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SYNTHESIS OF SOME HYDROXYNAPHTHAZARINS AND THEIR CARDIOPROTECTIVE EFFECTS UNDER ISCHEMIA-REPERFUSION *IN VIVO*

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Abstract. A series of hydroxynaphthazarins has been synthesized. Some of them were found in *in vivo* experiments to be protectors of myocardium under ischemia-reperfusion and to reduce the infarction zone by 50% without any adverse effect. All compounds exhibit a moderate or small toxicity and are active in low doses.

Introduction. Myocardial ischemia is a disease process characterized by a reduction in the flow of oxygenated blood to myocardium below that required to sustain normal aerobic metabolism in the cardiomyocyte[1]. Prompt reestablishment of coronary flow by clinical procedures is the most direct and effective means of limiting myocardial damage in ischemic heart[2]. However, reperfusion carries with it an injury component that may limit its therapeutic effectiveness and increase the extend of infarction[3]. In both phases, involvement of free-radical mechanism has been well documented[4]. Both natural and synthetic small-molecular antioxidants were tested for their ability to salvage reperfused myocardium and to reduce infarct size[4, 5]. Some of them have demonstrated positive effects in these tests. In this connection investigation of cardioprotective effects of hydroxynaphthazarins is of considerable interest because such natural compounds as echinochrome (7-ethyl-2,3,6-trihydroxynaphthazarin) and other quinonoid pigments of sea urchins possess high antioxidant activities[6]. However, these compounds are not easily accessible due to very low natural abundance of pigments[7]. In addition, the known methods of their synthesis are too ineffective to be practical[7,8]. We now describe the sufficiently effective syntheses of some hydroxynaphthazarins and briefly discuss their therapeutic potential against myocardial ischemic-reperfusion damage.

Synthesis. We have investigated two approaches to the synthesis of hydroxylated naphthazarins **(21-28)**. These approaches differ from each other in the formation of the key intermediates **8-13** (Scheme). The first of them utilizes a sequential functionalization of the naphthazarin nucleus of the starting substrate **1**.

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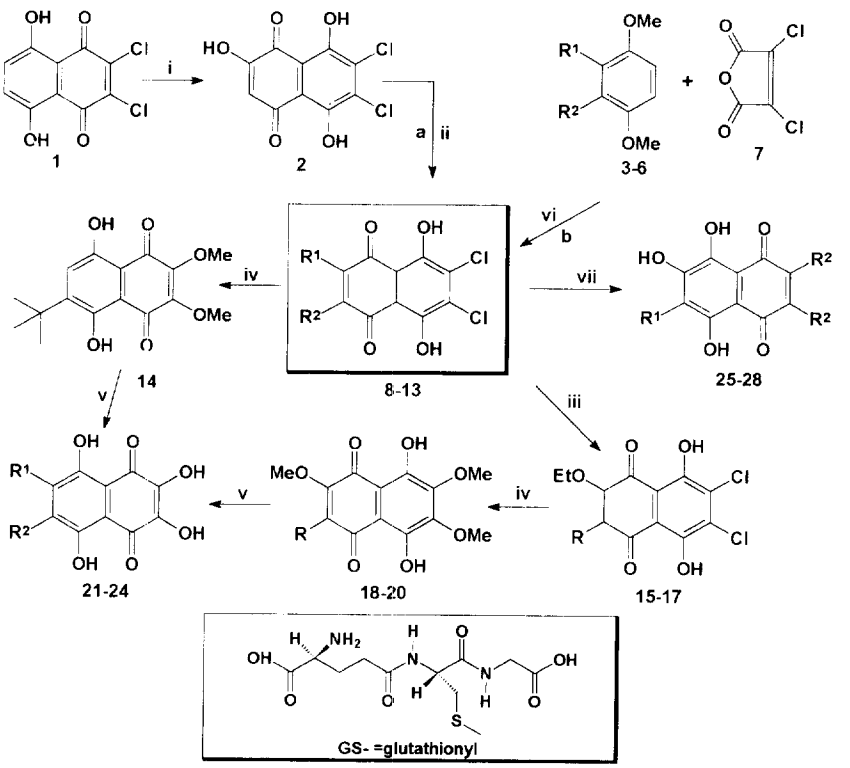
Dichloronaphthazarin (**1**)[9] was converted to 2-hydroxy-3-alkyl-6,7-dichloronaphthazarins **8-10** *via* oxidation with MnO_2 in concentrated H_2SO_4 followed by alkylation of the resulting compound **2**[10] with the corresponding acyl peroxides $(R^2COO)_2$ in boiling *t*-BuOH[11].

Scheme



3. R^1=t-Bu, R^2=H; **4**. R^1=OMe, R^2=Me; **5**. R^1=OMe, R^2=Et; **6**. R^1=R^2=OMe; **8**. R^1=OH, R^2=Et (**a:** 40%, **b:** 61%); **9**. R^1=OH, R^2=Pr (41%); **10**. R^1=OH, R^2=$(CH_2)_2COOH$ (38%); **11**. R^1=t-Bu, R^2=H (50%); **12**. R^1=OH, R^2=Me (65%); **13**. R^1=R^2=OH (42%); **15**. R=Et (81%); **16**. R=Pr (83%); **17**. R=$(CH_2)_2COOEt$ (70%); **18**. R=Et (74%); **19**. R=Pr (69%); **20**. R=$(CH_2)_2COOMe$ (51%); **21**. R^1=t-Bu, R^2=H (94%); **22**. R^1=OH, R^2=Et (91%); **23**. R^1=OH, R^2=Pr (93%); **24**. R^1=OH, R^2=$(CH_2)_2COOH$ (85%); **25**. R^1=Me, R^2=$S(CH_2)_2OH$ (88%); **26**. R^1=Me, R^2=GS (78%) (GS-= glutathionyl); **27**. R^1=Et, R^2=GS (82%); **28**. R^1=OH, R^2=GS (71%).

Reagents and conditions: (**i**) MnO_2, H_2SO_4, r.t., 2 h; (**ii**) $(R^2COO)_2$ (R^2=Et, Pr, $HOOC(CH_2)_2$-), t-BuOH, reflux; (**iii**) $(EtO)_3CH$, reflux, 3 h; (**iv**) MeOH/CsF/Al_2O_3, in closed reaction vessel, 90-95°C, 8 h; (**v**) $AlCl_3$, $PhNO_2$, 80°C, 4 h; (**vi**) $AlCl_3$, NaCl, 190°C, 5 min; (**vii**) R^2H, MeOH, OH^-, r.t., 10 min (if R^2=$HO(CH_2)_2S$-) and 24 h (if R^2=GS-).

In accordance with the second way the key intermediates **8**, **11-13** were formed as a result of cycloacylation of the substituted 1,4-dimethoxybenzenes **3-6** with dichloromaleic anhydride (**7**) (Scheme)[12]. This approach was already used by Scheuer and coworkers for the synthesis of tetrahydroxynaphthazarin (spinochrome E) through intermediate **13**[13]. Contrary to the first, the possibilities of the second approach are

limited by the stability of the starting 1,4-dimethoxybenzenes in the rigid conditions of cycloacylation. Substrate **3** was commercially available and substrates **4** and **6** were prepared from 2-methyl resorcinol dimethyl ether and pyrogallol trimethyl ether[14,15], respectively. The methyl ether **5** was obtained from 1,2,4-trimethoxybenzene[16].

The nucleophilic substitution of chlorine atoms with methoxy groups in dichloronaphthazarins **8-13** by the action of MeONa in MeOH is made difficult by ion-pairing interaction and the resulting poor nucleophilicity of quinone phenoxide anions[13]. However, the use of the complex reagent such as MeOH/CsF/Al_2O_3 provided good results[17]. So, reaction of substrate **11** with this reagent afforded dimethoxynaphthazarin **14** in high yield (78%)[18]. In the case of substrates **8-10**, bearing free β-OH group which shows strong electron-donating properties in the basic media, the selective protection of this group has to be used to effect the simultaneous substitution of all chlorine atoms on methoxy groups by the action of MeOH/CsF/Al_2O_3 reagent. The protection of β-hydroxy group of substrates **8-10** was realized through reaction with triethylorthoformate[19] to yield the corresponding ethoxy derivatives **15-17**. Lastly, substitution of chlorine atoms of substrates **15-17** in the above-mentioned conditions produced the corresponding polymethoxynaphthazarins **18-20**[20] in good yields (Scheme). All the ethers prepared (**14**, **18-20**) were easily converted into the corresponding polyhydroxynaphthazarins **21-24**[21] by the action of anhydrous $AlCl_3$ in nitrobenzene[9].

Thiols such as 2-mercaptoethanol and L-glutathione reduced are strong nucleophiles in basic media, and therefore nucleophilic substitution of chlorine atoms on alkylthio groups in dichloronaphthazarins **8**, **12** and **13** by the action of these reagents easily carried out at room temperature to give the corresponding thioderivatives **25-28**[22] (Scheme).

All compounds prepared had the expected spectral (^{1}H NMR, MS) characteristics and all new compounds gave satisfactory elemental analyses.

Biological Results. Cardioprotective effects of the substituted hydroxynaphthazarins **21-28** were evaluated in an animal model of myocardial infarction (MI). Myocardial infarction was induced in male Chinchilla rabbits (2.5-3.5 kg) by ligating the left anterior descending coronary artery for 30 min which was followed by a 7 day reperfusion. The operation was performed under nembutal anesthesia (30-40 mg/kg, i.v.). The MI development was signaled by cyanosis and ST segment elevation (lead I, AVL, pericardial leads). Reperfusion was started by cutting the ligature and confirmed by rapid disappearance of cyanosis. The thorax was rinsed with antibiotics and closed.

Effects of hydroxynaphthazarins **21-28** were compared with those of verapamil and nitroglycerol. The preparations were administered in the marginal ear vein as follows: **a**. hydroxynaphthazarins at an optimal dose of 1 mg/kg b.w. as a water solution containing 1 mg/ml 0.9% NaCl (for the glutathionyl derivatives **26-28**) or 1 mg/ml histidine (25 mM) and 0.04 mM EDTA (for **21-25**), a bolus injection 5 min before reperfusion; **b**. verapamil at a dose of 0.5 mg/kg in 0.9% NaCl, a bolus injection 5 min before reperfusion; **c**. nitroglycerol (650 μg per animal weighing about 3 kg) was infused at a rate of 10 μg/min for 65 min as a mixture of 2 ml 1%

nitroglycerol in ethanol and 200 ml normal saline. The infusion was started 5 min before occlusion and completed in 30 min thereafter. Control rabbits were given equal volumes of normal saline (**a**, **b**) and ethanol (2 ml)/water mixture (200 ml) (**c**).

Rabbits were killed by decapitation 7 days after the experiment. The left ventricle was isolated. Cardiotropic effects of compounds **21-28** were evaluated morphometrically by assessing the size of the postinfarctional scar. Myocardial infarction size was assessed planimetrically by measuring the scar area. The left ventricle was dissected in 5 transverse blocks and frozen at -20°C. Each block was cut into 10 μm sections which were analyzed for the succinate dehydrogenase activity in a reaction with nitroblue tetrazolium[23]. The total area and the scar area were measured using a JBAS-1 analyzer (Opton). The MI size was calculated as a percentage of the infarct area to the total area of the left ventricle. The results obtained are presented in Table.

Table. Effects of hydroxynaphthazarins 21-28 on MI size in rabbits

Compound	Number of animals	LD_{50} (mg/kg)*	MI size (% of the left ventricle)
Control	10		14.02±2.41
21	10	170	10.18±2.9
22	10	87	7.76±1.42**
23	10	80	10.08±1.18
24	10	95	7.90±1.02**
25	10	150	9.97±1.66**
26	10	1350	7.15±0.97**
27	10	1480	8.35±1.58**
28	10	1210	12.37±2.31
verapamil	10		6.51±0.62**
nitroglycerol	10		15.23±3.02

*The acute toxicity of these compounds (a bolus intraperitonea) was evaluated in white crossbred mice weighing 20-25 g. **P<0.05.

As can be seen from Table, in experimental model, echinochrome (**22**), its carboxyl analog **24**, and the diglutathionyl derivatives **26** and **27** exhibit high protective effects in ischemic-reperfusion myocardium and reduce the infarction zone by 50%. At therapeutic doses all aforementioned compounds with a high cardioprotective activity produce no side effects. It is notable that a prominent structure-activity relationship may be observed by correlation of the results obtained (Table). So, the replacement of the ethyl substituent in echinochrome (**22**) for the propyl one (compound **23**) resulted in a reduction of the cardioprotective activity. Similar result was noted when the methyl group in the diglutathionyl derivative **26** was replaced for the ethyl group (compound **27**). On the other hand, the replacement of the alkyl substituents in compounds **26** and **27** or

hydroxyl (compound **28**) resulted in a drastic reduction of cardioprotective activity. All compounds **21-28** exhibit a moderate or small toxicity at a single administration to white crossbred mice. It should be noted, that among all hydroxynaphthazarins obtained only compounds **26-28**, bearing glutathionyl residues, are of a good solubility in water.

Verapamil also reduced the MI size, and its efficiency was equal to that of compound **26** (the most potent agent among compounds examined). At the same time, verapamil impaired cardiac conductivity in 30% of cases (1-3 degree of the transient antrioventricular blockade). Nitroglycerol infusion did not decrease in the MI zone.

By this means, the foregoing results show that hydroxynaphthazarins are of most interest for further investigation as very promising cardioprotective preparations.

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10. Procedure and analytical data for **2**: To a stirred solution of 3.0 g (11.6 mmol) of dichloronaphthazarin (**1**) in 60 ml of conc. H_2SO_4 was carefully added by portions 2.3 g of MnO_2. Care should be taken so that a temperature of the reaction mixture was <40°C. The reaction mixture was stirred for about 2 h at room temperature, monitored by TLC (hexane-acetone, 3:1) every 20 min and then poured into 240 ml of water. To decompose the excess of MnO_2, 1.0 g of oxalic acid was added to the hot mixture. The mixture was stirred for 1 h and then allowed to stand for the night. The precipitate was separated by centrifugation, washed with brine (3x20 ml) and water (20 ml) and dried. The crude product was purified by sublimation at 165°C (1 mmHg) and crystallization from chloroform to give pure 2-hydroxy-6,7-dichloronaphthazarin (**2**) (2.1 g, 66%), m.p. 174°C (dec.); ^{1}H NMR (250 MHz, $CDCl_3$): δ; 6.45 (s, 1H, H^3), 12.05 (s, 1H, OH), 13.38 (s, 1H, OH); eims m/z, 12 eV (rel. int.): 274/276/278 $[M]^+$ (100).
11. Compounds **8-10** were synthesized from **2** in a manner analogous to that described by Fieser, L.F.; Oxford, A.E. *J. Am. Chem. Soc.* **1942**, *64*, 2060-2065. Selected spectral data: **8:** ^{1}H NMR ($CDCl_3$): δ 1.18 (t, 3H, *J* 7.7 Hz, Me), 2.66 (q, 2H, *J* 7.7 Hz, CH_2), 9.77 (br s, 1H, OH), 12.07 (s, 1H, OH), 13.60 (s, 1H, OH); eims m/z, 12 eV (rel. int.): 302/304/306 $[M]^+$ (100); **9:** ^{1}H NMR ($CDCl_3$): δ 1.00 (t, 3H, *J* 7.0 Hz, Me), 1.60 (m, 2H, CH_2), 2.62 (t, 2H, *J* 7.0 Hz, CH_2), 7.36 (br s, 1H, OH), 12.07 (s, 1H, OH), 13.58 (s, 1H, OH); eims m/z, 12 eV (rel. int.): 316/318/320 $[M]^+$ (100); **10:** ^{1}H NMR (DMSO-d_6): δ 2.26 (t, 2H, *J* 7.1 Hz, CH_2COOH), 2.57 (t, 2H, *J* 7.1 Hz, CH_2), 8.32 (s, 1H, OH),

12.43 (br s, 1H, OH), 13.08 (br s, 1H, OH).

12. Compounds **8**, **11**, and **12** were synthesized from the corresponding substrates **5**, **3**, and **4** in a manner analogous to that described by Scheuer P.J. et al. for the synthesis of **13** (see below, Ref. 13.). Selected spectral data: **11:** ^{1}H NMR ($CDCl_3$): δ 1.46 (s, 9H, CMe_3), 7.15 (s, 1H, H^7), 12.70 (s, 1H, OH), 13.59 (s, 1H, OH); **12:** ^{1}H NMR ($CDCl_3$): δ 2.16 (s, 3H, Me), 9.61 (br s, 1H, OH), 12.07 (s, 1H, OH), 13.55 (s, 1H, OH); eims m/z, 12 eV (rel. int.): 288/290/292 $[M]^+$ (100).
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18. Selected spectral data for **14:** ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H, CMe_3), 4.14 (s, 3H, OMe), 4.16 (s, 3H, OMe), 7.19 (s, 1H, H^7), 12.50 (s, 1H, OH), 13.63 (s, 1H, OH); eims m/z, 12 eV (rel. int.): 306 $[M]^+$ (100).
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20. Selected spectral data: **18**: ^{1}H NMR ($CDCl_3$): δ 1.17 (t, 3H, *J* 7.1 Hz, Me), 2.73 (q, 2H, *J* 7.1 Hz, CH_2), 4.08 (s, 3H, OMe), 4.10 (s, 3H, OMe), 4.14 (s, 3H, OMe), 12.98 (s, 1H, OH), 13.13 (s, 1H, OH); **19:** ^{1}H NMR ($CDCl_3$): δ 1.01 (t, 3H, *J* 7.2 Hz, Me), 1.57 (m, 2H, CH_2), 2.67 (t, 2H, *J* 7.2 Hz, CH_2), 4.05 (s, 3H, OMe), 4.09 (s, 3H, OMe), 4.12 (s, 3H, OMe), 13.00 (s, 1H, OH), 13.16 (s, 1H, OH); **20:** ^{1}H NMR ($CDCl_3$): δ 2.58 (t, 2H, *J* 7.6 Hz, CH_2COOMe), 3.04 (t, 2H, *J* 7.6 Hz, CH_2), 3.70 (s, 3H, COOMe), 4.10 (s, 6H, 2xOMe), 4.12 (s, 3H, OMe), 12.95 (s, 1H, OH), 13.10 (s, 1H, OH); eims m/z, 12 eV (rel. int.): 366 $[M]^+$ (100).
21. Selected spectral data: **21:** ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H, CMe_3), 6.78 (br s, 2H, 2xOH), 7.18 (s, 1H, H^7), 11.75 (s, 1H, OH), 12.90 (s, 1H, OH); eims m/z, 12 eV (rel. int.): 278 $[M]^+$ (100); **23:** ^{1}H NMR ($CDCl_3$): δ 1.00 (t, 3H, *J* 7.2 Hz, Me), 1.60 (m, 2H, CH_2), 2.70 (q, 2H, *J* 7.2 Hz, CH_2), 6.42 (br s, 1H, OH), 6.57 (br s, 1H, OH), 6.86 (br s, 1H, OH), 12.10 (s, 1H, OH), 12.30 (s, 1H, OH); eims m/z, 12 eV (rel. int.): 280 $[M]^+$ (100); **24:** ^{1}H NMR (DMSO-d_6): δ 2.38 (t, 2H, *J* 8.0 Hz, CH_2COOH), 2.78 (t, 2H, *J* 8.0 Hz, CH_2), 10.37 (br s, 2H, 2xOH), 11.00 (br s, 1H, OH), 12.14 (br s, 1H, COOH), 12.73 (br s, 1H, OH), 13.20 (s, 1H, OH); eims m/z, 70 eV (rel. int.): 310 $[M]^+$ (52), 292 (44), 264 (100).
22. Selected spectral data: **25:** ^{1}H NMR (DMSO-d_6): δ 1.99 (s, 3H, Me), 3.21 (t, 2H, *J* 6.6 Hz, SCH_2), 3.29 (t, 2H, *J* 6.6 Hz, SCH_2), 3.53 (m, 4H, 2xCH_2OH), 12.89 (s, 1H, OH), 14.09 (s, 1H, OH); eims m/z, 70 eV (rel. int.): 372 $[M]^+$ (43), 327 (100); **26:** ^{1}H NMR (D_2O): δ 1.83 (s, 3H, Me), 2.16 (m, 4H, 2xCH_2), 2.53 (m, 4H, 2xCH_2), 3.39 (d, 1H, *J* 6.5 Hz, SCH), 3.45 (d, 1H, *J* 6.5 Hz, SCH), 3.61 (d, 1H, *J* 6.5 Hz, SCH), 3.66 (d, 1H, *J* 6.5 Hz, SCH), 3.74 (s, 4H, 2xCH_2COOH), 3.78 (t, 1H, *J* 6.5 Hz, CH), 3.81 (t, 1H, *J* 6.5 Hz, CH), 4.61 (t, 1H, *J* 7.6 Hz, CHCOOH), 4.64 (t, 1H, *J* 7.6 Hz, CHCOOH); **27:** ^{1}H NMR (D_2O): δ 1.02 (t, 3H, *J* 7.0 Hz, Me), 2.16 (m, 4H, 2xCH_2), 2.39 (t, 2H, *J* 7.0 Hz, CH_2), 2.52 (m, 4H, 2xCH_2), 3.42 (d, 1H, *J* 6.4 Hz, SCH), 3.47 (d, 1H, *J* 6.4 Hz, SCH), 3.59 (d, 1H, *J* 6.4 Hz, SCH), 3.64 (d, 1H, *J* 6.4 Hz, SCH), 3.71 (s, 4H, 2xCH_2COOH), 3.78 (t, 1H, *J* 6.4 Hz, CH), 3.79 (t, 1H, *J* 6.4 Hz, CH), 4.58 (t, 1H, *J* 7.7 Hz, CHCOOH), 4.60 (t, 1H, *J* 7.7 Hz, CHCOOH), **28:** ^{1}H NMR (D_2O): δ 2.17 (m, 4H, 2xCH_2), 2.55 (m, 4H, 2xCH_2), 3.44 (d, 4H, *J* 6.5 Hz, 2xSCH_2), 3.73 (s, 4H, 2xCH_2COOH), 3.80 (t, 2H, *J* 6.5 Hz, 2xCHCONH), 4.60 (t, 2H, *J* 7.7 Hz, 2xCHCOOH).
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