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Synthesis, characterization and *in vitro* biological studies of novel Nitrilo derivatives of N-substituted phenyl piperazine

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Abstract

Nitrilo derivatives of N-alkyl and N-aryl phenyl piperazine have been synthesized and screened for antibacterial and antifungal activities. All the synthesized compounds showed the antibacterial activity against pathogenic strains of *Staphylococcus aureus* (MTCCB 737), *Pseudomonas aeruginosa* (MTCCB 741),*Streptomyces epidermidis* (MTCCB 1824) and *Escherichia coli*(MTCCB 1652) and antifungal activity against pathogenic strains of *Aspergillus fumigatus* (ITCC 4517), *Aspergillus flavus* (ITCC 5192) and *Aspergillus niger* (ITCC 5405). All compounds showed mild to moderate antimicrobial activity. However, compounds **3c**, **4a** and **6** showed potent antibacterial activity against pathogenic strains used in the study. Compounds **3a**, **3b**, **4b**, and **4d** showed mild to moderate antifungal activity against *Aspergillus* pathogenic strains.

الخلاصة

يهدف هذا البحث الى تحضير مشتقات جديده من نتريلو فنيل ببرازين المعوضه على ذرات النتروجين حلقة الفنيل ببرازين وذلك بادخال مجاميع من الاكيل و الاريل فكانت نسب النواتج تتراوح ٩٤-٧٦% تم تشخيص تلك المركبات الجديده المحضره من خلال تقنيات Mass spectrophotometer, T.L.C , ^{13}C-NMR , ^{1}HNMR ,IR) chromotography) تم في هذا اللبحث دراسة الفعاله البايلوجيه (الفعاليه ضد البكتريا والفطريات) للمركبات المحضره وذلك باستخدام ثلاثة انواع من سلالات البكتريا

(*Pseudomonas_aeruginosa* (MTCCB 741),*Streptomyces epidermidis* (MTCCB 1824) and *Escherichia coli*(MTCCB 1652) (*Staphylococcus aureus* (MTCCB 737 و ثلاثة انواع من سلالات الفطريات

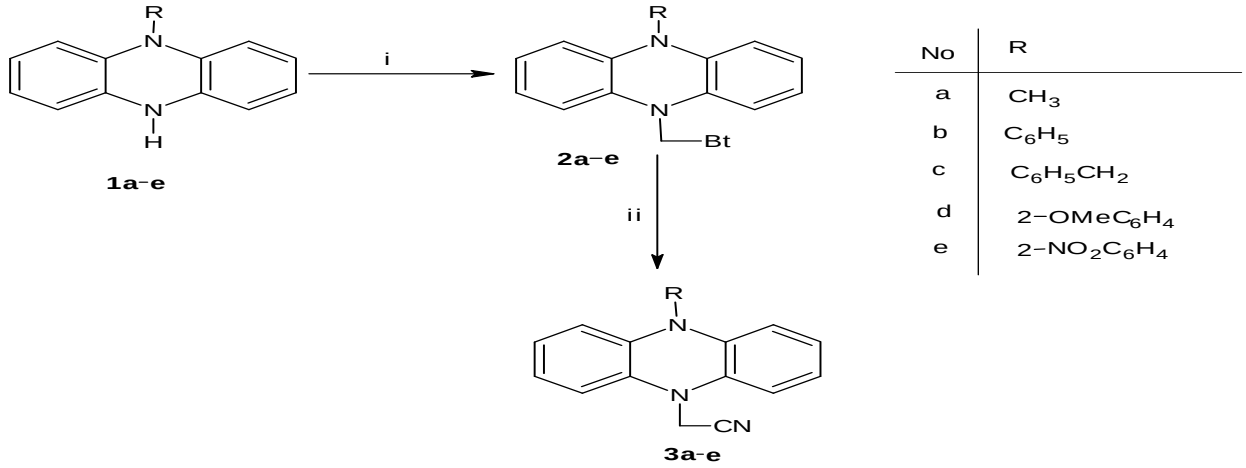
Aspergillus fumigatus (ITCC 4517), *Aspergillus flavus* (ITCC 5192) ,*Aspergillus niger* (ITCC 5405) . اظهرت نتائج الدراسه ان كل المركبات المحضره كان لها تاثيرات كمضادات للبكتريا والفطريات.المركبات **3c** **6** , **4a**, اظهرت فعاليه عاليه ضد سلالات البكتريا المعده للدراسه وكذلك المركبات **3a** ,**3b**,**4b**, **4d** ضد سلالات الفطريات.

Introduction

In recent decades, the problems of multi-drug resistant microorganisms have reached on alarming level in many countries around the world [1-3]. Several antibiotics have been prescribed and found to be effective on various infectious disorders. For the treatment of these intractable infections, new anti-infections agents are needed. Quinolone antibiotics are widely prescribed drugs because of their safety, good tolerance, broad antibacterial spectrum and less resistance [4-7]. Macrolide antibiotics are an important [1] therapeutic class against Gram-positive organisms [8]. Oxazolidinone's antibacterial agents are newer class of synthetic antibacterial agents with activity againstGram-positive bacteria [9].nitrilo derivatives of phenyl piperazine have been known for their uses in the synthesis of pharmaceutical intermediates, peptide analogues, antibiotics and other biologically active molecules and drugs [10-12]. The present investigation describes the synthesis of a series of nitrilo derivatives of N-alkyl and N-aryl phenyl piperazine. By (IR, ^{1}HNMR,^{13}C-NMR.Massspectrophotometer, T.L.C chromotography)techniques.The derivatives were assessed for their *in vitro* antimicrobial activity (zone of inhibition and minimum inhibitory concentration (MIC) activity) against a number of bacterial strains and anti-*Aspergillus* activity (minimum inhibitory concentration (MIC) activity).

Experimental and material

All reagents used were of AR grade.THF was distilled from sodium/benzophenone prior to use. Melting points were determined using a Thomas Hoover melting point apparatus and are uncorrected. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker 300 NMR spectrometer in $CDCl_3$ (with TMS for ^{1}H and chloroform-*d* for ^{13}C NMR as internal references) unless otherwise stated. Mass spectrum was recorded on Hybrid Quadrupole-TOF LC/MS/MS mass pectrometer (Q. Star XL). Column chromatography was performeds on silica gel (230-400 mesh). Microanalyses were obtained with an Elemental Analysensysteme GmbH VarioEL V3.00 element analyser. The reactions were monitored by thin layer chromatography (TLC) using aluminium sheets with silica gel 60 F_{254} (Merck). All of the reactions were carried out under nitrogen atmosphere



No	R
a	CH_3
b	C_6H_5
c	$C_6H_5CH_2$
d	$2-OMeC_6H_4$
e	$2-NO_2C_6H_4$

Scheme 1. Reagent and conditions: (i) CH_2O/Benzotriazole (1:1), MeOH/H_2O (9:1), 25^{o}C; (ii) NaCN, DMSO, 25^{o}C**.**

General procedure for the synthesis of benzotriazolyl derivatives of N-alkyl and N-aryl Phenyl piperazines (**2a-d**):

To a solution of N-alkyl and N-aryl phenyl piperazines **1a-d**, (0.35 g, 2 mmol) and benzotriazole (0.21g, 2 mmol) in CH_3OH/H_2O (9:1, 10 ml) was added formaldehyde (37% aqueous solution, (4mmol).The mixture was stirred at room temperature for 6-8 h. The precipitate formed was filtered and washed with cold Et_2O to gave pure product [(5-methyl phenyl piperazin-10-yl)methyl]-10H-1,2,3-benzotriazole **2a**, [(5-phenyl phenyl piperazin-10-yl) methyl]-10H-1,2,3-benzotriazole**2b**[(5-benzylphenylpiperazin-10-yl)ethyl]-10H-1,2-benzotriazol **2c** and {[5-(2-methoxyphenyl) phenyl piperazin-10-yl] methyl}-10H-1,2,3-benzotriazole **2d** in 87-94% yields as a sole bt1 isomer, which was directly used for subsequent reactions. For microanalysis purposes, the precipitate was recrystallized from $CHCl_3$/hexanes (1:1). Spectral data of the synthesized compounds **2a-d** were consistent with the reported values.

General procedure for the replacement of benzotriazole group from 2a-d with sodium cyanide:

A solution of benzotriazolyl derivative of N-alkyl and N-aryl Phenyl piperazines **2a-e** (1.0 mmol) and NaCN (2.0 mmol) was stirred in DMSO (10 ml) for 8-12 h, after removal of the solvent in vacuo; the residue was diluted with EtOAc. The mixture was washed with brine and dried over Na_2SO_4 Evaporation of the solvent was done in vacuo and the residue was purified by column chromatography with hexanes/EtOAc (6:1 to 3:1) as an eluent gave **3a-e** in 76-88% yields. Spectral data of the synthesized compounds **3a-d** were consistent with the reported values.

(5-Methyl-phenyl piperazin-10-yl)-acetonitrile 3a:

The general synthetic method described above affords **3a** as semisolid m.p 84^{o}C, yield 88-97%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.38 (s, 2H), 2.27 (s, 3H), 1.50 (m, 4H), 1.35 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 113.5, 54.9, 50.05, 40.23, 30.02; LCMS m/z found 240.3 (M+1),213,238. IR(cm^{-1}) 3095 (C-H aromatic),1600(C═C), 2920(C-H aliphatic),2252 (C≡N),1268 (C-N).

(5-Phenyl-phenyl piperazin-10-yl)-acetonitrile 3b:

The general synthetic method described above affords **3b** as dirty white solid m.p 123^{o}C, yield 88%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31-7.25 (m, 2H), 6.95-6.86 (m, 3H), 3.58 (s, 2H), 3.24(t,J = 4.8 Hz, 4H), 2.76 (t, J = 4.9 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ150.9, 129.1, 120.1, 116.3, 114.5, 51.8, 48.9, 45.9; LCMS m/z found 302.3 (M +1).

IR (cm^{-1})3100 (C-H aromatic),1622(C═C), 2820(C-H aliphatic),2250 (C≡N),1253(C-N)..

(5-Benzyl phenyl piperazin-10-yl)-acetonitrile 3c:

The general synthetic method described above affords **3d** as creamy white solid m.p 95^{o}C, yield; 88%;^{1}HNMR (300 MHz, $CDCl_3$): δ 7.19-7.12 (m, 5H), 3.39 (s, 2H), 3.34 (s, 2H), 2.47-2.46 (m, 4H), 2.39-2.37 (m, 4H); ^{13}C NMR (75 MHz, CDCl3): δ 137.6, 128.6, 127.9, 126.8, 114.5, 62.3, 52.1, 51.4, 45.5; LCMS m/z found 329.3 (M + 1).

IR (cm^{-1})3087 (C-H aromatic), 1562(C═C), 2823(C-H aliphatic), 2241 (C≡N),1250(C-N).

[5-(2-Methoxyphenyl)phenyl piprazin-10-yl]-acetonitrile 3d:

The general synthetic method described above affords **3d** pale solid m.p 110^{o}C, yield: 89%; ^{1}H NMR (300 MHz, $CDCl_3$): as

δ 7.86 (m, 2H), 6.87-6.89 (m, 2H), 3.869(s, 3H), 3.57 (s, 2H), 3.14 (m, 4H), 2.81 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ140.8, 125.9, 123.2, 121.0, 118.3, 114.8, 77.0, 55.4, 52.1, 45.9; LCMS *m/z* found 317.2 (M+1).

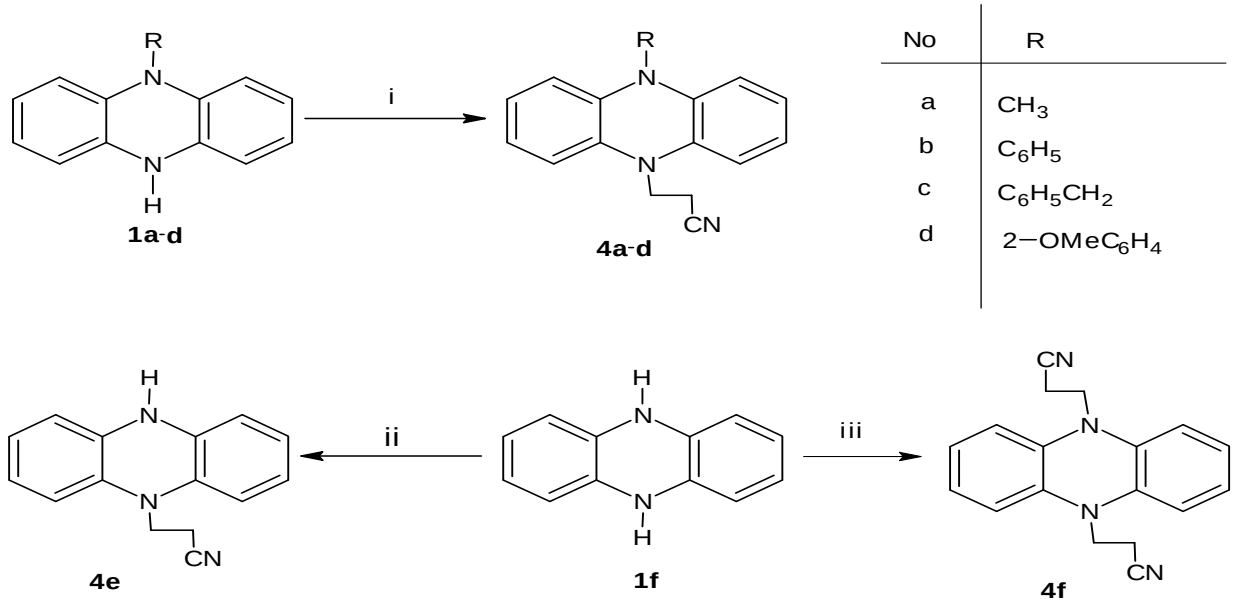
The general synthetic method described above affords **3e** as yellow crystals m.p 135^{o}C, yield 93%;^{1}H NMR (300 MHz, $CDCl_3$): δ7.38-7.24 (m, 5H), 3.57-3.42 (m, 6H), 2.59-2.61 (m, 5H), 2.53-2.48 (m, 4H);^{13}C NMR (75 MHz, $CDCl_3$): δ 137.9, 132.9, 129.2, 128.1, 125.2, 77.0, 52.8, 52.0, 41.4, 29.7, 3.5;LCMS *m/z* found: 247.3 (M + 1) peak
IR (cm$^{-1)}$3020 (C-H aromatic),1554 (C═C),2887(C-H aliphatic),2240 (C≡N), 1255(C-N),1250 (N-O).

General procedure for the aza-Michael reaction of N-alkyl and N-aryl phenyl piperazines (Scheme 2):

IR(cm^{-1}3)091(C-H aromatic), 1615(C═C), 2926(C-H aliphatic),2244(C≡N),1277(C-N).

[5-(4-Nitro-phenyl)-phenyl piperazin-10-yl]-acetonitrile 3e:

To a stirred solution of amine (1.0 equiv) and acrylonitrile (1.2 equiv) in THF (30 ml), Cu- nanoparticles (14-17 nm, 10 mol%) were added at room temperature and stirring was continued for 8-16 h under nitrogen. After completion of the reaction (TLC), THF was removed in vacuo; the reaction mixture was treated with water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and after the removal of the solvent in vacuo, the residue was purified by column chromatography (silica gel 250-400 mesh size).



Scheme 2. Reagent and conditions: (i) and (ii) 1.5 equiv acrylonitrile, 10 mol% Cu-nanoparticles (14-17 nm), THF, 25 ^{o}C; (iii) 2.8 equiv acrylonitrile, 15 mol% Cu-nanoparticles (14-17 nm), THF, 25 ^{o}C.

Results and discussion

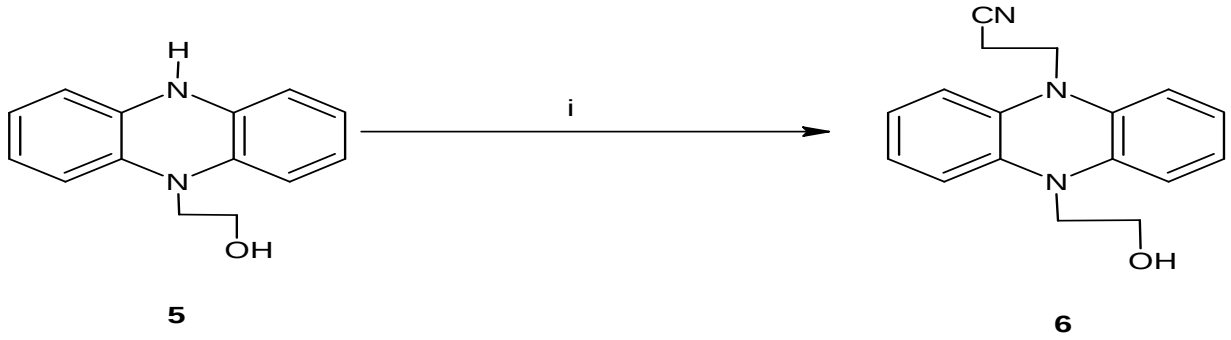
We report the synthesis, characterization and in vitro antimicrobial activity of nitrilo derivatives of N-alkyl and N-aryl phenyl piperazine.Nitrilo derivatives of N-alkyl and N-aryl substituted phenyl piperazine **3a-e** have been prepared by substituting benzotriazolyl group with cyano anion from the key benzotriazolyl intermediates **2a-e** (Scheme 1). The condensation of 5-methyl phenyl piperazine **1a**,(5-phenyl) phenyl piperazine **1b**, 5-benzyl phenyl piperazine **1c**, 5-(2-methoxyphenyl) phenyl piperazine **1d**, and 5-(4-nitrophenyl) phenyl piperazine **1e** with 1equiv of benzotriazole and 1 equiv of formaldehyde (37% aqueous solution) in MeOH/H_2O (9:1) at 25 ^oC gave benzotriazolyl intermediate **2a-e** in 87-94% yields as a sole isomer [13].Treatment of benzotriazolyl intermediate **2a-e** with 1.5 equiv of sodium cyanide in DMSO at room temperature replaced the benzotriazole group with cyanide group to afford (5-methyl phenyl piperazin-10-yl)-acetonitrile **3a**,[(5-phenyl) phenyl piperazin-10-yl]-acetonitrile **3b**, [(5-benzyl) phenyl piperazin-10-yl]-acetonitrile **3c**, [5-(2-methoxyphenyl) phenyl piperazin-10-yl]-acetonitrile **3d** and [5- (4-nitrophenyl)-phenyl piperazin-10-yl]-acetonitrile **3e** in 76-88% yields (Scheme1).The reactive C-N bond of the key benzotriazolyl intermediates **2a-e** allows easy replacement of benzotriazolyl group with cyano anion to afford nitrilo derivatives **3a-e** in good to excellent yields via nucleophilic substitutions [13].

In vitro antibacterial activity

The in vitro antibacterial activity was tested by disc diffusion method [15] and microbroth dilution technique [16] using pathogenic strains of *Staphylococcus aureus* (MTCCB 737), *Pseudomonas aeruginosa* (MTCCB 741), *Streptomyces epidermidis* (MTCCB 1824) and Escherichia coli (MTCCB 1652). The experimental result of antibacterial activity indicated variable degree of efficacy of the compounds against different strains of bacteria (Table 1). The zone of inhibition and MIC's values of phenyl piperazine derivatives were determined by disc diffusion method [15] and microbroth dilution technique [16].

We have synthesized these nitrilo derivatives of N-alkyl and N-aryl phenyl piperazine for antimicrobial activity. (5-Benzyl-phenyl piperazin-10-yl)-acetonitrile**3c**shows potent antibacterial activity against *S. aureus* with MIC value at 19.5 µg/ml and zone of inhibition at 18 mm, however, it did not show significant effect on other strains of bacteria used in experiment Similarly 5-(4 methyl-phenyl piperazin-10-yl)-propanitrile **4a** was effective against *P. aeruginosa, E. coli* only, the MIC being 19.5 µg/ml and 39.06 µg/ml with zone of inhibition of 19 mm and 18 mm, respectively (Table1). 3-[5-(2-Hydroxy-ethyl) phenyl piperazin-10- yl] -acetonitrile **6** shows potent antibacterial activity against *S. aureus* with MIC value 19.5 µg/ml and zone of inhibition 18 mm (Table 1),

but moderate activity against *P. aeruginosa E. coli* with MIC at 39.1µg/ml (Table 1). Compounds **3c** and **4a** showing better activity in comparison to other compounds used in study might be due to the presence of benzyl and methyl groups at 5-position of phenyl piperazine nucleus. Enhanced group at 5-position of phenyl piperazine nucleus. Other compounds appeared as broad spectrum, as they show mild to moderate effect on most of the strains used in the experiment.



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Scheme 3. Reagent and conditions: 2.5 equiv acrylonitrile, 10 mol% Cu-nanoparticles (14-17 nm), THF, 25o

Table 1: Antibacterial activities of nitrilo derivatives of N-alkyl and N-aryl phenyl piperazine showing zone of inhibition (mm) and MIC values (µ/ml) against selected pathogenic strains

Compound	*S. aureus*	*S. epidermidis*	*P. aeruginosa*	*E. coli*
3a	12/310.5	8/625.0	9/625.0	12/310.5
3b	10/310.5	12/156.3	13/156.3	9/310.5
3c	18/19.5	10/310.5	13/156.3	9/625.0
3d	9/625.0	8/625.0	8/625.0	10/310.5
3e	--	--	--	
4a	10/310.5	11/156.3	19/19.5	18/39.1
4b	13/156.3	12/156.3	9/625.0	12/156.3
4c	--	--	--	
4d	12/156.3	9/625.0	9/625.0	11/156.3
4e	--	--	--	
4f	11/310.5	9/625.0	11/156.3	10/625.0
6	18/19.5	11/310.5	15/39.1	15/39.1
Gentamicin	20/9.8	18/19.5	19/19.5	18/19.5

In vitro antifungal activity

Nine nitrilo derivatives of N-alkyl and N-aryl phenyl piperazine have also been examined for antifungal activity against pathogenic strains of *Aspergillus fumigatus* (ITCC 4517), *Aspergillus* flavus (ITCC 5192) and *Aspergillus Niger* (ITCC 5405). The *anti-Aspergillus* activity of all the synthesized have been evaluated by the disc diffusion (DDA), microbroth dilution (MDA) [17] and percentage spore germination inhibition (PSGI) [18]; the results are given in Table 2.

Table 2. Antifungal activites of nitrilo derivatives of N-alkyl and N-aryl phenyl piperazine

Compound	MIC								
	DDA(µg/disc)			MDA(µg/ml)			PSGI ((µg/ml)		
	A flavus	*Aniger*	*Afumigatus*	*A flavus*	*Aniger*	*Afumigatus*	*A flavus*	*Aniger*	*Afumigatus*
3a	93.8	93.7	187.5	500	500	500	250	250	500
3b	187.5	187.5	187.5	500	500	500	500	250	250
3c	–	–	–	–	–	–	–	–	–
4a	–	–	–	–	–	–	–	–	–
4b	187.8	187.5	93.8	500	500	250	250	250	250
4d	375.5	–	187.5	1000	–	500	500	–	500
4f	–	–	–	–	–	–	–	–	–
4g	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–
Amphotericin B	2.5	2.5	2.5	5	5	5	5	5	5

Result of in vitro *anti-Aspergillus* activity demonstrates that **3a**, **3b**, **4b** and **4d** show mild to moderate *anti-Aspergillus* activity against pathogenic strains. Compound (5-methyl phenyl piperazin-10-yl)-acetonitrile **3a** has MIC90 at 93.8 mg/disc in DDA assay against *A.flavus* and *A. Niger*. The next most active compound is 3-(5-phenyl- phenyl piperazin-10-yl)-propanitrile **4b**, which exhibited activity at 93.8 mg/disc in DDA against *A. fumigatus*.

The structure of compound **3a** has methyl group at 10-position chain at 5-position of phenyl piperazine nucleus.Compound **4b** has phenyl group at 10-position of phenyl piperazine nucleus and cyano group at 3-position of 5-position of phenyl piperazine nucle.

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