

Article

Understanding Chemistry and Unique NMR Characters of Novel Amide and Ester Leflunomide Analogues

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Abstract: A series of diverse substituted 5-methyl-isoxazole-4-carboxylic acid amides, imide and esters in which the benzene ring is mono or disubstituted was prepared. Spectroscopic and conformational examination was investigated and a new insight involving steric interference and interesting downfield deviation due to additional diamagnetic anisotropic effect of the amidic carbonyl group and the methine protons in 2,6-diisopropyl-aryl derivative (**2**) as conformationally restricted analogues Leflunomide was discussed. Individual substituent electronic effects through π resonance of p-substituents and most stable conformation of compound (**2**) are discussed.

Keywords: leflunomide derivatives; 2,6-diisopropylphenyl anilide chemical shift abnormalities; 5-methyl-4-isoxazole derivatives

1. Introduction

It is known that isoxazole derivatives show diverse biological activity and are known for their potential use against a broad array of diseases including infectious diseases, parasitic infection and for the area of oncology therapeutics [1]. For example, Leflunomide (Avara), is an immunomodulator which is used to treat the symptoms associated with rheumatoid arthritis RA and psoriatic arthritis (Figure 1) [2]. Leflunomide, as a small, low molecular-weight isoxazole derivative, is one of the most potent but associated with serious side effects [3].

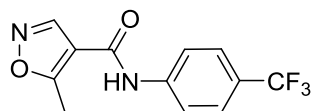


Figure 1. Leflunomide (Avara).

The importance of the amide group for living organisms can be correlated to some of its chemical properties such as planarity [4], relatively high barrier of rotation around the C–N bond, and its hydrogen bonding donor and acceptor properties. These are the key factors related to determining the conformations of protein-protein complexes, enzymes and other biopolymers like DNA and RNA. Studies on amide derivatives have led to many speculations [5]. As NMR provides one of the most sensitive biophysical techniques, NMR studies and utilization of chemical shift parameters are

increasingly being used to tackle more challenging biological problems. It is well known that the chemical shift depends on electronic and molecular environments [6].

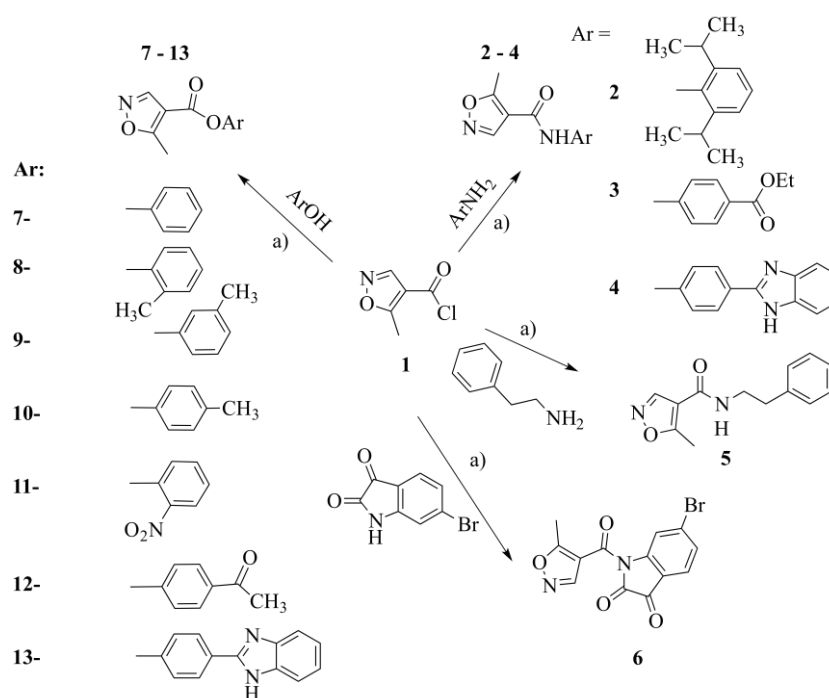
A high rotational barrier due to the partial double-bond character of tertiary amides leads to the geometric and magnetic nonequivalence of the nitrogen-attached groups even when both are the same. Amide and related functional groups are planar and exhibit E/Z (Rotational) isomerism [7].

There is a known preference of *N*-aryl amides to exist in an E (Ar and C=O anti) geometry. The N–Ar rotation barrier of a 2-phenylacetamide analog was reduced from 31 kcal mol^{−1} in the precursor to 17 kcal mol^{−1} in the enolate. This dramatic barrier reduction has implications of both N–Ar and amide C–N rotations [8].

Functional groups with ‘*N*sp²–Ar’ as *N*-aryl amides often prefer twisted geometries. Both the geometry of the N–Ar bond and its rotation barrier are crucial features in areas as diverse as enzyme/substrate binding [9–11].

Selectively deshielded aromatic protons in some *ortho*-substituted acetanilides [12] exhibit signals at unusually low fields for the aromatic proton adjacent to the acetamido group and for the amido proton itself.

Based on these facts, in this study, we synthesized novel Leflunomides, which are based on bioisosterism [13], by changing the substitution pattern at the 4-position of isoxazole ring of Leflunomide to: confer different conformations and electronic environment at the amide group that would exert some effect on the lipophilicity and enhance the activity of the target molecules. New substituents are applied, like for example replacing the lipophilic *p*-CF₃ group with the other electron withdrawing group, adding the electron donating group at either *ortho* or *para* position, replacing the entire ring with phenylethyl ring, or adopting hybrid pharmacophore like isatin and benzimidazole nucleus or isosteric replacement of amide by ester (Scheme 1).



Scheme 1. Synthetic route for novel Leflunomide derivatives.

As part of our research aiming at the synthesis and pharmacological evaluation of diverse functionalized aryl amide and isosteric analogues of leflunomide, new compounds have been investigated for further structure–activity relationship (SAR) studies. Our first publication in this series indicated that many Leflunomide analogues showed better antifibrotic activity than Leflunomide [14].

2. Results and Discussion

2.1. Synthetic Chemistry

In this study, the starting compounds assembled by coupling the key intermediate: 5-methylisoxazole-4-carbonyl chloride **7** and anilines, aryl ethylamine, isatin or phenols, in dichloromethane (DCM) using trimethylamine (TEA) as base [15] to afford the final products **2–13** in moderate to high yields (40–91%) (Scheme 1, Table 1). The desired benzimidazole derivative for preparation of **4** and **13** was obtained in a good yield starting from heating o-phenylenediamine (OPDA) with p-amino ethylbenzoate in the presence of a strong dehydrating agent such as polyphosphoric acid (PPA) [16] or with 4-hydroxybenzaldehyde in dimethyl formamide (DMF) using sodium metabisulfite as oxidizing agent [17], respectively. The structures of compounds **2–13** were approved on the basis of spectral data (IR, mass and NMR) and elemental analysis. All spectral data were in good agreement with the proposed structures.

Table 1. Synthesis of 5-methyl-4-isoxazole derivatives (**2–13**).

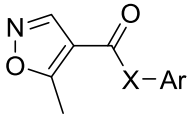
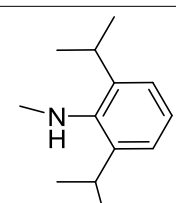
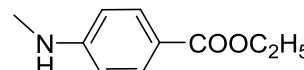
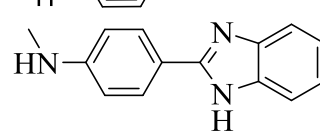
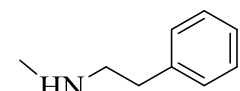
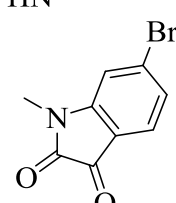
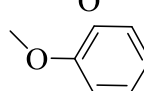
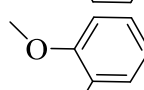
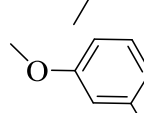
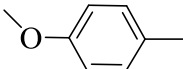
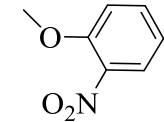
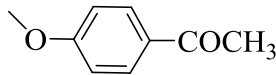
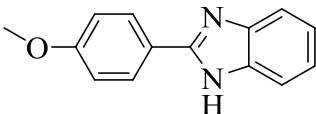
				
Products	-X-Ar *	Yield (%)	Melting Points (°C)	Colour
2		58	145–146	Yellow
3		68	130–131	red
4		71	185–186	gray
5		91	148–149	white
6		55	190–191	red
7		41	89–90	yellow
8		45	100–101	yellow
9		40	95–96	yellow

Table 1. Cont.

10		48	80–81	white
11		42	124–125	white
12		51	188–189	Buff
13		40	150–151	White

* General structure is provided on top of the table

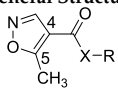
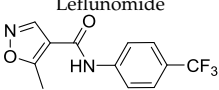
2.2. Spectroscopic Examination

Dimethyl sulfoxide- d_6 (DMSO) was the solvent of choice, not only for its excellent solvation properties, but also for the fact that amide proton chemical shifts in DMSO were clearly separable from the aromatic region. The downfield chemical shifts in DMSO (about 12.8 ppm in case of 2–5) are undoubtedly due to hydrogen bonding of the amide proton with solvent. The substituents exert relatively small influences on the δ of the N–H proton as the anisotropy effect depends on the spatial arrangement, but it is independent of the nuclei being observed [18].

The chemical shift of C_5 CH_3 and C_3 H in 1H -NMR and C_4 C=O in ^{13}C -NMR having nearly the same chemical shift value meaning a similar special arrangement like 5-Methylisoxazole-4-carboxylic acid [19]. Due to planar delocalization, ^{13}C -NMR chemical shifts for the amidic CON indicates (amidic sp^2 carbon near 188 Hz) due to electronic interactions and steric effects over these atoms.

Amides 2–4 and 6 exert the same sign for angle Θ between carbonyl and isoxazole or aromatic ring like leflunomide (cis relation between isoxazole and aromatic rings). While, 5 and 7–13 exert one opposite signs (trans relation between isoxazole and aromatic rings) as shown in (Table 2). In compound 6, N is imidic so the lone pair of electrons are delocalized over 2 C=O groups and thus the aryl protons are more deshielded.

Table 2. Dihedral angle between C=O and phenyl ring, and Dihedral angle between C–O and isoxazole ring.

General Structure 	Dihedral Angle between C=O and Phenyl Ring	Dihedral Angle between C–O and Isoxazolering
Leflunomide 	−30.03°	−173.3°
2	0.09°	179.6°
3	24.5°	7.17°
4	19.21°	160.2°
5	−63.3°	8.84°
6	19.21°	160.2°
7	129.3°	−164.3°
8	−97.88°	176°
9	−74°	155.6
10	−47°	16.5°
11	−64°	177.6°
12	−3.08°	−20.9°
13	33.9°	4.93°

The contributions for different anilide groups in the surroundings of our system is relative to corresponding chemical shift- for Hbase of acetanilide. For example, compound **3** which has substitution in p-position creates a push and pull effect which leads to more relevant long-range effect on the chemical shift and makes extra stability of the negative charge due to extended resonance (highlighted by arrows, as O atom stabilize –ve charge more than N atom in indicated R groups). As the presence of conjugation normally leads to upfield shift of *o*-protons (Figure 1). While in **4** the *o*-protons is more deshielded due to the –I effect of the positively charged nitrogen. The added *para*-group should not significantly affect either barriers or rotamer populations, and it is present simply as an analogue of leflunomide (Figure 2). It is reported that the ^1H chemical shift is not as sensitive as ^{15}N or ^{13}C to conjugation, and presence of the amide group at the end of the conjugation in this case can have the higher hand in effect on the ^1H chemical shift value.

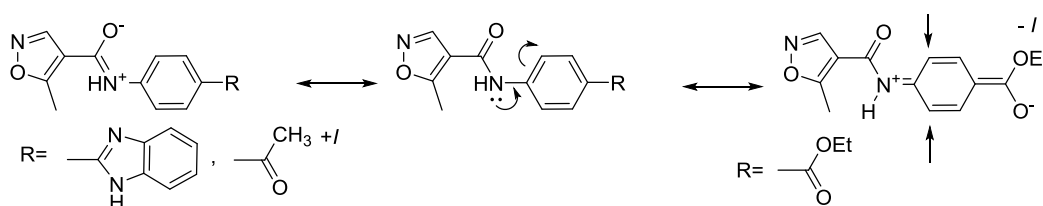


Figure 2. Effect of conjugation and para-substitution on the barrier or rotamer population.

Structures **3**, **4**, **10**, **12** and **13** have magnetically equivalent p-substituted phenyl with high ortho coupling (J value between 8 and 8.4 Hz) like leflunomide. While the others have magnetically non-equivalent phenyl or substituted phenyl. Structure **11** contains NO_2 group which may have a small anisotropic effect similar to that of $\text{C}=\text{O}$ group in Structure **12**, with a deshielding region in the plane of aromatic ring. The ortho proton(s) relative to nitro or acetyl group is strongly downfield, in part due to this interaction.

Structure **2** has symmetrically ortho disubstituted but contains magnetically non-equivalent meta aryl protons (aromatic protons is away from the carbonyl, and is shifted downfield by 0.3 ppm which is generally proposed by dispersion interactions. In addition, the 2-ortho di-isopropyl methine protons are non-equivalent (no plane of symmetry); chemical shift is indicated at two multiplets at 4.84 and 5.15. The spectrum shows how dramatic the effect can be, indicating a quite large downfield shift relative to the corresponding known practical range or calculated values. The prediction of chemical shift of the isopropyl CH group was calculated using the Curphy-Morrison Additivity Constants for Proton NMR [20].

The predicted ^1H -NMR chemical shift = $1.55 + 1.45 = 3$ ppm. The actual value was 4.84–5.15. So in case of the upfield value, $\Delta\delta = 4.84 - 3 = 1.84$, while in case of the downfield value, $\Delta\delta = 5.15 - 3 = 2.15$. To the best of our knowledge, this is the first spectacular report of such deviation and further study in this area is needed. In addition, examination of anticancer activity of compound **2** using Swiss Target Prediction software [21] indicated a very high susceptibility to cytochrome P450.

The secondary amide is adopting trans conformation, this means more rigidity and conformational stability [22] Conformational analysis of compound **2** using Marvin Suit software (Marvin 16.7.18.0, 2016, ChemAxon, <http://www.chemaxon.com>) showed dihedral angle between the plane of the aromatic ring and $\text{C}=\text{O}$ is -93.64° .

The compound (**2**) has three different π systems. The $\pi 2$ can be conjugated with $\pi 1$ as anilide (**2a**), loss of conjugation between nitrogen and aryl leads to the fact that the compound behaves as amide rather than anilide due to the bulky ortho 2,6-diisopropyl groups, or $\pi 3$ as carbonyl moiety (**2b**), very unstable, has possibility of diverse dipolar interactions (Figure 3).

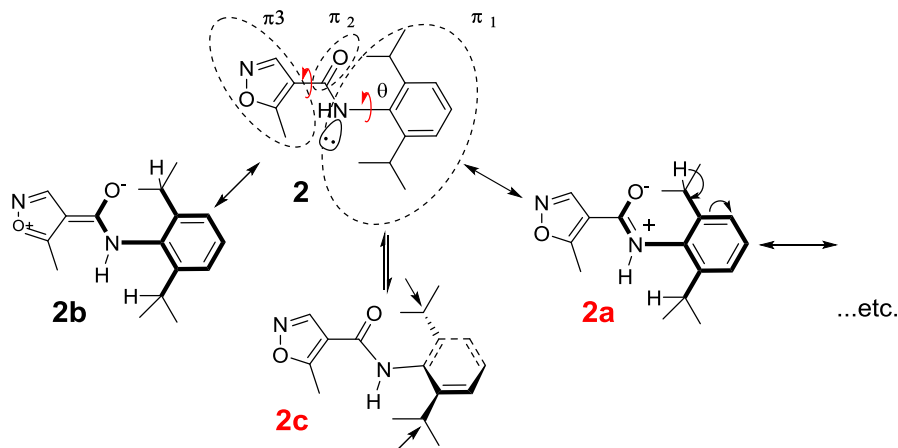


Figure 3. Diverse possible dipolar interaction (**2a** and **2b**) and the most stable conformation with planar carbonyl group \perp the *o*-diisopropyl phenyl ring (**2c**).

In addition, the relatively ^1H -NMR deshielded methine proton on 3° isopropyl carbon (indicated by \rightarrow) in **2** cannot be explained by co-planarity or private dipolar structure **2a** & **2b** or by hyperconjugation of methine proton as indicated by curved arrow in **2a**. The diamagnetic anisotropy of the conjugated planar carbonyl group which is nearly perpendicular to the aromatic ring, as indicated by **2c** which creates two additive environments of diamagnetic anisotropy, is in close contact to the **2** methine protons. Conformational analysis of compound **2** using 3D molecular model examination resulted in a better insight (Figure 4).

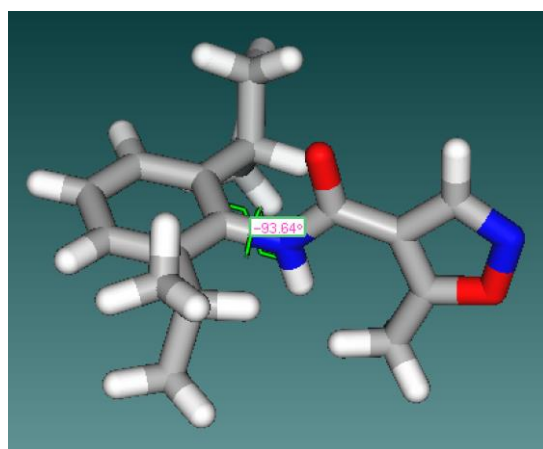


Figure 4. 3D structure of compound **2**, showing the dihedral angle between C=O group and the benzene ring.

The very small difference of Θ angle 3.46° is responsible for the non-equivalence and difference in chemical shift of the two methine protons. The added *ortho*-diisopropyl groups serves as the lock by raising the N–Ar rotation barrier; and is proposed [23,24]. Therefore, individual substituent electronic effects through well-defined π -resonance units indicate that these units behave both as isolated and as conjugated fragments, depending on the substituents.

Linear free energy relationships (LFER) were applied to the ^1H -NMR spectral data of compounds **7–13** and IR spectral. A variety of substituents were employed for phenyl substitution and fairly good correlations were obtained using the simple Hammett and the Hammett–Taft dual substituent parameter equations [25,26]. The correlation results of the substituent induced ^1H -NMR chemical

shifts (SCS) of the CH₃ at C₅ isoxazole spins indicated different sensitivity with respect to electronic substituent effects. The following equation was applied.

$$S = \rho\sigma + h$$

S is substituent dependent value (absorption frequency in cm⁻¹, or chemical shift), ρ is the proportionality constant, σ is Hammett constant, h is the intercept (Table 3 and Figure 5).

Table 3. Summary of results of simple Hammett equation fit.

Parameter	Value
ρ	-12.24 ± 1.419
R	0.9370
SD	1.419
h	0.169
Sy.x	0.266

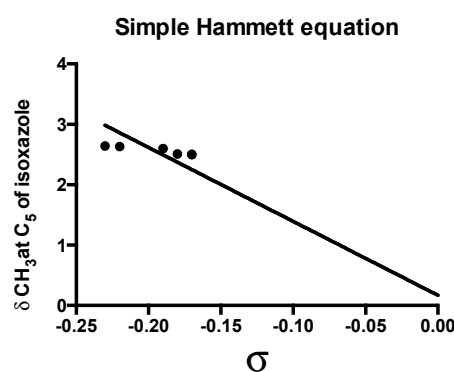


Figure 5. Hammett equation fit of the chemical shift of CH₃ at C₅ of isoxazole of compounds 7–12.

Detailed studies of this new observation with other *o*-substituted anilines and conformational determination is essential for biological correlations.

2.3. Antioxidant Activity

The antioxidant activity of the final compounds 2–13 had been tested using L-ascorbic acid as reference assay in triplicate and average values were considered. The ABTS antioxidant assay [26] is applied as follows:

1. 900 μ L of (ABTS/MnO₂ mix) was transferred to cuvette of spectrophotometer (SPEKOL11) and the absorbance ($A_{control}$) was measured at 734 nm against blank (methanol/phosphate buffer (1:1); reading ca. 0.2).
2. 900 μ L of mix was transferred to 100 μ L standard ascorbic acid in cuvette and the absorbance was measured against blank (methanol/phosphate buffer (1:1) + 100 μ L of ascorbic acid).
3. 900 μ L of mix to 100 μ L of sample was transferred in cuvette and the absorbance (A_{test}) was measured against blank (methanol/phosphate buffer (1:1) + 100 μ L of sample).
4. % Inhibition was calculated using the following equation; % Inhibition = $([A_{control} - A_{test}]/A_{control}) \times 100$.

The results of the preliminary qualitative antioxidant screening of 21 compounds are listed in (Table 4).

Table 4. Results of the preliminary qualitative antioxidant screening.

Compound	A	% Inhibition
ABTS Control	0.480	0.00
Ascorbic acid	0.059	87.71
Leflunomide	0.315	34.38
2	0.360	25.00
3	0.331	31.04
4	0.252	47.50
5	0.351	26.88
6	0.355	26.04
7	0.372	22.50
8	0.385	19.79
9	0.397	17.29
10	0.406	15.42
11	0.332	30.83
12	0.355	26.04
13	0.239	50.2

Most of compounds beside Leflunomide showed moderate antioxidant activity. In general:

1. Changing the amide linkage with ester linkage decreased the antioxidant activity more than most of the amide Leflunomide analogues.
2. The benzimidazole derivatives of Leflunomide **4** and **13** showed higher % of inhibition of radical production than Leflunomide. Benzimidazole derivatives are considered to be good chelating agents [27,28], therefore, our finding can pave the way for further in vivo studies of compounds **4** and **13**. In addition, this also shows the linkage between antifibrotic and antioxidant activity which has been reported in literature [29].

3. Experimental Section

General

Melting points were recorded using a Mel-Temp 3.0 melting point apparatus (Barnstead, Dubuque, IA, USA). IR spectra were performed on a Mattson 5000 FT-IR spectrometer (Fremont, CA, USA) in KBr disks at the Faculty of Pharmacy, Mansoura University. ^1H and ^{13}C -NMR spectra were obtained using a Bruker 400 MHz spectrometer (Billerica, MA, USA) and DMSO- d_6 as solvent. Mass spectra (m/z) were obtained from the Cairo University Mass Spectrometry Laboratory, Cairo, Egypt. High resolution mass (HRMS) were obtained from Georgia State University, Atlanta, GA 30303-3083, USA. Elemental analysis was done at the Microanalysis Centre, Cairo University, Egypt from a CHNS Elemental Analyser. The major chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Fluka (St. Louis, MO, USA).

5-Methylisoxazole-4-carbonyl chloride (1). [18,30]. Thionylchloride (3.53 g, 0.0278 mol) was added to a solution of 5-Methylisoxazole-4-carboxylic acid (2.7 g, 0.0185 mol) in anhydrous dichloromethane (50 mL) with catalytic drops of DMF. The reaction was heated under reflux for 12 h then followed by removing the solvent under reduced pressure. DCM (20 mL) was added and evaporated 3 times to produce a brown oil that was used directly in the next step.

General procedure for the synthesis of compounds (2–13). To a stirred solution of the amines or phenols (0.0024 mol) and trimethylamine (0.0025 mol) in dichloromethane (40 mL), 5-methylisoxazole-4-carbonyl-chloride (0.003 mol) was added dropwise at 0–5 °C. Then, reaction mixture was refluxed at 40 °C for 24 h. After completion of the reaction as indicated from the TLC, the solvent was evaporated under vacuum and the residue was purified using preparative TLC.

N-(2,6-Diisopropylphenyl)-5-methylisoxazole-4-carboxamide (**2**). Using 2,6-diisopropylaniline. IR (KBr) ν/cm^{-1} : 3268, 1606, 1532; $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ 1.31 (br t, 12H), 2.64 (s, 3H), 4.84–5.15 (2q, 2H), 7.74 (d, $J = 8.4$ Hz, 1H), 7.81 (d, $J = 8.4$ Hz, 1H), 7.86 (s, 1H), 8.77 (s, 1H), 12.8 (br s, 1H, D_2O exchangeable); m/z : Calcd. for $\text{C}_{17}\text{H}_{23}\text{N}_2\text{O}_2$: 287.1754; Found: 287.1756 $[\text{M} + \text{H}]^+$; Anal. Calcd. For $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_2$ (286.37): C, 71.30; H, 7.74; N, 9.78, Found: C, 71.23; H, 7.79; N, 9.75.

Ethyl 4-(5-methylisoxazole-4-carboxamido)benzoate (**3**). Using ethyl aniline p-carboxylate. IR (KBr) ν/cm^{-1} : 3307, 1713, 1639, 1547; $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ 1.25 (t, $J = 6.8$ Hz, 3H), 2.64 (s, 3H), 4.18–4.12 (m, 2H), 6.58 (d, $J = 8.4$ Hz, 2H), 7.64 (d, $J = 8.4$ Hz, 2H) Compare with previous **2a**, 8.77 (s, 1H), 12.8 (br s, 1H, D_2O exchangeable). $^{13}\text{C-NMR}$ (DMSO- d_6 , 100 MHz): δ 24.52, 46.06, 61.27, 110.36, 119.91, 120.23, 130.76, 131.41, 141.99, 166.75, 174.26, 187.71; ESI-HRMS: m/z Calcd. For $\text{C}_{14}\text{H}_{15}\text{N}_2\text{O}_4$: 275.1105; Found: 275.1107 $[\text{M} + \text{H}]^+$. Anal. Calcd. For $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_4$ (274.27): C, 61.31; H, 5.14; N, 10.21, Found: C, 61.35; H, 5.24; N, 10.11.

N-(4-(1H-Benzo[d]imidazol-2-yl)phenyl)-5-methylisoxazole-4-carboxamide (**4**). Using 4-(1H-Benzo[d]imidazol-2-yl)aniline. IR (KBr) ν/cm^{-1} : 3420, 3345, 1602, 1548; $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ 2.51 (s, 3H), 6.57 (s, 2H), 7.23–7.25 (m, 1H), 7.65 (s, 1H), 8 (d, $J = 8$ Hz, 2H), 8.34 (d, $J = 8$ Hz, 2H), 8.76 (s, 1H), 12.8 (brs, 1H, D_2O exchangeable).; (MS-EI): m/z 318 $[\text{M}^+]$, 49.64%. Anal. Calcd. For $\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_2$ (318.33): C, 67.91; H, 4.43; N, 17.60, Found: C, 67.85; H, 4.46; N, 17.68.

Methyl-*N*-phenethylisoxazole-4-carboxamide (**5**). Using phenylethyl amine. IR (KBr) ν/cm^{-1} : 3345, 1593, 1555; $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ 2.51 (s, 3H), 3.11 (t, $J = 7.2$ Hz, 2H), 3.72 (t, $J = 7.2$ Hz, 2H), 7.23–7.31 (m, 5H), 8.77 (s, 1H), 12.8 (br s, 1H, D_2O exchangeable); $^{13}\text{C-NMR}$ (DMSO- d_6 , 100 MHz): δ 22.26, 35.14, 41.14, 116.76, 126.69, 128.84, 129.08, 138.83, 139.36, 169.29, 188.79; (MS-EI): m/z 230 $[\text{M}^+]$, 39.46%; ESI-HRMS: m/z Calcd. for $\text{C}_{13}\text{H}_{13}\text{N}_2\text{O}_2\text{Na}$: 275.0772; Found: 275.0769 $[\text{M} - \text{H} + \text{Na}]^+$; Anal. Calcd. For $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_2$ (230.26): C, 67.81; H, 6.13; N, 12.17, Found: C, 67.88; H, 6.23; N, 12.19.

Bromo-1-(5-methylisoxazole-4-carbonyl)indoline-2,3-dione (**6**). Using isatin. IR (KBr) ν/cm^{-1} : 3307, 1650, 1639, 1547; $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ 2.64 (s, 3H), 8.11 (s, $J = 8.4$ Hz, 1H), 8.24 (d, $J = 8.4$ Hz, 1H), 8.76 (s, 1H), 8.83 (s, 1H). Anal. Calcd. For $\text{C}_{13}\text{H}_7\text{BrN}_2\text{O}_4$ (335.11): C, 46.59; H, 2.11; N, 8.36, Found: C, 46.69; H, 2.17; N, 8.3.

Phenyl 5-methylisoxazole-4-carboxylate (**7**). Using phenol. IR (KBr) ν/cm^{-1} : 1688, 1575; $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ 2.64 (s, 3H), 6.58 (s, 1H), 7.32 (d, $J = 8.4$ Hz, 1H), 7.65 (s, 1H), 7.7 (d, $J = 8$ Hz, 1H), 7.9 (s, 1H), 8.77 (s, 1H). $^{13}\text{C-NMR}$ (DMSO- d_6 , 100 MHz): δ 22.84, 116.57, 119.25, 122.45, 126.58, 129.9, 150.73, 163.64, 188.98; (MS-EI): m/z 203 $[\text{M}^+]$, 5.71%; Anal. Calcd. For $\text{C}_{11}\text{H}_9\text{NO}_3$ (203.19): C, 65.02; H, 4.46; N, 6.89, Found: C, 64.90; H, 4.33; N, 6.84.

o-Tolyl 5-methylisoxazole-4-carboxylate (**8**). Using *o*-cresol. IR (KBr) ν/cm^{-1} : 1683, 1570; $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ 2.13 (s, 3H), 2.5 (s, 3H), 7.08 (d, $J = 7.6$ Hz, 1H), 7.15–7.25 (m, 2H), 7.29 (d, $J = 6.8$ Hz, 1H), 8.77 (s, 1H); (MS-EI): m/z 217 $[\text{M}^+]$, 13.88%; Anal. Calcd. For $\text{C}_{12}\text{H}_{11}\text{NO}_3$ (217.22): C, 66.35; H, 5.10; N, 6.45, Found: C, 66.45; H, 5.19; N, 6.50.

m-Tolyl 5-methylisoxazole-4-carboxylate (**9**). Using *m*-cresol. IR (KBr) ν/cm^{-1} : 1721, 1602; $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ 2.32 (s, 3H), 2.5 (s, 3H), 6.92–7.03 (m, 2H), 7.08 (d, $J = 7.6$ Hz, 1H), 7.29 (d, $J = 7.6$ Hz, 1H), 8.77 (s, 1H); (MS-EI): m/z 217 $[\text{M}^+]$, 13.3%; Anal. Calcd. For $\text{C}_{12}\text{H}_{11}\text{NO}_3$ (217.22): C, 66.35; H, 5.10; N, 6.45, Found: C, 66.30; H, 5.08; N, 6.41.

p-Tolyl 5-methylisoxazole-4-carboxylate (**10**). Using *p*-cresol. IR (KBr) ν/cm^{-1} : 1676, 1596; $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ 2.3 (s, 3H), 2.51 (s, 3H), 7.01 (d, $J = 8$ Hz, 2H), 7.2 (d, $J = 8$ Hz, 2H), 8.77 (s, 1H); (MS-EI): m/z 217 $[\text{M}^+]$, 12.65%; Anal. Calcd. For $\text{C}_{12}\text{H}_{11}\text{NO}_3$ (217.22): C, 66.35; H, 5.10; N, 6.45, Found: C, 66.21; H, 5.05; N, 6.48.

2-Nitrophenyl 5-methylisoxazole-4-carboxylate (**11**). Using 2-nitrophenol. IR (KBr) ν/cm^{-1} : 1703, 1528, 1348; $^1\text{H-NMR}$ (DMSO- d_6 , 400 MHz): δ 2.6 (s, 3H), 7.61 (t, $J = 8$ Hz, 1H), 8.02 (d, $J = 8$ Hz, 1H), 8.21 (d,

$J = 8$ Hz, 1H), 8.34 (s, 1H), 8.73 (s, 1H); (MS-EI): m/z 248 [M^+ , 1.61%]; Anal. Calcd. For $C_{11}H_8N_2O_5$ (248.19): C, 53.23; H, 3.25; N, 11.29, Found: C, 53.32; H, 3.28; N, 11.34.

4-Acetylphenyl 5-methylisoxazole-4-carboxylate (12). Using p-hydroxyacetophenone. IR (KBr) ν/cm^{-1} : 1702, 1679, 1591; 1H -NMR (DMSO- d_6 , 400 MHz): δ 2.63 (s, 3H), 2.64 (s, 3H), 8 (d, $J = 8$ Hz, 2H), 8.09 (d, $J = 8$ Hz, 2H), 8.77 (s, 1H). ^{13}C -NMR (DMSO- d_6 , 100 MHz): δ 22.71, 26.91, 115.97, 119.92, 122.56, 126.58, 132, 154.75, 163.97, 187.3, 197.1; (MS-EI): m/z 245 [M^+ , 3.88%]; Anal. Calcd. For $C_{13}H_{11}NO_4$ (245.23): C, 63.67; H, 4.52; N, 5.71, Found: C, 63.52; H, 4.44; N, 5.59.

4-(1H-Benzol[d]imidazol-2-yl)phenyl 5-methylisoxazole-4-carboxylate (13). Using 2-(4-hydroxyphenyl) benzimidazole (0.5 g, 0.0024 mol) as phenol; IR (KBr) ν/cm^{-1} : 3307, 1639, 1547; 1H -NMR (DMSO- d_6 , 400 MHz): δ 2.64 (s, 3H), 6.92 (d, $J = 8.4$ Hz, 2H), 7.17 (d, $J = 3.2$ Hz, 2H), 7.54 (d, $J = 3.2$ Hz, 2H), 8.01 (d, $J = 8.4$ Hz, 2H), 8.78 (s, 1H). Anal. Calcd. For $C_{18}H_{13}N_3O_3$ (319.31): C, 67.71; H, 4.10; N, 13.16, Found: C, 67.74; H, 4.19; N, 13.13.

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