Med Chem Res (2015) 24:2461–2475
DOI 10.1007/s00044-014-1311-7

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, structure–activity relationships, and bioactivity evaluation of 6-bromo-quinazolinone derivatives

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Received: 24 May 2014 / Accepted: 7 December 2014 / Published online: 13 December 2014

Abstract 6-Bromo-quinazolinone derivatives were prepared and evaluated for the ability to inhibit cyclooxygenase-2 (COX-2). An extensive structure–activity relationship work was carried out, thus some potent and selective COX-2 inhibitors were identified. The key compound isothiocyanate was prepared through a simple and ecological method using di-2-pyridyl thionocarbonate in substitution of the thiophosgene, a potential air pollutant. The cyclization reaction of intermediate derivatives was developed through the methods reporting by Wamhoff. The anti-inflammatory activity of the derivatives **(5–12)** was evaluated by determining (by Western blot) the expression of cyclooxygenase (COX)-2, of inducible NO synthase (iNOS) and of intercellular adhesion molecule-1 (ICAM-1). The biological assays showed that the derivatives **7**, **9**, **10**, **12** act as potent inhibitors of COX-2, iNOS, and ICAM-1 expression in human keratinocytes NCTC-2544 cells. This work showed that the new derivatives could be used as a novel class of anti-inflammatory agents.

Keywords Quinazolinone · Synthesis · Anti-inflammatory · Cell · Keratinocytes

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Introduction

Inflammation is a complicated process involving several cell types and many putative mediators and modulators (Zambre *et al.,* 2007). It is well documented that several nonsteroidal anti-inflammatory drugs (NSAIDs) exert anti-inflammatory and analgesic effects through the inhibition of prostaglandin (PG) synthesis, by blocking cyclooxygenase (COX) activity (Vane, 1971). Nonsteroidal anti-inflammatory drugs (NSAIDs) have been used as first-line therapy for relieving inflammation and pain associated with arthritic conditions. Chronic usage of these drugs has been associated with the propensity for side effects such as gastrointestinal irritation (GI) (Allison *et al.,* 1992) and suppression of renal function (Clive and Stoff, 1984). Most NSAIDs inhibit the cyclooxygenase required for conversion of arachidonic acid to endoperoxide intermediate PGG_2 and PGH_2. Subsequently, PGH_2 is converted by a series of metabolizing enzymes into bioactive lipids, which include prostaglandins and thromboxane (Marnett and DuBois, 2002). COX activity originates from two distinct and independently regulated enzymes, termed COX-1 and COX-2 (DeWitt and Smith, 1988; Hla and Neilson, 1992). COX-1 is the constitutive isoform and is mainly responsible for the synthesis of cytoprotective prostaglandins in the gastrointestinal tract (GI) and of the proaggregatory thromboxane in blood platelets (Allison *et al.,* 1992). Differently, COX-2 is inducible and short-lived; its expression is stimulated in response to endotoxin, cytokines, and mitogens (Kujubu *et al.,* 1991; O'Sullivan *et al.,* 1993). COX-2 plays a major role in prostaglandin biosynthesis in inflammatory cells (monocytes/macrophages) and in the central nervous system (Smith *et al.,* 1998). The different tissue distribution of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) provides a rationale for the development of selective COX-2

inhibitors as anti-inflammatory/analgesic agents that lack in the GI side effects usually exhibited by NSAIDs (Masferrer *et al.,* 1994; Hawkey *et al.,* 2000). However, the simplified paradigm of constitutive COX-1 and inducible COX-2 has many exceptions: COX-1 can be regulated during development (Smith and Langenbach, 2001; Rocca *et al.,* 1999), whereas COX-2 is constitutively expressed in the brain (Yamagata *et al*., 1993), reproductive tissues (Kniss, 1999), and kidney (Harris *et al*., 1994; Ferreri *et al.*, 1999). The inhibition of COX-2 is thought to mediate the therapeutic actions of nonselective NSAIDs, whereas the inhibition of COX-1 results in unwanted side effects, particularly in the GI tract (Fitzgerald and Patrono, 2001; Patrono *et al.,* 2001). NSAIDs, including COX-2 inhibitors, may cause significant increase in heart attacks and strokes. This risk is particularly noteworthy in patients with risk factors for heart disease, in patients with related conditions and in those with longer duration of drug use. Some of these cause higher risks than any other does. However, celecoxib is still available to treat pain or inflammation caused by several conditions such as arthritis and ankylosing spondylitis (Miao *et al.*, 2014). It seemed worthwhile to look for candidates acting on more than one pathway involved in inflammatory conditions. Thus, in this study, we describe the synthesis and biological evaluation of a group of compounds containing 4(3*H*)-quinazolinone ring system as selective COX-2 inhibitors with anti-inflammatory and analgesic activities. The synthesis of these quinazolinone derivatives is not only of interest from an economical point of view, but in many cases also offers considerable advantages in terms of yield, selectivity, and simplicity of the reaction procedure. Quinazoline-4(3*H*)-ones are versatile nitrogen heterocyclic compounds, displaying a broad spectrum of biological and pharmacological activities such as anti-fungal (Liu *et al.,* 2006), anti-histaminic (Lemura *et al.*, 1989), anti-cancer (Abbas *et al.*, 2012), anti-viral (Dinakaran *et al.*, 2003), anti-inflammatory (Amin *et al.,* 2010), and anti-bacterial (Kini and Grover, 2006). Recent studies assert that some sulfonamides also work as anti-inflammatory drugs. Indeed, celecoxib works as a COX-2 inhibitor (Gassani *et al.*, 2010) and acetazolamide works by a diuretic mechanism (Jaiswal *et al.*, 2004). On light of these findings, we planned to prepare drug compounds as hybrid molecules. These molecules contain a quinazolinone ring system fused with a sulfonamide derivative in order to form a group of compounds resembling and collecting both features (nitrogen heterocyclic and sulfonamide moiety). These derivatives (**5**–**12**), containing functional groups present in some other selective inhibitors of COX-2 (Dannhart and Kiefer, 2001), have been obtained through the methods of cyclization reported by Wamhoff, starting from heteroaromatic β-enaminoesters (Wamhoff and Lichtenthaler, 1978). For the biological evaluation of the anti-inflammatory activity, **5**–**12** were evaluated on in vitro human keratinocyte cell line NCTC-2544 exposed to interferon (IFN-γ) and histamine. This cell model, particularly useful for reproducing the mechanisms involved in inflammation, was successfully used in our lab as reported in previous published studies (Cardile *et al.,* 2010; Barone *et al.,* 2013).

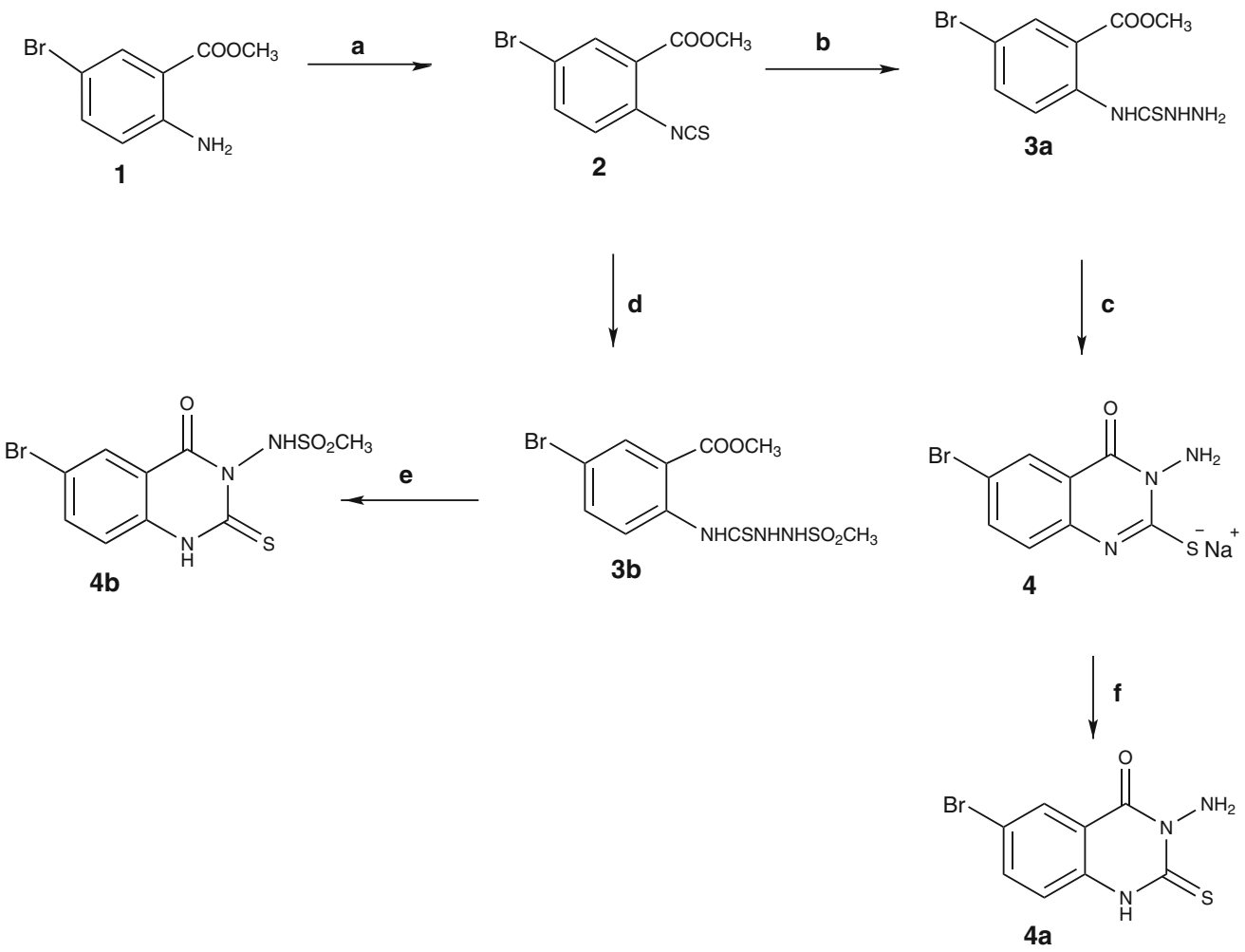
Results and discussion

Chemistry

As reported in literature, the presence of a quinazoline moiety, which can undergo substitution at the heteroatom or the distal aromatic ring, is a necessary requirement for anti-inflammatory activities. Moreover, quinazoline derivatives with the appropriate substituent mainly amine or substituted amine at position 4, as well as halogen (or electron rich substituent) at position 3, 6, or 8, are known to act against bacteria and inflammation (Tiwari *et al.*, 2006). In view of the previous rationale, it was reasonable to study the effects of two pharmacophoric moieties like quinazolinone and sulfonamide in a single molecule on inflammatory processes. The target compounds have been designed to contain different substituents with different electronic environments (Zayed and Hassan, 2014). As shown in Scheme 1, the starting compound was isothiocyanate **2** prepared, with high yield, from the reacting amino ester **1** in dichloromethane at room temperature for 24 h with di-2-pyridyl thionocarbonate (DPT), in substitution of the tedious previous method using thiophosgene, a potential air pollutant (Hodgkins and Ettilinger, 1956). The structure of product **2** was confirmed by means of the N=C=S group band in the regions 2,140–2,090 cm^{-1} of the infrared spectrum.

The reaction of isothiocyanate **2** with hydrazine or mesyl hydrazine (under stirring for 5 h) gave the thiosemicarbazide **3a** and methylsulfonyl hydrazine **3b**, respectively. The cyclization of these thiosemicarbazide derivatives, according to the methods reported by Wamhoff (Wamhoff and Lichtenthaler, 1978), was accomplished by treatment of compounds **3a** and **3b** with a solution of sodium hydroxide for 3 h and subsequent acidification with hydrochloric acid to obtain the versatile intermediates amino-thioxo **4a** and thioxo-methanesulfonamide **4b**. Analytical and spectral data of all the above key intermediates were in agreement with the reported structures (see Experimental section).

The reaction of methyl derivative **4e** with methanesulfonyl chloride gave the disulfonate **4d** (Scheme 2); by subsequent alkaline hydrolysis, the monomesyl derivative **4c** was obtained. The same product was obtained from methylation of methanesulfonamide derivative **4b**. The two independent preparations of **4c** confirmed the proposed



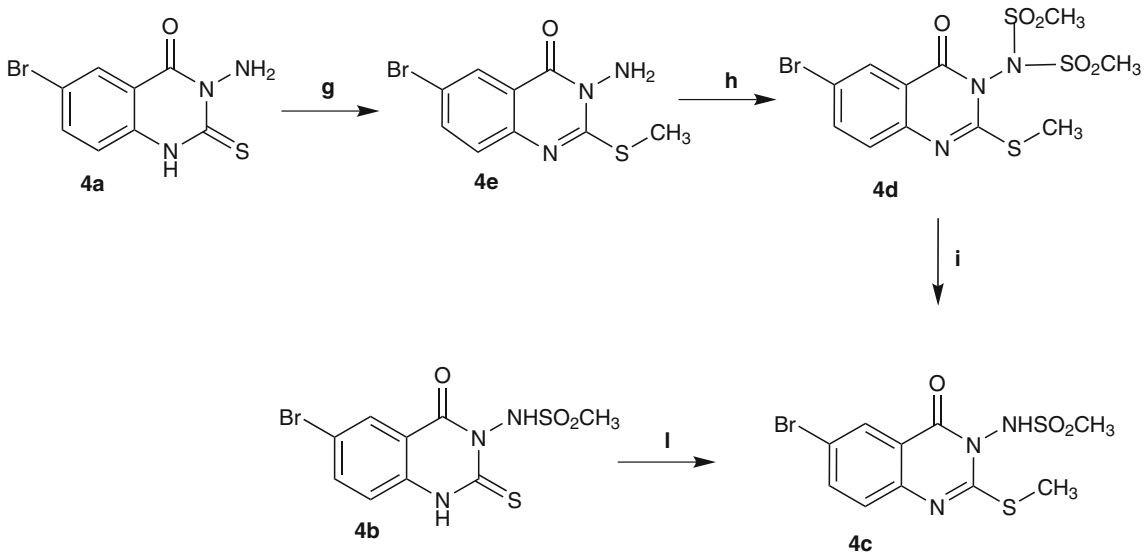
Scheme 1 Reagents and conditions: ***a*** DPT, CH_2Cl_2, r.t. stirring; *b* NH_2NH_2, CH_2Cl_2, r.t. stirring; *c* NaOH/EtOH, reflux; ***d*** $NH_2NHSO_2CH_3$, CH_2Cl_2, r.t. stirring; *e* **1** NaOH/H_2O, reflux; **2** H^+, r.t; ***f*** H^+, r.t

sulfonamide structure of **4b** and also that sulfur was more reactive than nitrogen adjacent to the sulfonic group.

The formation of aryl-sulfur bonds represents a key step in the synthesis of many molecules that are of biological, of pharmaceutical, and of materials interest. The general approach for the C–S bond coupling procedures required the use of a catalytic amount of copper, usually between 5 and 10 mol%, always in the presence of a ligand (10–20 mol%) and a base (1.5–2.5 equiv), under relative mild conditions (80–110 °C for 18–24 h) (Sperotto *et al.*, 2008). Therefore, the reaction of intermediate derivative **4b** with a variety of aryl and heterocyclic iodides in water/ethanol, under refluxing in basic conditions and in the presence of catalytic quantitative of copper powder and copper iodide for 6 h, gave the corresponding 6-bromo-quinazolinone derivatives (**5–12**) in good yields (Scheme 3). Alternatively, the derivative **10** was obtained in dimethylformamide at 80 °C in the presence of potassium carbonate, from the reaction of versatile derivative **4b** with cyclohexyl iodide. Further experiments were performed to find the optimal reaction temperature and reaction time. An excellent yield (95 %) was obtained after 6 h of reaction at 70–80 °C. It was noted that a small decrease in temperature of only 15 °C caused a significant decrease in derivatives (**5–12**) yield to 75 %. It is noteworthy of the fact that a good yield was obtained using water as solvent and NaOH as base.

Finally, as reported in literature, the influence of the amount of CuI catalyst was evaluated. CuI was the chosen halide salt, because of its stability in air. Differently, the Cu(II) salts tested ($CuBr_2$, $CuSO_4{\cdot}5H_2O$, $CuCl_2$) were found to be less efficient as catalysts (yields 25–35 %) than the Cu(I) salts (Sperotto *et al.*, 2008).

The proposed structures were confirmed by elemental analysis, IR, ^{1}H NMR, and Mass Spectrometry. Specifically, ^{1}H NMR spectrum in the region of 11.0–11.6 ppm showed a typical singlet attributable to NH of the methanesulfonamide group of derivatives **5–12**. In addition, the Mass Spectrum showed a prominent molecular ion peak



Scheme 2 *Reagents and conditions*: *g*) 1. CH_3I, KOH/H_2O, r.t.; *h*) $(C_2H_5)_3N$, $ClSO_2CH_3$, CH_2Cl_2, r.t.; *i*) **1**. KOH, THF; **2** H^+, r.t.. *l*) CH_3I, KOH/H_2O, r.t

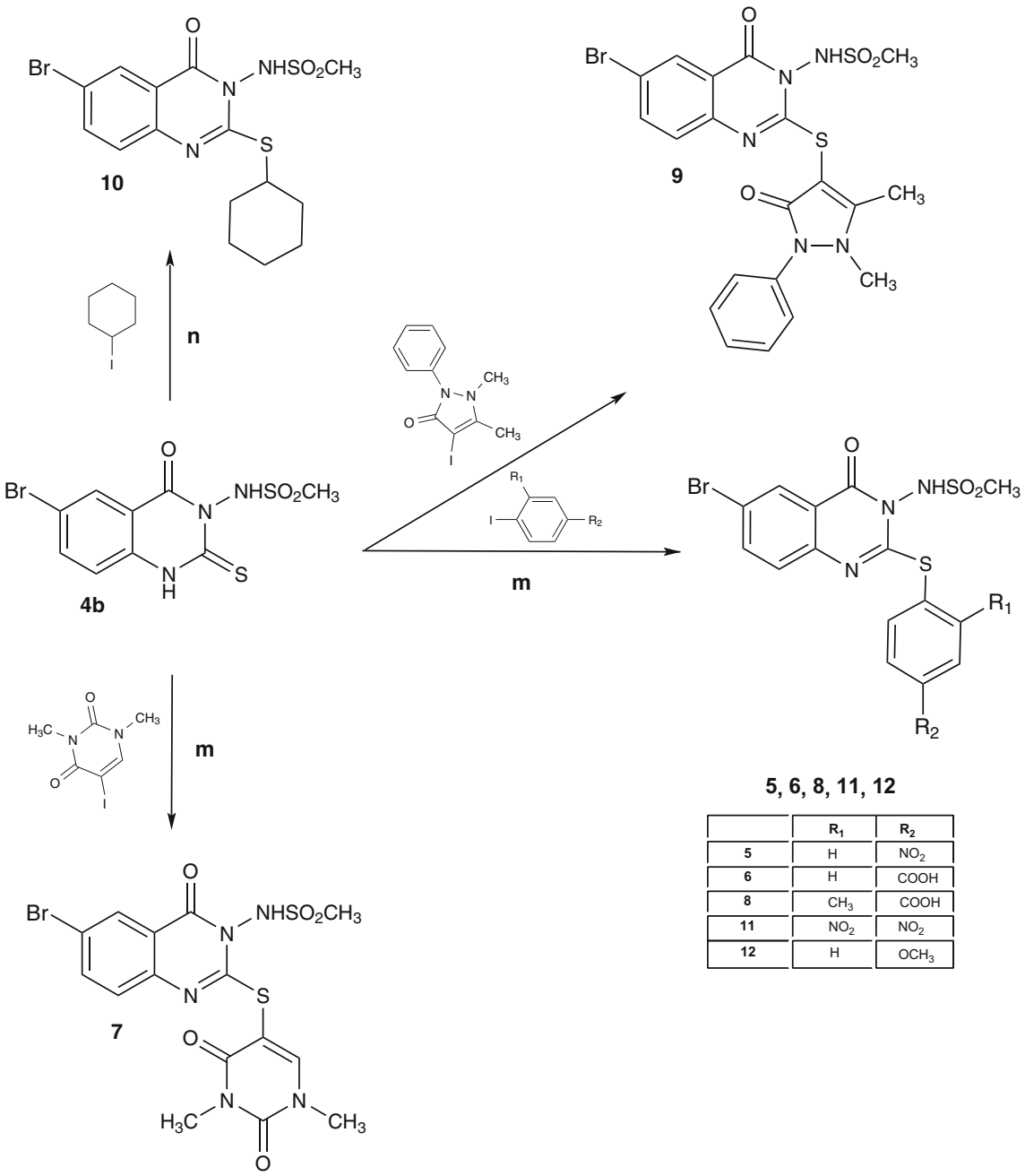
$[M^+]$ as the base peak and a fragmentation pattern consistent with their structures.

Biological evaluation

The purpose of this study was to analyze the anti-inflammatory activity of eight new 6-bromo-quinazolinone derivatives. To simulate an inflammatory model in vitro, normal human keratinocyte cell line NCTC-2544 was exposed to interferon-γ (IFN-γ), a cytokine that amplifies the inflammatory reactions, as well as to histamine, the latter is used to strongly augment the IFN-γ-induced keratinocyte activation (Albanesi *et al.*, 1998; Giustizieri *et al.*, 2004). Keratinocytes make up about 95 % of the cell mass of human epidermis, and are responsible for the biochemical and physical integrity of skin via their production of numerous high molecular weight molecules such as cytokeratins and mucopolysaccharides. Under normal homoeostatic conditions, the epidermis protects the body against external injury. In certain disease states, it used to be presumed that keratinocytes were passive targets for immunological attack from infiltrating T cells, while epidermal immune responses were actively directed by dendritic, antigen-presenting, major histocompatibility complex (MHC) class II-expressing Langerhans cells. Over the past decade, however, it has become clear that keratinocytes participate actively in such immune responses (Nickoloff, 1988). In many inflammatory skin diseases characterized by accumulation of T lymphocytes, keratinocytes aberrantly express MHC class II HLA-DR antigens (Aubock *et al.*, 1986), intercellular adhesion molecule1(ICAM-I) (Griffiths *et al.*, 1989), and CD36 antigen (Barker *et al.*, 1989), whereas in psoriasis, keratinocytes additionally produce interleukin 6 (IL-6) and IL-8 (Grossman *et al.*, 1989). In vitro, mononuclear-cell-derived cytokines, such as interferon gamma (IFN-γ) and tumor-necrosis factor alpha (TNF-α), stimulate keratinocytes to produce HLA-DR, ICAM-I, IL-1, IL-6, IL-8, monocyte chemotactic and activating factor (MCAF), and transforming growth factor alpha (TGF-α) (Nickoloff *et al.*, 1989). These data demonstrate that keratinocytes possess the capacity to interact with infiltrating mononuclear cells, either by release of pro-inflammatory cytokines or via intercellular adhesion reactions.

IFN-γ is an essential cytokine in amplifying inflammatory reactions because it stimulates iNOS and COX-2 gene transcription and the synthesis of chemokines that attract inflammatory cells, and induces the expression of molecules important for the retention and activation of T cells, such as ICAM-1 (Salvemini *et al.*, 1995). Histamine is released from mast cells and keratinocytes in the early stage of inflammation and is known to stimulate production of NO (Carlos *et al.*, 2006), (Sirois *et al.*, 2000), PGE_2 (Sirois *et al.*, 2000), (Staszak and Goodwin, 1980), and cytokines such as chemokines (Caron *et al.*, 2001), IL-1 (Dohlsten *et al.*, 1988). On the other hand, it can inhibit the production of several other cytokines such as TNF-α (Bissonnette, 1996) and also IFN-γ (Krouwels *et al.*, 1998), (Lagier *et al.*, 1997).

Celecoxib (10 μg/mL) was used as a reference anti-inflammatory drug. Although celecoxib is considered to exert its therapeutic effects through inhibition of the catalytic activity of COX-2, it was demonstrated that the drug can also block COX-2 expression in phorbol ester-stimulated mouse skin (Chun *et al.*, 2004). Moreover, celecoxib



	R_1	R_2
5	H	NO_2
6	H	COOH
8	CH_3	COOH
11	NO_2	NO_2
12	H	OCH_3

Scheme 3 *Reagents and conditions*: m) **1** H_2O/EtOH, Cu, CuI, reflux; **2** H^+, r.t.; n) **1** DMF, K_2CO_3, reflux; **2** H^+, r.t

inhibited serum NO production, iNOS, COX-2 expression, and PGE2 production in cigarette smoking-induced inflammation in rat lungs (Roh *et al.*, 2010). Celecoxib has been reported to reduce expression of ICAM-1 in experimental inflammatory models such as experimental colitis (Cuzzocrea *et al.*, 2001).

The untreated NCTC-2544 cells have undetectable levels of ICAM-1, and very low levels of iNOS and of COX-2, while incubation with IFN-γ and histamine led a strong expression of ICAM-1, iNOS, and COX-2. The addition of derivatives (**5**–**12**) at a concentration of 10 μg/mL with IFN-γ and histamine on NCTC-2544 cells produced a significant inhibition of the expression of iNOS (Fig. 1), ICAM-1 (Fig. 2), and COX-2 (Fig. 3).

Among the eight compounds tested, four (**7**, **9**, **10,** and **12**) were identified as excellent inhibitors of inflammatory markers, such as iNOS, COX-2, and ICAM-1. The synthesis of derivatives (**5**–**12**) was planned in order to confer different electronic environment that would affect the lipophilicity, and hence the activity of the derivatives. Thus, the objective of forming these hybrids was an attempt to generate an active anti-inflammatory agent with

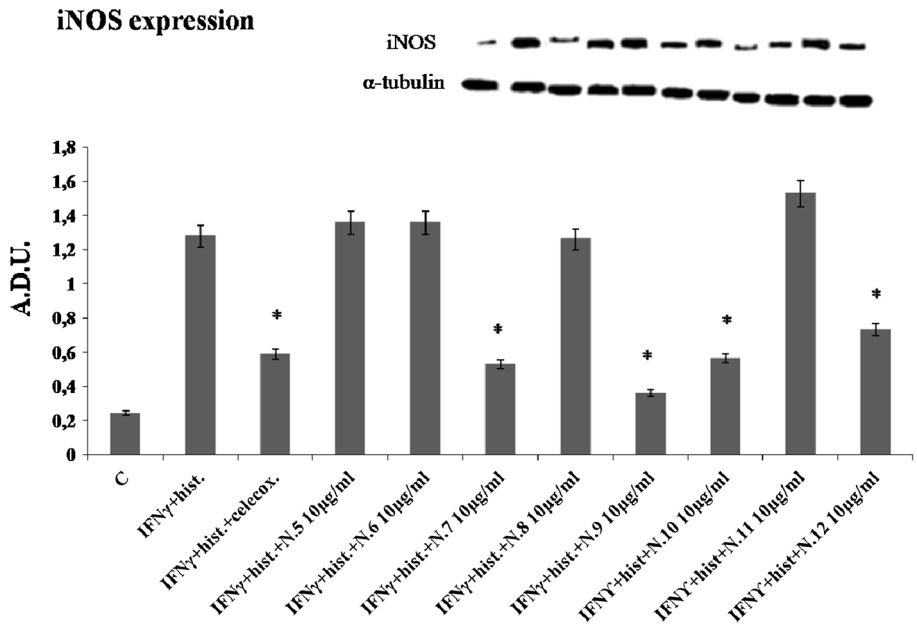


Fig. 1 Effects of 10 μg/mL derivatives (**5–12**) and celecoxib on iNOS protein expression induced by IFN-γ (200 U/mL) and histamine (10^{-4} M) on keratinocyte cell line NCTC-2544 determined by Western blot analysis. Data show the relative expression (mean ± SEM) of iNOS calculated as arbitrary densitometric units (A.D.U.) collected from three independent experiments. $^{*}P < 0.05$ compared to interferon-γ plus histamine-induced iNOS

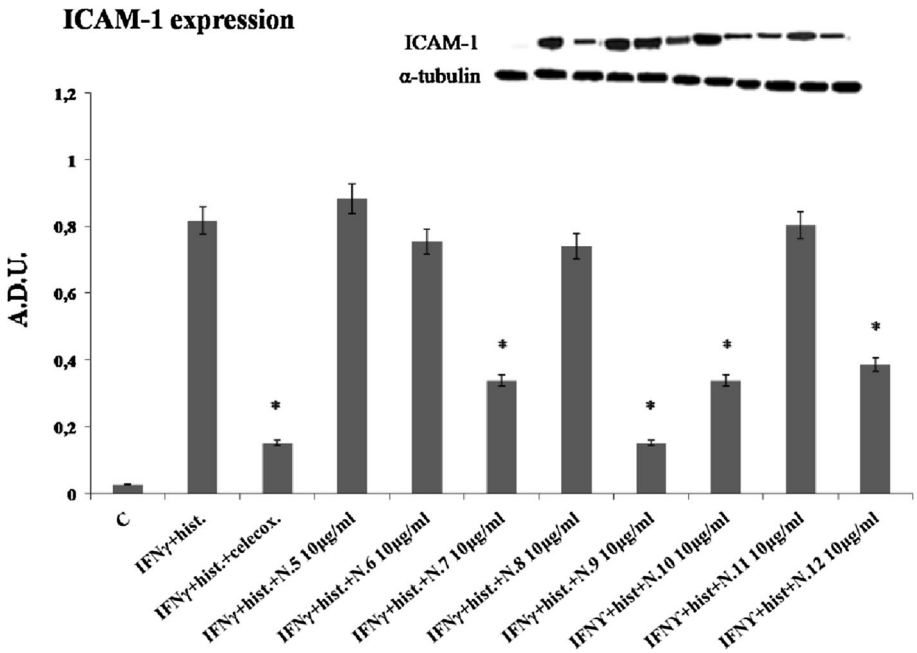


Fig. 2 Effects of 10 μg/mL derivatives (**5–12**) and celecoxib on ICAM-1 protein expression induced by IFN-γ (200 U/mL) and histamine (10^{-4} M) on keratinocyte cell line NCTC-2544 determined by Western blot analysis. Data show the relative expression (mean ± SEM) of ICAM-1 calculated as arbitrary densitometric units (A.D.U.) collected from three independent experiments. $^{*}P < 0.05$ compared to interferon-γ plus histamine-induced ICAM-1

potentiated activity and selectivity toward COX-2. Thus, the introduction of 1,3-dimethyluracil (**7**), antipyrine (**9**), cyclohexyl (**10**), and 4-MeO-phenyl (**12**) produced compounds with effective anti-inflammatory proprieties. In particular, the compound **9** containing antipyrine acts more efficiently than celecoxib blocking some pro-inflammatory actions of IFN-γ plus histamine on keratinocytes.

In fact, antipyrine was the first pyrazolin-5-one derivative used as an analgesic, antipyretic, and anti-inflammatory drug. Review of the literature in the last decades affirms that bioactive antipyrine derivatives have been synthesized and evaluated as potent anti-inflammatory, analgesic, and antipyretic (Bekhit and Abdel-Aziem, 2004).

The compound **9** was the most powerful of the derivatives (**5–12**) in the inhibition of inflammatory parameters. The compound **9** showed a higher potency than celecoxib. Instead, derivatives **5**, **6**, **8**, and **11** did not show any anti-inflammatory activity in NCTC-2544-treated cells.

Our results demonstrated that the derivatives **7**, **9**, **10,** and **12** act as excellent inhibitors of inflammatory markers expression: iNOS, COX-2, and ICAM-1.

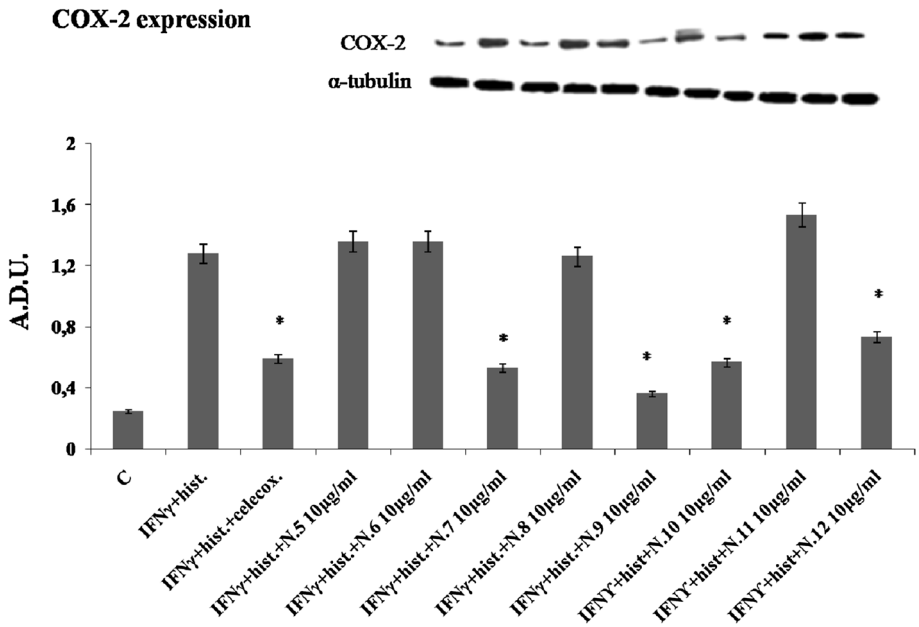


Fig. 3 Effects of 10 μg/mL derivatives (**5–12**) and celecoxib on COX-2 protein expression induced by IFN-γ (200 U/mL) and histamine (10^{-4} M) on keratinocyte cell line NCTC-2544 determined by Western blot analysis. Data show the relative expression (mean ± SEM) of COX-2 calculated as arbitrary densitometric units (A.D.U.) collected from three independent experiments. *$P < 0.05$ compared to interferon-γ plus histamine-induced COX-2

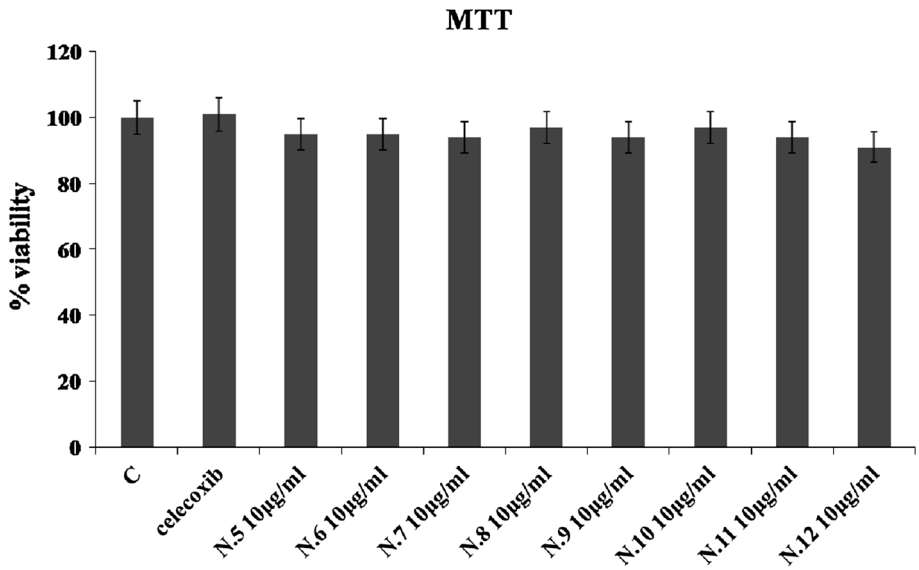


Fig. 4 Keratinocytes NCTC-2544 cell viability measured with tetrazolium salt assay (MTT) after 48 h of treatment with 10 μg/mL derivatives (**5–12**). The values of optical density measured at $\lambda = 550$ nm are reported as percentage with respect to the optical density registered for untreated control, the latter considered as 100 % of cell viability. The values are the mean ± SEM of three experiments performed in triplicate

The results of the MTT assay indicated that the derivatives (**5–12**) have no effect on cell viability, because the treatment of cells with 10 μg/mL of **5–12** did not reduce the ability of the NCTC-2544 cells to metabolize tetrazolium salts (Fig. 4).

Conclusion

The results of the present research indicate that, compared to celecoxib, the derivatives **7**, **9**, **10**, and **12** exhibit good anti-inflammatory properties, expressed by their capacity to counteract some pro-inflammatory effects induced by IFN-γ plus histamine in normal human keratinocyte NCTC-2544 cells. These findings could be useful for the development of new drugs for the treatment of various inflammatory pathologies.

Experimental

Materials and methods

^{1}H and ^{13}C NMR spectra were recorded with a Varian Unity INOVA instrument at 500 and 125 MHz, respectively, in the reported solvent; chemical shifts (δ) are reported in ppm from tetramethylsilane as an internal standard; coupling constants (J) are in Hertz (Hz). Melting

points were determined in open capillary tubes on a SMP1 apparatus (Stuart Scientific Staffordshire) and are uncorrected. The purity of substances was checked by thin layer chromatography (TLC) on Merck silica gel 60 F-254 plates. Mass spectra were recorded by Perkin Elmer Turbo Mass Clarus 560 Mass Spectrometer HRESIMS (high resolution MS), with a 70 eV working ionization energy, source temperature 250 °C, and trap current 90 μA. All commercial chemicals were purchased from Aldrich, Fluka, Merk, Lancaster, and Carlo-Erba and were used without further purification.

*General procedure for the preparation of methyl 5-bromo-2-isothiocyanatobenzoate (**2**)*

A solution of methyl 2-amino-5-bromobenzoate **1** (3.05 g, 13.04 mmol) in dichloromethane (40 mL) was added dropwise at room temperature to a solution of di-2-pyridyl thionocarbonate (DPT) (3.0 g, 13.03 mmol) in dichloromethane (40 mL) in substitution of the tedious previous method using thiophosgene, a potential air pollutant (Hodgkins and Ettilinger, 1956). The mixture was stirred at room temperature for 24 h. The resulting solution was evaporated under reduced pressure, to give a residue that was dissolved with propan-2-one and added in water. The resulting solid was collected, washed with water, dried, and crystallized from petroleum ether to give isothiocyanate **2** as yellow crystals.

*Methyl 5-bromo-2-isothiocyanatobenzoate (**2**)*

Yellow crystals; yield: 90 %; m.p. 120 °C (Hodgkins and Ettilinger, 1956); IR (KBr): ν_{max} 3100, 2920, 2140, 2090, 1710, 1450, 607 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ 8.08–7.57 (m, 3H, aromatic H), 3.89 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ 164.28 (C=O), 140.9 (C=S), 137.24, 134.70 and 130.85 (aromatic CH), 128.22, 122.37 and 122.00 (aromatic C), 51.50 (–OCH_3); HRESIMS *m/z* (pos): 270.9321 C_9H_6BrNS (calcd 270.9389); Anal. Calcd. for C_9H_6BrNS: C, 39.72; H, 2.22; N, 5,15. Found: C, 39.79; H, 2.24; N, 5.17.

The analytical data are in accord with literature values (Carpenter *et al.*, 2007).

*General procedure for the preparation of methyl 5-bromo-2-[(2 (methylsulfonyl)hydrazinecarbothioamido)]benzoate (**3b**)*

Methyl 5-bromo-2-isothiocyanatobenzoate **2** (1.05 g, 3.73 mmol) was added dropwise to a solution of mesylhydrazide (0.40 g, 98 %, 3.73 mmol) in dichloromethane (20 mL). The mixture was stirred at room temperature for 5 h. The resulting solid was collected, washed with dichloromethane, and crystallized from ethanol to give **3b** as white crystals (Wamhoff and Lichtenthaler, 1978).

*Methyl 5-bromo-2-[(2 (methylsulfonyl) hydrazinecarbothioamido)]benzoate (**3b**)*

White crystals; yield: 77 %; m.p. 223 °C; IR (KBr): ν_{max} 3294, 3243, 1657, 1324, e 1146 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ 10.67 (s, 2H, NH), 9.95 (s, 1H, NH), 7.65–6.51 (m, 3H, aromatic H), 3.08 (s, 3H, CH_3), 1.26 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ 168.34 (C=O), 161.29 (C=S), 140.50, 139.34 and 133.67 (aromatic CH), 122.35, 122.00 and 119.50 (aromatic C), 51.50 (–OCH_3), 43.92 (–SCH_3); HRESIMS *m/z* (pos):380.9501 $C_{10}H_{12}BrN_3S_2$ (calcd. 380.9520); Anal. Calcd. for $C_{10}H_{12}BrN_3S_2$: C, 31.42; H, 3.16; N, 10.99. Found: C, 31.53; H, 3.18; N, 11.03.

*General procedure for the preparation of sodium 3-amino-6-bromo-4-oxo-3,4-dihydroquinazoline-2-thiolate (**4**)*

A solution of compound **2** (2.50 g, 9.25 mmol) in dichloromethane (30 mL) was added dropwise at room temperature to a solution of hydrazine (0.28 mL, 9.25 mmol) in dichloromethane (50 mL). The mixture was stirred at room temperature for 2 h. The resulting solid (a mixture of various derivatives including compound **3a**) was filtered, collected, washed with dichloromethane, and dried to give a white powder (2.30 g). This powder was heated for 1 h under reflux while stirring in a solution of sodium hydroxide 0.1 M (190 mL). The resulting solid was collected, washed with warm 1,4-dioxane, and dried to give **4** as white powder.

*Sodium 3-amino-6-bromo-4-oxo-3,4-dihydroquinazoline-2-thiolate (**4**)*

White powder; yield: 67 %; m.p.: >310 °C dec.; IR (KBr): ν_{max} 3230, 3135, 1640 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ 8.22–7.64 (m, 3H, aromatic H), 6.33 (s, 2H, NH_2); ^{13}C NMR (DMSO, 125 MHz): δ 160.9 (C=O), 159.87 (=C–S), 145.90 (=C–NH), 136.30, 132.30 and 124.46 (aromatic CH), 123.45 and 121.32 (aromatic C). Anal. Calcd. for $C_8H_5BrN_3S$: C, 32.67; H, 1.71; N, 14.29. Found: C 32.66; H, 1.72; N, 14.32.

*General procedure for the preparation of 3-amino-6-bromo-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**4a**)*

Sodium 3-amino-6-bromo-4-oxo-3,4-dihydroquinazoline-2-thiolate **4** (1.98 g, 6.78 mmol) was dissolved in water (200 mL), and the resulting solution was acidified with concentrated hydrochloric acid to pH 3–4. The resulting solid was collected, washed with water, dried, and crystallized from dioxane to give **4a** as white powder.

*3-amino-6-bromo-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (**4a**)*

White powder; yield: 60 %; m.p.: >280 °C; IR (KBr): ν_{max} 3230, 3135, 1640 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ 7.97–6.58 (m, 3H, aromatic H), 6.33 (s, 2H, NH_2); ^{13}C NMR (DMSO, 125 MHz): δ 179.80 (C=S), 160.85 (C=O), 138.59 (=C–NH), 131.47, 130.90 and 122.45 (aromatic CH), 122.10 and 119.32 (aromatic C). HRESIMS *m/z* (pos): 270.9341 $C_8H_6BrN_3S$ (calcd. 270.9380); Anal. Calcd. for $C_8H_6BrN_3S$: C, 35.57; H, 2.22; N, 15.44. Found: C, 35.63; H, 2.24; N, 15.51.

*General procedure for the preparation of N-(6-bromo-4-oxo-2-thioxo-1,2-dihydroquinazolin-3(4H)-yl) methanesulfonamide (**4b**)*

A solution of compound **3b** (1.04 g, 2.63 mmol) and sodium hydroxide (0.10 g, 2.63 mmol) in water (40 mL) was refluxed under stirring for 3 h. The solution, containing the sodium salt was filtered and acidified with concentrated hydrochloric acid to pH 3–4: the resulting solid was collected, washed with water, dried, and crystallized from dimethylformamide/water to give **4b** as a white powder.

*N-(6-bromo-4-oxo-2-thioxo-1,2-dihydroquinazolin-3(4H)-yl) methanesulfonamide (**4b**)*

White powder; yield: 80 %; m.p. 270 °C dec.; IR (KBr): ν_{max} = 3214, 1708, 1345, 1158 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ = 11.00 (s, 1H, NH), 7.87–6.58 (m, 3H, aromatic H), 4.0 (s, 1H, NH), 3.26 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ = 179.53 (C=S), 160.64 (C=O), 138.10 (aromatic C), 131.55 (aromatic CH), 130.38 (aromatic CH), 119.28 (aromatic C), 44.51 (–CH_3) ppm; HRESIMS *m/z* (pos): 348.2003 $C_9H_8BrN_3S_2$ (calcd. 348.2097); Anal. Calcd. for $C_9H_8BrN_3S_2$: C, 30.87; H, 2.30; N, 12.00. Found: C, 30.84; H, 2.32; N,12.07.

*General procedure for the preparation of N-(6-bromo-2-(Methylthio)-4-oxoquinazolin-3(4H-)yl] methanesulfonamide (**4c**)*

A mixture of dimesyl derivative **4d** (0.10 g, 0.22 mmol) in a solution of potassium hydroxide (1 M) in water (10 mL) and tetrahydrofuran (90 mL) was stirred for 1 h at room temperature; the resulting solution was acidified with concentrated hydrochloric acid until pH 4–5 and then extracted with ethyl acetate. The organic phase was concentrated under vacuum to give a residue that was collected and dissolved in a sodium hydroxide 10 % aqueous solution. The resulting solution was filtered and acidified with concentrated hydrochloric acid until pH 4–5. The solid was collected, washed with water, and dried to give the monomesyl derivative **4c** as a white powder.

*N-(6-bromo-2-(methylthio)-4-oxoquinazolin-3(4H-)yl] methanesulfonamide (**4c**)*

White powder; yield: 86 %; m.p. 235 °C. IR (KBr): ν_{max} 3245, 1695, 1345, and 1155 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ 11.16 (s, 1H, NH), 8.11–7.45 (m, 3H, aromatic H), 3.31 (s, 3H, CH_3), 2.46 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ 159.64 (C=O), 158.95 (=C–S), 145.89 (=C–N), 133.08 and 129.95 (aromatic CH) 123.66, 123.50 and 122.05 (aromatic C), 45.62 (–CH_3), 15.84 (–CH_3); HRESIMS *m/z* (pos): 354.9334 $C_{10}H_{10}BrN_3S_2$ (calcd 354.9321); Anal. Calcd. for $C_{10}H_{10}BrN_3S_2$: C, 32.97; H, 2.77; N, 11.54. Found: C, 32.84; H, 2.80; N, 11.64.

*General procedure for the preparation of N-(6-bromo-2-(methylthio)-4-oxoquinazolin-3(4H)-yl)-N-(methylsulfonyl)methanesulfonamide (**4d**)*

A solution of methanesulfonyl chloride (0.22 mL, 2.85 mmol, 99.5 %, d = 1.480) in dichloromethane (10 mL) was added slowly dropwise at room temperature to a stirred solution of methyl derivative **4e** (0.23 g, 0.8 mmol) and triethylamine (1 mL) in dichloromethane (20 mL). The mixture was stirred at room temperature for 3 h; the organic phase was washed with water, dried on sodium sulfate, filtered, and concentrated under vacuum to give a solid, which was collected and washed with diethyl ether to give derivative **4d** as a pale yellow solid.

*N-(6-bromo-2-(methylthio)-4-oxoquinazolin-3(4H)-yl)-N-(methylsulfonyl)methanesulfonamide (**4d**)*

Yellow solid; yield: 60 %; m.p. 235 °C. IR (KBr): ν_{max} 1160, 1380, 1710 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ 8.14–7.49 (m, 3H, aromatic H), 3.77 (s, 6H, 2 × CH_3SO_2), 2.43 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ 160.05 (C=O), 159.94 (C=S), 145.88, 136.08, 132.28, 124.62, 123.03, 121.72 (aromatic C), 38.83 (–CH_3), 13.82 (–CH_3); HRESIMS *m/z* (pos): 440.9331 $C_{11}H_{12}BrN_3S_3$ (calcd. 440.9368); Anal. Calcd. for $C_{11}H_{12}BrN_3S_3$: C, 29.87; H, 2.73; N, 9.50. Found: C, 29.96; H, 2.75; N, 9.53.

*General procedure for the preparation of 3-amino-6-bromo-2(methylthio)-2,3-dihydroquinazolin-4(1H)-one (**4e**)*

A mixture of compound **4a** (1.33 g, 4.9 mmol) and methyl iodide (0.85 ml, 99 %, d = 2.27) in water (50 mL) was stirred at room temperature for 2 h. The resulting solid was

collected, washed with water, dried, and crystallized from ethanol/water to give **4e** as amorphous white solid.

*3-amino-6-bromo-2(methylthio)-2,3-dihydroquinazolin-4(1H)-one (**4e**)*

White solid; yield: 44 %; m.p. 180 °C. IR (KBr): ν_{max} 3315, 3210, 1680 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ 8.14–7.49 (m, 3H, aromatic H), 5.83 (s, 2H, NH_2), 2.43 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ 160.78 (C=O), 158.93 (C=S), 146.88, 135.08, 131.28, 129.95, 127.64 and 121.64 (aromatic C), 12.85 (–CH_3); HRESIMS *m/z* (pos): 286.9327 $C_9H_8BrN_3S$ (calcd. 286.9356); Anal. Calcd. for $C_9H_8BrN_3S$: C, 37.77; H, 2.82; N, 14.69. Found: C, 37.77; H, 2.81; N, 14.65.

*General procedure for the preparation of N-{6-bromo-2-[(4-nitrophenyl)sulfanyl]-4-oxoquinazolin-3(4H)-yl}methanesulfonamide (**5**)*

To a solution of compound **4b** (0.14 g, 0.40 mmol) and potassium hydroxide (0.04 g, 0.40 mmol) in ethanol/water 1:1 (40 mL) was added 1-iodo-4-nitrobenzene (0.09 g, 0.40 mmol) in the presence of copper powder (20 mg) and copper iodide (15 mg). The mixture was refluxed under stirring for 5 h at 80 °C and filtered while hot. The resulting solution was acidified with concentrated hydrochloric acid to pH 3–4; the resulting solid was collected, washed with water, dried, and crystallized from ethanol/water to give **5** as yellow crystals (Sperotto *et al.*, 2008).

*N-{6-bromo-2-[(4-nitrophenyl)sulfanyl]-4-oxoquinazolin-3(4H)-yl}methanesulfonamide (**5**)*

Yellow crystals; yield: 90 %; m.p. 170 °C. IR (KBr): ν_{max} 3220, 1705, 1345, 1155 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ 11.60 (s, 1H, NH), 8.22–7.64 (m, 7H, aromatic H), 3.16 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ 161.01 (C=O), 160.44 (–CS), 151.86 and 147.36 (aromatic C), 145.88 (C–NO_2), 138.41, 135.08, 133.28, 126.57, 126.13, 125.59 and 125.10 (aromatic CH), 120.67 (aromatic C), 119.95 (–CBr), 45.10 (SO_2–CH_3); HRESIMS *m/z* (pos): 469.9404 $C_{15}H_{11}BrN_4S_2$ (calcd. 469.9468); Anal. Calcd. for $C_{15}H_{11}BrN_4S_2$: C, 38.23; H, 2.35; N, 11.89.. Found: C, 38.33; H, 2.36; N, 11.92.

*General procedure for the preparation of 4-({6-bromo-3-[(methylsulfonyl)amino]-4-oxo-3,4-dihydroquinazolin-2-yl}thio)benzoic acid (**6**)*

To a solution of compound **4b** (0.15 g, 0.43 mmol) and potassium hydroxide (0.04 g, 0.43 mmol) in ethanol/water 1:1 (40 mL) was added 4-iodobenzoic acid (0.107 g, 0.43 mmol) in the presence of copper powder (20 mg) and copper iodide (15 mg). The mixture was refluxed under stirring for 5 h at 80 °C and filtered while hot. The resulting solution was acidified with concentrated hydrochloric acid to pH 3–4; the resulting solid was collected, washed with water, dried, and crystallized from ethanol/water to give **6** as white crystals (Sperotto *et al.*, 2008).

*4-({6-Bromo-3-[(methylsulfonyl)amino]-4-oxo-3,4-dihydroquinazolin-2-yl}thio)benzoic acid (**6**)*

White crystals; yield: 90 %; m.p. 215 °C; IR (KBr): ν_{max} 3220, 1705, 1345, 1155 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ 13.01 (s, 1H, COOH), 11.37 (s, 1H, NH), 8.05–7.21 (m, 7H, aromatic H), 3.28 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ 168.71 (–COOH), 160.04 (C=O), 159.60 (–CS), 146.13 (aromatic C), 138.40 and 136.85 (aromatic CH), 135.51, 131.66, 130.39 and 129.81 (aromatic C), 126.73, 126.46 and 126.36 (aromatic CH), 119.36 (–CBr), 47.78 (–CH_3); HRESIMS *m/z* (pos): 468.9411 $C_{16}H_{12}BrN_3S_2$ (calcd. 468.9487); Anal. Calcd. for $C_{16}H_{12}BrN_3S_2$: C, 40.86; H, 2.57; N, 8.93. Found: C, 40.98; H, 2.58; N, 8.96.

*General procedure for the preparation of N-{6-bromo-2-[(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)thio)-4-oxoquinazolin-3(4H)-yl}methanesulfonamide (**7**)*

To a solution of compound **4b** (0.13 g, 0.39 mmol) and potassium hydroxide (0.044 g, 0.39 mmol) in ethanol/water 1:1 (40 mL) was added 5-iodo-1,3-dimethylpyrimidine-2,4(1*H*, 3*H*)-dione (0.10 g, 0.39 mmol) in the presence of copper powder (19 mg) and copper iodide (14 mg). The mixture was refluxed under stirring for 5 h at 80 °C and filtered while hot. The resulting solution was acidified with concentrated hydrochloric acid to pH 3–4; the resulting solid was collected, washed with water, dried, and crystallized from ethanol/water to give **7** as brown crystals (Sperotto *et al.*, 2008).

*N-{6-bromo-2-[(1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)thio)-4-oxoquinazolin-3(4H)-yl}methanesulfonamide (**7**)*

Brown crystals; yield: 90 %; m.p. 275 °C; IR (KBr): ν_{max} 3059, 1690, 1640, 1600, 1351, 1154 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ 11.58 (s, 1H, NH), 8.42–7.56 (m, 4H, aromatic H), 3.43 (s, 3H, CH_3), 3.39 (s, 3H, CH_3), 3.25 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ 162.40 (C=O), 159.33 (–CS), 157.42 and 156.64 (C=O uracyl), 154.10 (aromatic C), 148.34 (CH-uracyl), 133.96 (aromatic CH), 131.14 (C-uracyl), 130.83 and 124.99 (aromatic CH), 123.17 (aromatic C), 121.15 (–CBr), 42.30 (–CH_3), 36.42

(N–CH_3), 29.03 (N–CH_3); HRESIMS *m/z* (pos): 486.9627 $C_{15}H_{14}BrN_5S_2$ (calcd 486.9684). Anal. Calcd. for $C_{15}H_{14}BrN_5S_2$: C, 36.89; H, 2.89; N, 14.34. Found: C, 36.99; H, 2.90; N, 14.39.

*General procedure for the preparation of 4-({6-bromo-3-[(methylsulfonyl)amino]-4-oxo-3,4-dihydroquinazolin-2-yl}thio)-3-methylbenzoic acid (**8**)*

To a solution of compound **4b** (0.16 g, 0.47 mmol) and potassium hydroxide (0.05 g, 0.47 mmol) in ethanol/water 1:1 (40 mL) was added 4-iodo-3-methylbenzoic acid (0.12 g, 0.47 mmol) in the presence of copper powder (20 mg) and copper iodide (15 mg). The mixture was refluxed under stirring for 5 h at 80 °C and filtered while hot. The resulting solution was acidified with concentrated hydrochloric acid to pH 3–4; the resulting solid was collected, washed with water, dried, and crystallized from ethanol/water to give **8** as white crystals (Sperotto *et al.*, 2008).

*4-({6-bromo-3-[(methylsulfonyl)amino]-4-oxo-3,4-dihydroquinazolin-2-yl}thio)-3-methylbenzoic acid (**8**)*

White crystals; yield: 90 %; m.p. 265 °C; IR (KBr): ν_{max} 3225, 1695, 1345, 1155 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ 13.01 (s, 1H, COOH), 11.58 (s, 1H, NH), 8.22–7.48 (m, 6H, aromatic H), 3.43 (s, 3H, CH_3), 3.25 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ 168.66 (COOH), 162.42 (C=O), 159.33 (–CS), 145.93 (aromatic C), 141.94 and 138.85 (aromatic CH), 136.96 (aromatic C), 132.32, 130.83, 129.23, 127.65, and 124.65 (aromatic CH), 123.06 (aromatic C), 121.77 (–CBr), 42.40 (–CH_3), 21.21 (–CH_3); HRESIMS *m/z* (pos): 482.9628 $C_{17}H_{14}BrN_3S_2$ (calcd. 482.9670); Anal. Calcd. for $C_{17}H_{14}BrN_3S_2$: C, 42.16; H, 2.91; N, 8.68. Found: C, 42.27; H, 2.93; N, 8.70.

*General procedure for the preparation of N-{6-bromo-2-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)thio]-4-oxoquinazolin-3(4H)-yl}methanesulfonamide (**9**)*

To a solution of compound **4b** (0.138 g, 0.40 mmol) and potassium hydroxide (0.045 g, 0.40 mmol) in ethanol/water 1:1 (40 mL) was added 4-iodo-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one(0.210 g, 0.40 mmol) in the presence of copper powder (20 mg) and copper iodide (14 mg). The mixture was refluxed under stirring for 5 h at 80 °C and filtered while hot. The resulting solution was acidified with concentrated hydrochloric acid to pH 3–4; the resulting solid was collected, washed with water, dried, and crystallized from ethanol/water to give **9** as yellow crystals (Sperotto *et al.*, 2008).

*N-{6-bromo-2-[(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)thio]-4-oxoquinazolin-3(4H)-yl}methanesulfonamide (**9**)*

Yellow crystals; yield: 95 %; m.p. 200 °C; IR (KBr): ν_{max} 3224, 3038, 1694, 1650, 1344, 1155 cm^{-1}; ^1H NMR (DMSO, 500 MHz): δ 11.51 (s, 1H, NH), 8.22–6.90 (m, 8H, aromatic H), 3.81 (s, 3H, CH_3), 3.38 (s, 3H, CH_3), 2.36 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ 167.37 (C=O), 157.10 (C=O), 156.80 (–CS), 154.61 (antipyrine C), 143.05 (aromatic C), 138.67 and 135.01 (aromatic CH), 134.21 (aromatic C), 132.83 (aromatic CH), 130.01 (N–C-aromatic), 128.29, 125.99, 125.17 and 124.08 (aromatic CH), 121.72 (–CBr), 119.15 (aromatic CH), 99.83 (antipyrine C), 42.30 (SO_2–CH_3), 34.52 (N–CH_3), 14.03 (–CH_3); HRESIMS *m/z* (pos): 535.0011 $C_{20}H_{18}BrN_5S_2$ (calcd. 535.0059); Anal. Calcd. for $C_{20}H_{18}BrN_5S_2$: C, 44.78; H, 3.38; N, 13.06. Found: C, 44.90; H, 3.40; N, 13.09.

*General procedure for the preparation of N-[6-bromo-2-(cyclohexylthio)-4-oxoquinazolin-3(4H)-yl]methanesulfonamide (**10**)*

The compound **4b** (0.180 g, 0.52 mmol), iodocyclohexane (0.110 g, 0.52 mmol) and potassium carbonate (0.072 g, 0.52 mmol) were added to dimethylformamide (2 mL). The mixture was refluxed under stirring for 12 h at 80 °C, acidified with concentrated hydrochloric acid to pH 3–4; the resulting solid was filtered, collected, washed with water, dried, and crystallized from petroleum ether to give **10** as white crystals.

*N-[6-bromo-2-(cyclohexylthio)-4-oxoquinazolin-3(4H)-yl]methanesulfonamide (**10**)*

Yellow crystals; yield: 90 %, ^1H NMR (DMSO, 500 MHz): δ 11.14 (s br, 1H, NH), 8.10–7.38 (m, 3H, aromatic H), 3.72 (s, 1H, S–CH), 3.29 (s, 3H, CH_3), 2.05–1.42 (m, 10H, cyclohexyl); ^{13}C NMR (DMSO, 125 MHz): δ 160.03 (C=O), 159.34 (–CS), 145.95 (aromatic C), 132.33, 124.62 and 123.53 (aromatic CH), 123.04 (aromatic C), 121.72 (–CBr), 42.41 (SO_2–CH_3), 38.22 (CH_2), 33.33 (CH_2), 25.51 and 25.32 (CH_2); HRESIMS *m/z* (pos): 431.0022 $C_{15}H_{18}BrN_3S_2$ (calcd. 431.0024); Anal. Calcd. for $C_{15}H_{18}BrN_3S_2$: C, 41.67; H, 4.20; N, 9.72. Found: C, 41.70; H, 4.22; N, 9.75.

*General procedure for the preparation of N-{6-bromo-2-[(2,4-dinitrophenyl)thio]-4-oxoquinazolin-3(4H)-yl}methanesulfonamide (**11**)*

To a solution of compound **4b** (0.15 g, 0.44 mmol) and potassium hydroxide (0.04 g, 0.44 mmol) in ethanol/water 1:1 (40 mL) was added 1-iodo-2,4-dinitrobenzene (0.12 g,

0.44 mmol) in the presence of copper powder (23 mg) and copper iodide (14 mg). The mixture was refluxed under stirring for 5 h at 80 °C and filtered while hot. The resulting solution was acidified with concentrated hydrochloric acid to pH 3–4; the resulting solid was collected, washed with water, dried, and crystallized from ethanol/water to give **11** as yellow crystals (Sperotto *et al.*, 2008).

*N-{6-bromo-2-[(2,4-dinitrophenyl)thio]-4-oxoquinazolin-3(4H)-yl}methanesulfonamide (**11**)*

Yellow crystals; yield: 90 %; m.p. 152 °C; IR (KBr): ν_{max} 3220, 1705, 1345, 1155 cm^{-1}; ^{1}H NMR (DMSO, 500 MHz): δ 11.60 (s, 1H, NH), 8.80–7.13 (m, 6H, aromatic H), 3.16 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ 161.01 (C=O), 160.44 (–CS), 151.86 and 147.36 (aromatic C), 145.88 (C–NO_2), 138.41 (C–NO_2), 135.08, 133.28, 126.57, 126.13, 125.59, and 125.10 (aromatic CH), 120.67 (aromatic C), 119.95 (–CBr), 45.10 (SO_2–CH_3); HRESIMS *m/z* (pos): 514.9631 $C_{15}H_{10}BrN_5S_2$ (calcd. 514.9608); Anal. Calcd. for $C_{15}H_{10}BrN_5S_2$: C, 34.89; H, 1.95; N, 13.56. Found: C, 34.98; H, 1.96; N, 13.60.

*General procedure for the preparation of N-{6-bromo-2-[(4-methoxyphenyl)thio]-4-oxoquinazolin-3(4H)-yl}methanesulfonamide (**12**)*

To a solution of compound **4b** (0.17 g, 0.49 mmol) and potassium hydroxide (0.05 g, 0.49 mmol) in ethanol/water 1:1 (40 mL) was added 1-iodo-4-methoxybenzene (0.11 g, 0.49 mmol) in the presence of copper powder (21 mg) and copper iodide (15 mg). The mixture was refluxed under stirring for 5 h at 80 °C and filtered while hot. The resulting solution was acidified with concentrated hydrochloric acid to pH 3–4; the resulting solid was collected, washed with water, dried, and crystallized from ethanol/water to give **12** as orange crystals (Sperotto *et al.*, 2008).

*N-{6-bromo-2-[(4-methoxyphenyl)thio]-4-oxoquinazolin-3(4H)-yl}methanesulfonamide (**12**)*

Orange crystals; yield: 90 %; m.p. 240 °C; IR (KBr): ν_{max} 3220, 1705, 1345, 1155 cm^{-1}; ^{1}H NMR (DMSO, 500 MHz): δ 11.37 (s, 1H, NH), 8.22–7.48 (m, 7H, aromatic H), 3.87 (s, 3H, CH_3), 3.28 (s, 3H, CH_3); ^{13}C NMR (DMSO, 125 MHz): δ 160.04 (C=O), 159.60 (–CS), 157.7, 145.13 (aromatic C), 138.40 and 136.85 (aromatic CH), 134.51 (aromatic C), 132.66, 129.81, 127.73, 123.64 (aromatic CH), 121.36 (–CBr), 55.81 (–OCH_3), 42.41 (–CH_3); HRESIMS *m/z* (pos): 454.9636 $C_{16}H_{14}BrN_3S_2$ (calcd. 454.9658); Anal. Calcd. for $C_{16}H_{14}BrN_3S_2$: C, 42.11; H, 3.09; N, 9.21. Found: C, 42.24; H, 3.11; N, 9.24.

Cell cultures

The (non-immortalized) human keratinocyte cell line NCTC-2544 was provided by Interlab Cell Line Collection (Genoa, Italy) and routinely maintained in Minimum Essential Medium (MEM) (Sigma-Aldrich, Italy) supplemented with 10 % fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, and incubated at 37 °C in a humidified, 95 % air/5 % CO_2 atmosphere. The medium was changed every 2–3 days. 24 h before experiments, the cells were trypsinized, and plated either in 100 mm Petri dishes for Western blot. Keratinocytes were stimulated or not (untreated controls) with 200 U/mL (corresponding to a concentration of 10 μg/mL) of IFN-γ (PeproTech EC, London, England) and 10^{-4} M of histamine (Sigma-Aldrich, Italy), in order to reproduce the mechanisms involved in the pathogenesis of inflammatory processes. This was made in the absence or presence of the derivatives (**5–12;** 10 μg/mL) or celecoxib (10 μg/mL); the latter was used as a reference anti-inflammatory drug. After 48 h, each sample was tested for the expression of iNOS, COX-2, and ICAM-1.

Western blot

The expression of iNOS, COX-2, and ICAM-1 was evaluated by Western blot analysis. Briefly, the untreated and treated keratinocytes were washed twice with ice-cold PBS and collected with lysing buffer (10 mM Tris–HCl plus 10 mM KCl, 2 mM $MgCl_2$, 0.6 mM PMSF, and 1 % SDS, pH 7.4). After cooling for 30 min at 0 °C, the cells were sonicated. Sixty micrograms of total protein, present in the supernatant, was loaded on each lane and separated by 4–12 % Novex Bis–Tris gel electrophoresis (NuPAGE, Invitrogen, Italy). Proteins were then transferred to nitrocellulose membranes (Invitrogen, Italy) in a wet system. The transfer of proteins was verified by staining the nitrocellulose membranes with Ponceau S and the Novex Bis–Tris gel with Brilliant blue R. The membranes were blocked in Tris-buffered saline containing 0.01 % Tween-20 (TBST) and 5 % non-fat dry milk at 4 °C overnight. Mouse monoclonal anti-ICAM-1 (1H4: sc-51632, Santa Cruz Biotechnology) (1:200 dilution), anti-NOS2 (N-20, sc-651, Santa Cruz Biotechnology) (1:300 dilution), anti-COX-2 (N-20, sc-1746, Santa Cruz Biotechnology) (1:100 dilution), and anti–α tubulin (T9026; Sigma–Aldrich) (1:5000 dilution) antibodies were diluted in TBST, and the membranes incubated for 2 h at room temperature. Antibodies were detected with horseradish peroxidase conjugated secondary antibody using the enhanced chemiluminescence detection Supersignal West Pico Chemiluminescent Substrate (Pierce Chemical Co., Rockford, IL). Bands were measured densitometrically and their relative density was calculated based on the density of the α-tubulin bands in each sample.

Cell viability

The MTT proliferation assay is based on the conversion by mitochondrial dehydrogenases of a substrate containing a tetrazolium ring into blue formazan, detectable spectrophotometrically. The level of formazan blue is then used as an index of cell viability. Briefly, cell cultures (8×10^3 cells/microwell) were set up in flat-bottomed 200 µl microplates, incubated at 37 °C in a humidified atmosphere of 95 % air/5 % CO_2 and after 24 h (60–70 % confluence) treated with 10 µg/mL of quinazolinone derivatives or celecoxib (10 µg/mL) for 48 h. Four hours before the end of the culture period, 20 µL of 0.5 % MTT in PBS was added to each microwell. After incubation with the reagent, the supernatant was removed and replaced with 100 µL of DMSO. In order to determine that the cells are not dying during the treatment, 6-hour longer time points were tested (data not shown). The optical density of each sample was measured using a microplate spectrophotometer (Titertek Multiskan; DAS) at $\lambda = 550$ nm. Each sample was tested in quadruplicate ($n = 12$).

Statistical analysis

Each experiment was repeated at least three times in triplicate and the mean ± SEM for each value was calculated. Statistical analysis of results [Student's *t* test for paired and unpaired data; variance analysis (ANOVA)] was performed using the statistical software package SYSTAT, version 11 (Systat Inc., Evanston IL, USA). A difference was considered significant at $p < 0.05$.

References

Abbas ES, Awadallah MF, Ibrahim AN, Said GE, Kamel MG (2012) New quinazolinone–pyrimidine hybrids: synthesis, anti-inflammatory, and ulcerogenicity studies. Eur J Med Chem 53: 141–149. doi:10.1016/j.ejmech.2012.03.050

Albanesi C, Pastore S, Fanales-Belasio E, Girolimoni G (1998) Cetirizine and hydrocortisone differentially regulate ICAM-1 expression and chemokine release in cultured human keratinocytes. Clin Exp Allergy 28:101–109

Allison MC, Howatson AG, Torrence CJ, Lee FD, Russel RI (1992) Gastrointestinal damage associated with the use of nonsteroidal anti-inflammatory drugs. New Engl J Med 327:749–754. doi:10.1056/NEJM199209103271101

Amin MK, Kamel MM, Anwar MM, Khedr M, Syam MY (2010) Synthesis, biological evaluation and molecular docking of novel series of spiro [(2*H*,3*H*) quinazoline-2,10-cyclohexan]-4(1*H*)-one derivatives as anti-inflammatory and analgesic agents. Eur J Med Chem 45:2117–2131. doi:10.1016/j.ejmech.2009.12.078

Aubock J, Romani N, Grubauer G, Fntsch P (1986) HLA-DR expression by keratinocytes is a common feature of diseased skin. Br J Dermatol 114:467–472

Barker JNWN, Markey AC, Allen MH, MacDonald DM (1989) Keratmocyte expression of OKM5 antigen in inflammatory cutaneous disease. Br J Dermatol 120:613–618

Barone M, Graziano ACE, Marrazzo A, Gemmellaro P, Santagati A, Cardile V (2013) Synthesis and biological evaluation of new benzo-thieno[3,2-d]pyrimidin-4-one sulphonamide thio-derivatives as potential selective cyclooxygenase-2 inhibitors. Mol Divers 17:445–458. doi:10.1007/s11030-013-9443-0

Bekhit AA, Abdel-Aziem T (2004) Design, synthesis and biological evaluation of some pyrazole derivatives as anti-inflammatory-antimicrobial agents. Bioorg Med Chem 12(8):1935–1945. doi:10.1016/j.bmc.2004.01.037

Bissonnette EY (1996) Histamine inhibits tumor necrosis factor a release mast cells through H2 and H3 receptors. Am J Respir Cell Mol Biol 14:620–626. doi:10.1165/ajrcmb.14.6.8652190

Cardile V, Frasca G, Rizza L, Rapisarda P, Bonina F (2010) Antiinflammatory effects of a red orange extract in human keratinocytes treated with interferon-gamma and histamine. Phytother Res 24(3):414–418. doi:10.1002/ptr.2973

Carlos D, Sá-Nunes A, de Paula L, Matias-Peres C, Jamur MC, Oliver C, Serra MF, Martins MA, Faccioli LH (2006) Histamine modulates mast cell degranulation through an indirect mechanism in a model IgE- mediated reaction. Eur J Immunol 36:1494–1503. doi:10.1002/eji.200535464

Caron G, Delneste Y, Roelandts E, Duez C, Herbault N, Magistrelli G, Bonnefoy JY, Pestel J, Jeannin P (2001) Histamine induces CD86 expression and chemokine production by human immature dendritic cells. J Immunol 166:6000–6006. doi:10.4049/jimmunol.166.10.6000

Carpenter RD, Lam KS, Kurth MJ (2007) Microwave-mediated heterocyclization to benzimidazo[2,1-*b*]quinazolin-12(5*H*)-ones. J Org Chem 72(1):284–287. doi:10.1021/jo0618066

Chun KS, Kim SH, Song YS, Surh YJ (2004) Celecoxib inhibits phorbol ester-induced expression of COX-2 and activation of AP-1 and p38 MAP kinase in mouse skin. Carcinogenesis 25(5): 713–722

Clive DM, Stoff JS (1984) Renal syndromes associated with nonsteroidal anti-inflammatory drugs. New Engl J Med 310: 563–572. doi:10.1056/NEJM198403013100905

Cuzzocrea S, Mazzon E, Serraino I, Dugo L, Centorrino T, Ciccolo A, Sautebin L, Caputi AP (2001) Celecoxib, a selective cyclooxygenase-2 inhibitor reduces the severity of experimental colitis induced by dinitrobenzene sulfonic acid in rats. Eur J Pharmacol 431(1):91–102

Dannhart G, Kiefer W (2001) Cyclooxygenase inhibitors-current status and future prospects. Eur J Med Chem 36:109–126. doi:10.1016/S0223-5234(01)01197-7

DeWitt DL, Smith WL (1988) Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. Proc Natl Acad Sci USA 85: 1412–1416

Dinakaran M, Selvam P, Declercq E, Sridhar SK (2003) Synthesis, antiviral and cytotoxic activity of 6-bromo-2,3-disubstituted-4(3*H*)-quinazolinones. Biol Pharm Bull 26:1278–1282. doi:10.1248/bpb.26.1278

Dohlsten M, Kalland T, Sjoergen HO, Carlson R (1988) Histamine inhibits interleukin 1 production by lipopolysaccharide-stimulated human peripheral blood monocytes. Scand J Immunol 27: 527–532

Ferreri NR, An SJ, McGiff JC (1999) Cyclooxygenase-2 expression and function in the medullary thick ascending limb. Am J Physiol 277:F360–F368

Fitzgerald GA, Patrono C (2001) The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med 345:433–442. doi:10.1056/NEJM200108093450607

Gassani AB, Rezende MR, Lima PP, Alves LD, Dosreis GW, Bakhle SY, Francischi NJ (2010) Is the sulphonamide radical in the celecoxib molecule essential for its analgesic activity? Pharmacol Res 62:439–443. doi:10.1016/j.phrs.2010.06.007

Giustizieri ML, Albanesi C, Fluhr J, Gisondi P, Norgauer J, Girolomoni G (2004) H1 histamine receptor mediates inflammatory responses in human keratinocytes. J Allergy Clin Immunol 114:1176–1182

Griffiths CEM, Voorhees JJ, Nickoloff BJ (1989) Characterization of intercellular adhesion molecule-l and HLA-DR m normal and inflamed skin: modulation by interferon-gamma and tumor necrosis factor. J Am Acad Derrnatol 20:617–629

Grossman M, Kruegar J, Younsh D et al (1989) Interleukm 6 is expressed m high levels in psoriatic skin and stimulates proliferation of cultured human keratmocytes. Proc Natl Acad Sci USA 86:6367–6371

Harris RC, McKanna JA, Akai Y, Jacobson HR, Dubois RN, Breyer MD (1994) Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. J Clin Invest 94:2504–2510. doi:10.1172/JCI117620

Hawkey C, Laine L, Simon T, Beaulieu A, Maldonado CJ, Acevedo E, Shahane A, Quan H, Bolognese J, Mortensen E (2000) Comparison of the effect of rofecoxib (a cyclooxygenase-2 inhibitor), ibuprofen and placebo on the gastroduodenal mucosa of patients with osteoarthritis: a randomized, double-blind, placebo-controlled trial. The rofecoxib osteoarthritis endoscopy multinational study group. Arthritis Rheum 43:370–377. doi:10.1002/1529-0131(200002

Hla T, Neilson K (1992) Human cyclooxygenase-2 cDNA. Proc Natl Acad Sci USA 89:7384–7388

Hodgkins JE, Ettilinger MG (1956) The synthesis of isothiocyanates from amines. J Org Chem 21:404–405. doi:10.1021/jo01110a006

Jaiswal M, Khadikar VP, Supuran TC (2004) Topological modeling of lipophilicity, diuretic activity, and carbonic inhibition activity of benzenesulfonamides: a molecular connectivity approach. Bioorg Med Chem Lett 14:5661–5666. doi:10.1016/j.bmcl.2004.08.051

Kini GS, Grover G (2006) Synthesis and evaluation of new quinazolone derivatives of nalidixic acid as potential antibacterial and antifungal agents. Eur J Med Chem 41:256–262. doi:10.1016/j.ejmech.2005.09.002

Kniss DA (1999) Cyclooxygenases in reproductive medicine and biology. J Soc Gynecol Invest 6:285–292

Krouwels FH, Hol BE, Lutter R, Bruinier B, Bast A, Jansen HM, Out TA (1998) Histamine affects interleukin-4, interleukin-5, and interferon-g production by human T cell clones from the airways and blood. Am J Respir Cell Mol Biol 18:721–730. doi:10.1165/ajrcmb.18.5.2909

Kujubu DA, Fletcher BS, Varnum BC, Lim RW, Herschman HR (1991) TIS10, a phorbol ester tumor promoter inducible mRNA from swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. J Biol Chem 266:12866–12872. http://www.jbc.org/content/266/20/12866

Lagier B, Lebel B, Bousquet J, Péne J (1997) Different modulation by histamine of IL-4 and interferon-g (IFN-g) release according to the phenotype of human Th0, Th1 and Th2 clones. Clin Exp Immunol 108:545–551. doi:10.1046/j.1365-2249.1997.3791276.x

Lemura R, Manabe H, Todayaki S (1989) Bioisosteric transformation of H1-antihistaminic benzimidazole derivatives. Chem Pharm Bull 37:2723–2726

Liu J, Wilson JC, Ye P, Sprague K, Sargent K, Si Y, Beletsky G, Yohannes D, Chung S (2006) Privileged structure based quinazolinone natural product-templated libraries: identification of novel tubulin polymerization inhibitors. Bioorg Med Chem Lett 16:686–690. doi:10.1016/j.bmcl.2005.10.022

Marnett LJ, DuBois RN (2002) COX-2: a target for colon cancer prevention. Annu Rev Pharmacol Toxicol 42:55–80. doi:10.1146/annurev.pharmtox.42.082301.164620

Masferrer JL, Zweifel BS, Manning PT, Hauser SD, Leahy KM, Smith WG, Isakson PC, Seibert K (1994) Selective inhibition of inducible cyclooxygenase-2 in vivo is anti-inflammatory and nonulcerogenic. Proc Natl Acad Sci USA 91:3228–3232

Miao XP, Li JS, Ouyang Q, Hu RW, Zhang Y, Li HY (2014) Tolerability of selective cyclooxygenase 2 inhibitors used for the treatment of rheumatological manifestations of inflammatory bowel disease. Cochrane Database Syst Rev. doi:10.1002/14651858.CD007744.pub2

Nickoloff BJ (1988) The role of gamma interferon in epidermal traffickmg of lymphocytes with emphasis on molecular and cellular adhesion events. Arch Dermatoll 124:1835–1843. doi:10.1001/archderm.1988.01670120051010

Nickoloff BJ, Mitra RS, Elder JT, Fisher GJ, Voorhees JJ (1989) Decreased growth inhibition by recombinant gamma interferon is associated with increased production of transforming growth factor alpha m keratinocytes cultured from psonatic lesions. Br J Dermatoll 121:161–174

O'Sullivan MG, Huggins EM Jr., Mccall CE (1993) Lipopolysaccharide—induced expression of prostaglandin H synthase-2 in aveolar macrophages is inhibited by dexamethasone but not by aspirin. Biochem Biophys Res Commun 191:1294–1300. doi:10.1006/bbrc.1993.1358

Patrono C, Patrignani P, Garcìa Rodriguez LA (2001) Cyclooxygenase—selective inhibition of prostanoid formation: transducing biochemical selectivity into clinical read-outs. J Clin Invest 108:7–13. doi:10.1172/JCI13418

Rocca B, Spain LM, Pure E, Langenbach R, Patrono C, FitzGerald GA (1999) Distinct roles of prostaglandin H synthases 1 and 2 in T-cell development. J Clin Invest 103:1469–1477. doi:10.1172/JCI6400

Roh GS, Yi CO, Cho YJ, Jeon BT, Nizamudtinova IT, Kim HJ, Kim JH, Oh YM, Huh JW, Lee JH, Hwang YS, Lee SD, Lee JD (2010) Anti-inflammatory effects of celecoxib in rat lungs with smoke-induced emphysema. Am J Physiol Lung Cell Mol Physiol 299(2):L184–L191. doi:10.1152/ajplung.00303.2009

Salvemini D, Manning PT, Zeifel BS, Seibert K, Curie MG, Needleman P, Masferrer JL (1995) Dual inhibition of nitric oxide and prostaglandin production contributes to the anti-inflammatory properties of nitric oxide synthase inhibitors. J Clin Invest 96:301–308. doi:10.1172/JCI118035

Sirois J, Ménard G, Moses AS, Bissonnette EY (2000) Importance of histamine in the cytokine network in the lung through H2 and H3 receptors: stimulation of IL-10 production. J Immunol 164:2964–2970. doi:10.4049/jimmunol.164.6.2964

Smith WL, Langenbach R (2001) Why there are two cyclooxygenase isozymes. J Clin Invest 12:1491–1495. doi:10.1172/JCI13271

Smith CJ, Zhang Y, Koboldt CM, Muhammad J, Zweifel BS, Shaffer A, Talley JJ, Masferrer JL, Seibert K, Isakson PC (1998) Pharmacological analysis of cyclooxygenase-1 in inflammation. Proc Natl Acad Sci USA 95:13313–13318

Sperotto E, van Klink G, de Vriev J, van Koten G (2008) Ligand free copper-catalyzed C–S coupling of aryl iodides and thiols. J Org Chem 73:5625–5628. doi:10.1021/jo800491k

Staszak C, Goodwin JS (1980) Is prostaglandin a mediator for the inhibitory action of histamine, hydrocortisone, and isoproterenol? Cell Immunol 54:351–361

Tiwari AK, Mishra AK, Bajpai A, Mishra P, Sharma RK, Pandey VK, Singh VK (2006) Synthesis and pharmacological study of novel pyrido-quinazolone analogues as anti-fungal, antibacterial, and anticancer agents. Bioorg Med Chem Lett 16:4581–4585. doi:10.1016/j.bmcl.2006.06.015

Vane JR (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol 231:232–235

Wamhoff H, Lichtenthaler L (1978) Heterocyclische β-enaminoester, 22: pyrido[2,3-*d*]pyrimidine aus 2-amino-3-ethoxycarbonyl-1,4,5,6-tetrahydropyridin und isocyanaten, isothiocyanaten, imidsäureestern, formamid und lactimethern. Chem Ber 111:2297–2306

Yamagata K, Andreasson KI, Kaufmann WE, Barnes CA, Worley PF (1993) Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. Neuron 11:371–386. doi:10.1016/0896-6273(93)90192-T

Zambre AP, Ganure AL, Shinde DB, Kulkarni VM (2007) Perspective assessment of COX-1 and COX-2 selectivity of nonsteroidal anti-inflammatory drugs from clinical practice: use of genetic function approximation. J Chem Inf Model 47:635–643. doi:10.1021/ci6004367

Zayed MF, Hassan MH (2014) Synthesis and biological evaluation studies of novel quinazolinone derivatives as antibacterial and anti-inflammatory agents. Saudi Pharm J 22:157–162. doi:10.1016/j.jsps.2013.03.004