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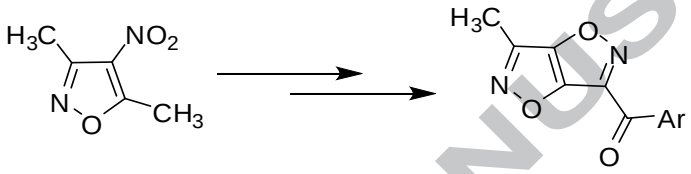

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A B S T R A C T

Synthesis of novel 6-methylisoxazolo[5,4-*d*]isoxazol-3-yl-aryl-methanones **5** has been achieved *via* nitro-nitrite rearrangement by utilizing vinylogous nitroaldol adducts as synthons under mild conditions. Furthermore, the new series of compounds **5a-**i were assessed for molecular properties prediction, drug-likeness by Molinspiration (Molinspiration, 2008) & MolSoft (MolSoft, 2007) softwares, lipophilicity and solubility parameters using ALOGPS 2.1 program. The new series of compounds **5a-i** were screened for their anti-inflammatory activity.
Keywords: 6-Methylisoxazolo[5,4-*d*]isoxazol-3-yl-aryl-methanones; Molecular properties prediction; Drug-likeness; Anti-inflammatory activity

In today's era of drug development, shaped by high throughput screening and combinatorial chemistry, fast bioavailability screening of virtual libraries consisting of hundreds of thousands even millions of molecules is required. In recent years, several new parameters have been introduced for absorption prediction, including molecular size and shape descriptors, hydrogen-bonding capabilities, and surface properties[1-6]. High oral bioavailability is often an important consideration for the development of bioactive molecules as therapeutic agents. Thus, an important goal for drug research is to gain sufficient understanding of the molecular properties that limit oral bioavailability to facilitate the design of viable new drug candidates.

The Henry reaction is one of the most important versatile and fundamental base–catalyzed carbon-carbon bond[7] forming reaction for the preparation of β-nitroalcohol adducts in synthetic organic chemistry. β-Nitroalcohol adducts are useful intermediates for building of natural products, such as nitroalkenes, 2-nitroketones, β-aminoalcohols, carboxylic acids, azides, α-hydroxy ketones, aldehydes and sulfides[8,9].

A major discovery in the search of novel anti-inflammatory agents without deleterious side effects exhibited by the conventional NSAIDs came from the identification of two different isoforms of the cyclooxygenase (COX) enzymes known as COX-1 and COX-2. COX-1 is a constitutive isoform that is involved in normal cellular functions whereas COX-2 is an inducible isoform that is expressed only after inflammatory stimulus[10,11]. Isoxazoles bearing sulfone and carboxamide moieties demonstrated to have significant pharmacological applications. For example, cyclooxygenase-2 (COX-2) selective inhibitors, Celecoxib[12], Rofecoxib[13] and Valdecoxib[14] are currently prescribed for the treatment of arthritis and inflammatory diseases. These COX-2 inhibitors exhibited anti-inflammatory activity with reduced gastrointestinal side effects. The isoxazoles possessing aryl and carboxamide moieties were also shown to have potent *in vivo* antithrombiotic efficacy[15].

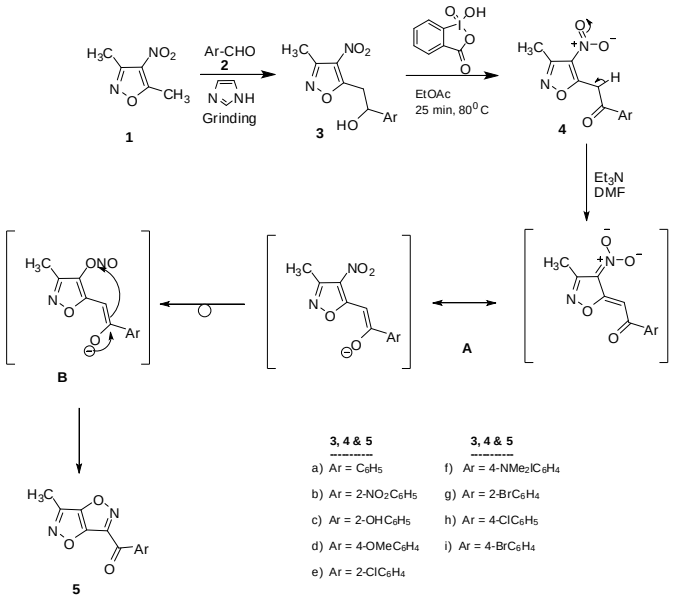
Based on the significance of pharmacological profile of isoxazole derivatives, and as a sequel to our work on the synthesis of biologically important isoxazolyl heterocycles[16-19] we, herein, describe the green route for synthesis of novel 6-methylisoxazolo[5,4-*d*]isoxazol-3-yl-aryl-methanones **5** ***via*** vinylogous nitroaldol adducts as synthons. The new compounds **5** were subjected to molecular properties prediction and drug likeness by Molinspiration (2008) and MolSoft (2007) softwares. Further lipophilicity and solubility parameters were assessed by ALOGPS 2.1 program and investigated for their anti-inflammatory activity.

The synthsis of title compound's **5a-i** was accomplished by synthetic sequence shown in **Scheme 1**. The reaction was first explored with two test experiments by grinding together 3,5-dimethy-4-nitroisoxazole **1** (3 mmol) and freshly distilled benzaldehyde **2** (1 mmol) with 5 mol% and 10 mol% of imidazole as mild Lewis base catalyst respectively. The reaction furnished vinylogous nitroaldol adducts **3**. The best overall yield (98%) was obtained with 10 mol% of imidazole catalyst and the reaction is found to be more effective in terms of short reaction time 5 min[20].

To investigate the scope of imidazole catalyzed synthesis of 2-(3-methyl-4-nitro-5-isoxazolyl)-1-aryl-1-ethanols **3**, several aromatic aldehydes were examined in this reaction. In all the cases, the products were obtained in excellent yields. The great advantages of the present procedure using imidazole in Henry reaction are the mild condition, high yields, short reaction times, simple experimental set-up and workup without any undesired by-products. Besides this, it is equally efficient and is compatible with different functional groups (electron releasing and electron

withdrawing groups) and the approach proved to be of general application.

With the finding of Henry reaction resulting in vinylogous nitroaldol adducts **3**, we utilized these synthons **3** as building blocks for the synthesis of novel 6-methylisoxazolo[5,4-*d*]isoxazol-3-yl-aryl-methanones **5** *via* nitro-nitrite rearrangement.



Scheme 1. Synthesis of 6-methylisoxazolo[5,4-*d*]isoxazol-3-yl-aryl-methanones **5a-i**.

The 2-(3-methyl-4-nitro-5-isoxazolyl)-1-phenyl-1-ethanone **4 a** (1 mmol) was stirred at 90 ^{o}C for 2 h with catalytic amount of Et_3N in DMF. The anionic intermediate **A** generated *in situ* by interaction of 2-(3-methyl-4-nitro-5-isoxazolyl)-1-phenyl-1-ethanone **4 a** with Et_3N undergoes nitro-nitrite rearrangement to yield corresponding nitrite like intermediate **B**, which in turn undergoes cyclization to yield the title compound *viz.*, 6-methylisoxazolo[5,4-*d*]isoxazol-3-yl-aryl-methanone **5 a** in good yield[22]. The reaction is extended to differently substituted ethanones 4 b-i , and in each case the product was obtained **5 b-i** in good yield.

The structures of all newly synthesized compounds (**3**, **4**, and **5**) are herein reported were confirmed by analytical and spectral data (IR, ^{1}H NMR, ^{13}C NMR and MS).

*In Silico study of Drug likeness and molecular properties:*Analysis of the structures of orally administered drugs, and of drug candidates, as pioneered by Lipinski, has so far been the primary guide to correlating physical properties with successful drug development[23,24]. This analysis has been very useful and has led to a set of rules relating to the importance of lipophilicity (octanol-water partition), molecular weight (MW), and the number of hydrogen bond donors and acceptors. Nonetheless, there are limitations on it as it relates to oral bioavailability. These include the lack of quantitative assessment of oral bioavailability in the data analyzed, a need to assume that all orally administered drugs are intended to be absorbed, the assumption that oral bioavailability is generally high for orally administered drugs. Good bioavailability can be achieved with an appropriate balance between solubility and partitioning properties. Thus in order to achieve good oral drugs we have subjected a series of 6-methylisoxazolo[5,4-*d*] isoxazol-3-yl-aryl-methanones **5 a-i** for the prediction of lipophilicity, solubility and Lipinski "Rule of Five".

Lipophilicity: The ALOGPS method is part of the ALOGPS 2.1 program[24] was used to predict lipophilicity[25] and aqueous solubility[26,27] of the compounds. The lipophilicity calculations within this program are based on the associative neural networking approach and the efficient partition algorithm. The LogKow (Kow-WIN) program[28] estimates the log octanol/water partition coefficient (logP) of organic chemicals and drugs by an atom/fragment contribution method developed at Syracuse Research Corporation[29]. The XLOGP2 is an atom-additive method applying corrections[30,31]. Computed partition coefficients for the studied compounds by XLOGP2 method varied between 2.67–3.57 and 1.79–2.86 by KoWWIN method (**Table 1**). The LogKoW (KoW-WIN) method is best supported for the most of the compounds on the basis of lipophilicity (≤5) to consider an oral drug/lead.

Table 1

Calculated partition coefficients and solubilities of 6-methylisoxazolo[5,4-*d*]isoxazol-3-yl-aryl-methanones (**5a-i)**

Compd.	ALOGPS	KoW-WIN	XLOGP2
5a	-3.16(16.00 mg/l)	1.97	2.78
5b	-3.63(64.40 mg/l)	1.79	2.67
5c	-2.90(31.00 mg/l)	2.27	2.80
5d	-3.25(14.00 mg/l)	2.05	2.69
5e	-3.51(80.35 mg/l)	2.61	3.40
5f	-3.23(16.00 mg/l)	2.15	2.98
5g	-3.41(12.00 mg/l)	2.86	3.57
5h	-3.50(82.77 mg/l)	2.61	3.40
5i	-3.40(12.00 mg/l)	2.86	3.57

Solubility: Drug solubility is an important factor that affects the movement of a drug from the site of administration in to the blood. It is known that insufficient solubility of drug can lead to poor absorption[32]. Investigation of the rate-limiting steps of human oral absorption of 238 drugs[32] showed that the absorption of a drug is usually very low if the calculated solubility is <0.0001 mg/l. According to that, the investigated compounds **5a-i** were found to fulfill the requirements of solubility (ALOGPS) and could be considered as a drug candidate for oral absorption.

Absorption, polar surface area, and "Rule of five" properties: High oral bioavailability is an important factor for the development of bioactive molecules as therapeutic agents. Good intestinal absorption, reduced molecular flexibility (measured by the number of rotatable bonds), low polar surface area or total hydrogen bond count (sum of donors and acceptors), are important predictors of good oral bioavailability[4,33]. Molecular properties such as membrane permeability and bioavailability is always associated with some basic molecular descriptors such as logP (partition coefficient), molecular weight (MW), or hydrogen bond acceptors and donors counts in a molecule[32]. Lipinski[6] used these molecular properties in formulating his "Rule of Five". The rule states that most molecules with good membrane permeability have logP ≤5, molecular weight ≤500, number of hydrogen bond acceptors ≤10, and number of hydrogen bond donors≤5. This rule is widely used as a filter for drug-like properties. **Table 2** contains calculated percentage of absorption (%ABS), molecular polar surface area (PSA) and Lipinski parameters of the

investigated compounds of the series (**5a-i**). Magnitude of absorption is expressed by the percentage of absorption. Absorption percent was calculated[29] using the expression: %ABS = 109 - 0.345 PSA. Polar surface area (PSA) was determined by the fragment-based method of Ertl and coworkers[34,35]. A poor permeation or absorption is more likely when there are more than 5H bond donors, 10 H-bond acceptors. Hydrogen-bonding capacity has been also identified as an important parameter for describing drug permeability[33]. The series (**5a-i**) under investigation has most of the compounds possessing less number of hydrogen bond donors (≤5) but do possess considerable number of acceptors (≤10) as shown in **Table 3**. Number of rotatable bonds is important for conformational changes of molecules under study and ultimately for the binding of receptors or channels. It is revealed that for passing oral bioavailability criteria, number of rotatable bonds should be $\leq 10^4$. The compounds in this series (**5a-i**) in general possess high number of rotatable bonds (2-3) and therefore, exhibit conformational flexibility. Molecular polar surface area (PSA) is a very useful parameter for the prediction of drug transport properties. PSA is a sum of surfaces of polar atoms (usually oxygen, nitrogen and attached hydrogen) in a molecule. PSA and volume is inversely proportional to %ABS. Compound **5e**, **5g, 5h,** and **5i** have maximum absorption (88.79%) as their corresponding polar surface area and volume are least among the series.

Table 2

Physico-Chemical properties calculations of 6-methyl isoxazole [5,4-*d*]isoxazol-3-yl-aryl-methanones (**5a-i**)

Compd.	%ABS	Volume (A3)	PSA (A2)	NROTB	HBA	HBD	log P, Calcd.	Formula weight
5a	88.78	211.62	58.29	2	5	0	2.75	228
5b	77.01	236.73	92.74	3	7	0	2.62	273
5c	77.01	222.72	75.83	2	6	1	2.58	244
5d	85.89	243.47	66.98	3	6	0	2.80	258
5e	88.79	226.87	58.59	2	5	0	3.35	262
5f	87.77	261.13	61.55	3	5	0	3.01	271
5g	88.79	232.56	58.59	2	5	0	3.30	306
5h	88.79	228.82	58.59	2	5	0	3.43	262
5i	88.79	233.48	58.59	2	5	0	3.64	306

Table 3

Drug-likeness of 6-methylisoxazolo[5,4-*d*]isoxazol-3-yl-aryl-methanones (**5a-i**)

Compd.	GPCRL	ICM	KI	NRL	PI	EI
5a	-0.18	0.04	-0.18	-0.17	-0.44	-0.04
5b	-0.24	-0.05	-0.20	-0.16	-0.45	-0.16
5c	-0.10	-0.06	-0.06	0.05	-0.32	0.04
5d	-0.12	-0.05	-0.10	-0.05	-0.36	-0.05
5e	-0.17	0.01	-0.12	-0.12	-0.52	-0.12
5f	-0.02	0.01	0.03	0.03	-0.27	-0.00
5g	-0.32	-0.04	-0.26	-0.23	-0.57	-0.12
5h	-0.12	0.04	-0.14	-0.12	-0.42	-0.06
5i	-0.26	-0.06	-0.18	-0.25	-0.52	-0.11

GPCRL: GPCR Ligand; ICM: Ion Channel Modulator; KI: Kinase Inhibitor; NRL: Nuclear Receptor Ligand; PI: Protease Inhibitor; EI: Enzyme Inhibitor.

Anti-inflammatory analysis: Anti-inflammatory activity was determined by carrageenan induced paw edema method[36]. Wistar rats of either sex weighing 150-200 g were divided into 6 groups (n=6) and they were fasted 18 h before the experiment with water *ad libitum*. Group-I received 1% sodium CMC (negative control), Group-II received *ibuprofen* at a dose of 100 mg/kg (positive control) and Group-III to VI were given the compounds **5a-i** (100 mg/kg). All the compounds **5a-i** were given in oral route. After 30 minutes, 0.1 mL of 1% carrageenan suspension in normal saline was injected into the subplantar region of the left hind paw of each rat to induce edema. The edema volumes of the injected paw measured with the help of plethysmograph at the interval of 0, 1, 2, 4 and 6 h. The difference between the paw volumes of treated animals were compared with that of the control group and the mean edema volume was calculated. Percentage inhibition was calculated as per the formula, %inhibition= [Vo-Vt)/Vo] x 100, where Vo = volume of the paw control at time t, Vt = volume of the paw of drug treated at time t.

In vitro cylcooxygenase inhibition studies: The compounds, **5a-i** were tested for their ability to inhibit *in vitro* COX-1 and COX-2 using a colorimetric COX (ovine) inhibitor screening kit (Catalog No. 760 112, Cayman Chemicals Inc., Ann Arbor, MI,USA) using the previously established method[37].

The anti-inflammatory activity of the title compounds, **5a-i** were evaluated by carrageenan-induced paw edema method in rats[36] at a dose of 100 mg/kg body weight using *ibuprofen* as a reference drug. Results were expressed as a mean ± S.E. The anti-inflammatory properties were recorded at successive intervals of 0, 1, 2, 4 and 6 h and compared with that of standard *ibuprofen*. The anti-inflammatory activity data (**Table 4**) indicated that all the compounds **5a-i** exhibited significant activity by decreasing the paw volume that was produced by carrageenan. Among all the compounds **5 a-i** tested, it is interesting to note that the compounds **5e, 5g, 5h** and **5i** showed better anti-inflammatory activity. This may be due to the presence of chloro and bromo substitutions on the benzene ring, besides the presence of isoxazolo [5,4-*d*]isoxazole frame work**.** The presence of electron donating methyl and methoxy groups on benzene ring (**5c** and **5d**) did not influence the activity much.

Table 4

Anti-inflammatory activity of 6-methylisoxazolo[5,4-*d*]isoxazol-3-yl-aryl-methanones [#] (**5a-i**)

	Paw volume (mL of Hg)[b]				
Group[a]	0h	1h	2h	4h	6h
5a	0.36±0.01	0.80±0.03	0.86±0.08^{ns}	0.82±0.06**	0.63±0.03**
5b	0.37±0.03	0.75±0.05	0.83±0.05^{ns}	0.81±0.03**	0.63±0.01**
5c	0.34±0.03	0.82±0.01	0.93±0.04^{ns}	0.80±0.02**	0.61±0.03**
5d	0.31±0.04	0.78±0.1	0.70±0.03	0.83±0.03**	0.62±0.05**
5e	0.22±0.08	0.51±0.06	0.68±0.03	0.51±0.02	0.50±0.01**
5f	0.35±0.02	0.78±0.08	0.83±0.03^{ns}	0.63±0.03*	0.55±0.03**
5g	0.22±0.03	0.50±0.02	0.68±0.03	0.65±0.04*	0.51±0.02**
5h	0.21±0.01	0.53±0.04	0.65±0.02^{ns}	0.67±0.03**	0.41±0.03***
5i	0.23±0.04	0.52±0.01	0.69±0.05	0.54±0.03**	0.32±0.01***
Control	0.36±0.03	0.90±0.05	1.07±0.08	1.2±0.05	0.96±0.03
Ibuprofen	0.30±0.05	0.66±0.03*	0.70±0.05***	0.60±0.05***	0.40±0.05***

[a]Dose levels: test compound (100 mg/kg b.wt) Ibuprofen (100 mg/kg b.wt)
[b]Values are expressed as mean ± S.E.
Statistically significant compound to respective control value
*P<0.05, **P<0.01, ***P<0.001
^{ns}Nonsignificant, compared to control.
[#]n=6, number of animals used in each group

The preliminary *in vitro* cyclo oxygenase inhibition studies of 6-methylisoxazolo[5,4-*d*] isoxazol-3-yl-arylmethanones **5 a-i** have evidenced that **5e, 5g, 5h** and **5i** are more selective and active than compounds **5a, 5b, 5c, 5d,** and **5f**. To study the activity, different substitutents are introduced on the benzene ring. Results indicated that compounds **5e, 5g, 5h** and **5i** having chloro and bromo substitutents on benzene ring showed better activity and they are selective towards cyclooxygenase-2 enzyme. Other compounds showed good anti-inflammatory activity but they are less selective cox-2 inhibitors, when compared with standard drug *Celecoxib*. The possible improvement of selective COX-2 inhibitory activity of this basic isoxazolo[5,4-*d*] isoxazole moiety through modulation of ring substituents and/or additional functionalization warrants further investigations **[Table 5]**

Table 5

In vitro cylcooxygenase inhibition studies of 6-methylisoxazolo [5,4-*d*]isoxazol-3-yl-aryl-methanones [#] (**5a-i**)

	%inhibition[a,b]	
Compound	COX-1(10 μM)	COX-2 (10 μM)
5a	29.6	30.1
5b	27.4	28.3
5c	36.9	40.0
5d	40.1	51.6
5e	17.8	86.1
5f	35.6	44.2
5g	18.3	80.3
5h	16.4	79.3
5i	19.3	72.7
Celecoxib	2.8	100

[a]Values are acquired using *in vitro* ovine COX-1/COX-2 assay kit (Catalog No. 760 112, Cayman Chemicals Inc., Ann Arbor, MI).
[b]Experiments were carried out in duplicate and have less than 10% error.
[c]Test compounds and Celecoxib were administered orally at the dose of 10 mg/kg.

In conclusion, we have demonstrated a convenient, facile, efficient and environmentally benign protocol for the synthesis of novel 6-methylisoxazolo[5,4-*d*]isoxazol-3-yl-aryl-methanones *via*. vinylogous nitroaldol adducts as synthons The newly synthesized compounds **5 a-i** were evaluated for their anti- inflammatory activity. Compounds **5 e, 5 g, 5 h** and **5 i** exhibited significant activity as that of standard drug. This happen to be the first report to study the molecular properties prediction and drug-likeness on isoxazole compounds . These studies indicated that most of the compounds behave as an oral absorption drugs.

Acknowledgments

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References and notes

1. Clark, D.E.; Pickett, S.D. *Drug Discovery Today*, **2000**, *5*,

49.
2. Stenberg, P.; Luthman, K.; Artursson, P. *J Control Relat*. **2000**, *65*, 231.
3. Van de Waterbeemd, H.; Camenisch, G.; Folkers, G.; Raevsky, O.A. *Quant Struct-Act Relat*. **1996**, *15*, 480.
4. Veber, D.F.; Johnson, S.R.; Cheng, H.Y.; Smith, B.R.; Ward, K.W.; Kapple, K.D. *J Med Chem*. **2002**, *45*, 2615.
5. Winiwarter; S., Bonham, N.M.; Ax, F.; Hallberg, A.; Lennernas, H.; Karlen, A. *J Med Chem*. **1998,** *41*, 4939.
6. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. *Adv. Drug Delivery Rev*. **1997**, *23*, 3.
7. Henry, L.C.R. *Hebd. Seances Acad. Sci*. **1895**, *120*, 1265.
8. Rosini, G.; Ballini, R. *Synthesis*. **1988**, *11*, 833.
9. Barco, A.; Benetti, S.; Risi, C.; Polloni, G. *Tetrahedron Lett*. **1996**, *37*, 7599.
10. Marnett, J.L.; Rowlinson, S.W.; Goodwin, D.C.; Kalgutkar, A.S.; Lanzo, C.A. *J Biol Chem.* **1999**, *274*, 22903.
11. Dannhardt, G.; Kiefer, W. *Eur J Med Chem.* **2001**, *36*, 109.
12. Penning, T.D.; Talley, J.J.; Bertenshaw, S.R.; Carter, J.S.; Collins, P.W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J.W.; Miyashiro, J.M.; Rogers, R.S.; Rogier, D.J.; Yu, S.S.; Anderson, G.D.; Burton, E.G.; Cogburn, J.N.; Gregory, S.A.; Koboldt, C.M.; Perkins, W.E.; Seibert, K.; Veenhuizen, A.W.; Zhang, Y.Y.; Isakson, P.C. *J Med Chem.* **1997**, *40*, 1347.
13. Prasit, P.; Wang, Z.; Brideau, C.; Chan, C.C.; Charleson, S.; Cromilish, W.; Ethier, D.; Evans, J.F.; Ford-Hutchinson, A.W.; Gauthier, J.Y.; Gordon, R.; Guay, J.; Gresser, M.; Kargman, S.; Kennedy, B.; Leblanc, Y.; Leger, S.; Mancini, J.; O'Neill, G.P.; Ouellet, M.; Percival, M.D.; Perrier, H.; Riendeau, D.; Rodger, I.; Tagari, P.; Therien, M.; Vickers, P.; Wong, E.; Xu, L.J.; Young, R.N.; Zamboni, R.; Boyce, S.; Rupniak, N.; Forrest, M.; Visco, D.; Patrick, D. *Bioorg Med Chem Lett*. **1999**, *9*, 1773.
14. Talley, J.A.; Brown, D.L.; Carter, J.S.; Masferrer, M.J.; Perkins, W.E.; Rogers, R.S.; Shaffer, A.F.; Zhang, Y.Y.; Zweifel, B.S.; Seibert, K. *J Med Chem*. **2000**, *43*, 775.
15. Pruitt, J.R.; Pinto, D.J.; Estrella, M.J.; Bostrom, L.L.; Knabb, R.M.; Wong, P.C.; Wright, M.R.; Wexler, R.R. *Bioorg Med Chem Lett*. **2002**, *10*, 685.
16. Rajanarendar, E.; Nagi Reddy, M.; Rama Murthy, K.; Surendar, P.; Reddy, R.N.; Reddy, Y.N. *Bioorg Med Chem Lett*. **2012**, *22*, 149.
17. Rajanarendar, E.; Nagi Reddy, M.; Rama Krishna, S.; Govardhan Reddy, K.; Reddy, Y.N.; Rajam, M.V. *Eu J Med Chem*. **2012**, *50*, 344.
18. Rajanarendar, E.; Raju, S.; Nagi Reddy, M.; Rama Krishna, S.; Hari Kiran, L.; Ram Narasimha Reddy, A.; Narasimha Reddy, Y. *Eu J Med Chem.* **2012**, *50*, 274.
19. Rajanarendar, E.; Nagi Reddy, M.; Rama Murthy, K.; Govardhan Reddy, K.; Raju, S.; Srinivas, M.; Praveen, B.; Srinivasa Rao, M. *Bioorg Med Chem Lett*. **2010**, *20*, 6052.
20. *General procedure for the environmentally benign synthesis of 2-(3-methyl-4-nitro-5-isoxazolyl)-1-aryl-1-ethanols (**3a-l**)*
A mixture of 3,5-dimethyl-4-nitroisoxazole **1** (3 mmol), aromatic aldehyde **2** (1 mmol), and 10 mol% of Imidazole was gently ground by hand using a motar and pestle of an appropriate size. The progress of the reaction was monitored by TLC. (Analytical TLC was performed on Merck precoated 60 F_{254} silica gel plates. Visualization was done by exposure to iodine vapour). The reaction mixture became sticky paste during the course of the reaction. Finally, it was diluted with 10 mL of ice-cold water. The solid separated was filtered and dried. The products were purified by recrystallization from ethyl acetate.
21. *General procedure for the synthesis of 2-(3-methyl-4-nitro-5-isoxazolyl)-1-aryl-1-ethanones **(4a-i)***
A mixture of 2-(3-methyl-4-nitro-5-isoxazolyl)-1-aryl-1-ethanols **3** (1 mmol) and *o*-iodoxybenzoic acid (IBX) (1 mmol) in EtOAc (10 mL) was refluxed at 80 ^{o}C for 4 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled to room temperature and filtered. The separated solid was washed with water and recrystallized from ethanol.
22. *General procedure for the synthesis of 6-methylisoxazolo[5,4-d]isoxazol-3-yl-aryl-methanones **(5a-i)***
To a solution of 2-(3-methyl-4-nitro-isoxazol-5-yl)-1-aryl-ethanones 4 1 mmol) in DMF (5 mL), catalytic amount of Et_3N was added and the contents were stirred at 90 ^{o}C for 2 h. After completion of the reaction (monitored by TLC), the reaction mixture was poured on to crushed ice and the separated solid mass was filtered, washed with water and recrystallized from ethyl acetate to afford the pure products **5**.
23. Lipinski, C.A. *J Pharmacol Toxicol Methods*. **2000**, *44*, 235.
24. Tetko, I.V.; Tanchuk, V.Y. *J Chem Inf Comput Sci*. **2002**, *42*, 1136.
25. Tetko, I.V.; Tanchuk, V.Y.; Kasheva, T.N.; Villa, A.E.P. *J Chem Inf Comput Sci*. **2001**, *41*, 1488.
26. Balakin, K.V.; Savchuk, N.P.; Tetko, I.V. *Curr Med Chem*. **2006**, *13*, 223.
27. Meylan, W.M.; Howard, P.H. *J Pharm Sci*. **1995**, *84*, 83.
28. Remko, M. *J Mol Struct Theo chem*. **2009**, *897*, 73.
29. Wang, R.; Fu, Y.; Lai, L. *J Chem Inf Comput Sci*. **1997**, *37*, 615.
30. Wang, R.X.; Gao, Y.; Lai, L.H. *Perspect Drug Discov Des*. **2000**, *19*, 47.
31. Zhao, Y.H.; Abraham, M.H.; Le, J.; Hersey, A.; Luscombe, C.N.; Beck, G.; Sherborne, B.; Cooper, I. *Pharm Res*. **2002**, *19*, 1446.
32. Muegge, I. *Med Res Rev*. **2003**, *23*, 302.
33. Refsgaard, H.H.F.; Jensen, B.F.; Brockhoff, P.B.; Padkjaer, S.B.; Guldbrandt, M.; Christensen, M.S. *J Med Chem*. **2005**, *48*, 805.
34. Ertl, P.; Rohde, B.; Selzer, P. *J Med Chem*. *2000*, *43*, 3714.
35. <http://www.molinspiration.com>. 10/06/2008.
36. Winter, C.A.; Risley, E.A.; Nuss, G.W. *Proc Soc Expt Biol Med*. **1990**, *111*, 544.
37. Black, W.C.; Bayly, C.; Belley, M.; Chan, C.C. *Bioorg Med Chem Lett*. **1996**, *6*,725.

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