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DESIGN AND SYNTHESIS OF HETEROCYCLIC ANALOGUES OF MYCOPHENOLIC ACID AS POTENTIAL CHEMOTHERAPEUTIC AGENTS

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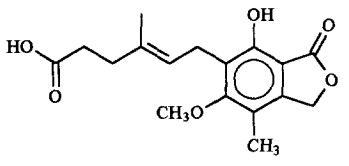
Abstract: A series of heterocyclic analogues **3-10** of mycophenolic acid were designed as metabolically stable congeners possibly targeted for inosine monophosphate dehydrogenase. The synthesis, cytotoxic and anti-HIV activity of these compounds are described.

Mycophenolic acid (MPA) **1**, NSC 129185, is a potent inhibitor of *inosine monophosphate dehydrogenase* (IMPD, IMP:NAD^+ *oxidoreductase*, EC 1.1.1.205), a rate-limiting enzyme in guanine nucleotide biosynthesis.[2] The compound may bind in the NAD^+/NADH domain of the IMPD active site similar to other IMPD inhibitors such as tiazofurin **2**, NSC 286193, and selenazofurin.[3] It has been shown to possess antineoplastic, antifungal, antiviral, antiinflammatory, and immunosuppressive activity.[4] The synthesis and structure activity relationship of MPA have been extensively studied.[4,5,6] Attractive features of this drug include low toxicity and the reversibility of toxicity upon withdrawl of drug. However, a major problem with MPA in humans is the rapid conjugation of the C-7 phenolic hydroxyl group with glucuronic acid, thereby preventing the accumulation of therapeutic levels of the drug.[7]



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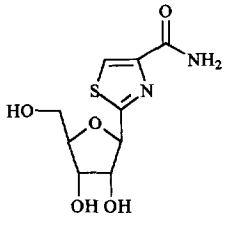
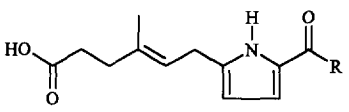


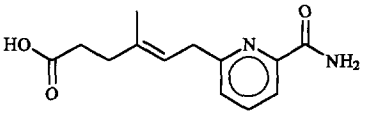
2

As one approach toward solving the problem of conjugation, we tried to replace the metabolically labile phenolic hydroxyl in MPA with more stable moieties which can act as hydrogen bonding groups. First, the phenolic hydroxyl of MPA was replaced by bio-isofunctional heterocyclic groups such as pyrrole **3-4** and pyridine **5** which can function as either a hydrogen bond acceptors or donors in the active site of enzyme.

These heterocycles are called "fixed geometry" hydrogen bonding groups because the colinear hydrogen geometry is more rigid than that of the phenol. The thiazole analogue **6** was also designed based on tiazofurin that is converted to tiazofurin adenine dinucleotide (TAD) *in vivo* mimicking the NAD^+ inverse regulation of IMPD.[8] MPA side chain of this heterocyclic analogue may occupy the ribose phosphate portion of NAD^+ in enzyme.



3 R = NH_2
4 R = OMe



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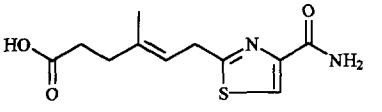
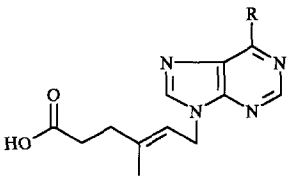
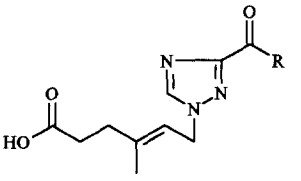


6

According to molecular modeling studies in this laboratory, the superimposition of mycophenolic acid (MPA) and NAD^+ shows that the MPA side chain, (E)-4-methyl-4-hexenoic acid, acts as a ribose phosphate equivalent.[9] The carboxylate moiety of the MPA side chain is overlapped on the NAD^+ phosphate, with the 2′,3′-double bond being well aligned with the C4′-O4′ bond of ribose. A similar relationship can be found in the structures of the naturally occuring neplanocins and the acyclic neplanocin analogues.[10] Also, substitution of the phosphate group with isosteric carboxylic acids in drug design has been successful in other systems. Therefore, the ribotides of 6-chloropurine ribotides and ribavirin 5′-phosphate, IMP analogues as IMPD inhibitors, are replaced by the MPA side chain, (E)-4-methyl-4-hexenoic acid, to provide target compounds **7-10**. These target compounds may bind to the IMP site in IMPD, and not to the NAD^+ site, where MPA and tiazofurin bind.



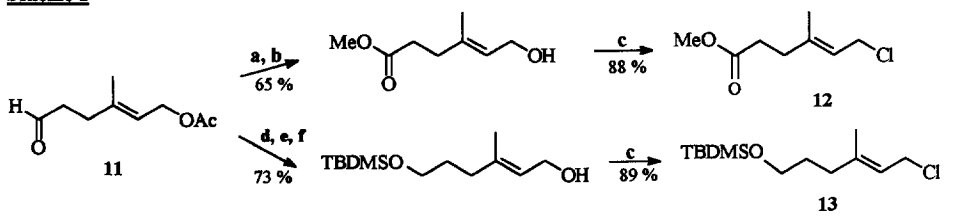
7 R = Cl
8 R = OH



9 R = NH_2
10 R = OH

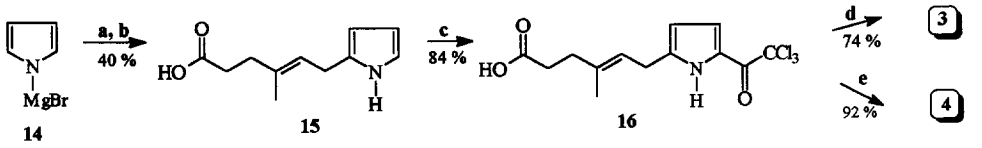
As synthons of the MPA side chain, two allylic chlorides of (E)-4-methyl-4-hexenoic acid, **12** and **13**, were synthesized from aldehyde **11** which was prepared from geraniol acetate by literature procedures.[11] (**Scheme 1**). Synthesis of pyrrole analogues, **3** and **4**, was accomplished via the direct incorporation of the MPA side chain synthon **12** on the pyrrole ring as a key step (**Scheme 2**). Alkylation of pyrrylmagnesium

Scheme 1



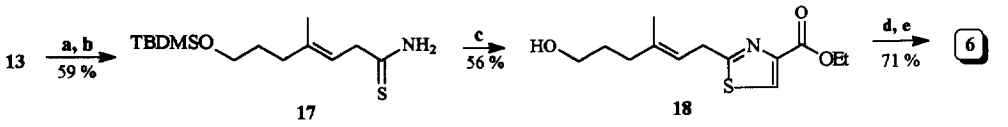
Reagent: (a) $AgNO_3$, KOH, EtOH-H_2O (b) CH_2N_2, ether (c) LiCl, MsCl, 2,6-lutidine, DMF, 0 °C, (d) $NaBH_4$, EtOH (e) TBDMSCl, NEt_3, DMAP, CH_2Cl_2 (f) K_2CO_3, MeOH

Scheme 2



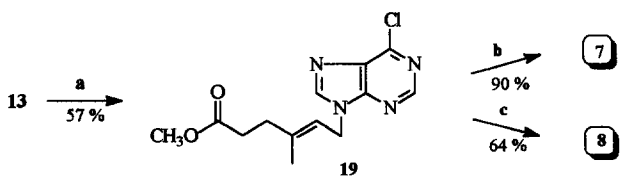
Reagent: (a) **12**, toluene, -40 °C - r.t (b) KOH, EtOH-H_2O (c) Cl_3COCl, ether, 0 °C - r.t (d) NH_3, MeOH (e) NaOMe, MeOH

Scheme 3



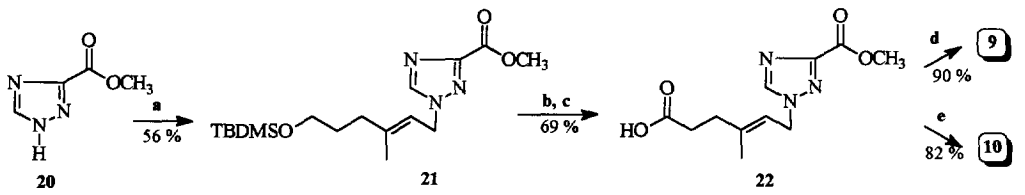
Reagent: (a) LiCN, DMF, r.t (b) H_2S, KOH, MeOH, 90 °C (c) $BrCH_2C(=O)CO_2Et$, CH_3CN, 0 °C - r.t (d) CrO_3, H_2SO_4, acetone (e) NH_3, MeOH

Scheme 4



Reagent: (a) 6-chloropurine, K_2CO_3, DMSO, r.t. (b) 0.5 N KOH, EtOH-H_2O, r.t (c) 0.1 N HCl, reflux

Scheme 5



Reagent: (a) NaH, DMF, then **13** (b) Bu_4NF, THF (c) CrO_3, H_2SO_4, acetone (d) NH_3, MeOH (e) KOH, EtOH-H_2O

bromide **14**[12] with allylic chloride **12** followed by ester hydrolysis, gave 2-alkylated pyrrole **15** as the major product (40%) along with 3-alkylated pyrrole (15%). Ring acylation of **15** with trichloroacetyl chloride[13] gave the expected 2,5-disubstituted pyrrole **16** as the only regioisomer.[14] Ammonolysis of **16** gave the amide analogue **3**, while treatment of **16** with sodium methoxide afforded the ester analogue **4**. Synthesis of pyridine analogue **5** was previously described in detail.[15] Synthesis of tiazofurin analogue **6** was based on the cyclization of the thioamide of the MPA side chain **13** with an α-halo ketone (**Scheme 3**). Allylic chloride **13** was converted to the corresponding nitrile, which was treated with potassium hydroxide and methanol-saturated hydrogen sulfide in a sealed bomb to give thioamide **17** without any deprotection of the sily group. Thioamide **17** was cyclized to the thiazole carboxylate **18** with ethyl bromopyruvate[16], with simultaneous cleavage of the silyl protecting group under the acidic conditions. Oxidation of alcohol **18** followed by ammonolysis produced target compound **6**. Two purine analogues, **7** and **8**, were synthesized as IMP analogues by N-9 alkylation of 6-chloropurine with a MPA side chain synthon **12** (**Scheme 4**). Mild basic hydrolysis of N-9 alkylated **19** afforded 6-chloropurine analogue **7**, while acid hydrolysis of **19** gave the inosine analogue **8**. Synthesis of ribavirin analogues, **9** and **10**, was based on the N-alkylation of methyl 1,2,4-triazole-3(5)-carboxylate **20**[18] with a synthon of the MPA side chain **13** (**Scheme 5**). Alkylation of **20** with allylic chloride **13** at room temperature afforded the 1,3-isomer **21** as the major isomer (56%) along with the 1,5-isomer (12%). Both structures were assigned based on a comparison of chemical shifts in each isomer according to a previous report.[19] Deprotection of **21** followed by Jone's oxidation gave acid **22**. Ammonolysis of **22** produced amide analogue **9**, while basic hydrolysis of **22** gave acid analogue **10**. All synthetic compounds were fully characterized by spectroscopy and elemental analysis.[20]

The lead compound, mycophenolic acid **1**, and all final compounds **3-10** were tested *in vitro* against a number of organ specific human solid tumor cell lines using a sulforhodamine colormetric assay.[21] IC_{50} values of MPA are given in the parentheses: L1210 murine lymphocytic leukemia (<0.2 μM), A549 human NSCLC lung carcinoma (0.4 μM), HT-29 human colon adenocarcinoma (0.9 μM), MCF7 human breast adenocarcinoma (0.5 μM), and PSN-1 human pancreatic carcinoma (0.7 μM). However, none of all prepared compounds showed a significant antitumor activity (IC_{50} = > 100 μM). MPA exhibited low activity (only 20

percent protection at 0.4 μM) against HIV infected T4 lymphocytes (CEM cell line) and was cytotoxic to the uninfected cell with an IC_{50} = 0.9 μM. Among tested compounds, only compound **3** and **4** showed a moderate activity to T4 lymphocytes with IC_{50} = 0.73 mM and 0.36 mM, respectively.

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20. Compound **3**: white solid: mp 129-130 ^{0}C; ^{1}H NMR (acetone-D6) δ 10.7 (bs, 1 H), 10.0 (bs, 1 H), 7.00 (bs, 2 H), 6.77 (m, 1 H), 5.90 (m, 1 H), 5.45 (t, 1 H), 3.40 (d, 2 H), 2.40 (m, 4 H), 1.70 (s, 3 H); IR (KBr) 3465 (NH), 3320, 3209 (OH), 2977, 1712 (C=O), 1633 (C=O), 1591, 1492, 1453, 1407, 1266 cm^{-1}. Anal. Calcd for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 60.85; H, 6.88; N, 11.79.

    Compound **4**: white solid: mp 128 ^{0}C; ^{1}H NMR ($CDCl_3$) δ 9.6 (bs, 1 H), 8.8 (bs, 1 H), 6.83 (m, 1 H), 5.93 (m, 1 H), 5.43 (t, 1 H), 3.80 (s, 3 H), 3.37 (d, 2 H), 2.50 (m, 4 H), 1.70 (s, 3 H); IR (KBr) 3301 (NH, OH), 2984, 1680 (C=O), 1488, 1331, 1302, 1210 cm^{-1}. Anal. Calcd for $C_{13}H_{17}NO_4$: C, 62.13; H, 6.82; N, 5.57. Found: C, 62.12; H, 6.86; N, 5.59.

Compound **6**: white solid: mp 144-145 °C; ^{1}H NMR (DMSO-d_6) δ 8.07 (s, 1 H), 7.3-7.8 (bd, 2 H), 5.50 (t, 1 H), 3.70 (d, 2 H), 2.1-2.6 (m, 4 H), 1.70 (s, 3 H); IR (KBr) 3365 (NH), 3107 (OH), 2921, 1702 (C=O), 1677 (C=O), 1658, 1521, 1475, 1304, 1213 cm^{-1}. Anal. Calcd for $C_{11}H_{14}N_2O_3S$: C, 51.95; H, 5.55; N, 11.01; S, 12.61. Found: C, 52.04; H, 5.58; N, 11.04; S, 12.58.

Compound **7**: white solid: mp 139-140 °C; UV (EtOH): λmax 264.7 (ε 12,000); ^{1}H NMR (DMSO-d6) δ 8.80 (s, 1 H), 8.67 (s, 1 H), 5.52 (t, 1 H), 4.93 (d, 2 H), 2.1-2.6 (m, 4 H), 1.87 (s, 3 H).; IR (KBr) 3112 (OH), 3078, 2907, 2725, 2542, 1710 (C=O), 1599, 1570, 1451, 1404, 1347, 1244, 1176 cm^{-1}. Anal. Calcd for $C_{12}H_{13}ClN_4O_2$: C, 51.34; H, 4.67; Cl, 12.63; N, 19.96. Found: C, 51.44; H, 4.68; Cl, 12.59; N, 19.92.

Compound **8**: white solid: mp decomp. >213 °C; UV (50% EtOH): λmax 249.9 (ε 12,800); ^{1}H NMR (DMSO-d6) δ 12-12.5 (bs, 1 H), 8.07 (bs, 2 H), 5.45 (t, 1 H), 4.70 (d, 2 H), 2.9-4.0 (m, 4 H), 1.82 (s, 3 H).; IR (KBr) 3121 (OH), 3057, 2914, 1713 (C=O), 1694, 1589, 1552, 1412, 1341, 1298, 1217, 1191, 1118 cm^{-1}. Anal. Calcd for $C_{12}H_{14}N_4O_3(+H_2O)$: C, 51.42; H, 5.76; N, 19.99. Found: C, 51.30; H, 5.78; N, 19.87.

Compound **9**: white solid: mp 122-123 °C; ^{1}H NMR (DMSO-d6) δ 8.53 (s, 1 H), 7.53-7.8 (bd, 2 H), 5.43 (t, 1 H), 4.85 (d, 2 H), 2.2-2.6 (m, 4 H), 1.77 (s, 3 H).; IR (KBr) 3345 (NH), 3128 (OH), 3090, 1705 (C=O), 1660 (C=O), 1595, 1490, 1433, 1270 cm^{-1}. Anal. Calcd for $C_{10}H_{14}N_4O_3$: C, 50.41; H, 5.92; N, 23.52. Found: C, 50.36; H, 5.95; N, 23.34.

Compound **10**: white solid: mp 169-170 °C; ^{1}H NMR (DMSO-d6) δ 8.60 (s, 1 H), 7.35-7.8 (bd, 2 H), 5.43 (t, 1 H), 4.85 (d, 2 H), 2.1-2.6 (m, 4 H), 1.77 (s, 3 H).; IR (KBr) 3113 (OH), 1697 (C=O) 1653 (C=O), 1648, 1446 cm^{-1}. Anal. Calcd for $C_{10}H_{13}N_3O_4$: C, 50.20; H, 5.48; N, 17.57. Found: C, 50.14; H, 5.51; N, 17.54.

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