> 20
3u	> 20	ND	4.8 ± 1.06	15.0 ± 0.0	1.7 ± 0.92
10	> 20	> 20	> 20	ND	> 20
12	> 20	> 20	> 20	> 20	> 20
nocodazole	0.075 ± 0.007	0.075 ± 0.007	0.080 ± 0.014	0.060 ± 0.0	0.061 ± 0.035

[a] Values are the mean ± SD of at least two independent experiments; ND: not determined.

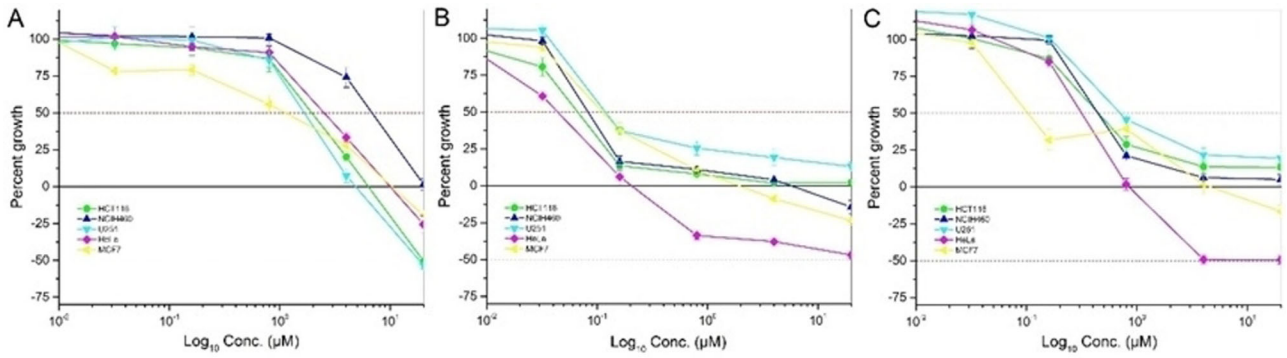


Figure 1. Growth inhibition (dose–response) profiles of EDOT derivatives A) **3e**, B) **3p**, and C) **3r** in a panel of five cancer cell lines after treatment for 48 h. HCT116 (green), NCI-H460 (blue), U251 (cyan), HeLa (magenta), and MCF7 (yellow).

and 5-positions blocked. Also, symmetrical substitution of EDOT at the 2- and 5-positions with trimethoxyphenyl vinyl ketone moieties (**3s**) maintained the antiproliferative potential ($GI_{50} < 2$ μM).

The cytotoxicity was completely abolished if the 3,4,5-trimethoxyphenyl group were replaced with a 3,4-dimethoxyphenyl group (**3q**) or with the absence of vinyl group in the spacer (**3t**). Also, the EDOT derivatives in which the cyclized vinyl ketone functionality was converted into heterocycles (**10** and **12**) were inactive analogues, thus highlighting the importance of the vinyl ketone spacer for anticancer properties.

Effect of EDOT derivatives on the clonogenicity of cancer cells

Having established the cytotoxic potential of EDOT derivatives, the long-term impact of **3e**, **3p** and **3r** on the colony-forming ability of HCT116 and NCI-H460 cells was studied using a clonogenic cell survival assay, in which the anticancer alkaloid camptothecin served as a standard (Figure 2). The derivatives **3p** and **3r** effectively abrogated colony formation even at concentrations as low as 0.5 μM. However, **3p** was more potent, inhibiting colony growth at a concentration of 0.25 μM. Compound **3e**, which showed moderate cytotoxicity, could attenuate the formation of colonies, albeit at concentrations above

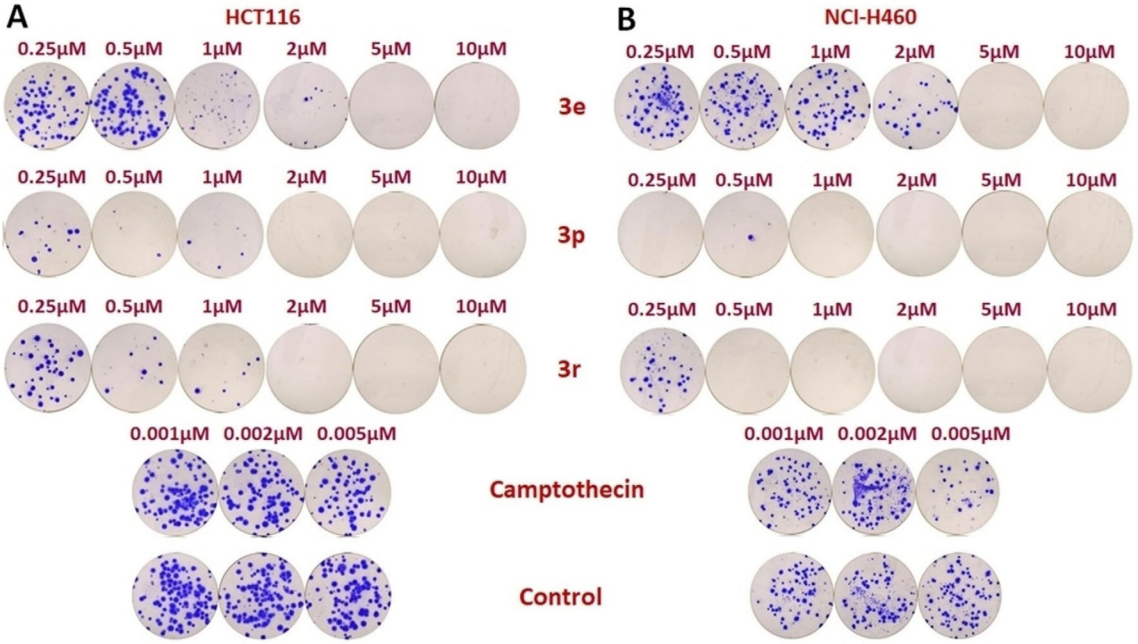


Figure 2. Effect of EDOT derivatives on the clonogenicity of A) HCT116 and B) NCI-H460 cells. Cells were seeded in six-well plates, allowed to adhere overnight, then treated with various concentrations (0.25–10 μM) of enones **3e**, **3p**, and **3r**. After 48 h, the cells were washed with Dulbecco's phosphate-buffered saline and grown for 14 days in drug-free medium. Cell colonies were stained with crystal violet and photographed.

1 μM in both cell lines. Thus, the results of the preliminary cytotoxicity assay were confirmed by the long-term clonogenic cell survival assay.

Cell-cycle effects of EDOT derivatives in HCT116 cells

The time-dependent cellular effects of the promising analogues **3p** and **3r** on HCT116 cells was studied using flow cytometry (Figure 3). The cell-cycle analysis with 2 μM of the propenone **3e** at 24 and 48 h showed only marginal changes in the population of cells in different phases. The compound **3p**, which emerged as a potent analogue from the cytotoxicity analysis, caused a significant arrest of HCT116 cells in the G2/M phase (60%) and the subsequent appearance of polyploid cells with >4N DNA content (15.8%) after 24 h treatment.

However, an increase in cell death as evident from the appearance of a sub-G1 population of cells (16.5%) was seen in **3p**-treated cells at 48 h. Similarly, in the case of **3r**, there was arrest of cells in the G2/M phase with an increase in the number of polyploid cells (10.5%) at 24 h. Compound **3p** was found to be more potent at causing cell death both at 24 and 48 h, whereas **3r** produced stronger G2/M arrest than **3p** in HCT116 cells. The occurrence of G2/M arrest following treatment with **3r** and **3p** suggests the activation of the mitotic checkpoint, whereas an increase in the numbers of polyploid cells indicates abnormal chromosomal segregation during mitosis, which is characteristic of agents that interfere with tubulin dynamics.[29]

Effect of EDOT derivatives 3p and 3r on human topoisomerase activity

DNA topoisomerases are class of enzymes involved in catalyzing changes in DNA topology, thus playing an essential role in DNA metabolism. In humans, there are two classes of topoisomerases causing either single-strand DNA cleavage (topoisomerase I) or cleavage of both strands (topoisomerase II) to release topological strain. Many small molecules are reported to influence the catalytic activities of topoisomerases, causing cell-cycle arrest or cell death and are recognized as antitumor drugs.[30] Several chalcone derivatives have been shown to inhibit different enzymes crucial to cell replication.[31] Also, it is reported that tricyclic planar pyrroloquinoline-conjugated chalcones inhibit the relaxation of supercoiled DNA by topoisomerase II.[32] Hence, the ability of the promising chalcone-like **3p** and **3r** to interfere with DNA topoisomerase activity was examined in a plasmid relaxation assay. Compound **3p** could partially inhibit hTopI activity at 200 μM, whereas no inhibition of hTopII was observed at this concentration (Figure 4). In contrast, **3r** failed to inhibit the relaxation activities of either topoisomerase at 200 μM.

Molecular docking of EDOT derivatives and inhibiting interactions with Chk2 kinase

Many bifunctional 2-aminopyridine analogues have been widely reported as kinase inhibitors. Crystallographic studies of Chk2 kinase and such inhibitors indicated binding of the 2-aminopyridine group into the ribose-binding pocket, with 3,5-

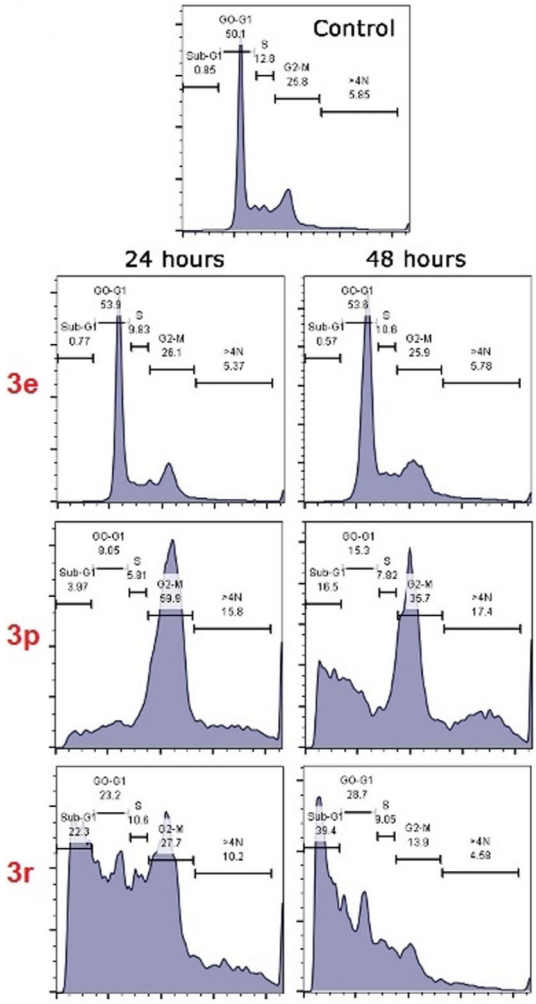


Figure 3. Time-dependent arrest of cells in EDOT-derivative-treated HCT116 cells. Cell-cycle analysis of human colon HCT116 cells treated with EDOT derivatives **3e**, **3p**, and **3r** (2 μM) for 24 and 48 h. Each panel shows the mean percentage of cells in the G0/G1, S, and G2/M phases of the cell cycle; figures are representative of two independent experiments.

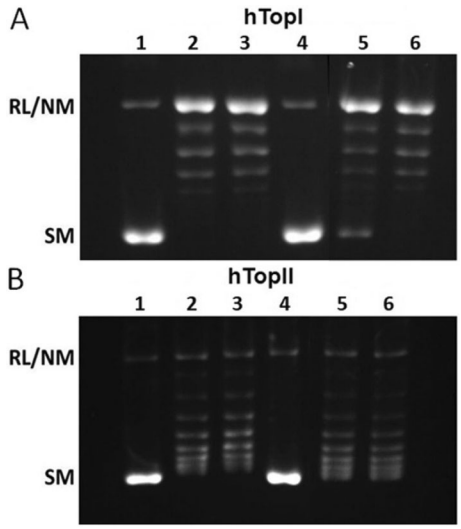


Figure 4. Effects of EDOT derivatives on recombinant human topoisomerases I and II. Relaxation of negatively supercoiled pBS (SK+) DNA with either purified A) hTopI or B) hTopII at a molar ratio of 3:1 under identical assay conditions. Lanes 1: 90 fmol pBS (SK+) DNA; lanes 2: same as lanes 1, but with 30 fmol hTopI or hTopII incubated for 30 min at 37 °C; lanes 3: same as lanes 2, but in the presence of DMSO (2% *v/v*); lanes 4: same as lanes 2, but in the presence of 25 μM camptothecin (A) or 25 μM etoposide (B) as positive controls; lanes 5 and 6: same as lanes 2, but in the presence of 200 μM **3p** and **3r**, respectively. Positions of supercoiled monomer (SM) and relaxed and nicked monomer (RL/NM) are indicated. All experiments were carried out in triplicate. The images shown here are representative of one set of those experiments.

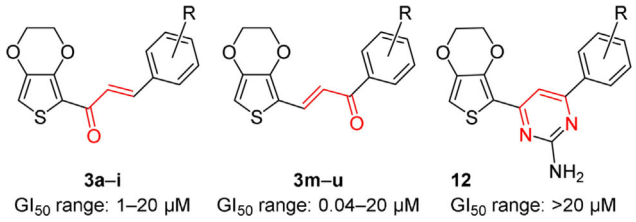


Figure 5. General structures of EDOT derivatives **3a–i** and **3m–u** and diaryl 2-aminopyrimidine analogue **12**.

diaryl substituents serving as hinge-binding motifs.[26a] In the present study, the resemblance of aminopyrimidine **12** and the isoxazole **10** to the ATP ligand raises the possibility of their accommodation into the ribose-binding site of the Chk2 kinase, and their substituents might serve as hinge-binding motifs. Thus, to examine the orientation of 4,6-diaryl-substituted 2-aminopyrimidine **12** and the 3,5-diaryl-substituted isoxazole **10** in the active ATP-binding site of the Chk2 kinase, an induced-fit docking protocol was adopted using the coordinates from the reported[26a] co-crystal structure of a related EDOT-substituted 2-aminopyrimidine bound to the Chk2 kinase (PDB ID: 2WTJ). We investigated whether the EDOT-substituted diaryl propenone interacts with the ATP-binding pocket of the Chk2 kinase as their structural contours resemble those of diaryl-substituted 2-aminopyridines (Figure 5).

The binding energy, glide score, and the key interacting residues of selected compounds are displayed in Table 2, and Figure 6 shows their corresponding 2D plots. The dihydrodioxin ring of 2-aminopyrimidine **12** reached the surface defined by residues Leu303 and Met304 to provide a better fit to this region of the Chk2 kinase, which was not observed with the corresponding five-membered isoxazole derivative **10**. A common striking feature of the vinyl ketones was the interaction of the carbonyl group with the Met304 residue in the hinge region of the kinase. The symmetrical bis-trimethoxyphenyl EDOT derivative **3s** emerged as the most promising analogue with the lowest Glide score, and its methoxy functional group forms hydrogen bonds with the key amino acid residues of the ATP-binding site. The compounds **3r** and **3p**, which displayed potent growth inhibition activity in the preliminary cytotoxicity assay, exhibited low binding free energy (-54.952 kcal mol^{-1}) with their trimethoxyphenyl groups making hydrophobic interactions with the binding pocket. In contrast, the 3-phenylpropenone analogues **3b**, **3n**, and **3e** displayed comparatively weak binding within the active site, al-

Table 2. Induced-fit docking of EDOT analogues with Chk2 kinase.

Compound	ΔG [kcal mol^{-1}]	Glide energy [kcal mol^{-1}]	H-bonding interactions	Hydrophobic interactions
3s	−9.578	−72.022	N–H···O (Met304) N–H···O (Lys224) N–H···O (Lys245) N–H···O (Asp368)	Ile251, Ile250, Leu301, Ile286, Leu226, Ala247, Leu354, Met304, Leu303, Leu236, Val234
3r	−8.215	−54.952	N–H···O (Met304) N–H···O (Thr367)	Leu354, Leu301, Ile286, Val234, Met304, Leu303, Ala247, Leu226
10	−6.113	−53.767	N–H···O (Met304) N–H···O (Lys249)	Ile286, Leu301, Val234, Ala247, Met304, Leu226, Leu303, Leu354
3p	−8.118	−52.025	N–H···O (Met304)	Ile299, Ala247, Leu301, Ile248, Met304, Leu303, Leu226, Leu354, Val234
3b	−6.603	−51.692	N–H···O (Met304) N–H···O (Lys249)	Leu354, Ile286, Leu301, Ala247, Leu303, Met304, Leu226, Val234
12	−6.951	−50.152	N–H···O (Met304) C–O···H (Glu308)	Val234, Leu354, Leu301, Ile286, Ala247, Met304, Leu236, Val246, Leu303, Leu226
3n	−7.762	−49.620	N–H···O (Met304)	Leu354, Ile299, Ala247, Ile286, Leu301, Val300, Ile248, Met304, Leu303, Leu226, Val234
3e	−7.021	−48.784	N–H···O (Met304)	Leu301, Ala247, Val234, Met304, Leu303, Leu236, Leu226, Leu354
Co-crystal[26a]	−9.031	−62.126	N–H···N (Met304) C–O···H (Met304) C–O···H (Glu308)	Val234, Leu301, Ile286, Ala247, Leu354, Phe310, Met304, Leu303, Leu226

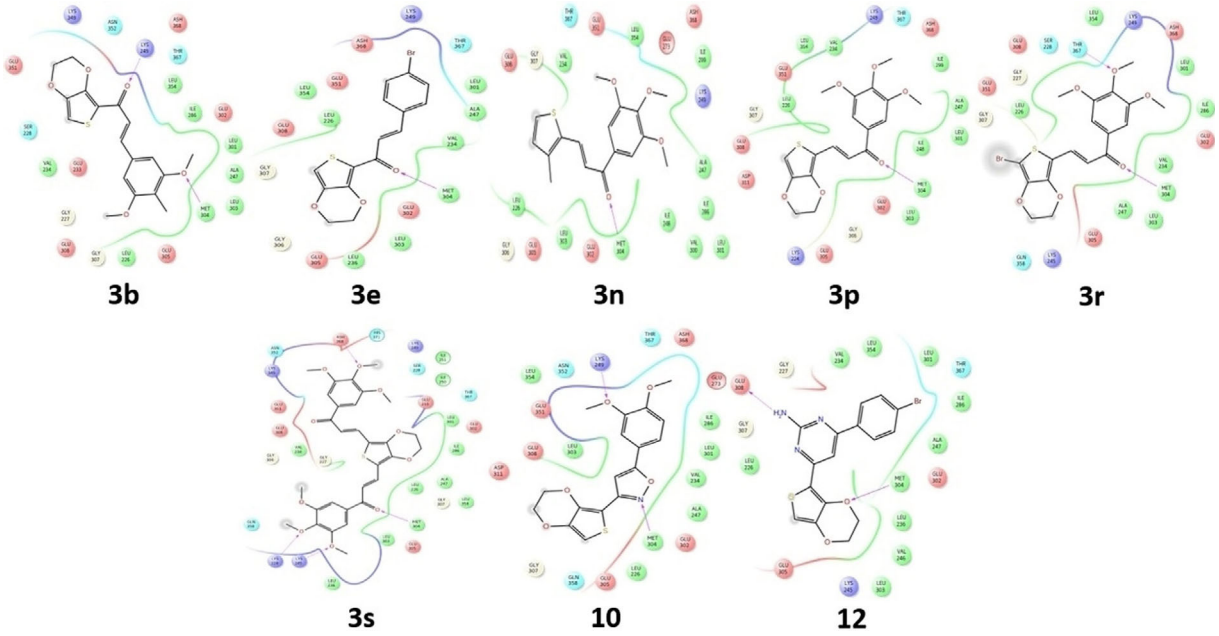


Figure 6. Key interacting residues of the ATP-binding domain of Chk2 kinase with the reported analogues.

though the carbonyl group of the enone maintains an essential hydrogen bond with the hinge region residue Met304. Thus, from these docking studies, it is clear that the reported derivatives also interacted with the ribose-binding domain of Chk2 kinase in a similar manner to 2-aminopyridines, which are known to inhibit the kinase activity of Chk2.

Conclusions

In summary, we synthesized various types of thiophene derivatives (**3a**–**u**) in good to excellent yields. The cytotoxic potencies of the reported EDOT derivatives were investigated in a panel of five human cancer cell lines. The structure–activity relationships determined with 23 compounds showed the functional requirement of the phenyl ring and the importance of the relative positions of the ketone and vinyl spacer for optimal cytotoxicity. Among the investigated compounds, **3p** and **3r** emerged as potent analogues displaying cytotoxicity at sub-micromolar concentrations on the tested tumor cell lines. In particular, **3p** exhibited antiproliferative activity similar to that of the reference antimitotic agent nocodazole. Another important observation was the 2,5-disubstituted EDOT **3r** having a cytotoxicity profile similar to that of 2-substituted EDOT **3p**, thus providing the possibility of avoiding the toxicological issues associated with unsubstituted thiophene analogues.[33] Consistent with the cytotoxicity assay, **3p** severely

affected the clonogenic potential of HCT116 cells with no colony growth observed at concentrations as low as 250 nM.

Cell-cycle analysis demonstrated the capacity of **3p** to arrest cells in the G2/M phase with a significant increase in the number of cells with >4N DNA content, indicating interference with mitotic function.[34] Interestingly, experiments with human topoisomerases identified **3p** as weakly inhibiting the hTopI-mediated relaxation of supercoiled DNA, thus representing an opportunity for the development of EDOT-enone-based topoisomerase inhibitors for cancer therapy. Finally, docking results suggest that the reported EDOT analogues interfere with the ATP binding pocket of Chk2 kinase catalytic domain in the manner reported for 2-aminopyrimidines. Furthermore, this study identified trimethoxyphenyl EDOT derivative **3p** as a lead compound with the potential for future drug development.

Experimental Section

Chemistry

All melting points are reported uncorrected. Progression of the reactions was monitored by thin-layer chromatography using hexane/ethyl acetate as an eluent. Column chromatography was carried out on silica gel (230–400 mesh, Merck) by using an eluent of increasing polarity. The ^{1}H, ^{13}C, and DEPT-135 NMR spectra were recorded in $CDCl_3$ using tetramethylsilane as an internal standard on a 300 MHz (Bruker Avance II) spectrometer at room temperature. Chemical shift values are quoted in parts per million (ppm) and coupling constants J are expressed in hertz (Hz). Elemental analysis data were recorded with a Elementar Vario Series Analyser instrument. Commercially available EDOT was purchased from Sigma–Aldrich and used as received in synthetic transformations. EDOT-2-carboxaldehyde (**5a**) and 2-acetyl-EDOT (**1**) were prepared using published procedures.[35]

General procedure for the synthesis of EDOT derivatives: The substituted benzaldehydes (1.0 mmol) were added to stirred solutions of 2-acetyl-EDOT (1, 1.0 mmol) in 10% NaOH in ethanol/water (2:1, 15 mL), and the reaction mixtures were heated at reflux for 1 h. Solid products precipitated and were filtered and washed with ethanol (10 mL) to give the respective 1,3-diaryl-2-propenone derivatives in good to excellent yields.

(*E*)-1-(2,3-Dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (3a): Condensation of **1** (0.18 g, 1.08 mmol) with veratraldehyde (**2a**, 0.20, 1.08 mmol) according to the general procedure afforded **3a** as a yellow solid (0.302 g, 84%). R_f = 0.28 (15% EtOAc/hexane); mp: 142–146 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.77 (d, J = 15.6 Hz, 1H, vinylic CH), 7.50 (d, J = 15.6 Hz, 1H, vinylic CH), 7.28–7.22 (m, 1H, ArH), 7.13 (s, 1H, ArH), 6.89 (d, J = 8.4 Hz, 1H, ArH), 6.72 (s, 1H, ArH), 4.44–4.41 (m, 2H, OCH_2), 4.29–4.27 (m, 2H, OCH_2), 3.94 (s, 3H, OCH_3), 3.92 ppm (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.1 (CO), 151.2 (C), 149.1 (C), 144.2 (C), 143.1 (CH),141.9 (C), 128.2 (C), 122.7 (CH), 121.8 (CH), 121.5 (C),111.2 (CH), 110.9 (CH), 108.9 (CH), 65.5 (OCH_2), 63.9 (OCH_2), 55.9 ppm (OCH_3); DEPT-135 NMR (75 MHz, $CDCl_3$): δ = 143.1, 122.7, 121.8, 111.2, 110.9, 108.9, 65.5, 63.9, 55.9 ppm; HRMS (ESI): m/z: calcd for $C_{17}H_{17}O_5S$: 333.0797 $[M+H]^+$, found: 333.0783l; elemental analysis calcd (%) for $C_{17}H_{16}O_5S$: C 61.43, H 4.85, found: C 61.62, H 4.67.

(*E*)-1-(2,3-Dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3b): Condensation of **1** (0.155 g, 0.93 mmol) with 3,4,5-trimethoxybenzaldehyde (**2b**, 0.20 g, 0.93 mmol) according to the general procedure afforded **3b** as a yellow solid (0.298 g, 88%). R_f = 0.25 (20% EtOAc/hexane); mp: 204–206 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.74 (d, J = 15.3 Hz, 1H, vinylic CH), 7.51 (d, J = 15.3 Hz, 1H, vinylic CH), 6.86 (s, 2H, ArH), 6.74 (s, 1H, ArH), 4.43–4.29 (m, 4H, OCH_2), 3.92 (s, 6H, OCH_3), 3.90 ppm (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.9 (CO), 153.4 (C), 144.3 (C), 143.1 (CH), 141.9 (C), 140.4 (C), 130.7 (C), 123.2 (CH), 121.3 (C), 109.1 (CH), 105.9 (CH), 65.5 (OCH_2), 63.9 (OCH_2), 60.9 (OCH_3), 56.3 ppm (OCH_3); HRMS (ESI): m/z: calcd for $C_{18}H_{19}O_6S$: 363.0902 $[M+H]^+$, found: 363.0916; elemental analysis calcd (%) for $C_{18}H_{18}O_6S$: C 59.66, H 5.01, found: C 59.83, H 4.88.

(*E*)-3-(4-Chlorophenyl)-1-(2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)prop-2-en-1-one (3c): Condensation of **1** (0.236 g, 1.42 mmol) with 4-chlorobenzaldehyde (**2c**, 0.20 g, 1.42 mmol) according to the general procedure afforded **3c** as a yellow solid (0.352 g, 86%). R_f = 0.32 (15% EtOAc/hexane); mp: 152–154 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.69 (d, J = 15.6 Hz, 1H, vinylic CH), 7.53 (d, J = 15.9 Hz, 1H, vinylic CH), 7.48 (d, J = 8.4 Hz, 2H, ArH), 7.29 (d, J = 8.4 Hz, 2H, ArH), 6.68 (s, 1H, ArH), 4.38–4.21 ppm (m, 4H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.8 (CO), 144.5 (C), 141.9 (C), 141.4 (CH), 136.1 (C), 133.7 (C), 129.7 (CH), 129.1 (CH), 124.3 (CH), 121.3 (C), 109.5 (CH), 65.5 (OCH_2), 63.9 ppm (OCH_2); HRMS (ESI): m/z: calcd for $C_{15}H_{12}ClO_3S$: 307.0196 $[M+H]^+$, found: 307.0209; elemental analysis calcd (%) for $C_{15}H_{11}ClO_3S$: C 58.73, H 3.61, found: C 58.93, H 3.76.

(*E*)-3-(4-Bromophenyl)-1-(2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)prop-2-en-1-one (3d): Condensation of **1** (0.179 g, 1.08 mmol) with 4-bromobenzaldehyde (**2d**, 0.20 g, 1.08 mmol) according to the general procedure afforded **3d** as a yellow solid (0.303 g, 84%). R_f = 0.30 (15% EtOAc/hexane); mp: 170–172 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.73 (d, J = 15.9 Hz, 1H, vinylic CH), 7.61 (d, J = 15.6 Hz, 1H, vinylic CH), 7.54–7.46 (m, 4H, ArH), 6.74 (s, 1H, ArH), 4.46–4.43 (m, 2H, OCH_2), 4.29–4.26 ppm (m, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.8 (CO), 144.5 (C), 141.8 (C), 141.4 (CH), 134.1 (C), 132.0 (CH), 129.8 (CH), 124.4 (C), 124.3 (CH), 121.2 (C), 109.5 (CH), 65.5 (OCH_2), 63.9 ppm (OCH_2); HRMS (ESI): m/z: calcd for $C_{15}H_{12}BrO_3S$: 350.9691 $[M+H]^+$, found: 350.9689; elemental analysis calcd (%) for $C_{15}H_{11}BrO_3S$: C 51.30, H 3.16, found: C 51.48, H 3.28.

(*E*)-3-(3-Bromo-4-fluorophenyl)-1-(2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)prop-2-en-1-one (3e): Condensation of **1** (0.18 g, 0.98 mmol) with 3-bromo-4-fluorobenzaldehyde (**2e**, 0.20 g, 0.98 mmol) according to the general procedure afforded **3e** as a yellow solid (0.317 g, 87%). R_f = 0.27 (15% EtOAc/hexane); mp: 176–178 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.83–7.81 (m, 1H, ArH), 7.70 (d, J = 15.6 Hz, 1H, vinylic CH), 7.57–7.52 (m, 2H, ArH), 7.17–7.12 (m, 1H, ArH), 6.76 (s, 1H, ArH), 4.47–4.45 (m, 2H, OCH_2), 4.28–4.27 ppm (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.6 (CO), 143.3 (d, $^{1}J_{CF}$ = 204.8 Hz), 140.1 (CH), 133.1 (CH), 133.0 (d, $^{4}J_{CF}$ = 2.6 Hz), 129.3 (d, $^{2}J_{CF}$ = 7.1 Hz), 124.7 (d, $^{3}J_{CF}$ = 2.7 Hz), 121.2 (C), 116.9 (d, $^{2'}J_{CF}$ = 22.7 Hz), 109.7 (d, $^{3'}J_{CF}$ = 5.3 Hz), 65.6 (OCH_2), 63.9 ppm (OCH_2); DEPT-135 NMR (75 MHz, $CDCl_3$): δ = 140.1, 133.1, 129.3 (d, $^{3'}J_{CF}$ = 7.1 Hz), 124.7 (d, $^{3}J_{CF}$ = 2.7 Hz), 116.9 (d, $^{2'}J_{CF}$ = 22.7 Hz), 109.7, 65.6, 64.0 ppm; HRMS (ESI): m/z: calcd for $C_{15}H_{11}BrFO_3S$: 368.9596 $[M+H]^+$, found: 368.9610; elemental analysis calcd (%) for $C_{15}H_{10}BrFO_3S$: C 48.80, H 2.73, found: C 48.96, H 2.90.

(*E*)-3-(4-Chloro-3-fluorophenyl)-1-(2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)prop-2-en-1-one (3 f): Condensation of **1** (0.23 g, 1.26 mmol) with 4-chloro-3-fluorobenzaldehyde (**2 f**, 0.20 g, 1.26 mmol) according to the general procedure afforded **3 f** as a yellow solid (0.348 g, 85 %). R_f = 0.29 (15 % EtOAc/hexane); mp: > 300 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.82 (d, J = 15.6 Hz, 1 H, vinylic CH), 7.51 (d, J = 15.9 Hz, 1 H, vinylic CH), 7.36–7.19 (m, 3 H, ArH), 6.68 (s, 1 H, ArH), 4.22–4.19 (m, 2 H, OCH_2), 4.39–4.37 ppm (m, 2 H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.5 (CO), 158.3 (d, $^1J_{CF}$ = 247.7 Hz), 144.7 (C), 141.9 (C), 140.3 (d, $^4J_{CF}$ = 2.5 Hz), 135.9 (d, $^{3'}J_{CF}$ = 6.8 Hz), 131.0 (CH), 125.4 (C), 125.3 (d, $^3J_{CF}$ = 3.4 Hz), 122.7 (d, $^2J_{CF}$ = 17.9 Hz), 121.2 (C), 115.4 (d, $^{2'}J_{CF}$ = 21.4 Hz), 109.8 (CH), 65.6 (OCH_2), 63.9 ppm (OCH_2); DEPT-135 NMR (75 MHz, $CDCl_3$): δ = 140.3 (d, $^4J_{CF}$ = 2.5 Hz), 131.0, 125.3 (d, $^3J_{CF}$ = 3.4 Hz), 125.2, 115.4 (d, $^{2'}J_{CF}$ = 21.4 Hz), 109.9, 65.6, 63.9 ppm; HRMS (ESI): *m/z*: calcd for $C_{15}H_{11}ClFO_4S$: 325.0101 $[M+H]^+$, found: 325.0119; elemental analysis calcd (%) for $C_{15}H_{10}ClFO_3S$: C 55.48, H 3.10, found: C 55.63, H 3.28.

(*E*)-3-(2,6-Dichlorophenyl)-1-(2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)prop-2-en-1-one (3 g): Condensation of **1** (0.21 g, 1.14 mmol) with 2,6-dichlorobenzaldehyde (**2 g**, 0.20 g, 1.14 mmol) according to the general procedure afforded **3 g** as a yellow solid (0.32 g, 82 %). R_f = 0.32 (15 % EtOAc/hexane); mp: > 300 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.89 (d, J = 15.6 Hz, 1 H, vinylic CH), 7.80 (d, J = 15.9 Hz, 1 H, vinylic CH), 7.37–7.18 (m, 3 H, ArH), 6.76 (s, 1 H, ArH), 4.38 (s, 2 H, OCH_2), 4.26 ppm (m, 4 H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.7 (CO), 144.9 (C), 141.9 (C), 136.0 (CH), 135.3 (C), 132.9 (C), 131.8 (CH), 129.6 (CH), 128.8 (CH), 121.0 (C), 109.8 (CH), 65.4 (OCH_2), 63.9 ppm (OCH_2); HRMS (ESI): *m/z*: calcd for $C_{15}H_{11}Cl_2O_3S$: 340.9806 $[M+H]^+$, found: 340.9818; elemental analysis calcd (%) for $C_{15}H_{10}Cl_2O_3S$: C 52.80, H 2.95, found: C 53.01, H 2.79.

(*E*)-1-(2,3-Dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)-3-(thiophen-2-yl)prop-2-en-1-one (3 h): Condensation of **1** (0.33 g, 1.78 mmol) with thiophene-2-carboxaldehyde (**2 h**, 0.20 g, 1.78 mmol) according to the general procedure afforded **3 h** as a red solid (0.413 g, 83 %). R_f = 0.38 (15 % EtOAc/hexane); mp: 134–136 °C (lit. 133–134 °C[36]); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.93 (d, J = 15.3 Hz, 1 H, vinylic CH), 7.44 (d, J = 15.6 Hz, 1 H, vinylic CH), 7.39 (d, J = 4.8 Hz, 1 H, ArH), 7.33 (d, J = 3.3 Hz, 1 H, ArH), 7.09–7.06 (m, 1 H, ArH), 6.72 (s, 1 H, ArH), 4.45–4.42 (m, 2 H, OCH_2), 4.28–4.26 ppm (s, 2 H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.6 (CO), 144.3 (C), 141.9 (C), 140.7 (C), 135.4 (CH), 131.6 (CH), 128.4 (CH), 128.3 (CH), 122.9 (CH), 121.3 (C), 109.1 (CH), 65.5 (OCH_2), 63.9 ppm (OCH_2); HRMS (ESI): *m/z*: calcd for $C_{13}H_{11}O_3S_2$: 279.0150 $[M+H]^+$, found: 279.0165; elemental analysis calcd (%) for $C_{13}H_{10}O_3S_2$: C 56.10, H 3.62, found: C 56.30, H 3.52.

(*E*)-1,3-Bis(2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)prop-2-en-1-one (3 i): Condensation of **1** (0.22 g, 1.17 mmol) with EDOT-2-carboxaldehyde (**5 a**, 0.20 g, 1.17 mmol) according to the general procedure afforded **3 i** as a yellow solid (0.308 g, 78 %). R_f = 0.25 (20 % EtOAc/hexane); mp: 196–198 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.86 (d, J = 15.3 Hz, 1 H, vinylic CH), 7.36 (d, J = 15.3 Hz, 1 H, vinylic CH), 6.67 (s, 1 H, ArH), 6.42 (s, 1 H, ArH), 4.41–4.22 ppm (m, 8 H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 180.7 (CO), 144.0 (C), 143.6 (C), 142.3 (C), 141.9 (C), 132.1 (CH), 121.7 (C), 120.6 (CH), 115.7 (C), 108.3 (CH), 102.7 (CH), 65.4 (OCH_2), 65.1 (OCH_2), 64.6 (OCH_2), 64.1 ppm (OCH_2); HRMS (ESI): *m/z*: calcd for $C_{15}H_{13}O_5S_2$: 337.0204 $[M+H]^+$, found: 337.0221; elemental analysis calcd (%) for $C_{15}H_{12}O_5S_2$: C 53.56, H 3.60, found: C 53.71, H 3.73.

(*E*)-3-(3,4-Dimethoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (3 j): Condensation of 2-acetylthiophene (**4 a**, 0.15 g, 1.20 mmol) with **2 a** (0.20 g, 1.20 mmol) according to the general procedure afforded **3 j** as a yellow solid (0.28 g, 85 %). R_f = 0.24 (10 % EtOAc/hexane); mp: 76–78 °C (lit.102–103 °C[37]); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.87 (d, J = 3.6 Hz, 1 H, ArH), 7.79 (d, J = 15.3 Hz, 1 H, vinylic CH), 7.66 (d, J = 4.8 Hz, 1 H, ArH), 7.32 (s, 1 H, ArH), 7.25–7.15 (m, 3 H, ArH), 6.89 (d, J = 8.1 Hz, 1 H, ArH), 3.95 (s, 3 H, OCH_3), 3.92 ppm (s, 3 H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 182.0 (CO), 151.5 (C), 149.2 (C), 145.7 (C), 144.2 (CH), 133.6 (CH), 131.6 (CH), 128.2 (CH), 127.7 (C), 123.2 (CH), 119.5 (CH), 111.2 (CH), 110.3 (CH), 56.0 (OCH_3), 55.9 ppm (OCH_3); HRMS (ESI): *m/z*: calcd for $C_{15}H_{15}O_3S$: 275.0742 $[M+H]^+$, found: 275.0750; elemental analysis calcd (%) for $C_{15}H_{14}O_3S$: C 65.67, H 5.14, found: C 65.80, H 5.07.

(*E*)-3-(4-Chlorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one (3 k): Condensation of **4 a** (0.18 g, 1.42 mmol) with **2 c** (0.20 g, 1.42 mmol) according to the general procedure afforded **3 k** as a colorless solid (0.295 g, 83 %). R_f = 0.34 (10 % EtOAc/hexane); mp: 112–114 °C (lit. 110–111 °C[38]); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.88 (d, J = 3.3 Hz, 1 H, ArH), 7.78 (d, J = 15.6 Hz, 1 H, vinylic CH), 7.69 (d, J = 4.8 Hz, 1 H, ArH), 7.57 (d, J = 8.4 Hz, 1 H, ArH), 7.41–7.36 (m, 3 H, ArH), 7.18 ppm (t, J = 4.2 Hz, 1 H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.8 (CO), 145.4 (C), 142.6 (CH), 136.5 (C), 134.1 (CH), 133.2 (C), 131.9 (CH), 129.7 (CH), 129.3 (CH), 128.4 (CH), 122.1 ppm (CH); HRMS (ESI): *m/z*: calcd for $C_{13}H_{10}ClOS$: 249.0141 $[M+H]^+$, found: 249.0135; elemental analysis calcd (%) for $C_{13}H_9ClOS$: C 62.78, H 3.65, found: C 62.89, H 3.54.

(*E*)-3-(3-Bromo-4-fluorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one (3 l): Condensation of **4 a** (0.12 g, 0.98 mmol) with **2 e** (0.20 g, 0.98 mmol) according to the general procedure afforded **3 l** as a colorless solid (0.245 g, 80 %). R_f = 0.29 (10 % EtOAc/hexane); mp: 142–144 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.79 (d, J = 3.6 Hz, 1 H, ArH), 7.76–7.73 (m, 1 H, ArH), 7.65–7.60 (m, 2 H, ArH), 7.47–7.42 (m, 1 H, ArH), 7.25 (d, J = 15.6 Hz, 1 H, vinylic CH), 7.11–7.04 ppm (m, 2 H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 181.4 (CO), 160.7 (d, $^1J_{CF}$ = 251.1 Hz), 145.1 (C), 141.1 (CH), 134.2 (CH), 133.0 (CH), 132.4 (d, $^3J_{CF}$ = 3.9 Hz), 132.0 (CH), 129.3 (d, $^{3'}J_{CF}$ = 7.6 Hz), 128.3 (CH), 122.4 (d, $^4J_{CF}$ = 2.3 Hz), 117.0 (d, $^{2'}J_{CF}$ = 22.7 Hz), 109.8 ppm (d, $^2J_{CF}$ = 21.5 Hz); DEPT 135-NMR (75 MHz, $CDCl_3$): δ = 141.1, 134.4, 133.1, 132.1, 129.3 (d, $^{3'}J_{CF}$ = 7.6 Hz), 128.4, 122.4 (d, $^3J_{CF}$ = 2.3 Hz), 117.0 ppm (d, $^{2'}J_{CF}$ = 22.7 Hz); HRMS (ESI): *m/z*: calcd for $C_{13}H_9BrFOS$: 310.9542 $[M+H]^+$, found: 310.9549; elemental analysis calcd (%) for $C_{13}H_8BrFOS$: C 50.18, H 2.59, found: C 50.29, H 2.48.

(*E*)-3-(Thiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3 m): Condensation of **2 b** (0.38 g, 1.78 mmol) with **2 h** (0.20 g, 1.78 mmol) according to the general procedure afforded **3 m** as a red solid (0.455 g, 84 %). R_f = 0.30 (20 % EtOAc/hexane); mp: 82–84 °C (lit. 69–71 °C[39]); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.95 (d, J = 15.3 Hz, 1 H, vinylic CH), 7.43 (d, J = 5.1 Hz, 1 H, ArH), 7.38 (d, J = 3.6 Hz, 1 H, ArH), 7.31–7.26 (m, 3 H, ArH), 7.11 (t, J = 4.2 Hz, 1 H, ArH), 3.95 ppm (s, 9 H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.4 (CO), 153.0 (C), 142.5 (C), 140.2 (C), 136.9 (CH), 133.3 (C), 131.8 (CH), 128.6 (CH), 128.2 (CH), 120.3 (CH), 106.0 (CH), 60.8 (OCH_3), 56.3 ppm (OCH_3); HRMS (ESI): *m/z*: calcd for $C_{16}H_{17}O_4S$: 305.0848 $[M+H]^+$, found: 305.0860; elemental analysis calcd (%) for $C_{16}H_{16}O_4S$: C 63.14, H 5.30, found: C 63.33, H 5.11.

(*E*)-3-(3-Methylthiophen-2-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3 n): Condensation of **2 b** (0.33 g, 1.59 mmol) with 3-methyl-thiophene-2-carboxaldehyde (0.20 g, 1.59 mmol) according to the general procedure afforded **3 n** as a red oil (0.435 g, 86 %).

R_f = 0.33 (20% EtOAc/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.04 (d, J = 15.0 Hz, 1H, vinylic CH), 7.31 (d, J = 5.1 Hz, 1H, ArH), 7.26–7.20 (m, 3H, ArH), 6.92 (d, J = 4.8 Hz, 1H, ArH), 3.96 (s, 6H, OCH_3) 3.94 (s, 3H, OCH_3), 2.41 ppm (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.6 (CO), 153.1 (C), 142.8 (C), 142.4 (C), 135.5 (CH), 134.5 (C), 133.6 (C), 131.5 (CH), 127.3 (CH), 119.5 (CH), 106.0 (CH), 60.9 (OCH_3), 56.4 (OCH_3), 14.3 ppm (CH_3); HRMS (ESI): *m/z*: calcd for $C_{17}H_{19}O_4S$: 319.1004 $[M+H]^+$, found: 319.1018; elemental analysis calcd (%) for $C_{17}H_{18}O_4S$: C 64.13, H 5.70, found: C 64.32, H 5.86.

(*E*)-3-[(2,2′-Bithiophen)-5-yl]-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3o): Condensation of **2b** (0.22 g, 1.03 mmol) with 2,2′-bithiophene-5-carboxaldehyde (0.20 g, 1.03 mmol) according to the general procedure afforded **3o** as a yellow solid (0.35 g, 88%). R_f = 0.30 (20% EtOAc/hexane); mp: 132–134 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.90 (d, J = 15.0 Hz, 1H, vinylic CH), 7.31–7.26 (m, 6H, ArH), 7.18 (d, J = 3.9 Hz, 1H, ArH), 7.07–7.05 (m, 1H, ArH), 3.97 (s, 6H, OCH_3) 3.94 ppm (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.3 (CO), 153.2 (C), 142.5 (C), 140.8 (2C), 138.9 (C), 136.9 (CH), 136.7 (C), 133.5 (CH), 128.2 (CH), 125.9 (CH), 124.9 (CH), 124.6 (CH), 119.9 (CH), 106.0 (CH), 60.9 (OCH_3), 56.4 ppm (OCH_3); HRMS (ESI): *m/z*: calcd for $C_{20}H_{19}O_4S_2$: 387.0725 $[M+H]^+$, found: 387.0740; elemental analysis calcd (%) for $C_{20}H_{18}O_4S_2$: C 62.16, H 4.69, found: C 62.36, H 4.59.

(*E*)-3-(2,3-Dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3p): Condensation of **6a** (0.25 g, 1.18 mmol) with EDOT-2-carboxaldehyde (**5a**, 0.20 g, 1.18 mmol) according to the general procedure afforded **3p** as a yellow solid (0.365 g, 86%). R_f = 0.25 (20% EtOAc/hexane); mp: 148–150 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.85 (d, J = 15.3 Hz, 1H, vinylic CH), 7.27–7.23 (m, 3H, ArH), 6.50 (s, 1H, ArH), 4.36–4.34 (m, 2H, OCH_2), 4.26–4.24 (m, 2H, OCH_2), 3.95 (s, 6H, OCH_3), 3.93 ppm (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.9 (CO), 153.1 (C), 143.8 (2C), 142.2 (C), 133.9 (CH), 133.9 (C), 118.3 (CH), 115.2 (C), 106.0 (CH), 103.4 (CH), 65.1 (OCH_2), 64.5 (OCH_2), 60.9 (OCH_3), 56.4 ppm (OCH_3); HRMS (ESI): *m/z*: calcd for $C_{18}H_{19}O_6S$: 363.0902 $[M+H]^+$, found: 363.0919; elemental analysis calcd (%) for $C_{18}H_{18}O_6S$: C 59.66, H 5.01, found: C 59.80, H 5.11.

(*E*)-3-(2,3-Dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (3q): Condensation of **6b** (0.21 g, 1.17 mmol) with **5a** (0.20 g, 1.17 mmol) according to the general procedure afforded **3q** as a yellow solid (0.332 g, 85%). R_f = 0.28 (15% EtOAc/hexane); mp: 190–192 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.84 (d, J = 15.3 Hz, 1H, vinylic CH), 7.67–7.61 (m, 2H, ArH), 7.34 (d, J = 15.3 Hz, 1H, ArH), 6.92 (d, J = 8.4 Hz, 1H, ArH), 6.48 (s, 1H, ArH), 4.36–4.34 (m, 2H, OCH_2), 4.27–4.24 (m, 2H, OCH_2), 3.96 ppm (s, 6H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.2 (CO), 153.0 (C), 149.2 (C), 143.5 (C), 142.2 (C), 133.2 (CH), 131.6 (C), 122.8 (CH), 118.3 (CH), 115.4 (C), 110.8 (CH), 109.9 (CH), 103.0 (CH), 65.1 (OCH_2), 64.5 (OCH_2), 56.1 (OCH_3), 56.0 ppm (OCH_3); HRMS (ESI): *m/z*: calcd for $C_{17}H_{17}O_5S$: 333.0797 $[M+H]^+$, found: 333.0787; elemental analysis calcd (%) for $C_{17}H_{16}O_5S$: C 61.43, H 4.85, found: C 61.63, H 4.73.

7-Bromo-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-carboxaldehyde (7): *N*-Bromosuccinimide (0.46 g, 2.59 mmol) was added to a stirred solution of **5a** (0.40 g, 2.35 mmol) in DMF (10 mL), and the mixture was stirred (shielded from light) at room temperature for 14 h. After completion of reaction, the mixture was poured over crushed ice (50 g) and stirred for 10 min. The resulting solid was filtered, washed with water, and dried. The crude **7** (0.45 g) was used in the next step without further purification.

(*E*)-3-(7-Bromo-2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3r): Condensation of **6a** (0.17 g, 0.80 mmol) with crude **7** (0.20 g, 0.80 mmol) according to the general procedure afforded **3r** as a green solid (0.275 g, 78%). R_f = 0.23 (25% EtOAc/hexane); mp: 168–170 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.97 (d, J = 15.3 Hz, 1H, vinylic CH), 7.23 (s, 2H, ArH), 7.15 (d, J = 15.3 Hz, 1H, vinylic CH), 4.34 (s, 4H, OCH_2), 3.95 (s, 6H, OCH_3), 3.93 ppm (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.5 (CO), 153.1 (C), 142.9 (C), 142.4 (C), 140.6 (C), 133.7 (C), 132.7 (CH), 118.3 (CH), 115.4 (C), 106.0 (CH), 92.2 (CBr), 65.0 (OCH_2), 64.9 (OCH_2), 60.9 (OCH_3), 56.4 ppm (OCH_3); HRMS (ESI): *m/z*: calcd for $C_{18}H_{18}BrO_6S$: 441.0007 $[M+H]^+$, found: 441.0019; elemental analysis calcd (%) for $C_{18}H_{17}BrO_6S$: C 48.99, H 3.88, found: C 49.18, H 3.74.

(2*E*,2′*E*)-3,3′-(2,3-Dihydrothieno[3,4-*b*][1,4]dioxine-5,7-diyl)bis[1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one] (3s): Condensation of **6a** (0.45 g, 2.12 mmol) with EDOT-2,5-dicarboxaldehyde (0.20 g, 1.01 mmol) according to the general procedure afforded **3s** as a yellow solid (0.435 g, 74%). R_f = 0.29 (40% EtOAc/hexane); mp: 246–248 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.86 (d, J = 15.0 Hz, 2H, vinylic CH), 7.34–7.26 (m, 6H, ArH), 4.41 (s, 4H, OCH_2), 3.96 ppm (s, 18H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.4 (CO), 153.2 (C), 143.7 (C), 142.6 (C), 133.5 (C), 132.7 (CH), 120.3 (CH), 117.8 (C), 106.2 (CH), 65.0 (OCH_2), 60.9 (OCH_2), 56.5 (OCH_3), 56.4 ppm (OCH_3); HRMS (ESI): *m/z*: calcd for $C_{30}H_{31}O_{10}S$: 583.1638 $[M+H]^+$, found: 583.1650; elemental analysis calcd (%) for $C_{30}H_{30}O_{10}S$: C 61.85, H 5.19, found: C 61.99, H 5.08.

(2,3-Dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)(3,4,5-trimethoxyphenyl)methanone (3t): *N*,*N*-Dimethylformamide (1 drop) was added to a solution of EDOT-2-carboxylic acid (0.25 g, 1.34 mmol) in thionyl chloride (0.15 mL, 2.0 mmol) at 0 °C. The reaction mixture was then heated in a water bath (90 °C) for 30 min. Subsequent removal of excess thionyl chloride under reduced pressure gave the crude acid chloride as a colorless liquid (0.24 g), which was used in the next step without further purification.

Tin(IV) chloride (0.14 mL, 1.19 mmol) was slowly added to a solution of the crude EDOT acid chloride (0.24 g) in anhydrous CH_2Cl_2 (10 mL) at 0 °C. To this, a solution of 3,4,5-trimethoxybenzene (0.225 g, 1.34 mmol) in anhydrous CH_2Cl_2 (5.0 mL) was added and the mixture was stirred at room temperature for 3 h. After completion of the reaction (monitored by TLC), the mixture was poured into ice-water (15 mL) containing conc. HCl (5.0 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2×5 mL). The combined organic layers were washed with water and dried (Na_2SO_4). Purification of the crude product by column chromatography (silica gel, 20% ethyl acetate in hexane) afforded **3t** as a green oil (0.315 g, 70%). R_f = 0.40 (20% EtOAc/hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.10 (s, 1H, ArH), 6.96 (s, 2H, ArH), 4.30–4.28 (m, 4H, OCH_2), 3.94 (s, 3H, OCH_3) 3.89 ppm (s, 6H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 184.3 (CO), 155.9 (C), 152.2 (C), 144.3 (C), 141.8 (C), 138.3 (C), 127.4 (C), 123.8 (CH), 106.6 (CH), 65.1 (OCH_2), 64.4 (OCH_2), 62.2 (OCH_3), 60.9 (OCH_3), 56.1 ppm (OCH_3); HRMS (ESI): *m/z*: calcd for $C_{16}H_{17}O_6S$: 337.0746 $[M+H]^+$, found: 337.0752; elemental analysis calcd (%) for $C_{16}H_{16}O_6S$: C 57.13, H 4.79, found: C 57.31, H 4.60.

7-(3,4-Dimethoxyphenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine-5-carboxaldehyde: 3,4-Dimethoxyphenylboronic acid (0.145 g, 0.80 mmol), $Pd(PPh_3)_4$ (0.093 g, 0.08 mmol), K_2CO_3 (0.22 g, 1.60 mmol), and H_2O (2 drops) were added to a solution of 5-bromo-EDOT-2-carboxaldehyde (0.20 g, 0.80 mmol) in DMF (15 mL). The reaction mixture was degassed and stirred at 80 °C for 2 days. The resulting black solution was passed through a Celite pad and the pad was washed with CH_2Cl_2 (10 mL). The filtrate was washed

with cold water (20 mL) and the aqueous layer was extracted with CH_2Cl_2 (2×5 mL). The combined organic layers were washed with water and dried (Na_2SO_4). Evaporation of solvent followed by column chromatographic purification (silica gel, 25% ethyl acetate in hexane) afforded 5-(3,4-dimethoxyphenyl)EDOT-2-carboxaldehyde as a red solid (0.184 g, 75%). R_f = 0.36 (15% EtOAc/hexane); mp: 190–192 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.91 (s, 1H, CHO), 7.39 (d, J = 8.4 Hz, 1H, ArH), 7.31 (s, 1H, ArH), 6.90 (d, J = 8.4 Hz, 1H, ArH), 4.41–4.39 (m, 4H, OCH_2), 3.92 ppm (s, 6H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.4 (CHO), 149.7 (C), 149.1 (C), 149.1 (C), 136.9 (C), 129.3 (C), 124.6 (C), 120.1 (CH), 114.9 (C), 111.3 (CH), 110.2 (CH), 65.2 (OCH_2), 64.6 (OCH_2), 56.0 ppm (OCH_3); HRMS (ESI): m/z: calcd for $C_{15}H_{15}O_5S$: 307.0640 $[M+H]^+$, found: 307.0653.

(*E*)-3-[7-(3,4-Dimethoxyphenyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-yl]-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (3u): Condensation of **6a** (0.10 g, 0.49 mmol) with 5-(3,4-dimethoxyphenyl)EDOT-2-carboxaldehyde (0.15 g, 0.49 mmol) according to the general procedure afforded **3u** as a red solid (0.20 g, 82%). R_f = 0.29 (25% EtOAc/hexane); mp: 172–174 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.93 (d, J = 14.7 Hz, 1H, vinylic CH), 7.31–7.18 (m, 5H, ArH), 6.90 (d, J = 7.8 Hz, 1H, ArH), 4.39 (m, 4H, OCH_2), 3.96 ppm (s, 15H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.6 (CO), 153.1 (C), 149.1 (C), 144.7 (2C), 142.2 (C), 137.7 (C), 134.1 (C), 133.6 (CH), 125.2 (C), 121.7 (C), 119.5 (CH), 117.3 (CH), 112.3 (C), 111.4 (CH), 109.9 (CH), 106.0 (CH), 64.9 (OCH_2), 64.7 (OCH_2), 60.9 (OCH_3), 56.4 (OCH_3), 56.0 ppm (OCH_3); HRMS (ESI): m/z: calcd for $C_{26}H_{27}O_8S$: 499.1427 $[M+H]^+$, found: 499.1443; elemental analysis calcd (%) for $C_{26}H_{26}O_8S$: C 62.64, H 5.26, found: C 62.84, H 5.12.

3-(2,3-Dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)-5-(3,4-dimethoxyphenyl)isoxazole (10): Hydroxylamine hydrochloride (**9**, 0.05 g, 0.72 mmol) and K_2CO_3 (0.17 g, 1.23 mmol) were added to a solution of enone **3a** (0.20 g, 0.60 mmol) in THF (15 mL). Then, the reaction mixture was heated at reflux for 2 h. After completion of reaction, the mixture was poured into ice-water (20 mL) and stirred for 10 min. The resulting solid was filtered and triturated with methanol (10 mL) to give isoxazole **10** as a yellow solid (0.146 g, 70%). R_f = 0.24 (10% EtOAc/hexane); mp: 110–112 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38 (s, 1H, ArH), 7.29 (d, J = 8.1 Hz, 1H, ArH), 6.89 (d, J = 8.1 Hz, 1H, ArH), 6.67 (s, 1H, ArH), 6.43 ppm (s, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.2 (C), 162.6 (C), 150.6 (C), 149.3 (C), 141.7 (C), 140.3 (C), 121.9 (C), 120.0 (CH), 111.1 (CH), 109.5 (CH), 104.8 (C), 101.9 (CH), 97.3 (CH), 65.2 (OCH_2), 64.5 (OCH_2), 56.1 (OCH_3), 55.9 ppm (OCH_3); HRMS (ESI): m/z: calcd for $C_{17}H_{16}NO_5S$: 346.0749 $[M+H]^+$, found: 346.0762; elemental analysis calcd (%) for $C_{17}H_{15}NO_5S$: C 59.12, H 4.38, N 4.06, found: C 59.31, H 4.49, N 4.19.

4-(4-Bromophenyl)-6-(2,3-dihydrothieno[3,4-*b*][1,4]dioxin-5-yl)-pyrimidin-2-amine (12): Guanidine hydrochloride (**11**, 0.08 g, 0.84 mmol) was added to a solution of enone **3d** (0.20 g, 0.57 mmol) in 10% NaOH in ethanol/water (2:1, 30 mL), and the reaction mixture was heated at reflux for 2 h. The resulting precipitate was filtered and washed with ethanol (5.0 mL) to give pyrimidine **12** as a yellow solid (0.12 g, 60%). R_f = 0.30 (25% EtOAc/hexane); mp: >300 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.84 (d, J = 8.4 Hz, 2H, ArH), 7.57 (s, 1H, ArH), 7.53 (d, J = 8.7 Hz, 2H, ArH), 6.46 (s, 1H, ArH), 5.0 (s, 2H, NH_2), 4.37–4.34 (m, 2H, OCH_2), 4.23–4.20 ppm (m, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5 (C), 162.9 (C), 159.5 (C), 142.6 (C), 142.0 (C), 136.8 (C), 131.8 (CH), 128.7 (CH), 124.8 (C), 117.1 (C), 103.8 (CH), 103.7 (CH), 65.3 (OCH_2), 64.3 ppm (OCH_2); HRMS (ESI): m/z: calcd for $C_{16}H_{13}BrN_3O_2S$: 389.9912 $[M+H]^+$, found: 389.9926; elemental analysis calcd (%) for $C_{16}H_{12}BrN_3O_2S$: C 49.24, H 3.10, N 10.77, found: C 49.42, H 3.29, N 10.94.

Biological studies

Cell lines and cytotoxicity assays: Human cancer cell lines selected for the study—HCT116 (colon cancer), NCI-H460 (lung cancer), U251 (glioblastoma), HeLa (cervical cancer), and MCF7 (breast cancer)—were purchased from ATCC (Manassas, VA, USA) and cultured following the manufacturer's protocol. The above tumor cell lines were grown in DMEM medium containing 10% fetal bovine serum supplemented with L-glutamine (2 mM) and penicillin–streptomycin antibiotic solution at 37 °C with 5% CO_2. For cytotoxicity assays, cells were seeded at densities of 1500–3000 cells per well in 96-well cell culture plates. Stock concentrations of EDOT analogues (20 mM) were made in DMSO and diluted in the culture media to working concentrations starting from 20 μM. Thus, test compounds at concentrations ranging from 20 μM to 1.2 nM were added to overnight adhered cells and incubated for a further 48 h at 37 °C. The antiproliferative effects of the test compounds were assessed using a sulforhodamine B assay[40] following the protocol developed by the NCI.[41] The growth inhibitory parameter GI_{50} value represents the time-zero-corrected drug concentration required to cause 50% growth inhibition relative to that of vehicle control. The standard deviation from at least two independent experiments were calculated using Microsoft Excel.

Clonogenic cell survival assay: Clonogenic assays were performed on HCT116 and NCI-H460 cells according to the reported procedure.[42] In brief, EDOT analogues at different concentrations were incubated with cells seeded at a very low density (100 cells per well of a six-well plate), followed by washing with Dulbecco's phosphate-buffered saline (DPBS) and culturing the drug-treated cells for a further 12 days in DMEM culture medium. The emerging colonies at the end of the assay were stained with crystal violet and photographed.

Flow cytometry: Cell-cycle analysis was performed on HCT116 cells collected at the end of 24 and 48 h post-treatment with EDOT enones (2 μM). The collected cells were washed with DPBS and fixed using 70% ethanol solution. Immediately prior to staining, the cells were washed twice with DPBS and stained with propidium iodide (20 μg mL^{-1}) in DPBS containing Triton X-100 (0.1% *v*/*v*) and RNase A (0.2 mg mL^{-1}) for 1 h at room temperature. The populations of cells in different phases was analyzed using a Beckman–Coulter MoFlo XDP Cell Sorter flow cytometer.

Plasmid relaxation assay: The activities of human topoisomerases I and II were assayed by analyzing the retarded mobility of the relaxed isomers of supercoiled pBS (SK+)[pBluescript(SK+)] DNA on a 1.2% agarose gel. Recombinant human topoisomerase I (hTopI) was expressed in Baculoviral Sf9 insect cell system and purified using a reported procedure,[43] whereas the human topoisomerase I (hTopII) was purchased from TopoGEN Inc. (Human Topo IIa, TopoGEN Inc.). The plasmid relaxation assay was carried out in relaxation buffer (25 mM Tris-HCl, pH 7.5, 5% glycerol, 0.5 mM DTT,10 mM $MgCl_2$, 50 mM KCl, 25 mM EDTA, and 150 μg mL^{-1} BSA) for hTopI,[44] and by following the manufacturer's protocol for hTopII. Prior to the addition of enzyme to the reaction mixture, the temperature of the DNA and buffer mixture was adjusted to 37 °C. The concentration of the EDOT analogues **3p** and **3r** was 200 μM. After 30 min, the enzyme reaction was stopped using stop solution (5% SDS, 15% Ficoll, and 0.25% bromophenol blue) and the sample was kept on ice until the resolution of plasmid DNA in

1.2% agarose. The gels were stained with ethidium bromide (0.5 μg mL^{-1}) and imaged under UV light.

Chk2 kinase molecular docking: The synthesized analogues were sketched, and energy minimized using the LigPrep module of Schrödinger 09 (LigPrep 2.3, Schrödinger Suite 2009).[45] The protein target, the crystal structure of Chk2 kinase bound to a 2-aminopyridine inhibitor (PDB ID: 2WTJ),[26a] was imported into the protein preparation wizard of Schrödinger 09, energy minimized, and the bound small molecule was removed from the coordinate file. Molecular docking of EDOT analogues with the resulting PDB coordinates was carried out using Schrödinger's Glide XP 5.8 program.[46] Based on their docking score and binding interactions with the catalytic residues, enones were selected for induced-fit docking. The best poses for hydrogen bonding, hydrophobic and π–π interactions were analyzed using the Chimera Visualization tool, PyMOL version 1.3 (The PyMOL Molecular Graphics System),[47] Glide (Schrödinger, LLC, New York, NY, USA), and LigPlot programs.[48]

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Conflict of interest

The authors declare no conflict of interest.

Keywords: Chk2 inhibitors · cytotoxicity · G2/M cell-cycle arrest · thiophenes · topoisomerase I inhibitors

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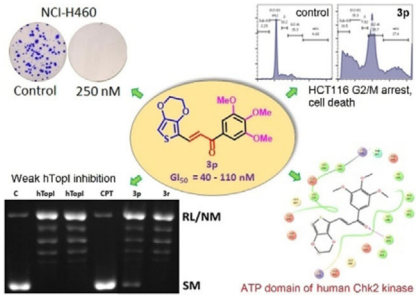
Divergent Synthesis and Evaluation of the in vitro Cytotoxicity Profiles of 3,4-Ethylenedioxythiophenyl-2-propen-1-one Analogues



Better on vinyl: A series of thiophene-containing vinyl ketones inspired by bioactive chalcone derivatives was synthesized and evaluated for their anticancer activity. Molecular docking with Chk2 kinase and a variety of cell-based and biochemical assays showed the most promising compound to be a vinyl ketone flanked by 3,4-ethylenedioxythiophene and 3,4,5-trimethoxyphenyl moieties.