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BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# SPIRO-IMIDAZO[1,2-*a*]INDENO[1,2-*e*]PYRAZINE-4-ONE DERIVATIVES ARE MIXED AMPA AND NMDA GLYCINE-SITE ANTAGONISTS ACTIVE *IN VIVO*.

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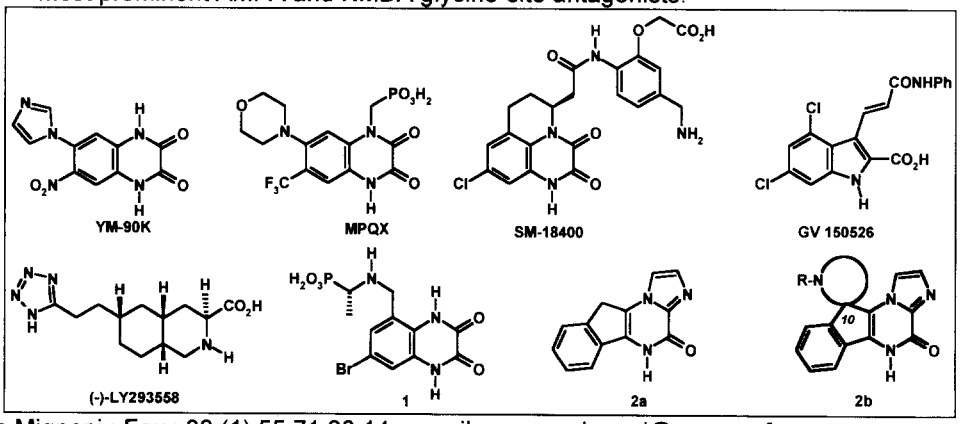
**Abstract:** *Original spiro-imidazo[1,2-a]indeno[1,2-e]pyrazine-4-one derivatives were synthesised and led to the identification of* ***3e*** *which showed good affinities for both the AMPA and the NMDA glycine-site receptors, and displayed good anticonvulsant effects after i.p. and i.v. administrations in the electroshock-induced convulsion assay in mice. The corresponding dextrorotatory isomer* ***(+)-3e*** *was notably more potent than the levorotatory isomer* ***(-)-3e*** *in in vitro and in vivo assays.* 

Excitotoxicity, or the overstimulation of glutamate receptors has been proposed as a pathological phenomenon in a number of neuronal degenerative diseases such as brain ischemia, anoxia and hypoglycemia, traumatic brain and spinal injury, Parkinson's and Huntington's disease.[1] Glutamate excitotoxicity would be mediated by NMDA, AMPA and kainate-preferring glutamate receptor subtypes.[2] Blocking their activation would thus be expected to have a neuroprotective effect. AMPA and NMDA antagonists are of particular interest, and several compounds belonging to various chemical families have been shown to be effective glycine-site NMDA or AMPA antagonists.[3] Representative examples are the AMPA antagonists **YM-90K**[4], **MPQX**[5] and **(-)-LY293558**[6], and the NMDA glycine-site antagonists **SM-18400**[7] and **GV 150526**[8] (Figure 1). To our knowledge, *N*-phosphonoalkyl-5-aminomethylquinoxaline-2,3-dione derivatives such as **1** are the only compounds reported to have high affinities for both the AMPA and the glycine/NMDA receptors.[9] A novel series of competitive AMPA receptor antagonists represented by the 5H, 10H-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one **2a** has been described by us.[10] Compound **2a** displayed significant anti-convulsant properties in

**Figure 1:** Most prominent AMPA and NMDA glycine-site antagonists.

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mice and rats and activity in models of global cerebral ischemia in the gerbil and focal cerebral ischemia and neurotrauma in the rat.

In this paper, we present original spiro-tricyclic antagonists with the general chemical structure **2b** (Figure 1) such as **3a-p**, **4a-d** and **5a-c** (Scheme 1), their affinities for the AMPA receptor and the glycine-binding site of NMDA receptors, and their anticonvulsant effects in electroshock-induced convulsion assays in mice (MES) following *i.p.* administration as shown in Table 2. SAR of the resulting 10,10-disubstituted imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-ones **2** will be discussed.

***Chemistry:*** The targeted imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one derivatives **3a-p**, **4a-d** and **5a-c**[9] were synthesized from **2a**[10] according to the sequences outlined in Scheme 1.

***Synthesis of 3b***. Compound **2a** reacted with *tert*-butoxy-bis(dimethylamino)methane followed by hydrolysis using HCl and then reduction by $NaBH_4$ to give **8** with 10% overall yield. Then, this compound was dehydrated using NaOH to give **9** with a 75% yield. As the key step, regioselective [3+2] cycloaddition reaction of **9** with the non-stabilized azomethine ylide **10**[11] obtained *in situ* by action of TFA with *N*-benzyl,*N*-*n*-butoxymethyltrimethylsilylmethylamine, gave **11** with a 41% yield. Finally, *N*-deprotection of **11** was carried out under standard experimental conditions giving **3b** with a 33% yield.

***Synthesis of 3a, 3e-h, 3j-l and 3m-o***. The *N*-substituted derivatives **3a, 3e-h**, **3j-l** and **3m-o** were prepared by treatment of **2a** with *i*$AmNO_2$ in the presence of NaH followed by the action of Zn (powder) in acetic acid giving the key amino intermediate **13** with 6% overall yield. Then, *N*-acetyl deprotection and finally *N*-Boc formation gave **15** with 11% overall yield. Reaction of **15** with 1-chloro-3-bromopropane as electrophile in the presence of NaH afforded **3l** with a 55% yield. Then, **3l** was *N*-deprotected using TFA to give **3a** with a 48% yield. Reductive alkylation of **3a** with formaldehyde and formic acid afforded **3e** with a 54% yield, whereas the *N*-ethylation and *N*-propylation were carried out by action of acetic acid and propionic acid in the presence of $NaBH_4$ giving **3f** and **3g** with 26% and 47% yield respectively. *N*-Benzylation of **3a** was carried out under standard experimental reaction conditions with benzylbromide in the presence of KOH as base giving **3h** with 55% yield. **3j** was synthesized using the condensation of *N*-phthaloylglycine chloride followed by the action of hydrazine with a 10% overall yield. Direct condensation of **3a** with succinic anhydride in acetic acid medium gave **3k** with a 29% yield. The synthesis of the ureas **3m-o** was carried out by the condensation of **3a** with the corresponding isocyanates with good yields (>77%).

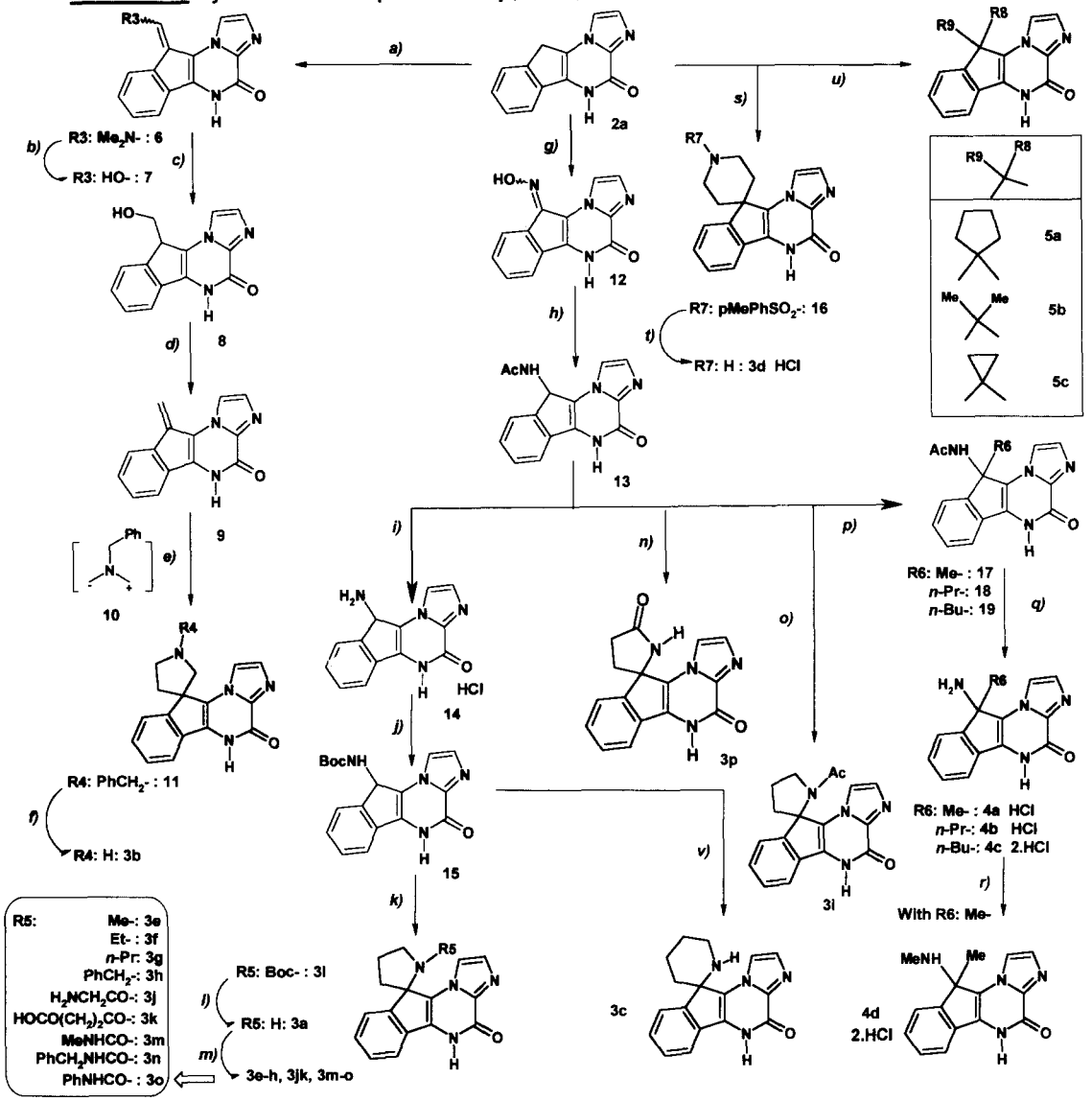
***Synthesis of 3c, 3p and 3i :*** **3c**, **3p** and **3i** were obtained following the same synthetic pathway as the preparation of **3l**. Thus, starting from **15**, condensation of 4-chloro-bromobutane in the presence of NaH followed by *N*-Boc deprotection using TFA gave **3c** with a 60% overall yield, while the spiro-derivatives **3p** and **3i** were obtained with 4% and 11% yield respectively by the condensation of **13** with either methyl 3-bromopropionate or 1-chloro-2-bromoethane, also in the presence of NaH.

***Synthesis of 3d:*** The synthesis of **3d** was carried out by the condensation of **2a** with *N*,*N*-bis-(2-chloroethyl)-*p*-toluenesulfonamide in the presence of NaH followed by *N*-deprotection with a 7.5% overall yield.

***Synthesis of 4a-d:*** **4a-d** was acheived starting from **13.** Alkylation of **13** with methyl iodide, *n*-propyl iodide or *n*-butyl iodide under standard experimental reaction conditions provided the corresponding 10-alkylated derivatives **17**, **18** and **19** with 34.5%, 54% and 45% yield respectively. Then, *N*-deacetylation led to **4a**, **4b** and **4c** with 40%, 59% and 59% yields respectively. The reaction of di-*tert*-butyl dicarbonate with **4a**, followed by *N*-methylation and *N*-deacylation under standard experimental reaction conditions gave **4d** with a 7.5% overall yield.

***Synthesis of 5a-c:*** the spiro-derivatives **5a-c** were prepared from **2a** using catalytic phase transfer conditions in the presence of NaOH and TBAB with 12%, 8% and 31% yield respectively.

**Scheme 1:** Synthesis of compounds **3a-p**, **4a-d**, and **5a-c**.




**Reaction conditions:** ***a)*** tBuOCH(NMe)$_2$, rt, 0.5h ***b)*** 5N HCl, rt, 0.5h ***c)*** $NaBH_4$, MeOH, rt, 2h ***d)*** 1N NaOH, MeOH/DMSO, rt, 16h then 1N HCl ***e)*** *n*$BuOCH_2N(CH_2Ph)CH_2SiMe_3$, cat. TFA, DMF, rt, 3h then 60°C, 1h ***f)*** AcOH, $H_2$ (147 psi), cat. $Pd(OH)_2/C$, 60°C, 3h **g)** NaH, *i*AmNO2, DMSO, rt, 1h ***h)*** Zn, AcOH, 80°C, 2h ***i)*** 2N HCl, reflux, 2h ***j)*** *di-tert*-butyl dicarbonate, $Et_3N$, DM, 25°C, 20h ***k)*** NaH, $Br(CH_2)_3Cl$, rt, 2.5h ***l)*** TFA, 20°C, 2.5h ***m)*** **3e**: HCOH (37%), $HCO_2H$, 28°C, 1h **3f**: AcOH, $NaBH_4$, 45°C, 16h **3g**: n-$C_3H_7CO_2H$, $NaBH_4$, 45°C, 16h **2h**: $PhCH_2Br$, EtOH, KOH, rt, 12h **2j**: *i) N*-phthaloylglycine chloride, $Et_3N$, DMF, 25°C, 16h *ii)* $H_2NNH_2.H_2O$, MeOH, reflux, 16h **3k**: succinic anhydride, AcOH, rt, 48h **3m**: MeNCO, DMF, rt, 2h **3n**: $PhCH_2NCO$, DMF, rt, 2h **3o**: PhNCO, DMF, rt, 2h ***n)*** $Br(CH_2)_2CO_2Me$, NaH, DMSO, rt, 2.5h ***o)*** $Cl(CH_2)_2Br$, NaH, DMSO, rt, 2.5h ***p)*** NaH, DMSO, rt, 16h, **17**: MeI, **18**: *n*-PrI, **19**: *n*-BuI, ***q)*** 2N HCl, reflux 1.5-2h ***r)*** *ii) di-tert*-butyl dicarbonate, DMF, 60°C, 20h *ii)* NaH, MeI, DMF, rt, 12h *iii)* TFA, rt, 1.5h then 1.3N HCl, MeOH, rt, 1h ***s)*** *p*-$MePhSO_2N[(CH_2)_2Cl]_2$, NaH, DMSO, rt, 20h ***t)*** HBr (47%), reflux, 5h then 1N HCl ***u)*** TBAB, NaOH, DMSO, rt, 6h, **5a**: $Br(CH_2)_4Br$, **5a**: MeI, **5b**: $Br(CH_2)_2Br$.

***Biological activitiy:***

***In vitro studies:*** The affinities for AMPA receptors and the glycine modulatory site on the NMDA receptor were evaluated in an *in vitro* binding assay using [$^3$H]-AMPA[12a] and [$^3$H]-5,7-dichlorokynurenate ([$^3$H]-DCKA) [12b] as selective $^3$H-ligands on rat cortical membrane preparations. Results for compounds **2a**, **3a-p**, **4a-d**, **5a-c** and **YM-90K** and **(-)-LY-293558** for the AMPA receptors and the glycine-binding site of NMDA receptors are shown in Table 1.

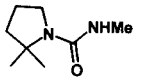
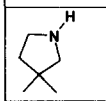
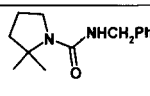
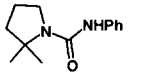
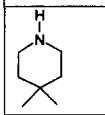
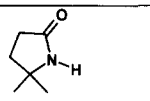
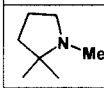
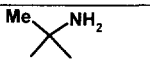
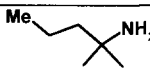
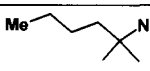
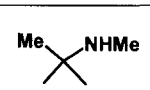
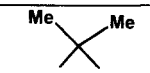
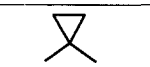
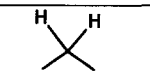
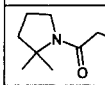
Structure-activity relationships for both receptors (AMPA and NMDA glycine site) were examined: ❶ Introduction in the 10-position of the imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one cycle of **2a** of alkyl groups such as dimethyl, spiro-cyclopentyl or spiro-cyclopropyl moieties reduced the affinity for the AMPA receptors (**5a-c** *vs* **2a**). Substitution of one of two methyl groups of **5b** by an amino group or a methylamino group moderately increased the AMPA binding potency (**4a** and **4d** *vs* **5b**). Substitution of the methyl group of **4a** by a butyl group (**4c** *vs* **4a**) or a propyl group (**4b** *vs* **4a**) increased both AMPA and glycine binding potencies. **4b** was the starting point for the preparation of the corresponding spiro-analogue **3a** which showed similar potency at both receptor subtypes ($IC_{50}$ ~ 250 nM) being at least 3 times more potent than **2a** for the AMPA receptors. ❷ Substitution of the five-membered ring of **3a** by a six-membered ring reduced potency at both receptor subtypes (**3c** *vs* **3a**), whereas the displacement of the nitrogen atom in β-position reduced the potency only at the glycine receptor (**3b** *vs* **3a**). A similar result was obtained in the six-spiro derivative series (**3d** vs **3c**). On the basis of these data, we decided to take **3a** as the lead compound. ❸ Introduction of a methyl group on the nitrogen of **3a** retained the potency on both receptor subtypes (**3e** *vs* **3a**) while introduction of either an ethyl or a *n*-propyl or a benzyl group decreased the potency at both receptor subtypes (**3f**, **3g** and **3h** vs **3a**). ❹ Introduction of either an acetyl (**3i**), an aminoacetyl (**3j**) or a carboxypropionyl (**3k**) chain reduced potency at both receptor subtypes, although this was most dramatic on the glycine-binding site (**3i**, **3j** and **3k** *vs.* **3a**). ❺ Introduction of a Boc function on the nitrogen atom of **3a** reduced potency at both receptor subtypes (**3l** *vs.* **3a**) as did the introduction of various urea groups (**3m-o** *vs.* **3a**) except for the phenyl urea **3o** on the glycine site, and the insertion of a carbonyl group in the α–position of the nitrogen atom of **3a** (**3p** *vs.* **3a**).

The most active compounds **3a-e, 3k** and **4b** showed similar AMPA binding potency to **YM-90K** and a two fold higher potency than **(-)-LY-293558**. The compounds **3k**, **YM-90K** and **(-)-LY-293558** displayed high discrimination for the AMPA receptors *vs* the glycine-binding site (at least 30 fold) whereas **3a-e** and **4b** showed lower selectivity (1-5 fold).

The good AMPA and glycine-binding site affinities of the racemic **3e** prompted us to examine the enantiomers **(+)-3e** and **(-)-3e** of this compound. The two enantiomers were prepared in optically pure form by HPLC using a column packed with a chiral stationary phase (Chiracel OC phase). Four runs were necessary for the separation, starting from 3.2g of **(+/-)-3e** [mobile phase: ethanol, flow-rate: 30 ml/min, detection: UV (265 nm), column diameter: 60mm]. Enantomeric homogeneity of both enantiomers (>99%) was evaluated by analytical HPLC using the same chiral phase [**(+)-3e :** $\alpha_D^{20}$ = +32.4 (AcOH, c = 0.5) ; [**(-)-3e :** $\alpha_D^{20}$ = -32.0 (AcOH, c = 0.5)]. As shown in Table 1, the dextrorotatory isomer **(+)-3e** displayed about 50-fold and 7-fold greater potency at the AMPA receptors and glycine site of NMDA receptors respectively than did the levorotatory molecule **(-)-3e** ($IC_{50}$ [$^3$H]-AMPA = 86 *vs.* 4200 nM, $IC_{50}$ [$^3$H]-DCKA = 172 *vs.* 1160 nM respectively).

***In vivo studies:*** The most active spiro-derivatives *in vitro* [**3abc**, **3ef**, **(+)-3e**, **(-)-3e**] were evaluated for *in vivo* activity in MES[13] assays in mice after *i.p.* administration (1% tween in water) and 30 minutes pretreatment time in comparison with **2a**, **YM-90K** and **(-)-LY29558**. In addition, **3j** and **3k** were selected in order to determine the influence of polar functions such as amino or carboxylic acid groups, whereas **4a** and **4b** were selected in order to determine the influence of a primary amine in position 10 of the imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one cycle.

**Table 1:** Binding studies of **2a**, **3a-p**, **4a-d**, **5a-c**, **YM-90K**, and **(-)-LY293558**.

| R1, R2 (10) | Cpd | Binding [$^3$H]-AMPA ($IC_{50}$ nM)[a] | Binding [$^3$H]-DCKA ($IC_{50}$ nM)[a] | R1, R2 (10) | Cpd | Binding [$^3$H]-AMPA ($IC_{50}$ nM)[a] | Binding [$^3$H]-DCKA ($IC_{50}$ nM)[a] |
|---|---|---|---|---|---|---|---|
|  | 3a | 250 | 280 |  | 3m | 4000 | 1700 |
|  | 3b | 200 | 640 |  | 3n | 10000 | 900 |
|  | 3c | 390 | 1180 |  | 3o | 7100 | 130 |
|  | 3d | 380 | 2000 |  | 3p | 1500 | 1700 |
|  | 3e | 200 | 420 |  | 4a | 2500 | 1400 |
|  | (+)-3e | 86 | 172 |  | 4b | 420 | 520 |
|  | (-)-3e | 4900 | 1160 |  | 4c | 1700 | 300 |
|  | 3f | 620 | 530 |  | 4d | 3400 | 2740 |
|  | 3g | 4300 | 470 |  | 5a | 1600 | 1400 |
|  | 3h | 10000 | 1300 |  | 5b | 4400 | 1000 |
|  | 3i | 610 | 560 |  | 5c | 2300 | 600 |
|  | 3j | 830 | 2800 |  | 2a | 760 | 3000 |
|  | 3k | 360 | 10000 | YM-90K | | 350 | 10400 |
|  | 3l | >10000 | 600 | (-)-LY293558 | | 600 | >10000 |

a : $IC_{50}$ values (nM) are the mean of at least 3 determinations each with at least 3 concentrations of test compounds in triplicate.

Among these compounds, **3b**, **3a**, **3c** and **3e** showed moderate *in vivo* activities ($ED_{50}$[14] = >80, 80, 45 and 31 mg/kg respectively). Furthermore, the dextrorotatory isomer **(+)-3e** was found to be a good anticonvulsant ($ED_{50}$ = 17 mg/kg), unlike the levorotatory isomer **(-)-3e** which was 5-fold less potent ($ED_{50}$ = 80 mg/kg). The isomer **(+)-3e** was 4-fold more potent than **2a** ($ED_{50}$ = 62 mg/kg), displayed the same level of potency as **YM-90K** ($ED_{50}$ = 12 mg/kg), and was 4-fold less active than **(-)-LY293558** ($ED_{50}$ = 4 mg/kg). Compound **3f** ($ED_{50}$ = 54 mg/kg), showing similar potency then **3j** ($ED_{50}$ = 70 mg/kg), was about 2-fold less active than **3e** thus correlating with the decrease of *in vitro* activity observed for the AMPA binding. A very interesting result was also obtained with the acidic spiro-derivative **3k** which displayed an $ED_{50}$ of 10 mg/kg. The lengthening of the alkyl chain of **4a** ($ED_{50}$ = 30 mg/kg) on position 10 decreased 2.5-fold the *in vivo* activity ($ED_{50}$ = 80 mg/kg, **4b** *vs* **4a**). In addition, compounds **3e** and **(+)-3e** showed good anticonvulsant effects in MES tests by *i.v.* route (vehicle: 1 eq. 0.1N HCl, pretreatment time : 5 minutes) with $ED_{50}$'s of 10 and 7 mg/kg respectively.

In conclusion, **(+)-3e** is representative of an original chemical family of both AMPA and NMDA (glycine) antagonists. It displayed moderate affinities for these two receptors with good *in vivo* activities after *i.p.* and *i.v.* administrations.

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**References and Notes**

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