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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, anti-inflammatory and analgesic activities of arylidene-2-(3-chloroanilino)nicotinic acid hydrazides

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Abstract A new series of 2-(3-chloroanilino)nicotinic acid hydrazides **12a**–**p** were synthesized and evaluated for their anti-inflammatory and analgesic activities. Most of the compounds have shown anti-inflammatory activity with a moderate-to-excellent activity range. Among them, 3-chloro **12d** and 4-methoxyphenyl derivatives **12i** exhibited the most potent anti-inflammatory activity relative to niflumic acid as the reference drug (95, 87, and 81 % reductions in inflammation, respectively). The compounds with the highest anti-inflammatory activity were subjected to analgesic assay and showed moderate-to-excellent analgesic activities. The 2,4-dimethoxyphenyl derivative (**12j**) exhibited the highest analgesic activity relative to niflumic acid (99.6 and 68 % activity, respectively).

Keywords Hydrazone · Anti-inflammatory · Analgesic

Introduction

The nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for treatment of pain, pyrexia, inflammation, rheumatoid arthritis, and osteoarthritis (Bacchi *et al*., 2012; Rubio-Perez and Morillas-Ruiz, 2012). NSAIDs block the biosynthesis of prostaglandins by inhibiting enzyme prostaglandin H_2 endoperoxide synthase or cyclooxygenase (Vane and Botting, 1998). Its constitutive isoform, namely COX-1, is related to homeostatic functions, such as gastric mucosal cytoprotection, renal blood flow regulation, and vascular antithrombotic activity; while its inducible isoform, COX-2, is overexpressed under stress conditions, although constitutive in a few tissues (Vane and Botting, 1998; Simmons *et al*., 2004). Since most of the currently available NSAIDs in the markets show greater selectivity for COX-1 than for COX-2 (Jackson and Hawkey, 1999), long-term usage of NSAIDs is associated with significant side effects of gastrointestinal, bleeding, and nephrotoxicity (Vandraas *et al*., 2010) which cause some patients to abandon NSAID therapy. Therefore, the discovery of new safer anti-inflammatory drugs represents a challenging goal for such a research area.

Some pyridine compounds such as aminopyridylmethanols and aminomethyl-pyridinamines (Sondhi *et al*., 2002) have been found useful as analgesic, as well as anti-inflammatory agents, and for treating Alzheimer's disease (Effland and Klein, 1991). The pyridine nucleus is present in niflumic acid (**1**) and flunixin (**2**), the two traditional NSAIDs belonging to the class of fenamates (Fig. 1). These drugs are derivatives of *N*-phenyl (or heteroaryl) anthranilic acid, which are clinically prescribed for their analgesic, anti-inflammatory, and anti-pyretic properties. Unlike aspirin and most NSAIDs, the fenamates do not affect platelet aggregation and bleeding time, but are still endowed of most of the adverse effects induced by NSAIDs, particularly gastrointestinal bleeding, ulceration, and perforation (Cocco *et al*., 2004).

Regarding GI damage of NSAIDs, two factors are generally considered: local irritation by the direct contact of carboxylic acid (–COOH) moiety of NSAIDs with GI mucosal cells (topical effect), and decreased tissue

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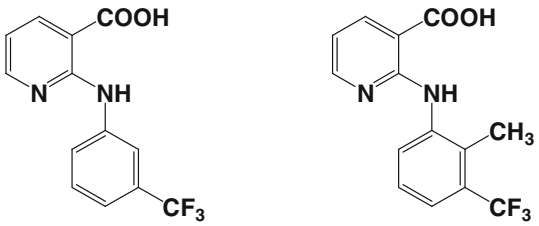
prostaglandin production in tissues which attenuates the physiological cytoprotective role of prostaglandins in maintaining GI health and homeostasis (Smith *et al*., 1998; Hawkey *et al*., 2000).

Synthetic approaches based on chemical modification of NSAIDs have been considered with the aim of improving safety profile of these NSAIDs. Several studies have described the derivatization of carboxylic acid function with amide or *N*-acylarylhydrazone moieties having less acidic amide hydrogen (Fig. 2) (Bala *et al*., 2013; Almasirad *et al*., 2005; Almasirad *et al*., 2006). On the other hand, some evidences suggest that the hydrazone moiety possesses a pharmacophoric character for the inhibition of cyclooxygenase and the presence of a *N*-heteroaromatic ring having less hydrophobic character in *N*-acyl moiety (**3**–**4**) improves their analgesic activity (Fig. 2) (Matheus *et al*., 1991; Simmons *et al*., 2004; Lacerda, 2012).

Considering the above approach, in recent studies, we have found that niflumic acid-based *N*-acylhydrazone derivatives (**6**) show significant anti-inflammatory and analgesic activities, comparable with niflumic acid as the reference drug (Kheradmand *et al*., 2013). Hence, as a part of our ongoing program (Almasirad *et al*., 2005, 2006; Moradi *et al*., 2010; Kheradmand *et al*., 2013), we describe herein the synthesis, and biological evaluation of a novel diverse group of *N*-acylarylhydrazone derivatives of 2-(3-chloroanilino)nicotinic acid with different substituents on the terminal phenyl (or heteroaryl) ring as anti-inflammatory and analgesic agents. Therefore, the effect of substitution of trifluoromethyl (Kheradmand *et al*., 2013) with chlorine group on aniline moiety, which provides slightly less lipophilic compounds, on their anti-inflammatory and analgesic activities was investigated.



1 (Niflumic acid) **2 (Flunixin)**

Fig. 1 Representative examples of pyridine derivatives of fenamates

Results and discussion

Chemistry

The synthetic reactions leading to the substituted arylidene-2-(3-chloroanilino)nicotinic acid hydrazides (**12a**–**p**) are outlined in Scheme 1.

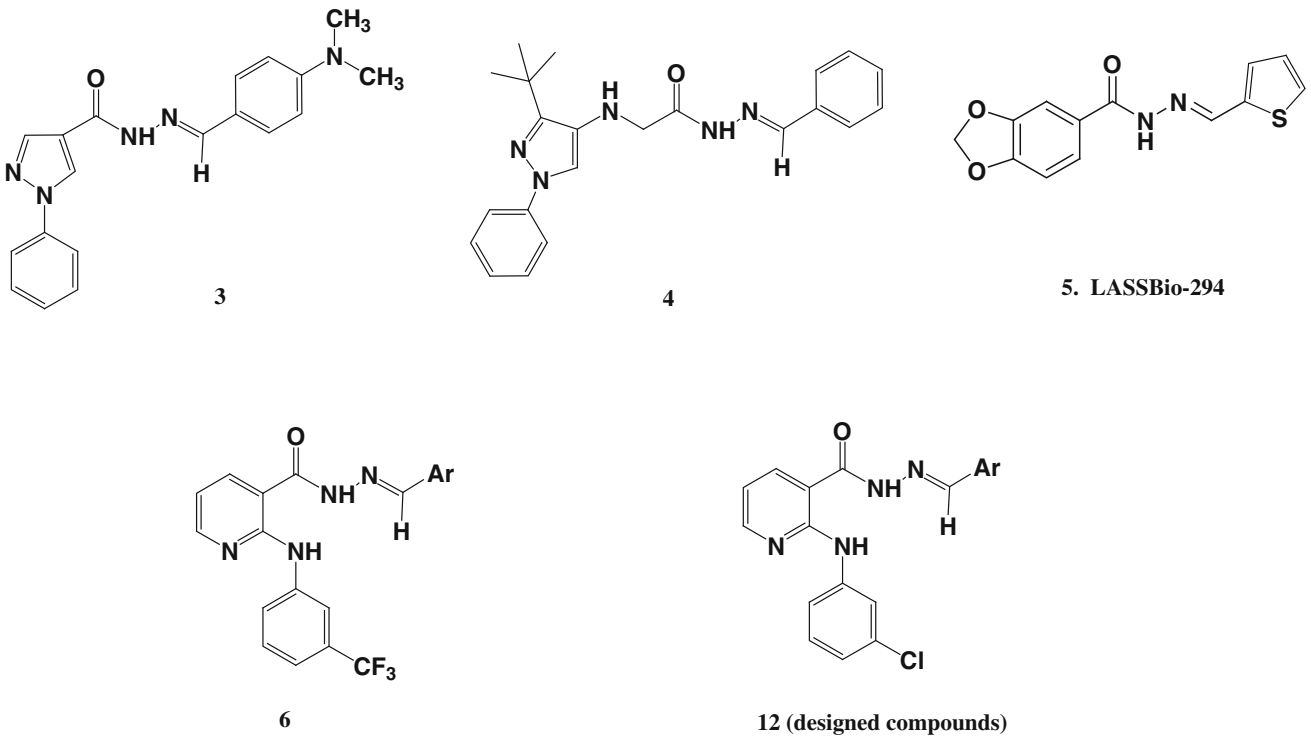
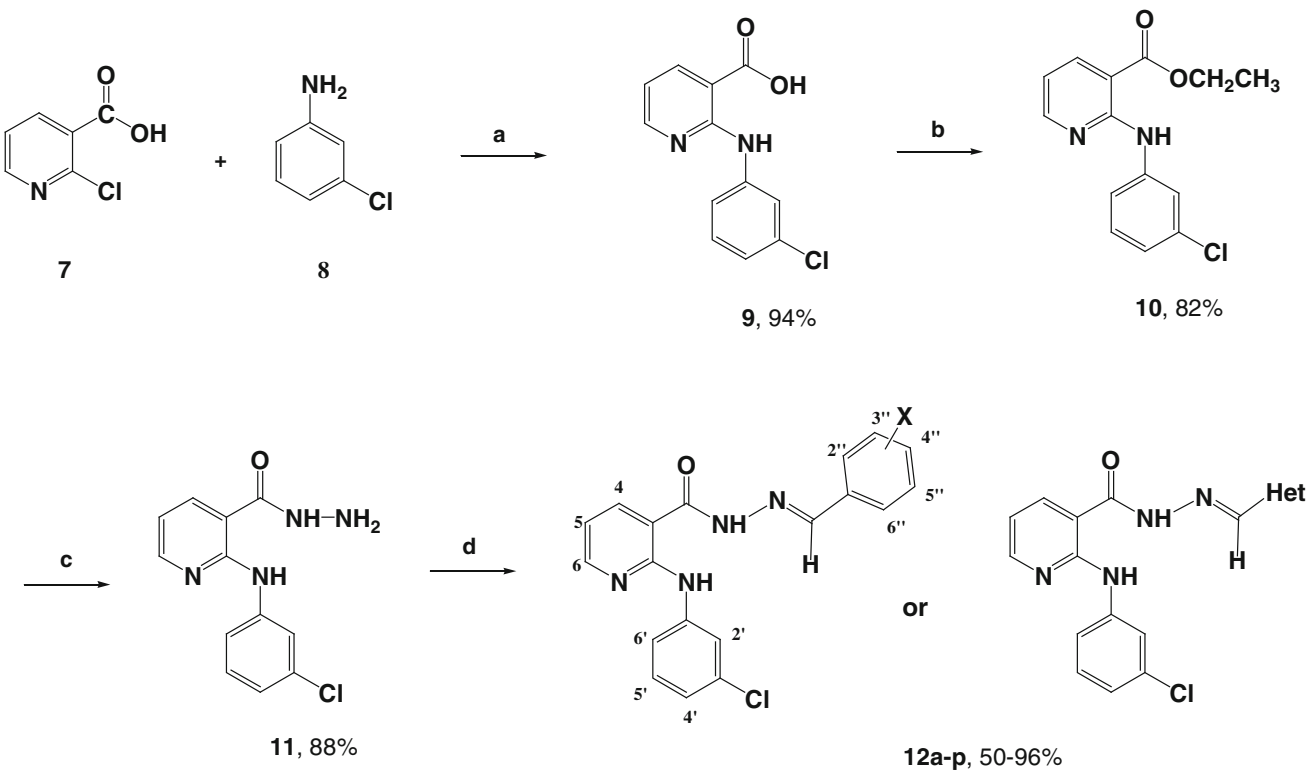


Fig. 2 Representative examples of hydrazone derivatives (**3**–**6**) and designed compounds **12**



Scheme 1 Reagents and conditions: **a** NaI 140 °C, 1 h, then 100 °C, 2 h; and **b** H_2SO_4, abs. EtOH, reflux, 72 h; **c** $N_2H_4{\cdot}H_2O$, abs. EtOH, r.t., 24 h; **d** Ar-CHO, abs. EtOH, HCl (2 drops), r.t., 24 h

2-(3-Chloroanilino)nicotinic acid hydrazide (**11**) is the key intermediate for the production of the title compounds **12**. The starting material, 2-(3-chloroanilino)nicotinic acid (**9**), was prepared by the reaction of 2-chloronicotinic acid (**7**) with an excess amount of 3-chloroaniline (**8**) in the presence of catalytic amount of sodium iodide (Hoffmann and Faure, 1968). Esterification of **9** using general acid-catalyzed method afforded the ethyl ester **10** (Kamal *et al*., 2007). Treatment of **10** with hydrazine hydrate in absolute ethanol generated the required hydrazide **11** (Kamal *et al*., 2007).

Title hydrazone compounds (**12a**–**p**) were prepared by the condensation of hydrazide **11** with different substituted aromatic aldehydes in absolute ethanol.

Anti-inflammatory activity

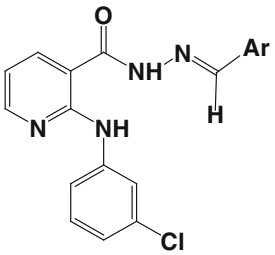
In vivo pharmacological evaluation of **12a**–**p** was carried out to assess their potential anti-inflammatory activity. Qualitative structure–activity relationship data, acquired using the carrageenan induced rat paw edema assay (Al-Haboubi and Zeitlin, 1983), showed that this group of arylidene-2-(3-chloroanilino)nicotinic acid hydrazides exhibit anti-inflammatory activity with moderate-to-excellent activity range (11–95 % inhibition at different time intervals) in comparison with niflumic acid as the reference drug (47–80 % inhibition, Table 1).

Interestingly, the compounds were shown to have different pharmacokinetic parameters, and hence, these compounds have shown their maximum activity at different time intervals. Therefore, they have been compared at their maximum anti-inflammatory activity (Table 1, last column).

First, the substituents on the terminal phenyl moiety, attached to the imine functional group of title compounds **12a**–**p** were rationally selected to acquire information about the influence of electronic and physicochemical parameters on pharmacological activities. The analysis of the results revealed that the presence of any substituent, electron-donating or electron-withdrawing, produced compounds with similar to noticeably improved activity relative to the unsubstituted one (**12a**). Moreover, introduction of small lipophilic fluoro, chloro, or methoxy substituents produced compounds with improved anti-inflammatory activities (47–95 % reduction in inflammation), while hydrophilic hydroxyl-substituted compounds (**12f**–**g**) have shown less to similar activities (35–48 % reduction in inflammation) for an *i.p.* dose of 10 mg/kg in comparison with **12a**.

The effect of changing the position of substituents on the phenyl ring was next investigated. In general, most of the meta-substituted compounds (except for **12h**–**i**) were preferred over para-substituted ones.

Table 1 Anti-inflammatory activities of compounds **12a–p**



Compound	Ar	AI acticity (%)[a]				
		At 30 m	At 1 h	At 2 h	At 3 h	Max. activity
12a	Ph	38.30 ± 9.41	38.96 ± 4.42	**39.35 ± 7.72**	32.62 ± 7.98	**39.35 ± 7.72**
12b	3-F-C_6H_4	**77.92 ± 4.04**[b]	67.83 ± 3.90	55.34 ± 11.77	56.06 ± 6.72	**77.92 ± 4.04**
12c	4-F-C_6H_4	36.92 ± 6.79	**47.18 ± 7.57**	30.10 ± 5.55	28.37 ± 6.37	**47.18 ± 7.57**
12d	3-Cl-C_6H_4	**95.37 ± 4.45**	71.94 ± 16.32	59.07 ± 9.14	43.17 ± 8.59	**95.37 ± 4.45**
12e	4-Cl-C_6H_4	**51.52 ± 8.46**	34.42 ± 8.38	33.01 ± 7.52	25.32 ± 4.98	**51.52 ± 8.46**
12f	3-OH-C_6H_4	35.31 ± 5.70	**47.94 ± 5.15**	39.65 ± 9.04	46.31 ± 2.75	**47.94 ± 5.15**
12g	4-OH-C_6H_4	10.03 ± 6.70	**35.57 ± 9.14**	24.89 ± 10.15	28.62 ± 10.77	**35.57 ± 9.14**
12h	3-OMe-C_6H_4	**79.07 ± 5.60**	61.21 ± 12.16	54.96 ± 8.75	40.51 ± 8.01	**79.07 ± 5.60**
12i	4-OMe-C_6H_4	49.29 ± 20.42	47.05 ± 10.33	**87.48 ± 5.04**	63.46 ± 5.61	**87.48 ± 5.04**
12j	2,4-$(OMe)_2$-C_6H_3	56.33 ± 18.23	40.66 ± 15.38	**75.76 ± 4.47**	61.66 ± 5.13	**75.76 ± 4.47**
12k	3,4-$(OMe)_2$-C_6H_3	**62.98 ± 7.70**	38.51 ± 13.17	28.98 ± 12.43	33.71 ± 12.65	**62.98 ± 7.70**
12l	3,4,5-$(OMe)_3$-C_6H_2	**45.18 ± 16.82**	10.52 ± 5.26	25.33 ± 10.57	16.95 ± 6.33	**45.18 ± 16.82**
12m	3-Pyridyl	36.23 ± 3.01	**37.38 ± 7.47**	32.43 ± 2.68	23.58 ± 14.24	**37.38 ± 7.47**
12n	4-Pyridyl	**72.44 ± 12.67**	45.89 ± 19.24	23.15 ± 12.75	15.23 ± 6.27	**72.44 ± 12.67**
12o	2-Furyl	33.80 ± 5.01	30.30 ± 4.76	48.30 ± 7.17	**57.94 ± 18.87**	**57.94 ± 18.87**
12p	2-Thienyl	33.20 ± 0.82	47.49 ± 13.89	**49.34 ± 7.39**	35.85 ± 6.06	**49.34 ± 7.39**
Niflumic acid		47.25 + 3.26	**81.38 + 15.62**	70.85 + 10.04	59.07 + 5.78	**81.38 + 15.62**

[a] The compounds are administered at the dose of 10 mg/kg

[b] The highest anti-inflammatory activity for each compound is shown as bold

Then, substitution of phenyl moiety with any heterocycle (pyridyl, thienyl, or furyl) produced compounds with similar to improved activities (49–72 % reduction in inflammation).

Finally, among these analogs, (3-chlorobenzylidene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12d**), exhibited the highest anti-inflammatory activity relative to niflumic acid as the reference drug (95 and 80 % reduction in inflammation postdrug administration, respectively).

Moreover, the analysis of the anti-inflammatory activities of **12a–p** and a comparison of their activities with previously synthesized analogs of these compounds (Kheradmand *et al*., 2013) having 3-trifluoromethylanilino moiety instead of 3-chloroanilino group have revealed that both groups showed noticeable activity relative to niflumic acid as the reference drug. In both groups 3-chlorobenzylidene derivatives have shown the highest anti-inflammatory activity, while **12d** showed more improved activity (95 %, relative to niflumic acid, 80 % reduction in inflammation) than this analog from the previously published compounds (78 %, relative to niflumic acid; 70 % reduction in inflammation). Also, in both group of compounds, meta-substitution provided compounds with higher anti-inflammatory activity than the para ones.

Regarding the other analogs, the influence of substituents on the anti-inflammatory activity was not similar in these two groups. In the present analogs, hydroxyphenyl (1**2f**, **g**) or pyridyl derivatives (**12m**, **n**) had shown weaker activities than niflumic acid, while similar substitutions in the previously published analogs provided compounds with similar to improved activities. On the contrary, the methoxy (**12h, i**) or 3-fluoro derivatives (**12b**) in the present group of compounds have shown similar to improved activities than niflumic acid, while similar analogs in the previously published ones revealed weaker activities.

Table 2 Analgesic activities of the selected **12** derivatives

Compound	R	Number of writhing[a]	Inhibition (%)
	CMC	48.75 ± 5.73	
12b	3-F-C_6H_4	20.85 ± 3.3*	52.23*,[b]
12d	3-Cl-C_6H_4	24.65 ± 4.47*	49.43*
12h	3-OMe-C_6H_4	19.65 ± 5.04*	59.69*
12i	4-OMe-C_6H_4	1.33 ± 0.88***	97.27***
12j	2,4-$(OMe)_2$-C_6H_3	0.2 ± 0.2***	99.59***
12n	4-pyridyl	4.6 ± 1.5***	90.56***
Niflumic acid		15.45 ± 6.9**	68.31**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to CMC group

[a] The compounds are administered at the dose of 10 mg/kg

[b] Analgesic activity relative to niflumic acid. *, ** and *** differed from control (CMC) group $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively

Analgesic activity

The compounds which exhibited significant anti-inflammatory activities comparable to that of the niflumic acid were subjected to analgesic assay.

The analgesic activities of the compounds were studied by using acetic acid-induced abdominal constriction test (writhing test) (Whittle, 1964). Qualitative structure–activity relationship data, acquired using the analgesic assay, showed that most of the selected arylidene-2-(3-chloroanilino)nicotinic acid hydrazides were able to reduce the AcOH-induced constrictions with moderate-to-excellent activity range (49–99.6 % inhibition) in comparison with niflumic acid as the reference drug (68 % inhibition) (Table 2).

Interestingly, among substituted analogs, 4-pyridyl derivatives (**12n**), 4-methoxyphenyl (**12i**) and 2,4-dimethoxyphenyl (**12j**) exhibited the most potent analgesic activity with 91, 97, and 99.6 % of inhibition, respectively, relative to the reference drug niflumic acid with 68 % of inhibition activity. This phenomenon can be explained by possible hydrogen bond formation between the nitrogen of 4-pyridyl or C-4 methoxy substituent on the phenyl ring with amino acids of target-binding site.

The analysis of analgesic activities of the selected compounds and comparison of their activities with the previously published analogs (Kheradmand *et al*., 2013) showed both groups as having significant analgesic activities relative to niflumic acid as the reference drug.

Conclusion

Various substituted arylidene-2-(3-chloroanilino)nicotinic acid hydrazides were synthesized and screened for their potential anti-inflammatory and analgesic activities. Most of the compounds have shown significant pharmacological activities. In carrageenan-induced rat paw edema assay, 3-chloro derivative **12d** exhibited the most potent anti-inflammatory activity. Selected compounds were subjected for writhing test and among them, 4-methoxy (**12i**) and 2,4-dimethoxyphenyl derivatives (**12j**) showed the most potent analgesic activities. Therefore, these new structures can be considered interesting for further modification as anti-inflammatory and analgesic agents.

Experimental protocols

Chemistry

Melting points were determined with a Reichert-Jung hot-stage microscope (Reichert-Jung, Germany) and are uncorrected. Infrared spectra were recorded on a Nicolet Magna 550-FT spectrometer (Nicolet, Madison, WI, USA). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker FT-500 MHz spectrometer (Bruker Bioscience, USA) in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard. Elemental microanalyses were carried out using a Perkin-Elmer 240-C apparatus (Perkin-Elmer, Beaconsfield, UK) and were within ±0.4 % of the theoretical values for C, H, and N. Male NMRI mice and male Wistar rats, used in the analgesic and anti-inflammatory screens, respectively, were purchased from Pasteur Institute (Karaj, Iran), and experiments were carried out using protocols approved by the ethics committee of Tehran University of Medical Sciences.

*Ethyl 2-(3-chloroanilino)nicotinate (**10**)*

2-(3-Chloroanilino)nicotinic acid **9** (24.8 g, 0.1 mol) and H_2SO_4 (5 mL) were taken in absolute ethanol (300 mL) and refluxed for 72 h. Then, the reaction mixture concentrated under reduced pressure; 100 mL of aqueous sodium carbonate solution (10 %, w/v) was added to the residue, and the ester was extracted in chloroform. Then, the solvent was dried over anhydrous Na_2SO_4, evaporated, and the remaining was recrystallized from ethanol to yield **10** (22.05 g) as a yellowish white solid.

Yield 82 %. m.p. 46–48 °C. IR (KBr) 3431 (NH), 1690 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 10.35 (bs, 1H, NH), 8.39 (dd, $J = 2.0$, 4.5 Hz, 1H, H_6), 8.25 (dd, $J = 2.0$, 8.0 Hz, 1H, H_4), 7.96 (t, $J = 1.5$ Hz, 1H, $H_{2'}$), 7.49 (dd, $J = 1.5$, 8.0 Hz, 1H, $H_{4'}$), 7.23 (t, $J = 8.0$ Hz, 1H, $H_{5'}$), 7.00 (dd, $J = 1.5$, 8.0 Hz, 1H, $H_{6'}$), 6.75 (dd, $J = 4.5$, 8.0 Hz, 1H, H_5), 4.38 (q, $J = 7.2$ Hz, 2H, CH_2), 1.41 (t, $J = 7.2$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 167.4 (C=O), 155.7 (C_2), 152.9 (C_6), 141.1 ($C_{1'}$), 140.1 (C_4), 134.4 ($C_{3'}$), 129.7 ($C_{5'}$),

122.4 ($C_{4'}$), 120.2 ($C_{2'}$), 118.4 ($C_{6'}$), 113.8 (C_5), 107.6 (C_3), 61.4 (CH_2), 14.2 (CH_3).

Anal. Calcd. for $C_{14}H_{13}ClN_2O_2$: C, 60.77; H, 4.74; N, 10.12. Found: C, 60.58; H, 4.87; N, 10.22.

*2-(3-Chloroanilino)nicotinic acid hydrazide (**11**)*

A mixture of ethyl 2-(3-chloroanilino)nicotinate **10** (20.75 g, 75 mmol) and hydrazine hydrate (375 mmol) in ethanol (200 mL) was stirred at room temperature for 24 h. Then, the reaction mixture concentrated in vacuo and left for overnight. The resulting crystals were filtered and re-crystallized from ethanol to yield acid hydrazide **11** (17.3 g) as a light lemon yellow solid.

Yield 88 %. m.p. 169–171 °C. IR (KBr) 3375, 3272 (NH), 1650 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 10.93 (bs, 1H, NH), 10.10 (bs, 1H, NH), 8.35 (d, $J = 4.5$ Hz, 1H, H_6), 8.09 (s, 1H, $H_{2'}$), 8.04 (d, $J = 7.5$ Hz, 1H, H_4), 7.38 (d, $J = 8.0$ Hz, 1H, $H_{4'}$), 7.30 (t, $J = 8.0$ Hz, 1H, $H_{5'}$), 6.99 (d, $J = 8.0$ Hz, 1H, $H_{6'}$), 6.90 (dd, $J = 4.5$, 7.5 Hz, 1H, H_5), 4.62 (bs, 2H, NH_2). ^{13}C NMR (DMSO-d_6) δ 167.0 (C=O), 154.3 (C_2), 150.8 (C_6), 142.2 ($C_{1'}$), 136.9 (C_4), 133.6 ($C_{3'}$), 130.8 ($C_{5'}$), 121.4 ($C_{4'}$), 118.6 ($C_{2'}$), 118.0 ($C_{6'}$), 114.7 (C_5), 111.3 (C_3).

Anal. Calcd. for $C_{12}H_{11}ClN_4O$: C, 54.87; H, 4.22; N, 21.33. Found: C, 54.73; H, 4.41; N, 21.45.

General procedure for synthesis of arylidene-2-(3-chloroanilino)nicotinic acid hydrazides (**12a**–**p**)

A mixture of hydrazide **11** (1 mmol) and corresponding aldehyde (1 mmol) in absolute ethanol (10 mL) in the presence of hydrochloric acid (2 drops) as the catalyst was stirred at room temperature for 24 h. The completion of the reaction was monitored by TLC. Then, water was added to the mixture, and the precipitate was filtered, washed, and recrystallized from ethanol to give **12**

*Benzylidene-2-(3-chloroanilino)nicotinic acid hydrazide (**12a**)*

It was obtained as a light lemon yellow solid, yield 50 %. m.p. 143-145 °C. IR (KBr) 3427, 3231 (NH), 1658 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 12.11 (bs, 1H, NH), 10.59 (bs, 1H, NH), 8.45 (bs, 1H, = CH–), 8.41 (d, $J = 4.5$ Hz, 1H, H_6), 8.21 (d, $J = 7.5$ Hz, 1H, H_4), 8.12 (s, 1H, $H_{2'}$), 7.78–7.70 (m, 2H, $H_{2'',6''}$), 7.52–7.40 (m, 4H, $H_{4'}$, $H_{3'',4'',5''}$), 7.31 (t, $J = 8.0$ Hz, 1H, $H_{5'}$), 7.03–6.94 (m, 2H, H_5 & $H_{6'}$). ^{13}C NMR (DMSO-d_6) δ 164.4 (C=O), 154.6 (C_2), 151.4 (C_6), 149.1 (=CH–), 142.1 ($C_{1'}$), 138.0 (C_4), 134.5 ($C_{3'}$), 133.6 ($C_{1''}$), 130.8 ($C_{5'}$), 130.7 ($C_{4''}$), 129.4 ($C_{2'',6''}$), 127.7 ($C_{3'',5''}$), 121.7 ($C_{4'}$), 119.0 ($C_{2'}$), 118.3 ($C_{6'}$), 114.6 (C_5), 111.5 (C_3).

Anal. Calcd. for $C_{19}H_{15}ClN_4O$: C, 65.05; H, 4.31; N, 15.97. Found: C, 65.18; H, 4.50; N, 15.87.

*(3-Fluorobenzylidene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12b**)*

It was obtained as a light lemon yellow solid, yield 82 %. m.p. 184–186 °C. IR (KBr) 3298 (NH), 1670 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 10.55 (bs, 1H, NH), 8.47 (s, 1H, =CH–), 8.45 (d, $J = 4.6$ Hz, 1H, H_6), 8.28 (d, $J = 7.5$ Hz, 1H, H_4), 8.09 (s, 1H, $H_{2'}$), 7.65–7.45 (m, 4H, $H_{2'',5'',6''}$ & $H_{4'}$), 7.38–7.25 (m, 2H, $H_{4''}$ & $H_{5'}$), 7.10-6.98 (m, 2H, H_5 & $H_{6'}$). ^{13}C NMR (DMSO-d_6) δ 164.4 (C=O), 162.9 (d, $J = 243.99$ Hz, $C_{3''}$), 154.5 (C_2), 151.1 (C_6), 147.7 (=CH–), 141.9 ($C_{1'}$), 138.4 (C_4), 137.2 (d, $J = 7.48$ Hz, $C_{5''}$), 133.6 ($C_{3'}$), 131.5 (d, $J = 7.55$ Hz, $C_{6''}$), 130.8 ($C_{5'}$), 124.1 ($C_{1''}$), 121.9 ($C_{4'}$), 119.3 ($C_{2'}$), 118.6 ($C_{6'}$), 117.5 (d, $J = 21.38$ Hz, $C_{4''}$), 114.7 (C_5), 113.6 (d, $J = 22.64$ Hz, $C_{2''}$), 111.6 (C_3).

Anal. Calcd. for $C_{19}H_{14}ClFN_4O$: C, 61.88; H, 3.83; N, 15.19. Found: C, 61.67; H, 3.91; N, 15.34.

*(4-Fluorobenzylidene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12c**)*

It was obtained as a light lemon yellow solid, yield 88 %. m.p. 199–200 °C. IR (KBr) 3355, 3215 (NH), 1679 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 12.22 (bs, 1H), 10.63 (bs, 1H), 8.51 (s, 1H, =CH–), 8.40 (d, $J = 4.8$ Hz, 1H, H_6), 8.30 (d, $J = 8.0$ Hz, 1H, H_4), 8.09 (s, 1H, $H_{2'}$), 7.82 (dd, $J = 6.5$, 8.0 Hz, 2H, $H_{2'',6''}$), 7.47 (d, $J = 7.2$ Hz, 1H, $H_{4'}$), 7.36–7.26 (m, 3H, $H_{5'}$ & $H_{3'',5''}$), 7.06–6.94 (m, 2H, $H_{6'}$ & H_5). ^{13}C NMR (DMSO-d_6) δ 164.1 (C=O), 163.7 (d, $J = 247.77$ Hz, $C_{4''}$), 154.2 (C_2), 150.2 (C_6), 148.1 (=CH–), 141.5 ($C_{1'}$), 138.9 (C_4), 133.7 ($C_{3'}$), 131.2 ($C_{1''}$), 130.9 ($C_{5'}$), 129.9 (d, $J = 7.55$ Hz, $C_{2'',6''}$), 122.3 ($C_{4'}$), 119.8 ($C_{2'}$), 119.0 ($C_{6'}$), 116.4 (d, $J = 21.38$ Hz, $C_{3'',5''}$), 114.6 (C_5), 112.0 (C_3).

Anal. Calcd. for $C_{19}H_{14}ClFN_4O$: C, 61.88; H, 3.83; N, 15.19. Found: C, 62.07; H, 3.99; N, 15.08.

*(3-Chlorobenzylidene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12d**)*

It was obtained as a light lemon yellow solid, yield 89 %. m.p. 198–200 °C. IR (KBr) 3443 (NH), 1671 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 12.37 (bs, 1H), 10.58 (bs, 1H, NH), 8.47 (s, 1H, =CH-), 8.42 (d, $J = 4.7$ Hz, 1H, H_6), 8.28 (d, $J = 8.0$ Hz, 1H, H_4), 8.11 (s, 1H, $H_{2'}$), 7.82 (s, 1H, $H_{2''}$), 7.72 (d, $J = 7.8$ Hz, 1H, $H_{6''}$), 7.55–7.45 (m, 3H, $H_{4'}$ & $H_{4'',5''}$), 7.33 (t, $J = 8.0$ Hz, 1H, $H_{5'}$), 7.08–6.95 (m, 2H, $H_{6'}$ & H_5). ^{13}C NMR (DMSO-d_6) δ 164.4 (C=O), 154.4 (C_2), 151.0 (C_6), 147.4 (=CH–). 141.8 ($C_{1'}$), 138.4 (C_4), 136.8 ($C_{3''}$), 134.2 ($C_{1''}$), 133.6 ($C_{3'}$), 131.3 ($C_{4''}$), 130.8

($C_{5'}$), 130.4 ($C_{5''}$), 126.9 ($C_{2''}$), 126.4 ($C_{6''}$), 122.0 ($C_{4'}$), 119.4 ($C_{2'}$), 118.7 ($C_{6'}$), 114.6 (C_5), 111.6 (C_3).

Anal. Calcd. for $C_{19}H_{14}Cl_2N_4O$: C, 59.24; H, 3.66; N, 14.54. Found: C, 59.03; H, 3.82; N, 14.31.

*(4-Chlorobenzylidene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12e**)*

It was obtained as a light lemon yellow solid, yield 90 %. m.p. 215–216 °C. IR (KBr) 3365 (NH), 1678 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 12.23 (bs, 1H, NH), 10.59 (bs, 1H, NH), 8.48 (s, 1H, =CH–), 8.41 (d, $J = 4.6$ Hz, 1H, H_6), 8.26 (d, $J = 8.2$ Hz, 1H, H_4), 8.10 (s, 1H, $H_{2'}$), 7.79 (d, $J = 8.0$ Hz, 2H, $H_{2'',6''}$), 7.54 (d, $J = 8.0$ Hz, 2H, $H_{3'',5''}$), 7.47 (d, $J = 8.0$ Hz, 1H, H_4'), 7.32 (t, $J = 8.0$ Hz, 1H, $H_{5'}$), 7.05–6.96 (m, 2H, $H_{6'}$ & H_5). ^{13}C NMR (DMSO-d_6) δ 164.3 (C=O), 154.3 (C_2), 150.5 (C_6), 147.9 (=CH–), 141.6 ($C_{1'}$), 138.7 (C_4), 135.2 ($C_{4''}$), 133.6 ($C_{3'}$), 133.5 ($C_{2'',6''}$), 130.8 ($C_{5'}$), 129.5 ($C_{1''}$), 129.3 ($C_{3'',5''}$), 122.2 ($C_{4'}$), 119.6 ($C_{2'}$), 118.9 ($C_{5'}$), 114.6 (C_5), 111.9 (C_3).

Anal. Calcd. for $C_{19}H_{14}Cl_2N_4O$: C, 59.24; H, 3.66; N, 14.54. Found: C, 59.09; H, 3.43; N, 14.67.

*(3-Hydroxybenzylidene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12f**)*

It was obtained as a yellowish gray solid, yield 90 %. m.p. 145–147 °C. IR (KBr) 3485 (OH), 3385 (NH), 1676 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 12.12 (bs, 1H, NH), 10.60 (bs, 1H, NH), 8.44–8.35 (m, 2H, H_6 & =CH–), 8.26 (d, $J = 8.0$ Hz, 1H, H_4), 8.10 (s, 1H, $H_{2'}$), 7.47 (d, $J = 8.0$ Hz, 1H, $H_{4'}$), 7.32 (t, $J = 8.0$ Hz, 1H, H_5'), 7.27 (t, $J = 7.2$ Hz, 1H, $H_{5''}$), 7.22 (s, 1H, $H_{2''}$), 7.12 (d, $J = 7.2$ Hz, 1H, $H_{6''}$), 7.10–6.86 (m, 2H, H_6' & H_5), 6.86 (d, $J = 7.2$ Hz, 1H, $H_{4''}$). ^{13}C NMR (DMSO-d_6) δ 163.8 (C=O), 158.2 ($C_{3''}$), 153.9 (C_2), 149.6 (C_6), 149.3 (=CH–), 141.1 ($C_{1'}$), 139.4 (C_4), 135.8 ($C_{1''}$), 133.8 ($C_{3'}$), 131.0 ($C_{5'}$), 130.4 ($C_{5''}$), 122.8 ($C_{4'}$), 120.3 ($C_{6''}$), 119.5 ($C_{2'}$), 119.3 ($C_{4''}$), 118.2 ($C_{6'}$), 114.5 (C_5), 113.3 ($C_{2''}$), 112.5 (C_3).

Anal. Calcd. for $C_{19}H_{15}ClN_4O_2$: C, 62.21; H, 4.12; N, 15.27. Found: C, 62.31; H, 3.97; N, 15.40.

*(4-Hydroxybenzylidene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12g**)*

It was obtained as a lemon yellow solid, yield 95 %. m.p. 159–161 °C. IR (KBr) 3459 (OH), 3385 (NH), 1664 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 12.05 (bs, 1H, NH), 10.70 (bs, 1H, NH), 8.40 (s, 1H, =CH–), 8.37 (d, $J = 4.9$ Hz, 1H, H_6), 8.28 (d, $J = 8.1$ Hz, 1H, H_4), 8.01 (s, 1H, $H_{2'}$), 7.58 (d, $J = 9.0$ Hz, 2H, $H_{2'',6''}$), 7.49 (d, $J = 8.0$ Hz, 1H, $H_{4'}$), 7.23 (t, $J = 8.0$ Hz, 1H, $H_{5'}$), 7.05–6.96 (m, 2H, $H_{6'}$ & H_5), 6.86 (d, $J = 9.0$ Hz, 2H, $H_{3'',5''}$). ^{13}C NMR (DMSO-d_6) δ 163.9 (C=O), 160.2 ($C_{4''}$), 154.3 (C_2), 150.3 (C_6), 149.6 (=CH–), 141.7 ($C_{1'}$), 138.4 (C_4), 130.8 ($C_{5'}$), 129.5 ($C_{2'',6''}$), 125.5 ($C_{1''}$), 122.1 ($C_{4'}$), 119.5 ($C_{2'}$), 118.8 ($C_{6'}$), 116.2 ($C_{3'',5''}$), 114.6 (C_5), 112.14 (C_3).

Anal. Calcd. for $C_{19}H_{15}ClN_4O_2$: C, 62.21; H, 4.12; N, 15.27. Found: C, 62.34; H, 4.19; N, 15.08.

*(3-Methoxybenzylidene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12h**)*

It was obtained as a light lemon yellow solid, yield 87 %. m.p. 193–195 °C. IR (KBr) 3338, 3184 (NH), 1655 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 12.22 (bs, 1H, NH), 10.60 (bs, 1H, NH), 8.46 (s, 1H, =CH–), 8.41 (d, $J = 4.4$ Hz, 1H, H_6), 8.26 (d, $J = 7.5$ Hz, 1H, H_4), 8.10 (s, 1H, $H_{2'}$), 7.49 (d, $J = 8.0$ Hz, 1H, $H_{4'}$), 7.40 (t, $J = 8.0$ Hz, 1H, $H_{5'}$), 7.35–7.25 (m, 3H, $H_{2'',5'',6''}$), 7.15–6.86 (m, 3H, $H_{4''}$ & $H_{6'}$ & H_5), 3.82 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6) δ 164.4 (C=O), 160.04 ($C_{3''}$), 154.6 (C_2), 151.3 (C_6), 149.0 (=CH–), 142.04 ($C_{1'}$), 138.1 (C_4), 136.0 ($C_{1''}$), 133.6 ($C_{3'}$), 130.7 ($C_{5'}$), 130.5 ($C_{5''}$), 121.7 ($C_{6''}$), 120.7 ($C_{4''}$), 119.1 ($C_{2'}$), 118.4 ($C_{6'}$), 117.0 ($C_{2''}$), 114.7 (C_5), 111.6 (C_3), 55.7 (CH_3).

Anal. Calcd. for $C_{20}H_{17}ClN_4O_2$: C, 63.08; H, 4.50; N, 14.71. Found: C, 63.04; H, 4.31; N, 14.51.

*(4-Methoxybenzylidene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12i**)*

It was obtained as a lemon yellow solid, yield 95 %. m.p. 219–220 °C. IR (KBr) 3338 (NH), 1668 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 12.15 (bs, 1H, NH), 10.65 (bs, 1H, NH), 8.42 (d, $J = 4.5$ Hz, 1H, H_6), 8.39 (s, 1H, = CH-), 8.29 (d, $J = 7.5$ Hz, 1H, H_4), 8.10 (s, 1H, $H_{2'}$), 7.70 (d, $J = 8.5$ Hz, 2H, $H_{2'',6''}$), 7.48 (d, $J = 8.0$ Hz, 1H, $H_{4'}$), 7.33 (t, $J = 8.0$ Hz, 1H, $H_{5'}$), 7.04 (d, $J = 8.5$ Hz, 2H, $H_{3'',5''}$), 7.00 (d, $J = 8.0$ Hz, 1H, $H_{6'}$), 6.98 (dd, $J = 4.5, 7.5$ Hz, 1H, H_5), 3.82 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6) δ 164.0 (C=O), 161.5 ($C_{4''}$), 154.3 (C_2), 150.3 (C_6), 149.2 (=CH–), 141.7 ($C_{1'}$), 138.5 (C_4), 133.6 ($C_{3'}$), 130.8 ($C_{5'}$), 129.3 ($C_{2'',6''}$), 127.1 ($C_{1''}$), 122.1 ($C_{4'}$), 119.5 ($C_{2'}$), 118.8 ($C_{6'}$), 114.9 ($C_{3'',5''}$), 114.6 (C_5), 112.1 (C_3), 55.8 (CH_3).

Anal. Calcd. for $C_{20}H_{17}ClN_4O_2$: C, 63.08; H, 4.50; N, 14.71. Found: C, 63.18; H, 4.72; N, 14.58.

*(2,4-Dimethoxybenzylidene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12j**)*

It was obtained as a light lemon yellow solid, yield 92 %. m.p. 202–204 °C. IR (KBr) 3320, 3195 (NH), 1625 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6) δ 11.95 (bs, 1H, NH), 10.72 (bs, 1H, NH), 8.71 (s, 1H, =CH–), 8.40 (d, $J = 4.7$ Hz, 1H, H_6), 8.21 (d, $J = 7.5$ Hz, 1H, H_4), 8.12 (s, 1H, $H_{2'}$), 7.83 (d,

$J = 8.8$ Hz, 1H, $H_{6''}$), 7.49 (d, $J = 7.9$ Hz, 1H, $H_{4'}$), 7.23 (t, $J = 7.9$ Hz, 1H, $H_{5'}$), 7.05–6.93 (m, 2H, $H_{6'}$ & H_5), 6.86 (m, 2H, $H_{3'',5''}$), 3.87 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6) δ 164.0 (C=O), 163.1 ($C_{4''}$), 159.8 ($C_{2''}$), 154.6 (C_2), 151.2 (C_6), 144.7 (=CH–), 142.1 ($C_{1'}$), 137.8 (C_4), 133.6 ($C_{3'}$), 130.7 ($C_{5'}$), 127.1 ($C_{6''}$), 121.6 ($C_{4'}$), 118.9 ($C_{2'}$), 118.2 ($C_{6'}$), 115.3 ($C_{1''}$), 114.6 (C_5), 111.6 (C_3), 107.0 ($C_{5''}$), 98.8 ($C_{3''}$), 55.3 (CH_3), 55.9 (CH_3).

Anal. Calcd. for $C_{21}H_{19}ClN_4O_3$: C, 61.39; H, 4.66; N, 13.64. Found: C, 61.50; H, 4.51; N, 13.73.

*(3,4-Dimethoxybenzylidene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12k**)*

It was obtained as a light lemon yellow solid, yield 96 %. m.p. 214–215 °C. IR (KBr) 3320, 3200 (NH), 1650 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 12.05 (bs, 1H, NH), 10.60 (bs, 1H, NH), 8.41 (d, $J = 4.8$ Hz, 1H, H_6), 8.40 (s, 1H, =CH–), 8.21 (d, $J = 8.0$ Hz, 1H, H_4), 8.11 (s, 1H, $H_{2'}$), 7.49 (d, $J = 8.0$ Hz, 1H, $H_{4'}$), 7.37 (s, 1H, $H_{2''}$), 7.32 (t, $J = 8.0$ Hz, 1H, $H_{5'}$),7.23 (d, $J = 7.6$ Hz, 1H, $H_{6''}$), 7.05–6.96 (m, 3H, $H_{6'}$ & H_5 & $H_{5''}$), 3.84 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6) δ 164.2 (C=O), 154.5 (C_2), 151.4 ($C_{4''}$), 151.2 (C_6), 149.5 ($C_{3''}$), 149.3 (=CH–), 142.1 ($C_{1'}$), 137.9 (C_4), 133.6 ($C_{3'}$), 130.7 ($C_{5'}$), 127.2 ($C_{1''}$), 122.7 ($C_{4'}$), 121.6 ($C_{6''}$), 119.0 ($C_{2'}$), 118.3 ($C_{6'}$), 114.6 (C_5), 111.9 ($C_{2''}$), 111.7 (C_3), 108.5 ($C_{5''}$), 56.0 (CH_3), 55.9 (CH_3).

Anal. Calcd. for $C_{21}H_{19}ClN_4O_3$: C, 61.39; H, 4.66; N, 13.64. Found: C, 61.32; H, 4.41; N, 13.83.

*(3,4,5-Trimethoxybenzylidene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12l**)*

It was obtained as a lemon yellow solid, yield 92 %. m.p. 215–217 °C. IR (KBr) 3380, 3293 (NH), 1674 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 12.25 (bs, 1H, NH), 10.68 (bs, 1H, NH), 8.43 (s, 1H, =CH–), 8.40 (d, $J = 4.9$ Hz, 1H, H_6), 8.28 (d, $J = 8.0$ Hz, 1H, H_4), 8.10 (s, 1H, $H_{2'}$), 7.49 (d, $J = 7.8$ Hz, 1H, $H_{4'}$), 7.33 (t, $J = 7.8$ Hz, 1H, $H_{5'}$), 7.10–6.95 (m, 2H, $H_{6'}$ & H_5), 3.85 (s, 6H, OCH_3), 3.72 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6) δ 164.1 (C=O), 154.2 (C_2), 153.7 ($C_{3'',5''}$), 150.3 (C_6), 149.1 (=CH–), 141.7 ($C_{1'}$), 139.8 ($C_{4''}$), 138.7 (C_4), 133.6 ($C_{3'}$), 130.8 ($C_{5'}$), 130.1 ($C_{1''}$), 122.2 ($C_{4'}$), 119.6 ($C_{2'}$), 118.9 ($C_{6'}$), 114.6 (C_5), 112.0 (C_3), 104.8 ($C_{2'',6''}$), 60.6 (OCH_3-4″), 56.4 (OCH_3-3″,5″).

Anal. Calcd. for $C_{22}H_{21}ClN_4O_4$: C, 59.93; H, 4.80; N, 12.71. Found: C, 59.85; H, 4.66; N, 12.54.

*(Pyridin-3-ylmethylene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12m**)*

It was obtained as a lemon yellow solid, yield. 95 %. m.p. 229–231 °C. IR (KBr) 3187 (NH), 1660 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 12.85 (bs, 1H, NH), 10.62 (bs, 1H, NH), 9.01 (s, 1H, $H_{2''}$), 8.73 (bs, 1H, $H_{6''}$), 8.54 (s, 1H, =CH–), 8.59 (bs, 1H, $H_{4''}$), 8.42 (d, $J = 4.5$ Hz, 1H, H_6), 8.24 (d, $J = 7.5$ Hz, 1H, H_4), 8.10 (s, 1H, $H_{2'}$), 7.70 (bs, 1H, $H_{5''}$), 7.48 (d, $J = 8.0$ Hz, 1H, $H_{4'}$), 7.32 (t, $J = 8.0$ Hz, 1H, $H_{5'}$), 7.07–6.94 (m, 2H, $H_{6'}$ & H_5). ^{13}C NMR (DMSO-d_6) δ 164.8 (C=O), 154.6 (C_2), 151.5 (C_6), 144.2 (=CH–), 143.5 ($C_{6''}$), 142.6 ($C_{2''}$), 141.9 ($C_{1'}$), 140.9 ($C_{4''}$), 138.7 (C_4), 133.6 ($C_{3'}$), 130.7 ($C_{5'}$), 127.2 ($C_{3'',5''}$), 121.9 ($C_{4'}$), 119.3 ($C_{2'}$), 118.6 ($C_{6'}$), 114.6 (C_5), 110.0 (C_3).

Anal. Calcd. for $C_{18}H_{14}ClN_5O$: C, 61.46; H, 4.01; N, 19.91. Found: C, 61.27; H, 3.88; N, 20.04.

*(Pyridin-4-ylmethylene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12n**)*

It was obtained as a lemon yellow solid, yield 90 %. m.p. 227–229 °C. IR (KBr) 3520, 3459 (NH), 1677 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 12.85 (bs, 1H, NH), 10.55 (bs, 1H, NH), 8.81 (d, $J = 4.0$ Hz, 2H, $H_{3'',5''}$), 8.57 (s, 1H, =CH–), 8.44 (d, $J = 4.5$ Hz, 1H, H_6), 8.32 (d, $J = 8.0$ Hz, 1H, H_4), 8.09 (s, 1H, $H_{2'}$), 8.00 (d, $J = 4.0$ Hz, 2H, $H_{2'',6''}$), 7.49 (d, $J = 8.0$ Hz, 1H, $H_{4'}$), 7.23 (t, $J = 8.0$ Hz, 1H, $H_{5'}$), 7.07–6.94 (m, 2H, $H_{6'}$ & H_5). ^{13}C NMR (DMSO-d_6) δ 165.0 (C=O), 154.6 (C_2), 151.9 (C_6), 146.6 ($C_{2'',6''}$), 144.9 (=CH–), 142.0 ($C_{1'}$), 138.5 (C_4), 133.5 ($C_{3'}$), 133.5 ($C_{1''}$), 130.7 ($C_{5'}$), 122.9 ($C_{3'',5''}$), 121.8 ($C_{4'}$), 119.2 ($C_{2'}$), 118.5 ($C_{6'}$), 114.7 (C_5), 110.9 (C_3).

Anal. Calcd. for $C_{18}H_{14}ClN_5O$: C, 61.46; H, 4.01; N, 19.91. Found: C, 61.27; H, 4.20; N, 20.06.

*(Furan-2-ylmethylene)-2-(3-chloroanilino)nicotinic acid hydrazide **12o***

It was obtained as a greenish umber solid, yield 71 %. m.p. 104–106 °C. IR (KBr) 3298 (NH), 1687 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 11.72 (bs, 1H, NH), 10.51 (bs, 1H, NH), 8.42 (d, $J = 4.5$ Hz, 1H, H_6), 8.31 (s, 1H, =CH–), 8.16 (d, $J = 7.5$ Hz, 1H, H_4), 8.09 (s, 1H, $H_{2'}$), 7.88 (s, 1H, $H_{5''}$), 7.44 (d, $J = 8.0$ Hz, 1H, $H_{4'}$), 7.31 (t, $J = 8.0$ Hz, 1H, $H_{5'}$), 7.10–6.96 (m, 3H, $H_{6'}$ & H_5 & $H_{3''}$), 6.62 (s, 1H, $H_{4''}$). ^{13}C NMR (DMSO-d_6) δ 164.3 (C=O), 154.5 (C_2), 151.4 (C_6), 149.7 ($C_{2''}$), 146.0 ($C_{5''}$), 142.1 ($C_{1'}$), 138.8 (C_4), 137.9 (=CH–), 133.6 ($C_{3'}$), 130.7 ($C_{5'}$), 121.7 ($C_{4'}$), 119.0 ($C_{2'}$), 118.3 ($C_{6'}$), 114.7 ($C_{4''}$), 114.6 (C_5), 112.8 ($C_{3''}$), 111.5 (C_3).

Anal. Calcd. for $C_{17}H_{13}ClN_4O_2$: C, 59.92; H, 3.85; N, 16.44. Found: C, 59.82; H, 3.99; N, 16.28.

*(Thiophen-2-ylmethylene)-2-(3-chloroanilino)nicotinic acid hydrazide (**12p**)*

It was obtained as a yellowish white solid, yield 76 %. m.p. 227–229 °C. IR (KBr) 3341 (NH), 1656 (C=O) cm^{-1}. ^{1}H

NMR (DMSO-d_6) δ 10.83 (bs, 1H, NH), 10.69 (s, 1H, =CH–), 10.59 (bs, 1H, NH), 8.44 (dd, $J = 2.0$, 4.0 Hz, 1H, H_6), 8.19 (d, $J = 6.8$ Hz, 1H, H_4), 8.11 (t, $J = 2.4$ Hz, 1H, $H_{2'}$), 7.93 (d, $J = 4.0$ Hz, 1H, $H_{3''}$), 7.89 (d, $J = 4.8$ Hz, 1H, $H_{5''}$), 7.46 (dd, $J = 2.4$, 8.0 Hz, 1H, $H_{4'}$), 7.31 (t, $J = 8.0$ Hz, 1H, $H_{5'}$), 7.24 (dd, $J = 4.0$, 4.8 Hz, 1H, $H_{4''}$), 7.05–6.96 (m, 2H, $H_{6'}$ & H_5). ^{13}C NMR (DMSO-d_6) δ 167.6 (C=O), 161.5 ($C_{2''}$), 154.4 (C_2), 151.7 (C_6), 142.0 ($C_{1'}$), 137.6 (C_4), 137.3 ($C_{3''}$), 133.6 ($C_{3'}$), 132.5 ($C_{4''}$), 130.8 ($C_{5'}$), 129.8 ($C_{5''}$), 128.8 (=CH–), 121.8 ($C_{4'}$), 119.0 ($C_{2'}$), 118.3 ($C_{6'}$), 114.9 (C_5), 110.8 (C_3).

Anal. Calcd. for $C_{17}H_{13}ClN_4OS$: C, 57.22; H, 3.67; N, 15.70. Found: C, 57.39; H, 3.54; N, 15.66.

Pharmacology

Anti-inflammatory assay

The anti-inflammatory activity was determined in vivo using the carrageenan-induced rat paw edema test (Al-Haboubi and Zeitlin, 1983). Edema was induced in the right hind paw of male rats by subcutaneous injection of 0.1 mL of 1 % (w/v) carrageenan in saline into their footpads 30 min after *i.p.* administration of the suspension of compounds in vehicle (0.5 % methyl cellulose).

The paw thickness was measured from the ventral to the dorsal surfaces using a dial caliper immediately before and then 30 min, 1, 2, and 3 h after carrageenan injection. The edema was calculated as the thickness variation between thickness of paw before and after carrageenan injection. Anti-inflammatory activity was expressed as the percentage of inhibition of the edema compared with the control group and was calculated using the following formula:

$$\%\text{Inhibition} = (1 - T_t/T_c) \times 100$$

where T_t and T_c are defined as the thickness variations of test group and control group, respectively.

Analgesic assay

The analgesic activity of all compounds was determined in vivo by acetic acid-induced abdominal constriction test (writhing test) in mice (Whittle, 1964). An aqueous acetic acid solution (1 %; 0.1 mL/10 g) was administered 30 min after *i.p.* administration of the suspension of compounds in vehicle (0.5 % methyl cellulose). The number of writhes was counted for 30 min after acetic acid injection. The analgesic activity was expressed as the percentage of inhibition of constrictions when compared with the vehicle control group and was calculated using the following equation: %Inhibition = [(control mean − test mean)/control mean] × 100.

Statistics

The results are expressed as the mean ± SEM of 6 animals per group. The data were statistically analyzed by one way analysis of Variance (ANOVA) followed by Tukey multicomparison test. Differences with $P < 0.05$ between experiments group were considered statistically significant.

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