Studies on New Phosphodiesterase Inhibitors. I. Synthesis of 1-(2,3-Epoxypropoxy)-2(4)-fluorobenzenes and 1-(2-Hydroxy-3-morpholinopropoxy and Piperazino)fluorobenzene Derivatives

Nigel R. BAKER, Nick G. BYRNE, Apollo P. ECONOMIDES, and Tariq JAVED*

Division of Chemical and Biological Sciences, School of Natural and Environmental Sciences, Coventry University, Priory Street, Coventry, CV1 5FB, England. Received December 26, 1994; accepted March 7, 1995

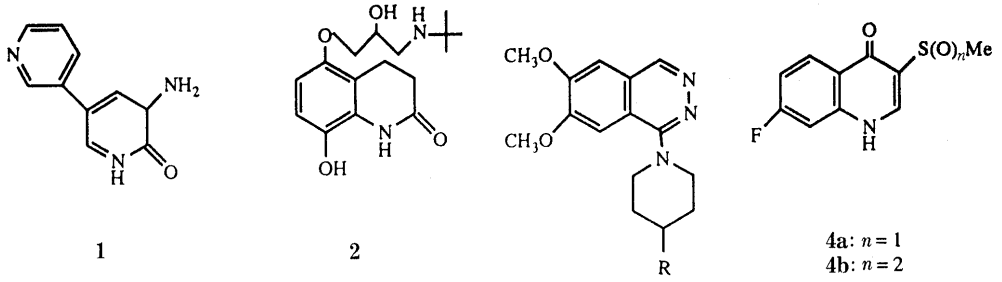
A series of 1-(2,3-epoxypropoxy)-2(4)-fluorobenzenes and their corresponding 1-(2-hydroxy-3-morpholino propoxy and piperazino)fluorobenzene derivatives (8a—f) were synthesised *via* a short synthetic route in good chemical yields and were evaluated for inotropic and chronotropic activity in isolated guinea-pig atria preparation. The compounds act as potential phosphodiesterase (PDE) inhibitors with desirable biological activity and have considerable promise in heart therapy.

Key words congestive heart failure; inotropic agent; phosphodiesterase inhibitor; 1-(4-fluorophenyl-3-morpholinopropoxy and piperazino)fluorobenzene

Congestive heart failure[1] is primarily a defect in the contractile pumping mechanisms of the heart which lead to a reduction in cardiac output. This produces atrial fibrillation and vasoconstriction. There is currently an intensive search for new and improved agents with positive inotropic and negative chronotropic vasodilatory activity. Present day therapy in clinical practice uses vasodilators and diuretics or a combination of these drugs. There is some concern with these inhibitors in that they increase cardiac contractility and potential arrhythmogenic activity. Digitalis, amrinone (**1**) and more recently 5-(3-*tert*-butylamino-2-hydropropoxy)-3,4-dihydro-8-hydroxy-2(1*H*)-quinolinone(8-hydroxycarteolol)[2] (**2**) and flosequinan (**4a**) and flosequinoxan (**4b**)[3] have been reported to act as positive inotropic and mixed arteriolar and venous vasodilator agents in cardiac failure. Carbazeran (**3a**) and buquineran (**3b**), are two particular types of phosphodiesterase inhibitors (PDE)[4] (Chart 1) that have been widely studied. However, almost all act as vasorelaxants having a narrow therapeutic ratio and increase the heart rate.

Although, numerous diverse structural classes of PDE III inhibitors have been reported, quantitative structure–activity relationships (QSAR) suggest many of these inhibitors may be responsible for multiple modes of binding to the PDE active site.[5] Thus, for example, there appears to be no clear distinction between competitive and non-competitive interactions or between high and low affinity forms of the enzyme. The requirement of relatively high dosage regimens of the inhibitors and their side-effect liability indicate that new isozyme-selective cyclic nucleotide PDE inhibitors as cardiotonic agents are still sought.[6] Such types of compounds would in principle need to possess inotropic and vasodilatory activity in a single molecule. Targeting of selective inhibitors to isozyme subtypes may achieve increased tissue selectivity and reduce side-effect liability, although there are no reports of this at present. This is a perennial problem that challenges synthetic medicinal chemists in the search for the dual pharmacological action which will produce an improvement in many of the haemodynamic parameters that are distorted in myocardial infarction.[7]

Thus, the prior art was not in general extremely helpful and therefore, in search for more promising compounds, we undertook a study to synthesise and investigate the biological activity of some selected derivatives of morpholine and piperazine. Due to the importance of PDE III in regulating cyclic adenosine monophosphate (cAMP) metabolism in the myocardium, the role of these novel compounds as selective PDE III inhibitors on the elevation of both intracellular cAMP and guanosine monophosphate (cGMP) was evaluated. Intracellular cAMP levels are generally enhanced by a stimulatory effect[8] of cardiac and vascular β-receptors as well as by agonists (*e.g.* pirbuterol) to provide haemodynamic benefits in heart failure.[9] It has been suggested that PDE inhibi-



1 2

4a: $n = 1$
4b: $n = 2$

3a: R = $OCONHC_2H_5$
3b: R = $NHCONHC_4H_9$

Chart 1

* To whom correspondence should be addressed.

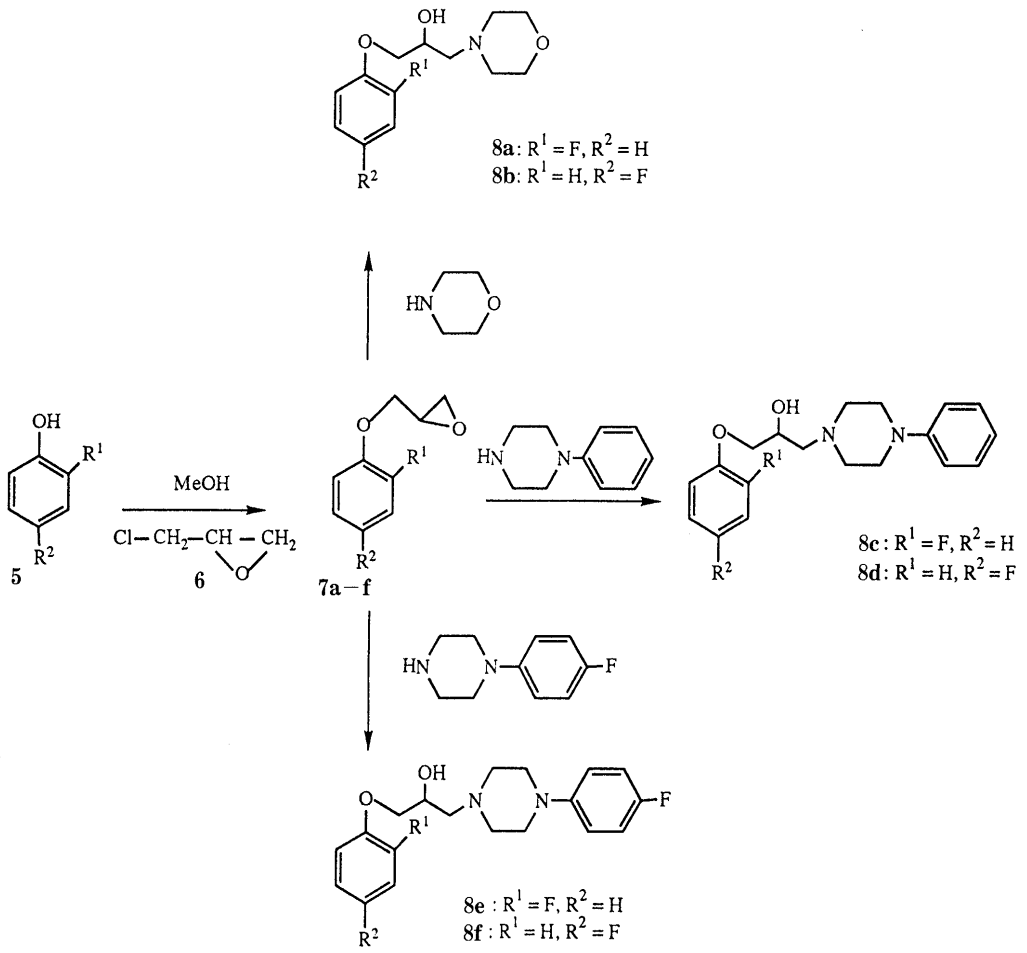


Chart 2

tion[10,11] may contribute to the favourable haemodynamic effects of amrinone.[12]

We now describe herein a short synthesis for the preparation of morpholino and piperazino derivatives and report their biological activity.

Synthesis

Various 1-(2,3-epoxypropoxy)fluorobenzenes (**7a—f**) and 2-hydroxypropoxymorpholino and piperazinofluorobenzenes (**8a—f**) were synthesised from the readily available fluorophenols (**5**) by treatment with epichlorohydrin (**6**) in methanol under reflux in the presence of sodium hydroxide for 6 h. Extraction and purification by vacuum distillation gave 1-(2,3-epoxypropoxy)fluorobenzenes in 65 to 69% yields as pale yellow oils. Ring opening of the oxirane precursors (**7a—f**) with morpholines and phenylpiperazines afforded a series of 1-(2-hydroxypropoxymorpholino and piperazino)fluorobenzenes (**8a—f**) in approximately 50 to 74% yields as white or pale brown solids after recrystallisation from methanol or ethanol as the solvent.

Biological Activity

The results of *in vitro* screening tests are shown in Table 1. The PDE inhibitory effects and chronotropic and inotropic potency of these compounds was initially compared with isoprenaline. The inotropic and chronotropic effect of a drug is commonly expressed by the tension-rate index, and thus a drug which has a tension-rate value of ≥ 3 is usually accepted as a potential cardiotonic agent. In our preliminary screening test, guinea

Table 1. Biological Activities of 1-(2,3-Epoxypropoxy)fluorobenzenes (**7a—f**) and Their 2-Hydroxy-3-morpholinepropoxy and Piperazino Derivatives (**8a—f**) on Isolated Guinea Pig Right Atria Preparation ($n=1$) and Papillary Muscle

Compd. No.	Inotropic effect	Chronotropic effect	IC_{50} cAMP PDE	IC_{50} cGMP PDE
7a	0.8	LE	NA	NA
7b	0.1	0.1	NA	NA
7c	0.3	0.4	NA	NA
7d	0.4	LE	NA	NA
7e	0.7	LE	NA	NA
7f	0.9	LE	NA	NA
8a	4.2	0.5	8.2	27
8b	4.4	LE	7.6	24
8c	4.9	LE	2.9	14.8
8d	5.1	0.6	2.7	10.9
8e	5.7	LE	1.5	7.5
8f	7.7	LE	0.9	8.7

The influence of variation of the fluoro substituents in the test compounds was evaluated with respect to the inotropic and chronotropic potency at doses ($ED_{50\%}$) producing the half-maximal response. Activity ratio of test compound = $ED_{50\%}$ of isoprenaline/dose of the test compound producing the same response as $ED_{50\%}$ of isoprenaline. The larger the activity ratio the more potent is the test compound. LE means lower than 0.1 activity ratio. IC_{50} values (cAMP PDE 5 μM, cGMP PDE 40 μM) *vs.* guinea pig heart[15] PDE. NA means not very active.

pig spontaneous beating right atria and papillary muscles were utilized. The initial outcome of our study has concluded that a variety of phenyl substituted preparations of morpholine and piperazine are suitable derivatives for displaying the desirable activity. Inotropic potency was immediately improved when the morpholine and piperazino ring systems were introduced. In attempting to improve inotropic and PDE inhibitory potency, fluoro analogues were prepared intentionally with the hope that they might interact with the phosphate binding site of the enzyme in a similar fashion to cAMP.

Clearly, there has been an indication of inotropic potency and this has been monitored in the fluoro analogues. N-phenyl piperazinyl compounds **8c** and **8d** were more potent as inotropes than the morpholino analogues **8a** and **8b**. Further increases in inotropic activity is obtained when an additional fluorine atom is introduced into the N-phenyl ring at the *para*-position in the piperazinyl derivatives, compounds **8e** and **8f**. In general cAMP PDE inhibitory activity increased with compounds **8a** to **8f**. Although inotropic potency is obviously important, the selection of compounds for further progression depends also on their overall pharmacologic profile. At a concentration of 0.5 μg/ml the tension-rate index was *ca.* 3.7. Increased concentrations prolonged right atrial rate and caused both an increase in papillary muscle and right atrial developed tension. There appeared to be no signs of hypersensitivity or tachyphylaxis after repeated treatment of the isolated tissues with these compounds.

Interestingly, Fujioka *et al.*[13] reported a study on novel positive inotropic agents *viz*: 6-(3-amino-2-hydroxypropoxy)-2(1*H*)-quinolinone derivatives, in which, they compared the inotropic and chronotropic effects of the 2(1*H*)-quinolinones with those of amrinone. Their studies showed a variable effect in increasing cardiac contractility without increasing heart rate through a mechanism of action that is not mediated through β-adrenoceptors. We find that fluorinated 4-piperazino and morpholino analogues are relatively stable and may therefore in similar fashion to quinazoline, piperidine and pthalazine derivatives act as substrates for the enzyme oxidase system. In developing a rationale enzyme-inhibitor interaction, it has been reported that the cyclic nucleotide may mediate negative inotropic and chronotropic responses[14] and may counteract cAMP mediated events. Although the ureido-function of isoprenaline analogues has been reported to occupy the cyclic phosphate recognition site[15] it may not be the optimal spatial arrangement on this assumption.

In summary, these novel and simple heretocycles are invariably seen to be effective inotropes having little intrinsic propensity to increase heart rate with a potential for use in clinical therapy. In view of the substantial improvements in force and rate which have been selectively realised in our original prototypes, it is possible that such agents may act through one of two types of reported mechanisms.[16]

Experimental

All melting points are uncorrected. Nuclear magnetic resonance (NMR) was recorded on a Bruker 250 MHz instrument in tetramethylsilane and deuterated chloroform as an internal standard. Mass spectra were recorded on a Kratos MS-30 spectrometer. Yields were not optimised.

Preparation of 1-(2,3-Epoxypropoxy)-2-fluorobenzene (7a) A mixture of 2-fluorophenol (11.2 g, 0.1 M), epichlorohydrin (28 g, 0.3 M) and sodium hydroxide (6.0 g, 0.2 M) were refluxed in 50 cm^3 of methanol for 6 h. The resultant mixture was allowed to cool and then concentrated *in vacuo*. Vacuum distillation gave the pure product as a pale yellow liquid (12.0 g, 69%), *Rf* 0.68 (benzene–ethanol, 15 : 1). NMR ($CDCl_3$) δ: 7.15—6.85 (m, 4H, ArF), 4.24—4.2 (d, 1H, OCH_2), 4.18—4.15 (d, 1H, OCH_2), 3.4—3.32 (m, 1H, CHO), 2.8—2.73 (dd, 1H, $CHOCH_2$), 2.87—2.85 (dd, 1H, OCH_2). MS *m/z*: 168, Found (M^+) 168. *Anal.* Calcd for $C_9H_9FO_2$: C, 64.28; H, 5.39. Found: C, 64.24; H, 5.38.

1-(2,3-Epoxypropoxy)-3-fluorobenzene (7b) In the same manner as described for **7a**, crude **7b** was prepared from 3-fluorophenol (11.2 g, 0.1 M). Distillation gave the product as a pale yellow liquid (12.0 g, 69%). NMR ($CDCl_3$) δ: 7.15—6.85 (m, 4H, ArF), 4.24—4.2 (d, 1H, OCH_2), 4.18—4.15 (d, 1H, OCH_2), 3.4—3.32 (m, 1H, CHO), 2.8—2.73 (dd, 1H, $CHOCH_2$), 2.87—2.85 (dd, 1H, OCH_2). MS *m/z*: 168, Found (M^+) 168. *Anal.* Calcd for $C_9H_9FO_2$: C, 64.28; H, 5.39. Found: C, 64.27; H, 5.36.

1-(2,3-Epoxypropoxy)-4-fluorobenzene (7c) In the same manner as described for **7a**, crude **7c** was prepared from 4-fluorophenol (11.2 g, 0.1 M). Distillation gave the product as a pale yellow liquid (11.6 g, 67%). NMR ($CDCl_3$) δ: 7.15—6.85 (m, 4H, ArF), 4.24—4.2 (d, 2H, OCH_2), 3.4—3.32 (m, 1H, CHO), 2.8—2.73 (q, 1H, $CHOCH_2$), 2.87—2.85 (t, 1H, OCH). MS *m/z*: 168, Found (M^+) 168. *Anal.* Calcd for $C_9H_9FO_2$: C, 64.28; H, 5.39. Found: C, 64.25; H, 5.38.

1-(2,3-Epoxypropoxy)-2,4-difluorobenzene (7d) In the same manner as described for **7a**, crude **7d** was prepared from 2,4-difluorophenol (11.4 g, 0.1 M). Distillation gave the product as a pale yellow liquid (11.0 g, 65%). NMR ($CDCl_3$) δ: 7.10—6.89 (m, 4H, ArF), 4.24—4.2 (d, 1H, OCH_2), 3.4—3.32 (m, 1H, CHO), 2.8—2.73 (q, 1H, $CHOCH_2$), 2.87—2.85 (t, 1H, OCH). MS *m/z*: 186, Found (M^+) 186. *Anal.* Calcd for $C_9H_8F_2O_2$: C, 58.07; H, 4.33. Found: C, 58.06; H, 4.31.

1-(2,3-Epoxypropoxy)-3,4-difluorobenzene (7e) In the same manner as described for **7a**, crude **7e** was prepared from 3,4-difluorophenol (11.4 g, 0.1 M). Distillation gave the product as a pale yellow liquid (11.0 g, 65%). NMR ($CDCl_3$) δ: 7.15—6.85 (m, 4H, ArF), 4.24—4.2 (d, 1H, OCH_2), 3.4—3.32 (m, 1H, CHO), 2.8—2.73 (q, 1H, $CHOCH_2$), 2.87—2.85 (t, 1H, OCH). MS *m/z*: 186, Found (M^+) 186. *Anal.* Calcd for $C_9H_8F_2O_2$: C, 58.07; H, 4.33. Found: C, 58.05; H, 4.32.

1-(2,3-Epoxypropoxy)-3-chloro-4-fluorobenzene (7f) In the same manner as described for **7a**, crude **7f** was prepared from 3-chloro-4-fluorophenol (11.4 g, 0.1 M). Distillation gave the product as a pale yellow liquid (10.5 g, 65%). NMR ($CDCl_3$) δ: 7.15—6.85 (m, 4H, ArF), 4.24—4.2 (d, 1H, OCH_2), 3.4—3.32 (m, 1H, CHO), 2.8—2.73 (q, 1H, $CHOCH_2$), 2.87—2.85 (t, 1H, OCH). MS *m/z*: 202.5, Found (M^+) 202. *Anal.* Calcd for $C_9H_8ClFO_2$: C, 53.35; H, 3.98. Found: C, 53.32; H, 3.97.

1-(2-Hydroxy-3-morpholinopropoxy)-2-fluorobenzene (8a) A mixture of **7a** (3.0 g, 19.0 mmol) and morpholine (3.2 g, 37 mmol) in methanol (15 cm^3) was refluxed for 4 h and then allowed to cool and concentrated *in vacuo* to give a white solid. Recrystallisation from methanol gave the title compound (3.55 g, 50%), mp 98—100 °C. NMR ($CDCl_3$) δ: 7.13—6.86 (m, 4H, ArF), 4.2—4.1 [m, 1H, CH(OH)], 4.1—4.05 (d, 2H, OCH_2), 3.8—3.7 (d, 2H, CN_2N), 3.5 (br s, OH), 2.8—2.6 [m, 4H, cycloO$(CH_2)_2$], 2.6—2.4 [m, 4H, cycloN$(CH_2)_2$]. MS *m/z*: 255, Found (M^+) 254, 144 [$CH_2CH(OH)CH_2$morpholino fragment]. *Anal.* Calcd for $C_{13}H_{18}FNO_3$: C, 61.16; H, 7.11; N, 5.49. Found: C, 61.14; H, 7.10; N, 5.47.

1-(2-Hydroxy-3-morpholinopropoxy)-4-fluorobenzene (8b) In the same manner as described for **8a**, crude **8b** was prepared from a mixture of **7c** (3.0 g, 19.0 mmol) and morpholine (3.2 g, 37.0 mmol) to give a white solid. Recrystallisation from ethanol (15 cm^3) gave the title compound (5.3 g, 73.7%), mp 92—93 °C. NMR ($CDCl_3$) δ: 7.13—6.86 (m, 4H, ArF), 4.2—4.1 [m, 1H, CH(OH)], 4.1—4.05 (d, 2H, OCH_2), 3.8—3.7 (d, 2H, CN_2N), 3.5 (br s, OH), 2.8—2.6 [m, 4H, cycloO$(CH_2)_2$], 2.6—2.4 [m, 4H, cycloN$(CH_2)_2$]. MS *m/z*: 255, Found (M^+) 255, 144 [$CH_2CH(OH)CH_2$morpholine fragment]. *Anal.* Calcd for $C_{13}H_{18}FNO_3$: C, 61.16; H, 7.11; N, 5.49. Found: C, 61.14; H, 7.10; N, 5.47.

1-(2-Hydroxy-3-phenylpiperazinopropoxy)-2-fluorobenzene (8c) In the same manner as described for **8a**, crude **8c** was prepared from a mixture of **7a** (3.0 g, 10.0 mmol) and phenylpiperazine (5.4 g, 36 mmol) to give a white solid. Recrystallisation from methanol (15 cm^3) gave the title compound (5.3 g, 57.6%), mp 110—113.5 °C. NMR ($CDCl_3$) δ: 7.3—7.2

(m, 4H, ArF), 7.1—6.8 (m, 5H, Ar), 4.2—4 [1m, 1H, CH(OH)], 4.05—4.03 (d, 2H, OCH_2), 3.5 (br s, OH), 3.42—3.4 (d, 2H, CN_2N), 3.25—3.1 [m, 4H, cyclo$(CH_2)_2$N], 3.05—2.8 [m, 4H, cyclo$(CH_2)_2$N]. MS *m/z*: 330, Found (M^+) 329, 175 [CH_2phenylpiperazine fragment]. *Anal.* Calcd for $C_{19}H_{23}FN_2O_2$: C, 69.07; H, 7.02; N, 8.48. Found: C, 69.05; H, 7.01; N, 8.46.

1-(2-Hydroxy-3-phenylpiperazinopropoxy)-4-fluorobenzene (8d) In the same manner as described for **8a**, crude **8d** was prepared from a mixture of **7c** (3.0 g, 19.0 mmol) and phenylpiperazine (5.4 g, 36 mmol) to give a white solid. Recrystallisation from methanol (15 cm^3) gave the title compound (4.99 g, 54.2%), mp 123—124 °C. NMR ($CDCl_3$) δ: 7.3—7.2 (m, 4H, ArF), 7.1—6.8 (m, 5H, Ar), 4.2—4 [1m, 1H, CH(OH)], 4.05—4.03 (d, 2H, OCH_2), 3.5 (br s, OH), 3.42—3.4 (d, 2H, CN_2N), 3.25—3.1 [m, 4H, cyclo$(CH_2)_2$N], 3.05—2.8 [m, 4H, cyclo$(CH_2)_2$N]. MS *m/z*: 330, Found (M^+) 329, 175 [CH_2phenylpiperazine fragment]. *Anal.* Calcd for $C_{19}H_{23}FN_2O_2$: C, 69.07; H, 7.02; N, 8.48. Found: C, 69.06; H, 7.01; N, 8.47.

1-[4-Fluorophenylpiperazinyl-2-hydroxypropoxy]-2-fluorobenzene (8e) In the same manner as described for **8a**, crude **8e** was prepared from a mixture of **7a** (3.0 g, 19.0 mmol) and fluorophenylpiperazine (6.0 g, 36 mmol) to give a white solid. Recrystallisation from ethanol (15 cm^3) gave the title compound (6.24 g, 64.3%), mp 115—117 °C. NMR ($CDCl_3$) δ: 7.13—6.86 (m, 8H, ArF), 4.2—4 [1m, 1H, CH(OH)], 4.1—4.05 (d, 2H, OCH_2), 3.5 (br s, OH), 3.2—3.1 (d, 2H, CN_2N), 2.9—2.8 [m, 4H, cyclo$(CH_2)_2$N], 2.7—2.6 [m, 4H, cyclo$(CH_2)_2$N]. MS *m/z*: 348, Found (M^+) 348, 329, 193 [CH_2-4-fluorophenylpiperazine fragment]. *Anal.* Calcd for $C_{19}H_{22}F_2N_2O_2$: C, 65.50; H, 6.36; N, 8.04. Found: C, 65.49; H, 6.35; N, 8.02.

3-[4-Fluorophenylpiperazinyl-2-hydroxypropoxy]-4-fluorobenzene (8f) In the same manner as described for **8a**, crude **8f** was prepared from a mixture of **7c** (3.0 g, 19.0 mmol) and fluorophenylpiperazine (6.0 g, 36 mmol) to give a white solid. Recrystallisation from ethanol (15 cm^3) gave the title compound (6.63 g, 68.4%). mp 143—146 °C. NMR ($CDCl_3$) δ: 7.13—6.86 (m, 8H, ArF), 4.2—4 [1m, 1H, CH(OH)], 4.1—4.05 (d, 2H, OCH_2), 3.5 (br s, OH), 3.2—3.1 (d, 2H, CN_2N), 2.9—2.8 [m, 4H, cyclo$(CH_2)_2$N], 2.7—2.6 [m, 4H, cyclo$(CH_2)_2$N]. MS *m/z*: 348, Found (M^+) 346, 193 [CH_2-4-fluorophenylpiperazine fragment]. *Anal.* Calcd for $C_{19}H_{22}F_2N_2O_2$: C, 65.50; H, 6.36; N, 8.04. Found: C, 65.48; H, 6.35; N, 8.02.

Pharmacological Evaluation Inotropic response was measured spontaneously in beating atria suspended at 32 °C in Kreb's solution under 1.75 g resting tension. The force was recorded isometrically and rate increments were measured by a ratemeter. Left ventricular pressure was measured in a sodium pentobarbitone 50 mg/kg anaesthetised parenteral administration using a Miller tip pressure transducer in the left ventricle inserted *via* the carotid artery. To see if any correlation between inotropic and enzyme inhibition exists, studies were focussed on compounds which increased the force of contraction rather than the rate of the heart, since an increase in heart rate leads to an elevation of myocardial oxygen consumption which is undesirable for patients in heart failure.

Acknowledgement The authors wish to thank Coventry University for a postgraduate research studentship to Nigel R Baker, Professor. Timothy J Mason for technical assistance throughout the study, the chemical toxicology group for biological assays and Mr. J. C. Bickerton for structural characterisation.

References

1) Braunwald E., "Heart Disease," 1st. Ed. 1980, Vol. 1, W. B. Saunders Company, Philadelphia, Chapter 13, p. 453.
2) Tominaga M., Ogawa H., Yo E., Yamashita S., Yabuuchi Y., Nakagawa K., *Chem. Pharm. Bull.*, **35**, 3699 (1987).
3) Birch A. M., Davies R. V., Maclean L. (the late), Robinson K., *J. Chem. Soc., Perkin Trans. 1*, **1994**, 387.
4) Weber K. T., *Am. J. Med.*, **72**, 655 (1982).
5) Robertson D. W., Jones N. D., Krushinski J. H., Pollock G. D., Swartzendruber J. K., Hayes J. S., *J. Med. Chem.*, **30**, 623 (1987).
6) Christensen S. B., Torphy T. J., "Annual Reports in Med. Chem.," Vol. 29, Academic Press Inc., San Diego, California, U.S.A., 1994, Chapter 19, Torphy T. J., *Rev. Clin. Basic Pharm.*, **6**, 61 (1987).
7) Erhardt P. W., *J. Med. Chem.*, **30**, 231 (1987).
8) Opie L. H., *Eur. Heart J.*, **4**, 199 (1983).
9) Amer M. S., McKinney G. R., *Life Sciences*, **13**, 753 (1973).
10) Amer M. S., Kreighbaum W. E., *J. Pharm. Sci.*, **64**, 1 (1975).
11) Coates W. J., Prain H. D., Reeves M. L., Warrington B. H., *J. Med. Chem.*, **33**. 1735 (1990).
12) Honerjäger P., Schäfer-Korting M., Reiter M., *Naunyn-Schmideberg's Arch. Pharmacol.*, **318**, 112 (1981).
13) Fujioka T., Teramoto S., Mori T., Hosokawa T., Tominaga M., Yabuuchi Y., *J. Med. Chem.*, **35**, 3607 (1992).
14) Goldberg N. D., Haddox M. K., Nicol S. E., Glass D. B., Sanford C. H., Kuehl F. A., Estensen R., *Adv. Cyclic Nucleotide Res.*, **5**, 307 (1975).
15) Alabaster C. T., Blackburn K. J., Joice J. R., Massingham R., Scholfield P. C., *Br. J. Pharmocol.*, **60**, 284 (1977).
16) Hosokawa T., Mori T., Fujiki F., Kinoshita S., Takemoto K., Imaizumi T., Noda T., Ohura M., Tominaga M., Yabuuchi Y., *Heart Vessels*, **7**, 66 (1992).