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Synthesis and Antimicrobial Evaluation of Novel Substituted Acetamido-4-subtituted-thiazole-5-indazole Derivatives

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ABSTRACT

A series of novel molecules containing thiazole and indazole ring structure were designed and synthesized. The compounds are synthesized on gram scale by using series of reactions having vicarious reaction and coupling reactions. We have optimized all the reaction steps for getting good yields. For first time we have synthesized substituted indazole acetic acid which is coupled with different substituted thiazoles. The structures of the synthesized compounds were elucidated and confirmed by ^{1}H NMR, ^{13}C NMR Mass spectrum and the purity was checked through HPLC analysis. Compounds 4a-4j are tested for antimicrobial activity, and results shows most of compounds showing promising antimicrobial activity.

INTRODUCTION

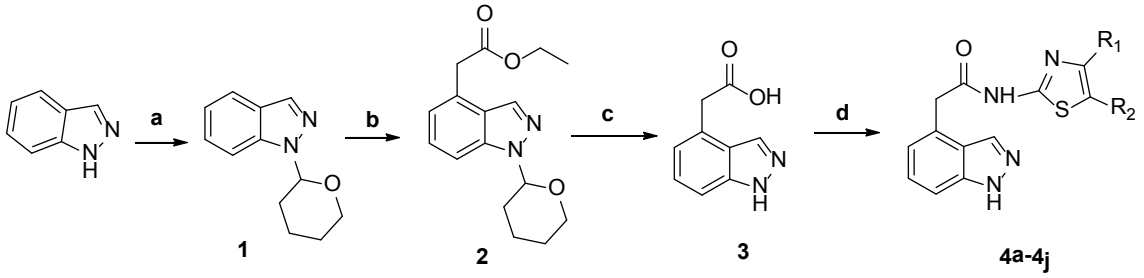
Indazole exhibits a major class of pharmaceuticals, agrochemicals, dyes and key intermediates for drugs. They act as key starting materials for the synthesis of many drugs like molecules. They acts as melanin concentrating hormones (MCH), orenigenic neuropeptide used as anti-obesity treatment, potential anticancer therpeuticals. They also used for inhibitors of nitric oxide synthesis (NOS) [1]. Inazole derivatives are showing promising activity for anti-HIV agents [2]. Indazole used for inhibitors for the treatment of cancer [3]. Indazole acts on highly potent and selective type I B-Raf kinase inhibitors [4]. Indazole Derivatives acts as a novel class of bacterial Gyrase B Inhibitors and inhibitors of PI3 kinase [5,6]. Indazole derivatives also act as selective and reversible monoamine oxidase B Inhibitors [7]. There are reports for synthesis of Indazoles in one pot 3 component synthesis [8]. Substituted indazole synthesized by using palladium catalyzed reactions [9]. Some reports are showing N1 and N2 protected indazoles are synthesized in region selective manner, some have done its borolyations [10,11]. Indazoles are showing good activity like anticancer against human lung carcinoma, antibacterial activity and antimicrobial activity [12-14]. In recent times, the applications of thiazoles were found in drug development for the treatment of allergies, hypertension, inflammation, schizophrenia, bacterial, HIV infections, and hypnotics and more recently for the treatment of pain, as fibrinogen receptor antagonists with antithrombotic activity and as new inhibitors of bacterial DNA gyrase B. Thiazole ring is an important pharmacophore and its coupling with other rings could furnish new biologically active compounds.

From all above references when we couple indazole with different groups it shows different activity so we planned to study the coupling of indazole with different thiazoles. The synthetic methods adopted for the preparation of the title compounds 4a-4j are depicted in the **Scheme 1** presented below.

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 ^{1}H 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. The following abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.



	R1	R2
4a	-COOEt	-Me
4b	-COOEt	-H
4c	-COOEt	-Et
4d	-COOMe	-H
4e	-COOMe	-Me
4f	-COOMe	-Et
4g	-Me	-Me
4h	-Me	-H
4i	-H	-Me
4j	-H	-H

Reagents and conditions: (a) $NaHCO_3$, DHP, H_2O at 0°C to RT; (b) KotBu, Ethyl chloroacetate, in DMSO, rt; (c) 6N aq. HCl; (d) Substituted-thiazoles, EDCI, DIPEA, DCM, rt.

Scheme 1. Synthesis of substituted-(2-(1H-indazol-4-yl)acetamido)-4,5-substituted-thiazoles **4a-4j.**

Synthesis of 1-(tetrahydro-2H-pyran-2-yl)-1H-indazole

To a stirred solution of 1H-indazole (10 g, 84.7 mmol) was dissolved in water (100 mL) then added Sodium bicarbonate (10.67 g, 127 mmol). Cool reaction mass to 0°C then added Dihydropyran (8.54 g, 101 mmol) drop wise. Reaction mixture was stirred at room temperature for 4 h. Evaporated reaction mixture under reduced pressure to obtain crude orange gummy mass. Purification of crude done by silica gel (100-200 mesh) column chromatography by using 10% EtOAc:Hexane to obtain 1-(tetrahydro-2H-pyran-2-yl)1H-indazole as yellow solid. Yield (15 g, 87.7%) MS (ESI) m/e $[M+H]^+$: 203 ^{1}H NMR (400 MHz, DMSO-d6): 1.59 (d, J=3.45 Hz, 2H); 1.68-1.83 (m, 1 H); 1.91-2.13 (m, 2H); 2.31-2.44 (m, 1 H); 3.71-3.82 (m, 1 H); 3.83-3.91 (m, 1 H); 5.87-6.02 (m, 1 H); 7.92 (d, J=9.26 Hz, 1 H); 8.16-8.31 (m, 2 H); 8.41 (s, 1 H); 8.81 (d, J= 1.45 Hz, 1 H).

Synthesis of Ethyl 2-(1-(tetrahydro-2H-pyran-2-yl)1H-indazol-4-yl)acetate

To a stirred solution of Potassium t-butoxide (41.58 g, 371 mmol) in RBF heat it using hot air gun to remove moisture then added dimethyl sulfoxide (100 mL). Again heat flask until dissolution of all the KOtBu. After dissolution of solid allow it to cool. In another RBF take compound 1-(tetrahydro-2H-pyran-2-yl)1H-indazole (15 g, 74.2 mmol) and ethyl chloro acetate (10 g, 81.6 mmol) in DMSO (30 mL). Add compound from RBF 2 to RBF 1 at 0°C immediate color change observed from colorless to blue. Stirred reaction mass at room temperature for 12 h. Poured reaction mass in 250 ml of chilled water and stirred it for 30 min. Solid precipitate out filter it wash it with water (200 mL); Hexane (100 mL); Toluene (100 mL) Dry it properly to obtain ethyl 2-(1-(tetrahydro-2H-pyran-2-yl)-1H-indazol-4-yl)acetate as yellow solid. Yield (19.7 g, 92%) MS (ESI) m/e $[M+H]^+$: 289 ^{1}H NMR (400 MHz, DMSO-d6) 1.37 (s, 9 H);1.59 (d, J=3.45 Hz, 2H); 1.68-1.83 (m, 1 H); 1.91-2.13 (m, 2H); 2.31-2.44 (m, 1 H); 3.71-3.82 (m, 1 H); 3.83-3.91 (m, 1 H); 4.02 (S, 2 h); 5.87-6.02 (m, 1 H); 7.92 (d, J=9.26 Hz, 1 H); 8.16-8.31 (m, 2 H); 8.41 (s, 1 H); 8.81 (d, J= 1.45 Hz, 1 H).

Synthesis of 2-(1H-indazol-4-yl) Acetic Acid

To a stirred solution of ethyl 2-(1-(tetrahydro-2H-pyran-2-yl)-1H-indazol-4-yl)acetate (10 g, 34.7 mmol) in 6N aqueous HCl (100 ml) was heated at 100°C for 4 h. Evaporated reaction mixture under reduced pressure to obtain crude compound. Crude was basified with aq. sodium bicarbonate solution and washed with 50 ml of ethyl acetate. Collected aqueous layer and acidified it up to pH 5 by using 2N aq. HCl. Solid precipitates out filtered it washed it with water (50 ml) and dry it to obtain 2-(1H-indazol-4-yl)acetic acid as white solid. Yield (5 g, 82%) MS (ESI) m\e $[M+H]^+$: 177 ^{1}H NMR (400 MHz, DMSO-d6) 4.02 (S, 2 h); 7.36 (d, J=8.8 Hz, 1 H); 7.48 (d, J=8.8 Hz, 1 H); 8.16-8.20 (m, 2 H); 12.52 (s, 1H); 13.21 (s, 1H).

Synthesis of Ethyl 2-(2-(1H-indazol-4-yl)acetamido)-4-methylthiazole-5-carboxylate 4a

To the stirred solution of 2-(1H-indazol-4-yl) acetic acid (0.5 g, 2.84 mmol) was treated with EDCl (0.82 g, 4.26 mmol), DIPEA (1.45 ml, 8.52 mmol) in DCM (10 ml). Then added ethyl 2-amino-4-methylthiazole-5-carboxylate (0.63 g, 3.41 mmol) and stirred RM at room temperature for 8 h. The reaction was monitored by TLC. Added 15 ml of cold water and stirred for 10 min. Then extracted it with 20 ml of DCM. Collected organic layer wash it with 1N aqueous HCl (10 ml) and washed with brine (10 ml). Evaporate the organic layer to obtained the compound with 75% purity ethyl 2-(2-(1H-indazol-4-yl)acetamido)-4-methylthiazole-5-carboxylate. Purification of crude done by silica gel (100-200 mesh) column chromatography by using 60% EtOAc: hexane to obtain ethyl 2-(2-(1H-indazol-4-yl)acetamido)-4-methylthiazole-5-carboxylate as white solid. Yield (0.8 g, 81%) MS (ESI) m/e $[M+H]^+$: 345 ^{1}H NMR (400 MHz, DMSO-d6): 1.24 (t, 3 h); 2.54 (s, 3 h); 3.87 (S, 2 h); 4.24 (q, 2 h); 7.38 (d, J=8.8 Hz, 1 H); 7.58 (d, J=8.8 Hz, 1 H); 8.16-8.20 (m, 3 H); 12.50 (s, 1H).

General Procedure for Synthesis of Substituted-(2-(1H-indazol-4-yl)acetamido)-4,5-substituted-thiazoles 4a-4j

To the stirred solution of 2-(1H-indazol-4-yl) acetic acid (1 eq.) was treated with EDCl (1.5 eq.), DIPEA (3 eq.) in DCM (10 ml). Then added substituted- 2-amino-4,5-substituted-thiazoles (1.2 eq.) and stirred RM at room temperature for 8 h. The reaction was monitored by TLC. Added cold water and stirred for 10 min. Then extracted it with DCM. Collected organic layer wash it with 1N aqueous HCl and washed with brine Evaporate the organic layer to obtained the compound with 75% to 80% purity of substituted-(2-(1H-indazol-4-yl)acetamido)-4,5-substituted-thiazoles. Purification of crude done by silica gel (100-200 mesh) column chromatography by using 60% to 80% EtOAc:Hexane to obtain **4a to 4j.**

Spectral Data

Ethyl 2-(2-(1H-indazol-4-yl)acetamido)-4-methylthiazole-5-carboxylate 4a

White solid, LC-MS *m/z* (%): 345 [M+H]. ^{1}H NMR (400 MHz, DMSO-d6): 1.24 (t, 3 h); 2.54 (s, 3 h); 3.87 (S, 2 h); 4.24 (q, 2 h); 7.38 (d, J=8.8 Hz, 1 H); 7.58 (d, J=8.8 Hz, 1 H); 8.16-8.20 (m, 3 H); 12.50 (s, 1H). HPLC-99.87% RT-6.93 min. ^{13}C NMR ($CDCl_3$, 100 MH_Z): 11, 15, 42, 65, 112, 120, 121, 127, 128, 141, 157, 158, 165, 171.

Ethyl 2-(2-(1H-indazol-4-yl)acetamido)thiazole-5-carboxylate 4b

White solid, LC-MS *m/z* (%): 331 [M+H]. ^{1}H NMR (400 MHz, DMSO-d6): 1.24 (t, 3 h); 3.87 (S, 2 h); 4.24 (q, 2 h); 7.38 (d, J=8.4 Hz, 1 H); 7.58 (m, 3 H); 8.20-8.23 (m, 2 H); 12.50 (s, 1H). HPLC-99.45% RT-6.52 min. ^{13}C NMR ($CDCl_3$, 100 MH_Z): 15, 42, 65, 112, 120, 121, 127, 128, 141, 147, 158, 165, 171.

Ethyl 2-(2-(1H-indazol-4-yl)acetamido)-4-ethylthiazole-5-carboxylate 4c

White solid, LC-MS *m/z* (%): 359 [M+H]. ^{1}H NMR (400 MHz, DMSO-d6): 1.24 (t, 3 h); 1.30 (t, 3h); 2.55 (q, 2 h); 3.87 (S, 2 h); 4.29 (q, 2 h); 7.38 (d, J=8.2 Hz, 1 H); 7.59 (d, J=8.4 Hz, 1 H); 8.15-8.21 (m, 3 H); 12.51 (s, 1H). HPLC-98.21% RT-7.30 min. ^{13}C NMR ($CDCl_3$, 100 MH_Z): 11, 15,17, 42, 46, 65, 112, 120, 121, 127, 128, 141, 157, 158, 165, 171.

Methyl 2-(2-(1H-indazol-4-yl)acetamido)thiazole-5-carboxylate 4d

White solid, LC-MS *m/z* (%): 317 [M+H]. ^{1}H NMR (400 MHz, DMSO-d6): 3.87 (S, 2 h); 3.91 (s, 3h); 7.36 (d, J=8.4 Hz, 1 H); 7.56 (m, 3 H); 8.20-8.23 (m, 2 H); 12.49 (s, 1H). HPLC-97.48% RT-5.99 min. ^{13}C NMR ($CDCl_3$, 100 MH_Z): 42, 55, 112, 120, 121, 127, 128, 141, 157, 158, 165, 171.

Methyl 2-(2-(1H-indazol-4-yl)acetamido)-4-methylthiazole-5-carboxylate 4e

White solid, LC-MS *m/z* (%): 317 [M+H]. ^{1}H NMR (400 MHz, DMSO-d6): 2.55 (s, 3h); 3.88 (S, 2 h); 3.90 (s, 3h); 7.36 (d, J=8.4 Hz, 1 H); 7.56 (m, 3 H); 8.20-8.23 (m, 1 H); 12.49 (s, 1H). HPLC-99.20% RT-.30 min. ^{13}C NMR ($CDCl_3$, 100 MH_Z): 17, 42, 55, 112, 120, 121, 127, 128, 141, 157, 158, 165, 171.

Methyl 2-(2-(1H-indazol-4-yl)acetamido)-4-ethylthiazole-5-carboxylate 4f

White solid, LC-MS *m/z* (%): 317 [M+H]. ^{1}H NMR (400 MHz, DMSO-d6): 1.31 (s, 3h); 2.55 (s, 3h); 3.88 (S, 2 h); 4.27 (s, 2h); 7.36 (d, J=8.4 Hz, 1 H); 7.56 (m, 3 H); 8.20-8.23 (m, 1 H); 12.49 (s, 1H). HPLC-98.92% RT-8.5 min. ^{13}C NMR ($CDCl_3$, 100 MH_Z): 15, 17, 42, 46, 55, 112, 120, 121, 127, 128, 141, 157, 158, 165, 171.

2-(1H-indazol-4-yl)-N-(4,5-dimethylthiazol-2-yl)acetamide 4g

White solid, LC-MS *m/z* (%): 287 [M+H]. ^{1}H NMR (400 MHz, DMSO-d6): 2.48 (s, 3h); 2.55 (s, 3h); 3.88 (S, 2 h); 7.38 (d, J=8.4 Hz, 1 H); 7.56-7.62 (m, 3 H); 8.20-8.23 (m, 1 H); 12.49 (s, 1H). HPLC-97.48% RT-5.99 min. ^{13}C NMR ($CDCl_3$, 100 MH_Z): 15, 17, 42, 112, 120, 123, 127, 128, 143, 157, 158, 165, 171.

2-(1H-indazol-4-yl)-N-(5-methylthiazol-2-yl)acetamide 4h

White solid, LC-MS *m/z* (%): 273 [M+H]. ^{1}H NMR (400 MHz, DMSO-d6): 2.48(s, 3h); 3.88 (S, 2 h); 7.38 (d, J=8.4 Hz, 1 H); 7.56-7.62 (m, 3 H); 8.20-8.23 (m, 2 H); 12.49 (s, 1H). HPLC-99.34% RT-6.23 min. ^{13}C NMR ($CDCl_3$, 100 MH_Z): 17, 42, 112, 120, 124, 126, 128, 141, 157, 158, 165, 171.

2-(1H-indazol-4-yl)-N-(4-methylthiazol-2-yl)acetamide 4i

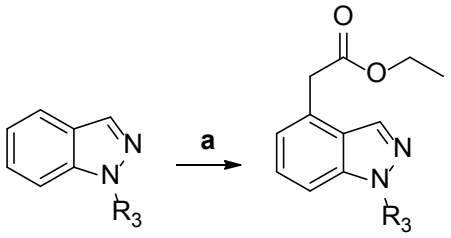
White solid, LC-MS *m/z* (%): 273 [M+H]. ^{1}H NMR (400 MHz, DMSO-d6): 2.58 (s, 3h); 3.88 (S, 2 h); 7.36 (d, J=8.4 Hz, 1 H); 7.46-7.64 (m, 3 H); 8.20-8.23 (m, 2 H); 12.49 (s, 1H). HPLC-99.02% RT-5.88 min. ^{13}C NMR ($CDCl_3$, 100 MH_z): 17, 42, 114, 120, 122, 127, 128, 142, 157, 158, 165, 171.

2-(1H-indazol-4-yl)-N-(thiazol-2-yl)acetamide 4j

White solid, LC-MS *m/z* (%): 259 [M+H]. ^{1}H NMR (400 MHz, DMSO-d6): 3.88 (S, 2 h); 7.36 (d, J=8.4 Hz, 1 H); 7.46-7.64 (m, 4 H); 8.20-8.23 (m, 2 H); 12.49 (s, 1H). HPLC-98.6%; RT-6.67 min. ^{13}C NMR ($CDCl_3$, 100 MH_z): 42, 113, 120, 122, 127, 128, 143, 157, 158, 165, 171.

RESULTS AND DISCUSSION

We have optimized condition for the preparation of our substituted products by varying different bases, varying solvents and reaction time. We presented optimization conditions for all the steps. For step (a) we have carried the THP protection by using organic bases using triethyl amine, diisopropyl amine and DMAP but in all these reactions there is formation of N1 and N2 substituted THP product formation. N1-substituted product formation is of 60%, 65% and 59% respectively. We have planned to use the inorganic bases like NaOH, KOH, Na_2CO_3 and $NaHCO_3$ in water as solvent. Among these reactions reaction with $NaHCO_3$ gives 87% exclusive N1 product formation in remaining cases there is again a mixture of product formation takes (NaOH-70%, KOH- 68% and Na_2CO_3-73%) place so reaction with sodium bicarbonate is the preferable one for step a **(Scheme 2).**



Reagents and conditions: (a) KotBu in DMSO dissolve, Ethyl chloroacetate and 1 in DMSO, added to KotBu, rt, 12 h.

Scheme 2. Synthesis ethyl 2-(1-substituted-1H-indazol-4-yl)acetate.

For step b we have done series of optimizations for finalizing the reaction conditions.

For step b we have protected compound 1 with DHP and methyl at N1 position.

We have done vicarious reactions by using literature procedure but we are failing to get yields more than 55% and tedious purifications are required so we have modified the condition. First we dissolve potassium t-butoxide in DMSO by heating then we have cooled it and added mixture of compound 1 and ethyl chloroacetate in DMSO solution. After addition of first drop of mixture the color of potassium t-butoxide changes from colorless to blue after 12 h there is completion of reaction. For work up we have to pour this reaction mixture in crushed ice solid precipitation occurs for both the N1-THP and methyl protected compounds, filter it and wash it with excess of water to obtain desired product as yellow solid with purity more than 90%, and yield is more than 90% for both the examples.

Step c is hydrolysis and deportation in one step by using 6N aqueous HCl solution to get substituted acetic acid derivative.

For step d we have done peptide coupling reactions with substituted thiazoles. We have used new conditions for peptide coupling which gives yields from 60% to 85% for different examples. We have avoided costly reagents and tedious work up for final peptide coupling for reactions of less reactive thiazoles.

We have synthesized derivatives from **4a to 4j** in good yields and set a method for the synthesis of substituted acetic acid. The details of compounds are shown **Table 1** below.

All the synthesized compounds were screened for *in vitro* antimicrobial activity. The antibacterial activity was evaluated against different bacterial strains such as *Staphylococcus aureus* (NCIM-2901), *Bacillus subtilis* (NCIM-2063) and *Escherichia coli* (NCIM-2256). Minimum inhibitory concentration (MIC, µg/mL) of antibacterial activity was determined using broth dilution methods per CLSI guidelines [15-18]. Levofloxacin was used as a standard drug for the comparison of antibacterial activity **(Table 2).** Fluconazole and miconazole were used as standard drugs for the comparison of antifungal activity. Dimethyl sulfoxide was used as solvent control. From the antimicrobial data, it is observed that all the newly synthesized compounds shows good to moderate level of antibacterial and antifungal activity **(Table 2).** The antimicrobial activity data reveals that compounds **(4b, 4e, 4f, 4h and 4j)**, are found to be most active and potent as antimicrobial agents among the series. The structure-activity relationship of the series can be explained as follows, the molecule gave increased antimicrobial activity due to the presence of less bulky group

on thiazol ring activity further increases when all thiazole ring is substituted with methyl and hydrogen atoms. The remaining compounds shows moderate to good antimicrobial activity.

Table 1. Synthesis of substituted-(2-(1H-indazol-4-yl)acetamido)-4,5-substituted-thiazoles 4a-4j.

Compound	Reactant (Thiazole)	Time	Melting point (°C)	Yield (%)
4a	Ethyl-2-amino-4-methylthiazole-5-carboxylate	8 h	123	81
4b	Ethyl-2-aminothiazole-5-carboxylate	7 h	118	80
4c	Ethyl-2-amino-4 ethylthiazole-5-carboxylate	6 h	153	78
4d	Methyl-2-aminomethylthiazole-5-carboxylate	6 h	107	82
4e	Methyl-2-amino-4-methylthiazole-5-carboxylate	7 h	113	68
4f	Methyl-2-amino-4-ethylthiazole-5-carboxylate	8 h	150	79
4g	4,5-Dimethylthiazol-2-amine	8 h	127	88
4h	5-Methylthiazol-2-amine	7 h	118	87
4i	4-Methylthiazol-2-amine	7 h	111	82
4j	Thiazol-2-amine	8 h	133	78

Table 2. Antimicrobial activity of the synthesized compounds (4a-4j).

Compound	MIC values[a] (µg/ml)					
	B. subtilis	***E. coli***	***S. aureus***	***C. albicans***	***A. flavus***	***A. niger***
4a	35	50	70	75	50	50
4b	28	27	28	50	12.5	12.5
4c	40	100	100	75	100	100
4d	35	60	50	100	50	50
4e	29	27	29	25	12.5	12.5
4f	25	27	28	50	25	25
4g	35	60	50	100	50	50
4h	28	29	30	25	12.5	12.5
4i	35	50	70	75	50	50
4j	28	29	30	25	25	25
Levofloxacin	29	29	28	-	-	-
Fluconazole	-	-	-	40	25	25
Miconazole	-	-	-	12.5	12.5	12.5

[a]Values are the average of three readings.

CONCLUSION

In conclusion by using this methodology, substituted-(2-(1H-indazol-4-yl)acetamido)-4,5-substituted-thiazoles were synthesized in gram scale. All the compounds are purified through column chromatography by using different proportions and ethyl acetate and hexane. We have developed simple and continent method for the synthesis of some novel indazole-thiazole coupled derivatives through a reaction of substituted thiazole carboxylates and indazole acetic acid by simple reaction steps. No costly reagents are required, no any pre-purification is needed and all the compounds synthesized were obtained in good yields. Most of compounds show promising antimicrobial activity against different bacterial strains.

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