ONE POT MULTICOMPONENT SYNTHESIS OF FUNCTIONALIZED PYRIDINES USING MORPHOLINE ORGANOBASE AT AMBIENT TEMPERATURE

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An atom efficient synthesis of polysubstituted dihydropyridine derivatives was accomplished by the one-pot four-component condensation of aldehydes, amines, dialkyl acetylene dicarboxylates and active methylene group-containing compounds such as malononitrile or ethyl cyanoacetate using morpholine as a catalyst at ambient temperature. Broad substrate scope, non-chromatographic purification, good yields of the products makes it be a useful and valuable methodology for employing the 1,4-dipolar intermediates in synthetic organic chemistry. Use of organobase as a single catalyst, room temperature conditions renders the method protocol as a significant addition to the existing methods for the synthesis of multifunctionalized dihydropyridines.

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INTRODUCTION

Multi-component reactions (MCRs) contribute significantly to the sustainable and diversity-oriented synthesis of various heterocyclic compounds in combinatorial and medicinal chemistry.[1] Reduced number of steps, high atom economy, energy efficient, time-saving and cost-effective nature of the MCRs make them highly desirable tools in synthetic organic chemistry. These reactions constitute important green tools for the synthesis of highly functionalized molecules in organic chemistry.

Cascade reactions, also known as domino reactions or tandem reactions, are chemical processes involving at least two consecutive reactions occurring, such that each subsequent reaction occurs due to the entity formed in the previous step, without the need to isolate the intermediate. These reactions thus assist in reducing the number of steps involved in the synthesis of biologically potent heterocyclic compounds[2]. Cost effective synthesis of highly functionalized and diverse heterocyclic compounds from readily available precursors is an enduring task for synthetic organic chemists. The formation of C-C, C-N and C-S bonds is also one of the significant challenges to the researcher community[3].

Dihydropyridines (DHPs) are abundant in various natural products as well as pharmaceutically important heterocyclic compounds; constituting the skeleton of drugs such as amlodipine, clevidipine, aranidipine and nifedipine (Figure 1). DHP is also a core component of calcium channel blockers used in the treatment of cardiovascular diseases, including hypertension and spastic smooth muscle diseases.[4-5] DHP derivatives exhibit a broad spectrum of biological activities and well-studied for applications in the treatment of Alzheimer's disease, cardiovascular diseases and hypertension.[6] Furthermore DHPs are also well known for antimicrobial,[7] anticancer,[8] antioxidant,[9] anti-inflammatory,[10] antidiabetic,[11] antitubercular[12] and analgesic[13] activities. These derivatives showed therapeutic properties including platelet antiaggregatory activities,[14] HIV protease inhibition[15] and neuroprotection.[16]

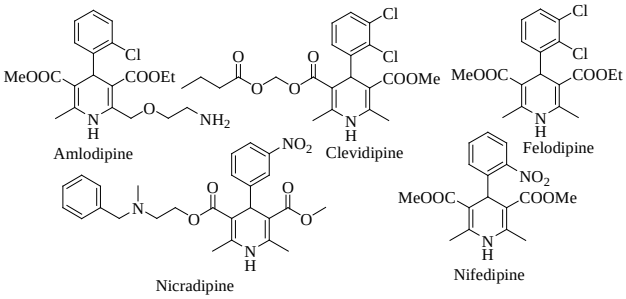
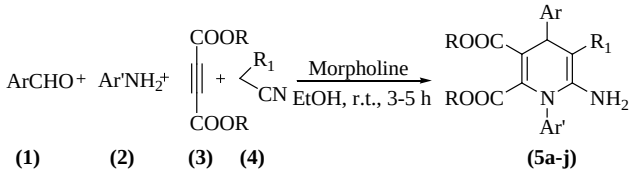


Figure 1. Some biologically active dihydropyridines

Thus, considering the therapeutic and pharmacological activities of DHP derivatives, considerable attention has been focused on designing efficient methodologies for the synthesis of these heterocyclic compounds. The classical, most simple and straightforward synthesis of these heterocycles is the Hantzsch's reaction of an aldehyde, β-ketoester and ammonia. However, only a few reports are available for the synthesis of highly functionalized 1,4-DHPs involving the use of Meglumine,[17] grinding technique,[18] triethylamine,[19] sodium hydroxide,[20] PEG-400,[21] ammonium hydrogen phosphate $(NH_4)_2HPO_4$,[22] yttrium triflate,[23] p-toluenesulfonic acid (p-TSA)[24] and L-proline.[25] However, many of these methods require prolonged reaction time, use of costly reagents and high reaction temperature.

Morpholine is a colorless, water-soluble organobase possessing both amine and ether functionalities. The presence of etheral oxygen withdraws electron density from the nitrogen, rendering morpholine less nucleophilic and less basic than piperidine. It is documented for the catalytic applications for the synthesis of various heterocyclic compounds such as α, β-unsaturated nitroalkenes[26], chromene core structured heterocyclic compounds[27]and

Hantzsch's polyhydroquinoline derivatives[28]. Thus, in continuation of our successful efforts in the development of new strategies for the synthesis of bioactive heterocyclic compounds[29]; herein we report the atom efficient synthesis of polysubstituted dihydropyridine derivatives by the one-pot four component condensation of aldehyde (**1**), amine (**2**), dialkyl acetylene dicarboxylate (**3**) and active methylene compound such as malononitrile or ethyl cyanoacetate (**4**) using morpholine as an organobase at room temperature in short reaction time to afford the corresponding products in high yields (**Scheme 1**).



Scheme 1. One pot synthesis of DHPs using morpholine organobase

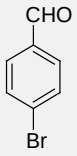
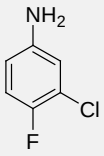
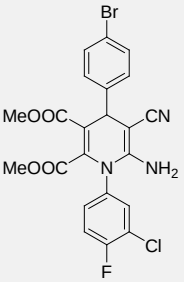
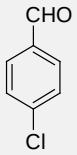
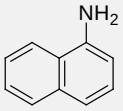
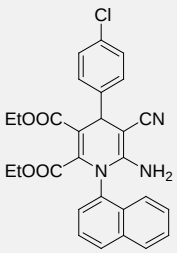
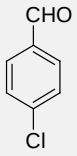
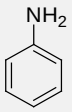
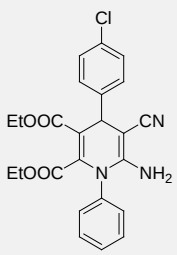
EXPERIMENTAL

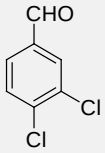
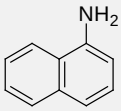
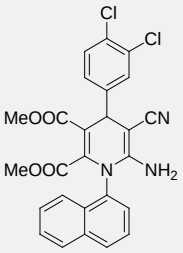
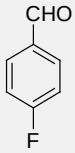
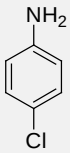
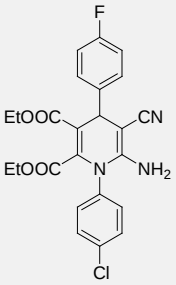
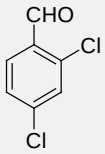
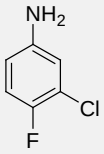
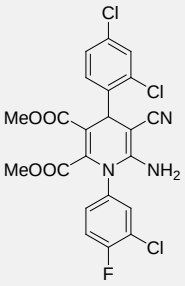
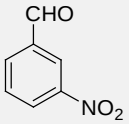
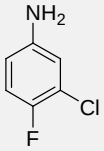
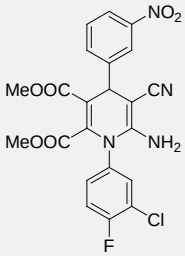
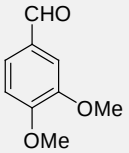
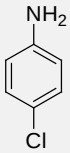
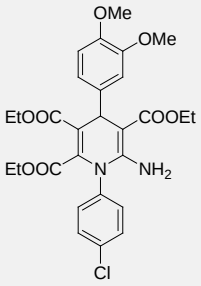
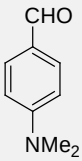
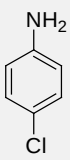
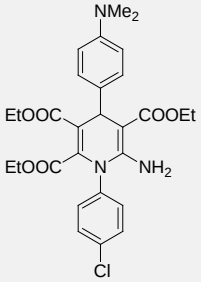
Chemicals used were SD fine or Sigma Aldrich made and used without further purification. The progress of the reaction was monitored on silica-gel coated aluminum TLC plates (Merck). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACF 200 spectrophotometer and chemical shifts were expressed in δ ppm in $CDCl_3$ with reference to TMS as the standard. IR spectra were recorded on Shimadzu FTIR (Prestige 21) spectrophotometer, mass spectra on a Shimadzu MS-Q spectrometer and melting points of the products were recorded on a digital melting point apparatus (Optics technology) using capillaries open at one end and were uncorrected.

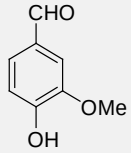
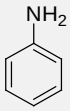
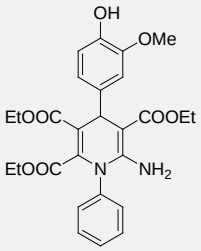
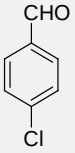
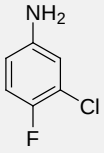
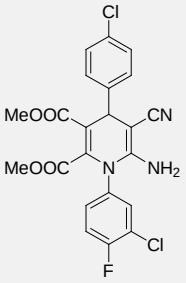
General procedure for the one pot four component synthesis of highly functionalized DHPs

A mixture of aldehyde (2 mmol), malononitrile (3 mmol) and morpholine (20 mol%) in ethanol (3 mL) was stirred at room temperature for 10 minutes; followed by dropwise addition of arylamine (2 mmol) and then dialkyl acetylene dicarboxylate (2 mmol). The contents were stirred at room temperature for an appropriate time as specified in Table 1. The progress of the reaction was monitored by thin layer chromatography (40 % ethyl acetate:n-hexane). After completion of the reaction, the reaction mass was concentrated, poured into ice-cold water and filtered off residue as the crude multifunctionalized dihydropyridine which was further purified by crystallization in ethanol.

Table 1. Yields, reaction time and physical constants of DHPs using morpholine organobase

Entry	Aldehyde	Amine	R	R_1	Product	Time, h	Yield (%)*	M. P. (°C)
1			Me	CN		4.0	78	108-109
2			Et	CN		4.5	85	137-138
3			Et	CN		3.0	89	176-177

4			Me	CN		4.5	80	101-102
5			Et	CN		3.5	82	183-184
6			Me	CN		4.0	76	117-118
7			Me	CN		3.0	86	120-121
8			Et	COOEt		4.0	80	142-143
9			Et	COOEt		5.0	78	115-116

10			Et	COOEt		5.0	77	109-110
11			Me	CN		4.0	84	112-113

(*For aldehyde: 2 mmol, active methylene compound: 3 mmol, DMAD or DEAD: 2 mmol, amine: 2 mmol, in ethanol in the presence of 20 mol % morpholine)

The spectral data of the synthesized compounds is mentioned below:

Dimethyl-6-amino-4-(4-bromophenyl)-1-(3-chloro-4-fluorophenyl)-5-cyano-1,4-dihydropyridine-2,3-dicarboxylate (Table 1, Entry 1):

Off white solid; M.P.=108-109 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 3.1 (s, 3H), 3.18 (s, 3H), 3.89 (s, 2H), 4.7 (s, 1 H), 7.48 (d, 4H), 8.02 (d, 3H); IR (neat) cm^{-1} 3332, 2959, 2172, 1741, 1696, 1641, 1562, 1372, 1210, 1032; Mass: 520.1$(M+1)^{+}$.

Diethyl-6-amino-4-(4-chlorophenyl)-5-cyano-1,4-dihydro-1-(naphthalen-1-yl)pyridine-2,3-dicarboxylate (Table 1, Entry 2):

Off white solid; M.P.=137-138 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.9 (t, 3H), 1.12 (t, 3H), 4.0 (s, 2H), 4.2 (q, 4 H), 4.8 (s, 1H), 7.3 (m, 7H), 7.5 (d, 4H); IR (neat) cm^{-1} 3461,3336, 2966, 2177, 1746, 1695, 1646, 1566, 1371, 1239, 1032; Mass: 502 (M+1)+.

Diethyl-6-amino-4-(4-chlorophenyl)-5-cyano-1,4-dihydro-1-phenylpyridine-2,3-dicarboxylate (Table 1, Entry 3):

Yellow solid; M.P.=176-177 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.9 (t, 3H), 1.1 (q, 3H), 3.9 (m, 2H), 4.1 (q, 4H), 4.7 (s, 1H), 7.39 (m, 6H), 7.5 (d, 3H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ ppm 13.42, 13.91, 38.27, 60.96, 62.19, 104.94, 120.44, 128.61, 128.88, 129.94, 130.48, 130.62, 132.85, 135.09, 141.76, 143.59, 149.81, 162.81, 162.88, 164.98; IR (neat) cm^{-1} 3382.9 (-NH_2), 2972.2 (C-H in -CH_3), 2187.9 (-CN), 1722.58 (-C=O), 1649.22; Mass: 452 $(M+1)^{+}$.

Dimethyl-6-amino-4-(3,4-dichlorophenyl)-5-cyano-1,4-dihydro-1-(naphthalen-1-yl)pyridine-2,3-dicarboxylate (Table 1, Entry 4):

Yellow solid; M.P.=101-102^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 3.12 (s, 3H), 3.22 (s, 3H), 4.01 (s, 2H), 4.88 (s, 1H), 7.49 (d, 3H), 7.22 (m, 7 H); IR (neat) cm^{-1} 3336, 2964, 2172, 1746, 1695, 1644, 1566, 1372, 1217, 1032; Mass: 508. $(M+1)^{+}$.

Diethyl-6-amino-1-(4-chlorophenyl)-5-cyano-4-(4-fluorophenyl)-1,4-dihydropyridine-2,3-dicarboxylate (Table 1, Entry 5)

Yellow solid; M.P.=183-184 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 1.05 (t, 3H), 1.1 (t, 3H), 3.9 (m, 2H), 4.05 (q, 4H), 4.7 (s, 1H), 7.1 (d, 2H), 7.3 (m, 4H), 7.5 (d, 2H); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ ppm 13.04, 13.57, 39.38, 60.27, 61.36, 104.61, 115.15, 120.54, 128.58, 129.37, 132.04, 133.88, 135.14, 141.35, 141.38, 150.34, 159.93, 162.36, 164.29; IR (neat) cm^{-1} 3338.7 (-NH_2), 2982.3 (C-H str. -CH_3), 2184.8 (-CN), 1744.41 (-C=O); Mass: 470 $(M+1)^{+}$.

Dimethyl-6-amino-1-(3-chloro-4-fluorophenyl)-4-(2,4-dichlorophenyl)-5-cyano-1,4-dihydropyridine-2,3-dicarboxylate (Table 1, Entry 6):

White solid; M.P.=117-118^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 3.1 (s, 3H), 3.15 (s, 3H), 4.01 (s, 2H), 4.78 (s, 1H), 7.4 (d, 4H), 7.62 (d, 2H); IR (neat) cm^{-1} 3334, 2970, 2179, 1749, 1698, 1631, 1572, 1370, 1203, 1044; Mass: 510.72 $(M+1)^{+}$.

Dimethyl 6-amino-1-(3-chloro-4-fluorophenyl)-5-cyano-1,4-dihydro-4-(3-nitrophenyl)pyridine-2,3-dicarboxylate (Table 1, Entry 7):

Off white solid; M.P.=120-121 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 3.21 (s, 3H), 3.28 (s, 3H), 4.12 (s, 2H), 4.73 (s, 1H), 7.81 (d, 3H), 8.07 (d, 4H); IR (neat) cm^{-1} 3340, 2969, 2273, 1749, 1648, 1568, 1372, 1219, 1033; Mass: 486.91 $(M+1)^+$.

Triethyl-6-amino-1-(4-chlorophenyl)-1,4-dihydro-4-(3,4-dimethoxyphenyl)pyridine-2,3,5-tricarboxylate (Table 1, Entry 8):

White solid; M.P.=142-143 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.9 (t, 3H), 1.03 (t, 3H), 1.12 (t, 3H), 3.02 (s, 3H), 3.08 (s, 3H), 3.96 (s, 2H), 4.12 (q, 4H), 4.19 (q, 2H), 4.7 (s, 1H), 7.53 (d, 3H), 7.90 (d, 4H); IR (neat) cm^{-1} 3335, 2957, 2176, 1744, 1694, 1638, 1560, 1372, 1215, 1033; Mass: 559.03 $(M+1)^+$.

Triethyl-6-amino-1-(4-chlorophenyl)-4-(4-(dimethylamino)-phenyl)-1,4-dihydropyridine-2,3,5-tricarboxylate (Table 1, Entry 9):

Yellow solid; M.P.=115-116 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.89 (d, 3H), 1.1 (t, 3H), 1.21 (t, 3H), 2.56 (s, 6H), 3.93 (s, 2H), 4.02 (q, 4H), 4.13 (q, 2H), 4.82 (s, 1H), 7.8 (d, 4H), 8.05 (d, 4H); IR (neat) cm^{-1} 3338, 2970, 2181, 1747, 1696, 1652, 1570, 1381, 1313, 1132; Mass: 541.9 $(M+1)^+$.

Triethyl-6-amino-1,4-dihydro-4-(4-hydroxy-3-methoxyphenyl)-1-phenylpyridine-2,3,5-tricarboxylate (Table 1, Entry 10):

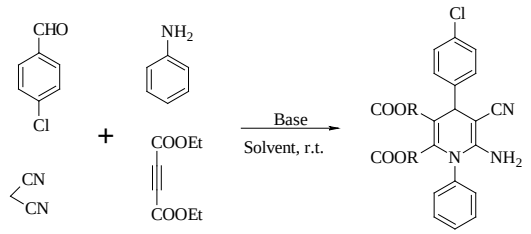
Faint solid; M.P.=109-110 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 0.99 (t, 3H), 1.1 (t, 3H), 1.2 (t, 3H), 3.2 (s, 3H), 3.92 (s, 2H), 4.03 (q, 2H), 4.14 (q, 2H), 4.19 (q, 2H), 4.78 (s, 1H), 5.9 (s, 1H, -OH), 7.27 (m, 5H), 7.43 (d, 3H); IR (neat) cm^{-1} 3328, 2803, 2184, 1748, 1690, 1643, 1562, 1370, 1213, 1032; Mass: 510.38 $(M+1)^+$.

Dimethyl-6-amino-1-(3-chloro-4-fluorophenyl)-4-(4-chloro-phenyl)-5-cyano-1,4-dihydropyridine-2,3-dicarboxylate (Table 1, Entry 11):

Faint yellow solid; M.P.=112-113 °C; 1H NMR ($CDCl_3$, 400 MHz) δ ppm 3.28 (s, 3H), 3.41 (s, 3H), 4.02 (s, 2H), 4.67 (s, 1H), 7.82 (d, 4H), 7.95 (d, 3H); IR (neat) cm^{-1} 3336, 2956, 2183, 1846, 1695, 1640, 1558, 1372, 1213, 1029; Mass: 476.01 $(M+1)^+$.

RESULTS AND DISCUSSION

Initially to optimize reaction conditions, the reaction of 4-chlorobenzaldehyde (1 mmol), malononitrile (1.5 mmol), diethyl acetylene dicarboxylate (1 mmol) and aniline (1 mmol) mixture was probed as a model condensation reaction using various bases and solvents at room temperature for the synthesis of diethyl-6-amino-4-(4-chlorophenyl)-5-cyano-1,4-dihydro-1-phenylpyridine-2,3-dicarboxylate (DHP-3) (Scheme 2, Table 2).



Scheme 2. Model reaction for optimization of reaction conditions

Table 2. Optimization of reaction conditions for the model reaction

No.	Conditions	Time,h	Yield,%@
1	N,N-DIPEA (10 mol %), EtOH, r.t.	5	36
2	Pyrrolidine (10 mol %), EtOH, r.t.	5	70
3	Imidazole (10 mol %), EtOH, r.t.	5	45
4	Cs_2CO_3 (10 mol %), EtOH: H_2O, r.t.	8	64
5	DABCO (10 mol %), EtOH, r.t.	8	53
6	Morpholine (10 mol %), EtOH, r.t.	3	84
7	Morpholine (20 mol %), r.t., EtOH	3	89
8	Morpholine (20 mol %), r.t., MeOH	3	81

@Reactions carried on 4-chlorobenzaldehyde (1 mmol), malononitrile (1.5 mmol), diethyl acetylenedicarboxylate (1 mmol) and aniline (1 mmol).

From Table 2, bases like N,N-diisopropyl(ethyl)amine (N,N-DIPEA), pyrrolidine, imidazole, DABCO or cesium carbonate (Cs_2CO_3) were not suitable to catalyze the transformation at room temperature after 5-8 h of reaction time. Among the several bases tried for the probe model reaction; use of morpholine (20 mol %) was found to be sufficient enough to carry out the transformation via one-pot synthesis of highly substituted 1,4-DHPs at ambient temperature. If no base was added, the reaction did not yield the desired product even after stirring for overnight (12 h). Morpholine was required in higher concentration (20 mol %) which could be most probably due to its decreased basicity and presence of an electron withdrawing oxygen atom in the ring. The reactions were found to be better in ethanol rather than methanol in terms of the yield of desired products.

Encouraged with these results, scope and generality of the catalytic efficiency of morpholine were extended by using diversely substituted aldehydes and amines (Table 1).

Furthermore, the use of dimethyl acetylene dicarboxylate instead of diethyl acetylene dicarboxylate and ethyl cyanoacetate in place of malononitrile also accomplished satisfactory results. Diethyl acetylenedicarboxylate showed slightly high reactivity as compared to dimethyl acetylene dicarboxylate. Aldehydes possessing electron donating as well as electron withdrawing substituents afforded the desired products without significant variation in yields. Thus, the domino one-pot reaction was successful with different active methylene compounds, dialkyl acetylene-dicarboxylate, aromatic aldehydes and amines (Table 1) revealing that the reaction works well and tolerates both electron-withdrawing and electron-donating substituents in

aromatic ring generating the target molecules in high yields with negligible variations in the product. It indicates that the present protocol has a broad substrate scope. After completion of the reaction as monitored by TLC, the reaction mass was concentrated on a rotary evaporator, poured onto crushed ice, stirred for 10 minutes and filtered off the resulting precipitated solid as a crude product which was further purified by crystallization with ethanol. Thus the work-up procedure for the morpholine catalyzed synthesis of DHPs was found to be convenient and straightforward. The products were confirmed by comparison of their physical constants with the literature values and analysis of spectroscopic data viz ^{1}H, ^{13}C NMR, IR and MS spectra.

A plausible mechanism for morpholine base catalyzed the synthesis of 1, 4-DHPs is outlined in Figure 2:

Step-1: The base abstracts proton from the acidic compound - malononitrile or ethyl cyanoacetate affording benzylidene intermediate (**I**) involving the Knoevenagel condensation.

Step-2: Treatment of DMAD or DEAD with the ethanolic solution of aromatic amine generates the 1,3-dipole intermediate (**II**).

Step-3: Michael addition of intermediate (**I**) with the dipolar intermediate (**II**), followed by migration of hydrogen, cyclization and tautomerization of the imino group (C=NH) to the amino (-C-NH_2) group results in the formation of the desired product.

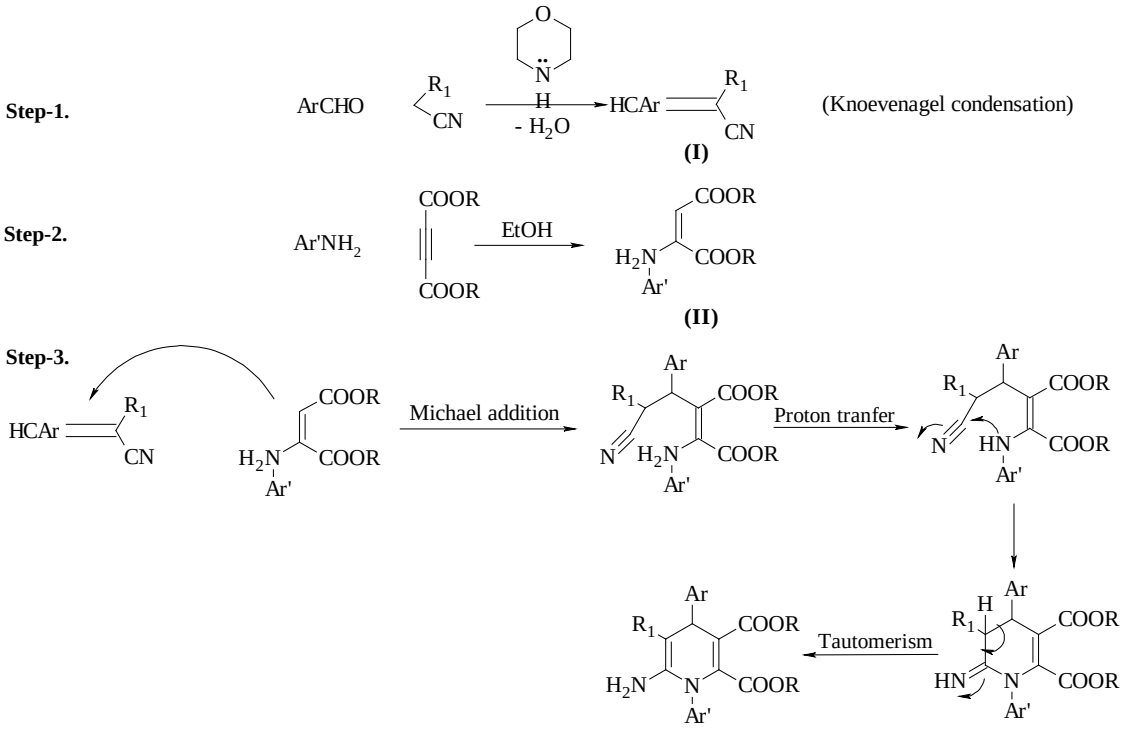


Figure 2. A plausible mechanism for the morpholine base catalyzed the synthesis of polysubstituted pyridines

CONCLUSION

In summary, an atom efficient, practical strategy and easy access to highly functionalized 1,4-DHP derivatives using morpholine as an organobase has been developed by one-pot four-component reaction of an aldehyde, acidic active methylene compound, DEAD or DMAD and various amines via tandem Knoevenagel, Michael and intramolecular nucleophilic additions at ambient temperature. Ease of work up, room temperature conditions, broad substrate scope, no need of column chromatographic purification and good yields make the present protocol attractive for the construction of DHPs.

It also provides a practical methodology for employing the 1,4-dipolar intermediate to design new multicomponent reactions in synthetic organic chemistry.

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REFERENCES

[1]Sunderhaus, J. D. and Martin, S. F., Applications of Multicomponent Reactions to the Synthesis of Diverse Heterocyclic Scaffolds, *Chem.,* **2009**, *15(6),* 1300. DOI: 101002/chem.200802140.

[2]Rotstein, B. H., Zaretsky, S., Rai, V., Yudin and A. K., Small Heterocycles in Multicomponent Reactions, *Chem. Rev.*, **2014**, *114(16),* 8323. DOI: 10.1021/cr400615v.

[3]Nairoukh, Z., Cormier, M. and Marek, I. *Merging C-H and C-C bond cleavage in organic synthesis*, **2017,** 0035.

[4]Haller, H., Effective management of hypertension with dihydropyridine calcium channel blocker-based combination therapy in patients at high cardiovascular risk, *Int J Clin Pract.* **2008**, *62(5)*, 781. DOI: 10.1111/j.1742-1241.2008.01713.x

[5]Opie L. H., Yusuf, S., Kübler, W., Current status of safety and efficacy of calcium channel blockers in cardiovascular diseases: a critical analysis based on 100 studies, *Prog. Cardiovasc. Dis.*, **2000**, *43(2)*,171. DOI: 10.1053/pcad.2000.7010

[6]Goyal, M., Dhanjal, J. K. Goyal, S, Tyagi, C., Hamid, R. and Grover, A., Development of Dual Inhibitors against Alzheimer's Disease Using Fragment-Based QSAR and Molecular Docking, *Biomed. Res. Int.*, **2014**, 979606. DOI: 10.1155/2014/979606.

[7]Olejníková, P., Švorc, L., Olšovská, D., Panáková, A., Vihonská, Z. , Kovaryová, K., Marchalín, Š., Antimicrobial activity of novel C2-substituted 1,4-dihydropyridine analogs, *Sci. Pharm.* **2014**, *82(2)*, 221. DOI: 10.3797/scipharm.1311-04.

[8]Bazargan, L., Fouladdel, S., Shafiee, A., Amini, M., Ghaffari, S. M., Azizi, E., Evaluation of anticancer effects of newly synthesized dihydropyridine derivatives in comparison to verapamil and doxorubicin on T47D parental and resistant cell lines in vitro, *Cell Biol. Toxicol.*, **2008**, *24(2)*, 165. DOI:10.1007/s10565-007-9026-x

[9]Velena, A., Zarkovic, N., Troselj, K. G., Bisenieks, E., Krauze, A., Poikans, J. and Dubur, G., 1,4-Dihydropyridine derivatives: Dihydronicotinamide analogues-model compounds targeting oxidative stress, *Oxidative Med. Cell. Longevity*, **2016**, Article ID 1892412, 35 pages. DOI: 10.1155/2016/1892412.

[10]Komoda, H., Inoue, T., Node, K., *Clin. Exp. Hypertens.* **2010**, *32(2)*, 121. Anti-inflammatory properties of azelnidipine, a dihydropyridine-based calcium channel blocker, DOI: 10.3109/10641960903254414.

[11]Briede, J., Stivrina, M., Vigante, B., Stoldere, D., Dubur, G., Acute effect of antidiabetic 1,4-dihydropyridine compound cerebrocrast on cardiac function and glucose metabolism in the isolated, perfused normal rat heart, *Cell Biochem. Funct.* **2008**, *26(2)*, 238. DOI:10.1002/cbf.1442

[12]Iman, M., Davood, A., Dehqani, G., Lotfinia, M., Sardari, S., Azerang, P., Amini, M., Design, synthesis and evaluation of antitubercular activity of novel dihydropyridine containing imidazolyl substituent., *Iran. J. Pharm. Res. Fall*, **2015**, *14(4)*, 1067.

[13]Gadotti, V. M., Bladen, C., Zhang, F. X., Chen, L., Gündüz, M. G., Şimşek, R., Şafak, C., Zamponi, G. W., Analgesic effect of a broad-spectrum dihydropyridine inhibitor of voltage-gated calcium channels, *Pflugers Arch.* 2015, *467(12)*, 2485. DOI: 10.1007/s00424-015-1725-1.

[14]Sunkel, C. E., Casa-Juana, M. F., Cillero, F. J., Priego, J. G., Ortega, M. P., Synthesis, platelet aggregation inhibitory activity, and in vivo antithrombotic activity of new 1,4-dihydropyridines, *J. Med. Chem.*, **1988**, *31(10)*, 1886. DOI: 10.1021/jm00118a004

[15]Hilgeroth, A., Dressler, C., Neuhoff, S., Spahn-Langguth, H., Langguth, P., Dimeric 4-aryl-1,4-dihydropyridines as novel HIV-1 protease inhibitors--affinities to intestinal P-glycoprotein, *Pharmazie.*, **2000**, *55(10)*, 784.

[16]Miyazaki, H., Tanaka, S., Fujii, Y., Shimizu, K., Nagashima, K., Kamibayashi, M., Uehara, T., Okuma, Y. and Nomura, Y., Neuroprotective effects of a dihydropyridine derivative, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid methyl 6-(5-phenyl-3-pyrazolyloxy)hexyl ester (CV-159), on rat ischemic brain injury, *Life Sci.*, **1999**, *64(10)*, 869.

[17]Chen, H. S., Guo, R. Y., Meglumine: an efficient, biodegradable, and recyclable green catalyst for one-pot synthesis of functionalized dihydropyridines, *Chem. Month.*, **2015**, *146(8)*, 1355. DOI: 10.1007/S00706-015-1416-9.

[18]Kumar, A. and Sharma, S., A grinding-induced catalyst- and solvent-free synthesis of highly functionalized 1,4-dihydropyridines via a domino multicomponent reaction, *Green Chem.*, **2011**, *13*, 2017. DOI: 10.1039/C1GC15223H.

[19]Sun, J., Xia, E. Y., Wu, Q. C. and Yan, G., Synthesis of polysubstituted dihydropyridines by four-component reactions of aromatic aldehydes, Malononitrile, Arylamines, and Acetylenedicarboxylate, *Org. Lett.*, **2010**, *12(16)*, 3678. DOI: 10.1021/ol101475b

[20]Pal, S., Choudhury, L. H. and Parvin T., One-Pot Multicomponent reactions for the efficient synthesis of highly functionalized dihydropyridines, *Synth. Comm.*, **2013**, *43(7)*, 986. DOI: 10.1080/00397911.2011.618283.

[21]Pal, S., Singh, V., Das, P. and Choudhury, L. H., PEG-mediated one-pot multicomponent reactions for the efficient synthesis of functionalized dihydropyridines and their functional group dependent DNA cleavage activity, *Bioorg. Chem.*, **2013**, *48*, 8. DOI: 10.1016/j.bioorg.2013.03.003

[22]Hadjebi, M., Hashtroudi, M. S. Bijanzadeh, H. R. and Balalaie, S., Novel four‐component approach for the synthesis of polyfunctionalized 1,4‐dihydropyridines in aqueous media, *Helv. Chim. Acta*, **2011**, *94*, 382. DOI: 10.1002/hlca.201000228.

[23]Rameshwar, N., Ramchander, J., Parthasarathy, T. and Reddy A. R., Yttrium triflate catalysed synthesis of 1,4-dihydropyridines under solvent-free conditions, *Hetero Lett.*, **2012**, *2(4)*, 455.

[24]Ping, W. U., Xia, E. Y., Jing, S. and Yan C. G., Synthesis of Polysubstituted 1,4-dihydropyridines via four-component reactions of arylamine, acetylenedicarboxylate, aromatic aldehyde and ethyl acetoacetate, *Chem. Res. Chin. Univ.*, **2012**, *28(4)*, 652.

[25]Jiang, H., Mai, R., Cao, H., Zhu, Q. and Liu, X., L-Proline-catalyzed synthesis of highly functionalized multisubstituted 1,4-dihydropyridines, *Org. Biomol. Chem.*, **2009**, *7*, 4943. DOI: 10.1039/B914659H

[26]Bandgar, B. P., Uppalla, L. S., and Sadavarte, V. S., Morpholine adsorbed on silica gel: A novel and mild basic catalyst for the synthesis of alpha,beta-unsaturated nitroalkenes, *Chem. Month.*, **2000**, *131(9)*, 949.

[27]Heravi, M. M., Zakeri, M. and Mohammadi, N., Morpholine catalyzed one-pot multicomponent synthesis of compounds containing chromene core in water, *Chin. J. Chem.*, **2011**, *29*, 1163.DOI: 10.1002/cjoc.201190217

[28]Heravi, M. M., Zakeri, M., Pooremamy, S. and Oskooie, H. A., Clean and efficient synthesis of polyhydroquinoline derivatives under solvent-free conditions catalyzed by morpholine, *Synth. Comm.*, **2010**, *41(1)*, 113. https://doi.org/10.1080/00397910903531862

[29]Joshi, V. M., Vyawahare, S. K., Tekale, S. U., Shinde, S. B., Uddin, M. F., Dake, S. A., Shisodia, S. U., An efficient synthesis of 3,4-dihydropyrimidin-2(1H)-ones using nanocomposite ferrite catalyst, **2013**, *2(7)*, 481.DOI: http://dx.doi.org/10.17628/ecb.2013.2.481-484

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SYNTHESIS OF NOVEL SUBSTITUTED-BENZO[*d*]THIAZOLE-2,4-DICARBOXAMIDES HAVING KINASE INHIBITION AND ANTI-PROLIFERATIVE ACTIVITY

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Keywords: Benzo[d]thiazoles; carboxamides; anticancer activity; kinase inhibitors.

A series of novel derivatives containing N^4-(4-fluorophenyl)-N^2-substitured-benzo[*d*]thiazole-2,4-dicarboxamides were synthesized via an efficient, mild and convenient multistep reaction protocol with excellent yields. The structure of the synthesize compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, mass spectra, elemental analysis and purity was checked by HPLC. All synthesized compounds were screened for anticancer activity against A-549 and Du-145 cancer cell lines by MTT assay. The preliminary bioassay suggests that most of the compounds show anti-proliferation with different degrees. The synthesized compound shows IC_{50} values in the range of 1.52-17.18 µM in both cell lines. The compounds having electron donating groups had higher anticancer activity compared compounds with electron withdrawing substitutions.

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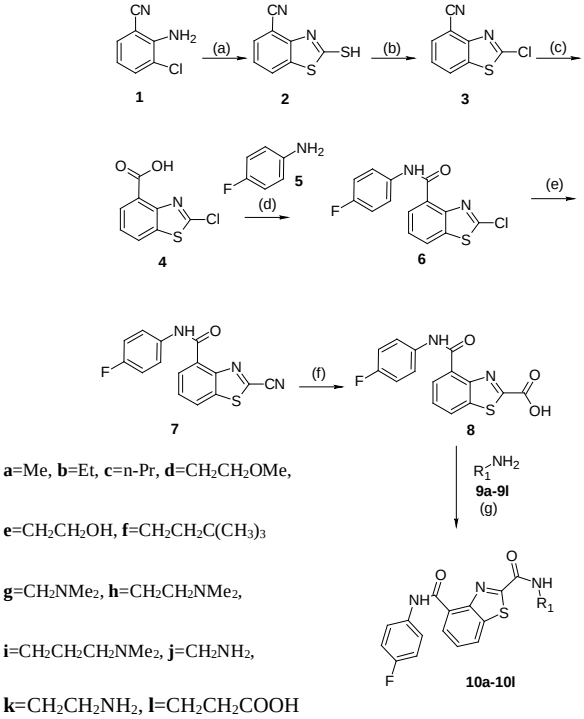
INTRODUCTION

The kinases plays important role in cell functioning. There are over 500 kinases comprising in the human kinome, and all are associated with the functioning of cells.[1] Different types of kinases are responsible for different functioning of cells, some kinases are target of rapamycin (TOR) signaling for cell growth.[2, 3] Some are protein tyrosine kinase inhibitors. By considering the importance of kinases we need to develop new kinase inhibitors with diversified activity.

In present work we have chosen substituted thiazol nuclei and its derivatives for cell line and kinases study. Substituted benzothiazole are known for diversified biological activities like anti-tubercular,[4] MAP kinase inhibitors.[5] Kinases plays key role in cancer initiation and progression.[6,7] Thiazoyl-sulfonamides act as carbonic anhydrase inhibitors[8] and anticancer.[9] Some derivatives comprising thiophene nuclei acts as anti-proliferative agents.[10-12] Neural precursor cell expressed, developmentally down-regulated 8 (NEDD8) activating small molecule-drug conjugates enzymes inhibitors,[13] Raf kinase inhibitor protein (RKIP),[14] poly-ADP-ribose polymerases (PARP) and topoisomerase (TOPO) inhibitors.[15] Some benzthiazole forms key building block of some of the biologically active derivatives.[16-17] By considering the diversified biological activity of benzo[*d*]thiazol and continuation of our research work,[18-20] we have synthesized a series of substituted -benzo[d]thiazole derivatives and all the synthesized compounds were tested for their biological activity in cell line and enzymatic study.

RESULTS AND DISCUSSION

We have synthesized a series N^4-(4-fluorophenyl)-N^2-substitured-benzo[*d*]thiazole-2,4-dicarboxamidey (**10a-10l**) starting from easily available 2-amino-3-chlorobenzonitrile (**1**).



Scheme 1. Synthesis of N4-(4-fluorophenyl)-N2-substitured-benzo[d]thiazole-2,4-dicarboxamide (**10a-10l**) :

We have optimized the entire step to get good yield, neat reaction profile, less harsh condition. The optimized steps are depicted in Scheme **1.** Reagents and conditions: (a): N-methylpiperidinone, 150 °C, 1 h, potassium O-ethyl carbodithioate, DMF, 180 °C, 12 h; (b): $POCl_3$, 100 °C, 2 h; (c): NaOH, THF, H_2O, 12 h; (d): 4-fluoroaniline (**5**), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), N,N-diisopropylethylamine (DIPEA), DMF, 0 °C- room temperature, 12 h ; (e): NaCN, MeOH, 12 h ; (f): NaOH, 100 °C, 2 h; (g): $SOCl_2$, 100 °C, 0.5h, substituted amines (**9a-9l**), 0 °C- room temperature, 12 h.

2-Mercaptobenzo[*d*]thiazole-4-carbonitrile (**2**) has already been prepared in DMF and N-methyl piperidione at high temperature.[21,22] We have used readily available 2-amino-3-chlorobenzonitrile (**1**) as a staring material. We have optimized step **a** by heating the compound **1** with N-methylpiperidinone at 150 °C for 1 h and adding the potassium ethylxanthate in DMF. The N-methylpiperidinone acts as base as well as high boiling solvent as higher temperature is required for the reaction to proceed. We heated that reaction mixture at 180 °C for 12 h to get >96 % yield. We have used mixture of solvents for solubility of compound and it helps in clean isolation of pure compound. The compound **2** was characterized as it shows clear S-C and S-H bonds in the IR spectrum at 730 cm^{-1} and 2560 cm^{-1}, respectively and in ^1H NMR the NH_2 protons were vanished.

In step **b** the compound **2** was chlorinated by using $POCl_3$ in 2 h at 100 °C to obtain crude compound with quantitative recovery, and the crude compound **3** was hydrolysed. The presence of compound **3** was confirmed with IR spectroscopy, the bands for C-S and C-H bonds were disappeared.

Compound **3** was hydrolyzed by using 20 % aqueous NaOH for 12 h at room temperature and later a routine acid-base treatment was used to isolate compound **4** with 90% isolated yield.[23] It has been used further without purification. Compound **4** was characterized by TLC and ^1H NMR as in TLC it shows trailing spot at base and in NMR it shows an acidic peaks at δ 12.2 ppm.

In step **d** compound **4** reacted with 4-fluoroaniline (**5**) by using peptide coupling condition to obtain compound **6** with 90 % yield. Formation of Compound **6** was confirmed with TLC, mass and ^1H NMR spectra, as its aromatic region shows A_2-B_2 pattern of peaks.

In step **e** we have done displacement reaction on compound **6** by using sodium cyanide in methanol to obtain compound **7** with 85 % yield. The presence of compound **7** conformed from IR spectrum as it shows distinct band at 2200 cm^{-1} for nitrile group.

The compound **7** was subjected to hydrolysis to get compound **8.** Using basic hydrolysis conditions led to quantitative yield of compound **8**. The compound **8** is a key intermediate for the synthesis of desired compounds **10a-10j**.

The compound **8** was converted to its acid chloride and lateer reacted with different amines **9a-9j** without base to obtain all the final compounds **10a-10j** with >80 % yields for all the derivatives. For the compounds **2** to **8** we have purified all the compounds by washing with different solvent combinations and avoided column purifications. For final compounds **10a-10j** we have used column purification to get required compounds with >95 % purity. The detailed experimental procedure and characterization of final compounds and key intermediates were given in experimental section.

EXPERIMENTAL SECTION

All chemicals, unless otherwise specified, were purchased from commercial sources and were used without further purification. The major chemicals were purchased from Sigma Aldrich and Avra labs. The development of reactions was monitored by thin layer chromatography (TLC) analysis on Merck pre-coated silica gel 60 F254 aluminum sheets, visualized by UV light. Melting points were recorded on SRS Optimelt, melting point apparatus and are uncorrected. The ^1H NMR spectra were recorded on a 400 MHz Varian NMR spectrometer. The ^{13}C were recorded on a 100 MHz Varian NMR spectrometer. The chemical shifts are reported as NMR spectra δ (ppm) units and standard is tetramethylsilane (TMS). The following abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br s). LC-MS mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.

Experimental procedure for the synthesis of containing N^4-(4-fluorophenyl)-N^2-substitured-benzo[*d*]thiazole-2,4-dicarboxamides (10a-10l)

Step-a: Synthesis of 2-mercaptobenzo[*d*]thiazole-4-carbonitrile (2)

In the reaction mixture of compound **1** (1.52 g, 0.01 mol) in N-methylpiperidinone (15 mL) was heated for 1 h at 150 °C. Potassium ethylxanthate (3.2 g, 0.02 mol) dissolved in DMF (7 mL) was added dropwise and the reaction mixture was stirred at 180° C for 12 h. Progress of reaction was monitored by TLC and LCMS. After completion the reaction, the reaction mixture was cooled to room temperature and poured into cold H_2O (15 mL). The reaction mixture was acidified by using 4 M aq. HCl solution up to pH 4, when a solid was precipitated. The formed precipite was filtered *in vacuo*, washed with cold water (15 mL) and diethyl ether (10 mL) to afford 2-mercaptobenzo[*d*]thiazole-4-carbonitrile (**2**, 1.85 g, crude) as a brown solid. The crude obtained was used further for the next reaction without purification.

Step-b: Synthesis of 2-chlorobenzo[*d*]thiazole-4-carbonitrile (3):

To the reaction mixture of compound **2** (1.92 g, 0.01 mol) in a round bottom flask, $POCl_3$ (1.9 mL) was added slowly at room temperature. The reaction mixture was stirred at 100 °C for 2 h. Progress of reaction was monitored by TLC and LCMS. The reaction mixture was cooled to room temperature and evaporated under reduced pressure to obtain 2-chlorobenzo[*d*]thiazole-4-carbonitrile (**3**, 2.5 g, crude) as gray semisolid compound. The crude obtained was used further for the next reaction without purification.

Step-c: Synthesis of 2-chlorobenzo[*d*]thiazole-4-carboxylic acid (4):

To a stirred solution of compound **3** (2.50 g) in a round bottom flask. 20 % aq NaOH solution (25 mL) was added at room temperature. The reaction mixture was stirred at room temperature for 12 h. Progress of reaction was monitored by TLC and LCMS.

The reaction mixture was poured into cold H_2O (10 mL) and extracted with DCM (15 mL). The aqueous layer was collected and acidified by using 4 M aq. HCl solution up to pH 3, when a solid was precipitated out. The formed precipitate was filtered off *in vacuo*, washed with water (25 mL), brine (20 mL) and diethyl ether (25 mL) to afford 2-chlorobenzo[*d*]thiazole-4-carboxylic acid (**4**, 2.52g, crude) as a white solid. The crude obtained was used further for the next reaction without purification.

Step-d: Synthesis of 2-chloro-N-(4-fluorophenyl)benzo[*d*]thiazole-4-carboxamide (6):

To a stirred solution of compound **4** (2.13 g, 0.01 mol) in DMF (10 mL) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (2.89 g, 0.015 mol) and N,N-diisopropylethylamine (DIPEA) (5.23 mL, 0.03 mol) were added at 0 °C. Compound **5** (1.66 g, 0.015 mol) was added at 0 °C and the reaction mixture was stirred at room temperature for 12 h. Progress of the reaction was monitored by TLC and LCMS. The reaction mixture was diluted with cold water (10 mL) and extracted with DCM (2 × 25 mL). The organic layer was separated, washed with 2 M aq. cold HCl (10 mL), brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product obtained was purified by washing with 25:75 of EtOAc:hexane (50 mL), cold diethyl ether (20 mL) and cold pentane (20 mL) to afford 2-chloro-N-(4-fluorophenyl)benzo[*d*]thiazole-4-carboxamide (**6**, 2.75 g, 90 %) as an off white solid.

Step-e: Synthesis of 2-cyano-N-(4-fluorophenyl)benzo[d]thiazole-4-carboxamide (7):

To a stirred solution of compound **6** (3.06 g, 1.0 mmol) in MeOH (25 mL) was carefully added NaCN (0.98 g, 2.0 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 h. Progress of reaction was monitored by TLC and LCMS. The reaction mixture was poured into cold H_2O (30 mL) and extracted with DCM (2 × 15 mL). The organic layer was separated, washed with H_2O (15 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to afford 2-cyano-N-(4-fluorophenyl)benzo[*d*]thiazole-4-carboxamide (**7**, 2.68 g, crude) as off white solid.The crude obtained was used further for the next reaction without purification.

Step-f: Synthesis of 4-(4-fluorophenylcarbamoyl)benzo[d]thiazole-2-carboxylic acid (8):

To a stirred solution of compound **7** (2.97 g) in a round bottom flask. 20 % aq. NaOH solution (25 mL) was added at room temperature. The reaction mixture was heated at 100 °C for 2 h. Progress of reaction was monitored by TLC and LCMS. The reaction mixture was poured in cold H_2O (10 mL) and extracted with DCM (15 mL), the aqueous layer was collected and acidified by using 2 M aq. HCl solution up to pH 3. A solid was precipitated out . The formed precipitate was filtered off *in vacuo*, washed with water (25 mL), brine (20 mL) and diethyl ether (25 mL) to afford 4-(4-fluorophenylcarbamoyl)benzo[*d*]thiazole-2-carboxylic acid (**8**, 2.85 g, crude) as a white solid. The crude product obtained was used further for the next reaction without purification.

Step-g: Synthesis of N^4-(4-fluorophenyl)-N^2-substituted-benzo[*d*]thiazole-2,4-dicarboxamide (10a-10l):

To a stirred solution of compound **8** (0.01 mmol) in DCM (5 mL) $SOCl_2$ (0.15 mmol) was added at 0 °C. The reaction mixture was heated at 100 °C for 0.5 h. In another round bottom flask the amine (**9a-9l)** (0.015 mmol) and DCM (5 mL) were mixed at 0 °C. The content of the flask one was added to the amine flask under inert atmosphere and the reaction mixture was stirred at room temperature for 12 h. Progress of the reaction was monitored by TLC and LCMS. The reaction mixture was diluted with cold water (5 mL) and extracted with DCM (2 × 10 mL). The organic layer was separated, washed with H_2O (5 mL) and brine (5 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product obtained was purified by silica gel (230-400 mesh, 5-35: 95-65 % of DCM:hexane) column chromatography to afford N^4-(4-fluorophenyl)-N^2-substitured-benzo[*d*]thiazole-2,4-dicarboxamide derivatives (**10a-10l**, 81-88 %) as a solids.

Spectral Data

N^4-(4-Fluorophenyl)-N^2-methylbenzo[*d*]thiazole-2,4-dicarboxamide (10a):

Yellow solid; M.p. 172-173 °C; Yield: 83 %; IR (KBr) (ν_{max}, cm^{-1}): 1627 (C=O), 1582 and 1520 (Ar); Anal. calc. for $C_{16}H_{12}FN_3O_2S$: C, 58.35; H, 3.67; N, 12.76; Found: C, 58.32; H, 3.70; N, 12.79. LC-MS m/z (%): 330.3 (M+H); HPLC-98.9 % RT-8.22 min; ^{1}H NMR (400 MHz, DMSO-d_6, ppm)= δ 12.8 (s, 1H, NH), 10.36 (s, 1H, NH), 8.23 (d, *J* = 8.2 Hz, 2H, ArH), 7.97 (d, *J* = 8.2 Hz, 2H, ArH), 7.66 (d, *J* = 7.6 Hz, 1H, ArH), 7.56 (d, *J* = 7.2 Hz, 1H, ArH), 7.24 (t, *J* = 8.4, 4.2 Hz, 1H, ArH), 2.68 (s, 3H, NH-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm)= δ 25.6, 115.2, 123.2, 123.4, 125.2, 126.4, 127.2, 131.2, 135.2, 158.4, 159.2, 162.4, 164.8, 168.3.

N^2-Ethyl-N^4-(4-fluorophenyl)benzo[*d*]thiazole-2,4-dicarboxamide (10b):

Yellow solid; M.p. 189-190 °C; Yield: 84 %; IR (KBr) (ν_{max}, cm^{-1}): 1622 (C=O), 1580 and 1524 (Ar); Anal. calc. for $C_{17}H_{14}FN_3O_2S$: C, 59.46; H, 4.11; N, 12.24; Found: C, 59.48; H, 4.10; N, 12.23. LC-MS m/z (%): 344.2 (M+H); HPLC-98.8 % RT-8.12 min.; ^{1}H NMR (400 MHz, MeOD, ppm)= δ 8.23 (d, *J* = 7.2 Hz, 1H, ArH), 8.21 (d, *J* = 8.0 Hz, 2H, ArH), 7.88 (d, *J* = 8.0 Hz, 2H, ArH), 7.68 (d, *J* = 7.2 Hz, 1H, ArH), 7.37 (t, *J* = 7.6 Hz, 1H, ArH), 2.58 (q, *J* = 15.2, 7.6 Hz, 2H, NH-CH_2), 1.24 (t, *J* = 8.4 Hz, 3H, CH_2-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm)= δ 14.4, 33.6, 115.2,

123.3, 123.4, 125.5, 126.6, 127.2, 131.3, 135.5, 158.4, 159.4, 162.2, 164.8, 168.3.

N4-(4-Fluorophenyl)-N2-propylbenzo[*d*]thiazole-2,4-dicarboxamide (10c):

Yellow solid; M.p. 197-198 °C; Yield: 86 %; IR (KBr) (ν_{max}, cm^{-1}): 1630 (C=O), 1570 and 1525 (Ar); Anal. calc. for $C_{18}H_{16}FN_3O_2S$: C, 60.49; H, 4.51; N, 11.76; Found: C, 60.48; H, 4.53; N, 11.78. LC-MS m/z (%): 358.3 (M+H); HPLC-98.3 %, RT-8.45 min; ^{1}H NMR (400 MHz, DMSO-d_6, ppm)= δ 8.23 (d, *J* = 8.4 Hz, 2H, ArH), 7.92 (d, *J* = 8.4 Hz, 2H, ArH), 7.84-7.76 (m, 2H, ArH), 7.36 (t, *J* = 7.2 Hz, 1H, ArH), 5.4 (s, 1H, NH), 3.63 (s, 1H, NH), 2.44 (t, *J* = 7.2 Hz, 2H, NH-CH_2), 1.62 (q, *J* = 15.2, 7.6 Hz, 2H, CH_2-CH_2), 0.88 (t, *J* = 8.4 Hz, 3H, CH_2-CH_3). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm)= δ 11.3, 23.2, 41.8, 115.2, 123.3, 123.6, 125.4, 126.2, 127.3, 131.5, 135.2, 158.6, 159.2, 162.4, 164.8, 168.3.

N^4-(4-Fluorophenyl)-N^2-(2-methoxyethyl)benzo[*d*]thiazole-2,4-dicarboxamide (10d):

White solid; M.p. 168-169 °C; Yield: 88 %; IR (KBr) (ν_{max}, cm^{-1}): 1632 (C=O), 1580 and 1535 (Ar); Anal. calc. for $C_{18}H_{16}FN_3O_3S$: C, 57.90; H, 4.32; N, 11.25; Found: C, 57.95; H, 4.31; N, 11.28. LC-MS m/z (%): 374.2 (M+H); HPLC-99.4 %, RT-8.24 min; ^{1}H NMR (400 MHz, DMSO-d_6, ppm)= δ 8.24 (d, *J* = 8.4 Hz, 2H, ArH), 7.98 (d, *J* = 8.4 Hz, 2H, ArH), 7.84-7.76 (dd, *J* = 3.6, 7.6 Hz, 2H, ArH), 7.38 (t, *J* = 6.8 Hz, 1H, ArH), 5.6 (s, 1H, NH), 3.66 (s, 1H, NH), 3.64 (t, *J* = 7.6 Hz, 2H, O-CH_2), 3.22 (s, 3H, O-CH_3), 2.68 (t, *J* = 8.4 Hz, 2H, NH-CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm)= δ 38.4, 58.6, 73.4, 115.6, 123.1, 123.1, 125.6, 126.4, 127.2, 131.5, 135.2, 158.8, 160.2, 162.2, 164.2, 168.8.

N^4-(4-Fluorophenyl)-N^2-(2-hydroxyethyl)benzo[*d*]thiazole-2,4-dicarboxamide (10e):

Off-White solid; M.p. 153-154 °C; Yield: 81 %; IR (KBr) (ν_{max}, cm^{-1}): 1622 (C=O), 1572 and 1520 (Ar); Anal. calc. for $C_{17}H_{14}FN_3O_3S$: C, 56.82; H, 3.93; N, 11.69; Found: C, 56.85; H, 3.91; N, 11.68. LC-MS m/z (%): 360.3 (M+H); HPLC-97.4 %, RT-8.75 min; ^{1}H NMR (400 MHz, DMSO-d_6, ppm)= δ 8.24 (d, *J* = 8.0 Hz, 2H, ArH), 7.97 (d, *J* = 8.0 Hz, 2H, ArH), 7.84-7.76 (dd, *J* = 3.6, 8.4 Hz, 2H, ArH), 7.38 (t, *J* = 7.6 Hz, 1H, ArH), 5.6 (s, 1H, NH), 4.78 (s, 1H, OH), 3.78 (dt, *J* = 8.4 Hz, 2H, O-CH_2), 3.36 (s, 1H, NH), 2.64 (dt, *J* = 7.6 Hz, 2H, N-CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm)= δ 40.2, 61.4, 115.4, 123.2, 123.3, 125.5, 126.4, 127.6, 131.7, 135.1, 158.5, 159.1, 162.1, 163.5, 168.1.

N^4-(4-Fluorophenyl)-N^2-(3,3-dimethylbutyl)benzo[*d*]thiazole-2,4-dicarboxamide (10f):

White solid; M.p. 211-212 °C; Yield: 86 %; IR (KBr) (ν_{max}, cm^{-1}): 1616 (C=O), 1580 and 1526 (Ar); Anal. calc. for $C_{21}H_{22}FN_3O_2S$: C, 63.14; H, 5.55; N, 10.52; Found: C, 62.88; H, 5.58; N, 10.58. LC-MS m/z (%): 400.4 (M+H); HPLC-97.7 %, RT-8.12 min; ^{1}H NMR (400 MHz, MeOD, ppm)= δ 8.16 (d, *J* = 8.4 Hz, 2H, ArH), 8.06 (d, *J* = 7.6 Hz, 1H, ArH), 7.78 (d, *J* = 8.4 Hz, 2H, ArH), 7.41 (d, *J* = 8.0 Hz, 1H, ArH), 7.06 (t, *J* = 7.6 Hz, 1H, ArH), 3.42 (dt, *J* = 7.6 Hz, 2H, NH-CH_2), 1.62 (t, *J* = 7.2 Hz, 2H, CH_2-CH_2), 0.98 (s, 9H, CH_2-$(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm)= δ 28.5, 30.2, 34.6, 43.8, 115.2, 122.8, 123.4, 125.4, 126.4, 127.7, 131.1, 135.4, 158.4, 158.8, 162.6, 164.5, 168.1.

N^2-((Dimethylamino)methyl)-N^4-(4-fluorophenyl)benzo[*d*]thiazole-2,4-dicarboxamide (10g):

Yellow solid; M.p. 198-199 °C; Yield: 82 %; IR (KBr) (ν_{max}, cm^{-1}): 1632 (C=O), 1571 and 1518 (Ar); Anal. calc. for $C_{18}H_{17}FN_4O_2S$: C, 58.05; H, 4.60; N, 15.04; Found: C, 58.08; H, 4.61; N, 15.08. LC-MS m/z (%): 373.4 (M+H); HPLC-96.8 %, RT-8.45 min; ^{1}H NMR (400 MHz, MeOD, ppm)= δ 8.24 (d, *J* = 7.2 Hz, 1H, ArH), 8.21 (d, *J* = 8.0 Hz, 2H, ArH), 7.86 (d, *J* = 8.0 Hz, 2H, ArH), 7.75 (d, *J* = 7.6 Hz, 1H, ArH), 7.38 (t, *J* = 7.2 Hz, 1H, ArH), 3.56 (s, 2H, NH-CH_2), 2.56 (s, 6H, N-$(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm)= δ 42.8, 68.6, 114.6, 122.8, 123.2, 125.3, 126.4, 127.2, 131.3, 135.2, 158.4, 159.6, 162.4, 163.8, 168.3.

N^2-(2-(Dimethylamino)ethyl)-N^4-(4-fluorophenyl)benzo[d]thiazole-2,4-dicarboxamide (10h):

Yellow solid; M.p. 208-209 °C; Yield: 86 %; IR (KBr) (ν_{max}, cm^{-1}): 1632 (C=O), 1570 and 1530 (Ar); Anal. calc. for $C_{19}H_{19}FN_4O_2S$: C, 59.05; H, 4.96; N, 14.50; Found: C, 59.08; H, 4.91; N, 14.55. LC-MS m/z (%): 387.3 (M+H); HPLC-99.2 %, RT-9.02 min; ^{1}H NMR (400 MHz, MeOD, ppm)= δ 8.18 (d, *J* = 8.4 Hz, 2H, ArH), 8.01 (d, *J* = 7.2 Hz, 1H, ArH), 7.84 (d, *J* = 8.4 Hz, 2H, ArH), 7.44 (d, *J* = 7.6 Hz, 1H, ArH), 7.10 (t, *J* = 7.6 Hz, 1H, ArH), 3.64 (td, *J* = 7.6 Hz, 2H, NH-CH_2), 2.78 (t, *J* = 7.2 Hz, 2H, N-CH_2), 2.38 (s, 6H, N-$(CH_3)_2$). ^{13}C NMR ($CDCl_3$, 100 $MHz_{,}$ ppm)= δ 36.4, 44.6, 57.8, 114.6, 122.8, 123.2, 125.4, 126.4, 127.2, 131.2, 135.5, 158.4, 159.5, 162.4, 163.8, 168.3.

N^2-(3-(Dimethylamino)propyl)-N^4-(4-fluorophenyl)benzo[*d*]-thiazole-2,4-dicarboxamide (10i):

Yellow solid; M.p. 218-219 °C; Yield: 84 %; IR (KBr) (ν_{max}, cm^{-1}): 1624 (C=O), 1574 and 1518 (Ar), Anal. calc. for $C_{20}H_{21}FN_4O_2S$: C, 59.98; H, 5.29; N, 13.99; Found: C, 59.94; H, 5.24; N, 14.03. LC-MS m/z (%): 401.3 (M+H); HPLC-97.35 %, RT-8.67 min; ^{1}H NMR (400 MHz, MeOD, ppm)= δ 8.22 (d, *J* = 7.6 Hz, 1H, ArH), 8.21 (d, *J* = 8.4 Hz, 2H, ArH), 7.84 (d, *J* = 8.4 Hz, 2H, ArH), 7.66 (d, *J* = 7.6 Hz, 1H, ArH), 7.36 (t, *J* = 7.2 Hz, 1H, ArH), 2.58 (t, *J* = 7.6 Hz, 2H, NH-CH_2), 2.42 (q, *J* = 7.2, 3.6 Hz, 2H, N-CH_2), 2.26 (s, 6H, N-$(CH_3)_2$), 1.90 (q, *J* = 8.8, 4.4 Hz, 2H, CH_2-CH_2). ^{13}C NMR ($CDCl_3$, 100 $MH_{Z,}$ ppm)= δ 24.8, 37.6, 44.9, 56.8, 114.6, 122.8, 123.1, 125.4, 126.4, 127.2, 131.4, 135.6, 158.4, 159.4, 162.4, 163.7, 168.1.

N^2-(Aminomethyl)-N^4-(4-fluorophenyl)benzo[*d*]thiazole-2,4-dicarboxamide (10j):

Brown solid; M.p. 148-149 °C; Yield: 81 %; IR (KBr) (ν_{max}, cm^{-1}): 1632 (C=O), 1586 and 1528 (Ar); Anal. calc. for $C_{16}H_{13}FN_4O_2S$: C, 55.80; H, 3.81; N, 16.27; Found: C, 55.84; H, 3.74; N, 16.31. LC-MS m/z (%): 345.3 (M+H); HPLC-99.1 %, RT-8.16 min; ^{1}H NMR (400 MHz, MeOD, ppm)= δ 8.22 (d, *J* = 8.4 Hz, 2H, ArH), 7.96 (d, *J* = 8.4 Hz,

2H, ArH), 7.92 (d, *J* = 7.6 Hz, 1H, ArH), 7.76 (d, *J* = 8.0 Hz, 1H, ArH), 7.26 (t, *J* = 7.6 Hz, 1H, ArH), 5.60 (s, 1H, NH), 4.28 (s, 2H, NH_2), 3.61 (s, 1H, NH), 3.56 (s, 2H, NH-CH_2). ^{13}C NMR ($CDCl_3$, 100 MH_Z, ppm)= δ 51.6, 115.5, 123.2, 123.5, 125.2, 126.5, 127.2, 131.5, 135.2, 158.4, 159.3, 162.4, 164.6, 168.4.

N^2-(2-Aminoethyl)-N^4-(4-fluorophenyl)benzo[*d*]thiazole-2,4-dicarboxamide (10k):

Brown solid; M.p. 154-155 °C; Yield: 84 %; IR (KBr) (ν_{max}, cm^{-1}): 1624 (C=O), 1576 and 1514 (Ar); Anal. calc. for $C_{17}H_{15}FN_4O_2S$: C, 56.97; H, 4.22; N, 15.63; Found: C, 56.84; H, 4.26; N, 15.58. LC-MS m/z (%): 359.4 (M+H); HPLC-96.84 %, RT-8.88 min; ^{1}H NMR (400 MHz, MeOD, ppm)= δ 8.24 (d, *J* = 8.4 Hz, 2H, ArH), 7.97 (d, *J* = 8.4 Hz, 2H, ArH), 7.84-7.76 (m, 2H, ArH), 7.36 (t, *J* = 7.6 Hz, 1H, ArH), 5.6 (s, 1H, NH), 4.12 (s, 2H, NH_2), 3.61 (s, 1H, NH), 3.04 (t, *J* = 7.6 Hz, 1H, NH-CH_2), 2.75 (t, *J* = 7.2 Hz, 1H, NH_2-CH_2). ^{13}C NMR ($CDCl_3$, 100 MH_Z, ppm)= δ 38.9, 42.4, 115.8, 123.2, 123.8, 125.2, 126.6, 127.2, 131.5, 135.2, 158.1, 159.4, 161.8, 164.4, 167.9.

3-(N^4-(4-Fluorophenyl)benzo[*d*]thiazole-2,4-dicarboxamido)-propanoic acid (10l):

Yellow solid; M.p. 221-222 °C; Yield: 88 %; IR (KBr) (ν_{max}, cm^{-1}): 1630 (C=O), 1578 and 1524 (Ar); Anal. calc. for $C_{16}H_{13}FN_4O_2S$: C, 55.81; H, 3.64; N, 10.85; Found: C, 55.85; H, 3.69; N, 10.81. LC-MS m/z (%): 388.3 (M+H); HPLC-99.2 % ,RT-8.86 min; ^{1}H NMR (400 MHz, DMSO-d_6, ppm)= δ 12.56 (br, 1H, COOH), 8.22 (d, *J* = 8.4 Hz, 2H, ArH), 7.98 (d, *J* = 8.4 Hz, 2H, ArH), 7.83-7.79 (m, 2H, ArH), 7.36 (t, *J* = 7.6 Hz, 1H, ArH), 5.67 (s, 1H, NH), 3.47 (s, 1H, NH), 2.72 (t, *J* = 7.2 Hz, 2H, NH-CH_2), 2.58 (t, *J* = 7.2 Hz, 2H, CO-CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm)= δ 34.8, 36.4, 115.5, 123.2, 123.4, 125.6, 126.2, 127.1, 131.2, 135.7, 158.4, 159.1, 161.6, 163.8, 167.8, 178.1.

Biological evaluation

All the synthesized compounds were tested for their in vitro anticancer activity against various cancer cell lines.

The anticancer activity test is performed according to the procedure developed by the National Cancer Institute (NCI, USA) in the 'In vitro Anticancer Drug Discovery Screen' that uses the protein-binding dye Sulforhodamine B (SRB) to assess cell growth.[24, 25]

All the newly synthesized compounds **10a-10j** are evaluated for their anti-proliferative activities against human lung cancer cell line (A-549) and human prostate cell line (DU-145). The results are summarized in Table 1. These values represent the concentration required to inhibit 50 % cell population compared with the control cells treated with DMSO and positive control Doxorubicin under similar conditions.

For lung cancer cell line (A549) the compounds **10a, 10d, 10g, 10h, 10i,** and **10j** are the most active with IC_{50} value in the range of 1.52 µM and 2.86 µM.

Table 1. In vitro anticancer screening of the synthesized compounds against cell lines (IC_{50}, µM ± SD, *n* = 3).

Sr. No.	A-549[a]	DU-145[b]
10a	2.86±0.12	3.17±0.16
10b	3.88±0.13	4.12±0.08
10c	8.17±0.21	9.06±0.04
10d	2.12±0.11	4.17±0.22
10e	3.16±0.08	2.16±0.06
10f	12.16±0.11	17.18±0.08
10g	1.52±0.24	1.68±0.12
10h	1.74±0.18	1.81±0.08
10i	1.98±0.08	1.97±0.12
10j	2.12±0.22	2.58±0.08
10k	3.18±0.16	4.12±0.16
10l	12.17±0.11	11.13±0.11
Doxorubicin	1.71±0.18	1.82±0.06

[a]A-549: Human lung cancer cell line; [b] DU-145: Human prostate cancer cell line; IC_{50}- The concentration required to inhibit 50% of cell population.

The remaining compounds are moderately to less active with IC_{50} values in the range of 3.18 to 12.17 µM. For Du-145 cell line the compounds **10e, 10g, 10h, 10i** and **10j** are the most active with IC_{50} value in the range of 1.68 to 2.58 µM. The remaining compounds are moderate to less active with IC_{50} value is in the range of 3.17 to 17.18 µM.

Compounds **10g** is the most active for A-549 and Du-145 cell line, **10g** is 1.125 and 1.083 times more effective than the Doxyrubicin standard. The compounds having strongly electron donating groups like t-butyl (**10f)** or strongly electron withdrawing group (**10l)** seem to be very less active.

The SAR can be drawn like compound having methyl, ethyl, propyl etc. substituents are moderate to less active as they all are having electron donating tendency. For the remaining groups as the electron donating groups are present along with some electron deficient nitrogen atom shows promising activity. The compounds only having electron withdrawing groups are also less active to inactive. The SAR can be drawn like that for better activity electron donating nature of substituent should be there along with some electron deficient atom to enhance the activity.

Table 2. Inhibitory activity of selected compounds against panel of eight human kinases.

Kinase	Inhibition, %		
	10g	**10h**	**10i**
Aurora-A	45	34	61
Aurora-B	28	40	38
CDK2/cyclin A	38	32	47
CDK2/cyclin E	27	36	51
CDK5/P25	26	19	31
EGFR	58	67	64
JNK	64	58	66
ERK1	43	51	49

The compounds **10g, 10h** and **10i** were found to be the most active in cell line studies, so further we have tested them for their activity against a panel of eight human kinase at 10 µM concentration.

The inhibition results are summarized in Table 2. Protein kinase plays a key role in cell proliferation, differentiation of cell, migration of cell, survival of cell and angiogenesis of cells. The compound **10g, 10h** and **10i** show inhibition in the range of 28 % to 61 % for Aurora-A and Aurora-B kinases, but the activity are not the same for both kind of Aurora kinases.

The compounds show inhibitions in the rage of 19 to 51 %. for CDK/cyclin A, CDK/cyclin E, CDK5/P25 kinases belong to CMGC kinase family. For MAP kinase family the all compounds show inhibition in the range of 49 to 66%. For EGFR and JNK kinases all three compounds shows inhibitions >58 %.

CONCLUSION

We have synthesized N4-(4-fluorophenyl)-N2-substitured-benzo[d]thiazole-2,4-dicarboxamide derivatives (**10a-10j**) from aniline through a series of reactions including benz-thiazol synthesis, chlorination, hydrolysis, cynation and amide coupling. We have reported simple reaction condition, easy workup, short reaction time and good to high yields. The synthesized compounds were screened for anticancer activity against A-549 and Du-145 cancer cell lines. Most of the compounds were active for tested cell lines with IC_{50} value in the range of 1.52 to 17.18 µM. The compounds **10g, 10h** and **10i** are most active with IC_{50} values in the range of 1.52-1.98 µM. The compounds **10g, 10h** and **10i** were shows promising inhibitions (> 58%) for EGFR and JNK human kinases. The compounds having electron donating substituents along with electron deficient atom shows promising activity and greater inhibitions for protein kinases.

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REFERENCES

[1]Felix, Y. F., Kothari, V., Driven to metastasize: Kinases as potential therapeutic targets in prostate cancer, *Proc. Natl. Acad. Sci*., **2016**, *113(3)*, 473-475. https://doi.org/10.1073/pnas.1522938113

[2]Abraham, R. T., Identification of TOR signaling complexes: more TORC for the cell growth engine, *Cell*, **2002**, *111*, 9-12. https://doi.org/10.1016/S0092-8674(02)01009-7

[3]Antonyak, M. A., Moscatello, D. K., Wong, A. J., Constitutive activation of c-Jun N-terminal kinase by a mutant epidermal growth factor receptor, *J. Biol. Chem*., **1998**, *273*, 2817-2822. https://doi.org/10.1074/jbc.273.5.2817

[4]Palmer, F. J., Trigg, R. B., Warrington, J. V., Benzothiazolines as antituberculous agents, *J. Med. Chem*., **1971**, *14*, 248-251. https://doi.org/10.1021/jm00285a022

[5]Liu, C., Lin, J., Pitt, S., Zhang, R. F., Leftheris, K., Benzothiazole based inhibitors of p38α MAP kinase, *Bioorg. Med. Chem. Lett*., **2008**, *18*, 1874-1879. https://doi.org/10.1016/j.bmcl.2008.02.011

[6]Keen, N., Taylor, S., Aurora-kinase inhibitors as anticancer agents, *Nat. Rev. Cancer*, **2004**, *4*, 927-936. https://doi.org/10.1038/nrc1502

[7]Matthews, N., Visintin, C., Hartzoulakis, B., Jarvis, A., Selwood, D. L., Aurora A and B kinases as targets for cancer: will they be selective for tumors?, *Expert Rev. Anticancer Ther*., **2006**, *6*, 109-120. https://doi.org/10.1586/14737140.6.1.109

[8]Carta, F., Birkmann, A., Buschmann, T. P. H., Schwab, W., Zimmermann, H., Maresca, A., Supuran, C. T., Lead Development of Thiazolylsulfonamides with Carbonic Anhydrase Inhibitory Action, *J. Med. Chem*., **2017**, *60*, 3154-3164. https://doi.org/10.1021/acs.jmedchem.7b00183

[9]Ronco, C., Millet, A., Plaisant, M., Abbe, P., Tekaya, N. H., Rocchi, S., Benhida, R., Structure activity relationship and optimization of N-(3-(2-aminothiazol-4-yl)aryl)benzenesulfonamides as anti-cancer compounds against sensitive and resistant cells, *Bioorg. Med. Che. Lett*., **2017**, *27*, 2192-2196. https://doi.org/10.1016/j.bmcl.2017.03.054

[10]Pawar, C. D., Pansare, D. N., Shinde, D. B., (Substituted)-benzo[b]thiophene-4-carboxamide synthesis and anti-proliferative activity study, *Lett. Drug. Des. Disc*., **2018**, https://doi.org/10.2174/1570180815666181004114125

[11]Pawar, C. D., Pansare, D. N., Shinde, D. B., Synthesis of new 3-(substituted phenyl)-N-(2-hydroxy-2-(substituted phenyl)ethyl)-N-methylthiophene-2-sulfonamide derivatives as antiproliferative agents, *Eur. J. Chem*., **2018**, *9(1)*, 13-21. https://doi.org/10.5155/eurjchem.9.1.13-21.1669

[12]Pawar, C. D., Chavan, S. L., Pawar, U. D., Pansare, D. N., Deshmukh, S. V., Shinde, D. B., Synthesis, anti-proliferative activity, SAR and Kinase inhibition studies of thiazol-2-yl-substituted sulfonamide derivatives, *J. Chin. Chem. Soc*., **2018**, https://doi.org/10.1002/jccs.201800312

[13]Zhuang, C., Guan, X., Ma, H., Cong, H., Zhang, W., Miao, Z., Small molecule-drug conjugates: A novel strategy for cancer-targeted treatment, *Eur. J. Med. Chem*., **2018**, *163*, 883-895. https://doi.org/10.1016/j.ejmech.2018.12.035

[14]El-Damasy, A. L., Lee, J., Seo, S. H., Cho, N., Keum, G., Design and synthesis of new potent anticancer benzothiazole amides and ureas featuring pyridylamide moiety and possessing dual B-RafV600E and C-Raf kinase inhibitory activities, *Eur. J. Med. Chem*., **2016**, *115*, 201-216. https://doi.org/10.1016/j.ejmech.2016.02.039

[15]Yuan, Z., Chen, S., Chen, C., Chen, J., Jiang, Y., Design, synthesis and biological evaluation of 4-amidobenzimidazole acridine derivatives as dual PARP and Topo inhibitors for cancer therapy, *Eur. J. Med. Chem*., **2017**, *138*, 1135-1146. https://doi.org/10.1016/j.ejmech.2017.07.050

[16]Satish, G., Reddy, K. H. V., Ramesh, K., Shankar, J., Nageswar, Y. V. D., An environmentally benign and efficient synthesis of 2-thio-substituted benzothiazoles, *Eur. J. Chem*., **2014**, 5, 291-295. https://doi.org/10.5155/eurjchem.5.2.291-295.861

[17]Ali, R., Siddiqui. N., Biological Aspects of Emerging Benzothiazoles: A Short Review, *J Chem.*, **2013**, https://doi.org/10.1155/2013/345198

[18]Pawar, C. D., Sarkate, A. P., Karnik, K. S., Pansare, D. N., Shinde, D. B., Synthesis and antiproliferative evaluation of new (4-substituted-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-yl)methane substituted sulfonamide derivatives, *Eur. J. Chem*., **2017**, *8*, 384-390.

https://doi.org/10.5155/eurjchem.9.1.13-21.1669

[19]Pawar, C. D., Pansare, D. N., Shinde, D. B., Synthesis and antiproliferative activity of 3-(substituted)-4,5,6,7-tetrahydro-6-(substituted)-1H-pyrazolo[3,4-c]pyridine Derivatives, *Eur. J. Chem*., **2017**, *8(4)*, 400-409. https://doi.org/10.5155/eurjchem.8.4.400-409.1645

[20]Pawar, C. D., Sarkate, A. P., Karnik, K. S., Shinde, D. B., Palladium catalyzed tricyclohexylphosphine ligand associated synthesis of N-(2-(pyridine-4-yl)-1H-pyrrolo[3,2-*c*]pyridin-6-yl-(substituted)-sulfonamide derivatives as

antiproliferative agents, *J. Hetero Chem.*, **2018**, *55(7)*, 1695-1701. https://doi.org/10.1002/jhet.3206

[21]Hoechst, A. G., Process for the preparation of 2-mercaptobenzothiazoles, **1984**, US4431813A

[22]Roger, S., O'Connor, S. J., Colsh, P., Derek, L., Clark, R. B., Stebbins, J., Campbell, A., Akuche, C., Shelekhin, T., Preparation and use of Biphenyl-4-yl-carbonylamino acid derivatives for the treatment of obesity, **2006**, WO 2006/044775.

[23]Pawar, C. D., Sarkate, A. P., Karnik, K. S., Shinde, D. B., Synthesis and evaluation of N-(Substituted phenyl)-2-(3-substituted) sulfamoyl) phenyl) acetamide derivatives as anticancer Agents, *Egyp. J. Basic and Applied Sci.*, **2017**, *4*, 310-314. https://doi.org/10.1016/j.ejbas.2017.09.001

[24]Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Jonathan, T. W., Heidi, B., Susan, K., Michael, R. B., New colorimetric cytotoxicity assay for anticancer-drug screening, *J. Natl. Cancer Inst.*, **1990**, *82*, 1107-1112. https://doi.org/10.1093/jnci/82.13.1107

[25]Vichai, V., Kirtikara, K., Sulforhodamine B colorimetric assay for cytotoxicity screening, *Nat. Protoc.*, **2006**, *1*, 1112-1116. https://doi.org/10.1038/nprot.2006.179

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X-RAY PHASE ANALYSIS OF IMPURITIES IN SILICON CRYSTALS MADE FROM METALLURGICAL SILICON WITH DIRECTIONAL CRYSTALLIZATION

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Keywords: Silicon; multi-element analysis; concentration of impurities; detection limits for impurities

The control of impurities at each stage of the purification process of Si is the primary topical problem in Si technology. The technique of determining the impurity composition should be multi-element and with low limit-detection of impurities. From this point of view, X-ray analysis of the phase composition of Si, based on the identification of X-ray diffraction lines is very attractive. Obtaining of Si directly from MG-Si is essential to reveal the physical possibilities of the directional crystallization. In this work, there are considered the options on a set of detectable impurities and the limits of their detection in such type of Si by X-ray diffraction method. It has been shown, that applicability of X-ray analysis of the phase composition of Si, based on the identification of X-ray diffraction lines, depends on the stage of Si purification.

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Introduction

Silicon is the main component of the modern efficient semiconductor devices including micro-, opto-, nanoelectronics. Silicon has many advantageous properties like the high photo and low temperature sensitivity, minimal reflection losses, therefore the 85 % of solar batteries are made from silicon. Nowadays and in the nearest future, silicon is an irreplaceable material and holds leading position among the semiconductor materials and optoelectronics.

The silicon mainly used in its crystalline or amorphous/crystalline thin film (epitaxial layers) forms on various substrates. The impurity composition of silicon request depends on the using field of silicon. Depending on the primary purpose of Si application there are three distinct Si product form: electronic quality Si, “solar” Si and metallurgical (technical) Si. Type of silicon must be supplied with exceptionally specified impurities to provide the defined expected parameters of Si.

Following and determination of these impurities are an essential step of the silicon production technology. Comparing the methods of silicon purity analysis, the number of simultaneously detectable impurities, the limit of their detection, the availability of the necessary equipment, the duration of the investigation and its cost have to take into consideration.[1-5]

We have developed a method which can be used for analysis of all kind of silicon products including metallurgical, "solar", integrated circuits and other silicons, etc., and in the most cases, which is multi-element and has low limit-detection of impurities. This method is based on [the] X-ray phase analysis of Si, based on the identification of X-ray diffraction lines. The goal of the present article is the investigation of the possibility to determine the composition of detrimental impurities in Si crystals, obtained by pulling directly from metallurgical grade Si (MG-Si) melt.

Experimental

In the experiments, metallurgical Si (*n*-MG-Si) with ~98.3 wt.% Si content and detrimental impurities of Fe, Al, P, Ca, Cu, Mg, Mn, Ni, and Ti was used. The Czochralski growth method of pulling crystals has been used for obtaining Si directly from MG-Si melt. Crystals have been grown from quartz crucible as described in our previous work.[6]

Microstructure has been examined under Neophont optical microscope. The specimens were chemically polished in a mixture of HNO_3 + HF (1:1) acids, washed with distilled water, and etched for 1–5 min in an alkaline solution of 30 % KOH at 50–100 °C.

Electrical properties (Hall effect and conductivity) measurements have been implemented by a standard dc bridge technique.

The content of contaminating impurities in *n*-MG-Si before and after the directional crystallization has been defined by X-ray diffraction method, micro X-ray spectral and emissive spectral analyses. X-ray investigations have been implemented on modernized X-ray diffractometer DRON–4–07 with software applying molybdenum radiation at the continuous regime of filming by 0.5 degrees min^{-1}.

Results and discussion

Initial experimental material of metallurgical silicon with 98.30 wt.% Si and undesirable impurities up to ~2 wt.% is the first step product of Si obtained by restoration from quartzite with reaction to carbon. MG-Si was a multiphase substance with n=1.2·10^{18} cm^{-3}. The appropriate microstructure and X-ray diffractogram of MG-Si are shown in Figs. 1 and 2, respectively.

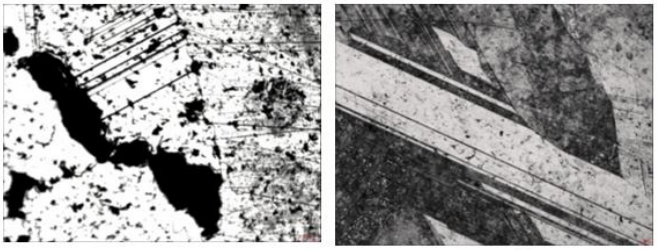

Figure 1. Microstructure (×100) of Si experimental samples of different purity. (a) *n*-MG-Si and (b) pulled with a rate of 0.3 mm min^{-1}.

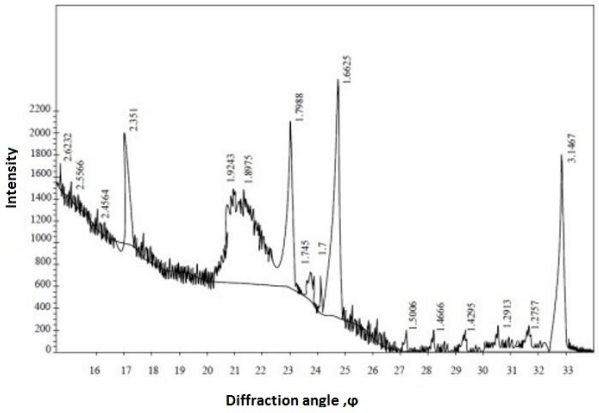

Figure 2. The x-ray diffraction pattern of *n*-MG-Si.

Fig.1a shows traces of inclusions of eutectic phases formed from impurities Mg, Mn, Cu, Fe, Ni, Ti Al, Ca and the silicon in MG-Si. The solubility of impurities in Si is much less in solid phase than in liquid state,[7] and their concentration exceeded the limit of their solubility in the silicon. As a result, impurities could not form solid solutions with silicon and precipitated as inclusions in the silicon matrix. Since phase composition cannot be judged from the chemical analysis data, X-ray structural phase analysis makes it possible to obtain the necessary information. The complex picture of the multiphase state of *n*-MG-Si is reflected in the X-ray diffractogram (Fig. 2).

The x-ray diffraction pattern of an experimental sample of MG-Si shown in Fig. 2 is typical for multiphase MG-Si having a significant number of impurities. Fig. 2 identifies each phase of multicomponent of MG-Si and makes it possible to determine their percentage.

After pulling of the crystal of MG-Si from the melt at ~0.30 mm per minute rate the experimental sample of *n*-MG-Si (n=1.2·10^{18}cm^{-3}) goes into *p*-type Si with 99.99 wt.% Si content, current carriers concentration was found to be 2·10^{16} cm^{-3} and mobility of them was 510 cm^2 V^{-1}·s^{-1}. It means that MG-Si has been purified from the majority of impurities by 1.5–3 orders up to 99.99 wt.% Si with impurity content of ~10^{-2} wt.%.This result could be confirmed by microstructure (Fig. 1b) and X-ray phase analyses (Fig. 3). Fig. 1b shows that after pulling of MG-Si from melt the tracks of inclusions of different phases, observed in the initial microstructure of MG-Si have disappeared and matrix become single-phase with dense structure.

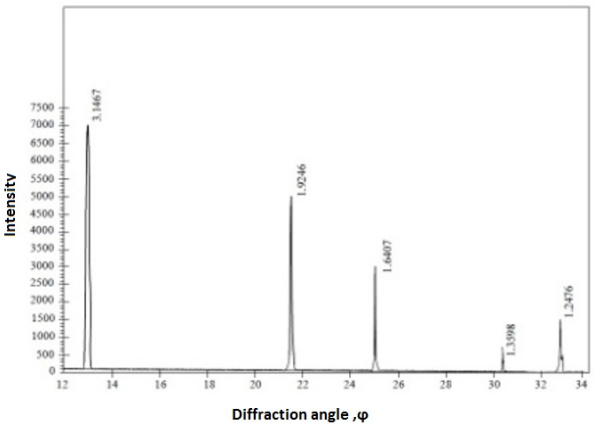

Figure 3. The x-ray diffraction pattern of purified pulled Si from MG-Si melt.

It is known, that a crystalline phase of the substance has the characteristic diffraction pattern that distinguishes it from other substances. Therefore, identification of the X-ray lines in the diffractogram (Fig. 3) has been conducted by the comparison of the diffractogram of experimental Si under study with the diffractogram of known Si standards (Fig. 4).[8] The interplanar distances *d* for all lines for different (*hkl*) has been calculated by the fundamental law of high-resolution X-ray spectroscopy (Wulff–Bragg‘s equation). The data of the interplanar distances and the intensity of the main lines on the X-ray diffractogram of Si pulled from the melt of *n*-MG-Si are placed in Table 1 in descending order.

Table 1. Interplanar distances and main lines intensity (in brackets) on X-ray diffractogram of Si pulled from a melt of *n*-MG-Si.

Si[8]	Si pulled from the melt			
	1	2	3	4
3.138 (100)	3.1467 (100)	3.1467 (100)	3.1486 (100)	3.1486 (100)
1.920 (60)	1.9246 (60)	1.9253 (60)	1.9260 (60)	1.9253 (60)
1.630 (35)	1.6407 (35)	1.6413 (35)	1.6413 (35)	1.6413 (35)
1.357 (8)	1.3598 (8)	1.3605 (8)	1.3609 (8)	1.3609 (8)
1.246 (13)	1.2476 (13)	1.2482 (13)	1.2482 (13)	–

The intensities of all the diffraction lines measured are expressed as a percentage of the strongest line to which the value of 100 % is attributed too. The intensity of diffraction

maxima depends on the chemical composition of the Si crystal.

The coincidence (within the measurement error) of the experimental and tabulated values of Reference Book of the interplanar distances and relative line intensities make it possible to identify the phases present in the material uniquely.

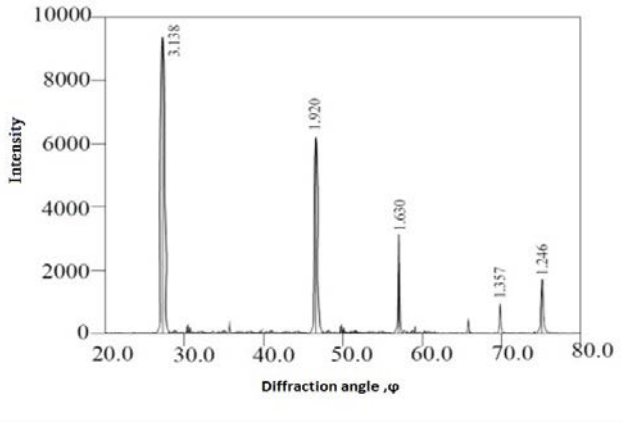

Figure 4. X-ray diffraction patterns of standard pure Si.

All basic diffraction maxima for pure standard Si (Fig. 4) are revealed in the X-ray diffraction pattern of purified Si (Fig.3).[8]

As can be seen from Fig. 3 and Fig. 4, pulled Si is single-phase, and the residual impurities in quantity of 10^{-2} wt.% cannot be fixed by X-ray diffraction analysis. Residual impurities are reflected in electrical properties (n=1.2·10^{16} cm^{-3}) and are detected by emission spectral analysis. This indicates, that the X-ray diffraction analysis of silicon purity is limited by 99.99 wt.% Si and does not have sufficient sensitivity to detect impurities in an amount of $<10^{-2}$ wt.%, thus X-ray diffraction method is suitable for analyses of Si purity only up to 99.99 wt.% Si level. For increasing the sensitivity of X-ray analysis, it is necessary to combine it with other methods.

Conclusion

In this paper, we considered the possibilities on a set of detectable impurities and the limits of their detection by X-ray diffraction method. The presented way is a multi-element analysis and one of the effective methods for simultaneous determination of the impurity composition of silicon. The limits of detection of the amount of detectable impurities are shown.

The application of X-ray diffraction method to Si depends on the stage of its purification. The content of contaminating impurities before and after purification has been established by X-ray spectral microanalyzer and emissive spectral analysis too.

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References

[1]Maximov, G. A., Pimenov, V. G., Timonin, D. A., Chemical-atomic absorption analysis of high-purity silicon with the detection limit of impurities on the level of 10–9–10–11%, *Vysokochist. Veshchestva*, **1993**(3), 127.

[2]Marcus, M. A., MacDowell, A. A., Celestre, R., Domning, E., Franck, K., Manceau, A., Morrison, G., Miller, T., Padmore, H. A., Sublett, R. E., Beamline 10.3.2 at ALS: a hard X-ray microprobe for environmental and materials sciences, *J. Synch. Rad.*, **2004**, 11, 239. https://doi.org/10.1107/S0909049504005837

[3]Cai, Z., Lai, B., Yun, W., McNulty, I., Khounsary, A., Maser, J., Ilinski, P., Legnini, D., Trakhtenberg, E., Xu, S., Tieman, B., Wiemerslage, G., Gluskin, E., Performance of a high-resolution x-ray microprobe at the Advanced Photon Source, *AIP Conf. Proc.*, **2000**, *521*, 31. https://doi.org/10.1063/1.1291754

[4]Fenning, D. P., Hofstetter, J., Bertoni, M. I., Hudelson, S., Rinio M., Lelièvre, J. F., Lai, B., del Canizo, C., Buonassisi, T., Iron distribution in silicon after solar cell processing: Synchrotron analysis and predictive modeling, *Appl. Phys. Lett*., **2011**, 98, 162103. https://doi.org/10.1063/1.3575583

[5]Huber A., Bohm G., Pahlke S., Industrial applications of instrumental neutron activation analysis, *J. Radioanal. Nucl. Chem.*, **1993**. 169, 93. https://doi.org/10.1007/BF02046787

[6]Kekua, M. G., Khutsishvili, E. V., *Germanium–Silicon Semiconductor Solid Solutions*, Tbilisi, Metsniereba, **1985**.

[7]Trumbore, F. A, Solid Solubilities of Impurity Elements in Germanium and Silicon, *Bell Syst. Tech. J.*, **1960**, 39, 205. https://doi.org/10.1002/j.1538-7305.1960.tb03928.x

[8]Ed. Umansky, Y. S., *Handbook of X-ray Diffraction Analysis of Polycrystals*, Moscow, Phys. Math. Lit. Press, **1961**.

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SYNTHESIS OF LINEAR OLEFINS FROM FATTY ACIDS: A COMPARISON OF NICKEL(I) AND RHODIUM(I) CATALYSTS IN LIQUID TRIPHENYLPHOSPHINE

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Keywords: Lauric acid; Fatty acids; $Ni(Ph_3P)_2Cl_2$; $Rh(Ph_3P)_3Cl$; Dehydration-decarbonylation; Linear olefins (LO); Linear α-olefins (LAO)

Linear α-olefins (LAO) with good selectivity toward linear olefins (LO) can be synthesized from widely available fatty acids (lauric, decanoic, miristic, palmitic and stearic acids) in high yield using a Ni(II) complex: $Ni(Ph_3P)_2Cl_2$ in liquid triphenylphosphine (TPP). It is shown spectrophotometrically, in liquid triarylphosphine that $Ni(Ph_3P)_2Cl_2$ assumes an octahedral coordination geometry which is believed to be the active species in the dehydration-decarbonylation reaction of fatty acids. For comparison also the Wilkinson's catalyst, i.e., the Rh(I) complex $Rh(Ph_3P)_3Cl$ was also studied as a fatty acid decarbonylation catalyst in liquid TPP. The Wilkinson's catalyst gives an extremely smooth >95% selectivity toward LAO. Thus, when high LAO selectivity is requested, $Rh(Ph_3P)_3Cl$ is the catalyst of choice. On the other hand, when economic LO/LAO mixtures are requested in high yields, for instance as in the case of hydroformylation feedstocks, then $Ni(Ph_3P)_2Cl_2$ is the recommended catalyst. The LO/LAO products were determined with GC-MS and the LAO selectivity was also determined with Raman spectroscopy.

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Introduction

Linear olefins (LO) are produced in multimillion ton per year worldwide. More in detail, linear α-olefins (LAO) which are the major products among the linear olefins and have reached a production of 5 million metric ton/year with sustained yearly growth.[1] LO and LAO are derived from petroleum mainly with three different processes. The most common process for LO/LAO production is based on ethylene oligomerization over Ziegler catalyst, although also alternative catalysts based on Ni-complexes are used as well.[2]

Alternative industrial processes for LO/LAO synthesis are based on paraffin thermal cracking, paraffin catalytic dehydrogenation as well as monochlorination and dechlorination of paraffins,[2] although the latter processes starting from paraffins are somewhat obsolete with respect to the ethylene oligomerization. The LO/LAO find many different applications ranging from co-monomers in commodity polymers i.e. different polyethylene LLDPE grades, to specialty polymers like the polyolefin elastomers or "plastomers", to polyalpha olefins (PAOs) oligomers suitable as lubricant oil and lubricant oil additives, to raw materials for surfactants and alkylation reactions and in the synthesis of other niche specialties.[1] In the surfactant application, the LO/LAO are hydroformylated and then hydrogenated to fatty alcohols. In the hydroformylation reaction, both LAO and LO with internal double bond are suitable raw materials since the hydroformylation catalyst causes the isomerization of LO to LAO.[2] Fatty alcohols are then sulfated to produce surfactants like sodium lauryl sulfate. LAO is also directly sulfonated or used to alkylate benzene and then produce alkylbenzene sulfonates surfactants.[2]

Fatty acids are widely available from a number of natural and renewable sources and the current world production exceeds the 20 million metric ton/year.[3,4] There is a growing interest in producing raw materials from renewable sources, to moderate the environmental impact of the excessive use of fossil sources of raw materials.[5-13] Recently, it has been demonstrated that fatty acids could be a valuable source of LO and LAO through a dehydration-decarbonylation reaction as comprehensively reviewed by Behr and Vorholt.[14] In other words, fatty acids can be converted in LO/LAO in a single reaction step and in high yields. In a steadily growing demand of LO/LAO there is certainly field to introduce a process based on renewable raw materials and in particular based on fatty acids dehydration-decarbonylation. The latter process is defined dehydration-decarbonylation because the fatty acid undergoes the release of water and carbon monoxide as two distinct products:[14]

$$R\text{-}CH_2\text{-}CH_2\text{-}COOH \rightarrow R\text{-}CH{=}CH_2 + CO + H_2O \quad (1)$$

rather than the CO_2 release in the true decarboxylation process, which could be schematically represented as:[8,15]

$$R\text{-}CH_2\text{-}CH_2\text{-}COOH \rightarrow R\text{-}CH_2\text{-}CH_3 + CO_2 \quad (2)$$

Initially, reaction (1) was achieved on fatty acid with the aid of an anhydride additive (like acetic anhydride) and Pd- or Rh-based complexes with good LAO selectivity:[14,16]

$$R\text{-}CH_2\text{-}CH_2\text{-}COOH + (CH_3CO)_2O \rightarrow R\text{-}CH{=}CH_2 + CO + 2CH_3COOH \quad (3)$$

More recently, it was shown that (3) can be performed effectively with metallic Pt or Pd on C preserving the high selectivity toward LAO.[17,18] The mechanism of such dehydration-decarbonylation reactions is discussed in detail by Eliasson et al.[19] The research on LAO formation from fatty acids involved also the adoption of pre-formed Pd-complexes which reduce the amount of triphenylphosphine (TPP) used as reaction solvent,[20] and a study on the effect of different phosphine ligand structures on the selectivity toward LAO or the conversion of fatty acids to LO/LAO with Pd complexes.[21-23] The research also moved toward the study of more economical and wide available catalysts. For example, a simple iron-based catalyst was found effective in LO/LAO production from fatty acids, but it was found effective only under CO overpressure.[24]

On the other hand, metallic nickel on carbon was found an effective decarboxylation catalyst for the production of fuels from fatty acids.[25] Remarkable results were obtained in LO/LAO synthesis from fatty acids using simple Ni(II) salts in TPP,[23] or in other phosphine ligands,[21] without the need to use anhydrides and without the need of CO or H_2 overpressure. Particularly interesting was also the demonstration of the regeneration of the TPP ligand, which acts as metal catalyst reducing agent and in the course of the dehydration-decarbonylation of fatty acids is converted into triphenylphosphine oxide (TPPO).[23]

As pointed out by Behr and Vorholt,[14] the anhydride formation is a crucial step in the dehydration-decarbonylation process of fatty acids. If the reaction is conducted at moderate temperatures, a light anhydride (like acetic anhydride) should be added and the selectivity in LAO is above 96%. On the other hand, operating at higher temperatures, acetic anhydride (as a promoter) is no more needed because the fatty acids anhydride formation is induced thermally. However, in such conditions, the selectivity to LAO is expected to drop considerably. Once the anhydride is formed there is the interaction with the metal catalyst, with oxidative addition of the metal, the decarbonylation step and the alkene formation and release.[19]

In literature the complex bis(triphenylphosphine)nickel(II) dichloride (i.e.$(Ph_3P)_2NiCl_2$) was reported as an effective decarbonylation reagent of certain cycloaliphatic anhydrides.[26] The present research is dedicated to the study of the mentioned Ni(II) complex as decarbonylation reagent of fatty acids at high temperatures, when the fatty acids anhydride formation is thermally-induced. For comparison, in similar reaction conditions, the mentioned Ni(II) complex was substituted with the Wilkinson catalyst (i.e. $(Ph_3P)_3RhCl$, a well-known catalyst for carbonylation and decarbonylation reactions. The dehydration-decarbonylation products of fatty acids were studied by GC-MS and Raman spectroscopy.

Experimental

Materials

All fatty acids, namely decanoic acid (≥ 98 % purity), myristic acid (≥ 98 % purity), palmitic acid (> 99 % pure) were purchased from Aldrich-Merck (St.Louis, MO), with the exception of lauric acid (> 99 % pure) which was obtained directly from Merck (Darmstadt, Germany) and stearic acid which was a commercial technical grade, being a mixture of saturated fatty acids from hydrogenated vegetable oils or from animal fats. The catalysts and ligands consisting in bis(triphenylphosphine)nickel(II) dichloride and tris(triphenylphosphine)rhodium(I) chloride as well as nickel dichloride, triphenylphosphine and diphenyl(p-tolyl)phosphine were purchased from Aldrich-Merck.

Methods

The electronic absorption spectra were recorded in a Shimadzu UV 2450 spectrophotometer with a temperature controlled cell holder TCC-240A. The complex $(Ph_3P)_2NiCl_2$ was dissolved in liquid diphenyl(p-tolyl)phosphine and the spectra were collected at 70 °C. For comparison also $NiCl_2$ was dissolved in diphenyl(p-tolyl)phosphine and the resulting spectra were recorded at 70 °C.

The analysis of the LO/LAO products was performed on a GC-MS (gas-chromatograph coupled with a mass spectrometer). The GC was model 7890A from Agilent Technologies equipped with 30 m capillary column type HP5-MS model 19091S-433. The MS was from Agilent Technologies model 5975C VL-MSD with triple axis detector. Typical injection volumes were about 5 µL. The inlet was kept at 300 °C and a ramp from 40°C to 300°C was employed in 3 min, then an isotherm at 300 °C for 60 min. The setpoint quad of the MS was kept at 150 °C while the setpoint of the source at 230 °C. The LO/LAO synthesized from carboxylic acids were diluted with n-hexane before injection in the GC-MS. Dilutions 1:10, 1:100 and 1:1500 were employed for each sample studied. The Raman spectra were recorded with a BWTEK spectrometer model BWS415i using its laser source at 785 nm. The spectra were acquired on liquid LO/LAO samples directly through Pyrex vials.

Synthesis of LO/LAO from fatty acids using $Ni(Ph_3P)_2Cl_2$

The set up employed for the olefin synthesis from fatty acids consists of an ordinary distillation apparatus connected with a vacuum pump. The reaction mixture was typically loaded in 50 mL round-bottomed reaction flask attached to the distillation apparatus and submerged in a sand bath kept in a Duran glass pot over an electrical heating plate. The reaction flask was typically loaded by 25 mmol of the fatty acid, 25 mmol of triphenylphosphine (TPP) and 0.84 mmol of $Ni(Ph_3P)_2Cl_2$ complex. It was heated to 330 °C (temperature measured with a thermocouple inside the sand bath) applying a vacuum initially kept at 90-150 Torr but brought gradually to 40-50 Torr during the LO/LAO distillation. The LO/LAO distillation resulted in quite a slow process, it was completed in a couple of hours.

Other variants in the molar ratio between the three components of the reaction mixture are summarized in Table 1 when lauric acid was employed as fatty acid in the dehydration-decarbonylation reaction. In Table 2 are reported the results relative to the dehydration-decarbonylation reaction for a series of selected fatty acid other than lauric acid.

Table 1. LO/LAO formation from lauric acid $Ni(Ph_3P)_2Cl_2$ vs. $Rh(Ph_3P)_3Cl$

(*)Batch #	Lauric acid	$Ni(Ph_3P)_2Cl_2$	TPP	Products yield, mol %	GC-MS			Raman	
					undecenes (LO+LAO)	undecane	1-undecene selectivity %	1-undecene selectivity %	trans/cis internal LO
1a	100	3.1	0	0					
1b	100	3.1	50	77	94.8	5.2	53.5	65.2	2.3
2a	100	7.6	40	85	97.2	2.8	34.8	46.9	2.6
2b	100	7.6	40	0					
2c	100	7.6	100	45				76.2	1.0
2d	100	7.6	100	22				64.0	1.2
3a	100	3.3	100	97				78.6	1.5
3b	100	3.3	100	26	95.6	4.4	43.1	44.5	1.6
(*) Batch #	Lauric acid	$Rh(Ph_3P)_3Cl$	TPP	Product yield % mol	undecenes (LO+LAO)	Undecane	1-undecene selectivity %	1-undecene selectivity %	Trans/cis internal LO
4a	100	0.9	0	0					
4b	100	0.9	50	50	99.5	traces	>95	>95	n.a.
4c	100	1.3	100	71	99.5	traces	>95	>95	n.a.
Ref. [23]	Nonanoic acid	$NiCl_2$	TPP	Product yield, mol %	Alkenes (LO+LAO)	Alkanes	LAO selectivity %	LAO selectivity %	Trans/cis internal LO
(**)	100	10	100	86	>95%	n.d.	14	n.d.	n.d.

(*) Results of the present work. The reagents are reported in molar ratio making 100 lauric acid; reaction conditions 320-340°C in sand bath, vacuum applied 40-90 Torr. Product yield is calculated on the moles of the starting fatty acid. Amount of LO, LAO and alkane determined by GC-MS. LAO selectivity and trans/cis ratio determined by Raman spectroscopy.

Synthesis of LO/LAO from fatty acids using $Rh(Ph_3P)_3Cl$ (Wilkinson catalyst)

The same reaction set-up and reaction conditions described in the previous section was also employed in the lauric acid dehydration-decarbonylation reaction using the Wilkinson catalyst, i.e., $Rh(Ph_3P)_3Cl$ in place of the Ni-complex. The results are summarized at the bottom of Table 1. The Rh(I) complex was used at about 1/3 the concentration of the Ni-complex.

Results and discussion

The work of John, Hillmayer and Tolman,[23] on the dehydration-decarbonylation reactions of fatty acids was impressive, since excellent results were achieved without the need to use acetic anhydride as a promoter of fatty acid anhydride formation and without the need of any other additive than simple nickel (II) salts. However, it was reported that the Ni-complex $(Ph_3P)_2NiCl_2$ is an active dehydration-decarbonylation catalyst on carboxylic acid anhydrides.[26] Since $(Ph_3P)_2NiCl_2$ is commercially available and not expensive, this prompted us to study its activity in the dehydration-decarbonylation reaction of fatty acids aiming to better yields and better selectivity than simple NiI_2 and $NiCl_2$ catalysts reported previously.

Spectrophotometric study of $(Ph_3P)_2NiCl_2$ in liquid diphenyl(p-tolyl)phosphine

Among the first-row transition metals, Ni(II) is unique in the behavior of its complexes which interconvert into different stereoisomers rather easily.[27] For instance, $(Ph_3P)_2NiCl_2$ has a tetrahedral and paramagnetic structure, but its crystallization from chlorinated solvents convert the complex into the square planar diamagnetic stereoisomer.[27-29] Furthermore, also in solution the equilibrium between the tetrahedral and the square planar structure has been detected for several Ni(II) complexes.[27-29]

Fig. 1 shows the electronic absorption spectrum of $(Ph_3P)_2NiCl_2$ dissolved in diphenyl(p-tolyl)phosphine (DPTP), a ligand and solvent analogous to TPP but having a lower melting point than TPP (67°C vs. 80°C respectively). Thus, DPTP was used as a solvent at 70°C in the temperature controlled cell holder. The spectrum of $(Ph_3P)_2NiCl_2$ in liquid DPTP is characterized by three electronic transitions. The transition next to the near infrared occurs at 906 nm and has approximately the same intensity of the transition in the visible at 540 nm. The transition next to the UV-A occurs at about 400 nm and its peak is too strong to be observed in the spectrum of Fig. 1, but it was observed at lower $(Ph_3P)_2NiCl_2$ concentration in DPTP.

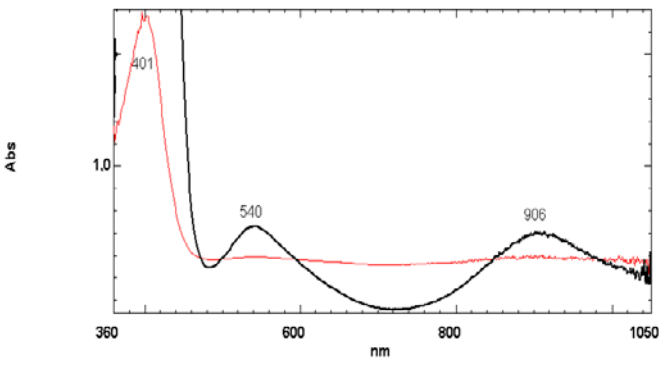

Figure 1. Electronic absorption spectra in liquid diphenyl(p-tolyl)phosphine at 70-72 °C: $(Ph_3P)_2NiCl_2$ (black line) and $NiCl_2$ (red line).

Such electronic spectral band pattern is typical of the octahedral structure of the nickel complexes which are paramagnetic.[27-29] Thus, it should be admitted that an equilibrium of the type

$$(Ph_3P)_2NiCl_2+2(o\text{-}MeC_6H_4)Ph_2P \longleftrightarrow ((o\text{-}MeC_6H_4)Ph2P)_2(Ph_3P)_2NiCl_2 \quad (4)$$

occurs in the DPTP medium. The originally tetrahedral complex $(Ph_3P)_2NiCl_2$ assumes an octahedral and paramagnetic structure in a coordinating solvent like TPP or DPTP. Thus, it is believed that the actual catalytically active species in the dehydration-decarbonylation reaction of the fatty acids is this octahedrally coordinated nickel complex.

Fig. 1 also shows the electronic spectrum of $NiCl_2$ dissolved in DTPT at 70 °C. The spectrum is characterized by a unique absorption band at about 400 nm accompanied by a broad and weak feature at about 550 nm. This spectrum suggests that at 70 °C $NiCl_2$ is able to coordinate DPTP, but the electronic transitions indicate essentially a square planar diamagnetic geometry. There is no evidence for an eventual square planar – tetrahedral equilibrium and no evidence at all for the formation of the octahedral coordination structure of Ni(II). It should be assumed that $NiCl_2$ reacts with DPTP and hence also with TPP at higher temperatures than 70°C and longer time to produce the effective active species which is able to catalyze the dehydration-decarbonylation reaction on fatty acids. With these premises, it is expected a better catalytic performance from $(Ph_3P)_2NiCl_2$ in the dehydration-decarbonylation of fatty acids with respect to $NiCl_2$ in liquid TPP.

LO/LAO synthesis from lauric acid and $(Ph_3P)_2NiCl_2$ in TPP

Lauric acid (or dodecanoic acid) which is obtained from coconut oil and palm kernel in multimillion metric ton/year,[3] was the preferred fatty acid to study the dehydration-decarbonylation reaction to LO/LAO in the present study. The results relative to lauric acid are reported in Table 1 while the results on a selection of other fatty acids dehydration-decarbonylation are summarized in Table 2.

Other authors,[23] have already reported that $NiCl_2$ in TPP is able to convert nonanoic acid into products with 89 % yield but low selectivity in LAO products which is reported as limited to 14 % only. These results are also reported at the bottom of Table 1 as a reference, suggesting the low selectivity of $NiCl_2$ toward LAO.

As shown in Table 1, TPP is an essential solvent and ligand in the dehydration-decarbonylation with $(Ph_3P)_2NiCl_2$ complex. Run #1a in Table 1 shows that 3.1 % mol of $(Ph_3P)_2NiCl_2$ in lauric acid alone is not able to produce any dehydration-decarbonylation reaction on lauric acid. However, the addition of 50 % mol TPP over lauric acid (run #1b) provides 77 % mol yield of products. The products were composed of 94.8% of LO/LAO undecenes and the undecane represents only 5.2 %. The selectivity to the LAO 1-undecene was found at 53.5 % by GC-MS and 65.2 % by Raman spectroscopy. A discussion on Raman spectroscopy for the analysis of LO/LOA is covered in detail in the last section of this paper.

Run #2a shows that an increase of $(Ph_3P)_2NiCl_2$ concentration over lauric acid improves the overall products yield from 77.0 % to 85 %, suppresses the formation of the alkane undecane to 2.8 % but reduces the selectivity considerably to LAO down to 34.8 % by GC-MS and 46.9 % by Raman, increasing the yield of LO with internal double bond. In run #2b, the consumed lauric acid in the exhausted run #2a was reconstituted. However, no reaction products were obtained at all, probably because of the oxidation of TPP to triphenylphosphine oxide (TPPO) as already noticed by other authors.[23] Indeed, GC-MS analysis of the white condensate found in the head of the distillation apparatus has revealed the presence to TPPO accompanied by lower amounts of TPP.

Run #2c was made after the addition of a new amount of TPP so that the total TPP already present from run #2a (and transformed to TPPO) and the newly added TPP were in an equimolar amount with the lauric acid. Run #2c is characterized by a relatively low yield in the LO products which was limited to 44.5 % mol while the selectivity to LOA was found at 76.2 % by Raman spectroscopy. A further replacement of the consumed lauric acid, produced the run #2d, where the overall products yield dropped further to only 22 % by mol over lauric acid while the selectivity to LOA remained quite good, 64 % by Raman. The latter permits the determination of the trans/cis isomers ratio for the olefins with an internal double bond. While in batches #1a and #2a the trans olefins amount was more than double the cis olefins amount, in run #2c and #2d the trans and cis olefins were found at an approximately equimolar ratio.

When an equimolar mixture of lauric acid and Ph_3P are melted together, they form a clear and homogeneous liquid phase. Such a mixture seems to have all the characteristics of a deep eutectic solvent.[30] In such a solvent, it is believed that the fatty acid decarbonylation reaction may be facilitated. To test this hypothesis run #3a was designed with the use of an equimolar mixture of lauric acid and TPP while the $(Ph_3P)_2NiCl_2$ concentration was brought back to the levels employed in run #1b. The yield in overall products was exceptionally high, i.e., 97 % mol over the starting lauric acid, while the alkane undecane was only 4.4% of the products and 95.6 % were LO. The LAO selectivity, i.e., 1-undecene content in the products was found at 78.6 %, a good result. Indeed, other authors[23] have reported that certain Ni(0) complexes such as $Ni(Ph_3P)_4$ as well as $(Ph_3P)_2Ni(CO)_2$ are able to increase the selectivity considerably toward LAO in nonanoic acid deoxygenation reporting values of 66-70 % but at the expenses of low yields in the conversion of nonanoic acid i.e. 25-30 %. Instead, with Ni(II) complex $(Ph_3P)_2NiCl_2$ and the conditions of run #3a, the yields in lauric acid conversion were nearly quantitative, and the selectivity to LAO (1-undecene) was quite impressive, reaching 78.6 %.

The following run #3b was started after the reconstitution of the lauric acid added on top to the reaction mixture remained from run #3a. This time, the yield in the overall products stopped at 26 % by mol, probably because the complete oxidation of TPP to TPPO was reached before the complete conversion of all the available lauric acid. The LOA selectivity dropped to 43.1 % as determined by GC-MS and 44.5 % as derived from Raman spectroscopy.

All the data in Table 1 show that the expected better catalytic performances of the complex $(Ph_3P)_2NiCl_2$, are confirmed both in terms of higher conversion of lauric acid into LO/LAO and moreover in terms of much higher selectivity in LAO production than $NiCl_2$. Furthermore, the Raman spectroscopy has assessed that the undecenes with internal double bonds in all the batches 1-3 reported in Table 1 occur most frequently with an excess of trans isomer over the cis isomer. Thermodynamically the trans isomers are more stable than the cis isomers while α-olefins are thermodynamically the least stable.[31] Thus, the dehydration-decarbonylation reaction is supposed to produce first the α-olefins for kinetics reasons, which in their turn isomerize into the thermodynamically more stable cis and trans olefins with the internal double bond. The prevalence of trans olefins (over the cis olefins) may be explained with the thermodynamic argument while the selectivity in LAO has certainly a mechanistic and kinetics basis.

LO/LAO synthesis from lauric acid and $(Ph_3P)_3RhCl$ in TPP

The Wilkinson's catalyst is an Rh(I) complex very effective as homogeneous hydrogenation catalyst of alkene and alkynes in mild conditions and as hydroformylation catalyst for olefins.[32] The chemical structure of $(Ph_3P)_3RhCl$ is square planar with tetrahedral distortion[32] and the complex was reported as an active decarboxylation catalyst.[33] Thus, in this study, the Wilkinson's catalyst was tested in the dehydration-decarbonylation reaction of lauric acid in comparison with the results obtained with $(Ph_3P)_2NiCl_2$ and using similar reaction conditions. The results are summarized at the bottom of Table 1.

Run #4a shows that $(Ph_3P)_3RhCl$ is completely ineffective in the dehydration-decarbonylation reaction of lauric acid in the absence of TPP, exactly as in the case of $(Ph_3P)_2NiCl_2$ in run #1a.

Thus, TPP plays a key role in this reaction irrespective to the nature of the central metal complex involved. Run #4b shows that the addition of 50% mol of TPP over lauric acid makes $(Ph_3P)_3RhCl$ effective in producing undecenes with very high selectivity. Undecane by-product is obtained in trace amounts. Run #5b also shows that $(Ph_3P)_3RhCl$ ensures a very high selectivity toward LAO (1-undecene) formation with >95% content overall undecenes as detected by Raman and confirmed by GC-MS.

In run #4c the $(Ph_3P)_3RhCl$ complex was acting over an equimolar mixture of lauric acid and TPP. A remarkable increase in the undecenes yield was observed with respect to run #4b (from 50 to 71% by mol over lauric acid), while the selectivity toward 1-undecene was preserved above 95%.

In summary, the Wilkinson's catalyst $(Ph_3P)_3RhCl$ presents the merit over $(Ph_3P)_2NiCl_2$ of very high selectivity toward LAO production while the by-product alkane, undecane, is produced only in trace amounts. However, the overall products yield of LO/LAO from lauric acid is lower with the Rh(I) complex in comparison to the overall yields achieved with the Ni(II) complex.

LO/LAO synthesis from a selected fatty acid and $(Ph_3P)_2NiCl_2$ in TPP

The Ni(II) complex $(Ph_3P)_2NiCl_2$ was also studied in the decarbonylation-dehydration reaction of a series of selected fatty acids as reported in Table 2. Using the equimolar ratio between the fatty acid and TPP, the complex $(Ph_3P)_2NiCl_2$ ensures in all cases a high conversion of the fatty acids into the products LO/LAO.

Table 2. LO/LAO formation from fatty acids (other than lauric) and $Ni(Ph_3P)_2Cl_2$ catalyst

(*) Batch #	Fatty acid Type	Molar ratio	$Ni(Ph_3P)_2Cl_2$	TPP	Product yield, mol %	GC-MS			RAMAN	
						Alkenes (LO+LAO)	Alkanes	Selectivity LAO %	Selectivity LAO %	trans/cis internal LO
5	Decanoic	100	3.4	100	71				80.3	2.3
6	Myristic	100	3.4	100	93	93.6	6.4	57.6	47.6	2.9
7	Palmitic	100	3.4	100	90	86.3	13.7	43.2	35.0	3.6
8	Stearic	100	3.9	100	83	91.1	8.9	43.6	34.0	3.8

(*) Results of the present work. The reagents are reported in molar ratio making 100 lauric acid; reaction conditions 320-340 °C in sand bath, vacuum applied 40-90 Torr. Product yield is calculated on the moles of the starting fatty acid. Amount of LO, LAO and alkane determined by GC-MS. LAO selectivity and trans/cis ratio determined by Raman spectroscopy.

Table 3. Distribution of products LO/LAO as determined by Raman and GC-MS

	Run #	Catalyst	% LAO	% LO with internal C=C	% cis-LO	% trans-LO
by GC-MS	3b	$Ni(Ph_3P)_2Cl_2$	45.0	55.0	n.d.	n.d.
by Raman	3b	$Ni(Ph_3P)_2Cl_2$	44.5	55.5	30	60
by GC-MS	4c	$Rh(Ph_3P)_3Cl$	>95	4	n.d.	n.d.
by Raman	4c	$Rh(Ph_3P)_3Cl$	>95	n.d.	n.d.	n.d.

Note: the alkanes are excluded from the calculation, only the olefins are considered

The by-product formation, i.e., alkanes remains significant also with higher fatty acids homologs than lauric acid. In the case of palmitic and stearic acid, the alkane fraction (respectively pentadecane and heptadecane) reached about 10 % or more of the overall products. Regarding the LAO content, the maximum selectivity of 80 % was detected with Raman on 1-nonene produced from decanoic acid and remained quite high also on the higher homologs of fatty acids. For instance, it is remarkable the LAO selectivity of 57.6 % observed on the products from myristic acid. In the case of the olefins obtained from palmitic and stearic acid, the LAO selectivity stopped at about 43 % (GC-MS).

It is quite remarkable that the trans/cis ratio is found higher in the LO with internal double bonds produced from higher fatty acids homologs whereas thermodynamics favor the trans isomer with the higher olefins homologs.

LO/LAO selectivity determination with Raman spectroscopy

In addition to the GC-MS analysis of the products obtained from the dehydration-decarbonylation reaction of fatty acids, also the Raman spectroscopy is a suitable analytical tool which is able to distinguish the amount of LAO over the total LO. Regarding LO with internal double bonds, Raman spectroscopy is able to detect both the amount of cis-LO and trans-LO. Raman spectroscopy is very sensitive to the geometry of the double bonds, and their substitution degree.[34] In the Raman spectra the alkane by-products were not determined. Thus, the focus is only on the LO/LAO olefins.

Fig. 2 shows the Raman spectra of the LO produced from lauric acid and Ni(II) complex (run #3b). Three distinct peaks can be observed at 1640, 1656 and 1670 cm^{-1}. The peak at a lower frequency is due to the LAO 1-undecene, the peak at 1656 cm^{-1} to cis-undecenes with an internal double bond and the peak at 1670 cm^{-1} to trans-undecenes with an internal double bond. From the integrated absorptivity of each of these peaks, it is possible to determine the relative amount of the three mentioned LO isomers.

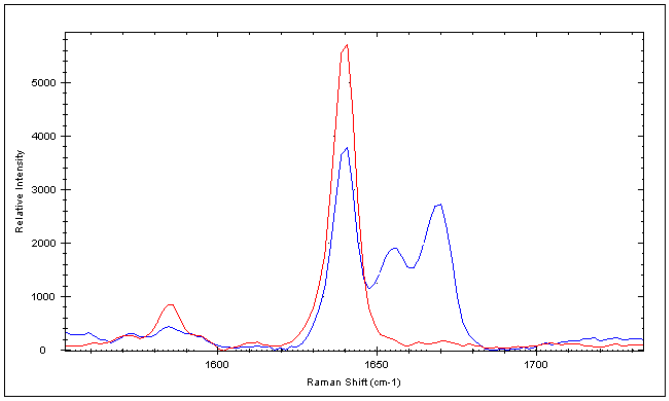

Figure 2. Raman spectra of the undecene mixture obtained from the lauric acid decarbonylation-dehydration reaction. The blue trace is due to the undecenes produced with Ni(II) catalyst from run #3a. The peak at 1640 cm^{-1} is due to 1-undecene, the band at 1656 cm^{-1} to cis-undecenes with an internal double bond and the peak at 1670 cm^{-1} to trans-undecenes with an internal double bond. The red trace is essentially due to 1-undecene produced from Rh(I) catalyst and run #6.

The calculations are summarized in Table 3 and compared with GC-MS results, recalculated by excluding the alkane components present in the mixture of products.

For the LO from lauric acid and Ni(II) complex (run #3b), the LAO content determined by GC-MS was 45.0% in fair agreement with 44.5% determined from the Raman spectrum of Fig. 2. Furthermore, among the LO with an internal double bond which represents more than 50% of the total olefins, the trans isomers are preponderant representing 60% of the LO. However, as shown in Table 1 and 2, the coincidence in the LAO values found with GC-MS and estimated with Raman spectroscopy are seldom precisely the same. However, Raman spectroscopy is a rapid screening tool for the analysis of the LO/LAO while GC-MS remains the most useful analytical tool in this case.

Fig. 2 also shows the Raman spectrum of the LO/LAO obtained from lauric acid and Rh(I) complex (run #4c). In this case, only a single and sharp band can be observed at 1640 cm^{-1} suggesting that the LAO 1-undecene was by far the main product. In fact, from the Raman spectrum, it is possible to determine an LAO content of >95% which is in good agreement with the value of >95% determined by GC-MS. Because of the very low content of LO with an internal double bond, in the case of the products from run #4c, it was not possible to determine the trans/cis ratio as done in the case of the products from batch #3b. All the results are summarized in Table 3.

Conclusions

It was shown that starting from lauric or dodecanoic acid, widely available from coconut and palm kernel oil it is possible to synthesize in a single step and high yields a mixture of linear olefins (LO) i,e, a mixture of undecenes with a good selectivity toward the linear α-olefin (LAO) 1-undecene. The synthesis can be achieved with the easily accessible and economic Ni(II) complex $(Ph_3P)_2NiCl_2$ using liquid triphenylphosphine (TPP) as a solvent. The best yields were reached when an equimolar mixture of lauric acid and TPP were heated with $(Ph_3P)_2NiCl_2$. The reaction starting from fatty acids and leading to LO/LAO was defined as a dehydration-decarbonylation reaction,[14] since it involves firstly the formation of the fatty acid anhydride with the release of water, followed by the decarbonylation with CO release (see Eqns. 1) and (3) as assessed experimentally.[23] As secondary product also the alkane undecane was detected by GC-MS in very low yield and undoubtedly due to the decarboxylation reaction of lauric acid according to Eqn. (2).

The Ni(II) complex $(Ph_3P)_2NiCl_2$ is an effective dehydration-decarbonylation in liquid TPP and it works well also with other fatty acids than lauric acid. As shown in Table 2, the mentioned complex is able to produce LO/LAO in high yield and good LAO selectivity also with decanoic, myristic, palmitic and stearic acid, confirming the general purpose effectiveness of the proposed catalyst. The electronic absorption spectra of $(Ph_3P)_2NiCl_2$ in liquid triarylphosphine has revealed that Ni(II) assumes an octahedral coordination structure which is believed to be the active species in the dehydration-decarbonylation reaction. Conversely, $NiCl_2$ liquid triarylphosphine does not show the

same spectrum as that displayed by $(Ph_3P)_2NiCl_2$ at 70 °C and it is already known to be less effective in the dehydration-decarbonylation reaction of fatty acids, giving lower yields and low to negligible selectivity toward LAO.[23]

The catalytic performances of $(Ph_3P)_2NiCl_2$ in the dehydration-decarbonylation reaction were also compared with those of the expensive Rh(I) complex $Rh(Ph_3P)_3Cl$ known as Wilkinson's catalyst. The Rh(I) catalyst shows an extremely high selectivity in LAO formation with yields >95 % as determined both with Raman spectroscopy and confirmed by GC-MS. Furthermore, the alkane by-product is produced in trace amounts. The unique drawback of the Wilkinson's catalyst is the lower yields in the lauric acid conversion to LO/LAO in comparison to those achieved with the Ni(II) complex.

In conclusion, $Rh(Ph_3P)_3Cl$ is the catalyst of choice if >95 % LAO selectivity is required with no other by-products. However, if the industrial goal is to produce a mixture of LO/LAO in high yields without the need of high selectivity to LAO, as in the case of feedstock for hydroformylation reaction, then the catalyst of choice is the easily accessible and economic Ni(II) complex $(Ph_3P)_2NiCl_2$. TPP plays a key role both as ligand and as redox solvent.

References

[1]Morgan, M., Linear alpha olefins – A question of balance, *Chem. Week*, **2016**, *March 14*. https://chemweek.com/CW/Document/77664

[2]Weissermel, K., Arpe, H. J., *Industrial Organic Chemistry*, 3rd ed. Wiley-VCH, Weinheim, **2008**, pp. 74-83. https://books.google.it/books?hl=it&lr=&id=AO5HyPl_X0wC&oi=fnd&pg=PP2&dq=2Weissermel,+K.,+Arpe,+H.+J.+Industrial+Organic+Chemistry,+3rd+ed.+WileyVCH,+Weinheim,+2008,+pp.+7483&ots=uMQ8crEHIG&sig=qqTFFJfhD6JLUmdACUNJeL1NfGc&redir_esc=y#v=onepage&q&f=false

[3]Gunstone, F. D., *Fatty Acid and Lipid Chemistry*. Springer, Dordrecht, **2012**. DOI: https://doi.org/10.1007/978-1-4615-4131-8

[4]Ahmad, M. U., (ed.). *Fatty Acids. Chemistry, Synthesis and Applications*. Elsevier, Amsterdam, **2017**.

https://books.google.it/books?hl=it&lr=&id=QioUDgAAQBAJ&oi=fnd&pg=PP1&dq=Ahmad,+M.U.,+(ed.).+Fatty+Acids.+Chemistry,+Synthesis+and+Applications.+Elsevier,+Amsterdam,+2017.&ots=6oe3K7HHpn&sig=fJpiQO9QRZSA5Qi2AZaXj5dwtQ&redir_esc=y#v=onepage&q=Ahmad%2C%20M.U.%2C%20(ed.).%20Fatty%20Acids.%20Chemistry%2C%20Synthesis%20and%20Applications.%20Elsevier%2C%20Amsterdam%2C%202017.&f=false

[5]Corma, A., Iborra, S., Velty, A., Chemical Routes for the Transformation of Biomass into Chemicals, *Chem. Rev.*, **2007**, *107*, 2411. https://pubs.acs.org/doi/abs/10.1021/cr050989d

[6]Metzger, J. O., Fats and oils as renewable feedstock for chemistry, *Eur. J. Lipid Sci. Technol.*, **2009**, *111*, 865. https://doi.org/10.1002/ejlt.200900130

[7]de Espinosa, L. M., Meier, M. A., Plant oils: The perfect renewable resource for polymer science?! *Eur. Polym. J.* **2011**, *47*, 837. https://doi.org/10.1016/j.eurpolymj.2010.11.020

[8]Dawes, G. J. S., Scott, E. L., Le Nôtre, J., Sanders, J. P., Bitter, J. H., Deoxygenation of biobased molecules by decarboxylation and decarbonylation – a review on the role of heterogeneous, homogeneous and bio-catalysis, *Green Chem.*, 2**015**, *17*, 3231. DOI: 10.1039/C5GC00023H

[9]Deuss, P. J., Barta, K., de Vries, J. G., Homogeneous catalysis for the conversion of biomass and biomass-derived platform chemicals, *Catal. Sci. Technol.*, **2014**, *4*, 1174. DOI: 10.1039/C3CY01058A

[10]Gandini, A., Lacerda, T. M., From monomers to polymers from renewable resources: Recent advances, *Progr. Polym. Sci.*, **2015**, *48*, 1. https://doi.org/10.1016/j.progpolymsci.2014.11.002

[11]Beller, M., Centi, G., Sun, L., Chemistry Future: Priorities and Opportunities from the Sustainability Perspective, *ChemSusChem*, **2017**, *10*, 6. https://doi.org/10.1002/cssc.201601739

[12]Lozano, F. J., Lozano, R., Freire, P., Jiménez-Gonzalez, C., Sakao, T., Ortiz, M. G., Trianni, A., Carpenter, A., Viveros, T., New perspectives for green and sustainable chemistry and engineering: Approaches from sustainable resource and energy use, management, and transformation, *J. Cleaner Prod.*, **2018**, *172*, 227. https://doi.org/10.1016/j.jclepro.2017.10.145

[13]Clarke, C. J., Tu, W. C., Levers, O., Brohl, A., Hallett, J. P., Green and Sustainable Solvents in Chemical Processes, *Chem. Rev.* **2018**, *118*, 747. https://pubs.acs.org/doi/abs/10.1021/acs.chemrev.7b00571

[14]Behr, A., Vorholt, A. J., *Homogeneous Catalysis With Renewables* (Vol. 39 of the Springer's book series: *Catalysts by Metal Complexes*), 2**017**, Springer, Cham, Switzerland. DOI: https://doi.org/10.1007/978-3-319-54161-7_12

[15]Schwarz, J., König, B., Decarboxylative reactions with and without light – a comparison, *Green Chem.* **2018**, *20*, 323. DOI : 10.1039/C7GC02949G

[16]Miller, J. A., Nelson, J. A., Byrne, M. P.. A Highly Catalytic and Selective Conversion of Carboxylic Acids to 1-Alkenes of One Less Carbon Atom, *J. Org. Chem.* **1993**, *58*, 18-20. https://pubs.acs.org/doi/abs/10.1021/jo00053a008?journalCode=joceah

[17]Lopez-Ruiz, J. A., Davis, R. J., Decarbonylation of heptanoic acid over carbon-supported platinum nanoparticles, *Green Chem.* **2014**, *16*, 683. DOI: 10.1039/C3GC41287C

[18]Chatterjee, A., Jensen, V. R., Heterogeneous Catalyst for the Transformation of Fatty Acids to α-Olefins, *ACS Catal.* **2017**, *7*, 2543. https://pubs.acs.org/doi/abs/10.1021/acscatal.6b03460

[19]Eliasson, S. H. H., Chatterjee, A., Occhipinti, G., Jensen, V. R., The Mechanism of Rh-Catalyzed Transformation of Fatty Acids to Linear Alpha-olefins, *Inorganics*, **2017**, *5*, 87. https://doi.org/10.3390/inorganics5040087

[20]Chatterjee, A., Hopen Eliasson, S. H., Törnroos, K. W., Jensen, V. R., Palladium Precatalysts for Decarbonylative Dehydration of Fatty Acids to Linear Alpha Olefins, *ACS Catal.* **2016**, *6*, 7784. https://pubs.acs.org/doi/abs/10.1021/acscatal.6b02460

[21]John, A., Miranda, M. O., Ding, K., Dereli, B., Ortuño, M. A., LaPointe, A. M., Tolman, W. B. *Organometal.* **2016**, *35*, 2391. https://pubs.acs.org/doi/abs/10.1021/acs.organomet.6b00415

[22]John, A., Dereli, B., Ortuño, M. A., Johnson, H. E., Hillmyer, M. A., Cramer, C. J., Tolman, W. B., Nickel Catalysts for the Dehydrative Decarbonylation of Carboxylic Acids to Alkenes, *Organometallics*, **2017**, *36*, 2956. https://pubs.acs.org/doi/abs/10.1021/acs.organomet.7b00411

[23]John, A., Hillmyer, M. A., Tolman, W. B., Anhydride-Additive-Free Nickel-Catalyzed Deoxygenation of Carboxylic Acids to Olefins, *Organometal.* **2017**, *36*, 506. https://pubs.acs.org/doi/abs/10.1021/acs.organomet.6b00940

[24]Maetani, S., Fukuyama, T., Suzuki, N., Ishihara, D., Ryu, I., Iron-catalyzed decarbonylation reaction of aliphatic carboxylic acids leading to a-olefins, *Chem. Comm.* **2012**, *48*, 2552. DOI : 10.1039/C2CC18093F

[25]Wu, J., Shi, J., Fu, J., Leidl, J. A., Hou, Z., Lu, X., Decarboxylation of Fatty Acids to Aviation Fuels over Nickel Supported on Activated Carbon, *Sci. Rep.*, 2**016**, *6*, 27820 (8 pages). https://doi.org/10.1038/srep27820

[26]Trost, B. M., Chen, F., Transition metal mediateliminations in in anhydrides and thioanhydrides, *Tetrahedron Lett.* **1971**, *12*, 2603. https://doi.org/10.1016/S0040-4039(01)96930-8

[27]Nicholls, D. « *Nic.*» ». In Bailar, J.C., Emeleus, H.J., Nyholm, R., Trotman-Dickenson, A.F. (Eds.), *Comprehensive Inorganic Chemistry*, chapter 42, Pergamon Press, Oxford, **1973**.

[28]Greenwood, N. N., Earnshaw, A. *Chemistry of the Elements*, 2nd, Elsevier, Amsterdam, 2004.

[29]Cotton, A. F., Wilkinson, G., Bochmann, M., Murillo, C. A. *Advanced Inorganic Chemistry*. Wiley, New York, **1999**. https://www.wiley.com/enus/Advanced+Inorganic+Chemistry%2C+6th+Edition-p-9780471199571

[30]Smith, E. L., Abbott, A. P., Ryder, K. S., Deep Eutectic Solvents (DESs) and Their Applications, *Chem. Rev.* **2014**, *114*, 11060. **DOI:** 10.1021/cr300162p

[31]Anslyn, E. V., Dougherty, D. A. *Modern Physical Organic Chemistry*. University Science books. Sausalito, California, **2006**.https://books.google.it/books?hl=it&lr=&id=gYSxijk_tMC&oi=fnd&pg=PR23&dq=Anslyn,+E.+V.,+Dougherty,+D.+A.+Modern+Physical+Organic+Chemistry.+University+Science+books.+Sausalito,+California,+2006.&ots=evwHbfGiXd&sig=es56OD0HWB2AjWqSqhPmyxX1bNA&redir_esc=y#v=onepage&q&f=false

[32]Cotton, A. F. *Chemistry of Precious Metals*. Chapman & Hall, London, **1997**, pp.91-132.

[33]Cornils, B., Herrmann, W.A., Schlogl, R., Wong, C.H. (Eds.). Catalysis from A to Z. A Concise Encyclopedia. Wiley-VCH, Weinheim, **2000**, p. 159.

[34]Freeman, S. K. Applications of Laser Raman Spectroscopy. John Wiley and Sons, New York, **1974**.

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ANTIOXIDANT ACTIVITY OF COMPOUNDS ISOLATED FROM *PYCNANTHUS ANGOLENSIS* (WELW.) WARB AND *BYROPHYLLUM PINNATUM* (LAM.) OKEN

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Keywords: extracts; isolates; antioxidant; *Pycnanthus angolensis; Byrophyllum pinnatum*

Pycnanthus angolensis (Welw.) Warb and *Byrophyllum pinnatum* (Lam.) Oken are two herbal recipes employed in native medicine for the treatment /management of gastrointestinal disorders, skin infections and more especially cancers, wounds and many other inflammatory conditions. Before now, seven compounds (**NG-1b, NG-2**, **NG-3b, NG-4c, NG-5a**, **KF-1a** and **KF-2)** have been obtained from the extracts of these plants by column and /or preparative thin-layer chromatographies. The search for new and more active antioxidant agents necessitated the screening of these compounds for potential activity. Generally the isolates gave (IC_{50}) of between 0.50 and 0.60 µg mL^{-1}. However, **NG-1b** demonstrated a significant activity at 0.48 µg mL^{-1} which compare favourably with that shown by Vitamin A (a standard antioxidant drug) at 0.49 µg mL^{-1}. These compounds, most especially **NG-1b** could be further worked on with the aim of improving on the observed antioxidant activity through *in-vitro* structural activity relationship studies (SARS) and probably *in-vivo* clinical trials.
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Introduction

Free radicals occur in the human body in the forms of reactive oxygenated species (ROS), super-oxides, peroxides, hydroxyl and nitrogenous species. These chemical species are natural by-products of cellular metabolism and also form an integral part normal physiology.[1] Furthermore, they can also be produced by bacterial leucocytes, atmospheric pollutants, drugs, xenobiotics and in mitochondrial respiration. In addition, chemical conversion of fats during lactation, exercise, fever, infections and even fasting can result in increased radical activity leading to damage of the immune and nervous systems. The stress hormones (adrenalin and noradrenalin) secreted by the adrenal glands under conditions of continuing and excessive emotional stress are metabolized into simpler, albeit, free radical molecules which can attack biological moieties such as proteins, lipids, enzymes, DNA and RNA resulting in cellular or tissue or organ injury associated with degenerative diseases.[2] The consumption of fruits and vegetables containing antioxidants has been found to offer protection against these diseases.[3] Besides playing an important role in the physiological systems, antioxidants have been used in the food industry to prolong the shelf life of foods especially those rich in polyunsaturated fats. The consumption of natural antioxidants has been reported to have potential health benefits.[4-6] However, the continued use of synthetic antioxidants is a source of concern [7-8] due to the health risks and associated toxicity.[1, 7-8]

Phytochemicals such as flavonoids, terpenes and polyphenols have been reported to inhibit free radical reactions, protect the human body from disease [9-10] and retard lipid oxidative rancidity.[11] Therefore, researches are presently focused on obtaining antioxidants from plant sources. Two of such plants namely, *Pycnanthus angolensis* (Welw.) Warb and *Byrophyllum pinnatum* (Lam.) Oken are employed in folkloric medicine for the treatment of gastrointestinal disorders, skin diseases and more especially cancers, ulcers, wounds and inflammations. Previous studies by the lead author and co-workers have led to seven compounds namely, ethyl cinnamate **(**cinnamic acid, ethyl ester) (**NG-1b**), 3-ethoxy-3,7-dimethyl-1,6-octadiene (ethyl linalool) (**NG-2**), 9-oximino-2,7-diethoxyfluorene (2,7-diethoxy-9H-fluoren-9-one oxime) (**NG-3b)**, 1,2-benzenedicarboxylic acid diethyl ester (diethyl phthalate) (**NG-4c**), ethyl 1,6-dihydro-2-methyl-4-hydroxy-6-oxo-3-pyridine carboxylic acid, ethyl ester (1,6-dihydro-2-methyl-4-hydroxy-6-oxo-3-nicotinic acid, ethyl ester (**NG-5**a), 1-ethoxy-2- hydroxy-4-prophenyl guaethol **(KF-1a)** and 17-(1′-cyclobutan)-2′-one-3-hydroxy)-(3β,17β)-spiro(andorst-5-ene, (**KF-2**) from the plants.[12-15] This present study was done by subjecting the crude extracts and the seven isolated compounds to antioxidant tests with a view of confirming or disproving the reported uses in ethno-medicine for the treatment/management of inflammatory conditions which arise from the debilitating incidence of free-radicals.

Experimentals

Fresh leaves of *P. angolensis* and *B. pinnatum* were collected in the month of July, 2016 within the University of Uyo Campus, Nigeria. The plants had previously been identified in studies [16-17] for which voucher specimens No. H 045 and No. H 047 were deposited in the Herbarium Unit of the Faculty of Pharmacy. Immediately after collection, the plant materials were separately dried in a laboratory oven

(Gallenkamp, England) at 40 ^{0}C for 48 h and the resultant materials powdered on an electric mill (Uniscope, England).

Extraction and processing of plant material

The powders were extracted with cold 96 % ethanol at room temperature (27 ± 2 ^{0}C) for 72 h. The obtained filtrates were then evaporated to dryness *in-vacuo* on a rotary evaporator (R205D, Shensung BS & T, China). The extracts, **NG-1b, NG-2**, **NG-3b, NG-4c, NG-5a**, **KF-1a** and **KF-2** previously isolated from chromatographic separations of organic fractions of the two plants [12-15] were kept in appropriately labelled amber bottles and then stored in a refrigerator at -4 ^{0}C prior to the antioxidant tests.

DPPH (2, 2-Diphenyl-1-picryhydrazyl hydrate) assay

The purple colour of the methanolic solution of DPPH is bleached when it accepts hydrogen or electrons from extract/isolate/standard antioxidant drug. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) molecule is noted for its stable free radical nature and when mixed with a substance that can donate a hydrogen atom or electrons results in its reduced form, 1,1-diphenyl-2-picrylhydrazine. The tests were done by developing the spotted samples of crude extracts, **NG-1b, NG-2**, **NG-3b, NG-4c, NG-5a**, **KF-1a** and **KF-2** in ethyl acetate: methanol (1:2) solvent mixture in duplicates. Ascorbic acid (Gemini Chemicals, Nigeria) was spotted along to serve as positive control. The developed chromatograms were sprayed with 0.1 % w/v methanolic solution of DPPH reagent (Sigma-Aldrich, Germany). The plates were irradiated with ultra-violet light at λ_m 366 nm for 15 minutes. Spots which appeared white against a purple background were taken as evidence of positive tests indicating antioxidant activity. [18-21]

Spectrophotometric determination of antioxidant activity using DPPH reagent

Substances which are capable of donating electrons or hydrogen atoms (free-radical scavengers) can convert the purple-coloured DPPH radical (2, 2-diphenyl-1-picrylhydrazyl hydrate) to its yellow-coloured non-radical form (1,1-diphenyl-2-picryl hydrazine).[2, 22] This reaction can be monitored by spectrophotometry. This is the most widely employed method of screening for antioxidant activity in plants.[23-26]

DPPH (4 mg) was weighed and dissolved in methanol (100 mL) to produce the stock solution (0.004 % w/v). Serial dilutions of the stock solution were carried out. The absorbance of each of the sample was taken at λ_m 512 nm using the UV-VIS spectrophotometer (Jenway 6405, USA). A solution of methanol without DPPH served as the blank.

Determination of the antioxidant activity of crude extract / isolate

In every case, 2 mg of the extract/isolate was dissolved in 50 mL of methanol. Serial dilutions were done to obtain the following concentrations; 0.0008 mg mL^{-1}, 0.0016 mg mL^{-1} and 0.0024 mg mL^{-1} using methanol. 5 mL of each concentration was incubated with 5 mL of 0.004 % w/v methanolic DPPH solution for optimal analytical accuracy. After an incubation period of 30 minutes in the dark at room temperature (25 ± 2 ^{0}C), observation was made for a change in the colour of the mixture from purple to yellow. The absorbance of each of the test samples was then taken at λ_m 512 nm. The Radical Scavenging Activity (RSA %) or Percentage Inhibition (PI %) of free radical DPPH was thus calculated:

$$RSA\ \%\ (PI\ \%) = [(A_{blank} - A_{sample})/\ A_{blank}] \times 100$$

where

A_{blank} is the absorbance of the control reaction (DPPH solution without the test sample and

A_{sample} is the absorbance of DPPH incubated with the extract/isolate /anti-oxidant drug.

Extract/isolate /standard antioxidant drug concentration providing 50 % inhibition (IC_{50}) was calculated using a graph of inhibition percentage against the concentration of the extract/isolate/standard antioxidant drug.[27-28]

DPPH assay of standard antioxidant drugs

Standard antioxidants namely, vitamin A (Emzor Drugs, Nigeria), vitamin C (Greenfield Drugs, Nigeria) and vitamin E (Gemini, Nigeria) were used. While vitamin C was in a tablet dosage form, vitamins A and E were formulated as gelatine capsules. The estimated weight of the formulations containing 2 mg of the standard antioxidant drugs were determined by proportionality and then diluted. Methanol was used to dissolve vitamin C, while n-hexane was used to dissolve vitamins A and E because solubility problems encountered with these two vitamins. Thus, methanolic and hexane solutions of 0.004 % w/v DPPH were used for incubation of vitamin C, vitamin A and E respectively for 30 minutes.

The absorbance value of each of the drugs was taken at wavelength at λ_m 512 nm and the IC_{50} determined.

Results and discussion

The plants were identified and collected observing basic rules of plant collection. Furthermore, the principles governing extraction and processing of extract/isolate were strictly adhered to, thus preventing any changes to the chemical composition of the samples.[29-30] Phytochemical investigations on the plant extracts indicated the presence of alkaloids, saponins, tannins, terpenes, flavonoids and cardiac glycosides while anthraquinones and cyanogenic glycosides were absent.[31] The crude extracts and resultant sub-fractions were separately put through silica chromatographic separations and the exercises led to the isolation of compounds coded as NG-1b, NG-2, NG-3b, NG-4c, NG-5a, KF-1a and KF-2 respectively. The identities of these compounds have been established to be ethyl cinnamate (cinnamic acid, ethyl ester), 3-ethoxy-3,7-dimethyl-1,6-octadiene (ethyl linalool), 9-oximino-2,7-diethoxyfluorene

(2,7-diethoxy-9H-fluoren-9-one oxime), 1,2-benzenedicarboxylic acid diethyl ester (diethyl phthalate), ethyl 1,6-dihydro-2-methyl-4-hydroxy-6-oxo-3-pyridine carboxylic acid, ethyl ester (1, 6-dihydro-2-methyl-4-hydroxy-6-oxo-3-nicotinic acid, ethyl ester, 1-ethoxy-2-hydroxy-4-prophenyl guaethol and 17-(1′-cyclobutan)-2′-one-3-hydroxy)-(3β,17β)-spiro(androst-5-ene, respectively using a combination of MS and IR spectral techniques.[12-15]

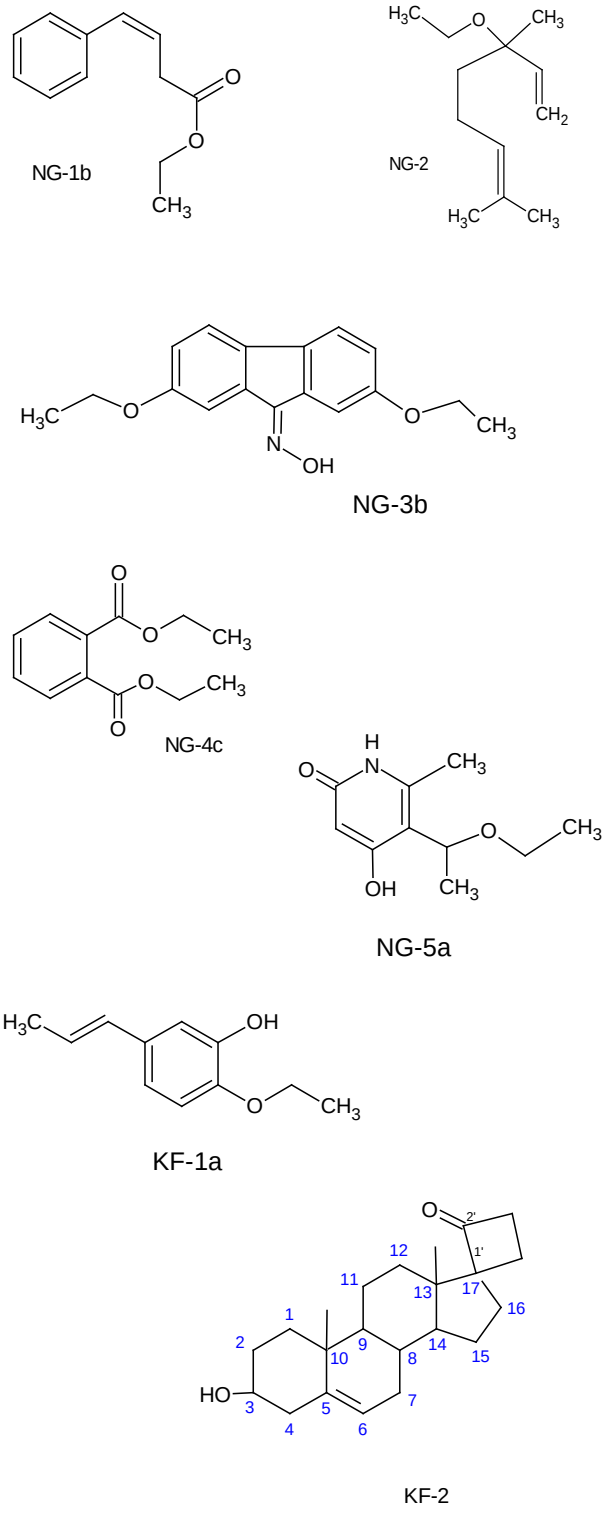

Figure 1. Structures of the studied compounds

Table 1. Absorbance of samples incubated with DPPH at different concentrations λ_{max} (512 nm)

Sample	Absorbance		
	0.0008 mg mL^{-1}	**0.0016 mg mL^{-1}**	**0.0024 mg mL^{-1}**
P. angolensis	0.538	0.532	0.520
B. pinnatum	0.324	0.322	0.276
NG-1b	0.299	0.282	0.280
NG-2	0.270	0.263	0.259
NG-3b	0.194	0.188	0.181
NG-4c	0.166	0.164	0.161
NG-5a	0.192	0.183	0.179
KF-1a	0.202	0.189	0.184
KF-2	0.115	0.107	0.096
Vitamin A	0.298	0.267	0.257
Vitamin C	0.115	0.098	0.078
Vitamin E	0.163	0.157	0.152

NG-1b=ethyl cinnamate (cinnamic acid, ethyl ester); NG-2=3-ethoxy-3,7-dimethyl-1,6-octadiene (ethyl linalool); NG-3b=9-oximino-2,7-diethoxyfluorene (2,7-diethoxy-9H-fluoren-9-one oxime); NG-4c=1,2-benzenedicarboxylic acid diethyl ester (diethyl phthalate); NG-5a = ethyl 1,6-dihydro-2-methyl-4-hydroxy-6-oxo-3-pyridine carboxylic acid, ethyl ester (1,6-dihydro-2-methyl-4-hydroxy-6-oxo-3-nicotinic acid, ethyl ester; KF-1a=1-ethoxy-2-hydroxy-4-prophenyl guaethol; KF-2 = 17-spiro(1′-cyclobutan)-2′-one-3-hydroxy)-(3β, 17β)-androst-5-ene; RSA % (PI %) = Radical scavenging activity (Percentage inhibition); IC_{50} = concentration at which 50 % of DPPH is scavenged or inhibited; NR=Not regressed (value could not be regressed from the % inhibition-concentration curve).

Table 2. Radical scavenging activity (percentage inhibition) of samples at different concentrations (mg mL^{-1}) and IC_{50} of samples (blank absorbance of 0.004 % w/v methanolic DPPH reagent: 0.613)

Sample	RSA % (PI %)			IC_{50} µg mL^{-1}
	0.0008	**0.0016**	**0.0024**	
P. angolensis	12.23	13.21	15.17	NR
B. pinnatum	47.14	47.47	54.97	1.17
NG-1b	51.22	54.00	54.32	0.48
NG-2	55.95	57.10	57.75	0.55
NG-3b	68.35	69.33	70.47	0.58
NG-4c	72.93	73.24	73.74	0.59
NG-5a	68.67	70.15	79.79	0.54
KF-1a	67.05	69.17	69.98	0.50
KF-2	81.24	82.54	84.40	0.59
Vitamin A	51.39	56.44	58.07	0.49
Vitamin C	81.57	84.01	87.28	0.67
Vitamin E	73.41	74.39	75.20	0.60

Rapid thin-layer chromatographic analysis for antioxidant activity

The extracts, isolates and ascorbic acid showed white spots on purple background when the chromatogram was sprayed DPPH reagent. The observation of white spots (irrespective of initial spotted colour) was the evidence of reduction of DPPH reagent (discoloration) by the by free-radical scavenger in the samples.

Determination of the antioxidant activity of extract/ isolate/ antioxidant drug

The Beer-Lambert Law is the basis of all absorption spectrophotometry. Therefore, a plot of absorbance against concentration for a cell of unit thickness (1 cm) should give a straight line passing through the origin. The reduction of the DPPH radical was determined by taking its absorption at a wavelength of λ_m 512 nm. It was observed that the absorbance of DPPH decreased as the concentration of added free radical scavenger extract /isolate /standard antioxidant drug) increased which suggested that the DPPH reagent was being reduced. The results of the reduction are as presented in **Table 1**. The Radical Scavenging Activity (RSA %) or percentage inhibition (PI %) and the IC_{50} values of extract and standard antioxidant drugs were computed as **Table 2** shows.

The RSA % is an indicator of the antioxidant activity of extract/ isolate /standard antioxidant drug.[32] All the isolated compounds gave largely moderate antioxidant activity (IC_{50}) of between 0.50 μg mL^{-1} and 0. 60 μg mL^{-1}. However, **NG-1b** elicited a remarkable activity at 0.48 μg mL^{-1}. It should be stated that this activity compare favourably with that demonstrated by Vitamin A (a standard antioxidant drug) at 0.49 μg mL^{-1}. Furthermore, the antioxidant activities demonstrated by the isolated compounds were not surprising because different preparations of the plants are used in ethno-medicine to treat /manage disease conditions such as wounds, inflammations and tumours amongst so many others.[33-38] In addition, secondary metabolites such as flavonoids or flavonoid-like, terpenes and phenols or hydroxylated compounds (such as **NG-2, NG-3b**, **NG-4c**, **NG-5a, KF-1a and K-2**) have been reported to inhibit free radical reactions and protect the human body from disease. [9, 18, 39-45] Also, the importance of the radical scavenging ability of some phytochemical compounds have found useful applications in the extension of shelf-life and control of deterioration of fatty foods, nutriceuticals and spices. [46-49] Aside the DPPH assay, other methods for determining the antioxidant activity of plants include the hydrogen peroxide, nitric oxide, conjugated diene, superoxide, phosphomolybdenum, peroxynitrile and xanthine oxidase assay methods amongst many others. [50-51]

Conclusion

The results of this present study indicate that compounds isolated from the plants have demonstrated antioxidant activities which can compare with those of standard antioxidant drugs in clinical practice. Also, these compounds are to be further investigated (*in-vitro* SARS) in our laboratories with a view to improving on the observed activities.

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References

[1]Wong, S. P., *Antioxidant activities of aqueous extract of selected plants*. Food science and technical program, Department of Chemistry, National University of Singapore, Singapore, **2005**.

[2]Nagalapur, S. K., Paramjyothi, S., In vitro antioxidant activity of *Launaea pinntifida*, *The Bioscan*, **2010**, *5(1)*, 105-108.

[3]Halliwell, B., Protection against tissue damage in vivo by desferrioxamine: What is its mechanism of action ? *Free Radical Biol. Med.*, **1989**, *7*, 645-657. https://doi.org/10.1016/0891-5849(89)90145-7

[4]Di Carlo, G., Maxolo, N., Izzo, A., Capasso F., Flavonoids: old and new aspects of a class of natural therapeutic drugs, *Life Sci.*, **1999**, *65, 337-353.* https://doi.org/10.1016/s0024-3205(99)00120-4

[5]Pulido, R., Bravo, L., Savana-Calixto, F., Antioxidant Activity of Dietary Polyphenols As Determined by a Modified Ferric Reducing/Antioxidant Power Assay, *J. Agric. Food Chem.*, **2000**, *48(8)*, 3396-3402. DOI: 10.1021/jf9913458

[6]Sumino, M., Sekine, T., Ruangrungsi, N., Igarashi, R., Ikegami, F., Ardisiphenols and Other Antioxidant Principles from the Fruits of *Ardisia colorata*, *Chem. Pharm. Bull.*, **2002**, *50(7)*, 1484-1487. https://doi.org/10.1248/cpb.50.1484

[7]Kahl, R., Kappus, A., Toxikologie der synthetischen Antioxidantien BHA und BHT im Vergleich mit dem natürlichen Antioxidans Vitamin E, *Z. Lebensmit.-Untersuch. Forsch.*, **1993**, *196(4)*, 329-338. https://doi.org/10.1007/BF01197931

[8]Valentao, P., Fernandes, E., Carvalho, F., Andrade, P. A., Seabra, R. M., Bastos, M. L., Antioxidative Properties of Cardoon *(Cynara cardunculus L.)* Infusion Against Superoxide Radical, Hydroxyl Radical, and Hypochlorous Acid, *J. Agric. Food Chem.*, **2002**, *50*, 4989-4993. DOI: 10.1021/jf020225o

[9]Kinsella, J. E., Frankel, E., German, B. J., Kanner, J., Possible mechanisms for the protective role of antioxidants in wine and plant foods, *Food Tech.*, **1993**, *47*, 85-89.

[10]Terao, J., Piskula, M. K., *Flavonoids as inhibitions of lipid peroxidation in membranes*. Inca Rice Evans and L. Packer (Eds.), **1997**.

[11]Duthie, G. G., Lipid Peroxidation. *Eur. J. Clin. Nutr*., **1993**, *47(11)*, 759-746.

[12]Oladimeji, H. O., Attih, E. E., Onu, O. N., Ethyl linalool and diethyl phthalate from *Pycnanthus angolensis* (Welw.) Warb. *Eur. Chem. Bull.*, **2017**, *6(2)*, 76-78. DOI: 10.17628/ecb.2017.6.76-78

[13]Oladimeji, H. O., Onu, O. N., Isolation of ethyl cinnamate and substituted fluorene from *Pycnanthus angolensis* (Welw.) Warb, *Eur. Chem. Bull*., **2017**, *6(9)*, 421-423, DOI: 10.17628/ecb.2017.6.421-423.

[14]Oladimeji, H. O., Eberefiak, K. E., Isolation and antimicrobial analysis of a steroidal terpene from the butanol fraction of *Byrophyllum pinnatum* (Lam.) Oken *Eur. Chem. Bull*., **2017**, *6(7)*, 292-294. DOI: 10.17628/ecb.2017.6.292-294

[15]Oladimeji, H. O., Onu, O. N., Antimicrobial activities of compounds isolated from *Pycnanthus angolensis* (Welw.) Warb and *Bryophyllum pinnatum* (Lam) Oken. *Eur. Chem. Bull*., **2018**, *7(7)*, 190-193. DOI: 10.17628/ecb.2018.7.190-193.

[16]Oladimeji, H. O., Ubulom, P. M. E., Igboasoiyi, A. C., Ndukwe, K., Nia, R., Some biological activities of *Pycnanthus angolensis* (Welw.) Warb. *J. Pharm. Biores*., **2006a**, *3(1)*, 49-55. https://doi.org/10.4314/jpb.v3i1.32092

[17]Oladimeji, H. O., Nia, R., Edoho, E. J., Investigation into the larvicidal and antimicrobial activity *Bryophyllum pinnatum* (Lam.) Oken. *Afr. J. Pharm. Res. Dev*., **2006b**, *2(2)*, 102-108.

[18]Alemika, T. E., Onawunmi, G. O., Olugbade, T. A., Isolation and characterization of incensole from *Boswellia dalzielli* stem bark. *J. Pharm. Biores.*, **2004,** *1(1),* 7-11. https://doi.org/10.4314/jpb.v1i1.32041

[19]Bondet, V., Brand-Williams, W., Berset, C., Kinetic and mechanisms of antioxidant activities using the DPPH free-radical method, *Lebensmitt. Wissenschaft. Tech.*, **1997**, *30,* 609-615.

[20]Cuendet, M., Hostettmann, K., Potterat, O., Indoid glycosides with free-radical scavenging properties from *Fragranea blumei***.** *Helv. Chim. Acta*, **1997**, *80(8),* 1144-1152. https://doi.org/10.1002/hlca.19970800411

[21]Kirby, A. J., Schmidt, R. J., Antioxidant activity of Chinese herbs for eczema and placebo herbs. *Int. J. Pharmacol.*, **1997**, *56,* 103-108. https://doi.org/10.1016/s0378-8741(97)01510-9

[22]Guangrong, H., Jiaxin, J., Dehui, D., Anti-oxidative and antibacterial activity of the methanol extract of *Artemisia anomala* S. Moore , *Afr. J. Biotech.*, **2008**, *7(9),* 1335-1338.

[23]Hu, C., Kitts, D. D., Studies on the antioxidant activity of Echinacea root extract, *J.* Agric. *Food Chem.*, **2000,** *48,* 1466-1472.

[24]Khaled, F. E., Ahmed, H. E, Amr, F., Antioxidant activity of volatile components of Egyptian *Artemisia judaica* L. *Food Chem.*, **2002**, *78(3),* 331-336.

[25]Singh, R. P,. Chidambara, M. K.,Jayaprakash,, G. K., Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in-vitro* model. *J. Agric. Food Chem.*, **2002**, *50,* 81-86. https://doi.org/10.1021/jf010865b

[26]Oladimeji, H. O., Akpan, B. C., Antioxidant potential of some Nigerian herbal recipes**.** *J. Pharm. Biores*., **2014**, *11(2),* 77-85.

[27]Lebeau, J., Furman, G., Bernier, J. L., Durietz, P., Teissier, E., Cotelle, N., Antioxidant properties of di-tert-butylhydroxylated flavonoids. *Free-Radicals Biol. Med*., **2000**, *29(9),* 900-912. https://doi.org/10.1016/s0891-5849(00)00390-7

[28]Leitao, G. G., Leitao, S. G., Vilegas, W., Z. Quick preparative separation of natural naphthoquinones with antioxidant activity by high speed counter current chromatography, Z. *Naturforsch*, **2002**, *57C,* 1051-1055. https://doi.org/10.1515/znc-2002-11-1217

[29]Odebiyi, O. O., Sofowora, A., Phytochemical screening of Nigerian medicinal plants-Part I*. Lloydia*, **1978,** *41, 234.*

[30]Odebiyi, O. O., Sofowora, A., Phytochemical screening of Nigerian medicinal plants-Part II. 2nd OAU/STRC Inter-African symposium. *Traditional pharmacopoeia of African medicinal plants.* OAU/STRC publishers, **1979**, *115*, 216.

[31]Oladimeji, H. O., Usifoh, C. O., Phytochemical and antimicrobial studies on some Nigerian medicinal plants. *J. Pharm.Biores.*, **2015**, *12(2),* 156-164. https://doi.org/10.4314/jpb.v12i2.11

[32]Meir, S., Kanner, J., Akiri, B., Determination and involvement of aqueous reducing compounds in oxidative defence systems of various senescing leaves. *J. Agric. Food Chem*., **1995,** *43,* 1813-1815. https://doi.org/10.1021/jf00055a012

[33]Dalziel, J. M., *Useful plants of West Tropical Africa. Crown agents for overseas governments and administrations*, **1956**, 122.

[34]Oliver, B., *Medicinal Plants in Nigeria I. Nigerian college of arts, Science and Technology*, **1959**, 21.

[35]Oliver, B., *Medicinal plants in Nigeria II. Nigerian college of arts, science and technology*, **1960**, 70-72.

[36]Watt, J. M., Breyer-Brandwijk, M. G., *The medicinal and poisonous plants of Southern and Eastern Africa. E. S. Livingstone limited*, **1962**, 59.

[37]Sofowora, A., *Medicinal plants and traditional medicine in Africa. Spectrum book limited*, **2008**, 112-114.

[38]Evans, W. C., *'Pharmacognosy'. 16th edition. WB Saunders company*, **2009**, 248-249.

[39]Tsimidou, M., Boskou, D., Antioxidant activity of essential oils from the plants of Laminaceae family. In: G. Charalampous spices, herbs and edible fungi. Elsevier publishers, **1994**, 273-284.

[40]Lagouri, V., Boskou, D., *Screening for antioxidant activity of essential oils obtained from spices. In: Charalampous G. food flavors. Generation, analysis and process influence*. Elsevier, **1995,** 869-874.https://doi.org/10.1016/s0167-4501(06)80203-7

[41]Yokosawa, T., Dong, E., Liu, Z. W., Shimizu, M., Antioxidative Activities Flavones and Flavonols. *In-vitro*, **1997**, *11*, 446-449.

[42]Daniel, R. S., Bifu, C. M., Devi, K. S., Augusti, K. T., Antioxidant effects of two flavonoids from the bark of *Ficus bengalensis* Linn., in hyperlipidemic rats *Ind. J. Expt. Biol*., **1998**, *36*, 902-906.

[43]Grassmann, J., Schneider, D., Weiser, D., Elsmer, E. F., Antioxidative effect of lemon oil and its components on copper induced oxidation of LDL *Arzneimittel- Forschung*, **2001**, *51*, 799-805. https://doi.org/10.1055/s-0031-1300118

[44]Malaya, G., Upal, K. M., Ramanathan, S. K., Thangavel, S., Madgula, L., Mohan, V., Anti-tumour activity and antioxidant status of *Caesalpinia bonducella* against Ehrlich ascites carcinoma in Swiss Albino Mice, *J. Pharmacol. Sci*., **2004**, *94*, 177-184. https://doi.org/10.1254/jphs.94.177

[45]Svoboda, K., Brooker, J. D., Zrustova, J., Antibacterial and antioxidant properties of essential oils. their potential applications in food industries *Acta. Horticul. (SHS)*, **2006**, *709(1),* 35-43. https://doi.org/10.17660/actahortic.2006.709.4

[46]Thomas, P. G., Wade, A. M., *Official gazette of US trade mark office patents*, **2001**, 1253, 2.

[47]Braca, A., Polili, M., Sanogo, R., Sanou, H., Morelli, I., Pizza, C., De-Tommasi, N., Chemical composition and antioxidant activity of phenolic compounds from wild and cultivated *Sclerocerya birrea* (Anacardiaceae) leaves *J. Agric. Food Chem.*, **2003**, *51(23),* 6689-6695. https://doi.org/10.1021/jf030374m

[48]Shahidi F., Antioxidants in food and food antioxidants. *Nahrung*, **2000**, *44*, 158-163. https://doi.org/10.1002/1521-3803(20000501)44:3<158::aid-food158>3.0.co;2-l

[49]Liyana-Pathirana, C. M., Shahidi, F., Antioxidant properties of commercial salt and hard water wheat (*Trilicum aestivum* L.) and their Milling Fractions. *J. Sci. Food Agric*., **2006**, *86(3),* 477-485. https://doi.org/10.1002/jsfa.2374

[50]Bunta**,** G. W., Ingold, R. U., β-carotene: An unusual type of lipid antioxidant. *Science*, **1984**, *224*, 569-573. https://doi.org/10.1126/science.6710156

[51]Dapkevicius, A., Vanskulons, R., Van-Beek, T. A., Linssen, J. P., Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *J. Sci. Food Agric*., **1998**, *77(1),* 140-146. https://doi.org/10.1002/(sici)1097-0010(199805)77:1<140::aid-jsfa18>3.3.co;2-b

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SYNTHESIS OF 1,8-DIOXOOCTAHYDROXANTHENE AND 3,3-ARYLIDENE BIS(4-HYDROXYCOUMARIN) DERIVATIVES

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Keywords: Solvent-free reaction; 1,8-dioxooctahydroxanthene; 3,3-arylindene bis (4-hydroxycoumarin) derivatives ; reusable promoting material

A rapid, green and efficient method for the synthesis of 1,8-dioxooctahydroxanthene and 3,3-arylidenebis(4-hydroxycoumarin) derivatives through a one-pot condensation from various aromatic aldehydes is described using manganese ferrite ($MnFe_2O_4$) and cobalt ferrite ($CoFe_2O_4$) as promoting material under solvent-free conditions which can easily be recovered and reused. Compared with other synthetic methods, this new method has advantages such as milder reaction conditions, good to excellent yields, short reaction times, and environmentally benign procedure.

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INTRODUCTION

Synthesis of heterocyclic compound has a huge importance in chemistry, biochemistry, modern drug design, and these compounds are widely distributed in nature. Nowadays there are a lot of heterocyclic pharmaceuticals, these are used widely as antitumor, antiviral, antibiotic, anti-HIV pharmaceuticals although there is a large number of literatures about the synthesis of heterocyclic compounds have potential biological activity, but preparation of novel compounds and more efficient and economic methods means challenge for organic chemists.

Xanthenes and their substituted derivatives are useful targets for chemical synthesis as they have been associated with a diverse range of therapeutic and pharmacological properties such as antiviral[1] and antibacterial activity.[2] Apart from these applications, they are used in photodynamic therapy.[3] view of the general observation that the biological activities are invariably associated with1,8-dioxooctahydroxanthenes and 3,3-arylidene bis(4-hydroxycoumarin) derivatives, in this work we describe a new method and promoter to prepare some derivatives belong to these compound classes.

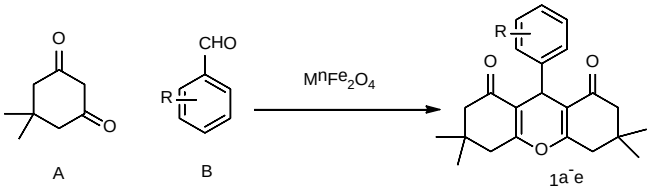
RESULT AND DISCUSSION

Synthesis of 1,8-dioxooctahydroxanthene derivatives

Many procedures for the synthesis of xanthenes and benzoxanthenes have been reported in the literature, including the reaction of For this purpose, react two molecules of dimedone (5,5-dimethyl-1,3-cyclohexane dione) with various aromatic aldehydes,[4] by using of different Lewis acid catalysts such as triethylbenzyl ammonium chloride[5], p-dodecyl benzenesulfonic acid[6], diammonium hydrogen phosphate under various conditions,[7] sulfonic acid under ultrasonic irradiation,[8] ionic liquids,[9] Amberlyst-15,[10] $NaHSO_4$-SiO_2 or silica chloride.[11]

In continuation of our work,[12-17] we have developed the new protocol that using nanosized manganese ferrite ($MnFe_2O_4$) is an efficient and reusable promoter for the synthesis of 3,3,6,6-tetramethyl-9-aryl-1,8-dioxooctahydro xanthene derivatives. The salient features of this protocol include the use of a small amount of the $MnFe_2O_4$, good yields, operational simplicity, short reaction times, promoter separation from the reaction medium. Moreover, the use of environmentally benign catalyst and avoidance of hazardous organic solvents are important features of this method.

To optimize the reaction conditions, the reaction of 5,5-dimethyl-1,3-cyclohexanedione (2 mmol) and benzaldehyde (1 mmol) under solvent-free conditions was selected as a model. After many studies on the above model reaction, we found that when less than 1 mmol of $MnFe_2O_4$ was applied the corresponding products obtained in lower yields and require more time, whereas use of more than 1 mmol $MnFe_2O_4$ did not improve the yield and require the same time. This was due to the fact that beyond a certain concentration, there exist an excess of $MnFe_2O_4$ sites over what is actually required by the reactant molecules and hence, the additional $MnFe_2O_4$ does not increase the rate of reaction. Therefore, in all further reactions 1 mmol of $MnFe_2O_4$ was used.



Scheme 1. Synthesis of 1,8-dioxooctahydroxanthene

In order to evaluate the generality of the process, we carried out a series of reactions using 5,5-dimethyl-1,3-cyclohexanedione (2 mmol) and various aromatic aldehydes

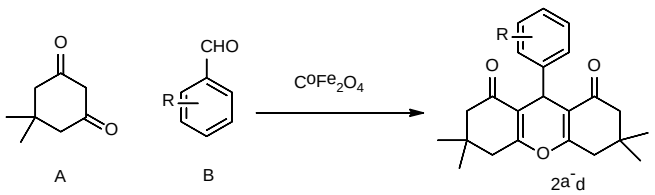
(1 mmol) in presence of $MnFe_2O_4$ (1 mmol) at 110 °C under solvent-free conditions. Most importantly, aromatic aldehydes with substituent's bearing either electron-donating or electron-withdrawing groups as well as heterocyclic aldehydes reacted successfully in the presence of $MnFe_2O_4$. In all these reactions expected products were obtained in good to excellent yields. The results are shown in Table 1. The suggested mechanism for the $MnFe_2O_4$ promoted synthesis of 1,8-dioxooctahydro xanthenes is shown in Scheme. Concerning the reaction mechanism, we suggest that, initially activation of the carbonyl group of aldehyde by $MnFe_2O_4$ facilitates nucleophilic attack of dimedone in its enol form and form the corresponding carbocation. This carbocation then reacts with these activated dimedone to give intermediate, which then undergo dehydration to give the final product.

Table 1. Synthesis of 1,8-dioxooctahydroxanthene by condensation of aldehydes and 5,5-dimethyl-1,3-cyclohexanedione using $MnFe_2O_4$.

R	Product	Time, min	% Yield	M.P., ^{0}C	
				Found	Reported
H	1a	45	90	203-204	204-205*
3-Cl	1b	45	95	180-182	182-184*
4-Cl	1c	60	92	225-227	226-228*
4-NO_2	1d	50	94	223-225	224-225*
4-OH	1e	60	92	245-246	247-248*

Synthesis of 3,3-arylidene bis(4-hydroxycoumarin) derivatives

An efficient method was proposed for the condensation of aldehydes with 4-hydroxycoumarin, which led to the corresponding 3,3-arylidene bis-(4-hydroxycoumarin) and different aldehydes in the presence of $CoFe_2O_4$. Initially, the systematic evaluation of different solvents fo rthe model reaction of 3-nitro benzaldehyde and 4-hydroxycoumarin in the presence of $CoFe_2O_4$ in water at reflux was focused on. Attempts were made to study and optimize the reaction conditions in order to show that performing the reaction in H_2O with low yield while using the amounts of EtOH in the media produced satisfactory results. These results revealed that the highest yield was obtained with the water/ethanol (1:1) solvent system.



Scheme 2. Synthesis of 1,8-dioxooctahydroxanthenes in the presence of cobalt ferrite ($CoFe_2O_4$)

In order to check the viability of this protocol in obtaining a series of 3,3-arylidene bis (4-hydroxycoumarin) derivatives, arrange of dicoumarols was synthesized using different aldehydes and 4-hydroxycoumarin under the standardized reaction condition (Table 2). Regardless of the nature of the substitution (electron donating and electron withdrawing) of the aromatic aldehydes, the products were obtained in good to excellent yields. In these reactions, there was no need for the column purification of the products. The obtained solid products were just filtered off from the reaction mixture, dissolved in hot ethanol, refiltered to separate solid mixed oxide residue and finally recrystallized from the filtrate to obtain pure dicoumarols.[6]

According to the proposed mechanism, the formation of 3,3-arylidene bis(4-hydroxycoumarin) could be rationalized. From the Knoevenagel condensation of aromatic aldehydes with 4-hydroxycoumarin in the presence of $CoFe_2O_4$ and followed by Michele addition of the second 4-hydroxycoumarin (Scheme 2).

Table 2. Synthesis of 3,3-arylidene bis(4-hydroxycoumarin) derivatives bycondensationofaldehydes and 4-hydroxycoumarin using $CoFe_2O_4$

R	Pro-duct	Time, min	Yield, %	M.P., ^{0}C	
				Found	Reported
H	**2a**	45	96	232-234	230-232*
4-OMe	**2b**	45	95	249-251	246-248*
4-Cl	**2c**	45	92	258-260	256-258*
4-NO_2	**2d**	45	90	237 - 240	232-234*

EXPERIMENTAL

Melting points were determined on an electrothermal apparatus, and the temperature was not calibrated. IR spectra were recorded as thin films on KBr using a Perkin-Elmer 1700 spectrophotometer. The NMR spectra were recorded on a Bruker ARX-300 spectrometer. Sample solutions were prepared in dimethylsulfoxide (DMSO) containing tetramethylsilane (TMS) as an internal reference. Mass spectra were recorded on a JMS-DX300 at 70 eV.
All chemical reagents were commercially available and purified with standard methods before use. Solvents were dried in routine ways and redistilled.

General procedure for the synthesis of 1,8-dioxooctahydro-xanthene Derivatives:

The 5,5-dimethyl-1,3-cyclohexanedione (2 mmol), an aromatic aldehyde (1 mmol) and $MnFe_2O_4$ (1 mmol) was heated in the oil bath at 110°C for the appropriate time. The progress of reaction was monitored by thin layer chromatography (TLC). Upon completion, the reaction mixture was cooled to room temperature and ethanol (10 ml) was added. The $MnFe_2O_4$ was recovered from filtrate. The residue was washed with ethanol (95%) to give compounds **3a-l** in high yields. Recovered $MnFe_2O_4$ was washed with diethyl ether (10 ml) and calcined at 120 °C for 1 h, before reusing.

3,3,6,6-Tetramethyl-9-phenyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (1a).

^{1}H NMR ($CDCl_3$, 500 MHz): 0.99 (s, 12H), 1.90 (s, 4H), 1.92 (s, 4H), 3.90 (s, 1H), 7.11–7.32 (m, 5H, Ar-H); IR (KBr) : 1710, 1622, 1545, 1509, 1120 cm^{-1}; MS (70 eV) m/z (%): 351.45 (M^++1, 100).

9-(3-Chlorophenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (1b).

^{1}H NMR ($CDCl_3$, 500 MHz): 0.98 (s, 12H), 1.92 (s, 4H), 1.94 (s, 4H), 3.93 (s, 1H), 7.11–7.13 (d, 2H, Ar-H); 7.30–7.32 (d, 2H, Ar-H); IR (KBr): 1712, 1620, 1542, 1504, 1122 cm^{-1}; MS (70 eV) m/z (%):385.90 (M^++1, 100).

9-(4-Chlorophenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (1c).

^{1}H NMR ($CDCl_3$, 500 MHz): 0.98 (s, 12H), 1.92 (s, 4H), 1.94 (s, 4H), 3.92 (s, 1H), 7.30–7.32 (m, 4H, Ar-H); IR (KBr): 1710, 1622, 1545, 1509, 1120 cm^{-1}; MS (70 eV) m/z (%): 385.90 (M^++1, 100).

3,3,6,6-Tetramethyl-9-(4-nitrophenyl)-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (1d).

^{1}H NMR ($CDCl_3$, 500 MHz): 0.99 (s, 12H), 1.90 (s, 4H), 1.92 (s, 4H), 3.91 (s, 1H), 7.48–7.50 (d, 2H, Ar-H); 8.14–8.16 (d, 2H, Ar-H); IR (KBr): 1710, 1622, 1545, 1509, 1120 cm^{-1}; MS (70 eV) m/z (%): 396.45 (M^++1, 100).

9-(4-Hydroxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (1e).

^{1}H NMR ($CDCl_3$, 500 MHz): 0.99 (s, 12H), 1.90 (s, 4H), 1.92 (s, 4H), 3.88 (s, 1H), 5.50 (s, 1H), 6.80–6.82 (d, 2H, Ar-H); 7.14–7.16 (d, 2H, Ar-H); IR (KBr): 1710, 1622, 1540, 1506, 1115 cm^{-1}; MS (70 eV) m/z (%): 367.45 (M^++1, 100).

General procedure for the synthesis of 3,3-arylidene bis(4-hydroxycoumarin) Derivatives

A mixture of 4-hydroxycoumarin (2 mmol, 0.324 g), substituted benzaldehydes (1 mmol, 0.106 g), and cobalt ferrite ($CoFe_2O_4$, 1 mmol, 0.326 g) was stirred at reflux in 5 ml ethanol-water mixture (1:1). The progress of the reaction was monitored by TLC. After the reaction completion and upon its cooling, the solid material was precipitated from the solution. The precipitates were filtered off, washed with water, and were recrystallized from EtOH to obtain pure 3,3-arylidenebis(4-hydroxy-*2H*-chromen-2-ones) derivatives as yellow-white solids.[8]

3,3,6,6-Tetramethyl-9-phenyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (2a).

^{1}H NMR ($CDCl_3$, 500 MHz): 0.99 (s, 12H), 1.90 (s, 4H), 1.92 (s, 4H), 3.90 (s, 1H), 7.11–7.32 (m, 5H, Ar-H); IR (KBr) : 1710, 1622, 1545, 1509, 1120 cm^{-1}; MS (70 eV) m/z (%): 351.45 (M^++1, 100).

9-(4-Methoxyphenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (2b).

^{1}H NMR ($CDCl_3$, 500 MHz): 0.98 (s, 12H), 1.92 (s, 4H), 1.94 (s, 4H), 3.80 (s, 3H), 3.92 (s, 1H), 7.10–7.12 (d, 2H, Ar-H); 7.30–7.32(d, 2H, Ar-H); IR (KBr): 1710, 1622, 1545, 1509, 1120 cm^{-1}; MS (70 eV) m/z (%): 381.50 (M^++1, 100).

9-(4-Chlorophenyl)-3,3,6,6-tetramethyl-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (2c).

^{1}H NMR ($CDCl_3$, 500 MHz): 0.98 (s, 12H), 1.92 (s, 4H), 1.94 (s, 4H), 3.92 (s, 1H), 7.30–7.32 (m, 4H, Ar-H); IR (KBr): 1710, 1622, 1545, 1509, 1120 cm^{-1}; MS (70 eV) m/z (%): 385.90 (M^++1, 100).

3,3,6,6-Tetramethyl-9-(4-nitrophenyl)-3,4,5,6,7,9-hexahydro-1H-xanthene-1,8(2H)-dione (2d).

^{1}H NMR ($CDCl_3$, 500 MHz): 0.99 (s, 12H), 1.90 (s, 4H), 1.92 (s, 4H), 3.91 (s, 1H), 7.48–7.50 (d, 2H, Ar-H); 8.14–8.16 (d, 2H, Ar-H); IR (KBr): 1710, 1622, 1545, 1509, 1120 cm^{-1}; MS (70 eV) m/z (%): 396.45 (M^++1, 100).

CONCLUSION

In conclusion, manganese ferrite ($MnFe_2O_4$) and cobalt ferrite ($CoFe_2O_4$) were proved to be efficient promoter for the synthesis of dicoumarols and 1,8-dioxooctahydroxanthenes, respectively. These conditions had advantages such as shorter reaction time, simpler work-up, inexpensive and non-toxic promoter, environmental benignity and excellent yields. The protocol described herein is advantageous in terms of preclusion of hazardous organic solvents, low amount of prooter, shorter reaction time, good yields, recovery and reusability of the promoter.

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REFERENCES

[1]Lambert, R. W., Martin, J. A., Merrett, J. H., Parkes, K. E. B., Thomas, G. J., PCT Int. Appl. WO 9706178; *Chem. Abstr*., **1997**, *126*, 212377y. http://dx.doi.org/10.4236/me.2014.51005

[2]Hideo, T., Tokkyo Koho JP 56005480; *Chem. Abstr*., **1981**, *95*, 80922b. https://doi.org/10.1080/17518253.2011.584217

[3]Ion, R. M., Frackowiak D., Planner, A., Wiktorowicz, K., The incorporation of various porphyrins into blood cells measured via flow cytometry, absorption and emission spectroscopy. *Acta. Biochim. Pol*., **1998**, *45*, 833-845. http://www.actabp.pl/pdf/3_1998/833.pdf

[4](a)Horning, E. C., Horning, M. G., Methone derivatives of aldehydes *J. Org. Chem*., **1946**, *11*, 95-99 https://doi.org/10.1021/jo01171a014 ; (b) Tu, S. J., Zhou, J. F., Lu, Z. S., Deng, X., Shi, D. Q. Wang, S. H., Condensation of aromatic aldehydes with 5,5-dimethyl- 1,3-cyclohexanedione without catalyst *Synth. Commun*., **2002**, *32*, 3063-3067 DOI:10.1081/SCC-120012999; (c) Jin, T. S., Zhang, J. S., Wang, A. Q., Li, T. S., Solid ‐ State Condensation Reactions Between Aldehydes and 5,5 ‐ Dimethyl ‐ 1,3 ‐ cyclohexanedione by Grinding at Room Temperature *Synth. Commun*., **2005**, *35*, 2339. DOI:10.1080/00397910500187282

[5]Wang, X. S., Shi, D. Q., Li, Y. L., Chen, H., Wei, X. Y., Zong, Z. M., A Clean Synthesis of 1 ‐ Oxo ‐ hexahydroxanthene Derivatives in Aqueous Media Catalyzed by TEBA *Synth. Commun*., **2005**, *35*, 97. DOI:10.1081/SCC-200046510

[6]Jin, T. S., Zhang, J. S., Xiao, J. C., Wang, A. Q., Li, T. S., Clean Synthesis of 1,8-Dioxo-octahydroxanthene Derivatives Catalyzed by p-Dodecylbenezenesulfonic Acid in Aqueous Media *Synlett*., **2004**, *5*, 866. DOI:10.1055/s-2004-820022

[7]Darvish, F., Balalaei, S., Chadegani, F., Salehi, P., Diammonium Hydrogen Phosphate as a Neutral and Efficient Catalyst for Synthesis of 1,8-Dioxooctahydroxanthene Derivatives in Aqueous Media *Synth. Commun*., **2007**, *37*, 1059-1067. DOI:10.1080/00397910701196520

[8]Jin, T. S., Zhang, J. S., Wang, A. Q. and Li, T. S., Ultrasound-assisted synthesis of 1,8-dioxo-octahydroxanthene derivatives catalyzed by p-dodecylbenzenesulfonic acid in aqueous media *Ultrason Sonochem*., **2006**, *13*, 220. DOI:10.1016/j.ultsonch.2005.04.002

[9]Dabiri, M., Baghbanzadeh, M., Arzroomchilar, E., 1-Methylimidazolium triflouroacetate ([Hmim]TFA): An efficient reusable acidic ionic liquid for the synthesis of 1,8-dioxo-octahydroxanthenes and 1,8-dioxo-decahydroacridines *Catal. Commun*., **2008**, *9*, 939-942. DOI:10.1016/j.catcom.2007.09.023

[10]Das, B., Thirupathi, P., Mahender, I., Reddy, V. S., Rao, Y. K., mberlyst-15: An efficient reusable heterogeneous catalyst for the synthesis of 1,8-dioxo-octahydroxanthenes and 1,8-dioxo-decahydroacridines *J. Mol. Catal. A. Chem*., **2006**, *247*, 233. DOI:10.1016/j.molcata.2005.11.048

[11]Das, B., Thirupathi, P., Mahender, I., Reddy, K. R., Ravikanth, B., Nagarapu, L., An efficient synthesis of 1,8-dioxo-octahydroxanthenes using heterogeneous catalysts *Catal. Commun*., **2007**, *8*, 535. DOI:10.1016/j.catcom.2006.02.023

[12]Pansare, D. N., Shelke, R. N., Pawar, C. D., A facile synthesis of (Z)-2-((5-(4-chlorobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)substituted acid using microwave irradiation and the conventional method. *Lett. Org. Chem*., **2017,** *14(7)*, 517. DOI: 10.2174/1570178614666170524142722

[13]Pansare, D. N., Shelke, R. N., Shinde, D. B., A facial synthesis and anticancer activity of (Z)-2-((5-(4-nitrobenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino)-substituted acid, *J. Het. Chem*., **2017**, *54(6)*, 3077. DOI: 10.1002/jhet.2919

[14]Pansare, D. N., Shinde, D. B., A facile synthesis of novel series (Z)-2-((4-oxo-5-(thiophen-2-yl methylene)-4,5-dihydro thiazol-2-yl)amino) substituted acid, *J. Saudi. Chem. Soc*., **2017**, *21*, 434. https://doi.org/10.1016/j.jscs.2015.10.005

[15]Pansare, D. N., Shelke, R. N., Khade, M. C., Jadhav, V. N., Pawar, C. D., Jadhav, R. A., Bembalkar, S. R., New thiazolone derivatives: design, synthesis, anticancer and antimicrobial activity. *Eur. Chem. Bull.* **2019**, *8(1)*, 7-14. DOI: 10.17628/ecb.2019.8.7-14

[16]Pansare, D. N., Shelke, R. N., Khade, M. C., Jadhav, V. N., Pawar, C. D., Deshmukh, S.U., Dhas, A. K., Chavan, P. N., Sarkate, A. P., Pawar, R. P., Shinde, D. B., Thopate, S. R., Synthesis and anticancer evaluation of new benzenesulfonamide derivatives. *Eur. Chem. Bull*. **2019**, *8(1)*, 1-6. DOI: 10.17628/ecb.2019.8.1-6

[17]Shelke, R. N., Pansare, D. N., Khade, M. C., Jadhav, V. N., Pawar, C. D., Deshmukh, S.U., Sarkate, A. P., Gore N. S., Pawar, R. P., Shinde, D. B., Thopate, S. R., Synthesis of 2-((5-benzylidene-4-oxo-4,5-dihydrothiazol-2-yl)-substituted amino acids as anticancer and antimicrobial agents. *Eur. Chem. Bull.* **2019**, *8(2)*, 63. DOI: 10.17628/ecb.2019.8.63-70

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ONE POT, FOUR-COMPONENT SYNTHESIS 2-AMINO-6-(1,4-DIOXO-3,4-DIHYDROPHTHALAZIN-2(1H)-YL)-4-ARYL-4H-PYRAN-3,5-DICARBONITRILES IN AN IONIC LIQUID

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Keywords: Diethyl phthalate, benzaldehydes, ethyl cyanohydrazide active methylene compounds; 1,8-diazabicyclo[5.4.0]-undec-7-en-8-ium acetate

[DBUH][OAc] (1,8-diazabicyclo[5.4.0]-undec-7-en-8-ium acetate) mediated, green synthesis of 2-amino-6-(1,4-dioxo-3,4-dihydrophthalazin-2(1H)-yl)-4-aryl-4H-pyran-3,5-dicarbonitriles have been synthesized by condensing diethyl phthalate, ethyl cyanohydrazide, benzaldehydes and malononitrile in [DBUH][OAc] medium, at 60-65 ºC for 2 h. Particularly valuable features of this method include high yield, broad substrate scope, shorter reaction times and straightforward procedure

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INTRODUCTION

Non-volatile, room-temperature ionic liquids (RTILs) have been extensively used as solvents and catalysts in ‘‘green chemistry’’,[1] a major driving force motivating organic chemists to develop environmentally benign methods of preparation of organic compounds.[2] Ionic liquids are widely used in many fields of chemistry and industry.[3] Their use as catalysts has attracted much attention in organic synthesis because product isolation and catalyst recycling are straightforward; occasionally, improvements in reaction rate and/or selectivity are also observed.[4]

Multi-component reactions (MCRs) are one-pot processes in which three or more compounds react in a single reaction vessel to form a product containing substantial components of all the reactants.[5] Thus, design of highly efficient chemical reaction sequences that provide maximum structural complexity and diversity with a minimum number of steps in the synthesis of compounds with interesting properties is important for drug discovery and synthesis of natural products.[6] MCRs have attracted much attention in combinatorial and medicinal chemistry and have been designed to produce biologically active compounds.[7] One of the main challenges in medicinal chemistry is the design and synthesis of biologically active molecules.[8]

Phthalazines are important heterocycles that are known to possess multiple biological activities such as antimicrobial, anticonvulsant, antifungal, anticancer and anti-inflammatory.[9] Carling *et* al. reported[10] the synthesis of 3-phenyl-6-(2-pyridyl)methyloxy-1,2,4-triazolo[3,4-*a*]phthalazines and analogs which were found to be a key structural element of certain CNS - active drugs. Jain *et* al. reported.[11] the synthesis of keto-glutamine tetrapeptide analogs containing a 2-oxo-pyrrolidine ring as a glutamine side chain mimic which showed improved inhibition against hepatitis A virus 3C proteinase. Grasso *et* al. reported[12] the synthesis of 6,7-methylenedioxyphthalazin-1(2*H*)-ones which were found to be potent anticonvulsant agents. Nomoto *et* al. reported[13] the synthesis of 6,7-dimethoxyphthalazine derivatives which showed relatively potent cardiotonic activity comparable to that of amrinone. Watanabe *et* al. reported[14] the synthesis of 4-benzylamino-1-chloro-6-substituted phthalazine which was found to be vasorelaxant activities and a number of methods have been reported for the synthesis of phthalazine derivatives.[15] Therefore, it was considered worthwhile to synthesize phthalazine moiety containing 4*H*-pyrans.

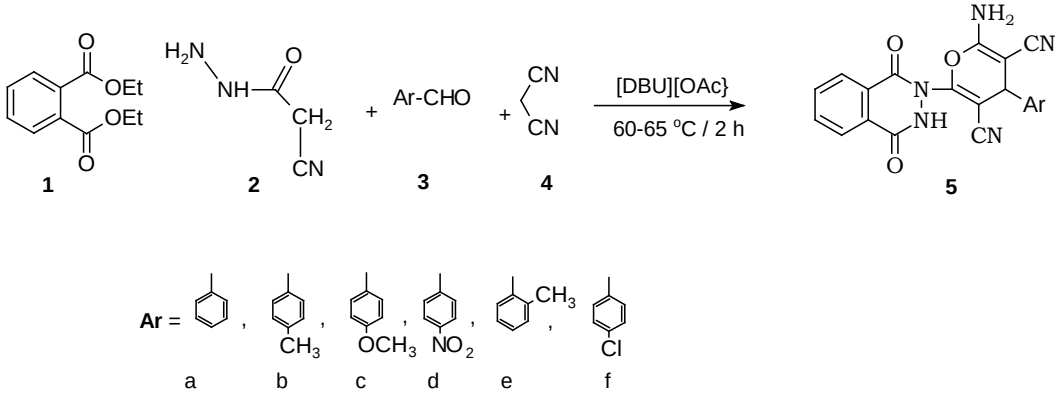
Keeping these results in our mind, we now wish to report one-pot, three-component synthesis of title compounds in weakly basic [DBUH][OAc] medium.

RESULTS AND DISCUSSION

Initially, one pot, four-component reaction of diethyl phthalate **1** (1 mmol), ethyl cyanohydrazide[16] **2** (1 mmol), benzaldehyde **3a** (1 mmol), and malononitrile **4** (1 mmol) in different ionic liquid medium (([DBUH][OAc], [bmim][Br] and [bmim][OH]) at 60-65 ºC to form 2-amino-6-(1,4-dioxo-3,4-dihydrophthalazin-2(1H)-yl)-4-phenyl-4H-pyran-3,5-dicarbonitrile **5a** have been taken as a model reaction. However, it was found that the one-pot reaction of in the presence of [DBUH][OAc] as a medium for 2 h at 60-65 ºC gave the highest yield (92 %) and the clean product **5a** (Table 1, entry 1). Here, initially compound **1** was reacted with **2** in [DBUH][OAc] at 60-65 ºC for 20 min to form 3-(1,4-dioxo-3,4-dihydrophthalazin-(1H)-yl)-3-oxo-propanenitrile as intermediate **6** (confirmed by TLC that means the absence of starting materials). Then to this reaction mixture added **3a** and **4** and again heated at 60-65 ºC for 1.5 h to form 2-amino-6-(1,4-dioxo-3,4-dihydrophthalazin-2(1H)-yl)-4-phenyl-4H-pyran-3,5-

dicarbonitrile **5a**. The product i.e 2-amino-6-(1,4-dioxo-3,4-dihydrophthalazin-2(1H)-yl)-4-phenyl-4H-pyran-3,5-dicarbonitrile **5a** were obtained in excellent yield (92%) on simple work-up of reaction mixture. The structure of the compound **5a** has been confirmed by ^{1}H-NMR, IR and mass spectroscopy.

Encouraged by above optimization results conditions, the one-pot reaction has been carried out at different temperature (RT, 40, 60 and 80 ^{o}C) in the presence of [DBUH][OAc] mediated to get desired compound **5a**. However, it was found that the one-pot reaction of **1** (1 mmol), **2** (1 mmol), **3a** (1 mmol) and **4a** (1 mmol)] in the presence of [DBUH][OAc] as medium (1 mmol) for 120 min at 60-65 ^{o}C gave the highest yield (92 %) and the clean product **5a** (Table 1, entry 1). In order to examine the quantity of [DBUH][OAc], the one-pot reaction has been carried out at different quantity (0.5, 1 and 2 mmol) of [DBUH][OAc] with respect of diethyl phthalate **1**. However, it was found that the one-pot reaction of [1 (1 mmol), 2 (1 mmol), 3a (1 mmol) & 4a (1 mmol)] in the presence of [DBUH][OAc] as medium (1 mmol) for 2 h at 60-65 ^{o}C gave the highest yield (92%) (Table 2, entry 2).

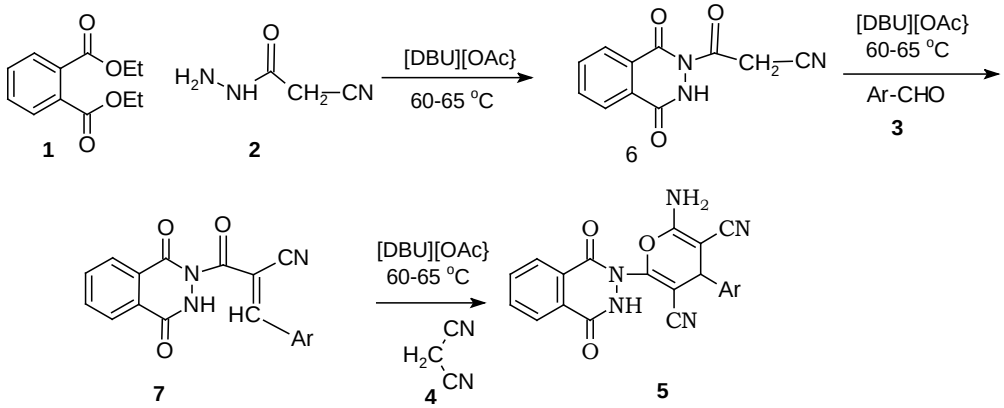


Scheme 1. Preparation of compound **5** by one-pot synthesis

After having optimized the reaction conditions, the generality of the reaction was confirmed by carrying out the condensation of several others **3b-3f** respectively in [DBUH][OAc] medium at 60-65 ^{o}C for 2 h giving **5b-5f** very good yields and no side product formation was detected. It was found that this method works with a wide variety of substrates. It is worthy of mentioning that the reaction of compounds **1**, **2**, **3a-3f** and **4** could get higher yield and require shorter reaction time for the formation of **5a-5f**.

The synthesis of **5** could also be achieved in step-wise syntheses. Thus, a mixture of 1 and 2 was heated at 60-65 ^{o}C for 0.5 h in [DBUH][OAc] medium to form intermediate **6**.[17] Then, 6 was reacted with **3** at 60-65 ^{o}C for 0.5 h in [DBUH][OAc] medium to form intermediate **7**[18] followed by **7** was reacted with **4** at 60-65 ^{o}C for 0.5 h in [DBUH][OAc] medium to form **5**. The reaction was monitored by TLC. The structures of these products have been established earlier based on their spectral data (Scheme 2).

Furthermore, compound **5** was assigned E-configuration on the presumption that bulky groups in a trans position would confer thermal stability on the molecule. This is the case by a careful examination of the Frame-work molecular models of both E and Z-configurations of **5** wherein it was observed that there was a minimum number of steric interactions in the E-configuration.



Scheme 2. Stepwise synthesis of compound **5**.

Table 1. Effect of ionic liquid, the temperature on the reaction of compounds **1**, **2**, **3a** and **4** to yielding **5a**.

Entry	Ionic liquid	T, °C	Time, h	5a, %
1	[DBUH][OAc]	60-65	2	92
2	[bmim][Br]	60-65	5	75
3	[bmim][OH]	60-65	3	78
4	[DBUH][OAc]	RT	12	81
5	[DBUH][OAc]	40-45	3.5	71
6	[DBUH][OAc]	80-85	1.5	62

Table 2. The effect of the amount of [DBUH][OAc] in the preparation of compounds **5a** from **1, 2**, **3a** and **4**.

Entry	[DBUH][OAc], mmol/mmol of 1	Time, h	5a, %
1	0.5	4	85
2	1	2	90
3	2	2	80

EXPERIMENTAL SECTION

Melting points are uncorrected and were determined in open capillary tubes in sulphuric acid bath. TLC was run on silica gel – G and visualization was done using iodine vapour or UV light. IR spectra were recorded using Perkin – Elmer 1000 instrument in KBr pellets. ^{1}H NMR spectra were recorded in DMSO–d_6 using TMS as internal standard at 400 MHz operating frequency. Mass spectra were recorded on Agilent-LCMS instrument. All reagents were purchased from Merck or Aldrich and used without further purification. [DBUH][Ac] was prepared as reported elsewhere.[17]

Preparation of compounds 5a-5f from compounds 1, 2, 3a-3f and 4 by one-pot synthesis:

A mixture of **1** (1 mmol) and **2** (1 mmol) were heated at 60-65 °C for 0.5 h in [DBUH][OAc] (1 mmol) for 0.5 h. (until no starting materials could be detected on thin-layer chromatography (TLC)). To this reaction mass added compounds **3** and **4** and heated again at at 60-65 °C for 1.5 h. (until no starting materials could be detected on thin-layer chromatography). After the reaction was complete, cold water was added to the reaction mixture and solid part was separated by filtration. The product was recrystallised from ethanol solvent to obtain compounds **5.**

5a: Mp: 139–141 °C; IR (KBr): 3306-3401 cm^{-1} (broad, medium, -NH-), 2218 cm^{-1} (sharp, strong, -CN-), 1706 cm^{-1} (sharp, strong, -CO- of amide group), 1659 cm^{-1} (sharp, strong, -CO- of amide group); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 6.1 (s, 1H, -CH), 7.5-8.0 (m, 9H, Ar-H), 9.6 (s, 2H, –NH_2), 11.6 (s, 1H, -NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6, 400 MHz): δ 48.5, 75.5, 86.3, 114.6, 115.1, 124.5, 127.9, 128.6, 129.7, 130.7, 134.1, 136.0, 155.8, 157.4, 163.0, 163.6; HRMS calcd for $C_{21}H_{13}N_5O_3$ $[M+H]^+$: 384.0427. Found: 384.0424.

5b: Mp: 181–182 °C; IR (KBr): 3309-3405 cm^{-1} (broad, medium, -NH-), 2210 cm^{-1} (sharp, strong, -CN-), 1716 cm^{-1} (sharp, strong, -CO- of amide group), 1656 cm^{-1} (sharp, strong, -CO- of amide group); ^{1}H- NMR (DMSO-d_6, 400 MHz): δ 6.1 (s, 1H, -CH), 7.5-8.0 (m, 9H, Ar-H), 8.1 (s, 2H, –NH2), 11.6 (s, 1H, -NH, D2O exchangeable); ^{1}H- NMR (DMSO-d_6, 400 MHz): δ 2.2 (s, 1H, -CH_3), 6.2 (s, 1H, -CH), 7.5-8.1 (m, 8H, Ar-H), 9.7 (s, 2H, –NH_2), 11.4 (s, 1H, -NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6, 400 MHz): δ 33.4, 47.3, 74.3, 82.9, 110.2, 111.3, 123.5, 124.3, 125.7, 128.3, 131.5, 135.3, 137.1, 153.4, 155.2, 163.3, 164.5; M^++1 =398.

5c: Mp: 141–143 °C; IR (KBr): 3303-3405 cm^{-1} (broad, medium, -NH-), 2217 cm^{-1} (sharp, strong, -CN-), 1704 cm^{-1} (sharp, strong, -CO- of amide group), 1658 cm^{-1} (sharp, strong, -CO- of amide group); ^{1}H- NMR (DMSO-d_6, 400 MHz): δ 6.1 (s, 1H, -CH), 7.5-8.0 (m, 9H, Ar-H), 9.4 (s, 2H, –NH_2), 11.6 (s, 1H, -NH, D_2O exchangeable); ^{1}H- NMR (DMSO-d_6, 400 MHz): δ 3.2 (s, 1H, -OCH_3), 6.3 (s, 1H, -CH), 7.5-8.2 (m, 8H, Ar-H), 9.3 (s, 2H, –NH_2), 11.4 (s, 1H, -NH, D_2O exchangeable); 13C-NMR (DMSO-d_6, 400 MHz): δ 38.5, 43.4, 73.4, 84.2, 111.4, 112.4, 123.4, 125.3, 127.3, 128.3, 131.6, 133.4, 134.3, 156.3, 157.3, 163.1, 164.1; M^++1 =414.

5d: Mp: 162–163 °C; IR (KBr): 3304-3402 cm^{-1} (broad, medium, -NH-), 2211 cm-1 (sharp, strong, -CN-), 1704 cm^{-1} (sharp, strong, -CO- of amide group), 1655 cm^{-1} (sharp, strong, -CO- of amide group); ^{1}H- NMR (DMSO-d_6, 400 MHz): δ 6.1 (s, 1H, -CH), 7.5-8.0 (m, 9H, Ar-H), 8.1 (s, 2H, –NH_2), 11.6 (s, 1H, -NH, D_2O exchangeable); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 6.2 (s, 1H, -CH), 7.4-8.0 (m, 8H, Ar-H), 9.1 (s, 2H, –NH_2) 11.6 (s, 1H, -NH, D2O exchangeable); ^{13}C-NMR (DMSO-d_6, 400 MHz): δ 49.3, 74.3, 85.3, 113.1, 114.6, 123.4, 124.5, 127.3, 128.4, 131.1, 133.2, 135.3, 145.4, 150.2, 163.1, 163.3; M^++1 =429.

5e: Mp: 181–183 °C; IR (KBr): 3306-3404 cm^{-1} (broad, medium, -NH-), 2214 cm^{-1} (sharp, strong, -CN-), 1702 cm^{-1} (sharp, strong, -CO- of amide group), 1655 cm^{-1} (sharp, strong, -CO- of amide group); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 6.1 (s, 1H, -CH), 7.5-8.0 (m, 9H, Ar-H), 8.1 (s, 2H, –NH2), 11.6 (s, 1H, -NH, D_2O exchangeable); 1H- NMR (DMSO-d_6, 400 MHz): δ 2.3 (s, 1H, -CH3), 6.1 (s, 1H, -CH), 7.4-8.1 (m, 8H, Ar-H), 9.2 (s, 2H, –NH_2), 11.3 (s, 1H, -NH, D2O exchangeable); ^{13}C-NMR (DMSO-d_6, 400 MHz): δ 28.3, 43.2, 74.1, 80.0, 109.5, 110.4, 118.4, 120.8, 125.4, 127.6, 131.6, 134.3, 135.2, 150.1, 153.2, 164.1, 164.7; M^++1 =398.

5f: Mp: 178–180 °C; IR (KBr): 3302-3401 cm^{-1} (broad, medium, -NH-), 2217 cm^{-1} (sharp, strong, -CN-), 1707 cm^{-1} (sharp, strong, -CO- of amide group), 1653 cm^{-1} (sharp, strong, -CO- of amide group); 1H- NMR (DMSO-d_6, 400 MHz): δ 6.1 (s, 1H, -CH), 7.5-8.0 (m, 9H, Ar-H), 8.1 (s, 2H, –NH_2), 11.6 (s, 1H, -NH, D2O exchangeable); ^{1}H- NMR (DMSO-d_6, 400 MHz): δ 6.1 (s, 1H, -CH), 7.5-8.0 (m, 8H, Ar-H), 9.3 (s, 2H, –NH_2), 11.3 (s, 1H, -NH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6, 400 MHz): δ 42.3, 74.4, 85.2, 110.5, 114.3, 122.4, 126.5, 128.6, 129.1, 131.4, 134.4, 136.3, 155.6, 157.4, 163.3, 163.9; M^+.+1 =418.

Preparation of compound 6 from compounds 1 and 2 via step-wise reaction

A mixture of diethyl phthalate **1** and ethylcyanohydrazide **2** were heated at 60-65 °C in [DBUH][OAc] (1 mmol) for 0.5 h. After the completion of the reaction as monitored by TLC, the reaction mixture was poured into ice-cold water. The product was precipitated out, filtered, washed with water, dried and recrystallised from ethanol. Yield=72 %. M.P. 152-154˚C [Lit M.P. 150-152 °C].[17]

Preparation of compounds 7 from compound 6 and compounds 3

A mixture of **6** (1 mmol), **3a-3f** (1 mmol) and [DBUH][OAc] (1 mmol) were heated at 60-65 °C for 0.5 h. After the completion of the reaction as monitored by TLC, the reaction mixture was poured into ice-cold water. The product was precipitated out, filtered, washed with water, dried and recrystallised from ethanol. Yield =88 %.

7a: Mp: 200–202 °C; IR (KBr): 3267-3518 cm^{-1} (broad, medium, -NH-), 2258 cm^{-1} (sharp, strong, -CN-), 1749 cm-1 (sharp, strong, -CO- group), 1683 cm^{-1} (sharp, strong, -CO- of amide group),1613 cm-1 (sharp, strong, -CO- of amide group); ^{1}H- NMR (DMSO-d_6, 400 MHz): δ 7.6-8.4 (m, 10H, Ar-H and NC-C=CH), 11.4 (s, 1H, -OH, D_2O exchangeable); 13C-NMR (DMSO-d_6, 400 MHz): δ 35.5, 81.3, 81.4, 117.2, 122.9, 124.0, 127.2, 128.4, 128.5, 129.0, 129.1, 129.6, 133.0, 136.1, 143.4, 164.4, 164.5, 164.8; HRMS calcd for C18H11N3O3 $[M+H]^+$: 318.0423. Found: 318.0426.

7b: Mp: 195–197 °C; IR (KBr): 3266-3513 cm^{-1} (broad, medium, -NH-), 2253 cm^{-1} (sharp, strong, -CN-), 1742 cm^{-1} (sharp, strong, -CO- group), 1684 cm^{-1} (sharp, strong, -CO- of amide group),1617 cm^{-1} (sharp, strong, -CO- of amide group); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 2.8 (s, 3H, CH_3), 7.6-8.4 (m, 9H, Ar-H and NC-C=CH), 11.5 (s, 1H, -OH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6, 400 MHz): δ 34.4,101.5, 106.3, 107.3, 114.4, 116.6, 122.2, 125.3, 131.4, 132.1, 133.1, 134.3, 138.3, 139.2, 140.4, 143.9, 154.8, 161.8, 164.9; M^+.+1 =332.

7c: Mp: 228–230 °C; IR (KBr): 3263-3515 cm^{-1} (broad, medium, -NH-), 2252 cm^{-1} (sharp, strong, -CN-), 1743 cm^{-1} (sharp, strong, -CO- group), 1682 cm^{-1} (sharp, strong, -CO- of amide group), 1616 cm^{-1} (sharp, strong, -CO- of amide group); ^{1}H- NMR (DMSO-d_6, 400 MHz): δ 4.0 (s, 3H, OCH_3), 7.1-8.3 (m, 9H, Ar-H and NC-C=CH), 11.3 (s, 1H, -OH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6, 400 MHz): δ 44.5, 100.2, 104.2, 105.5, 110.6, 113.7, 121.3, 123.6, 128.4, 131.2, 132.8, 134.2, 135.6, 138.3, 141.3, 144.2, 158.1, 161.4, 163.5; M^+.+1 =348.

7d: Mp: 220–222 °C; IR (KBr): 3263-3512 cm^{-1} (broad, medium, -NH-), 2251 cm^{-1} (sharp, strong, -CN-), 1740 cm^{-1} (sharp, strong, -CO- group), 1681 cm-1 (sharp, strong, -CO- of amide group),1616 cm^{-1} (sharp, strong, -CO- of amide group); ^{1}H- NMR (DMSO-d_6, 400 MHz): δ 7.4-8.3 (m, 9H, Ar-H and NC-C=CH), 11.0 (s, 1H, -OH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6, 400 MHz): δ 101.4, 103.2, 104.2, 109.1, 111.6, 118.3, 120.4, 124.6, 129.1, 132.4, 134.6, 135.7, 138.4, 140.2, 144.3, 158.1, 162.3, 165.3; M^+.+1 =363.

7e: Mp: 230–232 °C; IR (KBr): 3263-3512 cm^{-1} (broad, medium, -NH-), 2250 cm^{-1} (sharp, strong, -CN-), 1741 cm^{-1} (sharp, strong, -CO- group), 1681 cm^{-1} (sharp, strong, -CO- of amide group), 1618 cm-1 (sharp, strong, -CO- of amide group); ^{1}H- NMR (DMSO-d_6, 400 MHz): δ 2.9 (s, 3H, CH3), 7.6-8.4 (m, 9H, Ar-H and NC-C=CH), 11.6 (s, 1H, -OH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6, 400 MHz): δ 34.6,100.3, 104.2, 105.3, 113.4, 114.6, 121.2, 126.3, 130.3, 133.4, 134.5, 135.6, 138.5, 139.2, 140.4, 143.6, 156.4, 161.6, 164.4; M^+.+1 =332.

7f: Mp: 170–172 °C; IR (KBr): 3267-3518 cm^{-1} (broad, medium, -NH-), 2258 cm^{-1} (sharp, strong, -CN-), 1749 cm-1 (sharp, strong, -CO- group), 1683 cm^{-1} (sharp, strong, -CO- of amide group), 1613 cm^{-1} (sharp, strong, -CO- of amide group); ^{1}H- NMR (DMSO-d_6, 400 MHz): δ 7.4-8.3 (m, 9H, Ar-H and NC-C=CH), 11.1 (s, 1H, -OH, D_2O exchangeable); ^{13}C-NMR (DMSO-d_6, 400 MHz): δ 101.3, 103.4, 105.5, 108.3, 110.5, 119.2, 121.3, 123.5, 129.1, 131.4, 132.6, 134.7, 135.6, 141.2, 144.2, 158.4, 161.4, 166.2; M^+.+1 =352.

Preparation of compound 5 from compounds 7 and 4

A mixture of **7** (1 mmol), **4** (1 mmol) and [DBUH][OAc] (1 mmol) were heated at 60-65 °C for 1.0 h . After the completion of the reaction as monitored by TLC, the reaction mixture was poured into ice-cold water. The product was precipitated out, filtered, washed with water, dried and recrystallized from ethanol to form **5**. Yield =85%.

CONCLUSION

In summary, we have successfully adapted a simple one pot as well as step-wise and tandem process for the synthesis of novel 2-amino-6-(1,4-dioxo-3,4-dihydrophthalazin-2(1H)-yl)-4-phenyl-4H-pyran-3,5-dicarbonitrile with simple work up procedures in green methods.

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REFERENCES

[1]Inamuddin, A. M.; Green Solvents I: Properties and Applications of Ionic Liquids, Springer, United Kingdom, **2012**, https://www.springer.com/gp/book/9789400717114

[2]Inamuddin, A. M.; *Green Solvents II: Properties and Applications of Ionic Liquids*, Springer, United Kingdom, **2012,** https://www.springer.com/gp/book/9789400728905

[3]Tundo, P., Perosa, A., Zecchini, F., *Methods and Reagents for Green Chemistry: An Introduction*, Wiley, New Jersey, **2007**. doi:10.1002/9780470124086

[4]Wasserscheid, P., Welton, P., *Ionic Liquids in Synthesis* ,Wiley–VCH, Germany, **2003**. DOI:10.1002/9783527621194

[5]Montagne, C., Shiers, J. J., Shipman, M. Rapid generation of molecular complexity using "sequenced" multi-component reactions: one-pot synthesis of 5,5′-disubstituted hydantoins from methyleneaziridines, *Tetrahedron Lett*., **2006**, *47*, 9207. https://doi.org/10.1016/j.tetlet.2006.10.135

[6]Bienayme, H.; *Multicomponent Reactions*, Wiley–VCH, Weinheim, **2005**, 311-341. https://doi.org/10.1016/j.tetlet.2006.10.135

[7]Bra¨se, S., Gil, C, Knepper, K. The recent impact of solid-phase synthesis on medicinally relevant benzoannelated nitrogen heterocycles, *Bioorg. Med. Chem*., **2002**, *10*, 2415. https://doi.org/10.1016/S0968-0896(02)00025-1

[8]Hailes, H. C., Reaction Solvent Selection: The Potential of Water as a Solvent for Organic Transformations, *Org. Process Res. Dev*., **2007**, *11*, 114. DOI: 10.1021/op060157x

[9]El-Sakka, S. S.; Soliman, A. H.; Imam, A. M. Afinidad. 2009, 66, 167. Synthesis, antimicrobial activity and Electron Impact of Mass Spectra of Phthalazine-1,4-dione Derivatives, *Afinidad.* **2009**, *66*, 167. https://core.ac.uk/download/pdf/39152404.pdf

[10]Carling, R. W., Moore, K. W., Street, L. J., Wild, D., Isted, C., Leeson, P. D., Thomas, S., O'Conner, D., McKernan, R. M., Quirk, K., Cook, S. M., Atack, J. R., Wafford, K. A., Thompson, S. A., Dawson, G. R., Ferris, P., Castro, J. L. 3-Phenyl-6-(2-pyridyl)methyloxy-1,2,4-triazolo[3,4-a]phthalazines and Analogues: High-Affinity γ-Aminobutyric Acid-A Benzodiazepine Receptor Ligands with α2, α3, and α5-Subtype Binding Selectivity over α1, *J. Med. Chem*., **2004**, *47*, 1807; DOI: 10.1021/jm031020p

[11]Jain, R., Vederas, J. C., Structural variations in keto-glutamines for improved inhibition against hepatitis A virus 3C proteinase, *Bioorg. Med. Chem. Lett*., **2004**, *14*, 3655. https://doi.org/10.1016/j.bmcl.2004.05.021

[12]Grasso, S., DeSarro, G., Micale, N., Zappala, M., Puia, G., Baraldi, M., Demicheli, C., Synthesis and Anticonvulsant Activity of Novel and Potent 6,7-Methylenedioxyphthalazin-1(2H)-ones, *J. Med. Chem*., **2000**, *43*, 2851; DOI: 10.1021/jm001002x

[13]Nomoto, Y., Obase, H., Takai, H., Teranishi, M., Nakamura, J., Kubo, K., Studies on Cardiotonic Agents. II. : Synthesis of Novel Phthalazine and 1, 2, 3-Benzotriazine Derivatives, *Chem. Pharm. Bull (Tokyo)*, **1990**, *38*, 2179. https://doi.org/10.1248/cpb.38.2179

[14]Watanabe, N., Kabasawa, Y., Takase, Y., Matsukura, M., Miyazaki, K., Ishihara, H., Kodama, K., Adachi, H., 4-Benzylamino-1-chloro-6-substituted Phthalazines: Synthesis and Inhibitory Activity toward Phosphodiesterase 5, *J. Med. Chem*., **1998**, *41*, 3367. DOI:10.1021/jm970815r

[15]Sheradsky, T., Moshenberg, R., Bridgehead hydrazines. Unusual photorearrangement of 1,4-diphenylpyridazino[1,2-b]phthalazine-6,11-dione, *J. Org. Chem*., **1986**, *51*, 3123. DOI: 10.1021/jo00366a008

[16]Volmajer, J., Toplak, R., Bittner, S., Marechala, A. M. L. Regioselective combination of 1,3-dinucleophiles such as 2mercaptobenzimidazole, 5-mercapto-3-phenyltriazole and potassium thiocyanate with 2,2-dicyanooxiranes *ARKIVOC*. **2003**, *14*, 49. DOI: http://dx.doi.org/10.3998/ark.5550190.p008.002

[17]Kumar, P. P., Reddy, Y. D., Reddy, Ch. V. R., Devi, B. R., Dubey, P. K., One pot, three-component synthesis of novel 3,4-dihydrophthalazin-2(1H)-yl-4-phenyl-4H-pyrans, *Tetrahedron Lett.*, **2014**, *55*, 2177. https://doi.org/10.1016/j.tetlet.2014.02.020

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