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Potential inhibitors of plasmodial heme oxygenase; an innovative approach for combating chloroquine resistant malaria

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Abstract

Syntheses of imidazo-pyridines and substituted prolines and their effect on heme oxygenase activity of *Plasmodium yoelii* and corresponding infected host have been studied. Six compounds in vitro and one in vivo showed selective inhibition of parasite enzyme which may be further exploited in the development of resistant reversal agents.

1. Introduction

Malaria caused by the protozoan parasite plasmodia is still an unconquered disease for mankind. The main reason is the emergence of plasmodial resistance towards the routinely used antimalarials like chloroquine. This has resulted in a resurgence in malaria and has thrown up challenges for innovating a remedy to combat the situation. Efforts being made in this direction include biochemical and immunological approaches. Some encouraging experimental observations like increased vacuolar pH, permease, protease, or plasmodial glycoprotein levels have been analysed [1] to determine reasons for drug resistance in plasmodia. However, no specific and conclusive biochemical change(s) could be specified for explaining chloroquine resistance in plasmodia. Compared to the chloroquine-sensitive parasite, our observation on the enhanced heme oxygenase and decreased heme/hemozoin levels in chloroquine-resistant plasmodia [2] have projected the possibility for combating chloro-quine-resistant malarial parasite. Heme oxygenase is responsible for heme and hemozoin degradation generated during the intraerythrocytic development of malarial parasites of different species, viz *P. falciparum*, *P. knowlesi*, *P. yoelii* and *P. berghei* [3]. Heme itself is toxic but in conjugation with chloroquine it is more lethal to parasites [4]. This clearly indicates that if the heme milieu is minimised, there will be less opportunity for chloroquine to impart its lethal effect on the parasite, and compounds capable of inhibiting heme degradation will help to combat chloroquine resistance if given in conjugation with chloroquine. In continuation of our earlier studies [5,6] in this direction the need arose to identify new molecular structures associated with selective inhibition of parasite heme oxygenase. This led to the evaluation of imidazo [1,2-a]pyridines and substituted prolines as selective inhibitors of cell-free malarial parasites heme oxygenase. The details are presented here.

2. Chemistry

The strategy for the syntheses of substituted imidazo[1,2-a]pyridines involves the condensation of 2-aminopyridine and substituted phenacyl bromides to yield the 2-(substituted)phenylimidazo[1,2-a]pyridines(1–7) (Scheme 1, Table 1) [7,8]. Compounds **3–5** on O-alkylation with dialkylamino alkylchlorides/alkyl halide in the presence of sodium hydride yielded **8**, **9**, **10–16** and **17**, respectively. Selective nitrosation at position 3 in compound **2** and **6** gave the nitroso derivatives

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18, **19**, respectively. Reduction of compound **19** with Zn/CH_3COOH furnished the amine **20** (Scheme 1). The substituted prolines (**21–29**) were prepared by following the method reported in the literature [9]. Structures of all new compounds were in full agreement with their mass, NMR, IR and elemental analysis data.

3. Results

The compounds belonging to imidazopyridine and substituted prolines were taken in order to monitor their effect on heme oxygenase activity of the cell-free parasite *Plasmodium yoelii* as well as for control measures infected hepatic host enzymes. For compounds which were water insoluble, a proper control of the corresponding solvent was introduced and their effect predetermined on the enzyme to neutralize the alteration (if any). Almost all the compounds were DMSO-soluble except compound **23** which was soluble in water.

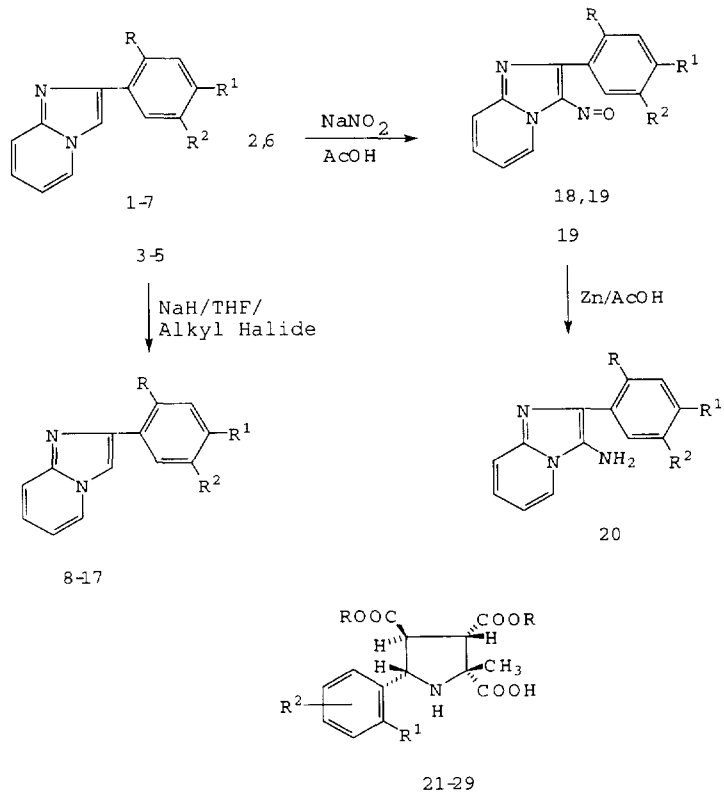
The data obtained showed that the compounds tested at different concentrations displayed inhibition of the parasite as well as the corresponding host enzyme (Table 1). Further, it was also observed that some compounds selectively inhibited the parasite enzyme with different extents of inhibition, while the host enzyme remained unaffected or there was negligible effect of the compounds on the same for compounds **8**, **14**, **15**, **16**, **24** and **25**.

The compounds which showed their inhibitory effect at lower concentration seem to be better inhibitors of the enzyme. However, compound **20** remained ineffective in arresting the parasite as well as the host enzyme. Compounds **21**, **22**, **23** and **26** equally inhibited the host and the parasite enzyme to almost the same extent.

The effect of three compounds, **8**, **14** and **25**, which showed selective or potential inhibitory effects on HO activity of the parasite in vitro, were also tested in vivo for the inhibition of the enzyme. The results obtained show that out of the three compounds only **25** completely inhibited the HO activity of the parasite in vivo (Table 2).

4. Discussion

Heme plays a crucial role in controlling the physiological function as well as resistance property of the malarial parasite. The lethal effect of the chloroquine–heme adduct depends upon the action of the key



Scheme 1.

Table 1
In vitro effect of different compounds on heme oxygenase activity of cell free parasite *P. yoelii* and corresponding infected host

Compound no.	R	R^1	R^2	Concentration (μM)	% inhibition in HO	
					Parasite	Host
1	H	Cl	H	10	Nil	Nil
				50	Nil	Nil
				100	20	12
2	H	Br	H	10	Nil	Nil
				50	Nil	Nil
				100	Nil	Nil
3	H	OH	H	10	20	Nil
				50	30	Nil
				100	80	Nil
4.	H	H	OH	10	Nil	Nil
				50	Nil	Nil
				100	Nil	Nil
5.	CH_3	OH	$CH(CH_3)_2$	10	12	15
				50	33	59
				100	72	60
6.	H	H	OCH_3	10	Nil	Nil
				50	Nil	Nil
				100	52	Nil
7.	H	OCH_3	H	10	Nil	Nil
				50	20	Nil
				100	20	Nil
8.	H	$O(CH_2)_2N\ Et_2$	H	10	100	Nil
				50	100	Nil
				100	100	Nil
9.	H	$O(CH_2)_2Pip^a$	H	10	Nil	Nil
				50	20	Nil
				100	20	Nil
10.	H	H	$O(CH_2)_2NMe_2$	10	Nil	Nil
				50	Nil	Nil
				100	Nil	Nil
11.	H	H	$O(CH_2)_2NEt_2$	10	Nil	Nil
				50	Nil	Nil
				100	Nil	Nil
12.	H	H	$O(CH_2)_2Pip^a$	10	Nil	15
				50	Nil	47
				100	Nil	100
13.	CH_3	$O(CH_2)_2NMe_2$	$CH(CH_3)_2$	10	Nil	Nil
				50	100	Nil
				100	100	Nil
14.	CH_3	$O(CH_2)_2NEt_2$	$CH(CH_3)_2$	10	100	Nil
				50	100	Nil
				100	100	5.9
15.	CH_3	$O(CH_2)_2Pyr^b$	$CH(CH_3)_2$	10	100	Nil
				50	100	Nil
				100	100	10.4
16.	CH_3	$OCH_2)_2Pip^a$	$CH(CH_3)_2$	10	12	Nil
				50	60	Nil
				100	100	Nil
17.	CH_3	OCH_3	$CH(CH_3)_2$	10	Nil	Nil
				50	Nil	Nil
				100	Nil	Nil
18.	H	Br	H	10	Nil	Nil
				50	Nil	Nil
				100	Nil	Nil

Table 1—*contd*

Compound no.	R	R^1	R^2	Concentration (μM)	% inhibition in HO	
					Parasite	Host
19.	H	H	OCH_3	10	15	Nil
				50	75	Nil
				100	100	Nil
20.	H	H	OCH_3	10	Nil	Nil
				50	Nil	Nil
				100	Nil	Nil
21.	CH_2CH_3	OH	H	10	Nil	Nil
				50	100	100
				100	100	100
22.	CH_3	OH	H	10	12	Nil
				50	50	31
				100	70	60
23.	CH_2CH_3	OH	5-NO_2	10	Nil	Nil
				50	15	10
				100	34	20
24.	CH_3	OH	5-NO_2	10	71	Nil
				50	100	5
				100	100	6
25.	CH_3	H	H	10	100	Nil
				50	100	Nil
				100	100	4
26.	CH_3	OCH_3	H	10	Nil	Nil
				50	34	Nil
				100	60	41
27.	CH_3	H	3-NO_2	10	Nil	Nil
				50	Nil	Nil
				100	50	Nil
28.	CH_3	OCH_3	5-OCH_3	10	Nil	Nil
				50	35	Nil
				100	70	Nil
29.	CH_3	OH	5-OCH_3	10	12	Nil
				50	29	5
				100	50	6

[a]Pip = piperdin-1-yl.
[b]Pyr = pyrrolidin-1-yl.

enzyme, heme oxygenase, which regulates the heme milieu of the parasite. Another possible pathway has been discussed by Slater et al. (1992) [12] which was later modified by Dorn et al. (1995) [13] stating the formation of hemozoin which was responsible for showing the resistant property of the parasite. Srivastava et al. (1995) [14] also reported that even the preformed hemozoin can be acted upon by heme oxygenase.

Compounds **8**, **14**, **15**, **16**, **24** and **25**, which selectively block the parasite heme oxygenase activity in vitro (and, with compound **25**, in vivo), indicate that they can intercept the intraerythrocytic heme catabolism and provide ample toxic heme environment for effective chloroquine-heme adduct formation which can make the resistant strain of the parasite susceptible. It is likely that if such compounds are administered with chloroquine, it may act as a resistant reversal agent [12]. The effective compounds having the selective inhibition of parasite enzyme open further avenues for development of resistant reversal agents based on the above-mentioned concept.

Table 2
In vivo[a] effect of potential compounds on heme oxygenase activity of the cell free *P. yoelii*

S. no.	Compound no.	% inhibition in heme oxygenase activity
1.	8	Nil
2.	14	57
3.	25	100

[a]Compounds were fed at the dose of 15 mg/kg b.wt ×5 days to *P. yoelii* infected mice and the parasites isolated from the animals were subjected to Heme oxygenase assay.

5. Conclusion

In conclusion, the present study confirms that imidazo [1,2-a] pyridines and substituted prolines as classes of compounds are the new molecular structures which selectively inhibit the parasite heme oxygenase in vitro and in vivo, thereby opening new avenues for the syntheses of different classes of potential resistant reversal agents, helpful in reversing the resistance property of the malarial parasites which when given in combination with chloroquine or other antimalarial(s) may eradicate the resistant parasites.

6. Biological evaluation

6.1 Materials and methods

Plasmodium yoelii nigeriensis (multidrug-resistant) infection was maintained in Swiss albino mice having chloroquine tolerance up to 120 mg/kg b.wt. Infection/parasitaemia was ascertained by monitoring the giemsa-stained blood smears of infected animals microscopically. The isolation of parasites was carried out from infected animals' blood through density gradient centrifugation and saponin lysis as described earlier [2]. Heme oxygenase activity was assayed following the bilirubin formation [10] in the post-mitochondrial fraction. Different concentrations (10–100 μM) of tested compounds were supplemented in an assay system of heme oxygenase before starting the enzymatic reaction. For the evaluation of the efficacy of the compounds in vivo, the compounds which selectively inhibited the heme oxygenase activity of the parasite were orally administered (15 mg/kg b.wt for 5 days) to *P. yoelii* infected mice. The parasites from each compound-treated infected animals were isolated and used for enzyme assay [2,10].

7. Experimental

All reagents and starting materials were from commercial sources and used without further purification. All melting points were recorded on hot stage apparatus and are uncorrected. ^{1}H-NMR was measured on a Perkin–Elmer R-32 or Bruker 400 FT or Bruker 300 FT NMR instrument using TMS as a standard and mass spectra on a JEOL- JMS-D-300 or JEOL SX 102/DA-6000 mass spectrometer. Chemical analyses were carried out on Carlo Erbo 1103 analyzer.

Representative procedure for the preparation of imidazo [1,2-a]pyridines:

7.1 2-[(4-Hydroxy-5-isopropyl-2-methyl)phenyl] imidazo[1,2-a]pyridine (5)

A solution of 1 g (3.89 mmol) of 4-hydroxy-3-isopropyl-6-methyl phenacyl bromide and 0.36 g (3.89 mmol) of 2-aminopyridine in dry chloroform (100 ml) was refluxed for 8 h. The solvent was removed under reduced pressure, and the residual solid was kept at 100°C under vacuum for 1 h. The solid obtained after neutralisation with sodium bicarbonate solution was filtered and recrystallised from methanol–chloroform. Yield, 80%; m.p. 214°C; MS; *m/z* 266 (M^+); ^{1}H NMR (CD_3OD): δ 1.12 (d, $J = 9$Hz,6H, $HC(C\underline{H}_3)_2$), 2.36 (s, 3H, $C\underline{H}_3$), 3.28 (m, 1H, $\underline{H}C(CH_3)_2$), 6.65 (s, 1H, H-3′), 6.9 ($\underline{dd}_e$, 1H, H-6), 7.3 ($\underline{dd}_e$, 1H, H-7), 7.48 (s, 1H, H-6′), 7.52 (d, $J = 9$ Hz, 1H, H-8), 7.85 (s, 1H, H-3), 8.4 (d, $J = 8$ Hz, 1H, H-5). Anal. calcd. for $C_{17}H_{18}N_2O.1/2\ H_2O$: C, 74.18; H, 6.90; N, 10.18; Found: C, 74.15; H, 6.98; N, 10.22%.

Compounds (**1–4**) and (**6,7**) were prepared following the same procedure.

7.1.1 2-(3-Methoxy phenyl)imidazo[1,2-a]pyridine (7)

Yield, 72%; m.p. 72°C [$CHCl_3$]; MS; *m/z* 224 (M^+); ^{1}H NMR ($CDCl_3$): δ 3.92 (s, 3H, $O\underline{CH}_3$), 6.78–7.65 (m, 6H, Ar–H), 7.52 (bs, 1H, H-2′), 7.88 (s, 1H, H-3), 8.12 (d, $J = 9$ Hz, 1H, H-5). Anal. calcd. for $C_{14}H_{12}N_2O$: C, 75.00; H, 5.35; N, 12.5; Found: C, 74.87; H, 5.31; N, 12.68%.

*7.1.2 2-[4-(2-*N,N*-Diethylamino)ethoxyphenyl]imidazo [1,2-a]pyridine (8)*

A mixture of 3.2 g (9.52 mmol), 2-*N,N*-diethylaminoethylchloride hydrochloride 1.63 g (9.52 mmol) and sodium hydride 0.68 g (28.57 mmol) in dry THF (50 ml) was heated under reflux for 10 h. Then the solvent was distilled off. The residual oil was taken up in chloroform, washed with water till neutral and dried over Na_2SO_4. The solvent was distilled off to give the product. Recrystallised from chloroform–methanol, yield (64%); m.p. 80°C; MS; *m/z* 309 (M^+); ^{1}H NMR ($CDCl_3$): δ 1.10 (t, $J = 8$ Hz, 6H, $-N(CH_2C\underline{H}_3)_2$), 2.4–3.0 (m, 6H, $3\times NC\underline{H}_2$), 4.15 (t, $J = 6.5$ Hz, 2H, $-OC\underline{H}_2$), 6.66–8.2 (m, 9H, Ar-H). Anal. calcd. for $C_{19}H_{23}N_3O.H_2O$: C, 69.72; H, 7.64; N, 12.84; Found: C, 69.73; H, 7.50; N, 12.87%.

7.1.3 2-[4-(2-Piperidin-1-yl)ethoxyphenyl]imidazo [1,2-a]pyridine (9)

Yield, 80%; m.p. 116°C [$CHCl_3$-MeOH]; MS: *m/z* 321 (M^+); ^{1}H NMR ($CDCl_3$): δ 1.5 (m, 6H, $3\times C\underline{H}_2$), 2.5 (m, 4H, $2\times NC\underline{H}_2$), 2.8 (t, $J = 6$ Hz, $C\underline{H}_2N$), 4.1(t, $J = 6$ Hz, $-OC\underline{H}_2$), 6.6–8.05 (m, 9H, Ar-H). Anal. calcd. for $C_{20}H_{23}N_3O.H_2O$: C, 70.79; H, 7.3; N, 12.3; Found: C, 70.92; H, 7.58; N, 12.35%.

*7.1.4 2-[3-(2-*N,N*-Dimethylamino)ethoxyphenyl] imidazo[1,2-a]pyridine (10)*

Yield, 80%; m.p. 172°C (oxalate)[MeOH-Ether]; MS: *m/z* 281 (M^+); ^{1}H NMR ($CDCl_3$): δ 2.5 (s, 6H, $N(C\underline{H}_3)_2$), 2.7(t, $J = 6$ Hz, 2H, $-C\underline{H}_2N$), 4.1(t,

$J = 6$ Hz, 2H, $-OCH_2$), 6.6–8.0 (m, 9H, Ar-H). Anal. calcd. for $C_{17}H_{19}N_3O.(COOH)_2.3H_2O$: C, 53.64; H, 6.35; N, 9.88; Found: C, 53.52; H, 6.37; N, 9.92%.

*7.1.5 2-[3-(2-N,N-Diethylamino)ethoxyphenyl]imidazo [1,2-a]pyridine (**11**)*

Yield, 80%; m.p. 165°C (oxalate)[MeOH-Ether]; MS: *m/z* 309 (M^+); ^1H NMR ($CDCl_3$): δ 1.02 (t, $J = 7$ Hz, 6H, $2\times CH_3$), 2.6 (m_e, 4H, $2\times NCH_2$), 2.85 (t, $J = 7$ Hz, 2H, CH_2N), 4.08 (t, $J = 7$ Hz, 2H, $-OCH_2$), 6.6–8.0 (m, 9H, Ar-H). Anal. calcd. for $C_{19}H_{23}N_3O.(COOH)_2.3H_2O$: C, 53.50; H, 6.58; N, 8.91; Found: C, 53.52; H,6.69; N, 8.98%.

*7.1.6 2-[3-(2-Piperidin-1-yl)ethoxyphenyl]imidazo [1,2-a]pyridine (**12**)*

Yield , 80%; m.p. 148°C (oxalate)[MeOH-Ether]; MS: *m/z* 321 (M^+); ^1H NMR ($CDCl_3$): δ 1.5 (m, 6H, $3\times CH_2$), 2.5 (m, 4H, $2\times NCH_2$), 2.75 (t, $J = 6$ Hz, 2H, $-CH_2N$), 4.15 (t, $J = 6$ Hz, 2H, $-OCH_2$), 6.6–8.0 (m, 9H, Ar-H). Anal. calcd. for $C_{20}H_{23}N_3O(COOH)_2.3H_2O$: C, 56.77; H, 6.6; N, 9.03; Found: C, 56.98; H, 6.38; N, 9.16%.

*7.1.7 2-[4-(2-N,N-Dimethylamino)ethoxy-5-isopropyl-2-methylphenyl]imidazo[1,2-a]pyridine (**13**)*

Yield, 76%; m.p. 111°C (oxalate)[MeOH-Ether]; MS: *m/z* 337 (M^+); ^1H NMR ($CD_3OD + CDCl_3$): δ 1.15(d, $J = 9.2$ Hz, 6H, $HC(CH_3)_2$), 2.25 (s, 6H, $N(CH_3)_2$, 2.36 (s, 3H, CH_3), 2.72 (t, $J = 6$ Hz, CH_2N), 3.25 (m, 1H, $HC(CH_3)_2$), 4.05 (t, $J = 6$ Hz, 2H, $-OCH_2$), 6.7 (s, 1H, H-3′), 6.78 (dd_e, 1H, H-6), 7.2 (dd_e, 1H, H-7), 7.45 (d, $J = 9$ Hz, 1H, H-8), 7.5 (s, 1H, H-6′), 7.76 (s, 1H, H-3), 8.3 (d, $J = 7$ Hz, 1H, H-5). Anal. calcd. for $C_{21}H_{27}N_3O.(COOH)_2.3H_2O$: C, 57.38; H, 7.27; N, 8.73; Found: C, 57.39; H, 7.37; N, 8.78%.

*7.1.8 2-[4-(2-N,N-Diethylamino)ethoxy-5-isopropyl-2-methylphenyl]im idazo[1,2-a]pyridine (**14**)*

Yield, 77%; m.p. 67°C (oxalate)[MeOH-Ether]; MS : *m/z* 365 (M^+); ^1H NMR (D_2O): δ 0.90 (d_e, 6H, $CH(CH_3)_2$), 1.15 (t_e, 6H, $2\times CH_2CH_3$), 2.0 (s, 3H, CH_3), 2.85 (m, 1H, $HC(CH_3)_2$), 3.1 (q_e, 4H, $2\times NCH_2CH_3$), 3.35 (bs, 2H, CH_2N), 3.95 (bs, 2H, $O\text{-}CH_2$), 4.05 (t, $J = 6$ Hz, 2H, $-OCH_2$), 6.6–8.0 (m, 7H, Ar-H). Anal. calcd. for $C_{23}H_{31}N_3O.(COOH)_2.H_2O$: C, 63.42; H, 7.38; N, 8.87; Found: C, 63.55; H, 7.42 ; N, 9.09%.

*7.1.9 2-[4-(2-Pyrrolidin-1-yl)ethoxy-5-isopropyl-2-methylphenyl]imidazo[1,2-a]pyridine (**15**)*

Yield, 75%; m.p. 120°C [$CHCl_3$-MeOH]; MS: *m/z* 363 (M^+) ; ^1H NMR ($CDCl_3$): δ 1.25 (d, $J = 8$ Hz, 6H, $HC(CH_3)_2$, 1.75 (m, $4H(CH_2)_2$, 2.46 (s, 3H, CH_3), 2.6 (m, 4H, $2\times N\text{-}CH_2$), 2.90 (t, 2H, $J = 6.5$ Hz, CH_2N), 3.3 (m, 1H, $HC(CH_3)_2$), 4.1 (t, $J = 6.5$ Hz, 2H, $-OCH_2$), 6.6–8.0 (m, 7H, Ar-H). Anal. calcd. for $C_{23}H_{29}N_3O.H_2O$: C, 72.44; H, 8.13; N, 11.02; Found: C, 72.66; H, 8.18; N, 11.12%.

*7.1.10 2-[4-(Piperidin-1-yl)ethoxy-5-isopropyl-2-methylphenyl]imidazo[1,2-a]pyridine (**16**)*

Yield , 56%; m.p. 216°C (oxalate)[MeOH-Ether]; MS: *m/z* 377 (M^+); ^1H NMR ($CDCl_3$): δ 1.15 (d, $J = 9$ Hz, 6H, $HC(CH_3)_2$, 1.5 (m, 6H, $3\times CH_2$), 2.36 (s, 3H, CH_3), 2.5 (m, 4H, $2\times NCH_2$), 2.85 (t, $J = 6$ Hz, CH_2N), 3.3 (m, 1H, $HC(CH_3)_2$), 4.1 (t, $J = 6$ Hz, 2H, $-OCH_2$), 6.6 (s, 1H, H-3′), 6.78 (dd_e, 1H, H-6), 7.2 (dd_e, 1H, H-7), 7.45 (d, $J = 9$ Hz, 1H, H-8), 7.5 (s, 1H, H-6′), 7.76 (s, 1H, H-3), 8.4 (d, $J = 6$ Hz, 1H, H-5). Anal. calcd. for $C_{24}H_{31}N_3O.(COOH)_2.2H_2O$: C, 62.02; H, 6.9; N, 8.34; Found: C, 61.93; H, 6.88; N, 8.36%.

*7.1.11 2-[5-Isopropyl-4-methoxy-2-methylphenyl] imidazo [1,2-a]pyridine (**17**)*

Yield, 48%; Viscous mass; MS : *m/z* 280 (M^+); ^1H NMR ($CDCl_3$): δ 1.26 (d, $J = 7$ Hz, 6H, $HC(CH_3)_2$, 2.53 (s, 3H, CH_3), 3.30 (m, 1H, $HC(CH_3)_2$), 3.86 (s, 3H, OCH_3), 6.75 (s, 1H, H-3′), 6.80 (dd_e, 1H, H-6), 7.19 (dd_e, 1H, H-7), 7.63 (s, 1H, H-6′), 7.70 (s, 1H, H-3), 7.75 (d, $J = 9$ Hz, H-8), 8.14 (d, $J = 7$ Hz, 1H, H-5). Anal. calcd. for $C_{18}H_{20}N_2O$: C, 77.14; H, 7.14; N, 10.0; Found: C, 77.12; H, 7.12; N, 9.89%.

*7.1.12 2-(3-Methoxyphenyl)-3-nitrosoimidazo[1,2-a]pyridine (**19**)*

To the solution of **7** (1.0 g (4.46 mmol)), in glacial acetic acid (10 ml), aqueous solution of sodium nitrite 0.3 g (4.46 mmol) was added dropwise maintaining temperature at 0°C. Stirring was continued at room temperature for another 3 h. Green solid separated was filtered and washed with chilled water, crystallised from methanol-chloroform (73%), m.p. 160°C: MS; *m/z* 253 (M^+); ^1H NMR ($CDCl_3$) δ 3.92 (s, 3H,OCH_3), 7.15–9.95 (m, 8H, Ar-H) Anal. calcd. for $C_{14}H_{11}N_3O_2$: C, 66.40; H, 4.34; N, 16.60; Found: C, 66.43; H, 4.50; N, 16.61%.

*7.1.13 3-Amino-2-(3-methoxyphenyl)imidazo[1,2-a] pyridine (**20**)*

To the solution of **19** (1.0 g (3.95 mmol)) in acetic acid-water (150:75 ml) mixture, zinc powder 2.31 g (35.5 mmol) was added in portions maintaining temperature at 0°C. Stirring was continued at room temperature for another 4 h. Zn dust was filtered and solid obtained after neutralisation with aq. ammonia was filtered, dried and crystallised from ethyl acetate (40%) m.p. 120°C: MS: *m/z* 239 (M^+); ^1H NMR ($CDCl_3$): δ 3.45 (bs, 2H, NH_2), 3.89 (s, 3H, $-OCH_3$), 6.78–7.6 (m, 7H, Ar-H), 8.0 (d, $J = 9$ Hz, 1H, H-5) Anal. calcd. for $C_{14}H_{13}N_3O$: C, 70.29; H, 5.4; N, 17.57; Found: C, 70.49; H, 5.5; N, 17.72%.

*7.1.14 Dimethyl/ethyl-2-methyl-c-5-(2-substituted-phenyl)-pyrrolidine -c-3,t-4- dicarboxylate-r-2-carboxylic acid (**21–29**)*

These compounds were prepared by the method reported in literature [9], and spectral data for new compounds are given below:

7.1.14.1 Compound 21. Yield, 60%; m.p. 217°C [MeOH]; MS: *m/z* 366 ($M^+ + 1$); ^{1}H NMR (Py.d_5): δ 1.04 (t, 3H, $CH_2C\underline{H}_3$), 1.14 (t, 3H, $CH_2C\underline{H}_3$), 3.92 (d, $J = 11$ Hz, 1H, H-3), 4.14 (q, 2H, $C\underline{H}_2CH_3$), 4.24 (q, 2H, $C\underline{H}_2CH_3$), 4.31 (t, 1H, H-4), 5.15 (d, $J = 10$ Hz, 1H, H-5), 6.88–7.46 (m, 4H, ArH). Anal calcd. for $C_{18}H_{23}NO_7$: C, 59.17; H, 6.30; N, 3.86 Found: C, 59.28; H, 6.50; N, 3.80.

7.1.14.2 Compound 23. Yield, 60%; m.p. 205–7°C [MeOH]; MS: *m/z* 411 ($M^+ + 1$); ^{1}H NMR (Pyd$_5$): δ 1.10 (t, 3H, $CH_2C\underline{H}_3$), 1.16 (t, 3H, $CH_2C\underline{H}_3$), 2.12 (s, 3H, $C\underline{H}_3$), 3.86 (d, $J = 11$ Hz, 1H, H-3), 4.12 (q, 2H, $C\underline{H}_2CH_3$), 4.16 (q, 2H, $C\underline{H}_2CH_3$), 4.22 (t, 1H, H-4), 5.32 (d, $J = 10$ Hz, 1H, H-5), 7.10–8.14 (m, 3H, ArH). Anal. calcd. for: $C_{18}H_{22}N_2O_9$: C, 52.68; H, 5.36; N, 6.82; Found: C, 52.70 ; H, 5.47 ; N, 6.78%.

7.1.14.3 Compound 24. Yield, 60%; m.p. 188–90°C [MeOH]; MS: *m/z* 383 ($M^+ + 1$), ^{1}H NMR (Py.d_5): δ 2.10 (s, 3H, CH_3), 3.68 (s,6H, $(OC\underline{H}_3)_2$), 3.88 (d, $J = 11$ Hz, 1H, H-3), 4.24 (t, 1H, H-4), 5.40 (d, $J = 10$ Hz, 1H, H-5), 7.10–8.88 (m, 3H, Ar-H); Anal. calcd: For $C_{16}H_{18}N_2O_9$: C, 50.26; H, 4.69; N, 7.28; Found: C, 50.30; H, 4.60; N, 7.28%.

7.1.14.4 Compound 27. Yield, 50%; m.p. 230–35°C [MeOH]; MS : *m/z* 367 ($M^+ + 1$); ^{1}H NMR (Pyd$_5$): δ 2.10 (s, 3H, $C\underline{H}_3$), 3.66 (s, 3H, $OC\underline{H}_3$), 3.68 (s, 3H, $OC\underline{H}_3$), 3.95 (d, $J = 11$ Hz, 1H, H-3), 4.22 (t, 1H, H-4), 4.98 (d, $J = 10$ Hz, 1H, H-5), 7.38–8.86 (m, 4H, ArH). Anal. calcd. For $C_{16}H_{18}N_2O_8$: C, 52.48; H, 4.91; N, 7.62; Found: C, 52.38; H, 4.82; N, 7.60%.

7.1.14.5 Compound 28. Yield, 68%; m.p. 210°C [MeOH]; MS : *m/z* 381 ($M^+ + 1$); ^{1}H NMR (Py.d_5) : δ 2.93 (s, 3H, $C\underline{H}_3$), 4.48 (s, 3H, $OC\underline{H}_3$), 4.52 (s, 3H, $OC\underline{H}_3$), 4.58 (s, 6H, $OC\underline{H}_3)_2$), 4.81 (d, $J = 11$ Hz, 1H, H-3), 5.01 (t, 1H, H-4), 6.22 (d, $J = 10$ Hz, 1H, H-5), 7.26–8.62 (m, 3H, ArH). Anal. calcd. For $C_{18}H_{23}NO_8$: C, 56.69; H, 6.08; N, 3.89; Found: C, 3.67; H, 5,90, N, 3.80%.

7.1.14.6 Compound 29. Yield, 62%; m.p. 202–4°C [MeOH]; MS: *m/z* 368 ($M^+ + 1$); ^{1}H NMR (Pyd$_5$): δ 2.10 (s, 3H, $C\underline{H}_3$), 3.61 (s, 3H, $OC\underline{H}_3$), 3.64 (s, 3H, $OC\underline{H}_3$), 3.68 (s, 3H, $OC\underline{H}_3$), 3.86 (d, $J = 11$ Hz, 1H, H-3), 4.34 (t, 1H, H-4), 4.92 (d, $J = 10$ Hz, 1H, H-5), 6.90–7.34 (m, 4H, ArH). Anal. calcd. For $C_{17}H_{21}NO_8$: C, 55.58; H, 5.71; N, 3.81; Found: C, 55.67; H, 5.63; N, 3.83%.

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