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Synthesis and Biological Evaluations of Condensed Pyridine and Condensed Pyrimidine-Based HMG-CoA Reductase Inhibitors

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Abstract—A series of 3,5-dihydroxyheptenoic acid derivatives containing pyrazolopyridine, isoxazolopyridine, thienopyridine, and pyrazolopyrimidine as a key scaffold was synthesized from condensed pyridine and condensed pyrimidine carboxylic acid esters by homologation, aldol condensation with ethyl acetoacetate dianion, and stereoselective reduction of the 5-hydroxyketone. Several compounds in the series were found to have potent HMG-CoA reductase inhibitory activities in vitro and marked cholesterol biosynthesis inhibitory activities in vivo. It has been shown that these scaffolds can be used as a suitable replacement for the hexahydronaphthalene ring present in naturally occurring HMG-CoA reductase inhibitors.

Introduction

3-Hydroxy-3-methylglutaryl-coenzyme A reductase is the major rate-limiting enzyme in cholesterol biosynthesis. The inhibitors of this enzyme, ranked as hypocholesterolemics, have become the most important reagents for prevention of atherosclerotic diseases. Since the discovery of the naturally occurring fungal metabolites compactin[1] and mevinolin, the microbially transformed pravastatin, a plethora of artificial inhibitors with simple aromatic and heteroaromatic rings has been synthesized to mimic these inhibitors with the structurally complicated hexahydronaphthalene ring system. This report describes the design, synthesis, and biological evaluations of a series of condensed pyridine- and condensed pyrimidine-based inhibitors, and the structure–activity relationships found in the approach.

Design and Synthesis

As a result of an early search on HMG-CoA reductase inhibitors, biphenyl compound **1**[2] was found to be a potent inhibitor, and the essential structure for inhibitory activity was proposed. Namely, it has been widely known that the desmethylmevalonic acid chain moiety, which corresponds to the natural substrate HMG-CoA, and an adjacent 4-(4-fluorophenyl) group and an alkyl side chain afford a high affinity for the target enzyme. A plethora of alicyclic, aromatic, and mono- and polycyclic heteroaromatic rings satisfying this structural requirement has been synthesized to evaluate their substantial activities and the pharmacokinetic profiles.[3]

We chose a scaffold for research making much of the following points: (i) a nucleus which can meet the structural requirement around the desmethylmevalonic acid chain to afford a high affinity to the target enzyme; (ii) a nucleus to which can be introduced various kinds of substituents to examine the substituent effect on substantial activity; (iii) a nucleus in which the lipophilicity can be easily adjusted, which is important in providing an appropriate pharmacokinetic profile such as bioavailability in the oral dosage, and cell membrane permeability. We selected a series of scaffolds, five-member ring-fused nitrogen compounds containing pyridine and pyrimidine, namely, pyrazolopyridine, isoxazolopyridine, thienopyridine, and pyrazolopyrimidine, which can satisfy the conditions above (Fig. 1). As previous studies

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suggested that the 4-(4-fluorophenyl) and 2-isopropyl substitutions afforded optimum potency, attention was focused on variations at other positions of the nucleus. In addition, most of the known artificial inhibitors have the isopropyl substituent for R^1. Since an isopropyl group on an aromatic ring can be readily oxidized metabolically to give an aromatic carboxylic acid,[4] we also tried to replace it with a structurally similar but metabolically more stable cyclopropyl group.

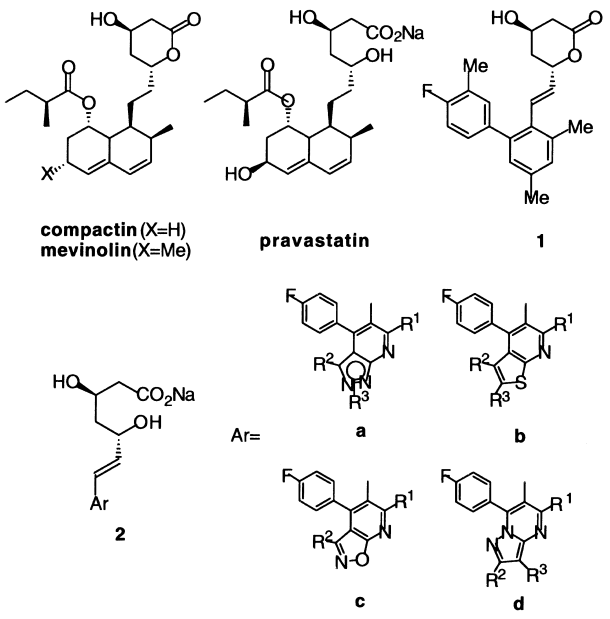
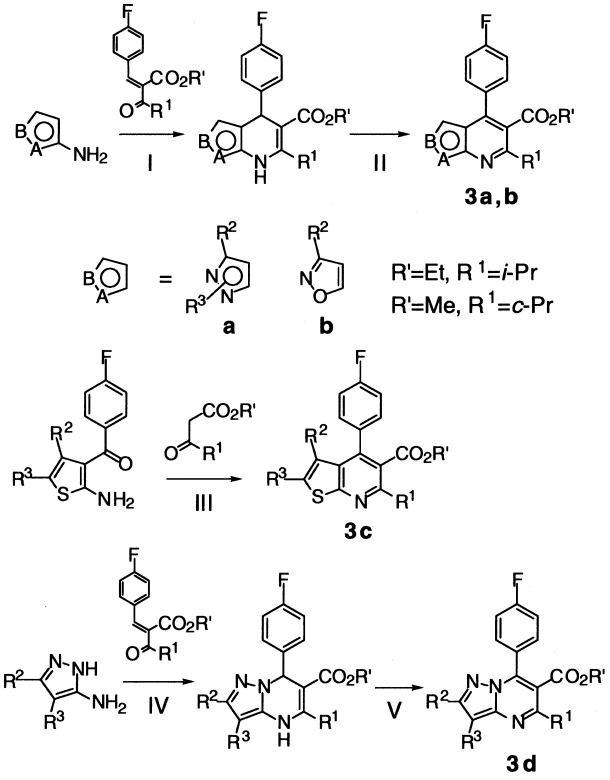


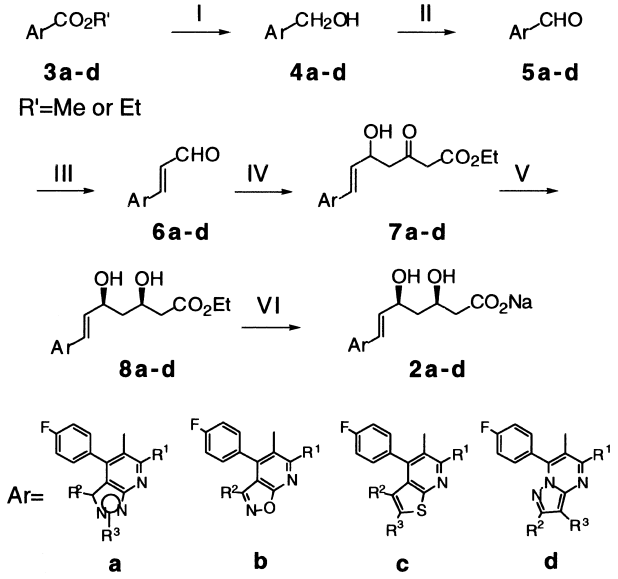
Figure 1.

Scheme 1 delineates the synthesis of the key intermediates, condensed pyridine and condensed pyrimidinecarboxylic acid esters **3a–d**.[5] Five-member ring-fused dihydropyridines were prepared according to literature procedures by Hanche reaction of aminopyrazoles and aminoisoxazoles with benzylideneketoesters respectively. Each compound was aromatized to give pyrazolopyridine derivatives **3a** or isoxazolopyridine derivatives **3b** by various kinds of oxidation methods, depending upon the nature of the compounds. We developed a convenient oxidation method with a sulfur and air oxidation process under basic conditions.[6] Regarding the preparation of the thienopyridine scaffold, Friedlander reaction of benzoyl-aminothiophene with 3-ketoesters proved to be a useful method to obtain **3c**. Pyrazolopyrimidine derivatives **3d** were prepared by condensation of position 2 unsubstituted aminopyrazoles with benzylideneketoesters, followed by manganese oxidation.

Scheme 2 shows the elaboration of 3,5-dihydroxyheptenoic acid derivatives **2a–d** starting from **3a–d**. The Dibal reduction of **3a–d**, followed by PCC oxidation of the resulting alcohol **4a–d** provided aldehydes **5a–d**. Homologation of **5a–d** to propenal **6a–d** was accomplished by utilizing *cis*-(2-ethoxyvinyl)lithium and subsequent hydrolysis with *p*-toluenesulfonic acid.[7] An aldol condensation of **6a–d** with the sodium/lithium dianion of ethyl acetoacetate afforded the racemic 3-keto-5-hydroxy esters **7a–d**. Partially *syn* stereoselective



Scheme 1. Synthesis of **3a–d**. Reagents and conditions: (I) neat, 130 °C or *t*-BuOH, 80–90 °C or DMA, 120 °C; (II) $KMnO_4$, acetone, rt or cromic anhydride, glacial AcOH, rt or S, DMA, 120 °C, 2.5 h or air, K_2CO_3, DMA, 120 °C, 3 h; (III) cH_2SO_4, glacial AcOH, 120 °C, 5 h or PTS, toluene, reflux; (IV) ^{t}BuOH or DMF, 90–120 °C; (V) $KMnO_4$ or MnO_2, acetone, rt.



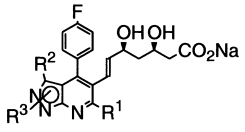
Scheme 2. Synthesis of **2a–d**. Reagents and conditions: (I) DIBAH, toluene, 0–10 °C; (II) PCC, AcONa, CH_2Cl_2, rt; (III) *n*-$Bu_3SnCH{=}CHOEt$, *n*-BuLi, THF, −78 °C; (ii) PTS, THF H_2O, rt; (IV) $CH_3COCH_2CO_2Et$, NaH, *n*-BuLi, THF, −15 °C; (V) Method A: $NaBH_4$, EtOH, rt; Method B: $Zn(BH_4)_2$, Et_2O, −78 °C; (VI) (i) NaOH aq, EtOH, rt; (ii) freeze dry.

reductions of the 3-keto group of **7a–d** were conducted with $NaBH_4$ at room temperature or with $Zn(BH_4)_2$ at −78 °C to give the desired racemic *erythro*-3,5-dihydroxyesters **8a–d** (*syn*:*anti* ratio approximately 7:3 and 9:1, respectively).[8] Finally, **8a–d** were saponified with aqueous NaOH and freeze-dried to afford the corresponding sodium salts **2a–d**.

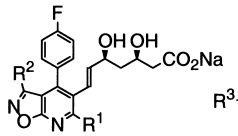
Results and Discussion

The 3,5-dihydroxyheptenoic acid sodium salts **2a–d** listed in Table 1 were evaluated for their ability to inhibit sterol synthesis in cell free system using a mixture of microsome fraction and semipurified-cytosolic fraction isolated from rat liver as the enzyme sources[9] and to

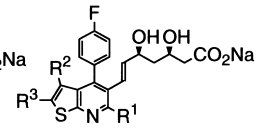
Table 1. Biological activities of **2a–d**



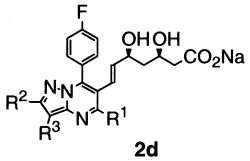
2a



2b



2c



2d

Compound	R^1	R^2	R^3	Method	In vitro HMG-CoA reductase inhibitory activity[a] relative potency		In vivo cholesterol synthesis inhibitory activity[b] % inhibition	
					Cell free	Hep G2 cell	0.2 (mg/kg)	2.0 (mg/kg)
2a–1	*i*-Pr	Me	1-Me	A	71	15	41.1	48.1
2a–2	*c*-Pr	Me	1-Me	A	228	118	35.5	74.3
2a–3	*i*-Pr	Me	1-*t*-Bu	A	100	31	51.8±4.3	86.0±2.1
2a–4	*c*-Pr	Me	1-*t*-Bu	B	168	11	4.5	72.2
2a–5	*i*-Pr	Ph	1-Me	A	21	6	37.0±16.3	71.1±9.0
2a–6	*c*-Pr	Ph	1-Me	B	300	14	−54.4	11.5
2a–7	*i*-Pr	H	1-Et	A	71	39		
2a–8	*i*-Pr	H	1-Ph	A	228	10		
2a–9	*i*-Pr	Me	1-Ph	A	264	1	3.3	19.9
2a–10	*i*-Pr	Me	1-(*p*-MeO-Ph)	A	154	149	−27.5	48.7
2a–11	*c*-Pr	Me	1-(2-Pyridyl)	B	229	4		
2a–12	*i*-Pr	Me	1-CH_2Ph	A	63	17	16.4	45.1
2a–13	*i*-Pr	*c*-Pr	1-Me	A	42	1	42.9	71.3
2a–14	*c*-Pr	*c*-Pr	1-*t*-Bu	B	98	2		
2a–15	*i*-Pr	*c*-Pr	1-Ph	A	55	7		
2a–16	*c*-Pr	Ph	1-*t*-Bu	B	75	<1		
2a–17	*i*-Pr	*p*-Cl-Ph	1-Me	A	93	26		
2a–18	*i*-Pr	Me	2-Ph	A	162	12		
2b–1	*i*-Pr	Me		B	170	1		
2b–2	*i*-Pr	Ph		B	83	1	33.7	52.3
2c–1	*c*-Pr	H	H	B	229	60	38.1±19.5	70.6±6.6
2c–2	*i*-Pr	H	Me	A	68	22	−21.5±22.8	34.2±23.6
2c–3	*i*-Pr	H	*i*-Pr	A	52	62	−71.5±54.4	−19.3±30.3
2c–4	*i*-Pr	H	Ph	A	<1	1		
2c–5	*i*-Pr	Et	Me	A	35	51	7.9±27.8	20.8±27.1
2c–6	*c*-Pr	Et	Me	A	65	38		
2c–7	*i*-Pr	Ph	Me	A	<1	1		
2c–8	*i*-Pr		–$(CH_2)_3$–	A	58	49	−73.6	−17.5
2c–9	*c*-Pr		–$(CH_2)_3$–	A	63	60	−58.6	−45.3
2c–10	*i*-Pr		–$(CH_2)_4$–	A	48	19	−89.0	−49.5
2c–11	*c*-Pr		–$(CH_2)_5$–	B	23	2		
2d–1	*i*-Pr	Me	H	A	27	3	−6.1	−45.1
2d–2	*c*-Pr	Me	H	A	24	2	−27.1	6.5
2d–3	*c*-Pr	*i*-Pr	H	B	6	1	21.8	−19.4
2d–4	*i*-Pr	*t*-Bu	H	A	4	<1		
2d–5	*i*-Pr	Ph	H	A	12	3	−1.4	24.1
2d–6	*i*-Pr	2-Furyl	H	B	13	<1		
2d–7	*c*-Pr	Me	Me	B	25	3		
2d–8	*c*-Pr	Me	Ph	B	64	3		
2d–9	*i*-Pr		–$(CH_2)_4$–	B	30	1		
Pravastatin[c]					100	1	35.4	27.8

[a]Potencies were obtained by comparison of IC_{50} values of **2a–d** with that of the internal standard pravastatin. Pravastatin was assigned a value of 100 in cell free system, 1 in Hep G2 cell culture system. Parameters were calculated using a logistic curve fit of dose response data from 3 or 5 dose points. The response at each dose is the mean response of triplicate determinations.

[b]Each value represents percent inhibition of cholesterol biosynthesis versus concomitantly assayed vehicle control group. Results are presented as the mean±SEM of at least two separate experiments each carried out with 5 animals/group. Values are expressed as mean±SEM when the number of experiments was 3 or more, or the mean only when the number of experiments was 2.

[c]The mean IC_{50} value of pravastatin was 4.2 nM in cell free system, 1.37 μM in cell culture system (see ref 10).

inhibit the cellular steroidgenesis in Hep G2 cells (human hepatoma cell line) cultured with 5% lipoprotein deficient serum containing medium for 48 h in vitro. All biological tests were also conducted under the same experimental conditions with pravastatin as reference for direct comparison. The activities were determined by decreased incorporation of sodium [2-^{14}C] acetate into non-saponifiable lipids. Selected compounds were further evaluated for their ability to inhibit hepatic cholesterol synthesis in a male Sprague–Dawley rat after oral administration, as determined by decreased incorporation of intraperitoneally injected sodium [2-^{14}C] acetate into serum non-saponifiable lipids.

In a limited number of compounds prepared, potent inhibitors which exceeded pravastatin's activity in vitro and showed marked steroidgenesis inhibitory activities in vivo were found largely in pyrazolopyridine series **2a**. In general, replacement of the isopropyl substituent for R^1 by cyclopropyl resulted in an appreciable increase in potency (**2a–1** vs **2a–2**, **2a–3** vs **2a–4**, **2a–5** vs **2a–6**, **2c–5** vs **2c–6**, **2c–8** vs **2c–9**, **2d–1** vs **2d–2**). Summary of results obtained in each series: (i) Pyrazolopyridine (**2a–1** to **2a–18**); many compounds in this series were exceedingly potent inhibitors in cell free test; their IC_{50} values were in the order of nM. Some compounds were more active than pravastatin by factors of over 100 in the Hep G2 test (**2a–2** and **2a–10**). (ii) Isoxazolopyridine series (**2b–1**, **2b–2**); although the compounds in this series were almost equipotent or more potent than pravastatin in cell free system, their inhibitory activities diminished in Hep G2 system and in vivo. (iii) Thienopyridine series (**2c–1** to **2c–11**); whereas significant loss of activity was observed by incorporation of phenyl into the scaffold (**2c–4**, **2c–7**), many compounds in this series were nearly half as active as pravastatin in cell free test and were more active by a factor of over 20 in the Hep G2 test. Alkyl or phenyl substituents for R^2,R^3 were not essential to increase the in vitro potency. The most potent compound in this series was R^2,R^3 unsubstituted analogue **2c–1**, which showed marked inhibitory activity in vivo. (iv) Pyrazolopyrimidine series (**2d–1** to **2d–9**); introduction of the bulky alkyl group or aryl group for the R^2 substituent led to a slight reduction in potency in cell free test (cf. **2d–1** and **2d–2** with **2d–3**, **2d–4**, **2d–5**, and **2d–6**). Generally, the compounds in this series showed diminished activities in Hep G2 tests.

Conclusion

1. In agreement with literature findings, prominent activity resides in compounds possessing the 4-(4-fluorophenyl) and isopropyl (cyclopropyl) substituent in these scaffolds. Most compounds in these series were equipotent or more potent than pravastatin in vitro and some of them showed marked inhibitory activities in vivo. Therefore, these five-member ring-fused nitrogen-containing pyridine and pyrimidine compounds can be used as a surrogate for the structurally complex hexahydronaphthalene ring present in the naturally occurring HMG-CoA reductase inhibitors.
2. However, the variations at other positions of the nucleus affected their substantial activities in a different manner in each series. In particular, many compounds with a variety of substituents for R^2 and R^3 showed significant activities in the pyrazolopyridine series. This work shows that further modulation and improvement in potency at inhibiting HMG-CoA reductase may be obtained with a variety of additional substituents in this series. On the other hand, the optimum potency was retained in the simplest compound with no R^2 and R^3 substituents in the thienopyridine series. This fact may allow the design of a compound with high potency without any introduction of metabolically unfavorable substituents.
3. While no clear correlation exists between the substantial activity in cell free system, inhibitory activity in cell culture, and the in vivo potency of these sets of compounds, the pharmacokinetic properties of the compounds such as bioavailability, membrane permeability, and metabolic degradation do have an effect on these parameters.

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