



Published in final edited form as:

Bioorg Med Chem. 2016 October 15; 24(20): 4779–4786. doi:10.1016/j.bmc.2016.04.050.

Synthesis and evaluation of duocarmycin SA analogs incorporating the methyl 1,2,8,8a-tetrahydrocyclopropa[c]imidazo[4,5-e]indol-4-one-6-carboxylate (Clmi) alkylation subunit

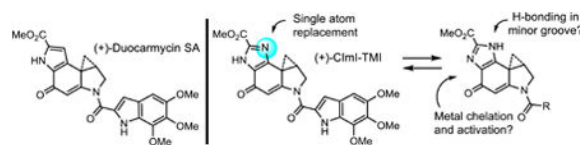
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Abstract

The design, synthesis, and evaluation of methyl 1,2,8,8a-tetrahydrocyclopropa[c]imidazo[4,5-e]indol-4-one-6-carboxylate (Clmi) derivatives are detailed representing analogs of duocarmycin SA and yatakemycin containing an imidazole replacement for the fused pyrrole found in the DNA alkylation subunit.

Graphical abstract



Keywords

Duocarmycin; Yatakemycin; CC-1065; DNA alkylation

1. Introduction

Duocarmycin SA (**1**),¹ yatakemycin (**2**),² and CC-1065 (**3**)³ are the parent members of a class of compounds that derive their antitumor activity from their ability to alkylate DNA (Figure 1).⁴ The study of the natural products,^{5–8} their synthetic unnatural enantiomers,⁹ and key analogs¹⁰ has defined structural features that control their DNA alkylation selectivity,^{4,10} efficiency, rate, reversibility,¹¹ and catalysis,¹² providing a detailed understanding of the relationships between structure, reactivity, and biological activity.¹³

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Supplementary Data. ¹H NMR and ¹³C NMR are provided. The supplementary data associated with this article can be found in the online version at doi:

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Central to these studies have been modifications in the DNA alkylation subunit of the natural products and the establishment of the impact that the deep-seated changes have on their functional reactivity and biological properties.¹³ Herein, we report the total synthesis and examination of the methyl 1,2,8,8a-tetrahydrocyclopropa[*c*]imidazo[4,5-*e*]indol-4-one-6-carboxylate (CImI) alkylation subunit and its incorporation into key analogs of the natural products (Figure 1). The CImI alkylation subunit incorporates a single heavy atom change (aryl CH to N) to the alkylation subunit of duocarmycin SA and yatakemycin, replacing the fused pyrrole with a fused imidazole and complementing a now growing series of such heterocyclic analogs.¹⁴⁻²² In addition to the impact this change may have on the alkylation subunit intrinsic reactivity and reaction regioselectivity, the tautomeric imidazole presents the opportunity for H-bonding on the DNA bound face of the molecule (H-bond donor or acceptor) potentially impacting the DNA alkylation selectivity, and offers the potential for selective metal cation complexation and activation of the reactive cyclopropane analogous to the pyridine analogue CPyI.¹⁷ Finally, this change represents the composite structure of the duocarmycin SA and *iso*-duocarmycin SA alkylation subunits,²⁰ the latter of which is a potent but isomeric variant of the natural alkylation subunit.

The alkylation subunits incorporate a vinylogous amide, which provides stability to what would otherwise be a reactive cyclopropane.^{10,12} Disruption of this vinylogous amide occurs upon a minor groove binding-induced conformational change, which brings the cyclopropane into conjugation with the cyclohexadienone and activates it for nucleophilic attack. Thus, the compounds are ordinarily unreactive, but are activated for adenine N3 alkylation upon target DNA binding.^{13,23} Moreover, this reactivity is still attenuated,⁹ allowing selective reaction by appropriately positioned adenines within the preferred AT-rich non-covalent binding sites such that it is the non-covalent binding selectivity of the compounds that controls the alkylation site selectivity.²⁴ It was not apparent how the substitution of the fused imidazole of CImI for the pyrrole would impact this key structural feature of the natural products.

2. Results and discussion

2.1. Synthesis of CImI²⁵

O-Benzoylation of 2-hydroxy-4-nitroaniline (K₂CO₃, BnBr, acetone, reflux, 8 h, 88%) followed by iodination with ICl (THF, reflux, 3 h, 86%) provided **4**²⁶ following a reported synthesis (Scheme 2). Acid-catalyzed amidine formation (4 N HCl, dioxane, 23 °C, 12 h, 88%) with methyl cyanofornate (4 equiv) cleanly and directly provided **5** in a reaction that is of special note for its effectiveness given the non-nucleophilic and hindered nature of the reacting amine. Intramolecular Cu(I)-catalyzed closure of **5** to the benzimidazole with C-N bond formation cleanly provided **6** in excellent conversion (0.05 equiv CuI, 0.1 equiv 1,10-phen, 1.25 equiv Cs₂CO₃, DME, 80 °C, 16 h).²⁷ *N*-Benzyl protection (1.5 equiv Cs₂CO₃, 2 equiv BnBr, DMF, 23 °C, 12 h, 70%) of the imidazole **6**, permitting protection during a later stage alkylation, provided a 1.6:1 mixture of isomeric *N*-benzyl products (**7** + isomer) that in principle could both be carried through to the final products. The aryl nitro group of the isomeric mixture was reduced to the corresponding amine **8** (10 equiv Zn-nanopowder, 15 equiv NH₄Cl, acetone/H₂O, 23 °C, 10 min), the resulting anilines were chromatographically

separated, and isomer **8** was protected to provide **9** (50% from **7**) using Boc₂O (2 equiv, THF, 70 °C, 12 h). Perhaps unnecessary, this did provide isomerically pure materials simplifying product characterizations and the work was carried forward using the major N3 isomer **9** depicted in Scheme 2. Regioselective C4-iodination using *N*-iodosuccinimide (1.2 equiv NIS, 0.1 equiv TsOH, THF/MeOH 1:1, 23 °C, 12 h, 86%) afforded **10**. A single crystal X-ray structure determination conducted with **10** confirmed the assigned structure and unambiguously established the structures of the two *N*-benzyl regioisomers.²⁸ Carbamate **10** was alkylated with 1,3-dichloropropene (2 equiv, 1.5 equiv NaH, DMF, 23 °C, 5 h, 56%) to afford **11**. The final 5-membered ring of the alkylation subunit was constructed using a tris(trimethylsilyl)silane-mediated 5-*exo-trig* aryl radical–alkene cyclization (2 equiv (Me₃Si)₃SiH, 0.5 equiv AIBN, benzene, 80 °C, 4 h, 69%) of **11** to afford **12**.²⁹ An analogous Bu₃SnH-mediated aryl radical–alkene cyclization (1.4 equiv Bu₃SnH, 0.1 equiv AIBN, benzene, 70 °C, 7 h, 73%) also provided **12** in comparable conversions, but was more sensitive to the quality of commercial reagent available. Treatment of **12** with 10% Pd/C under an atmosphere of hydrogen gas (50 psi H₂, THF, 25 °C, 2 h, 82%) effectively cleaved both the *O*- and *N*-benzyl protecting groups, affording **13**. This could also be accomplished using transfer hydrogenolysis (20% w/w Pd(OH)₂, 50 equiv 1,4 cyclohexadiene, EtOH, 80 °C, 2 h, 75%), albeit with a greater degree of competitive dechlorination. Resolution of **13** into its enantiomers was possible at this stage on a ChiralPak AD semi-preparative HPLC column (2 × 25 cm, 10% *i*-PrOH/hexane, 7 mL/min, α = 1.30). Spirocyclization was accomplished under exceptionally mild conditions upon treatment of **13** with aqueous 1 M NaHCO₃ in THF (23 °C, 4 h, 59%) to afford **14** (Scheme 1, natural enantiomer shown).

Boc deprotection of **13** (4 N HCl/EtOAc, 23 °C, 2 h) followed by direct coupling of the resulting indoline hydrochloride salt with 5,6,7-trimethoxyindole-2-carboxylic acid³⁰ (**19**, 1.3 equiv, 3.0 equiv EDCI, DMF, 23 °C, 48 h, 61%) afforded **15** (Scheme 2, natural enantiomer shown) that was spirocyclized under mild conditions (aqueous 1 M NaHCO₃, THF, 23 °C, 4 h, 65%) to afford **16** (CImI-TMI). Similarly, sequential Boc deprotection of **13** (4 N HCl/EtOAc, 23 °C, 2 h), coupling with carboxylic acid **20** (1.3 equiv, 3.0 equiv EDCI, DMF, 23 °C, 48 h, 69%), and spirocyclization of **17** provided **18** (CImI-indole₂, aqueous 1 M NaHCO₃, THF, 23 °C, 4 h, 60%) in good conversions.

2.2. Reactivity and reaction regioselectivity

Both the intrinsic reactivity of the alkylation subunits as assessed by the rate of acid-catalyzed solvolysis and the regioselectivity of addition to their activated cyclopropane have proven key to understanding the behavior of this class of compounds. The compared rates of solvolysis provide insights into the structural features that stabilize the reacting cyclopropane as well as into the source of catalysis for the DNA alkylation reaction, and their study defined a fundamental parabolic relationship between the alkylation subunit intrinsic reactivity and biological potency.³¹ The regioselectivity of the ring opening reaction involved in the DNA alkylation is controlled by the stereoelectronic alignment of the cyclopropane bonds with the cyclohexadienone π-system.³²

The solvolysis reactivity of *N*-Boc-CImI (**14**) was followed spectrophotometrically by UV in pH 7 and pH 3 phosphate buffer (Figure 2). No measurable solvolysis was observed at pH 7

and it was found to exhibit a solvolysis rate constant of $k = 5.52 \times 10^{-6} \text{ s}^{-1}$ and $t_{1/2} = 34.7 \text{ h}$ at pH 3. This reactivity is on par with that observed with *N*-Boc-MeCPI ($k = 5.26 \times 10^{-6} \text{ s}^{-1}$, $t_{1/2} = 37 \text{ h}$),³¹ the alkylation subunit of CC-1065. It is however, more reactive than *N*-Boc-DSA, which is also stable at pH 7, and exhibits an even lower solvolysis reactivity at pH 3 ($k = 1.08 \times 10^{-6} \text{ s}^{-1}$, $t_{1/2} = 177 \text{ h}$).¹⁰ Thus, the relative reactivity of CImI is essentially equal with that of CPI and it is 5-times more reactive than DSA toward acid-catalyzed solvolysis.

Treatment of **14** with 4 N HCl in EtOAc at $-78 \text{ }^\circ\text{C}$ (1.5 h) provided a single chloride addition product **13** in near quantitative yield (Scheme 3). Exclusive chloride addition to the less substituted cyclopropane carbon was observed, and no ring expanded 6-membered ring addition product, resulting from attack on the more hindered carbon, was detected. Treatment of **14** with 0.35 equiv of $\text{CF}_3\text{SO}_3\text{H}$ in MeOH at $0 \text{ }^\circ\text{C}$ slowly (24 h) led to cyclopropane ring opening, providing a 3:1 ratio of products **21:22**, representing nucleophilic addition to the least substituted and more substituted cyclopropane carbons, respectively, and displaying a reaction regioselectivity under these conditions that is similar to that of *N*-Boc-DSA (4–6.5:1)^{10b} but lower than that of *N*-Boc-CBI (>20:1).³²

2.3. Metal-catalyzed nucleophilic addition

In a past study, we disclosed the metal-catalyzed nucleophilic addition reactions of CPyI, a similar alkylation subunit that possesses a fused pyridine in place of the imidazole of CImI or pyrrole of DSA. That alkylation subunit and analogs incorporating CPyI were unreactive toward conventional nucleophiles at pH 7, but were capable of metal cation-catalyzed reaction with nucleophiles. Moreover, this reactivity was tunable by the choice of metal and displayed relative rates that reflected the relative stability constants of the product 8-hydroxyquinoline metal complexes ($\text{Cu}^{+2} > \text{Ni}^{+2} > \text{Zn}^{+2} > \text{Mn}^{+2} > \text{Mg}^{+2}$).¹⁷ Such behavior is not observed with the DSA alkylation subunit or CBI, indicating that it was intrinsic to the 8-ketoquinoline structure of CPyI. We anticipated that CImI might display this same behavior but exhibit an altered trend in reactivity, reflecting the relative stability constants of 7-hydroxybenzimidazole metal complexes ($\text{Cu}^{+2} > \text{Pb}^{+2} > \text{Cd}^{+2} > \text{Zn}^{+2} > \text{Ni}^{+2}$).³³ However, we found that *N*-Boc-CImI (**14**) failed to undergo solvolysis catalysis by any metal cation examined, rather that it behaved identical to *N*-Boc-DSA, being unaffected by treatment with $\text{Cu}(\text{acac})_2$, $\text{Ni}(\text{acac})_2$, $\text{Zn}(\text{acac})_2$, $\text{Pb}(\text{acac})_2$, or $\text{Cd}(\text{acac})_2$ (1 equiv) at $23 \text{ }^\circ\text{C}$ in MeOH.

2.4. Cell growth inhibition

Both enantiomers of compounds **14**, **16**, and **18** were assayed for cell growth inhibition (cytotoxic) activity against the L1210 tumor cell line (mouse leukemia cell line) that has been traditionally used initially to examine members in this class and the results are presented in Figure 3 alongside the comparison DSA-based compounds. The natural enantiomers of the compounds were found to display potent activity with IC_{50} 's of 70 nM, 50 pM and 40 pM, respectively. Although this represents a 5–10 fold drop in potency when compared to the corresponding DSA compounds themselves¹⁰ (IC_{50} 's = 6 nM, 10 pM, and 4 pM, respectively), the activity is on par with that observed with the CBI-based (e.g., CBI-TMI, $\text{IC}_{50} = 30 \text{ pM}$)³⁴ or CPI-based analogues and is consistent with the observation that

CImI is five times more reactive than DSA. The unnatural enantiomers were ca. >20-fold less active, similarly following trends observed in prior alkylation subunit analogs.

Moreover, this level of cytotoxic activity with the natural enantiomers is in line with expectations based on the relative stability of compounds and follows trends depicted in a parabolic relationship between reactivity and cytotoxic activity (Figure 4).³¹ This parabolic relationship, derived from data from more than 30 deep-seated modifications that span a 10^4 – 10^6 range of reactivity and activity, simply reflects the fact that the compound must be sufficiently stable to reach its biological target yet remain sufficiently reactive to alkylate DNA once it does.

2.5. DNA alkylation

The DNA alkylation selectivity and efficiency of the CImI analogues were examined within a 150 base-pair segment of DNA described previously (w794).³⁵ The alkylation site identification and the assessment of the relative selectivity among the available sites were obtained by thermally-induced strand cleavage of the singly 5'-end-labeled duplex DNA after exposure to the compounds as detailed.³⁵ The examination of (+)-CImI-TMI (**16**), conducted with the equivalent seco precursor **15**, demonstrated that it alkylated the same single site as (+)-duocarmycin SA in w794, displaying the same characteristic DNA alkylation selectivity (Figure 5). In addition, the natural enantiomer (+)-**16** alkylated DNA with an efficiency only 2-fold less than (+)-duocarmycin SA (data not shown), consistent with its 5-fold less potent cytotoxic activity. Like duocarmycin SA, the unnatural enantiomer of **16** proved less efficient at alkylating DNA than its natural enantiomer, and both unnatural enantiomers alkylated the same single major site. The results of these studies indicate that the incorporation of the additional nitrogen atom of the benzimidazole on the face of the compound that extends into the bottom of the minor groove does not alter the DNA alkylation selectivity despite its potential capabilities to serve as either a H-bond acceptor or H-bond donor, and that it has only a minor impact on the relative efficiency of DNA alkylation.

3. Conclusions

A single heavy atom modification to the alkylation subunit of duocarmycin SA was examined with the synthesis and evaluation of analogs containing the CImI alkylation subunit, replacing the fused pyrrole with a fused imidazole and complementing a now growing series of such heterocyclic analogs. In addition to the impact this change could have on the alkylation subunit intrinsic reactivity and reaction regioselectivity, the tautomeric imidazole presented the opportunity for H-bonding on the DNA bound face of the molecule (H-bond donor or acceptor) potentially impacting the DNA alkylation selectivity, and offered the potential for selective metal cation complexation and activation of the reactive cyclopropane. It was found that this modification reduced the intrinsic stability 5-fold, correspondingly reduced the cell growth inhibition properties 5–10 fold, had no impact on the DNA alkylation selectivity but slightly reduced its efficiency, and provided a heterocyclic alkylation subunit that was not subject to a metal-cation catalyzed reactivity. Nonetheless, the CImI-based analogs remain remarkably potent (e.g., (+)-CImI-TMI IC_{50} =

50 pM), displaying activity on par with that observed with MeCPI-based analogs (e.g., CC-1065) and the widely used CBI-based analogs.

4. Experimental

4.1. Methyl 2-((2-(Benzyloxy)-6-iodo-4-nitrophenyl)amino)-2-iminoacetate (5)

A solution of **4** (5.71 g, 15.4 mmol) in 4 N HCl in dioxane (87 mL) was treated dropwise with methyl cyanofornate (5.25 g, 61.7 mmol). The reaction mixture was stirred at room temperature overnight and the solvent was removed under a stream of N₂. The solid residue was dissolved and partitioned between EtOAc and saturated aqueous NaHCO₃, and the organic phase was washed with saturated aqueous NaCl and dried (Na₂SO₄). The solvent was removed in vacuo and the residue was purified by column chromatography (SiO₂, 30% EtOAc/hexanes) to afford **5** (6.20 g, 13.6 mmol, 88%) as an orange solid: ¹H NMR (CD₃OD, 400 MHz) δ 8.32 (d, *J* = 1.5 Hz, 1H), 7.89 (d, *J* = 1.6 Hz, 1H), 7.43 (d, *J* = 4.7 Hz, 2H), 7.35 (t, *J* = 4.7 Hz, 2H), 7.30 (t, *J* = 4.8 Hz, 1H), 5.18 (s, 2H), 3.91 (s, 3H); ¹³C NMR (CD₃OD, 100 MHz) δ 163.0, 150.4, 149.0, 147.7, 146.0, 137.6, 129.6, 129.1, 128.4, 127.6, 109.2, 92.1, 72.0, 53.9; IR (film) ν_{max} 3467, 3357, 3090, 2952, 1737, 1656, 1510, 1330, 1260 cm⁻¹; ESI-TOF HRMS *m/z* 456.0054 (M+H⁺, C₁₆H₁₄IN₃O₅ requires 456.0051).

4.2. Methyl 7-(Benzyloxy)-5-nitrobenzimidazole-2-carboxylate (6)

A suspension of **5** (7.92 g, 17.4 mmol), Cs₂CO₃ (7.08 g, 21.7 mmol), CuI (165 mg, 0.87 mmol), 1,10-phenanthroline (326 mg, 1.8 mmol) in DME (170 mL) under Ar was stirred and warmed at 80 °C for 16 h. The reaction mixture was cooled to 0 °C and 93 mL of aqueous 0.4 N HCl was slowly added. The aqueous phase was extracted with EtOAc and the combined organic extracts were washed with saturated aqueous NaCl, dried (Na₂SO₄) and concentrated in vacuo. The crude product (quantitative yield) was typically advanced to next step without further purification. Material loss due to adhesion to SiO₂ was observed if the reaction product was purified by column chromatography. For the reaction above, chromatography (SiO₂, 20–40% EtOAc/hexane gradient) afforded **6** as a solid (3.66 g, 11.2 mmol, 64%): ¹H NMR (DMSO-*d*₆, 500 MHz, 70 °C) δ 8.13 (br s, 1H), 7.71 (s, 1H), 7.57 (d, *J* = 7.4 Hz, 2H), 7.43 (t, *J* = 7.0 Hz, 2H), 7.36 (t, *J* = 7.3 Hz, 1H), 5.47 (s, 2H), 3.99 (s, 3H); ¹³C NMR (DMSO-*d*₆, 150 MHz) δ 158.9, 150.8, 145.2, 144.6, 137.6, 136.1, 134.4, 128.5, 128.1, 127.9, 102.5, 99.8, 70.4, 52.9; ESI-TOF HRMS *m/z* 328.0932 (M+H⁺, C₁₆H₁₃N₃O₅ requires 328.0928).

4.3. Methyl 1-Benzyl-7-(benzyloxy)-5-((*tert*-butyloxycarbonyl)amino)benzimidazole-2-carboxylate (9)

A solution **6** (1.03 g, 3.1 mmol) in DMF (20 mL) was treated with Cs₂CO₃ (1.55 g, 4.8 mmol) and the reaction mixture was stirred for 30 min at 23 °C. The resultant solution was treated with benzyl bromide (1.08 g, 6.3 mmol) and the reaction mixture was stirred at 23 °C overnight. The reaction mixture was diluted by addition of water and the aqueous portion was extracted with EtOAc. The combined organic extracts were washed with water, saturated aqueous LiCl and saturated aqueous NaCl, dried (Na₂SO₄) and concentrated in vacuo. Flash column chromatography afforded **7** (SiO₂, 0.92 g, 2.2 mmol, 70%) as a 1:1.6 (minor: major (**7**) respectively) mixture of regioisomers.

The mixture of **7** and its isomer (1.0 g, 2.4 mmol) was dissolved in acetone (35 mL) and H₂O (7 mL) containing NH₄Cl (1.92 g, 35.9 mmol). This solution was treated with Zn-nano powder (1.56 g, 23.9 mmol) and the reaction mixture was vigorously stirred at 23 °C for 5–10 min. The reaction mixture was filtered and most of the acetone was removed in vacuo. The mixture was diluted with EtOAc and filtered again. The aqueous layer was separated and the organic phase was washed with saturated aqueous NaCl, dried (Na₂SO₄) and concentrated. The more polar isomer was isolated as a solid by column chromatography (SiO₂, 1% MeOH/CH₂Cl₂). The amine product (472 mg, 1.2 mmol) was dissolved in THF (6.5 mL) and Boc₂O (1.06 g, 4.9 mmol) was added. The reaction mixture was warmed at 70 °C overnight and cooled to room temperature. The solvent was removed in vacuo and the residual oil was purified by flash column chromatography (SiO₂, 30% EtOAc/hexanes) to afford **9** (590 mg, 1.2 mmol, 50%) as a light yellow solid: ¹H NMR (CDCl₃, 400 MHz) δ 7.47 (br s, 1H), 7.26–7.32 (m, 3H), 7.18–7.20 (m, 3H), 7.13–7.15 (m, 3H), 6.87–6.89 (m, 2H), 6.66 (s, 1H), 6.10 (s, 2H), 5.09 (s, 2H), 3.96 (s, 3H), 1.53 (s, 9H); ¹³C NMR (CDCl₃, 100 MHz) δ 160.1, 153.1, 146.8, 143.5, 140.9, 138.1, 135.8, 135.7, 128.6, 128.5, 128.4, 128.1, 127.2, 126.2, 123.1, 102.6, 101.1, 80.6, 70.9, 53.0, 50.2, 28.5 IR (film) ν_{max} 2976, 1720, 1597, 1553, 1482, 1453 cm⁻¹; ESI-TOF HRMS *m/z* 488.2178 (M+H⁺, C₂₈H₂₉N₃O₅ requires 488.2180).

4.4. Methyl 1-Benzyl-7-(benzyloxy)-5-((*tert*-butyloxycarbonyl)amino)-4-iodobenzimidazole-2-carboxylate (**10**)

A solution of **9** (684 mg, 1.4 mmol) and *p*-TsOH·H₂O (27 mg, 0.14 mmol) in THF (10.5 mL) and MeOH (10.5 mL) was treated with NIS (378 mg, 1.7 mmol). The reaction vessel was covered in aluminum foil and the mixture was stirred overnight at 23 °C. A saturated solution of Na₂S₂O₃ (6 mL) was added to the reaction mixture and stirred for 30 min. The reaction mixture was extracted with EtOAc and the combined organic phase was washed with saturated aqueous NaCl, dried (Na₂SO₄) and concentrated in vacuo. The residue was purified by flash column chromatography (SiO₂, 2% MeOH/CH₂Cl₂) to afford **10** (742 mg, 1.2 mmol, 86%) as a pale yellow solid: ¹H NMR (acetone-*d*₆, 400 MHz) δ 7.74 (s, 1H), 7.50 (s, 1H), 7.30–7.33 (m, 5H), 7.22–7.26 (m, 3H), 6.94 (d, *J* = 4.8 Hz, 2H), 6.10 (s, 2H), 5.20 (s, 2H), 3.92 (s, 3H), 1.53 (s, 9H); ¹³C NMR (acetone-*d*₆, 100 MHz) δ 160.8, 153.9, 147.8, 145.4, 141.9, 139.2, 137.7, 136.8, 129.3, 129.3, 129.1, 129.1, 128.0, 127.9, 127.0, 123.1, 104.6, 80.9, 71.6, 53.0, 51.2, 28.5; IR (film) ν_{max} 1726, 1532, 1484, 1267, 1158 cm⁻¹; ESI-TOF HRMS *m/z* 614.1144 (M+H⁺, C₂₈H₂₈IN₃O₅ requires 614.1146).

4.5. Methyl (*Z*)-1-Benzyl-7-(benzyloxy)-5-((*tert*-butyloxycarbonyl)(3-chloroallyl)amino)-4-iodo-benzimidazole-2-carboxylate (**11**)

A solution of **10** (640 mg, 1.04 mmol) in DMF (9.5 mL) was treated with NaH (60% dispersion in mineral oil, 60 mg, 1.5 mmol) at 0 °C. After stirring at this temperature for 30 min, *cis*-1,3-dichloropropene (231 mg, 2.1 mmol) was added and the mixture was stirred for 5 h at 23 °C. H₂O (63 mL) was added to the reaction mixture and the mixture was extracted with EtOAc. The combined organic extracts were washed with H₂O, saturated aqueous LiCl and saturated aqueous NaCl, dried (Na₂SO₄) and concentrated in vacuo. The residue was purified by flash column chromatography (SiO₂, 20–35% EtOAc/hexanes, gradient) to afford **11** (398 mg, 0.58 mmol 56%) as a white solid: ¹H NMR (CDCl₃, 500 MHz, mixture

of rotamers) δ 7.26–7.31 (m, 3H), 7.22 (s, 3H), 7.11 (d, J = 7.1 Hz, 2H), 6.90 (s, 1.4H) & 6.84 (s, 0.6 H), 6.75 (s, 0.3H) & 6.66 (s, 0.7H), 6.13 (s, 1.4H) & 6.10 (s, 0.6H), 5.98 (s, 2H), 5.10 (d, J = 11.4 Hz, 1H), 5.04 (d, J = 11.7 Hz, 1H), 4.50 (d, J = 13.5 Hz, 1H), 4.29 (d, J = 12.8 Hz, 0.7H) & 4.16 (d, J = 12.8 Hz, 0.3H), 3.97 (s, 3H), 1.56 (s, 3H) & 1.33 (s, 6H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 160.0, 154.1, 147.2 & 146.7, 145.3 & 145.1, 141.5, 141.3 & 141.0, 137.7, 135.4, 128.8, 128.6, 128.5, 128.0 & 127.7, 127.5, 127.4, 126.2, 124.5 & 124.3, 120.7 & 120.2, 109.4 & 109.2, 84.5, 81.3 & 80.8, 71.0, 53.6 & 53.1, 50.8, 47.5 & 46.3, 28.6 & 28.3; IR (film) ν_{max} 2976, 1725, 1699, 1579, 1482, 1451, 1367 cm^{-1} ; ESI-TOF HRMS m/z 688.1070 ($\text{M}+\text{H}^+$, $\text{C}_{31}\text{H}_{31}\text{ClN}_3\text{O}_5$ requires 688.1070).

4.6. 3-(*tert*-Butyl) 7-Methyl 6-Benzyl-5-(benzyloxy)-1-(chloromethyl)-1,2-dihydro-3*H*-imidazo[4,5-*e*]indole-3,7-dicarboxylate (12)

Method A: Benzene (10 mL) degassed by the freeze-pump-thaw method three times was added to a sealed flask charged with **11** (200 mg, 0.29 mmol) and AIBN (20 mg, 0.12 mmol). $(\text{Me}_3\text{Si})_3\text{SiH}$ (145 mg, 0.58 mmol) was added and the flask was sealed and heated at 80 °C for 4 h. After cooling, the solvent was removed under a stream of N_2 gas. The reaction mixture was purified by column chromatography (SiO_2 , 20% EtOAc/hexanes) to afford **12** (113 mg, 0.20 mmol, 69%) as a pale yellow solid: ^1H NMR (CDCl_3 , 500 MHz) δ 7.85 (br s, 1H), 7.26–7.33 (m, 3H), 7.20 (t, J = 2.9 Hz, 3H), 7.14 (d, J = 7.1 Hz, 2H), 6.90 (d, J = 3.7 Hz, 2H), 6.11 (s, 2H), 5.10 (s, 2H), 4.28 (d, J = 11.0 Hz, 1H), 4.17–4.23 (m, 2H), 4.12 (br, 1H), 3.98 (s, 3H), 3.70 (br, 1H), 1.58 (s, 9H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 160.1, 147.3, 141.4, 139.8, 138.2, 135.8, 128.7, 128.6, 128.4, 128.3, 127.3, 126.2, 123.1, 97.5, 81.0, 71.2, 53.2, 50.2, 47.2, 41.1, 29.9, 28.6; IR (film) ν_{max} 1726, 1697, 1455, 1419, 1339, 1264 cm^{-1} ; ESI-TOF HRMS m/z 562.2104 ($\text{M}+\text{H}^+$, $\text{C}_{31}\text{H}_{32}\text{ClN}_3\text{O}_5$ requires 562.2103).

Method B: Benzene (42 mL) degassed by the freeze-pump-thaw method three times was added to a sealed flask charged with **11** (770 mg, 1.1 mmol) and AIBN (18 mg, 0.1 mmol). Bu_3SnH (460 mg, 1.6 mmol) was added and the flask was sealed and heated at 80 °C for 7 h. After cooling, the solvent was removed under a stream of N_2 gas. The reaction mixture was purified by column chromatography (SiO_2 , 30% EtOAc/hexanes) to afford **12** (457 mg, 0.81 mmol, 73%) as a yellow solid identical to the material described above.

4.7. 3-(*tert*-Butyl) 7-Methyl 1-(Chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-imidazo[4,5-*e*]indole-2,6-dicarboxylate (13)

A solution of **12** (50 mg, 0.089 mmol) in THF (2 mL) under Ar was charged with 10% Pd/C (18 mg, 0.017 mmol) and placed under 50 psi pressure of H_2 . The reaction mixture was agitated in a Parr Shaker apparatus for 2 h at 23 °C. The reaction mixture was filtered, concentrated in vacuo and purified by flash chromatography (SiO_2 , 30% acetone/hexanes) to afford **13** (28 mg, 0.073 mmol, 82%) as a white solid: ^1H NMR (acetone- d_6 , 400 MHz) δ 12.31 (br s, 1H), 8.96 (br s, 1H), 7.54 (br s, 1H), 4.25–4.31 (m, 1H), 4.10–4.18 (m, 1H), 4.06 (dd, J = 3.4, 10.7 Hz, 1H), 3.92–3.96 (m, 1H), 3.96 (s, 3H), 3.67 (t, J = 10.1 Hz, 1H), 1.56 (s, 9H); IR (film) ν_{max} 1745, 1366 cm^{-1} ; ESI-TOF HRMS m/z 382.1175 ($\text{M}+\text{H}^+$, $\text{C}_{17}\text{H}_{20}\text{ClN}_3\text{O}_5$ requires 382.1164).

Resolution: Racemic **13** was resolved into its enantiomers by HPLC on a ChiralPak AD semi-preparative column (2×25 cm, 10% *i*-PrOH/hexane, 7 mL/min, $\alpha = 1.3$). Peak 1: $[\alpha]_D^{20} -52$ (c 0.39, MeOH) for natural enantiomer; Peak 2: $[\alpha]_D^{20} +50$ (c 0.42, MeOH) for unnatural enantiomer.

4.8. 2-(*tert*-Butyl) 6-Methyl 1,2,8,8a-Tetrahydrocyclopropa[c]imidazo[4,5-*e*]indol-4-one-2,6-dicarboxylate (N-Boc-CImI, **14**)

A solution of **13** (8 mg, 0.021 mmol) dissolved in THF (0.8 mL) was treated with aqueous 1 M NaHCO₃ (0.8 mL, 0.8 mmol). The reaction mixture was stirred at room temperature for 4 h before it was extracted with EtOAc, dried with anhydrous Na₂SO₄, filtered, and concentrated. The solid product was purified by PTLC (SiO₂, 80% EtOAc/hexane) to yield **14** (4.3 mg, 0.012 mmol, 59%): ¹H NMR (CDCl₃, 400 MHz) δ 11.21 (br, 1H), 6.83 (s, 1H), 3.98–4.06 (m, 5H), 3.04 (q, $J = 5.1, 7.5, 4.8$ Hz, 1H), 2.10 (dd, $J = 3.9, 7.8$ Hz, 1H), 1.54 (s, 9H), 1.43 (t, $J = 4.6$ Hz, 1H); ¹³C NMR (CDCl₃, 125 MHz) δ 178.0, 161.2, 158.9, 151.6, 148.6, 139.4, 129.3, 109.5, 83.7, 54.0, 53.4, 33.4, 28.3, 25.9, 23.6; IR (film) ν_{\max} 2923, 2852, 1721, 1603, 1393, 1370, 1345, 1277, 1206 cm⁻¹; ESI-TOF HRMS m/z 346.1401 (M + H⁺, C₁₇H₁₉N₃O₅ requires 346.1397). $[\alpha]_D^{20} +115$ (c 0.27, MeOH) for natural enantiomer; $[\alpha]_D^{20} -115$ (c 0.27, MeOH) for unnatural enantiomer.

4.9. Methyl 1-(Chloromethyl)-5-hydroxy-3-(5,6,7-trimethoxyindole-2-carbonyl)-1,2-dihydro-3*H*-imidazo[4,5-*e*]indole-2-carboxylate (**15**)

An oven dried vial containing **13** (5.0 mg, 0.013 mmol) was treated with 4 N HCl in EtOAc (1.6 mL). The reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated under a stream of N₂. Additional EtOAc was added and the solvent was evaporated again. The residue was placed under vacuum overnight. EDCI·HCl (7.4 mg, 0.039 mmol), 5,6,7-trimethoxyindole-2-carboxylic acid (**19**, 4.2 mg, 0.017 mmol) and DMF (140 μ L) were added to the amine salt. The reaction mixture was stirred for 2 d at room temperature. The solvent was removed under a stream of N₂. The crude product was purified by column chromatography (SiO₂, 3% MeOH/CH₂Cl₂) to yield **15** (4.1 mg, 0.0079 mmol, 61%): ¹H NMR (DMSO-*d*₆, 500 MHz) δ 10.33 (s, 1H) 7.65 (br, 1H), 7.00 (s, 1H), 6.96 (s, 1H), 4.70 (t, $J = 10.2$ Hz, 1H), 4.40 (dd, $J = 3.3, 11.2$ Hz, 1H), 4.09–4.12 (m, 1H), 3.80–3.96 (m, 2H, embedded within –OMe singlets), 3.95 (s, 3H), 3.93 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H); ESI-TOF HRMS m/z 515.1327 (M + H⁺, C₂₄H₂₃ClN₄O₇ requires 515.1328). $[\alpha]_D^{26} -14$ (c 0.08, MeOH) for natural enantiomer; $[\alpha]_D^{26} +15$ (c 0.06, MeOH) for unnatural enantiomer.

4.10. Methyl 2-(5,6,7-Trimethoxy-1*H*-indole-2-carbonyl)-1,2,8,8a-tetrahydrocyclopropa[c]imidazo[4,5-*e*]indole-4-one-6-carboxylate (**16**)

A sample of **15** (2.0 mg, 3.88 μ mol) was dissolved in THF (0.2 mL). Aqueous 1 M NaHCO₃ (0.2 mL) was added and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was extracted with EtOAc and the combined organic phase was dried with anhydrous Na₂SO₄, filtered, and concentrated. The solid product was purified by PTLC (SiO₂, 8% MeOH/CH₂Cl₂) to yield **16** (1.20 mg, 2.5 μ mol, 65%): ¹H NMR (CDCl₃, 400 MHz) δ 11.02 (br, 1H), 9.26 (s, 1H), 7.12 (s, 1H), 6.95 (d, $J = 2.3$ Hz, 1H), 6.78 (s, 1H),

4.51 (dd, $J = 4.9, 10.4$ Hz, 1H), 4.45 (d, $J = 10.4$ Hz, 1H), 4.08 (s, 3H), 4.04 (s, 3H), 3.94 (s, 3H), 3.90 (s, 3H), 3.19–3.15 (m, 1H), 2.24 (dd, $J = 4.1, 7.6$ Hz, 1H), 1.58 (1H, embedded within H₂O signal; appears as a triplet in CD₃OD, 600 MHz at δ 1.71 (t, $J = 5.4$ Hz, 1H)); ESI-TOF HRMS m/z 479.1562 (M+H⁺, C₂₄H₂₂N₄O₇ requires 479.1561).

4.11. Methyl 6-(5-(Indole-2-carboxamido)indole-2-carbonyl)-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3H-imidazo[4,5-e]indole-2-carboxylate (17)

An oven dried vial containing **13** (4.9 mg, 0.013 mmol) was treated with 4 N HCl in EtOAc (1.5 mL). The reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated under a stream of N₂. Additional EtOAc was added and the solvent was evaporated again. The residue was put under vacuum overnight. EDCI-HCl (7.4 mg, 0.038 mmol), **20** (5.3 mg, 0.017 mmol) and DMF (135 μ L) were added to the amine salt. The reaction mixture was stirred for 2 d at room temperature. The solvent was removed under a stream of N₂. The crude product was purified by column chromatography (SiO₂, 4% MeOH/CH₂Cl₂) to yield **17** (5.14 mg, 0.009 mmol, 69%) and was carried forward without further characterization.

4.12. Methyl 2-(5-(Indole-2-carboxamido)indole-2-carbonyl)-1,2,8a-tetrahydrocyclopropa[c]imidazo-[4,5-e]indole-4-one-6-carboxylate (18)

A sample of **17** (1.58 mg, 2.71 μ mol) was dissolved in THF (0.16 mL). Aqueous 1 M NaHCO₃ (0.16 mL) was added and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was extracted with EtOAc and the combined organic phase was dried with anhydrous Na₂SO₄, filtered, and concentrated. The solid product was purified by PTLTLC (SiO₂, 8% MeOH/CH₂Cl₂) to yield **18** (0.89 mg, 1.62 μ mol, 60%): ¹H NMR (CD₃OD, 400 MHz) δ 8.16 (d, $J = 1.8$ Hz, 1H) 7.74 (d, $J = 8.0$ Hz, 1H), 7.63 (dd, $J = 2.0, 8.8$ Hz, 1H), 7.57 (d, $J = 8.8$ Hz, 1H), 7.40 (s, 1H), 7.33 (t, $J = 7.6$ Hz, 1H), 7.26 (s, 1H), 7.18 (t, $J = 7.6$ Hz, 1H), 6.92 (s, 1H), 4.65 (dd, $J = 4.6, 10.5$ Hz, 1H), 4.58 (d, $J = 10.6$ Hz, 1H), 4.00 (s, 3H), 3.21 (dd, $J = 4.8, 11.9$ Hz, 1H), 2.15 (dd, $J = 3.8, 7.6$ Hz, 1H), 1.68 (t, $J = 4.6$ Hz, 1H); ESI-TOF HRMS m/z 547.1721 (M+H⁺, C₃₀H₂₂N₆O₅ requires 547.1724). [α]_D²⁰ +74 (c 0.073, MeOH) for natural enantiomer; [α]_D²⁰ -72 (c 0.068, MeOH) for unnatural enantiomer.

4.13. Aqueous Solvolysis of *N*-Boc-CIml (pH 3 Phosphate Buffer)

N-Boc CIml (**14**, 190 μ g) was dissolved in MeOH (1.5 mL) and pH 3.0 buffer (1.5 mL, prepared by mixing 4:1:20 (v:v:v) 0.1 M citric acid, 0.2 M Na₂HPO₄, and water, respectively). After mixing of the reagents, the UV spectrum of the solution was measured against the reference solution containing MeOH (1.5 mL) and pH 3 phosphate buffer (1.5 mL) and this reading was used for the initial absorbance value (A_i). The UV spectrum was measured at regular intervals (every 1 h in first 8 h period and every 12 h for the remaining time) until no further change in absorbance was observed (A_f). The decrease in the long-wavelength absorption at 331 nm and increase in the short-wavelength absorption at 262 nm were monitored. The solvolysis rate constant ($k = 5.52 \times 10^{-6} \text{ s}^{-1}$) and half-life ($t_{1/2} = 34.7 \text{ h}$) were calculated from the least-squares treatment of the slope of the plot of time versus $\ln [(A_f - A_i)/A_f A]$.

4.14. Addition of HCl to *N*-Boc-Clml

An oven-dried flask containing **14** (3.8 mg, 0.011 mmol) dissolved in THF (0.25 mL) at $-78\text{ }^{\circ}\text{C}$ was treated with 4 N HCl in EtOAc (5.5 μL , 22 μmol) dropwise. The reaction mixture was stirred at $-78\text{ }^{\circ}\text{C}$ for 1.5 h. Upon disappearance of **14** by TLC, the reaction mixture was concentrated by a stream of N_2 keeping it at a cold temperature ($-78\text{ }^{\circ}\text{C}$). The residue was purified by flash column chromatography (SiO_2 , 30% acetone/hexane) to yield **13** (3.8 mg, 0.01 mmol, 90%) identical with authentic material.

4.15. Acid-Catalyzed Addition of CH_3OH to *N*-Boc-Clml

An oven-dried vial containing **14** (2.6 mg, 7.5 μmol) in MeOH (750 μL) was cooled to $0\text{ }^{\circ}\text{C}$ and TfOH (0.08 μL , 0.903 μmol) was added. The reaction mixture was stirred at $0\text{ }^{\circ}\text{C}$ for 3 h. Additional TfOH was added (0.08 μL , 0.903 μmol) and the reaction was stirred for another 3 h. Finally, additional TfOH was further added (0.08 μL , 0.903 μmol) and the mixture was stirred for another 18 h. At this point, no further change in TLC was observed. The reaction mixture was concentrated under a stream of N_2 and the product was purified by PTLC (250 μm , SiO_2 , 2.5% MeOH/ CH_2Cl_2 , eluted 3 times). The purification provided **21** (1.7 mg, 4.50 μmol , 60%) and **22** (0.5 mg, 1.32 μmol 18%) and recovery of starting material (0.5 mg, 1.44 μmol , 19%).

For **21**: ^1H NMR (CD_2Cl_2 , 600 MHz) δ 10.49 (s, 1H), 7.53 (s, 1H), 7.20 (s, 1H), 4.22 (t, J = 11.0 Hz, 1H), 3.99 (s, 3H), 3.80–3.84 (m, 1H), 3.70 (dd, J = 4.1, 8.5 Hz, 1H), 3.48–3.55 (m, 5H), 1.55 (s, 9H); ^{13}C NMR (CD_2Cl_2 , 150 MHz) δ 159.8, 152.5, 149.2, 143.9, 139.4, 132.2, 129.6, 106.8, 98.1, 76.9, 59.6, 53.5, 51.3, 39.2, 30.3, 28.7; ESI-TOF HRMS m/z 378.1667 ($\text{M}+\text{H}^+$, $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_6$ requires 378.1660).

For **22**: ^1H NMR (CD_2Cl_2 , 600 MHz) δ 7.17 (s, 1H), 3.93–4.24 (m, 4H), 3.81 (s, 1H), 3.64 (d, J = 13.2 Hz, 1H), 3.44 (s, 3H), 3.14 (dd, J = 5.7, 16.7 Hz, 1H), 2.88 (d, J = 16.7 Hz, 1H), 1.53 (s, 9H); ESI-TOF HRMS m/z 378.1669 ($\text{M}+\text{H}^+$, $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_6$ requires 378.1660).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge the financial support of the National Institutes of Health (CA042056) and the Skaggs Institute for Chemical Biology. We especially wish to thank Yam Poudel and Dimitri Hirsch-Weil for intermediate efforts in the work, and Professor Arnold L. Rheingold and Dr. Curtis E. Moore (UCSD) for the single crystal X-ray structure determination of **10**.

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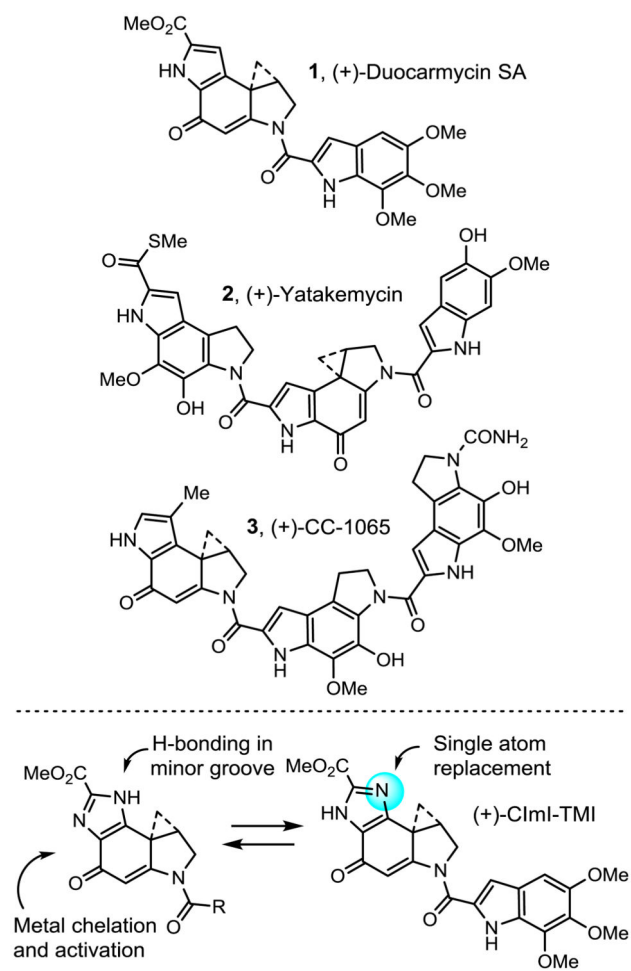


Figure 1.
Representative natural products in class and structure of ClmI.

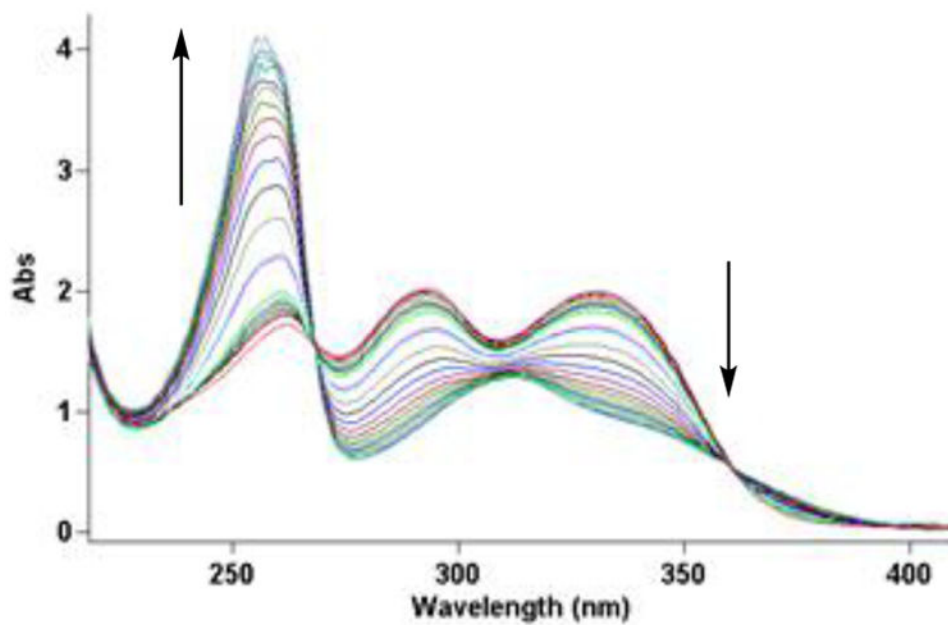


Figure 2. Solvolysis study (UV spectra) of *N*-Boc-CImI (**14**) in 50% MeOH–aqueous buffer (pH 3, 4:1:20 (v/v/v) 0.1 M citric acid, 0.2 M NaH₂PO₄, and H₂O, respectively). The spectra were recorded at regular intervals from 0–187 h.

Compound	IC ₅₀ (L1210, pM)
(+)- <i>N</i> -Boc-DSA	6,000
<i>ent</i> -(-)- <i>N</i> -Boc-DSA	60,000
(+)- 14 , (+)- <i>N</i> -Boc-Clml	70,000
(-)- 14 , <i>ent</i> -(-)- <i>N</i> -Boc-Clml	1,100,000
(+)- 1 , (+)-duocarmycin SA	10
(-)- 1 , <i>ent</i> -(-)-duocarmycin SA	100
(+)- 16 , (+)-Clml-TMI	50
(-)- 16 , <i>ent</i> -(-)-Clml-TMI	2,200
(+)-DSA-indole ₂	4
<i>ent</i> -(-)-DSA-indole ₂	150
(+)- 18 , (+)-Clml-indole ₂	40
(-)- 18 , <i>ent</i> -(-)-Clml-indole ₂	700

Figure 3.
In vitro cytotoxic activity.

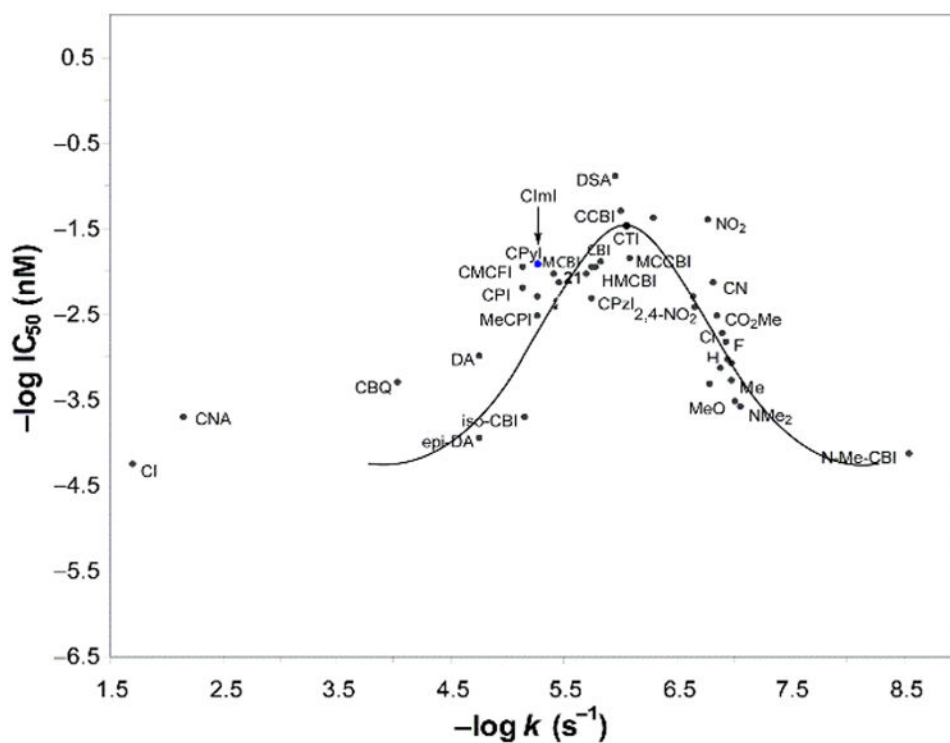


Figure 4. Plot of $-\log IC_{50}$ (nM) vs $-\log k$ (s^{-1}) illustrating the parabolic relationship between reactivity and cytotoxic activity and the placement of ClImI in this relationship.

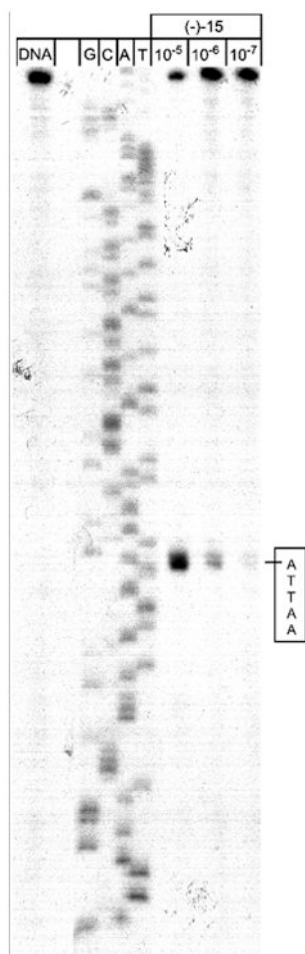
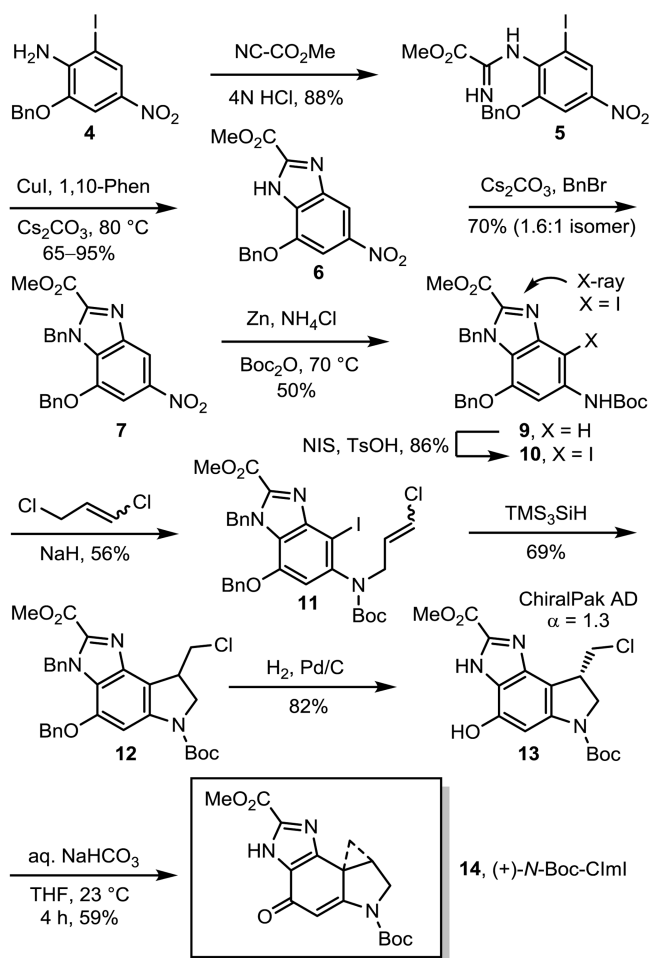
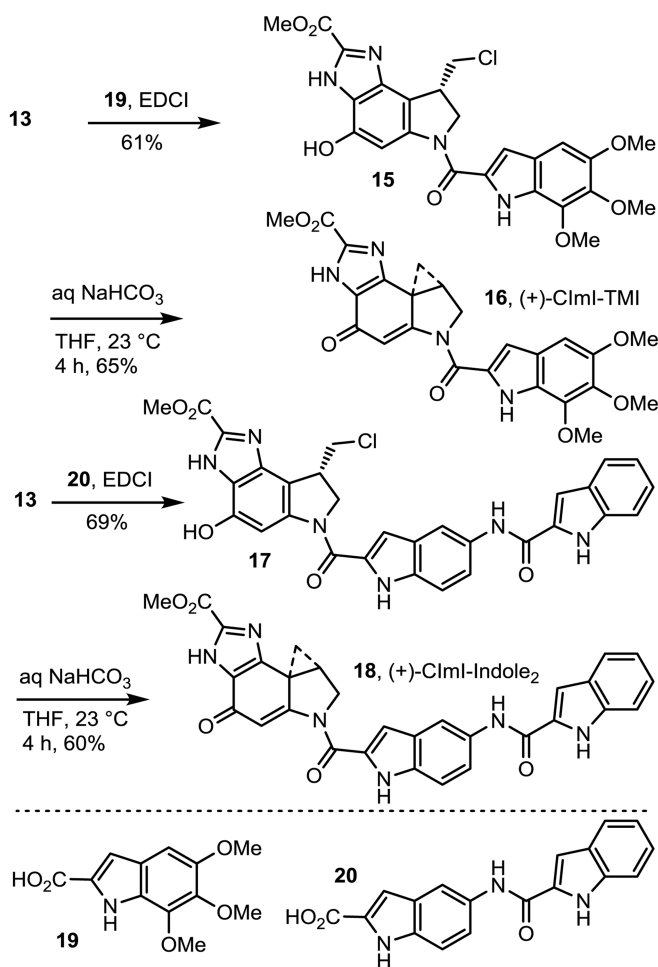


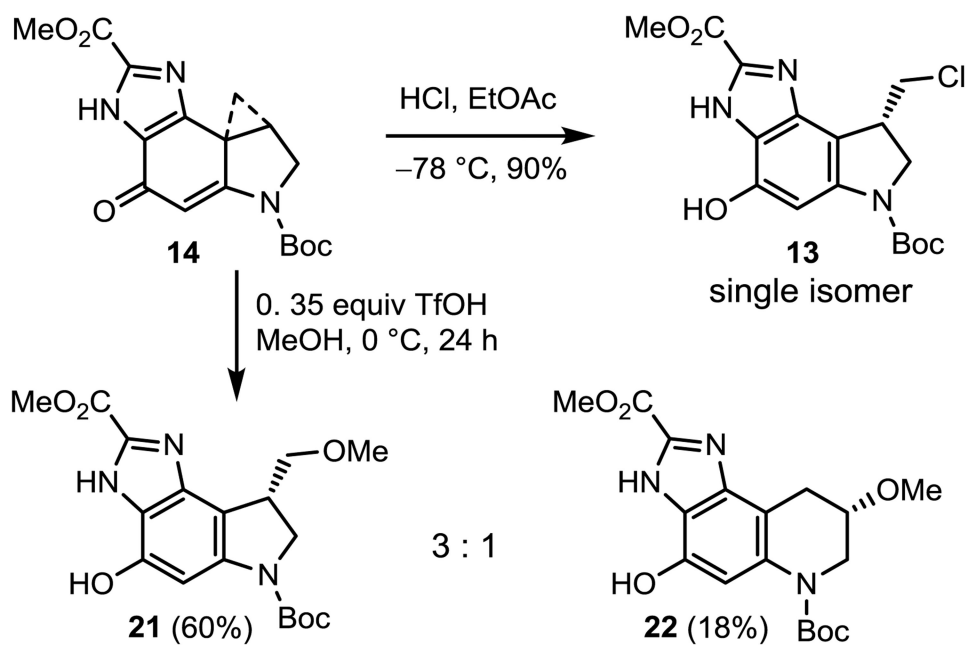
Figure 5. Thermally-induced strand cleavage of w794 DNA following DNA alkylation; DNA-agent incubation at 23 °C for 24 h, removal of unbound agent by EtOH precipitation, and 30 min of thermolysis (100 °C) followed by 8% denaturing PAGE and autoradiography. Lane 1, control DNA; lanes 2–5, Sanger G, C, A, and T sequencing reactions; lanes 6–8, (–)-**15** (natural enantiomer at 1×10^{-5} , 1×10^{-6} , and $1 \times 10^{-7} \text{ M}^{-1}$).



Scheme 1.
Synthesis of N-Boc-ClmI.



Scheme 2.
Synthesis of ClmI-TMI and ClmI-indole₂.



Scheme 3.
Acid-catalyzed Addition Reactions.