

# Design, synthesis and biological evaluation of indolizine derivatives as HIV-1 VIF–ElonginC interaction inhibitors

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**Abstract** The HIV-1 viral infectivity factor (VIF) protein is essential for viral replication. VIF recruits cellular ElonginB/C-Cullin5 E3 ubiquitin ligase to target the host antiviral protein APOBEC3G (A3G) for proteasomal degradation. Thus, the A3G-Vif–E3 complex represents an attractive target for the development of novel anti-HIV drugs. In this study, we describe the design and synthesis of indolizine derivatives as VIF inhibitors targeting the VIF–ElonginC interaction. Many of the synthesized compounds exhibited obvious inhibition activities of VIF-mediated A3G degradation, and 5 compounds showed improvement of activity compared to the known VIF inhibitor VEC-5 (1) with IC<sub>50</sub> values about 20 μM. The findings described here will be useful for the development of more potent VIF inhibitors.

**Keywords** VIF–ElonginC interaction inhibition · VEC-5 · Anti-HIV-1 · Indolizine derivatives · Structure-activity relationship

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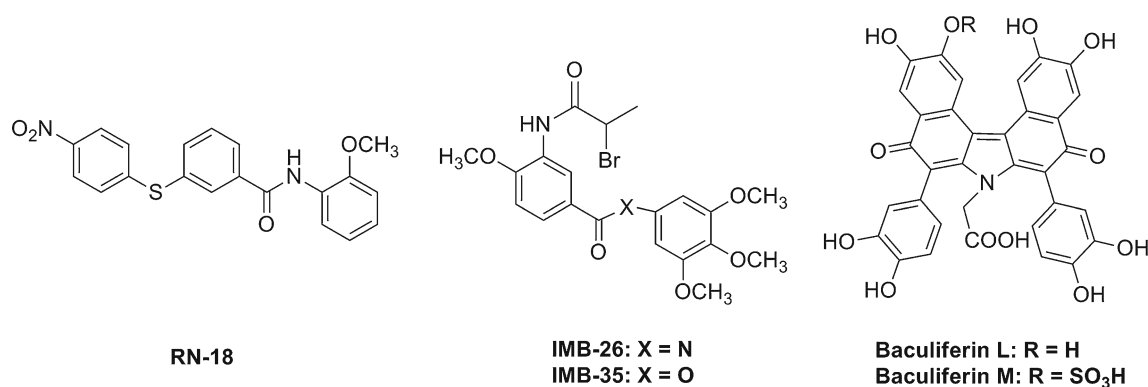
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## Introduction

Since the first cases of AIDS were identified in 1981, remarkable medical advances have been made in the prevention and treatment of HIV/AIDS. The Food and Drug Administration (FDA) approved that anti-HIV drugs can be divided into six categories: nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleotide reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors (FIs), co-receptor inhibitors (CRIs), and integrase inhibitors (INIs) [1]. Combined application of these antiretroviral drugs has proven to be exceedingly effective at reducing viral load and improving the clinical status of many patients. However, viral resistance and toxicity associated with these inhibitors have created an urgent need to develop new anti-HIV drugs with novel targets and mechanisms [2–4]. As all currently approved anti-HIV drugs target HIV-1 Pol and Env-encoded proteins (i.e., reverse transcriptase (RT), protease (PR), and integrase (IN, gp41), widening the molecular targets of HIV therapy by additionally targeting regulatory and accessory proteins may lead to new effective therapies with expanded treatment options [5,6].

Viral infectivity factor (VIF) is an HIV accessory protein, the primary function of which is to negate the action of APOBEC3G—a naturally occurring cellular inhibitor of HIV replication. In the absence of HIV-1 VIF, A3G is packaged into HIV-1 virions and introduces G-to-A hypermutations in viral minus-strand DNA during reverse transcription, which leads to the production of non-functional proviruses [7–10]. However, A3G-imposed replication block is primarily overcome by HIV-1 VIF that triggers the degradation of A3G by 26S proteasome through polyubiquitination. A normal cellular Elongin–Cullin–SOCS-box (ECS) ubiquitin ligase is comprised of ElonginB, ElonginC, Cullin5, Rbx2, and a cellular suppressor of cytokine signaling



**Fig. 1** Structures of HIV-1 VIF inhibitors

box (SOCS-box) protein. VIF targets A3G for polyubiquitination by mimicking SOCS-box protein that includes the BC-box, which binds ElonginB and ElonginC, and the Cullin box that has been proposed to participate in the binding of either Cullin5 or Cullin2 [11]. In this process, the N-terminal region of VIF binds to the N-terminal region of A3G, the zinc-binding region formed by a conserved HCCH motif interacts with Cullin5, and the BC-box region (residues 144 to 155) in the C-terminal of VIF binds to ElonginC [12–14]. Crystal structure of HIV VIF BC-box in complex with ElonginB and ElonginC shows more detailed interactions between side chains (Val142, Leu145, Leu148, Ala149, Ala152, and Leu153) of VIF residues and those of helix 3 and helix 4 of ElonginC, and the protein surface of ElonginC shows binding pockets for VIF Val142 and Leu145 [15]. Therefore, disrupting these binding interactions within the A3G-VIF-E3 complex presents new opportunities for therapeutic intervention. Several small molecule inhibitors of HIV-1 VIF (**RN-18**, **IMB-26/35**, and Baculiferins) have been identified (Figure 1) [16–19]. **RN-18** and **IMB-26/35** were identified by cell-based screening of chemical libraries, while Baculiferins were isolated from the marine sponge *Istrochota Baculifera*. **RN-18** was shown to inhibit VIF–A3G interaction through reduction of VIF in the host cells, and optimization of **RN-18** has generated new inhibitors with improved activities [19]. **IMB-26/35** analogs were shown to specifically bind to A3G, thereby stabilizing A3G expression and Baculiferins containing N-acetic acid group showed potent binding affinities toward both VIF and APOBEC3G.

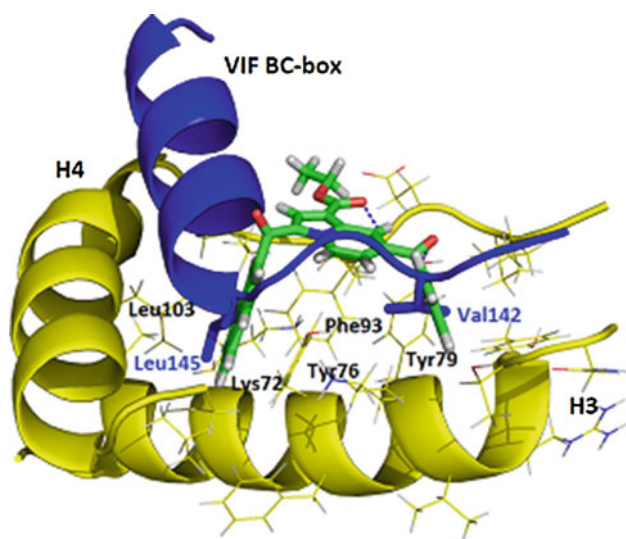
To discover novel HIV-1 VIF inhibitors, our research was focused on identifying small molecule inhibitors of VIF–ElonginC interaction. A virtual screening was carried out based on ElonginC, and a small molecule **1** (**VEC-5**) with an IC<sub>50</sub> of 36.1 μM against HIV-1 VIF was obtained. Further biological investigation confirmed that **VEC-5** protects A3G from VIF-mediated degradation through blocking interaction between VIF and ElonginC [20,21]. Such a mechanism is different from reported HIV-1 VIF inhibitors and may lead to new approaches for anti-HIV-1 treatment.

Compound **1** is an indolizine derivative. Indolizine derivatives have been applied in drug discovery due to some unique properties of the scaffold [22,23]. The biological activities of indolizines include antimicrobial, antioxidant, anti-inflammatory, anticonvulsant activities, etc. Many attractive methodologies of synthesis of indolizines have been developed. These methods could be classified as the Tschitschibabin reaction, 1, 3-dipolar cycloaddition, cyclisation reaction, etc., depending on the position of bond formation [22,23]. For the optimization of compound **1**, a series of indolizine derivatives have been synthesized in this study. The inhibition of VIF-mediated A3G-degradation and antiviral activities have been investigated. The determination of the structure-activity relationship (SAR) will be helpful for the discovery of more potent antiviral agents.

## Results and discussion

### Design

The binding model of **1** in complex with the crystal structure of human ElonginC (PDB code: 3DCG) was analyzed (Fig. 2). The model suggests that **1** largely mimics the conformation of a loop region of VIF BC-box. The naphthalene ring is stretched into the large hydrophobic cavity formed by Lys72, Val73, Tyr76, Pro94, Ile95, Pro97, Ile99, Pro97, Leu103, and Ala107, mimicking the interactions of Leu145 of VIF. The benzene ring of **1** is occupying another small hydrophobic groove mimicking the interactions of Val142 of VIF, while the ethyl moiety is extended outward from the ElonginC surface. There is no interaction between the indolizine ring and ElonginC and it was considered as a linker to provide proper angles and distances for the two aromatic moieties to stretch into the two hydrophobic pockets. Ester and 3-carbonyl moieties of **1** both act as hydrogen-bonding acceptors and form hydrogen bonds with Phe93 and Tyr79 of ElonginC, respectively. Overall, the most important



**Fig. 2** Superposition of the predicted binding model of compound **1** in complex with ElonginC on the crystal structure of VIF BC-box in complex with ElonginC. The VIF BC-box and compound **1** are shown in blue and green, respectively, and the nitrogen and oxygen atoms in **1** are shown in blue and red, respectively. The ElonginC protein is shown in yellow, for clarity, only helices 3 and 4 of ElonginC are shown. Hydrogen bonds are shown in dashed light blue lines

interaction seems to be the  $\pi - \pi$  stacking between the naphthalene ring of **1** and Tyr76 of ElonginC.

With the desire to improve potency, modification of compound **1** was initially focused on the substituents at position 3 and 7. Aralkylamine was introduced to mimic the naphthalene as well as to reduce the rigidity of compounds **2**. It was found that such replacement of naphthalene was tolerable; therefore, further modifications were based on compounds **2**. Modification was further carried out by exchanging the 3-position and 7-position substituents of the indolizine core structure, which generated compounds **3** and **4**. The length of the amide linker and the R substituent in compounds **3**, as well as the acyl group in compounds **4** were investigated. The next modification was carried out by variation of the substitution pattern of the indolizine scaffold. The point of attachment of the the aroyl group in compounds **2** was moved from 7-position to 8-position of the indolizine core structure generated compounds **5**, while the position of attachment of the 7-amide group in compound **4a** was also hopped to 8-position of the indolizine ring-generated compound **6**. The final modification was performed on the indolizine core structure by migrating the nitrogen atom position, which generated compound **7**, or by insertion of one nitrogen atom to afford compound **8** (Fig. 3).

## Chemistry

**Compounds 2/5:** These two series of analogs were synthesized by the following procedure (Scheme 1): Com-

pounds **9** were reacted with benzyl 2-bromoacetate to afford pyridinium **10**, then compounds **11** were obtained by constructing the indolizine ring through 1,3-dipolar cycloaddition of pyridinium **10** and ethyl propiolate [23,24]. The benzyl-protecting group of compounds **11** was selectively removed using LiOH to afford the corresponding carboxylic acid intermediates **12**, which were then condensed with various aryl alkylamines to afford analogs **2** and **5**.

**Compounds 3/4:** 2-Bromo-1-phenylethanone was reacted with protected isonicotinic acids **13** to afford the corresponding pyridinium bromides **14**, and then the indolizine ring was constructed by the same way mentioned above to afford **15**. After deprotection, **16** was condensed with aralkylamines to afford analogs **3** and **4** (Scheme 2). The 3-aroyle was also replaced by several small ring amino acyl groups and the synthetic route was shown in Scheme 3. The 3, 7-bis-protected dicarboxylic acid indolizine **19** was synthesized by the same procedure as mentioned above. The *tert*-butyl- protecting group of compound **19** was first removed and the pyridin-2-yl-methanamine was introduced to afford compound **21**. Then, the benzyl group was removed to get compound **22**, which was then condensed with heterocyclic amines to afford compounds **4z**.

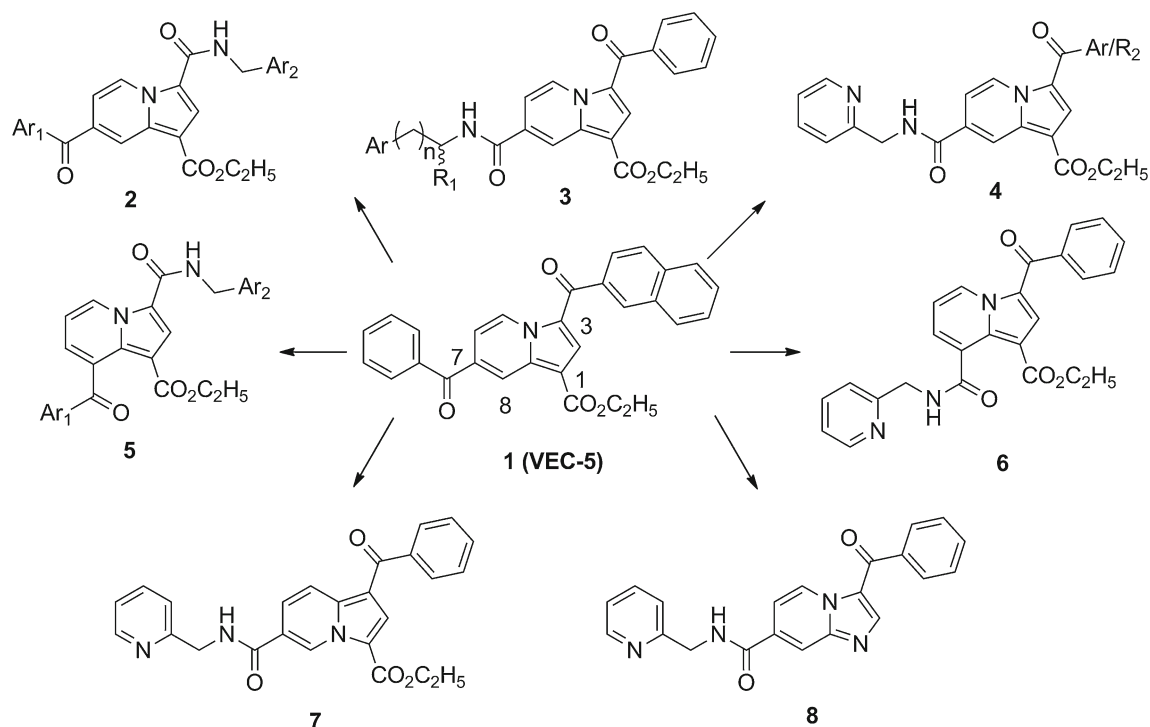
**Compounds 6:** The pyridinium bromide **24** was obtained by the reaction of 2-bromo-1-phenylethanone and *tert*-butyl nicotinate, which was subsequently reacted under typical indolizine construction conditions to afford **25**. Then, the *tert*-butyl group was removed using TFA to afford the corresponding carboxylic acid **26**, which was subsequently converted to the target compound **6** by condensing with pyridin-2-yl-methanamine (Scheme 4).

**Compound 7:** Compound **27** was reacted with 1-phenylprop-2-yn-1-one to afford the corresponding pyridinium bromide and then the indolizine core structure was constructed to give compound **29**. The benzyl-protecting group of compound **29** was removed to afford the corresponding carboxylic acid **30**. The target compound **7** was finally obtained by condensation of **30** with pyridin-2-yl-methanamine (Scheme 5).

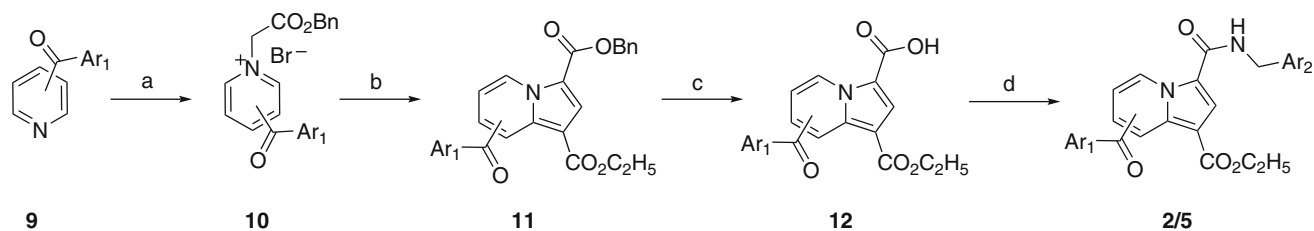
**Compound 8:** The carboxyl group of compound **31** was protected as methyl ester to afford compound **32**, which was then reacted with 1, 1-dimethoxy-*N,N*-dimethylmethanamine to afford compound **33**. The imidazo[1,2-*a*]pyridine core structure was constructed to afford **34** [25]. Deprotection of the ester group afforded compound **35**, which was then condensed with pyridin-2-yl-methanamine to afford the target compound **8** (Scheme 6).

## Biological activities and SARs

All the synthesized compounds were preliminarily evaluated for inhibiting VIF-mediated degradation of A3G effect



**Fig. 3** Design and structures of analogs of **1**



**Scheme 1** Reagents and conditions: *a* Benzyl 2-bromoacetate, ethyl acetate, reflux, overnight; *b* K<sub>2</sub>CO<sub>3</sub>, CH<sub>3</sub>CN, 0 °C, 30 min, then ethyl propionate, rt, 72 h; *c* LiOH, THF/C<sub>2</sub>H<sub>5</sub>OH/H<sub>2</sub>O, rt, 2 h; *d* Ar<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>, EDC, HOBt, DIPEA, THF, rt, overnight

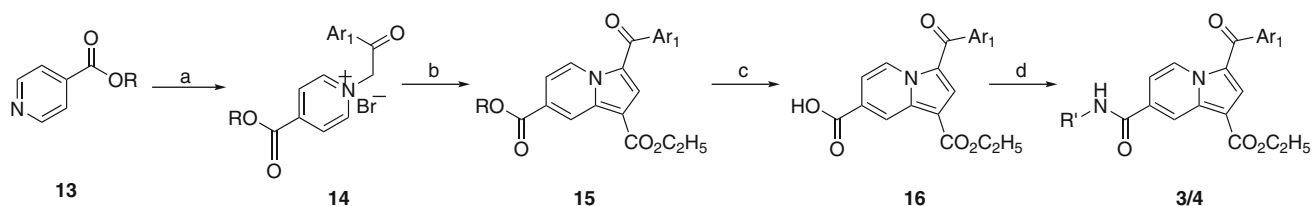
at a single concentration of 50 μM. The values are presented as the normalized intensity relative to the value in the HxB<sub>2</sub>VIF-cmyc or untreated pEYFP-A3G group. The results are summarized in Table 1. Based on the preliminary biological results, 23 representative compounds were selected for anti-HIV-1 activity assay. The anti-HIV-1 activities were determined by a p24 antigen detection assay and the cytotoxicities of these compounds were evaluated by MTT assay. The results are summarized in Table 2.

#### Modification on the substituents at position 3 and 7

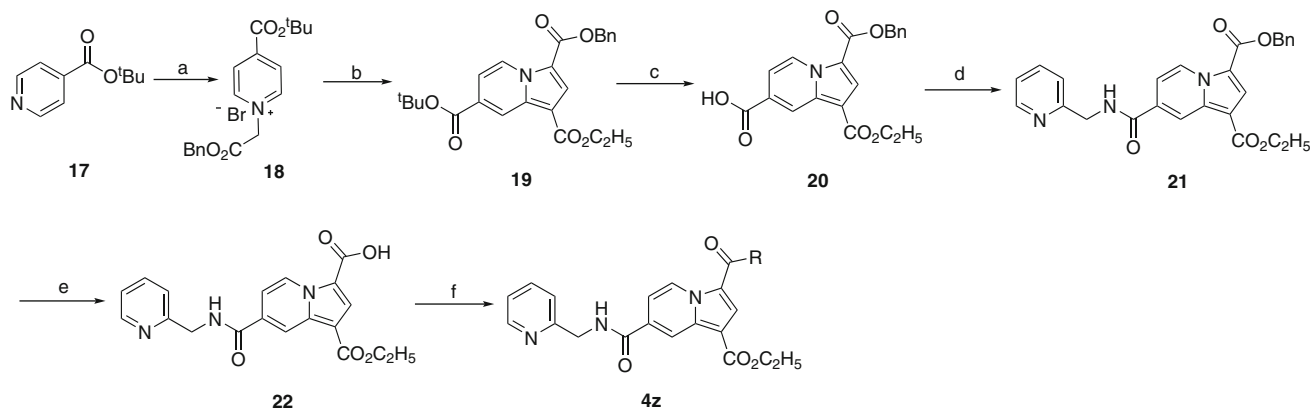
**Compounds 2:** In an effort to increase the solubility of the compounds, aralkylamines were introduced to mimic the β-naphthalene ring at position 3 of compound 1 as well as to reduce the rigidity of compounds 2. It was found that when the substituent at position 7 was maintained as

unsubstituted aryl, the replacement of β-naphthalene ring with aralkylamines (**2a–2e**) improved the activity of the compounds in the preliminary assay. The introduction of an electron-donating group (**2f**, **2g**) to the aryl led to reduction in potency, while an electron-withdrawing group gave variable results. Compound **2h** showed reduced activity, while **2i** showed improved activity in the preliminary assay. Further, anti-HIV-1 activity assay showed that compounds **2d** and **2e** exhibited slightly reduced activity compared to compound **1**, which suggested that the replacement of the β-naphthalene ring by aralkylamines is tolerable to some extent.

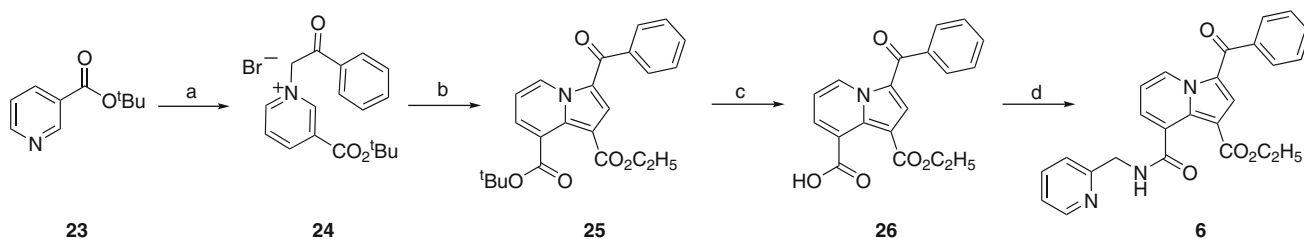
**Compounds 3/4:** In these two series of compounds, modifications were made to substituents at position 3 and 7 simultaneously based on the hypothesis that the terminal aryl groups of the 3,7-substituents were critical for interaction with target, and the potency can be retained as long as the distance and orientation of the two aryl groups were retained.



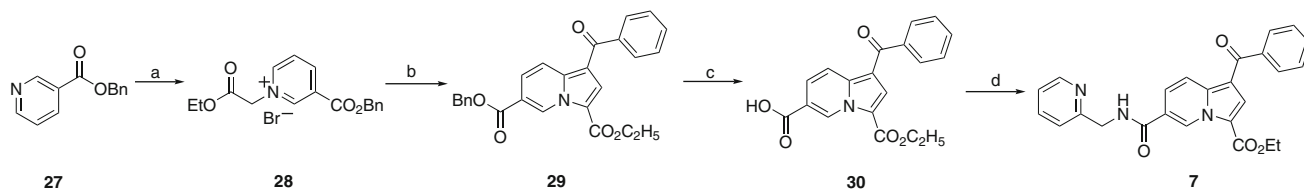
**Scheme 2** Reagents and conditions: *a* Ar<sub>1</sub>COCH<sub>2</sub>Br, ethyl acetate, reflux, overnight; *b* K<sub>2</sub>CO<sub>3</sub>, CH<sub>3</sub>CN, 0 °C, 30 min, then ethyl propiolate, rt, 72 h; *c* (1) R = *tert*-butyl: TFA, DCM, rt, overnight; (2) R = benzyl: TBAF, THF, rt, overnight; or LiOH, THF/C<sub>2</sub>H<sub>5</sub>OH/H<sub>2</sub>O, rt, 2 h *d* EDC, HOBT, DIPEA, THF, rt, overnight.



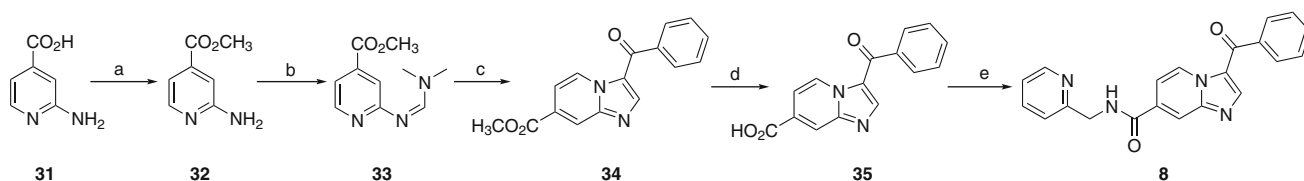
**Scheme 3** Reagents and conditions: *a* Ethyl acetate, reflux, overnight; *b* Alkynyl acid acetic, K<sub>2</sub>CO<sub>3</sub>, CH<sub>3</sub>CN, 0 °C-rt, 72 h; *c* TFA, DCM, rt, overnight; *d* Pyridin-2-yl-methanamine, EDC, HOBT, DIPEA, THF, rt, overnight; *e* LiOH, THF/EtOH/H<sub>2</sub>O, rt, 2 h; *f* EDC, HOBT, DIPEA, R<sub>1</sub>NR<sub>2</sub>, THF, rt, overnight



**Scheme 4** Reagents and conditions: *a* PhCOCH<sub>2</sub>Br, ethyl acetate, reflux, overnight; *b* Alkynyl acid acetic, K<sub>2</sub>CO<sub>3</sub>, CH<sub>3</sub>CN, 0 °C-rt, 72 h; *c* TFA, DCM, rt, overnight; *d* EDC, HOBT, DIPEA, THF, rt, overnight



**Scheme 5** Reagents and conditions: *a* Ethyl bromoacetate, ethyl acetate, rt, overnight; *b* 1-phenylprop-2-yn-1-one, K<sub>2</sub>CO<sub>3</sub>, CH<sub>3</sub>CN, 0 °C-rt, 72 h; *c* LiOH, THF/CH<sub>3</sub>OH/H<sub>2</sub>O, rt, 2 h; *d* EDC, HOBT, DIPEA, THF, rt, overnight



**Scheme 6** Reagents and conditions: (a) SOCl<sub>2</sub>, CH<sub>2</sub>OH, rt-reflux, overnight; (b) DMF-DMA, reflux, overnight; (c) EtOH, reflux, overnight; (d) LiOH, THF/CH<sub>3</sub>OH/H<sub>2</sub>O, rt, 1 h; (e) EDC, HOBT, DIPEA, THF, rt, overnight.

**Table 1** Structures of synthesized compounds and their activities to inhibit VIF-mediated A3G degradation

| Cmpd      | Structure | A3G (%) | Cmpd      | Structure | A3G (%)         |
|-----------|-----------|---------|-----------|-----------|-----------------|
| <b>1</b>  |           | 77      | <b>2a</b> |           | 93 <sup>a</sup> |
| <b>2b</b> |           | >100    | <b>2c</b> |           | 100             |
| <b>2d</b> |           | >100    | <b>2e</b> |           | >100            |
| <b>2f</b> |           | 14      | <b>2g</b> |           | 39              |
| <b>2h</b> |           | 19      | <b>2i</b> |           | 82              |
| <b>3a</b> |           | >100    | <b>3b</b> |           | >100            |
| <b>3c</b> |           | >100    | <b>3d</b> |           | 78              |
| <b>3e</b> |           | 86      | <b>3f</b> |           | >100            |
| <b>3g</b> |           | >100    | <b>3h</b> |           | >100            |
| <b>3i</b> |           | 75      | <b>3j</b> |           | >100            |
| <b>3k</b> |           | >100    | <b>3l</b> |           | ND <sup>b</sup> |
| <b>3m</b> |           | 42      | <b>3n</b> |           | 0               |

Table 1 continued

| Cmpd | Structure | A3G (%) | Cmpd | Structure | A3G (%) |
|------|-----------|---------|------|-----------|---------|
| 3o   |           | 92      | 4a   |           | ND      |
| 4b   |           | 63      | 4c   |           | 27      |
| 4d   |           | 66      | 4e   |           | 64      |
| 4f   |           | >100    | 4g   |           | 76      |
| 4h   |           | >100    | 4i   |           | >100    |
| 4j   |           | 99      | 4k   |           | 44      |
| 4l   |           | 85      | 4m   |           | >100    |
| 4n   |           | 69      | 4o   |           | >100    |
| 4p   |           | >100    | 4q   |           | 73      |
| 4r   |           | 78      | 4s   |           | 0       |
| 4t   |           | 32      | 4u   |           | 41      |
| 4v   |           | >100    | 4w   |           | >100    |

Table 1 continued

| Cmpd | Structure | A3G (%) | Cmpd | Structure | A3G (%) |
|------|-----------|---------|------|-----------|---------|
| 4x   |           | >100    | 4y   |           | 32      |
| 4z-1 |           | ND      | 4z-2 |           | >100    |
| 4z-3 |           | 18      | 4z-4 |           | 48      |
| 5a   |           | 0       | 5b   |           | 0       |
| 5c   |           | 11      | 5d   |           | ND      |
| 5e   |           | 2       | 5f   |           | 0       |
| 6    |           | 96      | 7    |           | 92      |
| 8    |           | 32      |      |           |         |

a Compounds were tested in triplicates and the data was presented as the mean value

b Not detected due to poor solubility

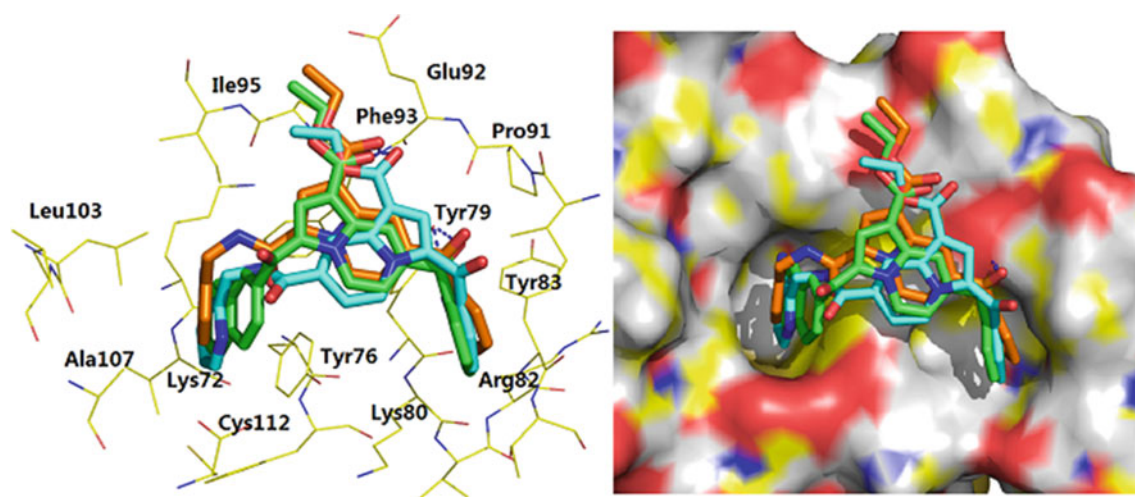
Therefore, compounds **3/4** were obtained by exchanging the substituents at position 3 and 7.

Compounds **3** were designed to investigate the effect of aralkylamine for activity in detail, while the aroyl at position 3 was maintained as benzoyl group. Various types of aralkylamine were introduced and the generated compounds showed similar to improved activities when  $n = 0$  (**3a–3k**). The introduction of **R** substituents showed no adverse effect on activity considering the chirality of the substituent (**3f–3h**). However, the activity of compounds decreased when  $n = 1$  (**3l–3n**), with the exception of compound **3o**, which indicated that the 3-atom separation between the **Ar** and the indolizine ring was favorable for activity. The same trend was observed in the anti-HIV-1 activity, 6 of the compounds bearing 3-atom separation between the **Ar** and the indolizine ring showed an  $IC_{50}$  of about  $50 \mu\text{M}$  while the 4-atom sep-

aration compound **3o** showed an  $IC_{50}$  of  $103 \mu\text{M}$ . The most potent compounds in this series were **3c** and **3j** with  $IC_{50}$  values of 19.3 and  $20.1 \mu\text{M}$ , respectively, and both showed low cytotoxicities.

Compounds **4** were designed to investigate the effect of 3-position aroyl on activity when pyridin-2-yl-methanamine was maintained at position 7. Various substituents were introduced to the phenyl ring of the benzoyl group to identify the optimal substituent. In general, introduction of an electron-withdrawing group (Cl, Br,  $\text{CF}_3$  and  $\text{NO}_2$ ) to the ortho-position of the phenyl ring decreased activity, while the introduction of an electron-donating (**4f**) group improved activity. Compound **4f** also showed comparable anti-HIV-1 activity ( $IC_{50} = 45.5 \mu\text{M}$ ) compared to **3a** ( $IC_{50} = 47.0 \mu\text{M}$ ). The introduction of substituents to the meta-position of the phenyl ring resulted in compounds with similar to





**Fig. 4** Superimposition of binding modes of compounds **1**, **2a**, and **3a** docked into the binding site of ElonginC. The carbon atoms of compounds **1**, **2a**, and **3a** are shown in green, orange, and cyan, respectively,

and the nitrogen and oxygen atoms in all ligands are shown in blue and red, respectively. Hydrogen-bonding interaction between ligand and residues are shown with blue dotted lines

**Table 2** Anti-HIV-1 activities of selected synthesized compounds

| Compound <sup>a</sup> | IC <sub>50</sub> <sup>b</sup> ( $\mu$ M) | CC <sub>50</sub> <sup>c</sup> ( $\mu$ M) | SI <sup>d</sup> |
|-----------------------|--|--|-----------------|
| <b>1</b>              | 36.1                                     | 167.4                                    | 4.6             |
| <b>2d</b>             | 42.1                                     | 107.3                                    | 2.5             |
| <b>2e</b>             | 55.2                                     | 233.5                                    | 4.2             |
| <b>3a</b>             | 47.0                                     | 151.0                                    | 3.2             |
| <b>3b</b>             | 54.3                                     | 114.1                                    | 2.1             |
| <b>3c</b>             | 19.3                                     | 98.2                                     | 5.1             |
| <b>3d</b>             | 50.9                                     | 91.4                                     | 1.8             |
| <b>3f</b>             | 41.0                                     | 216.0                                    | 5.3             |
| <b>3h</b>             | 49.5                                     | 229.0                                    | 4.6             |
| <b>3j</b>             | 20.1                                     | 250.5                                    | 12.5            |
| <b>3o</b>             | 103.2                                    | 139.0                                    | 1.3             |
| <b>4f</b>             | 45.5                                     | 247.5                                    | 5.4             |
| <b>4i</b>             | 57.8                                     | 54.0                                     | 0.93            |
| <b>4l</b>             | 22.3                                     | 133.5                                    | 6.0             |
| <b>4m</b>             | 54.4                                     | 81.6                                     | 1.5             |
| <b>4n</b>             | 98.1                                     | 142.2                                    | 1.4             |
| <b>4p</b>             | 20.5                                     | 349.9                                    | 17.1            |
| <b>4q</b>             | 20.5                                     | 99.4                                     | 4.8             |
| <b>4r</b>             | 108.7                                    | 159.5                                    | 1.7             |
| <b>4v</b>             | 223.8                                    | 139.2                                    | 0.62            |
| <b>4w</b>             | 48.7                                     | 89.7                                     | 1.8             |
| <b>4x</b>             | 124.4                                    | 53.4                                     | –               |
| <b>4z-2</b>           | > 500                                    | 236.8                                    | –               |
| <b>6</b>              | 69.7                                     | 154.2                                    | 2.2             |

<sup>a</sup> Compounds were tested in triplicate

<sup>b</sup> The concentration that affords 50 % inhibition of p24 production in the H9 cells

<sup>c</sup> 50 % cell toxicity concentration determined by MTT assay

<sup>d</sup> Selectivity index calculated as CC<sub>50</sub>/IC<sub>50</sub>

improved activities with the exception of compound **4k**. The meta-amine compound **4l** (IC<sub>50</sub>= 22.3  $\mu$ M) exhibited improved anti-HIV-1 activity and lower toxicity compared to compound **1**, while the bromine at the meta-position (**4i**) increased cytotoxicity. Furthermore, neither the introduction

of an electron-withdrawing group nor an electron-donating group provided similar to improved activity. The anti-HIV-1 activity of the para-substituted compounds correlated well with the electron density of the aryl ring, the poorer electron density of the aryl ring, the higher activity the compound showed (NO<sub>2</sub>  $\approx$  CF<sub>3</sub> > F > Cl > NH<sub>2</sub>). Compounds **4p** and **4q** both showed improved activity compared to **1** with IC<sub>50</sub> of 20.5  $\mu$ M. Introduction of two substituents to the phenyl ring decreased the antiviral activity in general except for compound **4v**. But, the anti-HIV-1 activity of compound **4v** decreased significantly compared to **1**. The compounds were tested with further modification of the phenyl group into its bioisosteres, such as furan and thiophene. The resulted compounds showed comparable activities to **3a**, but did not show improved potency compared to compound **1**. Introduction of large aryl group (**4y**) was also disfavor for activity.

The 3-position aroyl was further modified by replacing the phenyl ring with saturated heterocycles (**4z**). This group only produced one active compound (**4z-2**), but compound **4z-2** had weak anti-HIV-1 activity.

Variation of the substitution pattern of the indolizine scaffold

**Compounds 5/6:** The substituent at position 7 of compounds **2** was moved to position 8, while the 3-position substituent was maintained as amide group which generated compounds **5**. The 7-position amide group of compound **3a** was also investigated by changing the substitution site to the 8-position of the indolizine ring which generated compound **6**. The resulted compounds **5** showed significant decrease activities, while compound **6** showed increased activity in the preliminary assay. However, **6** showed about onefold

decrease of anti-HIV-1 activity compared to compound **1** suggesting that the position of the aroyl group is important for activity.

#### Replacement of the indolizine moiety

**Compounds 7/8:** Finally, two mimics of the indolizine core structure were synthesized and evaluated in the preliminary assay. When the N atom of the indolizine was migrated, the resulted compound **7** showed comparable activity. Then, an additional N atom was incorporated to the indolizine ring, which resulted in the imidazo[1, 2-a]pyridine core structure compound **8** with the carbethoxy group being eliminated. The activity of compound **8** decreased compared to **1**, which suggested that the carbethoxy group may play an important role in maintaining activity.

#### General conclusions about the SAR

Compounds **2** demonstrated that the naphthalene ring can be replaced by aralkylamines. The exchange of substituents at position 3 and 7 was well tolerated as shown in compounds **3** and **4**. Also, the substitution pattern of the indolizine scaffold was important, moved the 7-position substituent to the 8-position resulted in decrease in activity as shown in compounds **5** and **6**. The isosteric replacement of the indolizine moiety was also tolerated, as long as the substituents were retained.

**Molecular docking:** To elucidate the binding model of compounds with ElonginC, three representative compounds (**1**, **2a**, and **3a**) were selected for docking simulation studies.

Superimposition of the docked conformations of compounds **1**, **2a**, and **3a** to ElonginC shows the similarities of their bindings (Fig. 4). Both of the aryl rings of the aralkylamine group in compounds **2a** and **3a** are almost parallel to Tyr76 and form  $\pi - \pi$  stacking and hydrophobic interactions which are similar to compound **1**'s naphthalene ring. The aroyl groups of both compounds are located in the small hydrophobic pocket where compound **1**'s benzoyl group was located. The ethoxycarbonyl moiety of both compounds is extended outward from the ElonginC surface just like compound **1**. These interactions were thought to be crucial for ligands to bind to the receptor, which may be the reasons for these derivatives to maintain activity.

#### Conclusions

This study has identified new amide indolizine derivatives as HIV-1 VIF inhibitors based on the lead compound **1**. Studies were directed mainly at finding better replacement and suitable substitution site of the amide functionality present in the novel amide indolizine derivatives. The aroyl group was also investigated in an effort to improve anti-HIV-1 activity.

Among all the 62 synthesized derivatives of compound **1**, 5 compounds with the 3-aroyle, 7-amide indolizine structure showed improvement of activity compared to **1** with IC<sub>50</sub> values about 20  $\mu$ M. Overall, these indolizine derivatives show potential as effective HIV-1 VIF–ElonginC interaction inhibitors and the detailed study for structure-activity relationship may lead us to find more potent inhibitors.

#### Experimental section

##### Chemistry

All chemicals and solvents were purchased from commercial suppliers of analytical grade and used without further purification unless otherwise indicated. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded at 400 and 100 MHz, respectively, on a Bruker Avance III 400 NMR spectrometer. Chemical shifts are reported as values from an internal tetramethylsilane standard. High-resolution mass spectra (HRMS) were recorded on a Bruker Apex IV FTMS. LC/MS analyses were performed on an Agilent 1200-6110 instruments. Elemental analyses were performed on a Vario EL III instrument and are  $\pm 0.3$  of theoretical. Melting points were determined on a MelTemp apparatus and were uncorrected.

##### General procedure for the synthesis of compounds **2/5**

A mixture of 4-aroyle-pyridin **9** (10 mmol) and benzyl 2-bromoacetate (12 mmol) in THF was heated to reflux overnight. The solvent was evaporated and the residue was used for the next reaction without further purification.

The pyridinium salt **10** (10 mmol) was suspended in CH<sub>3</sub>CN (60 mL) at 0 °C and then potassium carbonate (20 mmol) was added portionwise to maintain the temperature at 0 °C. After 30 min, ethyl propiolate (11 mmol) was added dropwise at 0 °C, and the resulting mixture was stirred at room temperature for 3 days. The solvent was evaporated under reduced pressure and the residue was diluted with water, extracted three times with CH<sub>2</sub>Cl<sub>2</sub>. The organic layers were combined, washed with brine, dried over anhydrous sodium sulfate, and evaporated. The crude product was purified by silica gel chromatography to afford **11**.

LiOH·H<sub>2</sub>O (2 mmol) was added to the solution of compound **11** (1 mmol) in THF (10 mL), EtOH (10 mL) and H<sub>2</sub>O (3 mL). The reaction mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and 1N HCl solution was added to the residue. The precipitated product was filtered and washed with water to afford **12**. The crude product was used for the next reaction without further purification or characterization.

The crude carboxylic acid **12** (0.3 mmol) was dissolved in THF (15 mL), then EDC (0.45 mmol), HOBT (0.45 mmol),

DIPEA (0.6 mmol), and aralkylamine (0.36 mmol) were added. The reaction mixture was stirred overnight at room temperature. After the solvent was removed, the residue was extracted with  $\text{CH}_2\text{Cl}_2$ . The combined organic extracts were washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was concentrated and the residue was purified by silica gel chromatography to afford **2**.

*Ethyl 7-picolinoyl-3-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (2a)*

Yellow solid; Yield: 65.5%; Mp: 165–167 °C;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.75 (d,  $J = 6.1$  Hz, 1H, indolizine-H-5), 9.20 (s, 1H, indolizine-H-2), 8.78 (s, 1H, ArH), 8.61 (s, 1H, ArH), 8.11 (d,  $J = 7.3$  Hz, 1H, ArH), 8.00–7.85 (m, 2H, ArH), 7.77–7.67 (t,  $J = 7.0$  Hz, 1H, ArH), 7.66–7.47 (m, 3H, ArH), 7.37 (d,  $J = 7.3$  Hz, 1H, ArH), 7.30–7.25 (s, 1H, NH), 4.79 (d,  $J = 5.1$  Hz, 2H, Ar- $\text{CH}_2$ ), 4.38 (q,  $J = 6.0$  Hz, 2H,  $\text{CH}_2$ ), 1.38 (t,  $J = 6.0$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  191.24, 164.11, 161.25, 156.10, 154.83, 149.21, 148.77, 137.47, 137.25, 136.23, 131.30, 127.85, 126.73, 125.34, 124.98, 122.81, 122.41, 120.19, 119.42, 113.51, 109.11, 60.47, 44.34, 14.66; ESI-MS  $m/z$ : 429.0  $[\text{M}+\text{H}]^+$ ; HRMS (ESI-TOF $^+$ )  $m/z$  Calcd. for  $\text{C}_{24}\text{H}_{20}\text{N}_4\text{O}_4$   $[\text{M}+\text{H}]^+$ : 429.1485; Found: 429.1553.

*Ethyl 7-picolinoyl-3-(pyridin-3-ylmethylcarbamoyl)indolizine-1-carboxylate (2b)*

Yellow solid; Yield: 78.1%; Mp: 135–137 °C;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.72 (d,  $J = 7.4$  Hz, 1H, indolizine-H-5), 9.18 (s, 1H, indolizine-H-2), 8.76 (d,  $J = 3.0$  Hz, 1H, ArH), 8.63 (s, 1H, ArH), 8.54 (d,  $J = 2.9$  Hz, 1H, ArH), 8.11 (d,  $J = 7.8$  Hz, 1H), 7.95 (t,  $J = 7.7$  Hz, 1H), 7.81 (s, 1H, indolizine-H-8), 7.75 (d,  $J = 7.8$  Hz, 1H, ArH), 7.61 (d,  $J = 7.3$  Hz, 1H, indolizine-H-6), 7.54 (t,  $J = 5.9$  Hz, 1H, ArH), 7.30 (t,  $J = 5.5$  Hz, 1H, ArH), 6.90 (t,  $J = 4.3$  Hz, 1H, NH), 4.69 (d,  $J = 5.1$  Hz, 2H, Ar- $\text{CH}_2$ ), 4.33 (q,  $J = 6.9$  Hz, 2H,  $\text{CH}_2$ ), 1.34 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$  191.2, 164.0, 161.3, 154.7, 149.5, 149.3, 148.7, 137.5, 136.4, 135.9, 134.1, 131.6, 127.8, 126.8, 125.2, 125.0, 123.9, 120.1, 118.9, 113.7, 109.0, 60.5, 41.2, 14.6; ESI-MS  $m/z$ : 429.0  $[\text{M}+\text{H}]^+$ ; Anal. Calcd. for  $\text{C}_{24}\text{H}_{20}\text{N}_4\text{O}_4$ : C, 67.00; H, 4.69; N, 12.93; Found: C, 67.28; H, 4.71; N, 13.08.

*Ethyl 3-(benzylcarbamoyl)-7-picolinoylindolizine-1-carboxylate (2c)*

Yellow solid; Yield: 78.1%; Mp: 170–173 °C;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.74 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 9.18 (s, 1H, indolizine-H-2), 8.77 (d,  $J = 3.3$  Hz, 1H, ArH), 8.10 (d,  $J = 7.8$  Hz, 1H, ArH), 7.94 (t,  $J = 7.8$  Hz, 1H, ArH), 7.74 (s, 1H, indolizine-H-8), 7.60 (d,  $J =$

7.4 Hz, 1H, indolizine-H-6), 7.56–7.48 (m, 1H, ArH), 7.41–7.28 (m, 5H, ArH), 6.45 (t,  $J = 4.3$  Hz, 1H, NH), 4.66 (d,  $J = 4.9$  Hz, 2H, Ar- $\text{CH}_2$ ), 4.33 (q,  $J = 6.9$  Hz, 2H,  $\text{CH}_2$ ), 1.34 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$  191.2, 164.0, 161.1, 154.8, 148.7, 138.2, 137.5, 136.3, 131.4, 129.1, 128.1, 127.9, 127.9, 126.8, 125.3, 125.0, 119.7, 119.2, 113.6, 109.0, 60.5, 43.7, 14.6; ESI-MS  $m/z$ : 428.0  $[\text{M}+\text{H}]^+$ ; Anal. Calcd. for  $\text{C}_{25}\text{H}_{21}\text{N}_3\text{O}_4$ : C, 70.09; H, 4.94; N, 9.76; Found: C, 70.25; H, 4.95; N, 9.83.

*Ethyl 7-(furan-2-carbonyl)-3-(pyridin-2-yl-methylcarbamoyl)indolizine-1-carboxylate (2d)*

Yellow solid; Yield: 69.3%; Mp: 166–169 °C;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.75 (d,  $J = 7.4$  Hz, 1H, indolizine-H-5), 9.10 (s, 1H, indolizine-H-2), 8.61 (d,  $J = 4.4$  Hz, 1H, ArH), 7.91 (s, 1H, ArH), 7.76 (s, 1H, ArH), 7.73 (t,  $J = 7.8$  Hz, 1H, ArH), 7.69–7.62 (m, 1H, NH), 7.49 (d,  $J = 7.5$  Hz, 1H, indolizine-H-6), 7.40 (d,  $J = 2.5$  Hz, 1H, ArH), 7.37 (d,  $J = 7.9$  Hz, 1H, ArH), 7.26 (d,  $J = 7.7$  Hz, 2H, ArH), 6.65 (s, 1H, ArH), 4.79 (d,  $J = 4.4$  Hz, 2H, Ar- $\text{CH}_2$ ), 4.42 (q,  $J = 6.9$  Hz, 2H,  $\text{CH}_2$ ), 1.44 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$  179.8, 164.1, 161.2, 156.1, 152.4, 149.2, 147.5, 137.2, 136.3, 132.1, 128.2, 122.8, 122.5, 120.8, 120.1, 119.4, 112.9, 112.7, 108.6, 60.5, 44.3, 14.7; ESI-MS  $m/z$ : 418.0  $[\text{M}+\text{H}]^+$ ; Anal. Calcd. for  $\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_5$ : C, 66.18; H, 4.59; N, 10.07; Found: C, 66.10; H, 4.70; N, 10.04.

*Ethyl 7-(furan-2-carbonyl)-3-(pyridin-3-yl-methylcarbamoyl)indolizine-1-carboxylate (2e)*

Yellow solid; Yield: 93.3%; Mp: 134–136 °C;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.72 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 9.05 (s, 1H, indolizine-H-2), 8.63 (s, 1H, ArH), 8.53 (s, 1H, ArH), 7.86 (s, 1H, ArH), 7.80–7.71 (m, 2H, ArH+NH), 7.48 (d,  $J = 7.4$  Hz, 1H, indolizine-H-6), 7.42–7.36 (m, 1H, ArH), 7.28 (s, 1H, ArH), 7.18 (t,  $J = 4.6$  Hz, 1H, ArH), 6.65 (s, 1H, ArH), 4.69 (d,  $J = 5.5$  Hz, 2H, ArH), 4.35 (q,  $J = 7.0$  Hz, 2H,  $\text{CH}_2$ ), 1.38 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$  179.7, 164.0, 161.3, 152.3, 149.4, 149.1, 147.5, 136.4, 136.0, 132.3, 128.2, 123.9, 122.4, 120.9, 120.1, 119.0, 113.0, 112.7, 108.6, 60.6, 41.1, 14.6; ESI-MS  $m/z$ : 418.1  $[\text{M}+\text{H}]^+$ ; Anal. Calcd. for  $\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_5$ : C, 65.92; H, 4.62; N, 10.10; Found: C, 66.18; H, 4.59; N, 10.07.

*Ethyl 7-(3-methoxybenzoyl)-3-(pyridin-2-yl-methylcarbamoyl)indolizine-1-carboxylate (2f)*

Yellow solid; Yield: 54.6%; Mp: 152–153 °C;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.77 (d,  $J = 7.5$  Hz, 1H, indolizine-H-5), 8.69 (s, 1H, indolizine-H-2), 8.61 (s, 1H, ArH), 7.91 (s, 1H, indolizine-H-8), 7.75–7.69 (t,  $J = 7.6$  Hz, 1H, ArH), 7.68–7.60 (br, 1H, NH), 7.45–7.33 (m, 3H, ArH), 7.36 (d,

$J = 7.6$  Hz, 1H, indolizine-H-6), 7.25 (d,  $J = 7.0$  Hz, 1H, ArH), 7.16 (d,  $J = 7.0$  Hz, 1H, ArH), 4.79 (d,  $J = 4.4$  Hz, 2H, ArCH<sub>2</sub>), 4.34 (q,  $J = 6.8$  Hz, 2H, CH<sub>2</sub>), 3.87 (s, 3H, OCH<sub>3</sub>), 1.33 (t,  $J = 6.9$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  194.3, 164.0, 161.2, 159.9, 156.1, 149.3, 138.4, 137.1, 136.1, 132.5, 129.6, 128.2, 123.5, 122.7, 122.3, 120.1, 119.4, 114.3, 113.3, 108.6, 60.5, 55.7, 44.4, 14.5; ESI-MS  $m/z$ : 458.1 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>26</sub>H<sub>23</sub>N<sub>3</sub>O<sub>5</sub>: C, 68.16; H, 5.12; N, 9.17; Found: C, 68.26; H, 5.07; N, 9.19.

*Ethyl 7-(3-methoxybenzoyl)-3-(pyridin-3-yl methylcarbamoyl)indolizine-1-carboxylate (2g)*

Yellow solid; Yield: 43.8%; Mp: 128–130 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.74 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.65 (s, 1H, indolizine-H-2), 8.61 (s, 1H, ArH), 8.53–8.48 (m, 1H, NH), 7.90 (s, 1H, indolizine-H-8), 7.75 (d,  $J = 7.5$  Hz, 1H, ArH), 7.46–7.34 (m, 5H, ArH + indolizine-H-6), 7.27 (t,  $J = 6.3$  Hz, 1H, ArH), 7.16 (d,  $J = 7.5$  Hz, 1H, ArH), 4.68 (d,  $J = 5.3$  Hz, 2H, ArCH<sub>2</sub>), 4.27 (q,  $J = 6.7$  Hz, 2H, CH<sub>2</sub>), 3.86 (s, 3H, OCH<sub>3</sub>), 1.26 (t,  $J = 6.7$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  194.3, 164.0, 161.4, 159.9, 149.4, 149.1, 138.3, 136.2, 135.9, 134.3, 132.7, 129.6, 128.1, 123.9, 123.4, 122.7, 120.3, 119.4, 119.1, 114.4, 113.4, 108.5, 60.5, 55.6, 41.1, 14.4; ESI-MS  $m/z$ : 458.0 [M+H]<sup>+</sup>.

*Ethyl 7-(2-chloroisonicotinoyl)-3-(pyridin-2-yl methylcarbamoyl)indolizine-1-carboxylate (2h)*

Yellow solid; Yield: 50.1%; Mp: 187–190 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.79 (d,  $J = 7.4$  Hz, 1H, indolizine-H-5), 8.67–8.59 (m, 3H, ArH), 7.92 (s, 1H, indolizine-H-2), 7.79–7.69 (m, 2H, ArH+NH), 7.68 (s, 1H, indolizine-H-8), 7.56 (d,  $J = 5.0$  Hz, 1H, ArH), 7.42 (d,  $J = 7.4$  Hz, 1H, indolizine-H-6), 7.36 (d,  $J = 7.5$  Hz, 1H, ArH), 7.30–7.25 (m, 1H, ArH), 4.79 (d,  $J = 4.5$  Hz, 2H, Ar-CH<sub>2</sub>), 4.35 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.34 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  191.5, 163.7, 161.0, 155.9, 152.6, 150.6, 149.3, 147.1, 137.2, 135.5, 130.1, 128.6, 124.3, 123.8, 122.8, 122.4, 121.6, 120.3, 120.2, 112.0, 109.8, 60.7, 44.4, 14.5; ESI-MS  $m/z$ : 463.1 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>24</sub>H<sub>19</sub>ClN<sub>4</sub>O<sub>4</sub>: C, 62.01; H, 4.22; N, 11.95; Found: C, 62.27; H, 4.14; N, 12.10.

*Ethyl 7-(2-chloroisonicotinoyl)-3-(pyridin-3-yl methylcarbamoyl)indolizine-1-carboxylate (2i)*

Yellow solid; Yield: 9.4%; Mp: 189–192 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.78 (d,  $J = 7.4$  Hz, 1H, indolizine-H-5), 8.67–8.58 (m, 3H, ArH), 8.55 (s, 1H, ArH), 7.85 (s, 1H, indolizine-H-2), 7.76 (d,  $J = 7.8$  Hz, 1H, ArH), 7.67 (s, 1H, indolizine-H-8), 7.56 (d,  $J = 4.7$  Hz, 1H, ArH), 7.45 (d,  $J = 7.4$  Hz, 1H, indolizine-H-6), 7.32 (t,  $J = 4.9$  Hz, 1H,

ArH), 6.99 (t,  $J = 5.3$  Hz, 1H, NH), 4.70 (d,  $J = 5.3$  Hz, 2H, Ar-CH<sub>2</sub>), 4.32 (q,  $J = 6.9$  Hz, 2H, CH<sub>2</sub>), 1.31 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  191.4, 163.6, 161.1, 152.7, 150.7, 149.5, 149.3, 146.9, 136.0, 135.7, 134.0, 130.4, 128.7, 124.3, 124.0, 123.8, 121.5, 120.3, 119.7, 112.3, 109.8, 60.8, 41.2, 14.5; ESI-MS  $m/z$ : 463.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>24</sub>H<sub>19</sub>ClN<sub>4</sub>O<sub>4</sub>: C, 62.01; H, 4.22; N, 11.95; Found: C, 62.27; H, 4.14; N, 12.10.

*Ethyl 8-benzoyl-3-(pyridin-2-yl methylcarbamoyl)indolizine-1-carboxylate (5a)*

Off white solid; Yield: 90.9% over two steps; Mp: 173–175 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.91 (d,  $J = 7.0$  Hz, 1H, indolizine-H-5), 8.60 (s, 1H, indolizine-H-2), 7.92–7.80 (m, 3H, ArH), 7.71 (t,  $J = 7.6$  Hz, 1H, ArH), 7.65–7.59 (m, 1H, NH), 7.56 (t,  $J = 6.53$  Hz, 1H, ArH), 7.43 (t,  $J = 7.3$  Hz, 2H, ArH), 7.36 (d,  $J = 7.7$  Hz, 1H, ArH), 7.26–7.17 (m, 2H, ArH + indolizine-H-7), 6.97 (t,  $J = 6.9$  Hz, 1H, indolizine-H-6), 4.79 (s, 2H, Ar-CH<sub>2</sub>), 4.00 (q,  $J = 6.9$  Hz, 2H, CH<sub>2</sub>), 1.02 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  193.49, 163.53, 161.49, 156.27, 149.19, 137.39, 137.16, 133.74, 133.31, 131.70, 129.90, 129.85, 128.63, 125.85, 122.72, 122.25, 120.37, 118.24, 112.97, 106.51, 60.12, 44.35, 14.27; ESI-MS  $m/z$ : 428.1 [M+H]<sup>+</sup>.

*Ethyl 8-benzoyl-3-(pyridin-3-yl methylcarbamoyl)indolizine-1-carboxylate (5b)*

Yellowish green pale solid; Yield: 93.7%; Mp: 196–196 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.86 (d,  $J = 6.8$  Hz, 1H, indolizine-H-5), 8.73–8.36 (m, 2H, ArH), 7.90 (s, 1H, indolizine-H-2), 7.84 (d,  $J = 7.4$  Hz, 2H, ArH), 7.74 (d,  $J = 7.0$  Hz, 2H, ArH), 7.55 (t,  $J = 7.0$  Hz, 1H, ArH), 7.42 (t,  $J = 7.3$  Hz, 2H, ArH), 7.25 (br, 1H, NH), 7.21 (d,  $J = 6.7$  Hz, 1H, indolizine-H-7), 6.95 (t,  $J = 6.7$  Hz, 1H, indolizine-H-6), 4.64 (s, 2H, Ar-CH<sub>2</sub>), 3.87 (q,  $J = 6.4$  Hz, 2H, CH<sub>2</sub>), 0.89 (t,  $J = 6.7$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  193.6, 163.6, 161.6, 148.9, 148.4, 137.1, 135.9, 133.7, 133.4, 131.5, 129.8, 129.8, 128.6, 125.9, 120.7, 118.0, 112.9, 106.3, 60.0, 40.9, 14.0; ESI-MS  $m/z$ : 428.0 [M+H]<sup>+</sup>, 854.8 [2M+H]<sup>+</sup>; Anal. Calcd. for C<sub>25</sub>H<sub>21</sub>N<sub>3</sub>O<sub>4</sub>: C, 70.07; H, 4.96; N, 9.74; Found: C, 70.25; H, 4.95; N, 9.83.

*Ethyl 8-picolinoyl-3-(pyridin-2-yl methylcarbamoyl)indolizine-1-carboxylate (5c)*

Yellow solid; Yield: 15.9%; Mp: 64–66 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.91 (d,  $J = 6.9$  Hz, 1H, indolizine-H-5), 8.60 (s, 1H, ArH), 8.51 (s, 1H, ArH), 8.29 (d,  $J = 7.5$  Hz, 1H, ArH), 7.90 (t,  $J = 7.4$  Hz, 1H, ArH), 7.81 (s, 1H, indolizine-H-2), 7.71 (t,  $J = 7.6$  Hz, 1H), 7.53 (s, 1H, NH), 7.42 (t,  $J = 4.8$

Hz, 1H, ArH), 7.39–7.31 (m, 2H, ArH), 7.24 (t,  $J = 4.8$  Hz, 1H, ArH), 7.02 (t,  $J = 6.8$  Hz, 1H, indolizine-H-6), 4.78 (d,  $J = 3.9$  Hz, 2H, Ar-CH<sub>2</sub>), 3.97 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.07 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 193.3, 163.8, 161.6, 156.5, 154.7, 149.3, 149.0, 137.0, 134.7, 131.8, 130.0, 126.8, 126.7, 126.4, 123.2, 122.6, 122.2, 119.9, 117.9, 113.2, 106.2, 60.0, 44.4, 14.4; ESI-MS  $m/z$ : 429.1 [M+H]<sup>+</sup>, 879.1 [2M+Na]<sup>+</sup>.

*Ethyl 8-picolinoyl-3-(pyridin-3-yl methylcarbamoyl)indolizine-1-carboxylate (5d)*

Yellow solid; Yield: 60.7%; Mp: 158–160 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.86 (d,  $J = 7.0$  Hz, 1H, indolizine-H-5), 8.53 (s, 1H, ArH), 8.51–8.42 (m, 2H, ArH), 8.27 (d,  $J = 7.8$  Hz, 1H, ArH), 7.89 (t,  $J = 7.7$  Hz, 1H, ArH), 7.73–7.66 (m, 2H, NH+indolizine-H-2), 7.41 (t,  $J = 5.1$  Hz, 1H, ArH), 7.34 (d,  $J = 6.9$  Hz, 1H, indolizine-H-7), 7.28–7.21 (m, 2H, ArH), 7.01 (t,  $J = 6.9$  Hz, 1H, indolizine-H-6), 4.58 (d,  $J = 5.1$  Hz, 2H, Ar-CH<sub>2</sub>), 3.87 (q,  $J = 6.9$  Hz, 2H, CH<sub>2</sub>), 0.97 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 193.3, 163.6, 161.6, 154.6, 149.5, 149.4, 149.3, 148.9, 148.8, 137.1, 136.0, 135.8, 134.7, 134.5, 132.4, 131.8, 130.0, 126.8, 126.5, 123.9, 123.8, 123.3, 120.1, 117.5, 113.3, 106.1, 59.9, 40.9, 14.2; ESI-MS  $m/z$ : 429.0 [M+H]<sup>+</sup>, 857.0 [2M+H]<sup>+</sup>.

*Ethyl 8-(furan-2-carbonyl)-3-(pyridin-2-yl methylcarbamoyl)indolizine-1-carboxylate (5e)*

Yellow solid; Yield: 100%; Mp: 179–181 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.92 (d,  $J = 7.0$  Hz, 1H, indolizine-H-5), 8.61 (s, 1H, ArH), 7.87 (s, 1H, indolizine-H-2), 7.71 (t,  $J = 7.5$  Hz, 1H, ArH), 7.63 (s, 1H, ArH), 7.53 (s, 1H, NH), 7.36 (t,  $J = 8.3$  Hz, 2H, ArH), 7.24 (t,  $J = 6.9$  Hz, 1H, indolizine-H-6), 7.03–6.94 (m, 2H, ArH), 6.53 (s, 1H, ArH), 4.78 (d,  $J = 3.2$  Hz, 2H, Ar-CH<sub>2</sub>), 4.12 (q,  $J = 6.8$  Hz, 2H, CH<sub>2</sub>), 1.17 (t,  $J = 6.7$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 181.1, 163.7, 161.4, 156.2, 152.9, 149.3, 147.3, 137.1, 133.5, 130.6, 130.4, 126.2, 122.7, 122.2, 120.4, 119.4, 118.3, 112.9, 112.5, 106.5, 60.4, 44.4, 14.3; ESI-MS  $m/z$ : 418.0 [M+H]<sup>+</sup>.

*Ethyl 8-(furan-2-carbonyl)-3-(pyridin-3-yl methylcarbamoyl)indolizine-1-carboxylate (5f)*

Yellow solid; Yield: 96.2%; Mp: 189–191 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.87 (d,  $J = 6.9$  Hz, 1H, indolizine-H-5), 8.60 (s, 1H, indolizine-H-2), 8.49 (s, 1H, ArH), 7.86 (s, 1H, ArH), 7.73 (d,  $J = 7.5$  Hz, 1H, ArH), 7.63 (s, 1H, ArH), 7.45 (s, 1H, ArH), 7.36 (d,  $J = 6.8$  Hz, 1H, indolizine-H-7), 7.26 (t,  $J = 5.2$  Hz, 1H, ArH), 7.01 (s, 1H, ArH), 6.96 (t,  $J = 6.9$

Hz, 1H, indolizine-H-6), 6.52 (s, 1H, ArH), 4.66 (d,  $J = 5.4$  Hz, 2H, Ar-CH<sub>2</sub>), 4.00 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.05 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 181.0, 163.7, 161.6, 152.7, 149.3, 148.9, 147.4, 135.8, 134.4, 133.4, 130.4, 130.3, 126.3, 123.8, 120.6, 119.7, 117.9, 112.9, 112.5, 106.4, 60.3, 41.0, 14.1; ESI-MS  $m/z$ : 418.0 [M+H]<sup>+</sup>, 835.0 [2M+H]<sup>+</sup>.

General procedure for the synthesis of compounds **3/4**

A mixture of Ar<sub>1</sub>COCH<sub>2</sub>Br (20 mmol) and **13** (22 mmol) in EtOAc (40 mL) was heated to reflux over night. The precipitate was filtrated to afford **14** as a white solid, which was used for the next reaction without further purification.

The pyridinium salt **14** (20 mmol) was suspended in CH<sub>3</sub>CN (100 mL) at 0 °C and then potassium carbonate (40 mmol) was added portionwise to maintain the temperature at 0 °C. After 30 min, ethyl propiolate (22 mmol) was added dropwise at 0 °C and the resulting mixture was stirred at room temperature for 3 days. The solvent was evaporated under reduced pressure and the residue was diluted with water and extracted three times with CH<sub>2</sub>Cl<sub>2</sub>. The organic layers were combined, washed with brine, dried over anhydrous sodium sulfate, and evaporated. The crude product was purified by silica gel chromatography to afford compound **15**. LiOH·H<sub>2</sub>O (2.0 mmol) was added to a solution of compound **15** (1.0 mmol) in THF (15 mL), EtOH (15 mL), and H<sub>2</sub>O (5 mL). The reaction mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and 1N HCl solution was added to the residue. The precipitated product was filtrated and washed with water to afford **16** which was used for the next reaction without further purification.

The crude carboxylic acid **16** (0.25 mmol) was dissolved in THF (10 mL), then EDC (0.375 mmol), HOBt (0.375 mmol), DIPEA (0.5 mmol), and aralkylamine (0.275 mmol) were added. The reaction mixture was stirred overnight at room temperature. After the solvent was removed, the residue was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic extracts were washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was concentrated and the residue was purified by silica gel chromatography to afford **3/4**.

*Ethyl 3-benzoyl-7-(pyridin-2-yl methylcarbamoyl)indolizine-1-carboxylate (3a)*

Yellow solid; Yield: 52.7%; Mp: 174–177 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.97 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.86 (s, 1H, indolizine-H-2), 8.60 (d,  $J = 4.2$  Hz, 1H, ArH), 8.00–7.90 (m, 1H, NH), 7.87 (s, 1H, indolizine-H-8), 7.83 (d,  $J = 7.3$  Hz, 2H, ArH), 7.72 (t,  $J = 7.4$  Hz, 1H, ArH), 7.64–7.57 (m, 2H, ArH), 7.53 (t,  $J = 7.3$  Hz, 2H, ArH), 7.36 (d,  $J = 7.4$  Hz, 1H, indolizine-H-6), 7.29–7.18 (m, 1H, ArH),

4.82 (d,  $J = 4.3$  Hz, 2H, Ar-CH<sub>2</sub>), 4.41 (d,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.44 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) $\delta$  186.0, 165.1, 164.0, 155.8, 149.3, 139.7, 138.7, 137.1, 132.8, 132.0, 129.4, 129.2, 128.7, 123.5, 122.8, 122.4, 118.2, 113.7, 108.6, 60.6, 45.0, 14.7; ESI-MS  $m/z$ : 428.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>25</sub>H<sub>21</sub>N<sub>3</sub>O<sub>4</sub>: C, 70.00; H, 4.93; N, 9.77; Found: C, 70.25; H, 4.95; N, 9.83.

*Ethyl 3-benzoyl-7-(pyridin-3-ylmethylcarbamoyl)indolizine-1-carboxylate (3b)*

Yellow solid; Yield: 58.5%; Mp: 209–212 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) $\delta$  9.88 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.75 (s, 1H, indolizine-H-2), 8.60 (s, 1H, ArH), 8.52 (d,  $J = 4.0$  Hz, 1H, ArH), 7.81 (s, 1H, indolizine-H-8), 7.80–7.77 (m, 2H, ArH + NH), 7.74 (d,  $J = 7.9$  Hz, 1H, ArH), 7.60 (t,  $J = 7.3$  Hz, 1H, ArH), 7.55–7.48 (m, 3H, ArH + indolizine-H-6), 7.42 (t,  $J = 5.5$  Hz, 1H, ArH), 7.29–s7.25 (m, 1H, ArH), 4.69 (d,  $J = 5.8$  Hz, 2H, Ar-CH<sub>2</sub>), 4.32 (q,  $J = 7.2$  Hz, 2H, CH<sub>2</sub>), 1.35 (t,  $J = 7.2$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) $\delta$  186.0, 165.5, 163.9, 149.6, 149.3, 139.5, 138.6, 136.1, 133.8, 132.4, 132.1, 129.2, 129.1, 129.1, 128.7, 123.9, 123.5, 117.9, 113.7, 108.4, 60.6, 41.9, 14.6; ESI-MS  $m/z$ : 428.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>25</sub>H<sub>21</sub>N<sub>3</sub>O<sub>4</sub>: C, 70.00; H, 4.93; N, 9.77; Found: C, 70.25; H, 4.95; N, 9.83.

*Ethyl 3-benzoyl-7-(furan-2-ylmethylcarbamoyl)indolizine-1-carboxylate (3c)*

White solid; Yield: 60.1%; Mp: 172–174 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) $\delta$  9.90 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.73 (s, 1H, indolizine-H-2), 7.82 (s, 1H, indolizine-H-8), 7.82–7.78 (m, 2H, ArH), 7.60 (t,  $J = 7.3$  Hz, 1H, ArH), 7.57–7.46 (m, 3H, ArH), 7.39 (s, 1H, NH), 6.88 (t,  $J = 4.4$  Hz, 1H, ArH), 6.41–6.30 (m, 2H, ArH), 4.69 (d,  $J = 5.3$  Hz, 2H, Ar-CH<sub>2</sub>), 4.36 (q,  $J = 7.2$  Hz, 2H, CH<sub>2</sub>), 1.38 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) $\delta$  186.0, 165.0, 164.0, 150.8, 142.7, 139.6, 138.6, 132.6, 132.0, 129.2, 129.2, 128.7, 123.5, 117.8, 113.7, 110.8, 108.4, 108.3, 60.6, 37.4, 14.6; ESI-MS  $m/z$ : 417.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>24</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub>: C, 69.19; H, 4.86; N, 6.64; Found: C, 69.22; H, 4.84; N, 6.73.

*Ethyl 3-benzoyl-7-(benzylcarbamoyl)indolizine-1-carboxylate (3d)*

White solid; Yield: 35.2%; Mp: 196–198 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) $\delta$  9.90 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.73 (s, 1H, indolizine-H-2), 7.82 (s, 1H, indolizine-H-8), 7.82–7.78 (m, 2H, ArH), 7.60 (t,  $J = 7.3$  Hz, 1H, ArH), 7.56–7.46 (m, 3H, ArH), 7.39 (s, 1H, NH), 6.89 (t,  $J = 4.4$  Hz, 1H, ArH), 6.41–6.29 (m, 2H, ArH), 4.69 (d,  $J = 5.3$  Hz, 2H, Ar-CH<sub>2</sub>), 4.36 (q,  $J = 7.2$  Hz, 2H, CH<sub>2</sub>), 1.38 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>);

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) $\delta$  186.0, 165.0, 164.0, 150.8, 142.7, 139.6, 138.6, 132.6, 132.0, 129.2, 129.2, 128.7, 123.5, 117.8, 113.7, 110.8, 108.4, 108.3, 60.6, 37.4, 14.6; ESI-MS  $m/z$ : 427.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>26</sub>H<sub>22</sub>N<sub>2</sub>O<sub>4</sub>: C, 73.29; H, 5.18; N, 6.47; Found: C, 73.23; H, 5.20; N, 6.57.

*Ethyl 3-benzoyl-7-(3-fluorobenzylcarbamoyl)indolizine-1-carboxylate (3e)*

White solid; Yield: 32.2%; Mp: 208–210 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) $\delta$  9.93 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.75 (s, 1H, indolizine-H-2), 7.87–7.78 (m, 3H, ArH + indolizine-H-8), 7.61 (t,  $J = 7.1$  Hz, 1H, ArH), 7.58–7.49 (m, 3H, ArH), 7.38–7.28 (m, 1H, ArH), 7.16 (d,  $J = 7.4$  Hz, 1H, indolizine-H-6), 7.09 (d,  $J = 9.4$  Hz, 1H, ArH), 7.00 (t,  $J = 8.3$  Hz, 1H, ArH), 6.97–6.88 (m, 1H, NH), 4.68 (d,  $J = 5.4$  Hz, 2H, Ar-CH<sub>2</sub>), 4.36 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.38 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) $\delta$  186.0, 165.2, 164.1, 140.5, 139.6, 138.7, 132.5, 132.1, 130.6, 130.6, 129.3, 129.1, 128.7, 123.7, 123.5, 117.7, 115.2, 115.0, 114.9, 114.8, 113.8, 108.4, 60.7, 44.0, 14.7; ESI-MS  $m/z$ : 445.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>26</sub>H<sub>21</sub>FN<sub>2</sub>O<sub>4</sub>: C, 70.26; H, 4.76; N, 6.30; Found: C, 70.36; H, 4.97; N, 6.08.

*(R)-Ethyl 3-benzoyl-7-(1-phenylethylcarbamoyl)indolizine-1-carboxylate (3f)*

White solid; Yield: 79.1%; Mp: 175–178 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) $\delta$  9.92 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.71 (s, 1H, indolizine-H-2), 7.87–7.78 (m, 3H, ArH + indolizine-H-8), 7.60 (t,  $J = 7.0$  Hz, 1H, ArH), 7.53 (t,  $J = 7.2$  Hz, 3H, ArH), 7.46–7.41 (m, 2H, ArH), 7.38 (t,  $J = 7.3$  Hz, 2H, ArH), 7.31 (d,  $J = 7.2$  Hz, 1H, ArH), 6.66 (d,  $J = 7.3$  Hz, 1H, indolizine-H-6), 5.43–5.30 (m, 1H, Ar-CH), 4.37 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.66 (s, 3H, CH<sub>3</sub>), 1.39 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) $\delta$  186.0, 164.3, 164.1, 142.9, 139.6, 138.7, 133.0, 132.0, 129.2, 129.2, 129.1, 128.7, 127.9, 126.5, 123.5, 117.5, 113.9, 108.2, 60.6, 49.9, 21.9, 14.7; ESI-MS  $m/z$ : 445.0 [M+H]<sup>+</sup>, 881.3 [2M+H]<sup>+</sup>; Anal. Calcd. for C<sub>27</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>: C, 73.37; H, 5.39; N, 6.32; Found: C, 73.62; H, 5.49; N, 6.36.

*(S)-Ethyl 3-benzoyl-7-(2-methoxy-2-oxo-1-phenylethylcarbamoyl)indolizine-1-carboxylate (3g)*

White solid; Yield: 72.4%; Mp: 183–185 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) $\delta$  9.92 (d,  $J = 7.2$  Hz, 1H, indolizine-H-5), 8.80 (s, 1H, indolizine-H-2), 7.87–7.83 (m, 2H, ArH), 7.82 (s, 1H, indolizine-H-8), 7.60 (t,  $J = 6.9$  Hz, 1H, NH), 7.56–7.45 (m, 6H, ArH), 7.44–7.36 (m, 3H, ArH), 5.81 (d,  $J = 6.8$  Hz, 1H, Ar-CH), 4.38 (d,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 3.80 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 1.40 (t,  $J = 6.8$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) $\delta$  186.0, 171.4, 164.5, 163.9, 139.6, 138.5,

136.3, 132.1, 131.9, 129.4, 129.3, 129.2, 129.0, 128.7, 127.7, 123.6, 118.3, 113.6, 108.7, 60.7, 57.3, 53.2, 14.7; ESI-MS  $m/z$ : 485.2 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>28</sub>H<sub>24</sub>N<sub>2</sub>O<sub>6</sub>: C, 69.47; H, 5.02; N, 5.94; Found: C, 69.41; H, 4.99; N, 5.78.

*(R)*-Ethyl 3-benzoyl-7-(2-methoxy-2-oxo-1-phenylethylcarbamoyl)indolizine-1-carboxylate (**3h**)

Brown solid; Yield: 100%; Mp: 188–190 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.92 (d,  $J = 7.2$  Hz, 1H, indolizine-H-5), 8.80 (s, 1H, indolizine-H-2), 7.87–7.82 (m, 2H, ArH), 7.82 (s, 1H, indolizine-H-8), 7.60 (t,  $J = 6.9$  Hz, 1H, NH), 7.57–7.44 (m, 6H, ArH), 7.44–7.36 (m, 3H, ArH + indolizine-H-6), 5.81 (d,  $J = 6.8$  Hz, 1H, Ar-CH), 4.38 (q,  $J = 6.8$  Hz, 2H, CH<sub>2</sub>), 3.80 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 1.40 (t,  $J = 6.8$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 186.0, 171.4, 164.5, 163.9, 139.6, 138.5, 136.3, 132.1, 131.9, 129.4, 129.3, 129.2, 129.0, 128.7, 127.7, 123.6, 118.3, 113.6, 108.7, 60.7, 57.3, 53.2, 14.7; ESI-MS  $m/z$ : 485.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>28</sub>H<sub>24</sub>N<sub>2</sub>O<sub>6</sub>: C, 69.47; H, 5.02; N, 5.94; Found: C, 69.41; H, 4.99; N, 5.78.

Ethyl 7-(benzo[d][1,3]dioxol-5-ylmethylcarbamoyl)-3-benzoylindolizine-1-carboxylate (**3i**)

Off white solid; Yield: 99.3%; Mp: 181–183 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.91 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.72 (s, 1H, indolizine-H-2), 7.83 (s, 1H, indolizine-H-8), 7.82–7.79 (m, 2H, ArH), 7.61 (t,  $J = 7.3$  Hz, 1H, ArH), 7.53 (t,  $J = 7.4$  Hz, 3H, ArH), 6.89–6.80 (m, 3H, ArH + indolizine-H-6), 6.77 (d,  $J = 7.9$  Hz, 1H, ArH), 5.95 (s, 2H, ArO-CH<sub>2</sub>), 4.58 (d,  $J = 5.3$  Hz, 2H, Ar-CH<sub>2</sub>), 4.35 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.38 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 186.0, 165.1, 164.1, 148.3, 147.5, 139.6, 138.7, 132.8, 132.0, 131.7, 129.2, 129.1, 128.7, 123.5, 121.6, 117.6, 113.8, 108.8, 108.6, 108.3, 101.4, 60.6, 44.5, 14.7; ESI-MS  $m/z$ : 470.9 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>27</sub>H<sub>22</sub>N<sub>2</sub>O<sub>6</sub>: C, 68.75; H, 4.68; N, 5.94; Found: C, 68.93; H, 4.71; N, 5.95.

Ethyl 3-benzoyl-7-(naphthalen-2-ylmethylcarbamoyl)indolizine-1-carboxylate (**3j**)

White solid; Yield: 84.0%; Mp: 156–158 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.88 (d,  $J = 7.2$  Hz, 1H, indolizine-H-5), 8.74 (s, 1H, indolizine-H-2), 7.89–7.73 (m, 7H, ArH + indolizine-H-8), 7.65–7.56 (m, 1H, ArH), 7.56–7.41 (m, 6H, ArH + indolizine-H-6), 7.09–6.98 (m, 1H, NH), 4.83 (d,  $J = 5.1$  Hz, 2H, Ar-CH<sub>2</sub>), 4.32 (q,  $J = 6.9$  Hz, 2H, CH<sub>2</sub>), 1.35 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 186.0, 165.2, 164.0, 139.6, 138.7, 135.3, 133.6, 133.1, 132.8, 132.0, 129.2, 129.2, 128.9, 128.7, 127.9, 127.9, 127.0, 126.6, 126.3, 126.2, 123.5, 117.8, 113.8, 108.3, 60.6, 44.8, 14.7; ESI-MS

$m/z$ : 477.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>30</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>: C, 75.61; H, 5.08; N, 5.88; Found: C, 75.53; H, 5.19; N, 5.01.

Ethyl 3-benzoyl-7-(2,3-dihydro-1H-inden-2-ylcarbamoyl)indolizine-1-carboxylate (**3k**)

White solid; Yield: 66.4%; Mp: 217–219 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.91 (d,  $J = 7.4$  Hz, 1H, indolizine-H-5), 8.66 (s, 1H, indolizine-H-2), 7.83 (s, 1H, indolizine-H-8), 7.82–7.79 (m, 2H, ArH), 7.60 (t,  $J = 7.2$  Hz, 1H, ArH), 7.56–7.49 (m, 3H, ArH), 7.31–7.24 (m, 1H, ArH), 7.24–7.16 (m, 2H, 2H), 6.73 (d,  $J = 7.2$  Hz, 1H, ArH), 5.05–4.90 (m, 1H, CH), 4.35 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 3.48 (d,  $J = 7.2$  Hz, 1H, Ar-CH<sub>2</sub>), 3.44 (d,  $J = 7.2$  Hz, 1H, Ar-CH<sub>2</sub>), 3.02 (d,  $J = 3.8$  Hz, 1H, Ar-CH<sub>2</sub>), 2.98 (d,  $J = 4.1$  Hz, 1H, Ar-CH<sub>2</sub>), 1.36 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 186.0, 165.1, 164.1, 140.9, 139.6, 138.7, 133.0, 132.1, 129.2, 128.7, 127.2, 125.1, 123.5, 117.6, 113.9, 108.3, 60.6, 51.7, 40.3, 14.7; ESI-MS  $m/z$ : 453.0 [M+H]<sup>+</sup>; HRMS (ESI-TOF<sup>+</sup>)  $m/z$  Calcd. for C<sub>28</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>[M+H]<sup>+</sup>: 453.1814; Found 453.1813.

Ethyl 3-benzoyl-7-(phenethylcarbamoyl)indolizine-1-carboxylate (**3l**)

White solid; Yield: 34.8%; Mp: 198–200 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.89 (d,  $J = 7.2$  Hz, 1H, indolizine-H-5), 8.65 (s, 1H, indolizine-H-2), 7.8 (s, 1H, indolizine-H-8), 7.72 (d,  $J = 7.2$  Hz, 2H, ArH), 7.48 (d,  $J = 7.3$  Hz, 1H, ArH), 7.44 (t,  $J = 7.3$  Hz, 1H, ArH), 7.34–7.32 (m, 2H, ArH), 7.30–7.25 (m, 3H, ArH), 6.46–6.35 (m, 1H, NH), 4.37 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 3.76 (q,  $J = 6.3$  Hz, 2H, ArCH<sub>2</sub>-CH<sub>2</sub>), 2.98 (t,  $J = 6.8$  Hz, 2H, Ar-CH<sub>2</sub>), 1.38 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 186.0, 165.3, 164.0, 139.6, 138.8, 138.7, 133.1, 132.0, 129.2, 129.2, 129.0, 128.7, 126.9, 123.4, 117.6, 113.6, 108.3, 60.6, 41.7, 35.9, 14.7; ESI-MS  $m/z$ : 441.1 [M+H]<sup>+</sup>, 881.2 [2M+H]<sup>+</sup>.

*(S)*-Ethyl 3-benzoyl-7-(1-methoxy-1-oxo-3-phenylpropan-2-ylcarbamoyl)indolizine-1-carboxylate (**3m**)

Yellowish green solid; Yield: 34.8%; Mp: 113–115 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.88 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.70 (s, 1H, indolizine-H-2), 7.84 (s, 1H, indolizine-H-8), 7.84–7.81 (m, 2H, ArH), 7.61 (t,  $J = 7.2$  Hz, 1H, ArH), 7.54 (t,  $J = 7.3$  Hz, 2H, ArH), 7.39 (d,  $J = 7.2$  Hz, 1H, ArH), 7.37–7.31 (m, 2H, ArH), 7.29 (d,  $J = 5.6$  Hz, 1H, ArH), 7.22 (d,  $J = 7.3$  Hz, 2H, ArH), 7.06 (d,  $J = 7.5$  Hz, 1H, NH), 5.14 (q,  $J = 6.3$  Hz, 1H, ArCH<sub>2</sub>-CH), 4.38 (q,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>), 3.81 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.41–3.22 (m, 2H, Ar-CH<sub>2</sub>), 1.39 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 185.9, 172.0, 164.8, 163.7, 139.5, 138.4, 135.9, 132.1, 132.0, 129.9, 129.4, 129.2, 129.1, 128.9, 128.6,

127.5, 123.5, 118.2, 113.3, 108.6, 60.6, 54.0, 52.7, 38.1, 14.6; ESI-MS  $m/z$ : 499.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>29</sub>H<sub>26</sub>N<sub>2</sub>O<sub>6</sub>: C, 69.87; H, 5.26; N, 5.62; Found: C, 68.90; H, 5.14; N, 5.46.

(*S*)-Ethyl 7-(3-(1*H*-imidazol-5-yl)-1-methoxy-1-oxopropan-2-ylcarbamoyl)-3-benzoylindolizine-1-carboxylate (**3n**)

Yellow solid; Yield: 57.4%; Mp: 161–163 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.91 (d,  $J = 7.2$  Hz, 1H, indolizine-H-5), 9.00 (d,  $J = 5.7$  Hz, 1H, NH), 8.95 (s, 1H, indolizine-H-2), 7.82 (s, 2H, ArH), 7.80 (s, 1H, indolizine-H-8), 7.72 (s, 1H, ArH), 7.63–7.57 (m, 1H, ArH), 7.57–7.48 (m, 3H, ArH), 6.88 (s, 1H, ArH), 4.99 (q,  $J = 5.3$  Hz, 1H, ArCH<sub>2</sub>-CH), 4.38 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 3.71 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.25 (qd,  $J = 14.9, 4.7$  Hz, 2H, Ar-CH<sub>2</sub>), 1.39 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 186.0, 171.9, 165.0, 163.9, 139.7, 138.8, 135.7, 132.3, 132.0, 129.4, 129.2, 129.0, 128.7, 123.4, 118.8, 114.9, 113.5, 108.7, 60.6, 53.5, 52.6, 29.0, 14.7; ESI-MS  $m/z$ : 489.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>26</sub>H<sub>24</sub>N<sub>4</sub>O<sub>6</sub>: C, 63.93; H, 4.95; N, 11.47; Found: C, 63.77; H, 5.140; N, 11.65.

(*S*)-Ethyl 7-(3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-ylcarbamoyl)-3-benzoylindolizine-1-carboxylate (**3o**)

Light yellow solid; Yield: 74.6%; Mp: 191–193 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.85 (d,  $J = 7.2$  Hz, 1H, indolizine-H-5), 8.71 (s, 1H, indolizine-H-2), 8.34 (br, 1H, NH), 7.86–7.81 (m, 2H, ArH), 7.81 (s, 1H, indolizine-H-8), 7.64–7.49 (m, 4H, ArH), 7.38–7.33 (m, 2H, ArH), 7.16 (d,  $J = 7.4$  Hz, 1H, ArH), 7.13–7.07 (m, 2H, ArH), 6.94 (m, 1H, ArH), 5.16 (q,  $J = 7.0$  Hz, 1H, ArCH<sub>2</sub>-CH), 4.36 (d,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 3.75 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.49 (t,  $J = 4.3$  Hz, 2H, Ar-CH<sub>2</sub>), 1.36 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 186.0, 172.3, 164.9, 163.9, 139.6, 138.5, 136.4, 132.3, 132.1, 129.3, 129.2, 129.1, 128.7, 127.7, 123.5, 123.2, 122.6, 120.1, 118.7, 118.5, 113.3, 111.6, 110.1, 108.6, 60.6, 53.7, 52.8, 27.9, 14.6; ESI-MS  $m/z$ : 538.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>31</sub>H<sub>27</sub>N<sub>3</sub>O<sub>6</sub>: C, 69.02; H, 4.99; N, 7.62; Found: C, 69.26; H, 5.06; N, 7.82.

Ethyl 3-(2-fluorobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (**4a**)

Yellow solid; Yield: 97.0%; Mp: 178–181 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.03 (d,  $J = 7.2$  Hz, 1H, indolizine-H-5), 8.86 (s, 1H, indolizine-H-2), 8.59 (d,  $J = 4.3$  Hz, 1H, ArH), 8.03–7.95 (br, 1H, NH), 7.76–7.69 (m, 2H, ArH + indolizine-H-8), 7.63 (d,  $J = 7.3$  Hz, 1H, indolizine-H-6), 7.61–7.49 (m, 2H, ArH), 7.37 (d,  $J = 7.8$  Hz, 1H, ArH), 7.33–7.18 (m, 4H, ArH), 4.82 (d,  $J = 4.0$  Hz, 2H, Ar-CH<sub>2</sub>), 4.39 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.42 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>);

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 182.0, 165.0, 163.8, 159.7 (d,  $^1J_{C-F} = 251.3$  Hz), 155.7, 149.2, 139.0, 137.3, 133.2, 132.7 (d,  $^3J_{C-F} = 8.4$  Hz), 132.7, 130.3, 129.8, 129.9, 128.2 (d,  $^2J_{C-F} = 15.8$  Hz), 124.5, 124.0, 122.9, 122.5, 118.3, 116.7 (d,  $^2J_{C-F} = 21.6$  Hz), 114.1, 109.1, 60.7, 45.0, 14.7; ESI-MS  $m/z$ : 446.2 [M+H]<sup>+</sup>.

Ethyl 3-(2-chlorobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (**4b**)

White solid; Yield: 52.0%; Mp: 169–171 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.06 (d,  $J = 7.4$  Hz, 1H, indolizine-H-5), 8.87 (s, 1H, indolizine-H-2), 8.59 (d,  $J = 4.6$  Hz, 1H, ArH), 8.00 (br, 1H, NH), 7.73 (t,  $J = 7.7$  Hz, 1H, ArH), 7.64 (d,  $J = 7.2$  Hz, 1H, ArH), 7.56 (s, 1H, indolizine-H-8), 7.55–7.43 (m, 4H, ArH), 7.43–7.34 (m, 2H, ArH), 7.26 (d,  $J = 6.9$  Hz, 1H, ArH), 4.82 (d,  $J = 4.0$  Hz, 2H, Ar-CH<sub>2</sub>), 4.39 (q,  $J = 7.4$  Hz, 2H, CH<sub>2</sub>), 1.42 (t,  $J = 7.2$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 184.2, 165.0, 163.8, 155.7, 149.2, 139.1, 139.0, 137.3, 133.4, 131.6, 131.3, 130.5, 130.0, 129.4, 129.3, 126.9, 123.6, 122.9, 122.5, 118.3, 114.2, 109.2, 100.2, 60.7, 45.0, 14.7; ESI-MS  $m/z$ : 462.0 [M+H]<sup>+</sup>; HRMS (ESI-TOF<sup>+</sup>)  $m/z$  Calcd. for C<sub>25</sub>H<sub>20</sub>ClN<sub>3</sub>O<sub>4</sub>[M+H]<sup>+</sup>: 462.1221; Found 462.1210.

Ethyl 3-(2-bromobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (**4c**)

Yellow solid; Yield: 87.1%; Mp: 156–158 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.08 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.89 (s, 1H, indolizine-H-2), 8.62 (d,  $J = 3.4$  Hz, 1H, ArH), 8.04 (br, 1H, NH), 7.79 (t,  $J = 7.2$  Hz, 1H, ArH), 7.71 (d,  $J = 7.8$  Hz, 1H, ArH), 7.67 (d,  $J = 6.1$  Hz, 1H, ArH), 7.56 (s, 1H, indolizine-H-8), 7.50–7.36 (m, 4H, ArH), 7.35–7.29 (m, 1H, ArH), 4.85 (d,  $J = 4.3$  Hz, 2H, Ar-CH<sub>2</sub>), 4.41 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.44 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 185.0, 165.0, 163.8, 155.7, 149.3, 141.0, 139.2, 137.2, 133.6, 133.4, 131.4, 130.1, 129.4, 129.2, 127.4, 122.8, 122.4, 120.1, 118.3, 114.2, 109.2, 60.7, 45.0, 14.7; ESI-MS  $m/z$ : 505.9 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>25</sub>H<sub>20</sub>BrN<sub>3</sub>O<sub>4</sub>: C, 59.20; H, 4.00; N, 8.19; Found: C, 59.30; H, 3.98; N, 8.30.

Ethyl 3-(2-trifluorobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (**4d**)

White solid; Yield: 48.5%; Mp: 143–145 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.04 (d,  $J = 7.2$  Hz, 1H, indolizine-H-5), 8.88 (s, 1H, indolizine-H-2), 8.60 (d,  $J = 4.5$  Hz, 1H, ArH), 8.07 (br, 1H, NH), 7.83 (d,  $J = 6.8$  Hz, 1H, ArH), 7.76 (t,  $J = 7.7$  Hz, 1H, ArH), 7.72–7.61 (m, 3H, ArH), 7.56 (d,  $J = 7.4$  Hz, 1H, ArH), 7.50 (s, 1H, indolizine-H-8), 7.40 (d,  $J = 7.8$  Hz, 1H, ArH), 7.29 (t,  $J = 6.0$  Hz, 1H, ArH),



4.84 (d,  $J = 4.4$  Hz, 2H), 4.39 (q,  $J = 7.1$  Hz, 2H), 1.42 (t,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  184.6, 165.0, 163.7, 155.5, 148.9, 139.1, 138.1, 137.5, 133.4, 131.7, 130.2, 129.9, 129.4, 128.9, 128.2, 127.1, 123.5, 122.9, 122.6, 118.4, 114.2, 109.1, 60.7, 44.8, 14.6; ESI-MS  $m/z$ : 495.9  $[\text{M}+\text{H}]^+$ ; Anal. Calcd. for  $\text{C}_{26}\text{H}_{20}\text{F}_3\text{N}_3\text{O}_4$ : C, 63.01; H, 4.16; N, 8.42; Found: C, 63.03; H, 4.07; N, 8.48.

*Ethyl 3-(2-nitrobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4e)*

Yellow solid; Yield: 76.0%; Mp: 162–164 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.98 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.85 (s, 1H, indolizine-H-2), 8.59 (d,  $J = 4.1$  Hz, 1H, ArH), 8.25 (d,  $J = 8.2$  Hz, 1H, ArH), 8.02 (br, 1H, NH), 7.80 (t,  $J = 7.3$  Hz, 1H, ArH), 7.72 (q,  $J = 6.7$  Hz, 2H, ArH), 7.65 (d,  $J = 7.3$  Hz, 1H, indolizine-H-6), 7.60 (d,  $J = 7.4$  Hz, 1H, indolizine-H-6), 7.44 (s, 1H, indolizine-H-8), 7.36 (d,  $J = 7.7$  Hz, 1H, ArH), 7.29–7.23 (m, 1H, ArH), 4.82 (d,  $J = 4.1$  Hz, 2H, Ar- $\text{CH}_2$ ), 4.37 (q,  $J = 7.1$  Hz, 2H,  $\text{CH}_2$ ), 1.40 (t,  $J = 7.1$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  182.5, 164.9, 163.6, 155.7, 149.2, 146.9, 139.1, 137.2, 135.9, 134.1, 133.4, 130.9, 129.5, 129.3, 128.4, 125.1, 123.1, 122.8, 122.4, 118.4, 114.2, 109.2, 60.7, 45.0, 14.6; ESI-MS  $m/z$ : 473.0  $[\text{M}+\text{H}]^+$ ; Anal. Calcd. for  $\text{C}_{25}\text{H}_{20}\text{N}_4\text{O}_6$ : C, 63.38; H, 4.31; N, 11.75; Found: C, 63.56; H, 4.27; N, 11.86.

*Ethyl 3-(2-aminoobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4f)*

Brown solid; Yield: 68.2%; Mp: 73–75 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.68 (d,  $J = 7.2$  Hz, 1H, indolizine-H-5), 8.81 (s, 1H, indolizine-H-2), 8.62–8.55 (m, 1H, ArH), 8.01–8.00 (br, 1H, NH), 7.75 (s, 1H, indolizine-H-8), 7.70 (t,  $J = 7.5$  Hz, 1H, ArH), 7.62 (d,  $J = 7.7$  Hz, 1H, ArH), 7.51 (d,  $J = 7.2$  Hz, 1H, indolizine-H-6), 7.35 (d,  $J = 7.8$  Hz, 1H, ArH), 7.32–7.26 (m, 1H, ArH), 7.26–7.20 (m, 1H, ArH), 6.78–6.69 (m, 2H, ArH), 5.76–5.30 (br, 2H,  $\text{NH}_2$ ), 4.80 (d,  $J = 3.9$  Hz, 2H, Ar- $\text{CH}_2$ ), 4.38 (q,  $J = 6.9$  Hz, 2H,  $\text{CH}_2$ ), 1.42 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  186.9, 165.3, 164.1, 155.9, 149.4, 149.2, 138.3, 137.2, 133.4, 132.4, 132.1, 128.8, 128.6, 124.2, 122.8, 122.4, 120.9, 118.1, 117.1, 116.6, 113.2, 108.1, 60.5, 45.0, 14.7; ESI-MS  $m/z$ : 443.0  $[\text{M}+\text{H}]^+$ .

*Ethyl 3-(3-fluorobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4g)*

Off white solid; Yield: 73.3%; Mp: 169–171 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.94 (d,  $J = 6.5$  Hz, 1H, indolizine-H-5), 8.86 (s, 1H, indolizine-H-2), 8.60 (d,  $J = 4.0$  Hz, 1H, ArH), 8.04–7.94 (br, 1H, NH), 7.86 (s, 1H, indolizine-H-8), 7.72 (t,  $J = 7.5$  Hz, 1H, ArH), 7.61 (d,  $J = 6.3$  Hz, 2H,

ArH+ indolizine-H-6), 7.53 (d,  $J = 8.3$  Hz, 2H, ArH), 7.36 (d,  $J = 7.8$  Hz, 1H, ArH), 7.34–7.23 (m, 2H, ArH), 4.81 (d,  $J = 3.9$  Hz, 2H, Ar- $\text{CH}_2$ ), 4.41 (q,  $J = 6.8$  Hz, 2H,  $\text{CH}_2$ ), 1.44 (t,  $J = 7.0$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  184.2, 165.1, 164.2 (d,  $^1J_{\text{C}-\text{F}} = 274.0$  Hz), 162.8, 155.7, 149.3, 141.7, 138.9, 137.1, 133.1, 130.4 (d,  $^3J_{\text{C}-\text{F}} = 7.7$  Hz), 129.4, 129.2, 125.0 (d,  $^3J_{\text{C}-\text{F}} = 3.0$  Hz), 123.1, 122.8, 122.4, 119.0 (d,  $^2J_{\text{C}-\text{F}} = 21.3$  Hz), 118.2, 116.1 (d,  $^2J_{\text{C}-\text{F}} = 22.7$  Hz), 113.9, 108.8, 60.7, 45.0, 14.7; ESI-MS  $m/z$ : 446.1  $[\text{M}+\text{H}]^+$ ; Anal. Calcd. for  $\text{C}_{25}\text{H}_{20}\text{FN}_3\text{O}_4$ : C, 67.24; H, 4.52; N, 9.36; Found: C, 67.41; H, 4.53; N, 9.43.

*Ethyl 3-(3-chlorobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4h)*

Light yellow solid; Yield: 86.2%; Mp: 157–160 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.94 (d,  $J = 7.2$  Hz, 1H, indolizine-H-5), 8.87 (s, 1H, indolizine-H-2), 8.60 (d,  $J = 4.1$  Hz, 1H, ArH), 8.00 (br, 1H, NH), 7.84 (s, 1H, indolizine-H-8), 7.80 (s, 1H, ArH), 7.76–7.67 (m, 2H, ArH), 7.61 (d,  $J = 7.2$  Hz, 1H, indolizine-H-6), 7.57 (d,  $J = 7.9$  Hz, 1H, ArH), 7.47 (t,  $J = 7.7$  Hz, 1H, ArH), 7.36 (d,  $J = 7.8$  Hz, 1H, ArH), 7.29–7.23 (m, 1H, ArH), 4.81 (d,  $J = 4.1$  Hz, 2H, Ar- $\text{CH}_2$ ), 4.41 (q,  $J = 7.1$  Hz, 2H,  $\text{CH}_2$ ), 1.44 (t,  $J = 7.1$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  184.2, 165.0, 163.9, 155.7, 149.3, 141.3, 138.9, 137.2, 134.9, 133.2, 131.9, 130.0, 129.4, 129.2, 129.1, 127.3, 123.1, 122.8, 122.5, 118.2, 114.0, 108.8, 60.7, 45.0, 14.7; ESI-MS  $m/z$ : 461.9  $[\text{M}+\text{H}]^+$ ; Anal. Calcd. for  $\text{C}_{25}\text{H}_{20}\text{ClN}_3\text{O}_4$ : C, 65.01; H, 4.36; N, 9.10; Found: C, 65.14; H, 4.44; N, 9.08.

*Ethyl 3-(3-bromobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4i)*

Yellow solid; Yield: 57.3%; Mp: 124–127 °C;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.90 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.85 (s, 1H, indolizine-H-2), 8.59 (d,  $J = 4.6$  Hz, 1H, ArH), 8.07 (t,  $J = 4.2$  Hz, 1H, NH), 7.94 (s, 1H, indolizine-H-8), 7.82 (s, 1H, ArH), 7.72 (t,  $J = 5.8$  Hz, 3H, ArH), 7.59 (dd,  $J = 7.3, 1.8$  Hz, 1H, indolizine-H-6), 7.40 (t,  $J = 7.8$  Hz, 1H, ArH), 7.36 (d,  $J = 7.8$  Hz, 1H, ArH), 7.27–7.22 (m, 1H, ArH), 4.81 (d,  $J = 4.6$  Hz, 2H, Ar- $\text{CH}_2$ ), 4.40 (q,  $J = 7.2$  Hz, 2H,  $\text{CH}_2$ ), 1.43 (t,  $J = 7.2$  Hz, 3H,  $\text{CH}_3$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  184.0, 165.0, 163.7, 155.8, 149.2, 141.5, 138.9, 137.1, 134.8, 133.1, 131.9, 130.2, 129.3, 129.1, 127.7, 123.0, 122.9, 122.8, 122.4, 118.2, 113.9, 108.8, 60.7, 45.0, 14.6; ESI-MS  $m/z$ : 505.9, 507.9  $[\text{M}+\text{H}]^+$ ; Anal. Calcd. for  $\text{C}_{25}\text{H}_{20}\text{BrN}_3\text{O}_4$ : C, 59.23; H, 3.97; N, 8.35; Found: C, 59.30; H, 3.98; N, 8.30.

*Ethyl 3-(3-trifluorobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4j)*

Light yellow solid; Yield: 85.9%; Mp: 184–186 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.97 (d, *J* = 7.3 Hz, 1H, indolizine-H-5), 8.88 (s, 1H, indolizine-H-2), 8.60 (d, *J* = 4.3 Hz, 1H, ArH), 8.09 (br, 1H, NH), 8.01 (d, *J* = 7.8 Hz, 1H, ArH), 7.97 (s, 1H, indolizine-H-8), 7.86 (d, *J* = 7.7 Hz, 1H, ArH), 7.81 (s, 1H, ArH), 7.73 (t, *J* = 7.9 Hz, 1H, ArH), 7.68 (t, *J* = 7.9 Hz, 1H, ArH), 7.63 (d, *J* = 7.3 Hz, 1H, indolizine-H-6), 7.36 (d, *J* = 7.9 Hz, 1H, ArH), 7.27–7.24 (m, 1H, ArH), 4.82 (d, *J* = 4.0 Hz, 2H, Ar-CH<sub>2</sub>), 4.42 (q, *J* = 7.2 Hz, 2H, CH<sub>2</sub>), 1.44 (t, *J* = 7.1 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 184.2, 165.0, 163.8, 155.7, 149.3, 140.4, 139.1, 137.2, 133.3, 132.3, 129.4, 129.3, 128.5 (q, <sup>3</sup>*J*<sub>C-F</sub> = 3.6 Hz), 126.0 (q, <sup>3</sup>*J*<sub>C-F</sub> = 3.9 Hz), 123.0, 122.8, 122.5, 118.2, 114.1, 109.0, 60.7, 45.0, 14.6; ESI-MS *m/z*: 496.1 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>26</sub>H<sub>20</sub>F<sub>3</sub>N<sub>3</sub>O<sub>4</sub>: C, 63.94; H, 4.17; N, 8.50; Found: C, 63.03; H, 4.07; N, 8.48.

*Ethyl 3-(3-nitrobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4k)*

Light yellow solid; Yield: 63.6%; Mp: 172–174 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.95 (d, *J* = 6.7 Hz, 1H, indolizine-H-5), 8.88 (s, 1H, indolizine-H-2), 8.66 (s, 1H, ArH), 8.60 (d, *J* = 4.4 Hz, 1H, ArH), 8.45 (d, *J* = 8.2 Hz, 1H, ArH), 8.15 (d, *J* = 7.5 Hz, 1H, ArH), 8.08 (s, 1H, NH), 7.80 (s, 1H, indolizine-H-8), 7.74 (t, *J* = 8.0 Hz, 2H, ArH), 7.65 (d, *J* = 7.0 Hz, 1H, indolizine-H-6), 7.38 (d, *J* = 7.8 Hz, 1H, ArH), 7.30–7.26 (m, 1H, ArH), 4.82 (d, *J* = 4.3 Hz, 2H, Ar-CH<sub>2</sub>), 4.41 (q, *J* = 6.7 Hz, 2H, CH<sub>2</sub>), 1.43 (t, *J* = 7.0 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 182.9, 164.9, 163.6, 155.7, 149.2, 148.4, 141.2, 139.2, 137.3, 134.7, 133.6, 129.9, 129.4, 129.3, 126.3, 124.0, 122.9, 122.6, 122.5, 118.3, 114.3, 109.3, 60.8, 45.0, 14.7; ESI-MS *m/z*: 473.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>25</sub>H<sub>20</sub>N<sub>4</sub>O<sub>6</sub>: C, 63.36; H, 4.33; N, 11.75; Found: C, 63.56; H, 4.27; N, 11.86.

*Ethyl 3-(3-aminoobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4l)*

Light yellow solid; Yield: 80.3%; Mp: 168–171 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.88 (d, *J* = 7.2 Hz, 1H, indolizine-H-5), 8.82 (s, 1H, indolizine-H-2), 8.58 (d, *J* = 3.7 Hz, 1H, ArH), 8.08 (s, 1H, NH), 7.87 (s, 1H, indolizine-H-8), 7.71 (t, *J* = 7.6 Hz, 1H, ArH), 7.54 (d, *J* = 7.3 Hz, 1H, NH), 7.36 (d, *J* = 7.8 Hz, 1H, ArH), 7.32–7.20 (m, 2H, ArH), 7.16 (d, *J* = 7.5 Hz, 1H, ArH), 7.11 (s, 1H, ArH), 6.88 (d, *J* = 7.8 Hz, 1H, ArH), 4.80 (d, *J* = 3.7 Hz, 2H, Ar-CH<sub>2</sub>), 4.38 (q, *J* = 7.0 Hz, 2H), 3.68 (s, 2H, NH<sub>2</sub>), 1.41 (t, *J* = 7.1 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 186.1, 165.2,

164.0, 156.0, 149.2, 146.9, 140.7, 138.5, 137.1, 132.6, 129.5, 129.3, 129.1, 123.5, 122.7, 122.4, 119.5, 118.5, 118.1, 115.2, 113.5, 108.3, 60.6, 45.1, 14.7; ESI-MS *m/z*: 443.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>25</sub>H<sub>22</sub>N<sub>4</sub>O<sub>4</sub>: C, 67.82; H, 4.91; N, 12.57; Found: C, 67.86; H, 5.01; N, 12.66.

*Ethyl 3-(4-fluorobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4m)*

White solid; Yield: 67.4%; Mp: 179–181 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.88 (d, *J* = 7.2 Hz, 1H, indolizine-H-5), 8.84 (s, 1H, indolizine-H-2), 8.58 (d, *J* = 3.5 Hz, 1H, ArH), 8.11 (s, 1H, NH), 7.90–7.82 (m, 2H, ArH), 7.81 (s, 1H, indolizine-H-8), 7.72 (t, *J* = 7.6 Hz, 1H, ArH), 7.57 (d, *J* = 7.3 Hz, 1H, indolizine-H-6), 7.36 (d, *J* = 7.8 Hz, 1H, ArH), 7.27–7.15 (m, 3H, ArH), 4.81 (d, *J* = 3.3 Hz, 2H, Ar-CH<sub>2</sub>), 4.39 (q, *J* = 7.0 Hz, 2H, CH<sub>2</sub>), 1.42 (t, *J* = 7.1 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 184.4, 165.1 (d, <sup>1</sup>*J*<sub>C-F</sub> = 251.0 Hz), 165.0, 163.8, 155.9, 149.2, 138.6, 137.1, 135.8 (d, <sup>3</sup>*J*<sub>C-F</sub> = 2.9 Hz), 132.8, 131.7, 131.6, 129.0, 128.7, 123.2, 122.7, 122.4, 118.2, 115.9, 115.7 (d, <sup>2</sup>*J*<sub>C-F</sub> = 22.0 Hz), 113.7, 108.6, 60.6, 45.0, 14.6; ESI-MS *m/z*: 446.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>25</sub>H<sub>20</sub>FN<sub>3</sub>O<sub>4</sub>: C, 67.41; H, 4.53; N, 9.43; Found: C, 67.27; H, 4.61; N, 9.27.

*Ethyl 3-(4-chlorobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4n)*

Yellow solid; Yield: 97.6%; Mp: 180–182 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.96 (d, *J* = 7.4 Hz, 1H, indolizine-H-5), 8.89 (s, 1H, indolizine-H-2), 8.62 (d, *J* = 4.5 Hz, 1H, ArH), 7.98–7.89 (m, 1H, NH), 7.85 (s, 1H, indolizine-H-8), 7.84–7.78 (m, 2H, ArH), 7.74 (td, *J* = 7.7, 1.7 Hz, 1H, ArH), 7.62 (dd, *J* = 7.3, 1.9 Hz, 1H, indolizine-H-6), 7.57–7.51 (m, 2H, ArH), 7.37 (d, *J* = 7.9 Hz, 1H, ArH), 7.30–7.25 (m, 1H, ArH), 4.83 (d, *J* = 4.5 Hz, 2H, Ar-CH<sub>2</sub>), 4.44 (q, *J* = 7.2 Hz, 2H, CH<sub>2</sub>), 1.46 (t, *J* = 7.1 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 184.6, 165.1, 163.9, 155.7, 149.3, 138.9, 138.4, 138.1, 137.1, 133.1, 130.6, 129.2, 129.0, 123.2, 122.8, 122.4, 118.2, 113.9, 108.8, 60.7, 45.0, 14.7; ESI-MS *m/z*: 462.1 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>25</sub>H<sub>20</sub>ClN<sub>3</sub>O<sub>4</sub>: C, 64.93; H, 4.37; N, 9.20; Found: C, 65.01; H, 4.36; N, 9.10.

*Ethyl 3-(4-bromobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4o)*

Off white solid; Yield: 91.1%; Mp: 182–184 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.91 (d, *J* = 7.3 Hz, 1H, indolizine-H-5), 8.85 (s, 1H, indolizine-H-2), 8.59 (d, *J* = 3.9 Hz, 1H, ArH), 8.03 (s, 1H, NH), 7.81 (s, 1H, indolizine-H-8), 7.75–7.63 (m, 5H, ArH), 7.59 (d, *J* = 7.2 Hz, 1H, indolizine-H-6), 7.37 (d, *J* = 7.7 Hz, 1H, ArH), 7.30–7.22 (m, 1H, ArH), 4.81 (d, *J* = 3.7 Hz, 2H, Ar-CH<sub>2</sub>), 4.40 (q, *J* = 6.7 Hz, 2H, CH<sub>2</sub>),

1.43 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 184.6, 165.0, 163.8, 155.8, 149.2, 138.8, 138.4, 137.2, 133.0, 132.0, 130.7, 129.2, 129.1, 126.8, 123.1, 122.8, 122.5, 118.2, 113.9, 108.7, 60.7, 45.0, 14.7; ESI-MS  $m/z$ : 505.9 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>25</sub>H<sub>20</sub>BrN<sub>3</sub>O<sub>4</sub>: C, 59.30; H, 3.98; N, 8.30; Found: C, 59.25; H, 4.10; N, 8.11.

*Ethyl 3-(4-trifluorobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4p)*

Off white solid; Yield: 42.4%; Mp: 197–199 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.98 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.88 (s, 1H, indolizine-H-2), 8.60 (d,  $J = 4.2$  Hz, 1H, ArH), 8.01 (s, 1H, NH), 7.93 (d,  $J = 7.8$  Hz, 2H, ArH), 7.84–7.78 (d,  $J = 6.9$  Hz, 3H, ArH), 7.74 (t,  $J = 7.7$  Hz, 1H, ArH), 7.63 (d,  $J = 7.3$  Hz, 1H, indolizine-H-6), 7.37 (d,  $J = 7.7$  Hz, 1H, ArH), 7.31–7.23 (m, 1H, ArH), 4.83 (d,  $J = 7.7$  Hz, 2H, Ar-CH<sub>2</sub>), 4.41 (q,  $J = 7.2$  Hz, 2H, CH<sub>2</sub>), 1.44 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 184.5, 165.0, 163.8, 155.6, 149.2, 142.8, 139.1, 137.3, 133.3, 129.5, 129.4, 129.3 (q, <sup>2</sup> $J_{C-F} = 26$  Hz), 125.8 (q, <sup>3</sup> $J_{C-F} = 3.5$  Hz), 123.0, 122.9, 122.5, 118.2, 114.2, 109.0, 60.8, 45.0, 14.7; ESI-MS  $m/z$ : 496.1 [M+H]<sup>+</sup>.

*Ethyl 3-(4-nitrobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4q)*

Yellow solid; Yield: 76.2%; Mp: 208–210 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.99 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.89 (s, 1H, indolizine-H-2), 8.60 (d,  $J = 4.3$  Hz, 1H, ArH), 8.40 (d,  $J = 8.0$  Hz, 2H, ArH), 8.01 (s, 1H, NH), 7.98 (d,  $J = 8.2$  Hz, 2H, ArH), 7.79 (s, 1H, indolizine-H-8), 7.75 (t,  $J = 7.7$  Hz, 1H, ArH), 7.67 (d,  $J = 7.3$  Hz, 1H, indolizine-H-6), 7.38 (d,  $J = 7.8$  Hz, 1H, ArH), 7.31–7.24 (m, 1H, ArH), 4.83 (d,  $J = 4.1$  Hz, 2H, Ar-CH<sub>2</sub>), 4.42 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.44 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 183.5, 164.8, 163.6, 155.5, 149.7, 149.2, 145.1, 139.3, 137.3, 133.7, 130.0, 129.6, 129.3, 124.0, 122.9, 122.8, 122.5, 118.3, 114.4, 109.3, 60.9, 44.9, 14.7; ESI-MS  $m/z$ : 473.2 [M+H]<sup>+</sup>.

*Ethyl 3-(4-aminoobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4r)*

Yellow solid; Yield: 62.5%; Mp: 217–219 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.82 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.83 (s, 1H, indolizine-H-2), 8.59 (d,  $J = 4.0$  Hz, 1H, ArH), 7.92 (s, 1H, NH), 7.87 (s, 1H, indolizine-H-8), 7.79–7.68 (m, 3H, ArH), 7.51 (d,  $J = 7.3$  Hz, 1H, indolizine-H-6), 7.35 (d,  $J = 7.8$  Hz, 1H, ArH), 7.28–7.21 (m, 1H, ArH), 6.73 (d,  $J = 7.7$  Hz, 2H, ArH), 4.81 (d,  $J = 3.6$  Hz, 2H, Ar-CH<sub>2</sub>), 4.41 (q,  $J = 6.7$  Hz, 2H, CH<sub>2</sub>), 4.20 (br, 2H, NH<sub>2</sub>), 1.44 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)

δ 184.6, 165.4, 164.2, 155.9, 150.8, 149.3, 138.2, 137.1, 132.0, 131.9, 129.9, 129.0, 128.1, 123.9, 122.8, 122.4, 118.2, 114.1, 113.1, 107.9, 60.5, 45.0, 14.7; ESI-MS  $m/z$ : 443.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>25</sub>H<sub>22</sub>N<sub>4</sub>O<sub>4</sub>: C, 67.65; H, 5.06; N, 12.43; Found: C, 67.86; H, 5.01; N, 12.66.

*Ethyl 3-(2,4-dihydroxybenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4s)*

Yellow solid; Yield: 39.2%; Mp: 238–240 °C; <sup>1</sup>H NMR (400 MHz, DMSO) δ 10.97 (s, 1H, OH), 10.34 (s, 1H, OH), 9.64 (d,  $J = 7.1$  Hz, 1H, indolizine-H-5), 9.54 (t,  $J = 5.8$  Hz, 1H, NH), 8.84 (s, 1H, indolizine-H-2), 8.55 (d,  $J = 3.9$  Hz, 1H, ArH), 7.79 (t,  $J = 7.1$  Hz, 1H, indolizine-H-6), 7.67 (d,  $J = 9.3$  Hz, 2H, ArH), 7.53 (d,  $J = 8.6$  Hz, 1H, ArH), 7.39 (d,  $J = 7.8$  Hz, 1H, ArH), 7.34–7.25 (m, 1H, ArH), 6.45 (d,  $J = 8.6$  Hz, 1H, ArH), 6.41 (s, 1H, ArH), 4.64 (d,  $J = 5.6$  Hz, 2H, Ar-CH<sub>2</sub>), 4.34 (q, 6.9 Hz, 2H, CH<sub>2</sub>), 1.35 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, DMSO) δ 185.1, 164.3, 162.9, 160.8, 158.3, 148.9, 137.5, 136.7, 132.7, 132.0, 128.2, 127.0, 123.1, 122.1, 121.1, 118.1, 115.5, 113.1, 107.6, 106.9, 102.9, 59.9, 44.9, 14.3; ESI-MS  $m/z$ : 460.1 [M+H]<sup>+</sup>; HRMS (ESI-TOF<sup>+</sup>)  $m/z$  Calcd. for C<sub>25</sub>H<sub>21</sub>N<sub>3</sub>O<sub>6</sub>[M+H]<sup>+</sup>: 460.1509; Found 460.1496.

*Ethyl 3-(4-hydroxy-3-(hydroxymethyl)benzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4t)*

Light green solid; Yield: 81.3%; Mp: 202–204 °C; <sup>1</sup>H NMR (400 MHz, DMSO) δ 10.36 (br, 1H, OH), 9.76 (d,  $J = 7.4$  Hz, 1H, indolizine-H-5), 9.56 (t,  $J = 5.9$  Hz, 1H, NH), 8.86 (s, 1H, indolizine-H-2), 8.54 (d,  $J = 4.0$  Hz, 1H, ArH), 7.89 (d,  $J = 2.2$  Hz, 1H, ArH), 7.82–7.75 (m, 1H, ArH), 7.72 (s, 1H, indolizine-H-8), 7.68 (dd,  $J = 7.5$ , 1.9 Hz, 1H, indolizine-H-6), 7.67–7.63 (m, 1H, ArH), 7.39 (d,  $J = 7.8$  Hz, 1H, ArH), 7.33–7.26 (m, 1H, ArH), 6.96 (d,  $J = 8.4$  Hz, 1H, ArH), 5.17 (br, 1H, OH), 4.64 (d,  $J = 5.8$  Hz, 2H, Ar-CH<sub>2</sub>), 4.56 (s, 2H, CH<sub>2</sub>), 4.34 (q,  $J = 7.2$  Hz, 2H, CH<sub>2</sub>), 1.34 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, DMSO) δ 184.0, 164.4, 163.0, 158.3, 158.2, 148.9, 137.5, 136.7, 132.1, 129.5, 129.0, 128.6, 128.3, 126.9, 123.1, 122.2, 121.1, 118.2, 114.3, 113.2, 106.8, 59.9, 57.8, 44.9, 14.3; ESI-MS  $m/z$ : 474.2 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>26</sub>H<sub>23</sub>N<sub>3</sub>O<sub>6</sub>: C, 65.75; H, 4.91; N, 8.93; Found: C, 65.95; H, 4.90; N, 8.87.

*Ethyl 3-(3,4-dichlorobenzoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4u)*

Yellow solid; Yield: 56.5%; Mp: 192–195 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.92 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.87 (s, 1H, indolizine-H-2), 8.60 (d,  $J = 4.0$  Hz, 1H, ArH), 7.99 (s, 1H, NH), 7.92 (s, 1H, ArH), 7.83 (s, 1H, indolizine-

H-8), 7.75 (t,  $J = 7.5$  Hz, 1H, ArH), 7.69–7.60 (m, 3H, ArH + indolizine-H-6), 7.38 (d,  $J = 7.5$  Hz, 1H, ArH), 7.31–7.24 (m, 1H, ArH), 4.82 (d,  $J = 3.6$  Hz, 2H, Ar-CH<sub>2</sub>), 4.42 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.45 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 183.0, 165.0, 163.8, 155.6, 149.1, 139.3, 139.1, 137.4, 136.5, 133.4, 133.3, 131.0, 130.8, 129.2, 128.3, 122.9, 122.8, 122.6, 118.3, 114.1, 109.1, 60.8, 44.9, 14.7; ESI-MS  $m/z$ : 497.9 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>25</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>4</sub>: C, 60.32; H, 3.85; N, 8.43; Found: C, 60.50; H, 3.86; N, 8.47.

*Ethyl 3-(benzo[d][1,3]dioxole-5-carbonyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4v)*

Off white solid; Yield: 67.4 %; Mp: 174–176 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.87 (d,  $J = 7.2$  Hz, 1H, indolizine-H-5), 8.86 (s, 1H, indolizine-H-2), 8.60 (d,  $J = 4.6$  Hz, 1H, ArH), 8.01–7.91 (m, 1H, NH), 7.89 (s, 1H, indolizine-H-8), 7.76 (t,  $J = 7.8$  Hz, 1H, ArH), 7.57 (d,  $J = 7.6$  Hz, 1H, ArH), 7.45 (d,  $J = 7.7$  Hz, 1H, ArH), 7.40 (d,  $J = 7.3$  Hz, 1H, indolizine-H-6), 7.36 (s, 1H, ArH), 7.30 (d,  $J = 5.8$  Hz, 1H, ArH), 6.94 (d,  $J = 7.8$  Hz, 1H, ArH), 6.10 (s, 2H, OCH<sub>2</sub>), 4.83 (d,  $J = 3.8$  Hz, 2H, Ar-CH<sub>2</sub>), 4.43 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.45 (t,  $J = 7.0$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 184.2, 165.2, 163.9, 156.0, 151.1, 149.2, 148.1, 138.5, 137.1, 133.7, 132.5, 128.9, 128.6, 125.2, 123.4, 122.7, 122.4, 118.2, 113.4, 109.4, 108.2, 108.1, 102.0, 60.5, 45.0, 14.6; ESI-MS  $m/z$ : 472.1 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>26</sub>H<sub>21</sub>N<sub>3</sub>O<sub>6</sub>: C, 66.00; H, 4.57; N, 8.75; Found: C, 66.24; H, 4.49; N, 8.91.

*Ethyl 3-(furan-2-carbonyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4w)*

Yellow solid; Yield: 27.0 %; Mp: 164–166 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.03 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.86 (s, 1H, indolizine-H-2), 8.59 (d,  $J = 4.7$  Hz, 1H), 8.55 (s, 1H, indolizine-H-8), 7.92 (s, 1H, NH), 7.72 (t,  $J = 6.8$  Hz, 2H, ArH), 7.59–7.53 (m, 1H, ArH), 7.40 (d,  $J = 3.5$  Hz, 1H, ArH), 7.35 (d,  $J = 7.8$  Hz, 1H, ArH), 7.29–7.21 (m, 1H, ArH), 6.63 (d,  $J = 5.2$  Hz, 1H, ArH), 4.81 (d,  $J = 4.5$  Hz, 2H, Ar-CH<sub>2</sub>), 4.45 (q, 7.1 Hz, 2H, CH<sub>2</sub>), 1.48 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 171.4, 165.2, 164.1, 155.8, 153.4, 149.3, 146.3, 138.5, 137.1, 132.8, 129.3, 128.2, 122.8, 122.4, 118.3, 118.2, 113.7, 112.5, 109.1, 60.7, 45.0, 14.8; ESI-MS  $m/z$ : 418.0 [M+H]<sup>+</sup>; HRMS (ESI-TOF<sup>+</sup>)  $m/z$  Calcd. for C<sub>23</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub> [M+H]<sup>+</sup>: 418.1403; Found 418.1404

*Ethyl 7-(pyridin-2-ylmethylcarbamoyl)-3-(thiophene-2-carbonyl)indolizine-1-carboxylate (4x)*

Yellow solid; Yield: 63.6 %; Mp: 168–170 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.83 (d,  $J = 6.4$  Hz, 1H, indolizine-H-5), 8.84 (s, 1H, indolizine-H-2), 8.59 (s, 1H, indolizine-H-8), 8.18

(s, 1H, NH), 7.96–7.80 (m, 2H, ArH), 7.70 (d,  $J = 4.3$  Hz, 2H, ArH), 7.54 (d,  $J = 5.9$  Hz, 1H, ArH), 7.35 (d,  $J = 6.9$  Hz, 1H, ArH), 7.30–7.13 (m, 2H, ArH), 4.81 (s, 2H, Ar-CH<sub>2</sub>), 4.43 (q,  $J = 6.7$  Hz, 2H, CH<sub>2</sub>), 1.46 (t,  $J = 6.7$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 176.8, 165.1, 164.0, 155.8, 149.3, 144.1, 138.6, 137.1, 132.8, 132.6, 129.0, 128.1, 127.6, 123.2, 122.8, 122.4, 118.3, 113.6, 108.7, 60.7, 45.0, 14.7; ESI-MS  $m/z$ : 433.9 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>23</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S: C, 63.52; H, 4.67; N, 9.69; Found: C, 63.73; H, 4.42; N, 9.69.

*Ethyl 3-(1-naphthoyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4y)*

Yellow solid; Yield: 36.4 %; Mp: 163–165 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.19 (d,  $J = 7.3$  Hz, 1H, indolizine-H-5), 8.88 (s, 1H, indolizine-H-2), 8.60 (d,  $J = 4.6$  Hz, 1H, ArH), 8.12 (d,  $J = 8.0$  Hz, 1H, ArH), 8.02 (d,  $J = 8.2$  Hz, 1H, ArH), 8.00–7.91 (m, 2H, ArH), 7.76–7.65 (m, 3H, ArH), 7.64 (s, 1H, indolizine-H-8), 7.60–7.49 (m, 3H, ArH), 7.36 (d,  $J = 7.9$  Hz, 1H, ArH), 7.28–7.22 (m, 1H, ArH), 4.83 (d,  $J = 4.6$  Hz, 2H, Ar-CH<sub>2</sub>), 4.35 (q,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>), 1.38 (t,  $J = 7.2$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 187.2, 165.1, 163.9, 155.8, 149.3, 139.0, 137.3, 137.1, 134.0, 133.1, 131.1, 131.0, 130.1, 129.4, 128.6, 127.4, 127.0, 126.7, 125.6, 124.8, 124.7, 122.8, 122.4, 118.2, 114.0, 108.7, 60.6, 45.0, 14.6; ESI-MS  $m/z$ : 478.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>29</sub>H<sub>23</sub>N<sub>3</sub>O<sub>4</sub>: C, 72.77; H, 4.84; N, 8.92; Found: C, 72.94; H, 4.85; N, 8.80.

*Ethyl 7-(pyridin-2-ylmethylcarbamoyl)-3-(pyrrolidine-1-carbonyl)indolizine-1-carboxylate (4z-1)*

Brown solid; Yield: 48.7 %; Mp: 68–70 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.41 (d,  $J = 7.4$  Hz, 1H, indolizine-H-5), 8.73 (s, 1H, indolizine-H-2), 8.55 (d,  $J = 4.1$  Hz, 1H, ArH), 7.99 (s, 1H, NH), 7.73–7.62 (m, 2H, ArH), 7.33 (dd,  $J = 7.5, 1.9$  Hz, 2H, ArH), 7.24–7.16 (m, 1H, ArH), 4.77 (d,  $J = 4.9$  Hz, 2H, Ar-CH<sub>2</sub>), 4.38 (q,  $J = 7.1$  Hz, 2H, CH<sub>2</sub>), 3.87–3.58 (br, 4H, pyrrolidine-2CH<sub>2</sub>), 2.05–s1.90 (br, 4H, pyrrolidine-2CH<sub>2</sub>), 1.43 (t,  $J = 7.2$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 165.6, 164.3, 161.0, 156.2, 149.2, 137.0, 136.1, 130.1, 128.5, 122.6, 122.3, 121.4, 119.4, 118.2, 111.7, 106.7, 60.3, 49.6, 47.0, 45.0, 26.6, 24.1, 14.7; ESI-MS  $m/z$ : 421.2 [M+H]<sup>+</sup>.

*Ethyl 3-(morpholine-4-carbonyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4z-2)*

Brown solid; Yield: 56.9 %; Mp: 90–92 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.95 (d,  $J = 7.4$  Hz, 1H, indolizine-H-5), 8.73 (s, 1H, indolizine-H-2), 8.57 (d,  $J = 4.4$  Hz, 1H, ArH), 7.96 (s, 1H, NH), 7.70 (t,  $J = 7.7$  Hz, 1H, ArH), 7.48 (s, 1H,

indolizine-H-8), 7.42–7.32 (m, 2H, ArH), 7.27–7.19 (m, 1H, ArH), 4.78 (d,  $J = 4.8$  Hz, 2H, Ar-CH<sub>2</sub>), 4.40 (q,  $J = 7.2$  Hz, 2H, CH<sub>2</sub>), 3.89–3.82 (m, 4H, morpholine-2CH<sub>2</sub>), 3.81–3.74 (m, 4H, morpholine-2CH<sub>2</sub>), 1.45 (t,  $J = 7.1$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 165.5, 164.3, 161.8, 156.1, 149.2, 137.1, 136.4, 130.2, 127.8, 122.7, 122.4, 121.0, 118.3, 117.8, 112.1, 106.8, 67.1, 60.4, 45.7, 45.0, 14.7; ESI-MS  $m/z$ : 437.0 [M+H]<sup>+</sup>; HRMS (ESI-TOF<sup>+</sup>)  $m/z$  Calcd. for C<sub>23</sub>H<sub>24</sub>N<sub>4</sub>O<sub>5</sub>[M+H]<sup>+</sup>: 437.1825; Found 437.1814.

*Ethyl 3-(piperazine-1-carbonyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4z-3)*

Light yellow solid; Yield: 52.6%; Mp: 72–74 °C; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 8.73 (d,  $J = 6.8$  Hz, 1H, indolizine-H-5), 8.62 (s, 1H, indolizine-H-2), 8.50 (s, 1H, NH), 7.82 (t,  $J = 7.0$  Hz, 1H, ArH), 7.47 (d,  $J = 9.4$  Hz, 2H, ArH), 7.32 (s, 1H, indolizine-H-8), 7.27 (d,  $J = 6.8$  Hz, 1H, indolizine-H-6), 4.72 (s, 2H, Ar-CH<sub>2</sub>), 4.35 (q,  $J = 6.5$  Hz, 2H, CH<sub>2</sub>), 3.90–3.77 (br, 4H, piperazine-2CH<sub>2</sub>), 3.06–2.90 (br, 4H, piperazine-2CH<sub>2</sub>), 1.39 (t,  $J = 6.4$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ 167.9, 165.45, 163.1, 159.1, 149.9, 139.0, 137.3, 130.81, 128.5, 123.9, 123.2, 121.7, 119.9, 119.5, 112.4, 107.7, 61.5, 46.3, 46.2, 15.0; ESI-MS  $m/z$ : 436.1 [M+H]<sup>+</sup>; HRMS (ESI-TOF<sup>+</sup>)  $m/z$  Calcd. for C<sub>23</sub>H<sub>25</sub>N<sub>5</sub>O<sub>4</sub>[M+H]<sup>+</sup>: 436.1985; Found 436.1974.

*Ethyl 3-(4-methyl-1,4-diazepane-1-carbonyl)-7-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (4z-4)*

Brown solid; Yield: 45.0%; Mp: 62–64 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.01 (d,  $J = 5.9$  Hz, 1H, indolizine-H-5), 8.71 (s, 1H, indolizine-H-2), 8.57 (s, 1H, ArH), 8.01 (s, 1H, NH), 7.69 (t,  $J = 7.3$  Hz, 1H, ArH), 7.51 (s, 1H, indolizine-H-8), 7.34 (d,  $J = 7.4$  Hz, 2H, ArH), 7.23 (t,  $J = 5.1$  Hz, 1H, ArH), 4.78 (d,  $J = 3.5$  Hz, 2H, Ar-CH<sub>2</sub>), 4.39 (q,  $J = 6.9$  Hz, 2H, CH<sub>2</sub>), 3.98–3.77 (br, 4H, 1-methyl-1,4-diazepane-2CH<sub>2</sub>), 2.89–2.73 (br, 2H, 1-methyl-1,4-diazepane-CH<sub>2</sub>), 2.70–2.57 (br, 2H, 1-methyl-1,4-diazepane-CH<sub>2</sub>), 2.43 (s, 3H, NCH<sub>3</sub>), 2.12–1.98 (br, 2H, 1-methyl-1,4-diazepane-CH<sub>2</sub>), 1.44 (t,  $J = 6.9$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 165.6, 164.3, 162.8, 156.2, 149.2, 137.0, 136.1, 130.0, 127.9, 122.7, 122.3, 121.0, 118.7, 118.2, 111.8, 106.6, 60.4, 58.2, 46.7, 45.0, 27.7, 14.7; ESI-MS  $m/z$ : 464.2 [M+H]<sup>+</sup>; HRMS (ESI-TOF<sup>+</sup>)  $m/z$  Calcd. for C<sub>25</sub>H<sub>29</sub>N<sub>5</sub>O<sub>4</sub>[M+H]<sup>+</sup>: 464.2298; Found 464.2287.

*Ethyl 3-benzoyl-8-(pyridin-2-ylmethylcarbamoyl)indolizine-1-carboxylate (6)*

Brown solid; Yield: 65.6%; Mp: 126–129 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.99 (d,  $J = 6.9$  Hz, 1H, indolizine-H-5), 8.48 (d,  $J = 4.6$  Hz, 1H, ArH), 7.81 (s, 1H, indolizine-

H-2), 7.79 (s, 2H, ArH), 7.68 (t,  $J = 7.6$  Hz, 1H, ArH), 7.58 (d,  $J = 7.0$  Hz, 2H, ArH + indolizine-H-7), 7.51 (t,  $J = 7.1$  Hz, 3H, ArH), 7.38 (d,  $J = 7.8$  Hz, 1H, ArH), 7.19 (t,  $J = 6.0$  Hz, 1H, ArH), 7.06 (t,  $J = 6.9$  Hz, 1H, indolizine-H-6), 4.82 (d,  $J = 4.3$  Hz, 2H, Ar-CH<sub>2</sub>), 4.16 (q,  $J = 7.0$  Hz, 2H, CH<sub>2</sub>), 1.23 (t,  $J = 7.2$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 185.7, 167.2, 163.3, 156.0, 148.9, 139.8, 136.9, 135.1, 131.8, 130.2, 129.9, 129.3, 129.1, 128.6, 127.3, 122.7, 122.5, 122.4, 114.4, 108.2, 60.4, 45.0, 14.4; ESI-MS  $m/z$ : 428.0 [M+H]<sup>+</sup>; Anal. Calcd. for C<sub>25</sub>H<sub>21</sub>N<sub>3</sub>O<sub>4</sub>: C, 69.99; H, 4.92; N, 9.65; Found: C, 70.25; H, 4.95; N, 9.83.

*Ethyl 1-benzoyl-6-(pyridin-2-ylmethylcarbamoyl)indolizine-3-carboxylate (7)*

Yellow solid; Yield: 75.3%; Mp: 151–153 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 10.16 (s, 1H, indolizine-H-5), 8.64 (d,  $J = 9.3$  Hz, 1H, indolizine-H-8), 8.60 (d,  $J = 9.3$  Hz, 1H, indolizine-H-7), 7.88–7.81 (m, 5H, ArH), 7.72 (t,  $J = 7.1$  Hz, 1H, ArH), 7.60 (t,  $J = 7.2$  Hz, 1H, ArH), 7.54 (t,  $J = 7.3$  Hz, 2H, ArH), 7.37 (d,  $J = 7.8$  Hz, 1H, ArH), 7.27–7.22 (m, 1H, ArH), 4.82 (d,  $J = 4.6$  Hz, 2H, Ar-CH<sub>2</sub>), 4.41 (q,  $J = 7.2$  Hz, 2H, CH<sub>2</sub>), 1.41 (t,  $J = 7.2$  Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 190.6, 164.9, 161.1, 156.0, 149.3, 140.2, 140.1, 137.1, 131.8, 129.2, 128.6, 128.6, 127.1, 125.2, 123.6, 122.7, 122.4, 120.4, 116.0, 113.6, 60.9, 45.0, 14.7; ESI-MS  $m/z$ : 428.1 [M+H]<sup>+</sup>; HRMS (ESI-TOF<sup>+</sup>)  $m/z$  Calcd. for C<sub>25</sub>H<sub>21</sub>N<sub>3</sub>O<sub>4</sub>[M+H]<sup>+</sup>: 428.1610; Found 428.1599.

*3-Benzoyl-N-(pyridin-2-ylmethyl)imidazo[1,2-a]pyridine-7-carboxamide (8)*

Off white solid; Yield: 87.2%; Mp: 195–197 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.80 (d,  $J = 7.2$  Hz, 1H, imidazo[1,2-a]pyridine-H-5), 8.61 (d,  $J = 4.5$  Hz, 1H, ArH), 8.36 (s, 1H, imidazo[1,2-a]pyridine-H-2), 8.31 (s, 1H, imidazo[1,2-a]pyridine-H-8), 8.12 (s, 1H, NH), 7.96–7.88 (m, 2H, ArH), 7.74 (td,  $J = 7.7, 1.6$  Hz, 1H, ArH), 7.71–7.62 (m, 2H, ArH), 7.57 (t,  $J = 7.5$  Hz, 2H, ArH), 7.37 (d,  $J = 7.8$  Hz, 1H, ArH), 7.30–7.26 (m, 2H, ArH), 4.83 (d,  $J = 4.5$  Hz, 2H, Ar-CH<sub>2</sub>); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 185.2, 164.8, 155.4, 149.3, 148.5, 146.5, 139.2, 137.2, 135.1, 132.6, 129.1, 129.0, 128.9, 124.3, 122.9, 122.4, 116.6, 113.6, 44.9; ESI-MS  $m/z$ : 357.0 [M+H]<sup>+</sup>.

## Docking procedures

The crystal structure of ElonginC in a complex with ElonginB and VIF BC-box (PDB code: 3DCG) was used to predict the binding models of chemicals bound to ElonginC [26,27]. The binding poses of compounds with ElonginC were predicted with the GOLD 3.0.1 program (Cambridge Crystallographic Data Center, Cambridge, UK, 2006). All

the water molecules were removed from the main protein. Then, the protein file was prepared using the Clean Protein Tool in DiscoveryStudio 2.5 package (Accelrys, San Diego, CA). All the ligands were drawn in ChemDraw Ultra 11.0 (Cambridge Soft Corporation, Cambridge, 2008) and then prepared using Prepare Ligands Module in Discovery Studio 2.5. In this process, the compounds were protonated at pH 7.4 and their low-energy conformations were generated. In the docking simulation, VIF “Val142-Leu145” peptide was used for defining binding site, and the radius of the binding site was defined as 8 Å, sufficient to cover all the binding pockets. The ChemScore fitness function was utilized to evaluate the binding ability of various conformations. The genetic algorithm parameters were set as follows: number of operations 100,000, migrate 0, mutate 95, crossover 95, other parameters were set as default. Each ligand was docked 50 times and each pose was ranked according to its ChemScore fitness function.

## Biology

### Preliminary screening for VIF inhibitors

293T cells were seeded into 24-well plates in DMEM supplemented with 10% PBS and incubated overnight at 37 °C. Then, cells were transfected with pEYFP-A3G plus HxB<sub>2</sub>VIF-cmyc or a control plasmid VR1012 using Lipofectamine 2000 (Invitrogen) as recommended by the manufacturer. Cells were then cultured with different samples (final concentration at 50 μM) or DMSO. Cells were harvested at 48 h post transfection. YFP fluorescence was monitored using a Fluoroskan Ascent FL plate reader (Thermo Scientific) with excitation at 485 nm and emission at 538 nm. YFP expression transfected with pEYFP-A3G plus VR1012 and treated with DMSO was set to 100%.

### Antiviral assay

Exponentially growing H9 cells were incubated with wild-type HIV-1 virus at 37 °C for 3 h. After removal of the inocula and three extensive washings, cells were seeded into 96-well plates at a density of  $1 \times 10^4$ /well in 100 L of RPMI 1640. A 100 μL aliquot of compound solutions (ranging from 0 to 200 μM) were added and the cells were incubated for 7 days at 37 °C under 5% CO<sub>2</sub>. At day 7, p24 levels in each wells were evaluated using a p24 ELISA kit (Perkin Elmer, Norwalk, CT, USA). The IC<sub>50</sub> (concentration inhibiting HIV-1 by 50%) value was determined by plotting the p24 antigen contents against the drug concentration. To test the cytotoxicity of the compounds, exponentially growing uninfected H9 cells were identically seeded and cultured to that in the HIV antiviral assay. At day 7, cytotoxicity was evaluated with a

3-(4, 5-dimethylthiazol -2-yl)-2, 5-diphenyltetrazolium bromide colorimetric assay (MTT assay). CC<sub>50</sub> represents the compound concentration that decreases the cell viability by 50%.

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