Bioorganic & Medicinal Chemistry Letters 20 (2010) 2115–2118

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluations of novel bendazac lysine analogues as potent anticataract agents

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ARTICLE INFO

Article history:
Received 17 November 2009
Revised 12 February 2010
Accepted 13 February 2010
Available online 19 February 2010

Keywords:
Bendazac lysine analogues
Anticataract agents
Aqueous solubility

ABSTRACT

Novel bendazac analogues and their salts have been designed and prepared. The resulting compounds (**13c**–**d**, **15c**, **17c**) showed very good aqueous solubility (>100 mg/mL). An in vitro assay showed that most of the resulting compounds had potent protective activity against the oxidative damage. Particularly, compound **13d** was also able to enhance the WSP and T-AOC level in the $H_2O_2/FeCl_3$-induced oxidative damage model, indicating the resulting compound may protect the lens through an antioxidant pathway.

Cataract, often causing severe loss of vision and eventually blindness, is a multifactorial disease process, and is induced by various toxic factors, environmental stressors, and gene mutations.[1] Surgery, with lens extraction and intraocular lens implantation, is the only currently available treatment, but the high operation cost limits its applications in developing countries.[2] In addition, posterior capsule opacification (**PCO**) hinders the ultimate success of cataract surgery as well. It is of necessary need to develop novel agents preventing or delaying the appearance of **PCO** in patients submitted to cataract surgery.[3] So cataract prevention or medical treatment that could slow the progression of cataract is an important task. Among the few drugs currently available for the medical treatment of cataract, bendazac lysine (**BDL**) (Fig. 1) is a topical non-steroidal anti-inflammatory drug.[4,5]

Bendazac is one of agents that have been introduced for the treatment of cataracts. It can protect patients from the loss of vision, thus delaying the need for surgical intervention.[6] Bendazac and its main metabolite, the 5-hydroxy derivative, provide antioxidant effects as scavengers of oxygen-derived free radicals. Hence both of them endowed with protein-stabilizing properties.[7–12] It was shown that bendazac protected lens crystallins against denaturation, glycation, and carbamylation.[13–16] It is generally thought that bendazac stabilizes proteins by binding to specific sites, but a direct antioxidant activity was also demonstrated.[17–21] The most common bendazac-related adverse reactions were laxative activity and other gastrointestinal disturbances; furthermore a few cases of hepatotoxicity have been reported.[22–24]

In this paper a series of novel bendazac analogues have been designed, synthesized and evaluated for their anticataract activity. Among these analogues, a malonic acid group was connected to the bendazac skeleton by replacing the original acetic acid functional group. The presence of this dicarboxylic group is expected to increase the polarity of the resulting compound for the activity. Furthermore, different salts of the dicarboxylic acids with inorganic bases (NaOH, KOH) and amino acids (L-histidine, L-lysine) have also been prepared. The conversion from the free acid to the corresponding salts will probably give a great increase of the aqueous solubility and decrease the irritation of bendazac. The benzyl substituted indazolone, which is believed to be essential for the activity of bendazac, is maintained in the backbone of the analogues. Another structural modification is that different substituent groups have been introduced to the benzyl ring. Through this

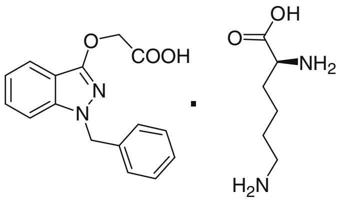

Figure 1. Bendazac lysine (**BDL**).

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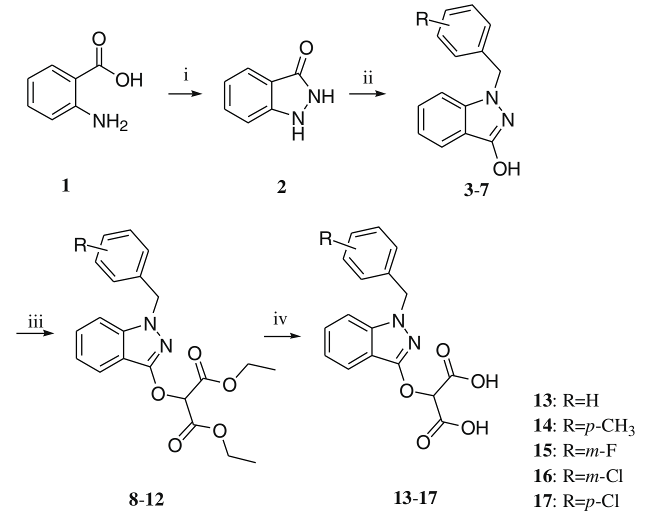
doi:10.1016/j.bmcl.2010.02.061



Scheme 1. Preparation of the study compounds (**13**–**17**). Reagents and conditions: (i) HCl and $NaNO_2$; (ii) NaOH, substituted benzyl chloride, 70 °C, 2 h; (iii) $BrCH(COOEt)_2$, potassium carbonate and TEBA, reflux, 4 h; (iv) KOH, reflux, 2 h.

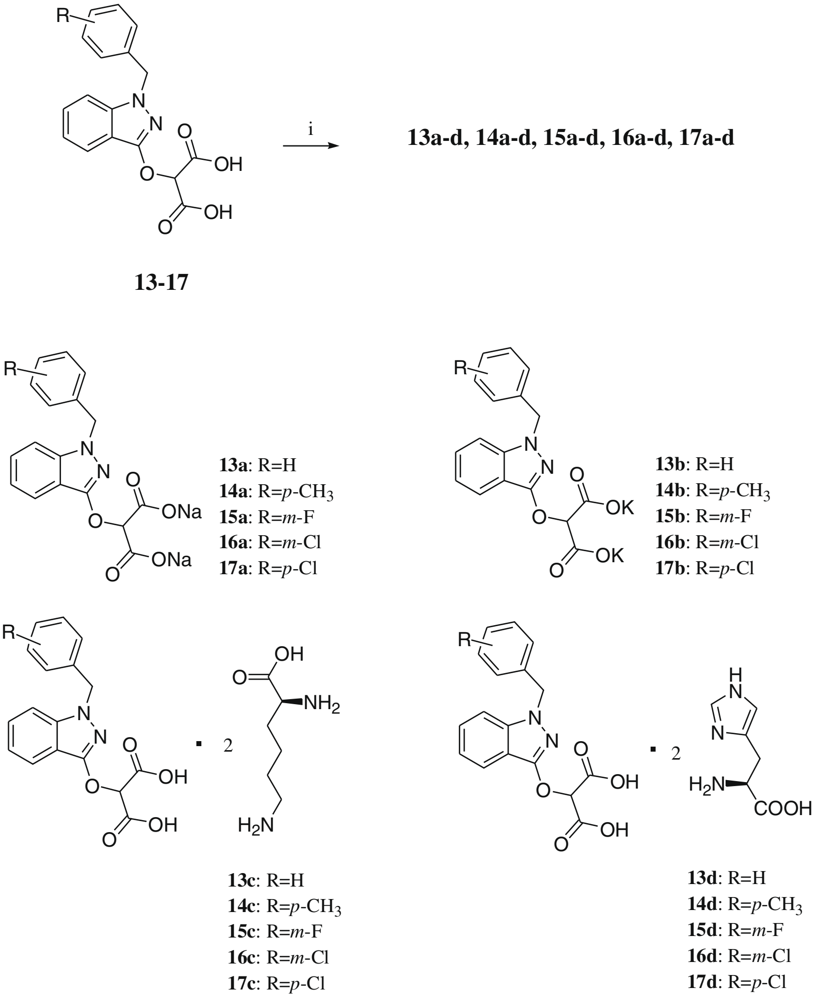
way the structure–activity relationship of the benzyl moiety can be studied. In order to investigate the anticataract activity of the analogues, some compounds (**13a**–**d**, **15a**, **15c**, and **16c**) have been evaluated in vitro.

The synthetic route of new compounds is described in Scheme 1. Indazolone was prepared by diazotization of anthranilic acid, followed by reduction to the hydrazine and ring closure,[25] then indazolone reacting with (un)substituted benzyl chlorine formed a series of N-substituted indazolone.[26] Followed by substitution with diethyl 2-bromomalonate and then hydrolyzation, compounds **13**–**17** were obtained (Scheme 1). These diacids were reacted with NaOH, KOH, L-lysine, and L-histidine, respectively, to generate their corresponding salts (Scheme 2). However, attempts to prepare their amine salts like those of triethylamine and tributylamine with the resulting diacids failed, only unstable compounds, which could hardly be isolated and purified, were obtained.

The aqueous solubility of compounds **13**–**17** and their salts has been measured, which is given in Table 1.

It was found that the solubility of the corresponding amino acid salts was quite good (all over 50 mg/mL), while that of the corresponding sodium or potassium salts was rather low. Among all these compounds, **17c** showed the best solubility, unfortunately, obvious moisture absorption was also found. Moreover, **15** expressed better solubility than other diacids and bendazac. In order to study the antioxidant activity of the resulting compounds, **13a**–**d**, **15a**, **15c**, and **16c** were selected as representative samples to perform further in vitro evaluations.

The ability of compounds to retard $H_2O_2/FeCl_3$-induced cell damage in isolated lenses cultured in Dulbecco's Modified Eagle Medium (DMEM) was tested, using the recently modified Lou's



Scheme 2. Preparation of the study compounds (**13a**–**d**, **14a**–**d**, **15a**–**d**, **16a**–**d**, and **17a**–**d**). Reagents and conditions: (i) corresponding base or amino acid, H_2O, rt, 2 h.

Table 1
The aqueous solubility of compounds

Compounds	Solubility (mg/mL)
Bendazac	<1.0
BDL	93.8
13	<1.0
13a	3.0
13b	2.2
13c	123.2
13d	180.0
14	<1.0
14a	<1.0
14b	<1.0
14c	57.4
14d	71.0
15	2.0
15a	3.2
15b	2.4
15c	134.4
15d	56.6
16	<1.0
16a	1.2
16b	<1.0
16c	195.2
16d	115.2
17	<1.0
17a	<1.0
17b	<1.0
17c	312.0
17d	51.0

protocol.[27] Transparent cultured lenses were randomly divided into control group and different sample groups. Control group lenses were incubated in DMEM supplemented with $FeCl_3$ (0.02%) and hydrogen peroxide (2% for rabbit lenses and 0.5% for rat lenses) which induced cataract formation. For the sample groups, tested compounds and **BDL** were added into culture media of transparent isolated lenses, respectively. $FeCl_3$ (0.02%) and hydrogen peroxide (2% for rabbit lenses and 0.5% for rat lenses) were added into culture media posterior to an hour. After 24 h, all lenses were photographed and the degree of opacification was graded as follows:

−, when there was absence of opacification;
+, when there was a slight degree of opacification;
++, when there was a middle degree of opacification;
+++, when there was extensive mature nuclear opacity.

As seen in Table 2, the order of activities of protecting isolated rabbit lenses against oxidation is **15a > BDL > 13d > 13a > 13b > 13c > > 16c**, and for rat lenses is **13d > 13a > 15a > 15c > 16c > BDL > 13c > > 13b**.

The activities of the resulting compounds and salts are related to the nature of the cation group. It has been shown that the salts with histidine have higher activity than those with lysine, sodium or potassium cations. For example, the activity order is **13d > 13a > 13b > 13c**.

Compound **13d**, which showed potent activity on both rabbit and rat lenses, was selected for further test. Different concentrations of **13d** (5, 10, and 15 mM) were tested for its protective effect on rabbit lenses.

As shown in Table 3, both **BDL** and **13d** have shown protective activities against oxidation compared with the control group. A clear dose-dependent manner of **13d** has been observed, the higher concentration is, the better activity shows. It is also worthwhile to note that the activity of **13d** is higher than **BDL** at the same concentration (10 mM).

Furthermore, the levels of water-solvent protein (WSP), total-antioxidant capacity (T-AOC) and malondialdehyde (MDA, a marker of lipid peroxidation) in lens homogenate of each group were also detected with the reagent kit individually.

As shown in Table 4, the levels of WSP and T-AOC of **BDL** and **13d** group were, as expected, much higher than that of the control group, while the level of MDA was obviously lower. Both **BDL** and **13d** showed positive protective activities. It was observed that at the equal molar dose **13d** showed a better activity than **BDL** as judging from all the three different investigations. Besides, a clear dose-dependent manner of the WSP-protective activity of **13d** was also found. Altogether, the biological results indicate that the resulting compounds, especially **13d**, are potent antioxidants and can effectively protect lens from the oxidative damage.

In conclusion, a series of novel bendazac analogues, which are characterized by replacing the original acetic acid group with the malonic acid group as well as introducing different substituent groups onto the benzyl ring, have been synthesized,[28,29] and their salts[30] are evaluated for the antioxidant activity in isolated lenses. The aqueous solubility of the compounds has been measured, and it was found most of them possessed quite good solubility, especially **15** showed better solubility (2.0 mg/mL) than bendazac (<1.0 mg/mL). The pharmacological results indicated that most compounds exhibited better activities than **BDL** in protecting lens against the oxidative damage. In particular, compound **13d**, bis(L-histidine) 2-(1-benzyl-1*H*-indazol-3-yloxy) malonate, was found to be able to enhance WSP and T-AOC level in the $H_2O_2/FeCl_3$-induced oxidative impairment model, indicating the resulting compound may protect the lenses through a antioxidant pathway.

Table 2
Ability of compounds on protecting lens against oxidative damage in vitro

Group[*]	Percentage of different degree of opacification[a] (%)				Percentage of different degree of opacification[b] (%)			
	−	+	++	+++	−	+	++	+++
Control	0	30	40	30	0	20	45	35
BDL	0	75	25	0	0	55	40	5
13a	0	60	40	0	0	80	20	0
13b	0	30	40	30	0	0	50	50
13c	0	30	35	35	0	15	70	15
13d	0	65	35	0	0	85	15	0
15a	0	80	20	0	0	60	35	5
15c	–	–	–	–	0	55	45	0
16c	0	0	35	65	0	55	30	15

[*] The concentration of tested sample is 0.5 mM.
[a] Tested by isolated rabbit lenses.
[b] Tested by isolated rat lenses.

Table 3
Antioxidant activity of different concentrations of **13d**

Group	Percentage of different degree of opacification[a] (%)			
	–	+	++	+++
Control	0	30	40	30
BDL 10 mM	20	65	15	0
13d 5 mM	30	70	0	0
13d 10 mM	90	10	0	0
13d 15 mM	100	0	0	0

[a] Tested by isolated rabbit lenses.

Table 4
Levels of WSP, T-AOC, and MDA in lens homogenate of each group

Group	WSP mg/mL	T-AOC u/mg protein	MDA nmol/mg protein
Control	7.40 ± 0.73	0.04 ± 0.05	0.46 ± 0.27
BDL 5 mM	9.34 ± 1.65	0.17 ± 0.05*	0.22 ± 0.10
13d 5 mM	12.99 ± 1.95*	0.21 ± 0.12*	0.04 ± 0.02*
13d 10 mM	16.78 ± 5.96*	0.64 ± 0.03*	0.03 ± 0.02*
13d 15 mM	19.04 ± 1.03*	0.34 ± 0.16*	0.08 ± 0.04*

* Compared with control group, $p < 0.01$.

Acknowledgments

The authors would like to thank Zhejiang Sharp Eyes Pharmacy Corporation for the financial support to this research. We are grateful to Dr. Fang Lei for his kind help in the preparation of this Letter.

References and notes

1. Shinohara, T.; White, H.; Mulhern, M. L.; Maisel, H. *Med. Hypotheses* **2007**, *69*, 669.
2. Toh, T.; Morton, J.; Coxon, J.; Elder, M. J. *Clin. Exp. Ophthalmol.* **2007**, *35*, 664.
3. Soldo, L.; Ruggieri, A.; Milanese, C.; Pinza, M.; Guglielmotti, A. *Ophthalmic Res.* **2004**, *36*, 145.
4. Saso, L.; Silvestrini, B. *Med. Hypotheses* **2001**, *56*, 114.
5. Harding, J. J. *Drug Aging* **1992**, *2*, 287.
6. Yin, X.; Zhang, Y.; Shen, J.; Wu, H.; Zhu, X.; Li, L.; Qiu, J.; Jiang, S.; Zheng, X. *Acta Pharmacol. Sin.* **2005**, *26*, 721.
7. Horwitz, J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 10449.
8. Jaenicke, R. *FASEB J.* **1996**, *10*, 84.
9. Jaenicke, R. *Naturwissenschaften* **1994**, *81*, 423.
10. Takemoto, L.; Boyle, D. *Int. J. Biol. Macromol.* **1998**, *22*, 331.
11. Derham, B. K.; Harding, J. J. *Prog. Retin. Eye Res.* **1999**, *18*, 463.
12. Guglielmotti, A.; de Joannon, A. C.; Cazzolla, N.; Marchetti, M.; Soldo, L.; Cavallo, G.; Pinza, M. *Pharm. Res.* **1995**, *32*, 369.
13. Silvestrini, B.; Catanese, B.; Barillari, G.; Iorio, E.; Valeri, P. *Int. J. Tissue React.* **1983**, *5*, 217.
14. Lewis, B. S.; Harding, J. J. *Exp. Eye Res.* **1988**, *47*, 217.
15. Marques, C.; Ramalho, J. S.; Pereira, P.; Mota, M. C. *Doc. Ophthalmol.* **1995**, *90*, 395.
16. Lewis, B. S.; Rixon, K. C.; Harding, J. J. *Exp. Eye Res.* **1986**, *43*, 973.
17. Musci, G.; Silvestrini, B. *Drugs Exp. Clin. Res.* **1987**, *13*, 289.
18. Schmut, O.; Hofmann, H. *Wien. Klin. Wochenschr.* **1985**, *97*, 819.
19. Mira, M. L.; Azevedo, M. S.; Manso, C. *Free Radical Res.* **1987**, *4*, 125.
20. Woollard, A. C. S.; Wolff, S. P.; Bascal, Z. A. *Free Radical Biol. Med.* **1990**, *9*, 299.
21. Crabbe, M. J. C.; Hoe, S. T. *Enzyme* **1991**, *45*, 188.
22. Balfour, J. A.; Clissold, S. P. *Drugs* **1990**, *39*, 575.
23. Prieto de Paula, J. M.; Rodríguez Rodríguez, E.; Villamandos Nicás, V.; Sanz de la Fuente, H.; Prada Mínguez, A.; del Portillo Rubí, A. *Rev. Clin. Esp.* **1995**, *195*, 387.
24. Laporte, J. R.; Capellà, D. *Med. Clin. (Barc)* **1995**, *104*, 396.
25. Baiocchi, L.; Corsi, G.; Palazzo, G. *Synthesis* **1978**, *9*, 633.
26. Gordon, D. W. *Synlett* **1998**, 1065.
27. Wang, G.-M.; Raghavachari, N.; Lou, M. F. *Exp. Eye Res.* **1997**, *64*, 693.
28. *Preparation of compounds* **8–12**. 32 g (0.134 mol) diethyl 2-bromomalonate was added into a solution of **3–7 (**0.054 mol), 18 g (0.131 mol) potassium carbonate and 1 g TEBA in 150 mL of 1,2-dimethoxyethane, followed refluxing for 4 h. After cooled to room temperature, the precipitate was filtered and the volatiles were evaporated in vacuo. The crude product was column chromatographed (*n*-hexane/ethyl acetate = 3:1). *Data for* **8**. Yield: 59%, colorless oil. IR (ν, cm^{-1}): 2981 m, 1768s, 1748s, 1619 m, 1235s, 744 m. ^{1}H NMR ($\mathrm{CDCl_3}$/TMS), δ (ppm): 1.29–1.33 (t, 6H, $\mathrm{CH_2}\mathit{CH_3}$, $J = 7.1$ Hz), 4.29–4.33 (q, 4H, $\mathit{CH_2}\mathrm{CH_3}$, $J = 7.1$ Hz), 5.34 (s, $\mathit{CH_2}\mathrm{C_6H_5}$), 5.77 (s, $\mathrm{C}\mathit{H}\mathrm{(COOH)_2}$), 7.08–7.83 (m, 9H, 2Ar). EI-MS: *m*/*z* $[\mathrm{M}]^+ = 382$.
29. *Preparation of compounds* **13–17**. 16 mmol **8–12** was added to an aqueous solution of KOH (1.8 g/35 mL). After refluxing for 2 h, the pH was adjusted to 2 with diluted hydrochloric acid, and the resulting precipitate was filtered. *Data for* **13**. Yield: 93%, white solid, mp: 182–186 °C (decomposed). IR (ν, cm^{-1}): 3033 m, 2934 m, 1741s, 1620 m, 1257s, 746 m, 711 m. ^{1}H NMR ($\mathrm{D_2O}$/TMS), δ (ppm): 5.30 (s, $\mathit{CH_2}\mathrm{C_6H_5}$), 5.36 (s, $\mathrm{C}\mathit{H}\mathrm{(COOH)_2}$), 7.00–7.73 (m, 9H, 2Ar). ESI-MS: *m*/*z* $[\mathrm{M{-}H}]^- = 325$.
30. *Data for* **13a**. Yield: 75%, white solid, mp: >280 °C. IR (ν, cm^{-1}): 2983w, 1643s, 1334 m, 741 m, 723 m. ^{1}H NMR ($\mathrm{D_2O}$/TMS), δ (ppm): 5.21(s, $\mathrm{C}\mathit{H}\mathrm{(COO^-)_2}$), 5.33 (s, $\mathit{CH_2}\mathrm{C_6H_5}$), 7.03–7.79 (m, 9H, 2Ar). *Data for* **13b**. Yield: 86%, white solid, mp: >280 °C. IR (ν, cm^{-1}): 2978w, 1639s, 1328 m, 740 m, 721 m. ^{1}H NMR ($\mathrm{D_2O}$/TMS), δ(ppm): 5.23 (s, $\mathrm{C}\mathit{H}\mathrm{(COO^-)_2}$), 5.35 (s, $\mathit{CH_2}\mathrm{C_6H_5}$), 6.97–7.78 (m, 9H, 2Ar). *Data for* **13c**. Yield: 86%, white solid, mp: 204–206 °C. IR (ν, cm^{-1}): 3031s, 2941s, 1615s, 1325 m, 741 m. ^{1}H NMR ($\mathrm{D_2O}$/TMS), δ (ppm): 1.30–1.43 (m, $\mathrm{2H_2NC_2H_4}\mathit{CH_2}\mathrm{CH_2}$ of lysine), 1.60–1.63 (m, $\mathrm{2H_2NC_2H_4CH_2}\mathit{CH_2}$ of lysine), 1.78–1.82 (m, $\mathrm{2H_2NCH_2}\mathit{CH_2}\mathrm{C_2H_4}$ of lysine), 2.90–2.93 (m, $\mathrm{2H_2N}\mathit{CH_2}\mathrm{CH_2C_2H_4}$ of lysine), 3.65 (m, $\mathrm{2C}\mathit{H}\mathrm{(NH_2)COOH}$ of lysine), 5.24 (s, $\mathrm{C}\mathit{H}\mathrm{(COO^-)_2}$), 5.36 (s, $\mathit{CH_2}\mathrm{C_6H_5}$), 7.09–7.81 (m, 9H, 2Ar). *Data for* **13d**. Yield: 92%, white solid, mp: 212–214 °C. IR (ν, cm^{-1}): 3128 m, 3026 m, 1618s, 1329 m, 1259w, 744 m. ^{1}H NMR ($\mathrm{D_2O}$/TMS), δ (ppm): 3.10–3.24 (m, $\mathrm{2C}\mathit{H_2}$ of histidine), 3.89–3.94 (m, $\mathrm{2C}\mathit{H}\mathrm{NH_2COOH}$ of histidine), 5.28 (s, $\mathrm{C}\mathit{H}\mathrm{(COO^-)_2}$), 5.34 (s, $\mathit{CH_2}\mathrm{C_6H_5}$), 7.05–7.80 (m, 11H, 9H of 2Ar and 2H of 2 imidazole), 8.27 (s, 2H of 2 imidazole).