RSC Advances

ROYAL SOCIETY OF CHEMISTRY

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Molybdate Sulfuric acid (MSA): an efficient reusable catalyst for the synthesis of tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones under solvent-free conditions and evaluation for their *in vitro* bioassay

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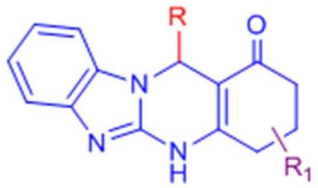
Received (in XXX, XXX) XthXXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

An efficient green synthesis of tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones has been achieved under solvent-free conditions of the reaction of 1H-benzo[d]imidazol-2-amine, various aldehydes and 1,3-dicarbonyl compounds in the presence of molybdate sulfuric acid (MSA) as a catalyst. Higher product yields were isolated easily with this reusable MSA catalyst and environmentally benign reaction conditions in shorter reaction time are the merits of this reaction. All the newly synthesized compounds were tested for their anti-oxidant and anticancer activities. Most of them showed good to excellent bio-activity in both experiments.

Now-a-days worldwide the second most cause of death is a class of cancer diseases by uncontrolled cell growth.[1] Even though more than 50 years past of the toxic action discovery of nitrogen mustards on cancer cells still there is a need to development of effective cytotoxic agents. On the other hand, reactive oxygen species (ROS) are the oxygen centred free radicals, which are generated in the human body and would cause damage to lipids, proteins and DNA and thus may lead to various diseases such as carcinogens, drug-associated toxicity, and inflammation. Furthermore, radical reactions play a significant role in the development of life limiting chronic diseases such as cancer, ageing, diabetes, arteriosclerosis and others.[2] Herein, the anti-oxidants are molecules either natural or synthetic have capable of interacting with free radicals and stopping their chain reactions before essential vital molecules are damaged.[3] Thus, they are recently fabricated as the drug candidates to counter these multifarious diseases to protect organisms and cells from these problems during metabolism.

Nitrogen-containing heterocycles played a major role in the pharmaceutical and agrochemical industries due to their often potent physiological properties, which have resulted in numerous applications, such as antibacterial,[4] antifungicidal,[5] antiviral,[6] antioxidant,[7] anti-inflammatory agents[8] and anticancer activity.[9] Among a large variety of N-containing heterocyclic compounds, tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones (A) have received considerable attention because of their pharmacological properties and clinical applications.[10] Moreover, they were found to possess multiple biological activities, such as antimicrobial,[11] antifungal,[12] anticancer,[13] anti-inflammatory,[14] anticonvulsant,[15] antihypertensive,[16] antihistaminic,[17] analgesic[18] and anti-HIV[19] activities.



(A)

Therefore, the synthesis of the tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones have attracted much attention in organic synthesis. So far, only a few methods have been reported for the synthesis of tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones.[20] However, these procedures also are limited in scope, because of relatively long reaction times and the use of an organic solvent, expensive ionic liquids and catalysts. Therefore, the search continues for a better green catalyst for the synthesis of heterocycles containing tetrahydrobenzo-[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones ring fragment in term of operational simplicity, inexpensive, environmentally benign practical procedure, economic viability and greater selectivity.

In this context, multi-component one-pot syntheses under solvent-free conditions have materialized as an efficient and powerful strategy in the modern synthetic organic chemistry because of synthesis of complex organic molecules from simple and readily available substrates can be achieved in a very fast and efficient manner without isolation of any intermediates.[21] The multi-component reactions (MCRs) contribute to these requirements of an environment friendly process by reducing the synthesis steps, energy consumption and waste production. Therefore, development of new MCRs and improvement of

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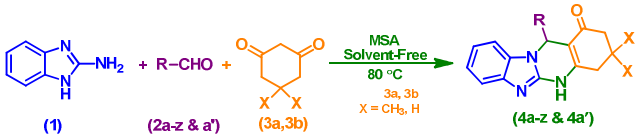
known MCRs are popular areas of research in the current synthetic organic chemistry.

On the other hand, sulfonic acid-containing catalysis has developed considerable interest in the various disciplines of science, including organic synthesis due to the prime advantage that, in most of the cases, these catalysts can be recovered without measurable changes in catalytic activity and selectivity and straightforward work-up, availability, eco-friendly reaction conditions, reusability and ability to promote a wide range of reactions.[22] Therefore, they can be used in continuous flow reactions. In this context molybdate sulfuric acid (MSA) being an proficient proton source was found synthetically useful in modern organic reactions.[23] It has numerous compensation over conventional acid catalysts, such as ease of handling, stability, less cost, easy recyclability due to insolubility in most of the organic solvent. Thus, it has been selected as a solid heterogeneous alternative to sulfuric acid.

Our literature survey at this stage revealed that there are no reports on the synthesis of tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones by MSA catalysis under solvent-free conditions. Therefore, the health risks stated above and the need for development of more effective anti-oxidant and anticancer drug molecules encourage us to design and synthesis of new chemical compounds with high efficiency, low toxicity and broad spectrum of bio-activity.

In continuation of our efforts to develop a better synthetic procedure in terms of operational simplicity, economic viability, greater selectivity and as well as the interest in applications of heterogeneous-catalyzed organic reactions[24] in green chemical synthetic approaches, herein, we report the results of tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones (**4a-z & 4a′**) in one-pot synthesis by coupling of 1H-benzo[d]imidazol-2-amine (**1**),various aldehydes (**2a-z & a'**) and 1,3-dicarbonyl compounds (3a, 3b) by using MSA as a reusable catalyst at 80 °C under solvent-free condition (**Scheme 1**) and studied their anticancer and anti-oxidant activities. In this study, we successfully identified the potential in vitro anti-oxidants and anticancer in synthesized compounds.



Scheme 1: Synthesis of tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones catalyzed by MSA under solvent-free condition and studied their anticancer and anti-oxidant activities.

Chemistry

For our initial investigation the reaction of 1H-benzo[d]imidazol-2-amine (1, 1 mmol), 2-pyridinecarboxaldehyde (2a, 1mmol) and dimidine (3a, 1 mmol) under neat condition at 80 °C (Scheme 1) run to standardize the experimental conditions. In the absence of catalyst we could not isolate any desired product even after 6 h stirring. After 10 h of stirring a trace amount of corresponding product was isolated (Table1, entry 1). After that, the same set of reaction was performed in the presence of 5 mol% of the PS/PTSA. Within 4 h the corresponding title product 4a, was isolated in 40% product yield (Table 1, entry 2).The product 4a was confirmed by usual spectroscopic techniques. Encouraged by this result, we attempted to optimize the yield of the reaction by screening the same set of reaction with various sulfonic acid containing catalysts such as glucose sulfonic acid (GSA), Phospho sulfonic acid (PSA), PEG-SO3H and MSA at even less than 3 h and the results are summarized in Table 1. Among all the screened catalysts MSA was found superior with respect to reaction time and product yield (Table 1, entry 6). Moreover, we found that the yields were obviously affected by the amount of MSA loaded. When 1 mol%, 2 mol%, 5 mol% and 10 mol% of MSA were used the yields were 65, 78, 92 and 92%, respectively (Table 1, entries 6-9). Therefore, 5 mol% of MSA was sufficient and no more significant improvement in the reaction rate and product yield was observed while increasing the amount of the catalyst from 5 to 10 mol% (Table 1, entry 9).

Table 1 Influence of the catalyst for the synthesis of 3,3-dimethyl-12-(pyridin-2-yl)-3,4,5,12-tetrahydrobenzo[4,5]imidazo[2,1-*b*]quinazolin-1(2*H*)-one (**4a**).[a]

Entry	Catalyst (mol%)	Time (min)	Yield[b] (%)
1	Neat	600	Trace
2	PT/*P*TSA (30 mg)	240	40
3	GSA (5)	170	65
4	PSA (5)	130	66
5	PEG-SO_3H (30 mg)	110	75
6[c]	MSA (5)	20	92, 90, 89, 86
7	MSA (1)	60	65
8	MSA (2)	40	78
9	MSA (10)	20	92

[a]Reaction of 1*H*-benzo[*d*]imidazol-2-amine (**1**, 1 mmol), Pyridine carboxaldehyde (**2a**, 1mmol) and dimidine (**3**, 1 mmol) under neat condition at 80 °C; [b]Isolated yield; [c]catalyst was reused four times.

Then we investigated the influence of various organic solvents at different reaction temperatures on the model reaction with 5 mol% of MSA and without catalyst. Among the various solvents such as toluene, THF, CH_2Cl_2, CH_3CN, and DMF rate of the reaction is sparingly slow and resulted in lower product yields (Table 2, entries 1-6). Conducting the same reaction in ethanol improved both the reaction rate as well as product yield (Table 2, entry 7). However, the better the product yield was observed in solvent-free conditions (Table 2, entry 8) and could be explained by a uniform distribution of the eutectic mixture of reactants, being in closer proximity to react them.

Table 2 Optimization of reaction conditions of solvent and temperature for the synthesis of 3,3-dimethyl-12-(pyridin-2-yl)-3,4,5,12-tetrahydrobenzo[4,5]imidazo[2,1-*b*]quinazolin-1(2*H*)-one (**4a**).[a]

Entry	Solvent	Temp (°C)	Yield[b] (%)	
			Catalyst-free	MSA(5 mol%)
1	Toluene	110	32.4	48.3
2	Chlorobenzene	120	35.2	55.7
3	CH_2Cl_2	75	39.2	58.4
4	CCl_4	75	30.4	45.1
5	THF	65	43.2	54.2
6	Acetonitrile	65	38.8	55.4
7	Ethanol	70	65.4	80.4
8	Solvent-free	80	25.3	92.0

[a]Reaction Conditions: 1*H*-benzo[*d*]imidazol-2-amine (**1**, 1 mmol), pyridinecarboxaldehyde (**2a**, 1 mmol) and dimidine (**3**, 1 mmol) at 20 min, [b]Isolated yield.

In order to investigate the catalytic activity and the possibility of the catalyst recyclability and reusability, the MSA was recovered from the reaction mixture by simple filtration in ethyl acetate. The separated catalyst was dried in vacuum oven at 100 °C and was reused as such for subsequent experiments under similar reaction conditions (Table 1, entry 6). The results showed that the catalyst could be effectively reused for at least four consecutive cycles without much appreciable loss in its catalytic activity. The recyclability data demonstrate that high stability of the catalyst under the reaction conditions (**Figure 2**).

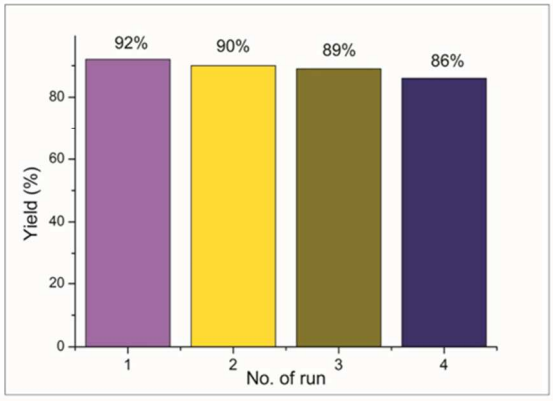


Figure 2: Reusability of the MSA catalyst.

In addition to solvent effect, herein, the temperature variations are also tested (Table 2, entries 1-7). It concluded that, the irrespective nature of the solvent medium observed only optimum conversion of products even at their boiling temperature (**Figure 1**), but under solvent-free conditions achieved maximum conversion of product yield at 80 °C (Table-2, entry 8). The lower yields in solvent medium due to may be the salvation of the reaction medium.

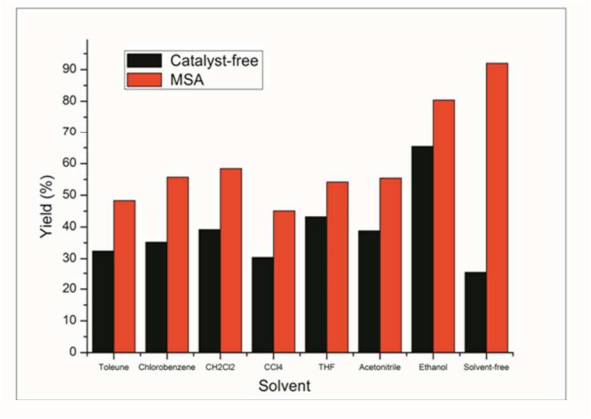


Figure 1: Effect of solvent on product yields on model reaction.

To compare the advantage of the use of MSA over the reported procedure the model reaction was considered a representative example (Table 3). While in most of these cases, comparative yields of the desired product were obtained following the MSA catalyzed procedure (Table 3, entry 5). But the reported procedures required high catalyst loading or expensive ionic liquids or catalysts and/or organic solvent. These results clearly demonstrate that MSA is more efficient catalyst for this three-component reaction. It is noteworthy that no more reports of the synthesis of tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones from aliphatic and heterocyclic aldehydes and studied their anticancer and anti-oxidant activities.

Table 3 Comparison of various reported catalysts with MSA

Entry	Catalyst/ solvent/ temperature (°C)	Catalyst load (mol%)	Time (min)	Yield (%)	Reference
1	$[bimm^+][BF_4]^-$ / 90	3mL	420	90	20f
2	Iodine/acetonitrile/ 80	10	15	88	20d
3	Sulfamic acid/ acetonitrile/ Reflux	5	20	90	20e
4	Silica gel/ MW/ ethanol/ 120	--	6	92	20b
5	MSA/ Solvent-free/ 80	5	20	92	Present work

Having the optimized reaction conditions for the synthesis of tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones (4a-z& 4a′) using MSA as the catalyst under solvent-free conditions, we subsequently applied for various aldehydes to explore the generality of the reaction system. As shown in Table 4, MCRs produced excellent product yields for a wide range of aromatic aldehydes bearing both electron-donating and electron-withdrawing substituents. In all cases the pure products were isolated by simple filtration without chromatography or a cumbersome work-up procedure. After the reaction, the catalyst

can be easily separated from the product and reused without a significant decrease in its catalytic activity. All of the new structures were characterized by ^{1}H NMR, ^{13}C NMR and HRMS. 4z and 4a' compounds data coincide with reported compounds.

Pharmacological screening

In the recent years an increased interest has been developed to use antioxidants for medical purposes. Thus, drugs are considered for preventing and/or treatment of diseases that are having anti-oxidant and free radical scavenging properties. The effective anti-oxidant that protects our body from free radicals that cause oxidative stress or "cellular rust" that can lead to a host of severe medical conditions. Since the anti-oxidants help to prevent cancer and other cardiovascular diseases, herein, we screened the anti-oxidant activity and followed by the cytotoxic activity of all the synthesized titled compounds.

Anti-oxidant activity

In many bioorganic redox processes generated free radicals may damage in various components of the body by inducing oxidative process and it has been implicated in a number of life-limiting chronic diseases and aging. The high reactivity of free radicals within the body can be neutralized by an electron or hydrogen radical that are donated by the anti-oxidants and thus free radical scavengers (Scheme 2). To explore the free radical scavenging ability of the newly synthesized compounds we carried out different type of in vitro assay experiments, such as, 1,1-diphenyl-2-picrylhydrazyl (DPPH),[25] hydroxyl (H_2O_2)[26] and reducing power (RP)[27] radical scavenging activity methods in a hope to develop potent anti-oxidants. Herein, we are measured the in vitro inhibitory concentration of 50% (IC_{50}) (Eq.-1) with reference to Vitamin-C as standard anti-oxidant. Most of the titled compounds exhibited good in vitro anti-oxidant activities in represented methods (Table 5).

$$\% \text{ of scavenged} = \frac{(A_{cont.} - A_{test})}{A_{cont.}} \times 100 \quad \textbf{(Eq.-1)}$$

Where, Acont. is the absorbance of the control (containing all reagents except the test compound, blank sample) and A test is the absorbance of the test compound.

DPPH scavenging activity

One of the widely accepted and often used tools for estimating free radical scavenging activity of anti-oxidants are nitrogen centred stable DPPH free radical which is purple in colour because it is a rapid, simple and inexpensive method. In the determination of radical scavenging activity, it can accept an electron or hydrogen radical from anti-oxidant and forms a non-radical DPPH (Scheme 2) resulting de-colorization which is in stoichiometry. It has been used to measure anti-oxidant properties by a change in the absorbance produced in this reaction.

In this view, most of the titled compounds showed good to excellent DPPH radical scavenging activity (Table 5). These compounds have good radical and/or hydrogen donating capacity (Scheme 2). In them **4c, 4b, 4t, 4f, 4n, 4o, 4d, 4i, 4p,** and **4a** showed highest scavenged activity of 88.7, 87.9, 87.2, 87.1, 86.4, 86.1, 82.8, 81.4, 81.2 and 80.9 respectively when compare with other compounds. The remaining compounds also exhibited good DPPH radical scavenging activity whencompared with the positive control standard ascorbic acid (89.8%) (**Figure -3**).

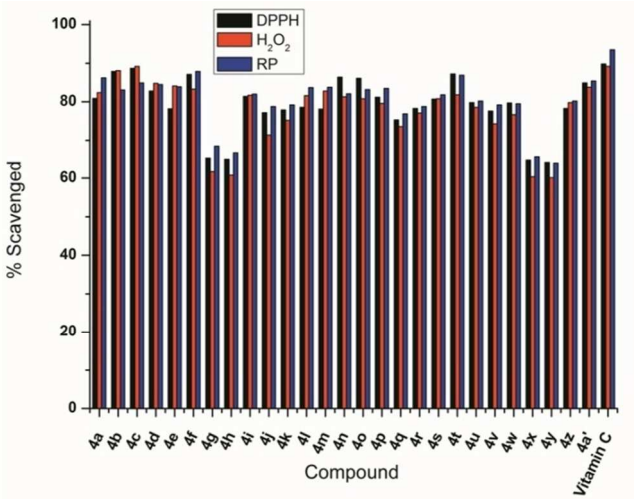


Figure 3: Anti-oxidant activity of the titled compounds - All the radical scavenging activities of the titled compounds represent with the standard Vitamin C. All data are expressed as mean ± S.D. (n = 3).

Hydroxyl radical (H_2O_2) scavenging activity

One of the most ROS that attacks almost every molecule in the body is that hydroxyl radical. It was measured based on competition between hydroxyl radicals generate by compounds and deoxyribose. Most of the newly synthesized titled compounds showed good to excellent hydroxy radical scavenging activity (Table 5).When compare with other, compounds **4c, 4b, 4d, 4e, 4f, 4m, 4a, 4t, 4i** and **4l** were showed excellent OH radical scavenging activity of 89.2, 88.1, 84.8, 84.1, 83.3, 82.8, 82.4, 81.8, 81.7 and 81.6% respectively. All the remaining compounds exhibited good hydroxyl radical scavenging activity values indicate that the title compounds can be considered as promising anti-oxidants when compare to the positive control ascorbic acid (89.2%) (**Figure -3**).

Reducing power (RP) scavenging activity

The potential anti-oxidant activity of a compound may indicate by its significant reducing power capability. The reducing power of titled compounds (Table 5) increased with increasing ready availability of either non-bonding electrons or labile hydrogen. The results showed (Figure 3) that hydroxy substituted compounds, **4f, 4t, 4a, 4c, 4d, 4e, 4m, 4l, 4p & 4o** are exhibited more scavenging activity of 87.9, 86.9, 86.2, 84.9, 84.5, 83.9, 83.8, 83.2, 83.5 & 83.2% respectively. But the aliphatic moiety as side group compounds, **4g, 4h, 4x, & 4y** showed least scavenging activity of 68.5, 66.8, 65.8 & 64.1% respectively when compare with the positive control ascorbic acid (93.5%).

Table 5 Anti-oxidant activity of test compounds (4a-z & 4a′)

Compound	Inhibition %		
	DPPH	H_2O_2	RP
4a	80.9	82.4	86.2
4b	87.9	88.1	83.1

4c	88.7	89.2	84.9
4d	82.8	84.8	84.5
4e	78.2	84.1	83.9
4f	87.1	83.3	87.9
4g	65.4	61.7	68.5
4h	65.1	60.8	66.8
4i	81.4	81.7	82.0
4j	77.2	71.4	78.8
4k	77.9	75.2	79.2
4l	78.6	81.6	83.7
4m	78.1	82.8	83.8
4n	86.4	81.3	82.1
4o	86.1	80.8	83.2
4p	81.2	79.6	83.5
4q	75.3	73.6	76.9
4r	78.3	77.1	78.8
4s	80.7	80.8	81.8
4t	87.2	81.8	86.9
4u	79.8	78.6	80.2
4v	77.6	74.3	79.2
4w	79.7	76.7	79.5
4x	64.9	60.4	65.8
4y	64.3	60.1	64.1
4z	78.3	79.8	80.2
4a′	84.9	83.8	85.4
Vitamin - C	89.8	89.2	93.5

Anticancer activity

Now-a-days frequently reported the anticancer activity of many N-containing heterocyclic/ acyclic compounds against various anticancer cell lines. In such a way once the titled compounds shown excellent anti-oxidant activities, our attention was turned to estimate their anticancer activity, because of they are auxiliary drugs co-administered with various formulations.

All the newly synthesized compounds were assessed in vitro cytotoxic activity against HeLa (human cervical cancer) and SK-BR-3 (human breast adenocarcinoma) cell lines. The numbers of live cells were measured after 24 h of treatment (MTT assay) and determined their cytotoxic activity[28] by calculating of the half maximal inhibitory concentrations (IC_{50}) values are presented in Table 6.

The results showed that compounds **4h, 4f, 4a, 4g, 4x, and 4y** possessed closer anti-proliferative activity against the two cell lines compared with that of standard anticancer drugs, Etoposide and Camptothecin. Among them, compounds **4h** and **4f** has the highest activity (13.54±0.60 and 14.41±0.52 µg/ mL for HeLa and 11.79±0.60 and 13.01±0.39 µg/ mL for SK-BR-3 cells). Almost closer level of activity was observed for the remaining compounds. But the compounds **4n, 4r, 4t, 4v and 4w** showed less activity on both cell lines.

Table 6 Cytotoxic activity* of test compounds (4a-z & 4a′) against HeLa and SK-BR-3 Celllines[a]

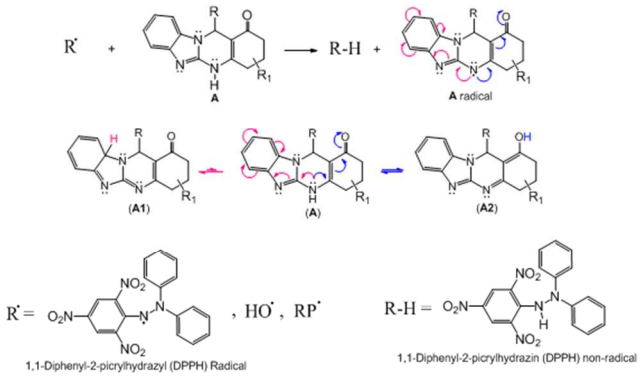
Compound	(IC_{50} in µg/mL)	
	HeLa	SK-BR-3
4a	16.00±0.35	14.80±0.58
4b	35.95±0.45	26.44±0.69
4c	27.41±0.46	26.66±0.58
4d	30.91±0.61	30.56±0.66
4e	26.49±0.75	25.63±0.53
4f	14.41±0.52	13.02±0.52
4g	17.23±0.73	13.01±0.39
4h	13.54±0.60	11.79±0.60
4i	26.01±0.62	25.01±0.70
4j	36.39±0.48	37.93±0.61
4k	39.00±0.75	37.81±1.02
4l	47.68±1.01	46.22±0.80
4m	47.75±0.98	43.70±0.62
4n	65.50±0.47	65.95±0.73
4o	39.93±0.80	38.04±0.68
4p	36.99±0.84	32.86±0.58
4q	41.48±1.03	36.50±0.60
4r	79.66±1.60	73.67±0.51
4s	49.07±1.29	42.53±0.58
4t	89.65±1.52	86.69±0.57
4u	41.13±1.07	35.81±0.66
4v	57.93±0.91	55.84±0.70
4w	66.21±1.66	54.65±0.50
4x	21.31±0.88	17.32±0.40
4y	23.34±0.82	17.20±0.79
4z	29.59±0.98	19.89±0.82
4a′	27.92±1.28	18.56±0. 91
Etoposide	9.75±0.55	5.32±0.42
Camptothecin	1.86±0.33	1.48±0.11

*With different concentrations of test compounds were treated on exponentially growing cells for 24 h and cell growth inhibition was analyzed (MTT assay); amean percentage decrease in cell number of five independent experiments was used to calculate the linear regression equation; §50% concentration decrease of cell number in the absence of

an inhibitor as compared with that of the control cultures. The values, inmean±SE (SE = standard error) are five individual observations.

Structure activity relationship (SAR)

The potent nature of titled N-containing heterocyclic compounds were expected to be more active due to the presence of heteroatoms which are contained non-bonding electron pairs or labile hydrogen on heteroatom (Scheme 2,A→ A1/ A2) and they can interact easily with ROS/cancerous cell lines.



Scheme 2: Radical scavenging activity of the titled compounds (4a-z & 4a′) due to labile nature of hydrogen by tautomerisation of unpaired electrons on nitrogen atom.

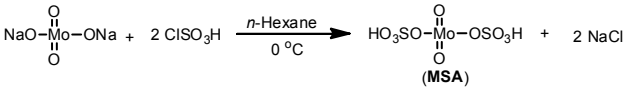
The anti-oxidant bioassay results showed that the compounds that are possessed with good electron donors/ labile hydrogens on the basic core structure (A) could affect the ability of titles compounds to interact with the peripheral and thereby influence the scavenging activity on ROS. On the other hand, the compounds, **4g, 4h, 4x, and 4y,** which are possess an aliphatic moiety on core structure, A, shown least scavenging activity. But while coming to the anti-proliferative activity of these compounds shows highest inhibitory activity. From these observations may conclude that the aliphatic side groups also exhibiting good activity in both types (anti-oxidant and anticancer) of the bioassay. The other compounds are also exhibited good anticancer activity.

In summary, we have demonstrated a green and efficient method for the synthesis of a series of tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones (**4a-z & 4a′**) in excellent yields via one-pot multi-component reaction by using a catalytic amount of MSA under solvent-free condition. The important features of this protocol, environmental acceptability, economic viability, the less reaction time, high yields, purification of products by non-chromatographic method, cleaner reaction profiles, atom efficiency and recyclability of MSA are qualifying it as the best method for the synthesis of tetrahydrobenzo[4,5]imidazo[2,1-b]quinazolin-1(2H)-ones (**4a-z & 4a′**). The tilled compounds were screened for their in vitro anti-oxidant and found to be most of the compounds are effective against ROS. The majority of them also have excellent in vitro anticancer activity on two human cancer cell lines, HeLa and SK-BR-3, compare with that of standard drugs.

Experimental

Preparation of MSA

To dry *n*-hexane (25 mL) in a 100 mL round bottom flask equipped with overhead stirrer and kept in an ice bath was added a suspension of anhydrous sodium molybdate (20 mmol, 4.118 g). To this solution drop wise added chlorosulfonic acid (0.266 mL, 40 mmol) during 30 min and stirred for 1.5 h (**Scheme 3**). The reaction mixture was gradually poured into 25 mL of chilled distilled water with stirring. MSA was separated by filtration andit was washed 5-6 times with cold distilled water until its filtrate tests negative for chloride ions. It was dried at 120 °C for 5 h, and obtained in 91% yield as bluish powder.



Scheme 3: Synthesis of MSA

Molybdate sulfuric acid which showed good thermal stability decomposed at 354 °C. The overlaid FT-IR spectra of sodium molybdate and molybdate sulfuric acid (MSA) are shown in **Figure S1.** As the spectrum of MSA demonstrates, the characteristic bands of both anhydrous sodium molybdate and OSO_3 group are shifted evidently to the higher wave numbers. The well defined bands at 3600–3000 cm^{-1} is related to OH stretching, the band at 1635 cm^{-1} is the H-O-H bending mode of the lattice water, and the bands at 1300-1100 cm^{-1} might be the asymmetric and symmetric stretching modes of S=O. A strong band at 827 cm^{-1} in the FT-IR spectrum of sodium molybdate is assigned to the stretching mode of Mo-O. This band is shifted to 1100 cm^{-1} and appeared as an overlapped band with S=O stretching bands in spectrum of MSA. Broadening of the absorbance band positioned at 3600–3000 cm^{-1} is due to the rapid exchanges of acidic hydrogens via H-bonding and reveals the formation of MSA.

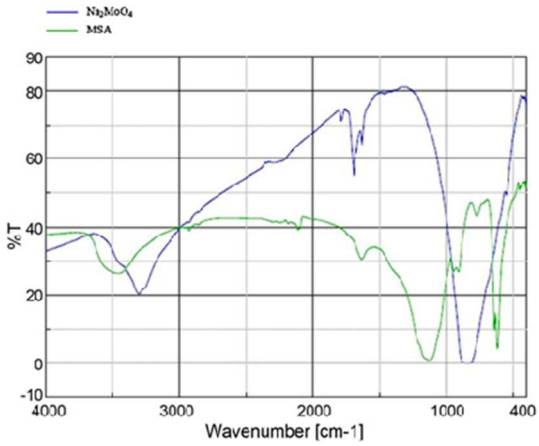
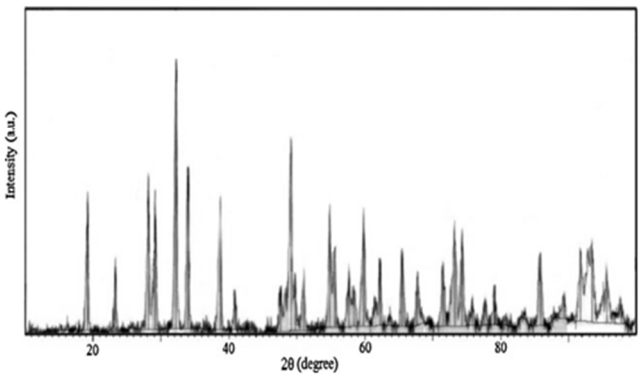


Figure S1: Overlaid FT-IR spectra of Na_2MoO_4 and $H_3OSO(MoO_2)OSO_3H$ (MSA).

The commercial Na_2MoO_4 presents the main diffraction peaks at 16.88, 27.78, 32.68, 48.98, 52.18 and 57.18 of 2 a.u, referred to the 111, 220, 311, 422, 511 and 440 diffraction plans. The XRD pattern of the prepared $(SO_3H)_2MoO_4$ shows a series of new peaks (**Figure S2**), randomly distributed crystallites by planes, and a large shift in the original peaks of Na_2MoO_4. These can be attributed to the formation of MSA as a new phase system, whereas broadening of all peaks shows less crystallization structure and more amorphous shape of MSA.



FigureS2: The powder X-ray diffraction pattern of the MSA.

Synthesis of 3,3-dimethyl-12-(pyridin-2-yl)-3,4,5,12-tetrahydrobenzo [4,5]imidazo[2,1-b]quinazolin-1(2H)-one (4a).

A mixture of 1H-benzo[d]imidazol-2-amine (1, 1 mmol), pyridinecarboxaldehyde (2a, 1 mmol) and dimidine (3a, 1 mmol) and MSA (5 mol %) was stirred at 80 °C under solvent-free condition for 20 min (Table 4, entry 1). The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was washed with ethyl acetate and filtered to recover the catalyst. The filtrate was evaporated, and the crude product was recrystallized from ethanol to afford pure 4a in excellent yield (92%). The spent MSA catalyst from different experiments was combined, washed with ether and dried overnight in a vacuum oven and reused.Compounds 4b-z & 4a′ were also synthesized by adopting this procedure.

Biological assays

DPPH free radical reduction method

The nitrogen centered stable free radical DPPH gives a strong absorption maximum at λ= 517 nm that is suitable for spectrophotometric studies. The test compounds in solutions (100 µM) were added to dioxane/ethanol solution (100 µM) of DPPH. The tubes were kept for 20 min at ambient temperature and measured the absorbance at λ= 517 nm. The % scavenging of the DPPH radical was expressed using **Eq.-1.**

Determination of Hydrogen Peroxide (H_2O_2) Scavenging Activity

Hydrogen peroxide scavenging activity of compounds was determined using a 40 mM solution of H_2O_2 that is prepared in phosphate buffered saline (50 mM, PBS, pH 7.4). The absorbance value of H_2O_2 mixture was determined at 230 nm using a spectrophotometer. To the solution of hydrogen peroxide-PBS (0.6 mL) added 100 µM compound solution in 4 mL distilled water. Absorbance of analyte mixturewas determined at λ= 230 nm after 10 min against a blank solution (parent compound with PBS) without H_2O_2. In similar way took Ascorbic acid in place of test compound and measured the absorbance after 10 minutes against a blank solution.

Reducing Power

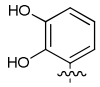
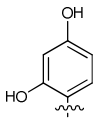
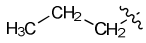
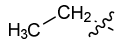
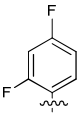
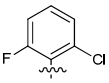
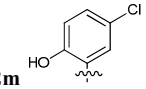
The principle involved here is that increase in the absorbance of the reaction mixtures indicates an increase in the anti-oxidant activity. Herein to forms coloured complexes the test compounds (at 25, 50, 75, and 100 mg/mL concentrations in methanol) were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassiumferricyanide [$(K_3Fe\ (CN)_6$] (1% w/v).The resulting mixture was incubated for 20 min at 50 °C, then added 2.5 mL of trichloro acetic acid (TCA, 10% w/v). After centrifuged the mixture for 10 min at 3000 rpm added 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%, w/v) to the upper layer of the solution (2.5 mL) and determined the UV absorbance at 700 nm against blank sample using a spectrophotometer. For each compound measured the mean values from three independent samples and was found less than 2% standard deviations.

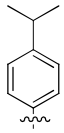
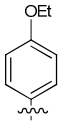
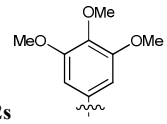
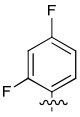
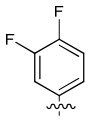
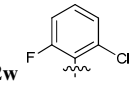
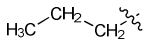
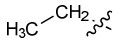
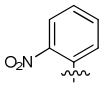
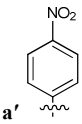
Anticancer activity

Cell culture

Cell proliferation was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasolium bromide (MTT) assay. HeLa cells were cultured at 37 °C in T-75 tissue culture flasks (Nunc, Denmark) in a 5% CO_2 humidified incubator using appropriate media supplemented with DMEM containing 10% heat-inactivated FBS. Similarly, SK-BR-3 cells were cultured in RPMI media. In 96 well microtiter plate each well with 100 µL medium were seeded the cells at identical conditions at a final density of 2×10^4 cells/well. The cells were treated with test compounds in different concentrations of (0.1–100 µg/mL) or DMSO (carrier solvent) after overnight incubation with three replicates each in a final volume of 200 µL. 10 µL of MTT (5 mg/ mL) was added to each well after 24 h and the plate was incubated for 4 h at 37 °C in the dark. The formazan crystals after removing the media along with MTT were solubilized in DMSO (100 µL/well). Finally, MTT reduction was measured by reading the absorbance at 570 nm using GENios® microplate reader (Tecan Austria GmbH, Austria). Effects of the tilted compounds on cell viability were measured by untreated cells added with DMSO as control. Linear regression analysis was subjected on the collected data and the regression lines were plotted for the best straight-line fit. By using the respective regression equation the IC_{50} concentrations were calculated.

Table 4 Synthesis of tetrahydrobenzo[4,5]imidazo[2,1-*b*]quinazolin-1(2*H*)-ones.[a]

Entry	Aldehyde (R)	Compound	Yield[b](%)	Time (min)	mp (°C)
1	 **2a**	**4a**	92	20	340-342
2	 **2b**	**4b**	94	26	282-284
3	 **2c**	**4c**	90	30	270-272
4	 **2d**	**4d**	91	26	301-303
5	 **2e**	**4e**	92	28	296-298
6	 **2f**	**4f**	89	30	310-312
7	**2g** 	**4g**	92	25	240-242
8	**2h** 	**4h**	91	24	271-273
9	 **2i**	**4i**	89	30	288-290
10	 **2j**	**4j**	88	35	308-310
11	 **2k**	**4k**	89	32	221-223
12	 **2l**	**4l**	88	30	278-280
13	 **2m**	**4m**	92	35	305-307
14	 **2n**	**4n**	90	31	298-300

15	 **2o**	**4o**	91	29	311-313
16	 **2p**	**4p**	93	25	309-311
17	 **2q**	**4q**	92	30	353-355
18	 **2r**	**4r**	93	25	378-380
19	 **2s**	**4s**	85	37	312-314
20	 **2t**	**4t**	91	32	375-377
21	 **2u**	**4u**	88	38	304-306
22	 **2v**	**4v**	89	40	378-380
23	 **2w**	**4w**	85	35	36-367
24	 **2x**	**4x**	90	30	310-312
25	 **2y**	**4y**	89	35	258-260
26	 **2z**	**4z**	91	20	355-357
27	 **2 a′**	**4a′**	92	14	325-327

[a]Reaction of 1*H*-benzo[*d*]imidazol-2-amine (**1**, 1 mmol), various aldehydes (**2a-z** & **a**' 1 mmol), 1,3-dicarbonyl compounds (**3**, 1 mmol) catalysed by MSA under solvent-free at 80 °C.; [b]Isolated yield.

Supporting Information

Analytical and spectral data and NMR spectra were provided as supplementary data for all Compounds.

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