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Synthesis, molecular modeling, and evaluation of nonphenolic indole analogs of mycophenolic acid

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Abstract—Based on the promising activity of an indole-3-carboxamide derivative, a nonphenolic analog of mycophenolic acid (MPA), we report herein the synthesis of a compound containing two important features for the activity of MPA, the ring methoxy and methyl. The synthesis was accomplished using two strategies; a method dependent on stepwise building of the hexenoate side chain followed by the indolecarboxamide ring system, and a convergent route that depended on 1,3-sigmatropic rearrangement as a key step. Docking experiments on both Chinese Hamster and Human Type-II *inosine monophosphate dehydrogenase* (IMPDH) showed that this compound has potential binding interactions with the NAD site. The analogs showed no activity against MCF7-S, MCF7-R, or IGR-OV1 cancer cells.

1. Introduction

Mycophenolic acid (MPA, Fig. 1) is a potent inhibitor of human *inosine 5′-monophosphate dehydrogenase* (IMPDH), a key enzyme in the *de novo* biosynthesis of guanine nucleotide. Its prodrug, mycophenolate mofetil (MMF, CellCept®), is used successfully as an immunosuppressive to prevent kidney allograft rejection. MPA has also been shown to possess in vitro anti-cancer activity,[1] however rapid glucuronide conjugation of the phenol moiety limits in vivo anticancer activity.[2] MPA is an uncompetitive inhibitor of IMPDH; it binds to the NAD (cofactor) site to form a ternary complex with the enzyme and the oxidized, covalently bound IMP (the substrate).[3] Other NAD site IMPDH inhibitors have been explored, for example, *C*-nucleosides (NAD analogs)[4] exemplified by tiazofurin[5] and aryloxazoles[6] such as VX-497 (merimempodib).[7]

These compounds are currently under investigation for potential application in leukemia[8] and hepatitis C infection.[9] Derivatization of MPA remains an intriguing target for antineoplastic agents because of its high potency combined with relatively low toxicity. The major side effect of MPA, gastrointestinal irritation, arises from hepatic excretion of its glucuronide metabolite,[10] but replacement of this metabolically liable phenol with bioisosteres results in significant loss of activity.[11]

We prepared the indole-3-carboxamide **1** in an effort to develop metabolically stable analogs of MPA[12] in which the indole N–H bioisosterically replaced the metabolically vulnerable MPA phenol. The indole showed reproducible activity against NCI tumor cell panels (mean $GI_{50} = 3.5\ \mu M$) and was active against ovarian cancer cell lines IGR-OV1 at sub-micromolar levels ($GI_{50} = 0.44\ \mu M$).[13] Indole **1** lacked two important MPA structural features essential for the control of the conformation of the juxtaposed hexenoate side chain[9]—the 6-methoxy and 5-methyl groups. Modeling studies predicted that the hexenoate side chain in the 5-methyl-6-methoxy analog **2** could adopt a favorable MPA-like conformation.

2. Chemistry

The indole **3** represents a key intermediate for the synthesis of the title compound **2** and two potential

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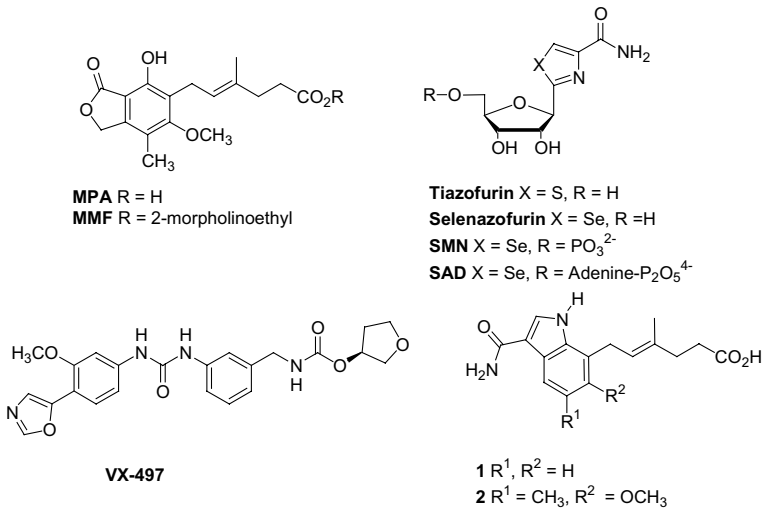
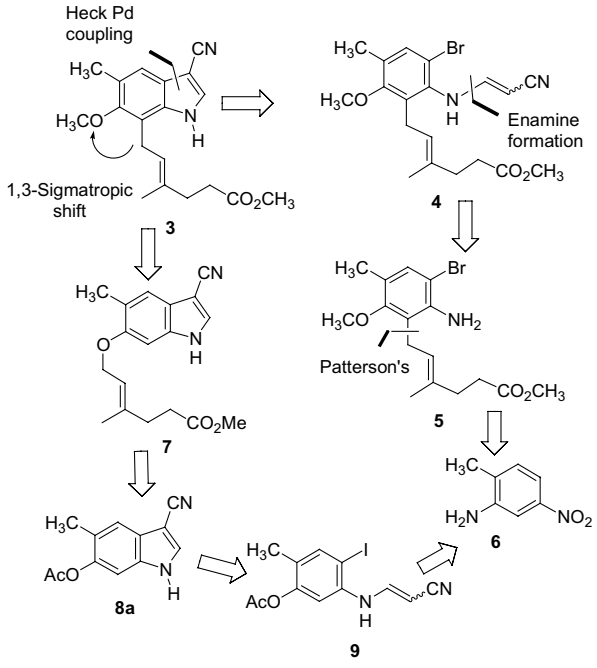


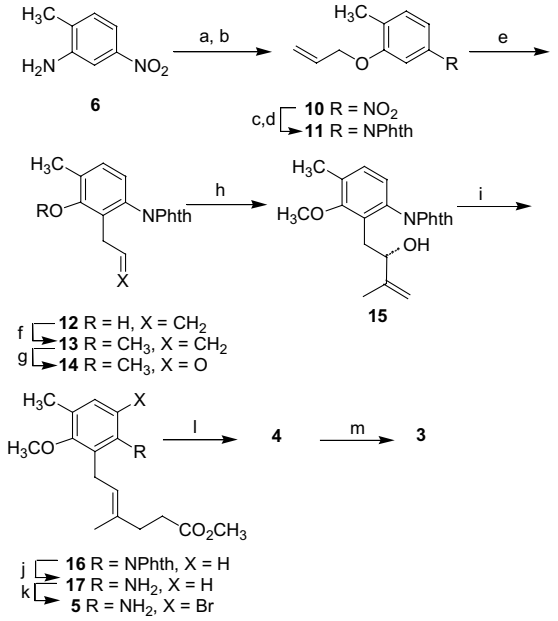
Figure 1. Inhibitors of human IMPDH and indole analogs of MPA.

approaches are summarized in Scheme 1. In one retrosynthetic plan, the hexenoic acid side chain is elaborated using Patterson's double Claisen strategy;[14] this is followed by construction of the 3-cyanoindole nucleus via intramolecular Heck Pd coupling[15] of the enamine **4** derived from the bromoaniline **5**. The second strategy builds the 3-cyanoindole compound **8a** followed by introduction of the hexenoate side chain via Dauben's 1,3-sigmatropic rearrangement.[16,17]

In the first approach, the starting compound **6** was converted to the *N*-protected allyloxyaniline **11**; Claisen rearrangement proceeded in high yield and the phenol **12** was directly methylated to give **13**. Ozonolysis (quenched with dimethylsulfide) of the allylic moiety afforded the aldehyde **14**. Treatment of **14** with isopropenyl MgBr provided the allylic alcohol **15**, the substrate for the second Claisen rearrangement. This acid catalyzed rearrangement was effected by treatment of **15** with trimethyl orthoacetate to give **16**. Cleavage of the phthalate moiety gave aniline **17** and bromination furnished the advanced intermediate **5** (Scheme 2).



Scheme 1. Retrosynthetic strategies for **3**.



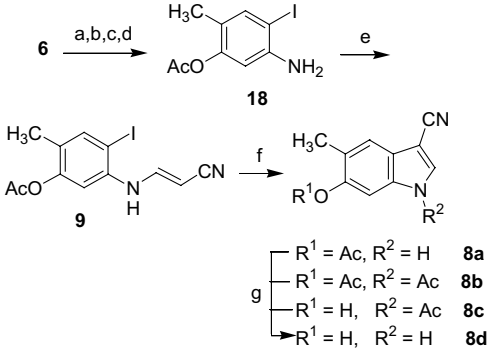
Scheme 2. Reagents and conditions: (a) i. $NaNO_2$, H_2SO_4, 0–5 °C; ii. H_2SO_4, 120 °C, 20 min; (b) allyl Br; K_2CO_3, acetone, reflux; (c) $SnCl_2{\cdot}2H_2O$, EtOAc, 65–70 °C; (d) phthalic anhydride, DMF, reflux; (e) *N*,*N*-diethylaniline, 200 °C; (f) $(CH_3)_2SO_4$, K_2CO_3, acetone, reflux; (g) i. O_3, CH_2Cl_2, MeOH, Pyridine, −78 °C; (ii) DMS; (h) isopropenyl MgBr, THF, −70 °C; (i) $CH_3C(OCH_3)_3$, $C_2H_5CO_2H$, 100 °C; (j) hydrazine hydrate, MeOH, 25 °C; (k) NBS, DMF; (l) acrylonitrile, 1,4-benzoquinone, $Pd(CH_3CN)_2Cl_2$, LiCl, THF, 50 °C; (m) $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Et_3N, DMF, 120 °C.

The conversion of the bromoaniline **5** into the enamine **4** represented a critical step in this synthesis because of the sterically hindered amino group and the deactivation by the *o*-bromo group. Two conditions for enamine formation were investigated (based on model reaction, data not shown). In the first approach, a mixture of **5** and 3,3-diethoxypropionitrile (DEPN) was heated in the presence of an acid catalyst, or, in a variation DEPN was converted to cyanoacetaldehyde in situ (TFA), then treated with **5** at room temperature. Neither of these procedures afforded the desired enamine **4**. Alternatively, Hegedus' method for oxidative Pd coupling of the aniline with acrylonitrile furnished **4** in low yield (8–12%).[18] The purification of **4** was difficult and incomplete because the β-cyanovinylogous anilines was a dynamic mixtures of *E* and *Z* isomers that streaked over the silica gel column.[19] Intramolecular Heck reaction of **4** provided the desired indole-3-nitrile **3**.

The poor yields in the last two crucial steps led us to search for an alternative strategy for the synthesis of **3**. We investigated Dauben's conditions for 1,3-sigmatropic rearrangement used to synthesize side chain analogs of MPA.[8] The plan, as illustrated in Scheme 1, was based on a convergent scheme: the synthesis of the indole **8a** followed by attachment to the hexenoate side chain to give the 1,3-sigmatropic substrate **7**. Thus, the iodoaniline **18** (prepared from **6**) was treated with cyanoacetaldehyde (freshly prepared by acid hydrolysis of 3,3-diethylpropionitrile) to give the Heck substrate **9** (Scheme 3).

The iodoenamine **9** precipitated from solution exclusively as a stable *E* isomer. Cyclization of **9** under Pd catalysis gave the indole **8a** in excellent yields along with the *N*- and *O*-acetylated indoles **8b**–**8c**. The acetyl rearrangement presumably was catalyzed by the presence of the base, Pd acetate, and heat. All the acetylated products were easily converted to **8d**.

Direct reaction of the phenol **8d** with the alcohol **21a** (prepared according to literature procedure, see Fig. 2) under Mitsunobu conditions consistently gave poor yields of **7** under different conditions (DEAD/Ph_3P/



Scheme 3. Reagents and conditions: (a) i. $NaNO_2$, H_2SO4, 0–5 °C; (ii) H_2SO4, heat; (b) Ac_2O, pyridine; (c) $SnCl_2{\cdot}2H_2O$, EtOAc, 70 °C; (d) NIS, DMF; (e) cyanoacetaldehyde, TFA, EtOAc; (f) $Pd(OAc)_2$, Et_3N, DMF, heat; (g) K_2CO_3, H_2O, MeOH.

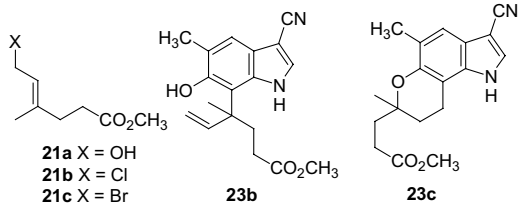
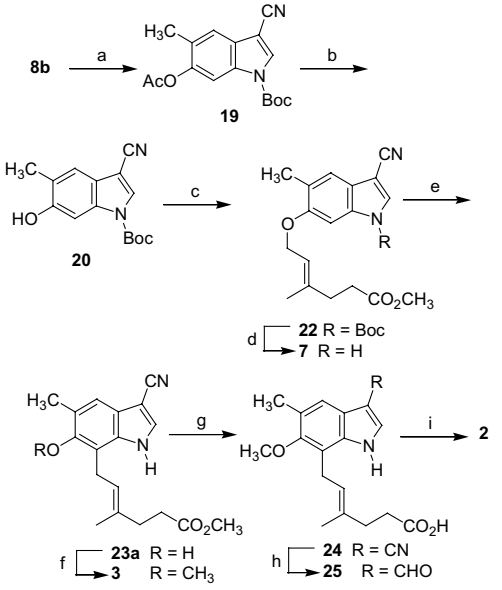


Figure 2. Structure of side chain intermediates (**21a**–**21c**) and products from attempts of 1,3-sigmatropic rearrangement under different conditions (**23b** and **23c**).

THF, or AzobisDMF/Bu_3P/THF, DIAD/Bu_3P/THF or benzene and ADDP/Bu_3P/benzene).[20] The alternative approach, S_N2 alkylation of the phenol, was not chosen originally because selective nucleophilic *O*-alkylation (vs *N*-alkylation) of 6-hydroxyindoles having an electron-withdrawing group at C-3 was found to be problematic in both the literature and in model alkylation of **8d**.

In the S_N2 alkylation approach, it was necessary to protect the indole NH selectively before *O*-alkylation. The protection step was complicated due to similarities in the pK_a of the NH and OH of **8d**. Thus, **8d** was acetylated to **8b**, which was converted into *N*-Boc-*O*-acetyl **19** with Boc anhydride in the presence of DIEA as an *O*-acetyl 'friendly' base (Scheme 4). Selective hydrolysis of the acetate with a buffer mixture of Na_2CO_3 and $NaHCO_3$ in aqueous dioxane gave **20**. Stronger basic conditions caused partial to complete loss of the Boc group. Ether formation was carried out successfully at room temperature using the 6-chlorohexenoate **21b**; subsequent cleavage of the Boc moiety gave the key intermediate **7**.



Scheme 4. Reagents and conditions: (a) $(Boc)_2O$, 2% DIEA in MeCN; (b) Na_2CO_3, $NaHCO_3$, aq Dioxane; (c) **21b**, K_2CO_3, acetone, 25 °C; (d) NaOMe, MeOH; (e) Montmorillonite KSF, toluene, reflux, 20 min; (f) diazomethane, MeOH, ether, 25 °C; (g) LiOH, MeOH, H_2O; (h) i. Dibal-H, CH_2Cl_2, −78 °C; ii. Tartaric acid, H_2O; (i) NH_3, NaCN, MnO_2, 2-propanol, 0–5 °C.

The next step in the synthesis was the 1,3-sigmatropic rearrangement to place the side chain in position 7 of the indole (compound **23a**). The best results were obtained when a solution of **7** in toluene was heated in the presence of montmorillonite KSF. Other catalysts were less effective: Florisil, a reported catalyst for the synthesis of MPA analogs, afforded a very low amount (about 10%) of a substance thought be the 3,3-sigmatropic (Claisen) product **23b** (^{1}H NMR data); $BF_3{\cdot}Et_2O$ at room temperature was excessively powerful and induced formation of the benzopyran **23c**; $BF_3{\cdot}Et_2O/CH_2Cl_2$ at 0 °C and $ZnCl_2/CH_2Cl_2$ at reflux furnished the phenol **23a** and significant amounts of the dealkylated side product **8d**. During the optimization of the montmorillonite KSF-catalyzed rearrangement, the interstitial water in the clay was critical to the reaction outcome. If the clay was dried (toluene azeotrope) before adding **7**, the 1,3-sigmatropic rearrangement was much slower (TLC). Moreover, the yield of the desired phenol **23a** was lower and the amount of dealkylation side product **8d** was higher compared to the use of the catalyst without predrying.

The unstable phenol **23a** was methylated promptly to give the critical intermediate **3**. In model experiments with **8d**, TMS-diazomethane afforded selective *O*-methylation to give **26** (structure in Scheme 5). In a similar manner, treatment of **23a** with diazomethane afforded **3** but the reaction mixture must contain a protogenic solvent such as MeOH. Dry ethereal solutions of diazomethane consistently showed no reactivity with this phenol.

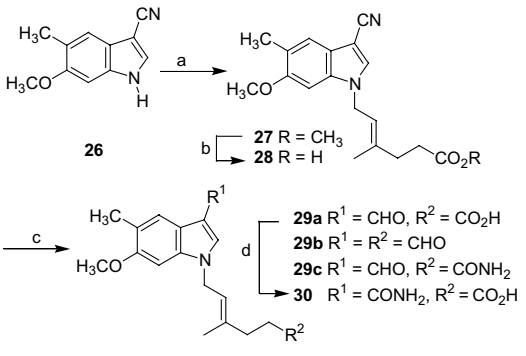
The next steps involved the conversion of the ester and nitrile in **3** into a carboxylic acid and a carboxylic amide, respectively. Neither alkaline nor acidic hydrolysis gave the amide nitrile. A different approach, following the method used in our synthesis of **1**, involved reduction of the nitrile to an aldehyde followed by oxidative amination to give the amide. Model studies were carried out with **27**, prepared via reaction of **26** with **21c** (Scheme 5).

The ester **27** was converted to the carboxylic acid **28** to prevent its reduction with Dibal-H. The cyano acid **28** was treated with Dibal-H[21] in CH_2Cl_2 at 0 °C followed by quenching with aqueous potassium sodium tartrate. Three products were isolated: the desired aldehydic acid **29a** (23%), the dialdehyde **29b** (13%), and a compound that was identified to be the aldehydic amide **29c** (22%). The structure of **29c** was assigned based on an upfield shift in the ^{1}H NMR signals for the 2′- and 3′-methylenes of the hexenoyl side chain and with the appearance of amidic NH_2. Other evidence for the assignment of **29c** was an aliphatic amide-1 C=O stretching peak in the IR spectrum at 1650 cm^{-1} (cf. 1724 cm^{-1} for the acid). No further characterization of this unstable byproduct was conducted.

The only nitrogen source for this unexpected reaction was the nitrile group. It was hypothesized that a hyperactive aluminum amide entity[22] $(i\text{-butyl})_2AlNH_2$ was formed by the action of water during the quench with the weakly basic Rochelle salt (Fig. 3). Therefore, the reaction mixture was quenched in acidic pH, (tartaric acid instead of potassium sodium tartrate) to suppress the formation of **29c** via instantaneous deactivation of the diisobutyl aluminum amide before attacking the activated aluminum carboxylate group. Two changes in the reaction conditions, performing the reaction at very low temperature (−78 °C) followed by a tartaric acid quench, led to complete disappearance of both side products (**29b** and **29c**) and formation of the desired aldehyde **29a** in a good yield. This aldehyde was oxidized into the corresponding amide **30** in a modest yield along with a significant amount of the cyano acid **28**. Compound **2** was prepared in three steps from **3** (Scheme 4) by the same procedure.

3. Structure-based molecular modeling

Structure-based computer modeling studies were conducted to explore possible binding modes of **2** with IMPDH. Vertex's Chinese Hamster IMPDH was selected as our first target from several described mammalian and microbial crystal structures of IMPDH due to its similarity with Human type-II (99% homology). In addition, it is bound to MPA as a ligand in the NAD



Scheme 5. Reagents and conditions: (a) **21c**, K_2CO_3, acetone, reflux; (b) KOH, MeOH, H_2O; (c) i. Dibal-H, CH_2Cl_2, 0 °C; (ii) Rochelle salt; (d) NH_3, NaCN, MnO_2, 2-propanol, 0–5 °C.

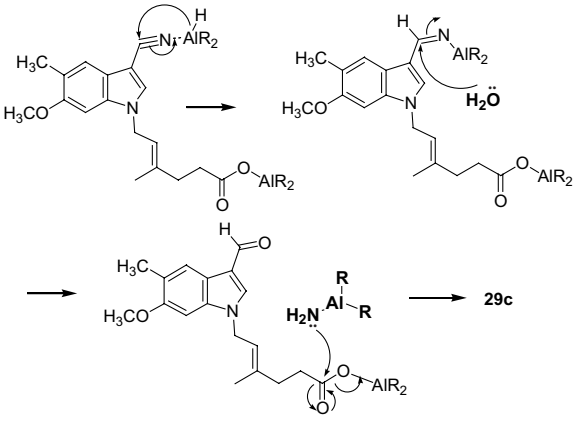


Figure 3. Proposed mechanism for formation of **29c**.

site.[23] The design of the indoles **1** and **2** was based on MPA as a lead structure so, as a first step, it was assumed that all would exhibit similar binding. The crystal structure of MPA with the IMPDH revealed that three major areas establish direct contacts for binding inside the active site. These are: (1) the π–π stacking interaction of the aromatic phthalide nucleus and the hypoxanthine nucleus of the IMP; (2) the network of H-bonding of C-7 phenol and lactone with Thr-333, Gln-441, and Gly-326 in which the phenol, the hydroxyl group of Thr-333 and the Gln-441 NH form a triangle of H-bonding while the lactone establishes two sets of bifurcate H-bonding with Gly-326 and Thr-333; (3) ionic and H-bonding interactions of the side chain carboxylic acid with Ser-276.

The indole **5** was built (using the SYBYL molecular modeling package, see Experimental) by modifying the structure of MPA extracted from the crystal structure of the complex. An overlay of both structures (Fig. 4A) reveals a good fit of the MPA lactone with the indole **2** amide and the carboxylic acids with one another.

The next experiment was to use the BUILD and DOCK techniques of SYBYL to investigate indole-3-carboxamide **2** binding with IMPDH. In the BUILD method, the MPA phthalide nucleus was modified into the indole **2** inside the active site. In the second method, the MPA structure was extracted outside the active site, modified to the indole **2** and docked into the NAD binding pocket in a way that maintained a good visual binding and a low SYBYL docking energy. In all DOCK experiments a priority was given to the aromatic π-stacking with the IMP and the interaction of the hexenoate side chain carboxylate because they are common features between both MPA and **2**.

Both DOCK and BUILD provided similar results in the examination of the interaction of the indole-3-carboxamide with the active site. However, the C2 of indole approached the side chain of Thr-333 unfavorably (1.9 Å) (Fig. 6A).

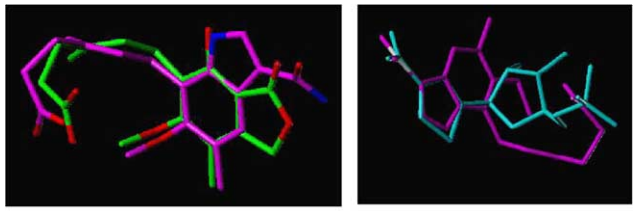


Figure 4. Overlay of **2** with MPA and SAD. The indole **2** is shown in magenta, MPA is green (A), and SMN is shown in cyan (B).

The question is, therefore, whether the protein will mobilize to accommodate this ligand. This depends on the mobility of this part of the protein. In this regard Thr-333 is one of the residues in a very mobile strand of the enzyme, the active site loop. This residue moved 7.2 Å when the active site loop shifted to react with the 6-Cl IMP, an inhibitor in the Human II enzyme crystal structure (Fig. 5). However, when we ran energy optimization (100 iterations), the protein strand was not forced to move away from the ligand. The SYBYL force field preferred slight adjustments in the ligand conformation (Fig. 6c). Consequently, the interaction profile of **2** differed from that of MPA after the computational

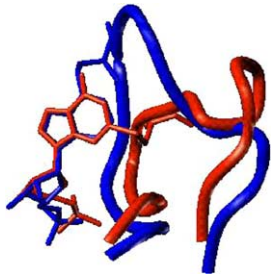


Figure 5. Comparison of the active site loops covalently attached to a substrate site ligand. The Chinese Hamster IMPDH is shown in red and the Human II IMPDH is shown in Blue. The IMP C2-Cys-331 adduct seen in the Hamster complex is replaced by a C6-Cys-331 adduct in Human II enzyme. This displaces the loop to the opposite site of purine.

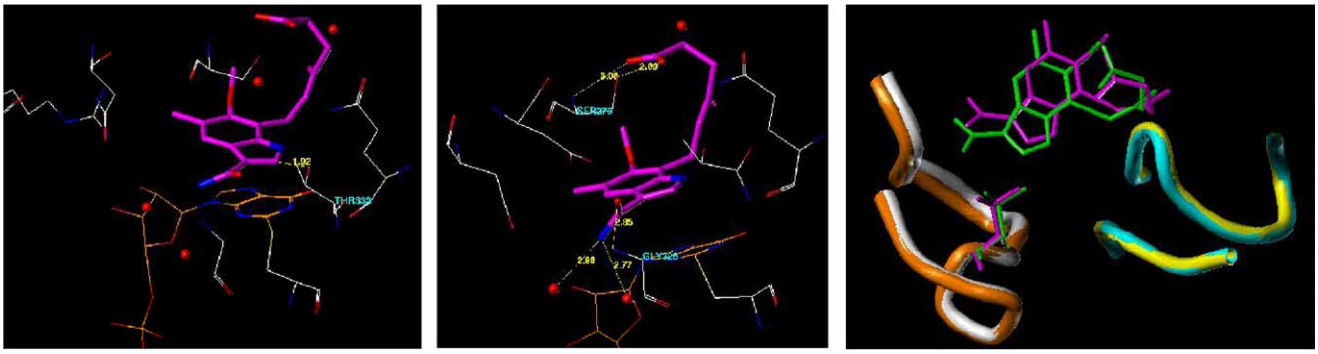


Figure 6. Docking of **2** into Chinese Hamster IMPDH. (A) The indole **2** (magenta) built via modification of MPA inside Chinese hamster IMPDH active site. Note the steric interaction between the C2 on indole and the side chain of the Thr-333. The IMP is highlighted in orange. (B) The model illustrated in A after energy minimization. Water molecules are shown as red spheres. The distances shown are attributed to the H bonding of the 3-carboxamide and the side chain carboxylic acid group with the active site. (C) Active site loop (orange tube) and flap (cyan tube) in A (before minimization) compared with B. The loop in B is shown in white and the flap is shown in yellow. The indole **2** and Thr-333 are colored magenta (before minimization) and green (after minimization). Note the slight movement of the loop toward the modeled ligand **2** to establish new H-bonds with the amide group.

optimization. The indole NH appeared to be unable to establish interaction with Thr-333. The loops of the protein were not severely shifted. The carboxamide of the indole has the potential to make H-bonds with Gly-326 and two molecules of water in the active site.[24]

Although results of binding experiments with the Chinese Hamster IMPDH were satisfactory, we needed to model our compound with the Human type-II IMPDH.[25] There are similarities between **2** and selenazofurin monophosphate (SMN, Fig. 1), a truncated analog of SAD (an inhibitor occupying the NAD site). Both compounds have a carboxamide attached to an aromatic heterocyclic ring. This carboxamide group was an essential component for the activity of **1**, the first generation indole MPA analogs. Replacement of this amide with ester or hydrogen resulted in complete loss of activity. An overlay of SMN with **2** in a way that superposes both the carboxamide group with the five-membered heterocyclic ring and the carboxamide groups of both structures led to a good fit of the carboxylate group of **2** with the phosphate group of SMN (Fig. 4B). However, in the Human-II IMPDH crystal structure, the carboxamide of SMN does not interact with the same amino acid residues that bind with the lactone of the MPA. It interacts principally with Asn-303 side chain amide, the ribose 2′-OH of the IMP, and the Gly-324 carbonyl.

When **2** was docked into the active site of Human type-II IMPDH, the 3-carboxamide and the side chain carboxylate showed potential interactions with the Asn-303 and Ser-276. Moreover, there were no significant steric conflicts with any part of the enzyme except a nonsignificant approach of the side chain with Gln-441 side chain.

4. Biological testing on cancer cell lines

The target compound **2**, **24**, and **30** were tested against three different cancer cell lines, MCF7-S (human breast cancer), MCF7-R (multi-drug resistance cancer), and IGR-OV1 (human ovarian cystoadenocarcinoma). None of the indoles showed significant inhibitory activity on cell proliferation (Table 1).

5. Conclusion

Promising activity of an earlier analog, convincing design, and modeling studies encouraged us to undertake the synthesis of **2**, an analog of the natural product MPA. The synthesis was accomplished using two strategies. The convergent route (utilizing the 1,3-sigmatropic rearrangement) was more efficient than the method centered on building the compound using Patterson's double Claisen and palladium catalyzed indole synthesis. Without data against the isolated enzyme, we cannot rationalize the lack of activity.

Table 1. Testing of indoles **2**, **24**, and **30** on cancer cell lines

Compound	IC_{50} μM ± SE (% growth inhibition at highest concentration tested)		
	MCF7-S	MCF7-R	IGR-OV1
MPA	0.098 ± 0.004	0.48 ± 0.03	0.34 ± 0.03
2	>100 (0)	>100 (0)	>100 (0)
24	>100 (15)	>100 (0)	>100 (0)
30	>100 (25)	>100 (6)	>100 (0)

6. Experimental

6.1. Instrumentation

Melting points were determined in an open capillary and are uncorrected. IR spectra were determined with a Mattson FT-IR interferometer. ^{1}H NMR spectra were recorded with Varian Inova-500, Inova-400, and Gemini-300 MHz spectrometers at 500, 400, and 300 MHz, respectively. ^{13}C NMR spectra were recorded on 125, 75 MHz. High-resolution (HRMS) and low resolution (LRMS) mass spectra were obtained from the University at Buffalo, Chemistry Department, mass spectrometry facility. Combustion analyses were performed at Atlantic Microlabs, Altanta, GA. Ozonolyses were done on a Welsbach Ozonizer.

6.2. Compound 2

A mixture of the aldehyde **25** (44 mg, 0.140 mmol), NaCN (35 mg, 0.71 mmol), and 2-propanol (1.5 mL) was placed in a glass pressure tube. The mixture was cooled to −40 °C and purged with ammonia. MnO_2 (304 mg, 3.5 mmol) was added, the tube was capped tightly with a Teflon screw cap and the mixture was stirred at 3 °C for 48 h. The reaction mixture was cooled to −40 °C, the tube was uncapped, and the mixture was filtered through Celite. The isopropanol was removed in vacuo and the solid residue was treated with saturated sodium chloride solution containing 10% 2 M HCl (pH 3–4). The product was extracted with EtOAc. The organic layer was washed with brine, dried, and concentrated in vacuo. The crude product was subjected to flash column chromatography (1% formic acid in CH_2Cl_2–EtOAc, 1:1) to give starting material(20 mg, 45%), the side product **24** (4 mg, 9%) and **2** (19 mg, 41%) as white solid: Mp 201.5–203 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.22 (br, 1H), 7.92 (d, 2H, $J = 2.5$ Hz), 7.78 (s, 1H), 7.41–7.19 (br, 1H), 6.81–6.60 (br, 1H), 5.24 (t, 2H, $J = 6.5$ Hz), 3.62 (s, 3H), 3.50 (d, 2H, $J = 6.5$ Hz), 2.28 (s, 3H), 2.24 (t, 2H, $J = 8.0$ Hz), 2.16 (t, 1H, $J = 8.0$ Hz), 1.77 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 177.35, 171.03, 153.82, 136.42, 135.80, 130.18, 126.03, 124.49, 123.86, 121.01, 118.37, 110.96, 61.50, 35.85, 34.01, 25.19, 17.09, 16.39; IR (KBr pellet) 3394, 3330, 3224, 1627, 1575 cm^{-1}; HRMS (EI): Required M^+ for $C_{18}H_{22}N_2O_4$, 330.1578; Found, 330.1578.

6.3. Compound 3

6.3.1. Method A . A solution of the enamine **4** (165 mg, 0.405 mmol) in DMF (0.3 mL) was placed in an ampoule

under an argon atmosphere and treated with Pd acetate (4.5 mg, 0.02 mmol), tris-(*o*-tolyl)phosphine (61 mg, 0.2 mmol), and triethylamine (0.112 mL). The ampoule was sealed and heated in an oven at 120 °C for 6.5 h. The mixture was cooled, partitioned between ether and water, and the organic layer was washed, dried (Na_2 SO_4), and evaporated in vacuo. The product was purified by flash column chromatography to give **3** (45 mg, 34%) as a white solid.

6.3.2. Method B. A solution of **23a** (588 mg, 1.88 mmol) in MeOH–ether (1:1, 6 mL) was treated with the diazomethane solution[26] and stirred at room temperature for 24 h. The solvent was removed in vacuo and the residue was purified by flash chromatography (hexanes–EtOAc, 3:1) to furnish a colorless mass of **3** (464 mg, 76%), which solidified (white solid) upon standing for a few days: Mp 102–104 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.43 (br, 1H), 7.65 (d, 1H, $J = 2.8$ Hz), 7.39 (s, 1H), 5.40 (t, 1H, $J = 7.2$ Hz), 3.72 (s, 3H), 3.69 (d, 2H, $J = 7.2$ Hz), 3.58 (s, 3H), 2.52 (t, 2H, $J = 8.2$ Hz), 2.59 (s, 3H), 2.54 (t, 2H, $J = 8.2$ Hz), 1.85 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.51, 153.41, 135.92, 133.76, 132.27, 126.30, 123.84, 123.24, 118.53, 117.00, 116.40, 85.99, 61.01, 51.67, 34.40, 31.32, 24.56, 16.73, 15.86; IR (neat) 3305, 3123, 2949, 2217, 1734, 1528, 1450 cm^{-1}; Anal. Calcd for ($C_{19}H_{22}N_2O_3$): C, 69.92; H, 6.79; N, 8.58. Found: C, 69.87; H, 6.80; N, 8.51.

6.4. Compound 4

A solution of **5** (880 mg, 2.47 mmol) in THF (10 mL) was treated successively with acrylonitrile (0.229 mL, 3.46 mmol), benzoquinone (267 mg, 2.47 mmol), LiCl (anhydrous, 1.047 g, 24.7 mmol), and $PdCl_2(CH_3CN)_2$ (64 mg, 0.24 mmol) at room temperature under argon atmosphere. The mixture was stirred at 50 °C for 18 h. The solvent was removed in vacuo. The product was dissolved in $CHCl_3$ (100 mL), washed with water, dried, and evaporated in vacuo. The resulting residue was subjected to flash chromatography (10:1 isopropyl ether–EtOAc) to give **4** (119 mg, 12%) as a yellowish brown oil: ^{1}H NMR (signals for *E* isomer) δ 7.32 (s, 1H), 7.16 (dd, 1H, $J = 13.8$ and 8.1 Hz), 6.31 (d, 1H, $J = 8.1$ Hz), 5.00 (t, 1H, $J = 6.0$ Hz), 3.92 (d, 1H, $J = 13.8$ Hz), 3.98 (s, 3H), 3.54 (s, 3H), 3.32 (d, 2H, $J = 6.0$), 2.42 (t, 2H, $J = 6.6$ Hz), 2.28 (t, 2H, $J = 6.6$ Hz), 1.75 (s, 3H), LRMS (FAB) 409.24 (91.2%, M+2), 408.24 (90.5%, M+1), 407.24 (100%, M), 406.24 (74.1%, M−1); IR (neat) 2204, 1726 cm^{-1}.

6.5. Compound 5

N-Bromosuccinimide (308 mg, 1.73 mmol) was added to a solution of the aniline **17** (400 mg, 1.44 mmol) in DMF (11 mL) and the mixture was stirred at room temperature for 24 h. The mixture was then partitioned between Et_2O and water. The organic layer was dried and concentrated in vacuo. The residue was subjected to flash column chromatography (Et_2O–Hexanes, 2:1) to afford 418 mg (81%) of **5** as reddish brown oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.11 (s, 1H), 5.06 (t, 1H, $J = 6.0$ and 1 Hz), 3.94 (br, 2H), 3.63 (s, 3H), 3.59 (s, 3H), 3.34 (d, 2H, $J = 6.0$ Hz), 2.40 (t, 2H, $J = 6.9$ Hz), 2.31 (t, 2H, $J = 6.9$ Hz), 2.15 (s, 3H), 1.79 (s, 3H).

6.6. Compound 7

A stirred solution of **22** (412 mg, 1 mmol) in THF (5 mL) under argon was cooled to 0 °C and treated with NaOMe (1 mL of 25% solution in MeOH). The temperature was raised to ambient after 10 min and the reaction was continued for 1.5 h. The mixture was poured into cold 0.2 M HCl to produce a precipitate. The product was extracted with EtOAc and the combined extract was washed with water, dried, and evaporated in vacuo. The product was purified using flash column chromatography (CH_2Cl_2–hexanes–EtOAc, 3:2:1) to furnish **7** (292 mg, 94%) as a white solid: Mp 114 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.50 (br, 1H), 7.57 (d, 1H, $J = 2.4$ Hz), 7.48 (s, 1H), 6.85 (s, 1H), 5.51 (t, 1H, $J = 6.0$ Hz), 4.57 (d, 2H, $J = 6.0$ Hz), 3.58 (s, 3H), 2.49 (t, 2H, $J = 7.2$ Hz), 2.41 (t, 2H, $J = 7.2$ Hz), 2.33 (s, 3H), 1.76 (s, 3H); IR (KBr pellet) 3289, 2216, 1730 cm^{-1}; Anal. Calcd for ($C_{18}H_{20}N_2O_3$): C, 69.21; H, 6.45; N, 8.97. Found: C, 69.04; H, 6.41; N, 8.98.

6.7. Compound 8a

A solution of **9** (22 g, 63.74 mmol) in DMF (35 mL) was treated with Pd acetate (716 mg, 3.15 mmol) and Et_3N (26.6 mL, 0.19 mol). The reaction was heated to 80 °C for 20 h under argon atmosphere. The mixture was cooled, EtOAc (500 mL) was added and the precipitated solids filtered. The organic layer was washed with water, dried, and evaporated in vacuo to give a brown solid. Chromatography (hexanes–EtOAc gradient, 2:1 to 1:1) gave (according to the order of elution) the diacetate **8b** (180 mg, 1%), the *N*-acetylphenol **8c** (1.2 g, 9%), **8a** (9.37 g, 69%), and the deacylated compound **8d** (1.93 g, 17%). Compound **8a** was obtained as off-white crystals: Mp 177–179 °C; ^{1}H NMR ($CDCl_3$) δ 8.85 (br, 1H), 7.56 (s, 1H), 7.54 (d, 1H, $J = 3.0$ Hz), 7.06 (s, 1H), 2.38 (s, 3H), 2.29 (s, 3H); IR (KBr pellet) 3304, 2218, 1739 cm^{-1}. Anal. Calcd for ($C_{12}H_{10}N_2O_2$): C, 67.28; H, 4.71; N, 13.08. Found: C, 67.28; H, 4.72; N, 12.96.

6.8. Compound 8b

A mixture of **8d** (4.7 g, 27.29 mmol), acetic anhydride (100 mL), and pyridine (50 mL) was stirred for 12 h at ambient temperature. The solution was concentrated in vacuo and the residue was treated with petroleum ether (40–60, 200 mL). The resulting solid was filtered and the crude product was subjected to flash column chromatography (CH_2Cl_2) to give 6.00 g (85%) of **8b**: Mp 178–178.5; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.11 (s, 1H), 7.90 (s, 1H), 7.54 (s, 1H), 2.65 (s, 3H), 2.91 (s, 3H), 2.05 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.28, 169.84, 148.58, 137.57, 133.00, 128.36, 125.86, 120.69, 114.77,

110.94, 91.30, 24.16, 21.20, 16.59; IR (KBr pellet) 1751, 1734 cm^{-1}; HRMS (EI) Required M^+ for $C_{14}H_{12}N_2O_3$: 256.0848; Found: 256.0853.

6.9. Compound 8c

This compound was obtained as white crystals: Mp 231–234; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.28 (s, 1H), 8.57 (s, 1H), 7.87 (s, 1H), 7.33 (s, 1H), 7.33 (s, 1H, disappeared when D_2O was added), 2.61 (s, 3H), 2.19 (s, 3H); IR 3255, 2221, 1747, 1623; Anal. Calcd for ($C_{12}H_{10}N_2O_2$): C, 67.28; H, 4.71; N, 13.08. Found: C, 67.32; H, 4.69; N, 13.08.

6.10. Compound 8d

A mixture of **8a** (1.16 g, 5.42 mmol), 10% aqueous K_2CO_3 (20 mL), and methanol (20 mL) was stirred until it became a clear solution (about 5 h at ambient temperature). Ice cooled 2 M HCl was added until the pH became 2–3 then the mixture was extracted with EtOAc (3 × 100 mL). The combined organic layers was washed with water, dried, and evaporated in vacuo to give **8d** (0.91 g, 97%) as a light brown solid (purity >98%). A small portion of the phenol was crystallized from CH_2Cl_2–methanol: Mp 246–249 °C (dec); ^1H NMR (DMSO-d_6, 400 MHz) δ 11.64 (br, 1H), 9.34 (s, 1H), 7.92 (d, 2H, $J = 2.8$ Hz), 7.24 (s, 1H), 6.87 (s, 1H), 2.17 (s, 3H); IR (KBr pellet) 3408, 3305, 2222 cm^{-1}; Anal. Calcd for ($C_{10}H_8N_2O$): C, 69.76; H, 4.68; N, 16.27. Found: C, 69.48; H, 4.77; N, 16.12.

6.11. Compound 9

A mixture of 3,3-diethoxypropionitrile (13.5 mL, 90 mmol), trifluoroacetic acid (41 mL), water (13.6 mL), and $CHCl_3$ (13.6 mL) was stirred for 15 h then added to a solution of **18** (26.19 g, 90 mmol) in EtOAc (200 mL). The mixture was stirred for 7 h, the precipitated product (>95% pure) was collected by filtration and washed with fresh EtOAc. The filtrate was washed (saturated aqueous $NaHCO_3$), dried, and concentrated. Flash column chromatography afforded the desired enamine **9** as a yellow solid (combined yield 19.9 g, 65%): Mp 155–156 °C; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.71 (d, 1H, $J = 11.2$ Hz), 7.70 (s, 1H), 7.54 (dd, 1H, $J = 13.6$ and 11.2 Hz), 7.06 (s, 1H), 4.91 (d, 1H, $J = 13.6$ Hz), 2.27 (s, 3H), 2.00 (s, 3H); IR (KBr pellet) 3314, 2192, 1747; Anal. Calcd for ($C_{12}H_{11}IN_2O_2$): C, 42.13; H, 3.24; I, 37.09; N, 8.19. Found: C, 42.17; H, 3.25; I, 37.15; N, 8.16.

6.12. Compound 10

A suspension of 2-methyl-5-nitroaniline **6** (22.8 g, 0.15 mol) in a mixture of sulfuric acid (33 mL), water (25 mL), and crushed ice (90 g) was stirred in an ice bath. Sodium nitrite solution (10.9 g, 0.16 mol in 26 mL water) was added dropwise from an addition funnel while maintaining the temperature between 0 and 5 °C. After completion of the addition, the mixture was stirred for 30 min at the same temperature range until it became a clear solution. The mixture was poured into heated (120 °C) sulfuric acid (100 mL) and water (75 mL) and the heating was continued for 20 min before cooling to 5 °C. The phenol separated as a light brown crystalline solid that was filtered and rinsed with water until neutral by pH paper. The solid was dried in a vacuum oven at 50 °C for 3 h to give 21.3 g (93%) of crude phenol with purity estimated to be about 95% from the ^1H NMR. The phenol was used for the next reaction without further purification: Mp 110–112 °C; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.37 (s, 1H), 7.57 (complex, 2H), 7.32 (d, 1H, $J = 8.4$ Hz), 2.20 (s, 3H).

A stirred mixture of 2-methyl-5-nitrophenol (37.68 g, 0.25 mol), acetone (310 mL), potassium carbonate (32.82 g, 0.24 mol), and allyl bromide (23.4 mL, 0.27 mol) was heated at reflux for 4 h. The mixture was cooled and filtered to remove the inorganic salt. The filtrate was concentrated in vacuo and the crude oily product was purified by flash column chromatography (CH_2Cl_2) to give **10** (46.0 g, 90% in two steps) as a yellow crystalline solid: Mp 35–37 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.76 (dd, 1H, $J = 8.4$ and 2.1 Hz), 7.63 (d, 1H, $J = 2.1$ Hz), 7.25 (d, 1H, $J = 8.4$ Hz), 6.06 (m, 1H), 5.46 (dd, $J = 17.1$ and 1.2 Hz), 5.33 (dd, $J = 10.5$ and 1.2 Hz), 4.62 (d, $J = 5.1$ Hz), 2.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.66, 147.00, 135.15, 132.22, 130.49, 117.83, 115.68, 105.73, 68.99, 16.57.

6.13. Compound 11

A solution of **10** (19.3 g, 0.1 mol) in EtOAc (200 mL) was treated with $SnCl_2 2H_2O$ (126 g, 0.5 mol) and heated at 65–70 °C for 1 h. EtOAc (1 L) and Na_2CO_3 (10% solution) were added until no more precipitation occurred. The gelatinous precipitate was filtered through Celite. The solid cake was stirred with EtOAc (30 mL) and re-filtered. The combined filtrate was washed with Na_2CO_3, water, dried, and evaporated in vacuo to give 15.5 g (90%) of the aniline as yellowish brown oil. The TLC (CH_2Cl_2–EtOAc, 5:1) and the ^1H NMR showed that the product was pure. The product was used for the next step: ^1H NMR (DMSO-d_6, 300 MHz) δ 6.75 (d, 1H, $J = 7.9$ Hz), 6.21 (d, 1H, $J = 2.0$ Hz), 6.10 (dd, 1H, $J = 7.9$ and 2.0 Hz), 6.04 (m, 1H), 5.39 (dd, 1H, $J = 17.2$ and 1.8 Hz), 5.22 (dd, 1H, $J = 10.5$ and 1.8 Hz), 4.77 (br, 2H), 4.43 (d, 2H, $J = 5.5$ Hz), 2.01 (s, 3H). A solution of this aniline (14.70 g, 0.090 mol) and phthalic anhydride (16.00 g, 0.108 mol) in DMF (18 mL) was heated at reflux for 2 h under argon. The mixture was cooled and poured into dilute HCl at 0 °C. The brown precipitate was collected by filtration and crystallized from methanol to give **11** as a brownish solid (16.22 g). The filtrate from the crystallization was chromatographed (CH_2Cl_2) to give an additional 2.6 g of **11** as a yellowish white solid. The pure product weighed 18.82 g (71%): Mp 153–153.5 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.86 (m, 2H), 7.71 (m, 2H), 7.19 (d, 1H, $J = 8.0$ Hz), 6.86 (dd, 1H, $J = 8.0$ and 1.5 Hz), 6.80 (d,

1H, $J = 1.5$ Hz), 6.00 (m, 1H), 5.37 (dd, 1H, $J = 17.5$ and 1.5 Hz), 5.22 (dd, 1H, $J = 10.5$ and 1.5 Hz), 4.49 (d, 2H, $J = 5$ Hz), 2.22 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.38, 156.84, 134.29, 133.02, 131.73, 130.72, 129.23, 127.28, 123.63, 118.67, 117.27, 109.83, 68.79, 16.11; IR (KBr pellet) 1723 cm^{-1}; Anal. Calcd for ($C_{18}H_{15}NO_3$): C, 73.71; H, 5.15; N, 4.77. Found: C, 73.81; H, 5.14; N, 4.77.

6.14. Compound 13

A solution of **11** (12.00 g, 40.91 mmol) in *N*,*N*-diethylaniline (320 mL) in a three-neck round bottom flask was degassed via stirring under vacuum for 20 min then passing argon. The mixture was heated under argon at 195–205 °C for 6 h then cooled to room temperature. The mixture was poured into ice/water-cooled HCl (2 L of 10% solution) to give a light brown precipitate. The solid was collected by filtration and washed several times with dilute HCl and water. The crude phenol **12** (9.85 g, 82%) was used in the next reaction without further purification: Mp 184–187 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.93 (m, 2H), 7.75 (m, 2H), 7.13 (d, 1H, $J = 8.1$ Hz), 6.73 (d, 2H, $J = 8.1$ Hz), 5.91 (m, 1H), 5.36 (s, 1H), 3.29 (d, 2H, $J = 6.3$ Hz), 2.25 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) 167.79, 164.98, 153.68, 135.37, 134.32, 131.89, 129.43, 128.82, 126.16, 123.76, 123.62, 120.93, 117.04, 30.99, 16.07; IR(KBr pellet) 3476, 1711 cm^{-1}.

The phenol **12** (10.83 g, 36.92 mmol) was mixed with iodomethane (11.1 mL; 0.177 mol) and 5.11 g (0.037 mol) of potassium carbonate in acetone (350 mL). The mixture was heated at reflux for 9 h. The mixture was cooled to room temperature and the inorganic solid was filtered. The acetone was evaporated in vacuo to provide the methoxy ether as a brown solid. Purification by flash column chromatography (4% EtOAc in CH_2Cl_2) furnished 9.62 g (85%) of **13** as a yellowish white solid: Mp 112–113 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.91 (m, 2H), 7.76 (m, 2H), 7.18 (d, 1H, $J = 8.0$ Hz), 6.89 (d, 1H, $J = 8.0$ Hz), 5.70 (m, 1H), 4.74 (m, complex, 2H), 3.75 (s, 3H), 3.37 (d, 2H, $J = 6.5$ Hz), 2.34 (s, 3H); Anal. Calcd for ($C_{19}H_{17}NO_3$): C, 74.25; H, 5.58; N, 4.56. Found: C, 74.18; H, 5.63; N, 4.49.

6.15. Compound 14

A solution of **13** (9.25 g, 30 mmol) in methanol–CH_2Cl_2 (420 mL; 1:1) containing pyridine (1 mL) was cooled to −78 °C and ozonized oxygen bubbled through until the solution became blue. Nitrogen was bubbled for 5 min to remove excess ozone, methyl sulfide (6.6 mL, 125 mmol) was added and the mixture was stirred at room temperature overnight. The mixture was concentrated in vacuo and flash chromatography (10% EtOAc in CH_2Cl_2) gave 8.34 g (90%) of **14** as a white solid: Mp 129–132 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.56 (d, 1H, $J = 2.2$ Hz), 7.95 (m, 2H), 7.81 (m, 2H), 7.30 (d, 1H, $J = 8.1$ Hz), 7.00 (d, 1H, $J = 8.1$ Hz), 3.75 (s, 3H), 3.53 (d, 2H, $J = 2.2$Hz), 2.40 (s, 3H).

6.16. Compound 15

A solution of the aldehyde **14** (1.57 g, 5.08 mmol) in THF (23 mL) under argon was cooled to −70 °C. Isopropenyl MgBr (0.923 g, 6.3 mmol as 12.6 mL of 0.5 M solution in THF) was added dropwise over 25 min. The mixture was stirred for 20 min at −70 °C, and then 4 mL of Grignard reagent was added. The reaction flask was warmed to −40 °C and stirring was continued for 30 min until the TLC showed disappearance of starting material. The reaction was quenched by addition of a saturated solution of ammonium chloride (50 mL). The mixture was partitioned between Et_2O (100 mL) and water (100 mL). The organic layer was washed with fresh water, dried, and concentrated in vacuo. The product was purified with flash column chromatography (CH_2Cl_2–EtOAc, 10:1) to provide the alcohol **15** (1.6 g, 90%) as white solid: Mp 113–116 °C (reported as an oil); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.93 (m, 2H), 7.80 (m, 2H), 7.23 (d, 2H, $J = 8.1$ Hz), 6.90 (d, 2H, $J = 8.1$ Hz), 4.83 (s, 1H), 4.67 (s, 1H), 4.17 (m 1H), 3.81 (s, 3H), 2.79 (dd, 1H, $J = 13.0$ and 3.0 Hz), 2.66 (dd, 1H, $J = 13.0$ and 9.5 Hz), 2.36 (s, 2H), 2.15 (s, 1H), 2.04 (d, 1H, $J = 12.9$ Hz), 1.57 (s, 3H); Anal. Calcd for ($C_{21}H_{21}NO_4$): C, 71.78; H, 6.02; N, 3.99. Found: C, 71.68; H, 5.99; N, 3.96.

6.17. Compound 16

The alcohol **15** (1.35 g, 3.84 mmol) was mixed with freshly distilled trimethyl orthoacetate (27 mL) and of propionic acid (0.057 mL). The mixture was stirred at 105–110 °C under an argon atmosphere for 10 h. The mixture was cooled, Et_2O was added, and the organic layer was washed with dilute $NaHCO_3$ solution followed by brine. The ethereal layer was dried (Na_2SO_4) and evaporated in vacuo to provide (1.38 g, 88%) of **16** as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.90 (m, 2H), 7.76 (m, 2H), 7.16 (d, 2H, $J = 8.0$ Hz), 6.87 (d, 2H, $J = 8.0$ Hz), 4.94 (t, 1H, $J = 6.4$ Hz), 3.72 (s, 3H), 3.57 (s, 3H), 3.38 (d, 2H, $J = 6.4$ Hz), 2.33 (s, 3H), 2.12 (t, 2H, $J = 8.5$ Hz), 1.92 (t, 2H, $J = 8.5$ Hz), 1.31 (s, 3H).

6.18. Compound 17

A solution of **16** (1.38 g, 3.39 mmol) in MeOH (20 mL) was treated with hydrazine hydrate (0.49 mL, 10.17 mmol) and the mixture was allowed to stand for 24 h at ambient temperature. The white solid was filtered and the solvent was evaporated in vacuo. The crude product was dissolved in Et_2O and washed with water, dried, and the ether was removed in vacuo. Flash column chromatography (CH_2Cl_2–EtOAc, 4:1) yielded 0.82 g (87%) of **17** as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.25 (d, 1H, $J = 8.1$ Hz), 6.38 (d, 1H, $J = 8.1$ Hz), 5.12 (t, 1H, $J = 6.5$ Hz), 3.65 (s, 3H), 3.59 (s, 3H), 3.1-3.4 (br, 2H), 3.31 (d, 2H, $J = 6.5$ Hz) 2.39 (t, 2H, $J = 6.9$ Hz), 2.31 (t, 2H, $J = 6.9$ Hz), 2.18 (s, 3H), 1.80 (s, 3H); Anal. Calcd for ($C_{16}H_{23}NO_3$): C, 69.29; H, 8.36; N, 5.05. Found: C, 69.29; H, 8.39; N, 5.07.

6.19. Compound 18

A mixture of 2-methyl-5-nitrophenol (22 g, 0.143 mol) in acetic anhydride–pyridine (440 mL; 1:1) was stirred at ambient temperature for 24 h then the solution was concentrated in vacuo. The residue was treated with petroleum ether (40–60) with stirring and scratching. A crystalline light orange solid precipitated (24.0 g, 86%): Mp 69–70 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.01 (dd, 1H, $J = 8.7$ and 2.5 Hz), 7.90 (d, 1H, $J = 2.4$ Hz), 7.39 (d, 1H, $J = 8.7$ Hz), 2.35 (s, 3H), 2.26 (s, 3H). A stirred solution of this product (24 g, 0.123 mol) in EtOAc (240 mL) was heated to 65–70 °C and treated portionwise with $SnCl_2{\cdot}2H_2O$ (138 g, 0.615 mol). The reaction was stirred for 6 h, cooled, EtOAc (1 L) was added and the organic solution was treated with cold Na_2CO_3 (10% solution). The organic layer was separated from the turbid aqueous layer and washed with water containing a few drops of 2 N HCl followed by water. The aqueous layer was filtered through Celite and the filtrate was washed with EtOAc (3×250 mL). The organic layer was washed with water acidified with a few drops of HCl. The combined organic solution was dried and evaporated to give the aniline (16 g; 79%) as an oil that was used without further purification: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.97 (d, 1H, $J = 7.1$ Hz), 6.47 (dd, 1H, $J = 2.4$ and 7.2 Hz), 6.35 (d, 1H, $J = 2.4$ Hz), 3.57 (br, 2H), 2.57 (s, 3H), 2.05 (s, 3H).

A solution of the aniline (12.00 g, 72.6 mmol) in DMF (125 mL) cooled to 0 °C was treated portionwise with *N*-iodosuccinimide (16.35 g, 72.6 mmol) over 30 min. The mixture (protected from light) was stirred for 12 h at ambient temperature, diluted with EtOAc (800 mL), and washed with 10% $Na_2S_2O_3$ then water. The organic layer was dried, concentrated, and purified (flash chromatography, hexanes–EtOAc, 3:1) to give **18** as off-white flaky crystals (14.25 g, 67%): Mp 57–58 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.49 (s, 1H), 6.44 (s, 1H), 4.00 (br, 2H), 2.29 (s, 3H), 2.02 (s, 3H); IR (KBr pellet) 3431, 3344, 1739 cm^{-1}. Anal. Calcd for ($C_9H_{10}INO_2$): C, 37.14; H, 3.46; I, 43.60; N, 4.81. Found: C, 37.24; H, 3.47; I, 43.64; N, 4.81.

6.20. Compound 19

A solution of **8b** (650 mg, 2.53 mmol) and $(Boc)_2O$ (641 mg, 2.79 mmol) in MeCN (10 mL) was treated with DIEA (0.2 mL). The mixture was stirred at ambient temperature for 2 days. The precipitate was filtered and rinsed with petroleum ether (25 mL). The solvent was removed from filtrate in vacuo. The solid residue was stirred with petroleum ether (25 mL) for 30 min then filtered. The combined product (755 mg, 95%) was greater than 98% pure (^{1}H NMR) and needed no further purification. A small portion was chromatographed (CH_2Cl_2–EtOAc, 10:1) to give **19** as a bright white powder: Mp 152–152.5 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) 8.04 (s, 1H), 7.89 (s, 1H), 7.55 (s, 1H), 2.36 (s, 3H), 2.29 (s, 3H), 1.66 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) 169.25, 148.22, 147.86, 133.39, 132.85, 127.39, 125.94, 120.97, 113.89, 109.49, 91.84, 86.00, 27.91, 20.70, 16.41; IR (KBr pellet) 2228, 1747, 1684 cm^{-1}. HRMS (EI) Required M^+ for $C_{17}H_{18}N_2O_4$: 314.1272; Found: 314.1266.

6.21. Compound 20

A solution of **19** (2.05 g, 6.52 mmol) in 1,4-dioxane, (20 mL) was treated with 20 mL of a 3:1 mixture of Na_2CO_3 (10% solution) and $NaHCO_3$ (saturated solution). The mixture was stirred at ambient temperature until all the starting material had disappeared (TLC, CH_2Cl_2). The precipitated solid product was filtered, washed with cold water, and dried. The filtrate was carefully neutralized with ice-cooled HCl (0.5 M solution) until pH 4–5. The precipitated solid was filtered, washed, and dried. The product (1.76 g, 99%) contained about 3–5% of **8d** but was pure enough to be used for the next reaction. For analytical purpose, a small portion was purified by flash column chromatography (CH_2Cl_2) to give **20** as a white powder: Mp 248–251 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.87 (s, 1H), 8.34 (s, 1H), 7.60 (s, 1H), 7.35 (s, 1H), 2.21 (s, 3H), 1.61 (s, 9H); IR (KBr pellet) 3437, 1682 cm^{-1}. HRMS (EI) Required M^+: 272.1161; Found M^+: 272.1179.

6.22. Compound 22

A solution of **20** (824 mg, 3.02 mmol) in acetone (12 mL) was treated with **21b**[12d] (641 mg, 3.63 mmol) and K_2CO_3 (829 mg, 6 mmol) under argon atmosphere. The mixture was stirred for 36 h then the solids (product mixed with inorganic salts) was filtered. This solid solution was dissolved in EtOAc and washed with water, dried, and evaporated in vacuo to furnish pure **25** (303 mg). The filtrate was concentrated in vacuo and subjected to flash column chromatography (1% MeOH in benzene) to give 417 mg of **22** (combined yield 720 mg, 58%): Mp 118–122.5 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.89 (s, 1H), 7.64 (s, 1H), 7.39 (s, 1H), 5.52 (t, 1H, $J = 6.3$ Hz), 4.58 (d, 2H, $J = 6.3$ Hz), 3.62 (s, 3H), 2.47 (t, 2H, $J = 6.6$ Hz), 2.39 (t, 2H, $J = 6.6$ Hz), 2.28 (s, 3H) 1.75 (s, 3H), 1.65 (s, 9H). HRMS (EI): m/z 312.15 (M^+−Boc).

6.23. Compound 23a

A suspension of montmorillonite KSF (12 g) in toluene (120 mL) was heated to reflux. The phenyl ether **22** (1.19 g, 3.80 mmol) was added to the hot mixture and the mixture was heated at reflux was for 20 min. The mixture was cooled to room temperature and the clay was removed by filtration. The mixture was left to stand at room temperature for 30 min, the side-product **8d** (156 mg, 24%) precipitated and it was removed by filtration. The filtrate was concentrated in vacuo and purified using flash column chromatography (CH_2Cl_2–hexanes–EtOAc 12:8:3) to afford the phenol **23a** (588 mg 50%) as a transparent oil, which acquired a brownish color upon standing: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.24 (br, 1H), 7.61 (d, 1H, $J = 3.0$ Hz), 7.37 (s, 1H), 5.47

(t, 1H, $J = 7.0$ Hz), 4.77 (s, 1H), 3.65 (d, 2H, $J = 7.0$ Hz), 3.62 (s, 3H), 2.55 (t, 2H, $J = 7.0$ Hz), 2.42 (t, 2H, $J = 7.0$ Hz), 2.37 (s, 3H), 1.83 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.40, 149.11, 136.40, 133.89, 131.35, 122.38, 121.00, 120.54, 117.78, 116.75, 109.51, 85.57, 51.63, 34.29, 31.50, 25.44, 16.80. 15.87; IR ($CHCl_3$) 3462, 3357, 2220, 1728 cm^{-1}.

6.24. Compound 24

A magnetically stirred solution of **3** (326 mg, 1 mmol) in MeOH (10 mL) was treated with a solution of LiOH (168 mg, 7 mmol in 10 mL H_2O) by dropwise addition with a dropping funnel. The turbid mixture was stirred at ambient temperature until the mixture became homogeneous (about 1.5 h). The mixture was transferred into a beaker and ice/H_2O. HCl solution was added cautiously until pH$\sim$4 then the liberated product was extracted with EtOAc (3×50 mL). The organic layer was washed with water, dried, and evaporated in vacuo. The crude product was purified using flash chromatography (1% formic acid in 5:1 CH_2Cl_2–EtOAc) to yield 220 mg (70%) of **24** as a sticky material that solidified after several days of storage at 5 °C: Mp 42–45 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.22 (br, 1H), 7.64 (d, 1H, $J = 3.0$ Hz), 7.38 (s, 1H), 5.43 (t, 1H, $J = 6.0$ Hz), 3.72 (s, 3H), 3.69 (d, 2H, $J = 6.0$ Hz), 2.56 (t, 2H, $J = 7.0$ Hz), 2.41 (t, 2H, $J = 7.0$ Hz), 2.39 (s, 3H), 1.85 (s, 3H); IR (neat) 3282, 2219, 1706 cm^{-1}; Anal. Calcd for ($C_{18}H_{20}N_2O_3$): C, 69.21; H, 6.45; N, 8.97. Found: C, 69.00; H, 6.69; N, 8.68.

6.25. Compound 25

A solution of **24** (155 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (6 mL) was cooled into −78 °C under argon. Dibal-H (3 mL of 1 M solution in CH_2Cl_2) was added dropwise over 20 min and the mixture was stirred for 90 min. The reaction was quenched by pouring it into 20 mL of 10% solution of tartaric acid (containing four drops of 2 N HCl) while stirring. The mixture was stirred for 30 min. The organic layer was separated, washed, and dried. The aqueous layer was washed with EtOAc (3×25 mL). The EtOAc extract was washed, dried, and combined with the CH_2Cl_2 extract. The combined extract was evaporated in vacuo to give the crude aldehyde, which was purified by flash column chromatography (0.75% formic acid in 3:1 mixture of CH_2Cl_2 and EtOAc) to furnish 56 mg (36%) of the aldehyde **25** as a white powder: Mp 170–171.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.27 (br, 1H), 9.70 (s, 1H), 7.93 (s, 1H), 7.84 (d, 1H, $J = 3.2$ Hz), 5.56 (t, 1H, $J = 6.0$ Hz), 3.74, (d, 2H, $J = 6.0$), 3.73 (s, 3H), 2.65 (t, 2H, $J = 7.2$ Hz), 2.44 (t, 2H, $J = 7.2$ Hz), 2.39 (s, 3H), 1.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 186.24, 176.87, 153.82, 136.33, 136.02, 127.33, 123.16, 121.29, 121.07, 117.52, 117.04, 60.85, 34.51, 30.35, 24.88, 16.72, 15.473; IR (KBr pellet) 3308, 1703 cm^{-1}; Anal. Calcd for ($C_{18}H_{21}NO_4$): C, 68.55; H, 6.71, N, 4.44. Found C, 68.56; H, 6.76, N, 4.49.

6.26. Compound 27

This compound was synthesized using a procedure adopted for preparation of **22**. Purification by flash column chromatography (hexanes–CH_2Cl_2–EtOAc, 2:1:1) provided **27** (1.81 g, 87%) as yellowish crystals: Mp 68–69 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.47 (s, 1H), 7.43 (s, 1H), 6.70 (s, 1H), 5.39 (t, 1H, $J = 6.6$ Hz), 4.66 (d, 2H, $J = 6.6$ Hz), 3.88 (s, 3H), 3.61 (s, 3H), 2.45 (m, complex, 4H), 2.31 (s, 3H), 1.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.05, 155.97, 140.26, 134.52, 132.35, 123.02, 121.19, 120.50, 118.60, 116.37, 91.46, 84.67, 55.45, 51.59, 44.53, 34.17, 32.20, 16.68, 16.46; IR (KBr pellet) 2209, 1736 cm^{-1}; HRMS (EI): Required M^+ for $C_{19}H_{22}N_2O_3$, 326.1630; Found, 326.1614. Anal. Calcd for ($C_{19}H_{22}N_2O_3$): C, 69.92; H, 6.79; N, 8.58. Found: C, 69.90; H, 6.73; N, 8.56.

6.27. Compound 28

0.5 M KOH (50 mL) was added drop-wise at 0 °C to a solution of **27** (1.57 g, 4.81 mmol) in MeOH (40 mL). The turbid mixture was stirred at room temperature for 2 h until it became clear. The solution was cooled to 0 °C and 0.5 M HCl was added dropwise until the pH became 3–4. The product was extracted with EtOAc (500 mL then 2×100 mL). The combined organic extract was washed with water, dried, and evaporated in vacuo. The crude solid was purified with flash column chromatography (1% formic acid in CH_2Cl_2–EtOAc, 2:1) to afford the acid **28** (0.90 g, 59%) as white solid: Mp 127.5–128.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.44 (s, 1H), 7.40 (s, 1H), 6.71 (s, 1H), 5.18 (t, 1H, $J = 6.9$ Hz), 4.65 (d, 2H, $J = 6.9$ Hz), 3.88 (s, 3H), 2.55 (t, 2H, $J = 7.2$ Hz), 2.44 (t, 2H, $J = 7.2$ Hz), 2.32 (s, 3H), 1.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.84, 156.04, 140.14, 134.61, 132.32, 123.06, 121.23, 120.45, 118.68, 116.40, 91.51, 84.57, 55.45, 44.40, 33.79, 32.05, 16.62, 16.38; IR (KBr pellet) 3200-2750, 2214, 1727 cm^{-1}. HRMS (EI): Required M^+ for $C_{18}H_{20}N_2O_3$, 312.1473; Found: 312.1518; Anal. Calcd: C, 69.21; H, 6.45; N, 8.97. Found: C, 68.81; H, 6.46; N, 8.77.

6.28. Compound 29a

This compound was prepared using the procedure for the preparation of **25**. Purification using flash column chromatography (0.75% formic acid in 3:1 mixture of CH_2Cl_2–EtOAc) furnished 56 mg (36%) of the aldehyde **29a** as a white powder: Mp 170–171.5 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.27 (br, 1H), 9.86 (s, 1H), 8.01 (s, 1H), 7.57 (s, 1H), 6.69 (s, 1H), 5.48 (t, 1H, $J = 6.0$ Hz), 4.67, (d, 2H, $J = 6.0$), 3.88 (s, 3H), 2.53 (t, 2H, $J = 7.2$ Hz), 2.44 (t, 2H, $J = 7.2$ Hz), 2.31 (s, 3H), 1.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 184.88, 177.73, 156.05, 140.57, 136.84, 136.67, 123.60, 122.96, 118.71, 118.50, 117.76, 91.39, 55.45, 44.39, 33.97, 32.02, 16.60, 16.46; IR (KBr pellet) 3308, 1703 cm^{-1}.

6.29. Compound 30

This compound was prepared using the procedure for the preparation of **2** starting from 133 mg (0.42 mmol) of

29a. The crude mixture was purified using flash column chromatography (1% formic acid in CH_2Cl_2–EtOAc, 2:1) to give the amide **30** (28 mg, 20%), starting material **29a** (51 mg, 38%), and the cyano side product **28** (20 mg, 15%). The amidic acid **30** was a white powder, Mp 186.5–188 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.06 (br, 1H), 7.85 (s, 1H), 7.76 (s, 1H), 7.21 (s, br, 1H), 6.92 (s, br, 1H), 5.35 (t, 1H, $J = 6.8$ Hz), 4.72 (d, 2H, $J = 6.8$ Hz), 3.81 (s, 3H), 2.36 (t, 2H, $J = 8.0$ Hz), 2.26 (t, 2H, $J = 8.0$ Hz), 2.20 (s, 3H), 1.82 (s, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 173.93, 166.29, 154.23, 138.99; 135.43, 128.93, 122.09, 120.10, 119.83, 119.38, 109.34, 91.84, 55.25, 43.62, 33.89, 32.20, 16.75, 16.22; IR (KBr pellet) 3019 (3200–2850), 1701, 1628 cm^{-1}; HRMS (EI) Required M^+ for $C_{18}H_{22}N_2O_4$: 330.1579; Found: 330.1593.

6.30. Molecular modeling

All the molecular modeling studies were carried out using the SYBYL molecular modeling package[27] running on an SGI Indigo workstation. MPA and NAD were extracted from Chinese Hamster IMPDH[28] and glutathione reductase[29] respectively. SAD was extracted from the crystal structure of Human type-II IMPDH.[30]

The structure of **2** was constructed using the BUILD/EDIT option of SYBYL via modifying the bound structure of the MPA as follows: The lactone ring and the phenolic OH were removed and pyrrole ring imported from the SYBYL fragment library were fused with the benzene structure. On the C-3 of the produced indole, a carboxamide was attached. The structure was given Gasteiger-Marsili charges. The heteroaromatic ring along with the carboxamide group was subjected to a geometry optimization (MAXIMIN2 minimizer, gradient energy change, 0.01 kcal/Å mol; rms displacement, 0.001 Å; nonbonded cutoff, 8.000 Å; dielectric function, distant dependent; dielectric constant, 1.00; iteration: until convergence). The hexenoic acid side chain was kept at the same conformation as the crystal structure.

6.31. Growth inhibition assay[31]

Assessment of cell growth inhibition was determined according to the methods of Skehan et al. (1). Briefly, cells were plated between 800 and 1500 cells/well in 96 well plates and incubated at 37 °C 15–18 h prior to drug addition to allow cell attachment. Compounds to be tested were solubilized in 100% DMSO and further diluted in RPMI-1640 containing 10 mM HEPES. Each cell line was treated with 10 concentrations of iodopaclitaxel (5 log range). After a 72 h incubation, 100 μL of ice-cold 50% TCA was added to each well and incubated for 1 h at 4 °C. Plates were then washed five times with tap water to remove TCA, low-molecular-weight metabolites and serum proteins. 50 μL of 0.4% sulforhodamine B (SRB), an anionic protein stain, was added to each well. SRB Staining changes linearly with increases or decreases in number of cells and protein concentrations at cell densities ranging from very sparse to supraconfluent. These staining characteristics provide an accurate assessment of cell growth (1). Following 5-min incubation at room temperature, plates were rinsed five times with 0.1% acetic acid and air dried. Bound dye was solubilized with 10 mM Tris Base (pH 10.5) for 5 min on a gyratory shaker. Optical density was measured at 570 nm.

Data were fit with the Sigmoid-Emax concentration-effect model (2) with nonlinear regression, weighted by the reciprocal of the square of the predicted response. The fitting software was developed at RPCI with MicroSoft FORTRAN, and uses Marquardt (3) algorithm as adapted by Nash (4) for the nonlinear regression. The concentration of drug, which resulted in 50% growth inhibition (IC50) was calculated.

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