European Journal of Medicinal Chemistry 77 (2014) 288–297

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

New polyfunctional imidazo[4,5-*C*]pyridine motifs: Synthesis, crystal studies, docking studies and antimicrobial evaluation

Gilish Jose [a], T.H. Suresha Kumara [a,b,*], Gopalpur Nagendrappa [a], H.B.V. Sowmya [a], Jerry P. Jasinski [c], Sean P. Millikan [c], N. Chandrika [a], Sunil S. More [d], B.G. Harish [e]

[a] P.G. Department of Chemistry, Jain University, 127/2, Bull Temple Road, Chamarajpet, Bangalore 560026, India
[b] Department of Chemistry, U.B.D.T. College of Engineering, Davangere, Karnataka 577004, India
[c] Department of Chemistry, Keene State College, Keene, N.H. 03435-2001, USA
[d] P.G. Department of Biochemistry, C.P.G.S., Jain University, Jayanagar 3rd block, Bangalore 560011, India
[e] Department of Biotechnology, M.S. Ramaiah Institute of Technology, Bangalore 560054, India

ARTICLE INFO

Article history:
Received 19 October 2013
Received in revised form
12 February 2014
Accepted 6 March 2014
Available online 11 March 2014

Keywords:
Imidazo[4,5-*c*]pyridine
Microwave-assisted organic synthesis
X-ray crystal studies
Antimicrobial activity
Molecular docking studies

ABSTRACT

New antimicrobial agents, imidazo[4,5-*c*]pyridine derivatives have been synthesized. We have developed a new synthetic protocol for the final reaction, an efficient microwave-assisted synthesis of imidazo[4,5-c]pyridines from substituted 3,4-diaminopyridine and carboxylic acids in presence of DBU mediated by T3P. The chemical structures of the new compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectral analysis and elemental analysis. In addition, single crystal X-ray diffraction has also been recorded for compound **9c**. The *in vitro* antimicrobial activities of the compounds were conducted against various Gram-negative, Gram-positive bacteria and fungi. Amongst the tested compounds **9c**, **9e**, **9g**, **9k** and **9l** displayed promising antimicrobial activity. The molecular docking of GlcN-6-P synthase with newly synthesized compounds was carried out.

1. Introduction

Infections caused by bacteria and fungi remain a major health concern due to the development of resistance to existing antimicrobial agents. The increasing incidences of Gram-positive and Gram-negative bacterial resistance to antibiotics such as chloramphenicol, streptomycin, tetracycline, sulfadiazine, bacitracin and azithromycin have caused life-threatening infectious diseases [1,2]. On the other hand, the systemic and dermal fungal infections such as Candidiasis, Cryptococcosis and Aspergillosis have significantly increased due to the resistance to the currently available antifungal azoles [3,4]. The emergence of multiple drug resistant microorganisms has caused major health concern worldwide. Therefore, there is an increasing need to design and synthesize new antimicrobial agents with broad spectrum of activities.

The imidazopyridine moiety is one of the most ubiquitous heterocyclic scaffolds present in many natural and unnatural compounds with significant biological applications such as antimicrobial [5–7], anti-inflammatory [8], anticancer [9,10], antiviral [11], antimitotic [12] and inotropic activity [13,14]. Thus, the synthesis of polyfunctional imidazopyridine derivatives is an important area of research. We report herein, synthesis, crystal studies, docking studies and antimicrobial evaluation of polyfunctional imidazo[4,5-*c*]pyridine derivatives. The molecular docking of newly synthesized compounds was carried out with the enzyme, L-glutamine: D-fructose-6-phosphate amidotransferase (EC 2.6.1.16), known under the trivial name of glucosamine-6-phosphatesynthase (GlcN-6-P synthase), as such new target for antimicrobial studies [15].

2. Results and discussion

2.1. Chemistry

We describe here an efficient method for the synthesis of novel imidazo[4,5-*c*]pyridine derivatives (**9a**–**l**) (Scheme 1). The commercially available **1** (2-Chloro-4-amino pyridine) was iodinated with ICl and KOAc in glacial acetic acid at 70 °C to afford a mixture of iodopyridines **2** (2-chloro-5-iodopyridin-4-amine), **3** (2-

* Corresponding author. Department of Chemistry, U.B.D.T. College of Engineering, Davangere, Karnataka 577004, India.
E-mail addresses: suresha.kumara@rediffmail.com, suresha.kumara@gmail.com (T.H. Suresha Kumara).

http://dx.doi.org/10.1016/j.ejmech.2014.03.019
0223-5234/

Abbreviations

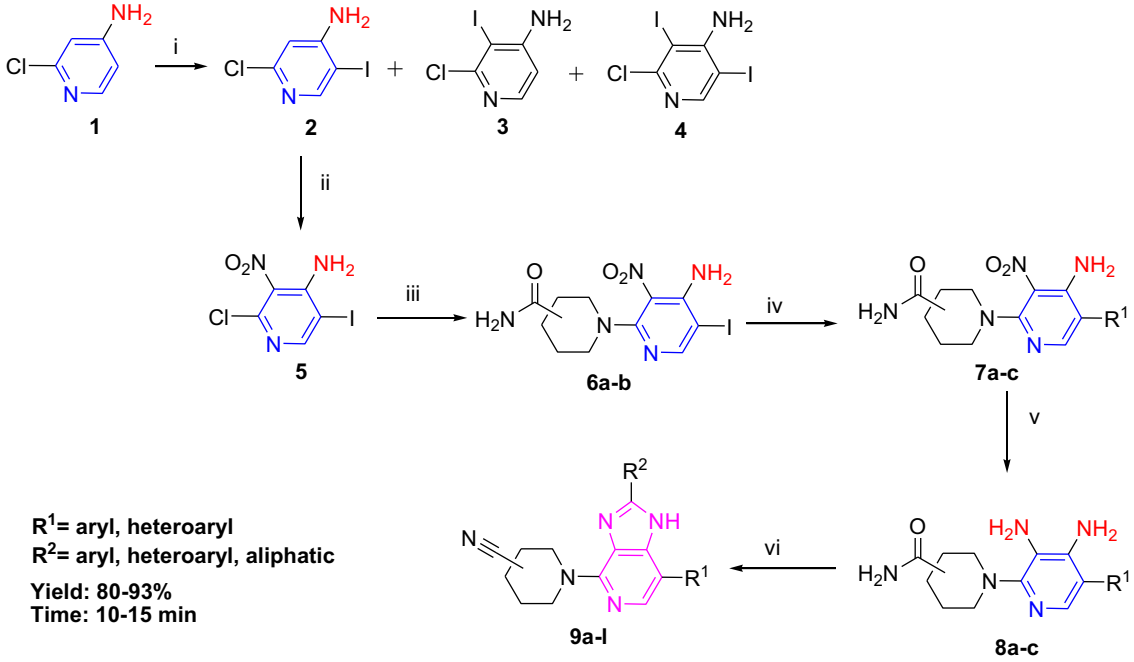
T3P	propyl phosphonic anhydride
DBU	1,8-diazabicycloundec-7-ene
TEA	triethylamine
DIPEA	N,N-diisopropylethylamine
ADME-Tox or ADMET	absorption, distribution, metabolism and excretion–toxicity
RMSD	root-mean-square deviation

chloro-3-iodopyridin-4-amine) and **4** (2-chloro-3,5-diiodopyridin-4-amine) in the ratio 45:45:10. The iodopyridines were isolated by preparative HPLC (normal phase). The structures of iodopyridines were assigned on the basis of ^{1}H NMR, mass spectral analysis and elemental analysis. ^{1}H NMR spectrum of elution 1 (**4**) showed two singlet peaks at δ 8.17 and 6.51 ppm corresponds to aromatic hydrogen at C6 and NH_2 group; elution 2 (**2**) showed three singlet peaks at δ 8.18, 6.65, 6.50 ppm corresponds to aromatic protons at C3, C6 and NH_2 group, whereas elution 3 (**3**) showed two doublet peaks and a single peak at δ 7.73, 6.52 and 6.50 corresponds to aromatic protons at C5, C6 and NH_2 group. Treatment of **2** with nitrating mixture (Conc.H_2SO_4/fuming HNO_3) gave **5**. Nucleophilic displacement of chloro group at C2 in **5** with piperidine carboxamides using 1,4-dioxane-water mixture (7:3) at 120 °C gave **6a**–**b**. The Suzuki reaction between **6** and aryl or heteroaryl boronic acids (R^1) catalysed by Pd(0) complex gave **7a**–**c**. The reduction of nitro group at C3 in **7a**–**c** by hydrogenation using 10% palladium on charcoal gave **8a**–**c**. ®T3P promotes cyclization of **8** with carboxylic acids (R^2-COOH) in presence of DBU under microwave irradiation to imidazo[4,5-*c*]pyridine derivatives (**9a**–**l**). The one pot procedure is efficient and allows short reaction times, easy work-up, and good yields. ®T3P [16,17] is a reactive cyclic anhydride and a powerful water scavenger, widely employed as a coupling agent for the synthesis of peptides [18–20]. T3P serves as a powerful dehydrating agent to build various heterocycles [21–23]. It offers several advantages over other reagents with higher yields, shorter reaction duration, ease of isolation of the products, slight side reactions, inexpensive, and nontoxic nature.

The compound **9a** was selected as a prototype compound to screen the final reaction conditions. We first conducted reaction with compound **8a** and (phenylthio) acetic acid in DMF in presence of TEA and T3P (50% solution in DMF) (3 equivalent) under conventional silicone oil bath heating at 150 °C. The reaction was completed after 20 h with 30% yield. The reaction monitoring data (by LCMS) revealed that $CONH_2$ group was not fully dehydrated into C≡N group. Then, heated at 180 °C observed that cyclization and dehydration were completed simultaneously after 15 h with 50% yield (entry 1, Table 1). IR spectrum of **9a** revealed that appearance of bands characteristic for imidazole NH at 3269 cm^{-1} and C≡N group at 2241 cm^{-1}, while it's ^{1}H NMR spectrum exhibited a broad singlet at δ 10.06 ppm assigned for imidazole NH proton. Mass spectra of **9a** showed expected *m/z* 444.20 $[M + H]^+$ corresponding with proposed isotopic mass (443.16). The obtained elemental analysis values are in consonance with theoretical data.

Next, we explored the effects of different bases such as DIPEA and DBU on the rate of the reaction or yield (Table 1). The reaction was completed after 12 h with 60% yield in presence of DIPEA (entry 2, Table 1). In presence of DBU, the total conversion was achieved after 6 h with 75% yield. Thus, DBU is acting as an efficient base to achieve complete conversion (entry 3, Table 1).

With the aim to reduce the reaction time, we conducted the reaction under microwave irradiation (solvent free) with different power variables such as 50 W, 75 W and 100 W. We first evaluated the effect of changes at 180 °C during 10 min (entries 1–3, Table 2). Total conversion was not achieved at 50 W (entry 1, Table 2) and at 100 W the yield was reduced to 78%, because of decaying the product (entry 3, Table 2). An excellent yield of 88% was gained at



Scheme 1. Synthesis of Imidazo[4,5-*c*]pyridine derivatives; Reagents and conditions: (*i*) ICl and KOAc in glacial acetic acid at 70 °C; (*ii*) Conc.H_2SO_4 and fuming HNO_3; (*iii*) piperidine carboxamide in 1,4-dioxane-water (7:3) at 120 °C; (*iv*) R^1-boronic acid, K_2CO_3 and $[PdCl_2(dppf)]CH_2Cl_2$ in 1,2-dimethoxyethane–water (9:1) at 120 °C; (*v*) H_2 (40 psi), Pd/C in ethanol-THF (7:3); (*vi*) R^2-COOH, DBU, T3P, MW, 75 W at 180 °C.

Table 1
Optimization of conditions for synthesis of **9a**.

Sl. no.	Base	Classical heating[a]	
		Time (h)	Yield (%)
1	TEA	15	50
2	DIPEA	12	60
3	DBU	6	75

[a] DMF as sovent at 180 °C.

75 W (entry 2, Table 2). The temperature decrease to 150 °C was resulted in lower yield (entries 4 and 5, Table 2). A temperature of 180 °C and a power of 75 W were proved to be the best irradiation parameters.

The synthesis of imidazo[4,5-*c*]pyridine derivatives (**9a**–**l**) from carboxylic acids (R^2) and 3,4-diaminopyridine **8** in presence of DBU mediated by T3P under microwave irradiation. After screening the conditions, we found that microwave irradiation (75 W) at 180 °C for 10–15 min in presence of DBU and T3P (solvent free) gave the best results in terms of conversion, purification/isolation, and yield. The scope of this new protocol was investigated with all substrates shown in Table 3 and synthesized imidazo[4,5-*c*]pyridine derivatives (**9a**–**l**) in 80–93% yield. The structures of the final products were assigned on the basis of their IR, ^{1}H NMR, ^{13}C NMR and mass (LCMS) spectral analysis as well as elemental analysis.

2.2. X-ray crystallographic analysis

The structure of the compound **9c** (Fig. 1) was unequivocally established by X-ray crystallographic analysis. Single crystal of **9c** was obtained through the slow evaporation of its ethanol solution. A suitable crystal ($C_{25}H_{22}N_5OF$, compound **9c**) was selected and analysed on a Xcalibur, Eos, Gemini diffractometer. The crystal was kept at 173(2) K during data collection. Using Olex2 [24], the structure was solved the Superflip [25] structure solution program using Charge Flipping and refined with the ShelXL [26] refinement package using Least Squares minimization.

Crystal data for $C_{25}H_{22}N_5OF$ (M = 427.47): monoclinic, space group $P2_1/a$ (no. 14), a = 10.5330(5) Å, b = 16.8672(7) Å, c = 12.9704(6) Å, β = 111.127(6)°, V = 2149.46(18) Å^3, Z = 4, T = 173(2) K, μ(CuKα) = 0.731 mm^{-1}, D_{calc} = 1.321 g/mm^3, 13,948 reflections measured (8.996 ≤ 2Θ ≤ 145.056), 4227 unique (R_{int} = 0.0398) which were used in all calculations. The final R_1 was 0.0739 ($I > 2\sigma(I)$) and wR_2 was 0.2092 (all data) (Table 4).

In 1-(7-(3-fluorophenyl)-2-(phenoxymethyl)-1*H*-imidazo[4,5-*c*]pyridin-4-yl)piperidine-3-carbonitrile ($C_{25}H_{22}N_5OF$, **9c**), the 6-membered piperidine ring is in a slightly distorted chair configuration with Cremer and Pople [27] parameters Q = 0.573(5), Å, θ = 171.7(4)°, φ = 176.804(7)° (Fig. 1). The dihedral angle between the mean planes of the 9-membered *1H-imidazo[4,5-c]pyridine* and two phenyl rings is 44.9(8)° and 75.6(3)°. Bond lengths are in normal ranges [28] (Table 5). In the crystal, an N···H…N hydrogen bond links the molecules into chains along [010] (Table 6).

Table 2
Screening irradiation parameters for the synthesis of **9a**.

Entry	Power (W)	Temp (°C)	Time (min)	Conversion (%)	Yield (%)[a]
1	50	180	10	80	75
2	75	180	10	100	88
3	100	180	10	100	80
4	75	150	10	85	78
5	75	150	25	100	85

[a] Isolated yield.

2.3. Biology

All the newly synthesized compounds were evaluated *in vitro* for their antimicrobial activity against various pathogenic bacteria and fungal stains by agar well diffusion method. The antibacterial activities are carried out against two Gram-positive bacteria stains, *Bacillus cereus* (NCIM-2155) and *Staphylococcus aureus* (MTCC-4102) and two Gram-negative bacteria stains, *Escherichia coli* (NCIM-2931) and *Pseudomonas aeruginosa* (MTCC 4727). For the antifungal activity, compounds were screened against *Candida albicans* (MTCC-183) and *Saccaromyces cerevisiae* (NCIM-3044). Streptomycin and Fluconazole were used as antibacterial and antifungal references, respectively. The *in vitro* antibacterial activity of the synthesized compounds was found to be moderatively active, slightly active or inactive compared to standard drugs (Table 7). The inhibition zone diameter data analysis indicated that compounds **9c**, **9e**, **9g**, **9k** and **9l** moderately or strongly inhibited the growth of the tested microorganisms.

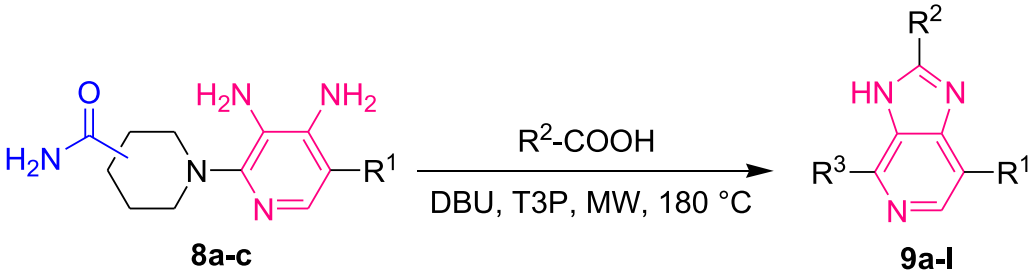
Five compounds (**9c**, **9e**, **9g**, **9k** and **9l**) from the above result were tested for antimicrobial activity (MIC) against respective microorganisms and their IC_{50} values were calculated (Table 8). Compounds **9c** and **9g** were showed good anti-yeast activity against *S. cerevisiae*. Compound **9g** showed significant anti-yeast activity with IC_{50} 98.8 ± 1.03 μg/mL. Compounds **9e** and **9k** exerted good antifungal activity against *C. albicans*. Compound **9k** exerted good antifungal activity against *C. albicans* with IC_{50} 73.7 ± 0.06 μg/mL. Compound **9g** showed significant antibacterial activity against Gram-negative bacteria *E. coli* with IC_{50} 74.48 ± 1.19 μg/mL. Compound **9l** exerted good antibacterial activity against Gram-positive bacteria *S. aureus* with IC_{50} 100 ± 1.03 μg/mL. Concerning the antibacterial and anti-yeast activity compound **9g** was found to be the most active in the series against *E. coli* and *S. cerevisiae*.

2.4. Molecular docking studies

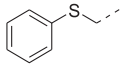
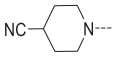
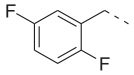
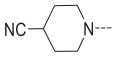
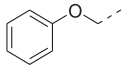
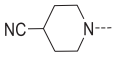
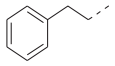
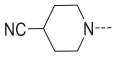
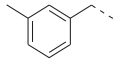
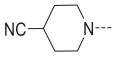
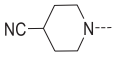
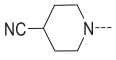
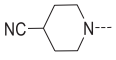
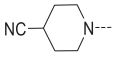
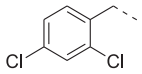
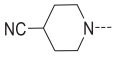
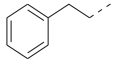
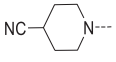
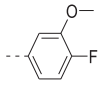
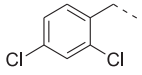
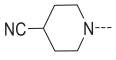
All twelve compounds were predicted for different properties like drug-likeliness, human intestinal absorption, and *in vitro* skin permeability using PreADMET server [29,30]. The results are summarized in Table 9. The molecular docking of glucosamine-6-phosphate synthase (GlcN-6-P synthase) with the new imidazo [4,5-*c*]pyridine motifs and the standard drug fluconazole yielded best possible conformations with parameters including the docking energy, inhibition constant and RMSD (Table 9). Compounds **(9a–l)** showed very good docking energy ranging from −11.24 kcal/mol to −13.18 kcal/mol because halogen atoms have formed intermolecular bonds with the active site amino acids of GlcN-6-P synthase in a fashion that resembles the H-bonds [31–33]. The compounds containing halogen atoms will also improve the oral absorption, skin penetration and also increase membrane permeability [34].

The minimum docked energy was found in the analogue **9c** (−13.18 kcal/mol) with an estimated inhibition constant of 3.53 × 10^{-9} and RMSD 0.48. Whereas, docked energy of the standard drug fluconazole was −5.67 kcal/mol with an inhibition constant of 5.53 × 10^{-5} and RMSD 1.97. In *in vitro* antimicrobial studies the compound **9c** showed significant inhibition of *S. cerevisiae*. So it can be predicted as the activity may be due to inhibition of enzyme GlcN-6-P synthase, which catalyses a complex reaction involving ammonia transfer from L-glutamine to Fru-6-P followed by isomerisation of the formed fructosamine-6-phosphate to glucosamine-6-phosphate.

Table 3
Synthesis of imidazo[4,5-c]pyridine derivatives (**9a**–**l**).



8a-c **9a-l**

Compound	R^1	R^2	R^3	Microwave irradiation	
				Time (min)	Yield (%)
9a				10	88
9b				10	90
9c				10	87
9d				10	89
9e				10	91
9f				12	82
9g				15	80
9h				12	85
9i				12	86
9j				10	89
9k				10	86
9l				10	93

3. Conclusions

In summary, we have synthesized new antimicrobial leads with potent activity against various Gram-positive, Gram-negative bacterial and fungal stains. Most of the synthesized compounds (e.g. **9c**, **9e**, **9g**, **9k** and **9l**) showed moderate to good antimicrobial activity compared to the control antibiotic (*Streptomycin*) and the antifungal drug (*Fluconazole*). The structure-antimicrobial activity relationship of these derivatives was explained by molecular docking. These compounds can be considered as an initial leads for the development of better antibacterial and antifungal agents.

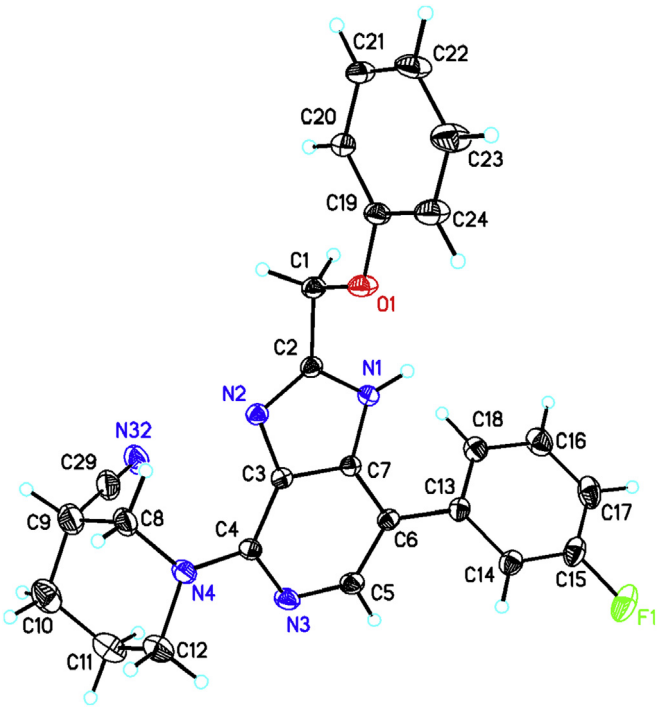


Fig. 1. ORTEP drawing of **9c** shows the labelling scheme and 30% probability displacement ellipsoids.

4. Experimental section

4.1. General methods

All reagents and solvents were used as purchased from commercial suppliers without further purification. All reactions performed under an inert atmosphere of dry nitrogen using dry solvents. Compounds **9a**–**l** synthesized by using a Biotage® Initiator microwave synthesizer. Reactions monitored by TLC analysis using silica gel 60 F_{254} thin layer chromatography plates. Column chromatographic separations were performed using silica gel 60–120 and 230–400 mesh; Ethyl acetate, Petroleum ether, Dichloromethane and Methanol as the solvent system. All the final compounds were characterized by IR, NMR (^{1}H and ^{13}C), LCMS, CHNS analysis and melting point analysis (see Supporting Information). The NMR spectra was recorded at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR), referenced to an internal standard (TMS) or residual solvent protons and chemical shifts (δ) reported in ppm. The splitting patterns were designated as s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; dt, doublet of triplet; m, multiplet; brs, broad singlet. The coupling constant *J* reported in Hz. The CHN analysis was recorded in Elementar vario MICRO cube. The mass recorded in Agilent LCMS 1100 series instrument using ESI-APCI positive scan. IR spectra were recorded on an infrared Fourier Transform spectrometer. Melting points of samples were determined in open capillary tubes using Buchi Melting point B-540 apparatus and are uncorrected.

Table 4
Crystal data and structure refinement for **9c**.

Identification code	**9c**
Empirical formula	$C_{25}H_{22}N_5OF$
Formula weight	427.47
Temperature/K	173(2)
Crystal system	Monoclinic
Space group	$P2_1/a$
a/Å	10.5330(5)
b/Å	16.8672(7)
c/Å	12.9704(6)
α/°	90
β/°	111.127(6)
γ/°	90
Volume/Å^3	2149.46(18)
Z	4
ρ_{calc}mg/mm^3	1.321
m/mm^{-1}	0.731
F(000)	896
Crystal size/mm^3	0.44 × 0.36 × 0.22
2Θ range for data collection	8.996–145.056°
Index ranges	$-9 \le h \le 12$, $-20 \le k \le 19$, $-15 \le l \le 10$
Reflections collected	13,948
Independent reflections	4227[R(int) = 0.0398]
Data/restraints/parameters	4227/0/294
Goodness-of-fit on F^2	1.028
Final R indexes [$I \ge 2\sigma$ (I)]	$R_1 = 0.0739$, $wR_2 = 0.2026$
Final R indexes [all data]	$R_1 = 0.0803$, $wR_2 = 0.2092$
Largest diff. peak/hole/e Å^{-3}	1.16/−0.39

4.2. Experimental procedure

4.2.1. Experimental procedure for the synthesis of 2-chloro-5-iodopyridin-4-amine (*2*)

A mixture of 2-chloro-4-amino pyridine **1** (20 g, 0.15 mol), potassium acetate (22.9 g, 0.23 mol) and ICl (27.7 g, 0.17 mol) in glacial acetic acid (200 mL) was heated to 70 °C for 4 h. The solvent was concentrated under reduced pressure. The residue was neutralized with 10% $NaHCO_3$ solution (250 mL) and extracted with two 300-mL portions of EtOAc. The combined organic extracts were washed with brine (200 mL) and dried over anhydrous Na_2SO_4. The solvent was removed in vacuum. The crude product showed a mixture of iodopyridines **2**, **3** and **4** in the ratio 45:45:10. The required compound **2** (elution 2) was isolated via normal phase preparative HPLC (mobile phase: 60:40, 0.1% TFA in hexane-IPA; column: SunFire Silica 19 * 150 mm, 5 μm; Flow rate: 18.0 mL/min) in 49.7% yield (19.7 g) as an off white solid.

4.2.2. Experimental procedure for the synthesis of 2-chloro-5-iodo-3-nitropyridin-4-amine (*5*)

The compound **2** (19.5 g, 0.07 mol) was mixed in Conc.H_2SO_4 (58 mL) and cooled in an ice bath; fuming HNO_3 (5.8 mL) was added drop wise via a syringe. The reaction was stirred for 4 h at room temperature. The reaction mixture was slowly poured onto the crushed ice cubes, stirred well and neutralized with aqueous ammonia (20 mL). The solid formed was collected by filtration, washed with water and dried to provide compound **5** in 85% yields (19.5 g) as a yellow solid.

4.2.3. General experimental procedure for the synthesis of compounds (*6a*–*b*)

An oven-dried Pressure tube was charged with compound **5** (10 g, 0.03 mol), piperidine carboxamide (0.06 mol) and 7:3 mixture of 1,4-dioxane-water (150 mL). The Pressure tube was capped with a Teflon screw cap. The resulting mixture was heated to 120 °C for 6 h. The solvent was concentrated under reduced pressure. The residue was neutralized with 10% $NaHCO_3$ solution (100 mL) and extracted with two 150-mL portions of CH_2Cl_2. The combined organic extracts were washed with of brine (75 mL) and dried over anhydrous Na_2SO_4. The solvent was removed in vacuum. The crude product was purified via column chromatography on silica gel (60–120 mesh) to provide the title compound.

Table 5
Selected Crystal Bond Lengths (Å), Bond angles (°), and Torsion angles (°) for $C_{25}H_{22}N_5OF$.

$C_{25}H_{22}N_5OF$			
N···C2	1.365(3)	N1···C7	1.368(3)
N2···C2	1.309(3)	N2···C3	1.393(3)
N3···C4	1.335(3)	N3···C5	1.347(3)
N4···C4	1.376(3)	N4···C8	1.427(3)
N4···C12	1.467(4)	N32···C29	1.137(4)
O1···C1	1.424(3)	O1···C19	1.375(3)
C1···C2	1.493(3)	C9···C29	1.481(5)
C19···O1···C1	117.17(19)	C2···N2···C3	104.32(19)
C4···N4···C8	124.5(2)	C8···N4···C12	113.0(2)
O1···C1···C2	106.47(19)	N2···C2···N1	113.3(2)
N2···C3···C7	110.2(2)	C7···C3···C4	118.3(2)
N3···C4···N4	117.0(2)	N3···C4···C3	118.9(2)
N4···C4···C3	123.9(2)	N3···C5···C6	126.5(2)
N32···C2···C9	178.6(4)	N4···C8···C9	107.2(2)
N2···C3···C4···N3	−177.6(2)	N2···C3···C4···N4	−2.5(4)
N4···C8···C9···C10	53.8(3)	N4···C8···C9···C29	−66.8(3)
C4···N4···C8···C9	96.9(3)	C4···N4···C12···C11	−95.3(3)
C5···N3···C4···N4	−173.5(2)	C5···N3···C4···C3	2.0(4)
C8···N4···C4···N3	−160.5(3)	C8···N4···C4···C3	24.3(4)
C8···N4···C12···C11	66.3(3)	C12···N4···C8···C9	−63.7(3)

4.2.4. General experimental procedure for the synthesis of compounds (**7a**–**c**)

An oven-dried Schlenk tube was charged with compound **6** (10 g, 25.56 mmol), boronic acid (38.34 mmol), powdered K_2CO_3 (7 g, 51.12 mmol) and 9:1 mixture of 1,2-dimethoxyethane-water (150 mL). The Schlenk tube was capped with a rubber septum, evacuated and backfilled with argon (this sequence was carried out four times). 1,1′-Bis(diphenylphosphino)ferrocene-palladium(II) dichloride-dichloromethane complex (1.0 g, 1.27 mmol) was added and the Schlenk tube was sealed with a Teflon screw cap. The reaction mixture was heated to 120 °C for 3 h. The reaction mixture was filtered through a thin pad of celite (eluted with CH_2Cl_2) and the eluent was concentrated under reduced pressure. The crude product was purified via flash column chromatography on silica gel (230–400 mesh) to provide the title compound.

4.2.5. General experimental procedure for the synthesis of compounds (**8a**–**c**)

The Parr shaker bottle was charged with compound **7** (1 g) and 7:3 mixture of ethanol-THF (20 mL). 10% Palladium on carbon (0.2 g, 20 wt %) was added and the reaction mixture was hydrogenated (40 psi) at room temperature for 2 h. The reaction mixture was filtered through celite bed and the filtrate was concentrated under reduced pressure to provide the title compound.

4.2.6. General experimental procedure for the synthesis of compounds (**9a**–**l**)

1-(7-(3-fluorophenyl)-2-(phenylthiomethyl)-1*H*-imidazo[4,5-*c*]pyridin-4-yl)piperidine-3-carbonitrile (**9a**) as an example: The microwave tube was charged with compound **8a** (250 mg, 0.76 mmol), phenylthio acetic acid (165.9 mg, 0.98 mmol), DBU (462.2 mg, 3.04 mmol) and T3P (50% solution in DMF, 1.3 mL, 2.27 mmol). The sealed microwave tube was irradiated (75 W) at 180 °C in a microwave initiator for 10 min. The reaction mixture was dissolved in 15 mL of EtOAc and the organic layer extracted with 8 mL of 10% $NaHCO_3$ solution and 5 mL of brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and the solvent was removed in vacuum. The crude product was purified via flash column chromatography on silica gel (230–400 mesh) to provide compound **9a** in 88% yield (296.2 mg) as a pale brown solid.

Table 6
Hydrogen bond interactions for $C_{25}H_{22}N_5OF$ [Å and °].

D-H…A	d(D-H)	d(H…A)	d(D…A)	<(DHA)
N1···H1…N32#1	0.88(3)	2.11(4)	2.933(3)	155(3)

Symmetry transformations used to generate equivalent atoms: #1 1−x, 1−y, 2−z.

Table 7
Antimicrobial activity of compounds (**9a**–**l**).

Compound	Conc. (μg/mL)	Antibacterial activity[a]				Antifungal activity[a]	
		E. coli	*P. aeruginosa*	*B. cerus*	*S. aureus*	*C. albicans*	*S. cerevisiae*
9a	500	–	5.2	–	–	–	–
	1000	–	11.0	–	–	–	–
9b	500	–	–	–	–	–	–
	1000	–	–	–	–	–	–
9c	500	–	–	2.8	–	–	8.7
	1000	–	–	6.0	–	–	19.0
9d	500	–	–	6.7	–	–	–
	1000	–	–	13.0	–	–	–
9e	500	–	6.2	–	–	6.8	–
	1000	–	13.0	–	–	15.0	–
9f	500	6.0	–	–	–	–	–
	1000	12.5	–	–	–	–	–
9g	500	8.0	–	–	–	–	6.9
	1000	16.0	–	–	–	–	15.0
9h	500	–	–	–	–	–	–
	1000	–	–	–	–	–	–
9i	500	–	–	–	–	–	–
	1000	–	–	–	–	–	–
9j	500	–	–	–	–	–	–
	1000	–	–	–	–	–	–
9k	500	5.4	–	–	4.2	12.3	–
	1000	12.0	–	–	10.0	22.0	–
9l	500	4.1	3.5	–	10.5	–	–
	1000	9.0	8.0	–	23.0	–	–
Streptomycin	10	21.4	20.9	20.2	23.8	–	–
Fluconazole	10	–	–	–	–	25.0	24.0

[a] Zone of inhibition in mm, hyphen denotes no activity.

4.2.7. In vitro antimicrobial procedure

All the newly synthesized compounds were evaluated *in vitro* for their antimicrobial activity. The antimicrobial activities are carried out against two Gram-positive bacterial strains *S. aureus* (MTCC-4102) and *B. cereus* (NCIM-2155), two Gram-negative bacteria stains *E. coli* (NCIM-2931) and *P. aeruginosa* (MTCC-4727) and two fungal stains *C. albicans* (MTCC-183), *S. cerevisiae* (NCIM-3044) using the nutrient agar disc diffusion method [35] at 500 μg/mL and 1000 μg/mL concentration. DMSO was used as blank exhibited no activity against any of the used organisms. The antimicrobial activity was determined by measuring of the inhibition zone, after 20 h of incubation at 37 °C for bacterial strains and 4 days at 37 °C for fungal strains. The diameters of the inhibition zones were measured in mm. Streptomycin and Fluconazole were used as standard drugs against bacterial and fungal strains, respectively at 10 mg/mL concentration.

MIC values for the synthesized compounds were determined by using broth micro dilution [36]. Standard and isolated stain of *E.*

Table 8
Antimicrobial activity MIC and IC_{50} of compounds (**9c**, **9e**, **9g**, **9k** and **9l**).

Compound	Microorganism	MIC (μg/mL)	IC_{50} (μg/mL)
9c	*Saccaromyces cerevisiae*	384	192 ± 0.05
9e	*Candida albicans*	404.2	206.4 ± 0.08
9g	*Escherichia coli*	197.6	74.48 ± 1.19
	Saccaromyces cerevisiae	296.4	98.8 ± 1.03
9k	*Candida albicans*	195.4	73.7 ± 0.06
9l	*Staphylococcus aureus*	200	100 ± 1.03

Table 9
Molecular docking of imidazo[4,5-*c*]pyridine motifs with glucosamine-6-phosphate synthase.

Entry	Human intestinal absorption (%)	*In vitro* skin permeability (logKp, cm/hour)	Docking energy (kcal/mol)	Inhibition constant (M)	Amino acid residue involved in H-bond	Bond length (Å)
9a	95.94	−2.78	−12.27	5.17×10^{-8}	His 77:HE2::**9a**:NAC	1.71
9b	94.96	−3.14	−12.98	2.60×10^{-9}	His 77:HE2::**9b**:NAC	2.13
9c	95.10	−3.07	−13.18	3.53×10^{-9}	His 77:HE2::**9c**:NAC	2.13
9d	95.04	−2.62	−12.82	5.08×10^{-9}	His 77:HE2::**9d**:NAC	1.85
9e	95.04	−2.72	−12.54	1.82×10^{-9}	His 77:HE2::**9e**:NAC	2.15
9f	94.36	−3.29	−12.35	4.59×10^{-9}	Arg 73:HH21::**9f**:NA	2.03
9g	94.32	−3.59	−11.89	4.96×10^{-9}	His 77:HE2::**9g**:NAC	1.98
9h	94.22	−3.09	−11.24	2.58×10^{-8}	Arg 73:HH21::**9h**:NAV	1.92
					His 77:HE2::**9h**:NAX	2.05
9i	94.21	−2.95	−12.30	6.96×10^{-9}	No H-Bond	–
9j	95.95	−3.42	−11.95	1.15×10^{-8}	Gly 99:HN::**9j**:NAU	1.78
9k	94.82	−3.27	−11.85	2.45×10^{-8}	His 77:HE2::**9k**:NAP	1.96
					Gly 99:HN::**9k**:NAT	1.72
9l	95.98	−3.07	−11.21	3.81×10^{-8}	No H-Bonds	–
Fluconazole	–	–	−5.36	5.53×10^{-5}	Arg 73:HH21::DF:OA	2.20
	–	–			Arg 73:HE::DF:OA	2.09
	–	–			His 77:HE2::Dp10:NA	1.87

coli, *S. aureus* were used to determine the antibacterial activity and *S. cerevisiae*, *C. albicans* were used to determine the antifungal activity as the compounds showed the activity against the above mentioned organism. Streptomycin and fluconazole were used as the references. The bacterium was cultivated in Mueller-Hinton agar and was diluted with Mueller-Hinton broth. All fungi were cultivated in Sabouraud dextrose agar and were diluted with sabouraud broth. The synthesized compounds and references were dissolved in DMSO at a concentration of 1000 μg/mL. Two fold dilutions of the synthesized compounds and reference compound were added to the wells (550… 4.3 μg/mL). Then a suspension of microorganism was inoculated into the wells. Final inoculums concentrations in the wells were 100 cfu/mL for bacteria and 2.5 × 100 cfu/mL for fungi. The plates were incubated at 37 °C for 24 h for the antibacterial activity and at 37 °C for 48 h for antifungal activity. At the end of the incubation period, MIC values were recorded as the lowest concentration of the substances that has no visible turbidity.

4.2.8. Molecular docking studies procedure

The protein-drug interaction was studied by automated docking to determine the orientation of inhibitors bound to the active site of target protein GlcN-6-P synthase. A genetic algorithm method, implemented in the program AutoDock 4.2, was employed [37]. The 2D structures (.mol) of all the twelve compounds are converted to 3D structure (.pdb) using Openbable software tool. The 3D coordinates (.pdb) of each molecule were loaded on to PRODRG server [38] and PreADMET server for energy minimization and drug likeliness prediction respectively. The protein structure file 1Jxa was downloaded from Protein Data Bank (www.rcsb.org/pdb) was edited by removing the heteroatom's, adding C-terminal oxygen [39]. For docking calculations, Gasteiger–Marsili partial charges [40] were assigned to the inhibitors and non-polar hydrogen atoms were merged. All torsions were allowed to rotate during docking. The grid map was centred at the residues of the protein predicted from the CASTp server [41]. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization using default parameters. The number of docking runs was 50, the population in the genetic algorithm was 250, the number of energy evaluations was 100,000, and the maximum number of iterations 10,000. The docking results for inhibitors against glucosamine-6-phosphate synthase [PDB Id: 1jka], showed minimum docking energy, binding energy, inhibition constant, intermolecular energy with RMS as documented. The computer specification used-Operating system: Microsoft Windows XP, Processor: Intel Pentium 3.40 GHz, RAM: 2 GB, Hard disk: 500 GB, Python: 2.4.

4.3. Characterization data for the compounds

4.3.1. 2-Chloro-5-iodopyridin-4-amine (**2**)

Off white solid (49.7%); mp 98.9–100.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.18 (s, 1H), 6.65 (s, 1H), 6.50 (s, 2H); MS (*m/z*): 254.9 $[M + H]^+$. Anal. calcd. for $C_5H_4ClIN_2$: C, 23.60; H, 1.58; N, 11.01; found: C, 23.58; H, 1.57; N, 10.98.

4.3.2. 2-Chloro-5-iodo-3-nitropyridin-4-amine (**5**)

Yellow solid (85%); mp 156.2–158.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.40 (s, 1H), 7.21 (s, 2H); MS (*m/z*): 299.9 $[M + H]^+$. Anal. calcd. for $C_5H_3ClIN_3O_2$: C, 20.05; H, 1.01; N, 14.03; found: C, 19.98; H, 0.995; N, 13.98.

4.3.3. 1-(4-Amino-5-iodo-3-nitropyridin-2-yl)piperidine-3-carboxamide (**6a**)

Yellow solid (90%); mp 168.5–170.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (s, 1H), 7.30 (s, 1H), 6.95 (s, 2H), 6.81 (s, 1H), 3.68–3.65 (m, 2H), 2.94–2.87 (m, 2H), 2.34–2.31 (m, 1H), 1.73–1.71 (m, 2H), 1.60–1.50 (m, 2H); MS (*m/z*): 392.0 $[M + H]^+$. Anal. calcd. for $C_{11}H_{14}IN_5O_3$: C, 33.78; H, 3.61; N, 17.90; found: C, 33.72; H, 3.59; N, 17.89.

4.3.4. 1-(4-Amino-5-iodo-3-nitropyridin-2-yl)piperidine-4-carboxamide (**6b**)

Yellow solid (89%); mp 185.1–186.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (s, 1H), 7.29 (s, 1H), 6.95 (s, 2H), 6.81 (s, 1H), 3.68–3.65 (m, 2H), 2.93–2.86 (m, 2H), 2.33–2.31 (m, 1H), 1.74–1.70 (m, 2H), 1.59–1.51 (m, 2H); MS (*m/z*): 392.1 $[M + H]^+$. Anal. calcd. for $C_{11}H_{14}IN_5O_3$: C, 33.78; H, 3.61; N, 17.90; found: C, 33.74; H, 3.62; N, 17.92.

4.3.5. 1-(4-Amino-5-(3-fluorophenyl)-3-nitropyridin-2-yl) piperidine-3-carboxamide (**7a**)

Orange solid (80%); mp 155.3-156.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.70 (s, 1H), 7.53–7.45 (m, 1H), 7.35 (s, 1H), 7.23–7.19 (m, 3H), 6.87 (s, 1H), 6.71 (s, 2H), 3.73–3.60 (m, 2H), 2.98–2.84 (m, 2H), 2.38–2.31 (m, 1H), 1.91–1.87 (m, 1H), 1.70–1.48 (m, 3H); MS (*m/z*): 360.2 $[M + H]^+$. Anal. calcd. for $C_{17}H_{18}FN_5O_3$: C, 56.82; H, 5.05; N, 19.49; found: C, 56.84; H, 5.07; N, 19.51.

*4.3.6. 1-(4-Amino-5-(1-methyl-1H-pyrazol-4-yl)-3-nitropyridin-2-yl)piperidine-4-carboxamide (**7b**)*

Orange solid (81%); mp 212.5-214.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.86 (s, 1H), 7.73 (s, 1H), 7.54 (s, 1H), 7.28 (s, 1H), 6.79 (s, 1H), 6.62 (s, 2H), 3.87 (s, 3H), 3.68–3.65 (m, 2H), 2.93–2.86 (m, 2H), 2.33–2.31 (m, 1H), 1.74–1.70 (m, 2H), 1.59–1.51 (m, 2H); MS (*m/z*): 346.3 $[M + H]^+$. Anal. calcd. for $C_{15}H_{19}N_7O_3$: C, 52.17; H, 5.55; N, 28.39; found: C, 52.15; H, 5.56; N, 28.41.

4.3.7. 1-(4-Amino-5-(4-fluoro-3-methoxyphenyl)-3-nitropyridin-2-yl)piperidine-4-carboxamide (7c)

Orange solid (84%); mp 205.1-207.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.70 (s, 1H), 7.53–7.45 (m, 1H), 7.35 (s, 1H), 7.23–7.19 (m, 2H), 6.71 (s, 2H), 3.94 (s, 3H), 3.73–3.60 (m, 2H), 2.98–2.84 (m, 2H), 2.38–2.31 (m, 1H), 1.91–1.87 (m, 1H), 1.70–1.48 (m, 3H); MS (*m/z*): 390.2 $[M + H]^+$. Anal. calcd. for $C_{18}H_{20}FN_5O_4$: C, 55.52; H, 5.18; N, 17.99; found: C, 55.54; H, 5.195; N, 17.98.

*4.3.8. 1-(3,4-Diamino-5-(3-fluorophenyl)pyridin-2-yl)piperidine-3-carboxamide (**8a**)*

Brown solid (91%); mp 155.1–157.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (s, 1H), 7.50–7.43 (m, 1H), 7.29 (s, 1H), 7.21–7.12 (m, 4H), 6.81 (s, 2H), 5.00 (s, 2H), 3.09–3.05 (m, 2H), 2.86–2.79 (m, 2H), 2.71–2.59 (m, 1H), 1.78–1.58 (m, 4H); MS (*m/z*): 330.2 $[M + H]^+$. Anal. calcd. for $C_{17}H_{20}FN_5O$: C, 61.99; H, 6.12; N, 21.26; found: C, 61.97; H, 6.115; N, 21.24.

*4.3.9. 1-(3,4-Diamino-5-(1-methyl-1H-pyrazol-4-yl)pyridin-2-yl) piperidine-4-carboxamide (**8b**)*

Brown solid (86%); mp 208.0–210.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.13 (s, 1H), 7.84 (s, 1H), 7.56 (d, J = 2.0 Hz, 1H), 7.39 (d, J = 2.4 Hz, 1H), 7.26 (s, 2H), 6.74 (s, 1H), 5.00 (s, 2H), 3.87 (s, 3H), 3.68–3.65 (m, 2H), 2.93–2.86 (m, 2H), 2.33–2.31 (m, 1H), 1.74–1.70 (m, 2H), 1.59–1.51 (m, 2H); MS (*m/z*): 316.3 $[M + H]^+$. Anal. calcd. for $C_{15}H_{21}N_7O$: C, 57.13; H, 6.71; N, 31.09; found: C, 57.14; H, 6.71; N, 31.07.

*4.3.10. 1-(3,4-Diamino-5-(4-fluoro-3-methoxyphenyl)pyridin-2-yl) piperidine-4-carboxamide (**8c**)*

Brown solid (89%); mp 218.2–220.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.34 (s, 1H), 7.30–7.26 (m, 2H), 7.13 (d, J = 8.4 Hz, 1H), 6.91–6.88 (m, 2H), 6.77 (s, 2H), 4.57 (s, 2H), 3.85 (s, 3H), 3.23–3.20 (m, 2H), 2.70–2.66 (m, 2H), 2.22–2.20 (m, 1H), 1.79–1.78 (m, 4H); MS (*m/z*): 360.4 $[M + H]^+$. Anal. calcd. for $C_{18}H_{22}FN_5O_2$: C, 60.15; H, 6.17; N, 19.49; found: C, 60.12; H, 6.18; N, 19.46.

*4.3.11. 1-(7-(3-Fluorophenyl)-2-(phenylthiomethyl)-1H-imidazo [4,5-c]pyridin-4-yl)piperidine-3-carbonitrile (**9a**)*

Pale brown solid (88%); mp 95.2–96.9 °C; IR (KBr, ν_{max}, cm^{-1}): 3269 (NH), 3019 (C-H_{arom}), 2922 (C-H_{aliph}), 2241 (C≡N), 1587 (C=C_{arom}); ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.06 (s, 1H), 7.91 (s, 1H), 7.39 (t, J = 2.4 Hz, 1H), 7.37–7.36 (m, 1H), 7.34 (t, J = 6.4 Hz, 1H), 7.30 (d, J = 1.2 Hz, 1H), 7.28 (d, J = 1.6 Hz, 1H), 7.24–7.22 (m, 1H), 7.15 (d, J = 7.6 Hz, 1H), 7.08 (d, J = 9.2 Hz, 1H), 7.00–6.95 (dt, J = 8.4, 2.4 Hz, 1H), 4.76 (d, J = 12.0 Hz, 1H), 4.42 (d, J = 10.4 Hz, 1H), 4.28 (s, 2H), 3.95 (q, J = 9.2 Hz, 1H), 3.69 (t, J = 10.0 Hz, 1H), 2.89–2.83 (m, 1H), 2.12–2.08 (m, 1H), 1.98–1.92 (m, 1H), 1.91–1.82 (m, 1H), 1.66–1.60 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.8, 162.4, 150.2, 148.8, 138.9, 138.4, 138.3, 134.7, 131.3, 130.6, 129.7, 128.1, 127.8, 123.4, 121.4, 114.7, 114.5, 113.4, 49.1, 47.1, 32.3, 28.9, 27.9, 23.9; MS (*m/z*): 444.20 $[M + H]^+$; Anal. calcd. for $C_{25}H_{22}N_5SF$: C, 67.70; H, 5.00; N, 15.79; found: C, 67.64; H, 4.99; N, 15.81.

*4.3.12. 1-(2-(2,5-Difluorobenzyl)-7-(3-fluorophenyl)-1H-imidazo [4,5-c]pyridin-4-yl)piperidine-3-carbonitrile (**9b**)*

Brown solid (90%); mp 105.9–108.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.08 (s, 1H), 7.88 (s, 1H), 7.41–7.36 (m, 1H), 7.23 (d, J = 7.2 Hz, 1H), 7.15 (d, J = 9.2 Hz, 1H), 7.05–6.96 (m, 3H), 6.94–6.91 (m, 1H), 4.79 (d, J = 12.0 Hz, 1H), 4.52–4.39 (m, 1H), 4.24 (s, 2H), 4.20–4.06 (m, 1H), 3.90–3.75 (m, 1H), 2.97–2.90 (m, 1H), 2.15–2.12 (m, 1H), 2.00–1.89 (m, 2H), 1.73–1.67 (m, IH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.8, 162.4, 160.3, 158.3, 157.9, 155.9, 149.5, 131.4, 131.3, 128.1, 125.5, 125.4, 125.3, 125.3, 123.5, 121.1, 118.0, 117.9, 117.8, 117.7, 117.1, 117.0, 116.9, 116.8, 116.1, 116.0, 115.8, 115.7, 114.9, 114.8, 114.7, 114.6, 113.5, 49.3, 47.5, 29.1, 28.8, 27.9, 23.4; MS (*m/z*): 448.20 $[M + H]^+$; Anal. calcd. for $C_{25}H_{20}N_5F_3$: C, 67.11; H, 4.51; N, 15.65; found: C, 67.06; H, 4.45; N, 15.57.

*4.3.13. 1-(7-(3-Fluorophenyl)-2-(phenoxymethyl)-1H-imidazo[4,5-c]pyridin-4-yl)piperidine-3-carbonitrile (**9c**)*

Off white solid (87%); mp 187.5–189.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.15 (s, 1H), 7.96 (s, 1H), 7.44–7.38 (m, 1H), 7.31 (t, J = 1.2 Hz, 2H), 7.28 (s, 1H), 7.05–6.97 (m, 4H), 5.30 (s, 2H), 4.84 (d, J = 12.0 Hz, 1H), 4.42 (d, J = 10.4 Hz, 1H), 3.98–3.94 (m, 1H), 3.69 (t, J = 10.4 Hz, 1H), 2.89–2.83 (m, 1H), 2.15–2.11 (m, 1H), 1.99–1.88 (m, 2H), 1.71–1.66 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 162.5, 158.0, 150.3, 147.8, 138.7, 138.2, 138.2, 131.4, 131.4, 130.2, 128.1, 123.5, 122.5, 121.3, 115.1, 114.9, 114.7, 113.6, 64.3, 49.2, 47.2, 28.9, 27.9, 23.9; MS (*m/z*): 428.10 $[M + H]^+$; Anal. calcd. for $C_{25}H_{22}N_5FO$: C, 70.24; H, 5.19; N, 16.38; found: C, 69.93; H, 5.11; N, 16.30.

*4.3.14. 1-(7-(3-Fluorophenyl)-2-phenethyl-1H-imidazo[4,5-c] pyridin-4-yl)piperidine-3-carbonitrile (**9d**)*

White solid (89%); mp 128.5–130.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.69 (s, 1H), 9.69 (s, 1H), 7.87 (s, 1H), 7.35–7.30 (m, 1H), 7.29–7.27 (t, J = 6.0 Hz, 2H), 7.24–7.22 (m, 1H), 7.21–7.17 (m, 2H), 7.10 (d, J = 6.8 Hz, 1H), 7.04 (d, J = 8.8 Hz, 1H), 6.99–6.94 (dt, J = 8.0, 2.0 Hz, 1H), 4.76 (d, J = 12.0 Hz, 1H), 4.42 (d, J = 10.4 Hz, 1H), 3.98–3.94 (m, 1H), 3.69 (t, J = 10.4 Hz, 1H), 3.18–3.07 (m, 4H), 2.92–2.88 (m, 1H), 2.15–2.11 (m, 1H), 1.97–1.84 (m, 2H), 1.69–1.64 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 162.4, 151.9, 150.4, 140.9, 138.5, 138.5, 131.2, 131.1, 129.2, 128.9, 128.1, 127.1, 123.5, 121.5, 114.7, 114.5, 114.3, 113.5, 49.2, 47.2, 34.5, 31.1, 28.9, 27.8, 23.8; MS (*m/z*): 426.20 $[M + H]^+$; Anal. calcd. for $C_{26}H_{24}N_5F$: C, 73.39; H, 5.69; N, 16.46; found: C, 73.21; H, 5.66; N, 16.41.

*4.3.15. 1-(2-(3-Methylbenzyl)-7-(3-fluorophenyl)-1H-imidazo[4,5-c]pyridin-4-yl)piperidine-3-carbonitrile (**9e**)*

Off white solid (91%); mp 94.8–96.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.86 (s, 1H), 7.88 (s, 1H), 7.37–7.31 (m, 1H), 7.19 (t, J = 7.6 Hz, 2H), 7.12–7.05 (m, 4H), 6.98–6.94 (dt, J = 8.4, 2.4, 1H), 4.84 (d, J = 10.8 Hz, 1H), 4.57–4.42 (m, 1H), 4.20 (s, 2H), 4.05 (t, J = 12.0 Hz, 1H), 3.82–3.70 (m, 1H), 2.95–2.89 (m, 1H), 2.29 (s, 3H), 2.15–2.11 (m, 1H), 2.00–1.88 (m, 2H), 1.73–1.66 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.8, 162.4, 151.9, 150.1, 139.1, 138.2, 136.4, 131.3, 131.2, 129.9, 129.9, 129.3, 129.9, 128.5, 126.2, 123.5, 121.3, 114.8, 114.7, 114.6, 114.5, 49.3, 47.4, 35.8, 28.9, 27.9, 23.9, 21.8; MS (*m/z*): 426.20 $[M + H]^+$; Anal. calcd. for $C_{26}H_{24}N_5F$: C, 73.39; H, 5.69; N, 16.46; found: C, 73.31; H, 5.67; N, 16.42.

*4.3.16. 1-(2-Cyclohexyl-7-(3-fluorophenyl)-1H-imidazo[4,5-c] pyridin-4-yl)piperidine-3-carbonitrile (**9f**)*

Off white solid (82%); mp 99.6–101.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.69 (s, 1H), 7.89 (s, 1H), 7.44–7.39 (m, 1H), 7.27–7.26 (m, 1H), 7.18 (d, J = 9.2 Hz, 1H), 7.05–7.00 (dt, J = 8.4, 2.0 Hz, 1H), 5.01–4.85 (m, 1H), 4.70–4.49 (m, 1H), 4.20–3.91 (m, 1H), 3.85–3.61 (m, 1H), 2.98–2.86 (m, 2H), 2.13–2.06 (m, 2H), 1.98–1.94 (m, 1H), 1.93–1.91 (m, 1H), 1.88–1.84 (m, 2H), 1.75–1.72 (m, 2H), 1.66–1.60 (m,

2H), 1.43–1.24 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.5, 162.1, 149.4, 138.1, 137.5, 130.9, 130.8, 123.1, 120.8, 114.4, 114.3, 114.2, 114.1, 48.9, 46.9, 38.1, 31.7, 31.6, 29.6, 28.5, 27.4, 25.8, 25.7, 23.5; MS (*m/z*): 404.10 $[M + H]^+$; Anal. calcd. for $C_{24}H_{26}N_5F$: C, 71.44; H, 6.49; N, 17.36; found: C, 71.32; H, 6.47; N, 17.32.

4.3.17. 1-(7-(3-Fluorophenyl)-2-(furan-2-yl)-1H-imidazo[4,5-c] pyridin-4-yl)piperidine-3-carbonitrile (**9g**)

Brown solid (80%); mp 90.8–92.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.75 (s, 1H), 7.97 (s, 1H), 7.54–7.44 (m, 2H), 7.34–7.24 (m, 2H), 7.18–7.05 (m, 1H), 6.59–6.58 (m, 1H), 4.98 (d, *J* = 12.0 Hz, 1H), 4.70–4.66 (m, 1H), 3.96–3.91 (m, 1H), 3.74–3.67 (m, 1H), 2.96–2.93 (m, 1H), 1.90–1.69 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.6, 162.2, 150.3, 144.8, 143.7, 141.1, 139.5, 131.0, 130.9, 123.1, 120.9, 114.4, 114.3, 114.2, 114.1, 112.5, 110.8, 48.7, 46.6, 28.6, 27.4, 23.5; MS (*m/z*): 388.10 $[M + H]^+$; Anal. calcd. for $C_{22}H_{18}N_5FO$: C, 68.21; H, 4.68; N, 18.08; found: C, 68.02; H, 4.69; N, 18.04.

4.3.18. 1-(7-(3-Fluorophenyl)-2-neopentyl-1H-imidazo[4,5-c] pyridin-4-yl)piperidine-3-carbonitrile (**9h**)

Pale brown solid (85%); mp 100.9–102.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.79 (s, 1H), 7.87 (s, 1H), 7.44–7.38 (m, 2H), 7.27 (s, 1H), 7.16 (d, *J* = 9.2 Hz, 1H), 7.04–6.99 (dt, *J* = 8.4, 2.0 Hz, 1H), 4.98–4.83 (m, 1H), 4.60–4.48 (m, 1H), 4.18–4.09 (m, 1H), 3.88–3.84 (m, 1H), 2.95–2.93 (m, 1H), 2.76 (s, 2H), 2.29–2.12 (m, 1H), 2.03–1.91 (m, 3H), 1.06 (s, 9H); MS (*m/z*): 392.30 $[M + H]^+$; Anal. calcd. for $C_{23}H_{26}FN_5$: C, 70.56; H, 6.69; N, 17.89; found: C, 70.59; H, 6.72; N, 17.90.

4.3.19. 1-(7-(3-Fluorophenyl)-2-(pentan-3-yl)-1H-imidazo[4,5-c] pyridin-4-yl)piperidine-3-carbonitrile (**9i**)

Pale brown solid (86%); mp 110.9–112.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.31 (s, 1H), 7.88 (s, 1H), 7.44 (q, *J* = 7.6 Hz, 1H), 7.28 (s, 1H), 7.17 (d, *J* = 9.2 Hz, 1H), 7.06–7.02 (dt, *J* = 8.4, 2.0 Hz, 1H), 5.01–4.90 (m, 1H), 4.71–4.50 (m, 1H), 4.18–4.09 (m, 1H), 3.88–3.84 (m, 1H), 2.95–2.82 (m, 2H), 2.16–2.14 (m, 1H), 2.01–1.95 (m, 3H), 1.84–1.80 (m, 4H), 0.89 (t, *J* = 7.6 Hz, 6H); MS (*m/z*): 392.30 $[M + H]^+$; Anal. calcd. for $C_{23}H_{26}FN_5$: C, 70.56; H, 6.69; N, 17.89; found: C, 70.61; H, 6.74; N, 17.90.

4.3.20. 1-(2-(2,4-Dichlorobenzyl)-7-(1-methyl-1H-pyrazol-4-yl)-1H-imidazo[4,5-c]pyridin-4-yl)piperidine-4-carbonitrile (**9j**)

Off white solid (89%); mp 135.9–138.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.53 (s, 1H), 7.87 (s, 1H), 7.56–7.51 (m, 2H), 7.31 (s, 1H), 7.17 (s, 2H), 4.50–4.42 (m, 2H), 4.32 (s, 2H), 3.86–3.79 (m, 5H), 2.88–2.83 (m, 1H), 2.04–1.93 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.9, 150.0, 139.8, 138.0, 137.6, 134.7, 134.2, 133.6, 131.9, 129.8, 128.2, 128.0, 126.2, 122.2, 117.2, 105.0, 45.5, 39.4, 33.0, 28.9, 27.2, 23.9, 21.8; MS (*m/z*): 468.20 $[M + 2H]^+$; Anal. calcd. for $C_{23}H_{21}N_7Cl_2$: C, 59.23; H, 4.54; N, 21.02; found: C, 59.14; H, 4.54; N, 20.93.

4.3.21. 1-(7-(1-Methyl-1H-pyrazol-4-yl)-2-phenethyl-1H-imidazo [4,5-c]pyridin-4-yl)piperidine-4-carbonitrile (**9k**)

Off white solid (86%); mp 145.1–147.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.69 (s, 1H), 7.86 (s, 1H), 7.45 (s, 1H), 7.36 (s, 1H), 7.32 (t, *J* = 1.6 Hz, 1H), 7.30 (d, *J* = 1.6 Hz, 1H), 7.25 (s, 1H), 7.22 (d, *J* = 1.2 Hz, 1H), 7.20 (s, 1H), 4.55–4.45 (m, 2H), 3.95–3.78 (m, 5H), 3.24–3.13 (m, 4H), 2.91–2.84 (m, 1H), 2.08–1.96 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.5, 150.4, 141.0, 138.7, 138.1, 137.6, 133.6, 131.9, 129.2, 129.0, 128.3, 127.8, 127.1, 122.3, 117.4, 105.5, 45.4, 39.4, 34.6, 31.2, 30.1, 29.0, 27.3, 23.3; MS (*m/z*): 412.30 $[M + H]^+$; Anal. calcd. for $C_{24}H_{25}N_7$: C, 70.05; H, 6.12; N, 23.83; found: C, 69.95; H, 6.10; N, 23.21.

4.3.22. 1-(2-(2,4-Dichlorobenzyl)-7-(4-fluoro-3-methoxyphenyl)-1H-imidazo[4,5-c]pyridin-4-yl)piperidine-4-carbonitrile (**9l**)

Off white solid (93%); mp 164.0–165.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.41 (s, 1H), 7.90 (s, 1H), 7.42 (d, *J* = 2.0 Hz, 1H), 7.25 (t, *J* = 2.0 Hz, 1H), 7.23–7.20 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.14 (q, *J* = 8.4 Hz, 1H), 7.02–6.99 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.96–6.94 (m, 1H), 4.55–4.51 (m, 2H), 4.32 (s, 2H), 3.95–3.85 (m, 5H), 2.92–2.88 (m, 1H), 2.06–1.95 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.4, 151.0, 150.6, 148.5, 139.4, 138.9, 134.6, 134.5, 133.4, 132.2, 129.9, 128.3, 122.2, 119.9, 119.8, 117.3, 117.2, 113.4, 113.0, 56.7, 45.2, 33.2, 30.1, 29.0, 27.3, 23.1; MS (*m/z*): 510.2 $[M + H]^+$; Anal. calcd. for $C_{26}H_{22}N_5Cl_2FO$: C, 61.18; H, 4.34; N, 13.72; found: C, 61.06; H, 4.33; N, 13.67.

Acknowledgements

The authors are thankful to the authorities of Jain University for encouragement and financial support for this work. The authors are also thankful to Indian Institute of Science, Bangalore, India for providing spectral data. JPJ acknowledges the NSF–MRI program (Grant No: CHE-1039027) for funds to purchase the X-ray diffractometer. THS thankful to the Principal, UBDT College of Engineering, Davanagere, Karnataka, India for the support and encouragement.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.03.019. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] D.T.W. Chu, J.J. Plattner, L. Katz, New directions in antibacterial research, Journal of Medicinal Chemistry 39 (1996) 3853–3874.
[2] J. Davies, Bacteria on the rampage, Nature 383 (1996) 219–220.
[3] C.A. Hitchock, Resistance of *Candida albicans* to azole antifungal agents, Biochemical Society Transactions 21 (1993) 1039–1047.
[4] F.C. Odds, Resistance of yeasts to azole-derivative antifungals, Journal of Antimicrobial Chemotherapy 31 (1993) 463–471.
[5] C.C. Cheng, G.W. Shipps Jr., Z. Yang, B. Sun, N. Kawahata, K.A. Soucy, A. Soriano, P. Orth, L. Xiao, P. Mann, T. Black, Discovery and optimization of antibacterial AccC inhibitors, Bioorganic & Medicinal Chemistry Letters 19 (2009) 6507–6514.
[6] R.W. Bürli, P. Jones, D. McMinn, Q. Le, J.X. Duan, J.A. Kaizerman, S. Difuntorum, H.E. Moser, DNA binding ligands targeting drug-resistant Gram-positive bacteria. Part 2: C-terminal benzimidazoles and derivatives, Bioorganic & Medicinal Chemistry Letters 14 (2004) 1259–1263.
[7] B.C. Bishop, E.T.J. Chelton, A.S. Jones, The antibacterial activity of some fluorine-containing benzimidazoles, Biochemical Pharmacology 13 (1964) 751–754.
[8] T.A. Krenitsky, J.L. Rideout, E.Y. Chao, G.W. Koszalka, F. Gurney, R.C. Crouch, N.K. Cohn, G. Wolberg, R. Vinegar, Imidazo[4,5-c]pyridines (3-deazapurines) and their nucleosides as immunosuppressive and antiinflammatory agents, Journal of Medicinal Chemistry 29 (1986) 138–143.
[9] K. Ramasamy, N. Imamura, N.B. Hanna, R.A. Finch, T.L. Avery, R.K. Robins, G.R. Revankar, Synthesis and antitumor evaluation in mice of certain 7-deazapurine (pyrrolo[2,3-d]pyrimidine) and 3-deazapurine (imidazo[4,5-c] pyridine) nucleosides structurally related to sulfenosine, sulfinosine, and sulfonosine, Journal of Medicinal Chemistry 33 (1990) 1220–1225.
[10] C. Temple Jr., J.D. Rose, R.N. Comber, G.A. Rener, Synthesis of potential anticancer agents: imidazo[4,5-c]pyridines and imidazo[4,5-b]pyridines, Journal of Medicinal Chemistry 30 (1987) 1746–1751.
[11] H. Berner, H. Reinshagen, M.A. Koch, Antiviral. 1. 2-(.alpha.-Hydroxybenzyl) imidazo[4,5-c]pyridine, Journal of Medicinal Chemistry 16 (1973) 1296–1298.
[12] C. Temple Jr., Antimitotic agents. Synthesis of imidazo[4,5-c]pyridin-6-ylcarbamates and imidazo[4,5-b]pyridin-5-ylcarbamates, Journal of Medicinal Chemistry 33 (1990) 656–661.
[13] D.W. Robertson, E.E. Beedle, J.H. Krushinski, G.D. Pollock, H. Wilson, V.L. Wyss, J.S. Hayes, Structure-activity relationships of arylimidazopyridine cardiotonics: discovery and inotropic activity of 2-[2-methoxy-4-(methylsulfinyl)

phenyl]-1H-imidazo[4,5-c]pyridine, Journal of Medicinal Chemistry 28 (1985) 717–727.
[14] P. Barraclough, J.W. Black, D. Cambridge, V.P. Gerskowitch, R.A.D. Hull, R. Lyer, W.R. King, C.O. Kneen, M.S. Nobbs, G.P. Shah, S. Smith, S.J. Vine, M.V. Whiting, Inotropic activities of imidazopyridines, Archiv der Pharmazie 323 (2006) 501–505.
[15] S. Milewski, Glucosamine-6-phosphate synthase-the multi-facets enzyme, Biochimica et Biophysica Acta 1597 (2002) 173–192.
[16] A.L.L. García, T3P: a convenient and useful reagent in organic synthesis, Synlett (2007) 1328–1329.
[17] M. Schwarz, *n*-Propane phosphonic acid anhydride – a condensation reagent, Synlett (2000) 1369.
[18] H. Wissmann, H.J. Kleiner, New peptide synthesis, Angewandte Chemie International Edition in English 19 (1980) 133–134.
[19] R. Escher, P. Bünning, Synthesis of *N*-(1-carboxy-5-aminopentyl)dipeptides as inhibitors of angiotensin converting enzyme, Angewandte Chemie International Edition in English 25 (1986) 277–278.
[20] F. Burkhart, M. Hoffmann, H. Kessler, Stereoselective synthesis of a c-glycosidic analog of *N*-glucoasparagine, Angewandte Chemie International Edition in English 36 (1997) 1191–1192.
[21] J.K. Augustine, R. Kumar, A. Bombrun, A.B. Mandal, An efficient catalytic method for the Beckmann rearrangement of ketoximes to amides and aldoximes to nitriles mediated by propylphosphonic anhydride (T3P®), Tetrahedron Letters 52 (2011) 1074–1077.
[22] S. Poojari, P.P. Naik, G. Krishnamurthy, One-pot synthesis of thieno [2,3-*d*] pyrimidin-4-ol derivatives mediated by polyphosphonic anhydride, Tetrahedron Letters 53 (2012) 4639–4643.
[23] X. Wen, J.E. Bakali, R.D. Poulain, B. Deprez, Efficient propylphosphonic anhydride (®T3P) mediated synthesis of benzothiazoles, benzoxazoles and benzimidazoles, Tetrahedron Letters 53 (2012) 2440–2443.
[24] O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A.K. Howard, H. Puschmann, OLEX2: a complete structure solution, refinement and analysis program, Journal of Applied Crystallography 42 (2009) 339–341.
[25] L. Palatinus, G. Chapuis, SUPERFLIP – a computer program for the solution of crystal structures by charge flipping in arbitrary dimensions, Journal of Applied Crystallography 40 (2007) 786–790.
[26] G.M. Sheldrick, A short history of SHELX, Acta Crystallographica A64 (2008) 112–122.
[27] D. Cremer, J.A. Pople, General definition of ring puckering coordinates, Journal of the American Chemical Society 97 (1975) 1354–1358.
[28] F.H. Allen, O. Kennard, D.G. Watson, L. Brammer, A.G. Orpen, R. Taylor, Tables of bond lengths determined by *X*-ray and neutron diffraction. Part 1. Bond lengths in organic compounds, Journal of the Chemical Society, Perkin Transactions 2 2 (1987) S1–S19.
[29] D.E. Clark, *In silico* prediction of blood-brain barrier permeation, Drug Discovery Today 8 (2003) 927–933.
[30] R. Didziapetris, P. Japertas, A. Avdeef, A.J. Petrauskas, Classification analysis of P-glycoprotein substrate specificity, Drug Target 11 (2003) 391–406.
[31] S. Buchini, A. Buschiazzo, S.G. Withers, A new generation of specific *Trypanosoma cruzi* trans-sialidase inhibitors, Angewandte Chemie International Edition 47 (2008) 2700–2703.
[32] C. Bonnefous, J.E. Payne, J. Roppe, H. Zhuang, X. Chen, K.T. Symons, P.M. Nguyen, M. Sablad, N. Rozenkrants, Y. Zhang, L. Wang, D. Severance, J.P. Walsh, N. Yazdani, A.K. Shiau, S.A. Noble, P. Rix, T.S. Rao, C.A. Hassig, N.D. Smith, Discovery of inducible nitric oxide synthase (iNOS) inhibitor development candidate KD7332, part 1: Identification of a novel, potent, and selective series of quinolinone iNOS dimerization inhibitors that are orally active in rodent pain models, Journal of Medicinal Chemistry 52 (2009) 3047–3062.
[33] A.C. Leite, D.R. Moreira, M.V. Cardoso, M.Z. Hernandes, V.R.A. Pereira, R.O. Silva, A.C. Kiperstok, S.L. Mda, M.B. Soares, Synthesis, Cruzain docking, and in vitro studies of aryl-4-oxothiazolylhydrazones against Trypanosoma cruzi, ChemMedChem 2 (2009) 1339–1345.
[34] C.L. Gentry, R.D. Egleton, T. Gillespie, T.J. Abbruscato, H.B. Bechowski, V.J. Hruby, T.P. Davis, The effect of halogenation on blood-brain barrier permeability of a novel peptide drug, Peptides 20 (1999) 1229–1238.
[35] B.A. Arthington-Skaggs, M. Motley, D.W. Warnock, C.J. Morrison, Comparative evaluation of PASCO and National Committee for Clinical Laboratory standards M27-A broth microdilution methods for antifungal drug susceptibility testing of yeasts, Journal of Clinical Microbiology 38 (2000) 2254–2260.
[36] T.H. Al-Tel, R.A. Al-Qawasmeh, R. Zaarour, Design, synthesis and *in vitro* antimicrobial evaluation of novel Imidazo[1,2-a] pyridine and imidazo[2,1-b][1,3]benzothiazole motifs, European Journal of Medicinal Chemistry 46 (2011) 1874–1881.
[37] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility, Journal of Computational Chemistry 16 (2009) 2785–2791.
[38] A.K. Ghose, G.M. Crippen, Atomic physicochemical parameters for three-dimensional-structure-directed quantitative structure-activity relationships. 2. Modeling dispersive and hydrophobic interactions, Journal of Chemical Information and Computer Science 27 (1987) 21–35.
[39] T.A. Binkowski, S. Naghibzadeg, J. Liang, Computed atlas of surface topography of proteins, Nucleic Acids Research 31 (2003) 3352–3355.
[40] J. Gasteiger, M. Marsili, Iterative partial equalization of orbital electronegativity, Tetrahedron 36 (1980) 3219–3228.
[41] T. Reya, H. Clevers, Wnt signalling in stem cells and cancer, Nature 434 (2005) 843–850.