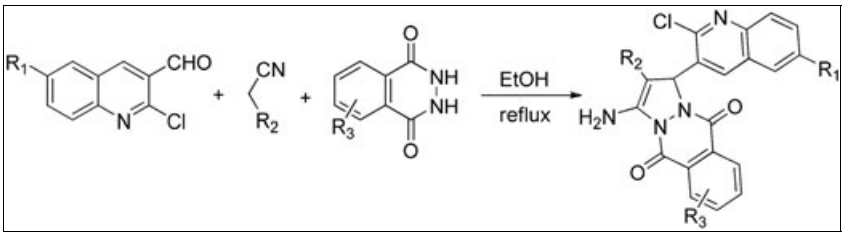
An Efficient and Facile Synthesis of 1*H*-Pyrazolo[1,2-*b*] phthalazine-5,10-dione Derivatives of Biological Interest

Nimesh M. Shah, Manish P. Patel, and Ranjan G. Patel*

Department of Chemistry, S P University, Vallabh Vidyanagar-388120, Gujarat, India
*E-mail: patelranjanben@yahoo.com

Received December 23, 2010
DOI 10.1002/jhet.918
View this article online at wileyonlinelibrary.com.



A simple and efficient method has been developed for the synthesis of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione derivatives through a one-pot three-component condensation reaction of 2-chloro-3-formyl quinolines, malononitrile/ethyl cyanoacetate and 2,3-dihydro-1,4-phthalazinedione using a catalytic amount of piperidine in refluxing ethanol. All the synthesized compounds were screened for their antibacterial activity against a panel of pathogenic strains of bacteria and fungi.

J. Heterocyclic Chem., **49**, 1310 (2012).

INTRODUCTION

Quinoline and its derivatives have been known to possess diverse pharmacological activities, such as, antibacterial, antifungal, antimycobacterial, antidepressant, antimalarial, anticonvulsant, antiviral, anticancer, antiproliferative, and anti-inflammatory activities [1–10]. Because of their interesting physiological properties, quinoline derivatives in particular are extremely important and are frequently found in privileged structures (pharmacophore), in numerous biologically active compounds.

Heterocycles containing the phthalazine ring are important targets in synthetic and medicinal chemistry, because this fragment is a key moiety in numerous biologically active compounds [11, 12]. Phthalazine derivatives, which have two bridgehead nitrogen atoms in a fused ring system, possess cytotoxic [13], antimicrobial [14], anticonvulsant [15], antifungal [16], anticancer [17], and anti-inflammatory [18] activities. Phthalazine-containing compounds are also highly potent inhibitors of vascular endothelial growth factor receptor II (VEGFR-2) [19–21].

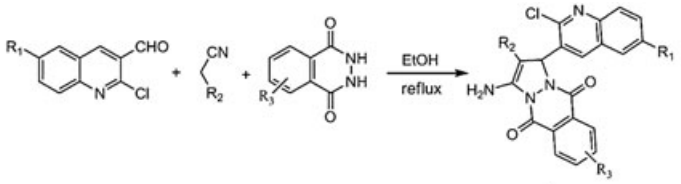
Literature survey reveals few studies concerning pyrazolo [1,2-*b*]phthalazine-dione [22, 23] derivatives of aromatic aldehyde, wherein not a single reference was found where 2-chloro-3-formyl quinoline is used and evaluated for their biological profile. As part of our continued interest on quinolines [24] and development of highly expedient methods for the synthesis of diverse heterocyclic compounds of biological importance, we report herein the synthesis of 1*H*-pyrazolo [1,2-*b*]phthalazine-5,10-dione derivatives using a three component one pot protocol under mild conditions. The structures of compounds were established using analytical technique FT-IR, ^{1}H and ^{13}C NMR, elemental analysis, and some selected compounds confirmed by mass spectrometry. All the compounds were evaluated for their *in vitro* antimicrobial activity against eight human pathogens, of which three Gram-positive bacterial pathogens *Streptococcus pneumoniae*, *Clostridium tetani*, *Bacillus subtilis*, three Gram-negative bacterial pathogens *Salmonella typhi*, *Vibrio cholerae*, *Escherichia coli*, and two fungal pathogens *Aspergillus fumigatus and Candida albicans*, using broth microdilution MIC (Minimum Inhibitory Concentration) method [25].

RESULTS AND DISCUSSION

In this work, a new series of sixteen compounds was synthesized. Scheme 1 illustrates the synthetic pathway used for the preparation of target compounds. The starting compounds 2-chloro-3-formyl quinolines **1a–d** were prepared according to literature method [26]. The one-pot three-component condensation reaction of 2-chloro-3-formyl quinolines **1a–d** with malononitrile or ethyl cyanoacetate **2a-b** and 2,3-dihydro-1,4-phthalazinedione **3a-b** proceeded smoothly in ethanol in the presence of piperidine as a catalyst to give the corresponding 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione **4a-p** derivatives in good to excellent yields.

To choose the most appropriate medium in this heterocyclization reaction, the reaction of 2-chloro-3-formyl quinolines **1a–d**, malononitrile or ethyl cyanoacetate **2a-b** and 2,3-dihydro-1,4-phthalazinedione **3a-b**, various reaction conditions were investigated. To search for the optimal reaction solvent, the reaction was examined in ethylene glycol, DMF, HOAc, THF, and ethanol as solvent under reflux, respectively. The reaction in ethanol resulted in

Scheme 1. Synthetic pathway for 1*H*-pyrazolo[1,2-b]phthalazine-5,10-dione derivatives **4a-p**.



1a-d 2a-b 3a-b 4a-p

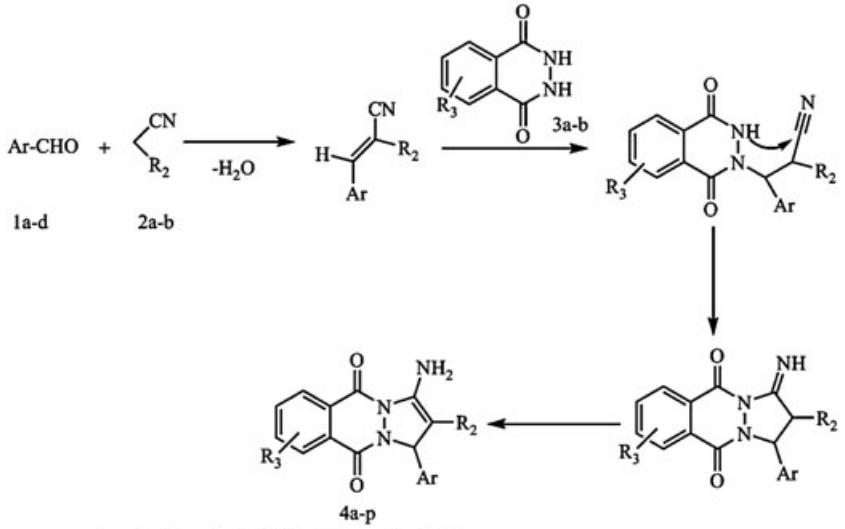
Compd	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l	4m	4n	4o	4p
R_1	H	CH_3	OCH_3	Cl	H	CH_3	OCH_3	Cl	H	CH_3	OCH_3	Cl	H	CH_3	OCH_3	Cl
R_2	CN	CN	CN	CN	COOEt	COOEt	COOEt	COOEt	CN	CN	CN	CN	COOEt	COOEt	COOEt	COOEt
R_3	H	H	H	H	H	H	H	H	NO_2	NO_2	NO_2	NO_2	NO_2	NO_2	NO_2	NO_2

higher yields and shorter reaction time than others. So ethanol was chosen as the appropriate solvent. Moreover, to further improve the reaction yields, different bases like NaOH, K_2CO_3, DMAP, Et_3N, and piperidine were examined in ethanol. The base piperidine afforded the target product **4a** in an 82% yield. Therefore, piperidine was chosen as the most suitable base for all further reactions.

A possible mechanism for the reaction is outlined in Scheme 2. The reaction may occurs via initial Knoevenagel condensation of **1a-d** and **2a-b** in presence of piperidine to give intermediate heterylidenenitrile which on subsequent Michael-type addition of the 2,3-dihydro-1,4-phthalazinedione **3a-b**, followed by cyclization and tautomerization affords the corresponding 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione derivatives **4a-p**.

The structures of the obtained compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, and FT-IR spectral data and molecular weight of some selected compounds confirmed by mass spectrometry. In the IR spectra, some significant stretching bands due to NH_2, C=O, and C=N were at about 3460–3195 cm^{-1}, 1710–1645 cm^{-1}, 2220–2190 cm^{-1}, respectively. In the ^{1}HNMR spectra, the signal due to C1H-pyr proton present in all compounds, appeared at 6.52–6.59 ppm as singlet. All the other aromatic and aliphatic protons were observed at the expected regions. All compounds gave satisfactory elemental analyses. Mass spectra of the compounds showed a $[M+H]^+$ peaks in agreement with their exact mass or molecular weight. All spectroscopic data have been given in experimental section.

Scheme 2. Possible mechanism for the formation of 1*H*-pyrazolo[1,2-b]phthalazine-5,10-dione derivatives **4a-p**.



1a-d 2a-b 4a-p

Ar = 2-Chloro-6-substituted-3-formyl-quinoline

R_2 = CN, COOEt

R_3 = H, 3-NO_2

EVALUATION OF ANTIMICROBIAL ACTIVITY

The *in vitro* antimicrobial activity of all the synthesized compounds and standard drugs were assessed against three representative of Gram-positive bacteria *viz*. *Streptococcus pneumoniae* (MTCC 1936), *Clostridium tetani* (MTCC 449), *Bacillus subtilis* (MTCC 441), three Gram-negative bacteria *viz*. *Salmonella typhi* (MTCC 98), *Vibrio cholerae* (MTCC 3906), *Escherichia coli* (MTCC 443) and two fungi *viz*. *Aspergillus fumigatus* (MTCC 3008) *and Candida albicans* (MTCC 227) by the Broth Microdilution MIC method recommended by National Committee for Clinical Laboratory Standards (NCCLS) [23]. The strains employed for the activity were procured from (MTCC – Micro Type Culture Collection) Institute of Microbial Technology, Chandigarh. Inoculum size for test strain was adjusted to 10^8 CFU mL^{-1} (Colony Forming Unit per milliliter) by comparing the turbidity (turbidimetric method). Mueller Hinton Broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose Broth used for fungal nutrition. Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, and Norfloxacin were used as standard antibacterial drugs, whereas Griseofulvin and Nystatin was used as standard antifungal drugs. DMSO was used as diluents/vehicle to get desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains. Serial dilutions were prepared in primary and secondary screening. Each synthesized compound and standard drugs were diluted obtaining 2000 μg mL^{-1} concentration, as a stock solution. In primary screening 1000, 500, and 250 μg mL^{-1} concentrations of the synthesized drugs were taken. The active synthesized compounds found in this primary screening were further diluted to obtain 200, 100, 62.5, and 50 μg mL^{-1} concentrations for secondary screening to test in a second set of dilution against all microorganisms. The control tube containing no antibiotic is immediately sub cultured [before inoculation] by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism. The tubes are then put for incubation at 37°C for 24 h for bacteria and 48 h for fungi. The highest dilution (lowest concentration) preventing appearance of turbidity is considered as minimal inhibitory concentration (MIC, μg mL^{-1}) i.e., the amount of growth from the control tube before incubation (which represents the original inoculum) is compared. A set of tubes containing only seeded broth and the solvent controls were maintained under identical conditions so as to make sure that the solvent had no influence on strain growth. The result of

Table 1

Antimicrobial activity of the compounds **4a-p**.

	Minimum inhibitory concentration (MIC, μg mL^{-1})							
	Gram-positive bacteria			Gram-negative bacteria			Fungi	
	Streptococcus pneumoniae	*Clostridium tetani*	*Bacillus subtilis*	*Salmonella typhi*	*Vibrio cholerae*	*Escherichia coli*	*Aspergillus fumigatus*	*Candida albicans*
Compounds	MTCC 1936	MTCC 449	MTCC 441	MTCC 98	MTCC 3906	MTCC 443	MTCC 3008	MTCC 227
4a	250	200	200	250	200	250	1000	1000
4b	250	250	100	100	100	62.5	500	500
4c	500	500	500	200	500	200	>1000	>1000
4d	500	250	250	200	250	250	500	500
4e	250	500	500	200	200	200	500	500
4f	500	200	250	100	100	100	>1000	>1000
4g	150	100	62.5	100	200	150	250	250
4h	250	250	500	250	500	250	>1000	500
4i	100	200	200	250	200	200	1000	1000
4j	500	250	250	500	250	250	>1000	>1000
4k	250	200	250	200	100	250	1000	500
4l	200	250	250	100	62.5	100	250	200
4m	250	250	500	200	200	100	1000	500
4n	200	500	200	200	200	62.5	250	100
4o	200	500	200	500	500	250	1000	500
4p	500	500	250	250	250	250	>1000	1000
Ampi.	100	250	250	100	100	100	–	–
Chlorm.	50	50	50	50	50	50	–	–
Cipro.	50	100	50	25	25	25	–	–
Genta.	0.5	5	1	5	5	0.05	–	–
Norfl.	10	50	100	10	10	10	–	–
Grise.	–	–	–	–	–	–	100	500
Nyst.	–	–	–	–	–	–	100	100

this is much affected by the size of the inoculum. The test mixture should contain 10^8 CFU mL^{-1} organisms. The protocols were summarized in Table 1 as the minimal inhibitory concentration (MIC, μg mL^{-1}).

The antimicrobial screening results are summarized in Table 1. The results revealed that most of the compounds showed fairly good antibacterial and antifungal activity when compared with standard drugs Ampicillin and Griseofulvin. Against Gram-positive pathogen *S. pneumoniae*, compound **4i** were found to exhibit comparable activity, to Ampicillin. The compounds **4a, 4f, 4g, 4i,** and **4k** found to be more efficient whereas, **4b, 4d, 4h, 4j, 4l,** and **4m** were found equally potent to Ampicillin towards *C. tetani*. The compound **4g** were found equally potent to Ciprofloxacin towards *C. tetani*. The compounds **4a, 4b, 4g, 4i, 4n,** and **4o** shows better activity where as, **4d, 4f, 4j-l,** and **4p** found equally potent, to Ampicillin against *B. subtilis*. The compound **4g** found to be more efficient whereas, **4b** were found equally potent to Norfloxacin against *B. subtilis*. Towards Gram-negative strain *S. typhi*, compounds **4b, 4f-g,** and **4l** were equally active to Ampicillin. The Compound **4l** found to be more efficient whereas, **4b, 4f,** and **4k** found equipotent to Ampicillin against *V. cholerae*. The compound **4b** and **4n** shows better and **4f** and **4l-m** were found to exhibit comparable activity to Ampicillin towards *E. coli*. Against fungal pathogen *C. albicans*, compound **4g, 4l,** and **4n** found better activity where as, **4b, 4d-e, 4h, 4k, 4m,** and **4o** were found to be equipotent compared to Griseofulvin. Rest of the compounds showed less activity against all the microorganisms tested. Under these conditions, control *N,N*-dimethylformamide (DMF) did not show any antibacterial and antifungal activity.

CONCLUSIONS

A simple and efficient one-pot procedure has followed for generation of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-dione derivatives. The advantages of this approach is that the reaction procedure is convenient, involves simple experimental procedures and product isolation is easy. It is one-pot reaction which allows the construction of relatively complicated nitrogen containing heterocyclic systems using simple starting materials. It can be concluded from antimicrobial screening (Table 1), against panel of human pathogens, that some of the synthesized compounds were found to be highly active, compared to standard drugs, against bacterial pathogens. Among them, many compounds were found to be the most active against *Clostridium tetani* and *Bacillus subtilis* compared with rest of the employed species. Antifungal activity of the compounds shows that many compounds are found to be potent against *C. albicans*. It suggests that this class of compounds may be selectively targeted to microbial growth and could be a good starting point to find new lead compounds.

EXPERIMENTAL

The reagents used in this work were obtained from Aldrich and were used without purification. All used solvents were of analytical grade. All melting points were taken in open capillaries and are uncorrected. Thin-layer chromatography (TLC, on aluminium plates precoated with silica gel, 60 F_{254}, 0.25 mm thickness) (Merck, Darmstadt, Germany) was used for monitoring the progress of all reactions. UV radiation and/or iodine were used as the visualizing agents. Elemental analysis (% C, H, N) was carried out by Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA). The IR spectra were recorded in KBr on a Perkin-Elmer Spectrum GX FT-IR Spectrophotometer (Perkin-Elmer, USA) and only the characteristic peaks are reported in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using solvent peak as internal standard at 400 MHz and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm). Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan).

General procedure for the synthesis of 1*H*-Pyrazolo[1,2-*b*] phthalazine-5,10-dione derivatives 4a-p. A mixture of 2-Chloro-3-formyl quinoline (1a-d, 1 mmol), malononitrile or ethyl cyanoacetate (2a-b, 1 mmol) and 2,3-dihydro-1,4-phthalazinedione (3a-b, 1 mmol) in ethanol (10 mL) containing few drops of piperidine was heated under reflux for 3–4 h. On completion of reaction, monitored by TLC (ethyl acetate: hexane::3:7), the reaction mixture was cooled to room temperature and the solid separated was filtered, washed with ethanol, dried, and recrystallized to give the desired product. Analytical and spectroscopic characterization data of the synthesized compounds 4a-p are given below.

3-Amino-1-(2-chloroquinolin-3-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (4a). Pale yellow powder; Yield: 82%; mp 274–276°C. IR (KBr, v, cm^{-1}): 3380 and 3185 (NH_2), 2210 (CN), 1690 (C═O), 1675 (C═O);^1H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 6.59 (s, 1H, C1H-pyr), 7.66-8.10 (m, 7H, Ar-H), 8.25-8.34 (m, 2H, Ar-H), 8.81 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 59.89 (C1-pyr), 61.07 (C2-pyr), 116.12, 124.60, 127.25, 127.88, 128.09, 128.24, 128.50, 128.82, 129.35, 131.93, 134.46, 135.30, 147.16, 151.90, 154.21 (Ar-C), 157.25 (C═O), 158.32 (C═O). MS: *m/z* = 402 $[M+H]^+$. Anal. Calcd for $C_{21}H_{12}ClN_5O_2$ (401.81 g/mole): C, 62.77; H, 3.01; N, 17.43%. Found: C, 62.60; H, 2.89; N, 17.54%.

3-Amino-1-(2-chloro-6-methylquinolin-3-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (4b). Pale yellow powder; Yield: 69%; mp 232–234°C; IR (KBr, v, cm^{-1}): 3385 and 3175 (NH_2), 2200 (CN), 1680 (C═O), 1660 (C═O); ^1H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 2.47 (s, 3H, CH_3), 6.57 (s, 1H, C1H-pyr), 7.68-8.09 (m, 6H, Ar-H), 8.23-8.34 (m, 2H, Ar-H), 8.69 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 21.58 (Ar-CH_3), 60.13 (C1-pyr), 61.87 (C2-pyr), 116.11, 124.75, 127.27, 127.79, 127.88, 128.83, 129.36, 131.04, 133.96, 134.52, 135.32, 137.93, 145.72, 151.89, 154.11 (Ar-C), 157.23 (C═O), 158.17 (C═O). Anal. Calcd for $C_{22}H_{14}ClN_5O_2$ (415.83 g/mole): C, 63.54; H, 3.39; N, 16.84%. Found: C, 63.42; H, 3.55; N, 16.94%.

3-Amino-1-(2-chloro-6-methoxyquinolin-3-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (4c). Pale yellow powder; Yield: 71%; mp 220–222°C; IR (KBr, v, cm^{-1}): 3390 and 3185 (NH_2), 2205 (CN), 1685 (C═O), 1670 (C═O);

^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 3.82 (s, 3H, OCH_3), 6.56 (s, 1H, C1H-pyr), 7.28-8.07 (m, 6H, Ar-H), 8.09-8.33 (m, 2H, Ar-H), 8.73 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 55.92 (Ar-OCH_3), 61.24 (C1-pyr), 62.72 (C2-pyr), 106.02, 117.17, 124.72, 127.16, 127.73, 128.57, 128.89, 131.15, 133.66, 135.13, 137.07, 145.73, 151.0, 154.93, 157.12 (Ar-C), 157.43 (C=O), 158.32 (C=O). Anal. Calcd for $C_{22}H_{14}ClN_5O_3$ (431.83 g/mole): C, 61.19; H, 3.27; N, 16.22%. Found: C, 61.42; H, 3.09; N, 16.34%.

3-Amino-1-(2,6-dichloroquinolin-3-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (4d). Pale yellow powder; Yield: 81%; mp 204–206°C; IR (KBr, v, cm^{-1}): 3370 and 3175 (NH_2), 2195 (CN), 1680 (C=O), 1665 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 6.58 (s, 1H, C1H-pyr), 7.86-8.08 (m, 6H, Ar-H), 8.25-8.34 (m, 2H, Ar-H), 8.77 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 59.23 (C1-pyr), 61.30 (C2-pyr), 117.02, 124.69, 126.97, 127.13, 127.85, 128.14, 128.78, 131.37, 133.21, 133.54, 134.47, 135.16, 147.16, 151.90, 154.21 (Ar-C), 157.25 (C=O), 158.32 (C=O). Anal. Calcd for $C_{21}H_{11}Cl_2N_5O_2$ (436.25 g/mole): C, 57.82; H, 2.54; N, 16.05%. Found: C, 57.97; H, 2.23; N, 16.28%.

Ethyl 3-amino-1-(2-chloroquinolin-3-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (4e). Pale yellow powder; Yield: 76%; mp 248–250°C; IR (KBr, v, cm^{-1}): 3450 and 3335 (NH_2), 1705 (C=O), 1665 (C=O), 1640 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 1.01 (t, 3H, J = 9.1 Hz, CH_3), 3.98 (q, 2H, J = 7.7 Hz, OCH_2), 6.53 (s, 1H, C1H-pyr), 7.62-8.08 (m, 7H, Ar-H), 8.30-8.35 (m, 2H, Ar-H), 8.78 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 14.56 (CH_3), 59.47 (C1-pyr), 63.67 (OCH_2), 81.97 (C2-pyr), 124.34, 126.97, 127.09, 127.23, 127.28, 127.71, 128.14, 129.53, 131.38, 134.07, 135.93, 146.48, 151.08, 151.87 (Ar-C), 156.06 (C=O), 158.63 (C=O), 164.27 (COOEt). Anal. Calcd for $C_{23}H_{17}ClN_4O_4$ (448.86 g/mole): C, 61.54; H, 3.82; N, 12.48%. Found: C, 61.39; H, 4.09; N, 12.67%.

Ethyl 3-amino-1-(2-chloro-6-methylquinolin-3-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (4f). Pale yellow powder; Yield: 79%; mp 204–206°C; IR (KBr, v, cm^{-1}): 3445 and 3340 (NH_2), 1705 (C=O), 1660 (C=O), 1635 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 1.06 (t, 3H, J = 7.8 Hz, CH_3), 2.43 (s, 3H, CH_3), 3.97 (q, 2H, J = 7.7 Hz, OCH_2), 6.55 (s, 1H, C1H-pyr), 7.65-802 (m, 6H, Ar-H), 8.27-8.33 (m, 2H, Ar-H), 8.72 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 14.38 (CH_3), 21.43 (Ar-CH_3), 59.13 (C1-pyr), 63.31 (OCH_2), 81.88 (C2-pyr), 124.72, 127.33, 127.49, 127.74, 128.91, 129.07, 131.07, 133.71, 134.63, 135.18, 136.97, 145.23, 151.11, 153.57 (Ar-C), 156.36 (C=O), 158.13 (C=O), 164.43 (COOEt). Anal. Calcd for $C_{24}H_{19}ClN_4O_4$ (462.89 g/mole): C, 62.27; H, 4.14; N, 12.10%. Found: C, 62.03; H, 4.32; N, 11.91%.

Ethyl 3-amino-1-(2-chloro-6-methoxyquinolin-3-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (4g). Pale yellow powder; Yield: 70%; mp 265–267°C; IR (KBr, v, cm^{-1}): 3460 and 3340 (NH_2), 1700 (C=O), 1670 (C=O), 1640 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 0.97 (t, 3H, J = 8.8 Hz, CH_3), 3.88 (s, 3H, OCH_3), 3.95 (q, 2H, J = 7.9 Hz, OCH_2), 6.57 (s, 1H, C1H-pyr), 7.31-8.07 (m, 6H, Ar-H), 8.31-8.33 (m, 2H, Ar-H), 8.54 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 14.52 (CH_3), 55.94 (Ar-OCH_3), 59.23 (C1-pyr), 64.01 (OCH_2), 82.17 (C2-pyr), 106.07, 123.79, 127.23, 127.79, 128.91, 129.09, 129.37, 132.43, 134.27, 135.22, 142.74, 148.06, 150.78, 153.55 (Ar-C), 157.29 (C=O), 158.05 (C=O), 164.30 (COOEt). MS m/z = 479.1 $[M+H]^+$. Anal. Calcd for $C_{24}H_{19}ClN_4O_5$ (478.88 g/mole): C, 60.19; H, 4.00; N, 11.70%. Found: C, 60.03; H, 3.84; N, 11.48%.

Ethyl 3-amino-1-(2,6-dichloroquinolin-3-yl)-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (4h). Pale yellow powder; Yield: 79%; mp 257–259°C; IR (KBr, v, cm^{-1}): 3455 and 3345 (NH_2), 1710 (C=O), 1665 (C=O), 1645 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 0.99 (t, 3H, J = 9.2 Hz, CH_3), 3.98 (q, 2H, J = 7.8 Hz, OCH_2), 6.59 (s, 1H, C1H-pyr), 7.84-8.06 (m, 6H, Ar-H), 8.22-8.33 (m, 2H, Ar-H), 8.73 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 14.27 (CH_3), 59.63 (C1-pyr), 63.87 (OCH_2), 82.18 (C2-pyr), 124.14, 124.88, 127.21, 127.54, 128.05, 128.63, 131.49, 132.39, 133.16, 133.94, 135.18, 146.75, 151.23, 154.03 (Ar-C), 157.12 (C=O), 158.51 (C=O), 164.37 (COOEt). Anal. Calcd for $C_{23}H_{16}Cl_2N_4O_4$ (483.30 g/mole): C, 57.16; H, 3.34; N, 11.59%. Found: C, 56.98; H, 3.15; N, 11.83%.

3-Amino-1-(2-chloroquinolin-3-yl)-6(or 9)-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (4i). Pale yellow powder; Yield: 73%; mp 218–220°C; IR (KBr, v, cm^{-1}): 3375 and 3180 (NH_2), 2205 (CN), 1685 (C=O), 1670 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 6.53 (s, 1H, C1H-pyr), 7.64-8.07 (m, 4H, Ar-H), 8.16-8.60 (m, 4H, Ar-H), 8.89 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 59.37 (C1-pyr), 60.94 (C2-pyr), 116.31, 125.93, 126.88, 127.35, 127.78, 127.97, 128.18, 128.83, 129.46, 129.67, 131.13, 134.27, 134.87, 146.39, 147.76, 151.94, 154.69 (Ar-C), 157.84 (C=O), 158.37 (C=O). Anal. Calcd for $C_{21}H_{11}ClN_6O_4$ (446.80 g/mole): C, 56.45; H, 2.48; N, 18.81%. Found: C, 56.14; H, 2.69; N, 18.52%.

3-Amino-1-(2-chloro-6-methylquinolin-3-yl)-6(or 9)-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (4j). Pale yellow powder; Yield: 69%; mp 231–233°C; IR (KBr, v, cm^{-1}): 3390 and 3175 (NH_2), 2210 (CN), 1690 (C=O), 1665 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 2.53 (s, 3H, CH_3), 6.59 (s, 1H, C1H-pyr), 7.58-8.01 (m, 3H, Ar-H), 8.18-8.56 (m, 4H, Ar-H), 8.76 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 21.61 (Ar-CH_3), 59.86 (C1-pyr), 61.31 (C2-pyr), 116.16, 124.98, 125.63, 126.27, 127.80, 128.53, 128.78, 129.28, 131.73, 132.17, 133.26, 134.68, 135.86, 146.04, 147.20, 151.36, 154.82 (Ar-C), 157.93 (C=O), 158.29 (C=O). MS m/z = 461.8 $[M+H]^+$. Anal. Calcd for $C_{22}H_{13}ClN_6O_4$ (460.83 g/mole): C, 57.34; H, 2.84; N, 18.24%. Found: C, 57.57; H, 2.57; N, 18.05%.

3-Amino-1-(2-chloro-6-methoxyquinolin-3-yl)-6(or 9)-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (4k). Pale yellow powder; Yield: 71%; mp 273–275°C; IR (KBr, v, cm^{-1}): 3380 and 3185 (NH_2), 2200 (CN), 1680 (C=O), 1665 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 3.78 (s, 3H, CH_3), 6.52 (s, 1H, C1H-pyr), 7.23-8.07 (m, 3H, Ar-H), 8.26-8.60 (m, 4H, Ar-H), 8.68 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 55.90 (Ar-OCH_3), 60.83 (C1-pyr), 61.71 (C2-pyr), 106.09, 116.32, 124.23, 127.22, 128.04, 128.74, 129.18, 129.58, 131.36, 133.48, 135.53, 137.08, 141.48, 145.85, 150.06, 154.32, 157.54 (Ar-C), 158.19 (C=O), 158.74 (C=O). Anal. Calcd for $C_{22}H_{13}ClN_6O_5$ (476.83 g/mole): C, 55.42; H, 2.75; N, 17.62%. Found: C, 55.21; H, 2.49; N, 17.87%.

3-Amino-1-(2,6-dichloroquinolin-3-yl)-6(or 9)-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carbonitrile (4l). Pale yellow powder; Yield: 81%; mp 224–226°C; IR (KBr, v, cm^{-1}): 3385 and 3175 (NH_2), 2205 (CN), 1685 (C=O), 1670 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 6.59 (s, 1H, C1H-pyr), 7.81-8.10 (m, 3H, Ar-H), 8.20-8.63 (m, 4H, Ar-H), 8.73 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 60.35 (C1-pyr), 61.58 (C2-pyr), 116.43, 124.17, 127.24, 127.82, 128.67, 128.83, 129.13, 129.78, 131.62, 132.75, 133.20, 134.75, 135.27, 144.16, 147.36, 151.84, 154.56 (Ar-C), 157.78 (C=O), 158.33

(C=O). Anal. Calcd for $C_{21}H_{10}Cl_2N_6O_4$ (481.85 g/mole): C, 52.41; H, 2.09; N, 17.46%. Found: C, 52.58; H, 2.00; N, 17.73%.

Ethyl 3-amino-1-(2-chloroquinolin-3-yl)-6(or 9)-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (4m). Pale yellow powder; Yield: 76%; mp 260-262°C; IR (KBr, v, cm^{-1}): 3450 and 3335 (NH_2), 1705 (C=O), 1665 (C=O), 1640 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 1.04 (t, 3H, J = 8.9 Hz, CH_3), 3.95 (q, 2H, J = 7.9 Hz, OCH_2), 6.57 (s, 1H, C1H-pyr), 7.56-8.06 (m, 4H, Ar-H), 8.16-8.64 (m, 4H, Ar-H), 8.83 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 14.53 (CH_3), 60.03 (C1-pyr), 63.48 (OCH_2), 82.76 (C2-pyr), 125.03, 126.87, 127.13, 127.46, 127.89, 128.55, 129.19, 129.47, 129.91, 131.21, 134.17, 135.83, 145.76, 146.33, 151.28, 151.83 (Ar-C), 156.17 (C=O), 158.45 (C=O), 164.21 (COOEt). Anal. Calcd. for $C_{23}H_{16}ClN_5O_6$ (493.86 g/mole): C, 55.94; H, 3.27; N, 14.18%. Found: C, 55.73; H, 3.04; N, 14.42%.

Ethyl 3-amino-1-(2-chloro-6-methylquinolin-3-yl)-6(or 9)-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (4n). Pale yellow powder; Yield: 80%; mp 276–278° C; IR (KBr, v, cm^{-1}): 3445 and 3340 (NH_2), 1705 (C=O), 1660 (C=O), 1635 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 1.09 (t, 3H, J = 8.9 Hz, CH_3), 2.47 (s, 3H, CH_3), 3.93 (q, 2H, J = 9.2 Hz, OCH_2), 6.53 (s, 1H, C1H-pyr), 7.69-8.03 (m, 3H, Ar-H), 8.22-8.65 (m, 4H, Ar-H), 8.77 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 14.44 (CH_3), 21.36 (Ar-CH_3), 59.84 (C1-pyr), 63.07 (OCH_2), 82.41 (C2-pyr), 124.23, 125.77, 127.05, 127.67, 128.81, 129.17, 129.63, 131.29, 131.84, 133.61, 135.24, 136.44, 144.93, 145.62, 151.18, 151.32 (Ar-C), 155.86 (C=O), 158.04 (C=O), 164.48 (COOEt). MS m/z = 508.6 $[M+H]^+$. Anal. Calcd for $C_{24}H_{18}ClN_5O_6$ (507.88 g/mole): C, 56.76; H, 3.57; N, 13.79%. Found: C, 56.59; H, 3.36; N, 13.97%.

Ethyl 3-amino-1-(2-chloro-6-methoxyquinolin-3-yl)-6(or 9)-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (4o). Pale yellow powder; Yield: 83%; mp 244–246 °C; IR (KBr, v, cm^{-1}): 3460 and 3340 (NH_2), 1700 (C=O), 1670 (C=O), 1640 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 0.93 (t, 3H, J = 7.7 Hz, CH_3), 3.78 (s, 3H, OCH_3), 3.91 (q, 2H, J = 6.8 Hz, OCH_2), 6.59 (s, 1H, C1H-pyr), 7.28-8.03 (m, 3H, Ar-H), 8.18-8.55 (m, 4H, Ar-H), 8.62 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 14.27 (CH_3), 55.62 (Ar-OCH_3), 59.74 (C1-pyr), 63.22 (OCH_2), 82.64 (C2-pyr), 106.17, 123.18, 127.33, 128.01, 128.66, 129.18, 129.53, 133.37, 134.81, 135.27, 136.89, 142.06, 145.83, 148.69, 151.03, 155.26 (Ar-C), 157.13 (C=O), 158.34 (C=O), 164.53 (COOEt). Anal. Calcd for $C_{24}H_{18}ClN_5O_7$ (523.88 g/mole): C, 55.02; H, 3.46; N, 13.37%. Found: C, 54.71; H, 3.75; N, 13.58%.

Ethyl 3-amino-1-(2,6-dichloroquinolin-3-yl)-6(or 9)-nitro-5,10-dioxo-5,10-dihydro-1H-pyrazolo[1,2-b]phthalazine-2-carboxylate (4p). Pale yellow powder; Yield: 81%; mp 234=236°C; IR (KBr, v, cm^{-1}): 3455 and 3345 (NH_2), 1710 (C=O), 1665 (C=O), 1645 (C=O);^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 0.96 (t, 3H, J = 8.9 Hz, CH_3), 3.95 (q, 2H, J = 9.0 Hz, OCH_2), 6.54 (s, 1H, C1H-pyr), 7.82-8.05 (m, 3H, Ar-H), 8.20-8.60 (m, 4H, Ar-H), 8.77 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 14.21 (CH_3), 60.23 (C1-pyr), 63.46 (OCH_2), 82.53 (C2-pyr), 124.34, 127.15, 127.72, 128.31, 128.86, 129.16, 129.64, 131.22, 132.54, 133.02, 133.59, 135.38, 145.63, 146.11, 151.19, 152.62 (Ar-C), 155.58 (C=O), 158.36 (C=O), 164.46 (COOEt). Anal. Calcd for $C_{23}H_{15}Cl_2N_5O_6$ (528.30 g/mole): C, 52.29; H, 2.86; N, 13.26%. Found: C, 52.46; H, 3.09; N, 13.01%.

Acknowledgments. The authors are thankful to Head, Department of Chemistry, Sardar Patel University for providing ^{1}H NMR and ^{13}C NMR spectroscopy and research facilities. We are also thankful to Oxygen Healthcare Research Pvt. Ltd., Ahmedabad, for providing mass spectroscopy facilities, Vaibhav Laboratories, Ahmedabad, Gujarat, India for the FT-IR, SICART, Vallabh Vidyanagar, for elemental analysis and Dhanji P. Rajani, Microcare Laboratory, Surat, Gujarat, India for antimicrobial screening of the compounds reported herein. One of the authors is grateful to UGC, New Delhi for Research Fellowship in Sciences for Meritorious Students.

REFERENCES AND NOTES

[1] Reddy, G. V.; Kanth, S. R.; Maitraie, D.; Narsaiah, B.; Rao, P. S.; Kishore, K. H. Eur J Med Chem 2009, 44, 1570.

[2] Abdel-Mohsen, S. A. Bull Korean Chem Soc 2005, 26, 719.

[3] Melendez Gomez, C. M.; Kouznetsov, V. V.; Sortino, M. A.; Alvarez, S. L.; Zacchino, S. A. Bioorg Med Chem 2008, 16, 7908.

[4] Dinakaran, M.; Senthikumar, P.; Yogeeswari, P.; China, A.; Nagaraja, V.; Sriam, D. Bioorg Med Chem Lett 2008, 18, 1229.

[5] Via, L. D.; Gia, O.; Gasparotto, V.; Ferlin, M. G. Eur J Med Chem 2008, 43, 429.

[6] Li-Ping, G.; Qing-Hao, J.; Guan-Rong, T.; Kyu-Yun, C.; Zhe-Shan, Q. J Pharma Pharmaceut Sci 2007, 10, 254.

[7] Charris, J. E.; Dominguez, J. N.; Gamboa, N.; Rodrigues, J. R.; Angel, J. E. Eur J Med Chem 2005, 40, 875.

[8] Clemence, F.; Le, M. O.; Delevallee, F.; Benzoni, J.; Jouanen, A.; Jouquey, S. J Med Chem 1988, 31, 1453.

[9] Sawada, Y.; Kayakiri, H.; Abe, Y.; Mizutani, T.; Inamura, N.; Asano, M.; Hatori, C.; Aramori, I.; Oku, T.; Tanaka, H. J Med Chem 2004, 47, 2853.

[10] Ko, T. C.; Hour, M. J.; Lien, J. C.; Teng, C. M.; Lee, K. H.; Kuo, S. C. Bioorg Med Chem Lett 2001, 11, 279.

[11] Khalil, A. M.; Berghot, M. A.; Gouda, M. A. Eur J Med Chem 2009, 44, 4448.

[12] Kim, J. S.; Lee, H.-J.; Suh, M.-E.; Choo, H.-Y.P.; Lee, S. K.; Park, H. J.; Kim, C.; Park, S. W.; Lee, C.-O. Bioorg Med Chem 2004, 12, 3683.

[13] Kim, J. S.; Rhee, H.-K.; Park, H. J.; Lee, S. K.; Lee, C.-O.; Choo, H.-Y.P. Bioorg Med Chem 2008, 16, 4545.

[14] Butnariu, R. M.; Caprosu, M. D.; Bejan, V.; Ungureanu, M.; Poiata, A.; Tuchilus, C.; Florescu, M.; Mangalagiu, I. I. J Heterocycl Chem 2007, 44, 1149.

[15] Zhang, L.; Guan, L.-P.; Sun, X.-Y.; Wei, C.-X.; Chai, K.-Y.; Quan, Z.-S. Chem Bio Drug Des 2009, 73, 313.

[16] Ryu, C.-K.; Park, R.-E.; Ma, M.-Y.; Nho, J.-H. Bioorg Med Chem Lett 2007, 17, 2577.

[17] Li, J.; Zhao, Y.-F.; Yuan, X.-Y.; Xu, J.-X.; Gong, P. Molecules 2006, 11, 574.

[18] Zelenin, K. N.; Bezhan, I. P.; Pastushenkov, L. V.; Gromova, E. G.; Mel'nikova, L. F.; Lesiovskaja, E. E.; Chakchir, B. A. Arzneimittel Forsch.s/Drug Res 1999, 49, 843.

[19] Tonra, J. R.; Surguladze, D.; Mitelman, S.; Kussie, P.; Bohlen, P.; Doody, J. F. Bioorg Med Chem 2009, 17, 731.

[20] Piatnitski, E. L.; Duncton, M. A. J.; Kiselyov, A. S.; Katoch-Rouse, R.; Sherman, D.; Milligan, D. L.; Balagtas, C.; Wong, W. C.; Kawakami, J.; Doody, J. F. Bioorg Med Chem Lett 2005, 15, 4696.

[21] Duncton, M. A. J.; Piatnitski, E. L.; Katoch-Rouse, R.; Smith, L. M.; Kiselyov, A. S.; Milligan, D. L.; Balagtas, C.; Wong, W. C.; Kawakami, J.; Doody, J. F. Bioorg Med Chem Lett 2006, 16, 1579.

[22] (a) Aziz Elassar, A. Z. A.; Elkholy, Y. M.; Elnagdi, M. H. Die Pharmazie 1996, 51, 714; (b) Ghahremanzadeh, R.; Shakibaei, G. I.; Bazgir, A. Synlett 2008, 1129.

[23] Nabid, M. R.; Rezaei, S. J. T.; Ghahremanzadeh, R.; Bazgir, A. Ultrason Sonochem 2010, 17, 159.

[24] (a) Thumar, N. J.; Patel, M. P. Arch Pharma Pharma Med Chem 2010, 2, 91; (b) Mungra, D. C.; Patel, M. P.; Patel, R. G. Med Chem Res DOI: 10.1007/s00044–010-9388–0; (c) Ladani, N. K.; Patel,

M. P.; Patel, R. G. Phosphorus Sulfur Silicon 2010, 185, 658; (d) Nirmal, J. P.; Patel, M. P.; Patel, R. G. Indian J Chem 2010, 49B, 587; (e) Thumar, N. J.; Patel, M. P. ARKIVOC 2009, XIII, 363; (f)] Mungra, D. C.; Patel, M. P.; Patel, R. G. ARKIVOC 2009, XIV, 64; (g) Ladani, N. K.; Patel, M. P.; Patel, R. G. ARKIVOC 2009, X, 292; (h) Thumar, N. J.; Patel, M. P. Phosphorus Sulfur Silicon 2009, 184, 2720; (i) Shah, N. K.; Patel, M. P.; Patel, R. G. Phosphorus Sulfur Silicon 2009, 184, 2704; (j) Ladani, N. K.; Patel, M. P.; Patel, R. G. Indian J Chem 2009, 48B, 261; (k) Shah, N. K.; Patel, M. P.; Patel R. G. Indian J Chem 2009, 48B, 1170; (l)] Nirmal, J. P.; Patel, M. P.; Patel R. G.; Indian J Chem B 2009, 48, 712; (m) Patel, N. A.; Patel, R. G.; Patel, M. P. J Environ Res Dev 2009, 3, 851; (n) Patel, N. A.; Surti, S. C.; Patel, R. G.; Patel, M. P. Phosphorus Sulfur Silicon 2008, 183, 2191; (o) Thakor, S. F.; Parmar, P. V.; Patel, M. P.; Patel, R. G. Saudi Pharma J 2008, 16, 64; (p) Thakor, S. F.; Patel, D. M.; Patel, M. P.; Patel, R. G. Saudi Pharma J 2007, 15, 48; (q) Patel, B. B.; Patel, R. G.; Patel, M. P. J Serb Chem Soc 2006, 71, 1015; (r) Patel, R. D.; Patel, M. P.; Patel, R. G. Indian J Chem B 2005, 44, 1944.

[25] National Committee for Clinical Laboratory Standards (NCCLS), 940, West Valley Road, Suite 1400, Wayne, Pennsylvania 19087–1898, USA. Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Supplement (ISBN 1–56238- 454 -6), 2002, M100-S12 (M7).

[26] Cohn, O. M.; Narine, B.; Tarnowaski, B. J Chem Soc Perkin Trans 1981, 1, 1520.