ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 54 (1999) 780–784

IL FARMACO

4-Quinazolinones: synthesis and reduction of prostaglandin E_2 production

Natale Alfredo Santagati *, Ennio Bousquet, Angelo Spadaro, Giuseppe Ronsisvalle

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Catania, Viale Andrea Doria 6, 95125 Catania, Italy

Received 19 July 1999; accepted 28 September 1999

Abstract

We synthesized and evaluated the anti-inflammatory activity of a series of 4-quinazolinone derivatives. Two approaches were used to yield the title compounds. A first group of quinazolinone derivatives was obtained by the appropriate substituted anthranilates. A second group of quinazolinone compounds was prepared through the benzoxazin-4-ones intermediate. The pharmacological results reveal that the synthesized derivatives exhibit a significant anti-inflammatory effect in an experimental ocular inflammation model. In fact, all the tested compounds lowered the prostaglandin E_2 (PGE_2) production with respect to the control group ($P < 0.05$). The 3-cyclohexyl-6-chloro-quinazolin-4(3H)-one and 3-cyclohexyl-quinazolin-4(3H)-one derivatives were the most active compounds. These compounds significantly reduced PGE_2 levels even more than the reference drug tolmetin and significantly lower protein concentration and polymorphonuclear leukocytes number compared to the control group ($P < 0.05$). Therefore, these compounds may be useful to prevent ocular inflammatory reactions.

Keywords: Quinazolinones; Synthesis; Antiinflammatory; Prostaglandins; Ocular model

1. Introduction

A large number of compounds with a 4-quinazolinonic structure have been synthesized for biological screening, and a variety of interesting pharmacological properties were observed [1]. The most important activities regard their anticonvulsant [2–5] and their anti-inflammatory actions [6–10]. Moreover, several 4-quinazolinone compounds have shown analgesic [11] and antihypertensive [12,13] activities. Recently, a centrally 5-HT_{2A} antagonist activity [14] and a good affinity for cholecystokinin CCK-B receptors [15,16] have been shown from different derivatives with quinazolinonic structure.

As part of an ongoing research project regarding the development of substances with anti-inflammatory activity [17] we have synthesized a series of 4-quinazolinone derivatives with different substituents at the 2, 3 and 6 positions in order to evaluate the decrease of PGE_2 levels after treatment with arachidonic acid (AA).

Interaction of AA with cycloxygenase produces initially the cyclic endoperoxide prostaglandin G_2 (PGG_2) and thence, through its peroxidase activity, to prostaglandin H_2 (PGH_2). The action of prostaglandin endoperoxide isomerase on PGH_2 produces PGE_2, an important mediator on the inflammatory process [18].

2. Results and discussion

2.1. Chemistry

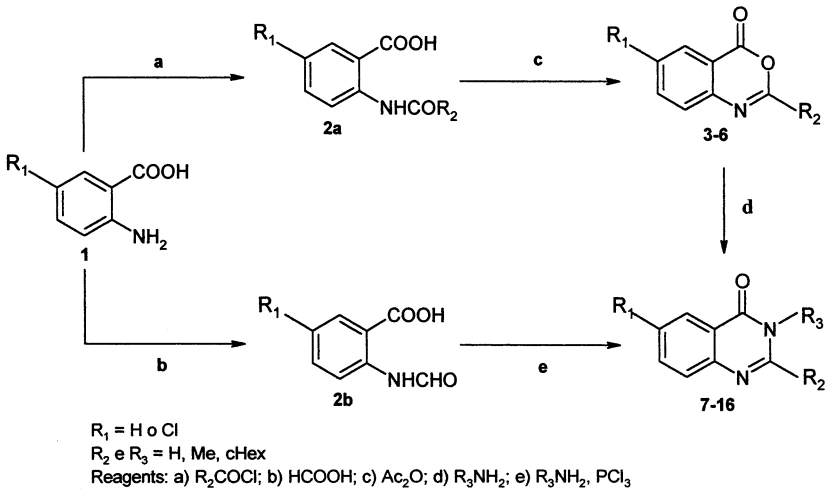
The synthetic route to the quinazolin-4(3H)-one derivatives is summarized in Scheme 1.

The anthranilic acids (**1**) were coupled with the appropriate acid chloride generating the corresponding substituted anthranilates (**2a**), which underwent cyclization by treatment with boiling acetic anhydride to form the intermediate benzoxazin-4-ones (**3–6**). The substituted benzoxazinones by treatment with ammonia or methylamine solutions yielded the quinazolinone derivatives **7–12**. To prepare the compounds **13–16** we followed a different synthesis method. The two intermediate anthranilates (**2b**), obtained by refluxing the acids

* Corresponding author. Tel.: +39-095-738 4004; fax: +39-095-222 239.

E-mail address: santagat@mbox.unict.it (N. Alfredo Santagati)

0014-827X/99/$ - see front matter
PII: S0014-827X(99)00102-0



Scheme 1. Synthetic routes to benzoxazinones (**3–6**) and quinazolin-4(3H)-ones (**7–16**).

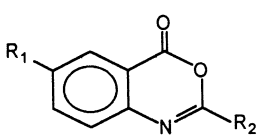
(**1**) in benzene with 98% formic acid, were treated with methylamine or cyclohexylamine and subsequently with phosphorus tricloride, to yield the final derivatives **13–16**. The structures of the synthesized compounds were confirmed by elemental analysis, IR and ^{1}H NMR. Structures, yields, melting points and recrystallization solvents of the benzoxazinones and the quinazolinones are presented in Tables 1 and 2, respectively.

2.2. Pharmacology

The anti-inflammatory activity of the synthesized 4-quinazoline derivatives was evaluated in an ocular inflammation model previously described [19]. An inspection of pharmacological data, reported in Table 3, reveals that all the compounds tested lowered the PGE_2 production with respect to the control group ($P < 0.05$).

We also evaluated the activity of benzoxazin-4-ones intermediate compounds but we did not record any activity. This result may be explained by the fact that benzoxazin-4-ones in aqueous environment undergo a rapid hydrolytic reaction of the lactonic ring, as demonstrated by high performance liquid chromatographic analysis (data not shown). The 3-cyclohexyl-6-chloroquinazolin-4(3H)-one (**16**) and 3-cyclohexylquinazolin-4(3H)-one (**14**) derivatives were the most active compounds. In fact, these compounds significantly reduced PGE_2 levels even more than the reference drug with 5.5- and 3.8-fold decrease, respectively. In addition these compounds showed a significant reduction in protein concentration, and PMNS number compared to the control group ($P < 0.05$). The compounds **10** and **12** also reduced the PGE_2 production even more than the reference drug with a 3.3- and 2.5-fold decrease, respectively. However, these compounds showed a non-significant reduction in protein concentration, and PMNS number with respect to the control group ($P > 0.05$). Regarding the compounds **7**, **8**, **9**, **11**, **13**, **14** and **15** we observed a decrease of PGE_2 production following arachidonate administration with respect to the control

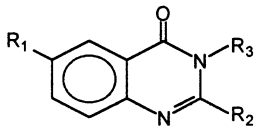
Table 1
Structures, yields and melting points of the prepared benzoxazinone derivatives [a]



Compound	R_1	R_2	Yield (%)	M.p. (°C)	Formula
3	H	Me	88	86–87	$C_9H_7NO_2$
4	H	cHex	78	67–69	$C_{14}H_{15}NO_2$
5	Cl	Me	92	124–125	$C_9H_6NO_2Cl$
6	Cl	cHex	85	104–06	$C_{14}H_{14}NO_2Cl$

[a] All compounds were recrystallized from petroleum ether (b.p. 80–100°C).

Table 2
Structures, yields and melting points of the prepared quinazolinones derivatives



Compound	R_1	R_2	R_3	Yield (%)	M.p. (°C)	Formula
7 [a]	H	Me	H	91	238–239	$C_9H_8N_2O$
8 [a]	H	cHex	H	85	225–226	$C_{14}H_{16}N_2O$
9 [a]	Cl	Me	H	90	282–283	$C_9H_7N_2OCl$
10 [a]	Cl	cHex	H	87	262	$C_{14}H_{15}N_2OCl$
11 [a]	H	cHex	Me	88	111–112	$C_{15}H_{18}N_2O$
12 [a]	Cl	cHex	Me	80	155–156	$C_{15}H_{17}N_2OCl$
13 [b]	H	H	Me	56	106	$C_9H_8N_2O$
14 [a]	H	H	cHex	48	114	$C_{14}H_{16}N_2O$
15 [b]	Cl	H	Me	52	148	$C_9H_7N_2OCl$
16 [a]	Cl	H	cHex	41	158	$C_{14}H_{15}N_2OCl$

[a] Recrystallized from ethanol 95°C.
[b] Recrystallized from cyclohexane.

group. These compounds were less active than the reference drug tolmetin. In addition these derivatives were not able to reduce the protein concentration and PMNS number compared to the control group.

It has been well demonstrated [19] that topical administration of arachidonic acid causes a production of PGE_2 and leukotriene B_4 (LTB_4) in rabbit aqueous humor as well as a breakdown of the blood–aqueous barrier. LTB_4 is a potent chemiotactic agent for PMNs. The treatment with some of the synthesized compounds decreased the number of PMNs in the aqueous humor but not in significant measure, except for compounds **14** and **16**. This could be due to the presence of LTB_4 whose biosynthesis could be inhibited in a different way from the drugs tested. Furthermore, the presence of proteins in the aqueous humor indicates a breakdown of the blood–aqueous barrier which was significantly contrasted by compounds **16** and **14**.

The pharmacological results showed that the presence of the cyclohexylic group was important for the anti-inflammatory activity with respect to the methyl group. In particular, the activity is greater when cycloaliphatic substituent was found in position 3 rather than in position 2. The improvement in the activity of the 3-cyclohexyl-derivatives was not due to the blocking of the tautomerism of the –NH–CO– group as demonstrated by the lower activity of the corresponding 3-methyl-derivatives **12**, **11**, **5** and **13**. In all synthesized derivatives the introduction of a chlorine atom in position 6 caused a remarkable increment of the activity with respect to the non-substituted analogues. The decrease of PGE_2 levels after drug treatment is particularly interesting because this mediator plays an important role in the ocular inflammation process. The results of this study suggest that the compounds 3-cyclohexyl-6-chloro-quinazolin-4(3H)-one (**16**) and 3-cyclohexyl-quinazolin-4(3H)-one (**14**) may be employed topically to prevent ocular inflammatory reactions.

Table 3
Prostaglandin E_2 levels, protein concentration and number of polymorphonuclear leukocytes in the aqueous humor of albino rabbit 2 h after arachidonate instillation in the eye pre- and post-treated with quinazolin-4(3H)-ones (**7**–**16**) or reference drug tolmetin

Compound	PGE_2 [a] (ng/g)	PMNs [a] ($PMNs/mm^3$)	Protein [a] (mg/ml)
7	7.60 ± 1.50 *	1480 ± 220	24.5 ± 5.1
8	4.00 ± 0.96 *	1420 ± 300	24.6 ± 2.9
9	6.50 ± 1.34 *	1450 ± 195	24.8 ± 3.1
10	0.75 ± 0.02 *	1350 ± 200	23.2 ± 2.7
11	4.15 ± 0.95 *	1380 ± 420	24.1 ± 4.1
12	1.00 ± 0.15 *	1390 ± 320	23.6 ± 2.8
13	6.00 ± 1.35 *	1400 ± 250	24.2 ± 3.1
14	0.65 ± 0.15 *	1200 ± 250 *	20.3 ± 3.1 *
15	5.50 ± 0.91 *	1380 ± 400	24.5 ± 6.1
16	0.45 ± 0.11 *	1100 ± 200 *	19.5 ± 2.8 *
Tolmetin	2.50 ± 0.58 *	700 ± 150	10.1 ± 4.1
Control	22.2 ± 4.31	1500 ± 200	24.7 ± 3.7

[a] $n = 8$ eyes.
* $P < 0.05$ with respect to control group.

3. Experimental

3.1. Chemistry

2-Amino-benzoic acid and 2-amino-5-chloro-benzoic acid are commercially available from Fluka Chimica (Milan). All other chemicals or solvents were of reagent grade.

IR spectra were recorded on a Perkin–Elmer 1720 spectrophotometer as KBr disks. Melting points were determined with a Büchi 510 apparatus and are not corrected. NMR spectra were performed on a Varian Inova 200 MHz spectrometer. Elemental analyses for C, H and N were obtained on a Carlo Erba 1106 analyser and were within $\pm 0.4\%$ of theoretical values. Thin-layer chromatography (TLC) was carried out on Merck Silica Gel 60 F_{254} (0.25 mm thickness). Column chromatography was performed by the flash procedure.

3.1.1. General procedure for the synthesis of benzoxazinones (3–6)

The intermediate benzoxazin-4-ones **3–6** were prepared according to the literature method [20]. A mixture of the opportune anthranilate derivative of general structure **2a** (50 mmol) and acetic anhydride (45 ml) was refluxed for 3 h. The solution was concentrated under reduced pressure until an oily residue was obtained, which, after cooling at room temperature (r.t.), solidified. IR (cm^{-1}): 1735–1760 (C=O), 1590–1600 (C=N) (see Table 1).

3.1.2. General procedure for the synthesis of quinazolin-4(3H)-ones (7–12)

The title compounds were prepared according to a literature method [21]. A mixture of opportune benzoxazinone derivative **3–6** (40 mmol) in absolute ethanol (20 ml) was treated with an excess of NH_3 33% solution (40 ml) or methylamine 40% solution (40 ml) and left at r.t. for 48 h. The solid, obtained as light-thin crystals, was filtered, dried, and crystallized by opportune solvent and purified by flash chromatography (toluene:methanol, 95:5 v/v). IR (cm^{-1}): 1665–1700 (C=O), 1590–1600 (C=N) (see Table 2).

3.1.2.1. 2-Methyl-4(3H)-quinazolinone (7). ^{1}H NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 7.41–8.10 (m, 4H, ArH), 12.22 (s, 1H, NH).

3.1.2.2. 2-Cyclohexyl-4(3H)-quinazolinone (8). ^{1}H NMR ($CDCl_3$): δ 1.40–2.09 (m, 10H, CH_2–cHex), 2.69–2.80 (m, 1H, CH–cHex), 7.42–8.31 (m, 4H, ArH), 11.78 (s, 1H, NH).

3.1.2.3. 6-Chloro-2-methyl-4(3H)-quinazolinone (9). ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 7.57–8.00 (m, 3H, ArH), 12.38 (s, 1H, NH).

3.1.2.4. 6-Chloro-2-cyclohexyl-4(3H)-quinazolinone (10). ^{1}H NMR (DMSO-d_6): δ 1.17–1.93 (m, 10H, CH_2–cHex), 2.51–2.56 (m, 1H, CH–cHex), 7.59–8.01 (m, 3H, ArH), 12.24 (s, 1H, NH).

3.1.2.5. 2-Cyclohexyl-3-methyl-4(3H)-quinazolinone (11). ^{1}H NMR ($CDCl_3$): δ 1.59–2.13 (m, 10H, CH_2–cHex), 2.54–2.75 (m, 1H, CH–cHex), 3.52 (s, 3H, CH_3), 7.11–8.01 (m, 4H, ArH).

3.1.2.6. 6-Chloro-2-cyclohexyl-3-methyl-4(3H)-quinazolinone (12). ^{1}H NMR ($CDCl_3$): δ 1.34–1.98 (m, 10H, CH_2–cHex), 2.49–2.70 (m, 1H, CH–cHex), 3.56 (s, 3H, CH_3), 7.41–8.09 (m, 3H, ArH).

3.1.3. General procedure for the synthesis of quinazolin-4(3H)-ones (13–16)

A solution of compound type **2b** (0.1 mol) in pyridine (50 ml) was treated with an excess of methylamine 40% solution (20 ml) or cyclohexylamine (0.1 mol) and PCl_3 (0.12 mol). The mixture was kept in a water bath at 40°C for 90 min. The pyridine excess was then eliminated at reduced pressure, the residue was dissolved by hot HCl (10%). After filtration and cooling at r.t., the filtrate was neutralized by NaOH (20%) and then left to precipitate the final compounds, which were dried and crystallized by opportune solvents. IR (cm^{-1}): 1670–1700 (C=O), 1600–1610 (C=N) (see Table 2).

3.1.3.1. 3-Methyl-4(3H)-quinazolinone (13). ^{1}H NMR ($CDCl_3$): δ 3.59 (s, 3H, CH_3), 7.25–8.10 (m, 4H, ArH), 7.39 (s, 1H, CH).

3.1.3.2. 3-Cyclohexyl-4(3H)-quinazolinone (14). ^{1}H NMR ($CDCl_3$): δ 1.20–2.03 (m, 10H, CH_2–cHex), 4.74–4.87 (m, 1H, CH–cHex), 7.44–8.33 (m, 4H, ArH), 8.12 (s, 1H, CH).

3.1.3.3. 6-Chloro-3-methyl-4(3H)-quinazolinone (15). ^{1}H NMR ($CDCl_3$): δ 3.90 (s, 3H, CH_3), 7.40 (9s, 1H, CH, 7.48–8.11 (m, 3H, ArH).

3.1.3.4. 6-Chloro-3-cyclohexyl-4(3H)-quinazolinone (16). ^{1}H NMR ($CDCl_3$): δ 1.01–1.87 (m, 10H, CH_2–cHex), 4.56–4.63 (m, 1H, CH–cHex), 7.61–8.03 (m, 3H, ArH), 8.10 (s, 1H, CH).

3.2. Pharmacology

Arachidonic acid and Tolmetin (used as control drug) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Ketamine HCl (Ketalar®) was obtained from Parke-Davis (Milan, Italy). The prostaglandin E_2 (PGE_2) enzyme immunoassay kit was purchased from Amersham Life Science (Milan, Italy) and Coomassie solution was obtained from Pierce (Rockford, IL, USA).

3.2.1. Drug formulations

All drugs tested were formulated at a concentration of 0.02 M in the same isotonic vehicle with a pH of 7.20 (carboxymethylcellulose 1%, NaCl, phosphate buffer). Tolmetin in this condition was soluble whereas all the 4-quinazolone derivatives tested were present as a suspension.

3.2.2. Animals

Female New Zealand albino rabbits (Charles River, Calco, Italy), 1.8–2.2 kg, free of any signs of ocular inflammation or gross abnormality, were used. Animal procedures conformed to the ARVO (Association for Research in Vision and Ophthalmology) resolution on the use of animals in research.

3.2.3. Ocular inflammation

Ocular inflammation was induced as described previously [19] by topical administration (50 μl) of 0.5% sodium arachidonate dissolved in phosphate-buffered saline (pH 7.4). Drug formulations (50 μl) were instilled into the conjunctival sac 180, 120, 90 and 30 min before induction of ocular inflammation by arachidonate (pretreatment) and then again 60 min thereafter (post-treatment). Two hours after the arachidonate instillation the rabbits were anesthetized by intravenous injection of 20 mg/kg of Ketamine HCl. Aqueous humor was withdrawn by a tuberculin syringe and divided into three aliquots in order to evaluate the content of PGE_2 by enzyme immunoassay, the protein concentration by Coomassie solution and polymorphonuclear leukocyte (PMNs) levels by an improved Neubauer chamber. The experimental plan included a group of four animals for each drug tested including the reference group (treated with the reference drug tolmetin) and the control group that received no treatment.

3.2.4. Statistical analysis

Results are expressed as the mean $\pm$ SD Student's *t*-test was used to evaluate the significance between the groups of animals. The statistical significance was fixed at $P < 0.05$.

Acknowledgements

The authors are grateful to the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST) for financial assistance.

References

[1] W.L.F. Armarego, Quinazolines, in: A.R. Katritzky, A.J. Boulton (Eds.), Adv. Het. Chem, vol. 24, Academic Press, New York, 1979, pp. 56–60.

[2] M.A. Aziza, M.K. Ibrahim, A.G. El-Helby, Pirazolo[3,4-d]-pyrimidines: C_4, C_6 substitution leads to adenosine A_1 receptor selectivity, Al-Azhar J. Pharm. Sci. 13 (1994) 66–73.

[3] A.E. Ossman, S.E. Barakat, Synthesis and anticonvulsant activity of same new 3-(*p*-sulphamoylphenyl)-4(3H)-quinazolinones, Arzneim. Forsch. 44 (1994) 915–919.

[4] M. Shrimali, R. Kalsi, K.S. Dixit, J.P. Barthwal, Substituted quinazolones as potent anticonvulsants and enzyme inhibitors, Arzneim. Forsch. 41 (1991) 514–519.

[5] M. Hori, R. Iemura, H. Hara, A. Ozaki, T. Sukamoto, H. Ohtaka, Novel 4-substituted 2-piperazinylquinazolines as potent anticonvulsive and antihypoxic agents, Chem. Pharm. Bull. 38 (1990) 1286–1291.

[6] A. Bekhit, M.A. Khalil, Non-steroidal anti-inflammatory agents: synthesis of novel benzopyrazolyl, benzoxazolyl and quinazolinyl derivatives of 4(3H)-quinazolinones, Pharmazie 53 (1998) 539–543.

[7] A. Hitkari, M. Bhalla, A.K. Saxena, M. Verma, M.P. Gupta, K. Shanker, Substituted quinazolinones and their anti-inflammatory activity, Boll. Chim. Farm. 134 (1995) 609–615.

[8] S. Plescia, G. Daidone, D. Raffa, M.L. Bajardi, Synthesis and pharmacological study of some 3-(isoxazol-5-yl)-quinazolin-4(3H)-ones, Farmaco 47 (1992) 465–475.

[9] A. Kumar, R.S. Verma, B.P. Jaju, J.N. Sinha, Quinazolinylpyrazolines as antiinflammatory agents, J. Indian Chem. Soc. 67 (1990) 920–921.

[10] A.M. Farghaly, I. Chaaban, M.A. Khalil, A.A. Bekhit, Non-steroidal antinflammatory agents. Synthesis of novel 2-pyrazolyl-4(3H)quinazolinones, Arch. Pharm. 323 (1990) 833–836.

[11] L. Fisnerova, J. Grimova, Z. Roubal, E. Maturova, B. Brunova, Ester of 3-(2-hydroxyethyl)-4(3*H*)quinazolinone with an analgesic effect, Cesk. Farm. 35 (1986) 447–450.

[12] E.E. Allen, S.E. Delaszlo, S.X. Huang, C.S. Quagliato, W.J. Greenlee, R.S.L. Chang, T.-B. Chen, K.A. Faust, V.J. Lotti, Quinazolinones-1: design and synthesis of potent quinazolinone-containing AT(1)-selective angiotensin-II receptor antagonists, Biorg. Med. Chem. Lett. 3 (1993) 1293–1298.

[13] S. Botros, S.F. Saad, Synthesis, antihypertensive and β-adrenoreceptor antagonist activities of 3-[4-[3-(4-aryl-1-piperazinyl)-isopropanoloxy]phenyl]-4(3H)-quinazolones, Eur. J. Med. Chem. 24 (1989) 585–590.

[14] F. Claudi, G. Giorgioni, L. Scoccia, R. Ciccocioppo, I. Panocka, M. Massi, 3-[2-[4-(4-Fluorobenzoyl)piperidin-1-yl]ethyl]-5,6,7,8-tetrahydro-4(3H)-quinazolinones: serotonin 5-HT_{2A} receptor antagonists endowed with potent central action, Eur. J. Med. Chem. 32 (1997) 651–659.

[15] A. Varnavas, L. Lassiani, E. Luxich, M. Zacchigna, E. Boccu, Quinazolinones derivatives: synthesis and binding evaluation on cholecystokinin receptors, Farmaco 51 (1996) 333–339.

[16] K. Rasmussen, M.J. Yu, J.F. Czachura, Quinazolinone cholecystokinin(CCK)-B antagonists decrease midbrain dopamine unit activity, Synapse 17 (1994) 278–282.

[17] N.A. Santagati, A. Caruso, V. Cutuli, F. Caccamo, Synthesis and pharmacological evaluation of thieno[2,3-d]pyrimidin-2,4-dione and 5H-pyrimido[5,4-b]indol-2,4-dione derivatives, Farmaco 50 (1995) 689–695.

[18] R.F. Borne, in: W.O. Foye, T.L. Lemke, D.A. Williams (Eds.), Principles of Medicinal Chemistry, Williams and Wilkins, USA, 1995, pp. 538–542.

[19] S. Spampinato, A. Marino, C. Bucolo, T. Bachetti, S. Mangiafico, Effects of sodium naproxen eye drops on rabbit ocular inflammation induced by sodium arachidonate, J. Ocular Pharmacol. 7 (1991) 125–133.

[20] L.A. Errede, H.T. Oien, D.R. Yarian, Acylanthranils 3. The influence of ring substituents on reactivity and selectivity in the reaction of acylanthranils with amines, J. Org. Chem. 42 (1977) 12–18.

[21] H.W. Grimmel, A. Guenther, J.F. Morgan, A new synthesis of 4-quinazolones, J. Am. Chem. Soc. 68 (1946) 542–545.