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C-2-Aryl O-substituted HI-236 derivatives as non-nucleoside HIV-1 reverse-transcriptase inhibitors

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Abstract

Several novel thiourea derivatives of the NNRTI HI-236 substituted at the C-2 oxygen of the phenyl ring have been synthesized and evaluated for their inhibitory activity against HIV-1 (IIIB) replication in MT-2 cell cultures. The compounds were synthesized in order to fine-tune the activity of HI-236 as well as to gain insight into spatial characteristics in the pocket pertaining to the positional choice of tether in the design of [NRTI]-tether-[HI-236] bifunctional inhibitors. Two of the thiourea derivatives bearing a butynyl (**6c**) or hydroxyethyl tether (**6n**) were endowed with improved anti-HIV activity compared to HI-236. NNRTI activity was confirmed by a cell-free RT assay on six of the derivatives in which **6c** returned an IC_{50} of 3.8 nM compared to 28 nM for HI-236, establishing it as an improved lead for HI-236. The structure-activity profile is discussed in terms of potential interactions in the NNRTI pocket as suggested by a docking model using AutoDock, which have a bearing on the bifunctional drug design.

Keywords

NNRTI; HI-236; AutoDock; Thiourea; HIV; Bifunctional inhibitors

1. Introduction

The HIV-1 reverse-transcriptase enzyme is responsible for converting the genomic single-stranded RNA of HIV into a double-stranded DNA and continues to be a major target for anti-HIV drug discovery.[1] Inhibitors fall into two distinct classes as nucleoside analogues (NRTIs) and non-nucleoside analogues (NNRTIs), and their modes of action are distinct and well documented.[2] Thus, while NRTIs act as competitive substrates at the substrate-binding site, the NNRTIs work allosterically in an adjacent pocket, interfering with reverse transcription by altering the conformational mobility of RT, which results in non-competitive inhibition. To date, more than 30 structurally diverse NNRTIs have been identified, which have been comprehensively reviewed by several workers.[3] NNRTIs are vulnerable to HIV's high mutation rate, and to circumvent this, they are currently used in combination with NRTIs. An alternative strategy that has been investigated by a number of groups over the last decade or so has been to combine an NRTI and an NNRTI into a single bifunctional molecule.[4] Such inhibitors may be classified into two types: cleavable[5] and non-cleavable.[6] The former involve NRTI–linker–NNRTI systems that are designed to release the two drugs into the cytoplasm via enzymatic

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hydrolysis in the hope of promoting synergistic inhibitory action. Conversely, the latter, based on an original suggestion by Nanni[7] and coworkers, are designed to promote inhibition at both sites cooperatively through the individual drugs in the same entity, on the basis that the NRTI and NNRTI drug–target sites are in close proximity of 10–15 Å.[8] The latter, ambitious strategy has produced some inventive combinations, but true bifunctional character has yet to be comprehensively established. In the design of such bifunctional drugs, it is important to identify attachment points on each drug as well as to choose an appropriate tether regarding size and functional character.

We have been interested in developing non-cleavable bifunctional NRTI/NNRTI compounds involving d4T as the NRTI and the PETT (phenethylthiazolyl) derivative HI-236 as the NNRTI. These compounds were selected in view of their excellent profiles as antiviral agents, and it was decided to attach the linker on the NRTI side of the molecule to C-5 of the pyrimidine base in view of anticipated low interference with base pairing.[9] On the HI-236 side, it was decided to use the C-2 phenolic oxygen in view of studies conducted by Uckun[10] regarding the binding mode of HI-236 in the RT pocket. Our recently published[11] first prototype, [d4T]-butyne-[HI-236], returned an EC_{50} of 250 nM against HIV-1 (IIIB) in MT-2 cell culture using an MTT assay. This result compares favourably with Ladurée's bifunctional,[12] also based on d4T but conjugated to a PETT derivative through a glycylsuccinyl cleavable linker, which turned out to be inactive (Fig. 1).

PETT (phenethylthiazolyl) derivatives such as those shown in Figure 1 were first introduced by the Eli Lilly group in 1995.[13] Changing the thiazole ring to a pyridine ring as *N*-(5-bromo-2-pyridinyl)-*N*′-(2-pyridylethyl)-thiourea (named as Trovirdine), resulted in enhanced activity and a detailed structural analysis of it carried out by the Uckun group revealed abundant sterically usable space surrounding the pyridyl group as well as the ethyl linker.[14,15] They postulated that an efficient use of this space would lead to more potent anti-HIV agents with higher affinity for the NNRTI binding pocket, and this resulted in several more potent derivatives including the highly potent HI-236,[10,15] with a much superior inhibition profile in which the pyridyl ring of trovirdine was replaced by a 2,5-dimethoxyphenyl group, Figure 2.

Structural studies around HI-236 revealed, as with other PETT derivatives, that the thiourea portion interacts in Wing 1 towards the front of the pocket via H-bonding with K101.[10,15] Conversely, the C-5-methoxy group forges cooperative C–H_{π} interactions with the highly conserved W229, while the phenyl ring interacts with Y188. The methoxy group at C-2 was found to lie underneath the ethyl linker and was considered to occupy some of the vacant space near Y188 in Wing 2, an issue which assumes a greater weight of importance for drug-resistant strains where there is an increase in pocket size due to mutated residues of smaller size such as Y181C and Y188L. The realization of the [d4T]-butynyl-[HI-236] bifunctional prototype mentioned previously[11] prompted a study of the influence of the nature of the C-2 tether of HI-236 on its biological activity. In this paper, we present the synthesis and biological activity of a small library of HI-236 derivatives involving substitution of the C-2 phenolic methyl group, the objective being not only to shed light on NNRTI attachment at the C-2 oxygen for the bifunctional design, but also to probe the possibility of further binding in the region of the pocket just mentioned in an attempt to improve the activity of HI-236 even further. Tethers attached to the C-2 phenolic oxygen were chosen to probe length, functionality and polarity. Bearing in mind that a Sonogashira connection[16] between C-5 of d4T and a terminal alkyne was used for the bifunctional connection chemistry, some, but not all of the derivatives studied were selected with an alkyne terminus.

2. Chemistry

Synthesis of the tethered derivatives utilized phenolic amine **1** as a key intermediate that was readily available in multigram quantities using chemistry reported by Glennon[17] via a three-step sequence from commercially available 2-hydroxy-5-methoxybenzaldehyde, Scheme 1. In our case, it was found it easier to isolate **1** as an *N*-Boc carbamate **2**. Yields for the various steps in the sequence to carbamate **2** were high and spectroscopic data was in accordance with literature values for all of the known compounds, Scheme 1.

Thereafter, following benzyl group deprotection to **3**, the various O-alkylations at the C-2 phenolic oxygen could be carried out with introduction of the thiourea moiety last for each case. A more convergent approach involving converting amine **1** first into the thiourea derivative, debenzylating and then carrying out the C-2 phenolic alkylation reaction to generate a small library of targets was deemed to be unattractive in view of the anticipated incompatibility of the thiourea functionality with the alkylation chemistry.

Alkylation of carbamate **3** (Scheme 2 and Table 1) to afford a library of alkylated *N*-Boc derivatives **4** proceeded efficiently with bromide or tosylate electrophiles using K_2CO_3 in acetonitrile at reflux for around 20 h and proved to be superior to a Mitsunobu reaction with the corresponding alcohol. Yields of chromatographed products were generally in excess of eighty percent (see Section 4). In most cases, the electrophile was readily available either commercially or via a one-step derivatisation.

The exceptions came for the alkynyl tethers used to probe the question of the influence of tether length and aqueous solubility of the bifunctional compounds. These involved alkynyl PEG (ethylene glycol) chains and were synthesized by the following sequence shown in Scheme 3 in which the tether was developed divergently. Thus, standard phenolic alkylation of a monobenzylated glycol bromide afforded the alkylated product, which was committed to a two-step sequence involving hydrogenolytic debenzylation followed by tosylation to afford tosylates **5a** and **5b**. Nucleophilic displacement with propargyloxide anion then furnished the products **4i** and **4j**. The latter[18] turned out to be superior to hydroxyl-group alkylation with propargyl bromide. Similarly, alcohols **4n** and **4o** were obtained from hydrogenolysis of their benzyl ethers obtained from alkylation of **3** as described.

Table 1 summarises the various products of C-2 phenolic alkylation of Boc carbamate **3**.

All derivatives **4a**–**o** returned acceptable NMR spectra together with acceptable combustion analysis data (solids) and/or HRMS mass spectral data. Notable in the NMR were the triad of signals for the three aromatic protons in the ^{1}H NMR spectrum integrating correctly against the *N*-Boc *t*ert-butyl singlet. The ^{13}C NMR spectra returned the correct number of carbon singlets in each case.

Finally, synthesis of the target thioureas **6** was accomplished via a Boc-deprotection, condensation sequence shown in Scheme 4.

Thus, each derivative **4** was reacted with trifluoroacetic acid (TFA) in DCM at 0 °C for 1–3 h until TLC revealed complete deprotection to a polar amine spot. In view of the amine's anticipated water solubility, the final step was conducted without using an aqueous work-up. Thus, following complete removal of all volatiles including any excess TFA, the residue was redissolved in THF, Hünig's base (EtN(*i*-Pr)$_2$) was added to liberate the free amine and the thiocarbonyl reagent **7** added according to the original procedure. As described by the Eli Lilly group,[13] the latter could be readily prepared by reacting 2-amino-5-bromopyridine with 1,1′-thiocarbonyldiimidazole in acetonitrile at room temperature for 12 h to afford a precipitate that

was used without purification. Attempted recrystallization of **7** from methanol resulted in a product with imidazole substituted by methoxy (Scheme 5).

Condensation between the amine and **7** could be realized in DMF at 100 °C as described by Bell and coworkers[13] to afford the targets **6a**–**o** in an overall yield of around 30% for the two steps. However, later on, it was discovered that condensation could be carried out under much milder conditions in THF or DMF at room temperature to increase the two-step yield to around 60%. All of the targets were isolated by silica-gel column chromatography as crystalline solids that were crystallized to a constant, sharp melting point to return acceptable combustion analysis data. A full spectroscopic analysis using ^{1}H and ^{13}C NMR spectroscopy was also carried out on each derivative to return acceptable data. Notably, the aromatic region in the ^{1}H NMR spectra provided convenient markers for the aromatic and heteroaromatic rings to demonstrate that condensation had taken place. The thiourea N–H's could be discerned downfield as two separate resonances. The thiourea thiocarbonyl carbon could be identified using ^{13}C NMR, resonating at around 179 ppm. The retention of bromine in the pyridine ring was confirmed by ^{13}C NMR (δ_{C-Br} ~112.6 ppm) as well as microanalytical and IR data. Table 2 summarises yields for the two-step sequence of **4**–**6** in Scheme 4.

3. Biological results, modeling, and discussion

The inhibitory activity of compounds **6a**–**o** was measured against HIV-1 (IIIB) replication in MT-2 cell culture using an MTT assay.[19] HI-236 was included as a reference compound. The results are shown in Table 3.

In order to lend support that these HI-236 derivatives act as NNRTIs, six of the derivatives (**6c**, **6d**, **6i**, **6k**, **6n**, and **6o**) covering the most (**6n**) to the least active (**6k**) were subjected to an in vitro steady-state RT inhibition assay, the results from which are shown in Table 4. The results present strong support for all of the derivatives in this study to be acting as NNRTIs since they have no possibility of being phosphorylated for NRTI activity against RT. This is also in keeping with the published findings on HI-236. The activities ranged from 3.8 to 100 nM, and as expected were superior to those from the cell-culture results except for **6n**, which was slightly lower. Such improvements likely reflect the lipophilicity of the derivatives and their poor solubility in the aqueous cell-culture medium. Notably, the ester **6k**, which was effectively inactive in cell culture (12 μM), revealed a 120-fold improvement to 100 nM in the RT assay, possibly due to ester hydrolysis in the cell-culture medium.[20] Importantly, the alkyne **6c** returned a value of 3.8 nM in the RT assay, which was more active compared to that of the alcohol **6n** (19 nM), in spite of the reverse being true in cell culture (26 nM vs 12 nM, respectively). The most active derivative **6c** was then evaluated in cell culture against the Y181C resistant strain and retained activity much better than HI-236 (8-fold decrease against 25 for HI-236).

Regarding a general comparison of all derivatives, the results from the cell culture shown in Table 3 were taken to reveal important trends within classes of different tether (polar vs lipophilic). Four of the compounds returned activities equal to HI-236 (**6b** and **6m**) or greater (**6c**, 2-fold increase; **6n**, 4-fold increase). In order to assist with interpretation of the results, docking studies were carried out using AutoDock 3.05[21] based on the recently published HIV-1 RT protein crystal structure of *N*-(5-chloro-2-pyridinyl)-*N*′-[2-(4-ethoxy-3-fluoro-2-pyridinyl) ethyl]-thiourea as template.[22] The conformation of the PETT derivatives studied was set to accommodate the well documented[14,22,23] intramolecular hydrogen bond between the hydrogen of the thiourea nitrogen attached to the alkyl side and the nitrogen of the pyridinyl ring to form a flat six-membered pseudo-ring. This protocol resulted in a strong preference for one low energy conformation which dominated 153 of the 250 possible conformations. HI-236

was docked first, and we were able to establish parity of result with that obtained by Uckun. The two structures are shown in Figure 3.

Thus, as established by Uckun, the inhibitor adopts the characteristic butterfly-shaped orientation with the thiourea moiety embedded in Wing 1 in which hydrogen bonding between the thiourea hydrogen on the *N*-pyridyl side and the carbonyl oxygen of K101 is clearly visible. Wing 2 accommodates the phenyl ring of HI-236 close to the Y181 and Y188 region with the 5-methoxy group interacting with W229. The 2-alkoxy substituent positions into a well-containing residues Y181, Y188, F227, V106, and V179. This feature had significance for the aims of this study. The only difference between our structure and that of Uckun's was the orientation of W229 relative to Y181. In Uckun's model, the six-membered ring of the indole of W229 overlapped Tyr181, whereas the model obtained from our calculations had the five-membered ring of the indole overlapping Tyr181. This could have been due to differences in the static protein structure used in our docking calculations as compared with the structure of the HIV-1 RT binding pocket used in the studies by Uckun. Attention was then focused on docking two of the derivatives, chosen as the ester **6k** and the alcohol **6o**. The parity of **6k** and **6o** with our docked HI-236 in terms of overall topology in the pocket was first investigated, as illustrated in Figure 4 for **6k**. The elongation of the 2-alkoxy substituent can be seen but the positioning of the rest of the PETT structure essentially stayed the same, revealing that EC_{50} differences are likely to be based on interactions in the Wing 2 region as postulated. Alcohol **6o** gave a similar result.

Examination of the way in which the 2-alkoxy substituents of **6k** and **6o** positioned in the hydrophobic region of Wing 2 as shown in Figures 5 and 6 taken from each end of the pocket for both cases, gave insights into the possible interactions responsible for fluctuation of biological activity.

Both structures suggest the possibility of interaction with V109 or, to a lesser extent V179, towards the floor of the pocket. A cooperative hydrogen bond for **6o** would nicely explain the much enhanced activity of alcohol **6n** (EC_{50} = 0.012 μM) with a shorter (two-carbon) chain, in which presumably the distance between hydrogen-bond donor and acceptor is optimal. **6n** returned an activity four times higher than HI-236 in cell culture, and almost twice that in the RT assay and thus presents itself as an interesting candidate for the problematic V106A mutation if the hydrogen bonding postulate is correct.

Similarly, for the alkyl and alkynyl compounds **6a**–**f**, the results show that the hydrophobic pocket is accommodating but with the only significant cooperative interaction being with the triple bond. In this regard, the butynyl chain of **6c**, rather than the propargyl (**6b**) or pentynyl substituents (**6d**) appears to be optimal, with **6c** indicating a 2-fold increase in activity compared to HI-236 in cell culture and 7-fold in the RT assay. Although this classical force field does not include an explicit π–π term, the charge distribution on the aromatic rings mimics this fundamentally quantum effect surprisingly well. Therefore we suggest that the increased activity of **6c** is an optimal cooperative π–π face-to-face interaction between the aromatic residues of Y181, Y188, and the triple bond. In addition, a much lower fold reduction in activity for **6c** (8×) was observed against the Y181C[24] mutant strain compared to that of HI-236 (25×), (see Table 4). The PEG derivatives predictably returned lower activities, but not overly so given their bulk. Thus **6j**, with 10 atoms in the substituent, returned an activity of 0.39 μM as only 8-fold less potent than HI-236. By comparison, the benzyl derivative **6h** is too bulky to be satisfactorily accommodated. Finally, the nitrile derivative **6m** indicated some level of cooperation in the pocket being as active as HI-236, in spite of its five-atom substituent. Once again, π–π and/or hydrogen bonding possibilities appear to be likely explanations.

In summary, it is important to note that of the 15 compounds tested in cell culture, two (**6c** and **6n**) were more active than HI-236 and two (**6b** and **6m**) were as active, while this picture improved in the RT assay in which of the six derivatives tested, two (**6c** and **6n**) were more active and two were as active as HI-236 (**6d** and **6o**). Such results endorse the conclusions drawn from the study by Uckun[15] that the PETT derivatives have unused available pocket volume with good potential for drug-development, and have identified tethered butynyl derivative **6c** as an advanced lead. Further fine-tuning is worthwhile pursuing on developing side chains that can cope with mutated residues contained in resistant strains,[25] and the modelling results suggest that this might be possible to achieve by adding further substitution at the C-3 position of the aromatic ring *ortho* to the C-2 O-tether. In addition, the study has generated important insights regarding the choice of the C-2 oxygen as the attachment point for the tether in the bifunctional compounds, and the likelihood of a tether at this position providing a route from the pocket to the NRTI binding site. In this regard, a comprehensive study of elongated alkylated bifunctional double-drugs in order to shed light on the origin of biological activity for the prototype in Figure 1[11] will be communicated in a forthcoming paper.

4. Experimental

4.1. Docking aspects

The binding conformations of HI-236 (**1**) and its ester (**6k**) and alcohol (**6o**) derivatives bound to HIV-1 Reverse Transcriptase (RT) were modelled using AutoDock 3.05[21] based on the published HIV-1 RT protein crystal structure of *N*-(5-chloro-2-pyridinyl)-*N*′-[2-(4-ethoxy-3-fluoro-2-pyridinyl)ethyl]-thiourea.[22] Each ligand was built in Gaussview,[26] and optimised using Gaussian 98 to relax bond lengths and angles that were not varied in the docking simulation. Polar hydrogen atoms were added to the protein and Kollman united-atom partial charges assigned using the AutoDockTools package. For each ligand, Gasteiger-Marsili[27] partial charges were assigned, as implemented in AutoDockTools. Autodock 3.05 utilises an empirical scoring function[21] to calculate binding free energies, which incorporates five energy terms, including a Lennard–Jones 12-6 term and a directional 12-10 hydrogen bond term, for which the default parameters distributed with AutoDockTools were used. Electrostatic interactions were calculated using a distance-dependent dielectric constant.[28] Atomic solvation parameters and fragmental volumes were assigned using the AddSol utility, from which the desolvation contribution to the binding free energy is calculated. A 61 × 61 × 61 grid map was used in all docking calculations, with a grid spacing of 0.375 Å. Given the known location of the NNRTI binding site, the grid was centred on the coordinates for the equivalent atom of PETT-lig corresponding to the default selected root atom of each ligand investigated. Docked conformations were generated using the Lamarckian genetic algorithm (LGA) with an initial population size of 150 structures. Translation, quaternion and torsional step sizes were set to 2 Å, 5.0° and 5.0° respectively for HI-236 and 0.1 Å, 1.0° and 1.0° for ligands **9k** and **9o**. Further parameters were set to their default values. A total of 250 runs were performed for each ligand, and the resulting conformations clustered using a root mean-squared deviation criterion of 0.5 Å in *x*, *y*, z positional coordinates. Bond rotation from the pyridinyl ring across to the thiourea moiety was disallowed, and the conformation was set to accommodate the well documented[14,21,22] intramolecular hydrogen bond between the hydrogen of one of the thiourea nitrogens and the nitrogen of the pyridinyl ring to form a flat six-membered pseudo-ring. This protocol resulted in a strong preference for one low energy conformation which dominated 153 of the 250 possible conformations. Although using a static protein structure in the docking simulation and limiting the rotation of certain bonds, the binding conformation of HI-236 in this study compared well with that obtained by Uckun.[15]

4.2. General procedures for synthesis

Microanalyses were obtained with a Fisons EA 110 CHN Elemental Analyser. Infrared (IR) absorptions were measured on a Perkin-Elmer Spectrum One FT-IR spectrometer. ^{1}H NMR spectra were recorded on a Varian Mercury Spectrometer at 300 MHz and a Varian Unity Spectrometer at 400 MHz with Me_4Si as internal standard. ^{13}C NMR spectra were recorded at 75 MHz on a Varian Mercury Spectrometer or at 100 MHz on a Varian Unity Spectrometer with Me_4Si as internal standard. High resolution mass spectra were recorded on a VG70 SEQ micromass spectrometer. Melting points were determined using a Reichert-Jung Thermovar hot-stage microscope and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on silica-gel 60 F_{254} (Merck). Column chromatography was performed with Merck silica-gel 60 (70–230 mesh). HI-236[10] was synthesized by the same sequence as for derivatives **6** and returned acceptable NMR data.

4.3. Procedure for tosylates 5a and 5b

The phenol **3** was O-alkylated with the appropriate PEGbromide as its monobenzyl ether according to the procedure below for formation of the alkylated derivatives **4**. The alkylated products were subjected to hydrogenolysis in ethanol using Pd–C (10 mol%) at atmospheric pressure for 18 h. Following filtration through Celite, the alcohols were purified by column chromatography in yields in excess of 80% before being tosylated with *p*-toluenesulfonyl chloride (1.5 equiv) in dichloromethane using triethylamine (2 equiv) and DMAP (cat). Following a conventional work-up, the product was isolated by column chromatography. Solid products were generally recrystallized from ethyl acetate/hexane mixtures.

4.3.1. *N*-(*tert*-Butoxycarbonyl)-2-[5-methoxy-2-(2-*p*-toluenesulfonyloxyethoxy) phenyl]ethylamine (5a)—76% Yield as a colourless oil; IR ($CHCl_3$) ν_{max} 3690, 3451, 1706, 1367, 1164 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.75 (2H, d, J = 8.2 Hz), 7.28 (2H, d, J = 8.2 Hz), 6.62 (3H, m), 4.77 (1H, br s), 4.30 (2H, t, J = 4.7 Hz), 4.05 (2H, t, J = 4.7 Hz), 3.68 (3H, s), 3.22 (2H, q, J = 6.8 Hz), 2.65 (2H, t, J = 6.8 Hz), 2.38 (3H, s), 1.38 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.9, 154.1, 150.2, 145.0, 132.9, 129.9, 129.3, 127.8, 116.7, 112.0, 111.9, 78.8, 68.4, 66.4, 55.5, 40.5, 30.9, 28.4, 21.5; EI-HRMS: m/z; found: 465.18130 (M^+). $C_{23}H_{31}NO_7S$ (M^+) requires 465.18212.

4.3.2. *N*-(*tert*-Butoxycarbonyl)-2-{5-methoxy-2-[2-(2-*p*-toluenesulfonyloxyethoxy)ethoxy]phenyl}ethylamine (5b)—83% Yield as a colourless oil; IR ($CHCl_3$) ν_{max} 3693, 3453, 1706, 1367, 1168 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (2H, d, J = 7.9 Hz), 7.28 (2H, d, J = 7.9 Hz), 6.73 (1H, d, J = 8.8 Hz), 6.68 (2H, m), 4.80 (1H, br s), 4.17 (2H, t, J = 4.7 Hz), 3.98 (2H, t, J = 4.7 Hz), 3.75 (4H, m), 3.73 (3H, s), 3.29 (2H, q, J = 6.7 Hz), 2.74 (2H, t, J = 6.7 Hz), 2.39 (3H, s), 1.39 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.9, 153.9, 150.9, 144.8, 133.1, 129.8, 129.3, 127.9, 116.6, 113.0, 112.0, 78.8, 70.0, 69.3, 68.8, 68.3, 55.6, 40.6, 31.0, 28.4, 21.5; EI-HRMS: m/z; found: 509.20675 (M^+). $C_{25}H_{35}NO_8S$ (M^+) requires 509.20834.

4.4. Procedure for the *N*-Boc O-alkylated derivatives 4

The alkylating agent as a tosylate or bromide (2.0 mmol) in dry acetonitrile (5 mL) was added dropwise over 1 h to a refluxing and stirring mixture of the phenol **3** (1.0 mmol) and anhydrous potassium carbonate (4.0 mmol) in dry acetonitrile (15 mL). The reaction was refluxed for 20 h. The mixture was filtered, the acetonitrile evaporated, and the residue subjected to silica-gel column chromatography (10% EtOAc/pet ether) to afford the product generally as a colourless solid. Entries **4n** and **4o** were derived via hydrogenolysis of their corresponding benzyl ethers, while entries **4i** and **4j** were obtained via substitution of tosylates **5a** and **5b** respectively using

propargyloxide ion in refluxing THF (5 h). Yields cited in the text refer to the final step in each case.

4.4.1. *N*-(*tert*-Butoxycarbonyl)-2-(5-methoxy-2-propoxyphenyl)ethylamine (4a)—95% Yield, mp 52–54 °C; IR ($CHCl_3$) ν_{max} 3684, 3452, 3002, 2977, 1706, 1502 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 6.76 (1H, d, J = 9.2 Hz), 6.70 (2H, m), 4.78 (1H, br s), 3.88 (2H, t, J = 6.4 Hz), 3.75 (3H, s), 3.35 (2H, q, J = 6.6 Hz), 2.78 (2H, t, J = 6.6 Hz), 1.80 (2H, m), 1.42 (9H, s), 1.04 (3H, t, J = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.9, 153.5, 151.3, 129.5, 116.8, 112.3, 112.0, 79.8, 70.2, 55.7, 40.9, 30.9, 28.4, 22.8, 10.7; EI-HRMS: *m*/*z*; found: 309.19401 (M^+). $C_{17}H_{27}NO_4$ (M^+) requires 309.19400. Anal. Found: C, 66.13; H, 8.80; N, 3.91. $C_{17}H_{27}NO_4$ requires; C, 65.99; H, 8.80; N, 4.53.

4.4.2. *N*-(*tert*-Butoxycarbonyl)-2-(5-methoxy-2-propargyloxyphenyl)ethylamine (4b)—75% Yield, mp 49–50 °C; IR ($CHCl_3$): ν_{max} 3691, 3454, 3308, 3022, 2124, 1707, 1501 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.90 (1H, d, J = 9.3 Hz), 6.72 (2H, m), 4.65 (3H, d, J = 2.4 Hz), 3.75 (3H, s), 3.35 (2H, q, J = 6.8 Hz), 2.79 (2H, t, J = 6.8 Hz), 2.47 (1H, t, J = 2.4 Hz), 1.42 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9, 154.3, 149.8, 129.6, 116.7, 113.6, 112.0, 79.0, 79.0, 56.7, 55.6, 40.6, 30.9, 28.4; EI-HRMS: *m*/*z*; found: 305.16244 (M^+). $C_{17}H_{23}NO_4$ (M^+) requires 305.16271. Anal. Found: C, 66.90; H, 7.54; N, 4.54. $C_{17}H_{23}NO_4$ requires C, 66.86; H, 7.59; N, 4.59.

4.4.3. *N*-(*tert*-Butoxycarbonyl)-2-[2-(3-butynyl-1-oxy)-5-methoxyphenyl] ethylamine (4c)—61% Yield, mp 76–77 °C; IR ($CHCl_3$): ν_{max} 3455, 3309, 2413, 1707, 1602 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.75 (3H, m), 4.70 (1H, br s), 4.05 (2H, t, J = 6.8 Hz), 3.75 (3H, s), 3.36 (2H, q, J = 6.6 Hz), 2.79 (2H, t, J = 6.6 Hz), 2.66 (2H, dt, J = 2.7, 6.8 Hz), 2.03 (1H, t, J = 2.7 Hz), 1.42 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.9, 153.9, 150.6, 129.3, 116.8, 112.8, 112.0, 80.7, 78.9, 69.8, 66.8, 55.6, 40.7, 30.9, 28.4, 19.7; EI-HRMS: *m*/*z*; found: 319.17756 (M^+). $C_{18}H_{25}NO_4$ (M^+) requires 319.17836. Anal. Found: C, 67.10; H, 7.66; N, 3.67. $C_{18}H_{25}NO_4$ requires C, 67.89; H, 7.89; N, 4.39.

4.4.4. *N*-(*tert*-Butoxycarbonyl)-2-[5-methoxy-2-(4-pentynyl-1-oxy)phenyl] ethylamine (4d)—93% Yield; mp 69–71 °C; IR ($CHCl_3$) ν_{max} 3696, 3452, 3307, 2980, 2344, 1707, 1502, 1218, 1164 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 6.79 (1H, d, J = 9.6 Hz), 6.70 (2H, m), 4.72 (1H, br s), 4.02 (2H, t, J = 6.5 Hz), 3.75 (3H, s), 3.34 (2H, q, J = 6.5 Hz), 2.78 (2H, t, J = 6.5 Hz), 2.41 (2H, td, J = 2.7, 6.5 Hz), 1.99 (2H, m), 1.96 (1H, t, J = 2.7 Hz), 1.43 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.9, 153.7, 151.0, 129.0, 116.7, 112.5, 112.0, 83.4, 79.3, 68.9, 66.9, 55.7, 40.8, 30.9, 28.4, 28.4, 15.4. Anal. Found: C, 68.44; H, 8.16; N, 4.20. $C_{19}H_{27}NO_4$ requires; C, 68.53; H, 8.28; N, 3.87.

4.4.5. *N*-(*tert*-Butoxycarbonyl)-2-[2-(2-butynyl-1-oxy)-5-methoxyphenyl] ethylamine (4e)—92% Yield; mp 53–55 °C; IR ($CHCl_3$) ν_{max} 3681, 3449, 3014, 2246, 1703, 1501, 1205 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 6.90 (1H, d, J = 9.6 Hz), 6.71 (2H, m), 4.65 (1H, br s), 4.60 (2H, q, J = 2.3 Hz), 3.76 (3H, s), 3.35 (2H, q, J = 6.0 Hz), 2.79 (2H, t, J = 6.0 Hz), 1.83 (3H, t, J = 2.3 Hz), 1.43 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.9, 154.0, 150.1, 129.4, 116.5, 113.6, 111.9, 83.3, 78.6, 74.5, 57.3, 55.6, 40.6, 30.6, 28.4, 3.6. Anal. Found: C, 67.39; H, 7.97; N, 4.12. $C_{18}H_{25}NO_4$ requires C, 67.69; H, 7.89; N, 4.23.

4.4.6. *N*-(*tert*-Butoxycarbonyl)-2-(2-allyloxy-5-methoxyphenyl)ethylamine (4f)—99% Yield; mp 54–56 °C; IR ($CHCl_3$) ν_{max} 3682, 3449, 3201, 1502, 1703, 1210 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 6.78 (1H, d, J = 9.0 Hz), 6.71 (2H, m), 6.05 (1H, ddt, J = 5.1 Hz, 10.5 Hz, 17.3 Hz), 5.39 (1H, dq, J = 1.6 Hz, 17.3 Hz), 5.26 (1H, dq, J = 1.6 Hz, 10.5 Hz), 4.71 (1H, br s), 4.50 (2H, dt, J = 1.6 Hz, 5.1 Hz), 3.76 (3H, s), 3.40 (2H, q, J = 6.5 Hz), 2.80 (2H,

t, J = 6.5 Hz), 1.43 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 156.0, 153.7, 150.8, 133.6, 129.2, 117.1, 116.7, 112.9, 112.0, 79.0, 69.5, 55.7, 40.8, 30.9, 28.4. Anal. Found: C, 66.41; H, 8.21; N, 4.40. $C_{17}H_{25}NO_4$ requires C, 66.43; H, 8.20; N, 4.56.

4.4.7. *N*-(*tert*-Butoxycarbonyl)-2-(2-benzyloxy-5-methoxyphenyl)ethylamine (4g)—89% Yield; mp 103-104°C; IR ($CHCl_3$): ν_{max} 3691, 3453, 1707, 1503 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.41 (5H, m), 6.84 (1H, d, J = 8.1 Hz), 6.71 (2H, m), 5.03 (2H, s), 4.70 (1H, br s), 3.76 (3H, s), 3.37 (2H, q, J = 6.4 Hz), 2.83 (2H, t, J = 6.4 Hz), 1.42 (9H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 155.9, 153.9, 151.0, 137.4, 128.6, 128.0, 127.9, 127.3, 116.8, 113.0, 112.0, 79.0, 70.9, 55.7 (OCH_3), 40.8 (C-1), 30.9 (C-2), 27.8 ($OC(CH_3)_3$); EI-HRMS: m/z; found: 301.13383 [(M^+–*tert*-butyl) + H]. $C_{21}H_{27}NO_4$ requires 301.13409 [(M^+ – *tert*-butyl) + H]. Anal. Found: C, 70.50; H, 7.62; N, 3.86. $C_{21}H_{27}NO_4$ requires C, 70.56; H, 7.61; N 3.92.

4.4.8. *N*-(*tert*-Butoxycarbonyl)-2-[2-(2-benzyloxyethyl-1-oxy)-5-methoxyphenyl] ethylamine (4h)—85% Yield; mp 56–58 °C; IR ($CHCl_3$) ν_{max} 3673, 3449, 3000, 2398, 1701, 1501 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40 (5H, m), 6.79 (1H, d, J = 8.8 Hz), 6.70 (2H, m), 4.78 (1H, br s), 4.63 (2H, s), 4.10 (2H, t, J = 4.8 Hz), 3.82 (2H, t, J = 4.8 Hz), 3.75 (3H, s), 3.37 (2H, q, J = 6.5 Hz), 2.80 (2H, t, J = 6.5 Hz), 1.42 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.9, 153.7, 151.0, 138.1, 129.2, 128.4, 127.7, 127.6, 116.6, 112.9, 111.9, 78.7, 73.2, 68.7, 68.4, 55.5, 40.7, 30.9, 28.4; EI-HRMS: m/z; found: 401.22044 (M+). $C_{23}H_{31}NO_5$ (M+) requires 401.22022. Anal. Found: C, 68.90; H, 7.80; N, 3.37. $C_{23}H_{31}NO_5$ requires C, 68.80; H, 7.78; N, 3.49.

4.4.9. *N*-(*tert*-Butoxycarbonyl)-2-[5-methoxy-2-(2-propargyloxyethoxy)phenyl] ethylamine (4i)—74% Yield from tosylate **5a** with propargyloxide; colourless oil; IR ($CHCl_3$): ν_{max} 3674, 3452, 3307, 2121, 1703 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.78 (1H, d, J = 7.8 Hz), 6.69 (2H, m), 4.79 (1H, br s), 4.26 (2H, d, J = 2.3 Hz), 4.10 (2H, m), 3.88 (2H, m), 3.75 (3H, s), 3.35 (2H, q, J = 6.7 Hz), 2.80 (2H, t, J = 6.7 Hz), 2.45 (1H, t, J = 2.3 Hz), 1.42 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 156.0, 153.9, 150.9, 129.4, 116.7, 113.0, 112.0, 79.6, 78.9, 74.7, 68.4, 68.3, 58.5, 55.7, 40.8, 31.0, 28.4; EI-HRMS: m/z; found: 349.18937 (M^+). $C_{19}H_{27}NO_5$ (M^+) requires 349.18892.

4.4.10. *N*-(*tert*-Butoxycarbonyl)-2-{5-methoxy-2-[2-(2-propargyloxyethoxy) ethoxy] phenyl}ethylamine (4j)—91% Yield from tosylate **5b** with propargyloxide; colourlesss oil; IR ($CHCl_3$): ν_{max} 3693, 3607, 3453, 3308, 3012, 2980, 2934, 2120, 1706, 1502; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.74 (1H, d, J = 8.6 Hz), 6.66 (2H, m), 4.90 (1H, br s), 4.17 (2H, d, J = 2.4 Hz), 4.05 (2H, t, J = 4.9 Hz), 3.80 (2H, t, J = 4.9 Hz), 3.71 (3H, s), 3.70 (4H, m), 3.31 (2H, q, J = 6.7 Hz), 2.76 (2H, t, J = 6.7 Hz), 2.42 (1H, t, J = 2.4 Hz), 1.39 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.0, 153.8, 151.0, 129.4, 116.6, 113.1, 112.0, 79.6, 78.7, 74.6, 70.5, 69.8, 69.1, 68.5, 58.3, 55.6, 40.7, 31.0, 28.4; EI-HRMS: m/z; found: 393.21448 (M^+). $C_{21}H_{31}NO_6$ (M^+) requires 393.21514.

4.4.11. *N*-(*tert*-Butoxycarbonyl)-2-(2-methoxycarbonylmethyloxy-5-methoxyphenyl)ethylamine (4k)—98% Yield; mp 66–68 °C; IR ($CHCl_3$) ν_{max} 3681, 3449, 1758, 1704, 1501, 1227 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.67 (3H, m), 4.84 (1H, br s), 4.61 (2H, s), 3.78 (3H, s), 3.74 (3H, s), 3.40 (2H, q, J = 6.5 Hz), 2.90 (2H, t, J = 6.5 Hz), 1.41 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.6, 156.0, 154.3, 150.1, 129.5, 116.9, 112.4, 112.0, 78.8, 66.0, 55.6, 52.1, 40.7, 30.9, 28.4. Anal. Found: C, 60.37; H, 7.36; N, 3.91. $C_{17}H_{25}NO_6$ requires C, 60.16; H, 7.42; N; 4.13.

4.4.12. *N*-(*tert*-Butoxycarbonyl)-2-(2-cyanomethoxy-5-methoxyphenyl) ethylamine (4l)—96% Yield; mp 98–102 °C; IR ($CHCl_3$) ν_{max} 3681, 3449, 2240, 1703, 1501, 1226 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 6.89 (1H, d, J = 9.6 Hz), 6.74 (2H, m), 4.73 (2H, s), 4.61 (1H, br s), 3.76 (3H, s), 3.35 (2H, q, J = 6.8 Hz), 2.80 (2H, t, J = 6.8 Hz), 1.43 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.9, 155.4, 148.9, 130.1, 117.1, 115.4, 114.0, 112.2, 79.2, 55.6, 54.9, 40.5, 31.4, 28.4. Anal. Found: C, 62.87; H, 7.09; N, 8.30. $C_{16}H_{22}N_2O_4$ requires C, 62.73; H, 7.24; N, 9.14.

4.4.13. *N*-(*tert*-Butoxycarbonyl)-2-[2-(3-cyanopropyl-1-oxy)-5-methoxyphenyl] ethylamine (4m)—96% Yield; mp 69–71 °C; IR ($CHCl_3$) ν_{max} 3681, 3449, 3014, 2246, 1703 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 6.74 (3H, m), 4.73 (1H, br s), 4.03 (2H, t, J = 5.8 Hz), 3.75 (3H, s), 3.32 (2H, q, J = 6.8 Hz), 2.77 (2H, t, J = 6.8 Hz), 2.60 (2H, t, J = 7.0 Hz), 2.14 (2H, m), 1.42 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.9, 154.0, 150.6, 128.9, 119.3, 116.8, 112.5, 112.0, 79.5, 66.3, 55.7, 40.8, 31.2, 28.4, 25.7, 14.4; EI-HRMS: *m*/*z*; found: 334.1924 (M^+). $C_{18}H_{26}N_2O_4$ (M^+) requires 334.1893. Anal. Found: C, 63.62; H, 7.82; N, 8.06. $C_{18}H_{26}N_2O_4$ requires C, 64.65; H, 7.84; N, 8.38.

4.4.14. *N*-(*tert*-Butoxycarbonyl)-2-[2-(2-hydroxyethoxy)-5-methoxyphenyl] ethylamine (4n)—93% Yield via hydrogenolysis of **4h**; mp 90–91 °C; IR ($CHCl_3$) ν_{max} 3681, 3615, 3449, 3021, 2405, 1700, 1501, 1165 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 6.74 (3H, m), 4.87 (1H, br s), 4.00 (4H, m), 3.88 (1H, br s), 3.75 (3H, s), 3.31 (2H, q, J = 6.7 Hz), 2.78 (2H, t, J = 6.7 Hz), 1.42 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.5, 153.9, 151.4, 128.9, 117.1, 112.6, 112.0, 79.7, 70.6, 61.6, 55.9, 41.0, 32.6, 28.6; EI-HRMS: *m*/*z*; found: 311.17278 (M+). $C_{16}H_{25}NO_5$ (M^+) requires 311.17327. Anal. Found: C, 61.70; H, 8.03; N, 4.14. $C_{16}H_{25}NO_5$ requires C, 61.72; H, 8.09; N, 4.50.

4.4.15. *N*-(*tert*-Butoxycarbonyl)-2-[2-(3-hydroxypropyl-1-oxy)-5-methoxyphenyl]ethylamine (4o)—100% and 95% Yields in the alkylation and hydrogenolysis steps respectively; mp 44–46 °C; IR ($CHCl_3$) ν_{max} 3681, 3623, 3449, 2399, 1700, 1505, 1205 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 6.80 (1H, d, J = 9.6 Hz), 6.70 (2H, m), 4.78 (1H, br s), 4.06 (2H, t, J = 5.9 Hz), 3.87 (2H, t, J = 5.9 Hz), 3.75 (3H, s), 3.34 (2H, q, J = 6.6 Hz), 2.76 (2H, t, J = 6.6 H), 2.37 (1H, br s), 2.03 (2H, quin, J = 5.9 Hz), 1.41 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.9, 153.6, 151.1, 128.9, 116.8, 112.4, 112.0, 79.0, 66.9, 65.9, 55.7, 40.7, 31.0, 29.9, 28.4. Anal. Found: C, 62.97; H, 8.35; N, 4.13. $C_{17}H_{27}NO_5$ requires C, 62.75; H, 8.36; N, 4.30.

4.5. Procedure for the synthesis of thioureas 6a–o

Trifluoroacetic acid (0.2 mL) was added to a solution of the *N*-Boc carbamate **4** (1 mmol) in CH_2Cl_2 (2 mL) at 0 °C, and the solution stirred for 2 h. Diisopropylethylamine (0.4 mL) was added, the solvent evaporated *in vacuo* and the crude amine dried under vacuum for 1 h. Thiocarbonyl reagent **7** (1.3 mmol) was added to the crude amine in DMF (5 mL) and the mixture stirred at 100 °C for 16 h. The mixture was then poured into ice-cold water (5 mL) and stirred for 30 min. The precipitate formed was filtered and washed with cold water (2× 5 mL) or alternatively extracted into ethyl acetate and the residue purified by column chromatography using ethyl acetate/light petroleum mixtures as eluent. Alternatively, the condensation reaction with **7** could be carried out in THF (5 mL) at room temperature for 20 h. Following evaporation of solvent, the residue was subjected directly to column chromatography to afford thioureas **6**.

4.5.1. *N*-(5-Bromo-2-pyridinyl)-*N′*-[2-(5-methoxy-2-propyl-1-oxyphenyl)ethyl]-thiourea (6a)—Using THF/rt, (65%); mp 162–163 °C; IR ($CHCl_3$) ν_{max} 3696, 3413 (NH), 3167, 2963 (C–H), 1512, 1472 (C=S), 1212 (C–N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 11.18

(1H, br s, NH), 8.95 (1H, br s, NH), 8.08 (1H, d, *J* = 2.4 Hz), 7.67 (1H, dd, *J* = 2.4 Hz, 8.8 Hz), 6.81 (1H, d, *J* = 3.2 Hz), 6.78 (1H, d, *J* = 8.8 Hz), 6.74 (1H, dd, *J* = 3.2, 8.8 Hz), 6.72 (1H, d, *J* = 8.8 Hz), 4.02 (2H, m), 3.87 (2H, t, *J* = 6.4 Hz), 3.75 (3H, s), 2.99 (2H, t, *J* = 6.4 Hz), 1.80 (2H, m), 1.04 (3H, t, *J* = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 179.0 (C=S), 153.3, 151.7, 151.5, 146.8, 141.1, 128.5, 117.7, 113.2, 112.6, 112.2, 111.5, 70.2, 55.6, 45.8, 30.0, 22.8, 10.7. Anal. Found: C, 50.77; H, 5.21; N, 9.50; S, 7.14%. $C_{18}H_{22}BrN_3O_2S$ requires C, 50.95; H, 5.23; N, 9.90; S, 7.55.

4.5.2. *N*-(5-Bromo-2-pyridinyl)-*N′*-[2-(5-methoxy-2-propargyloxyphenyl)ethyl]-thiourea (6b)—Using DMF/100 °C, (17%); mp 121–122°C; IR ($CHCl_3$) ν_{max} 3691, 3416 (NH), 3307 (≡CH), 3174, 1602, 1137 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 11.13 (1H, br s, NH), 8.29 (1H, br s, NH), 8.12 (1H, d, *J* = 2.4 Hz), 7.69 (1H, dd, *J* = 2.4, 8.8 Hz), 6.95 (1H, d, *J* = 8.8 Hz), 6.82 (1H, d, *J* = 3.1 Hz), 6.76 (1H, dd, *J* = 3.1, 8.8 Hz), 6.59 (1H, d, *J* = 8.8 Hz), 4.67 (1H, d, 1H, *J* = 2.2 Hz), 4.01 (1H, q, *J* = 6.7 Hz), 3.76 (3H, s), 3.01 (2H, t, *J* = 6.7 Hz), 2.47 (1H, t, *J* = 2.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 179.1 (C=S), 154.2, 151.7, 150.0, 146.7, 141.1, 129.2, 117.6, 113.5, 113.3, 112.6, 111.5, 79.0, 75.3, 56.8, 55.5, 45.7, 29.8. Anal. Found: C, 51.23; H, 4.50; N, 8.62%. $C_{18}H_{18}BrN_3O_2S$ requires C, 51.44; H, 4.32; N, 10.00.

4.5.3. *N*-(5-Bromo-2-pyridinyl)-*N′*-{2-[2-(3-butynyl-1-oxy)-5-methoxyphenyl] ethyl}-thiourea (6c)—Using DMF/100 °C, (25%); mp 155–156 °C; IR ($CHCl_3$) ν_{max} 3691, 3415, 3308 (≡CH), 3176, 3048, 2360 (C≡C), 1591, 1511, 1138 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 11.13 (1H, br s, NH), 8.44 (1H, br s, NH), 8.10 (1H, d, *J* = 2.4 Hz), 7.68 (1H, dd, *J* = 2.4, 8.7 Hz), 6.76 (3H, m), 6.61 (1H, d, *J* = 8.7 Hz), 4.03 (4H, m), 3.75 (3H, s), 3.00 (2H, t, *J* = 6.6 Hz), 2.68 (2H, td, *J* = 2.6, 6.9 Hz), 2.04 (1H, t, *J* = 2.6 Hz); ^{13}C NMR (75*δ*MHz, $CDCl_3$) *δ*: 179.2 (C=S), 153.8, 151.6, 150.9, 146.9, 141.2, 128.9, 117.8, 113.1, 112.8, 112.7, 111.6, 80.8, 69.9, 66.9, 55.6, 45.8, 30.0, 19.8. Anal. Found: C, 52.01; H, 4.46; N, 9.00%. $C_{19}H_{20}BrN_3O_2S$ requires C, 52.54; H, 4.64; N, 9.67.

4.5.4. *N*-(5-Bromo-2-pyridinyl)-*N′*-{2-[5-methoxy-2-(4-pentynyl-1-oxy)phenyl] ethyl}-thiourea (6d)—Using THF/rt, (69%); mp 140–141 °C; IR ($CHCl_3$) ν_{max} 3684, 3415 (NH), 3308 (≡CH), 3152, 3018, 2956, 2245 (C≡C), 1512, 1468 (C=S), 1226 (C–N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 11.18 (1H, br s, NH), 8.83 (1H, br s, NH), 8.10 (1H, d, *J* = 2.4 Hz), 7.68 (1H, dd, *J* = 2.4 Hz, 8.8 Hz), 6.81 (1H, d, *J* = 9.0 Hz), 6.81 (1H, d, *J* = 3.0 Hz), 6.75 (1H, dd, *J* = 3.0, 9.0 Hz), 6.70 (1H, d, *J* = 8.8 Hz), 4.01 (4H, m), 3.75 (3H, s), 2.98 (2H, t, *J* = 6.8 Hz), 2.41 (2H, td, *J* = 2.4, 6.8 Hz), 2.02 (2H, m), 1.96 (1H, t, *J* = 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 179.1 (C=S), 153.5, 151.7, 151.2, 146.7, 141.1, 128.5, 117.7, 113.2, 112.6, 112.3, 111.5, 83.5, 68.9, 66.9, 55.6, 45.7, 30.0, 28.4, 15.4. Anal. Found: C, 53.54; H, 4.78; N, 7.81; S, 5.62%. $C_{20}H_{22}BrN_3O_2S$ requires C, 53.58; H, 4.95; N, 9.37; S, 7.15.

4.5.5. *N*-(5-Bromo-2-pyridinyl)-*N′*-{2-[2-(2-butynyl-1-oxy)-5-methoxyphenyl] ethyl}-thiourea (6e)—Using THF/rt, (60%); mp 149–150 °C; IR ($CHCl_3$) ν_{max} 3690, 3410 (NH), 3018, 2239 (C≡C), 1510, 1469 (C=S), 1207 (C–N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 11.12 (1H, br s, NH), 8.58 (1H, br s, NH), 8.11 (1H, d, *J* = 2.4 Hz), 7.68 (1H, dd, *J* = 2.4 Hz, 8.8 Hz), 6.93 (1H, d, *J* = 8.8 Hz), 6.81 (1H, d, *J* = 3.2 Hz), 6.75 (1H, dd, *J* = 3.2 Hz, 8.8 Hz), 6.65 (1H, d, *J* = 8.8 Hz), 4.61 (2H, q, *J* = 2.4 Hz), 4.01 (2H, m), 3.75 (3H, s), 3.00 (2H, t, *J* = 6.8 Hz), 1.83 (3H, t, *J* = 2.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 179.1 (C=S), 153.9, 151.6, 150.4, 146.8, 141.1, 129.1, 117.5, 113.6, 113.1, 112.6, 111.6, 83.4, 74.5, 57.5, 55.6, 45.8, 29.8, 3.7. Anal. Found: C, 52.51; H, 4.53; N, 8.95; S, 6.31%. $C_{19}H_{20}BrN_3O_2S$ requires C, 52.54; H, 4.64; N, 9.67; S, 7.38.

4.5.6. *N*-(5-Bromo-2-pyridinyl)-*N′*-[2-(2-allyloxy-5-methoxyphenyl)ethyl]-thiourea (6f)—Using THF/rt, (67%); mp 153–154 °C; IR ($CHCl_3$) ν_{max} 3681, 3413 (N–H),

3041, 1509 (C=C), 1422 (C=S), 1212 (C–N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 11.11 (1H, br s, NH), 8.13 (1H, br s, NH), 8.10 (1H, d, J = 2.4 Hz), 7.69 (1H, dd, J = 2.4, 8.7 Hz), 6.82 (1H, d, J = 2.9 Hz), 6.80 (1H, d, J = 9.0 Hz), 6.74 (1H, dd, J = 2.9, 9.0 Hz), 6.55 (1H, d, J = 8.7 Hz), 6.05 (1H, ddt, J = 5.1, 10.5, 17.3 Hz), 5.39 (1H, dq, J = 1.6, 17.3 Hz), 5.26 (1H, dq, J = 1.6, 10.5 Hz), 4.49 (2H, dt, J = 1.6, 5.1 Hz), 4.01 (2H, m), 3.76 (3H, s), 3.01 (2H, t, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 179.1 (C=S), 153.5, 151.5, 151.0, 146.9, 141.1, 133.6, 128.7, 117.7, 117.0, 113.0, 112.8, 112.6, 111.5, 69.5, 55.6, 45.8, 30.0. Anal. Found: C, 51.32; H, 4.62; N, 9.56%. $C_{18}H_{20}BrN_3O_2S$ requires C, 51.18; H, 4.78; N, 9.95.

4.5.7. *N*-(5-Bromo-2-pyridinyl)-*N′*-[2-(2-Benzyloxy-5-methoxyphenyl)ethyl]-thiourea (6g)—Using THF/rt, (37%); mp 141–142 °C as a yellow solid; IR ($CHCl_3$) ν_{max} 3691, 3416, 1602, 1505, 1138 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 11.18 (1H, br s, NH), 8.80 (1H, br s, NH), 8.03 (1H, d, J = 2.6 Hz), 7.65 (1H, dd, J = 2.6, 8.7 Hz), 7.37 (5H, m, ArH), 6.86 (1H, d, J = 9.0 Hz), 6.84 (1H, d, J = 3.2 Hz), 6.74 (1H, dd, J = 3.2, 9.0 Hz), 6.67 (1H, d, J = 8.7 Hz), 5.02 (2H, s), 4.03 (2H, m), 3.75 (3H, s), 3.03 (2H, t, J = 6.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 179.0 (C=S), 153.6, 151.6, 151.1, 146.7, 141.1, 137.3, 128.8, 128.5, 127.8, 127.2, 117.7, 113.2, 112.9, 112.6, 111.5, 70.7, 55.6, 45.7, 30.0; HRMS (EI) m/z; found: 471.0594 (M^+), $C_{22}H_{22}BrN_3O_2S$ (M^+) requires 471.0616. Anal. Found: C, 55.48; H, 4.68; N, 8.59; S, 6.17%. $C_{22}H_{22}BrN_3O_2S$ requires C, 55.94; H, 4.69; N, 8.90; S, 6.79.

4.5.8. *N*-(5-Bromo-2-pyridinyl)]-*N′*-{2-[2-(2-Benzyloxyethyl-1-oxy)-5-methoxyphenyl]ethyl}-thiourea (6h)—Using THF/rt, (58% yield); mp 110–111 °C; IR ($CHCl_3$) ν_{max} 3692, 3416 (NH), 3175, 3016, 1506, 1475 (C=S) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 11.09 (1H, br s, NH), 8.38 (1H, br s, NH), 8.04 (1 H, d, J = 2.4 Hz), 7.66 (1H, dd, J = 2.4 Hz, 8.8 Hz), 7.31 (5H, m, ArH), 6.81 (1H, d, J = 8.8 Hz), 6.80 (1H, d, J = 3.2 Hz), 6.74 (1H, dd, J = 3.2 Hz, 8.8 Hz), 6.58 (1H, d, J = 8.8 Hz), 4.63 (2H, s), 4.11 (2H, t, J = 4.8 Hz), 4.03 (2H, m), 3.84 (2H, t, J = 4.8 Hz), 3.75 (3H, s), 3.01 (2H, t, J = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 179.0 (C=S), 153.6, 151.8, 151.3, 146.6, 141.0, 138.1, 128.8, 128.5, 127.7, 127.7, 117.7, 113.4, 112.8, 112.9, 111.6, 73.4, 68.9, 68.5, 55.6, 45.7, 30.0. Anal. Found: C, 56.07; H, 4.86; N, 7.37; S, 5.80%. $C_{24}H_{26}BrN_3O_3S$ requires C, 55.82; H, 5.07; N, 8.14; S, 6.21.

4.5.9. *N*-(5-Bromo-2-pyridinyl)]-*N′*-{2-[5-methoxy-2-(2-propargyloxyethoxy) phenyl]ethyl}-thiourea (6i)—Using DMF/rt, (62%); mp 128–130 °C; IR ($CHCl_3$) ν_{max} 3693, 3416, 3307, 3165, 2961, 1506, 1475, 1223 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 11.19 (1H, br s, NH), 9.24 (1H, br s, NH), 8.07 (1H, d, J = 2.6 Hz), 7.66 (1H, dd, J = 2.6, 8.7 Hz), 6.81–6.77 (3H, m), 6.72 (1H, dd, J = 2.9, 9.2 Hz), 4.25 (2H, d, J = 2.4 Hz), 4.09 (2H, t, J = 4.8 Hz), 4.01 (2H, q, J = 6.6 Hz), 3.88 (2H, t, J = 4.8 Hz), 3.74 (3H, s), 2.99 (2H, t, J = 6.6 Hz), 2.45 (1H, t, J = 2.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 178.9 (C=S), 153.6, 151.7, 151.1, 146.6, 141.0, 128.8, 117.6, 113.4, 112.9, 112.6, 111.5, 79.5, 74.7, 68.4, 68.2, 58.5, 55.5, 45.6, 29.9; HRMS (EI) m/z; found: 463.0578 (M^+). $C_{20}H_{22}BrN_3O_3S$ (M^+) requires 463.0565. Anal. Found: C, 51.76; H, 4.72; N, 8.66; S, 6.86%. $C_{20}H_{22}BrN_3O_3S$ requires C, 51.73; H, 4.78; N, 9.05; S, 6.91.

4.5.10. *N*-(5-Bromo-2-pyridinyl)-*N′*-{2-[5-methoxy-2-(2-(2-propargyloxyethoxy) ethoxy)phenyl]ethyl}-thiourea (6j)—Using DMF/rt, (60%); mp 91–92 °C; IR ($CHCl_3$) ν_{max} 3691, 3416, 3307, 3166, 2935, 1506, 1475, 1266 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ: 11.11 (1H, br s, NH), 8.67 (1H, br s, NH), 8.07 (1H, d, J = 2.4 Hz), 7.67 (1H, dd, J = 2.4, 8.7 Hz), 6.81–6.78 (2H, m), 6.73 (1H, dd, J = 3.0, 8.7 Hz), 6.67 (1H, d, J = 8.7 Hz), 4.21 (2H, d, J = 2.1 Hz), 4.07 (2H, t, J = 4.9 Hz), 4.01 (2H, q, J = 6.6 Hz), 3.85 (2H, t, J = 4.9 Hz), 3.73 (7H, m), 2.99 (2H, t, J = 6.6 Hz), 2.42 (1H, t, J = 2.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 179.1 (C=S), 153.6, 151.6, 151.2, 146.8, 141.1, 128.8, 117.7, 113.2, 112.8, 112.6, 111.5, 79.6, 74.6,

70.6, 70.0, 69.2, 68.4, 58.5, 55.6, 45.7, 30.0; HRMS (EI) *m*/*z*; found: 507.0821 (M^+). $C_{22}H_{26}BrN_3O_4S$ (M^+) requires 507.0827. Anal. Found: C, 52.02; H, 4.10; N, 7.87; S, 6.24%. $C_{22}H_{26}BrN_3O_4S$ requires C, 51.97; H, 5.15; N, 8.26; S, 6.31.

4.5.11. *N*-(5-Bromo-2-pyridinyl)-*N′*-[2-(2-methoxycarbonylmethyloxy-5-methoxyphenyl)ethyl]-thiourea (6k)—Using THF/rt, (50%); mp 161–164 °C; IR ($CHCl_3$) ν_{max} 3681 (N–H), 3015, 1519, 1469 (C=S), 1212 (C–N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 11.12 (1H, br s, NH), 8.32 (1H, br s, NH), 8.11 (1H, d, *J* = 2.4 Hz), 7.69 (1H, dd, *J* = 2.4, 9.1 Hz), 6.83 (1H, d, *J* = 2.4 Hz), 6.71 (2H, m), 6.61 (1H, d, *J* = 9.1 Hz), 4.62 (2H, s), 4.05 (2H, m), 3.78 (3H, s), 3.74 (3H, s), 3.06 (2H, t, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$ ppm) δ: 179.2 (C=S), 169.6, 154.2, 151.5, 150.3, 146.9, 141.2, 129.2, 117.8, 113.0, 112.7, 112,6, 111.6, 66.3, 55.5, 52.2, 45.7, 29.9; HRMS (ES) *m*/*z*; found: $[M+H]^+$, 454.0437. Calcd. for $C_{18}H_{21}BrN_3O_4S$ (M+H), 454.0436.

4.5.12. *N*-(5-Bromo-2-pyridinyl)-*N′*-[2-(2-cyanomethyloxy-5-methoxyphenyl) ethyl]-thiourea (6l)—Using THF/rt, (68%); mp 155–156 °C; IR ($CHCl_3$) ν_{max} 3681, 3413, 3022 (NH), 2239 (C≡N), 1505, 1469 (C=S), 1216 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 11.20 (1H, br s, NH), 8.72 (1H, br s, NH), 8.10 (1H, d, *J* = 2.4 Hz), 7.70 (1H, dd, *J* = 2.4 Hz, 8.8 Hz), 6.92 (1H, d, *J* = 8.8 Hz), 6.85 (1H, d, 3.2 Hz), 6.78 (1H, dd, *J* = 3.2, 8.8 Hz), 6.69 (1H, d, *J* = 8.8 Hz), 4.75 (2H, s), 3.99 (2H, q, *J* = 6.8 Hz), 3.76 (3H, s) 3.01 (2H, t, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 179.4 (C=S), 155.4, 151.6, 149.0, 146.7, 141.3, 129.8, 117.9, 115.4, 114.0, 113.3, 112.8, 111.9, 55.6, 54.9, 45.6, 29.6. Anal. Found: C, 48.38; H, 4.04; N, 12.38; S; 6.76%. $C_{17}H_{17}BrN_4O_2S$ requires C, 48.46; H, 4.07; N, 13.30; S, 7.61.

4.5.13. *N*-(5-Bromo-2-pyridinyl)]-*N′*-{2-[2-(3-cyanopropyl-1-oxy)-5-methoxyphenyl]ethyl}-thiourea (6m)—Using THF/rt, (53%); mp 165–166 °C; IR ($CHCl_3$) ν_{max}, 3413 (NH), 3167, 2964 (C–H), 2246 (C≡N), 1505, 1465 (C=S), 1239 (C–N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 11.26 (1 H, br s, NH), 9.34 (1H, br s, NH), 8.08 (1H, d, *J* = 2.4 Hz), 7.67 (1H, dd, *J* = 2.4 Hz, 8.8 Hz), 6.82 (1H, d, *J* = 3.2 Hz), 6.81 (1H, d, *J* = 8.8 Hz), 6.77 (1H, d, *J* = 8.8 Hz), 6.74 (1H, dd, *J* = 3.2 8.8 Hz), 3.99 (4H, m), 3.74 (3H, s), 2.98 (2H, t, *J* = 6.8 Hz), 2.62 (2H, t, *J* = 7.0 Hz), 2.14 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 179.0 (C=S), 153.7, 151.7, 150.6, 146.5, 141.1, 128.4, 119.2, 117.7, 113.4, 112.7, 112.2, 111.5, 66.0, 55.5, 45.6, 29.9, 25.6, 14.4. Anal. Found: C, 50.61; H, 4.78; N, 12.37; S, 6.92%. $C_{19}H_{21}BrN_4O_2S$ requires C, 50.78; H, 4.71; N, 12.47; S, 7.13.

4.5.14. *N*-(5-Bromo-2-pyridinyl)]-*N′*-{2-[2-(2-hydroxyethoxy)-5-methoxyphenyl] ethyl}-thiourea (6n)—Using THF/rt, (65%); mp 162–163 °C; IR ($CHCl_3$) ν_{max} 3623, 3413, 3181 (NH, OH), 2928, 1505 (C–H), 1472 (C=S) 1227 (C–N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 11.16 (1H, br s, NH), 8.47 (1H, br s, NH), 8.12 (1H, d, *J* = 2.4 Hz), 7.70 (1H, dd, *J* = 2.4, 8.8 Hz), 6.81 (1H, d, *J* = 2.8 Hz), 6.77 (1H, d, *J* = 8.8 Hz), 6.73 (1H. dd, *J* = 2.8, 8.8 Hz), 6.66 (1H, d, *J* = 8.8 Hz), 3.99 (6H, m), 3.77 (3H, s), 3.03 (2H, t, *J* = 7.2 Hz), 2.66 (1H, br s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 179.4 (C=S), 153.7, 151.6, 151.3, 146.7, 141.3, 128.3, 117.4, 113.4, 112.8, 112.5, 111.7, 70.3, 61.5, 55.6, 45.7, 30.3. Anal. Found: C, 47.96; H, 4.61; N, 9.77; S, 7.01%. $C_{17}H_{20}BrN_3O_3S$ requires C, 47.89; H, 4.75; N, 9.86; S, 7.52.

4.5.15. *N*-(5-Bromo-2-pyridinyl)]-*N′*-{2-[2-(3-hydroxypropyl-1-oxy)-5-methoxyphenyl]ethyl}-thiourea (6o)—Using THF/rt, (51%), mp 163–165 °C; IR ($CHCl_3$) ν_{max} 3681 (N–H), 3406 (O–H) 3014, 1509, 1472 (C=S), 1216 (C–N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 11.15 (1H, br s, NH), 8.46 (1H, br s, NH), 8.10 (1H, d, *J* = 2.4 Hz), 7.68 (1H, dd, *J* = 2.4, 8.8 Hz), 6.81 (1H, d, *J* = 8.8 Hz), 6.81 (1H, d, *J* = 2.9 Hz), 6.74 (1H, dd, *J* = 2.9, 8.8 Hz), 6.62 (1H, d, *J* = 8.8 Hz), 4.05 (2H, t, *J* = 5.9 Hz), 4.00 (2H, m), 3.87 (2H, q, *J* = 5.9 Hz), 3.76 (3H, s), 2.98 (2H, t, *J* = 6.6 Hz), 2.05 (2H, quin, *J* = 5.9 Hz), 2.04 (1H, br s,

OH); ^{13}C NMR (101 MHz, $CDCl_3$ ppm) δ: 179.1 (C=S), 153.5, 151.6, 151.3, 146.8, 141.2, 128.4, 117.7, 113.2, 112.7, 112.4, 111.6, 65.9, 60.1, 55.6, 45.8, 32.3, 30.0. Anal. Found: C, 49.37; H, 4.88; N, 9.08; S, 6.60%. $C_{18}H_{22}BrN_3O_4S$ requires C, 49.09; H, 5.04; N, 9.54; S, 7.28.

4.6. Anti-HIV evaluation

The following reagents were obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: MT-2 cells and Nevirapine-Resistant HIV-1 (N119) from Dr. Douglas Richman and HTLV-III$_B$/H9 from Dr. Robert Gallo.

Antiviral activity and cellular toxicity were determined using the MTT colorimetric method.[19] MT-2 cells[29] at a concentration of 1×10^5 cells per millilitre were infected with wild-type HIV-IIIB[30] or Y181C mutant virus[31] at a multiplicity of infection (MOI) of 0.1. Infected and mock-infected cells were incubated in growth medium (RPMI 1640, 10% dFBS, kanamycin) for 5 days with varying concentrations of each compound being tested in triplicate in a 96-well plate. MTT, a cell-permeable tetrazolium dye was then added to each well. After 5 h, acidified isopropanol was added to lyse the cells and stop the reaction. The plates were gently shaken overnight, and the absorbance measured at 595 nm on a plate reader. The average of these triplicate samples was then plotted versus inhibitor concentration to generate dose–response curves. The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) of the compounds were defined as the concentrations required to inhibit viral replication and to reduce the number of viable cells by 50%, respectively.

4.7. Steady-state IC_{50} determination

6 nM RT (active sites based on pre-steady-state active site determination) was pre-incubated for at least 15 min with 1 μM 5′-radiolabeled primer/template prior to mixing with appropriate concentrations of inhibitor and allowed to incubate for a minimum of 15 additional minutes on ice. DMSO concentrations were kept constant at less than 2%. DMSO alone was added as a no inhibitor control for each set of experiments. Reactions were initiated with the addition of 5 μM dTTP and 10 mM $MgCl_2$ and were quenched after 15 min at 37 °C with 0.3 MEDTA. All concentrations represent final concentrations after mixing. Reaction products were subjected to 20% denaturing polyacrylamide gel-electrophoresis and quantitated on a Bio-Rad Molecular Imager FX. Product formation was plotted as a function of inhibitor concentration and fitted to a hyperbola to generate IC_{50} curves. IC_{50} values are defined as the concentration of inhibitor that inhibits steady-state single nucleotide incorporation by 50%.

Supplemental Material

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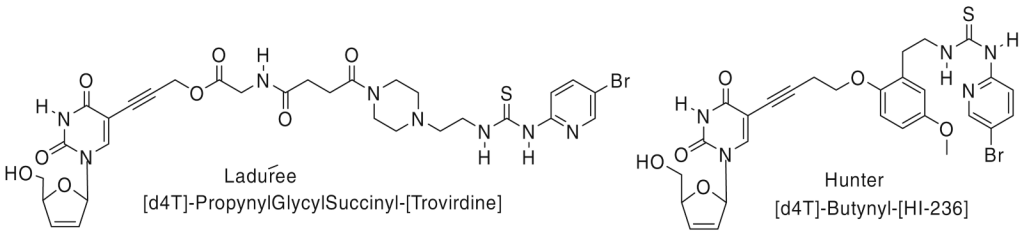


Figure 1.
Structures of two recent bifunctional inhibitors based on PETT compounds with d4 T.

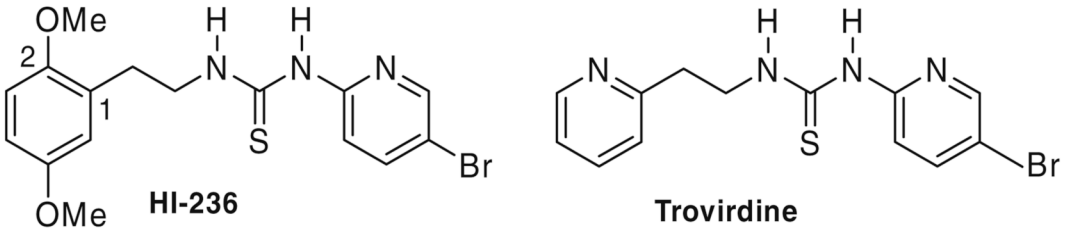
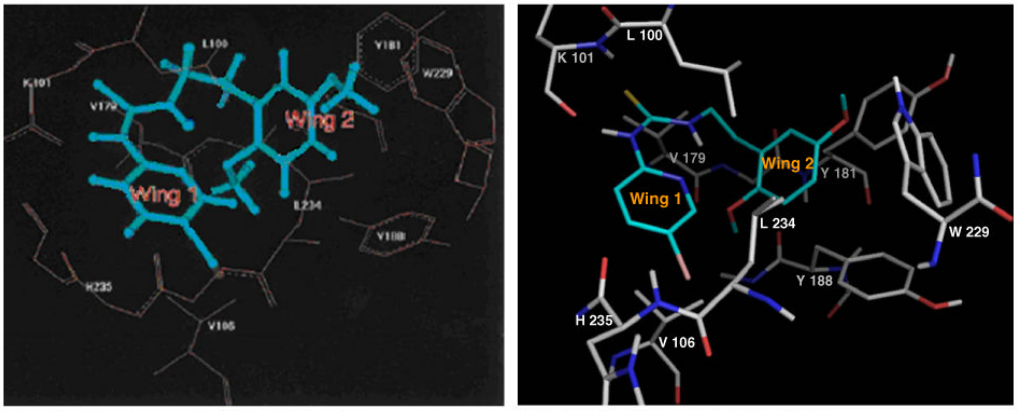


Figure 2.
Structures of HI-236 and Trovirdine.



Uckun's HI-236 docked into the HIV pocket

Our HI-236 docked by AutoDock 3.05

Figure 3.
HI-236 docked into the HIV pocket.

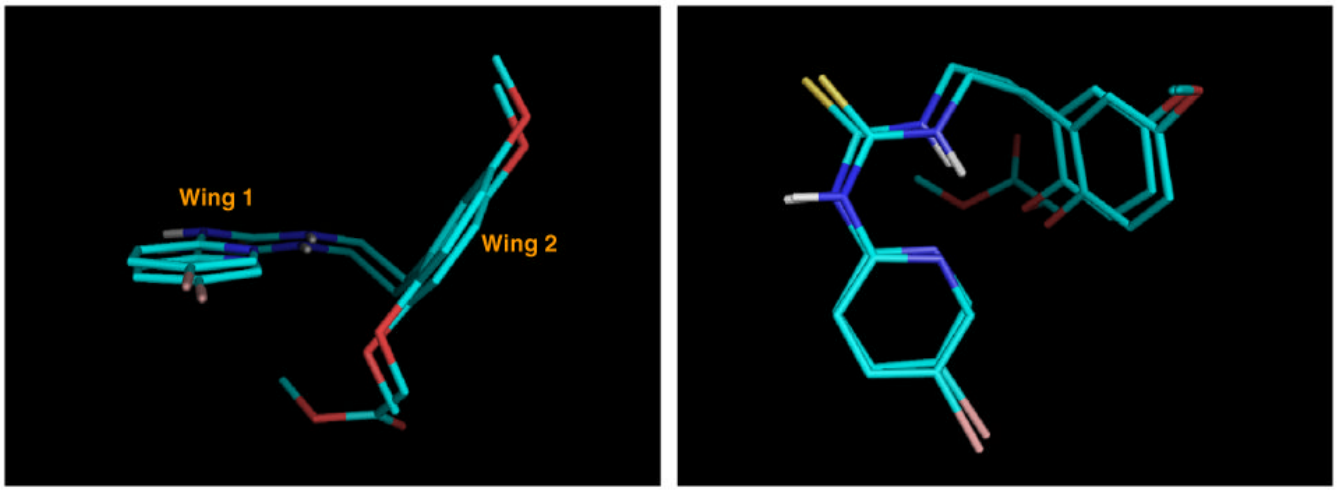


Figure 4.
The similarities in orientation between the AutoDock models of HI-236 and **6k**.

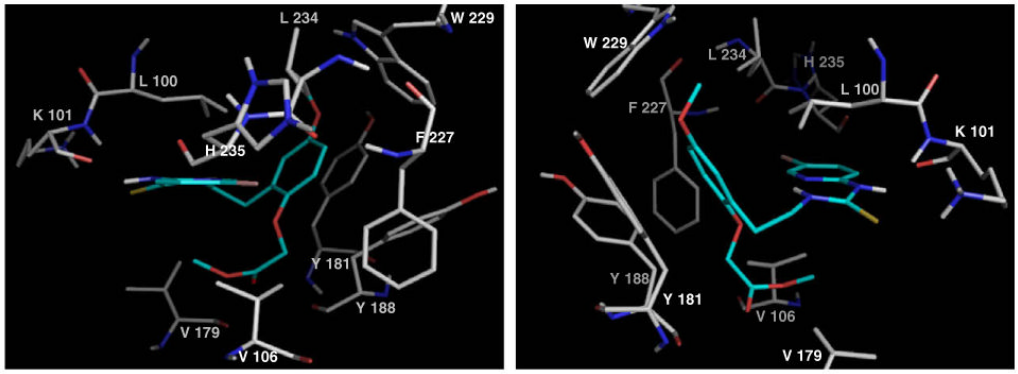


Figure 5.
Docking of ester **6k**.

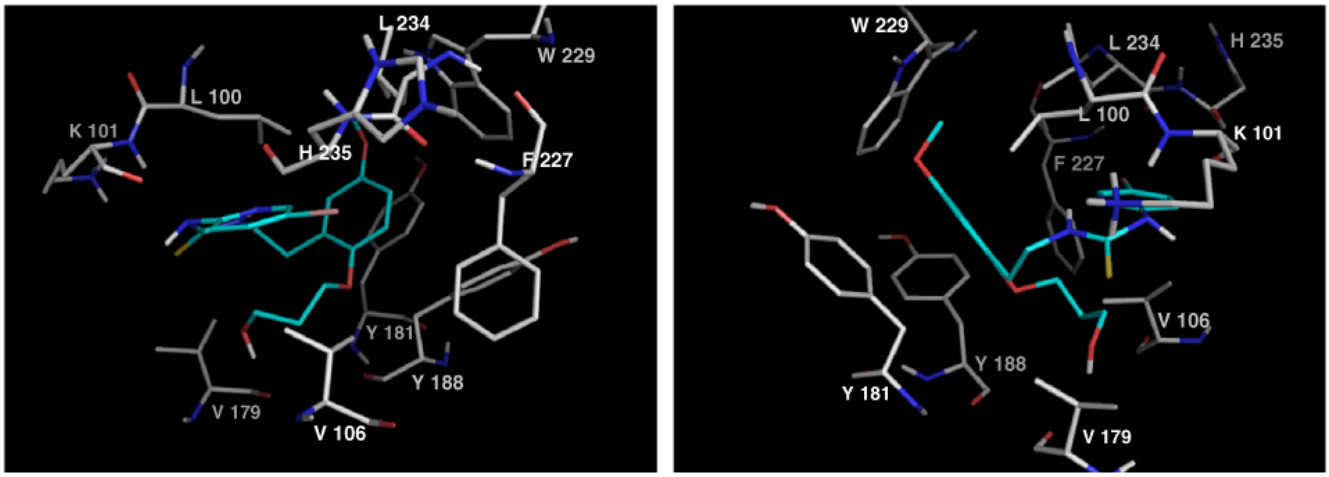
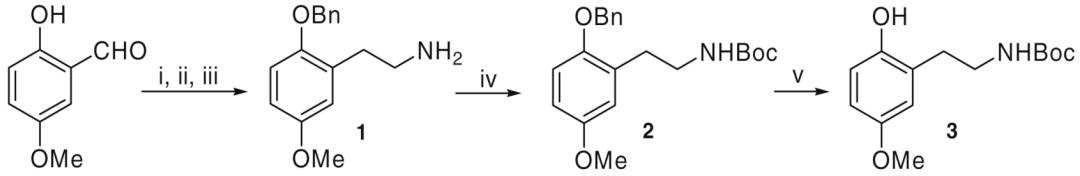
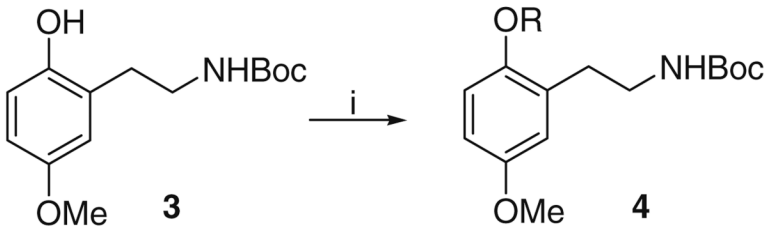


Figure 6.
Docking of alcohol **6o**.

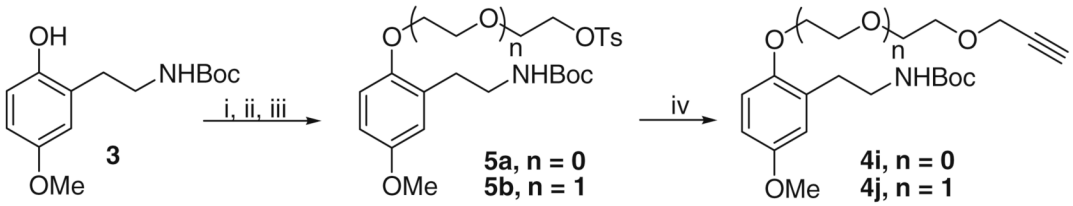


Scheme 1.

Reagents and conditions: (i) BnBr, K_2CO_3, EtOH, reflux, 16 h; 95%; (ii) CH_3NO_2, NH_4OAc, 70 °C, 14 h; 75%; (iii) $LiAlH_4$, THF, reflux, 4 h; (iv) $(Boc)_2O$, Et_3N, CH_3CN, rt, overnight; 76% (2 steps); (v) H_2, Pd/C, EtOH, rt, 5 h; 66%.

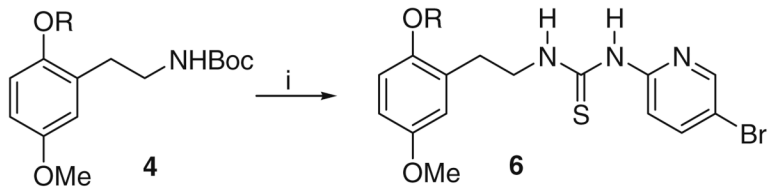


Scheme 2.
Reagents and conditions: (i) K_2CO_3, CH_3CN, 80 °C, 20 h or NaH, DME, 80 °C, 20 h with ROTs.



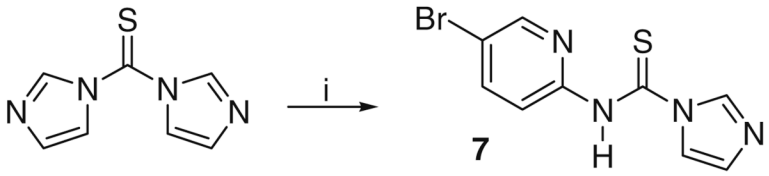
Scheme 3.

Reagents and conditions: (i) BnPEGBr (*n* = 0 or 1), K_2CO_3, CH_3CN, 80 °C, 20 h or NaH, DME, 80 °C, 20 h; (ii) H_2, Pd/C, EtOH, rt, 18 h (iii) TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C–rt, 20 h (iv) propargyl alcohol, NaH, THF, 70 °C, 5 h.



Scheme 4.

Reagents and conditions: (i) CF_3COOH, CH_2Cl_2, 0 °C, 1 h and then DIEA, 0 °C, 10 min, followed by **8**, THF, rt, 2 h.



Scheme 5.
Reagents and conditions: (I) 2-amino-5-bromopyridine, CH_3CN, rt, 120.

No.	R
4i	N≡—$CH_2CH_2CH_2$
4j	

No.	
4m	
4n	

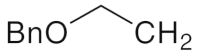
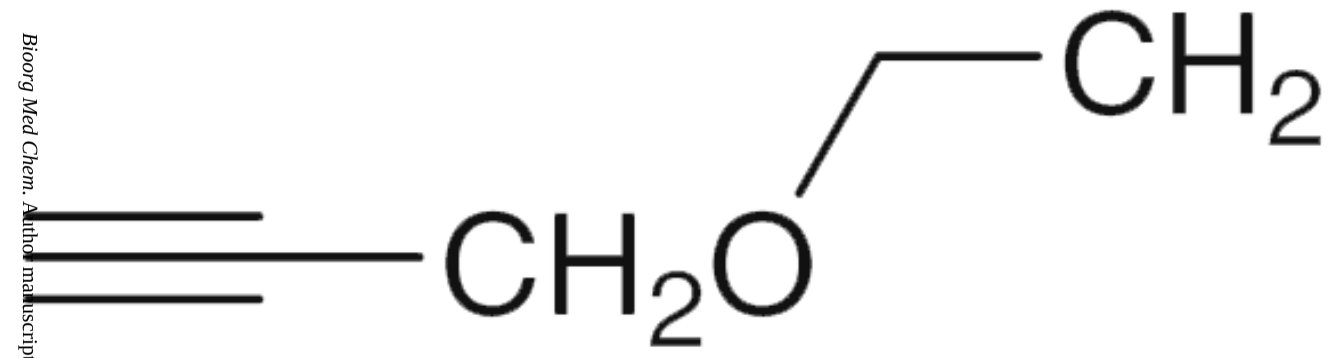
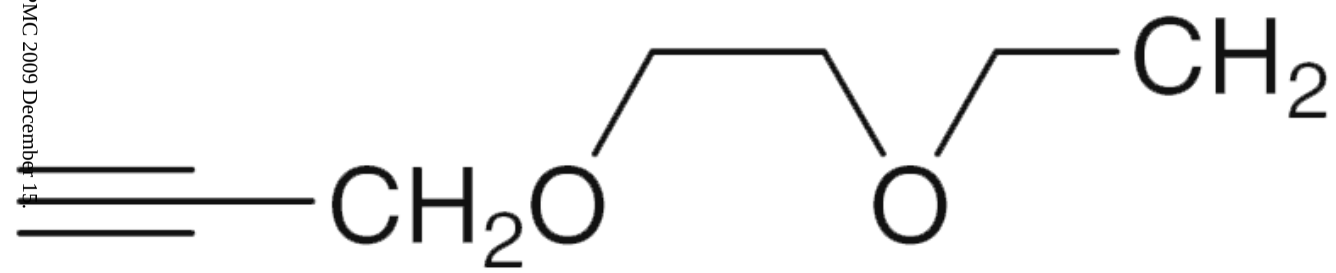
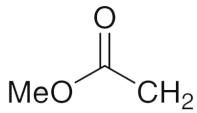
No.	R
4k	
4l	
4o	

Table 2

Yields for converting **4**–**6**.

Compound	Yield	Compound	Yield	Compound	Yield	Compound	Yield
6a	65	**6e**	60	**6i**	62	**6m**	53
6b	17[a]	**6f**	67	**6j**	60	**6n**	65
6c	25[a]	**6g**	37	**6k**	50	**6o**	51
6d	69	**6h**	58	**6l**	68		

[a]DMF at 100 °C; the others involved THF at rt.

)	T.I[d]	R	EC$_{50}$[b] (μM)	CC$_{50}$[c] (μM)	T.I[d]
	21	 **6h**	2.0	5.8	3
	1	 **6i**	0.095	9.5	100
	2	 **6j**	0.39	6.8	17
	38	 **6k**	12	48	4

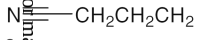
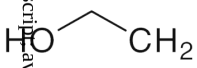
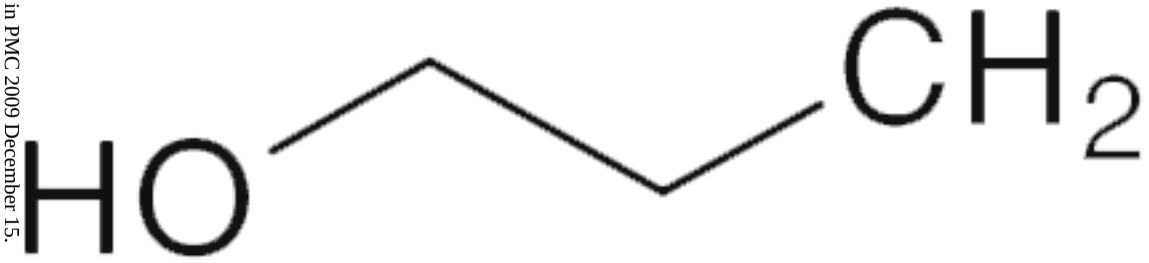
)	T.I[d]	R	EC$_{50}$[b] (μM)	CC$_{50}$[c] (μM)	T.I[d]
	40		0.12	5.5	46
	74		0.05	3.8	76
	55		0.012	1.0	83
	18		0.098	8.5	94

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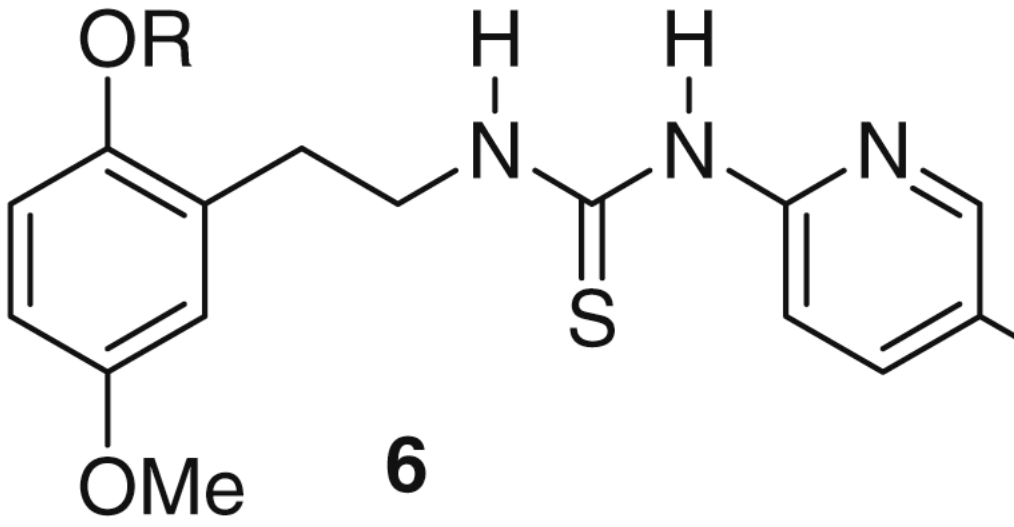
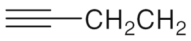
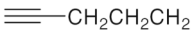
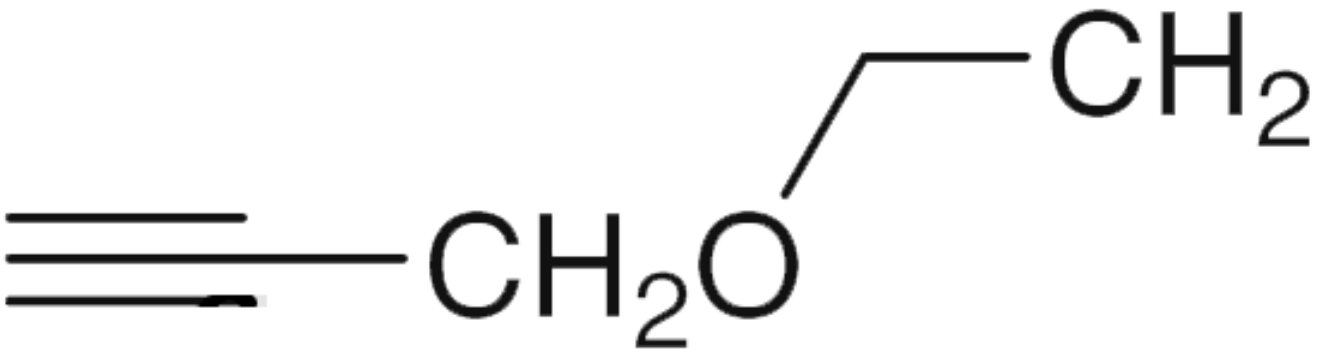
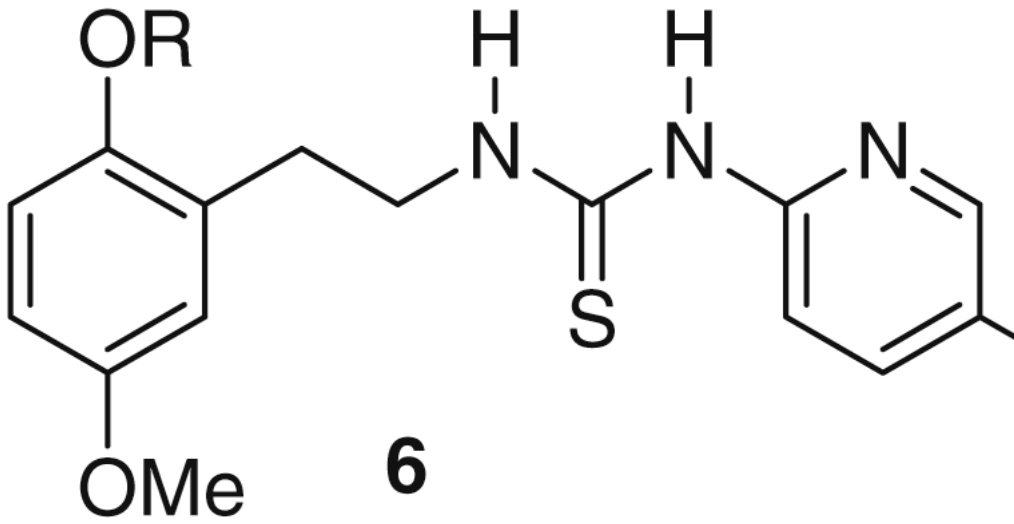
Table 4

Comparison of cell culture versus in vitro RT inhibition (nM) for selected derivatives of **6**.

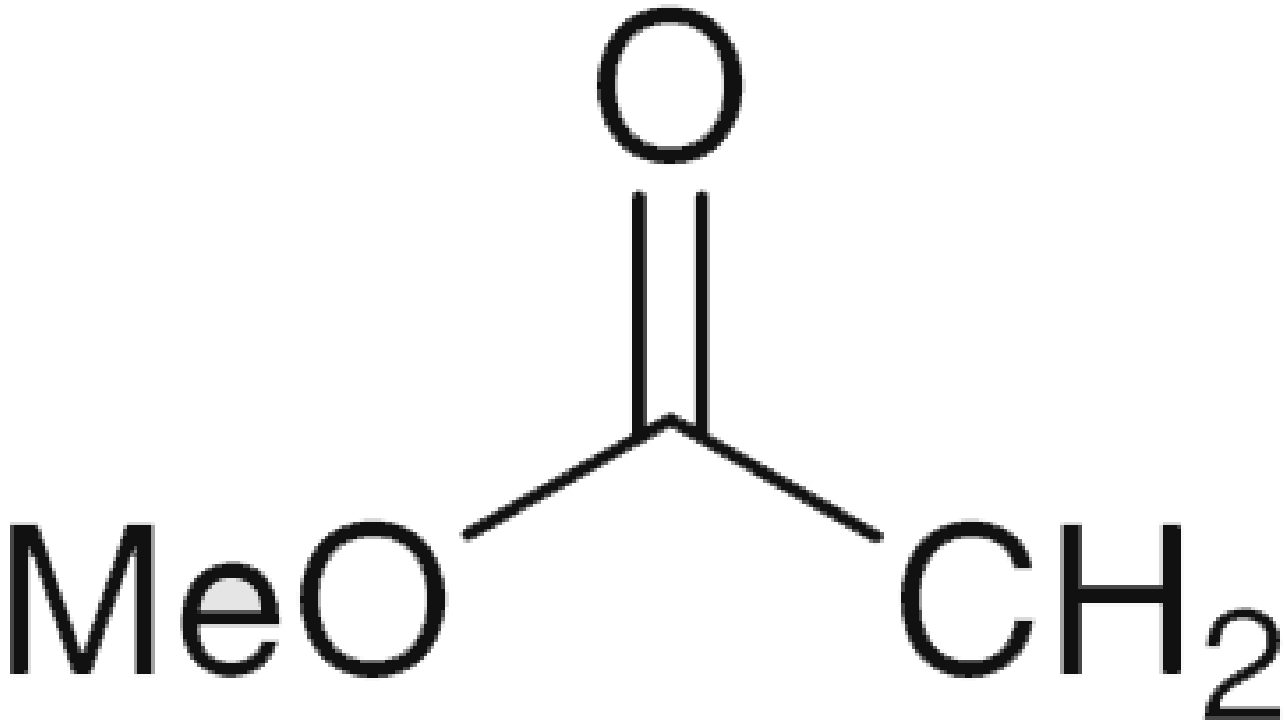


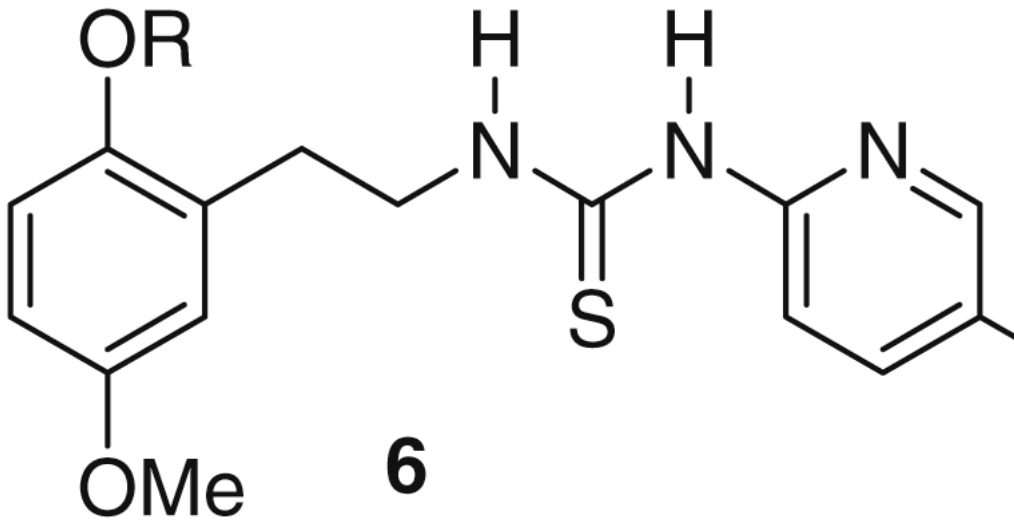
6

R	An act EC (nM
Me (HI-236)	48
 **6c**	26
 **6d**	250
 **6i**	95

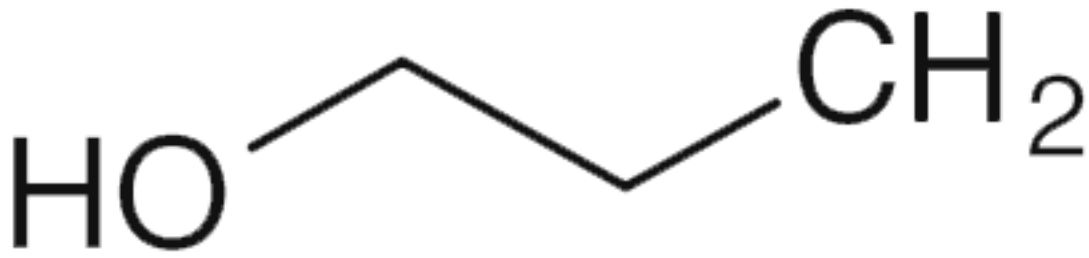


6

R	An act EC (nM
	12,
6k	
	12
6n	



6

R	An act EC (nM
 6o	98