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SAR analysis of a series of acylthiourea derivatives possessing broad-spectrum antiviral activity

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ABSTRACT

A series of acylthiourea derivatives were designed, synthesized, and evaluated for broad-spectrum antiviral activity with selected viruses from *Poxviridae* (vaccinia virus) and two different genera of the family *Bunyaviridae* (Rift Valley fever and La Crosse viruses). A compound selected from a library screen, compound **1**, displayed submicromolar antiviral activity against both vaccinia virus (EC_{50} = 0.25 μM) and La Crosse virus (EC_{50} = 0.27 μM) in cytopathic effect (CPE) assays. SAR analysis was performed to further improve antiviral potency and to optimize drug-like properties of the initial hits. During our analysis, we identified **26**, which was found to be nearly fourfold more potent than **1** against both vaccinia and La Crosse viruses. Selected compounds were further tested to more fully characterize the spectrum of antiviral activity. Many of these possessed single digit micromolar and sub-micromolar antiviral activity against a diverse array of targets, including influenza virus (*Orthomyxoviridae*), Tacaribe virus (*Arenaviridae*), and dengue virus (*Flaviviridae*).

We live in a world surrounded by a myriad of diverse viruses which are found in every ecosystem. A majority of these are harmless to humans but an extensive list of highly infectious viral families capable of generating high morbidity and mortality are included under the umbrella of NIAID Category A, B, and C pathogens.[1] Many of these viruses pose threats through weaponization as well as being endemic in areas where military personnel may be deployed. These pathogens include viral families such as *Poxviridae* (variola virus), *Filoviridae* (Ebola and Marburg viruses), and *Bunyaviridae* (Rift Valley fever and La Crosse viruses). Given the rather large number of these more virulent pathogens from such diverse viral families, an approach targeting broad-spectrum antiviral activity would be preferable to targeting each individual virus. Currently, there are limited options of efficacious broad-spectrum antivirals on the market. Ribavirin is active against a number of RNA and DNA viruses including influenzas, flaviviruses, and agents of many viral hemorrhagic fevers. This purine nucleoside analog likely functions through lethal mutagenesis, although multiple direct and indirect mechanisms have been proposed.[2] Ribavirin co-administered with PEGylated interferon-alpha (INF-α) is used as a treatment for hepatitis C virus infections.[3] However, this combination treatment has inherent issues such as serious side effects including hemolytic anemia.[4,5] This treatment is also quite costly, making it unfeasible for widespread use. Additionally, ribavirin exhibits teratogenicity in some animal species.[6,7]

In order to uncover molecular scaffolds possessing potential broad-spectrum antiviral activity, high-throughput screening (HTS) was performed on a library of 206,000 small molecules. HTS assayed activity against Rift Valley fever virus (RVFV) and La Crosse virus (LACV), which represent two distinct genera in the genetically diverse *Bunyaviridae* family. Molecules which displayed inhibitory activity against either of these viruses at a fixed concentration (5 μM) and contained chemically viable scaffolds were subsequently tested against both RVFV and LACV across a range of concentrations (0.009–25 μM) to characterize activity. During this screening process, two hits were identified, compounds **1** and **2** (Fig. 1), which shared an acylthiourea moiety. Acylthiourea derivatives have been reported in the literature for possessing a broad

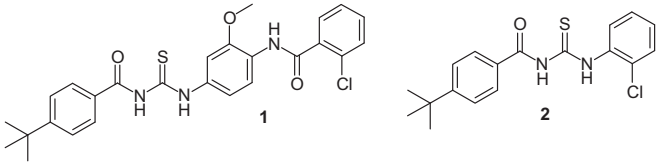

Figure 1. Broad-spectrum antiviral hits discovered via high-throughput screening.

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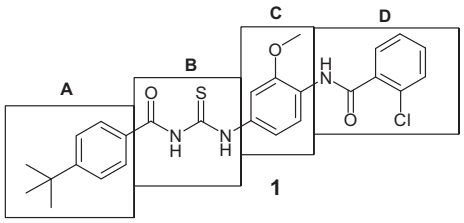


Figure 2. Segmentation of the acylthiourea scaffold—segments A, B, C, and D.

range of biological activities including antibacterial,[8] antifungal,[9] anticancer,[10] antiviral,[11] and antithyroid[12] activities. Compound **1** was further tested against other viral families and showed submicromolar antiviral potency against vaccinia virus (*Poxviridae*), Ebola virus (*Filoviridae*), influenza virus (*Orthomyxoviridae*), human immunodeficiency virus type 1 (*Retroviridae*), Tacaribe virus and lymphocytic choriomeningitis virus (*Arenaviridae*), encephalomyocarditis virus (*Picornaviridae*), Sindbis virus (*Togaviridae*), dengue virus (*Flaviviridae*), Andes virus (*Bunyaviridae*), and respiratory syncytial virus (*Paramyxoviridae*).

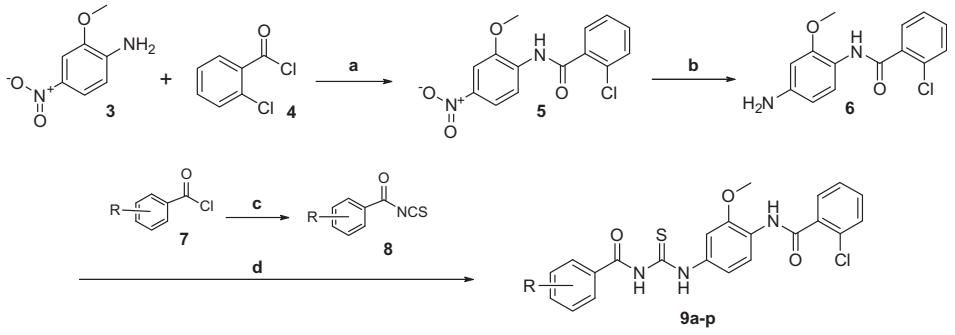
Concerning the SAR analysis of this acylthiourea scaffold shared by **1** and **2**, a strategy was employed involving the segmentation of the molecule as shown in Figure 2. As illustrated, **2** is very similar to **1** with the major difference being the addition of the chlorobenzamide portion (segment D). The majority of the efforts were concentrated on changing one segment at a time. Initially, changes were made in order to probe the chemical space of the scaffold and determine the effect on antiviral potency. This included incorporating groups of various size, electron donating/accepting capability, as well as polar and ionizable groups. This study also made it possible to uncover which moieties of the HTS hits might be crucial for activity. To ensure subsequent analogs displayed broad-spectrum activity, they were screened against both vaccinia virus (VV) and LACV. Compound **1** displayed superior activity against VV (**1**, EC_{50} = 0.25 μM; **2**, EC_{50} = 0.5 μM), while LACV activity was similar (**1**, EC_{50} = 0.27 μM; **2**, EC_{50} = 0.28 μM).[13] Analogs were also tested for cytotoxicity to make certain the observed antiviral activity was not due to cellular toxicity. Beyond the initial probing of the scaffold, more advanced SAR studies focused on the design of compounds with more drug-like properties by improving the various ADMET characteristics of the molecules, while retaining broad-spectrum antiviral potency.

The overall SAR strategy involved probing one particular segment of each HTS scaffold at a time, while essentially keeping the other segments fixed. The majority of the segment A analogs were based on the scaffold of **1** since this compound was about twofold more potent than **2** against VV. The general synthetic pathway utilized to prepare analogs **9a–p** is outlined in Scheme 1. The chemistry was straightforward with an amide coupling followed by reduction of the nitro group to provide intermediate **6**. This intermediate was then reacted with thioisocyanate **8**, which was formed in situ, to afford the final products.

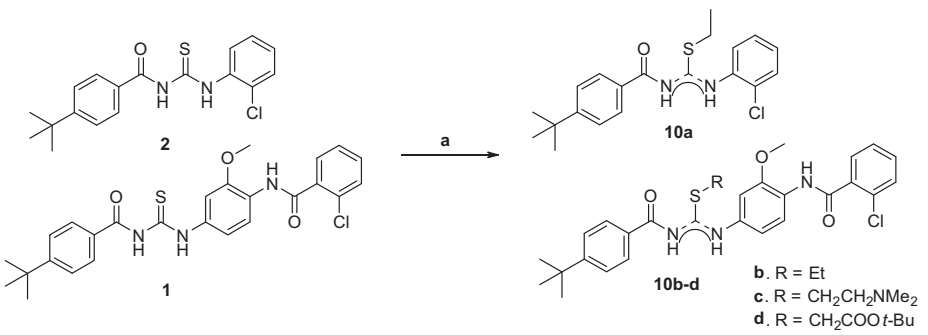
Concerning segment B chemistry, sulfur alkylated compounds **10a–d** were prepared using the general procedure outlined in Scheme 2. As will be discussed, the intended chemistry was alkylation of the amide nitrogen of segment B, as opposed to the observed sulfur alkylation. The alkylation procedures involved the use of an alkyl halide and sodium hexamethyldisilazane (NaHMDS) or potassium carbonate which resulted in the same *S*-alkyl products. For **1**, alkylation beyond *S*-ethyl was explored with alkyl groups containing amino and ester functionalities.

Additional segment B analogs were prepared which incorporated an amidine moiety as shown in Scheme 3. The synthesis involved separate treatment of thiocyanate compounds **11** and **12** with 4-*tert*-butylbenzamidine and NaHMDS to yield **13a** and **13b**. For **13a**, there was enough material synthesized to prepare the corresponding HCl salt form.

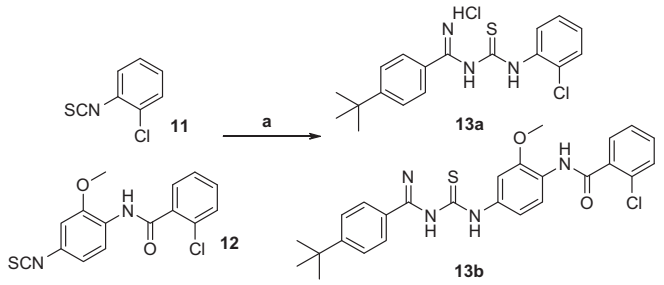
Continuing with segment B, the thiourea group was replaced with a guanidine moiety for compound **16** as shown in Scheme



Scheme 1. Reagents and conditions: (a) TEA, 4:1 DCM/THF, 0 °C to rt; (b) $SnCl_2$, MeOH, 65 °C; (c) NH_4SCN, acetone; (d) DCM, rt.



Scheme 2. Reagents and conditions: (a) RX, 1 M NaHMDS in THF, THF, −60 °C to rt or RX, K_2CO_3, DMF, rt to 50 °C.



Scheme 3. Reagents and conditions: (a) 4-*tert*-butylbenzamidine, NaHMDS, THF, 60 °C, 4 h.

4. The synthesis involved a three-step procedure initiated by reacting compound **6** with Boc-protected methylisothiourea **14** in the presence of mercuric chloride to produce **15**. Following deprotection and acylation with 4-*tert*-butylbenzoylchloride, compound **16** was isolated.

We took advantage of the ease of sulfur alkylation in segment B to install a cyanoguanidine moiety in place of the thiourea as illustrated in Scheme 5. After synthesizing the corresponding thioether (**17**) of compound **1**, a microwave-mediated reaction with sodium hydrogen cyanamide afforded compound **18**.

The majority of the segment C analogs were based on compound **2** due to ease of preparation and a general synthetic pathway is illustrated in Scheme 6.[14] These analogs were mostly synthesized by coupling compound **8** with the appropriate aniline derivatives (**19**). In some cases, an additional modification was required, such as deprotection, to obtain the final product.

A few segment C analogs were based on the extended scaffold of compound **1** including **26**, for which the synthetic pathway is outlined in Scheme 7. Following the initial amide coupling with the Boc-protected aniline derivative (**23**), the nitro group was reduced using iron powder and ammonium chloride to afford compound **25**. This material was reacted with thioisocyanate derivative **8**, deprotected with TFA and treated with HCl to afford **26**. As will be discussed, **26** turned out to be our most potent compound against both VV and LACV.

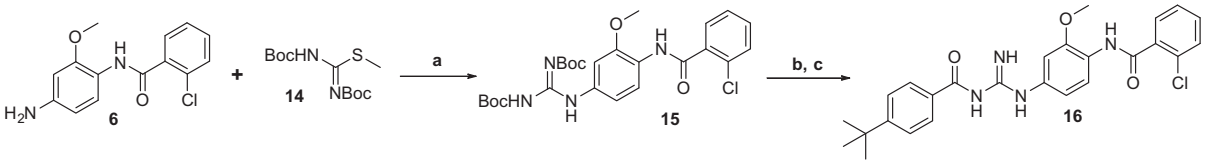
For segment D analogs **30a–o**, Scheme 8 outlines the chemistry used to prepare these targets. Various reaction conditions were utilized for coupling the corresponding carboxylic acid starting material since the amide couplings proved to be very problematic. In some cases, the corresponding acyl chloride starting material was utilized, as opposed to the carboxylic acid, in order to isolate the compounds in a reasonable yield. For some of the analogs (**30h–l** and **30o**), following the amide bond formation it was necessary to deprotect the amino moiety either through removal of a Boc group or reduction of a nitro moiety. For **30j–l** and **30o**, the corresponding HCl salt forms were prepared.

The chemistry outlined in Scheme 9 was used to isolate **32**, which involved reductive alkylation of **29** with 2-chlorobenzaldehyde. Amidine derivative **35** was synthesized following Scheme 10. Imido thioester **34** was generated first, via alkylation of the corresponding thioamide, and then reacted with intermediate **29** to produce the final product.

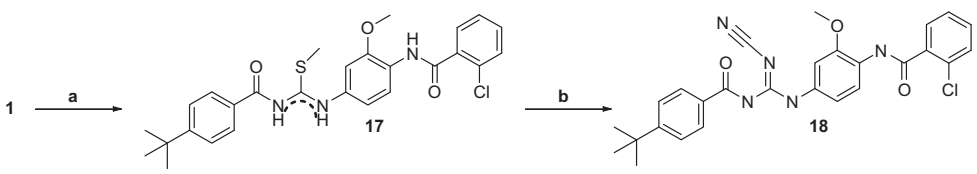
Some of the analogs which contained structural changes in multiple segments included the amino-linked pyridyl analogs **39a–c** (Scheme 11). The synthesis of these compounds involved a palladium-catalyzed amination reaction, followed by reduction of the nitro moiety with tin chloride to form **38**. These intermediates were reacted with thioisocyanate derivative **8** followed by HCl salt formation to generate **39a–c**.

A systematic substitution strategy was employed to probe segment A using a variety of groups aimed at replacement of the *tert*-butyl moiety in an attempt to reduce overall lipophilicity of the molecule. Substitutions were made at all three positions, *ortho*, *meta* and *para*, using hydrogen, chloro, nitro, methyl, methoxy, and amino for compounds **9a–p** (Fig. 3). These groups were chosen to determine the effects of size and electronic character on this segment. Unfortunately, all substitutions resulted in a complete loss of activity against both VV and LACV. Another series of molecules included substitution of the 4-*tert*-butylphenyl ring with a 2-, 3-, or 4-pyridyl ring, which would allow for ionization and a potential increase in solubility. All of these compounds were inactive as well. Two analogs were also tested in which a saturated ring was substituted in place of the phenyl ring, both with and without the 4-*tert*-butyl substituent. Interestingly, with the *tert*-butyl group present there was no antiviral potency observed, while removal of this moiety resulted in an analog with a single digit micromolar VV EC_{50} value. A possible explanation may involve disfavored positioning of this bulky *tert*-butyl substituent due to the preferred conformation of the saturated ring. From this study of segment A, it was ascertained that a bulky lipophilic group was necessary to retain antiviral potency attained by the HTS hits. There was an analog (Fig. 4, R = 4-CH_3) which contained a methyl group in segment A and displayed submicromolar activity against both VV (EC_{50} = 0.77 μM) and LACV (EC_{50} = 0.44 μM).[13] In this instance, the bulky, lipophilic *tert*-butyl group was instead present in segment D. It was hypothesized that there could be an inversion of the scaffold at the location of biological interaction, thus filling the presumed hydrophobic pocket. Interestingly, subsequent analogs possessing this scaffold inversion did not show any antiviral activity and therefore further work was not pursued.

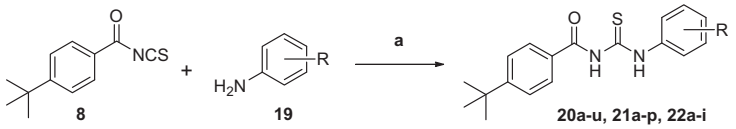
At the outset, changes to segment B, the acylthiourea portion, were not attempted as we believed this portion was crucial for



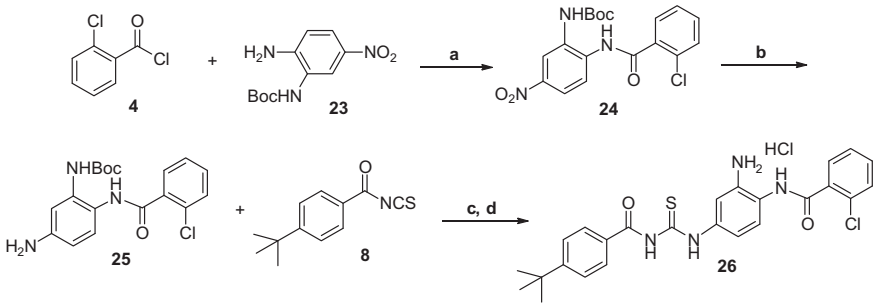
Scheme 4. Reagents and conditions: (a) $HgCl_2$, DIEA, DCM, rt, 18 h; (b) TFA, DCM, rt, 18 h; (c) 4-*tert*-butylbenzoylchloride, DIEA, DCM, rt, 18 h.



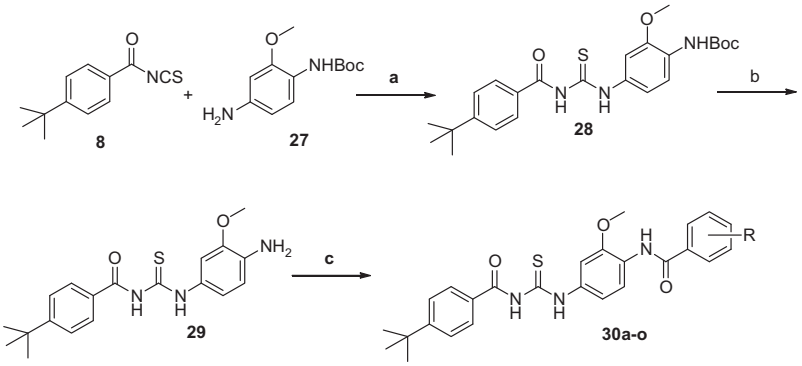
Scheme 5. Reagents and conditions: (a) MeI, K_2CO_3, DMF, rt, 4 h; (b) NaNHCN, *i*PrOH, ω (300 W), 80 °C, 10 min.



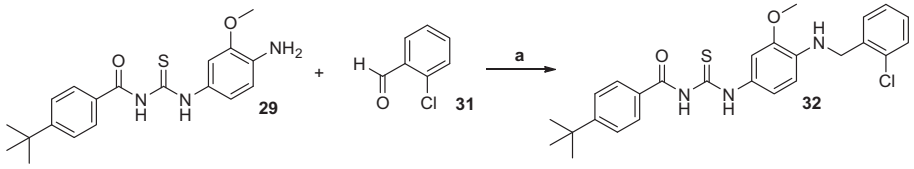
Scheme 6. Reagents and conditions: (a) acetone, rt, 2 h.



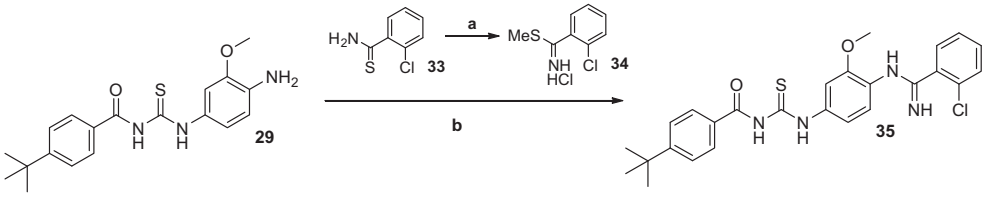
Scheme 7. Reagents and conditions: (a) DIEA, THF, rt, 3 h; (b) iron powder, NH_4Cl (aq), THF, rt, 3 days; (c) acetone, rt, 2 h; (d) TFA/DCM (1:1), DCM, 0 °C, then 2 M HCl in diethyl ether, rt, 3 h.



Scheme 8. Reagents and conditions: (a) acetone, rt, 2 h; (b) TFA/DCM, rt, 2 h; (c) RCOOH, Ghosez reagent, pyridine, DCM, 0 °C to rt, 6 h or RCOOH, PyBOP, *N*-methylmorpholine, DMF, rt, 18 h or RCOOH, HATU, DMF, rt, 5 h or RCOCl, TEA or pyridine, DCM, rt.

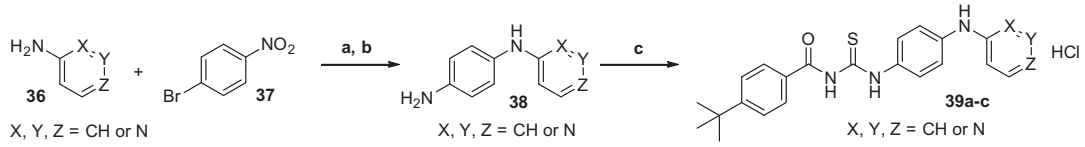


Scheme 9. Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, DCE, rt.



Scheme 10. Reagents and conditions: (a) MeI, acetone, reflux, 5 h; (b) DIEA, EtOH, reflux, 2 h.

activity due to the presence of this group in both HTS hits. During formulation studies, it was determined that the amide of the acylthiourea was labile to basic conditions and we observed subsequent hydrolysis. Therefore, molecules were designed to reduce this liability including methylene or amidine replacement of the carbonyl along with alkylation of the proximal amide-nitrogen to make the region more sterically hindered (Table 1). When alkylation was performed as the final step in order to install steric bulk to the amide-nitrogen of **1** and **2**, the major products that were isolated were in fact **10a** and **10b**, stemming from sulfur alkylation. It was interesting to discover that these compounds had similar potency to the parent compounds. We tried to exploit this feature of



Scheme 11. Reagents and conditions: (a) $Pd_2(dba)_2$, BINAP, Cs_2CO_3, DMF, 80 °C, 18 h; (b) $SnCl_2$, EtOAc, MeOH/H_2O, reflux, 18 h; (c) 8, acetone, rt, 2 h then 2 M HCl in Et_2O.

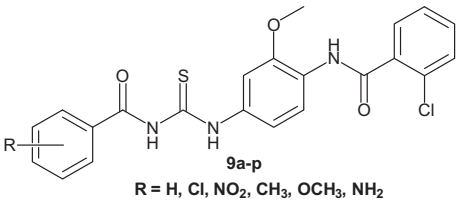


Figure 3. Systematic SAR study of segment A.

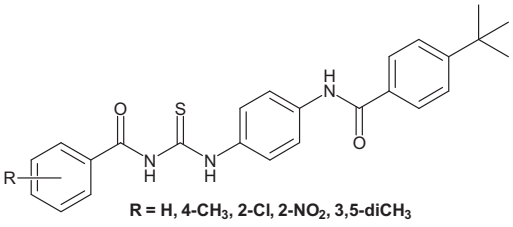


Figure 4. Inversion of Scaffold.

sulfur alkylation and install polar groups that may improve the solubility, as for analogs **10c** and **10d**; however, these molecules did not display any antiviral potency. Installation of an ethyl group onto the other nitrogen atom of segment B was successful (**40**), but this analog displayed a dramatic decrease in antiviral potency against VV and was inactive against LACV. From the results of methylene (**41**) and amidine replacement (**13a** and **13b**) of the carbonyl moiety of segment B, it was apparent that this group may be required due to the dramatic decrease in antiviral activity. Another analog involved replacement of the thiourea moiety with a guanidine group (**16**) in an attempt to improve solubility characteristics and reduce the liability of potential in vivo sulfur oxidation. Unfortunately, this molecule was inactive against both viruses. Another analog that was synthesized involved bioisosteric replacement of the thiourea portion of **1** with a cyanoguanidine moiety (**18**). This compound failed to display antiviral activity against either virus at 25 μM, which was the highest concentration tested.

A large portion of the acylthiourea analogs were focused on changes to segment C as this portion appeared to be the most amenable to substitution based upon preliminary studies. Initially, a systematic study was performed in an analogous manner to the one used for segment A which involved phenyl ring substitutions with hydrogen, chloro, nitro, carboxylic acid, methyl, methoxy, and amino (Table 2). In addition, replacement of the phenyl ring with a pyridine or cyclohexane ring was also explored. In this study, the activity of **2** was used as a benchmark since these analogs most closely resembled this HTS hit. While these substitutions did not uncover analogs that offered superior antiviral potency, the study did reveal which positions and types of functionalities may be tolerated. Overall, substitution with electron donating groups, such as methyl (**20j–l**), methoxy (**20m–o**), and amino (**20p–r**), led to active analogs. Groups offering electron withdrawing capability through resonance (**20d–i**) were less tolerated, resulting in many inactive analogs. It was interesting to note that chloro substitution gave very different results based upon the position of attachment. Compared to **2**, compound **20b** showed a fivefold decrease in activity, while compound **20c** displayed a complete loss of antiviral activity. The 2- and 3-pyridyl ring analogs (**20s** and **20t**) were inactive while the synthesis of the 4-pyridyl analog was unsuccessful. The inactivity of these pyridine ring compounds is in contrast with the unsubstituted phenyl (**20a**) and cyclohexane (**20u**) rings which displayed single digit micromolar activity.

As previously discussed, substitution of segment C in the *ortho*- or *meta*-positions with chloro or methyl produced active analogs (**2**, **20b**, **20j** and **20k**). With this in mind, analogs were prepared in which disubstitution of segment C was explored and the results are shown in Table 3. A couple analogs were tested that incorporated a trifluoromethyl group (**21c** and **21d**), as opposed to a simple methyl moiety, to reduce potential oxidative metabolism of this ring and the alkyl group itself. Compounds containing a hydroxyl group (**21o** and **21p**) were also included in this study as this group may increase solubility through hydrogen bonding. Some fairly potent analogs were identified and activity usually tracked with the pattern of chloro substitution in the 2-position in combination with a bulky lipophilic group at the 3-, 4-, or 5-positions. Unfortunately, none of these substitution patterns resulted in an analog that surpassed the potency of the parent compound (**2**). It should also be noted that the hydroxyl analogs (**21o** and **21p**) showed signs of cytotoxicity with single digit micromolar CC_{50} values.

Continuing with segment C, it was observed that an amino group was well tolerated in the *meta*- and *para*-positions (**20q** and **20r**) for the scaffold of **2**. In addition to antiviral potency against both VV and LACV, these analogs offered the advantage of improved solubility, at least in acidic conditions, due to the addition of this ionizable group. It was interesting to note that the combination of these two primary amine groups (**22a**), which independently display activity, did not possess potency against the selected viruses. It was evident from **22b** that the methoxy group of **1** may not have a dramatic effect on activity since this compound displayed similar potency to **20r**, which lacked the methoxy moiety. When the 4-NH_2 group was combined with 2-chloro substitution (**22c**), the result was a dramatic increase in antiviral potency when compared to **20r**. This is in agreement with the trend observed in Table 3 which illustrated advantageous substitution patterns involving the 2-chloro moiety. When we attempted to further improve solubility by installing an aminomethyl group, as for compounds **22d** and **22e**, we observed a dramatic increase in solubility, but with complete loss of antiviral activities. Additional SAR studies led to monoalkylated (**22f**) and dialkylated (**22g**) amino groups installed at the *para*-position which possessed increased basicity, for ease of salt formation, along with the potential to reduce in vivo oxidation. These groups were also combined with 2-chloro substitution which led to an increase in potency for **22h**, but did not have much effect for compound **22i**. In all cases, the HCl salt forms of these compounds were synthesized in an attempt to increase water solubility. All of these results are summarized in Table 4.

The extended scaffold of **1** was also used in segment C SAR studies involving replacement of the methoxy group (Table 5). One such analog was derived by substituting the methoxy moiety with an amino group (**26**) which offered improved solubility through presence of an ionizable group and made salt formation possible.

While it was shown later that solubility was only modestly increased, the antiviral activity was significantly improved against both VV (EC_{50} = 0.06 μM) and LACV (EC_{50} = 0.05 μM) and this molecule turned out to be the most potent analog identified.

Table 1
Changes to segment B

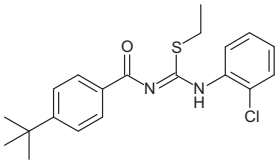
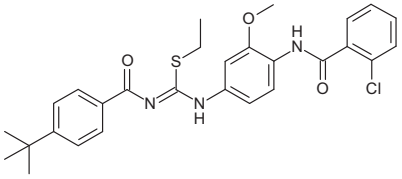
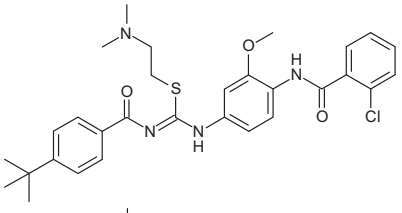
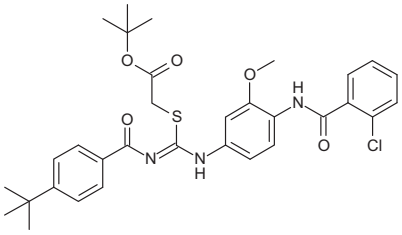
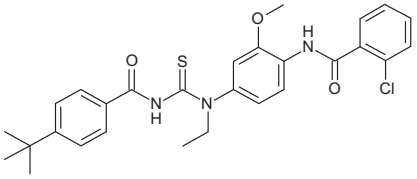
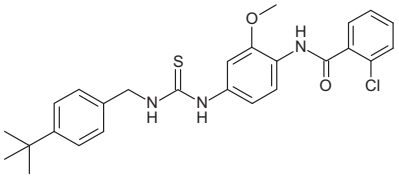
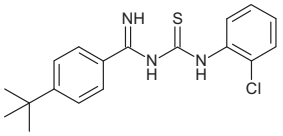
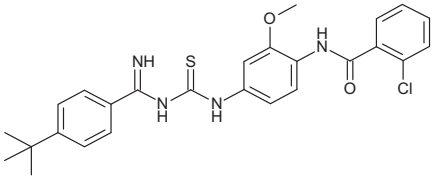
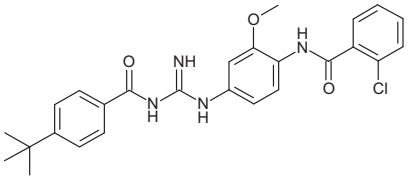
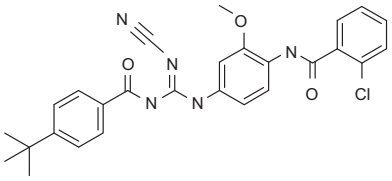
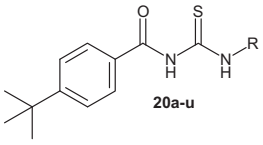
Compound	Structure	VV EC_{50} (μM)	LACV EC_{50} (μM)
10a		1.91[a]	0.51[a]
10b		0.23	0.30
10c		>25	>25
10d		>25	>25
40		10.1	>25
41		>25	>25
13a		>25	>25
13b		7.64[b]	3.01
16		>25	nd

Table 1 (*continued*)

Compound	Structure	VV EC_{50} (μM)	LACV EC_{50} (μM)
18		>25	nd

nd = not determined.
[a] Average value of multiple experiments.
[b] One experiment displayed inactivity at highest concentration tested (25 μM).

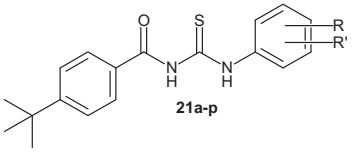
Table 2
Systematic SAR analysis of segment C



Compound	R	VV EC_{50} (μM)	LACV EC_{50} (μM)
20a	Ph	5.57[a]	5.01[b]
2	2-ClPh	0.35	0.28
20b	3-ClPh	1.58	4.16
20c	4-ClPh	>25	>25
20d	2-NO_2Ph	3.64[a]	3.70[a]
20e	3-NO_2Ph	>25	>25
20f	4-NO_2Ph	4.08	>25
20g	2-CO_2HPh	>25[c]	>25
20h	3-CO_2HPh	>25	>25
20i	4-CO_2HPh	>25	>25
20j	2-CH_3Ph	0.83[a]	0.63[a]
20k	3-CH_3Ph	1.01[a]	1.33[a]
20l	4-CH_3Ph	1.91[a]	4.86[a]
20m	2-OCH_3Ph	1.39	1.15
20n	3-OCH_3Ph	4.07	>25
20o	4-OCH_3Ph	2.39	>25
20p	2-NH_2Ph	>25	>25
20q	3-NH_2Ph	2.72[a,b]	2.19[a]
20r	4-NH_2Ph	4.41[a,b]	2.27[a]
20s	2-Pyridyl	>25	>25
20t	3-Pyridyl	>25	>25
20u	Cyclohexyl	4.92	3.71

[a] Average value of multiple experiments.
[b] Single experiment displayed inactivity at highest concentration tested (25 μM).
[c] Single experiment showed activity of 2.29 μM.

Table 3
Disubstitution study of segment C



Compound	R	R′	VV EC_{50} (μM)	LACV EC_{50} (μM)
21a	2-Cl	3-CH_3	1.83[a]	1.17
21b	2-Cl	5-CH_3	0.66[a]	1.67[b]
21c	2-Cl	5-CF_3	1.00[a]	0.6
21d	2-Cl	3-CF_3	1.83	0.85
21e	2-Cl	5-OCF_3	1.9	>25
21f	2-Cl	6-CH_3	2.49[a]	0.94[a]
21g	2-Cl	5-Cl	2.42[b]	2.45[b]
21h	2-Cl	4-Cl	>25	>25
21i	2-CH_3	4-CH_3	1.06[a]	0.88[a]
21j	2-CH_3	3-CH_3	1.83[a]	2.59[a]
21k	2-CH_3	3-Cl	6.47[b]	0.919[b]
21l	2-CH_3	5-CH_3	2.86[a]	>25
21m	3-CH_3	5-CH_3	3.48[a]	4.43[b]
21n	3-CH_3	4-CH_3	3.93	1.23
21o	2-OH	5-*t*-butyl	0.98[a]	3.42
21p	2-OH	5-CH_3	>25	>25

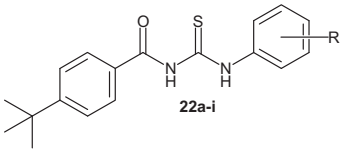
[a] Average value of multiple experiments.
[b] Single experiment displayed inactivity at highest concentration tested (25 μM).

Interestingly, the hydroxyl analog (**42**), which could be a potential metabolite of **1**, displayed very similar potency to the parent. Overall, the data that was gathered regarding segment C suggested that the extended scaffold of **1** may be involved in additional binding interactions, when compared to compound **2**, leading to an increase in antiviral potency.

Analogs were also synthesized which probed the chemical space around segment D with the hopes of improving drug-like properties. Multiple attempts to incorporate an ionizable moiety into segment D, for reducing lipophilicity and improving solubility, are summarized in Table 6. One of these studies involved replacement of the 2-chlorophenyl ring with aromatic heterocycles containing one or multiple nitrogen atoms. It was observed that 3- and 4-pyridyl ring containing compounds (**30b** and **30c**) displayed decent potency against VV, while the presence of a nitrogen atom in the 2-position led to a dramatic decrease in activity (**30a**, **30d**, and **30e**). It was also shown that replacement of the phenyl group with an imidazole ring (**30f** and **30g**) led to loss of activity. The incorporation of amino (NH_2) and methylamino (CH_2NH_2) substituents was attempted for **30h–30l**, which did show signs of VV activity in most cases, but were also mostly inactive against LACV. While acceptable antiviral potency was observed against VV for **30k** and **30l**, these compounds also displayed cytotoxicity with single digit micromolar CC_{50} values. In addition, two analogs were prepared that incorporated carboxylic acid group substitution onto the phenyl ring of segment D (**30m** and **30n**). Unfortunately, these compounds did not show any activity against either virus tested at 25 μM. The same was true for the benzimidazole derivative (**30o**). For compounds **30a–o**, we did not observe any activity against LACV. The overall trend indicated that segment D was not amenable to substitution with ionizable groups when broad-spectrum activity is desired.

Attempts to replace the phenyl ring of segment D with an alkyl chain linked to a terminal polar functionality [$(NCH_3)_2$, CO_2CH_3, COOH] was shown to be unsuccessful at retaining antiviral potency (**43a–e**). In addition, the dimethylamino analogs (**43a** and **43b**) showed signs of cytotoxicity with single digit micromolar CC_{50} values. In contrast, substitution with a simple *N*-acetyl group (**43f**) displayed good potency against both viral targets. The potency observed for a smaller, polar functionality in this region was not

Table 4
Amino group substitution in segment C

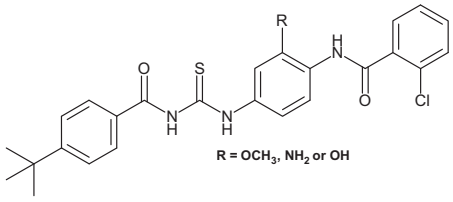


22a-i

Compound	R groups	VV EC_{50} (μM)	LACV EC_{50} (μM)	Solubility (μg/mL)		
				H_2O	SGF[a]	CTB[b]
22a	3,4-DiNH_2[c]	>25[d]	>25	2.9	4.2	2.0
22b	3-OCH_3, 4-NH_2	2.09[e,f]	1.69[e,f]	ND	53	0.4
22c	2-Cl, 4-NH_2	0.62[f]	0.75[f]	0.1	6.4	0.5
22d	2-CH_2NH_2	>25	>25[g]	121	261	11
22e	3-CH_2NH_2	>25	>25	258	248	24
22f	4-$NHCH_3$	1.16[f]	1.45	1.9	190	0.3
22g	4-$N(CH_3)_2$	2.43	nd	1.0	36	ND
22h	2-Cl, 4-$NHCH_3$	0.33[f]	0.24[f]	0.2	3.2	0.1
22i	2-Cl, 4-$N(CH_3)_2$	3.26[f]	1.55[f]	ND	ND	ND

nd = not determined.
ND = not detectable.
[a] SGF = simulated gastric fluid.
[b] CTB = Caco-2 transport buffer.
[c] Di-HCl salt was synthesized.
[d] Single experiment showed activity of 7.78 μM.
[e] Two experiments displayed inactivity at highest concentration tested (25 μM).
[f] Average value of multiple experiments.
[g] Single experiment showed activity of 6.93 μM.

Table 5
Methoxy group substitution in segment C

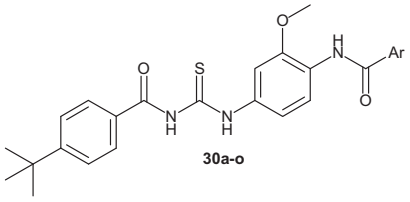


R = OCH_3, NH_2 or OH

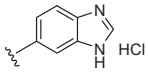
Compound	R	VV EC_{50} (μM)	LACV EC_{50} (μM)
1	OCH_3	0.25[a]	0.27[a]
26	NH_2	0.06[a]	0.05[a]
42	OH	0.23	0.21

[a] Average value of multiple experiments.

Table 6
Ionizable groups incorporated into segment D



30a-o

Compound	Ar	VV EC_{50} (μM)	LACV EC_{50} (μM)
30a	2-Pyridyl	>25	>25
30b	3-Pyridyl	1.72	nd
30c	4-Pyridyl	2.03	nd
30d	Pyrazyl	>25	>25
30e	Pyrimidyl	>25	>25
30f	2-Imidazole	>25	>25
30g	4-Imidazole	>25	>25
30h	2-Aminophenyl	2.61	>25
30i	3-Aminophenyl	>25	>25
30j	4-Aminophenyl	19.96[a]	>25[a]
30k	3-Methylaminophenyl	1.63[b]	>25
30l	4-Methylaminophenyl	0.81[b,c]	>25
30m	3-Benzoic acid	>25	nd
30n	4-Benzoic acid	>25	>25
30o		>25	>25

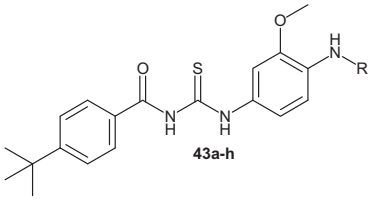
nd, not determined.
[a] High variability for this analog with single digit micromolar potency observed for one experiment.
[b] Average value of multiple experiments.
[c] One experiment displayed inactivity at highest concentration tested (25 μM).

repeated for the urea (**43g**) or guanidine (**43h**) analogs. All of these results are summarized in Table 7.

Substitutions of the amide group in segment D were explored and summarized in Table 8. The carbonyl group was substituted with a methylene for **32** and this analog had nearly the same activity as the parent compound (**1**). This discovery provided the potential for a backup series of compounds sharing this same substitution if amide hydrolysis were to become an issue for segment D. Replacement of the amide group with an amidine (**35**) provided an ionizable moiety, while the thioamide (**44**) derivative was synthesized to represent a closely related surrogate with the potential to reduce possible hydrolysis at this position. Both compounds resulted in a decrease in activity when compared to **1**.

Some of the alterations that were examined incorporated changes to both segments C and D and are summarized in Table 9.[15] For compounds purchased from commercial sources, it was difficult to find molecules to probe only one specific part of the acylthiourea scaffold. One set of analogs included rather substantial changes including replacement of the entire segment D portion with saturated heterocycles including piperidine, piperazine, and morpholine (**45a–e**). The incorporation of these groups offered the potential for improved solubility through both ionizable groups

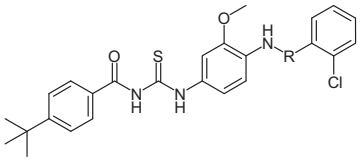
Table 7
Polar group appended alkyl chains in segment D



Compound	R	VV EC_{50} (μM)	LACV EC_{50} (μM)
43a	$CO(CH_2)_2N(CH_3)_2$	>25	>25
43b	$CO(CH_2)_4N(CH_3)_2$	>25	>25
43c	$COCH_2CO_2CH_3$	18.61	>25
43d	$COCH_2CO_2H$	>25	>25
43e	$CO(CH_2)_2CO_2H$	>25	>25
43f	$COCH_3$	3.27[a]	1.35
43g	$CONH_2$	>25	4.86
43h	$C(=NH)NH_2$	>25	>25

[a] Average value of multiple experiments.

Table 8
Amide group substitution of segment D



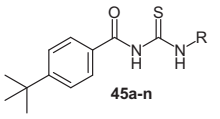
Compound	R	VV EC_{50} (μM)	LACV EC_{50} (μM)
1	C=O	0.25[a]	0.27[a]
32	CH_2	0.34[a]	0.20[a]
35	C=NH	1.75[a]	2.06[a]
44	C=S	0.99	0.64

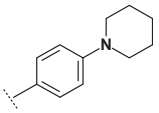
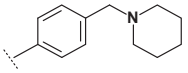
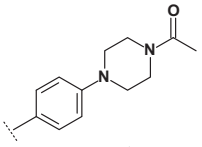
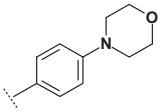
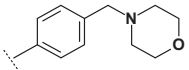
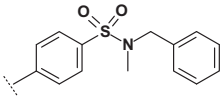
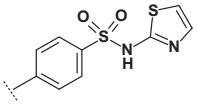
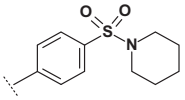
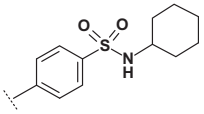
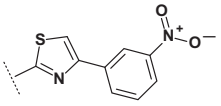
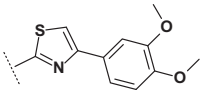
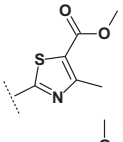
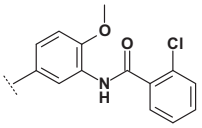
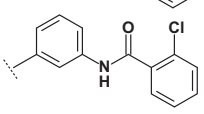
[a] Average value of multiple experiments.

and structural flexibility of the non-planar rings. However, these molecules did not display any antiviral activity in our assays, which may not be surprising given the structural disparity compared to **1**. In addition, they also appeared to be cytotoxic. Another batch of compounds examined the replacement of the amide linker of segment D with a sulfonamide linker (**45f–i**). The sulfonamide substitution included linkage to a benzyl substituent (**45f**), thiazole (**45g**), piperidine (**45h**) and cyclohexane (**45i**). In all cases there was a complete loss of antiviral activity. A series of compounds was also tested which included replacement of the segment C phenyl ring with a thiazole ring (**45j–l**) and these were inactive as well. It was apparent that the location of the amide portion of segment D was very important given the inactivity of **45m** and **45n**, with a large decrease in antiviral activity observed when this group is moved away from the *para*-position. Overall, it appeared that these relatively dramatic changes to segments C and D were not tolerated even though segments A and B remained unchanged.

Interestingly, we found that we were able to omit certain moieties of **1** without having a substantial impact on activity (**46a** and **46b**, Table 10).[16] Compound **46a**, which is lacking the chloro and methoxy phenyl substituents, retained the broad-spectrum activity of the parent compound. The addition of 2-chloro substitution (**46b**) did not appear to have much of an effect on antiviral potency when compared to **46a**. Compound **46c** contained an amino linker in segment D, as opposed to the amide linker of **1**, and there

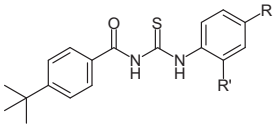
Table 9
Significant changes to segments C and D

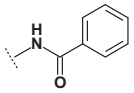
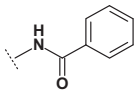
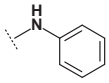
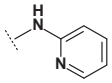
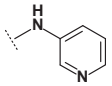
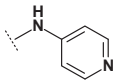


Compound	R	VV EC_{50} (μM)	LACV EC_{50} (μM)
45a		>25	>25
45b		>25	>25
45c		>25	>25
45d		>25	>25
45e		>25	>25
45f		>25	>25
45g		>25	>25
45h		>25	>25
45i		>25	>25
45j		>25	>25
45k		>25	>25
45l		>25	>25
45m		>25	>25
45n		>25	>25

was only a 2- to 3-fold decrease in activity. Structural modifications of **46c** were performed and involved the addition of an

Table 10
Key group probing of segment D



Compound	R	R′	VV EC_{50} (μM)	LACV EC_{50} (μM)
46a		H	0.17[a]	0.15[b]
46b		Cl	0.16	0.08
46c		H	0.82[a]	1.7[a]
39a[c]		H	>25	>25
39b[c]		H	24.5	>25
39c[c]		H	>25	7.7[a]

[a] Average value of multiple experiments.
[b] One experiment displayed inactivity at highest concentration tested (25 μM).
[c] HCl salt form of compound.

ionizable pyridyl ring to segment D in an attempt to improve solubility (**39a–c**). While solubility was increased, the analogs were rendered inactive in our antiviral assays against both VV and LACV. It was reasonable to expect at least modest activity against VV based upon results from pyridyl ring incorporation into segment D, as observed for **30b** and **30c**. However, the absence of the carbonyl linker appears to have greatly changed this interaction.

In summary, we have shown that the acylthiourea scaffold, uncovered through HTS hits **1** and **2**, displays broad-spectrum activity against a variety of viral families. Through our SAR studies, it was evident that a bulky, lipophilic group (i.e., *tert*-butyl moiety) in segment A and acylthiourea moiety in segment B were required for broad-spectrum antiviral activity. As a result, it appeared that the portions of the scaffold that were most amenable to structural modifications were segments C and D. The activity data suggested that the extended scaffold of **1** may be picking up additional binding interactions, when compared to **2**, leading to an increase in potency. In the end, we were able to improve potency as well as drug-like properties of the acylthiourea scaffold derived from the HTS hits.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.035. These data include MOL files and InChiKeys of the most important compounds described in this article.

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13. VV and LACV antiviral activity values were averages from multiple experiments for compounds **1, 2** and the compound in Figure 4 (R = CH_3).
14. Scheme 6 was used to prepare compounds **20a**, **20b**, **20f**, **20m**, **20n**, **20p–r**, **20u**, **21a**, **21b**, **21d–f**, **21i**, **21j**, **21l**, **21m**, **22a–i**. Please note that for compounds **22a–i**, additional modifications were required beyond those described in Scheme 6 such as deprotection and reductive amination. The other compounds contained in Tables 2 and 3 were purchased from commercial sources.
15. For the analogs in Table 9, all of these were purchased from commercial sources except for **45m**.
16. For Table 10, compounds **46a** and **46c** were purchased from commercial sources.