RESEARCH ARTICLE

Synthesis and Pharmacological Study of Thiophene Derivatives

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ABSTRACT

In this study, the Gewald reaction was used to develop target compounds (RAA_1-RAA_9) in the quest for potentially active novel compounds with anti-cancer and antioxidant properties. The physicochemical and spectroanalytical studies of the synthesized derivatives verified their molecular structures. All synthesized compounds were chosen as prototypes by the NCI and tested for anti-cancer activity against a panel of cancer cell lines. The anti-cancer efficacy of the compounds was observed to be quite variable. Compound RAA_5 was selected for a five-dose assay after showing strong anti-cancer activity in primary screening against all the cell lines.

Additionally, the antioxidant activity of the compounds was determined by using a stable DPPH free radical as a radical scavenger. Compounds RAA_5 and RAA_7 exhibited excellent antioxidant activity, while other compounds of the series displayed satisfactory antioxidant activity compared to ascorbic acid. Our findings established the anti-cancer activity of novel thiophene derivatives, suggesting their potential for use in the development of new anti-cancer therapeutics.

Keywords: 60 cell lines, Anticancer, Antioxidant, Gewald Reaction, NCI, SAR, Synthesis, Thiophene.

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INTRODUCTION

Cancer is the most prevalent, particularly complex and deadly illness in the modern medical field.[1] It has been one of the world's main sources of mortality over the last decade. In 2018, an approximated 9.6 million cancer deaths were reported by the World Health Organization.[2-3] The medical, scientific community faces a major challenge to evolve medications, remedies, and care for better and more effective treatment and cure for cancer.[4] Such neoplasm tumor cells are diverse and heterogeneous, with a proclivity for rapid proliferation. These malignant neoplasms may invade or propagate through blood circulation and lymphatic networks to other areas of the body.[5]

One available cure for various forms of cancer is chemotherapeutic agents. However, certain limitations, including drug tolerance, systemic cytotoxicity and a limited therapeutical index, are correlated with these agents.[6-9] To address these drawbacks, novel chemotherapeutic agents with established mechanisms must be developed. Anti-cancer chemotherapy is now developed by discovering cytotoxic molecules or compounds that can kill cancer cells. Such medications boost the survival and wellbeing of cancer patients.[10-11] Various molecules having heterocyclic rings, especially those containing thiophene rings, have already displayed considerable antiproliferative potential. Due to numerous properties, thiophene derivatives remains unique among biomolecules utilized in research to evaluate biological activity.[12]

Thiophene derivatives offer better specificity and safety profiles due to diverse synthesis pathways.[13] Particularly, the 2-amino-thiophenes have gained a huge interest. Because of advances in their synthetic routes, stability, availability, and structural simplicity, 2-amino-thiophenes have received considerable attention, enabling them to be essential moiety for pharmaceuticals.[14-15] A large spectrum of biological properties has been identified for thiophene and its analogous, including antifungal,[16] antimicrobial,[17-18] antileishmanial,[19] anxiolytic,[20] anti-inflammatory,[21] antiplatelet,[22] antioxidant,[23] antiandrogenic activities,[24] and anti-diabetes[25] activities.

Reactive oxygen species (ROS) involvement in various pathological conditions has been well known, including cancer, inflammatory diseases, liver and vascular disease, rheumatoid arthritis, and aging. An increased free radical consumption or a decreasing antioxidant concentration that impacts the cell membranes and other components, such as DNA, lipid, proteins, and lipoproteins, is associated with oxidative stress.[26] For example, excess hydroxyl radicals and peroxynitrite, which

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cause damage to cell membranes and lipoproteins may trigger lipid peroxidation. This process generates malondialdehyde and conjugated diene products, all of which are cytotoxic and mutagenic. Once initiated, it spreads quickly and affects a wide range of lipid molecules.[27] 8-OH-G is the most extreme known DNA variation associated with oxidative stress, and it seems to be a potential carcinogenesis marker.[28] To generate carbonyl function, ROS can oxidize the backbone of proteins and the protein side chains that bind with other amino acid side chains.[29]

ROS is known to cause a variety of human cancers, and thiophene-based compounds have certainly been intensively widely for their anti-cancer properties, considering the severe nature of the condition.[30] As a result of the above observation, the present research utilized the Gewald reaction to synthesize thiophene derivatives and evaluate their therapeutic potential to continue our quest for novel anti-cancer and antioxidant agents.

MATERIALS AND METHODS

Chemicals and Instrumentations

Scheme 1 outlines the synthetic route for a series of thiophene derivatives. From authorized suppliers, all the chemicals were procured and used without any further purification. For tracking the reaction progress, glass plates coated with silica gel G and eluents including ethyl acetate/benzene (1:1) and ethyl acetate/n- hexane (1:2) were used for TLC. The plates were visualized in iodine chamber. In an open capillary melting point apparatus, the melting points were recorded and reported without any corrections. IR spectra (in KBr) were acquired using the DRS 8000A accessory technique on a Shimadzu IR Affinity-1 FTIR spectrophotometer. With tetramethylsilane (TMS) as an internal standard, ^{1}H and ^{13}C NMR spectra in $CDCl_3$ were acquired on a Bruker Avance-II 400 NMR Spectrometer running at 500 MHz indicating chemical shift values (δ) on ppm scale. Using Waters Q-TOF (ESI–MS) micromass, mass spectra were recorded. SAIF, Panjab University, Chandigarh conducted spectral analysis, including mass spectroscopy and NMR studies.

Experimental Methods

Synthesis of ethyl 5-acetyl-2-amino-4-methylthiophene-3-carboxylate (4):

Sulphur (0.06mol) was added with stirring into an equimolar (0.05 mol) mixture of ethyl cyanoacetate and acetylacetone at room temperature. Diethylamine (0.05 mol) was transferred to this heterogeneous mixture in a dropwise manner. For 4hrs, the reaction mixture was stirred at a temperature of 40–50°C. Later at room temperature, the mixture was kept overnight. After filtering and drying, the precipitate was recrystallized using ethanol. Yield: 34%; R_f= 0.66; M.P.: 150-152°C; IR (KBr, cm^{-1}): 3408 (N-H str.), 1257 (C-O-C str.), 1666 (C=O str.), 2968 (C-H str.), 1583 (C=C str.), 785 (C-S-C str.).

Synthesis of 2-chloro-N-(substitutedphenyl)acetamides (7a-i):

An appropriate substituted aromatic amine (0.05 mol) was dissolved in a saturated sodium acetate solution (25 mL). If the substance is not completely dissolved, the mixture is warmed up until absolutely dissolved. It was subsequently cooled in an ice bath with stirring. To this reaction mixture, chloroacetyl chloride (0.07 mol) was added dropwise to avert vigorous reaction. Later at room temperature, the mixture was kept for 5–6 hours. After filtering, washing with cold water, and drying, the precipitate was recrystallized using aqueous ethanol. Analytical data of 2-chloro-*N*-(substituted phenyl) acetamides is included in Table 1.

Synthesis of ethyl 2-((substitutedphenylcarbamoyl) methylamino)-5-acetyl-4-methylthiophene-3-carboxylate (RAA_1-RAA_9):

In 1,4-dioxane (15 mL), various *N*-substituted *α*-chloroacet--anilides (7a-i) and compound 4 as synthesized above, were mixed in equimolar proportions (0.05 mol). The reaction mixture was refluxed for 2 hours after the addition of triethylamine solution (0.005 mol). Afterward, the reaction mixture was allowed to cool before being poured over crushed ice. The product obtained was filtered, washed with potassium bicarbonate (1%), and dried. Using the ethyl acetate/n-hexane solvent system (1:2), the reaction was monitored and R_f values for RAA_1-RAA_9 were calculated.

Ethyl 2-((phenylcarbamoyl)methylamino)-5-acetyl-4-methylthiophene-3-carboxylate (RAA_1)

Creamy solid (73.33%); R_f= 0.66; M.P.: 130-132°C; IR (KBr, cm^{-1}): 3405 (N-H str. coupled), 3297 (N-H str.), 3097 (Ar-H str.), 1663 (C=O str.), 1618 (C=C str.), 1499 (C-N str.), 1284 (C-O str.), 859 (C-C str.), 786 (C-S str.), 691 (Monosubsti. Ring); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.0507-7.5584 (m, 5H, Ar-H), 6.6102 (s, 1H, -CONH), 4.1974 (s, 2H, CH_2), 1.3650-1.3935 (t, 3H, OCH_2CH_3), 4.3059-4.3486 (q, 2H, OCH_2CH_3), 3.7043 (s, 1H, NH), 2.6994 (s, 3H, $-COCH_3$), 2.4113 (s, 3H, $-CH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.15, 166.60, 166.17, 163.79, 146.42, 136.67, 129.15, 125.27, 120.89, 120.13, 109.27, 77.28, 77.02, 60.26, 42.88, 30.19, 16.87, 14.35; ESI-MS (m/z): 361.61 (M+1).

Ethyl 2-((4-chlorophenylcarbamoyl)methylamino)-5-acetyl-4-methylthiophene-3-carboxylate (RAA_2)

Buff colored solid (92%); R_f= 0.67; M.P.: 122-124°C; IR (KBr, cm^{-1}): 3408 (N-H str. coupled), 3297 (N-H str.), 3085 (Ar-H str.), 1665 (C=O str.), 1606 (C=C str.), 1474 (C-N str.), 1275 (C-O str.), 863 (C-C str.), 826 (C-Cl str.), 778 (C-S str.); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.0607-7.3588 (m, 4H, Ar-H), 1.3651-1.3937 (t, 3H, OCH_2CH_3), 6.6101 (s, 1H, -CONH), 4.4479 (s, 2H, CH_2), 4.3055-4.3484 (q, 2H, OCH_2CH_3), 3.7041 (s, 1H, NH), 2.6694 (s, 3H, $-COCH_3$), 2.4142 (s, 3H, $-CH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.20, 166.72, 166.14, 163.97, 146.52, 137.79, 134.73, 129.11, 125.30, 120.80, 120.22, 118.05, 109.18, 60.30, 41.84, 30.21, 16.76, 14.30; ESI-MS (m/z): 396.16 (M+1).

Ethyl 2-((4-bromophenylcarbamoyl)methylamino)-5 -acetyl-4-methylthiophene-3-carboxylate (RAA_3)

Light Yellow (93%); R_f= 0.6; M.P.: 134-136°C; IR (KBr, cm^{-1}): 3410 (N-H str. coupled), 3300 (N-H str.), 3081 (Ar-H str.), 1663 (C=O str.), 1606 (C=C str.), 1488 (C-N str.), 1250 (C-O str.),

Table 1: Analytical Data of 2-chloro-N-(substituted phenyl)acetamides

Compound Code	*Mol. Formula*	*Mol. Weight*	*Color*	*% Yield*	*R_f Value**	*Melting Point***	*IR (KBr, cm^{-1})*
7a	C_8H_8ClNO	169	White	86.35	0.71	110–112	3267(N-H str.), 3099-3145(C-H str. aromatic), 1672(C=O str.), 1556(C=C str. in ring), 750(C-Cl str.).
7b	$C_8H_7Cl_2NO$	204	White	76.07	0.74	108–110	3263(N-H str.), 3082-3130(C-H str. aromatic), 1666(C=O str.), 1556(C=C str. in ring), 777(C-Cl str. aromatic).
7c	$C_8H_7BrClNO$	248	Light Brown	76.04	0.72	118–122	3265(N-H str.), 3126(C-H str. aromatic), 1672(C=O str.), 1552(C=C str. in ring), 780(C-Cl str.), 499(C-Br str. aromatic).
7d	$C_8H_7Cl_2NO$	204	White	24.53	0.67	112–114	3269(N-H str.), 3116(C-H str. aromatic), 1678(C=O str.), 1548(C=C str. in ring), 770(C-Cl str. aromatic).
7e	$C_8H_7ClN_2O_3$	214	Brown	40.18	0.71	106–110	3304(N-H str.), 3086(C-H str. aromatic), 1681(C=O str.), 1531(C=C str. in ring), 1350(C-NO_2 str. aromatic).
7f	$C_8H_7ClN_2O_3$	214	Creamy White	10.90	0.68	104–06	3307 (N-H str.), 3018 (C-H str. aromatic), 1693 (C=O str.), 1517 (C=C str. in ring), 1338 (C-NO_2 str. aromatic).
7g	$C_8H_7Cl_2NO$	204	White	23.82	0.7	118–120	3043 (C-H str. aromatic), 3267 (N-H str.), 1672 (C=O str.), 1531 (C=C str. in ring), 758 (C-Cl str. aromatic).
7h	$C_8H_6Cl_2N_2O_3$	249	Yellow	43.79	0.67	104–106	3373 (N-H str.), 1693 (C=O str.), 1502 (C=C str. in ring), 1321 (C-NO_2 str. aromatic), 750 (C-Cl str. aromatic).
7i	$C_8H_6Cl_2N_2O_3$	249	Orange	48.63	0.6	124–126	3354 (N-H str.), 3093 (C-H str. aromatic), 1687 (C=O str.), 1504 (C=C str. in ring), 1342 (C-NO_2 str. aromatic), 770 (C-Cl str. aromatic).

* R_f Value (Solvent System: Ethyl acetate : Benzene (1:1))
** Melting Point in °C

862 (C-C str.), 773 (C-Br str. coupled), 657 (C-S str.); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.3693-7.4143 (m, 4H, Ar-H), 1.3619-1.3924 (t, 3H, OCH_2CH_3), 6.6721 (s, 1H, -CONH), 4.2411 (s, 2H, CH_2), 4.3134-4.3464 (q, 2H, OCH_2CH_3), 3.7040 (s, 1H, NH), 2.6960 (s, 3H, $-COCH_3$), 2.4292 (s, 3H, $-CH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.27, 166.77, 166.32, 163.09, 146.43, 137.21, 130.79, 129.79, 125.45, 120.19, 120.18, 118.15, 109.19, 60.50, 41.88, 30.24, 16.11, 14.62; ESI-MS (m/z): 440.61 (M+1), 441.41 (M+2).

Ethyl 2-((3-chlorophenylcarbamoyl)methylamino)-5-acetyl-4-methylthiophene-3-carboxylate (RAA_4)

Brown Solid (77.7%); R_f= 0.7; M.P.: 108-110°C; IR (KBr, cm^{-1}): 3408 (N-H str. coupled), 3296 (N-H str.), 3087 (Ar-H str.), 1664 (C=O str.), 1603 (C=C str.), 1477 (C-N str.), 877 (C-C str.), 819 (C-Cl str.), 785 (C-S str.); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.2624-7.4005 (m, 4H, Ar-H), 1.3538-1.3898 (t, 3H, OCH_2CH_3), 6.6608 (s, 1H, -CONH), 4.1830 (s, 2H, CH_2), 4.3009-4.3331 (q, 2H, OCH_2CH_3), 3.7052 (s, 1H, NH), 2.6976 (s, 3H, $-COCH_3$), 2.4326 (s, 3H, $-CH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.21, 166.70, 166.16, 163.96, 146.51, 137.84, 134.83, 130.12, 125.30, 120.85, 120.21, 118.06, 109.24, 60.26, 42.84, 30.19, 16.87, 14.35; ESI-MS (m/z): 396.37 (M+1).

Ethyl 2-((3-nitrophenylcarbamoyl)methylamino)-5-acetyl-4-methylthiophene-3-carboxylate (RAA_5)

Light Brown solid (67%); R_f= 0.66; M.P.: 122-124°C; IR (KBr, cm^{-1}): 3415 (N-H str. coupled), 3295 (N-H str.), 2986 (Ar-H str.), 1684 (C=O str.), 1526 (N–O asymmetric stretch), 1476 (C-N str.), 1310 (C-NO_2 str. aromatic), 1273 (C-O str.), 835 (C-C str.), 786 (C-S str.); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.5230-8.0346 (m, 4H, Ar-H), 7.2704 (s, 1H, -CONH), 1.3658-1.3943 (t, 3H, OCH_2CH_3), 4.2416 (s, 2H, CH_2), 4.3057-4.3485 (q, 2H, OCH_2CH_3), 3.7073 (s, 1H, NH), 2.6978 (s, 3H, $-COCH_3$), 2.4388 (s, 3H, $-CH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.29, 166.77, 166.14, 164.42, 148.61, 146.61, 137.96, 130.01, 125.73, 120.85, 119.76, 114.95, 109.25, 60.27, 42.82, 30.18, 16.89, 14.34; ESI-MS (m/z): 406.24 (M+1).

Ethyl 2-((2-nitrophenylcarbamoyl)methylamino)-5-acetyl-4-methylthiophene-3-carboxylate (RAA_6)

Light Yellow solid (91.7%); R_f= 0.67; M.P.: 110-112°C; IR (KBr, cm^{-1}): 3410 (N-H str. coupled), 3295 (N-H str.), 2986 (Ar-H str.), 2854 (C-H str.), 1665 (C=O str.), 1588 (C=C str.), 1513 (N–O asymmetric stretch), 1458 (C-N str.), 1311 (C-NO_2 str. aromatic), 1271 (C-O str.), 1257 (C-N str.); 847 (C-C str.), 738 (C-S str.); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.5111-8.0341 (m, 4H, Ar-H), 7.2701 (s, 1H, -CONH), 1.3644-1.3956 (t, 3H, OCH_2CH_3), 4.2420 (s, 2H, CH_2), 4.3052-4.3482 (q, 2H, OCH_2CH_3), 3.7071 (s, 1H, NH), 2.6977 (s, 3H, -$COCH_3$), 2.4390 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.27, 166.18, 166.14, 164.40, 148.43, 137.53, 130.90, 130.67, 125.13, 120.45, 120.07, 114.94, 109.20, 60.50, 42.83, 30.14, 16.81, 14.61; ESI-MS (m/z): 406.1 (M+1).

Ethyl 2-((2-chlorophenylcarbamoyl)methylamino)-5-acetyl-4-methylthiophene-3-carboxylate (RAA_7)

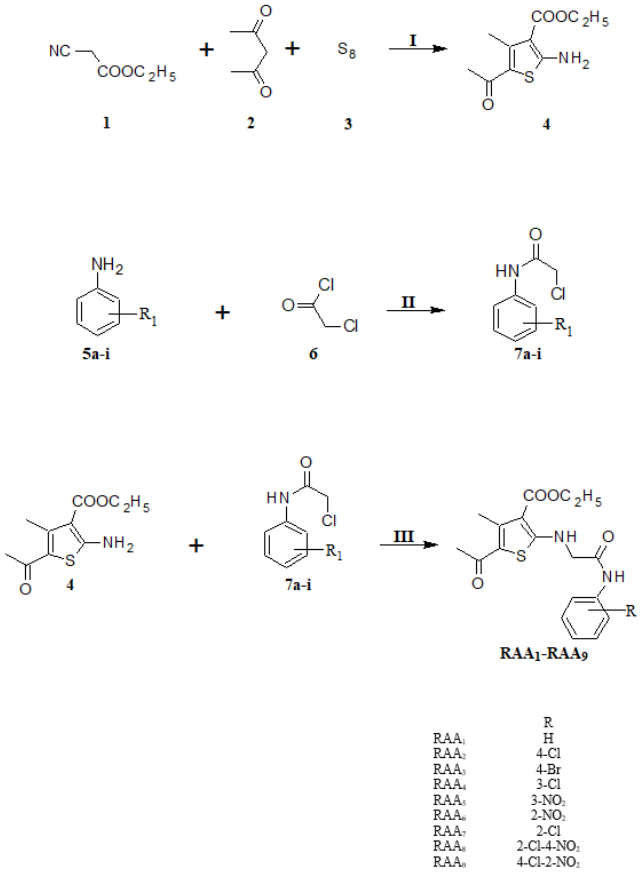
Light Brown solid (75.44%); R_f= 0.72; M.P.: 120-122°C; IR (KBr, cm^{-1}): 3410 (N-H str. coupled), 3296 (N-H str.), 3070 (Ar-H str.), 2986 (C-H str.), 1665 (C=O str.), 1604 (C=C str.), 1504 (C-N str.), 1256 (C-O str.), 836 (C-C str.), 810 (C-Cl str.), 758 (C-S str.); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.2690-7.4133 (m, 4H, Ar-H), 1.3624-1.3939 (t, 3H, OCH_2CH_3), 6.6823 (s, 1H, -CONH), 4.2401 (s, 2H, CH_2), 4.3035-4.3462 (q, 2H, OCH_2CH_3), 3.7043 (s, 1H, NH), 2.6965 (s, 3H, -$COCH_3$), 2.4294 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.16, 166.71, 166.17, 163.94, 146.47, 133.66, 129.21, 127.79, 125.52, 123.52, 121.31, 120.83, 109.20, 60.25, 43.13, 30.18, 16.87, 14.35; ESI-MS (m/z): 393.42 (M+1).

Ethyl 2-((2-chloro-4-nitrophenylcarbamoyl)methylamino)-5-acetyl-4-methylthiophene-3-carboxylate (RAA_8)

Yellow solid (88.6%); R_f= 0.60; M.P.: 118-120°C; IR (KBr, cm^{-1}): 3496 (N-H str. coupled), 3376 (N-H str.), 3117 (Ar-H str.), 1665 (C=O str.), 1588 (C=C str.), 1510 (N-O asymmetric stretch), 1310 (C-NO_2 str. aromatic), 1262(C-O str.), 947 (C-C str.), 893 (C-Cl str.), 786 (C-S str.); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.5610-8.2108 (m, 3H, Ar-H), 6.6210 (s, 1H, -CONH), 1.3653-1.3941 (t, 3H, OCH_2CH_3), 4.4219 (s, 2H, CH_2), 4.3254-4.3482 (q, 2H, OCH_2CH_3), 3.7010 (s, 1H, NH), 2.6692 (s, 3H, -$COCH_3$), 2.4146 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 191.27, 166.73, 166.12, 163.09, 146.46, 140.32, 130.52, 126.45, 125.64, 123.01, 121.34, 120.95, 109.18, 60.50, 43.88, 30.24, 16.17, 14.31; ESI-MS (m/z): 440.73 (M+1).

Ethyl 2-((4-chloro-2-nitrophenylcarbamoyl)methylamino)-5-acetyl-4-methylthiophene-3-carboxylate (RAA_9)

Yellowish Orange solid (94.1%); R_f= 0.66; M.P.: 130-132°C; IR (KBr, cm^{-1}): 2993 (Ar-H str.), 3410 (N-H str. coupled), 3296 (N-H str.), 2873 (C-H str.), 1661 (C=O str.), 1589 (C=C str.), 1455 (N–O asymmetric stretch), 1254 (C-O str.) 914 (C-C str.), 891 (C-Cl str.), 786 (C-S str.); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.5521-8.1106 (m, 3H, Ar-H), 6.6112 (s, 1H, -CONH), 1.3652-1.3938 (t, 3H, OCH_2CH_3), 4.4221 (s, 2H, CH_2), 4.3252-4.3485 (q, 2H, OCH_2CH_3), 3.7013 (s, 1H, NH), 2.6680 (s, 3H, -$COCH_3$), 2.4135 (s, 3H, -CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 192.26, 166.32, 166.18, 163.49, 146.43, 137.39, 135.03, 130.07, 129.91, 126.45, 126.41, 125.12, 109.16, 60.52, 43.89, 30.24, 15.11, 14.51; ESI-MS (m/z): 440.51 (M+1).



Scheme 1: Conditions and Reagents: (I) Stirring at 40-50°C for 4 hours, Diethyl amine; (II) Stirring at 0-5°C, Saturated Sodium Acetate Solution; (III) Reflux for 2hrs, Dioxane, Triethylamine.

Biological Assessment of the Synthesized Derivatives

Assessment of Anticancer Activity

Nine compounds were recognized to possess anti-cancer activity in the complete NCI 60 human tumor cell screen protocol at the National Cancer Institute (NCI). The molecules were first examined on about 60 cancer cell lines at a concentration of 10^{-5} M, including breast (BC), CNS (CNSC), colon (CC), leukemia (L), melanoma (M), lung (NSCLC), ovarian (OC), prostate (PC) and renal (RC) cancer. A mean graph of percent growth of treated cells revealed the selected compounds' behavior. The percentage growth was recorded using spectrophotometry in contrast to controls that were not given the experimental entities. Cell survival and proliferation were assessed throughout the 48 hours continuous drug exposure procedure utilizing a Sulforhodamine B (SRB) protein assay.[31-33] Further research was conducted at five concentrations (10^{-4} to 10^{-8} M) after compound RAA_5 showed substantial growth inhibition. GI_{50}, TGI, and LC_{50} were used as dose-response parameters for

compound evaluation. GI_{50} value (Growth inhibition of 50%) reveals the concentration of compound inhibiting 50% net cell growth. LC_{50} value (Lethal concentration that gives 50% cell kill) denotes cytotoxicity and is the compound concentration leading to a 50% net loss of initial cells after an incubation period of 48 hours. TGI value (Total Growth Inhibition) denotes the concentration of compound that inhibits total growth and denotes cytostatic impact.

The formula to compute the percent growth curve is:

$$\frac{(T - T_o)}{(C - T_o)} \times 100 \qquad \text{Eq. (1)}$$

where;
C is the vehicle control (without drug) cell count,
T denotes the day 3 cell count at test concentration, and
T_o denotes number of cells at day 0.
Drug concentrations resulting in 50% and 0% growth after 48 hours of intake of drug are used to derive GI_{50} and TGI values.

For GI_{50} Value: $\frac{(T - T_o)}{(C - T_o)} \times 100 = 50$ Eq. (2)

For TGI Value: $\frac{(T - T_o)}{(C - T_o)} \times 100 = 0$ Eq. (3)

For LC50 Value: $\frac{(T - T_o)}{(C - T_o)} \times 100 = -50$ Eq. (4)

when $T < T_o$.

The methodology outlined by the NCI/NIH Development Therapeutic Program was utilized to generate the results. After determining log GI_{50} values, full panel mean-graph midpoint (MG_MID) values were calculated. These are more significant for evaluating the activity since they are expressed in concentration values. Based on the test procedure, the observed data are logarithmic concentration values indicating inhibition of 50%. The compound is inactive. if the value is greater than -4.[34]

Assessment of Antioxidant Activity

The free radical scavenging capabilities of synthesized compounds in comparison to ascorbic acid were evaluated using the Shimada technique, which is based on the principle of scavenging the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.[35] In methanol, various concentrations of synthesized derivatives and ascorbic acid (10-100 µg/mL) were prepared. To 1-mL of 0.1 mM DPPH solution, 1-mL of each concentration of test substance and ascorbic acid was added. After vigorous shaking, the mixture was kept at room temperature in a dark place for 30 minutes, and the absorbance was measured using UV at 517 nm.[36] The % scavenging of the free radical DPPH was calculated as follows:

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \qquad \text{Eq. (5)}$$

where,
Where A_0 is the absorbance of control, and
A_1 is the absorbance of standard/ sample.

To determine the IC_{50} value for each compound, a graph showing percent inhibition against different concentrations of synthesized compounds was utilized. Compound concentration at which a 50% decrease in the original DPPH concentration occurs is regarded as IC_{50} value. A lower mean inhibitor concentration value indicates a greater free radical scavenging activity.

RESULT AND DISCUSSION

Chemistry

Using Gewald synthesis, nine novel thiophene compounds (RAA_1-RAA_9) were synthesized in this study. Ethyl cyanoacetate, acetylacetone, and sulfur were reacted in the presence of diethylamine as a base to synthesize intermediate (4). Aromatic amines and chloroacetyl chloride were reacted under cold conditions to give 2-chloro-*N*-(substituted phenyl) acetamides (7a-i). By reacting equimolar amounts of 4 and different 2-chloro-*N*-(substituted phenyl)acetamides in the presence of triethylamine, target products were obtained in 75–86% yields. Various spectroscopic techniques were used to characterize the synthesized derivatives, all of which agreed with their assigned chemical structures.

Biological Assessment of the Synthesized Compounds

Anti-cancer Activity

The anti-cancer potential of chosen derivatives (RAA_1-RAA_9) was tested on 60 human cancer cell lines at the NCI, USA. Compound behavior is shown in Table 2 as a mean graph of

Table 2: Anti-cancer screening data as a percentage of growth at a single dose (10^{-5} M) assay

Compound	*NSC Code (provided from NCI)*	*CNSC*	*NSCLC*	*OC*	*BC*	*RC*	*M*	*L*	*CC*	*PC*	*Mean Growth Percent*
RAA_1	D-827787 / 1	96.90	95.26	107.95	96.45	96.91	106.32	107.17	103.23	101.97	101.25
RAA_2	D-827788 / 1	99.03	95.43	109.69	98.75	103.30	104.20	107.83	106.51	105.77	103.03
RAA_3	D-827789 / 1	101.92	98.46	115.09	102.96	102.23	105.22	108.22	107.13	107.16	105.00
RAA_4	D-827790 / 1	101.62	97.12	108.03	100.07	101.24	105.17	105.72	104.14	109.38	102.99
RAA_5	D-827791 / 1	-10.39	-10.88	-8.52	-8.53	-30.48	-32.72	-2.67	-23.53	-14.22	-16.98
RAA_6	D-827792 / 1	99.63	96.63	106.70	100.76	97.70	102.18	107.01	104.01	101.34	101.56
RAA_7	D-827793 / 1	96.97	98.06	106.49	96.47	94.68	102.93	107.07	104.55	109.23	101.09
RAA_8	D-827794 / 1	96.26	98.72	107.10	96.28	97.81	100.82	100.74	108.20	112.77	101.18
RAA_9	D-827795 / 1	95.86	91.34	99.49	94.28	95.63	99.11	106.57	97.66	93.46	97.11

***** *CNSC:* Central Nervous System Cancer; *NSCLC:* Non-Small Cell Lung Cancer; *OC:* Ovarian Cancer*; BC:* Breast Cancer; *RC:* Renal Cancer; *M:* Melanoma; *L:* Leukemia; *CC:* Colon Cancer; *PC***:** Prostate Cancer

treated cell growth percentage. RAA_5 showed the highest mean growth percent activity of -16.98%. The most susceptible cancer cell lines were breast, renal, lung, leukemia, and CNS cancer (Table 3). After suppressing cell growth in several cell lines at 10^{-5} M concentrations, compound RAA_5 was examined further using a 5-log dosage molar range. GI_{50} values reported for RAA_5 ranged from 0.411 to 2.8μM (Table 4). For the selected compound, all leukemia cancer cell lines had TGI values greater than 100 mM. MG_MID values were estimated after calculating their log GI_{50} values. Substantial activity was seen in compound RAA_5 with MG_MID value -5.82. One Dose Mean Graph of RAA_5 is presented in Figure 1.

Table 3: Synthesized compound anti-cancer action against most sensitive cell line

Compound	*Range of Growth %*	*Cell line with the highest sensitivity*	*Most susceptible cell line's growth percentage*	*Growth inhibition (GI%)*
RAA_1	76.33 to 128.42	NSCLC (EKVX)	76.33	23.67
		RC (UO-31)	83.25	16.75
		CNSC (SNB-75)	84.12	15.88
		NSCLC (HOP-92)	85.54	14.46
		BC (MCF7)	89.82	10.18
RAA_2	74.65 to 127.22	NSCLC (EKVX)	74.65	25.35
		BC (MCF7)	85.98	14.02
		CNSC (SNB-75)	90.21	9.79
		RC (UO-31)	90.30	9.70
		NSCLC (NCI-H522)	91.13	8.87
RAA_3	85.70 to 133.34	CC (HCT-116)	85.70	14.30
		NSCLC (NCI-H23)	89.40	10.60
		NSCLC (EKVX)	89.48	10.52
		RC (CAKI-1)	90.00	10.00
		NSCLC (NCI-H522)	91.36	8.64
RAA_4	85.32 to 119.49	CNSC (SNB-75)	85.32	14.68
		NSCLC (EKVX)	86.00	14.00
		RC (UO-31)	88.87	11.13
		NSCLC (HOP-92)	90.22	9.78
		BC (MCF7)	91.25	8.75
RAA_5	-86.48 to 95.02	L (SR)	0.31	99.69
		NSCLC (EKVX)	0.60	99.40
		OC (OVCAR-5)	1.06	98.94
		M (SK-MEL-28)	2.62	97.38
		CC (SW-620)	3.64	96.36
		CNSC (SNB-19)	9.50	90.50
		BC (MDA-MB-231/ ATCC)	10.25	89.75
RAA_6	71.26 to 116.47	RC (UO-31)	71.26	28.74
		RC (CAKI-1)	81.62	18.38
		NSCLC (HOP-92)	86.26	13.74
		NSCLC (HOP-62)	91.50	8.50
		BC (MCF7)	92.12	7.88
RAA_7	81.11 to 120.60	RC (UO-31)	81.11	18.89
		NSCLC (EKVX)	81.61	18.39
		CNSC (SNB-75)	83.30	16.70
		BC (BT-549)	84.33	15.67
		NSCLC (HOP-92)	86.02	13.98
RAA_8	77.94 to 120.83	RC (UO-31)	77.94	22.06
		CNSC (SNB-75)	82.04	17.96
		BC (BT-549)	85.04	14.96
		NSCLC (HOP-92)	88.44	11.56
		RC (CAKI-1)	88.64	11.36
RAA_9	70.82 to 112.29	RC (UO-31)	74.18	25.82
		NSCLC (EKVX)	76.89	23.11
		CNSC (SNB-75)	80.05	19.95
		NSCLC (HOP-92)	82.44	17.56
		CC (HCT-116)	83.26	16.74

*60 cell lines assay in 1 dose 10^{-5} M conc.

Table 4: Five-dose assay of compound RAA5 against 60 human cancer cell lines

		RAA_5			
Panel	*Cell line*	GI_{50}	*TGI*	LC_{50}	*MG_MID***
	MCF7	1.44	5.21	44.9	
	MDA-MB-231/ ATCC	2.12	4.77	20.4	
BC	HS 578T	2.05	5.97	>100	-5.73
	BT-549	1.44	4.85	43.8	
	T-47D	2.31	6.70	86.8	
	MDA-MB-468	1.85	4.53	34.00	
PC	PC-3	0.794	3.63	>100	-5.88
	DU-145	2.21	5.91	>100	
	786-0	1.97	4.21	9.01	
	A498	1.70	3.31	6.46	
	ACHN	1.50	2.90	5.62	
RC	CAKI-1	1.27	3.65	11.4	-5.80
	RXF 393	1.21	2.51	5.23	
	SN12C	1.84	5.40	35.6	
	TK-10	2.10	6.81	>100	
	UO-31	1.27	2.62	5.38	
	IGROV1	0.414	1.62	6.85	
	OVCAR-3	2.29	5.69	51.4	
	OVCAR-4	2.23	6.36	>100	
OC	OVCAR-5	1.42	4.19	16.7	-5.82
	OVCAR-8	2.80	-	>100	
	NCI/ADR-RES	1.77	5.39	>100	
	SK-OV-3	1.20	3.46	-	
	SF-268	2.11	5.16	>100	
	SF-295	2.17	5.11	16.1	
	SF-539	1.82	4.48	13.1	
CNSC	SNB-19	2.31	6.50	60.3	-5.67
	SNB-75	2.03	5.61	>100	
	U251	2.36	9.14	43.8	
	COLO 205	1.65	4.60	30.6	
	HCC-2998	1.98	3.91	7.73	
	HCT-116	1.71	5.02	50.9	
CC	HCT-15	0.845	5.35	58.5	-5.90
	HT29	0.797	3.26	39.6	
	KM12	2.61	7.99	62.5	
	SW-620	0.491	7.69	75.9	
	A549/ATCC	2.57	8.02	>100	
	EKVX	2.10	5.52	27.6	
	HOP-62	2.50	6.58	>100	
	HOP-92	1.68	4.56	17.3	
NSCLC	NCI-H226	1.64	4.46	>100	-5.76
	NCI-H23	1.14	3.45	>100	
	NCI-H322M	2.16	5.85	25.9	
	NCI-H460	1.21	3.26	8.78	
	NCI-H522	1.28	4.12	36.9	
	LOX IMVI	0.411	1.67	4.82	
	MALME-3M	1.51	3.06	6.16	
	M14	1.50	3.28	7.14	
	MDA-MB-435	1.68	3.10	5.71	
M	SK-MEL-2	2.01	4.84	27.8	-5.87
	SK-MEL-28	1.78	3.71	7.74	
	SK-MEL-5	1.68	3.17	6.00	
	UACC-257	2.50	7.47	>100	
	UACC-62	0.574	2.14	6.10	
	CCRF-CEM	0.585	>100	>100	-5.92
	HL-60(TB)	2.57	14.2	>100	
	K-562	1.89	>100	>100	
L	MOLT-4	0.570	>100	>100	
	RPMI-8226	2.51	6.79	>100	
	SR	0.646	>100	>100	

*Values are in µM

**Full Panel Mean Graph Midpoint

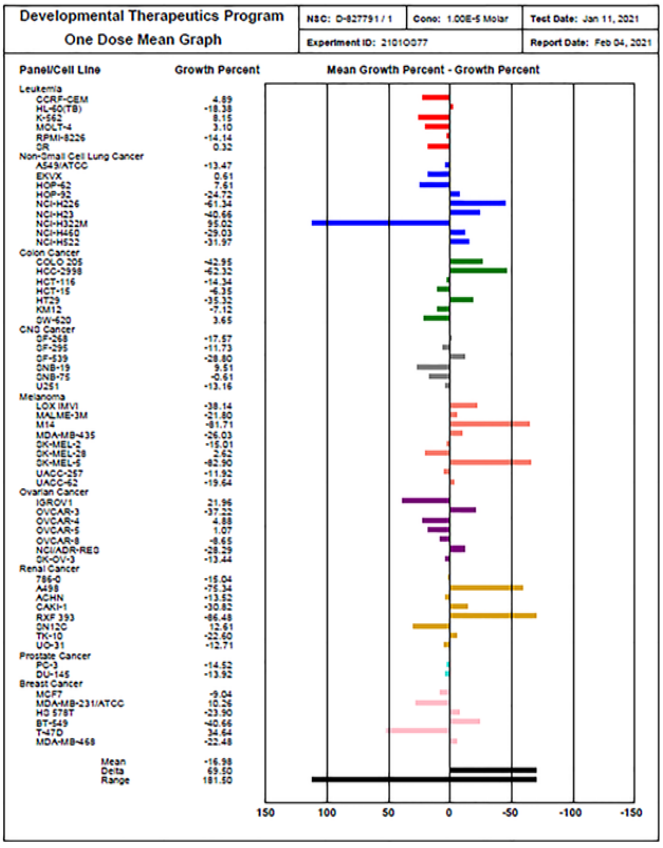


Figure 1: One Dose Mean Graph of compound RAA_5

Antioxidant Activity

The antioxidant potential of the synthesized compounds was evaluated *in vitro* using the DPPH assay in terms of percentage (%) inhibition (Figure 2). The IC_{50} value of synthesized compounds was obtained by plotting concentrations against percent inhibition of the test compound. (Figure 3).

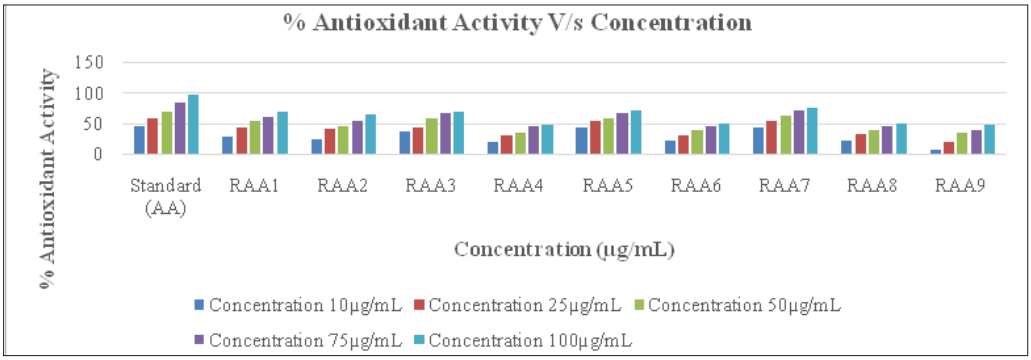


Figure 2: DPPH Scavenging free radical activity of the synthesized thiophene derivatives

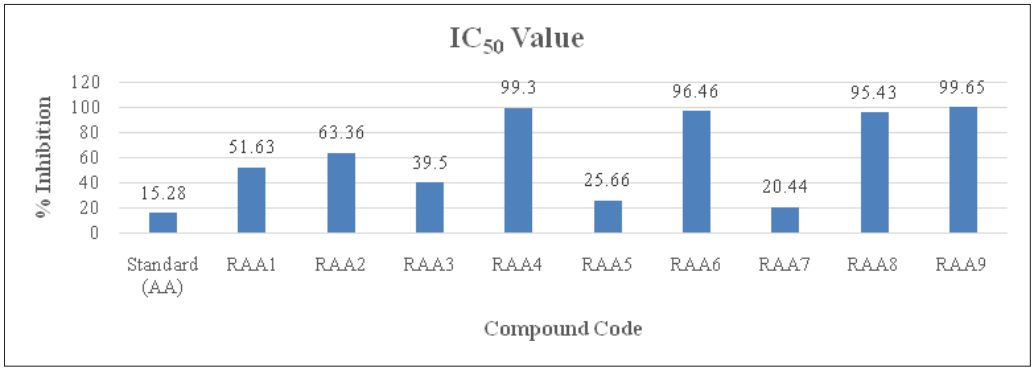


Figure 3: IC_{50} values of synthesized derivatives

Table 5: Radical scavenging properties of synthesized derivatives

Compound Code	*Concentration (µg/mL)*					
	10	*25*	*50*	*75*	*100*	*IC_{50}*
Standard*	45.50	57.61	68.61	84.22	97.26	15.28
RAA_1	27.24	42.4	53.09	60.18	67.87	51.63
RAA_2	22.71	41.69	45.67	53.22	64.04	63.36
RAA_3	37.26	43.71	57.34	65.6	69.17	39.5
RAA_4	19.85	29.32	34.77	45.09	47.97	99.3
RAA_5	41.83	53.32	57.48	66.78	71.43	25.66
RAA_6	22.16	29.32	38.34	44.78	49.12	96.46
RAA_7	42.58	53.52	62.79	71.7	74.15	20.44
RAA_8	22.47	32.42	37.62	45.16	49.79	95.43
RAA_9	6.76	19.45	34.27	39.55	47.21	99.65

*Ascorbic Acid

According to the outcomes, only a few synthesized compounds (RAA_5 and RAA_7) showed substantial antioxidant activity in comparison to ascorbic acid, while others exhibited moderate to strong antioxidant properties. The results of the antioxidant screening are indicated in Table 5.

CONCLUSION

To summarize, this paper discusses the synthesis and pharmacological potentials of novel thiophene derivatives. Compound RAA_5 presented excellent anti-cancer and antioxidant activity. Presence of the electron-withdrawing group at benzylidene ring conferred upon it the highest anti-cancer and antioxidant activity. These possible, encouraging biological screening findings of synthesized derivatives will provide a substantial foundation in this domain, perhaps contributing to the development of effective remedies.

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