Chemistry of Heterocyclic Compounds, Vol. 42, No. 8, 2006

SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF 2,3-DIARYL-4(3H)-QUINAZOLINONES

M. R. Yadav, S. T. Shirude, A. Parmar, R. Balaraman, and R. Giridhar

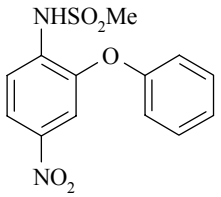
2,3-Diaryl-4(3H)-quinazolinones containing various substituents on diaryl rings have been synthesized and evaluated for their cyclooxygenase-2 inhibitory activity by the colorimetric COX (ovine) inhibitor screening assay and anti-inflammatory activity by the carrageenan-induced rat paw edema assay. 2-(4-Nitrophenyl)-3-(4-tolyl)-4(3H)-quinazolinone showed a maximum COX-2 inhibition of 27.72% at 22 μM concentration in the present series and exhibited a mild anti-inflammatory activity at a dose of 50 mg/kg in carrageenan-induced rat paw edema assay.

Keywords: 4(3H)-quinazolinones, COX-2 inhibitors.

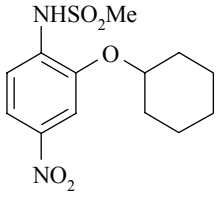
One of the aims of medicinal chemists is to develop drugs that would hit single targets. Selective drug action is intended to minimize undesired side effects. By optimizing binding to a selected target protein, safe and efficacious drug molecules could be developed for the treatment of a disease. The discovery of isoforms of cyclooxygenase enzyme as COX-1 and COX-2 showed that the side effects of NSAIDs were due to their unwanted inhibition of COX-1 along with the desired blockade of COX-2 enzyme. This discovery led to the development of selective COX-2 inhibitors, which are almost free of gastric side effects and possess potent anti-inflammatory activity [1]. The COX-2 inhibitors have been successful in treating such inflammatory diseases as acute pain, rheumatoid arthritis, and osteoarthritis; a few of them are also being studied for treating different types of cancers and Alzheimer's disease [2]. Despite a few latest cautionary reports, COXIB treatment has a high degree of benefit over risk, and the strategies for the use of NSAIDs have been described [3].

Unlike classic NSAIDs, which have a diverse class of chemical structures, the selective COX-2 inhibitors can be classified into two classes as (i) acidic methane sulfonamides containing diphenyl ethers, e.g., nimesulide **1** [4] and NS-398 **2** [5], and (ii) vicinal diaryl heterocycles with sulfamoyl or methylsulfonyl substitution, e.g., etoricoxib [6], celecoxib [7], refocoxib [8], and valdecoxib [9]. A central carbo/heterocyclic ring system bearing two vicinal aryl moieties is a prerequisite for selective COX-2 inhibitors. A wide variety of heterocycles can serve as a template for COX-2 inhibitors, i.e., pyrazole (celecoxib), isoxazole (valdecoxib, paracoxib sodium), furan (rofecoxib), oxazole (JTE-522), thiophene (DuP-697), pyridine **3** (etoricoxib), pyrazine **4** [10], and quinoxaline **5** [10].

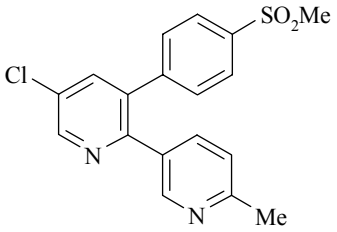
Pharmacy Department, Faculty of Technology and Engineering, Kalabhavan, The M. S. University of Baroda, Vadodara-390 001, India; e-mail: mryadav11@yahoo.co.in. Published in Khimiya Geterotsiklicheskikh Soedinenii, No. 8, pp. 1198-1205, August, 2006. Original article submitted May 17, 2006.



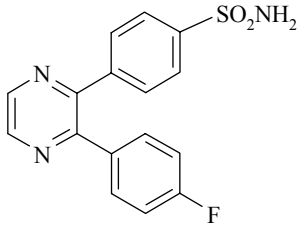
Nimesulide **1**



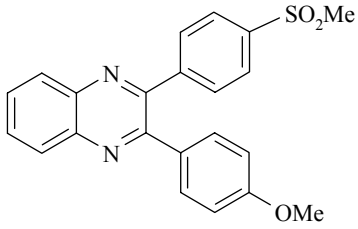
NS-398 **2**



Etoricoxib **3**



Pyrazine derivative **4**

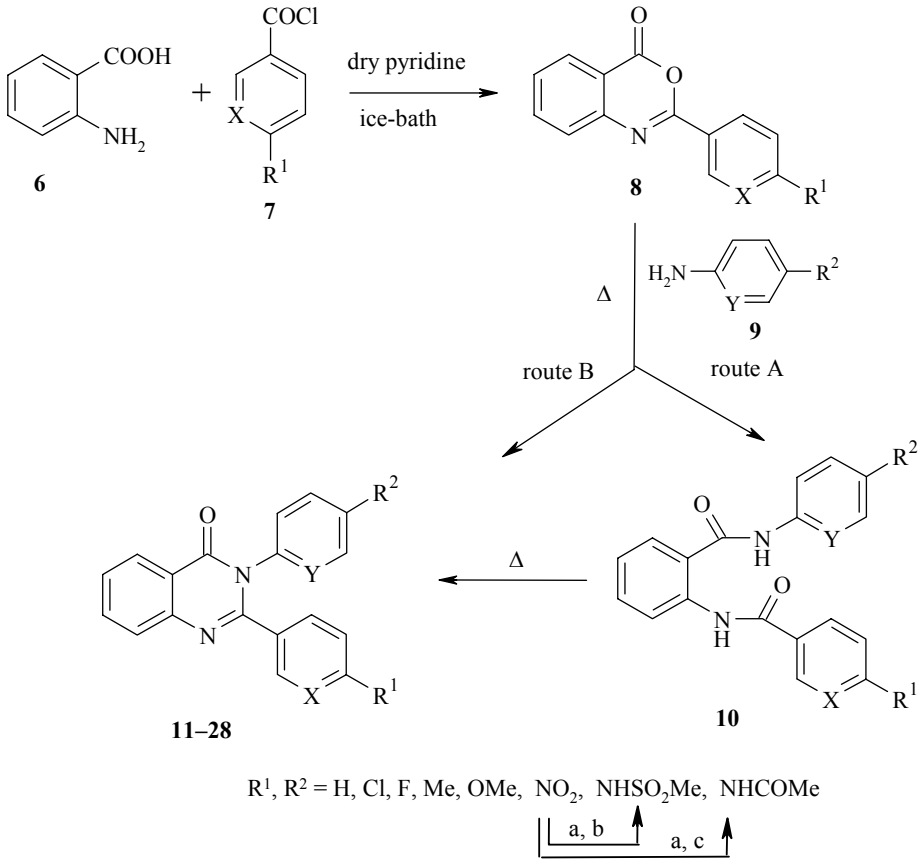


Quinoxaline derivative **5**

In vicinal diaryl heterocyclic COX-2 inhibitors, along with five-membered central heterocyclic rings, six-membered heterocyclic rings have been used as a template. But there is only one report [10] on the use of fused-ring system like quinoxaline as a central template for COX-2 inhibitors. It was planned in this work to use 4(3H)-quinazolinone as a central fused heterocyclic ring in place of quinoxaline for the synthesis of potential COX-2 inhibitors.

Aryl acid chlorides **7**, the required starting materials for the synthesis of benzoxazin-4(3H)-ones, were obtained by treating the corresponding aryl acids with thionyl chloride, while benzoyl chloride was commercially available. Anthranilic acid **6** reacted with these acid chlorides under basic conditions to afford the substituted 2-aryl-3,1-benzoxazin-4(3H)-ones **8** following the reported [11] procedure (Scheme). Formation of 3,1-benzoxazin-4(3H)-ones was confirmed by matching the melting points with the reported values. The IR spectra of compounds **8** showed the presence of characteristic stretching vibrations at about 1770 cm^{-1} for the carbonyl of lactone.

Substituted diamides **10**, important intermediates in the preparation of 4-quinazolinones, were prepared by heating substituted 2-aryl-3,1-benzoxazin-4(3H)-ones **8** with aryl amines **9** following the reported procedure [11]**.** When the reaction mixture of compounds **8** and amines **9** did not melt at 100°C, the temperature was raised to 200°C. Interestingly, it was observed that heating the mixture of 2-phenyl-3,1-benzoxazin-4(3H)-one **8** and 2-aminopyridine **9** yielded diamide **10** under milder conditions (at lower temperatures, 100°C), while at higher temperatures (200°C) the cyclized product 4(3H)-quinazolinone **24** was isolated (Route B). This observation is restricted to compounds having the pyridyl ring either in the 3,1-benzoxazin-4(3H)-one **8** component or in amine **9** component. With the rest of the substituents (H, Me, Cl, F, OMe and NO_2) only amides **10** were obtained even at higher temperatures. For their cyclization into the final quinazolinones, a catalytic amount of anhydrous zinc chloride (Route A) was used. Compounds **23** and **24** were synthesized by both routes, i.e., with the isolation of diamides **10** and directly in the fused form, while compound **25** was synthesized without the isolation of diamide **10**. The IR spectra of compounds **10** demonstrated the presence of characteristic NH stretching vibrations and amide peaks at about 3300 and 1655 cm^{-1}, respectively. The IR spectra of compounds **11**-**25** showed the presence of characteristic carbonyl stretching vibrations at about 1680 cm^{-1}.



Reagents and conditions: a) iron powder and NaCl in aq. MeOH, reflux 7 h on water bath; b) mesyl chloride, pyridine 4 h; c) acetic anhydride, pyridine 12 h.

11 $R^1 = R^2 = H$, **12** $R^1 = Cl$, $R^2 = H$, **13** $R^1 = NO_2$, $R^2 = H$, **14** $R^1 = H$, $R^2 = NO_2$, **15** $R^1 = Cl$, $R^2 = NO_2$, **16** $R^1 = H$, $R^2 = Me$, **17** $R^1 = Cl$, $R^2 = Me$, **18** $R^1 = NO_2$, $R^2 = Me$, **19** $R^1 = H$, $R^2 = OMe$, **20** $R^1 = Cl$, $R^2 = OMe$, **21** $R^1 = F$, $R^2 = OMe$, **22** $R^1 = H$, $R^2 = F$, **23**, **24** $R^1 = R^2 = H$, **25** $R^1 = Cl$, $R^2 = H$, **26** $R^1 = NHSO_2Me$, $R^2 = H$, **27** $R^1 = H$, $R^2 = NHSO_2Me$, **28** $R^1 = NHCOMe$, $R^2 = H$; **11-22**, **24-28** X = C, **23** X = N; **11-23**, **26-28** Y = C, **24**, **25** Y = N

Nitroquinazolinones **13** and **14** were reduced to the corresponding amine derivatives by iron powder and sodium chloride in boiling aqueous methanol. These amino derivatives were treated with mesyl chloride under basic conditions to afford methanesulfonamide compounds **26** and **27**. Similarly, the amino derivative obtained from compound **13** was also treated with acetic anhydride to yield amide derivative **28**. The physical data for substituted 2,3-diaryl-4(3H)-quinazolinones **11-28** are given in Table 1 and the spectral data of compounds **11-28** are listed in Table 2.

All the synthesized compounds were evaluated for their cyclooxygenase-2 inhibitory activity by the colorimetric COX (ovine) inhibitor screening assay [12] and anti-inflammatory activity by the carrageenan-induced rat paw edema assay [13] (Table 3). Compounds **26** and **27** with a well-known COX-2 pharmacophore were found to be inactive at 22 μM concentration. Compound **18** with an electron-withdrawing group (NO_2) was found to be the most potent compound in the series with an inhibition of 27.72% at 22 μM concentration; in comparison, valdecoxib, a well-known clinically used COX-2 inhibitor, showed 87.2% inhibition at this concentration. Compound **18** exhibited a mild anti-inflammatory activity at a dose of 50 mg/kg in the carrageenan-induced rat paw edema assay. This study showed that 4(3H)-quinazolinone may not be a good bioisostere of the five/six-membered central ring present in the clinically used COX-2 inhibitors.

TABLE 1. The Spectral and Analytical Data for Substituted 2,3-Diaryl-4(3H)-quinazolinones **36-53**

Compound	Molecular formula	Found, % / Calculated, %			mp, °C (Lit. [11] mp)	*Rf**	Yield % (Route)
		C	H	N			
11	$C_{20}H_{14}N_2O$	—	—	—	158-159 (156-157)	0.70	22 (A)
12	$C_{20}H_{13}ClN_2O$	—	—	—	177-178 (177)	0.83	63 (A)
13	$C_{20}H_{13}N_3O_3$	—	—	—	224-225 (224-225)	0.64	38 (A)
14	$C_{20}H_{13}N_3O_3$	69.66 / 69.97	3.69 / 3.82	12.42 / 12.24	204-205	0.74	32 (A)
15	$C_{20}H_{12}ClN_3O_3$	63.24 / 63.59	2.96 / 3.20	11.34 / 11.12	224-225	0.81	29 (A)
16	$C_{21}H_{16}N_2O$	81.02 / 80.75	4.89 / 5.16	8.67 / 8.97	182-183	0.68	46 (A)
17	$C_{21}H_{15}ClN_2O$	72.58 / 72.73	4.02 / 4.36	7.79 / 8.08	190-192	0.62	57 (A)
18	$C_{21}H_{15}N_3O_3$	71.02 / 70.58	3.94 / 4.23	11.87 / 11.76	218-219	0.60	31 (A)
19	$C_{21}H_{16}N_2O_2$	77.09 / 76.81	4.65 / 4.91	8.36 / 8.53	—	0.80	10 (A)
20	$C_{21}H_{15}ClN_2O_2$	69.72 / 69.52	4.42 / 4.17	7.49 / 7.72	168-170	0.84	31 (A)
21	$C_{21}H_{15}FN_2O_2$	72.64 / 72.82	3.98 / 4.37	8.25 / 8.09	167-169	0.60	42 (A)
22	$C_{20}H_{13}FN_2O$	76.51 / 76.82	4.12 / 3.99	8.22 / 8.53	148-150	0.65	30 (A)
23	$C_{19}H_{13}N_3O$	—	—	—	175-177 (175-177)	0.62	47 (A) 27 (B)
24	$C_{19}H_{13}N_3O$	75.86 / 76.24	4.12 / 4.38	13.89 / 14.04	188-189	0.67	64 (A) 56 (B)
25	$C_{19}H_{12}ClN_3O$	68.63 / 68.37	3.92 / 3.62	12.26 / 12.59	182-183	0.69	32 (B)
26	$C_{21}H_{17}N_3O_3S$	64.76 / 64.44	4.59 / 4.38	10.53 / 10.73	144-145	0.23	48
27	$C_{21}H_{17}N_3O_3S$	64.71 / 64.44	4.62 / 4.38	10.96 / 10.73	>280	0.29	31
28	$C_{22}H_{17}N_3O_2$	74.25 / 74.35	3.95 / 4.82	11.92 / 11.82	246-247	0.60	48

* 5% methanol in chloroform.

TABLE 2. Spectral Data for Substituted 2,3-Diaryl-4(3H)-quinazolinones **11-28**

Compound	IR spectrum, ν, cm^{-1}	UV spectrum (MeOH), λ_{max}, nm (log ε)	^1H NMR spectrum, δ, ppm
1	2	3	4
11	1680 (C=O)	229 (4.55)	—
12	1680 (C=O)	279.5 (4.04)	—
13	1682 (C=O), 1519 (NO_2 asym.), 1350 (NO_2 sym.)	225 (4.53)	—
14	1694 (C=O), 1519 (NO_2 asym.), 1350 (NO_2 sym.)	278.5 (4.55)	7.22-8.36 (13H, m, ArH)

TABLE 2 (continued)

1	2	3	4
15	1679 (C=O), 1521 (NO_2 asym.), 1334 (NO_2 sym.)	278.5 (4.58)	7.23-8.35 (13H, m, ArH)
16	1678 (C=O)	225 (4.44)	2.30 (3H, s, CH_3); 7.00- 8.36 (13H, m, ArH)
17	1678 (C=O)	225 (4.49)	2.33 (3H, s, CH_3); 7.00- 8.35 (12H, m, ArH)
18	1683 (C=O), 1521 (NO_2 asym.), 1347 (NO_2 sym.)	293 (4.39)	2.34 (3H, s, CH_3); 6.99- 8.35 (12H, m, ArH)
19	1682 (C=O), 1250 (Ar–O), 1025 (O–Me)	—	3.81 (3H, s, OCH_3); 6.85-8.35 (13H, m, ArH)
20	1685 (C=O), 1248 (Ar–O), 1024 (O–Me)	230.5 (4.53)	3.79 (3H, s, OCH_3); 6.87-8.33 (12H, m, ArH)
21	1679 (C=O), 1250 (Ar–O), 1022 (O–Me)	230.5 (4.65)	3.78 (3H, s, OCH_3); 6.82-8.35 (12H, m, ArH)
22	1675 (C=O)	228.5 (4.58)	6.96-8.33 (13H, m, ArH)
23	1676 (C=O)	280 (4.31)	—
24	1682 (C=O)	229 (4.44)	7.16-8.46 (13H, m, ArH)
25	1683 (C=O)	228.5 (4.75)	7.12-8.45 (12H, m, ArH)
26	3267 (NH, b), 1686 (C=O), 1331 (SO_2 asym.), 1146 (SO_2 sym.)	282.5 (4.47)	2.91 (3H, s, CH_3); 6.85 (1H, br.s, NH); 6.98-8.34 (13H, m, ArH)
27	3269 (NH, b), 1684 (C=O), 1332 (SO_2 asym.), 1150 (SO_2 sym.)	—	2.96 (3H, s, CH_3); 6.48 (1H, br.s, NH); 7.14-8.36 (13H, m, ArH)
28	3385 (NH), 1685 (C=O)	279.5 (4.34)	2.10 (3H, s, CH_3); 7.13-8.34 (14H, m, ArH, NH)

TABLE 3. *In vitro* COX-2 Inhibition and *in vivo* Anti-inflammatory Data for Substituted 2,3-Diaryl-4(3H)-quinazolinones **11-28**

Compound	COX-2 % inhibition*	Anti-inflammatory activity	
		Dose, mg/kg	% Edema inhibition*[2]
1	2	3	4
11	IA	50	5.2
12	IA	50	4.1
13	8.6	50	10.1
14	5.3	50	8.7
15	IA	50	3.37
16	IA	50	4.2
17	IA	50	6.2
18	27.72	50	35.7
19	IA	50	0.0
20	IA	50	5.9

TABLE 3 (continued)

1	2	3	4
21	IA	50	8.9
22	8.82	50	18.5
23	IA	50	7.6
24	IA	50	6.9
25	12	50	22.8
26	IA	50	8.2
27	8.05	50	16.2
28	IA	50	9.36
Valdecoxib	87.2	25	70

* Compounds were screened for inhibitory activity on COX-2 at a concentration of 22 μM. IA: inactive at a concentration of 22 μM.
*[2] Carrageenan-induced rat paw edema assay.

EXPERIMENTAL

The yields reported here are unoptimized. Melting points were determined using a heating block-type melting point apparatus and are uncorrected. Purity of the compounds and reactions were monitored by thin-layer chromatography (TLC) on silica gel plates (60 F_{254}, Merck), visualizing with ultraviolet light or iodine vapors. The UV spectra were recorded on a Shimadzu UV-1601 spectrophotometer, and the values in parentheses indicate the log of molar extinction coefficients. The IR spectra were recorded using the KBr disc method on a Shimadzu FT-IR model 8300. The ^{1}H NMR spectra were recorded on a Brucker 300 MHz spectrometer in $CDCl_3$. The final compounds were purified by passing through a silica gel H (100-200 mesh) purifying column, using a mixture of ethyl acetate and petroleum ether or chloroform alone as eluents before submitting for elemental analysis.

Syntheses of Substituted 2-Aryl-3,1-benzoxazin-4(3H)-ones 8. Representative preparation of 2-(4-fluorophenyl)-3,1-benzoxazin-4(3H)-one. A mixture of 4-fluorobenzoic acid (5 g, 35.69 mmol) and thionyl chloride (5 ml) was refluxed in a round bottom flask (50 ml) for 3 h under anhydrous conditions. Excess of thionyl chloride was removed under vacuum. A solution of anthranilic acid **6** (5.0 g, 36.50 mmol) in dry pyridine (15 ml) was added dropwise to a stirred solution of the above acid chloride in dry pyridine (15 ml) at a temperature below 10°C over a period of 15 min under anhydrous conditions. After complete addition the reaction mixture was stirred for 4 h at room temperature. The reaction mixture was poured into crushed ice (500 g). The precipitated solid was filtered, washed with aqueous sodium bicarbonate (5%) followed by water, and dried. The crude product was crystallized from acetone to yield the title compound (6.00 g, 68%); mp 174-176°C. R_f 0.65 (chloroform). IR spectrum, ν, cm^{-1}: 1762 (carbonyl of lactone), 1625, 1591, 1507, 1472, and 772. UV spectrum (MeOH), λ_{max}, nm (log ε): 271 (4.12).

Syntheses of Substituted Diamides 10. 2-(4-Fluorobenzamido)-N-(4-methoxyphenyl)benzamide. A mixture of 2-(4-fluorophenyl)-3,1-benzoxazin-4(3H)-one (1.0 g, 4.15 mmol) and 4-anisidine (2.0 g, 16.26 mmol) was heated on a water bath for 4 h. The reaction mixture was suspended in methanol, and the solid was filtered, washed repeatedly with methanol, and dried to yield the title compound: 1.10 g, 73%. IR spectrum, ν, cm^{-1}: 3294 (NH stretching), 1656 (amide peak), 1236, 1180, 1033, 825, and 750.

Syntheses of Substituted 2,3-Diaryl-4(3H)-quinazolinones 11-24. A. Representative preparation of 2-(4-fluorophenyl)-3-(4-methoxyphenyl)-4(3H)-quinazolinone **21**. 2-(4-Fluorobenzamido)-N-(4-methoxyphenyl)-benzamide (0.5 g, 1.37 mmol) was fused in the presence of anhydrous zinc chloride (0.1 g) on a diethylene

glycol (digol) bath at its boiling point for 10 min. The reaction mixture was suspended in water, and the resulting solid was filtered and dried. The product so obtained was passed through a purifying column of silica gel (100-200 mesh) using chloroform as a mobile phase. The product was crystallized from methanol to give compound **21**. The physical and analytical data are given in Tables 1 and 2.

2-(4-Chlorophenyl)-3-(2-pyridyl)-4(3H)-quinazolinone 25. B. A mixture of 2-(4-chlorophenyl)-3,1-benzoxazin-4(3H)-one (0.6 g, 2.33 mmol) and 2-aminopyridine (0.25 g, 2.66 mmol) was fused on digol bath for half an hour. The crude product obtained from the reaction mixture was crystallized from methanol to afford compound **25**.

2-(4-Methanesulfonamidophenyl)-3-phenyl-4(3H)-quinazolinone 26. Sodium chloride (1.0 g) and iron powder (1.0 g) were added in parts to a refluxing solution of 2-(4-nitrophenyl)-3-phenyl-4(3H)-quinazolinone **13** (0.5 g, 1.46 mmol) in aqueous methanol (200 ml, 95%). Refluxing was continued further for 7 h. The reaction mixture was filtered through a filtering aid (high flow supercel), and the filtrate was concentrated *in vacuo* to remove methanol. The resulting aqueous solution was neutralized by adding sodium bicarbonate and extracted with chloroform (3 × 25 ml). The combined organic extract was dried and the solvent removed to obtain a sticky residue. This residue was dissolved in dry pyridine (2 ml) and cooled on an ice bath, and mesyl chloride (0.4 ml) was added dropwise with stirring. The stirring was continued for 4 h at room temperature. The reaction mixture was poured into crushed ice (50 g) containing concentrated hydrochloric acid (5 ml). The solid so obtained was filtered, dried, and crystallized from methanol to yield compound **26**.

Compound 27 was prepared similarly starting with compound **14,** and compound **28** was prepared by replacing mesyl chloride with acetic anhydride.

Assay of *in vitro* COX-2 Inhibition [12]. The final compounds were evaluated for their ability to inhibit ovine COX-2 enzyme (percent inhibition at 22 μM). Inhibition of the enzyme was determined using a colorimetric COX (ovine) inhibitor screening assay kit (Catalog No. 760111, Cayman Chemicals, Ann Arbor, MI, USA) following the procedure described in the catalog.

Assay of *in vivo* Carrageenan-induced Rat Paw Edema. Anti-inflammatory activity was determined using the carrageenan-induced rat paw edema method [13] applying a plethysmometer (UGO-Basil, Italy).

The authors are grateful to the All India Council for Technical Education (AICTE) for the award of a National Doctoral Fellowship (NDF) to S.T.S.

REFERENCES

1. R. J. Flower, *Nature Rev. Drug Discovery*, **2**, 179 (2003).
2. T. D. Warner and J. A. Mitchell, *FASEB J.*, **18**, 790 (2004).
3. E. M. Antman, D. DeMets, and J. Loscalzo, *Circulation*, **112**, 759 (2005).
4. G. Cignarella, P. Vianello, F. Berti, and G. Rossoni, *Eur. J. Med. Chem.*, **31**, 359 (1996).
5. R. Davis and R. N. Brogden, *Drugs*, **48**, 431 (1994).
6. D. Riendeau, M. D. Percieval, C. Brideau, S. Charleson, D. Dube, D. Ethier, J. P. Falgueyret, R. W. Friesen, R. Gordon, G. Greig, J. Guay, J. Mancini, M. Ouellet, E. Wong, L. J. Xu, S. Boyce, D. Visco, Y. Girard, P. Prasit, R. Zamboni, I. W. Rodger, M. Gresser, A. W. Ford-Hutchinson, R. N. Young, and C. C. Chan, *J. Pharmacol. Exp. Ther.*, **296**, 558 (2001).
7. T. D. Penning, J. J. Talley, S. R. Bertenshaw, J. S. Carter, P. W. Collins, S. Docter, M. J. Graneto, F. J. Lee, J. W. Malecha, J. M. Miyashiro, R. S. Rogers, D. J. Rogier, S. S. Yu, G. A. Anderson, F. G. Burton, J. N. Cogburn, S. A. Gragory, C. M. Koboldt, W. E. Perkins, K. Seibert, A. W. Veenhuizen, Y. Y. Zhang, and P. C. Isakson, *J. Med. Chem.*, **40**, 1347 (1997).

8. P. Prasit, Z. Wang, C. Brideau, C. C. Chan, S. Charleson, W. Cromlish, D. Either, J. F. Evans, A. W. Ford-Hutchinson, J. Y. Gauthier, R. Gordon, J. Guay, M. Gresser, S. Kargman, B. Kennedy, Y. Leblanc, S. Leger, J. Mancini, G. P. O'Neil, M. Ouellet, M. D. Percieval, H. Perrier, D. Riendeau, I. Rodger, P. Tagari, M. Therien, P. Vickers, E. Wong, L. J. Xu, R. N. Young, R. Zamboni, S. Boyce, N. Rupniak, M. Forrest, D. Visco, and D. Patrick, *Bioorg. Med. Chem. Lett*., **9**, 1773 (1999).
9. J. J. Tally, D. L. Brown, J. S. Carter, M. J. Graneto, C. M. Koboldt, J. L. Masferrer, W. E. Perkins, R. S. Rogers, A. F. Shaffer, Y. Y. Zhang, B. S. Zweifel, and K. Seibert, *J. Med. Chem*., **43**, 775 (2000).
10. S. K. Singh, V. Saibaba, V. Ravikumar, S. V. Rudrawar, P. Daga, C. S. Rao, V. Akhila, P. Hegde, and Y. K. Rao, *Bioorg. Med. Chem.*, **12**, 1881 (2004).
11. D. T. Zentmyer and E. C. Wagner, *J. Org. Chem.*, **14**, 967 (1949).
12. H. Sano, T. Noguchi, A. Tanatani, Y. Hashimoto, and H. Miyachi, *Bioorg. Med. Chem.*, **13**, 3079 (2005).
13. C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).