European Journal of Medicinal Chemistry 44 (2009) 2184–2189

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design, synthesis and characterization of novel 2-(2,4-disubstituted-thiazole-5-yl)-3-aryl-3*H*-quinazoline-4-one derivatives as inhibitors of NF-κB and AP-1 mediated transcription activation and as potential anti-inflammatory agents

Rajan S. Giri [a,*], Hardik M. Thaker [b], Tony Giordano [c], Jill Williams [c], Donna Rogers [c], Vasudevan Sudersanam [b], Kamala K. Vasu [b]

[a] Department of Medicinal Chemistry, University of Minnesota, 308 Harvard Street, SE, MN 55445, USA
[b] B. V. Patel Pharmaceutical Education and Research Development Centre, Sarkhej-Gandhinagar Highway, Thaltej, Ahmedabad 380 054, Gujarat, India
[c] Feist-Weiller Cancer Center, Louisiana State University Health Sciences Center at Shreveport, 1501 Kings Highway Shreveport, LA 71130, USA

ARTICLE INFO

Article history:
Received 24 September 2008
Received in revised form
28 October 2008
Accepted 29 October 2008
Available online 5 November 2008

Keywords:
Thiazole
Inhibitors of NF-κB and AP-1 mediated transcriptional activation
Anti-inflammatory agents and transcription inhibitors

ABSTRACT

A series of 2-(2,4-disubstituted-thiazole-5-yl)-3-aryl-3*H*-quinazoline-4-one derivatives were designed and synthesized. Synthesized molecules were further evaluated for their inhibitory activity towards transcription factors NF-κB and AP-1 mediated transcriptional activation in a cell line based in vitro assay as well as for their anti-inflammatory activity in in vivo model of acute inflammation. This series provides us with selective and dual inhibitors of NF-κB and AP-1 mediated transcriptional activation which also exhibit significant efficacy in in vivo model of inflammation. Two of the compounds **9m** and **9o** turned out to be the most promising dual inhibitors of NF-κB and AP-1 mediated transcriptional activation with an IC_{50} of 3.3 μM for both. **9n** ($IC_{50} = 5.5$ μM) and **9p** ($IC_{50} = 5.5$ μM) emerged as selective inhibitors of NF-κB mediated transcriptional activation and **9c** ($IC_{50} = 5.5$ μM) and **9d** ($IC_{50} = 5.5$ μM) were found to be more selective inhibitor of AP-1 mediated transcriptional activity. Though the relationship between the activities shown by these compounds in in vivo and in vitro model is still to be established, these results suggest the suitability of the designed molecular framework as a potential anti-inflammatory molecular framework which also exhibits the inhibitory activity towards NF-κB and AP-1 mediated transcriptional activation. This will be worth studying further to explore its complete potential particularly in chronic inflammatory conditions. The structure activity relationship (SAR) of this series has been discussed herein.

© 2008 Elsevier Masson SAS. All rights reserved.

1. Introduction

Inflammation remains a common and, all too often poorly controlled clinical problem which can be life threatening in extreme form of allergy, autoimmune diseases and rejection of transplanted organs. The treatment options available for inflammatory diseases are unsatisfactory and complicated due to their lack of efficacy and adverse effect profile. Due to the failure of single targeted therapy, and in view of the polygenic nature of inflammatory diseases which involves a number of pathways acting in series and in parallel it seemed appropriate to look for candidates acting on more than one pathway involved in inflammatory conditions [1].

Transcription factors are regulatory proteins which bind to specific DNA sequences in the gene promoter or enhancer regions and activate or inhibit transcription of genes [2]. Transcription factor nuclear factor-κB (NF-κB) and the activating factor (AP-1) are key transcription factors that orchestrate expression of many genes involved in inflammatory conditions. NF-κB regulates the transcription of genes including TNF-α, interleukin-1 (IL-1), IL-2, IL-6, adhesion molecules and chemokines, among others [3,4]. Similarly, (AP-1) regulates the production of the cytokines TNF-α, IL-1, IL-2, granulocyte-macrophage colony stimulating factor (GM-CSF) and matrix metalloproteases (MMP) [5]. Therefore, the selective inhibition of transcription factors like NF-κB and AP-1 involved in inflammatory diseases may thus control a plethora of mediators of inflammation [6]. This may also overcome some of the redundancy that is inherent to biological systems and possibly responsible for the failure of single-targeted therapy [7,8]. It is also reported that compounds which shows their anti-inflammatory effect through inhibiting the activation NF-κB and AP-1 does not cause gastrointestinal mucosal damage and other side effects associated with long term NSAIDs and glucocorticoid use [11]. Based on these observations, it appears that inhibition of NF-κB and/or AP-1

* Corresponding author. Tel.: +1 318 655 3063.
E-mail address: girix007@umn.edu (R.S. Giri).

0223-5234/$ – see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.10.031

transcriptional activation may represent an attractive target in the development of novel anti-inflammatory agents [9,10].

SPC-839 (Fig. 1) reported by Palanki and his coworkers is one of the very few compounds which demonstrate their anti-inflammatory activity by inhibiting the activation of NF-κB and AP-1 mediated transcriptional activation [12–14]. Taking **SPC-839** as a chemical lead [15] and by using the concept of bioisosterism [16], we have designed a novel scaffold (2-(2,4-disubstituted-thiazole-5-yl)-3-aryl-3*H*-quinazolin-4-one (**9**, Fig. 1)) for its evaluation as inhibitor of NF-κB and/or AP-1 mediated transcriptional activation. Herein, we report the synthesis of various analogs of designed scaffold and their evaluation as inhibitors of NF-κB and AP-1 mediated transcriptional activation and as anti-inflammatory agents. Thus the structure activity relationship (SAR) of this series has been discussed.

2. Chemistry

The synthesis of designed compounds is outlined in Scheme 1. The thiourea derivatives (**3**) were prepared by treating different amidines (**2**) with suitable isothiocyanates in toluene or hexane. 1-amidino-3-substituted thiourea derivatives (**5**) were prepared by treating guanidine hydrochloride (**4**) with suitable isothiocyanates in aqueous basic media [17]. Amidines (**2**) were prepared from commercially available acetonitrile or benzonitrile (**1**) [18]. The synthesis of 2-chloromethyl-3-aryl-3*H*-quinazoline-4-one (**8**) was furnished by using modified Niementowski synthesis, where we began with chloro-acetylation of antranilic acid (**6**) to get corresponding 2-(2-choroacetyl amino) benzoic acid (**7**). Acid (**7**) was then treated with trichlorophosphate (PCl_3) to convert into acid chloride which was immediately treated with substituted aniline *insitu* to substitute chloro group of acid chloride with anilines. This was then refluxed in toluene to get cyclised product (**8**) [19–21]. In the final step, we treated thiourea derivatives (**3** and **5**) in acetonitrile with 2-chloromethyl-3-aryl-3*H*-quinazolin-4-one derivatives (**8**) in acetonitrile at 75–80 °C for 2–4 h to furnished desired 2-(2-alkylamino/arylamino-4-methyl/amino/phenyl-thiazole-5-yl)-3-aryl-3*H*-quinazolin-4-one derivatives (**9**) [22,23]. The synthesized compounds were characterized by their ^{1}H NMR chemical shift, LC-MS (m/e), and elemental analysis.

In this synthesis, functionalized thiourea derivatives **3** and **5** provide two ring carbon atoms and both the heteroatom of resultant thiazole ring; the remaining carbon atom (C-5) was supplied by the methylene group of 2-chlormethyl group at the second position of quinazolinone ring.

The mechanism involves the S-alkylation of the –SH group of amidine by $–CH_2–$ of chloromethyl at the second position of quinazolinone ring followed by the intramolecular cyclization by the nucleophilic attack of active methylene group. Presumably this nucleophilic attack may take place due to initial tautomerization of quinazolinone double bond to generate an enamine like intermediate; the quinazolinone nitrogen then serves as an electron source

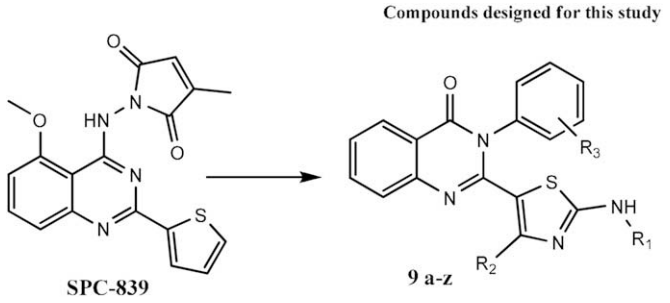
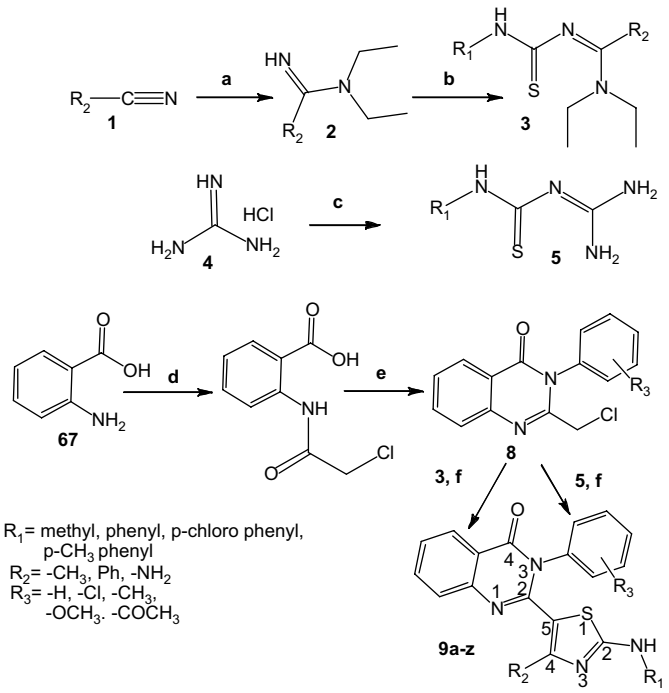


Fig. 1. Chemical lead based approaches for the discovery of novel quinazolinone derivatives as inhibitors of NF-κB and AP-1 mediated transcriptional activation.



Scheme 1. Reactions and conditions: a) $AlCl_3$, diethyl amine, 120–140 °C, b) R_1-NCS, toluene or hexane 0–5 °C, c) R_1-NCS, NaOH, 0–5 °C, d) Chloroacetylchloride (ClAcCl), TEA, 0–5 °C for 30 min then on RT of 3 h, e) Arylamine, PCl_3, toluene, 110 °C, 5–6 h, f) Acetonitrile, 80 °C 2–4 h.

for the nucleophilic attack. The driving force for the thiazole formation is the elimination of the diethyl amine residue in case of intermediate **3** (in case of intermediate **5** ammonia will be liberated) and the formation of the stable five membered aromatic heterocyclic ring. The mechanism is summarized in Scheme 2.

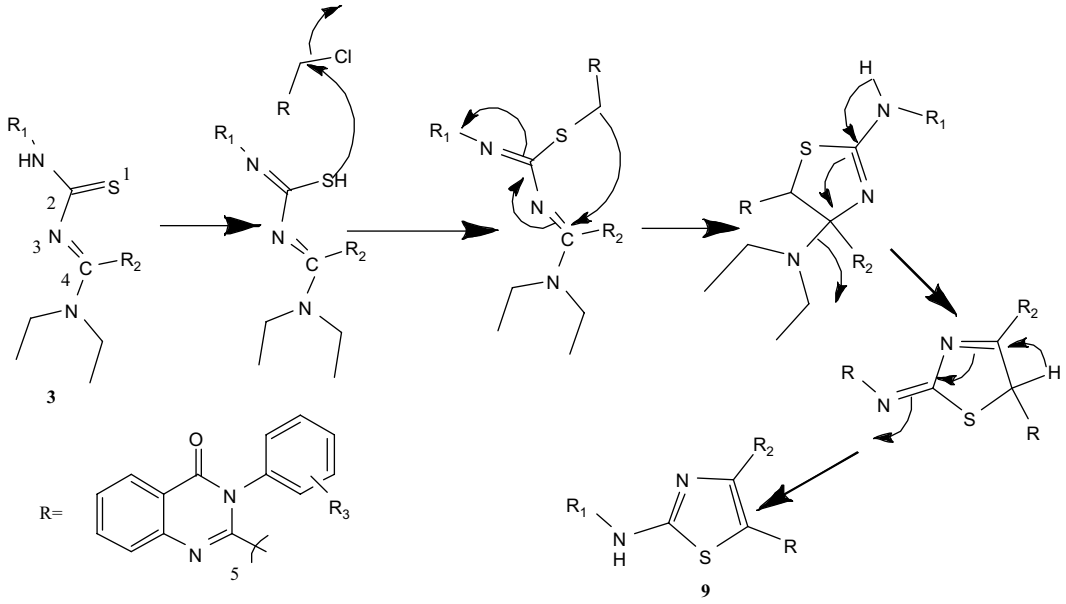
3. Results and discussion

All of the 26 compounds synthesized were evaluated for their activities. A substantial number of compounds have been identified exhibiting moderate to excellent inhibitory activity towards NF-κB and/or AP-1 mediated transcriptional activation in a cell line based in vitro assay at 10 μM concentration. IC_{50} was determined for the compounds showing more than 50% inhibition at 10 μM (Table 2). These compounds were also found to exhibit a significant protective activity in the carrageenan induced rat paw edema model (in vivo, 50 mg/kg, p.o.) in comparison to the existing anti-inflammatory drug ibuprofen (Table 1).

On the basis of the biological activity exhibited by the designed series we can classify this set of compounds into three classes. (1) Compounds selectively efficacious as inhibitors of NF-κB mediated transcriptional activation and also showing good in vivo activity like, compounds **9l**, **9n** and **9p** (Table 1). (2) Compounds selectively efficacious as inhibitors of AP-1 mediated transcriptional activation and also showing in vivo activity, like compounds **9c**, **9d** and **9g** (Table 1). (3) Compounds that are dual inhibitors of both NF-κB and AP-1 mediated transcriptional activation and are also active in in vivo model of acute inflammation like **9m** and **9o** (Table 1).

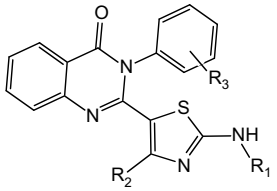
Our findings suggest that selectivity of the compound for these transcriptional factors is primarily dependent on the combination of substitutions at the 2nd position of the thiazole (R_1), 4th position of the thiazole (R_2) and on the phenyl ring at the 3rd (R_3) position of the quinazolinone ring.

For selectivity towards inhibition of NF-κB mediated transcriptional activation, both arylamino and methylamino have shown comparable selectivity at the 2nd position of thiazole, while



Scheme 2. Plausible mechanism for the synthesis of designed compounds.

Table 1
Evaluation of synthesized compounds for their inhibitory activity towards NF-κB and AP-1 mediated transcriptional activation and as anti-inflammatory agents.



Compounds	R_1	R_2	R_3	% Protection[a] in vivo (50 mg/kg)	% Inhibition in vitro (10 μM)	
					NF-κB	AP-1
9a	Ph	$-CH_3$	–H	12	25	16
9b	*p*-Cl Ph	$-CH_3$	–H	4	0	0
9c	$-CH_3$	$-CH_3$	2-CH_3	35	16	67
9d	Ph	$-CH_3$	2-CH_3	42	21	60
9e	*p*-Cl Ph	$-CH_3$	2-CH_3	45	*n.d*	*n.d*
9f	$-CH_3$	$-CH_3$	3-CH_3	*n.d*	10	20
9g	*p*-Cl Ph	$-CH_3$	3-CH_3	*n.d*	2	39
9h	*p*-Cl Ph	$-CH_3$	4-CH_3	37	12	2
9i	*p*-Cl Ph	–CH3	2-OCH_3	27	*n.d*	*n.d*
9j	*p*-Cl Ph	–CH3	4-OCH_3	5	0	0
9k	Ph	–CH3	2-Cl	*n.d*	6	21
9l	*p*-Cl Ph	–CH3	2-Cl	58	60	21
9m	$-CH_3$	$-CH_3$	4-Cl	71	93	82
9n	Ph	–CH3	4-Cl	72	79	9
9o	*p*-Cl Ph	$-CH_3$	4-Cl	38	96	84
9p	$-CH_3$	$-CH_3$	4-$COCH_3$	42	71	0
9q	–Ph	$-CH_3$	4-$COCH_3$	*n.d*	15	7
9r	*p*-Cl Ph	$-CH_3$	4-$COCH_3$	13	0	2
9s	*p*-Cl Ph	$-NH_2$	4-Cl	37	0	0
9t	*p*-Cl Ph	$-NH_2$	4-$COCH_3$	53	12	2
9u	*p*-Cl Ph	–Ph	–H	*n.d*	0	27
9v	*p*-Cl Ph	–Ph	2-CH_3	51	27	32
9w	*p*-Cl Ph	–Ph	2-Cl	61	24	0
9x	*p*-CH_3 Ph	–Ph	4-Cl	*n.d*	0	0
9y	$-CH_3$	–Ph	4-$COCH_3$	*n.d*	4	15
9z	*p*-Cl Ph	–Ph	4-$COCH_3$	23	0	31
Ibuprofen	–	–	–	52	–	–

n.d = Not determined.

[a] Oral administration for all test compounds for in vivo anti-inflammatory activity, $p < 0.05$, Student's unpaired *t*-test versus controls. For the standard drug ibuprofen, dose and % protection were 50 mg/kg, 52% respectively.

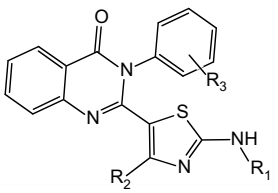
electron withdrawing groups like –Cl and –$COCH_3$ are more favorable on the *para* position of phenyl ring at the third position of the quinazolinone ring.

For selectivity towards inhibition of AP-1 mediated transcriptional activation, both arylamino and methylamino have shown comparable selectivity at the 2nd position of thiazole. However, electron-releasing groups like –CH_3 on *ortho* position of the phenyl ring at the third position of quinazolinone ring are more desirable.

Dual inhibitory activity towards NF-κB and AP-1 mediated transcriptional activation was accomplished when R_1 was methyl and *p*-Cl phenyl in combination with *p*-Cl substitution at the phenyl ring at the 3rd position of quinazolinone. Change in the position of –Cl group from the *para* position (**9o**) to the *meta* position (**9l**) of the phenyl ring at 3rd position of the quinazolinone ring decreases the potency and increases the selectivity of the compounds towards inhibition of NF-κB mediated transcriptional activation.

These results also suggest that the presence of electron withdrawing or releasing group on phenyl ring at 3rd position of quinazolinone is very important for the biological activities discussed above as absence of these groups at this position make the

Table 2
IC_{50} value determined for compounds showing more than 50% inhibition in in vitro assay.



Compounds	R_1	R_2	R_3	IC_{50} (in vitro)	
				NF-κB	AP-1
9c	$-CH_3$	$-CH_3$	2-CH_3	*n.d*	5.5 μM
9d	Ph	$-CH_3$	2-CH_3	*n.d*	5.5 μM
9l	*p*-Cl Ph	–CH3	2-Cl	5.5 μM	*n.d*
9m	$-CH_3$	$-CH_3$	4-Cl	3.3 μM	3.3 μM
9n	Ph	–CH3	4-Cl	5.5 μM	*n.d*
9o	*p*-Cl Ph	$-CH_3$	4-Cl	3.3 μM	3.3 μM
9p	$-CH_3$	$-CH_3$	4-$COCH_3$	5.5 μM	*n.d*

compounds only mildly active or virtually inactive in all the biological assays mentioned (**9a**, **9b** and **9u**, Table 1).

Replacement of $-CH_3$ group at the 4th position of the thiazole ring (**9o**) with an $-NH_2$ (**9s** and **9t**) group or phenyl group (**9u**–**9z**) was found to be detrimental for the inhibitory activity towards NF-κB and AP-1 mediated transcriptional activation. However, in both cases where an $-NH_2$ group was introduced instead of a $-CH_3$ group at the 4th position of the thiazole ring, the compounds retained their anti-inflammatory activity in in vivo system (**9s** and **9t**, Table 1). In most cases in vivo and in vitro results exhibited by designed series are reasonably comparable. A notable exception is the in vivo result of **9o** which shows less inhibition compared to the other hits even though it has emerged as the most potent dual inhibitor of NF-κB and AP-1 mediated transcriptional activation. This suggests that the designed compounds may also interfere with some other pathways involved in the inflammatory cascade. The experiments for concluding the actual molecular aspect of the anti-inflammatory activity of the compounds are currently underway where we are trying to see the relationship between the activities shown by these compounds in in vivo and in vitro models.

We have hereby generated a novel molecular framework, 2-(2,4-disubstituted-thiazole-5-yl)–3-aryl-3*H*-quinazoline-4-one by utilizing the chemical lead based approach and concept of bioisosterism. Compounds based on this framework have found to be active as an inhibitor of NF-κB and AP-1 mediated transcriptional activation (in vitro) and, also shown anti-inflammatory activity in acute model of inflammation (in vivo). These results may open up new avenues in designing candidates acting on more than one rate-limiting step along the inflammatory cascade.

4. Conclusion

In an attempt to discover novel inhibitors of NF-κB and AP-1 mediated transcriptional activation and potent anti-inflammatory agents, a series of 2-(2,4-disubstituted-thiazole-5-yl)-3-aryl-3*H*-quinazoline-4-one derivatives were designed, synthesized and evaluated for its biological activity. This series eventually turned out to be very promising as it provides us with selective and dual inhibitors of NF-κB and AP-1 mediated transcriptional activation. All these inhibitors also demonstrated significant in vivo efficacy compared to the known anti-inflammatory drug ibuprofen. Though the relationship between the activities shown by these compounds in in vivo and in vitro model is still to be established, these results suggest the suitability of the designed molecular framework as a potential anti-inflammatory lead. This will be worth studying further to explore its complete potential particularly in chronic inflammatory conditions. Additional characterization of these compounds and related analogs will be reported in due course.

5. Experimental

Unless mentioned otherwise all the starting materials and solvents were purchased from commercially available sources. Melting points were determined on a Toshniwal melting point apparatus using open glass capillary tubes and represent the uncorrected values. Proton NMR spectra were measured on a Bruker spectrometer using the specified solvents at RSIC Chandigarh. LC-MS analysis was done on a Perkin Elmer Applied Bio-Sciences API-165 in the analytical laboratory of B. V. Patel PERD Centre, Ahmedabad. Elemental analysis was carried out at RSIC, IIT Powai on Perkin Elmer 2400 CHN elemental analyzer. All the reactions were monitored using thin layer chromatography (TLC) using glass plate coated with silica gel G or GF_{254}. TLC plates were developed in iodine and toluene:acetonitrile (7:3), taken as mobile phase, unless mentioned otherwise.

5.1. General synthesis of 2-(2-alkylamino/arylamino-4-alkyl/phenyl/amino-thiazole-5-yl)-3-aryl-3H-quinazolin-4-one derivatives

The general procedure for synthesis of designed compounds (**9**) is as follows. 0.01 mole of thiourea derivatives (**3**) or 1-amidino-3-substituted thiourea derivatives (**5**) in 5 ml of acetonitrile was added to a stirred solution of 0.01 mole of the respective 2-chloromethyl-3-aryl-3*H*-quinazoline-4-one (**8**) in 5 ml of acetonitrile at 75–80 °C. This reaction mixture was stirred at this temperature for 2–4 h and then cooled to room temperature. The compound that precipitated was filtered and recrystalized in methanol to obtain solid crystals. In case the solid did not separate from the reaction mixture, the completion of the reaction was confirmed on TLC, the solvent was evaporated completely and chilled water was added to it and triturated till the solid separated.

5.2. Characterization of synthesized compounds

5.2.1. 2-(4-Methyl-2-phenylamino-thiazol-5-yl)-3-phenyl-3H-quinazolin-4-one (**9a**)

Yield: 72.36%, M.P.: 210–12 °C, R_f: 0.51, M.F.: $C_{24}H_{18}N_4OS$, LC-MS (m/e): 411 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.27 (s, 3H), 7.21–7.90 (m, 13H), 8.11–8.14 (d, 1H, J_{ortho}7.6 Hz), 10.32 (s, 1H), Anal. Calcd. For. $C_{24}H_{18}N_4OS$: C, 70.22; H, 4.42; N, 13.65, Found: C, 70.19; H, 4.51; N, 13.67.

5.2.2. 2-[2-(4-Cholro-phenylamino)-4-methyl-thiazol-5-yl]-3-phenyl-3H-quinazoline-4-one (**9b**)

Yield: 39%, M.P.: 230–2 °C, R_f: 0.32, M.F.: $C_{24}H_{17}N_4OSCl$, LC-MS (m/e): 445 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.25 (s, 3H), 7.23–7.87 (m, 12H), 8.10–8.15 (d, 1H, J_{ortho}7.2 Hz), 10.35 (s, 1H), Anal. Calcd. For. $C_{24}H_{17}N_4OSCl$: C, 64.79; H, 3.85; N, 12.59, Found: C, 64.81; H, 3.86; N, 12.61.

5.2.3. 2-(4-Methyl-2-methylamino-thiazol-5-yl)-3-o-tolyl-3H-quinazolin-4-one (**9c**)

Yield: 30%, M.P.: 218–20 °C, R_f: 0.63, M.F.: $C_{20}H_{18}N_4OS$, LC-MS (m/e): 363 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.35 (s, 3H), 2.42 (s, 6H), 7.21–7.92 (m, 7H), 8.12–8.14 (d, 1H, J_{ortho}7.4 Hz), 10.32 (s, 1H), Anal. Calcd. For. $C_{20}H_{18}N_4OS$: C, 66.28; H, 5.01; N, 15.46, Found: C, 66.25; H, 4.98; N, 15.48.

5.2.4. 2-(4-Methyl-2-phenylamino-thiazol-5-yl)-3-o-tolyl-3H-quinazolin-4-one (**9d**)

Yield: 49%, M.P.: 227–8 °C, R_f: 0.64, M.F.: $C_{25}H_{20}N_4OS$, LC-MS (m/e): 425 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.18 (s, 3H), 2.28 (s, 3H), 6.98–7.76 (m, 12H), 8.13–8.15 (d, 1H, J_{ortho}7.6 Hz), 10.21 (s, 1H), Anal. Calcd. For. $C_{25}H_{20}N_4OS$: C, 70.73; H, 4.75; N, 13.20, Found: C, 70.69; H, 4.77; N, 13.27.

5.2.5. 2-[2-(4-Chloro-phenylamino)-4-methyl-thiazol-5-yl]-3-o-tolyl-3H-quinazolin-4-one (**9e**)

Yield: 54%, M.P.: >275 °C, R_f: 0.63, M.F.: $C_{25}H_{19}N_4OSCl$, LC-MS (m/e): 459 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.19 (s, 3H), 2.26 (s, 3H), 7.08–7.75 (m, 11H), 8.11–8.12 (d, 1H, J_{ortho}7.2 Hz), 10.27 (s, 1H), Anal. Calcd. For. $C_{25}H_{19}N_4OSCl$: C, 65.42; H, 4.17; N, 12.21, Found: C, 65.39; H, 4.16; N, 12.22.

5.2.6. 2-(4-Methyl-2-methylamino-thiazol-5-yl)-3-m-tolyl-3H-quinazolin-4-one (**9f**)

Yield: 30%, M.P.: >275 °C, R_f: 0.49, M.F.: $C_{20}H_{18}N_4OS$, LC-MS (m/e): 363 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.18 (s, 6H), 2.28 (s, 3H), 6.98–7.76 (m, 7H), 8.13–8.15 (d, 1H, J_{ortho}7.2 Hz),

10.21 (s, 1H), Anal. Calcd. For. $C_{20}H_{18}N_4OS$: C, 66.28; H, 5.01; N, 15.46, Found: C, 66.21; H, 4.93; N, 15.42.

*5.2.7. 2-[2-(4-Chloro-phenylamino)-4-methyl-thiazol-5-yl]-3-*m*-tolyl-3H-quinazolin-4-one (**9g**)*

Yield: 51%, M.P.: 236–8 °C, R_f: 0.65, M.F.: $C_{25}H_{19}N_4OSCl$, LC-MS (m/e): 459 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.17 (s, 3H), 2.24 (s, 3H), 7.15–7.89 (m, 11H), 8.01–8.11 (d, 1H, J_{ortho} 7.5 Hz), 10.5 (s, 1H), Anal. Calcd. For. $C_{25}H_{19}N_4OSCl$: C, 65.42; H, 4.17; N, 12.21, Found: C, 65.36; H, 4.15; N, 12.19.

*5.2.8. 2-[2-(4-Chloro-phenylamino)-4-methyl-thiazol-5-yl]-3-*p*-tolyl-3H-quinazolin-4-one (**9h**)*

Yield: 55%, M.P.: >275 °C, R_f: 0.61, M.F.: $C_{25}H_{19}N_4OSCl$, LC-MS (m/e): 459 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.3 (s, 3H), 2.47 (s, 3H), 7.21–7.86 (m, 11H), 8.11–8.14 (d, 1H, J_{ortho}7.4 Hz), 10.30 (s, 1H), Anal. Calcd. For. $C_{25}H_{19}N_4OSCl$: C, 65.42; H, 4.17; N, 12.21, Found: C, 65.40; H, 4.15; N, 12.17.

*5.2.9. 2-[2-(4-Chloro-phenylamino)-4-methyl-thiazol-5-yl]-3-(2-methoxy-phenyl)-3H-quinazolin-4-one (**9i**)*

Yield: 41%, M.P.: 225–6 °C, R_f: 0.61, M.F.: $C_{25}H_{19}N_4O_2SCl$, LC-MS (m/e): 475 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.34 (s, 3H), 3.75 (s, 3H) 6.95–7.88 (m, 11H), 8.11–8.14 (d, 1H, J_{ortho}7.5 Hz), 10.33 (s, 1H), Anal. Calcd. For. $C_{25}H_{19}N_4O_2SCl$: C, 63.22; H, 4.03; N, 11.80, Found: C, 63.26; H, 4.10; N, 11.81.

*5.2.10. 2-[2-(4-Chloro-phenylamino)-4-methyl-thiazol-5-yl]-3-(4-methoxy-phenyl)-3H-quinazolin-4-one (**9j**)*

Yield: 38%, M.P.: >275 °C, R_f: 0.7, M.F.: $C_{25}H_{19}N_4O_2SCl$, LC-MS (m/e): 475 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.28 (s, 3H), 3.71 (s, 3H), 6.92–7.92 (m, 11H), 8.11–8.14 (d, 1H, J_{ortho}7.4 Hz), 10.33 (s, 1H), Anal. Calcd. For. $C_{25}H_{19}N_4O_2SCl$: C, 63.22; H, 4.03; N, 11.80, Found: C, 63.25; H, 4.07; N, 11.78.

*5.2.11. 3-(2-Chloro-phenyl)-2-(4-methyl-2-phenylamino-thiazol-5-yl)-3H-quinazolin-4-one (**9k**)*

Yield: 44%, M.P.: 228–30 °C, R_f: 0.4, M.F.: $C_{24}H_{17}N_4O_2SCl$, LC-MS (m/e): 446 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.48 (s, 3H), 6.90–7.93 (m, 12H), 8.14–8.17 (d, 1H, J_{ortho} 7.5 Hz), 10.2 (s, 1H), Anal. Calcd. For. $C_{24}H_{17}N_4O_2SCl$: C, 64.79; H, 3.85; N, 12.59, Found: C, 64.77; H, 3.84; N, 12.61.

*5.2.12. 3-(2-Chloro-phenyl)-2-[2-(4-chloro-phenylamino)-4-methyl-thiazol-5-yl]-3H-quinazolin-4-one (**9l**)*

Yield: 41%, M.P.: 237–8 °C, R_f: 0.51, M.F.: $C_{24}H_{16}N_4OSCl$, LC-MS (m/e): 480.3 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.26 (s, 3H), 7.23–7.87 (m, 11H), 8.10–8.15 (d, 1H, J_{ortho}7.2 Hz), 10.35 (s, 1H), Anal. Calcd. For. $C_{24}H_{16}N_4OSCl$: C, 60.13; H, 3.36; N, 11.69, Found: C, 60.11; H, 3.35; N, 11.71.

*5.2.13. 3-(4-Chloro-phenyl)-2-(4-methyl-2-methylamino-thiazol-5-yl)-3H-quinazolin-4-one (**9m**)*

Yield: 41%, M.P.: 195–7 °C, R_f: 0.61, M.F.: $C_{19}H_{15}N_4OSCl$, LC-MS (m/e): 384 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.33 (s, 3H), 2.82 (s, 3H), 7.17–7.82 (m, 7H), 8.28–8.30 (m, 1H), 9.37 (s, 1H), Anal. Calcd. For. $C_{19}H_{15}N_4OSCl$: C, 59.60; H, 3.95; N, 14.63, Found: C, 59.61; H, 3.97; N, 14.65.

*5.2.14. 3-(4-Chloro-phenyl)-2-(4-methyl-2-phenylamino-thiazol-5-yl)-3H-quinazolin-4-one (**9n**)*

Yield: 51%, M.P.: 277–80 °C, R_f: 0.84, M.F.: $C_{24}H_{17}N_4O_2SCl$, LC-MS (m/e): 446 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.46 (s, 3H), 6.94–7.85 (m, 12H), 8.23–8.25 (dd, 1H, J_{ortho}8.4 Hz, J_{meta}1.0), 9.91 (s, 1H), Anal. Calcd. For. $C_{24}H_{17}N_4O_2SCl$: C, 64.79; H, 3.85; N, 12.59, Found: C, 64.75; H, 3.86; N, 12.57.

*5.2.15. 3-(4-Chloro-phenyl)-2-[2-(4-chloro-phenylamino)-4-methyl-thiazol-5-yl]-3H-quinazolin-4-one (**9o**)*

Yield: 35%, M.P.: 267–8 °C, R_f: 0.48, M.F.: $C_{24}H_{16}N_4OSCl_2$, LC-MS (m/e):480 (M + 1), ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] = 2.29 (s, 3H), 7.29–7.9 (m, 11H), 8.12–8.15 (d, 1H, J_{ortho}7.8 Hz), 10.33 (s, 1H), Anal. Calcd. For. $C_{24}H_{16}N_4OSCl_2$: C, 60.13; H, 3.36; N, 11.69, Found: C, 60.26; H, 3.29; N 11.65.

*5.2.16. 3-(4-Acetyl-phenyl)-2-(4-methyl-2-methylamino-thiazol-5-yl)-3H-quinazolin-4-one (**9p**)*

Yield: 30%, M.P.: 245 °C, R_f: 0.46, M.F.: $C_{21}H_{18}N_4O_2S$, LC-MS (m/e): 391 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.38 (s, 6H), 2.56 (s, 3H), 6.98–7.76 (m, 7H), 8.13–8.15 (d, 1H, J_{ortho}8.0 Hz), 10.21 (s, 1H), Anal. Calcd. For. $C_{21}H_{18}N_4O_2S$: C, 64.60; H, 4.65; N, 14.35, Found: C, 64.61; H, 4.67; N, 14.32.

*5.2.17. 3-(4-Acetyl-phenyl)-2-(4-methyl-2-phenylamino-thiazol-5-yl)-3H-quinazolin-4-one (**9q**)*

Yield: 48%, M.P.: 271–3 °C, R_f: 0.51, M.F.: $C_{26}H_{20}N_4O_2S$, LC-MS (m/e): 453.5 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.35 (s, 3H), 2.62 (s, 3H), 7.09–8.03 (m, 12H), 8.31–8.33 (dd, 1H, J_{ortho}8.6 Hz, J_{meta}1.2), 9.87 (s, 1H), Anal. Calcd. For. $C_{26}H_{20}N_4O_2S$: C, 69.01; H, 4.45; N, 12.38, Found: C, 69.9; H, 4.48; N, 12.35.

*5.2.18. 3-(4-Acetyl-phenyl)-2-[2-(4-chloro-phenylamino)-4-methyl-thiazol-5-yl]-3H-quinazolin-4-one (**9r**)*

Yield: 43%, M.P.: 209–10 °C, R_f: 0.52, M.F.: $C_{26}H_{19}N_4O_2SCl$, LC-MS (m/e): 488 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.32 (s, 3H), 2.61 (s, 3H), 7.27–8.0 (m, 11H), 8.13–8.16 (d, 1H, J_{ortho}7.5 Hz), 10.35 (s, 1H), Anal. Calcd. For. $C_{26}H_{19}N_4O_2SCl$: C, 64.13; H, 3.93; N, 11.51, Found: C, 64.11; H, 3.92; N, 11.53.

*5.2.19. 2-[4-Amino-2-(4-chloro-phenylamino)-thiazol-5-yl]-3-(4-chloro-phenyl)-3H-quinazolin-4-one (**9s**)*

Yield: 34%, M.P.: 240–2 °C, R_f: 0.44, M.F.: $C_{23}H_{15}N_5OSCl_2$, LC-MS (m/e): 481 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 7.23–7.76 (m, 9H), 7.88–8.48 (m, 5H), 10.35 (s, 1H). Anal. Calcd. For. $C_{23}H_{15}N_5OSCl_2$: C, 57.51; H, 3.15; N, 14.58, Found: C, 57.57; H, 3.1; N, 14.3.

*5.2.20. 3-(4-Acetyl-phenyl)-2-[4-amino-2-(4-chloro-phenylamino)-thiazol-5-yl]-3H-quinazolin-4-one (**9t**)*

Yield: 32%, M.P.: 261–2 °C, R_f: 0.56, M.F.: $C_{25}H_{18}N_5O_2SCl$, LC-MS (m/e): 488 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.67 (s, 3H), 7.21–7.70 (m, 9H), 7.91–8.44 (m, 5H), 10.32 (s, 1H), Anal. Calcd. For. $C_{25}H_{18}N_5O_2SCl$: C, 61.54; H, 3.72; N, 14.35, Found: C, 61.52; H, 3.69; N, 14.37.

*5.2.21. 2-[2-(4-Chloro-phenylamino)-4-phenyl-thiazol-5-yl]-3-phenyl-3H-quinazolin-4-one (**9u**)*

Yield: 71%, M.P.: >275 °C, R_f: 0.68, M.F.: $C_{29}H_{19}N_4OSCl$, LC-MS (m/e): 507 (M + 1), ^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 6.59–7.84 (m, 17H), 8.33–8.34 (dd, 1H, J_{ortho}8.5 Hz, J_{meta}1.1 Hz), 9.38 (s, 1H), Anal. Calcd. For. $C_{29}H_{19}N_4OSCl$: C, 68.70; H, 3.78; N, 11.05, Found: C, 68.65; H, 3.68; N, 11.14.

*5.2.22. 2-[2-(4-Chloro-phenylamino)-4-phenyl-thiazol-5-yl]-3-*o*-tolyl-3H-quinazolin-4-one (**9v**)*

Yield: 57%, M.P.: >275 °C, R_f: 0.70, M.F.: $C_{30}H_{21}N_4OSCl$, LC-MS (m/e): 521 (M + 1), ^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 2.1 (s, 3H), 6.92–7.87 (m, 16H), 8.30–8.32 (dd, 1H, J_{ortho}7.8 Hz, J_{meta}1.12 Hz), 9.65 (s, 1H), Anal. Calcd. For. $C_{30}H_{21}N_4OSCl$: C, 69.16; H, 4.06; N, 10.75, Found: C, 69.11; H, 3.98; N, 10.69.

*5.2.23. 3-(2-Chloro-phenyl)-2-[2-(4-chloro-phenylamino)-4-phenyl-thiazol-5-yl]-3H-quinazolin-4-one (**9w**)*

Yield: 51%, M.P.: 242–3 °C, R_f: 0.77, M.F.: $C_{29}H_{18}N_4OSCl_2$, LC-MS (m/e): 542 (M + 1), ^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 7.41–8.21

(m, 16H), 8.22–8.23 (m, 1H), 10.62 (s, 1H), Anal. Calcd. For. $C_{29}H_{18}N_4OSCl_2$: C, 64.33; H, 3.35; N, 10.35, Found: C, 64.29; H, 3.38; N, 10.26.

*5.2.24. 3-(4-Chloro-phenyl)-2-(4-phenyl-2-*p*-tolylamino-thiazol-5-yl)-3*H*-quinazolin-4-one (**9x**)*

Yield: 27%, M.P.: 225–7 °C, R_f: 0.61, M.F.: $C_{30}H_{21}N_4OSCl$, LC-MS (m/e): 521 (M + 1), ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] = 2.3 (s, 3H), 6.46–6.48 (dd, 2H, J_{ortho}7.2 Hz, J_{meta}2.04 Hz), 7.0–7.29 (m, 11H), 7.51–7.55 (m, 1H), 7.77–7.82 (m, 2H), 8.28–8.30 (dd, 1H, J_{ortho}8.9 Hz, J_{meta}1.0 Hz), 8.62 (s, 1H), Anal. Calcd. For. $C_{30}H_{21}N_4OSCl$: C, 69.16; H, 4.06; N, 10.75, Found: C, 69.21; H, 3.99; N, 10.78.

*5.2.25. 3-(4-Acetyl-phenyl)-2-(2-methylamino-4-phenyl-thiazol-5-yl)-3H-quinazolin-4-one (**9y**)*

Yield: 44%, M.P.: 239–40 °C, R_f: 0.53, M.F.: $C_{26}H_{20}N_4O_2S$, LC-MS (m/e): 453 (M + 1), ^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm] = 2.55 (s, 3H), 2.83 (s, 3H), 6.64–7.85 (m, 12H), 8.28–8.31 (dd, 1H, J_{ortho}8.8 Hz, J_{meta}1.0), 9.38 (s, 1H), Anal. Calcd. For. $C_{26}H_{20}N_4O_2S$: C, 69.01; H, 4.45; N, 12.38, Found: C, 68.92; H, 4.41; N, 12.29.

*5.2.26. 3-(4-Acetyl-phenyl)-2-[2-(4-chloro-phenylamino)-4-phenyl-thiazol-5-yl]-3H-quinazolin-4-one (**9z**)*

Yield: 44 %, M.P.: 218–20 °C, R_f: 0.49, M.F.: $C_{31}H_{21}N_4O_2SCl$, LC-MS (m/e): 549 (M + 1), ^{1}H NMR (300 MHz, DMSO-d_6) δ [ppm] = 2.64 (s, 3H), 6.93–7.64 (m, 16H), 8.2–8.3 (d, 1H, J_{ortho}7.4 Hz), 10.54 (1H), Anal. Calcd. For. $C_{31}H_{21}N_4O_2SCl$: C, 67.81; H, 3.86; N, 10.20; Found: C, 67.88; H, 3.82; N, 10.15.

5.3. Biology

5.3.1. In vivo acute model of inflammation

The carrageenan induced rat paw edema test was used for the determination of in vivo anti-inflammatory activity [24]. Sprague-Dawley rats (bred in the animal colony at the B. V. Patel PERD, Centre, Ahmedabad) of either sex, in the weight range of 150–250 g were used for the study. Animals were fasted for 18 h prior to the experiment. Each group designating a single compound was tested against a control group receiving only the vehicle treatment ($n = 4$/group). All compounds were administered orally (50 mg/kg body weight) in 0.2% agar suspension prepared freshly prior to use. One hour post dosing, 0.1 ml of 1% carrageenan solution in normal saline (ripened for 7 days) was injected in the subplantar region of the right hind paw of each rat. After 3 h of the carrageenan injection, the reduction in the paw volume compared to vehicle control was measured plethysmometrically. All experimental protocols were reviewed and accepted by the Institutional Animal House Ethics Committee (IAEC) prior to the initiation of the experiment.

All the final compounds were tested simultaneously with the vehicle control to evaluate their anti-inflammatory activities. The percentage protection at 50 mg/kg dose was calculated according to the following formula, % Protection = [(Control−Test)/Control] × 100.

5.3.2. In vitro cell line based assays for NF-κB and AP-1

The human embryonic kidney cell line, (HEK293), from Panomics, Inc, Freemont, CA, was used for the in vitro cell line based screening for NF-κB and AP-1 activity. These cells stably expressed either plasmids containing a minimal promoter and tandem copies of the NF-κB transcriptional element (5'-AGTTGAGGGGACTTTCC-CAGGC-3') or the AP-1 transcriptional element (TGACTAA) regulating luciferase expression. Cells were plated at 5×10^4 cells/well in 96 well plates at 37 °C in 5% CO_2 for 24 h. Test compounds were dissolved in 0.1% DMSO, added at desired concentrations (10 μM to 1 nM) to replicate wells, and incubated at 37 °C in 5% CO_2 for an additional 24 h. For induction of transcription and to test the biological activity of the promoters, the HEK/NF-κB cell line was stimulated with TNF-α (1 ng/μl) and the HEK/AP-1 cell line was stimulated with PMA (1 ng/μl) and the cells were then incubated again at 37 °C in 5% CO_2 for 20–24 h. After incubation, the cell lysis buffer containing luciferase substrate (Bright-Glo Luciferase Assay System, Promega) was added to each well. Luminescence was immediately measured using Rosys Anthos Lucy II or Victor III (Perkin Elmer) luminometer. The luminescence of each test compound was reported as relative light units (RLU) by taking the mean of the replicate wells and normalizing them to the maximal control values. In separate assays, viability of the cells incubated with similar concentrations of compound was analyzed to confirm that changes in RLU that were not due to cytotoxicity (data not shown).

Acknowledgement

The authors are thankful to the Industrial Commissionerate of Gujarat for providing grant for carrying out this work.

References

[1] S.L. Hickenbottorn, J. Grotta, Semin. Neurol. 18 (1998) 485–492.
[2] C. Kevin, R. Nikolaus, Nat. Rev. Genet. 8 (2007) 93–103.
[3] M. Karin, Nature 441 (2006) 431–436.
[4] R. Piva, G. Belardo, M. Santoro, Antioxid. Redox Signal 8 (2006) 478–486.
[5] R. Eferl, E. Wagner, Nat. Rev. Cancer 3 (2003) 859–868.
[6] M. Roth, J.L. Black, Curr. Drug Targets 7 (2006) 589–595.
[7] C.C. Gallen, J. Ame. Soc. Exp. Neuro. Ther. 1 (2004) 165–180.
[8] M.L. Handel, Inflamm. Res. 46 (1997) 282–286.
[9] A.M. Manning, A.J. Lewis, Rheum. Arthritis 1 (1997) 65–73.
[10] M.S. Palanki, Curr. Med. Chem. 9 (2002) 219–227.
[11] I. Tegeder, E. Niederberger, E. Israr, H. Guhring, K. Bruce, C. Euchenhofer, S. Grosch, G. Geisslinger, FASEB J. 15 (2001) 1–4.
[12] M.S. Palanki, P.E. Erdmann, M. Ren, Bioorg. Med. Chem. Lett. 13 (2003) 4077–4080.
[13] M.S. Palanki, P.E. Erdmann, M. Manning, Bioorg. Med. Chem. Lett. 10 (2000) 1645–1648.
[14] M.S. Palanki, L.M. Gayo, G.I. Shevlin, Bioorg. Med. Chem. Lett. 12 (2002) 2573–2577.
[15] K.H. Bleicher, H.J. Bohm, K. Muller, Nat. Rev. Drug Discov. 2 (2003) 369–378.
[16] M.L. Lidia, J.B. Eliezer, Curr. Med. Chem. 12 (2005) 23–49.
[17] Y. Okamota, A. Takada, T. Ueda, Chem. Pharm. Bull. 19 (1971) 764–769.
[18] R.P. Hullin, J. Miller, W.F. Short, J. Chem. Soc. (1947) 394–396.
[19] D.P. Acharya, S. Chattopadhyay, Ind. J. Het. Chem. 7 (1997) 101–104.
[20] P.S.N. Reddy, C.H. Nagaraju, Syn. Common 21 (1991) 173–181.
[21] M. Kidwai, S. Rastogi, R. Mohan, Ruby, Croat. Chem. Acta 76 (2003) 365–369.
[22] S. Rajappa, V. Sudarsanam, V.G. Yadav, B.V. Gaikwad, Proc. Indian Acad. Sci. (Chem. Sci.) 91 (1982) 451–456.
[23] P.X. Franklin, A.D. Pillai, P.D. Rathod, S. Yerande, M. Nivsarkar, H. Padh, K. Vasu, V. Sudarsanam, 1 (2008) 129–134.
[24] C.A. Winter, E.A. Risley, G.W. Nuss, Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547.